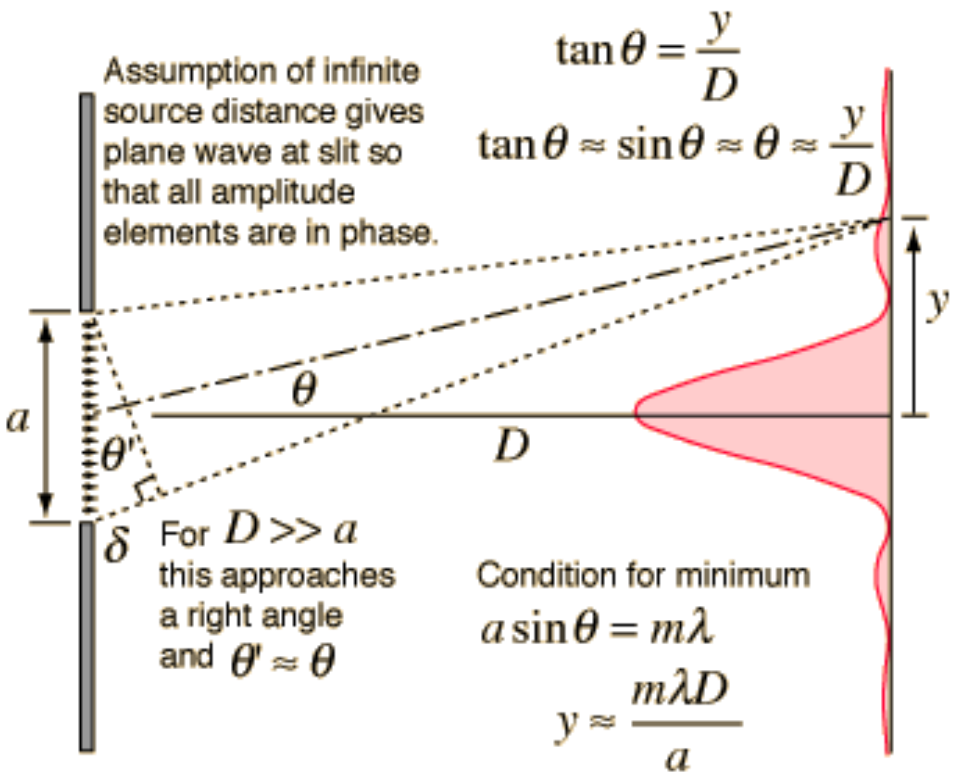
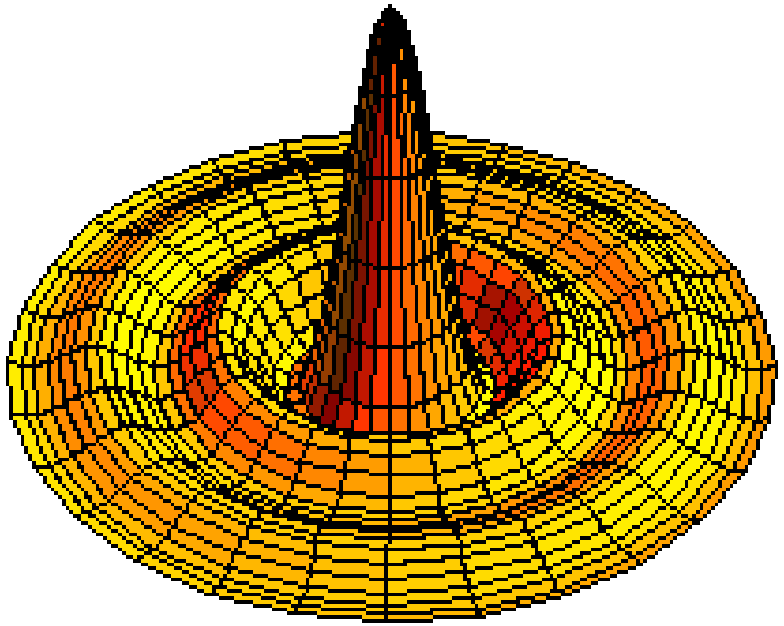
Optical Resolution



