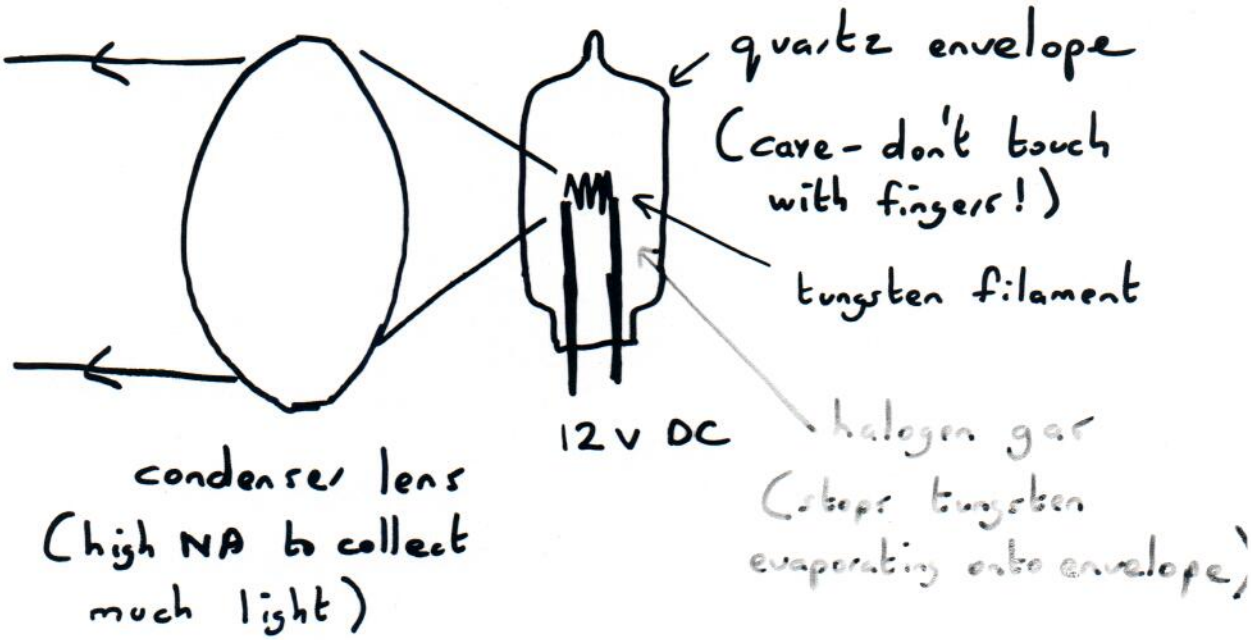
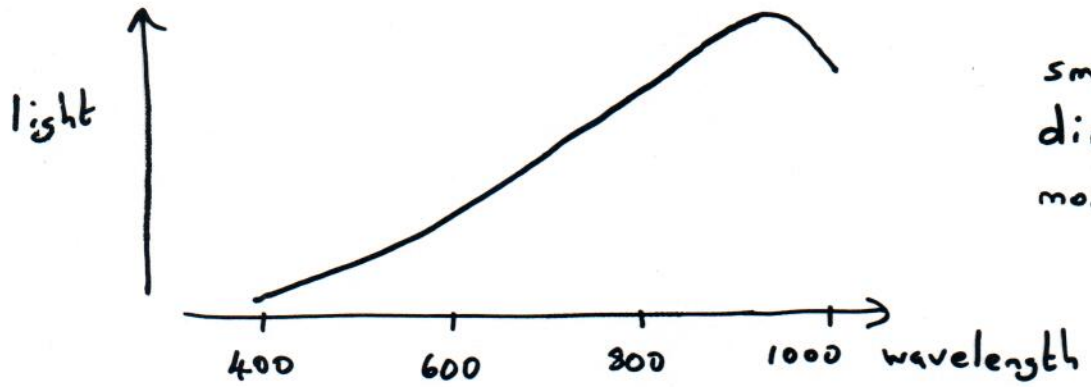
Generating Light

