Combining Imaging and Spectroscopy: Solving Problems with Near Infrared Chemical Imaging

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Infrared Microscopy

Historically near-infrared (NIR) spectroscopy has enjoyed significant success as the industrial 'cousin' of the mid-infrared FTIR spectrometer. Most NIR spectrometers are significantly more rugged than their MIR counterparts, and are therefore suited for use as a QA/QC tool. Sample preparation is trivial or non-existent for most solid-state or liquid samples and data collection is rapid, while still achieving excellent signal to noise. All these factors combine to produce an analytical measurement technology with widespread applicability. Until relatively recently, its capabilities as a microspectroscopy and chemical imaging tool has been largely underutilized. Most NIR spectra have been collected from bulk, not microspectroscopic samples.

Mid-infrared (MIR) microscopy, based on a Fourier Transform infrared (FTIR) spectrometer, has been the infrared microspectroscopy technique most readily available up to now. It is a broadly employed chemical identification tool that is used to complement traditional light microscopy. Since the first description of the coupling of an infrared (IR) spectrometer to a microscope[1], the method has enjoyed widespread use in a variety of research and industrial applications. In practice, the method couples light microscopy and chemical identification of a spatially defined area. Typically the light microscope is used to locate the region of interest, and then it is non-invasively and non-destructively sampled with infrared radiation to provide chemical identification of the desired microscopic area within the sample. Infrared mapping[2] techniques provided the first step toward bringing the methodology closer to imaging by translating the sample under the microscope while simultaneously recording infrared spectra. This approach slowly builds a low resolution chemical map of a pre-defined area, in contrast to the single spatially resolved data point obtained with the conventional, non-mapping infrared microscope. Global IR spectroscopic imaging, in contrast to mapping, uses a two-dimensional detector sensitive to IR radiation, and was first implemented in the mid 1990's.[3,4] The technique closely resembles digital imaging with a light microscope, and was enabled by the commercialization of the infra-red, focal-plane array (FPA) detector, which had previously been available only for military use. The high cost, limited availability and fragility of two-dimensional mid-infrared FPAs, even a decade after they were first employed for chemical imaging, ensures that most commercially available IR imaging systems still use small focal planes (64x64 pixels) resulting in marginal image quality, particularly when contrasted with light microscope images collected with even a modest CCD camera. This and other impediments preclude its widespread use, particularly in industrial settings. A compromise implementation has recently been introduced that uses a linear array based FTIR system that is more rugged and less expensive to manufacture. It also has limitations in terms of data acquisition speed and, therefore, image resolution is typically limited by data collection time.

Near Infrared Chemical Imaging

With the ready availability of near infrared FPA detectors developed for the telecommunications and thermal imaging market, NIR chemical imaging is rapidly emerging as an extremely practical, useful, and robust chemical imaging microanalysis tool. Commercially available instrumentation can collect imaging data sets containing 81,920 full-range NIR spectra (1200-2450 nm) in less than one minute. These instruments combine two-dimensional infrared focal plane arrays and solid-state tunable filters to produce compact instruments that operate with no-moving parts. As a result the instruments are equally at home in the laboratory environment or in an industrial process setting. While the data we will show in this article are microanalytical measurements the methodology performs equally well, or better, as a macroscopic imaging tool.

Examples

Figure 1a shows the basic data construct for infrared and near infrared imaging systems. The data set, commonly referred to as a hypercube, consists of a sequence of images of a sample recorded over a series of infrared wavelengths or frequencies. The data can be viewed as a collection of frequency resolved images (figure 1b) or, as a spectroscopist would, as a series of spatially resolved spectra (figure 1c). In either case the data can be collected one image at a time, one spectrum at a time, or in the case of a one-dimensional array detector, one line at a time. In the near-infrared imaging system described in this article an image plane contains 320x256

