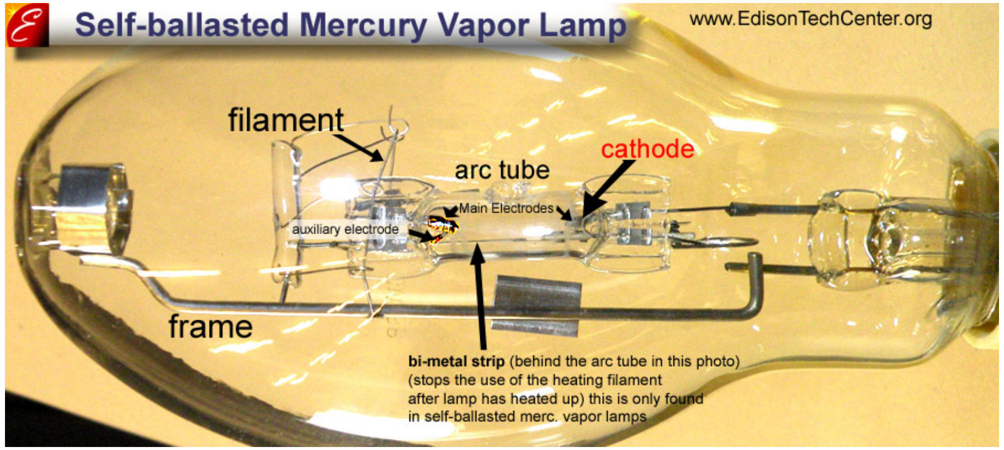
Light sources, phase contrast

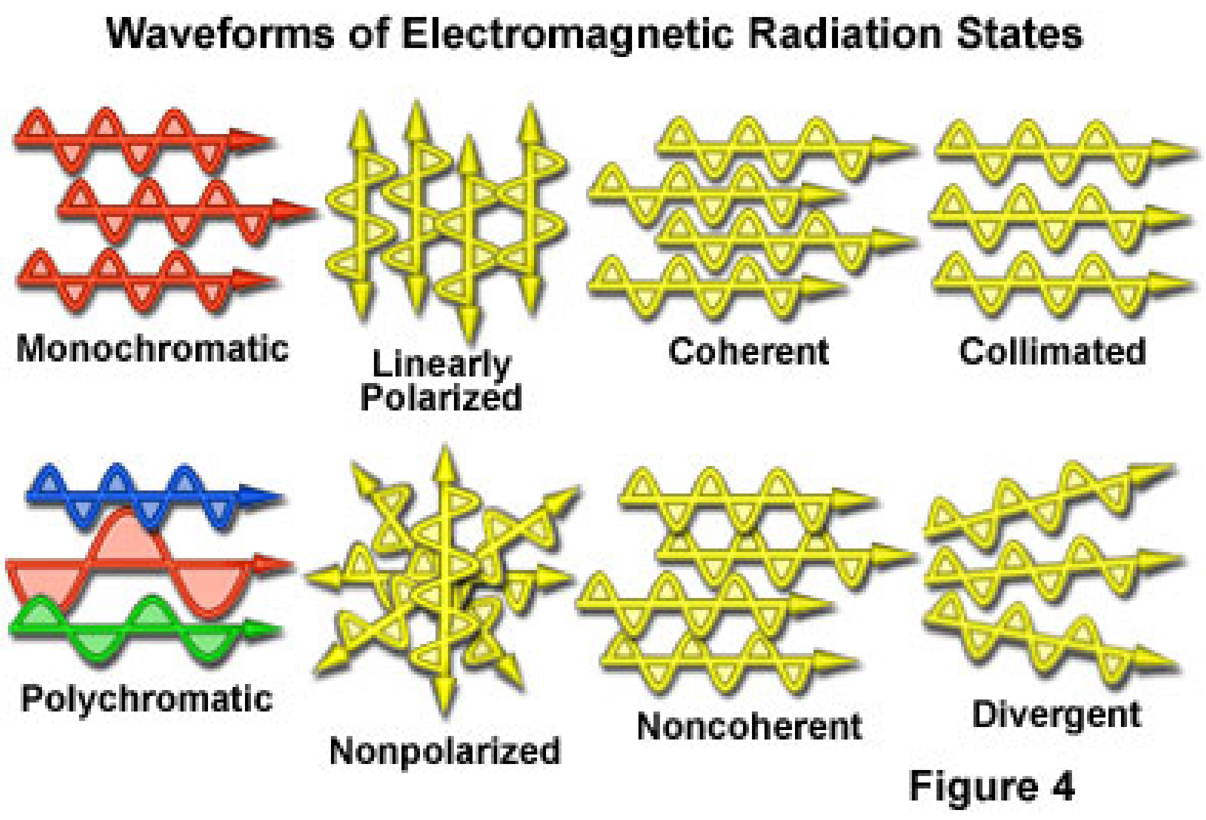
- Last class
  - Staining and contrast
  - Phase contrast
  - Darkfield
- This class
  - Polarization of light
  - Birefringent materials
  - Polarization microscopy

Light source options

- Broadband sources (filaments)
- Light emitting diodes
- Lasers



Properties of light

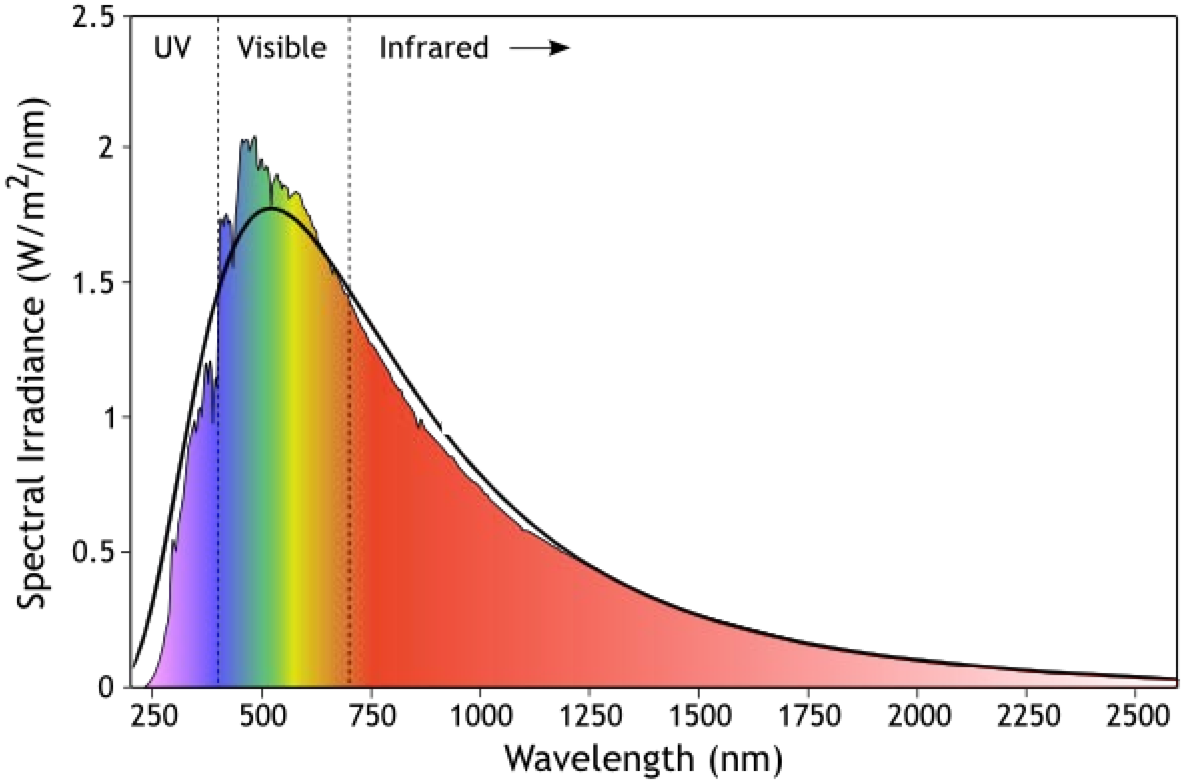


Other things to consider:

