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QUANTITATIVE IMAGING ION MICROSCOPY: A SHORT REVIEW

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Abstract

A short review of recent efforts being made in the quantification of images in ion microscopy is given. Special aspects of instrumentation, detection and acquisition, which are unique to direct imaging secondary ion mass spectrometry, are discussed in relation to the successful application of traditional empirical quantification schemes. Application of such quantification schemes requires proper sample preparation, standardization, analysis, and quite often, special techniques in image processing and the correlation of ion microscopy with other microscopies. Quantification within this technique is a difficult goal which can only be realized if the analyst pays strict attention to every step of the analytical process.

Key Words: Ion microscopy, secondary ion mass spectrometry (SIMS), direct imaging SIMS, quantitative imaging, quantification, image analysis, correlative microscopy, video detection.

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Introduction

The ability of dynamic Secondary Ion Mass Spectrometry (SIMS) to map depth and lateral elemental distributions in a substrate make it a useful tool in virtually every field requiring the analysis of solid materials. An analytical goal of many research laboratories utilizing SIMS has been in establishing and improving the quantitative aspects of the technique; most quantitative schemes having been developed for the microprobe mode of SIMS analysis. In the microprobe mode, a small area is analyzed by either using a very small diameter (≤ 1 μm) primary beam or by aperturing the secondary beam, in a broad-beam instrument, so that only a small area (1-10 μm^2) of the sample is analyzed. This should be compared to ion microscopy (IM), in which a broad ($\geq$ 100 μm) primary beam impinges on the sample. In this mode, the secondary ions are extracted and mass filtered in a parallel, stigmatic fashion, followed by detection using a two-dimensional position sensitive detector, yielding a direct image of the lateral distribution of the analyte. (The small-spot ion microprobe can build images by scanning the probe in a raster fashion over the sample (Levi Setti, et al., 1986).) While most, if not all, quantification schemes developed for microprobe SIMS are applicable to IM, the acquisition and quantitative analysis of IM images require extra considerations in sample selection-preparation, analytical conditions, detector selection-characterization, and post-acquisition processing. The intent here is to give the reader a short review of the efforts and progress being made in the quantification of IM images in a number of fields utilizing SIMS; the emphasis will be on those considerations needed to successfully apply quantification schemes to IM images.

Aspects of IM Quantification

The goal of quantification, determining the lateral (x,y) and depth (z) concentration of an analyte with accuracy and precision, is achieved by the

transformation of data collected in intensity space to that of concentration space; this is conceptualized in Figure 1. It is up to the analyst to determine the function, f( ), required to carry out the transformation. The determination of the transformation function can be either theoretical (Benninghoven, et al., 1987b) or empirical.

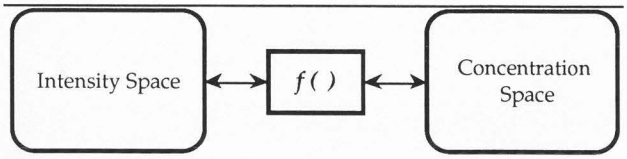

Figure1. The function f( ) transforms intensity space to concentration space.

The difficulty in determining such a functionality is shown in Figure 2. The signal intensity recorded by the detector is not a simple function of concentration, as it is in atomic spectroscopy, for example. The signal intensity in SIMS is a complex function, F( ), of sample matrix, sample topography, matrix-primary beam interactions, instrumental transmission, and detector response (see for example: Williams, 1985). The signal intensity is not a linear combination of each of these parameters because the above factors are coupled in a complex manner, e.g. the primary beam may induce a particular topography on the sample due to preferential sputtering. This topography, in turn, may change the instrumental transmission as the secondary extraction field is perturbed. Thus a change in signal intensity would not be a result of some change in analyte concentration. A number of empirical techniques have been developed to reduce the functionality of the signal intensity/ concentration relationship so that quantitative analysis may be carried out. Because of the many inherent difficulties in the process, much of the work preformed in IM is qualitative or semi-quantitative. Semi-quantitative measurements may involve the determination of ion ratios as a guage of sample-to-sample variations in analyte concentration. Semi-quantitative IM offers a wealth of information for observing changes in concentration profiles without the need to establish absolute values.

Empirical Quantification Schemes

Empirical quantification schemes have provided success in microprobe SIMS yielding quantitative results within ±10%. The bulk of the work has been done in the electronic materials industry, as evidenced in the proceedings of the SIMS conferences (Benninghoven, et al., 1986, 1987a, 1989). Most empirical methods rely on the relative sensitivity factor (RSF) approach (Werner, 1980). RSF's are often used in combination with external standards or, methods based upon standard additions or isotope dilution using in situ doping techniques

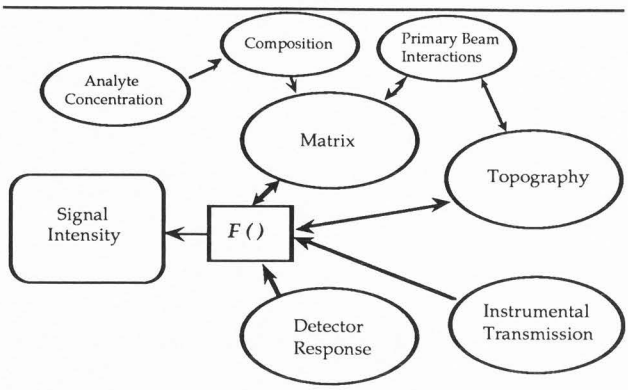

Figure 2. The function F( ) produces a given signal intensity based upon a variety of experimental and phenomenological factors common in SIMS.