① Tungsten/halogen lamp



black-body radiation – color temp. ~ 3000 °K.



smooth spectral distribution – more red than blue

(why your car headlights look yellow)

Pro -

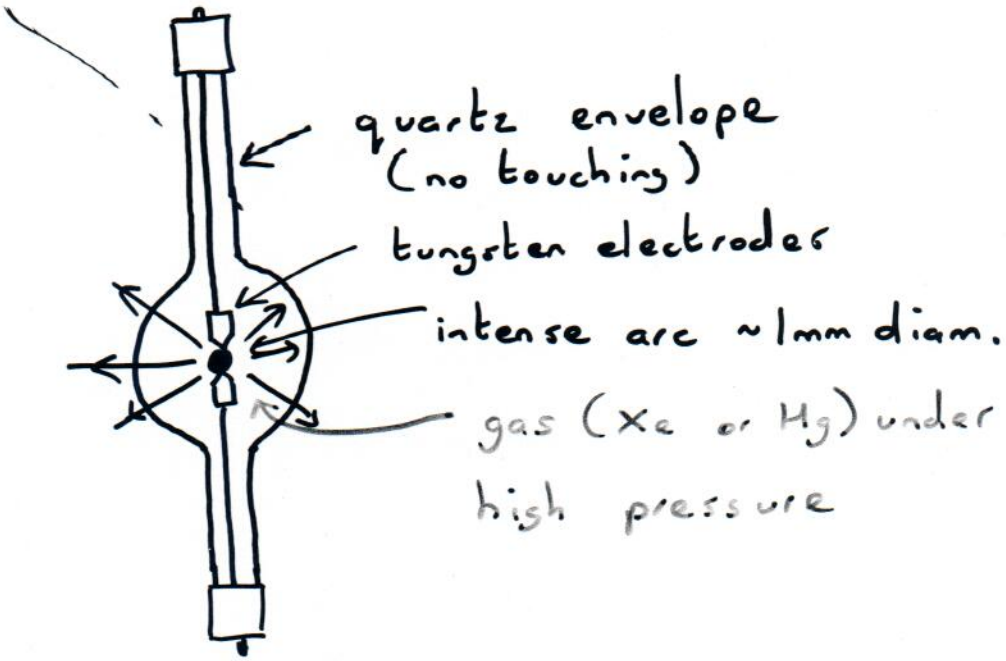
Cheap and simple
Very stable output (if good power supply)

Con -

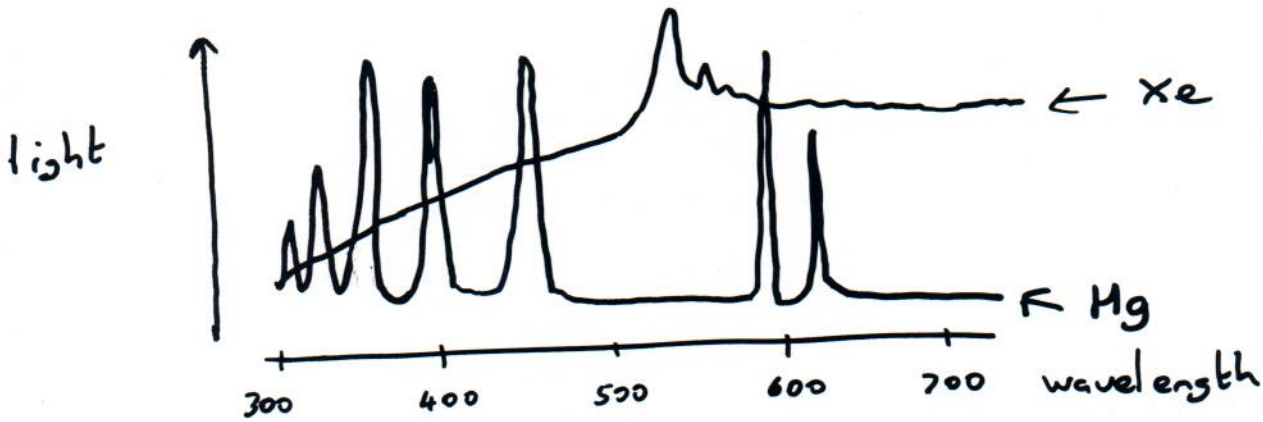
Not much output in blue/UV : not good for fluorescence.