Intensity
Total cost
Compatibility with existing setup
Lifetime
Alignment

Sunlight

- The original light source
- Free, 12 hours a day
- Blackbody radiator
- Polychromatic
- incoherent
- Variable intensity (clouds)



$$I(\nu, T) = \frac{2h\nu^3}{c^2}\frac{1}{e^{\frac{h\nu}{kT}} - 1}$$

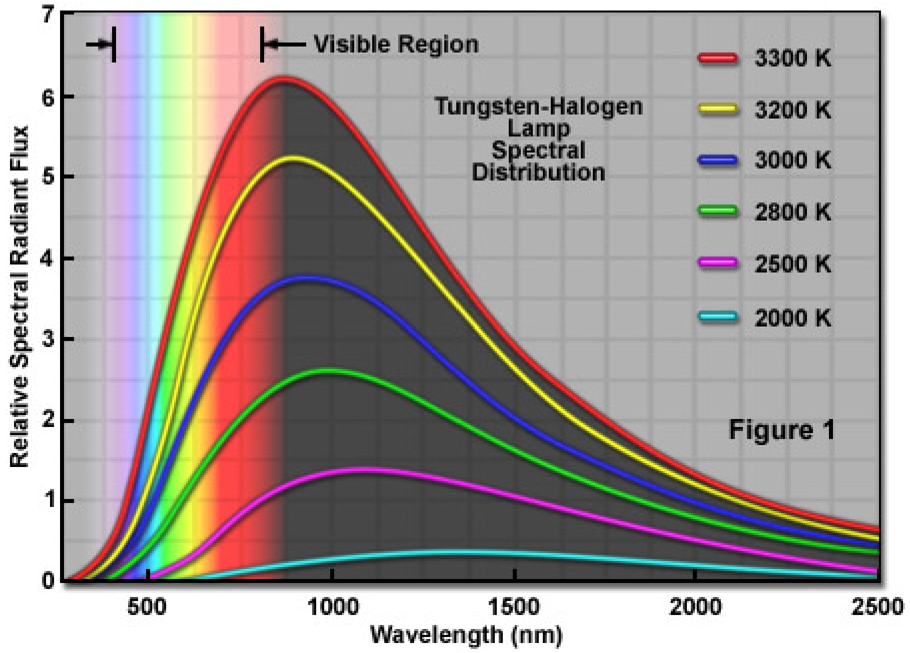
$$\lambda_{\max} = \frac{b}{T}$$

k = Boltzmann's constant = 1.4x10^{-23} J/K
h = Planck's constant = 6.3x10^{-34} J*s

b = 2.9 x10^{-3} K m

Incandescent bulbs

- Cheap – relatively dim
- Broad spectrum
- Long lifetime
- Low power
- Unpolarized
- Incoherent
- Diverging



$$I(\nu, T) = \frac{2h\nu^3}{c^2}\frac{1}{e^{\frac{h\nu}{kT}}-1}$$

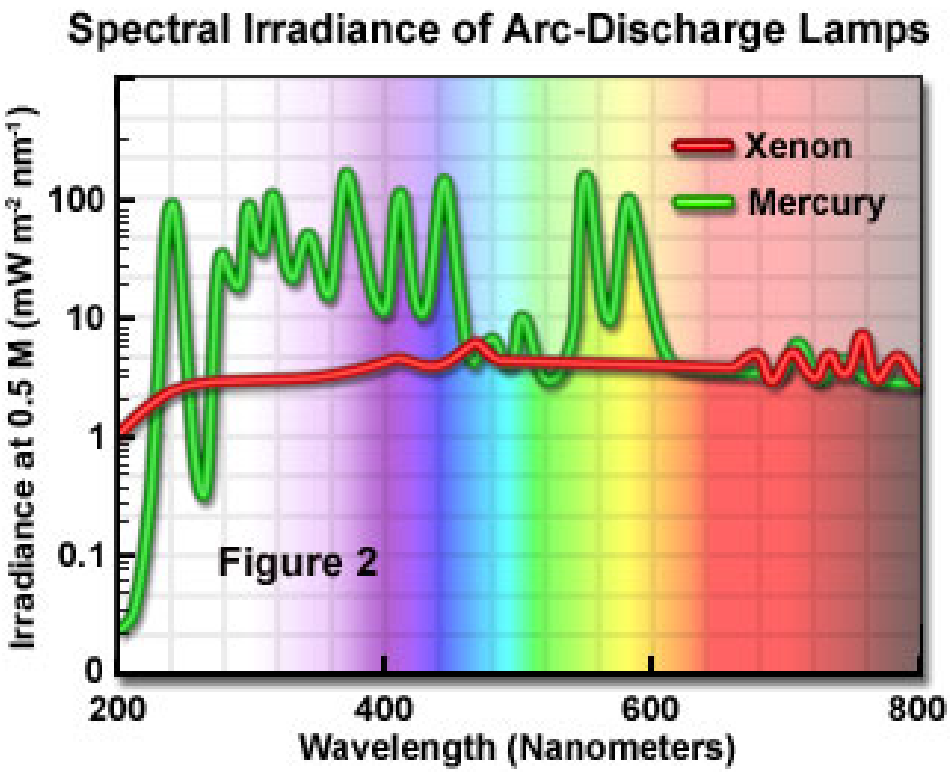
$$\lambda_{\max} = \frac{b}{T}$$

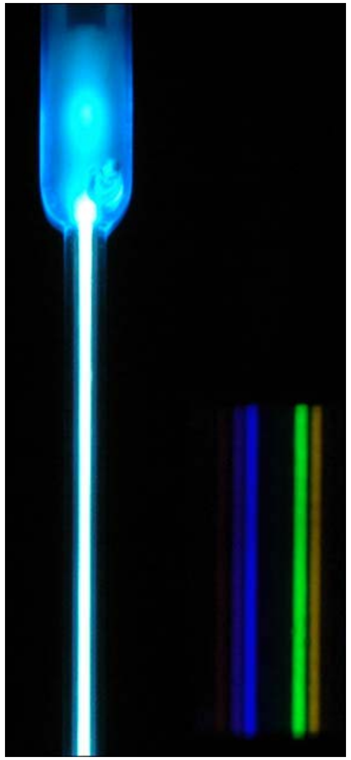
b = 2.9 x10^{-3} K m

Black body emitter – spectrum and intensity dependent on temperature, which changes slowly with time. These are typically nice stable sources, but most of the light is in the IR

