

M5052

CHARACTERIZATION OF MATERIALS AND NANOMATERIALS

Graduate Program in Nanotechnology

INTRODUCTION TO MICROSCOPY

(FOR NANOSCIENCE AND MATERIALS

CHARACTERIZATION)

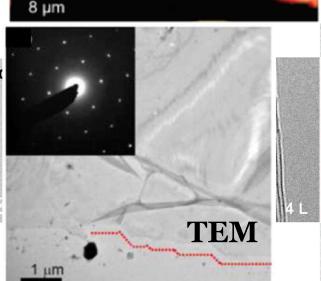
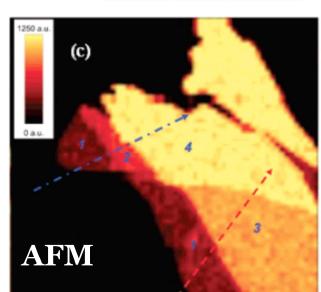
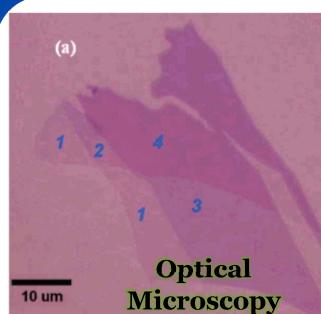
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Investigador, Grupo de Enfoque de Nanotecnología para Diseño de Dispositivos



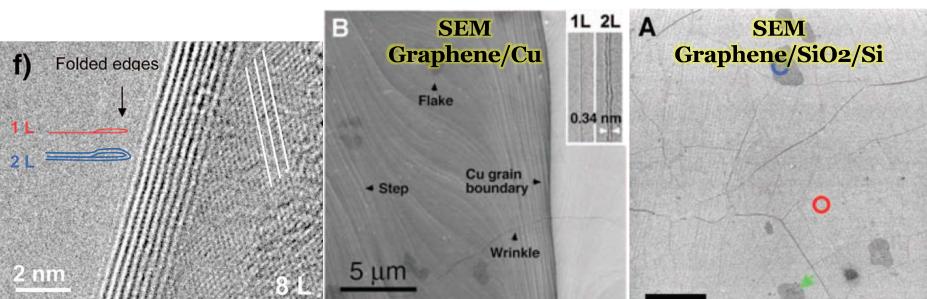
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M5052 – Characterization of Materials



Example: Microscopies in the Characterization of Graphene

- Graphene: atomic monolayer of sp^2 carbon; thickness: 0.34 nm
 - Visible with optical microscopy: Absorbance of 2.29 %, enough for a contrast difference detectable by eyesight
- In atomic force microscopy (AFM) number of layers can be identified directly by height
- Transmission electron microscopy (TEM) allows to determine if sheets are individual or if it is few-layer graphene)
 - Electron diffraction pattern confirms the 2D crystal nature of graphene
- Scanning electron microscopy (SEM) is used to observe graphene over large area substrates



Brief Introduction to Optical Microscopy



Leeuwenhoek Optical Microscope



Cuff style Compound Optical Microscope from 1750

Image sources:

Leeuwenhoek Microscope: <http://arsmachina.com/loeuwenhoek.htm>
Cuff Microscope: <http://arsmachina.com/cuff1527.htm>

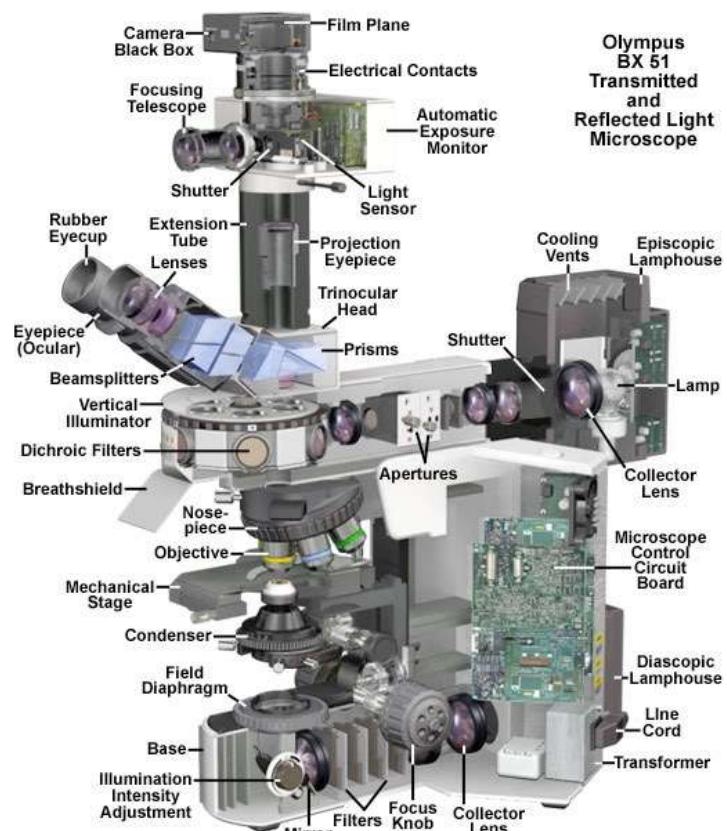
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Some Applications of Optical Microscopy in Materials

- Thin Sections of Materials
- Polymeric Films
- Fibers
- Morphology of materials
 - Powders
 - Foam materials
 - Dispersions
 - Etc.
- Grain size and inclusions in metals and ceramics
- Fractography
- Etc.



<http://olympusmicro.com/primer/anatomy/bx51cutaway.html>

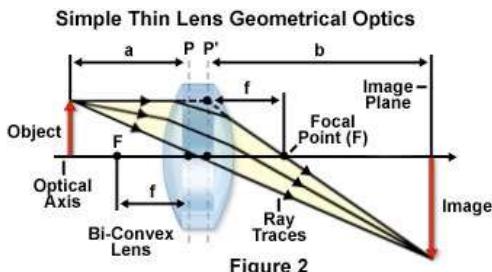
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Basics of Optical Microscopy

- A lens magnifies an object by bending light and projecting it to a larger visual angle



- Magnification = (Image Height)/(Object Height)

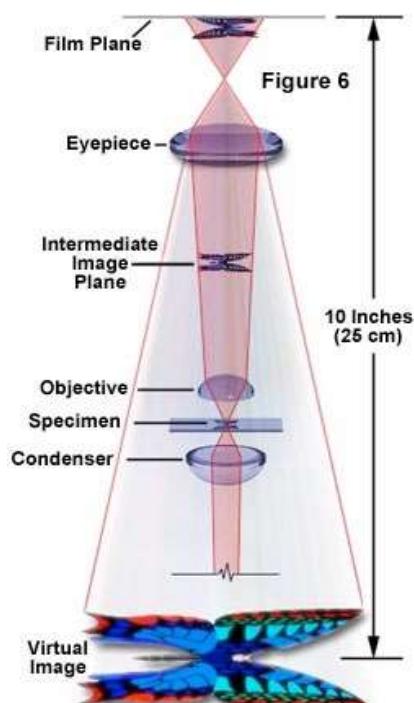
$$M = b/a \quad 1/a + 1/b = 1/f$$

- Most microscopes are compound microscopes

- Objective Lens and Eyepiece lens
- Additionally a condenser lens focuses light on the sample
- Eyepiece magnification usually 10X
- Total magnification is $M_{\text{objective}} \times M_{\text{eyepiece}}$

Images taken from:

<http://www.olympusmicro.com/primer/lightandcolor/lensesintro.html>
<http://www.olympusmicro.com/primer/anatomy/magnification.html>



