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Interferometric Phase-Based Dual Wavelength Tomography

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ABSTRACT

We describe our phase-sensitive interferometry technique implemented as phase dispersion microscopy (PDM)/ optical tomography (PDOT). The technique is based on measuring the phase difference between fundamental and second harmonic low coherence light in a novel interferometer. We attain high sensitivity to subtle refractive index differences due to dispersion with a differential optical path sensitivity of 5 nm. Using PDM, we show that ballistic light in a turbid medium undergoes a phase velocity change that is dependent on scatterer size. We demonstrate that the microscopy technique performs better than a conventional phase contrast microscope in imaging dispersive and weakly scattering samples. The tomographic implementation of the technique (PDOT) can complement Optical Coherence Tomography (OCT) by providing phase information about the scanned object.

Keywords: phase, interferometry, microscopy, tomography

1. INTRODUCTION

We have recently developed a robust and highly sensitive imaging technique which we have implemented as phase dispersion microscopy (PDM) [1] and phase dispersion optical tomography (PDOT) [2]. The technique uses low coherence interferometry to measure the optical phase of light transmitted through or backscattered by the target sample. The key feature is the use of a pair of harmonically related low coherence light sources to eliminate motional artifacts in the interferometer and the target, which usually prevent accurate measurements of phase information.

This technique allows measurement of subtle refractive index differences, or equivalently phase velocity variations, due to dispersion. This sensitivity permit us to observe a phase velocity change in the ballistic light transmitted through a turbid medium that varies with scatterer size [3]. This dependence cannot be explained through the photonic model, which is extensively used in optical tomography [4]. In this model, ballistic propagation is pictured as photons which are undeflected in transmission.

Like phase contrast microscopy (PCM) [5], this technique in its microscopy implementation can be used to study unstained tissue sections by rendering subtle refractive index difference visible. In addition, PDM outperforms PCM in its ability to image weakly scattering specimens and provide quantitative measurements.

The implementation of this phase technique that provides depth resolution, PDOT, can complement optical coherence tomography (OCT) [6]. OCT is a valuable technique for imaging in vivo biological tissues, which provides information about the scattering properties of sub-surface structures through measurement of the amplitude of a backscattered electric field. It has been recently demonstrated that interferometric methods which measure phase information can provide additional information about the birefringence [7], dispersion [1] and spatial phase variation [8] of a sample. By combining the ability of PDM to measure dispersion related phase information with the capability of OCT to obtain depth-resolved images, PDOT can reveal dispersion based differences which are otherwise not detectable with OCT.

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2. THE TECHNIQUE

Our novel phase sensitive technique is based on a modified low-coherence Michelson interferometer (Fig. 1a shows setup for 2D imaging; Fig. 1b shows setup for depth resolved imaging). The input light is a pair of overlapped beams of laser light at the fundamental and second harmonic frequencies. The source is a low coherence Ti:sapphire laser producing 150 fs pulses at 800 nm, and the second harmonic is generated by a standard frequency doubler. The composite beam is split into two at the beam splitter. One part is input to the target sample while the other is reflected by a moving reference mirror. The motion of the mirror induces a Doppler shift in the returning beam. The two composite beams then are recombined, separated by their wavelength components with a dichroic mirror, and measured separately by photodetectors. The resulting heterodyne signals at both wavelengths are measured and digitized. Each digitized signal is bandpassed around its center heterodyne frequency, as given by the Doppler shift. The filtered signals are then Hilbert transformed to yield their respective phases, Ψ_1 and Ψ_2 [9].

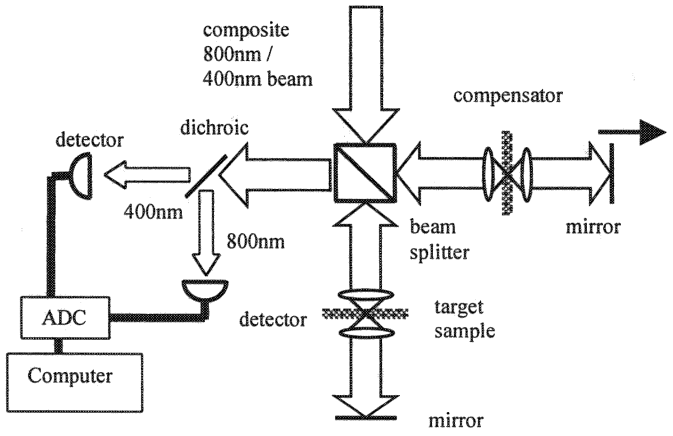

Figure 1a: Phase Dispersion Microscope (2D)

