The amplitude and the phase or: Measuring directional and random motion with optical coherence tomography
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Photonic force phase imaging

Abstract

We report on a method to measure the height profile of non-scattering soft (bio-
logical) samples in liquid environments using a combination of optical tweezers and
optical coherence tomography. An optical tweezer is used to trapped a 3 μm in di-
ameter polystyrene sphere that is used as a probe to scan the surface of a sample. A
phase-sensitive optical coherence tomography system is used to detect the backscat-
tered radiation from the probe. In this way, the measured displacement of the probe
is used to measure the height profile of the sample with nanometer resolution. The
method is validated by measuring the height profile of a silicone mold of a precision
diffraction grating. As an application example for profilometry of soft non-scattering
samples we measured the height profile of a smooth muscle mouse aorta fixed cell.

This chapter contains unpublished data.
6.1 Introduction

The established technique to measure the height profile of samples at sub-μm resolution is the Atomic Force Microscope (AFM) \[94\]. AFM can achieve resolutions in the order of 1 Å using cantilever spring constants in the order of 0.1…1 N/m. When measuring soft biological material using a sharp AFM tip in combination with relatively high spring constants can result in damage of the sample. Moreover, the application of AFM in liquid environments is not straightforward. A solution to this problem is presented by the Photonic Force Microscope (PFM) \[95\]. In PFM, an optical tweezer is used to trap a dielectric particle and probe the surface of a sample. Typically, the forward scattered light of the trapped particle is used to measure the position of the particle inside the trapping volume. In the case that the trapped particle interacts with a sample, the trapped particle will be deflected and the variations in the scattered intensity can be used to measure the height profile of the sample. This principle is similar to AFM, however the spring constants involved are two to three orders of magnitude smaller than those of the AFM cantilever \[96\]. Furthermore, the PFM probe is easier to characterize when compared to the AFM cantilever.

Typically, the forward scattered and unscattered radiation by the probe particle trapped in an optical tweezer is detected by using a quadrature photodiode \[96\]. The integrated intensity over each detector quadrant can be related to the three-dimensional position of the particle. However, the intensity detection dynamic range is typically limits the position detection to 400 nm in the propagation direction of the trapped beam and 200 nm in the transverse direction for a 1064 nm trapping laser \[96\].

Here, we report on a method based on optical coherence tomography (OCT) to measure the displacement in the propagation direction of the trapping beam of an optically trapped dielectric particle. We use the high sensitivity of the phase of the OCT signal to quantify the height profile of a non-scattering silicone diffraction grating and a fixed smooth muscle mouse aorta cell.

6.2 Materials and methods

The experiments are performed with a fixed stage microscope (BX51WI, Olympus) and a home built fiber-based swept-source OCT system. A schematic of the experimental set-up is shown in Fig. 6.1. The optical tweezer set-up is built around the microscope and is based on a 1070 nm laser (IPG Photonics), a beam expander (BE02-05C, Thorlabs), an acousto-optic deflector (DTD-274HD6M, IntraAction), and a water immersion microscope objective (UPlanApo 60x/1.20w, Olympus). The probes used in the experiment are polystyrene spheres with a diameter of 3 μm (Thermo Scientific). The OCT system operates at a center wavelength of 1312 nm with a bandwidth of 92 nm and a sweep frequency of 50 kHz (Axsun Technologies). The average output power is 20.9 mW and the duty cycle is 59.4%. Data is sampled (ATS9350, AlazarTech) with an interferometrically derived external clock signal at equidistant wavenumber intervals. To ensure phase stability each sweep is triggered by the signal of a fiber Bragg grating centered at 1266 nm (OE Land) \[42\]. The in-
6.2. Materials and methods

![Figure 6.1: Schematic of the OCT and optical tweezer set-up. PD: photodetector, FBG: fiber Bragg grating, PC: polarization controllers, C: collimating lens, F: focusing lens, M: mirror, AOD: acusto-optical deflector, IRF: infra-red filter, IRM: infra-red mirror, and MO: microscope objective.](image)

