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Contrast-phase Imaging of Fixed-Cells through Micro-Cavity Scanning Microscopy

Abstract: Contrast phase imaging at infrared wavelengths is achieved through an extrinsic Fabry-Perot cavity in optical fiber. The micro-cavity is realized by approaching a cleaved fiber to a distance of about few tens of microns from the surface under test. The probe is a single mode fiber and is fed by a low-coherence source. The information is extracted from the reflected spectrum, that starts to be modulated by the interference when the fiber begins to interact with the sample. The measurement of the reflected optical intensity provides a map of the sample reflectivity, whereas from the analysis of the spectrum in the time/spatial domain, it is possible to extract topography and refractive index variations. This information is entangled in the contrast phase image obtained. In this work we review the system proposed in [19] in order to extract topography and local surface permittivity of biological samples. The system displays tridimensional images with a transverse resolution that is not limited by the numerical aperture NA of the scanning probe (as suggested by the Rayleigh limit), but it is related to the transverse field behavior of the electromagnetic field inside the micro-cavity. Differently, the source bandwidth, demodulation algorithm and optical spectrum analyzer resolution affect the resolution in the normal direction.

Keywords: scanning microscopy, micro-cavity, low-coherence interferometry

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Due to their high sensitivity, simple structure and immunity to electromagnetic interference Fabry-Perot optical cavities have always been applied in the realization of sensors [1–7]. Recently, optical micro-cavities were exploited to measure refractive index of optical glasses [8], to realize wide-range displacement sensors with sub-nanometer resolution [9], and in addition, due to their integrability, in the realization of miniaturized multi-cavity biosensors to study self-assembled thin-film layers [10]. In scanning probe microscopy, they were used to monitor the displacement of cantilevers and this has lead to an improvement in the electromagnetic analysis of the cavity behavior in presence of multi reflections, angular misalignments or highly reflective surfaces [11]. Analytical models have been reported, in recent works [12], that analyze the asymmetrical spectral response, the sensitivity, the fringe contrast and the dynamic range of low-coherence interferometers based on fiber Fabry-Perot. This work describes a scanning probe microscopy technique based on optical fiber micro-cavity, in which a cleaved single mode fiber is approached to a sample. The interaction between the sample and probe occurs at distance of tens of microns whereas a cleaved fiber is used as probe. The interfering signal directly comes from the micro-cavity in which the diffracted field experiences multiple reflections and interferences. The contrast phase images, reflectivity and topographic information can be investigated by means of the optical fiber itself without the use of lens [15]. Changes of the surface permittivity and cavity dimensions lead to a variation of the cavity response. The main electromagnetic parameters change periodically making the relation between them and the physical quantity to be measured, non-linear. Different algorithms can be used for the image reconstruction, borrowed from Spectral Optical Radars, Synthetic Aperture or Ground Penetrating Radars [13, 14, 16]. In proposed work, the spectrum acquired at each point is analyzed in the time domain, following a method similar to the one introduced in Scanning Microwave Microscopy and in a previous work [17, 18]. By recording the behavior of the peak of the cavity response in the time domain, we can acquire contrast phase information. The latter is a function of the effective refractive index of the sample, as well as of the

