Superresolution Optical Fluctuation Imaging (SOFI) using quantum dots

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Dertinger et al., PNAS 2009; Optics Express 2010; Angew Chemie 2010

Superresolution Optical Fluctuation Imaging (SOFI)

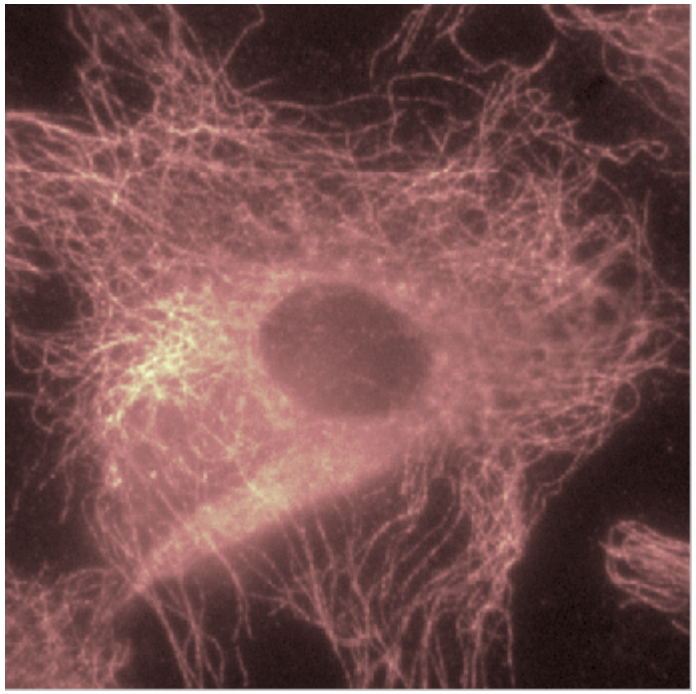
1. The fluorescent label has to exhibit at least two different emission states. For example, these states can be a fluorescent and a non-fluorescent one, but in principle any two or more states which are optically distinguishable will do.

2. Different emitters have to switch between states repeatedly and independently from each other.

Thomas Dertinger, Ryan Colyer, Joerg Enderline

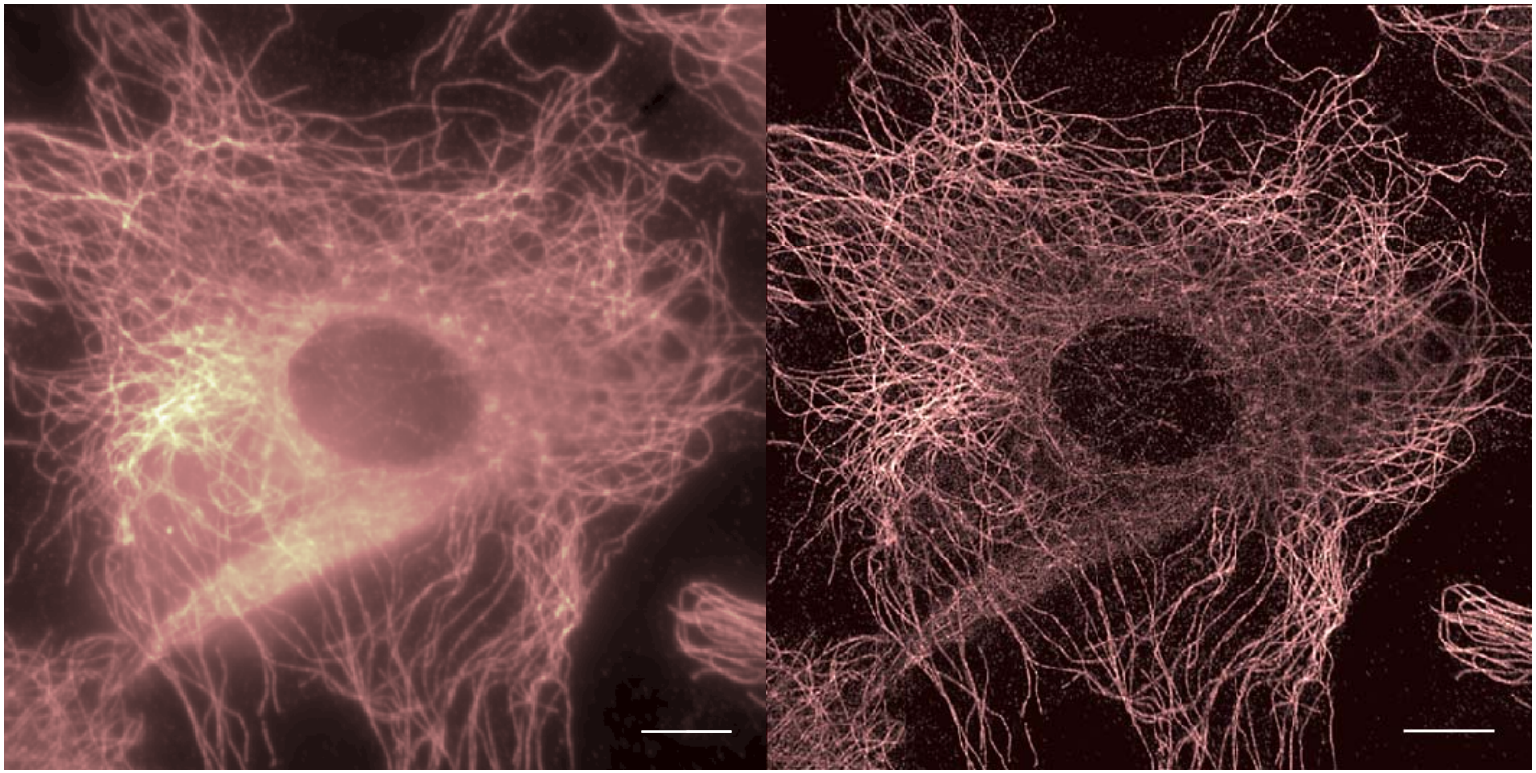
SOFI

Acquire movie on a fluctuating signal (any probe fluctuation)



3T3 fibroblast; quantum dot labeled tubulin network

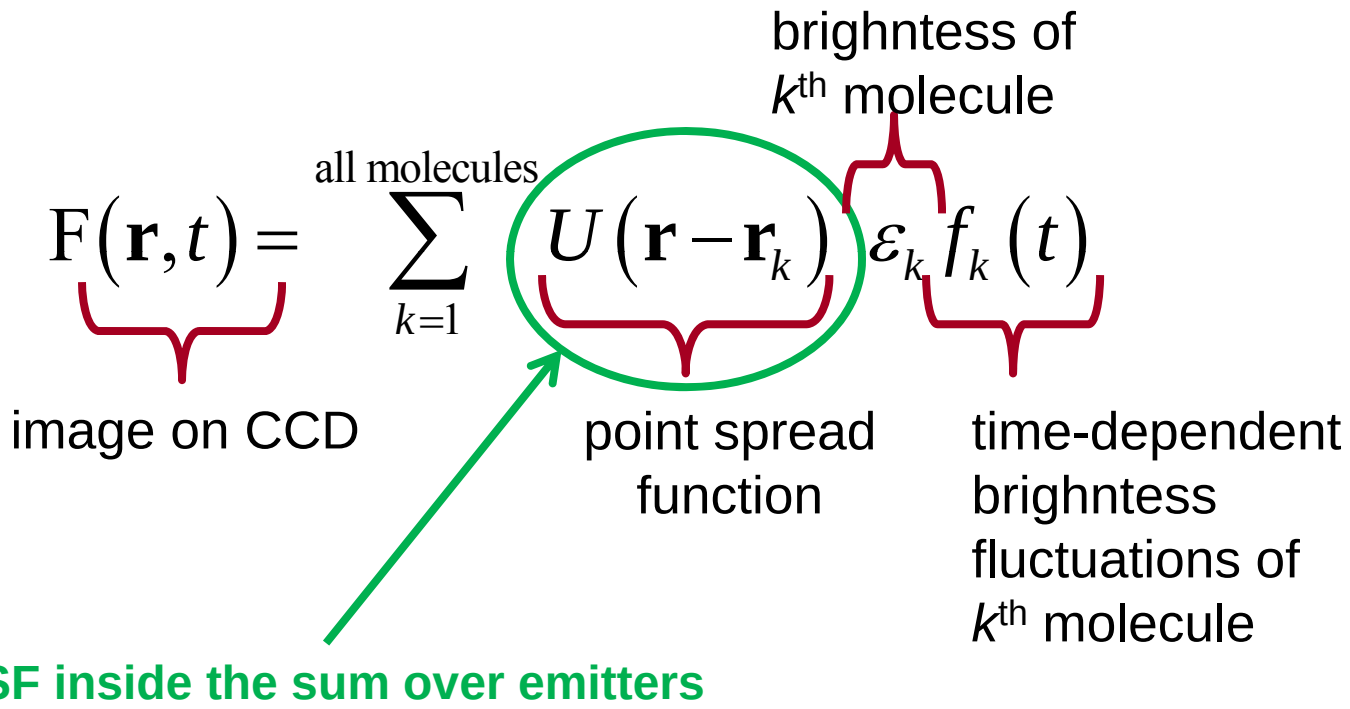
SOFI features Super-resolution in 3D



Resolution enhancement: 2x in 3D

Scalebar: 10 µm

image formation



Correlation Analysis

To do SOFI, we correlate the fluctuations:

$$\delta F(t) = F(t) - \langle F(t) \rangle_t$$

$$G_2(\mathbf{r},\tau) = \langle \delta F(\mathbf{r},t+\tau) \cdot \delta F(\mathbf{r},t) \rangle_t =$$

$$= \sum_{k,l}^{\text{all molecules}} U(\mathbf{r}-\mathbf{r}_k) U(\mathbf{r}-\mathbf{r}_l)\, \varepsilon_k\, \varepsilon_l \langle \delta f_k(t+\tau)\, \delta f_l(t) \rangle_t$$

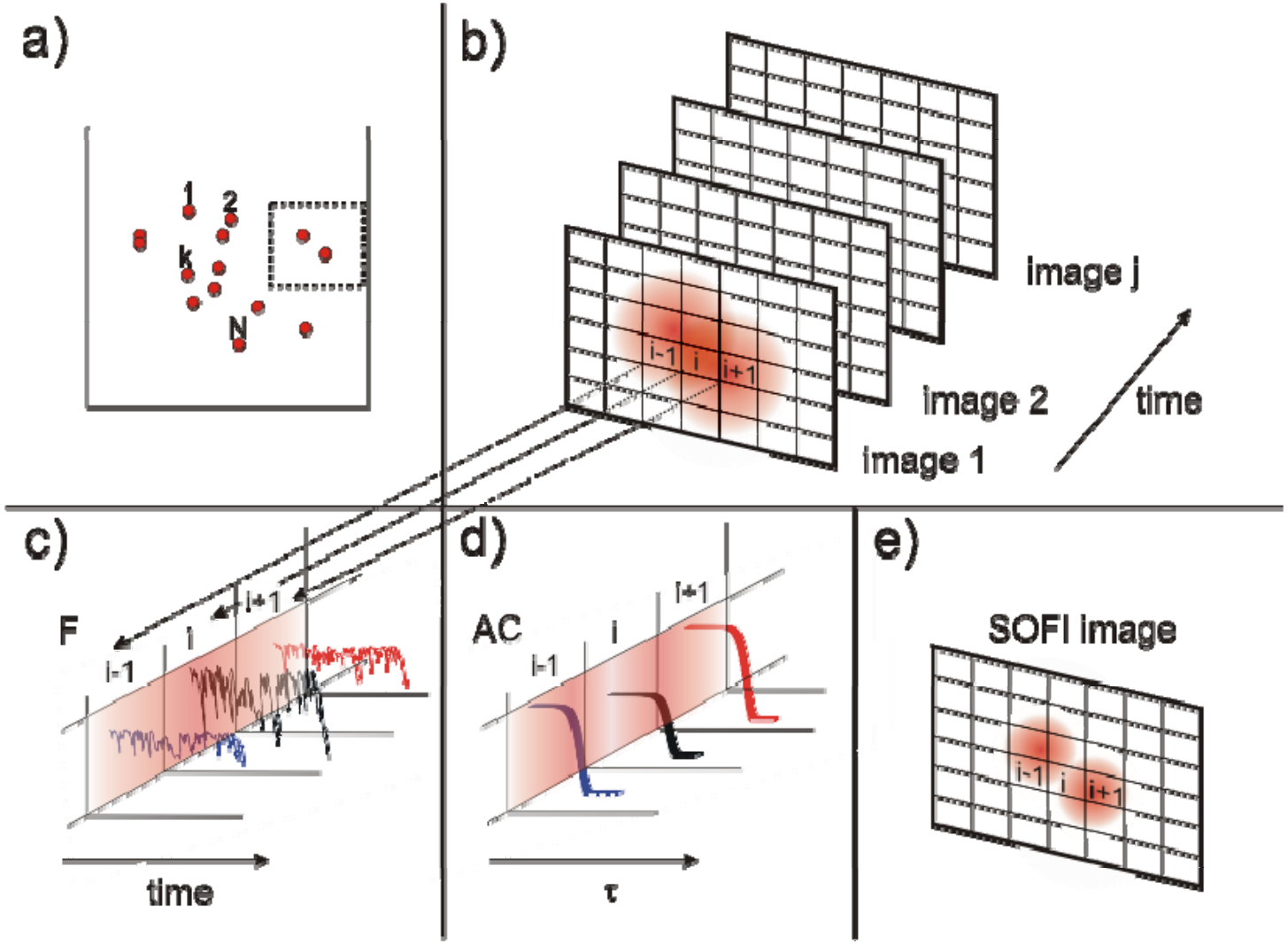
emitters fluctuate independently:
i.e. emitter k correlates only with itself

$$G_2(\mathbf{r},\tau) = \sum_{k=1}^{\text{all molecules}} U^2(\mathbf{r}-\mathbf{r}_k)\, \varepsilon_k^2 \langle \delta f_k(t+\tau)\, \delta f_k(t) \rangle_t$$