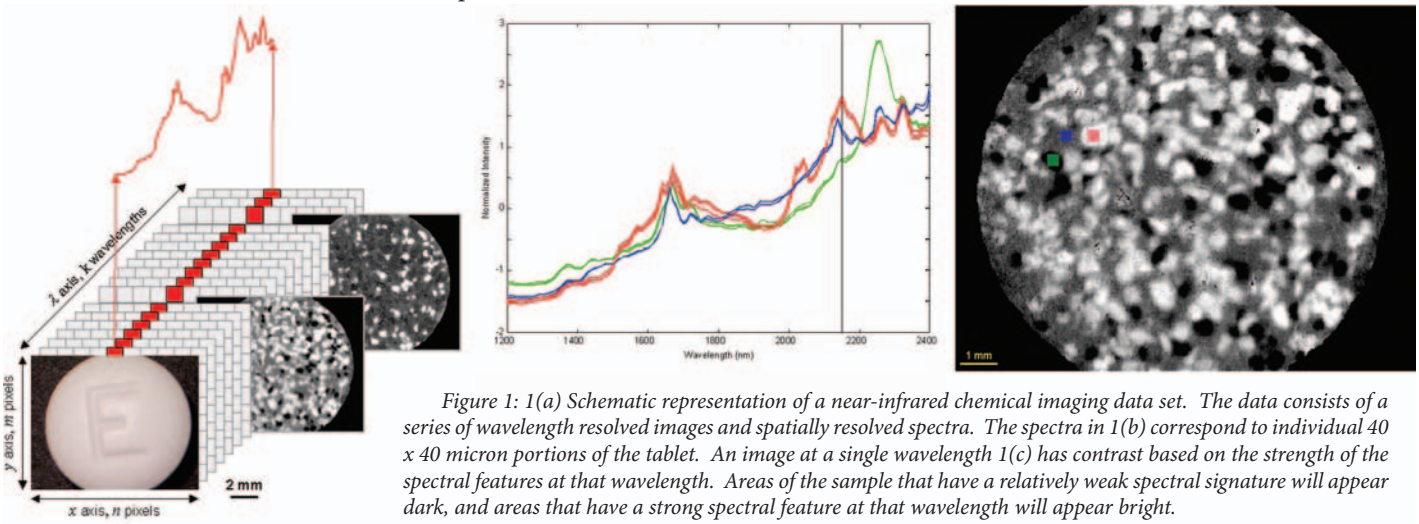

Figure 1: 1(a) Schematic representation of a near-infrared chemical imaging data set. The data consists of a series of wavelength resolved images and spatially resolved spectra. The spectra in 1(b) correspond to individual 40 x 40 micron portions of the tablet. An image at a single wavelength 1(c) has contrast based on the strength of the spectral features at that wavelength. Areas of the sample that have a relatively weak spectral signature will appear dark, and areas that have a strong spectral feature at that wavelength will appear bright.

