A 'Different' Kind of Microscopy

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At the August M&M-2006 meeting in Chicago, we were standing next to our poster titled A Different Kind of Microscopy: Analyzing Features with an Automated Electron Beam when an acquaintance with long experience in electron microscopy wandered by. After a glance at the poster title, he challenged: "What's different about that?" Upon hearing our summary he asked (with what we took to be an encouraging tone of voice): "Are you going to publish this?" We had enough similar reactions from others to make that seem a good idea, and this is the result.

Why "Different"?

This discussion should begin by noting that the operative word in the title is "different," not "new." In point of fact, the foundations for the technique were laid in the 1970s when some workers began putting scanning electron microscopes and microprobes under software control by interfacing them to the "minicomputers" that powered the computerized x-ray analyzer units then entering the market. Even prior to this, there were a few "hard-wired" image analyzers that mechanized the process of extracting information from microscope images.[1] Thus, automated analysis of features via an electron beam instrument is hardly a *new* concept.

However, the reality is that instruments of the type discussed in this article are frequently found in places where you wouldn't expect a traditional SEM, and consequently there are many microscopists who are unaware of how the technology has evolved, where it is being used, and what a modern optimized unit can do. It is not unusual to encounter otherwise knowledgeable people who think of automated analysis only as another accessory capability to supplement the traditional manual techniques. However, automated analysis has also proven to be a uniquely powerful turn-key technique for specialized tasks and environments where the practice can be quite different from the conventional kind of microscopy that most readers of this publication are customarily engaged in. That being said, however, there is a common foundation of theory and technology on which both types of microscopy are based, and both types are often needed to provide the complete picture. So, although there are indeed significant distinctions, it is important that there be both awareness and appreciation between these different but complementary kinds of microscopy. The differences are conveniently summarized under the headings of: (1) Motivation; (2) Instrumentation; and (3) Quality Control.

Motivation

Conventional microscopy is typically concerned with in-depth understanding of individual instances, whereas automated microscopy is concerned with characterizing populations. That distinction becomes quite obvious when a conventional microscopist is asked statistically loaded questions such as: "What is the size distribution of this kind of feature?"; "How often does that kind of particle occur?"; or "How do I know that my process is consistently producing a pure product?" Unless one is willing to extrapolate from rather small sample sizes, answers to such questions require analysis of statistically significant numbers of both features and specimens. Meaningful answers may involve accurate characterization of tens of thousands, hundreds of thousands, or even millions of instances, a prospect that clearly is not amenable to manual techniques, but is the *forte* of an automated instrument.

If conventional microscopy is an "art," then the practice of automated microscopy described here is more like "accounting." Indeed, the repetitive tasks at which an automated instrument excels would be incredibly boring for a microscopist. The applications themselves, however, can be both interesting and important. Thanks to criminal investigation shows such as the popular CSI franchise, there is an increased awareness that microscopic gun shot residue (GSR) particles found on a suspect are evidence of being in proximity when a firearm was discharged. Finding and identifying these characteristic Pb/Ba/Sb particles was originally developed with manual SEM/EDX units. However, as the particles can be submicron and the sampling surface so large (typically a set of three or four half-inch diameter stubs), automation of the search process is a practical necessity. In this case, the motivation is simply to automate a particularly tedious part of the overall analysis. Once characteristic GSR particles have been located for inspection, a trained forensic microscopist makes the final determination, operating the SEM/EDX in a conventional fashion. While the automation efficiently detects and classifies potential GSR particles, it is ultimately the credibility of a human operator that makes this analysis a viable form of evidence.

