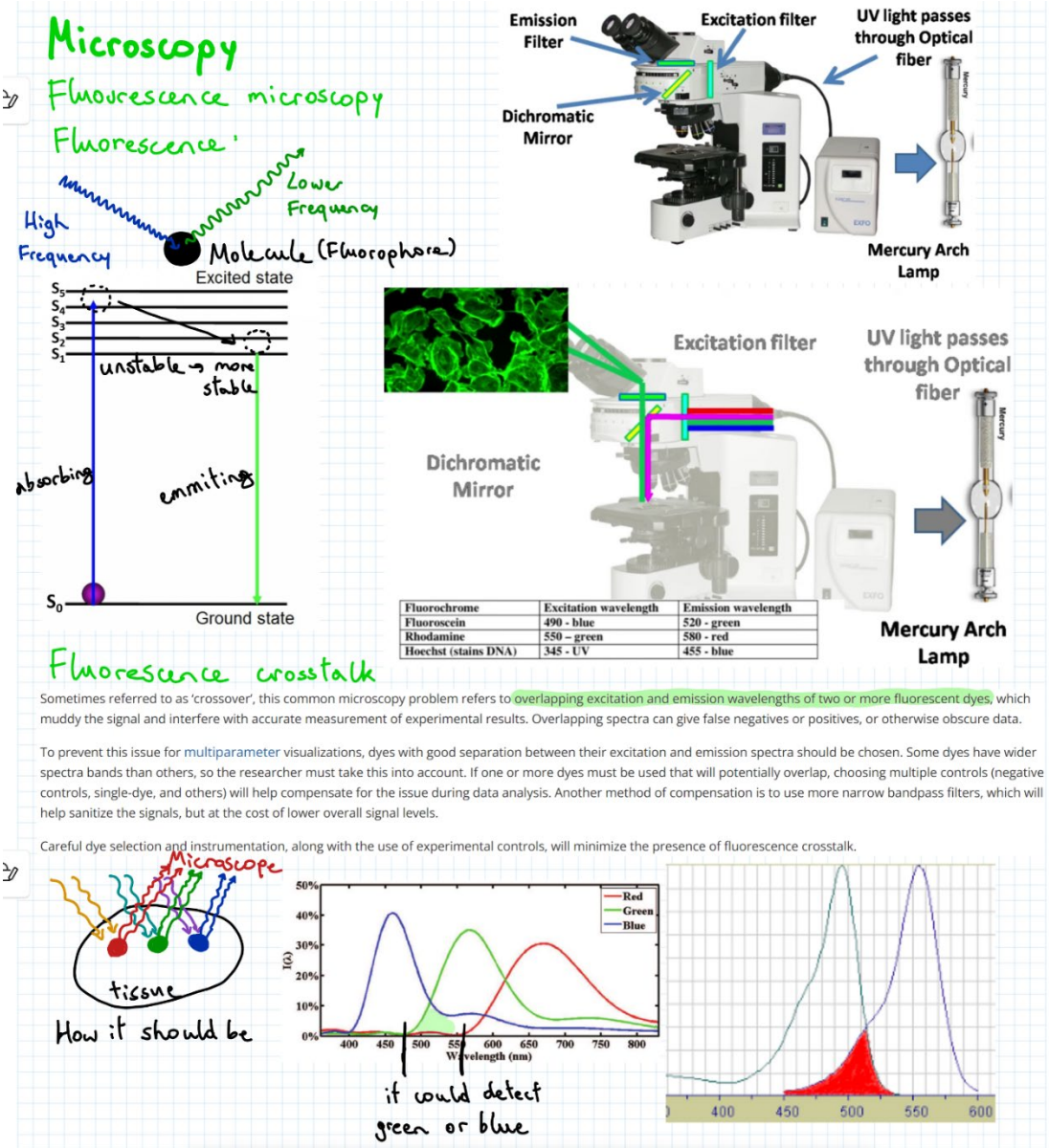
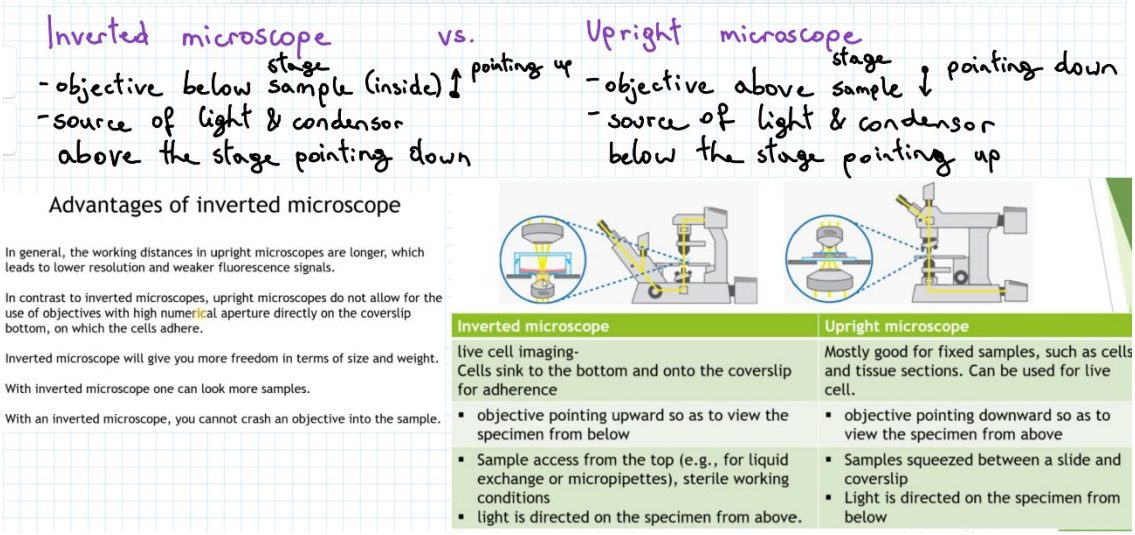
Bsp 2.: Tag 6: Verschiedene Arten von Mikroskopen kennenlernen und recherchieren, wie sie funktionieren:





