Generating light

1. Tungsten/halogen lamp

- Condenser lens (high NA to collect much light)
- Quartz envelope (care—don’t touch with fingers!)
- Tungsten filament
- Halogen gas (stops tungsten evaporating onto envelope)
- 12 V DC

Black-body radiation - color temp. ~ 3000 °K.

Light intensity vs. wavelength:
- Smooth spectral distribution - more red than blue
- Why your car headlights look yellow
Pro-

Cheap and simple

Very stable output (if good power supply)

Con-

Not much output in blue/UV: not good for fluorescence.

Fairly large source (filament): cannot focus down to very intense spot.
High pressure arc-lamps

- Quartz envelope (no touching)
- Tungsten electrodes
- Intense arc ~1mm diam.
- Gas (Xe or Hg) under high pressure

Spectral output depends on whether Xe or Hg.

- Xe - fairly even output: very bright in visible, but weak UV. (why the headlights of a Lexus look blue)

- Hg - intense lines in UV and blue: very good for UV fluorescence excitation at $\nu 350$ nm.
Pro -

Very intense, small source.
Can be focused to fairly small spot
Much better than halogen lamp for fluorescence excitation

Con -

Expensive (bulbs ~ $160, power supply 1-2k$)
Voltage surge to ignite Xenon arc (kv) can damage computers/electronics
Bulbs have short life and are prone to explode!

Warnings

Arc lamps emit lots of UV - don't look at light, and avoid skin exposure
If Hg bulb explodes leave room immediately (Hg vapor toxicity)
Laser

Light emission by stimulated emission of radiation

energy of fluorescent molecule

excited state

incident photon

stimulates drop in energy level

photon emitted

pump molecule to higher energy level (e.g. by electric current or flashlamp)

incident photon

emitted photon

Emitted photon is in same direction and phase as incident photon.
Inside a laser

Laser cavity

Output beam

Partly-transmitting lasing medium

(can be gas, liquid, crystal)

Fully-reflecting mirror

Characteristics of laser beam

1. Almost perfectly parallel beam
2. Monochromatic (usually): single \( \lambda \)
3. Coherent: all light waves in same phase

(Actually a nuisance for fluorescence imaging, not an advantage)
Pro -

* Entire energy of laser beam can be focused to diffraction-limited spot. (possible to achieve unbelievable peak energies: >1TW cm⁻²)

Monochromatic

Con

Expensive (1-100k$ or more)

Only certain specific wavelengths available

eg: 488nm - argon ion laser
532nm - tripled Nd:YAG
584nm - yellow HeNe

Depending on type -
short lifetime
noisy
room heater
Argon-ion laser

Used for most biological laser scan imaging applications

![Diagram of Argon-ion laser with beam, laser head (~2ft long), cooling fan, and power supply connected to regular wall outlet.]

Emits at 457, 488, 514 nm
Select desired λ with interference filter

Laser Safety

Don’t look at laser beam !!!
keep beam covered, and below eye level
Don’t peek into equipment
Be especially scared of invisible beams (UV and IR)
Detecting Light

General issues

Quantum efficiency (QE). What % of incident photons contribute to the signal.

Dark noise. How many "photons" does the detector appear to see in complete darkness. Sets ultimate limit to sensitivity.

Wavelength dependence. Is QE higher at some \( \lambda \) than others.

Added noise. Eg does readout from detector add noise? Does a photon always give the same output signal.
The real world -

No detector is perfect, but some are pretty good.

Two main types:

Photodiode (and variants - e.g. CCD camera) to be covered by R.F.

Photomultiplier

Remaining vestige of vacuum tube technology

![Diagram of photomultiplier](image)

Phocone $\rightarrow$ electron $\rightarrow$ etc.

-ve $\rightarrow$ dynode

Voltage on photocathode/dynodes progressively more +ve, so electrons accelerated.

Photoelectric effect (Einstein) followed by electron multiplication.
Characteristics of photomultipliers:

Can detect single photons - but QE only 20% at best; i.e. only 1 in 5

Dark noise can be very low (<1 "photon" s⁻¹)

Respond very fast (10⁹ photons s⁻¹ or more)

Linear response over enormous range.

Better sensitivity in blue than red.
Image intensifiers

Works on same principle as photomultiplier, but produces amplified image.

Can place in front of regular camera (c.c.d.) to greatly enhance sensitivity to faint fluorescence signals.