Ion Arc Lamps

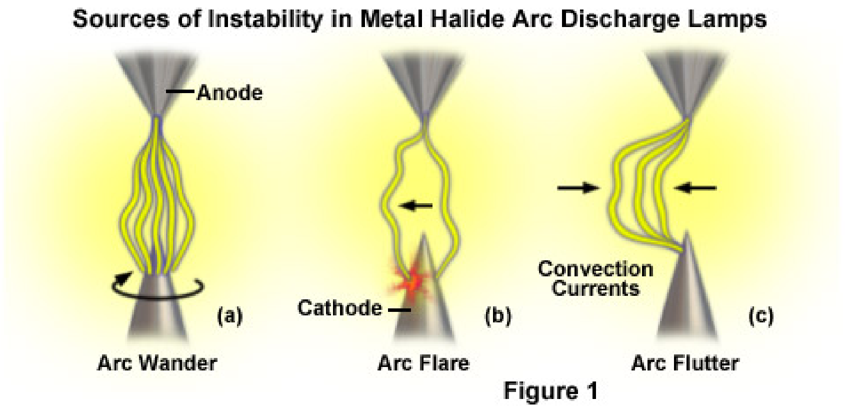
- More expensive
- 10-100x brighter
- Short lifetime
- Unpolarized
- Incoherent
- Diverging





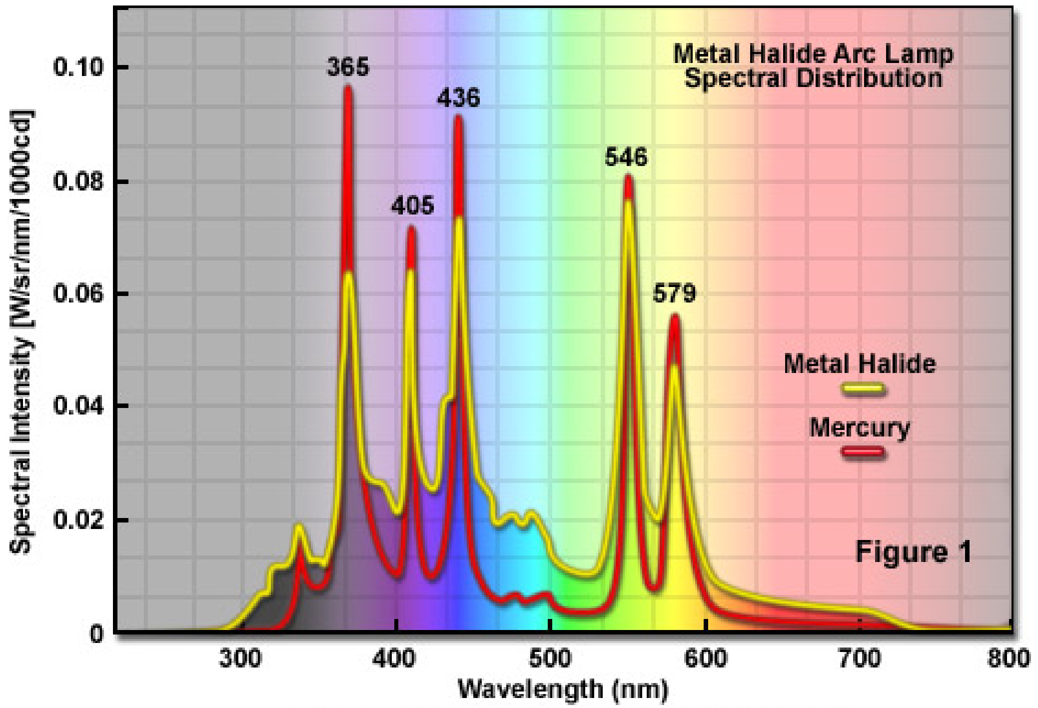
Cost is ~50c per hour
They take a long time to stabilize
Lots of UV, don't look at them directly

Excited by electron orbitals changing states; apply enough voltage, and you can flip an electron out, and it will fall back in. Gives same energy (color) photons each times an atom falls back in

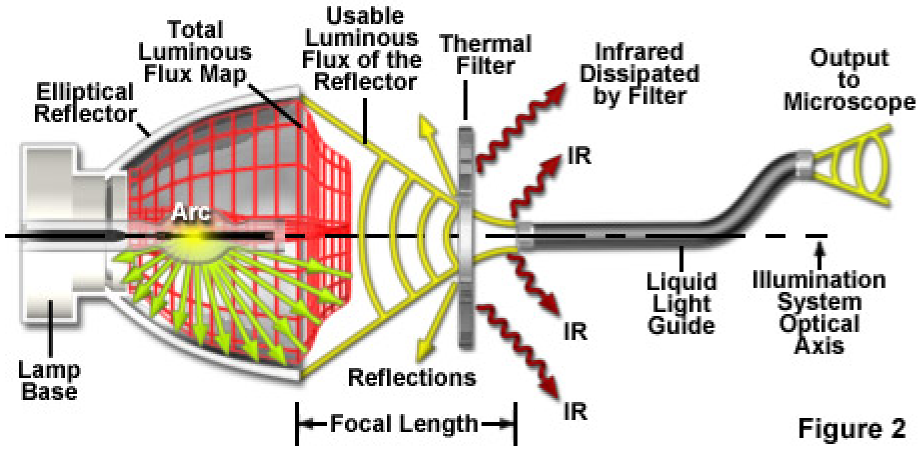


Metal Halide

- Add sodium iodide which dissociates and adds background colors to the mercury
- Expensive-ish
- Broader spectrum
- Very bright
- Longer lifetime
- Risk of explosion
- Incoherent



Metal Halide Arc Discharge Reflector Lamp



LEDs – Not just for TVs

- Uses a semiconductor
- High output intensities
- Directed emission
- Spectrally narrow (compared to tungsten and halide sources)
- Can be very rapidly turned on and off
- Last forever (as long as you thermally protect them)
- Frighteningly stable

