

The author(s) shown below used Federal funds provided by the U.S. Department of Justice and prepared the following final report:

Document Title: Application of Laser-induced Breakdown Spectroscopy to Forensic Science: Analysis of Paint and Glass Samples

Author: Michael E. Sigman

Document No.: 232135

Date Received: October 2010

Award Number: 2004-IJ-CX-K031

This report has not been published by the U.S. Department of Justice. To provide better customer service, NCJRS has made this Federally-funded grant final report available electronically in addition to traditional paper copies.

Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

FINAL TECHNICAL REPORT

Application of Laser-induced Breakdown Spectroscopy to Forensic

Science: Analysis of Paint and Glass Samples

Michael E. Sigman

¹National Center for Forensic Science and Department of Chemistry, University of
Central Florida, PO Box 162367, Orlando, FL 32816-2367

Award number 2004-IJ-CX-K031

Office of Justice Programs

National Institute of Justice

Department of Justice

Points of view in this document are those of the authors and do not necessarily represent the official position of the U.S. Department of Justice. The work was done at the national Center for Forensic Science.

Table of Contents

Section	Page
1. Executive Summary	3
2. Background	4
2.1 Forensic Glass Analysis	4
2.2 Laser Induced Breakdown Spectroscopy.....	5
2.3 Data Analysis Methods.....	6
3 Samples Analyzed	9
4 Experimental Approach	10
4.1 Instrumentation	10
4.2 Sample Analysis	12
5 Results and Discussion	18
5.1 Discrimination based on Emission Ratios	18
5.2 Discrimination by Full Spectral and Peak Correlation Methods	23
5.2.1 Analysis by Sorenson’s Equation.....	26
5.2.2 Analysis by Linear Correlation	28
5.2.3 Analysis by Rank Correlation	29
5.2.4 Analysis by Full Spectral Euclidean Distance	31
6 Conclusions	32
References	34
Appendix I: Scientific Presentations Resulting from this Award	37
Appendix II: Sample Statistical Analysis	39

1. Executive Summary: A commercial single 1064 nm pulse laser-induced breakdown spectroscopy (LIBS) instrument was purchased with the funding provided under this award and the instrument was utilized to evaluate the potential for using LIBS in the forensic analysis of glass samples. LIBS is a less expensive technology than laser ablation – inductively coupled plasma – mass spectrometry (LA-ICP-MS), which has been shown to provide high discriminating power in the forensic analysis of glass. Results from these studies reveal that LIBS, when used in conjunction with refractive index (RI), provided high (>90%) discriminating power for several glass types, including beverage glass, automobile headlamp glass, and float glass from automobile side and rear windows. LIBS and RI exhibited a lower discriminating power for automobile side-mirror glass, a glass type commonly found in forensic casework. A subset of the side-mirror glass which exhibited a small variance in measured RI values was highly discriminated (>90%) by LIBS+RI. A comparative assessment of LIBS and LA-ICP-MS for several common sets of glass samples, found LA-ICP-MS to be a more highly discriminating analytical technique, although the analysis time is longer for LA-ICP-MS.

Single pulse LIBS, used in conjunction with RI, can provide an inexpensive screening for forensic glass samples and may also have utility in other trace analyses. However, since all glass samples used in these studies were known to have come from different sources, failure to achieve 100% discrimination constitutes a Type II error (false inclusion) which has a high associated cost factor in the forensic sciences. It is recommended, based on this study, that failure to discriminate questioned and known samples by LIBS or LIBS+RI, constitutes grounds for reanalysis by a more highly

discriminating technique. This study did not address the use of the evolving dual pulse LIBS techniques, which may prove to be more highly discriminating for glass analysis.

The research conducted under this award was performed in collaboration with Lt. Joseph Powell of the South Carolina Law Enforcement Department (SLED). The research resulted in one research publication,¹ one manuscript submitted, data for a third manuscript and 10 presentations at national and international meetings (see Appendix I).

2. Background: This section of the report gives a short background on forensic glass analysis, a brief introduction to LIBS, and a description of the data analysis methods used to determine the discriminating power of LIBS.

2.1 Forensic Glass Analysis: Forensic analysis of glass has historically been based on refractive index, dispersion and density analyses². In 1991, the statistical discrimination of flat glass by neutron activation analysis of the sample's elemental composition was demonstrated³. Up to 70 different elements have been determined in glass by ICP-MS⁴. The forensic significance of glass analysis has been shown to be enhanced by combining elemental analysis and refractive index⁵. It has been estimated that the probability of two unrelated glass specimens having indistinguishable elemental compositions and refractive indices lies between extremes of 10^{-5} – 10^{-13} . Elemental analysis of glass samples has been evaluated for forensic purposes using ICP-AES,⁵ and ICP-MS.^{6,7} The utility of these techniques in casework involving glass evidence has recently been reported⁸. Research by Almirall et al. has recently focused on the use of LA-ICP-MS^{9, 10} and a comparison of LIBS with other techniques for the discrimination of glass fragments. Research by

Almirall,^{8,9,10} Koons^{5,6} and Duckworth and Baynes⁷ has been established a statistical framework for interpreting forensic glass evidence.

2.2 Laser-Induced Breakdown Spectroscopy (LIBS): Laser-induced breakdown spectroscopy (LIBS) is an analytical technique that allows for the determination of a sample's elemental composition based on laser ablation followed by atomic, ionic, and molecular emission processes coming from elements transferred into the plasma as a result of laser-induced breakdown. Recent advances in instrumentation have produced commercially available LIBS spectrometers that are inexpensive compared to instrumentation required for comparable techniques. Characteristics of LIBS, including rapid analysis time, lack of required sample preparation, potential for field portability, and cost effectiveness, make this relatively non-destructive method of analysis very attractive for forensic applications.

When a high power laser pulse, generally in the 1-10 MW/cm² range, is focused tightly, dielectric breakdown occurs leading to a plasma composed of elemental and molecular fragments from the atmosphere and other objects in the immediate vicinity. As the plasma cools, the excited atoms emit light at characteristic wavelengths in the 200 – 900 nm spectral region. The emission lines are spectrally resolved and recorded, typically about 1 micro-second after the laser pulse, to give information on the elemental composition of the sample. Several reviews of recent developments in the field of LIBS research address the multitude of applications for which this burgeoning spectroscopic technique has been used.^{11,12,13,14}

The determination of relative element concentrations in a sample is a task for which LIBS is well suited². Absolute concentration determination by LIBS is complicated by

laser shot-to-shot variations, variations in sample physical properties and related calibration issues. The difficulties associated with determining the absolute concentration of elements in a glass sample by LIBS do not prohibit the use of this analytical technique for discrimination of glass and other trace evidence samples, since a comparison of samples from questioned and known origins requires only knowledge of the relative elemental concentrations or associated emission intensities from each sample.¹⁵

A direct comparison of LA-ICP-MS and LIBS has previously been reported for the analysis of trace metals in a solid matrix of KBr and two oxides.¹⁶ There have been previous reports of glass analysis by LIBS for non-forensic applications.^{17,18,19,20,21,22,23,24,25}

2.3 Data Analysis Methods: The approach taken under this program for the analysis of LIBS and LA-ICP-MS data follows from approaches for LA-ICP-MS data analysis that have previously been published and are gaining acceptance in the forensic community.^{5,6,7,8,9,10, 29} The data analysis approach involved identifying a set of elemental emissions from glass samples and constructing a set of emission intensity ratios from each spectrum. The ratios calculated for spectra taken from different glass samples were compared and discrimination was achieved when any single intensity ratio from two samples differed by a value larger than the combined confidence intervals. A similar approach was taken to compare ratios of isotopic abundances measured from LA-ICP-MS data. It is important to note that alternative approaches to the analysis of LIBS data have also been reported, notably including spectral correlation,^{26,27} and multivariate statistical approaches such as principal components analysis (PCA)^{27,28}. We have

examined some of these approaches for the analysis of float glass samples and found them to be of less value than a direct comparison of elemental emission intensity ratios. Spectral correlation (full spectral correlation or rank correlation analysis) is hampered by the fact that a highly digitized spectrum (i.e. a glass LIBS spectrum) over the 200 – 900 nm range may contain 14,000 data points and only 100 – 150 spectral peaks (with 3 – 5 points per peak). This leaves the vast majority of the points in the baseline, which significantly weights any correlation, and eliminates the possibility of a rank correlation analysis. Multivariate techniques, such as PCA, are designed primarily for classification based on a simple linear combination model. The models may work well for general classification (i.e. determining that an unknown powder is dry milk versus a lyophilized biological warfare agent), but are less successful in discriminating between two highly similar materials.

