

Today's class:

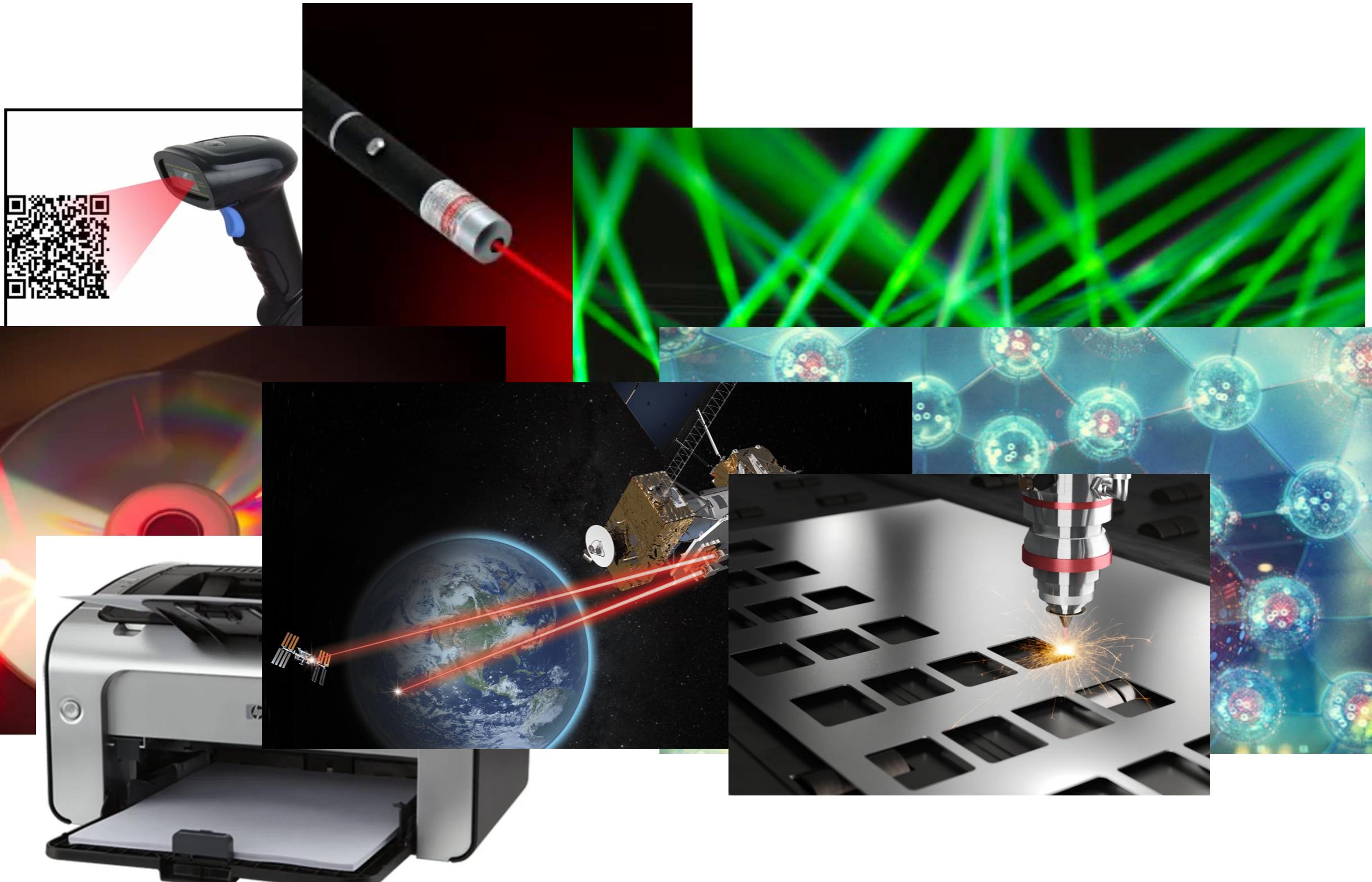
Lasers and Light Microscopy

This lecture follows the materials from the following books

- *Physical Chemistry for Life Sciences, by PW Atkins and JD Paula, Oxford, 2006*
- *Prescott's Principles of Microbiology, McGraw-Hill 2009*

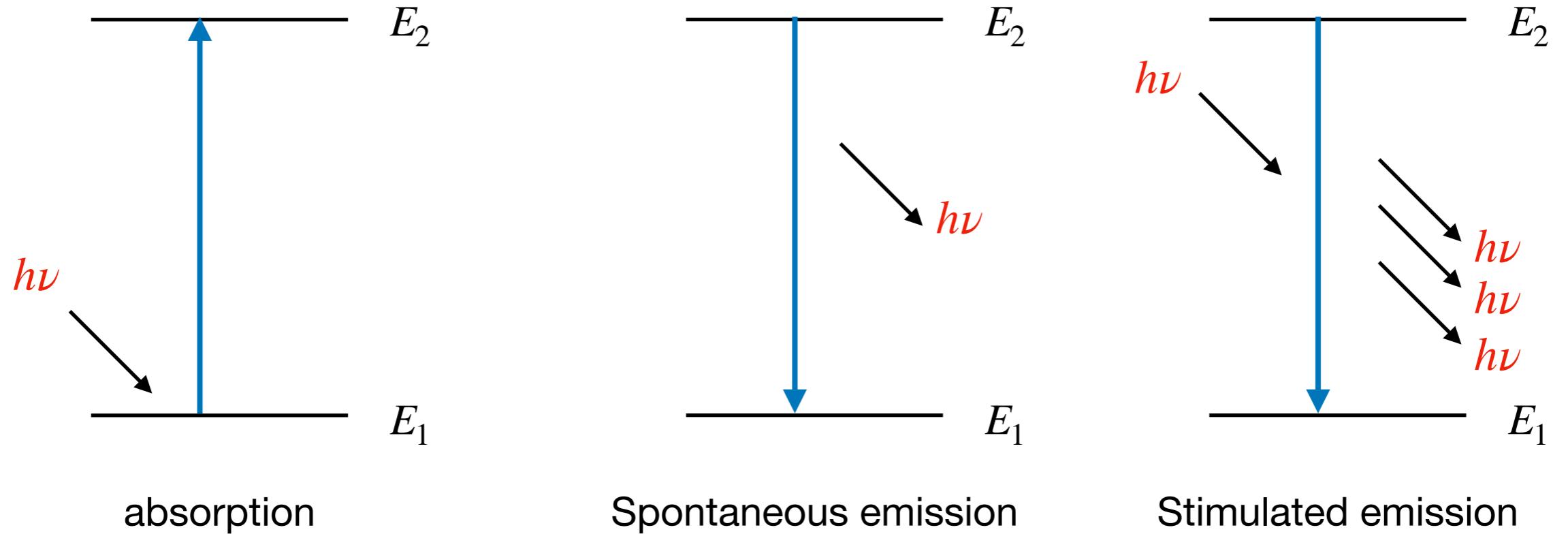
Why the interest in lasers?

Lasers have made our life easy and fun!



What is laser?

