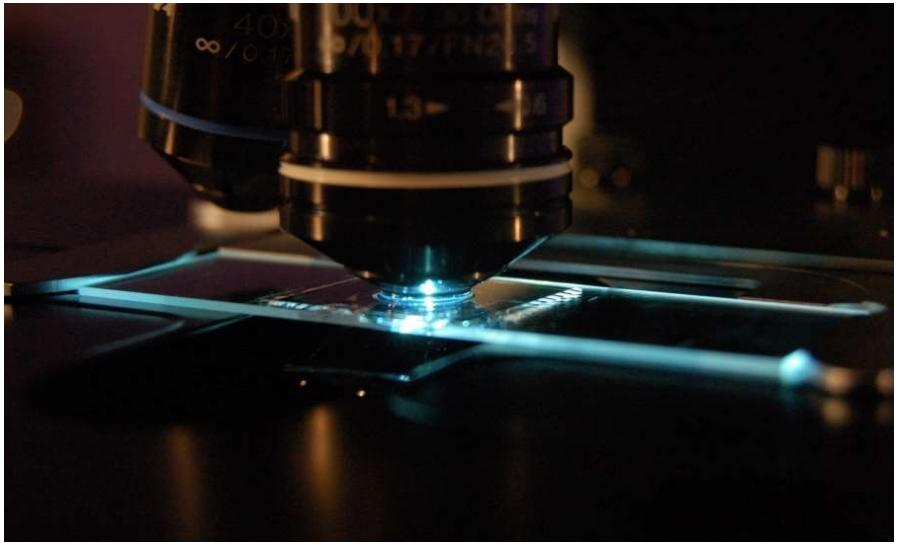
Label free imaging of nanoparticles in cells by Enhanced Darkfield Hyperspectral Microscopy



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Schaefer SEE srl

Nano Innovation 2020
Rome, 15-18 September
Conference & Exhibition

Technology Overview

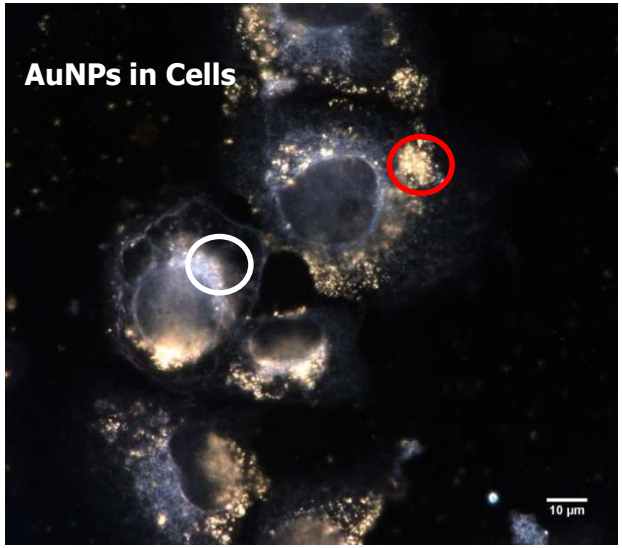
- ✓ Patented enhanced darkfield optics
- ✓ Hyperspectral Imaging

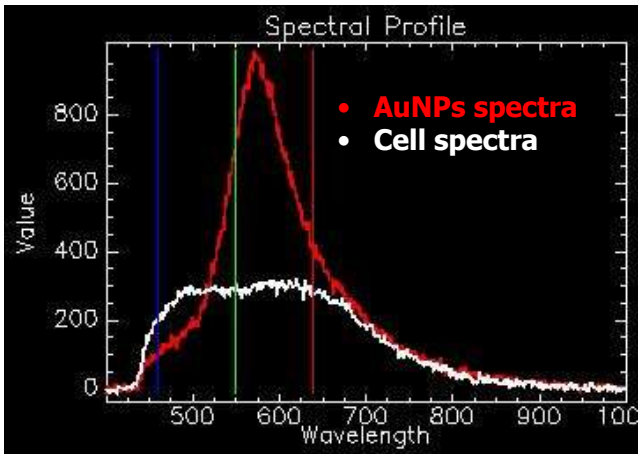
Application Examples

- ✓ Silica AuNPs
- ✓ AuNPs in cells
- ✓ AgNP bacteria interaction
- ✓ Polystyrene plastic in ex-vivo tissue
- ✓ Polymer Nanoparticle – small molecule drug loading
- ✓ Quantum Dots in Neuronal Cell

Key Features of
Enhanced Darkfield Hyperspectral Microscopy

- Patented enhanced darkfield optics provide high signal-to-noise images to optically detect nanoscale features within a sample.
- Hyperspectral imaging captures the optical spectrum within each nanoscale pixel of the sample image.
- Quantification data: Spectral analysis and spectral mapping can be quickly conducted within each pixel of a large sample area.