The Tukey honestly significant difference (HSD) test was used to analyze the LIBS, LA-ICP-MS and RI data collected under this program to facilitate determining the discrimination power of these techniques. The Tukey HSD test was utilized to ensure that the probability of a Type I error was held constant during the multiple pairwise comparisons within the data set⁷. An analysis of variance (ANOVA) was performed to evaluate the variance in each isotope ratio, refractive index measurement and emission intensity ratio, both within a set of replicate measurements and between sets of replicate measurements on different glass samples. An ANOVA allowed for selection of the emission or isotopic ratios that gave the highest discrimination between samples based on the F-test which compares the variance between samples with the variance within replicate measurements from each sample. Parameters giving favorable F-test results

were compared by a Pearson correlation analysis to find a set of parameters that were not linearly correlated, thereby increasing the information content in the parameter set. The ANOVA and Pearson correlation analysis was followed by a Tukey HSD post test. Under this test, the average parameter (refractive index, isotopic or emission ratio) for two glasses are significantly different at a chosen experimentwise error rate (α) if Tukey's HSD comparison, Equation 1, holds.

$$|Avg(1) - Avg(2)| \geq \left(\frac{S_w}{\sqrt{n}} \right) \cdot Q(\alpha, P, df) \quad (1)$$

In Equation 1, Avg(i) is the average of n measurements of the ratios in question for sample i, S_w is the within-group standard deviation from the ANOVA test, and $Q(\alpha, P, df)$ is the critical value of a Studentized range distribution at the α percentile point for P averages and df degrees of freedom used to estimate the standard deviation S_w .⁷ A detailed example of how each data set was analyzed is given in Appendix II.

The isotope concentration ratios from LA-ICP-MS (along with the RI values) and the emission ratios from LIBS (also along with the RI values) were analyzed for correlation within each data set. Highly correlated ratios were eliminated so as to maximize the information content. Two ratios were deemed to be sufficiently independent to be retained in the data set if the correlation coefficient (r) was less than the cutoff-criteria of an absolute value of 0.8.

Several other data analysis methods were examined which involved comparisons of either individual peak intensities, or correlation of emission intensities at each wavelength across the full spectral profile. One approach utilized Sorenson's equation. Sorenson's equation, Equation 2, counts the number of peaks occurring in both of two

spectra under comparison (multiplied by a factor of two) and divides by the total number of spectra in both spectra. The calculated distance parameter, P, can vary from zero to one.

$$P = \frac{2(n_a \cup n_b)}{n_a + n_b} \quad (2)$$

Individual peaks extracted from spectra of individual glass samples were also compared by linear correlation and rank correlation, wherein the peaks located in both of the two spectra are ranked according to intensity and assigned integer values ranging from 1 for the least intense peak through n for the largest of n peaks in the spectrum. Full spectral comparison methods including linear correlation and calculation of a distance metric, D_E , based on Euclidian distance, Equation 3, were also examined for sample discrimination. In Equation 3, S_a and S_b refer to the spectra under comparison, with the vector dot product of the two spectra calculated in the numerator and the same-spectrum vector dot products calculated in the denominator. The spectra are normalized to unit intensity (maximum intensity of one) before calculation of the dot products in Equation 3.

$$D_E = \sqrt{2} * \left[\sqrt{1 - \frac{(S_a \bullet S_b)}{\sqrt{S_a \bullet S_a} \sqrt{S_b \bullet S_b}}} \right] \quad (3)$$

3. Samples Analyzed:

Under this award, LIBS has been evaluated as a method for the forensic analyses of glass, an evidentiary item where elemental composition can provide valuable discriminating information. A review of the current status of forensic glass comparison

has been given elsewhere, along with a general description of the elemental analysis methods mentioned above and their application to glass analysis.²⁹ The objective of this research has been to determine if LIBS is of sufficient discriminating power to be useful in forensic comparisons of glass evidence. This evaluation of LIBS as a viable forensic approach was conducted by analyzing the following sample sets:

- A. 23 Automobile glass samples (side windows and rear windows); analyzed on the float side by two techniques, LIBS and LA-ICP-MS. Refractive index measurements were used in combination with both LIBS and LA-ICP-MS.
- B. 27 Automobile glass samples (side windows and rear windows); analyzed on the non-float side by LIBS. Refractive index measurements were also used in combination with LIBS.
- C. 15 Automobile headlamp samples, analyzed by LIBS and LA-ICP-MS. Refractive index measurements were used in combination with both LIBS and LA-ICP-MS.
- D. 15 Brown beverage glass samples; analyzed by LIBS. Refractive index measurements were also used in combination with LIBS.
- E. 34 Automobile side-mirror samples (analysis of non-mirrored side) by LIBS and LA-ICP-MS. Refractive index measurements were used in combination with both LIBS and LA-ICP-MS.

4. Experimental Approach:

4.1 Instrumentation: The LIBS instrument used in this research was an Ocean Optics (Dunedin, FL, USA), model LIBS2000+, equipped with a Q-switched Nd-YAG pulsed laser (Big Sky Lasers, model CFR200, Bozeman, Montana, USA). The laser 1064 nm

output pulse width was 9 ns. Spectra were collected at a laser output energy of 98 mJ/pulse, and detector delay optimized in the range of 3 – 15 μ s to allow for background continuum decay. The emission intensities (200 – 900 nm) were collected by a fiber optic bundle connected to seven linear CCD array spectrometers. The LIBS sample chamber was comprised of a plastic box fitted with an x,y-adjustable sample stage, inert gas purge line and a safety interlock to prevent laser operation when the chamber door was open, see Figure 1. The laser pulse entered the chamber from the top and was focused on the sample with a 7 cm focal length lens, which was adjustable along the vertical axis to accommodate samples of variable thickness. The emission collection optic was held in a fixed position relative to the focusing lens and translated vertically with the lens. Data acquisition and some data analysis were performed using the Ocean Optics OOILIBS software.

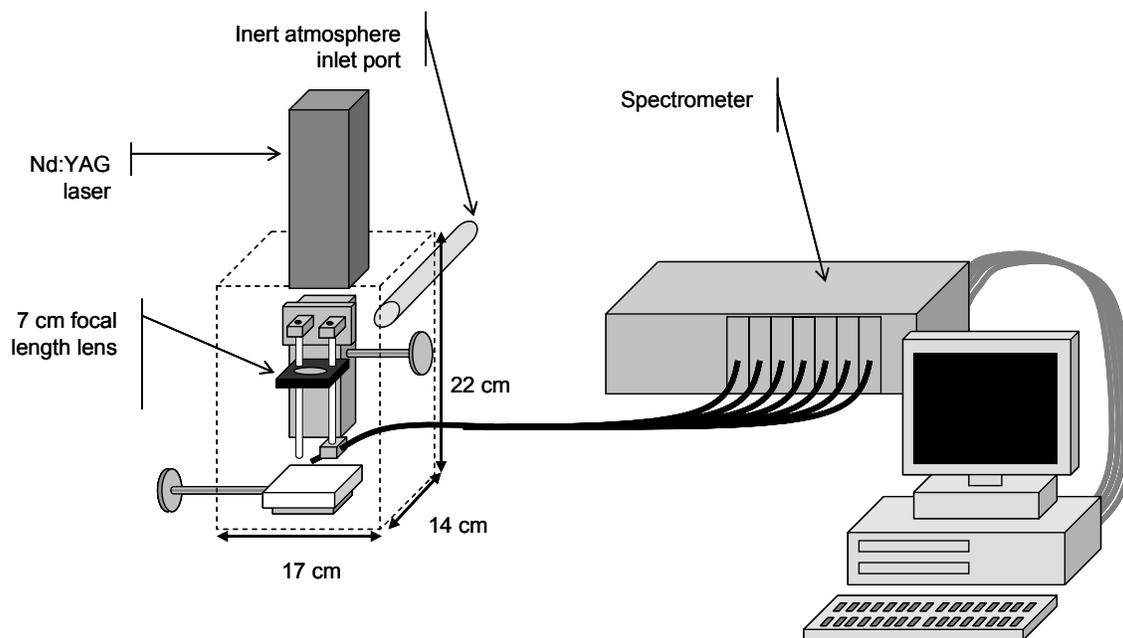


Figure 1. Diagram of the LIBS sample chamber, spectrometer and computer control.

The LA-ICP-MS system used in this study was equipped with a laser ablation unit (New Wave Research/Mechantek Fremont, CA, USA) model LUV 213 and an ICP-MS (Agilent Technologies, Palo Alto, CA, USA) model 7500s. The 213-nm output obtained from a Q-switched Nd-YAG laser (pulse width of 3-5 ns) was focused to an adjustable spot size of 5 – 400 μm^2 . Maximum laser energy output was 6 mJ/pulse, but sample incident energy is reduced to 3 mJ/pulse due to losses from optical components. A full set of experimental parameters for the LA-ICP-MS are given elsewhere¹.

The glass refractive index measurement (GRIM3) instrument (Foster and Freeman, Evesham, Worcestershire, UK) utilizes the refractive index-temperature variation method. The instrument uses a 589-nm lamp and Mettler hot stage to determine the refractive index of each glass sample.

A scanning electron microscope (LEO 1450 VP, Thornwood, N.Y., USA) with variable pressure secondary electron detector and energy dispersive spectrometer operating at 30 KV (Oxford INCA software, High Wycombe, U.K.) were used for scanning electron microscope – energy dispersive x-ray (SEM-EDX) analysis. Copper was used as an external standard for EDX calibration.

4.2 Sample Analysis: The LIBS sample chamber was purged with argon gas for 45 seconds at a rate of approximately 80 ml/min to remove air and to enhance spectral intensity. During each sampling acquisition, the laser was focused on the surface of the sample with a 50 mm focal length lens and the laser was fired at one spot. Ten spectra were taken at a frequency of 1 Hz, and averaged to give a single “average spectrum”. Average spectra were collected at five different positions on the glass surface for each sample. The total time required to analyze each glass sample was approximately two

minutes. The elemental emission wavelengths and emission line ratios used for discrimination of the glass sample sets are given in Table 1. LIBS analysis of each glass sample was found to impart minimal damage on the glass surface. This is important for preserving the integrity of the sample and facilitation of small sample analysis. An SEM image showing the impact on a glass surface resulting from of a single LIBS event is shown in Figure 2.

