Setting up a beam path – from excitation to detection

Antonia Göhler

January 2015

Fluorescence Microscopy

- non-invasive
- highly selective and sensitive: spatial information at the cellular level not easily available from biochemistry
- quantitative
- live cell applications: time resolution
- allows experimental manipulation

Why do we want home-build microscopes?

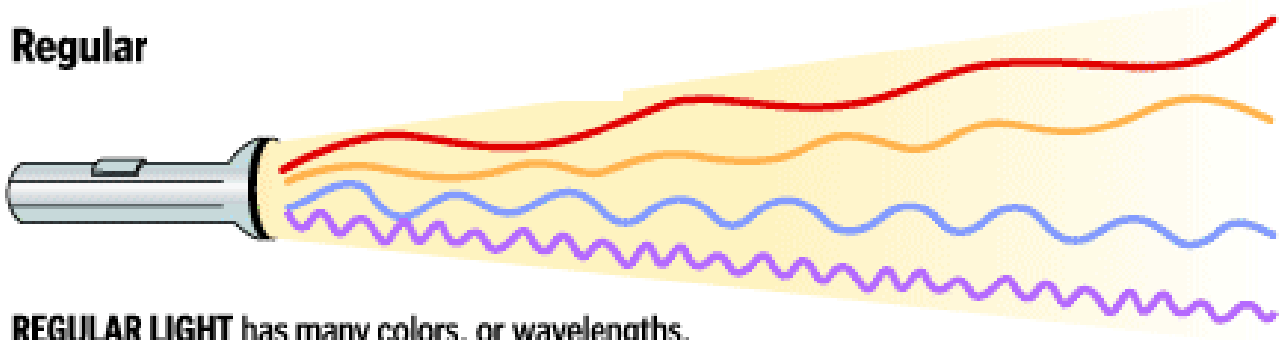
- special application: flexibility to perform new and varied experiments
- combination of different techniques (wide-field, SIM, STORM...)
- better understanding of what’s going on: microscope is no longer a black box
- better control/access to components
- optimisation opportunity for local experiments
- changeability: dichroics, inverted vs upright...
- independent of companies/engineer

- it’s fun :-)

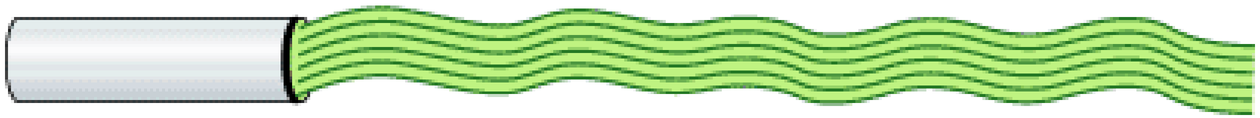
Main Components of a Microscope

- Light Source
  - LED, Laser
- Optical components to separate excitation and emission light
- Detectors (sCMOS, CMOS, CCD, EMCCD, PMT, MCP)

Light Sources – Regular Light vs Laser Light



REGULAR LIGHT has many colors, or wavelengths, mixed together, creating white light. The light waves spread out as they travel.



LASER LIGHT is of the same wavelength, with all of the waves in phase, or in step, with one another. A laser is always a single color because the waves are the same length. Because the waves are parallel, a laser light stays in a tight beam for long distances.

