



Best Practice Manual for the Forensic Examination of Fibres

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Acknowledgements

Over 20 years have passed since the publication of the first version of the Manual of Best Practice for the Forensic Examination of Fibres (2001). In 2003 the efforts of those involved in its production were already officially recognised by the ENFSI board. This was the first 'Manual of Best Practice' produced by any of the ENFSI working groups and it became the template which others used to produce similar documentation. After 10 years a revised version was made available online (2011) and its structure was amended by splitting it up to several single guidelines. During this process also the original European Fibre Working Group (EFG) has changed into European Textile and Hair Group (ETHG). Inspired by the Fibre manual some members of the ETHG have produced a first version of the Best Practice Manual for the Microscopic Examination and Comparison of Human and Animal Hair (2015). The present version of the Fibre BPM is based on the ENFSI official template and it can be seen as a subtle merging between past and present, between established knowledge and recent needs for standardisation and uniformisation in the Forensic community.

Many individuals have contributed to the Manual of Best Practice but it is especially important to acknowledge the efforts of the original sub-group chairpersons as well as those responsible for the previous guidelines:

- Mike Grieve, Weine Drotz, Walter Brueschweiler, Roger Robson, Ruth Griffin, Ken Wiggins, Jolanta Was-Gubala and Kornelia Nehse (2001)

- Peter Adolf, Henk Leijenhorst, Bridget Fleming, Chris Gannicliffe, Ray Palmer, Joachim Hellwig, Pavel Kolar, Peveline Drummond, Georg Jochem, William Hutchinson, Jolanta Was-Gubala, Avner Rosengarten, Genevieve Massonnet, Laurent Lepot, Aija Kampa, Ieva Gutovska, Elina Dubro and Ulrike Decke (2011).

Without the enthusiasm of these individuals the former guidelines would never have existed. Sadly, some of the original members who were instrumental in the inception of the guidelines are no longer with the group. The Steering Committee of the ETHG would like to thank them for their participation and dedicate this latest revision to the memories of those who have passed.

The present BPM version was prepared by:

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Due to the corona virus pandemic, the preparation of the present document took over two years and involved over 20 video conferences. The completion of this document is a result of the considerable amount of time and work being dedicated by the above individuals, without whom this version of the BPM would not have been possible.

The team developing the present BPM version also received support from the ETHG Steering Committee, especially ETHG chairperson Jan-Eric Grunwald (Bayerisches Landeskriminalamt Bavarian State Criminal Police Office – Munich, Germany).

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1 AIMS

This Best Practice Manual (BPM) aims to provide a framework for procedures, quality principles, training processes and approaches to the forensic examination of textile fibres. This BPM can be used by Member laboratories of ENFSI and other forensic science laboratories to establish and maintain working practices in the field of forensic examination of fibres that will deliver reliable results, maximize the quality of the information obtained and produce robust evidence. The use of consistent methodology and the production of more comparable results will facilitate interchange of data between laboratories.

The term BPM is used to reflect the scientifically accepted practices at the time of creation. The term BPM does not imply that the practices laid out in this manual are the only good practices used in the forensic field. In this series of ENFSI Practice Manuals the term BPM has been maintained for reasons of continuity and recognition.

2 SCOPE

This Best Practice Manual (BPM) provides guidelines for the entire forensic process of fibre examination (including recovery at scenes of crime and in the laboratory), laboratory examination (identification, comparison and analysis), evidential evaluation and interpretation, and presentation of evidence. This BPM applies to criminal cases as well as to accident investigation cases (e.g., fibre plastic fusion).

It does not address other textile examinations such as textile damage analysis or textile imaging. This BPM is intended for experts in the field and assumes prior knowledge in this discipline. It is not a standard operating procedure (SOP) and addresses the requirements of the judicial systems in general terms only. Relevant SOPs and method descriptions are attached in the appendices.

This document also encompasses the requirements for systems, procedures, personnel, equipment and facilities for the forensic process of fibre examination.

3 DEFINITIONS AND TERMS

For the purposes of this Best Practice Manual (BPM), the relevant terms and definitions given in ENFSI documents, the ILAC G19 “Modules in Forensic Science Process”, and in standards such as ISO 9000, ISO 17000, 17020 and 17025 are applied. Other relevant and specific definitions, which assist in the interpretation of this manual, are listed below.

Activity Level

The degree of support that can be assigned that an alleged activity is responsible for the transfer of a particular trace item (or items) between two surfaces or items. For this type of evaluation information regarding the framework of circumstances of the case is needed.

Associated items

Two or more items that have material present on them that appears to have originated from a common source.

Background trace

Trace(s) collected that appear to have no relevance to the crime.

Brushing

Surface debris collection technique using a brush; this is not recommended as a routine method for fibre recovery by the ETHG.

Case assessment

Also referred to as 'initial case assessment' or 'case pre-assessment'. It is an exercise involving the evaluation of circumstances and exhibits relating to a submitted case. Aspects to be considered are: the question being asked by the customer, the examination priorities and strategy, the potential examination outcomes and their associated evidential value in order to increase the effectiveness of said examination.

Case file

The physical (may also include electronic) documentation of an individual case containing all the relevant information required and generated, in order to produce a report for use by the court (e.g., the circumstances of the case itself, communications with submitting officer(s), details of continuity, results of the laboratory examinations and the final report itself).

Chain of custody

The total physical (and electronic) documentation detailing the continuity of the physical handling of items and samples, within a sphere of responsibility e.g., from submission to the forensic laboratory until return to the submitting agency.

Characterisation

The determination and documentation of features such as surface morphology, cross sectional shape, colour, damage and the results of instrumental analyses, which distinguish a particular fibre or population of fibres from other fibre populations. 'Identification' (see below) may be considered as part of this process.

Clippings

Collecting technique using scissors; usually of fingernails.

Collection

The physical recovery and packaging of an item/sample/sub-sample after its detection.

Collective

A group or population of fibres that appear to have a common origin due to sharing comparable characteristics.

Contamination

Introduction of material to an item which is not 'crime – related' and has been inadvertently introduced to it either at the crime scene, laboratory or during its storage and transportation.

Controlled environment

A location controlled in respect of physical environment, access and cleanliness, e.g., examination rooms in a laboratory.

Crime scene

Any location where illegal activity has taken place – may also be called "the scene".

Detection

The finding of an item/sample/character that is of evidential value.

Documentation

A written description of e.g., an item, event, circumstances on paper, drawings, diagrams or photographic images (Including those in an electronic format). For full validity documentation should be signed and dated by all concerned.

ETHG E-learning platform

Created by the European Textile and Hair Group (ETHG), this platform gives a practical demonstration of the best practices in forensic fibre examination through videos and illustrated

presentations, made by experienced practitioners in the field. Access is limited outside the ETHG working group and the European forensic community. <https://e-learning.ethg.eu>

Evidence

Established nature of an object or event that provides support to a hypothesis and is of interest to the court.

Evidential value

The judged, calculated, assessed or estimated supporting level that a finding provides to a hypothesis or proposition.

Evaluative reporting

Providing an opinion on the degree to which the laboratory findings in a case corroborate or refute a particular set of hypotheses or propositions placed before the court.

Evidentiary item

An item of physical evidence; an object with an established nature that provides evidential value.

Exhibit

An object under investigation which may bear evidence. See 'Item' .

Identification

The classification of a particular fibre type according to its morphology and/or chemical composition. In some fibres (e.g., polyacrylonitrile) the presence of co-polymers may allow a more specific identification (i.e., a 'sub-classification'). 'Identification' may be considered as part of the process of 'characterisation'.

Item

An object under investigation which may bear evidence. See 'Exhibit' .

Fibre Distribution Map

A diagram showing the areas where crime relevant fibres were recovered on a body or item. Such information can be generated using 'one to one' or 'zonal' taping and can be useful in corroborating an alleged activity responsible for the fibre transfer in question.

FTIS (Fibre Type Information System)

Created by the European Textile and Hair Group (ETHG), FTIS is a database and an information platform for the characterisation of common and rare fibre types and set up on a web-based platform with easy accessibility for the European Forensic Fibre Community. Access is limited outside the ETHG working group and the European forensic community. <https://ftis.kontrollwerk.com/>

Known sample

A sample taken from a known source for comparison against recovered samples. (Also known as a 'control sample', 'reference sample', 'exemplar', 'target fibre').

Locard's Exchange Principle

A principle proposed by Edmond Locard, stating that objects in physical contact will reciprocally exchange material.

Loss

Undesired release e.g., of transferred fibres from a textile surface.

Man-made fibre

Man-made fibres can be classified into two groups, those derived from natural organic polymers such as cellulose (regenerated cellulosic fibres e.g., viscose, modal, lyocell etc.) or protein and those synthesized from organic chemicals found in coal and oil (e.g. polyester, polyamide, acrylic etc.), often referred to as 'synthetic fibres'.

Object

An entity of variable size that itself is defined as physical evidence. From the object you may recover traces that also may be defined as physical evidence.

One-to-one taping

A detailed collection technique involving surface debris taping, where the area of the tape exactly represents the same area on the surface being taped. Any recovered material from the tapes can then be associated with an exact location on the body or item, in order to construct a fibre distribution map. See also 'Taping', 'Zonal Taping' and 'Fibre distribution map'.

Packaging

Physical covering of items and samples, providing security to the contents and preventing contamination or loss, with a unique ID.

Peer review

The process of checking casework notes and associated report(s) for factual errors and/ or inaccuracies. Where evaluative opinions are expressed in a report, these should also be reviewed to check the 'robustness' and underlying logic. These checks should be performed by a suitably qualified colleague or associate.

Persistence

The degree to which an object retains trace evidence (e.g., fibres on a garment) relative to factors such as time, handling and treatment since transfer.

Picking-off

Removal of fibres manually e.g., with forceps.

Practitioner

A person who is suitably qualified and trained to carry out one or more functions in a forensic science laboratory (e.g., technician, court reporting scientist etc.).

Preservation

Action taken to ensure the integrity of an exhibit or trace evidence.

Questioned

Disputed e.g., by the prosecutor and/or the defence.

Recommendation

A particular action or methodology which is deemed to be desirable or best practice in a particular circumstance, but not mandatory.

Recovered sample

Sample obtained from a crime scene or exhibit with the aim of identification or comparison with known samples.

Recovery

The detection, collection and preservation of an item or a sample.

Sample

A representative part of an item or a trace removed for separate analysis; further sub-samples may be removed from the sample.

Scraping

Removal of fibres by manually scraping an exhibit with a blunt edge; not routinely recommended for fibre recovery by the ETHG.

Searching

Action to find single fibres or fibre tufts of interest in traces recovered from items or exhibits. Can be carried out visually or via microscopy.

Shedding capacity

An assessment of the transfer potential of a textile. Also called shedding potential or sheddability test.

Shaking

Removal of particulate traces by manually shaking items over a recipient medium in which the material can be collected and secured. It is often used in combination with 'Scraping' (see above). It is not recommended as a routine method of fibre recovery by the ETHG.

Source level

The degree of support that can be assigned that a particular trace item has originated from a putative source based on lab results.

Synthetic fibre

A term describing a fibre that is synthesized from organic chemicals found in coal and oil and whose polymeric/chemical structure does not occur in nature. The ETHG considers '*synthetic fibres*' to be a sub-set of 'man-made fibres'.

Taping or tape lifting

Action where clear adhesive tapes are pressed onto a surface to collect trace evidence present in surface debris. The tapes are then pressed onto a clear sheet (or folded back on themselves) to secure the material and maintain its integrity. See also 'one-to-one taping' and 'zonal taping'.

Target fibre

A fibre type in a fabric or defined from the background traces and chosen after an evidential assessment to be used in active searching for crime relevant fibres (see 'Known sample'). A fibre(s) of unknown provenance from a fibre population/collective may also be used for such purposes.

Textile imaging

A process to identify clothing from video and images for comparison purposes with questioned items of clothing.

Textile reference sample

A sample used to determine the fibre type in a textile and to be used to identify possible target fibres (see 'Known sample').

Trace

A smaller or larger physical part of a physical object (item) that can be further analysed for evidential purposes.

Traceability

The recognition of the ability/possibility to trace events in the total documentation (records and labelling) e.g., possibility to follow all the steps in handling and timing and identifying staff involved in an examination sequence.

Transfer

The phenomenon of displacement of material from one item, surface or person to another item, surface or person. Such displacement of material can occur from; one surface to another ('One-way transfer'), reciprocally ('Two-way transfer') or from one surface to another by an intermediary surface ('Secondary transfer').

Transporting

Physical transfer of items or persons from one location to another.

Vacuuuming

A technique for collection by the use of a vacuum cleaner and special filters. This is not recommended by the ETHG as a routine fibre recovery method, however, there may be circumstances where taping is ineffective and this method could be considered (e.g., surface heavily soiled with particulate debris). Where this method is employed, the apparatus and filters must be scrupulously cleaned and be free of potential contaminants.

Zonal taping

A method of tape lifting where the body or item being taped is divided into distinct areas or 'zones'. Each zone is systematically taped so that any recovered material can be associated with a particular zone in order to construct a fibre distribution map. Whilst a fibre distribution map generated by this method is less detailed than 'one-to-one' taping, it is much less time consuming both at the scene and in the laboratory. Usually, zonal taping generates sufficient information. A combination of both methods may also be used.

4 RESOURCES

4.1 Personnel

For the purposes of this BPM the term 'practitioner' is used for any trained and competent individual undertaking any stage of textile fibres examination, including recovery and examination, evidential evaluation and interpretation, and presentation of evidence.

Skill levels for different tasks are listed in 4.1.1 – 4.1.4. In different organisations individuals can cover one or all of the tasks listed, for example most organisations have court reporting practitioners and non-court reporting practitioners.

Practitioner competence should be regularly assessed by proficiency tests and/or collaborative exercises. Organisations should maintain a record of the education and job-specific training, assessment and ongoing competency of their practitioners.

4.1.1 Recovery and reference sampling

For those practitioners recovering fibres at crime scenes and from items in the laboratory, and practitioners taking reference samples, the following skills are necessary:

- Familiarity with relevant health and safety issues;
- Appropriate knowledge of textile fibres;
- Awareness of contamination risks;
- Awareness of other trace materials;
- Competence in trace evidence techniques in accordance with the requirements at the examining laboratory (e.g., method of recovery, packaging, documentation).

4.1.2 Sample preparation

For those practitioners preparing samples in the laboratory for further testing (e.g., mounting) the following skills are necessary:

- Familiarity with relevant health and safety issues;
- Appropriate knowledge of textile fibres;
- Awareness of contamination risks;
- Awareness of other trace materials;
- Familiarity with the relevant laboratory protocols.

4.1.3 Examination and microscopy

For those practitioners examining samples in the laboratory the following skills are necessary:

- Familiarity with relevant health and safety issues;
- Awareness of contamination risks;
- Awareness of other trace materials;
- Competence in operating relevant instruments and equipment;
- Competence in fibres examination and comparison;
- Familiarity with the relevant literature.

4.1.4 Evaluation, interpretation and reporting of examinations and microscopy

For those practitioners evaluating, interpreting and reporting the results of textile fibre examinations the following skills are necessary:

- Competence in assessing case requirements, devising and directing examination strategies and evaluating examination outcomes;
- Competence in examination and comparison of textile fibres;
- Understanding of the relevance of other trace materials;
- Knowledge of contamination risks;
- Knowledge of methodology and relevant instruments and equipment;
- Knowledge of the relevant literature;
- Competence in preparing and presenting evidence (written or verbal) for the relevant jurisdiction.

Note: This BPM has been written primarily from the viewpoint of forensic laboratory personnel, but it is accepted that in some organisation's other individuals, including Police Officers, Crime Scene Technicians, Medico-legal Experts, and Medical Examiners, may play a role in recovery of textile fibres. These practitioners must have the relevant education and training listed in section 4.1.

4.2 Equipment

4.2.1 At the crime scene

Recovery techniques on the crime scene should be focused on a systematic collection as fibre traces can be invisible to the naked eye. The widely used method for the collection of fibre traces at the crime scene is by means of transparent adhesive tapes. The collection technique should allow an easy search of the collected traces in the laboratory as well as a safe long-term storage (refer to Section 8.1 for equipment and materials).

4.2.2 In the laboratory

In order to achieve the highest discriminating power, it is important to use a combination of different methods that can differentiate fibres from each other. Many techniques involve different forms of microscopy. The nature and extent of the fibre examinations required, and also access to different equipment in the organisation, will dictate which types of analyses are used.

The equipment for the techniques used for fibre identification/comparison should be applicable to the smallest sample sizes, be highly discriminating and, where possible, be non-destructive. The equipment of the methods detailed below fulfils these criteria and are currently available to forensic fibre practitioners. The equipment should be used in accordance with the recommendations in the methods and appendices in this Manual (refer to Section 5).

4.2.3 Microscopy

Low power microscopy

- Objectives and eyepieces, range of magnification up to approximately 100X.
- Accessories for polarization and fluorescence (optional).

High power microscopy

- Can be standalone microscopes or comparison microscopes with different applications such as stated below.

Brightfield microscopy

- Objectives and eyepieces, range of magnification approximately 40X to at least 400X.
- Ocular micrometer scale or validated computer software.

Polarising microscopy

- Often an application to a brightfield/comparison microscope, see equipment above.
- A rotating stage.
- Analyser, polariser and a slot for compensators or lambda plate.
- Eyepiece cross-hair graticule when birefringence is calculated (optional).

Fluorescence microscopy

- Often an application to a brightfield/comparison microscope, see equipment above.
- A selection of broadband excitation filters covering the range from ultra-violet to violet, blue and green.

Miscellaneous techniques

- For interference microscopy, cross sectioning, solubility, melting point determination and scanning electron microscopy, see Appendix 1, Microscopy of Textile Fibres.

4.2.4 Microspectrophotometry

- A microscope suited for purpose.
- A magnification range / resolution suited for purpose.
 - Used in the visual range; apochromatic fluorite objectives.
 - Used in the UV and visual range; special lens objectives made from quartz.
- Photometer devices.
 - For scanning microspectrophotometers; a light source, a monochromator and a photomultiplier.
 - For multichannel microspectrophotometers (MCS); a light source, a polychromator and a diode array detector (DAD) or a charge-coupled device (CCD).
- An appropriate computer system and software for the generation, visualisation and comparison of MSP-spectra.

For more information, see Appendix 2, Microspectrophotometry of Textile Fibres.

4.2.5 Infrared spectroscopy

- A microscope suited for purpose.
- A magnification range / resolution suited for purpose.
- A detector.
 - Utilised with a microscope, e.g., mercury cadmium telluride (MCT) detectors.

- Utilised on the FTIR main bench, e.g., deuterated triglycine sulphate (DTGS) detectors.
- Different applications/sampling devices such as ATR, diamond anvil cell, golden gate etc.
- An appropriate computer system and software for the generation, visualisation and comparison of IR-spectra.

For more information, see Appendix 3, Infrared Spectroscopy of Textile Fibres.

4.2.6 Raman spectroscopy

- A microscope suited for purpose.
- A magnification range / resolution suited for purpose.
- A dispersive spectrometer using a grating-based dispersive unit and a charge-coupled device (CCD) detector.
- Lasers, preferably more than one, for example 514 or 532 nm (green) and 785 nm (NIR).
- Rejection filters suitable for the laser wavelengths used.
- An appropriate computer system and software for the generation, visualisation and comparison of Raman-spectra.

For more information, see Appendix 4, Raman Spectroscopy of Textile Fibres.

4.2.7 Other techniques

For other techniques such as thin layer chromatography (TLC) or High Performance Liquid Chromatography (HPLC), Pyrolysis Gas Chromatography (PyGC or PyGCMS) or pyrolysis/mass spectrometry (PyMS), see Appendix 5, Chromatographic Techniques and Appendix 6, Other Analytical Techniques.

4.3 Equipment maintenance and performance checks

Any maintenance, problems, calibration and performance checks must be documented and dated.

4.3.1 Maintenance

An equipment record should be maintained that records the manufacturer, model, serial number, software and firmware version, the date of acquisition, the date placed in service and the location for each piece of equipment used in the examination and comparison of fibres.

The practitioner should be familiar with and follow the manufacturer's operating manual and maintenance recommendations for each piece of equipment used for the examination. These should be readily available, together with any repair and/or general maintenance documents.

Any technical maintenance or recalibration should be executed by a suitably qualified and accredited engineer.

4.3.2 Performance and calibration checks

All equipment must be correctly set up as detailed in the manufacturer's instructions and all users must be fully trained in their operation.

It is recommended that the performance of the equipment is checked against appropriate working standards every time it is used. The checks against appropriate standards should also be performed if anything is changed in the optical paths of any equipment.

Only equipment that is operating properly should be employed in casework, and then only within the limits of its performance check.

4.4 Reference materials

4.4.1 Textile fibre collections

Laboratories should hold some form of reference collections of known synthetic fibres, natural fibres and animal fibres to assist in fibre identification.

Where possible the collections should be authenticated and traceable. It is advisable that the laboratory puts into place appropriate control measures to ensure that the integrity of any authenticated samples is maintained.

If the collections are not authenticated or traceable, they should be used with caution and referred to for guidance and indication purposes only. Those collections could potentially be authenticated by cross-analysis between several forensic laboratories.

4.4.2 Calibration and working standards

All working standards should be calibrated by an accredited agency.

4.4.3 Microscopy

Calibrated stage micrometer: A slide with a linear scale of known dimensional divisions, against which the 'working standard' ocular micrometer/eyepiece graticule/validated software programme can be compared.

Optical balance fibre reference slides for comparison microscopy (e.g., brightfield and fluorescence): Paired slides with a uniformly coloured sample of fibres typically cut in half and mounted on slides.

Polarisation identification slides: Slides with cross-checked synthetic fibres.

For miscellaneous techniques such as interference microscopy, cross-sectioning, solubility, melting point determination and scanning electron microscopy, see Appendix 1, Microscopy of Textile Fibres.

4.4.4 Microspectrophotometry

Certified holmium and/or didymium filter: Wavelength accuracy check.

Certified absorption filters/ neutral density filters: Absorption accuracy check.

4.4.5 Infrared (IR) spectroscopy

Certified polystyrene or indene: Wavelength accuracy check.

4.4.6 Raman spectroscopy

Raman shift standard, for example certified polystyrene or silicon: Wavelength accuracy check.

4.4.7 Other techniques

For chromatographic techniques such as Thin Layer Chromatography (TLC) or High Performance Liquid Chromatography (HPLC), Pyrolysis Gas Chromatography (PyGC) or

PyGCMS) or pyrolysis/mass spectrometry (PyMS), see Appendix 5, Chromatographic Techniques and Appendix 6, Other Analytical Techniques.

4.5 Facilities and environmental conditions

4.5.1 At the crime scene

The environmental conditions vary considerably for every crime scene (see Section 8.1).

4.5.2 In the laboratory

Laboratories used for the examination of items for fibres should be designed for efficient and effective working (see Section 8.2). Particular consideration should be given to the need for avoidance of contamination (see Section 8.1.1).

To minimise the chance of contamination, some consideration should be given to the following:

- Laboratories, equipment and sampling materials used for the examination and comparison of fibres should be cleaned thoroughly before and after use.
- Laboratories should provide a minimum of two purpose designed search rooms with limited access, for searching items. This allows for physical separation of associated items from different sources, such as suspects, victims and the scene. If different examiners and/or rooms cannot be used for associated items, there must be a clear, documented time gap and evidence of relevant decontamination procedures between searches.
- In situations where evidence types other than fibres may be of potential significance the laboratories should provide accommodation to allow for the effective recovery of the different evidence types.

Regarding environmental conditions and accommodation needed for specific equipment, the manufacturer's operating manual and maintenance recommendations for each piece of equipment should be followed.

4.6 Materials and reagents

4.6.1 At the crime scene

Materials used in recovery/sampling fibres at the crime scene can include the use of clear adhesive tape and backing, forceps and scissors.

The choice of personal protective equipment (PPE) such as protective coats, disposal gloves and facemasks will be determined by the aims of the examiner and just as importantly, the hazard assessment of the scene itself (see Sections 8.1.1 and 14.1). For the purposes of anti-contamination, it is recommended that a protective overall, disposal gloves and facemasks should be worn as a minimum.

4.6.2 In the laboratory

All materials and chemicals used for the examination and comparison of fibres should be of suitable quality and demonstrated to be fit for purpose.

All chemicals and reagents, whether manufactured internally or obtained from external suppliers, should be labelled with their identity, concentration (if appropriate), date of preparation or receipt, date of opening, date of expiry and any special storage or safety requirements, if applicable, to comply with laboratory policy and/or appropriate regulations. The identity of the individual preparing reagents produced in the laboratory should also be recorded together with records of the preparation procedures.

Materials used in recovery and/or examination of fibres at the laboratory can include the following:

- forceps (preferable smooth, non-serrated tips);
- clear adhesive tape and backing;
- glass microscope slides and cover slips;
- mounting media and solvents;
- ruler/measure marked in mm increments (this need not be calibrated as measurements are rarely critical).

Many suitable mounting media are available for preparing temporary and permanent fibre mounts, and the selection of an appropriate mounting medium will be influenced by the particular microscope technique used, see relevant appendices. Solvent-based mounting media may affect the morphology or dyeing of certain type of fibres especially considering long-term storage (reference fibre collection).

The choice of personal protective equipment (PPE) such as protective coats, disposal gloves and facemasks will be determined by the aims of the examiner and the hazards associated with a particular exhibit (e.g. body fluid contamination) (see Sections 8.2.1 and 14.2).

5 METHODS

Before any examination is carried out, a case examination strategy should be defined as described in Section 9, Initial Assessment. Prior to any analyses using a dedicated technique, the relevant performance checks should be carried out as described in Section 4.2, Equipment. It is paramount that maintenance on a regular basis is also valuable for the proper functioning of the equipment. Moreover, a validation of the methods used is also highly recommended according to criteria developed in Section 6.1, Validation.

The following techniques are currently available to forensic fibre practitioners. It is advisable to be carried out in accordance with the recommendations in the Appendices to this Manual. The techniques used for fibre identification/comparison should, where possible, be non-destructive, applicable to the smallest sample sizes, and be highly discriminating. The methods detailed below generally fulfil these criteria.

For comparison purposes it is desirable to use appropriate techniques for colour comparison and fibre polymer and chemical composition determination. Fibre comparison should be made between questioned and reference fibres or between questioned fibres that potentially originate from the same source. This is usually carried out using low and high power microscopy, colour comparison techniques (e.g. Microspectrophotometry) and chemical composition analytical methods (e.g. FTIR). The use of microscopic techniques may be sufficient for elimination purposes in some cases when the differences are obvious.

5.1 Examination techniques

Macroscopic and low power stereomicroscopic examinations are useful for recovering fibres from tapings/samples or directly on pieces of evidence. Recovery and sampling are further described in Section 8, Handling items.

This is an important preliminary step for determining whether a questioned item is a textile fibre or not and for observing fibre characteristics, such as colour, shape and texture.

Low power microscopic examination can also be used to define the type of fibre (e.g. natural versus man-made) and the colour (i.e. a colour block description such as red/orange,

blue/violet, grey/black, etc.). It is not suitable for accurate identification of fibre type and objective description of fibre colour.

Based on the previous steps, low power microscopy can also be used for assessing which questioned fibres are suitable or not for further comparison. Where there is doubt, those questioned fibres should be included in the subsequent comparison sequence.

5.2 Microscopy of textile fibres

Microscopic examinations are employed in forensic fibre characterisation, identification, and comparison. Several types of light microscopes may be used, and the nature and extent of the fibre evidence will dictate which techniques are selected and performed.

A side-by-side, point-by-point microscopic comparison of morphological features provides a fundamental discriminating method for determining if two or more fibres are distinguishable or not.

The use of microscopic techniques may be sufficient for elimination purposes when the differences are obvious, however, where an elimination is uncertain, further tests may be required.

For questioned and reference comparisons with the use of high power microscopy, a fibre or multiple fibres may be mounted on a uniquely labelled glass microscope slide, with an appropriate cover slip, and the same mounting medium must be used for both questioned and reference fibres. Each mounted fibre must be clearly visible and correctly labelled.

Before use, microscopes must be correctly set up as detailed in the manufacturer's instructions and all users fully trained in their operation and adjustment. Any microscope used to measure dimensions should be calibrated.

More theoretical, technical and practical aspects are detailed in the Appendix 1, Microscopy of Textile Fibres. Sample preparation is briefly mentioned in Section 8.2, In the laboratory and further described in the Appendix.

5.2.1 Brightfield microscopy

Brightfield microscopy is the basic technique used for observation of morphological characteristics in all fibre types. Physical characteristics of man-made, natural and inorganic fibres are detailed in the Appendix, Microscopy of Textile Fibres, the FTIS database and scientific literature.

Comparison using only one microscope has the advantage that both the questioned and reference samples are viewed under exactly the same physical and optical conditions.

However, examination using a single microscope requires extremely meticulous work routine with exact recording of all steps taken (e.g., including photographs of the fibres being examined so that questioned and reference fibres can be clearly differentiated).

5.2.2 Comparison microscopy

Comparison microscopy is used for simultaneous viewing of questioned and reference fibres, enabling comparisons of the morphological characteristics and colour of fibres to be made. However, the colour comparison is subjective, so additional complementary techniques are recommended (Microspectrophotometry, Raman spectroscopy).

The use of a comparison microscope has the advantage that the questioned fibres can be easily compared with a wide range of fibres from the reference material, thus taking into account variation that may occur within it (e.g., in fibre diameter; depth of dyeing). The

questioned and reference fibres are examined with separate light beams, and depending on the microscope, there may be one or two light sources. Both microscopes must be balanced so that the background appears the same. The visual responses from two fibres known to have originated from the same source, and mounted on separate microscope slides, must be the same colour, brightness, and clarity; a balanced neutral background colour is optimal.

5.2.3 Polarising microscopy

Polarising microscopy is used for determination/measurement of optical characteristics, (e.g., sign of elongation and birefringence) leading to preliminary identification of the generic type of man-made fibres. Optical characteristics are detailed in the Appendix 1, Microscopy of Textile Fibres. Polarising microscopy cannot be used to identify exact chemical composition of fibres, but the presumptive polymer type can be determined.

5.2.4 Fluorescence microscopy

Fluorescence microscopy is used for examining/comparing materials applied in the dyeing and finishing of textile fibres. It is always recommended to use this technique, if Microspectrophotometry is used only in the visible range.

Fluorescence may arise from fibres themselves or from dyes and other additives, such as washing powder and optical brighteners. Fibres should be mounted in a non-fluorescent medium, and examination using various combinations of excitation and barrier filters is desirable (mostly used: UV, Violet, Blue, Green). At each excitation wavelength, the colour and intensity or absence of fluorescence emission should be noted. Care should be employed to ensure that questioned fibres are not excluded because of fluorescence caused by contaminants, or the effects of localised conditions, that is not exhibited by the reference fibres. It is also possible to measure emission fluorescence spectra.

Samples should not be exposed to UV excitation for too long under a high magnification as this could result in a change in fluorescence, peak intensity and colour of the fibre, possibly due to the bleaching effects.

5.2.5 Miscellaneous techniques

Other techniques, such as interference microscopy, cross sectioning, solubility, melting point determination and scanning electron microscopy, which may yield additional physical and analytical information on examined fibres, are detailed in the Appendix 1, Microscopy of Textile Fibres.

5.3 Microspectrophotometry

Microspectrophotometry (MSP) provides an objective means of colour comparison. It may be used in the visible range (MSP-Vis), or in the UV and visible range (MSP UV-Vis), the latter normally requiring the use of non-UV absorbing materials (e.g., quartz) and temporary preparation of the sample.

This technique has been tried and tested by forensic fibre experts with the undoubted conclusion that, when performed on a modern instrument, it is highly discriminating. What is of particular importance to an operator is a clear understanding of the capabilities of the instrument. However, it is just as important to be able to recognise its limitations and to use other discriminating techniques when necessary. Obvious examples where other techniques should be considered include heavily dyed fibres that produce bland, featureless spectra and very pale coloured fibres with nondescriptive spectra.

The aim of the operator is to demonstrate, by using the instrument to its full effect, the full range of variation of dye intensity and colour measurement artefacts present in the reference sample prior to analysing the questioned fibres.

Man-made fibres, as opposed to natural fibres, usually demonstrate little intra-sample variation in terms of their absorption or transmission of light. However, it should be remembered that fibres are not plane objects so their characteristics (cross-sectional shape, texturisation, levels of delustrant etc.) will all have an effect on the result. When selecting measuring areas these factors should be taken into account and the choice made carefully and consistently.

Samples should not be exposed to UV excitation for too long in a high magnification as this could result in a change in fluorescence, peak intensity and colour of the fibre, possibly due to the bleaching effects.

More theoretical, technical and practical aspects are detailed in the Appendix 2, Microspectrophotometry of Textile Fibres. Sample preparation is briefly mentioned in Section 8.2 In the laboratory and further described in the Appendix.

5.4 Infrared spectroscopy

Fourier Transform Infrared (FTIR) microspectroscopy allows accurate identification of fibre polymers from very small samples. Fibre identification is made by comparison of the fibre spectrum with laboratory reference spectra (as well as authenticated spectral data gathered within forensic networks) or by following guidelines for the interpretation of spectra. Infrared spectral libraries made commercially available may also be used.

Successful identification of fibre polymers by IR spectra depends on experience and familiarity with fibre reference spectra. The spectra must be acquired and examined carefully. The effects of pressure, diffraction, scattering, artefacts, noise, interference, fibre flatness and instability of the detector should be recognised when present in the spectrum. The % transmission (no less than approximately 70% is recommended) and baseline drift should be considered when interpreting the spectra as these will affect any library searches.

There is some potential for obtaining additional compositional information (fibre generic class and subclass) by using infrared spectroscopy in addition to polarizing light microscopy (PLM). Because of the large number of sub-generic classes, forensic examination of fibres containing e.g. polyacrylonitrile is likely to benefit significantly from infrared spectral analysis. The spectral information due to the presence of dye(s) is usually low but may also be used, e.g. in acrylic fibres, for comparison purposes. The extent to which infrared spectral comparison is indicated cannot be generalized and will vary with specific sample and case evaluations.

The generic class of man-made textile fibres and the sub-generic class of synthetic manufactured fibres may be identified. Sub-generic classes of e.g., polyester, polyamide, acrylic and modacrylic fibres can be discriminated by IR spectroscopy. It may be desirable to confirm the identification by other methods such as PLM or melting point determination.

Natural fibres may also be analysed by IR spectroscopy; however, other than assisting with classification of the fibres, little additional compositional information is provided over that yielded by light microscopy. Dyes may be detectable in these fibres by subtraction of the undyed fibre. It may be desirable to confirm the identification by other characteristics such as morphological features or Herzog test.

More theoretical, technical and practical aspects are detailed in the Appendix 3, Infrared Spectroscopy of Textile Fibres. Sample preparation is briefly mentioned in Section 8.2 In the laboratory and further described in the Appendix.

5.5 Raman spectroscopy

Raman spectroscopy is, among other techniques (such as MSP, TLC and FTIR), a possible method for analysing and characterizing dyed (or pigmented) fibres. At the moment it is not widely used as a routine technique for fibre identification and comparison, because most of the forensic laboratories can rely on information provided by the conventionally used techniques (microscopy, MSP and FTIR). Raman spectroscopy allows the in-situ analysis of single fibres and offers the advantage of being almost non-destructive.

At present the dedicated literature is incomplete regarding the potential and limitations of this method. The added value of Raman spectroscopy is still under debate and is highly dependent on the fibre type and colour. Raman analyses are mostly carried out in a comparative way between reference and questioned samples. The technique provides an additional and complementary means of colour comparison.

The dyes (or pigments) signal is usually dominant in the Raman spectrum and may partially or totally hide the information from the fibre substrate (generic class, sub-class). Pigments are usually easily detected in fibres and can be identified using a spectral database. The spectral response of a dye varies depending on the dye itself and its concentration in the fibre. Dye identification may be complicated due to the large number of dyes present on the market, the use of their multi-component mixtures and also because different dyes can provide similar Raman response.

For man-made fibres Raman spectroscopy can also be used to identify fibre generic classes and in some cases sub-classes. Fibre substrate identification can be made by comparison with laboratory reference spectra. The analytical information provided is usually equivalent or sometimes less detailed than when using FTIR.

More theoretical, technical and practical aspects are detailed in the Appendix 4, Raman Spectroscopy of Textile Fibres. Sample preparation is briefly mentioned in Section 8.2 In the laboratory and further described in the Appendix.

5.6 Other techniques

There are other analytical methods, which can be applied to fibre examinations. A number of these techniques are currently being developed and may prove to be useful in examination and comparison of single fibres. Some of the techniques included here involve the use of hazardous chemicals, and the possible safety hazards or precautions associated with their application should be taken into account. It is the responsibility of the user of these documents to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to use.

5.6.1 Chromatographic techniques

Chromatographic methods, especially in combination with mass spectrometry, are characterized by high sensitivity and very good identification parameters. Metameric coloration of fibres can be detected using UV/visible Microspectrophotometry, but if this technique is restricted to the visible range only, differences in dye components may remain undetected. Further differentiation may be possible with the use of additional chromatographic techniques, as thin layer chromatography (TLC) or High Performance Liquid Chromatography (HPLC). Pyrolysis Gas Chromatography, with or without mass spectrometry (PyGC or PyGCMS) or pyrolysis/mass spectrometry (PyMS) can be used to identify the generic type of an unknown fibre and in some/many cases may identify sub-classes within a generic class.

5.6.1.1 *Thin layer chromatography (TLC)*

TLC is an inexpensive, simple, well-documented technique that can be used to complement the use of Microspectrophotometry in comparisons of fibre colorants. The application of TLC may serve to discriminate between fibres, or it may confirm their similarity. Since the technique involves classification of the dye and visualisation of mixture components, it may provide very useful information in intelligence led investigations involving industrial enquiries. However, in the era of Green Chemistry, this method is losing importance and popularity. TLC method has some disadvantages as a large amount of sample is required for pale colours (e.g., yellow), the quantification is not accurate, and due to the variation of retention factor between TLC runs there is difficulty in making a spectral database of dyes.