Table 1: LIBS emission line ratios and associated wavelengths used for glass discrimination on all samples.

(A) Float Glass – float Side	(B) Float Glass – non-float Side	(C) Headlamp Glass
Mg II(280.271)/Sn (283.998)	Si (252.851)/Mn II (257.61)	B (208.957)/Si (221.089)
Si (288.158)/Al (309.271)	Fe (373.713)/U II (383.146)	B (249.772)/ Si (250.69)
Sn (328.233)/Na (330.237)	Na (819.479)/ Cl (822.174)	Mg II (279.553)/Pb (280.2)
Na (330.237)/Ti II (336.123)	Nd (393.591)/Ca (422.67)	Th II (339.203)/Zr II (343.823)
Ca (422.673)/Ti (428.335)	Si (221.174)/ Nb (243.538)	U (394.382)/Al (396.152)
Cr (428.973)/Ti (430.839)	Si (288.18)/ Ca II (317.93)	Al (394.382)/Ca II (396.152)
Mg (518.360)/Ti (521.038)	Cr (391.568)/ Nd (393.591)	Na (558.995)/H (656.273)
Sn (326.233)/Ti II (336.123)	Ca II (527.018)/Mn (534.94)	Si (742.36)/N (746.831)
Fe II (259.94)/Mg (277.983)	Fe II (849.787)/N II (856.814)	Na (819.479)/Cl (821.204)
Ca (649.378)/Ba (649.876)	N II (856.814)/Nd (859.461)	Na (819.479)/Cl (822.174)
(D) Brown Container Glass	(E) Side-Mirror Glass	
Si (221.806)/Fe II (238.204)	Fe (373.713)/U II (383.146)	
Pb (280.2)/Mg (285.213)	Cr (390.568)/Nd (393.591)	
Na (285.281)/Si (288.158)	Nd (393.591)/Ca (422.67)	
Mg (518.36)/Ca (558.876)	N II (856.814)/Nd (859.461)	
Ca (616.217)/Cd (643.847)	Na (285.281)/Si (288.158)	
Cd (643.847)/ H (656.273)	Si (288.158)/Ca II (317.93)	
Si (742.35)/N (744.229)	Mg (383.829)/Ca II (396.847)	
Si (742.35)/N (746.831)	Fe (559.47)/Na (568.864)	
Na (818.326)/Cl (822.174)	Mg (518.36)/Ca (558.875)	
Sn II (558.882)/Na (588.995)	Mg II (280.271)/Sn (283.998)	

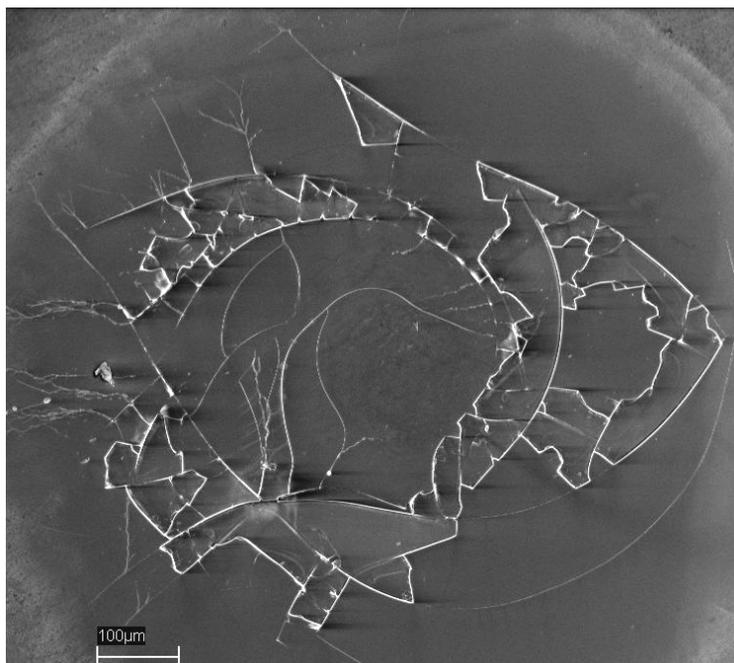


Figure 2. SEM image of the burn pattern and minimal destruction from a single LIBS event initiated by a 1064 nm laser pulse impinging on a float glass surface.

A typical example of a LIBS spectrum from a sample of float glass is shown in Figure 3. Some of the emission lines are labeled to indicate the element responsible for the emission. The quality of float glass produced commercially is tightly controlled to maintain the refractive index within a narrow range. The LIBS spectra for float glass samples were found to be highly similar, especially with regard to the most intense emission peaks. Attempts to discriminate between samples based on full-spectra correlation and multivariate techniques, such as principle components analysis, were unsuccessful. Discrimination was achieved primarily based on elements having very weak emission intensities. Only isolated and nearly Gaussian weak emission intensities were used to construct discrimination emission ratios.

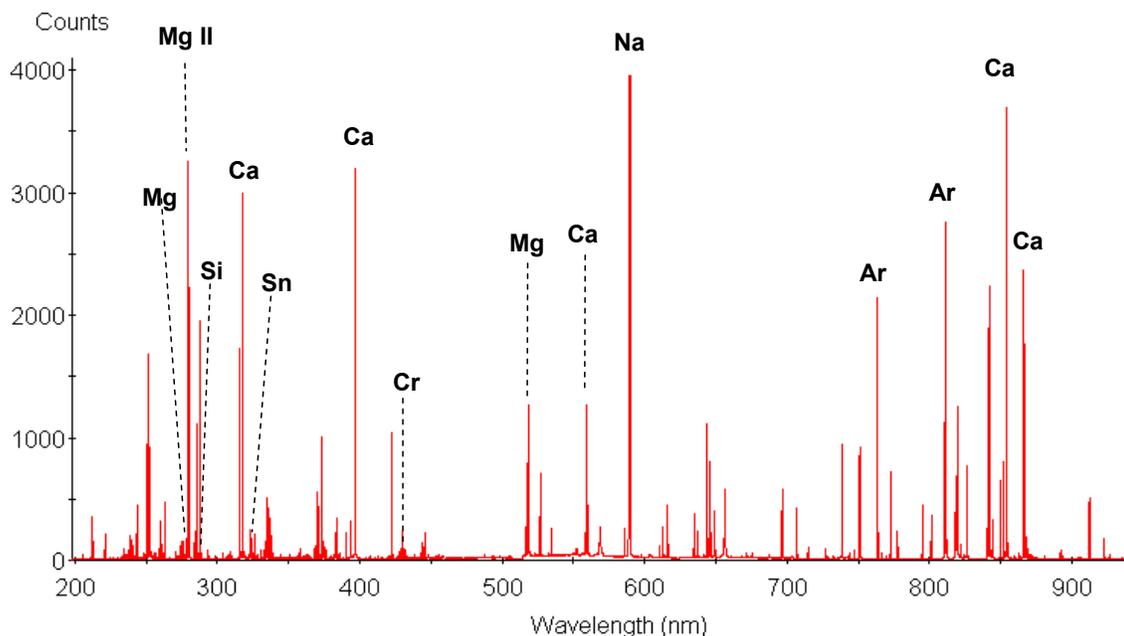


Figure 3. Typical LIBS spectrum of a float glass sample.

From the spectra collected, 18 ionic and atomic emission lines present in the majority of samples were chosen for discrimination of the glasses. The 10 emission ratios were created based on the spectral proximity of two emission lines, which were confined to a single spectrometer in the train of seven spectrometers. Not all emission lines were observed in all of the float glass samples studied, but all emission lines were present in most of the samples. The absence of specific emission lines in a given float glass sample spectrum was used as an additional sample-discriminating factor.

The short single-sample analysis time required for LIBS allowed for spectra on all 23 glass samples to be collected over a 12 hour period. In order to account for possible instrumental instabilities in the LIBS system, the precision of LIBS analysis of the automobile glass samples was measured by repetitive (hourly) LIBS analysis of a single float glass sample. The “single day” %RSD obtained for each emission line ratio varied

from 0.8% to 15.0%. LIBS data on all 23 float glass samples was acquired over the course of a single day, thereby avoiding possible day-to-day instrumental fluctuations.

The LA-ICP-MS sample chamber was purged with argon gas prior to ablation. Data was collected using one of two techniques, the “Rastering” technique and the “Drill-Down” technique. “Raster” Technique: Four layers were ablated; the data from the first layer was discarded while the next three layers were analyzed by the ICP-MS. Each layer ablated was approximately 5 μm deep. The ablation process was accompanied by rastering the sample to produce an ablated area of approximately 1.5 mm x 1.5 mm. The isotope ratios chosen for discrimination of the sample sets (A through E) are given in Table 2, and were chosen, in part, based on previous work,⁹ and previous experience by the South Carolina Law Enforcement Department (SLED). Once the sample is ablated the released particles are carried by an argon gas flow to the inductively couple plasma. Ions produced in the plasma are then carried sequentially to a quadrupole mass analyzer and detector. The rastering method of collecting LA-ICP-MS was more destructive to the glass surface than LIBS analysis; however, single-spot LA-ICP-MS analysis is far less destructive than the method employed here. An SEM image of the surface following LA-ICP-MS analysis is shown in Figure 4. “Drill-Down” Technique: Each sample was analyzed at five positions 225 μm apart, with each laser ablation producing a 50 μm well in the glass. Each spot was pre-ablated for 1 second and then analyzed over a 41 second period. Repositioning of the laser from one ablation spot to the next took approximately 13 seconds. Drill-down analysis was performed with a laser power of 0.03 mJ (65%). Each “drill-down” was comprised of a single scan which incorporated 12 acquisitions.

