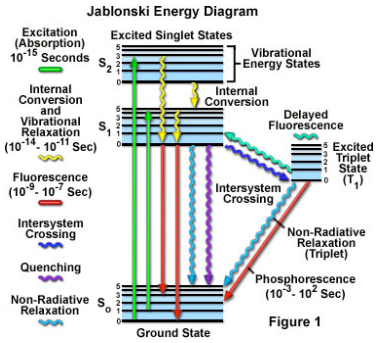
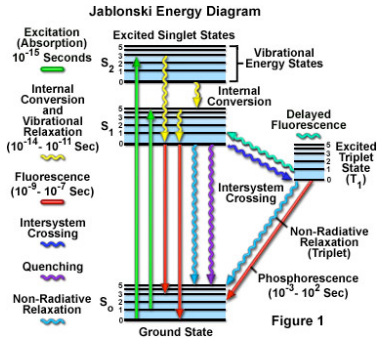


Transition to the Dark Side

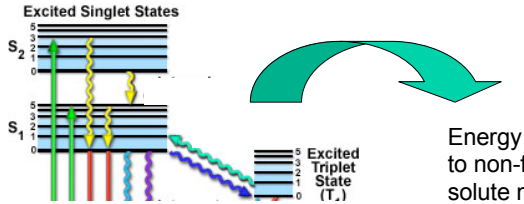


Triplet Blinking
- stable triplet state
- $S_1 \rightarrow T_1$ due to solvent
  - -halogens
  - -transition metals

Quenching

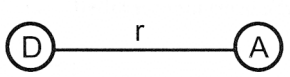
Collision with solutes

Resonance energy transfer

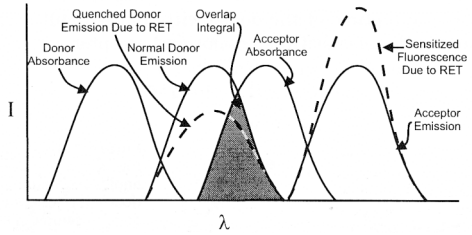


Energy transferred to non-fluorescent solute molecule

Fluorescence Resonance Energy Transfer



r < 50 A



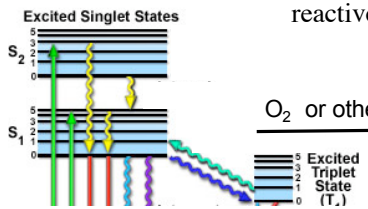
Death of a Fluor

Photobleaching

T_1 is highly reactive

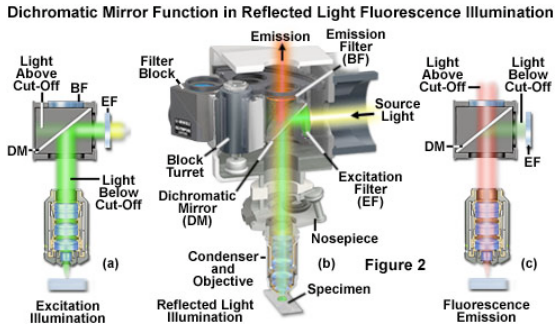
O_2 likes T_1 electrons

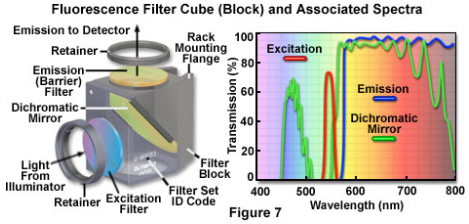
Oxygen radicals are highly reactive & cytotoxic

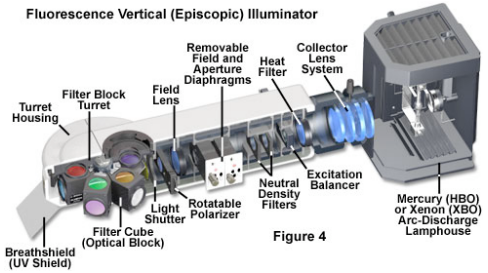


O_2 or other solute → New compound (disrupted conjugation)