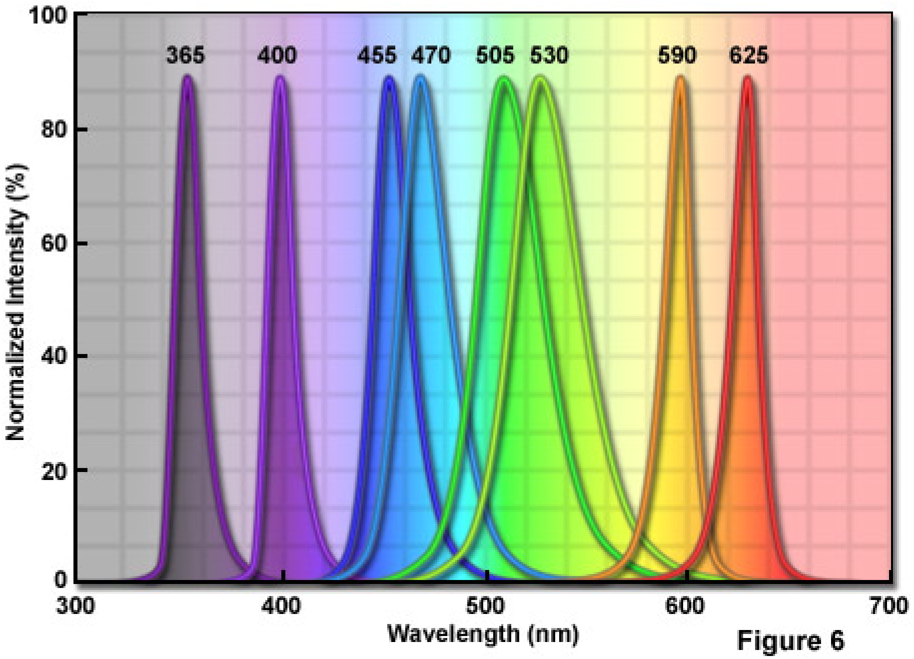
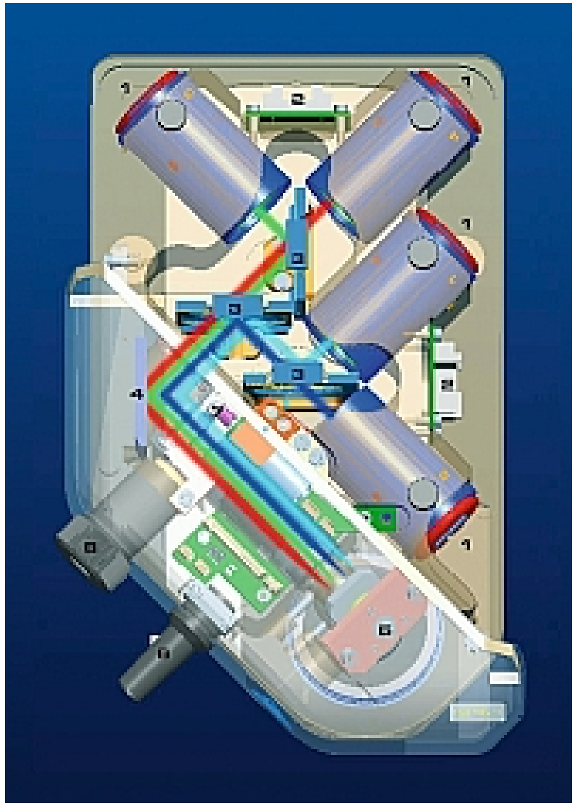
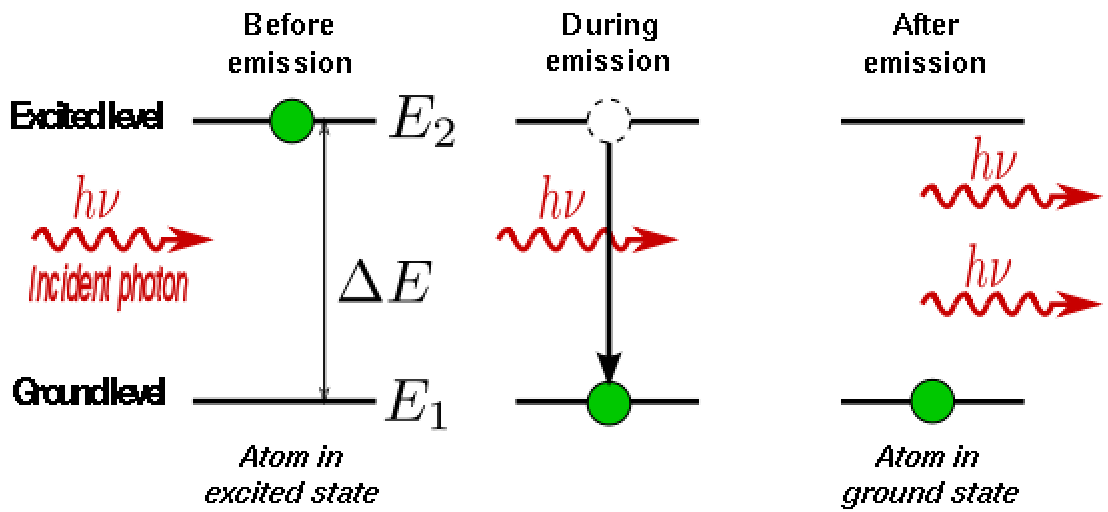


Figure 6



Lasers

- Put out coherent, monochromatic light
- Only way to focus to a diffraction limited spot
- Low energy (mW typically), but it is monochromatic, and can be very tightly focused. Can lead to very high widefield intensities.



$$E_2 - E_1 = \Delta E = h\nu$$

Laser excites electrons using voltage, then when a single photon hits that electrons, 2 photons come out completely coherent

Lasers

- Can be very stable
- Figures of merit are
  - Intensity
  - Mode
  - Polarization
  - Divergence
  - Poynting stability
- Diode lasers can be rapidly modulated

Model Name	Wavelength (nm)	Power (mW)	Power (mW)
OBIS 355 LG	355	20	-
OBIS 375 LX	375	16 and 50	-
OBIS 405 LX	405	50, 100, 140 and 200	50 and 100
OBIS 422LX	422	100	-
OBIS 445 LX	445	75	45
OBIS 458 LX	458	75	-
OBIS 473LX	473	75	50
OBIS 488 LS	488	20, 60, 80, 100 and 150	15, 40, 60, 80 and 120
OBIS 488 LX	488	50 and 150	30 and 100
OBIS 505 LX	505	50	50
OBIS 514 LS	514	20	15
OBIS 514 LX	514	40	30
OBIS 520 LX	520	40	25
OBIS 532 LS	532	20, 50, 80, 100 and 150	20,40, 60, 80 and 120
OBIS 552 LS	552	20, 60, 80, 100 and 150	15, 40, 60, 80 and 120
OBIS 561 LS	561	20, 50, 80, 100 and 150	40, 60, 80 and 120
OBIS 594 LS	594	20, 60 and 100 *NEW	40
OBIS 637 LX	637	140	100
OBIS 640 LX	640	40 and 100	75
OBIS 647 LX	647	120	100
OBIS 660 LX	660	100	75
OBIS 685 LX	685	40	-
OBIS 730 LX	730	30	-
OBIS 785 LX	785	100 *NEW	-

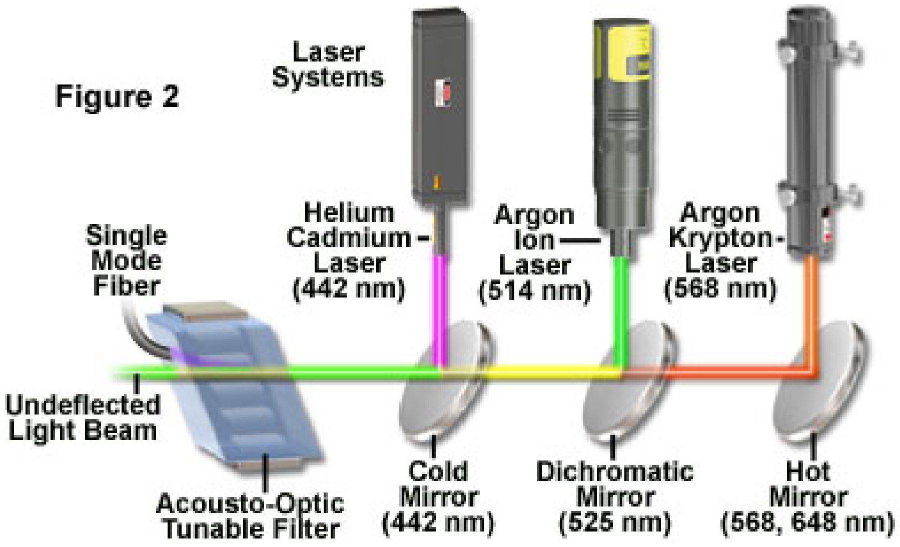
Lasers have VERY long coherence lengths, speckle can be a problem