Tissues, cells, and the smaller structures inside cells (organelles) are mostly water and are therefore transparent. Imaging tiny see-through bags of water results in images that don't contain a lot of information, and in microscopy, it is vital to have some sort of **contrast** or **stain** that will give areas of the sample **color** and make them far easier to see. In addition, what if you only want to image **some** of the smaller structures inside a cell, like a nucleus or a cell membrane? Coloring the entire cell would make it impossible to localize the areas you are interested in.

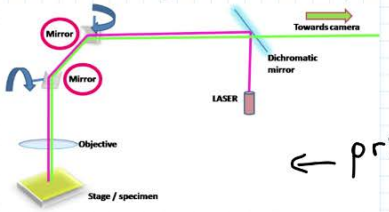
Fluorescence solves both these issues of contrast and localization. Fluorescence is where an object will **emit light after absorbing light**. Many different objects exhibit fluorescence, such as minerals (the word fluorescence coming from the mineral **fluorite**), deep-sea fish (most famously the jellyfish ***Aequorea victoria***, from which **green fluorescent protein (GFP)** was discovered), plants, chemicals and many more.

Fluorescent molecules (known as **fluorophores**) are used to label samples, and fluorophores are available that emit light in **virtually any color**. In a **fluorescent microscope**, a sample is labeled with a fluorophore, and then a bright light (**excitation light**) is used to illuminate the sample, which gives off fluorescence (**emission light**). In this manner, samples are **highly contrasted** to the black background as the fluorophore emits a bright-colored light. By **localizing** these fluorophores to the area of interest a clear image of any part of a cell can be taken, making fluorescence microscopy a **powerful tool** for life sciences.

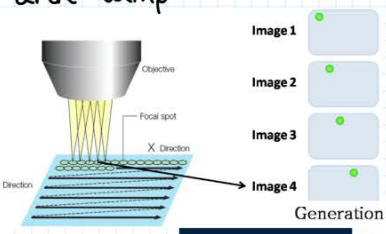
confocal microscope

- is widely used for fluorescence imaging in the life sciences
- produce a point source of light & reject out-of-focus light
  ↳ image deep into tissues with high resolution & 3D reconstructions of imaged samples
- invented due to drawbacks of fluorescence microscopy (continuous exposure of samples to high intensity UV light → photo bleaching & fluorescent molecules out of the focal plane are also obtained → blurred image)
- used laser light instead of mercury arch lamp
- digital camera with pinhole
  ↳ light of only one focal plane to be focused on digital camera

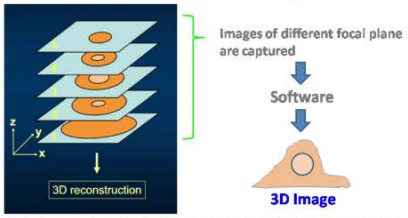
mirrors can be adjusted to focus the next region of the specimen