Table 2: LA-ICP-MS isotope ratios and associated wavelengths used for glass discrimination on all samples.

${}^7\text{Li} / {}^{53}\text{Cr}$ (Sample sets A, D and E)	${}^{57}\text{Fe} / {}^{55}\text{Mn}$ (Sample sets A, D and E)
${}^{23}\text{Na} / {}^{24}\text{Mg}$ (Sample sets A, D and E)	${}^{55}\text{Mn} / {}^{238}\text{U}$ (Sample sets A and E)
${}^{27}\text{Al} / {}^{47}\text{Ti}$ (Sample sets A, D and E)	${}^{66}\text{Zn} / {}^{60}\text{Ni}$ (Sample sets A, D and E)
${}^{29}\text{Si} / {}^{39}\text{K}$ (Sample sets A and E)	${}^{118}\text{Sn} / {}^{44}\text{Ca}$ (Sample sets A, D and E)
${}^{27}\text{Al} / {}^{29}\text{Si}$ (Sample sets A, D and E)	${}^{232}\text{Th} / {}^{238}\text{U}$ (Sample sets A and E)
${}^{88}\text{Sr}/{}^{90}\text{Zr}$ (Sample set D)	${}^{85}\text{Rb}/{}^{232}\text{Th}$ (Sample set D)
${}^{139}\text{La}/{}^{178}\text{Hf}$ (Sample set D)	

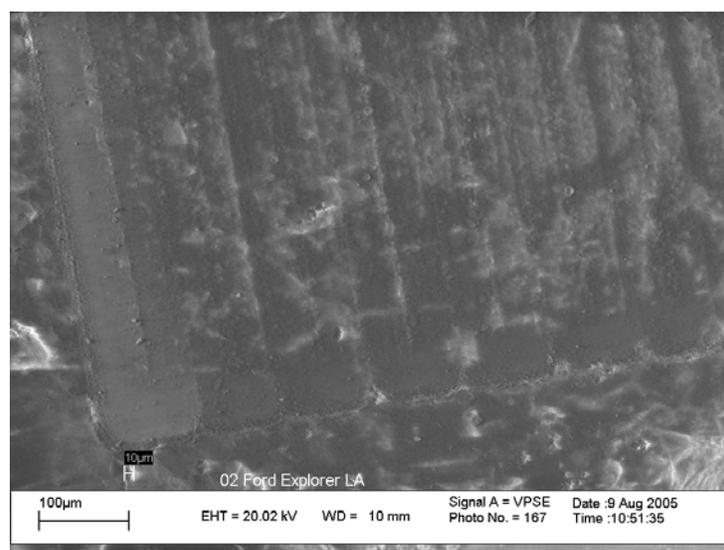


Figure 4. SEM image of a float glass sample following analysis by LA-ICP-MS using the rastering technique. The raster pattern can be seen in the image.

The intensities measured by LA-ICP-MS were optimized for the 16 analyte isotopes of interest in the NIST SRM sample 612. The NIST sample was analyzed each day as a quality control measure to ensure reproducibility in the instrumental performance. In instances where day-to-day variations in the isotopic abundances

exceeded +/- 15%, the LA-ICP-MS was re-optimized in order to obtain the isotopic abundances originally observed for SRM 612.

The refractive index (RI) was obtained by using standard immersion oils B and C from Locke Scientific (Hants, UK). The calibration curves for immersion oils were generated using Locke Scientific (Hants, UK) glass reference samples B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, B11, B12, C1 and C2. The RI values were averaged over several fragments of each glass studied. A Mettler hot stage was used to heat and cool the immersion oil surrounding each fragment for RI measurement for which the iso-refractive temperature was visually determined. The GRIM3 software allowed the simultaneous determination of four RI values. Samples for the refractive index (RI) measurements were washed in an ultrasonic bath containing deionized water for five minutes. Once dry, the samples were crushed into small shards. Two drops of refractive index immersion oil (B or C) were placed on a glass microscope slide. The shards of crushed glass were placed into the oil and then topped with a cover slip.

5. Results and Discussion:

5.1 Discrimination Based on Emission Ratios: Table 3 gives the tabulated results for each dataset. The table lists the number of emission or isotopic ratios used in the comparison for each data set and the percent discrimination obtained by each method, followed by the confidence level in parentheses. Percent discrimination was determined by combining LA-ICP-MS or LIBS with RI for some samples. In other cases, the discrimination capability has been determined LA-ICP-MS or LIBS without the use of RI data. Both results are given in the table. Table 3 also lists the percent discrimination that was achievable based only on RI data for each sample set.

Table 3. Discrimination for each sample set (A-F) by LIBS, LA-ICP-MS and RI.

LIBS	LA-ICP-MS	RI
(A) Float Glass (23 samples, 253 comparisons, analysis of float side)		
<i>10 Emission Ratios + RI</i> 100(90), 99.2(99)		95.7(90), 94.9(99) ‡
<i>10 Emission Ratios</i> 99.4(90), 82.2(99)	<i>10 Isotopic Ratios</i> * 100.0(90), 100.0(99)	
(B) Float Glass (27 Samples, 351 comparisons, analysis of non-float side)		
<i>10 Emission Ratios + RI</i> 97.4(90), 98.9(99)		
<i>10 Emission Ratios</i> 82.3(90), 71.8(99)		
(C) Automobile Lamps (15 Samples, 105 comparisons, analysis of outside surface only)		
<i>10 Emission Ratios + RI</i> 100(90), 100(99)	<i>10 Isotopic Ratios + RI</i> † 93.3(90), 91.4(99)	80.0(90), 76.2(99)
<i>10 Emission Ratios</i> 98.1(90), 98.1(99)	<i>10 Isotopic Ratios</i> † 72.4(90), 61.0(99)	
(D) Brown Drinking Glass (15 Samples, 105 comparisons)		
<i>10 Emission Ratios</i> 100(90), 100(99)		81.9(90), 75.2(99)
(E) Automobile Side Mirror Glass (34 Samples, 561 comparisons, analysis of non-coated side only)		
<i>10 Emission Ratios + RI</i> 79.1(90), 71.5(99)	<i>10 Isotopic Ratios + RI</i> * 100(90), 99.8(99)	46.5(90), 42.3(99)
<i>10 Emission Ratios</i> 62.0(90), 53.1(99)	<i>10 Isotopic Ratios</i> * 100(90), 99.6(99)	

* Sample analysis by “Rastering” technique.

† Sample analysis by “Drill-Down” technique.

‡ Based on 351 comparisons from 27 samples

Some comments are required concerning interpretation of the RI data. A standard deviation of 2.2×10^{-4} has been reported for repetitive automated RI measurements within a single pane of float glass.²⁹ When adopting this value for determining the discriminating power of RI for float glass, as was done in previous studies which

combined RI and LA-ICP-AES,⁷ at a 90% confidence level 86.6% of the 351 pairwise comparisons could be discriminated by RI alone and 82.6% of the float glass samples could be discriminated with a 99% confidence level. The values given for the float glass samples in Table 1 were obtained from RI measurements that we made using a GRIM3 instrument, which gave a smaller standard deviation (1×10^{-5}), and afforded a somewhat higher discrimination of the float glass samples. In fact, discrimination by RI alone exceeded discrimination by LIBS alone for the float glass sample sets A and B.

Discrimination by RI decreased for sample-sets C, D and E, due to a larger variance of the refractive index within a glass sample. This change is reflected in the refractive index F values of 4,054 and 36, for sample sets A and E respectively. The lower F value for the side-mirror glass (set E) is the result of an increase of the within-group variance by three orders of magnitude, while the between-group variance increased by only one order of magnitude. The refractive index measurements for some, but not all, of the side-mirror glass samples exhibited large variations in the measured value. Approximately 11 of the 34 samples gave excessively large variations in RI, and the remaining samples exhibited a variance in the refractive index measurement which approximated the variance in RI measurements for the other sets of glass samples.

Table 4 gives the total discrimination across all sample sets, as determined by LA-ICP-MS and LIBS without the use of RI data to aid in the discrimination. Combining the RI data with either LIBS or LA-ICP-MS data provides higher discrimination. Table 5 gives the total discrimination across all sample sets when LA-ICP-MS and LIBS data are used in conjunction with RI data.

Table 4. Total discrimination across all sample sets (A-E) by LIBS and LA-ICP-MS without the use of refractive index data.