More theoretical, technical and practical aspects concerned with the classification and TLC of textile fibre dyes are detailed in the Appendix 5, Chromatographic Techniques.

5.6.1.2 *High performance liquid chromatography*

An alternative for TLC is high performance liquid chromatography (HPLC), a fast and sensitive technique which can be applied for analysis of dyes in forensic science. HPLC systems have been developed to analyse a small number of dyes, a dye mixture and its components, a particular dye class, and a combination of dye classes. However, there are many dye classes used to colour fibres, and it is difficult to separate them on a single chromatographic system. More theoretical, technical and practical aspects are detailed in the Appendix 5, Chromatographic Techniques.

5.6.1.3 *Pyrolysis-GC(MS) and pyrolysis-MS*

Some laboratories conduct pyrolysis gas chromatography with or without mass spectrometry (PyGC or PyGCMS) or pyrolysis/mass spectrometry (PyMS) as a method for forensic fibre examination. More theoretical, technical and practical aspects are detailed in the Appendix 5, Chromatographic Techniques. The information contained in it are concerned with the pyrolysis of single fibres and fibres from bulk material, classification of the generic class of polymer, and interpretation of the resulting pyrograms and mass spectra. The protocols and equipment mentioned in this document are not meant to be totally inclusive or exclusive.

5.6.2 Other selected techniques

Brief details of numerous other less well-known methods, which are sometimes mentioned in connection with forensic fibre examination, as fibre density measurement, solubility tests, thermal methods, methods for elemental determination and miscellaneous techniques, are described in the Appendix 6, Other Analytical Techniques. They are available but are not routinely used due to problems associated with sample size and/or interpretation of the results. Some of them are methods using costly research grade equipment but may occasionally be useful for specific purposes.

5.7 Overview of the instrumental methods

Table 1 is intended to give the reader an overview of the instrumental methods available for fibre examination and comparison. Some of these methods are already well-known and used routinely and others may be used to collect extra information when trying to identify or compare fibre samples. In practice it is not possible to use all the methods listed below but the reader is now aware of their existence and properties.

Some additional information is provided for each method so the reader can make her/his own opinion about the usefulness and relevance of the technique in her/his analytical procedure or particular casework. Depending on instruments available in her/his own laboratory the reader

could choose to combine several techniques in order to collect valuable information about fibre type, colour and characteristics.

When choosing an instrumental method, some properties may be taken into account:

- (A) The method is non-destructive;
- (B) The method is applicable to single fibres, as well as short fibres;
- (C) The method is known to be discriminatory;
- (D) Sample preparation is minimal (in situ measurement is possible);
- (E) The method is fast;
- (F) The method is well documented (in literature or by the end-user).

Table 1: Overview of instrumental methods

| Instrumental methods | (A) | (B) | (C) | (D) | (E) | (F) | Analytical information (* = sometimes) |
|-------------------------------------|-----|-----|-----|-----|-----|-----|---|
| Optical microscopy | | | | | | | |
| Brightfield microscopy (BF) | ++ | ++ | ++ | ++ | ++ | ++ | Morphology |
| Polarising microscopy (PLM) | ++ | ++ | + | ++ | ++ | ++ | Fibre generic class, dichroism |
| Fluorescence microscopy (FLUO) | ++ | ++ | + | ++ | ++ | ++ | Fluorescence properties (dyes and finishes) |
| Birefringence | ++ | ++ | + | ++ | + | ++ | Fibre polymer orientation |
| Darkfield microscopy (DF) | ++ | ++ | - | ++ | ++ | - | Presence of pigments/delustrant |
| Interference microscopy | ++ | ++ | - | ++ | ++ | - | Fibre classes |
| Comparison microscopy | ++ | ++ | ++ | ++ | ++ | + | Morphology, polarisation and fluorescence |
| Microspectrophotometry (MSP) | | | | | | | |
| Visible range (MSP-Vis) | ++ | ++ | ++ | ++ | ++ | ++ | Absorption spectrum (colour) |
| UV visible range (MSP-UV) | ++ | ++ | ++ | + | ++ | ++ | Absorption spectrum (dyes and finishes) |
| Fluorescence (MSP-FLUO) | ++ | ++ | + | ++ | ++ | + | Fluorescence spectrum (dyes and finishes) |
| Linear dichroism (MSP-PPL) | ++ | ++ | + | ++ | ++ | ++ | Linear dichroism (LD) spectrum |
| Vibrational spectroscopy | | | | | | | |
| Raman | ++ | ++ | ++ | ++ | ++ | + | Fibre generic class (and subclass*), dyes/pigments* |
| FT-IR | + | ++ | + | + | ++ | + | Fibre generic class and subclass |
| Chromatography techniques | | | | | | | |
| TLC | - | + | ++ | - | - | + | Number of dye components (+ behaviour) |
| HPLC | - | ++ | ++ | - | + | - | Number and ID* of dye components |
| Pyr-GC/MS | - | ++ | ++ | + | + | - | Fibre class, dye composition |
| Elemental composition | | | | | | | |
| SEM/EDX | ++ | ++ | ++ | + | ++ | + | Elemental composition; detailed morphology |
| ICP/MS | - | | ++ | + | ++ | - | Elemental composition |
| Other methods | | | | | | | |
| Hot stage microscopy | + | + | + | + | + | + | Range of melting point |
| Solubility tests | + | + | - | + | ++ | - | Fibre subclass |
| Microtomy | + | + | - | + | - | + | Cross-sectional pattern |
| Microchemical tests | - | + | ++ | ++ | + | - | Fibre chemical class |
| IR/MS | - | - | + | + | - | - | Isotope ratio |

(A) Nature of the method: (++) non-destructive; (+) partly destructive, a short segment of the fibre is used; (-) destructive.

(B) Applicability on single fibres: (++) always; (+) sometimes; (-) never.

(C) Discrimination power (based on scientific literature): (++) high; (+) medium; (-) weak.

(D) Sample preparation: (++) none/minimal; (+) limited; (-) hard preparation step.

(E) Rapidness: (++) very fast; (+) normal; (-) slow.

(F) Appropriate methods: (++) routinely used; (+) sometimes used; (-) rarely used.

5.8 Peer review

Peer review is part of a laboratory's quality management system and includes technical, critical and the evaluation of evidence.

The outcome of a fibre examination should be reviewed prior to the issuing of any report detailing the findings and/or any associated interpretation of microscopic or instrumental analysis.

This process takes the form of a review of technical findings and critical findings during which a second competent practitioner:

- reviews the documented results
- carries out an independent examination of the fibre samples when necessary
- reviews any evidence evaluation and opinion

The reviewing process should be traced in the case and may be outlined in the report. A standard check sheet may be an option for ensuring that all relevant issues have been covered. The laboratory should have a procedure in place to address and resolve any differences in opinion between two practitioners.

Other specific requirements during the examination process of textile fibres may be applicable depending on some legal jurisdictions, international standards or institution-specific requirements.

5.8.1 Review of the technical findings

In fibre examination cases, 'technical findings' are considered those findings not related to opinion, such as, the observation and documentation of any macroscopic and microscopic characteristics of the questioned and/or reference fibres. These may also include the results of other analytical measurements or findings. Except spectral data, some characteristics (e.g., colour) may be described differently between two practitioners, but it is paramount that each practitioner remains consistent when describing the same characteristics among various samples.

The technical review can be made based on detailed photographs, microscopic images and display of spectral data or other findings using tables and/or charts.

When necessary, the second practitioner may examine the fibre samples again or collect additional raw data for identification or comparison purposes.

5.8.2 Review of the critical findings

In fibre examination cases, 'critical findings' are considered those findings which are based on interpretation or opinion. For example:

- the identification of the fibre generic class or sub-class
- the comparison of microscopic characteristics
- the comparison spectral data
- the initial location of questioned fibres, where significant evidential value will be attached to where the fibres were recovered (for example, fibres located on the edge of a knife blade or on the neck of a strangled victim).

Most of these interpretative steps will help in reporting an opinion on whether or not questioned and reference fibres are indistinguishable or, in case of questioned fibres comparison, could have originated from the same source. A second opinion can be given by a practitioner with technical competencies on the techniques used for comparison/ identification purposes.

The relative rarity of the fibre type (source level) or the location and the number of questioned fibres (activity level) can lead to evidence evaluation (see Section 12, Evaluation and interpretation). In this particular case the technical competences are not mandatory to be able to give a second opinion, but the reviewer has to be educated with this kind of evaluative reasoning and with relevant literature.

6 VALIDATION AND ESTIMATION OF UNCERTAINTY OF MEASUREMENT

6.1 Validation

For the general requirements of validation, the reader is referred to the ENFSI document “Guidelines for the Single Laboratory Validation of Instrumental and Human Based Methods in Forensic Science”.

Validation should focus on the process of characterisation and comparison of textile fibres and does not normally extend to evaluating the significance of fibre comparison results.

Fibre characterisation is based upon well-established scientific principles supported by scientific literature extending back over a few decades. Fibre characterisation is soundly based on scientific peer-reviewed methodology in forensic examination of textile fibres.

Fibre characterisation is based on a combination of morphological details (human based method), colour measurement (instrumental method) and determination of chemical composition (instrumental methods) using established analytical techniques (see Section 5, Methods).

The FTIS database of the ETHG working group can be used for finding trusted information on morphology (microscopic images, morphological and optical properties) and chemical composition (infrared spectra), as well as for other additional properties (thermal behaviour, solubility tests).

Forensic comparison of fibres has been used and scientifically accepted worldwide for many decades. The literature dealing with fibre characteristics and the reliability of forensic fibre comparison is extensive. The comparison is based on the observation of similarities as well as differences in morphological details and in analytical data (i.e., the presence or absence of discriminant details in (UV) Vis, infrared and/or Raman spectra).

The quality of fibre comparisons depends both on the instrumental performance and on the judgment and experience of the practitioner, which may be achieved by scientific education, training and continued professional development.

Fibre characterisation and comparison are a combination of instrumental and human-based methods of examination. Therefore, validation and verification should comprise a thorough testing of all instrumental methods used and, on the other hand, practitioners demonstrating competence by showing that they can provide consistent, reproducible and valid results that are compatible with the results of other competent practitioners (see Sections 4, Resources and 7, Quality Assurance). The latter can be achieved by testing and comparing several practitioners within the same laboratory and/or by taking part in PT/CE tests.

Validation should be done and documented in compliance with the laboratory quality management system of the institution. This applies for the methods described in Section 5, Methods.

6.2 Estimation of uncertainty of measurement

Uncertainties of measurement in fibre examinations can firstly relate to those arising from the use of instruments, equipment and reagents. Secondly, as a human-based method, practitioner competence forms the basis of reliable and reproducible results. In addition, fibre examinations are often reliant on adequate sampling and the nature and variation of fibre samples.

6.2.1 Instrumentation / equipment / reagents

Uncertainties of measurement attributable to instrumental techniques can be minimised by servicing and maintenance of the equipment according to the manufacturer's specifications. In addition, regular performance and calibration checks should be performed.

Uncertainties may arise with various techniques, but other complementary techniques could be used to compensate the known limitations. In any case using several complementary techniques is always desirable for minimizing uncertainty. Table 2 provides a troubleshooting guide for the most conventional techniques used in fibre examination.

Table 2: Troubleshooting guide for conventional fibre examination techniques

| Technique | Limitation(s) | Additional techniques |
|----------------------------|---|---|
| Microscopy | Observation of morphological details (e.g., longitudinal view, cross-section, polarisation colours, wool scale pattern) with deeply coloured fibres | Cross-sectioning, spectroscopy for polymer identification, scale casting or SEM for scale pattern |
| MSP | Poor quality spectra with very pale (faint absorption) and deeply coloured fibres (saturation of spectral details) | Raman spectroscopy, chromatographic techniques |
| IR | Identification of natural fibres with a very similar cellulosic composition | Microscopy (morphological details), Herzog test, cross-sectioning, staining tests e.g., Billingham's test |
| Raman | Absence of response (due to extensive fluorescence emission); limited detection of single dye or dyes in mixtures | Multiple Raman laser sources (to try to bypass fluorescence); MSP, chromatographic techniques |
| Chromatographic techniques | Analysis of single fibres | MSP |

Uncertainties of measurements attributable to reagents (e.g., mounting media, solvents for extraction) can be minimised by the selection of reagents of appropriate quality and/or refractive index. Failure to carry out these steps can have an impact on the practitioner's ability to visualise and recognise key characteristics / features.

Macroscopic measurements, such as fibre length, are rarely critical and are typically used as direct comparison. Therefore any uncertainty of measurement is unlikely to be of significance.

Uncertainties will increase when comparing questioned fibres to fibre collectives instead of comparing to known fibres originating from the same textile source material.

6.2.2 Practitioner competence

As a human-based method, the reliability and reproducibility of results of fibre examinations are highly reliant upon practitioner competence. Well-trained and competent practitioners participating in regular proficiency testing and collaborative exercises pose minimal risk of error and uncertainty.

The e-Learning platform of the ETHG working group can be used as a guidance for educating and training individuals with trusted best practices.

Practitioner competence extends in some cases to reporting (see Section 12, Evaluation and Interpretation).

6.2.3 Fibre samples

Each of the following has an impact on the ability of the practitioner to attribute a fibre to a source with any degree of certainty:

- Non representative reference sampling (e.g. surface fibres vs core fibre, warp and weft yarns)
- Highly variable fibre samples (e.g. labels with high content of 'other (re-use) fibres')
- Fibre samples with limited or non-distinguishable characteristics / features (typically undyed fibres and especially the natural ones)
- No authenticated source material (comparison to fibre collectives)
- Irregular fibre population (single trace, damaged traces, traces contaminated with body fluids, small sample sizes, etc.).

7 QUALITY ASSURANCE

7.1 Proficiency testing / collaborative exercises

Proficiency tests (PTs) and collaborative exercises (CEs) should be used to test and assure the quality of identification and comparison of fibres.

Proficiency tests and collaborative exercises could form a part of the maintenance of a practitioner's competence. A list of currently available PT/CE schemes as composed by the QCC is available at the ENFSI website.

“Guidance on the Conduct of Proficiency Tests and Collaborative Exercises within ENFSI – QCC-PT-001” provides information for the ENFSI Expert Working Groups (EWGs) on how to organise effective proficiency tests and collaborative exercises for their members.

Proficiency tests in fibre examination are usually set annually by the ENFSI Textile and Hair Group (ETHG). Such tests typically involve:

- Fibre identification
- Fibre comparison
- Identification of the fibre origin
- Description of findings (conclusion)

In addition, these may be supplemented by tests provided by collaborations of laboratories or within an organisation.

The performance in proficiency testing and collaborative exercises must be reviewed by the organisations in a timely manner and any inconsistent results should be investigated and addressed with appropriate corrective actions according to the institute's quality management system.

7.2 Quality controls (QC)

It is essential to carry out calibration and performance checks for instrumental analysis. All calibration and performance checks must be documented and dated.

For further information please see appendices of this BMP. In the following subchapters calibration adjustments are carried out during an instruments servicing by a qualified engineer, which is usually conducted at defined intervals. Regular performance checks as outlined below should be completed prior to an instrument's use (See Section 4.3 and 4.4).

Hard copies of the instrumental results of recovered fibre and known fibre should be printed and placed in the case file. The practitioners involved should sign and date the printout. This verification process can also be completed electronically.

7.2.1 Microscopy

Before use, microscopes must be correctly set up as detailed in the manufacturer's instructions and all users fully trained in their operation and adjustment. In particular, bright field microscopes should always be adjusted for Köhler illumination and a comparison microscope must additionally always be balanced using paired slides of fibres from the same source. Any microscope used to measure dimensions should have its eyepiece graticule accurately checked at least annually (or if any part of the microscope is changed) using a slide micrometer. Instruments used to establish refractive index, birefringence or melting point should be checked to ensure that the expected results are obtained from known materials.

For miscellaneous techniques such as interference microscopy, cross-sectioning, solubility, melting point determination and scanning electron microscopy, see Appendix 1, Microscopy of Textile Fibres.

7.2.2 Microspectrophotometry

Microspectrophotometers (MSP) and microscopes interlinked with spectrometers should be checked regularly. This ensures that the instrument is operating to expected standards.

Before any form of calibration is undertaken the system must demonstrate absolute consistency in the optical path so results are comparable. The microscope should be set up for Köhler illumination; normal operating parameters should be defined, and the lamp allowed to warm up and stabilise according to manufacturers' instructions.

Demonstration of the accuracy of wavelength and absorbance are important and if colorimetry is used as a comparative feature, then that too should be standardised using traceable commercial standards.

Wavelength accuracy over the visible range can be checked with the aid of holmium and/ or didymium filters. As the exact position of the absorption bands vary from batch to batch it is important that the filter is authenticated and traceable to original data from the manufacturer. Resolution can also be checked routinely using these filters.

Demonstration of standardisation of absorbance is also important especially if undertaking colorimetry. It also acts as a check on linearity, it is important that these standards are authenticated and traceable to original data from the manufacturer. Absorption filters will normally be placed in the sample plane, but if they are not, a blank slide, plus mountant and cover slip must be placed in the sample plane to assist with Köhler illumination.

Consistency may be monitored using the lamp energy and 100% transmittance line. This is acquired by measuring an area without the analyte; because there is no absorbing substance in the optical path, the transmittance at all wavelength steps should be 100% +/- 1 %. It can also be used to indicate aberrant behaviour of the lamp especially with the xenon lamp.

Calibration limits of each standard are supplied with authenticated samples direct from the manufacturer.

It is suggested that the calibration checks are performed and recorded for reference regularly or adjusted accordingly to frequency of use.

If your laboratory is using an MSP or MCS on a daily basis it is suggested that you should undertake at least one calibration checks regularly to demonstrate reproducibility. During

operation the operator may run the 100% line and/ or a background scan for each cover slip. Necessary adjustments can be made to demonstrate instrument consistency.

7.2.3 Infrared spectroscopy

An infrared spectrometer with a microscope (or camera) attachment is recommended. All infrared spectrometers should be quality controlled regularly.

It is essential that instrument performance and calibration is monitored routinely according to the manufacturers' recommendations. It is recommended that the energy of the detector is checked through the object and the microscope before use. Instrument performance records may be maintained on hard copy and/or computer disk. These should include calibration and alignment checks, maintenance records, wavelength checks (e.g., polystyrene spectra), and should be signed and dated. Examples of the performance evaluation method for FTIR include:

- System throughput to check for optimal performance i.e., both the microscope and interferometer are properly aligned, and the S/N is comparable to normal manufacturer recommendations.
- The interferogram size, position ($800\text{-}1200\text{cm}^{-1}$) and shape are examined to ensure that there is maximum signal, and a stable, smooth signal, as per the manufacturers' recommendations. The sampling mode, the sample type, and the instrument alignment affect the peak height.
- Single-beam spectrum of the background should be examined to ensure that there are no interfering absorbances from the surface where the background spectrum is acquired, the water and carbon dioxide absorbances are minimised i.e., the instrument has stabilised.
- The spectral shape and the noise should be examined.
- S/N checks by recording the 100% line, in absorbance, at the $2000 - 2600\text{ cm}^{-1}$ region i.e., where no interfering absorbing atmospheric bands occur in the spectrum. $S/N = 100/\text{max-min}$ at this area or P-P $S/N = 1/\text{max-min}$ (peak to peak).
- 100% transmittance (T) line. Ideally an IR spectrum should be composed of a collection of smooth curves emanating from a flat baseline, at 100% transmission (or zero absorbance). If the IR beam travels through more than $10\text{-}15\mu\text{m}$ thickness of fibre, then 0% transmission will be obtained in some regions of the spectrum.
- Peaks may be offset because of lack of frequency calibration. Since infra-red spectroscopy is mainly used for structure determination it is important that frequencies are accurate and reproducible. Drifting of wavelength occurs in dispersive instruments. This is not a factor in the FTIR because the laser provides a continual internal accuracy alignment.
- By measuring a thin organic film on polished metal plates, or by using a flattened microfibre to identify the spatial adjustment of the system.
- Polystyrene or Indene are used for wavelength accuracy as they produce an elaborate many-featured spectrum. Interference fringes may distort polystyrene spectra.

Where used, the microscope should be set for Köhler illumination each day before use if used in transmittance.

7.2.4 Raman spectroscopy

Raman spectrometers and microscopes interlinked with spectrometers should be calibrated regularly. This ensures that the instrument is operating to expected standards.

It is essential that instrument performance and calibration are evaluated routinely according to manufacturers' recommendations. Instrument performance records should be maintained and should be signed and dated. Examples of the performance check method for Raman include:

- Raman shift calibration check. As small changes in true Raman shift can be scientifically informative, it is very important to proceed to a Raman shift calibration check. In order to determine the Raman shift, both the frequency of the laser and the Raman scattering should be known. Usually, visible lasers are very stable, but the frequency of diode laser is less accurate and may even vary with the time.
- Frequency calibration with absolute frequency standards. In this case, the atomic emission lines of gases are used (neon, argon or mercury lamps). The atomic source is placed near the sample position. The laser must correspond to zero Raman shift. The plasma lines from the laser itself can also be used if the bandpass filter is removed. This calibration is usually done by the manufacturer.
- Frequency calibration with Raman shift standards: Raman shift standards does not depend on accurate knowledge of the laser frequency provided it is constant. The ASTM committee collected and tabulated the results for a set of 8 standards [ASTM E 1840-96, 1998]. ASTM Raman shift standards are available with a standard deviation of $< 1\text{cm}^{-1}$. These shifts were determined with 514 and 1064 nm lasers. Changes in observed frequencies are possible at other wavelengths due to resonance effect. The instrument response has to be controlled by checking the magnitude of the Raman signal under identical analytical conditions. For example, the intensity of the silicon band at 520 cm^{-1} (silicon is used to set the Raman shift in dispersive instruments) permits the detection of instrumental changes. Amongst the different substances proposed, polystyrene is also suitable for forensic application. It is in solid form, stable and nontoxic. It shows Raman shifts between 620 and 2904 cm^{-1} .

7.2.5 Other techniques

For other techniques such as Thin Layer Chromatography (TLC) or High Performance Liquid Chromatography (HPLC), Pyrolysis Gas Chromatography (PyGC or PyGCMS) or pyrolysis/mass spectrometry themselves (PyMS), see Appendix, Chromatographic techniques.

7.3 Data collection for control, monitoring and trend analysis peer review

Data collection should be documented according to the regulatory requirements and quality assurance system of each laboratory.

If applicable, it is recommended to carry out trend analysis relating to the results of performance checks in order to perform risk assessment and preventive measures.

Any irregularity observed during maintenance, performance checks or calibration should be monitored. It is advisable to perform root-cause-analysis if applicable.

7.3.1 MSP data processing

Commercially available MSPs have incorporated the requirements of operational settings, data control, processing and recording with the support of a system processor. As such they all carry their own software routines to cope with the tasks. In addition, there are further software routines available for use with these systems.

Through the science of colour coding (colorimetry) it is also possible to numerically determine and specify colours. However, modern computer software allows spectral searching and comparison.

7.3.2 MSP databases

Some laboratories now accrue the data: some in the form of spectra, others include physical characteristics and manufacturers details as well. Modern software packages offer the capabilities to store the information in blocks of colour. As long as these databases are kept up to date and used in conjunction with other information (e.g., peer review, literature, practitioner experience) they can offer valuable information both in terms of evidential value and as an intelligence aid. To use a database effectively a considerable amount of data is required.

7.3.3 IR spectra

It is recommended that spectra of the known fibres are saved to a hard disk or a CD, as per laboratory procedures. It is generally useful to save all data on disk just after it is generated and prior to any modification. If any data manipulation has been carried out the raw data must be saved, and the data manipulation carried out on a copy of the spectrum. Data that is damaged during subsequent processing can then be restored from the saved files. Any manipulation carried out, e.g., baseline correction is carried out in absorbance and should be noted on the hard copy of the spectrum. The saved spectra can be retrieved and compared with pre-stored library spectra at a later date.

7.3.4 Use of IR libraries

Reference (IR) spectra are essential for the identification of the fibre composition. These can be in the form of literature references or a spectral library. Do not attempt to identify spectra by Trade name, but only by chemical composition.

A computer-assisted search can be used to provide valuable information. In addition to indicating the identity of the fibre the library search may compute a probability of match based on band position and intensity. This can be an approximate technique due to noise, environmental conditions, varying peak heights etc. The results of a database search should be treated with caution as the database may not contain all the variations in fibre polymer structures.

An in-house spectral library built from a collection of authenticated specimens representing the generic types and sub types is recommended. This should be created using the same technique and instrument used within the laboratory for the unknown fibre.

Any problems due to sample preparation, instrument etc. are overcome by comparison of spectra prepared in house on the laboratory IR spectrometer. For more information, refer to Appendix 3, Infrared Spectroscopy of Textile Fibres

8 HANDLING ITEMS

8.1 At the crime scene

The following recommendations apply to the examination of fibres relating to a crime scene, as well as fibres relating to victim(s), suspect(s) and other person(s) relevant to the case.

8.1.1 Avoidance of contamination

The accessibility to the crime scene, to other relevant areas and to corpses should be restricted and any access to these by individuals should be documented.

Items from different sources must be physically separated from one another after seizure to reduce the potential for contamination, e.g., clothing and other articles from the scene, suspect(s) and of the victim(s) must be kept separate from one another. Seized items from different incident related individuals should be packed by different personnel if possible.

Any situation that could cause contamination or otherwise compromise the trace evidence examinations should be documented and communicated.

In order to protect fibre traces or their substrates from loss and/or biological, chemical, or physical contaminants, personal protective equipment (PPE) such as protective coats, disposal gloves and facemasks should be worn by the practitioner and respective attendees at the scene. The choice of PPE will be determined by the aims of the examiner.

PPE should not only protect fibres but also personnel from possible generic hazards that may apply to any crime scene, for example risks associated with exposure to body fluids, trip hazards, etc. (refer to Section 14.1, Health and Safety / At the crime scene).

The area for examination at the crime scene may require protection against environmental conditions and other disturbing factors, e.g., tents to protect against adverse weather, crowds of onlookers, etc.

Items at a crime scene which bear visible but easily lost traces, or items that are impractical to transport, should be appropriately documented, and the traces collected by an appropriate technique.

To prevent the loss of evidence, consideration should be given to the recovery of fibres at the scene by an appropriate method (refer to Section 8.1.2, Search and recovery). Alternatively, items can be removed from the scene and fibres recovered later in the laboratory. If this is the case, these items should be packaged immediately in an appropriate manner (refer to Section

8.1.4. Preservation and packaging)

Steps should be taken to avoid post-incident contamination between the scene(s), victim(s) and suspect(s), e.g., by the use of different personnel or PPE. In addition, suspects or other incident related individuals should be transported in different police cars.

8.1.2 Search and recovery

Items at a crime scene which bear visible but easily lost traces, or items that are impractical to transport, should be appropriately documented, and the traces collected by an appropriate technique.

Depending on the circumstances, here are some general guidelines pertinent to securing fibre evidence at a crime scene with deceased victims:

- 1:1 Taping or Zonal Taping should be carried out on the bodies and/or clothes of the victim(s) at the crime scene providing they are undisturbed (e.g., a deposition site may not be appropriate). This will assist in providing an exact record of the original location and density of transferred fibre traces. Wet bodies or bodies covered with fresh blood may affect the efficiency of the tape lifting recovery and a subsequent taping may be required after a drying step.
- Removal of clothes from the body at the scene, and immediate packaging of the clothing to preserve trace evidence. These items should be packed in separate bags and transported to the laboratory as soon as possible.
- Bodies should be removed immediately only if circumstances do not first permit inspection and evidence recovery, e.g., due to imminent danger, extreme hindrance to traffic, no possibility of working without being overlooked by persons who are not involved (crowds of onlookers).

- The body must always be transported in such a way as to preserve any potential evidence. A new body bag must always be used. When no fibre recovery on the body was possible at the scene, the inside of the body bag or any appropriate containers (paper sacks, etc.) should be taped.
- (Naked) wet bodies should be transported to the relevant facility in order to dry for fibre recovery.

Small or manageable items at a crime scene which bear visible, firmly attached traces or which are expected to bear invisible significant fibre traces, should be documented, packaged and sealed individually for transport to a laboratory for examination.

Items and traces should be kept secure, in a sealed package, protected from loss, damage and contamination, until the item is examined in the laboratory.

Quality control samples, that can be used to satisfactorily verify a contaminant-free recovery environment, may be taken.

Depending on the case circumstances standard procedures regarding fibre recovery at scenes cannot always be followed. However, it is important to recover as many fibres as possible and any non-standard methods (e.g., for damp items, heavily contaminated items) employed should be documented together with the reasons for the use of the non-standard methods.

8.1.3 Target fibre selection

Known samples of fibres from a specific source, with which it would be helpful to establish that the offender(s) and/or victim(s) and other person(s) relevant to the case may have come into contact, should be recovered. These should include all component fibre types and colours (including faded and unfaded areas, worn and less worn areas, etc.). Tufts of fibres or an excised piece of material is preferred to tapings.

In absence of a reference garment, questioned single fibres of an unknown provenance, recovered on different surfaces, can also be analysed in order to do a trace comparison.

“Background” fibres may also be obtained from areas with which the offender is not thought to have had contact, but to help establish whether any ‘collectives’ of fibres found on a victim could have originated from the victim’s own environment or they could relate to textiles worn by the suspect. The background fibre population at the crime scene is also helpful in identifying if there are textiles in the area that would be an obvious source of questioned fibres (bright colour/good shedding potential) which could have been transferred to the offender.

8.1.4 Preservation and packaging

The method of preservation and packaging of fibres and items to be examined for fibres will vary. Packaging must prevent loss, deterioration or contamination of the fibres.

Minute or loose trace evidence must be secured under tape, on microscope slides or be placed in small clean containers such as small paper folds, petri dishes, tubes etc. Large items, such as whole garments, should preferably be sealed individually in clean, unused packaging.

Wet recovered items should be air dried as soon as possible, without exposure to heat or sunlight, in a secured area in a manner that will prevent loss or contamination of the fibres.

8.1.5 Labelling and documentation

The techniques used for detection, collection, and preservation of the items should be recorded together with the location from which the items are removed.

The labelling of any items recovered should follow the local jurisdiction guidelines.

The minimum details that should be recorded and be directly and unequivocally attributed to each package are:

- The location of the scene, from where or from whom, the item was seized.
- The date (and time, when appropriate) the item or trace was recovered.
- The name of the person recovering the item or trace.
- A short description of the recovered item or trace, plus appropriate remarks about any special observations/circumstances that may apply.
- A unique identifying mark for each item or trace, e.g., case number and item number.
- The position of relevant items (e.g., documented by notes, sketches, measurements and/or photographs, 3D scan).

8.1.6 Transport

There are no particular problems or risks associated with the transportation of fibre samples provided that they have been packaged appropriately as to avoid any risk of contamination or tampering.

8.2 In the laboratory

8.2.1 Anti-contamination precautions

The accessibility to examination areas should be controlled and documented.

Items should preferably be searched in purpose designed rooms with restricted access and air filtration.

Every effort must be made to use different rooms to search items from different scenes or people and these rooms should be physically located some distance from each other. Ideally, different examiners should examine items from victim(s) and from suspect(s). When this is not possible, there must be a clear, documented time gap and evidence of decontamination between searches.

Once the fibres are recovered, secured and labelled the risk of inter-sample contamination is minimised. To demonstrate the integrity of the examination, notes should include details of item packaging and the examination undertaken, together with the examination date, time and location and name of examiner.

Any situation that could cause contamination or otherwise compromise the trace evidence examination should be documented and communicated.

In order to protect fibre traces or their substrates from loss and/or biological, chemical, or physical contaminants, personal protective equipment (PPE) such as protective coats, disposal gloves and facemasks should be worn by the practitioner. The choice of PPE will be determined by the examination strategy.

PPE should not only protect fibres but also personnel from possible generic hazards that may apply to any fibre examination, for example risks associated with exposure to body fluids, etc. (refer to Section 14.2, Health and Safety).

Equipment used for collection and/or storage of evidence, e.g., adhesive tapes, acetate sheets, forceps and scissors, should be maintained in a manner so as to avoid contamination, either between items or between different cases.

If it is deemed necessary, clean any packaging of exhibits immediately prior to opening to prevent any contamination. Measures should be taken as to avoid contamination between the

item and the outside of the packaging of the items, e.g., by opening the packaging of the items close to, but not on, the examining bench and changing to a fresh pair of gloves before moving the item to the bench, or by cleaning the outside of the packaging before opening it to prevent any contamination.

Laboratories, examination areas and equipment should be cleaned according to laboratory anti-contamination protocols before and after examinations are carried out.

8.2.2 Search and recovery

Examination areas should have adequate lighting.

Fibres may be recovered in the laboratory by taping and picking off the fibres or fibre tufts individually with forceps, respectively.

Clear adhesive tape is usually used to recover transferred fibres. The width and degree of adhesiveness of the tape should be varied according to the nature of the surface being taped. After collection of the fibres, it is recommended that the tapes should be placed sticky side down on a transparent carrier (e.g., an acetate sheet) and clearly labelled with the unique identifying mark and their place of origin. Alternatively, the tapes may be sealed by folding them over on their own sticky surface.

Combing could also be used as an alternative to taping for removal of fibres from head hair. A new fine-toothed comb should be used, and the hair should be combed over a large sheet of paper. Alternatively, a sterile gauze could be inserted in the teeth of the comb as a carrier for collecting fibres.

Vacuuming (by use of a vacuum cleaner and special filters) or shaking could also be applied as an alternative to taping for fibre recovery in exceptional circumstances. However, these methods are not recommended by the ETHG as routine methods for fibre recovery.

Depending on the case circumstances standard procedures regarding fibre recovery in the laboratory cannot always be followed. However, it is important to recover as many fibres as possible and any non-standard methods (e.g., for damp items, heavily contaminated items) employed should be documented together with the reasons for the use of the non-standard method.

Quality control samples, that can be used to satisfactorily verify a contaminant-free recovery environment (as far as is practically possible), should be taken.

8.2.3 Reference samples

Reference samples of fibres comprising fabrics from known sources (e.g., clothing, carpets, upholstery, bedding or other textile fabrics present in a particular environment), with which it may be helpful to establish that relevant offender(s) / victim(s) / other person(s) may have come into contact, should be recovered. These should include all component fibre types and colours (including faded and unfaded areas, worn and less worn areas, etc.). Tufts of fibres or an excised piece of material is preferred to tapings. If tapings are to be used as reference samples from the known source, care must be taken in order to avoid any confusion with background fibres from other sources.

In addition, a taping should be made to assess the shedding potential of the items sampled. These should be taken from a representative area, including any areas of obvious damage and/ or wear.

In absence of a reference garment, questioned single fibres of an unknown provenance, recovered on different surfaces can also be analysed in order to carry out a trace comparison.

8.2.4 Storage

Any items recovered from the scene, victim(s), suspect(s) or others should be stored in a manner which prevents loss, deterioration, or contamination, e.g., tape lifts should be secured in clean, unused containers or packaging. Ideally, fibres should be stored in a dry and dark environment.

A record of movement including storage should be held.

9 INITIAL ASSESSMENT

The significance and evidential value of fibre evidence as associative evidence relies heavily on the correct detection, collection and preservation of this evidence and the appropriate sequence of examination both at the scene and in the laboratory (refer to Section 10, Prioritisation and Sequence of Examinations). Furthermore, the success of fibre examinations and interpreting the evidential value of fibre evidence (refer to Section 12, Evaluation and Interpretation) is strongly dependent on the case assessment and the forensic examination strategy, devised by the relevant personnel involved.

Prior to starting any examinations, the requirements of the customer must be clearly defined and documented. Detailed information about the alleged circumstances of the case is usually necessary to initially assess any potential limitations in the examinations proposed and their effect on the perceived outcome. This is particularly important when evaluative reporting on activity level is involved.

Factors to be considered when establishing the customer's requirement include:

- What area(s) and level of expertise is required.
- Whether the laboratory has the capacity, suitable resources, facilities and equipment available to perform the necessary examinations.
- Whether there is any time or other constraints that might affect the overall examination strategy, for example, where other evidence types are involved in the examination sequence.
- Whether the request put forward by the customer can be executed.
- If the request includes a proposition, it should be assessed whether the proposition can be tested.
- If yes, at least one alternative proposition favourable to the defence should be formulated.
- In relation to fibre evidence specifically, here are some general guidelines pertinent to case assessment:
 - What is suspected or known to have occurred before, during and after the incident;
 - What persons (or objects or garments) are involved;
 - The sequence of events and the time frames involved, including those associated with the recovery of items submitted for examination;
 - If an article has been worn for a considerable length of time subsequent to the offence, a fibre examination for fibres transferred to the recipient may not be of value, but the shedding capacity of the item should still be considered;
 - Particular garments or areas of garments can be prioritized depending on the circumstances of the case;
 - Prioritise those fibres and garments likely to be of the most value.

Considering fibre recovery, the following guide should be used when deciding which garments should be taped, and which items are appropriate as potential sources of questioned fibres:

- Garments that shed low numbers of fibres are easily taped but are poor as potential sources of questioned fibres.