such as ion implantation. The RSF approach has been widely applied in both imaging and microprobe SIMS. The general RSF equation is defined (Werner, 1980):

$$RSF_{x/ref} = (i_x/i_{ref})(C_{ref}\, f_{ref}/C_x\, f_x) \qquad (1)$$

where i is signal intensity, C is the elemental concentration, f is the isotopic abundance, x indicates the analyte, and ref indicates the reference species. The reference species is usually chosen to be a homogeneously distributed matrix elemental ion or cluster ion. Once the RSF is determined from analysis of an appropriate standard, the RSF can be used to correlate signal intensity to concentration in an unknown:

$$C_x=(i_x/i_{ref})(C_{ref}\, f_{ref}/f_x\, RSF_{x/ref}) \qquad (2)$$

By taking the ratio of signal intensities, one removes factors such as changes in instrumental transmission or detector sensitivity in sample-to-sample and day-to-day measurements. Thus once an RSF is determined for a given analyte within a given matrix the RSF remains a valid constant from analyst to analyst and even from one "identical" instrument to another. The RSF approach is limited in that it can only take into account instrumental changes that occur after the SIMS ionization event takes place. A major limitation in the RSF method is the need for the standard and unknown to be analyzed under identical analytical conditions as small changes in environment, e.g., sample chamber atmosphere, can readily effect ion yields, hence destroying analytical certainty. Consider a standard which was analyzed under sample chamber conditions rich in oxygen. For many combinations, oxygen greatly increases the ion yield of the analyte and not the ion yield of the reference species. All subsequent analysis of the unknown must be carried out under identical oxygen

concentration or the RSF will not be valid as the reference/analyte yield ratio will vary. The RSF method alone can only correct for instrumental changes that effect the transmission, detection, or yield, of all the ions used in the scheme, equilaterally.

Other empirical methods extend the basic RSF method. Michiels, et al., (1990) applied RSF's and a modified RSF method, matrix ion species ratios (MISR) in the quantification of IM images. Developed for microprobe SIMS, the MISR method (Ganjei, et al., 1978) allows for the correction of fluctuations in analysis conditions and small changes in matrix composition. The analysis consists of measuring the ion ratios, of a standard, for three or more matrix ions under a variety of analytical conditions. Congruently, an RSF is determined for each new analysis; the series of RSF's so constructed are correlated with the matrix species ratios. This creates an internal reference system capable of self-correcting matrix effects, in which the selection of the appropriate RSF value is based upon the measurement of the matrix species ratio in the unknown.

Many groups in SIMS have contributed to the recent literature on the general subject and philosophy of quantitative analysis; we present a few of the highlights and important articles here. Grasserbauer, et al., (1989) outlined an analytical strategy for quantitative trace analysis based on the optimization of analytical technique and empirical standardization. The basic outline is good, common sense analytical chemistry: (1) reduction of systematic and random error, (2) calibration with proper reference standards, and (3) assessment of accuracy. The most important problems encountered in analysis are: spectral interferences, charge compensation, and the chemical matrix effect. Adams, et al., (1989) reviewed recent efforts in quantitative SIMS, focusing on empirical methods comparing the traditional RSF method to MISR. Deng and Williams (1989) assessed factors influencing the accuracy and precision in quantitative SIMS measurements based on an empirical technique using external standards. They determined that differences in the energy distribution between the reference species and analyte, as well as misalignments in sample positioning are significant sources of error in the RSF method. They suggest improving comparative SIMS analysis by choosing a matrix species that has a similar energy distribution to that of the analyte, and care in the reproducible positioning of samples within the instrument chamber. Williams (1985) discussed the limitations of quantitative SIMS with respect to instrumentation and standards. Friedbacher, et al., (1990) explored the transferability of RSF's in a variety of metals for semiquantitative analysis.

The standard additions approach, based largely on ion implantation, has also done quite well in microprobe SIMS as a standardization technique. The main advantage to this type of standardization lies in the fact that the standard is internal and hence, "standard" and "unknown" are analyzed under identical analytical conditions. Leta and Morrison (1980a) investigated the utility of using ion implants for the quantification of solid matrices and developed a large number of standards for semiconducting matrices (Leta and Morrison, 1980b; Chu and Morrison, 1982a). The main utility in the implantation/standard addition approach to image quantification lies in the ability to perform image depth profiling (IDP) (Patkin and Morrison, 1982). The IDP is basically a time course experiment in which images are collected at a series of selected masses. Image processing is then carried out to reconstruct a three dimensional representation of the substrate (Bryan and Linton, 1986; Lee, et al., 1990). If the IDP is combined with ion implantation the scheme can be made quantitative. Chu, et al., (1982b) used IDP for the analysis of thin layers. Patkin, et al., (1982) considered differential sputtering corrections in IDP for the analysis of biological tissue. Bryan and Linton, (1986) made considerable improvements in automated acquisition IDP with a high dynamic range using a CID camera for acquisition and quantitative analysis. Cox, et al., (1987) carried out IDP in the analysis of individual coal particles for the determination of trace elemental distributions. Novak, et al., (1990b) used IDP for the characterization of lithium niobate waveguides and also reviewed the technique's utility in materials analysis (Novak, 1990a). Gillen, et al., (1991) used IDP for the characterization of light element diffusion in single crystal yttrium barium copper oxide superconductors.

Applications and Problems in Applying Analytical Schemes to IM Images

The major problem in applying these empirical approaches to IM lies in the production of good, representative standards. Due to the complexity of matrix effects, it is desirable to have a standard that has an identical matrix to that of the unknown sample, with analyte distributions that are well known in three dimensions. While this may be possible for some matrices, e.g. in the semiconductor field, the analyst must frequently resort to using or fabricating a standard that has a representative matrix composition using a homogeneously distributed analyte. Quantitative imaging in metallography has been carried out by Thorne, et al., (1987), Michiels, et al. (1990). Fragu, et al.,(1989) used IM for the relative quantification of ^{127}I in human thyroid tissue. Gibson, et al., (1989) applied quantitative IM imaging to the study of boron diffusion in silicon-carbide thin films. Work in the authors' own laboratory has focused on quantitative imaging of cultured, freeze

dried animal cells (Ausserer, et al., 1989) for the study of physiological transport of diffusible ions (Chandra, et al., 1989, 1991, 1992) and quantitative intracellular drug uptake (Bennett, et al., 1992).