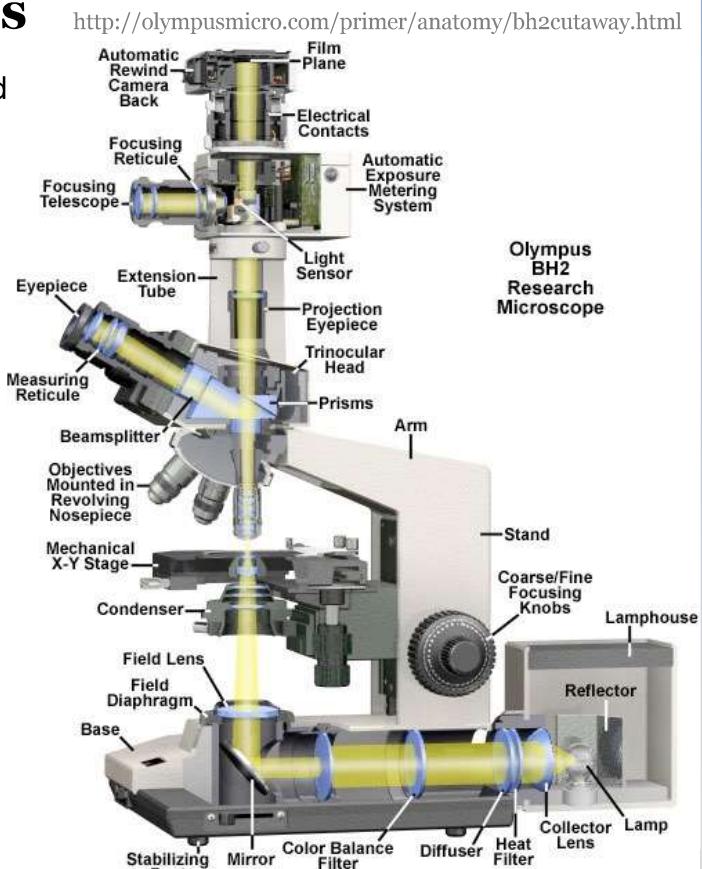
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Objective Lens Types

- Objective lenses are usually designed to work with a cover glass
 - Aberration corrections take into account light refraction by thin (0.17 mm) cover glass
 - Not using a cover glass or a thicker one may result in deterioration of image
- Objective lenses designed to work immersed
 - Oil or Water Immersion, depending on the lens
 - Used for high numerical aperture lenses
- Lenses designed to work "dry"
 - Without any cover glass or liquid between the lens and the specimen to be examined
 - More suitable for most materials applications



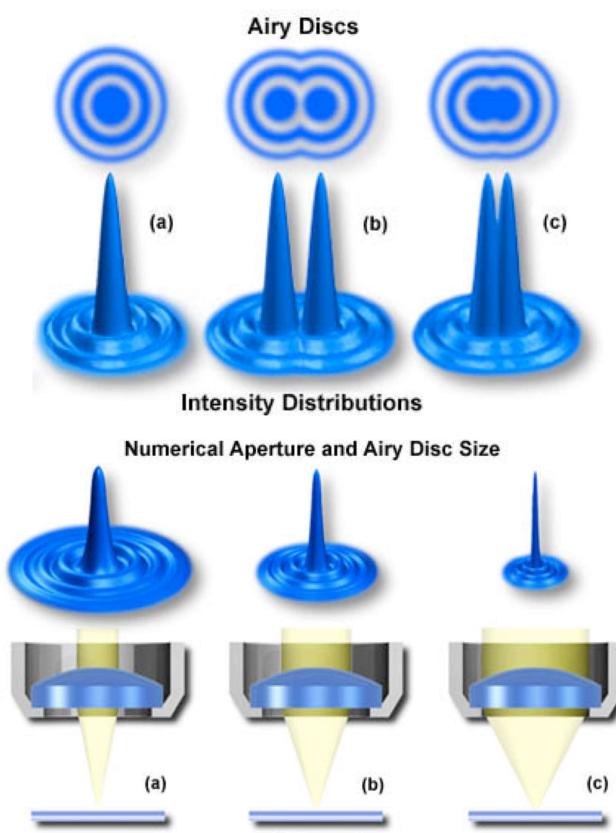
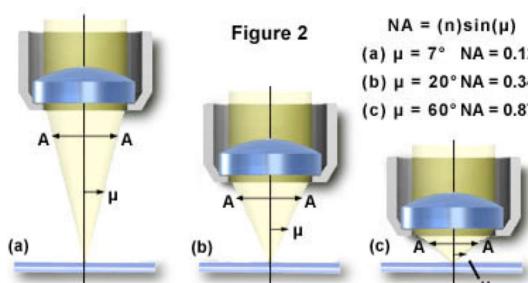
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Resolution

- Resolution is the ability to distinguish (resolve) two objects
- Airy Disk: light is not focused to a point due to diffraction
 - When central points of Airy disks overlap, they can not be resolved
- Numerical Aperture influences resolution
- Larger N.A. reduces Airy disk size
 - Larger N.A. gives better resolution (ability to resolve smaller features)



Images taken from: <http://olympusmicro.com/primer/anatomy/numaperture.html>

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Scanning tunneling microscopy (STM)

Resolutions of Different Microscopies

- This figure from 1982 shows how different microscopy techniques are suitable for different observation scales
- Conventional Optical microscopy is quite limited compared to scanning probe and electron microscopies
 - In the three decades since then, advanced optical microscopy techniques have broken the resolution limit shown here

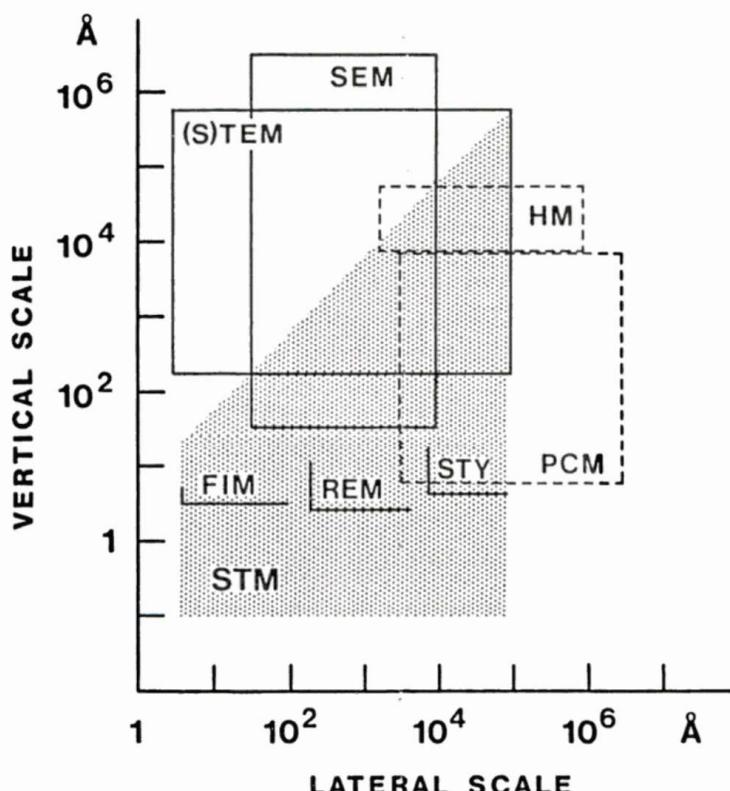


Image taken from: Roland Wiesendanger (Ed.) Scanning Probe Microscopy and Spectroscopy : Methods and Applications, Cambridge University Press, Cambridge, (1994).

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Fig. 1.43. Comparison of resolutions of different microscopes. STM: shaded area. HM: high-resolution optical microscope. PCM: phase-contrast microscope, (S)TEM: (scanning) transmission electron microscope, SEM: scanning electron microscope, REM: reflection electron microscope, and FIM: field ion microscope (Binnig and Rohrer, 1982).

