ADVANCED MICROSCOPY WITH DIFFRACTIVE OPTICAL ELEMENTS

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In modern microscopy, traditional wide-field illumination and image-formation are often replaced with patterned excitation and non-imaging photon collection. In many instants, diffractive illumination schemes can substitute for conventional reflective or refractive designs. In particular, the availability of programmable diffractive optical elements (PDOEs) has made this an attractive alternative, since PDOEs permit highly versatile microscope designs and support an increase in the overall spatio-temporal resolution.

We have developed several microscope systems that employ acousto-optic devices (AODs) as PDOEs. Two examples of such PDOE-based advanced microscopy will be presented and discussed with respect to traditional designs. Theses schemes are: random-access multiphoton (RAMP) microscopy and standing wave (SW) microscopy.

In RAMP microscopy, we use AODs to rapidly position a focused laser beam laterally for two-dimensional (2D) scanning [1] and also axially in the three-dimensional scheme (3D-RAMP) [2,3,4]. This design makes obsolete any moving parts, resulting in inertia-free laser scanning systems. Together with multiphoton excitation of fluorescent labels and indicators, such arbitrary 3D high-speed scan patterns allow for unique flexibility of structural and functional imaging in live biological tissue.

In SW microscopy, we can employ AODs to control phase, orientation and period of 2D and 3D standing waves. This approach is different from traditional structured illumination schemes, in that the back focal plane of the objective lens is scanned with multiple coherent focused beams [5,6]. This design results in interfering collimated dynamic beams at the focal plane, generating 2D and 3D illumination pattern that support fast, stable and flexible super-resolution biological imaging.

In summary, PDOEs have been demonstrated to be highly versatile building blocks that can replace conventional schemes for dynamic wave front-shaping. The resulting microscopes contain no moving parts, which makes them well-suited for advanced imaging at high spatio-temporal resolution.

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