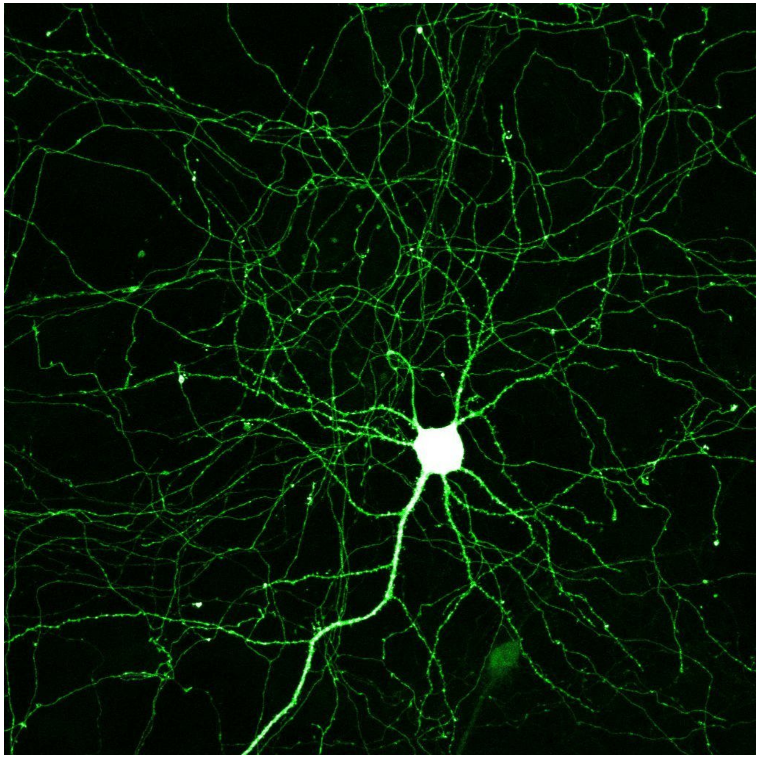
Imaging



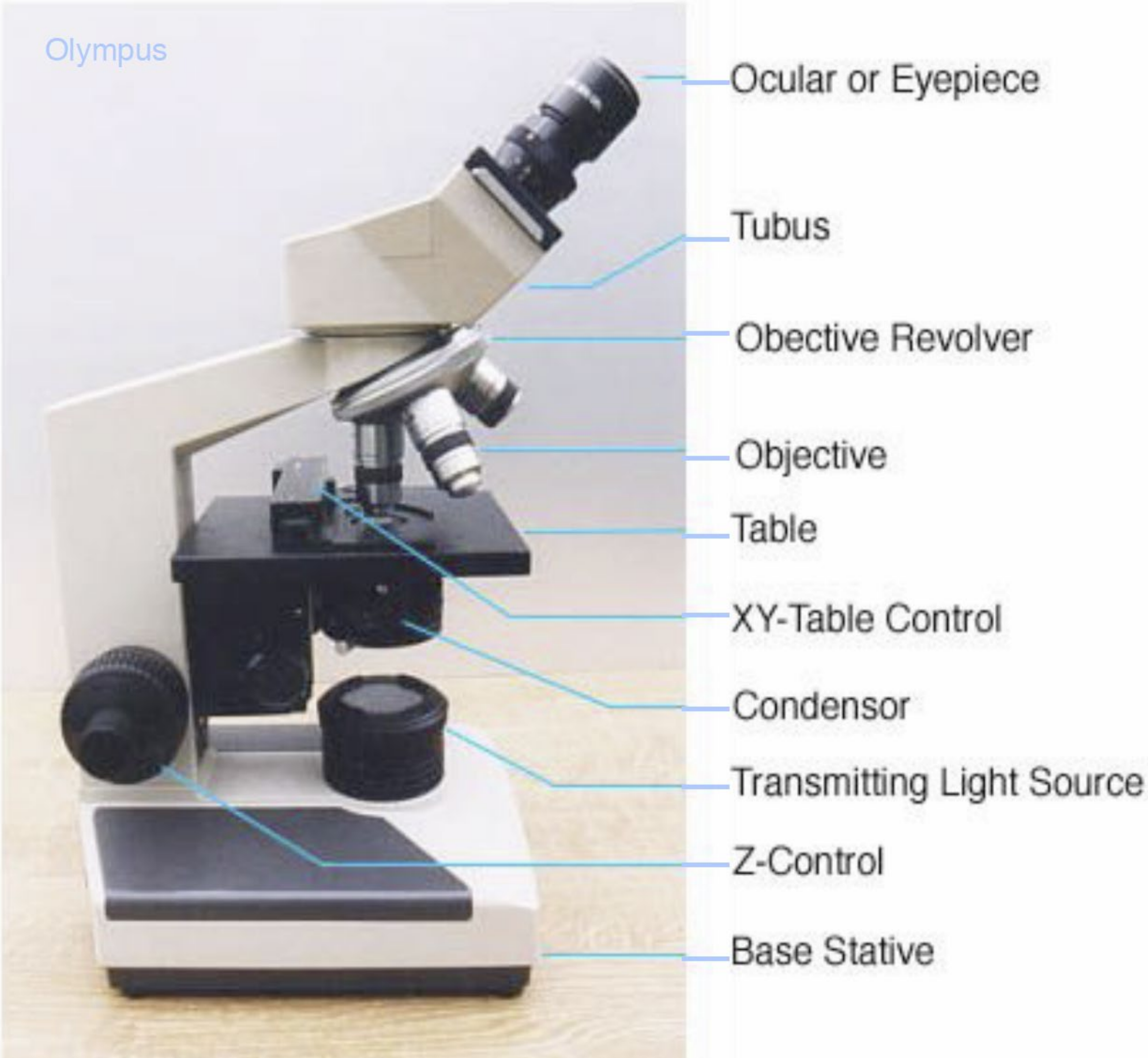
Darcy S. Peterka

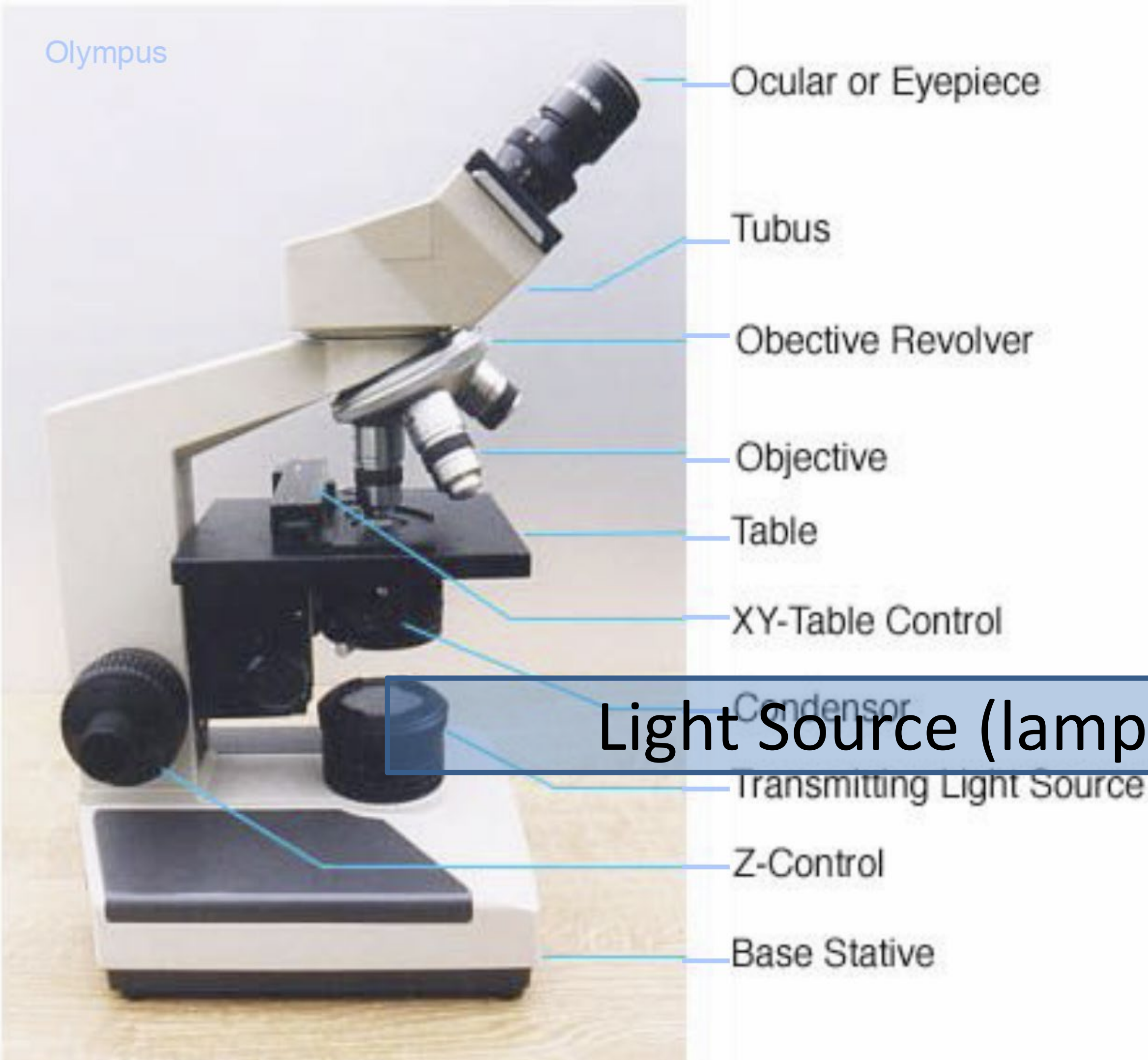
COLUMBIA | Zuckerman Institute
MORTIMER B. ZUCKERMAN MIND BRAIN BEHAVIOR INSTITUTE

ISFNS 2018

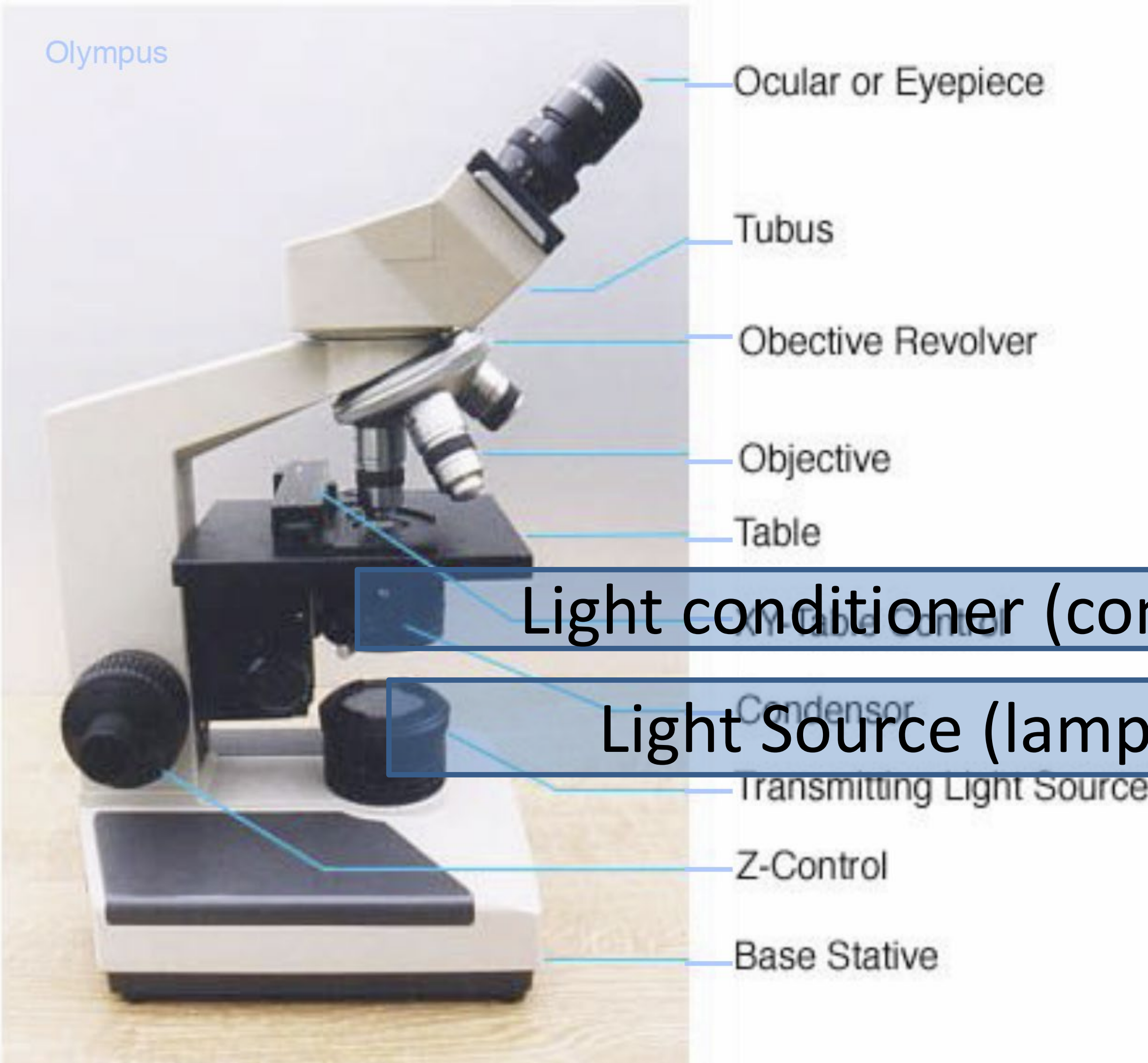
The "simple" microscope

Leeuwenhoek Microscope
(circa late 1600s)


Olympus
Ocular or Eyepiece
Tubus
Obective Revolver
Objective
Table
XY-Table Control
Condensor
Transmitting Light Source
Z-Control
Base Stative

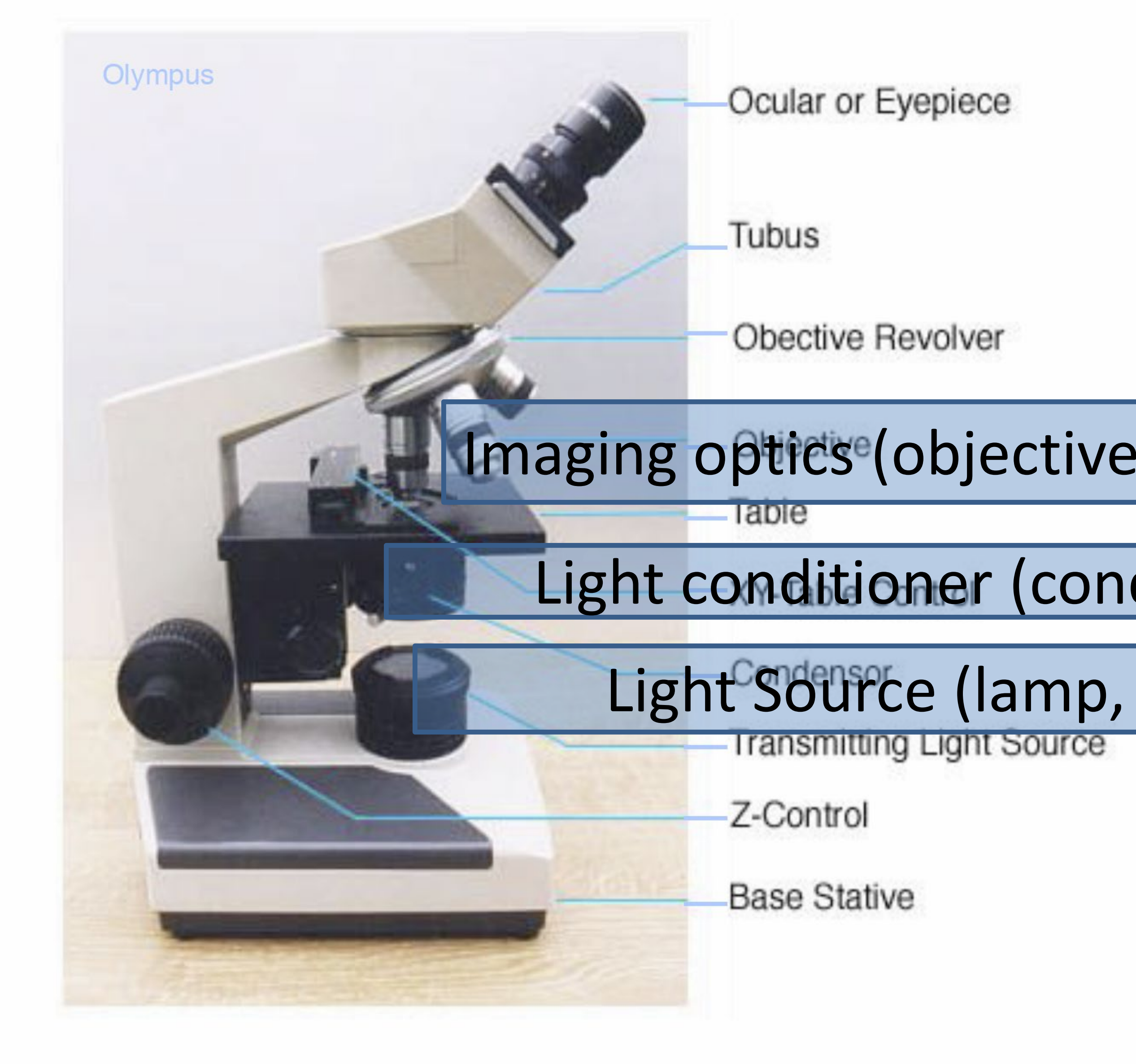


Light Source (lamp, laser, etc)



Light conditioner (condenser, etc)

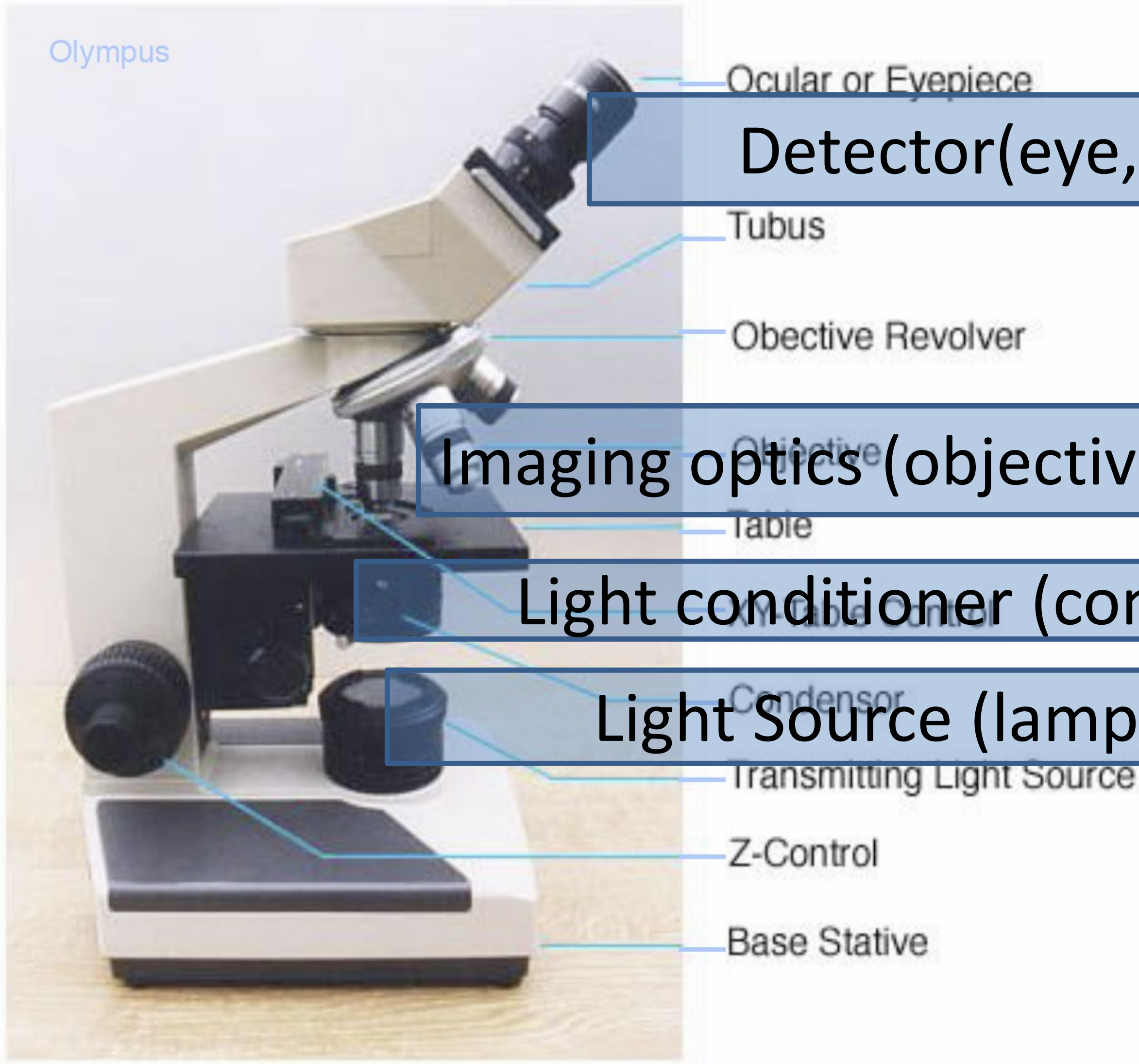
Light Source (lamp, laser, etc)



Imaging optics (objective, tube lens, etc)

Light conditioner (condenser, etc)

Light Source (lamp, laser, etc)

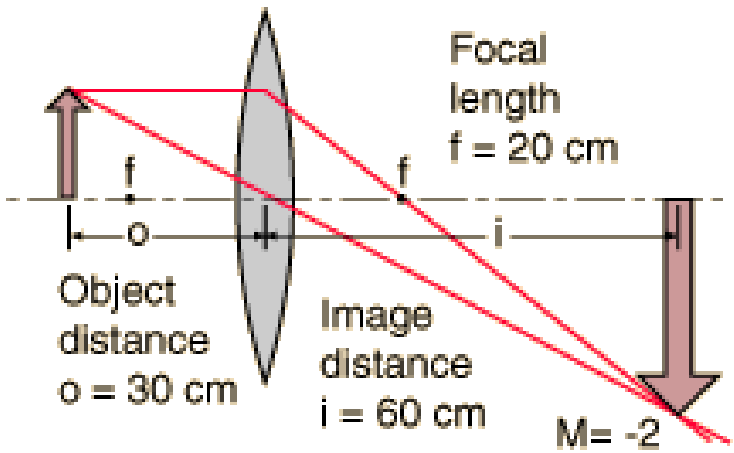


Detector(eye, ccd, pmt)

Imaging optics (objective, tube lens, etc)

Light conditioner (condenser, etc)

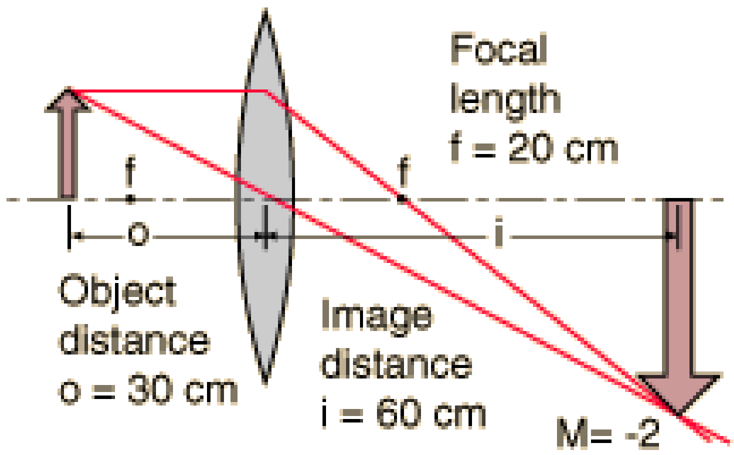
Light Source (lamp, laser, etc)



$$\frac{1}{o} + \frac{1}{i} = \frac{1}{f}$$

$$\frac{1}{\text{object distance}} + \frac{1}{\text{image distance}} = \frac{1}{\text{focal length}}$$

Thick lens from Melles Griot Optics Guide – link here:
http://www.astro.caltech.edu/~lah/ay105/pdf/Fundamental-Optics.pdf



$$\frac{1}{o} + \frac{1}{i} = \frac{1}{f}$$

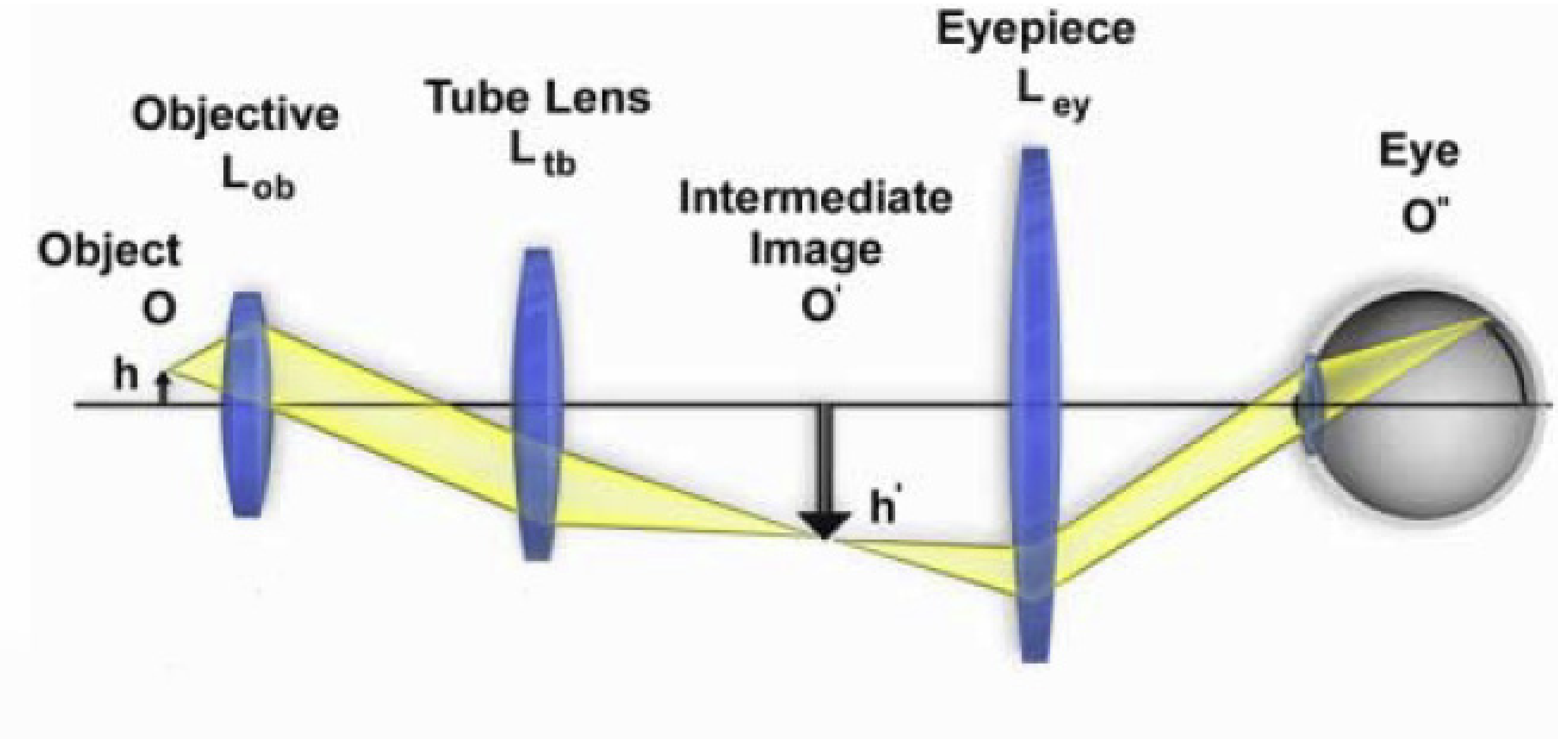
$$\frac{1}{\text{object distance}} + \frac{1}{\text{image distance}} = \frac{1}{\text{focal length}}$$

$$\left|\text{Mag}\right|_{lateral} = \frac{\text{image distance}}{\text{object distance}}$$

$$\left|\text{Mag}\right|_{axial} = M^2$$

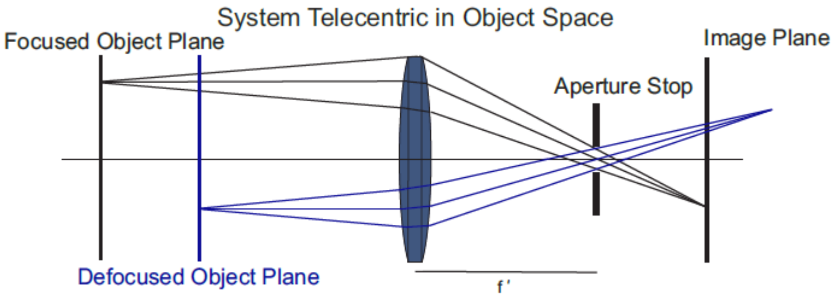
Thick lens from Melles Griot Optics Guide – link here:
http://www.astro.caltech.edu/~lah/ay105/pdf/Fundamental-Optics.pdf

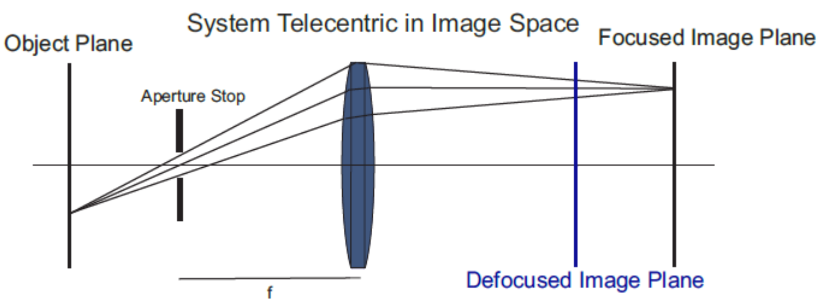
essential optical layout of a modern microscope…

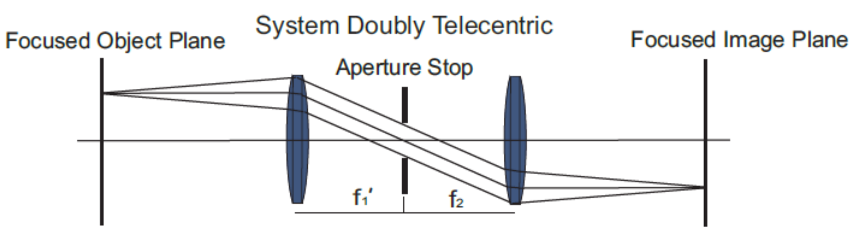


$$M_{total} = M_{objective} \times M_{eyepiece}$$

Can you put the tube lens anywhere? Yes, but…



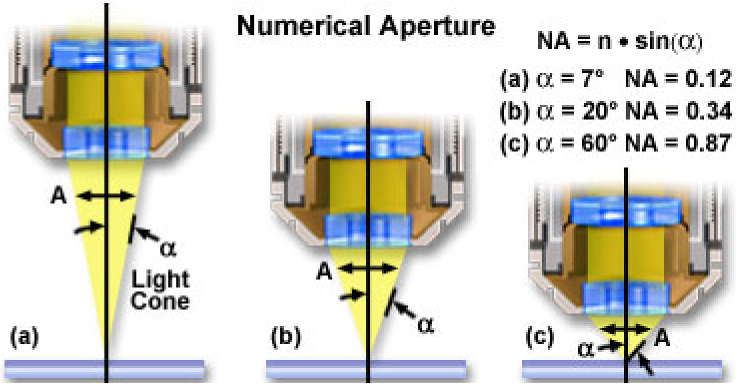




For building systems, and the lens spacing in infinity corrected (or other) systems, "telecentricity" is a nice design goal. If you look at the rays, you can see the central line are parallel to the optical axis, and this means When things move or blur, you don't see lateral shifts. Also, it means you see the same view, not A perspective view…

Magnification is different from resolution. Magnification describes the apparent size of an object, and can be made arbitrarily large

Resolution describes the ability to distinguish two nearby objects and is fixed by the wavelength of light and the numerical aperture of the objective*



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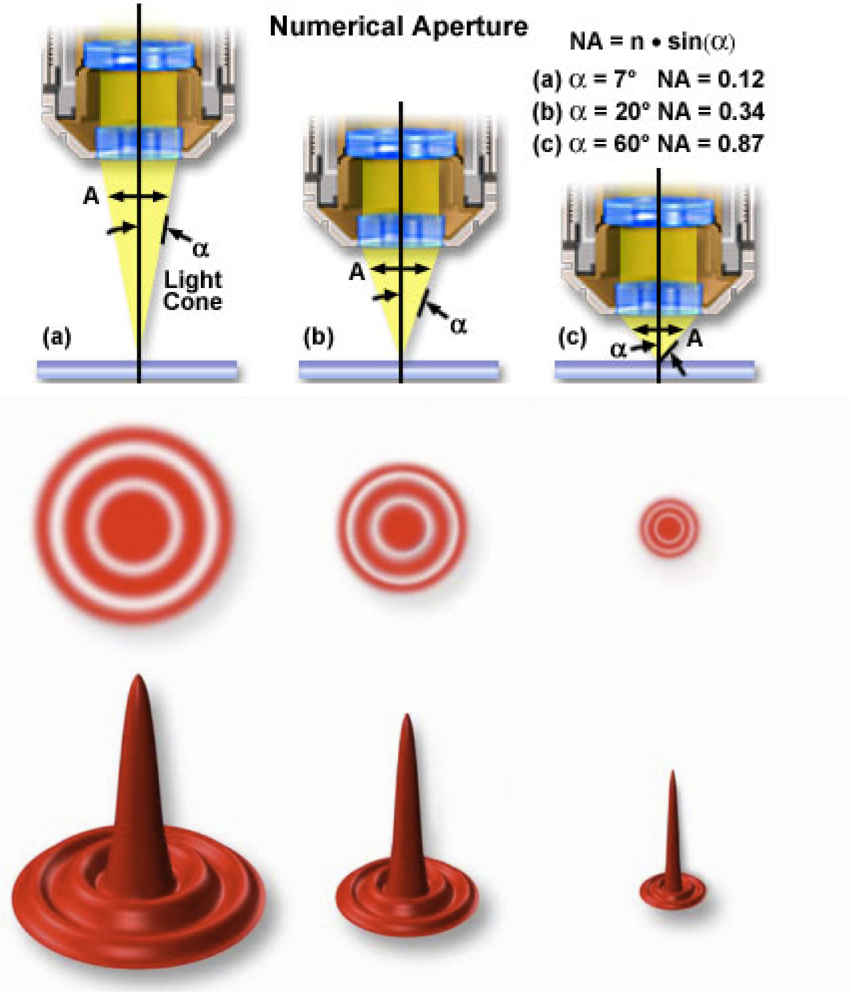

