

NIR chemical imaging—near infrared spectroscopy on steroids

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Introduction

NIR chemical imaging collects tens of thousands of spatially distinct NIR spectra simultaneously. These spatially resolved data provide qualitative and quantitative insight into the functionality of heterogeneous samples such as pharmaceuticals tablets, polymer laminates or agricultural and biological materials. An often-overlooked advantage of this technique is the ability to perform high-throughput measurements on samples, in effect ignoring the spatial dependence of the information. Rather than focusing on characterising the heterogeneity of a single sample, the high-throughput application is used to compare the chemical signature of multiple (hundreds to thousands) of individual samples. In this article we present examples of NIR chemical imaging, both in assessing heterogeneity within a single sample, and as a high-throughput technique comparing multiple samples. The examples presented both employ NIR chemical imaging as a primary (direct measurement) method for assessing samples, in contrast to a secondary (calibration-based) analytical technique. We will also emphasise the quantitative, reproducible and robust nature of these analyses.

Although the quantitative nature of chemical imaging has always been an integral part of the technique, the analytical approaches for dealing with this novel data construct were developed as applications evolved. As with all new analytical techniques, early developments emphasised technology and hardware improvements, to determine robust, stable and relatively economical instrument and experimental configurations based on available technology. Also contributing substantially to the increasing capabilities of chemical imaging has been the tremendous strides in desktop computing power, which has increased the size of the data files that can be routinely handled while simultaneously increasing the speed and complexity of the types of analyses that can be applied. The latest developments in chemical imaging have focused on advances in numerical strat-

egies, algorithms and routine software tools to enhance the information that can be extracted from chemical imaging data sets.

Although it is normal for novel analytical techniques to begin their lifecycle with the mystique that the technique and analysis is more of an art-form than a science, successful techniques move into the mainstream by providing robust, reliable and automated *data collection* capabilities, coupled tightly with quantitative, statistical, objective, reproducible and automated *data processing* tools. NIR chemical imaging has moved rapidly in these directions. Additionally, for many applications, NIR imaging information is elucidating novel critical product performance attributes that has had the effect of transforming NIR chemical imaging from a specialised technology into a routine and highly desirable quantitative analytical tool.

Instrumentation and data collection protocol

Data presented were collected on the Sapphire[®] NIR Chemical Imaging System and processed using ISys[®] software (both from Spectral Dimensions Inc., Olney, MD, USA). The Sapphire system is a solid state, no-moving parts NIR chemical imaging spectrometer. Samples are illuminated in reflectance with broadband NIR light from four quartz tungsten halogen sources, filtered to pass wavelengths applicable to the spectral range of the system (1200–2450nm). After interacting with the sample, the resulting reflected NIR radiation is collected with imaging optics that can be readily changed depending on the desired field of view for a particular application or experiment. Wavelength selection is performed with a high-resolution Liquid Crystal Tunable Filter (LCTF), and the resulting wavelength selected radiation (9nm bandpass at 1900nm) is focused onto a Stirling cooled Indium Antimonide (InSb) focal plane array with 320×256pixels. This

global imaging implementation enables 81,920 NIR spectra (each comparable to single point spectra from conventional NIR spectrometers) to be collected over the complete spectral range in under 5min.

Background scans are collected with the same collection parameters used for the sample, but with the sample replaced with a piece of high reflectance white ceramic. Spectralon[™] has been found to have spatial heterogeneities that make it non-ideal as a reference for global imaging. In addition to a background correction, because the experimental implementation is a direct **staring** experiment, dark current from the detector must be subtracted from both the sample and background scans to properly correct the data. The optical configuration of the instrument, with sample illumination impinging on the sample at an oblique angle, results in very little specular reflection collected by the system. For the most part, only diffusely reflected light makes it through the optical train and onto the detector. Therefore, dark scans are easily collected by using a mirrored surface in the place of the sample. This is equivalent to having no sample in the field of view. New background and dark image cubes need only be collected if the data collection parameters are changed (spectral range or wavelength spacing) or if the instrument environment (temperature) has changed enough for the instrument to move outside of an equilibrium state. Data is processed as follows:

$$\text{reflectance} = \frac{(\text{sample} - \text{dark})}{(\text{background} - \text{dark})}$$

Most data presented is in the form $\log(1/R)$, where R is reflectance.

Recently, an implementation has been designed that enables the sample, dark and background data to be collected in an interleaved manner by mounting an automated reference accessory that places them into the field of view of the experiment under computer control.¹ In this configuration, data collection is totally automatic, and sample, background and dark scans are collected sequentially at each wavelength, without operator intervention. This automation could be a particularly important

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innovation for process measurements. Additionally, other materials can be added to the holder for automatic wavelength or linearity calibration.

