Spectral-domain phase microscopy with improved sensitivity using two-dimensional detector arrays

K. Singh,[1,2] C. Dion,[1,2] M. R. Lesk,[1,3] T. Ozaki,[1,2] and S. Costantino[1,3,4]
[1]*Centre de Recherche, Hôpital Maisonneuve-Rosemont, Montréal, Quebec, Canada*
[2]*Institut National de la Recherche Scientifique, Énergie, Matériaux et Télécommunications, Varennes, Quebec, Canada*
[3]*Département d'Ophtalmologie, Université de Montréal, Montréal, Quebec, Canada*
[4]*Institut de Génie Biomédical, Université de Montréal, Montréal, Quebec, Canada*

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In this work we demonstrate the use of two-dimensional detectors to improve the signal-to-noise ratio (SNR) and sensitivity in spectral-domain phase microscopy for subnanometer accuracy measurements. We show that an increase in SNR can be obtained, from 82 dB to 105 dB, using 150 pixel lines of a low-cost CCD camera as compared to a single line, to compute an averaged axial scan. In optimal mechanical conditions, phase stability as small as 92 μrad, corresponding to 6 pm displacement accuracy, could be obtained. We also experimentally demonstrate the benefit of spatial-averaging in terms of the reduction of signal fading due to an axially moving sample. The applications of the improved system are illustrated by imaging live cells in culture. © *2011 American Institute of Physics*. [doi:10.1063/1.3556787]

I. INTRODUCTION

Improved sensitivity to measure subnanometer displacements is highly desirable in several applications ranging from the study of cellular dynamics[1–3] and accurate surface profiling [4–7] to material inspection.[5,6,8,9] Determining the coatings thickness is essential in numerous industrial processes and enhanced real-time monitoring of their growth would significantly help the fabrication procedure.[8,10–12] In addition, membrane displacements in cells which can be voltage induced or triggered by mechano-sensitive channels are of the order of 1 nm^3 and to measure such small volume changes requires techniques with subnanometer accuracy.

Various phase measurement methodologies have been developed and used in different studies for high-accuracy cell dynamics measurements.[1,2,13–17] In spectral-domain phase microscopy (SDPM), phase measurements of a few tens of picometers which correspond to submilliradian precision have been achieved[15,17–19] and used to monitor membrane motion[15,16] and cellular volume changes.[15,18,19] Overall, the subnanometer accuracy yielded by phase change tracking methods in interferometric measurements is unparalleled among noninvasive far field optical approaches.

As an extension of Fourier-domain optical coherence tomography (FDOCT), SDPM can track subwavelength displacements of the sample by inspecting the phase of interference patterns. A detailed analysis of the high signal-to-noise ratio (SNR) obtained with SDPM is explained by the large number of pixels in the detector array.[20] After a Fourier transformation is performed, the signal distributed throughout all pixels of the linear detector adds up coherently whereas the noise adds up incoherently, hence improving the SNR and, thus, the phase sensitivity. To further improve phase sensitivity in SDPM, it is essential to optimize the SNR of the detection system.[16,18]

Similar interferometric schemes have used two-dimensional (2D) detectors to, for example, remove mirror images in optical coherence tomography (OCT) experiments.[21] This also yielded a 5 dB gain in SNR due to the use of a larger number of pixel points in reconstructing the signal spectrum. In addition, 2D detectors have also been utilized extensively for parallel detection in FDOCT[22–24] and time-domain OCT.[25–30] Nevertheless, the use of full 2D array detectors for increasing SNR and phase sensitivity has not been investigated. In this work, we demonstrate the use of a low-cost 2D detector (CCD) to improve the SNR and sensitivity of SDPM, demonstrating measurements of live biological samples with subnanometer accuracy.

II. THEORETICAL BACKGROUND

To understand the basic principles of SDPM, this methodology is typically implemented on a Michelson scheme in which a broadband light source is used to illuminate a reference surface and a sample. The reflected beams are recombined producing an interference pattern on a diffraction grating and the spectrum is observed using a linear photodetector array. The intensity distribution along the k-axis (wavenumber-axis) $I(k)$ can be described as the light source spectrum, $I_0(k)$, with a sinusoidal modulation at the exact frequency of the optical path difference (OPD) nd, which can be written as

$$I(k) = I_r(k) + I_s(k) + 2\sqrt{I_r(k)I_s(k)}\mathrm{Cos}(ndk + \phi_0). \quad (1)$$

Here, d is the geometric path difference between both arms of the interferometer, n is the refractive index of the medium through which light propagates, ϕ_0 is a phase constant, and I_r and I_s are the light intensities back-reflected from the reference and the sample, respectively.