LASER = Light Amplification by Stimulated Emission of Radiation

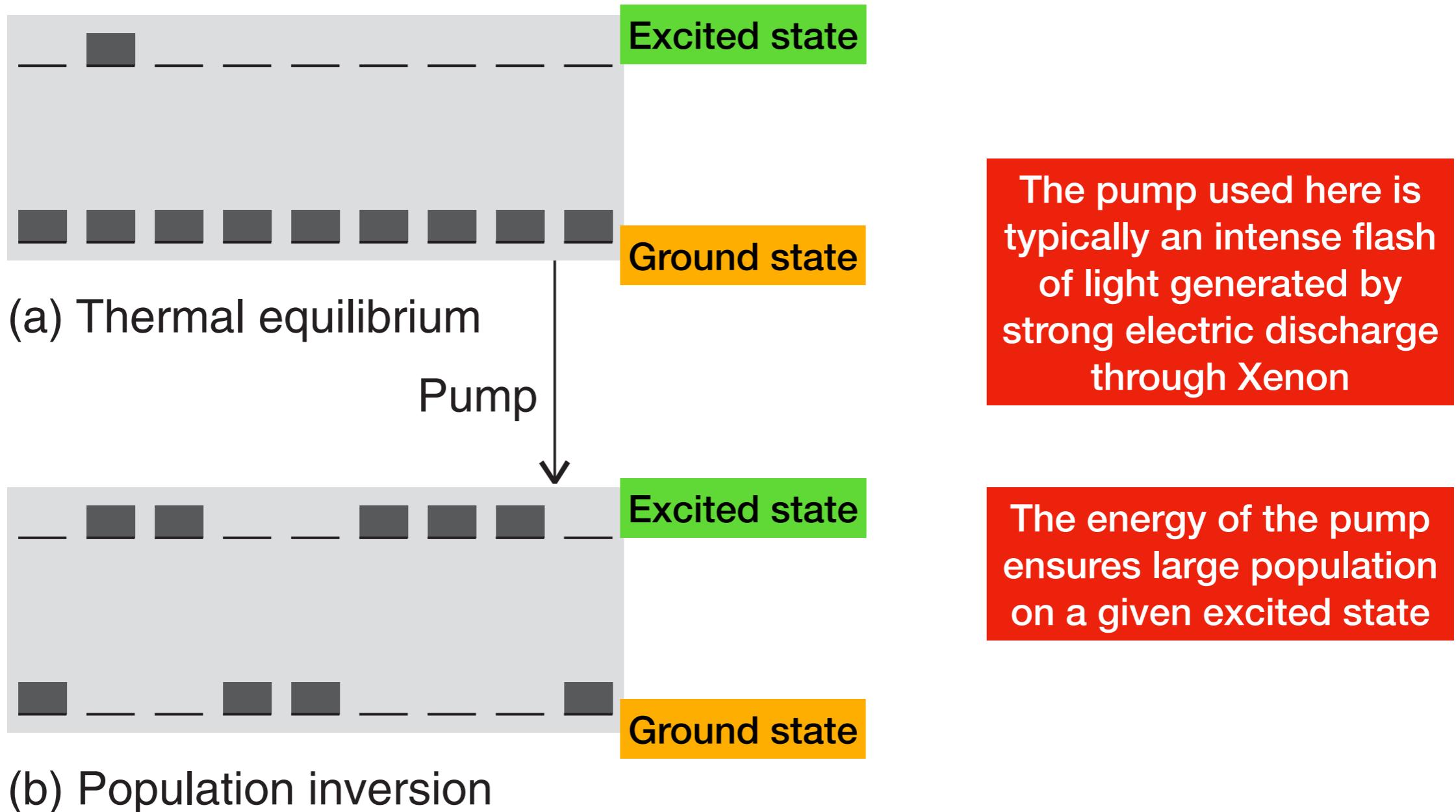


Requirements for laser action

- The lifetime of the excited state is very large
- Population at the excited state has to be larger than in the ground state

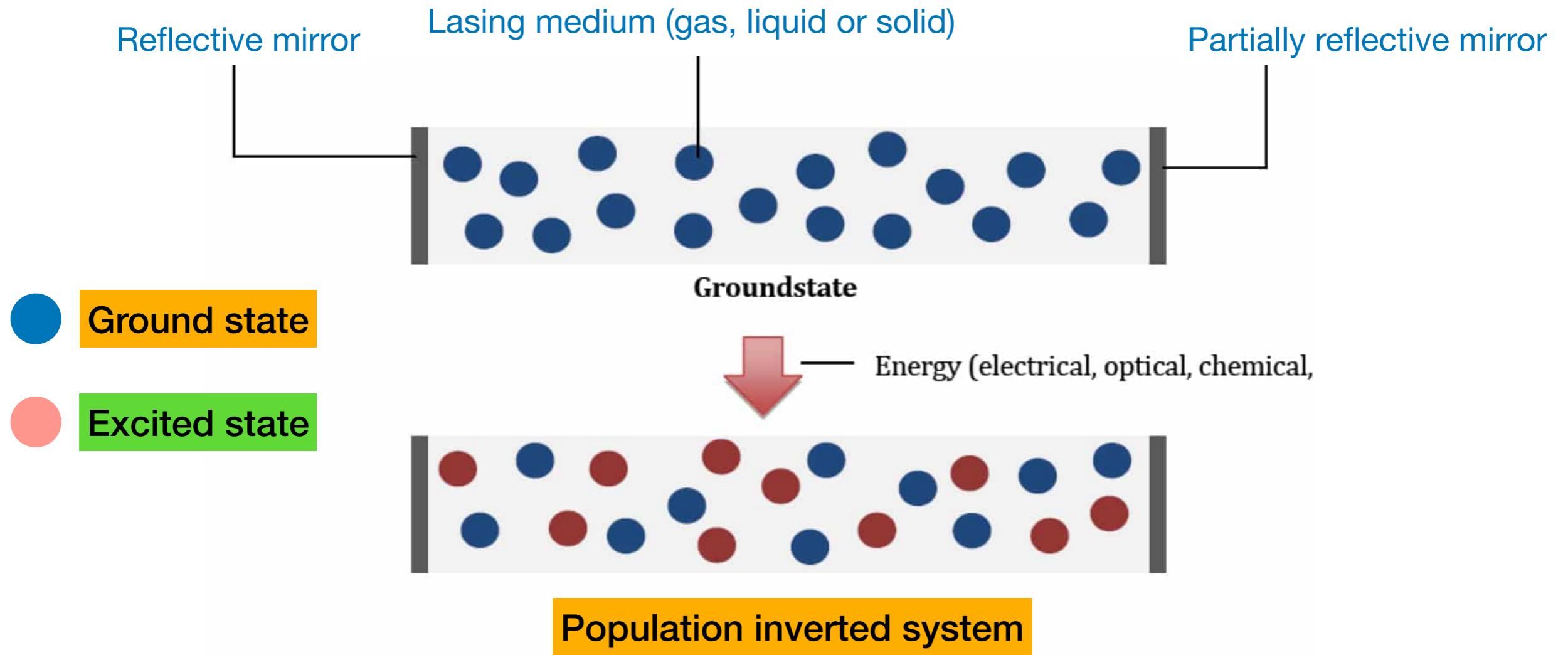
This can be achieved by a phenomenon called ‘population inversion’