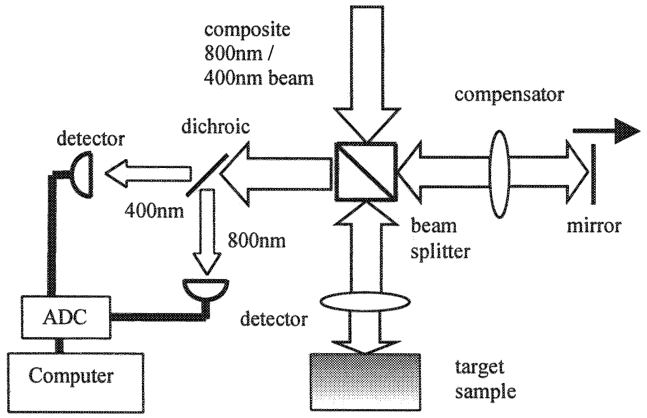

Figure 1b: Phase Dispersion Optical Tomography (depth resolved)

It can be seen that a jitter of magnitude, Δx, in either the signal or reference arm length will vary the phases, Ψ_1 and Ψ_2, by $k_1\Delta x$ and $k_2\Delta x$ respectively, with k_1 (k_2) the free space wavenumber of the fundamental (second harmonic) light. As k_2 is exactly double k_1, the effect of this jitter can be totally eliminated by subtracting twice Ψ_1 from Ψ_2. Due to the fact that phase measurements are inherently limited to modulus 2π, such elimination of phase artifacts is only possible when one wavelength is an integer multiple of the other. This operation yields $\Delta OL_{k_2,k_1}$, the optical path length difference experienced by the two wavelengths in the interferometer, with great sensitivity:

$$\Delta OL_{k_2,k_1} = \frac{(\Psi_2 - 2\Psi_1)}{k_2} . \quad (1)$$

The sensitivity achieved is about 5 nm in optical path length difference or, equivalently, about 9×10^{-2} rad for phase difference with respect to the second harmonic light. Note that phase measurements are inherently limited to modulus 2π; therefore measurements of longer path differences require appropriate phase-unwrapping techniques such as those described in Ref. 10.

3. ANOMALOUS PHASE VELOCITY OF BALLISTIC PROPAGATION

The sensitivity of the technique to optical path length difference can be exploited to measure subtle phase velocity changes of ballistic light propagating through a turbid medium [3]. We modify the interferometer setup for this purpose by replacing the focusing lens array and sample in Fig. 1a with a 10 mm thick cuvette filled with turbid media. The corresponding compensator arrangement is replaced by a water filled cuvette of the same thickness. We achieve a differential phase velocity sensitivity of 40m/s through this arrangement. Measurements are taken as polystyrene microspheres of a given size are gradually added to the signal arm cuvette. The fractional volume of microspheres, η, is varied from 8×10^{-6} to 3×10^{-3}. The relative refractive index of the microspheres is 1.20 at 800 nm and 1.23 at 400 nm, with respect to that of water. Each measurement of optical path difference is then used to find the fractional phase velocity difference, $\frac{\Delta v_2}{v_o} - \frac{\Delta v_1}{v_o}$, between the two wavelengths in the cuvette:

$$\frac{\Delta v_2}{v_o} - \frac{\Delta v_1}{v_o} = -\frac{\Delta OL_{k_2,k_1}}{2Ln_o}, \quad (2)$$

with v_o the speed of light in water, n_o the refractive index of water and L the thickness of the cuvette. Note that the insignificantly small second order corrections due to dispersion of water are omitted. Measurements are made for a succession of microspheres varying in radius, a, from 10 nm to 10 μm. The data points of Fig. 2 show the measured fractional difference in phase velocities as a function of scatterer size. The experimental observations are best discussed by comparison with the following theoretical phase velocity analysis of the ballistic light.

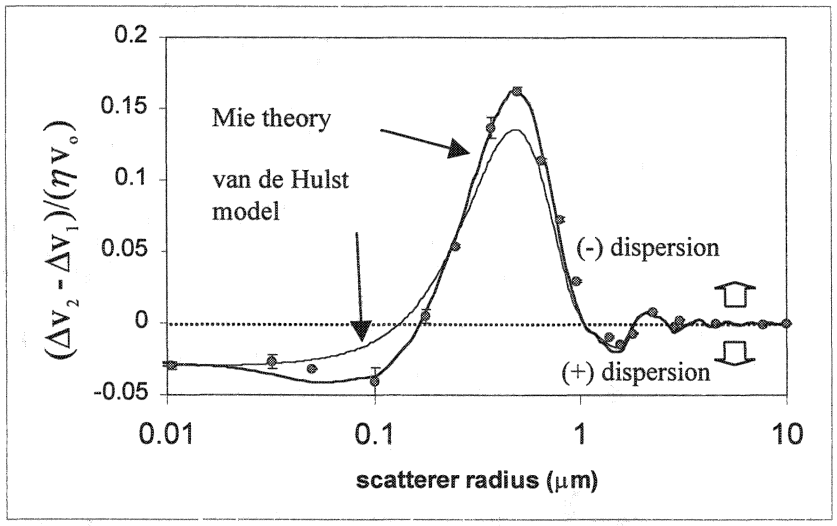

Figure 2: Experimental plot of differential phase velocity variation with scatterer size.

