OMX workshop - Oxford Feb 2020

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Micron OXFORD

A short history of super-resolution microscopy (SRM)

► **Axial resolution**

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The needs of biology drive new development

SRM - a multidimensional challenge

Schermelleh et al. *Nat Cell Biol*, 2019

SRM - a multidimensional challenge

- **Microscope:** detection efficiency, sensor dynamic range
- **Fluorescence labelling:** brightness, density, specificity
- **Sample/target properties:** sparse or dense, high or flat?

→ **Photon budget**

Lateral, axial
Spatial resolution

Limiting factors: ***Contrast***
Signal-to-noise / signal-to-background ratio
(minimal requirement method-specific)

To consider / affects:
- *Axial (z) resolution increase, yes or no?*
- *Localisation precision & labeling density (SMLM)*
- *Throughput of high frequency information (=fine details)*
- *Signal-to-noise ratio improvement*

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3D, depth, multicolour
Context

Aberrations
Spherical, chromatic, sample-induced

Imaging depth
[µm]

- *Single-, 2- or multicolour?*
- *Single plane or volumetric?*
- *Out-of-focus blur / background suppression*

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Acquisition — Speed

Detector
Quantum efficiency, readout, parallel vs. point detection

Temporal resolution
[frame^{-1}]*

- *Throughput*
- *Imaging area*
- *Imaging volume*

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[frame⁻¹]*

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Low — Photodamage

Efficiency
Resolution gain per illumination light dose

Light intensity
[W/cm²]

- *Photobleaching/-stability*
- *Toxicity/thermal damage*
- *Number of time points*
- *Overall imaging duration*

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Microscopy has become very complicated

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One technique does not meet all requirements

Schermelleh et al. *Nat Cell Biol*, 2019

SRM - a multidimensional challenge

Microscopy has become very complicated

One technique does not meet all requirements

Central resources, expert knowledge, guidance

Schermelleh et al. *Nat Cell Biol*, 2019

How to choose the best SRM method

Schermelleh et al. *Nat Cell Biol*, 2019

How to choose the best SRM method

The biological question should guide the method choice

Schermelleh et al. *Nat Cell Biol*, 2019

How to choose the best SRM method

The biological question should guide the method choice

Biologists need more than one SRM option

Schermelleh et al. *Nat Cell Biol*, 2019

Comparison: Widefield vs 3D-SIM

Variants: 3D SIM, 2D SIM, TIRF-SIM

Schermelleh et al. *Nat Cell Biol*, 2019

From wide-field to 3D-SIM

Mouse C127 cell

From wide-field to 3D-SIM

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3D-SIM,
just another tool in the repertoire ?

It‘s not that simple!

The untold story

SI reconstruction artifacts

RPE-1 cell, DAPI staining

SI reconstruction artifacts

HeLa clell nuclei, chromatin staining

Stripes

Bleaching,
Drift or vibrations
Moving particles
(locally constrained)

High frequency noise

Low contrast-to-noise,
Low modulation contrast

Halo / Doubling

Spherical aberration,
Refractive index mismatch

SI reconstruction artifacts

SI reconstruction artifacts

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SI reconstruction artifacts

Quality control by Fourier analysis

SI reconstruction artifacts

Demmerle et al. 2017, *Nat Protocols*

Z-wrapping artefact

Z-wrapping artefact

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SIMcheck - Toolbox for Fiji/ImageJ

Ball et al. 2015, *Scientific Rep* 5

Tools and best practise protocols to get the maximum out of SIM

OPEN

SCIENTIFIC REPORTS | 5:15915 |

Received: 03 March 2015
Accepted: 01 October 2015
Published: 03 November 2015

SIMcheck: a Toolbox for Successful Super-resolution Structured Illumination Microscopy

Graeme Ball[1,†], Justin Demmerle[1], Rainer Kaufmann[1,2], Ilan Davis[1], Ian M. Dobbie[1] & Lothar Schermelleh[1]

PROTOCOL

Strategic and practical guidelines for successful structured illumination microscopy

Justin Demmerle[1,8], Cassandravictoria Innocent[1,8], Alison J North[2], Graeme Ball[1,7], Marcel Müller[3], Ezequiel Miron[1], Atsushi Matsuda[4,5], Ian M Dobbie[1], Yolanda Markaki[6] & Lothar Schermelleh[1]

NATURE PROTOCOLS | VOL.12 NO.5 | 2017

PROTOCOL

Quantitative 3D structured illumination microscopy of nuclear structures

Felix Kraus[1,5], Ezequiel Miron[2], Justin Demmerle[2], Tsotne Chitiashvili[1,5], Alexei Budco[1], Quentin Alle[2], Atsushi Matsuda[3,4], Heinrich Leonhardt[1], Lothar Schermelleh[2] & Yolanda Markaki[1,5]

NATURE PROTOCOLS | VOL.12 NO.5 | 2017

REVIEW ARTICLE | FOCUS
https://doi.org/10.1038/s41556-018-0251-8
nature cell biology

Super-resolution microscopy demystified

Lothar Schermelleh[1*], Alexia Ferrand[2], Thomas Huser[3], Christian Eggeling[4,5], Markus Sauer[6], Oliver Biehlmaier[2] and Gregor P. C. Drummen[7,8*]

Golden rules (not only) for SIM

- Labeling **Specificity**
- Modulation **Contrast**
- Spherical **Aberration**
- Channel **Alignment**

Imaging workflow: quality is paramount!