Population inversion

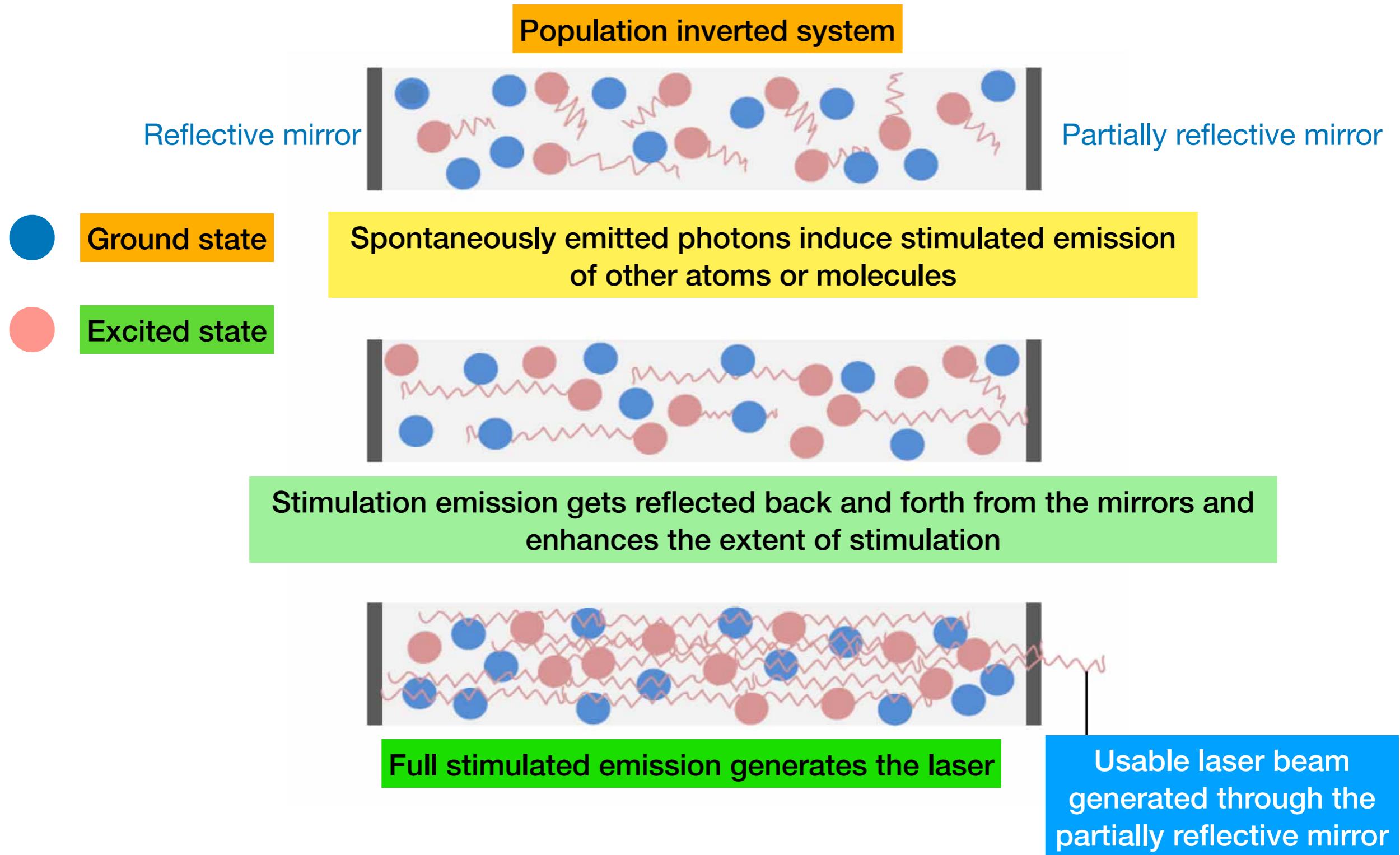


Laser intensity need to be very strong. How is that achieved?

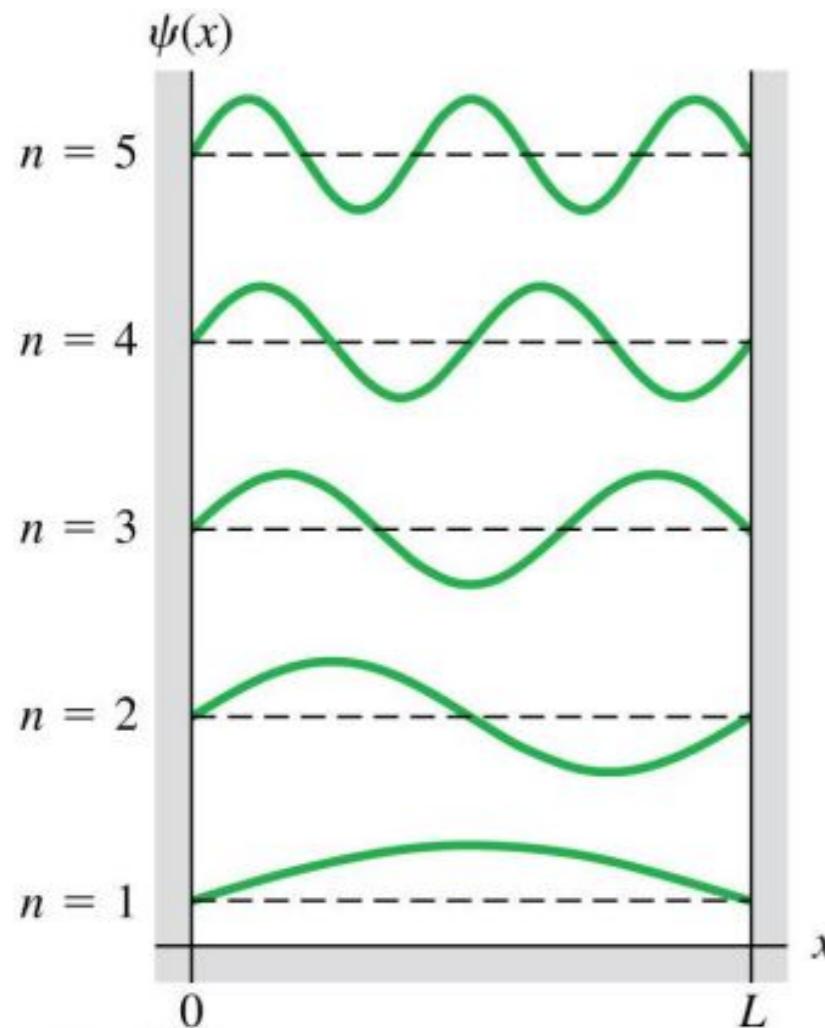
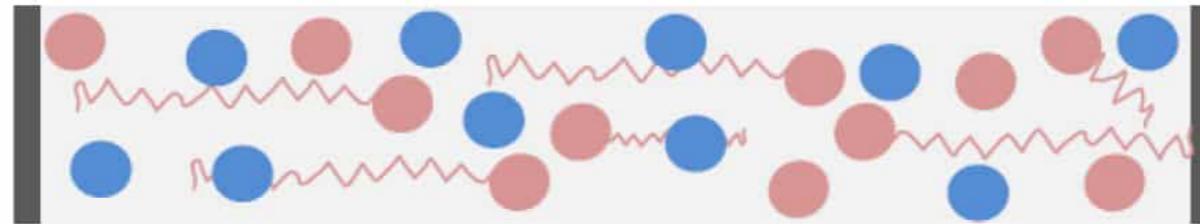
The laser cavity



