Confocal Microscopy

Outline

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- Optical Microscopy
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- Laser Scanning Confocal Microscopy
- Examples

Optical Microscopy

- Optical microscopy is the oldest type of microscope and uses visible light and a system of lenses to create an image of the sample
- Although not able to image nanostructures, optical microscopy is important in nanotechnology characterization
- Light microscopes can be used to image biological structures and inspection of lithography processes

Optical Microscopy

- The resolution of a light microscope (R) is a function of the numerical aperture of the lense (NA), and the wavelength of illumination (λ)

$$R = \frac{0.61\lambda}{NA}$$

- The numerical aperture of a lens is at its maximum close to 1. Wavelength of illumination $\lambda > 380$ nm for optical wavelengths. Thus resolution is limited to about 500 nm for an optical microscope

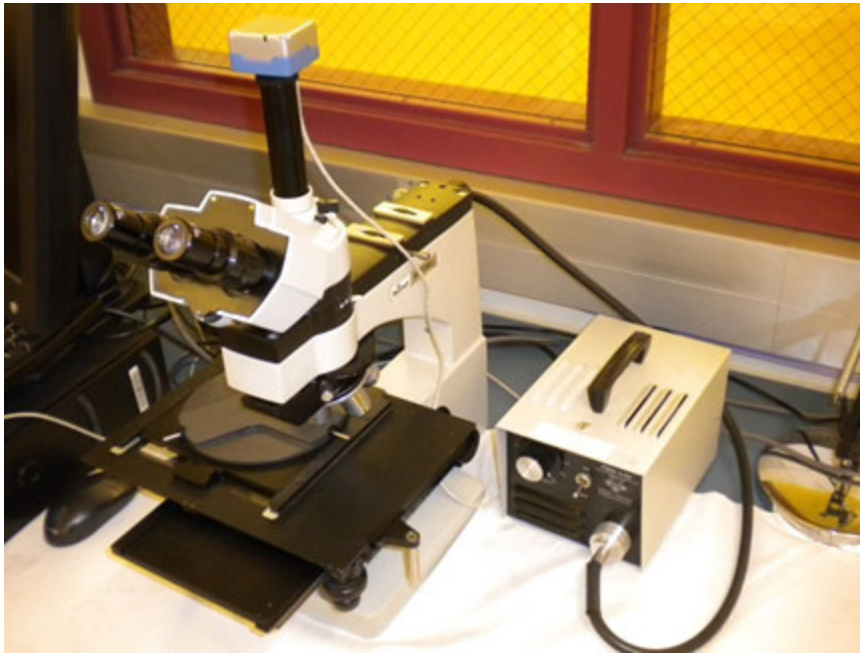
Leng, Yang. *Materials Characterization*. John Wiley and Sons, Singapore. 2008

Types of Optical Microscopes

- Bright-field illumination, light transmission, compound light microscopes
  - Light transmitted through a sample forms an image
- Dark-field illumination, episodic illumination, reflected light microscopes
  - Light reflected by the sample form an image
- Fluorescent light microscopes
  - Light emitted from the sample form an image