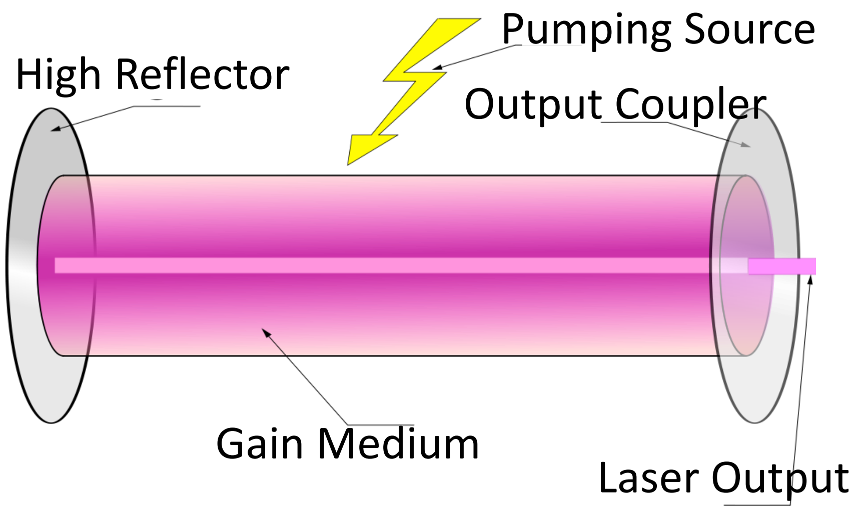
LASER – Light Amplification by Stimulated Emssion of Light

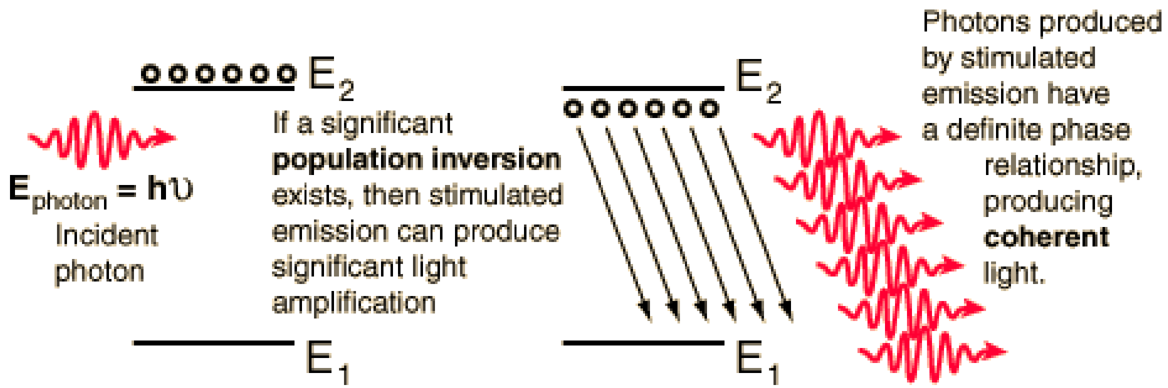
- “A laser is a device that emits light through a process of optical amplification based on the stimulated emission of electromagnetic radiation”
- differs from other sources of light because it emits light *coherently*
  - spatial coherence = focused into a tight spot over long distances (laser cutting, lithography, laser pointers)
  - temporal coherence = very narrow spectrum (emission of a single color, fs-pulses)

- important applications: optical disk drives, laser printers, barcode scanners, for both fiber-optic and free-space optical communication, in medicine for laser surgery and various skin treatments, and in industry for cutting and welding materials, in military and law enforcement devices for marking targets and measuring range and speed.

