BIOPHOTONICS - EXAMINATION

February 20, 2013

3 hours
Documents not allowed, computer not allowed, calculator allowed.

Part I by Arnaud Dubois (25%)
Part II by Nathalie Westbrook (25%)
Part III by Cedric Bouzigues (50%)

Number of pages: 6

PART I

1. In which region of optical wavelengths is the absorption of light by biological tissue minimal?

2. What is Rayleigh scattering?

3. What is the significance and typical value of these three parameters used to characterize light scattering in tissues:
    1. The scattering mean free path *l* ?
    2. The anisotropy parameter *g* ?
    3. The transport mean free path *l** ?

4. What is the best theoretical spatial resolution that can be achieved with a classical optical imaging technique?

5. Is it possible to reach this maximal theoretical spatial resolution at a depth of 1 cm in biological tissues? Why?

6. Explain briefly the principle of Optical Coherence Tomography (OCT).

7. What are the most significant application domains of OCT?

8. Which parameters determine the transverse resolution and the axial resolution in OCT?

PART II: Optical tweezers

The following figure represents an optical tweezers setup extracted from the article « A comparative study of living cell micromechanical properties by oscillatory optical tweezers », by Wei et al (Optics Express 2008).

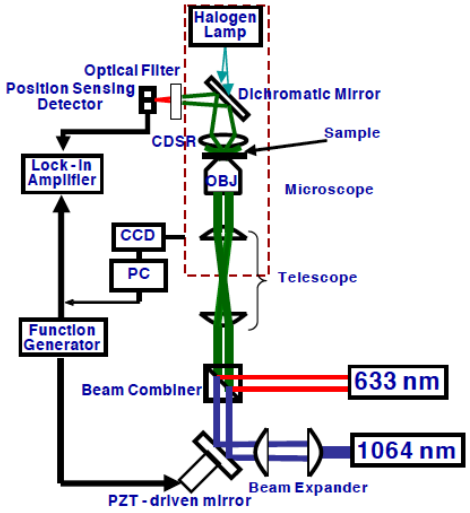


Fig. 2. A schematic diagram of the experimental setup. The area enclosed by the dashed lines represents an inverted optical microscope.

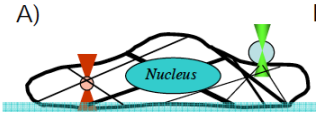
Extracted from the text of the article :

« An infrared laser (Nd:YVO4 1064nm diode-pumped solid-state cw laser, Spectra Physics) was steered by a mirror mounted on a PZT piezo-electric 2-axis (orthogonal) tip/tilt platform (S-330, Physik Instrumente) which was connected to a function generator (a built-in function of the lock-in amplifier, Stanford Research SR-830).The 106 nm and a 633 nm HeNe laser beams (Uniphase, 5mW) joined with a beam combiner cube into a collinear configuration were launched into the right-side-port of an inverted microscope (Olympus IX-81) via a telescope lens pair, and directed into the direction of microscope optical axis via a dichromatic mirror (not shown). An oil immersion objective lens OBJ (NA=1.3, 100X, Olympus) was used to focus both

Answer the following questions relative to this setup :

1) Where on this setup is the optical trap ?
2) Which of the two lasers must be used for the trap ? Justify your answer. What must be the role of the other laser ?
3) Explain which characteristic of the microscope objective is essential for the trap to work and why.
4) What is the role of the position sensing detector ? Where must it be located with respect to the condenser (CDSR) ?
5) The PZT-driven mirror is used to make the trap position oscillate. As you can see on the drawing, there is a 1:1 telescope (made of 2 lenses with the same focal length) on the beam path. What is the purpose of this telescope ?
6) The authors compare the effect of oscillatory forces applied inside the cell and on the membrane (see figure below). In the first case they trap intracellular organelles and in the second case they use silica beads (1,5µm diameter) attached via the integrins to the outside of the membrane. They measure the average diameter of the organelles (typ. 2µm) and estimate their index of refraction as well as that of the intracellular medium. They conclude that the trap stiffness is 61% lower when trapping the organelles compared to the silica beads.
   What must be the origin of this lower stiffness ?

A)



PART III: 3D-PALM

We are interested in this part in the development of methods for 3-dimensional imaging with subdiffraction resolution in all the three space directions based on PhotoActivated Light Microscopy (PALM) techniques. The four sections are mostly independent and can be treated separately.

Beyond Rayleigh criterion.

a) Recall what Rayleigh criterion is and note σ the resolution limit.
b) Explain what the Point Spread Function (PSF) of an optical system is.
c) We assume that acquisition is made by a photon-counting detector and is only limited by the shot noise. Give a probabilistic interpretation of the PSF.
d) We assume that the PSF is characterized by a standard deviation σ and note $\boldsymbol{x_i}$ the position at which the ith of a total number of *N* photons emitted by a single point source is detected. Demonstrate that $\overline{\boldsymbol{X}} = \frac{1}{N}\sum_{i=1}^{i=N} \boldsymbol{x_i}$ is an indicator of the actual position of the source $\boldsymbol{x_0}$ and give the standard deviation of $\overline{\boldsymbol{X}}$. Deduce the localization accuracy of a single point source and compare it to the Rayleigh criterion.