Fraunhofer Diffraction

Single slit & circular aperture

Circular aperture -> Airy disk



Airy Disk

Optical Resolution

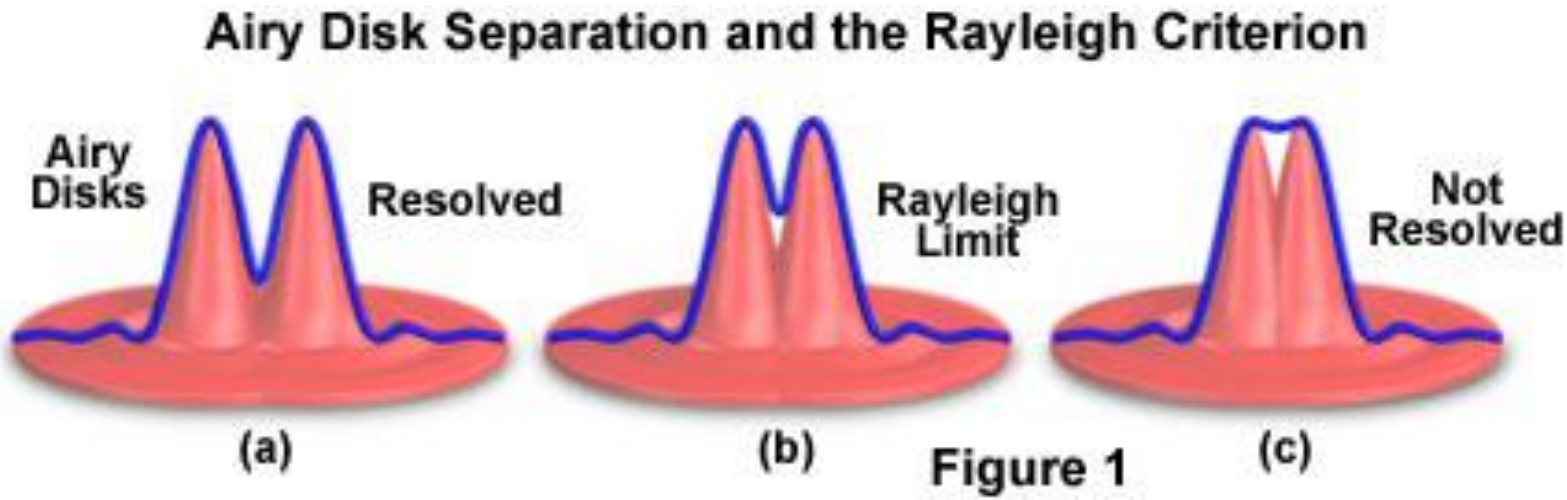


Figure 1

$$d = \frac{0.61\lambda}{NA}$$

d = Rayleigh Criterion length
λ = wavelength
NA : Objectvie Numerical Aperture

For visible wavelength
d ~ 250nm

Fast, background-free, 3D super-resolution optical fluctuation imaging (SOFI)

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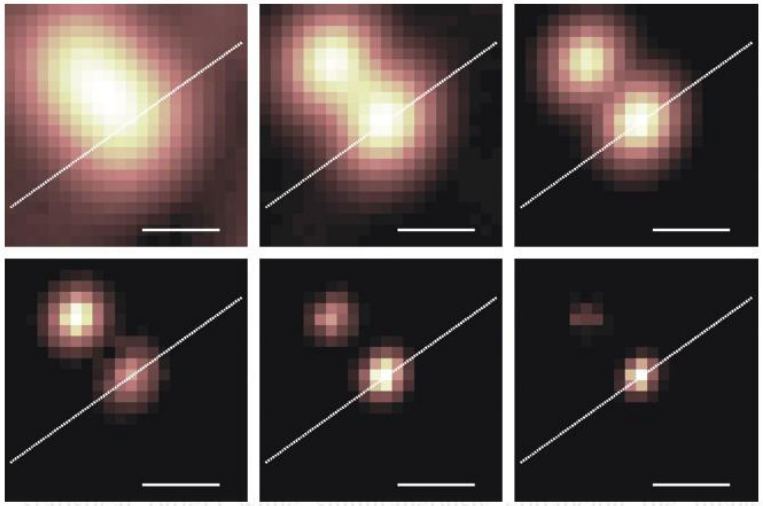
Super-resolution optical microscopy is a rapidly evolving area of fluorescence microscopy with a tremendous potential for impacting many fields of science. Several super-resolution methods have been developed over the last decade, all capable of overcoming the fundamental diffraction limit of light. We present here an approach for obtaining subdiffraction limit optical resolution in all three dimensions. This method relies on higher-order statistical analysis of temporal fluctuations (caused by fluorescence blinking/intermittency) recorded in a sequence of images (movie). We demonstrate a 5-fold improvement in spatial resolution by using a conventional wide-field microscope. This resolution enhancement is achieved in iterative discrete steps, which in turn allows the evaluation of images at different resolution levels. Even at the lowest level of resolution enhancement, our method features significant background reduction and thus contrast enhancement and is demonstrated on quantum dot-labeled microtubules of fibroblast cells.