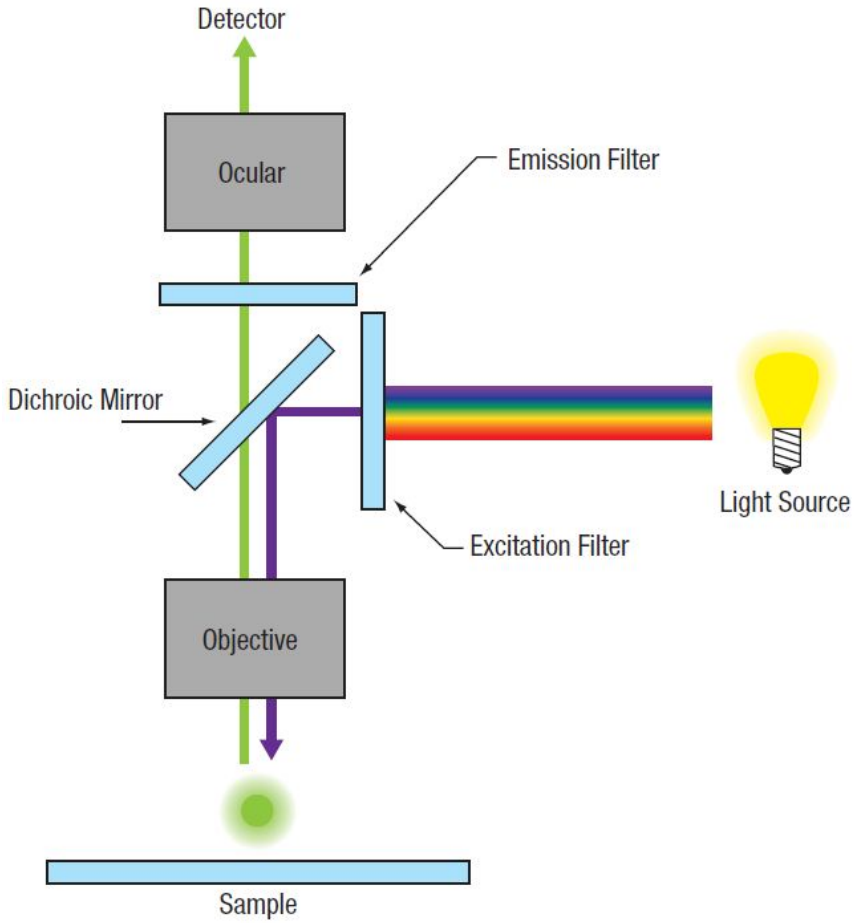
Reflection Microscopy

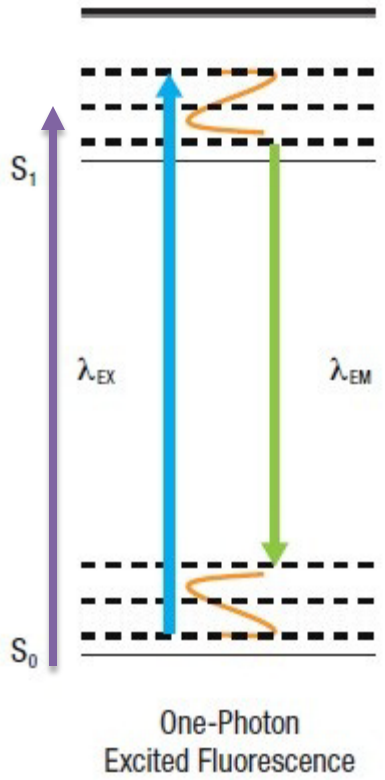
- Episodic illumination or reflective optical light microscope with still camera CCD attachment in the SHINE Nanotechnology Lab