Often solved by running through a fiber which will mix all the modes together

Acousto-Optic Tunable Filters (AOTF)

- Play laser light like a piano
- Uses acoustic modes to set up diffraction patterns
- Can turn on and off lasers in nanoseconds
- Can couple 8 lasers onto the same path

Acousto-Optic Tunable Filters in Confocal Microscopy



AOTF Selection of Specific Regions for Excitation in Confocal Microscopy

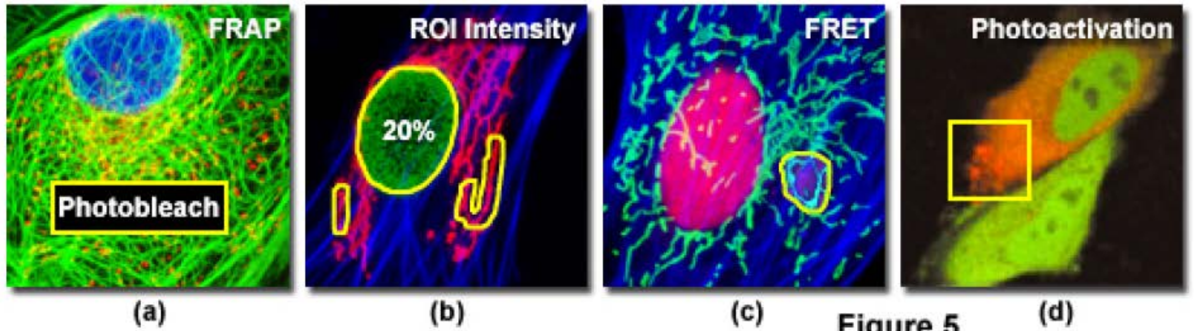
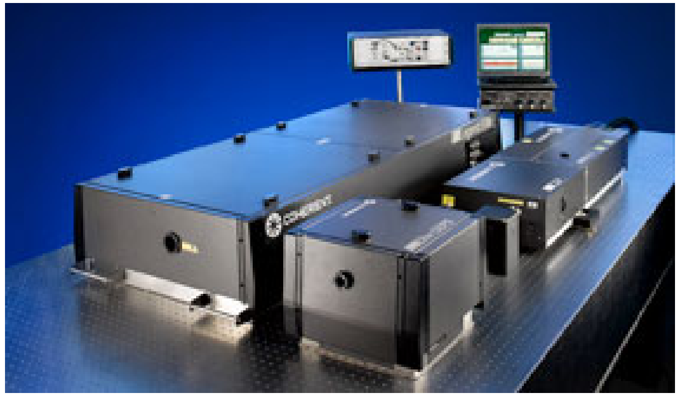
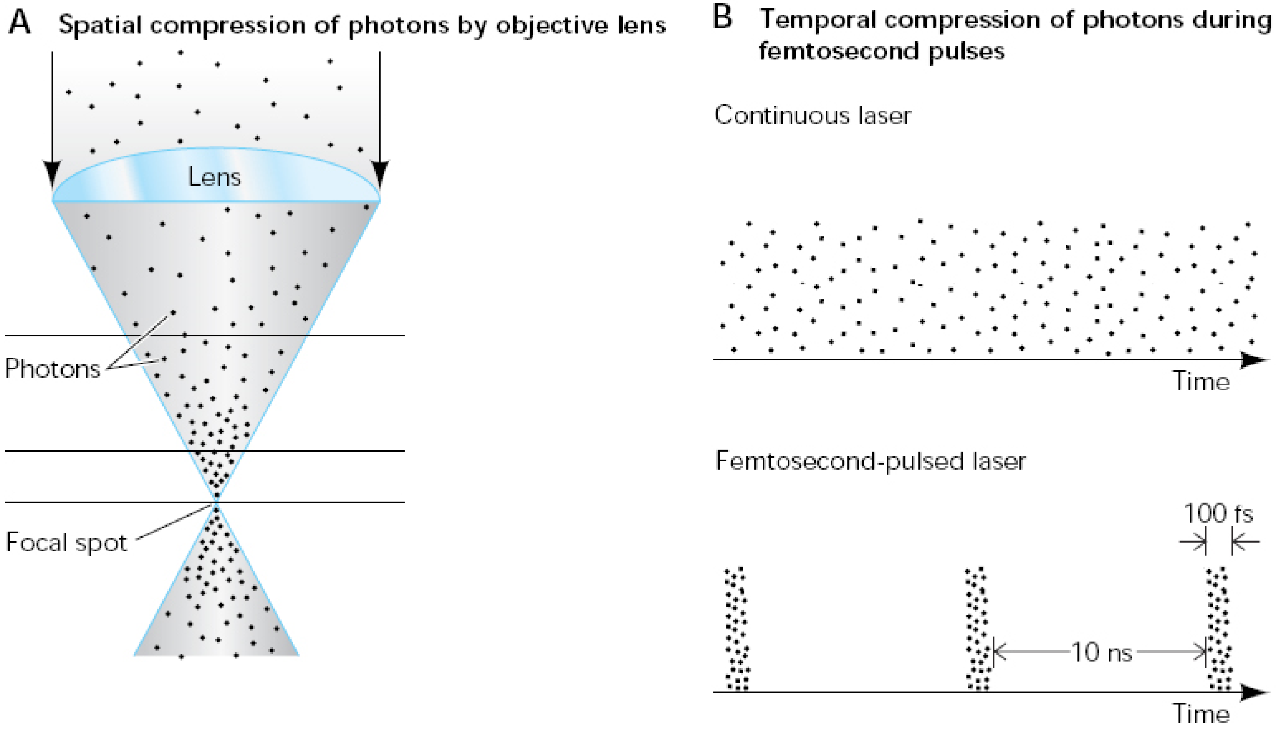


Figure 5

Ultrafast lasers

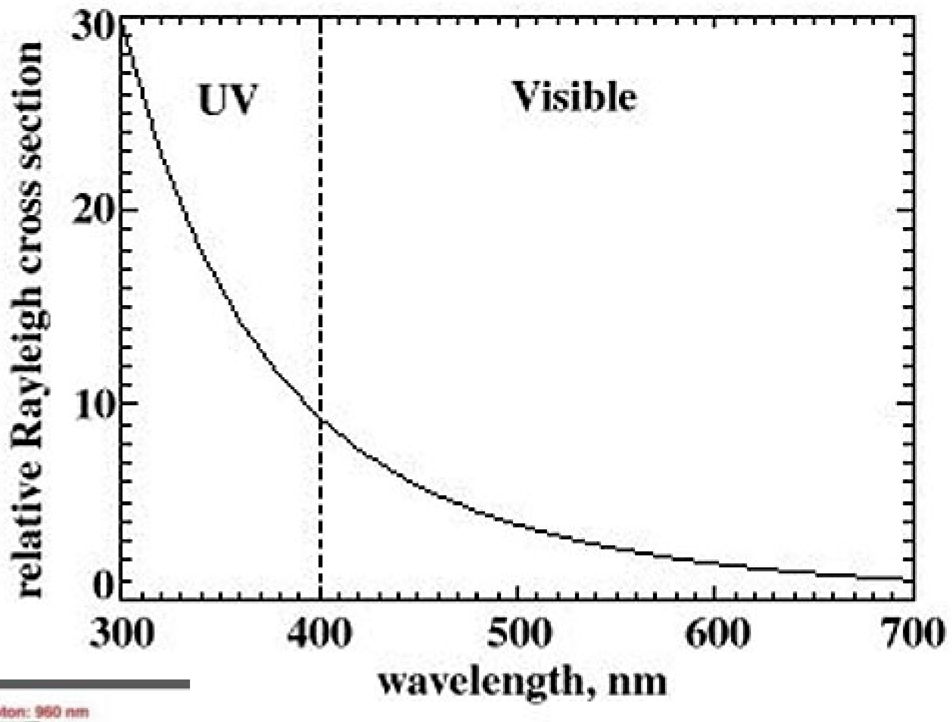
- Titanium sapphire laser
- Pulse duration (20 fs – 2 ps: .000000000002 s)
- Pulses at ~80MHz (80,000,000 per second)
- Wavelengths from 750-1400 nm
- Used to be VERY finicky, have significantly improved stability recently