At the other end of the spectrum, there are applications where extremely important analyses are being conducted entirely by an automated instrument. An example is the use of automated SEM/EDX to predict bearing failure in jet engines. Figure 1 shows a specially packaged and ruggedized version of an instrument that the US Air Force deploys around the world in support of aircraft squadrons. Following each sortie, metallic wear debris is removed from a magnetic chip detector located in the engine's lubrication stream, transferred to a suitable substrate, and placed in the automated Jet Engine Maintenance Monitor (JEMM™) for analysis. Based upon the number, size, and composition of observed fragments, a risk level is assigned that warns of the onset of bearing failure. This very important analysis has been credited with saving significant numbers

Figure 1. The JEMM is a self-contained and ruggedly packaged automated SEM/EDX unit that is deployed by the US Air Force in support of aircraft squadrons.

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of aircraft and is now required practice for certain airframe/engine configurations. An application like this is certainly not "microscopy" in a conventional sense, nor are the practitioners "microscopists." In fact, one of the most critical requirements for the JEMM unit is that it must produce highly reliable output when operated by enlisted personnel who have received only basic procedural training.

Somewhere in between these two extremes fall various industrial applications concerned with particulate contamination. In the automotive industry, leading manufacturers are employing automated SEM/EDX systems to validate the processes used to clean critical assemblies. As closer tolerances and finer orifices are becoming commonplace, particulate contamination is an increasing area of concern (Figure 2). Though the particles flushed out of these assemblies are typically of a size amenable to light-optical methods, x-ray identification of the material is critical to the application. Another growing application is the use of an automated SEM/EDX for monitoring of foreign particles in pharmaceuticals. The drug formulation is put into solution and the solid material extracted onto a membrane filter. After drying, the filter is analyzed using automated SEM/EDX. Electron beam analysis is being chosen for this kind of quality-control application because of the limitations of more traditional bench-type instruments, such as flow counters based on light-obscuration. Traditional light-optical systems cannot reliably detect and characterize the micron-scale particles that are of increasing concern.

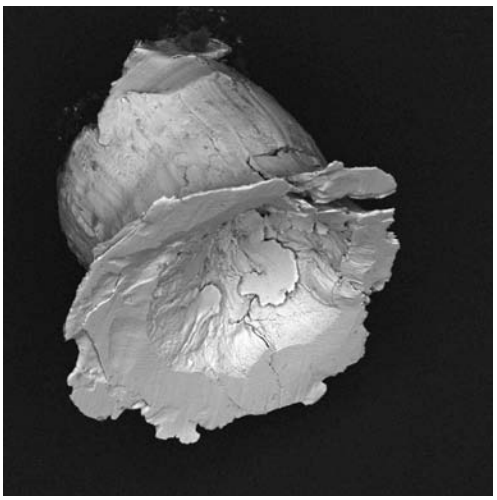

Figure 2. Foreign particles such as this one removed from the fuel injector of a diesel engine represent a major reliability issue for modern high-tolerance mechanisms (diameter ~100 microns).

This brief discussion is intended to illustrate that these are not the kinds of applications that would be welcome in a conventional microscopy laboratory. Indeed, all of the above would be quite impractical as routine procedures if performed by a human operator seated at a manual instrument.

It can be generalized that the fundamental distinction between the motivation for conventional microscopy and the automated kind discussed here is that the former views the SEM/EDX as a flexible instrument for investigation – a sensory extension of the human operator as it were – whereas users of the latter simply regard it as a tool that addresses a class of measurement problems.

Instrumentation

The optimal form for any tool will always follow from the function it is expected to perform. In the case of the automated instrument, the principal functional requirements can be summarized as throughput and information. *Throughput* refers to the ability to conduct the analysis with the speed and timeliness that the application requires (usually, "as fast as possible"). *Information* is used here as a generic term encompassing accuracy, detail, and precision (*i.e.*, "what one needs to know").

The twin requirements of information and throughput have important implications regarding how an automated SEM/EDX tool should be constructed. This might at first seem a puzzling statement, since, when it comes down to basic principles; doesn't one SEM operate pretty much like any other? That is certainly true when discussing conventional instruments that are all designed around the same central task – capturing and presenting pictorial information. In other words, a conventional SEM is employed as a kind of magnifying camera. But a SEM is not a camera! (Figure 3) It is efficient and natural for a camera to collect full image frames, whereas for an SEM the time-consuming rastering of the beam to acquire a full image is a concession to the human visual system. So when the human operator is removed from the equation, that is when the interpretation of information will be made by a digital computer, the optimal solution changes. Efficient automation of a SEM/EDX builds on the fact that the focused electron beam can be positioned dynamically and selectively to sense the presence and characteristics of features without collecting full image frames.