cumulants | fluorescence | quantum dots | superresolution microscopy | intermittency



contrast. We argue that no other super-resolution microscopy technique can compete with the simplicity of the SOFI approach and its undemanding requirements with regard to fluorescent labels, optics, and other hardware. The experimental procedure

BIOPHYSICS AND COMPUTATIONAL BIOLOGY

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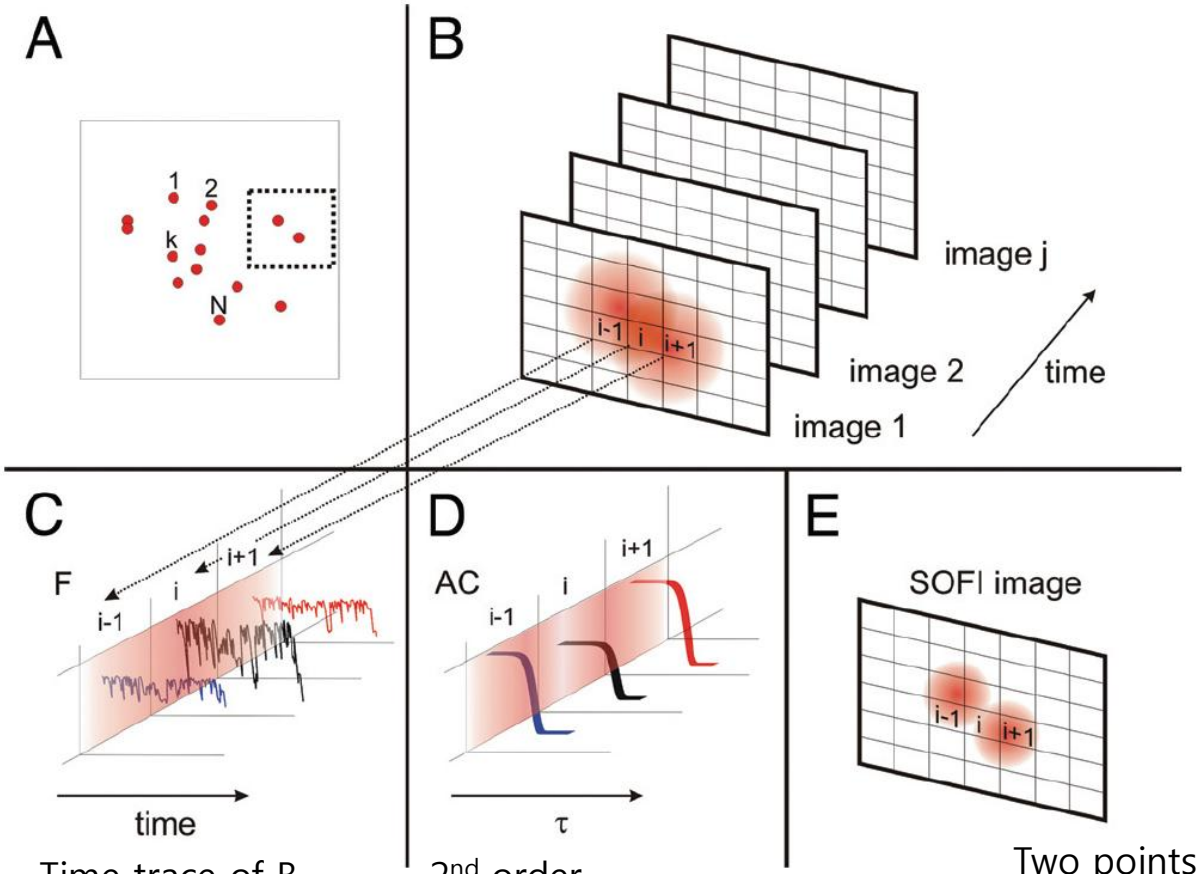
Department of Physics, Sogang University

Soft Matter Optical Spectroscopy Laboratory

Principle of SOFI

Emitter distribution
In the object plane

Magnified detail of A
: Conventional imaging cannot resolve two points



Time trace of B

2nd order
correlation function of C

Two points are
Resolved!

