Liquid crystal devices heighten resolution in biological cell microscopy

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Using liquid crystal devices to convert Gaussian beams to vector beams dramatically improves confocal and multi-photon laser microscopy in living tissues.

Laser scanning microscopy (LSM) is an essential tool for life-science and medical researchers because it enables them to observe events occurring in live cells or tissues. However, the spatial resolution of the technique is limited by the diffraction of light, which in conventional LSM measures ~300nm, and it relies on the size of the focal spot of the excitation laser light. In theory, the area of the focal spot cannot exceed roughly half the excitation wavelength. Despite recent developments in LSM that have created new applications for biology, these spatial resolution limitations make LSM undesirable for many researchers who want to elucidate the live dynamics of intracellular biomolecules and components.

Consequently, there is increasing demand for higher spatial-resolution technology that breaks the diffraction limit. To address this demand, we have developed a model that demonstrates how a vector beam can dramatically improve the lateral spatial resolution of confocal laser scanning microscopy (CLSM).

First, we developed a pair of liquid crystal devices (LCDs) designed to convert a linearly polarized (LP) Gaussian beam into a kind of vector beam: a radially polarized beam with six concentric rings that we called a higher-order radially polarized (HRP) beam (see Figure 1). We inserted the LCDs in front of an objective lens with a handmade adaptor. Using CLSM (Olympus BX5, FV1000), we introduced the LCDs and, using tiny fluorescent beads whose small diameter would ordinarily cause them to fall within the diffraction zone, experimented with the confocal aperture (CA) to measure point spread functions. We tightly focused the HRP beam at the conventional CA size to suppress the beam’s side lobes. Under optimized conditions, we were able to distinguish each of the aggregated 0.17μm beads, whereas in conventional CLSM we were unable to see them (see Figure 2). We also visualized the finer structures of networks of fluorescently labeled cytoskeleton microtubules in the COS-7 cell line—cells (C) simian in origin (O) containing simian (S) virus-40 genetic material—and primary cultures of murine neurons. Moreover, because LCDs encompass various wavelengths (including near-IR) we were able to use the HRP beam for improved multi-photon microscopy. The beam enabled us to see fine intracellular structures, not only in fixed cells stained with various dyes, but also in living cells expressing a fluorescent protein. Furthermore, HRP beams have a dramatically extended depth of field compared with an LP beam,

Another advantage of this method is that it can extend the capability of CLSM without making physical modifications to the microscope. Other approaches to super-resolution microscopy have been proposed recently, some of which have achieved
very high spatial resolution (below 20nm). However, these approaches have several disadvantages. For example, stimulated-emission-depletion microscopy requires an additional high-power laser and complex instrumentation for the dark region around the focal point. In addition, the kinds of suitable fluorescent dyes that can be used are limited. Our method, by contrast, can use a variety of fluorescent dyes because the LCDs can be used over a wider range of wavelengths.

By taking advantage of an HRP beam’s smaller focal spot, we have experimentally demonstrated enhanced lateral resolution in fluorescence imaging just by adding LCDs to the system. This result highlights the advantages of LCDs and vector beams for microscopy in life-science research for a variety of biological applications. We hope that our method will advance clinical research in the future by improving the resolution of confocal or two-photon endoscopy, which would assist the diagnosis and surgery of esophageal, gastric, or bowel cancer.

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