

## GaiaMicro HyperSpectral Imaging System

Hyperspectral microscope images appear very similar to a traditional optical microscope image with one important difference. Each pixel of a hyperspectral image provides the complete reflectance spectral response of that pixel's spatial area within the VNIR or SWIR spectral range. This enables nondestructive spectral measurements of nanoscale elements in the full spatial context of the sample image.

At 100x magnification, a hyperspectral microscope image may contain as many as 700,000 pixels, each as small as 128nm each. This hyperspectral data is recorded at approximately 2nm of spectral resolution in the VNIR range, enabling minute spectral differences to be measured from pixel to pixel within the image.



Imaging spectroscopy can be considered a happy combination of spectroscopy and image processing, and can be seen as an extension of classical image processing. GaiaMicro

HyperSpectral platform is based on a Metallurgical Microscopy, dual-mode optical device, which allows both image capture for hyperspectral imaging and direct view mode for high-resolution CCD image capture. The HyperSpectral data reveals the spectrum of every pixel in the image, and provides advanced analysis tools to extract quantitative and hidden information from within a sample. In Direct View mode, the system records image details under extremely low intensities and provides a finely detailed high-resolution and high-definition image.

Spectral Imaging describes image acquisition and analysis method which combines spectroscopy, multi-dimensional imaging and computing to delineate the way light reacts with a sample in order to quantify and analyze information that might otherwise be hidden. The

underlying principle is the simultaneous measurement of the detailed spectrum of every point of a given area (surface), or more specifically, of each pixel of a given CCD array. Spectral Imaging can be used to obtain fluorescence or bright field spectra, such as absorption, transmission, or reflection.

The spectral image allows you to precisely locate chemical constituents providing unique and unparalleled insights into the molecular origin, formulation and phase of the observed living entity. The sensitivity range of the spectral image measurement follows the sensitivity of the camera CCD or InGaAs and allows measurements between 400~1000 nm or 900-1700nm, thus covering the whole visible range as well as low near infrared range of electromagnetic radiation. A maximum spectral resolution, comparable with ~3 nm at short wavelengths and increasing to ~5 nm towards the near infrared range, is achieved.

Table:1

Instrument model	GaiaField-Pro-V10	GaiaField-Pro-V10E
Spectral Region(nm)	400-1000	400-1000
Spectral resolution(nm)	$3 \pm 0.5$ (with 30 $\mu$ m slit)	$2.8 \pm 0.2$ (with 30 $\mu$ m slit)
Slit Length(mm)	9.8	14.2
Slit Width( $\mu$ m)	30	30
Numerical Aperture	F/2.8	F/2.4
Light Transmission	>50%	
Spectral Bands	720(2X Binning)	720(2X Binning)
CCD Pixels	1936 $\times$ 1456	
Pixel Size( $\mu$ m)	4.54 $\times$ 4.54	
Digital OutPut(bits)	8 & 12	
Dynamic Range(dB)	>66	
Frame Number (fps)	15	
Exposure Time Range(ms)	0.0035-1200000	
Lens Connector	C-Mount	
Power	DC16.8V	
Power Consumption(W)	45 W cooled	
Cooled Mode	Wind Cooled	
Storage Temperature( $^{\circ}$ C)	-20 $\sim$ +50	
Running Temperature ( $^{\circ}$ C)	+5 $\sim$ +40	

Table:2

Instrument model	GaiaField-F-N17E-N3	GaiaField-F-N17E-HS
Spectral Region(nm)	900-1700	900-1700
Spectral resolution(nm)	5(with 30μm slit)	5(with 30μm slit)
Slit Length(mm)	14.2	14.2
Slit Width(μm)	30	30
Numerical Aperture	F/2.0	F/2.0
Light Transmission	>50%	>50%
Spectral Bands	100-200	200-300
Pixels	320x256	640x512
Pixel Size(μm)	30mm×30mm	20umx20um
Digital OutPut(bits)	14	14
Frame Number (fps)	100	100
Exposure Time Range(ms)	5us-1s	1us-40ms in high gain mode
Gain Mode	High/Low	High/Low
Signal Connector	Ethernet port/USB	USB2.0
Lens Connector	C-Mount	C-Mount
Cooled mode	Cooled TE3	Cooled TE3
Power (detector)	+12V	12VDC
Power Consumption(W)	< 4 W without TEC operation Max. 30 W with TE3-cooling	60
Storage Temperature(°C)	-45~+85	
Operating Temperature (°C)	-40~+70	



Hyperspectral microscope images can be captured of both biological and materials-based nanoscale samples. These nanoscale materials may be integrated in a wide range of biological or materials based environments. See below an example of lesion in glomerular cell. In this example, hyperspectral imaging makes it possible to quickly identify and map the difference present in the tissue and provides a class distribution confirming the total area where lesion are present.

