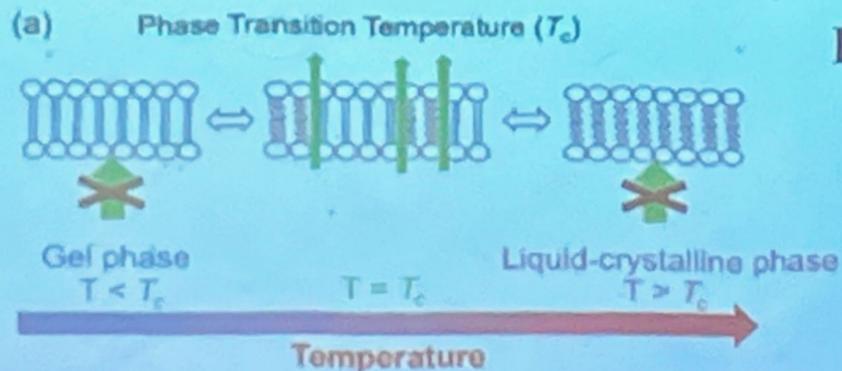


## Biophysical Properties for Drug delivery (Liposome fluidity)

The structure of lipid tails strongly influences the  $T_c$  of lipids, and controls their mechanical strength, lateral diffusion and permeability. The membrane permeability is the largest at  $T_c$  because of the coexistence and interconversion of the two phases, creating leaky phase boundaries (Fig. 3a).<sup>78,79</sup> Many thermal-responsive liposomal drug delivery systems have been developed



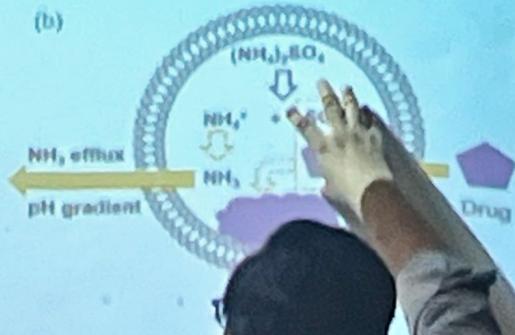
Recap

## Liposomes (Active Drug loading)

Active drug loading, also known as remote loading, refers to loading drugs into preformed liposomes. Active loading usually takes advantages of diffusion properties when a pH gradient is established across lipid bilayers.<sup>53</sup> This method requires drugs to have both an uncharged form and a charged form, where only the uncharged drugs can cross liposome membranes. Once diffused into liposomes, they become charged and membrane-impermeable and entrapped inside. The remote loading method has led to the successful development of many commercial formulations.

### Recap

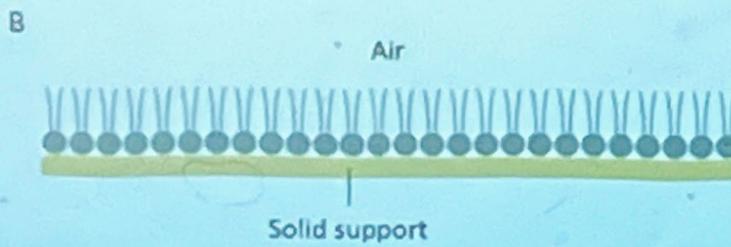
was generated by a transmembrane ammonium gradient. Ammonium salts could dissociate into protons. Since ammonia has a high membrane permeability, a pH gradient can also be created. Thus, DOX can influx and precipitate with ammonium counter ions to form membrane-impermeable drug complexes.



For Doxil®

## Lipid Monolayer (Solid Support)

The properties of lipid monolayers depend dramatically on their molecular density. They can form various phases in two dimensions which are controlled by temperature and by the surface pressure, analogous to the three-dimensional pressure [5].



### Recap

Solid-supported lipid monolayers offer an advantage over the non-immobilized monolayers since the surface immobilization limits the motion of phospholipids while still allowing the study of drug-lipid interactions. These immobilized monolayers also constitute more realistic models, since they better mimic the dynamics of the phospholipids in the biological membranes.

# Modelling LUNG SURFACTANT using Lipid Monolayer

Lipid monolayers can be used as models to study complex phenomena in biological systems. For example, lipid monolayer constitutes the outer layer of tear film in the eyes [22]. Lipid monolayer is the main structural element of lung surfactant, which has very low protein content.

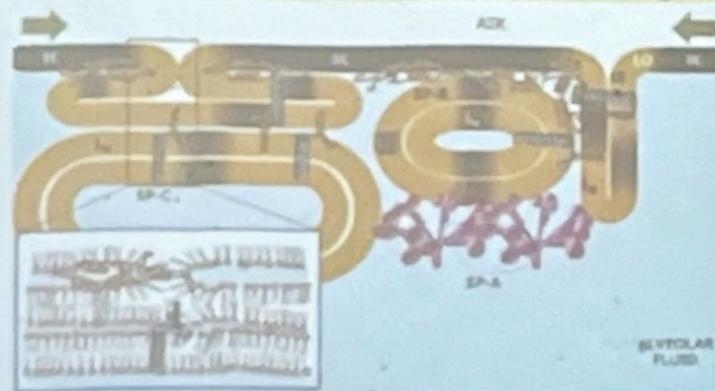


Fig: 3D structure of lung surfactant.

Lung surfactant is a mixture of lipids and proteins forming a monomolecular film at the gas exchange interface in the lung alveoli [8]. Lung surfactant facilitates breathing. Its deficiency or inhibition causes failure of lung function that leads to severe respiratory disorders.

Lung surfactant consists mainly of PC lipids (80% by weight), with DPPC as major component (40% w). Anionic PG and PI lipids account for 8–15% by weight. Lung surfactant also contains cholesterol (5–10% w), PE lipids, fatty acids, and other minor components. Overall, lung surfactant is characterized by a rather complex lipid composition.

## Recap



a. Supported Lipid Bilayer(SLB)

## Biophysical Methods

c. Introduction/Principle of Scanning Tunnel Microscopy (STM)

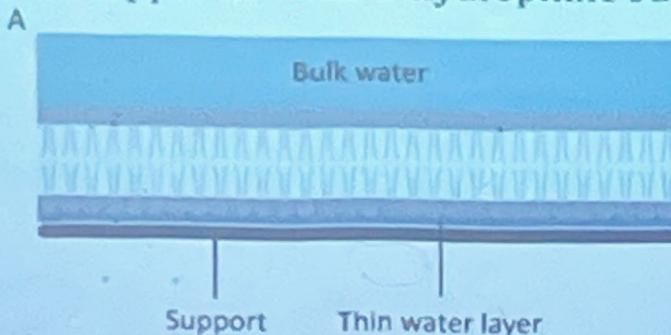
c. Atomic Force Microscopy (AFM)

d. Confocal Microscopy(CM)

Acknowledgment: Aggregated Lecture from lot of resources  
(Textbook/Journal Papers/Youtube lectures)