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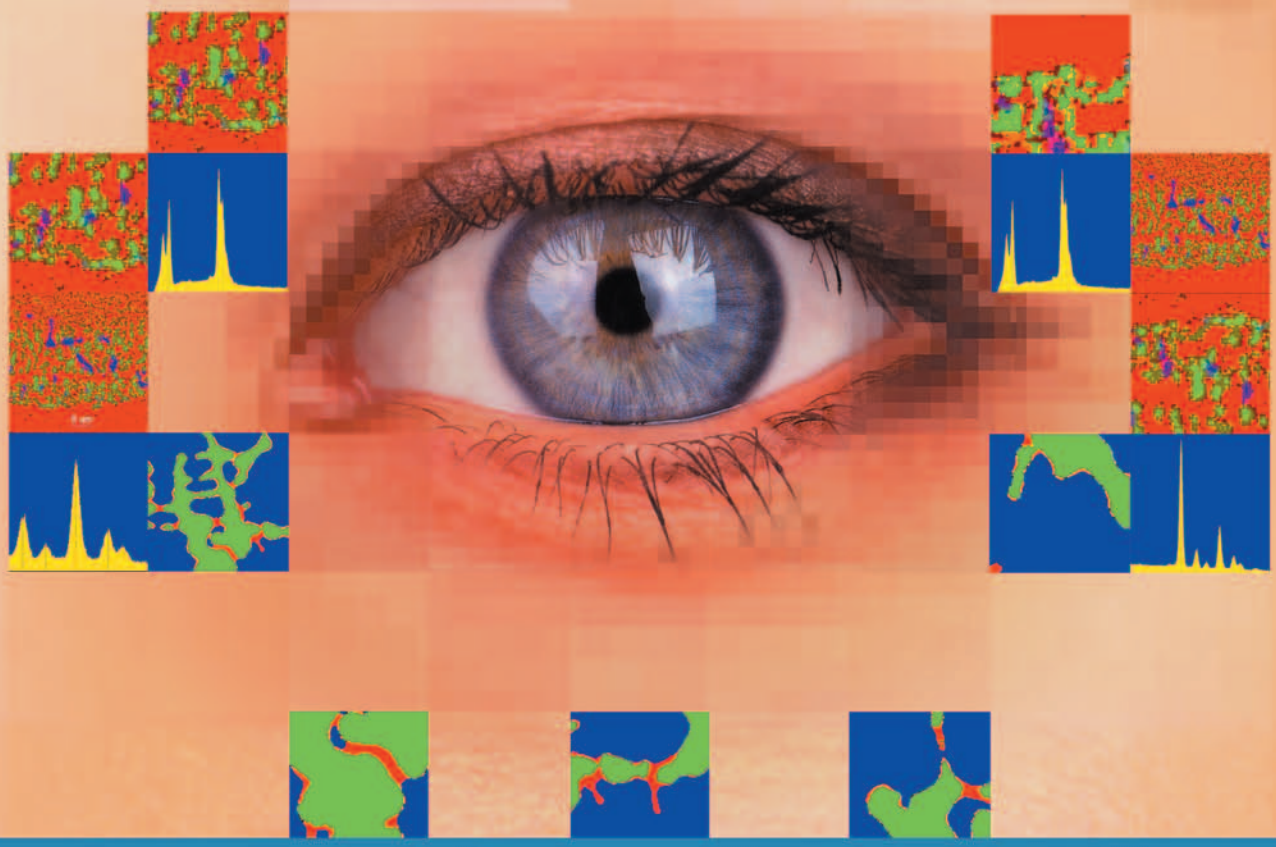

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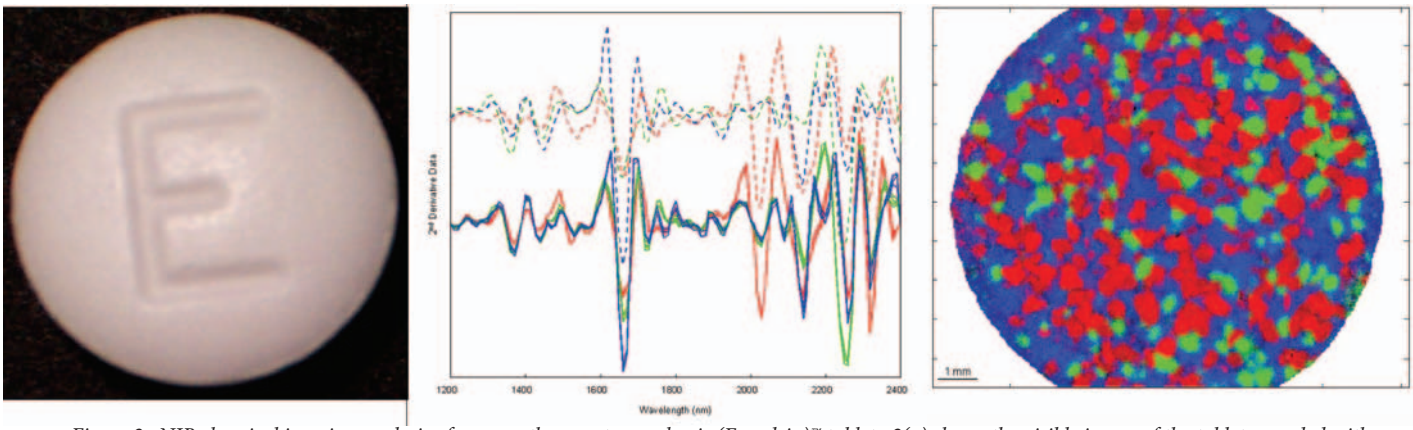

