Nonlinear optical spectroscopy and microscopy seminar

Co-organized by

Department of Physical Chemistry

&

Department of Applied Physics

“"Multicolour single-molecule made easy: a simpler approach to spectral separation""”

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14:15, Nov 4 (Tue), 2025

Science III, 1S081

Please contact Takuji.Adachi@unige.ch if there is any question. The next seminar will be in early 2026. Stay tuned.

"Multicolour single-molecule made easy: a simpler approach to spectral separation"

Multicolour and spectrally-resolved single-molecule microscopy can give considerable biological insight: fluorophores can undergo spectral changes in response to their local environment, providing information on the nanoscale hydrophobicity of oligomeric protein species;[1] coupling between fluorophores by FRET causes a colour change that gives insights into molecular dynamics;[2] and detecting multiple colours of fluorophore can enable super-resolution microscopy experiments to simultaneously image multiple biological targets, allowing interactions between biological structures to be uncovered on the nanoscale.[3] Current state-of-the-art instrumentation for multicolour experiments is often technically complex to implement, with the number of colours able to be deconvolved limited to about ~8. [4]

We have developed a new image analysis pipeline that works with off-the-shelf hardware. This image analysis algorithm, and the underlying information-theoretic approach, can be used to discriminate up to ~8 different fluorophores, achieving the state-of-the-art with a considerable reduction in experimental complexity. We will present experimental evidence that our method enables easy single-molecule FRET, and far greater multiplexing of multicolour super-resolution microscopy.

References

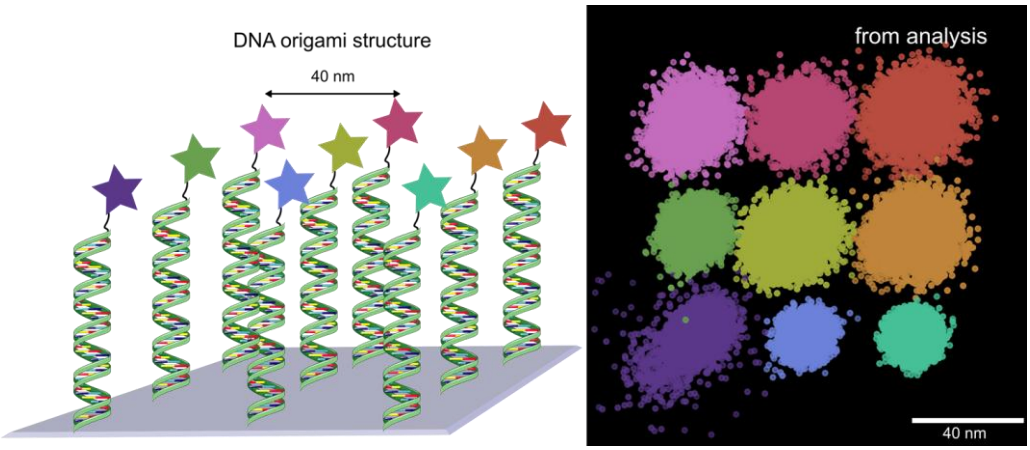
1. Bongiovanni, M. N. et al. Multi-dimensional super-resolution imaging enables surface hydrophobicity mapping. Nat Commun 7, 13544 (2016).

2. Hohng, S., Joo, C. & Ha, T. Single-Molecule Three-Color FRET. Biophys J 87, 1328–1337 (2004).

3. Dempsey, G. T., Vaughan, J. C., Chen, K. H., Bates, M. & Zhuang, X. Evaluation of fluorophores for optimal performance in localization-based super-resolution imaging. Nat Methods 8, 1027–1036 (2011).

4. Kumar, A. et. al. Multispectral live-cell imaging with uncompromised spatiotemporal resolution, biorxiv (2024) doi: 10.1101/2024.06.12.597784

Figure 1: We are able to



distinguish 9 different colours at sub-diffraction limited distances.