THE MICROSCOPE • Vol. 64:2, pp 51–60, 2016

The Differences Between Refractive Index Measurements of the External Surface and Bulk Area of Container Glass

Joseph Insana, B.S.
Microtrace, LLC [1]
Patrick Buzzini, Ph.D.
Department of Forensic Science, Sam Houston State University[2]

ABSTRACT

Refractive index (RI) is known to be a highly discriminatory optical property used in forensic comparative examinations of glass evidence. RI is known to vary at different locations on a given glass object. In addition to spatial heterogeneity, variation in RI measurements could be observed between the external surface and the bulk of a glass object. Considering the improvements of modern glass manufacturing processes, this study aimed to compare RI data from the external surfaces of glass containers to those collected from their bulk in order to determine if a significant difference exists. The body areas of eight glass containers were selected for this study. A novel methodology was developed to isolate the external surface layer of glass fragments from their bulk. A total of 560 measurements were carried out using the glass refractive index measurement (GRIM) system. The results show that differences were detected for three out of eight containers. Data produced in this study can be helpful to trace evidence examiners when evaluating potential differences observed during comparative examinations or while attempting to explain the dispersion of RI data as a consequence of a sampling method with respect to container glass.

Keywords: forensic science, trace evidence, glass evidence, container glass, sampling, refractive index measurement, glass refractive index measurement (GRIM), phase contrast microscopy (PCM), filing of glass (FoG) method

INTRODUCTION

Refractive index (RI) is a prime property of glass that is measured in the context of forensic comparative examinations of unknown glass fragments and reference glass from a putative source. The technique based on the combination of a hot plate with phase contrast microscopy has been known for several decades (1).

Glass is made primarily at the "hot end" of the factory, which is where the silicon is mixed with recycled glass or cullet along with stabilizers. Once the materials are melted together, the glob of molten glass flows through veins that lead to the molds where the molten glass is pressed into formation. Once glass is made into the desired container, it travels down the lair, a heat chamber in which the heat gradually decreases in order for the glass to be properly annealed. This allows every glass container to be subjected to the same annealing process, which aims to keep their uniformity; hence refractive indices as similar as possible. As the glass objects travel through the lair, a cold-end inspector will check the annealing process by using a Laminar Stress Measurement System to measure the excessive surface compression that will ultimately affect the overall quality of glass during the transition from the hot end to the cold end of the factory (2). In the mid-1970s, glass inspectors would use slides containing cross sections of glass with different degrees of stress to determine if the object or mold needed to be replaced. As manufacturing methods advanced, so did the inspection process, which

Note: Presented at Inter/Micro 2015, Chicago; the 2015 NIJ–RTI International Impression, Pattern, Trace Evidence Symposium (IPTES), San Antonio; and the 2016 68th meeting of the American Academy of Forensic Sciences, Las Vegas.

[1]790 Fletcher Drive, Suite 106, Elgin, IL 60123. [2]Chemistry and Forensic Science Building, 1003 Bowers Boulevard, 221G, Box 2525, Huntsville, TX 77341-2525. Corresponding author: Patrick Buzzini, patrick.buzzini@shsu.edu

allowed more uniformity in the glass-making process. Even though quality control has improved over the years, uncontrolled variation occurs in products that could cause differences in refractive indices among glass objects, more specifically within each glass container. Different factors such as mold interaction and lair transition could create subtle differences within each glass container. Glass objects can experience variation in RI through a multitude of situations. Thermal changes along with photoelastic constants of glass and atomic arrangement can intervene as well (3, 4). The cullet used during the formation of the molten glass as a flux can contain impurities that will slightly affect the chemical composition of a glass container (5). The wall of the furnace can also contribute to the resulting chemical composition of the glass products over time (6). These subtle differences can equate to creating a higher variance in refractive indices between the internal portion of the object and the external surface of the object.

As a consequence, a critical aspect to be considered while conducting RI measurements in a forensic context is the evaluation of intra-source variation of this property. For example, Bennett et al. (7) have shown that RI varies at different locations of float glass panes. However, in addition to spatial heterogeneity, previous studies indicate that differences in RI measurements could be observed between the external surface and the bulk area of a glass object. Davies et al. (8) have measured refractive indices of the bulk and surface of 20 float window glasses, 20 non-float window glasses, 20 patterned window glasses, and 20 toughened float windscreen glasses. In particular, they found that for window float glass, the RI of the tin-contact surface has always been higher than that of the bulk, whereas non-float sheet glass did not exhibit significant differences. Underhill (9) confirmed these findings by noting three layers of distinctly different RI values in various float glass samples. Locke and Hayes (10) observed that RI variations were larger in thick windows and toughened glasses but were also detectable in domestic window-panes. They applied laboratory annealing as well, which reduced RI variations. They also reported that, in general, toughened glass showed higher variations than non-toughened glass. Their study also measured fragments from two locations of a vodka bottle, and they observed large differences between the bulk and the exterior surface. Zoro et al. (11) studied the differences in RI between bulk and exterior surfaces of five flat float glass, five flat non-float glass, three bottles and three tableware objects. They reported differences as well. Finally, Suzuki et al. (12) have analyzed 16 brown beer bottles of similar aspect. They sampled the bottles in four areas, including the neck, shoulder, body, and bottom. They did not report any significant variation in RI values between different sampling depths at any of the sampled areas. However, it should be noted that their RI values were considered using four decimal places. The other studies described above, except for (9), as well as the present study, consider five decimal places of the RI values.

