

# Structured Illumination Microscopy

If? Why? How? → Theory / Microscopes / Data reconstruction

2020-02-11, Micron Oxford, Marcel Müller

# What is SIM? Should you use it?



# Super-resolution imaging

- Image beyond the diffraction limit → but how?
- “big three”: **SMLM**, **STED**, **SIM**
- *“Mapping (previously unobservable) spatial information into (almost exclusively) the temporal domain”*
- Various methods & combination
- Allow for trade-offs, typically
  - **Imaging speed**
  - **Resolution**
  - **Observation time**
  - Instrument complexity
  - Usable dyes

# Some confusion around terminology

## Gustaffson-(Heintzmann)-SIM:

- Use **sinusoidal modulation** of illumination light
- Allows for „**direct reconstruction**“
  - Number of phases set by sinusoids ( $2xN + 1$ ), allows to disentangle effects of illumination from sample structure
  - Based on direct decomposition in Fourier space (sinusoids become delta peaks, which allows to explicitly invert the sample/illumination convolution)
- Other approaches (using deconvolution-like algorithms) exist, often combined with more complex illumination schemes... we'll see in a few years what works best

## ISIM, MSIM, etc.:

- Also “structure” light, so why not call them SIM?
- The new “ISM” fits better to what they achieve

*Journal of Microscopy*, Vol. 198, Pt 2, May 2000, pp. 82–87.  
Received 12 January 2000; accepted 3 March 2000

### SHORT COMMUNICATION

#### Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy

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San Francisco, California 94143-0448, U.S.A.

**Key words.** Actin, cytoskeleton, fluorescence microscopy, interference, lateral resolution, moiré microscopy, optical transfer function, patterned excitation, resolution, structured illumination, super-resolution, wide-field microscopy.

*Biophysical Journal*, Volume 94, June 2008, 4957–4970

4957

#### Three-Dimensional Resolution Doubling in Wide-Field Fluorescence Microscopy by Structured Illumination

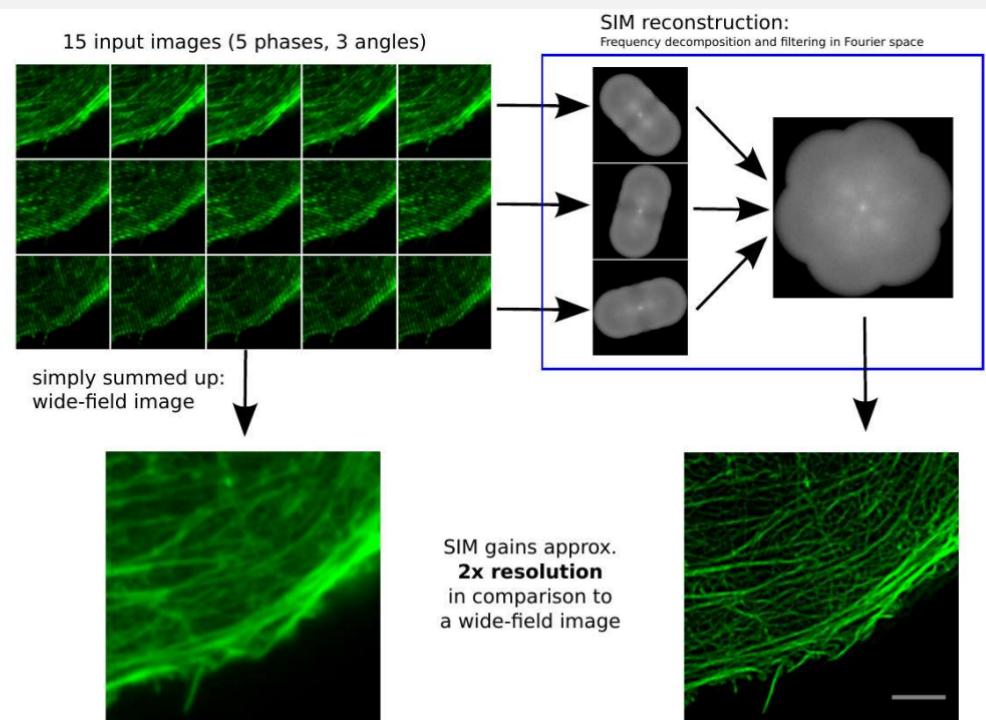
Mats G. L. Gustafsson,<sup>1</sup> Lin Shao,<sup>1</sup> Peter M. Carlton,<sup>1</sup> C. J. Rachel Wang,<sup>1</sup> Inna N. Golubovskaya,<sup>1</sup> W. Zacheus Cardozo,<sup>2</sup> Daniel A. Weisz,<sup>2</sup> and John W. Sedat<sup>1</sup>

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**ABSTRACT** Structured illumination microscopy is a method that can increase the spatial resolution of wide-field fluorescence microscopy. It uses a patterned illumination source to encode information about the specimen into the observed image. This method is applied in three dimensions to double the axial as well as the lateral resolution, with true optical sectioning. A grating is used to generate three mutually coherent light beams, which interfere in the specimen to form an illumination pattern that varies both laterally and axially. The spatially structured excitation intensity causes normally unreachable high-resolution information to become encoded into the observed images through spatial frequency mixing. This new information is computationally extracted and used to generate a three-dimensional reconstruction with twice as high resolution, in all three dimensions, as is possible in a conventional wide-field microscope. The method has been demonstrated on both test objects and biological specimens, and has produced the first light microscopy images of the synaptosomal complex in which the lateral elements are clearly resolved.

# SIM: Structured illumination microscopy

- “Abbe twice”
  - Resolution limit independent of light going in or out of the sample
- Structure the illumination light (fine pattern at res. limit)
- Take 9 or 15 images
- Reconstruct into super-resolved image
  - + compatible with many dyes
  - + very fast (60fps and more)
  - Complex instruments
  - Limited to 2x resolution



# Should you use SIM?

SIM<sup>(\*)</sup> gives you:

- 2x push in resolution
- Out-of-focus reduction (think: confocal)
- Really nice contrast for 150nm-ish sized structures
- Lots of speed (15fps OMX, 2x 30fps *in our lab*)

SIM requires:

- “stable” fluorescence (< 30% bleaching, no blinking)
- low aberrations in the sample (“cells good, tissue ‘meh’”)
- 5-10µm axial range, don’t image through too much material
- If you have demanding samples:
  - Experienced operators help a lot
  - Example: Immersion oil  
1.518 to 1.522 makes a difference

There is a really good Nat. Prot.:

J. Demmerle, [10.1038/nprot.2017.019](https://doi.org/10.1038/nprot.2017.019)

(\*) Linear SIM, on a commercial microscope (see next slides),  
w/o any “cool new tricks”

Alternatives:

- General: Airy-Scan
  - “like confocal, but better”
  - Resolution and contrast not as good as SIM, but far beyond widefield
  - Image quality degrades gracefully in demanding samples
- Thick samples: Two-photon
  - Typically confocal-like resolution
  - Excels in thick samples
- Fixed samples: STORM, STED, ...
  - (much) slower than SIM
  - Special / suitable dyes needed
  - Large push in spatial resolution

1. Choose the imaging method that suits your problem  
(probably start with a confocal...)
2. SIM excels at
  - Straight-forward resolution doubling on “nice” samples
  - Lots of speed for live-cell imaging

# Commercial systems: GE, Zeiss, Nikon

## GE Healthcare

OMX SE (current)

OMX v4 (previous, in wide use)

- fast (15fps single slice, 1s 1µm stack), multi-camera, rather complex systems
- TIRF-capable (only SE version)
- For excitation wavelength, check with facility, as these are configured individually
- Acquisition and post-processing is separated (fairSIM for 2D, full 3D in development)
- No eyepieces!

## Zeiss

Zeiss Elyra S.1

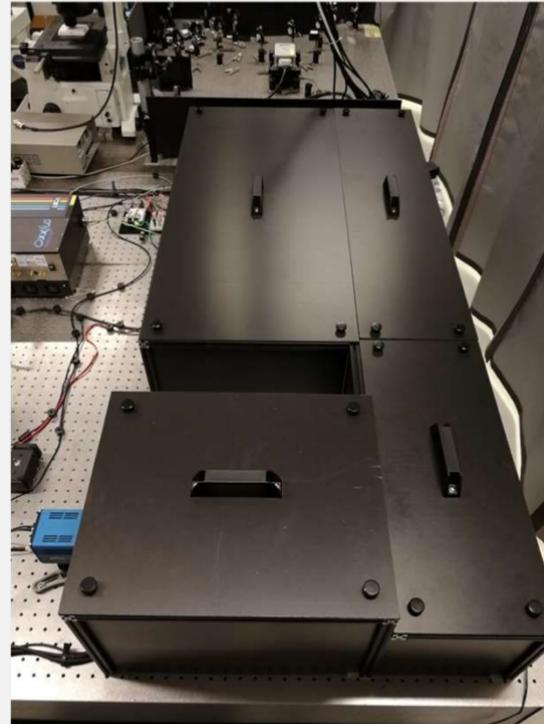
- Standard Zeiss research microscope, with SIM “attached”
- Slower than the OMX, especially for multi-color imaging
- Integrated software solution (Zeiss ZEN)
- “Compact” setup
- “Lattice SIM” → 5-beam interference

## Nikon

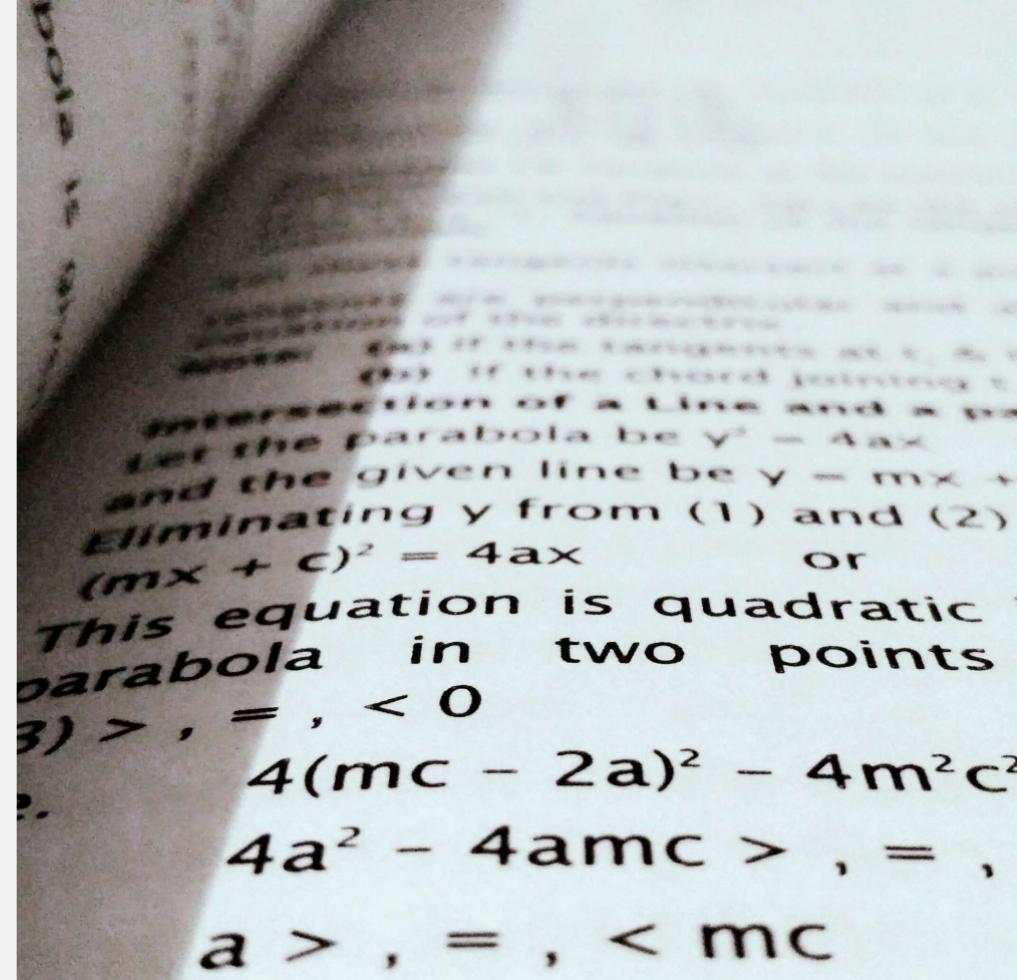
Nikon NSIM(-S)