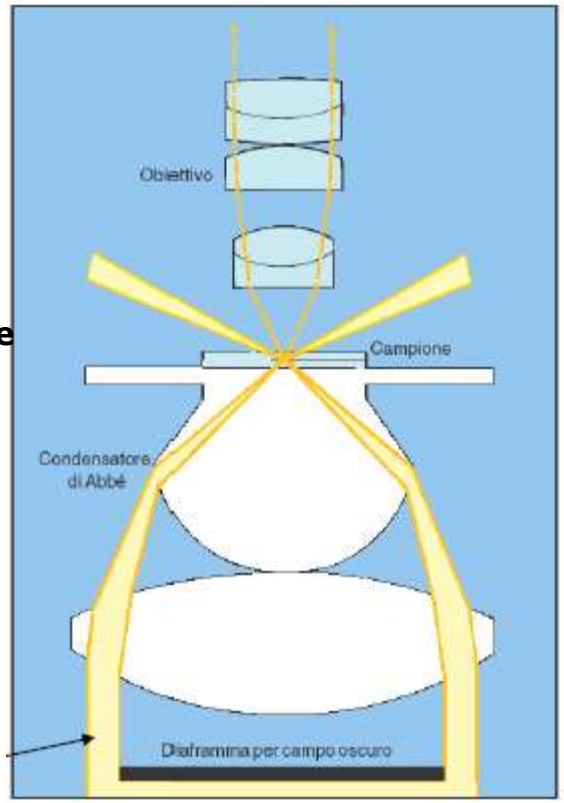




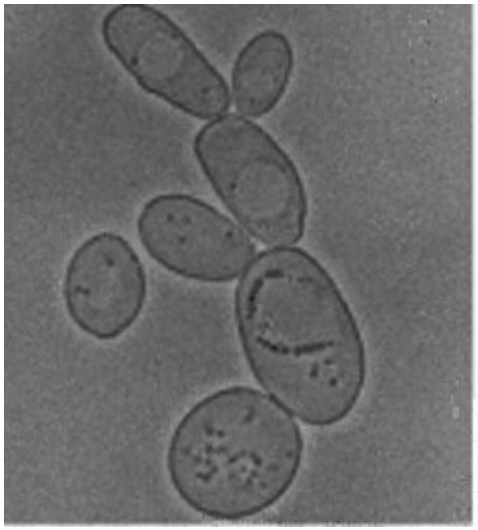
Standard Dark Field Microscopy

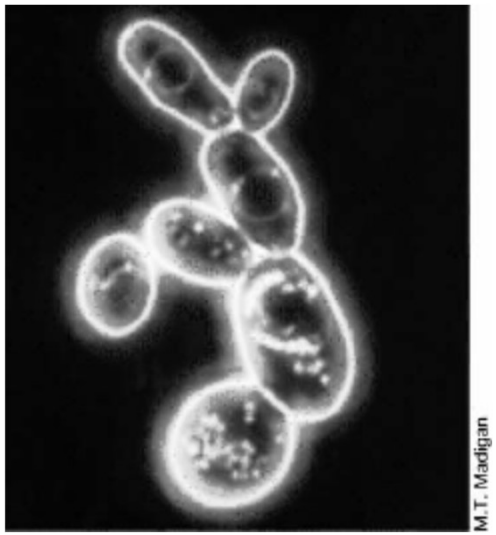
Illumination at an oblique angle

Light scattered from the sample goes to the objective background dark



Hollow light cone





Darkfield image

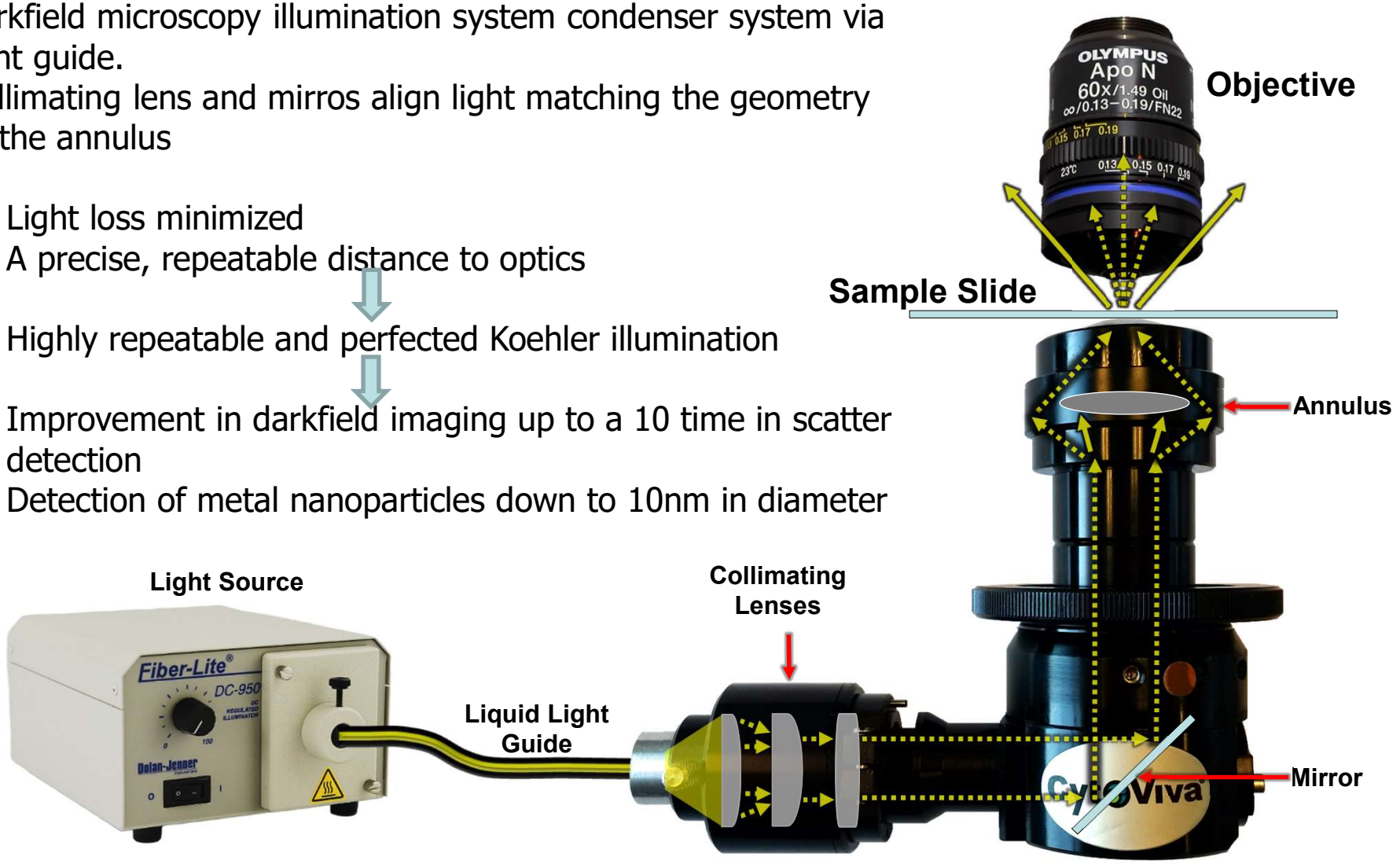
Inefficient
Light lost in optical path
Koehler illumination not guaranteed

as results weak light from the sample

Enhanced Darkfield Illumination Optics

The source illumination is directly coupled to the enhanced darkfield microscopy illumination system condenser system via light guide.
Collimating lens and mirros align light matching the geometry of the annulus

- ✓ Light loss minimized
- ✓ A precise, repeatable distance to optics
- ✓ Highly repeatable and perfected Koehler illumination
- ✓ Improvement in darkfield imaging up to a 10 time in scatter detection
- ✓ Detection of metal nanoparticles down to 10nm in diameter



Article
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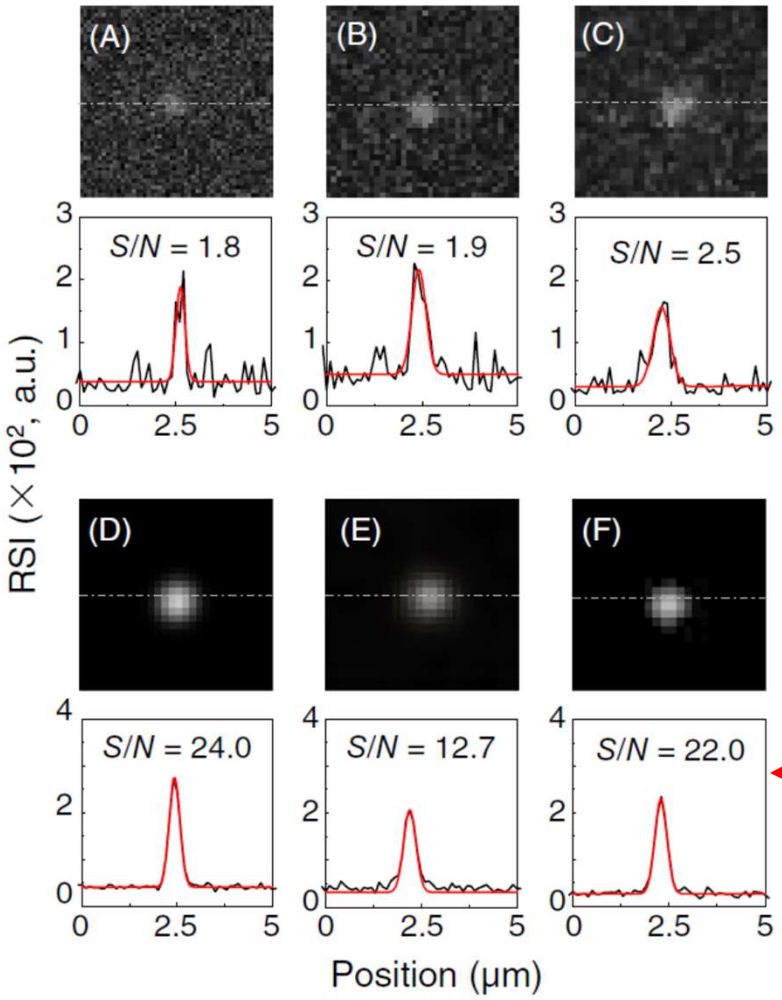
Microchip Electrophoresis with Enhanced Dark-Field Illumination Detection for Fast Separation of Native Single Super-Paramagnetic Nanoparticles

Peng Zhang,[†] Sangyoon Park,[‡] and Seong Ho Kang[†,§,*]