The laser action



Criteria for laser amplification



A photon of stimulated emission = particle in a box

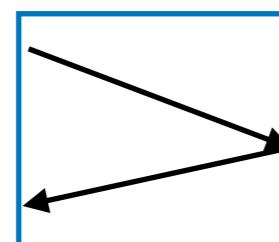
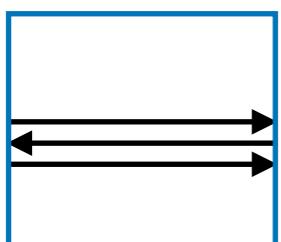
Therefore, only those emissions are sustained that satisfy

$$n \times \frac{\lambda}{2} = L, \quad n = 1, 2, 3, \dots$$

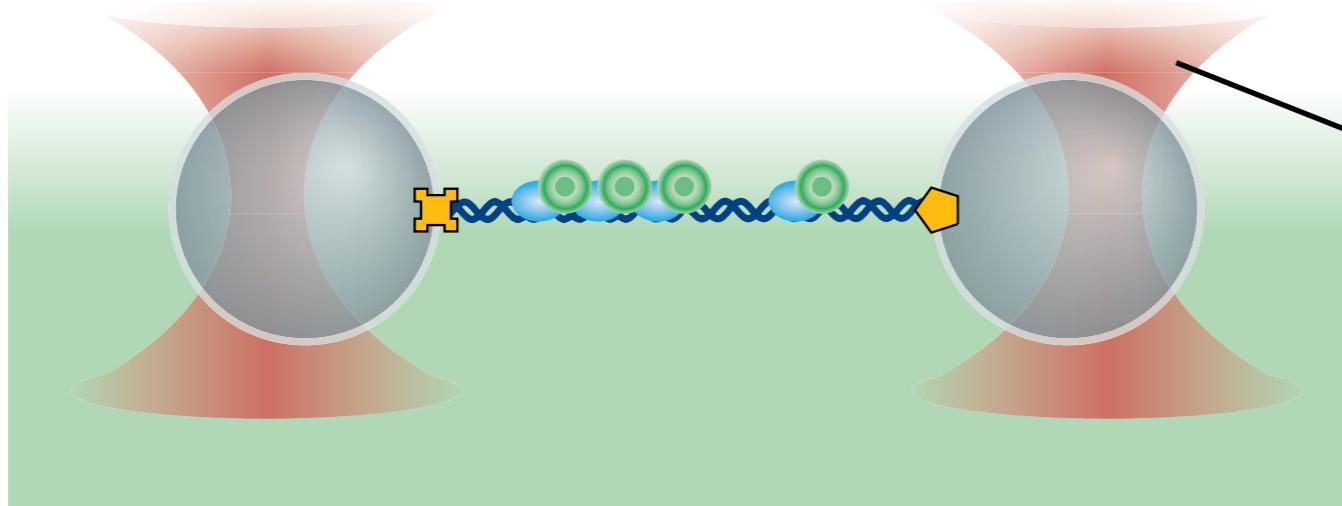
Rest of the emissions are lost by destructive interference

The sustained emissions are called 'resonant modes' of the laser.

Finally, photons that travel parallel to the axis of the cavity gets reflected back and forth several times and gets selected for amplification. Rest are lost.

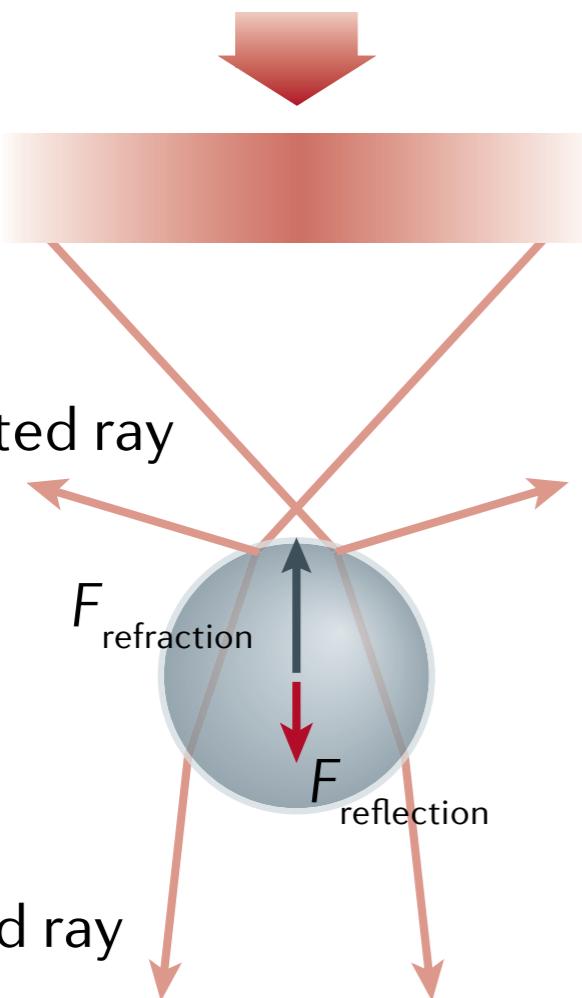


Applications of laser in biology: optical tweezers

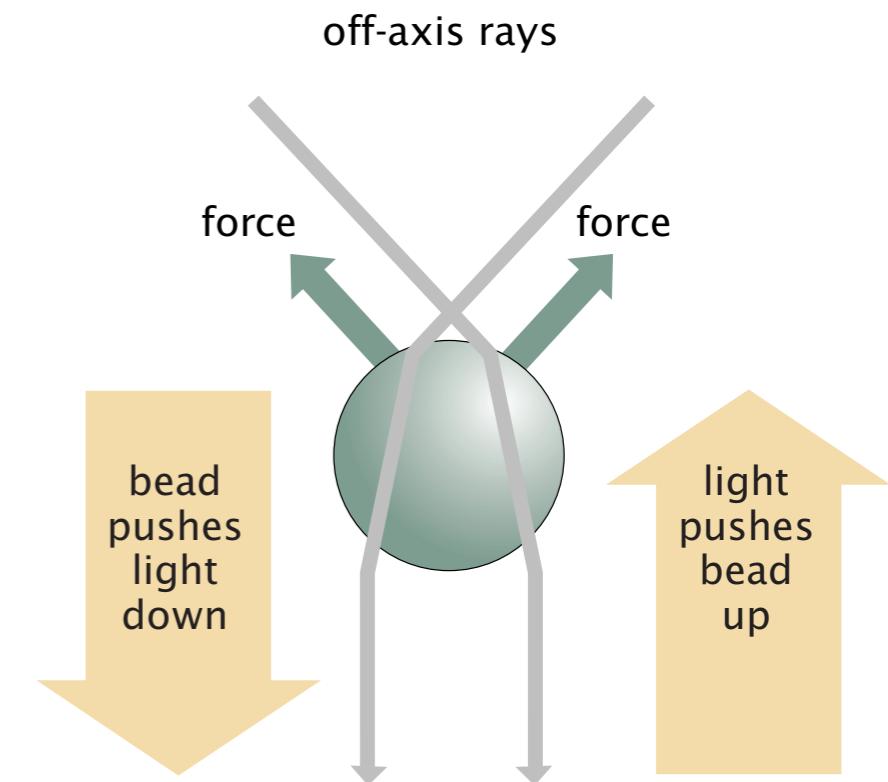
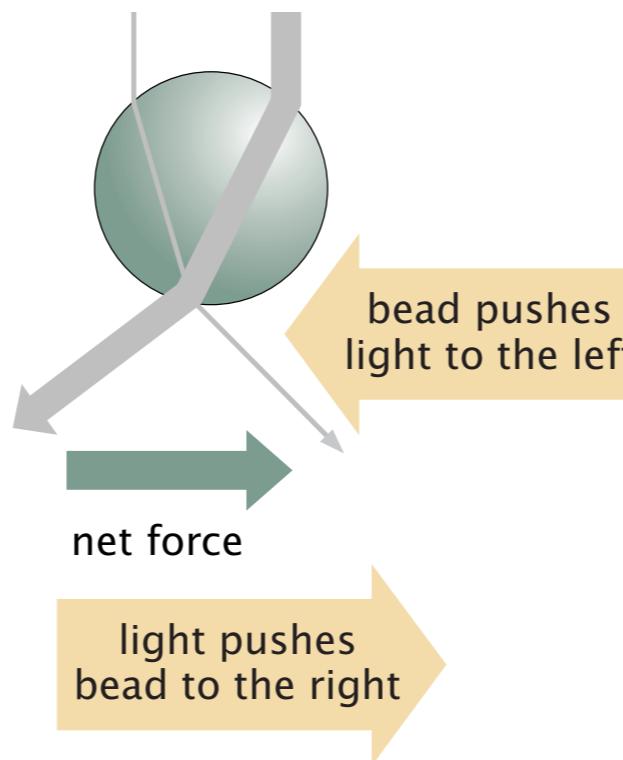