LASER – Light Amplification by Stimulated Emssion of Light

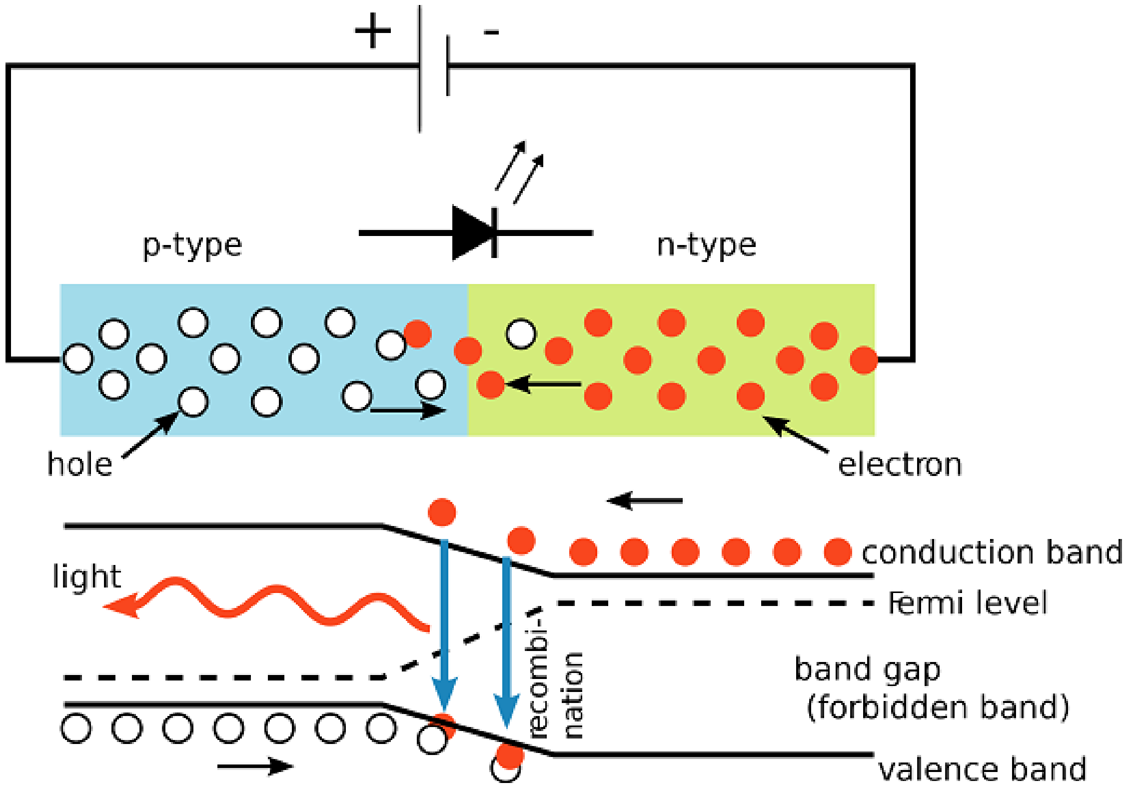
- active laser medium: material with properties that allow it to amplify light with a specific wavelength by stimulated emission (power increase)
- pumping process: supplying the gain medium with energy (electrical current, another laser or a flash lamp)
- Light bounces back and forth between two mirrors, passing through the gain medium and being amplified each time
- Typically one of the two mirrors, the output coupler, is partially transparent



