UAMS
UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES
Department of Neurobiology and Developmental Sciences
Center for Translational Neuroscience
College of Medicine

Neuronal Signals - NBDS 5161
Session 9: Imaging Neuronal Activity

Abdallah HAYAR

Lectures can be downloaded from
http://hayar.net/NBDS5161

Updated Tentative Schedule for Neuronal Signals (NBDS 5161)
One Credit–Hour, Summer 2010
Location: Biomedical Research Building II, 6th floor, conference room,
Time: 9:00 -10:20 am

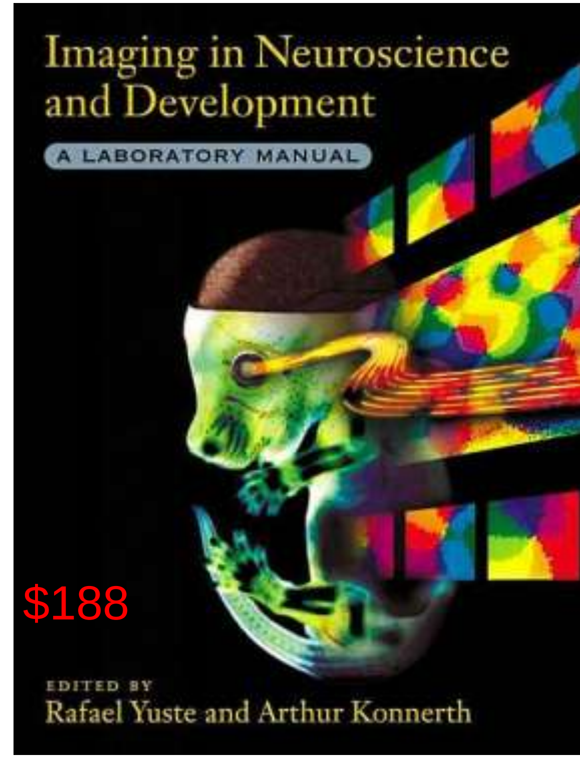
Session	Day	Date	Topic	Instructor
1	Tue	6/1	Design of an electrophysiology setup	Hayar
2	Thu	6/3	Neural population recordings	Hayar
3	Thu	6/10	Single cell recordings	Hayar
4	Fri	6/11	Analyzing synaptic activity	Hayar
5	Mon	6/14	Data acquisition and analysis	Hayar
6	Wed	6/16	Analyzing and plotting data using OriginLab	Hayar
7	Fri	6/18	Detecting electrophysiological events	Hayar
8	Mon	6/21	Writing algorithms in OriginLab®	Hayar
9	Wed	6/23	Imaging neuronal activity	Hayar
10	Fri	6/25	Exam and students' survey – Lab. demonstration	Hayar
11	Fri	7/9	Article presentation: Electrophysiology & Imaging	Hayar

Student List

	Name	E-mail	Regular/Auditor	Department	Position
1	Simon, Christen	CSimon@uams.edu	Regular (form signed)	Neurobiology & Developmental Sciences	Graduate Neurobiology – Mentor: Dr. Garcia-Rill
2	Kezunovic, Nebojsa	NKezunovic@uams.edu	Regular (form signed)	Neurobiology & Developmental Sciences	Graduate Neurobiology – Mentor: Dr. Garcia-Rill
3	Hyde, James R	JRHyde@uams.edu	Regular (form signed)	Neurobiology & Developmental Sciences	Graduate Neurobiology – Mentor: Dr. Garcia-Rill
4	Yadlapalli, Krishnapraveen	KYadlapalli@uams.edu	Regular (form signed)	Pediatrics	Research Technologist – Mentor: Dr. Alchaer
5	Pathan, Asif	APATHAN@uams.edu	Regular (form signed)	Pharmacology & Toxicology	Graduate Pharmacology – Mentor: Dr. Rusch
6	Kharade, Sujay	SKHARADE@uams.edu	Regular (form signed)	Pharmacology & Toxicology	Graduate Pharmacology – 4th year - Mentor: Dr. Rusch
7	Howell, Matthew	MHOWELL2@uams.edu	Regular (form signed)	Pharmacology & Toxicology	Graduate Interdisciplinary Toxicology - 3rd year - Mentor: Dr. Gottschall
8	Beck, Paige B	PBBeck@uams.edu	Regular (form signed)	College of Medicine	Medical Student – 2nd Year - Mentor: Dr. Garcia-Rill
9	Atcherson, Samuel R	SRAtcherson@uams.edu	Auditor (form signed)	Audiology & Speech Pathology	Assistant Professor
10	Detweiler, Neil D	NDDETWEILER@uams.edu	Auditor (form not signed)	Pharmacology & Toxicology	Graduate Pharmacology – 1st year
11	Thakali, Keshari M	KMThakali@uams.edu	Unofficial auditor	Pharmacology & Toxicology	Postdoctoral Fellow – Mentor: Dr. Rusch
12	Boursoulian, Feras	FBoursoulian@uams.edu	Unofficial auditor	Neurobiology & Developmental Sciences	Postdoctoral Fellow – Mentor: Dr. Hayar
13	Steele, James S	JSSTEELE@uams.edu	Unofficial auditor	College of Medicine	Medical Student – 1st Year – Mentor: Dr. Hayar
14	Smith, Kristen M	KMSmith2@uams.edu	Unofficial auditor	Neurobiology & Developmental Sciences	Research Technologist – Mentor: Dr. Garcia-Rill
15	Gruenwald, Konstantin	kjoachimg@gmail.com	Unofficial auditor	Neurobiology & Developmental Sciences	High school Student – Mentor: Dr. Hayar
16	Rhee, Sung	RheeSung@uams.edu	Unofficial auditor	Pharmacology & Toxicology	Assistant Professor
17	Light, Kim E	LightKimE@uams.edu	Unofficial auditor	Pharmaceutical Sciences	Professor

Electrophysiological and imaging setup

Microscopy & Imaging Resources



$188

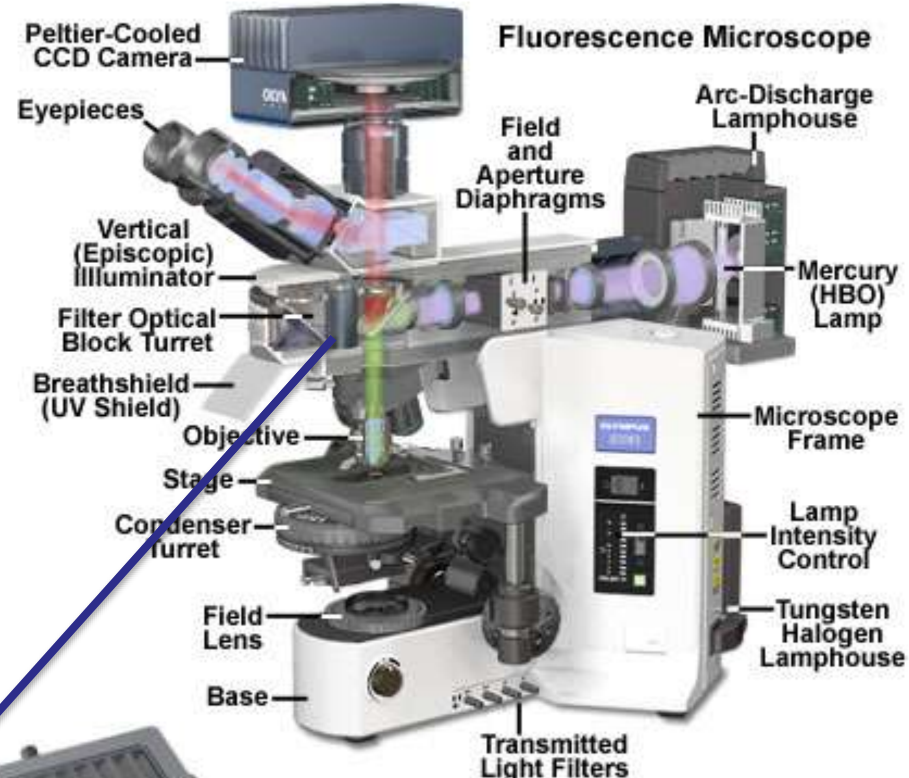
http://www.olympusmicro.com
http://www.chroma.com/sites/all/themes/chroma-blue/uploads/files/HandbookofOpticalFilters.pdf
http://search.barnesandnoble.com/Imaging-in-Neuroscience-and-Development/Rafael-Yuste/e/9780879696894/?itm=1&USRI=yuste+imaging

Optical Recording from Individual Neurons in Culture- Optical-Recording-Chap4.pdf

Optical Recording from Populations of Neurons in Brain Slices - Optical-Recording-Chap16.pdf

Anatomy of the Fluorescence Microscope

Figure 8



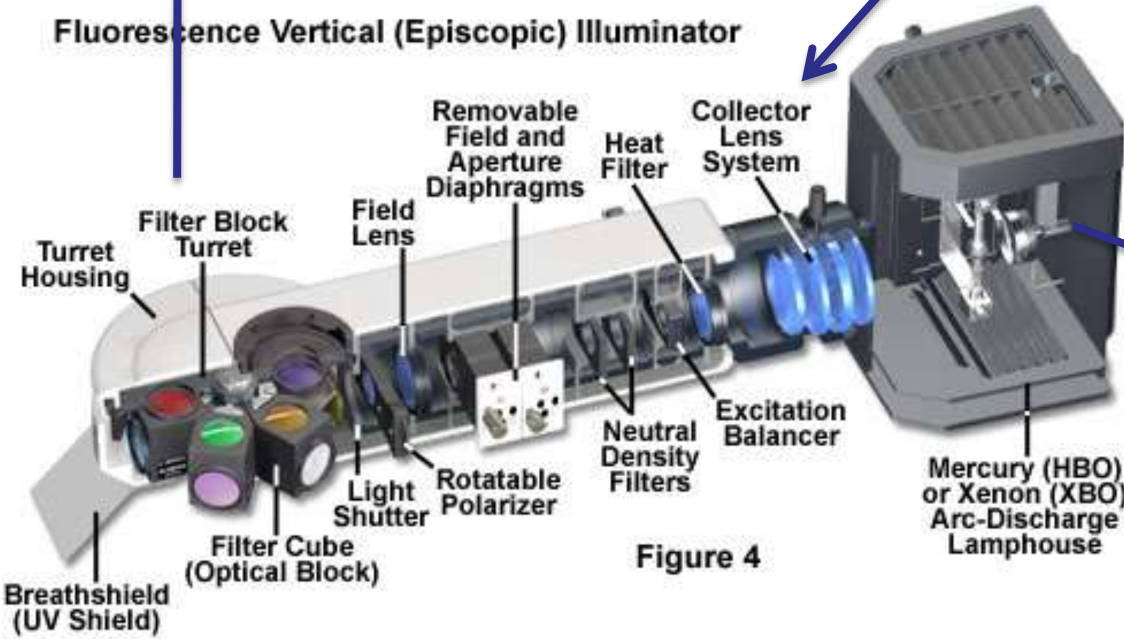


Figure 4

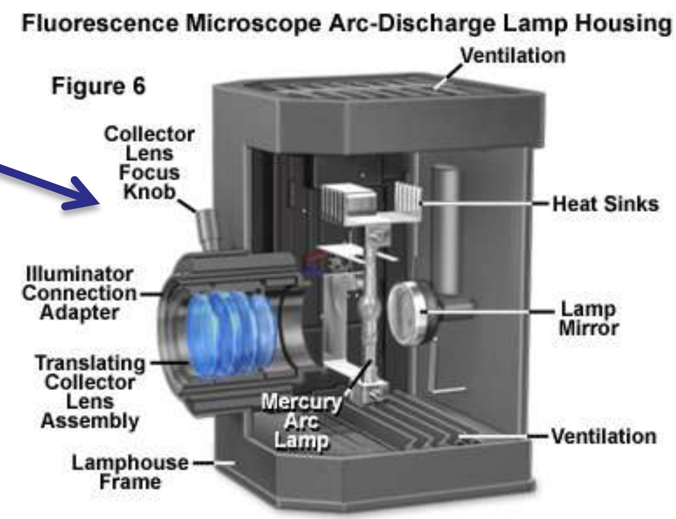


Figure 6

Electromagnetic waves

Electromagnetic waves are typically described by any of the following 3 physical properties: the frequency f, wavelength λ, or photon energy E.
The photon is the basic "unit" of light and all other forms of electromagnetic radiation and is also the force carrier for the electromagnetic force.