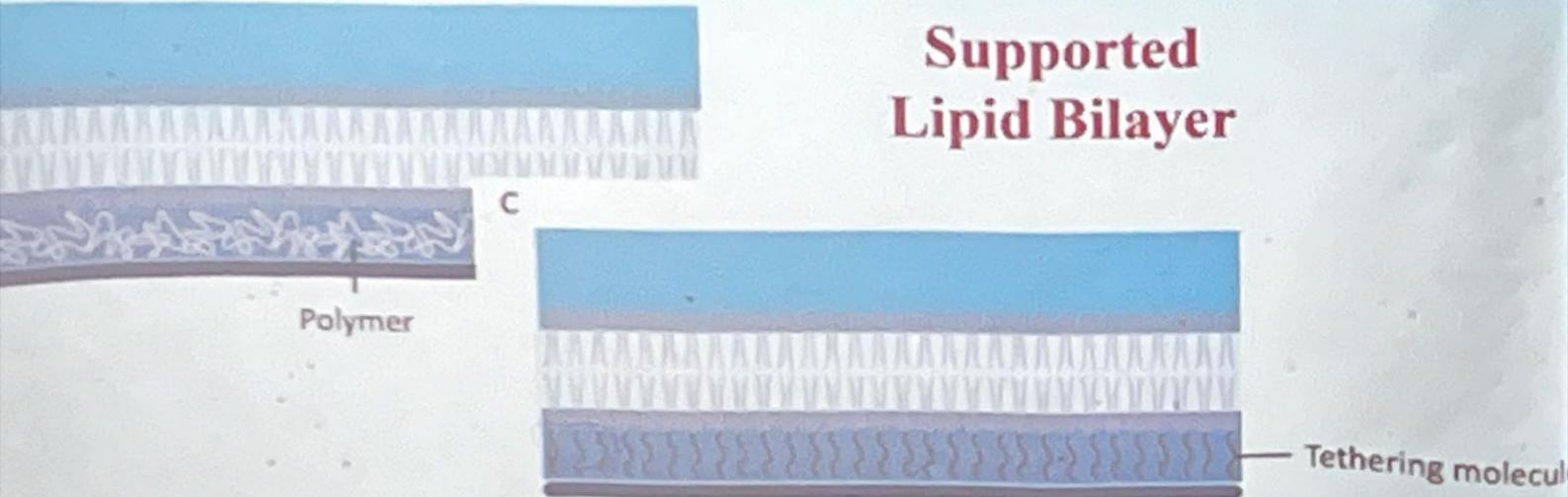
## Supported Lipid Bilayer(SLB)

**Supported lipid bilayers** (SLB) are biomimetic models where a bilayer is supported onto a hydrophilic surface (Fig. 3A)



**Supported lipid bilayer (SLB) coatings** are versatile, cell-membrane-mimicking biointerfaces that are useful for fundamental biophysical studies and for various applications such as biosensors, diagnostics, and antifouling surfaces.

## Supported Lipid Bilayer



Still, the use of SLB exhibits some drawbacks due to the proximity between the substrate and the lipid bilayer, which hampers the free diffusion of ions and accommodation of transmembrane proteins [17]. To overcome these different approaches for substrate-membrane stabilization have been proposed in the past, creating new variations of SLB such as polymer-supported bilayers (Fig. 3B) and tethered bilayers (Fig. 3C).

## Methods to form Supported Lipid Bilayer

# LANGMUIR

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Invited Feature Article

### Supported Lipid Bilayer Formation: Beyond Vesicle Fusion

Joshua A. Jackman and Nam-Joon Cho\*

# Methods to form Supported Lipid Bilayer

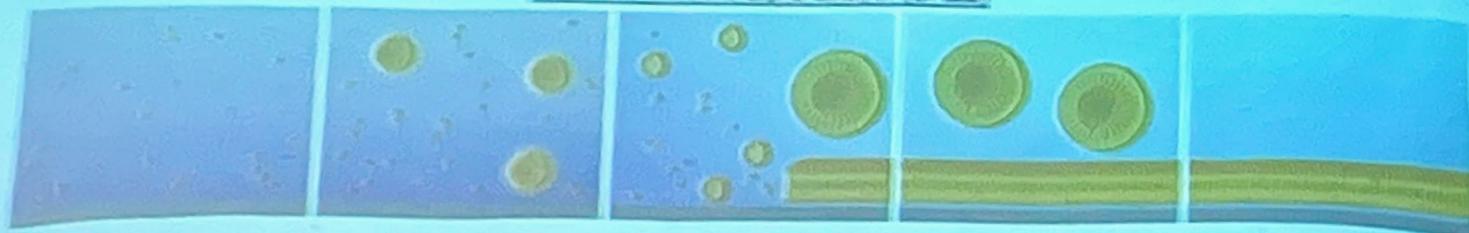


Currently, the vesicle fusion method is the most widely used one to fabricate SLBs and involves the adsorption and spontaneous rupture of lipid vesicles on a target surface.<sup>25–28</sup>

If there are attractive interactions between a contacting vesicle and a solid surface (e.g., van der Waals and electrostatic forces), then the vesicle will adsorb onto the surface.<sup>29,30</sup> Depending on the vesicle–substrate interaction strength, an adsorbed vesicle can become deformed and will either remain intact or eventually rupture due to a combination of vesicle–substrate and/or vesicle–vesicle interactions.<sup>31–33</sup>

## Methods to form Supported Lipid Bilayer

Solvent-Assisted Lipid Bilayer (SALB)



(Middle column) The SALB method is based on depositing long-chain phospholipids in a water-miscible organic solvent followed by a solvent-exchange step with aqueous solution. Initially, the phospholipid molecules in organic solvent self-assemble into inverted micelles and/or remain in the monomeric state and attach to the surface in an equilibrium with bulk lipids. During the solvent-exchange step, the bulk liquid transitions from predominately organic solvent to fully aqueous solution. Consequently, phospholipid molecules within the system begin to form lamellar-phase structures, leading to SLB formation on the surface.

