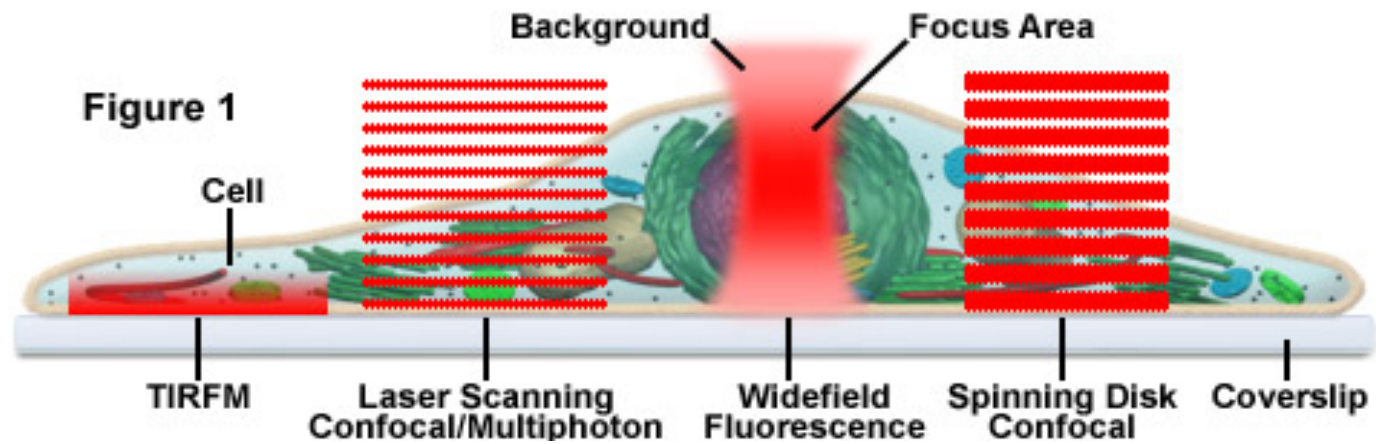


Types of Imaging

Fluorescence Imaging Modes in Live-Cell Microscopy



<http://zeiss-campus.magnet.fsu.edu/articles/livecellimaging/techniques.html>

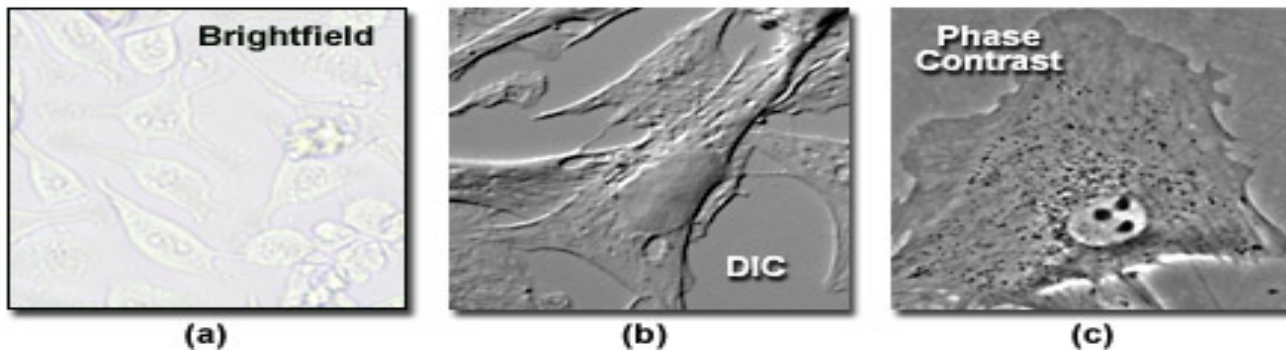
Frigault M M et al. J Cell Sci
2009;122:753-767



©2009 by The Company of Biologists Ltd

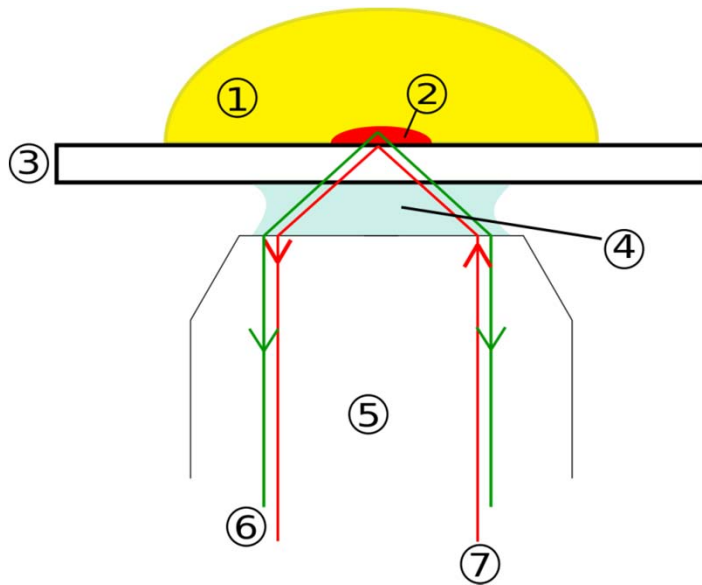


Transmitted Light Techniques in Live-Cell Imaging



<http://zeiss-campus.magnet.fsu.edu/articles/livecellimaging/techniques.html>

TIRF (Total Internal Reflection Microscopy)

