Systems Biology Imaging Platform
BERLIN INSTITUTE FOR MEDICAL SYSTEMS BIOLOGY

Spectral STORM
Multi-colour super-resolution imaging with a single excitation laser

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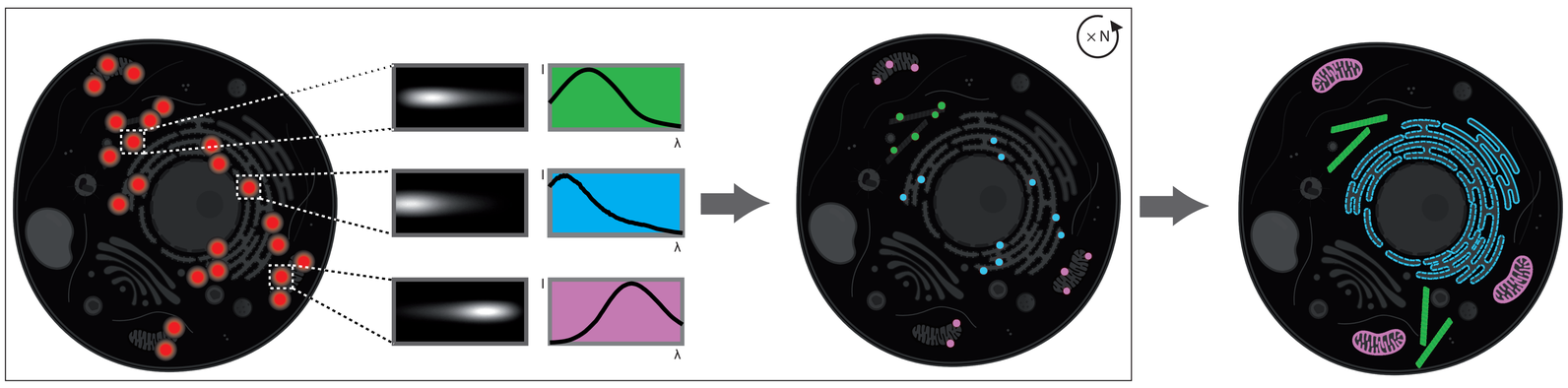
Concept



Stochastic blinking events of different far-red fluorophores are used for 3D localization

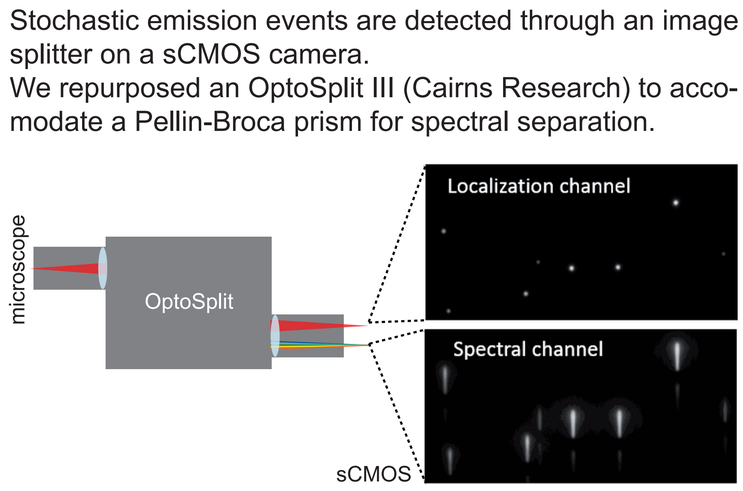
Analysis of spectral signature relative peak of each event in spectral channel

False **colour assignment and rendering** of multi-colour superresolution image from thousands of acquired frames

Summary

- Multi-colour localization microscopy approach for simultaneous acquisition of up to 4 different fluorophores
- A single excitation laser, objective and sCMOS camera on a standard inverted microscope
- Large field of view: 130 x 60 µm^2
- Spectral fingerprint of each emission event serves to classify fluorophore
- Dissolves problems with image registration, chromatic aberrations and sample drift for nanoscale co-localization analysis

Microscope setup



Flat-field Illumination

We use 638 nm diode laser and a microlens-based Köhler integrator[1] for **homogenous epi-illumination of the entire field of view**. This ensures field-independent image resolution[2].

Spectral Detection

Stochastic emission events are detected through an image splitter on a sCMOS camera.
We repurposed an OptoSplit III (Cairns Research) to accomodate a Pellin-Broca prism for spectral separation.

For 3D localization, a cylindrical lens introduces a **depth-dependent astigmatism** in one detection path.
(Alternatively, a double helix or tetrapod PSF could also be employed)

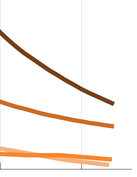
Subsequent custom **spectral analysis** assigns a false colour to each localized emission event based on its spectroscopic signature.

