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TECHNICAL NOTE

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Analytical Survey of Restorative Resins by SEM/EDS and XRF: Databases for Forensic Purposes*

ABSTRACT: Frequently in forensic cases, unknown substances must be identified. Automated databases can ease the burden of comparison as materials may be compared against many known standards in a relatively short period of time. It has been shown that dental resins can be named according to brand or brand group even in conditions as harsh as cremation. Databases are already in use for many materials, but no such database exists for dental resins. Thus, two databases were generated. One utilized a laboratory-based method, scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDS), in conjunction with the Spectral Library Identification and Classification Explorer (SLICE) software. The other was based on portable X-ray fluorescence (XRF). The ability to perform database comparison with portable instrumentation can thus be brought directly to the field. Both the SLICE and XRF databases were evaluated by testing unknown resins. EDS is a well-established technique and the SLICE program was demonstrated to be a good tool for unknown resin identification. Portable XRF is a relatively new instrument in this regard and its databases have been constructed mostly for metal alloy comparison and environmental soil testing. However, by creation of a custom spectral library, it was possible to distinguish resin brand and bone and tooth from other substances.

KEYWORDS: forensic science, forensic odontology, restorative resins, victim identification, SEM/EDS, XRF, database

The presentation of incinerated and fragmented remains can create a challenging situation for identification of the victim. Standard methods of identification, such as fingerprint comparison and DNA analysis may not be possible if the damage is extreme. Dental X-ray comparison may also not be feasible if the structural relationship of the dentition has been destroyed.

High temperatures can dramatically alter the physical appearance of the teeth as they become fragile and subject to shrinkage and fragmentation. The high temperatures however, will not destroy most dental materials. Dental resins, in particular, can survive conditions as harsh as cremation. Not only do they survive these conditions, they retain the ability to be named by brand or brand group (1,2). This may provide a valuable clue when little evidence exists. In circumstances when traditional methods have been depleted, any additional information that can be gained, such as brand name of dental resins, can be significant. The importance of this technique was demonstrated in a 1999 murder case. The ability to name the resin cement, which was found in an incinerated tooth, proved pivotal in identification of the victim. This information aided in conviction of the suspect (3).

In our previous work, we described the unique differences between 10 brands of resin currently available on the market. We have also shown that these resins are able to withstand conditions as harsh as cremation. Not only did they survive, they were retrievable and identifiable by brand name or brand group (1). The analytical method used in that study was scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDS). SEM/EDS is a reliable and reproducible technique that produces images of high resolution as well as an X-ray spectrum that represents an elemental fingerprint of that product. In a subsequent study, we showed that these materials can also be identified by the technique of X-ray fluorescence (XRF) (2). Both of these methods were used in the generation of spectral databases for dental resins with the intent of simplifying identification of these materials.

Restorative resins are principally composed of an organic matrix surrounding inorganic filler particles. The inorganic fillers are added mainly for physical properties and radiopacity purposes. Manufacturers try to achieve a radiopacity range that is greater than or equal to that of enamel. Heavy elements, such as Sr, Ba, Zr, and Yb are incorporated in unique combinations to satisfy this requirement. It is these inorganic elemental combinations that remain virtually unchanged even after exposure to cremation conditions and allow for the distinction between brands or brand groups.

When an individual has been incinerated, restorations can be found still in place, dislodged and hidden among debris or in microscopic amounts still adhering to enamel and dentin pieces (Fig. 1). When unknown materials are collected from a debris field, identification of important evidence can be difficult because these materials must be compared with a known standard. Automated databases can make rapid comparisons, thus avoiding time-consuming manual comparison of spectra, especially in situations in which a large variation in composition may be encountered. Many databases currently exist to aid in determining the identity of a wide variety of different materials. These databases are dependent on the spectroscopy utilized. Spectral databases are well established for techniques such as Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy, both of which are based on organic composition. A new database system has now been generated and is based on inorganic elemental composition using EDS spectra derived from SEM/EDS analysis (4,5).

