Slow deformation measurement of biological samples with 3D interferometric microscope

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Abstract—The 3D interference microscope is used to measure the mechanical and structural properties of biological samples. In this paper the pulsation of the eye of the zebrafish embryo and the motion of the neuron is measured. The measurement is often performed on a microsphere placed on a biological sample. The microsphere reflects a strong signal, and by applying a magnetic field the sphere can induce a controlled and varying pressure on the object. The deformation of the object and the motion of the sphere provide information about different mechanical properties of the biological sample. This paper specifically concentrates on the measurement of nanometric deformation at frequencies below 10Hz.

3D interferometric microscopes are commonly used to measure surface topography and shape with lateral resolution down to a fraction of a micrometer and vertical resolution down to a fraction of a nanometer [1]. The measurements are highly precise, fast and non-contact. These 3D microscopes are often used in production for quality control of precision machining, high brightness light emitting diodes (LEDs), solar, ophthalmic, semiconductor, medical devices as well as in academic research.

Measurement is based on collected interference fringes as scanned through focus with a specialized interferometric objective of the microscope. Typically, the samples measured by interferometric techniques are static or undergo harmonic motion at high frequencies on the order of tens to hundreds or thousands of megahertz like micro-electro-mechanical systems (MEMS) [2]. The motion of the sample creates additional shift and fringe deformation, which may lead to the total washout of fringes. Thus, for harmonic motion above 10Hz up to 2GHz stroboscopic illumination is synchronized with the motion of the sample in order to “freeze” the fringes, and the shape of the sample is measured multiple times at different phases of the harmonic motion providing information about the object’s motion and deformation over time.

During the last few years these interferometric systems have found more and more applications in biotechnology research. The challenge in measuring biological samples is different than of engineering samples like MEMS devices. The reflectivity of the biological sample is low, the motion’s amplitude is very small and often nonharmonic, the frequencies are on the order of a few Hz and the sample is often immersed in liquid. We will focus on these measurements in this paper.

Interferometric measurements have also started being used to study mechanical influences on cell and tissue growth, emerging areas of research in biology and biomedicine. It is now understood that mechanical cues are critical to structural development of tissues and cells during embryogenesis or cancer metastasis, and these cues also play a large role in processes in mature organisms where controlled tissue growth is required, such as wound healing.

The measurement of mechanical properties requires a force application at the point (often a varying force at low frequencies); measurement of the deformation occurs at this point or over the entire surface. In interferometry magnetic beads are placed on a culture of cells or tissue for force application, and the motion of the bead as well as the entire cell or tissue can then be observed at the same time [3]. The same beads can be placed on low reflectivity biological samples to measure sample motion.

Other methods are used to measure the motion or mechanical properties of cells and small animal samples, for example [4], an AFM (atomic force microscope), NSOM (near-field scanning optical microscopy), microrheology and other point by point measurement methods. The major drawback of these methods is that they are not fast enough to capture the dynamics over the surface area. Thus, an interferometric method is well-suited for examining biological measurement [3].
The measurement of a living animal and nerve cell

For measurements our colleagues from UCLA used Veeco’s (now Bruker) 3D interferometric microscope with a green LED and a 20X Michelson objective specially adapted to measure samples in liquid [3]. In this paper we will describe measurements of self-induced natural deformations of biological samples: small animal and nerve cell or neuron.

In an experiment with a small animal the behavior of the developing eye of a living zebrafish embryo (Danio rerio) was measured. While cultured cells provide a wealth of information, understanding higher animals is important since they reproduce human etiology better than the cultured cells.

In the first measurement a microsphere was placed on the eye of a three day old zebrafish embryo and the motion of this sphere was measured indicating the motion in the eye.

The microsphere, and thus the underlying eye structure, was moving sinuoidally up and down with an amplitude of about 200nm peak to valley at a frequency of 2.25Hz. This pulsation of the eye is related to the intraocular pressure pulsation associated with a cardiovascular flow. The intraocular pressure in humans is much larger and can be measured with other methods.