Microscopic hyperspectral imaging "no contact" and "no damage" detection on biological tissues. It can really realize the comprehensive analysis target of "where, what and how much" for

biological tissue and qualitative detection target, provide quantitative and positioning description, so as to realize auxiliary diagnosis of some diseases. With the improvement of system spatial resolution and spectral resolution, can analysis biological tissue biochemical from the angle of molecular, and find out the pathogenesis of response for some organizations achieve automatic analysis and auxiliary medical researchers, and quantitative evaluation effect of the medication.

Combined with blood cells morphological characteristics and the existing method of blood cell analysis, researchers research the data of the normal blood / leukemia blood of hyperspectral imaging. The characteristics of red blood cells, lymphocytes, leukemia cells were analyzed by microscopic hyperspectral imaging, and the implementation of the diseased cells segmentation and morphological parameter quantitative calculation.

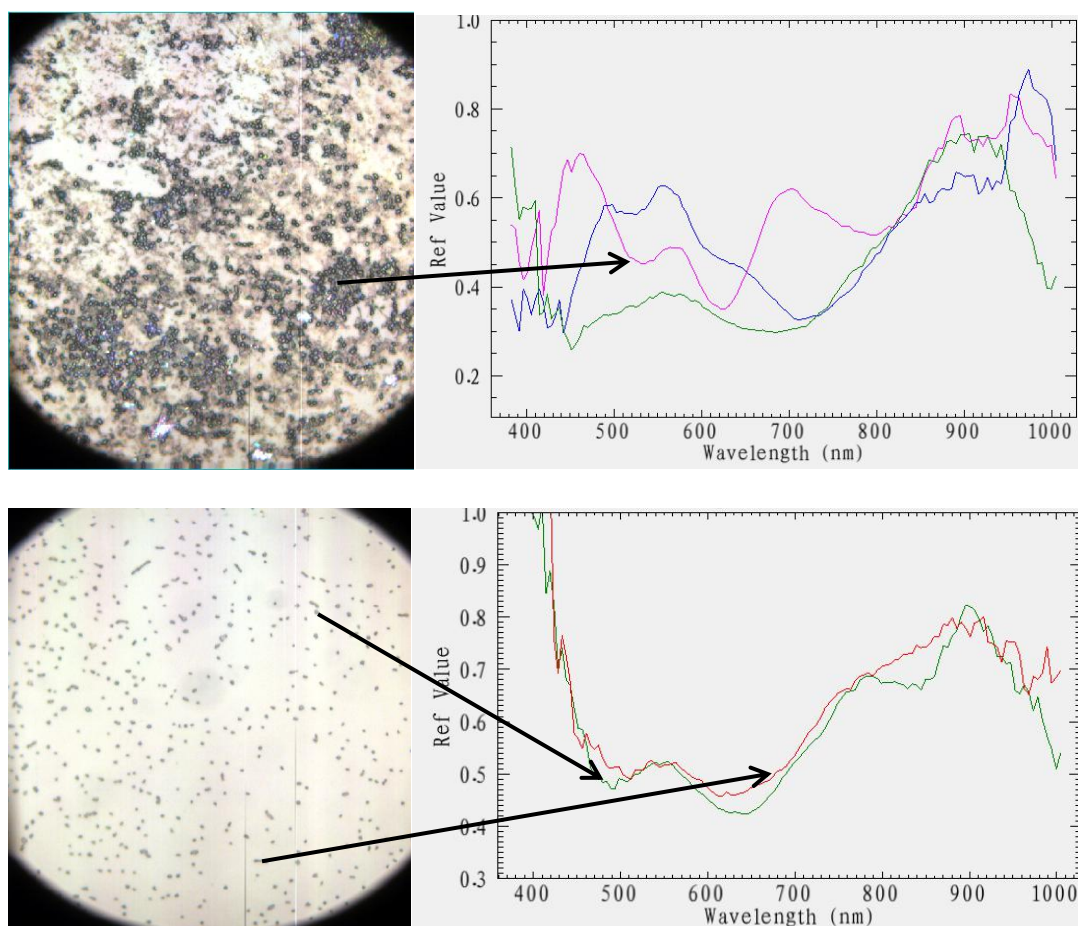


Figure Vis-NIR Microscopic hyperspectral imaging and spectral of bacillus cell

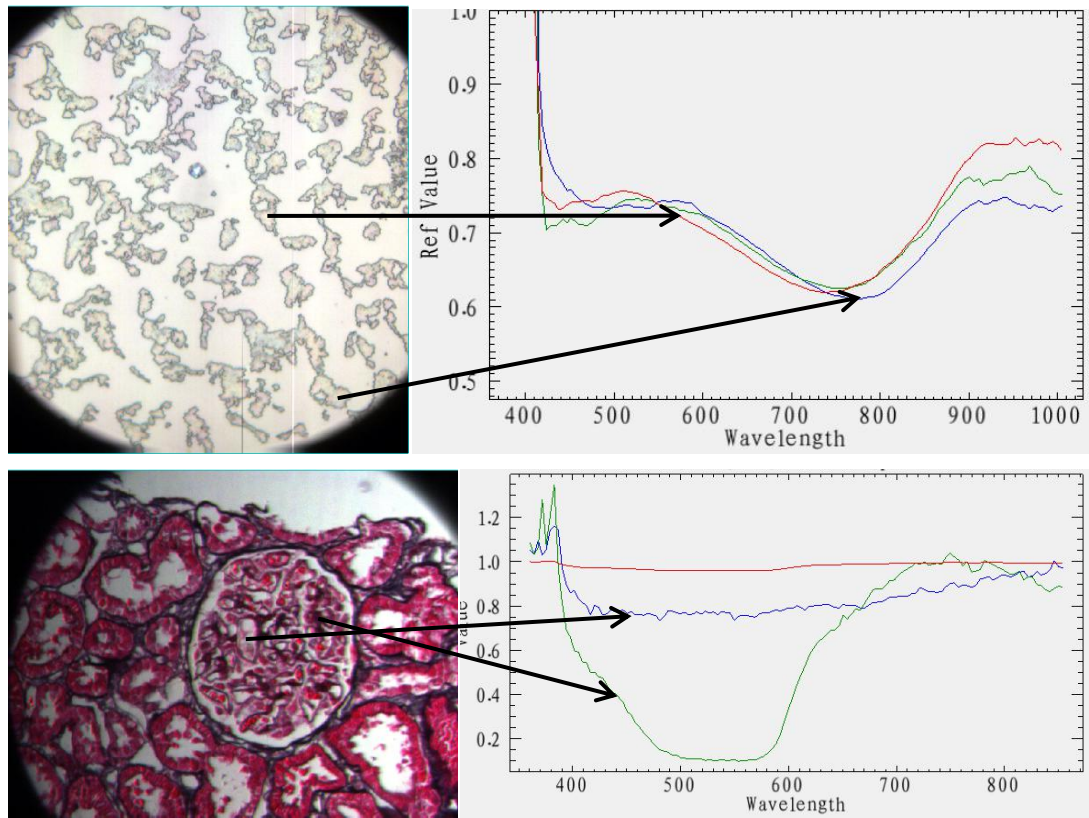


Figure Vis-Nri Microscopic hyperspectral imaging and spectral of glomerular cell

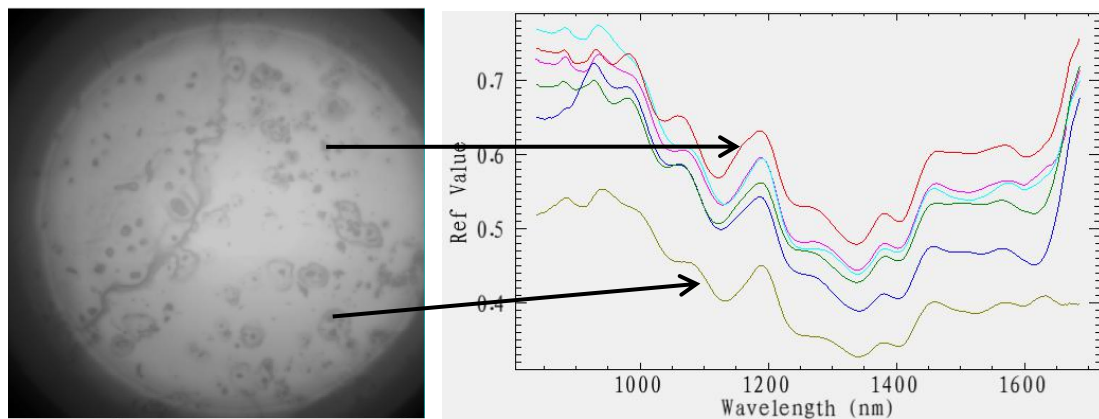
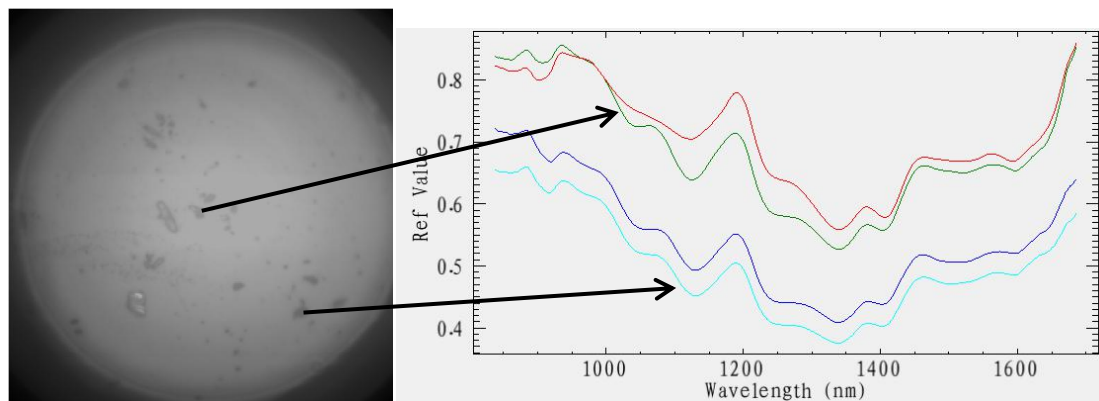
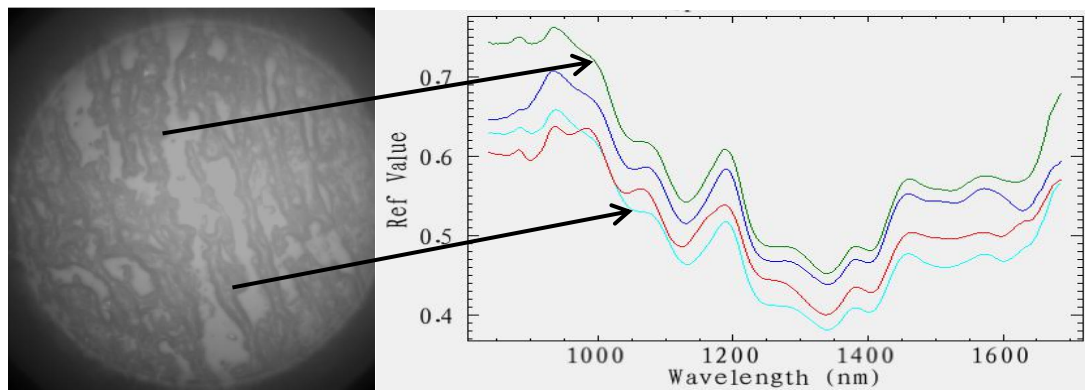
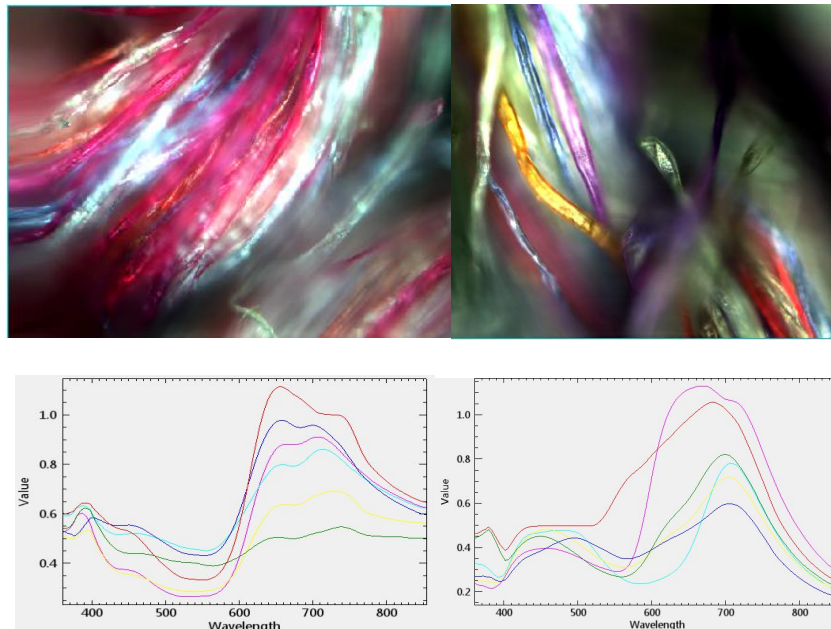


