Optical Observation - Hyperspectral Characterization of Nano-scale Materials In-situ
Research at the nanoscale is more effective, when research teams can quickly and easily observe and characterize a wide range of nanomaterials in a wide range of environments without labeling or other alteration of these materials...
Nanoscale Hyperspectral Microscopy

Is currently utilized by over 300 nano research labs worldwide engaged in...

Nano-materials development
Nano-toxicology
Nano-drug delivery

...it provides in-situ optical observation and spectral characterization of non-labeled nanomaterials in the environments they need to be studied.
Integrated optical microscopy & hyperspectral imaging...

- Patented, advanced darkfield microscopy (7x improvement in signal-to-noise over standard darkfield)

- Hyperspectral imaging enables pixel level spectral characterization of the sample imaged

...specifically designed to advance nano-scale research.
Current System Footprint

- VNIR Spectrophotometer
- Optical Camera
- Optical or Hyperspectral Image
- Hyperspectral Data
- 150w Halogen Light Source
- Motorized Stage
- CytoViva Dual Mode Fluorescence Module
- CytoViva Equipped Optical Microscope
Patented Darkfield-based Microscope Illumination Optics
Patented Microscope Optical Illumination System enables nano-scale imaging: US patents No. 7,542,203, 7,564,623

Functionality Enabling Improved Optical Performance:

1. **Pre-aligned Koehler illumination**: Precisely focuses the source light onto the entrance slit of the annular condenser

2. **Main feature of critical illumination**: Focuses the light precisely on the same plane of the sample as the focal point of the objective; result of pre-aligned Koehler illumination

* Annular illumination produces an improved point spread function. Through design enhancements in the alignment and focus of annular Illumination, CytoViva produces significantly improved optical performance over other comparable techniques including standard darkfield (annular) illumination.

Both images represent the identical field of view of 50nm colloidal gold captured under identical conditions: Olympus BX-41, 40X objective, Dage MTX color digital camera at a constant exposure and an X-Cite 120 light source.
Hyperspectral Imaging (HSI)
Hyperspectral Imaging (HSI)

How It Works

• The VNIR spectrophotometer mounts onto a microscope camera mount

• It captures the unique reflectance spectra of objects from the microscope field of view (VNIR spectrum from 400nm-1,000nm)

• The complete spectra for each pixel of the CCD detector is captured (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

• Detailed quantitative analysis of each nanoscale object in the field of view can be performed.
50nm AuNPs in RBC culture
Sample taken directly ex-vivo with no staining or other preparation required
Protein conjugated AuNPs in mamillan cells

Hyperspectral Image
Cells exposed to AuNPs

Hyperspectral Image: Identical cells not exposed to AuNPs
Spectral Mapping of AuNPs in Cancer 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

Library spectra from AuNP

Classification Distribution

File: rala_cells-slf-on_D_Rala6nmAu3
AgNPs in Solution

Aggregating AgNPs in water

Spectra from individual non-dispersed AgNPs

Dispersed AgNPs in ionic solution

Spectra from individual dispersed AgNPs
AgNPs in Algae

Hyperspectral image 100x:
Algae cell + AgNP
AgNPs in Algae

Mapping in red all pixels matching spectral library
Algae cell + AgNP

spectral library: AgNPs inside algae cell: chlorophyll modifies AgNP spectral response
Characterizing drug functional groups on nanoparticle surface

PEG coated NPs are spectrally different from non-PEG coated
TiO₂ Injected in Whole Animal Organism

Whole animal organism control

TiO₂ nanoparticles injected in whole animal organisms

TiO₂ nanoparticles in solution

Note: This spectra response incorporates characteristics of both samples.
MWCNTs inhaled by mouse in lung tissue

Aggregating CNTs on glass slide

Spectral signatures collected from the CNT sample and loaded into the spectral library
MWCNTs inhaled by mouse in lung tissue

Hyperspectral Scanned Image unstained 5um thick lung tissue and CNTs

The red areas are the pixels matching the CNT spectral profiles within the spectral library.
Nanomaterials
Application Examples
Hyperspectral measurements of single pixel areas from the three different samples quantitatively confirms different surface chemistry from each sample.
Hyperspectral Image Scan

Reference Spectra of GOx coated CNTs

CNTs Mapped in Sample

Scanned image and Zoom illustrating GOx coated CNTs adhering to fiber

Derived from previously scanned pure GOx coated CNT sample

Overlay image illustrating all areas in red containing the selected spectral profile and location of the GOx functionalized CNTs

CNT characterization application
Hyperspectral Image: 50x

Ag rods on opaque silicon substrate
(reflectance darkfield microscopy utilized)

Green pixels illustrate collection area for – silicon substrate mean spectra

Red pixels illustrate collection area for – Ag nanorods mean spectra