1 Motivation

Holographic optical tweezers (HOTs) have revolutionized the way we can perform experiments at the micron scale. HOTs use light focused by a high numerical aperture objective to trap, move and assemble a number of objects. Moreover, once calibrated, optical traps can be used to directly probe interactions and mechanical responses of those objects to mechanical stress. The applications are widespread in physics, chemistry and biology [1], [2]. On the other hand, diamond anvil cells (DACs) have provided optical access to high pressure fluids (up to hundreds of GPa) opening the way to many different physical quantitative measurements including optical absorption, reflectivity, Raman and Brillouin scattering, X-ray diffractometry, at low or high temperature conditions [3]. However DACs, as many other complex sample environments, only provide optical access within a small numerical aperture (NA), preventing the projection of stable optical traps within samples at extreme thermodynamic conditions. The possibility to manipulate objects and measure forces in high pressure conditions would open new experimental scenarios ranging from the physics of liquids to biology.

In the course of the PhD we intend to build the first experimental setup for holographic micromanipulation and visualization inside diamond anvil cells. With this setup we will be able to stably 3D-trap micro-particles by calibrated HOTs and 3D-track them in real-time through digital holographic microscopy (DHM), inside a high pressure cell. These trapped micro-particles will be used as local probes for micro-rheological measurements on simple and complex fluids and as micro force devices for the investigation of mechanical (membrane elasticity) and dynamical (motility) properties of self propelling unicellular organisms, under extreme pressure conditions.

2 Holographic Optical Tweezers (manipulation)

The electromagnetic radiation can exert forces on dielectric transparent materials. The origin of these forces can be easily understood in the framework of
Figure 1: HOT-DHM setup. L1, L2: beam expanding telescope; L3, L4: beam reducing telescope; DM: dichroic mirror for green light; in yellow bright field illumination; in red coherent illumination; L5 tube lens; F filter for green light; CMOS: digital camera.

geometrical optics taking into account the elementary processes of reflection and refraction of the rays which we can consider to constitute a light beam. Using a mW light beam we can exert forces of the order of pN which, despite being negligible in the macroscopic world, are adequate for manipulating in a precise and non invasive way matter at the mesoscopic scale (≈ µm). For example, a tightly focused laser-beam can stably trap a colloidal transparent dielectric sphere in the 3 spatial dimensions, in a point close to its focus. This “device”, commonly referred to as optical tweezer, was successfully demonstrated for the first time in 1986 by A. Ashkin [4], [5].

For our research we will need to create and move at wish several optical tweezers inside our samples in order to fulfill specific experimental needs. This will be made possible by the use of holographic techniques (HOTs) [6], [7], [8]. Through the use of a spatial light modulator (SLM) it is possible to dynamically sculpt the wavefront of a laser beam in a practically arbitrary way (with typical video resolution and refresh rate). This engineered wavefront is focused by a microscope objective into a desired light intensity pattern inside the sample volume (see Fig. 1).

It is then necessary to compute the shape to give to the wavefront by the SLM in order to obtain the desired light intensity inside the sample. This problem, in general, is not analytically solvable, but can be solved in real time (ms) using iterative algorithms running on GPUs [9].

3 High pressure: DAC and mirror trapping

We will use a diamond anvil cell (DAC) to generate the high pressures we will need for our research. DACs are essentially formed by two diamonds pushed
against each other by two metallic disks and separated by a thin (hundreds of μm) metallic gasket with a hole in the center to harbour the sample (see Fig. 2). These devices, can be rather small in size (2-3 cm), and allow to reach huge pressures (hundreds of GPa), with forces that can be applied by hand screwing. The DACs offer optical access to the sample pressured in between the diamonds, but, for geometrical reasons, they require the use of small numerical aperture objectives. This implies that axial trapping is not feasible using a single optical trap.