As an example of standard production and the quantitative imaging process, let us consider the steps taken in our own laboratory to produce standards for quantification of diffusible ions in cell cultures. The first step in the process was a qualitative evaluation of the severity of matrix effects in the freeze dried cell matrix (Chandra, et al., 1987; Ausserer, et al., 1988). It was already known that animal tissues prepared by traditional plastic embedding techniques exhibited strong matrix effects, that is, relatively homogeneous matrix species, such as C^+, exhibited heterogeneities in IM (Brenna and Morrison, 1986; Burns, 1986). The goal of Chandra and co-workers (1987) was three-fold. They first needed to produce a sample preparation technique that preserves the elemental and morphological integrity of a cell culture monolayer while producing a sample appropriate for SIMS analysis. They then needed to determine if matrix effects, in different cellular compartments, were not too severe to preclude quantitative imaging. The study of freeze dried cell cultures, however, revealed minor heterogeneity in matrix species such as H^+, C^+, CN^-, and O^-. Once a laterally homogeneous matrix was established it was felt that RSF's could be applied through the use of a secondary standard. Ausserer, et al., (1989) produced such a standard using cell homogenates quantified by inductively-coupled-plasma/atomic-emission-spectroscopy (ICP-AES). Quantification of boron, calcium, magnesium, potassium, and sodium was achieved in good agreement with X-ray microanalysis, a well established quantification technique in cell biology. Such work has allowed our laboratory to pursue the uptake, localization, and quantification of boronated drugs in cell cultures. Boronated drugs are of extreme importance in boron neutron capture therapy (BNCT), a newly developing treatment for brain tumors (Hatanaka, 1986). Present work involves testing boronated drugs for uptake in cancerous and normal cell lines to test selective uptake and intracellular localization. This work will help determine a drug's usefulness as a BNCT therapeutic agent. There is a great need for quantitative results in order to assess the specific concentration and concentration differences of boronated drugs, on a cellular level, in cancerous and non-cancerous tissues (Hatanaka, 1986). Localization of the drug within the cell is also of extreme importance as the effectiveness of BNCT is directly dependant upon localization of the drug within or near the nucleus. IM represents virtually the single tool which can be applied to this type of analysis.

The validity of the quantification scheme used here stresses the importance of sample preparation; it relies on the assumption that there are no serious SIMS matrix differences between the various parts of the cell, e.g. between the nucleus and cytoplasm, when a specific sample preparation is followed. If the SIMS matrix effects were significant, the homogenate standard would not be as analytically valid a standard. This illustrates the major problem in quantifying images; any change in the matrix of the unknown must be followed in the standard. The problem can become quite severe if the analyte concentration goes much above the 1% level, because SIMS nonlinear matrix effects can occur in this regime as the analyte becomes a significant part of the matrix(Williams, 1985). There are certainly a number of caveats in the approach of Ausserer, et al., notably that an element does not necessarily have to behave in the same manner as a homogeneous matrix ion. The question here is a matter of the chemical differences in various parts of the cell and whether or not these differences result in different practical ion yields for an element of interest under dynamic SIMS analysis at the spatial resolution attainable. An extremely important aspect of the validation of our calibration scheme comes from other microscopies, as well as the information available on the intracellular distribution of ions from non-microscopical techniques. The results of such work shows that the preparation technique (1) yields distributions of rapidly diffusible species that correlates well other techniques and (2) the concentrations that are quantified by IM are in good agreement. It is interesting to note that the major importance in our technique is not in the assignment of absolute concentrations of an element in a given cellular compartment. The true power of the scheme is the ability to compare the same cellular compartments, in cells that have undergone different treatments, for the exploration of topics in cell biology such as the localization of calcium stores in the Golgi apparatus (Chandra, et al. 1991), and isotope exchange studies for the determination of exchange kinetics within a cellular pool. As an example, consult figure 3. The ion microscope was used in conjunction with laser-scanning confocal fluorescence microscopy for the determination of the Golgi apparatus as an intracellular store of calcium. Through the use of a fluorescent label, the Golgi apparatus can be unambiguously assigned in the light microscope. Correlation of the same areas with IM reveals much higher calcium levels within Golgi. How do we know that this reflects a change in concentration rather than a SIMS matrix effect within the Golgi? Part of the evidence supporting a true concentration difference is shown in figure 3. The bottom row of photographs are of cells that have been exposed to a calcium ionophore, A23187. It is well known (Pressman, 1976) that this drug is a