$$f = \frac{c}{\lambda}, \quad \text{or} \quad f = \frac{E}{h},$$

c = 299,792,458 m/s is the speed of light in vacuum
h = 6.62606896(33)×10−34 J s

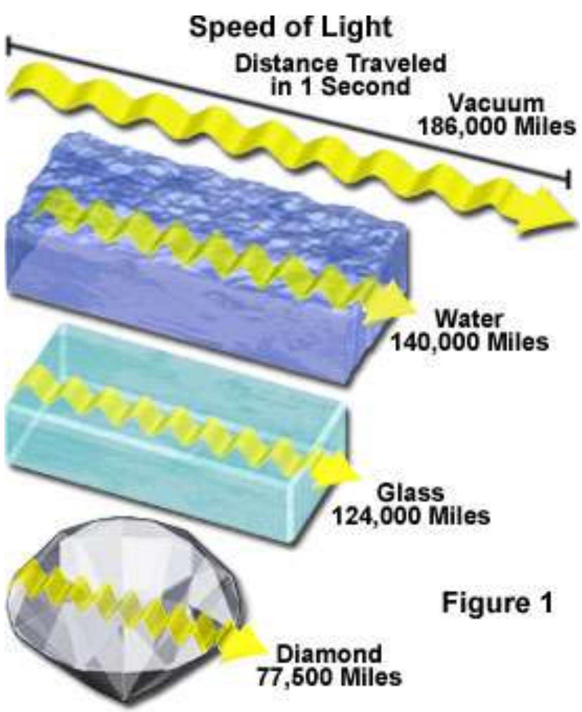
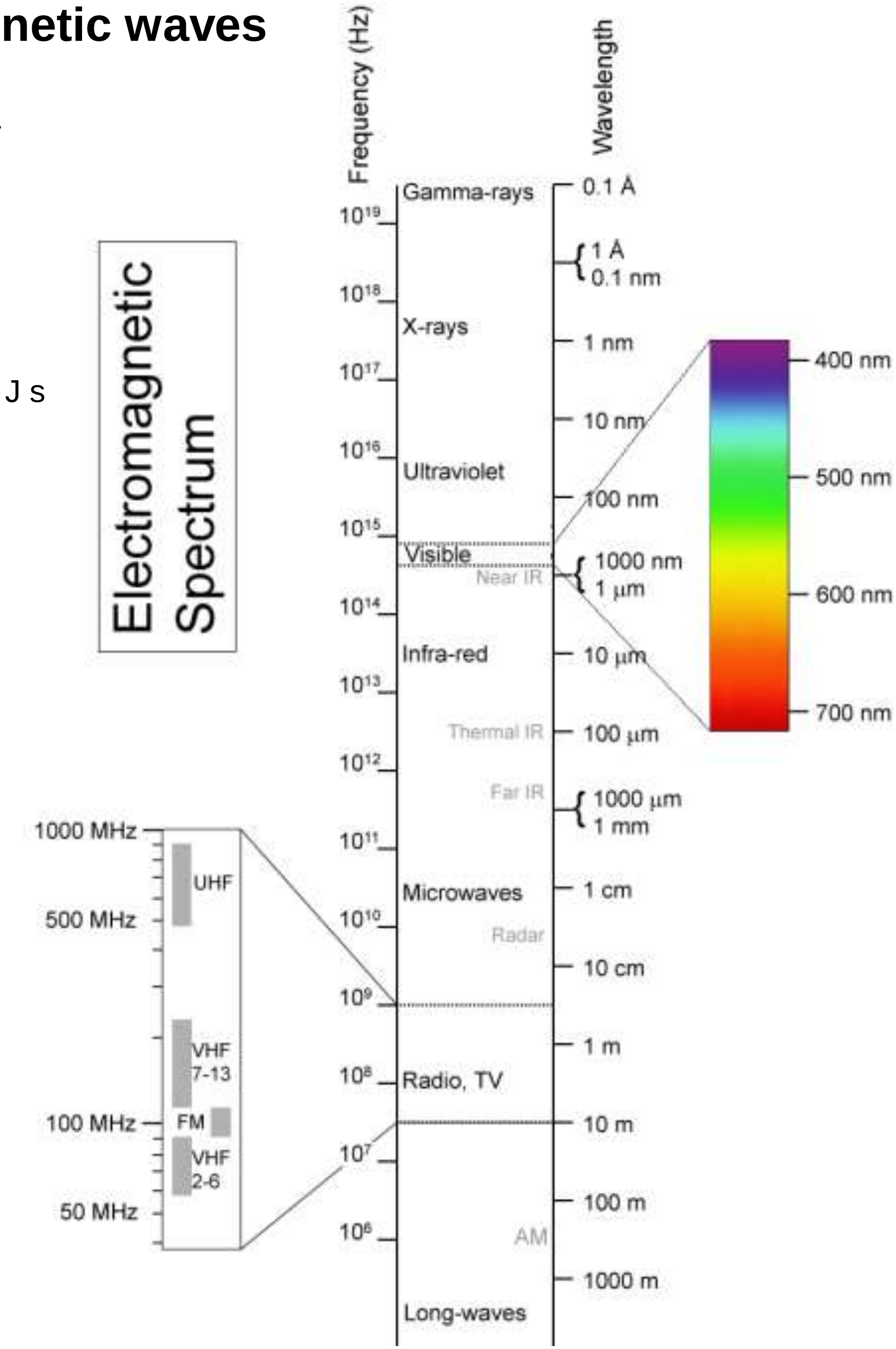


Figure 1



When light traveling in a vacuum enters a new transparent medium, such as air, water, or glass, the speed is reduced in proportion to the refractive index of the new material.

Mercury Arc lamps

Mercury Arc Lamp UV and Visible Emission Spectrum

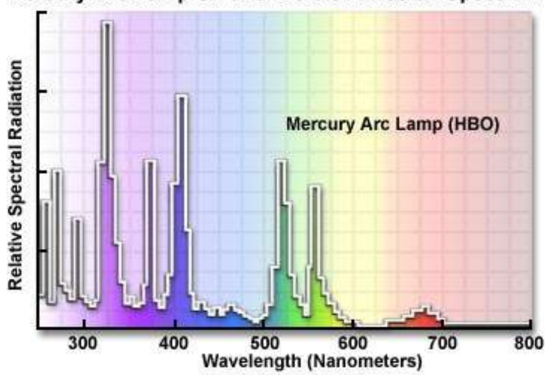


Figure 8

Arc Geometry in Discharge Lamps

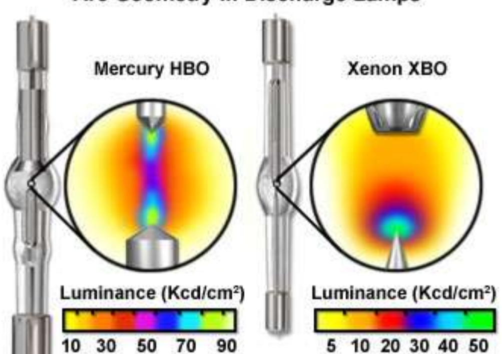
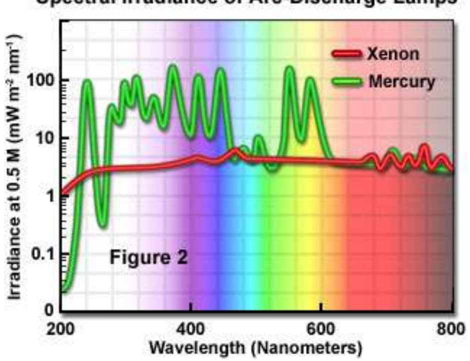


Figure 1

Spectral Irradiance of Arc-Discharge Lamps



Tungsten-halogen lamps

Tungsten Lamp Emission Spectrum

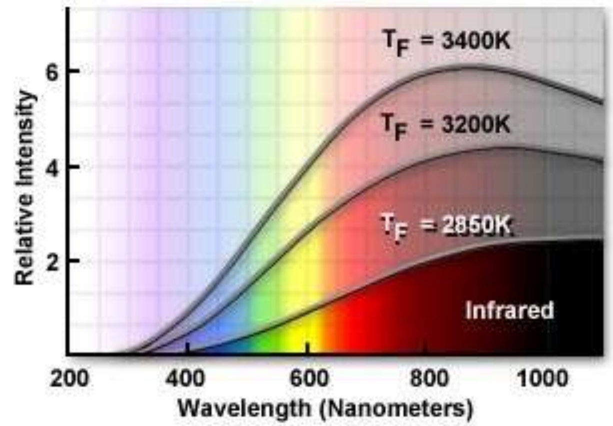


Figure 2

Incandescent Light Sources

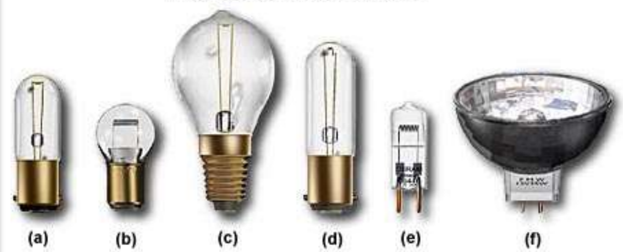


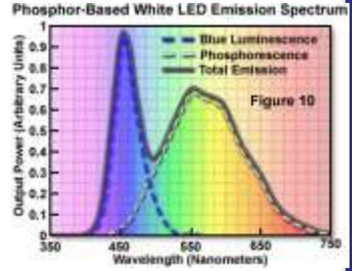
Figure 3

Light Emitting Diodes

Anatomy of a White Light Emitting Diode

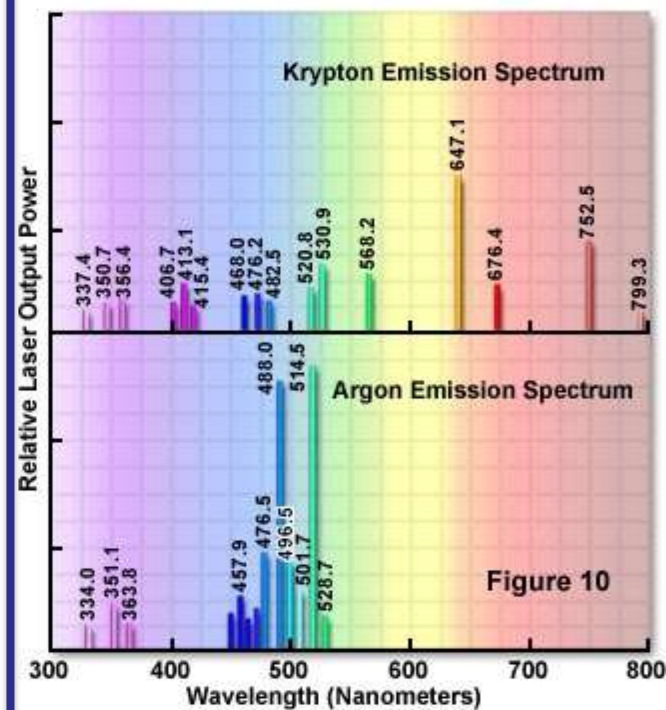
Figure 7

Phosphor-Based White LED Emission Spectrum



Laser Systems

Laser Illumination Source Emission Spectra



Spectra Physics Tsunami® Ti:Sapphire Mode-Locked Laser

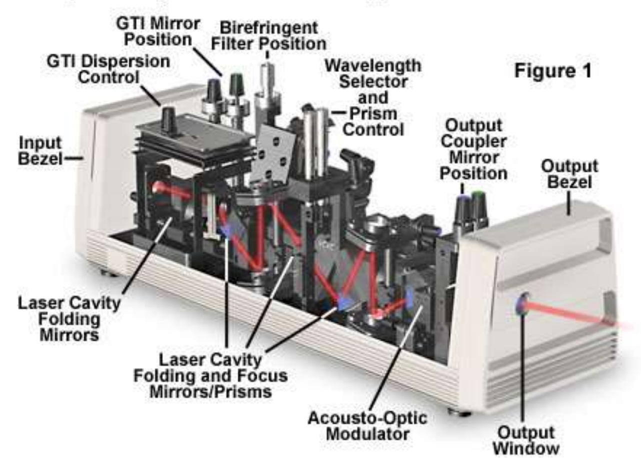


Figure 1

Radiation occur due to fact that electrons moving in orbits around the nucleus of an atom are arranged in different energy levels within their probability distribution functions.