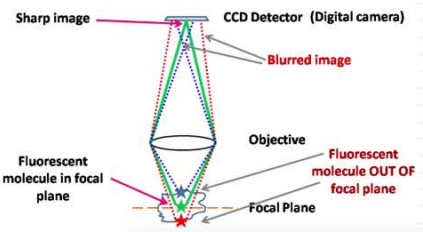


← principle

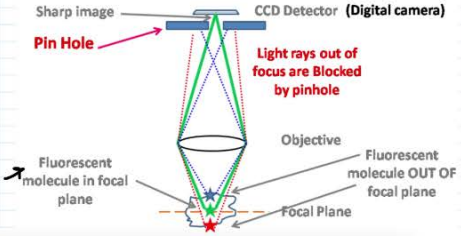


Generation of 3D image



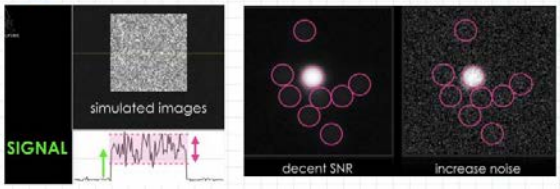


V.S.



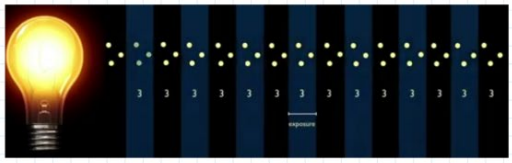
better

Signal to noise ratio (SNR)

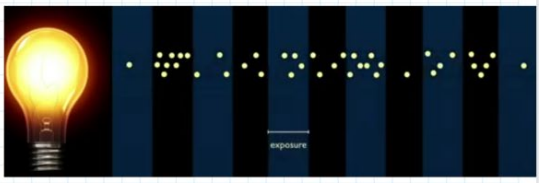


- if noise is low enough relative to the signal → distinguish different intensities
- if noise increases → larger differences in intensity to accurately differentiate objects
- influences:
  - detection ability
  - precision of intensity measurements
  - resolution

- Why is there noise?
  exposure time of sample differs
  ↳ Poisson Noise: Fundamental limitation on certainty of intensity measurements
  stdev = $\sqrt{photons}$ photons ↑ = poisson noise ↑ but $\frac{photon}{\sqrt{photon}}$ improves



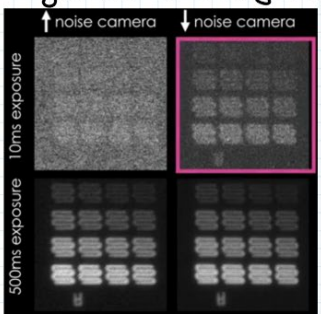
vs.



ideal emmission rate of photons reality

detectors add noise & low exposure time

- for collecting more signal: choice of fluorophore, filters, lens modality & detector
  FP-Base (compare)
  ↳ matching
- get rid of background, don't use plastic or mounting media that contain fluorophore (no DAPI)

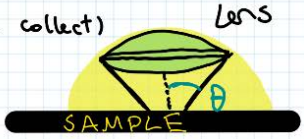


Numerical aperture

- limits resolution & image brightness
- Resolution: The distance by which two objects must be separated in order to distinguish them as separate from each other

  in epi-fluorescence microscopy: $d = \frac{0.61\lambda}{NA}$ — λ – emmission wavelenght (generate image); NA – Numerical aperture

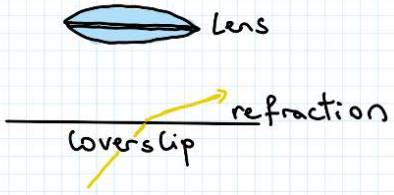
$NA = (n) \sin\theta$ – max. angle of acceptance (max. angle of light that objective can collect)

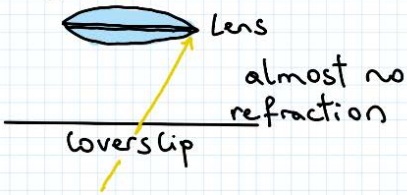


NA = 1 means can collect light with 90° of the specimen

↳ How do we get NA above 1?

A: raise refractive index (Brechungsindex) use immersion media (immersion oil)





only use immersion oil with oil immersion objective