# Nonlinear synchronous multi-color metabolic imaging microscopy

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## Abstract

Conventional multiphoton microscopy (MPM) has pioneered the field of bioimaging for deep tissue and label-free imaging. The ability to make slices and the reduced photoinduced damage make MPM an ideal tool for examining thick living specimens in clinical studies. The ExtreMed (Extreme Ultrashort Pulses for Advanced Medical Applications and Diagnostics) project [*] is at the forefront of research in the field of ultra-broadband pulsed lasers with few cycles below 10 fs deployed for MPM. We are developing a new generation of a user-friendly version of the patented SyncRGB-FLIM (synchronous red-green-blue fluorescence lifetime imaging microscopy) method [1, 2], that allows simultaneous observation of both native markers, such as NAD(P)H compounds as well as common fluorescence labels with emission across the entire visible spectrum in a single image scan configuration without tuning the laser wavelength.

For optimal performance, pulse compression via the dScan method [3] at the focus of the sample is crucial, which is performed here using a custom-developed measurement head that allows its characterization at the focus of the high-NA microscope objective, i.e., at the sample plane. To date, the SyncRGB-FLIM method has been successfully presented in a stage scanning microscope configuration [2], but not with fast beam scanning.

Here we present a SyncRGB-FLIM microscope based on fast beam scanning excitation, developed as part of the ExtreMed project, in which the few-cycle sub 10 fs laser pulses are maintained at the focus of a high NA microscope objective. Label-free applications of this novel SyncRGB FLIM microscope onto 2D and 3D live cancer cell models are shown, demonstrating its versatility for in vitro-based research applications, including the emerging 3D disease model-based diagnostics and therapeutic research.

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## References

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