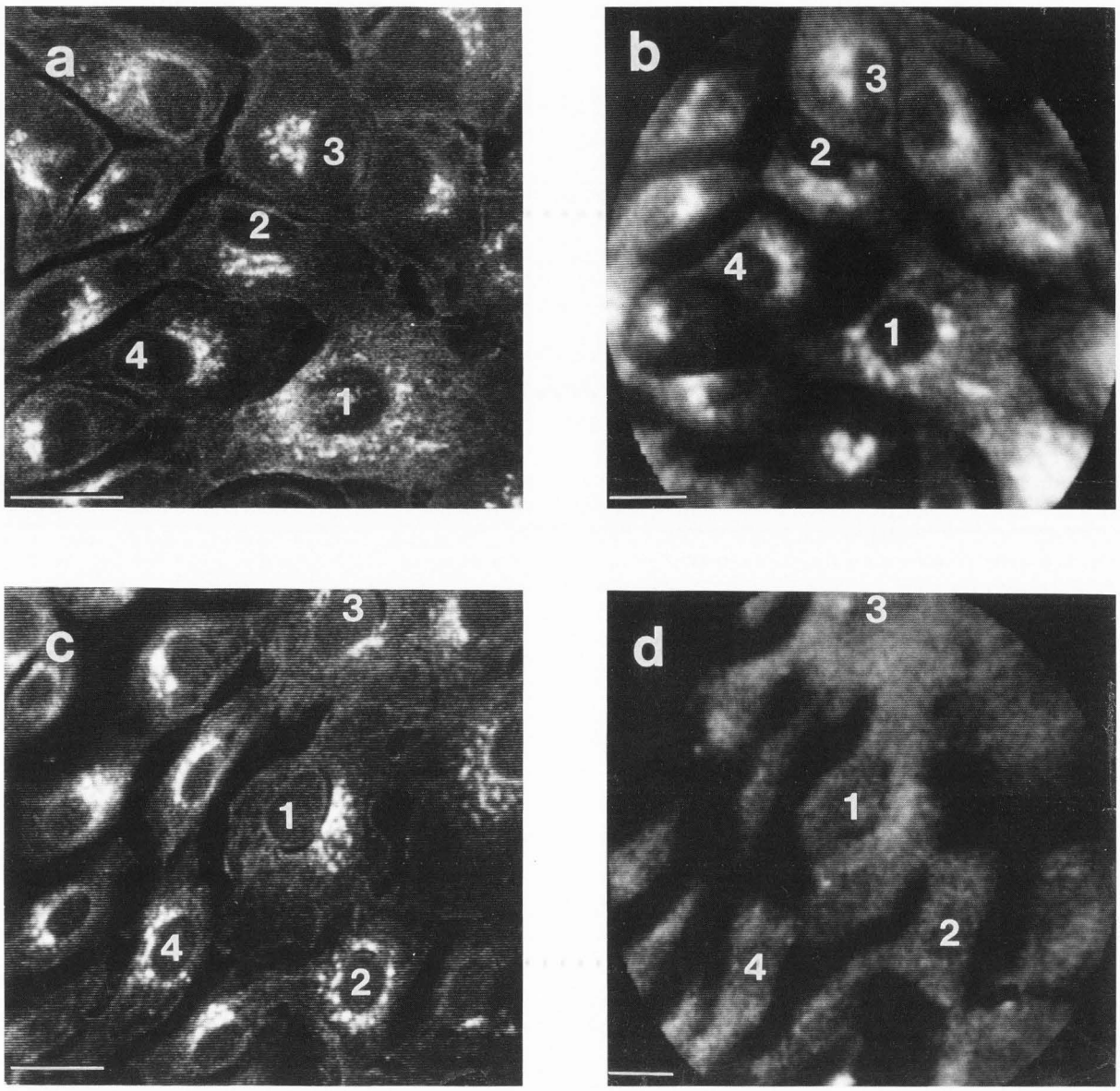

Figure 3. Correlative laser scanning confocal fluorescent and IM images of freeze-fractured, freeze dried LLC-PK$_1$ cells. Cells in the top row (a and b) represent cells that have been treated with C$_6$-NBD-ceramide, a fluorescent Golgi stain and exposed to a Ca^{2+} free media for 5 min in the presence of 2mM EGTA. Cells in the bottom row (c and d) were treated with the Golgi stain and were also exposed to 2 μm A23187 in Ca^{2+} free media for 5 min in the presence of 2 mM EGTA. Photos a and c are confocal fluorescent images, revealing the localization of the Golgi, while photos b and d are the corresponding IM images of $^{40}Ca^{+}$, showing the relative total concentration of calcium within the cell. A23187, a calcium ionophore is shown to release the calcium stores within the Golgi, observe the changes in intensity in the IM images while the Golgi structure is preserved in the fluorescent images. Note that the numbers in photos a and c match the same cells numbered in photos b and d respectively. Bar, 20 μm. (Reprinted with permission from *J. Cell Sci.* 100, 747-752 (1991)).

calcium transporting agent that moves calcium from areas of high to low concentration. Either the presence of the drug has changed the matrix and we are observing a "matrix effect" lowering the Ca signal, or we are observing a true concentration change of Ca within the Golgi. The fluorescence images, of these same cells, show that the drug does not effect the perinuclear organization of the Golgi; the Golgi structure is preserved. Since the drug is administered in a 2 μm concentration the alteration of the matrix is insignificant, and we must be observing true changes in concentration. Images of C, K, Na, and Mg (not shown) are relatively homogeneous, which also supports our conclusions

that the matrix effects in different cellular compartments are minimal. Further work in our laboratory, involving stable isotope exchange studies of Ca^{++}, has revealed Golgi as an important intracellular store of highly sequestered calcium (Chandra 1992, unpublished results). Even if the Golgi has some small difference in ion yield for Ca, relative to the rest of the cell, comparison of Golgi area to Golgi area among sister cells still allows for semiquantitative analysis in exchange studies. On-going work in our laboratory involves the extension this technique to other cellular compartments for a variety of diffusible ions.

