Spatial scanning of a sample with two-dimensional angle-resolved low-coherence interferometry for analysis of anisotropic scatterers

Ge Song, Zachary A. Steelman, Wesley Kendall, Han Sang Park, and Adam Wax

Department of Biomedical Engineering, Duke University, Durham, NC 27708, USA
*gs172@duke.edu

Abstract: Angle-resolved low-coherence interferometry (a/LCI) measures depth-resolved angular scattering for cell nuclear morphology analysis. 2D a/LCI, developed to collect across two scattering planes, is currently limited by the lack of spatial scanning. Here we demonstrate 2D a/LCI scanning across a three-dimensional volume using an image rotation scheme and a scanning mirror. Validation using various optical phantoms demonstrated excellent scatterer size determination over a 7.5 mm linear range, for a total accessible area of ∼44 mm². Measurements from anisotropic scatterers allowed accurate determination of sizes and computation of aspect ratios. This scanning system will facilitate analysis of scatterer structure across wider tissue areas.

1. Introduction

Angle-resolved low-coherence interferometry (a/LCI) is an optical technique that combines light scattering measurements with the depth resolution of low coherence interferometry [1]. By collecting angle-resolved scattered light across an angular range near the backscattering direction, a/LCI enables quantitative nuclear morphology measurements in biological tissue [2]. Angular scattering at various depth planes can be analyzed to recover tissue properties such as the nuclear refractive index and density for specific tissue layers. a/LCI uses inverse light scattering analysis (ILSA), a technique based on Mie theory, to determine structure from angle-resolved scattering measurements. Studies using a/LCI have demonstrated high diagnostic accuracy for precancer detection in the cervix [3,4], esophagus [5,6], and colon [7]. Recently, the development of 2D a/LCI [8,9] enabled collection of angular scattering over two transverse scattering planes at various depths within the sample using an angular scanning scheme. This advance extended the ability to study tissue structural information by extracting spatial correlation and fractal dimension through 2D Fourier analysis [8]. Previous work has demonstrated the technique’s capability to analyze retinal tissue structure and texture in ex vivo mouse models [8], even detecting signs of neurodegeneration through retinal measurements [10]. While 2D a/LCI accesses an additional dimension in the angular scattering data, which unlocks new types of analysis, it lacks the ability to spatially scan across a sample without repositioning the probe, a capability that is often crucial for clinical use. Here, we seek to strengthen 2D a/LCI by incorporating a spatial scanning mechanism, and by assessing its sensitivity to anisotropic scattering that is often present in complex biological tissue.

Introduction of a spatial scanning mechanism to traditional a/LCI overcomes the need for mechanical translation of the sample or probe, which limits the speed of data acquisition. The sampling area of the a/LCI beam (<0.5 mm²) is much smaller than the region of interest in most biological tissues, suggesting the need for a reliable scanning mechanism. a/LCI also exhibits specific beam-geometry requirements which make traditional beam-steering methods untenable.
Fortunately, a scanning a/LCI instrument has been introduced previously [11], which used a reflection-only three-optic rotator (ROTOR) prism and a two-axis scanning mirror appended to the sample arm. This spatial scanning scheme was used in a 1D a/LCI system, which only allowed collection along a single angular scattering plane. In this work, we seek to incorporate this spatial scanning capability into a 2D a/LCI system. The combination of the spatial and angular scanning in our system provides detection of angular scattering over a two-dimensional solid angle for each point within a three-dimensional volume of sample points.

Incorporation of the ROTOR prism into a/LCI also introduces the ability to control the polarization of the illumination beam, which may be used to study anisotropy in scatterers. Biological tissues often include scatterers with more complex geometries and orientations than simple spheres [12]. Rotation of the ROTOR prism allows for precise control of the polarization illumination relative to the sample [11]. By using orthogonal polarizations, it is possible to interrogate different dimensions of anisotropic scattering objects. For geometrically spheroidal scatterers, it has been shown that different linear incident light polarizations will interrogate different dimensions of the scattering object [13–15]. Because the ROTOR prism rotates both the illumination and collection axis, it can be used to facilitate measurements of anisotropic scatterers to shed light on their geometry.

