High Sensitivity Measurements of the Scattering Dispersion of Phantoms using Spectral Domain Optical Coherence Tomography*

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ABSTRACT

We demonstrate a novel technique to determine the size of Mie scatterers with high sensitivity. Our technique is based on spectral domain optical coherence tomography measurements of the dispersion that is induced by the scattering process. We use both Mie scattering theory and dispersion measurements of phantoms to show that the scattering dispersion is very sensitive to small changes in the size and/or refractive index of the scatterer.
Keywords: dispersion; Mie scattering; optical coherence tomography; relative group delay; spectral domain

1. INTRODUCTION

Dispersion is often viewed in terms of its deleterious effect on the resolution of optical coherence tomography (OCT) images. However, it has recently been proposed that measurements of dispersion could possibly be used for clinical diagnostics, for example, to characterize plaque morphology for heart disease screening [1]. We demonstrate that OCT-based measurements of dispersion have even broader potential and can possibly be applied with very high sensitivity to the measurement of the sizes of cell nuclei for applications such as early cancer detection.

Tissue dispersion has two components: material dispersion, which arises from the wavelength dependence of the refractive index, and scattering dispersion, which results from the wavelength dependence of the Mie scattering that typically occurs in tissue. Dispersion is often described by the relative group delay (RGD), which is defined as $d\phi/d\omega$, where ϕ is the phase of the electric field and ω is the angular frequency. We have developed Mie theory to predict the scattering RGD as a function of wavelength, simple phantoms to emulate the nuclei of cells, and a spectral domain OCT system capable of characterizing RGD as a function of wavelength with high resolution.

2. THEORY

It has previously been demonstrated that the intensity spectrum of the light that is Mie scattered from cell nuclei is strongly affected by the size of the nuclei [2]. Healthy nuclei have a characteristic diameter of 4 to 7 μm, while dysplastic nuclei can be as large as 20 μm [2]. In this paper, we show that phase of the scattered field is strongly affected by the diameter of the scatterer. Our Mie theory predicts the electric field (both magnitude and phase) backscattered both from a single sphere in a homogeneous medium and from a single sphere on a surface. We then calculate the RGD from the phase of the Mie scattered field. Fig. 1 shows the predicted RGD as we vary the sphere's refractive index and diameter, assuming a single sphere in a homogeneous medium (refractive index of 1.33) and that both the sphere and surrounding medium are lossless. This plot demonstrates that sphere size and/or refractive index could potentially be determined with high sensitivity from a measurement of RGD. The periodicity of the RGD spikes could be used to determine the diameter of the scatterer, while the location of the spikes could potentially be used to determine refractive index. Also shown in Fig. 1 is the material dispersion of 6 mm of water (representing a double pass

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through a 3 mm sample) calculated from Schiebener's formula [3]. This indicates that the scattering dispersion is likely much larger than the material dispersion of water-based samples such as tissue.