The experimental observations can be compared to previously overlooked aspects of van de Hulst and Mie

scattering theories[11]. Van de Hulst theory reveals the analytical form $\frac{\Delta v}{v_0}$ as:

$$\frac{\Delta v}{v_0} = 1 - n = -\frac{3\eta}{2a^3k^3}(ka)^2\left(\frac{\sin\rho}{\rho^2} - \frac{\cos\rho}{\rho}\right), \qquad (3)$$

with $\rho = 2ka(m-1)$ the normalized scatterer size, and *(m-1)* the relative refractive index difference between the scatterers and the surrounding medium. This model is stictly valid only when the scatterer size is large compared to the wavelength and the refractive index difference is small. Nevertheless, it provides important insights and is a good fit to the experimental data.

From the theory and the experiment, we can see that there are 3 regimes of ballistic light propagation, depending on the scatterer properties:

I. $\rho << 1$ - turbid medium as bulk medium.

In this limit, the phase velocity change, Δv , reduces to $-\eta v_0(m-1)$. This change arises only from bulk refractive index change due to the presence of small scatterers. From another perspective, when the phase lag through each scatterer is small, the net result is simply an overall change in phase velocity, as determined by the refractive index difference.

II. $\rho \approx 1$ - no simplification.

In this regime Eq. (5) cannot be simplified. The phase velocity is seen to oscillate with changing ρ. The net change in phase velocity is strongly dependent on whether the forward scattered light is in phase or out of phase with the input light. We note the existence of an anomalous phase velocity *increase* for some values of ρ, despite the fact that the scatterers have *higher* refractive index than water. Usually, adding a material with index higher at 400 nm than at 800 nm (normal dispersion) into water would cause the 400nm light to be slowed more than at 800nm. However, in the case of scatterers with $\rho \approx 1$, the opposite is observed. Thus, the medium exhibits anomalous dispersive effects.

III. $\rho >> 1$ - phase velocity is independent of turbidity.

In this limit, the phase velocity change, Δv , is zero. This is the only regime in which the photonic model provides a complete description. The phase velocity is thus independent of the presence of turbidity. Physically, we can understand this from the fact that when ρ is large, the phase of the transmitted light varies rapidly with increasing distance from the center of the sphere. The net result is that the phase shift of the transmitted light averages to zero. Therefore, large scatterers have no effect on the bulk refractive index for ballistic propagation.

This dependency of the ballistic light phase velocity on ρ implies that the ballistic light itself must carry phase information about the structure and composition of the turbid medium. The photonic model which ignores all phase considerations simply cannot explain this variation. This finding suggests that the incorporation of phase measurement techniques into optical tomography can obtain more information about a scanned specimen than a purely intensity based approach can obtain.

4. PHASE DISPERSION MICROSCOPY

As shown in Fig. 1a, the technique can be implemented in a transmission microscope geometry. In our implementation, the phase dispersion microscope (PDM), achromatic 10X microscope objectives focus the composite beam onto the sample with a FWHM of about 7 μm at both wavelengths; however, difficulty in aligning the returning path to overlap with the incoming path degrades the resolution to about 10 microns. (Note that finer resolution is achievable by using higher power objectives and improved alignment.) A similar lens array and a clear slide is inserted in the other interferometer arm as compensator.

For a given sample of known thickness L, we can evaluate the refractive index dispersion relative to that of the compensator medium, $(\Delta n_{400nm} - \Delta n_{800nm})$, between the wavelengths by.

$$\Delta n_{400nm} - \Delta n_{800nm} = \Delta OL_{k_2,k_1} / L, \qquad (2)$$

where Δn_{400nm} (Δn_{800nm}) is the difference in refractive index between the sample and the compensator medium at the wavelength of 400nm (800nm). The sensitivity of our system permits us to detect refractive index dispersion as small as 5×10^{-6} for a 1 mm sample. This sensitivity improves for thicker samples.

