All Fluorophores Welcomed: How to extend confocal microscopy with multicolor unmixing tools for advanced multiplexing and spatial omics

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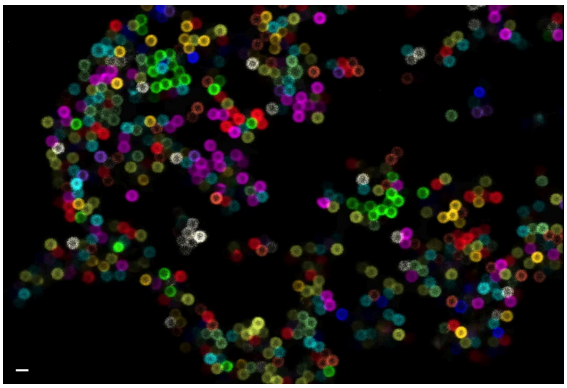
Abstract

The STELLARIS confocal platform is particularly well suited for high multiplexing strategies as it combines a freely tunable white light laser (WLL | 440nm -790 nm) excitation with up to 5 highly sensitive spectral detectors for complete flexibility of detection from 410 nm up to the NIR range[1]. This unique combination allows to fit a large palette of fluorophores and to optimise any possible combination. The availability of the WLL also in the lightsheet mode allows the same excitation freedom. It also enables to perfectly match the laser line to all filter combinations needed in each experiment.

In this workshop, we will introduce the different approaches to achieve multiplexing in a direct straightforward manner. We will show how it is possible to populate the complete visible and NIR spectrum with 11 signals and optimally image all the fluorophores present in the sample in one imaging round[2] In addition, we will discuss how the availability of lifetime-based tools in STELLARIS enable going beyond these spectral capabilities to extend the possibilities of detecting signals in higher order of multiplexing experiments, in both fixed and live imaging experiments.



Spectral separation of 11 fluorophores coupled to polystyrene beads on a STELLARIS confocal system

References

1. Application Note: The Power HyD family of detectors, Schweikhard et al. Nature Methods 2022 Oct, doi: d42473-020-00398-0

2. Multiplexing through Spectral Separation of 11 Colors, Hoffmann S. et al. https://www.leicamicrosystems.com/science-lab/life-science/multiplexing-through-spectral-separation-of-11-colors/ Oct 18, 2022