Conventional DF images (a–c) of 160nm native single SPMNPs

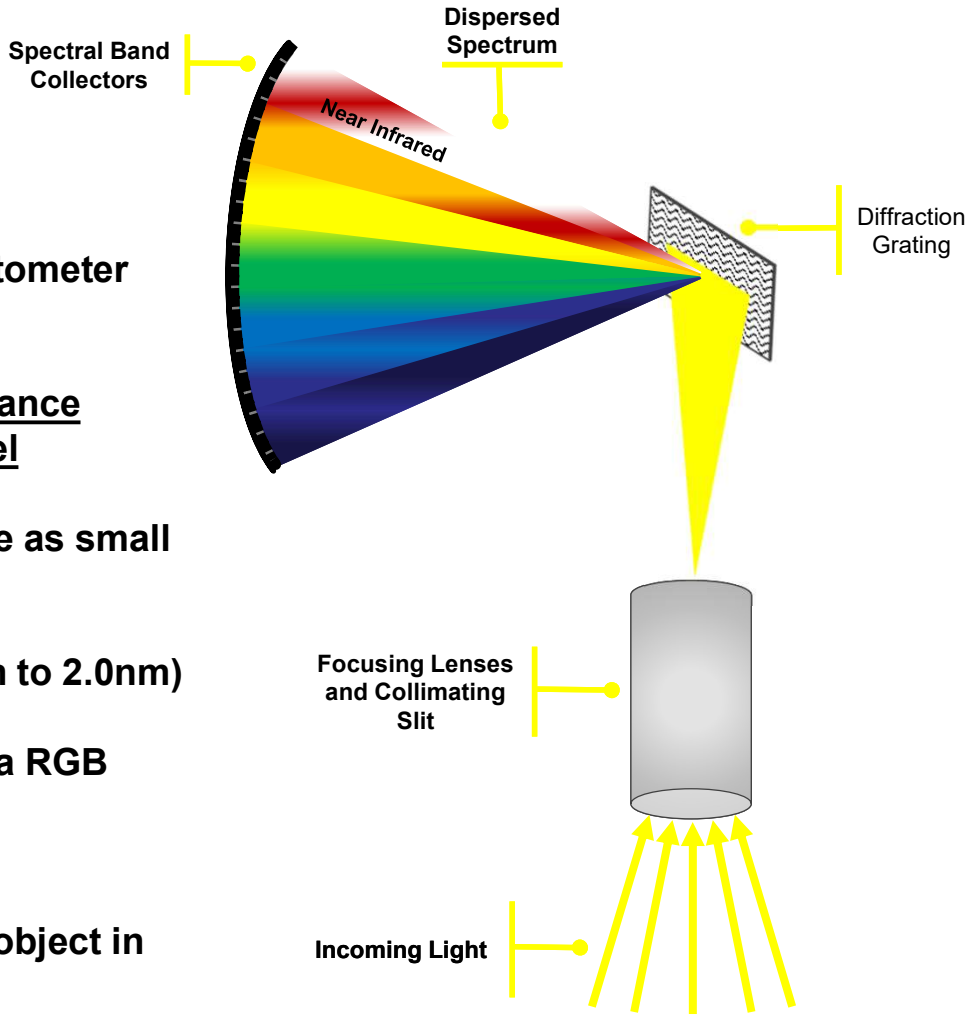
CytoViva EDF images (d–f) of 160-nm native single SPMNPs.

