

GUIDELINE FOR THE DETERMINATION OF REFRACTIVE INDICES OF GLASS FRAGMENTS

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GENERAL REMARK

This guideline assumes prior knowledge in the forensic discipline. It is based on consensus among the relevant forensic experts and reflects the accepted practices at the time of writing. The requirements of the judicial systems are addressed in general terms only.

Beside this guideline also the standard method ASTM E1967-19 should be referred to.

1. AIMS

This guideline describes the use of and the determination of refractive indices of glass fragments, using the oil immersion method and a phase contrast microscope, within the field of forensic examination of glass.

The guideline is aimed towards experts in the field of forensic examination of glass.

2. SCOPE

This guideline provides recommendations of sample preparation, technical methods, calibration routines and the determination of refractive index of glass samples. The guideline also outlines aspects of data analysis and its interpretation but relies on the assumption that each laboratory will have their own procedures on this topic in place; which will depend on national legal requirements.

LIMITATION: This guideline reflects the European Forensic Glass Community's recommendation at the time of writing. This guideline does not serve as a textbook in the field of comparison of glass by refractive index.

By application of the described procedure, refractive indices in the range of 1.46 – 1.56 of small glass fragments can be determined. The procedure described can be used for comparative glass analysis case work and, in some instances, to classify glass samples.

3. TERMS AND DEFINITIONS

For the purposes of this guideline, the relevant terms and definitions given in ENFSI documents, the ILAC G19 "Modules in Forensic Science Process", as in standards like ISO 9000, ISO 17020 and 17025 apply. Also, refer to the main document Best Practice Manual for the Forensic Identification and Comparison of Glass (PGT-BPM-002).

4. INTRODUCTION

Refractive index (RI) can be described as the ratio of the velocity of light in a vacuum to the velocity of light in a transparent medium.

Refractive index is frequently used in forensic laboratories to characterize and compare small, irregularly shaped glass fragments. As the refractive index of glass depends upon the raw materials used, the manufacturing process and subsequent thermal history, RI can be used to discriminate between glass objects of different origin. The method is quick, reproducible and does not change the intrinsic properties of the glass, although glass fragments may be broken to smaller parts during the examination. The particles following RI testing can still be further characterised, e.g., by laboratory annealing or elemental analysis.

The refractive index measurements described in this guideline are based upon the immersion technique whereby the RI of liquids alters far more with changing temperature than glass. Thus, if a glass fragment is immersed in a liquid, such as an oil, and illuminated with monochromatic light under a phase contrast microscope, a temperature can be found where the oil and glass have the same refractive index. At this point, i.e. the match temperature, the glass cannot or can merely be seen.

5. TECHNOLOGY

Several commercial instruments are available to perform the automated determination of refractive index of glass. This is conducted by the oil immersion method using a phase contrast microscope with monochromatic light, a hot stage and a control unit.

The refractive index of a material varies with the wavelength of the light and the temperature of the material. In forensic glass examinations, the refractive index is generally reported at 589 nm. Additional wavelengths, such as 488 nm and 656 nm, may be used to improve the discrimination capability of the method. The refractive indices are determined at their respective match temperatures and are generally not corrected to 20°C.

The system for determination of RI consists of the following units/parts:

Microscope: A microscope fitted with a phase contrast optical system. A x10 objective (with long working distance) is recommended.

Filters: An interference filter with the peak wavelength in question (589 nm, 488 nm, 656 nm etc.) and a bandwidth of 10 nm is recommended to provide monochromatic illumination. The filters must allow for sufficient transmission of the wavelength in question.

Light Source: An adjustable light source (equivalent to approx. 100 W when using a halogen light bulb) is recommended in order to have a bright video image without overloading the video camera.

Hot stage: A hot stage for the microscope slides with a minimum precision of 0.1°C in the temperature range of approx. 25°C – 120°C is required.

Digital Camera: A digital video camera connected to the microscope and to a computer is required to determine the phase contrast.

Computer: A computer (often in combination with additional control devices) is required to control the hot stage, record the video signal from the digital camera and to control and evaluate the measurements.

Immersion oils: Silicone oils covering the refractive index range between approximately 1.46 – 1.56 are required. The purpose made series A, B and C oils, attributed to Locke or similar suppliers, which have been proven to be suitable may be used. For example, the Locke B oil covers the refractive index range of approximately 1.51 to 1.53 and is therefore suitable for most glass samples encountered in forensic glass casework.

Calibration standards: Glass standards are required to calibrate the combination of instrument and immersion oil. These standards should be provided with tables showing the refractive index of the glass at intervals over a temperature range and at the chosen wavelength(s). The accuracy of the RI values should at least be guaranteed to the fourth decimal place. A series of glasses is available from the instrument manufacturer or from Schott AG.