- The more contrast there is between questioned fibres and reference fibres from the garment itself, the more easily target fibres can be discriminated (e.g., brightly coloured, coarse).
- Fibres, which fluoresce strongly under ultraviolet light are good, target fibres, particularly if the garment being taped does not fluoresce or fluoresces to a lesser extent.
- The less common the fibre type, the better it is as a target fibre.
- If a garment is damaged it may be a good source of transferred fibres, even when the sheddability of the overall garment is poor.
- Pay special attention to important areas of the garment as indicated by the circumstances of the case, e.g., the seat of a pair of trousers may be targeted if the person was believed to have been sitting on a car seat cover.
- If possible, it is always preferable to establish two-way contact or association.
- In addition to garments, other non-textile objects may also have to be examined for fibres, e.g., car parts in hit and run pedestrian accidents, window ledges in break and enter cases, etc. Bulky objects may need to be taped at the crime scene; however, it is preferable that items which can be easily transported without loss of evidence be taped within the laboratory, under controlled conditions.

In instances where more than one scientific discipline is involved and/or different evidence types need to be considered, a coordinated examination strategy will determine the actions to be taken and the most appropriate methods to be used for the search and recovery of fibres. This may involve a joint examination by practitioners from different disciplines.

The general principles and practices to prevent and control contamination and loss of evidence are applicable to both crime scene and laboratory.

When seizing, recovering, storing, transporting and examining items/samples/sub-samples from a scene, or when seizing clothing or textiles belonging to individuals potentially involved in the crime, care must be taken to prevent contamination and loss of trace materials. The investigator (police officers, forensic examiners etc.) should assess the situation carefully before acting and then take decisions on how to manage the actual case. This will often involve discussion with other experts and the officer in charge of the case.

Factors to be considered when assessing the potential risk of contamination by establishing whether:

- There was any opportunity for the transfer of fibres between the individuals, items and/or surfaces involved prior to the incident or the fibre recovery respectively.
- There was any opportunity for fibre transfer between the individual(s), items and/or surfaces involved following the incident or the fibre recovery respectively.
- The items relating to the individual(s), and/or items involved were properly handled during recovery and packaged appropriately.
- There was any opportunity for secondary transfer to individuals, items and/or surfaces involved, such as contact with other individuals and/or seating.

Factors to be considered when assessing the chance of recovery of fibre evidence:

- The opportunity for fibre transfer between textile items will depend on the area of contact, the duration of the contact and the pressure involved in the contact. It will also depend on the construction and nature of the surface of the textiles or other items involved.
- Transferred fibres are quickly lost or redistributed after the initial transfer. The rate of loss will depend on the nature of the recipient surface and the conditions to which it is subjected. Trials have shown that up to 80% of transferred fibres may be lost within the first two hours after contact. However, the original number of fibres transferred can run

into thousands, so it is possible that large numbers of fibres will still remain after this interval.

The retention of transferred fibres is also heavily influenced by the nature and duration of the post contact activity of the recipient. Where possible, information concerning this should be obtained. As well as useful for forming initial expectations of recovery, this may have subsequent importance in assessing the relevance of any fibre evidence.

The situation is largely better when considering fibre traces on a static dead body, however outdoor conditions could also affect the number of traces transferred and retained.

- Certain types of textiles, with smooth non-porous surfaces, have very limited capacity for donating or retaining fibres.

Factors to be considered when assessing the relevance of fibre evidence:

- Textiles are mass-produced articles. It is normally not possible to state that specific recovered fibres originated from a particular textile source to the exclusion of all others. Textiles are seldom unique, and the possibility of the specific recovered fibres having originated from another item made with the same material can never be ruled out completely.
- Nevertheless, fibres, especially man-made ones, exhibit a very high degree of variety and the matching of recovered fibres in a case can provide strong evidence.
- The combination of several characteristics of fibres such as colour, fibre type, alteration/bleaching etc. can improve the individuality of fibres and therefore increase the evidential value.
- However, some fibre types (e.g., colourless cotton and indigo dyed cotton) are so widely distributed that in most circumstances they will be of no evidential value. In addition, colourless fibres are not normally included in transfer examinations due to the difficulty in recovering them and their relative lack of comparative features. In special cases, e.g., traffic accidents involving fibre-plastic fusions, even these common types/colourless fibres may assume special significance due to case circumstances.
- In order to be able to assess the potential significance of any findings, it is necessary to have information on:
 - The distribution and frequency of occurrence of the different types of questioned fibres/fabrics involved in the case;
 - Transfer and persistence studies in relation to the types of questioned fibres/fabrics involved in the case.

When evaluative reporting is used the practitioner should assess the evidential value of the anticipated findings considering what is expected to find if the proposition or its alternative were correct or not (based on the relevance, the potential significance, the chance of recovery, transfer and persistence of the fibre evidence).

All of the issues regarding the evidential value of fibre evidence are discussed further in Section 12, Evaluation and Interpretation.

10 PRIORITISATION AND SEQUENCE OF EXAMINATIONS

The prioritisation and sequence of examinations is determined by the customer's requirement and the overall forensic examination strategy (refer to Section 9, Initial Assessment). Personnel should be aware that various types of evidence will be present during the processing of a crime scene or the examination of items submitted to the laboratory. Some types of evidence may be more significant to a particular case and therefore should be given higher priority. The situation has to be considered carefully before any examination takes place. All

potential forensic opportunities should be considered, and the appropriate experts should be consulted.

Consideration should be given to the following before commencing any recovery and examinations of fibres:

- The urgency and priority of the customer's need for information.
- Other types of forensic evidence, and the associated examinations, which may have to be carried out on the same items.
- Which items have the potential to provide the best evidence.
- Which items offer the best choice of questioned fibres, in terms of evidential value, colour and sheddability.

It is usually preferable to start by searching for the best choice of questioned fibres on the items where the finding of questioned fibres may have the most evidential significance (e.g., underclothes in a rape case).

To minimise the possibility of contamination it is preferable to examine all items relating to one individual or scene before commencing with items relating to others.

In general, the examination protocol might be as follows:

- Recovery of visible material, such as tuft of fibres, hairs, glass, etc., adhering loosely to the item.
- Recovery of any potential fibre evidence not visible to the naked eye. In some instances, a low power microscopical search of the item may be relevant (e.g., fibres on knives, under fingernails etc.). This method decreases any potential contamination and loss of unforeseen evidence.
- Fibre analysis and comparison using the agreed methodology (refer to Section 5, Methods).

Considering the potential value of the information from each technique we refer to Section 5, Methods.

11 RECONSTRUCTION

In some cases, it might be necessary to carry out experimentation to evaluate the findings. This might take the form of a simulation or reconstruction exercise designed to mimic the proposed causative actions. For example, tests to replicate heat-damaged fibres in cases of suspected arson, crush-damaged fibres in cases of assault.

Such tests should be designed so as to answer the issues raised during the examination process, or by the client. Any experiments or tests carried out should be well documented, including any assumptions made and limitations of the tests. Before carrying out destructive testing in reconstruction experiments it is advisable to consider the use of replicate samples. Where replicates are used (e.g., weapons) they should closely reflect the form and condition of the original item.

When carrying out any experimentation, it is preferable not to alter the original evidence (depending on the legal jurisdiction). Where it is altered by experimentation, this has to be clearly documented and appropriate permission should be sought.

The detection of Fibre Plastic Fusions and associated plastic coating marks may also be used to determine, with a great deal of confidence, who was driving a motor vehicle at the time of a collision.

12 ASSESSMENT OF RESULTS AND INTERPRETATION

Evaluation and interpretation of the findings should reflect the issues identified in the initial pre-assessment of the case and should address the specific requirements of the investigation within the specific framework of circumstances of the case itself (refer to Section 9, Initial Assessment). This is particularly important where a logical evaluative approach (such as a Bayesian framework) is employed in this process.

Case pre-assessment should provide a means of determining expectations concerning potential examination outcomes given the allegations of the prosecution and defence. The actual outcome of the examination can therefore be evaluated in this context and mitigate the potential for cognitive bias (see below).

The significance of the laboratory findings in a specific fibre case can essentially be considered in two distinct parts.

At the most fundamental level, this considers the results of the analysis and comparison of a fibre(s) considered to be related to a crime, with a putative source and the ‘confidence’ that can be attributed to any ‘match’ obtained. In a Bayesian framework (see below), this is referred to as a “source level” proposition.

Factors important in this evaluation include (but are not limited to), the relative ‘rarity’ of the fibres in question, the number of tests employed and the discriminating power of the analytical sequence.

In addition, peer reviewed published studies such as: fibre population studies, colour block studies, target fibre studies, as well as the practitioners own experience, must also be considered in this type of evaluation. Where a database is consulted in this process, caution should be employed when considering generated fibre frequency data – particularly where a limited or unrepresentative dataset is used and should (unless there are compelling reasons to do so) not be used as the sole means of information.

This most fundamental evaluation aids the practitioner in assisting the legal system in considering the question of how likely is it that the fibres in question are ‘crime related’ as opposed to be chance (‘adventitious’) matches.

In many legal systems there is a requirement to assist in addressing the question of how ‘crime related’ fibres became transferred to a particular garment or surface.

In adversarial legal systems (such as in the UK) it is common for the practitioner to be asked to assist in determining how likely it is that an activity alleged by the prosecution could account for the presence of such fibres, compared to an alternative activity alleged by the defence. For evaluative reporting, this is referred to as an “activity level” proposition.

Where this is a requirement of a particular legal system (adversarial or inquisitorial), examples of factors important in this type of evaluation include (but are not limited to): the relative rarity of the fibres, background, the nature and type of the donor and recipient surfaces, the ease with which they retain or shed fibres, the type, nature and duration of contact as well as post contact/ crime activity.

There are numerous peer reviewed published transfer and persistence studies providing data concerning the transfer and persistence of fibres on various substrates, and these can and should be used in order to inform such an evaluation. In addition, the practitioner's own casework experience is also invaluable in forming an opinion in this respect.

Where a very specific activity is alleged to be responsible for the presence of crime related fibres, it is, wherever possible and practical, desirable to carry out an experimental reconstruction of the alleged activity to inform the practitioners opinion.

Where possible, and where the legal system permits, interpretation and evaluation of the findings should be carried out using a logical evaluative reasoning approach.

For evaluative purposes, ENFSI recommends assessing the value of the findings by assigning a likelihood ratio. Guidance on this can be found in the published ENFSI document “Guideline for Evaluative Reporting in Forensic Science”.

This guideline essentially provides a robust, transparent, balanced and impartial methodology for the practitioner to evaluate how likely the findings are in a given case, given the prosecution version of events compared to those proposed by the defence.

It is beyond the scope of this document to provide examples of its use in practice, however, there are numerous published papers and texts on its application in this type of reporting.

This framework essentially provides a robust and impartial methodology for the practitioner to evaluate how likely the findings are in a given case, given the prosecution version of events compared to those proposed by the defence.

It is beyond the scope of this document to provide examples of its use in practice, however, there are numerous published papers and texts on the application of this method.

Whatever level or method of evaluation is employed by the practitioner, it is incumbent on them to ensure that any report provided for use by their legal system, should be written in a form which offers maximum transparency and justification regarding this evaluation (e.g., what tests have been performed, literature or other experimental data has been referred to, what propositions/ versions of events have been considered?) in a balanced and impartial manner.

'Confirmation bias' occurs when the practitioner sees what they expect to see, and this becomes particularly apparent where the findings are relatively ambiguous. Non-case relevant information e.g. police opinions, awareness of any particularly emotive aspects of a case ('contextual bias'), or knowledge of the opinion of any earlier examination by a colleague, can all influence the interpretation. The effects of such cognitive bias have been widely reported, but it should be stressed it has never been demonstrated or quantified in fibre examinations. Since all forensic examinations are context sensitive, it is vital that case relevant information is used in the evaluative process and that this is documented in the notes. The use of a documented case pre-assessment and a transparent, balanced and robust, peer reviewed evidence evaluation are effective methods of countering allegations of cognitive bias in fibre casework.

More detailed information regarding the evaluative reporting are given in the published ENFSI document “Guideline for Evaluative Reporting in Forensic Science”.

13 PRESENTATION OF RESULTS

The overriding duty of those providing expert testimony is to the Court and to the administration of justice.

Evidence can be presented to the court either orally or in writing. Only information which is supported by the examinations carried out should be presented. The presentation of evidence should clearly state the results of any evaluation and interpretation of the examination.

After issuing their report, the practitioner may review and alter their opinion based on new information given to them.

13.1 Presentation of written evidence

The practitioner's findings and opinion are normally provided in written form, as a statement of evidence or an analytical, investigative or evaluative report. These can be used by the investigator, the prosecutor, the defence or the court. Depending on the customer's requirements, either a shortened or a complete report can be issued.

Written reports should include all the relevant information in a clear, concise, structured and unambiguous manner, as required by the relevant legal process for the country of jurisdiction, as well as the guidelines of the institute. Where possible, reports should clearly state the outcomes of any evaluation and interpretation of the examination, and written reports must be peer reviewed.

13.2 Presentation of oral evidence

At Court, the practitioner should only respond to matters arising from their report, or those matters raised in Court, which fall within their area of expertise. Expert witnesses should resist responding to questions that will take them outside their field of expertise, unless specifically directed by the Court, and even then, a declaration as to the limitations of their expertise should be made.

14 HEALTH AND SAFETY

The relevant national health and safety regulations must be complied with.

14.1 At the crime scene

There are no specific hazards associated with the recovery of fibres at the crime scene.

Generic hazards that apply to any crime scene, for example risks associated with exposure to body fluids, trip hazards, etc., will always need to be considered and may be documented as part of the formal risk assessment process prior to scene entry. Control measures should be put in place to either remove the risk or minimise it to an acceptable safe working level.

No specific protective clothing is necessary for trace evidence recovery at crime scenes, and the usual anti-contamination clothing is adequate.

14.2 In the laboratory

Generic hazards that apply to any laboratory examination, for example risks associated with exposure to body fluids, etc., will always need to be considered. Control measures should be put in place to either remove the risk or minimise it to an acceptable safe working level.

No specific protective clothing is necessary for fibre recovery or examination at the laboratory, and the usual laboratory anti-contamination clothing (as dictated by local laboratory procedure) is adequate.

Appropriate safety equipment such as safety cabinets and eye baths, as outlined in the various procedures, should be made available near the work sites by the laboratory management. It is the responsibility of the laboratory personnel to use them where required.

Risks in the examination process at the laboratory include:

- Microscopic examination and comparison of fibres can be a lengthy process, during which time the operator may be seated for prolonged periods at the microscope. There is a risk of eye strain and postural discomfort, which should be alleviated by frequent breaks from the examination process.

- The laboratory may consider the need for a documented risk assessment of the workplace environment, to ensure that laboratory lighting is adequate and seating position is optimised, with seating providing the appropriate lumbar support and set at the appropriate height relative to the laboratory bench and microscope etc.
- Exposure to chemicals can also be an issue. Solvents may be used by some laboratories to remove fibres from tape lifts, whilst some laboratories may utilise solvent-based mountants for mounting fibres on microscope slides. The risks associated with the various chemicals, solvents and mountants used will vary.

The laboratory should undertake and document a risk assessment of the hazards associated with the use of any chemicals, solvents or mountants, in a specific activity and identify the precautions to be taken during their use in that activity to mitigate the risks. Some chemicals, solvents and mountants may have to be used in a fume hood or while wearing a suitable protective mask and safety spectacles. In some instances, certain personnel should avoid any exposure to the particular chemical. For example, xylene-based mountants may pose a risk to unborn children and exposure of pregnant female staff to these chemicals must be avoided.

All chemicals, biohazards and supplies should be stored and disposed of according to the appropriate government regulations and laboratory policy.

Firearms could also be an issue when recovering fibres. Therefore, caution should be taken when handling guns. If needed, firearm experts should be consulted before handling guns and assist when recovering fibres.

14.3 At court

There are no specific safety risks associated with fibre evidence where the materials may be brought into the public domain, such as Courts.

However, if the fibres are known to present a biohazard, for example, it is contaminated with body fluids, it should be appropriately packaged and clearly labelled to indicate the biohazard risk.

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16 AMENDMENTS AGAINST PREVIOUS VERSION

Not applicable.

17 APPENDICES

Appendices to this BPM provide additional information on methodology and analytical techniques. This information was already part of the previous guidelines [3]. For recent advances in the field the reader is intended to refer to dedicated literature or to references [9-10 & 14].

APPENDIX 1 – MICROSCOPY OF TEXTILE FIBRES

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1 INTRODUCTION

This document describes guidelines for microscopic examinations employed in forensic fibre characterisation, identification, and comparison. Several types of light microscopes are used including stereobinocular, brightfield, polarised light, comparison, fluorescence and interference. In certain circumstances, the scanning electron microscope may yield additional physical and analytical information. The nature and extent of the fibre evidence will dictate which tests or techniques are selected and performed.

The initial examination is made using reflected light stereomicroscopy and this usually consists of examining tapings that have been used to collect transferred fibres. Recovered fibres which, after this initial stage, cannot be distinguished from the known fibres are removed from the tapings and prepared on microscope slides, in a mounting medium under a coverslip, for further detailed microscopic examination. It is recommended that each recovered fibre be mounted under an individual coverslip to facilitate record keeping during subsequent examinations. The fibres are then examined microscopically with a combination of various illumination sources, filters, and attachments to determine their fibre type and record their appearance. At each stage of the examination known and recovered fibres are compared to determine if they exhibit the same microscopic characteristics and optical properties. Any recognisable difference that cannot be explained will result in that particular fibre being eliminated from the examination.

Microscopic examination of textile fibres provides the quickest, and least destructive means of determining their morphological features. The presumptive polymer type can be determined by using polarized light microscopy. Additionally, a side-by-side microscopic comparison provides a fundamental discriminating method for determining if two or more fibres are consistent with originating from the same source. It should be remembered that such a visual comparison gives a result that may be partially subjective and it is essential to conduct further tests that produce measurable results that are reproducible and objective.

2 METHODOLOGY

2.1 Mounting media

2.1.1

Many suitable media are available for temporary and permanent fibre mounts. The choice of mountant depends on availability, the particular application(s), and examiner preference; however, the important criteria discussed in [9-13] must be met:

2.1.2

Fibres that are to be compared microscopically must be mounted (see 2.3.1 and 2.3.3), and the same mountant must be used for both questioned and known fibres.

2.1.3

An examiner should be aware of the possible deleterious effects that a mounting medium (especially solvent-based media) may have on textile fibres, particularly when mounted for a long time. If fibres must be removed for further testing, the mounting medium should be removed with a solvent. It should be noted that certain solvents may affect the structure or composition of the fibre or dye.

2.1.4

If a solvent-based mounting medium is used for refractive index determination, the index of the mountant may be checked periodically against solid refractive index standards and, if necessary, readjusted to its proper value by the addition of solvent [14]. Alternatively, the refractive index of the medium can be measured directly and the value recorded by the examiner. If such a medium is used for permanent mounts, the examiner should be aware of a change of refractive index with time.

2.1.5

Liquids used for exact refractive index determinations should be known to within 0.0005 refractive index units at n_D . To make appropriate temperature corrections, values for the temperature coefficient (dn/dt) for each liquid should be available, and a thermometer covering the range 20-30°C, calibrated in tenths of a degree. High dispersion liquids ($V < 30$) are desirable for dispersion staining and the Becke line method [15]. Cargille refractive index liquids are suitable for this purpose and are recommended for refractive index measurements of fibres.

2.2 Microscope set up

Before use, microscopes must be correctly set up as detailed in the manufacturer's instructions and all users fully trained in their operation and adjustment. In particular, bright field microscopes should always be adjusted for Köhler illumination [1,2] and a comparison microscope must always be balanced using paired slides of fibres from the same source. Any microscope used to measure dimensions should have its eyepiece graticule calibrated using a slide micrometer. Instruments used to establish refractive index, birefringence or melting point should be checked to ensure that the expected results are obtained from known materials. All calibration and performance checks must be documented and dated.

2.3 Comparison microscopy

2.3.1

Recovered fibres can be mounted under individual cover slips to allow each one to be uniquely identified (see also 2.3.3)

2.3.2

When using a comparison microscope, the recovered and known fibres will be mounted on separate microscope slides and are therefore examined with separate light beams. Depending on the microscope, there may be one or two light sources. Both microscopes must be set up for Köhler illumination and balanced so that the background appears the same. Also, check the light balance by viewing two fibres known to have originated from the same source mounted on separate microscope slides before comparisons are carried out. The visual responses from the two samples must be the same colour, brightness, and clarity; a balanced neutral background colour is optimal. The use of a comparison microscope has the advantage that the recovered fibres can be easily compared with a wide range of fibres from the known material, thus taking into account variations (e.g. fibre diameter; depth of dyeing) that may occur within it.

2.3.3

Comparison using only one microscope has the advantage that both the recovered and known samples are viewed under exactly the same physical and optical conditions. However, examination using a single microscope requires an extremely meticulous work routine with exact recording of all steps taken (including photographs of the fibres being examined so that recovered and known fibres can be clearly distinguished) and should only be carried out by experienced personnel. Recovered and known fibres are mounted side by side under the same cover slip. The length of the known fibre should be chosen to clearly differentiate it from that of the recovered fibre. If the recovered fibres are of various lengths, groups of fibres of approximately the same length can be put together. Each group must be examined separately.

2.3.4

This side-by-side, point-by-point examination is the best technique to discriminate between fibres. The physical characteristics of the fibres (see 2.4 and 2.5) must be compared microscopically to determine if they are the same in the known and recovered samples. Photography may be recommended to capture the salient features for later demonstration.

2.3.5

Fluorescence is the emission of light of a certain wavelength by an object when excited by light of a shorter wavelength (higher energy). General information can be found in [41-42]. Fluorescence may arise from fibres themselves or from dyes and other additives, such as washing powder and optical brighteners [43]. Fibres should be mounted in a non-fluorescent medium to observe fluorescence [44]. Examination using various combinations of excitation and barrier filters is desirable (mostly used: UV, Blue, Green). At each excitation wavelength, the color and intensity or absence of fluorescence emission should be noted. Care should be employed to ensure that recovered fibres are not excluded because of fluorescence caused by contaminants, or the effects of localised conditions, that is not exhibited by the known fibres. It may be necessary to take several samples from the known material. It is also possible to measure emission fluorescence spectra [45-47].

2.4 Physical characteristics of manufactured fibres

2.4.1

Record all fibre characteristics that result from the manufacturing process (e.g. the same length of flock fibres or the distinctive shape of fibres from some fabrics) and fibre surface characteristics such as manufacturing striations, damage, and residual surface debris such as droplets, blood, or other foreign material. Surface characteristics are more apparent in a mounting medium of refractive index significantly different from those of the fibre.

2.4.2

Special attention should be paid to unusual morphological characteristics that may enhance the evidential value of fibres e.g. channels or voids, anti-static inclusions, flame retardant material, localized differences in colour or structure.

2.4.3

The diameter of round fibres can be easily measured using a calibrated eyepiece graticule. Fibres of other shapes require more careful consideration [16]. If fibre diameters are not

uniform within a sample, a determination of the range of diameters exhibited by the sample is recommended.

2.4.4

Colour may be uniform along the length of a fibre or it may vary. Variation in colour between fibres in a sample should be recorded. By adding colourant (mostly organic or inorganic pigments) to the polymer solution or melt before the fibres are spun it is possible to manufacture “producer-dyed” fibres. Under the microscope the colour is normally visible as more or less evenly distributed pigment particles.

2.4.5

The presence or absence of delustrant particles is a useful comparative feature. If present, the size, shape, distribution, relative abundance, and general appearance should be noted. Delustrant particles, while not indicative of any particular generic fibre type, can be characteristic of end use properties needed by a manufacturer. Also, delustrants serve to eliminate all but manufactured fibres. A distinction can be made between carbon black particles and titanium dioxide particles by examining the fibre under reflected light against a dark background. The latter will reflect and appear as bright spots.

2.4.6

When viewed longitudinally on glass slides in a suitable mountant, the apparent cross-sectional shape of fibres can often be determined by slowly focusing through the fibre (optical sectioning). It is important to view the shape along the length of the fibre. On occasions it may be helpful to do this under crossed polars. Actual fibre cross-sections provide the best information on cross-sectional shape. (See section 2.9.3)

2.4.7

It is desirable to have an accredited fibre reference collection with documented proof of the origin of the samples. Full details must be provided (e.g. Generic type, Manufacturer, Trade Name, shape, method of colouration, uses etc.)

2.5 Physical characteristics of natural fibres

2.5.1

Colour, diameter, and miscellaneous morphological/physical features should be noted for natural fibres. The following characteristics are important :

2.5.2

Cotton [80, 81] is the most common natural fibre, that grows around the seeds of the cotton plant (*Gossypium* sp.). In the raw mature state the longitudinal view of a cotton fibre shows irregular helical convolutions. The length of fibres ranges from 10 to 50 mm. The cross section mostly is flattened with lumen. Various shapes are possible, e.g. Bean-, U-shapes. Most of the time the fibre width ranges from 10 to 20 μm . Special treatments change the surface and cross section shape. Mercerization gives cotton fibres a lustrous appearance. They generally have smoothed surfaces and cylindrical shapes with few convolutions.

2.5.3

Silk [78, 79], a protein fibre produced by the silk-worm (*Bombyx mori*), has morphological features that differ from animal hairs. Some features of silk include cross-over marks, and most often a wedge to triangular cross section with rounded corners, dependent on the species. In textiles, silk may occasionally be seen as paired fibres cemented together (natural form), but is most often found as single fibres due to the manufacturing process. Fibrillation may be noticeable under high power magnification. Forms with other cross sectional shapes can be encountered (Tussah-, Anaphe Silk). In general animal hairs need to be compared with authentic specimens for identification.

2.5.4

The principal morphological features of **animal hairs** [17-19] are the root, medulla, cortex, and cuticle; shield size and subshield structures are also useful traits for species identification. Medullary and cortical structures are best observed on hairs mounted on a slide with a suitable mounting medium. Cuticular scales are best observed on replicas cast in a transparent polymer (scale casts). Scale counts (scales per 100 micrometers) can help distinguish specialty animal fibres.

2.5.5

Plant fibres [21-24, 35] may be encountered as the technical fibre (cordage, sacks, mats, etc.) or as individual cells (fabrics and paper). The examination of technical fibres should include a search for epidermal tissue and crystals and the preparation of a cross section; additionally, a chemical test for lignin may be done. Ashing may be necessary prior to examination for crystals [22]. Technical fibres should be macerated, fabrics teased apart, and paper repulped for the examination of individual cells. Relative thickness of cell walls and lumen, cell length, and the presence, type, and distribution of dislocations should be noted. The direction of twist of the cellulose in the cell wall can also be determined by the Herzog test [22,24]. Other characteristic cells should be noted and compared to authentic specimens.

2.5.6

It is desirable to have an authenticated collection of natural fibres. Information concerning their identity, origin, uses and colouration (if any) should be supplied.

2.6 Physical characteristics of inorganic fibres

2.6.1

Mineral fibres are commonly called asbestos, which is a general term for many naturally occurring fibrous hydrated silicate minerals. The asbestos minerals include chrysotile, amosite, crocidolite, fibrous tremolite/actinolite, and fibrous anthophyllite. Asbestos fibres alone or mixed with other components may occur in building materials, insulation products or as pressed sheets in gaskets. Take care when analysing asbestos fibres since they are a health hazard.

2.6.2

All asbestos minerals can be easily identified by their optical properties using polarised light microscopy. Although not considered essential, the dispersion staining technique is extremely helpful [25,26]. Scanning electron microscopy with energy dispersive spectrometry can also

be used to characterise the asbestos minerals. Non-microscopic techniques for asbestos identification include x-ray diffraction and infrared spectroscopy.

2.6.3

Glass fibres are often encountered in building materials and insulation products. Glass fibres are also called manmade vitreous fibres [27]. Based on the starting materials used to produce glass fibres, they can be placed into three categories; fibreglass (continuous and non-continuous), mineral wool (rock wool and slag wool), and refractory ceramic fibres (glass ceramic fibres). Single crystal and polycrystalline refractory fibres such as aluminum oxide, silicon carbide, zirconium oxide, and carbon are not included because they are not considered glass fibres.

2.6.4

Light microscopy together with classical immersion methods are used to determine the refractive index for the classification and comparison of glass fibres. The dispersion staining technique may be used when determining the refractive index and variation of the refractive index within a sample. Determination and comparison of the refractive index of noncontinuous (fibreglass wool), rock wool, and slag wool can also be accomplished by annealing the fibres and using the double variation method [28]. Solubility tests using 10% HCl should be conducted and the results noted. A binder resin may also be present on some glass wool products and may fluoresce under UV light.

2.6.5

Scanning electron microscopy with energy dispersive spectrometry may be used to provide elemental composition. Elemental ratios may be used for comparison purposes. It is necessary to eliminate any absorption effects when acquiring the energy dispersive spectrum or artificial variation in the elemental composition may be introduced [65].

2.6.6

It is desirable to have an authentic collection of inorganic fibre types. The same criteria apply as with other fibre types.

2.7 Optical characteristics

Detailed discussions of optical characteristics are provided by McCrone [1, 29], Heyn [30] McCrone, McCrone, and Delly [15], Patzett [31] Hartshorne and Stuart [32], and Stoiber and Morse [33]. Synthetic fibres can be classified into Generic types by determining their optical characteristics using Polarised Light Microscopy (PLM) [34-36]. A Table showing the behaviour of most generic types of man-made fibres under polarised light can be found in [34]. PLM can also be useful in identifying mercerized cotton and wool fibres.

2.7.1 Refractive index

The refractive index (n) of a transparent material is:

$$n = \frac{\text{speed of light in a vacuum}}{\text{speed of light in the material}}$$

All transparent fibres other than glass are anisotropic and display two principle refractive indices, one parallel to the long axis of the fibre (n_e), and one perpendicular to the long axis of the fibre (n_o). A third quantity, the isotropic refractive index (n_{iso}), can also be calculated from the formula:

$$n_{iso} = \frac{[n_e + 2(n_o)]}{3}$$

This approximates to the average refractive index of a fibre as if it were isotropic. Since refractive index varies with wavelength and temperature, a standard refractive index (n), is defined for all transparent materials as the refractive index at a wavelength of 589 nm (the D line of sodium) at 25 C°.

The refractive indices of a fibre may be determined by several methods. Whatever the method used, determination of n_e and n_o should be made using plane polarised light with the fibre aligned parallel and perpendicular to the privileged direction of the polariser, respectively.

Refractive index measurements may be relative or exact. A relative refractive index measurement involves: 1) determining whether an immersed object is higher or lower in refractive index than the immersion medium and 2) estimating the approximate refractive index based upon amount of contrast between the fibre and the medium. The contrast shows the amount of difference between the fibre and the medium. Exact numerical values for n_e and n_o of a fibre (at 589nm at 25 C°) can be determined by the Becke line method or by dispersion staining. Measurements using these methods have a precision of 0.001 refractive index units [16].

2.7.2 Birefringence

For a fibre displaying two refractive indices, birefringence is defined as $n_e - n_o$. Birefringence may be determined precisely by measuring n_e and n_o and then using the above formula. Alternatively retardation can be determined for a particular thickness of fibre and birefringence calculated from the following formula:

$$\frac{\text{Retardation (nm)}}{\text{Birefringence}} = 1000 \times \text{Thickness } (\mu\text{m})$$

The retardation can be roughly estimated by using a quartz wedge and observing the interference colour and order of colour at the point where the thickness of the fibre is measured and compensation has been achieved. Compensation is a situation where the retardation of light produced by the wedge is equal and opposite in sign to the retardation produced by the fibre. Under these circumstances the centre of the fibre will appear black. This can be difficult for fibres with high birefringence showing many orders of interference colours, as modern quartz wedges only cover 4 orders. A more accurate determination of retardation can be achieved by either cutting a wedge slice through the end of a fibre to count the orders [39] or by using various compensators such as the de Sénarmont, Ehringhaus and tilting (Berek) [37,38]. Compensators are birefringent and are adjustable which allows the amount of retardation to be varied. They may cover up to 30 orders. For a given thickness of compensator the interference colour in the fibre will be cancelled and this is the compensation point. At this point the background colour behind the fibre is noted and the thickness of the fibre measured. The birefringence can then be estimated from the Michel-Lévy chart. Compensation will occur in only one of the two 45° positions depending on whether the fibre has positive or negative elongation.[35,36]. When measuring retardation using a tilting compensator or quartz wedge, it must be ensured that no error has been introduced due to differences in dispersion of

birefringence between the compensator and the fibre [39]. Caution must be employed when interpreting results from deeply dyed fibres, as the dye can obscure the interference colors. The birefringence of non-circular fibres can be estimated by carefully measuring the appropriate thickness of fibre for a given compensation point [40].

2.7.3 Elongation

For a birefringent fibre, the sign of elongation is positive (+) if $n_e > n_o$ and negative (-) if $n_e < n_o$. It should be noted that all common manufactured fibres with a birefringence higher than 0.010 have a positive sign of elongation. Fibres with low birefringence (<0.005) will only appear a dull whitegrey colour; instead of showing bright interference colours. The sign of elongation is determined with a first order red plate (sensitive tint) or quarter wave compensator [34-36]. Very few man made fibres will show negative elongation, the commonest variety are the acrylic fibres.

2.7.4 Dichroism

Dichroism [34], a form of pleochroism, is the differential absorption of light by an object when viewed at different orientations relative to the vibration direction of plane polarised light. Certain dyed fibres and some mineral fibres may exhibit dichroism which is a useful additional point of comparison.

2.7.5 Interference microscopy

Interference microscopy [48] can be used to determine the refractive indices of fibres both parallel and perpendicular to the fibre axis. An interferometer is used to split a beam of polarized light, which recombines after passing through the object being examined. The fibre is successively immersed in Cargille refractive index liquids. Depending on the refractive indices of liquid and fibre, one beam will lag behind the other, producing an interferogram when the beams recombine. Depending on the pattern of the interferogram it can be determined whether the RI of the fibre is greater or less than that of the liquid. The refractive indices of the fibre can be plotted on a "Standort" diagram [34, 48]. The sign of elongation can be determined depending on whether the fibre lies above or below the zero birefringence line.

2.7.6 Dispersion staining

Dispersion staining [49] is an alternative method for determination of the refractive indices of man-made fibres. The fibres are mounted in a Cargille refractive index liquid with an R.I. of 1.525. The microscope must be equipped with a special condenser for dark field illumination [1] and a special dispersion staining objective is necessary. Using the central stop, coloured lines are visible at the periphery of the fibre. These will vary in the parallel and perpendicular positions depending on the composition of the fibre

2.8 Miscellaneous techniques

2.8.1 Cross sectioning

Examples of common cross sectional forms seen in man-made fibres are : round, roundrenallated, flat-ribbon, triangular, trilobal, irregular-lobed, bean, dogbone etc. A considerable variety of shapes exists. Some fibres may have internal channels along their length. The so-called Modification ratio [34] of trilobal fibres may help to link them to a particular manufacturer.

Physical cross sections can be prepared from fibres as short as 1mm [50-53]. Manufactured and vegetable fibres may be sectioned anywhere along their length. Animal hairs may be sectioned to yield additional identifying characteristics [17-19]. When observing manufactured fibre cross sections, the general shape, distribution of delustrant, and/or pigment particles; the presence and size of spherulites or voids; depth of dye penetration; and surface treatments should be recorded when present. The fibre dimensions measured from a cross section can be used for the calculation of birefringence and the determination of the modification ratio of trilobal fibres. Cross sections are also useful for confirming whether fibres are bicomponent.

2.8.2 Solubility (see also – Other Analytical Methods)

Solubility testing is a destructive method but can, however, provide supplemental information to non-destructive methods. Possible reactions of fibres to solvents include partial and complete solubility, swelling, shrinking, gelling, and colour change. If solubility tests are used as part of an identification scheme, appropriate knowns should be tested following the laboratory's QA/QC guidelines for a batch of solvents. It is essential to view known and questioned fibres simultaneously when comparing their solubilities [54,55, 58,62]

2.8.3 Melting point determination

A polarised light microscope equipped with a hot stage is recommended for observations of the effect of heat on thermoplastic fibres [56-60]. Using slightly uncrossed polars, one may observe droplet formation, contraction, softening, charring, and melting of fibres over a range of temperatures; these observations, including melting temperature(s), should be recorded. Since manufactured fibres are composed of mixtures of chemical compounds rather than pure polymers and are a combination of crystalline and amorphous regions, changes are observed over a temperature range rather than at a single melting point. Fibres should be mounted in an inert, heat-resistant medium, such as a high temperature stable silicone oil, to ensure reproducible melting behaviour [60]. Accurate and reproducible results are best obtained using a heating rate of no greater than 1-2 °C/minute when near the initial melting temperature. The hot stage should be calibrated using appropriate standards, following established guidelines. The recommended melting point apparatus should be adjustable for temperatures from ambient to at least 300 °C, in increments of 0.1°C , and should allow a heating rate of as low as 1 °C /minute.

2.8.4 Scanning electron microscopy / energy dispersive X-ray

SEM/EDX [63, 70] is used as an imaging and microanalytical tool in the characterisation of fibres. Fibre surface morphology can be examined with great depth of field at continually variable magnifications. Fibres and/or prepared cross sections are mounted to a specimen stub and may be conductively coated to prevent possible electron beam charging. The use of a suitable calibration standard is recommended for the accurate measurement of fibre cross sections.

Applications of SEM-EDX to fibre analysis may include the characterisation of fibre surface morphology [64] cross sections, [61] identification of pigments and delustrants by elemental analysis, fibre damage due to cuts and tears [65-69] trace debris and chemical residues on fibres and surface feature modifications. SEM-EDX can also be used to identify elements [62-63] originating from flame retardants (e.g. halogens, antimony, phosphorus) in fibres as well as tannins and dyes in leather fabrics. Surface imaging using the SEM as an aid in the identification of animal hair scale structure has been reported [71].