Reflected Light (Epifluorescence) Microscopy







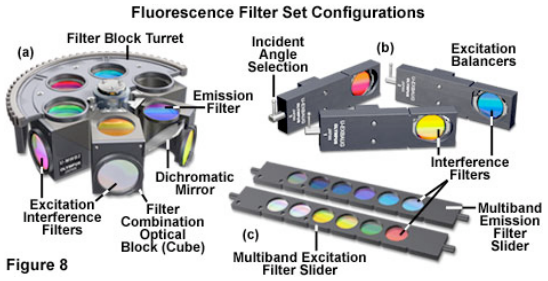


Figure 8

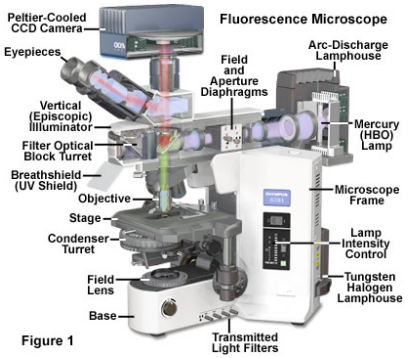


Figure 1

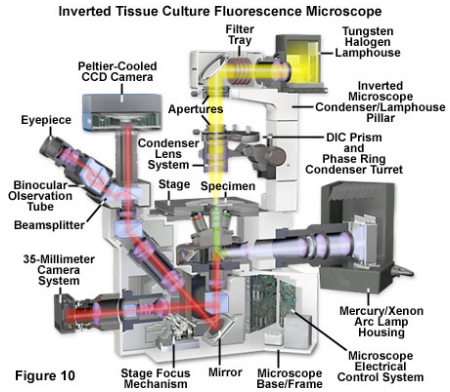


Figure 10

Transmission Fluorescence Microscopy

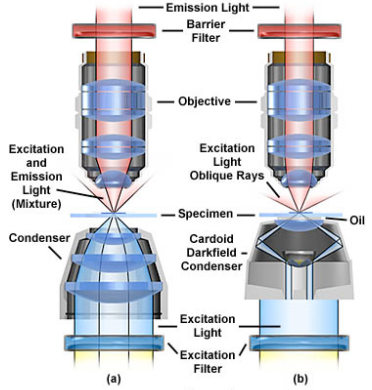
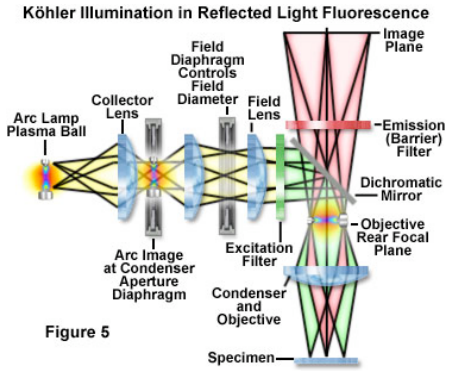
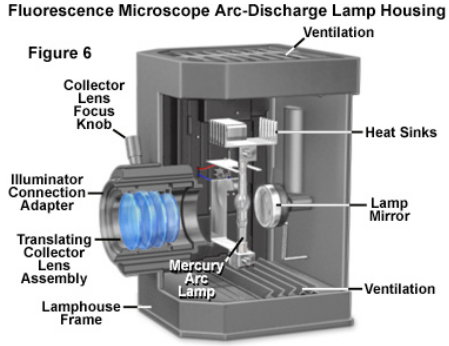
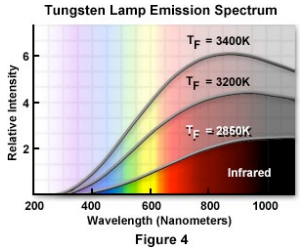
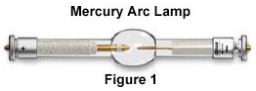


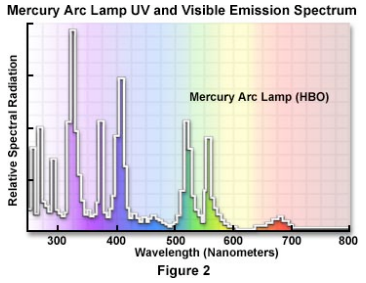
Figure 1

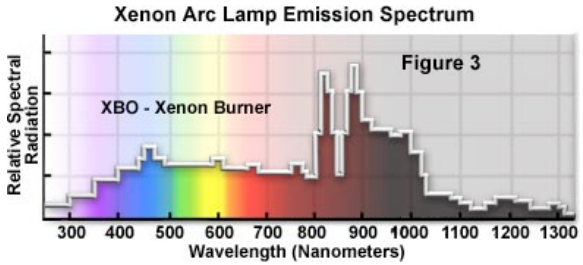

Köhler Illumination in Reflected Light Fluorescence
Image Plane
Field Diaphragm Controls Field Diameter
Collector Lens
Field Lens
Arc Lamp Plasma Ball
Emission (Barrier) Filter
Dichromatic Mirror
Objective Rear Focal Plane
Arc Image at Condenser Aperture Diaphragm
Excitation Filter
Condenser and Objective
Specimen
Figure 5

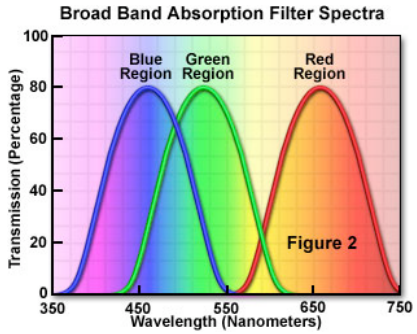

Fluorescence Microscope Arc-Discharge Lamp Housing
Figure 6
Ventilation
Collector Lens Focus Knob
Heat Sinks
Illuminator Connection Adapter
Lamp Mirror
Translating Collector Lens Assembly
Mercury Arc Lamp
Ventilation
Lamphouse Frame

