

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

## Microscopy

### Fluorescence microscopy

### Fluorescence

Labels: Emission Filter, Excitation filter, UV light passes through Optical fiber, Dichromatic Mirror, Mercury Arch Lamp.

Labels: High Frequency, Lower Frequency, Molecule (Fluorophore), Excited state, Ground state, absorbing, emitting.

Labels: Excitation filter, UV light passes through Optical fiber, Dichromatic Mirror, Mercury Arch Lamp.

Fluorochrome	Excitation wavelength	Emission wavelength
Fluorescein	490 - blue	520 - green
Rhodamine	550 - green	580 - red
Hoechst (stains DNA)	345 - UV	455 - blue

## Fluorescence crosstalk

Sometimes referred to as 'crossover', this common microscopy problem refers to overlapping excitation and emission wavelengths of two or more fluorescent dyes, which muddy the signal and interfere with accurate measurement of experimental results. Overlapping spectra can give false negatives or positives, or otherwise obscure data.

To prevent this issue for multiparameter visualizations, dyes with good separation between their excitation and emission spectra should be chosen. Some dyes have wider spectra bands than others, so the researcher must take this into account. If one or more dyes must be used that will potentially overlap, choosing multiple controls (negative controls, single-dye, and others) will help compensate for the issue during data analysis. Another method of compensation is to use more narrow bandpass filters, which will help sanitize the signals, but at the cost of lower overall signal levels.

Careful dye selection and instrumentation, along with the use of experimental controls, will minimize the presence of fluorescence crosstalk.

<h3>Inverted microscope</h3> <ul style="list-style-type: none"> <li>- objective below sample (inside) <math>\uparrow</math> pointing up</li> <li>- source of light &amp; condensor above the stage pointing down</li> </ul>	vs.	<h3>Upright microscope</h3> <ul style="list-style-type: none"> <li>- objective above sample <math>\downarrow</math> pointing down</li> <li>- source of light &amp; condensor below the stage pointing up</li> </ul>
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## Advantages of inverted microscope

In general, the working distances in upright microscopes are longer, which leads to lower resolution and weaker fluorescence signals.

In contrast to inverted microscopes, upright microscopes do not allow for the use of objectives with high numerical aperture directly on the coverslip bottom, on which the cells adhere.

Inverted microscope will give you more freedom in terms of size and weight.

With inverted microscope one can look more samples.

With an inverted microscope, you cannot crash an objective into the sample.

Inverted microscope	Upright microscope
live cell imaging- Cells sink to the bottom and onto the coverslip for adherence	Mostly good for fixed samples, such as cells and tissue sections. Can be used for live cell.
<ul style="list-style-type: none"> <li>objective pointing upward so as to view the specimen from below</li> <li>Sample access from the top (e.g., for liquid exchange or micropipettes), sterile working conditions</li> <li>light is directed on the specimen from above.</li> </ul>	<ul style="list-style-type: none"> <li>objective pointing downward so as to view the specimen from above</li> <li>Samples squeezed between a slide and coverslip</li> <li>Light is directed on the specimen from below</li> </ul>

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.

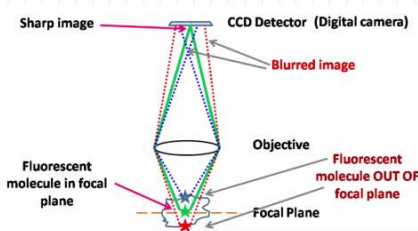
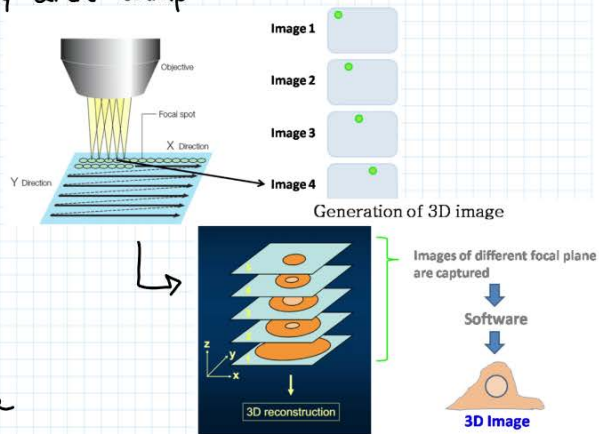
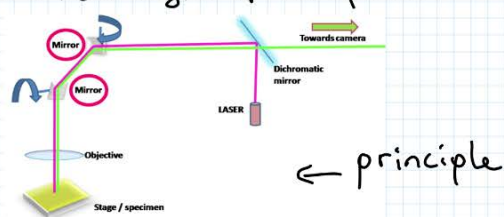
Fluorescence solves both these issues of contrast and localization. Fluorescence is where an object will **emit light after absorbing light**. Many different objects exhibit fluorescence, such as minerals (the word fluorescence coming from the mineral *fluorite*), deep-sea fish (most famously the jellyfish *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

