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Hyperspectral Imaging for the Life Sciences and Beyond

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Hyperspectral Imaging for the Life Sciences and Beyond

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Abstract

Hyperspectral imaging has evolved from a novel astronomical and remote sensing technique into a bona fide imaging methodology for the life sciences. Hyperspectral imaging's potential to observe and quantify multi-parameter events at the molecular and cellular level open avenues for insights into the basic mechanism of life and the realization of ground breaking diagnostic applications such as high content analysis. The current state of the art utilizes technologies ranging from interference filters and mechanical wheels to non-linear optics such as acousto-optic tunable filters, almost all available commercially as complete platforms. Each technology has its advantages and limitations and its merits must be evaluated in the context of the specific imaging application. We present an overview of some new and exciting applications of this technology in both conventional and novel life science imaging methods along with some observations on future trends in this field.

1.0 Growth of Multi-spectral and Hyperspectral Imaging

Multi-spectral and hyperspectral imaging techniques, originally developed for applications in remote sensing and astronomy, have slowly transformed many areas of life sciences imaging ranging from fluorescence microscopy to in-vivo imaging. Unlike imaging with standard color (RGB) cameras, multispectral imaging provides a wealth of information not discernable to the human eye that can be used for such diverse applications as food production quality assurance, monitoring of crop health and military surveillance. The images in Figure 1 are a comparison of camouflage and true vegetation using classified hyperspectral images.

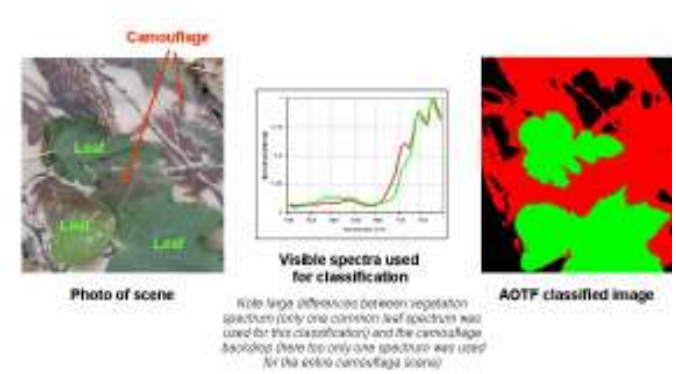


Figure 1 - Hyperspectral Data from a Comparison of Camouflage and True Vegetation

In multispectral imaging, a series of images of an object are acquired at many different wavelengths, producing what can be thought of as an "image cube." Figure 2 illustrates the acquisition of images at individual bandpasses indexed by wavelength and compiled to provide spectral absorbance profiles for determination of oxygen saturation through a cranial window in a mouse.

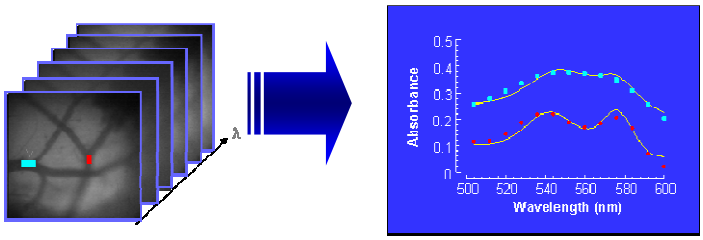


Figure 2 - Building Up an Image Cube

Increasingly, life sciences imaging involves simultaneous monitoring of multi-color labels such as fluorophores or quantum dots. Hyperspectral imaging is the fundamental technology that enables differentiating and quantitation of these labels, and provides the basis for studies of not only cellular components but entire systems.[1] The advent of new developments in non-linear optics has introduced faster, more flexible, sensitive and precise hyperspectral systems, which will not only provide new insight for researchers but also more timely and accurate diagnostic tools for clinical use.

2.0 Current State of the Art

A typical wide-field fluorescence microscope is comprised of an excitation source (often filtered), the sample stage, an objective magnification lens, a dielectric band-pass or "barrier" filter, dichroic filter, a final magnification lens and the imaging detection plane. In its most basic form, multi-spectral imaging can be realized with a set of interference filters and a mechanical filter wheel incorporated into the optical imaging path of a microscope, as in Figure 3. The sample can be a multi-labeled slide, illuminated by an excitation source such as mercury vapor lamp. The filter wheel's output at each spectral band-pass is detected by a single or multi-element detector such as a CCD and collected via computer. Such an approach confines the user to a limited number of fixed wavelength choices requiring a prior knowledge of the sample's spectral emission characteristics, and is mechanically restricted to change wavelengths in ~50 milliseconds.