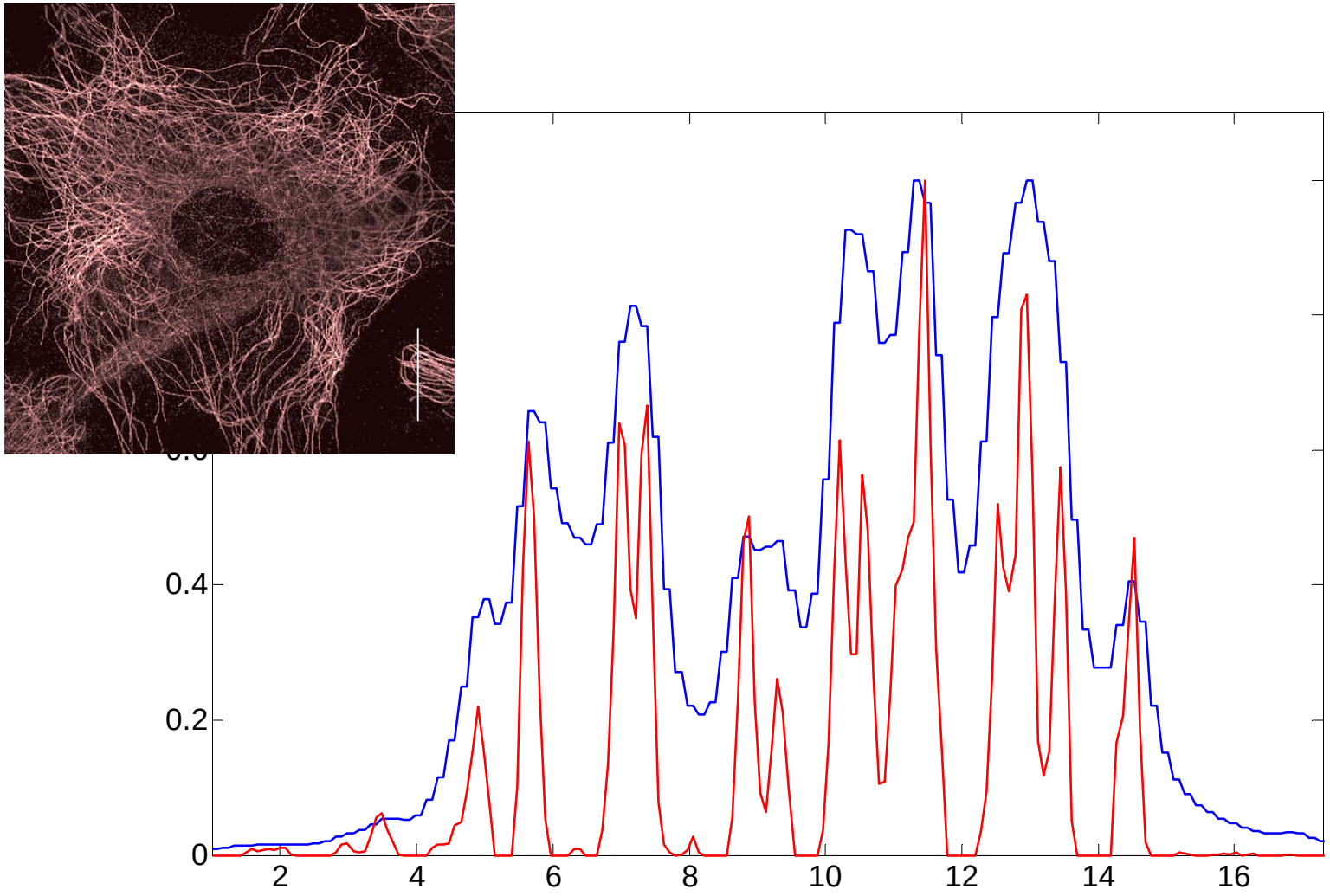
square of PSF inside the sum over emitters

Dertinger et al. , PNAS 2009

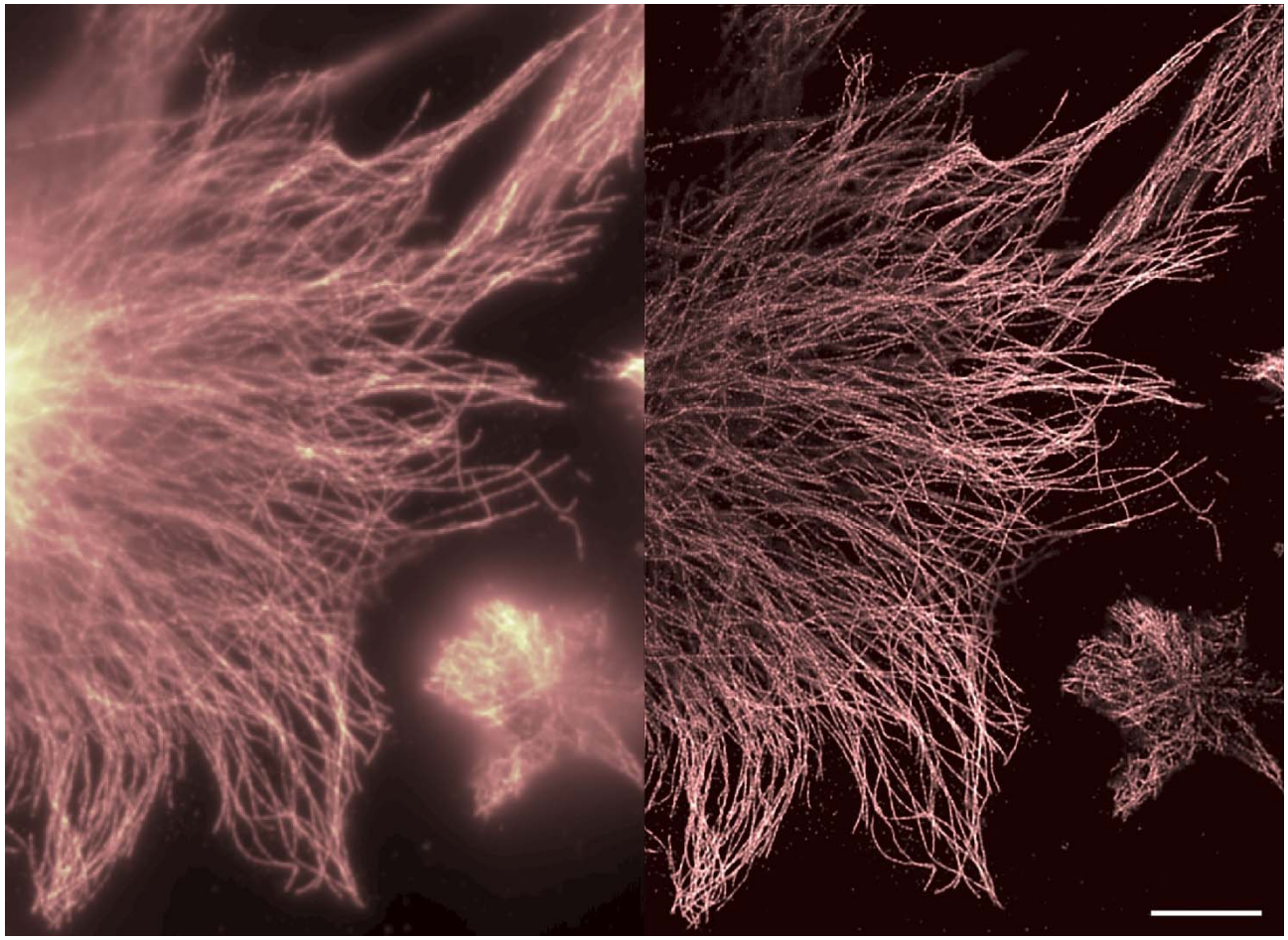
SOFI scheme


a)
1
2
k
N
b)
image j
image 2
image 1
time
i-1
i
i+1
c)
F
i-1
i
i+1
time
d)
AC
i-1
i
i+1
τ
e)
SOFI image
i-1
i
i+1

