Multifocus microscopy with precise color multi-phase diffractive optics applied in functional neuronal imaging.

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Public Summary:
Multifocus microscopy (MFM) allows high-resolution instantaneous three-dimensional (3D) imaging and has been applied to study biological specimens ranging from single molecules inside cells nuclei to entire embryos. We here describe pattern designs and nanofabrication methods for diffractive optics that optimize the light-efficiency of the central optical component of MFM: the diffractive multifocus grating (MFG). We also implement a "precise color" MFM layout with MFGs tailored to individual fluorophores in separate optical arms. The reported advancements enable faster and brighter volumetric time-lapse imaging of biological samples. In live microscopy applications, photon budget is a critical parameter and light-efficiency must be optimized to obtain the fastest possible frame rate while minimizing photodamage. We provide comprehensive descriptions and code for designing diffractive optical devices, and a detailed methods description for nanofabrication of devices. Theoretical efficiencies of reported designs is approximately 90% and we have obtained efficiencies of > 80% in MFGs of our own manufacture. We demonstrate the performance of a multi-phase MFG in 3D functional neuronal imaging in living C. elegans.

Scientific Abstract:
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