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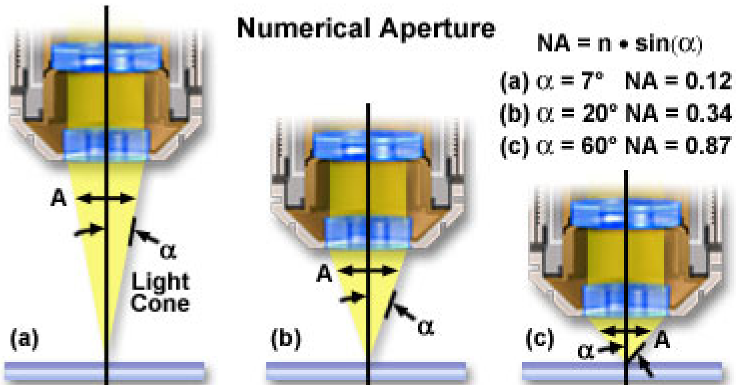

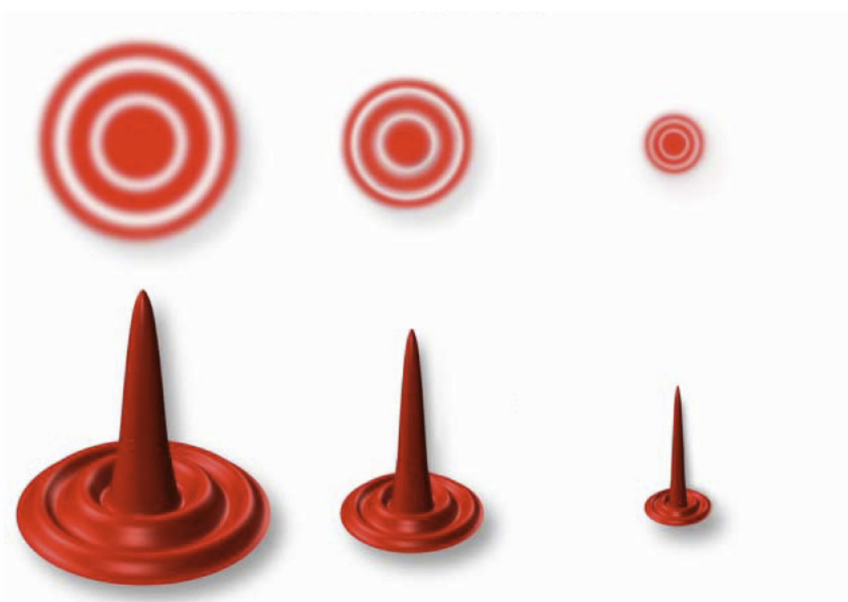

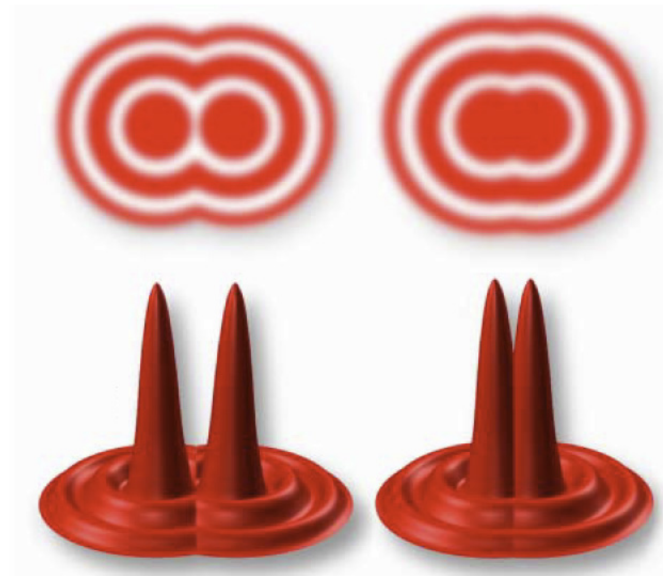

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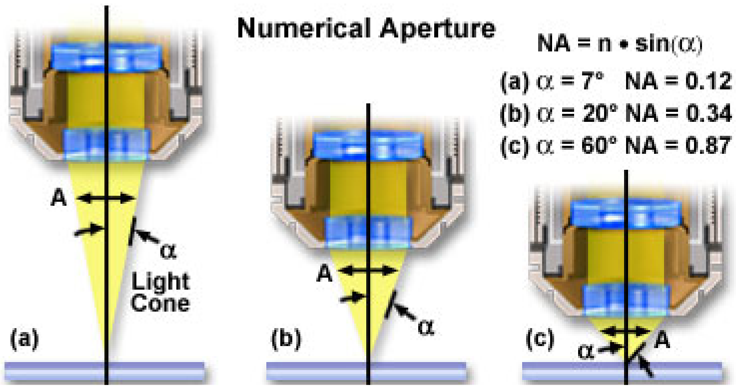
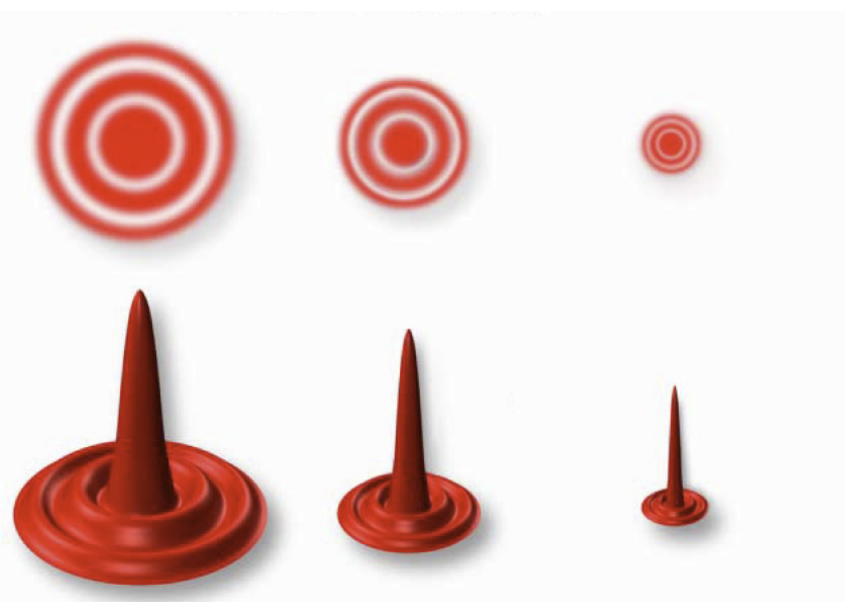
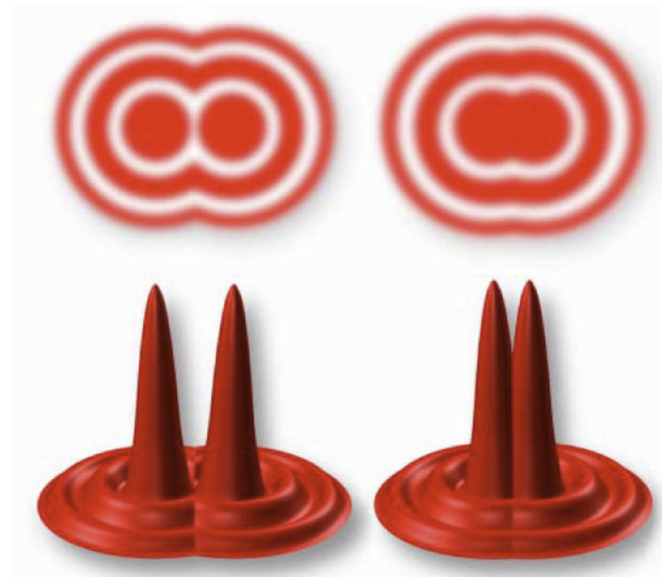
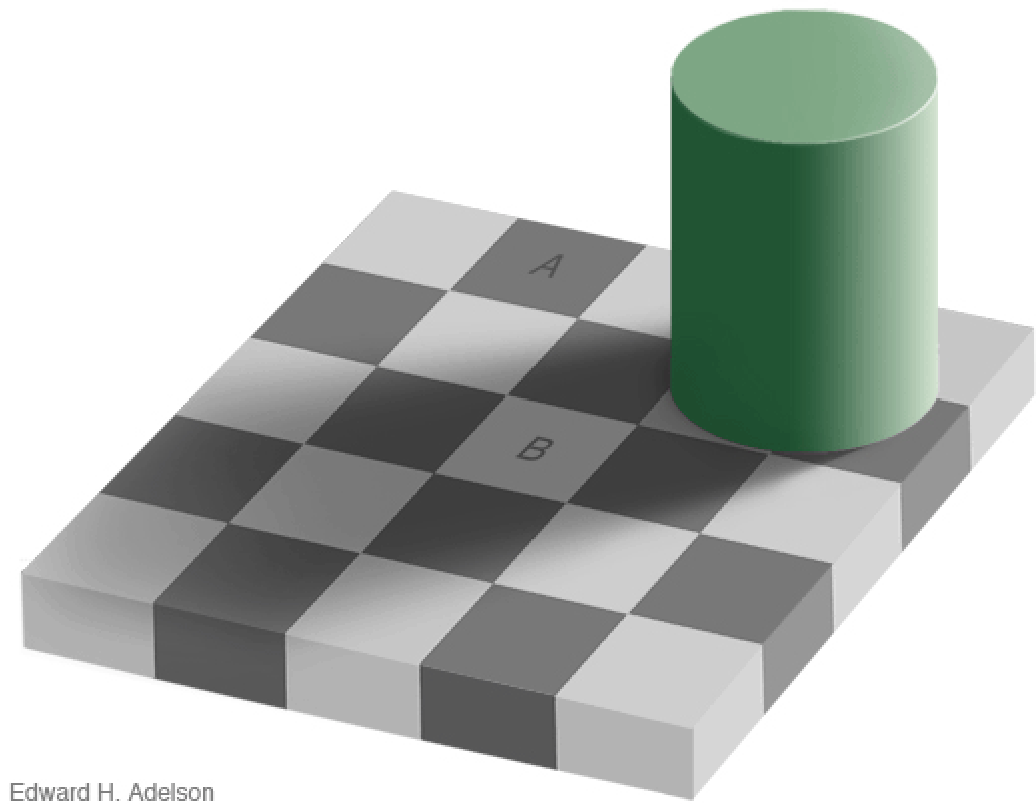






$$r_{\min} = \frac{0.61 \cdot \lambda}{\mathrm{NA}}$$

How do we see things?



C ONTRAST

Slide taken from D.J. Uhlinger

C ONTRAST

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

Slide taken from D.J. Uhlinger

C ONTRAST

50 Units 50 50

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

Slide taken from D.J. Uhlinger

0 Units

C ONTRAST

50 Units 50 50

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

Slide taken from D.J. Uhlinger

0 Units

100 Units

CONTRAST

50 Units

50

50

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

Slide taken from D.J. Uhlinger

0 Units 50 Units 100 Units

CONTRAST

50 Units 50 50

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

Slide taken from D.J. Uhlinger

0 Units 50 Units 100 Units

CONTRAST

50 Units 50 50

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

50 – 0 / 50 + 0 = 1

Slide taken from D.J. Uhlinger

0 Units 50 Units 100 Units

CONTRAST

50 Units 50 50

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

50 – 0 / 50 + 0 = **1**

50 – 100 / 50 + 100 = **-0.33**

Slide taken from D.J. Uhlinger

0 Units 50 Units 100 Units

CONTRAST

50 Units 50 50

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

50 – 0 / 50 + 0 = 1

50 – 50 / 50 + 50 = 0

50 – 100 / 50 + 100 = -0.33

Slide taken from D.J. Uhlinger

How do you get contrast?

It depends on the sample, and the method of observation…

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- Brightfield
- Darkfield
- Phase Contrast
- Polarized Light
- DIC (Differential Interference Contrast)
- Fluorescence (and related techniques)

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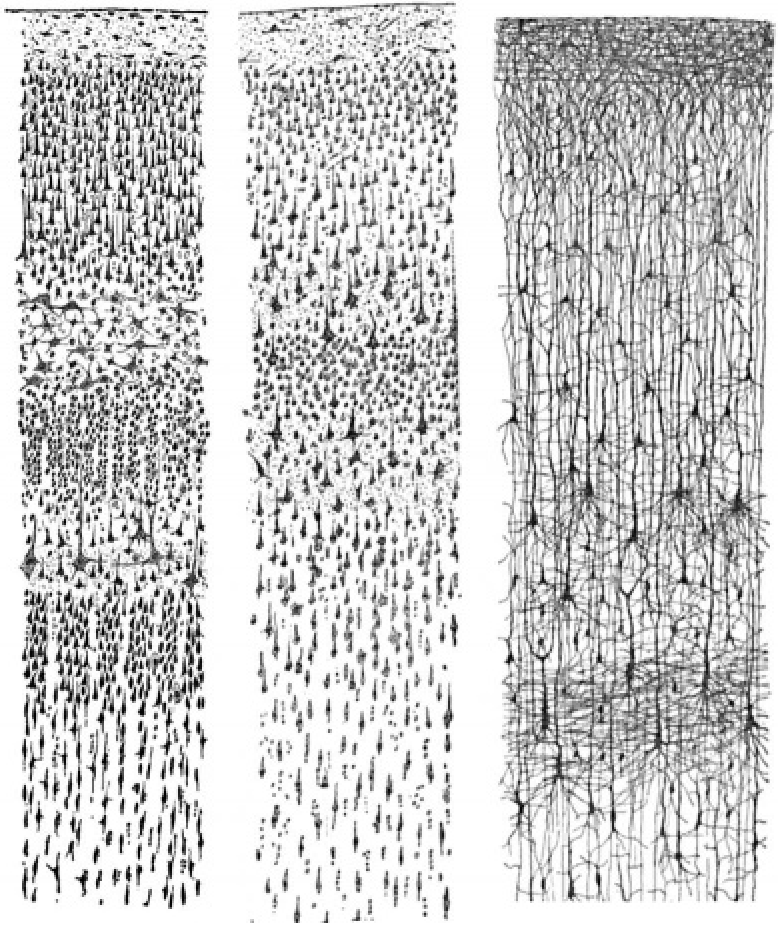
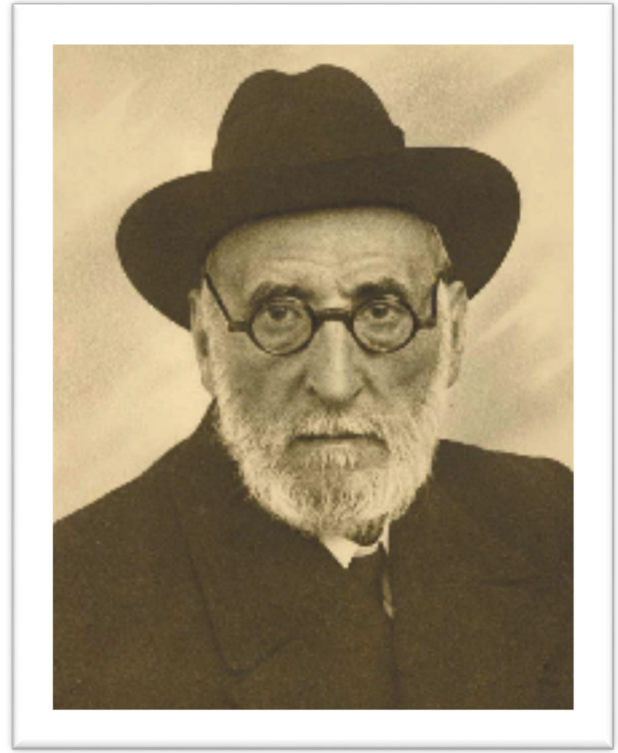
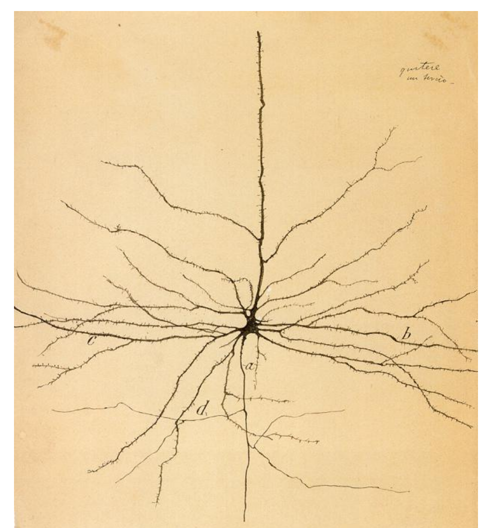
Brightfield illumination

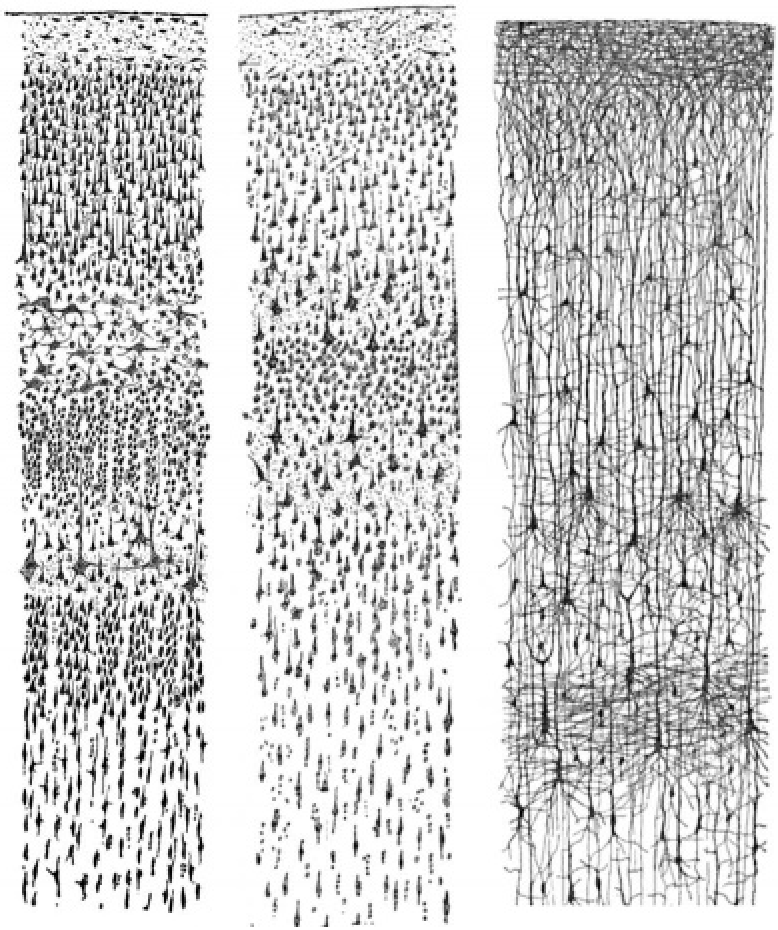


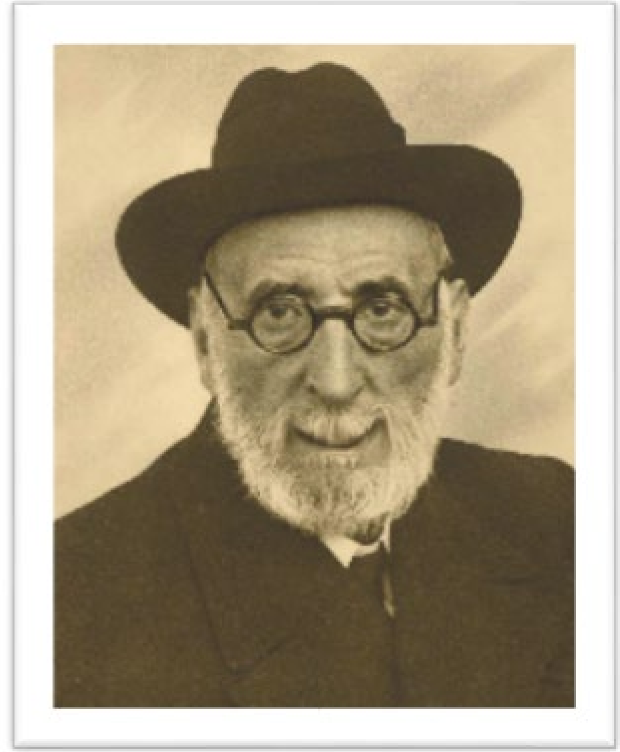


Ramon y Cajal

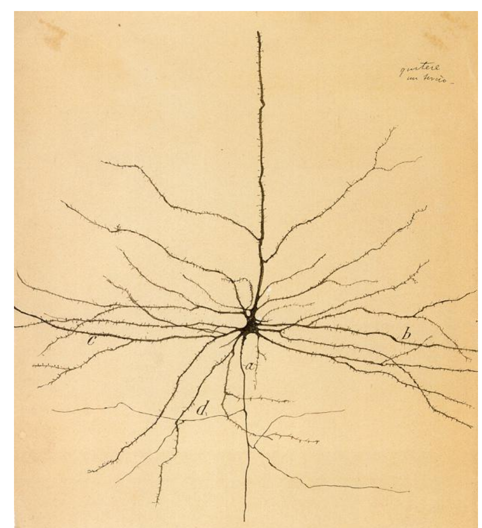


Brightfield illumination

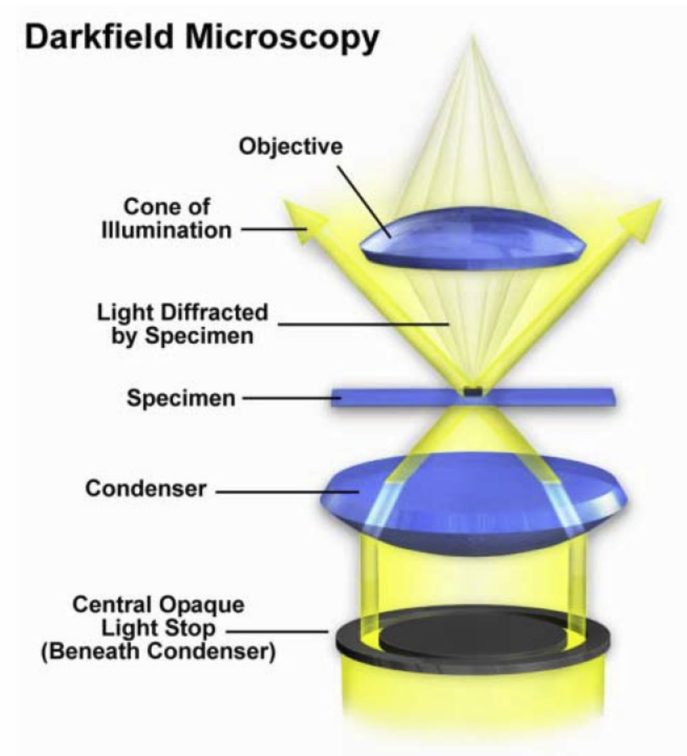




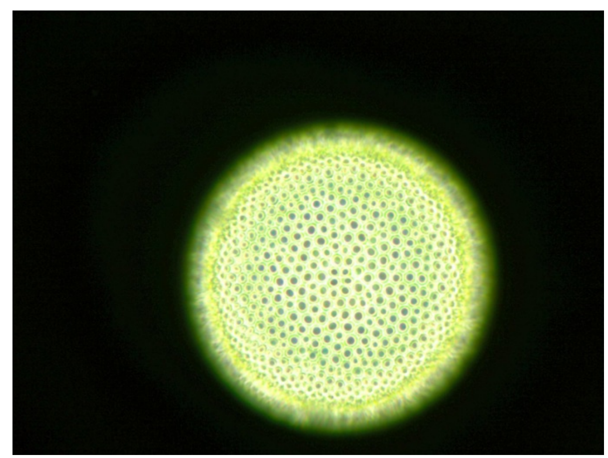
Ramon y Cajal



Darkfield illumination



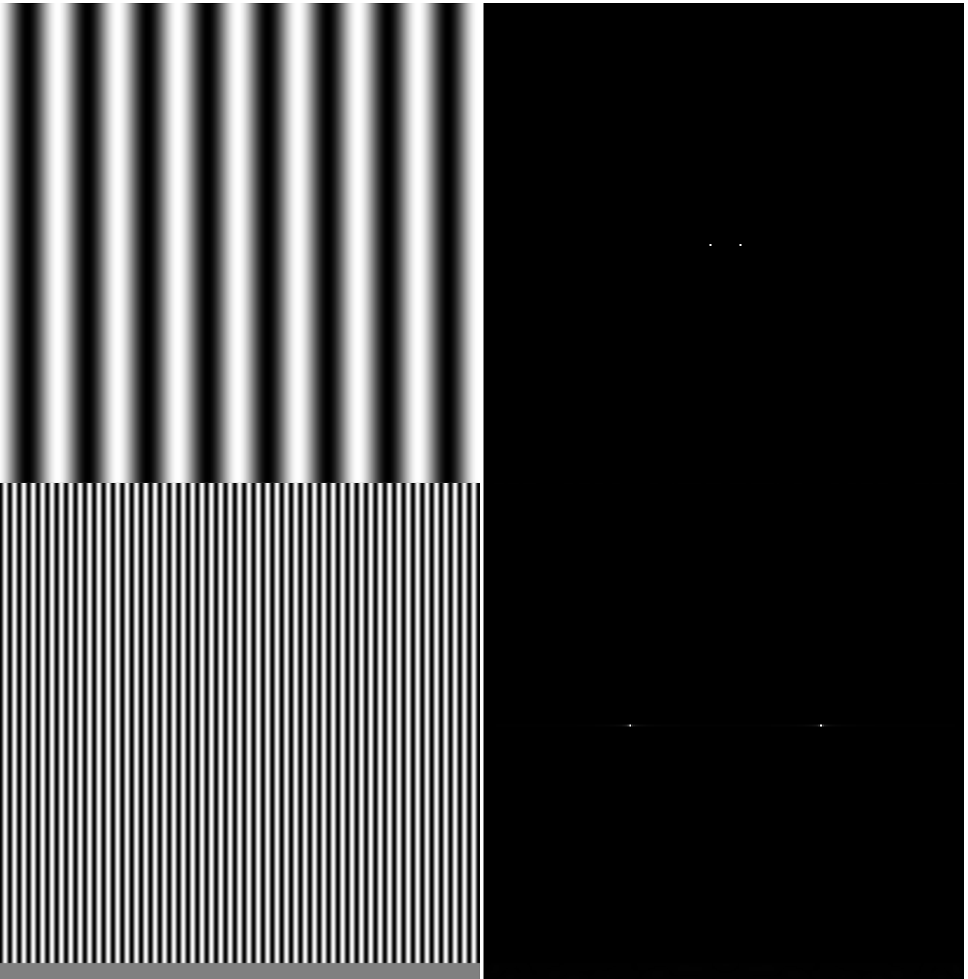
- Direct light through sample at only oblique angles, and block direct light
- Only light scattered, refracted, or reflected by sample makes it into the objective
- Bright object on dark background – sensitive to edges, outlines, and boundaries
- Sizes of features are not reliable



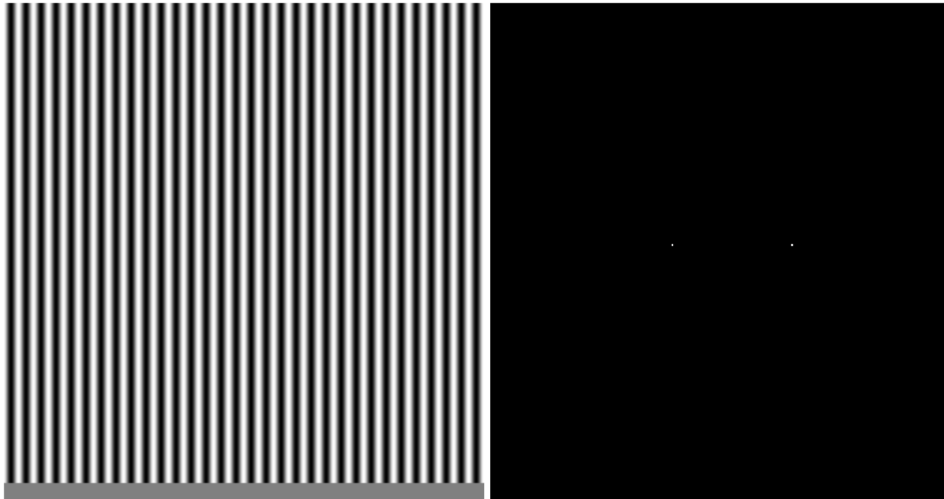
Fourier Transform… see https://en.wikipedia.org/wiki/Fourier_transform

$$\hat{f}\,(\xi) = \int_{-\infty}^{\infty} f(x)\; e^{-2\pi i x \xi}\, dx,$$

Fourier transform pairs…
Image on left, FT on right. Spatial description – you see sine waves of intensity.
The FT “image” shows you the frequency of the sine waves (two dots because + and - freq)
*** Why don’t you see the middle dot, like on my demo scope? Because in these pictures, I compute it for a “real” sine wave, meaning goes from -1 to 1, so average intensity is 0. In an Optical system, *some* light is always propagated, so you would see a middle dot.



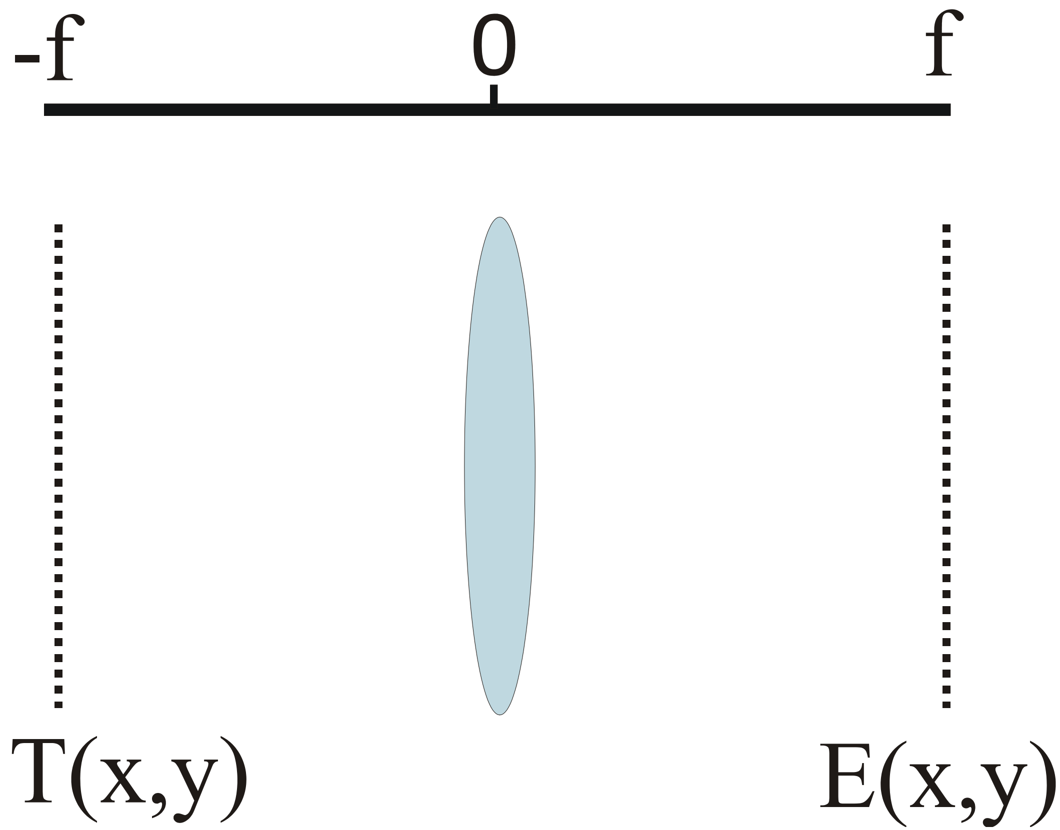
Also note that this is just showing the Amplitude^2 of the transform, and doesn’t show the phase. The phase part contains most of the information, re: where these frequency components match up in space… See next slide…



Matlab/imageJ

Under imagej/process/fft, can do ffts. What you see is the log scale image of the "power spectrum". That is it is the intensity of the particular frequency in the image. Each intensity would correspond to the coefficient in front of that freq in the Fourier decomposition of the image. The *phase* is NOT shown by default. Under fft options, you can have it displayed. Check it out….

