Applications of conical diffraction to super-resolution fluorescence microscopy

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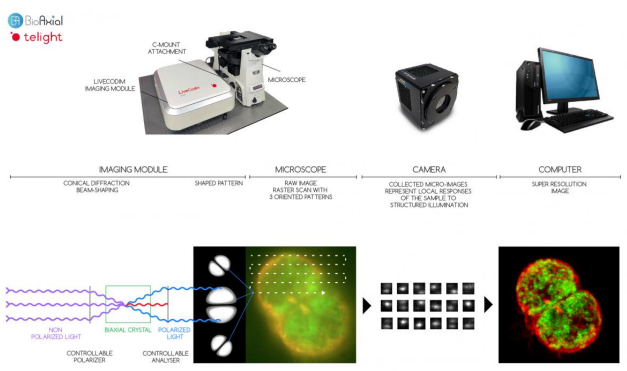
Abstract

Life sciences rely on the technological advancements of light microscopy to characterize dynamic processes that drive biology. While confocal microscopy has made tremendous strides to improving the spatial and temporal resolution of fluorescence microscopy, the advent of super-resolution imaging has been one of the most important breakthroughs of the last two decades.

The main limit to the resolution of laser-scanning microscopy (such as confocal) is the point-spread function (PSF) of the light spot used to scan the image and cause fluorescence emission. In conventional microscopy, it is constrained by the diffraction limit of light and its size is fixed and proportional to the wavelength, capping resolution at ~250 nm. Super-resolution microscopy techniques have enabled researchers to visualize structures at the nanometer scale with high precision, though even the most resolved techniques invite multiple drawbacks: specific dye preparations, higher photo-damage and long integration times are common. There is therefore a need for simple and fast super-resolution imaging methods.

Conical diffraction is a well-known optical phenomenon that has only recently drawn the attention of the microscopy community for its beam-shaping potential. Conical diffraction occurs when polarized light aligned into a biaxial crystal spreads to form a hollow cone within the crystal and emerges with cylindrical light patterns. By controlling the polarization of the light going in and out of the crystal, it is possible to selectively eliminate portions of the light and create precise patterns with sharp extinctions, such as “donut” or “double crescent” shapes [1]. This shaping occurs passively in the crystal while polarization control is accomplished by voltage changes, which can be done at kHz rates. This makes it possible to scan using shaped PSFs with features sharper than the diffraction limit without resorting to inhibition lasers or multi-laser interference.

Here, we present the conical diffraction phenomenon and some of its most useful properties for microscopy. We then show how it can be deployed and integrated into super-resolution fluorescence microscopy [1, 2]. We offer a perspective of its advantages and constraints, as well as of the principal use cases for it.



Example of the use of conical diffraction for super-resolution microscopy with the LiveCodim system (top row, left). From left to right: a laser beam is polarized in a controllable polarizer and enters a biaxial crystal, where it undergoes conical diffraction. An analyzer then removes light from the beam, resulting in the shown patterns, which are of the same size as an Airy diffraction-limited spot, but the region of darkness is smaller and sharper. Each point of a fluorescent cell sample is scanned with three different distributions with rotating orientations of the extinction, and the system's response to those patterns is acquired. A maximum-likelihood algorithm then reconstructs a super-resolved image using the high-frequency information obtained from the sample.

References

1. G. Sirat, US Patent US9250185 - Method and device for superresolution optical measurement using singular optics. (Bioaxial SAS, 2010-2017).
2. J. Caron et al., "Conical diffraction illumination opens the way for low phototoxicity super-resolution imaging." Cell Adhesion & Migration. Vol. 8 No.5. 2014. DOI: 10.4161/cam.29358.