Exam TFY 4265 2013 – Solutions

Oppgave 1

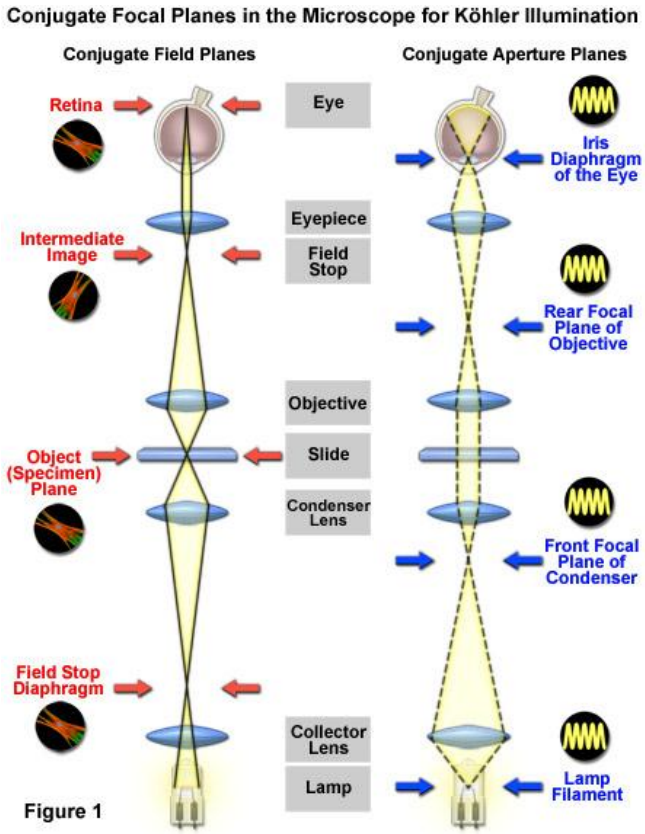


Figure 1

a) See figure

b) Two planes are conjugate if one plane is imaged in the other plane. In a microscope there are two sets of four conjugate planes which are illustrated above. Not especially that the lamp is not imaged in the objectplane (the lamp and specimen are NOT conjugate planes)

c)

- The field stop aperature is called the Field stop diaphragm in the figure. Notice that this is conjugate to the object plane so it controls the area of illumination.
- The condensor apperature is placed in the front focal plane of the condensor. By looking at the beam path on the left, we can see that this controls the angle of the incident light on the sample (affecting brightness, contrast and resolution)'
- The condensor annulus is placed in the front focal plane of the condensor and the phase plate in the back focal plane of the objective. We seee that these two planes are conjugate planes so the undiffracted light from the annulus is imaged on the partially transparant ring in the phase plate.

d) Necessary steps

- Fixation to stop biological changes (usually formaldehyde. Others: Alcohol, methanol, glutaraldehyd)
- Dehydration (to prepar for embedding material) in alchol.
- Embedding in parafin
- Sectioning on a microtome
- Mount on glass slide
- Deparaffinization
- Stain (DAPI is a good nuclear stain)
- Add mounting medium and coverslip
- Seal edges

Oppgave 2

a) FCS is based on measuring fluctuations in fluorescence as molecules diffuse in or out of the focal volume, or change their fluorescent properties. From this signal an autocorrelation curve is calculated. At low times there will be a high correlation and at low signals there will be a low correlation. The time-delay at which there is a drop in correlation is dependent on the diffusivity of the fluorescent particles. Decay at a shorter time-delay indicates faster diffusion and vice versa.

b)In figure 2 we see that the free particles diffuse fast (white circles) with drop off a short time-delay. We also see that for the particles (squares) the time constant is basically the same for the two pH values, indicating very little free fluorescent molecules released.