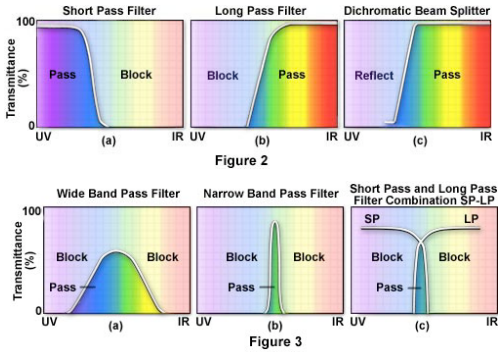

Tungsten Lamp Emission Spectrum
T_F = 3400K
T_F = 3200K
T_F = 2850K
Infrared
Relative Intensity
6
4
2
200
400
600
800
1000
Wavelength (Nanometers)
Figure 4

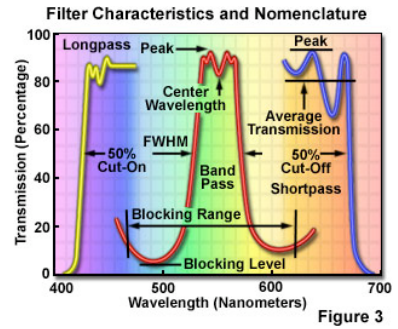

Mercury Arc Lamp
Figure 1


Mercury Arc Lamp UV and Visible Emission Spectrum
Mercury Arc Lamp (HBO)
Relative Spectral Radiation
300
400
500
600
700
800
Wavelength (Nanometers)
Figure 2


Xenon Arc Lamp Emission Spectrum
Figure 3
XBO - Xenon Burner
Relative Spectral Radiation
300 400 500 600 700 800 900 1000 1100 1200 1300
Wavelength (Nanometers)


Broad Band Absorption Filter Spectra
Blue Region
Green Region
Red Region
Figure 2
Transmission (Percentage)
100
80
60
40
20
0
350
450
550
650
750
Wavelength (Nanometers)


Short Pass Filter
Long Pass Filter
Dichromatic Beam Splitter
Transmittance (%)
100
0
Pass
Block
Block
Pass
Reflect
Pass
UV
(a)
IR
UV
(b)
IR
UV
(c)
IR
Figure 2
Wide Band Pass Filter
Narrow Band Pass Filter
Short Pass and Long Pass Filter Combination SP-LP
Transmittance (%)
100
0
Block
Block
Pass
Block
Block
Pass
SP
LP
Block
Block
Pass
UV
(a)
IR
UV
(b)
IR
UV
(c)
IR
Figure 3


Filter Characteristics and Nomenclature
Longpass
Peak
Peak
Center Wavelength
Average Transmission
FWHM
50% Cut-On
Band Pass
50% Cut-Off
Shortpass
Blocking Range
Blocking Level
Transmission (Percentage)
100
80
60
40
20
0
400
500
600
700
Wavelength (Nanometers)
Figure 3