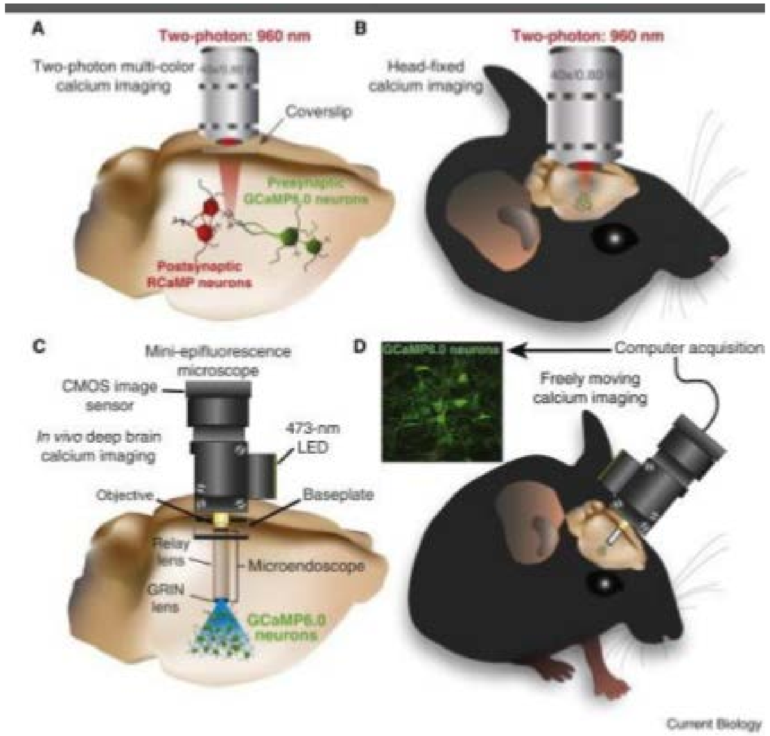


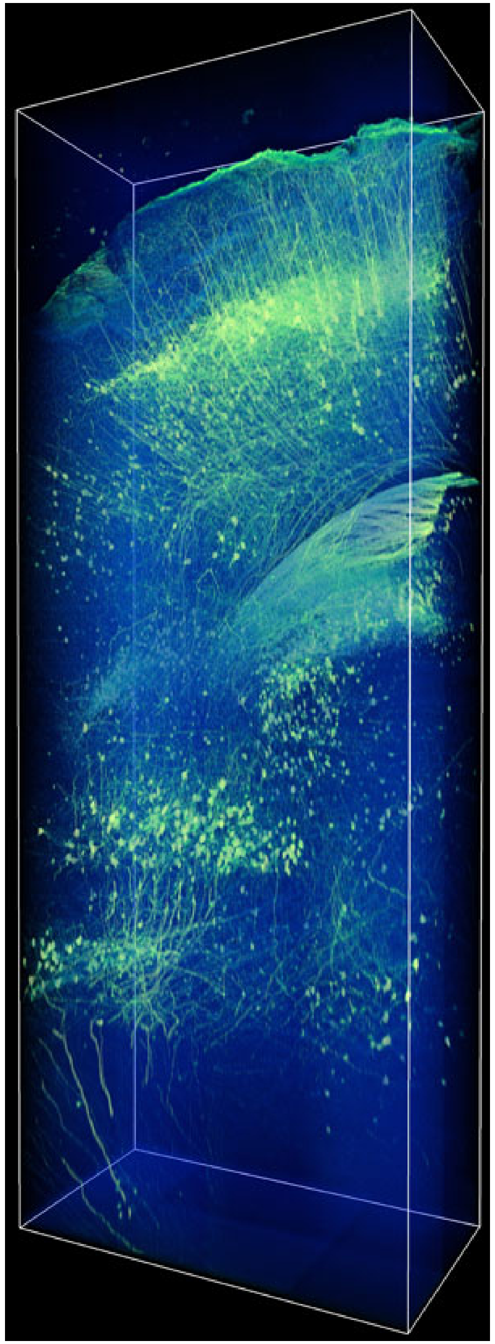


Ultra fast lasers

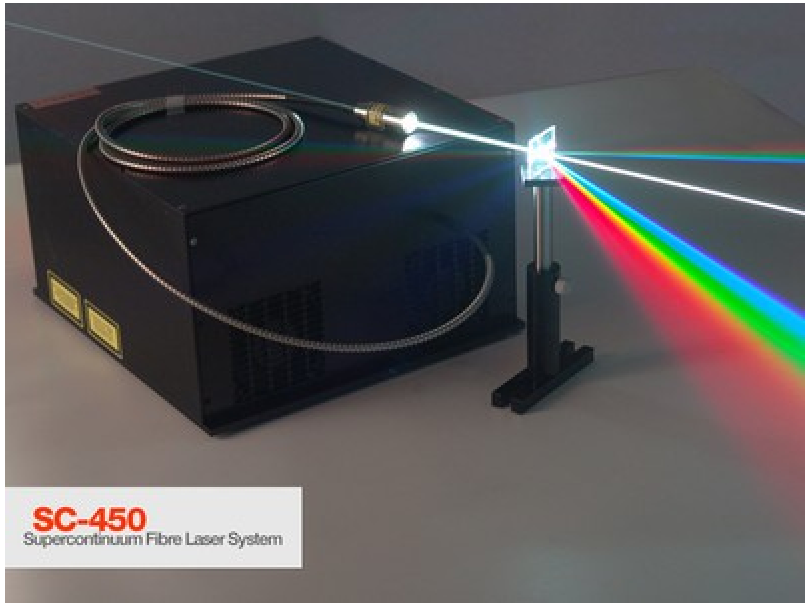
Pulses in the near IR allow for deep imaging of tissue.