Calibration verification standard(s): At least one additional, well-characterized and homogenous glass standard is required to monitor the quality of the measurements. It is

recommended to use the Schott/BKA K5 glass produced by Schott AG for the Bundeskriminalamt (BKA), Germany, as a calibration verification standard.

6. METHODOLOGY

A glass fragment, immersed in silicone oil on a microscope slide, is put into the hot-stage mounted on a phase contrast microscope, with a monochromator attached in the light-path. The microscope is aligned to produce an even illumination with maximum contrast. The microscope image is converted to a digital image by a video camera attached to an output port on the microscope. The temperature of the liquid is changed within the hot stage. The respective instrument software identifies the point of minimum contrast of the glass in the oil. The refractive index is calculated from the match temperature using the calibration that was previously performed on the same instrument, with the same oil using a specific set of reference glass standards.

7. SAMPLE PREPARATION

7.1 Selection of samples

7.1.1 Control (known) samples

The samples that are chosen for measurements should represent the RI variation within the control sample (within source variability). If possible, several samples from different regions of the broken glass object should be sampled. It is recommended to perform at least 12 measurements in total on at least four different fragments for most non-tempered glasses and at least 20 measurements on at least 4 fragments for tempered glasses.

In the case of glass bottles, samples from different regions should be taken (such as neck, bottom, front and back).

The original surfaces have refractive indices that can be different from the bulk material. Therefore, measurements on original surfaces should generally be avoided. The regions close to the original surfaces can also exhibit refractive indices deviating from the glass bulk in a systematic manner, especially in the case of tempered glass. Choosing fragments from the surface or the bulk of a glass sheet may be assisted by colouring the surfaces with a felt-tip pen before crushing.

It is important that the analyst is aware of surface effects and that there is a strategy to handle these.

7.1.2 Recovered (unknown) samples

When many glass fragments are recovered from a piece of clothing or another object, a suitable portion selected at random should be analysed.

The hypergeometric distribution may be used as a statistical tool to calculate the number of fragments that need to be analysed in order to determine a group of samples (with similar RI) of a certain supposed size with a given probability.

The subsample selected should be representative of the full sample, but fragments that can already be discriminated from all control samples by other properties (such as surface properties, colour and elemental composition) can be excluded from the subsample.

7.2 Sample cleaning

Prior to the analysis, glass fragments may be cleaned with water, organic solvents like ethanol or acetone, or with diluted acids, such as nitric acid. Cotton swabs, tissues, stainless steel needles and tweezers are tools that may be used to remove dirt from the surface. Cleaning of the samples can improve the quality of the refractive index measurements but includes the risk of losing the fragment. After cleaning, the fragments must be dried before measurement.

7.3 Sample preparation

In general, the glass fragment is embedded in a drop of silicone oil on a flat microscope slide. The fragment is then broken into smaller parts with a stainless-steel needle or a similar tool to create freshly broken edges. A cover slip is then placed on the drop of oil. Alternatively, a slide with a 'shallow well' may be used to accommodate glass fragments and oil; no cover slip is added.

In some instances, it may be possible to determine the refractive index without creating freshly broken edges.

7.4 Refractive index measurements

If possible, set up the microscope for Kohler illumination. Adjust the phase ring so it is aligned with the phase plate in the objective. View the image on the monitor to ensure there is sufficient light intensity in the field of view. Phase ring alignment and focus should be adjusted for each individual sample and also after any repositioning of a sample within the hot stage.

The condition of the lamp should be monitored when the microscope is aligned and adjusted at the start of a session. If the illumination is uneven or of low intensity, the bulb should be replaced.

Place the microscope slide with the sample in oil into the microscope hot stage such that the fragment is positioned in the field of view. Focus the microscope on the glass sample. The hot-stage temperature should be adjusted to a temperature approximately 2° – 3°C above the anticipated match temperature before selection of an edge(s) is made.

Select one or more glass edges that appear clean and sharp. A box shown on the monitor defines the monitored edge/area and should be positioned such that equal areas of the fragment and surrounding oil are covered. The size of the box can be adjusted in newer instruments to select a sharp, high contrast edge. Newer instrument generations also provide the opportunity to set several measurement boxes to record multiple edges. It is important that each edge must be in sharp focus to get good results.

'Poor' edges may appear very sharp when there is a significant RI difference between the fragment and oil. Check if the fragments edges are in focus and the illumination level is high.

Record a mean match temperature using the automated cooling and heating cycle at the ramping rate set by the laboratory's written procedures. Most instruments allow to adjust ramping rates, but generally 4°C/min are recommended.