In this study, we present the integration of the ROTOR spatial scanning mechanism into 2D a/LCI and validate the resulting system using various microsphere scattering phantoms. Because the system generates multidimensional data, spanning spatial and angular dimensions, a variety of phantom geometries were employed to characterize the capabilities of the device, including one- and two-dimensional microsphere arrays, anisotropic phantoms in different orientations, and three-dimensional composite phantoms comprising both isotropic and anisotropic media. The clinical relevance and utility of this scanning system are discussed.

2. Materials and methods

2.1. Angular scanning scheme

The 2D a/LCI system used in this study was described previously [8,9] and its schematic is shown in Fig. 1(a). Briefly, light from a Ti:Sapphire laser (Coherent Mira-900F) is coupled into a spool of polarization maintaining fiber (Corning, 50 m) for self-phase modulation. The spectrally broadened light ($\Delta\lambda = 28$ nm) was passed into the sample and reference arms of a Mach-Zehnder interferometer. Light in the sample arm illuminates the sample at plane P (Fig. 1(a)) with an oblique and collimated beam. The scattered light is relayed from the sample using 4f lens relays, and mixed with the reference beam using a beamsplitter before detection with an imaging spectrometer (Princeton Instruments, SP-2150). The slit of the imaging spectrometer serves to collect the primary axis of scattering, as the slit plane is in the Fourier plane of the sample. To acquire angular scattering in the orthogonal dimension, a galvanometer placed in the collection path scans the angle-resolved scattered field across the slit of the spectrometer. An illustration of the angular scanning scheme is shown in Fig. 1(b). A 640 $\times$ 480 CCD array (AVT, Pike F-032) is used as the sensor for the spectrometer and is able to detect a total field of view of 34° (-4° to 30° along the galvanometer scan direction) by 21° (-10.5° to 10.5° along the slit direction) of angle-resolved scattered light with an angular resolution of 0.137° [8].

This angular scanning scheme acquires two orders of magnitude more scattering data from a single scan compared to traditional a/LCI [8]. However, the physical size of the illumination beam (400 $\mu$m diameter) limits the spatial extent of the interrogated volume. To adequately sample a greater area, a spatial scanning mechanism was introduced after sample plane P. As shown in Fig. 1(a), the spatial scanning mechanism relayed the sample plane to a new location at P', while maintaining the illumination angle and collimation necessary for a/LCI.
2.2. Spatial scanning scheme

To perform spatial scanning with 2D a/LCI, a spatial scanning mechanism consisting of two 4f lens systems, a ROTOR prism, and a two-axis, gimbal-mounted scan mirror was appended after the original image plane, P. The ROTOR prism, described previously [11], acts as an image rotator, and consists of two elliptical mirrors and a spherical mirror mounted in a custom 3D printed housing (Fig. 2(b)). The housing allowed the prism to be attached on both ends to two 1-inch rotation mounts, enabling rotation over a full 360°. The 4f lens systems were used to maintain magnification, as well as illumination angle and collimation at the new image plane during transmission through the ROTOR prism. A schematic of the entire spatial scanning mechanism was simulated in OpticStudio (Zemax) and is shown in Fig. 2(a). Insertion of a gimbal-mounted scan mirror (Thorlabs, PF10-03-M01) prior to L4 allowed for line scans on the image plane, P'. To collect scattered light across a range of angles, the required aperture at the pupil plane will be larger, posing a modality-specific design challenge. However, this broad aperture requirement only occurs along the axis of collection and thus the pupil plane at L4 contains unused space along the orthogonal scanning axis. Scanning the beam across a two-dimensional area can therefore be achieved through rotation of both the collection and scanning axis of the system at L4 using the ROTOR prism. In this way, asterisk-shaped two-dimensional scans at the center of the sample plane P' may be acquired by iteratively rotating the linear scan axis (Fig. 2(c)). We note that rotation of the prism over an angle corresponds to a rotation that is twice that angle for the scanning and collection axis, similar to a Dove prism [16]. A rotation of 22.5° on the prism, for example, corresponds to a 45° rotation of the scanning axis at L4, enabling a diagonal line scan at P'. Scanning of the illumination beam is therefore achieved over a circular area with a diameter that is determined by its linear scan range. Significantly, the polarization of the illumination beam aligns with the collection axis, which is always perpendicular to the scanning axis [11]. As summarized in Fig. 2(c), incorporation of this spatial scanning mechanism allowed for iterative collection of 2D depth-resolved angular scattering profiles for different positions of the ROTOR prism.
Fig. 2. Spatial scanning scheme. (a) Zemax generated schematic. Two 4f systems, the ROTOR prism, and a scan mirror are appended after the original a/LCI image plane, P, to enable spatial scanning. This maps a new image plane at P' with L4 as the new a/LCI objective. (b) Drawing of the ROTOR prism, which consists of 3 mirrors mounted in a customized housing. (c) ROTOR allows for rotation of the scanning and collection axes at the L4 plane. Rotation of the ROTOR prism corresponds to double the scan angle at L4 Plane. The gimbal-mounted scan mirror allows for line scans at P'.