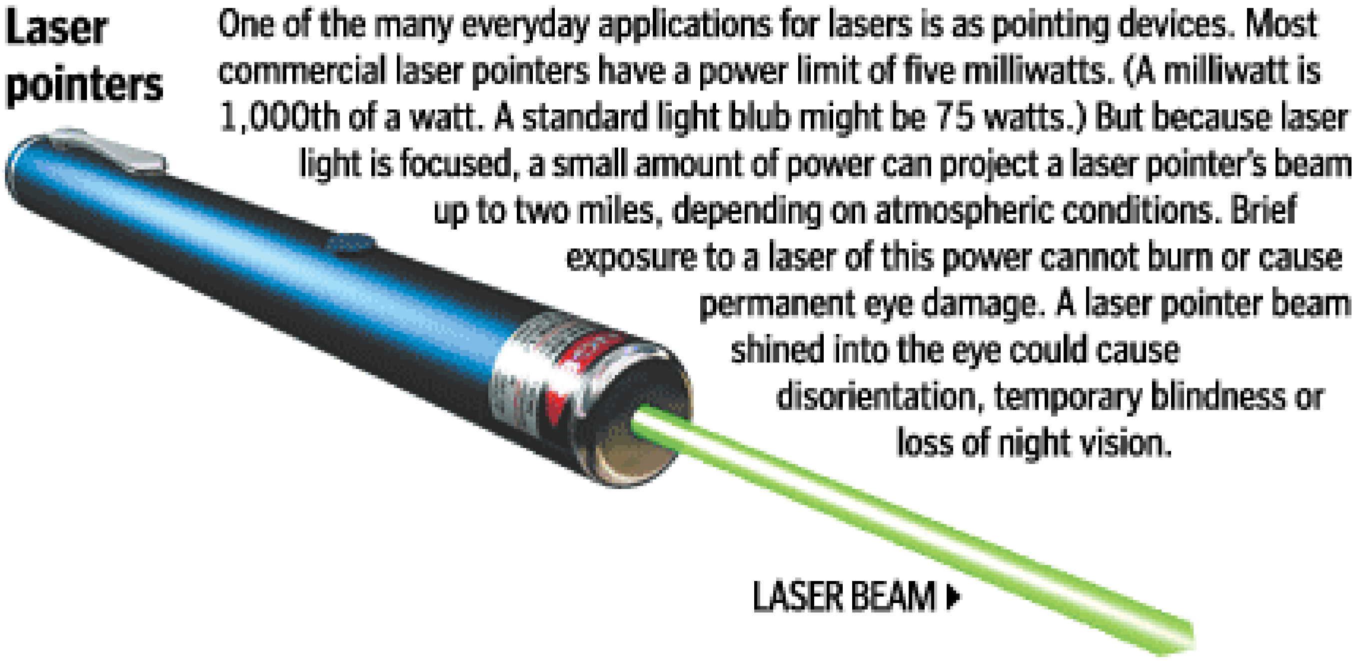


LED – Light Emitting Diode

- two-lead semiconductor light source (basic pn-junction diode)
- emits light when activated: by applying a suitable to the leads, electrons are able to recombine with electron holes within the device and to release energy in the form of photons
- the color of the light (corresponding to the energy of the photon) is determined by the energy band gap of the semiconductor

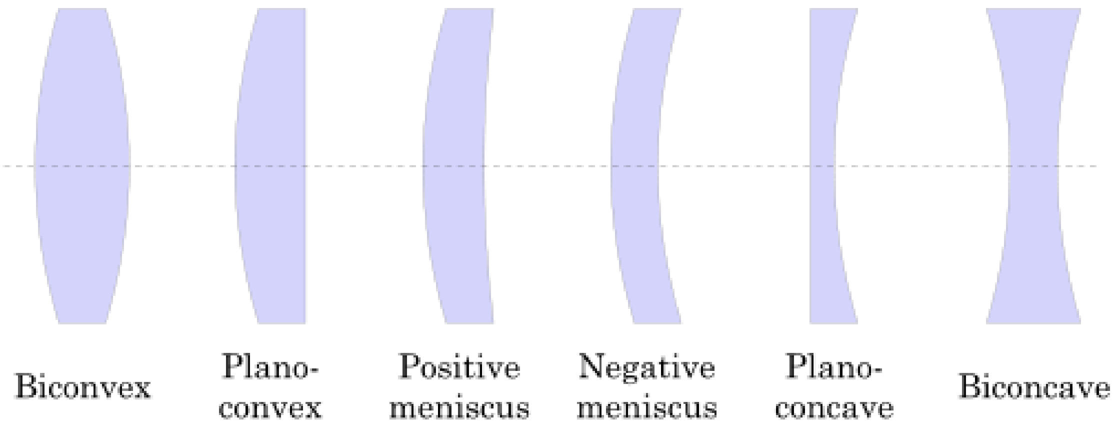


Laser Pointer

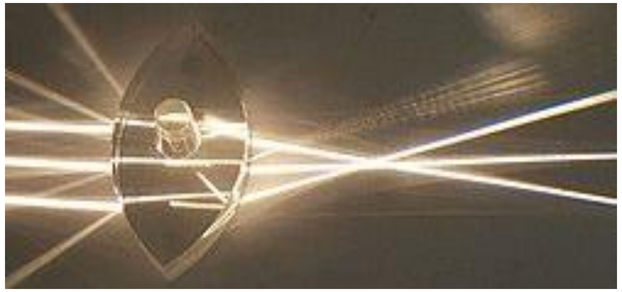


Types of Lenses...