Three Condition for SOFI

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

2. Different emitters have to switch between states repeatedly and independently from each other in a stochastic way.

3. For this approach, the image should be acquired with pixels smaller than the diffraction limit. Resolution less than the pixel size will be the topic of a future publication.

Theory 1 – Correlation Function

Fluorescence source distribution

$$\sum_{k=1}^{N} \delta(\mathbf{r} - \mathbf{r}_k)\cdot\varepsilon_k\cdot s_k(t)$$

ε_k : constant molecular brightness
$s_k(t)$: time-dependent fluctuation

Fluorescence signal at position **r** and time t

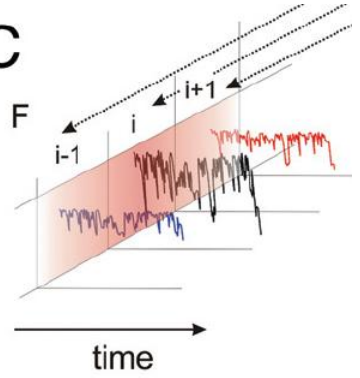
$$F(\mathbf{r}, t) = \sum_{k=1}^{N} U(\mathbf{r} - \mathbf{r}_k)\cdot\varepsilon_k\cdot s_k(t)$$

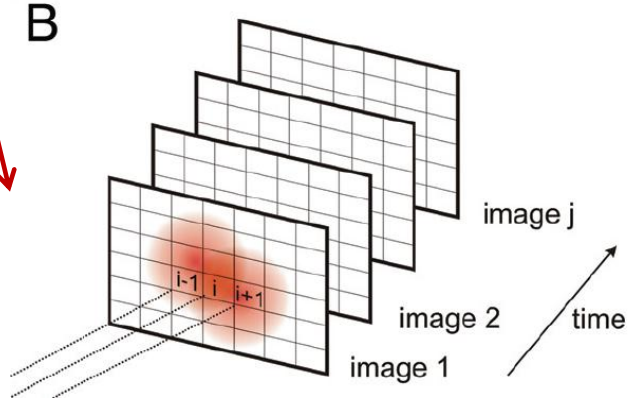
Fluorescence Fluctuation

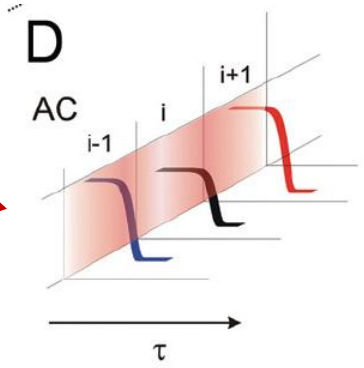
$$\begin{aligned}\delta F(\mathbf{r}, t) &= F(\mathbf{r}, t) - \langle F(\mathbf{r}, t)\rangle_t \\ &= \sum_k U(\mathbf{r} - \mathbf{r}_k)\cdot\varepsilon_k\cdot[s_k(t) - \langle s_k(t)\rangle_t] \\ &= \sum_k U(\mathbf{r} - \mathbf{r}_k)\cdot\varepsilon_k\cdot\delta s_k(t),\end{aligned}$$

2nd order correlation function

$$\begin{aligned}G_2(\mathbf{r}, \tau) &= \langle \delta F(\mathbf{r}, t + \tau)\cdot\delta F(\mathbf{r}, t)\rangle_t \\ &= \sum_{j,k} U(\mathbf{r} - \mathbf{r}_j)U(\mathbf{r} - \mathbf{r}_k)\cdot\varepsilon_j\cdot\varepsilon_k\cdot\langle \delta s_l(t + \tau)\delta s_k(t)\rangle \\ &= \sum_k U^2(\mathbf{r} - \mathbf{r}_k)\cdot\varepsilon_k^2\cdot\langle \delta s_k(t + \tau)s_k(t)\rangle\end{aligned}$$







Theory 2 – Correlation Function to Cumulant Function

PSF in 2nd order Image

$$U(\mathbf{r}) = \exp\left(-\frac{x^2+y^2}{2\omega_0^2} - \frac{z^2}{2\omega_{z0}^2}\right) \qquad \Rightarrow U^2(\mathbf{r}) = \exp\left(-\frac{x^2+y^2}{2\tilde{\omega}_0^2} - \frac{z^2}{2\tilde{\omega}_{0z}^2}\right)$$

