Dr Deirdre Kavanagh Microscope Officer University of Birmingham

dSTORM image of a-Tubulin Image courtesy of Abs Khan



COMPARE Advanced Imaging Systems

Selective plane illumination:

- Lattice Light Sheet Microscopy
- Dual Inverted SPIM (DiSPIM)

Single molecule localisation:

- dSTORM
- PALM











Is lattice light-sheet right for your project?

Applications

• You are interested in intracellular dynamics

Advantages

- Cell friendly
- High speed imaging (300 slices per sec)
- Excellent spatial resolution (230 x 230 x 370 nm x-y-z)

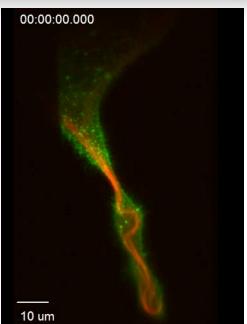
Considerations

- Coverslips 5 mm, FOV 50 μm
- Dipping objectives (8 ml bath)
- Computational heavy data
- Instrument set-up time

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The Universities of Birmingham and Nottingham





Malou Zuidscherwoude





Is dual inverted SPIM right for your project?

Applications

• You are interested in whole cell/small organism dynamics

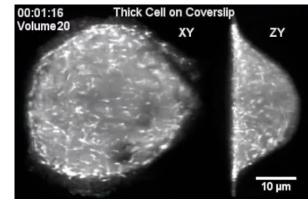
Advantages

- Cell friendly
- Isotropic Resolution (330 nm)
- Simple sample mounting (coverslips)

Considerations

- Dipping objectives
- Computational heavy data







Is single molecule imaging right for your project?

(dSTORM) Direct Stochastic Optical Reconstruction Microscopy

Biological Question

• Where endogenous proteins are localised and/or arranged

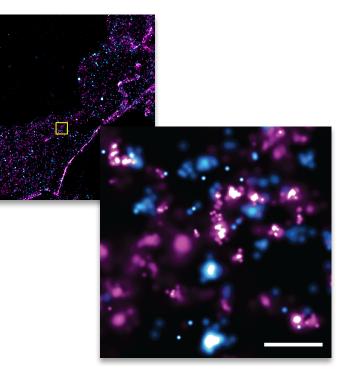
Advantages

- Amazing resolution (20 nm, x-y)
- Dual-colour
- TIRF and 3D localisation (z 50 nm)

Considerations

- Best results with Alexa-647
- Semi-quantitative technique
- STORM buffer optimisation

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Natalie Poulter



Is single molecule imaging right for your project?

PALM - Photoactivatable Localisation Microscopy

Biological Question

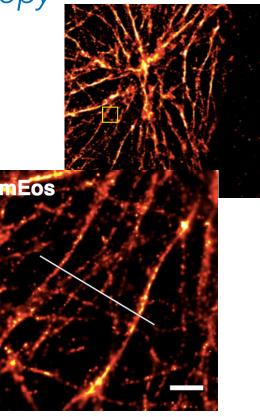
 Where exogenous proteins are localised and/or arranged

Advantages

- Amazing resolution (20 nm)
- Quantifiable -1:1 labelling
- Possible to do live PALM
- Image in PBS

Considerations

- Exogenous proteins
- Single colour (combine with STORM) IN PARTNERSHIP: The Universities of Birmingham and Nottingham



Abs Khan



Your biological question



IMAGING TIP: Don't get blinded by super-resolution! Super-Resolution is a fantastic tool BUT only if it is applied to the right question. Use the technique you can learn the most from.

IMAGING TIP: Keep it simple by starting with the basics! Use a Laser Scanning Confocal or an Epifluorescence microscope to get an idea of where your object is and/or is it moving.



Microscope access procedure

- If you would like to use our microscopes please submit a request for training (<u>https://ppms.eu/bham</u>) and we will schedule a meeting to discuss your project!
- Complete new project form also on Stratocore prior to meeting.
- E-mail: D.M.Kavanagh@bham.ac.uk