Fairly large source (filament) : cannot focus down to very intense spot.

High pressure arc-lamps



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 ~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 ~ $160, power supply 1-2 k$)

Voltage surge to ignite Xenon arc (kV) can damage computers/electronics

Bulbs have short life and are prone to explode!

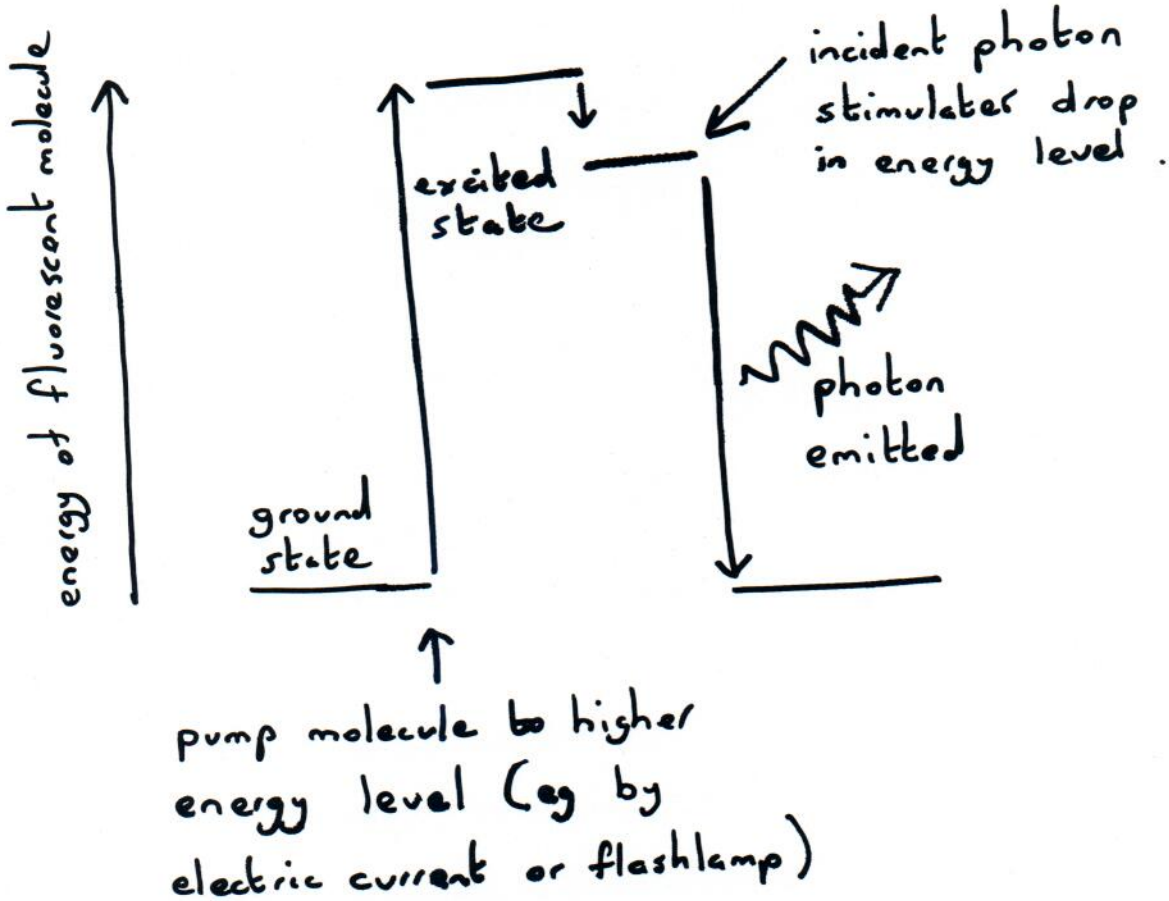
Warnings

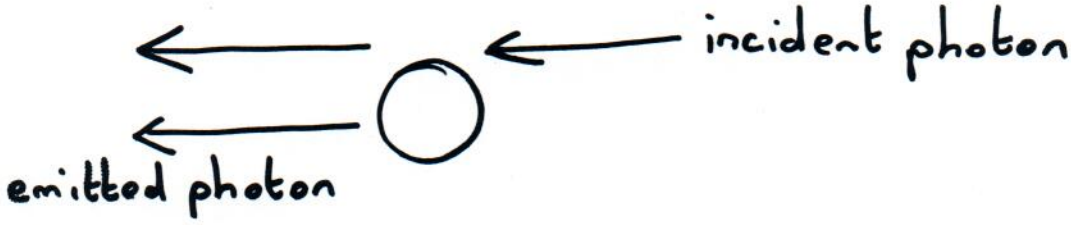
Arc lamps emit lots of UV - don't look at light, and avoid skin exposure

If Hg bulb explodes leave room immediately (Hg vapor toxicity)

Laser

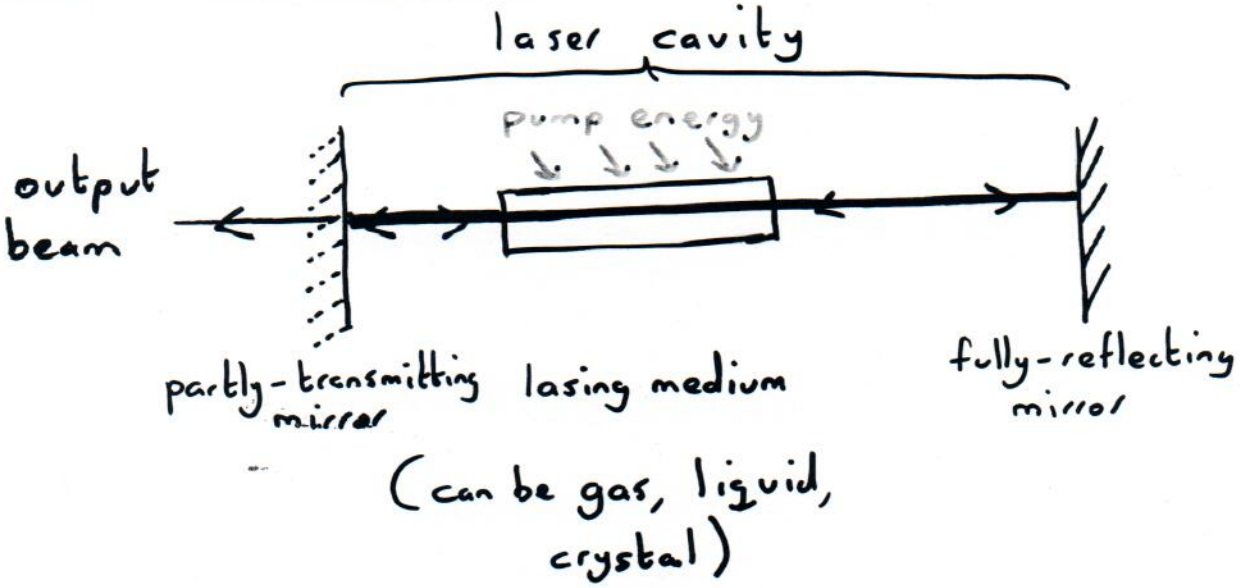
Light emission by stimulated emission of radiation





