STUDY OF PARTICLE – SURFACE INTERACTION USING HIGH-PRECISION FLUORESCENT PARTICLE TRACKING

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Single particle tracking has become an invaluable tool in many scientific fields ranging from cell biology and single molecule research to soft matter physics. Tracking of fluorescent particles plays a central role because its specificity allows one to study processes in complex environments, e.g. living cells. Moreover, using particles with different spectral signatures, molecular scale distances between objects can be precisely measured.

Recently, we presented a 3-D fluorescent particle tracking technique which is based on the use of a standard wide-field epifluorescence arrangement in an off-focus imaging mode [1]. The off-focus images of a point-like object have the form of complex ring intensity patterns and the distance of the object from the imaging focal plane (i.e. the axial object position \( z \)) is precisely encoded by the intensity pattern (number and diameter of rings, relative intensity of rings - see Fig. 1). To extract actual value of \( z \), we used radius \( r_0 \) of the outermost ring, which scales linearly with \( z \). The lateral coordinates \( x, y \) were then determined from the position of the pattern center in the image. By modulating the position of an immobilized 216 nm bead, we confirmed the possibility of achieving sub-nanometer tracking precision along all three axes simultaneously.

![Figure 1: Off-focus images of a 216 nm fluorescent bead. \( r_0 \) – radius of the outermost ring.](image)

With the technique described above, we have studied binding of various functionalized nanoparticles to glass surface. We observed a major difference in the surface interaction for carboxylate- and sulphate-modified beads. Generally, carboxylate beads bind more loosely and tend to detach from the surface (see Fig. 2). This can be directly related to different chemical properties of the particle coating. Since many particles can be tracked simultaneously, average binding properties of studied surface - particle combination are readily accessible. The technique, however, is not limited to the study of nonspecific interaction. Upon coating the beads and surface with molecules of interest, interaction of specific binding partners (e.g., antibody - antigen) can be analyzed.

![Figure 2: Repeated attachment / detachment of a 200 nm carboxylate bead to / from glass surface.](image)