Total number of discriminations and percent discrimination without RI	
<i>Total for LIBS</i> <i>1375 comparisons</i>	<i>Total for LA-ICP-MS</i> <i>919 comparisons</i>
<i>1076 discriminations = 78.3% discrimination at 90% confidence</i>	<i>890 discriminations = 96.8% discrimination at 90% confidence</i>
<i>790 discriminations = 57.5% discrimination at 99% confidence</i>	<i>858 discriminations = 93.3% discrimination at 99% confidence</i>

Table 5. Total discrimination across all sample sets (A-E) by LIBS and LA-ICP-MS with the use of refractive index data.

Total number of discriminations and percent discrimination with RI		
<i>Total for LIBS</i> <i>1270 comparisons</i>	<i>Total for LA-ICP-MS</i> <i>666 comparisons</i>	<i>Total for RI</i> <i>1122 comparisons</i>
<i>1148 discriminations = 90.4% discrimination at 90% confidence</i>	<i>659 discriminations = 99.0% discrimination at 90% confidence</i>	<i>767 discriminations = 68.3% discrimination at 90% confidence</i>
<i>1015 discriminations = 79.9% discrimination at 99% confidence</i>	<i>649 discriminations = 97.5% discrimination at 99% confidence</i>	<i>685 discriminations = 61.0% discrimination at 99% confidence</i>

Comparison of Tables 4 and 5 shows LA-ICP-MS to have an overall higher discrimination across the three sample sets examined by both LA-ICP-MS and LIBS under this program. LA-ICP-MS, when used in combination with RI, consistently provided greater than 90% discrimination at 90% and 99% confidence levels, with discrimination of 100% float glass and side-mirror glass samples at 90% confidence. LIBS, used in combination with RI gave greater than 90% discrimination in all data sets other than the side-mirror glass, and provided 100% discrimination of the automobile

headlamp and float glasses at 90% confidence. The overall performance of LIBS was decreased by the low discrimination percentage observed for the side-mirror data set (62% discrimination at the 90% confidence level without RI data). This was the data set identified above as having the largest within-group variance for the refractive index; however, LA-ICP-MS provided very good discrimination for this data set. The two “optical” methods (LIBS and RI) gave low discrimination for the side-mirror glass, and as noted above, 11 of the 34 glass samples exhibited the large variance in RI. When the 11 high-variance glasses are removed from the data set, the discrimination by RI increases to 88.9% (90% CI) and 87.4% (99% CI). The LIBS discrimination similarly increased to 66.8% (90% CI) and 51.8% (99% CI). The discrimination by LIBS+RI for the reduced side-mirror sample set increased to 94.9% (90% CI) and 93.7% (99% CI). These results demonstrate that the same glasses which exhibited large variances and low discrimination by RI, also exhibited the same trends in LIBS emission ratios. Notably, factors that led to the observed behavior in LIBS and RI results did not influence the sample discrimination by LA-ICP-MS.

When the side-mirror data set is completely eliminated from the comparison, the LIBS discrimination, at the 90% CI, increases to 99.7% in conjunction with RI and to 98.9% without RI. The discrimination, at the 99% CI, for LIBS+RI increases to 89.9%, while the discrimination for LIBS without RI increases to 74.4%.

An important parameter that was not examined for any of the sample sets is the potential of wavelength dependence for LIBS discrimination. A modified sampling methodology that employs a shorter wavelength laser that would be optically absorbed by the glass and result in ablation of a larger amount of sample might possibly increase the

discrimination for this set of glasses. Alternatively, the discriminating power may be increased by selection of an alternative set of emission ratios.

5.2 Discrimination by Full Spectral and Peak Correlation Techniques: The sample discrimination described above, in Section 5.1, relied on the use of different emission intensity ratios for each set of glass samples. A more straight forward method of spectral comparison would be desirable to facilitate questioned and known sample discrimination. Several discrimination methods were examined by comparing float glass sample spectra. The sample set chosen for comparison included samples that were discriminated based on peak intensity ratios (Section 5.1) and samples that could not be discriminated by that technique. In the comparison methods discussed below, two factors play a significant role in controlling the utility of the methods. Full spectral comparison methods are hampered by the baseline contributions. A full spectrum may contain in excess of 13,000 points, of which only 1,000 – 2000 total points may contribute to 100 – 200 peaks. In this situation, those points contributing to the baseline significantly influence correlation approaches. Likewise, rank correlation of full spectra is hampered by baseline contributions because the noise which contributes randomly to the baseline significantly broadens the distribution. Therefore, a more reasonable approach is to compare only selected peaks that meet specified criterion for peak selection (i.e. signal-to-noise ratio greater than three, or intensity greater than a specified cutoff, such as 10% normalized intensity). If two spectra under comparison both contain emission intensity at a given wavelength, and if that intensity exceeds the peak selection criterion in one spectrum but not in the other, the two spectra could be deemed different based on a missing peak in one spectrum. Under this scenario, sample discrimination is dependant on judging the significance of a

missing peak. Each of these challenges is addressed in the following spectral comparisons.

The statistical approach to comparing two samples was to use a t-test to determine if a test parameter comparing two different samples was statistically different from the same parameter when comparing repetitive analyses of each individual sample. For example, to determine if two glass samples are different based on a Euclidean distance measurement (D_E), replicate LIBS spectra were recorded for each of the two glass samples and then the replicate spectra were compared within the same sample and between samples. In the following discussion, we drop the subscript E, denoting Euclidean distance, for the sake of generality since D can correspond to any reproducible comparison parameter. The average distance between same-sample (replicate) analyses (\overline{D}_{SS}) and the average distance between different-sample analyses (\overline{D}_{DS}) were compared using a standard t-test to determine if the average D values differ, and therefore if the samples differ. For example, if sample X is analyzed in triplicate (giving analyses X1, X2 and X3) and sample Y is analyzed in triplicate (producing Y1, Y2 and Y3), same-sample comparisons (i.e. $D_{X1,X2}$, $D_{X1,X3}$, $D_{X2,X3}$, $D_{Y1,Y2}$, $D_{Y1,Y3}$, and $D_{Y2,Y3}$) can be calculated along with \overline{D}_{SS} based on all six values. All six values are used in the calculation of \overline{D}_{SS} since each D value represents a comparison between replicate measurements of samples from a common source and serve to establish an estimate of variance for replicate analyses of samples. Similarly, the different-sample distances (i.e. $D_{X1,Y1}$, $D_{X1,Y2}$, $D_{X1,Y3}$, $D_{X2,Y1}$, $D_{X2,Y2}$, $D_{X2,Y3}$, $D_{X3,Y1}$, $D_{X3,Y2}$, and $D_{X3,Y3}$) were calculated along with \overline{D}_{DS} based on all nine values. Each of the nine values represents a calculated distance between Sample X and Sample Y. When \overline{D}_{SS} and \overline{D}_{DS} are compared by a t-test, if the null

hypothesis is accepted, the samples may be said, with a stipulated confidence level, to have come from a common source. If the null hypothesis is rejected, the two samples can be asserted with known statistical significance to have come from different sources.

The appropriate form of the t-test for two data sets with differing numbers of points (n_{SS} and n_{DS}) and with unknown population variances that are possibly unequal is given in Equation 4. In Equation 4, \bar{D}_{SS} and \bar{D}_{DS} are as defined above, S_{DS} and S_{SS} are the standard deviations for the samples and n_{DS} and n_{SS} are the number of distance values in each respective data set.³⁰

$$t' = \frac{|\bar{D}_{DS} - \bar{D}_{SS}|}{\sqrt{\frac{S_{DS}^2}{n_{DS}} + \frac{S_{SS}^2}{n_{SS}}}} \quad (4)$$

The number of degrees of freedom (DF) is approximated by Equation 5. The DF will fall between smaller of $n_{DS}-1$ and $n_{SS}-1$, and their sum ($n_{DS} + n_{SS}$).

$$DF = \frac{\left(\frac{S_{DS}^2}{n_{DS}} + \frac{S_{SS}^2}{n_{SS}}\right)^2}{\frac{\left(\frac{S_{DS}^2}{n_{DS}}\right)^2}{n_{DS}-1} + \frac{\left(\frac{S_{SS}^2}{n_{SS}}\right)^2}{n_{SS}-1}} \quad (5)$$

The comparative method outlined in the previous paragraph was used to compare representative LIBS spectra from each of the sample sets (A-E) using Sorenson's equation (Equation 2), linear correlation, rank correlation, and Euclidean distance (Equation 3). The representative samples chosen are listed in Table 6, and includes three samples from Set A (designated A1 – A3), two samples from Set B (designated B1 and B2), and two samples each from sets C, D and E, each designated with the respective

sample letters (C1, C2, D1, D2, E1 and E2). Of the three samples from Set A, the pairs (A1,A3) and (A2,A3) were discriminated by the emission ratios method discussed in the previous section. The pair (A1, A2) was not discriminated by the emission ratios method. Sample B1 corresponds to the same glass sample as A1; however, A1 corresponds to analysis on the float side of the glass and B corresponds to analysis on the non-float side of the same glass. Each of the same-set pairs (B1,B2), (C1,C2), (D1,D2) and (E1,E2) were discriminated based on emission intensity ratios.

Table 6: Sample designations for comparison by Sorenson’s equation, linear correlation, rank correlation and Euclidean distance.