Note: up to 10x measured improvement in S/N ratio with CytoViva's EDF optics

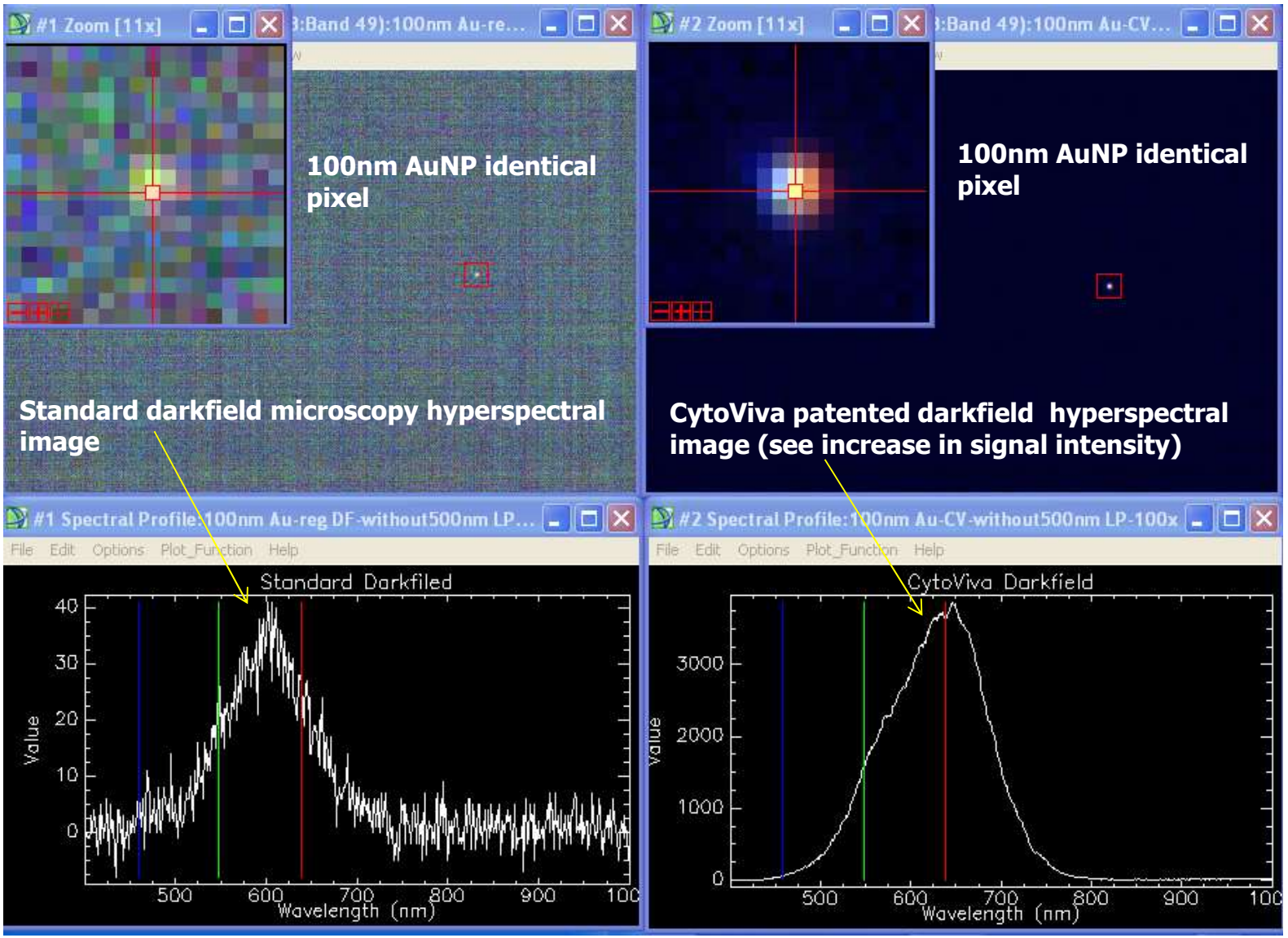
Hyperspectral Imaging

How It Works

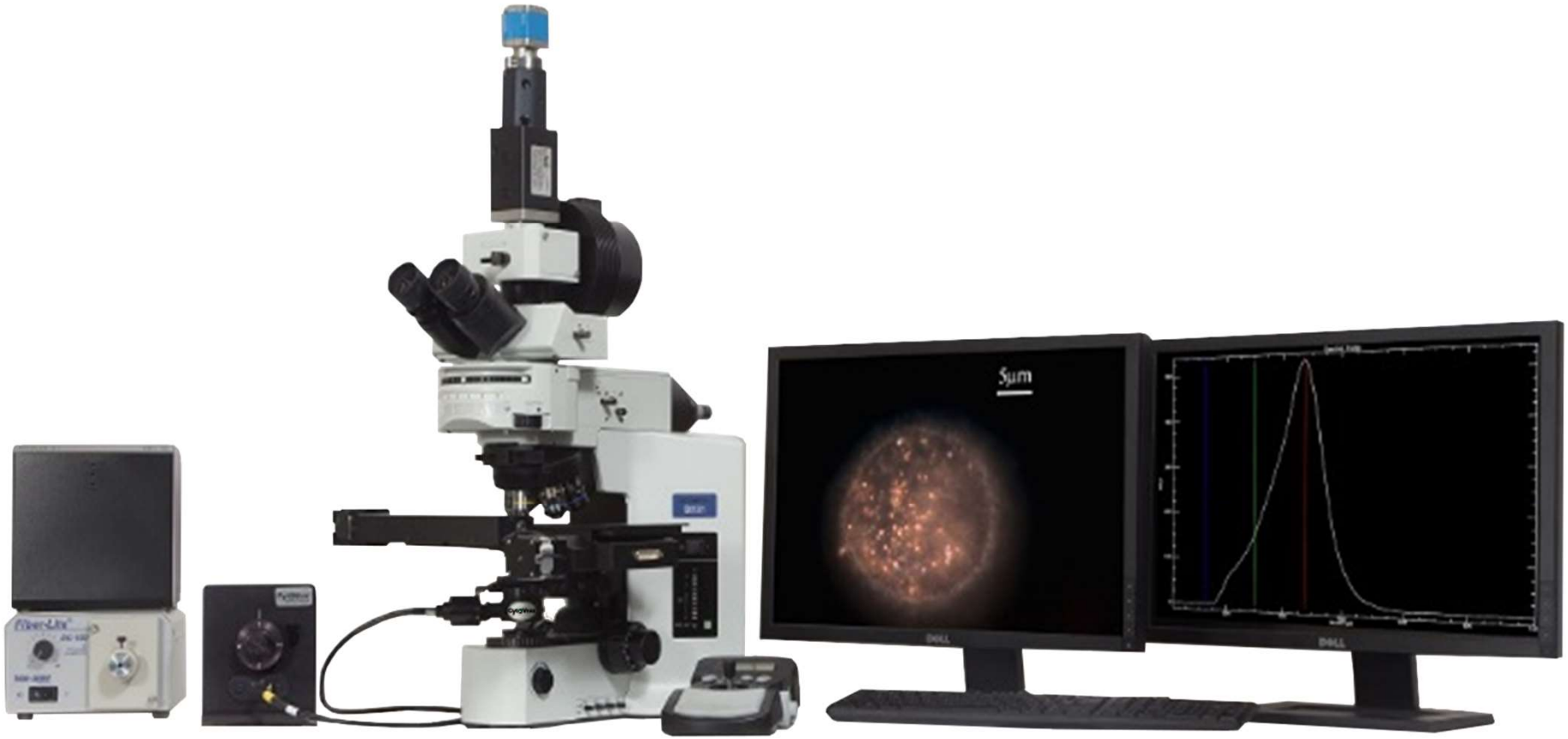
- The VNIR or SWIR diffraction grating spectrophotometer mounts onto a microscope camera mount
- By a scan moving across the filed of view, reflectance spectra, in VNIR range, are acquired for each pixel
- a map of reflectance spectra is created (pixel size as small as 128nm)
- Spectral data is reported in high resolution (down to 2.0nm)
- The data is presented as a spectral curve and as a RGB image.
- Hyperspectral image is acquired in few seconds
- Detailed quantitative analysis of each nanoscale object in the field of view can be performed
- A spectra library is created, and it is used to identify and quantify the nanoparticles in the image



Hyperspectral Image S/N Improvement



Enhanced Darkfield Hyperspectral Microscope
System Configuration



Identification of functionalized nanoparticles used as drug delivery

Figure 1. 200nm Uncoated Au Silica NPs (Au1)

Figure 2. 200nm Uncoated Au Silica NPs (Au2)

Figure 3. 200nm PEG Coated Au Silica NPs (Au3)

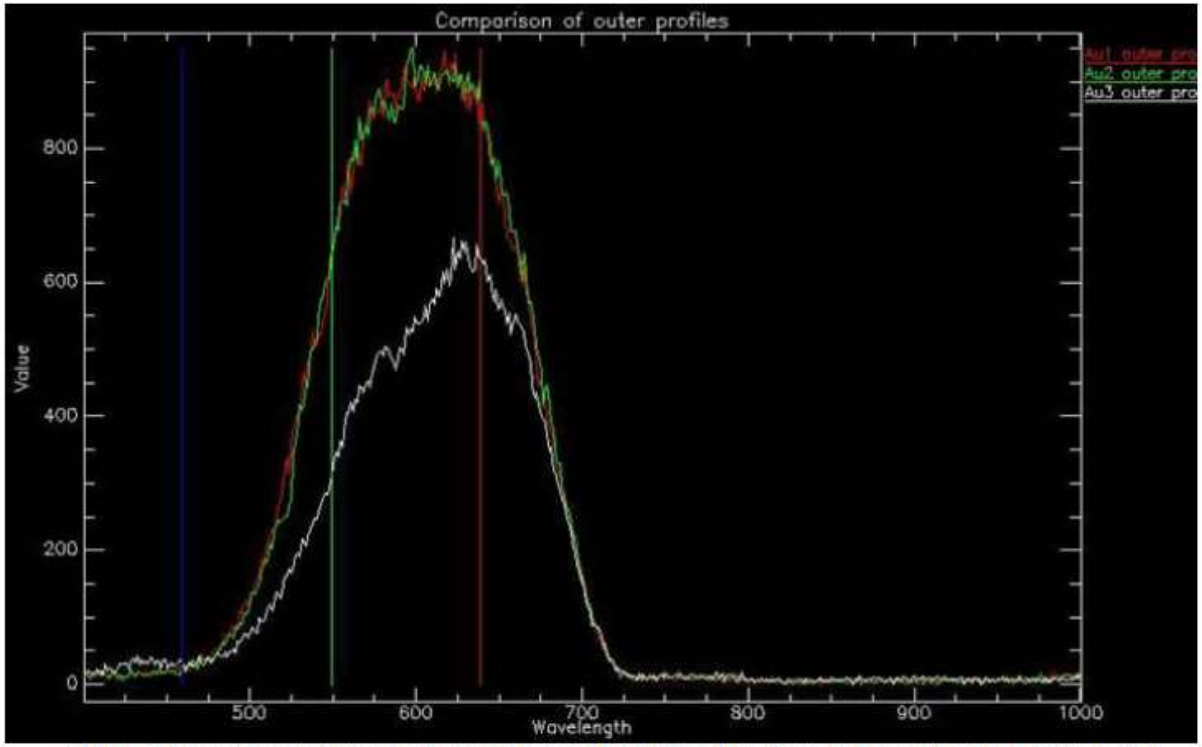
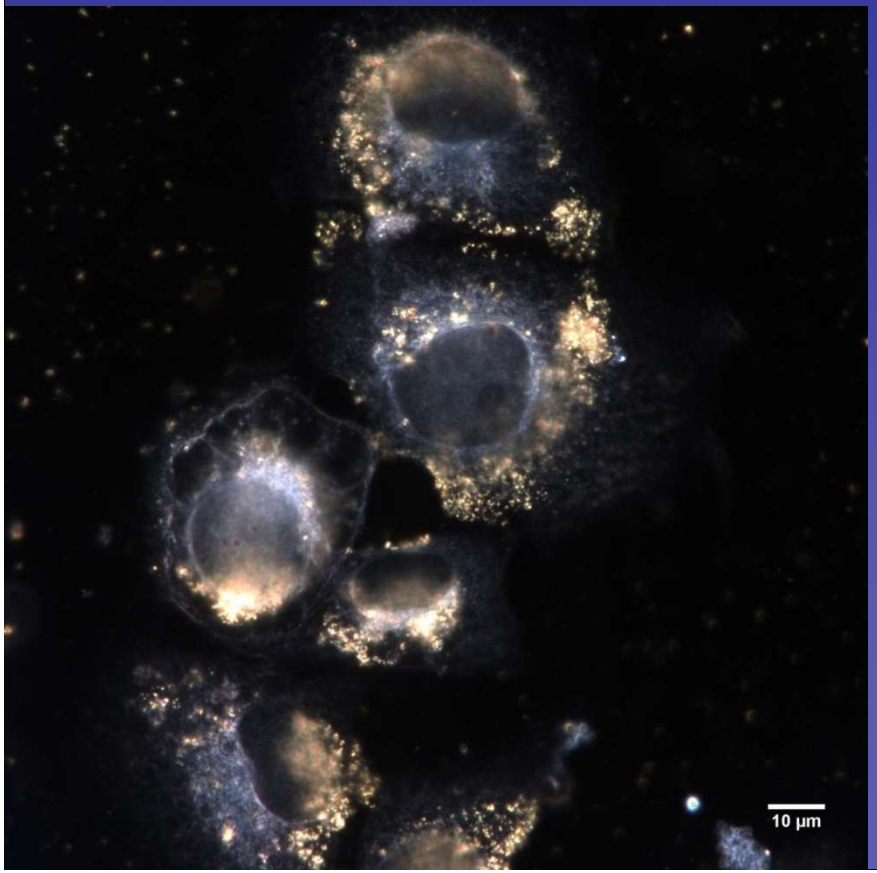