Figure Near infrared microscopic hyperspectral imaging and spectral characteristics of cells, nuclei, blood stains, bacillus cell and mutton tissue sections





*Figure Near infrared microscopic hyperspectral imaging and spectral characteristics of blood stains**Figure Near infrared microscopic hyperspectral imaging and spectral characteristics of mutton tissue**Figure A hyperspectral microscope shows the spectral and imaging of fabric (20x)*

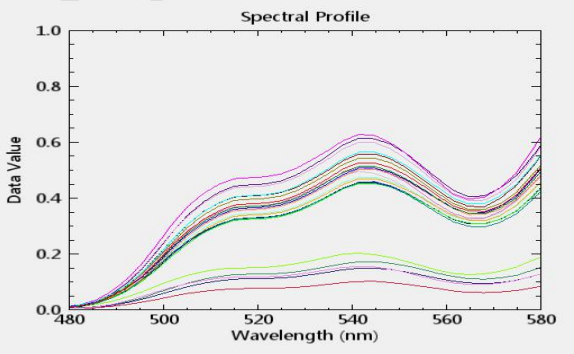
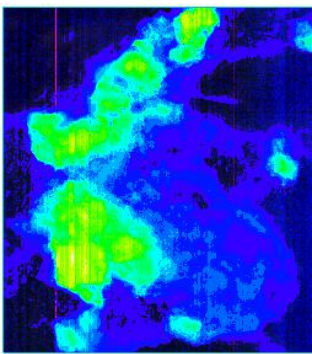
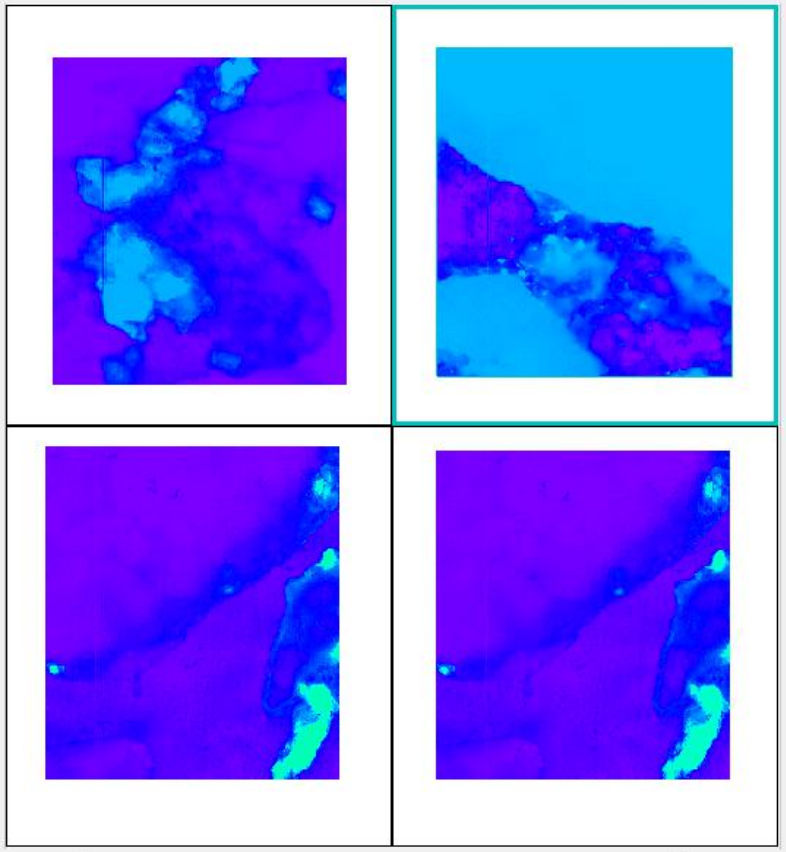