Many of the electrons can absorb additional energy from external sources of electromagnetic radiation, which results in their promotion to an inherently unstable higher energy level.

Eventually, the "excited" electron loses the extra energy by emitting electromagnetic radiation of lower energy and, in doing so, falls back into its original and stable energy level.

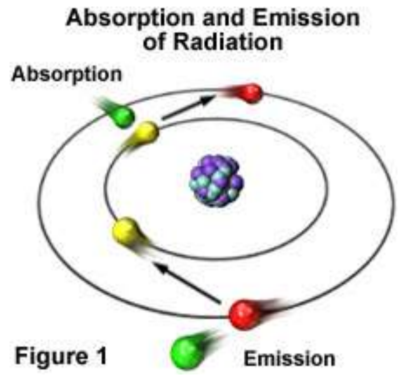


Figure 1

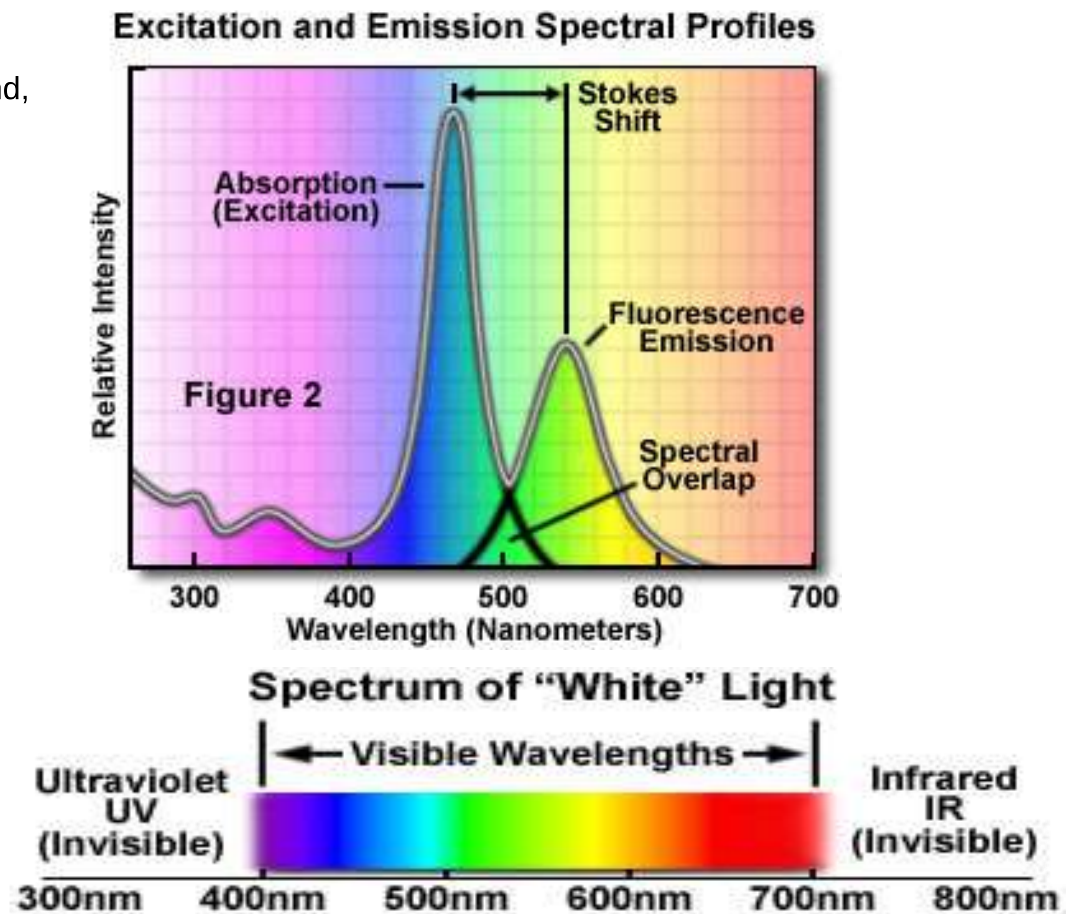


Figure 2

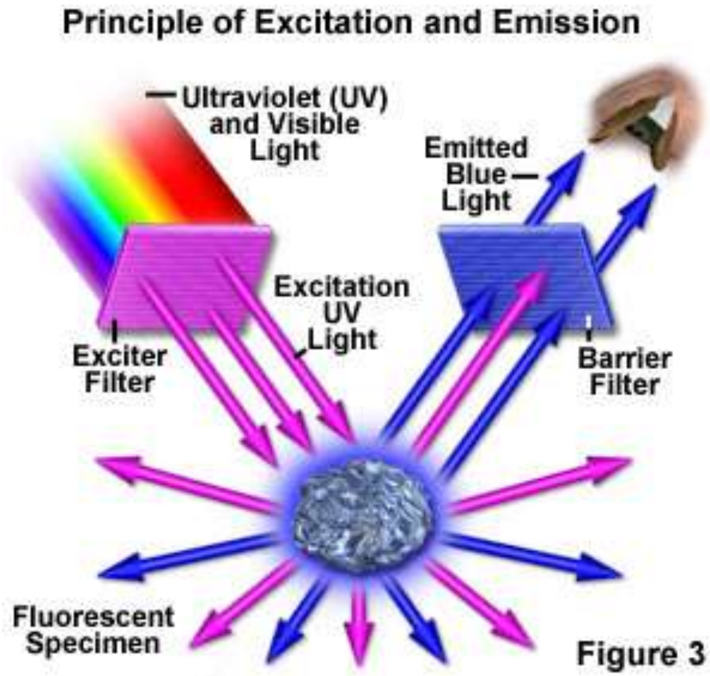
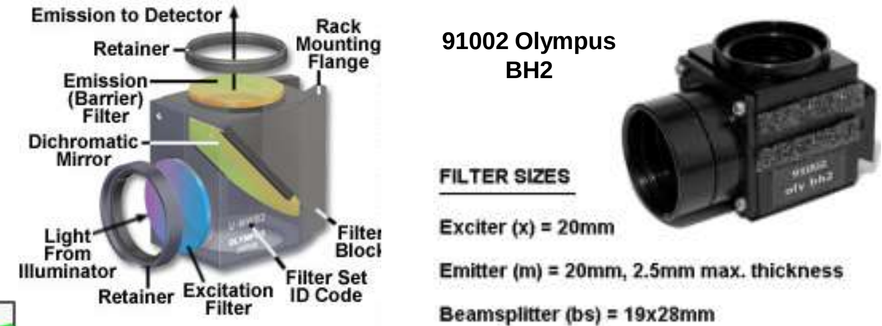
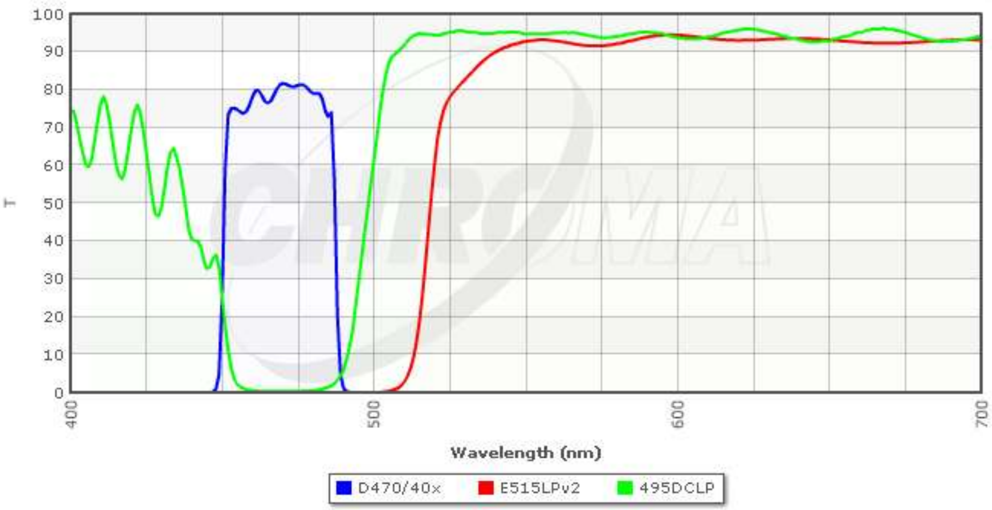
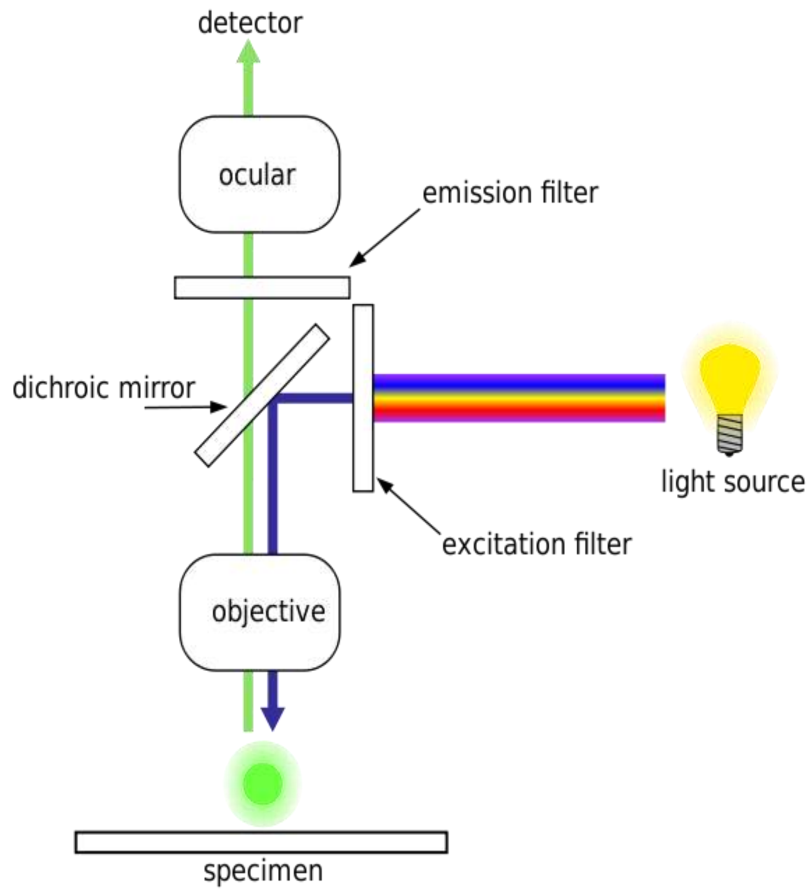


Figure 3

The primary filtering element in the epifluorescence microscope is the set of three filters housed in the fluorescence filter cube (also called the filter block): the excitation filter, the emission filter, and the dichroic beamsplitter.







1) The excitation filter (also called the exciter) transmits only those wavelengths of the illumination light that efficiently excite a specific dye.
2) The dichroic beamsplitter (also called the dichroic mirror or dichromatic beamsplitter) is a thin piece of coated glass set at a 45-degree angle to the optical path of the microscope. This coating has the unique ability to reflect one color, the excitation light, but transmit another color, the emitted fluorescence.
3) The emission filter (also called the barrier filter or emitter) attenuates all of the light transmitted by the excitation filter and very efficiently transmits any fluorescence emitted by the specimen. This light is always of longer wavelength (more to the red) than the excitation color.