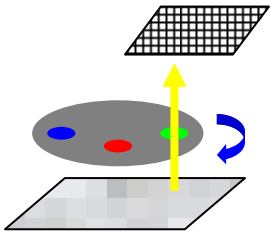


Figure 3 - Filter Wheel

Many hyperspectral imaging systems employ a dispersive element, such as a prism or a grating (transmissive or reflective). Grating or prism techniques are often referred to as 'push-broom' spectral imagers since the entire image is collected line-by-line by precise motion of the sample across the instrument's optical path. By either physically moving the sample or by directing the beam and detector field-of-view, a spectral record of the entire sample image can be collected. This technique collects entire spectral profiles of each pixel of the sample image, which can be time consuming, both in collection and process and storage, but has benefits in terms of data post processing. Figure 4 depicts a schematic of a push-broom system. Such systems are limited to static or relatively slowly changing phenomenon since they are restricted by the speed of motion of the mechanical stages used in moving the sample through the scan. Depending upon the size of the sample this can range from seconds to minutes.

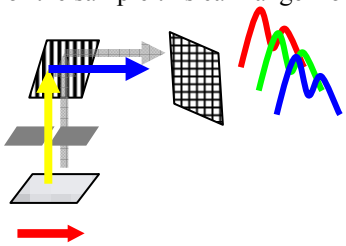


Figure 4 - Push-broom Grating Hyperspectral Imager

In Fourier Transform Imaging Spectroscopy (FTIS), the entire image is captured and, using a Sagnac interferometer and Fourier transforming the resulting images, a spectral profile is constructed. This system, shown in Figure 5, provides excellent spectral resolution across the visible range. The technique does require mechanical movement of one of the interferometer arms, and data processing requirements are considerable. Hence, its application to fast-moving dynamic phenomena is limited, and the acquired data is not immediately available for viewing.

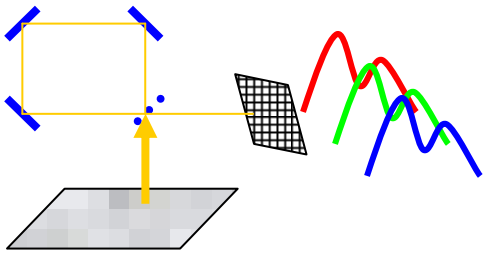


Figure 5 - Sagnac Interferometer Hyperspectral Imager

Tomographic imaging is another approach to spectral imaging. In this technique, the scene is dispersed off a diffraction grating or rotating prism, for example, and multiple orders of the spectrally distributed image are collected at once. This technique has the advantage of the full spatial and spectral information being acquired at one time. However, spatial resolution is limited (since the CCD chip must capture all order images) and, as for FTIS, there are significant processing requirements.

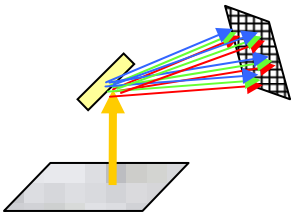


Figure 6 - Tomographic Hyperspectral Imager

Liquid Crystal Tunable Filters (LCTF), also known as Lyot filters, are created using alternating layers and thicknesses of birefringent crystal and liquid-crystal polarizers, which can be used to select out various band passes from a source spectrum, as shown in Figure 7. Lyot filters are band-sequential scanning systems with broad tuning capabilities from the visible through the infrared. Although LCTFs can be constructed with various bandwidths, this bandwidth cannot be changed once fabricated. Wavelength switching times are on the order of 100 - 150 milliseconds and transmission throughput can be quite low, particularly in the blue and for narrower bandwidths.

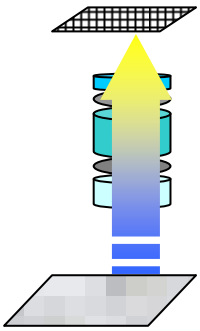


Figure 7 - Liquid Crystal Tunable Filter

Acousto-optic tunable filters (AOTFs) are crystals with optical properties that can be controlled by sending sound waves through them. These sound waves are generated by applying a radio-frequency electrical signal to a specially designed transducer that is bonded to the crystal. With AOTFs, both wavelength and bandwidth can be controlled electronically. Like tomographic imagers and LCTFs, there are no moving parts. Wavelength switching speeds are faster than other band sequential technologies with random access selection in ~50 microseconds. Although AOTFs were introduced over 30 years ago, their use in imaging has historically been limited due to difficulties in obtaining good image quality. This has recently been addressed, however, through the use of innovative transducer designs and long interaction-length crystals.