- (probably) similar to the Zeiss system
- Does not seem to have a large user base
- Never operated one myself
- S: Speed / SLM

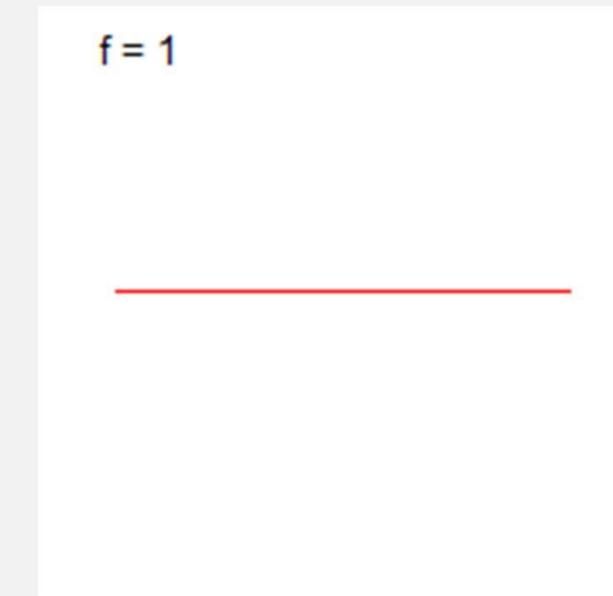
# Bespoke systems → later



# SIM math



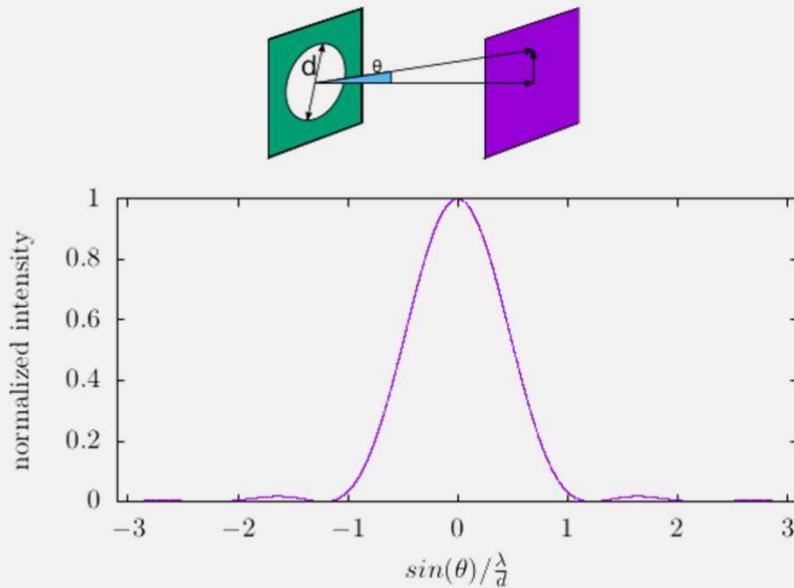
# Fourier transforms (adding frequencies)



# Fourier transforms decomposition



# PSF: resolution limited by diffraction

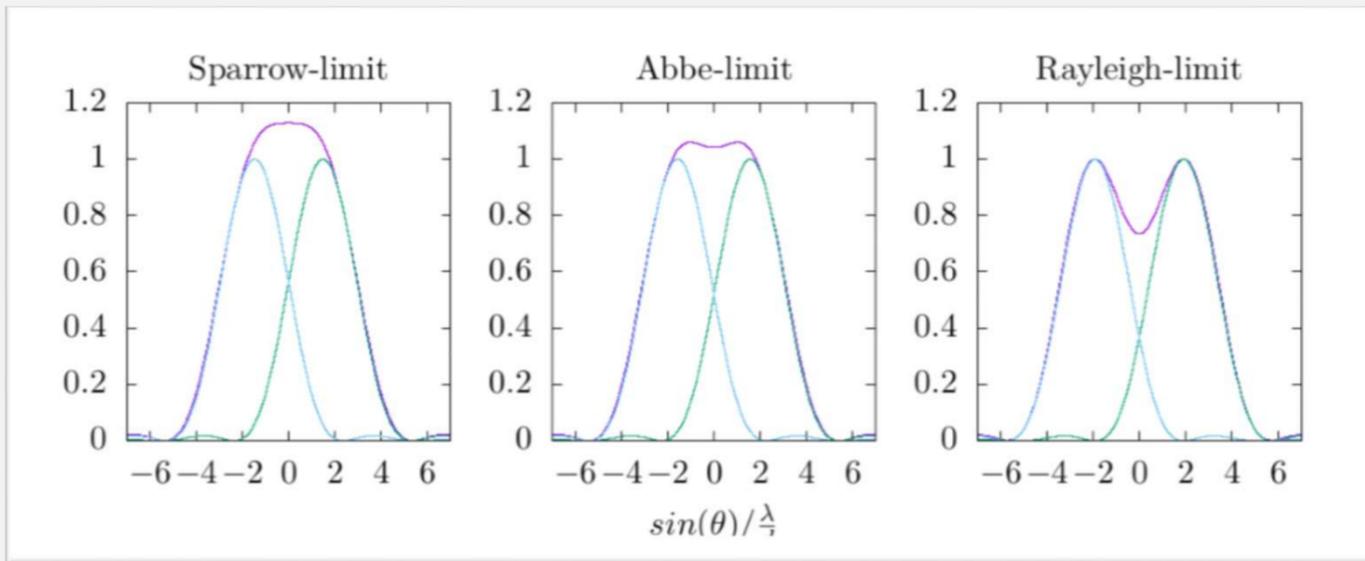


$$I(\theta) = \frac{J_1\left(\frac{2\pi a}{\lambda} \theta\right)}{\frac{2\pi a}{\lambda} \theta}$$

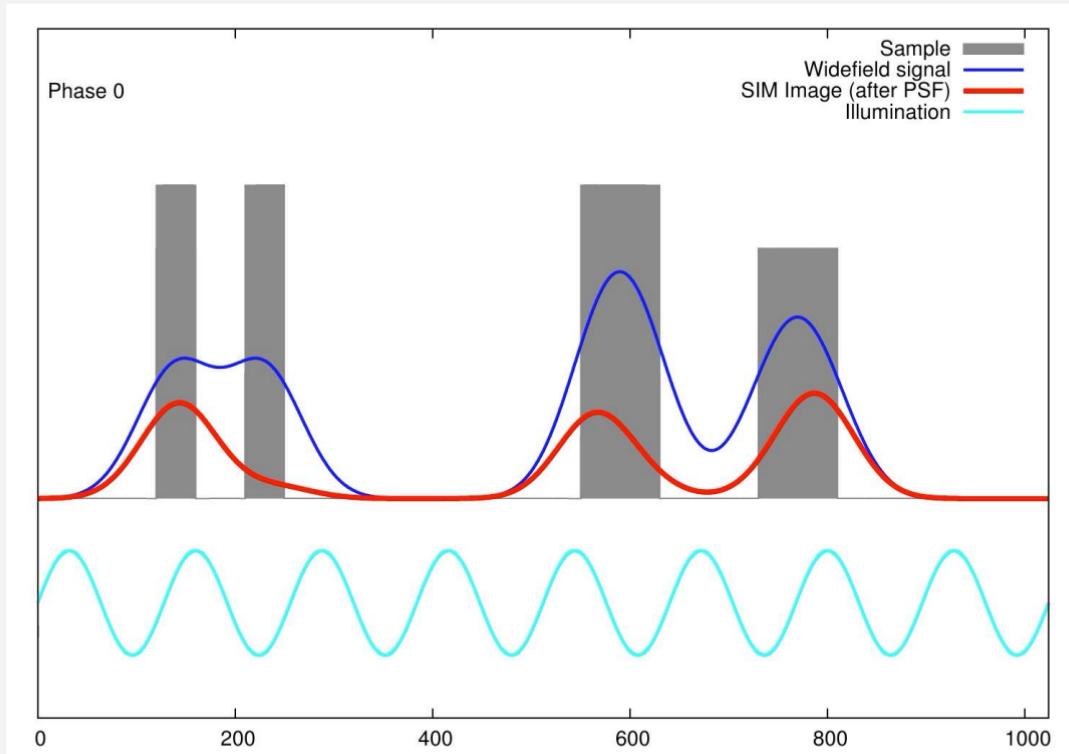
(for small angles  $\theta$ )

- It is a physical limit
- It is different for coherent and incoherent light
- Fluorescent emission: incoherent
- Fluorescent excitation:
  - LED: incoherent
  - Laser: coherent

# Typical definitions of resolution limits “distinguish points...”



**SIM extracts additional information**



# OTF: PSF in Fourier space

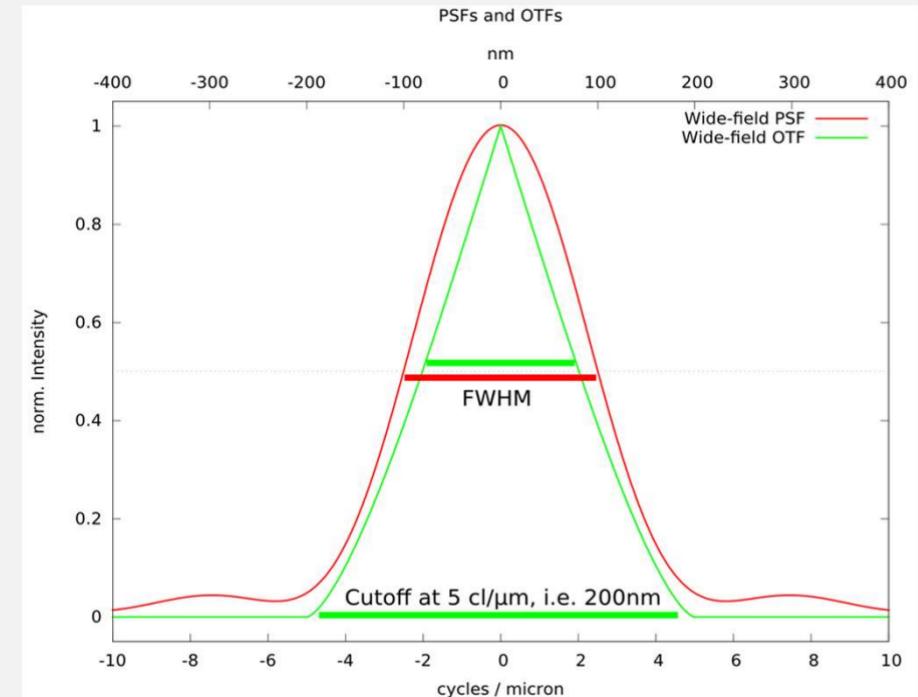
$$h(k) = \frac{2}{\pi} \left[ \arccos\left(\frac{k}{k_{max}}\right) - \frac{k}{k_{max}} \sqrt{1 - \left(\frac{k}{k_{max}}\right)^2} \right]$$

With Abbe:  $\frac{\lambda}{2NA} = k_{max}$

Keep in mind:

$$\tilde{h}(x) = FT(h(k))$$

It is the same function!  
(in a different basis)