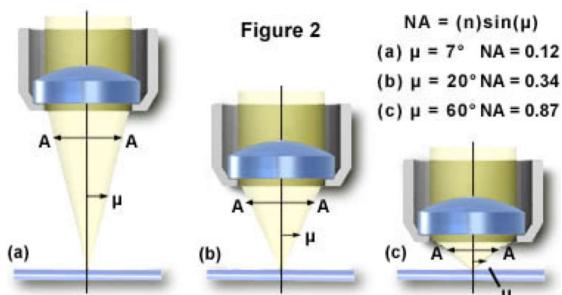
Optical Microscopy: The Good and the Bad

▪ Advantages

- Relative simplicity
- Only requires a source of light and magnifying lenses
- Little sample preparation required
- Convenient to use; relative low cost
- Non-destructive
- Non-invasive
- Versatile
- Reflected or Transmitted light modes
- Different techniques available to increase contrast
- Magnifications of several hundreds

▪ Disadvantages

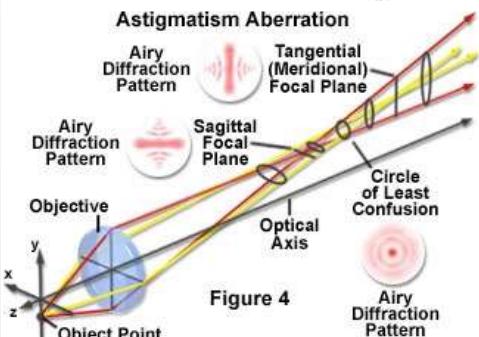
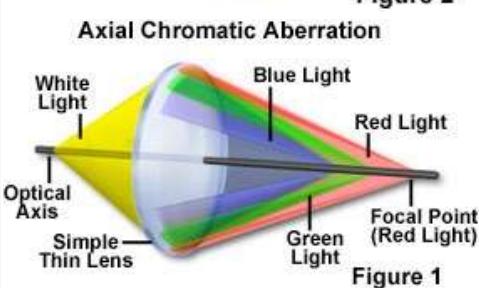
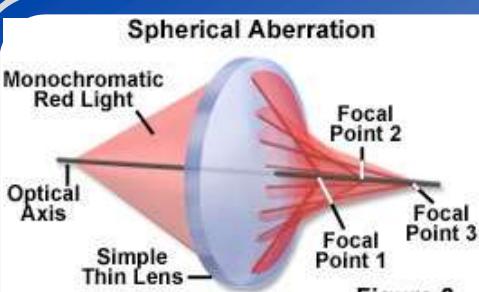
- Magnification of only up to several hundred times
- Limited depth of field
 - From $\sim 8 \mu\text{m}$ for N.A. 0.25 to $\sim 0.4 \mu\text{m}$ (N.A. 1.30)
- Maximum thickness of $\sim 5 \mu\text{m}$ in transmission mode
- Limited resolution due to diffraction limit in conventional optical microscopy techniques
 - Resolution = $\lambda/2\text{NA}$
 - $R \approx 0.2 \mu\text{m}$ is the best resolution possible
 - E.g. 360 nm, NA=0.95: $R=0.19 \mu\text{m}$



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Aberrations

▪ Spherical Aberration

- Curvature of lens focuses light rays at different points
 - Rays near center not refracted
 - Rays passing near edge refracted to a large degree

▪ Chromatic Aberration

- Refraction of light depends on wavelength resulting in dispersion
 - Blue light is refracted more
 - Red light is refracted the least
- Visible as colored fringes on image

▪ Astigmatism Aberration

- Light rays entering lens from different axes are refracted differently
- Off-focus images of points (lat. *stigma*) spread into ellipses

▪ Lens materials and microscope construction correct and minimize aberrations

- Only at the highest resolution these may be a problem with a modern, high quality microscope
- Appearance of aberrations may indicate need for maintenance

Images taken from:
<http://www.olympusmicro.com/primer/lightandcolor/opticalaberrations.html>

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Contrast in Optical Microscopy

▪ Percent Contrast

$$C(\%) = \frac{I_S - I_B}{I_B} \times 100$$

I_B : intensity of the background

I_S : specimen intensity.

- The human eye distinguishes differences in contrast larger than 2%
 - Differences in absorption of light by the observed objects in transmission microscopy
 - A graphene monolayer absorbs 2.3% of light, that is why an atomically thin sheet can be seen by optical microscopy

- Differences in color are also easily detected by the eye
 - Use of staining agents for samples

- Specimens that are transparent do not offer enough contrast
 - Differences in refractive index results in difference in *phase* of light but the eye can not detect it

▪ Different techniques are used to enhance contrast

Phase Contrast

- Phase of light is retarded when passing through a sample
 - Differences in refraction index mean differences in time for light to pass through an object
 - Usually phase of sample diffracted light is retarded by $\frac{1}{4}$ wavelength (90°)
- Additional displacement of phase of undeviated light by $+90^\circ$ results in destructive interference
 - Resulting differences in intensity increase contrast
 - Phase can also be displaced -90° (negative phase contrast)

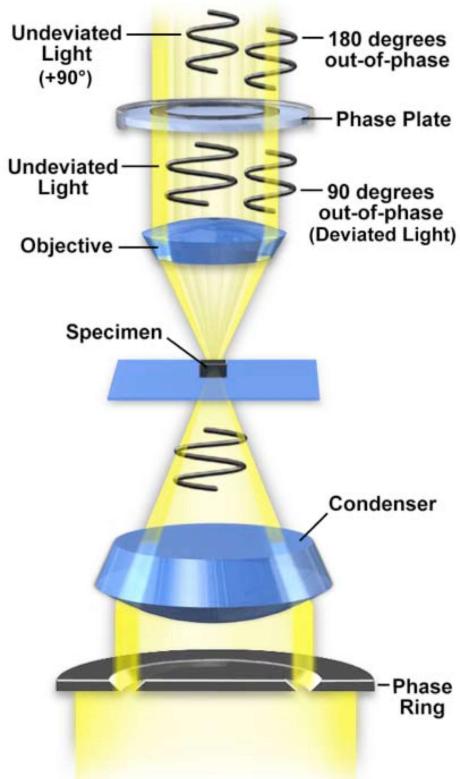
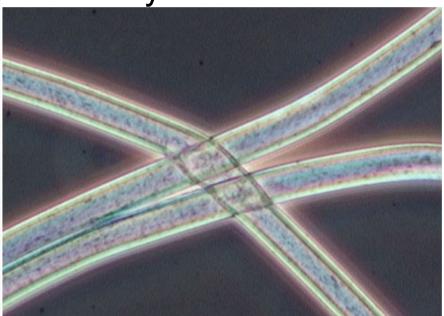


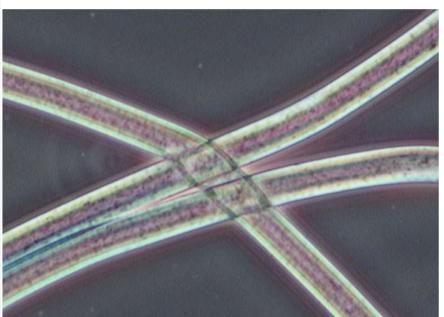
Image from: M.W. Davidson and M. Abramowitz, "Optical Microscopy", 1999
<http://micro.magnet.fsu.edu/primer/opticalmicroscopy.html>

Phase Contrast Microscopy of some Materials

Polyester Fibers



Positive Phase Contrast

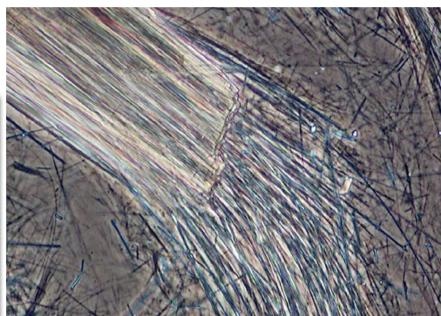


Negative Phase Contrast

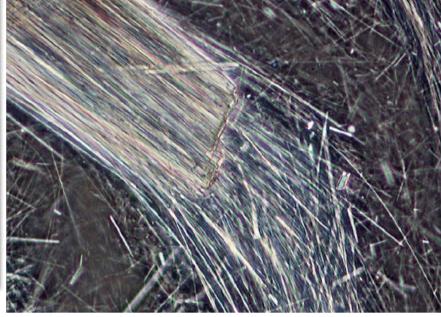
Butterfly Wing Scales
by Phase Contrast
Optical Microscopy