Figure A hyperspectral microscope shows different fluorescence of rock

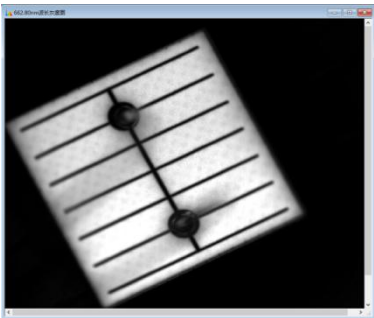
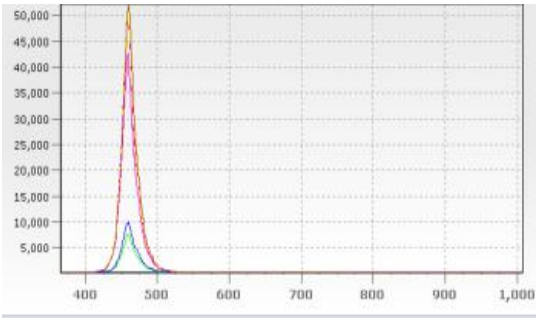


Figure A hyperspectral microscope shows the spectral analysis of an LED structure.

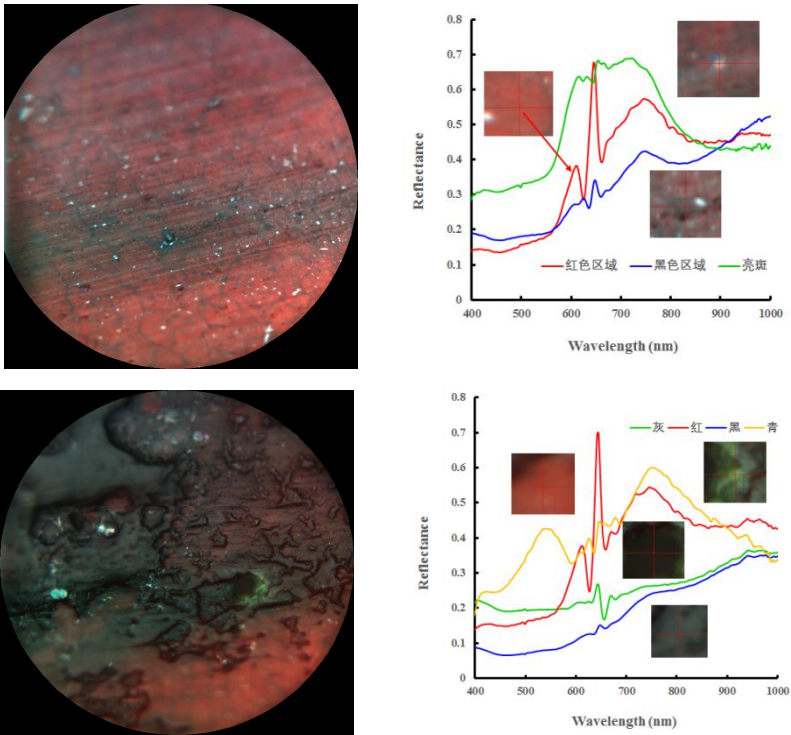


Figure A hyperspectral microscope shows different composition of rock

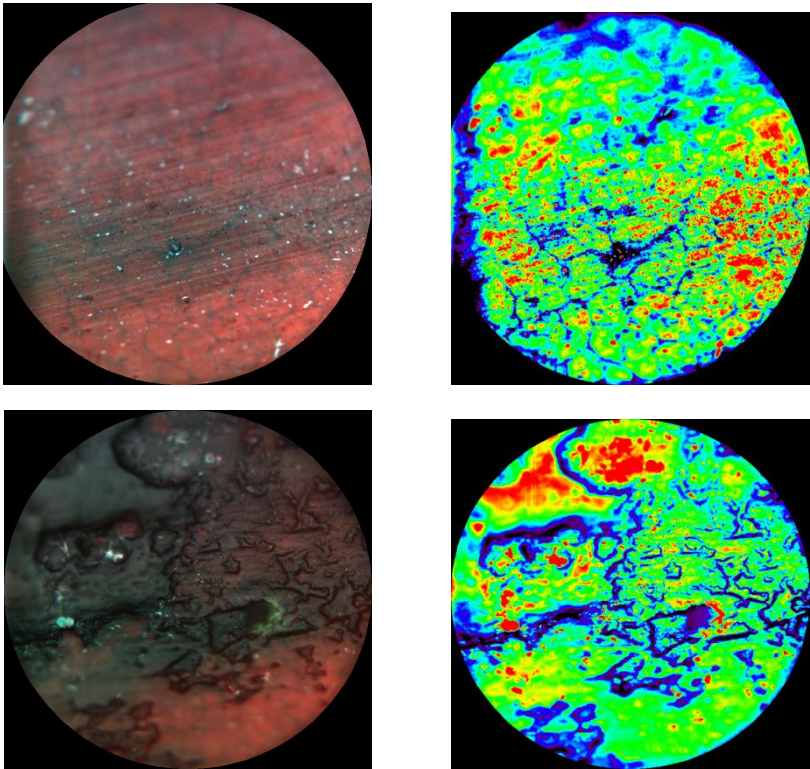


Figure the classification result of rock



Darkfield illumination is commonly used for the analysis of biological samples containing nanomaterials that significantly scatter light. When combined with hyperspectral imaging, it becomes an exceptional tool to also detect the composition and the location of nanomaterials embedded in cells. IMA™, Photon Etc.'s hyperspectral imager, can be equipped with a highly efficient darkfield condenser and generate high contrast images of biological samples.

The high throughput of Photon Etc.'s hyperspectral filter allows the rapid acquisition of spectrally resolved high-resolution images. Since the camera captures the whole area in the field of view, it is possible to collect spectral and spatial information in real time, with the possibility of recording spectrally resolved videos to follow the dynamics of cells and luminescent nanoscale components. PHySpec™, Photon Etc.'s software, enables principal component analysis (PCA) in order to identify the smallest variations of single and aggregated nanoparticles.

