SYNTHETIC APERTURE OPTICS

Technical Note

Introduction

Advances in high resolution light microscopy methods are opening exciting new frontiers for direct visualization of biologically important target molecules in their native environment. Quantification of individual molecules of DNA, RNA, and proteins is now possible at the single cell level while preserving important contextual information such as localization and morphology.

However, current high resolution microscopy methods for single-cell interrogations rely on expert practitioners, and are largely limited to small-scale research only, due to the inherently slow rate of data capture (~10 cells/min) of conventional high Numerical Aperture (NA) oil immersion lenses. The speed restriction in conventional high resolution optical imaging is imposed by the physics of the lens; that is, resolution cannot be improved without dramatically sacrificing field of view (FOV), depth of field (DOF), and working distance (WD) of imaging (See Figure 1 in the next page).

As the detection and quantification of biologically important target molecules inside cells and tissues become increasingly important in clinical research, personalized diagnostics, and drug development, there is a clear need for an improved high resolution microscopy platform that dramatically improves the speed and the ease of acquiring single-cell, single-molecular data.

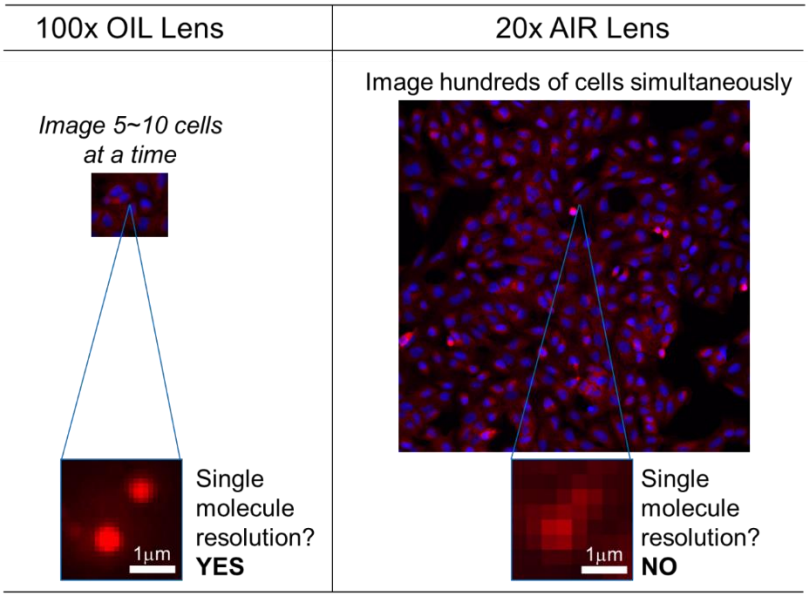

Figure 1. In conventional oil-immersion microscopy, the cost of high-resolution is paid with a limited field of view. This allows only ~5-10 cells in each field of view. In contrast, many cells can be imaged using a 20X air lens, however the resolution is insufficient for detailed analysis at the single cell level. This classical trade-off of resolution for field of view has limited optical microscopy to low-throughput analysis of biological data acquisition.

Our Solution

SAO is a next-generation fluorescence microscopy solution based on a 20x air lens with NA of 0.45 yet achieves lateral resolution comparable to a conventional 60x or 100x oil immersion lens, enabling ~25 times larger field of view (FOV), ~10 times greater depth of field (DOF), and ~60 times longer working distance (WD) than a 100x oil lens.

Fluorescently labeled target molecules in cells and tissues can now be imaged with an unprecedented combination of resolution, FOV, and DOF, leading to an approximately 100 times expansion of space that can be simultaneously imaged without repositioning the sample in x, y, or z.

The instrument is based on Optical Biosystems' proprietary optical imaging technology called Synthetic Aperture Optics (SAO). The term SAO derives from the fact that active illumination can be used to produce a *synthetic* NA that is significantly larger than the physical NA of the lens.