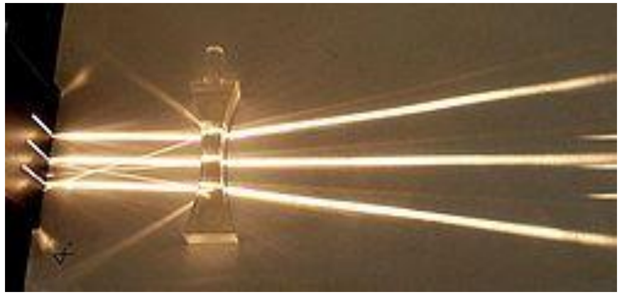
- ...are classified by the curvature of the two optical surfaces



- convex: a collimated beam of light passing through the lens will be converged (or *focused*) to a spot behind the lens (distance = focal length of the lens)

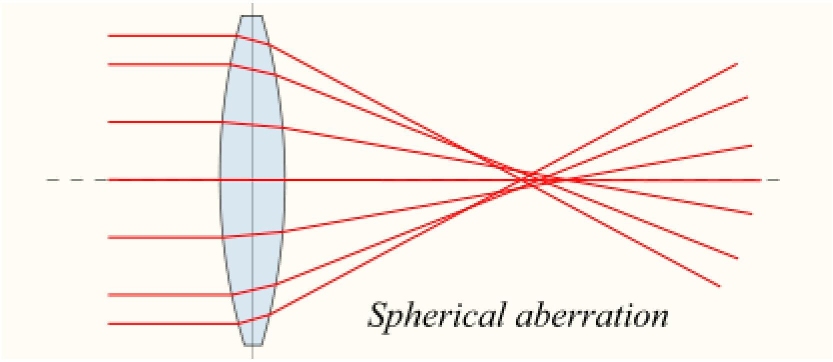


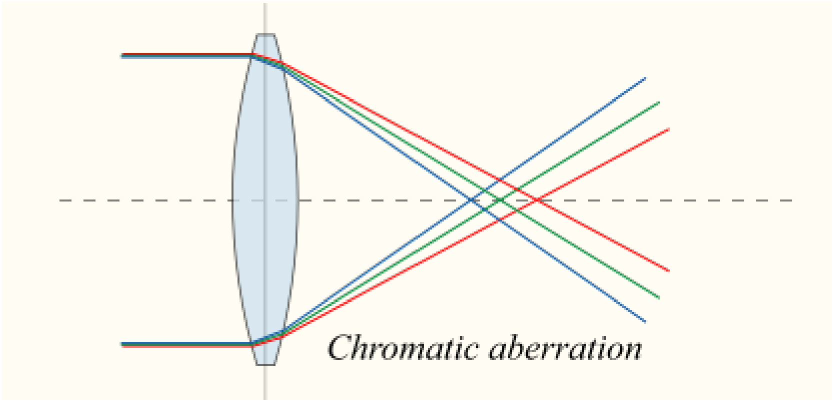
- concave types: collimated beam becaomes diverged (spread)