Asbestos Fibers



Positive Phase Contrast



Negative Phase Contrast

Fiber images from: <http://www.microscopyu.com/galleries/phasecontrast/index.html>

Butterfly Wing image from: <http://micro.magnet.fsu.edu/primer/techniques/phsegallery/butterflywing.html>

Dark Field Microscopy

- Sample illuminated at oblique angles
- Light diffracted by the sample is collected by the objective lens
- Light that does not interact with the sample passes through

Reflected Light Darkfield Configuration

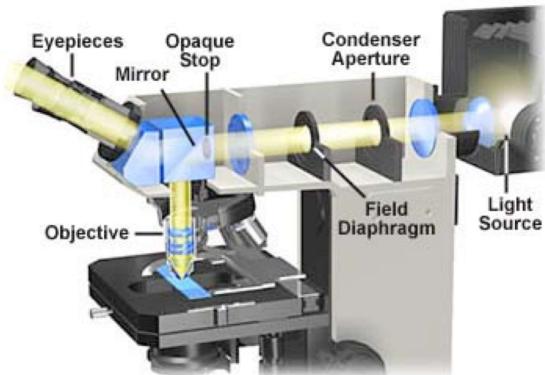
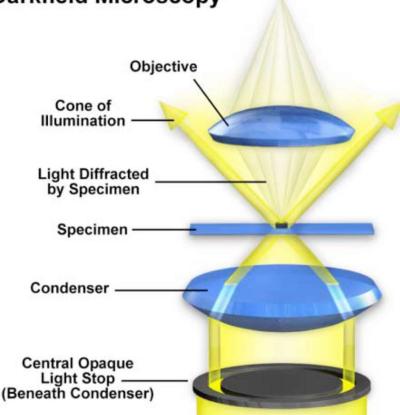


Figure 1

Images from: M.W. Davidson and M. Abramowitz, "Optical Microscopy", (1999) <http://micro.magnet.fsu.edu/primer/opticalmicroscopy.html>

Darkfield Microscopy



Reflected Light Darkfield Objective

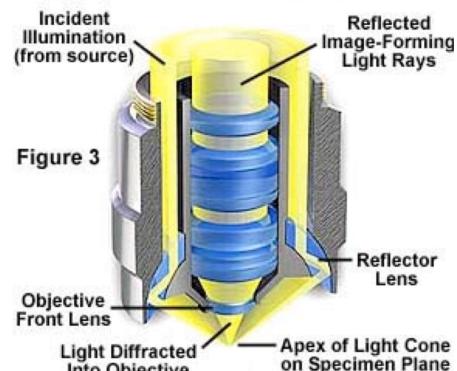
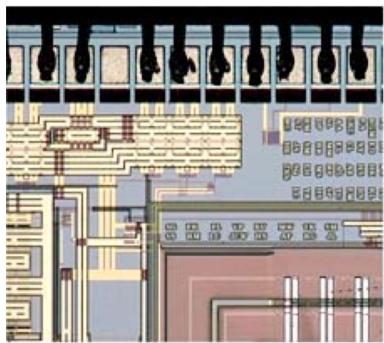
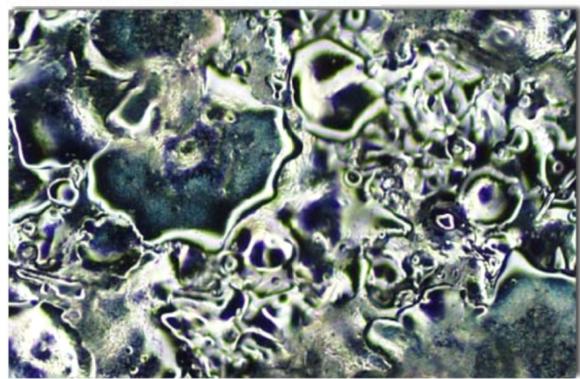


Figure 3

Brightfield and Darkfield Reflected Light Microscopy



Dark Field Micrograph of Butterfly Wing Scales



Dark Field Micrograph of Quasicrystals

Integrated Circuit image from: <http://olympusmicro.com/primer/techniques/darkfieldreflect.html>

Butterfly Wing image from: <http://micro.magnet.fsu.edu/primer/techniques/darkfieldgallery/butterfly.html>

Quasicrystals from: <http://micro.magnet.fsu.edu/primer/techniques/darkfieldgallery/quasi.html>

Polarized Light Microscopy

- Plane Polarized Light vibrates in one axis,
 - East-west plane of the optic axis
- Birefringent Materials split polarized light
 - Anisotropic crystals have different indices of refraction depending on the orientation of the crystal lattice with respect to the incident light
 - Rays have perpendicular polarizations
 - One of them is retarded with respect to the other
- Waves interfere constructively or destructively at the analyzer
 - Cross-polarized analyzer (XPL) perpendicular to polarization of illumination
 - This can result in appearance of a spectrum of colors dependent on crystal orientation or composition
 - Removing the analyzer allows analysis of plane-polarized light (PPL)

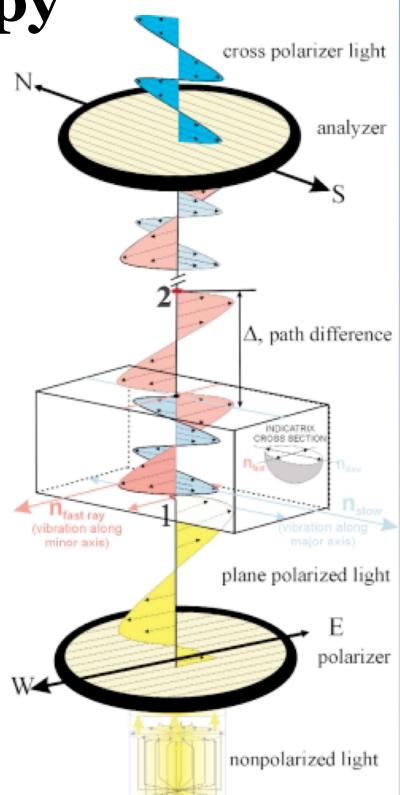
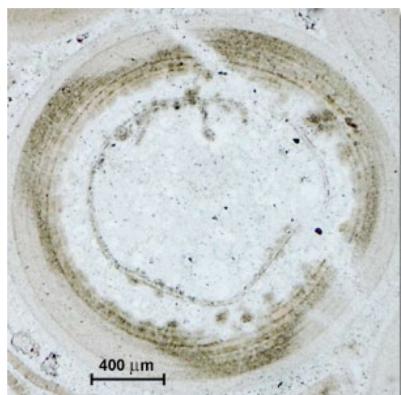
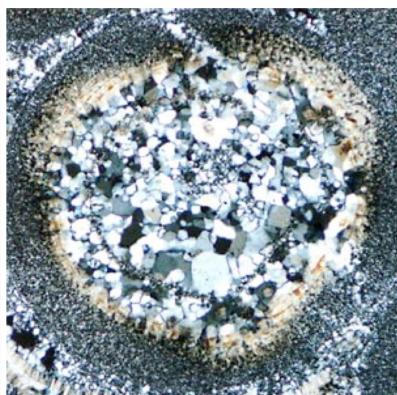


Image from R. Weaver "Rediscovering Polarized Light Microscopy", American Laboratory, October 2003, p. 55

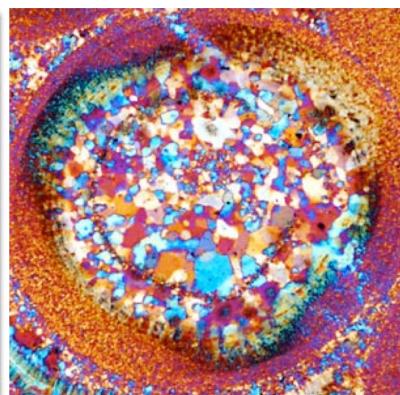
- Polarized Light Microscopy is used in analysis of grain size, orientation, etc. of Metals, Ceramics, Minerals



Oolite (a concentric layered spherical mineral) illuminated with plane-polarized light.