2.3. Sample preparation

For this study, two scatterer geometries were used as validation phantoms. Samples containing spherical polystyrene beads with diameters of 10, 12, or 15 µm were constructed using standard methods [17]. Polystyrene microspheres (Thermo Fisher Scientific) were centrifuged into pellets and dried in a vacuum chamber to remove residual water. Beads were then embedded in PDMS (Sylgard 184, Dow Corning) and placed to cure in a 3D-printed cubic mold at 60 °C. For spherical scatterers, a 3 by 3 grid phantom was constructed with 2.5 mm cubes to validate spatial scanning. The grid phantom contained 15 µm diameter beads in the center cube, 12 µm beads in the corners, and 10 µm beads on the sides. This phantom structure allowed for validation of both 1D line scanning and 2D spatial scanning using the ROTOR prism scheme.

To analyze system sensitivity to anisotropic scatterers, prolate spheroidal scatterers in PDMS were also constructed. This was accomplished by constructing a thin film of phantom material as discussed above, and suspending the PDMS film, which contained the spherical beads, by one end inside an oven. A uniaxial stress was placed on the film by attaching a weight to the other end. The phantom was then heated at 200 °C, which is above the glass transition temperature of the polystyrene spheres (Fig. 3(a)). After 45 mins, the phantom was cooled at room temperature with the weight still attached. This method stretched the polystyrene spheres in the PDMS to spheroidal structures with aspect ratios greater than 0.75. Figure 3(b) displays quantitative phase microscopy (QPM) images of the individual beads oriented in two directions as previously defined in [13–15], either in transverse electric (TE) or transverse magnetic (TM) orientation. Beads stretched with this method exhibited good uniformity in orientation when observed through focus with the QPM system, due to the uniaxial stress placed on the PDMS film. For this experiment, 12 µm and 15 µm spherical beads were stretched to a spheroidal shape that are defined by their minor axis, major axis, and aspect ratios. Using phase images acquired from a QPM system [18], aspect ratios of the anisotropic scatterers were measured. The sizes along their major and
minor axes, M and m, can be calculated using the expected aspect ratio, ε, and by assuming equal volume before and after stretching according to Eq. (1):

\[
\frac{4}{3} \pi \left( \frac{d}{2} \right)^3 = \frac{4}{3} \pi \left( \frac{M}{2} \right) \left( \frac{m}{2} \right) \left( \frac{m}{2} \right) \tag{1}
\]

Where \( m = \epsilon M \) and \( d \) is the diameter of the unstretched spheres. For a 12 µm diameter spherical bead that was stretched to an aspect ratio of 0.78, the equal-volume minor axis was calculated to be 11.0 µm and the equal-volume major axis yielded 14.2 µm. Similarly, for a 15 µm spherical bead that was stretched to an aspect ratio of 0.86, the equal-volume minor axis was 14.3 µm while the equal-volume major axis was 16.6 µm. Use of this method enabled construction of anisotropic scattering phantoms with high uniformity in size (~2-4% aspect ratio variation) and orientation distributions, as well as a composite 2 × 2 grid phantom which contains both spherical and spheroidal beads at specific orientations. Sampling of this phantom seeks to demonstrate sensitivity to anisotropic scattering with spatial variations.

