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Report:

In this report we describe a setup of an optical tweezer (OT), which is able to fix a single sample entity in a liquid filled capillary and to perform a scanning micro-diffraction experiment on it. As a demonstration experiment - an example for a colloidal system – a small cluster of multilamellar phospholipid vesicles was fixed in the X-ray beam and the diffraction of micron sized vesicle cluster is measured with a micrometer beam.

The sketch of the setup applied for the micro-diffraction X-ray experiments with optically trapped microparticles in front of the focused X-ray beam is shown in Fig. 1a. We use a single mode CW fiber laser that generates a collimated linear polarized beam at 1064 nm. The beam is directed to the active area of the spatial light modulator (SLM) to control the phase of the wave front by means of a pre-calculated diffractive optical element (DOE) which is computer addressed to the SLM. The DOE allows to control the convergence and the shape of the laser beam which is then reflected by the dichroic mirror to the microscope objective and focused inside a thin walled glass capillary of 100 μm diameter. Particles immersed in water are fluxed inside the capillary by a syringe pump and trapped at the location of the focused laser beam. The working space is imaged by a second microscope objective (40x) on a CCD. The focused X-ray beam (1 μm) is directed orthogonal to the trapped particle in the capillary.
Multilamellar vesicle dispersions of Palmitoyl-Oleoyl-Phosphatidylethanolamine (POPE) (20 w%) and water were prepared according to standard procedures. Prior to the experiment, the stock solution was diluted with a 0.1 mM $CaCl_2$ solution to obtain the final used concentration of 1 w%. This solution was transferred by the remote controlled syringe pump into the capillary held at room temperature. Using the optical tweezers the liposome vesicles were optical sorted until a cluster size of 10 μm had been reached and fixed afterwards in the X-ray beam. A typical diffraction pattern of the 10 μm large POPE cluster taken with an exposure time of 5 s is shown in Fig. 1b.

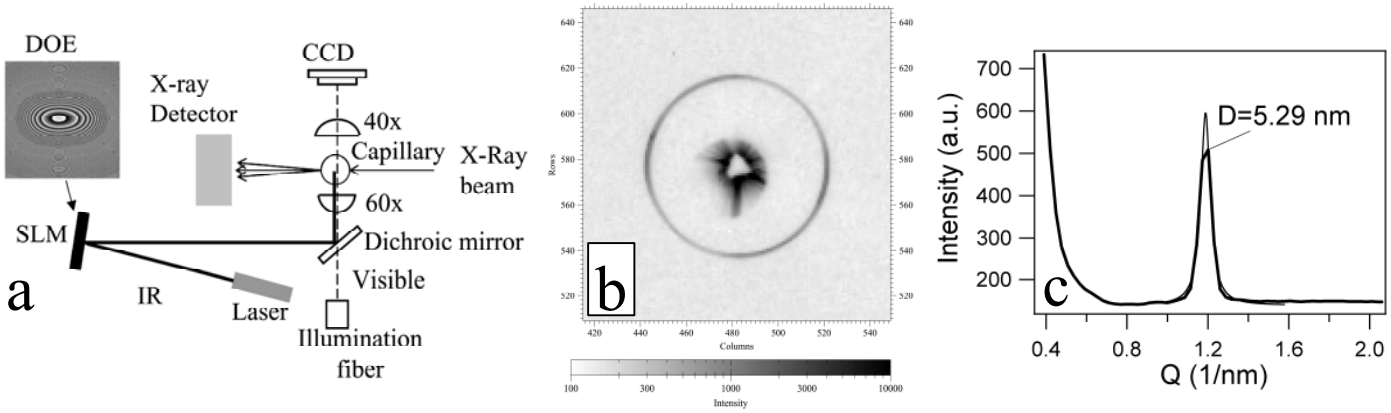

FIGURE 1. (a) Optical setup for trapping and aligning of the microparticles in front of the micro focused X-ray beam. The inlet is an example of diffractive optical element (DOE) displayed on the spatial light modulator (SLM). (b) 2D Diffraction pattern and (c) plot of the azimuthally integrated intensity of the trapped 10 x 10 x 10 μm^3 large cluster of POPE liposomes.