Oolite imaged through crossed polarized illumination.

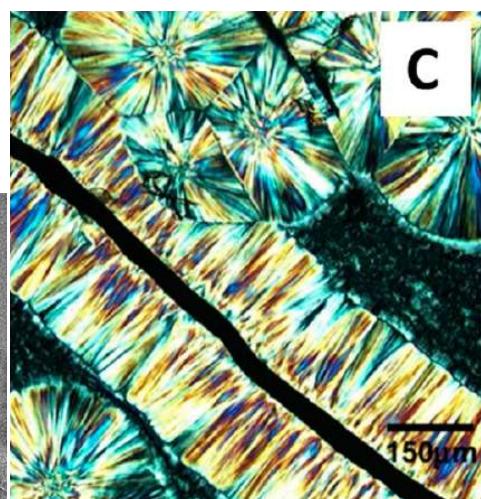
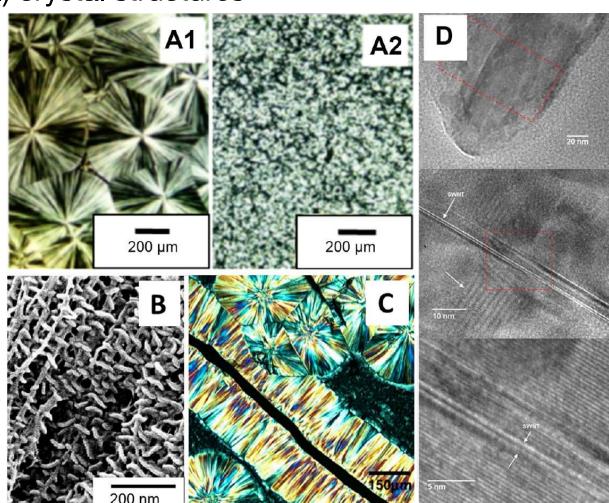


Oolite imaged with crossed polarizers and a full-wave (first order) retardation plate inserted between the specimen and analyzer.

Images from: P.C. Robinson, M.W. Davidson, "Optical Microscopy", 1999
<http://www.microscopyu.com/articles/polarized/oolite.html>

Polarized Light Microscopy of Polymer Fibers with Carbon NanoTubes (CNT)

- Carbon nanotubes act as nucleation sites for crystallization of polymers, crystals grow radially off the CNT instead of the regular spherulite (radial spherical) crystal structures



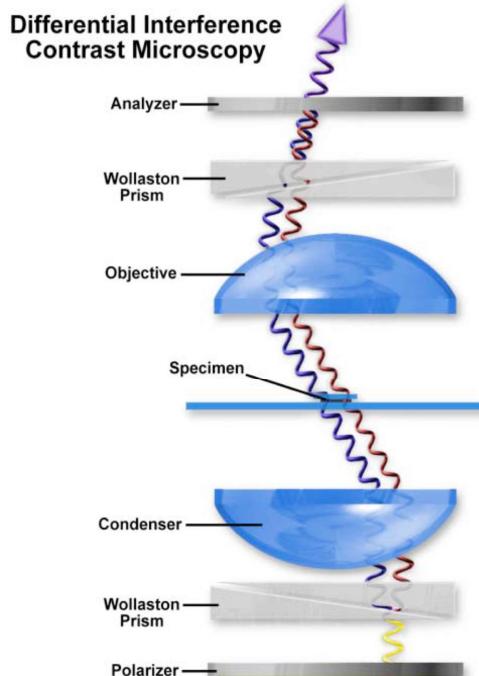
ACS Appl. Mater. Interfaces 6 (2014) 6069–6087

dx.doi.org/10.1021/am405136s |

Figure 2. Cross-polarized optical micrographs of (A1) PP and (A2) SWNT/PP with 1 wt % SWNT bulk samples melted at 220 °C and cooled to room temperature at a rate of 0.5 °C/min. Reprinted with permission from ref 26. Copyright 2008 Elsevier. (B) UHMWPE forms aligned shish-kebab structure on MWNTs after controlled solution crystallization. Reprinted with permission from ref 37. Copyright 2013 American Chemical Society. (C) Polarized optical micrograph of transcrystalline interphase layer of PP surrounding the CNT fiber. Reprinted with permission from ref 39. Copyright 2008 Elsevier. (D) Transmission electron micrographs of self-assembled PVA/SWNT nano fibril. Nano fibril diameter is about 120 nm. SWNT and PVA (200) lattice planes (d -spacing ~0.385 nm) parallel to SWNT axis can be observed. Reprinted with permission from ref 40. Copyright 2006 Elsevier.

Differential Interference Contrast

- Polarized light is split in two beams, with perpendicular polarizations, by a Nomarski prism (modified Wollaston prism)
 - The difference between beams paths is called “shear” and is small
 - Technique is sometimes called Nomarski interference contrast microscopy
- After interacting with the specimen and being collected by the objective lens another prism combines the beams
 - Interference results in differences in intensity and color



Differential Interference Contrast

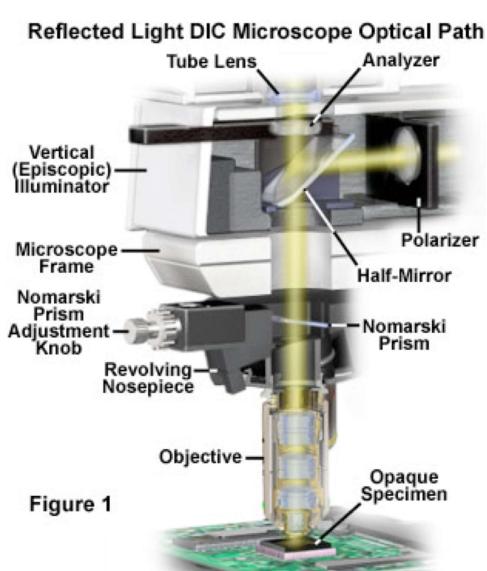


Figure 1

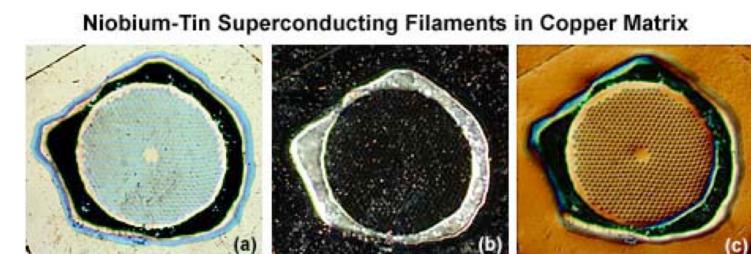
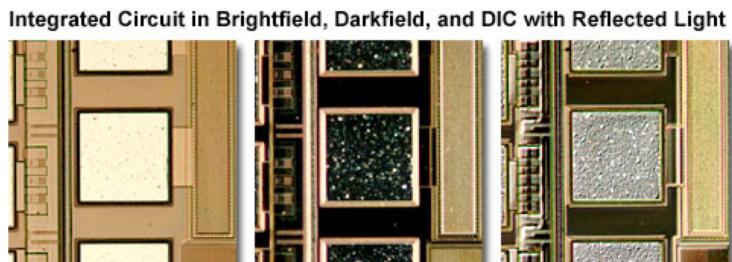
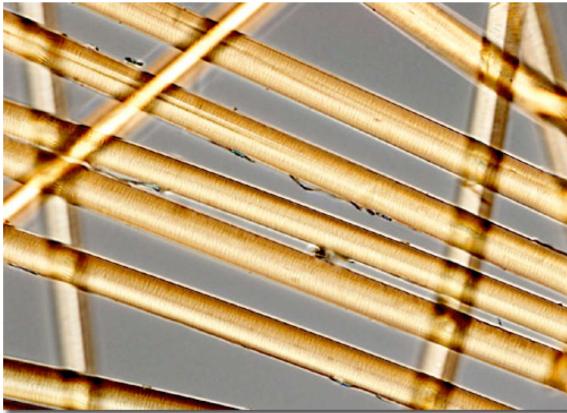