Every SEM operator quickly learns that the collection time and information content of micrographs can't be considered separately, but must be traded against each other. For example, to achieve a finer measurement at the same magnification, a smaller pixel size must be used and since this increases the number of pixels as the inverse square of the pixel dimension, the frame time also increases as the square. To make use of that smaller pixel size, a smaller beam spot must also be used and this worsens signal-to-noise, which can be offset only by increasing the pixel time. In other words, anything that a SEM operator does to increase information comes at the expense of throughput and vice versa. These effects are unique to the SEM in that exposure time in a camera is not intrinsically tied to the pixel resolution. The SEM is different, first because of the sequential point-wise exposure mechanism, and second because of the way the illumination of a pixel is limited by source physics. The bottom line is that, unlike a camera, each and every SEM pixel costs a separate and substantial exposure time that can be reduced only at the expense of information. This difference is important because automating the SEM as just another kind of camera that generates full frames of image information results in degraded throughput, degraded information, or both. Rather, optimal automation requires that the number of pixels be held to a minimum, but without sacrifice of information.

As a model of how this can be achieved, it's instructive to consider the way the human vision system collects information. Although we have the sense of looking at complete scenes, we know that we do not process the whole visual field uniformly. Rather, our brain dynamically directs our attention to those features that are of greatest interest and uses sophisticated pattern recognition to extract the relevant data quickly. Similarly, an efficient SEM-based implementation can make a relatively small number of measurements to determine where features are present, and then dynamically use this information to make more precise local measurements at each site of interest.

It is almost always desirable to perform feature measurements with a pixel resolution that is a fraction of the smallest feature size of interest. In a frame-based implementation, where the image is first captured and then analyzed as a static field, this requires that all pixels be acquired at the finest (measurement) resolution and with the same dwell interval. In a dynamic implementation, however, different conditions can be used while searching for features and

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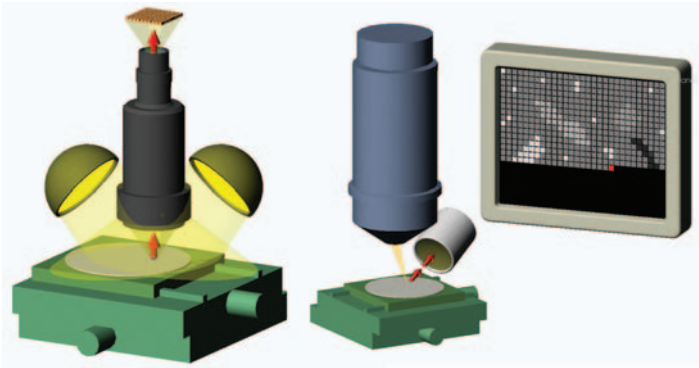

Figure 3. A camera system (left) employs broad illumination and focusing optics to capture all pixels of the field simultaneously on the sensing array. A SEM (right) uses a moving focused beam spot to illuminate the image pixels sequentially.

for characterizing them once found. For example, if features down to 1 micron in size are to be characterized with a resolution of 1/8 micron, the searching can be carried out with a pixel spacing of ~1 micron (*search* grid) and then switched to 1/8 micron spacing (*measure* grid) for determining the size and shape of the feature. The potential benefit of this dynamic adjustment is illustrated by the limiting case where there are no features of interest in the field. For this example there is a 64-fold difference between the time required to scan the entire field with the *measure* spacing and the time required to determine that there are no features of interest via the *search* spacing. This is the difference between a one-day measurement, and a half-hour measurement! Or if set up for the same total analysis time, this represents an eight-fold difference in the signal-to-noise of each pixel measured!