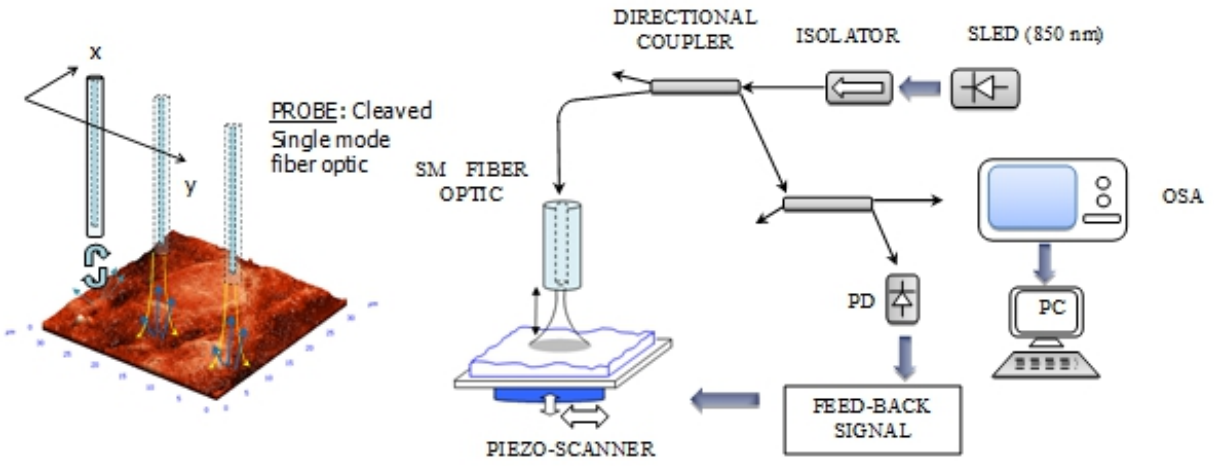

Fig. 1. Micro-Cavity Scanning Probe set-up.

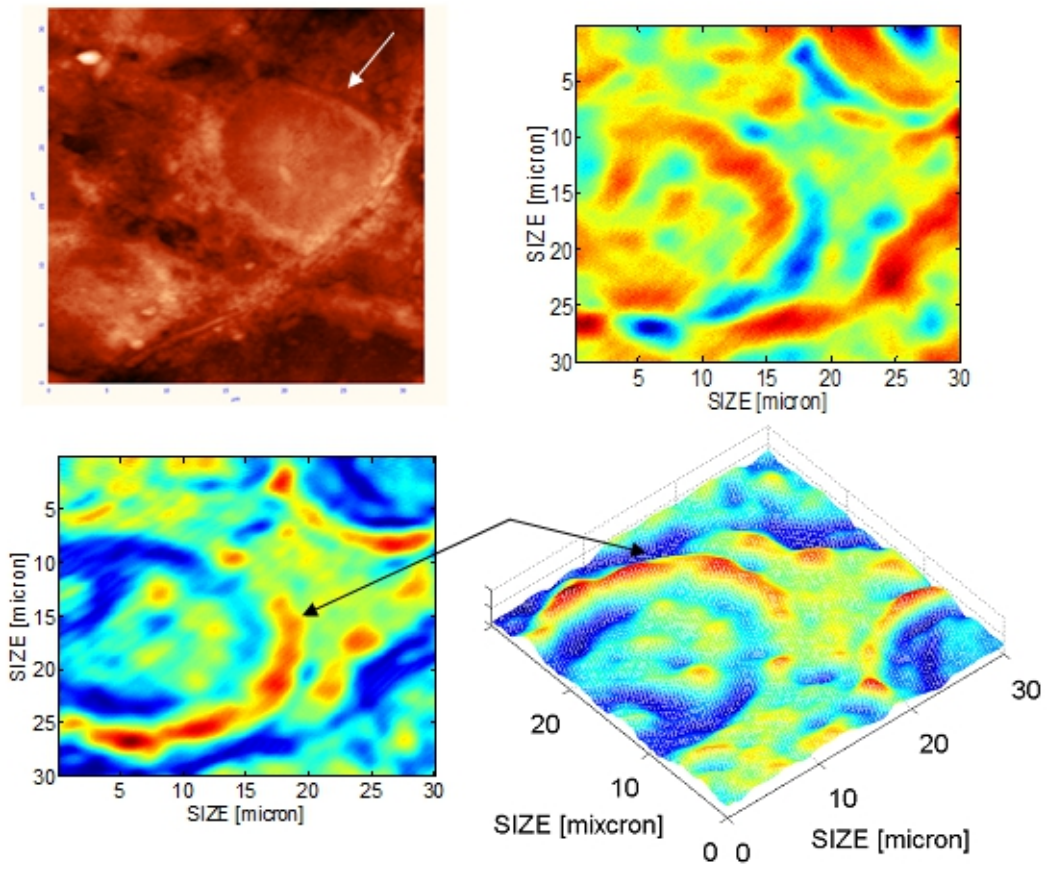

Fig. 2. C2C12 muscle cells fixed on glass substrate. (a) Example of cell morphology acquired by Atomic Force Microscope. (b) map of the infrared reflected intensity, (c,d) contrast phase images acquired at a fixed time instant (about 0.81 ps, the reference time is the fiber facet).

