Hyperspectral Identification of Multi-fluorescent Signals in a Single Image January 2021

CytoViva

Illuminating the Future

Advancements in immunofluorescence have created a large and highly stable supply of fluorescent labels with emission wavelengths ranging from the early visible to the near infrared. By combining this extensive fluorescence capability with emerging biomarker diagnostics for research, there is an opportunity for significant advancement in the diagnosis of cancer and a wide range of other diseases.

The options for non-invasive diagnostic testing using immunofluorescence in combination with disease bio-markers can be increased even further by the ability to accurately detect and measure multiple fluorophores in a single sample image. This capability enables quantitative identification of multiple potential disease biomarkers isolated from a single blood, saliva, urine or other sample.

Most established fluorescent imaging and detection modalities are limited in their ability to identify fluorescent labels. For example, confocal or wide-field fluorescence microscopy can typically identify labels in individual colors with up to a maximum of three colors (e.g. red, green, blue). Furthermore, imaging each color requires the use of multiple lasers or filters for excitation. Likewise, while conventional flow cytometry is a higher throughput method for fluorescence-based disease diagnosis, a typical detection limit of 200 nm – 300 nm can preclude its use for detection of many disease biomarkers such as exosomes, which can be below 200 nm in size.

CytoViva Hyperspectral Microscopy addresses these limitations with the ability to image multiple fluorescent labels conjugated specifically to different disease biomarkers in a single sample acquisition with a very high detection ability below 100 nm.[1] CytoViva utilizes highly unique hyperspectral microscopy technology that incorporates patented enhanced darkfield (EDF) condenser optics on the microscope. These optics project highly structured, oblique angle, broad spectrum light onto the sample resulting in a very high signal-to-noise ratio in the recorded image, thus providing superior detection capability. The broad range of wavelengths from halogen light (a continuum source free of spectral lines) supports excitation of fluorescent labels across the visible and near infrared spectral range. From this dark-field form of illumination, only fluorescence emission and light scatter from the specimen structure is recorded. As such, direct excitation is not detected by objective.

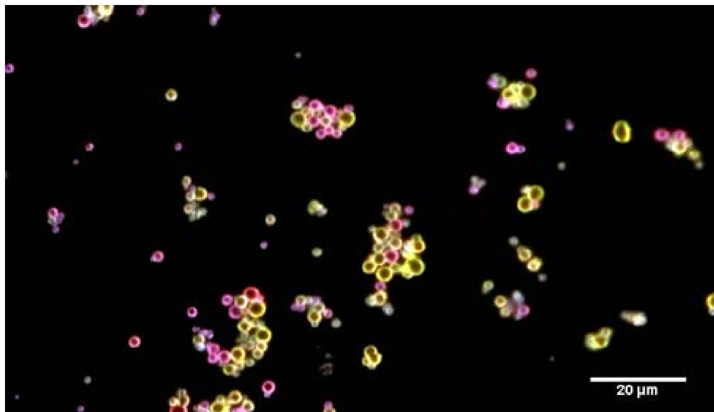


Figure 1: Enhanced darkfield hyperspectral image of beads labeled with fluorescence producing emission 510 nm, 600 nm and 655nm.

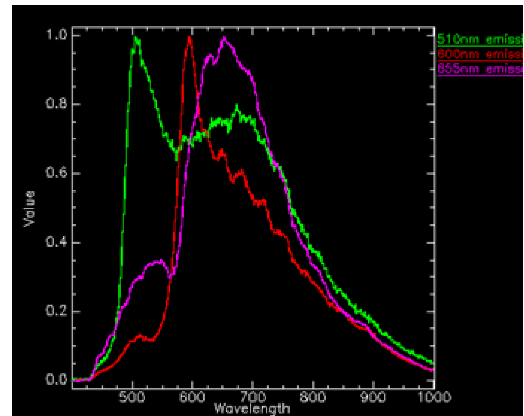
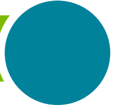


Figure 2: Spectra taken from image pixels corresponding to different labels on beads spread over the hyperspectral Image in Figure 1. Spectra include fluorescent label peaks as well as scattering and absorption features of the sample.



