Technology Offer

Simultaneous multi-color fluorescence microscopy with improved spatio-temporal resolution and image contrast

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Fluorescence microscopy is one of the key techniques in the life and materials sciences, with applications ranging from routine diagnostics to state-of-the-art research investigations. Key to this success has been its intrinsic advantages combined with continuous improvement in its fundamental features such as resolution, speed, and sensitivity.

Two key challenges are fast single-fluorophore imaging of multi-color samples. This need arises in a broad range of settings such as very high-resolution imaging, or in upcoming new diagnostics methodologies such as spatially-resolved transcriptomics based via methods such as MERFISH. The research and diagnostic potential of such methods is limited because these measurements easily take many minutes just to acquire a single type of fluorophore.

The KU Leuven and the Max Planck Institute have now developed a new technology that offers simultaneous multi-color fluorescence microscopy with single-molecule sensitivity. The invention consists of an improved optical module supported by state-of-the-art software, and enables three or more colors to be acquired simultaneously.

This technology represents a major leap forward by greatly enhancing the throughput and performance of the groundbreaking methodologies that are currently being used and developed in the field of molecular biology, diagnostics and others and that rely heavy on this type of imaging.

Concept

Our technology allows the encoding of the emission color of the fluorophores in the shape with which they appear in the image. The identity of the fluorophore can then easily be determined by visual or algorithmic inspection. In this way, three or more different types of fluorophores can be imaged at once, strongly enhancing the throughput of the measurement.

Our design consists of a novel compact optical module that can be easily integrated into an existing microscope or system under development. Our optical design uses only reflective optics, which has a number of crucial advantages:

- **Optimal conservation of imaging quality;**
- **minimal light loss;**
- **functional over the full visible and near-infrared spectrum;**
- **can be used in combination with any type of microscope and objective;**
- **compatible with other approaches that encode information into the imaging.**

In addition to its great capabilities in distinguishing the emitter color, the module could also be used to obtain additional information such as the fluorescence anisotropy.

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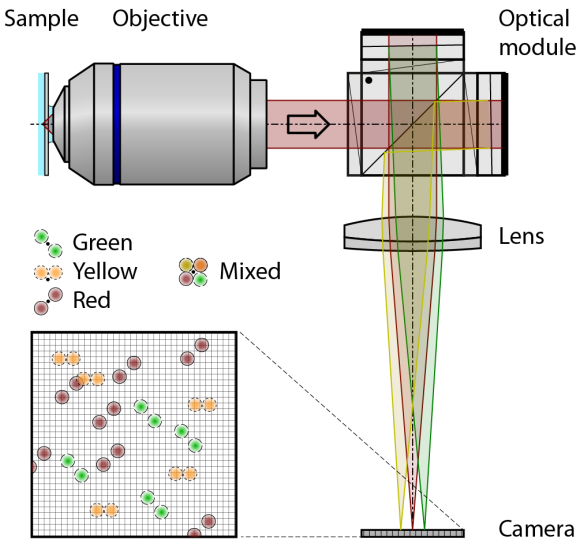
How does it work?



The optical module

An objective collects and collimates light from a sample. The collimated light beams are split into several sub-beams that are delivered at distinct mutual angles. A lens then forms a composite image consisting of shifted copies, one copy per sub-beam. These shifts are large enough to separate the image spots but small with respect to the overall image dimensions.

This results in the following improvements:

- Extension of a widefield (fluorescence) microscope
- Flat faces accept and deliver collimated beams
- Multi-color images with one grayscale camera
- Permanent alignment
- Minimal aberrations
- Conserved field size

The image analysis method

The emission of stochastically blinking fluorophores is recorded by a sequence of images. In each image, the light from bright fluorophores is distributed in characteristic sets of image spots, where the distribution of brightness among the image spots conveys the encoded information, for instance the colors of the fluorophores. The above described image acquisition technology can be combined with a wide variety of computational analysis methodology. The lab of KU Leuven disposes of a wide software and methodology library that depending on the specific application can easily be integrated with existing pipelines.

Applications

- Fast imaging with nanoscale spatial resolution.
- High-content/high-throughput imaging.
- Spatially-resolved transcriptomics and other ‘omics’ methods.
- Single-molecule pulldowns.
- Förster Resonance Energy Transfer.
- Single-particle tracking.
- Diagnostic instrumentation.

(non-exhaustive list)

In summary, our straightforward optical device allows ready integration with existing software and hardware and delivers a speed-up of at least a factor of three for multiplexed imaging, delivering a crucial advantage in throughput.

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Patent Information

- Priority document EP3690510A1 filed on 4 February 2019.
- Patent application WO2020/160893A1 filed on 24 January 2020.
- Patent application US17/393690 filed on 4 August 2021.
- Patent application EP20700911.9 filed on 24 August 2021.

Collabration

This technology has been developed in the lab of Nanobiology under the direction of **Prof. Dr. Peter Dedecker** at KU Leuven and by **Dr. Marcel Leutenegger** of the Lab for NanoBiophotonics at Max Planck Institute for Biophysical Sciences, Göttingen.

We are looking for interested optical design partners to license-in the offered technology.
Upon request, we will be happy to provide you with more detailed information on the technology.

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