3 FIBRE-PLASTIC FUSIONS/FIBRE-METAL FUSIONS

These are contact traces [72-77] produced almost exclusively in traffic accidents that allow determination of the seating arrangement of the occupants of the car at the moment of impact and the trajectories of the occupants during the accident. The traces occur due to the kinetic energy generated by the impact resulting in frictional heat causing local softening of thermoplastic material (fibres and plastic components inside the vehicle which are rubbed together). Transfer may occur in both directions. The following techniques are involved in the examination of these traces :

- Isolation and removal using stereomicroscopy
- Identification of fibres using brightfield/PLM/FTIR-microscopy
- Identification of plastic material by preparing a heat pressed film which can be examined using brightfield/darkfield/PLM and FTIR-microscopy.
- Fibres films and fragments may be found on bullets.

4 TERMINOLOGY

Anisotropic

The non-uniform spatial distribution of physical or optical properties e.g. in polarising microscopy this refers to the preferential orientation of optical properties with respect to the vibration plane of polarised light, leading to birefringence and pleochroism

Analyser

A polariser used after the object to determine the effect of the object on the illuminating light, usually polarised

Autofluorescence (primary fluorescence)

The inherent fluorescence of a substance

Barrier filter

Used in fluorescence microscopy to suppress unnecessary excitation light that has not been absorbed by the fibre and selectively transmits only the fluorescence

Becke line

The bright line near the boundary of a fibre (or other material) in a medium of different refractive index which moves as the object is focussed through the point of best focus

Becke line method

A method for determining the refractive index (RI) of a fibre relative to its mountant by noting the direction in which the Becke line moves. This is in the direction of the higher RI as the distance between the object and the objective is increased. The RI of the material can be precisely measured by immersing the sample in a series of liquids of known RI. Where the RIs are matched, the Becke line disappears

Birefringence (Δn)

The numerical difference between refractive indices in a fibre (or other anisotropic material). Birefringence (Δn) = refractive index parallel to fibre length (n_e) – refractive index perpendicular to fibre length (n_o)

Comparison microscopy

Two microscopes linked by an optical bridge that contains a series of prisms that present images from both microscopes to a single pair of eyepieces. The image field is split allowing

two specimens to be examined and compared simultaneously in either transmitted or reflected light.

Compensator

A retardation plate of variable optical path length used in polarising microscopy to measure optical path length differences in the object. Many types exist from the simple quartz wedge to other more complicated types that are often designated with the name of their originator e.g. de Sénarmont, Berek, Ehringhaus

Cortex

The main structural component of hair consisting of elongated and fusiform (spindle shaped) cells. The cortex may contain pigment grains, air spaces called cortical fusi, and ovoid bodies which are aggregations of pigment

Crimp

The waviness of a fibre

Cross-section

A thin section cut perpendicular to the fibre axis, to enable the cross-sectional shape to be determined

Cross-over marks

Oblique flattened areas along silk fibres caused by the overlapping of extruded silk fibres before they have dried completely

Cuticle

The layer of scales composing the outer surface of a hair shaft. Cuticular scales are normally classified into three basic types; coronal (crown-like), spinous (petal-like), and imbricate (flattened)

Delustrant

A white pigment, usually titanium dioxide, used to dull the luster of a manufactured fibre

Dichroism

An example of pleochroism occurring in some optically uniaxial materials. Visualised as the sample demonstrating two different colors when parallel and perpendicular to the privileged direction of the polariser

Dislocations

Concerning natural fibres (e.g., flax, ramie, jute, hemp) where distinct features in the shape of X, I, V's are present along the fibre cell wall. These features are often useful for identification

Dispersion of birefringence

The variation of birefringence with wavelength of light

Dispersion staining

A technique for refractive index determination that employs central or annular stops placed in the objective back focal plane of a microscope

Dyes

Soluble substances that add color to textiles (and other substrates). Dyes are classified into groups that have similar chemical characteristics (e.g., acid, basic, azo). They are incorporated into the fibre by chemical reaction, absorption, or dispersion

Excitation filter

A filter used in fluorescence microscopy that transmits specific bands or wavelengths of energy capable of inducing visible fluorescence in various substrates

Fluorescence

A form of photoluminescence which effectively ceases after the excitation illumination is removed. It actually persists for a very short time ($<10^{-8}$ s) but is not seen

Fluorescence microscopy

Microscopy in which the image is formed by fluorescence emitted by the object

Inorganic fibres

A class of fibres of natural mineral origin (e.g., chrysotile asbestos) and manmade mineral origin (e.g., fibreglass)

Interference colours

Colours produced by the interference of two out-of-phase rays of white light when a birefringent material is observed at a non-extinction position between crossed polars

Isotropic

Uniform spatial distribution of physical and optical properties of a material. In polarised light microscopy refers to uniformity of optical properties with respect to the vibration direction of plane polarised light

Isotropic refractive index (n_{iso})

The average refractive index of birefringent materials which approximates to the refractive index of the unoriented polymer.

Light microscope

A microscope that employs light in the visible or near-visible portion of the electromagnetic spectrum

Lignin

The majority noncarbohydrate portion of wood. It is an amorphous polymeric substance that cements cellulosic fibres together

Lumen

The cavity or central canal present in many natural fibres (e.g., cotton, flax, ramie, jute, hemp). Its presence and structure are often a useful aid in identification

Luster

The gloss or shine possessed by a fibre, resulting from its reflection of light. The luster of manufactured fibres is often modified by use of a delustering pigment

Manufactured Fibre

A class name for various families of fibres produced from fibre-forming substances which may be synthesized polymers, modified or transformed natural polymers and glass

Medulla

The central portion of a hair composed of a series of discrete cells or an amorphous spongy mass. It may be air-filled, and if so, will appear opaque or black using transmitted light or white using reflected light. In animal hair, several types have been defined: uniseriate or multiseriate ladder, cellular or vacuolated, and lattice

Michel-Lévy chart

A chart relating thickness, birefringence, and retardation (orders of Newton's colours) so that any one of these variables can be determined for an anisotropic fibre when the other two are known

Modification ratio

A geometrical parameter used in the characterisation of noncircular fibre cross-sections. The modification ratio is the ratio in size between the outside diameter of the fibre and the diameter of the core. It may also be called “aspect ratio”

Natural fibres

A class name of fibres of vegetable origin (e.g., cotton, flax, ramie), animal origin (e.g., silk, wool, and specialty furs) or of mineral origin (e.g., asbestos)

Pigment

A finely divided insoluble material used to deluster or color fibres (e.g., titanium dioxide, iron oxide)

Phosphorescence

A form of photoluminescence which persists for an appreciable time (usually $>10^{-8}$ s) after the cessation of the excitation

Photoluminescence

The selective absorption of radiation of short wavelengths by matter and the emission of radiation of longer wavelengths

Plane polarised light

Light that is vibrating in one plane

Pleochroism

The general term for the change in absorption color with the vibration direction of the illumination. In uniaxial fibres the proper term is dichroism

Polar

Any device which selects plane polarised light from natural light

Polarised light

A bundle of light rays with a single propagation direction and a single vibration direction. The vibration direction is always perpendicular to the propagation direction. It is produced by use of a polar, from ordinary light by reflection, or double refraction in a suitable pleochroic substance

Polarised light microscopy

A microscope equipped with two polars, one below the stage (the polariser) and one above the stage (the analyser)

Privileged direction (of a polar)

The direction of vibration to which light emerging from a polar has been restricted

Refractive index (n)

For a particular transparent medium, the ratio of the speed of light in a vacuum to the speed of light in that medium

Relative refractive index

The ratio of the speed of light in one medium to that in an adjacent medium

Retardation (r)

The actual distance of one of the doubly refracted rays behind the other as they emerge from an anisotropic fibre. Dependent upon the difference in the two refractive indices, $n_e - n_o$, and the thickness of the fibre

Retardation Plate

Optically anisotropic material inserted between crossed polars to produce a specific retardation

Sign of elongation

Referring to the elongation of a fibre in relation to refractive indices. If elongated in the direction of the high refractive index, the fibre is said to be positive; if elongated in the direction of the low refractive index, it is said to be negative

Spherulites

Spheres composed of needles or rods all oriented perpendicular to the outer surface, or a plane section through such a sphere. A common form of polymer crystallisation from melts or concentrated solutions

Stereomicroscope

A microscope containing two separate optical systems, one for each eye, giving a stereoscopic view of a specimen

Surface dye

A colorant bound to the surface of a fibre

Synthetic fibres

A class of manufactured polymeric fibres which are synthesised from chemical compounds (e.g., nylon, polyester)

Technical fibres

A bundle of natural fibres composed of individual elongated cells that can be physically or chemically separated and examined microscopically for identifying characteristics (e.g., hemp, jute, and sisal)

Thermoplastic fibre

A synthetic fibre that will soften or melt at high temperatures and harden again when cooled

Thickness (t)

The optical path through the fibre used for the calculation of birefringence, typically measured in microns (μm)

Uniaxial

Having one crystal optical axis

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APPENDIX 2 – MICROSPECTROPHOTOMETRY OF TEXTILE FIBRES

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1 INTRODUCTION

Colour is one of the most important characteristics to distinguish fibres. MSP (Microspectrophotometry), DAD- (Diode Array Detection) and CCD- (Charged Coupled Device) Spectroscopy offer highly discriminative methods for comparison and analysis.

Microspectrophotometers (MSPs) have been used conventionally in forensic science since the mid 1970's and now provide a quick, reproducible, highly discriminating method for colour analysis and comparison.

The comparison of two or more colours is no longer subjective as it is when using comparison microscopy. It is recognised that the human eyes' impression of a colour match depends on factors such as eye adaptation and fatigue and also on the change of light or light source. The colour perception of stimulus varies from one observer to another and from the same observer at different times. It is also imperative to be assured that two samples are dyed in the same way – metamerism is difficult to detect with the eye i.e. fibres that display the same hue but have been dyed with different dyes. MSP is well suited to objective analysis and comparison of samples and has a high degree of discrimination.

This technique has been tried and tested by forensic fibre experts with the undoubted conclusion that, when performed on a modern instrument, it is highly discriminating. What is of particular importance to an operator is a clear understanding of the capabilities of the instrument, especially those manufactured in the past ten years.

When undertaking comparisons with dedicated software the true potential of this instrument for colour analysis falls into perspective; however, it is just as important to be able to recognise its limitations and to use other discriminating techniques when necessary. Obvious examples where other techniques should be considered include heavily dyed fibres that produce bland, featureless spectra and very light coloured fibres with undescriptive spectra.

As the instruments are highly specialised it is recommended that specialists operate them in order to obtain the best results. This recommendation is of particular importance if the technique is to be used in the absence of other complementary techniques.

There are still a range of MSPs available in the laboratories and on the market. The following guidelines are laid out in a general format with a view to being applicable to older and newer instruments alike, inclusive of the more recent Diode Array Detector (DAD) and Charge Coupled Device (CCD) systems.

2 THEORY

2.1 Analyte structure

Within the Electromagnetic spectrum of radiation lies the near ultraviolet-visible region (UV-Vis) extending between approximately 240-800 nm. The interaction of light in this region with electrons found in coloured molecules can result in the excitation of the electrons. Dyes (or pigments) are substances with conjugated systems of excitable electrons; therefore Microspectrophotometry has been established as a method of measuring the interaction of the radiation of different wavelengths of light across the UV-Vis region in terms of absorption or transmission by a coloured analyte – inclusive of dyed/pigmented textile fibres.

An MSP has the ability to measure the attenuation of the radiation at each selected wavelength, across the UV-Vis region. The physical result of this interaction between the radiation and the substance being examined (the analyte) is always measured as a spectrum and is thus related to a part of the structure of the dye/pigment molecules within the analyte.

The areas of a colourant that are responsible for the absorption of light are called chromophores; these are simple unsaturated groups attached to e.g. a benzene ring. To increase the depth of colour of a colourant, and allow molecular bonding to fibres, chromophores are combined with salt-forming or reactive groups, known as auxochromes. Man-made fibres, as opposed to natural fibres, usually have a homogenous chemical

structure; thus the dye is often bonded to the fibre in an even manner and demonstrates little intra-sample variation in terms of its absorption or transmission of light.

2.2 Instrumentation

Generally there are two different methods for UV/VIS-Spectroscopy available: MSP's with step monochromator which allow only a defined area of light (with a certain bandwidth) to pass through the sample at a time (2 nm steps are often used) or DAD- or CCD Spectrometer where a single beam of light passes the optical path and the sample simultaneously and is split for detection only after passing the sample.

2.2.1 MSP's with monochromators

The MSP has been designed to meet the requirements of analysing the effects of colour and/or finishing products of an analyte in situ. In scanning spectroscopy an MSP consists of a microscope with light source, a monochromator, a photomultiplier and data processing devices. The main function of the microscope is to transmit light energy in the most efficient and reproducible format; this is wholly operator dependent and critical for production of quality spectra. Illumination should be provided with a 12v/100w halogen bulb for the visible region, a Xenon 75W or a combined halogen/deuterium light in the UV-Vis range and a HBO mercury bulb for making reflected light fluorescence measurements. The bulbs may be obtained from different manufacturers.

It is suggested that an apochromatic fluorite objective of $\times 40$, is used for fibre analysis in the visible region. A condenser with an aperture not exceeding 0.6 is also desirable. Objectives for UV-Vis are specialised, made from quartz glass or based on a Cassegrain (mirror) objective.

A monochromator produces monochromatic light of a certain spectral bandwidth. The different spectral bandwidths pass the analyte step by step and a spectrum is created point by point. This is known as scanning spectroscopy.

Monochromators consist of gratings, that is, they allow linear calibration of the wavelength scale. The grating varies from 600-1221 lines per mm. depending on whether the system is operating in the Vis and/or UV region. The gratings in UV-Vis monochromators are holographically blazed and may thus cause unwanted signal enhancement leading to the appearance of Wood's anomaly in the spectrum. This, and polarising effects caused by the analyte, can be suppressed with the insertion of a polarising filter in the optical path in front of the analyte. The polariser and the fibre are usually oriented in a north-south direction.

At the end of the optical path is a photosensitive device that transfers the photons of the light into electrical energy. This is called a Photo Multiplier Tube (PMT) and generally sensitive over the spectral range 240-800 nm.

2.2.2 Multichannel Spectrometers (MCS)

The use of multichannel spectroscopy (MCS) is meanwhile widely established in forensic fibre analysis. The measurement time is drastically reduced due to simultaneous multichannel detection. A DAD is composed of a grating where photosensitive diodes are placed side by side to detect only the light originating from certain wavelengths to scan, store and processed the information onto a computer. The use of a diode array is to monitor light that has passed through an analyte. The resolution will depend on the number of diodes. A sufficient number of diodes must be incorporated in the array to cover this range of wavelengths. A Charge Coupled Device (CCD) holds more than one row of diodes to detect and process the light of a certain wavelength. The resolution for detection depends extremely on the number of diodes used in a DAD or CCD system. Older systems sometimes lacked a sufficient number of diodes with a small bandwidth. Newer systems meanwhile offer a high number of diodes so that the quality of spectra is comparable to MSP. Besides XPO bulbs the use of a combined

Deuterium/Halogen light source has proven to produce very good and reliable results. The method is less time consuming and equally exact if the setup was performed correctly.

2.3 Data processing

Commercially available MSPs have incorporated the requirements of operational settings, data control, processing and recording with the support of a system processor. As such they all carry their own software routines to cope with the tasks. In addition there are further software routines available for use with these systems.

Through the science of colour coding (colorimetry) it is also possible to numerically determine and specify colours. Assuming that the colourant obeys Beer's Law and the data is acquired in absorbance then these values, termed complementary chromaticity coordinates (ccc's), may be collected.

3 METHODOLOGY

3.1 Sampling

Any garment which is a potential source of transferred textile fibres should be sampled in such a manner as to represent areas of the garment most likely to be the area of contact. The whole range of variation in this area should be covered.

The use of forceps is suggested for taking warp and weft samples from appropriate locations. Attention should be paid to local artefacts such as wear and tear or to any variations that may have resulted from localised physical and chemical changes.

A fibre lift of the appropriate area may be used and fibres removed from it to be truly representative of the shedding fibres.

The fibres should be mounted on a flat slide in a suitable mounting medium, with negligible or no fluorescence appropriate to the analysis. In general glass slides and coverslips can be used with various mounting medium (e.g. Entellan New, Phytostol or Histomount) for Vis analysis. Measurements in the UV range require the use of quartz slides and cover slips and a non-fluorescent mounting medium such as Glycerol or Zeiss Fluorfree. Questioned fibres and those from the reference sample must always be mounted in the same medium.

The mounting of fibres from the reference sample in a line parallel to the short edges of the slide can be very time saving when carrying out analysis in the UV range.

The tracking of fibres and demonstration of continuity should be adhered to throughout analysis. Each analyte should possess a unique identifier, inclusive of Case Number and Exhibit Number. The questioned fibres should also be identified in such a manner that no errors could be feasibly made by inadvertently analysing the wrong fibre on the wrong microscope slide.

It is suggested that the reference fibres are mounted under their own cover slips and questioned fibres are individually mounted under their own cover slip and labelled uniquely. Alternatively, the reference and questioned fibres can be mounted under the same cover slip, provided adequate steps are taken to ensure that they can be differentiated.

The aim of the operator is to demonstrate, by using the instrument to its full effect, the full range of variation of dye intensity and colour measurement artefacts present in the reference source sample prior to analysing the questioned fibres.

Due to the very nature of the analyte under investigation it is not recommended to set a definitive rule on the numbers of fibres that should be analysed (cf. Quality Assurance section of the BPM). The aim is to sample sufficient fibres from the potential source to allow meaningful conclusions to be drawn about the potential match of the questioned fibres.

It is suggested that a minimum of 5 spectra from individual fibres is collected from each reference colour and/or fibre type. This must be adjusted according to circumstances dependent on the degree of spectral consistency.

Generally there is a greater degree of intra-sample variation observed in dyed natural fibres, thus this wider variation in dye intensity should be demonstrated usually by analysing greater number of fibres when necessary.

It should be remembered that fibres are not plane objects so characteristics such as cross-sectional shape, levels of delustrant and presentation of the analyte will all have an effect on the result. When selecting measuring areas these factors should be taken into account and the choice made carefully and consistently.

3.2 Calibration

This ensures that the instrument is operating to expected standards. If your Laboratory is accredited or seeks accreditation it is paramount that the operator of the MSP can demonstrate to an arbitrator that the instrument is calibrated while in use.

Before any form of calibration is undertaken the system must demonstrate absolute consistency in the optical path so results are comparable. The microscope should be set up for Kohler illumination; normal operating parameters should be defined and the lamp allowed to warm up and stabilise according to manufacturers' instructions.

Demonstration of wavelength accuracy and absorbance are important and if colorimetry is used as a comparative feature then that too should be standardised using traceable commercial standards.

Wavelength accuracy over the visible range can be checked with the aid of holmium and/or didymium filters. As the exact position of the absorption bands vary from batch to batch it is important that the filter is authenticated and traceable to original data from the manufacturer. Resolution can also be checked routinely using these filters.

Spectral emission lines of a mercury lamp may be used for calibration as a yearly check as part of a maintenance service, usually provided by the manufacturer. A mercury lamp also has emission lines in the UV region suitable for calibration purposes. The check should also include a narrow band interference filter that checks the spectral resolution.

Demonstration of standardisation of absorbance is also important especially if undertaking colorimetry. It also acts as a check on linearity, it is important that these standards are authenticated and traceable to original data from the manufacturer. Absorption filters will normally be placed in the sample plane, but if they are not, a blank slide, plus mountant and cover slip must be placed in the sample plane to assist with Kohler illumination.

Consistency may be demonstrated using the 100% transmittance line. This is acquired by measuring an area without the analyte; because there is no absorbing substance in the optical path, the transmittance at all wavelength steps should be $100\% \pm 1\%$. It can also be used to indicate aberrant behaviour of the lamp especially with the xenon lamp.

Calibration limits of each standard are supplied with authenticated samples direct from the manufacturer.

It is suggested that the calibration standards are performed and recorded for reference at least once per month, or adjusted accordingly to frequency of use.

If your Laboratory is using an MSP or MCS on a daily basis it is suggested that you should undertake at least one calibration check to demonstrate reproducibility. During operation the operator may run the 100% line and/ or a background scan for each cover slip. Necessary adjustments can be made to demonstrate instrument consistency.

3.3 Scanning parameters

Measurement values may be adjusted using software applications. In general the lower the resolution value the better the spectral quality will be due to an enhancing of the signal to noise ratio; however MSPs/MCSs have individual characteristics which may involve altering the resolution, bandwidth, step width and number of scans. The wavelength range should reflect the maximum amount of information you are attempting to gather – depending on the instrument used this will be in the region of 240-780 nm. The measurement may be carried out

in Absorbance or Transmittance mode and the measuring slit adapted where possible to the analyte under investigation.

It is suggested that the parameters that are best suited to your instrument are discussed with the manufacturer. The step width and bandwidth should be set at 2.5 nm or less.

Those operators who traditionally undertake measurements in transmittance mode should consider using absorbance for deeply dyed or weakly dyed fibres. Absorbance mode must be used if colorimetry data is to be collected in order that the relationship between dye concentration and colour values (ccc values) is linear.

3.4 Measurement

It is standard practice to initially adjust the PMT/DAD/CCD-Spectrometer to operate at its best potential depending on the light energy it is receiving. It is also necessary to obtain a scan of the background reference absorption/transmission prior to scanning the analyte.

The reference scan should be made from an area immediately adjacent to the fibre on the same slide. The number of scans, the resolution, step width, bandwidth and number of measurements can be varied using the software. All parameters must remain the same between scans of background and analyte. The fibres should be measured in the same orientation (normally north south) to avoid false exclusion due to the effect of dichroism. Areas selected for analysis should be uniform and replicated in both the reference and questioned fibres – see Section 5, Suggested measuring areas.

If the fibres are particularly pale in colour the operator should consider closing down the lower field diaphragm to reduce stray light around the measuring slit and raising the resolution accordingly to reduce noise. The lower field diaphragm may also be used as an alternative measuring slit if ultrafluar objectives are used. If the fibres contain a deep coloured dye, which produces a bland featureless spectrum, then potentially more discriminating techniques should be considered.

If analysing in the UV region the fibre should be measured using monochromatic light of narrow bandwidth and the monochromator must be placed in front of the sample to prevent bleaching of the dye by exposure to a higher level of radiation. With a DAD/CCD instrument it is essential to prevent that the analyte is constantly placed in the light path of the light source used for analysis. Very fast scanning also ensures that bleaching is minimal.

3.5 Fluorescence emission measurement

Fluorescent fibre dyes and optical brighteners absorb light energy and then emit energy usually at a longer wavelength. This feature may be measured as an additional characteristic.

Fluorescence emission spectra can be measured in addition if fluorescence is observed and systems are equipped.

To carry out such an examination the microscope must be fitted with an incident fluorescence illuminator fitted with filter cubes for the excitation by the mercury lines at 365 nm and 436 nm. The use of plan-neofluar immersion objectives is recommended.

3.6 Linear dichroism measurement

Pleochroism is a well-known phenomenon in mineralogy. Some minerals show differences in colours when viewed under plane polarized light (PPL) with different orientations. In textile fibres, only two main orthogonal directions are considered, which correspond to the fibre long axis and to its perpendicular direction. Linear dichroism (LD) is the selective absorption of polarised light of different orientation, corresponding to these two main fibre directions. Fibres possessing LD show differences in hue and/or intensity when observed with PPL of different orientation. Microspectrophotometry (MSP) can also be used to measure the absorption of PPL in the two main fibre directions.

Linear dichroism is encountered in dyed fibres, such as polyesters, proteinic fibres (nylon, silk and wool), cellulosic fibres (cotton and viscose) and low birefringent fibres (acetates and

acrylics); and also in pigmented fibres. The measurement of LD with MSP-PPL can be used as an additional tool for comparing questioned and reference fibres.

To carry out such an examination the microscope must be fitted with a rotatable substage polariser. Two methods using a different measurement sequence can be used for MSP-PPL:

- The first method can be used for manmade fibres or straight natural fibres such as wool, silk or flax. Measurements in parallel position are performed with both fibre and polariser direction, oriented northsouth. Measurements in perpendicular position are performed with the fibre oriented northsouth and the polariser direction oriented eastwest.
- The second method can be used for cotton fibres as their morphology requires a different approach. Instead of changing the direction of the polariser, only the northsouth orientation is used. The measurement zone is set to a square of $10 \times 10 \mu\text{m}$. First, a reference spectrum is recorded next to the fibre. Then, the two measurements in parallel and perpendicular position are performed, the latter by rotating the fibre by 90° . Careful positioning must guarantee that the measurement in perpendicular position is performed at exactly the same area as for the one in parallel position.

4 INTERPRETATION AND DISCUSSION

There are a number of guiding principles which are used by spectroscopists that are just as applicable to this type of examination.

4.1 Spectral comparison / interpretation

Spectral comparisons may be undertaken either by overlaying the spectra of interest on a light box or using a dedicated software routine and comparing the spectra on screen. The first method is rapidly being replaced by software routines as they have the added advantage of providing the opportunity to expand and overlay bands. Modern software also allows for the examination of both the questioned and reference fibres as populations in their own right; mean spectra of populations may be compared and the spectra can be stored into colour block databases. Most systems also offer software to switch spectra between absorption and transmission. The first derivative of the absorbance spectra should also be taken into account for evaluation. First derivative spectra sometimes prove to be helpful for the interpretation of spectra with a broad and featureless appearance.

The interpretation of all spectra (absorbance, transmittance, first derivative) depends on the variety of fibres. The number of spectra need to be taken from a single fibre depends on the consistency of the fibre dye but it seems to be advisable to take more than one spectrum from a single fibre when the fibre shows lighter and darker areas.

Ideally to be considered indistinguishable the spectra under consideration should be congruent i.e. have identical shapes so that all parts correspond. A spectrum that demonstrates a considerable deviation away from the absorbance/transmittance range of the reference sample generally will be eliminated, or queried at an earlier stage through comparison microscopy.

Fundamental principles of comparison and interpretation include:

- Initial examination of the whole spectrum, followed by critical examination of specific bands (their shape and the absorption maxima and minima), troughs, shoulders and the curves between them.
- Gain sufficient appreciation of the range and variety of shape expressed in the reference sample (this is dependent on the sample size analysed).
- Comparison of each questioned spectrum with the reference source and if possible a comparison of the questioned population with that of the reference population.
- When analysing natural fibres a wider range is often observed in the reference source. This may be as a direct result of variation in dye uptake. This does not undermine the

value of the comparison and on occasion may enhance the value if the questioned fibres demonstrate the same characteristic. Standard deviations can prove particularly useful in these instances in determining the range.

- It is paramount that in cases where the discrimination power is considered to be insufficient other complimentary techniques are undertaken if possible.

4.2 Colorimetry comparison/interpretation

Data accrued by numerical colour coding has been adopted in some Forensic Labs as a further means of colour comparison. On such occasions the ccc values tend to be used and specific standard deviation parameters are adopted. The approach is to ascertain whether or not the ccc's obtained from a questioned fibre falls within the range demonstrated by the known sample.

It should be noted that a ccc figure should not be considered in comparative terms to be as specific as the spectrum but may be used to complement spectral results. Factors affecting the spectral comparison may all affect the data produced.

The use of Error Ellipses may be of value – not usually in the form of an aid to matching criteria – but more as a tool to demonstrate the degree of variation within the sample and to determine any subtle differences between populations.

More frequently the ccc data is used as a source of colour reference which can prove to be useful as a search parameter in a database.

It is suggested that the operator does not rely solely on colorimetry results when making comparisons. A spectral comparison should always be undertaken as different spectra may have similar ccc values.

A clear understanding of the technique – together with some well-founded and reproducible principles within the laboratory on the lines of those set out above – should give high quality results. It is desirable that the results be interchangeable between laboratories; however, where different instruments are being used this may not always be possible.

It should be noted that ccc data only refers to the results produced in the visible range. It is not advisable to rely only on ccc data.

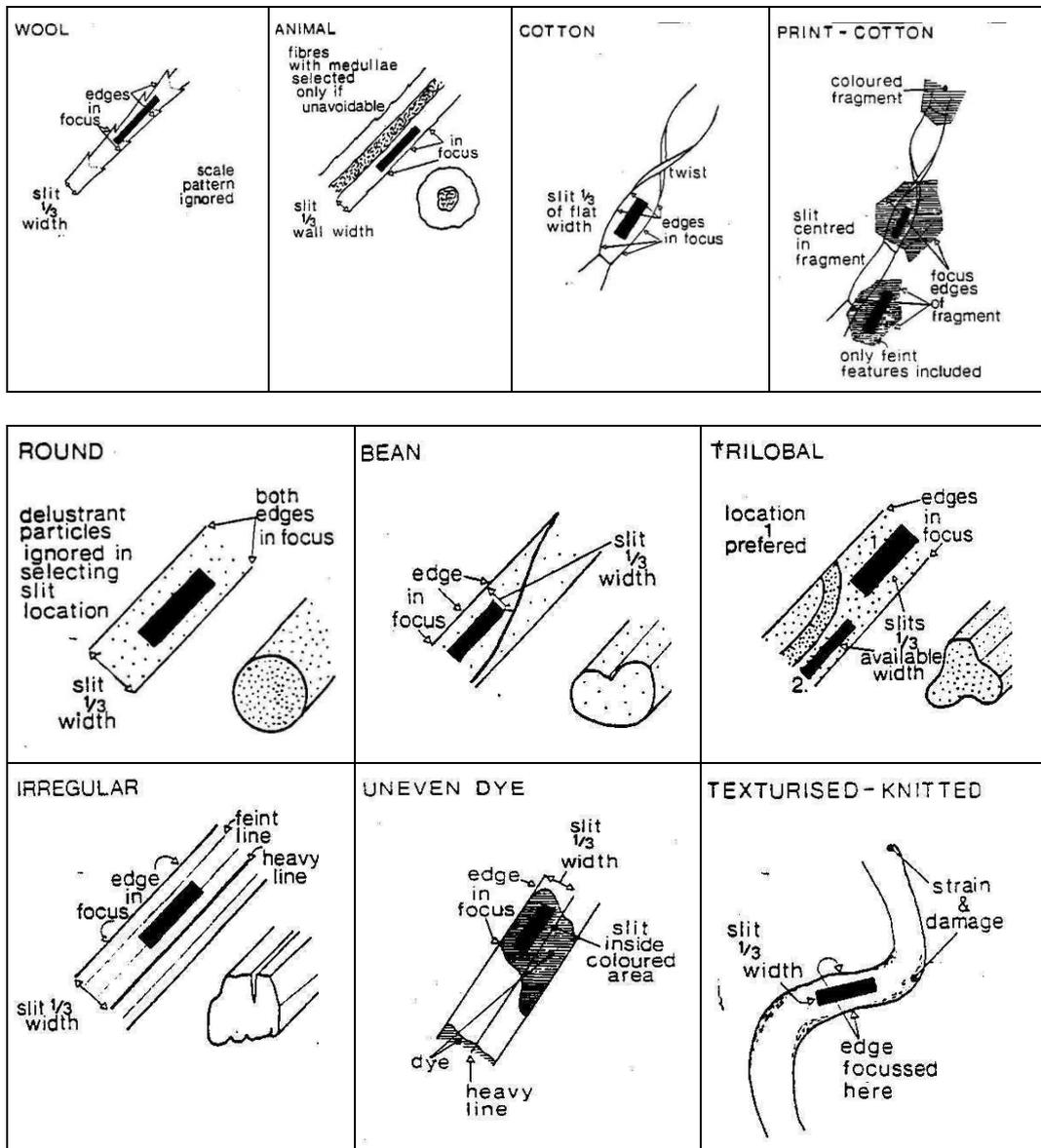
4.3 Databases

Some laboratories now accrue the data: some in the form of spectra, others include physical characteristics and manufacturers details as well. Modern software packages offer the capabilities to store the information in blocks of colour. As long as these databases are kept up to date and used wisely they can offer valuable information both in terms of evidential value and as an intelligence aid.

To use a database effectively a considerable amount of data is required.

5 SUGGESTED MEASURING AREAS

N.B. position of aperture is irrespective of the fibre shape.



6 TERMINOLOGY

Absorbance

The logarithm to the base 10 of the reciprocal internal transmittance expressed as a fraction and often referred to as optical density.

Absorption

The transformation of light to a different form of energy by interaction with matter. It may vary with wavelength giving rise to colour.

Analyte

A designated substance that is available for analysis.

Auxochrome

Colour bearing molecules (chromophores) may be modified and increased in colour intensity by the introduction of a variety of smaller groups into the molecule.

Bandwidth

Width of the monochromator exit slit.

Beer's Law

The basic quantitative expression of the absorbance of electromagnetic radiation. The absorbance of a solution is equal to the product of the absorptivity, the concentration of solute and the thickness of the layer through which the radiation passes.

Chromophore

The part of the molecular structure of an organic dye or pigment responsible for colour.

Complementary Chromaticity Coordinate (ccc)

A figurative expression of colour derived from normalising the perception of the colours red, green and blue; with the assumption of linearity through Beer's Law.

Error Ellipse

Variations in ccc values forming an elliptical shape when plotted in a chromaticity diagram.

Grating

A set of structures repeating regularly which when illuminated produce maxima and minima of intensity as a consequence of interference. These vary in position according to wavelength. Radiation of any given wavelength may thus be selected from complex radiation allowing the grating to be used as a monochromator.

Monochromator

A device designed to isolate monochromatic radiation from a complex radiation.

Photo Multiplier Tube

A photosensitive device that occurs at the end of the optical path which transfers the photons of the light into electrical energy.

Questioned fibre

These are the fibres, which have been removed, during the examination, for further analysis and comparison with the known fibre.

Reference fibre

Fibres with distinctive characteristics (e.g. generic type, morphology and colour) from a known textile source, e.g. a garment, which will be compared with the recovered fibres. The following terms are also used e.g. control, donor or target fibres.

Step width

Distance between two points of measurement in a spectrum.

Transmittance

The measure of transmission in terms of either the ratio of transmitted intensity to incident intensity (total transmittance) or the ratio of the intensity reaching the final or exit surface of the medium to that entering the medium (internal transmittance). Consequently, total transmittance is influenced by both absorption and reflection; internal transmittance is influenced by absorption only.

Wood's anomaly

The effect caused by a polarisation of defracted energy due to decreasing reflectance on the holographically-blazed monochromator. This can be observed in the form of a distinct transmission maximum within the spectrum.

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APPENDIX 3 – INFRARED SPECTROSCOPY OF TEXTILE FIBRES

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1 INTRODUCTION

Complete information about fibre composition is not always apparent from optical data, microchemical tests, or melting point. An additional method of analysis should be used such as infrared (IR) spectroscopy or e.g. pyrolysis gas chromatography. Infrared analysis offers the advantage of being the less destructive of these methods. It is an established technique e.g. for the identification and comparison of fibres and polymeric materials in forensic examinations. There is the potential for obtaining additional compositional information by using IR spectroscopy in addition to polarising light microscopy, PLM. Because of the large number of sub-generic classes, forensic examination of fibres containing e.g. polyacrylonitrile is likely to benefit significantly from IR spectral analysis. New fibres can be characterised and in some cases the identification of the fibre generic class may indicate the manufacturer. The spectral information due to the presence of dye or pigment, especially in man-made fibres, may also be used for comparison purposes. This guideline covers the identification of fibre composition by interpretation of absorption spectra obtained by infrared spectroscopy. Infrared spectra of fibres are obtained using an IR spectrometer which may be coupled with an IR microscope. The recommended point for IR analysis in a forensic fibre examination is following visible and comparison microscopy with fluorescence, PLM, visible or UV/visible spectroscopy, but before dye extraction for thin-layer chromatography. Additional spectra may be run after dye extraction. The use of IR spectra for comparison purposes is also discussed.

Fibre identification is made by comparison of the fibre spectrum with laboratory reference spectra or by following guidelines for the interpretation of spectra. Commercial IR spectral libraries may also be used. The extent to which IR spectral comparison is indicated will vary with specific sample and case evaluations.

This Appendix is written as a guideline for individuals and laboratories that conduct forensic fibre examinations and comparisons and is intended to be applicable to a wide range of infrared spectrometer and microscope configurations. The systems described relate to the effective application of infrared spectroscopy to the analysis of fibre evidence. These are applicable not only to the infrared analysis of polymeric materials and single fibres but also to the analysis of small particles. This list of analytical techniques is not meant to be totally inclusive or exclusive. Fibre samples may be prepared and mounted for infrared analysis by a variety of techniques some of which are described in this document. The minimum length of fibre necessary for the analysis should be used in case of a trace sample.

Each laboratory may use different systems and methods to meet their specific requirements. The general handling and tracking of samples should meet or exceed the requirements of the Best Practice Manual.

2 THEORY

IR spectroscopy is used to analyse and determine chemical composition by looking at the chemical bonds in a material thus providing information about the polymer composition i.e. the fibre generic class and sub class.

In Fourier Transform Infrared (FTIR) instruments all wavelengths pass through the optics simultaneously therefore light is brighter and easier to detect than in earlier dispersive instruments. An interferometer splits the incoming light beam into two parts, one part travels down a fixed path, and the other part travels to a moving mirror. These are then reflected back to the beam splitter. The beams recombine and pass through the sample to the detector. The second beam experiences a change in pathlength and hence the combined beams exhibit destructive and constructive interference. The entire IR spectrum can be measured very rapidly using a Michelson Interferometer and as a result many scans may be obtained in a short time and summed with a corresponding improvement in signal to noise (S/N) ratio. A Fourier Transform algorithm is used to convert the interferogram into the final output spectrum that is used for identification and comparison purposes.

3 METHODOLOGY

3.1 General

The use of IR microscopes coupled with FTIR spectrometers has improved the IR analysis of single fibres, making the technique feasible for routine use in the forensic laboratory. The microscope acts as a beam condenser and is also used to view the samples and to define the area for analysis. It is possible to analyse very small samples (1mm or less) and microcontaminants quickly, reproducibly, and with a high degree of sensitivity. The method is practically nondestructive and provides a wealth of information.