The aforementioned studies are more than 15 years old, and information about RI variations of container glass is based on a few numbers of samples of container glass or on a non-fully exploited RI data (i.e., four decimal places). Considering the improvements of modern glass manufacturing processes in the last several years, this study aims to expand knowledge on the potential RI differences between the external surfaces of glass containers and those collected from their bulk. The objectives of this study are the development of a methodology that 1) separates the exterior surface from the internal bulk, 2) produces representative RI data from the selected glass containers, and 3) applies a simple and robust statistic that informs about a potential difference between RI data from bulk and exterior of a given glass container.

MATERIALS AND METHODS

Sample Selection

Eight glass containers have been collected for this study. These consist of two green-colored beer bottles from Yuengling (samples 001 and 007), two green wine bottles from Valdobbiadene prosecco (samples 060 and 065), two brown beer bottles from Molson Canadian (samples 023 and 027), and two colorless honey pots from Breitsamer Honig (samples 062 and 064).

Preparation of the Fragments

Labels on the glass objects were removed by peeling them off using a flathead screwdriver and by applying isopropyl alcohol. Individual glass objects were placed in sealable polyethylene bags and then smashed with a crescent wrench. Fragments from the bottles were separated into three distinct sections corresponding to their main parts, namely the neck/shoulder, body, and base. Glass from container objects such as pots was also divided in the same way. The fragments from different areas were separated and stored in three different petri dishes. Only glass fragments from the body area were selected in this study. After a cleaning step using an ultrasonic bath, the fragments were then further broken to obtain an appropriate size for conducting RI measurements. Two different approaches were used to obtain fragments from the bulk and from the exterior surface of a given fragment. To isolate glass portions from the external surface of a fragment, a metal file with one set of teeth was utilized along with electrical tape and a mortar or similar cup-shaped receptacle. This process was named the Filing of Glass (FoG) method. A surface edge of a fragment is selected for extraction. Once selected, a section of electrical tape is cut to cover the entire side of the fragment. The placement of the tape needs to be about a centimeter below the surface and wrapped around the entire side of the fragment. Once the fragment is covered with tape, a flat file is held at a 45° angle and is pushed forward against the fragment then lifted off and repeated. This is done over a mortar to collect the filed glass shards (Figure 1).

A mortar or similar cup-shaped receptacle then collects the loose fragments, which are subsequently transferred onto a glass slide. A drop of Locke silicone oil type B mounting medium ($n^{20} = 1.537$) is then added. Smaller glass shards are obtained by further smashing them using a spatula. Another drop of mounting medium is added before fixing the preparation with a square glass coverslip. A mortar and pestle was used in order to further break glass fragments and isolate the internal bulk portion. Irregular edges of the smashed fragments were checked with a stereomicroscope. These fragments were also further smashed using a spatula while immersed in the type B silicon oil mounting medium. The preparation is finalized by adding another drop of mounting medium and placing a square glass coverslip over the fragments.

Refractive Index Measurements

Refractive index measurements of the selected glass fragments were analyzed using the hot plate method in conjunction with phase contrast microscopy. The instrument GRIM 3 by Foster + Freeman Ltd. was used for this purpose. The instrument is equipped with a Leica DM 2500 phase contrast microscope and a Mettler Toledo FP82HT hot stage. Software Glass 2.0.103 and Stage Manager 1.0.24 by Foster + Freeman Ltd. were used to manage measurements and data. Glass fragments were mounted on 76 mm × 19 mm boroslicate glass slides with a RI of about 1.5224 and fixed with 15 mm × 15 mm square coverslips. As mentioned above, a Locke silicone oil type B was used as a mounting medium. Performance checks of the instrument were carried out with Locke standard glasses B3 and B11.

Fourteen fragments were measured for each

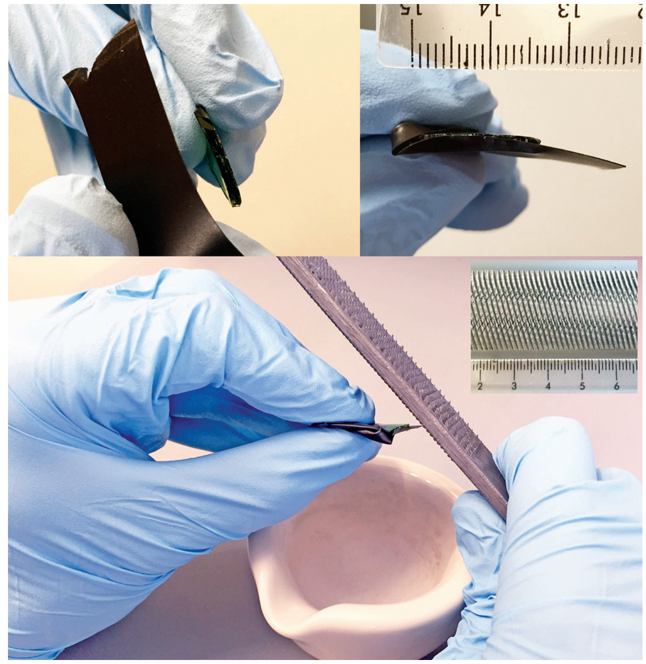

Figure 1. The Filing of Glass (FoG) method used for scraping debris from the exterior surface of glass objects. A section of electrical tape is applied and wrapped around the side of a sampled glass fragment. A file is held at a 45° angle and is then pushed forward against the fragment then lifted off and repeated. This is done over a mortar to collect the filed glass shards.

selected container: seven from the bulk area and seven from the exterior surface. Five RI measurements were conducted for each fragment resulting in 70 RI measurements per container for a total of 560 RI measurements.