It has been recently demonstrated the possibility of trapping with low NA objectives using “mirror traps” [10]. The mirror trapping consists in projecting two optical traps along the beam propagation axis in such a way that the reflection of one of them, by some interface, comes to be overlying the other trap; if the two traps have the same intensity there is no net force along the beam propagation direction, whereas the radial forces add up constructively, giving raise to 3D stable trapping. We propose to use the mirror trapping technique to achieve stable 3D trapping inside diamond anvil cells.

In order to obtain traps of the same intensity it is necessary to “send more light” to the trap that is reflected, to counterbalance the loss of intensity due to the reflection. After a first unsuccessful attempt using bare water-diamond interface as the mirror trapping reflective surface, we are now coating the surface of the DAC’s top diamond to raise the reflection coefficient to the value of 0.8. Although easy feasible in principle, possible issues can come from: heating due to absorption, water chemical stability of metallic coatings, low transmittance of illumination light (coming from above, whereas the laser beam comes from below). The solutions we are presently testing are ≈ 40 nm Ag and ≈ 17 nm Al.

4 Digital Holographic Microscopy (visualization)

In the Digital holographic microscopy (DHM) technique a coherent light source is used to illuminate a sample and the intensity fringes arising from the interference of the light scattered by the sample and the reference beam are recorded on a digital camera. Using Lorenz-Mie scattering theory [11] it is possible to numerically compute the light intensity on a plane perpendicular to the optical axis given the specific optical composition of the sample. Inverting the problem, we can reconstruct the full 3D configuration of objects in the sample from
Figure 3: Mirror trapping scheme inside the DAC. Diamonds and sample chamber in white, metallic gasket in brown. The green laser beam comes from below and is reflected at the sample-top diamond interface. From left to right: first trap, second (reflected) trap, both traps.

Figure 4: DHM experimental hologram (left) and DHM radial histogram (right) for a synthetic polymeric micro-bubble. In the right plot the measured bubble properties values are listed. The refractive indeces are relative to water refractive index, 1.33; “Distance” is the separation between the bubble plane and the recorded hologram plane.

We have started using DHM for the study of synthetic, air filled, polymeric micro-bubbles at ambient pressure conditions [13]. We have been able to track as well as to measure geometrical and optical properties of hundreds of bubbles [14]. For a typical bubble we obtained the following values for internal radius, shell thickness, shell refractive index: (1.200 ± 0.025)µm, (0.500 ± 0.045)µm, (1.401 ± 0.011), corrisponding to relative precisions of, respectively, 2%, 10% and 0.8%. Our next goal is to implement DHM inside the DAC for full 3D tracking and characterization of synthetic dielectric objects under high pressure conditions. DHM will also be an important tool for tracking 3D trajectories of motile cells at high pressure.
5 Project outline

Here I list the steps of my research project, in chronological order. The first three steps have been already tackled, even though no stable 3D trapping inside the DAC has been obtained yet. The last two are the applications we intend to focus on in the rest of the PhD time span.

- **Metallic coating of the DAC top diamond to achieve 3D stable trapping through the mirror trapping technique inside the DAC.** In this way we bring the reflectivity of the interface sample-top diamond to the optimal value of 0.8. The coatings we are examining are: 17 nm Al and 40 nm Ag, both on a 3 nm Ti substrate.

- **Development of 2D particle tracking techniques with bright field illumination.** We are developing Python scripts for automatically analyse the bright field camera images and track particles in 2D inside the DAC.

- **Development of Digital Holographic Microscopy (DHM) techniques for full 3D particle tracking.** We are using coherent laser illumination and holographic image analysis techniques for real time 3D particle tracking (≈ 100 KHz).

- **First micro-rheological studies in extreme pressure conditions.** Refractive index and rheological properties will be measured, for different pressures up to some GPa, for water and complex fluids, like gels and colloidal suspensions [15], [16].

- **Biomechanics and cellular motility at high pressures.** We will directly investigate how pressure affects the elastic properties of cellular membranes at high pressure [17]. We will study the viability of bacteria by monitoring their motility at increasing pressures [18].

References