# Biophysical Methods

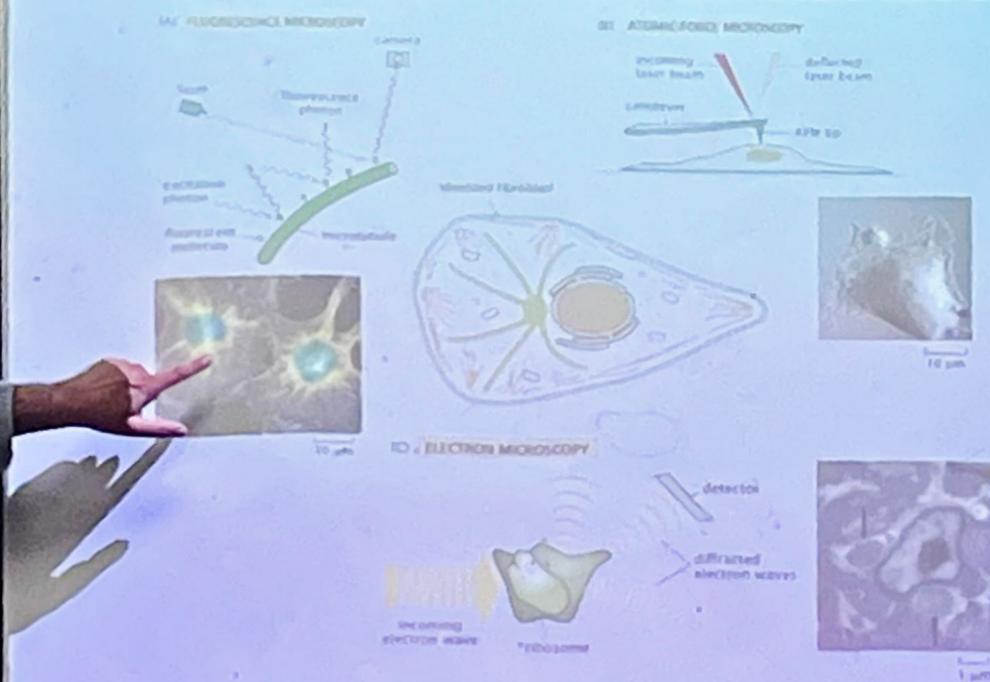


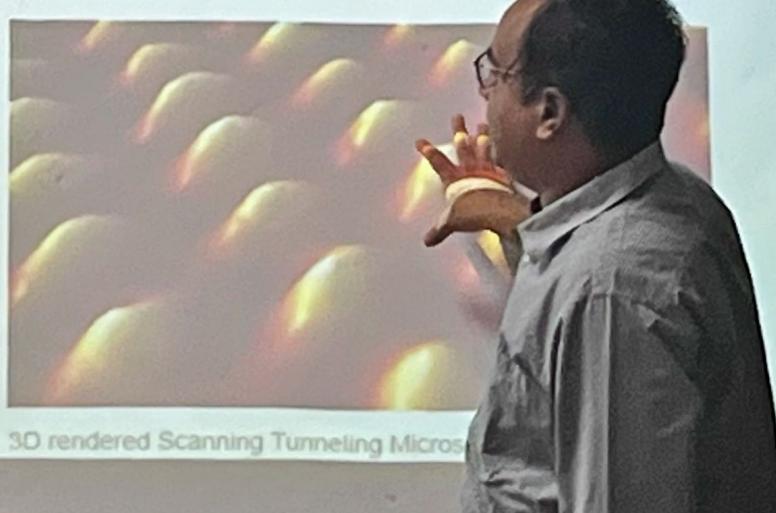
Figure 4.17 Experimental techniques that have revealed the structure of both cells and their organelles. (A) Fluorescence microscopy schematic and the associated image of a fibroblast with labeled microtubules (yellow) and DNA (green). (B) Atomic force microscopy schematic and image of the surface topography of a fibroblast. (C) Electron microscopy schematic and image of cross-section through a ribosome in an animal tissue. Arrows indicate bundles of collagen fibers. (A, courtesy of Tørrisen Wermann; B, adapted from M. Rademacher, *Meth. Cell Biol.* 83:347, 2007; C, adapted from D. E. Turk and A. L. Trinick, *J. Cell Biol.* 103:231, 1986.)

# Introduction to Scanning Tunnel Microscope

The development of the family of scanning probe microscopes started with the original invention of the STM in 1981. Gerd Binnig and Heinrich Rohrer developed the first working STM while working at IBM Zurich Research Laboratories in Switzerland. This instrument would later win Binnig and Rohrer the Nobel prize in physics in 1986.

## How an STM Works

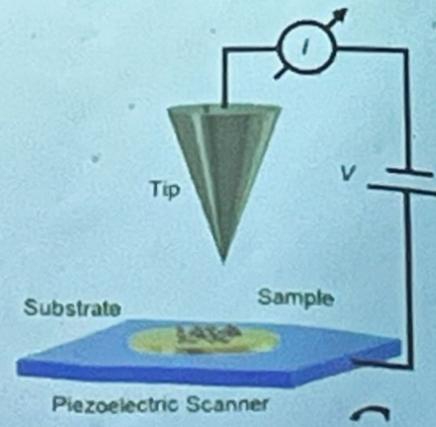
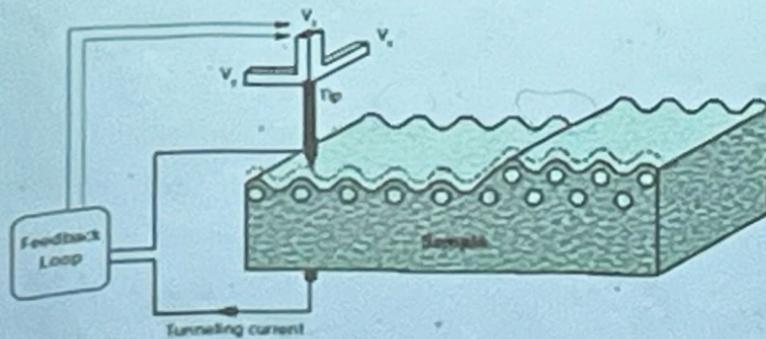
The scanning tunneling microscope (STM) works by scanning a very sharp metal wire tip over a surface. By bringing the tip very close to the surface, and by applying an electrical voltage to the tip or sample, we can image the surface at an extremely small scale – down to resolving individual atoms.



3D rendered Scanning Tunneling Microscopy

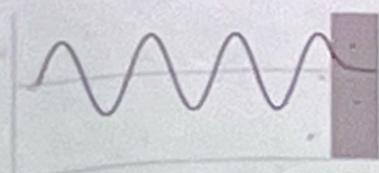
## Principle of Scanning Tunnel Microscope

The STM is based on several principles. One is the quantum mechanical effect of tunneling. It is this effect that allows us to "see" the surface. Another principle is the piezoelectric effect. It is this effect that allows us to precisely scan the tip with angstrom-level control. Lastly, a feedback loop is required, which monitors the tunneling current and coordinates the current and the positioning of the tip. This is shown schematically below where the tunneling is from tip to surface with the tip rastering with piezoelectric positioning, with the feedback loop maintaining a current setpoint to generate a 3D image of the electronic topography.

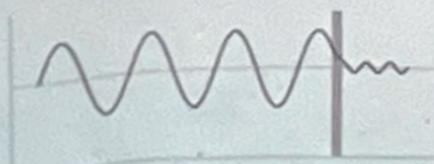


## Tunnelling in STM

Tunneling is a quantum mechanical effect. A tunneling current occurs when electrons move through a barrier that they classically shouldn't be able to move through. In classical terms, if you don't have enough energy to move "over" a barrier, you won't. However, in the quantum mechanical world, electrons have wavelike properties. These waves don't end abruptly at a wall or barrier, but taper off quickly. If the barrier is thin enough, the probability function may extend into the next region, through the barrier! Because of the small probability of an electron being on the other side of the barrier, given enough electrons, some will indeed move through and appear on the other side. When an electron moves through the barrier in this fashion, it is called tunneling.