Statistical Analysis

Due to the relatively small number of pairs of data sets to be compared, the Welch modification of the Student t-test was used. The Welch test is used when the two compared populations have different variances, and it is typically utilized in the context of comparisons of RI data from glass fragments (13). Due to the relatively small sample size of the collected measurements as well as the departure from normality for the majority of the distributions, the method of bootstrapping was applied to the collected data sets. Random sampling with replacement was conducted 10,000 times from the combined data of bulk and exterior surface for each sample, under the null hypothesis that these data sets cannot be differentiated. The Welch test was then applied again on the bootstrapped data. Tests have been carried out using the R statistical software (14).

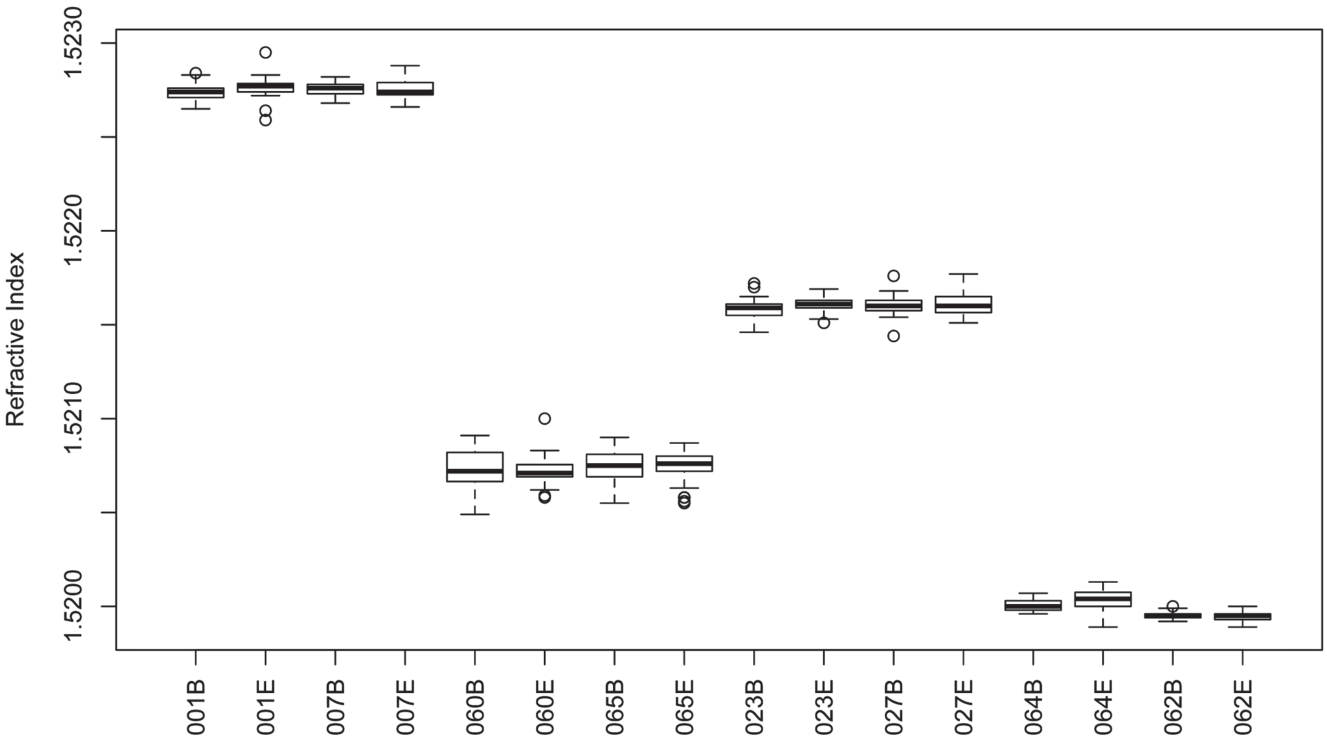

Figure 2. Box plots showing the distributions of the RI values of the bulk area (B) and the exterior surface (E) from the body areas of the eight container glass objects selected for tis study.

RESULTS

The measured RI values for the eight selected samples are separated into four main ranges of values. Glass objects from the same type and manufacturer exhibit overlapping RI values. The only exception to this seems to be the honey pots (samples 062 and 064), which appear to have different ranges of RI values. The box plot in Figure 2 shows the obtained RI data distributions.

The distributions of the data show clear differences between the RI values of the selected glass objects of different types and manufacturer. Following this preliminary visual inspection, the RI properties between the bulk area and exterior surface of the body areas exhibit overlapping values.

The data distributions for each container were observed by consideration of a histogram, which includes a probability density function as well as a normal quantile plot. The RI values for both bulk and exterior areas of sample 001 (green Yuengling bottle) display relatively normal distributions. Such distribution results in bimodal for the bulk area of the second Yuengling beer bottle (sample 007), whereas RI data from the exterior surface displays a normal distribution with a slight right skewness (Figure 3).

The RI distributions of both bulk and exterior areas of the green prosecco bottle (sample 060) show a tendency to a normal distribution in the sense that the majority of the observations are clustered in the center of the distribution. However, both distributions exhibit other areas presenting a clustering of observations, based primarily on the consideration of the probability density functions in the histograms. The second green bottle of prosecco (sample 065) exhibits a normal distribution of the data for the bulk area, while the exterior surface displays a bimodal distribution, although a central distribution is strongly emphasized. The normal quantile plot shows a strong departure from the normal line (Figure 4).