**Fig. 3.** Anisotropic scattering phantom. (a) A uniaxial stress is placed on the PDMS phantom and heated above the glass transition temperature of the polystyrene beads. This stretches the beads to give a “football”-like shape. (b) Representative phase images of a stretched 12 µm anisotropic scatterer with an expected aspect ratio of 0.78. Both the TE and TM configurations place their axis of symmetry along their major axes. Scale bars: 10 µm.

### 2.4. Mie fitting

To validate the performance of the system, we performed scans on the phantoms described earlier and determined the scatterer sizes using ILSA based on Mie theory. With a/LCI, the measured angular scattering are compared to a library of precomputed Mie scattering spectra using computational scattering software (MiePlot, [19]). Parameters included scatterer and PDMS refractive indices of 1.58 and 1.41, respectively. The central wavelength of the light source was set to its measured value of 792 nm. A Mie library with scatterer diameters ranging from 5-18 µm in 0.1 µm increments was generated, to which the measured data was fitted. Each Mie function assumed a slight (1%) distribution of scatterer diameters, which is sufficient to encompass naturally occurring distributions in both the scatterer size and source bandwidth [20]. Comparison of measured spectra to the library was performed as outlined by Brown et al., [2], which included spectrum normalization, detrending, low-pass filtering and determination of the lowest chi-squared error for scatterer size determination.
3. Results

To validate the spatial scanning capability of the system, a line scan and a 2D spatial scan were performed across the 3 by 3 grid scattering phantom grid, using the ROTOR and two-axis scan mirror to translate the interrogation point. The a/LCI beam was first spatially scanned across a 7.5 mm line on the center row of the grid phantom at 15 locations spaced at 500 µm intervals (Fig. 4(a)). Since the system is limited by the ∼400 µm diameter illumination beam, we defined a safe minimum linear increment that can be detected to be 500 µm. The resulting spectra were then compared to the Mie library and size determination of the scatterers was performed using an ILSA algorithm [2]. Figure 4(b) illustrates the scatterer diameters as measured using spectra collected from the spatial line scan. The one-dimensional scanning capability of the system appears robust, with microsphere diameters across the entire 7.5 mm range exhibiting less than a wavelength of error (< 0.8 µm) in each case, with a mean error of only 0.1 µm across all points. For all subsequent analysis, we defined size determination accuracy to be acceptable if it exhibits error less than the wavelength of the light source (< 0.8 µm). This limit is much smaller than the typical increase in nuclear diameter for precancer (∼2-4 µm) [4]. Representative angle-resolved scattering from 15 and 10 µm microspheres, along with their closest fits computed using Mie theory, are displayed in Fig. 4(c).

![Fig. 4. Line scan validation. (a) A 3 × 3 scattering phantom was constructed with different size polystyrene beads embedded in PDMS. The 2D angular scattering was spatially scanned at 15 locations spaced at 500 µm intervals across a 7.5 mm line. (b) Fitted bead diameters using Mie theory compared to the true diameters across the entire scan range. Dashed lines indicate ± wavelength of error in the fit. (c) Representative scattering profiles and closest fits of the 15 and 10 µm microspheres. Excellent agreement between the theoretical and measured profiles is observed.](image)