Figure 2: NIR chemical imaging analysis of an over the counter analgesic (Excedrin)™ tablet. 2(a) shows the visible image of the tablet recorded with a digital camera. The analysis proceeds by spectrally matching NIR library spectra of aspirin, acetaminophen and caffeine shown in 2(b) as dashed lines, with the spectra from each spatial location within the tablet. Representative spectra from several spatial locations across the tablet are shown as solid lines in the lower panel of 2(b). Comparison between the pure components and the areas assigned to the components by the spectral match indicates that the assignment is consistent. The spectra in 2(b) are color coded: red spectra correspond to acetaminophen, blue to aspirin, and green to caffeine. Figure 2(c) visualizes the distribution of the three components by color matching individual pixels using the spectral matching. The three color channels are overlaid to summarize the distribution of all three components simultaneously. Again, the same color scheme is maintained.

pixels and the wavelength can be rapidly and continuously varied over the conventional near infrared (1200-2450 nm) spectral range. A more complete description of this instrument (Sapphire™) is available elsewhere.[5,6] The chemical image in figure 1b is that of a whole tablet, demonstrating a field of view (FOV) of approximately 12x9mm. In this example, because the full data set contains 81,920 spectra, a single microspectrum samples a location on the tablet surface of approximately 40x40 microns. This magnification is configurable and can be increased or decreased by selection of the appropriate image formation optics.

Figure 2 shows the type of information that can be gleaned from NIR chemical imaging analysis. The sample is an over-the-counter analgesic (Excedrin™) that contains three active ingredients: aspirin, acetaminophen and caffeine. The visible image of the tablet is shown in Figure 2a. The data set (hypercube) has been processed so that the near infrared spectra of the pure components, stored in a spectral library are 'matched' to the 81,920 spectra in the complete data set. Representative spectra from the different components in the data set are shown in comparison to the pure component spectra in figure 2b. The resulting matched spectra for the spatial locations of the three active ingredients are highlighted in red for acetaminophen, green for caffeine or blue for aspirin (figure 2c). This RGB summary image reveals a tremendous amount of information about the composition of the tablet in terms of particle sizes of the three major components as well as their relative abundance and spatial distribution. For example, it is trivial and intuitively obvious that the caffeine component comprises the lowest concentration (9%). The entire data set is collected in approximately 5 minutes using a tablet removed straight from the bottle with no sample preparation.

The ability to detect and identify contaminants is not easily solved using bulk spectroscopy techniques, as the spectral signature of the contaminant is diluted by that of the sample. For contaminants that are spatially localized within the sample though, microspectroscopy can determine a spectral signature. As described earlier, a typical infrared miscrospectroscopy experiment proceeds by identifying an anomalous region of the sample using the light microscope and then guiding the spectrometer to obtain an infrared spectrum of that microscopic location. The single infrared spectrum recorded enables chemical identification of that specific region. For this approach to work and be useful, the anomalous region must be visible under the light microscope and its chemical identity unknown. This is obviously not always the case, and in the pharmaceutical industry is rarely the case, most white powders 'look' the same. Global imaging techniques though, are ideal for screening large areas for contaminants with no prior knowledge of contaminant location.