Both the bulk and exterior areas of the measured RI values of the brown beer bottle (sample 023) follow a normal distribution. Inspection of the normal quantile plots allows for appreciating a substantial deviation from normality in the regions of the tails of both distributions. The second brown bottle (sample 027) displays a normal distribution with respect to the RI values of the bulk area. However, some data are dispersed at the level of the tails of the distribution. Instead, the RI distribution for the external surface appears to be

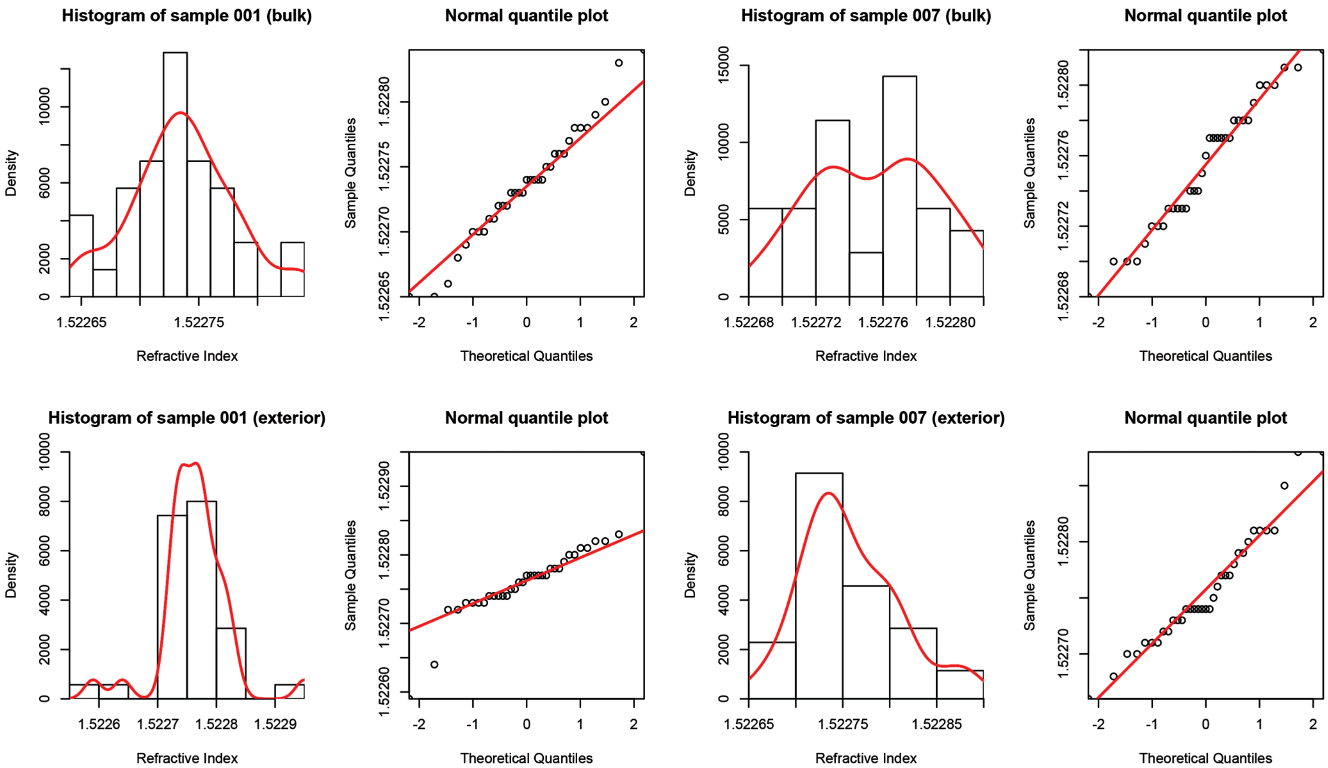

Figure 3. Histograms with probability density functions and normal quantile plots for checking the normality of the RI distributions for bulk and exterior areas of samples 001 (left) and 007 (right) (green Yuengling beer bottles).

slightly right-skewed (Figure 5).

The RI distribution of the bulk area of sample 064 (colorless honey pot) exhibits a skewness tendency on the right, while the RI distribution of its exterior surface exhibits a skewness tendency on the left. The bulk area of the other colorless honey pot collected in this study (sample 062) displays a right-skewed distribution, while the RI values for the exterior surface appear to be normally distributed (Figure 6).

For the Welch modification of the Student t-test, the null hypothesis is H_0: $\mu_x = \mu_y$ or $\mu_x - \mu_y = 0$, meaning that there is no difference between the sample means of the compared sets. The alternative hypothesis is H_0: $\mu_x \neq \mu_y$ or $\mu_x - \mu_y \neq 0$, meaning that there is a difference between the sample means of the two compared sets. Table 1 shows the calculated p-values for the tests carried out between the RI values of the bulk area and the exterior surface of for a given sample.

It can be observed that five samples out of eight (007, 027, 060, 062, and 065) have a p-value higher than 0.1. According to the scale by Curran (15), this means that the null hypothesis cannot be rejected. For samples 001 and 023, a p-value between 0.01 and 0.05 was calculated: following the scale, there is evidence against the null hypothesis. Finally, a p-value between 0.001 and 0.01 was calculated for sample 064, which is considered strong evidence against the null hypothesis. Also, note that differences between similar glass objects are observed. Only glass samples from the two selected bottles of prosecco yielded similar p-values.

Given the departures from normality observed in the majority of the distributions of the measured RI values within the collected samples, bootstrapping was applied to each sample. Figure 7 shows the comparisons between the theoretical t-distribution and the bootstrapped distributions for each sample after 10,000 draws with replacement. These distributions now have a normal shape.

Following the Welch test of the bootstrapped data, it can be noticed that the calculated p-values are very similar to those calculated with the measured RI values (Table 1).