Instrumentation

Here we will focus on the instrumentation involved in the detection of ion images, rather than IM instrumentation in general, because the subject is a critical aspect of quantitative IM. A brief introduction to IM instrumentation follows. The general IM instrument is a refinement of traditional mass spectrometry; having provisions for broad primary ion beam bombardment, stigmatic extraction of secondary ions from the primary beam/sample interaction, transfer optics for magnification and image aberration reduction, stigmatic mass selection, and two-dimensional detection. Here the term "stigmatic" refers to the focusing properties of the ion optics used in such instrumentation (Liebl, 1989); that is, the optics possess a cylindrical axis of symmetry, allowing for a 1:1 correspondence between positions of ions in the extraction field of the system, and the final image produced by the detector. The ion optics in this type of instrument are strictly analogous to those used in light and electron microscopes. The first ion microscope to use these principles was developed by Castaing and Slodzian(1962). Commercial instrumentation based on their work resulted in the highly successful Cameca IMS-300, 3f/4f/5f, and the new IMS-1270 line of instruments. There are many accounts of this instrumentation in the SIMS literature (see for example: Benninghoven, et al., 1987b; Vickerman, et al. 1989; Rouberol, et al., 1980) and the bulk of it will not be repeated here. The instruments evolving from the designs of Castaing and Slodzian are all based on magnetic sector instrumentation; either a magnetic prism (IMS-300) or standard geometry Nier-Johnson spectrometers (IMS-3f/4f/1270). The next significant step in the development of novel IM instrumentation has come from the recent work of Schueler, et al. (1990). The approach of this instrument is to combine stigmatic extraction and transfer optics with time-of-flight mass spectrometry. This instrumentation should widen the scope of SIMS applications as it possesses the ability to perform molecular, as well as elemental, imaging. Molecular imaging in dynamic SIMS using IM has been demonstrated by Gillen, et al., (1990). Both types of instruments have limited spatial resolutions, on the order of 0.2-1 μm, a result of aberrations in the initial extraction field and primary imaging lens, generally referred to as the immersion lens (Liebl, 1989).

Image Detection and Acquisition

The choice of an appropriate imaging detector is crucial for proper quantitative or semiquantitative work; it is probably the single most important instrumental factor in IM. In our own laboratory, good reproducible quantitative work was possible on a daily basis only after the implementation of a high quality solid state detection system. An ideal detector would be capable of single ion detection, possess absolute uniformity of lateral (x,y) sensitivity, possess a linear response with signal intensity, have a uniform sensitivity for ions of different mass and charge, have the capability to integrate signal with time, and allow for digital acquisition of image data. The dominant imaging detector used in ion microscopy is the microchannel plate/fluorescent screen assembly (MCP/FS) (Mantus and Morrison, 1990). The MCP serves the function of converting and amplifying an ion signal into an electron signal; each ion hitting the MCP results in a burst of electrons emitted from the opposite side of the plate. Each electron burst is then converted into a photon signal by a fluorescent screen. Images are then acquired by a wide variety of means using both photographic (Fassett, et al., 1977) and electronic detection. Video tube cameras as well as solid state imaging detectors, charge coupled devices (CCD) and charge injection devices (CID), and the resistive anode encoder (RAE) have been successfully used in the acquisition of ion images for quantitative measurements. Both types of acquisition (video tube and solid state) can be applied in a quantitative scheme, and the relative merits of each are discussed below.

The MCP as the primary image detector has a number of disadvantages and nonlinearities. Mantus and Morrison (1990) examined the performance of the MCP as a detector in IM paying particular attention to linearity, homogeneity, MCP/FS signal-noise relationships and mass dependence. They determined that the MCP is quite linear in response from total ion count rates of less than 10 count/s to over 10^8 count/s at fixed MCP gain. Their results are summarized in Table 1. They also confirmed the results of others (Michiels, et al., 1990; Ling, et al., 1988; Newbury and Bright, 1988a) in that the response of the MCP/FS at a fixed ion flux versus MCP gain is nonlinear.

The MCP/FS sensitivity is not uniform across its surface, the center of the plate being less sensitive than the edge (see also: Michiels, et al., 1990; Ling, et al., 1988). This nonuniformity is also found to degrade with time which is attributed to the fact that the center of the MCP receives more ion hits over a long period of time than the edge. They also describe a complex mass dependence in the detection of positive elemental ions which is roughly modeled

Table 1. Linearity data of MCP/FS at Four Different Gains[a]

% of full gain	slope(calc error)	corr coef	Range count/s max	Range count/s min
20	0.567 (0.008)	0.999728	10^9	500
40	26.7 (0.1)	0.999962	10^8	10
60	183 (1)	0.999923	10^7	1
80	530 (11)	0.999543	10^6	1

[a] Adapted from Mantus and Morrison (1990), the slope, slope error, and correlation coefficient were calculated using standard linear least-squares analysis. The range given represents the useful limits of the MCP/FS at the given % gain.

on a fit using the inelastic stopping powers of an ion impact in the MCP material. The mass dependence will, of course, be absorbed by any empirical quantitative scheme but qualitative analysis and comparison of ion images should take the mass dependence into account allowing for a more realistic interpretation of IM images. The mass dependence will also disappear in any system that relies on pulse counting of the MCP signal, as in the RAE, because the mass dependence is reflected as variations in intensity of the electron pulses rather than in the probability of pulse production (i.e. the sensitivity of detection) (see also Hellsing, et al., 1985). That is, it seems that the differences in stopping power result in the MCP producing different numbers of electrons in each electron burst. Different amounts of charge in each burst will produce different photon intensities from the FS as each electron in the burst is capable of participating in a quantum emission. It is our own experience that operating the MCP/FS at maximum gain reduces the mass dependence in a qualitative fashion.