1. Camera image  $D$  of sample  $S$  under constant illumination  $I$  (widefield), blurred by PSF  $\tilde{h}$

$$D(\vec{r}) = [S(\vec{r}) \cdot I(\vec{r})] * \tilde{h}(\vec{r})$$

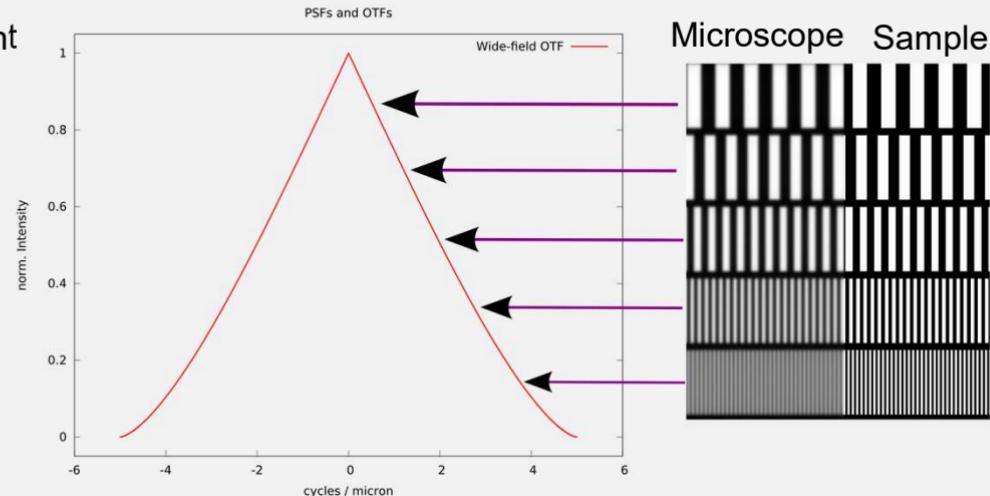
$$I(\vec{r}) = I_0 \rightarrow \text{const.}$$

2. Microscopy in Fourier space:  
fold with PSF  $\rightarrow$  multiplication with OTF

$$\tilde{D}(\vec{k}) = [\tilde{S}(\vec{k}) * \tilde{I}(\vec{k})] \cdot h(\vec{k})$$

$$\tilde{D}(\vec{k}) = [\tilde{S}(\vec{k}) * \delta(\vec{k}) I_0] \cdot h(\vec{k})$$

$$\tilde{D}(\vec{k}) = I_0 \cdot \tilde{S}(\vec{k}) \cdot h(\vec{k})$$



- OTF shows two effects
  - Band-limit, i.e. higher frequencies do not transmit
  - Medium frequencies get “dampened”  $\rightarrow$  that is what we perceive as “blurred”
- What this omits (at least, besides all practicalities)
  - Area integration of camera pixels
  - **Light quantisation (in SIM: every time someone says “filtering”)  $\rightarrow$  Shot noise**

1. Camera image D of sample S,  
but now under **sinusoidal SIM illumination**  $I(\vec{r})$ ,  
blurred by PSF  $\tilde{h}$

$$D_{q,n}(\vec{r}) = [S(\vec{r}) \cdot I(\vec{r})] * \tilde{h}(\vec{r})$$

$$I(\vec{r}) = I_0 \sum_{m=0}^M a_{q,m} \cos\{2 \pi m (\vec{r} \cdot \vec{p}_q + \phi_n)\}$$

$\vec{p}_q$  Wave vector, spacing and rotation of your SIM pattern

$\phi_n$  Phase shift of the SIM pattern

$m$  Number of harmonics

$a_{q,m}$  Modulation strength of a certain harmonic

Side note:  $m$  has to start at 0. This is “constant” and accounts for the fact that light intensities physically cannot be negative.

## 2. Fourier space

$$D_{q,n}(\vec{r}) = \left[ S(\vec{r}) \cdot I_0 \sum_{m=0}^M a_{q,m} \cos\{2 \pi m (\vec{r} \cdot \vec{p}_q + \phi_n)\} \right] * \tilde{h}(\vec{r})$$

$$\tilde{D}_{q,n}(\vec{k}) = \left( I_0 \sum_{m=0}^M a_{q,m} e^{\pm im\phi_n} \boxed{\delta(\vec{k} \mp m \cdot \vec{p}_q) * \tilde{S}(\vec{k})} \right) \cdot h(\vec{k})$$

## 3. Convolution with delta peak → shift of information

$$\tilde{D}_{q,n}(\vec{k}) = \left( I_0 \sum_{m=0}^M a_{q,m} e^{\pm im\phi_n} \tilde{S}(\vec{k} \mp m \vec{p}_q) \right) \cdot h(\vec{k})$$

#### 4. Construct a linear equation system.

**Vectors:**

$$\tilde{D}_{q,n}(\vec{k}) = \left( I_0 \sum_{m=0}^M a_{q,m} e^{\pm im\phi_n} \tilde{S}(\vec{k} \mp m\vec{p}_q) \right) \cdot h(\vec{k})$$

**Matrix:**

$$\bar{E} \rightarrow \bar{E}_{mn} = a_{q,m} e^{im\phi_n} .$$

Equation on the left becomes:

$$\sum_{n=1}^N \tilde{D}_{q,n}(\vec{k}) = \sum_{n=1}^N \sum_{m=-M}^M \bar{E}_{mn} \tilde{T}'_m(\vec{k})$$

$$\tilde{T}' \rightarrow \tilde{T}'_m(\vec{k}) = \tilde{S}(\vec{k} - m\vec{p}_q) h(\vec{k}) I_0$$

with entries  $m = -M .. -0, +0 .. M$

Also absorbs contributions of  $h(\vec{k})$  and  $I_0$

Multiple Measurements

$\tilde{D}'_q \rightarrow \tilde{D}_{q,n}(\vec{k})$  with  $n$  varying phases  $\phi_n$

$$\tilde{D}'_q(\vec{k}) = \bar{E} \tilde{T}'(\vec{k})$$

## 5. Solve the equation system: “*Band separation*”

$$\tilde{D}'_q(\vec{k}) = \bar{E} \tilde{T}_q'(\vec{k})$$

$$\tilde{T}_q'(\vec{k}) = \bar{E}^{-1} \tilde{D}'_q(\vec{k})$$

Remember: We've shifted information:

$$\tilde{T}' \rightarrow \tilde{T}_m(\vec{k}) = \tilde{S}(\vec{k} - m\vec{p}_q) \mathbf{h}(\vec{k}) I_0$$

Example for M=1, N=3 (3-phases 2-beam SIM)

$$\begin{pmatrix} 2 a_{q,0} & a_{q,1} e^{i\phi_1} & a_{q,2} e^{-i\phi_1} \\ 2 a_{q,0} & a_{q,1} e^{i\phi_2} & a_{q,2} e^{-i\phi_2} \\ 2 a_{q,0} & a_{q,1} e^{i\phi_3} & a_{q,2} e^{-i\phi_3} \end{pmatrix}^{-1} \begin{pmatrix} \tilde{D}_{q,\phi_1} \\ \tilde{D}_{q,\phi_2} \\ \tilde{D}_{q,\phi_3} \end{pmatrix} = \begin{pmatrix} \tilde{S}_q(\vec{k}) \mathbf{h}(\vec{k}) I_0 \\ \tilde{S}_q(\vec{k} - \vec{p}_1) \mathbf{h}(\vec{k}) I_0 \\ \tilde{S}_q(\vec{k} + \vec{p}_1) \mathbf{h}(\vec{k}) I_0 \end{pmatrix}$$

- right-hand side provides information beyond the diffraction limit, along  $\vec{p}_1$
- Multiple orientations ‘q’ to fill all 2D space
- Can be extended to higher harmonics M>1, by providing 2 extra phase shifts per harmonic
- This is the first step in the SIM reconstruction**

- Note: We don't need to know  $\vec{p}_n$  at this point
- Less obvious: We can correct for a global phase shift ( $\phi_n = \phi_{\text{global}} + \phi'_n$ ) later  
→ Here we only need to know relative phases

Why care? This is what makes parameter extraction work

## 7. Reassemble the bands

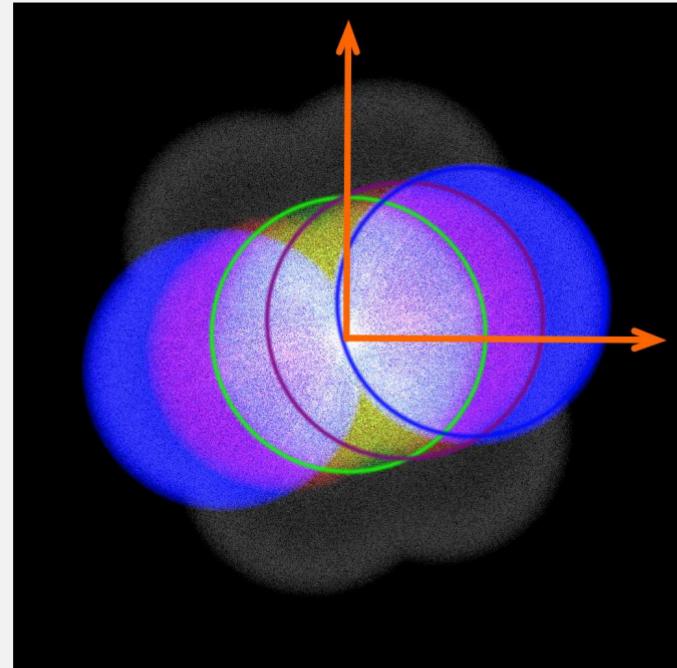
Shift the sample information

$$\tilde{S}_m(\vec{k} - m\vec{p}_q)h(\vec{k})I_0 \rightarrow \tilde{S}_m(\vec{k})h(\vec{k} + m\vec{p}_q)I_0$$

Sum it all up:

$$\tilde{S}'_{\text{SIM}}(\vec{k}) = \sum_{q=1}^Q \sum_{m=-M}^M \frac{\{\tilde{S}_m(\vec{k})h(\vec{k} + m\vec{p}_q)I_0\}}{h(\vec{k} + m\vec{p}_q)I_0}$$

This now clearly contains information beyond the diffraction limit, see shifted support of  $h(\vec{k} + m\vec{p}_q)$



## 8. Filter

$$\tilde{S}'_{\text{SIM}}(\vec{k}) = \sum_{q=1}^Q \sum_{m=-M}^M \frac{\{\tilde{S}_m(\vec{k}) h(\vec{k} + m\vec{p}_q) I_0\}}{h(\vec{k} + m\vec{p}_q) I_0}$$

**Problem:**

For all  $|\vec{k}| > k_{\max}$ , we are dividing by 0

(One) solution:  
Generalized Wiener filtering

$$\tilde{S}''_{\text{SIM}}(\vec{k}) = \sum_{q=1}^Q \sum_{m=-M}^M \frac{\{\tilde{S}_m(\vec{k}) h(\vec{k} + m\vec{p}_q) I_0\} \cdot h(\vec{k} + m\vec{p}_q)}{h^2(\vec{k} + m\vec{p}_q) + \omega^2}$$

... and apodization

$$\tilde{S}_{\text{SIM}}(\vec{k}) = \sum_{q=1}^Q \sum_{m=-M}^M \frac{\{\tilde{S}_m(\vec{k}) h(\vec{k} + m\vec{p}_q) I_0\} \cdot h(\vec{k} + m\vec{p}_q)}{h^2(\vec{k} + m\vec{p}_q) + \omega^2} \cdot A(\vec{k})$$

**At this point:**

- SIM reconstruction fully defined
- Linear equations, directly solvable

How do we know all the parameter?

$\vec{p}_q$  Wave vector, spacing and rotation of your SIM pattern

$\phi_{\text{global}}$ ,  $\phi'_n$  Phase shift of the SIM pattern  
 $m$  Number of harmonics

$a_{q,m}$  Modulation strength of a certain harmonic

Instrument and sample fluctuations too big to run with fixed  $\vec{p}_q$  and  $\phi_{\text{global}}$ .

$\phi'_n$  we can assume to be equidistant,  
or at least known.

$a_{q,m}$  we don't need to know as precisely,  
but will extract.

## Parameter estimation:

$\phi'_n$  known, which is all we need for band separation.