Laser beam focussed through a microscope objective of high numerical aperture

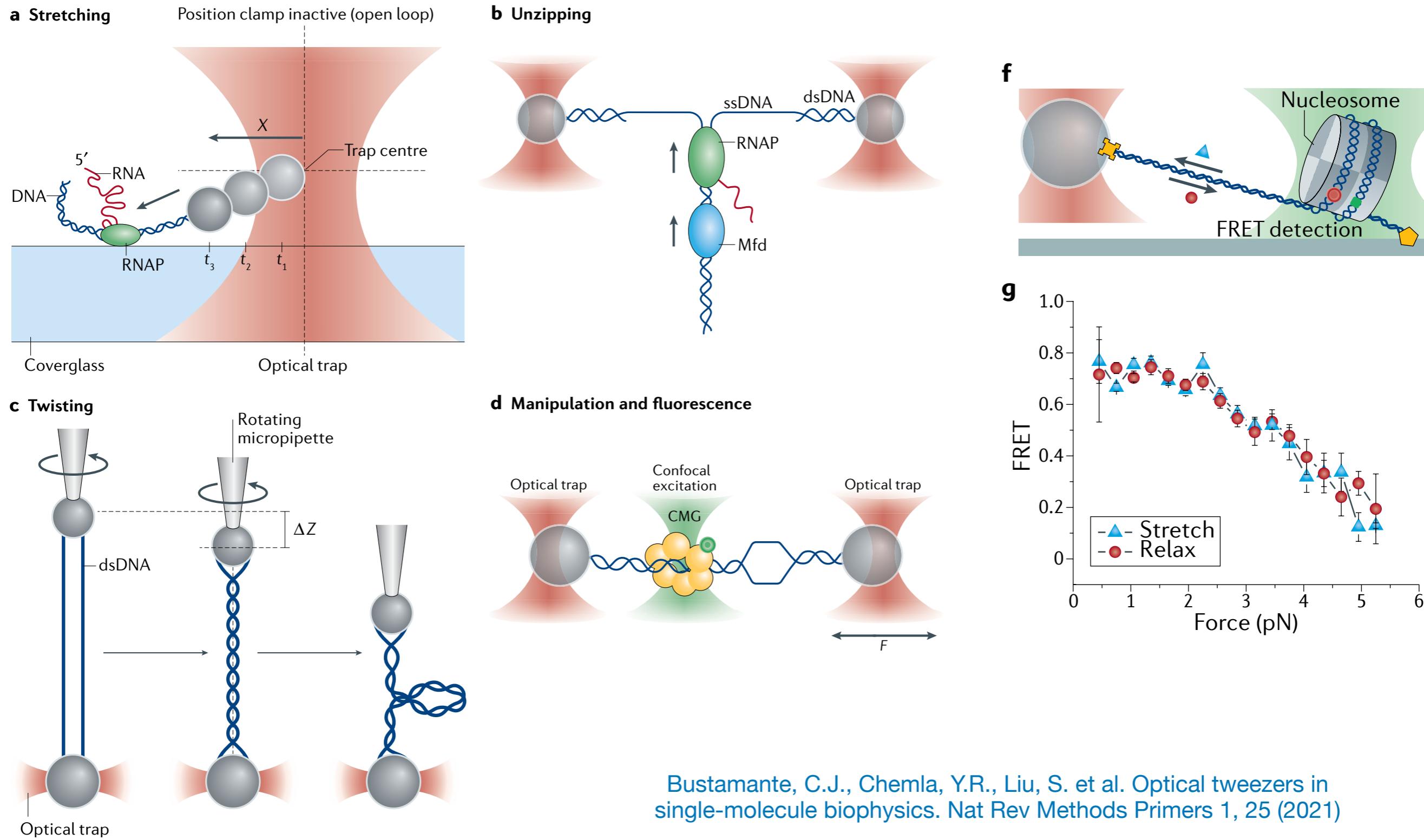
Glass or plastic beads larger than wavelength of light used



Beads are pushed by a restoring force towards the focus generated by light-bead momentum exchange