Emitted photon is in same direction and phase as incident photon

Inside a laser



Characteristics of laser beam

1. Almost perfectly parallel beam
2. Monochromatic (usually): single λ
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 : $> 1 TW cm^{-2}$)

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 -

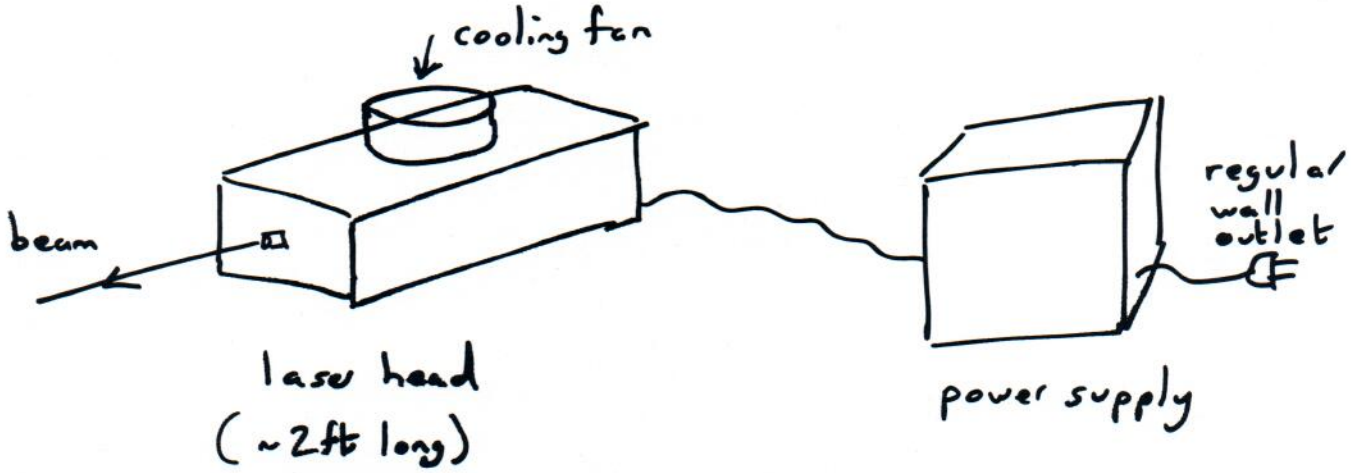
short lifetime

noisy

room heater

Argon-ion laser

Used for most biological laser scan imaging applications



Emits at 459, 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 λ than others.

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

The real world -

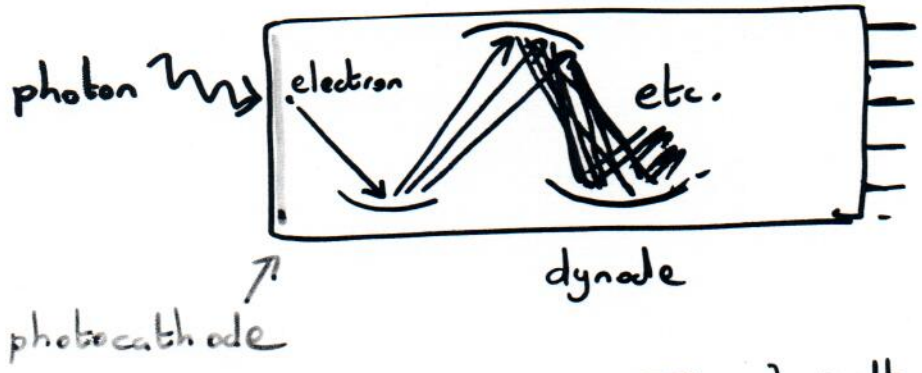
No detector is perfect, but some are pretty good.