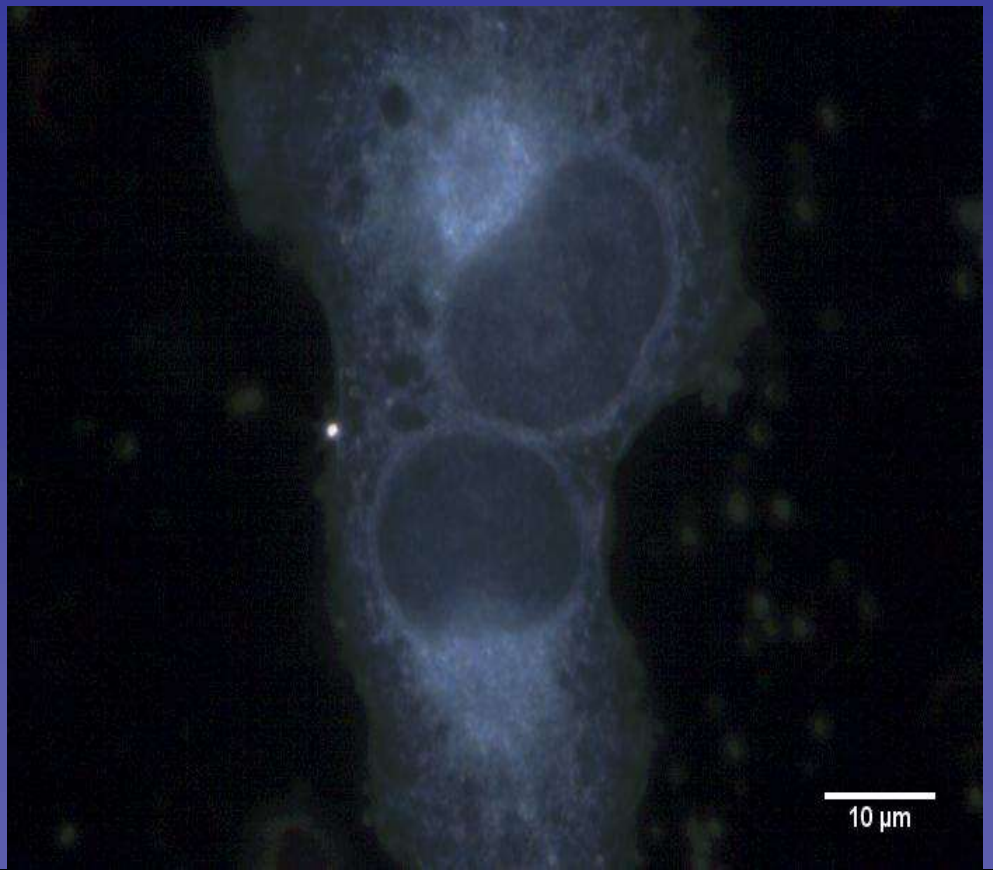
Figure 4. Mean Spectral Comparison of Uncoated Au Silica NPs (red and green) versus PEG coated Au Silica NPs(white)

AuNPs in cells

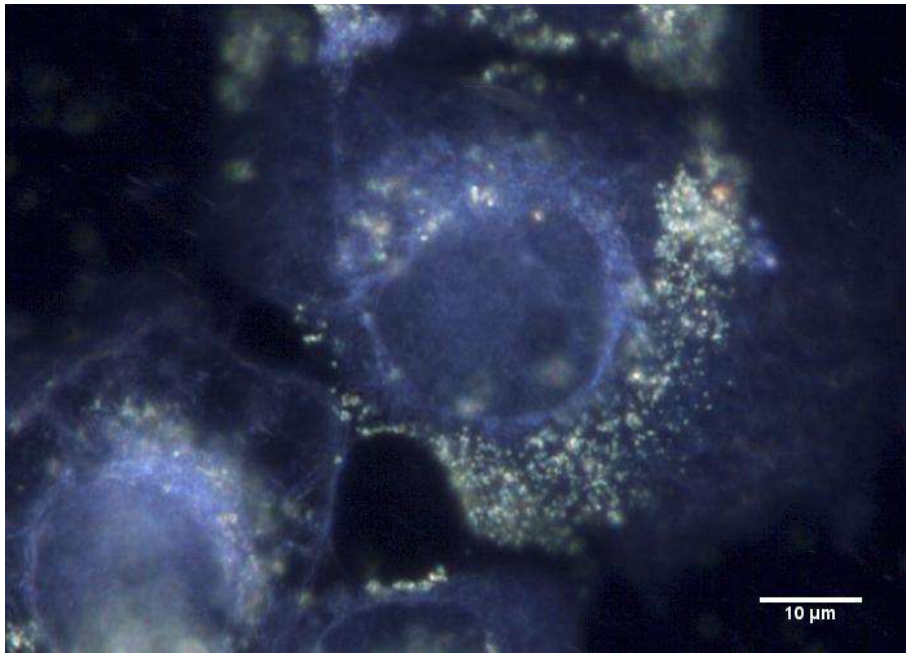
Hyperspectral Image
Cells exposed to AuNPs



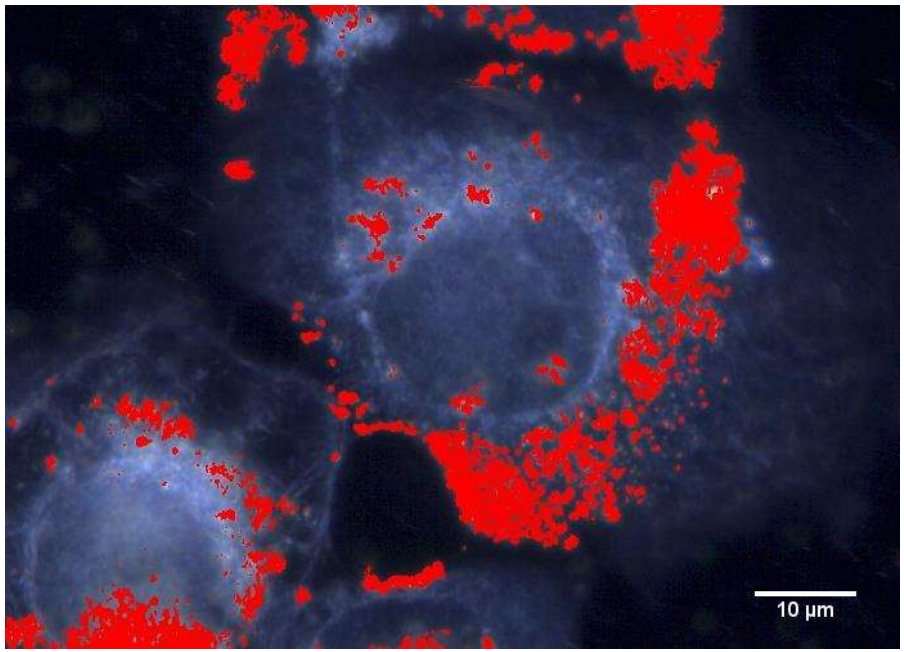
Hyperspectral Image:
Identical cells not exposed to AuNPs