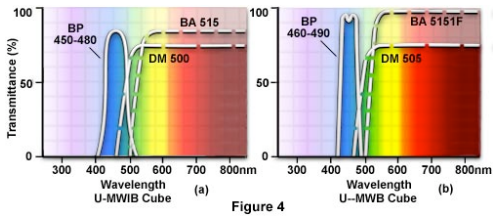


Figure 4

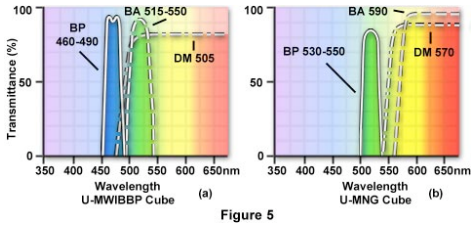


Figure 5

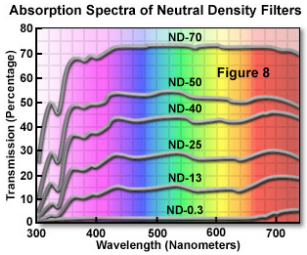


Figure 8

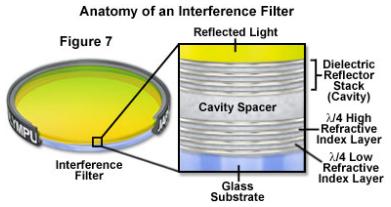


Figure 7

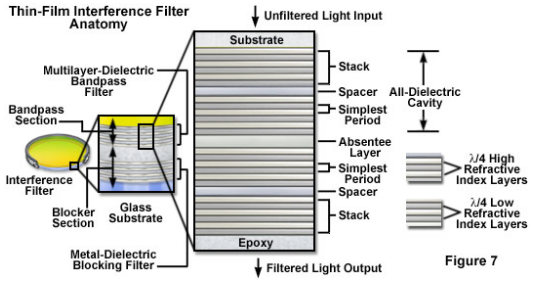
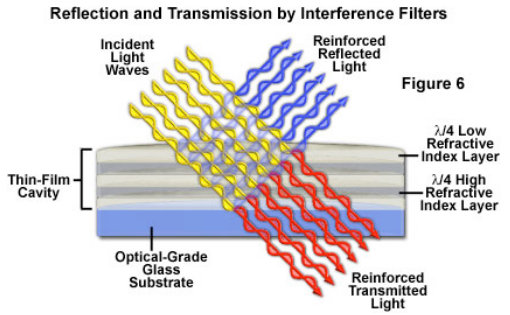


Figure 7



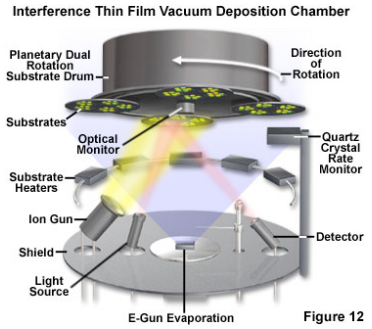
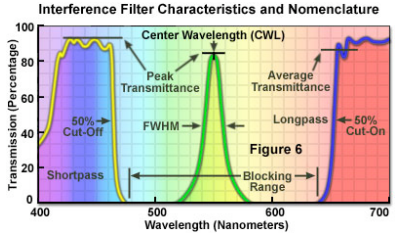
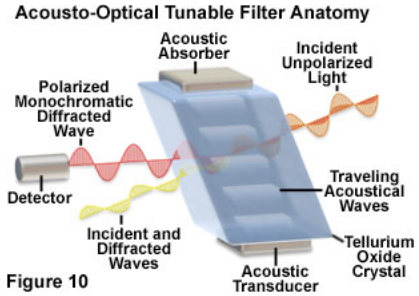
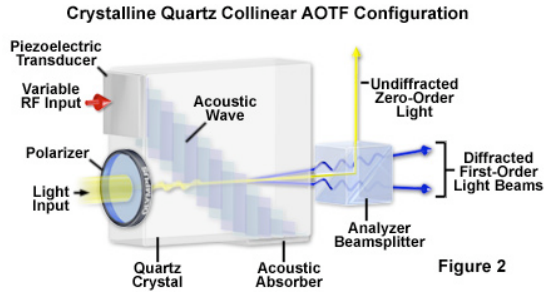


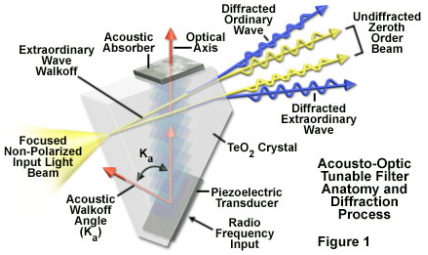
Figure 12

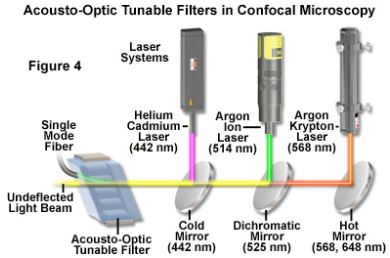


AOTF

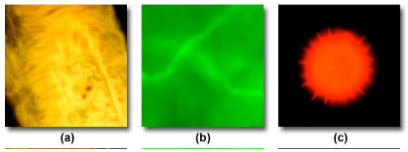

Acousto-Optical Tunable Filter Anatomy
Acoustic Absorber
Incident Unpolarized Light
Polarized Monochromatic Diffracted Wave
Detector
Traveling Acoustical Waves
Incident and Diffracted Waves
Tellurium Oxide Crystal
Acoustic Transducer
Figure 10


Crystalline Quartz Collinear AOTF Configuration
Piezoelectric Transducer
Variable RF Input
Acoustic Wave
Undiffracted Zero-Order Light
Polarizer
Diffracted First-Order Light Beams
Light Input
Analyzer Beamsplitter
Quartz Crystal
Acoustic Absorber
Figure 2