As it is an older technology (Inoue, 1986) video cameras were the first type of electronic imaging detector used in IM. Furman and Morrison (1980) applied a silicon-intensified-tube (SIT) camera to an IMS-300. More recently video tube camera technology has been used by Michiels, et al., (1989); Leta, (1986); Thorne, et al., (1986). There has been much debate in the literature as to the linearity of response for a video camera based system. Ling, et al. (1988) studied the critical behavior of the MCP/FS-Video Camera detector with emphasis on using the detector in a quantitative fashion. The study reported a number of nonlinearities in ion intensity with respect to both lateral response and ion signal as a function of concentration. They determined that a multiple term logarithmic linearization function was necessary to linearize the intensity-concentration response of this detector relative to an electron multiplier (EM). In this work it was not clear where the nonlinearities arose, in the camera, in the MCP/FS, or both. Michiels, et al.(1990) report their low light level SIT-MCP/FS system as having a linear signal response from 50 to 5 x 10^5 counts/s. This points to an inherent problem of nonlinearity in certain types of video cameras rather than the MCP/FS itself. The typical intra-image dynamic range for a high quality video camera is 3 to 4 orders of magnitude (i.e. 256:1 for a typical 8-bit digitization); the dynamic range can be further extended by using a multi-frame frame approach. Single frames can be acquired, digitized and integrated as a single image in the image processing system.(Michiels, et al., 1990; Furman and Morrison, 1980) The dynamic range here is limited by either the integrating capacity of the image processing system, or, by the dark read noise of the camera, the limiting factor being the smallest of the two. If the response of the detector is nonlinear however, the dynamic range is degraded by perhaps as much as an order of magnitude (Ling, et al. 1988).

Solid state image acquisition systems are gaining greater popularity and represent significant improvements in linearity, flexibility, and reproducibility over their video-tube counterparts. Solid state acquisition systems fall into two distinct classes: video rate and slow scan (Inoue, 1986). The video rate systems behave much like a tube camera in that images are read out of the camera at a video, near real time (TV), rate. Slow scan cameras are analogous to film cameras; the imaging element is exposed to the signal for a given length of time, via shuttering, after which one image is read out of the camera into an appropriate image analysis system(Sweedler, et al., 1988). Newbury and Bright (1988b) studied the image intensity/count-rate response of the CCD-TV camera relative to the MCP/FS. Hunter, et, al. (1989a, 1989b) describe an integrating CCD camera used in conjunction with a dual microchannel plate; single ion detection is also achieved. This device is a hybrid of the video rate and slow scan CCD cameras, allowing for integration times from 16 ms to over 5 s; data is output in a standard video format. Mantus and Morrison (1990) examined the characteristics of a slow-scan CCD with particular attention to separating MCP/FS characteristics from those of the camera. The slow-scan CCD is a highly flexible detector as it can be operated in a number of modes (Sweedler, et al., 1988) that allow for both imaging and spectrographic detection (see, for example: Mantus, et al. 1991). The different modes are achieved by on-chip summing of pixel intensities, referred to as binning (Epperson and Denton, 1989). Binning can also be used to change the effective pixel size on the detector, hence improving detector dynamic range at the cost of image resolution (Mantus and Morrison, 1990). The output of the slow-scan camera is not a video format, rather it is a data stream; image observation is achieved by transferring the CCD data to an image processor which converts the data to a video capable format. Intra-image dynamic range is 3 to 4 orders of magnitude (i.e. 16,384:1 for a 14-bit digitization) while on chip integration extends the dynamic range to the limit of the CCD's dark current. Since this type of detector (Janesick, et al., 1987) exhibits well

characterized noise and a high degree of linearity, they were able to separate and characterize "true" MCP/FS behavior. Some of their findings are discussed below.

Bryan, et al. (1985, 1986) used a CID in conjunction with a dual MCP. Like the CCD, the CID has some interesting detection properties (Sweedler, et al., 1988). Perhaps chief among these is the ability to read the data on the CID chip while the chip is acquiring information, without destroying that information. When a CCD is read, the charge (i.e. the image data) accumulated on the chip is destroyed; this is not the case with a CID. Such an ability could take much of the guesswork out of choosing a proper exposure time for the detector. One can monitor the charge built up on the chip as a function of time; when the charge is near the saturation (say ≈80-90%) of the detector the shutter is closed. In this way, one is assured of using the maximum dynamic range of the detector automatically while minimizing analysis time.

Not all detectors using an MCP rely upon the conversion of electrons to photons via a fluorescent screen. Chief among these is the resistive-anodic-encoder (RAE) first implemented as an imaging detector in SIMS by Odom, et al. (1983). The RAE is a position sensitive charge detector; each ion impact on the MCP which results in a burst of electrons is detected by the RAE. The RAE is normally operated in a pulse counting mode, each count on the RAE roughly corresponding to one ion impact on the MCP. The RAE, like the other solid state detectors, is highly linear; as a result this is an excellent detector for quantitative work. There is, however, one serious problem with the RAE, the upper count rate is limited to $\sim 10^5$ counts/s. The other solid state detectors do not exhibit this effect and can be operated at the maximum signal provided by the MCP/FS, approximately 10^9 counts/s.

An attempt to completely replace the MCP/FS with a solid-state detector was carried out by Turner, et al. (1988). They replaced the MCP/FS with a thinned, backside illuminated CCD detector. This detector exhibited good homogeneity and excellent linearity of response but suffered from low sensitivity and a severe mass dependence. Further work is needed in this area and should be encouraged because a sensitive, linear, homogeneous solid-state detector would significantly improve acquisition of images in IM. The MCP is a good detector but it's nonuniform lateral response and short analytical lifetime make it less than ideal. Replacement of the MCP with some solid state device that yields digital information directly, without having to go through multiple steps, would further improve acquisition.

There are certainly a wide variety of imaging detectors to use in IM and the choice of detector will largely depend on one's analytical needs. The RAE and slow-scan solid state detectors are the best choices as they are flexible and have linear, reproducible responses. They are, however, quite expensive to implement in the laboratory, especially the RAE. The slow scan CCD seems to have a slight advantage over the RAE in terms of a lower cost and a higher dynamic range. A video tube camera acquisition system will certainly be less expensive, and offers real time imaging, but will suffer with poorer linearity of response, greater inhomogeneity, and deterioration with age. (Inoue, 1986). If the proper steps are taken to characterize this type of detector, however, it can perform adequately in quantitative IM. A nice compromise are simpler, inexpensive direct video out CCD cameras. They combine the linearity of a solid state detector with the ease of implementation and real time behavior of video tube cameras. They generally lack the ability, however, to integrate signal intensities for long periods of time (greater than 10 seconds), a potential limitation in trace analytical work. It seems to the authors that the best choice for a state-of-the-art imaging detector would be use of a slow scan CCD chip that is directly coupled to a glass fiber bundle. These can be fabricated by a number of CCD camera manufacturers. The phosphor screen, which is also commonly fabricated on a glass fiber bundle plate, can be directly applied to the other end of the fiber bundle to which the CCD is attached. This will largely eliminate other sources of optical inhomogeneities and noise (Mantus and Morrison, 1990) which can arise in the coupling of a camera to the mass spectrometer.