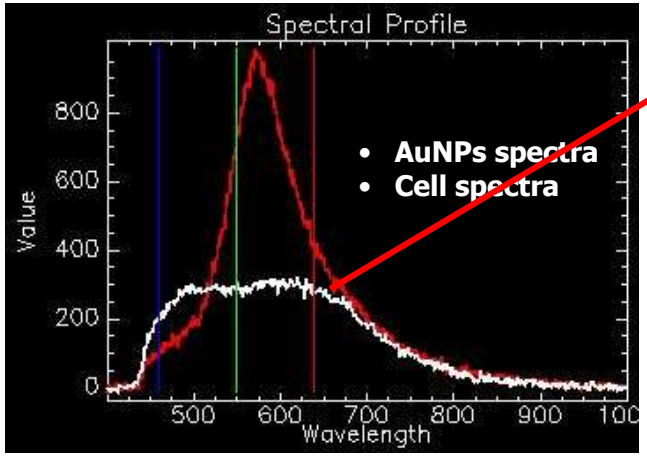
Spectral Mapping of AuNPs in Cells



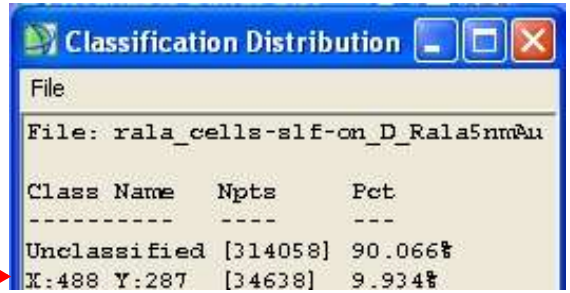
Hyperspectral image: Rala coated AuNP in cells



Pixels in red map each pixel matching the spectral response of aggregating Rala AuNP spectra in cells.



AuNP pixel match classification



AgNPs - Pathogen Interaction: antimicrobial focus

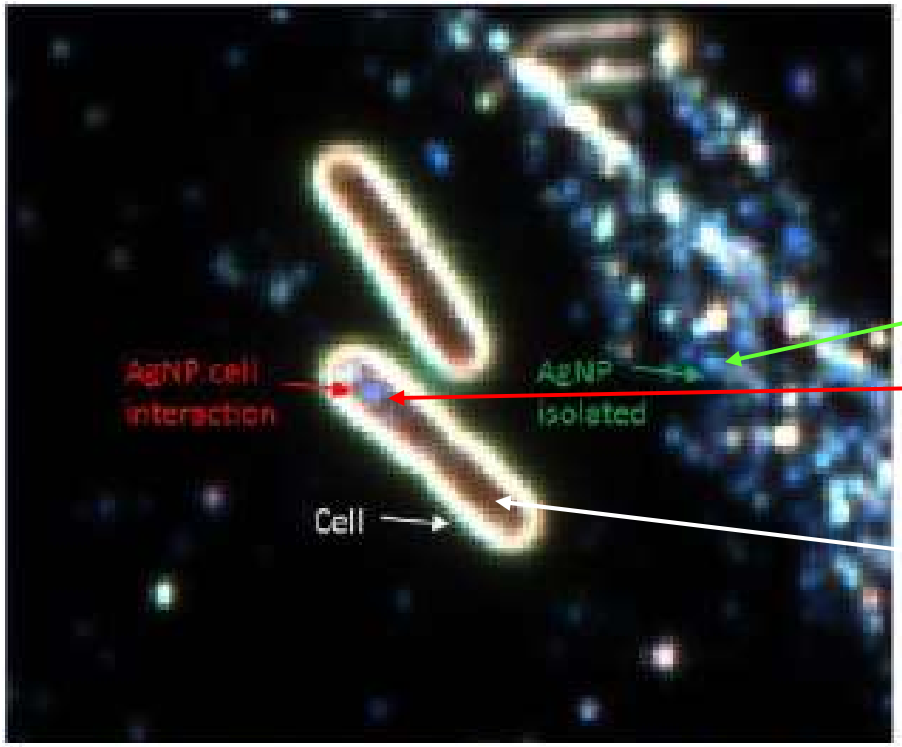


Figure 1: Enhanced Darkfield Hyperspectral Image of AgNP Bacterial Interaction

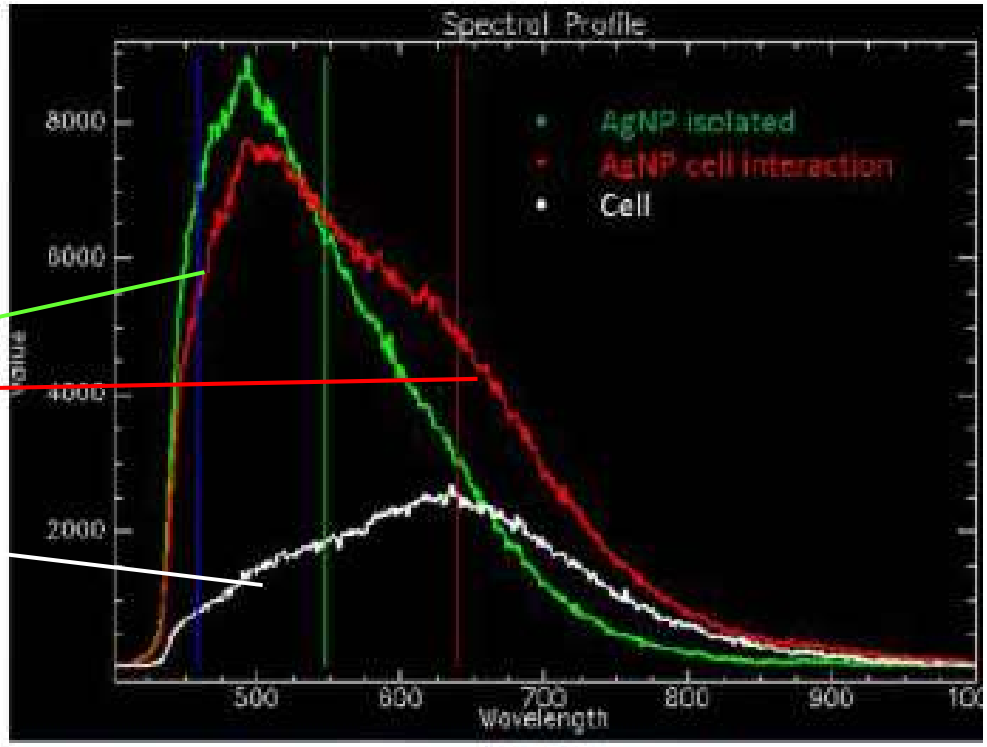
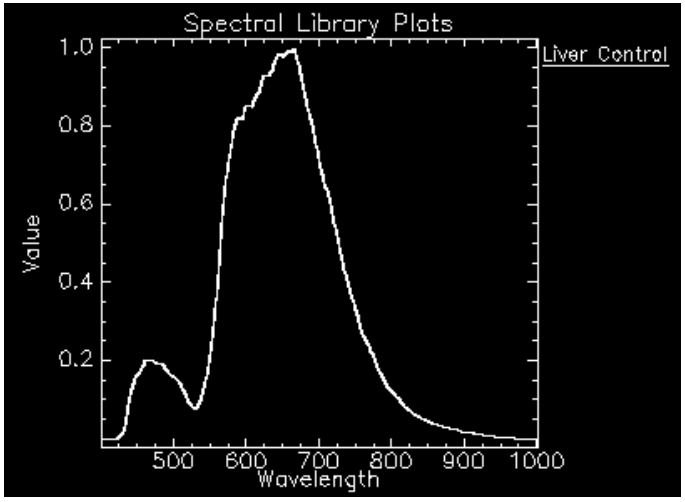


Figure 2: Spectral Response of AgNP, Bacteria and AgNP Bacterial Interaction

Polystyrene Plastic Ex-vivo Mouse Tissue Detection

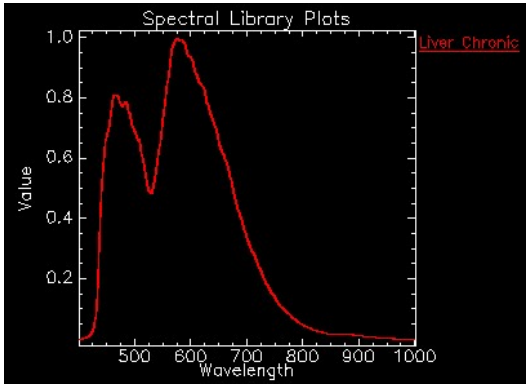
In the control cells it can be observed that there are areas of nanoscale particulate. This is normal due to either the tissue prep process or naturally occurring elements in the tissue. However this is not polystyrene. To spectrally characterize these nanoparticulates, a region of interest (ROI) was taken by selecting a diverse number of pixels from the nanoparticles and measuring the mean, or average, spectral response. This ROI is reflected in the spectral plot below.