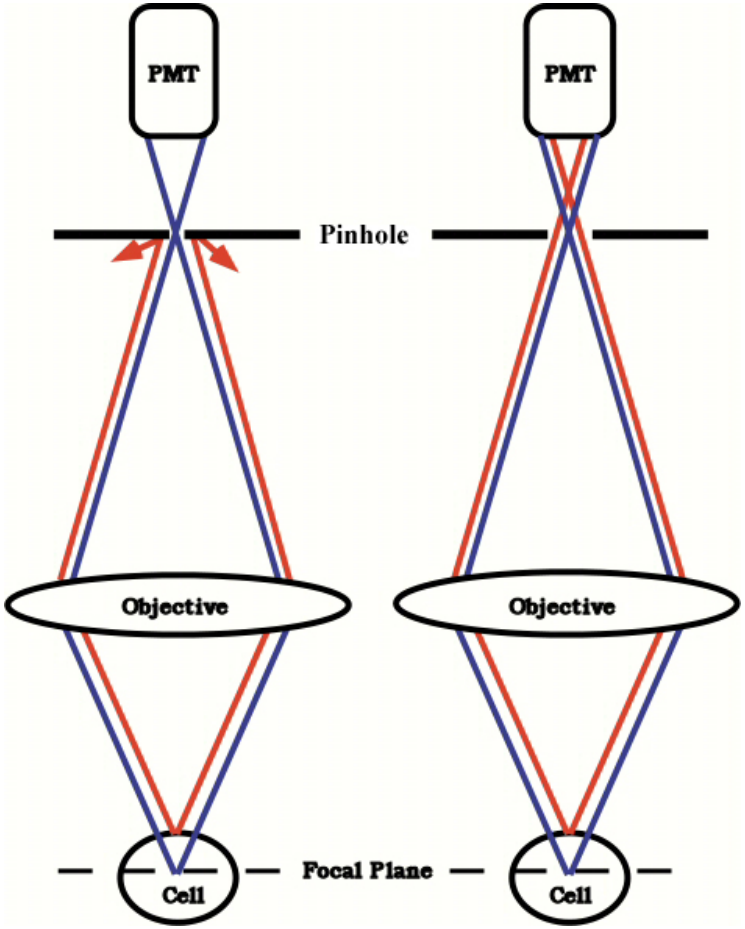
EpiStar 2560 Metallographic Microscope

Fluorescence Microscopy





Confocal Microscopy

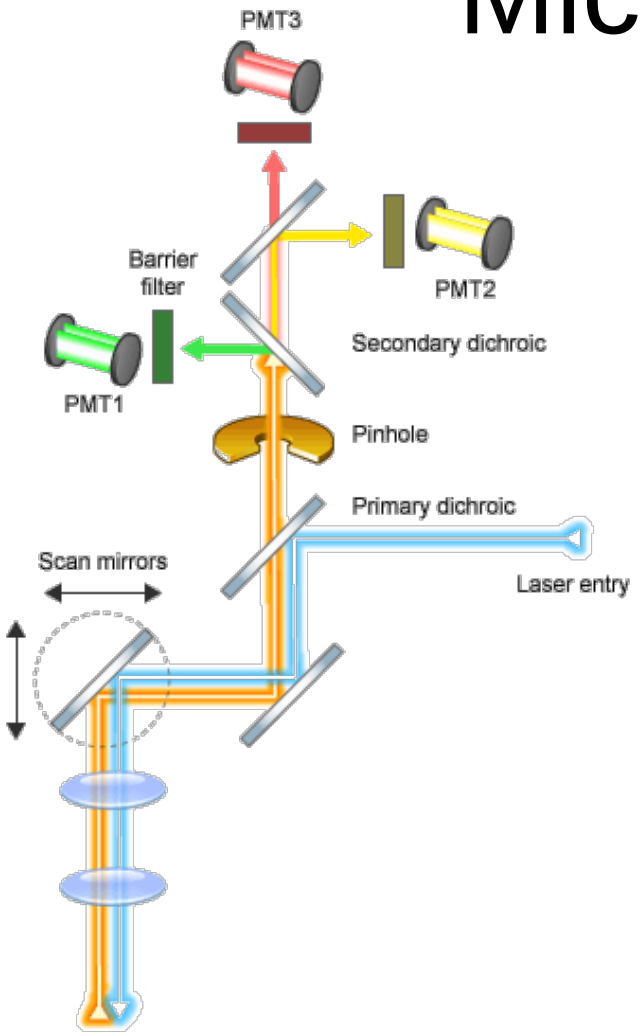


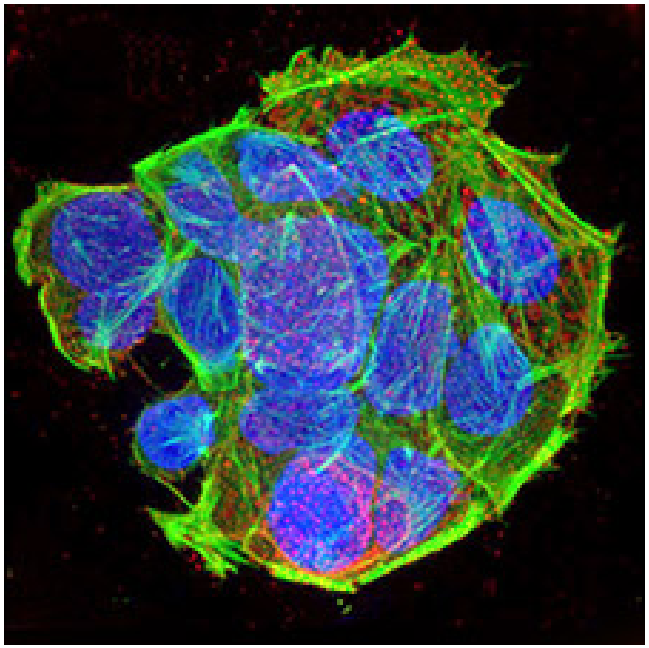
- The only light which reaches the detector is in-focus light from a single focal plane through the sample
- Out-of-focus light, from focal planes above and below the in-focus plane is blocked by the pinhole

How does confocal microscopy use nanotechnology?

- Nano particles and quantum dots are used as fluorescent markers
- Nanomaterials are used in drug delivery to cells which can be analyzed using confocal microcopy
- Nanomaterials are used as anti-bacterial agents. Bacteria can be imaged using confocal microscopy
- Nanomaterials are used in many other biological processes.