Thus, we can get to here:

$$\tilde{\mathbf{T}}' \rightarrow \tilde{T}_m(\vec{k}) = \tilde{S}_m(\vec{k} - m\vec{p}_q)h(\vec{k})I_0$$

Problem, for shifting (and recombining) we need  $\vec{p}_q$

Solution:

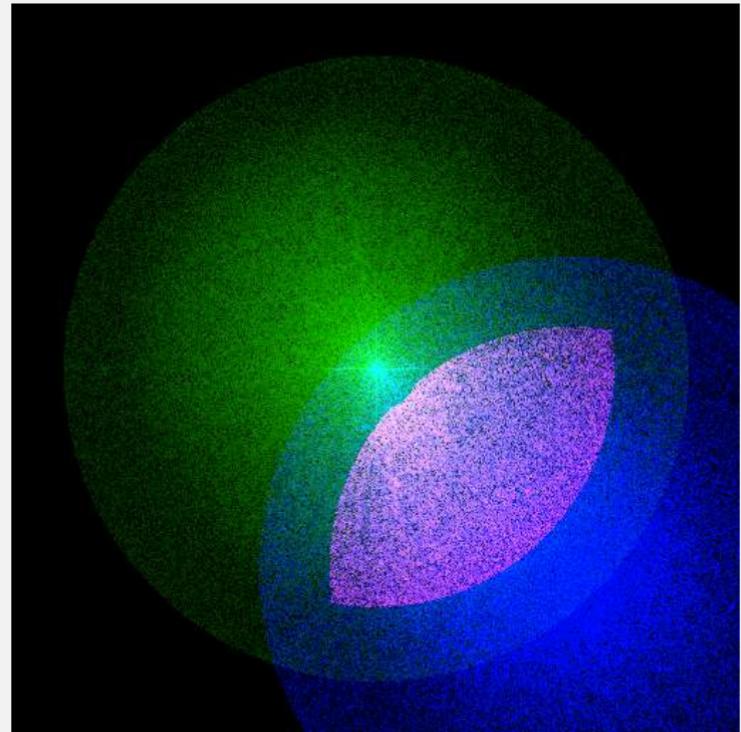
- (only) for the correct  $\vec{p}_q$

$$\frac{\tilde{T}_m(\vec{k} + m\vec{p}_q)}{h(\vec{k} + m\vec{p}_q)} = \frac{\tilde{T}_0(\vec{k})}{h(\vec{k})}$$

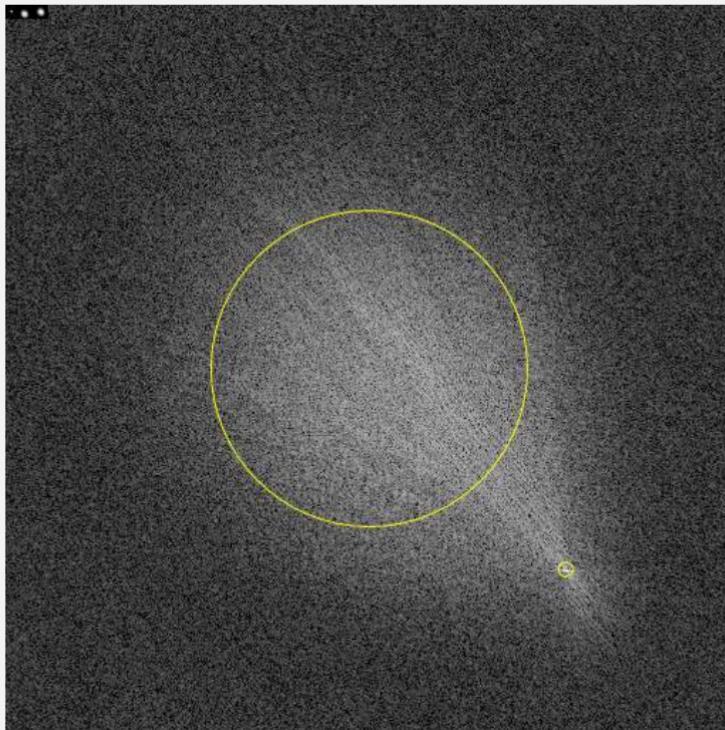
- Again, division by 0. Find regions  $(\vec{k})$  where both

$$h(\vec{k} + m\vec{p}_q) \gg 0 \text{ and } h(\vec{k}) \gg 0$$

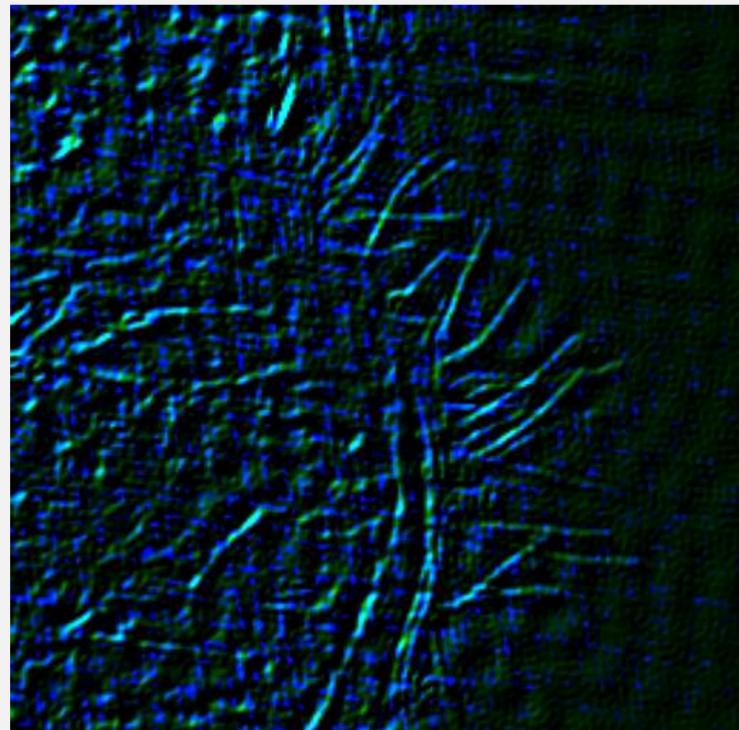
- Iteratively iterate  $\vec{p}_q$  until both bands give you the same signal.



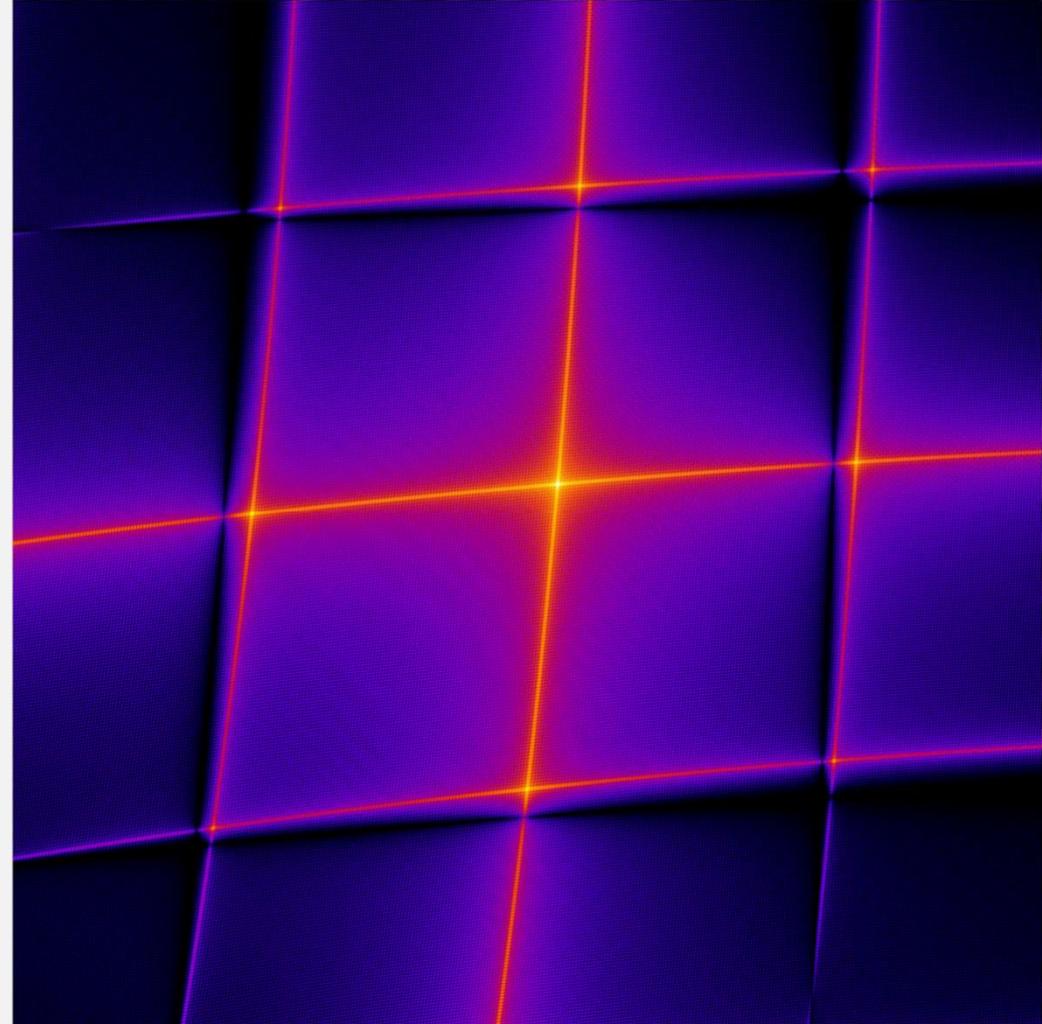
Peak finding visualized in fairSIM



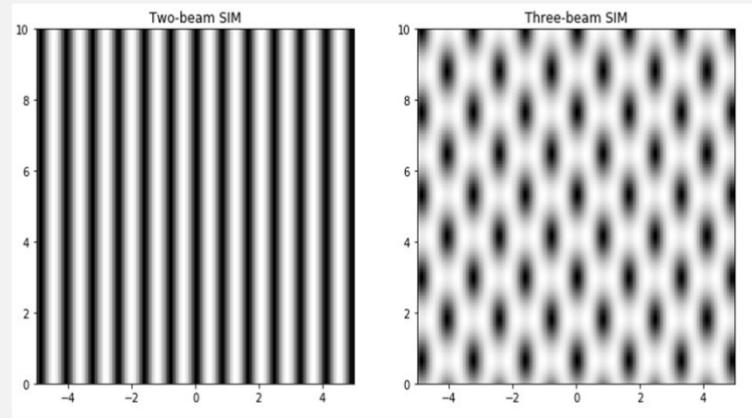
Medium freq. information content  
from two different bands  
(blue, green)



# SIM illumination modes + instruments



# SIM illumination modes



- TIRF systems
- Bespoke systems
- Faster (9 vs. 15 raw frames)
- Only lateral resolution enhancement
- Maintains optical sectioning
- All commercial systems
- Axial and lateral enhancement
- Complex opto-mechanics

# Who does what?

OMXv4 (and older):

**3-beam SIM**

“blaze” models:

Fixed grating, fast galvos

Older models:

Phase galvos, rotating grating

OMX SR:

**3-beam SIM**

**TIRF SIM**

“blaze” model:

Fixed grating,  
fast galvos

Zeiss Elyra S1

**3-beam SIM**

(variable pitch)

Rotating grating

Zeiss Elyra 7

**5-beam SIM (2x2x1)**

(variable pitch)

No rotation needed

Nikon NSIM(-E)