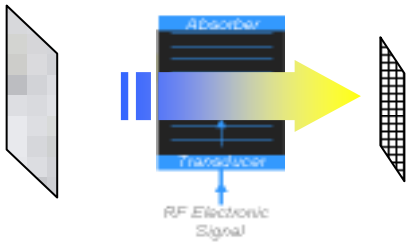


Figure 8 - Acousto-optic Tunable Filter

3.0 Life Science Imaging Applications of Hyperspectral Imaging

Hyperspectral imaging techniques can be applied to conventionally-stained slides viewed in transmission using a light microscope and have shown promise in detecting staining alterations (metachromasia) due to cancer-related changes in a cell's macromolecular components and quantifying light-absorbing chromophores (hemoglobin, bilirubin, etc.) beyond the capabilities of the naked eye. Clinically, such approaches can be utilized to confirm and quantify the presence of abnormal tissue on a conventional pathology slide, to resolve "look alike" questions, or to clarify tumor margins in surgical pathology. Figure 9 shows two examples of the potential power of spectral imaging to detect metachromasia in conventionally prepared H & E samples.

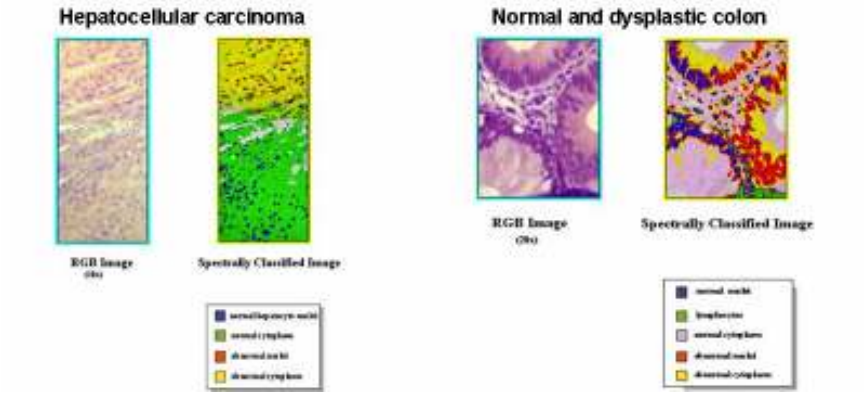


Figure 5 - Detecting Metachromasia in H&E Stained Samples

The images in Figure 10 illustrate the use of AOTF multispectral transmission imaging to identify normal and high-grade squamous intraepithelial lesions in cervical cell samples.

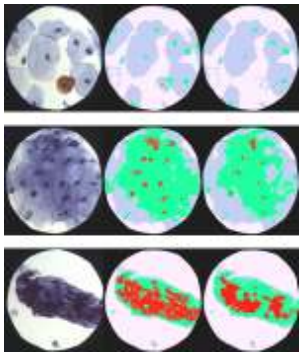


Figure 10 - Multispectral Transmission Imaging to Identify Normal (green) and High-grade Squamous Intraepithelial Lesions (red) in Cervical Cell Samples

One of the most exciting areas for hyperspectral imaging is multicolor fluorescence imaging of cells and tissues. Hyperspectral imaging has been applied to a number of fluorescence microscopy research techniques:

- Multi-parameter fluorescence (seven or more colors on a single slide)
- Multi-color fluorescence in-situ hybridization (FISH)
- Imaging of dynamics events in the neuron
- Ratio imaging
- Fluorescence (Forster) resonance energy transfer (FRET)
- Lifetime imaging microscopy
- Spectral karyotyping (SKY)

Let us consider the first of these techniques. Using conventional multichroic interference filters, four fluorescence probes can be routinely detected in a single cell. As additional probes are added, overlap of the probe emission spectra makes distinguishing them very difficult. By using spectral imaging techniques, multiple overlapping probes can be distinguished within a single filter passband. Figures 11 and 12 illustrate this technique using an AOTF spectral imaging device to distinguish three closely spaced green fluorescence dyes in mouse endothelioma (Fig. 11) and image seven fluorescence markers in breast cancer cells (Fig. 12).

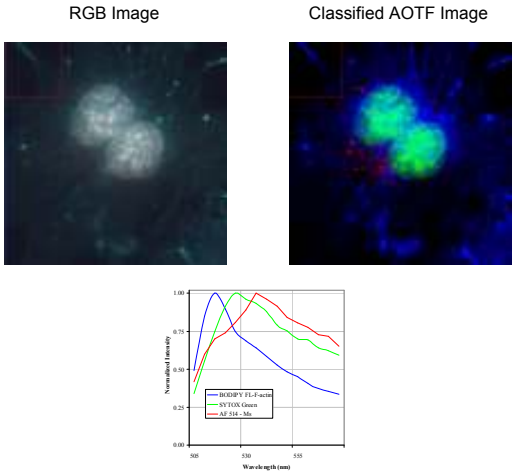
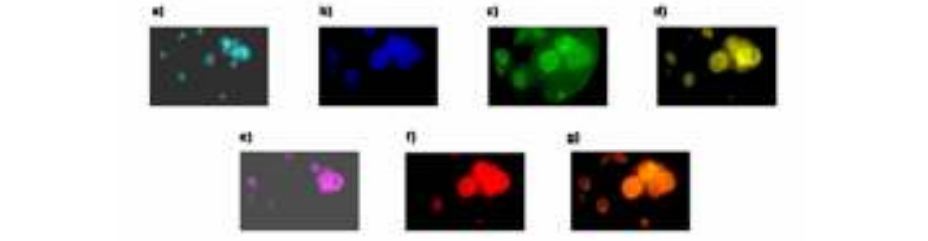