Polystyrene Plastic Ex-vivo Mouse Tissue Detection

In the polystyrene exposed tissue can be observed that there is an aggregation of nanoparticulates. To spectrally characterize these nanoparticles, a region of interest (ROI) was taken by selecting a diverse number of pixels from the nanoparticles and measuring the mean, or average, spectral response.

This ROI is reflected in the spectral plot below. This spectrum is unique to the polystyrene exposed mouse and is not found in the control mouse tissue. This is determined by using the Filter spectral library feature to compare all pixels in the PS exposed sample vs the negative control.



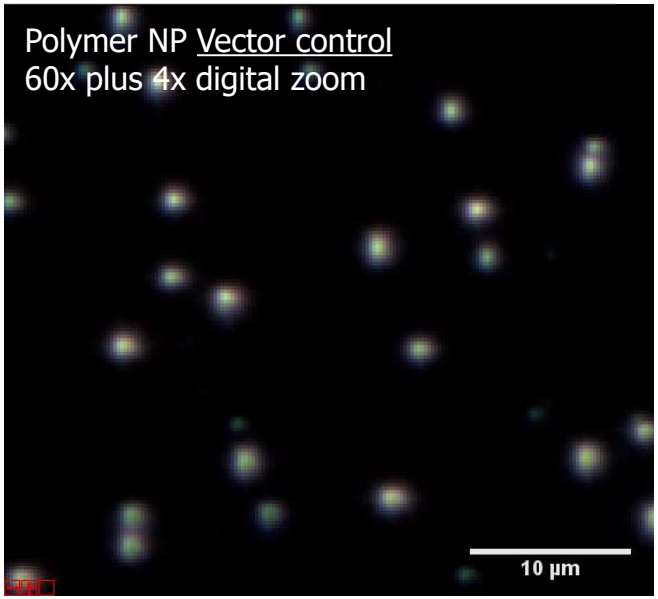
Spectral Mapping Polystyrene in Exposed Mouse Liver

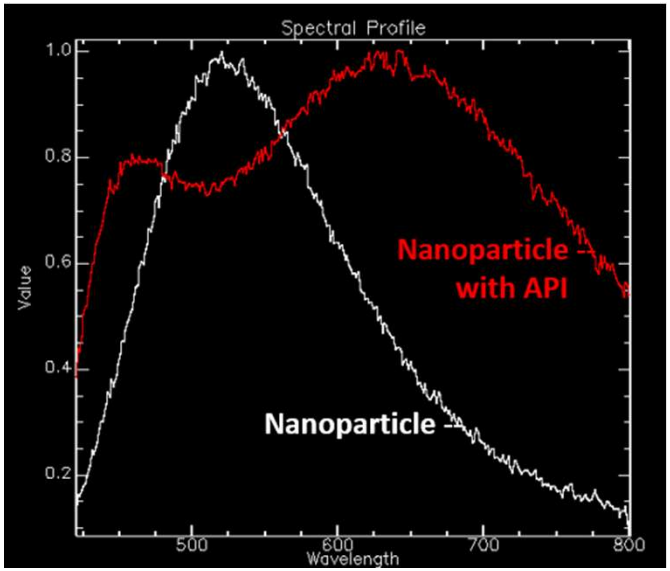
To conduct the spectral mapping in this image, we created a spectral library from the nanoparticles in the polystyrene exposed liver sample. Next, to eliminate false positives we filtered this Polystyrene exposed Liver spectral library against the Control Liver sample. Any spectrum matching in any single pixel of the Control Liver was removed.

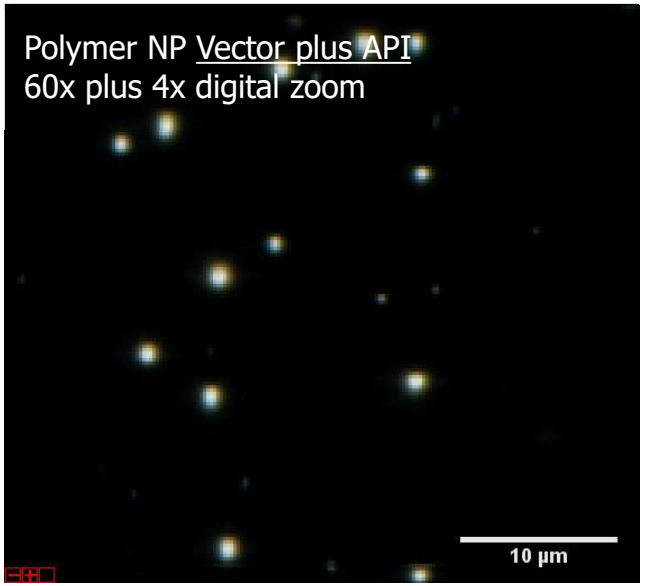
The remaining 'filtered' spectral library contains spectrum that is unique to the Polystyrene exposure of the liver sample.

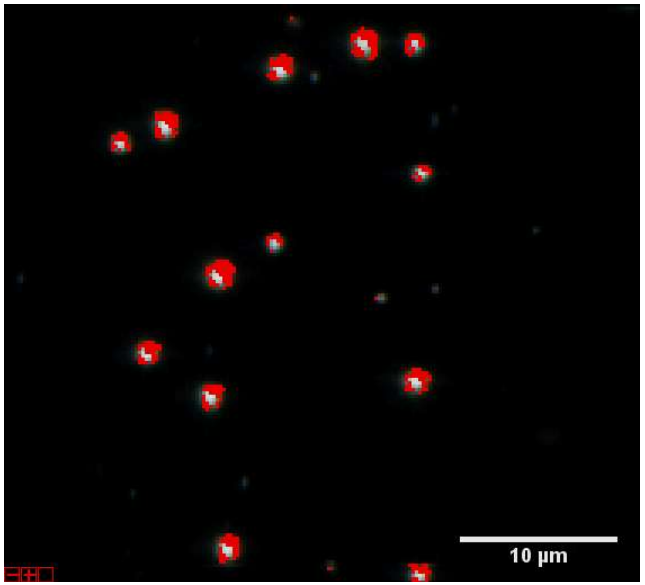
Using the Spectral Angle Mapper (SAM) utility, we match and pseudo color **red** any pixel that matches the filtered spectral library.