**3-beam SIM**

Rotating grating

Nikon NSIM-S

**3-beam SIM**

**2-beam SIM**

**TIRF SIM**

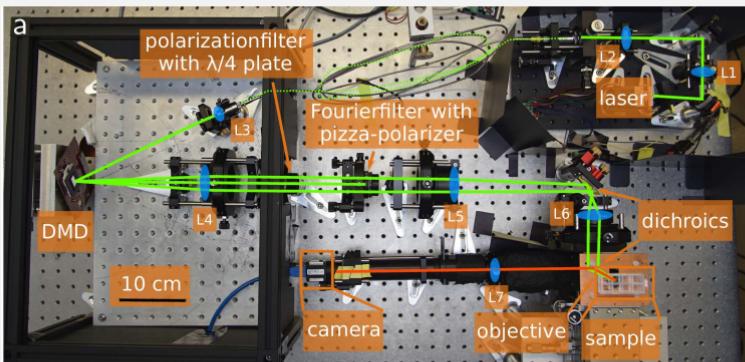
SLM

# Bespoke systems

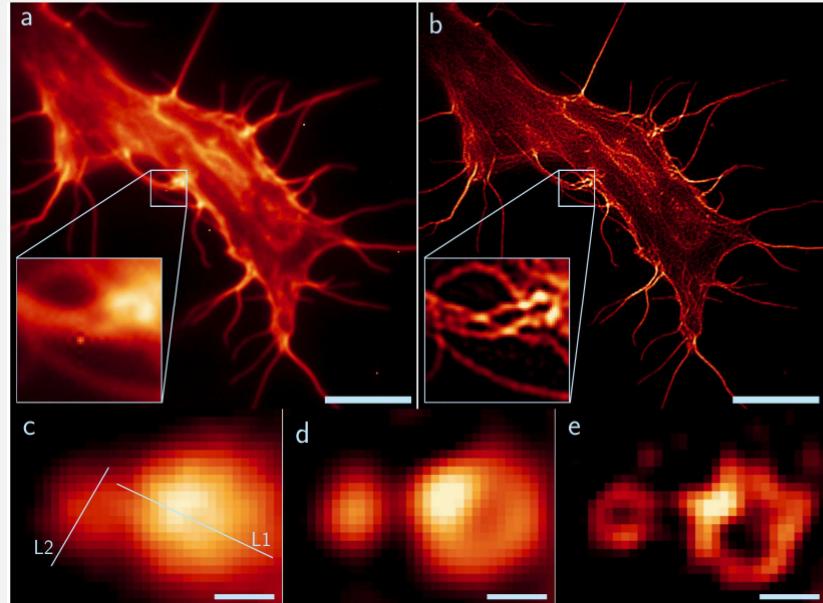
Two-beam is massively easier to implement, especially when optimizing speed and/or cost



A. Sandmeyer



\$15k-20k SIM microscope



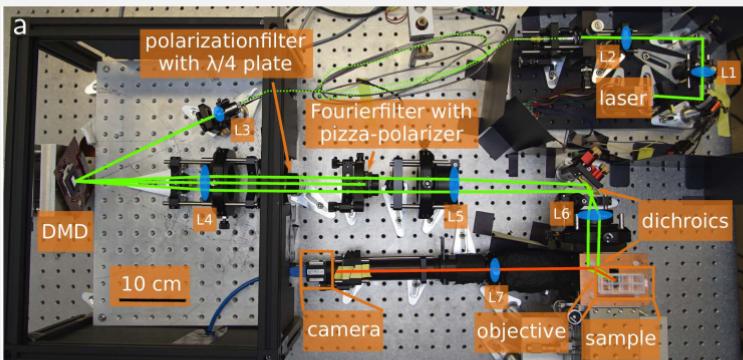
Fixed HEK293T cells transfected with the fluorescent protein mScarlet, to either label actin (a,b) or lysosomes (c-e). Closed labeled actin filaments cannot be distinguished in the WF image (a), but the SIM image reveals that more than one filament is present (b) (scale bar 5  $\mu\text{m}$ , inset 2.1  $\mu\text{m} \times 2.1 \mu\text{m}$ , exposure time per raw frame 50 ms). Lysosomes have different diameters and with the used plasmid, the outer membrane was stained. The membrane structure of bigger lysosomes (cross-section L1, plot e) can be revealed with conventional WF imaging (c) and additional filtering (d), but for smaller lysosomes (cross-section L2, plot f) SIM imaging is required (scale bar 250 nm, exposure time per raw frame 50 ms).

# Bespoke systems

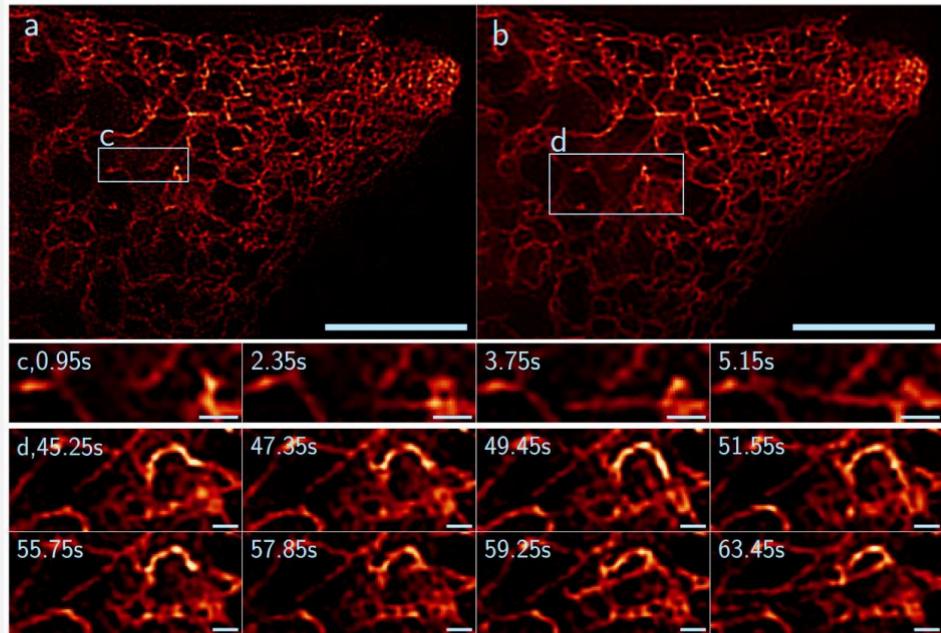
Two-beam is massively easier to implement, especially when optimizing speed and/or cost



A. Sandmeyer

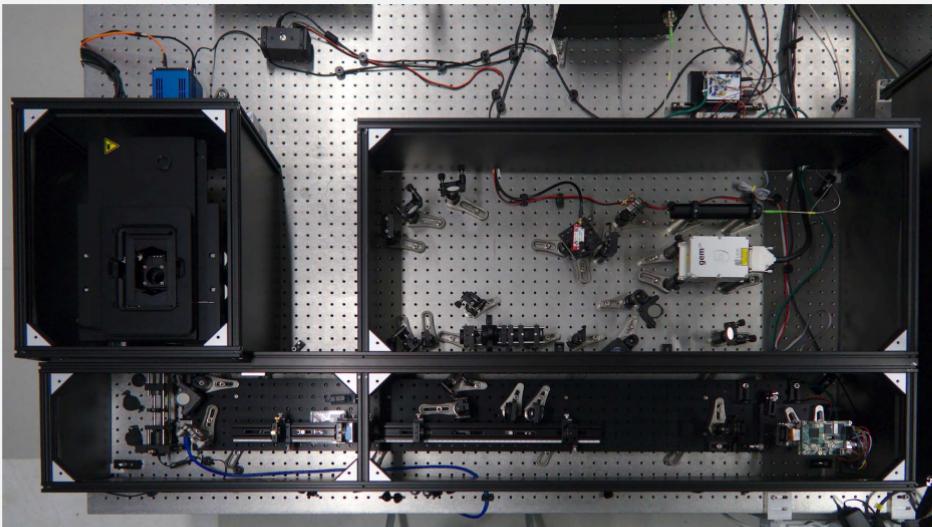


\$15k-20k SIM microscope



Endoplasmic reticulum (ER) network in a living U2OS cell. The organelle was stained with ER-Tracker Red and the data was recorded with a delay of 250 ms between each SIM sequence at room temperature. (a) SIM image at time point 0 s reveals many filaments. (b) Hessian denoising of the SIM frame reduces the background signal and smoothes the filaments (scale bar 5  $\mu$ m, exposure time per raw frame 50 ms). The time sequence (c) is at inset 1 and shows the elongation of a fiber (scale bar 500 nm). In addition, a detachment can be observed with a subsequent attachment to another point and a further elongation (d, inset 2, scalebar 500 nm).<sup>28</sup> Both insets display the Hessian denoised data.

# More bespoke systems

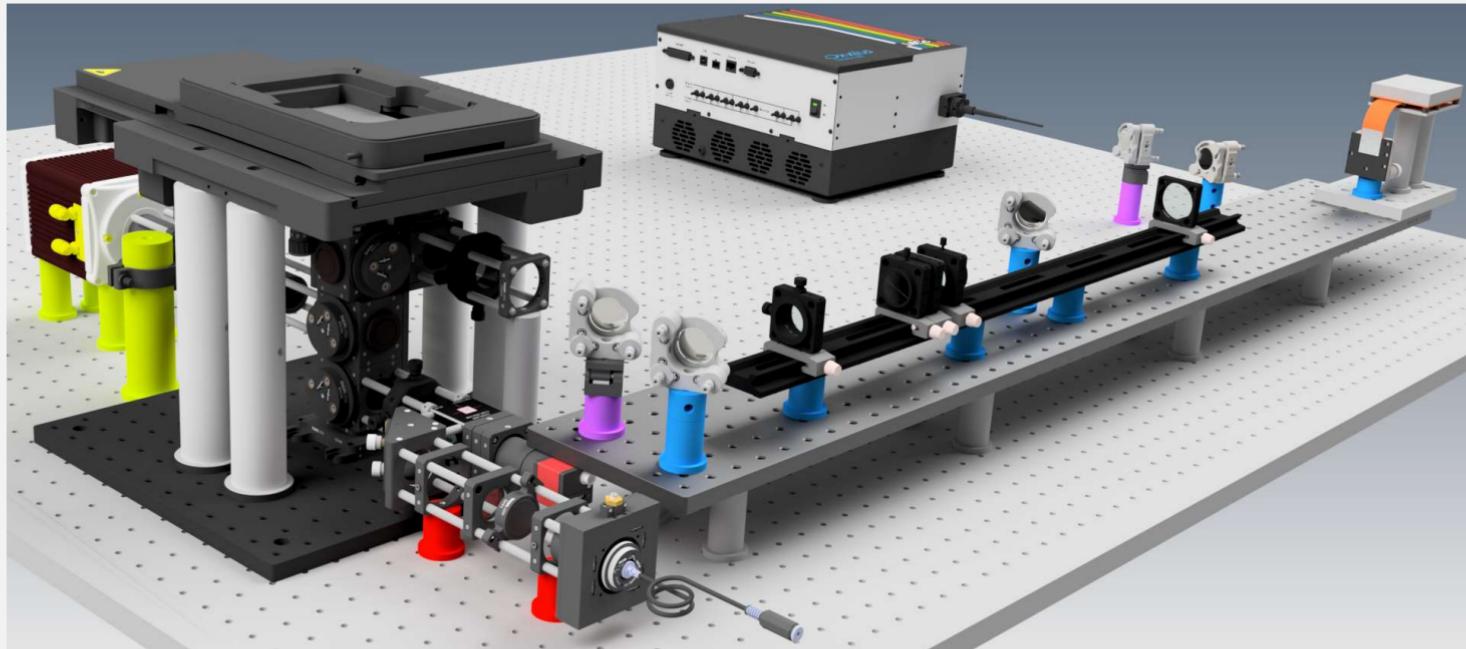


\$40k-60k SIM microscope

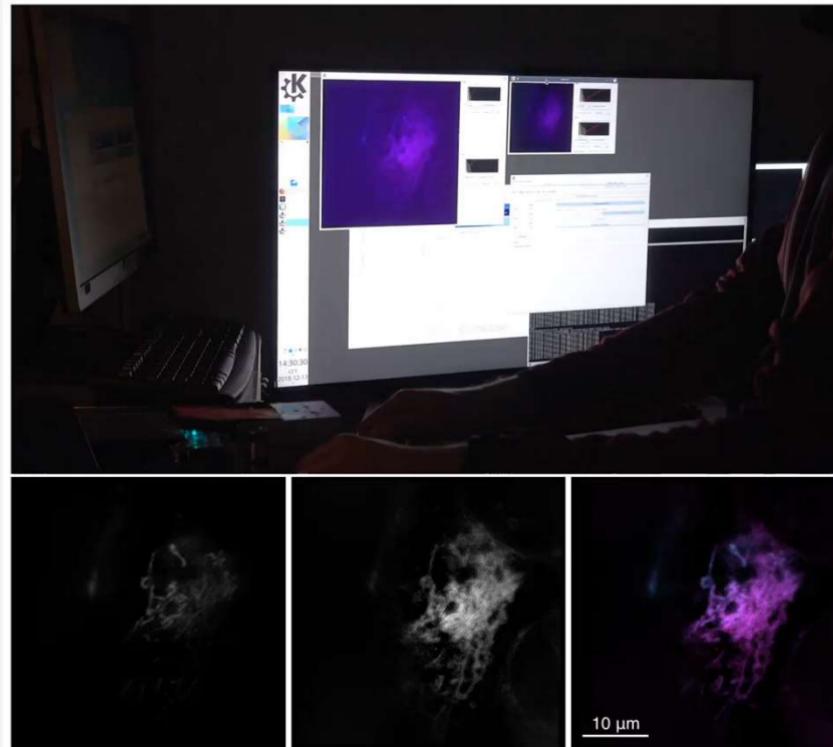
- 488 / 561 / 640 laser lines
- Fast stage with lots of travel
- IR focus tracking
- sCMOS camera
- ~20fps reconstructed SIM images
- 98% off-the-shelf parts (Thorlabs & co.)
- Full documentation (AutoCAD, part lists, various source codes)
- Designed for throughput and replication (multiple systems if project gets funded)