The second experiment was done on a five day old zebrafish embryo (Fig. 1). The microsphere was placed again on the eye; however, no microsphere was needed since by then the embryo had developed patches of the reflective pigment in the iris area of the eye and a strong signal was obtained. These silver and gold reflective inclusions are known as irridosomes and pteranosomes. Thanks to these multiple reflective inclusions a map of iris pulsation deformation could be created showing the nonuniformity of deformation amplitude, possibly indicating different tissue types developing within the eye. More details on these measurements and other measurements with pressure applied by the microsphere in order to determine Young’s modulus and other mechanical properties of the eye and their importance can be found in Ref. [5].

A similar experiment was done to measure the motion of a neuron, an electrically excitable cell that processes and transmits information by electrical signaling. The measured motion indicates the neuron’s electrical firing while the electrode was touching the neuron. The motion was observed by looking at the interference irradiance on the 10 micron sphere placed on the neuron. The nonharmonic motion at the nanometer level was detected using interferometric techniques. Figure 2 shows the observed region on the sphere for motion detection and Figure 3 shows the deformation as measured across the sphere over 20 pixels during 800ms period.

Fig. 1. Interferometric measurement of the eye of a living zebrafish embryo with a visible microsphere in a lower left corner of the eye. Patches of the reflective pigment in the iris area of the eye are visible.

The timescale of the vibrations of a sphere placed on a neuron. Vibrations are nonharmonic on nanometer order in the amplitude.
Analysis of fringes for small deformation

The challenge of these measurements was to determine the slow harmonic and nonharmonic motion on the order of only a few Hz and amplitude of only a few to a few tens of nanometers. Even with a measurement repeated every few hundred milliseconds, a common 90 degree phase shifting interferometry [6] measurement based on a set of a few frames collected with 60 frames per second camera is not fast enough to capture this motion. Rather, a measurement repeated every few tens of a millisecond over a period of about a second is needed. Phase shifting interferometry (PSI) can still be useful if applied a bit differently. The measurements requires one long scan that collects about 200-300 frames and applies the PSI algorithm to a set of frames starting with every new frame collected. This results in almost the same number of measurements as collected frames collected every 16ms. This method can be called a temporal carrier phase shift method since the carrier is created in time [7].

Even this slow motion of the sample creates additional phase shift and some fringe washout, but its effect on the measured motion is minimal. From simulations and experiments we conclude that with a 60fps camera and am illumination wavelength around 532nm and a 90 degree phase shift algorithm, this method can detect motions of the frequencies up to 10Hz. Different frequencies will have different limits of properly detected amplitude of vibrations; 1Hz amplitudes up to 450nm can be detected, while for 3Hz only around 150nm and for 9Hz 50nm. A camera with twice as many frames per second speed will allow for the measurement of harmonic motions that are twice as fast but not for higher amplitudes.

Figure 4 shows the induced harmonic motion of a mirror calculated at a single point at 7Hz with three different amplitudes. In addition to the harmonic motion, the present lower frequency amplitude is coming from the scanner’s nonlinearity. It may be important to understand performance of the scanner used, and if the motion of the scanner is repeatable, it can be characterized and subtracted [7].

The time over which the motion can be continuously monitored is limited by the axial spread of good contrast fringes. For small amplitudes up to 1/8 of the wavelength (60-70nm for visible wavelengths) a change of irradiance of the fringe over its linear range can be calibrated and scaled to the object’s motion. Such a method does not require a scanning mechanism and small motion can be observed over a long period of time.

In conclusions, the 3D interference microscope broadens its application to include the measurement of biological samples. Due to its high precision it starts becoming not just a qualitative but a quantitative method to measure a wide range of biological samples and a variety of mechanical and other parameters, which can be later tied to disease development, reaction to drug treatment or understanding cell or small animal basic functioning.

References