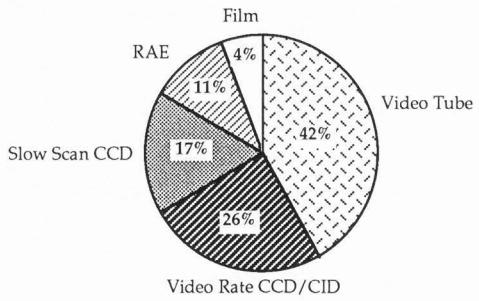

Figure 4. The proportion of various imaging detectors used in research publications cited in this review.

Methods Aiding Quantitative IM

Though not a direct means of quantifying IM images, there are a large number of techniques and methods that aid in the quantification scheme. Discussion of all the applicable methods is beyond the scope of this paper; a few highlights will be discussed in image processing techniques, applications of chemometrics, and correlative microscopy.

Image processing may be loosely defined as the application of analog and digital electronics for the enhancement, manipulation, and analysis of images (Inoue, 1986). Image processing is important in both qualitative and quantitative IM. It can be

said, with a high degree of confidence, that quantification in IM would be impossible without image processing. The acquisition of an image is only the first step in the quantification scheme; the analyst then needs to perform a series of operations, ranging from creating ratio images and image correlation, to contrast enhancement for display and communication of images. Image processing can be particularly useful in correcting both spatial and intensity distortions induced by the instrumentation common in IM (Drummer and Morrison, 1980; Bryan, et al., 1985; Ling, et al., 1987; Hunter, et al., 1989a; Olivo, et al., 1989). Image processing is important in both qualitative and quantitative IM. One particularly important aspect of quantitative imaging is image registration and correlation (Olivo, et al., 1990a). For example, a common operation taken in an empirical scheme is the ratioing of two images. For the ratio to accurately reflect pixel-to-pixel proportions, the two images must be properly aligned. This is a notably important point in magnetic sector instruments because images of different masses experience slight positional shifts relative to fixed points on the detector(Ling, et al. 1988). A particularly attractive aspect of image processing is image presentation in two and three-dimensions; a large number of algorithms have been devised for the presentation of three-dimensional IM elemental maps (Bryan and Linton, 1986; Lee, et al., 1990). Bright and Newbury (1991) have developed an effective technique for comparing and correlating two or three related images, referred to as concentration histogram imaging (CHI). CHI is particularly useful in determining inhomogeneities and, in testing the uniformity of correction procedures in the production of compositional maps.

Chemometrics and information theory have permeated virtually every aspect of analytical chemistry and IM is no exception. Multidimensional and principal component analysis allow for the extraction of meaningful information from systems with a large amount of complex data sets (Sharaf, et al., 1986). Linton, et al., (1989) used pattern recognition techniques to increase the useable amount of information that is available from imaging atomic and cluster ions. An image is acquired at each mass of interest; the approach then treats each pixel as a mass spectrum and picks out important correlations between pixels having similar mass spectra. The process yields correlations as to the chemical composition of heterogeneous inclusions in a matrix and should make quantitative imaging of molecular species in IM feasible. Work in the authors' laboratory are pushing toward similar goals under the integration of data acquisition systems and instrumental control. The ideal system would be capable of acquiring a large number of images at a variety of masses under automatic computer control followed by state-of-the-art statistical analysis and expert-systems control (Ling, 1989). An expert system would be capable of accessing a large data base that would be distilled from the knowledge acquired over the past decades of SIMS analysis, as well as having complete computer control of all instrumentation. It would allow users to operate instrumentation without having to worry about mundane tasks such as instrumental calibration and mass spectral tuning. A researcher can spend more time on acquisition and data analysis.

Correlative microscopy plays a particularly important role in most microanalytical laboratories because no one technique can yield all the information necessary to solve many analytical problems. Researchers have worked towards correlating IM to optical and electron microscopies. As already mentioned, in an earlier section, Chandra, et al. (1989) have successfully applied optical fluorescence microscopy and IM to quantification of free and total intracellular calcium in cultured cells. Olivo, et al., (1990b) used optical and ion image registration/correlation in the analysis of thyroglobulin chemical modification. Turner, et al., (1987) went into a detailed description of the process necessary for the direct superpositioning of scanning electron micrographs (SEM) and IM. Lee, et al., (1989) used an immunogold-silver stain to image antigen-antibody sites in kidney tissues with IM (the technique is common to and was correlated with transmission electron microscopy).

Bernardo, et al., (1988) developed a scattering model of dark field ion microscopy; a technique, when correlated with topographic information from SEM, that is capable of correcting topographical contrast effects in IM. The dark field technique relies upon collecting ion images using a displaced energy window. By measuring the energy distributions of ions that are scattered off a topographic feature contrast (intensity) corrections can be made to an image if the topography is determined by some other technique, such as SEM.

Conclusion

Quantitative IM necessitates all of the requisites of microprobe SIMS with the added complexities of two-dimensional acquisition and analysis. With the increased availability of advanced two-dimensional detection and image acquisition/processing systems, quantitative ion microscopy is a feasible application of secondary ion mass spectrometry. The technique, although limited by the complexity of SIMS matrix effects, can be successfully applied to a variety of biological and inorganic matrices as long as the analyst takes the proper care in selection/production of standards and equal care in the analysis of unknowns.