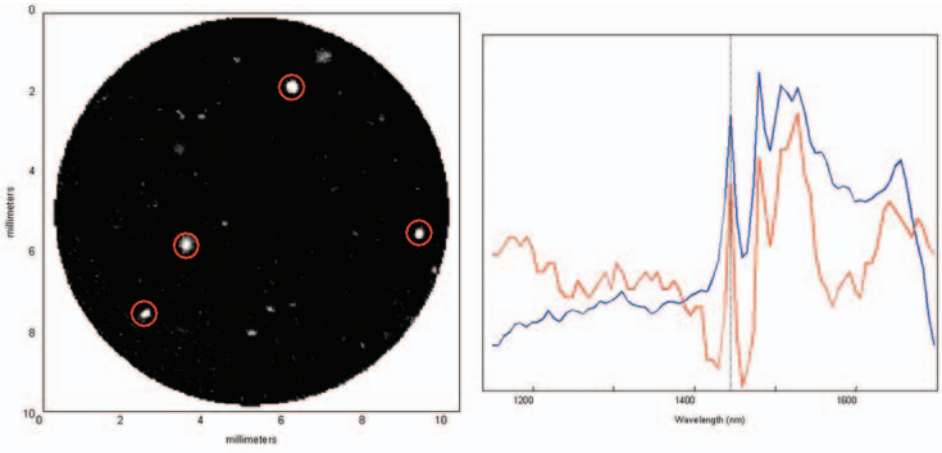

Figure 3: Near infrared chemical image of an intact pharmaceutical tablet containing a low level spatially localized contaminant. 3(a) is a single channel image at 1444 nm. 3(b) shows the spectral signature (in blue) of a known degradant of the active pharmaceutical ingredient (API). The spectrum in red is the spectrum of a contaminant pixel, after subtraction of the dominant substrate spectrum. The contaminant is therefore identified to be the API degradant. The contrast in the image 3(a) based on a strong spectral absorption feature at 1444 nm therefore highlights the distribution of the contaminant.

Figure 3 shows a NIR imaging application for locating low-level contaminants. Again a pharmaceutical product is used for the example, but the ubiquitous nature of NIR spectroscopy suggests that the method would work for many other sample types. In the tablet shown in figure 3a, neither the location nor identity of the contaminant can

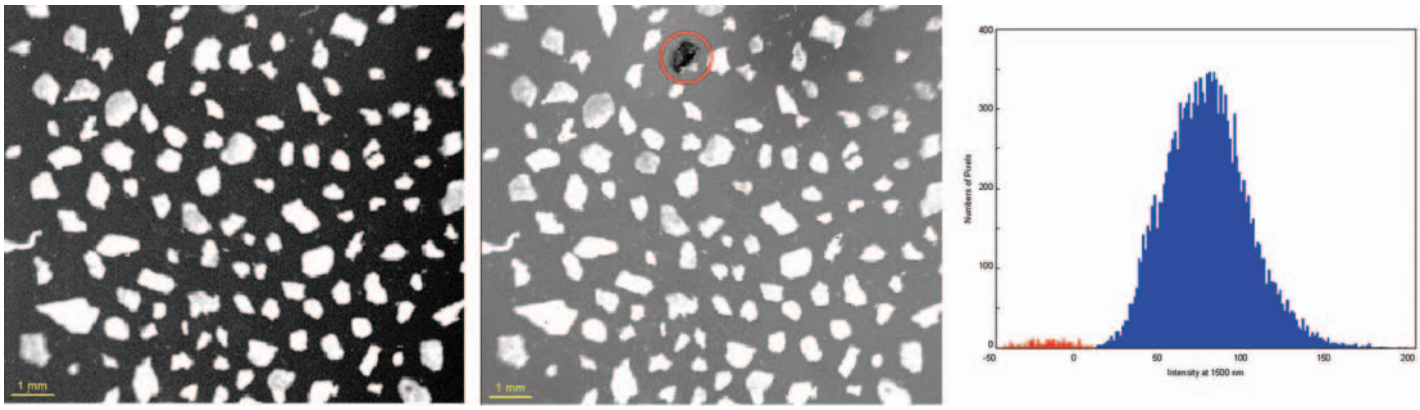

Figure 4: The brightfield image 4(a) of an array of cattle-feed particles. 4(b) shows the NIR chemical image highlighting a single localized particle. Although most of the particles comprising the sample are vegetable based, this pixel is identified as animal protein. This contrast is based on differences in the NIR spectrum of this particle relative to all the others. The histogram 4(c) shows the distribution of the 'chemical intensities' for all the particles, and can be used to quantify the area occupied by the contaminant (population highlighted in red in the histogram) and therefore its relative abundance in the whole sample.