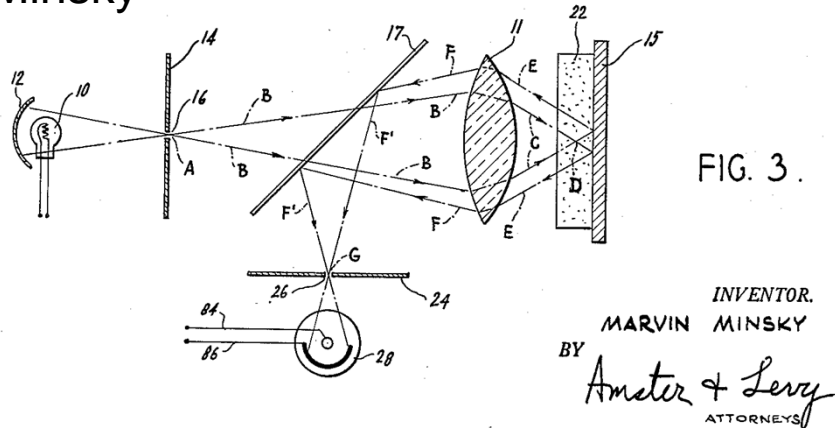


<http://en.wikipedia.org/wiki/File:Tirfm.svg>

- Works based on the generation of an evanescent wave of excitation that is limited to ~ 100 nm above the cover slip
 - Good for monitoring events on the plasma membrane
1. Specimen
 2. Evanescent wave range
 3. Cover slip
 4. Immersion oil
 5. Objective
 6. Emission beam (signal)
 7. Excitation beam

Laser Scanning Confocal

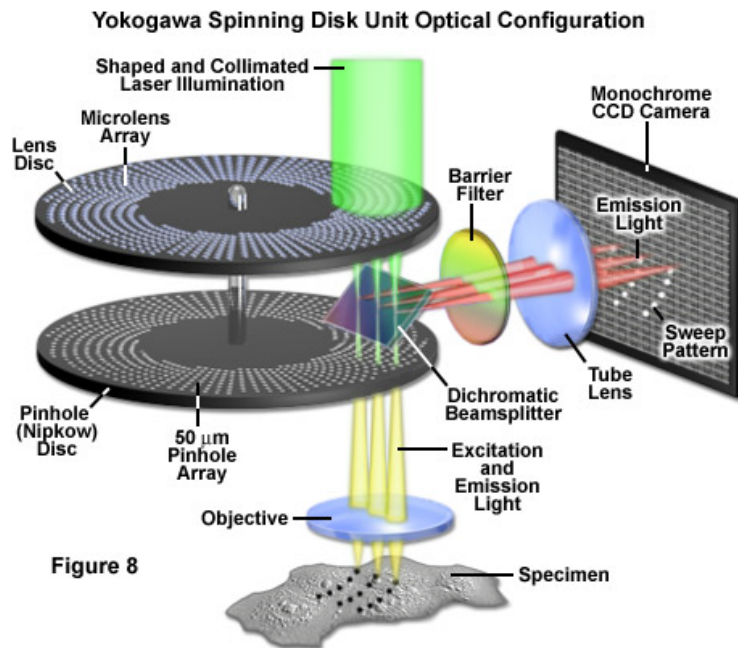
1957 patent by
Minsky



http://upload.wikimedia.org/wikipedia/commons/4/4b/Minsky_Confocal_Reflection_Microscope.png

- Illumination of only one spot at a time
 - Therefore to get a whole image one has to “raster” the illumination
- Only emission light from a selected plane is allowed to pass through to the detector—pinhole
- Leads to large losses of emission light so applicability for live-cell imaging can be limited
 - Photobleaching, phototoxicity, need very bright samples

Spinning Disc Confocal



- An array of many pinholes arranged in spiral shapes on a disc that spins
 - Allows multiple points of illumination that quickly scan over the specimen
- Quicker and more light efficient than laser scanning
 - Therefore generally regarded as better for live cell imaging
- However the tradeoff is generally reduced optical sectioning due to pinhole bleedthrough from out of plane light

<http://zeiss-campus.magnet.fsu.edu/articles/spinningdisk/introduction.html>

Live-Cell Imaging: Acquisition

- Ideal live-cell image acquisition system
 - sensitive enough to acquire superior images from weakly fluorescent specimens
 - Photobleaching, phototoxicity
 - fast enough to record all dynamic processes
 - sufficient resolution to capture fine specimen detail
 - Subcellular process or a reporter that uses the whole cell volume?
 - dynamic range capable of measuring relevant differences in intensity within and across cells
 - Keeps focal plane (autofocus) for multiple positions (motorized stage)
 - Looking at multiple cells over a time course
- Cameras and photomultipliers
 - Large body of knowledge on these, will not cover it in this course