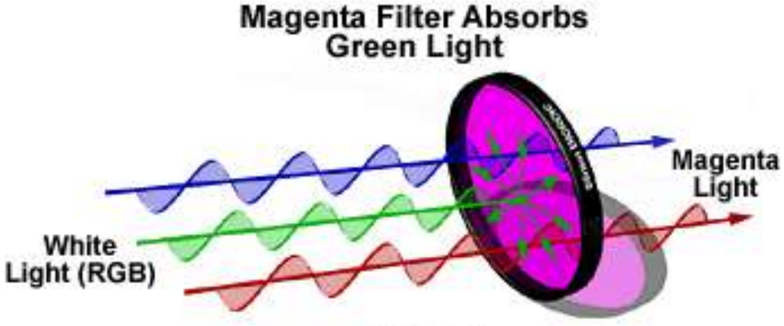


Figure 1

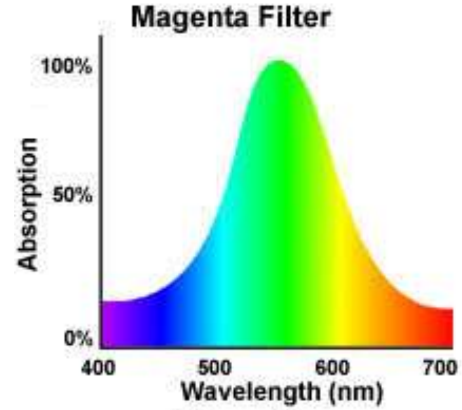


Figure 2

Absorption Filters - In Figure 1, the three incident waves are colored red, green, and blue but are intended to represent all the colors that comprise white light. The filter selectively transmits the red and blue portions of the incident white light spectrum, but absorbs most of the green wavelengths. As discussed in our section on primary colors, the color magenta is obtained by subtracting green from white light. The light-modulating properties of a typical color filter are illustrated in Figure 2.

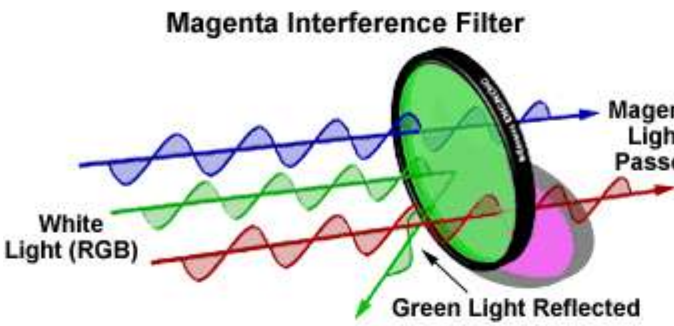
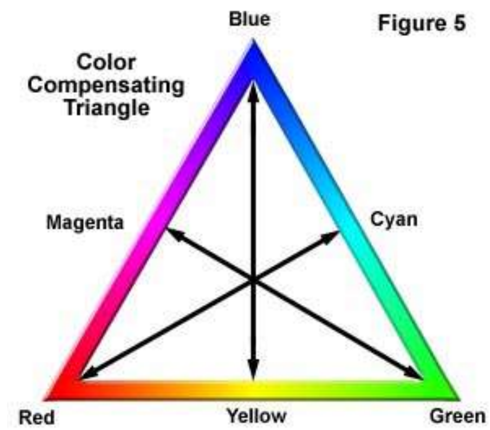


Figure 3

Figure 5

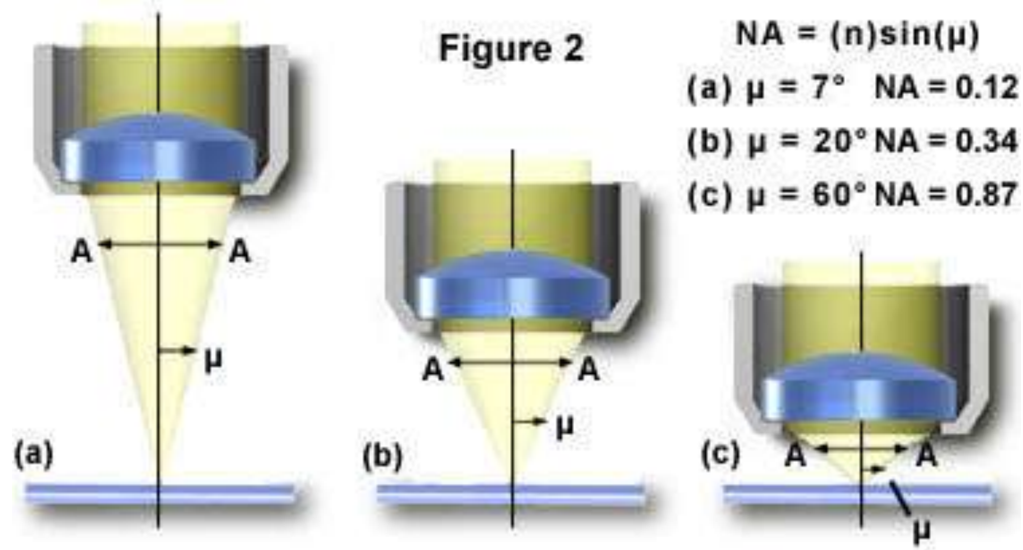


Interference Filters - These filters differ from absorption filters in the fact that they reflect and destructively interfere with unwanted wavelengths as opposed to absorbing them. The term dichroic arises from the fact that the filter appears one color under illumination with transmitted light and another with reflected light. In the case of the magenta dichroic filter illustrated below in Figure 3, green light is reflected from the face of the filter and magenta light is transmitted from the other side of the filter.

Figure 1

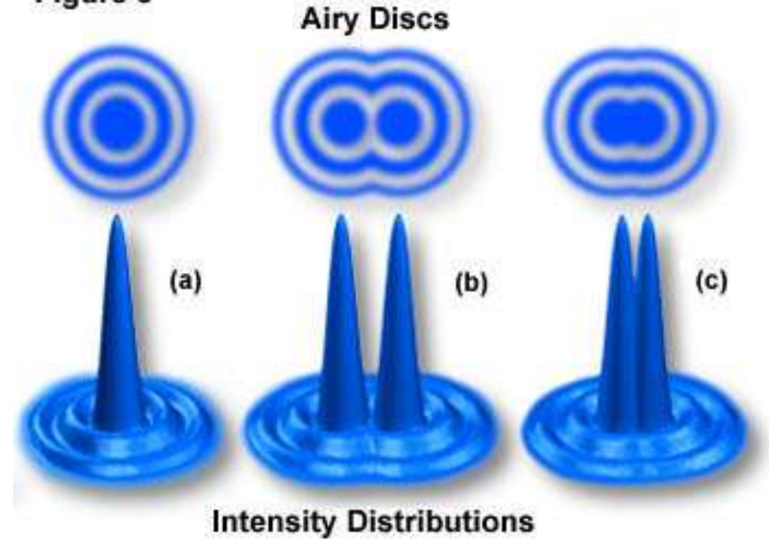
Figure 1

The angle **μ** is one-half the angular aperture (**A**) and is related to the numerical aperture through the following equation**:** **Numerical Aperture** (**NA**) **= n(sin μ)** where **n** is the refractive index of the imaging medium between the front lens of the objective and the specimen cover glass, a value that ranges from 1.00 for air to 1.51 for specialized immersion oils. From this equation it is obvious that when the imaging medium is air (with a refractive index, **n** = 1.0), then the numerical aperture is dependent only upon the angle **μ** whose maximum value is 90°. The sin of the angle **μ**, therefore, has a maximum value of 1.0 (sin(90°) = 1), which is the theoretical maximum numerical aperture of a lens operating with air as the imaging medium (using "dry" microscope objectives).



When light from the various points of a specimen passes through the objective and is reconstituted as an image, the various points of the specimen appear in the image as small patterns (not points) known as Airy patterns. This phenomenon is caused by diffraction or scattering of the light as it passes through the minute parts and spaces in the specimen and the circular back aperture of the objective. The central maximum of the Airy patterns is often referred to as an Airy disk, which is defined as the region enclosed by the first minimum of the Airy pattern and contains 84 percent of the luminous energy. These Airy disks consist of small concentric light and dark circles as illustrated in Figure 3. This figure shows Airy disks and their intensity distributions as a function of separation distance.

Figure 3



The smaller the Airy disks projected by an objective in forming the image, the more detail of the specimen that becomes discernible. Objectives of higher correction (fluorites and apochromats) produce smaller Airy disks than do objectives of lower correction. In a similar manner, objectives that have a higher numerical aperture are also capable of producing smaller Airy disks. This is the primary reason that objectives of high numerical aperture and total correction for optical aberration can distinguish finer detail in the specimen.

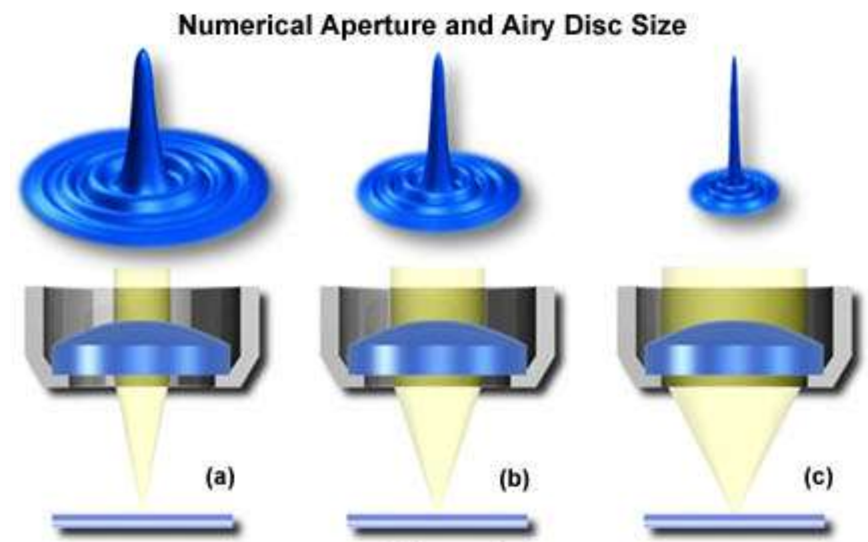


Figure 4

Resolution and Numerical Aperture by Objective Type

	Plan Achromat		Plan Fluorite		Plan Apochromat	
Magnification	N.A	Resolution (µm)	N.A	Resolution (µm)	N.A	Resolution (µm)
4x	0.10	2.75	0.13	2.12	0.20	1.375
10x	0.25	1.10	0.30	0.92	0.45	0.61
20x	0.40	0.69	0.50	0.55	0.75	0.37
40x	0.65	0.42	0.75	0.37	0.95	0.29
60x	0.75	0.37	0.85	0.32	0.95	0.29
100x	1.25	0.22	1.30	0.21	1.40	0.20

Resolution vs. Wavelength

Wavelength (nanometers)	Resolution (micrometers)
360	.19
400	.21
450	.24
500	.26
550	.29
600	.32
650	.34
700	.37

Optical Correction & Magnification0	Working Distance (mm)
ACH 10x	6.10
ACH 20x	3.00
ACH 40x	0.45
ACH 60x	0.23
ACH 100x (Oil)	0.13
PL 4x	22.0
PL 10x	10.5
PL 20x	1.20
PL 40x	0.56
PL 100x (Oil)	0.15
PL FL 4x	17.0
PL FL 10x	10.00
PL FL 20x	1.60
PL FL 40x	0.51
PL FL 100x (Oil)	0.10
PL APO 1.25x	5.1
PL APO 2x	6.20
PL APO 4x	13.00
PL APO 10x	3.10
PL APO 20x	0.65
PL APO 40x	0.20
PL APO 60x (Oil)	1.10
PL APO 100x (Oil)	0.10

Optical Corrections - These are usually listed as Achro and Achromat (achromatic), as Fl, Fluar, Fluor, Neofluar, or Fluotar (fluorite) for better spherical and chromatic corrections, and as Apo (apochromatic) for the highest degree of correction for spherical and chromatic aberrations.

Working Distance - This is the distance between the objective front lens and the top of the cover glass when the specimen is in focus. In most instances, the working distance of an objective decreases as magnification increases.