Of course, the situation of a "blank" field isn't typical. Thus, analysis by the dynamic method must also include the time required to characterize each feature once it is identified. Here again, substantial advantages may be realized by an appropriate choice of characterization algorithm. Just as it is unnecessary for a batter to visually assess every detail of a thrown baseball in order to hit it, it is rarely necessary to have a pixel-by-pixel rendition of a feature in order to appropriately classify it. Rather, the ideal is a "sparse" algorithm that quantifies the important feature parameters with adequate precision and reliability, but does so with a minimum number of pixels. Figure 4 illustrates the performance of one such sparse algorithm that has proven suitable for large classes of problems that involve basic polygonal or elliptical shapes. The lower trace is the time for an actual analysis of a test field via the algorithm, for five different *measure* dwell intervals. When this data is fitted to an equation of the form C+*nT* where C is a constant overhead and T is the pixel dwell time used for the sizing step, the parameter n, which represents the number of pixels used in the measurement, is found to be ~428,000, which is 1/88th of the 38 million pixels that would be required to collect full image frames with this same pixel resolution. In other words, a "pixel efficiency" of nearly two orders of magnitude is demonstrated for this actual example.

Complex feature shapes (such as curving strings and features with voids) may require denser algorithms. However, even when the chosen sizing algorithm collects every pixel of the identified feature, the resulting analysis is still far faster than a full-frame method since the high density pixels are collected only where feature information is present.

The above has focused on simple detection and size/shape analysis. An important part of many automated analyses also involves compositional characterization. Because the goal is to match the features with predefined classes rather than to perform a full quantitative analysis, EDX spectra with relatively small numbers of counts can be used. Spectral acquisition times of a few seconds are typical in this kind of application, and though collection is thus quite fast, this still drastically slows the rate at which individual particles can be processed to something like 10-50/minute (from the ~500/min that can be characterized by size/shape only). Consequently, various strategies are employed to minimize the number of particles that must be x-ray analyzed, and to intelligently allocate the time spent on each. (However, with the advent of the new breed of high-speed x-ray "drift" detectors, this aspect of the automated analysis procedure is also being vastly accelerated.)

A dynamic particle analysis implementation has an important advantage over a frame-based method in the area of compositional analysis. In a pure frame-based implementation, the image frame is first acquired, and then as features of interest are identified via the image analysis, the electron beam is returned to a feature to collect an x-ray spectrum. Consequently, considerable time can elapse between when the frame was collected and the return of the beam for EDX analysis, which can make accurate relocation of small features problematic. A dynamic implementation, on the other hand, collects the x-ray spectrum immediately after the feature is found, and the opportunity for drift-related errors is thus greatly reduced.

The sheer volume of data that can be generated by an automated instrument drives the need for efficient database capabilities and offline review tools. Unlike common laboratory particle sizing instruments that produce only size-range counts, an automated microscope produces detailed particle-by-particle data (including a "thumbnail" image of each particle if selected) and thus post-processing can "mine" information from previously acquired datasets. Previously characterized features can also be physically relocated for more extensive manual analysis.

An optimally automated SEM/EDX is thus seen to employ conventional capabilities in unconventional ways in order to analyze feature populations accurately and efficiently. Successful implementation of these techniques requires that the instrument platform be engineered with the tight integration necessary for efficient dynamic operation. It should also be apparent that many features of conven-