The top image shows us that when an electron (the wave) hits a barrier, the wave doesn't abruptly end, but tapers off very quickly - exponentially. For a thick barrier, the wave doesn't get past.



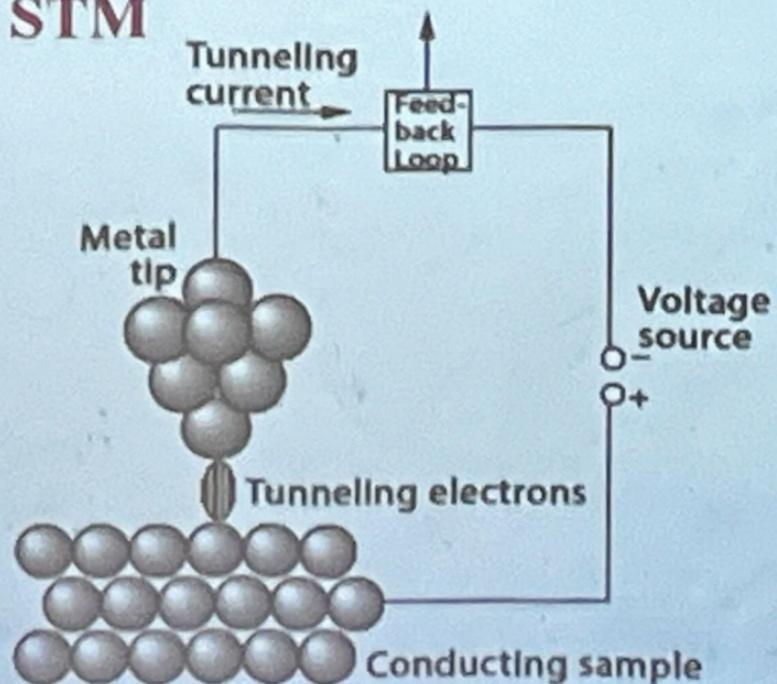
The bottom image shows the scenario if the barrier is quite thin (about a nanometer). Part of the wave does get through and therefore some electrons may appear on the other side of the barrier.

Because of the sharp decay of the probability function through the barrier, the number of electrons that will actually tunnel is very dependent upon the thickness of the barrier. The current through the barrier drops off exponentially with the barrier thickness.

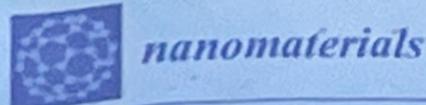
Schematic of electron wavefunction.

To extend this description to the STM: The starting point of the electron is either the tip or sample, depending on the setup of the instrument. The barrier is the gap (air, vacuum, liquid), and the second region is the other side, i.e. tip or sample, depending on the experimental setup. By monitoring the current through the gap, we have very good control of the tip-sample distance.

## Tunnelling in STM



# STM Application of Protein deposited on Au



Review  
**Scanning Tunneling Microscopy of Biological Structures:  
An Elusive Goal for Many Years**

Andrés Rodríguez-Galván <sup>1,\*</sup> and Flavio F. Contreras-Torres <sup>2</sup>

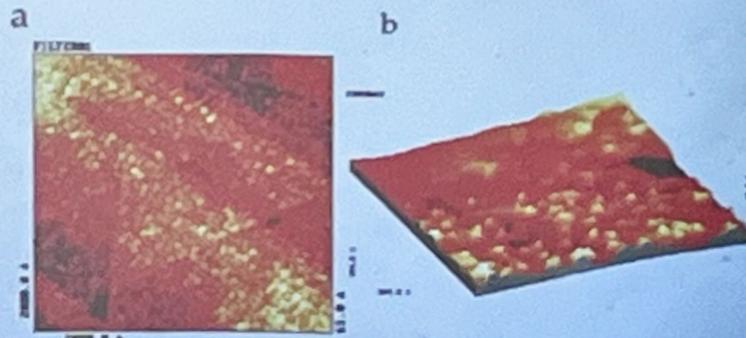


Figure 4. STM images of protein azurin deposited on Au(111). The spots represent individual azurin molecules (a). The 3D image shows the proteins exhibited a high central contrast that was interpreted as an area of high tunneling conductivity (b). STM analysis was performed in liquid. Reprinted with permission from Ref. [77]. Copyright (1999), National Academy of Sciences.

# STM Image of Lipid Bilayer



Nat. Acad. Sci. USA  
pp. 969-972, February 1987

## Images of a lipid bilayer at molecular resolution by scanning tunneling microscopy

(biological membranes/cadmium isocyanate/graphite)

D. P. E. SMITH\*, A. BRYANT\*, C. F. QUATE\*†, J. P. RABE‡§, CH. GERBER‡¶, AND J. D. SWALEN‡

Department of Applied Physics, Stanford University, Stanford, CA 94305; †IBM Almaden Research Center, San Jose, CA 95120; and ‡IBM Research Laboratory, Zürich, Switzerland

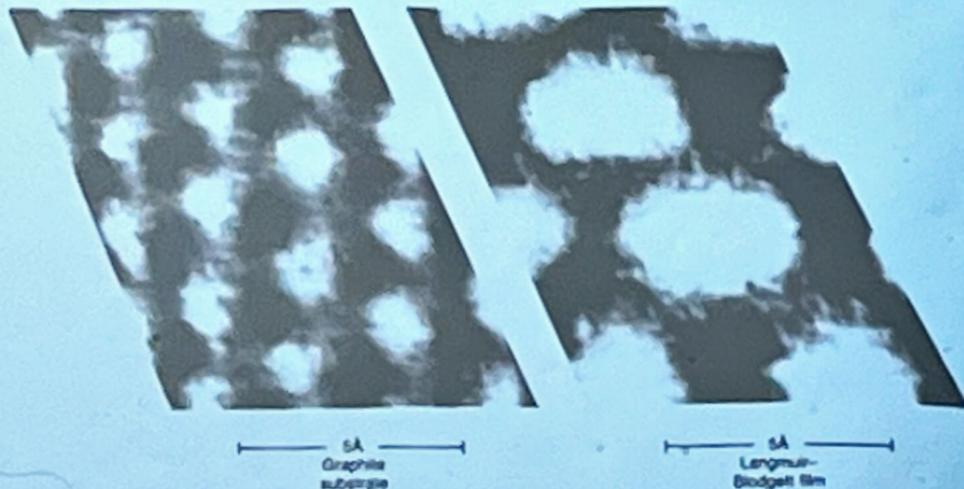


FIG. 4. Images of a cleaved graphite sample obtained with a STM. Left, pristine graphite. Right, graphite coated with CdACh bilayer. Brightness indicates higher tunneling current and therefore represents higher topography, or increased conductivity.

## Need for Atomic Force Microscopy (AFM)

Topography imaging alone does not always provide the answers that researchers need and the surface topology often does not correlate to the material properties. For these reasons, advanced imaging modes have been developed to provide quantitative data on a variety of surfaces. Now many material properties can be determined with AFM techniques, including friction, electrical forces, capacitance, magnetic forces, conductivity, viscoelasticity, surface potential, and resistance.