Fourier Optics (thin lens)



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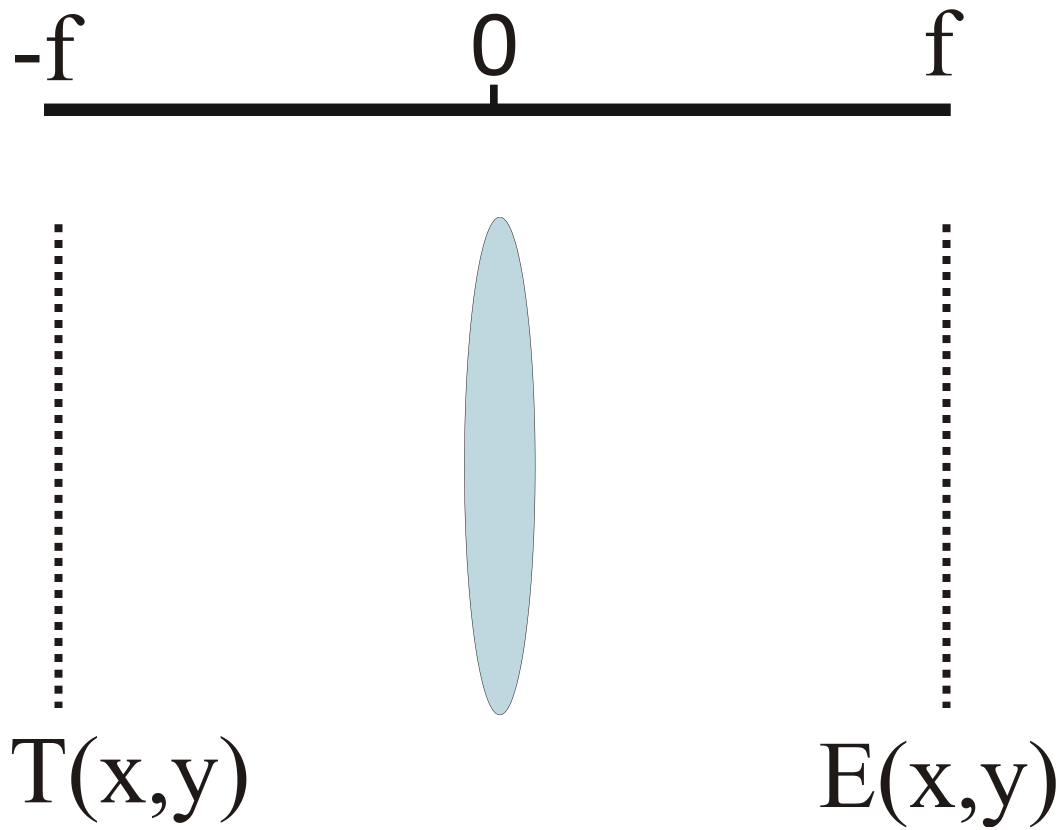


$$E_f\left(x,y\right)=C\iint T_{-f}\left(x_{-f},y_{-f}\right)\cdot\exp\left[-\frac{ik}{f}\left(x_{-f}x_f+y_{-f}y_f\right)\right]dx_{-f}dy_{-f}$$

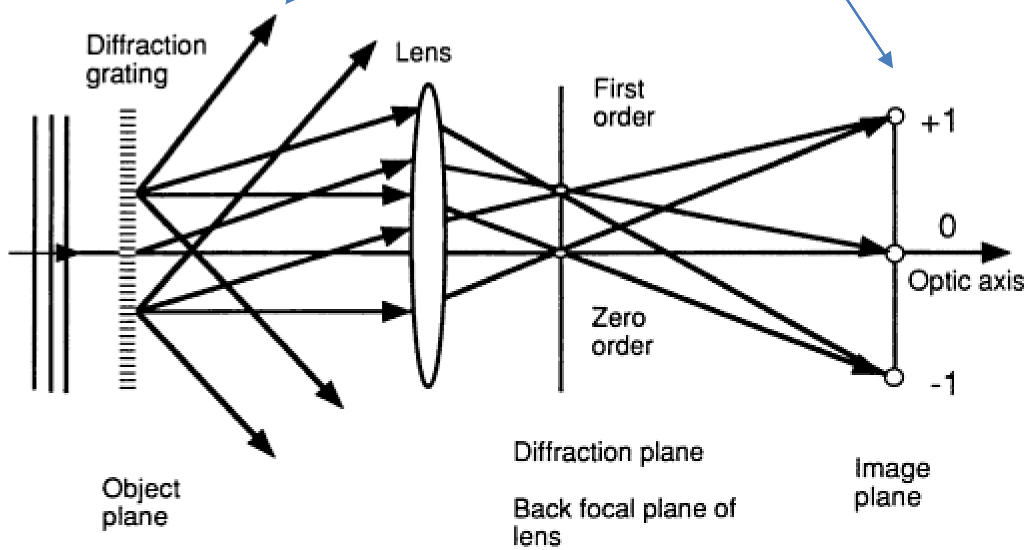
Why does a lens do a transform? Well, from Maxwell's Eq. and A bunch of approximations, we see that that math works…

See http://web.mit.edu/2.710/Fall06/2.710-wk10-a-sl.pdf

Good ref book, but pretty technical
https://www.macmillanlearning.com/college/us/product/Introduction-to-Fourier-Optics/p/1319119166

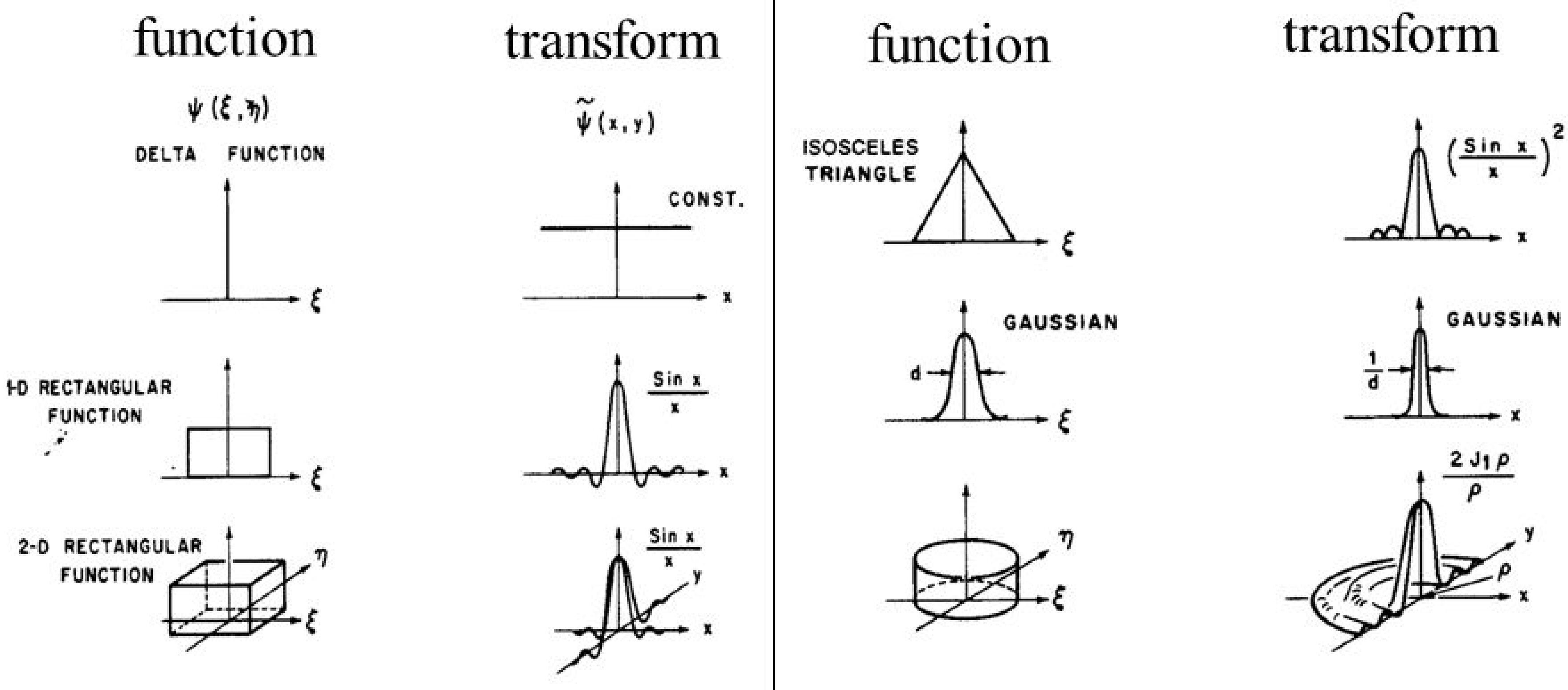
This light is lost

No frequencies above here…

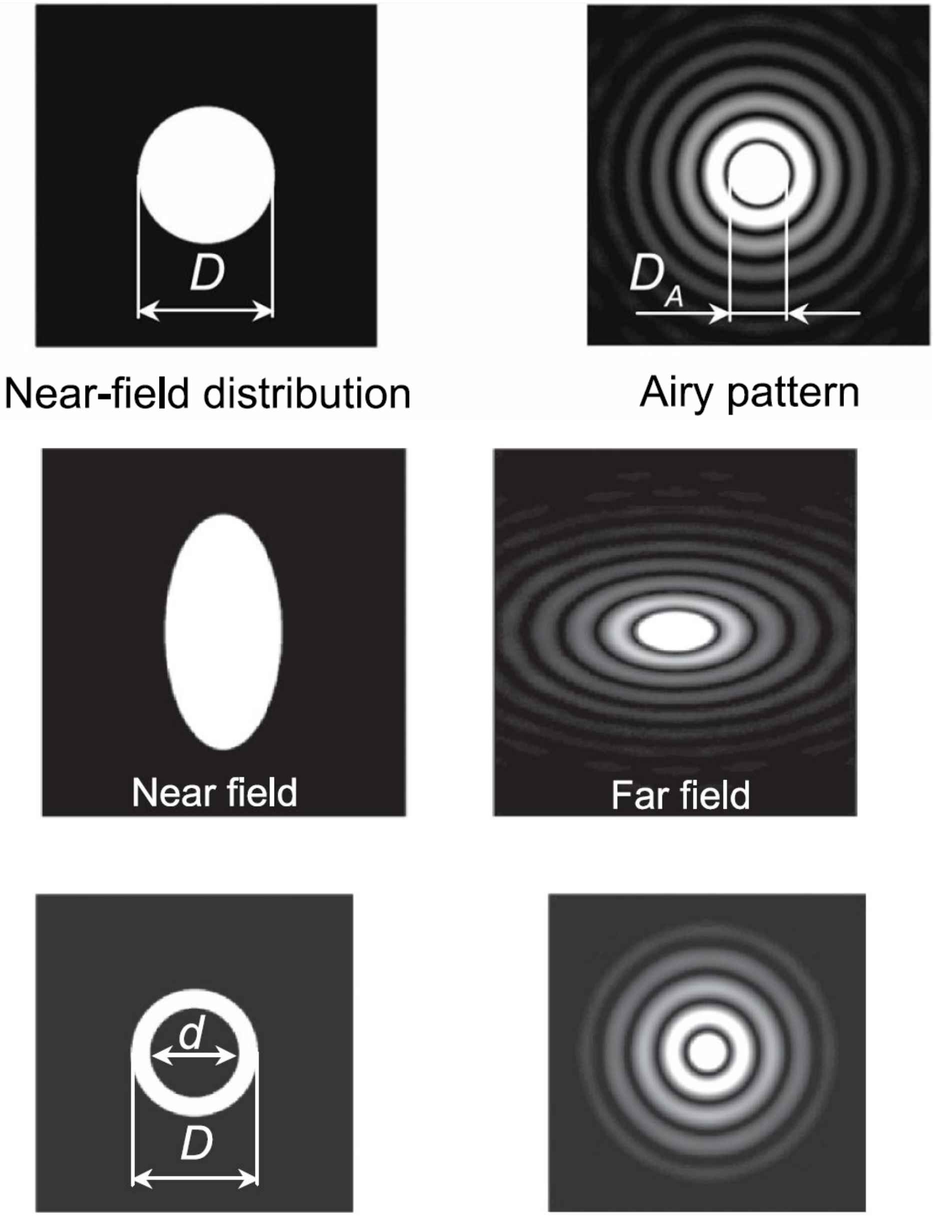


Why does NA affect resolution? If you imagine the diffraction of a small feature, this leads to wide angle scattering. If you don’t capture those rays, you can’t send them down your optical system. In terms of Fourier Optics, you don’t capture the high frequency components in the fourier plane, so you are effectively doing a low-pass filter of the image.

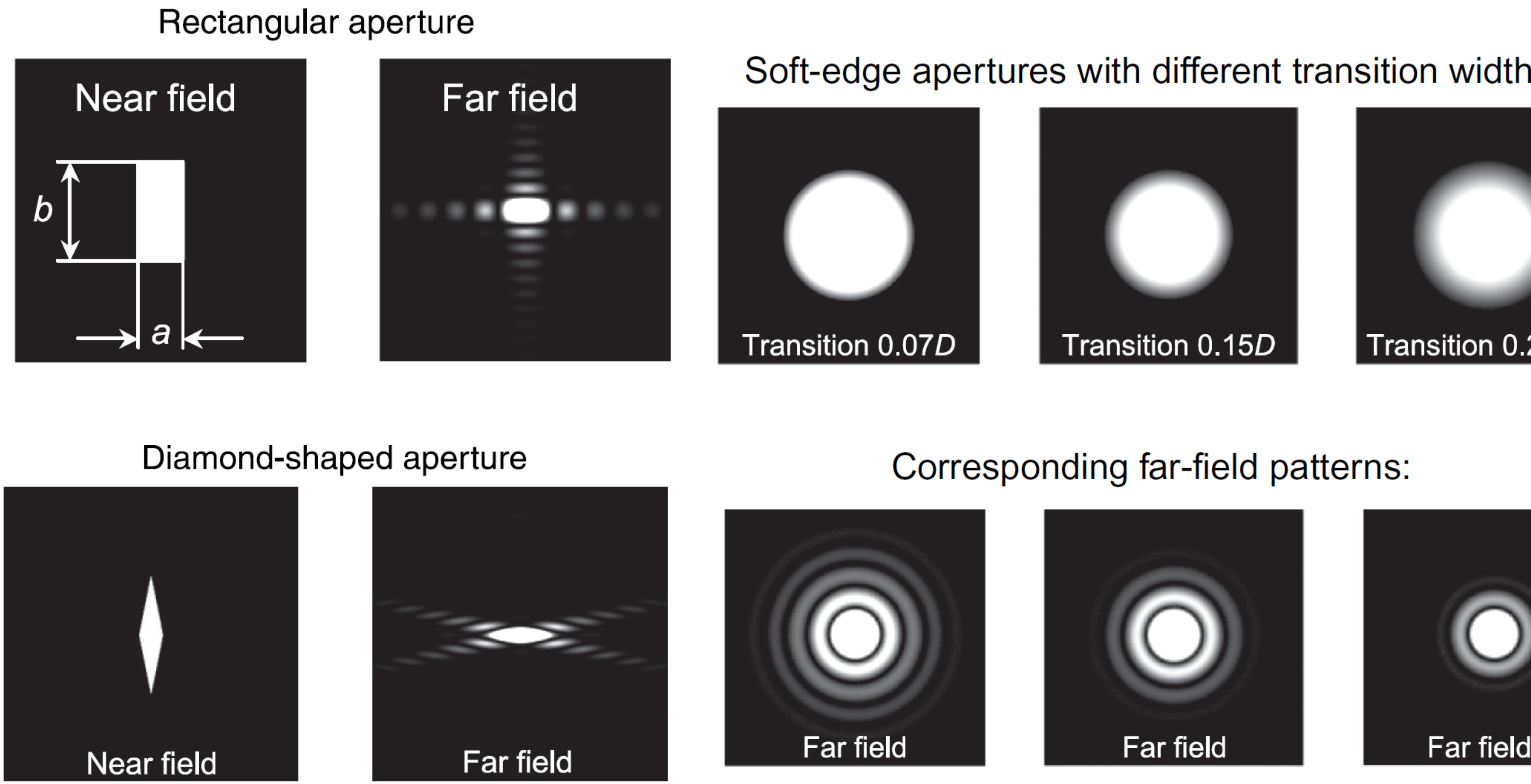
Some Fourier transform pairs (graphical illustration)



Source: *Physical Optics Notebook: Tutorials in Fourier Optics*, Reynolds, et. al., SPIE/AIP



Transform pairs.
Airy disk is the transform of a
Circular disk. Other shapes have
Different transforms…See next slide
Nothing inherently fundamental about
Airy disk in microscopy per se… it
is just convenient to build circularly
symmetric systems – both mechanically, and
conceptually…



Rectangular aperture
Near field
b
a
Far field
Soft-edge apertures with different transition width
Transition 0.07D
Transition 0.15D
Transition 0.
Diamond-shaped aperture
Near field
Far field
Corresponding far-field patterns:
Far field
Far field
Far field

	Field Plane	Fourier Plane
C	Field Amplitude, $E(x,y)$	$\tilde{E}(f_x, f_y)$
	Amplitude Point–Spread Function, $h(x,y)$ Coherent Point–Spread Function Point–Spread Function PSF, APSF, CPSF	Amplitude Transfer Function, $\tilde{h}(x,y)$ Coherent Transfer Function Transfer Function ATF, CTF
	$E_{image} = E_{object} \otimes h$	$\tilde{E}_{image} = \tilde{E}_{object} \times \tilde{h}$
I	Irradiance, $I(x,y)$	$\tilde{I}(f_x, f_y)$
	Incoherent Point–Spread Function, $H(x,y)$ Point–Spread Function PSF, IPSF	Optical Transfer Function, $\tilde{H}(f_x, f_y)$ OTF Modulation Transfer Function, $\left\lvert\tilde{H}\right\rvert$ MTF Phase Transfer Function, $\angle\tilde{H}$ PTF
	$I_{image} = I_{object} \otimes H$	$\tilde{I}_{image} = \tilde{I}_{object} \times \tilde{H}$

- ***Incoherent light, circular aperture***

$$OTF(\rho') = \frac{2}{\pi}\left[\operatorname{acos}(\rho') - \rho'\sqrt{1-\rho'^2}\right]$$

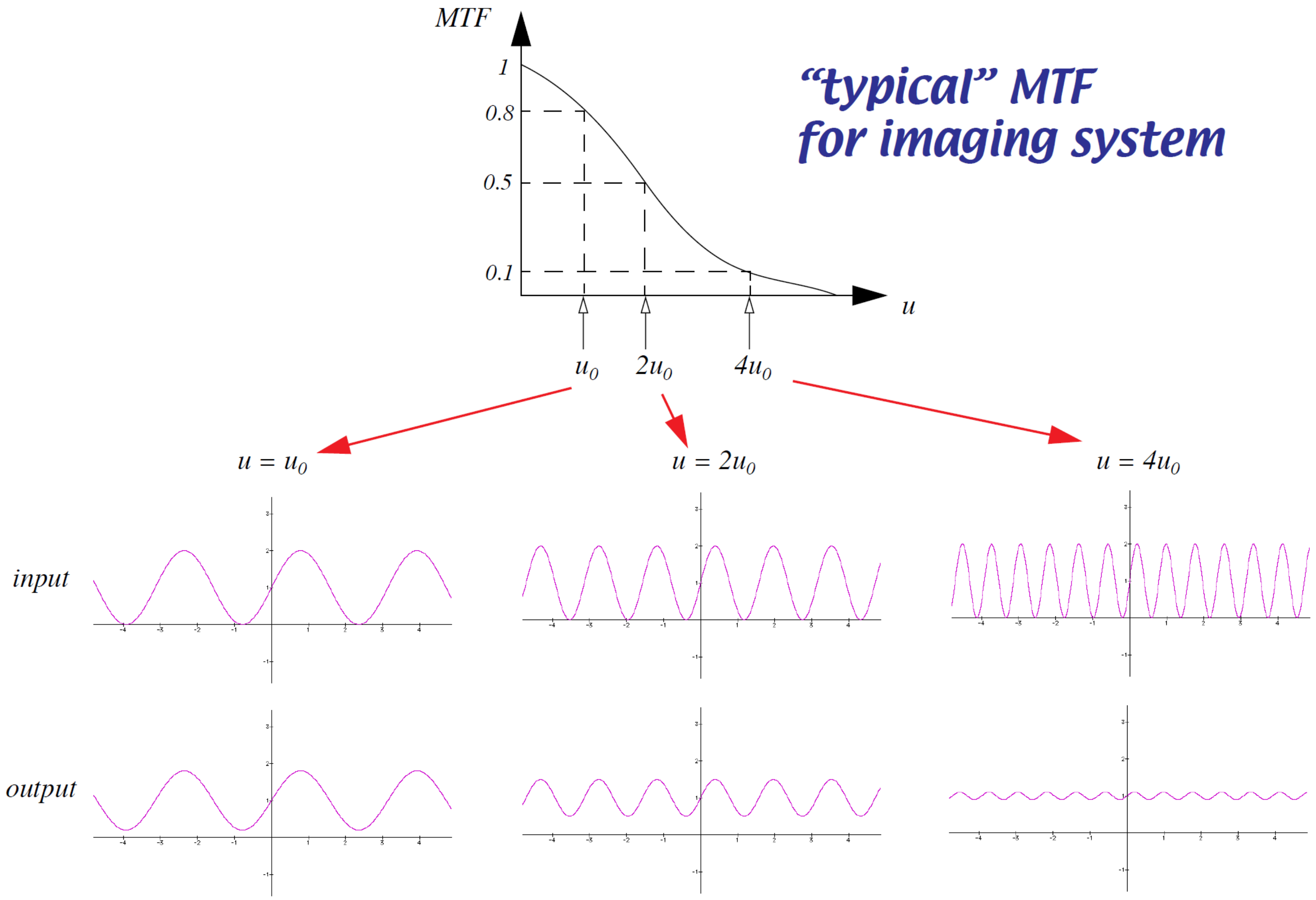
where the normalized radial spatial frequency is given by,

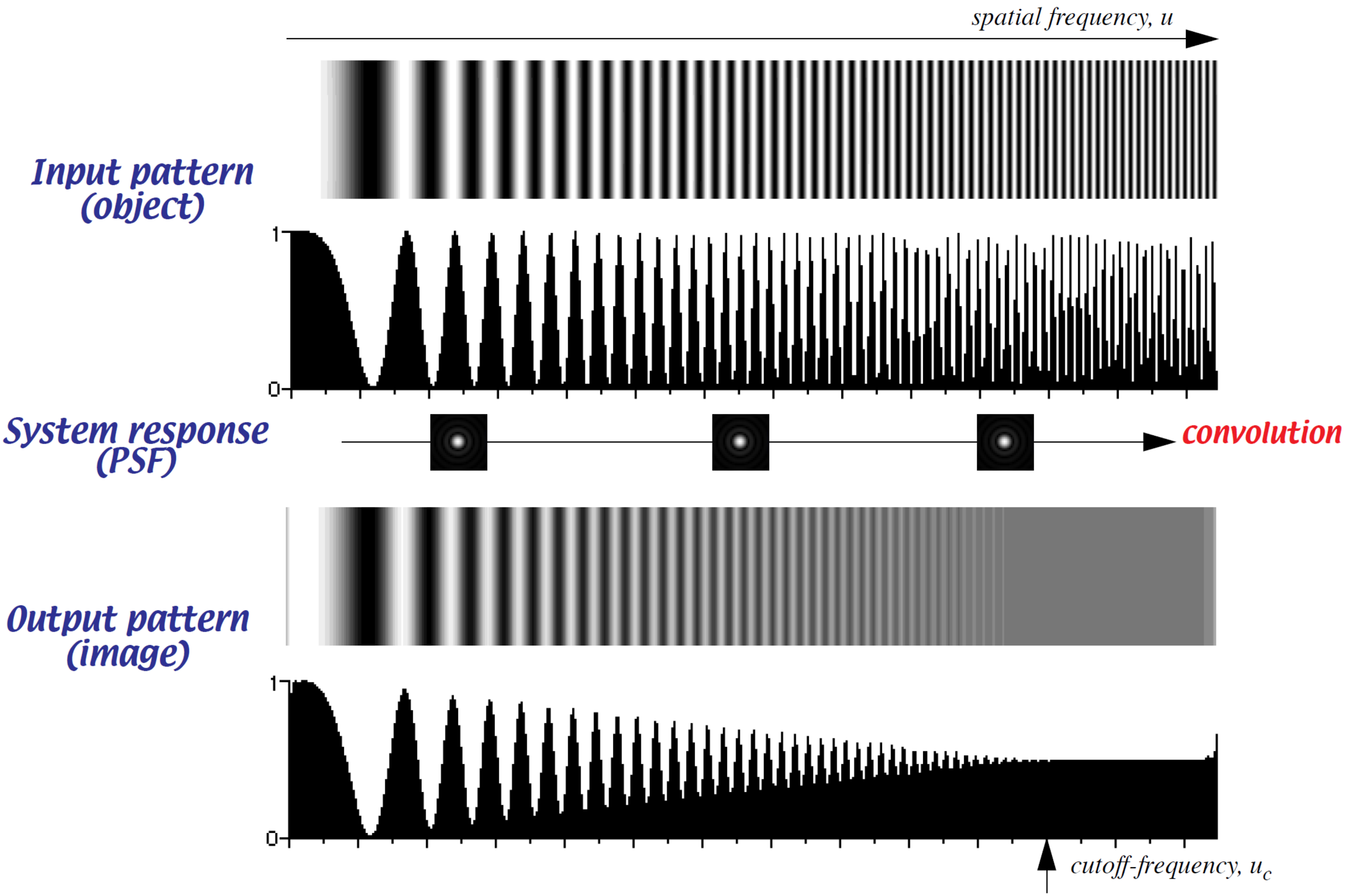
$$\rho' = \rho/\rho_c$$

and the cutoff frequency is given by,

$$\rho_c = \frac{D}{\lambda f} = \frac{1}{\lambda N} \quad \text{where} \quad \begin{aligned} D &= \text{aperture diameter} \\ f &= \text{focal length} \\ N &= \text{f-number} \\ \lambda &= \text{wavelength of light} \end{aligned}$$

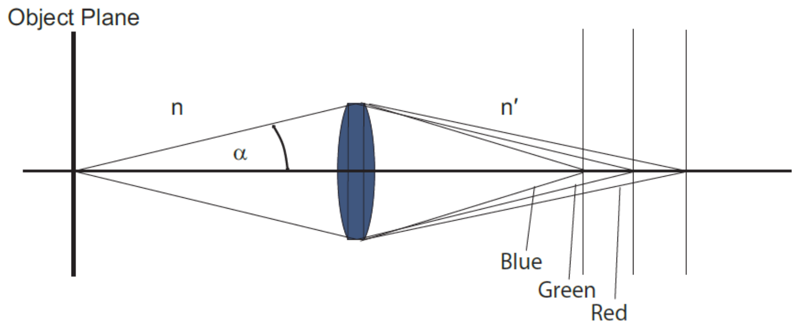
“typical” MTF for imaging system

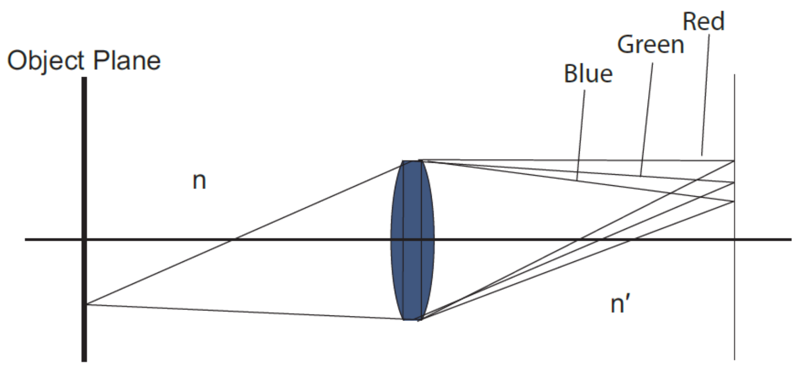




spatial frequency, u
Input pattern
(object)
1
0
System response
(PSF)
convolution
Output pattern
(image)
1
0
cutoff-frequency, u_c

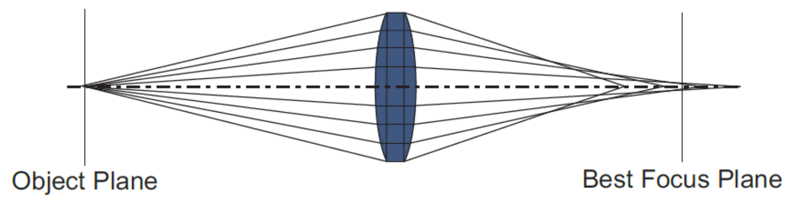
Aberrations…



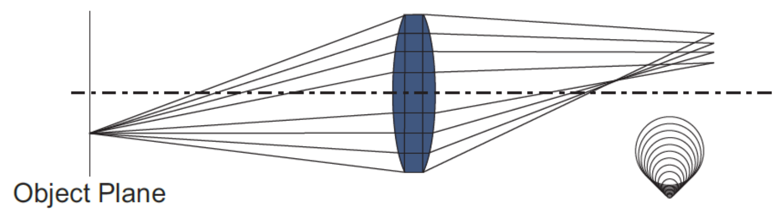


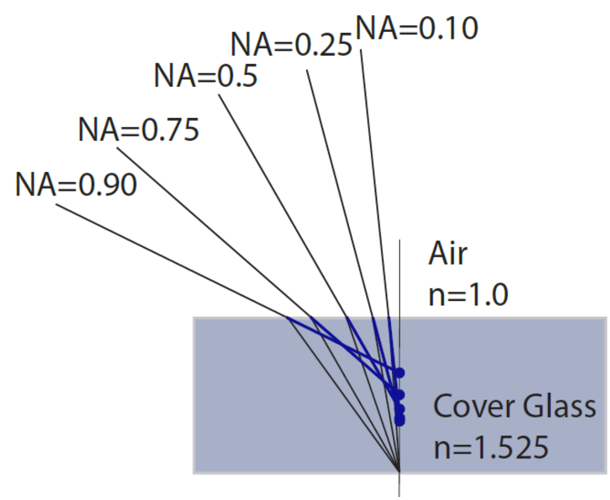
Chromatic Aberrations – because refractive index depends on Frequency (wavelength), lenses have different focal lengths for each color.

Spherical Aberration

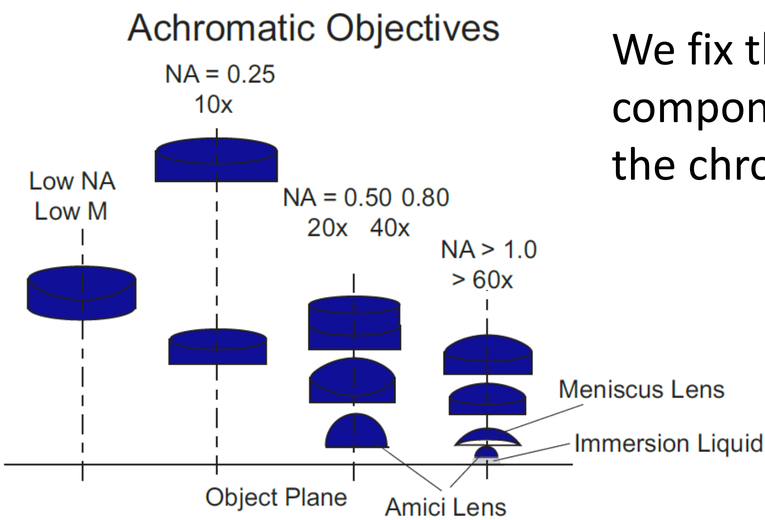


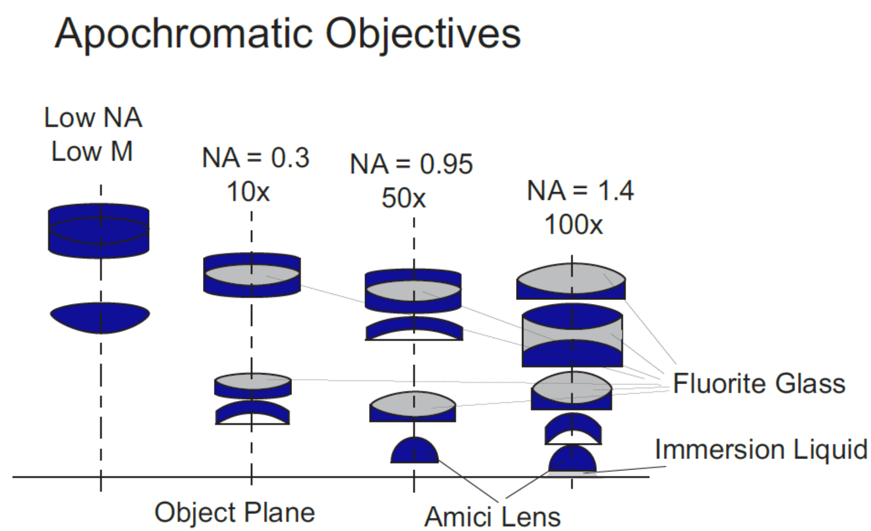
Coma





We fix that by making complex lenses that have multiple components, and we choose the shape and material so that the chromatic errors cancel out…

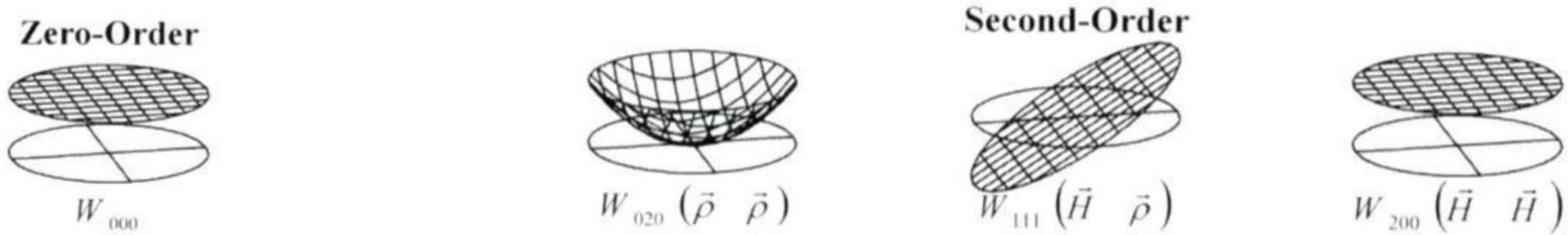




Plenty of other aberrations too.