This ability of PDM to provide quantitative information is one of the important differences between PCM and PDM. In addition, PDM can be applied to a wider range of specimens than PCM. PCM forms images by phase shifting the scattered light field from a specimen and interfering it with the unscattered light field. Therefore, it can be applied only to specimens that scattered a significant amount of light. In contrast, PDM directly measures the small phase shifts of the unscattered light and, thus, can be applied to specimens that are weakly scattering or do not scatter at all. Finally, it is difficult to separate the contributions from absorption and phase shift in an PCM image. In PDM, these two factors can be easily measured independently, as absorption affects only the amplitude of the detected heterodyne signals while the phase shift affects only the phase lags of the heterodyne signals.

As an illustration of the capabilities of PDM, we compare the performance of PDM to PCM on similarly prepared samples comprising a drop of water and a drop of DNA solution (1.0% vol. conc.) sandwiched between two cover slips (Fig. 3). The separation between the cover slips is 170 μm. As evident in Fig. 3, PDM can easily distinguish the two drops and provides a refractive index dispersion value for the DNA solution. In contrast, PCM does not distinguish between the two nearly transparent droplets nor does it provide any quantitative data. Interestingly, the refractive index dispersion measured in this experiment by PDM, $(1.3\pm0.2)\times10^{-4}$, differs from the value, 1.6×10^{-4}, extrapolated from an experiment with more dilute samples of DNA, based only on the ratio of the sample concentrations. This difference can be attributed to the fact that the refractive index depends on scatterer size, as well as concentration. Thus, at higher concentration, the formation of DNA aggregates, which behave as scatterers, effectively alters the refractive index. The above study on ballistic light propagation [3] has experimentally verified that the refractive index depends strongly on scatterer size.

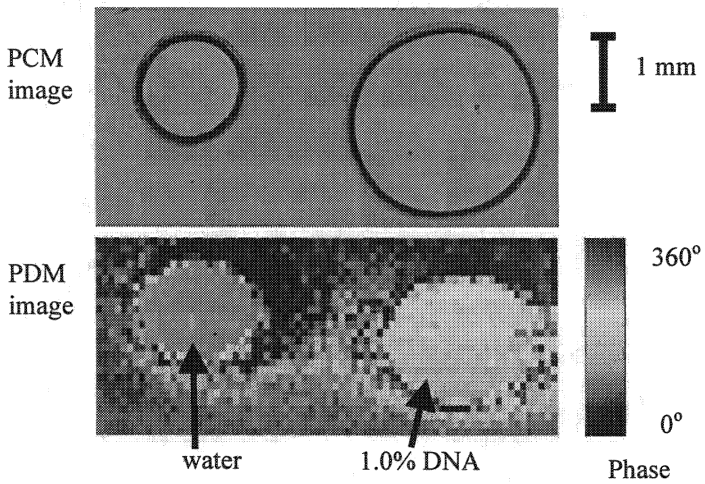

Figure 3: Images of a drop of 1.0% vol. conc. DNA solution and water. We measure the dispersion contribution due to DNA as $(1.3\pm0.2)\text{x}10^{-4}$.

To further demonstrate the capability of PDM, we compare PDM and PCM images of a brain tissue sample. A 16 μm thick sample was prepared from a frozen brain tissue block using a microtome. The sample was obtained from the autopsy material of an Alzhemier disease patient and sandwiched between two cover slips. A drop of glycerol was applied to keep the sample moist and to provide index matching. We use a compensator in the reference arm identical to the target sample but with no tissue. Figure 4 shows PCM and PDM images taken from the same sample. For comparison, a stained sample from an adjacent thin section is also shown. As can be seen, the PCM image reveals only a slight distinction between the gray and white matter; this is due to the relatively weak scattering of brain tissue. In comparison, the differences between the two are quite visible with PDM. This can be attributed to differences in the composition of the two tissue types, which give rise to a small but measurable refractive index dispersion change.