Figure 5 shows validation of the 2D spatial scanning capability of the system, using the 3 by 3 grid phantom. Rotation of the ROTOR prism at 0°, 22.5°, and 45° allowed for line scans at 0° (horizontal), 45° (diagonal) and 90° (vertical). Within each line scan, measurements were taken in 2.5 mm linear increments for cubes along the horizontal or vertical axis and ∼3.5 mm increments for cubes along the diagonal, such that a scan was taken at the center of each cube within the grid phantom. The resulting spectra were processed to predict the scatterer dimensions. Absolute and
Fig. 5. 2D spatial scanning validation. The $3 \times 3$ phantom is scanned at the center of each grid with the ROTOR prism rotated at $0^\circ$, $22.5^\circ$, and $45^\circ$, corresponding to scan axis rotation of $0^\circ$ (horizontal), $45^\circ$ (diagonal) and $90^\circ$ (vertical). All scans yielded errors less than a wavelength of light, with all percentage errors less than 3%.

Percentage errors of the resulting fits reveal that all scans yielded scatterer dimensions that were within a wavelength of error, and all fits were within 3% of the true sphere size.

A useful property of our system is the ability to rotate our illumination polarization using the ROTOR, and thus, to interrogate different axes of anisotropic scatterers. By rotating the ROTOR prism to $0^\circ$ and $45^\circ$, we effectively rotated the illumination beam’s polarization to $0^\circ$ and $90^\circ$, respectively.

Fig. 6. Representative 2D angle-resolved scattering from stretched 12 $\mu$m anisotropic scatterers in a TE (vertical) orientation. $\theta_x$ indicates angular scattering collected along the slit and $\theta_y$ indicates scattering along the galvanometer scan direction. Scans were taken with the ROTOR prism at $0^\circ$ and $45^\circ$, corresponding to the system’s polarization axis along the major and minor axes of the scatterers, respectively. Expected sizes are 11.0 $\mu$m along the minor axis and 14.2 $\mu$m along the major axis, which were measured to be 10.5 $\mu$m and 14.5 $\mu$m, respectively. Scattering patterns collected from the major axis exhibit greater frequencies in scattering intensities, indicating a greater size along that dimension.
corresponding to interrogation of either the major or minor axis of the anisotropic scatterers. To validate the system’s sensitivity to anisotropic scattering, angle-resolved scattering spectra were collected to determine the major and minor axes of the spheroidal beads. While Mie theory is most applicable to spherical scatterers, previous studies [12,13] have shown that the Mie approximation is empirically useful for providing an estimate of the spheroidal dimension parallel to the polarization axis. Figure 6 illustrates representative angle-resolved scattering acquired from a single scan for stretched 12 μm spheroidal beads in TE orientation using the ROTOR prism. Representative scattering patterns collected along each axis (i.e., with the polarization oriented along both the major and minor axes of the spheroids) as well as the resulting Mie fits are shown. As expected, scattering along the major axis exhibited greater angular oscillation frequencies, corresponding to a larger size along that dimension. Mie fitting of this single scan verified good size determination along the major and minor axes, with discrepancy between actual and measured size being less than a wavelength for both sample orientations.

Figure 7 shows the results of ILSA for stretched 12 μm and 15 μm spheroidal beads with expected aspect ratios of 0.78 and 0.86, respectively. For both the TE and TM orientations, 5 scans were performed with the polarization axis parallel and perpendicular to the sample, to interrogate both the major and minor axis for each sample orientation. The resulting measurements of the major and minor axes, performed by rotating the polarization of the illumination beam, yielded good size estimates. The average minor and major axes measured for the 12 μm spheroids