Wave front aberrations through order 6

Aberration name	Vector form	Algebraic form	*j*	*m*	*n*
Zero order					
Uniform piston	W_{000}	W_{000}	0	0	0
Second order					
Quadratic piston	$W_{200}(\vec{H}\ \ \vec{H})$	$W_{200}H^2$	1	0	0
Magnification	$W_{111}(\vec{H}\ \ \vec{\rho})$	$W_{111}H\rho\cos(\phi)$	0	1	0
Focus	$W_{020}(\vec{\rho}\ \ \vec{\rho})$	$W_{020}\rho^2$	0	0	1
Fourth order					
Spherical aberration	$W_{040}(\vec{\rho}\ \ \vec{\rho})^2$	$W_{040}\rho^4$	0	0	2
Coma	$W_{131}(\vec{H}\ \ \vec{\rho})(\vec{\rho}\ \ \vec{\rho})$	$W_{131}H\rho^3\cos(\phi)$	0	1	1
Astigmatism	$W_{222}(\vec{H}\ \ \vec{\rho})^2$	$W_{222}H^2\rho^2\cos^2(\phi)$	0	2	0
Field curvature	$W_{220}(\vec{H}\ \ \vec{H})(\vec{\rho}\ \ \vec{\rho})$	$W_{220}H^2\rho^2$	1	0	1
Distortion	$W_{311}(\vec{H}\ \ \vec{H})(\vec{H}\ \ \vec{\rho})$	$W_{311}H^3\rho\cos(\phi)$	1	1	0
Quartic piston	$W_{400}(\vec{H}\ \ \vec{H})^2$	$W_{400}H^4$	2	0	0
Sixth order					
Oblique spherical aberration	$W_{240}(\vec{H}\ \ \vec{H})(\vec{\rho}\ \ \vec{\rho})^2$	$W_{240}H^2\rho^4$	1	0	2
Coma	$W_{331}(\vec{H}\ \ \vec{H})(\vec{H}\ \ \vec{\rho})(\vec{\rho}\ \ \vec{\rho})$	$W_{331}H^3\rho^3\cos(\phi)$	1	1	1
Astigmatism	$W_{422}(\vec{H}\ \ \vec{H})(\vec{H}\ \ \vec{\rho})^2$	$W_{422}H^4\rho^2\cos^2(\phi)$	1	2	0
Field curvature	$W_{420}(\vec{H}\ \ \vec{H})^2(\vec{\rho}\ \ \vec{\rho})$	$W_{420}H^4\rho^2$	2	0	1
Distortion	$W_{511}(\vec{H}\ \ \vec{H})^2(\vec{H}\ \ \vec{\rho})$	$W_{511}H^5\rho\cos(\phi)$	2	1	0
Piston	$W_{600}(\vec{H}\ \ \vec{H})^3$	$W_{600}H^6$	3	0	0
Spherical aberration	$W_{060}(\vec{\rho}\ \ \vec{\rho})^3$	$W_{060}\rho^6$	0	0	3
Un-named	$W_{151}(\vec{H}\ \ \vec{\rho})(\vec{\rho}\ \ \vec{\rho})^2$	$W_{151}H\rho^5\cos(\phi)$	0	1	2
Un-named	$W_{242}(\vec{H}\ \ \vec{\rho})^2(\vec{\rho}\ \ \vec{\rho})$	$W_{242}H^2\rho^4\cos^2(\phi)$	0	2	1
Un-named	$W_{333}(\vec{H}\ \ \vec{\rho})^3$	$W_{333}H^3\rho^3\cos^3(\phi)$	0	3	0



W_{000}
piston

$W_{020}\rho^2$
defocus

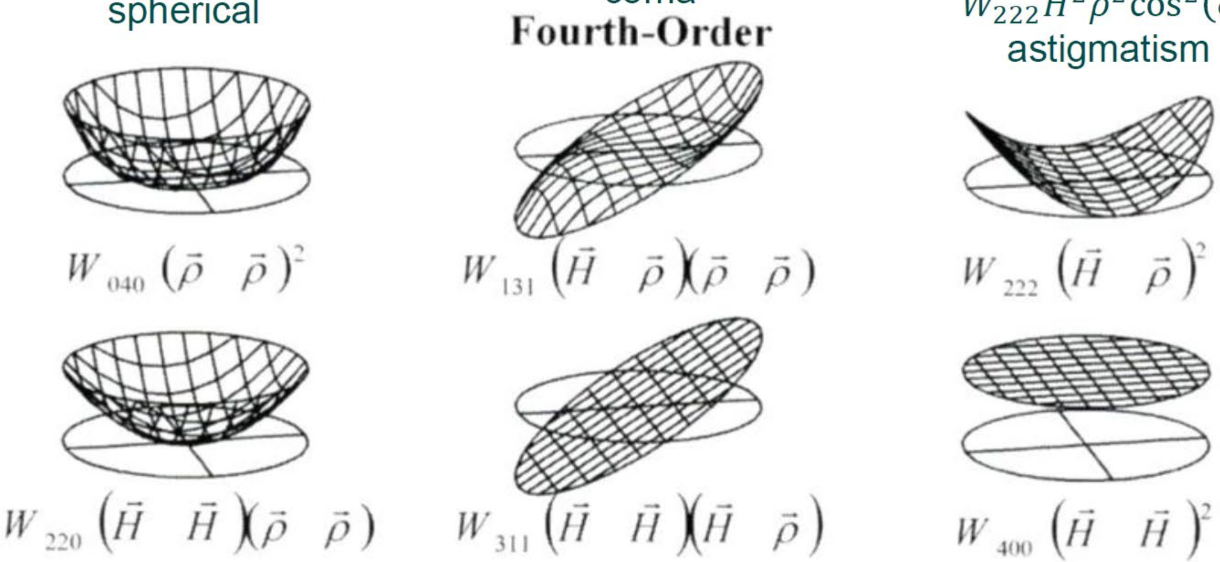
$W_{111}H\rho\cos\varphi$
tilt

$W_{200}H^2$
quadratic piston

$W_{040}\rho^4$
spherical

$W_{131}H\rho^3\cos\varphi$
coma

$W_{222}H^2\rho^2\cos^2(\varphi)$
astigmatism



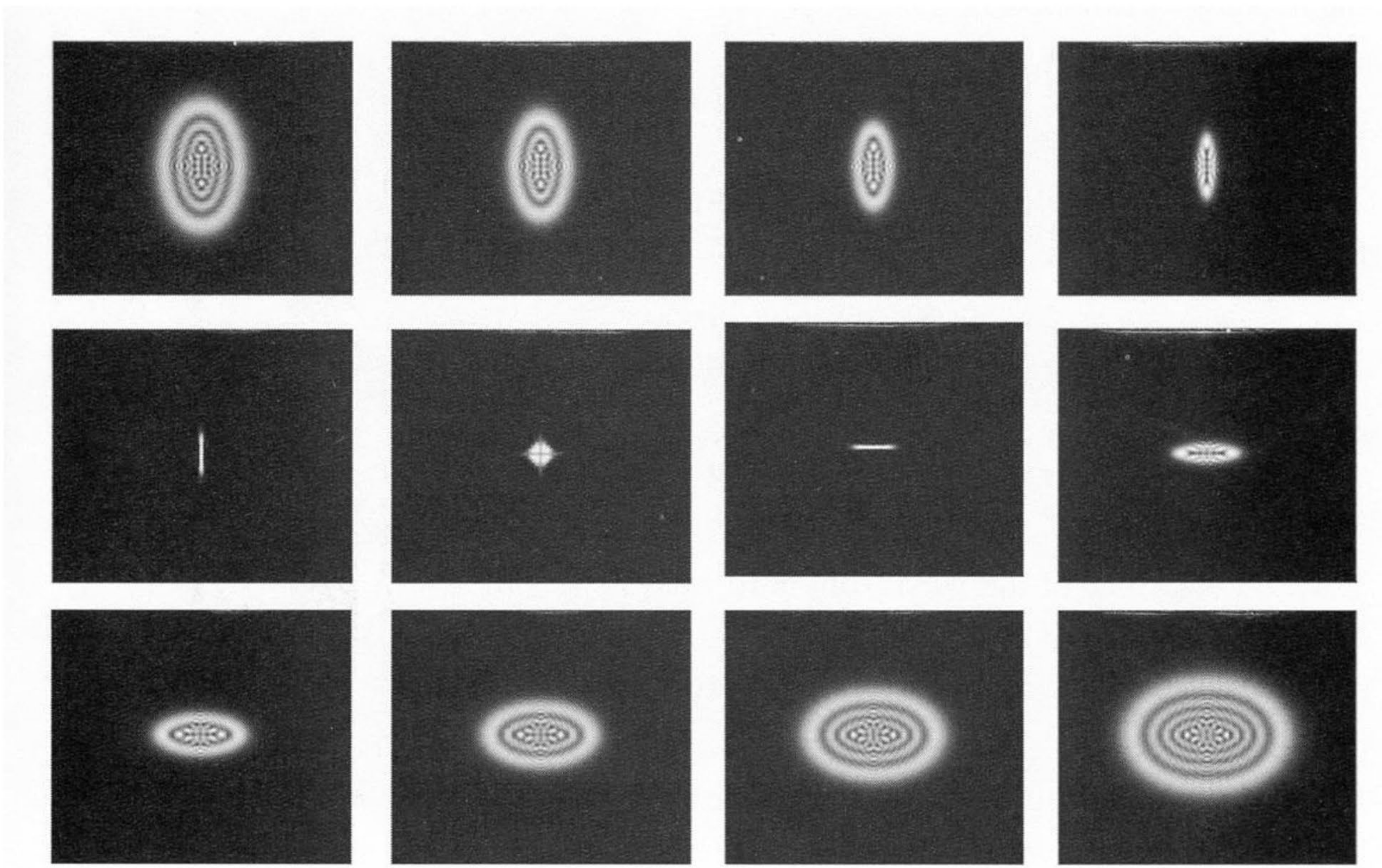
$W_{220}H^2\rho^2$
field curvature

$W_{311}H^3\rho\cos\varphi$
distortion

$W_{400}H^4$
quartic piston

Seidel aberrations

Through-focus images with pure astigmatism ($W_{222} = 2\lambda$)



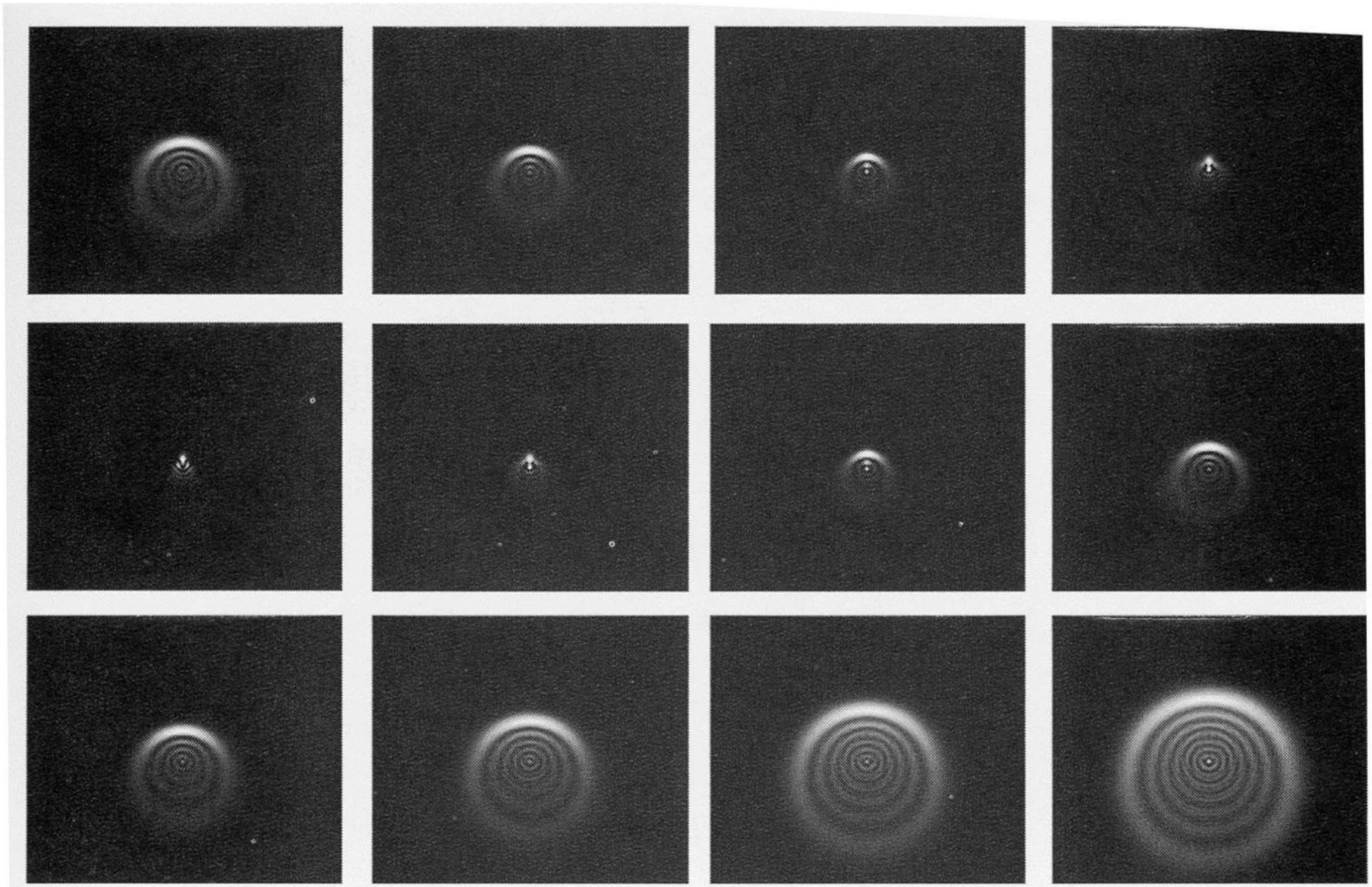
J. Sasian, *Introduction to aberrations in optical imaging systems,* Fig. 7.6

Through-focus images with pure coma ($W_{131} = 2\lambda$)

4λ 1λ

0 -3λ

-4λ -7λ



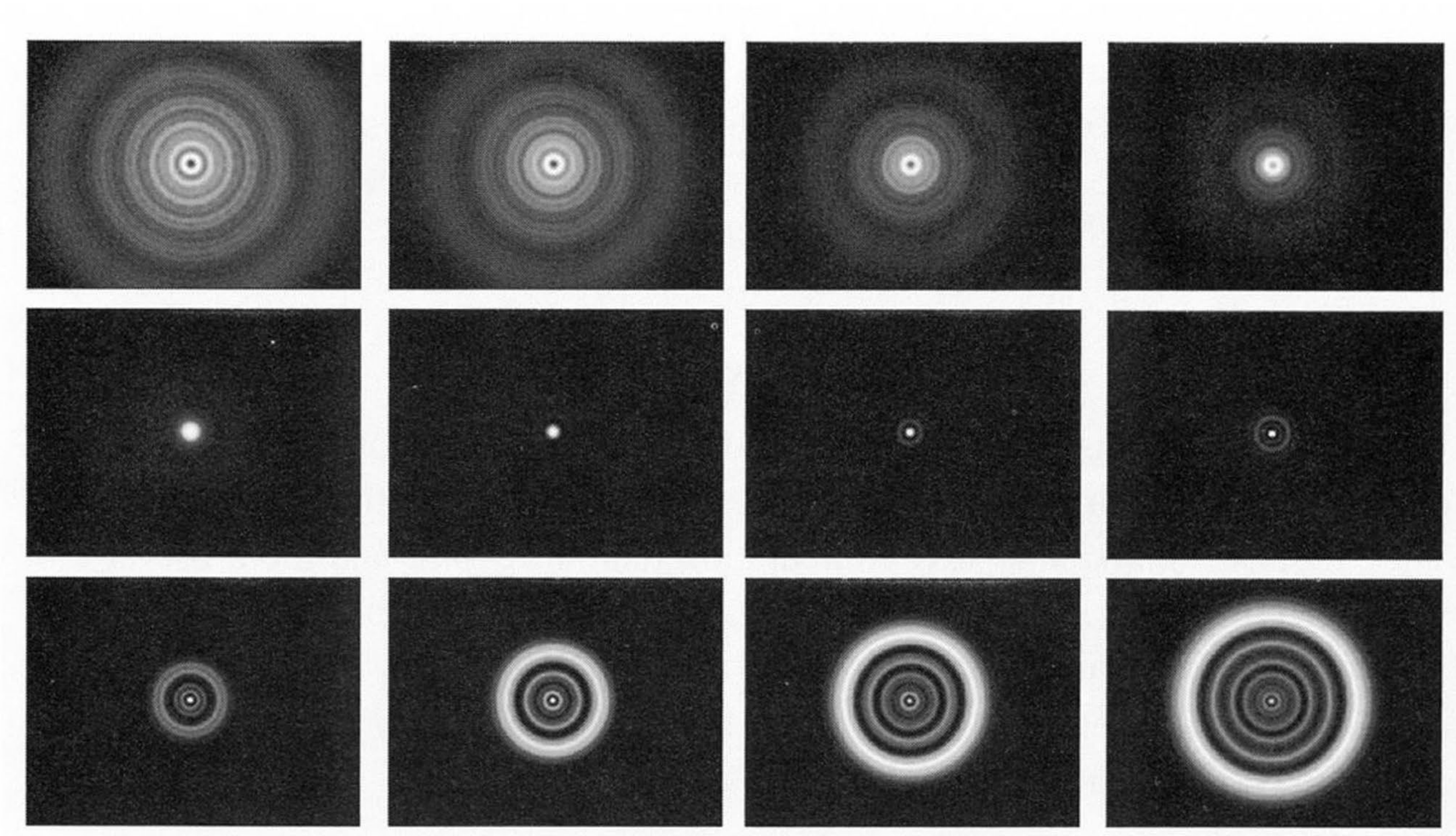
J. Sasian, *Introduction to aberrations in optical imaging systems,* Fig. 7.5

Through-focus images with pure spherical $(W_{040} = 2\lambda)$

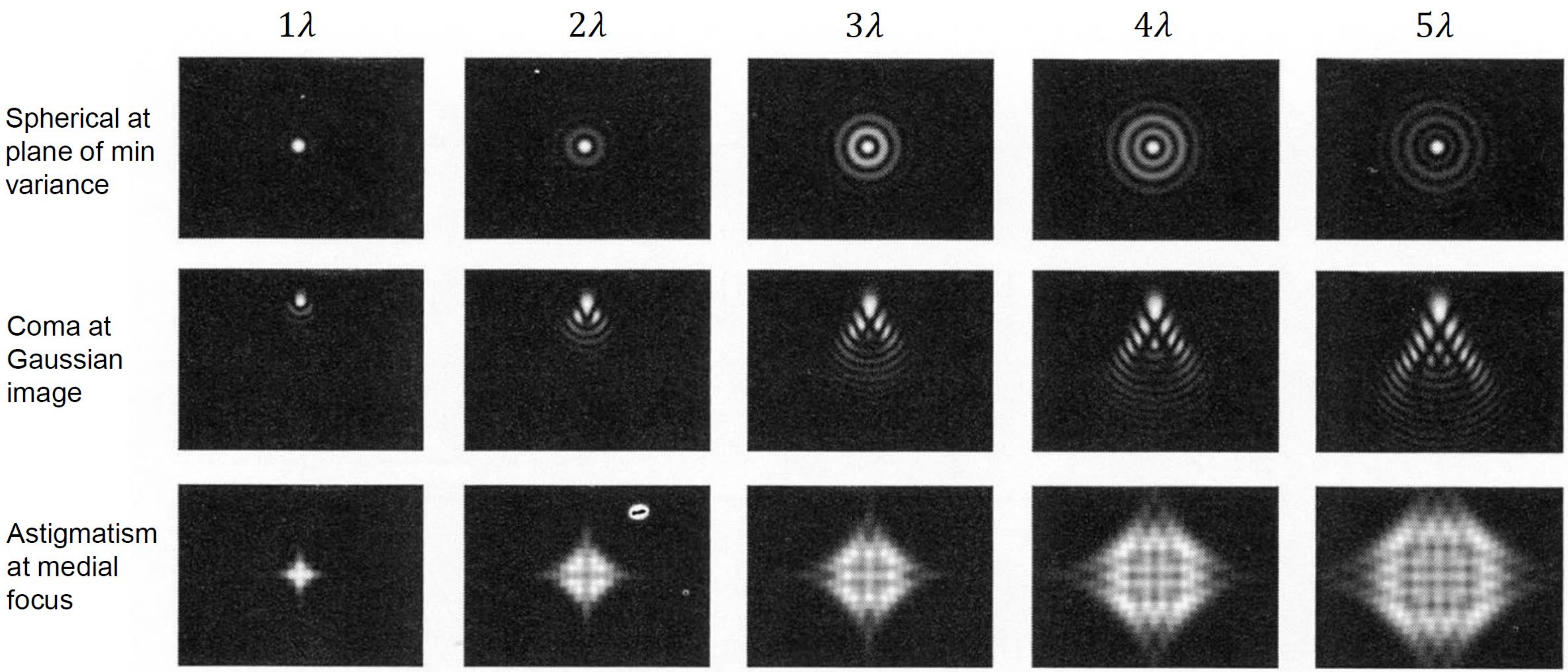
4λ 1λ

0 -3λ

-4λ -7λ

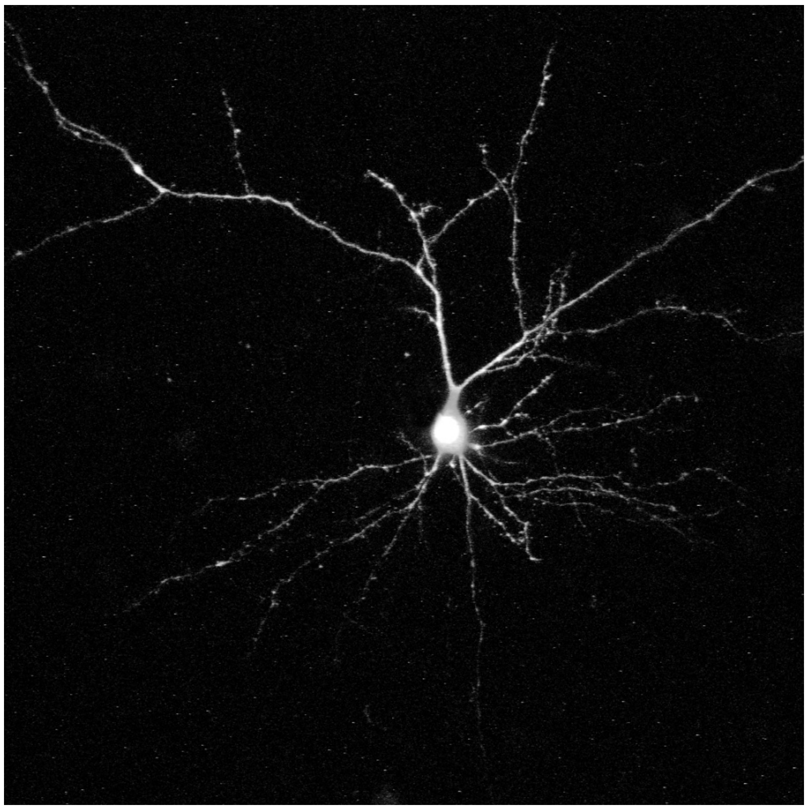


J. Sasian, *Introduction to aberrations in optical imaging systems,* Fig. 7.4

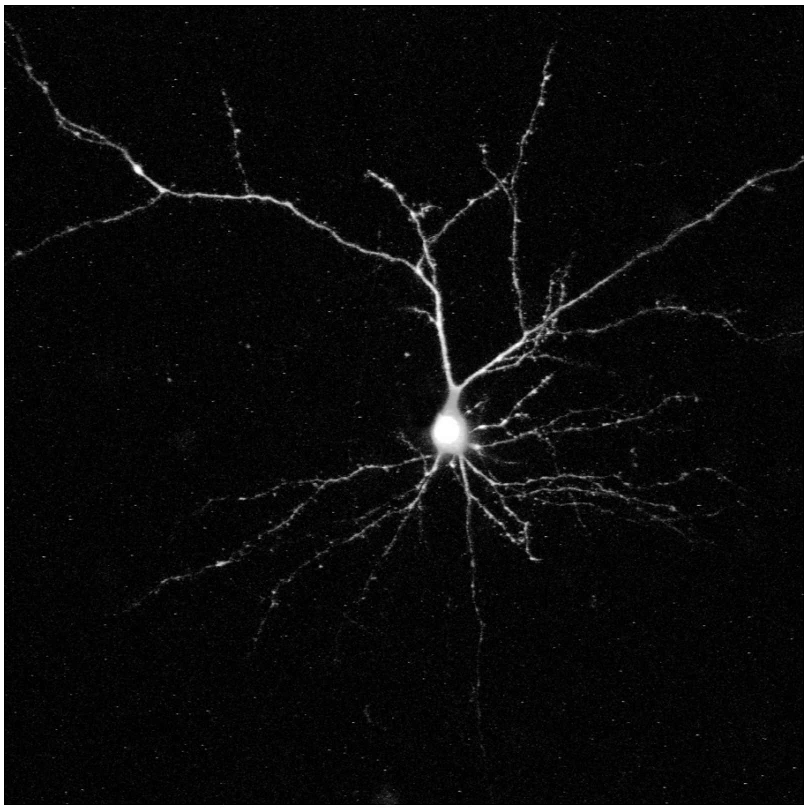


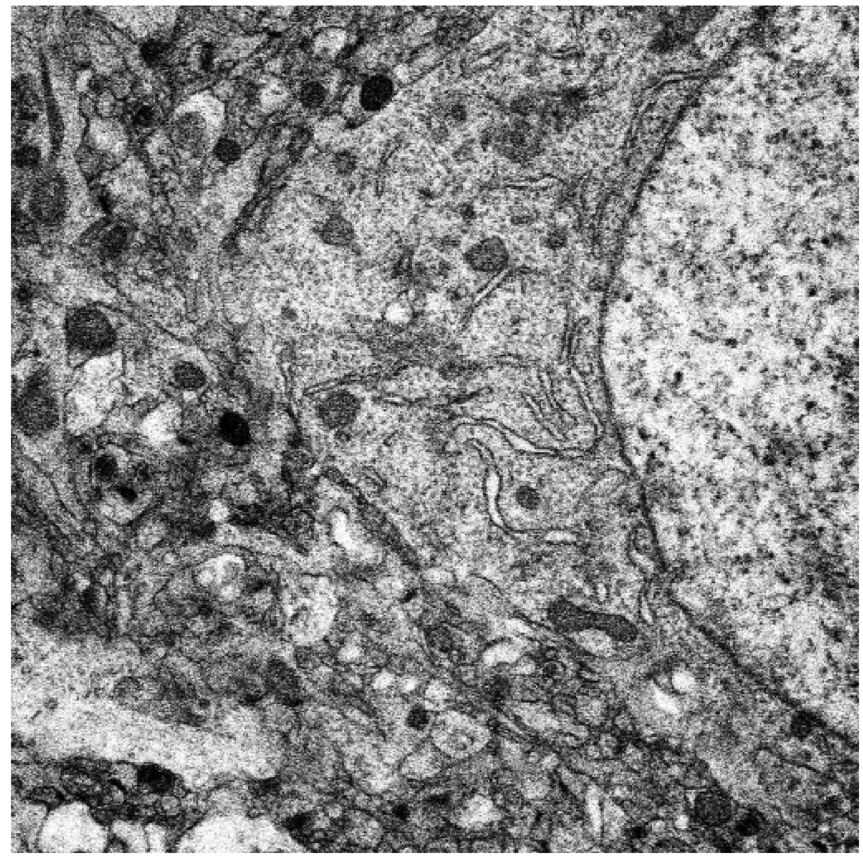
J. Sasian, *Introduction to aberrations in optical imaging systems,* Fig. 7.3

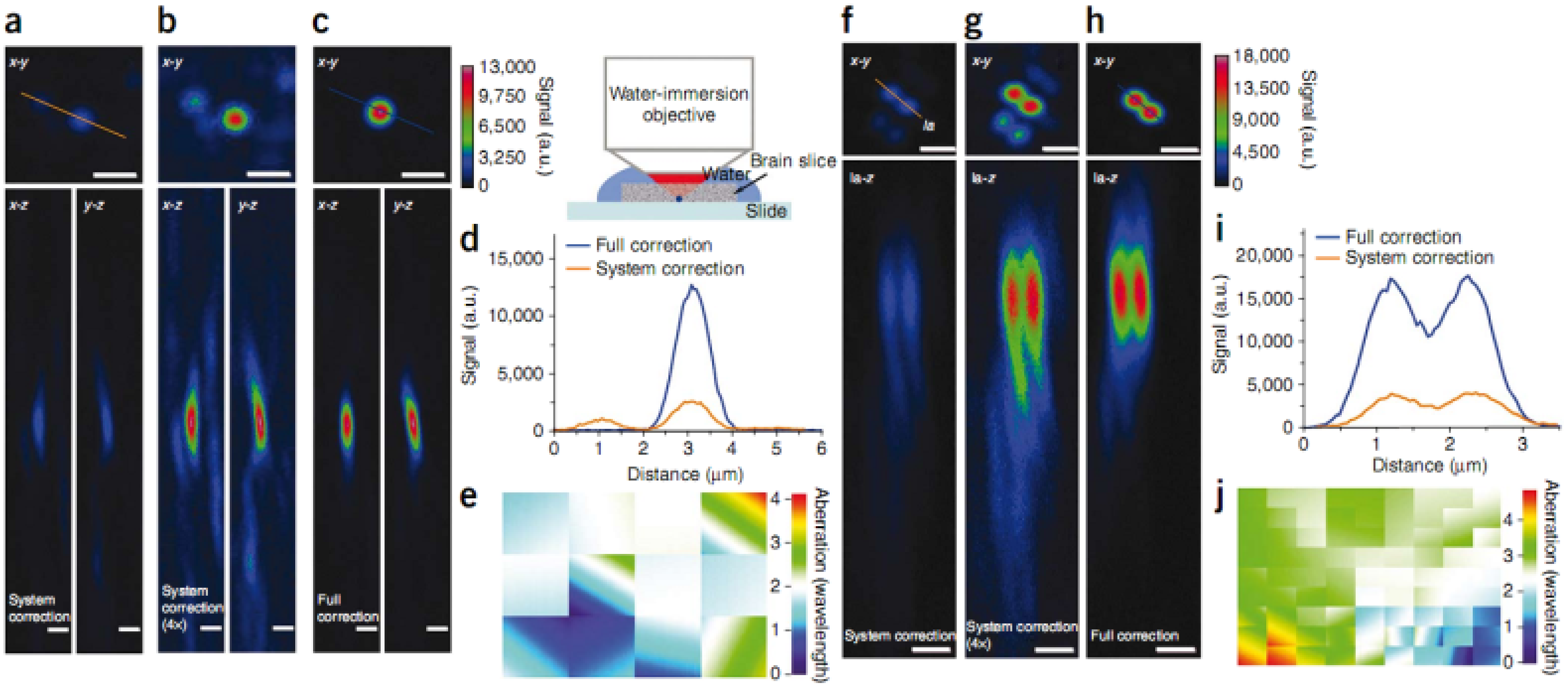
How deep can you go?



How deep can you go?







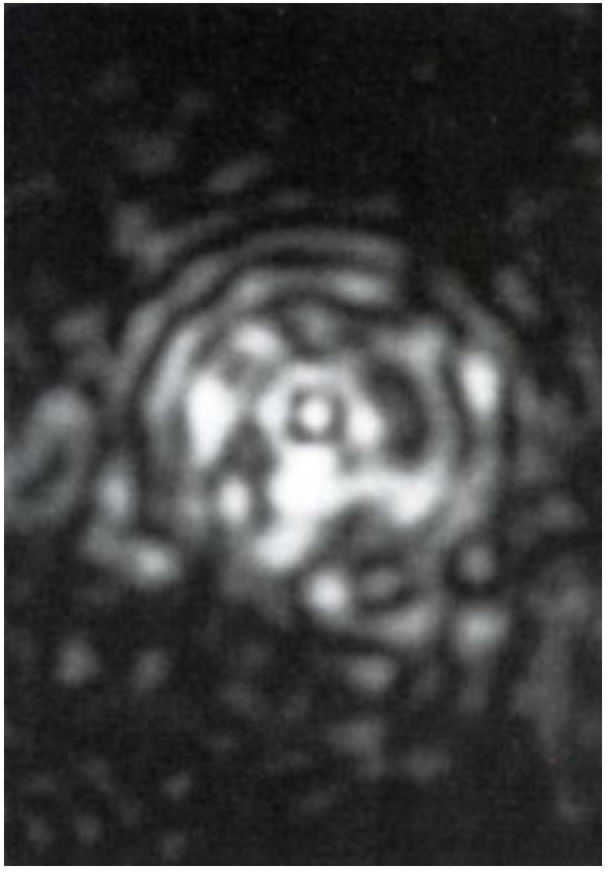
Adaptive optics via pupil segmentation for high-resolution imaging in biological tissues

Na Ji[1], Daniel E Milkie[2] & Eric Betzig[1]

NATURE METHODS | VOL.7 NO.2 | FEBRUARY 2010 |

Slice severely aberrates the PSF

Image of a sub-resolution fluorescent object
50 µm within the slice



7 January 2008 / Vol. 16, No. 1 / OPTICS EXPRESS 67

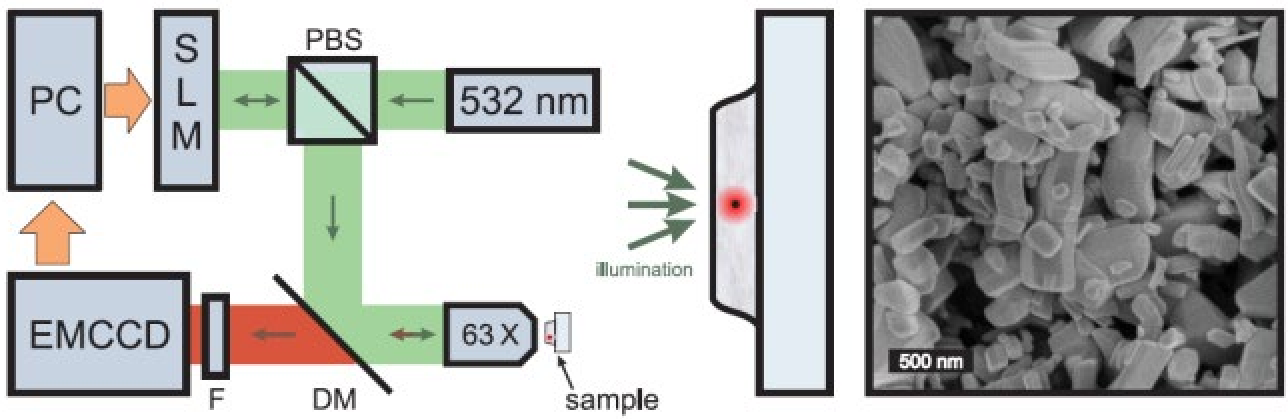
Demixing light paths inside disordered metamaterials

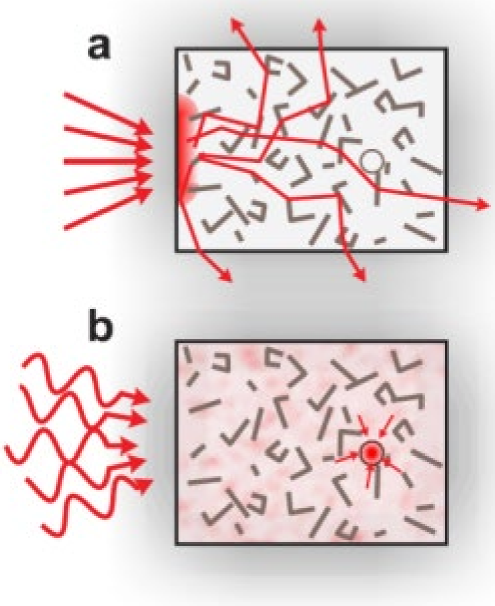
I. M. Vellekoop[1], E. G. van Putten[1], A. Lagendijk[1,2] and A. P. Mosk[1]

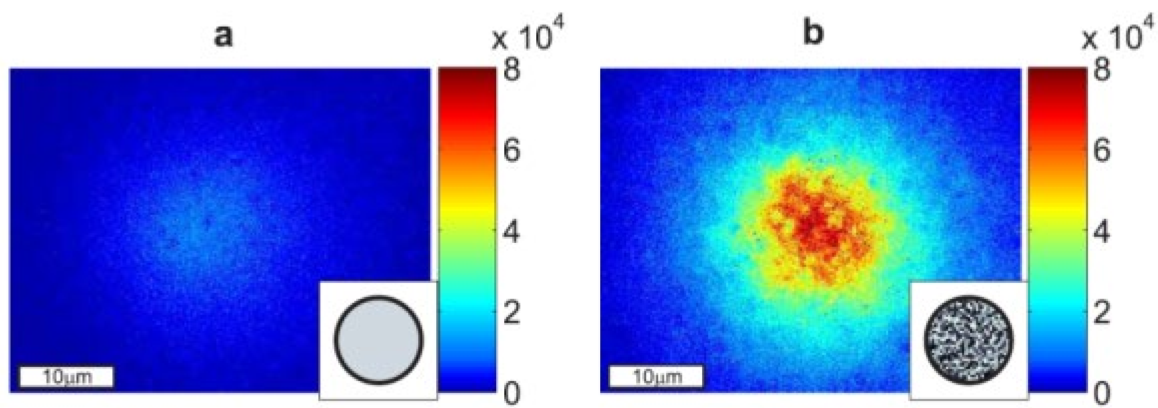
[1]*Complex Photonic Systems, Faculty of Science and Technology and MESA+ Institute for Nanotechnology, University of Twente, P.O.Box 217, 7500 AE Enschede, The Netherlands*