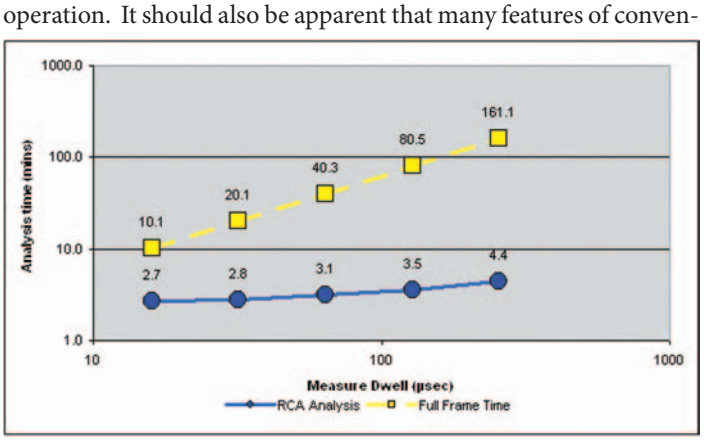

Figure 4. The graph depicts a test employing a 0.75 x 0.75 mm pattern of 1092 circular features of 1 to 100 micron diameter. A pixel size of 0.122 microns is employed for the measurement. The top trace is the time that would be required to acquire full frames (38 million pixels) using dwell times of 16, 32, 64, 128, and 256 microseconds respectively (exclusive of overhead and processing). The lower trace shows the actual time (including search overhead and processing) to perform the complete analysis using the same dwell times for the fine measure steps of the sizing algorithm.

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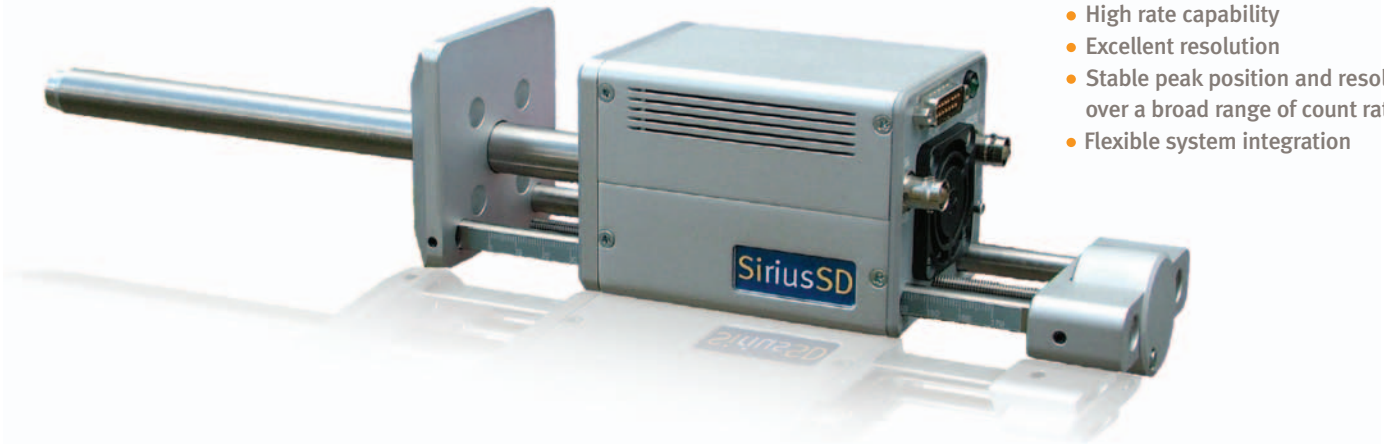

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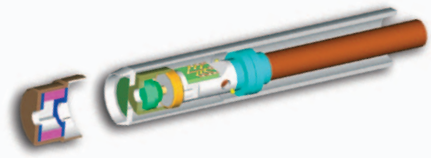

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Figure 5 – The PGS specimen is fabricated with five distinct analysis zones. The four corner zones each contain 1092 circular features ranging from 1 to 100 microns in diameter and are intended for quick system checks. When combined with the central array of elliptical features, a 4x4 mm zone of 5050 features is available for more comprehensive tests.

tional SEM performance that are of major importance in manual operation are of diminished relevance, and other factors such as robust motorized staging and stable long-term operation have a high priority in an automated instrument that is required to function reliably for long unattended periods. Thus the instrument that is designed ground-up for such automated operation will differ in a number of important ways from one designed for conventional microscopy.