DISCUSSION

Although differences in RI values of bulk and external surface have not been systematically observed within all the selected glass containers, this study

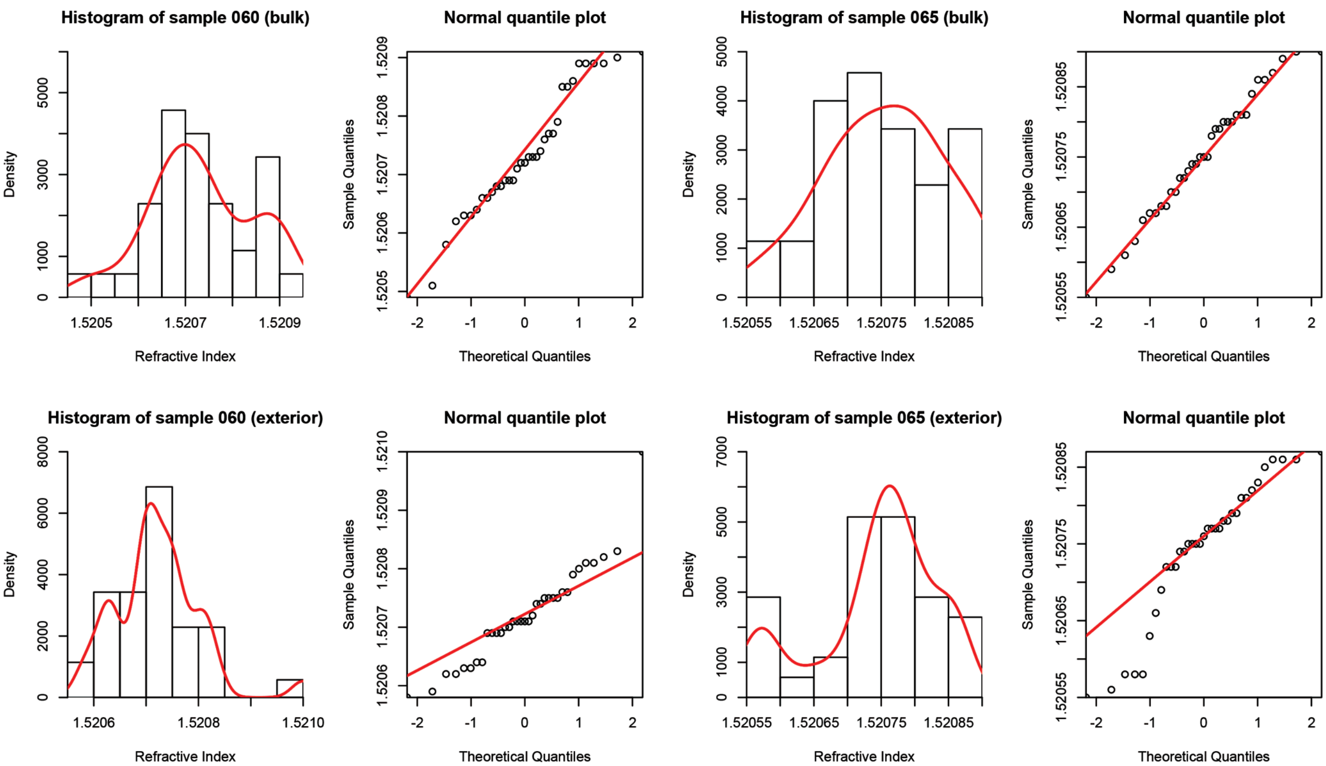

Figure 4. Histograms with probability density functions and normal quantile plots for checking the normality of the RI distributions bulk and exterior areas of samples 060 (left) and 065 (right) (green Valdobbiadene prosecco bottles).