[2]*FOM Institute for Atomic and Molecular Physics, Kruislaan 407, 1098 SJ Amsterdam, The*

a







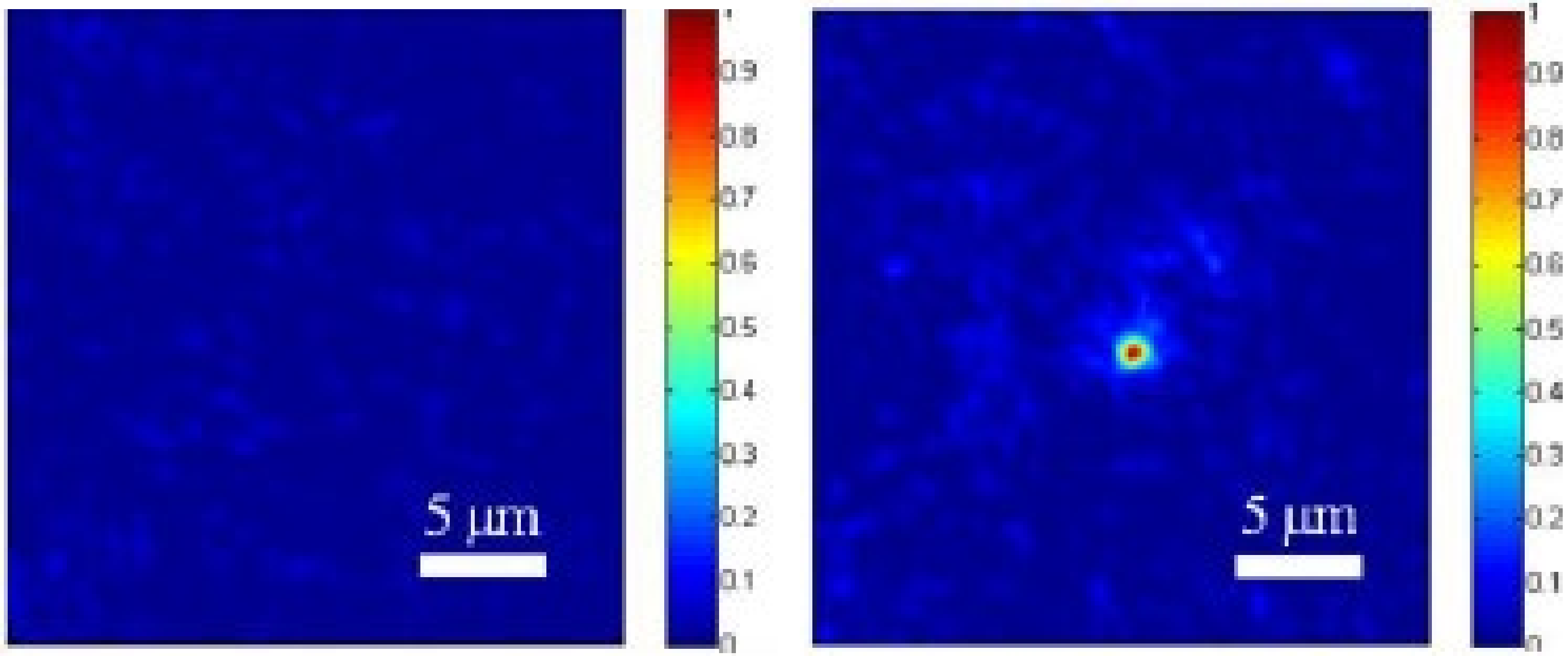
High-speed scattering medium characterization with application to focusing light through turbid media

Donald B. Conkey,* Antonio M. Caravaca-Aguirre, and Rafael Piestun

Department of Electrical, Computer, and Energy Engineering, University of Colorado, Boulder, Colorado 80309, USA

**Donald.Conkey@Colorado.edu*

16 January 2012 / Vol. 20, No. 2 / OPTICS EXPRESS 1739



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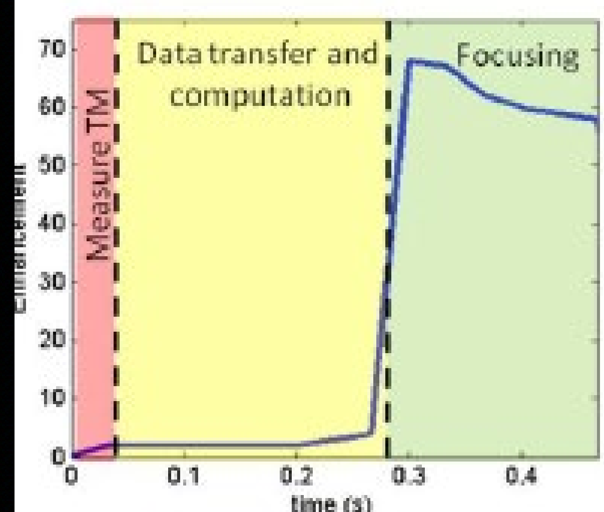
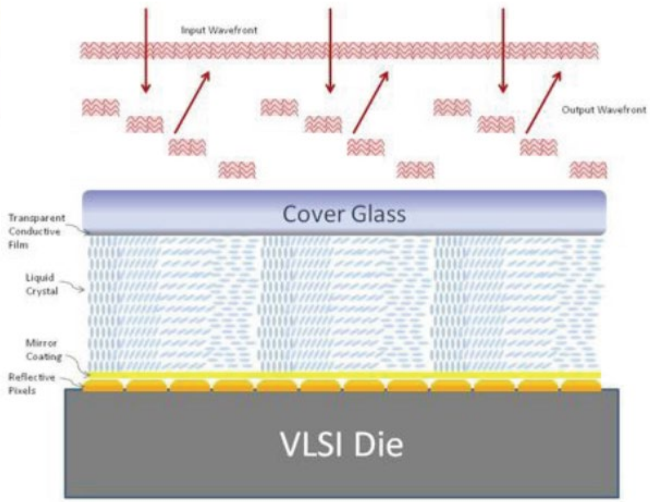
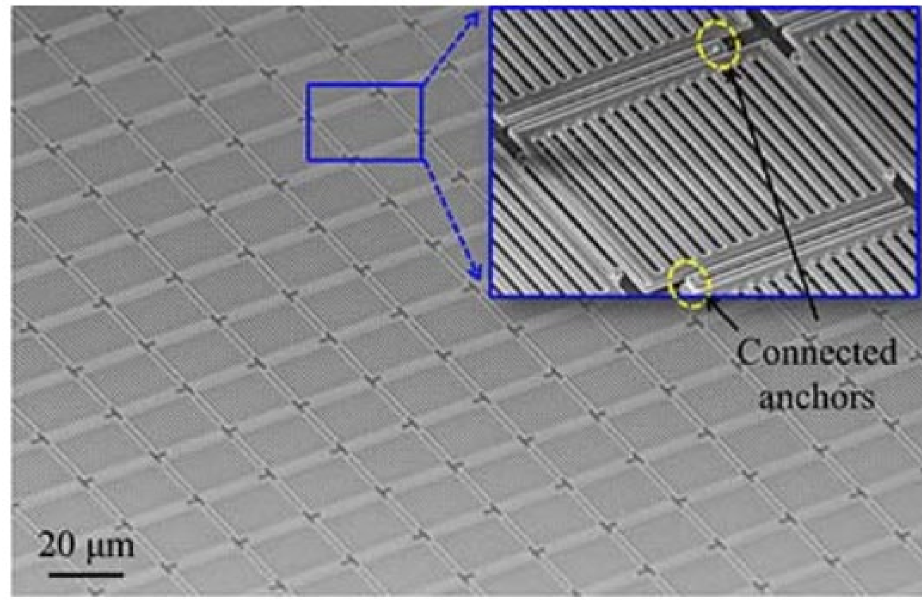


Spatial Light Modulator (SLM)



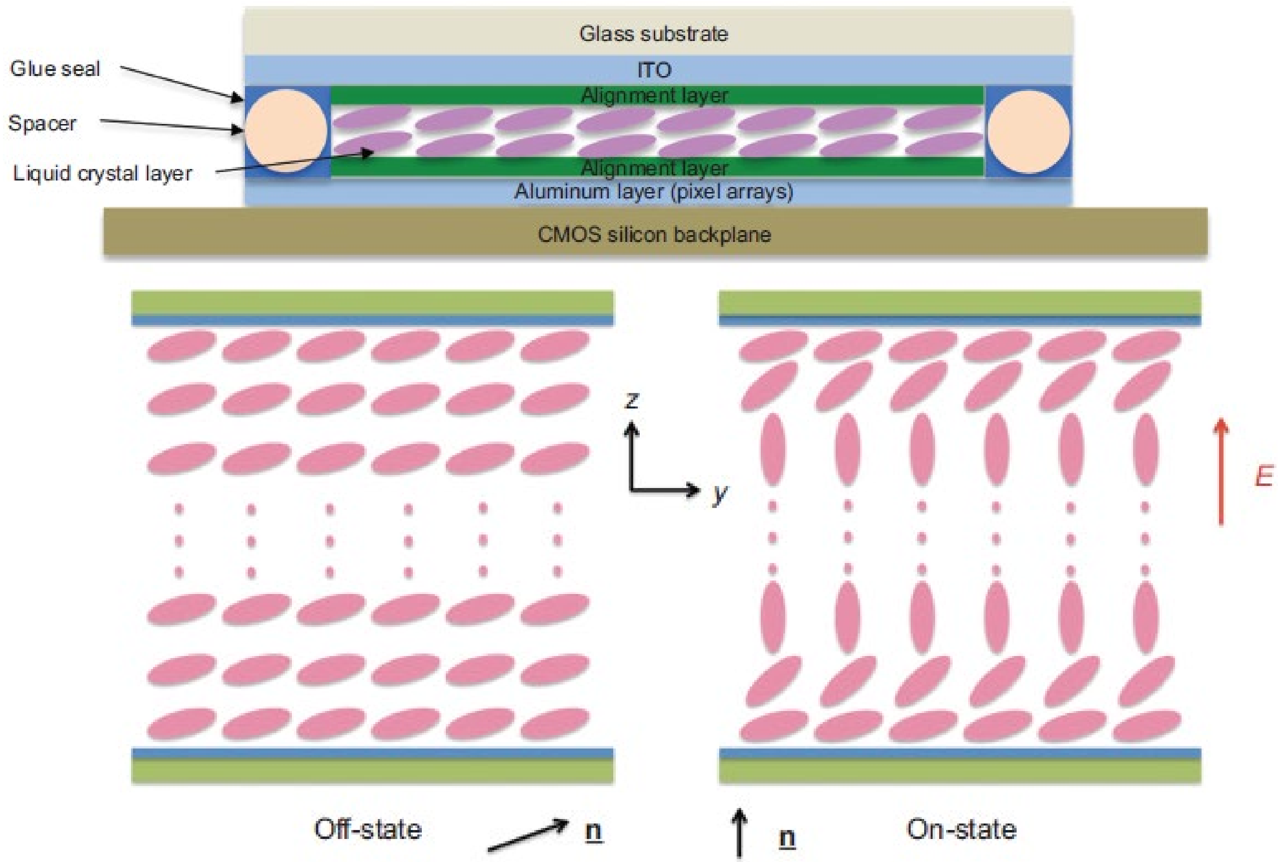


Liquid crystal array

Microelectromechanical Mirror Array

Flexible control of the phase of light – programmable diffraction

SLM limitations?



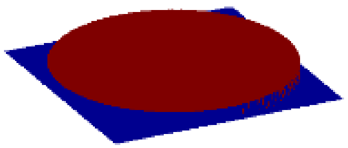
$\delta = 2\pi \Delta n d / \lambda$ $\delta = 2\pi \Delta n 2d / \lambda$ $d = \lambda / 2\Delta n$

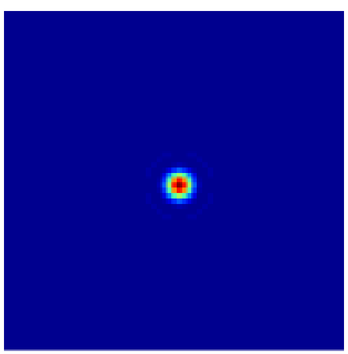
Fundamentals of phase-only liquid crystal on silicon (LCOS) devices

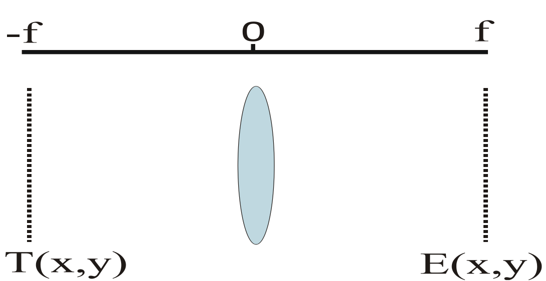
Light: Science & Applications (2014) **3**, e213; doi:10.1038/lsa.2014.94; published online 24 October 2014

Controlling light, flexibly

SLM Phase Pattern – mimics an arbitrary glass surface



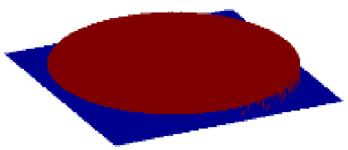


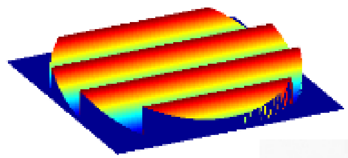


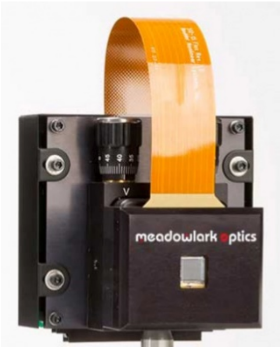
$$E_f(x,y) = C\iint T_{-f}\left(x_{-f},y_{-f}\right)\cdot \exp\left[-\frac{ik}{f}\left(x_{-f}x_f + y_{-f}y_f\right)\right]dx_{-f}dy_{-f}$$

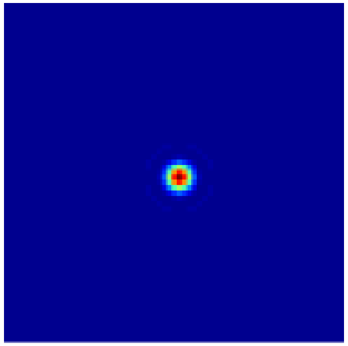
Controlling light, flexibly

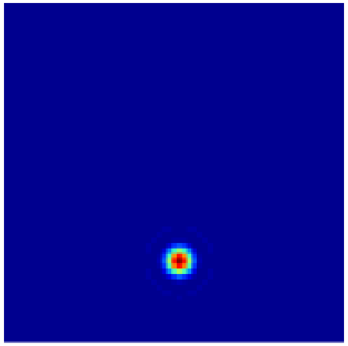
SLM Phase Pattern – mimics an arbitrary glass surface

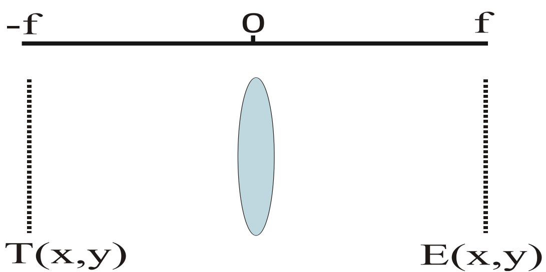








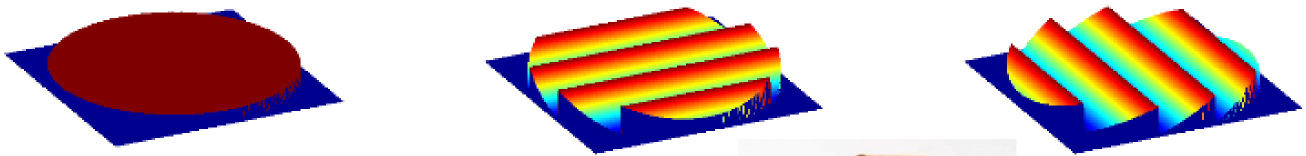


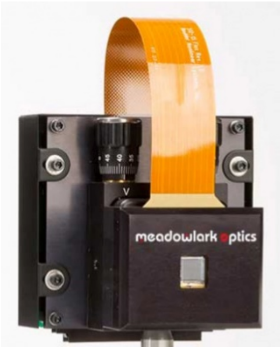


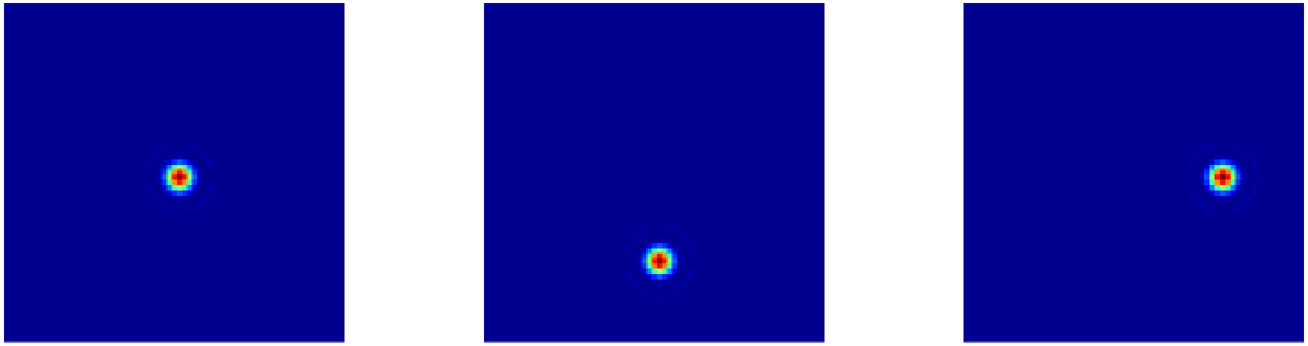
$$E_f(x,y) = C \iint T_{-f}(x_{-f}, y_{-f}) \cdot \exp\left[-\frac{ik}{f}\left(x_{-f}x_f + y_{-f}y_f\right)\right] dx_{-f}dy_{-f}$$

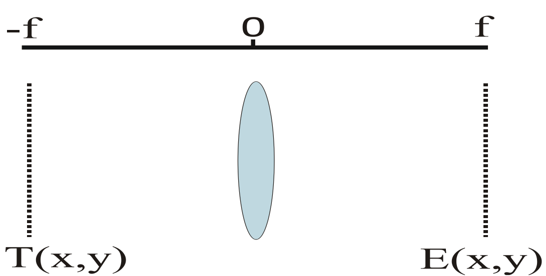
Controlling light, flexibly

SLM Phase Pattern – mimics an arbitrary glass surface









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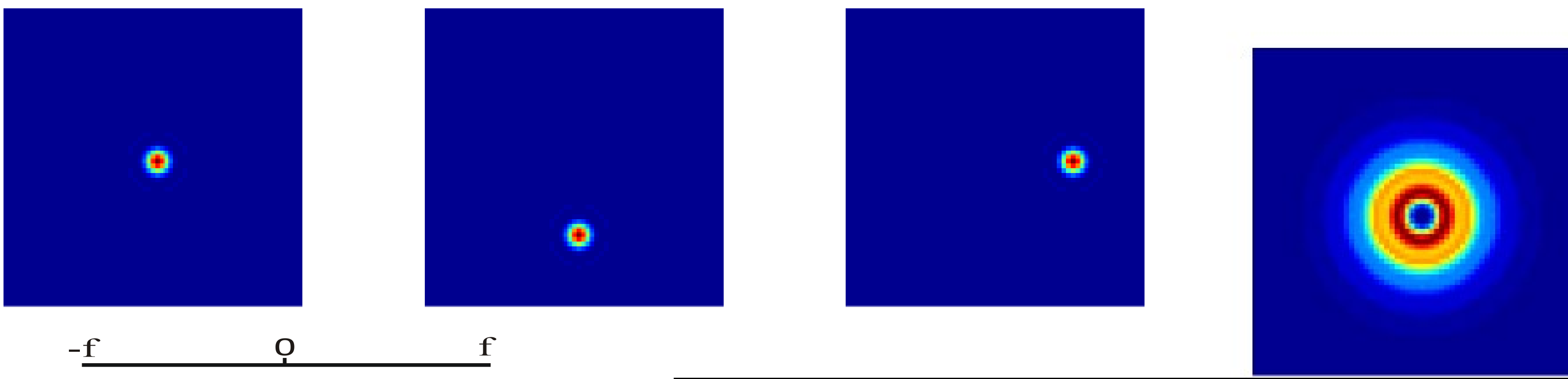

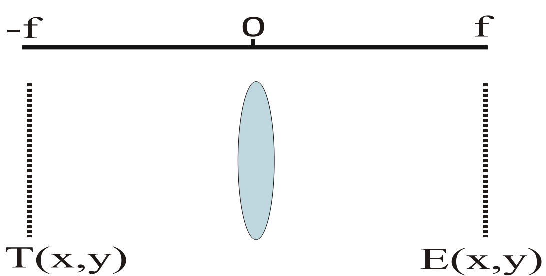

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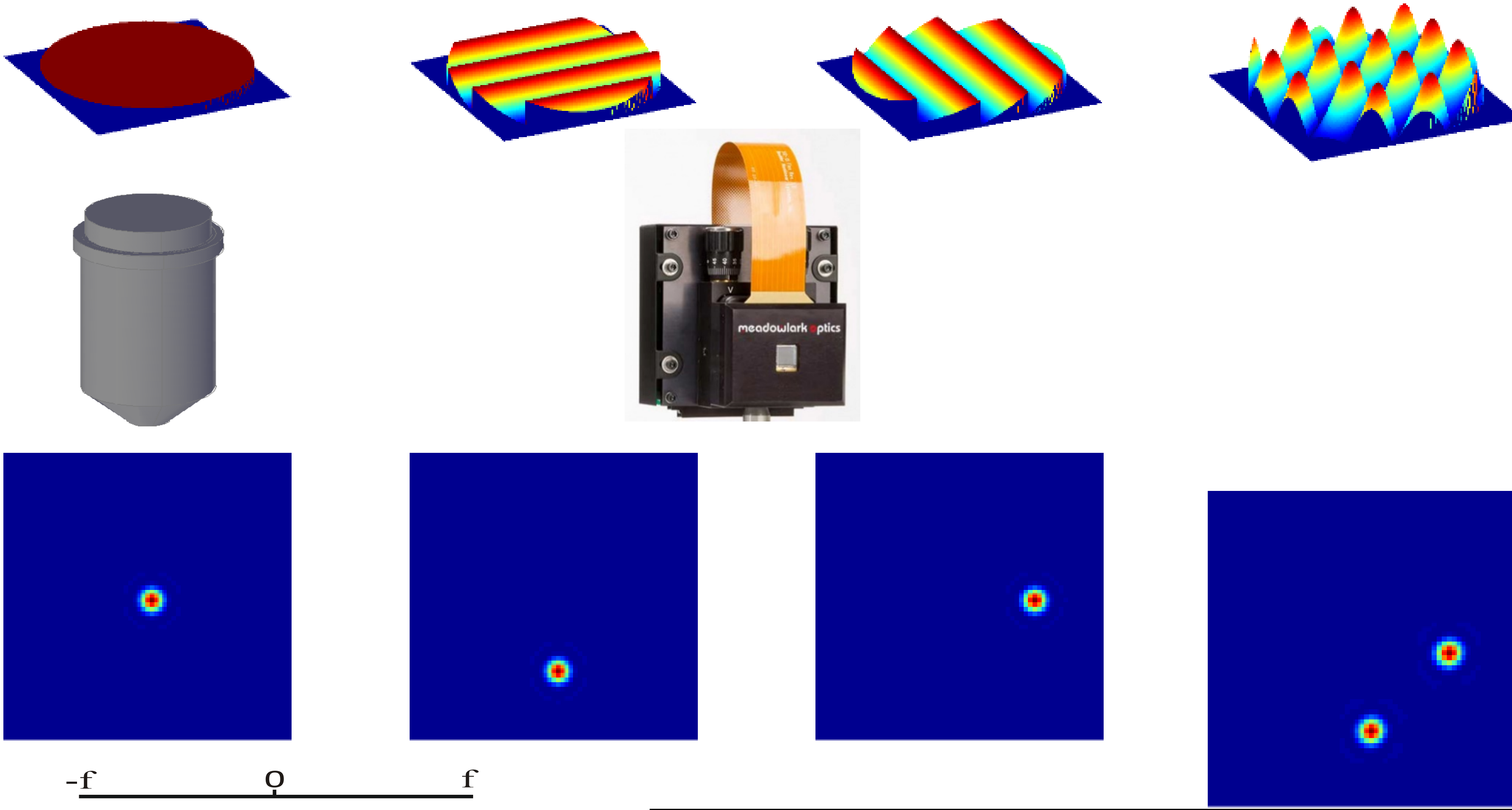
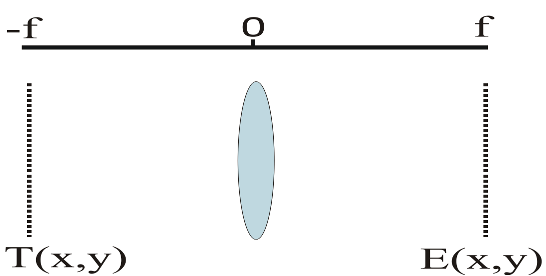
Controlling light, flexibly

SLM Phase Pattern – mimics an arbitrary glass surface





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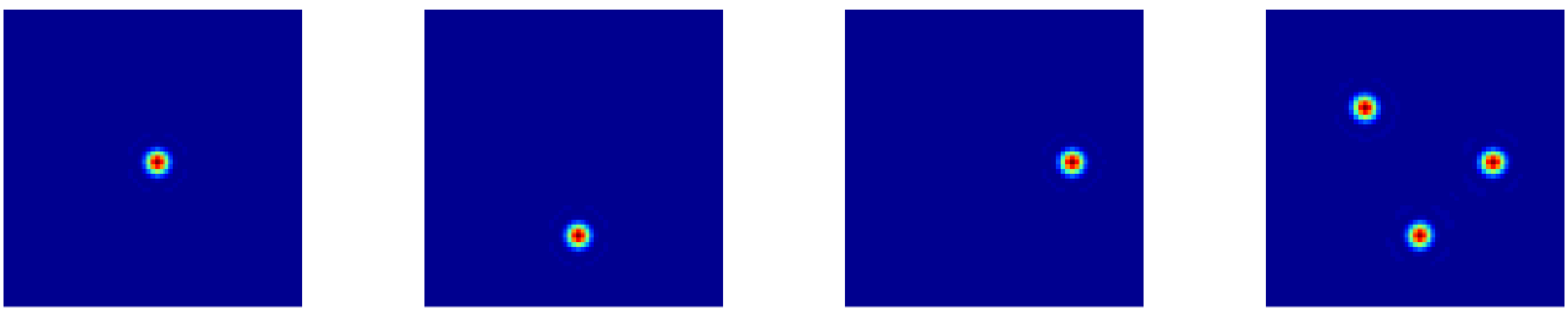

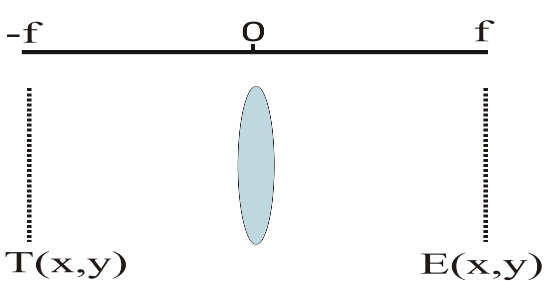

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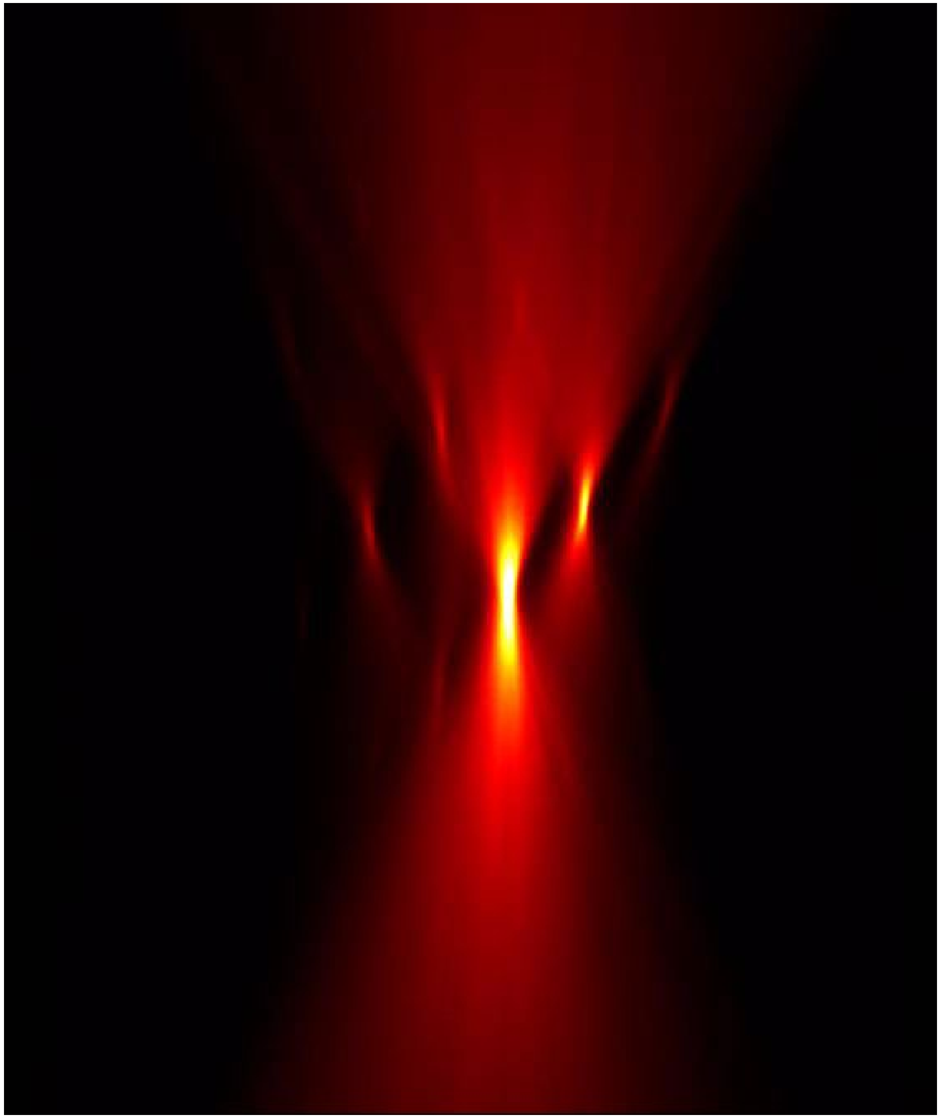
Spatial Light Modulators: arbitrary shaping of light 3D



XZ view

CONTRAST

Slide taken from D.J. Uhlinger

CONTRAST

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

Slide taken from D.J. Uhlinger

CONTRAST

50 Units 50 50

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

50 Units

Slide taken from D.J. Uhlinger

0 Units

CONTRAST

50 Units 50 50

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

50 Units

Slide taken from D.J. Uhlinger

0 Units

100 Units

CONTRAST

50 Units

50

50

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

50 Units

Slide taken from D.J. Uhlinger

0 Units 50 Units 100 Units

CONTRAST

50 Units 50 50

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

50 Units

Slide taken from D.J. Uhlinger

0 Units

50 Units

100 Units

CONTRAST

50 Units

50

50

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

50 – 0 / 50 + 0 = 1

50 Units

Slide taken from D.J. Uhlinger

0 Units · 50 Units · 100 Units

CONTRAST

50 Units · 50 · 50

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

50 Units

50 – 0 / 50 + 0 = 1

50 – 100 / 50 + 100 = -0.33

Slide taken from D.J. Uhlinger

0 Units 50 Units 100 Units

CONTRAST

50 Units 50 50

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

50 – 0 / 50 + 0 = 1

50 Units

50 – 50 / 50 + 50 = 0

50 – 100 / 50 + 100 = -0.33

Slide taken from D.J. Uhlinger

Our job: measuring grass

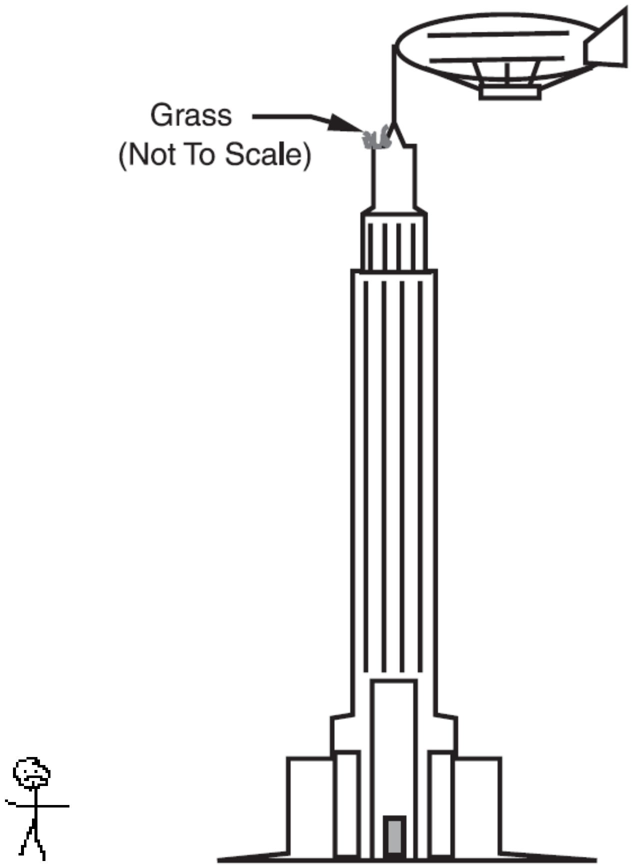


Figure 10.1 Background signals.