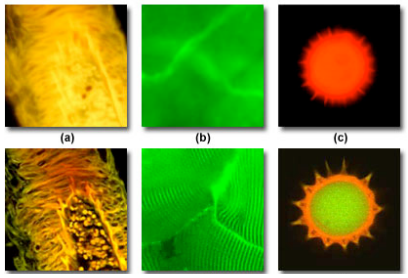

Diffracted Ordinary Wave
Undiffracted Zeroth Order Beam
Extraordinary Wave Walkoff
Acoustic Absorber
Optical Axis
Diffracted Extraordinary Wave
Focused Non-Polarized Input Light Beam
TeO_2 Crystal
K_a
Acoustic Walkoff Angle (K_a)
Piezoelectric Transducer
Radio Frequency Input
Acousto-Optic Tunable Filter Anatomy and Diffraction Process
Figure 1


Acousto-Optic Tunable Filters in Confocal Microscopy
Figure 4
Laser Systems
Single Mode Fiber
Helium Cadmium Laser (442 nm)
Argon Ion Laser (514 nm)
Argon Krypton Laser (568 nm)
Undeflected Light Beam
Acousto-Optic Tunable Filter
Cold Mirror (442 nm)
Dichromatic Mirror (525 nm)
Hot Mirror (568, 648 nm)

Widefield Fluorescence Microscopy

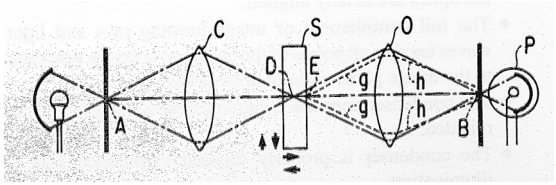


Widefield Fluorescence Microscopy



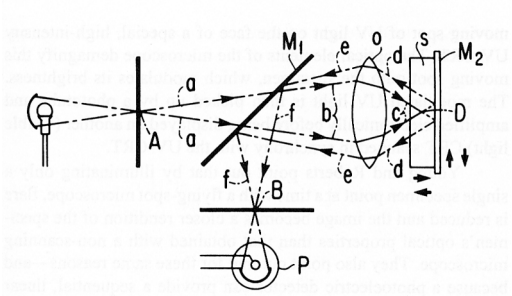
Confocal Fluorescence Microscopy

Marvin Minsky's Confocal Microscope



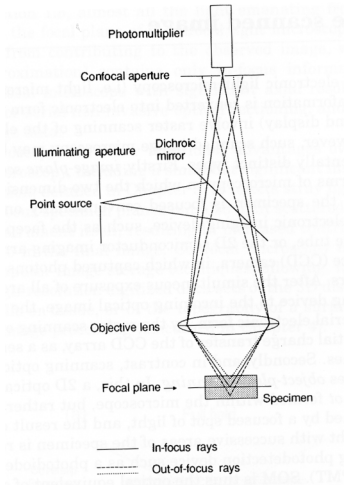
Patent application submitted in 1957 while Minsky was a post-doc at Harvard

Marvin Minsky's Confocal Microscope



Epi-illuminated design eliminated need for a condenser

Also incorporated the concept of stage scanning



Confocal Microscope

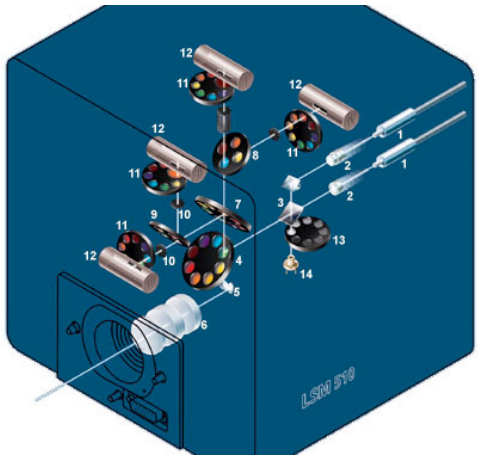
Invented in 50s

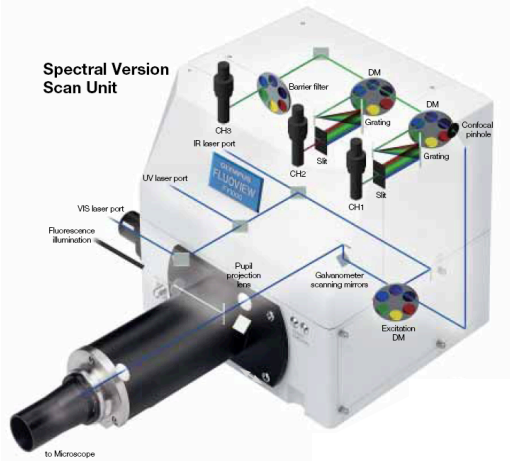
Not practical until availability of low cost lasers and high speed computers

First commercial devices available in the 80s

Uses pinhole to limit collected light to that which is "confocal" (i.e., shares conjugates planes of focus) with the object plane