R. Van den Eynde



# Run the reconstruction in real-time



# SIM reconstruction



# The fairSIM project

- 2014 – 2019 strong research focus on SIM
- ImageJ plugin (Nat. Commun. 2016)
- Video-rate, multi-color microscope with on-the-fly reconstruction (Nat. Commun. 2019)
- Comprehensive, open solutions for SIM imaging
- Foundation of various ongoing research projects

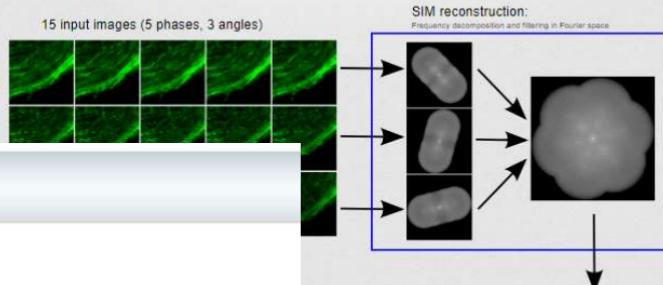


[View on GitHub](#)

Structured Illumination Microscopy (SIM) provides a fast and gentle super-resolution approach for fluorescence microscopy. The *fairSIM project* aims to provide a range of free and open-source tools and resources for scientists working with SIM.

## The fairSIM ImageJ / FIJI plugin

We provide an open-source implementation of the reconstruction algorithms used for SIM. It runs as a plugin to the popular image processing software [ImageJ](#) and the [FIJI](#) package.



## ARTICLE

Received 16 Oct 2015 | Accepted 8 Feb 2016 | Published 21 Mar 2016

[DOI: 10.1038/ncomms10980](https://doi.org/10.1038/ncomms10980)

OPEN

Open-source image reconstruction of super-resolution structured illumination microscopy data in ImageJ

Marcel Müller<sup>1</sup>, Viola Mönkemöller<sup>1</sup>, Simon Hennig<sup>1</sup>, Wolfgang Hübler<sup>1</sup> & Thomas Huser<sup>1,2</sup>



## ARTICLE

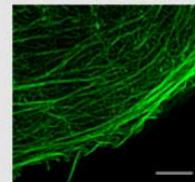
<https://doi.org/10.1038/ncomms12165>

OPEN

Video-rate multi-color structured illumination microscopy with simultaneous real-time reconstruction

Andreas Markwirth<sup>1</sup>, Mario Lachetta<sup>1</sup>, Viola Mönkemöller<sup>1,2</sup>, Rainer Heintzmann<sup>3,4</sup>, Wolfgang Hübler<sup>1</sup>, Thomas Huser<sup>1</sup> & Marcel Müller<sup>1</sup>

SIM gains approx.  
2x resolution  
in comparison to  
a wide-field image



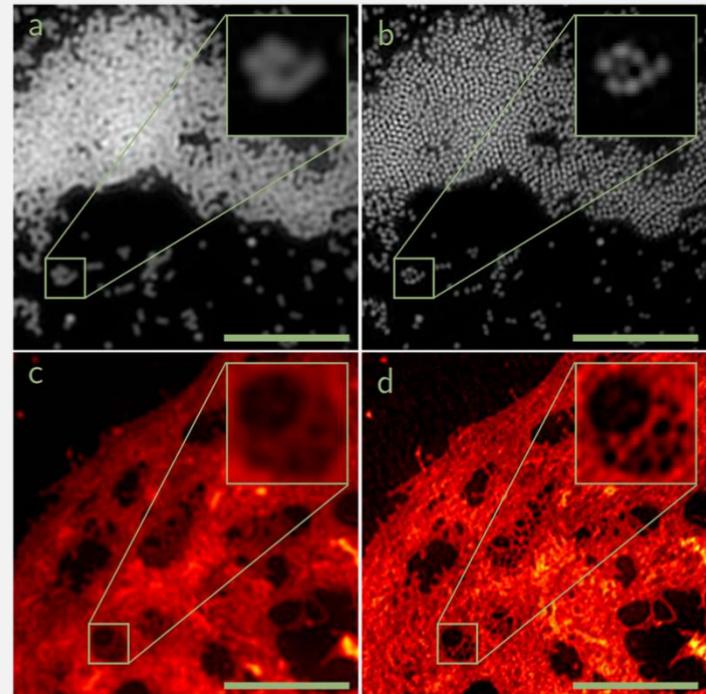
# Single-slice reconstruction (2-beam / 3-beam)

- Fast imaging
- Prime focus of fairSIM so far
- 2-beam / 3-beam both work in single slice
- 3-beam and 2-beam variations allow for background suppression

Single slice SIM reconstructions from fairSIM (data taken on a DeltaVision|OMX v4, excitation at 642nm):

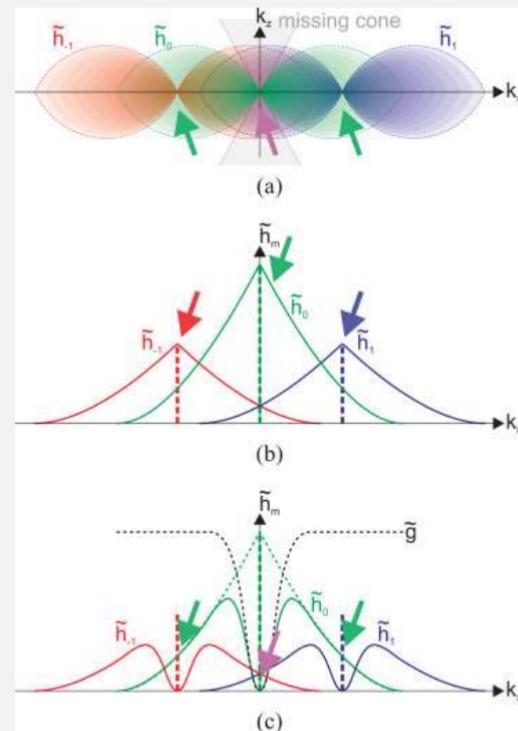
- 200nm fluorescent beads, wide-field (a) and SIM (b).
- CellMask Deep Red membrane stain on LSEC, wide-field (c) and SIM (d)

Scalebar 5µm



# Single-slice SIM: OTF attenuation / notch filtering

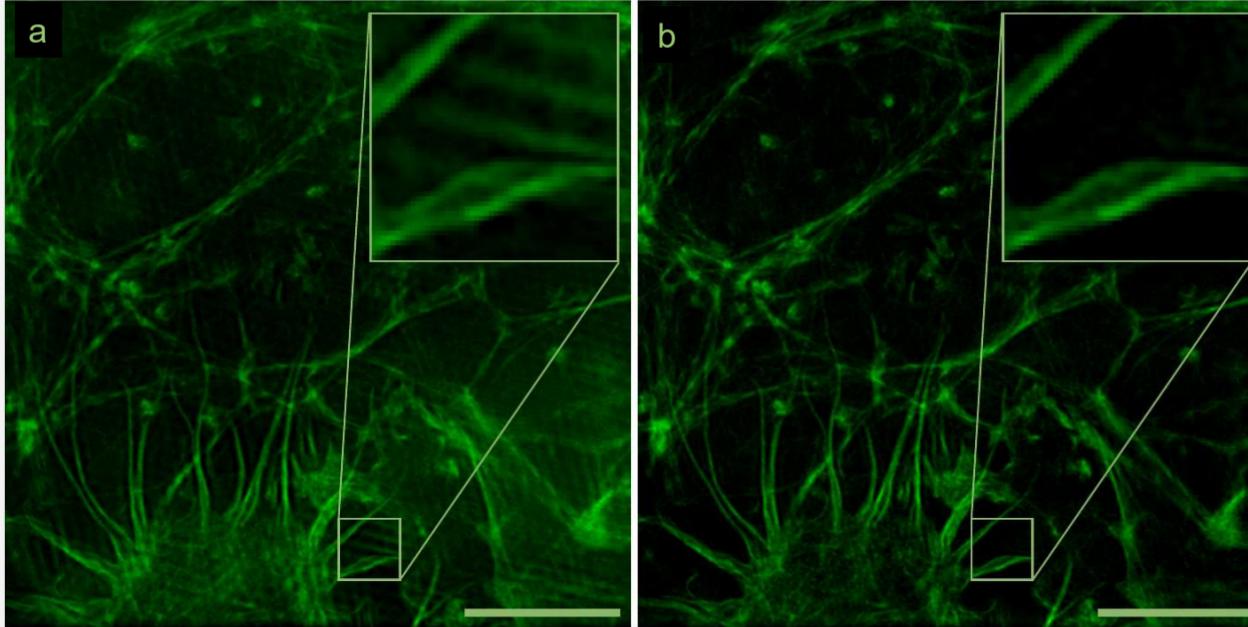
- Out-of-focus light („background“) leads to artifacts in the reconstruction
- In terms of OTFs: Missing cone problem in the 3D OTF
- Can be mitigated in 2D SIM (via 3-beam or lower lateral resolution)
- Almost required for non-TIRF datasets
- Freely configurable in fairSIM



K. Wicker, R. Heintzmann, Opt. Exp. 2013, doi:10.1364/OE.21.002032  
 K. O' Jolleran, M. Shaw, Biomed. Opt. Exp. 2014, doi:10.1364/BOE.5.002580  
 M. Shaw, K. O' Jolleran, Optics Letters, 2012, doi: 10.1364/OL.37.004603

K. Wicker, R. Heintzmann, Opt. Exp. 2013,  
 doi:10.1364/OE.21.002032, fig. 7

# Single-slice SIM: OTF attenuation / notch filtering



Actin filaments in U2OS cells, labelled with Phalloidin-Atto488, measured on a DeltaVision|OMX, excited at 488 nm wavelength.

- (a) reconstruction using a standard 2D OTF
- (b) reconstruction using the attenuated OTF (strength 0.995, FWHM 2 cycles/micron).