Our job: measuring grass

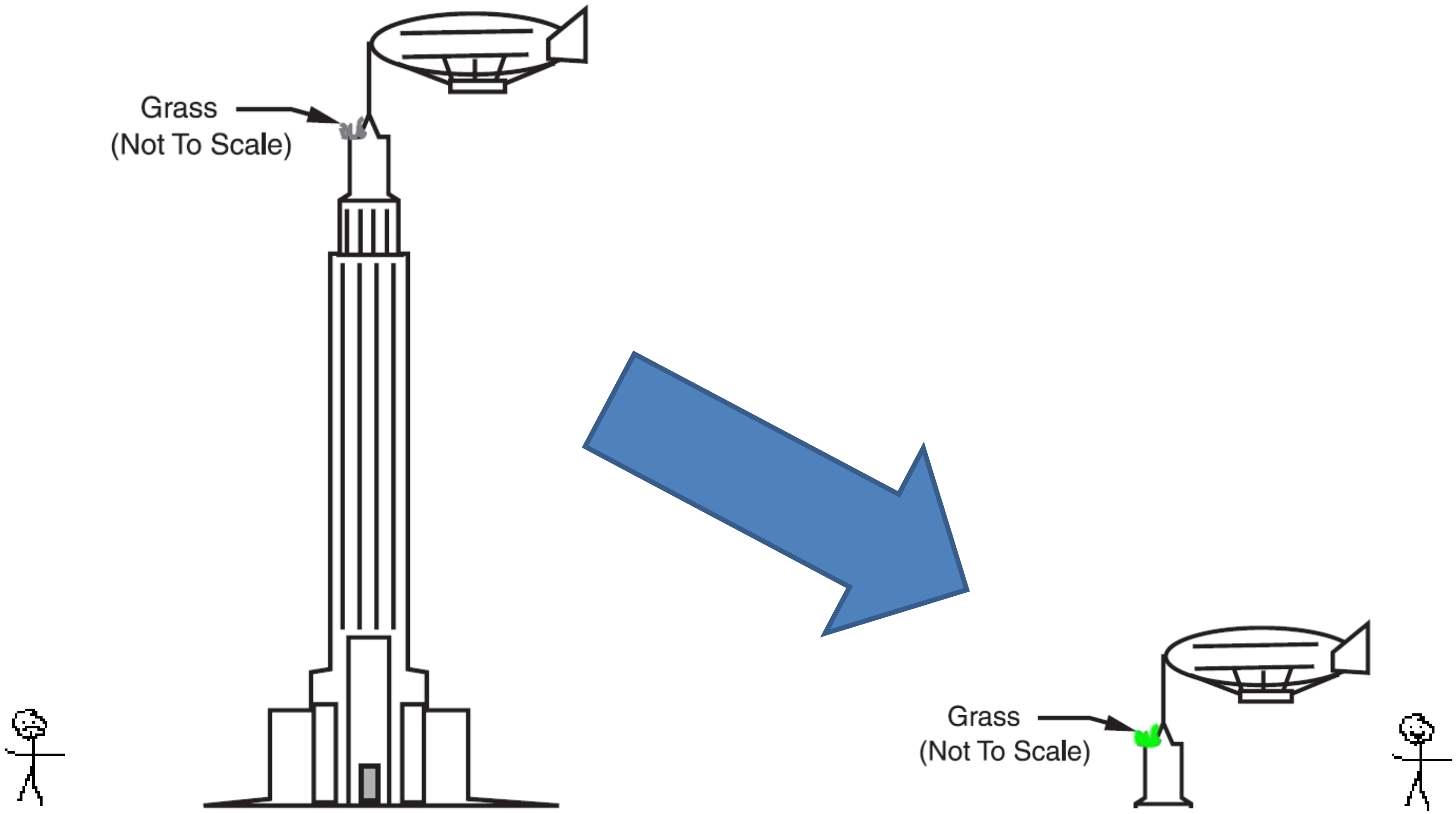
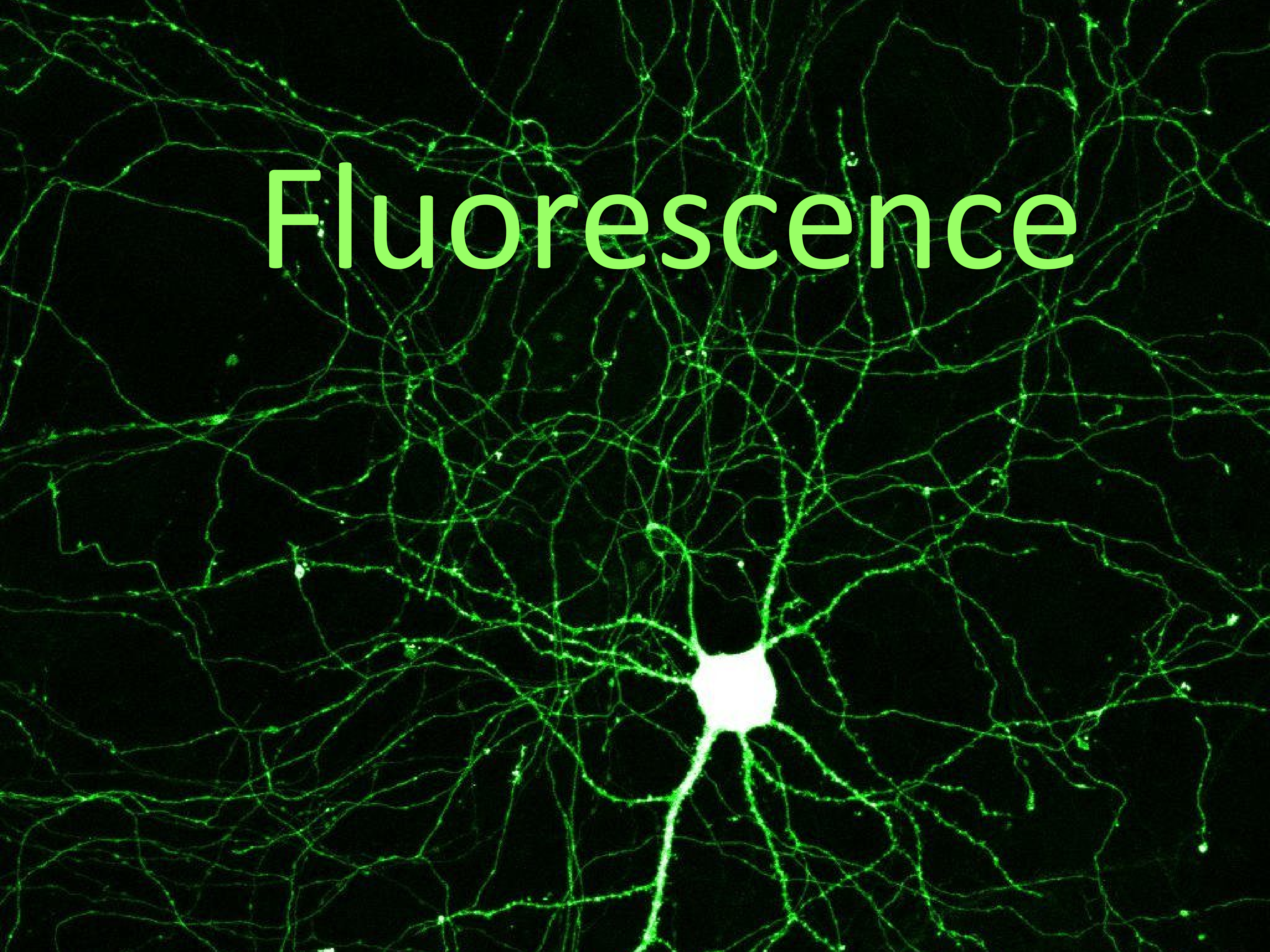
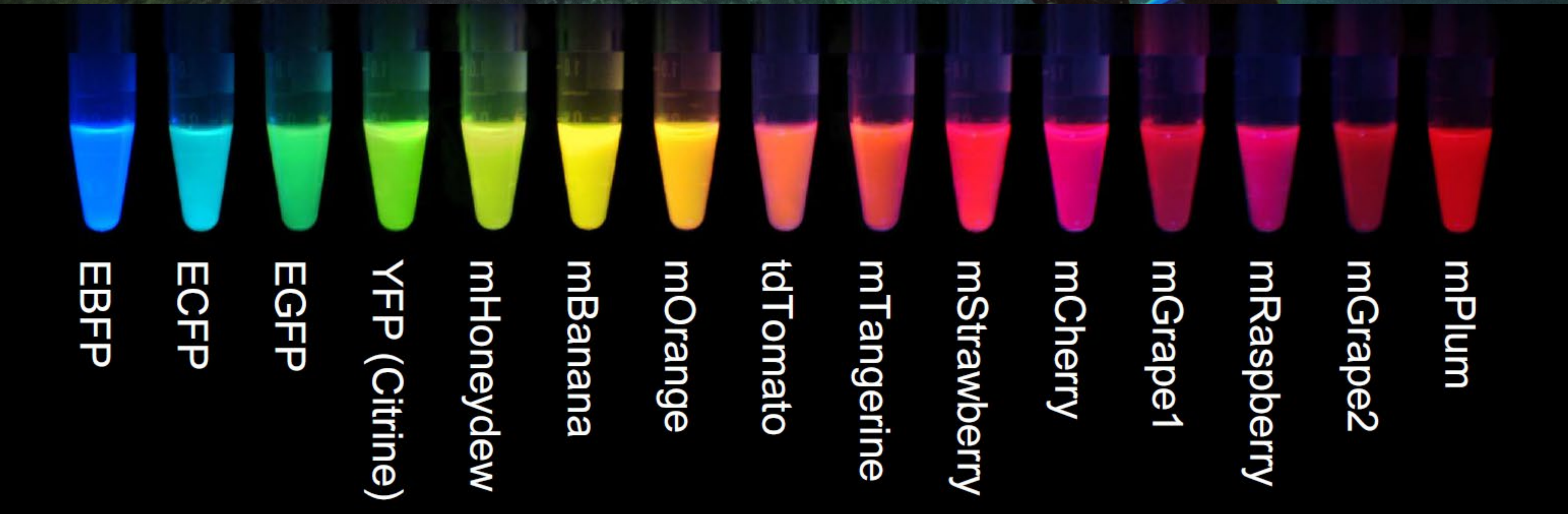
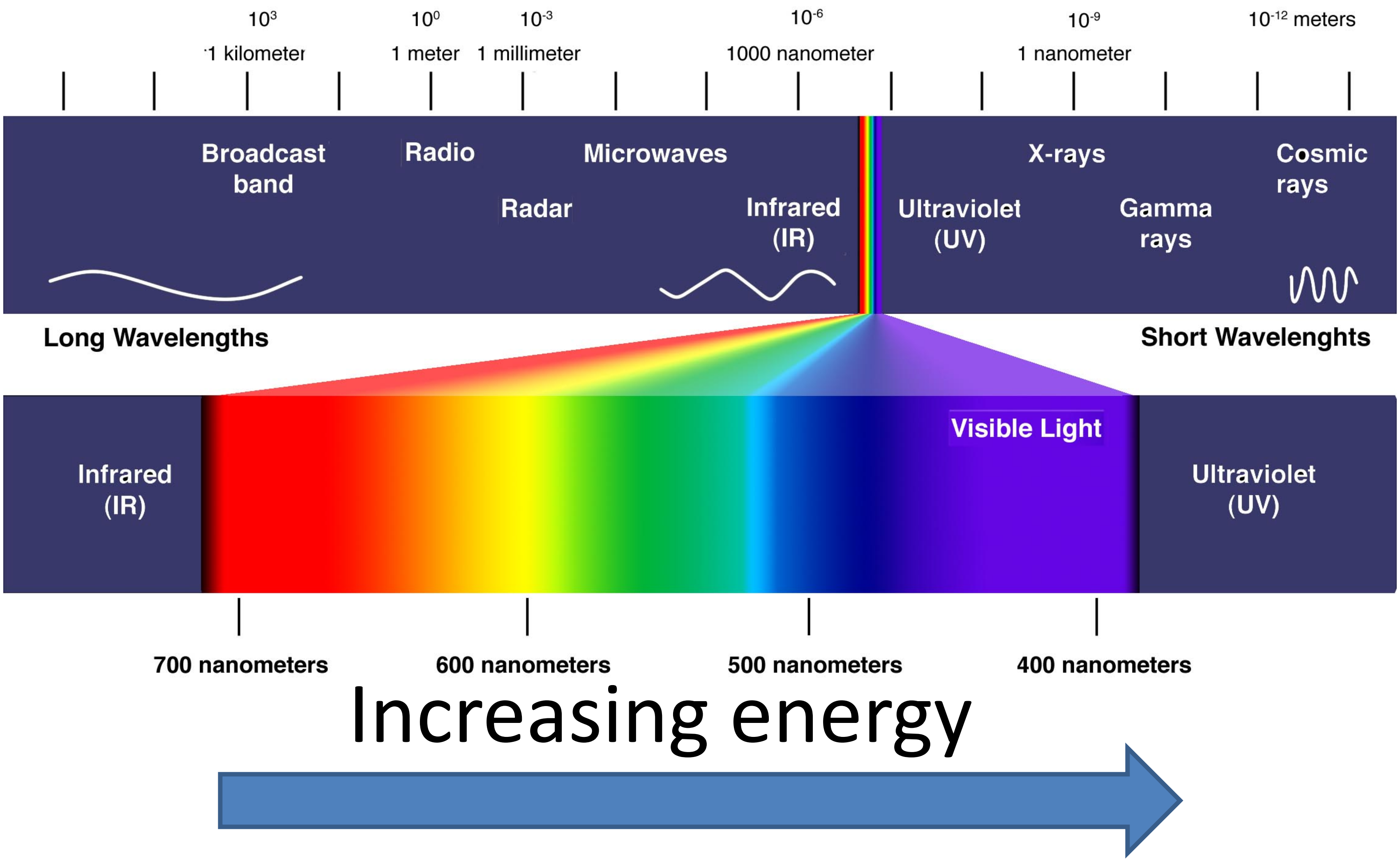


Figure 10.1 Background signals.

Fluorescence



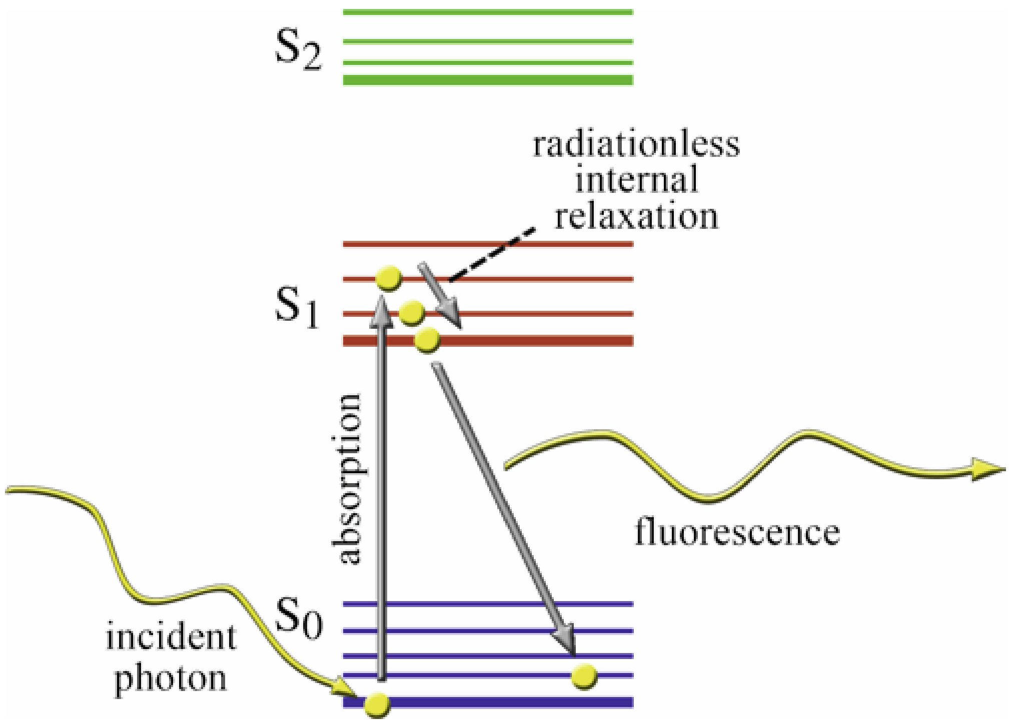

EBFP
ECFP
EGFP
YFP (Citrine)
mHoneydew
mBanana
mOrange
tdTomato
mTangerine
mStrawberry
mCherry
mGrape1
mRaspberry
mGrape2
mPlum



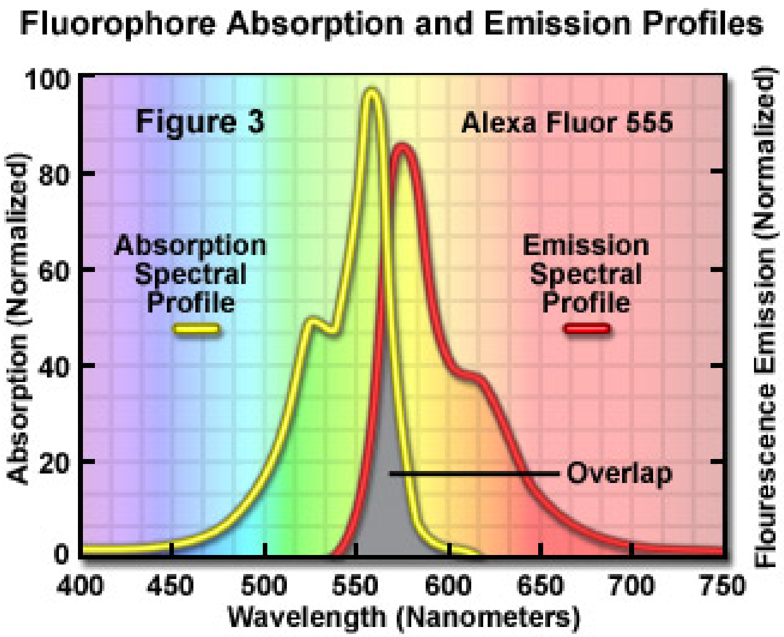
10^3
1 kilometer
10^0
1 meter
10^-3
1 millimeter
10^-6
1000 nanometer
10^-9
1 nanometer
10^-12 meters
Broadcast band
Radio
Microwaves
Radar
Infrared (IR)
X-rays
Cosmic rays
Ultraviolet (UV)
Gamma rays
Long Wavelengths
Short Wavelenghts
Infrared (IR)
Visible Light
Ultraviolet (UV)
700 nanometers
600 nanometers
500 nanometers
400 nanometers
Increasing energy

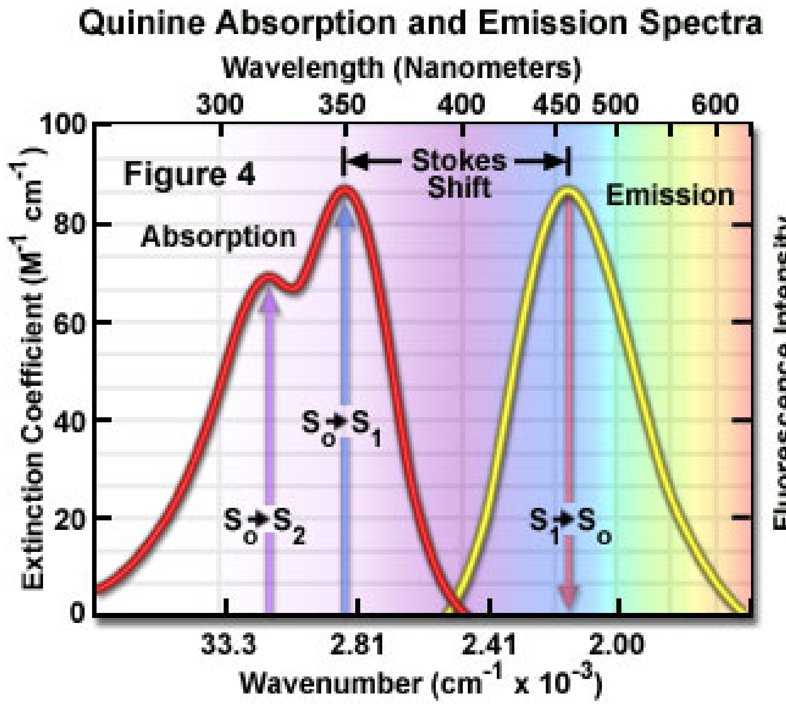
Principle of Fluorescence

1. Energy is absorbed by the molecule which becomes excited.
2. The electron jumps to a higher energy level.
3. Soon, the electron drops back to the ground state, emitting a photon (or a packet of light) - the molecule is fluorescing.

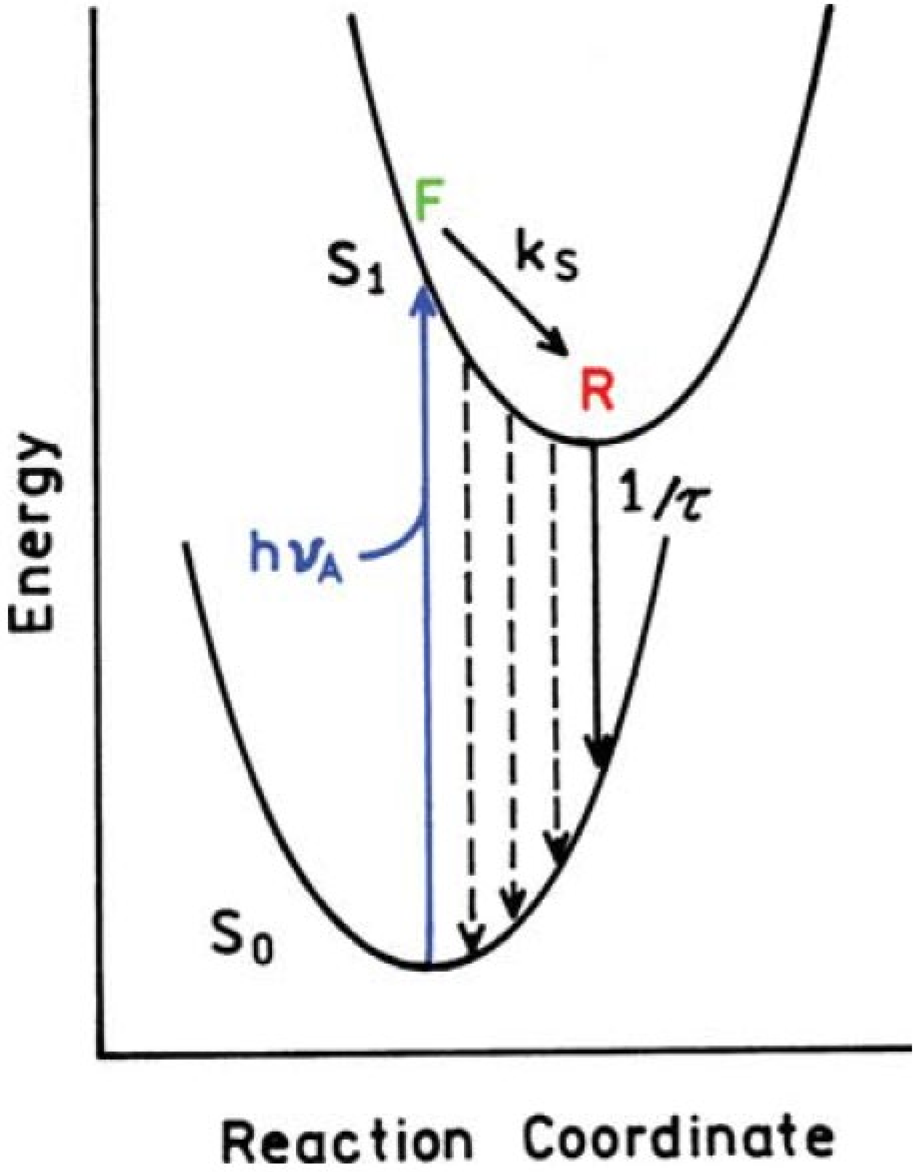


Fluorescence Stoke's shift

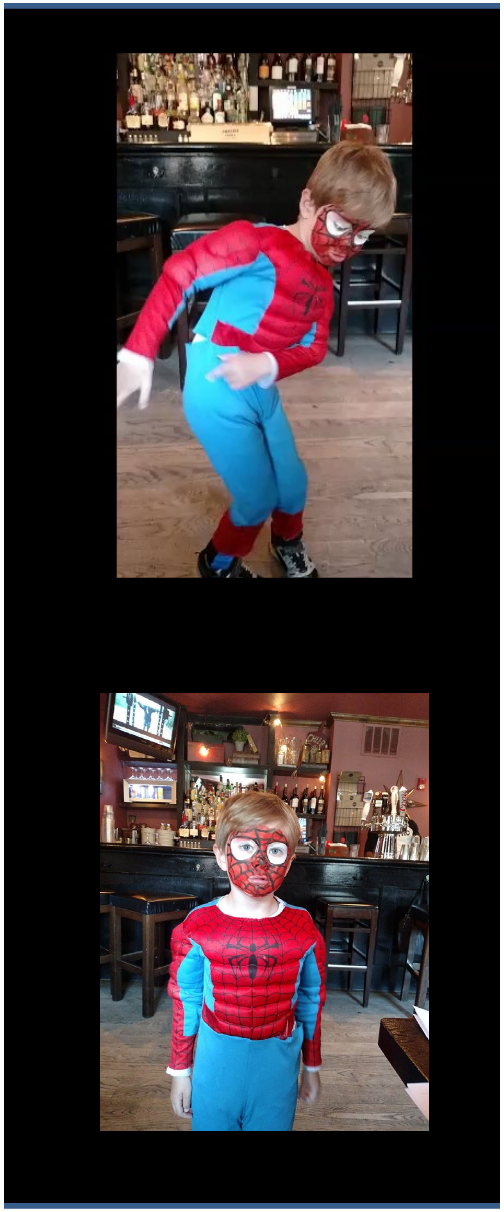


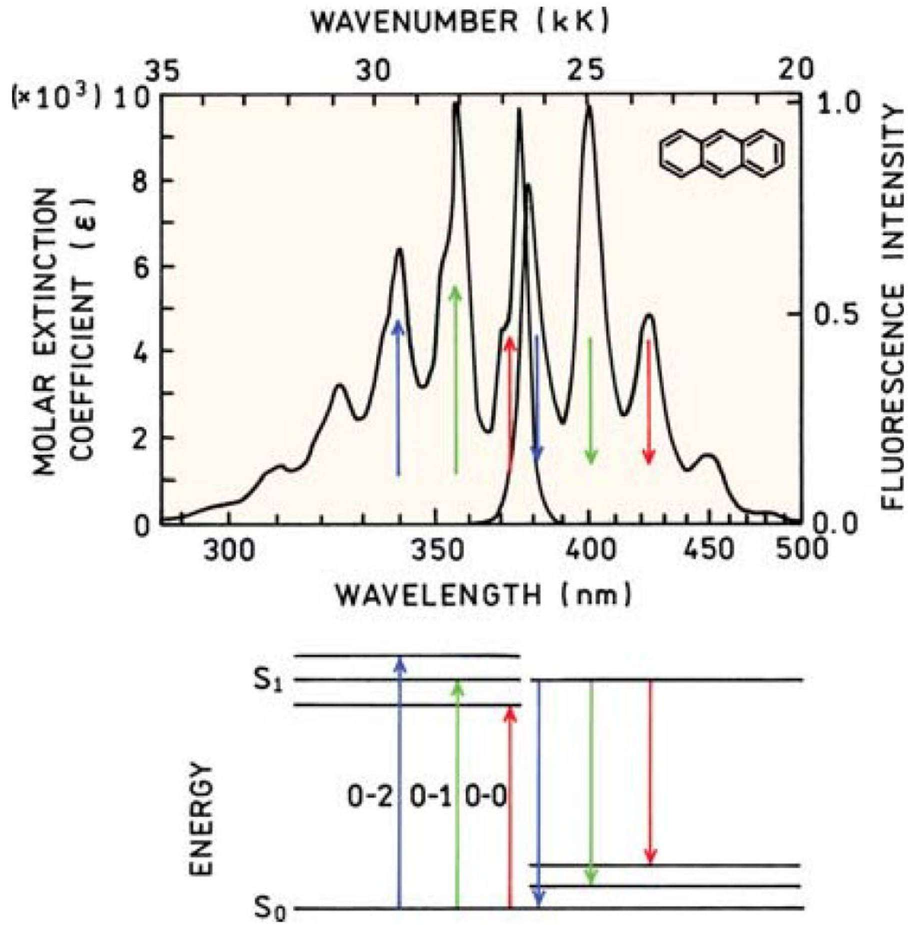


- Fluorescence emission peak wavelength is red-shifted with respect to absorption peak wavelength
- This shift may vary typically from 5 to more than 100 nm, depending on the electronic structure of the molecule



Energy
F
S_1
k_S
R
$1/\tau$
$h\nu_A$
S_0
Reaction Coordinate





WAVENUMBER (kK)
35
30
25
20
(×10³) 10
8
6
4
2
0
1.0
0.5
0.0
MOLAR EXTINCTION COEFFICIENT (ε)
FLUORESCENCE INTENSITY
300
350
400
450
500
WAVELENGTH (nm)
S₁
S₀
ENERGY
0-2
0-1
0-0

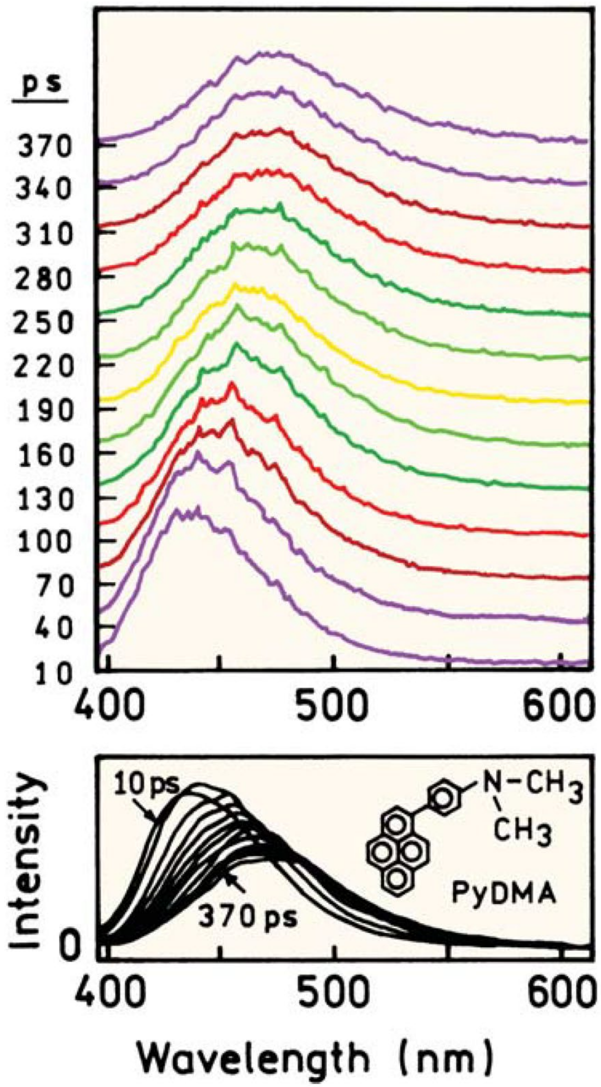


Figure 4.37. Time and wavelength-dependent intensity decays of dimethyl-(4-pyren-1-yl-phenyl)amine (PyDMA) measured with a streak camera. Revised from [161].

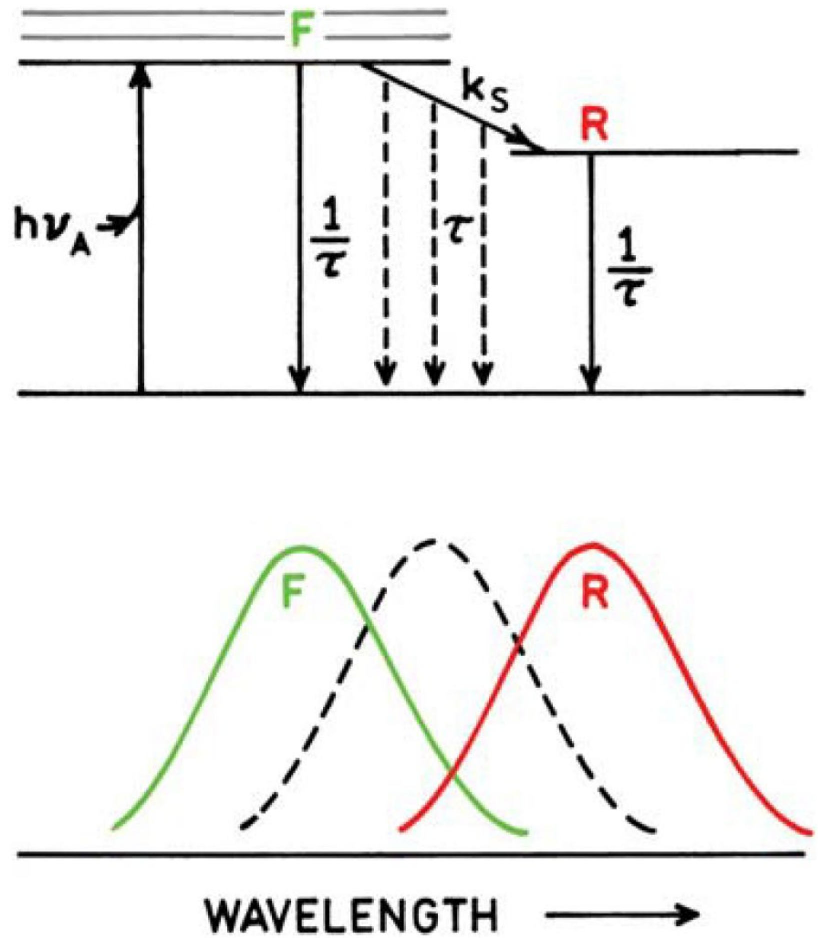


Figure 7.1. Jablonski diagram for continuous spectral relaxation.

In fluorescence, signal has inherent noise because you are counting discrete events – the arrival of photons. This noise is governed by Poisson statistics.

$$Signal = N$$

$$Std.Dev. = \sqrt{N}$$

Only 63% of the measurements are in the range

$$N \pm \sqrt{N}$$

Practical Implications?

To reliably detect activity with a S/N of 4…

fractional fluorescence change per unit activity		Required number of detected photons	
0.1%		16,000,000	
1%		160,000	
10%		1,600	
100%		16	

Required signal decreases with the square of the modulation!