be determined using light microscopy. The product is white and the contaminant is white. However, by using characteristic NIR absorption bands, the location of anomalous regions of the tablet can be quickly visualized with no *a priori* knowledge of their location or composition. Further, since the data set is actually constructed of a NIR spectrum for each image pixel, the spectrum of the contaminant can be easily compared to spectral libraries as a means to identify it. Figure 3b shows the spectrum of a known degradent of the active pharmaceutical ingredient (in blue) compared to the spectrum of the contaminant (in red), confirming its identity. In this particular approach the technique is used as a screening tool to chemically scan relatively large areas of a sample at modest spatial resolution (40x40 microns) in an attempt to search for localized contaminants. While conventional mapping approaches can also establish the presence of the contaminant, these methods are extremely laborious and would quickly become impractical for multiple samples, or larger field of views. Finally, it is worth noting that if the contaminant was not spatially localized, but instead homogeneously distributed throughout the sample, both microscectroscopy and global imaging would suffer from the dilution issue present in the bulk, and would be unable to detect the contaminant.

The same concept of looking for a needle in a haystack is again demonstrated in Figure 4, but here the goal is to identify a contaminant in multiple spatially isolated samples instead of within a single sample. The sample shown in figure 4a, is comprised of particles of cattle feed, and is in effect a series of randomly arranged samples for which multiple NIR spectra per particle are simultaneously measured. Using spectral libraries and other identification methodologies, the data set can be rapidly processed to identify anomalous particles based on their NIR spectral differences. These spectral differences are used to produce the chemical contrast shown in the figure. The lone dark particle in figure 4b is identified as animal protein while the other particles are vegetable based, a determination based on comparison of the spectral signatures of the individual particles to a library of feed component spectra. This application is of interest in the European Union for screening cattle feed for the presence of animal protein to prevent the spread of BSE.7 The image figure 4b can also be represented by a histogram, showing the contaminant as a distinct population in the overall distribution. This representation can be used to quantify the information that is qualitatively visualized in the image. By dividing the number of pixels in the contaminant population (261) by the total number of pixels containing sample (15191), a rough measure of contaminant abundance is calculated. In this example, the animal protein is calculated to be present at 1.72%. As described above, global imaging implementations do not suffer from the conventional 'dilution' problem associated with conventional assays, so the method can achieve high throughput and be made significantly more sensitive than a conventional NIR approach, two attributes of significant importance if the assay is to be deployed as a screening tool.

Figure 5 takes the concepts outlined earlier, so to speak, into another dimension, visualizing the volumetric distribution of an active pharmaceutical ingredient within a tablet. While the conventional data structure shown in figure 1 is three-dimensional (1 spectral and 2 spatial), the data shown in figure 5 has three