Scalebar 5 $\mu$ m, inset 2 $\mu$ m

# Single-slice SIM: RL-deconvolution

- Combine Richardson-Lucy deconvolution with SIM reconstruction
- Published by V. Perez
- Implementation straight forward, took a few hours
- Available since v.1.1.0-beta3
- Crisp images, but be aware of deconvolution

## SCIENTIFIC REPORTS

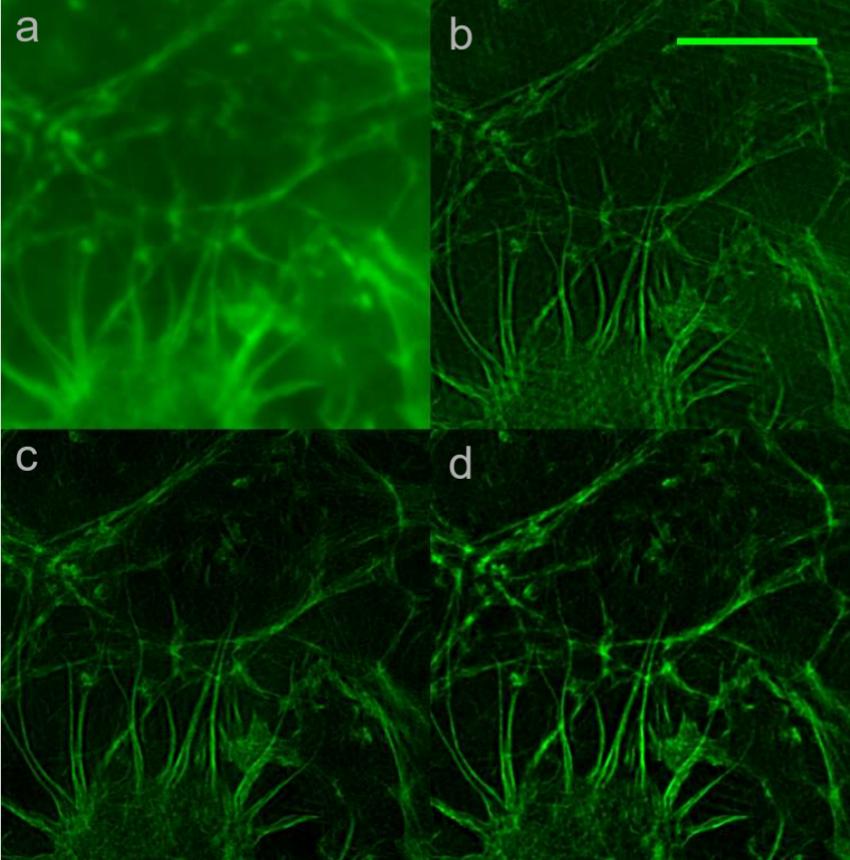
OPEN

Optimal 2D-SIM reconstruction by two filtering steps with Richardson-Lucy deconvolution

Received: 27 June 2016  
Accepted: 25 October 2016  
Published: 16 November 2016

Victor Perez, Bo-Jui Chang & Ernst Hans Karl Stelzer  
Structured illumination microscopy relies on reconstruction algorithms to yield super-resolution images. Artifacts can arise in the reconstruction and affect the image quality. Current reconstruction

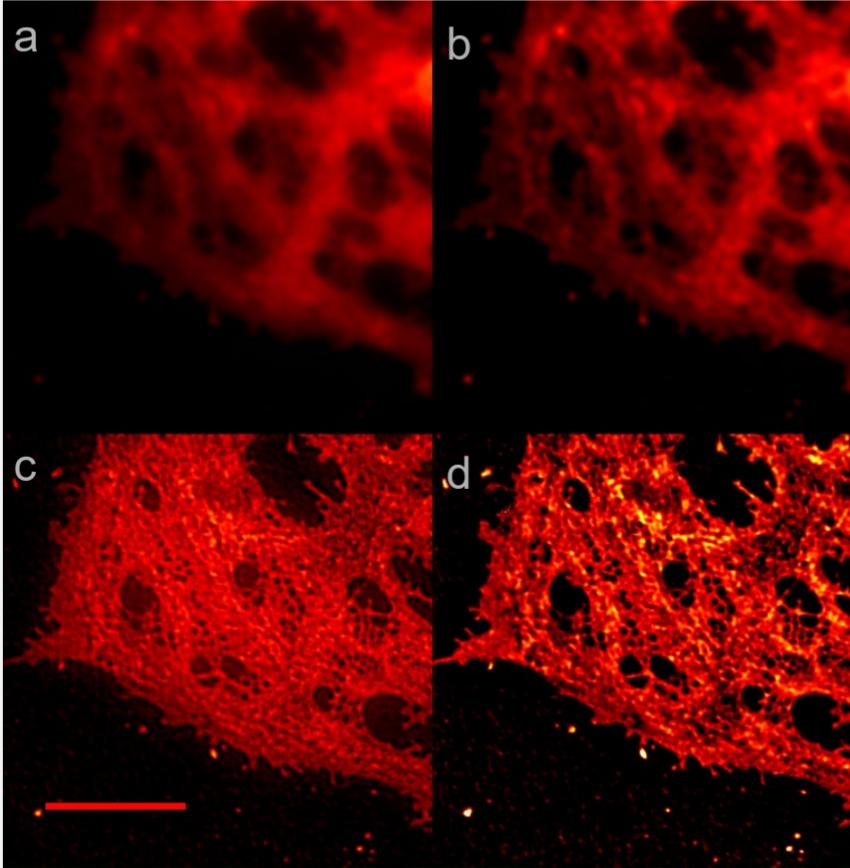
# Single-slice SIM: RL-deconvolution



Actin filaments in U2OS cells, labelled with Phalloidin-Atto488, measured on a DeltaVision|OMX, excited at 488 nm wavelength.

- (a) Wide-field
- (b) Reconstruction w. standard OTF
- (c) Reconstruction using the attenuated OTF (strength 0.995, FWHM 2 cycles/micron).
- (d) Reconstruction using Richardson-Lucy deconvolution (Scalebar 5 $\mu$ m)

# Single-slice SIM: RL-deconvolution



CellMask DeepRed membrane stain,  
LSECs, measured on a  
DeltaVision|OMX, excited at 642 nm  
wavelength.

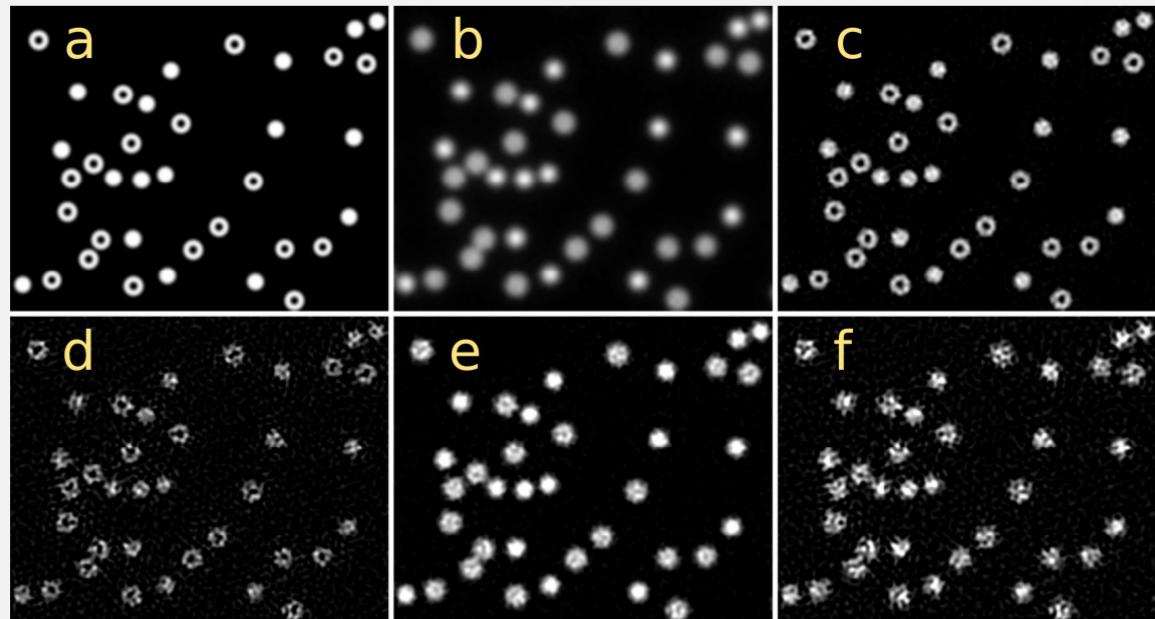
- (a) Wide-field
- (b) Reconstruction w. standard OTF
- (c) Reconstruction using the  
attenuated OTF (strength 0.995,  
FWHM 1.5 cycles/micron).
- (d) Reconstruction using  
Richardson-Lucy deconvolution

Scalebar 5 $\mu$ m

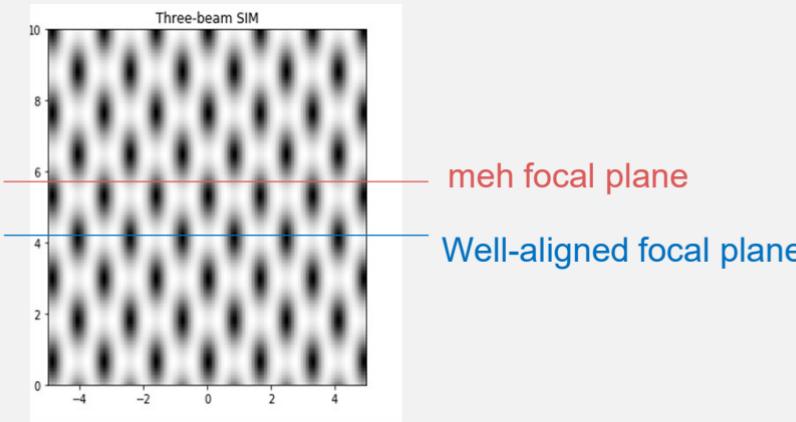
# Simulation, SNR, modulation depth

Degradation of SIM quality due to SNR and pattern contrast. A layer of fluorescent spheres, which are either solid or hollow, is simulated (a). The wide-field image does not allow to tell the hollow and solid spheres apart (b), while a SIM image at reasonable modulation depth of  $\sim 80\%$  and photon count of  $\sim 7000$  per sphere allows for it (c).

Reduction in photon count by  $10\times$  to  $\sim 700$  per sphere introduce typical SIM noise artifacts (d) and uncertainty about the sphere shape. A reduction in SIM pattern contrast to  $\sim 20\%$  does not cause noise, but the super-resolution information is lost (e). A combination of both effects heavily degrades signal quality (f).



# Full 3D reconstruction (usually 3-beam, OMX)



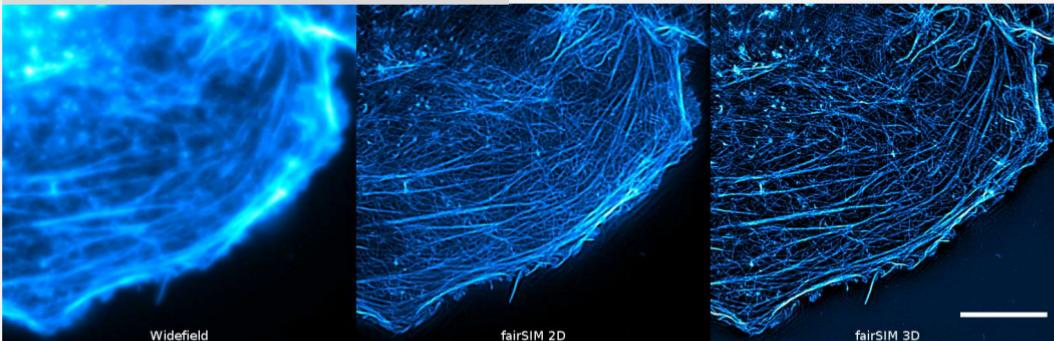
Peculiar way Gustafsson /  
SoftWORX / (currently)  
fairSIM handles “top-phase”

- Z is special: shift sample instead of pattern
- There's nothing special about z: we could do the same with x,y, or indeed not do this for z, but step through top phases. We don't, for historical(?) reasons
- We're encoding pattern alignment (*top phase*) into instrument calibration data (OTF). We wouldn't need to do this
- Not a lot of people look at this!

