Noncontact mechanical mapping of the optical nerve head with Brillouin microscopy

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INTRODUCTION

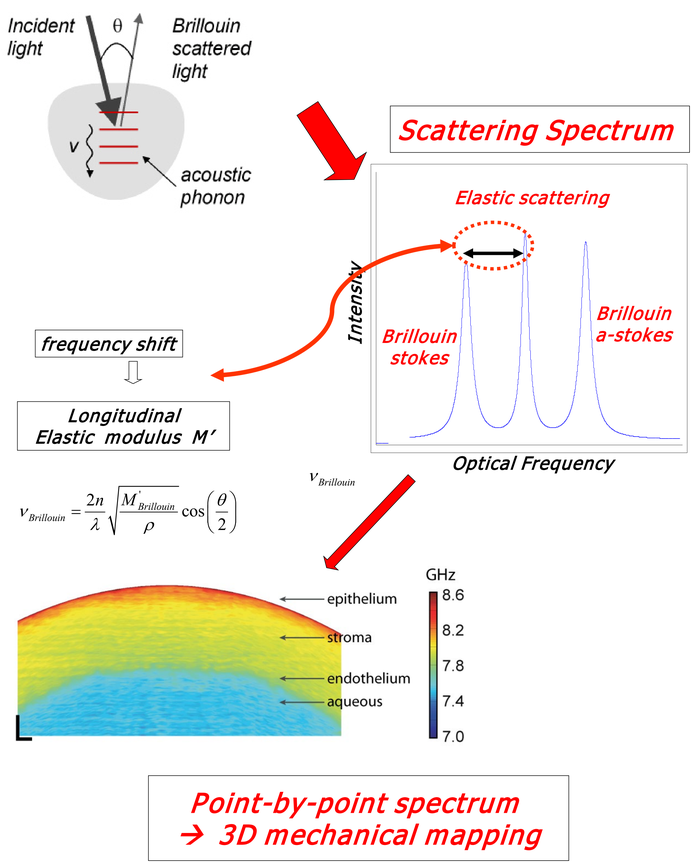
Glaucoma is characterized by chronic progressive degeneration of the optic nerve in the eye. The mechanism of glaucoma development is not understood, but a hallmark of glaucoma is the cupping or excavation of the optical nerve head (ONH). It is widely believed that these ONH structural changes are the result of a mechanical imbalance at the back of the eye between elevated intraocular pressure (IOP) and the stiffness of the ONH (and the lamina cribrosa (LC) within it) and its surrounding scleral tissue. Indeed, the evidence for a biomechanical role in glaucoma development is strong: histology studies show that mechanical stress from elevated IOP leads to remodeling of the extracellular matrix in the optic nerve head as well as mechanically-related increased collagen content and stiffness of the connective tissue; ONH zones with largest deformation present the most retinal ganglion cell axon injury. Tissues in the back of the eye are mechanically connected and IOP-induced strains on the ONH depend on the stiffness of the surrounding sclera; the ONH has been suggested as a weak spot, mechanically more susceptible to damage than surrounding sclera.

Mapping the mechanical properties at the back of the eye is crucial to test these hypotheses and improve our understanding of the mechanisms by which elevated IOP affects the tissues of the ONH. This could also enable testing new therapeutic procedures based on altering the stiffness of ONH/scleral tissues. Moreover, this improved understanding combined with the development of a noninvasive imaging technology assessing the biomechanical properties of the ONH/sclera will improve our ability to diagnose and manage eyes at risk of glaucoma based on the novel stiffness parameters.

Mechanical measurements to retrieve material stiffness generally require to apply a stress to a sample and measure its deformation; thus, standard tests are macroscopic and destructive i.e. not suitable for measurements within intact eyes. In the past years, the investigation of the mechanics of the eye posterior segment has been limited to histology, measuring structural changes after acute increases in IOP and to second harmonic generation/optical coherence tomography, which avoid tissue sectioning, but still only image structural deformations in the tissue architecture of the ONH and sclera in response to increases in IOP.

