Technology Offer

Simultaneous Multi-Color Fluorescence Microscopy with Enhanced Spatio-Temporal Resolution

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Abstract

This invention, developed jointly by the KU Leuven and the Max-Planck-Society, introduces a novel optical module enabling simultaneous multi-color fluorescence microscopy with single-molecule sensitivity and significantly improved throughput. Designed for easy integration with existing microscopy setups, the module utilizes a fully reflective optical system to encode emission color into spatial image features. Combined with advanced image processing algorithms, the system allows researchers to detect and distinguish multiple fluorophores in real-time using a single grayscale camera. This breakthrough dramatically shortens acquisition times, accelerates high-content imaging, and supports emerging applications such as spatial transcriptomics and nanoscale diagnostics. The technology represents a major advancement for molecular imaging in life sciences and diagnostics, offering broad compatibility and minimal optical loss.

Background

Fluorescence microscopy has become an indispensable tool in biology and materials science, with ongoing developments pushing the boundaries of resolution, sensitivity, and imaging speed. Traditional multi-color imaging, however, is limited by the sequential acquisition of different fluorophores, resulting in long experiment times and complex system alignment. These limitations hinder progress in single-molecule studies, high-throughput imaging, and new spatial ‘omics’ technologies like MERFISH, where rapid, multiplexed imaging is critical. A key need exists for a solution that combines high-resolution, multi-channel imaging with fast acquisition and easy system integration, all without sacrificing image quality or molecular specificity.

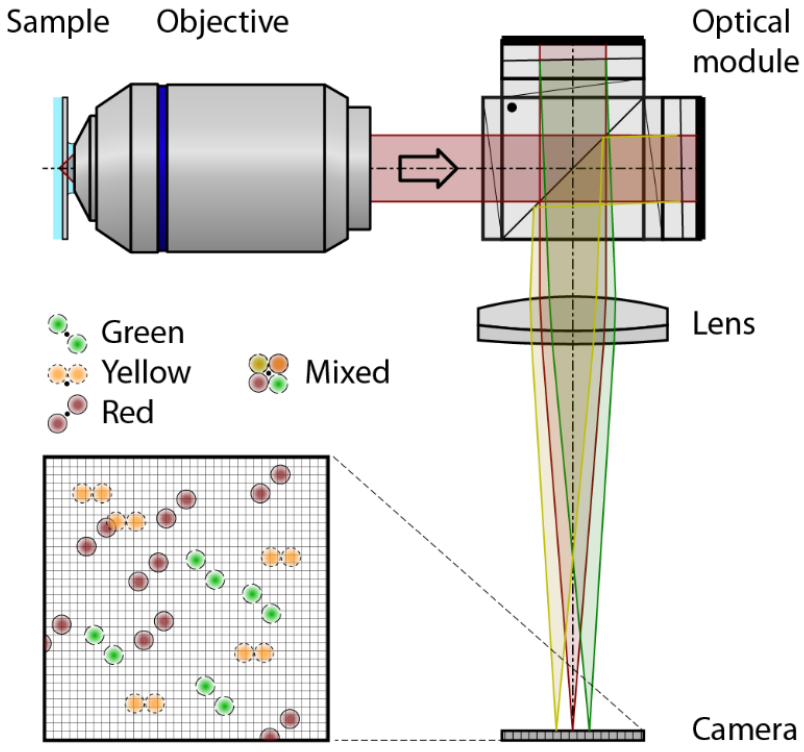

Figure 1: Multi-color fluorescence microscopy with spatial encoding. Fluorescence from the sample is collimated by the objective and enters a reflective optical module, where it is split into angled sub-beams by emission wavelength. A lens refocuses these into shifted image copies on a single grayscale camera. Each fluorophore (green, yellow, red) forms a unique spot pattern, enabling spectral identification. Mixed fluorophores produce overlapping patterns. This approach allows fast, simultaneous imaging of multiple colors without filters or sequential acquisition.

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The core of the invention is a compact, fully reflective optical module that enables real-time imaging of multiple fluorophores by encoding their spectral identity into spatially distinct image features. Traditional fluorescence microscopes rely on sequential imaging and multiple filters to separate emission wavelengths, resulting in time-consuming workflows and complex system configurations. In contrast, this system exploits a novel beam-splitting design that introduces angular separation of collimated light beams collected from the sample.

Light from the specimen is first collimated by the microscope objective and then passed through a reflective optical arrangement that splits the beam into several sub-beams, each emerging at a unique angle. These sub-beams are recombined through a lens to form shifted, yet spatially compact image replicas on a single grayscale camera. The distribution pattern of intensity across these shifted copies carries encoded spectral information, allowing differentiation between at least three fluorophores simultaneously.

Importantly, the use of only reflective components eliminates chromatic aberration and minimizes light loss, ensuring high imaging fidelity across the visible and near-infrared spectrum. This design is compatible with a wide range of microscope platforms and objectives. Furthermore, the spatial encoding approach is amenable to computational image analysis, enabling integration with advanced algorithms for localization, tracking, and multiplexed quantification of single-molecule signals.

Advantages

- **Simultaneous imaging of 3 or more fluorophores** with single-molecule precision using one camera
- **Drastically speed-up** for multiplexed imaging
- **Multi-color image acquisition** using a single grayscale camera
- **High optical quality** due to exclusive use of reflective optics (minimal aberration/light loss)
- **Easy integration** into existing microscopes; no need for complex realignment or calibration
- **Versatile compatibility** with various imaging modes and analysis algorithms

Potential applications

- High-throughput imaging for spatial transcriptomics and other ‘omics’ methods
- Nanoscale resolution imaging in live or fixed biological samples
- Single-molecule tracking in dynamic systems
- Enhanced diagnostic platforms
- Single-particle assays and Förster Resonance Energy Transfer (FRET) studies

Patent Information

PCT application *(WO2020160893A1)* (24.01.2020), active in US

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