Fig. 7. Mie fitting for anisotropic scatterers (n = 5 for each orientation). All bars indicate mean ± standard deviation. Boxplots on the left plot Mie fitting results and bar plots on the right show average absolute error of the resulting fits to the true dimensions. (a) stretched 12 μm beads with an expected aspect ratio of 0.78 (measured from phase images). Using Mie fitting of the major and minor axes dimensions, the measured aspect ratio is 0.74 ± 0.04 in TE orientation and 0.75 ± 0.04 in TM orientation. (b) stretched 15 μm beads with an expected aspect ratio of 0.86. Mie fitting of the two axes yielded a measured aspect ratio of 0.86 ± 0.03 in TE orientation and 0.86 ± 0.03 in the TM orientation.
were $10.8 \pm 0.6 \mu m$ and $14.5 \pm 0.2 \mu m$ in TE orientation and $10.8 \pm 0.6 \mu m$ and $14.4 \pm 0.1 \mu m$ in TM orientation. Similarly, average minor and major axes measured for the $15 \mu m$ spheroids were $13.9 \pm 0.2 \mu m$ and $16.2 \pm 0.5 \mu m$ in TE orientation and $14.1 \pm 0.3 \mu m$ and $16.4 \pm 0.3 \mu m$ in TM orientation. Subsequent computations yielded accurate aspect ratios (0.74 $\pm$ 0.04 in TE orientation and 0.75 $\pm$ 0.04 in TM configuration for the $12 \mu m$ spheroids; 0.86 $\pm$ 0.03 in TE configuration and 0.86 $\pm$ 0.03 in the TM configuration for the $15 \mu m$ spheroids) compared with their ground truth values of 0.78 and 0.86 as measured using QPM.

In addition to demonstrating the ROTOR prism’s ability to control polarization, and thus interrogate various axes of anisotropic scatterers, spatial scanning further augments the capacity of our device to examine spatial variation in anisotropic structures. Validation of spatial scanning capabilities with anisotropic scatterers is shown in Fig. 8. A 2 by 2 grid phantom was constructed using various spherical and spheroidal microspheres, with the spheroidal samples orientated at $+45^\circ$ and $-45^\circ$. Figure 8(a) illustrates the architecture of the grid phantom, which enabled validation of the device by performing diagonal line scans using the ROTOR prism. By rotating the prism at $22.5^\circ$ and $-22.5^\circ$, the scanning and collection axes are rotated to $45^\circ$ and $-45^\circ$ to measure angular scattering along the various dimensions of the polystyrene beads. While a line scan across grids 2 and 4 represent a change in spherical scatterer size, a similar scan across grids 1 and 3 represents a change in orientation of equal sized spheroidal scattering objects. Due to the nature of the scanning system and polarization orientation, our device is sensitive to both changes in size and orientation. Figure 8(b) shows the resulting Mie fits for the spectra collected from the grid phantom, with “actual size” referring to the axis interrogated by the system, in this case the major axis in grid 1 and the minor axis in grid 3 for the anisotropic scatterers. Incorporation of the spatial scanning mechanism into a/LCI preserved good sensitivity to anisotropic scatterers, with all average errors less than $\lambda$ and the largest single scan percent error measured at only 3.5%.

**Fig. 8.** Validation of spatial scanning with anisotropic scatterers. (a) A 2 $\times$ 2 phantom was constructed using spherical (grid 2: 15 $\mu$m; grid 4: 10 $\mu$m) and 15 $\mu$m spheroidal microspheres (grid 1: 16.6 $\mu$m major axis; grid 3: 14.3 $\mu$m minor axis). Spheroidal beads were oriented at $45^\circ$ to demonstrate the sensitivity of our system to a change in orientation of anisotropic scattering objects. The ROTOR prism was rotated at $22.5^\circ$ and $-22.5^\circ$, and 3 scans were taken at each of the 4 grids in the phantom, with the computed scatterer sizes averaged for each grid. (b) Resulting fits from the collected angle-resolved scattering. Average errors were all sub-wavelength (less than 0.8 $\mu$m) and the largest single scan percent error was 3.5%, demonstrating that our system is sensitive to changes in both scatterer size and scatterer orientation.
4. Discussion

In this work, we have designed and validated a scanning system for multidimensional collection of light scattering, enabling measurement of 2D scattering data from each point within a three-dimensional volume. The incorporation of the ROTOR prism and a gimbal-mounted scan mirror facilitated both line and area scanning, while depth resolution was obtained using low coherence interferometry. Applying this combined scanning system to various optical phantoms shows utility for sample morphology and anisotropy analysis, especially for complex samples which vary spatially in scatterer dimension or orientation. It is our hope that this increased utility will prove useful for evaluating biological tissues, whose microscopic nuclei often change size and orientation as an early indicator of pathology [13,21–23].