Sample Set	Designation	Description
A	A1	1996 Mazda 626, side window, float glass, analysis on the float side
A	A2	1998 Ford Escort, side window, float glass, analysis on the float side
A	A3	1984 GCM C1500, side window, float glass, analysis on the float side
B	B1	1996 Mazda 626, side window, float glass, analysis on the non-float side
B	B2	1994 Honda Accord, , side window, float glass, analysis on the non-float side
C	C1	Vehicle unidentified, headlamp glass
C	C2	1994 Oldsmobile Cutlass Ciera, headlamp glass, analyzed on the outer surface
D	D1	Theakston Old Peculiar, brown beer bottle glass, analyzed on inner surface
D	D2	Coors Light, brown beer bottle glass, analyzed on inner surface
E	E1	1989 Nissan Stanza, side mirror glass, float glass, analyzed on float side opposite the mirror coating
E	E2	2004 Ford Freestar, side mirror glass, float glass, analyzed on float side opposite the mirror coating

5.2.1 Analysis by Sorenson’s Equation: Sorenson’s equation, Equation 2, was used to compare representative LIBS spectra from Table 6. Each spectrum was the average of 10

single laser pulse spectra and three replicate analyses were made of each glass sample.

The peaks used in the comparison were defined as having a signal-to-noise ratio greater than three. Discrimination is based on peaks in the 200 – 800 nm range. Table 7 gives the results for the pairwise comparisons based on Sorenson’s equation and a summary of the discrimination for samples from the same set, different sets, and all samples, is given at the bottom of the table.

Table 7: Results for Sorenson’s equation pairwise comparison of samples from Table 6. The degrees of freedom (DF) from Equation 5, the calculated t’ from Equation 4, Student t-value at the 5% significance level, and the interpretation of (samples discriminated or not discriminated) are given in the table.

Comparison	DF	t’ (calculated)	t ($\alpha=0.05$)	Discriminated
A1 vs A2	25	0.770	2.06	No
A1 vs A3	20	0.996	2.09	No
A2 vs A3	13	1.805	2.13	No
A1 vs B1	13	1.775	2.16	No
A1 vs B2	13	1.538	2.16	No
A1 vs E1	10	1.528	2.23	No
A1 vs D1	10	28.600	2.23	Yes
A1 vs C1	12	4.116	2.18	Yes
B1 vs B2	12	0.514	2.18	No
B1 vs E1	13	3.051	2.16	Yes
B1 vs D1	11	29.090	2.20	Yes
B1 vs C1	11	3.229	2.18	Yes
B2 vs E1	12	1.353	2.18	No
B2 vs D1	11	5.354	2.20	Yes
B2 vs C1	10	2.529	2.23	Yes
E1 vs D1	11	28.854	2.22	Yes
E1 vs C1	11	3.532	2.22	Yes
D1 vs C1	6	10.086	2.45	Yes
C1 vs C2	12	0.325	2.18	No
D1 vs D2	9	0.126	2.26	No
E1 vs E2	7	0.433	2.37	No
Same Set Discrimination			0/7	0%
Different Set Discrimination			10/14	71%
All Comparisons Discrimination			10/21	48%

None of the same-set samples (A vs A, B vs B etc.) were differentiated by the method.

Furthermore, samples from sets A, B and E (all of which are float glass) were not

discriminated by the method, irrespective of whether the analysis was performed on the float or non-float side of the glass. The Sorenson's method was found to be the least discriminating of all the full spectral and peak correlation methods tested. The samples in sets A, B and E can not be discriminated by this measure which depends only on a count of the number of peaks and not on the relative intensities of the peaks.

5.2.2 Analysis by Linear Correlation: The spectra from Table 6 were also analyzed by performing a linear correlation of the extracted peaks common to both spectra. In this case, the comparison depends on the relative peak intensities and in order to remove potential errors associated with incorrectly identifying a peak as "missing", the correlation was performed only for those peaks common to both spectra, i.e. a Pearson's correlation with pairwise removal of missing data. Results from the calculation are shown in Table 8.

The linear correlation for repetitive analyses from the same-set samples A and B (A vs A and B vs B) did not discriminate the samples at the 95% confidence level. Notably, A1 vs A3 and A2 vs A3 were discriminated at both the 90% and 99% confidence levels using the emission ratios method. Same-set sample C, D and E were discriminated by the linear correlation method. The comparison of float glasses from sets A and B were not discriminated, even though the samples from Set A were analyzed on the float side and those from Set B were analyzed on the non-float side. The linear correlation was able to discriminate between samples from Set E and those from Set A and Set B. As with Sorenson's equation, spectra from very different glass types (headlamps and bottle glass) were readily discriminated. Incorporating relative peak intensity into the comparison through the use of a linear correlation technique gives some improvement over

comparison by Sorenson's equation, which is based only on a count of common peaks; however, failure to discriminate between analyses on float and non-float sides of the same glass indicate that this comparison technique would not be highly useful in the comparison of casework samples.

Table 8: Results for pairwise comparison by linear correlation of samples from Table 6. The degrees of freedom (DF) from Equation 5, the calculated t' from Equation 4, Student t-value at the 5% significance level, and the interpretation of (samples discriminated or not discriminated) are given in the table.

Comparison	DF	t' (calculated)	t ($\alpha=0.05$)	Discriminated
A1 vs A2	19	0.014	2.09	No
A1 vs A3	21	0.007	2.08	No
A2 vs A3	26	0.003	2.06	No
A1 vs B1	13	0.756	2.18	No
A1 vs B2	7	1.605	2.37	No
A1 vs E1	11	15.752	2.23	Yes
A1 vs D1	9	18.541	2.26	Yes
A1 vs C1	8	29.615	2.31	Yes
B1 vs B2	12	0.152	2.18	No
B1 vs E1	13	5.717	2.16	Yes
B1 vs D1	9	19.510	2.26	Yes
B1 vs C1	8	21.609	2.31	Yes
B2 vs E1	12	4.657	2.57	Yes
B2 vs D1	8	9.223	2.31	Yes
B2 vs C1	9	22.571	2.26	Yes
E1 vs D1	9	14.060	2.26	Yes
E1 vs C1	8	23.491	2.31	Yes
D1 vs C1	8	29.222	2.31	Yes
C1 vs C2	11	2.461	2.20	Yes
D1 vs D2	12	2.577	2.18	Yes
E1 vs E2	13	3.274	2.16	Yes
Same Set Discrimination			3/7	43%
Different Set Discrimination			12/14	86%
All Comparisons Discrimination			15/21	71%

5.2.3 Analysis by Rank Correlation: Rank correlation analysis can often give improved discrimination over a simple linear correlation. This comparison differs from the linear correlation by comparing the ranking of all of the intensities for peaks in two

spectra (Spearman rank correlation). In this comparison, as in the simple linear correlation, peaks were not included in the analysis if they were missing from one of the two spectra being compared (pairwise deletions). The data being compared here are the spectra for samples in Table 6. Results are given in Table 9, and represent an improvement over the discrimination obtained by linear correlation.

Table 8: Results for pairwise comparison by rank correlation of samples from Table 6. The degrees of freedom (DF) from Equation 5, the calculated t' from Equation 4, Student t -value at the 5% significance level, and the interpretation of (samples discriminated or not discriminated) are given in the table.

Comparison	DF	t' (calculated)	t ($\alpha=0.05$)	Discriminated
A1 vs A2	23	0.005	2.07	No
A1 vs A3	26	0.040	2.06	No
A2 vs A3	26	0.036	2.06	No
A1 vs B1	13	2.334	2.16	Yes
A1 vs B2	11	4.038	2.20	Yes
A1 vs E1	12	6.100	2.18	Yes
A1 vs D1	12	15.953	2.16	Yes
A1 vs C1	13	15.640	2.16	Yes
B1 vs B2	11	0.217	2.20	No
B1 vs E1	13	6.232	2.16	Yes
B1 vs D1	11	19.040	2.20	Yes
B1 vs C1	8	15.737	2.31	Yes
B2 vs E1	13	6.534	2.16	Yes
B2 vs D1	13	14.029	2.16	Yes
B2 vs C1	9	11.176	2.26	Yes
E1 vs D1	12	15.212	2.18	Yes
E1 vs C1	8	11.929	2.18	Yes
D1 vs C1	9	15.723	2.31	Yes
C1 vs C2	12	3.547	2.18	Yes
D1 vs D2	13	4.927	2.16	Yes
E1 vs E2	12	3.104	2.18	Yes
Same Set Discrimination			3/7	43%
Different Set Discrimination			14/14	100%
All Comparisons Discrimination			17/21	81%

Rank correlation does not discriminate the same-set A and B spectra (A vs A and B vs B); however, the same-set C, D and E spectra were discriminated. All of the spectra from different sample sets are discriminated at the 95% confidence level. This includes

discrimination of the float glass samples analyzed on the float and non-float sides. The rank correlation analysis gives the most robust discrimination of all of the spectral and peak correlation methods examined.

Table 10: Results for pairwise spectral comparison by Euclidean distance between samples from Table 6. The degrees of freedom (DF) from Equation 5, the calculated t' from Equation 4, Student t -value at the 5% significance level, and the interpretation of (samples discriminated or not discriminated) are given in the table.