This system is the Spectral Library Identification and Classification Explorer (SLICE) software that was developed under contract

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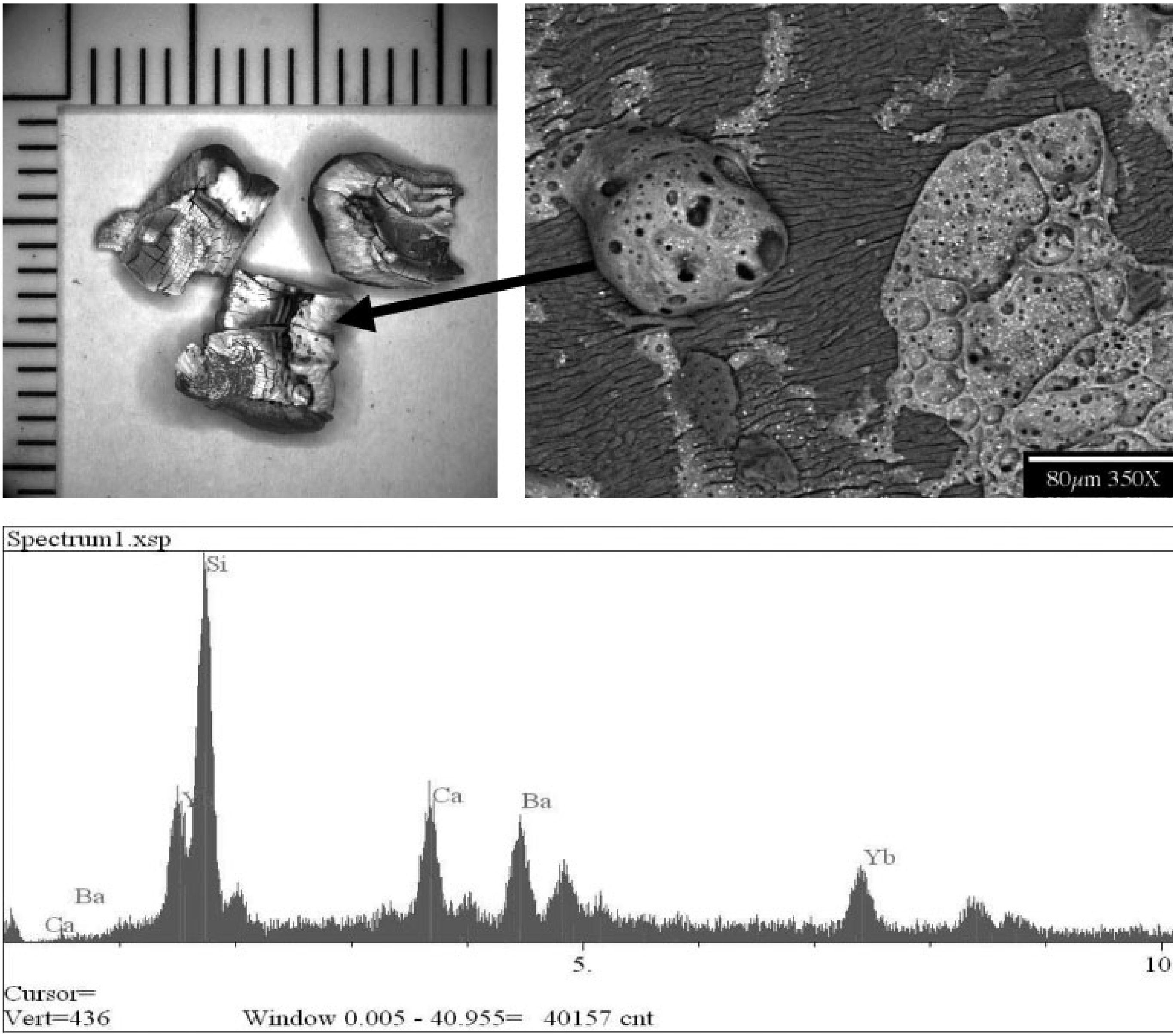

FIG. 1—*Incinerated enamel fragments with suspected resin particles remaining (upper left). Backscattered SEM image of suspected resin (upper right). EDS spectra showing resin to be consistent with Tetric Ceram or 4 Seasons.*

from the FBI. This database consists of storage, query, and display utilities. The advantages of SLICE are the ability to archive spectra, images, and information in true database architecture.