Figure 5

Microscope Configuration and Integrated Circuit from: <http://www.microscopyu.com/articles/dic/reflecteddic.html>
Nb-Sn filaments image from: <http://olympusmicro.com/primer/techniques/darkfieldreflect.html>

Differential Interference Contrast

Poly-paraphenylene terephthalamide
(Kevlar) Fibers



Kevlar Fiber:

<http://micro.magnet.fsu.edu/primer/techniques/dic/dicgallery/kevlarlarge.html>

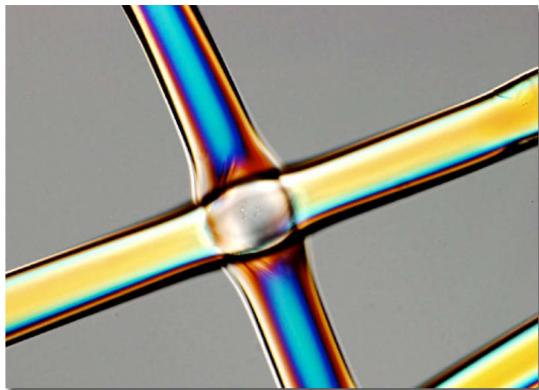
PP fiber:

<http://micro.magnet.fsu.edu/primer/techniques/dic/dicgallery/polypropylenefiberslarge.html>

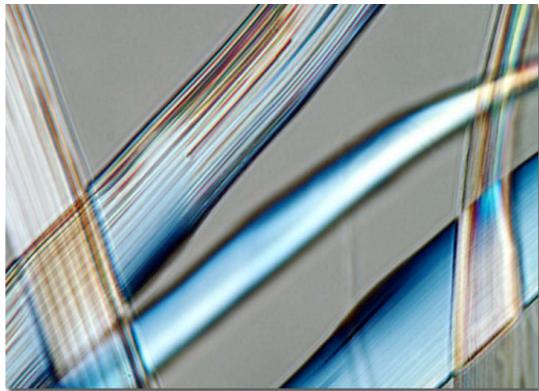
Silk Fiber:

<http://micro.magnet.fsu.edu/primer/techniques/dic/dicgallery/wildsilksmall.html>

Poly-propylene Fibers



Wild Silk Fibers

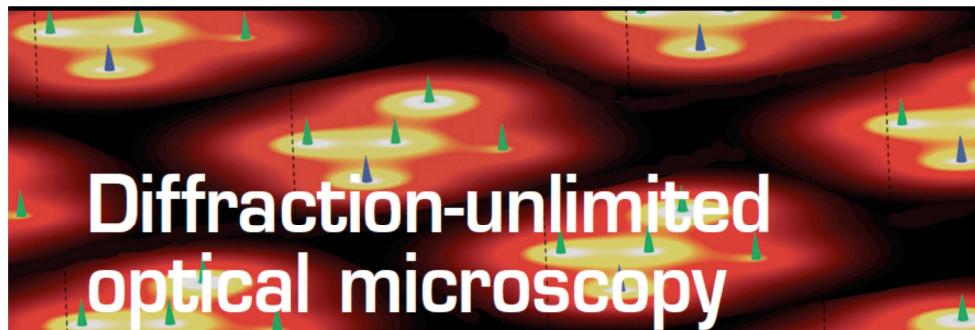


Advanced Microscopy Techniques

- Micro Raman
 - Raman spectroscope attached to a microscope
- Micro-FTIR
 - Fourier Transform Infrared spectroscopy through a microscope
- NOTE: both micro-Raman and micro-FTIR allow chemical mapping
- **Nanoscopy:** optical microscopy with resolution below the diffraction limit
- Different approaches used to surpass the diffraction limit, most of them use lasers and fluorescence
 - Confocal Microscopy
 - Imaging of fluorescent molecule markers, using an aperture to remove out of focus light
 - STED: stimulated emission depletion microscopy
- STORM: stochastic optical reconstruction microscopy
 - Stochastic: occurring randomly in time
- PALM: Photo-activated localization microscopy
- SNOM (scanning near field optical microscopy)
 - A type of scanning probe microscopy, related to AFM and STM
- There are many recent advances in microscopy techniques below the diffraction limit
 - Most of the applications relate to biology, but these techniques may also be used to characterize some nanostructures
- Optical microscopy *without lenses* is also a very active area of research
 - Metamaterials and nanostructures used to manipulate photons (instead of lenses)

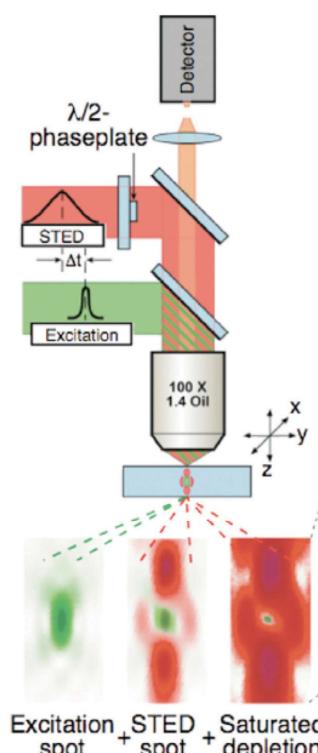
Advanced Optical Microscopy: Beyond the Diffraction Limit

- The use of fluorescence in imaging allows overcoming the wavelength related limitations of optical microscopy

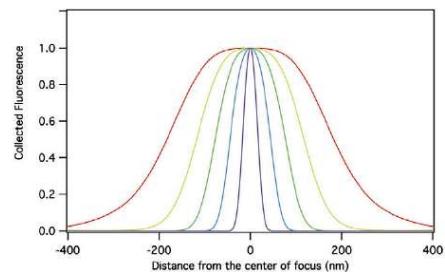
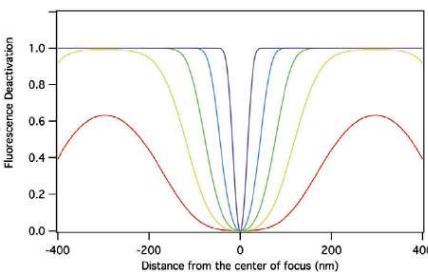
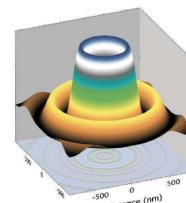
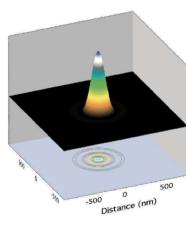


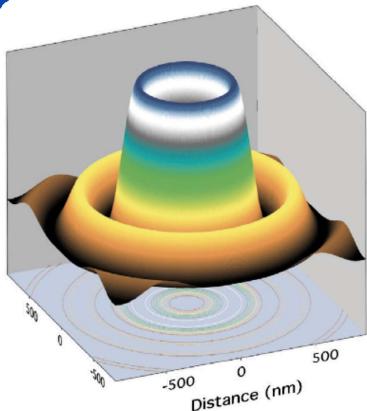
- P. Dedecker, J. Hofkens, J.-i. Hotta, "Diffraction-unlimited optical microscopy" Materials Today, **11**(supplement) (2008) 12–21 [DOI: 10.1016/S1369-7021(09)70003-3]
- Optional lecture, a good introduction to the topic if you want to learn more, but be aware that some limitations listed here are likely overcome in the 10 years since this was published