With the purpose of showing the capabilities of IMA™ to analyze nanomaterials in biological systems, a sample of MDA-MB-23 human breast cancer cells has been tagged with 60 nm gold nanoparticles (GNPs) and exposed to a dark field illumination on the entire field of view (Figure 1). With a 60X objective, an area of 150x112  $\mu\text{m}^2$  was imaged, with a step of 2 nm and an exposition time of 2 s per wavelength. The complete analysis took only a few minutes, for more than one million spectra, each of them covering the whole visible spectrum.

Cells typically have a flat scattering spectrum, whereas GNPs show a sharp peak around 550 nm. Figure 2 illustrates the 550 nm image extracted from the dark field hyperspectral cube of breast cancer. The GNPs are marked with a green colouring after PCA software processing. The magnification of a breast cancer cell (Figure 3a) and the spectra of the regions containing GNPs (some examples in Figure 3b) confirmed the presence of single 60 nm NPs (peak at 550 nm) and their aggregates (peaks red-shifted). The hyperspectral camera did not detect any GNPs in the areas between the cells.

Results kindly provided by David Rioux, Éric Bergeron and Michel Meunier, at École Polytechnique of Montreal, Quebec, Canada.

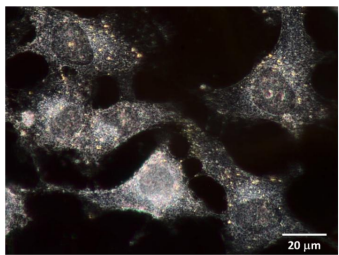


FIG.1 Dark-field image of human breast cancer cells tagged with gold nanoparticles (50 nm size).

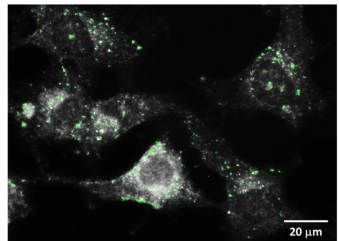


FIG.2 Monochromatic image at 550 nm. GNPs marked in green after PCA.

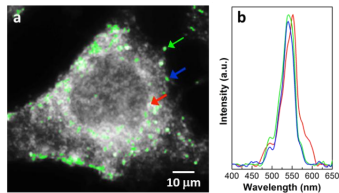


FIG.3 Magnification of a breast cancer cell (a) and spectra of GNPs in different areas (b).