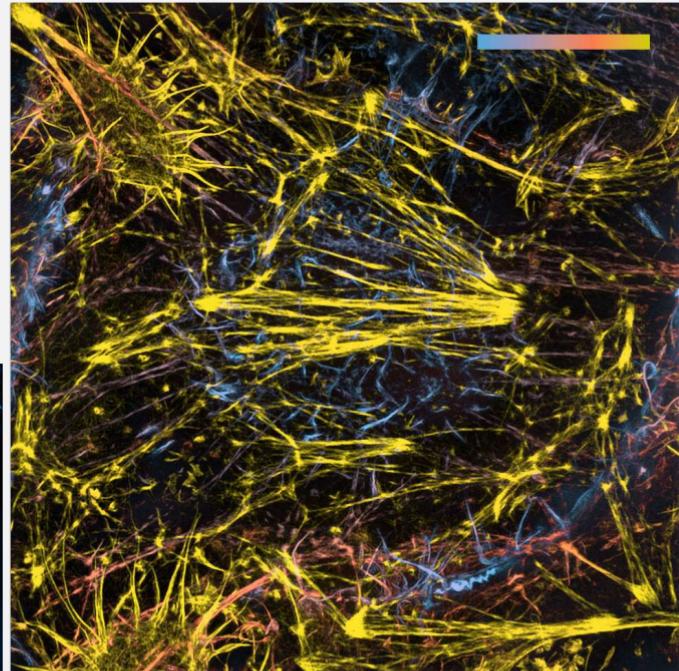
# fairSIM 3D – beta version

- Summer project at Micron Oxford:
- Full 3D reconstruction in fairSIM**
- ‘*develop-3D-SIM*’ branch (github)
- Working beta version since Sep. 2017
- **Known bugs**, and an MSCA-postdoc Nov. 2017-2019
- GPL’d version of (one of) the original Gustafsson codes
- ... back at fixing fairSIM since I moved back to Bielefeld

Actin filaments in LSEC cells, labelled with Phalloidin-Atto488, measured on a DeltaVision|OMX, excited at 488 nm. Scalebar 5µm, single slice vs. 2µm z-projection



Actin filaments in U2OS cells, labelled with Phalloidin-Atto488, measured on a DeltaVision|OMX, excited at 488 nm. Scalebar 5µm, z-color-coding 0µm – 6.25µm



# fairSIM 3D – new GUI, new concept

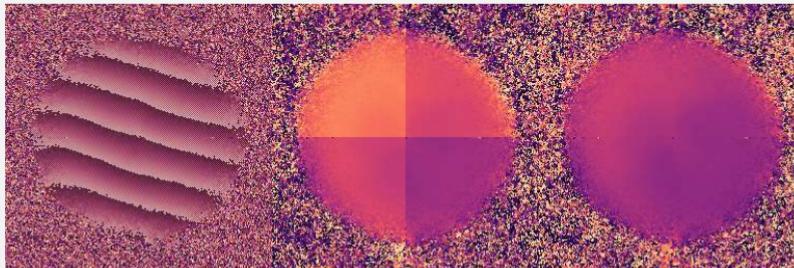
3D SIM processing requires more knowledge about the microscope (esp. OTFs → top phase)

- Editor for alignment data (stored in one XML-file)
- GUI for multi-channel, multi-timepoint reconstruction

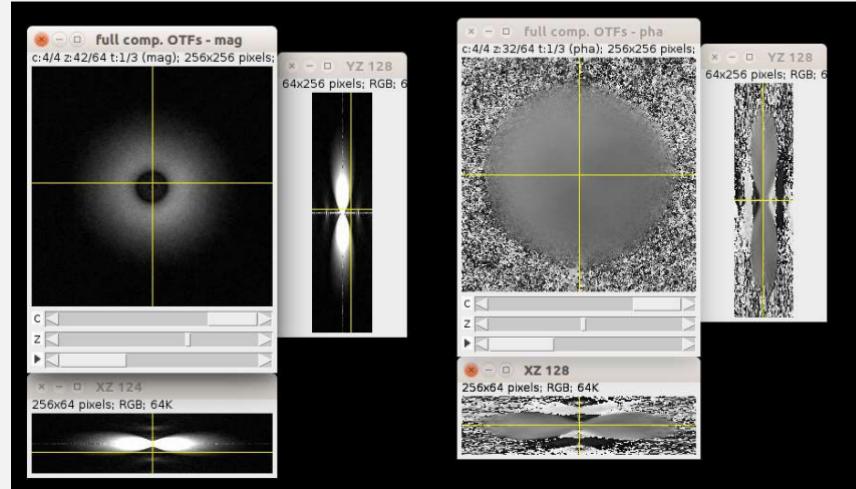
Both parts work, but are very “unpolished” currently.

# fairSIM 3D – OTF generation

- Single bead, multi-plane stack, under SIM illumination (see Lothar's protocol)
- Band-separate
- Compensate bead position (phase)
- Compensate artifacts



OTF phase unwrapping



ToDo:

- Compensate noise / average / smooth
- Have a (at least minimalistic) GUI
- Test and integrate

# Hessian SIM

- Use (spatial and temporal) “smoothness” as a filter
- Reasonable approach, especially for low-SNR, high-speed video
- Currently limited to 2D+time data, no reason the approach wouldn't work on 3D
- MATLAB-based implementation, *quite slow*
- Pure post-processing of reconstructed data: Run fairSIM first, follow up with Hessian filtering
- Implementation in our software: time

ARTICLES

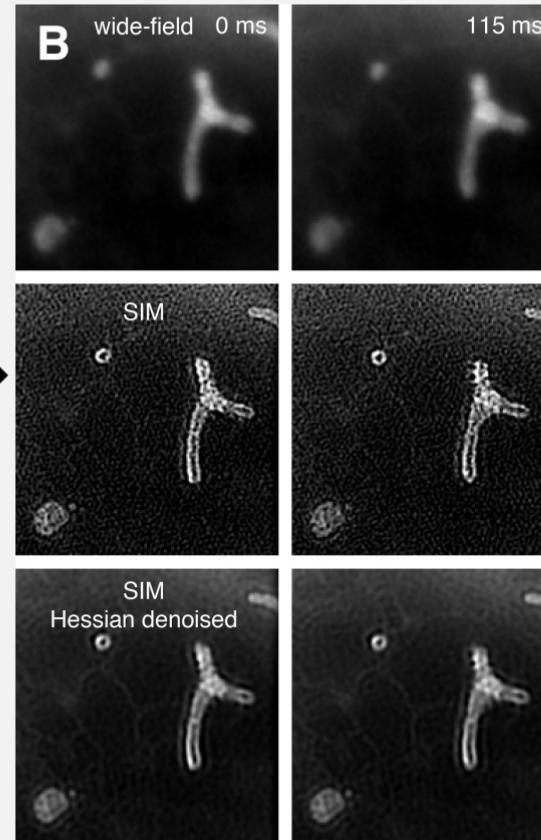
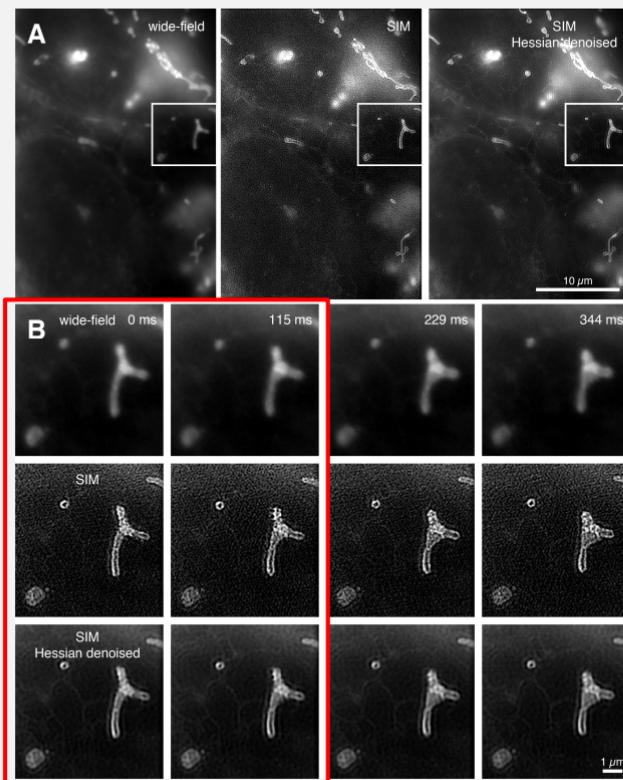
**nature  
biotechnology**

Fast, long-term, super-resolution imaging with Hessian structured illumination microscopy

Xiaoshuai Huang<sup>1,8</sup>, Junchao Fan<sup>2,8</sup>, Liuju Li<sup>1,8</sup>, Haosen Liu<sup>2</sup>, Runlong Wu<sup>3</sup>, Yi Wu<sup>4</sup>, Lisi Wei<sup>1</sup>, Heng Mao<sup>5</sup>, Amit Lal<sup>6</sup>, Peng Xi<sup>6</sup>, Liqiang Tang<sup>7</sup>, Yunfeng Zhang<sup>3</sup>●, Yanmei Liu<sup>1</sup>, Shan Tan<sup>2</sup> & Liangyi Chen<sup>1</sup>●

To increase the temporal resolution and maximal imaging time of super-resolution (SR) microscopy, we have developed a deconvolution algorithm for structured illumination microscopy based on Hessian matrices (Hessian-SIM). It uses the continuity of biological structures in multiple dimensions as a priori knowledge to guide image reconstruction and attains artifact-minimized SR images with less than 10% of the photon dose used by conventional SIM while substantially outperforming current algorithms at low signal intensities. Hessian-SIM enables rapid imaging of moving vesicles or loops in the endoplasmic reticulum without motion artifacts and with a spatiotemporal resolution of 88 nm and 188 Hz. Its high sensitivity allows the use of sub-millisecond excitation pulses followed by dark recovery times to reduce photobleaching of fluorescent proteins, enabling hour-long time-lapse SR imaging of actin filaments in live cells. Finally, we observed the structural dynamics of mitochondrial cristae and structures that, to our knowledge, have not been observed previously, such as enlarged fusion pores during vesicle exocytosis.

# Hessian SIM → fastSIM mitochondria data



Demonstration of the system in use for live-cell imaging of mitochondrial dynamics. Single-color imaging of U2OS cells with 5ms raw frame exposure time (see Fig. 2b), with overview (a) and time series of cut-out (b). The dynamics could be observed in real time and super-resolution by the system operator. The microscope hardware could easily provide higher speeds and continuous data acquisition, however, current dyes are limited in both brightness and photo-stability. Nonetheless, short exposure times are still very helpful in time-lapsed acquisition, as they suppress motion blur.

# Generalized solvers

1. Camera image  $D$  of sample  $S$  under constant illumination  $I$  (widefield), blurred by PSF  $\tilde{h}$

$$D(\vec{r}) = [S(\vec{r}) \cdot I(\vec{r})] * \tilde{h}(\vec{r})$$

2. Microscopy in Fourier space:  
fold with PSF  $\rightarrow$  multiplication with OTF

$$\tilde{D}(\vec{k}) = [\tilde{S}(\vec{k}) * \tilde{I}(\vec{k})] \cdot h(\vec{k})$$

$$\tilde{D}(\vec{k}) = \left[ \tilde{S}(\vec{k}) * \sum_{l=1}^L \delta(\vec{k} - \vec{p}_l) I_0 \right] \cdot h(\vec{k})$$

**There's no need to decompose & directly solve for  $S$ ...**

Instead, use an iterative linear equation solver (CG, etc.) to invert the equation

Still a linear equation, should convert nicely, etc.

Cannot recover information not there in the first place.

Might put arbitrary “stuff” in regions undefined by the original equation system.

→ Regularization! Think about noise.

→ People work on this, but “let’s all use XYZ” has not emerged yet.