In FTIR microspectrometers the detectors used are e.g. mercury cadmium telluride (MCT) while deuterated triglycine sulphate (DTGS) detectors are used on the FTIR main bench. Narrow range MCT detectors have a narrower spectral range starting at 750 cm^{-1} and are used for very small samples. The wide range MCT detectors work to a lower frequency i.e. 450 cm^{-1} . These MCT detectors are much more sensitive than the DTGS detectors, and as such need to be cooled using liquid nitrogen.

The techniques outlined below may demand considerable operator skill and require patience and practice in order to obtain a consistent result. Some methods (see Section 3.3) are time consuming, destructive, require a large amount of sample, and may require a long analysis time for obtaining good quality spectra. Correct optical alignment of accessories (e.g. microscopes, beam condensers, Attenuated Total Reflectance (ATR) objectives) is essential to get good spectra.

Between each fibre measurement the equipment should be cleaned sufficiently to ensure that no transfer of material from one fibre to the next, or from outside sources onto the fibre in question (contamination), occurs. Equipment should be handled carefully according to the manufacturer's instructions (surface coated/polished mirrors are particularly susceptible to damage). Salts used in mulls and IR windows should be kept dry. The work area around the IR instrument should also be kept clean. Any modifications to the techniques during analysis should be documented in the examination notes.

3.2 Commonly Used Techniques

3.2.1 Transmittance

The fibres can be measured in transmittance by placing the fibre across an aperture on an IR window, a hole in a cardboard or metal disk, or mounted on a self-adhesive double layer paper disk. The cross sectional shape is then preserved and there is no alteration in fine structure. IR windows (e.g. potassium bromide, KBr) can be used if the fibre is too small or too brittle to go across an aperture or is too small to sacrifice a 2 mm length. $100\text{ }\mu\text{m}$ of fibre can be analysed by flattening the fibre and placing it onto an IR window. Fibres may be flattened using an IR press, roller, scalpel blade etc. The spectrum of the fibre is ratioed against the background of the KBr window acquired adjacent to the sample.

A diamond cell (medium or high pressure) may be used to flatten the fibre. These are used with a beam condenser or microscope. Diamond itself is almost completely transparent to IR however it does absorb radiation around $1900\text{-}2000\text{ cm}^{-1}$ and can lead to loss of information near that frequency, especially in dispersive instruments. It is possible to remove the diamond crystalline interference by removing the fibre from the diamond and remounting it on a salt disc, or to decrease it by analysing the fibre on a half diamond cell.

The fibre sample can be sandwiched between 2 diamond windows. A spectrum is produced from the background adjacent to the sample or of the diamond faces in contact (this may scratch the diamond windows). A ratio of the sample in the diamond to air is obtained, followed by subtraction of the diamond spectra. Alternatively the diamond windows can be separated and a spectrum of the fibre attached to one of the windows can be acquired. Also if the sample

is very small a KBr crystal placed next to the fibre before flattening can be used as the background.

3.2.2 Attenuated Total Reflection (ATR)

Fibre samples may also be measured by ATR spectroscopy. ATR in general is a surface technique which is very useful e.g. for opaque or strongly absorbing samples, however, it can also be used for routine analysis of fibre samples. The beam interacts at the surface of the crystal through evanescent waves and multiple reflections occur. It is important to have the sample in intimate contact with the crystal as the depth of penetration and efficiency of sample contact affect the results of the analysis. The depth of penetration into the sample increases with wavelength therefore absorption bands at lower frequency are more intense than in transmission and enhancement of the fingerprint region occurs. Minor spectral differences may therefore be more readily distinguished. There may be a considerable shift in peak position with increased ATR pressure, e.g. a 5 cm^{-1} shift may occur due to compressive stress induced by pressing a flexible material on a harder surface. Therefore for quantitative analysis the same pressure should be applied for each sample. A pressure device is used for solids that presses the sample against the crystal. In Golden Gate ATR high pressure contact is achieved against a diamond by using a sapphire anvil assembly.

The positioning of the crystal on the fibres may sometimes be difficult for several reasons (thickness, section shape, etc.) and a preliminary flattening step may eventually help.

3.3 Other Techniques

For special applications other techniques, e.g. those listed below, may be used, but these are now largely obsolete:

- Micro internal reflection (MIR)
- Reflectance-absorbance (R/A): The flattened sample ($2\text{-}3\ \mu\text{m}$) is placed on a polished metal surface. The incident radiation passes through the sample, reflects off the metal surface and passes through the sample a second time, i.e. double pass transmission collected in a reflection mode
- Diffuse Reflectance Infrared Fourier Transform spectroscopy, DRIFTS

Other formerly used techniques include the formation of thin films by dissolving the fibre in appropriate solvents or the formation of salt discs transparent to IR radiation by mixing fibre material with e.g. KBr in a mull and compressing the mixture. The choice of salt depends on the application:

| Compound | KBr | NaCl | KCl | CsBr | CsI | ZnSe |
|------------------------------|-----|------|-----|------|-----|------|
| Cut off (cm^{-1}) | 320 | 650 | 400 | 200 | 150 | 454 |

3.4 Sampling of fibres

3.4.1

The quantity of fibre used and the number of fibre samples required will differ according to:

- Specific technique and sample preparation.
- Sample homogeneity.
- Condition of the sample.
- Other case dependent analytical conditions and/or concerns.

3.4.2

To ensure reproducibility two or three spectra from each single fibre may be measured.

3.4.3

A proportion of the questioned fibres may be analysed. A typical IR microscope is optimised for a 100 µm-spot size, thus little beam energy passes through a point which is farther than 50 µm from the centre of the field of view. Fibres of 100 µm in length can be analysed.

A fraction of the questioned fibre to be analysed may be retained, if required, for record purposes or independent examination. This will be dependent on the length of the questioned fibre and any further tests to be carried out.

3.4.4

Samples must be prepared in a uniform manner i.e. to produce reproducible spectra the sample preparation should be exactly the same for all fibres being compared. There should be strict attention to consistency of sampling procedures for reference and questioned fibres so that false matches are avoided. Questioned fibres should be mounted individually for analysis. For traceability and to prevent contamination it is not recommended that several questioned fibres are mounted on or in a single mount.

Several fibres from the reference sample can however be mounted on a single mount but they should be well separated (microscopically) so that their positions can be unambiguously documented for later retrieval and/or reanalysis and to prevent spectral contamination from stray radiation which might pass through another fibre. It may be necessary to remove both reference and questioned fibres from the same medium as any residual mounting medium, if this cannot be removed, may affect the fibre spectrum.

3.4.5

Each fibre type within the reference sample must be analysed at least once if a similar questioned fibre is to be identified, e.g. each different colour in the reference sample, both delustrated and bright fibres, etc. In the case of bicomponent fibres (which contain 2 separate polymer types along the fibre length permanently joined at an interface, side by side or sheath/core) IR spectra of different areas of the fibre should be taken to identify both polymer types present.

3.4.6

It is recommended that the IR analysis is carried out before TLC analysis, as additives and dyes can be detected in the FTIR spectrum. This information is lost on extraction for TLC analysis and the solvents used may cause contamination problems. Where possible IR analysis should be carried out on the same fibres that have been analysed for microspectrophotometry, and these fibres then used for TLC.

3.5 Sample preparation and handling

3.5.1

The preparation of the sample should be carried out under a low power microscope to reduce the risk of losing the fibre fragment. Useful sample preparation accessories may include, but are not limited to, sample supports, infrared windows, presses, dies, rollers, forceps and scalpels.

3.5.2

Fibres may be removed directly e.g. from a fibre tuft or thread, from under a coverslip, or from the TLC tube. The fibres are then placed in the sample holder. Generally only a portion of the fibre (2 mm or less) is required. The fibre should be washed using an appropriate solvent to remove the adhesive or mounting media.

3.5.3

Spectra from unaltered fibres may have distorted peak ratios, poor baselines, and/or weak signal to noise, S/N. The uneven thickness of the fibres, i.e. the circular cross section of round fibres or the irregular shape of some fibres, produce variable pathlengths of the IR light beam (diffraction of the IR light) due to deviations from Beer's Law. Therefore the ratio of the less intense bands to the stronger bands is greater.

A thin flat fibre produces more accurate spectra of good quality. Flattening the fibre provides an increased area for analysis therefore S/N is greater and diffraction at the fibre edges is reduced. A more uniform thickness is obtained which reduces the pathlength for fibres of high absorptivity. However, flattening the fibres alters the crystalline/amorphous structure of the fibre and may result in minor differences in peak frequencies and intensities. This effect must be taken into consideration when making spectral comparisons.

In certain situations, a combination of both approaches may be advisable. Thus it may be necessary to take 2 spectra i.e. with the fibre slightly compressed (to identify and preserve fine structure) and then with the fibre sufficiently compressed to flatten the fibre and give a good overall spectrum. The degree of flattening of the fibre may affect the spectra and interference fringes may occur in the spectrum. These fringes are most often observed in the spectra of acrylic fibres.

Flattening of the fibre may be achieved by e.g. rubbing the sample with a hardened steel roller on a hard surface (a frosted glass slide, or metal), pressing with a metal probe or pressing between two dies (The dies should be reserved for this purpose because indentations form on the die surface). As the sample is flattened it begins to appear transparent when it reaches the desired thickness. Film thickness should be approximately 0.5 to 5 μm . Flattening by these techniques is most useful for relatively soft materials that will retain their flat shape once the pressure is released.

If the fibre is too thick excess absorbance in the spectrum may result. A thin section can be sliced off the edge of the flattened fibre and this fragment is then further compressed. For harder fibres it is more reliable to use the diamond cell or ATR objective e.g. polyamide fibres are generally hard with high absorptivity therefore the roller pressure may not be sufficient and over absorption of the amide I and II bands occurs.

3.5.4

The fibre, either flattened or unflattened, may be mounted across an aperture, on an IR window, or between IR windows. The longitudinal plane (flattened surface) of the fibre should be as nearly parallel to the IR window or other mount as possible, i.e. as flat as possible on the surface. The choice of window material should not reduce the effective spectral range of the detector being used. Where the fibre is mounted between two IR windows, a small KBr crystal may be placed next to the fibre. The background spectrum may be acquired through this crystal to avoid interference fringes that can arise if the spectrum were acquired of an air gap between the two IR windows.

3.5.5

After IR analysis the fragment of fibre may be recovered from the sample holder using adhesive tape or forceps. This fragment may be stored under the adhesive tape, remounted on a slide, or used for TLC analysis. The adhesive tape or slide with this fragment, if recovered, should be labelled with the case number and fibre number according to laboratory procedures.

3.6 FTIR Analysis conditions

3.6.1

A mid-infrared FTIR spectrometer coupled to an infrared microscope, and compatible with the spectrometer, is recommended because of the sensitivity and ease of sample handling. However dispersive IR is not excluded.

The lower frequency cut off will vary with the microscope detector used (preferably no higher than 750 cm^{-1}). The range used will be dependent on the sample technique or the detector used (see Section 3.1).

3.6.2

All spectrometer and microscope components should be turned on and allowed to reach thermal stability prior to commencement of calibration and operational runs. The FTIR detectors should be allowed to settle after the addition of the liquid nitrogen (approximately 20 min, see manufacturers' recommendations). The overall stability, especially thermal, of the spectrometer over the entire background and sample collection time period must be maintained.

3.6.3

It is essential that instrument performance and calibration be evaluated routinely according to manufacturers' recommendations. It is recommended that the energy of the MCT be checked through the object and the microscope before use. Instrument performance records may be maintained on hard copy and/or computer disk. These should include calibration and alignment checks, maintenance records, wavelength checks (e.g. polystyrene spectra), and should be signed and dated. Examples of the performance evaluation method for FTIR include:

- System throughput to check for optimal performance i.e. both the microscope and interferometer are properly aligned and the S/N is comparable to normal manufacturer recommendations.
- The interferogram size, position ($800\text{-}1200\text{ cm}^{-1}$) and shape are examined to ensure that there is maximum signal, and a stable, well-shaped signal, as per manufacturers' recommendations. The sampling mode, the sample type, and the instrument alignment affect the peak height.
- Single-beam spectrum of the background should be examined to ensure that there are no interfering absorbances from the surface where the background spectrum is acquired, the water and carbon dioxide absorbances are minimised i.e. the instrument has stabilised.
- The spectral shape and the noise should be examined.
- S/N checks by recording the 100% line, in absorbance, at the $2000\text{ - }2600\text{ cm}^{-1}$ region i.e. where no interfering absorbing atmospheric bands occur in the spectrum. $S/N = 100/\text{max-min}$ at this area or P-P $S/N = 1/\text{max-min}$ (peak to peak).
- 100% T line. Ideally an IR spectrum should be composed of a collection of smooth curves emanating from a flat baseline, at 100% transmission (or zero absorbance). If the IR beam travels through more than $10\text{-}15\text{ }\mu\text{m}$ thickness of fibre then 0% transmission will be obtained in some regions of the spectrum.
- Peaks may be offset because of lack of frequency calibration. Since infrared spectroscopy is mainly used for structure determination it is important that frequencies are accurate and reproducible. Drifting of wavelength occurs in Dispersive instruments. This is not a significant factor in the FTIR because the laser provides a continual internal accuracy alignment.
- By measuring a thin organic film on polished metal plates, or by using a flattened microfibre to identify the spatial adjustment of the system.
- Polystyrene or Indene are used for wavelength accuracy as they produce an elaborate many-featured spectrum. Interference fringes may distort polystyrene spectra.

3.6.4

Radiation throughput, stray radiation reduction, and aperture focus in the sample image plane are some of the considerations in the selection of aperture parameters and positioning. Fibre

width, flatness, and linearity will usually limit the size of the illumination and the size of the apertures used for analysis.

The apertures that control the areas (fields) of sample illumination and detector measurement in an IR microscope may be of fixed circular, or variable size diaphragm, or a pair of knife edges which can be adjusted to a rectangular shape. The variable rectangular apertures can be more closely matched to the fibre shape.

Not all systems provide for the control of both illumination and detector measurement fields. The following recommendations can be modified to suit the constraints of a particular system design:

- The objective and/or condenser should be adjusted (if possible) for any IR window, which lies between the optic and the sample in the beam path. This compensation reduces spherical aberration and permits more accurate focus.
- Infrared spectrometers and microscopes exhibit a polarisation bias. This fact, coupled with the pleochroism associated with most fibres, makes it essential that fibre alignment be consistent throughout an analysis and preferably for all fibre analyses performed on a given system. A vertical or “northsouth” alignment is typically used.
- Samples should be focused as close to the centre of the sample volume as possible and centred on the optical axis of the system. The condenser may be focused and recentred if necessary (this is best accomplished using a circular field aperture, pin-hole).

In general, the illuminating and detector fields should lie within the boundaries of the fibre edges, i.e. the aperture should be smaller than the sample (inscribing the sample). The proper aperture size should be selected to avoid spectral contamination from the surroundings. The objectives should be focused correctly to avoid false focus, spectral shifts or increased noise. In transmittance the aperture before and after the sample are matched (Redundant Aperturing) so that the area is the same for objective and condenser. The lower aperture focuses the IR beam to illuminate only the fibre. In reflectance only the upper aperture is required.

The detector measurement aperture width should be adjusted to slightly less than the width of the fibre, but preferably not less than 10 μm . The aperture length may vary with sample geometry, but should not be so great as to allow the detector to be saturated when acquiring a background spectrum. The illuminating field aperture should be adjusted so that the image of its edges coincides with those of the detector measurement aperture.

The size and position of the apertures should not vary between sample and background data acquisition for a given analysis. If necessary, parameters can be subsequently modified and new sample and background spectra acquired.

3.6.5

FTIR spectroscopy uses a single beam, therefore two spectra (background and sample spectra) must be acquired to produce a pure sample spectra. The IR radiation travels through air as well as the sample. Purging of the FTIR instrument, using nitrogen or an air dryer, is strongly recommended to reduce the atmospheric carbon dioxide and water levels and to protect detector cell windows. Interference from the atmospheric contaminants is time dependent, i.e. there is variation in the atmospheric CO_2 and H_2O content for each spectrum acquired. Background spectra are therefore acquired immediately before or after the sample spectrum, depending on laboratory procedures and manufacturers' recommendations. A compensated spectrum is then produced. In dispersive instruments these atmospheric bands are not visible as 2 beams are used simultaneously (the reference and sample beams) and the reference beam is used to compensate for absorbance due to air.

A “background” spectrum refers to a reference absorption spectrum which includes the absorbance contributions of all system components except the sample of interest. It is used to eliminate any unsaturated spectral contributions from the environment or material holding the sample.

The system parameters for background spectra should be identical to the parameters used for sample spectra (with the possible exception of gain). These parameters include any automatic

instrument control, pre-set frequency limits for spectra, wavenumber range, resolution, mirror velocity, number of scans, and spectrometer aperture size.

3.6.6

Resolution should be set as per laboratory procedures, e.g. 4 cm^{-1} . Higher resolution may be used, however, the additional data points typically yield no further analytical information for polymer samples. Using a higher resolution will decrease S/N, will cause problems in the correction of the gas phase bands of water and will increase the time of the measurement. The apertures are adjusted to fit the sample, and hence it may be convenient to acquire the sample spectrum prior to acquiring the background spectrum. However, the gain settings are dependent on the aperture size. If the aperture is too large the instrument will be overloaded (on background) and outside the working parameters. Therefore the gain should be checked on an area of background beside the sample. Many FTIR spectrometers can alter the gain automatically.

3.7 Spectra

3.7.1

The quality of the spectra is dependent on the focus, the size of the window, the thickness of the fibre, nature of the fibre, delustrant etc.. Spectra of the fibres should be repeated if the baseline is noisy, % transmission low, diffraction effects are great, interference fringes are great. These hamper the identification of the fibre and comparison of the spectra.

3.7.2

Spectra may be plotted after the quality is assessed by visual examination on the screen. Peaks should be narrow. Distorted peaks, unnaturally broad or flat topped are usually due to the sample absorbing too much radiation. Subjecting the fibre to a second flattening may be necessary.

The spectra should all be printed/displayed to the same scale to enable comparison of all questioned and reference fibres. These are printed/displayed in either transmission or absorbance as per laboratory procedures.

3.7.3

Report documentation may include the date, the operator, the system parameters, and the original instrumental output data. Each spectrum should be labelled with at least the case number and item number, date and operator. It is also useful to add a description of the fibre colour and fibre shape if the reference sample contains mixed fibres.

3.7.4

Hard copies of the questioned fibre and reference fibre spectra may be printed and placed in the case file. The operator and Fibre Examiner should sign and date the printout. Digital copies (or printscreen) of the spectra may be copied into the case report and properly annotated.

3.7.5

It is recommended that raw spectra of the fibres are saved to a hard disk, as per laboratory procedures. It is generally useful to save all data on disk just after it is generated and prior to any modification. Each spectrum file should be labelled with at least item number and the case number; date and operator are usually saved in the data file by the acquisition software.

3.7.6

If any data manipulation has to be carried out the raw data must be saved first and the data manipulation carried out on a copy of the spectrum. Data that is damaged during subsequent processing can then be restored from the raw files. Any manipulation carried out, e.g. baseline correction is carried out in absorbance and should be noted on the hard copy of the spectrum or in the title of the spectrum file. Some manufacturer's file format may automatically store the manipulations of a spectrum and thus provide a mean of tracking any change. The raw spectra can be retrieved and compared with pre-stored library spectra at a later date.

4 INTERPRETATION AND DISCUSSION

Every feature of the spectrum contains information concerning the sample. The fibre composition can be obtained from the position of the absorption peak as the frequency of absorption is proportional to the bond strength. The infrared absorption spectrum of molecular compounds contains bands that can be associated with specific functional groups present. These functional groups, as well as any identifying peaks, co-monomers or dyes (or pigments), in the sample spectrum may be labelled, manually or automatically. Also any peaks that show significant difference between two fibres should be labelled on the spectrum. The generic type or sub class of the fibre polymer may be identified if necessary. Further detail e.g. inter-sample variability, different compositions etc. should be considered when comparing the spectra.

The spectra should be compared, any anomalies noted and further IR analysis carried out if necessary. The reference fibre spectrum is compared with the questioned fibre spectra, by visual comparison, or by overlaying of the spectra on screen or as printouts. The pattern of major and minor peaks, their relative position and absorption should be comparable. If peaks are the same for reference and questioned fibres then the questioned fibres are considered to contain the same constituents as the reference fibres. If peaks differ substantially in position ($> 10 \text{ cm}^{-1}$) or peaks are missing in one of the spectra then the fibres do not match.

4.1 Problems occurring in the IR spectra

4.1.1

Effects such as diffraction, interference, pleochroism etc. may be present in the spectra. These may usually be rendered acceptable by doing repeated measurements on different parts of the fibre.

4.1.2

The baseline may indicate different problems. A sloping baseline indicates that diffraction effects due to the microscope aperture may not have been compensated for e.g. if background and sample spectra were collected through different apertures. The baseline may be at high absorbance and the peaks look 'short'. This can be minimised by increasing the width of the sample and increasing the aperture to approx. 70% of the sample width.

Circular cross sections disperse the beam (refraction). This is frequency dependent therefore the effect is not consistent across the spectrum. If stray radiation is affecting the spectrum the baseline will be at a value less than 100% transmittance and strong peaks will appear weaker than expected.

4.1.3

If the sample is very thin or small then a noisy spectrum is obtained. This can be improved by taking a larger number of scans to increase S/N or by increasing the amount of sample.

4.1.4

Reflection will give spectral distortions and hence interference will occur between the transmitted beam and the reflected beam. Interference fringes are present in transmission and Reflectance/Absorbance (R/A) but absent in internal reflection. Internal reflection may also occur e.g. 4-8% of the radiation will be reflected from the surfaces of a thin film suspended in air. Band intensities can be distorted and this can interfere with the library searching.

4.1.5

Optical inhomogeneities will scatter radiation from areas outside the sampling area creating spectral interference. A thin supported film will produce interference fringe patterns superimposed on the fibres spectrum. They arise from constructive and destructive interference of internally reflected waves inside the sample. These interference fringes will affect peak intensities. This does not interfere with qualitative interpretation but will affect quantitative analysis or the searching of a database. These interference fringes can be minimised.

4.1.6

Diffraction is the most significant factor in reflectance measurements. This results when light strikes a high contrast edge. This can occur up to 40 μm away from an edge and therefore other fibres should be placed 50 μm or more away. Diffraction is also observed whenever radiation is forced to pass through a slit, aperture or past a high contrast edge. The angle through which radiation is diffracted is a function of frequency such that at high frequency the radiation is diffracted less than at low frequencies (longer wavelengths). Diffraction is worst when the dimensions of the object is similar to the wavelength of the IR radiation (2 – 15 μm), i.e. smaller than 10 μm , but is also noticeable in fibres up to approximately 100 μm diameter. Diffraction affects only the absorbance values not the resolution or frequency. Qualitative results are accurate but quantitative results are affected.

4.1.7

Dust particles and water vapour, opaque areas, particles of delustrant, or imperfections within the fibre may scatter light and the spectra will be distorted due to wavelength dependent scattering (shorter wavelength will be scattered more than longer wavelength).

4.1.8

Progressive deterioration of the resultant spectra can be due to detector defects in FTIR spectrometer.

4.2 Reporting the fibre composition

4.2.1

This analytical method covers manufactured textile fibres (with the exception of inorganic fibres) including but not limited to the following generic types:

* All cellulosic fibres e.g. Viscose, Lyocell, Modal and Cupro will produce the same spectra.

4.2.2

The following generic types of fibre are rarely encountered in routine forensic examinations:

- Polyimide
- Vinylal
- Elastane
- Polyether ether ketone (PEEK)
- Polytetrafluoroethylene (PTFE)
- Polyphenylenesulphide

- Novoloid
- Polycarbonate
- Polylactic acid (PLA)
- Polybenzimidazole (PBI)
- Polyvinylidenechloride

Exemplar data, reference standards, and/or examiner experience may be inadequate for their characterisation by microscopic and microchemical techniques. For these fibre types, IR spectroscopic confirmation of polymer type may be advisable.

4.2.3

Natural fibres may also be analysed by IR spectroscopy, however, no additional compositional information is provided over that yielded by light microscopy. Dyes (or pigments) may be detectable in these fibres by comparison with the undyed fibre.

4.2.4

Successful identification of fibre polymers by IR spectra depends on experience and familiarity with fibre reference spectra. The spectra must be acquired and examined carefully. The effects of pressure, diffraction, scattering, artefacts, noise, interference, fibre flatness and instability of the detector should be recognised when present in the spectrum. The % transmission (no less than approximately 70%) and baseline drift should be considered when interpreting the spectra as these will affect any library searches.

4.2.5

Spectral identification is accomplished by comparison of the positions of the absorption bands, according to wavelength or wavenumber, and their relative intensities with spectra of known reference standards. The generic class of manufactured textile fibres and the sub-generic class of synthetic manufactured fibres may be identified.

If the fibres match in generic class then the polymer composition can be determined by more detailed examination of spectra. Sub generic classes of e.g. polyester, polyamide, acrylic and modacrylic fibres can be separated by IR. It may be desirable to confirm the identification by other methods such as PLM or melting point determination.

4.2.6

Similarity or dissimilarity in the IR spectra can be noted when making a fibre comparison. A wide variety of materials may contribute additional bands to the IR spectrum e.g. solvent residues, dyes, dye receptor sites, and flame retardants.

If fibres have been mounted on slides residue of the mounting medium may be present. Blood and other body fluids may also be present. These additional bands are usually not considered in the comparison process because their level of contribution will obviously differ between questioned and reference fibres.

In certain instances a contaminant may eventually increase the evidential value if it is present on both questioned and reference fibres. Washing of the fibre will remove the mounting media and may also remove the contaminant.

If the spectra of the questioned and reference fibres match these criteria then the fibres can be said to be indistinguishable in chemical composition.

4.3 Data manipulation techniques

On board data systems are available on spectrophotometers which allow techniques such as spectral subtraction, data manipulation like baseline correction or smoothing, comparisons with library spectra, quantitation etc. to be carried out. If the data is recorded and stored, the manipulation, e.g. the addition and subtraction of spectra, is a matter of choosing the necessary computer programs. The spectra should first be converted to absorbance. Automatic correction of the spectra is carried out e.g. for ATR. Other data manipulations are carried out as required.

4.4 Use of IR libraries

4.4.1

Reference IR spectra are essential for the identification of the fibre composition. These can be in the form of literature references or a commercial spectral library identified by polymer composition. N.B. Such collections contain many duplicate spectra. Do not attempt to identify spectra by Trade name, but only by chemical composition.

4.4.2

A computer-assisted search can be used to provide valuable information. In addition to indicating the identity of the fibre the library search may compute a likelihood or probability of match based on band position and intensity. This can be an approximate technique due to noise, environmental conditions, varying peak heights etc. The results of a database search should be treated with caution as the database may not contain all the variations in fibre polymer structures.

4.4.3

An in-house spectral library built from a collection of authenticated specimens representing the generic types and sub types is recommended. This should be created using the same technique and instrument used within the laboratory for the unknown fibre. Any problems due to sample preparation, instrument etc. are overcome by comparison of spectra prepared in house on the laboratory IR spectrometer.

4.4.4

It is desirable that a basic fibre infrared spectral collection should contain, as a minimum, spectra from the following fibre types:

- Polyacrylonitrile PAN (at least the following)

PAN homopolymer

PAN/MA with and without DMF

PAN/MA with AMPS

PAN/VA

PAN/MMA

Example of a type including acrylamide e.g. PAN/MA/AA

PAN/MA/Itaconic acid

MA = methyl acrylate, MMA = methylmethacrylate, VA = vinyl acetate,

AMPS = 2-acrylamido-2-methylpropane sulphonic acid, DMF = dimethylformamide

- Modacrylic (at least the following)

Vinyl chloride co-polymer

Vinylidene chloride co-polymer

Example containing antimony oxide

Example containing aluminium trihydrate

- Polyamide (at least the following)

Nylon 6

Nylon-6,6

- Aramids

- Polyester (at least the following)

Polyethylene terephthalate, PET

Poly-1,4-cyclohexylene-dimethylene terephthalate, PCDT

Polybutylene terephthalate, PBT

Polytrimethylene terephthalate, PTT

- Polyolefins

Polyethylene

Polypropylene (basic type)

PE/PP co-polymer

- Cellulosic

Cotton

Viscose

Modal

Cupro

Lyocell

- Wool
- Silk
- Acetate
- Triacetate
- Polyvinyl chloride (e.g. Rhovyl)
- Vinyl chloride (e.g. Saran)
- PTFE
- Example of a Polyurethane
- Example of a Vinyl alcohol

5 SPECTRAL IDENTIFICATION CHARTS

5.1 Most frequent acrylic fibre types

CN triple bond stretch at 2245 cm^{-1}



C-O stretch 1220 cm^{-1}
and 1130 cm^{-1} present.

YES →

PAN/MMA
1

↓ NO

C-O stretch 1170 cm^{-1}
and 1204 cm^{-1} present

YES →

PAN/MA
1, 2, 3, 4, 5, 6, 8

↓ NO

C-O stretch 1235 cm^{-1} .
Minor peak at 940 cm^{-1}

YES →

PAN/VA
2, 3, 7

MMA = Methyl methacrylate

MA = Methylacrylate

VA = Vinyl acetate

Additional compounds may appear in the acrylic spectra as indicated above:

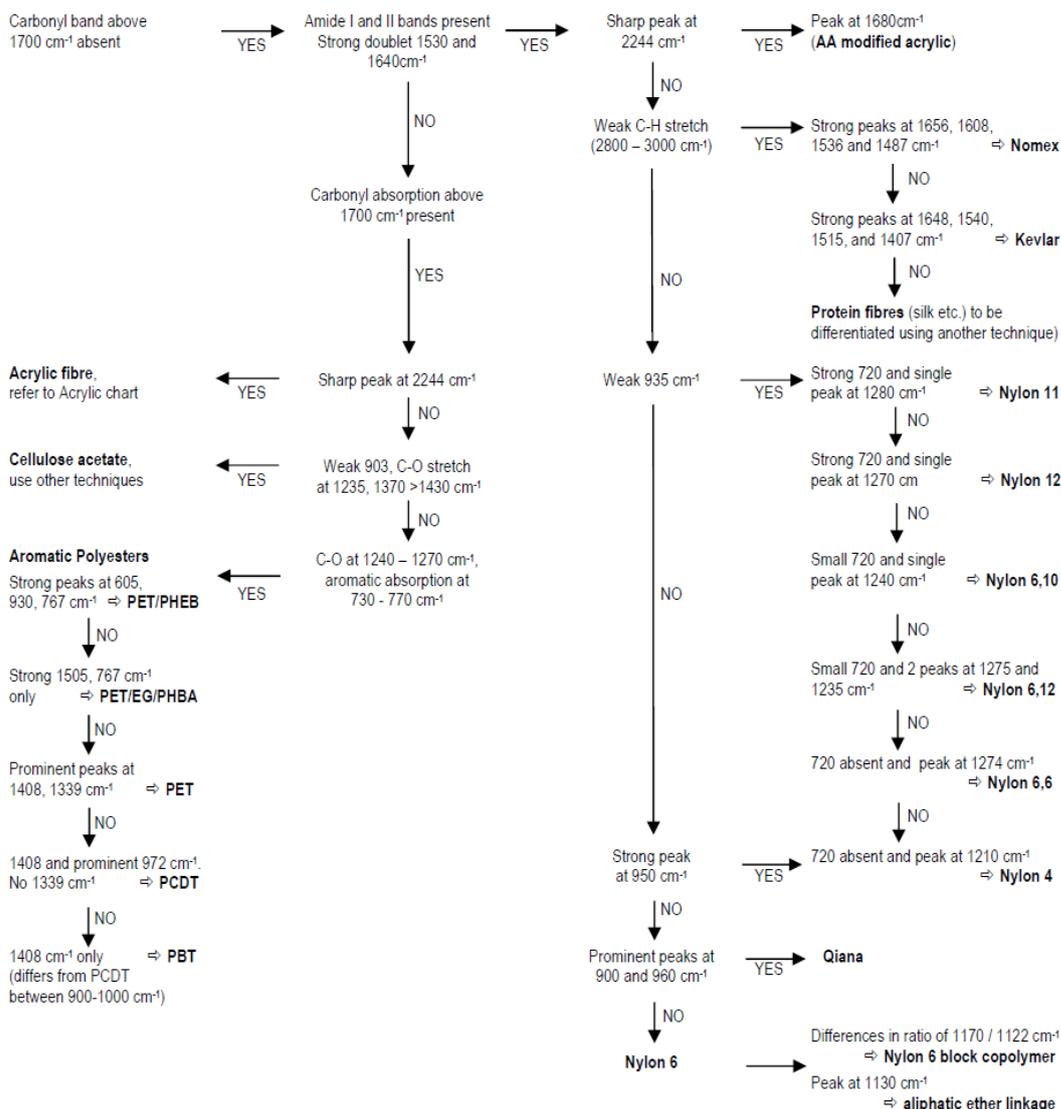
| | | | |
|------------------|-----------------------------|----------------------|---|
| Solvent residues | 1670 cm^{-1} | Dimethylformamide | 1 |
| | $1805, 1785\text{ cm}^{-1}$ | Ethylene carbonate | 2 |
| | 2053 cm^{-1} | Sodium thiocyanide | 3 |
| Terpolymers | $1580 - 90\text{ cm}^{-1}$ | Itaconic acid | 4 |
| | $1672, 1532\text{ cm}^{-1}$ | Aromatic sulphonate | 5 |
| | $1680, 1611\text{ cm}^{-1}$ | Acrylamide | 6 |
| | 1493 cm^{-1} | Methyl vinylpyridine | 7 |
| | 1042 cm^{-1} | Aliphatic sulphonate | 8 |

For further details on acrylic fibre spectra refer to:

Grieve, M.C. Another look at the classification of Acrylic fibres, Science & Justice, 1995, 35, p. 179-190.

Grieve, M.C. and Griffin, R.M.E. Is it a Modacrylic?, Science & Justice, 1999, 39, p. 151-162.

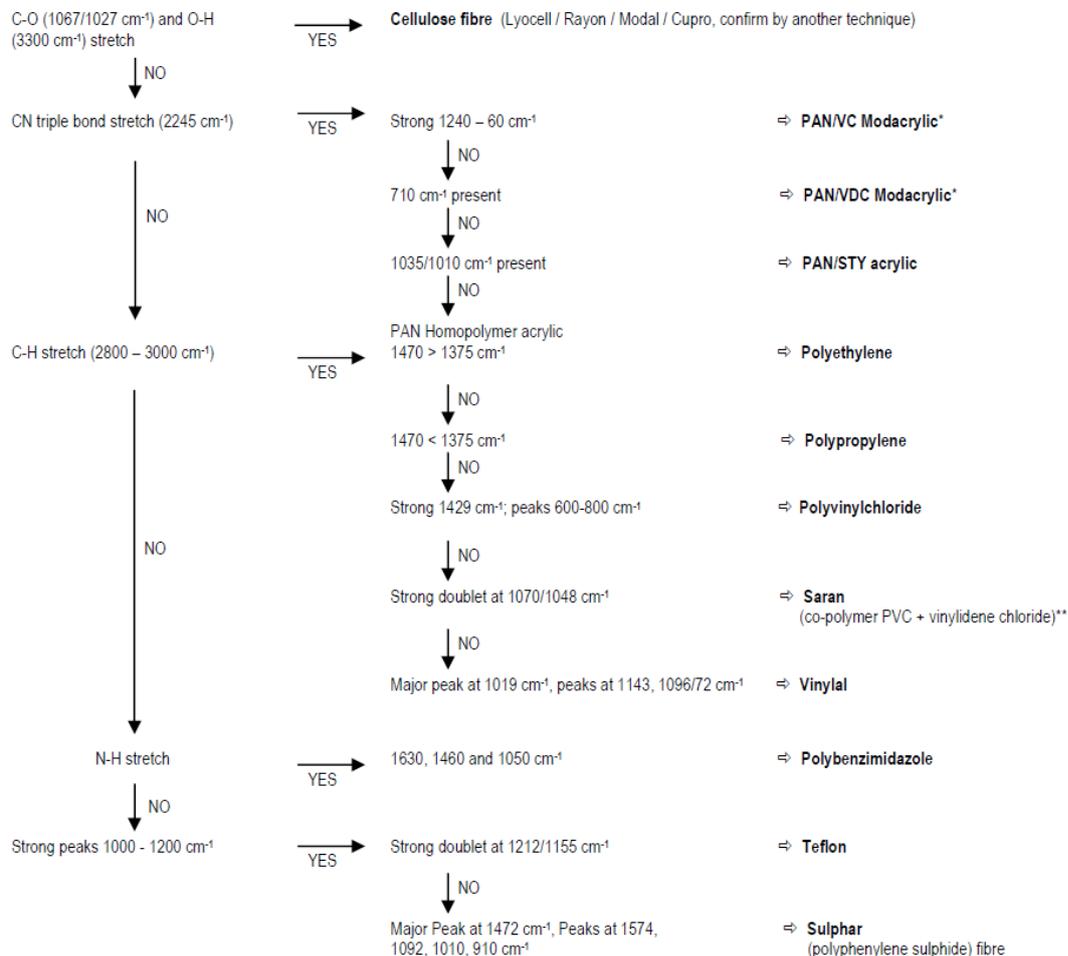
5.2 Strong carbonyl absorption present



Definitions:

- AA Acrylamide
- PET Poly(ethyleneterephthalate)
- PHEB Poly(hydroxyethoxybenzoate)
- EG Ethylene Glykol
- PHBA p-hydroxybenzoicacid
- PBT Poly(butyleneterephthalate)
- PCDT Poly-1, 4 -cyclohexylene-dimethyleneterephthalate

5.3 Strong carbonyl absorption (1730-1740 cm⁻¹) absent



For further details on Modacrylic fibres see:

* Grieve, M.C. & Griffin, R.M.E. Is it a Modacrylic? Science & Justice, 1999, 39, 151-162.