Zeiss LSM510





Marvin Minsky's Confocal Microscope

Reduced blurring of image by light scattering

Increased effective resolution

Improved signal-to-noise ratio

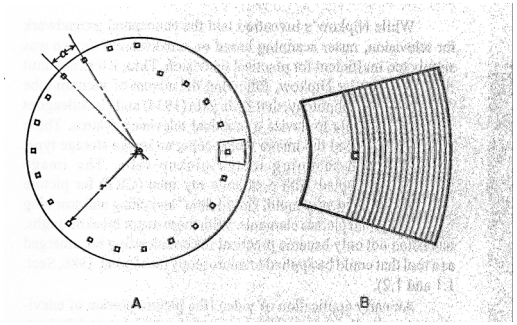
XY-scan possible across a wide area of the specimen

Inclusion of Z-scan possibility

Electronic adjustment of magnification

Possibility of quantitative measurements of optical properties of the specimen

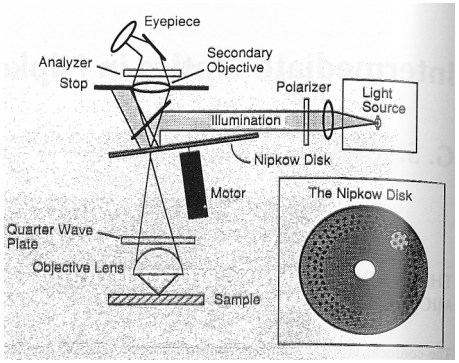
Nipkow Disk



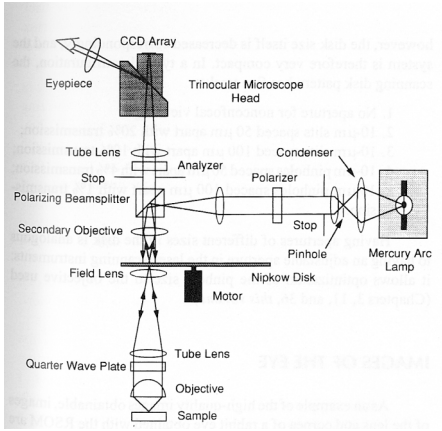
Invented in 1884 by Paul Nipkow,a contemporary of Abbe.

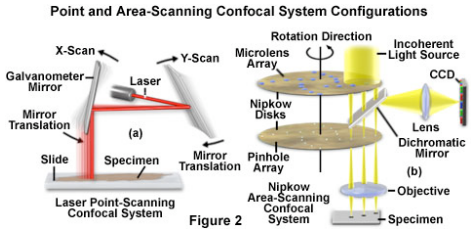
Central element of an early incarnation of the television.

Tandem Scanning Optical Scopes



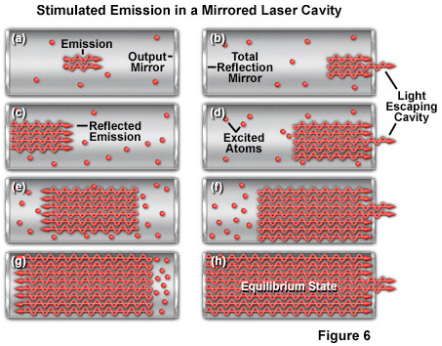
Tandem Scanning Optical Scopes

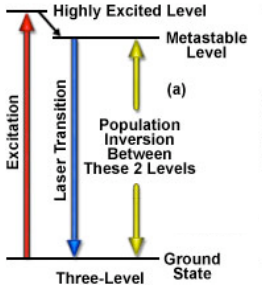




Light Amplification by Stimulated Emission of Radiation

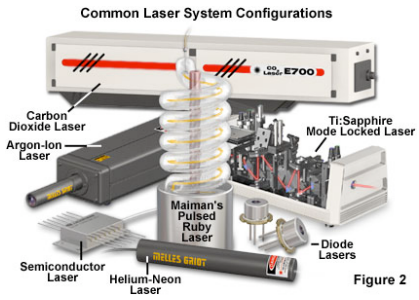
1917	Albert Einstein	stimulated emission possible
1950s	Charles Townes	built first "MASER"
	Gordon Gould	coined term LASER
1960	Theodore Maiman	built first LASER (ruby)

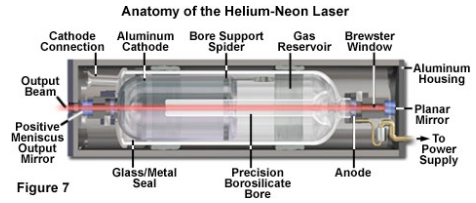




Stable LASER output requires establishment and maintenance of a "population inversion" of excitable electrons.