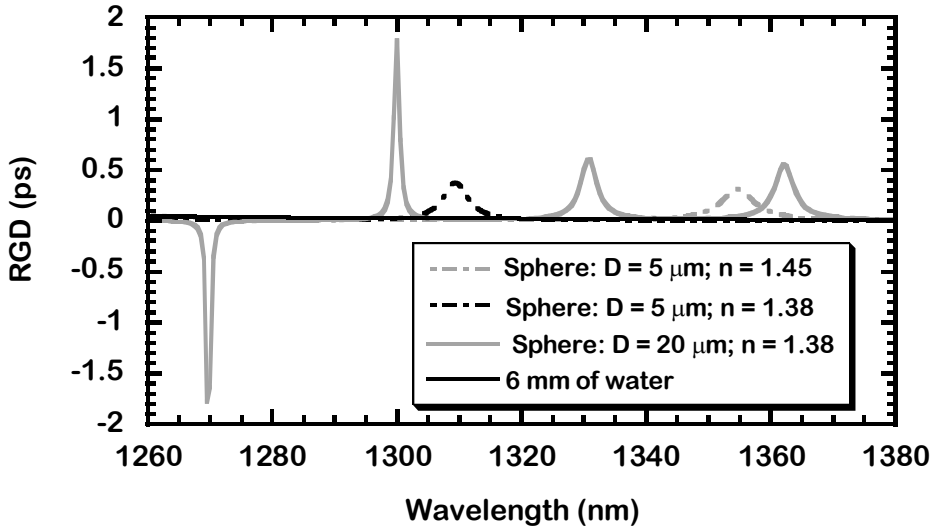

Figure 1. Group delay predicted from Mie theory for different sphere diameters (D) and refractive indices (n). The group delay of water is also shown for comparison.

3. MEASUREMENT

Our spectral domain OCT system is shown in Fig. 2. The system consists of two fiber-optic Michelson interferometers: a reference interferometer to track the wavelength of the tunable laser as it is swept, and a measurement interferometer. The phantom (sample) is placed in one arm of the measurement interferometer, and it is aligned by monitoring the light scattered off the sample with the microscope. The signal from the reference interferometer is sent to a zero crossing circuit, which is used to trigger sampling of the measurement interferometer signal.

Our measurement technique is similar to previously demonstrated spectral domain measurements of the RGD of optical fiber components, particularly fiber Bragg gratings [4]. Starting with the interferogram as function of frequency, we perform an inverse Fourier transform to obtain the time domain interferogram. We then use a window to remove the DC and autocorrelation terms and one of the complex conjugate impulse response terms. We then calculate the RGD from the following formula [5]:

$$t_g \equiv \frac{d\phi}{d\omega} = \mathrm{Re}\left\{\frac{FT(\tau I(\tau))}{FT(I(\tau))}\right\},$$

where t_g is the relative group delay, ϕ is the phase of the electric field, τ is the delay time, $I(\tau)$ is the windowed time-domain interferogram, and Re represents the real part of the function. The wavelength resolution of the RGD result is inversely proportional to the width of the window. The optimal window width is a tradeoff between wavelength resolution and RGD resolution; larger windows give better wavelength resolution, but include more noise, thereby degrading the RGD resolution.

We tested our system by measuring the RGD of a 5 cm thick sample of well-characterized commercial high-index glass. We compared our result with the change in RGD with wavelength predicted by using the Sellmeier equation for that glass and found agreement better than 20 fs over a 45 nm wavelength range (representing the central 65 % of the laser tuning range). Our RGD results are less accurate at the extreme ends of the tuning range, which likely results from

uncertainties created by the laser tuning as it starts and finishes a wavelength sweep combined with the limited frequency response of the zero-crossing trigger circuit.