Practical Implications

1- Increase the signal to noise ratio:

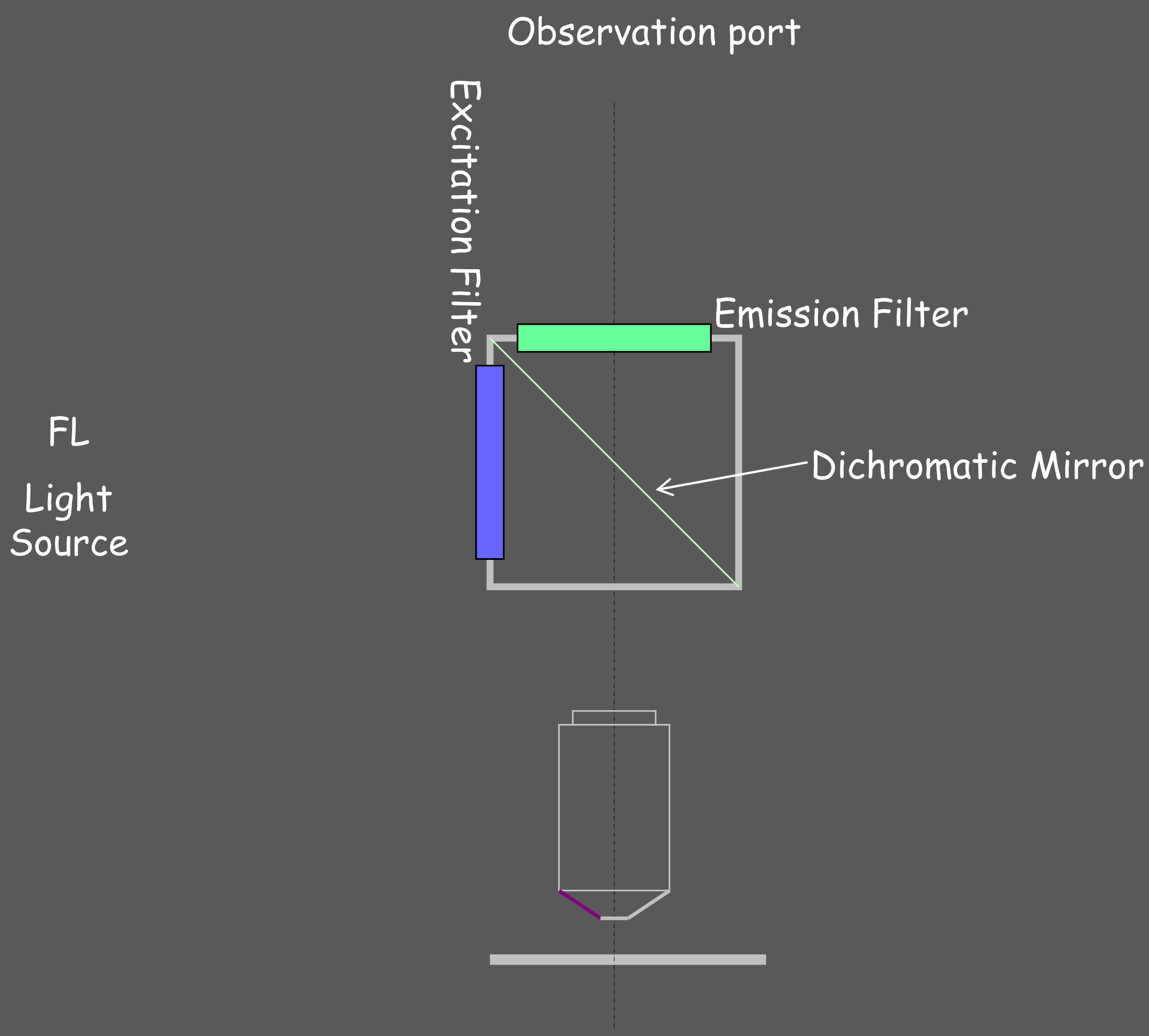
- optimize the excitation
- collect as many photons as possible
- decrease the noise to the shot-noise limit

2- Optimize the % of fluorescence modulation:

- avoid non-modulated signals (like tissue fluorescence or out-of focus fluorescence)
- resolve optically compartments with large signal dynamics

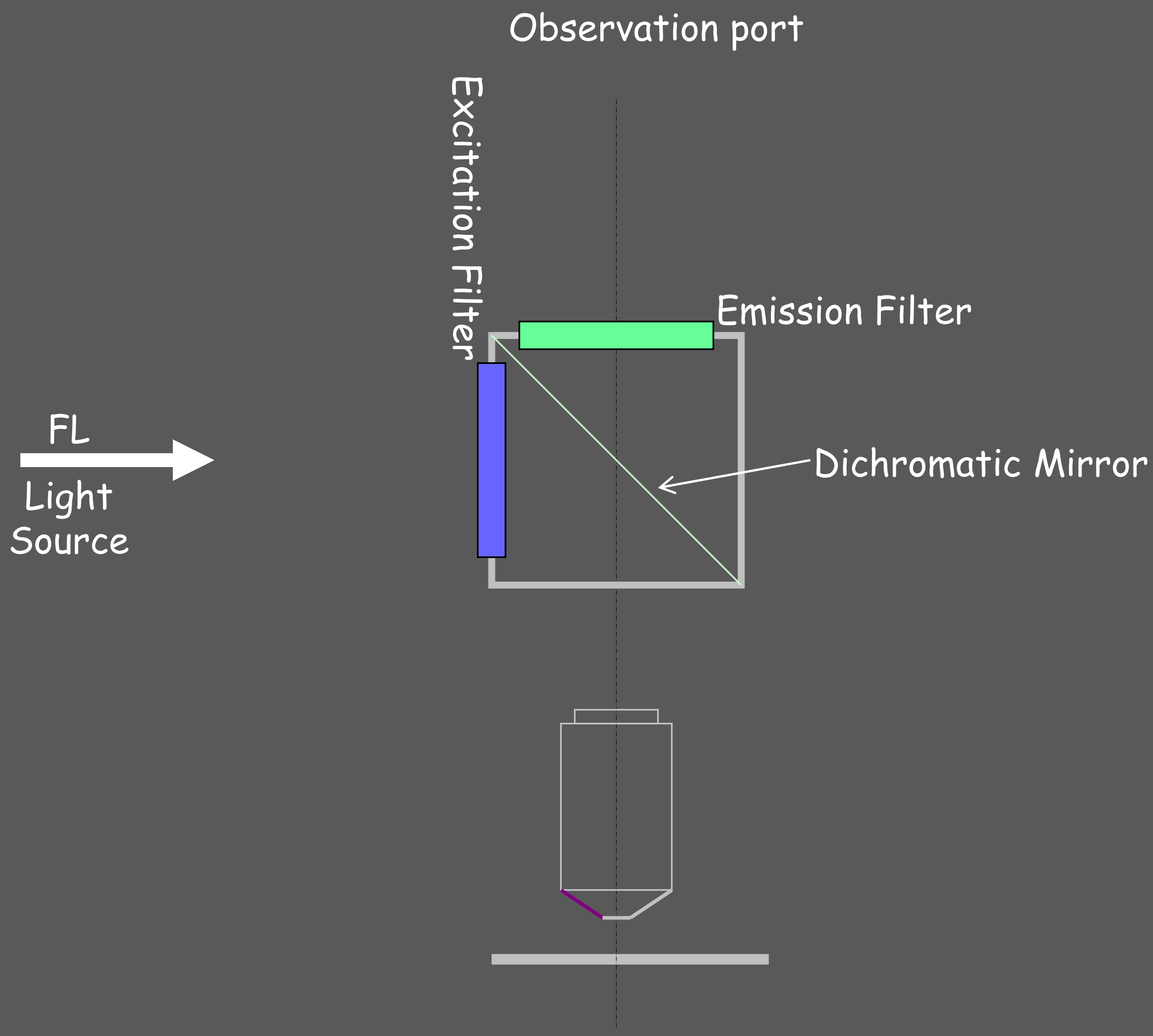
Epi - Fluorescence

(Specimen containing green fluorescing Fluorochrome)



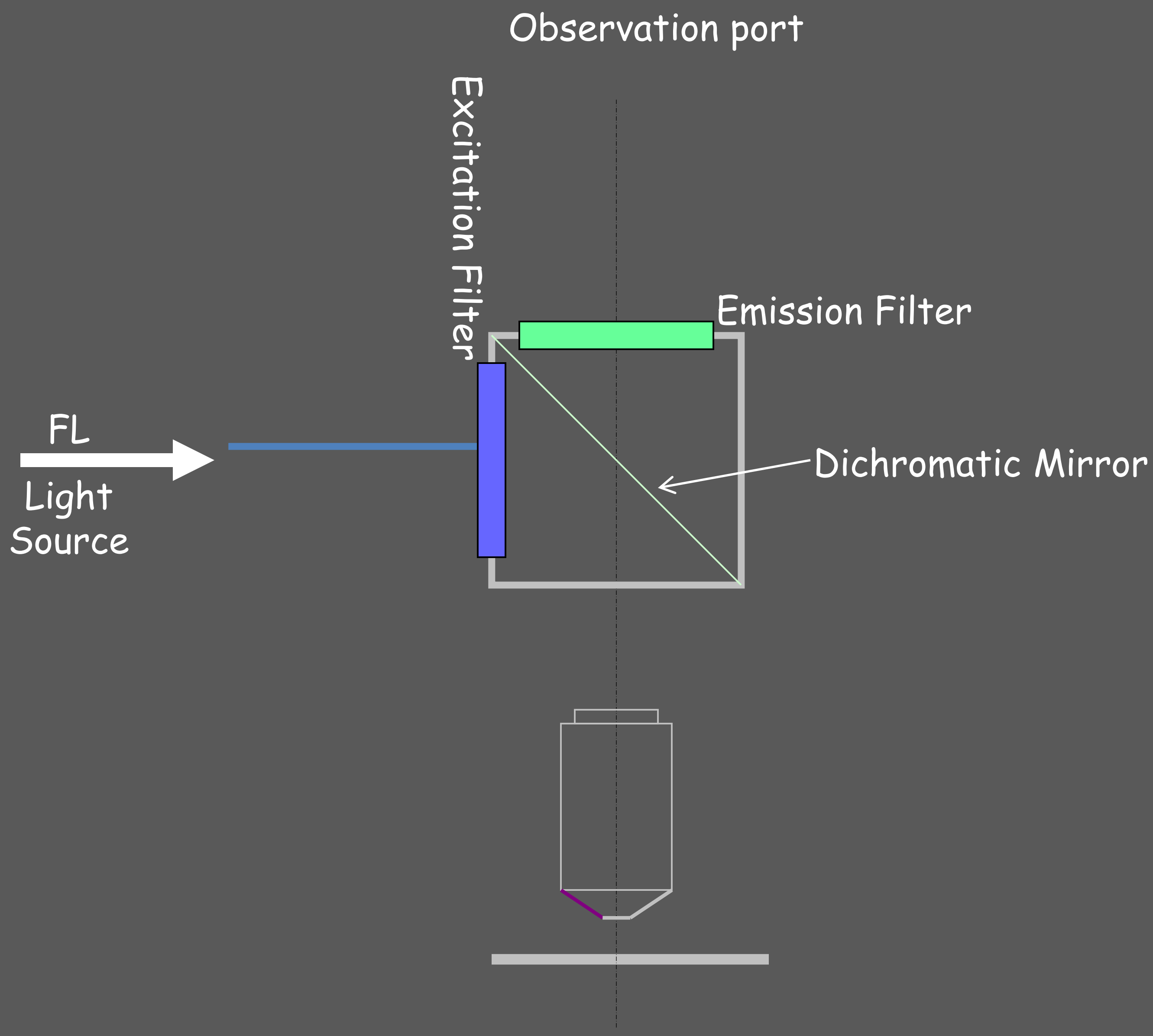
Epi - Fluorescence

(Specimen containing green fluorescing Fluorochrome)



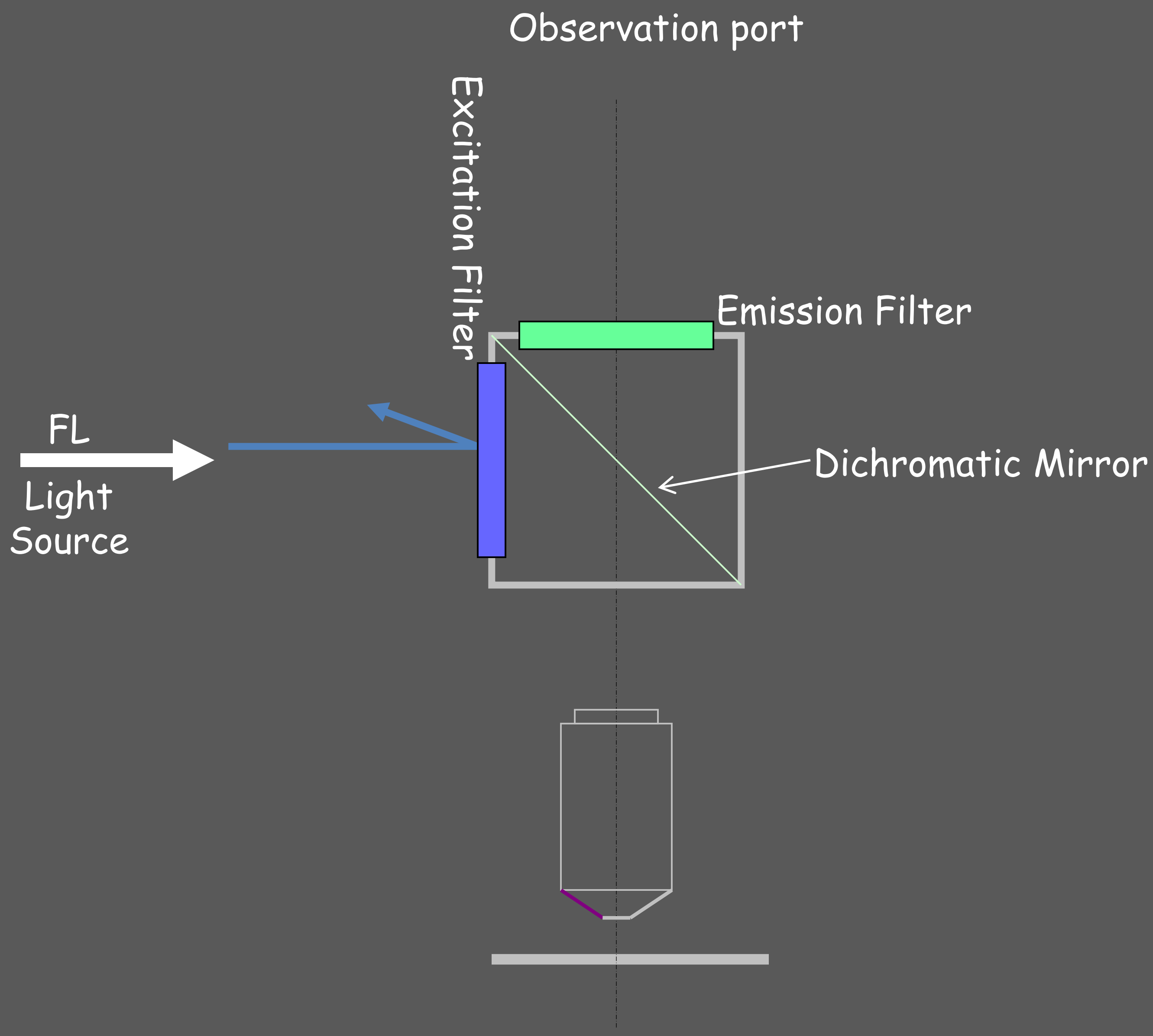
Epi - Fluorescence

(Specimen containing green fluorescing Fluorochrome)



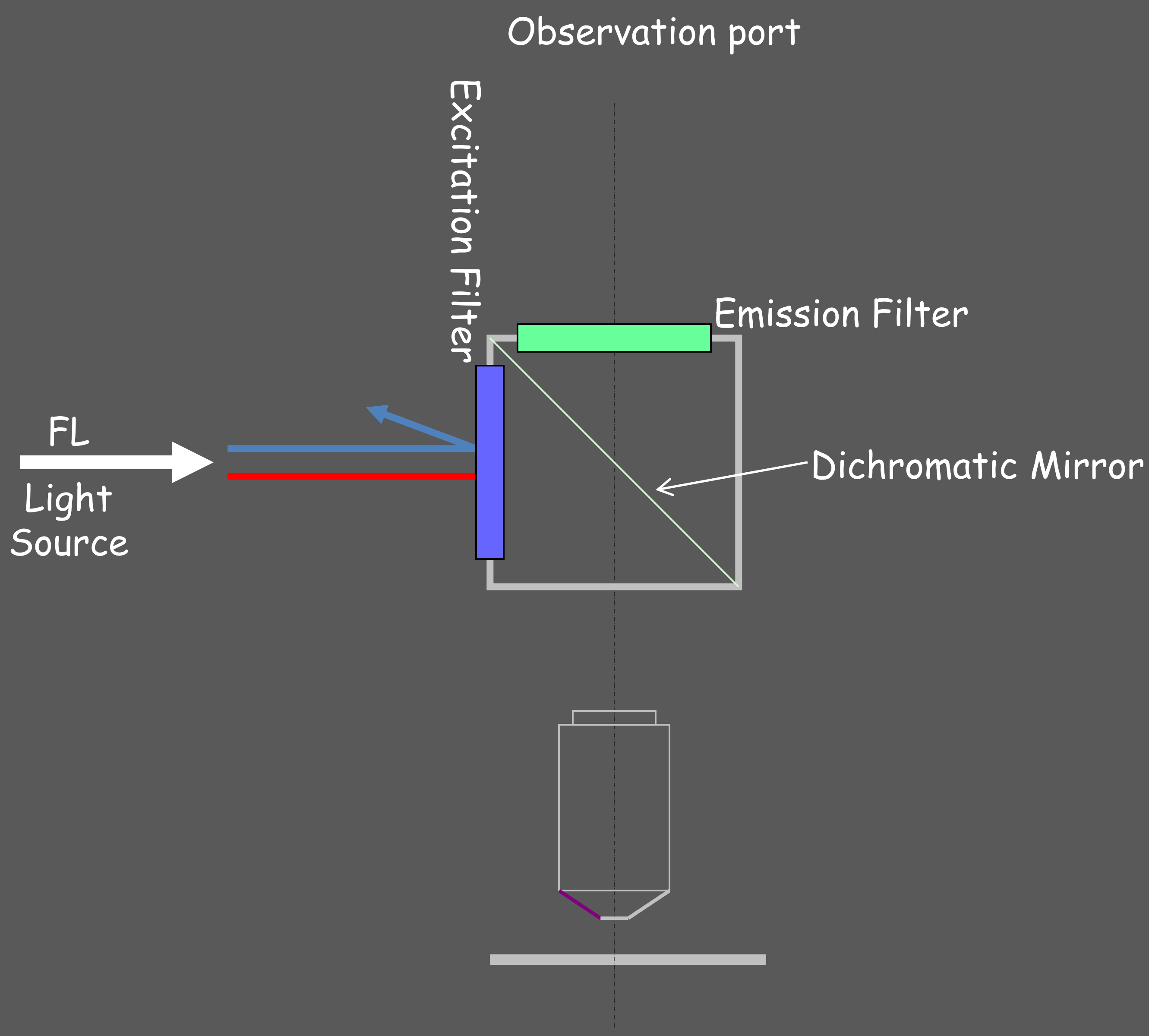
Epi - Fluorescence

(Specimen containing green fluorescing Fluorochrome)



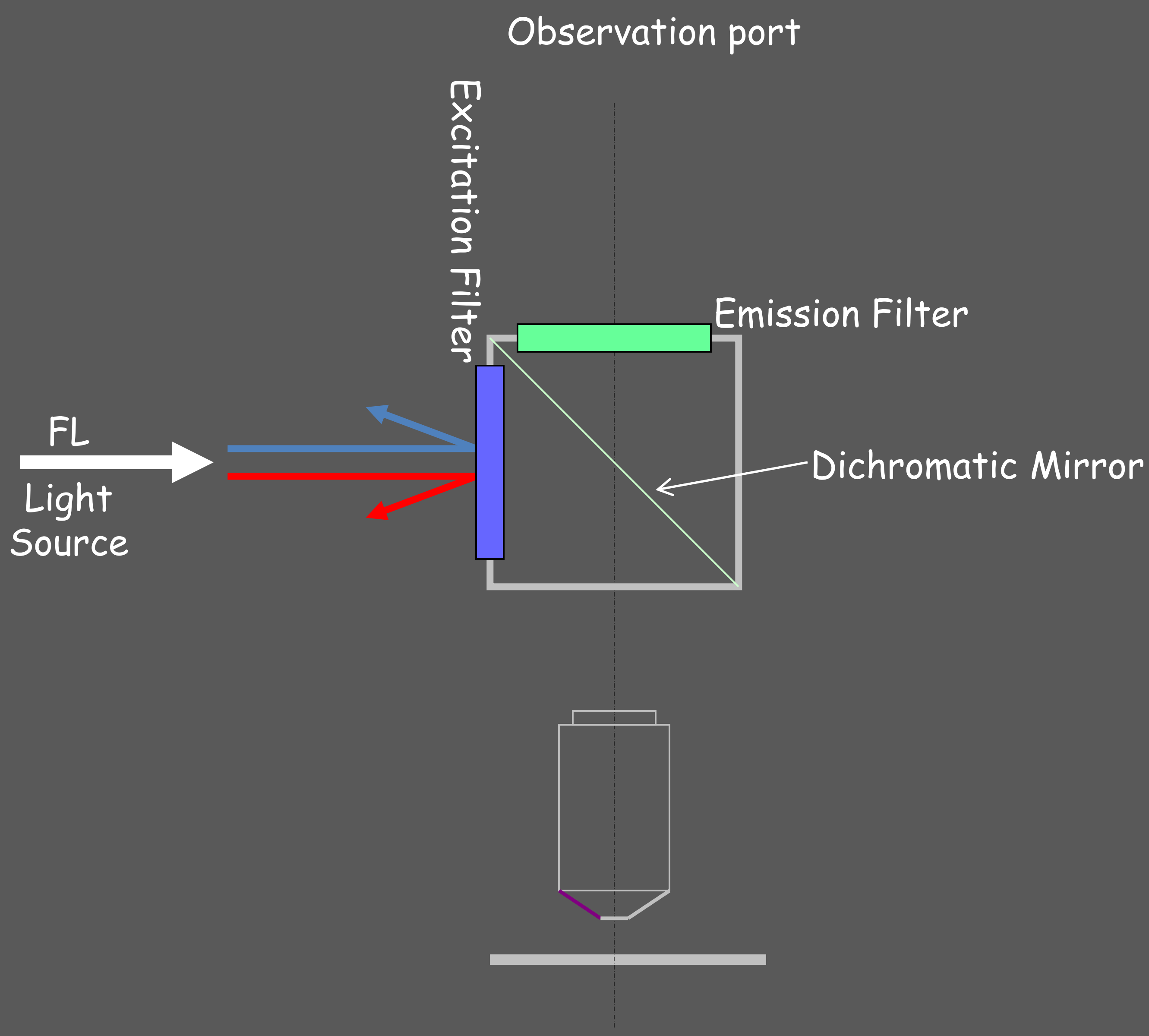
Epi - Fluorescence

(Specimen containing green fluorescing Fluorochrome)



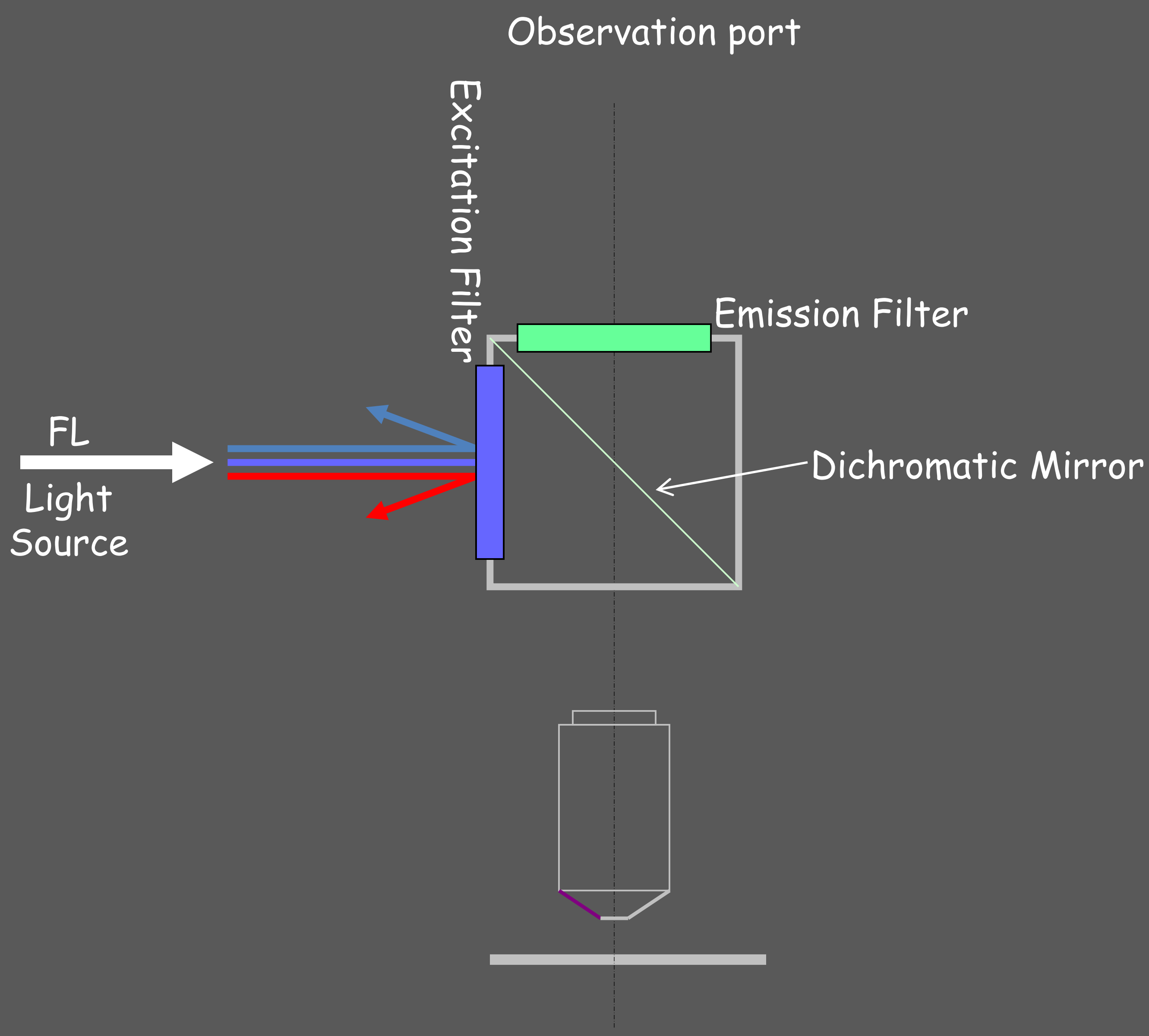
Epi - Fluorescence

(Specimen containing green fluorescing Fluorochrome)



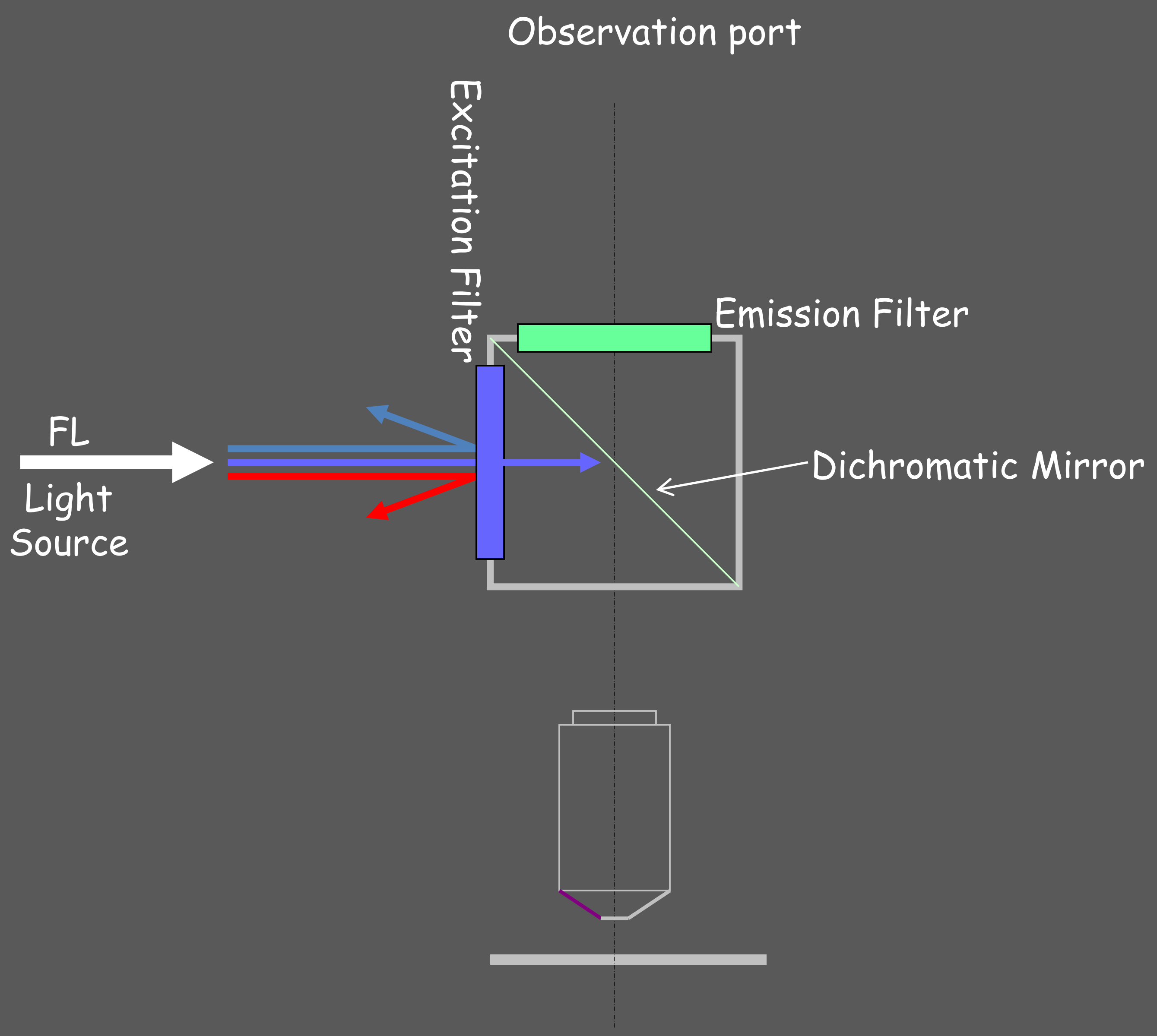
Epi - Fluorescence

(Specimen containing green fluorescing Fluorochrome)



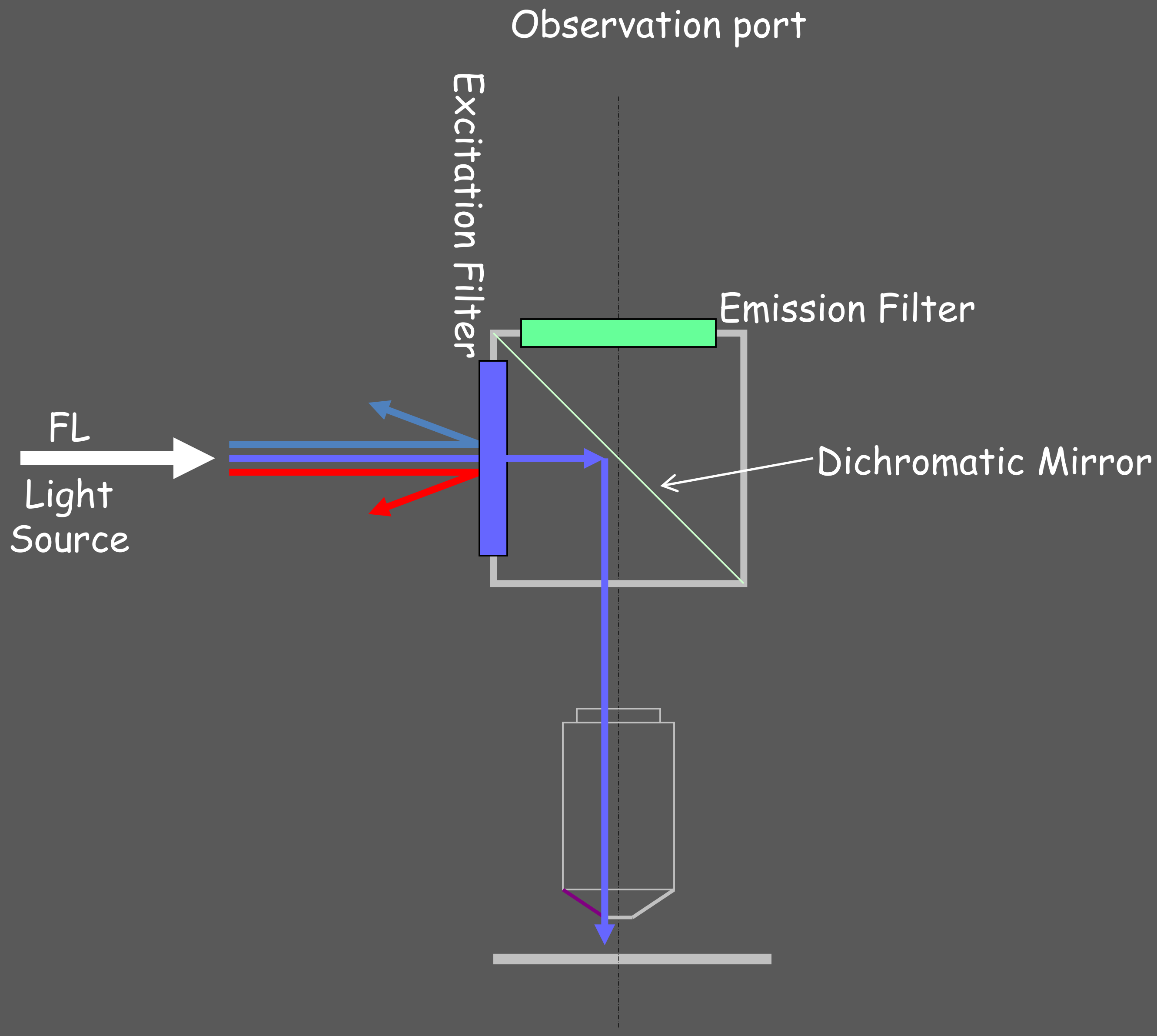
Epi - Fluorescence

(Specimen containing green fluorescing Fluorochrome)



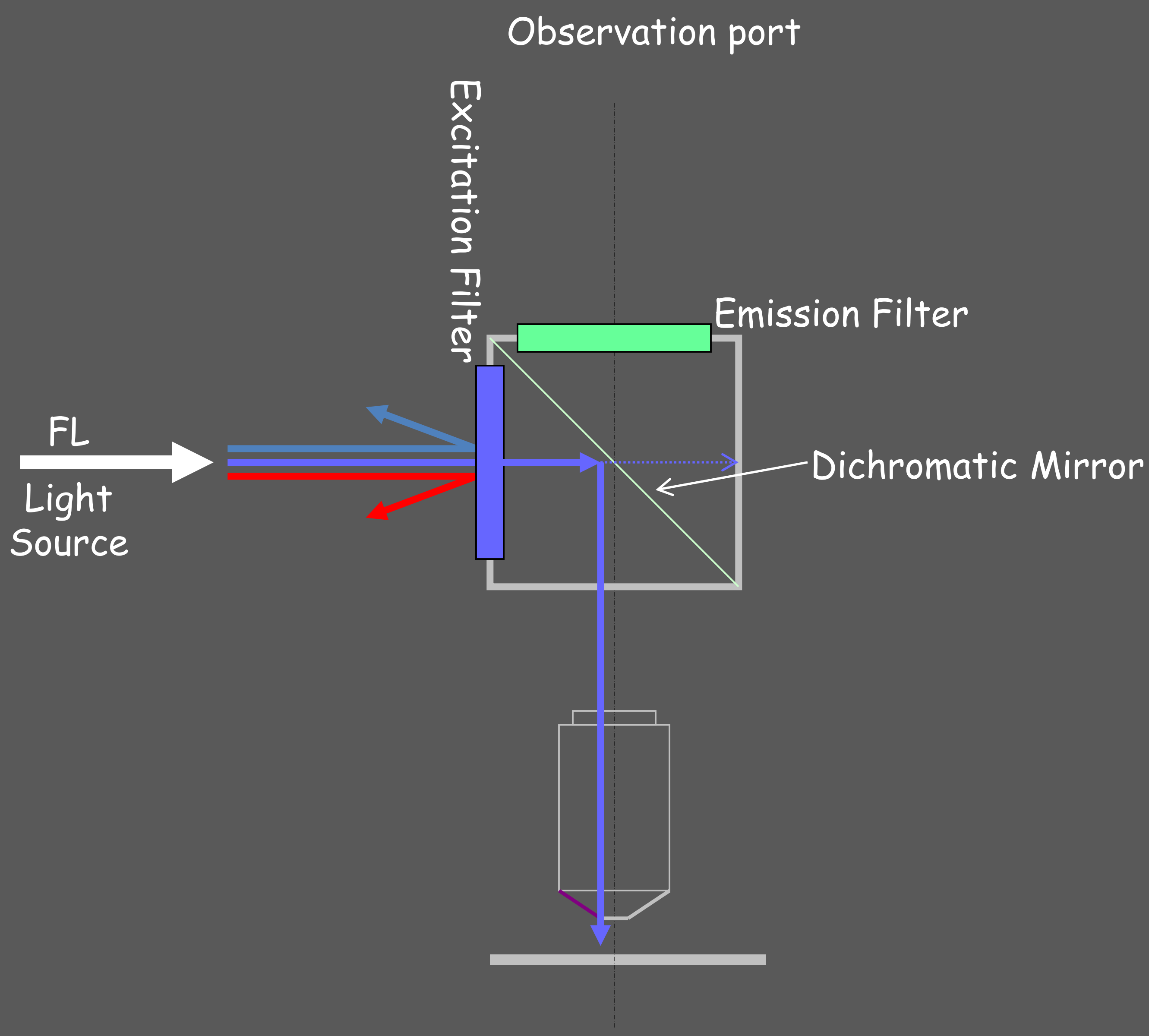
Epi - Fluorescence

(Specimen containing green fluorescing Fluorochrome)



