

# ***Multiphoton Microscopy in the Biomedical Sciences IX***

**Ammasi Periasamy**

**Peter T. C. So**

*Editors*

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## **STED microscopy made simple**

Andreas Schönle, Lars Kastrup, Katrin Willig, Dominik Wildanger, Benjamin Harke,  
Brian Rankin, Christian Eggeling, Alexander Egner, Stefan W. Hell

Max-Planck-Institut für Biophysikalische Chemie, Germany

### **ABSTRACT**

We present a simple, low-cost STED microscope setups entirely built from standard optical components and based on light sources which are available off the shelf. The devices have been optimized to allow fast alignment through a simple, robust protocol and thus stable operation even by non-expert users. In this configuration, we achieved routine resolutions of down to 70 nm, thus surpassing the diffraction limit 3-fold. This implementation of the STED-principle can therefore serve as a template for researchers seeking to apply non-invasive far-field sub-diffraction imaging or fluorescence fluctuation spectroscopy in nano-sized focal volumes to solve their scientific problems.

## **Optimization of fluorescence collection in multiphoton microscopy**

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### **ABSTRACT**

Efficient fluorescence collection is critically important to maximize image quality and depth in multiphoton microscopy. Here we present an optimized, large aperture fluorescence collection system for use with the Olympus  $20 \times 0.95\text{NA}$  objective and two Hamamatsu H7422P-40 GaAsP photomultiplier tubes. Using Zemax optical design software to model the fluorescence intensity distribution and collection geometry, we have designed and constructed an optimized, large aperture detector housing assembly, which provides a significant increase in collected fluorescent signal, image quality, and maximum imaging depth.