Quality Control

If one were to ask a typical microscopist to justify why his/her assessment of a sample was to be believed, the response would likely reduce to: "Because I know what I'm doing!" That's a legitimate response for a manual microscopic analysis, where it is the insight and integrity of the microscopist that ultimately determines the quality of the analysis. However, one can't rely on the skill of an operator when the instrument collects and interprets the data unattended. There must be objective means by which the credibility of the results can be gauged.

The "obvious" way to check the performance of an instrument is to employ standards, but that really hasn't been a viable option until recently, due to the absence of suitable particle-standard specimens. The problem involves what seem to be mutually exclusive requirements. On the one hand, to give a valid measure of performance, the standard should challenge the instrument with a large number of suitably diverse features, yet to be useful as a routine procedure, the test should be quick and simple. To be a useful test of instrument sensitivity, the standard should simulate the atomic number contrast of real samples, yet it must be stable and robust relative to handling and storage, which real-world particle samples are generally not. Most challenging of all is that ambient micron-scale particles in a typical laboratory environment will quickly contaminate any exposed surface, and thus the "true" particle counts of any standard are immediately open to question.

To address these challenges, an entirely new type of particle standard methodology has been developed, known as the Performance Grading System (PGS)[2]. What makes this system different from a conventional standard is that the physical specimen (Figure 5) is accompanied by a database that accurately describes the location, size, shape, and composition of each feature. The third component of the system is a special software program (MapMatch™) that accepts the output of an automated analysis performed on the specimen and matches the measured features with the known features in the database. In this way it is possible to reliably discriminate between fabricated features and extraneous material, and certifiable measures can be obtained. Since the backscatter contrast realistically simulates low-atomic-number materials on a filter-paper substrate, the specimen provides a realistic test of detection sensitivity.

The PGS was originally developed with the primary goal of providing a simple and quick yet highly credible system check that could be performed routinely, and it does this very well. A five minute test (~3 minutes for data collection and ~1.5 minutes for analysis) produces a comprehensive "pass/fail" assessment of instrument status. However, in addition to being a very practical system check for users, the PGS has also proven to be a powerful tool for development. The detailed metrics that are now available have made it possible to do the kind of in-depth studies that are vital for ongoing refinements of the technology.

In regulated environments, such as in the pharmaceutical and medical device industry, there are stringent requirements to ensure the integrity of reported data. One aspect of this is, of course, ensuring correct instrument performance. Though any responsible instrument manufacturer employs quality control measures, those required for automated products used in regulated industries necessarily differ in scope and rigor from those appropriate for manual scientific instruments. These measures begin with validation activities conducted during instrument design and culminate with specialized tests conducted at the user's site. In addition to instrument performance and validation, cGMP environments also share concerns for data integrity and security. This kind of consideration is, of course, quite foreign to traditional "honor system" data-handling philosophies as practiced in most microscopy laboratories.

Perhaps the greatest difference between the concept of "quality control" as practiced in conventional manual microscopy and that which must be practiced in an automated application is one of mindset. Conventional manual microscopy is indeed a subjective "art" and the "highest quality" data will be produced by those practitioners who can wring the best performance from their instrument. That approach is, however, a detriment to quality control in an automated application. The ideal in an automated application is that the same results will be obtained consistently, regardless of who is performing the analysis. Consequently, the design of an automated instrument will place great store on methodology to ensure consistent setup and operation.

Conclusion

We trust that we've made a convincing case that the practice of automated electron-beam analysis does indeed represent a different but complementary kind of microscopy from that conventionally practiced with manual SEM/EDX instruments. The differences begin with the nature and objectives of the applications being addressed, and are reflected in differences in the optimal configuration of the instrument, and the manner in which the quality of the results must be assured. ■

Endnotes

1 Deserving of particular mention are the pioneering efforts of the late Eugene White of Penn State University, whose team seems to have invented the principle of using a dynamic technique to locate and characterize features with an automated SEM.

2 Performance Grading System, PGS, JEMM, and MapMatch are trademarks of Aspex Corporation. Patents on the PGS technology are pending.

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