A complex valued depth profile of the sample, commonly called axial scan (A-Scan), can be obtained by calculating the fast Fourier transform (FFT) $\tilde{I}(z)$ of Eq. (1), whose magnitude has peak values at $z = \pm nd$. The phase at a particular OPD

can also be obtained from this complex Fourier transform[19] and tracked as a function of time (standard phase unwrapping algorithms[31,32] may be used for large displacements). Variations in the phase between two time points t and t_0 can be used to evaluate variations in the OPD of the sample using

$$\Delta z = \frac{\lambda_0}{4\pi n}\left[\phi_z(t) - \phi_z(t_0)\right], \tag{2}$$

where $\lambda_0$is the central wavelength of the light source.

The minimum phase variation that can be detected, i.e., the phase sensitivity is limited by the SNR of the system and if only shot noise is considered, it can be defined by[16,18]

$$\langle \Delta\phi^2 \rangle \approx \frac{1}{\mathrm{SNR}}. \tag{3}$$

Here, the SNR for a SDPM system is calculated as

$$\mathrm{SNR[dB]} = 20\ \log_{10}(|\tilde{I}|/\sigma), \tag{4}$$

where $|\tilde{I}|$ is the magnitude of the FFT ($\tilde{I}(z)$) peak obtained in the A-Scan of the sample and σ is the standard deviation (SD) of the noise signal away from the interference peak. Thus, an improvement of the phase sensitivity or displacement sensitivity, imperatively implies an increase in the SNR either through an increase of the signal or a reduction of the noise.

Considering the interference term in Eq. (1), the amplitude of the interference peak is proportional to both the root mean square of the reference and the sample beam intensities. Thus, one simple way to obtain better SNR is to increase the power from either of these reflected beams. However, the saturation limit of the detectors restricts the maximum total intensity that can globally be used. Another straightforward way to improve SNR is to increase the integration time of the camera to average out fluctuations due to shot noise but this limits the sampling frequency of the system. Other than saturation and long integration times, the combined signal from the reference surface and the sample surface can be spread on a 2D array detector. This workaround permits the total intensity to be spread over the multiple CCD lines, while still each line of the 2D detector carrying the same information about depth profile of the sample. Therefore, the spectrum from each line of the CCD camera can be Fourier transformed to obtain multiple A-Scans of the sample. By averaging these A-Scans, the signal is added coherently and the noise is cancelled out providing better SNR.

III. EXPERIMENTAL SETUP

We show in Fig. 1(a) schematic diagram of the optical setup for the SDPM used in this work. We used a fiber-based Michelson interferometer, consisting of a 2 × 1, 80:20 fiber coupler terminated with fiber collimators and a superluminescent diode (SLD, Superlum) with central wavelength at 930 nm and a 50 nm bandwidth. The inspection beam was focused onto the sample using a 20X (NA = 0.75) objective (Olympus UPLSAPO 20X). For detection, the signal was dispersed using a diffraction grating (1200 lines/mm) and focused on to a CCD camera (Apogee 2000) using a combination of cylindrical lenses L1 (focal length $f = 150$ mm) and L2 ($f = 100$ mm). The signal intensity at the detector was attenuated using

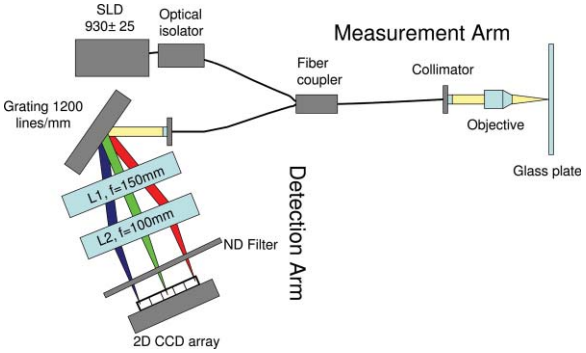

FIG. 1. (Color online) Schematic diagram of the optical setup.

a set of neutral density filters in order to keep the intensity constant, below the saturation level of the detector. The camera exposure time was set to 1 ms and images were processed using a 2.0 GHz computer with custom software (LABVIEW; National Instruments Inc.). The FFT of all CCD lines produced individual A-Scans from which an average A-Scan was calculated. The axial resolution for the averaged A-Scan was found to be 8.8 μm which is slightly higher than the theoretical axial resolution of 7.6 μm.