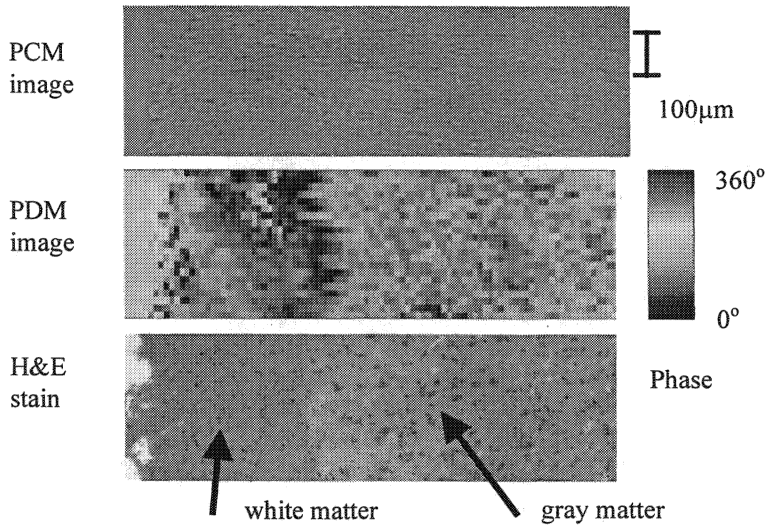

Figure 4: Images of an unstained white matter – gray matter interface in a 16 μm brain sample. An adjacent section stained with H&E is shown for comparison.

5. PHASE DISPERSION OPTICAL TOMOGRAPHY

The PDM system can be readily adapted into a depth resolved imaging system, by using a backscattering geometry, as is done in OCT [6]. The schematic of the phase dispersion optical tomography (PDOT) system is shown in Fig. 1b. The short durations (150 fs) of the light pulses provide the necessary broad spectral bandwidth for coherence gating. The measured depth resolution of the system is about 30 μm.

As PDOT provides depth resolution, it can be applied to multiple layer targets, such as epithelial tissues, to reveal dispersion information about each layer. We demonstrate this ability in a preliminary experiment on a sample consisting of gelatin and water sandwiched between a mirror and a cover slip. The gelatin layer induces a sufficient optical path change compared to an equal thickness of water that the reflection from the mirror shows a noticeable shift.

The PDOT system can also measure the intrinsic phase shift associated with an interface reflection or scattering by a Mie particle. In particular, the phase shift associated with scattering is a function of the size-to-wavelength ratio of the scatterer. The measured phase shift can then be processed to extract information about the optical properties of the reflector or scatterer. In particular, continuous phase shift measurement can reveal the deformation rate of cell nuclei undergoing structural changes. Alternatively, a spectral profile of the phase shift can be processed to determine the size and content of sub cellular organelles.

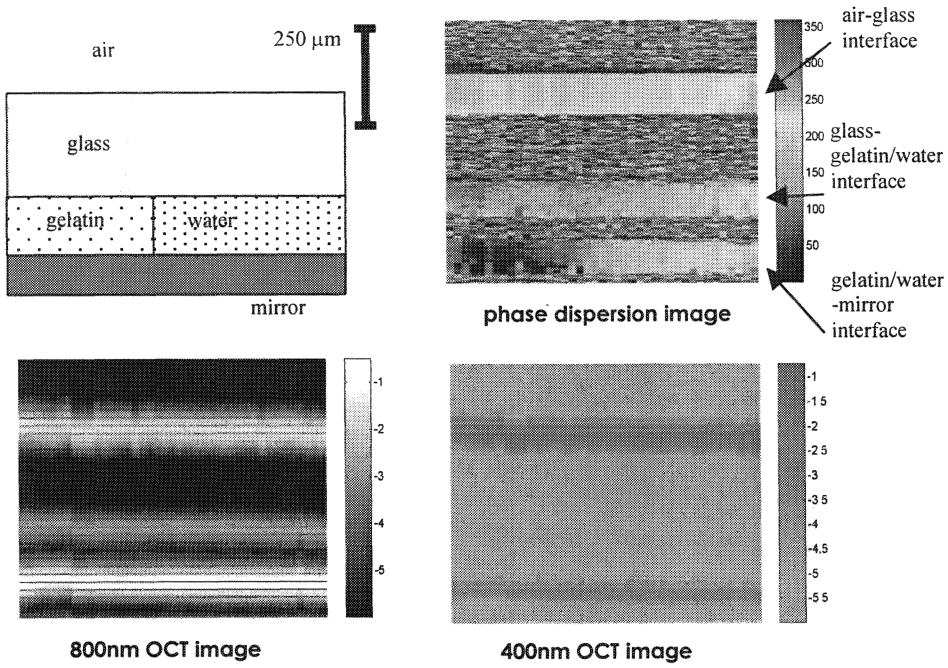

Figure 5: Phase Dispersion Optical Tomography image of a simple sample.

6. CONCLUSIONS

In summary, we have developed a robust phase sensitive technique, which completely eliminates phase noise due to interferomteric jitters. Our technique is highly sensitive and can be used to provide quantitative phase information about the target. The 2D implementation, PDM, can perform better than the current standard – phase contrast microscopy. The advantages of PDM include its ability to provide quantitative data and its wider range of imaging targets which includes non- or weakly scattering specimen. The tomographic implementation of the phase technique can complement OCT by providing dispersion based information.

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