We have developed a novel all-optical approach for biomechanical testing of biological tissue using Brillouin light scattering. However, so far, we have only applied Brillouin microscopy to transparent ocular tissues such as cornea and lens. To move the technology to the back of the eye, here we propose to combine it with Adaptive Optics and demonstrate its' ability to measure ONH/sclera region.

Brillouin Microscopy

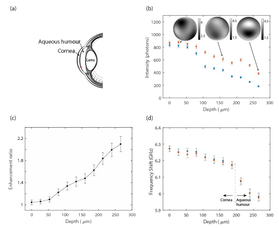


Point-by-point spectrum → 3D mechanical mapping

Adaptive Optics- Brillouin Microscopy (AO-BM)

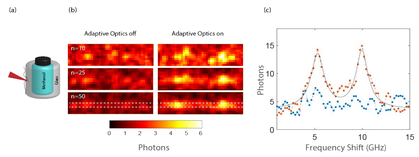
We successfully developed an adaptive optics (AO) configuration designed for Brillouin scattering spectroscopy to engineer the incident wavefront and correct for aberrations. Our configuration does not require direct wavefront sensing and the injection of a 'guide-star'; hence, it can be implemented without the need for sample pre-treatment.

Aberration corrections



Demonstration of AO-enhanced Brillouin measurements. (a) Structure of the porcine eye and the scanned region (red line). (b) The intensity of the Brillouin signal at various depths 27 microns apart throughout the cornea and aqueous humor of a fresh porcine eye. The optimization was performed at each location and three representative phase maps are shown. At higher depths the signal was enhanced by more than 2-fold when AO was applied (orange dots) vs the uncorrected signal intensity (blue dots). (c) Signal enhancement as a function of measurement depth. (d) The Brillouin shift at every location in (b), with (orange dots) and without (blue dots) AO correction. Error bars represent the standard deviation of twenty measurements.

Low- SNR measurements

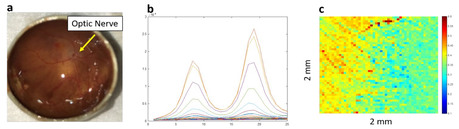


AO-enabled measurements at low SNR: (a) Schematics of the experimental configuration. (b) Average of many acquired spectra of glass without AO (left) and with AO (right). A Gaussian filter (3x3 pixel, σ=2) was applied to the data. (c) Line plot of the area between the white dashed lines in (b). The spectrum of glass is not visible even when 50 frames are averaged if AO is not applied (blue); however, with AO correction on, the spectrum can be easily measured (orange).

Overall, we demonstrated 2.5-fold enhancement in Brillouin signal strength and 1.4-fold improvement in axial resolution because of the correction of optical turbidity

Brillouin imaging of ONH-Sclera region

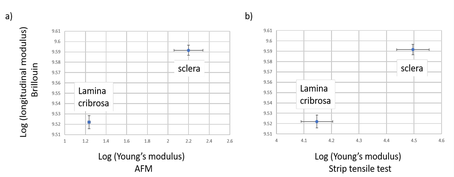
To demonstrate that our technology has enough mechanical sensitivity to measure mechanical changes relevant to glaucoma, we used the posterior pole of a glaucoma human donor (Fig. 4a). In the ONH area, our technology is able to retrieve high quality scans at a depth more than 100 microns (Fig. 4b). Mapping the ONH area, we can clearly detect the boundary between the sclera (stiff, on the left) and the ONH (soft, on the right).



Brillouin image of a glaucoma donor ONH region. a) Photograph of the posterior pole of the human donor sample; b) Brillouin signal strength at different depths; c) Brillouin map of the ONH region.