Figure 11 – Distinguishing Three Closely Spaced Green Dyes in Mouse Endothelioma



A - nucleus labeled with DAPI; B - *her-2/neu* labeled with FITC; C - *vegf* labeled with Cy3; D - *cyclin D1* labeled with Cy3.5; E - *ras* labeled with Cy5; F - *c-myc* labeled with Cy5.5; and G - *p53* labeled with Cy7.

Figure 12 - Detection of Seven Probes in Breast Cancer Cells

Unlike a color camera, spectral imaging provides detailed color information throughout the spectrum imaged. By using techniques such as linear pixel unmixing, quantitative information can be obtained even when there is strong colocalization of the various probes. One example of this approach, also acquired with an AOTF spectral imager, is shown in Figure 13. The three images in the center are the results of using linear pixel unmixing to separate out the blue, green, and red components, respectively. The image at the right is the pseudo-colored classified image based on these results. This image contains quantitative information as to the amount of each component present at every pixel. For illustrative purposes, the spectrum of the red fluorescence signal is shown in the inset. This technique has also been used to eliminate autofluorescence background from fluorescence samples.

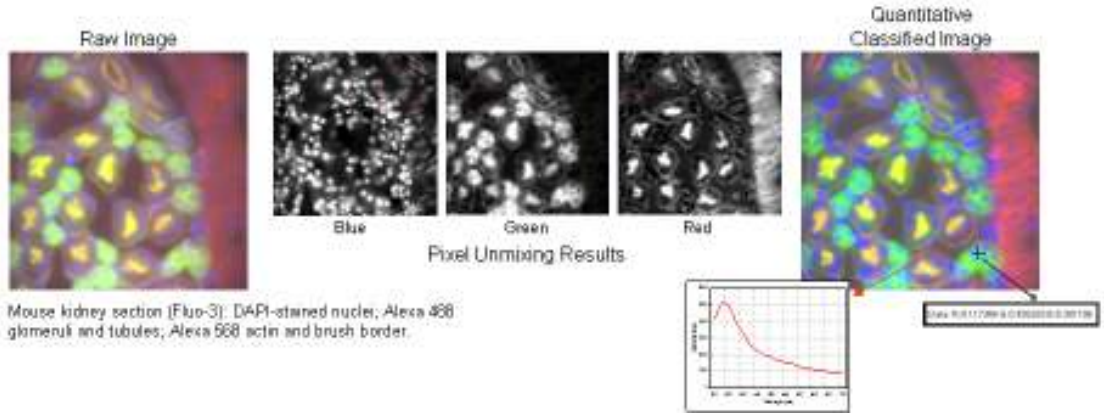


Figure 13 – Linear Pixel Unmixing in a Three Color Tissue Sample

4.0 Next Steps

Hyperspectral technologies are now beginning to become integrated with other imaging technologies in the life sciences—in brightfield and fluorescence microscopes, in confocal instruments and in macro imaging set-ups. Spectral imagers can be used with a variety of other microscope techniques to provide a wealth of information not available with conventional microscopy, and in particular, to allow a vast increase in the number of probes that can be imaged within a single sample.

Of particular interest for drug development are in vivo techniques which permit real time visualization of the take up and effectiveness of therapeutic agents. Investigators must select from among the variety of technology options for hyper and multispectral imaging available those that most closely address the event or phenomenon being observed. In the clinical sphere, automated imaging platforms incorporating conventional optical microscopes with hyperspectral imaging systems and intelligent software have the potential to transform diagnostic medicine as more and more diagnostically and therapeutically relevant targets are identified. Numerous other applications are being investigated, and it is likely that their successful development and implementation will require interdisciplinary efforts involving physicists, engineers, life scientists and physicians.

5.0 References and Acknowledgements

[1] Hiraoka, Shimi and Haraguchi, "Multispectral Imaging Fluorescence for Living Cells", Cell Structure and Function, 27, Japan Society for Cell Biology, pp. 367-374, 2002