SLICE has two main functions. The first is the archival storage of data with addition of any pertinent information. Thus, there are fields that can hold not only spectra, but also other kinds of data such as images or descriptive text. For resins, this could include microstructural information and XRF analysis, as well as general information such as manufacturer data, lot number, analysis parameters, and any other pertinent physical characteristics.

The second function is a query of the archives. Queries can be based on individual or multiple criteria. SLICE allows for comparison of unknown spectra to those stored in the database. Matches are determined based on similarity of the composition of the material. An order of ranking is produced according to a selected profile. Thus, the materials are listed according to their correspondence to the unknown.

The technique of XRF spectroscopy is similar to EDS in that an X-ray spectrum is obtained which represents an elemental fingerprint of the sample. The main difference between XRF and EDS is the excitation radiation. XRF uses an X-ray beam to generate characteristic X-rays, whereas EDS uses an electron beam. The characteristic X-rays constitute the spectra. One of the advantages of XRF is the ability to detect major, minor, and trace levels of an element, whereas EDS is limited to major and minor elemental concentrations. Therefore, the detection limit for XRF is around 10 part per million (p.p.m.) and EDS is around 1% (6,7).

In a previous study, we also demonstrated the usefulness of XRF analysis in resin brand discrimination (2). These units are small, lightweight, battery operated, and can be operated either by an onboard palm type computer or by an external laptop. XRF systems may come with standard reference libraries, the most common being metal alloys, but custom libraries can be created by the user from other substances, such as restorative resins. As a result, the unit can be used in "point and shoot" mode to rapidly identify presumptive evidence in the field. XRF has the advantage of being portable, thus allowing it to be brought directly to the site for analysis of materials *in situ.*

Materials and Methods

Thirty-two modern resins available in the U.S. were collected. Table 1 lists the name of resin and manufacturer. These materials were limited to restorative resins and did not include other classes of materials such as flowable resins, compomers, glass ionomers, and sealants, although in the future it is anticipated that these materials will be added to the database.

The resins used did include all categories of composite restorative resin, such as hybrids, micro-hybrids, micro-fills, macro-fills, nano-fills, and packable resins. Disks of resin 1 cm in diameter were prepared and light cured according to manufacturers' directions. The disks were embedded in acrylic. The surface was then polished with an automatic metallographic polishing unit to a final polish of 3-μm diamond.

Each disk was analyzed using SEM/EDS. An EDS spectrum was collected at 500× magnification with a collection time of 300 sec. This low magnification was selected to provide an analysis area sufficient to produce the average composition of each sample. As the spectrum was to be used as a reference, a long collection time was required in order to obtain the best signal to noise ratio. Representative backscattered electron images were also collected at 500×, 1000×, and 5000×. The spectra were then converted to the Electron Microscopy Society of America (EMSA) format that is an industry standard. This is internationally recognizable and universally formatted.

The EDS spectra were uploaded to the SLICE software, each forming the basis of an individual record. Descriptive text including manufacturer, lot number, expiration date, and trace element concentration were then added to the appropriate fields in each record. The backscattered images were also added to the page. This data entry sequence was repeated for each resin. Figure 2 shows a representative of an individual record page.

The same resins were analyzed by XRF to create a custom spectral reference library. The unit used for this study was the Alpha II, supplied by Innov-X Systems (Woburn, MA). The resin disks were analyzed in a laboratory test stand and reference spectra were generated. A sample of bone and tooth was also added to the library. This was performed by a function on the XRF that allows for addition of spectra into the custom spectral library in the unit. This mode was used for incorporation of all of the new spectra.

The resins were also analyzed using XRF to determine the trace elemental concentrations. The trace analysis revealed additional elements not displayed with EDS. In order for an element to be detectable in an EDS spectrum, it must be present in a concentration >1%. Thus for the resin brands that contained Ba, trace amounts of Sr were also detected using XRF. Trace amounts of Zr were seen for the Ivoclar (Amherst, NY) brands Tetric Ceram, Tetric Evo, and 4 Seasons. These three brands contained Zr at around 1%. EDS will not detect this low concentration if it happens to fall under 1%. It will, however, be detected readily with XRF. The results of these analyses were recorded for addition to the SLICE database, and provided for an additional discriminator between resin brands. Both the SLICE resin EDS database and the XRF custom spectral library were tested by analyzing a number of unknown samples of resin.

