マルチモーダル超解像イメージング

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ライブセル環境下で、一つ一つの細胞個性を分子レベルで解析する技術を開発する。

細胞をマルチモーダルに超解像観察する光学顕微鏡を開発、 細胞内分子を多角的に計測・分析し、細胞の個性を検出、定量化する。 細胞内の特定の分子の詳細な分布の測定と、分子振動検出による網羅的な細胞内分子検出、 および得られる膨大なデータの解析を組み合わせることで、細胞の振る舞いを決定している分子レベルの「要素」を探る。



High resolution and large field of view multi-modal imaging by SLI microscopy

High resolution Raman micro-spectroscopy of mouse brain tissue



High resolution *fluorescence* microscopy of fixed HeLa cell stained with ATTO532 phalloidin



1682 cm⁻¹ (amide-I); 2848 cm⁻¹ (CH2 stretching)



We have succeded in high-resolution Raman/fluorescence imaging of biological samples by developed SLI microscope.

Line-illuimination Raman/fluorescence microscopy

Raman scattering

contains rich chemical information - distribution, concentration, temperature, and environment of the molecule [1].



Structured line illumination (SLI) Microscopy Setup





We implemented structured illumination microscopy (SIM) [2] to line illumination (LI) microscope for the realization of multi-modal high-resolution imaging [3].



The mixing between SI and Raman scattering from the object allow access to the normally inaccessible high spatial frequency information.



Raman shift [cm⁻¹]

The spatial frequencies of LI and SLI Raman images of ϕ 330 nm polystyrene particles

The improvement of spatial resolution was confirmed for the all the detected Raman bands from the sample.

Slit-confocal: x) 320 nm; y) 380 nm SIM: x) 320 nm; y) 190 nm

References: [1] Almar F Palonpon et al., "Raman and SERS microscopy for molecular imaging of live cells", *Nature Protocols* **8**, 677-692 (2013) [2] M. G. L. Gustafsson, "Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy", Journal of Microscopy **198**, 82-87 (2000) [3] Watanabe et al., "Structured line illumination Raman microscopy" Nat. Commun. 6:10095 doi: 10.1038/ncomms10095 (2015) **‡** F. Yand et al., "The molecular structure of green fluorescent protein" *Nature Biotechnology* **14**, 1246-1251 (1996)