Interferometric signal is detected with a 150 MHz balanced photodetector (PDB450C, Thorlabs) and a 80 MHz low-pass filter (VLF-80+, Mini-Circuits). The trigger signal is detected with a 125 MHz photodetector (1811, New Focus). The optics of the sample and reference arms are composed of a collimating lens (PAF-X-18-C, Thorlabs) and an achromatic doublet focusing lens (AC254-040-C, Thorlabs) with a numerical aperture of 0.04. The power ratio of the sample and reference arms is 90/10. We measured $w_x = 10.8 \pm 0.2 \, \mu m$ and $w_z = 8.1 \pm 0.3 \, \mu m$ in air with a mirror reflector. The refractive index of the medium is $n = 1.32$. To isolate the high-power beam from the optical tweezer from OCT-set-up a long-pass filter was used (67299, Edmund Optics).

A silicone grating is fabricated by making a negative mold of a precision diffraction grating (53006BK01-942R, Richardson Gratings) using the recipe described by de Bruin et al. [90]. The diffraction grating has 14.3 grooves/mm and a blaze angles of $3.33^\circ$.

The minimum measurable longitudinal displacement is determined by the phase stability of the OCT system [42]. Here, we quantify the phase stability as the standard deviation of 1000 time adjacent acquisitions of the complex-valued OCT signal generated by a trapped bead. The phase stability $\delta \phi$ is related to the longitudinal displacement $\delta z$ by [42]:

$$\delta z = \frac{\lambda}{4\pi n} \delta \phi ,$$

with $\lambda$ the center wavelength of the source and $n$ the refractive index of the medium.
6.3 Results and discussion

Figure 6.2 shows a histogram of the measured phase difference from the OCT signal of a single trapped sphere. The calculated standard deviation of the phase values is 225 mrad. Using Eq. 6.1 results in a minimum measurable longitudinal displacement of 17.8 nm.

As a validation experiment we measured the profile of a silicone grating. Figure 6.3(a) shows a schematic of the experiment. The sphere is trapped on the surface of the silicone grating. The transversal displacement was generated by moving the silicone grating with a mechanical stage. As the silicone grating moves under the trapped sphere, the longitudinal movement of the sphere following the shape of the grating is measured by the OCT signal. Fig. 6.3(b) shows the measured longitudinal displacement of the trapped bead. The measured height of the grating is 3.5 μm. When compared to the height of the original grating there is a difference of 0.55 μm. This is attributed to imperfections in the production of the silicone mold. A further issue that should be taken into account is the finite size of the probe sphere when measuring steep walls. This can be overcome by using, e.g., triangular probes [95]. Figures 6.3(c) and (d) show microscopy images of the trapped sphere and the silicone grating corresponding to the positions shown by the arrows in Fig. 6.3(b). The difference in the grating height can also be seen by the sphere appearing out-of-focus in Fig. 6.3(d).

As an application example for profilometry of soft non-scattering samples we measured the height profile of a smooth muscle mouse aorta fixed cell. The experimental arrangement is analogous to the previous. The measured cell height profile is shown in Fig. 6.4. The inset shows a microscopy image of the cell and the probe sphere resting on its surface.
Figure 6.3: (a) Schematic of the experiment. The probe sphere is trapped from the top and is imaged from the bottom. The silicone gratings is move transversely by an electro-mechanic stage. (b) Measured longitudinal displacement of the trapped sphere. The arrows correspond approximately to the positions shown in (c) and (d).

6.4 Conclusion

We have combined optical tweezers and optical coherence tomography to measure the height profile of soft and non-scattering samples. Our experimental results show that by using the phase of the optical coherence tomography signal we are able to resolve sub-μm features of (biological samples). We anticipate that the presented method opens up new opportunities to measure profiles of non-scattering and soft samples in liquid environments.
Figure 6.4: Measurement of the longitudinal displacement of the probe sphere as a fixated cell is transversely moved by the stage. The inset shows an image of the cell and the probe sphere.