IV. RESULTS

We have first investigated the influence of the number of CCD lines used on the SNR and the phase stability of the average A-Scan. In this experiment, the beam was focused on a 200 μm-thick glass plate, where the two interfaces were respectively acting as reference and sample surfaces to produce the interference pattern. The data was acquired from the 2D detector with fixed exposure time of 1 ms at intervals of 100 ms which includes the time to send and save the data to the computer. The position of a cylindrical lens L1 was adjusted in order to spread the interferogram over a variable number of CCD lines. The SNR was evaluated using Eq. (4) from each average A-Scan obtained for a specific number of CCD lines illuminated and phase stability was evaluated from the standard deviation of the phase measured over time. Both results are shown in Fig. 2, where a 23 dB increase in SNR can be observed, from 82 dB to 105 dB, using 150 pixel lines, as compared to a single line. On the other hand, the phase stability was found to be 330 μrad for 1 line and 92 μrad for 150 lines, corresponding to a 3.6 times improvement. In com-

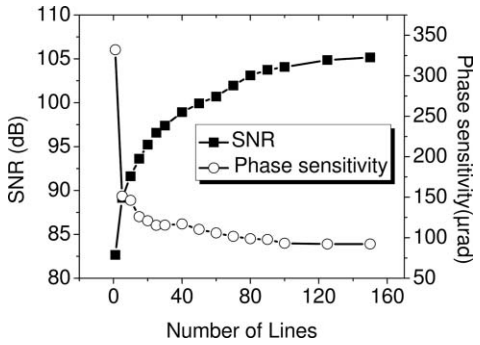

FIG. 2. SNR and measured phase stability as a function of the number of CCD lines considered in the average A-Scan.

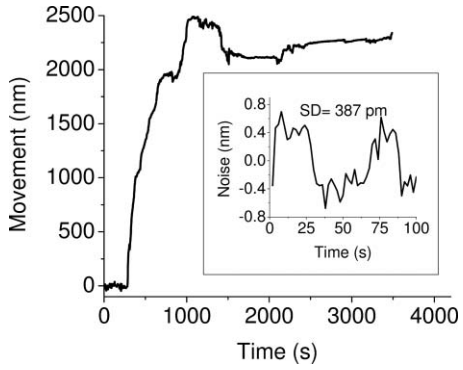

FIG. 3. Cell membrane position measured as a function of time after adding distilled water to the culture media. The inset shows the SD of the cover slip thickness used as a substrate for cells, simultaneously measured with SDPM.

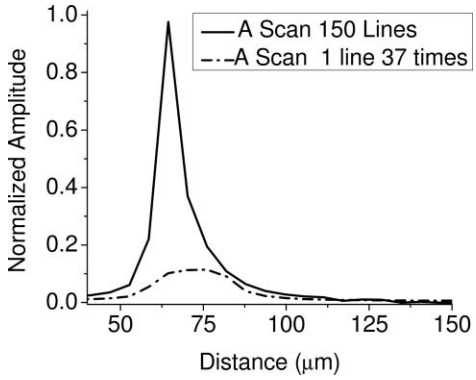

FIG. 4. Resultant A-Scan measured with 150 lines of a 2D detector and 37 lines measured sequentially with a 1D detector for same acquisition time.

parison, the phase sensitivity just due to shot noise calculated from Eq. (3) predicts 73 μrad for one line (SNR = 82 dB) and 6 μrad for 150 lines (SNR = 105 dB) which are significantly lower than the experimentally obtained values of phase stability. This disagreement between the expected and experimental values is attributed to mechanical noise due to vibrations and nonuniform thickness of the glass plate at the picometer level. Indeed, angular variations of only 10 millidegree between the cover slip and the laser beam produce a change of more than 5 pm in OPD or equivalently 70 μrad in phase.