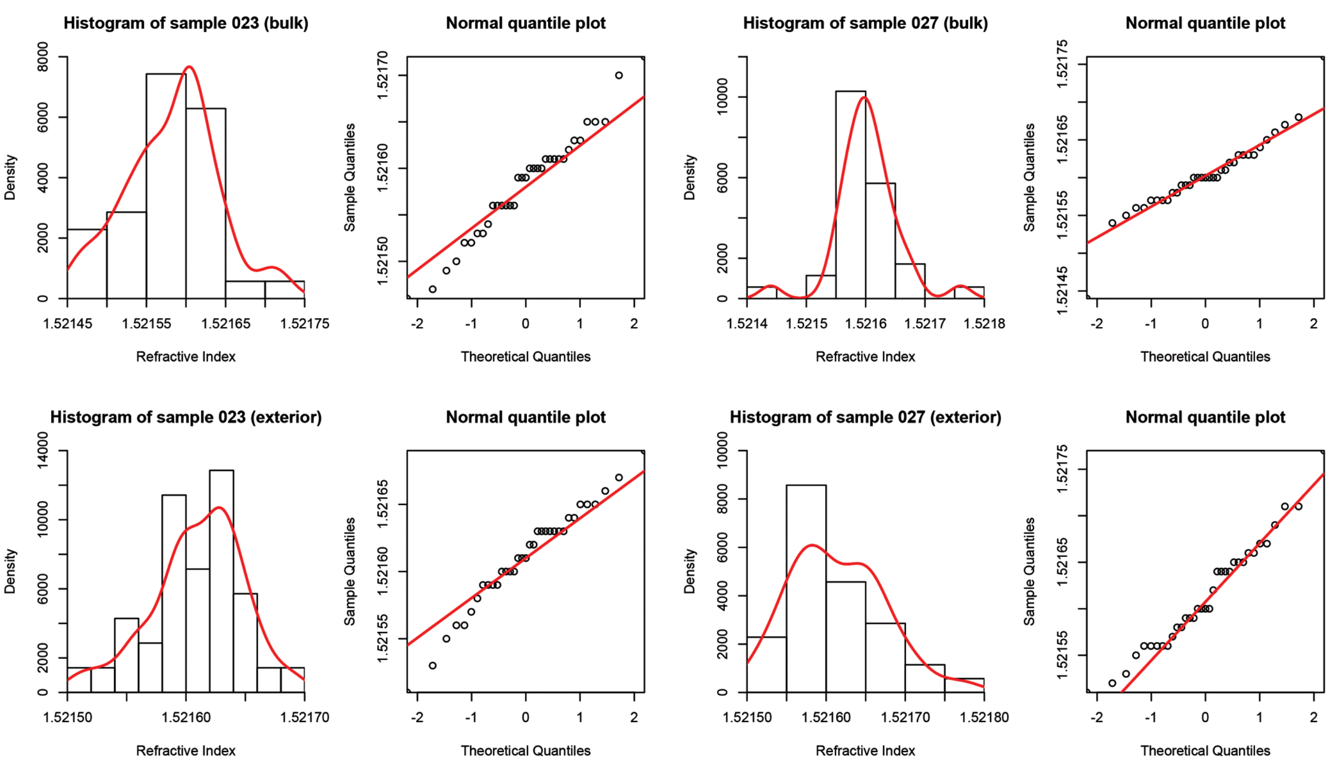

Figure 5. Histograms with probability density functions and normal quantile plots for checking the normality of the RI distributions for bulk and exterior areas of samples 023 (left) and 027 (right) (brown Molson Canadian beer bottles).

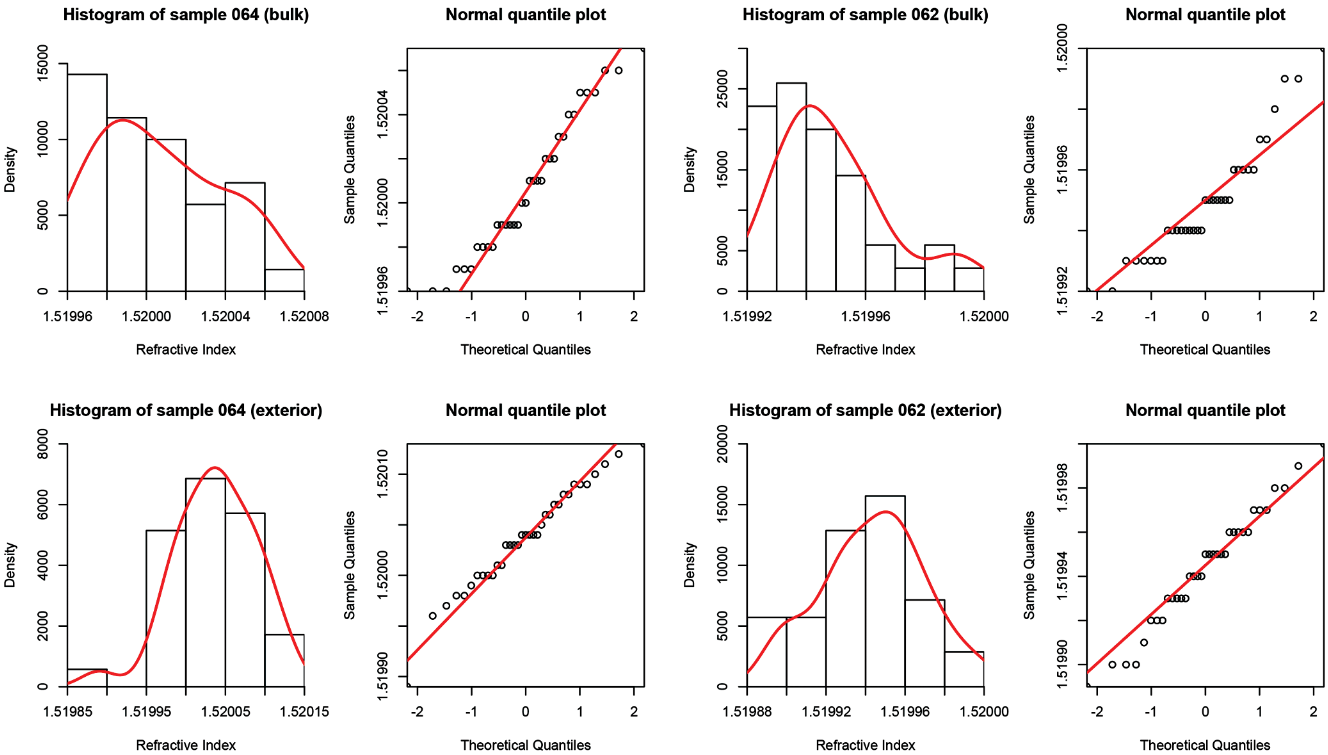

Figure 6. Histograms with probability density functions and normal quantile plots for checking the normality of the RI distributions for bulk and exterior areas of samples 062 (right) and 064 (left) (colorless Breitsamer Honig honey pots).

demonstrates that these differences exist. A method for isolating fragments from the external surface of a glass container was also devised for this study.