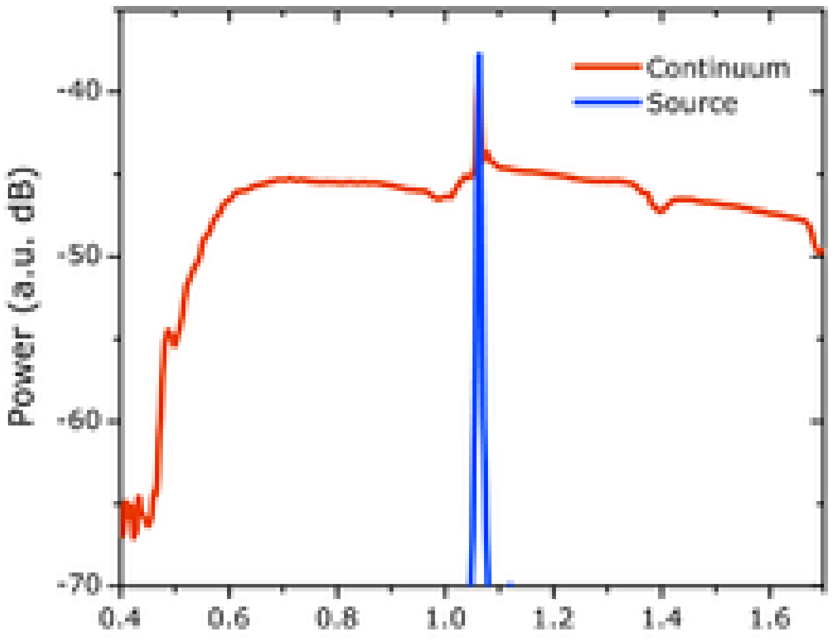


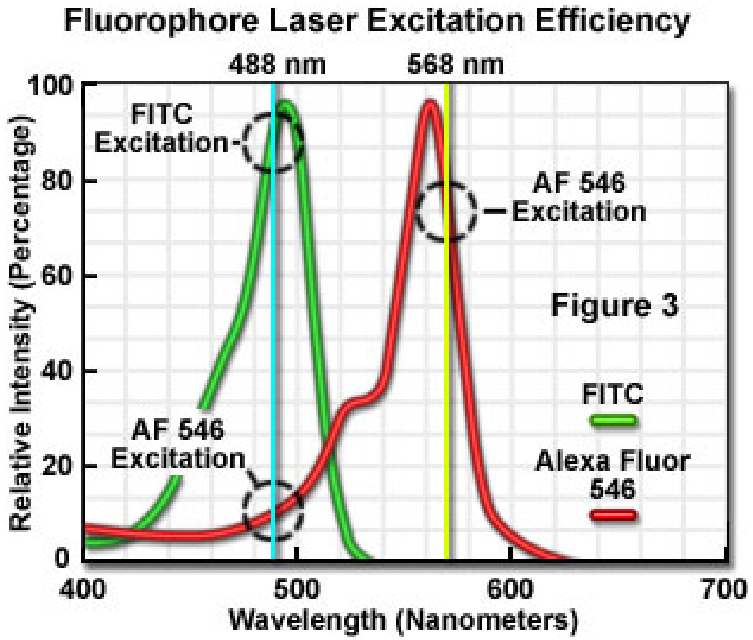




Supercontinuum laser

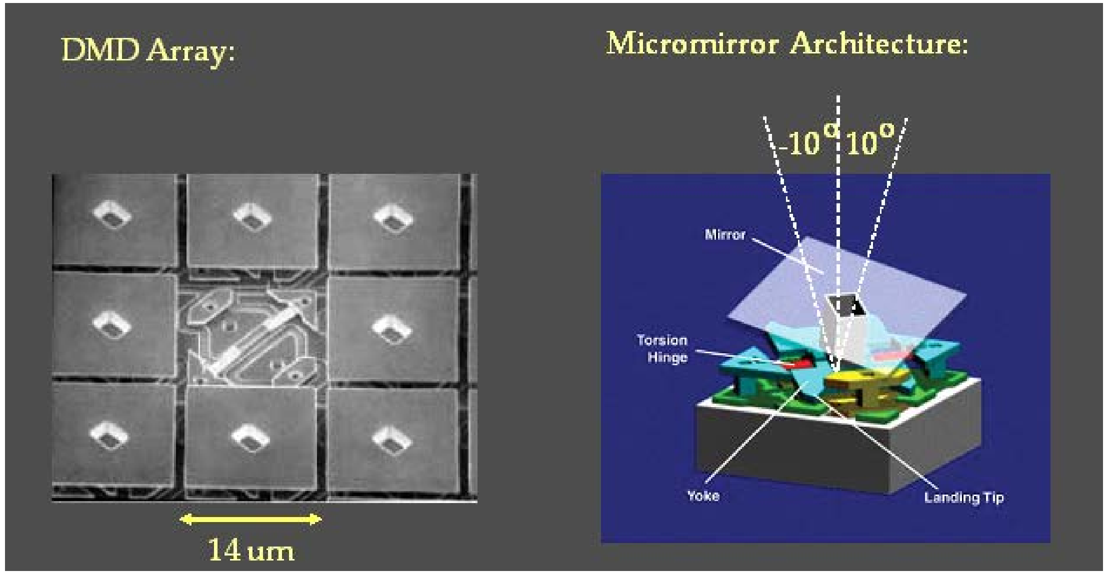


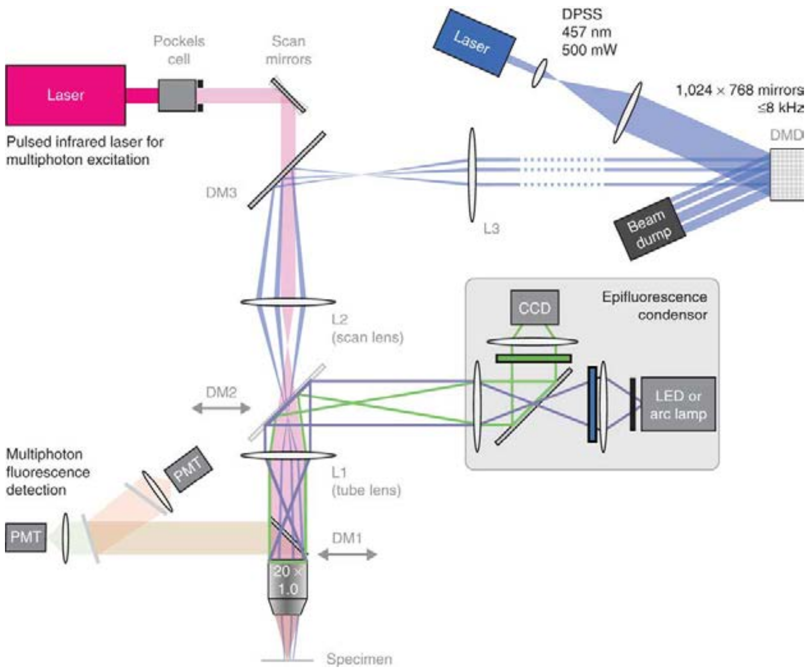




Ultrafast pulsed light source
Uses nonlinear fiber mixing to produce all wavelengths
Use AOTF to select for desired color