As an application of the improved SNR, we measured the membrane dynamics of HeLa cells placed in hypotonic solutions. The cells were cultured in glass bottom petri dish (Mattek) and were observed through an inverted microscope (IX71, Olympus) using a 40X (NA = 0.90) objective (Olympus UPLSAPO 40X). The SLD beam was coupled to the microscope using a side-port, reflected by a hot mirror and focused on the upper cell membrane. The interferometric signal, thus, presented two components generated by (*i*) the interference between the cell membrane and the air/glass interface and (*ii*) the interference between the two surfaces of the glass plate. The beam was focused on the sample in such a way that peaks in the A-Scan produced by the cell and the glass plate had equal amplitudes. The number of illuminated CCD lines was fixed to 150, yielding a SNR of 66 dB, corresponding to a 15 dB improvement as compared to using a single CCD line.

To introduce a change in the cell membrane position with respect to the glass surface we gradually added distilled water to the culture media, which increased the cellular volume by osmotic pressure. The cell membrane position as a function of time is presented in Fig. 3. Before water was added to the dish, the standard deviation in the cell membrane position was found to be about 10 nm, and after adding water the cell membranes overall moved approximately 2.5 μm. The SD of the cover slip thickness (sticking to the bottom of a petri dish filled with culture media) yielded 387 pm which also represents the error of the cell membrane position.

V. DISCUSSION AND CONCLUSION

Better phase sensitivity and SNR can always be obtained by averaging A-Scans, and this can be achieved either in space, from of each line of a 2D-CCD, or by averaging sequential A-Scans acquired with a linear CCD array. An adequate choice between linear and 2D detectors must take different parameters into account, where speed and cost play an important role. To understand the advantages of using 2D-CCD for improved phase measurement, we compared the proposed space-averaging with conventional time-averaging using single-line arrays. The CCD acquisition time consists of both exposure time plus the readout time. For 2D-CCD detectors, this total acquisition time interval to acquire N lines with x pixels per line is given by, $T_{2D} = \tau_e + N\,x\,\tau_r$, where τ_e is the exposure time and τ_r is the readout time per pixel. Similarly, for a 1D detector the total acquisition time of M measurements is $T_{1D} = M(\tau_e + x\,\tau_r)$, where x and τ_r are typically the same for both 1D and 2D CCDs. Therefore, for equal total acquisition times, assuming equal exposure and pixel readout times, the number of A-Scans that can be used for averaging will always be higher using 2D compared to 1D detectors (i.e., $N > M$), thus yielding better SNR. However, one must note that this conclusion holds true only if the light incident on each pixel of the 1D- and 2D-CCD is the same.

In addition, space-averaging can further be advantageous in the case of moving samples and to show this experimentally, we measured the average A-Scan of an axially moving mirror sample placed on a motorized translation stage. The sample velocity was set to move a distance equal to the axial resolution (8.8 μm) of our system in the time needed to read 150 lines of the 2D-detector, i.e $T_{2D} = 49$ ms, $\tau_e = 1$ ms, $\tau_r = 200$ ns, $x = 1600$ pixels. With these settings, the total number of lines that could be read using a 1D-detetctor is 37, and we compared in Fig. 4 the average A-Scans obtained with both modalities. The movement of the object leads to a decrease of the peak intensity an increase of the FWHM of the peak with time averaging, and thus worse SNR and axial resolution. For this specific example, peak intensity is reduced by a factor of eight, which corresponds to a decrease of 18 dB in SNR, while the FWHM or axial resolution is increased approximately by a factor of 3. This signal fading is due to the combined effect of the smaller total number of lines acquired and sequential spectra obtained at different sample positions.

Finally, to make a sound compromise choosing the detector geometry, considering that price is now approximately the same in both cases, an increased acquisition rate us-

ing 2D detectors can be achieved by binning pixels. Using this modality which today can lead hundreds of frames per second using standard cameras the acquisition rates are suitable for most biological experiments and cover a broad range of applications, simultaneously achieving a gain in the SNR.

In conclusion, using a 2D-detector array and simple modifications to the interferometer detection scheme, we demonstrated increased SNR and improved phase stability. The large number of pixels of a low-cost 2D-CCD camera allowed probing phase changes with 92 μrad stability, corresponding to a displacement measurement of approximately 6 pm in air, using a 50 nm-bandwidth superluminescent diode as light source. This improvement to the SDPM technology can be useful for precise phase and displacement measurement and we showed its applicability for imaging dynamic biological samples with low scattering characteristics. Finally, in addition to being easy to setup the proposed detection scheme is compatible with alternative implementations of SDPM[16,18] where increased SNR for subnanometer accuracy becomes essential.

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