TABLE 1—*List of modern resins and manufacturers in the spectral database.*

Product name	Manufacturer
Surefil	Dentsply Caulk (Milford, DE)
Esthet-X	Dentsply Caulk
TPH3	Dentsply Caulk
Prisma APH	Dentsply Caulk
Quixx	Dentsply Caulk
Filtek Supreme	3M (St. Paul, MN)
Filtek Z250	3M
Filtek Z100	3M
Filtek P60	3M
Premise	Kerr (Orange, CA)
Prodigy	Kerr
Herculite XRV	Kerr
Point 4	Kerr
ICE	SDI (Bensenville, IL)
ROK	SDI
Glacier	SDI
Tetric Evo Ceram	Ivoclar Vivadent (Amherst, NY)
Tetric Ceram	Ivoclar Vivadent
Heliomolar	Ivoclar Vivadent
4 Seasons	Ivoclar Vivadent
3-D Direct	Vident (Brea, CA)
Gradia	GC America (Alsip, IL)
Grandio	VOCO (Cukhaven, Germany)
EPIC-AP	Parkell (Farmington, NY)
Miris	Whaledent (Cuyahoga Falls, OH)
Synergy	Whaledent
Durafil	Heraeus Kulzer (Armonk, NY)
Venus	Heraeus Kulzer
Charisma	Heraeus Kulzer
Amelogen	Ultradent (South Jordan, UT)
Vit-l-escence	Ultradent
Estelite	Tokuyama (Tokyo, Japan)

Results

The search function of SLICE allows query of the database. Queries from the database may be made by selecting individual or multiple criteria from the search function. These criteria include keyword, composition, date, instrument parameters, and "best fit."

Keyword criteria will use any word in the text fields that are associated with the spectrum, including the notes page. The notes page exists for any additional written information associated with the product. The composition parameter searches the database for the presence of a certain element or percent of that element in the sample. The date category can include date of manufacture or date of analysis. Instrument parameters include accelerating voltage, detector type, or window material, which are conditions of analysis associated with the SEM. The "best fit" criteria can search the entire database or portions of it and spectra are chosen by closest comparison to the unknown. The results are displayed in a window that lists the spectra having the closest fit. The best-fit mode was the parameter used for the test samples of resin. In almost all of the test cases, when presented with an unknown, the SLICE software successfully returned the correct answer as the best match in this mode.

There were several combinations of resins that could not be distinguished with reproducibility based on EDS spectral analysis alone. In these circumstances, the other information on the query page, such as microstructure and XRF analysis, allowed distinction of brand. Only two brands of resin could not be distinguished as individuals. These were Tetric Ceram and 4 Seasons, both manufactured by Ivoclar. These resins were identical in all of the parameters used for this study. An appropriate notation was made in the