Generalize correlation function $\tilde{\omega}_{0z} = \omega_{0z}/\sqrt{2}$ and $\tilde{\omega}_0 = \omega_0/\sqrt{2}$

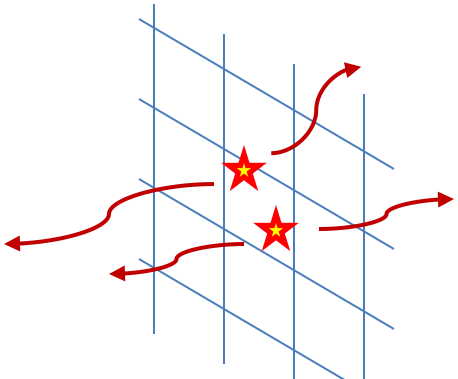
$$\mathrm{G}_n(\mathbf{r}, \tau_1, \ldots, \tau_{n-1}) = \langle \delta F(\mathbf{r}, t)\delta F(\mathbf{r}, t+\tau_1)\cdots\delta F(\mathbf{r}, t+\tau_{n-1})\rangle_t$$

Cumulant function $C_n(r,\tau_1,\ldots,\tau_{n-1})$

$$\mathrm{C}_n(\mathbf{r}, \tau_1, \ldots, \tau_{n-1}) = \sum_k U^n(\mathbf{r}-\mathbf{r}_k)\varepsilon_k^n w_k(\tau_1, \ldots, \tau_{n-1})$$

ω_k : correlation-based weighting function

All cross terms caused by lower-order correlation contributions are elimunated in cumlulants.



Consider 4 photons from two emitters in one pixel,

4th order correlation contribution is concealed by lower order (2nd order) contribution

Theory 3 – Cumulant Function

$$C_n(\mathbf{r}, \tau_1, \ldots, \tau_{n-1}) = \sum_k U^n(\mathbf{r} - \mathbf{r}_k)\varepsilon_k^n w_k(\tau_1, \ldots, \tau_{n-1})$$

Resolution is enhanced by a factor $\sqrt{n}$
n-fold larger brightness will appear 2^n times brighter in n^{th} order Image

$$\begin{aligned}
C_2(\mathbf{r}, \tau_1) &= G_2(\mathbf{r}, \tau_1) \\
C_3(\mathbf{r}, \tau_1, \tau_2) &= G_3(\mathbf{r}, \tau_1, \tau_2) \\
C_4(\mathbf{r}, \tau_1, \tau_2, \tau_3) &= G_4(\mathbf{r}, \tau_1, \tau_2, \tau_3) - G_2(\mathbf{r}, \tau_1)\cdot G_2(\mathbf{r}, \tau_3) \\
&\quad - G_2(\mathbf{r}, \tau_1 + \tau_2)\cdot G_2(\mathbf{r}, \tau_2 + \tau_3) - G_2(\mathbf{r}, \tau_1 + \tau_2 + \tau_3)\cdot G_2(\mathbf{r}, \tau_2)
\end{aligned}$$