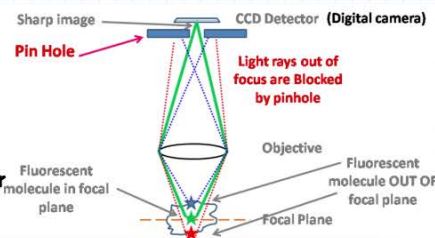
- is widely used for **fluorescence imaging** in the life sciences
- produce a point source of light & reject out-of-focus light
  - ↳ image deep into tissues with high resolution & 3D reconstructions of imaged samples
- invented due to drawbacks of fluorescence microscopy (continuous exposure of samples 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 arc 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

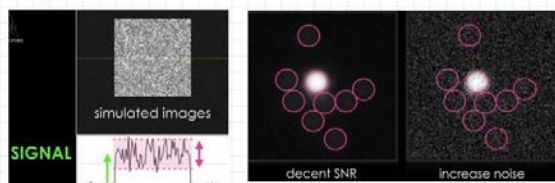


V.S.

better →



## Signal to noise ratio (SNR)

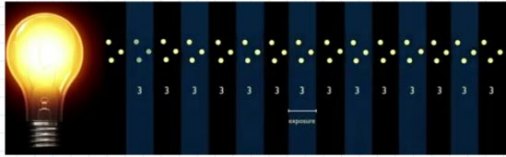


- if noise is low enough relative to the signal → distinguish different intensities
- if noise increases → larger differences in intensity to accurately differentiate objects
- influences:
  - detection ability
  - precision of intensity measurements
  - resolution

- Why is there noise?

exposure time of sample differs

↳ Poisson Noise: Fundamental limitation on certainty of intensity measurements  
 $stdev = \sqrt{\text{photons}}$  photons  $\uparrow$  = poisson noise  $\uparrow$  but  $\frac{\text{photon}}{\text{photon}}$  improves



VS.



ideal emission rate of photons

reality

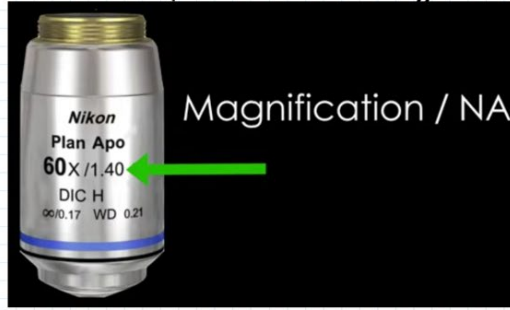
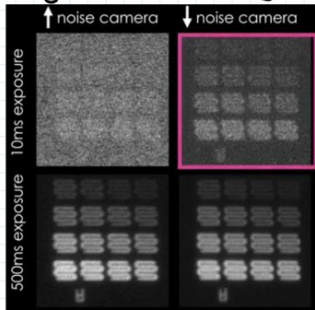
detectors add noise & low exposure time

FP-Base (compare)

& detector

- for collecting more signal: choice of fluorophore, filters, lens modality

- get rid of background, don't use plastic or mounting media that contain fluorophore (no DAPI)



## Numerical aperture

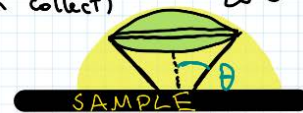
- limits resolution & image brightness

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

in epi-fluorescence microscopy:  $d = \frac{0.61 \lambda}{NA}$  - emission wavelength (generate image)  
 - Numerical aperture

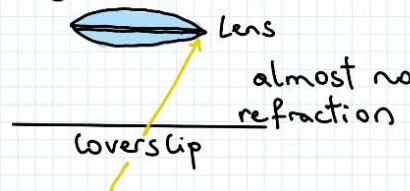
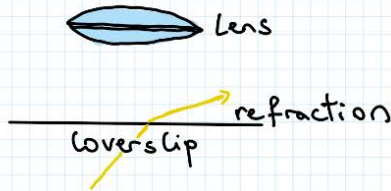
$NA = (n) \sin \theta$  - max. angle of acceptance  
 (max. angle of light that objective can collect)

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