STED: Stimulated-Emission Depletion



- Modification of the phase of the laser shapes the spot into a “donut-mode”
 - Intensity at center of the spot is zero
- Irradiate first with normal (Airy-disk shaped) beam
 - Puts all molecules irradiated in a fluorescent mode
- Irradiation with donut-mode beam stimulates emission except at the central focal point
 - Only the few molecules in the small central spot remain in a fluorescent state
 - The fluorescence of the other molecules is *depleted*
- Varying intensity of donut beam reduces size of central spot increasing resolution (figure below)

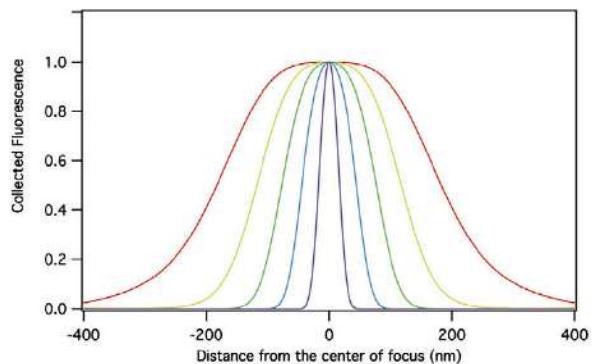
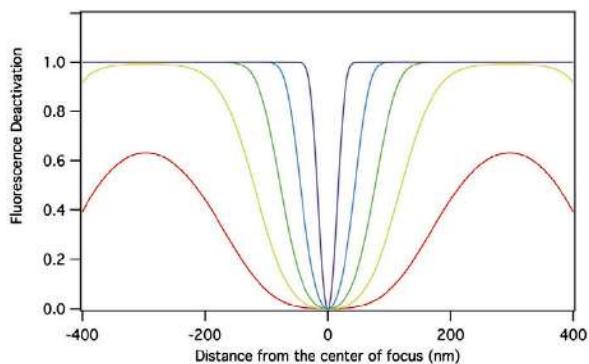
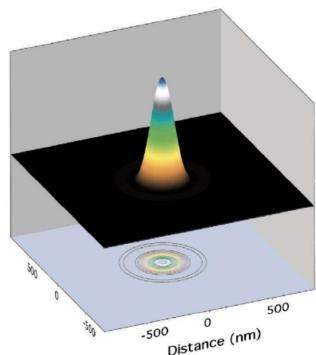




Intensity of light in a donut mode laser beam (color scale from 0:black to dark blue for maximum intensity)

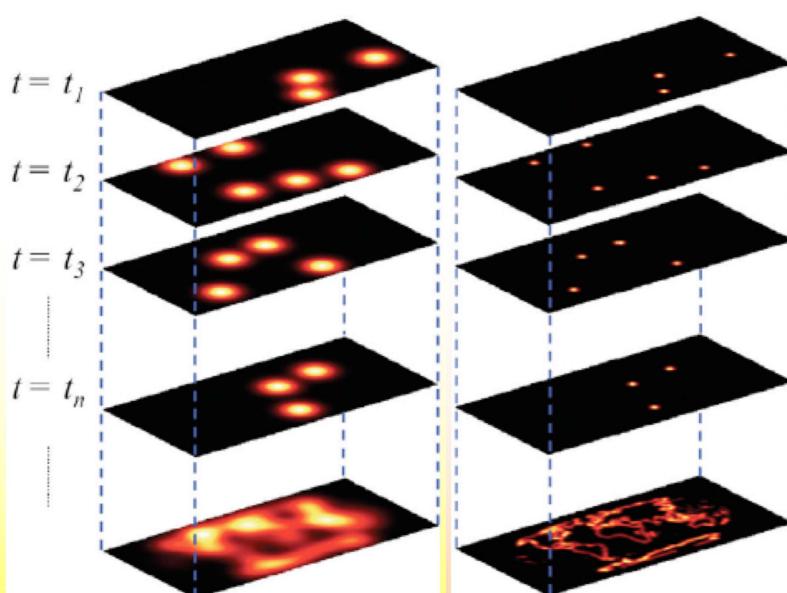
STED:

- Higher intensity of the “donut” laser beam reduces the diameter of the center spot, and thus reduces the area where fluorophores are not deactivated
- Intensity of the fluorescence is highest at the center of the spot



PALM

Photo-Activated Localization Microscopy



Resolution of fluorescence spots is still limited by optics, but only few spots fluoresce at a certain time

Center point locations recorded over time to build the image

- Fluorescent molecules excited in a way that guarantees that only a few molecules will fluoresce at any given time
- PALM = FPALM, fluorescence photoactivation localization microscopy
- Intensity distribution position is recorded
- Central point is taken as the location of the fluorophore molecule
- Repeated measurements used to map the distribution of fluorophores

STORM

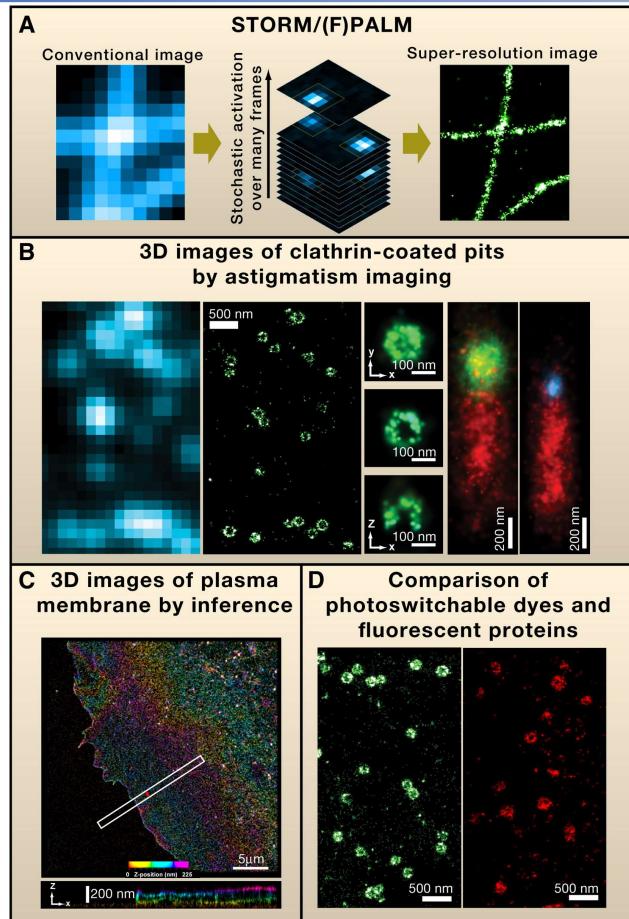
- Stochastic Optical Reconstruction Microscopy
 - A different name for PALM

(A) The STORM approach takes advantage of *photoswitching* of fluorophores *to temporally separate images of single molecules that overlap spatially*. At any time during image acquisition, only a sparse subset of fluorophores is activated to the fluorescence state, allowing these molecules to be imaged individually and thus localized. *After multiple iterations* of the activation and imaging processes, a *super-resolution image* is *constructed* from the localizations of many fluorophores.