Laser Scanning Confocal Microscopy





www.seattlenano.org

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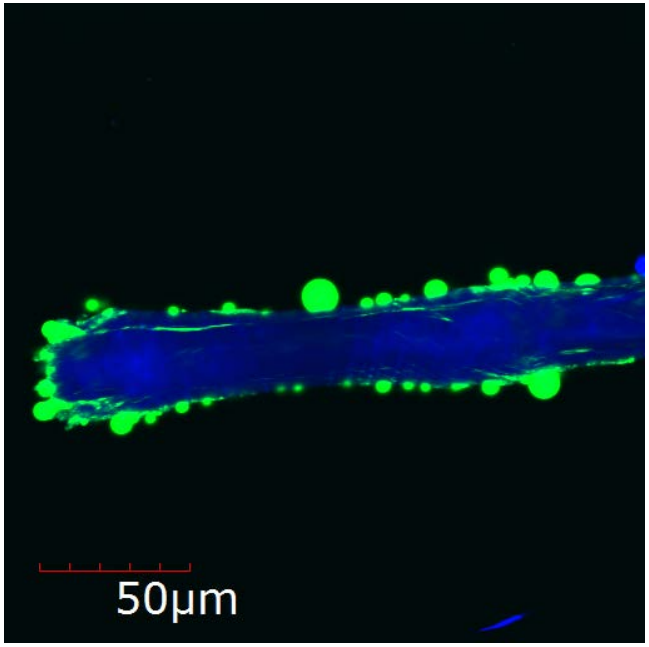
Laser Scanning Confocal Microscopy



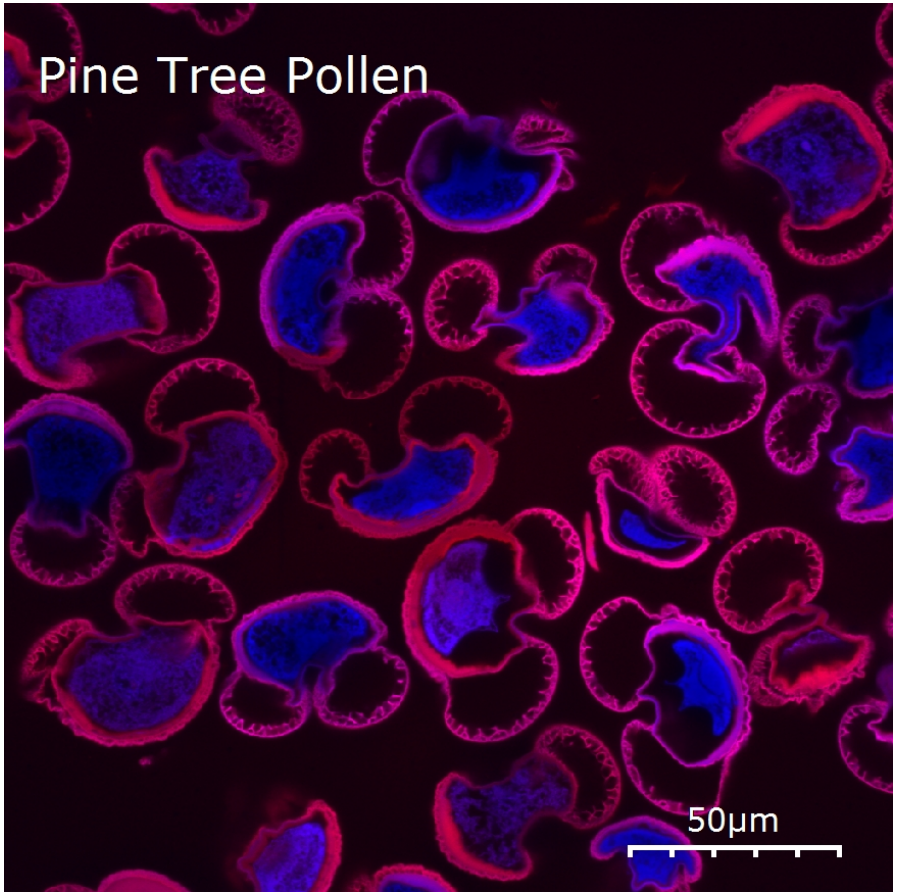
SHINE Student Image from a Laser Scanning Confocal Microscope