SOFI features Super-resolution in 3D

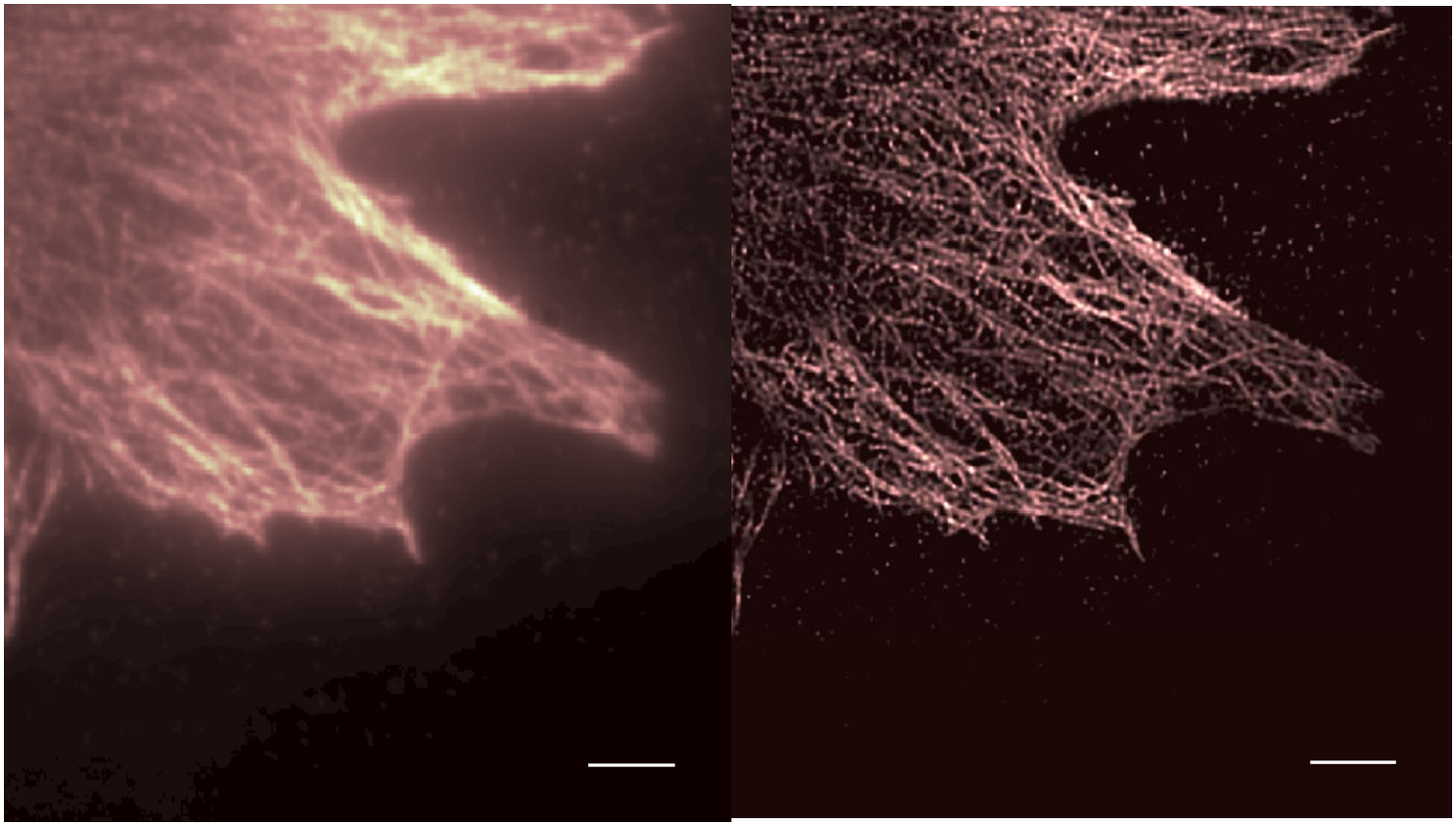


SOFI features background-reduction and contrast-enhancement



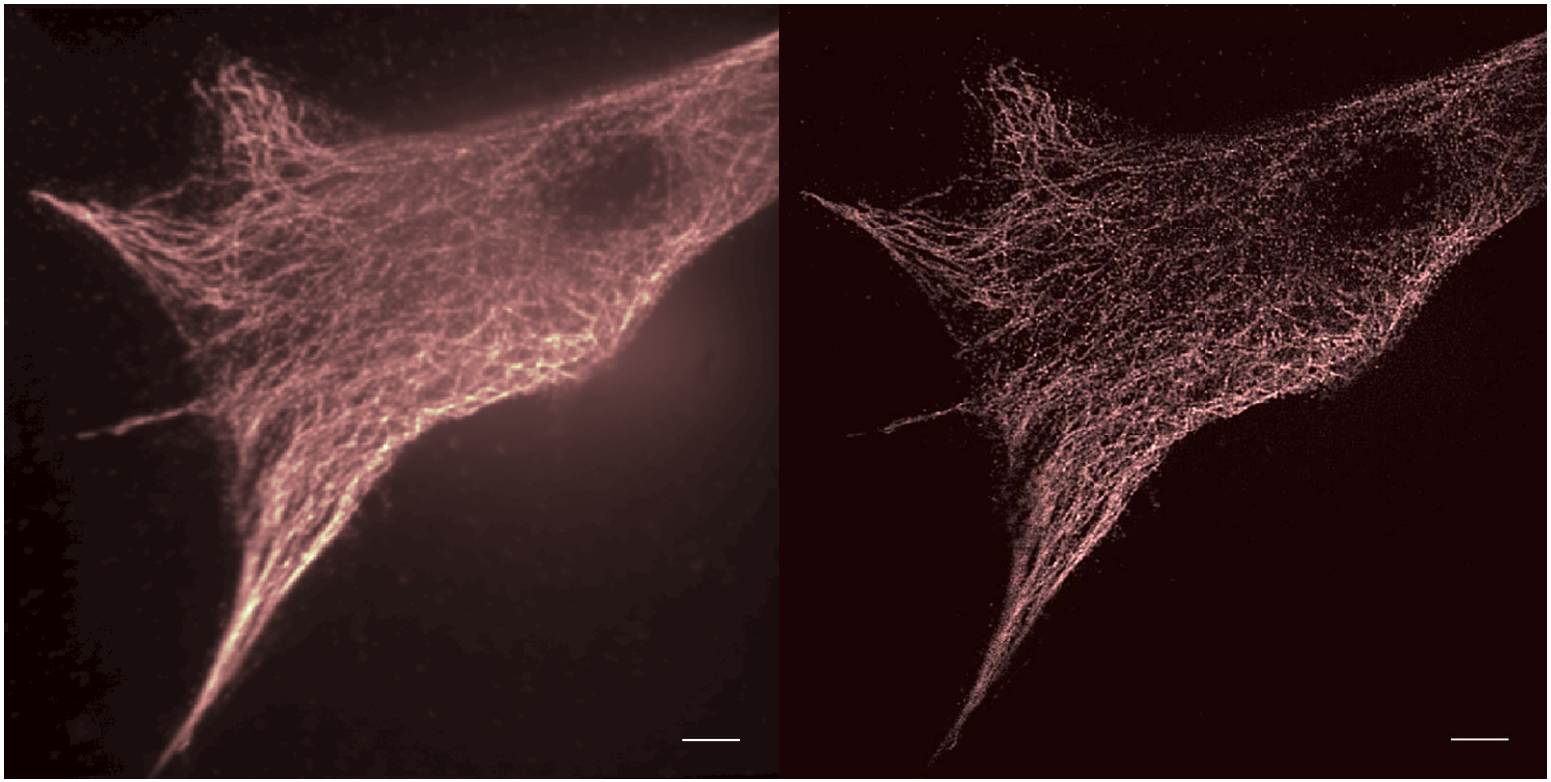
Scalebar: 10 µm

SOFI on TIRF



Scalebar: 5 µm

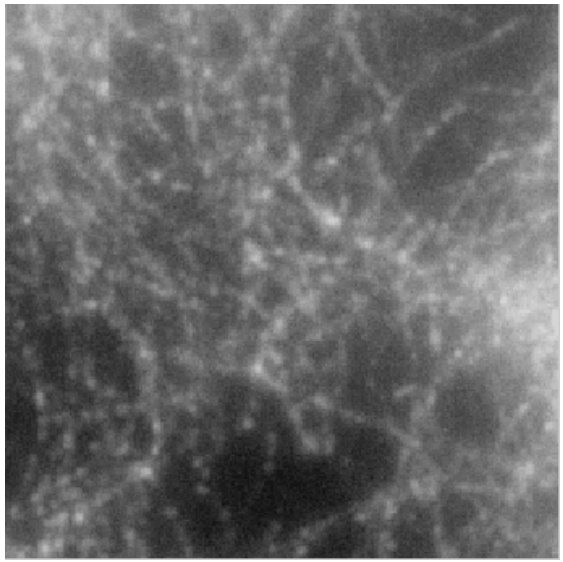
SOFI on Spinning Disk Confocal



Scalebar: 5 µm

SOFI with dyes

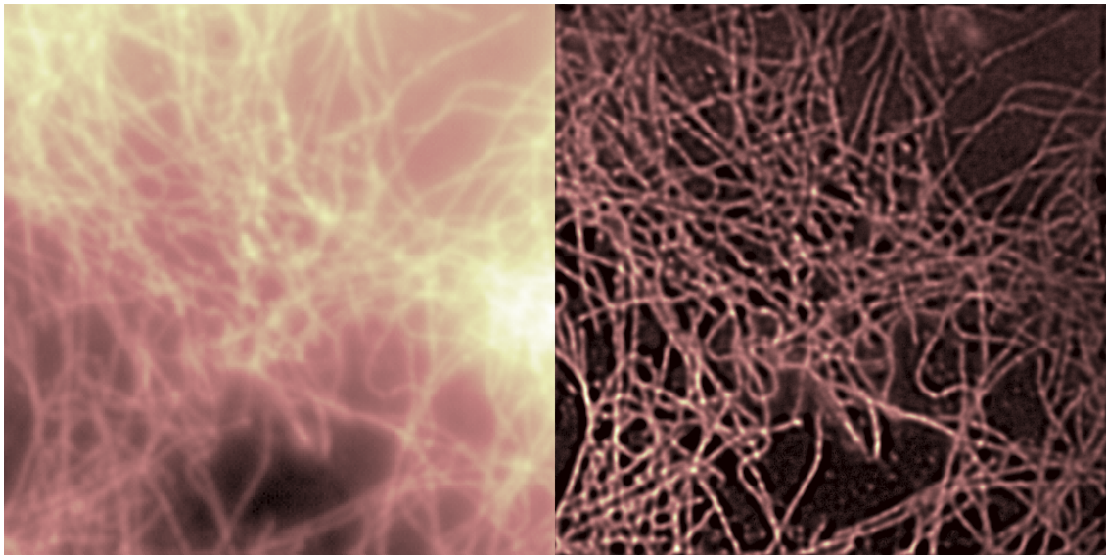
3T3 Fibroblasts, Alexa647 labeled tubulin network



In collaboration with Mike Heilemann, University of Bielfeld, Germany

SOFI with dyes

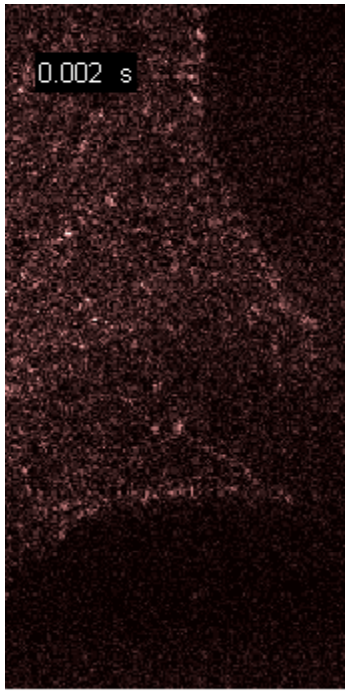
3T3 Fibroblasts, Alexa647 labeled tubulin network



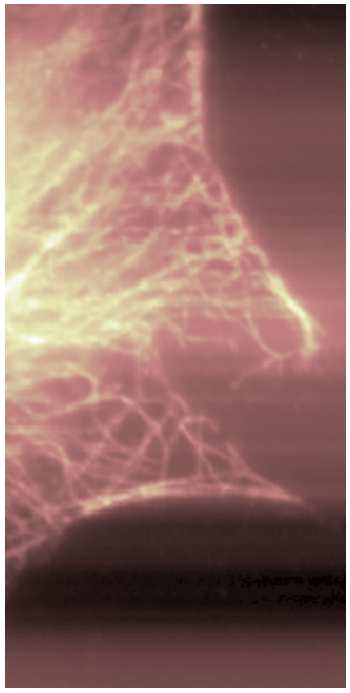
In collaboration with Mike Heilemann, University of Bielfeld, Germany

Fast SOFI

SOFI image, movie taken at 1000 frames/s

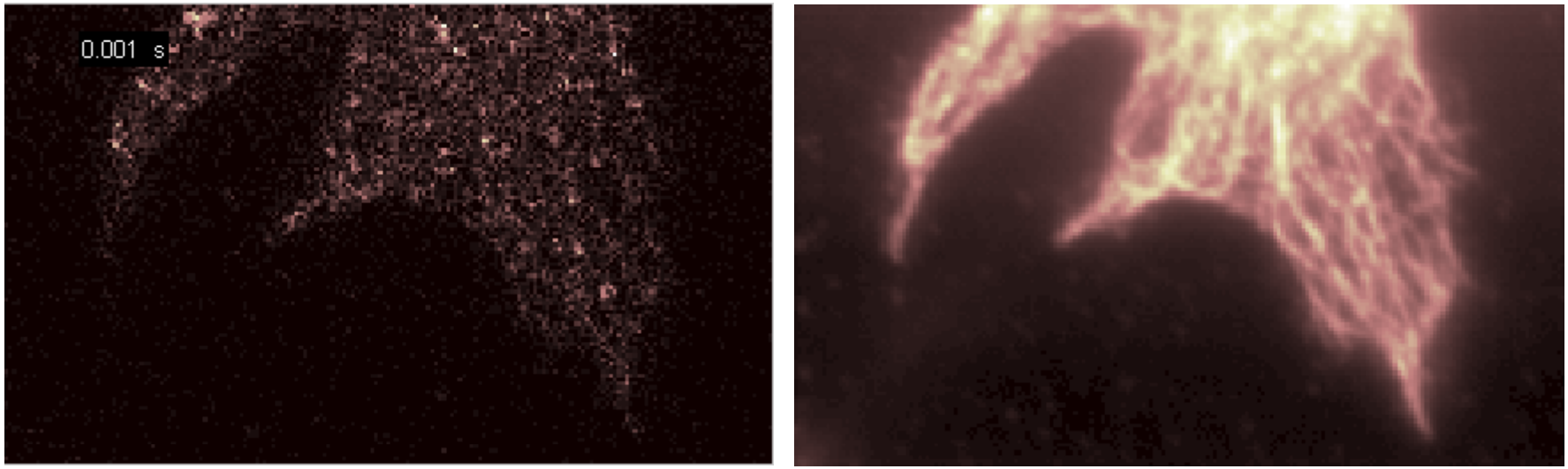


Original Image



Fast SOFI

SOFI image, movie taken at 2000 frames/s



Higher-order SOFI

Higher-order cumulants yield higher resolution

n-th order correlation function:

$$\mathrm{G}_n\left(\mathbf{r},\tau\right)=\left\langle \delta\mathrm{F}\left(\mathbf{r},t\right)\cdot\delta\mathrm{F}\left(\mathbf{r},t+\tau_1\right)\cdot\ldots\cdot\delta\mathrm{F}\left(\mathbf{r},t+\tau_{n-1}\right)\right\rangle_t$$

n-th order cumulant:

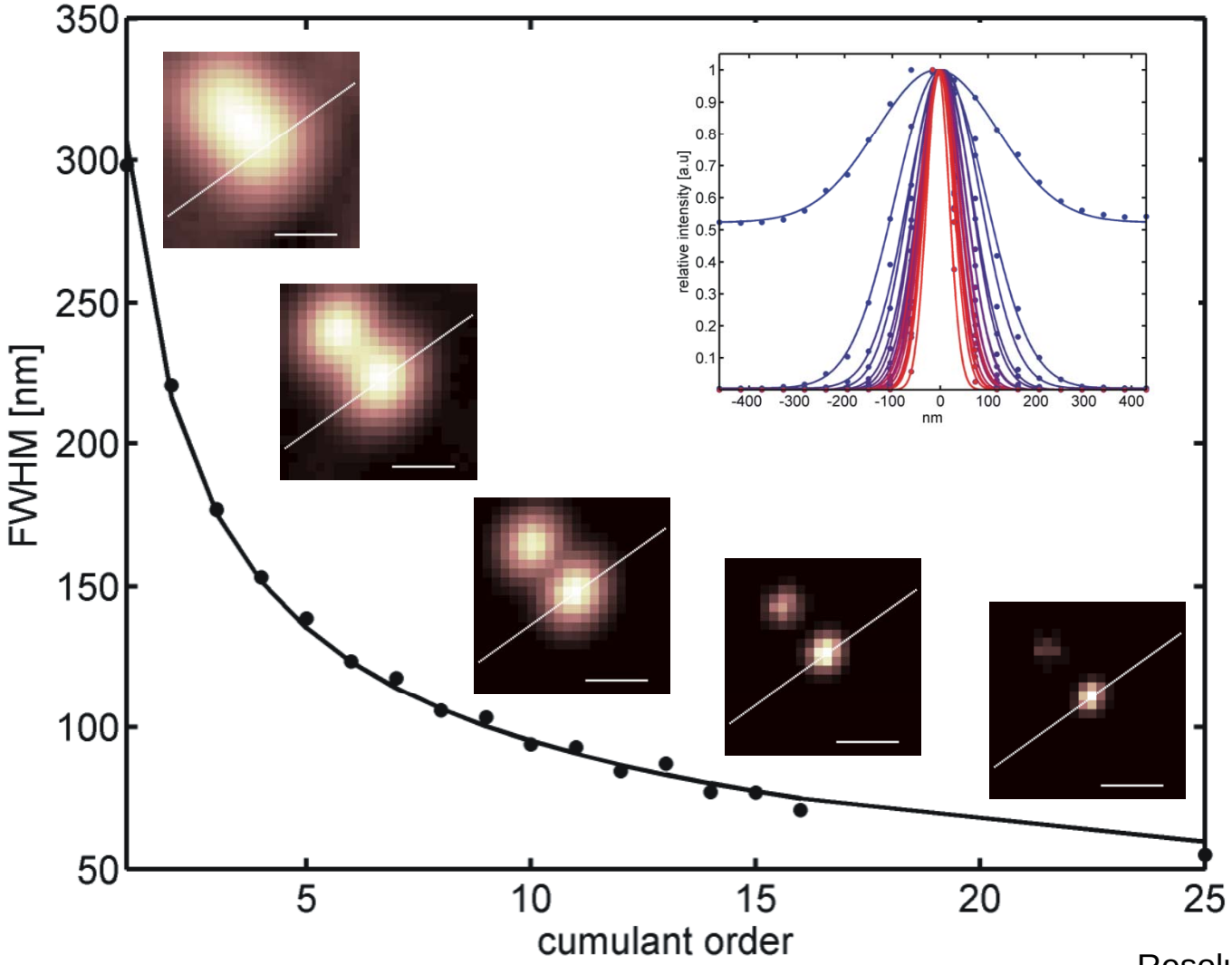
$$\mathrm{C}_n\left(\mathbf{r},\tau_1,\ldots\tau_{n-1}\right)=\mathit{function}\left(G_i\right)\propto\varepsilon^n U^n \qquad i\le n$$

$$\mathrm{C}_2\left(\mathbf{r},\tau_1\right)=\mathrm{G}_2\left(\mathbf{r},\tau_1\right)$$

$$\mathrm{C}_3\left(\mathbf{r},\tau_1,\tau_2\right)=\mathrm{G}_3\left(\mathbf{r},\tau_1,\tau_2\right)$$

$$\mathrm{C}_4\left(\mathbf{r},\tau_1,\tau_2,\tau_3\right)=\mathrm{G}_4\left(\mathbf{r},\tau_1,\tau_2,\tau_3\right)-\mathrm{G}_2\left(\mathbf{r},\tau_1\right)\cdot\mathrm{G}_2\left(\mathbf{r},\tau_3\right)-\mathrm{G}_2\left(\mathbf{r},\tau_1+\tau_2\right)\cdot\mathrm{G}_2\left(\mathbf{r},\tau_2+\tau_3\right)-\mathrm{G}_2\left(\mathbf{r},\tau_1+\tau_2+\tau_3\right)\cdot\mathrm{G}_2\left(\mathbf{r},\tau_2\right)$$

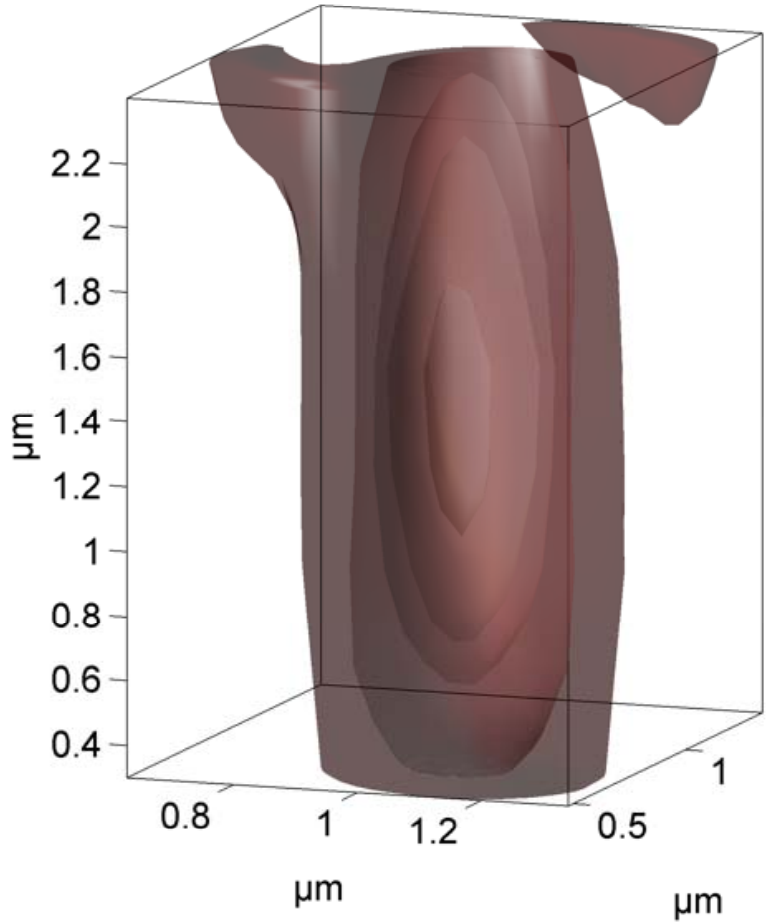
HIGHER-ORDER SOFI



Scalebar: 250 nm

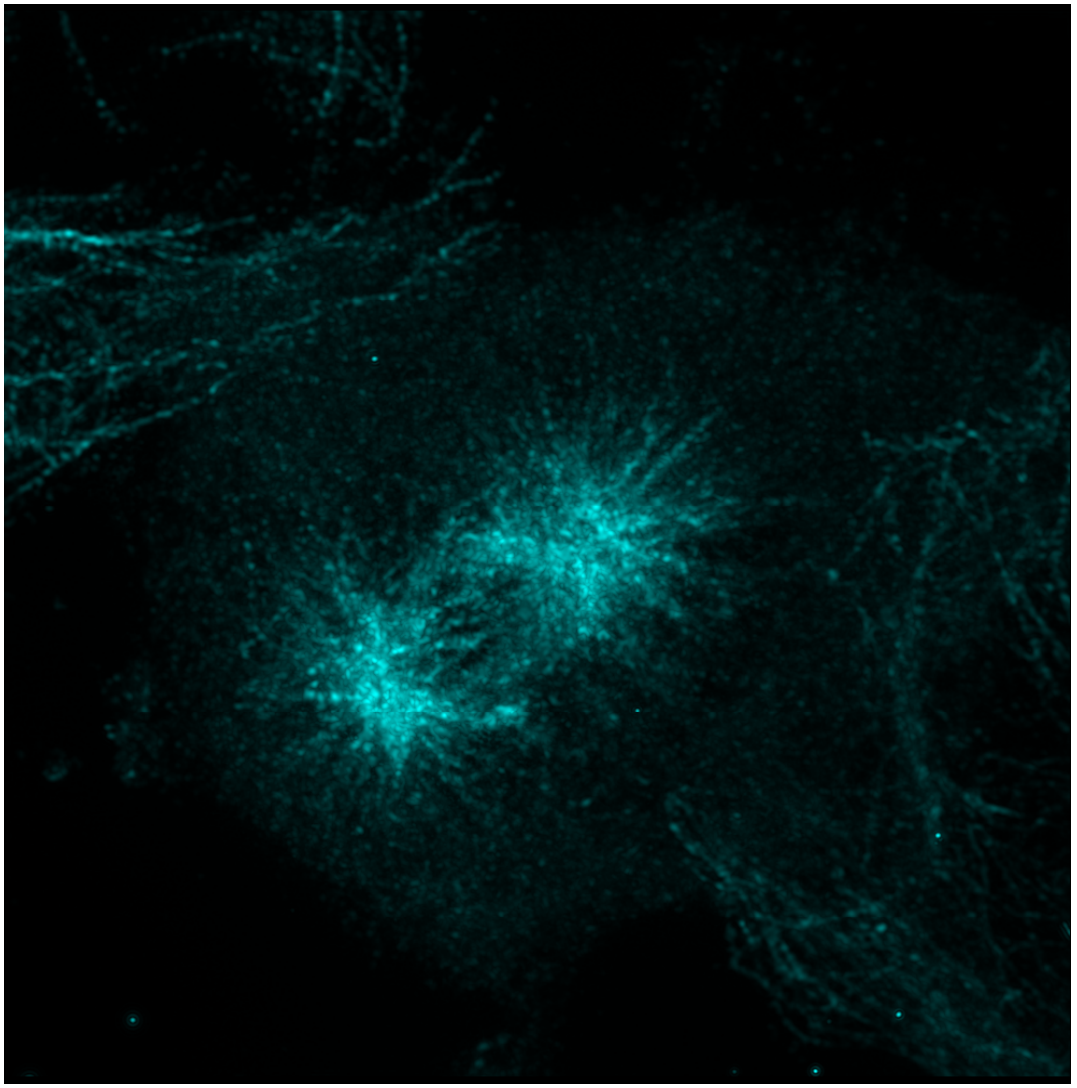
Resolution enhancement
5x = 60 nm

3D-SOFI



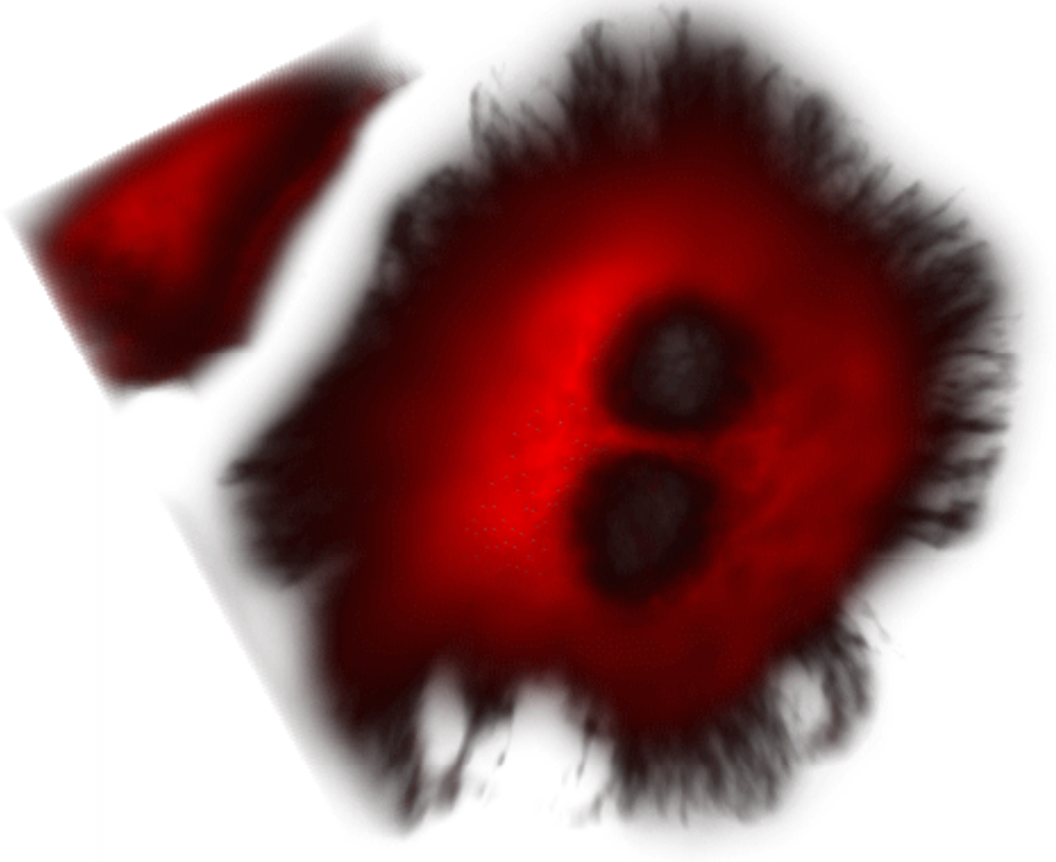
3D-SOFI

3D projection of ‘wide-field’ SOFI sections of QDs labeled μ-tubules in HeLa cell



