

Microscopy Problem Set 1

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Please hand in the solutions of this problem set next lecture on April 26th of 2019.

1 Your first microscope

In this task you will design a 4f system based light microscope using the following components

Objective lens		Nikon CFI Apo 40XW NIR (0.8NA) € 2000,00
Tube lens		Nikon 200mm Tube Lens (d=30 mm) € 250,00
Camera		Hamamatsu Orca Flash4.0 V2 QE: 82% (600 nm) 2048x2048 px Pixel Size: 6x6 μm € 13 000,00

and a fluorescent sample emitting at 500 nm.

In order to do so, follow these steps:

- a) First, make a sketch of a fluorescent wide field microscope (without illumination system). Indicate the position of the aperture stop. Trace the main light rays (chief ray and marginal ray) for both, on-axis and off-axis, fluorescence points in the sample.

Then calculate:

- b) Calculate the focal length f_{obj} of the given objective and the half opening cone angle α .
- c) Calculate the size of the field of view (FOV) limited by first: the tube lens and second: the camera. What is your conclusion/suggestion?.
- d) Estimate the lateral resolving power.
- e) Calculate the pupil diameter of the system.
- f) Is the image appropriately sampled by the camera?
- g) Explain the advantages of a infinity imaging between objective and tube lens.
- h) In order to use this system for non fluorescence samples, it should be equipped with decent illumination. Sketch a Köhler illumination system, and explain the advantages over direct illumination.