Digital micromirror device

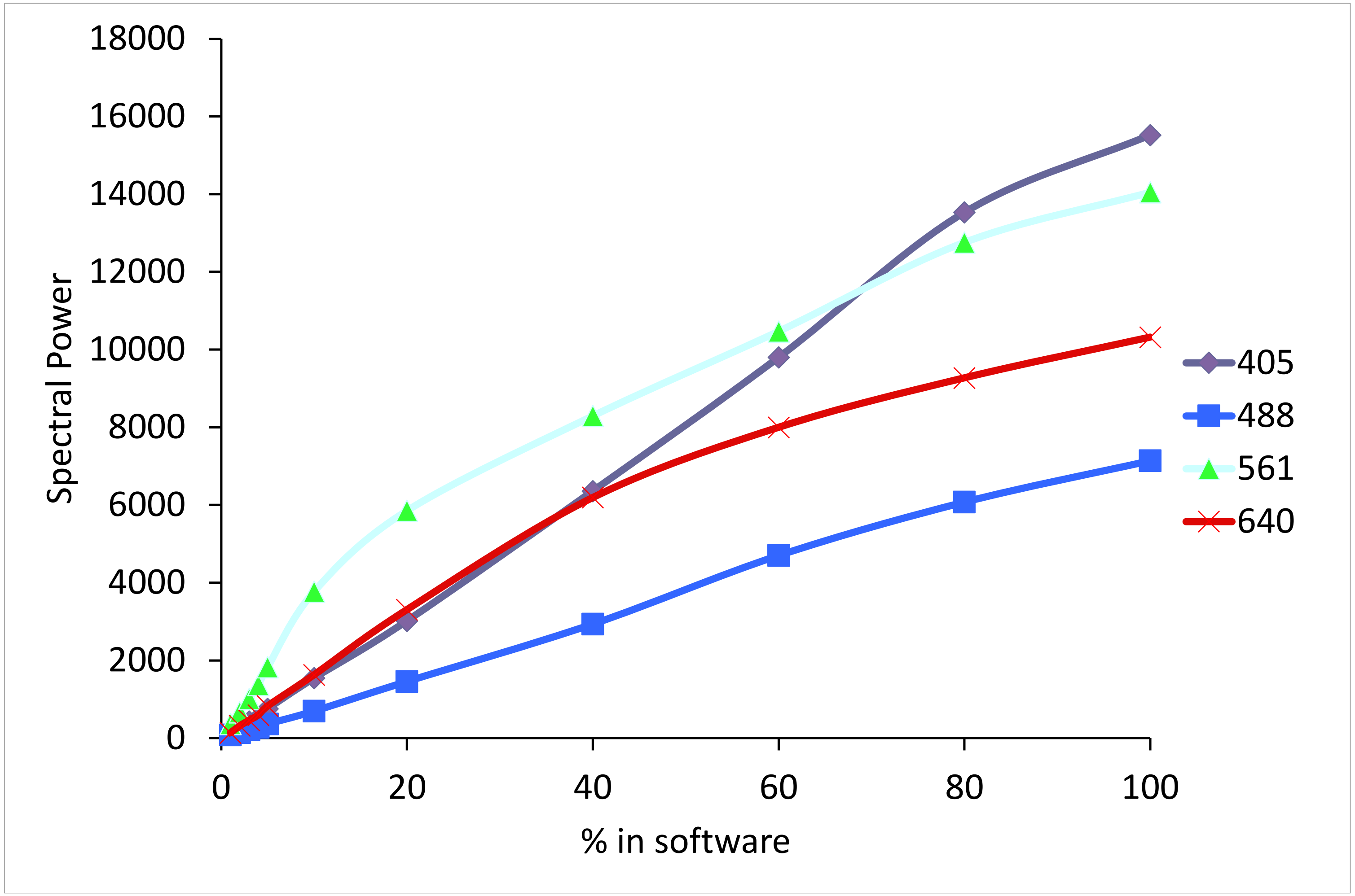




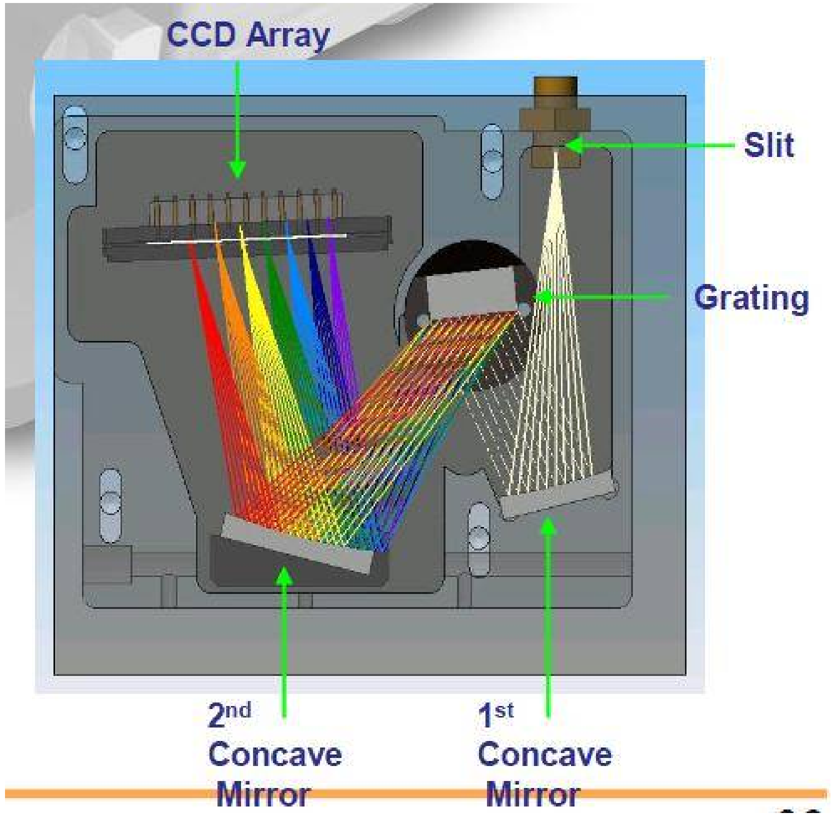
Used with RGB light to make projectors

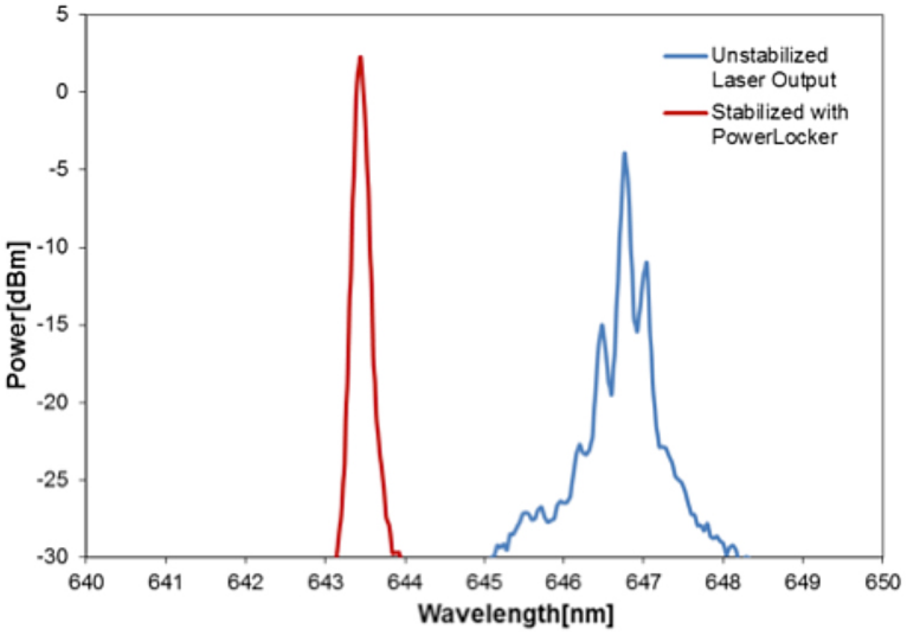
Arbitrarily pattern light in space and time

Measuring light intensity



Measuring light spectrum





On to Matlab…