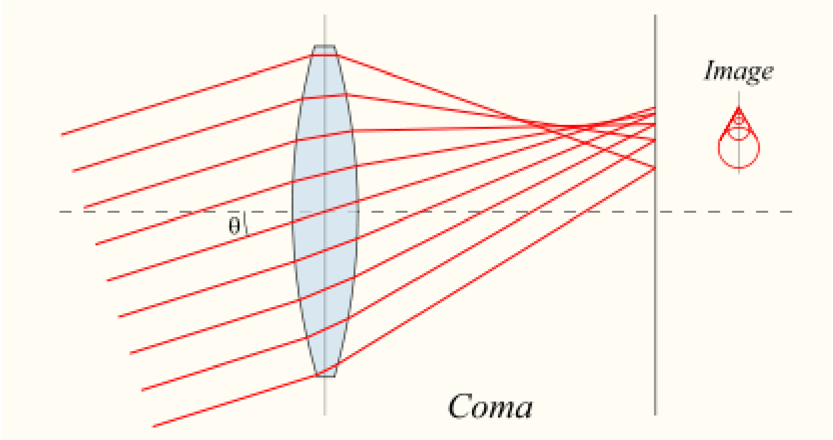


Aberrations

- spherical surfaces are not the ideal shape with which to make a lens
- *Spherical aberration*: beams are focused in a slightly different place resulting in a blurring of the image
- *Chromatic aberration* is caused by the dispersion of the lens material—the variation of its refractive index, *n*, with the wavelength of light and is seen as fringes of color around the image
- *Coma* occurs where rays pass through the lens at an angle to the axis θ. The image becomes a comet-like shape





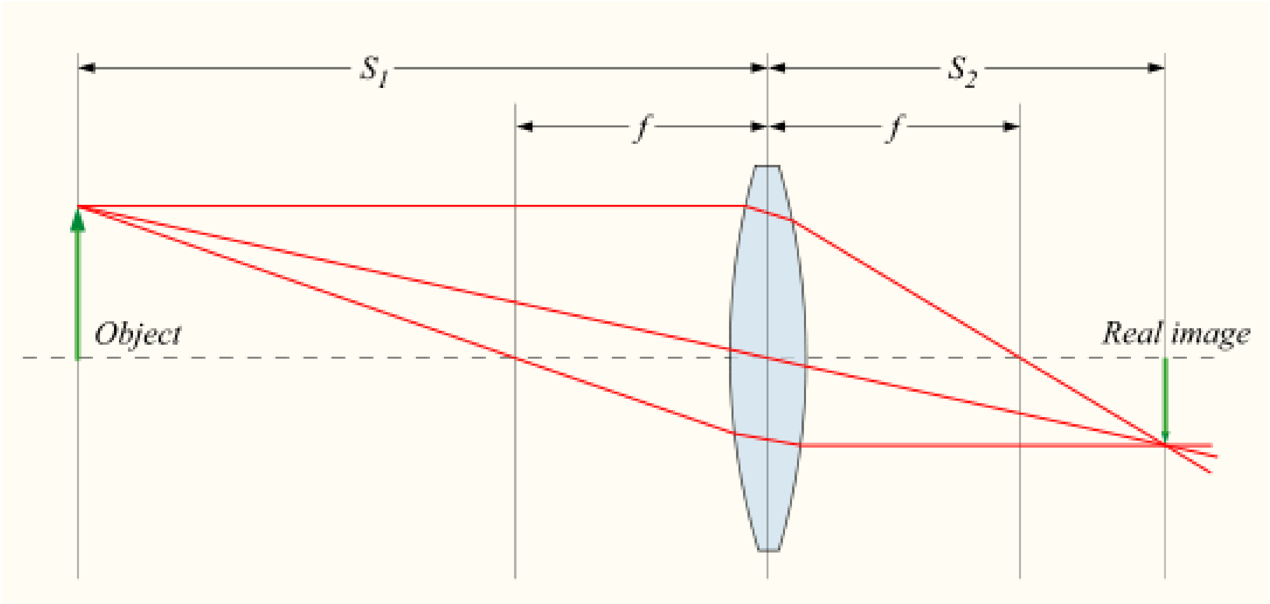


Lensmaker's equation and Imaging properties

$$\frac{1}{f} = (n-1)\left[\frac{1}{R_1} - \frac{1}{R_2} + \frac{(n-1)d}{nR_1R_2}\right]$$