Examples

A simple example of the application of NIR chemical imaging as a quantitative, primary analytical method is the direct measurement of coating thickness of a single time release microsphere. The microsphere studied in this example is approximately 1.2mm in diameter, and has been cross-sectioned for data collection. Figure 1(a) shows an absorbance NIR chemical image at 2080nm. The contrast in this image is based on the difference in NIR absorbance of the coating relative to the core. At 2080nm the core layer shown in red has a relatively stronger absorption. Representative single point spectra taken from within these two layers are displayed in Figure 1(b), with spectra taken from the core area coloured in red, and those from the coating coloured in blue, to correspond to the chemical image. Each single point spectra represents an area on the sample of $\sim 9 \times 9 \mu\text{m}$. Figure 1(c) is a binary representation of the microsphere, in which a threshold was chosen to differentiate between core and coating spectra. Pixels corresponding to coating spectra are set to “zero”, and appear blue in this colour map. Core pixels as determined by the threshold are set to “one”, and appear red. If we assume that each granule approximates a sphere with uniform density, we can calculate that the core accounts for approximately 22% of the total mass of each granule. Variability in coating thickness is also visualised, and the thickness at various points along the microsphere can be measured directly. Statistical measures of mean coating thickness as well as coating thickness variations are readily derived since thousands of measurements are made simultaneously.

One of the unique capabilities of NIR global imaging is the ability to perform high-throughput applications, using the parallel capabilities of the technique to evaluate multiple samples simultaneously. In this example, the optics have been changed to enable a larger field of view ($12.8 \times 10.2 \text{mm}$), with each pixel sampling $40 \times 40 \mu\text{m}$ across this area. The sample is a series of microspheres approximately 0.8mm in diameter that are the constituents of a time-release capsule. Data was collected over the spectral range 1200–2400nm with a 10nm data increment. At each wavelength, 16 images were co-added to produce a data cube in approximately 5min of data collection time. The raw data was dark and background cor-

Table 1

	Red particles	Blue particles	Overall
Number of particles	44	91	135
Mean particle size (mm^2)	0.477	0.536	0.517
Std. dev. particle size (mm^2)	0.059	0.095	0.089
Mean diameter (mm)	0.779	0.826	0.811
Std. dev. diameter (mm)	0.275	0.348	0.337

rected and converted to absorbance as described previously. The sample consists of two types of microspheres, with 135 individuals in the sampling area. Using the spectral differences for each microsphere type [the image at 2050nm highlighting one type in red, Figure 2(a), and the image at 2130nm highlighting the other type in blue, Figure 2(b)] qualitative comparisons can be made. The differences in their NIR absorption properties at these two wavelengths are directly due to the chemical differences in the coating. It also appears as though the blue particles are a little larger, and that there are more of them. In addition to these qualitative observations, quantitative information is readily derived using *Isys*[®]. Table 1 presents the primary, direct quantitative information resulting from the data set, supporting the qualitative observations, but providing values for the total number particles, the distribution between the two particle types, the mean particle size, the standard deviation for this value, the mean particle diameter and the standard deviation for this value.

If we again assume that each granule approximates a sphere with uniform density and further assume that the drug cores for each type are the same size, we can calculate that the uncoated granules account for approximately 32% of the total drug mass but only account for 29% of the total sample mass. In addition, this data set may also be considered as simply 135 micro NIR spectra recorded simultaneously for a randomly arranged sample. It is a relatively straightforward exercise for the software to just output 135 separate “average” spectra, one for each sphere.

Summary

NIR chemical imaging has evolved from a specialised laboratory-based analytical technique, providing mostly qualitative assessment of sample heterogeneity, into a robust process ready technique that provides reliable quantitative data derived *directly* or *indirectly* from *single* or *multiple* samples. Unlike a typical NIR measurement, neither of the

examples chosen for this article used any calibration data, library spectra or chemometrics to produce the numerical output.

However, it should be noted that not all samples are amenable to chemical imaging analysis and it is important to understand the reasons for this. For many samples and applications, single-point NIR spectroscopy will provide the required information in the form of a single qualitative or quantitative measurement; for example, bulk sample identification or quantitative determination of the composition of a mixture. Conversely, there are many samples and applications where only NIR chemical imaging can provide answers. Today NIR chemical imaging is being used to measure spatial heterogeneity and component distribution in powder blends, make direct measurements of coating thickness, particle sizes and associated distribution statistics in complex multi-component samples, locate and identify contaminants in single or multiple samples and perform conventional NIR spectroscopy in a high-throughput modality. In addition, all of this is being done with both microscopic and macroscopic optics on samples that differ in size by several orders of magnitude.

