Demanding CCD Applications: Spectral Imaging

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When high performance CCD arrays and cameras first became available, their applications could be neatly categorized into imaging or spectroscopy. In the past few years however, a new set of applications, mostly in biomedicine, have combined imaging and spectroscopy into various types of spectral imaging. In this article we examine this rapidly growing field. The text is based on conversations with Dr. D. Lansing Taylor of Carnegie-Mellon University and Dr. Neil Lewis of National Institutes of Health (NIDDK), two recognized experts in this important field.

How do you define spectral imaging?

Taylor: In conventional CCD-based imaging, an image is produced, by an optical instrument such as a microscope, telescope or endoscope, and digitally recorded on the CCD array. In spectral imaging, we not only record the light intensity at each image pixel, but also information about the spectrum of that light, i.e. the different wavelengths that it contains. Alternatively, we acquire images at different discrete wavelengths.

Lewis: In the ideal experiment, the light would be both spatially and spectrally resolved with each pixel recording a complete and characteristic spectrum.

What type of light is usually imaged?

Lewis: The light forming the image could result from laser induced sample fluorescence, Raman emission, near-infrared absorption or diffuse reflection.

What kinds of experiments are you involved with?

Taylor: In our lab, we work with fluorescence images and spectra as well as a variety of other contrast methods. We excite fluorescence using both lasers and spectrally filtered lamps. For this purpose, we introduce fluorescently labeled compounds into a living cell. These could be fluorescent analogs or physiological indicators. The fluorescent analogs are labeled molecular structures that are introduced into a cell and have characteristic emission spectra. By filtering the light with optical filters before it reaches the CCD camera we can record high contrast images indicating the location of these different materials and structures. High resolution molecular specific anatomy and chemistry if you like.

The physiological probes used to study cellular physiology are fluorescent compounds whose emission spectrum shifts with changes in the concentration of a specific agent such as calcium ions. Monitoring calcium ions is very popular because many cellular activities are associated with changes in Ca^{2+} concentration distributions.

Very recently, we have started working in the areas of 3-dimensional reconstruction microscopy. In this technique, a full 3D image of a translucent or transparent sample is reconstructed by a computer from a number of image slices.

Lewis: While we also work with fluorescent stains and indicators, the main thrust of our research efforts has been in developing CCD-based imaging microscopes to study Raman scattering and near-infrared (NIR) absorption. With these instruments, we are able to record images which simultaneously provide information on both the spatial and chemical properties of biological as well as other materials.

For Raman emission, we irradiate the sample with unfocused laser light and collect the scattered radiation with a modified light microscope. The light is spectrally filtered and imaged on to a high performance CCD camera. For NIR absorption the experimental arrangement uses a conventional white light source and an infrared focal plane array. In both cases, the different materials in the sample each have a characteristic spectrum. We use the spectral signature recorded at each pixel to create modified images which highlight the distribution of particular species or material within the sample.

How do you separate the wavelengths?

Lewis: Our novel imaging technique uses a device called an AOTF (acousto-optic tunable filter). This is a solid state device, with an aperture of 7 mm and a resolution of ± 2 nm, which gives virtually instantaneous tuning in either a random access or scanning mode.

Taylor: At the present time, we are using several coated optical filters mounted on an automated carousel. The other techniques don't yet deliver the image quality we require.

Why is it necessary to use a cooled CCD camera in this type of work?

Taylor: Other than the obvious advantages of recording digital images, the main reasons are linearity, high dynamic range and low noise. When you are dealing with any kind of optical spectroscopy, you need to be able to accurately measure the intensity of the light being recorded. We also deal with enormous intensity differences for which we need at least 14 bits of resolution.

Lewis: Our Raman imaging experiment would not be possible without the low noise characteristics of modern, cooled CCD cameras. This is because the Raman effect is notoriously weak; typically only 1 in 10^8 of the incident photons are Raman scattered.

What technological advances would have a strong positive impact on your work?

Taylor: Higher speed with no loss of resolution. At the moment, we often sacrifice resolution for speed by utilizing binning to speed up the CCD readout. What we would really like though is to utilize the full resolution of say a 1024^2 array with readout rates up to at least 10-20 frames/sec. This would allow us to look at a much wider range of fundamental cellular processes at the resolution we need to understand their mechanics.

Lewis: We are not so concerned in our work with speed. Our major problem is handling and analyzing the vast amounts of data we generate. We are dealing with complex overlapped spectra and we often need to collect images at hundreds of discrete wavelengths. When we use our CCD array at full resolution, in principle we are collecting over 200,000 spectra. While there are excellent image analysis and spectral analysis software packages on the market, there is no suitable commercial product at this time which handles all our needs. ■