Two main types:

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

Photomultiplier

Remaining vestige of vacuum tube technology



} 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 : ie only 1 in 5

Dark noise can be very low (<1 "photon" s^{-1})

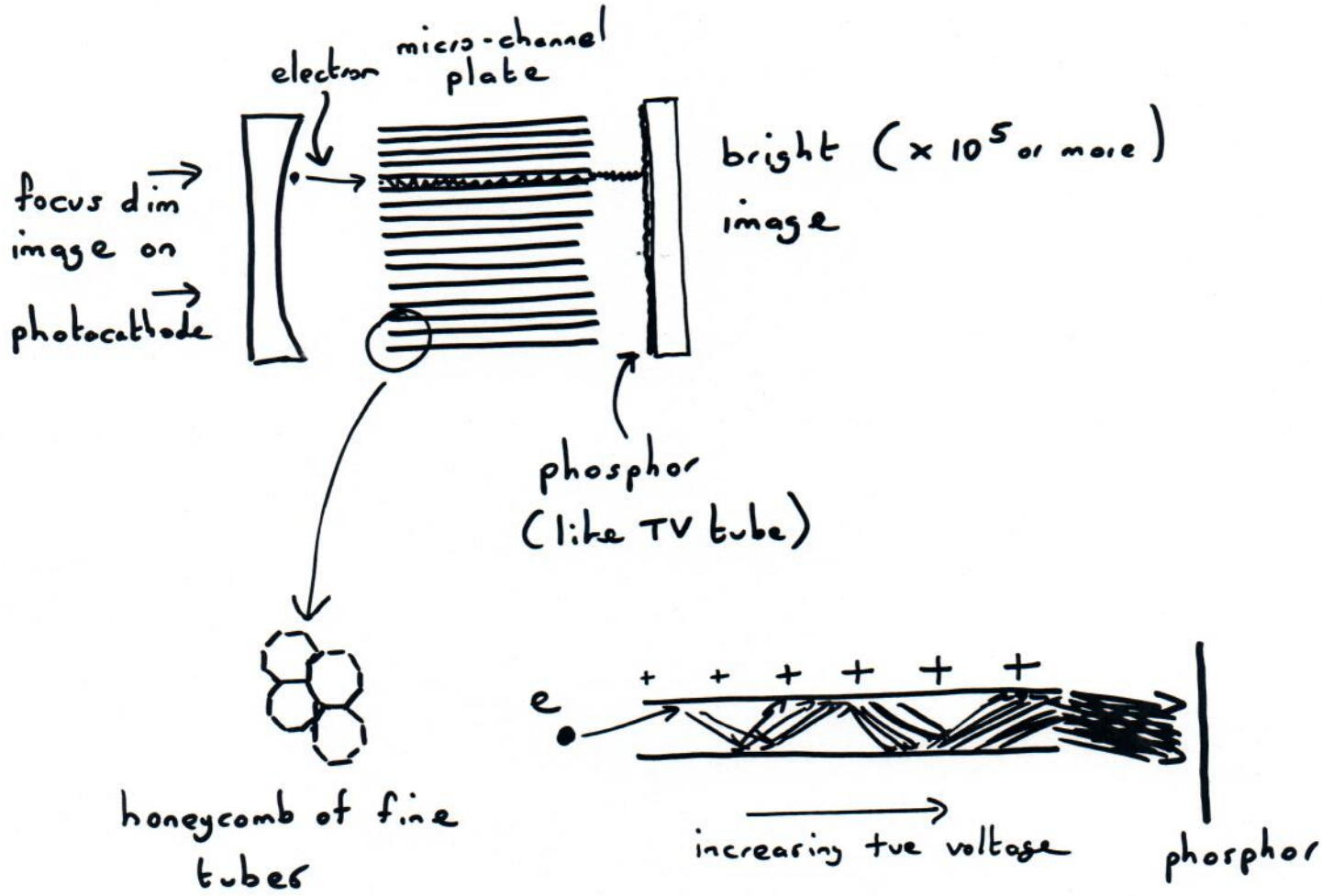
Respond very fast (10^9 photons s^{-1} or more)

Linear response over enormous range.

Better sensitivity in blue than red

Image intensifier

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.