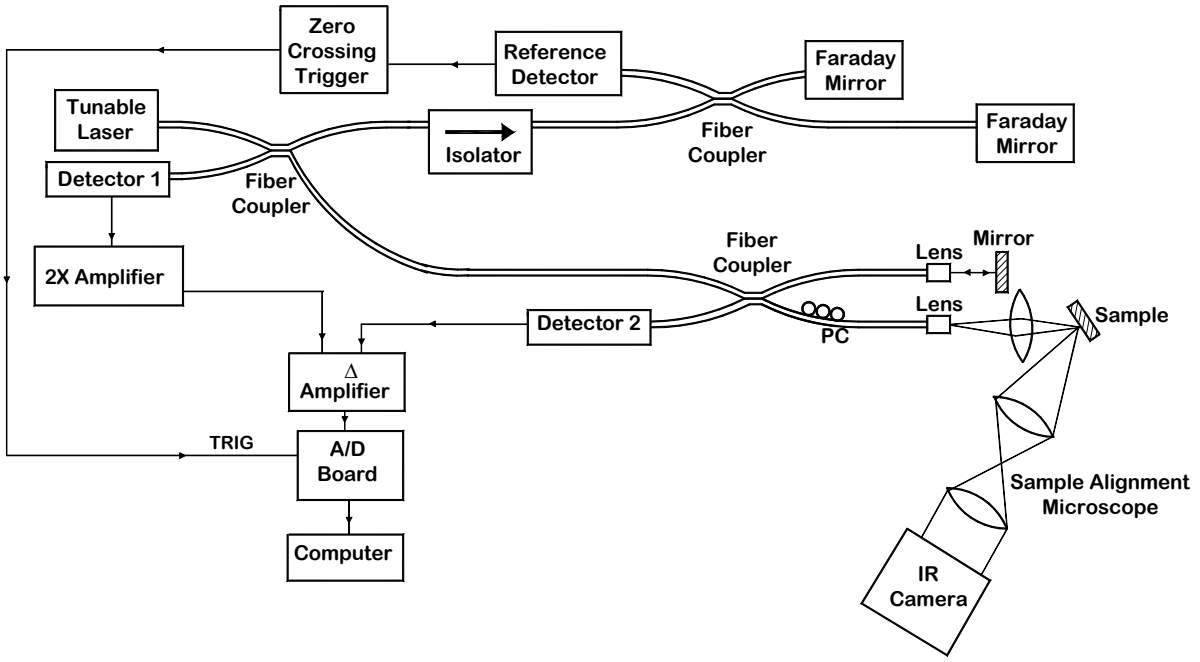

Figure 2. Diagram of the OCT system used for dispersion metrology. PC: polarization controller. Δ: difference

We first measured the RGD of scattering spheres on a microscope slide surrounded by air. We collimate the light from the sample arm of our OCT system to a 1.3 mm diameter, and then we focus on the sample using a 10X objective, giving a focal spot size of 20 μm with an effective numerical aperture of 0.04. The microscope slide is tilted with respect to the incident beam to avoid specular reflections from the slide. The scattering spheres were distributed on the slide with large separation between them to avoid multiple scattering events. In addition to the OCT measurement, we also measure the diameter of each sphere using a microscope with a reticule eyepiece. Fig. 3 shows the RGD measured from three different sizes of spheres surrounded by air. Although our RGD measurements agree qualitatively with the Mie scattering predictions (i.e., the periodicity of the RGD oscillations matches that predicted by theory), an exact comparison is difficult since the values we have for sphere size and refractive index include a large uncertainty (as large as 7 %), and the Mie scattering prediction is very sensitive to small changes in size or refractive index. Also, the wavelength scale of our RGD results may include an uncertainty as large as 1 nm.

We developed phantoms by embedding a low density of scattering spheres in porcine gelatin. We aligned the OCT light on one sphere as described above and measured the RGD of the light scattered from that sphere. The results for two different sized spheres in gelatin are shown in Fig. 4. Also shown is the Mie scattering prediction for a 18 μm sphere, assuming that sphere and gelatin are lossless, with refractive indices of 1.49 and 1.47, respectively. Again, an exact comparison of theory with experiment is difficult because the Mie theory is extremely sensitive to small changes in sphere diameter, refractive index, etc., but we are encouraged by the similarities between the Mie prediction and the measurement. We are still investigating the source of the sign difference near a wavelength of 1292 nm in Fig. 4, but early results indicate that small changes in alignment may affect the sign of the RGD. For determining the size of Mie scatterers, we think that the periodicity of the RGD signal is more important than its sign.

4. CONCLUSIONS

We have demonstrated a new technique for measuring the size of optical Mie scatterers that is based on OCT measurements of the dispersion of the light scattered from a sample. This technique is potentially very sensitive to small differences in the size and refractive index of scatterers, and may have better immunity to speckle than intensity-based measurements of the scattering spectra. These results may have application to the *in vivo* detection of precancerous dysplasia. Our future efforts will focus on extending the theory and measurement to include the interactions between multiple scatterers and adding the effects of focusing to our Mie theory. Additionally, we plan to improve our measurement technique with a better focusing geometry and a more powerful laser with a broader tuning range to enhance our ability to measure scatterers as small as 4-10 μm.