** In some Saran fibres PVC may be copolymerised with vinyl acetate resulting in a carbonyl peak (of varying size) appearing in the spectrum at c.1730 cm⁻¹.

Definitions:

VC = vinyl chloride

VDC = vinylidene chloride

Sty =sodium styrene sulphonate

6 TERMINOLOGY

Aberration (Spherical)

Radiation passing through the outer periphery of a lens does not come to the same focus as that passing through the centre. This effect can also be observed when curved mirrors are used as lenses. An additional effect can be observed when the edge of a lens acts as a prism and focuses different wavelengths differently (chromatic aberration).

100% Line – This is calculated by ratioing two background spectra taken under identical conditions. Ideally the result is a flat line at 100% transmittance. The slope and noise of 100% lines are used to measure the quality of the spectra and the performance of the instrument.

Absorbance, A

The logarithm to the base 10 of the reciprocal of the transmittance, T, $A = \log_{10} (1/T) = -\log_{10} T$.

Absorbance Spectrum

These are calculated by comparing a sample single beam spectrum to a background spectrum. Absorbance is linearly proportional to concentration (Beer's Law) and therefore is used in quantitative analysis.

Absorption band

A region of the absorption spectrum in which the absorbance passes through a maximum.

Absorption spectrum

A plot, or other representation, of absorbance, or any function of absorbance, against wavelength, or any function of wavelength.

Alignment (Optical)

The arrangement of optical parts, lenses and diaphragms, so that all of their axes are mutually coincident.

Aperture

An opening in an optical system that controls the amount of light passing through the system.

Attenuated Total Reflection (ATR)

Infrared radiation entering a prism made from a high refractive index IR transmitting material can be totally internally reflected. Crystals used include Diamond, ZnSe, ZnS, KRS5, Ge, etc. An evanescent wave extends beyond the surface of the crystal into the sample that is in contact with the crystal. ATR produces a very short pathlength for the IR light in the sample therefore the technique is ideal for highly absorbing materials.

Background

Apparent absorption caused by anything other than the substance for which the analysis is being made.

Baseline

Portion of the spectrum in which there is generally no band information. Normally flat. For various reasons it may be curved. This distorts the spectrum but may be removed using baseline correction facilities.

Baseline wander

Due to inequivalence in the components in the sample and reference beam affecting the balance of the beam at 100% transmittance end of the scale.

Beam condenser

A device which focuses the IR beam to a spot to concentrate the energy there and thus enlarge the image of the sample.

Beam splitter

Any optical device that reflects half the radiation impinging upon it and transmits the other half. These are used in Michelson interferometers to send infrared radiation to the moving mirror and fixed mirror respectively.

Beers Law

As thickness is increased absorbance levels for every peak increase by a proportional amount.

Cellulosic fibre

Fibres composed of polymers formed from glucose.

Diffraction

A modification that light undergoes when passing the edges of opaque bodies or through narrow slits. This results in light intensity appearing in the shadow of a high contrast edge.

Diffraction limited region

Energy through the system is governed by the aperture size as well as the source profile therefore imperative to measure both sample and background spectra through the same aperture size or else a sloped baseline will occur. Best way to avoid this is to measure the sample spectrum immediately after aperturing down then moving to the background.

Diffuse Reflectance Infrared Fourier Transform spectroscopy (DRIFTS)

Infrared radiation is focused onto the rough surface of a sample where it is transmitted, absorbed, scattered and reflected. The reflected energy leaves the sample in all directions after penetrating one or more particles, i.e. diffusely scattered, and is collected. Different physical interactions between light and the sample produce observable changes in the spectrum. This spectrum is therefore different from the transmission spectrum and hence a correction function is required. This technique can be used for cloth or fibres that can be abraded onto the cell.

Dispersive instrument

Usually double beam instruments, use a monochromator (a prism or diffraction grating). This contains an optical system of mirrors that focus an image of the entrance slit on to the exit slit. The dispersing device (prism or grating) spreads the image out into a spectrum in the exit slit plane according to the frequency of radiation present. Movement of the prism or grating will scan the spectrum. This is calibrated in reciprocal wavelength i.e. wavenumber or frequency, cm^{-1} . There is a trade-off between high resolution and noise, which can be helped by slower scan speeds.

Fourier transform infrared (FTIR) spectrometry

A form of infrared spectrometry in which an interferogram is obtained; this interferogram is then subjected to a Fourier transform to obtain an amplitude-wavenumber (or wavelength) spectrum. In FTIR spectroscopy, the Fourier transform converts a time function (the interferogram) to a frequency function (the infrared absorption spectrum). Spectral data are collected through the use of an interferometer, which replaces the monochromator found in the dispersive infrared spectrometer.

Generic class

A group of fibres having similar (but not necessarily identical) chemical composition. A generic name applies to all members of a group and is not protected by trademark registration. Generic names for manufactured fibres include, for example, rayon, nylon, and polyester (Generic names to be used in the United States for manufactured fibres were established as part of the Textile Fibre Products Identification Act enacted by Congress in 1954 (12)).

Infrared spectroscopy

Spectroscopy in the infrared region of the electromagnetic spectrum of wavelength ranging from 10 to 14,000 cm^{-1} . This region is of lower energy i.e. lower frequency than red visible light. The Far infrared region is between 10 and 400 cm^{-1} , the mid infrared region between 400 and 4000 cm^{-1} , and the near infrared region is between 4000 and 14,000 cm^{-1} .

Infrared spectrum

Plot of the intensity of absorbed infrared radiation (shown as either % Transmission or Absorbance) against the frequency of the IR radiation. The intensity varies with the frequency.

Interference fringes

The Christianson effect i.e. a sine wave baseline. Flat samples often show these when examined by reflectance technique. May also be visible in transmittance, rarely in ATR.

Interferogram

A plot of the intensity of the recombined light versus the difference in the 2 pathlengths of the recombined beam. Interferograms are Fourier transformed to obtain single beam spectra.

Internal Reflection Spectroscopy (IRS)

The technique of recording optical spectra by placing a sample material in contact with a transparent medium of greater refractive index and measuring the reflectance (single or multiple) from the interface, generally at angles of incidence greater than the critical angle. See also ATR.

Manufactured (man-made) fibre

Any fibre derived by a process of manufacture from any substance, which, at any point in the manufacturing process, is not a fibre.

MIR – Micro Internal Reflectance

Differs from Attenuated Total Reflectance (ATR) in that the infrared radiation is dependent upon the amount of sample in contact with surface of the prism.

Pleochroism

Occurs due to polarising effect of the lens in the microscope. Variation of the colour of materials based on their orientation under polarised light.

Quantitative analysis

The use of peak heights, band ratios, or peak areas to calculate the amount of a substance in an unknown sample.

Questioned fibre

These are the fibres, which have been removed, during the examination, for further analysis and comparison with the known fibre.

Redundant aperturing

System for masking the infrared energy going to and emerging from a sample in an infrared microscope. One aperture is located at a plane between the source and the sample and another aperture is located at a plane between the sample and the detector.

Reference fibre

Fibres with distinctive characteristics (e.g. generic type, morphology and colour) from a known textile source, e.g. a garment, which will be compared with the recovered fibres. The following terms are also used e.g. control, donor or target fibres.

Reflection/absorption (R/A) or double transmittance

A reflection sampling technique used on thin films. The infrared beam passes through the film, reflects off the metal and then passes through the film again. The incident and reflected beams are in the sample itself. It is used when the sample thickness is typically 2.5 to 15 μm , similar to the wavelength of radiation. If the sample thickness is less then grazing angle incidence is used which has greater sensitivity. R/A is a transmission type measurement collected in a reflection mode and so spectral distortions are few. Surface contaminants can be analysed using these methods.

Resolution (Spectral)

The ability of an instrument to distinguish between two spectral features that are close to each other in wavenumber. Resolution depends on the wavelength of radiant energy examined and spectral bandwidth of the system. In dispersive instruments the mirrors, gratings, entrance and exit slit widths, scan speed and detector system contribute to the resolution of the spectrum. In FTIR the resolution is inversely proportional to the displacement or optical path difference; high resolution is denoted by small numbers e.g. 0.01 cm^{-1} while low resolution is denoted by high numbers e.g. 64 cm^{-1} .

Scale

The range over which the spectra are collected or displayed. This should be the same for both the recovered and known fibres. The horizontal scale is shown as the frequency in

wavenumbers or cm^{-1} . The vertical scale shows either transmission or absorbance of the beam.

Signal to noise (S/N)

The ratio of signal in a spectrum to the noise in a spectrum. Noise is measured as the random fluctuations in the baseline. In FTIR the S/N is proportional to the square root of the number of spectra accumulated therefore, to halve the noise, it is necessary to quadruple the number of scans.

Smoothing

A spectral manipulation process. The noise is reduced at the expense of spectral resolution and there may be distortions in heavily smoothed spectra.

Spatial definition

Ability to define an area for analysis in order to obtain spectral purity.

Spatial resolution

The fineness of detail that is revealed by the microscope.

Spectrometer

Photometric device for the measurement of spectral transmittance, spectral reflectance, or relative spectral emittance.

Specular reflectance

The reflection from the smooth shiny surface of a material, without the beam penetrating the sample. The angle of incidence equals the angle of reflectance of the light.

Stray light

Any unwanted radiation measured with the required signal. The reference or background spectrum is collected to eliminate any unwanted spectral contributions from the environment or other substances on the examining surface e.g. on reflectance surface or on ATR crystal or diamond.

Sub generic class

A group of fibres within a generic class, which share the same polymer composition. Sub generic names include, for example, nylon-6, nylon-6.6, and poly(ethylene terephthalate).

Transmittance, T

The ratio of radiant power transmitted by the sample, I , to the radiant power incident on the sample, I_0 , i.e. $T = I/I_0$. Transmittance spectra are obtained by ratioing a sample single beam spectrum to a background spectrum. Transmittance is not linearly proportional to concentration.

Vibrational modes

Absorbed radiation taken up in energy levels associated with vibration of the molecules. The bonds in polyatomic molecules have spring like properties. The bond lengths and bond angles can vary to give stretching and bending movements, which are collectively referred to as vibrations. The frequency absorbed is the natural frequency of vibration of the particular bond, set of bonds, or of the whole molecule strength e.g. a triple bond takes more energy to break than a double bond.

Wavelength

The distance, measured along the line of propagation, between two points that are in phase on adjacent waves. It corresponds to the distance in the direction of propagation of a regular wave l , divided by the number of cycles of the wave in that direction N . $\lambda = l/N$. By convention it is the wavelength in vacuo, not the medium.

Wavenumber

Number of waves per unit length l , in a vacuum, usually given in reciprocal centimetres, cm^{-1} .

ZPD

Zero path difference where both mirrors of the interferometer are the same distance from the beam splitter.

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APPENDIX 4 – RAMAN SPECTROSCOPY OF TEXTILE FIBRES

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1 INTRODUCTION

At the present time, Raman spectroscopy is not widely employed as a routine technique for fibre identification in forensic science laboratories. This is because other existing techniques like microscopy and FTIR provide most of the information needed.

Raman spectroscopy is one of the possible methods to analyse and characterize fibre dyes or pigments like other existing techniques (MSP, TLC and FTIR). Like for all the other techniques, the added value provided by the method is depending on the fibre type and colour. At present, the literature does not provide a complete overview of the potential and limitation of the method. The provisions of this guide must therefore be considered to be general in nature.

Raman spectroscopy allows the in-situ analysis of fibres and offers the advantage of being almost not destructive. Raman spectroscopy measures vibrational energies of the molecules like Fourier transform infrared (FTIR) spectroscopy. However, these methods are based on different selection rules and thus complementary.

Raman spectroscopy may be mainly used to characterize coloured fibres providing information about their dyes or pigments content. Different surveys show that the dyes or pigments signals are usually dominant in the Raman spectrum and may hide the information from the fibre substrate (class, subclass). Analysis are mostly comparative between questioned and reference samples. Pigments are usually easily detected in fibres and can be identified using a reference database. The spectral response of a dye varies depending on the dye itself and its concentration in the fibre. The fibre class may also influence the global Raman spectrum. Dye identification may be complicated due to the large number of dyes present on the market and also because different dyes can provide similar Raman response.

For the identification of man-made fibres, FTIR will probably remain the method of choice. However, Raman spectroscopy can also be used to identify fibre classes and some subclasses. Fibre identification can be made by comparison of the fibre spectrum with laboratory reference spectra. The analytical information provided is usually equivalent or sometimes less detailed than when using FTIR. However, there are exceptions: for example, colourless cellulosic man-made fibres and colourless vegetable fibres. For these fibres, and under controlled analytical conditions, it is possible to differentiate or characterise more subclasses than by using FTIR.

This Appendix covers mainly the comparison of fibre samples using Raman spectroscopy. The possibility to use databases in order to identify fibre types, dyes or pigments will be discussed. The interpretation of Raman spectra to determine molecular structure is not covered in this Appendix.

The recommended point for Raman analysis in a forensic fibre examination is following visible and comparison microscopy with fluorescence, polarized light microscopy (PLM), visible or UV/visible microspectrophotometry (MSP) and infrared spectroscopy (FTIR). It should be used before dye extraction for thin-layer chromatography (TLC).

This document is written as a guideline for individuals and laboratories that conduct forensic fibre examinations and comparisons and is intended to be applicable to a wide range of Raman spectrometer and microscope configurations.

2 THEORY

Infrared and Raman spectroscopy both measure the vibrational energies of molecules but these methods are based on different selection rules. To be active in infrared, the dipole moment of the molecule must change. For a transition to be Raman active, there must be a change in polarizability of the molecule. Thus, Raman spectroscopy and infrared spectroscopy provide complementary information.

Raman spectroscopy is based on the scattering of light by matter. When a monochromatic light of energy $h\nu_0$ (0 referring to initial energy) encounters a sample, the most part of photons will have no interaction with the matter. Some part of the incident radiation will be scattered at this

same energy. In this case, the photons are elastically scattered: this process is called Rayleigh scattering.

In Raman scattering, the energy of the scattered photons differs from $h\nu_0$ by an amount equal to a vibrational transition $h\nu_s$ (s referring to scattered light). In this case, an inelastic scattering of photons by molecules occurs: this process is called Raman scattering. This effect is very weak and only about 1 in 10^{10} incident photons will undergo Raman scattering. The main difficulty with Raman spectroscopy is fluorescence emission from either the sample of interest itself or from impurities. Even weak fluorescence can be much stronger than Raman scattering, easily overwhelming the weak Raman signal. However, even if the fluorescence phenomenon is a continuous effect, it is localized in a part of the visible range for a considered sample. At the opposite, the Raman effect follows the excitation wavelength and a change of laser source can eventually help to solve the fluorescence problem.

There are two different types of Raman scattering depending on the molecule energy state:

- The molecule at the ground state is excited to a virtual energy state and relaxes into a vibrational excited state, which generates Stokes Raman scattering.
- Some (few) molecules are already in an exciting state, they will be excited to a virtual energy state and relax to the ground state, the Raman scattering is then called anti-Stokes Raman scattering.

The Stokes and anti-Stokes peaks are symmetrically positioned around the Rayleigh peak but their intensities are very different (see Figure 1). As Stokes peaks are more intense, Raman spectra are usually recorded in the Stokes region only.

A Raman spectrum consists of scattered intensity plotted vs. energy. Each peak corresponds to a given Raman shift from the incident energy $h\nu_0$, generally expressed in wavenumbers (cm^{-1}). Commercial softwares automatically set the laser line as the zero of the spectrum and display the Stokes Raman area where the Raman peaks are expressed in cm^{-1} .

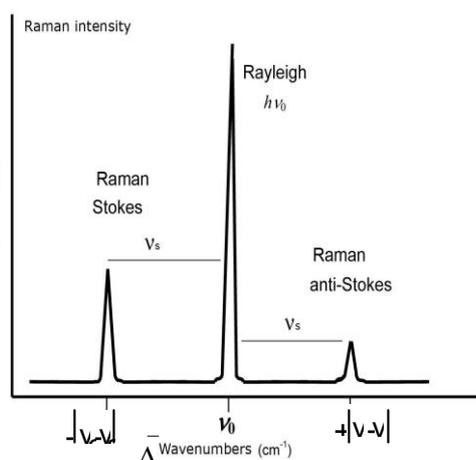


Figure 1: Raman spectrum showing the Rayleigh peak at frequency ν_0 , the Raman peak with the lower frequency (Stokes) is stronger. The Raman peak with the larger frequency (anti-Stokes) is weaker.

3 INSTRUMENTATION

Single fibres can only be measured using an instrument with a high magnification. For fibre analysis Raman spectrometer has to be coupled with a microscope. A standalone Raman spectrometer will only allow for the measurement of a piece of textile and reference dyes/pigments and is not adapted for forensic science casework. This guideline will only consider instruments equipped with a microscope.

3.1 Instrument types: dispersive vs. non dispersive spectrometers

The Raman market is divided into two types of instruments:

- Dispersive spectrometers separate the different wavelengths scattered by the sample generally using a grating-based dispersive unit and a charge-coupled device (CCD) detector. These instruments can be coupled with Raman microscopes and have good sensitivity. The spectral resolution depends on the grating used (number of lines per mm).
- Non dispersive spectrometers do not separate the different wavelengths scattered by the sample but modulate them in frequencies depending on their wavelengths. The result is a single beam detected by a single detector. These instruments are called FT-Raman. Fourier Transform interferometers are used to modulate the beam. High-sensitivity germanium (Ge) detectors (nitrogen cooled) can be used. FT-Raman are mostly bench instruments and they can lack of sensitivity when coupled to a Raman microscope.

At the moment of writing this document, all the forensic science surveys on fibres have used dispersive instrumentation.

3.2 Lasers

The choice of the laser (or lasers) wavelength(s) is of prime importance for Raman spectroscopy. A wide variety of laser wavelength are proposed, from the ultraviolet (UV < 200 nm) to near infrared (NIR, 1064 nm or above). Note that lasers have limited life time and they will have to be replaced after some years of use.

Since the Raman effect intensity is inversely proportional to the wavelength, short wavelengths can usually be detected with higher efficiency and less noise, improving the sensitivity. However, shorter wavelengths are more likely to excite fluorescence. So if fluorescence is anticipated, the laser wavelength should be as long as required to avoid fluorescence, but not so long that the signal strength and detector noise become problems.

There are considerable safety concerns with UV Lasers and their use tends to require significant investment, these wavelengths have not been fully tested in forensic science yet.

Owing to the impact of dispersive systems on signal to noise ratios at longer wavelengths, lasers shorter than 900nm tend to be favoured. FT systems, with their signal to noise advantage, can use lasers of much longer wavelengths, 1064 nm tends to be the standard.

Some of the most common lasers are listed below:

| | |
|-------|--|
| Blue | 488 nm, Argon ion |
| Green | 514 nm, Argon ion |
| Green | 532 nm, (Nd:YAG, frequency doubled) |
| Red | 633 nm, Helium-Neon (He-Ne) |
| NIR | 785 or 830 nm, diode lasers |
| NIR | 1064 nm, Neodymium-doped Yttrium Aluminium Garnet (Nd:YAG fundamental) |

Different surveys carried out in forensic science on several fibre samples show that at least two different wavelengths should be available in order to avoid fluorescence of most samples and obtain complementary information. A visible source in the green (514 or 532 nm) and a NIR source (for example 785 nm) are complementary and enable the measure of most samples.

3.3 Microscope

Any stable research microscope equipped for reflected light can be used if adapted to the spectrometer. However, optical objectives tend to be configured for optical systems and Raman performance using these objectives may vary. It would be recommended that any new objective's performance is checked on the Raman system prior to purchase. A range of infinity corrected objectives are used (ranging from 10x to 100x). Other options for the visualisation of the sample, for example transmitted light, polarization filters or dark field can be helpful to visualize transparent samples (like mounted fibres).

The spot size of the laser on the sample and the depth of focus will decrease as the magnification of the objective increases. Example with a 514 nm laser according to McCreery [2000, table 11.1, page 296]:

10x objective – laser spot diameter 33 μm – depth of focus 220 μm

50x objective – laser spot diameter 6.5 μm – depth of focus 10 μm

100x objective – laser spot diameter 3.3 μm – depth of focus 4 μm

The spot size of the laser on the sample and the depth of focus will also increase with the wavelength of the laser. Example with a 100x objective:

514 nm laser – laser spot diameter 3.3 μm

785 nm laser – laser line 1 \times 7 μm

These values may slightly differ from one instrument to the other.

The depth of focus may decrease if the microscope can be set in confocal mode. A confocal microscope has an additional aperture, called confocal hole which will prevent the out-of-focus rays to reach the detector. Confocal mode enables to reduce the depth of focus to less than 2 μm (514 nm laser, 100x objective lens) but also decreases the Raman signal. Confocal mode is used for the analysis of thin multilayer material and is also recommended by some authors to measure mounted fibres.

Note: the objectives should be adapted to the sample preparation used. If mounted fibres are used, objectives should be corrected for glass cover slips as it can be hard to visualise the fibres at high magnification. For in-situ measurements, the objectives should not be corrected.

3.4 Rejection Filters

Rejection filters are very important in a Raman spectrometer since they allow to separate the weak Raman signal from the intense Rayleigh component of the scattered light. Holographic notch filter, oxide coated edge filter or long pass filters can be used. After interaction of the laser with the sample, the rejection filter transmits the weak Raman light to the detector and reject the intense Rayleigh line. Rejection filters are adapted to one specific laser wavelength and has to be changed when changing the laser excitation wavelength.

4 METHODOLOGY

The use of Raman microscopes coupled with Raman spectrometers has enabled the analysis of single fibres, thus making the technique feasible for use in the forensic laboratory. It is possible to analyse very small samples (0.1 mm or less) quickly, reproducibly, and with a high degree of sensitivity. The method is practically nondestructive and sometimes provides a wealth of information. The exception may be the possible punctual burning of the fibre if the power of the laser source is too high. The diameter of the crater is usually a few microns.

Raman spectra are measured in reflectance. Some preparation procedures are mentioned below. This list is not meant to be totally inclusive or exclusive. The preparation of the sample should be carried out under a low power microscope to reduce the risk of losing the fibre fragment.

The laser should be turned on and allowed to reach stability prior to operational runs. The lasers needs approximately 20 min to reach stability (see manufacturers' recommendations).

4.1 Commonly Used Techniques, sample handling and preparation

4.1.1

It is recommended to measure fibres directly in-situ in reflectance mode without flattening. Fibres can be deposited on a microscopic slide and held on both sides with adhesive tape or fixed with a perforated sticker. The microscopic slide can be covered with an aluminium foil.

Fibre mounted on the diamond cell window prior to flattening for FTIR is handy as FTIR can then be performed immediately afterwards. One has to be mindful of the diamond contribution of course which is very intense but can be mitigated by adopting a confocal approach.

Fibres may be removed directly (e.g. from a fibre tuft or thread) or from under a coverslip. This should be carried out under a stereomicroscope using forceps so that the fibre is not lost. As the technique is almost not destructive, the whole fibre can be mounted for analysis. The fibre should be washed using an appropriate solvent to remove remains of the adhesive or mounting media if necessary.

4.1.2

It is also possible to measure mounted fibres directly. Ideally, the microscope should be set in confocal mode in order to focus on the fibre and avoid as far as possible contribution from the mounting medium and the coverslip. Interference with the mounting medium should be checked in all cases by measuring their Raman spectra as reference and compare it with the obtained result. A comparative survey in the US indicates that Norland Optical Adhesive #65 shows less interference than Permout® and Meltmount™. In Europe some laboratories are using Histomount (National Diagnostics) which shows little interference as well. Glass cover slip may cause problem (glass interference), especially when using NIR wavelengths. Quartz cover slip may be used instead.

4.1.3

Reference textile dyes may be diluted in water (or ethanol if the dye is not water soluble). The solution is deposited on an aluminium foil. Measurements are taken after solvent evaporation on a thin portion of the preparation. Direct measurements of dye powders are often difficult, provide poor quality spectra and are not always reproducible due to dye content and presence of additives.

4.1.4

Polarisation effects may influence the relative intensities of some Raman bands. In order to minimise these effects, it is recommended to keep the orientation of all compared fibres constant (for example Eastwest or Northsouth).

4.2 General considerations

4.2.1

Between each fibre measurement the equipment should be cleaned sufficiently to ensure that no transfer of material from one fibre to the next, or from outside sources onto the fibre in question (contamination), occurs. Equipment should be handled carefully according to the manufacturer's instructions. The work area around the Raman instrument should also be kept clean.

4.2.2

It is necessary that the Raman analysis is carried out before TLC analysis, as additives and dyes can be detected in the Raman spectrum. This information is lost on extraction for TLC analysis and the solvents used may cause contamination problems. Whenever possible, Raman analysis should be carried out on the same fibres that is also analysed by microspectrophotometry and FTIR.

4.2.3

The length of the fibres used and the number of fibre samples required may differ according to sample homogeneity. A minimum of two spectra at different location of an individual fibre is required in order to control the repeatability of the measurements and the sample

homogeneity. Sample homogeneity testing could also be performed using line maps and/or depth profiles of single fibres.

4.2.4

After the analysis, the fibre can be recovered and stored for further analysis. This fibre should be labelled with the case number and fibre number according to laboratory procedures.

4.3 Techniques for Special Applications, SERS/SERRS

4.3.1

SERS (Surface Enhanced Raman Scattering) or SERRS (Surface Enhanced Raman Resonance Scattering) present the advantage to enhance the Raman signal and to quench the fluorescence. However, only a limited number of molecules and conditions have been explored for the forensic analysis of fibres yet. The literature shows that excellent results can be obtained with pure dyes and some examples are presented with cotton fibres. However, there is a need of a good contact between the reagent and the sample in order to enhance the Raman signal. This is often difficult to obtain because many fibre dyes are embedded in the fibre substrate and thus not in direct contact with the reagent. Some authors recommend the extraction of the dye(s) followed by the application of SERRS. This method has not been applied yet to forensic science samples. A non-extractive hydrolysis technique suitable to the analysis of microscopic samples has been developed and effectively applied to the in-situ analysis of a single red wool fibre in an ancient textile.

4.3.2

Several procedures exist to prepare SERRS solutions and can be found in the related literature. Citrate-reduced silver colloid in a aqueous solution of poly(L-lysine) is the most commonly used.

4.4 Calibration and validation

4.4.1

It is essential that instrument performance and calibration are evaluated routinely according to manufacturers' recommendations. Instrument performance records may be maintained on hard copy and/or computer disk. These should include calibration and validation, maintenance records, wavelength checks (e.g. polystyrene spectra), and should be signed and dated. Calibration is used to relate observed spectral frequencies and intensities to their true values. Validation is a procedure to verify the calibration and control that instrument performance has not degraded over time. It is important that the calibration and validation protocols are established clearly. Examples of the performance evaluation method for Raman include:

- Raman shift calibration check: As small changes in true Raman shift can be scientifically informative, it is very important to proceed to a Raman shift calibration check. In order to determine the Raman shift, both the frequency of the laser and the Raman scattering should be known. Usually, visible lasers are very stable but the frequency of diode laser is less accurate and may even vary with the time.
- Frequency calibration with absolute frequency standards: In this case, the atomic emission lines of gases are used (neon, argon or mercury lamps). The atomic source is placed near the sample position. The laser must correspond to zero Raman shift. The plasma lines from the laser itself can also be used if the bandpass filter is removed. This calibration is usually done by the manufacturer.
- Frequency calibration with Raman shift standards: Raman shift standards does not depend on accurate knowledge of the laser frequency provided it is constant. The

ASTM committee collected and tabulated the results for a set of 8 standards [ASTM E 1840-96, 1998]. ASTM Raman shift standards are available with a standard deviation of $< 1\text{ cm}^{-1}$. Amongst the different substances proposed, polystyrene is particularly suitable for forensic application. It is in solid form, stable and nontoxic. It shows Raman shifts between 620 and 2904 cm^{-1} . These shifts were determined with 514 and 1064 nm lasers. Changes in observed frequencies are possible at other wavelengths due to resonance effect.

4.4.2

Validation: The instrument response has to be controlled by checking the magnitude of the Raman signal under identical analytical conditions. For example, the intensity of the silicon band at 520 cm^{-1} (silicone is used to set the Raman shift in dispersive instruments) permits the detection of instrumental changes.

Raman shift standard can also be used for validation by checking the standard deviation of the position and relative intensities of certain peaks under the same analytical conditions. The chosen peaks have to cover a wide range of Raman shifts.

4.5 Raman Analysis conditions

The analytical conditions should be optimized, and may probably differ, for each instrument and for each combination of fibre type and colour. Some set of parameters are mentioned in the literature for some given types of fibres and colours. This manual only provides global advices and will not get into too much detail.

- Wavelengths range: for most of the dyes and dyed fibres a range between 2000 and 200-300 cm^{-1} is sufficient. For some colourless man-made fibre the range can be extended to 4000 cm^{-1} in order to get all the polymer characteristics.
- Microscopic objective lens: the choice of the objective lens influences the size and the depth of the area measured. Different objectives should be tested on the questioned/reference samples. Generally, 50x or 100x objective provide good results. Higher magnifications can reduce fluorescence and increase S/N.
- Laser power (or intensity): the laser power should not saturate the detector or burn the sample. It is recommended to start with a low laser power and increase it depending on the result obtained. It should be noted that a high laser power may modify the molecule structure and thus the Raman spectra of heat sensitive samples (graphitization bands may appear as two large peaks round 1300 and 1600 cm^{-1}).
- Time of analysis, number of accumulation, number of measurements: the choice of these parameters depends on the quality of the first spectrum obtained. As a start, the time of analysis can be short (10 s), no accumulation, 1 measurement. If the S/N ratio is good, there is no need to ameliorate the spectrum. Otherwise these parameters may be changed and increased. The number of accumulations should be at least of 2 to highlight the presence of cosmic rays and to allow eventually the software to suppress them automatically. If possible, the same parameters are used for the reference and the questioned samples. Remark: it may not always be possible, as the questioned fibre could be contaminated with material (such as optical brighteners, etc.) that may require different conditions to obtain a spectrum.
- Grating: For some instruments, the resolution can also be set by changing the grating (from 300 to 1800 lines per mm). A 300 l/mm grating offers a low resolution but a fast measuring time as a 1800 lines/mm has a high resolution but a longer measuring time. A 1200 lines/mm grating is convenient to obtain a well resolved spectrum.
- Sample focus: The quality of the spectra may also be dependent on the correct focus on the sample. In confocal mode, it may allow to avoid interference from other materials (mounting medium, coverslip) for the analysis of mounted fibres.
- Fluorescence: The main concern is to avoid fluorescence in order to get a Raman spectrum. The easiest way to avoid fluorescence is to change the excitation

wavelength (laser). Another possibility is to use photobleaching. The latter is the irradiation of the sample with the laser for a long period of time (about 15 minutes to one hour). Provided that the sample is stable under these conditions this process can sometimes reduce or suppress fluorescence. SERRS may also be used to quench the fluorescence and enhance the Raman signal.

4.6 Spectra

4.6.1

The spectra should all be printed/displayed to the same scale to enable comparison of all questioned and reference fibres. Baseline correction is also recommended.

4.6.2

Report documentation may include the date, the operator, the system parameters, and the original instrumental output data. Each spectrum printed should be labelled with at least the case number and item number, date and operator. It is also useful to add a description of the fibre colour and fibre shape if the reference sample contains mixed fibres.

4.6.3

Hard copies of the questioned fibre and reference fibre spectra may be printed and placed in the case file. The operator and fibre examiner should sign and date the printout. Digital copies (or printscreen) of the spectra may be copied into the case report and properly annotated.

4.6.4

It is recommended that raw spectra of the fibres are saved to a hard disk, as per laboratory procedures. It is generally useful to save all data on disk just after it is generated and prior to any modification. Each spectrum file should be labelled with at least item number and the case number; date and operator are usually written in the data file by the acquisition software.

4.6.5

If any data manipulation has been carried out the raw data should be saved and the data manipulation carried out on a copy of the spectrum. Data that is damaged during subsequent processing can then be restored from the saved files. Any manipulation carried out, e.g. baseline correction should be noted on the hard copy of the spectrum or in the title of the spectrum file. Some manufacturer's file format may automatically store the manipulations of a spectrum and thus provide a mean of tracking any change. The saved spectra can be retrieved and compared with pre-stored library spectra at a later date. Smoothing algorithms can be applied, however it is recommended that they are applied sparingly and with considerable caution. Interpretation based on smoothed data should include comparisons of the spectra to the raw data. Derivatization of the data can be used to enhance spectral band positions.

5 INTERPRETATION AND DISCUSSION

5.1 Spectral comparison

First the homogeneity of the spectra of the questioned and reference samples should be observed.

For comparison purposes, baseline corrected spectra are superimposed to check if the same pattern is observed. First the presence of similar main bands at similar positions is controlled. If some spectra are especially noisy, it may be difficult to observe all the spectral details. This

should be taken into account when comparing spectra. A smoothing operation is also possible but should be used with care (disappearance or appearance of minor bands).

If the spectra have a good quality, every feature of the spectrum contains information concerning the sample. Examples of good quality spectrum, poor quality spectrum and spectrum overwhelmed by fluorescence are given in Figure 2. If the spectra are of good quality, any bands that show significant difference between two fibres should be labelled on the spectrum. The reference fibre spectrum is compared with the questioned fibre spectra by overlaying of the spectra on screen. The pattern of major and minor peaks, their relative position and intensities should be comparable at the same laser wavelength. If bands are the same for reference and questioned fibres, then these fibres can be said to be indistinguishable. If bands differ substantially in position or bands are missing in the questioned fibre spectra, then it can be concluded that the fibres do not match. Minor differences between two very similar spectra may eventually come from cosmic rays and new acquisitions of the spectra is helpful.

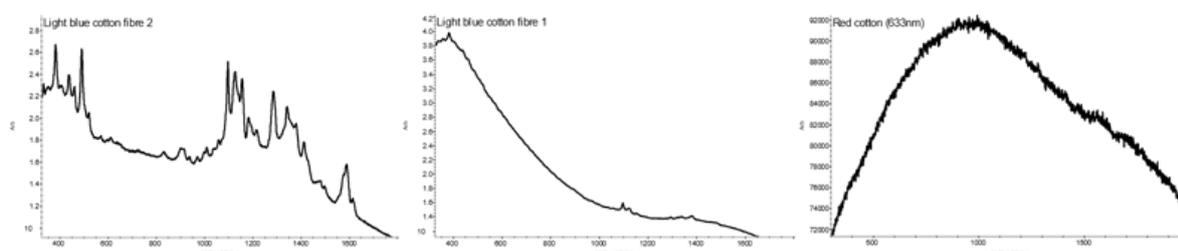


Figure 2: From left to right, Raman spectrum of good quality (light blue cotton fibre 2), of poor quality (light blue cotton fibre 1) and spectrum overwhelmed by fluorescence (red cotton).

5.2 Spectral interpretation and use of Raman library

A Raman spectrum is a combination of information from the fibre substrate, dye(s) (or pigments) and delustrant (if present). Depending on the sample, some of these constituents can be absent or present in the Raman spectra. Often one of them is dominant: it may be the only one detected or the one providing the most part of Raman bands characterising the spectrum. Globally, the contribution of the fibre substrate is important for colourless or pale fibres, but the dye(s) is (are) dominant in deeply coloured fibres. Pigments are usually dominant and well detected in pigmented fibres. For pigment and dye mixtures, respectively, one of the constituent is often dominant and may hide the contribution of the others.

- Fibre class/subclass: An in-house reference database of undyed fibres is important in order to determine if the measured spectrum corresponds to the fibre substrate, the dye(s) or pigment(s) or a mixture of both. In some cases it can also provide information about fibre class or subclass.
- Dyes: Several thousand of dyes are available on the market and a chemical structure is very difficult to determine based on the Raman spectrum obtained. A database is thus needed in order to identify dyes. The European Fibre Group (EFG) has created a database for some textile dyes (especially reactive blue/red/black dyes with 514, 532 and 785 nm lasers). It should be noted that dye identification is a difficult task for several reasons:
 - Different dyes can present very similar Raman spectra.
 - An exhaustive database is not available.
 - Interaction with information from the fibre substrate.
 - Interaction with other dyes or pigments present.
- It is recommended to use first a database created with the same laser as the one used for the questioned sample. The determination of the dye class based on TLC procedure may also reduce the number of possible hits.
- Pigments/delustrants: The number of pigments is limited to some hundreds. A Raman database of pigments was created by the European Paint Group (EPG) with 458, 514,

633 and 785 nm lasers. This database contains almost all the pigments available on the market. Each pigment has a unique Raman spectrum and pigment identification is relatively easy. It can be more complicated if a pigment mixture is present and both of them provide a Raman contribution. Using multiple laser sources may help in detecting different pigments apart or in better understanding the number of components in the pigment mixture.

6 TERMINOLOGY

Anti-stokes scattering

Some (few) molecules are already in an excited state, they will be excited to a virtual energy state and relax to the ground state, the Raman scattering is then called anti-Stokes Raman scattering.

Confocal settings of the microscope

A confocal microscope has an additional aperture, called confocal hole which will prevent the out-of-focus rays to reach the detector. Confocal mode reduces the depth of focus but also decreases the Raman signal.

Dispersive instruments

Dispersive instruments separate the different wavelengths scattered by the sample generally using a grating-based dispersive unit.

In-situ measurements

Measure of the sample in its original form, without manipulation or preparation.

Fluorescence

Emission of light from atoms or molecules when exposed to a radiation source (for example electromagnetic radiations). The atoms or molecules are excited by the source and emit photons to return to their ground states.

FT instruments

Fourier Transform (FT) instruments don't separate the different wavelengths scattered by the sample but modulate them in frequencies depending on their wavelengths. The result is a single beam detected by a single detector.