Must "pump" the medium with either electrical or photonic energy





~ 10,000 - 40,000 hours

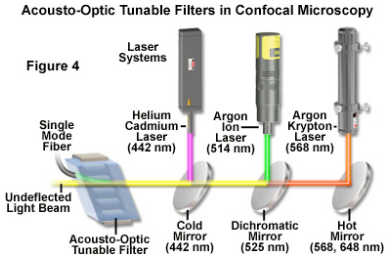
Laser Lines

Table 1.1. Principle emission lines of gas lasers useful for confocal laser scanning microscopy

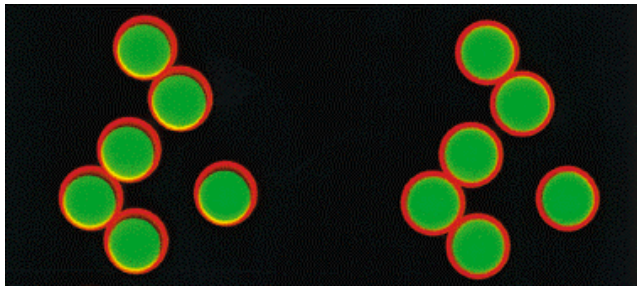
Laser	Wavelength (nm)			
	UV	Blue	Green	Red
Helium–cadmium	325	442		
Helium–cadmium (RGB)		442	534, 538	636
Low power argon ion		488	514	
Water-cooled argon ion	351, 364	457, 488	514, 528	
Argon–krypton mixed gas		488	568	647
Helium–neon (green)			543	
Helium–neon (red)				633

RGB, red, green and blue; UV, ultraviolet.

1152



Alignment



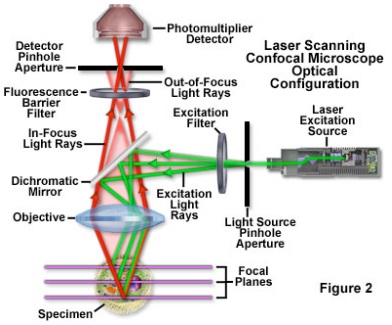


Figure 2

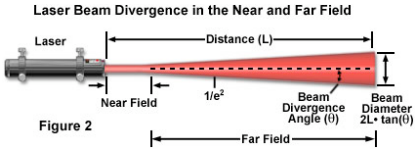


Figure 2

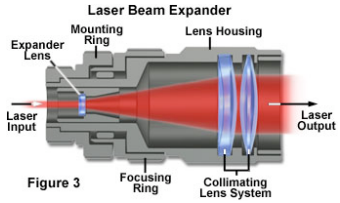
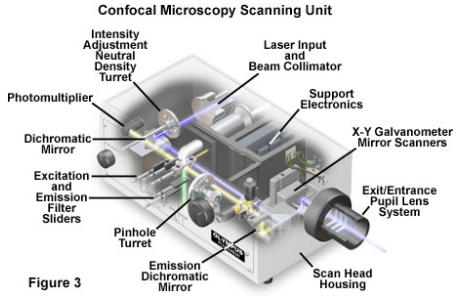
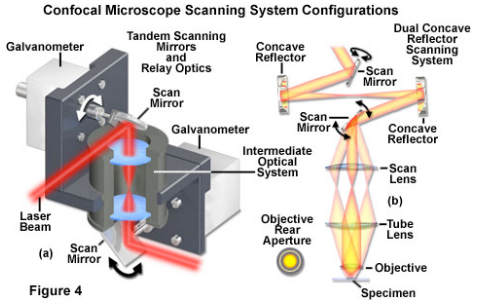
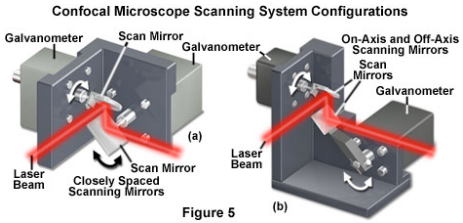
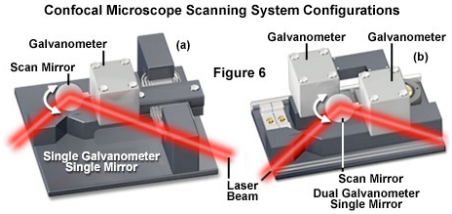


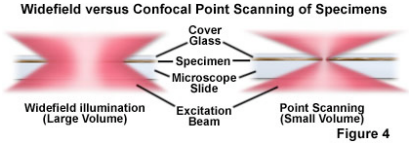
Figure 3


Confocal Microscopy Scanning Unit
Intensity Adjustment Neutral Density Turret
Laser Input and Beam Collimator
Photomultiplier
Support Electronics
Dichromatic Mirror
X-Y Galvanometer Mirror Scanners
Excitation and Emission Filter Sliders
Exit/Entrance Pupil Lens System
Pinhole Turret
Emission Dichromatic Mirror
Scan Head Housing
Figure 3


Confocal Microscope Scanning System Configurations
Galvanometer
Tandem Scanning Mirrors and Relay Optics
Scan Mirror
Galvanometer
Intermediate Optical System
Laser Beam
(a)
Scan Mirror
Concave Reflector
Dual Concave Reflector Scanning System
Scan Mirror
Scan Mirror
Concave Reflector
Scan Lens
(b)
Tube Lens
Objective Rear Aperture
Objective
Specimen
Figure 4