Lensmaker's equation for the focal length of a lens

$$\frac{1}{S_1} + \frac{1}{S_2} = \frac{1}{f}$$

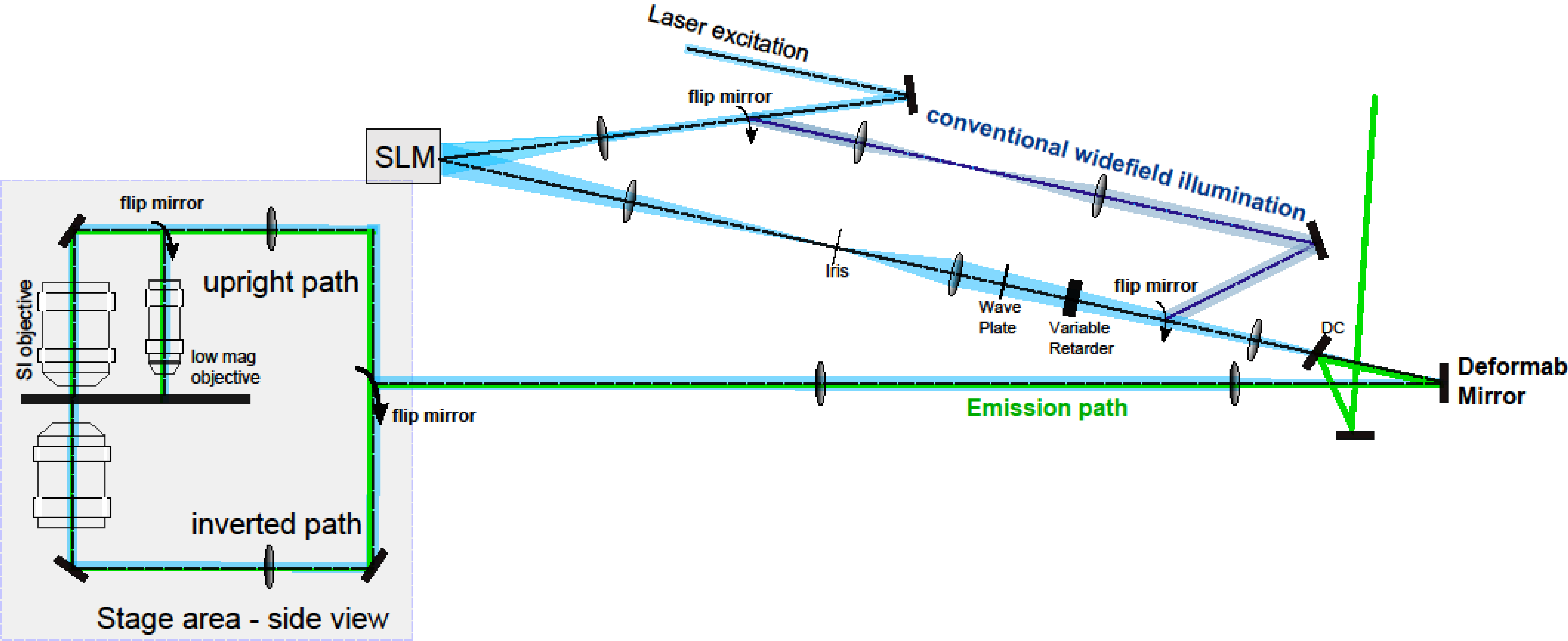


How to build a microscope?

- What kind of microscope is needed?
- Where do you want to build it? How much space do you have?
- What do you need?

Optics

- lenses
- mirrors
- posts
- holders
- iris
- light source
- dectetor (camera, screen)


Laser excitation
flip mirror
SLM
conventional widefield illumination
flip mirror
Iris
Wave
Plate
Variable
Retarder
flip mirror
DC
Deformab
Mirror
Emission path
upright path
SI objective
low mag
objective
flip mirror
inverted path
Stage area - side view