SAO is a breakthrough optical imaging method that decouples the resolution from the physics of the lens, allowing higher resolution without sacrificing throughput and ease of use. As illustrated in Figure 2, in SAO, a sample is illuminated by a sequence of high resolution light patterns formed by the interference of laser beams and a low resolution lens (e.g., 20x air lens) acquires a series of low resolution images.

The sequence of brightness values reported from a single pixel of a CCD imager encodes the target contrast pattern with sub-pixel resolution. Fourier domain components at spatial frequencies contained in the probing illumination patterns are recovered from the pixel brightness sequence to reconstruct the high-resolution image.

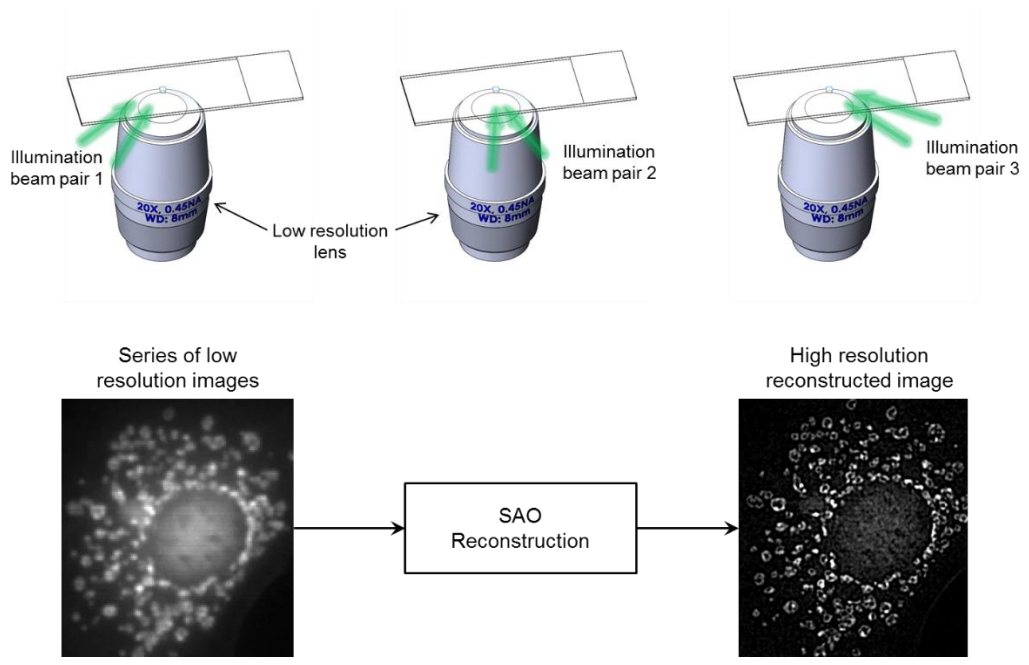

Figure 2. The SAO Concept. The resolution of a 20X air objective can be dramatically improved using SAO technology. The sample is illuminated by a series of high resolution light pairs (green arrows) that are created by the interference of the excitation laser beams. The low resolution lens captures the series of images, which are then reconstructed using proprietary software to generate a single image that is equivalent to the resolution of a 100X oil immersion objective. This combination of illumination and reconstruction breaks the long-standing resolution limits of long working distance air objectives.

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To demonstrate the resolution of SAO, 40nm beads were imaged and compared to traditional oil-immersion microscopy (Figure 3). The width of the point spread function, a measure of resolution, was equivalent to a 100X oil-immersion objective after reconstruction. In a biological application (Figure 4), microtubule structures are clearly defined with SAO and match the clarity of a 100X oil-immersion objective but without using oil.