3D-SOFI

Fitst: 3D-rendering – of original data set

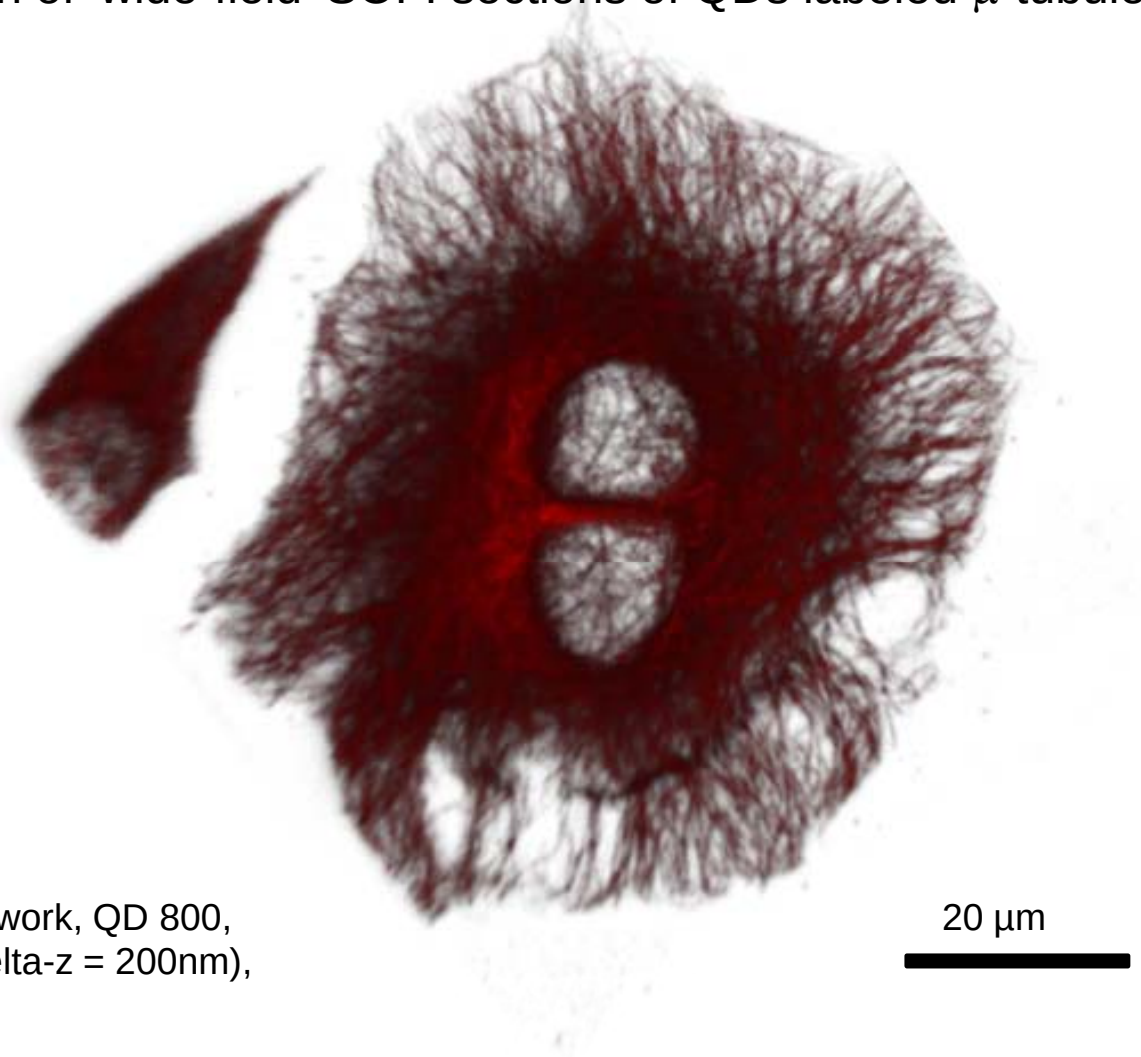


HeLa cell, tubulin network, QD 800,
32 stacks, 6.4 µm (delta-z = 200nm),
2000 frames / stack,
170 nm/pixel
(Displaying at stretched z-aspect-ratio)

20 µm

3D-SOFI

3D projection of ‘wide-field’ SOFI sections of QDs labeled μ-tubules in HeLa cell

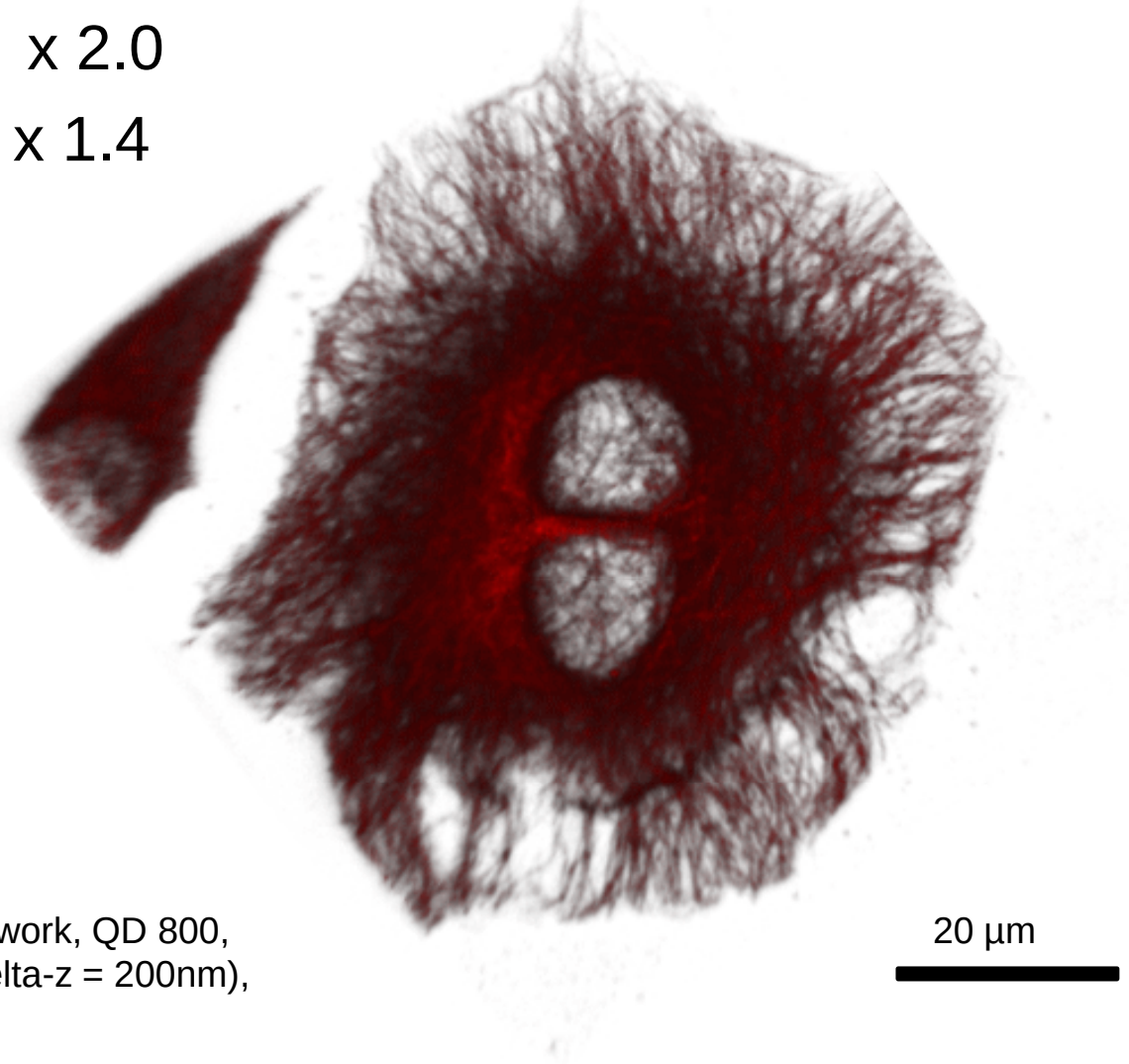


HeLa cell, tubulin network, QD 800,
32 stacks, 6.4 µm (delta-z = 200nm),
2000 frames / stack,
170 nm/pixel
(Displaying at stretched z-aspect-ratio)

3D-SOFI

Resolution enhancement:

- along x,y: x 2.0
- along z: x 1.4



HeLa cell, tubulin network, QD 800,
32 stacks, 6.4 µm (delta-z = 200nm),
2000 frames / stack,
170 nm/pixel
(Displaying at stretched z-aspect-ratio)

Cross-Correlation Math

The equations for SOFI cross-correlations are very similar to the autocorrelations, with a few important differences:

$$\mathrm{XC}_2\left(\mathbf{r}_1,\mathbf{r}_2,\tau\right)=\left\langle \delta F(r_1,t+\tau)\cdot\delta F(r_2,t)\right\rangle_t$$

$$=\sum_{k,l}U\left(\mathbf{r}_k-\mathbf{r}_1\right)U\left(\mathbf{r}_l-\mathbf{r}_2\right)\varepsilon_k\varepsilon_l\left\langle \delta f_k\left(t+\tau\right)\cdot\delta f_l\left(t\right)\right\rangle_t$$

We still have a PSF^2 term inside the sum

$$\mathrm{XC}_2\left(\mathbf{r}_1,\mathbf{r}_2,\tau\right)=U^2\left(\frac{\mathbf{r}_1-\mathbf{r}_2}{\sqrt{2}}\right)\cdot\sum_k U^2\left(\mathbf{r}_k-\frac{\mathbf{r}_1+\mathbf{r}_2}{2}\right)\cdot\varepsilon_k^2\cdot\left\langle \delta f_k\left(t+\tau\right)\cdot\delta f_k\left(t\right)\right\rangle_t$$

But now we have a weighting term out in front. This depends on the PSF and the distance between the two pixels.

We also have a new "**position**" term for th center of the resulting squared PSF!

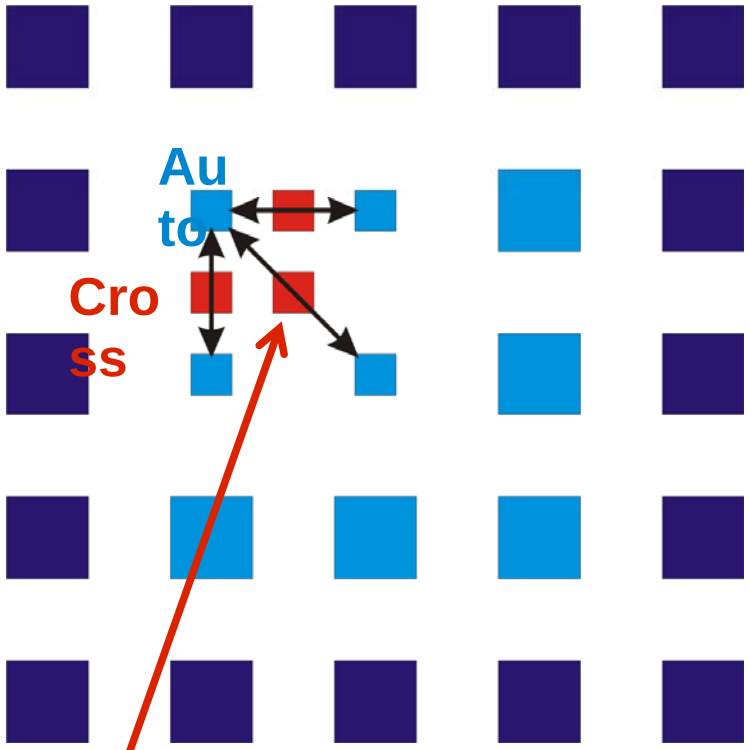
It's the average of the two pixels!

"Super-pixels"

- Each cross-correlation between neighboring pixels produces a new pixel with an effective "intermediate" location between them.

- When the autocorrelation and cross-correlations are combined, this results in 4 times the SOFI image pixels as the original image.

- This means we get new pixels for "free". It gives us pixels with the resolution information from between two camera pixels.



$$\mathrm{XC}_2\left(\mathbf{r}_1,\mathbf{r}_2,\tau\right)=U^2\left(\frac{\mathbf{r}_1-\mathbf{r}_2}{\sqrt{2}}\right)\cdot\sum_k U^2\left(\mathbf{r}_k-\frac{\mathbf{r}_1+\mathbf{r}_2}{2}\right)\cdot\varepsilon_k^2\cdot\left\langle\delta f_k\left(t+\tau\right)\cdot\delta f_k\left(t\right)\right\rangle_t$$

Dertinger, et al., Optics Express 18(18), 18875-85 (2010).

