Nano-bio experimental mechanics at the optical limit

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Abstract. Caustic signatures in an optical microscope have been used to track nano-entities with diameters substantially below the wavelength of visible light in both simple and biological fluids. This optical technique allows the diffusion of nano-entities, as small as 3 nm in diameter, to be quantified and their interaction with synthetic and biological surfaces to be investigated. Examples include the sedimentation of gold nanoparticles in the presence of cells and the early stages of the formation of biofilms by bacteria. The former is relevant to investigations of nanoparticles as drug delivery vehicles and the latter to the design of anti-bacterial surfaces. In recent research, the interaction of bacteriophages with bacterium has been investigated.

Possible Sessions

4, 12 or 19.

Introduction

The method of caustics, or the shadow spot technique, has been known in experimental mechanics for more than a century; however, it has not been widely or frequently used. For a brief period in the 1990s, it was used as a non-contact method of measuring stress intensity factors for surface cracks in metals [1] and for evaluating the parameters governing contact between solid bodies [2]. In the last decade, it has been developed as a non-invasive, real-time tracking technique for nano-entities interacting in a biological environment. In an optical microscope, small particles will generate caustics several orders of magnitude larger than themselves when the illumination is close to coherent [3]. Near-coherence can be achieved in a standard optical microscope by setting it for Kohler illumination, introducing a narrow-band filter, closing the aperture of its condenser lens to its minimum and moving the microscope slightly out of focus. This configuration allows particles as small as 3 nm in diameter to be tracked in three-dimensions and the characteristics for their diffusion through a range of homogeneous fluids to be evaluated [4], which are important for understanding both drug delivery and toxicological mechanisms. The real-time, non-invasive nature of the technique has allowed the settling dynamics of nanoparticles in a biological medium [5] and the dynamics of bacteria during their early stage formation of a biofilm[6] to be elucidated.

Methods & Results

The three-dimensional optical signatures generated by nano-entities in a standard optical microscope enable a powerful non-invasive real-time technology for monitoring the motion and interactions of a wide range of bodies, including synthetic and organic nanoparticles as well as viruses and bacteria. The technique is simple to apply and requires no permanent modification to a standard microscope. In recent work on biological systems, a standard optical microscope (Axio Observer Z1m, Carl Zeiss DE) has been employed with its condenser aperture closed leaving an approximately 1 mm diameter “pin-hole” and with a narrow bandwidth filter (central wavelength: 550 nm with 45 nm bandwidth, Olympus JP). A stage-top incubation system (Incubator PM S1, Heating Insert P S1, Temperature and CO2 module S1, Carl Zeiss DE) was used so that experiments could be conducted at biologically relevant temperatures. The microscope was set up for Kohler illumination and a camera (Axiocam z1.m Carl Zeiss DE) was used to record the data, typically at 40 fps and with a x40 objective lens.

In recent research, the motion of bacteriophages has been observed. Bacteriophages are probably the most abundant life forms on the planet; however, they are typically a few tens of nanometres across and a few hundreds of nanometres long [7]. These dimensions are smaller than the wavelength of visible light, which means they are not visible in an optical microscope; however, they generate a caustic when viewed in a microscope set-up as described in the previous paragraph. Fig 1. shows the caustic generated by a single bacteriophage in an optical microscope and by a bacterium being attacked by bacteriophages. Bacteriophages, or phages infect and destroy bacteria, thus offering an alternative route to managing bacterial infections as antibiotics become less effective. High resolution imaging solutions, such as electron microscopes, involve electro-magnetic radiation that is harmful to life forms which makes the optical method of caustics an attractive option for studying the motion and interaction of nano-entities, such as phages.

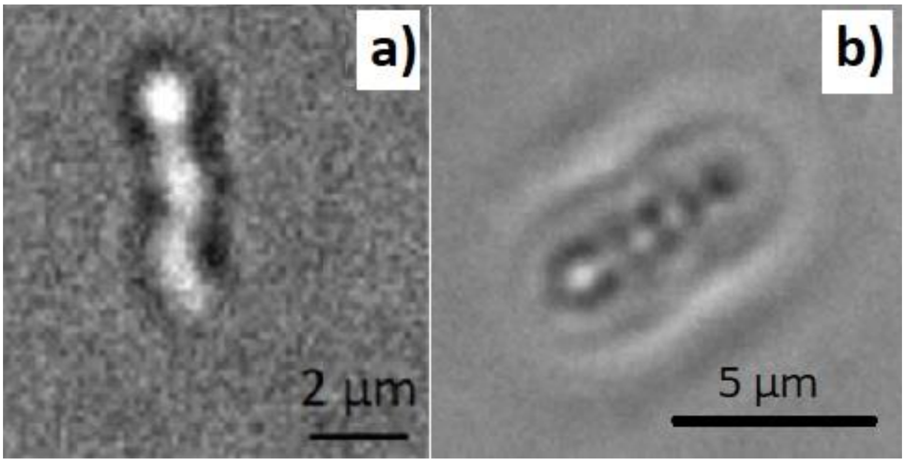

Fig. 1. Images of caustics generated in an optical microscope by (a) a bacteriophage and (b) a bacterium being attacked by bacteriophages.

Discussion & Conclusion

While the use of the optical method of caustics, sometimes referred to as the shadow spot technique, has almost disappeared in experimental fracture mechanics and experimental contact mechanics, it is emerging as powerful technique for monitoring the interaction of nano-entities in biology. The non-invasive, real-time features of the technique render it an attractive option for tracking the transport and interaction of nanoscale life forms and of synthetic nanoparticles immersed in biological media. Quantitative analysis of the diffusion of nano-entities in simple and complex fluids has been performed and provided new insights into dynamics at the nanoscale. Current research is focussed on understanding the transport of nano-entities through biological media and their interactions with synthetic and organic surfaces and with other life forms, including bacteria and human cells.

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