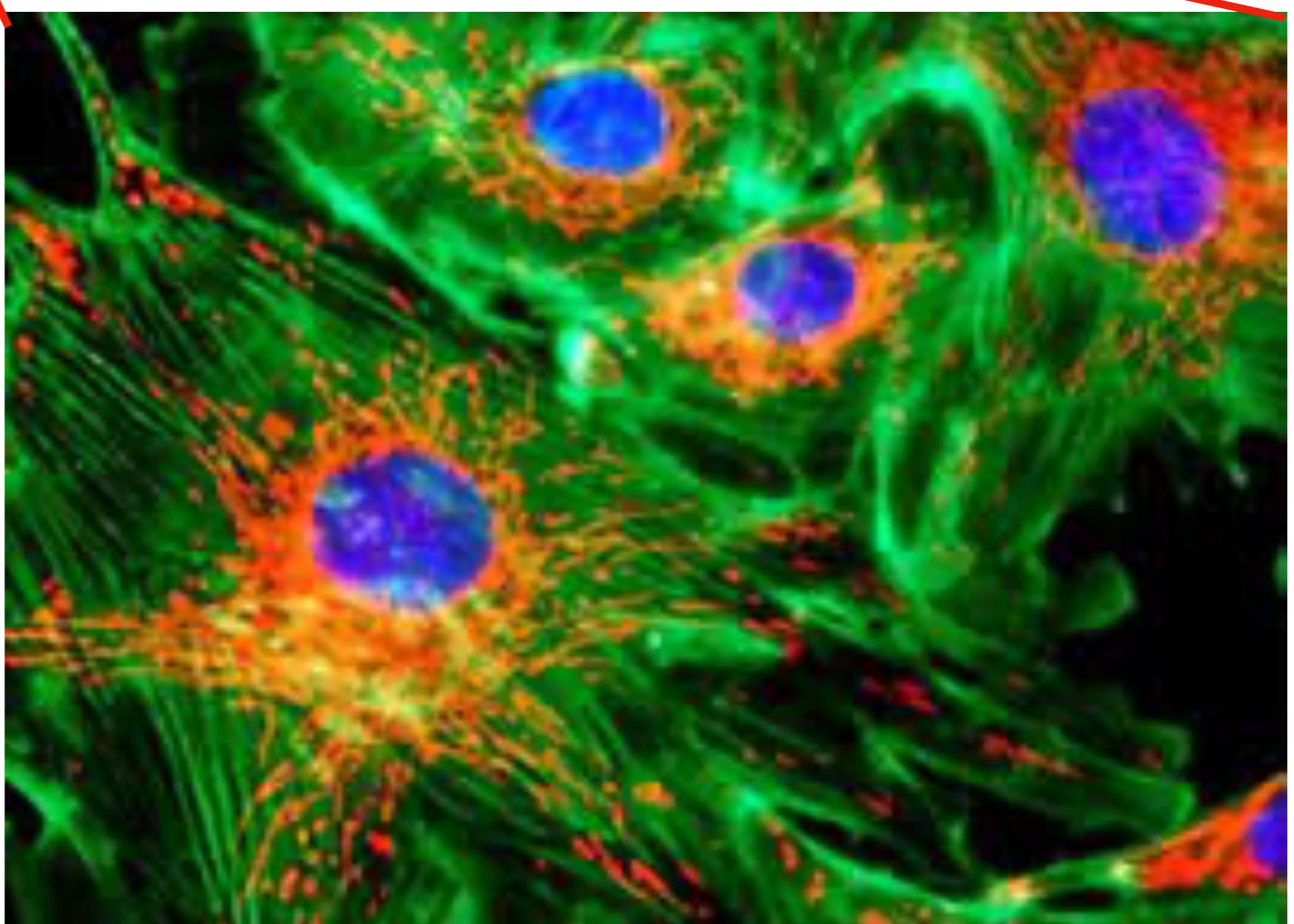


Applications of optical tweezers in biology

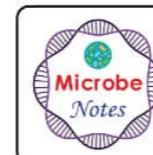
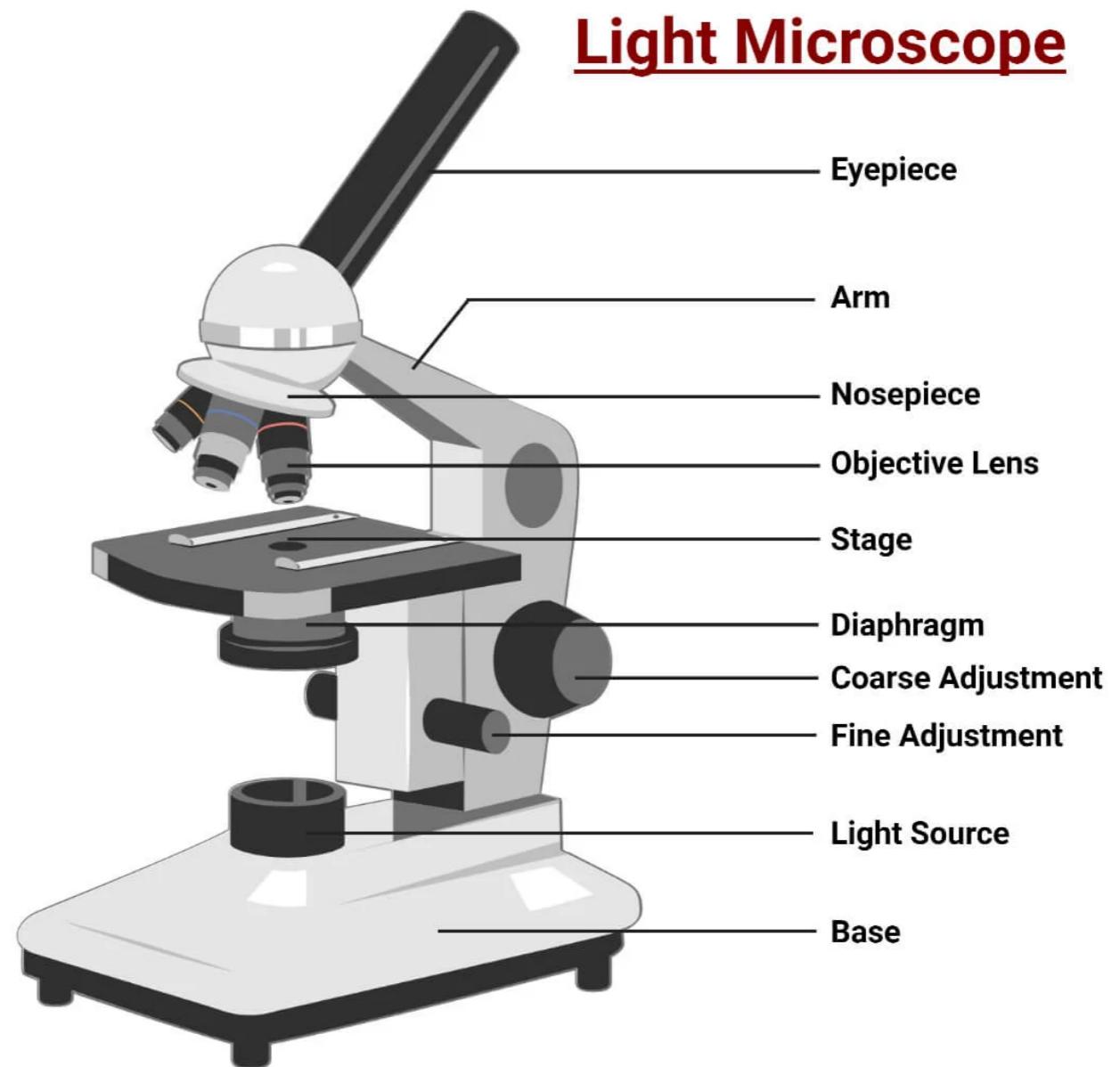
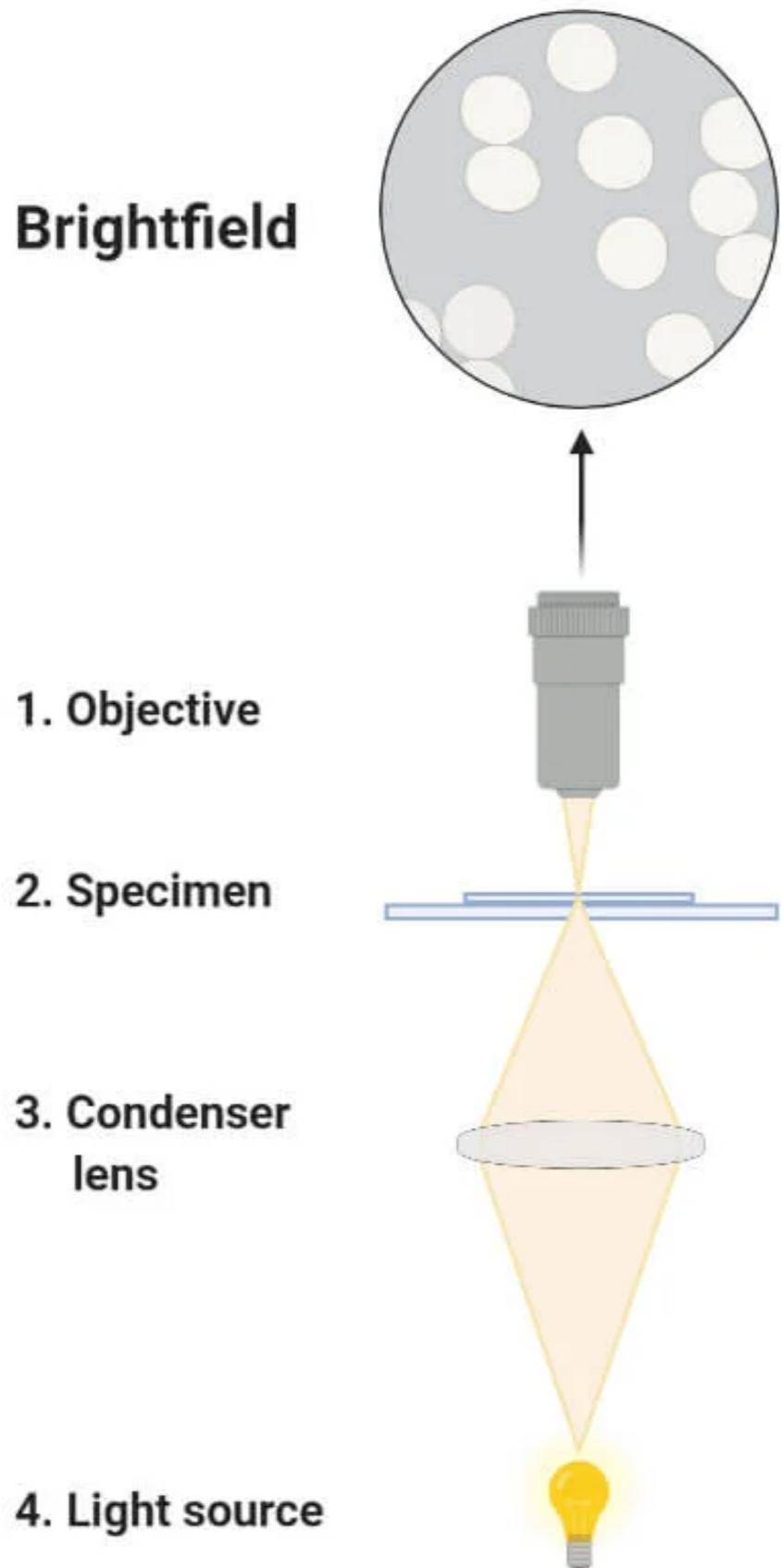