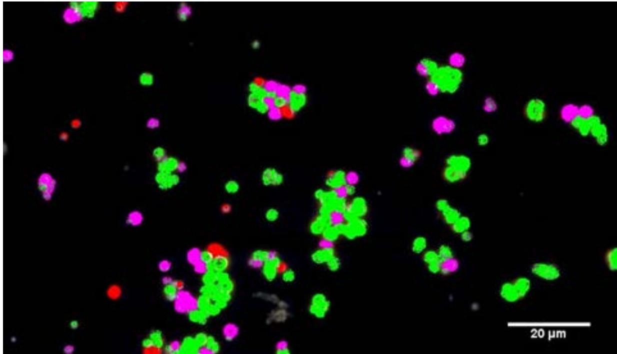


Figure 3: Mapping of fluorescent beads corresponding to emission spectrum of 510 nm (green), 600 nm (red) or 665 nm (magenta) as shown in Figure 2.

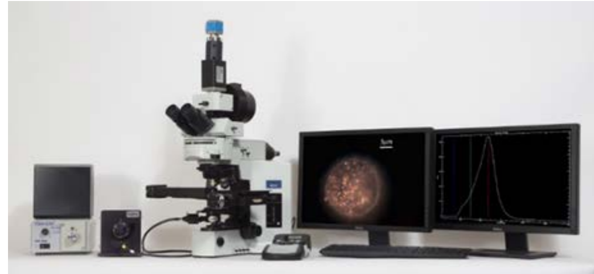


Figure 4: CytoViva Enhanced Darkfield Hyperspectral Microscope

The result is a recorded spectral image with low background noise which enables fluorescence emission or other scattering signals from isolated disease biomarkers below 100 nm in size to be identified.[2] CytoViva's enhanced darkfield hyperspectral microscope system provides a spectral resolution of approximately 2 nm across the visible to near infrared spectral range. This allows a large number of fluorescent labels to be identified in a single sample, including the identification and mapping of multiple fluorescence signals within a single visible color range.

The figures above illustrate how a mix of fluorescent labels are identified and characterized. Figure 1 is an enhanced darkfield hyperspectral image of beads that contain three different fluorescent labels. Two of the beads produce fluorescent emission in the red color range (600 nm and 655 nm) while one bead produces a green fluorescent emission (510 nm). This image was created using a full spectrum quartz halogen lamp. Note the dark, low-noise background produced by the enhanced darkfield optics versus the high signal from the beads. Figure 2 illustrates fluorescent emission spectral responses from three differently labeled fluorescent beads in the sample. The recorded emission spectral peaks of 510 nm, 600 nm and 655 nm are shown. Note that as broadband illumination is used to excite these fluorescent beads, the underlying polymer structure of the beads contributes to the spectral curve on a secondary basis along with the primary fluorescent peak.

Figure 3 shows differently labeled beads in the sample that have been identified using CytoViva's Spectral Angle Mapping feature. Each bead is shown in the color corresponding to that of the spectral curve produced by its own label (Figure 2). Figure 4 shows the CytoViva Enhanced Darkfield Hyperspectral Microscope used to capture these images and data.

To produce this data in an optimized manner, fluorescent structures such as beads or exosomal bio-markers, must be sufficiently isolated from other cell, tissue or biological structures, and have a stable fluorescent label. However, applications with exosomal bio-markers utilize isolation methods that are well established for a wide range of stable fluorophores.

CytoViva's enhanced darkfield microscope optics, combined with its hyperspectral imaging capabilities, create a unique opportunity to identify multiple fluorescently-labeled elements in a single sample image. To learn more about CytoViva's technology supporting this, and other related applications, please contact CytoViva at info@cytoviva.com. We would be pleased to discuss your research and organize a demonstration with your samples.

[1] Peng Zhang Sangyoon Park Seong Ho Kang (2015) Microchip Electrophoresis with Enhanced Dark-Field Illumination Detection for Fast Separation of Native Single Super-Paramagnetic Nanoparticles Bulletin of the Korean Chemical Society https://doi.org/10.1002/bkcs.10219

[2] Chaudhari, K., & Pradeep, T. (2014). Spatiotemporal mapping of three dimensional rotational dynamics of single ultrasmall gold nanorods. Scientific Reports, 4, 5948. http://doi.org/10.1038/srep05948