Grating or diffraction grating

Reflecting (or transmitting) material which present several equidistant parallel grooves on its surface. This material diffracts the light according to its wavelength.

Laser

Acronym for "Light Amplification by Stimulated Emission of Radiation". Monochromatic and coherent light source which present a high intensity.

NIR

Infrared light close to the visible light (about 0.7 to 1.4 μm wavelengths).

Polarizability

Polarizability describes a molecular property having to do with the symmetry of the molecule. Raman spectroscopy provides information concerning the depolarization ratio of a molecule which is the intensity ratio between the perpendicular component and the parallel component of the Raman scattered light.

Questioned fibre

These are the fibres, which have been removed, during the examination, for further analysis and comparison with the known fibre.

Raman scattering

In Raman scattering, the energy of the scattered photon differs from $h\nu_0$ by an amount equal to a vibrational transition. It is an inelastic scattering of photons by molecules. This effect is very weak and only about 1 in 10^{10} incident photons will undergo Raman scattering.

Raman shift

Difference in wavenumbers (cm^{-1}) between the incident energy $h\nu_0$ (laser source) and the energy of the Raman scattering band.

Raman spectrum

A Raman spectrum consists of scattered intensity plotted vs. energy. Each peak corresponds to a given Raman shift from the incident energy $h\nu_0$, generally expressed in wavenumbers (cm^{-1}).

Raman spectroscopy

Raman spectroscopy is based on the scattering of light by matter. The energy of the scattered photon differs from the energy of the exciting source (laser) by an amount equal to vibrational transitions of the molecules.

Rayleigh scattering

When a monochromatic light of energy $h\nu_0$ encounters a sample, there is a small probability that it will be scattered at the same frequency. In this case, the photons are elastically scattered, a process which is called Rayleigh scattering.

Reference fibre

Fibres with distinctive characteristics (e.g. generic type, morphology and colour) from a known textile source, e.g. a garment, which will be compared with the recovered fibres. The following terms are also used e.g. control, donor or target fibres.

Rejection filter

Filter that passes most wavelengths but attenuates some of them to a very low level. A rejection filter is used in Raman spectroscopy to reject the laser wavelengths (Rayleigh scattering) and let pass the other wavelengths (i.e. Raman scattering).

RR

Acronym for Resonance Raman. In resonance Raman spectroscopy, the energy of the laser coincides with an electronic transition of the molecule. In such case, the vibrational modes associated with this transition exhibit a great increase of the Raman scattering intensity.

SERS

Acronym for Surface Enhanced Raman Scattering. SERS is a surface sensitive technique that results in the enhancement of Raman scattering and can also quench the fluorescence. A colloid solution is usually used.

SERRS

Acronym for Surface Enhanced Resonance Raman Scattering. This technique takes advantages of both RR and SERS phenomena.

Stokes scattering

The molecule at the ground state is excited to a virtual energy state and relax into a vibrational excited state, which generates Stokes Raman scattering.

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APPENDIX 5 – CHROMATOGRAPHIC TECHNIQUES

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1 CLASSIFICATION AND THIN LAYER CHROMATOGRAPHY OF TEXTILE FIBRE DYES

1.1 Preface

Since the first edition of the manual in 2001, there have been virtually no technical or methodological changes to this inexpensive, highly discriminating, yet under-utilised technique. The reason for this is simple; over the years the methodology has been proven to be robust and entirely fit for purpose. The adage 'don't fix it if it isn't broken' very much applies in this circumstance. Whilst other methodologies for examining fibre dyes have been the subject of numerous studies, trials and evaluation (e.g. RAMAN), all have yet to show the same 'bang for the buck' as TLC – particularly when its results are combined with Microscopy, MSP and FTIR.

1.2 Introduction

Metameric coloration of fibres can be detected using UV/visible spectroscopy. If spectroscopy is restricted to the visible range only, differences in dye components may remain undetected. Further differentiation may be possible with the use of additional analytical techniques. One method of detecting additional components is to use thin layer chromatography (TLC). This is an inexpensive, simple, well-documented technique that can be used to complement the use of microspectrophotometry in comparisons of fibre colorants. The application of TLC may serve to discriminate between fibres, or it may confirm their similarity. Since the technique involves classification of the dye and visualisation of mixture components, it may provide very useful information in intelligence led investigations involving industrial enquiries.

This guideline is intended to advise and to assist individuals and laboratories that conduct forensic fibre examinations and comparisons in the effective application of TLC to the analysis of fibre evidence. It is concerned with the extraction of dyes from single fibres and bulk material, classification of the dye or colorant; application and development of the extractants on TLC plates using an optimal elution system and evaluation and interpretation of the resulting chromatograms. The protocols and equipment mentioned in this document are not meant to be totally inclusive or exclusive.

1.3 Theory

Forensic analysis of fibre colorants using TLC should only be considered for single fibre comparisons after all available non-destructive comparative techniques have been tried i.e. microscopy (brightfield and fluorescence), microspectrophotometry, FTIR.

Extraction procedures carried out prior to TLC analysis can provide useful information about the dye class involved. Similar colours made up of different dye components can be differentiated using this technique.

Dyes from the known material should first be characterised and eluent systems evaluated to achieve optimum separation of the extract. Taking single fibres from the known material that are of an equivalent length to the recovered fibres to be tested is the next stage. If acceptable results are obtained then the dye should be extracted from single known and recovered fibres of equivalent lengths.

Both the alkaline hydrolysis of dyed wool fibres and enzymatic digestion of dyed cotton fibres are destructive methods of analysis. Thus, analysts have to decide whether, in terms of evidential value in a particular case, the benefits of chromatographic analysis will outweigh the loss of the fibre and whether the courts will agree with the judgement made. In many cases, only a small proportion of the recovered fibres needs to be examined in this way and others will be available for re-examination if required. The question of 'destroying' fibres is far from being a new one. The choice between including or omitting TLC methods in forensic fibre dye comparisons is often a matter of local policy. Some laboratories have always regarded TLC, with its implied dye extraction (for acid, basic, disperse, metallized, direct and azoic dyes) to be a destructive technique, and as such they avoid it or use it only as a last resort.

Microspectrophotometry over the full spectral range may be sufficient, being both discriminating and non-destructive. Others say any additional discrimination offered by dye extraction and TLC is seen as too important to ignore. The minimal recommendation is that TLC should be used, provided the sample permits, as a complementary technique where microspectrophotometry has been used only in the visible range.

TLC eluent systems have been described for separating dyes used on polyester, polyamide and acrylic fibres (Beattie et al, 1981b), cellulosic fibres (Home and Dudley, 1981) and polypropylene fibres (Hartshorne and Laing, 1984). Eluents for wool fibre dyes had already been described (Macrae and Smalldon, 1979) and after a further ten years a scheme for azoic dyes on cotton fibres was developed by Laing et al (1990). Crabtree et al 1995 and Rendle et al 1994 described eluent systems for reactive dyes on wool and cotton respectively.

TLC is inherently non-reproducible due to the lack of stability of eluents and temperature although the use of automatic sample application and automatic development chamber can significantly improve this parameter. Standard dye mixtures should therefore be used to check eluent performance.

TLC has the following disadvantages: It is dependent on an adequate concentration of colorant being present in the material to be examined: short lengths of single fibre, or very pale fibres may be unsuitable. Dye extraction from some cellulosic and wool fibres may be difficult to achieve. Pigment granules (as used in some produced dyed fibres) cannot be extracted.

Chromatography is a widely applicable differential technique in which the flow of solvent or gas causes the components of mixtures to migrate differently from a narrow initial zone in a porous absorptive medium. Similar colours resulting from a mixture of different dyes can be distinguished using TLC.

Dye extraction/classification schemes have been developed which generally allow single fibres to be sequentially extracted with a range of solvents. These schemes determine the most efficient method for dye extraction and also allow the dye present to be classified (with a degree of caution). The fibre type can be identified using brightfield and polarised microscopy and/or FTIR spectroscopy. Once the fibre type is known the sequential classification schemes can be followed. The schemes involve both solvent extraction and chromatography; generally only a single fibre is necessary. However, a second fibre is required to complete the classification for cotton and viscose. When it is necessary to classify very pale fibres a small tuft will be needed. Care should be taken to only classify a dye as being equivalent to, or behaving as representative of, a particular dye class. This covers the eventuality of any new dyes being produced that may not conform to the present schemes.

Extraction and classification studies have been carried out on the dyes for wool, polyamide, polyacrylonitrile, polyester, cotton, viscose, polypropylene and acetate fibres. In 1979 Macrae and Smalldon developed a three part scheme for the major dye classes found on wool, i.e. acid, metallized and reactive. Schemes for the extraction and classification of dyes on single nylon, acrylic and polyester fibres (Beattie et al 1979), cellulose acetate fibres (Beattie et al 1981) and polypropylene fibres (Hartshorne and Laing 1984) followed. In 1991 Laing et al introduced a scheme for cotton and viscose fibres.

For many years acid, direct, metallized, disperse, basic and azoic dyes were considered as the only extractable dye classes, which were therefore amenable to TLC. The other major classes, reactive, vat, ingrain and sulphur were considered not to extract. In 1981 Home and Dudley reported that a 1.5% aqueous solution of sodium hydroxide would extract the majority of reactive dyes found on cotton. However subsequent workers have noted that success is limited and those that do extract undergo a colour change. Laing et al 1991 identified reactive dyes on cotton by an exclusionary procedure but were unable to extract them. Cheng et al 1991 worked on a similar extraction / classification procedure to Laing but used hydrazine in water as a solvent but still many dyes could not be extracted. The latest approach is to use enzymatic hydrolysis, Rendle et al 1994 reported that pre-swelling with sodium hydroxide, followed by digestion of the cotton fibres dyed with reactive dyes using cellulase, produces homogeneous coloured solutions. Typically the fibre disappears. They are not true dye extracts but probably contain dye chromophores bound to glucose molecules. These products are

amenable to separation by TLC. This process has proved to be successful for both bulk and single fibres.

Reactive dyes that are used on wool are also a problem to extract. The high fastness of the reactive dyes is likely to be due to covalent bonds between the dyestuff and the fibre (Macrae and Smalldon 1979). These processes are designed on the basis that the dye will react with nucleophilic groups, such as amino, thiol and hydroxyl functions in the amino acid residue side chains of the keratin fibres. Crabtree et al 1995 reported a method for the alkaline hydrolysis of wool fibres dyed with reactive dyes and subsequent analysis of the coloured products by TLC. The method has been successfully applied to single wool fibres and in 1996 Wiggins demonstrated that TLC analysis of reactive dyes yields important information over and above that obtained using techniques such as comparison microscopy and visible light microspectrophotometry.

1.4 Methodology

1.4.1 Sample Handling

Anti-contamination procedures including using clean glassware, washing forceps and ensuring that benches etc are clean must be followed.

The general handling and tracking of the samples should meet or exceed the requirements of both the Quality Assurance and Evidence Collection Guidelines.

Pre-treatment (mounting medium, washing solvent etc.) and sample preparation must be identical for all known and recovered fibres being compared on one TLC plate.

For removing single fibres from slide preparations the following procedure is recommended:

- Any traces of marker pen ink must be cleaned from the coverslip if necessary using an appropriate solvent, e.g. acetone.
- If a mounting medium has been used which solidifies the coverslip should be cracked all around the fibre and an appropriate solvent, which will dissolve the mountant, but not affect the fibre or the colorant, should be used.
- The fibre is removed and washed in the solvent.

1.4.2 Classification of Dyes

Dye classes can be classified into broad groups based on their chemical properties or method of application. The determination of the dye class of the known fibres is necessary to establish the best extractant, as well as to assist in the subsequent selection of the most efficient eluent system.

The ease of dye extraction and the particular extractant required will depend on the generic class of the fibre and the dye class present. The generic class of the known and recovered fibres must be determined prior to TLC analysis.

Dye classification can be done on single fibres or tufts of fibre removed from the known item. A new fibre or tuft can be used for each classification stage Extractants used for classification are not necessarily those used for single fibre extraction prior to TLC.

Documented extraction schemes can be used to determine the dye class of fibres of known generic classes, and thus the optimum extractant.

Examples of dye classification schemes are as follows:

WOOL Fibres

Stage 1: Pyridine/Water (4:3), 100°C, 10 min

- | | |
|-----------------------|-------------------|
| Good extraction | → ACID dye |
| Little/ no extraction | → Go to Stage 2 |

Stage 2: 2% aqueous oxalic acid, 100°C, 20 min, then pyridine/water (4:3), 100°C, 10 min

- | | |
|---------------------|-------------------------|
| Improved extraction | → METALLIZED Dye |
|---------------------|-------------------------|

Little/ no extraction → **REACTIVE** Dye

COTTON and VISCOSE Fibres

Stage 1: Glacial acetic acid, 100°C, 20 min

Good extraction → **AZOIC** Dye
Little/ no extraction → Go to Stage 2

Stage 2: Pyridine/Water (4:3), 100°C, 20 min

Good extraction → **DIRECT** Dye
Little/ no extraction → Go to Stage 3

Stage 3: Dithionite/polyvinylpyrrolidone, 100°C, 20 min. Check fibre colour. Apply extract to TLC plate; check colour of spot

Fibre colour changed. No coloured spot or spot not original fibre colour

→ **REACTIVE** Dye

Fibre colour unchanged. No coloured spot or spot not original fibre colour

→ **INGRAIN** Dye

Fibre colour changed and original coloured spot → Go to Stage 4

Stage 4: 10-14% Sodium hypochlorite, 100°C, 10 min (new fibre)

Fibre colour changed → **SULPHUR** Dye
Fibre colour unchanged → **VAT** Dye

ACRYLIC Fibres

Stage 1: Formic acid/water (1:1), 100°C, 20 min

Good extraction → Go to Stage 2

Stage 2: TLC procedure – methyl acetate eluent

Movement → **DISPERSE** Dye
No movement → Go to Stage 3

Stage 3: TLC procedure – methanol eluent

Sharp line at solvent front → **ACID** Dye
Little/ no movement/ smearing → **BASIC** Dye

POLYESTER Fibres

Stage 1: Chlorobenzene, 130°C, 10 min

Good extraction → **DISPERSE** Dye
Little/ no extraction → Go to Stage 2

Stage 2: Dimethyl formamide/formic acid (1:1), 100°C, 20 min

Good extraction → **BASIC** Dye.

POLYAMIDE Fibres

Stage 1: Chlorobenzene, 150°C, 15 min

Good extraction → **DISPERSE** Dye
Little/ no extraction → Go to Stage 2

Stage 2 : Pyridine/water (4:3), 100°C, 20 min

- Good extraction → Go to Stage 3
 Little/ no extraction → **REACTIVE** or **DIAZO** Dye

Stage 3: TLC procedure – methanol eluent

- Sharp line at solvent front → **ACID** Dye
 Little/ no movement/ smearing → **BASIC** Dye

POLYPROPYLENE Fibres

Stage 1: Methyl acetate/water/acetic acid (5:5:1), 100°C, 20 min

- Good extraction → **DISPERSE** Dye
 Little/ no extraction → Go to Stage 2

Stage 2: Pyridine/ water (4:3), 100°C, 20 min

- No extraction → **PIGMENT**
 Some extraction → Go to Stage 3

Stage 3: 2% aqueous oxalic acid, 100°C, 20 min, then pyridine/water (4:3), 100°C, 20 min

- Improved extraction → **METALLIZED** Dye
 No improvement → **ACID** Dye

ACETATE/TRIACETATE Fibres

Stage 1: Cellulose Acetate – pyridine/water (4:3), room temp. 15 min

Cellulose Triacetate – pyridine/water (4:3), 100°C, 20 min

- Little/no extraction → **DIAZO** Dye
 Good extraction → **DISPERSE** Dye

1.4.3 Thin Layer Chromatography of extractable fibre dyes

This section will deal with the TLC of the following dye classes: acid, azoic, basic, direct, disperse and metallized.

The choice and composition of some extraction solutions, examples of eluent composition and Standard Dye Mixtures are shown below.

Choice and composition of some extraction solutions:

| Dye class | Fibre Type | Extraction Solution |
|------------------|-------------------|-------------------------------|
| Acid | Wool | Pyridine / water 4:3 v/v* |
| | Silk | “ |
| | Polyamide | “ |
| | Protein | “ |
| | Polyacrylonitrile | “ |
| | Polypropylene | “ |
| Azoic | Cotton | “ |
| | Viscose | “ |
| Basic | Polyacrylonitrile | Formic acid / water 1:1 v/v** |

| | | |
|------------|-------------------|--|
| | Modified acrylic | “ |
| | Polyester | Pyridine / water 4:3 v/v* |
| | Polyamide | “ |
| Direct | Cotton | “ |
| | Viscose | “ |
| Disperse | Polyester | Pyridine / water 4:3 v/v* or Chlorobenzene |
| | Polyacrylonitrile | Pyridine / water 4:3 v/v* |
| | Polyamide | “ |
| | Polypropylene | “ |
| | Acetate | “ |
| | Triacetate | “ |
| Metallized | Wool | 2% aqueous oxalic acid*** then Pyridine / water 4:3 v/v* |
| | Polypropylene | “ |

* Pyridine/water 4:3 v/v, prepare 100ml and use until exhausted.

** Formic acid/water 1:1 v/v, prepare 100ml and use until exhausted.

Should either of the above solutions become contaminated or as can be the case with pyridine, it polymerises (the solution goes yellow) they should be discarded.

*** 2% aqueous oxalic acid (0.2g in 10ml water) use immediately and discard excess.

Examples of eluent composition for TLC analysis of fibre dyes:

| Eluent no. | Solvents* | Proportions (v/v) |
|------------|--|-------------------|
| 1 | n-Butanol, acetone, water, ammonia | 5:5:1:2 |
| 2 | Pyridine, amyl alcohol, 10% v/v ammonia | 4:3:3 |
| 3 | n-Butanol, ethanol, ammonia, pyridine, water | 8:3:4:4:3 |
| 4 | Methanol, amyl alcohol, water | 5:5:2 |
| 5 | Toluene, pyridine | 4:1 |
| 6 | Chloroform, ethyl acetate, ethanol | 7:2:1 |
| 7 | n-Hexane, ethyl acetate, acetone | 5:4:1 |
| 8 | Toluene, methanol, acetone | 20:2:1 |
| 9* | n-Butanol, acetic acid, water | 2:1:5 |
| 10 | n-Butanol, ethanol, ammonia, pyridine | 4:1:3:2 |
| 11 | Chloroform, butanone, acetic acid, formic acid | 8:6:1:1 |
| 12** | n-Butanol, acetic acid, water | 4:1:5 |

* The ethanol used is 99% and the ammonia is 0.880 S.G. unless stated. Eluents 6 and 11 should be discarded daily, all others on a weekly basis.

** This solvent mixture forms an upper and lower phase. Use the upper phase as the eluent.

In order to be sure that extraction solutions have not been contaminated and that eluents are performing as expected they should be checked before use. Mixtures of standard dyes should be used to check eluent performance. If the extractant solution track is clear, it is obvious that the extractant is free from contaminants and can be used. The standard dye chromatogram is

compared to previously stored chromatograms. If it is separating as normal, the eluent may be used for casework chromatography.

The list below suggests suitable mixtures but is not totally inclusive or exclusive. Any (simple) mixture of dyes separating in the eluent of choice may be used. Dyes can be purchased from a range of companies e.g. Aldrich.

Examples of Standard Dye Mixtures

Approximately 5mg of each dye component are made up to a final volume of 25ml with pyridine/water (4:3 v/v) or other suitable extractant. Use until the supply is exhausted.

- Solution A for eluents 1, 2, 3, 4, 9, 10, 12:
Solway green G (CI Acid Green 25), Solway blue RNS (CI Acid Blue 47) and Naphthalene fast orange 2GS (CI Acid Orange 10).
- Solution B for eluents 5, 7, 8:
Supracet fast orange G (CI Disperse Orange 3), Supracet fast violet B (CI Disperse Violet 8) and Supracet scarlet 2G (CI Disperse Orange 1).
- Solution C for eluent 6:
Supracet fast orange G (CI Disperse Orange 3) and Supracet fast violet B (CI Disperse Violet 8).
- Solution D for eluent 11:
Solway green G (CI Acid Green 25), Supracet fast orange G (CI Disperse Orange 3) and Supracet fast violet B (CI Disperse violet 8).

1.4.3.1 Chromatography – Testing of eluents and extraction solutions

Suggested TLC plates are Merck DC Alufolien Kieselgel 60F254 (7.5 x 5.0 cm). The plate should rest on a hot plate at 70°C approximately. The standard dye and extraction solution are applied to the origin line 1 cm from the lower edge of the TLC plate and 1cm in from the side using a finely drawn capillary to produce a spot of approximately 2mm diameter. Care should be taken to avoid scratching the silica surface. At least one known sample should be spotted on either side of the recovered samples. The order of the samples on the plate should be noted. The TLC plate is placed in an oven at approximately 100°C for 5 minutes to ensure it is completely dry as residual solvent may affect the separation/elution of the dyes.

Chromatograms can be developed vertically in a glass chamber, which may be as simple as a covered glass beaker. Commercial tanks are available. Twin trough tanks allow for the equilibration of the solvent mixtures in the tank and on the TLC plate prior to elution. They also allow the solvent to be transferred to the plate side without removing the cover, but extreme care must be taken when doing this to prevent splashing of the TLC plate which can result in uneven movement of the solvent front.

The eluent should be added to the tank and allowed to stand in a closed container for a few minutes before development, so that the chamber will be saturated with the solvent vapour (this will not be complete if a beaker is used. Equilibration is not critical when sample size is very small and the elution time is short).

Sufficient eluent is added to the tank such that the level of the eluent in a vertical tank is at least 0.5 cm below the origin/application spots on the TLC plate. The plate is removed from the oven and eluted until good resolution is achieved e.g. 2 – 3 cm from the origin. Occasionally, it may be desirable to focus the spots using a methanol pre-run prior to elution. The plate should then be removed and the position of the solvent front marked with pencil. It should then be dried in a hot air stream or carefully on a hotplate and the eluent discarded

1.4.3.2 Choice of Elution System

A tuft of fibres is placed into a glass Durham Tube (2.5cm x 0.3cm internal diameter) or similar and approximately 100µl of extraction solution is added. The tube may be sealed or left open and is placed in a sand bath or oven and heated to approximately 100°C. The extraction progress may be checked at 15-minute intervals for up to a maximum of 1 hour.

If a sample to be eluted is a mixture of fibre or dye types these must be dealt with separately. A few single fibres of each type are placed in glass tubes (2.5cm in length, with an internal diameter of 1.5mm approximately and sealed at one end), pushed down the tube with a fine wire and approximately 10µl of solvent is added using a finely drawn glass pipette or syringe. The tube is then heat sealed to avoid evaporation, and incubated for the relevant time and temperature in an oven. The colour of the solution and fibre may be noted after removal from the oven. The resulting extracts are spotted onto TLC as previously described. A minimum of two eluents should be evaluated for each dye extract. Examples of common combinations of fibre type and dye class, together with the eluents, which can give the best separation, are shown below.

Fibre type/dye class and appropriate eluent:

| Fibre type | Dye | Eluent nos |
|-------------------|--------------------|------------|
| Wool or silk | Acid or metallized | 1, 2 |
| Cotton or viscose | Direct | 1, 4, 3 |
| Cotton or viscose | Azoic | 5 |
| Polyester | Disperse | 6, 7, 8, 5 |
| Polyacrylonitrile | Basic | 11, 12, 1 |
| Polyamide | Acid | 9, 10. |

If other fibre type/dye class combinations are encountered, then the appropriate eluents for that dye class should be used. Once the plates have been developed with the use of the appropriate eluents, usually to a distance of 2 – 3 cm from the origin, five parameters are considered when selecting the optimum eluent:

- Separation of component bands
- Sharpness of bands
- Movement from the origin
- Components travelling at or close to the solvent front
- Strength of dye extract from recovered fibres

The best system is when separation is achieved in the first 60 –70% of the elution distance and the greatest number of clear bands are seen.

If the suggested eluents produce poor separation, others appropriate to the dye class should be evaluated. In exceptional circumstances eluents appropriate to other dye classes may be used.

1.4.3.3 Single fibre procedure

Both recovered and known fibres normally need to be removed from microscope slides following microscopy and visible microspectrophotometry. Any traces of marker pen ink should be cleaned from the coverslip using an appropriate solvent e.g. acetone.

The coverslip should be cracked around the fibre and an appropriate solvent should then be introduced to soften the mountant. The fibre is removed and washed in the solvent before placing it in a glass tube as previously described. The tube is labelled and stored in a covered petri dish or equivalent. Additional known and recovered fibres that require testing are prepared

in the same way. The known fibres should be chosen so as to be of an equivalent length and depth of dyeing to the recovered fibres and should be treated in an identical manner.

An appropriate extractant is added to the tubes and, after sealing, they are placed in an oven at an appropriate temperature and for a specific time interval to extract. A TLC plate is labelled ensuring that a standard dye is included and recovered fibre extracts are spotted between known fibre extracts. The extracts are spotted onto the plate and the plate is then eluted in a beaker or TLC chamber as previously described.

1.4.3.4 *Equivalent fibre testing*

If a recovered fibre to be tested is short in length, or pale in colour, there may be insufficient dye present to obtain a result from TLC. In these circumstances a known fibre, equivalent in all respects, should first be analysed. If a result is obtained from a known fibre that is equivalent in all respects, then the recovered fibre should also give a result if it originates from that or from another item made of identical fibre.

1.4.3.5 *Comparison of large fibre samples*

If large samples are available they can be extracted in Durham tubes or similar. A mixture of known and recovered extracts may be included and co-chromatographed. This can be particularly useful if it is thought that one of the samples is contaminated with solvent like substances e.g. petrol from a piece of material in an arson case.

1.4.3.6 *Examination of TLC plates*

Plates should be examined immediately after drying using visible and longwave ultraviolet light. Band position(s) and colour(s) should be noted. One method of enhancing TLC plates is as follows:

After any obvious visible and/or UV bands on the TLC plate have been noted fill a plastic petri dish or similar container with liquid nitrogen. Ensure all safe working practices are observed.

Using forceps, place the TLC plate; face up, in the petri dish. Switch on the UV light source and note the appearance of additional or enhanced UV bands.

Switch off the UV light and allow the liquid nitrogen to evaporate.

The colour/fluorescence of the spots and the distance from the centre of each spot to the origin may be measured and recorded. The method of documentation is a matter of individual laboratory preference. Plates and samples must be identifiable. Plates should be documented by photography or drawing and/or retained and stored out of direct sunlight in a manner designed to minimise fading.

1.4.4 Thin Layer Chromatography of reactive fibre dyes

This section will deal with reactive dyes encountered on wool and cotton fibres. Coloured solutions are released from a sample of the known garment by either chemical or enzymatic digestion for wool and cotton fibres respectively. The solution is tested with appropriate reagents to achieve optimum separation. Equivalent fibre testing is carried out as necessary. Dye is then obtained from single fibres and the component colours separated out using TLC. Recovered and known chromatograms are compared.

Fibre disruption solutions, suggested eluents, standard dyed fibres and standard dyes are listed below.

Composition of Fibre Disruption Solutions:

| | |
|-------------|---|
| Acetic Acid | 0.5M glacial acid (prepare 100ml and use until stock is exhausted) |
| Cellulase | 1.6mg/ml sodium acetate buffer (prepare 50ml and discard at the end of each week) |
| Citric Acid | 0.3M in methanol (prepare 10ml and discard at the end of each week) |

| | |
|-------------------------------|--|
| Sodium Acetate Buffer | 0.1M in water adjusted with consideration of the type of cellulase to pH5 ± 0.2 with glacial acetic acid (prepare 50ml and discard after one week) |
| Sodium hydroxide (for wool) | 0.75M in water (prepare 100ml and use until stock is exhausted or discard after one month) |
| Sodium hydroxide (for cotton) | 3M in water (prepare 100ml and use until stock is exhausted or discard after one month) |

Note: Cellulase (*Penicillium Funiculosum*) should be stored at -18°C and used until the stock is exhausted or the expiry date is reached.

Composition of Eluents:

| Eluent No | Solvents | Proportions (v/v) |
|-----------|--|-------------------|
| 1 | n-Butanol, acetone, water, ammonia | 5:5:1:2 |
| 2 | Pyridine, amyl alcohol, 10% ammonia | 4:3:3 |
| 3 | n-Butanol, ethanol, ammonia, pyridine, water | 8:3:4:4:3 |
| 4 | Methanol, amyl alcohol, water | 5:5:2 |
| 13 | Propan-1-ol, methanol, water, ammonia | 6:3:1:4 |
| 14 | n-Butanol, ethanol, ammonia, pyridine, water | 8:3:4:4:6 |
| | n-Butanol, ethanol, ammonia, pyridine, water | 6:3:2:6:6 |

Standard Dyed Fibres:

| Fibre Type | Dye | Supplier | Colour Index |
|------------|----------------------------|-----------|------------------|
| Wool | Drimalan brilliant red F-B | Sandoz | Reactive red 147 |
| Cotton | Xiron brilliant red B-HD | Chemic AG | Reactive red 24 |

1.4.4.1 Preparation of Standard Dye Mixtures

Solution A for Eluents 1, 2, 3, 4, 13, 14, 15:

- Solway green (CI Acid green 25)
- Solway Blue RNS (CI Acid blue 47)
- Napthalene fast orange 2GS (CI Acid orange 10)

Approximately 5mg of each dye component is made up to a final volume of 25ml with pyridine/water 4:3 v/v and used until the supply is exhausted.

Just before using the eluent it should be checked using a standard dye mixture, and the fibre disruption solution should also be tested to ensure that it has not been contaminated. The dissolution process must be checked to ensure that it is working correctly by analysing a standard dyed fibre sample.

Dye can be released from the standard fibres using the following methods:

- Wool – large fibre tufts
Place a tuft of fibres in a 500µl (Eppendorf type) centrifuge tube, add 100µl of 0.75M sodium hydroxide and incubate at 45°C for 24 hours with regular inversion. Add 66µl of 0.3M citric acid, mix and centrifuge at 7000rpm for 5 minutes.
- Wool – single fibres or small tufts
Push the fibres to the bottom of a glass tube as previously described. Add 3µl of 0.75M sodium hydroxide and seal the tube before incubating at 45°C for 24 hours with

continuous agitation. Open the tube and add 2µl of 0.3M citric acid, mix and centrifuge at 7000rpm for 5 minutes.

- Cotton – large fibre tufts

Place a tuft of fibres in a 500µl (Eppendorf type) centrifuge tube, add 50µl of 3M sodium hydroxide and keep at 0°C for 4 hours. The solution is then discarded. Resuspend the sample in 50µl of 0.5M acetic acid for 20 seconds and discard the solution. Wash the sample twice with 150µl of cellulase solution and discard the solution. Resuspend the sample in 150µl of cellulase solution and incubate at 45-60°C (depending of the time of cellulose) for 20 hours with regular inversion. Centrifuge at 7000rpm for 5 minutes. Transfer an aliquot of the dye solution to a new 500µl centrifuge tube; add an equal volume of methanol and mix.

- Cotton – single fibres or small tufts

Push the fibres to the bottom of a glass tube as previously described. Add 5µl of 3M sodium hydroxide and seal the tube. It is then kept at 0°C for 4 hours before discarding the solution. Resuspend the sample in 5µl of 0.5M acetic acid, for 20 seconds and discard the solution. Resuspend the sample in 3µl of cellulase solution, the tube is resealed and incubated at 45-60°C (depending of the time of cellulose) for 20 hours with continuous agitation. 3µl of methanol is added, mixed and centrifuged at 7000rpm for 5 minutes.

Once known fibre extracts have been prepared as described above, they should be evaluated in a minimum of two eluents.

Eluents 13, 1 and 2 can be used to separate reactively dyed wool whereas eluents 14, 15, 3 and 4 allow cotton extracts to be adequately separated. When the best eluent has been chosen using the parameters as listed for 'extractable dyes' above, equivalent fibres can be prepared and tested.

1.4.4.2 *Single Fibre Procedure*

The sample preparation is as previously described and once the single fibres are in tubes the method for cotton or wool single fibre dye release should be followed. Extracts are spotted onto TLC plates and eluted in the appropriate eluent. Large samples can be compared using co-chromatography.

1.4.4.3 *Examination of TLC plates*

After drying the TLC plates should be examined using the same conditions set out for 'extractable dyes' in the earlier section.

1.4.5 Non-extractable dyes

This section will deal with ingrain, sulphur and vat dyes.

If classification indicates that a non-extractable dye or pigment (rather than a reactive dye) is present then place one known and one recovered fibre in labelled capillary tubes. Add approximately 10µl pyridine/water 4:3 v/v and after sealing the tube attempt to extract at 100°C for one hour. If neither fibre extracts, a positive association is noted. If the recovered fibre extracts and the known does not the result is negative. If both known and recovered 'bleed' dye into solution there may be sufficient dye for analysis.

1.5 Interpretation/Discussion

Chromatograms of dyes from the same fibres run in different eluent systems or on different plate types cannot be compared. The spot colours/fluorescence, sequence, and position of the spots obtained from the dye of the questioned fibres are compared to those from the corresponding known fibres:

- The component dyes of known and recovered fibres are indistinguishable when the band colours/fluorescence, sequence and positions are consistent.

- An exclusion is noted when either the recovered and known band patterns show no similarities or where there are a number of coincident bands but one or more bands are missing from the recovered or known.
- An inconclusive association is noted when there are no bands on the TLC plate because insufficient colorant is present in the extract. If equivalent fibre testing is carried out, inconclusive results should only rarely occur.

Subtle differences between known and recovered fibres should be carefully considered before a negative association is reported. Testing of additional areas of the known garment may result in a positive association being found. This happens when dye batch variation is encountered (Wiggins et al 1988) In cases where the amount of extract is very small, the distance travelled by the eluent is very small or the spots are not defined, attempts to calculate the Retention Factor (RF) values can easily be inaccurate and are therefore meaningless.

The TLC methods used in the forensic comparison of fibre colorants must have been published in a recognised journal, other relevant textbooks, in an accredited laboratory manual or if new systems are developed accompanied by appropriate documentation.

Plates must be identifiable with respect to case number, sample source, examiner, and date. Case documentation on TLC must include the source of the samples, method of dye classification, extraction time and temperature, details of extractants/eluent systems tested and/or used, and the results. The use of standard dye mixtures as system performance checks is strongly recommended.

1.6 Terminology

Adsorbent

The stationary phase for adsorption TLC.

Adsorption

The attraction between the surface atoms of a solid and an external molecule by intermolecular forces.

Chamber

A glass chamber in which TLC development is carried out.

Chromatography

A method of analysis in which substances are separated by their differential migration in a mobile phase flowing through a porous, absorptive medium.

Co-chromatography

A proportion of the known and recovered fibre extracts are mixed and eluted alongside individual fibre extracts. Any additional spots or mismatching of dye will be visible in the cochromatography run.

Development

The movement of the mobile phase through the adsorbent layer after development.

Dye extraction

The removal of the dye from a fibre by incubating it in an appropriate extraction solvent(s).

Eluent

The solvent mixture which acts as the mobile phase in TLC.

Extract

The solution containing the dye components (solutes) which is obtained after treating the fibres with a suitable extractant.

Extractant

The extraction solution.

Metameric pair

Two colours that appear the same under one illumination, but different under other illumination.

Mobile phase

The moving liquid phase used for development.

Origin

The location of the applied sample or the starting point for the chromatographic development of the applied sample.

Oxidation

The addition of oxygen to a compound. More generally, any reaction involving the loss of electrons from an atom. It is always accompanied by reduction.

Qualitative Analysis

Identification of the constituents of a sample without regard to their relative amounts. It often refers to elemental analysis, but may also refer to the detection of acid – base or redox properties in a sample.

Reduction

Any process in which an electron is added to an atom or an ion. Four common types of reduction are removal of oxygen from a molecule, the liberation of a metal from its compounds and diminution of positive valency of an atom or ion. Always occurs accompanied by oxidation.

Resolution

The ability to visually separate two spots.

Retention Factor (Rf)

The ratio of the distance travelled by the solute spot's centre divided by the distance travelled by the solvent front, both measured from the origin.

Saturation

Chamber equilibration between the eluent's solvent and vapour phase prior to chromatography.

Solute

In TLC, a mixture of components to be separated.

Solvent front

The final point reached by the mobile phase as it flows up or across the TLC plate during development of the chromatogram.

Spot

A round zone of sample application at the origin: or in a chromatogram, a round zone caused by migration of a component of the solute.

Spotting

Applying an extract at the origin of the TLC plate.

Stationary phase

The solid adsorbant coating of a TLC plate.

Thin layer chromatogram

The series of spots visible on the adsorbent layer after development

Thin layer chromatography

TLC, a separation technique in which the flow of solvent causes the components of a mixture to migrate differentially from a narrow initial zone in a thinly applied porous adsorptive medium.

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1.8 TLC of Fluorescent or Optical Brightening Agents

1.8.1 Introduction

The world's annual production of fluorescent brightening agents represents about 10% of the business in dyestuff companies. About 80% of the brightening agents are stilbene derivatives. Brightening agents are used in detergent mixture (58%), paper brightening (25%), textile finishing (12%) and plastics (5%).