The FoG technique implemented in this research gathers fine fragments from the external surface in a uniform fashion. The fragment size is the major limitation to successfully separating bulk and exterior areas. This technique is recommended for fragments with edges of about 5 mm long or longer. This condition is most often fulfilled in the case of glass submitted as a reference. Instead, recovered fragments are usually of smaller size, and it is not always possible to determine if they are part of the bulk or from the external surface of the original object. However, in cases where it is possible to discern the external surface (i.e., surface fluorescence of float glass), and if the size of the fragments allows, it is recommended to determine if a RI difference exists between bulk and exterior areas in order to expand and more precisely control the study of intra-source variation of the questioned fragments.

Only the body area of glass containers was selected for this study. Clearly, collecting data from other areas would be informative to evaluate if RI differences occur in the entirety of a given glass object. For example, if differences are observed in a given area, then is it expected to observe them in other areas as well? The body area was prioritized because it constitutes the largest part of the selected glass objects.

In theory, the Welch test is exploited for normally distributed populations. In our data set, very few of the distributions were normal. However, this test was used because 1) the number of RI measurements for each glass container was relatively small (35 for each compared population), 2) the Welch test is known to be robust in cases of deviations from normality, and 3) it is possible to rely on the central limit theorem, which states that the distribution of the sum (or average) of a large number of independent, identically distributed variables will be approximately normal, regardless of the underlying distribution. The measured RI and bootstrapped data both yielded very close p-values demonstrating the robustness of the Welch test to deviations from normality. This test was also regarded as practical for the data treatment of a small number of the eight selected objects.

Traditionally, in the context of hypothesis testing, a conclusion in regard to the acceptance or the rejection of the null hypothesis is reached based on critical

Table 1. p-Values Calculated for the Welch Tests of the Original Measured RI Values and After the Bootstrapping of the Bulk Area and the Exterior Surface for a Given Sample

Sample No.	p-Value from Measured RI Values	p-Value from Bootstrapped Data
001	0.0358	0.0323
007	0.7700	0.7671
060	0.6401	0.6383
065	0.6616	0.6554
023	0.0254	0.0253
027	0.4126	0.4091
062	0.3095	0.3147
064	0.0036	0.0033

values referring to a designed significance level (usually $\alpha = 0.05$ or $\alpha = 0.01$) or cut-off point. Also, p-values are commonly utilized as measures of the strength of the evidence against the null hypothesis. This approach can be used for assessing significance at levels other than the traditional $\alpha = 0.05$ and $\alpha = 0.01$ as well. However, the rule-of-thumb that suggests the rejection of the null hypothesis whenever p-values smaller or equal to 0.05 are observed is often considered (16). In this study, thresholds or cut-off points were not chosen. No basis was identified to decide whether $\alpha = 0.05$ or $\alpha = 0.01$, or another significance level would be appropriate for the question at hand. Note that the tests for two samples (001 and 023) produced p-values between $\alpha = 0.01$ and $\alpha = 0.05$. It was preferred to follow the verbal scale used by Curran (15). This approach permits one to describe the amount of evidence against the null hypotheses in various degrees. In the present study, it was important to demonstrate that differences between bulk and exterior surfaces are plausible although not systematic and that such differences are not coherent between containers of the similar type. These gradual conclusions captured the cases where no particular difference was noted between bulk and exterior areas and cases where a difference may be present leading the glass examiner to the consideration of an in-depth variation of the RI values in addition to radial variation.

As stated earlier, differences between bulk and exterior surfaces have been observed for three containers. Also, differences have been observed between similar glass objects for three out of the four pairs (i.e., 001–007, 023–027, and 062–064). An explanation of these observations is advanced based on the random variation occurring during the glass manufacturing process where the different factors such as mold interactions and lair transitions could be responsible for subtle differences within each glass container. Another explanation could be that two glass containers may be manufactured from two different batches of cullet (5).

The results of this study indicate that the glass examiner should consider the distinction between bulk and exterior surface during the sampling process in order to evaluate the variation within recovered fragments as well as the intra-source variation of reference glass.

CONCLUSION

This study intends to provide objective information to glass examiners concerned with the understanding of RI in-depth variation that could be expected between bulk and external surface for container glass. These results can be valuable when examiners interpret potential differences observed during comparative examinations or when they attempt to explain the dispersion of RI data as a consequence of a sampling method. Despite the small number of glass objects analyzed in this study, results indicate that differences in RI values between the bulk areas and exterior surfaces occur and, therefore, the glass examiner should consider in-depth heterogeneity in addition to spatial heterogeneity during the sampling process.