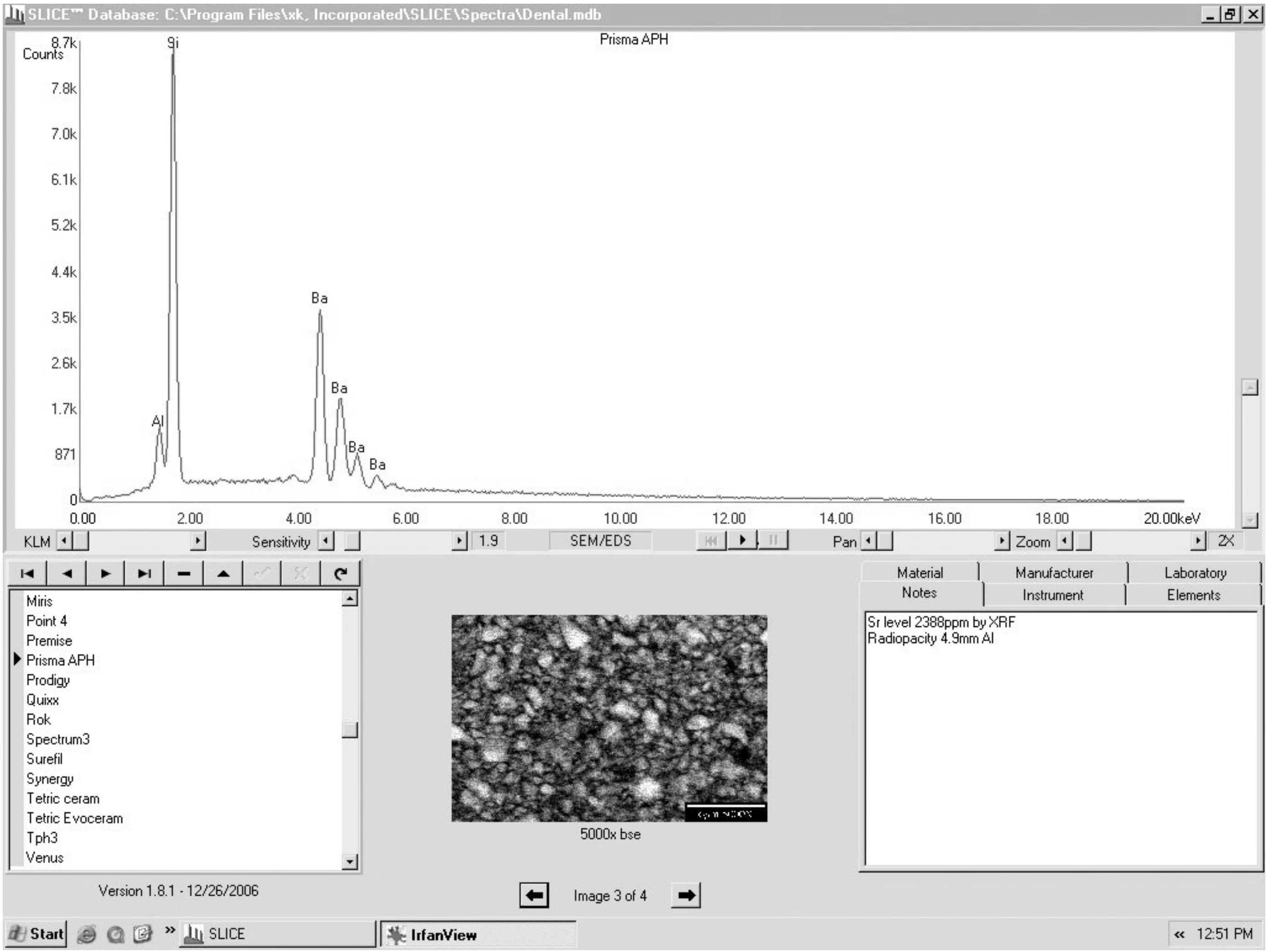

FIG. 2—*Data page from SLICE program.*

descriptive text section to alert investigators to the fact that these materials are the same.

The custom spectral library generated with the portable XRF was also tested by analyzing unknown resins. Similarly, in most test cases, the correct brand name was identified based on spectral fit. Again, the only two brands that could not be distinguished were Tetric Ceram and 4 Seasons. The machine was programmed to read that either of these two could be the appropriate choice.

An advantage of XRF was the speed and ease of analysis. The spectral database displayed the name of the resin brand in as quickly as 6–10 sec. Figure 3 shows a computer screen capture from the XRF analysis, listing the name of the unknown resin. It was also possible to distinguish bone and tooth from other substances in the same time frame. This analysis took place without introduction of a sample to a vacuum chamber, as would be the case for SEM/EDS. Thus, the overall time taken to perform this analysis was minimal. This illustrates the great potential of this technique for screening large numbers of samples, or use in the field.

Discussion

This paper describes database generation for dental materials using two analytical techniques. The database generated using EDS spectra and the SLICE software is now curated by the FBI and is available to the forensic community.

It is anticipated that this database will need updating on a yearly basis to incorporate new products as they come on the market. The most challenging point to the construction of the database was the initial collection of data from numerous products. With the baseline supplied from this study, however, expansion and upkeep of the database is relatively simple. The software allows for easy addition of new entries, and as new products come on the market they will be added. If products stay the same, a notation will be made under the descriptive text.