2. PALM

a) Recall the principles and properties of PALM, notably the required illumination sequence, the maximal density of visible fluorophores on each image and the accessible resolution.
b) In order to maximize acquisition speed, is it interesting to increase the power of (i) photoactivation beam, (ii) the imaging beam or (iii) both of them ?

3. 3D-PALM

a) By considering that the microscope PSF is given by a Gaussian, with width $\sigma(z) = \sigma_0\sqrt{1 + (\frac{z\lambda}{{\sigma_0}^2})}$ is it possible by using the PALM method described in the previous question to determine the distance or the absolute position of imaged fluorophores from the focal plane?
b) How would you like to shape the microscope PSF to obtain axial subdiffraction localization ?
c) Pavani et al. (PNAS 2009) propose the use of a double helix PSF (DH-PSF). The image of a single point source can thus be approximated by two equidistant spots (Figure 3). Explain how PALM experiments performed with this system can provide lateral and axial superresolution.
d) In these experiments, what is the contribution of out-of-focus (|z|>1 µm) fluorophores during photoactication and imaging/photobleaching steps? To what extent does it limit the performance of this method?

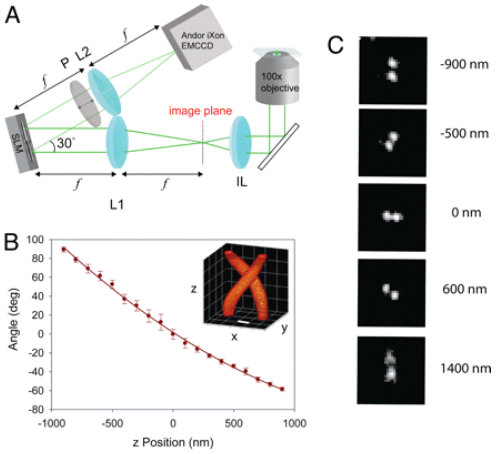


Figure 3. *A) Scheme of the experimental set-up. The DH-PSF is generated by a Spatial Light Modulator (SLM) placed in the Fourier plane. B) Angle between the two lobes of the helix in function of the defocusing. C) Images of a fluorescent bead at different z positions.*

4. Multi-layer PALM

a) One method to perform multi-layer PALM experiments would be to restrain photoactivation to a layer of a 3D-sample by using of two-photon or confocal photactivation for which activation is limited to a focal volume of typical radius ≈1 µm. What is the limitation of this approach to realize a stack of PALM images of a typically 15 µm x 15 µm x 15 µm sample?

b) Vaziri et al. (PNAS 2008) propose the use of temporal focusing: different spectral components of an ultrashort (≈10 fs) laser pulse are spatially dispersed by a grating and imaged on the sample (Figure 4). The temporal width of the pulse is minimal at the focal plane and two-photon excitation can thus only be elicited at this plane. This effect reduces the two-photon excitation in the z direction compared to standard spatial focusing. This results in the formation of a photoactivation volume with a 15 µm width in the x-y direction and a ≈2 µm depth in the z direction. Explain the interest of this activation pattern to perform multi-layer PALM and the advantage provided by temporal focusing compared to standard spatial focusing.

c) What are the lateral/axial resolutions in this experimental system? Does this technique enable 3D-superresolution?

d) Based on your analysis of the two considered PALM experiments (PALM with DH-PSF and temporal focusing PALM) propose a method to obtain 3D-superresolution.

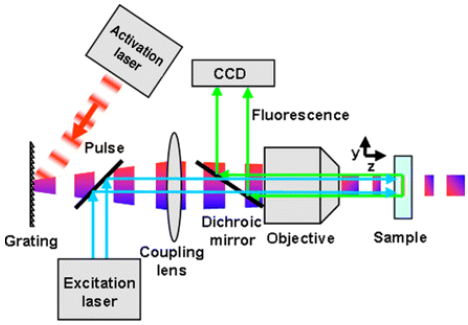


Figure 4. *Temporal focusing PALM experimental system. Ultrashort pulses from the activation laser are spatially dispersed by a grating and refocused on the sample through the objective.*

BIBLIOGRAPHY

*Wei et al. Opt.Exp **16**, 8594 (2008)*
*Pavani et al. Proc. Natl. Acad. Sci. **106**, 2995 (2009)*
*Vaziri et al. Proc. Natl. Acad. Sci. **105**, 20221 (2008)*

END