Principles of Confocal Microscopy

Coherent light emitted by the laser system (excitation source) passes through a pinhole aperture that is situated in a conjugate plane (confocal) with a scanning point on the specimen and a second pinhole aperture positioned in front of the detector (a photomultiplier tube). As the laser is reflected by a dichromatic mirror and scanned across the specimen in a defined focal plane, secondary fluorescence emitted from points on the specimen (in the same focal plane) pass back through the dichromatic mirror and are focused as a confocal point at the detector pinhole aperture.

The significant amount of fluorescence emission that occurs at points above and below the objective focal plane is not confocal with the pinhole (termed Out-of-Focus Light Rays) and forms extended Airy disks in the aperture plane. Because only a small fraction of the out-of-focus fluorescence emission is delivered through the pinhole aperture, most of this extraneous light is not detected by the photomultiplier and does not contribute to the resulting image. Refocusing the objective in a confocal microscope shifts the excitation and emission points on a specimen to a new plane that becomes confocal with the pinhole apertures of the light source and detector.

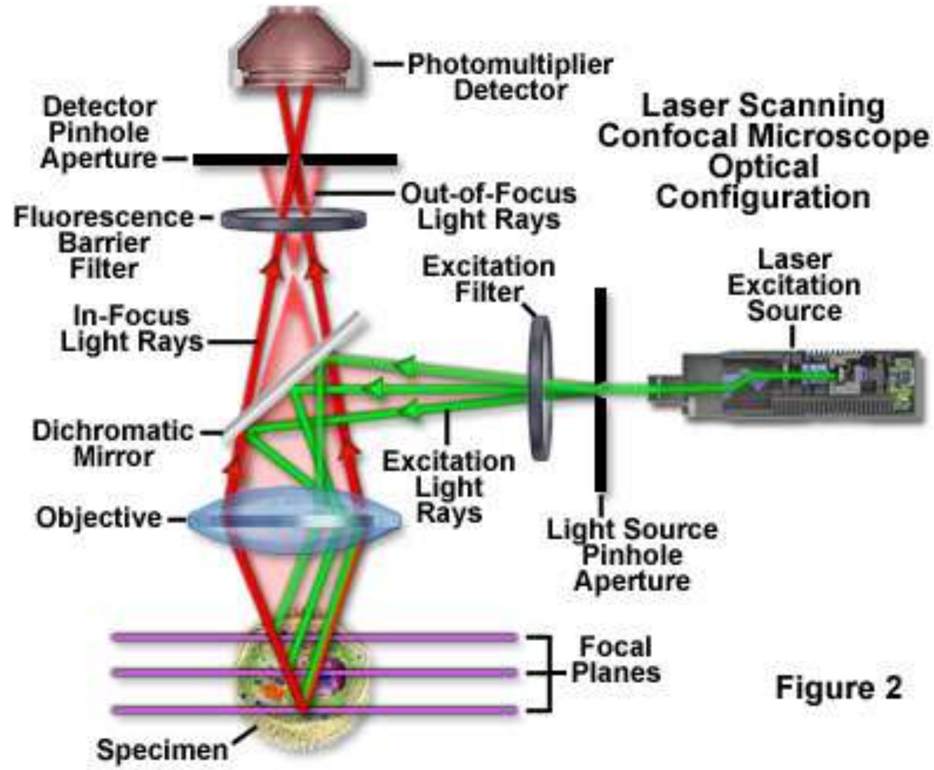


Figure 2

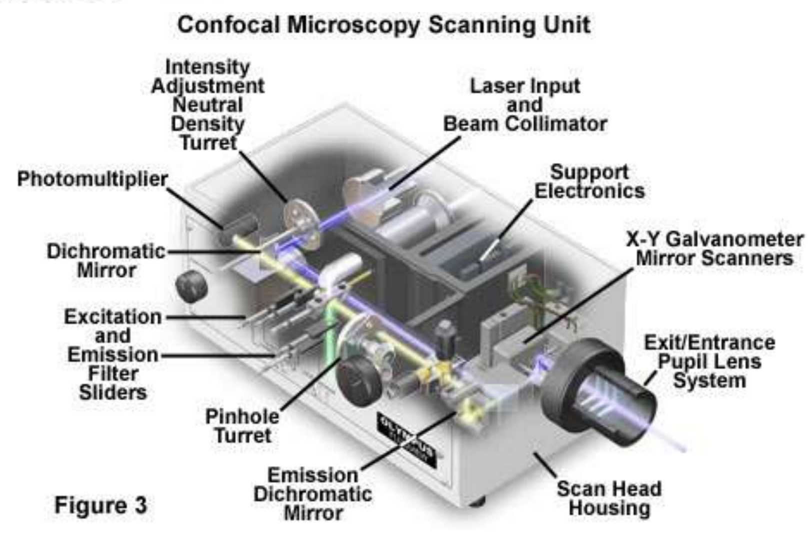


Figure 3

Confocal microscopy offers several advantages over conventional widefield optical microscopy, including the ability to control depth of field, elimination or reduction of background information away from the focal plane (that leads to image degradation), and the capability to collect serial optical sections from thick specimens. The basic key to the confocal approach is the use of spatial filtering techniques to eliminate out-of-focus light or glare in specimens whose thickness exceeds the immediate plane of focus.

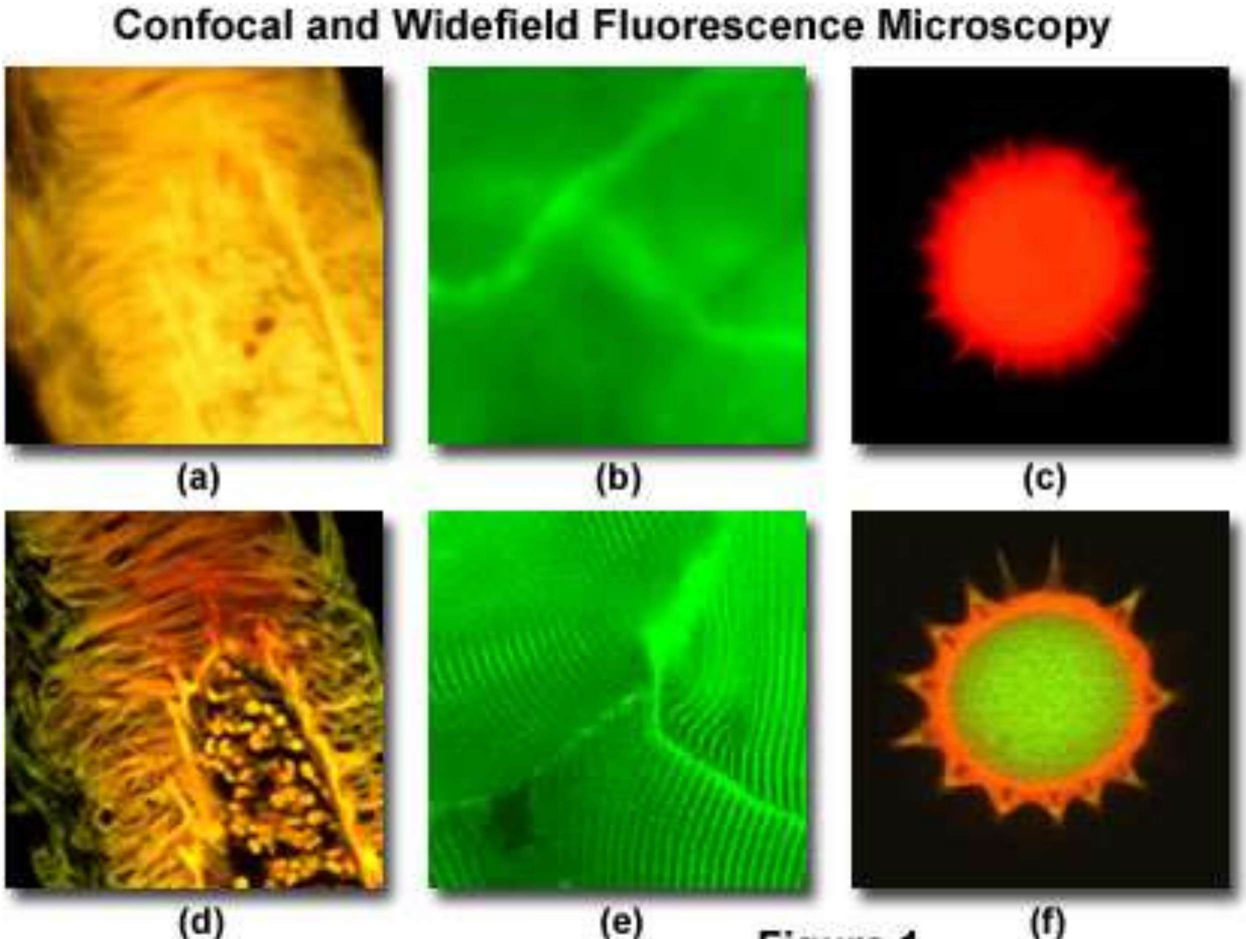
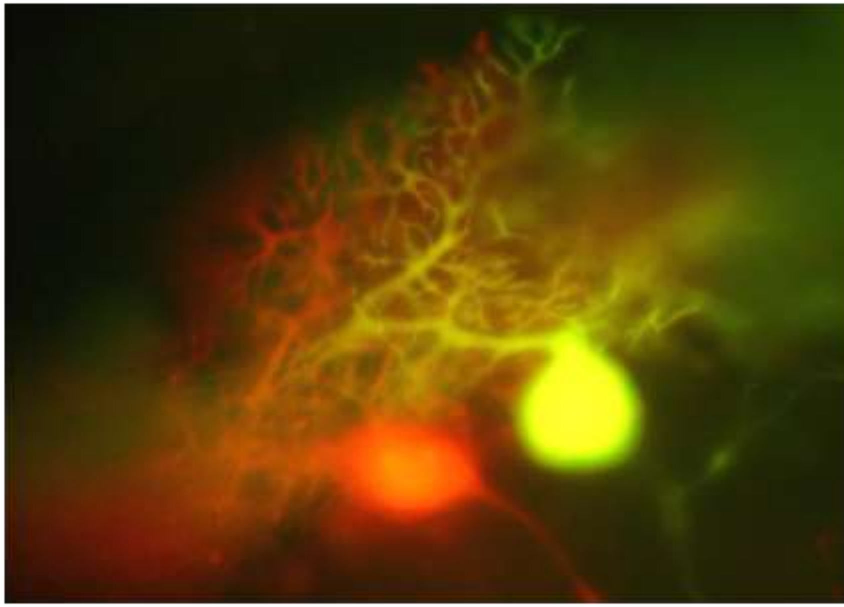
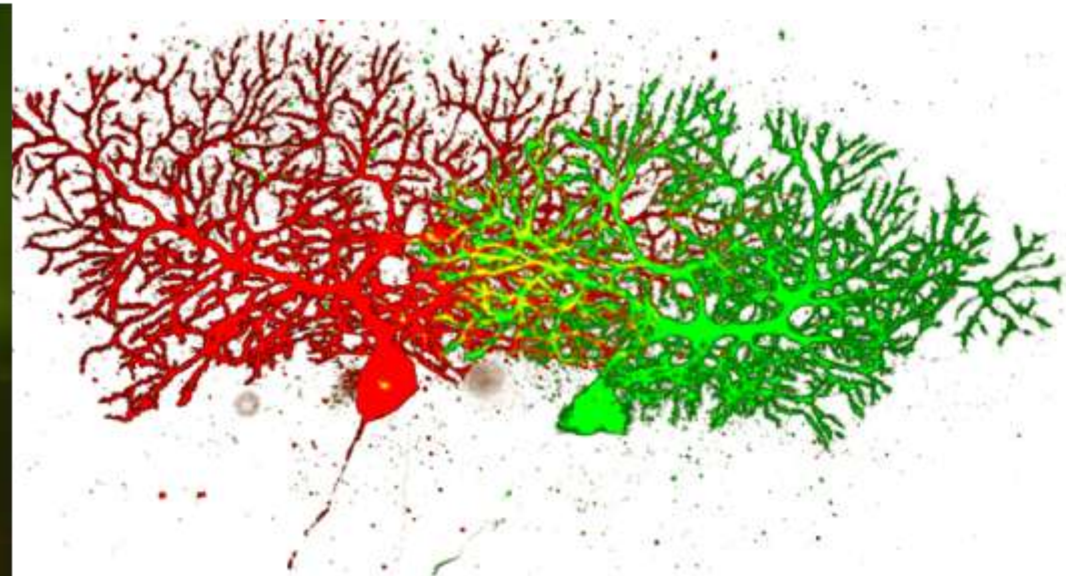
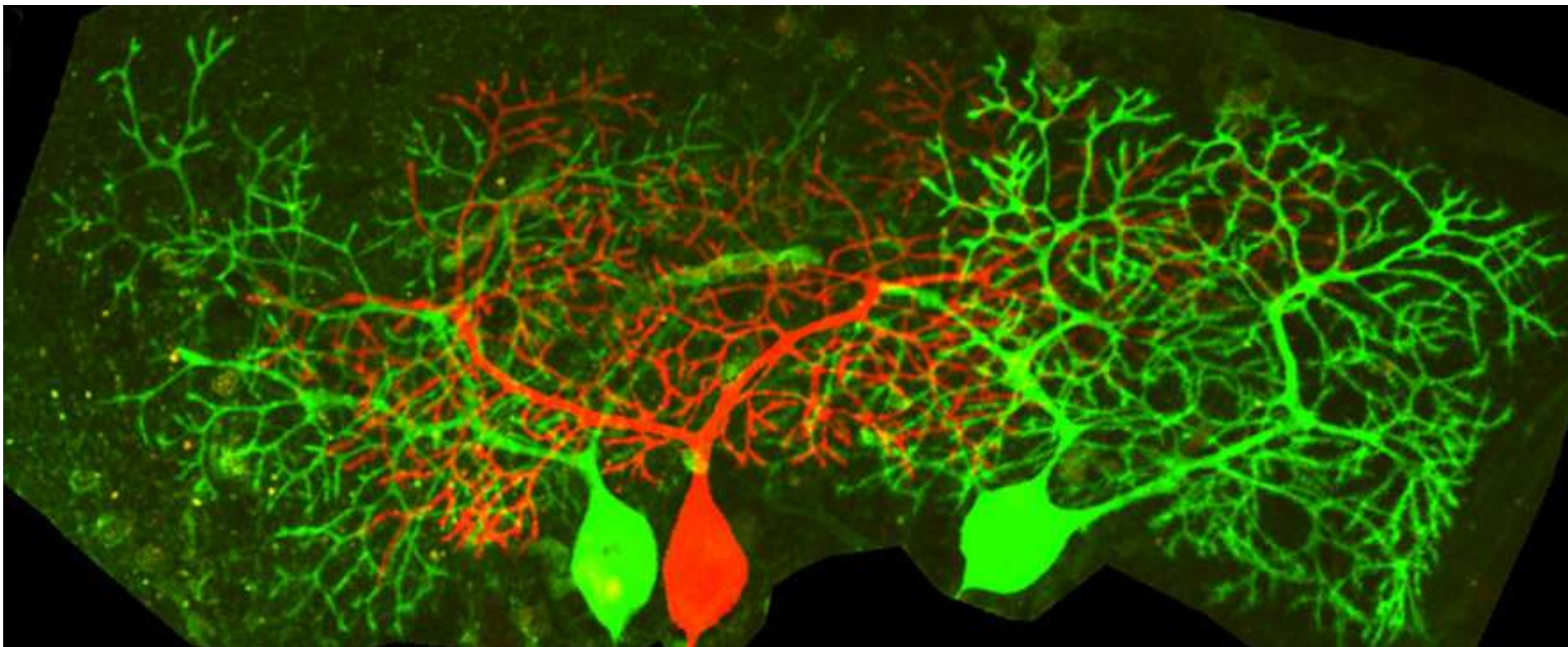