In figur 3 we see a much shorter time constant at pH 7 than at pH 5, indicating that more free molecules have been released. The curves are also flatter which could indicate a distribution of time constants, perhaps due to aggregation of particles.

c) Diffraction limits the acheivable resolution in optical microscopy. Light that passes through a finite apperature (the objective or the condensor) can only be focused down to a spot which has an Airy pattern where the minimum size is related to the size of the apperature, the wavelength of the light and the refractive index of the medium between the apperture and the focus.

d) In STED, fluorescent molecules are excited as in normal CLSM. Very quickly after the excitation, a second, doughnut shape pulse, deexcites the molecules in an outer ring through stimulated emission. This emission is at a longer wavelength than the detected fluorescence and is filtered out. Due to the nonlinear saturation of depletion, only a molecules in a small region (smaller than the diffraction spot) are left to give fluorescence.

Oppgave 3

a)

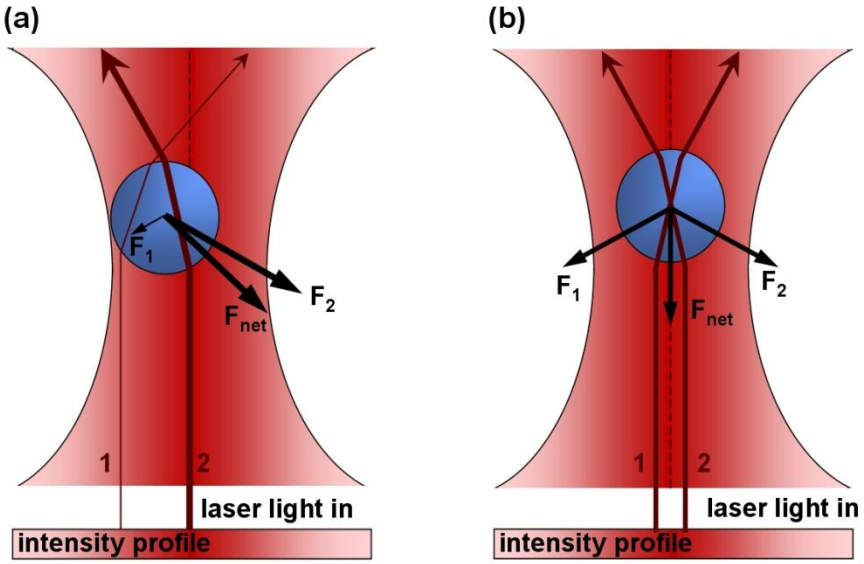
- In contact mode, the tip is so close to the surface the repulsive, steric (Pauli exclusion principle) is working. The sample is scanned under the tip and deflections in the tip are meausured as surface properties or topography.

- In non-contact mode the tip is further from the surface so that attractive van der Waals forces are acting. The tip is oscilating and the interaction is determined from changes in resonance frequency.
- In tapping mode the tip is oscillating with a larger amplitude and is mostly far from the surface but is touching the surface at the end maximum amplitude of the surface. The tipo thus feels both attractive and repulsive forces.

b)

- In constant height imaging the height of the sample is kept constant and changes in surface properties are measured as deflections of the cantilever.
- In constant force imaging the feebacksystem is moving the sample such that the deflection of the cantilever is kept at a constant level.

c)



d) There are several ways to calibrate the Optical tweezers trap. A popular method is to utilize the power spectrum of the brownian motion of the particle from which the calibration factors relating voltage to movement, and movement to force can be extracted. See compendium extract for details.

Oppgave 4

a)

- Secondary electrons are electrons from the atoms in the samples which are ionized and ejected by the high energy electrons from the excitation beam.
- Backscatterd electrons are electrons which are scattered at high angles due to interaction with nuclei in the sample.

SE are typically measured in SEM and sometimes BSE are also measured with special detectors. None of these are directly measured in TEM but both can contribute as a reduction in signal intensity as electrons are scattered away from the detector.

b) The electromagnetic lenses sends the electrons in a spiral. The magnification is changed by changing the magnetic field in the lenses which will change how large a fraction of a the circular movement the electron will complete. Thus rotating the image.

c)

- Fixation – stop biological process
- Post fixation – some staining and further stabilization
- Dehydration – prepare for embedding
- Embedd in resin
- Investigate semi-thin secitons
- Cut in ultrathin slices
- Mount on grid
- Image in TEM
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