Polymer Nanoparticle Drug Delivery Vectors





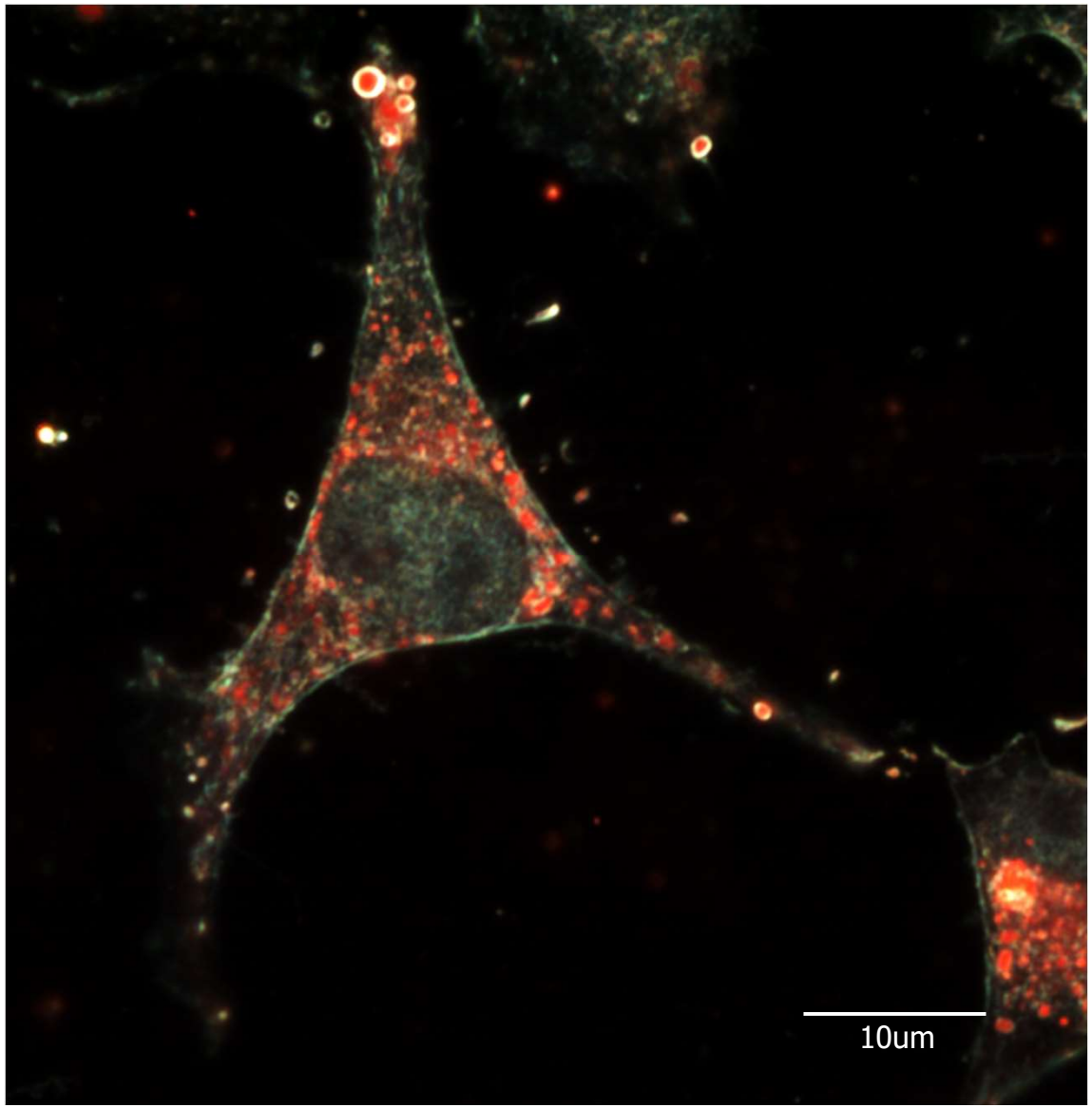


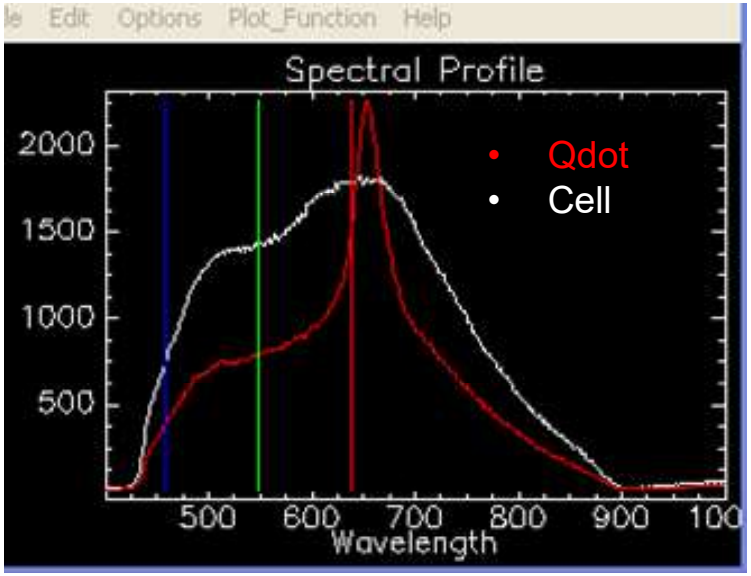


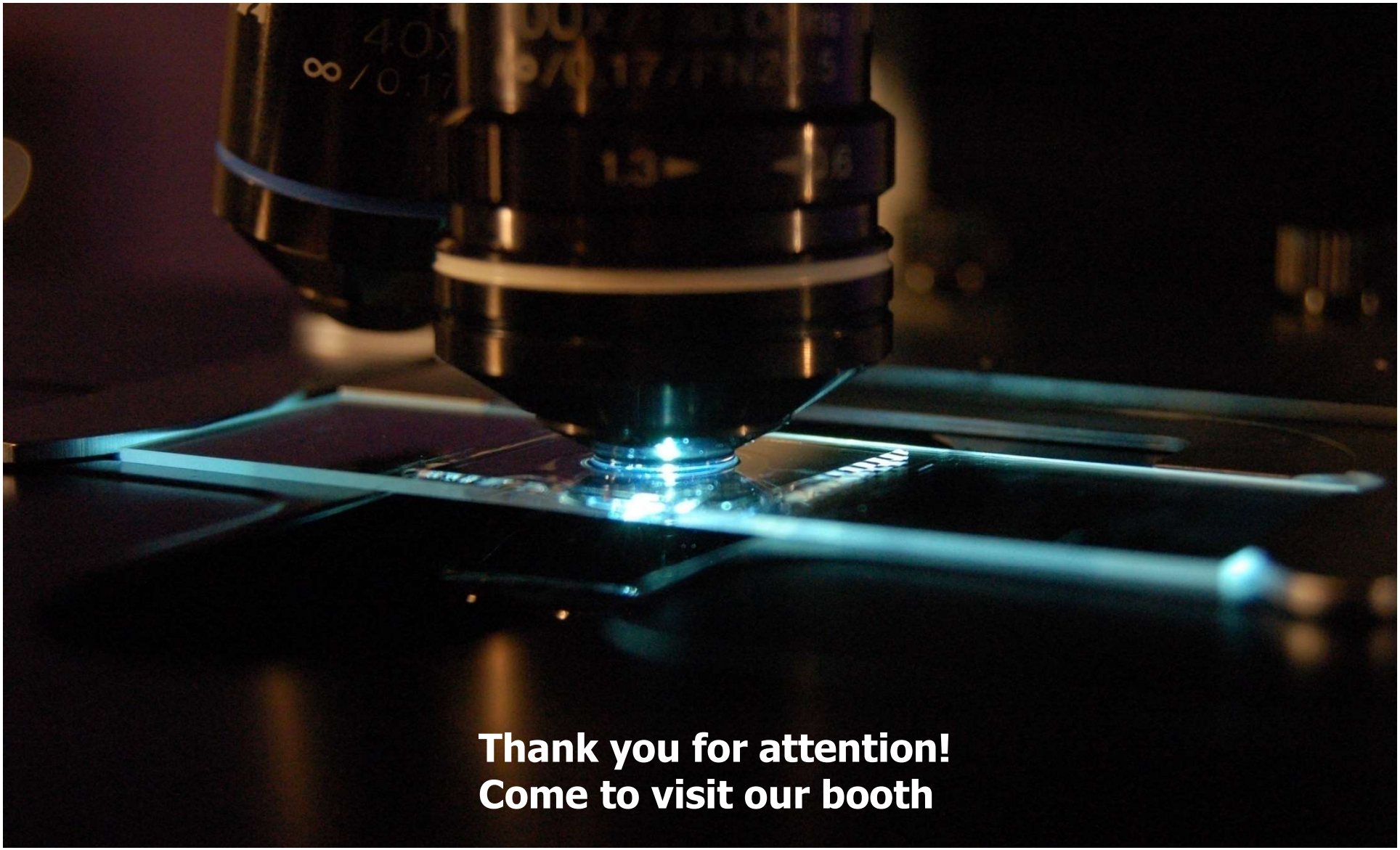
The API molecule does not change the appearance of the polymer NP vector, but changes its spectral response.

Spectral mapping of the drug load illustrates that the API is present on each nanoparticle in this field of view.

Quantum Dots in Neuronal Cell



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