High-resolution macroscopy uses nonlinear optical imaging

A novel approach to generating large-scale, 3D images allows tracking of individual collagen fibers and characterization of their structural organization.

Tissue shape and rigidity depend on the biomechanical strength of the material, which is defined by its collagen content and organization. To understand how collagen controls strength, better knowledge of its macroscopic organization is required. We have developed a novel approach to image collagen using high-resolution macroscopy that combines the sharpness of nonlinear optical (NLO) imaging with automated image-acquisition techniques to generate macroscale 3D data sets with microscopic detail.

Traditional imaging methods suffer from a lack of either detail or context when scanning large volumes or using high-resolution macroscopy to study small regions of interest, respectively. The sharp imaging enables resolving the substructure. However, the relation to the macrostructure and how changes in microstructure can influence macroscopic tissue parameters remain poorly understood. High-resolution macroscopy addresses these issues by allowing researchers to generate large-scale, 3D image mosaics (several millimeters or even centimeters across) while retaining the crispness obtained through NLO imaging.

NLO microscopy is unique in its ability to produce second-harmonic-generated (SHG) signals from noncentrosymmetric structures such as collagen when excited by very-fast-pulsed (such as femtosecond) laser light.1–4 This imaging paradigm exploits a nonlinear optical phenomenon by which two longer-wavelength photons interact simultaneously with collagen structures to form a single photon that has exactly half the wavelength and twice the energy of the two initial light particles. The process is structure specific and does not require stains or dyes. Thus, collagen fibers, which represent the predominant and most important structural element in the human body, can be excited by IR femtosecond-laser pulses to emit visible SHG light.

Figure 1. 3D reconstruction based on high-resolution macroscopic images of the human optic nerve head comprising about 5 billion voxels (3D pixels).

Here we report on two studies using SHG high-resolution macroscopy to generate large-scale 3D reconstructions of the collagenous matrix organization in the human cornea and optic nerve heads. We cut cross-sectional slices of approximately 0.3mm thick along the central meridian of human corneas in the superior-inferior direction and imaged them using a Zeiss laser-scanning microscope. Second-harmonic light was generated using a titanium-sapphire femtosecond laser. We used the same system to scan 2μm serial sections of the human optic nerve head.

Tissues are scanned as a series of individual z-stack images (a 3D data set composed of a series of uniformly spaced image planes throughout the 3D object). A computer-controlled stage moves the sample after each scan, generating a mosaic pattern of overlapping blocks. Depending on the size of the cross section, high-resolution macroscopy images of the cornea are 50–70 megapixels per slice. Current 3D stacks consist of 25–50 slices each. Figure 1 shows a high-resolution macroscopy-based reconstruction of the human optic nerve head, consisting of 400 planes.

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Figure 2. 3D reconstructed collagen fiber (purple) extracted from a 6 gigavoxel high-resolution macroscopy image of the human cornea. The individual collagen fiber was followed for 1mm along the anterior cornea. The fiber shows multiple branching within the anterior cornea and insertion into the anterior limiting lamina (green) at multiple sites. This structural organization is suggestive of the trusses or brackets used to stabilize buildings or bridges.

at 12 megapixels each for a total of approximately 5 gigavoxels (3D pixels).

Depending on the application, these images can either be viewed in full or downsampled to reduce the file size. Because of the high resolution and specificity of the SHG signal from collagen, individual fibers can be semiautomatically separated from the background. Using Amira visualization software, the structural organization and complexity of individual fibers can be reconstructed in 3D and overlaid on the original images (see Figure 2).

These reconstructions make it possible to study both micro- and macrostructure simultaneously by allowing us to traverse scales at will, zooming in and out of areas of interest across the entire sample. The resulting data sets can be further processed for quantitative analysis or for 3D visualization and reconstruction of macroscopic portions of the tissue at microscopic resolutions.

High-resolution macroscopy enables detailed investigations of whole tissues and allows researchers to analyze data on a much larger scale while still retaining the necessary magnification to resolve the fine details associated with tissue substructure. In doing so, the connection between macroscopic parameters and the microscopic elements that form tissues can be studied. The level of detail is limited only by the diffraction barrier.

The studies presented here focus on portions of the human eye. Yet they are merely one of many possible applications for high-resolution macroscopy. Given the abundance of collagen throughout the human body and the option of using other imaging modalities such as two-photon excited fluorescence, this imaging paradigm can be used to study other organs or tissues such as the brain or liver, and to reconstruct large portions or even entire tissues at extremely high resolution in 3D. Our future work will be using high-resolution macroscopy to study the cornea and optic nerve and relate the biomechanical properties of the tissues to their large- and small-scale structural organization.

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