The used exposure time is close to the limit of the radiation damage as tested on a small cluster fixed to the capillary wall. But only single exposures are taken in one position and consequently the radiation damage can be neglected for the used experimental conditions. The measured diffraction ring of the unoriented liquid crystalline phase L_α is demonstrated in Fig. 1b. From the azimuthally integrated 2D diffraction pattern (Fig. 1c) the d-spacing was determined to be 5.29 nm by fitting a Lorentzian onto the 1st order diffraction peak. The d-spacing corresponds well to published literature values[1]. The integrated intensity under the peak is a direct measure of the liposome concentration seen by the X-ray beam.
For demonstrating the capabilities of the OT and to measure the size of the cluster, a scanning diffraction experiment was performed with a step size of 2.5 x 5 µm. Fig. 2 shows the obtained results. The first two figures show the microscope images of the intense IR laser beam used for trapping (Fig. 2a) and the image taken immediately after turning off the trapping laser to visualize the size of the trapped laser (Fig. 2b). respectively. A cluster size of 10 x 10 μm^2 has been deduced in the horizontal plane. Additionally some small clusters are visible which are neither in the focal plane nor in the plane of the X-ray beam. Overmore the upper half of the images, the meniscus of the liquid inside the capillary is seen. The result of the scanning diffraction experiment is given in Fig 2c, which is performed in the vertical plane. Here the integrated intensity under the 1st order reflections is given as a function of the scanning position. The high intensity regime (dark) corresponds to the POPE cluster, of which the size has been determined with about 10 x 10 µm in vertical plane. The agreement with the cluster dimension measured with microscopy is good.

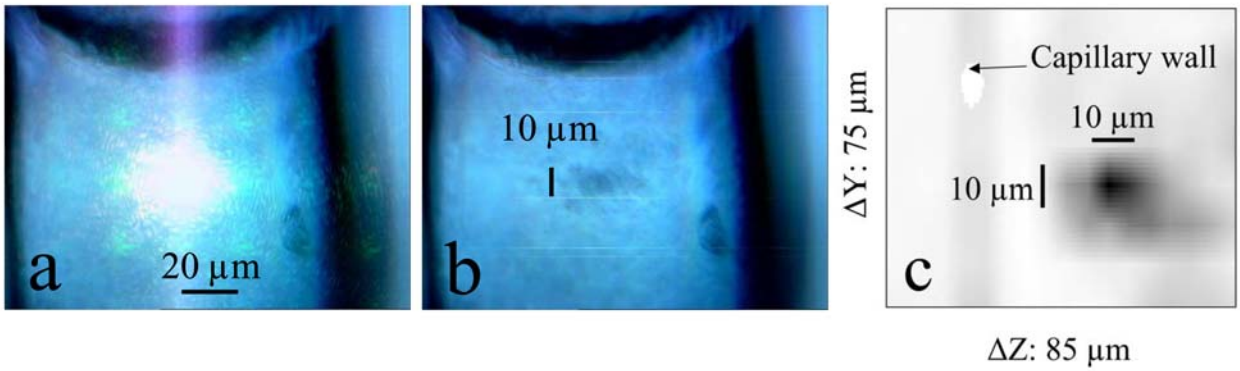

FIGURE 2 (a) Microscope snapshots of the trapping laser, (b) of the trapped cluster of POPE liposome immediately after shutdown of the trapping laser and (c) surface plot of the integrated intensity of the POPE versus Y-Z scanning position Y-Z with a scanning increment of 2.5 x 5 µm.

References

1. **M. Rappolt, A. Hickel, F. Bringezu, K. Lohner, *Biophys.J.* 84, 3111-3122 (2003).**