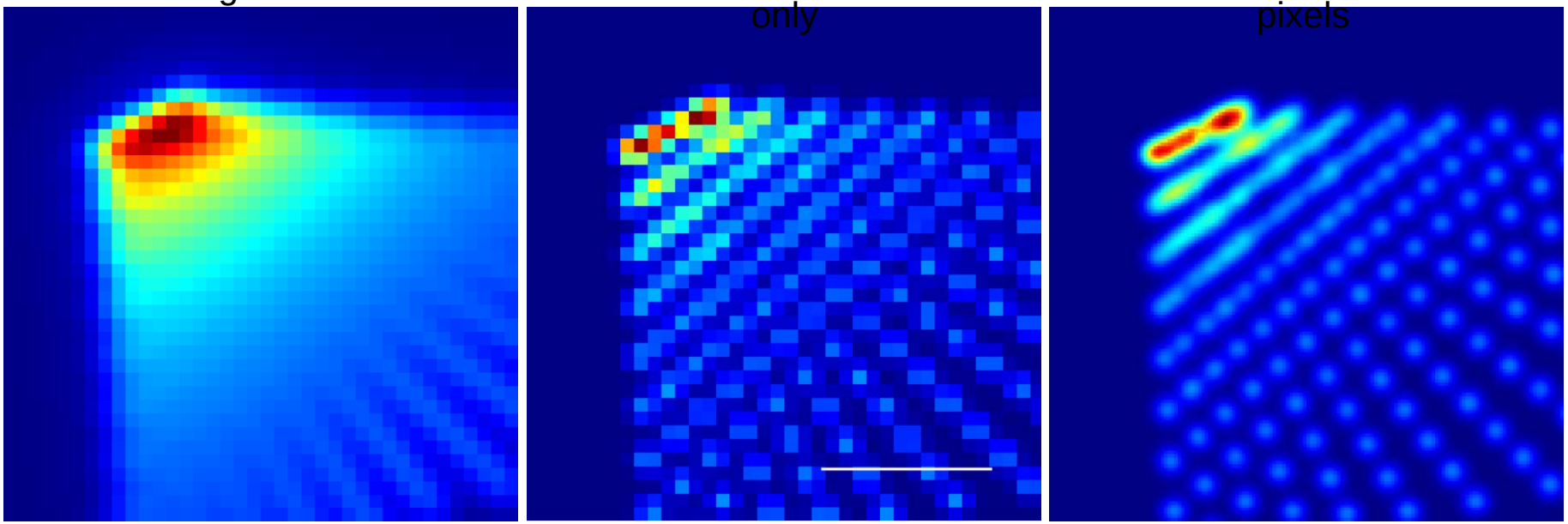
“Super-pixels” Show True Resolution

- Here is a simulation which shows that the cross-correlated pixels truly do show “intermediate” pixel information which is not present in the original pixels.

Original image

SOFI, 4th order autocorrelation only

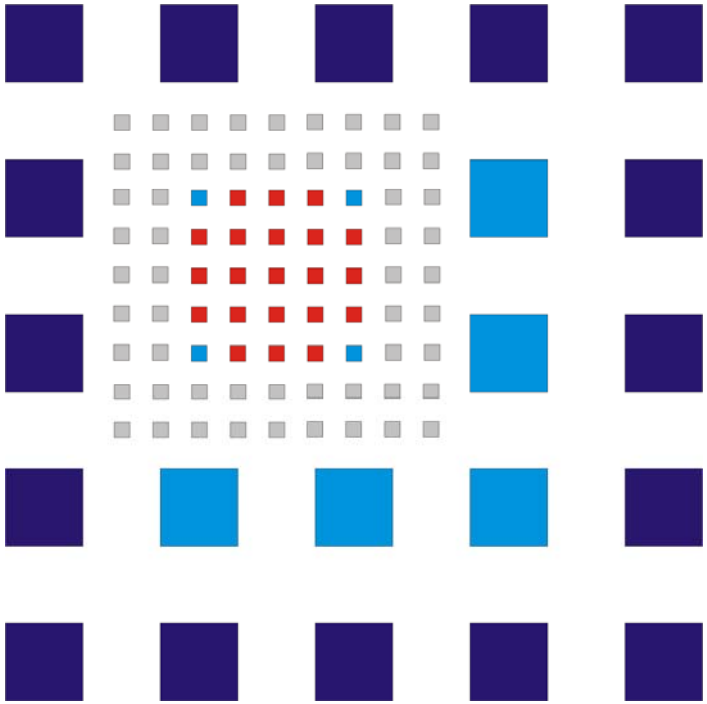
SOFI, 4th order, with super-pixels



Dertinger, et al., Optics Express 18(18), 18875-85 (2010).

“Super-pixels”, higher orders

- These super-pixels scale along as we go to higher cumulant orders.
- The fourth order yields 4x4 times as many pixels as the original image.



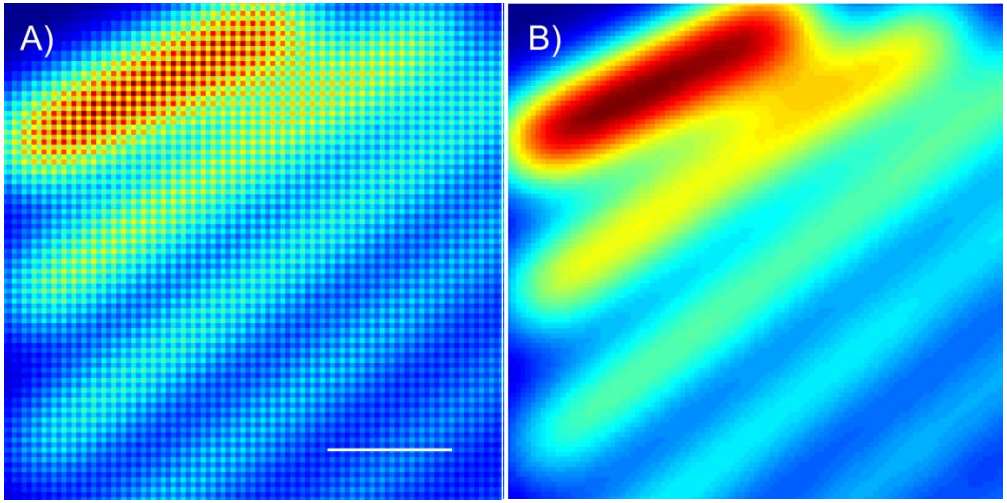
- This means that we never need to lose field of view as we gain resolution with SOFI!
- (Rule of thumb: Always choose a pixel size around half the PSF.)

Weighting the Cross-Correlation

- Remember that “weighting term” in the cross-correlation?

$$\mathrm{XC}_2\left(\mathbf{r}_1,\mathbf{r}_2,\tau\right)=U^2\left(\frac{\mathbf{r}_1-\mathbf{r}_2}{\sqrt{2}}\right)\cdot\sum_k U^2\left(\mathbf{r}_k-\frac{\mathbf{r}_1+\mathbf{r}_2}{2}\right)\cdot\varepsilon_k^2\cdot\left\langle\delta f_k\left(t+\tau\right)\cdot\delta f_k\left(t\right)\right\rangle_t$$

- This makes all the values of the cross-correlation pixels “less” than their autocorrelation neighbors!



← Minimize the variance of all the pixels.

- So we can do a fit of the weighting coefficients to turn image (A) into the corrected image (B).

Dertinger, et al., Optics Express 18(18), 18875-85 (2010).

Extracting the PSF Parameters

- As soon as we've done a fit of the weighting coefficients for a given sample, then we have found an experimental value of the this term.

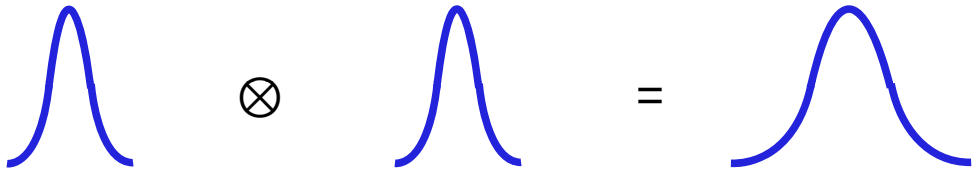
$$\mathrm{XC}_2\left(\mathbf{r}_1,\mathbf{r}_2,\tau\right) = U^2\left(\frac{\mathbf{r}_1-\mathbf{r}_2}{\sqrt{2}}\right)\cdot\sum_k U^2\left(\mathbf{r}_k-\frac{\mathbf{r}_1+\mathbf{r}_2}{2}\right)\cdot\varepsilon_k^2\cdot\left\langle\delta f_k\left(t+\tau\right)\cdot\delta f_k\left(t\right)\right\rangle_t$$

- And, we know r_1-r_2, because that's simply the distance between two pixels. Call it “1” for neighbors.
- Since *U* is the PSF function, that means that by our fit, we have experimentally measured the width of the PSF in units of pixels!
- We get the PSF width for free, with no extra measurements, as a byproduct of computing the cross-correlation components.
- So let's see what we can do with that PSF information...

Dertinger, et al., Optics Express 18(18), 18875-85 (2010).

The Optical Transfer Function under SOFI

- The Fourier transform of a Gaussian PSF is a Gaussian in frequency space.
- Squaring the PSF convolves the frequency space Gaussians:

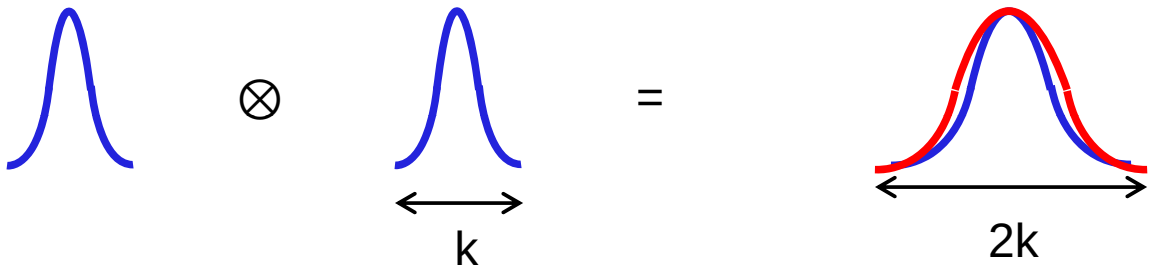


- The new Gaussian is only $\sqrt{2}$ wider by the FWHM.
- However, by the math of convolution, the “support”, or information content, of this optical transfer function (OTF) goes **twice** as far out as the original.

Dertinger, et al., Optics Express 18(18), 18875-85 (2010).

Improving Resolution with the “OTF”

- Since the support of the OTF goes twice as far out, and since we know the precise PSF dimensions from the cross-correlation weighting...



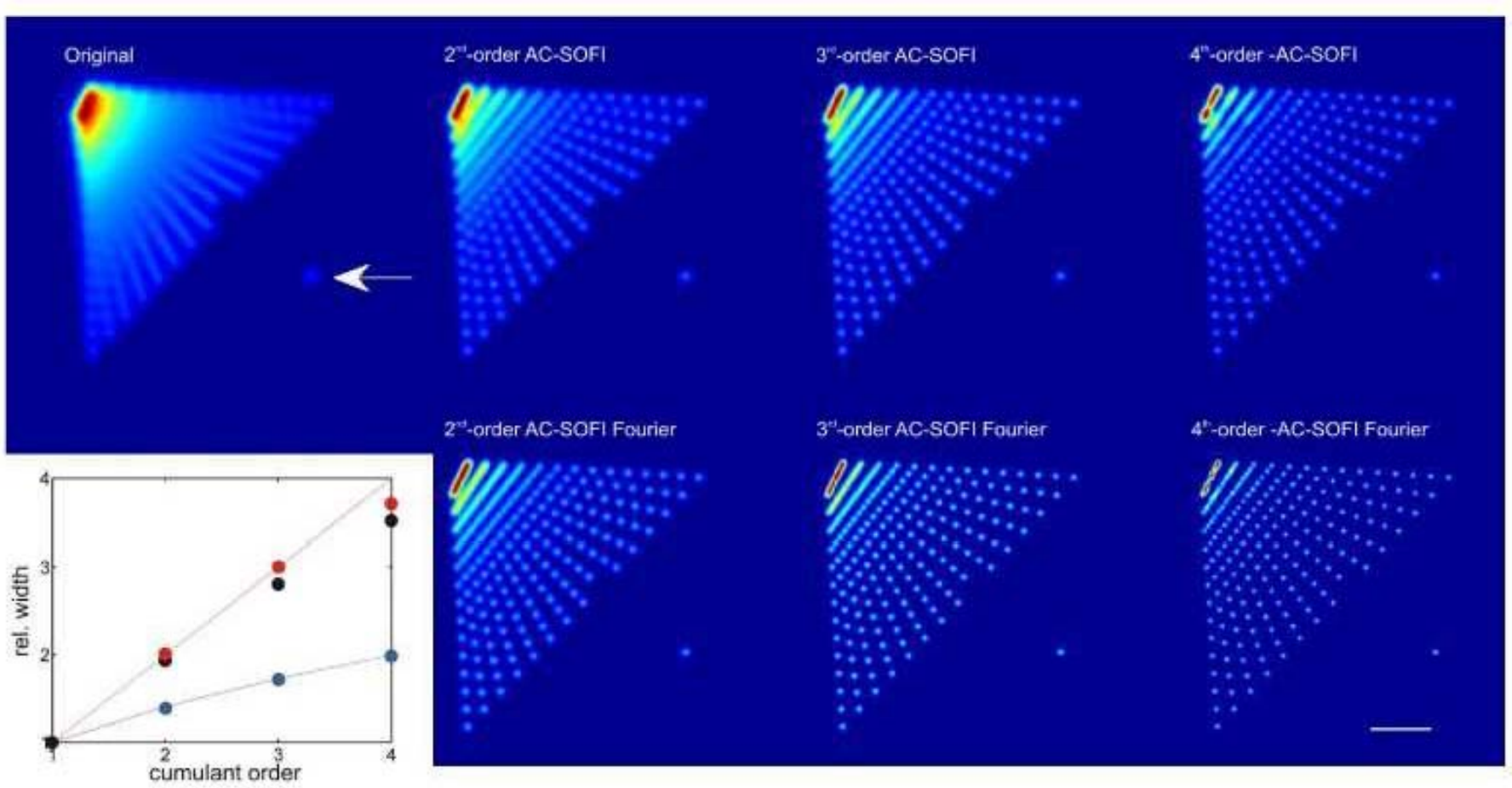
- If we simply reshape the frequency space Gaussian by multiplying it back to the full amplitude, we will recover the full information which is actually there, giving us twice the spatial frequency coverage.