## Working of Atomic Force Microscopy (AFM)

Analogous to how an Scanning Tunneling Microscope works, a sharp tip is raster-scanned over a surface using a feedback loop to adjust parameters needed to image a surface. Unlike Scanning Tunneling Microscopes, the Atomic Force Microscope does not need a conducting sample. Instead of using the quantum mechanical effect of tunneling, atomic forces are used to map the tip-sample interaction.

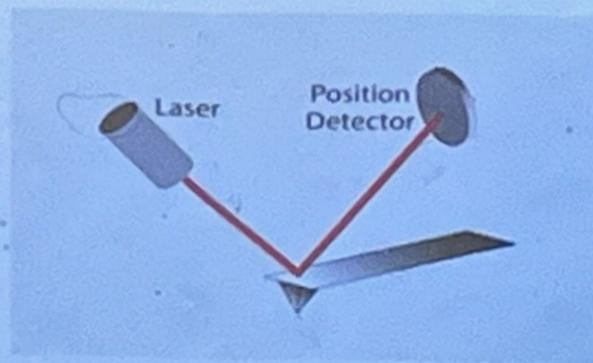
Often referred to as scanning probe microscopy (SPM), there are Atomic Force Microscopy techniques for almost any measurable force interaction – van der Waals, electrical, magnetic, thermal. For some of the more specialized techniques, modified tips and software adjustments are needed.

In addition to Angstrom-level positioning and feedback loop control, there are 2 components typically included in Atomic Force Microscopy: Deflection and Force Measurement.

# AFM Probe Deflection

## AFM Probe Deflection

Traditionally, most Atomic Force Microscopes use a laser beam deflection system where a laser is reflected from the back of the reflective AFM lever and onto a position-sensitive detector. AFM tips and cantilevers are typically micro-fabricated from Si or  $\text{Si}_3\text{N}_4$ . Typical tip radius is from a few to 10s of nm.



Laser beam deflection for atomic force microscopes

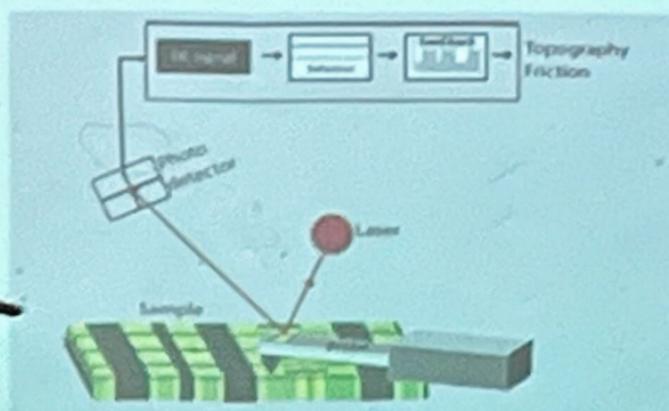


$\text{Si}_3\text{N}_4$  cantilever used in atomic force microscopes

# Feedback loop for AFM

## Feedback Loop for Atomic Force Microscopy

Atomic Force Microscopy has a feedback loop using the laser deflection to control the force and tip position. As shown, a laser is reflected from the back of a cantilever that includes the AFM tip. As the tip interacts with the surface, the laser position on the photodetector is used in the feedback loop to track the surface for imaging and measuring.



Schematic for contact mode Atomic Force Microscopy

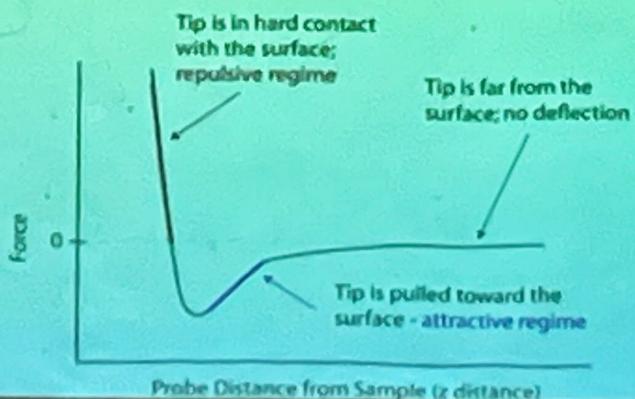
## Measuring Forces in AFM

because the Atomic Force Microscope relies on the forces between the tip and sample, these forces impact AFM imaging. The force is not measured directly, but calculated by measuring the deflection of the lever, knowing the stiffness of the cantilever.

Hooke's law gives:

$$F = -kz$$

where  $F$  is the force,  $k$  is the stiffness of the lever, and  $z$  is the distance the lever is bent.



## AFM Images

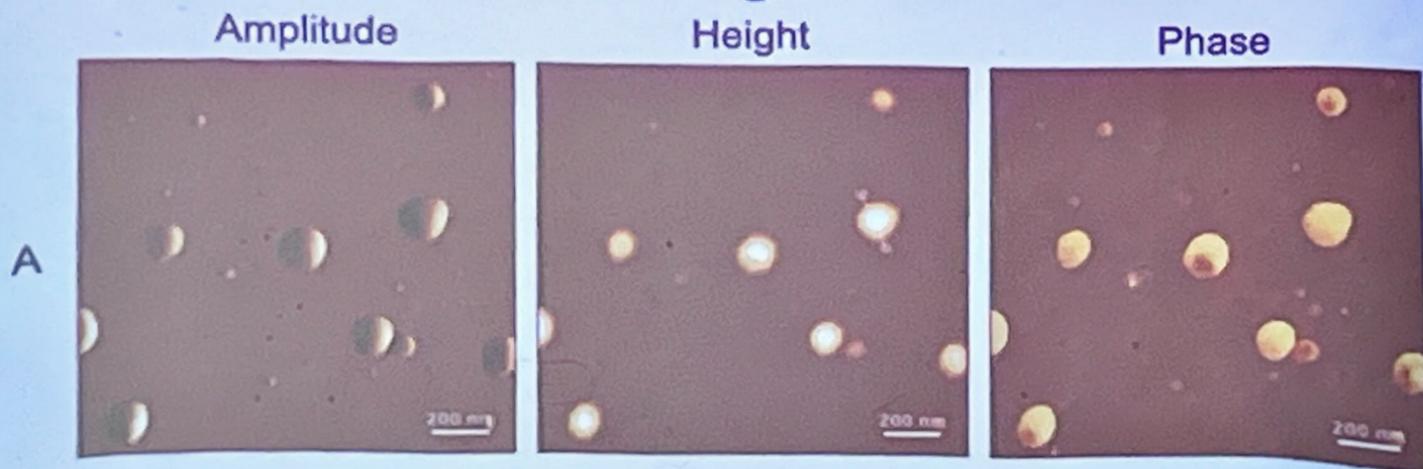


Fig. 2 Visualization of (a) GDNT/egPC/DOTAP liposomes with a molar ratio of 10/80/10 mol% on a  $1.5 \mu\text{m} \times 1.5 \mu\text{m}$  consisting of amplitude, height, and phase image:

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Gerard G.M. D'Souza

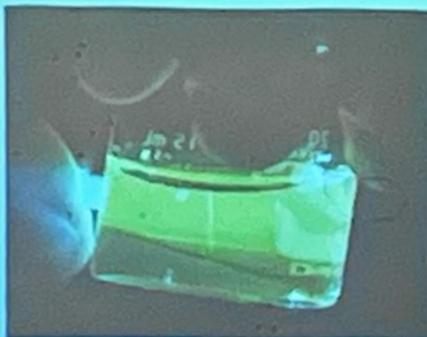
Hongwei Zhang *Editors*

Springer Protocols

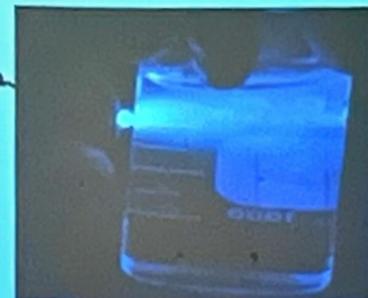
Molecules that are capable of fluorescing are called *fluorescent molecules*, *fluorescent dyes*, or *fluorochromes*.

## TAKE A STEP BACK TO FLORESCENCE MICROSCOPY

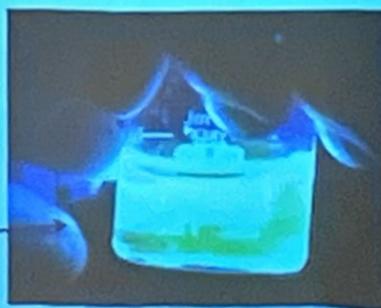
UV light in Tonic water emits blue light  
Eye is sensitive to blue and not UV



Blue light in Floursine Dye we get yellow fluorescence



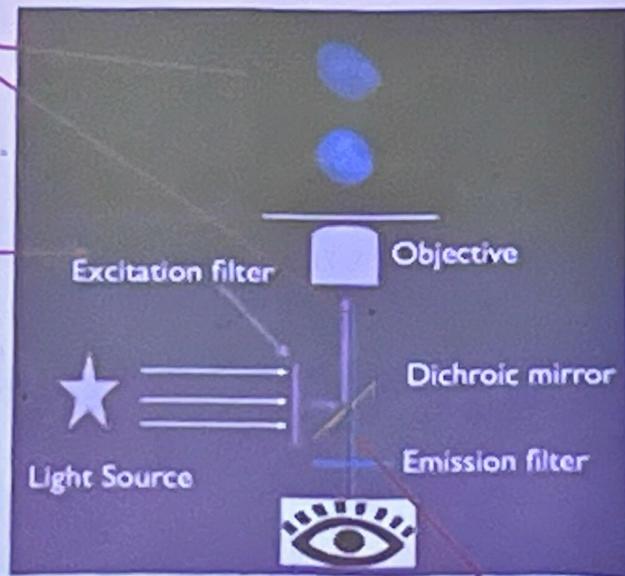
Shining green light in Rodemin dye you get cyan orangish fluorescence



# Fluorescence Microscope

Sample is excited  
Filters are very important  
and make it different from  
a normal microscope

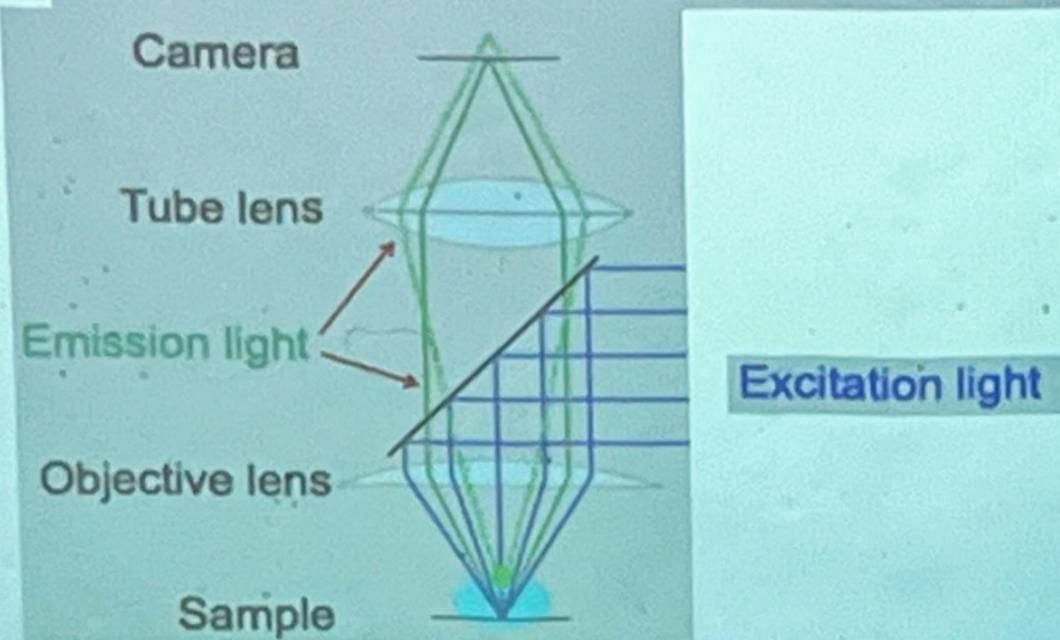
Filters out the source for  
wavelength needed



Fluorescence light travels back to eye  
through Emission filter

# NEED FOR CONFOCAL MICROSCOPY

## Fluorescence Illumination of a single point

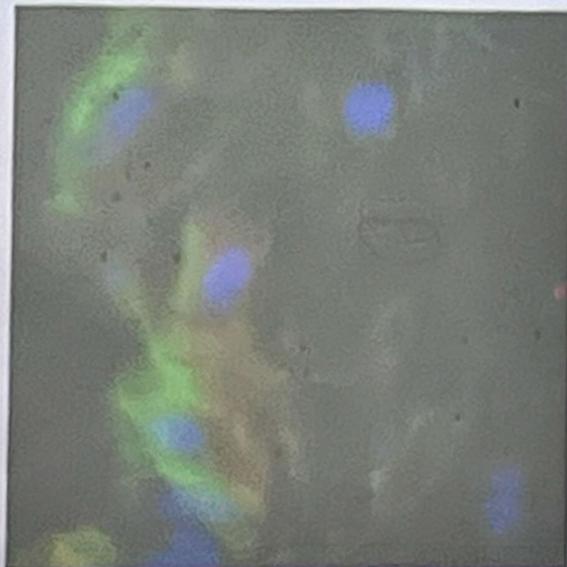


Problem – fluorescence is emitted along entire illuminated cone, not just at focus

## NEED FOR CONFOCAL MICROSCOPY

A problem: out of focus light

Conventional microscopes see both in-focus and out of  
focus light



Resulting Image for a  
mouse tissue

See lot of  
blurry/fuzzy out of  
focus light which  
reduces our ability to  
see specifics