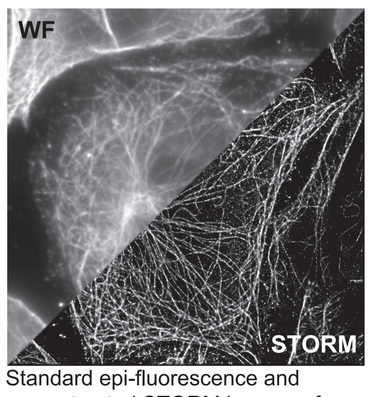
Microscope control and localization fitting are performed in open-source software µManger[3] and Fiji / ThunderSTORM[4,5].

Calibration

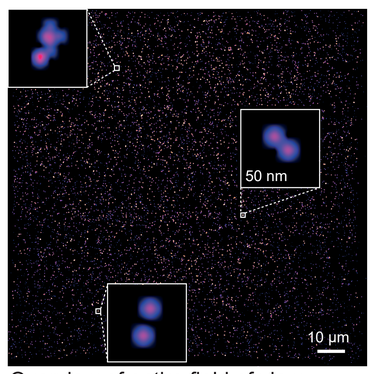
Lateral Resolution and Field of View

DNA Origami probes (GattaQuant) labelled with AlexaFluor-647 at 50 nm distance demonstrate field-independent nanometer resolution





Standard epi-fluorescence and reconstructed STORM images of microtubules in CHO cells immunolabelled with AlexaFluor647



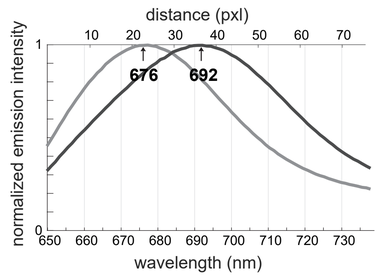
Overview of entire field of view (130 x 130 µm^2) and zoomed-in regions with individual DNA origami probes

Spectral resolution

We use immunolabelling with **standard organic dyes**, which makes this method applicable to a wide range of molecular biology samples.

	Emission peak [nm]	**Ext. coeff.** [L/mol cm]
DyLight 633	650 nm	170 000
Alexa Fluor 647	666 nm	203 000
Biotium CF660	685 nm	200 000
Biotium CF680	698 nm	210 000

Calibration of spectral separation with fluorescent beads from a FocalCheck slide demonstrates a spetral resolution of 1.1 nm/pxl



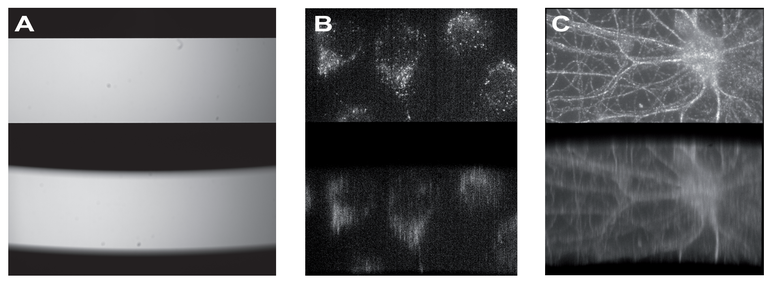
Discussion

Limitations and Ongoing Development

In practice, the **photon budget** is the biggest limitation. One challenge here is to find good **imaging conditions** and acquisition parameters for all dyes simultaneously.

High **transmission** along the entire optical path is crucial. **Spherical abberations** in one channel channel can lead to misinterpretation of spectra (Fig. A).

Sparse emission is necessary to separate spectra of individual events (Fig. B & C). Because the required **sparsity** is higher than in conventional STORM acquisitions, recording times can become longer.



False assignment of spectra leads to **crosstalk** in the final image. A robust goodness-of-fit measure have to be employed to filter out localizations with bad spectral fits.

Applicability & Outlook

In contrast to previous spectroscopic super-resolution microscopy efforts[6,7] we can achieve nanometer resolution in 3D with a single objective and camera over a field of view of 130 x 60 µm^2. This increases the applicability of this method for biomedical research compared to previous approaches.

The optical setup can be implemented on any epi-fluorescence microscope. We intend to publish the spectral analysis as an open source package in the near future.

References

1. Völkel, R. & Weible, K. J. Laser Beam Homogenizing: Limitations and Constraints. *Proc. SPIE* (2008).
2. Douglass et al. Super-resolution imaging of multiple cells by optimized flat-field epi-illumination. *Nat. Photonics* (2016).
3. Edelstein et al. Advanced methods of microscope control using µManager software. *J. Biol. Methods* (2014).
4. Ovesný et al. ThunderSTORM: a comprehensive ImageJ plug-in for PALM and STORM data analysis and super-resolution imaging. *Bioinformatics* (2014).
5. Schindelin et al. Fiji: an open-source platform for biological-image analysis. *Nat. Methods* (2012).
6. Dong et al. Super-resolution spectroscopic microscopy via photon localization. *Nat. Commun.* (2016).
7. Zhang et al. Ultrahigh-throughput single-molecule spectroscopy and spectrally resolved super-resolution microscopy. *Nat. Methods* (2015).