- When this goes back into real space, it means 2X resolution from the second order rather than the square root of two!

Dertinger, et al., Optics Express 18(18), 18875-85 (2010).

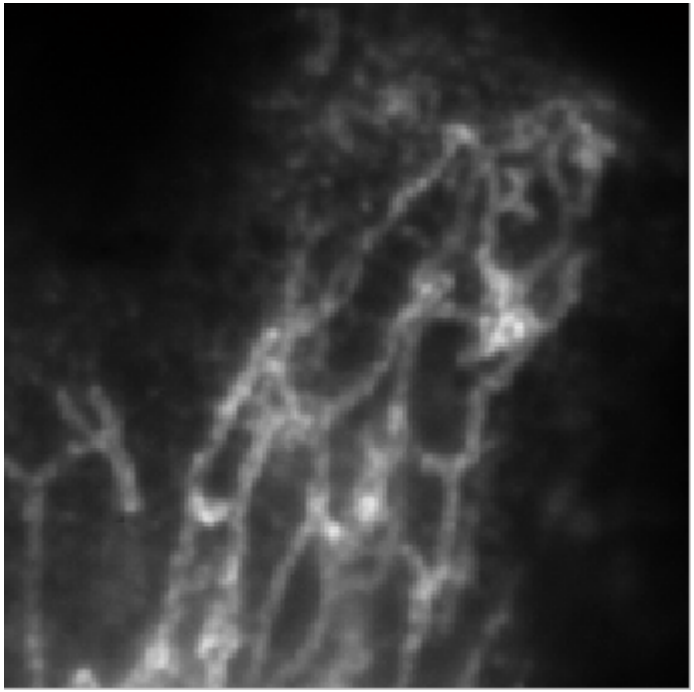
Superresolution Which Scales Linearly

- When the full OTF correction is performed, as in this simulation, the resolution scales linearly with cumulant order! 4th order = 4X.

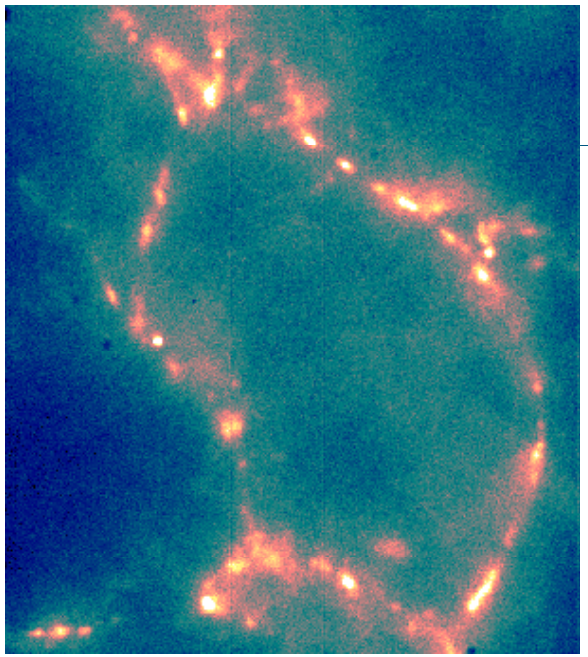


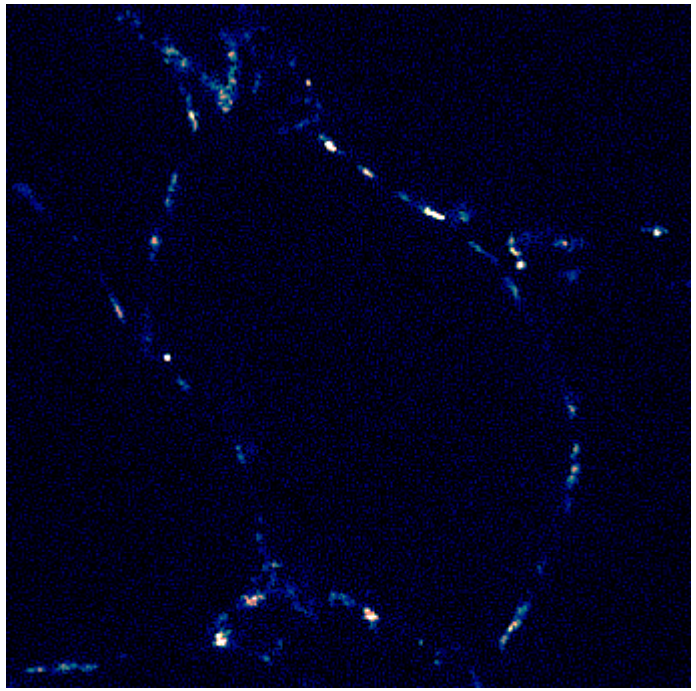
Dertinger, et al., Optics Express 18(18), 18875-85 (2010).

SOFI on tubulin network in live cells



SOFI on EGFR





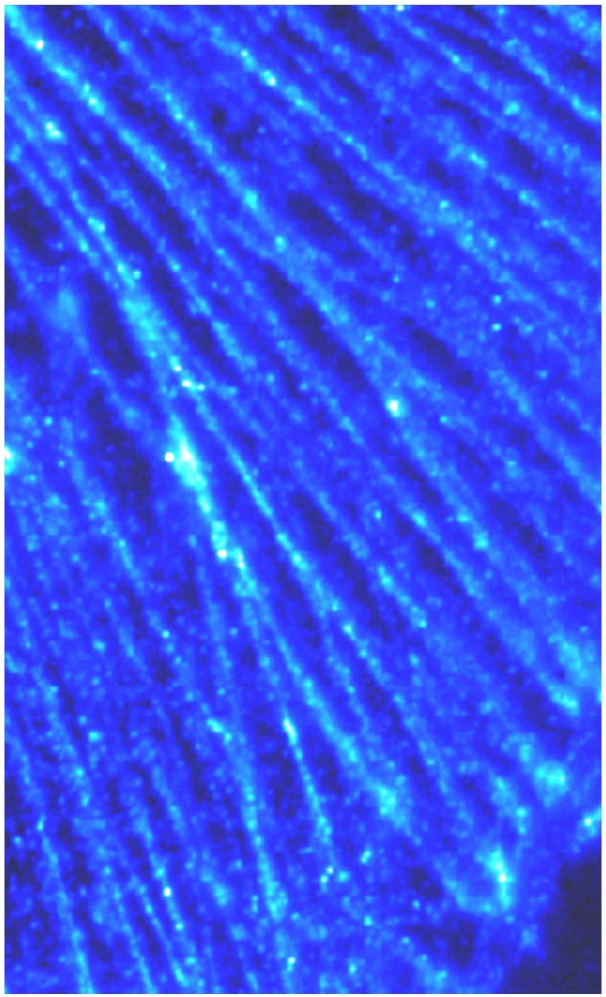
MCF7_EGFR_800__3, 500 frames blocklength 50, 70nm/pixel

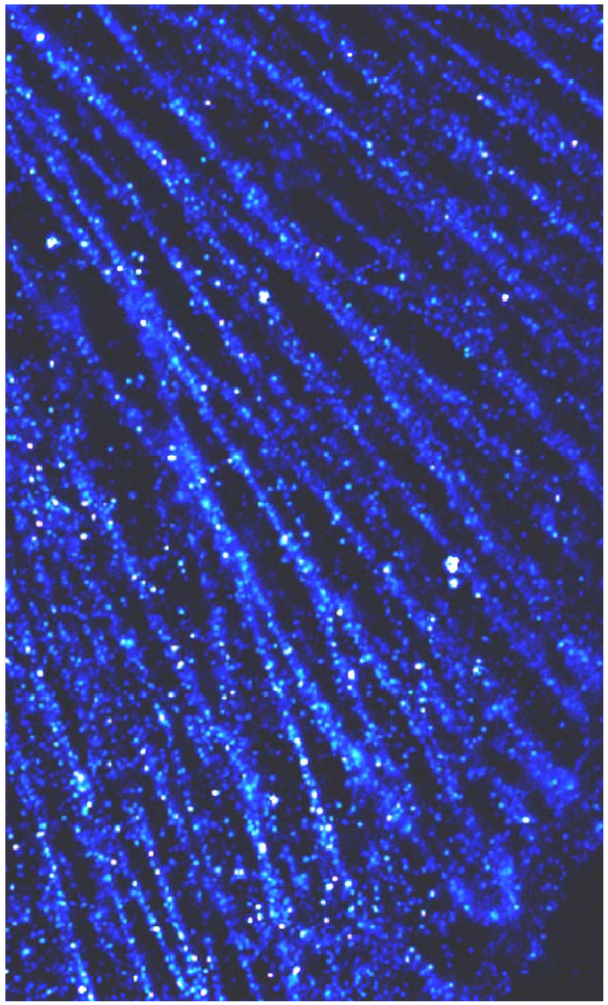
SOFI on FAPs

FAP:
Fluorogen Activating Protein

Single-chain antibodies evolved to bind malachite green dye.

Acquisition time: 200s
TIRF microscope.
150x magnification





In collaboration with Marcel Bruchez at Carnegie Mellon University

SOFI summary

- Implementation of SOFI is simple
- It is inherently 3D
- It is background-free
- It can be fast
- It works on all blinking probes (i.e. almost all probes)
- It works on all types of microscopes
- Resolution is not limited to pixel size

Acknowledgements

Thomas Dertinger

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Dertinger et al., PNAS 2009; Optics Express 2010; Angew Chemie 2010

A Novel Detector

Xavier Michalet

Ryan Colyer

Oswald Siegmund

Anton Tremsin

Paul Vallerga

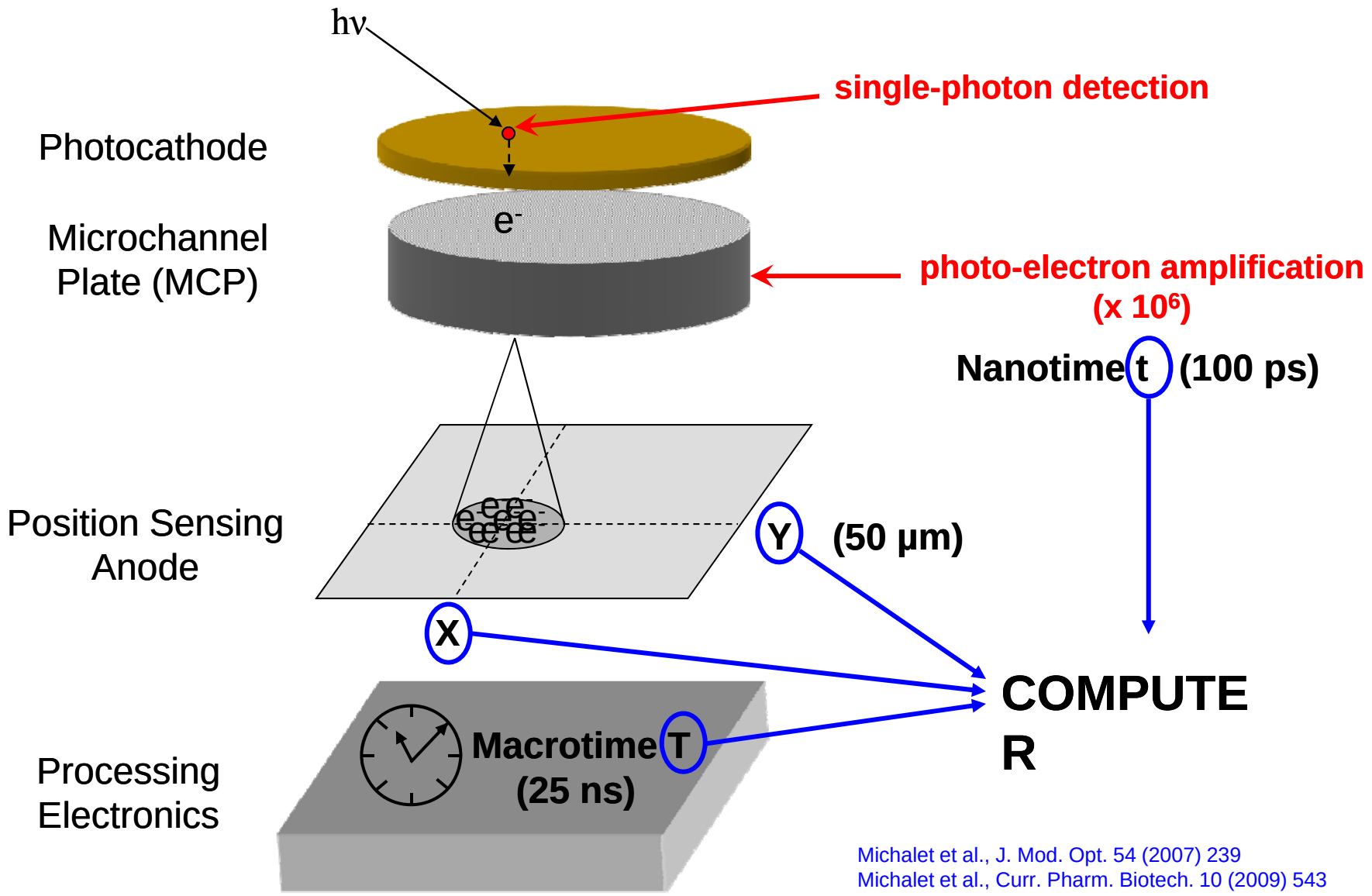
Paul Hinks (Photonis)