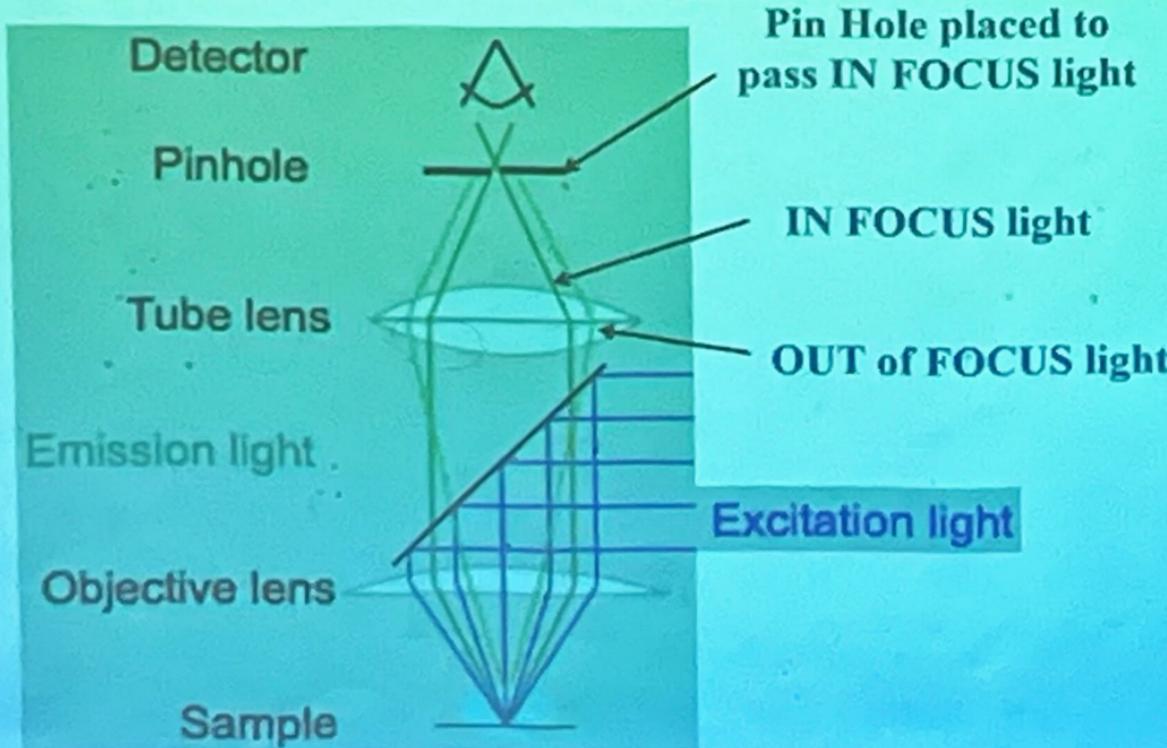
## NEED FOR CONFOCAL MICROSCOPY

Thick fluorescent specimens such as rounded cells and tissue sections can pose problems for conventional wide-field fluorescence optics, because bright fluorescent signals from objects outside the focal plane increase the background and give low-contrast images. Confocal and deconvolution microscopy solve the problem by rejecting signals from nearby sources above and below the focal plane.

Is a fluorescent signal distributed on a membrane surface or contained throughout the cytoplasm as a soluble factor? Within the limits of resolution of the light microscope, are different fluorescence signals colocalized on the same structure? What is the three-dimensional structure of the specimen?

# The Confocal Microscope

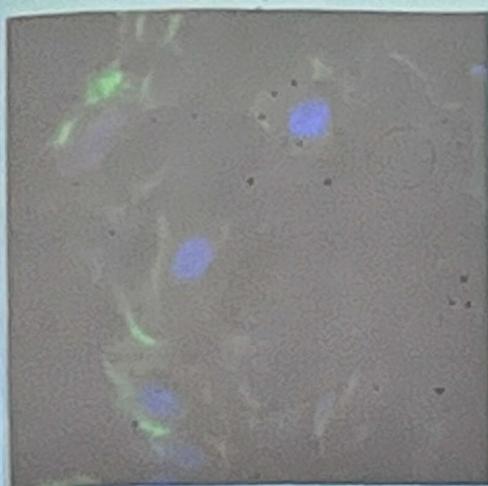
Use a pinhole to block out-of-focus light



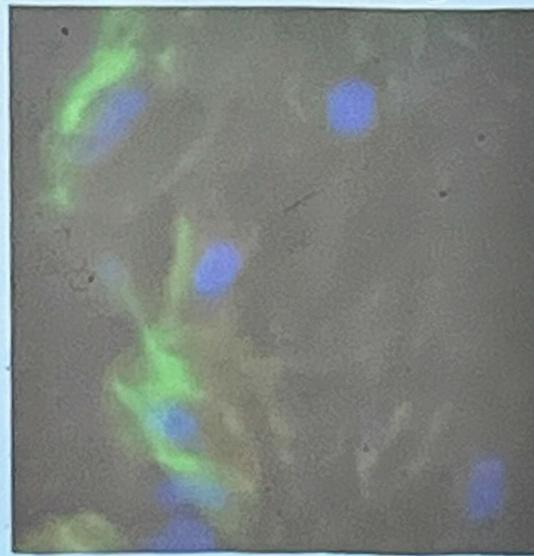
### A problem: out of focus light

A confocal microscope blocks the out of focus light

Confocal



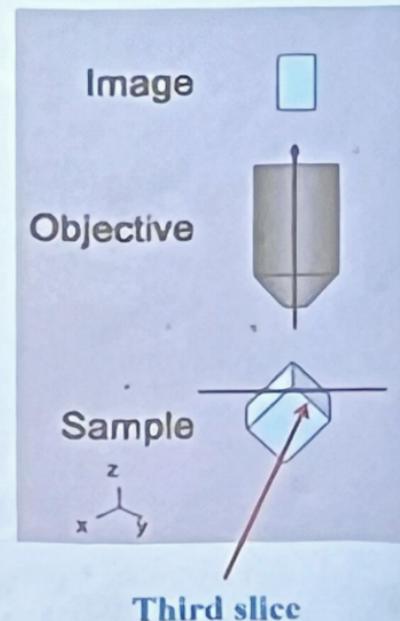
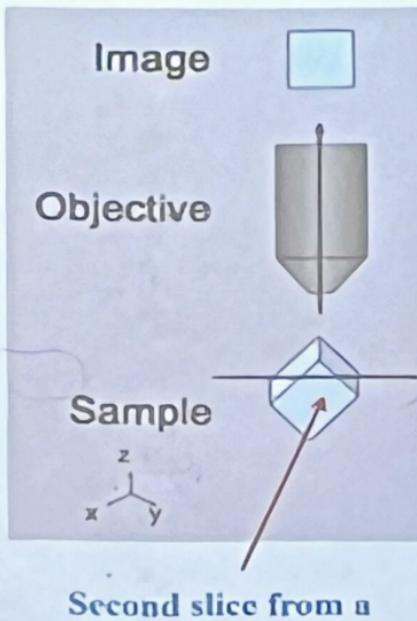
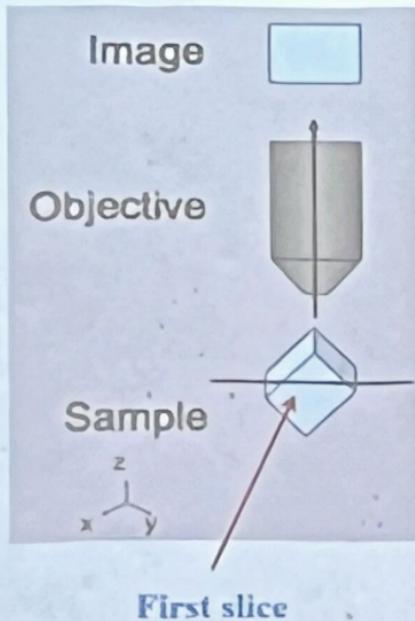
Crisper and sharper  
Image with specifics