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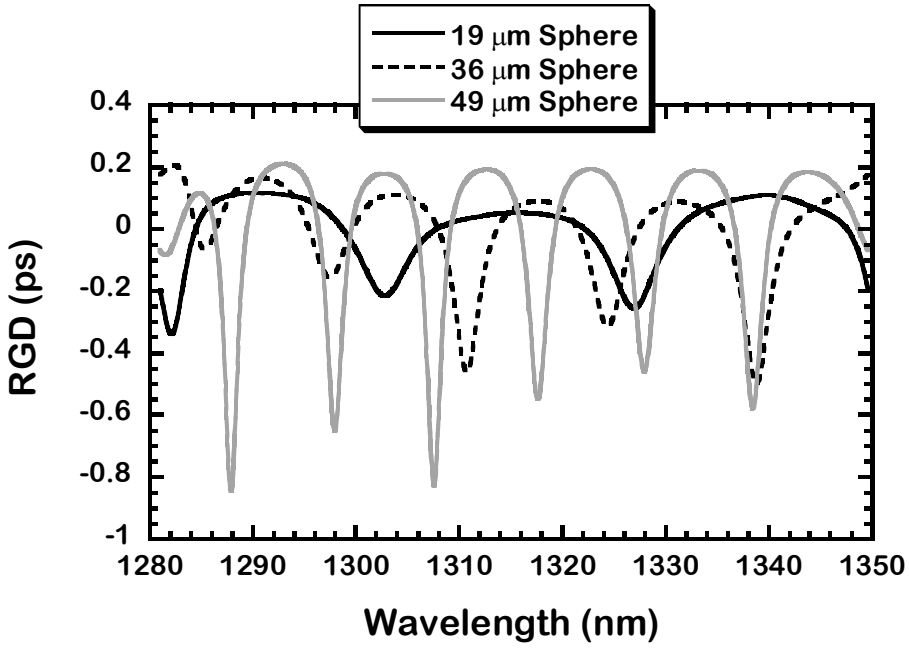

Figure 3. Comparison of measured group delay for three sizes of scattering spheres on a microscope slide surrounded by air.

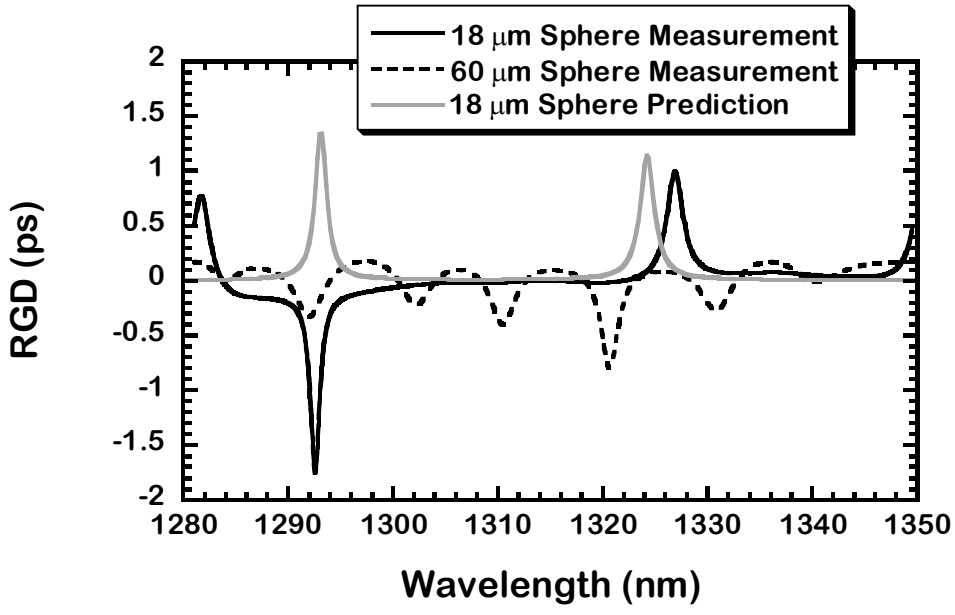

Figure 4. Comparison of measured and predicted group delay of phantoms constructed from scattering spheres embedded in porcine gelatin.