The image of the fragment on the monitor should be observed throughout the measurement process to ensure there are no optical artefacts in the measurement area and the edge(s) chosen represent those of the rest of the fragments visible on the monitor.

7.5 Calibration

At least 6 reference glasses of known RI should be used for the calibration of oil B (or similar). Different oils, such as oil A in the Locke set, can be used to cover the higher RI range to

approximately 1.56. Locke oil C or a similar silicone oil can be used to cover the lower range down to approximately 1.46. There are fewer reference glasses available within these RI ranges. Three reference glasses should be used for calibration of oil A, and two for oil C.

The software calculates a linear calibration function $RI = a + b \times T$ of refractive index against temperature.

The calibration should be checked by measuring at least one reference glass with known refractive index on each measurement day.

A new calibration must be performed when essential parts of the instrument (such as the hot stage) are replaced, when new oil or a new batch of oil is used, or when the values for the calibration verification standard(s) are not meeting defined quality criteria.

7.6 Laboratory Annealing

By controlled heating of glass fragments to approximately 550°C – 600°C and cooling them down slowly, thermal stress can be removed from the glass samples, and therefore the refractive index of the glass changes in a reproducible manner.

Laboratory annealing can be applied to classify glass or to compare glass samples.

The difference between the refractive indices determined before and after the annealing procedure ($\Delta RI = RI$ after annealing – RI before annealing) provides information about the thermal history of the glass. For example, tempered glass (thermally toughened glass) has a high ΔRI , while non-tempered sheet glass has a lower ΔRI . Some optical glass may even have a negative ΔRI , but it is unusual to encounter such glass in casework.

A more special application is the comparison of glass samples that have been exposed to high temperatures in a fire, such as glass from arson cases. When glass is exposed to a fire, the refractive index may change in an unpredictable, non-reproducible and non-uniform way, so that a comparison of a pair of control and recovered samples by RI is problematic. By applying the annealing procedure to the control and recovered samples, thermal stress is removed from all samples and a meaningful comparison by RI becomes possible.

There are several annealing schemes that can roughly be divided in two groups. Long schedules typically utilize muffle ovens with a schedule of heating to 550° – 600°C and slow cooling over approximately 16 – 24 hours. Short schedules typically utilize a tube oven, and the procedure typically takes approximately 4 – 12 hours. The absolute value of ΔRI depends on the applied annealing schedule, however, the difference in ΔRI ($\Delta RI(\text{sample A}) - \Delta RI(\text{sample B})$) between two different sources of glass is similar.

8. DATA CALCULATION

Mean values and standard deviations may be calculated from the replicate measurements of each sample. Replicate measurements of poor quality may be excluded from the calculations. Outliers may also be removed from the calculations.

9. COMPARISON OF SAMPLES

There are various approaches to the comparison of glass fragments by refractive index data. This guideline will only provide an overview on the available methods.

Several factors influence the result of a comparison.

Therefore, it is highly recommended that every laboratory should perform a validation of their procedure regarding false positive results (random matches) and false negative results.

9.1 Exclusion criteria

The most commonly applied and described criteria are:

- range overlap criteria
- N sigma criteria (some laboratories also use a minimum standard deviation)
- T tests (including the Welch modification)

As an alternative to exclusion criteria, Likelihood Ratio (LR) calculations at source level using the continuous approaches can be applied. These result in likelihood ratios instead of binary match/no match outcomes.

Several variations of the respective criteria are applied in different laboratories.

9.2 Sampling and replicate measurements

The number and selection of fragments from the control samples, including whether and how the original surfaces are represented, can influence how the within source variability is monitored in the refractive index results.

The numbers of replicate measurements for both recovered and control samples directly affect the degrees of freedom in T test-based exclusion criteria and therefore the result of the comparison. Additionally, these also have an influence on the results of calculations performed when applying continuous approaches.

Elimination of outliers and poor quality measurements will also have an influence on standard deviation and therefore on the final result of a comparison.

9.3 Grouping

Recovered fragments may be grouped using different methods (e.g. Evett Lambert modifications 1&2, Scott Knott modification) prior to comparing groups to the control sample(s). The results of the comparison of each individual recovered fragment to each control sample, separately, may differ from the results involving the comparison of groups of recovered fragments with each control sample.

10. VALIDATION

A validation of the method is included in the ASTM standard test method E1967-19.

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12. AMENDMENTS TO PREVIOUS VERSION

This Guideline is a complete rewrite of the Appendix C to the Best Practice Manual for Forensic Glass Examinations, approved by the Expert Working Group Paint & Glass in 2009.