Comparison	DF	t' (calculated)	t ($\alpha=0.05$)	Discriminated
A1 vs A2	25	0.060	2.06	No
A1 vs A3	26	0.107	2.06	No
A2 vs A3	23	0.423	2.07	No
A1 vs B1	11	0.369	2.02	No
A1 vs B2	9	2.964	2.26	Yes
A1 vs E1	12	13.867	2.18	Yes
A1 vs D1	8	9.776	2.31	Yes
A1 vs C1	12	40.099	2.18	Yes
B1 vs B2	9	0.200	2.31	No
B1 vs E1	9	4.096	2.26	Yes
B1 vs D1	11	10.031	2.20	Yes
B1 vs C1	12	16.494	2.18	Yes
B2 vs E1	6	8.696	2.45	Yes
B2 vs D1	9	9.223	2.26	Yes
B2 vs C1	11	33.655	2.23	Yes
E1 vs D1	8	10.209	2.31	Yes
E1 vs C1	9	35.665	2.26	Yes
D1 vs C1	8	19.341	2.31	Yes
C1 vs C2	13	6.542	2.16	Yes
D1 vs D2	11	0.705	2.20	No
E1 vs E2	11	6.156	2.20	Yes
Same Set Discrimination			2/7	29%
Different Set Discrimination			13/14	93%
All Comparisons Discrimination			15/21	71%

5.2.4 Analysis by Full Spectral Euclidean Distance: Discrimination was also attempted based on the full spectral profile by calculating the Euclidean distance, as given in Equation 3. This comparison method does not rely on identifying peaks and/or missing peaks and the required vector dot product is easily computed in a standard spreadsheet program. Results are given in Table 10. The full spectral comparison by Euclidean

distance did not discriminate same-set A, B and D samples; however, same-set C and E samples were discriminated. The method did discriminate between A1 and B2, which are two different float glass samples with A1 analyzed on the float side and B2 analyzed on the non-float side. However, samples A1 and B1 were not discriminated. This pair represents analysis of the same float glass sample on the float side (A1) and the non-float side (B1). This is the second best overall discrimination for the full spectral and peak correlation techniques examined.

6. Conclusion:

In this work, we have shown that the discriminating power of single (nanosecond) pulse 1064 nm LIBS is noticeably less than that of LA-ICP-MS for the analysis of glass samples drawn from four types commonly encountered in forensic casework. This conclusion is based on the analysis methodology that utilized a comparison of emission line ratios within each spectrum. The use of refractive index measurements in addition the LIBS spectra significantly increases the overall discriminating power of LIBS. The combined discriminating power of LIBS and RI exceeds that of either individual technique. A total of 1270 pairwise comparisons of different glass samples (each comparison of samples within the same sample set, thereby limited to comparison of the same glass type) resulted in 1148 discriminations (90.4%) by combining LIBS and RI at the 90% confidence limit. When the confidence limit was increased to 99%, the discrimination decreased to 79.9%. In contrast, LA-ICP-MS combined with RI analysis of a subset of the same glass samples resulted in 659 discriminations out of 666 comparisons (99% discrimination) at the 90% confidence limit. Increasing the confidence

level to 99% lowered the number of discriminations to 649 (97.5% discrimination). The discriminating power of LIBS in this study was lowered significantly by the results from a single set of glass samples, those from side-mirrors. A subset of the side-mirror samples exhibited high variance and low discrimination by RI and LIBS analysis. The combined LIBS+RI discrimination results for side-mirrors raise unanswered questions about the influence of sample heterogeneity on LIBS analysis of glass with 1064 nm light, which results in a very small amount of sample actually ablated into the plasma. When the side-mirror data is eliminated from the comparison, the LIBS+RI discrimination increases to 99.7% and discrimination by LIBS alone was 98.9% (90% confidence level). When excluding the side-mirror data, at the 99% confidence level LIBS+RI gave 89.9% discrimination LIBS alone gave 74.4% discrimination.

Direct comparison of questioned and known samples by linear correlation and rank correlation of peak intensities, as well as by Sorenson's peak counting parameter and a full spectral Euclidean distance calculation, failed to discriminate representative spectra of automobile side-window float glasses taken from the same sample sets. Spectra that were discriminated by selected emission intensity ratios were not discriminated by these less selective methods. The rank correlation method proved to be the best peak correlation method for discriminating between questioned and known samples. The utility of this method for casework samples will depend on further improvements in the data analysis method.

Based on the results of this investigation, single nanosecond pulse LIBS analysis of glass performed with 1064 nm light offers a potential discriminating capability when used in conjunction with refractive index. The method is nearly non-destructive, requires

little or no sample preparation and the analysis time is only a few minutes. LIBS offers an inexpensive glass analysis method affordable to most state and local crime laboratories, but care must be taken when the technique fails to discriminate between a questioned and known sample. Failure to discriminate in the sample sets examined here constitutes a Type II error, since the glasses are known to come from different sources. In Forensic Science, a Type II error, false inclusion or falsely linking an individual to a crime, is generally considered to be of greater social consequence than a Type I error, which constitutes false exclusion. In cases where two samples are not discriminated by LIBS or LIBS in combination with RI, a secondary analysis is recommended, preferably by LA-ICP-MS in combination with RI.

LIBS analysis techniques are constantly evolving and the newer dual pulse methods may offer a more reliable approach to forensic glass analysis. Dual pulse LIBS was not examined under this award, but is being addressed by other research groups. Samples that show greater diversity, for example automobile paints, may be more easily discriminated by single pulse LIBS. The analysis of paint samples is being addressed at the National Center for Forensic Science under funding from the National Institute of Justice.

References.

- 1 C. M Bridge, J. Powell, K. L. Vomvoris, J. M. MacInnis, M. R. Williams, M. E Sigman*, *Applied Spectroscopy*. **60**(10), 1181 (2006).
2. J. I. Thornton and D. Crim, *J. Forensic Sci.* **34**, 1323 (1989).
3. S. J. Pitts and B. J. Kratochvil, *J. Forensic Sci.* **36**, 122 (1991).

4. T. Parouchais, I. M. Warner, L. T. Palmer, and H. Kobus, *J. Forensic Sci.* **41**, 351 (1996).
5. R. D. Koons and J. Buscaglia, *J. Forensic Sci.* **44**, 496 (1999).
6. R. D. Koons and J. Buscaglia, *J. Forensic Sci.* **47**, 505 (2002).
7. D. C. Duckworth, S. J. Morton, C. K. Bayne, R. D. Koons, S. Montero, and J. R. Almirall, *J. Anal. Atomic Spec.* **17**, 662 (2002).
8. S. Montero, A. L. Hobbs, T. A. French, and J. R. Almirall, *J. Forensic Sci.*, **48**, 1101 (2003).
9. T. Trejos, S. Montero, and J. R. Almirall, *Anal. Bioanal. Chem.* **376**, 1255 (2003).
10. T. Trejos and J. R. Almirall, *Anal. Chem.* **76**, 1236 (2004).
11. Y.-I. Lee, J. Sneddon, *ISIJ International* **42**, S129 (2002).
12. W. Lee, J. Wu, Y. Lee, J. Sneddon, *Applied Spectroscopy Reviews* **39**, 27(2004).
13. D. W. Hahn, A. W. Miziolek, and V. Palleschi, *Applied Optics* **42**, 5937 (2003).
14. D. A. Rusak, B. C. Castle, B. W. Smith, J. D. Winefordner, *Trends in Analytical Chemistry* **17**, 453 (1998).
15. F. C. De Lucia, Jr., R. S. Harmon, K. L. McNesby, R. J. Winkel, Jr., A. W. Miziolek, *Applied Optics* **42**, 6148 (2003).
16. K. Meissner, A. Wokaun and D. Guenther, *Thin Solid Films* **453 – 454**, 316 (2004).
17. K. Muller and H. Stege, *Archaeometry* **45**, 421 (2003).
18. V. Lazic, R. Fantoni, F. Colao, A. Santagata, A. Morone and V. Spizzichino, *J. Anal. Atomic. Spectrom.* **19**, 429 (2004).
19. M. Ducreux-Zappa and J.-M. Mermet, *Spectrochimica Acta Part B* **51**, 321 (1996).
20. M. Ducreux-Zappa and J.-M. Mermet, *Spectrochimica Acta Part B* **51**, 333 (1996).

21. S. Klein, T. Stratoudaki, V. Zafirooulos, J. Hildenhagen, K. Dickmann and T. Lehmkuhl, *Applied Physics A* **69**, 441 (1999).
22. H. Kurniawan, K. Kagawa, M. Okamoto, M Ueda, T. Kobayashi and S. Nakajima, *Applied Spectroscopy* **50**, 299 (1996).
23. Y. I. Lee and J. Sneddon, *Analyst* **119**, 1441 (1994).
24. U. Panne, M. Clara, C. Haisch and R. Niessner, *Spectrochimica Acta Part B* **53**, 1969 (1998).
25. R. Russo, X. L. Mao, W. T. Chan, M. F. Bryant and W. F. Kinard, *J. Anal. Atomic Spectrom.* **10**, 295 (1995).
26. I. B. Gornushkin, B. W. Smith, H. Nasajpour and J. D. Winefordner, *Anal. Chem.* **71**, 5157 (1999).
27. C. A. Munson, F. C. De Lucia Jr., T. Pichler, K. L. McNesby and A. W. Miziolek *Spectrochimica Acta Part B* **60**, 1217 (2005).
28. M. Z. Martin, N. Labbe, T. G. Rials and S. D. Wullschleger *Spectrochimica Acta Part B* **60**, 1179 (2005).
29. R. D. Koons, J. Buscaglia, M. Bottrll and E. Miller. In R. Saferstein, editor. *Forensic Science Handbook* 2nd ed. Prentice Hall: Upper Saddle River, NJ, 2002; Vol. 1, 161-213.
30. Sachs, L. "Applied Statistics: A Handbook of Techniques," Springer Series in Statistics, Springer-Verlag, New York, 1984.