Light microscopy



Principles of light microscopy

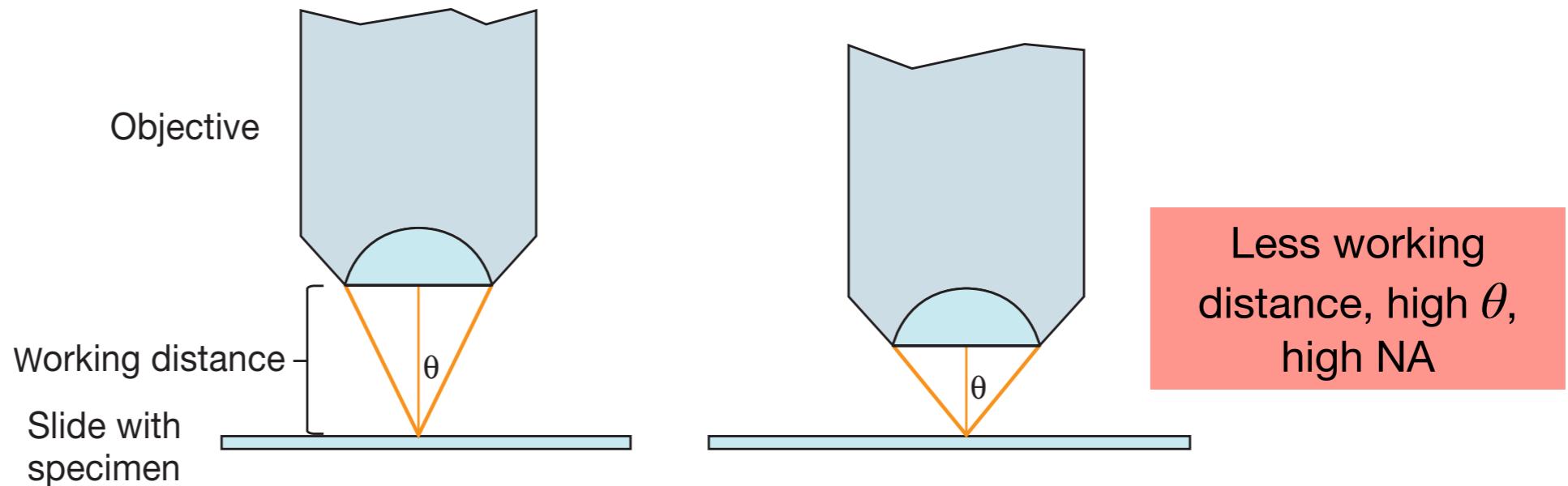


The
Biology
Notes

The
Chemistry
Notes

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Numerical aperture in microscopy



Numerical aperture of a lens, $NA = n \sin \theta$

n = refractive index of the medium in which the lens works

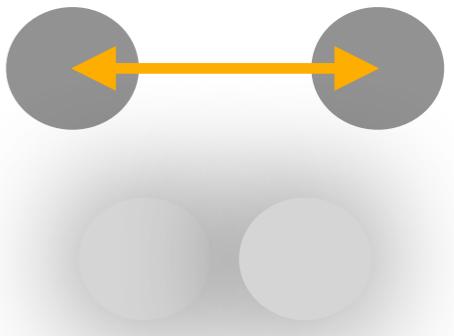
θ = 1/2 angle of the cone of light that enters the lens from the sample

Optical resolution

Abbe's optical theory of microscope design provides the definition of optical resolution

Abbe equation

$$d = \frac{\lambda}{2 \times NA} = \frac{\lambda}{2n\sin\theta}$$



Minimal distance between the two objects that reveals them as separate entities

λ = wavelength of light used to illuminate the specimen

Thus, best resolution is achieved with blue light

Now only way to improve resolution further is increasing the NA of the objective lens