Numerous problems have been encountered with the TLC of optical brightening agents and if the technique is used in forensic science great care must be taken:

- There is little forensic literature in relation to the TLC of optical brightening agents. Most of the references have been published by the textile industry and there is no indication that they could be applied to single fibre analysis. Problems already exist as in industry the level of brightening agent used may be extremely small (perhaps in the range of 0.01 – 0.1% – on weight of fibre) and the proportion of the agent to co-extracted fibrous material may become critical for satisfactory chromatography.
- If the material is coloured or has been previously washed interference will occur from the dyes and from the optical brightening agents in the detergent. In industry, if dyed material has been extracted, Schweppe recommends making a preliminary separation by column chromatography. The migration of the brightener zone can be monitored by illuminating the column with a UV lamp.
- TLC should only be carried out as a comparison exercise between fibres. Identification of brightening agents on fibres is often difficult. According to Brown: 'By means of TLC, unknown samples of fluorescent brightening agents can be identified by direct comparison with known standard samples. Identification of fluorescent brightening agents extracted from cellulosic fibres is more difficult because the pattern of bands produced is usually very different from that given by the original solid fluorescent brightening agent. Comparison can be made only with extracts of known dyeing, which adds considerably to the amount of work required to achieve an identification'. He concluded that 'the difficulties associated with the identification of extracts have not so far been overcome'.
- Schweppe also mentioned the following difficulties: ' In the TLC of optical brightening agents, difficulties arise that are not encountered in the chromatography of dyes. For instance, brightening agents of the stilbene and oxazol type are normally present in the trans form, but they undergo a partial conversion to the cis form on exposure to UV light. Since this conversion also take place in sunlight, such substances are chromatographed in the dark, or the spots of brightening agent applied to the plate are first irradiated with UV light to enable the cis-trans equilibrium to be established before an attempt is made to separate the substances. When the later mode is used, two principal spots are produced on the chromatogram. Another difficulty in the TLC of optical brightening agents arises from the multiplicity of secondary components, which contain mainly stilbene derivatives, which can vary considerably in amount. This may well make identification by TLC somewhat problematic'.

1.8.2 Terminology

Optical brighteners

Compounds that on addition to a white or coloured textile material increases its brightness by converting some of the UV radiation into visible light.

UV absorber

A type of ultraviolet stabiliser, soluble in the polymer it is protecting, which is a powerful absorber of ultraviolet radiation in the wavelength range which causes photo-degradation of the polymer. They are capable of dissipating the ultraviolet energy harmlessly as heat by internal conversion.

1.8.3 References

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1.9 Microchemical Analysis of Dyes

Microchemical analysis is a technique that can be considered for single fibre dye comparison and providing information about the chemical class of dye present on the fibre. Microchemical analysis of dye consists of qualitative reactions performed using specific reagents i.e. concentrated inorganic acids (sulphuric, nitric, hydrochloric) and reduction – oxidation reactions. Microchemical analysis procedures can be carried out on single fibres or fibre fragments including pale coloured fibres. This can be particularly useful if a laboratory does not have a microspectrophotometer. These reactions could be particularly important where fibres are too small for TLC. Microchemical analysis and interpretation requires both technical knowledge and experience to ensure that the end result is reliable. The technique is still used successfully in laboratories in Lithuania, Estonia, Latvia, Ukraine, Belorussia and Russia.

1.9.1 Qualitative reactions

Initially the known and recovered fibres are compared by monitoring fibre colour changes in specific concentrated inorganic acids i.e. sulphuric, nitric and hydrochloric. However, these reactions usually destroy the fibre. Only a short fragment of fibre, approximately 0.2mm, is required for each reaction. Both known and recovered fibres are mounted on standard glass microscope slides, side by side, and a drop of concentrated acid (sulphuric, nitric and hydrochloric) is added directly to the fibre using a micropipette. The colour changes, at room temperature, are observed over a five-minute period after the reagent is added using a stereomicroscope (x 20 -x 60). After using the sulphuric acid a drop of distilled water is added and any changes noted (has the original colour been restored or not).

Qualitative reactions using inorganic acids can be carried out on both man made and natural fibres. Additional reactions using concentrated formic acid and 10% sodium hydroxide can also

be performed on polyamide, acetate and polyester fibres. In the case of acetate fibres, dyed with disperse dyes, additional reactions using concentrated acetic acid and 10% sodium hydroxide in ethanol can be useful.

1.9.2 Reduction – oxidation reactions

Reduction – oxidation reactions can be carried out directly on single wool, cotton, bast and viscose fibres without any preliminary preparation. The known fibre (or few fibres) is mounted on the slide and the following procedures are carried out:

- 1-2 drops of 10% sodium hydroxide (5% NaOH for wool) is added;
- 1-2 drops of 10% sodium dithionite is added;
- This preparation is heated for 1-2 minutes to 70°-90°C and any colour changes are immediately noted.
- Fibres are removed from this preparation and placed in a drop of distilled water, finally they are removed from the water (air oxidation).
- A drop of 10% ammonium peroxodisulfate is added.

The colour changes at each stage are observed using a stereomicroscope (x20 – x60). The same procedures, as set out above, can be carried out on the recovered fibre(s). The colour changes for the control and recovered fibre(s) are compared. The reduction-oxidation reactions are often irreversible for natural fibres.

Viscose fibres are often dyed with the same types of dye as cotton fibres e.g. azoic, direct, sulphur and vat dyes are often encountered although this trend may be geographical.

If anthraquinone dyes have been used to dye the fibres then the dye class i.e. vat or sulphur can be determined using the following schemes. Fibre(s) are mounted on a microscope slide and 1-2 drops of each of 10% sodium carbonate and 10% sodium sulphide added. The preparation is heated to 70° – 90°C. Colour changes, if any, are noted with the help of a stereomicroscope. If there is a colour change then a sulphur dye is present if not a vat dye is present.

Producer dyed regenerated cellulose fibres i.e. viscose, cupro; modal do not show any colour changes in oxidation-reduction reactions. Surface pigmented regenerated cellulose fibres show the same colour changes in these reactions.

Acrylic fibres are mainly dyed with cationic (basic) dyes; the colour changes of these dyes are detailed below.

Unlike natural fibres, reduction-oxidation reactions for synthetic fibres require the fibre to be dissolved first in a specific solvent appropriate to the fibre type.

Synthetic fibres are placed on a piece of filter paper on a slide. A drop of solvent is added and the preparation heated until the solvent evaporates. This produces a spot of dye, which can be subjected to reduction-oxidation tests.

Extraction solvents for reduction-oxidation of dyes in the main synthetic fibre types are listed below:

| Fibre Type | Solvent |
|-------------------|---|
| Acrylic | Dimethyl formamide |
| Polyester | 80% phenol (preferably) or Phenol – chlorobenzene (1:1) Phenol – chloroform (3:2) Phenol – benzene (1:1) |
| Polyamide | 85% formic acid |
| Polyvinylchloride | Cyclohexanone |

The results obtained from reduction – oxidation reactions provide useful information about the chemical class of the dye present:

Reduction – oxidation reactions of dyes on natural fibres (wool, cotton, bast etc.)

| Chem. Composition of the dye | Fibre Colour Change | | | |
|------------------------------|--|-----------------------|-----------------|-----------------------------|
| | Reduction | | Oxidation | |
| | 10% sodium hydroxide +10% sodium dithionite | | air oxidation | 10% ammonium peroxdisulfate |
| | room temperature | heating (70° – 90°C) | orig. colour is | |
| Soluble Azo | colourless | colourless | not restored | not restored |
| Triaryl methane | colourless | colourless | not restored | restored |
| Nitro | colourless | colourless | not restored | not restored |
| Nitroso | colourless | colourless | not restored | not restored |
| Azine | yellow | colourless | restored | restored |
| Oxazine | colourless | colourless | restored | restored or becomes brown |
| Thiazin | colourless | colourless | restored | not restored or changed |
| Antraquinone | shade is changed | shade is changed | restored | restored |
| Soluble Vat | yellow | yellow | restored | restored |
| Sulphuric | yellow | yellow | restored | restored |
| Vat anthraquinone | colour is changed | dark colour | restored | restored |
| Insoluble Azo | colour is not changed | colourless | not restored | not restored |
| Phthalocyanines | colour is not changed | colour is not changed | changed | changed |

Changes of cationic dye colour under reduction – oxidation reactions

| Chemical dye class | Fibre Colour Change | | | |
|--------------------|--|----------------------|--------------------------------|--------------------------------|
| | Reduction | | Oxidation | |
| | 10% sodium hydroxide +10% sodium dithionite | | air oxidation | 10% ammonium peroxdisulfate |
| | room temperature | heating (70° – 90°C) | | |
| Azocarbocyanine | no change | no change | – | – |
| Diazocarbocyanine | no change | no change | – | – |
| Hemicyanine | orig. colour faded | orig. colour faded | orig. colour is part. restored | orig. colour is part. restored |
| Naphthostyrene | colourless | colourless | orig. colour is not restored | orig. colour is part. restored |
| Oxazine | colourless | colourless | orig. colour is restored | - |

| | | | | |
|--------------------|----------------------------------|--------------------------------|--------------------------------|---------------------------------|
| Anthraquinone | light orig. colour | faded orig. colour with yellow | orig. colour is part. restored | orig. colour with another shade |
| Polycyclic ketones | orig. colour changes | orig. colour changes | orig. colour | – |
| Triarylmethane | orig. colour faded or colourless | colourless | orig. colour is not restored | orig. colour is restored |
| Azo | orig. colour faded or colourless | colourless | orig. colour is not restored | orig. colour is not restored |

Polyester, polyamide and acrylic fibres can be dyed with disperse dyes. It is known that azo, anthraquinone and triaryl methane dyes are common among disperse dyes. The results of reductionoxidation indicate that differences due to chemical structure of the disperse dye could be detected and are the same as shown earlier for natural fibres.

The colour of producer dyed synthetic fibres, including those with carbon or other pigments will remain unchanged when tested either with inorganic acids or reduction – oxidation reactions. When dye mixtures are subjected to reduction-oxidation reactions they react as though each part of the mixture is being tested separately. The chemical composition of the dye cannot always be determined particularly when dye mixtures are involved. However, colour changes that result from specific tests can give useful information, which may allow fibres to be differentiated. When fibres are being compared identical colour changes could mean that these fibres are dyed with the same dye or dyes. If any differences are noted in the microchemical reactions, the compared fibres could not have been dyed with the same dyes.

1.9.3 Terminology

Microchemical analysis of dye

The testing of colorants on fibres by various reagents. The reagents should be chosen to produce a variety of chemical reactions on the dyestuffs or pigments.

Qualitative reactions

Specific reactions of colorants on fibres using concentrated inorganic acids.

Reduction – oxidation reactions

Reactions of colorants on fibres using a specific reducing agent (sodium dithionite) and oxidising agent (ammonium peroxodisulphate).

Producer dyed fibres

Fibres that are coloured when dye is added to the polymer prior to extrusion.

1.9.4 References

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2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF FIBRE DYES

2.1 Introduction

Thin layer chromatography (TLC) is common technique for dye analysis in forensic sciences, as it is fast and cheap. However this method has some disadvantages as a large amount of sample is required for pale colours (e.g. yellow), the quantification is not accurate, and due to the variation of retention factor between TLC runs there is difficulty in making a spectral database of dyes. An alternative for TLC is high performance liquid chromatography (HPLC), a fast and sensitive technique which can be applied in this field.

There are many dye classes used to colour fibres. It is difficult to separate them on a single chromatographic system. All of the proposed protocols should be applicable for single fibres.

2.2 Theory

HPLC systems have been developed to analyse a small number of dyes, a dye mixture and its components, a particular dye class, and a combination of dye classes.

Forensic analysis of fibre dyes using HPLC may be considered for single fibre comparisons. If this technique is used for simultaneous analysis of several fibres (i.e. yarn or a piece of fabric) then only comparison between similar samples can be made.

Important information about the dye composition can be obtained by the use of TLC extraction protocols i.e. classification of the dye type. This should be determined in order to select the appropriate chromatographic conditions. In most cases the identification of the dye will not be possible.

If enough material is available, the known sample should first be analysed in bulk in order to best establish the correct protocol for dye extraction and the chromatographic conditions, before proceeding to single fibre analysis.

Although HPLC has a better reproducibility than TLC, the use of standard dyes is of great importance to assess the performance of the system, especially if the chromatographic column is not thermostated.

2.3 Methodology

Methods have been developed for the HPLC, UPLC, Ion-pair HPLC analysis of fibre dyes. Most forensic science laboratories have HPLC units available, but very rarely are these dedicated to the forensic analysis of dyed fibres.

2.3.1

The sample handling for dye extraction in fibres is the same as in the TLC Guidelines. If any post-treatment of the extracted dye is necessary, this must be clearly described in the specific procedure.

2.3.2

The minimum length of the fibre required is approximately 3 mm, and the volume of the extract is 1 – 20µL.

2.3.3

To compare dye extracts from known and recovered fibres accurately it is essential that extraction procedures provide reproducible results and are identical for the known and recovered fibres.

2.3.4

The following protocols for the extraction of dyes from fibres were recommended among others by Speers et al (1994)

- Basic dyes: use of 5 µl Formic acid-water (1:1) at 100°C for 20 min. Diluted to 10 µl with acetonitrile – water 1:1 (i.e the HPLC eluent) containing internal standard 0,14 ng/µl.
- Disperse dyes: Extract with 5 µl of Chlorobenzene at 130°C for 30 min. Remove the fibre, evaporate the solvent and dilute with acetonitrile–water containing 0,2 ng/µl of internal standard.
- C: Acid dyes: Extract with 5 µl of Pyridine/water (4:3) v/v, at 90 °C for 20 min. Evaporate the solvent and dilute with 10 µl of acetonitrile-water containing 0,2 ng/µl of internal standard.

2.3.5

When a dye cannot be extracted using the TLC extraction methods it may be a reactive dye. In this case, an alkaline hydrolysis or enzymatic extraction should be performed, because these methods allow to break a covalent bond between reactive dye and fibre.

2.3.6

With use of a Diode Array Detector (DAD) it is possible to obtain a (UV)-visible spectrum of every detected dye component and it can simultaneously show the corresponding chromatograms from different wavelengths in one run. This means that it is possible to detect all components of the dye extract and ensures that in extracts containing many coloured dye components dyes do not go undetected because of incorrect choice of detector wavelength. A tuneable UV-visible detector can also be used. However to obtain minimal reliability with a tuneable wavelength UV-Vis detector it is necessary to carry out three chromatographic runs at three different wavelengths to detect all dye components. This requires a greater amount of sample than that usually available in the fibre extracts.

2.3.7

The choice of packing material and mode of separation for any particular type of dye can be decided upon after tests involving chromatographic efficiency, resolution of dye components and time of analysis.

2.3.8

The internal standards can be used to calculate relative retention times, RRT, for the dye components, e.g. Rhodamine Bm CI 45170 Aldrich Chemicals (a basic dye which is not known to be used in commercial fibre dyes).

2.3.9

A searchable database of the dyes can be developed using the relative retention time and UV visible spectra of known dyes.

2.3.10

Documentation should include the data obtained through the analytical process. The following instrumental variables (parameters) must be recorded in the laboratory and be accessible for later reference, or included in the case file:

- specific HPLC utilised
- type of HPLC column including:
 - length
 - internal

- diameter
- particle
- size
- stationary
- phase
- mobile phase
- flow and gradient (if applicable) of mobile phase
- temperature
- wavelength (or wavelength range if Diode Array) of data acquisition
- chart speed (if applicable)
- injection volume

2.4 Interpretation

The number and proportion of the dye components present in the extract, the retention time R_t or RRT, and the UV-visible spectrum of each dye component should be compared for the known and corresponding recovered fibres.

If a dye database is used the comparison between the visible spectrum and retention time for every peak should be documented.

2.5 Terminology

Chromatography

A method of analysis in which substances are separated by their differential migration in a mobile phase flowing through a porous, absorptive medium.

Chromatogram

The visual display of the progress of a separation achieved by chromatography. A chromatogram shows the response of a chromatographic detector as a function of time.

Column

The essential part of Chromatography is a Stainless Steel Tube containing the stationary phase and through which the mobile phase flows.

Diode Array Detector

A detector positioned after the column that is capable of making an UV-Visible spectrum in a very short time (more than one spectrum per second). All parts of the spectrum are scanned simultaneously.

Liquid-Liquid Chromatography

A separation process in which the stationary phase is a liquid and where the separation is based on selective partitioning between the mobile and stationary liquid phases.

Liquid-Solid Chromatography

A separation process in which the stationary phase is a solid and where the separation is based on selective partitioning between the mobile and stationary solid phases.

Mobile phase

The liquid used to sweep or elute the sample components along the planar surface or through the column. It may consist of a single component or of a mixture of components. The term eluent is often used for the preferred Mobile Phase.

Multi-solvent Delivery Pump

A pump that is capable of running with two or more solvents at the same time.

Stationary phase

The active immobile material on the planar surface or within the column that retards the passage of sample components by one of a number of processes or their combination. There are three types of stationary phase: Liquid Phases, Interactive Solids and Bonded Phases. Inert materials that merely provide physical support for the stationary phases are not part of the stationary phase.

2.6 References

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3 PYROLYSIS-GAS CHROMATOGRAPHY- (MASS SPECTROMETRY) AND PYROLYSIS-MASS SPECTROMETRY OF TEXTILE FIBRES

3.1 Introduction

This guide is intended to assist individuals and laboratories that conduct pyrolysis gas chromatography with or without mass spectrometry (PyGC or PyGCMS) or pyrolysis/mass spectrometry (PyMS) in their selection, application and evaluation of PyGC(MS) or PyMS as a method for forensic fibre case work.

The guideline is concerned with the pyrolysis of single fibres and fibres from bulk material, classification of the generic class of polymer, and interpretation of the resulting pyrograms and mass spectra. The protocols and equipment mentioned in this document are not meant to be totally inclusive or exclusive.

3.2 Theory

Pyrolysis is a destructive analytical method. Therefore, consideration must be given to the applicability of this procedure to each case depending on the sample size and the amount of sample consumption that can be tolerated [2]. Pyrolysis of polymers is the breaking apart of larger polymer chains into smaller fragments by the application of heat in an inert atmosphere [2]. When the heat energy applied to the polymer chains is greater than the energy of specific bonds in that polymer chain, these bonds will fragment in a predictable, reproducible way at a specific temperature. In PyGC, the fragments generated by pyrolysis are introduced into a Gas Chromatograph (GC) for separation and characterisation. PyGC can be used to identify the generic type of an unknown fibre and in some cases may identify subclasses within a generic class [1]. In PyGCMS after separation by GC the fragments generated by pyrolysis are introduced into a mass spectrometer for characterisation. PyGCMS can be used to identify subclasses within a generic class [2]. In PyMS the fragments generated by pyrolysis are directly introduced into the ionisation source of the MS. PyMS can be used to identify the generic type of an unknown fibre and in many cases may identify subclasses within a generic class.

It is recommended that each laboratory without a MS detector develops its own standard chromatograms of the different generic fibres if performing fibre identification analyses. These chromatograms demonstrate the analytical potential as well as the limitations of PyGC performed on fibres by a particular system. Using a MS detector commercially available mass spectrum libraries can be used to identify the pyrolysis fragments. The mass spectral fragmentation pattern of the pyrolysates can be likened to a 'fingerprint' which characterises the chemical composition of the specimen [7]. PyMS is suitable for the production of standardised reference spectra. By this inter-laboratory exchange of data and the development of 'fingerprint' libraries pertaining to fibres are possible [7].

The potential of pyrolysis gas chromatography of fibres include:

- Comparative analyses of two or more fibres,
- Identification analyses of known and/or recovered fibres. With regard to some fibre types, such as acrylics, PyGC can be used in conjunction with infrared spectroscopy to provide differentiation within generic classes [3] and

- The technique is extremely sensitive and can be used to analyse a wide variety of materials [5].

These points hold also for pyrolysis mass spectrometry; moreover this technique is very fast and is suitable for developing spectral 'fingerprint' libraries [7].

As with any instrument, PyGC has limitations to its application, the two most important of which are the number of parameters and the control of these parameters. First, PyGC encompasses a great variety of parameters. Fluctuation in any one of these parameters will produce pyrolysis product changes. These parameters may be related to the sample and its preparation, and may include: sample homogeneity, sample size, sample shape, sample placement within and contact with the quartz tube, and sample weight. Other variables related to the pyrolysis instrument include the temperature of the pyrolysis, the rate of temperature rise, the duration of the pyrolysis and the pyrolysis chamber atmosphere. Second, the variables must be controlled to ensure reproducible results. For more complex samples the reproducibility of replicate sample pyrograms becomes more involved. Therefore, users should establish their systems' capability to discriminate various copolymer ratios.

With PyMS reproducibility problems can occur with certain polymer types and interpretation difficulties are experienced with interpretation of the composition of the polymer and additives [2].

3.3 Methodology

The user is required to maintain authenticated, traceable reference standards of fibres for comparison and/or identification analyses. These known standards can include fibres obtained from testing services and/or manufacturers. Control samples should be routinely run as established by laboratory procedures.

3.3.1

Proper sample preparation and technique are prerequisites for obtaining reproducible results. Fibres being compared should be analysed using the same parameters and approximately the same sample size and shape [4, 8].

Samples may be prepared using low power magnification and cleaned tools must be used to handle the sample(s) and quartz tube [9].

3.3.2

A pyrolysis instrument must be able to heat a sample to a preset temperature at a known rate for a specific time. These conditions must be accurately reproducible [6]. The gas chromatograph used in fibre pyrolysis should:

- have a reproducible temperature profile and stable carrier gas flow rate,
- have a capillary column capable of distinguishing different fibre types and
- have the capability to reproducibly separate and identify pyrolysis products.

After establishing standard methods and protocols, standard pyrograms should be run to check the temperature setting and resulting pyrogram pattern. A polymer material such as low density polyethylene or polypropylene may be used for routine performance checks. The frequency of routine instrument performance checks should be established by each laboratory.

The instrument performance sample should be an easily obtainable material which yields reproducible chromatograms having peaks over the entire range of the pyrogram with major peaks near the start, in the middle and near the end.

The pyrolysis unit must be checked in conjunction with the gas chromatograph at routine intervals as established by each laboratory. The pyrolyser should be checked after the gas chromatograph has been checked.

An instrument performance sample should be introduced into the GC during routine performance checks as established by each laboratory. New instrument performance sample chromatograms must be compared with previous ones in order to establish relative sensitivity,

resolving power and baseline profiles. This ensures that the case samples and the reference library are still comparable. In some cases instrument performance will change sufficiently to require new reference standards to be generated. These instrument performance sample chromatograms should be kept in the instrument logbook for a predetermined length of time as established by laboratory protocol.

3.3.3

The following series of procedures must be followed for an analysis:

- Run an instrument performance sample according to laboratory procedures and ensure instrument is operating properly.
- Column head pressure and split flow rates should be adjusted in accordance with established procedures.
- Controls and blanks must be run as established by individual laboratory procedures. Allowable maximum peak to noise ratio in blank samples should be defined in laboratory procedures.

3.3.4

Run known and recovered samples under the same conditions and compare their chromatograms. Known samples should be run in duplicate to assess variations in the pyrograms and to ensure reproducibility and if sufficient recovered sample permits, this too should be subjected to duplicate analysis.

3.3.5

Run the known sample and recovered sample and compare their chromatograms to the reference library. The library chromatograms should originate from the same instrument and protocol used in the current analysis. All identifications must be confirmed by running an authenticated fibre reference standard at the time of analysis.

3.3.6

The following procedures should be performed as established by laboratory protocol:

- Check to ensure even spacing along the platinum coil on a pyroprobe coil unit. This coil should be visually inspected before each use.
- The pyrolysis quartz tube must be heat cleaned after each use. If the quartz tube is reused, each laboratory should develop, document and use a cleaning procedure that can be demonstrated to be noncontaminating to subsequent runs.
- Check gas cylinders and change when the pressure drops to a predetermined level. If gas line moisture traps, oxygen scrubbers, etc. are being utilised, these should be changed when tanks are changed or as necessary to maintain system performance.

3.3.7

Scheduled routine maintenance procedures must be performed per individual laboratory. Record performed maintenance in an instrument logbook. This must include cleaning the appropriate parts of the detector, reassembling the detector, changing GC septa and pyroprobe O-ring seals, cleaning injection port, checking glass liners during routine maintenance, other cleaning as needed, and performing any additional scheduled routine maintenance.

Instrument performance must be checked whenever a new column is installed or whenever repairs are done to the pyrolyser.

3.4 Documentation

Documentation must include data obtained through the analytical process. The following instrumental variables (parameters) must be recorded in the laboratory and be accessible for later reference, or included in the case file:

- specific GC utilised
- type of GC column including:
 - length
 - diameter
 - coating
 - coating thickness
 - type of carrier gas and detector
 - flow rates
 - split flow (if applicable)
- oven temperature program including:
 - initial temperature ramp rates
 - final temperature- temperature holding duration
 - injector and detector temperatures
 - specific pyrolyser unit utilised
- pyrolysis temperature including:
 - interface temperature
 - ramp rates
 - final temperature
 - temperature holding duration (interval)
- (if available) type of MS detector including:
- type of ionisation source
 - other parameters including scan parameters (e.g. low mass, high mass, threshold, sampling rate), ionisation mode (e.g. electron impact or chemical ionisation).

The data generated by PyGC is dependent upon various factors such as sample size, condition, and handling. Likewise, interpretation of PyGC(MS) or PyMS data is dependent upon the training and experience of the examiner. Awareness of the strengths and limitations of the technique must be considered. In addition, the examiner must assess the variability of the instrument and variations within the pyrograms. Therefore the examiner must complete a formalised training program which must include proficiency tests to establish competency in both the analysis and interpretation aspects of this technique, prior to undertaking casework.

3.5 Terminology

Gas Chromatography

A method of analysis in which substances are separated because of the differences in the affinity to the stationary phase.

Gas Chromatogram or Pyrogram

The visual display of the progress of a separation achieved by chromatography. A chromatogram shows the response of a chromatographic detector as a function of time.

Capillary Column

Long, narrow bore capillary tube (column), the inside walls of which are coated in a thin film of stationary phase.

Mobile phase

In gas chromatography, the mobile phase is the inert carrier gas that moves the volatile analytes through the length of the column.

Stationary phase

In a capillary (Wall Coated Open Tubular WCOT) column, the stationary phase is generally a modified or unmodified polysiloxane compound coating the walls of a fused silica column. Compounds are selectively retained based on their interaction with the coating's functional groups.

Mass Spectrometry

A method of analysis in which the molecules of substances are broken into fragment ions of different mass by ionisation. Analysing the distribution of molecule fragments sorted by mass results in a mass spectrum and definitive identification of the original molecular compound.

3.6 References

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APPENDIX 6 – OTHER ANALYTICAL TECHNIQUES

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1 INTRODUCTION

There are other analytical methods, which can be applied to the fibre examinations. A number of these techniques are currently being developed and may prove to be useful in examination and comparison of single fibres. This document is intended to assist individuals and laboratories that conduct forensic fibre examinations and comparisons in the effective application of the methods listed above to the analysis of fibre evidence.

Some of the procedures included here (fibre density determination and solubility tests) involve the use of hazardous chemicals. This guideline does not address the possible safety hazards or precautions associated with their application. It is the responsibility of the user of these documents to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to use.

In Section 4, brief details are given of numerous other less well known methods which are sometimes mentioned in connection with fibre examination. They are available but are not normally used due to problems associated with sample size or with interpretation of the results. The disadvantages are listed under each description. They are methods using costly research grade equipment, but may occasionally be useful for specific purposes.

2 FIBRE DENSITY

2.1 Introduction

The determination of fibre density can be used to distinguish between fibres of various generic types. If measured more accurately, it may allow differentiation between similar fibres of the same generic type. However, it is not usual to measure fibre densities in routine casework as information of equal value may be obtained more quickly by alternative methods. The determination of fibre density can be of interest to laboratories with limited instrumental equipment.

2.2 Theory

Fibre density is related to the degree of crystallinity of fibres. Crystallinity may be affected by manufacture processing and consumer uses such as laundering and outdoor exposure. As a result, accurate density measurements may offer the possibility for differentiation between two otherwise identical fibres of the same generic class. The determination of fibre density is particularly interesting for man-made fibres. For example, it allows a rapid discrimination between olefin and polyamide fibres, which sometimes appear similar under polarised light [1]. A Table of fibre densities is compiled in [1].

2.3 Methodology

Densities may be measured by immersion techniques either using a single liquid or a series of single liquids of known density (sink-float method), or by setting up of a density gradient column.

2.3.1

The Sink Float method is suitable for rapid screening. Although relative, this method allows the estimation of the density of the tested fibre.

1. A series of liquids of known densities are prepared in small test tubes, the following liquids have been suggested [2]:

| Liquid | Density g/cm ³ |
|-----------------------------------|---------------------------|
| Distilled water | 1.00 |
| Hexane/Carbon Tetrachloride 43:57 | 1.20 |

| | |
|-----------------------------------|------|
| Hexane/Carbon Tetrachloride 32:68 | 1.28 |
| Hexane/Carbon Tetrachloride 27:73 | 1.35 |
| Chloroform | 1.45 |
| Carbon Tetrachloride | 1.60 |

2. Known and recovered fibres to be tested should be unambiguously described and recorded in such a way that they can be recovered and submitted to subsequent testing.
3. A fraction of the known fibre is immersed in one of the tubes. Once the equilibrium is reached (allow up to 30 min), the fibre will either float or sink, unless it has the same density as the liquid.
4. A fraction of the recovered fibre is immersed in the same tube, and its behaviour is compared to that of the known fibre.
5. The test can be repeated in each of the liquids.
6. The known and recovered fibres can be discriminated if they show a different behaviour (i.e. one fibre sinks and the other floats).
7. If both the known and the recovered fibres behave the same way, they have a similar density and cannot be differentiated on this basis. Further testing is therefore required.

2.3.2

Another method uses a density gradient column containing calibrated glass density floats. The positions or levels of the floats in the column are measured and a calibration chart plotted of float positions (in cm) against float densities (in g/cm³). This calibration chart should give a linear curve that can be used to determine the unknown density of a specimen from its position in the column.

Although this method can be used, it has many disadvantages, and is very rarely applied in forensic casework.

2.3.3

Olefin fibres can be recognised because they will float on water. Polyethylene and polypropylene can be separated by immersion in isophorone (SG 0.92g/cm³); polyethylene will sink; polypropylene floats [3].

2.3.4

The appropriate Health and Safety precautions must be observed when using volatile liquids that can be harmful to health.

2.4 Terminology

Density

The mass of a substance per unit of volume

Relative density (r.d.) – The ratio of the density of a substance to the density of a reference substance. For liquids or solids it is the ratio of the density (usually at 20°C) to the density of water (at its maximum density). This quantity was formerly called specific gravity

Specific gravity – see relative density

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3 SOLUBILITY TESTS

3.1 Introduction

In the early days of forensic fibre identification solubility tests were the most powerful tool for the identification of tiny man-made fibre fragments. Even though it is a destructive technique these tests are still of interest, especially for laboratories with limited instrumental equipment.

3.2 Theory

The chemical properties of man-made fibres are important aids to identification of the fibre forming polymers. The procedures suggested in the literature [1-6] show several schemes of fibre identification. For the most commonly encountered manmade fibres the most useful solubility test still in common use today, is that for distinguishing between cellulose acetate and triacetate fibres [3].

3.3 Methodology

Following the procedures of solubility tests, appropriate controls of the solvents and reagents should be carried out by testing authenticated fibres.

It is recommended the test is run under the microscope. A fibre fragment is placed on a slide with a cover-slip. The solvent reaches the fragment by capillarity action under the cover-slip. Identification of known fibre should be the first step.

While comparing the possible reactions of the known and the recovered fibre both fibres must be viewed simultaneously. Possible reactions are: partial and complete solubility, swelling, shrinking, gelling and colour change.

3.4 Terminology

Man-made fibres

A class name for various families of fibres produced from fibre-forming substances which may be synthesised polymers or modified / transformed natural polymers (e.g. nylon, modal).

Known fibre

A subset of a larger population or sample originating from a verifiable source, collected as representative of that larger grouping. For example a section of the suspect's T-shirt.

Recovered fibre

Fibres collected as or from items of evidence which have a known location but an unknown originating source. For example, loose fibres collected from a victims clothing.

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4 OTHER METHODS NOT ROUTINELY USED IN FORENSIC FIBRE ANALYSIS

4.1 Thermal methods

Thermogravimetry (TG) or Thermogravimetric Analysis (TGA) [1-3] registers the mass changes of a specimen in response to heating/cooling. The resulting curve gives information concerning the thermal stability and composition of the sample.

Derivative Thermogravimetry (DTG) [1-3] monitors the first derivative of weight loss with respect to temperature dM/dT . It uses thermoscales with a built-in automatic differentiator.

Thermogravimetry-Mass Spectrometry (TG-MS) [4] A combination of TG with a detector (MS) allowing quantitative monitoring of any gases evolved.

Differential Thermal Analysis (DTA) [3] uses a thermocouple to measure the temperature between an inert reference material which is thermostable, and a sample which is to be identified. It provides data on physical and chemical transitions in matter which involve absorption or evolution of heat e.g. glass transition, crystallization, decomposition.

Differential Scanning Calorimetry (DSC) [2, 5] provides a measurement of the rate, magnitude and temperature at which chemical or physical changes occur in a substance during heating or cooling. The difference in electrical power required to maintain the reference and specimen pans at a linearly increasing or decreasing temperature is recorded as a function of temperature and is directly proportional to the energy involved in the thermal transitions.

Thermomechanical Analysis (TMA) [6] measures changes in the contraction or extension of a fibre quantitatively as a function of temperature.

Disadvantages:

- These methods are destructive.
- They require relatively large amounts of sample (0.1 – 100mg). With the exception of TMA there is no literature describing their use on single fibres. (With TMA, there is a length limitation – quoted as varying from 4-13mm).
- The qualitative capacity of thermal analyses is less than that of infrared microspectroscopy or pyrolysis techniques.

4.2 Methods for elemental determination

Neutron Activation Analysis was used on hairs during the 1960's. There is no documentation of it having been applied to, or having the necessary sensitivity for single fibre analysis. The technique involves the use of radioactive materials [7].

4.2.1 Emission Spectroscopy

No documentation in forensic literature. Not sensitive enough for single fibre analysis. The general problems of interpretation apply.

4.2.2 Atomic Absorption spectroscopy

As above

4.2.3 Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) [8]

Fibres are dissolved in conc. sulphuric acid, and passed through a 1 micron filter to form plasma "the technique is totally destructive and the quantity of fibre required (mg level) is unrealistic from a forensic point of view".

Inductively Coupled Plasma – Mass Spectroscopy (ICP-MS)

Similar to above, but coupled to an MS instrument. The usual interpretational difficulties apply.

4.2.4 Laser Microprobe Mass Analysis (LAMMA) [9-10]

A focussed laser beam is used to vaporize and partially ionise the sample. The ions are separated according to their mass to produce a spectrum, showing positive and negative ions, molecules and elements. Although the method is very sensitive (ppm range) the problem comes in interpretation of the results for the reasons stated under general disadvantages.

4.2.5 (Micro) X-ray Fluorescence (XRF) [11-12]

A rapid, non-destructive and sensitive method for elemental determinations in fibres a few millimetres long (carpet fibres).

Not quantitative, may be useful to identify manufacturers, by searching for specific elements.

4.2.6 Total Reflection X-ray Fluorescence (TRXF) [13]

Can be applied non-destructively to very small fragments of single fibre. Non-quantitative. Problem of removing any surface contamination from fibres. As yet, only tested on colourless fibres.

General disadvantage:

All of these methods suffer from the problem of interpreting the results, because detailed information concerning exposure of known material and recovered fibres (before & after the offence) is not available. Control and recovered fibres would have had to have been subjected to identical conditions in order for the results to have any meaning. It is not possible to cover the whole range of variation that may be expected in a known garment due to localised conditions/ staining, washing etc. There may even be a natural range of elemental variation in cotton or other natural fibres. The instrumentation required for many of these methods is very expensive.

4.3 Miscellaneous techniques

4.3.1 X-ray diffraction [14-15]

X-ray beam passed through divergence slit falls on powdered fibre sample. Radial scan shows characteristic diffraction pattern based on crystalline structure. Peak width depends on the size

of the crystallites in the sample. Measurement of the molecular orientation in the crystalline regions of the fibre can be accomplished. Each fibre type will give a characteristic peak depending on its crystal structure.

- Requires a bundle of fibres; cannot give information that is not more easily obtainable by FTIR microscopy or dispersion staining.

4.3.2 Small Angle Light Scattering (SALS) [16-19]

The fibre is placed in a holder and is rotated at various angles to a monochromatic light from a low power laser which has been passed through a polarising filter. A photographic image is made of the scattering effect.

- No commercial equipment available, complicated procedure, requiring darkened room and no fibre movement
- Difficulty in interpreting results with respect to fibre history and treatment (see [17]) – Acrylic discrimination possible using FTIR-microscopy

Fourier Transform Infrared Photoacoustic spectroscopy (PAS) [20]

Allows study of spectra in the near IR-region.

- Not applicable to single fibres, requires approx 5mg (powdered) fibre!
- Complex equipment
- Cannot provide information unavailable by other conventional techniques, except perhaps in special applications to cellulose fibres (ageing/sizing of cotton)

4.3.3 Atomic Force Microscopy (AFM) [21-22]

Probes a surface by sensing force or its gradient between the surface and a tip. The ability of AFM to provide high resolution topical images is now well developed and has been applied to textile fibres. Differences in longitudinal texture on wool fibres can potentially be used to differentiate between wool and other speciality animal hairs from different species.

4.3.4 Confocal Laser Scanning Microscopy (CLSM) [23]

This method offers the possibility of obtaining three dimensional pictures of fibres which can be useful in determining their cross sectional shape without the necessity to cut a cross section. It could also be useful for visualising surface morphology. The fibre must reflect light or must exhibit fluorescence, or both. The value of the technique is still being researched.

4.3.5 Capillary Zone Electrophoresis (CZE) [23-27]

One of a number of related methods that have been tried for separating dye components. Despite its exceptional separating power, there are numerous problems associated with its use for single fibre dye analysis in forensic work, not only because of the poor detection limits, but because of inherent difficulties with the equipment in its present stage of development. Organic solvents are required for dye extraction which are incompatible with the aqueous-based buffer systems used in CZE.

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