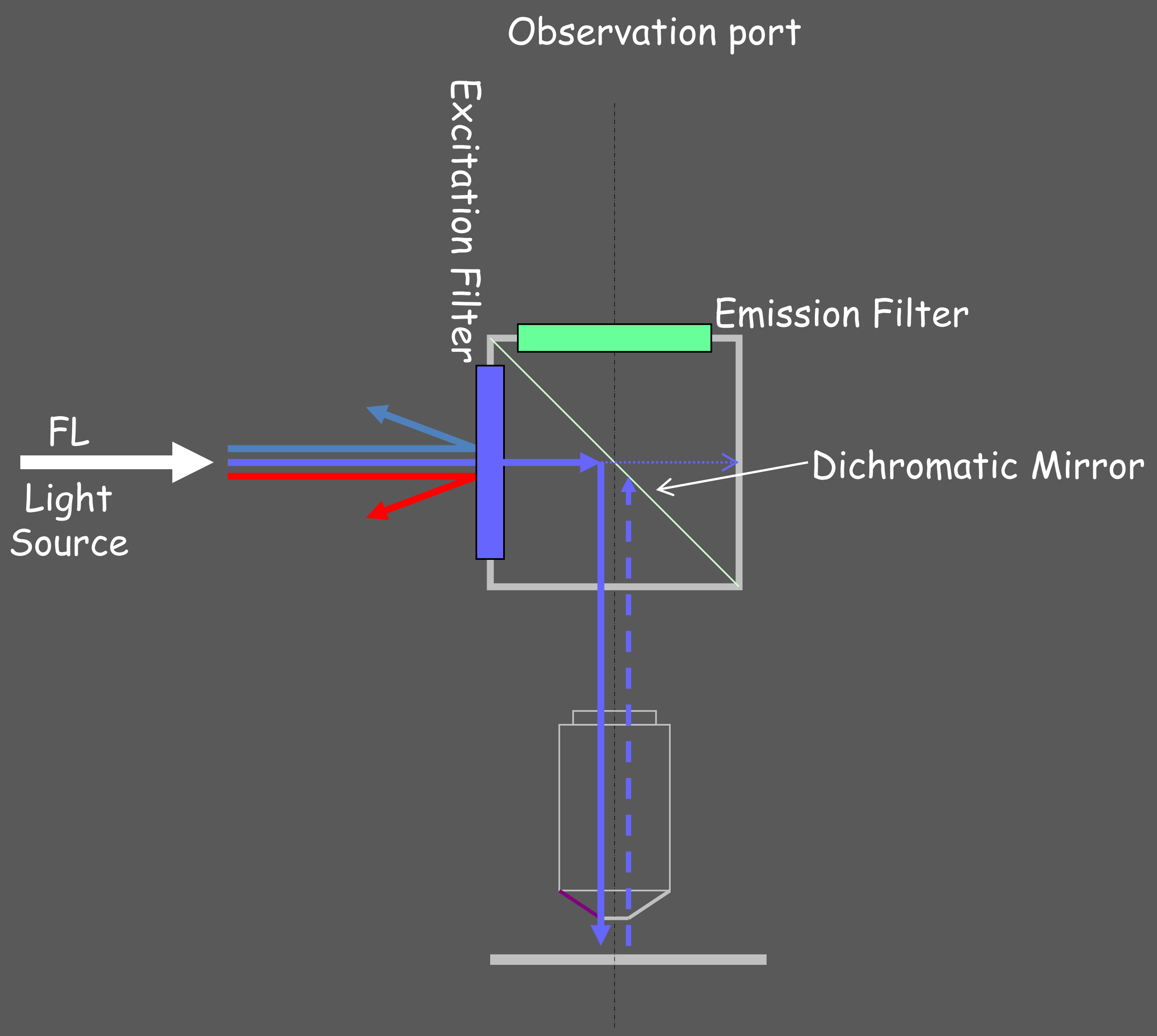
Epi - Fluorescence

(Specimen containing green fluorescing Fluorochrome)



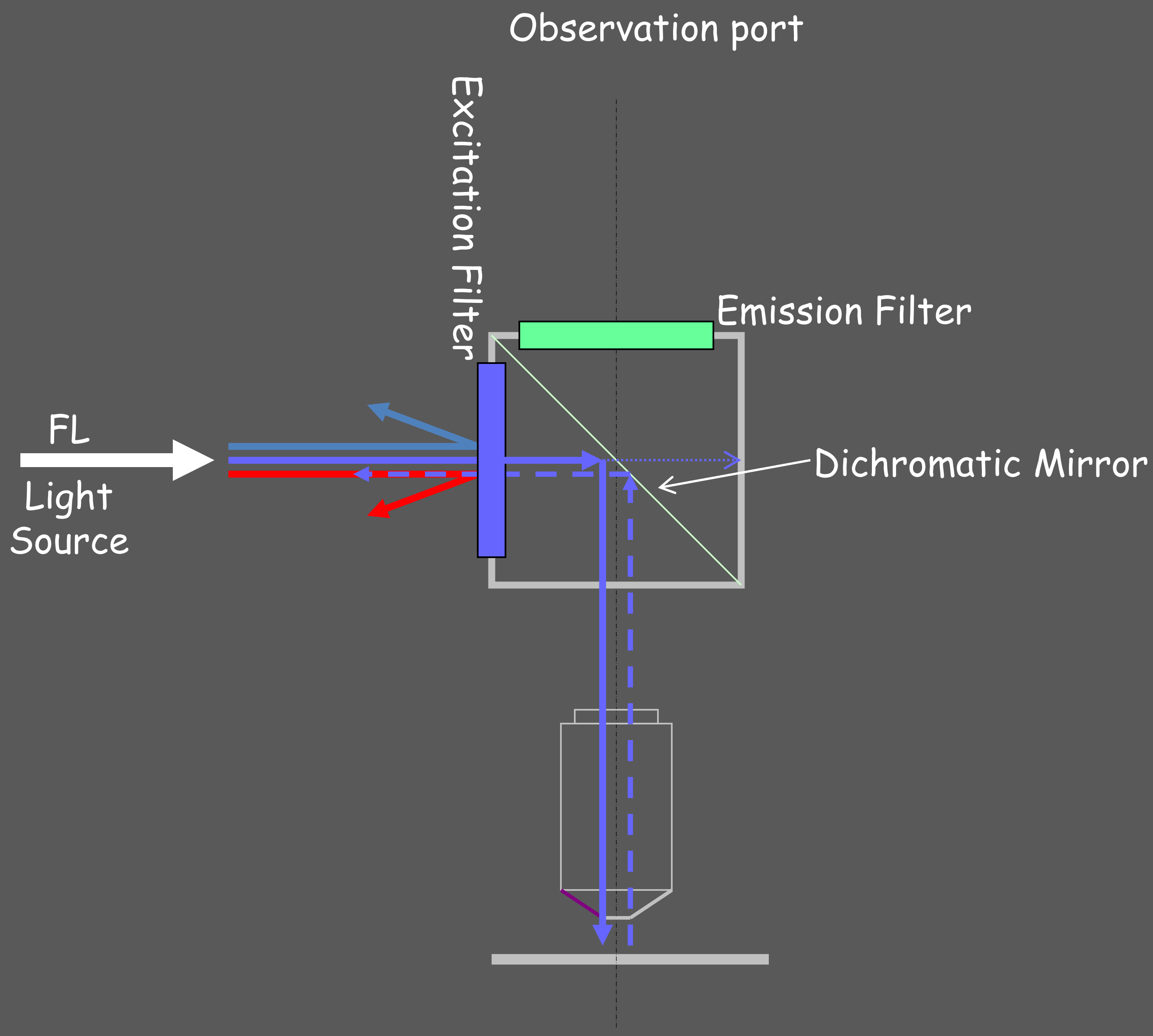
Epi - Fluorescence

(Specimen containing green fluorescing Fluorochrome)



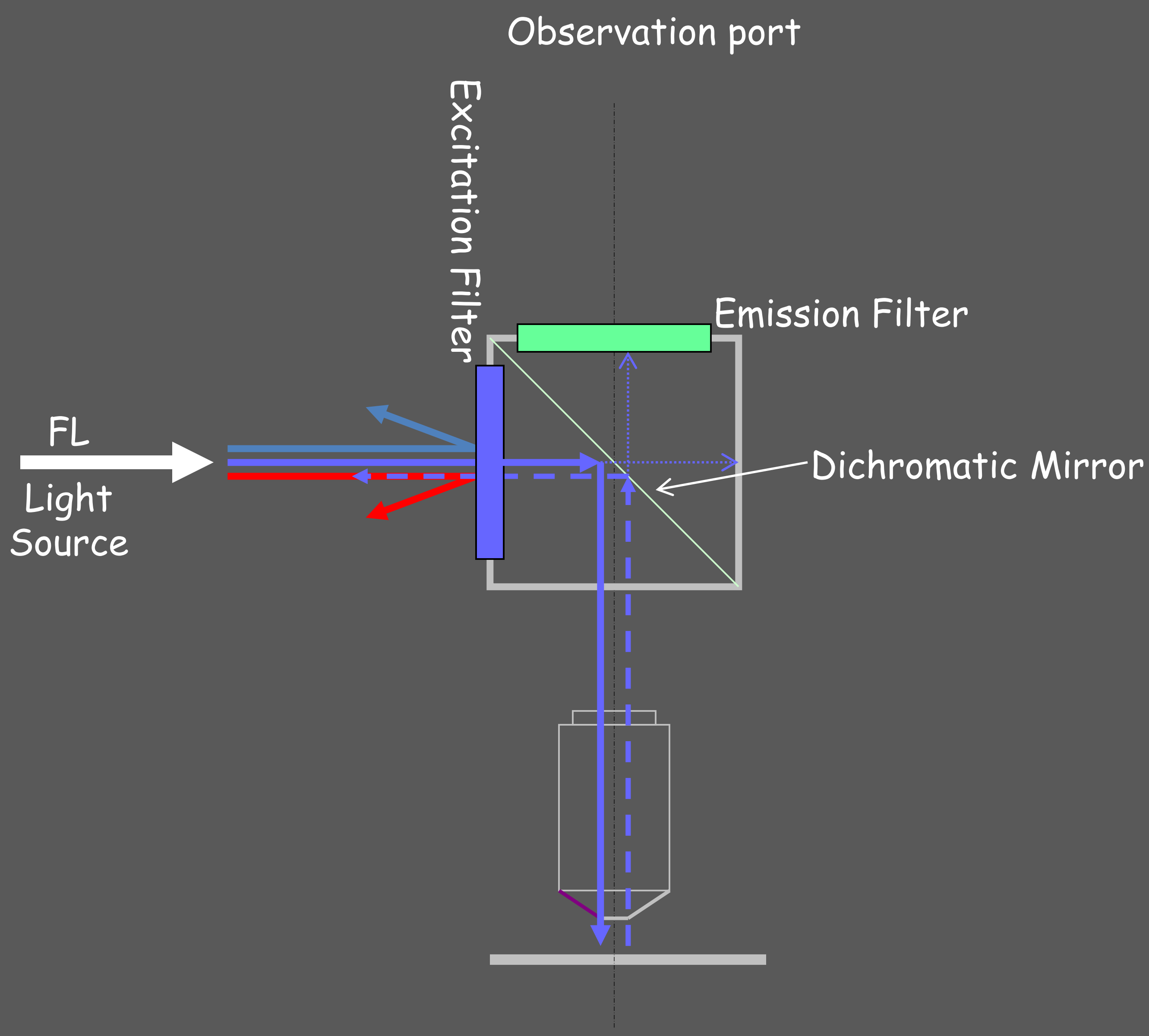
Epi - Fluorescence

(Specimen containing green fluorescing Fluorochrome)



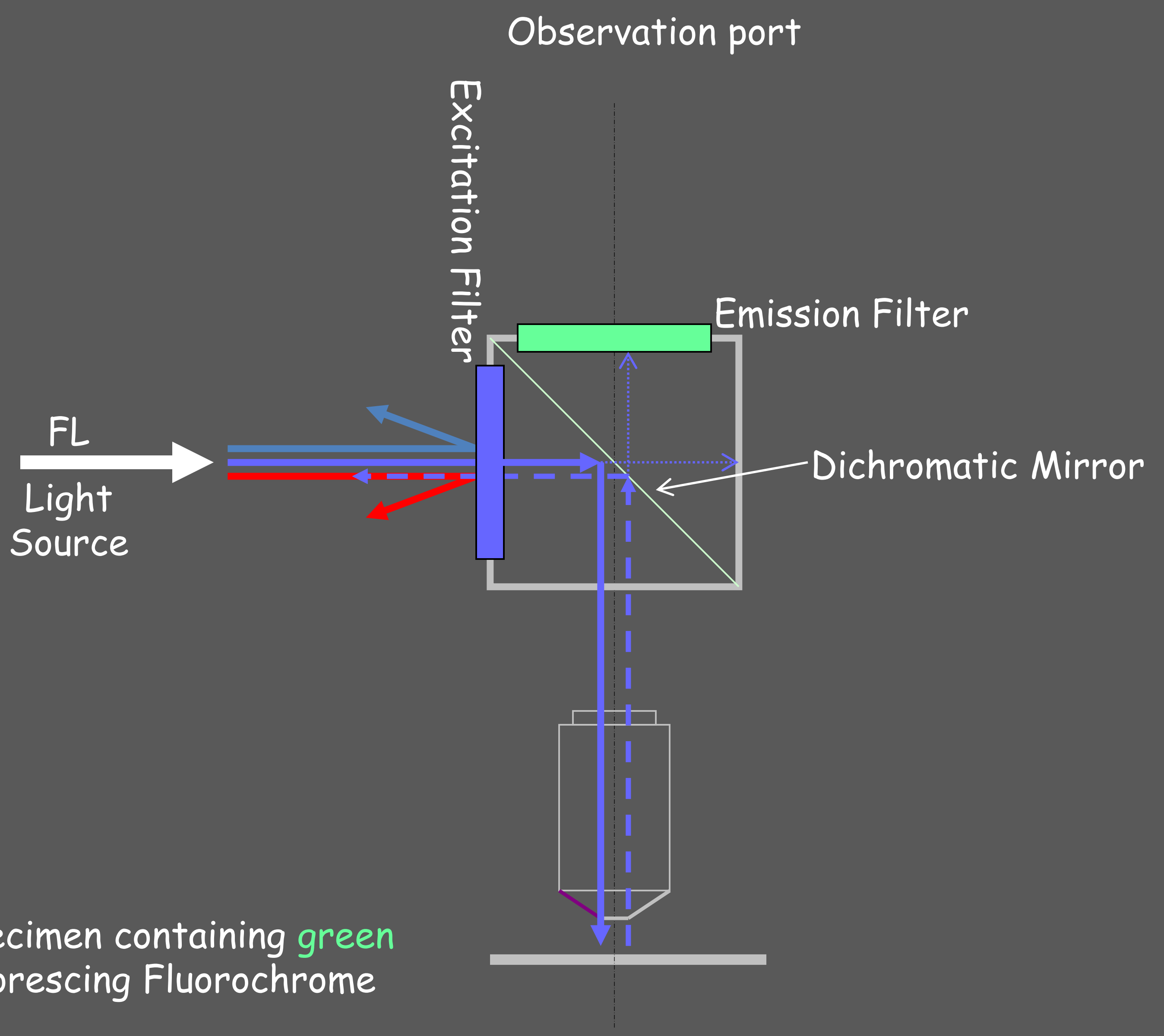
Epi - Fluorescence

(Specimen containing green fluorescing Fluorochrome)



Epi - Fluorescence

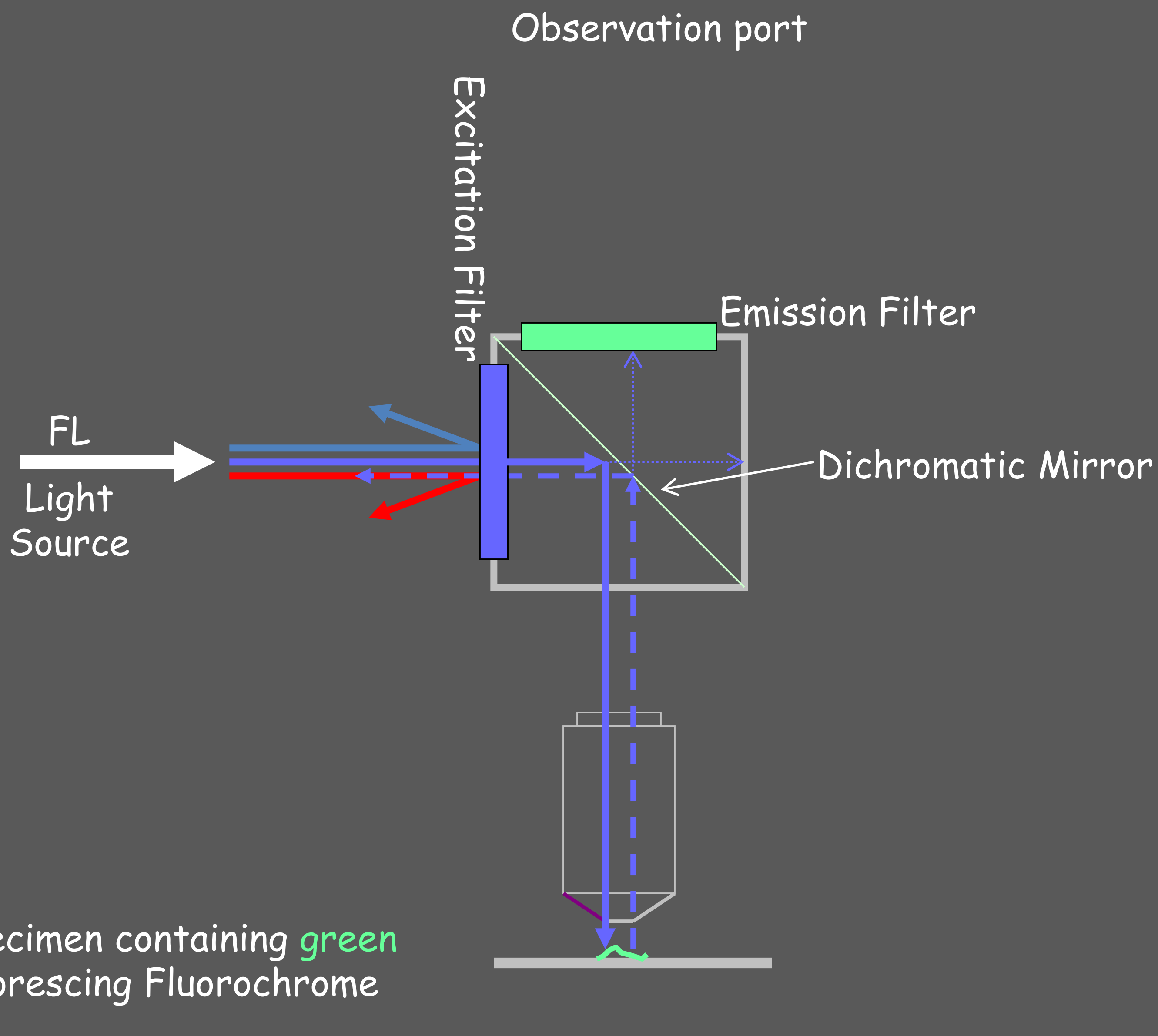
(Specimen containing green fluorescing Fluorochrome)



Specimen containing green fluorescing Fluorochrome

Epi - Fluorescence

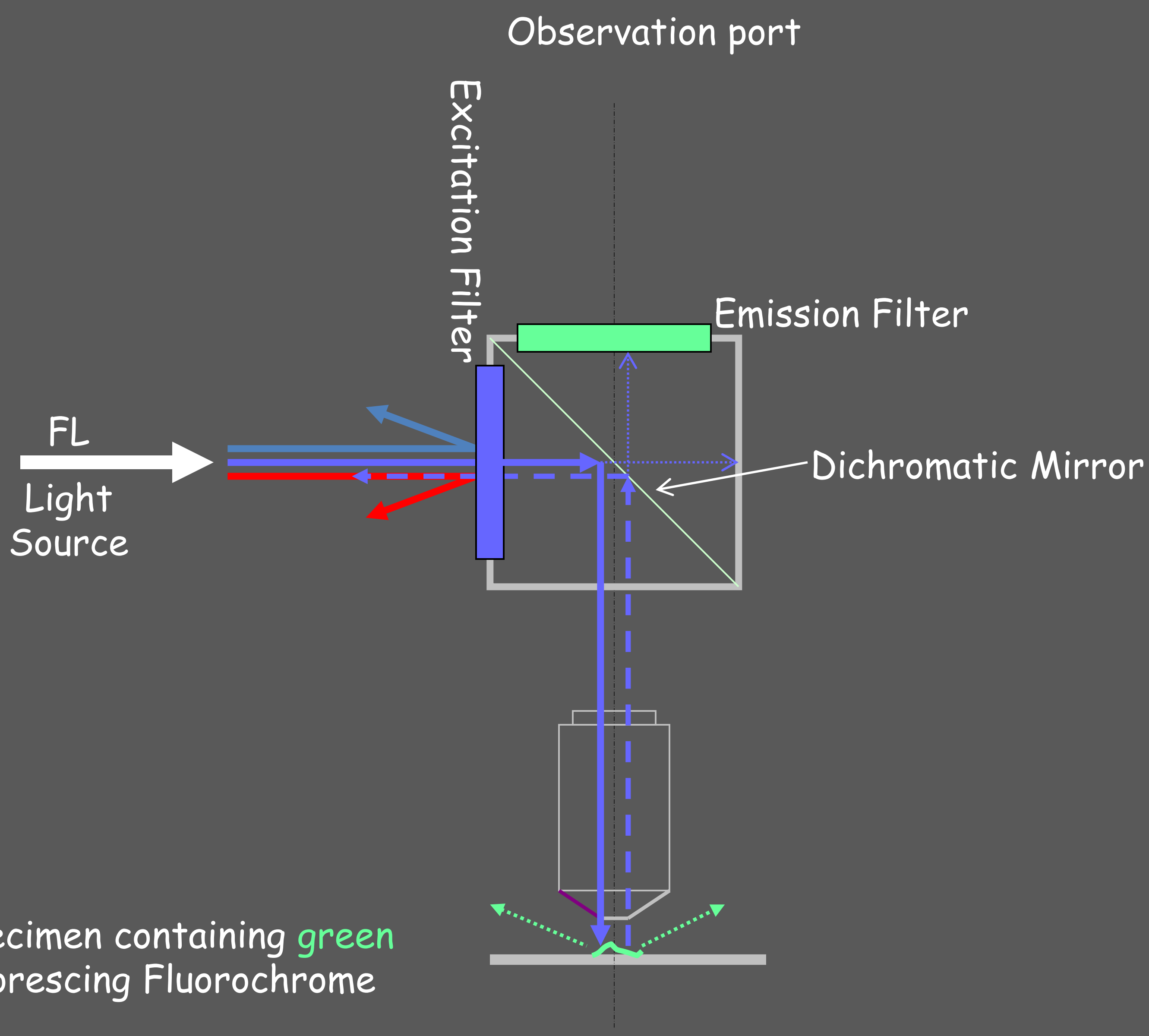
(Specimen containing green fluorescing Fluorochrome)



Specimen containing green fluorescing Fluorochrome

Epi - Fluorescence

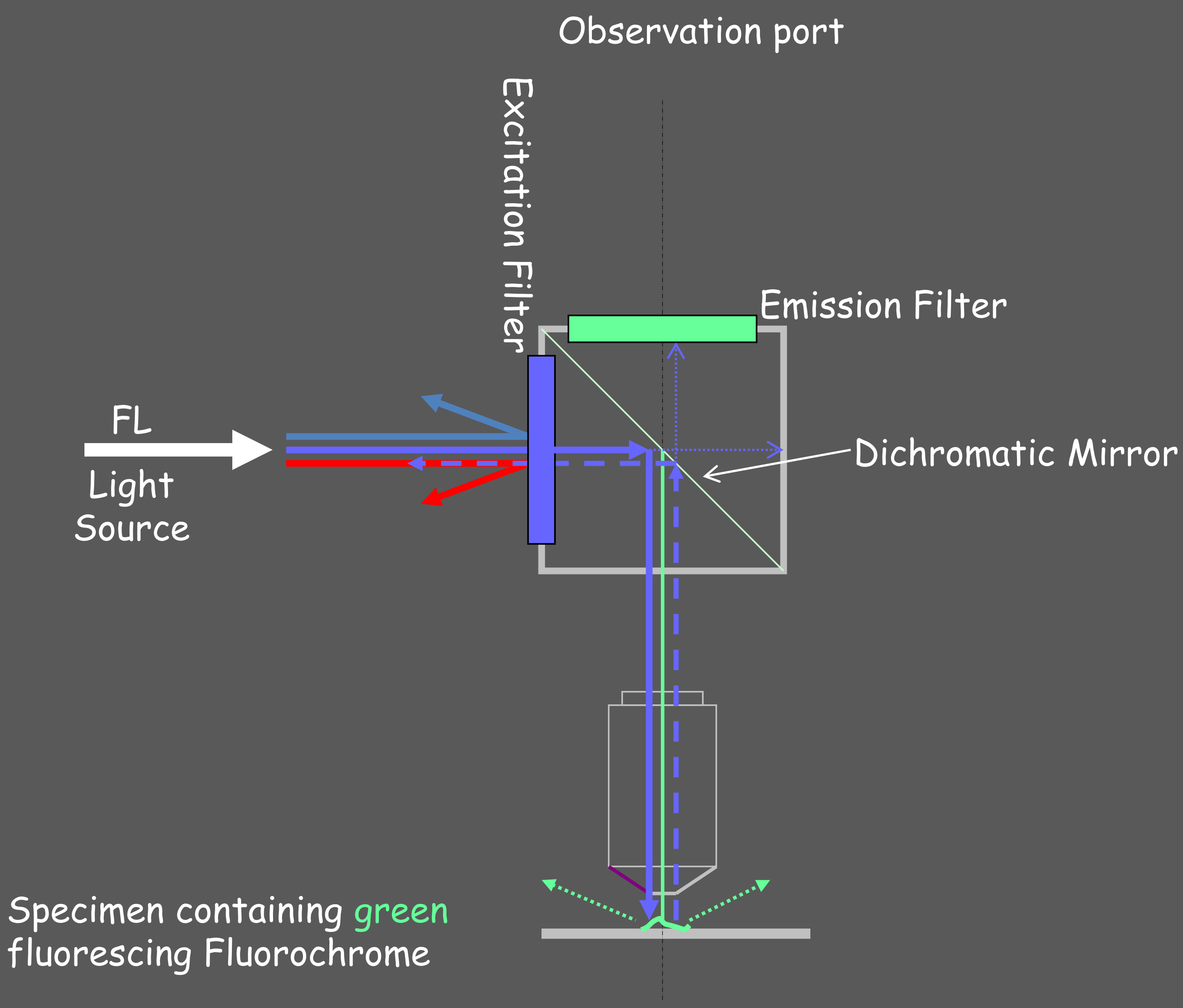
(Specimen containing green fluorescing Fluorochrome)



Specimen containing green fluorescing Fluorochrome

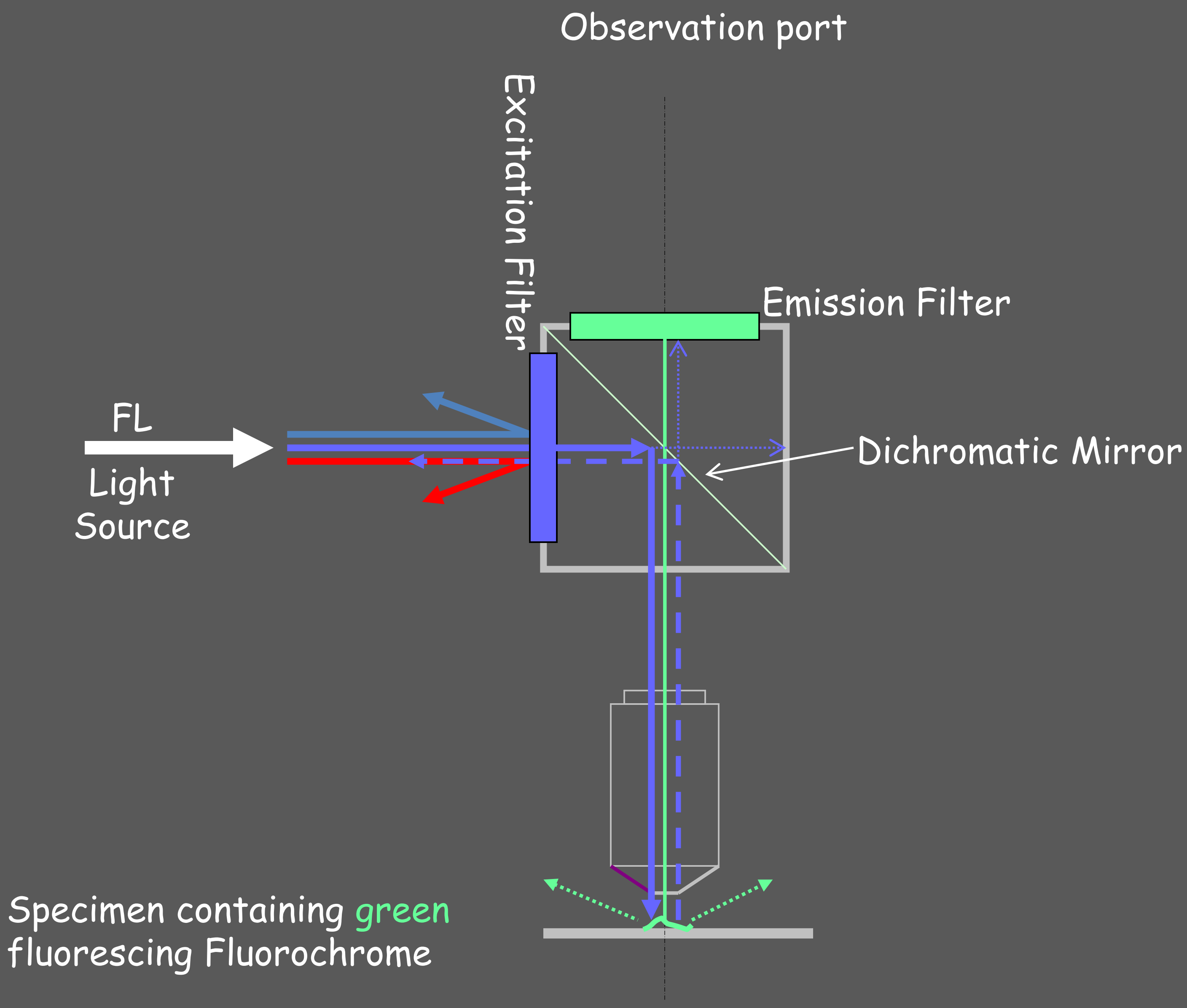
Epi - Fluorescence

(Specimen containing green fluorescing Fluorochrome)



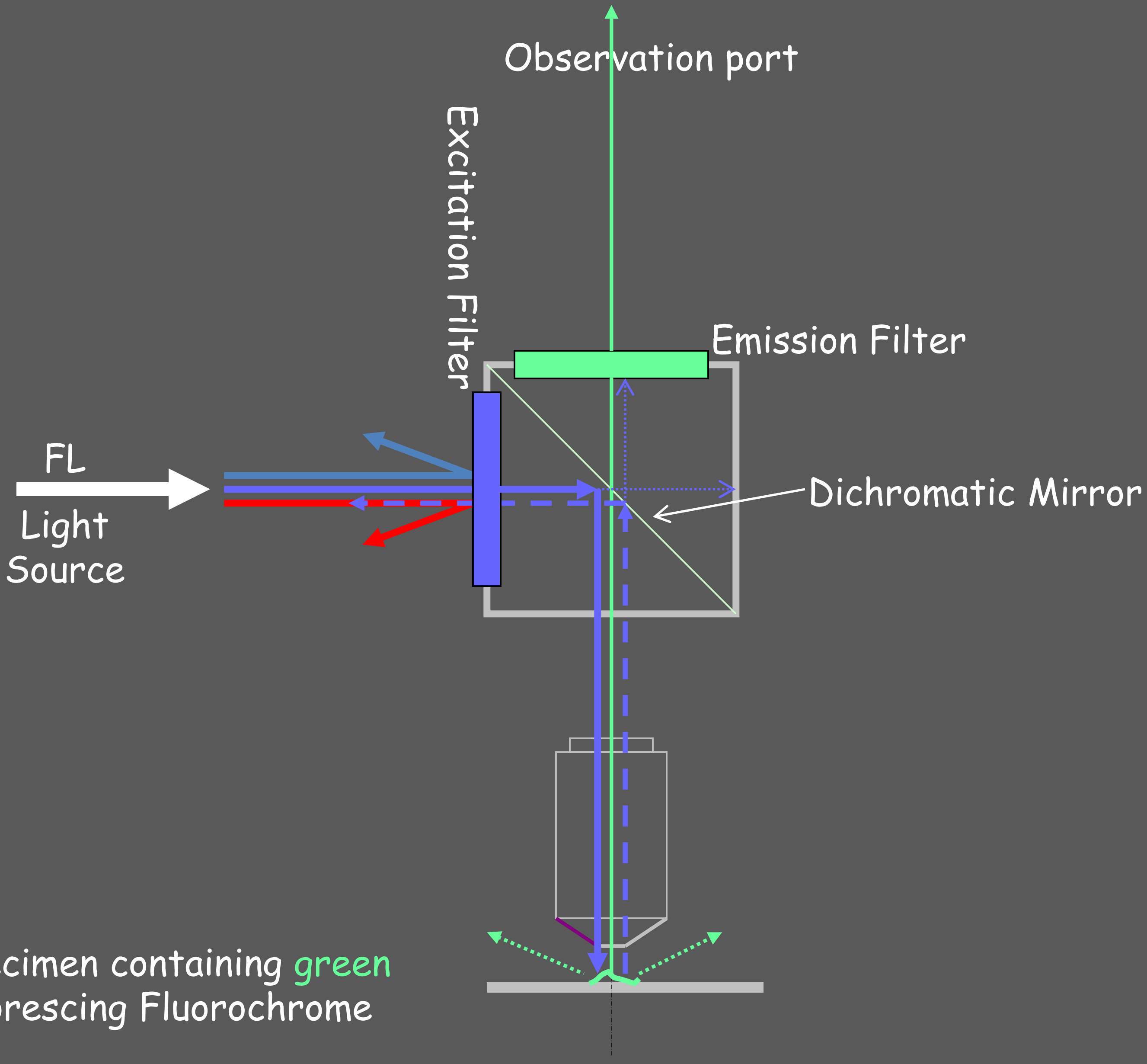
Epi - Fluorescence

(Specimen containing green fluorescing Fluorochrome)



Epi - Fluorescence

(Specimen containing green fluorescing Fluorochrome)



Specimen containing green fluorescing Fluorochrome

How do you make filters?
See https://www.alluxa.com/learning-center/what-are-thin-film-optical-filters/

https://www.photonics.com/Articles/Thin-Film_Optical_Filters_for_Phase_Control/a58006
https://www.photonics.com/Articles/Thin-Film_Coatings_A_Buyers_Guide/a42399


Thank you!!
$$$
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NIMH, NEI,NINDS
Kavli Foundation
Zuckerman Institute