Most of the generational changes noticed in these products can be seen in the microstructure of these resins, as there is a current trend to bring nano-filled materials to the market. The inorganic elemental composition of a given product, however, appears to be rarely altered. Some products remain virtually unchanged with time. Figure 4 shows the microstructure and EDS spectra comparing Heliomolar manufactured in 1996 to that manufactured in 2006. Twenty-three historical resins were examined, the earliest dating to 1971, to investigate how these materials have evolved. Table 2 lists 23 historical materials acquired to date. Once a more complete compilation of historical resins is collected, they too will be added to the database. Knowledge of the time frame that a product was on the market may have

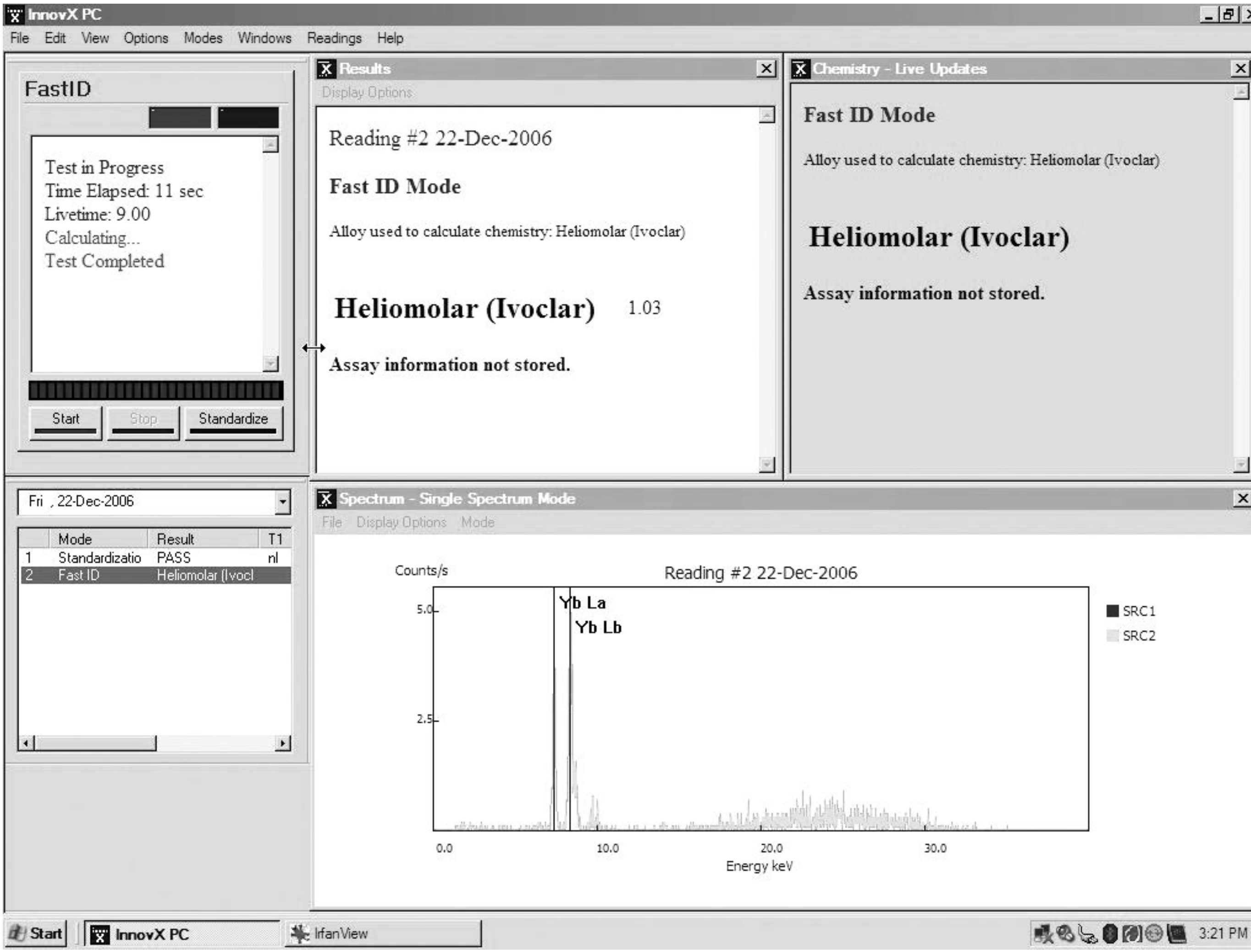

FIG. 3—*Screen capture from analysis with portable XRF. The unknown is correctly identified as Heliomolar.*

significance in placing remains within a certain historical window.