Validation

Brillouin spectroscopy measures the longitudinal modulus of material at GHz frequency. In biological tissue, due to low compressibility and modulus frequency dependence, this signature is different from the traditional Young's modulus. We have not been able yet to perform a one-to-one quantification of Brillouin vs Young's modulus on the same sample. However, we quantified the Brillouin shift values of lamina cribrosa region vs peripapillary sclera and compared our results with previous data in the literature. The comparison is only qualitative at this stage because it compares sheep samples (Brillouin) with human samples (AFM and tensile tests). However, it confirms the overarching point that in normal eyes the sclera surrounding the ONH is stiffer than the lamina cribrosa region, and that Brillouin signatures are clearly able to differentiate the two regions based on their mechanics and in the right direction.



Comparison of Brillouin-derived longitudinal modulus and traditional mechanical measurements. (a) Brillouin measured in our lab is compared with AFM results from IOVS 53, 2960 (2012); (b) Brillouin measured in our lab is compared with tensile tests results from IOVS 46, 1286 (2005).

CONCLUSIONS

- The mechanism by which elevated IOP levels induce degradation of the optical nerve head (ONH), the initial site of injury in glaucoma, is not yet understood but it is widely suspected that a main culprit is the lack of mechanical balance between IOP-induced strains and the stiffness of ONH and surrounding sclera. However, we do not have viable technology to test the stiffness of the back of the eye.
- We have developed a potential solution to this need, an all-optical approach to mechanical measurements using Brillouin light scattering. Our technology is already in human clinical trials for transparent ocular tissues (cornea, lens). In this grant we extended the reach of Brillouin microscopy to the back of the eye by using Adaptive Optics (AO).
- We have demonstrated that our new AO-Brillouin microscope enables imaging opaque tissues such as ONH and sclera, we have validated the instrument to show we can measure the expected mechanical differences at the back of the eye.
- Thus, this pilot grant provided proof-of-principle for the use of this new technology in glaucoma animal models to measure novel functional/mechanical parameters of the sclera and ONH.

NEXT STEPS

- Glaucoma is characterized by chronic progressive degeneration of the optic nerve in the eye. It is widely believed that the structural changes of the optical nerve head (ONH) associated with glaucoma are the result of a mechanical imbalance at the back of the eye between elevated intraocular pressure (IOP) and the stiffness of the ONH (and the lamina cribrosa (LC) within it) and its surrounding scleral tissue.
- Thus, mapping the mechanical properties at the back of the eye is crucial to test this important hypothesis and improve our understanding of the mechanisms by which elevated IOP affects the tissues of the ONH. This could also enable testing new therapeutic procedures based on altering the stiffness of ONH/scleral tissues.
- Moreover, this improved understanding combined with the development of a noninvasive imaging technology assessing the biomechanical properties of the ONH/sclera will improve our ability to diagnose and manage eyes at risk of glaucoma based on the novel stiffness parameters.
- Our technology results are impressive and have resulted in two publications. The application to glaucoma is still in progress. Our results are very encouraging but need to be confirmed with more samples and with auxiliary imaging modalities co-localized with Brillouin microscopy.
- We maintain an active protocol with NDRI tissue bank to provide samples of normal vs glaucoma posterior poles. This will enable to complete the study of glaucoma vs normal tissue to publish a paper that will introduce the technology to glaucoma researchers.
- This project has developed the first instrument to measure mechanical properties of ONH and sclera in situ. Once we collect definitive data on the difference in stiffness between healthy and glaucoma conditions, we will be able to perform power and sample-size calculations for future studies.
- Larger studies will test the hypothesis that ONH/sclera mechanical changes under elevated IOP are crucial for glaucoma development. To test this, small animal studies will be proposed where biomechanical models can be validated by Brillouin tissue stiffness measurements and deformation maps under controlled IOP. These studies will guide the development of novel therapeutics to repair scleral mechanical imbalance and may lead to a diagnostic modality to grade the risk of glaucoma development based on ONH/sclera stiffness.

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