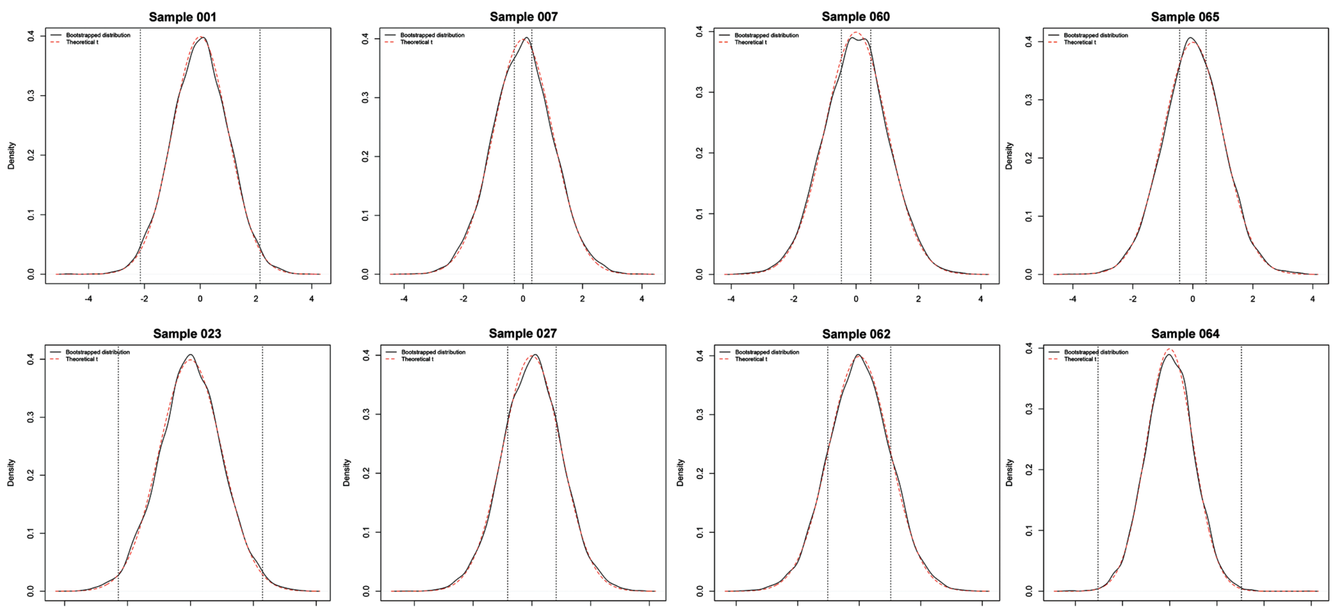

Figure 7. Comparisons between the theoretical t-distribution and the bootstrapped distributions for each sample (N = 10,000, bandwidth = 0.1449).

ACKNOWLEDGMENTS

The authors would like to thank Mr. Frank Insana, retired glass quality-control inspector, for sharing insightful information about the manufacturing process of container glass. The authors also would like to thank Professor James Curran from the University of Auckland, Auckland, New Zealand, for providing the R code for the bootstrapping method and his insightful discussions about glass evidence interpretation.

This project was conducted in the context of FIS 497 Independent Undergraduate Research with the Forensic and Investigative Science Department at West Virginia University during 2014 fall and 2015 spring semesters.

REFERENCES CITED

1. Ojena, S.M. and De Forest, P.R. "Precise Refractive Index Determination by the Immersion Method, Using Phase Contrast Microscopy and the Mettler Hot Stage," *Journal of Forensic Sciences*, 12, pp 315–319, 1972.

2. Glass Photonics. "Applications: Laminar Stress," 2012; http://www.glassphotonics.com/applications/residential_float/laminar.html (accessed Sept. 12, 2016).

3. Baak, T.; Schroeder, J.B.; Morse, S.E.; Hoogland, J.; Friedman, I.; and Lincoln, H.M. *Optics Athermalization*, Perkin-Elmer Corporation Rep. AFAL-TR-69-19 for the Air Force Avionics Laboratory, Wright-Patterson Air Force Base, Ohio, 1969.

4. Stoiber, R.E. and Morse, S.A. *Microscopic Identification of Crystals*, Ronald Press: New York, 1972.

5. Bottrell, M.C. "Forensic Glass Comparison: Background Information Used in Data Interpretation," *Forensic Science Communications*, 11:2, 2009; https://www2.fbi.gov/hq/lab/fsc/backissu/april2009/review/2009_04_review01.htm (accessed July 6, 2016).

6. Koons, R.D.; Buscaglia, J.; Bottrell, M.; and Miller, E.T. "Forensic Glass Comparisons" (Ch. 4), in *Forensic Science Handbook, Volume I* (Second Edition), R. Saferstein (ed.), Prentice Hall: Upper Saddle River, NJ, pp 161–213, 2002.

7. Bennett, R.L.; Kim, N.D.; Curran, J.M.; Coulson, S.A.; and Newton, A.W.N. "Spatial Variation of Refractive Index in a Pane of Float Glass," *Science & Justice*, 43:2, pp 71–76, 2003.

8. Davies, M.M.; Dudley R.J.; and Smalldon, K.W. "An Investigation of Bulk and Surface Refractive Indices for Flat Window Glasses, Patterned Window Glasses and Windscreen Glasses," *Forensic Science International*, 16:2, pp 125–137, 1980.

9. Underhill, M. "Multiple Refractive Index in Float Glass," *Journal of Forensic Science Society*, 20, pp 169–176, 1980.

10. Locke, J. and Hayes, C.A. "Refractive Index Variations Across Glass Objects and the Influence

of Annealing," *Forensic Science International*, 26:2, pp 147–157, 1984.

11. Zoro, J.A.: Locke, J.; Day, R.S.; Badmus, O.: and Perryman, A.C. "An Investigation of Refractive Index Anomalies at the Surfaces of Glass Objects and Windows," *Forensic Science International*, 39:2, pp 127–141, 1988.

12. Suzuki, Y.; Sugita, R.; Suzuki, S.; and Marumo, Y. "Forensic Discrimination of Bottle Glass by Refractive Index Measurement and Analysis of Trace Elements with ICP-MS," *Analytical Sciences — The Japan Society for Analytical Chemistry*, 16, pp 1195–1198, 2000.

13. Curran, J.M.; Hicks, T.N.; and Buckleton, J.S. *Forensic Interpretation of Glass Evidence*, Taylor & Francis, CRC Press: Boca Raton, FL, 2000.

14. R Core Team. R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria, 2015; http://www.R-project.org/ (accessed Sept. 12, 2016).

15. Curran, J.M. *Introduction to Data Analysis with R for Forensic Scientists*, CRC Press: Boca Raton, FL, 2011.

16. Navidi, W. *Statistics for Engineers and Scientists (Fourth Edition)*, McGraw Hill Education: New York, 2015. ■