In a nutshell—*NIR spectroscopy is an extremely capable industrial analytical measurement technology. NIR chemical imaging builds upon, and greatly extends this capability by opening up a whole new range of measurement modalities, data collection speeds and application possibilities. The next few years should witness significant growth in the number and types of NIR chemical imaging systems routinely deployed in both the laboratory and process environments.*

Reference

1. E.N. Lewis, *Things That Are New in Imaging*, presented at the Eastern Analytical Society annual meeting (2004).

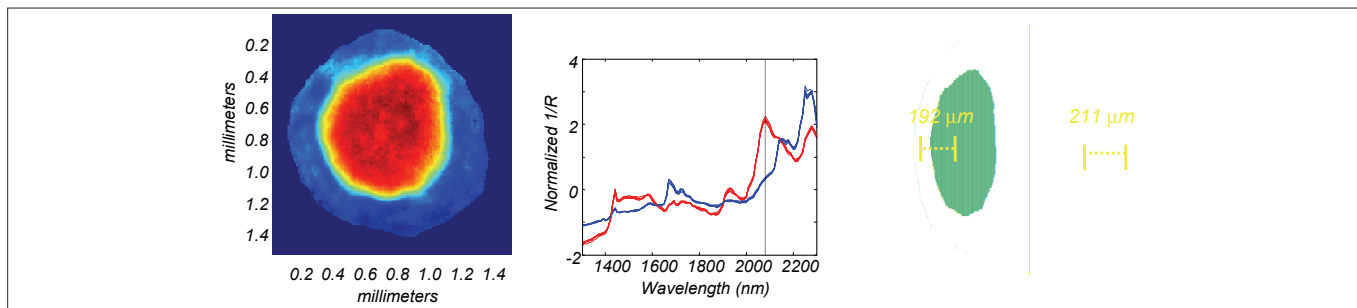


Figure 1. NIR chemical images and representative single pixel spectra of a single microsphere. (a) visualises the chemical differences between the core and the coating, with a single channel the image at 2080 nm. There are significant spectral differences between the core and coat materials at this wavelength, which provides the image contrast. These differences are highlighted in (b), where representative single pixel (each from a $9 \times 9 \mu\text{m}$ area on the sample) are shown. Spectra from the core area are displayed in red, and spectra from the coating layer are in blue. (c) is a binary image in which individual spectra are automatically set to zero or one based on a threshold that differentiates between core and coating spectra. This enable clear edges to be established between these layers, and quantitative information on coating thickness and regularity to be determined. Variation in coating thickness is observed.

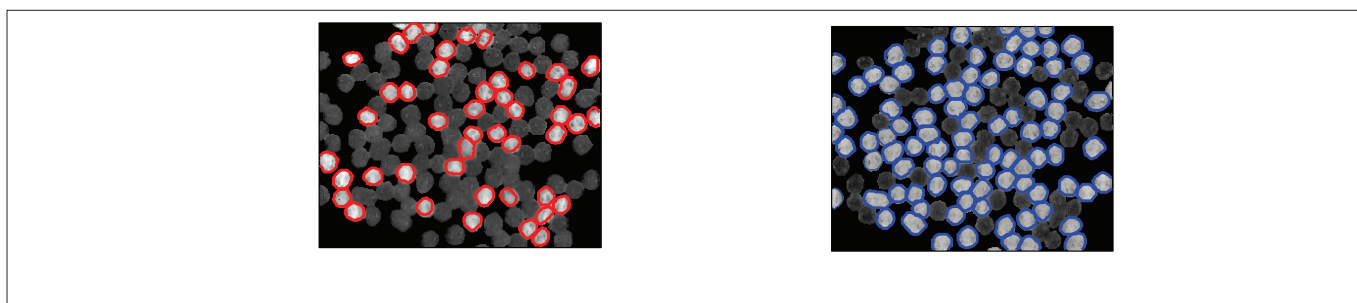


Figure 2. Single channel NIR chemical images of 135 microspheres, highlighting chemical and morphological differences between two bead types. (a) at 2050 nm highlights one type of microsphere—outlined in red. The image in (b) is at a different wavelength (2130 nm) that instead clearly visualises the second type of microsphere—outlined in blue. Visual inspection indicates qualitatively that the blue microspheres are slightly larger than their red counterparts, and that there are more of them. Table 1 in the text provides quantitative comparative assessment of the two microsphere types.