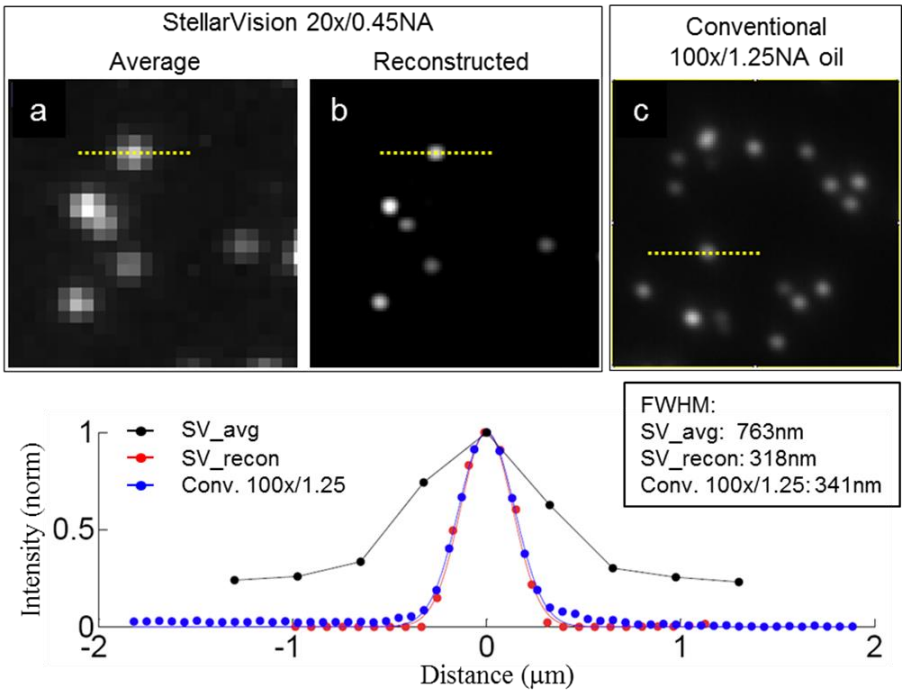

Figure 3. To demonstrate the resolution of SAO as compared to traditional, oil-immersion microscopy, 40nm diameter fluorescent beads (F8795, Thermo Fisher Scientific, Waltham, MA) were imaged by SAO and a conventional oil immersion lens. (a) Average of SAO's 12 raw low-resolution images, producing an image similar to a conventional wide-field image with the same 20x lens. (b) SAO reconstructed image for the same region as in (a). (c) Conventional wide-field image of a different region of the same slide acquired with 100x/1.25 NA oil immersion lens. The plot shows intensities along the yellow line for each of the three images and full width half maximum (FWHM) of a single 40 nm diameter bead.

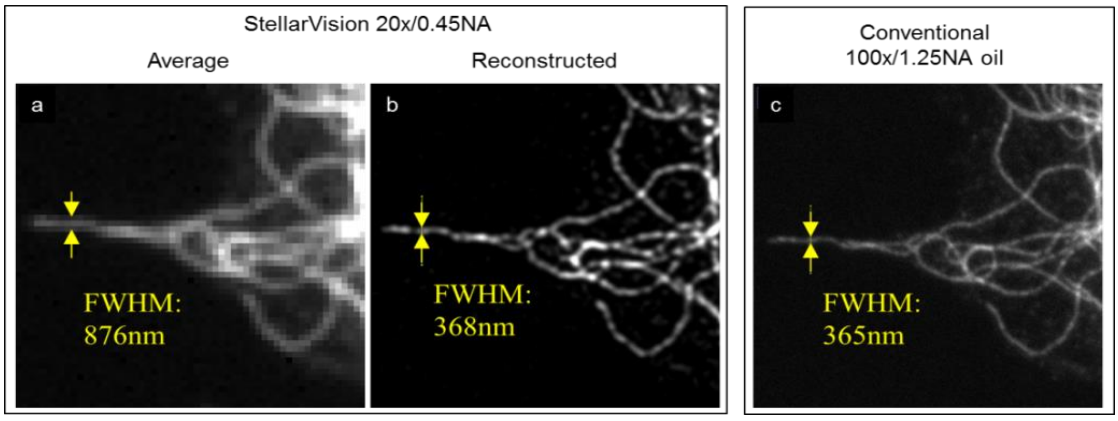

Figure 4. The resolution of SAO matches that of a conventional oil-immersion objective but does not require oil. Above, the full width half maximum of a microtubule polymer in bovine pulmonary artery endothelial cells was imaged with SAO and compared to a conventional microscope with a 100x/1.25 NA oil immersion lens (Slide: F14781, Thermo Fisher Scientific, Waltham, MA). The resolution of SAO's 20X air objective is improved to the equivalent resolution of a 100X oil-immersion