surface topography, according to the relation introduced in [19]. The frequency response of cavity, is affected by different effects: the diffraction of the guided wave at the fiber end, the presence of multiple reflections and mutual interferences, coupling with fiber guided mode, fiber optic and surface angular misalignment, and finally by the reflection characteristics of the sample. Each scattered wave has different propagation behaviors and can interfere with other waves inside the cavity. The complex reflection coefficient of the surface under investigation can also introduce losses and be described by a complex permittivity. Fig. 1 describes the set-up used to realize the micro-cavity scanning system. A SLED source at 850 nm (bandwidth FWHM = 40 nm) feeds a broad band directional coupler that directs provides separation between transmitted and reflected waves. An addition coupler shifts the wave into a port feeding a photo-detector and a spectrum analyzer. The latter is split by a further coupler that directs the signal to a photo-detector and an Optical Spectrum Analyzer (OSA) with a resolution of about 0.23 nm. The sample is placed on a piezo-scanner that controls the position with nanometric resolution in the plane and in the normal direction. The cleaved fiber (Numerical Aperture = 0.10 – 0.14, Mode Field Diameter = 5.6 μm) is mounted on a fixed head. The piezo approaches the sample to the fiber making a cavity with a dimension of tens of microns. Calibration measurements were performed over Atomic Force calibration gratings whose surfaces have good reflectivity at the working wavelength of 850 nm. The grating is realized in SiO_2 with a step height equal to 535 ± 4 nm, and a period of 3.00 ± 0.01 μm. The system has shown a resolution of about 1.12 μm in the transverse direction, whereas in the normal direction it is affected by different factors, such as the laser source bandwidth and fluctuations or the piezo-scanner drift, appearing during scans in the constant height mode. As reported in [19], before starting the scan and approaching the cleaved fiber to the sample, the reflected spectrum is measured at a distance at which there is no interaction between fiber facet and sample. This step allows to calibrate each spectrum acquired, in order to reduce the effects of spurious reflections, rising up in each junction of the fiber path, and the shape of broadband source, both affecting time domain response, mainly for small cavity dimensions [19]. The images acquired describes the changes of the peak-amplitude and peak-position of the time domain response of the micro-cavity, due to variations of the fiber-sample distance and surface permittivity. The interferometric system was tested over biological samples. In these applications, the low level of reflectivity has a direct effect in the image resolution due to the change induced in the quality factor of the optical cavity. The images reported in Fig. 2 are relative to muscle cells, grown on a carpet of nanotubes, and fixed on glass substrate. Two different pieces of information can be extracted from the time domain analysis of cavity response: topography and contrast phase images. The latter is more sensitive to surface permittivity variations and provides more details in comparison to reflectivity and topographic maps. Phase imaging measures optical thickness variations due to small changes in refractive index. Different values of refractive index are associated to differences in material density and very small refractive index changes can show a large effects in the phase images.

In conclusion, we have shown in this review how to exploit the high sensitivity of an extrinsic micro-cavity coupled with a common path Low-Coherence interferometer in the field of scanning microscopy. This system is able to characterize the topography and permittivity of biological samples, without damaging and introducing chemical agents [21]. The advantage with respect to a standard near field approach is the reduced interaction with the sample, an aspect relevant in biological applications. The system is also characterized by a compact and simple architecture that paves the way for the realization of a measurement system able to disentangle the refractive index from topography variations.

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