The additional parameters of the SLICE program can become useful when products have similar spectra and further distinction is necessary. Based on EDS elemental composition, the resins were separated into 11 distinct groups. The 11 groups were further subdivided based on elemental percent concentrations of the same elements. One particular group which all contained Si, Al, and Ba had elemental ratios that were very similar as analyzed using EDS. The "best fit" algorithm in the SLICE software was almost always able to discriminate between these samples. However, with brands that are similar, additional parameters could be utilized.

The combination of XRF and EDS analysis can be a valuable discriminator based on the ability of the XRF in separating these products based on p.p.m. concentration of Sr. The Si/Al/Ba group had p.p.m. Sr amounts ranging from 176 to 3700 p.p.m., and this analysis could further help to distinguish resins in this group.

One disadvantage of portable XRF is the inability to detect silicon. This instrument cannot detect below P in the periodic table because of the absorption of low-energy X-rays in air. As silicon is common to all resin brands, its absence from the analysis will not affect the outcome. It is only those resins that use only silica as the primary filler that will not be recognized. Portable XRF will therefore not be a useful technique to analyze such resins. This problem does not exist with SEM. Because samples are analyzed in a vacuum chamber, Si is readily detectable.

Another discriminator was microstructure. The microstructure of many of the resins varied considerably, even within the same classes of resins. For example, within the same categories as defined by the manufacturers, resins labeled nano-fill, hybrid, micro-fill, or packable varied greatly in microstructure.

When elemental composition and XRF is combined with microstructure, almost all of the resins can easily be distinguished as individuals. The only one pair that could not be distinguished was Tetric Ceram and 4 Seasons. These two resins were identical within all of the parameters encompassed in this study. It may be a logical assumption to conclude that these materials are the same, and are only marketed under different names. Organic analysis was not used, however, and by the use of techniques such as FTIR and Raman spectroscopy it may be possible to distinguish differences in the organic matrix of these two products.

These databases cannot only identify dental materials, but can also be a quick screening tool for biological material such as bone or tooth (6). Thus in a situation such as the World Trade Center disaster, when small particles of unknown substances must be screened, portable instrumentation can be a valuable quick