Confocal Microscope Scanning System Configurations
Galvanometer
Scan Mirror
Galvanometer
On-Axis and Off-Axis Scanning Mirrors
Scan Mirrors
Galvanometer
(a)
Laser Beam
Scan Mirror
Closely Spaced Scanning Mirrors
Laser Beam
(b)
Figure 5


Confocal Microscope Scanning System Configurations
Galvanometer
(a)
Scan Mirror
Single Galvanometer Single Mirror
Laser Beam
Galvanometer
Galvanometer
(b)
Scan Mirror
Dual Galvanometer Single Mirror
Figure 6


Widefield versus Confocal Point Scanning of Specimens
Cover Glass
Specimen
Microscope Slide
Widefield Illumination (Large Volume)
Excitation Beam
Point Scanning (Small Volume)
Figure 4

Pollen Grain Serial Optical Sections by Confocal Microscopy

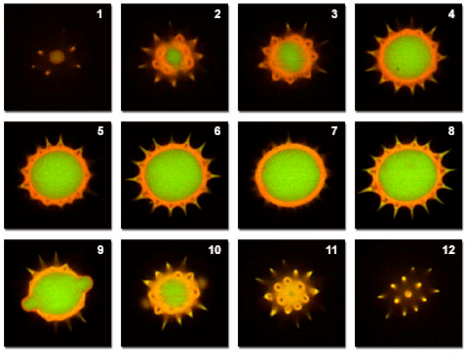
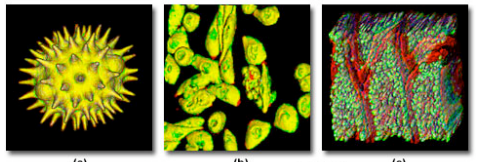


Figure 6

Three-Dimensional Volume Renders from Confocal Optical Sections



(a) (b) (c)

Figure 7

Side-On Photomultiplier Anatomy

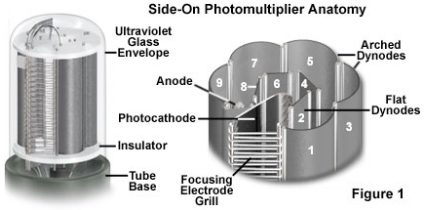


Figure 1

Common Photomultiplier Dynode Chain Configurations

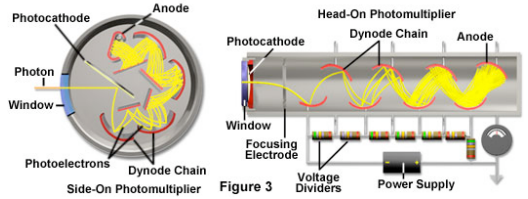
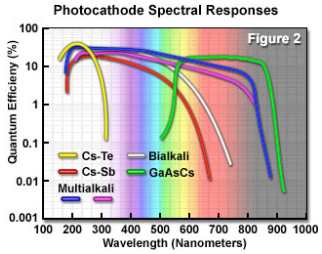
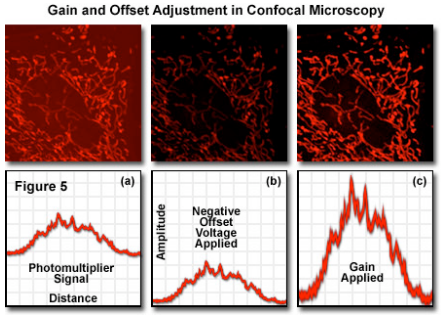


Figure 3


Photocathode Spectral Responses
Figure 2
Quantum Efficiency (%)
100
10
1
0.1
0.01
0.001
Cs-Te
Cs-Sb
Multialkali
Bialkali
GaAsCs
100 200 300 400 500 600 700 800 900 1000
Wavelength (Nanometers)


Gain and Offset Adjustment in Confocal Microscopy
Figure 5
(a)
Photomultiplier Signal
Distance
(b)
Amplitude
Negative Offset Voltage Applied
(c)
Gain Applied

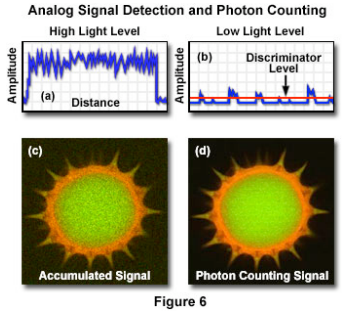

Analog Signal Detection and Photon Counting
High Light Level
Low Light Level
Amplitude
(a)
Distance
Amplitude
(b)
Discriminator Level
(c)
Accumulated Signal
(d)
Photon Counting Signal

Figure 6