Computational adaptive optics for high-throughput volumetric optical coherence microscopy

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Image formation based on computation and aberrated optical design provides a promising approach for volumetric imaging studies of biological dynamics at cellular and sub-cellular resolution.

High-throughput optical imaging is playing an increasingly important role in biological imaging. The technique enables biological dynamics to be studied at a variety of different spatiotemporal scales.¹⁻³ One area that is poised to benefit from this capability is the study of collective or emergent behavior in embryonic development, tissue regeneration, and cancer.⁴⁻⁵ Existing modalities for high-speed volumetric imaging at cellular or sub-cellular resolution are typically based on the detection of fluorescence signals. Consequently, they are subject to photobleaching and phototoxicity constraints and, as a result of this, have limited scope in settings that preclude the use of exogenous contrast agents (e.g., many clinical settings).

High-speed methods such as optical coherence tomography (OCT) could help to provide label-free imaging of biological dynamics, thereby filling this gap in biological imaging. Recent advances in ultrahigh-speed OCT have enabled the acquisition of volumetric datasets at video rates (i.e., one volume in 25ms).⁶ However, combining this high-throughput acquisition with cellular-resolution optical coherence microscopy (OCM) presents significant challenges. The main factor limiting the volumetric acquisition rate of OCM is the rapid degradation of resolution and signal strength at increased distance from the point of optical focus. There are a number of hardware approaches to volumetric cellular-resolution OCM. These include the acquisition of multiple OCM datasets, each with a different focus depth, and subsequent synthesis of a single ‘in-focus’ volume.⁷⁻⁹ The illumination and collection beam can also be engineered to provide an extended focal region.¹⁰ Of these approaches, focus-scanning methods offer the highest

![Image](image_url)

**Figure 1.** Volumetric optical coherence microscopy (OCM) and CAO-OCM (computational adaptive optics-OCM) reconstructions of a grape sample, imaged with an astigmatic system. (a) An image of the volume obtained via OCM and (b) the corresponding CAO-OCM reconstruction. Volume renderings show a depth range spanning from 220 to 1130µm below the sample surface. These reconstructions and volume renderings were performed offline. (c–e) Real-time en face OCM planes from three depths (380, 730, and 1030µm, respectively), obtained using a graphics processing unit. (f–h) The corresponding CAO-OCM en face planes, showing simultaneous real-time correction of defocus and astigmatism. Scale bars: 100µm.

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image quality. However, the total volumetric acquisition times can be on the order of 10 min,8 or require the acquisition of more than 10 volumetric datasets before a single in-focus volume can be obtained.9 This approach is therefore impractical for imaging biological-system dynamics on timescales of minutes or less. The use of Bessel beam illumination, on the other hand, can provide cellular-resolution OCM simultaneously over a given depth range.10 However, this approach is yet to demonstrate cellular-resolution OCM over a depth range spanning more than a few hundred micrometers.

Our approach for high-throughput volumetric OCM is designed to provide uniform high resolution and signal-to-noise ratio (SNR) over depth without requiring a time-consuming scan of the focus. We have employed an astigmatic optical system to equalize signal collection versus depth, combined with CAO to compensate the resolution penalty that usually accompanies imaging with astigmatic optics.

Interferometric detection with OCT allows the complex optical field that is backscattered from within a sample (due to spatial variations in the refractive index) to be measured. An OCT dataset can therefore be treated as a digital hologram.11 As in digital holography,12, 13 this allows optical image formation and optimization to continue after data acquisition.14–16 CAO provides a method by which aberration correction can be carried out via numerical manipulation of the Fourier domain of an OCT dataset.17 This is analogous to the operation of hardware adaptive optics (HAO), in which the phase of the Fourier domain signal is physically manipulated at the time of imaging. As with HAO, CAO can be applied in ‘sensorless mode’ by using image metrics to optimize the correction,17 in ‘guide-star mode’ (in which point-like scatterers in the sample are used to sense the aberrations present),18, 19 and via the implementation of a sub-aperture wavefront-sensing method.20

Figure 1 presents a volumetric CAO-OCM reconstruction of a grape sample. This reconstruction is based on a 3D dataset that we acquired using a spectral-domain OCT system (with ~2 μm axial resolution) and astigmatic sample-arm optics (with aberration-free focal plane resolution of ~3 μm). Our astigmatic optical system provides enhanced photon collection at two axially separated line foci, thereby equalizing SNR versus depth. Compared to the OCM volume, the CAO-OCM reconstruction shows grape cellular structures at significantly higher resolution throughout a volume spanning a depth range of nearly 1 mm. The figure also shows real-time CAO-OCM, implemented on a graphics processing unit.21 Our system achieves simultaneous correction of defocus and astigmatism for three en face planes at user-selectable depths within the volume. This highlights an advantage that CAO has compared to HAO, which itself would require a highly complicated multi-conjugate setup to achieve a similar multi-plane correction.22

We have found that CAO can also be leveraged to provide an alternative approach by which to address the problem of reduced signal collection with increased distance from the point of focus.17, 23 The ability to correct aberration effects after data is acquired relaxes the typical aberration-free design constraints on optical systems. These systems can instead be designed for optimal signal collection versus depth. We compared the resolution and signal strength of reconstructed data acquired with a standard Gaussian beam—see Figure 2(a)—to our astigmatic optical system: see Figure 2(b). Although the use of CAO-OCM reconstruction on both of these systems offers comparable resolution—see Figure 2(c)—the astigmatic system offers an overall SNR advantage for imaging over a relatively large (~1 mm) depth range: see Figure 2(d). This advantage occurs as a result of the ability to adjust the axial separation of the two astigmatic line foci and thereby enhance photon collection at

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Maximum-intensity projections of cross-sectional images from a volumetric dataset of a titanium-dioxide resolution phantom, obtained using (a) a standard Gaussian beam and (b) our astigmatic beam. Scale bars: 150 μm. (c) After CAO-OCM reconstruction, both the astigmatic and standard Gaussian optical systems support similar resolution. (d) A comparison of signal-to-noise ratio (SNR) vs. depth, demonstrating the more uniform depth-dependent signal that is obtained with an astigmatic beam compared to that achieved with a standard Gaussian beam. The depths marked with dotted blue lines correspond to the dotted yellow lines in (a) and (b). All results shown have undergone CAO-OCM processing.
these depths. Although photon collection is reduced at the nominal Gaussian beam focal plane, an SNR improvement can be seen at both shallower (<800 μm) and deeper (>1200μm) positions in the sample, resulting in an overall reduction to the signal dynamic range. This increases the depth range over which volumetric imaging with uniform high resolution and SNR can be achieved.

We have shown that CAO can help facilitate large-volume OCM imaging by leveraging aberrated optical-system design to optimize photon collection across all depths. This imaging paradigm could enable high-throughput volumetric OCM with isotropic cellular resolution over millimeter-scale 3D fields of view. With further work, this could enable the use of OCM for volumetric imaging of emergent behavior in embryonic development, tissue regeneration, and cancer. The system could also serve as a multimodal bridge by which to connect label-free methods and existing high-throughput fluorescence-imaging techniques. In our future work, we aim to leverage high-throughput volumetric OCM for the study of collective cell migration dynamics in 3D environments.

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References


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