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The SAO Advantage

Optical Biosystems has integrated it's SAO technology into an user-friendly and high-throughput system. SAO enables routine interrogation of biological samples across large areas at single-cell resolution, offering a clear advantage over traditional imaging approaches (Figure 5).

- A single oil-free 20X objective captures the equivalent resolution of a 100X oil objective
- Long working distance allows easy integration with any sample format
- High depth of field obviates most Z-stacking
- Wide field of view provides more cells per image and more powerful statistics
- Fully automated computer control allows remote operation from anywhere
- Compatible with the most common sample formats and fluorophores
- Non-expert ease of use enables widespread user accessibility
- Limited required training reduces core lab staff effort

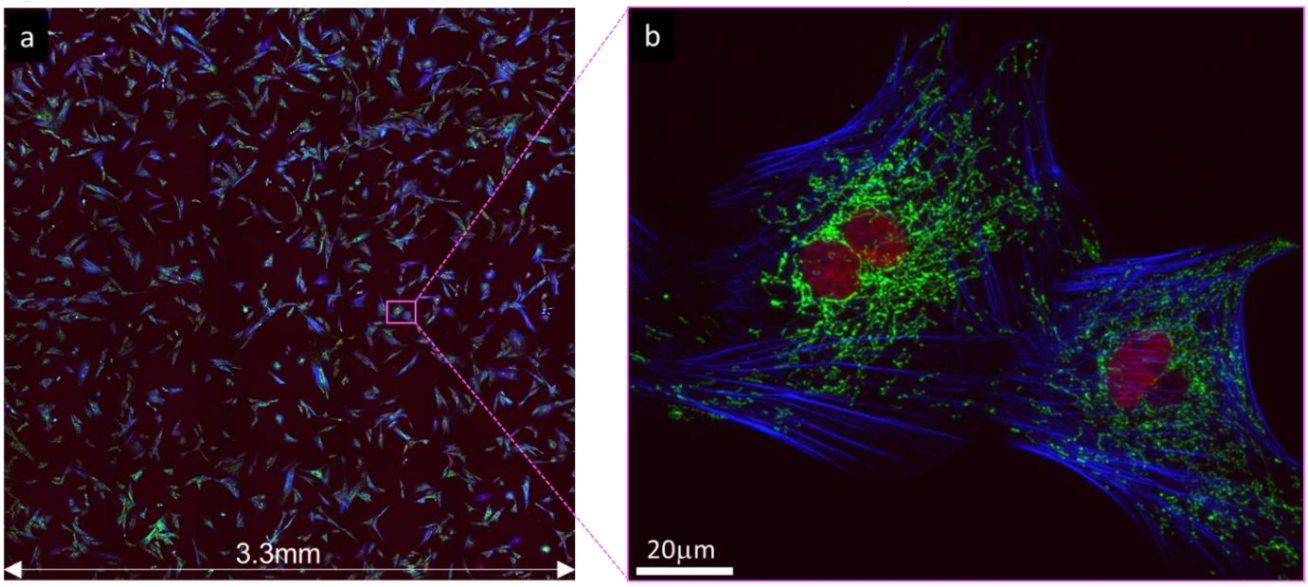

Figure 5. One hundred fields of view (10 x 10) covering a 3.3mm x 3.3mm area of a coverslip were automatically imaged on three channels using SAO at oil immersion resolution in about 10 minutes. Muntjac skin fibroblast cells were stained for actin (Alexa Fluor® 488 phalloidin, blue), mitochondria (Alexa Fluor® 555, green), and nuclei (TO-PRO®-3, red). The image to the right is a crop of the same image showing the high resolution signal across the entire area.

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