Imaging - what is it and how is it implemented?

Application Note

Introduction

The term imaging has various meanings depending on the audience. The dictionary definition of Imaging is To make or produce a likeness of an object. The physics definition is The science of producing, recording, storing, transmitting and/or displaying visual images by any system (photographic, video, facsimile, etc.) in any form. A quick search on the World Wide Web brings up topics such as film and digital photography, visible, atomic force and electron microscopy, cytology, radar, computer security, magnetic resonance imaging and positron emission tomography.

The term imaging has a different meaning in each field. As an investigation of the terms will show, the implementation of each “imaging technique” is quite different. Even within the infrared micro-spectroscopy field, the term “imaging” is used for many different techniques. The aim of this paper is to describe each of the various implementations of “imaging” and discuss the benefits and drawbacks of each technique.
Overview of technology

Visible Images
In many instances an infrared instrument capable of taking a visible picture of the sample has been called an “imaging” instrument. These visible images are generated using either film or digital photography. The visible picture produced is an image but not an infrared image.

Infrared Images
The acquisition of infrared pictures is also a form of imaging. These images can be taken using film or a CCD sensitive to short wavelength infrared (~1 micron). There is no wavelength specificity here and the picture is the overall response from all the wavelengths of light hitting the film or CCD chip.

Mapping
The spectroscopist is generally interested in obtaining wavelength specific images of his or her sample. Imaging to achieve these goals is conducted in a slightly different manner. The first and simplest technique to obtain a wavelength specific image is a “mapping” technique where a sample is moved under the objective of a microscope attached to an FTIR by a motorized stage and then, using an aperture or mask, individual spectra are collected from a grid of small areas on the sample. This technique could be considered as analogous to a pointillist painting. Although beautiful, a painting is not completed in a few minutes. A typical map, from the mapping experiment described, could take hours to days to run and the resulting data would be points that are separated by the amount that the stage has moved.

This mapping technique has a number of drawbacks and can be considered to be relatively inefficient.

1. Data collection times can be very long (hours/days). You must sequentially collect spectra from each element of the grid you define
2. For samples smaller than the detector element size, performance is poor because:
   a) The signal arises from only a small part of the detector
   b) But the noise results from the entire detector
3. There is a need to be able to visually discern the area of interest before doing the experiment - otherwise the time wasted in repeating the experiment is significant. Unfortunately, a chemically heterogeneous sample may not appear heterogeneous under visible light.
4. If the sample is changing with time the results will be an inaccurate representation of the sample.
5. The use of an aperture or mask enhances the diffraction limitation effect, thereby degrading the spatial resolution.

Line mapping using a linear array (raster scanning)
A slightly more efficient method of building a wavelength specific image in the infrared is line mapping or raster scanning. This technique is similar to how a television or CRT functions. A linear array of detectors collects spatially resolved spectra using an FTIR microscope, as shown in Figure 1. Then the sample is moved precisely (typically by a motorized stage) and a second set of data is collected. This is continued until either the entire sample is scanned or the end of travel on the stage is reached.

Figure 1. A series of interferograms is collected on one row at a time only, requiring the stage to move the sample across the detector array to collect an image of the entire sample
This linear array mapping technique has both advantages and disadvantages for collection of wavelength specific images. It is faster than the true single point mapping technique described above. If you have an array of 16 elements in your detector you will collect 16 data points at once.

On the other hand if each pixel in your line array acquires data from a 10 x 10 micron area and your sample is 160 microns on a side, you will need to collect 16 rows of data (at 16 separate time intervals to build up your image). Since the signal to noise in the FTIR experiment is proportional to the resolution and number of scans, as you go to higher resolution or better signal to noise your overall collection time increases, not just by the amount of time for a single data collect, but also by 16 times that number. For one experiment this time differential may not be significant but for many samples the increase in time can be dramatic, resulting in decreased productivity and possibly increased running costs.

**Spectrochemical imaging**

The most effective way to acquire a wavelength specific image of a sample is to take a picture using an infrared sensitive camera and an FTIR. Here the user will obtain a 2–dimensional image at all wavelengths in a single measurement. This is accomplished by combining the technologies of a Focal Plane Array (FPA) and the FTIR. A focal plane array is a 2–dimensional array of infrared sensitive detectors similar to a CCD, and measures a signal at every point from the sample area examined. The FTIR produces simultaneous spectral information at every frequency. The result is a spectrum at every point on the sample, **collected simultaneously**.

The sample is imaged on the whole array, and so each detector pixel collects the spectrum from one element of the “grid”. Effectively a full array of spectra is collected in a single scan. This is illustrated below in Figure 2.

**Discussion**

The three techniques discussed above are all capable of building an image of the sample. The benefit to the user, however, is efficiency and time to acquire the data. Table 1 below shows a hypothetical situation of a 160 x 160 micron area where a 1-minute data collection is used to calculate the theoretical time required to collect an entire set of data at constant resolution and with the same number of scans.
Table 1. Theoretical times for data collection of a 160 x 160 micron area using different imaging techniques

<table>
<thead>
<tr>
<th>Technique</th>
<th>Sample size (µm)</th>
<th>Detector element size (µm)</th>
<th>Spatial resolution (µm)</th>
<th>Number of collects (entire area)</th>
<th>Total data collect time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mapping</td>
<td>160 x 160</td>
<td>250</td>
<td>10</td>
<td>256</td>
<td>256</td>
</tr>
<tr>
<td>Linear Array</td>
<td>160 x 160</td>
<td>25</td>
<td>6.25</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Spectro-chemical Imaging</td>
<td>160 x 160</td>
<td>5.5</td>
<td>5.5</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

This calculation does not take into account a variety of factors that can impact the situation. In the single detector mapping experiment, when the sample is smaller than the detector element size, performance is poor because the signal arises from only a small part of the detector but the noise results from the entire detector.

An aperture is typically used to mask the sample to the area of interest. Therefore, to achieve the same signal to noise across the sample, one would most likely need to collect data for 15 minutes at each point when compared to 2-Dimensional arrays for which no aperture is required, giving a total data collection time of 64 hours.

In the raster scanning experiment, the detector is filled with IR light from the sample so the inherent noise problem of the mapping experiment goes away. Here you have another factor to take into account: stage travel. After each data collect, the stage needs to move PRECISELY to the next position. This motion takes time and requires a well-calibrated and precise stage. Most stage mechanisms have an overshoot component and errors typically quoted are on the order of 1 to 2 microns. Using a conservative estimate of 15 seconds to precisely move the stage to the next position, the overall experiment described above will take 20 minutes. Still a very long time.

Table 2 summarizes the time taken to collect the image taking into consideration the additional overhead times mentioned above.

Table 2. Estimated real times for data collection of a 160 x 160 micron area using different imaging techniques

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Conclusion

There are a number of ways in which an infra red image of a sample can be collected. By far, the most efficient and precise method for imaging a sample is taking an entire 2-dimensional snapshot of the sample.

In addition to the benefits of time and increased productivity, spectrochemical imaging is by far the best way to achieve high fidelity images with the best signal to noise in the minimum amount of time.

The approach can be used with all the common sampling techniques such as transmission, reflection absorption and Attenuated Total Reflection (ATR). It lends itself to a variety of applications including material science, food materials, pharmaceuticals and biological materials.
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