

# CHROMATIC MULTIPHOTON SERIAL (CHROMS) MICROSCOPY: MULTICOLOR IMAGING OF LARGE BRAIN TISSUE VOLUMES

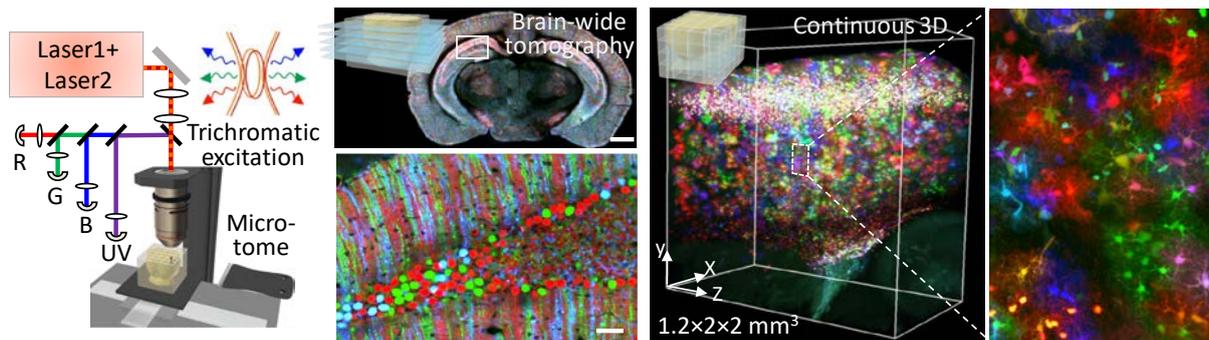
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Large-scale microscopy approaches generally lack efficient multicolor contrast modalities. We address this issue by demonstrating chromatic multiphoton serial (ChroMS) microscopy [1], a method combining trichromatic two-photon excitation through wavelength mixing [2], microtome-based serial block-face image acquisition [3], and color combinatorial labelling strategies [4]. This approach (Figure) delivers large-scale micrometric imaging of spectrally distinct fluorescent proteins with constant micrometer-scale resolution and sub-micron channel registration over the entire imaged volume. We obtain multicolor 3D imaging over continuous tissue volumes of several cubic millimeters as well as brain-wide serial 2D multicolor imaging. We illustrate the potential of this method for several types of measurements relevant for region-scale or whole brain studies: (i) color-based analysis of astrocyte morphology and clonal distribution across the mouse cerebral cortex [1,5], (ii) tracing of densely labeled neurons, and (iii) brain-wide mapping of axonal projections labeled with distinct tracers. Overall, ChroMS imaging enabled us to record for the first time large-scale images of multicolor-labeled tissue with resolution and quality appropriate for color-based morphological, clonal and connectivity analyses. This approach should find many applications for multiscale studies in neuroscience.



## References:

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