Figure and caption text (with emphasis added)

from: B. Huang, H. Babcock, X. Zhuang,
“Breaking the Diffraction Barrier: Super-Resolution Imaging of Cells” *Cell* 143 (2010) 1047-1058
DOI: 10.1016/j.cell.2010.12.002

- Xiaowei Zhuang, inventor of STORM was recognized with a 2019 Breakthrough Prize in Life Sciences “For discovering hidden structures in cells by developing super-resolution imaging – a method that transcends the fundamental spatial resolution limit of light microscopy.”
- <https://breakthroughprize.org/News/47>



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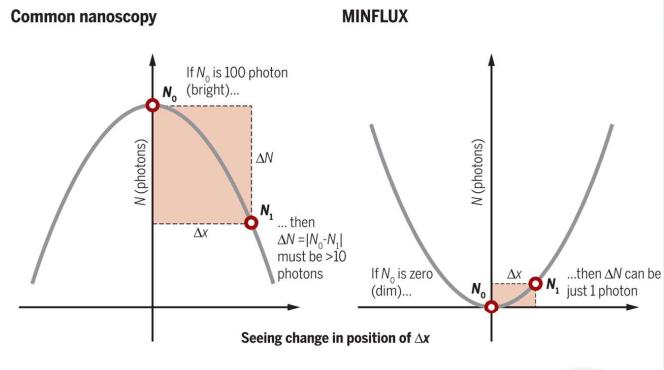
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MINFLUX

- Novel technique for nanometer resolution imaging of fluorophores
 - 6 nm resolution reported in 2017, ultimate resolution may be < 1 nm
 - One of the most advanced “nanoscopy” techniques
- Uses stochastic switching of fluorophores (as in PALM) combined with a donut shaped laser probe (as in STED)
- Position of zero intensity spot in donut shaped beam is well known and controllable
- Approximate position of fluorophore detected by moving around the spot
 - It will fluoresce only when the laser excites it
- Approximate position used to find the exact spot where there is no fluorescence
 - This is the exact position of that fluorophore molecule
- Minimal flux of light is needed, since only a few fluorescent photons need to be detected to know where the molecule is (not) precisely located

- Developed by the team of Stefan Hell
- Hell shared the Nobel Prize in Chemistry in 2014 for developing STED
- F. Balzarotti et al. “Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes” *Science* 355 (2017) 606–612
- DOI: 10.1126/science.aak9913



Implementing MINFLUX

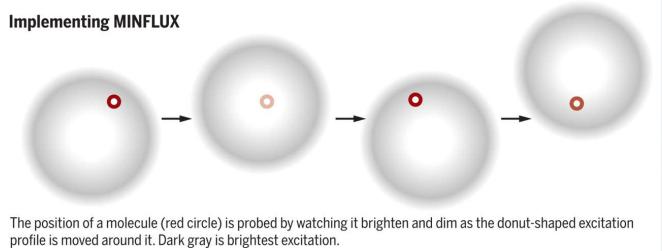


Image from: *Science* 355 (2017) 582-584; DOI: 10.1126/science.aam5409

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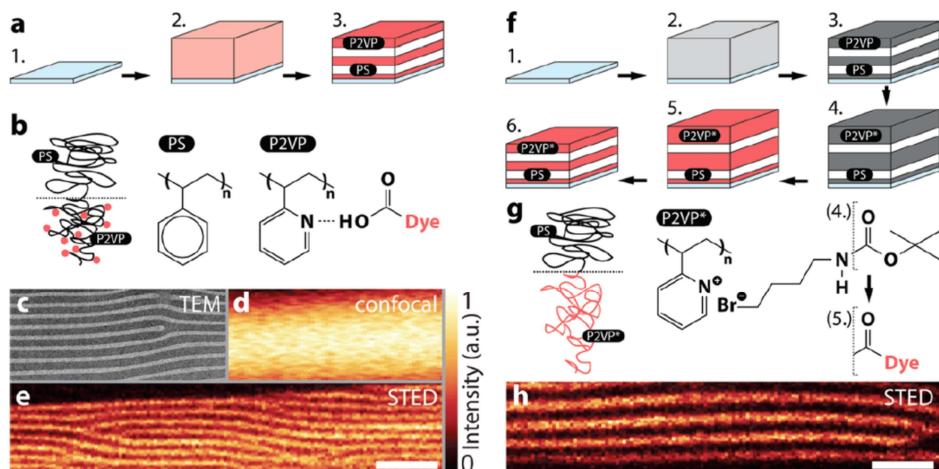
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Nanoscopy for Nanotechnology Example: 3D mapping of Self Assembled Block Copolymer Nanostructures by STED

- Block Copolymer: Incompatible blocks segregate into separate phases, but remain joined by covalent bond between blocks
 - Fluorescent dye selectively added to one polymer phase
- STED microscopy used to map in 3D, with nanometer resolution, the spatial

C.K. Ullal, R. Schmidt,
S.W. Hell, A. Egner "Block
Copolymer Nanostructures
Mapped by Far-Field
Optics" Nano Lett. 9
(2009) 2497
[DOI: 10.1021/nl901378e]



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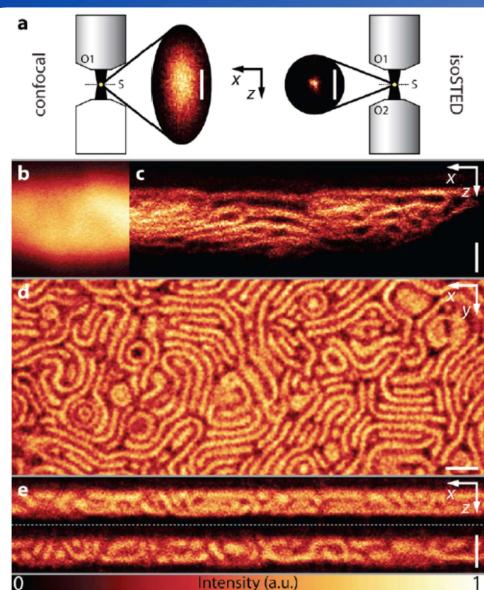


Figure 2. Imaging with an isotropic PSF. (a) xz sections of typical confocal (left) and isoSTED (right) PSFs mapped by a fluorescent bead ~ 40 nm in diameter. The isoSTED spot is created by the coherent use of the wavefronts of the two opposing objective lenses O1 and O2 which focus onto the same spot in the sample plane (S). (b) Confocal micrograph of a kinetically trapped structure seen in a PS-P2VP block copolymer is compared with (c) an isoSTED image from a contiguous region, recorded with a spherical spot <50 nm in diameter. The weight fraction of the P2VP block is 0.3. (d) xy and (e) two xz sections taken at different locations in a film of a PS-P2VP block copolymer, swollen with 30 wt % PS homopolymer. The weight fraction of the PS-P2VP block copolymer is 0.5. Scale bars 250 nm (a) and 500 nm (c, d, and e).

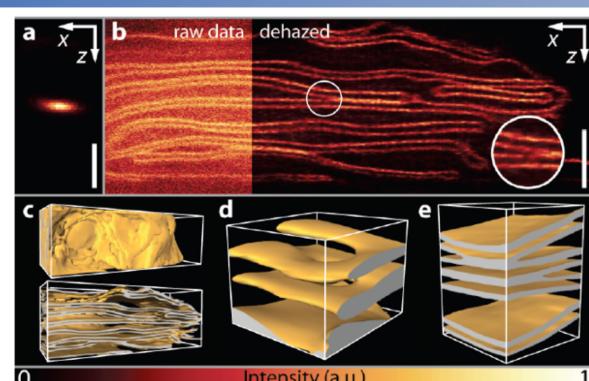


Figure 3. 3D reconstruction of swelling-induced mesoporous morphology recorded by STED microscopy. (a) Typical oblate PSF shape (43×120 nm), mapped by a fluorescent bead ~ 40 nm in diameter. (b) Raw (left) and dehazed (right) xz section, taken from a 3D data stack recorded with an oblate PSF. As seen in the highlighted and $2\times$ enlarged region, the unzipped layers lining the pores are on average over half the size of the intact ones. (c) Perspective views of the corresponding data stack, which was binarized to aid in the visualization. (d) A helicoidal screw dislocation neighboring a pore. (e) Selected area showing "unzipping" of the P2VP domains, forming pores. Scale bars, 250 nm (a) and 1 μm (b). Length of short edge was 2.5 μm (c), 0.6 μm (d), and 1.0 μm (e).

- C.K. Ullal, R. Schmidt, S.W. Hell, A. Egner
"Block Copolymer Nanostructures Mapped
by Far-Field Optics" Nano Lett. 9 (2009) 2497

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