A new single-molecule photon-counting camera

+

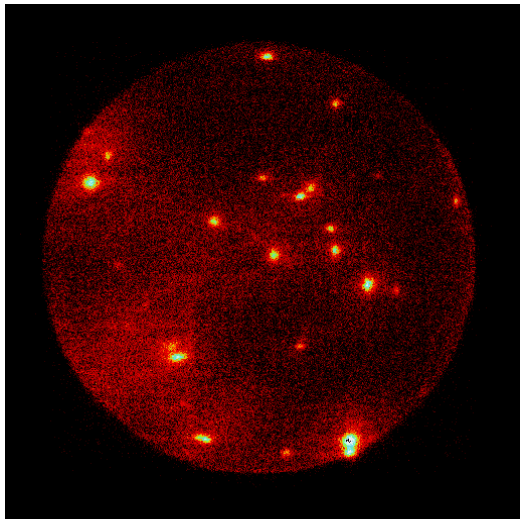
The H33D ("heed") Detector

High spatial, High temporal resolution, High throughput, 2+1D detector

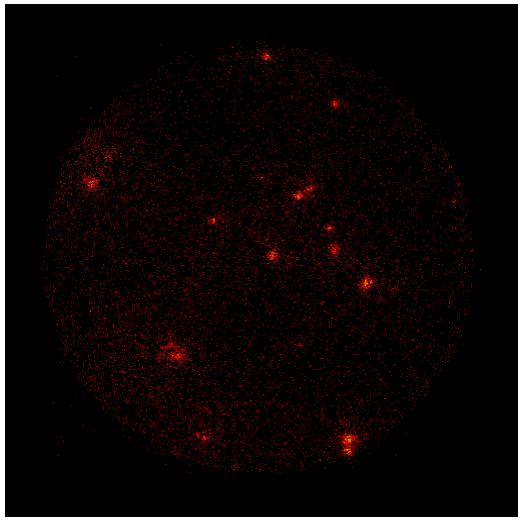


Michalet et al., J. Mod. Opt. 54 (2007) 239
Michalet et al., Curr. Pharm. Biotech. 10 (2009) 543

The H33D in action…



10 fps



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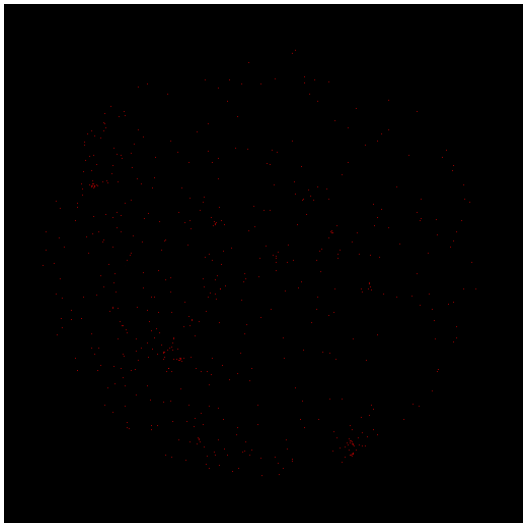
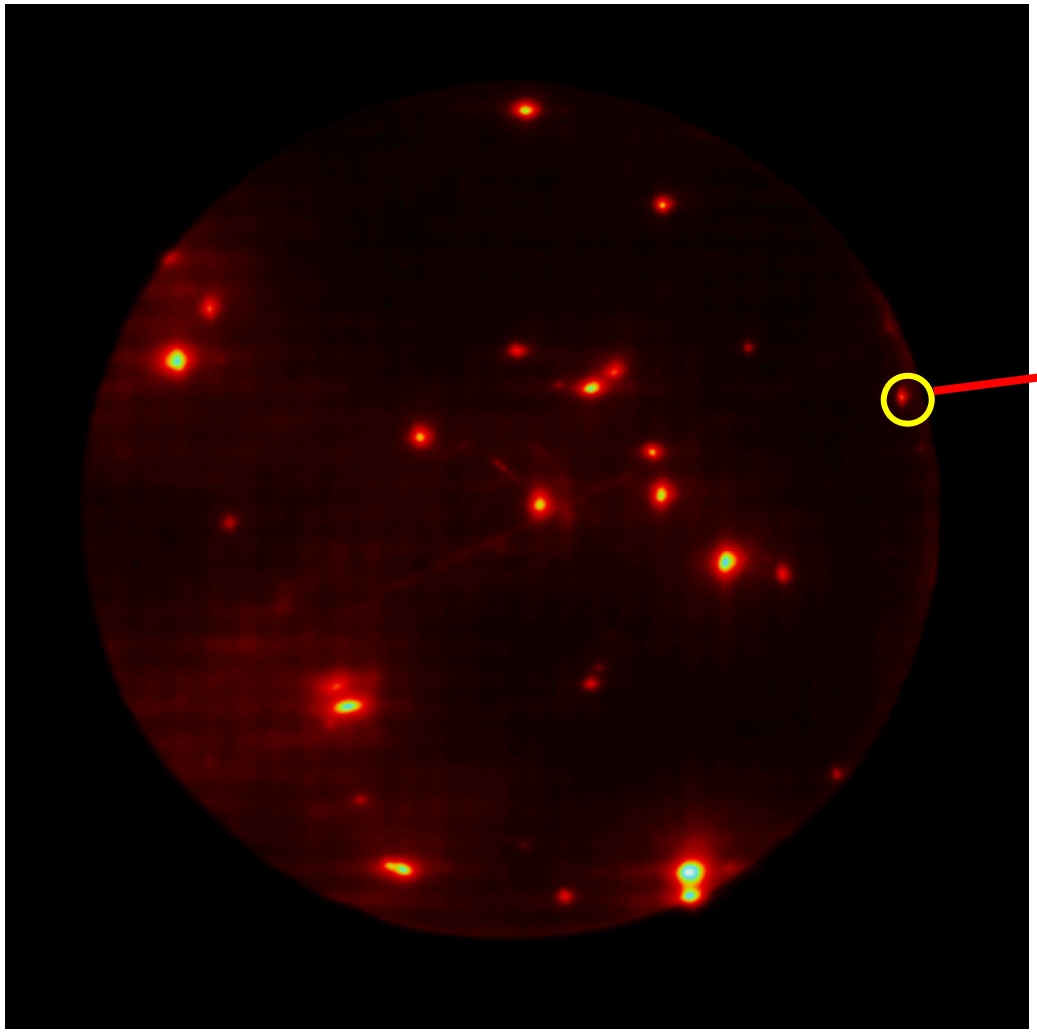
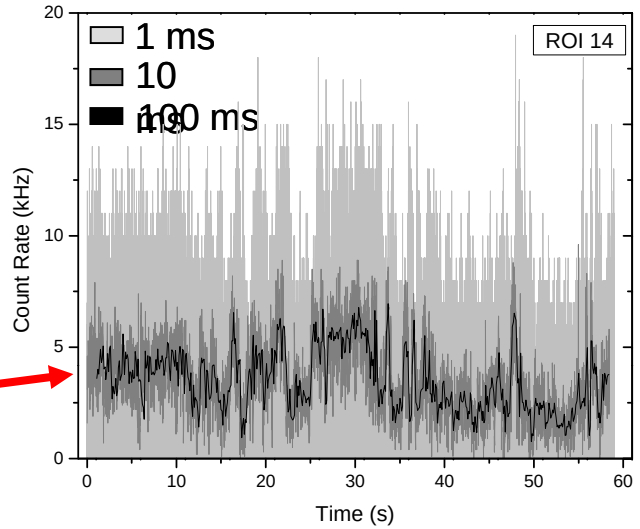


1000 fps

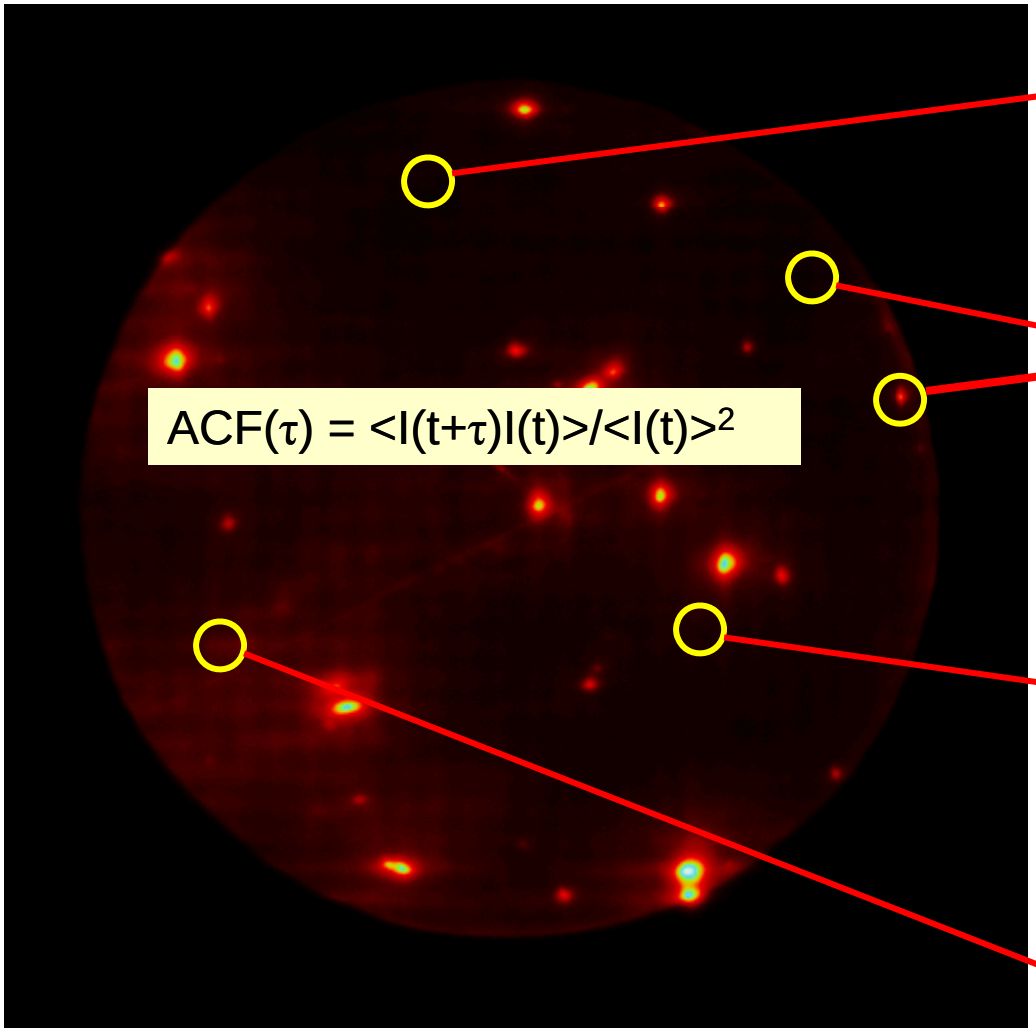
Xavier Michalet, Anton Tremsin, Paul Vallerga, Oswald Siegmund, Photonis

The H33D's detector timing resolution

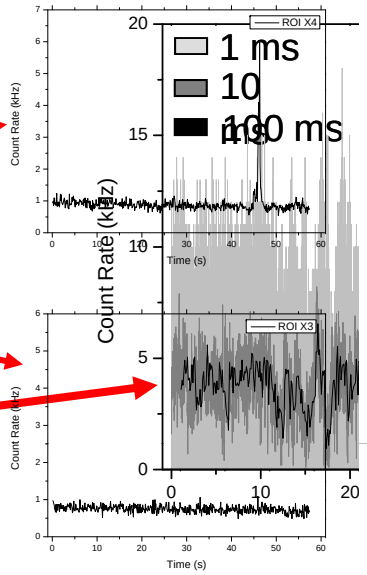


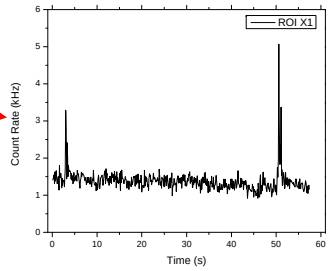


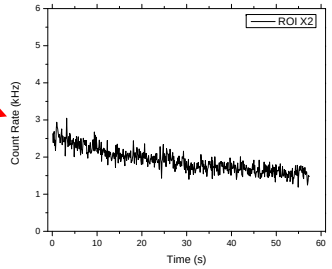
Beyond tracking and beyond single-molecule…

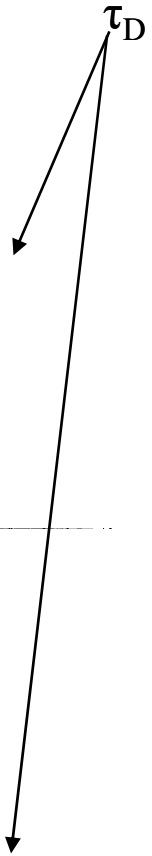


Time Trace









A Novel Detector

Xavier Michalet

Ryan Colyer

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