Labelling

- Dyes (spectra, photo-stability)
- Labelling method (FPs, IF, FISH,....)
- Labelling specificity (antibodies)
- Signal-to-noise / background

Think 'holistically'!

Imaging workflow: quality is paramount!

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Sample

- Optical quality (coverslip, cleanness)
- Refractive index mismatch
- Embedding medium, RI immersion
- Imaging depth

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Microscope

- Mechanical stability
- Photon efficiency
- Modulation contrast / calibration
- Camera: (EM)CCD / sCMOS

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Postprocessing

- PSF/OTF (λ-, depth-, RI-dependent)
- Channel alignment
- Quality control

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Dataset
x, y, z, λ,(t)

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Quantitative Analysis

- Quality control
- Co-localisation
- Segmentation
- Distances

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Every little helps!

Optimisations can be made on each step of the way and all adds up to obtain an optimal result.

Sample prep: Mounting medium matters !

Images (3D-SIM): Andreas Maiser (LMU München)

(Stripe) contrast is key!

Mapping of local modulation contrast variation (SIMcheck)

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Local modulation contrast variation

Local modulation contrast variation

CCD is superior for SIM with dense emitter and high background

CCD: lower read noise and greater well-depth -> higher dynamic range, but lower speed

Demmerle et al. 2017, Nat Prot

Nothing beats signal-to-background, but bleaching!

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Strike an optimal balance of sufficiently high dynamic range and photobleaching,
No more than ~50% bleaching!

Photon budget is limited – spend it wisely!

Imaging multiple time points, requires trade off in other areas, e.g. z-height.

OMX Blaze: 2 s / frame
(1.75 μm z-stack = 225 images /frame, 100 time points)

OMX Blaze 10 s / frame
(5 μm z-stack = 600 images / frame, 10 time points

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Quality control: Do not discard negatives!

Ball et al. 2015, *Scientific Rep* 5

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Min-to-Max-Ratio (MMR)

Ball et al. 2015, *Scientific Rep* 5

Quality control: Do not discard negatives!

Min-to-Max-Ratio (MMR)

Quality parameter ('signal-to-noise')

Ball et al. 2015, *Scientific Rep* 5

Quality control: Become familiar with Fourier space!

…. to verify effective resolution

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Quality control: Become familiar with Fourier space!

…. to identify artefacts

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Spherical aberration - correction by oil refractive index

PSF

‘bottom-heavy’ symmetric ‘top-heavy’

Courtesy of Dan White (GE)

Spherical aberration - correction by oil refractive index

Lambert&Waters 2016, *JBC*

Matching spherical aberration of OTF and sample

405 nm 488 nm 592 nm

$n_{Glycerol}$ = 1.47@23°C

RI_{OTF}	**1.512** (**RI_{ref488}**)		
PSF (→OTF)	/ **G** /		

Matching spherical aberration of OTF and sample

405 nm 488 nm 592 nm

$n_{Glycerol}$ = 1.47@23°C

RI_{OTF}	1.511	**1.512** (**RI_{ref488}**)
PSF (→OTF)	**B**	/ **G** /

Matching spherical aberration of OTF and sample

405 nm 488 nm 592 nm

$n_{Glycerol}$ = 1.47@23°C

RI_{OTF}	1.511	**1.512** (**RI_{ref488}**)	1.515
	B	/ **G** /	**R**
PSF (→OTF)	0 μm AI: 0	0	0

Matching spherical aberration of OTF and sample

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405 nm 488 nm 592 nm

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Matching spherical aberration of OTF and sample

If you can't avoid match spherical aberration OTF with sample!

C127 cell, DAPI
Glycerol mounting
~2 μm z-depth

Matching spherical aberration of OTF and sample

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Choice of oil RI for multicolour imaging

Demmerle et al. 2017, *Nat Protocols*

If you image 3D, register 3D!

- Biological 3D calibration sample to determine alignment parameter
- Adjust z-shift to optimally match in the center of the sample

Smeets et al., 2014, *Neuromethods*

Channel registration in 3D using “biological” calibration slide

Kraus et al, 2017 NatProtocols

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Kraus et al, 2017 NatProtocols

System calibration:
3D structured illumination pattern focus

Calibrating axial illumination pattern focus (top-phase)

3D-SIM - pros & cons

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+ Multiple colours with standard dyes
+ Lateral and axial resolution improvement
+ 3D optical sectioning with enhanced contrast

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Context

+ Light dosage lower than other SR-techniques
+ Linear response → quantitative
+ Relatively deep imaging (up to 10 µm, or 20 µm w/ Silicon)
+ Sensitivity and speed (SLM, Blaze) → live cell imaging

Versatility

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Versatility

- Only moderate lateral resolution improvement
- Mathematical reconstruction → artifacts
- High requirements on sample quality and system calibration

Challenges

Thanks!

“DNA drop” Wellcome Trust Image Award 2017 Winning Entry (by Ezequiel Miron)