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Discussion with Reviewers

R. W. Linton: Sector field and time-of-flight SIMS instruments are now commercially available which offer both microscope and microprobe imaging of secondary ions. Would the authors provide a brief summary of the relative merits of these two approaches to SIMS imaging? It would be appropriate to compare capabilities such as analysis time, lateral resolution, image field dimension, and detector considerations such as sensitivity and quantitative response.

R. Gijbels: Can the authors elaborate on the advantages and disadvantages of ion microscopy and scanning ion microprobe mass analysis as far as spatial resolution, sensitivity, time of flight analysis, quantification and imaging aspects are concerned?

Authors: The microprobe and microscope modes are complementary in nature, each strengthens the other's weaknesses. Generally speaking the microprobe mode offers advantages in higher sensitivity, submicron spatial resolution(better than 0.1 μm), and greater versatility with some disadvantages in acquisition time. The single largest

advantage to the microscope mode is parallel imaging; this is important in the analysis of samples that are very thin or sputter at a rapid rate. Microprobe mode can compete with the microscope mode in terms of analysis time when used with a time-of-flight (TOF) spectrometer because the entire mass spectrum is acquired at once, although there are TOF instruments that can operate in the microscope mode. Most TOF instruments, however, are dedicated to static rather than dynamic conditions. With recent advances in instrumentation the two modes compete fairly evenly in terms of image field size, and it seems that microprobe mode offers better "depth of field". Detection in the microscope mode is much more complicated and expensive. Overall the best choice in instrumentation today is likely to be found in the microprobe mode, although an instrument that can operate in either mode is the best choice of all.

R. W. Linton: The incorporation of post-ionization, in particular non-resonant multiphoton ionization using pulsed lasers, has been a recent advance for reducing matrix effects in quantitative elemental analysis using SIMS. The first examples of microprobe SIMS imaging using laser post-ionization are just now being presented. What are the prospects and potential limitations of laser post-ionization for both microprobe and microscope SIMS imaging and quantification?

Authors: Any techniques that reduce chemical matrix effects are welcome in the field and new processes such as laser post-ionization offer great promise to improve both the quantitative certainty of measurements as well as analytical sensitivity. The main application of such techniques, involving imaging SIMS, will more than likely come from microprobe mode SIMS. It has even been proposed by some workers in this field that detection limits will approach single atoms as ionization and collection efficiencies approach 1 for post-ionization time-of-flight instruments. The biggest limitation in using post-ionization techniques in ion microscopy is a likely loss of lateral resolution. The lateral resolution of IM is, of course, governed by the uniformity of the extraction field produced by the immersion lens, it is the single most important lens in an ion microscope. It is not clear at what exact point in time or space an ion is formed in post ionization as compared to a neutral's trajectory from the sputtered surface. (Of course it is not completely understood when a "native" secondary ion forms either.) These neutrals, which are not subject to the extraction field early in their trajectory, will suddenly be ionized and then pulled into the extraction lens. The image produced, after mass analysis, may therefore not reflect the neutral's native orientation in the sample, hence lateral resolution will decrease. It is not known what the magnitude of resolution loss will be, and it may even be within the aberrations of the lens, but the authors are not aware of any results as yet on this matter. For a post ionization technique to work in IM mode it must produce the neutral ionization as early as possible after a neutral is sputtered.

R.W. Linton: The authors state that the use of cell homogenates for standards relies "on the assumption that there is no significant matrix effect between the various parts of the cell, e. g. between the nucleus and cytoplasm. . .". However, it is known that substantial local variations in sputtering rate may be observed even for freeze dried cell monolayers. For example, nucleoli, nuclear envelopes and cell membranes may sputter more slowly than cytoplasm. Such phenomena suggest significant matrix effects on ion intensities within cells, as well as substantial topographic contrast artifacts for cells and tissues that have been extensively sputtered. It would be useful for the authors to address this point for quantitative ion imaging of biological specimens, including citing some of their own recent efforts involving this application.

Authors: Our laboratory has taken a great deal of time investigating SIMS analysis of biologically oriented samples. When one speaks of sputter yield and differences in those yields one must consider the nature of the various structures within the cell and how they relate to such topics such as instrumental lateral resolution in the X,Y, and Z planes. For example, even though a membrane may sputter more slowly than some other component of a cell, a membrane is on average 3-9 nm thick. Under dynamic conditions it is impossible to tell when one has sputtered through a membrane since a dynamic SIMS image is a integration of some sample volume from the cell. The difference in sputter yield results in a minor perturbation of the total, integrated signal. We can never say that there are no matrix effects going on, rather from experience we know that they are fairly inconsequential and that the elemental distributions we observe are physiologically correct and to a good degree, quantitative. We observe homogeneous signals for a wide variety of elements and fragments that are known to be homogeneously distributed throughout a cell. We also observe inhomogeneous distributions for those elements that are known to be inhomogeneously distributed. Our quantitative results from IM correlate well with information obtained from both light microscopy and X-ray microanalysis.

It is fairly easy to say that topographic contrast is a much larger concern than a chemical matrix effect, and not just sputter induced topography but the topology of the fresh sample surface. With a good knowledge of cell biology, one can use IM to both discriminate between properly prepared samples and improperly prepared samples

as well as carry out biological investigations. These matters have been discussed extensively in papers by Chandra, et al., (1987), Ausserer, et al., (1988), Chandra and Morrison (1992). The proceedings of the SIMS VIII conference should also be illuminating as Chandra, et al. provides a rather convincing argument for our case. The entire issue of quantification of biologicals in IM has essentially undermined it's utility and obscured what it is truly capable of as a tool to answer some unique questions in cell biology. There are many problems it can solve without the need to assign an absolute concentration number to every pixel in an image.