Appendix I: Scientific Presentations Resulting from this Award

1. Katie L. Vomvoris; Candice Bridge; Zachary M. Parker; Jean MacInnis; Michael Sigman “The Analysis of Commercial Blasting Agents by Laser Induced Breakdown Spectroscopy (LIBS), With Emphasis on Methods for Heterogeneous Samples” AAFS 58th Annual Meeting, February 20-25, 2006, Washington State Convention & Trade Center, Seattle, Washington.
2. Candice M. Bridge; Joseph Powell; Katie Vomvoris; Jean MacInnis; Michael E. Sigman, “Characterization of Automobile Float Glass With Laser Induced Breakdown Spectroscopy (LIBS) and Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS)” AAFS 58th Annual Meeting, February 20-25, 2006, Washington State Convention & Trade Center, Seattle, Washington.
3. Candice M. Bridge; Joseph Powell; Katie Vomvoris; Jean MacInnis; Michael E. Sigman, “Characterization of Automobile Float Glass With Laser Induced Breakdown Spectroscopy (LIBS) and Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS),” Florida American Chemical Society Meeting (FAME 2006) , May 11-13, 2006,Renaissance Orlando Hotel Airport, Orlando, FL.
4. Katie L. Steele, Candice M. Bridge and Michael E. Sigman “The analysis of Commercial Blasting Agents By Laser Induced Breakdown Spectroscopy (LIBS), with Emphasis on Methods for Heterogeneous Samples,” Florida American Chemical Society Meeting (FAME 2006) , May 11-13, 2006,Renaissance Orlando Hotel Airport, Orlando, FL.
5. Michael E. Sigman, Candice Bridge, Kate Vomvoris and Jean MacInnis, “LIBS: A New Tool for Forensic Science,” 2006 LACSEA, Optical Society of America, Incline Village, NV, February 9, 2006. **(INVITED)**
6. Michael E. Sigman “Application of Laser-Induced Breakdown Spectroscopy (LIBS) to Forensic Science: Analysis of Float Glass Samples,” General Forensics Technical Working Group, National Institute of Justice, Washington, DC, May 10, 2006.
7. Michael E. Sigman “Forensic Applications of Laser-Induced Breakdown Spectroscopy: Comparative Glass Analysis” 4th International Conference on Laser Induced Plasma Spectroscopy and Applications, Montréal, Canada, September 5-8, 2006.
8. Katie Steele, Candice M. Bridge and Michael E. Sigman "The Analysis of Commercial Blasting Agents by Laser-Induced Breakdown Spectroscopy (LIBS), with Emphasis on Methods for Heterogeneous Samples." Federation of Analytical Chemistry and Spectroscopy Societies, FACSS 2006, Orlando, FL, September 2006.
9. Candice M. Bridge, Katie Steele and Michael E. Sigman “Forensic Analysis of Glass by Laser-Induced Breakdown Spectroscopy.” Federation of Analytical Chemistry and

Spectroscopy Societies, FACSS 2006, Orlando, FL, September 2006.

- 10.** M. E. Sigman and C. M. Bridge, "Glass Sample Discrimination by Laser Induced Breakdown Spectroscopy (LIBS)." American Academy of Forensic Sciences, San Antonio, TX, February 19-24, 2007.

Appendix II: Sample Statistical Analysis

For each of h samples, some number n replicate measurements have been made. The number of replicates need not be the same for each sample.

The first step in the analysis is to perform an Analysis of Variance (ANOVA). Begin by calculating the standard deviation within groups, S_W , by the following equation;

$$S_W = \sqrt{\frac{\sum_{i=1}^h \sum_{j=1}^n (X_{i,j} - \bar{X}_i)^2}{N - h}}$$

where $X_{i,j}$ is the j^{th} replicate measurement of a parameter (emission ratio, refractive index, etc.) of the i^{th} sample, \bar{X}_i is the average value of the parameter for the i^{th} sample, and N is the total number of replicates. The total number of replicates may be calculated as $h * n$

if each of the h samples contains n replicates, otherwise N may be calculated as $\sum_{i=1}^h n_i$,

the number of replicates for each sample summed over all h samples.

To examine the discrimination potential of a ratio, we must also know the standard deviation between groups, which is calculated by the following equation;

$$S_B = \sqrt{\frac{\sum_{i=1}^h n_i (\bar{X}_i - \bar{\bar{X}})^2}{h - 1}}$$

where $\bar{\bar{X}}$ is the global mean and all other symbols are as defined above. From S_W and S_B ,

the F-value can be calculated as; $F = \frac{S_B^2}{S_W^2}$. If the calculated F exceeds $F_{\text{Critical}}(h-1, N-h, \alpha)$,

then at least two of the samples can be differentiated at the α significance level using the parameter for which F was calculated. The F_{Critical} values can be obtained from standard tables. In order to select a minimal set of parameters that will offer the highest overall discriminating power, it is important to choose parameters that do not contain the same information content. One way to make this selection is to choose a set of parameters that are not highly correlated. To achieve this goal, the Pearson correlation values, r , are calculated between each pair of parameters. The Pearson correlation value between parameters X and Y, denoted $r_{X,Y}$, is calculated as;

$$r_{X,Y} = \frac{\sum_{i=1}^h \left\{ (\bar{X}_i - \bar{X})(\bar{Y}_i - \bar{Y}) \right\}}{\left\{ \left[\sum_{i=1}^h (\bar{X}_i - \bar{X})^2 \right] \left[\sum_{i=1}^h (\bar{Y}_i - \bar{Y})^2 \right] \right\}}$$

where the symbols associated with parameter Y are defined analogous to those given above for parameter X. A matrix of $r_{X,Y}$ values is constructed, a Pearson's correlation matrix, and those parameters having a correlation less than some predefined cutoff value should be chosen as discriminating pairs. Typically the cutoff value will be approximately 0.6.

Once the set of discriminating parameters has been chosen, each parameter is used in pairwise comparisons between all samples to determine if each parameter discriminates between two samples. This comparison is made using a Tukey Honestly Significant Difference (HSD) test following the ANOVA analysis discussed above (i.e. this is a post-hoc test). Two samples (A and B for example) are considered to be different when the following condition holds;

$$|\bar{X}_A - \bar{X}_B| \geq \left(\frac{S_w}{\sqrt{n}} \right) * Q(\alpha, h, df)$$

where \bar{X}_A and \bar{X}_B are the average values for parameter X over samples A and B, S_w is the within group standard deviation calculated from the ANOVA analysis and $Q(\alpha, h, df)$ is the critical value for a significance level α , for h samples with df degrees of freedom calculated as $N-h$. The Tukey HSD test allows for a comparison between multiple samples within a group (i.e. between two samples or all pairwise comparisons between 20 samples) while controlling the significance level α for the entire set of comparisons.

The results from pairwise comparisons for each parameter are used to construct discrimination matrices. These matrices are symmetrical about the diagonal and therefore only $\frac{1}{2}$ of the matrix is required. Each entry in the matrix is binary, indicating that the two samples are discriminated (1) or they are not discriminated (0). The following two matrices are given as a simple example of the comparison of samples A – D using parameters X and Y. The third Matrix is the simple logical OR combination of the two matrices.

Discrimination Matrix

Parameter X

	A	B	C	D
A	0			
B	1	0		
C	0	1	0	
D	0	0	1	0

Discrimination Matrix

Parameter Y

	A	B	C	D
A	0			
B	1	0		
C	1	0	0	
D	1	0	0	0

Combined
Discrimination Matrix

	A	B	C	D
A	0			
B	1	0		
C	1	1	0	
D	1	0	1	0

In the discrimination matrix for Parameter X, sample pairs (B,A), (C,B) and (D,C) are discriminated by parameter X, as indicated by an entry of 1 in the appropriate position within the table. Each position in the table is designated as a (Row, Column) pair. The diagonal is composed entirely of 0 entries, since a sample can obviously not be discriminated from itself. Similarly, the discrimination matrix for Parameter Y indicates discrimination of sample pairs (B,A), (C,A) and (D,A). The logical OR combination of the two discrimination matrices results in the Combined Discrimination Matrix. For example, if (B,A) is 1 in either Parameter X – OR – Parameter Y discrimination matrices, the Combined Discrimination Matrix entry for (B,A) is a 1, otherwise the entry is a 0. Since there are four samples in this example ($h = 4$), the total number of unique pairwise comparisons, pc , is given by;

$$pc = \frac{h(h-1)}{2}$$

The fraction of pairwise discriminations which could be made utilizing both parameters X and Y is given by the sum over all entries in the Total Discrimination Matrix, divided by pc . The percent discrimination is obtained by multiplying the ratio by 100. In the

example given, the sum over all entries in the Total Discrimination Matrix is 5, $pc = 4(4 - 1)/2 = 6$, and the percent discrimination is $5/6 * 100 = 83.3\%$