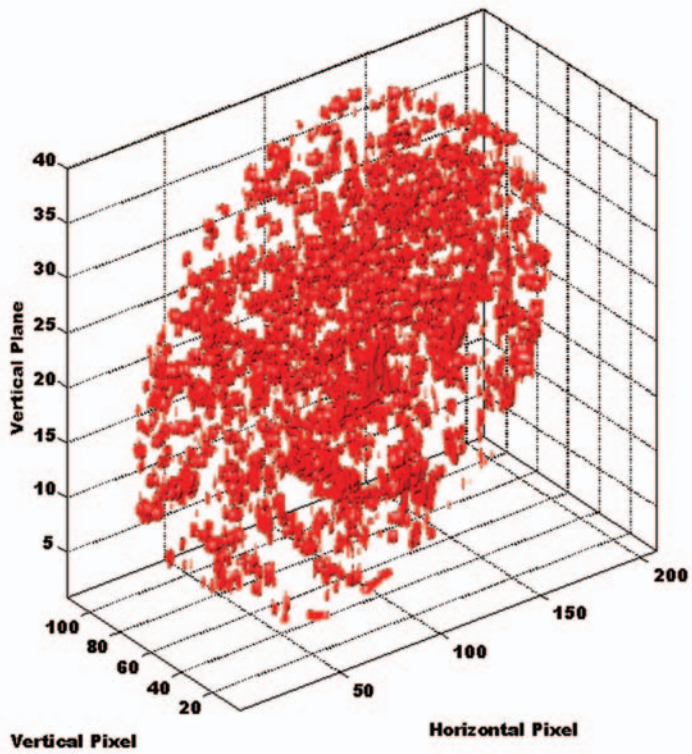

Figure 5: NIR volumetric chemical image showing the distribution of the active ingredient in a pharmaceutical tablet. This data set is actually a 4 dimensional data set containing 3 spatial and one spectral axis. As a result there is a discrete NIR spectrum for each volume element in the image.

spatial dimensions as well as the spectral dimension. Typically, the NIR imaging technique looks principally and non-destructively at the sample surface, but in this example the sample is physically sectioned in-situ to acquire spectral information throughout its volume. In operation, a single hypercube is recorded of the sample surface in reflectance mode and then that layer is shaved away by a sharp blade without moving the sample's overall position relative to the microscope. For this study, a ~250 micron slice of the sample is removed. The data collection process is continuously repeated on a newly exposed surface until the sample is completely exhausted. The final result is 50 hypercubes similar to the one shown in figure 1. Each single image cube is processed in a similar manner to that shown in figure 2 to reveal the spatial location (in two dimensions) of the active ingredient. The process is repeated for all the sections and the resulting 50 single channel images are reassembled into a single three-dimensional image. This image shows the distribution of the active ingredient domains throughout the tablet, not just on its surface. Because a complete spectral data set is recorded for each section, the process of visualizing the 3D distribution of the other components is readily accomplished. Multiple components can be visualized in three dimensions by constructing a RGB volume.

Conclusions

The general notion of a non-destructive, non-invasive chemical imaging tool to complement conventional light microscopy has significant appeal. NIR chemical imaging shares most of the tremendous attributes of conventional NIR spectroscopy, and is therefore an ideal industrial tool. In addition to being rugged and requiring limited to no sample preparation, the technique can rapidly produce high fidelity images detailing critical parameters of valuable products such as pharmaceuticals. We are already beginning to see the deployment of this kind of instrumentation into industrial environments where it can add significant real-time information and therefore value to a manufacturing operation. ■