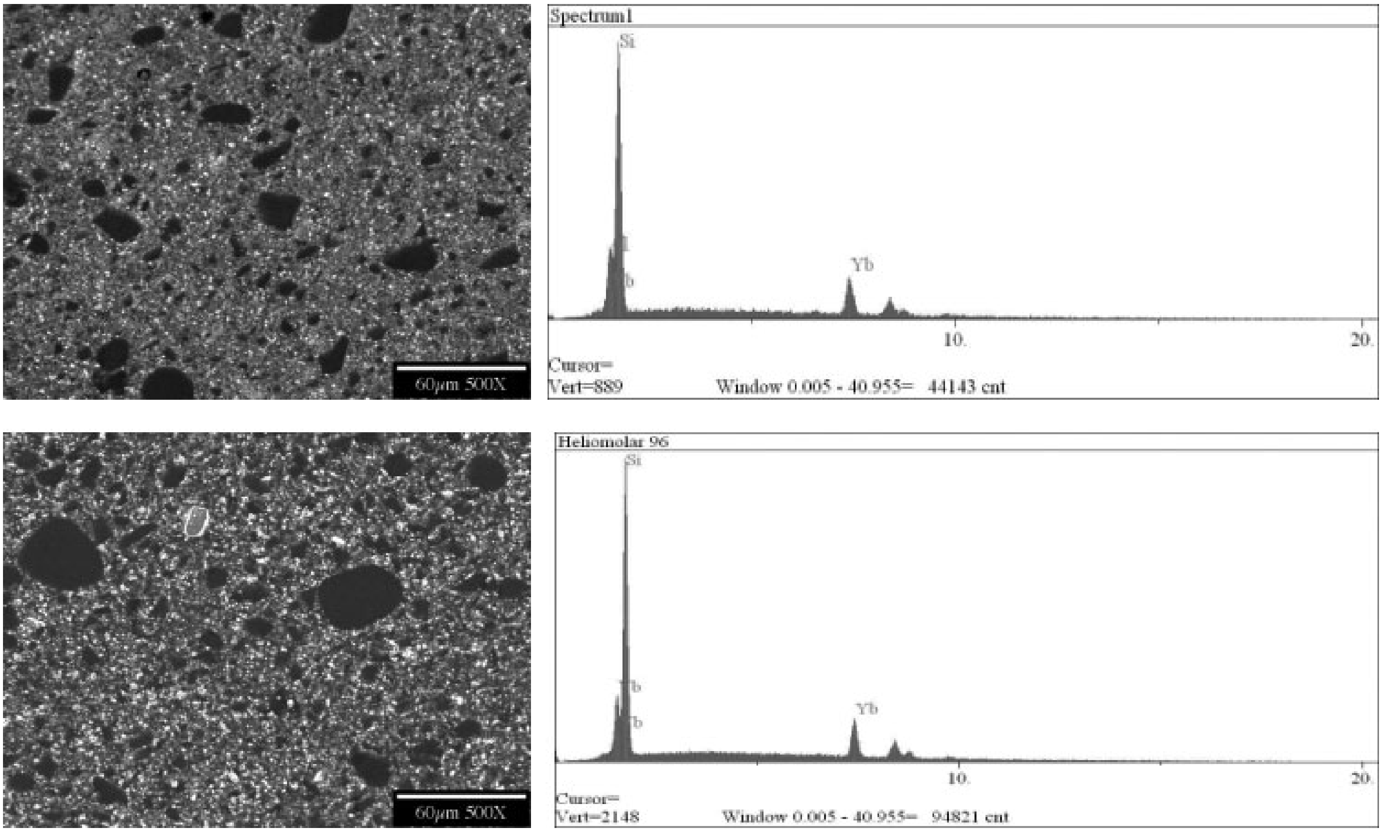

FIG. 4—*Microstructure and EDS spectra comparing Heliomolar from 1996 to that manufactured in 2006. Notice almost no difference between the two samples.*

TABLE 2—*List of historical resins and manufacturers acquired to date.*

Product name	Manufacturer
SMILE	Kerr (Orange, CA)
Command Ultrafine	Kerr
Adaptic HRI	Johnson and Johnson (East Windsor, NJ)
Aurafil	Johnson and Johnson
Distalite	Johnson and Johnson
Finesse	Caulk (Milford, DE)
Fulfil Surepac	Caulk
Concise	3M (St. Paul, MN)
Concise light cure	3M
P-10	3M
Silux	3M
Addent XV	3M
Isomolar	Vivadent (Schaan, Lichtenstein)
Visiofil	ESPE (Norristown, PA)
Visio Disperg	ESPE
LITE	Phasealloy (El Cajon, CA)
Opalux	ICI (Manchester, U.K.)
Bioglass	Dentsply (Milford, DE)
Prismafil	Dentsply
TPH Spectrum	Dentsply
Occlusion	COE laboratories (Chicago, IL)
Profile	Pennwalt (Philadelphia, PA)
Lumifor	Columbus Dent (St. Louis, MO)
Tetric Ceram 2002	Ivoclar Vivadent (Amherst, NY)
Heliomolar 1996	Ivoclar Vivadent

screening tool for presumptive analysis as it can rapidly distinguish tooth and/or bone from foreign substances.

For this database to be useful on an international basis, it is imperative to have materials from a global society added to the database. It is quite possible that resins manufactured in the U.S. are marketed under different names in other countries, and vice versa. This type of information could be added to the descriptive text section of the SLICE database. Also, other countries undoubtedly have materials that are unique to that country. The presence and recognition of such materials could provide clues as to the nationality of a victim. Knowledge of the nationality of the victim could be a key piece of information in order to establish identity. A database will only be as good as the amount of entries in it and it will be important to have international collaboration on such an endeavor.

It is the understanding of the authors that this database may only be used in rare circumstances. Nonetheless, the existence of such a database would be a valuable asset if needed. It provides essential additional information that forms the basis for case investigation and evidence collection, especially in situations when very few clues remain. Documentation and interpretation of evidence can provide the necessary profile to solve a complicated case. It must be stressed that in order for this technique to be of value, detailed documentation of procedures performed, including brand name of materials, must exist in the victim's dental chart.

It must also be stressed that this technique is not intended to replace conventional methodology, but serve as an aid when traditional methods are exhausted or when additional scientific corroboration is needed.

Acknowledgment

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