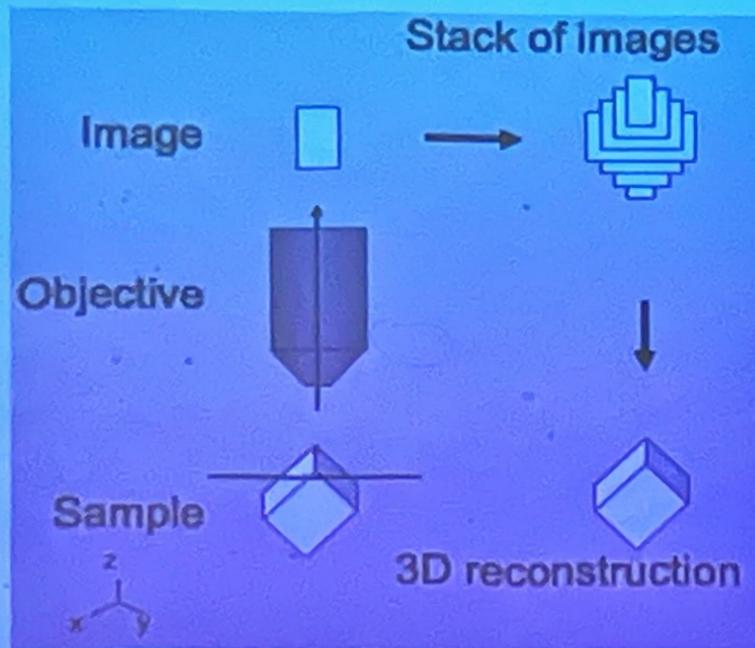
Blurry/fuzzy image

**HOW IS 3D RECONSTRUCTION DONE**

# How do we acquire optical sections?



## Optical sectioning and 3D reconstruction



# Determination of the Subcellular Distribution of Fluorescently Labeled Liposomes Using Confocal Microscopy

Melani A. Solomon

## Abstract

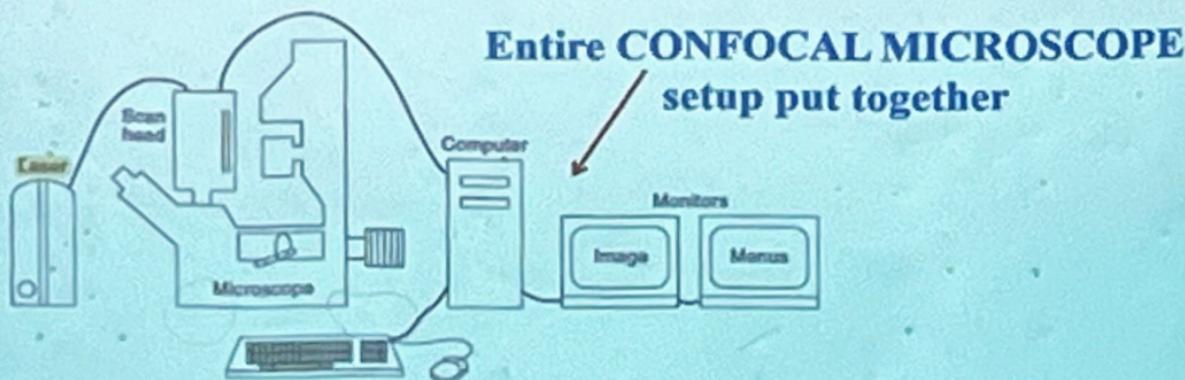
It is being increasingly recognized that [dearomatized] fluorescently labeled liposomes can be used to study cellular processes that can be studied by confocal imaging to determine their subcellular locations within the cell. Hence, the development of such technology requires a means of measuring subcellular distribution by utilizing imaging techniques that can visualize and quantitate the extent of this subcellular localization. [The apparent increase of resolution along the Z axis offered by confocal microscopy makes this technique suitable for such studies.] In this chapter, we will describe the application of confocal laser scanning microscopy (CLSM) to determine the subcellular distribution of fluorescently labeled mitochondriotropic liposomes.

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Hongwei Zhang *Editors*

Springer Protocols

Confocal microscopes are *integrated electronic microscope systems*, in which the light microscope is part of an electronic imaging system containing an electronic detector or camera, a computer and computer software, and electronic devices for image display, printing, and storage (Fig. 12-2).



Basic components of a confocal laser scanning microscope (CLSM). A laser provides a beam of light that is scanned across the specimen by the scan head under the control of a computer. The scan head also directs fluorescence signals from the specimen to its pinhole and photomultiplier tube (PMT). The computer holds the image in an image memory board until it is processed and displayed on a computer monitor. A second monitor displays software menus for image acquisition and processing. It is important to realize that a confocal image is never generated in the microscope. Instead, the image is built up electronically, point by point and over time, from fluorescent signals received by the PMT accumulated in the image memory board of the computer.

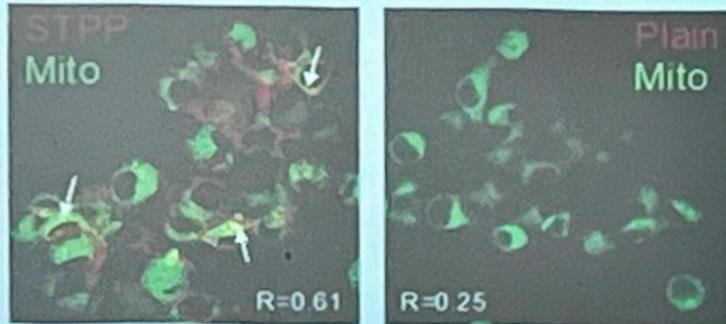
HDMI 2 / MHL



1080p 60Hz

Thanks for coming..Questions

**Prof Swaminathan will handle next three lectures**



**Fig. 1** Representative composite micrographs of BT-20 cells treated with rhodamine-labeled plain liposomes (Plain) or rhodamine-labeled STPP liposomes (STPP), mitochondria stained green with MitoFluor Green (Mito). The arrows indicate the areas of colocalization, while the  $R$  values indicate the observed Pearson's correlation coefficient