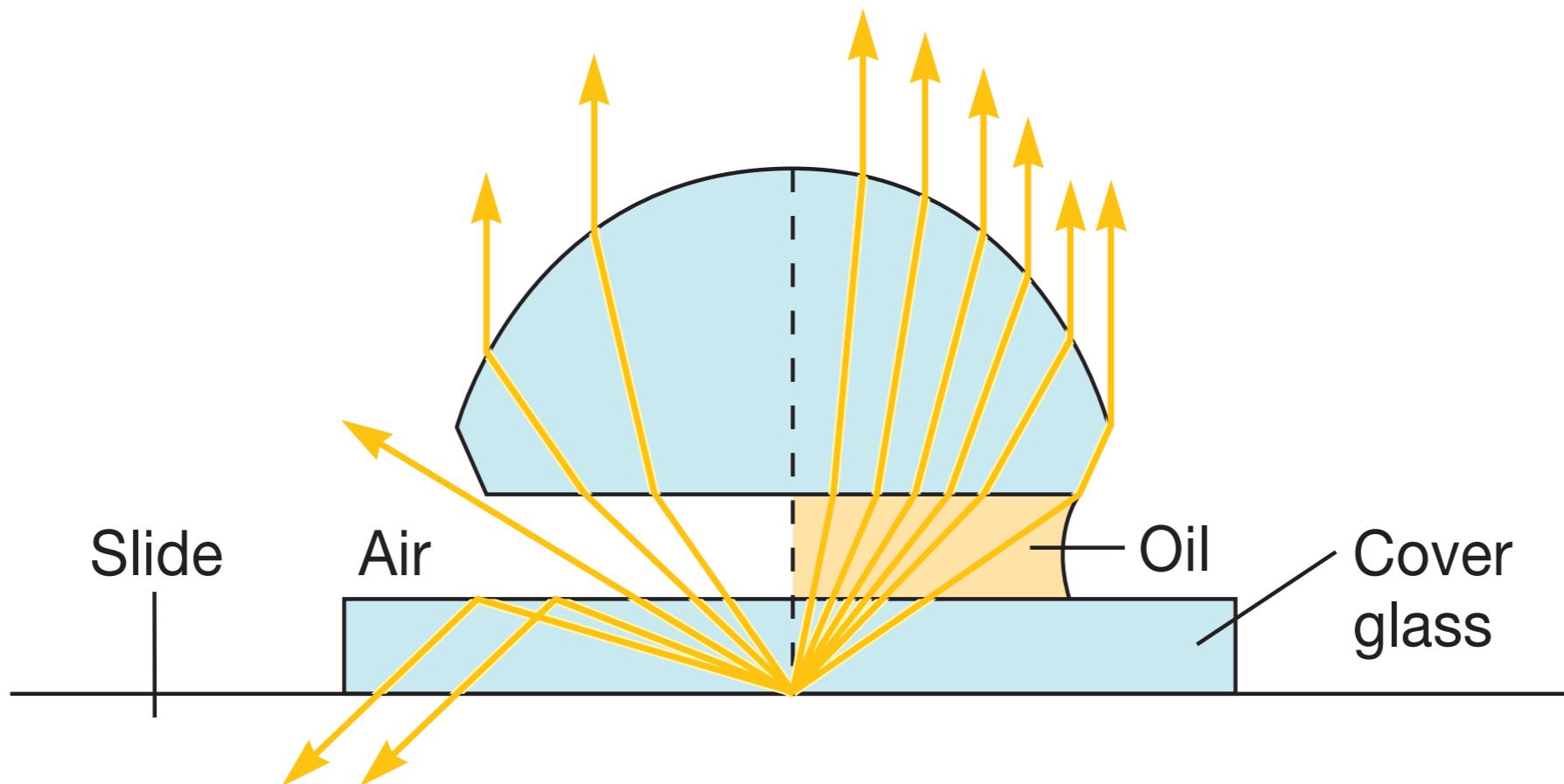
How to enhance NA of objective lens ?

Numerical aperture of a lens, $NA = n \sin \theta$

If operating in air: $n = 1$, maximum $NA = 1 \times \max(\sin \theta) = 1$

Most lenses have $NA \approx 0.9$

Replacing air with immersion oil can increase the NA of the lens



Types of light microscopy

The modern types of light microscope include

Bright field light microscopy

Phase contrast light microscopy

Dark field light microscopy

Fluorescence microscopy

Bright field light microscopy

Most basic type of microscopy - ‘naked microscopy’
Magnification achieved by combining an objective and an eyepiece lenses



Yeast cells under bright field microscope

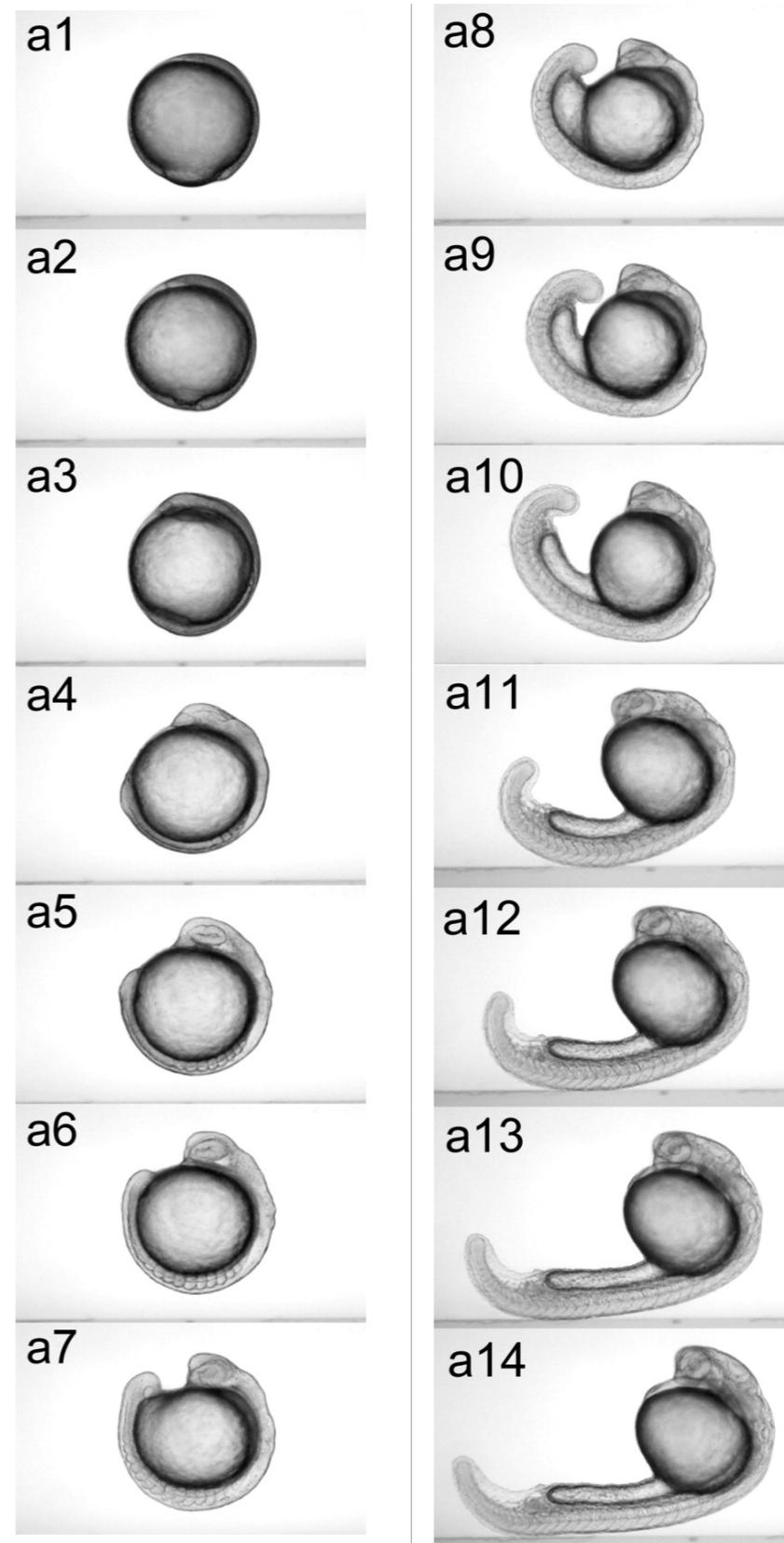
Advantages

- Inexpensive
- Little to no manipulation of the sample

Disadvantages

- Limited in resolution
- Low magnification power
- Transparent samples hard to resolve
- Staining often need for contrast and fixing (killing) the cells

Bright field images of a developing zebrafish



Resolution vs. magnification of bright field microscopy



Estimate the maximum resolving power by blue light ($\sim 500 \text{ nm}$) with NA = 1.25

Using Abbe equation

$$d = \frac{0.5 \times 500 \text{ nm}}{1.25} \approx 0.2 \mu\text{m}$$

- At best, a bright-field microscope can distinguish between two dots about $0.2 \mu\text{m}$ apart (\sim size of small bacterium).
- The vast majority of viruses ($\sim 20\text{-}100 \text{ nm}$) cannot be examined with a light microscope.

Now, human eyes can just see things of size $\sim 0.2 \text{ mm}$

Therefore, maximum magnification of bright field microscopy $\sim 1000\times$