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Endnotes

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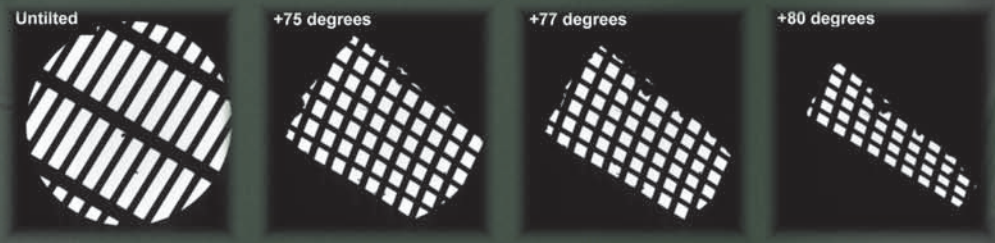
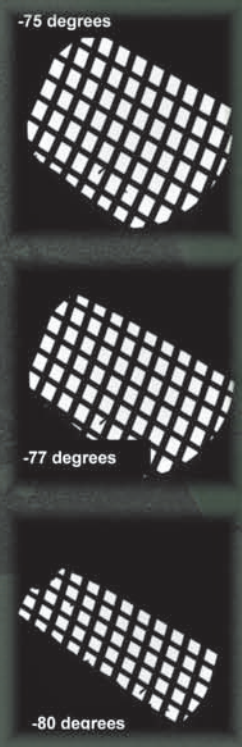
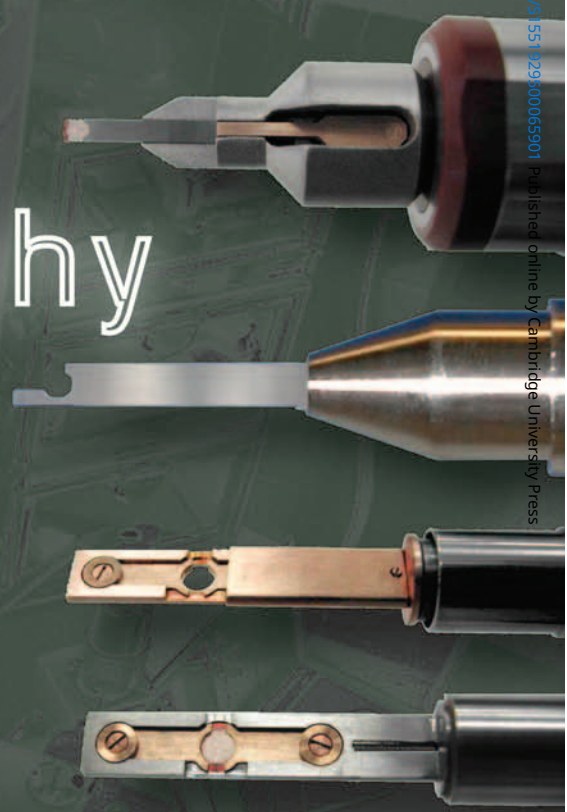


Images of a 3mm diameter 400 x 100 mesh grid taken using a Gatan 916 holder at various tilts.



Tomography Holders



TEM

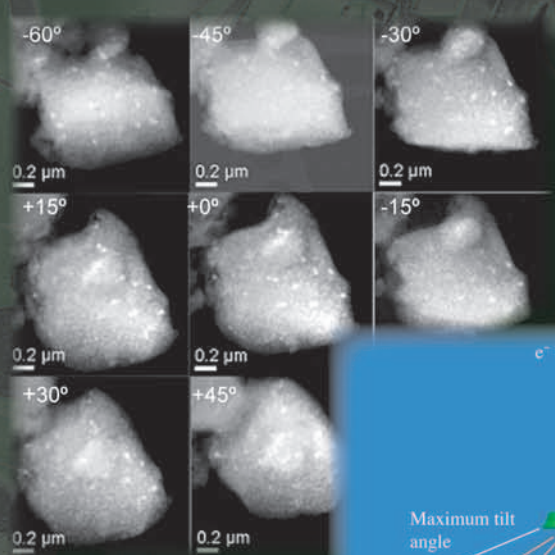
maximum tilt

largest field of view

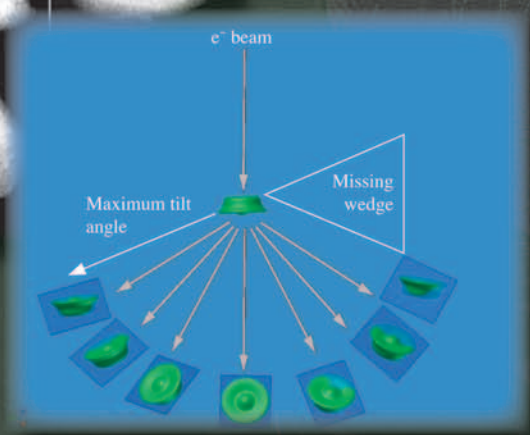
room temperature

cryo-transfer

narrow gap pole piece



Part of a tilt series taken in the TEM using the Gatan 912 Holder. Images courtesy of Dr. Guenter Moebus, Dept. of Engineering Materials, University of Sheffield, United Kingdom.



The Gatan 912 High Tilt Holder is designed to overcome the limitations of achieving very high tilt angles within a narrow gap pole piece and is available only for cantilever type goniometers. The holder reduces the size of the missing wedge in the acquired data set.

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