Figure 1

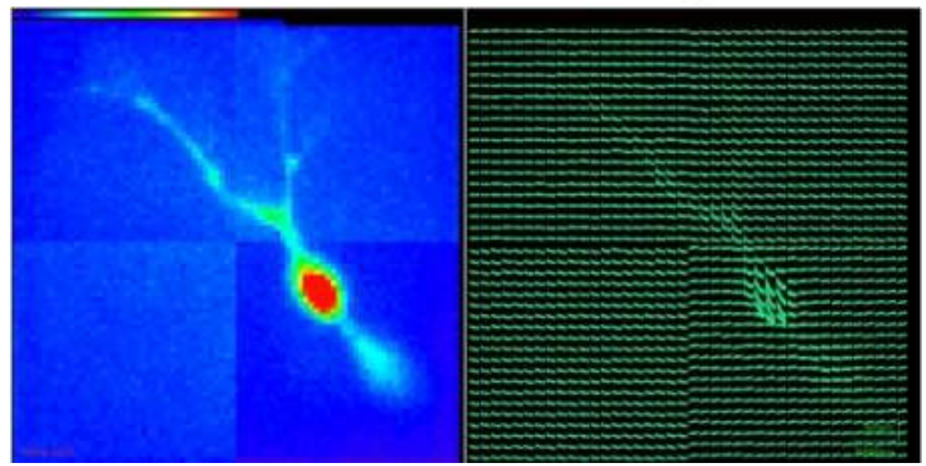
Series of images that compare selected viewfields in traditional widefield and laser scanning confocal fluorescence microscopy. A thick section of fluorescently stained human medulla in widefield fluorescence exhibits a large amount of glare from fluorescent structures above and below the focal plane (Figure 1(a)). When imaged with a laser scanning confocal microscope (Figure 1(d)), the medulla thick section reveals a significant degree of structural detail. Likewise, widefield fluorescence imaging of whole rabbit muscle fibers stained with fluorescein produce blurred images (Figure 1(b)) lacking in detail, while the same specimen field (Figure 1(e)) reveals a highly striated topography in confocal microscopy. Autofluorescence in a sunflower pollen grain produces an indistinct outline of the basic external morphology (Figure 1(c)), but yields no indication of the internal structure. In contrast, a thin optical section of the same grain (Figure 1(f)) acquired with confocal techniques displays a dramatic difference between the particle core and the surrounding envelope.

REDSHIRT IMAGING

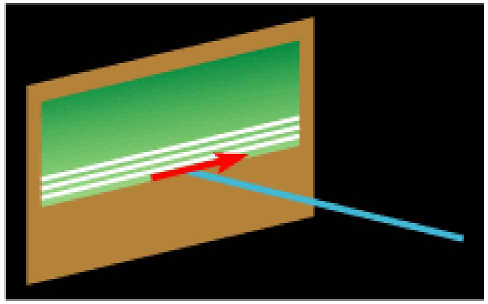
NEUROCCD-SMQ

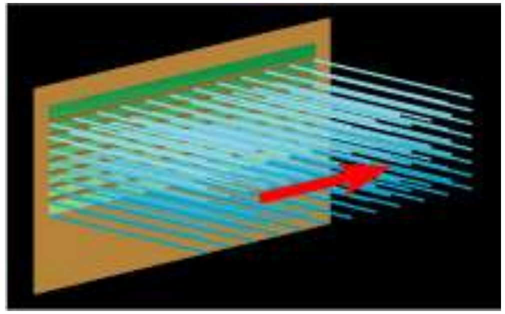
is capable of recording action potential propagation in individual neurons with outstanding temporal resolution:
80 x 80 pixels: 2000 frames/sec
3 x 3 bin: 5000 frames/sec



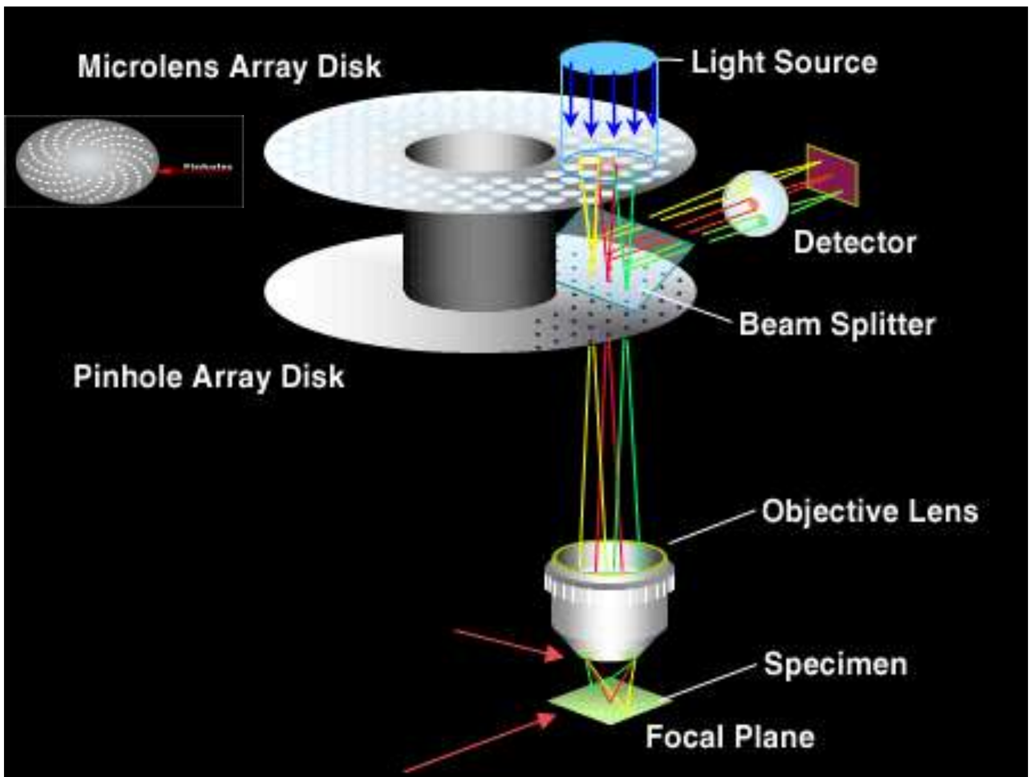
1 frame = 80 x 80 pixels = 6400 pixels or channels at 14 bits
+ 8 channels of electrical recording
2000 frames /sec = 25 Mbytes/sec
1 CD =700 Mbytes = 30 sec of data
1 DVD= 4.3 Gbytes = 3 min of data

Principles of the Microlens-enhanced Nipkow Disk Scanning Technology

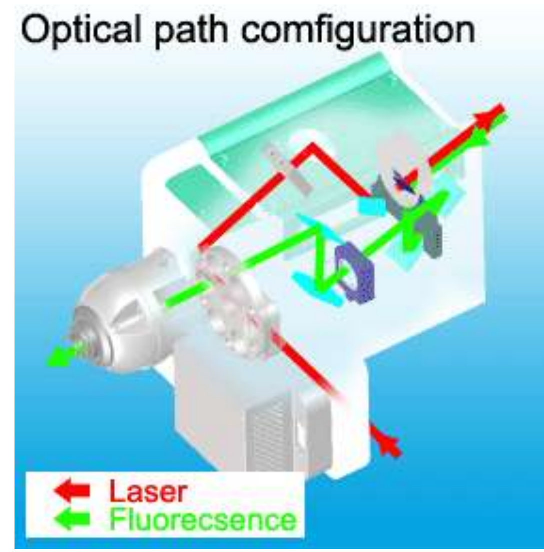




The most common conventional confocal microscopes use a single laser beam to scan a specimen, while the CSU scans the field of view with approximately 1,000 laser beams, by using microlens-enhanced Nipkow-disk scanning:



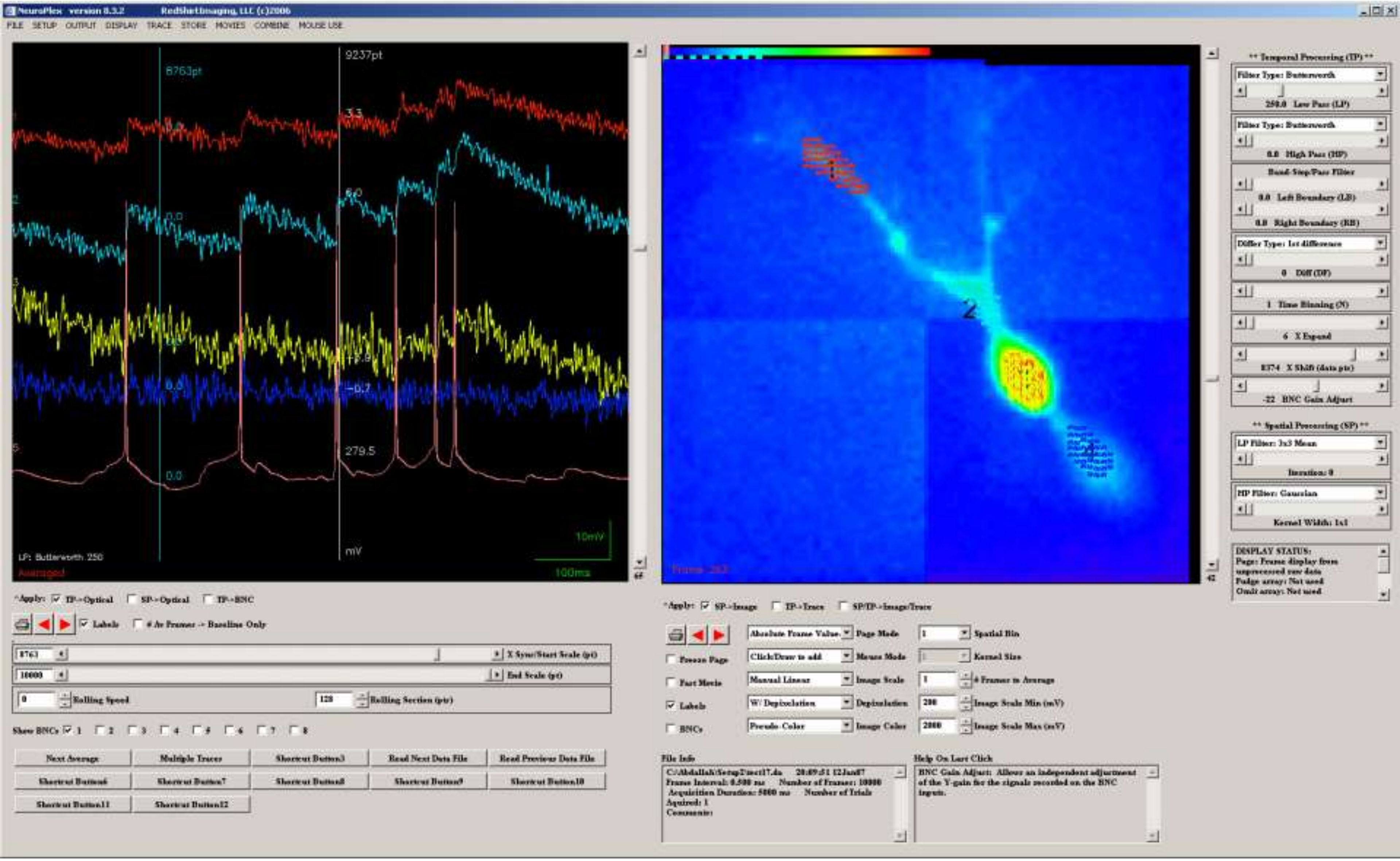
http://www.yokogawa.com/scanner/principles/principle1.htm



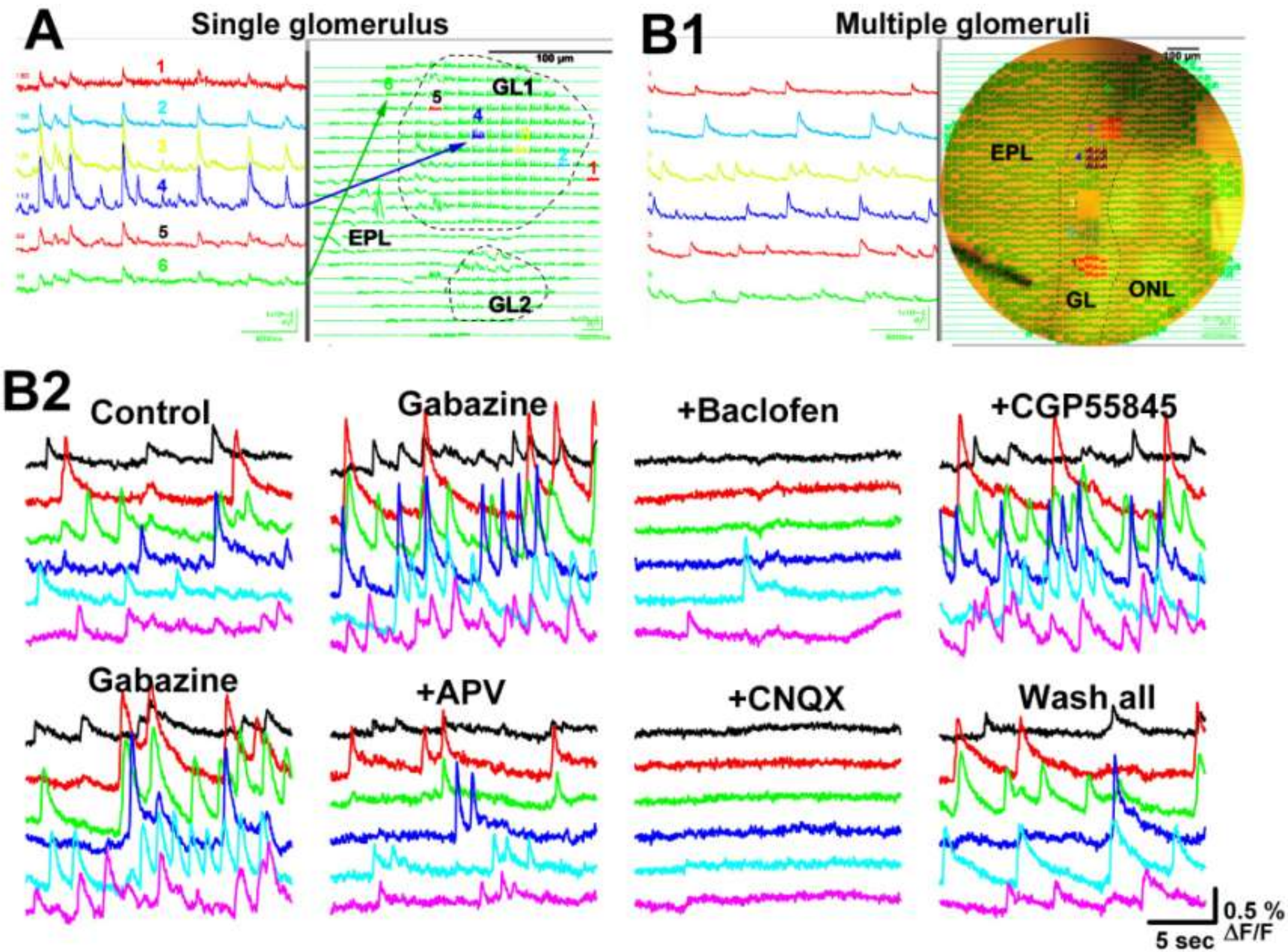
NEURO CCD-SMQ

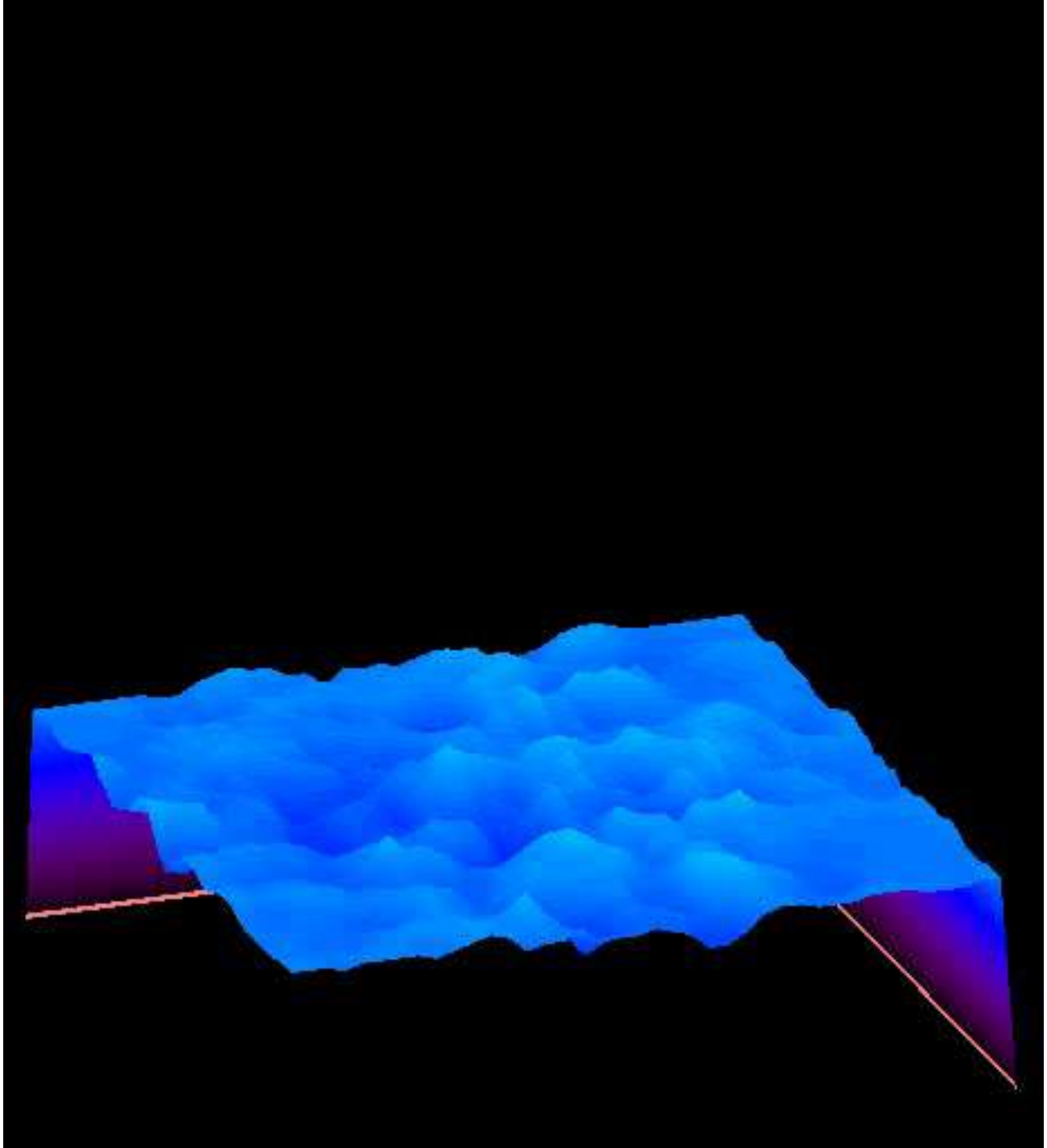
80 x 80 pixels
6400 pixels
14 bits resolution
2000 frames/sec

Fast optical recordings will help resolve the time of occurrence of action potentials