Samples consisting of polystyrene beads embedded in PDMS were used to validate spatial scanning. We demonstrated sub-wavelength accuracy in scatterer size determination when using this scheme to scan across a 7.5 mm range. Angular scattering collected along the major and minor axes of spheroidal beads also allowed for accurate computation of their aspect ratios. These results agree with previous studies [13,14], which showed that Mie theory may still be a suitable model for analysis of spheroids. During our analysis, we observed consistently accurate (errors < \( \lambda \)) fits along the minor axis for both TE and TM orientations. However, fits along the major axis, at least for the two phantoms tested, seemed to slightly favor the TM orientation. This is reflected in Fig. 7, which shows improved sizing capability along the major axis for TM orientations. This potential trend is magnified as the bead becomes less stretched (larger aspect ratio from Figs. 7(a) – 7(b)), and interestingly, the errors along the minor axis also decreases. A possible explanation can be attributed to the imprecision of using Mie theory for spheroidal scatterers. Beads with larger aspect ratios as defined here are more spherical in shape, which will yield more favorable fitting under the assumption that the scatterer is a Mie object. Similar observations when sampling the major axis have been made previously [13–15], though more analysis is needed to better understand the underlying effects of modeling anisotropic scattering using Mie theory. Nevertheless, our results still open the potential for application of this system to accurately assess structure across a large area while maintaining sensitivity to changes in scatterer geometry.

The scanning system’s sensitivity to anisotropic scattering is important when considering its application to measuring biological samples. Nuclear geometry and deformation often serve as important markers of cellular physiology and tissue structure [1,24–27]. a/LCI specifically has been shown to accurately profile mechanically induced changes in nuclear morphology of murine macrophages, as well as osmotically induced changes in porcine chondrocytes [12]. For retinal imaging in particular, anisotropic structural features of the nerve fiber layer (NFL) [28–31] could be explored with this scanning system in the future. Our recent study [10] used this 2D a/LCI system to sample various locations on the retina of a triple transgenic mouse model for Alzheimer’s disease, and extracted tissue textural information through light scattering analysis. The results showed that 2D a/LCI was able to detect changes in the light scattering of the retina as a potential biomarker of Alzheimer’s disease. With the addition of the spatial scanning mechanism from this work, multiple layers of the complex retinal tissue can now be analyzed at more quadrant-specific locations to generate large magnitudes of useful light scattering data over several dimensions.

Due to the addition of distal scanning optics to the 2D a/LCI system, the range of detected scattering angles is somewhat constrained. This effect has been outlined previously [11], where the gimbal-mounted scan mirror reduces the aperture when it is tilted to enable line scanning. Without the spatial scanning mechanism, the original 2D a/LCI system exhibited a 21° (~10.5° to 10.5° relative to backscattering) angular range along the dimension of the spectrometer slit, and a 34° (~4° to 30°) range along the galvanometer scan dimension. Due to the geometry of the spatial scanning scheme, the aperture condition reduces the range of scattering angles to be
approximately 20° (-6° to 14°) along the galvanometer scanning dimension but maintains the 21° angular range along the slit dimension. In this work, we are using only the angular range along the galvanometer scan axis for Mie fitting and thus, this implementation slightly reduces the amount of useful scattering information acquired. While a reduced angular range can modestly affect the accuracy of ILSA [32], our results indicate that our device was quite capable despite the reduced angular range, with no obvious loss in size determination accuracy.

To conclude, a scanning a/LCI system was presented to rapidly and accurately acquire 2D angular scattering information over a large sample area. Incorporation of the ROTOR prism and scanning mirror, in addition to the current angular scanning scheme in 2D a/LCI, enabled both line and area scanning without sample or probe translation. The system scanning capabilities were validated using measurements from various complex optical phantoms, illustrating the ability for imaging samples with anisotropic scattering. Future efforts will emphasize on miniaturization and automated control of the scanning elements for a faster and more viable method of investigating microscale alterations to enable measurements across wide areas of biological tissue.

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