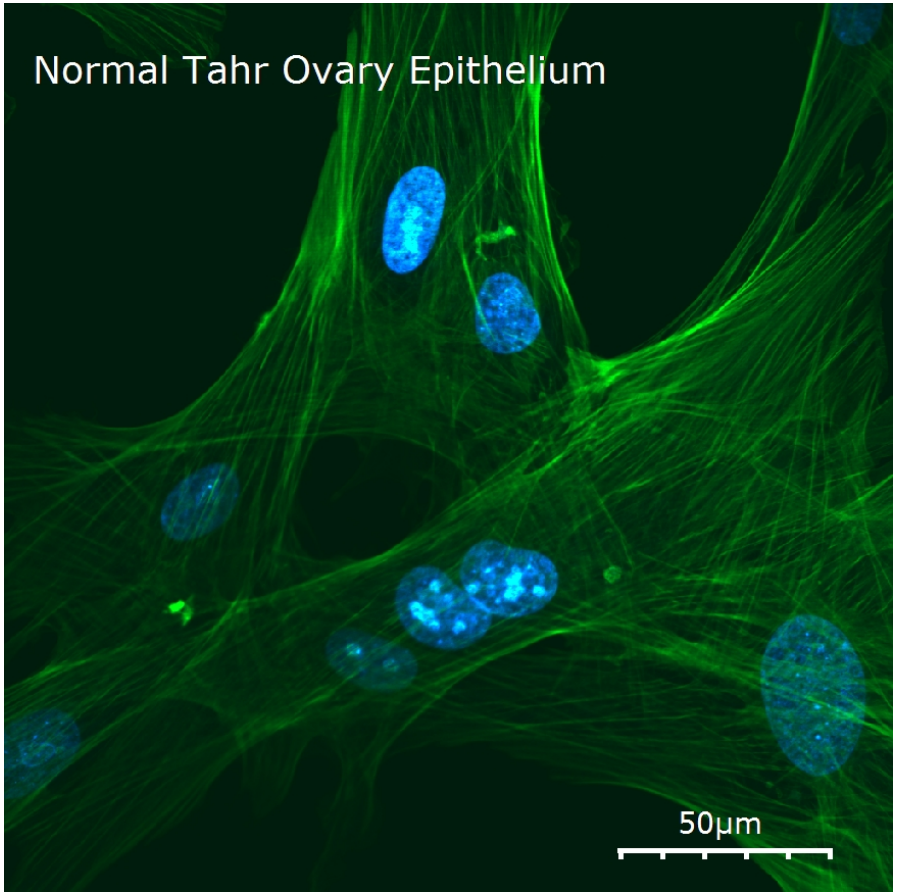
Olympus Fluoview FV10i Laser Scanning Confocal Microscope

SHINE's LSCM

Specifications	**(SHINE Nanotechnology Lab) Olympus Fluoview FV10i Confocal Microscope**
Illumination	**450 nm, 535 nm, 570 nm, and 620 nm excitation laser lines**
Objective Lenses	**10x and 60x oil-immersion lenses**
Detector	**Photomultiplier tube fluorescent photon detectors**
Chassis	**Fully enclosed, vibration isolated bench-mount system**
Operating Modes	**2-D images, multiple Z-stack 3-D images, z-stack over time, and multiple-area imaging modes**
Advantages	
Able to view a variety of samples, living samples	**Ease of use, samples on microscope slides or dishes**
High resolution optical images	**Ability to capture 2D and 3D images**
Disadvantages	
Limited magnification range (10x – ~1000x)	**Sample must be epi fluorescent or labeled with a fluorescent dye**
Imaging software requires manual adjustment	

Laser Scanning Confocal Microcopy Images





Student Images from SHINE's Laser Scanning Confocal Microscope

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Confocal Microscopy

Advantages and Disadvantages:

- Able to image living samples in situ
- Possible to make 3-D images
- Can image samples in water or other media. No vacuum required
- Requires an epi fluorescent sample or a fluorescent labeled sample
- Magnification is limited by the optical excitation wavelength. Practically can image cells, but not individual nanostructures

Confocal Microscopy Resources

http://www.ammrf.org.au/myscope/confocal/introduction/	Training site on fluorescence microscopy
http://www.microscopyu.com/articles/confocal/index.html	Great website with a lot of resources - theory, prep, comparisons to widefield fluorescence, and simulations
http://www.microscopyu.com/articles/confocal/confocalintropreparation.html	One of the links from above website; a lot more information under the confocal index
http://www.olympusfluoview.com/theory/confocalintro.html	Lots of theory; links to tutorials - similar to Nikon's MicroscopyU
http://www2.bioch.ox.ac.uk/microngroup/resources/	PDFs of Advanced Microscopy lectures, as well as other useful resources
http://depts.washington.edu/keck/links.htm	Links from the University of Washington Keck Center that has a list confocal references
http://depts.washington.edu/keck/confocalgate.htm	University of Washington Resource; theory and sample prep
http://www.microscopy-analysis.com/editorials/editorial-listings/confocal-microscopy-re-writes-origin-flowering-plants	Brief introduction, using auto fluorescence of microfossils
http://www.microscopy-analysis.com/editorials/editorial-listings/confocal-microscopy-uncovers-toxic-nanoparticles	LSCM and nanoparticles

This module is one of a series designed to be used by faculty members at post-secondary institutions in workshops, courses, and overview lectures to introduce nanotechnology and its applications.

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