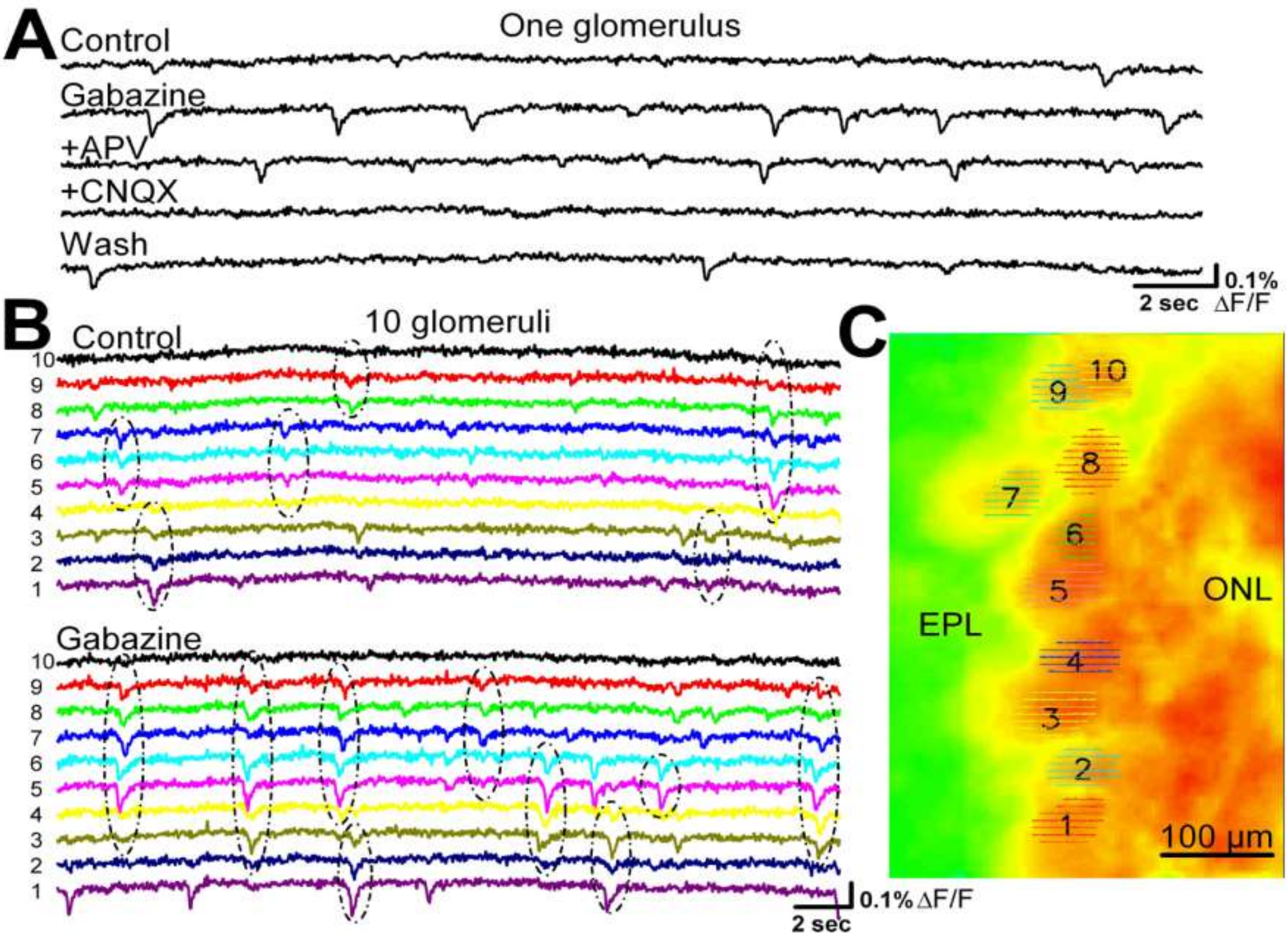


Spontaneous population calcium signals in individual glomeruli

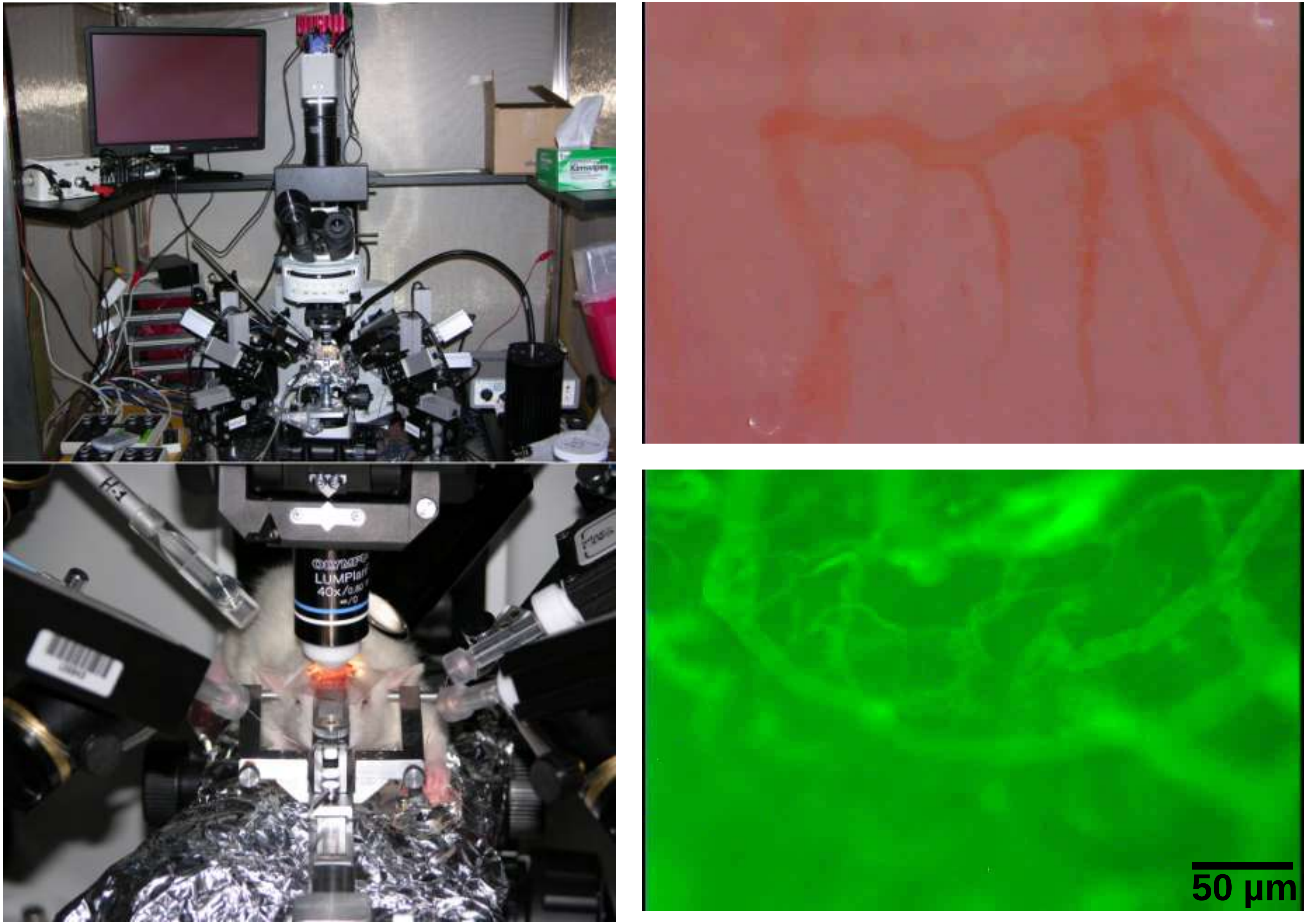




Voltage-sensitive dye imaging: Evidence for inter-glomerular interactions



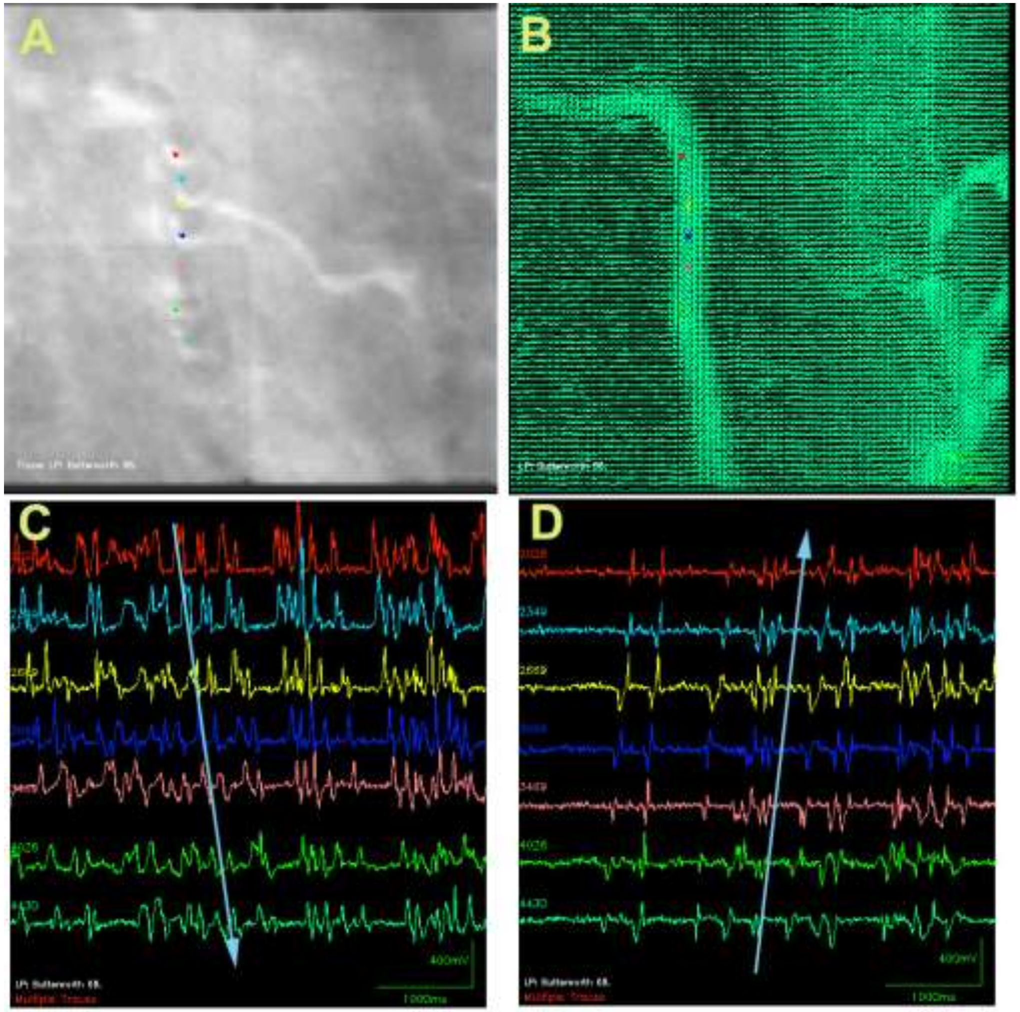
Video microscopy of cerebral blood flow



Ultra fast imaging of red blood cell flow in capillaries

X60 objective

10 µm



Flow = # of RBCs that cross a point per sec

Density = # of RBCs / mm

Velocity = distance traveled (mm)/ sec

Flow = Velocity x Density

SAMPLE TEST QUESTIONS:

1- In Clampex software, each sample of data is stored in:
A. 1 Kbyte
B. 16 bit
C. 16 bytes
D. 1 byte

2- A low-pass filter is used to
A. eliminate low frequencies
B. eliminate high frequencies
C. to amplify signals
D. to de-convolute a signal

3- A decimal number of 10 is translated into binary as:
A. 1111
B. 0010
C. 1010
D. 0101

4- A drug produces postsynaptic effect if
A. the paired-pulse ratio does not change
B. there is a decrease in the frequency on miniEPSCs
C. the paired-pulse ratio is decreased
D. the frequency of miniEPSC increases

5- The capacitance of the cell membrane can be used to
A. calculate its resistance
B. calculate its area
C. calculate the number of channels
D. calculate its conductance

6- The resultant of 2 resistors in series is the
A. difference between their resistances
B. The sum of the inverse of their resistance
C. The sum of their resistance
D. The product of their resistance

7- The unit pA indicates:
A. 10^{-15} Amp
B. 10^{-7} Amp
C. 10^{-12} Amp
D. 10^{-9} Amp

8- The capacitance is measured in
A. coulombs
B. mM
C. watts
D. Farad

9- GABAA receptors are
A. Chloride channels
B. cationic channels
C. permeable to calcium
D. voltage-dependent

10- During an action potential,
A. Calcium flows outside the cell
B. Potassium flows inside the cell
C. Chloride flows inside the cell
D. Sodium flows inside the cell