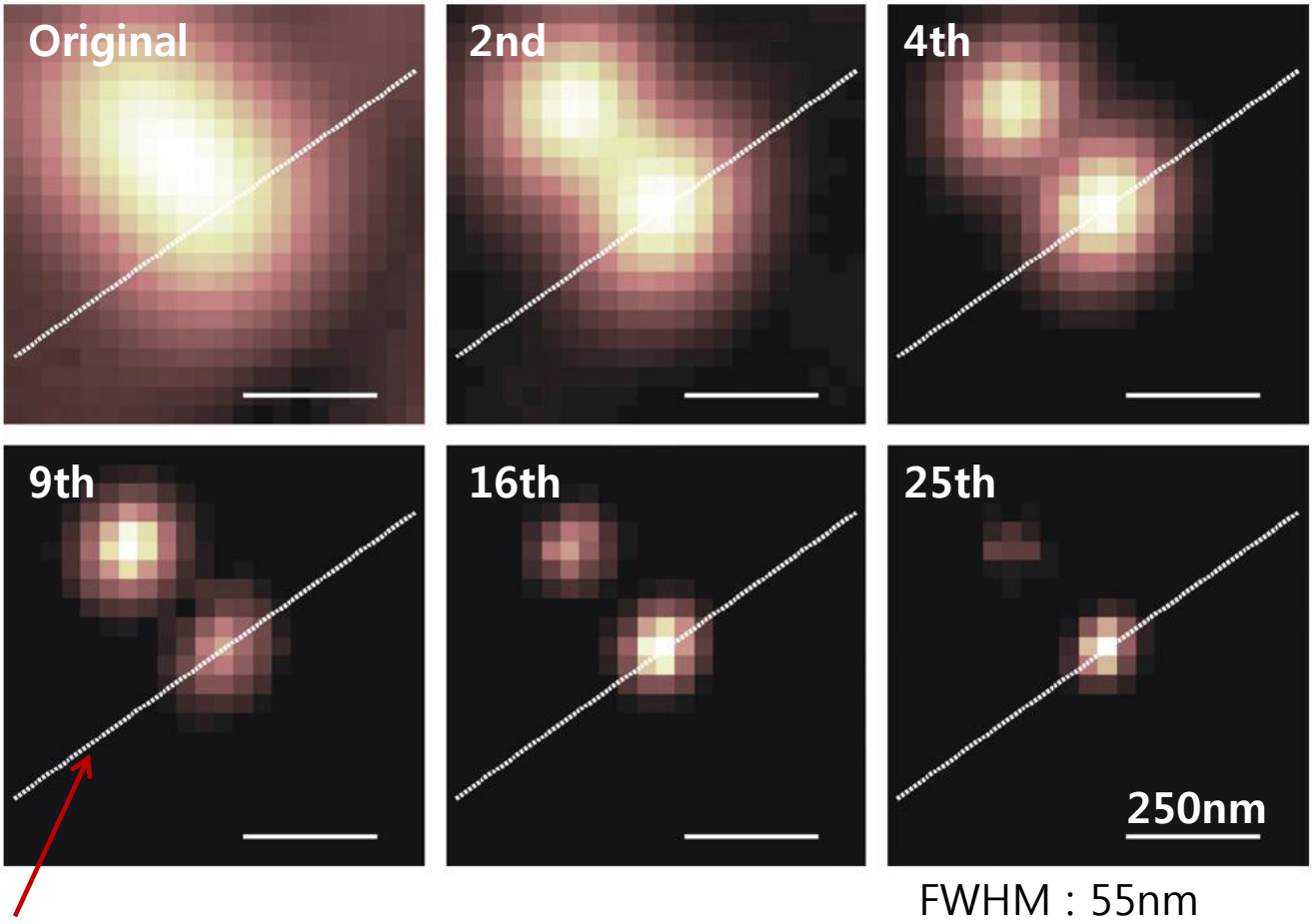
Setup

Microscope Setup and Data Analysis. Movies were taken on an inverted wide-field microscope (Olympus IX71, objective: UPlanApo 60×, 1.45, Oil). A 470-nm LED array device was used as a light source (Lumencor Aura Light Engine) and a CCD camera (Andor iXon + 885) was used to record the signal. Filter set was dichroic [505 DCXR, Chroma Technology; emission (D620/40, Chroma Technology]. Magnification was adjusted to obtain 35 nm per pixel. To generate and evaluate SOFI images, movies were analyzed by using a custom-written Matlab (Mathworks) code. The shortest accessible time lag is the frame integration time (time between two subsequent frames). We computed all SOFI images for the zero time lag only C_n (**r**,0,0,…0). In this case a computationally less expensive expression for the cumulants formula can be used (see *SI Text*). Cumulants of orders > 2 can turn negative depending on the underlying fluctuation pattern. SOFI images are therefore displayed as absolute values.

Results 1 – Images of Two Quantum Dots due to Correlation Orders

S/B : 1.9
FWHM : 289nm

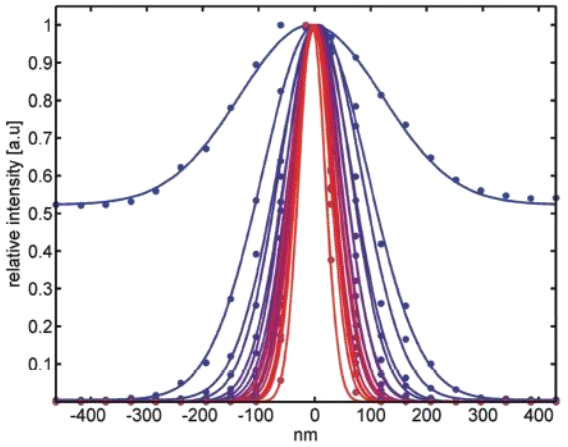
S/B : 250

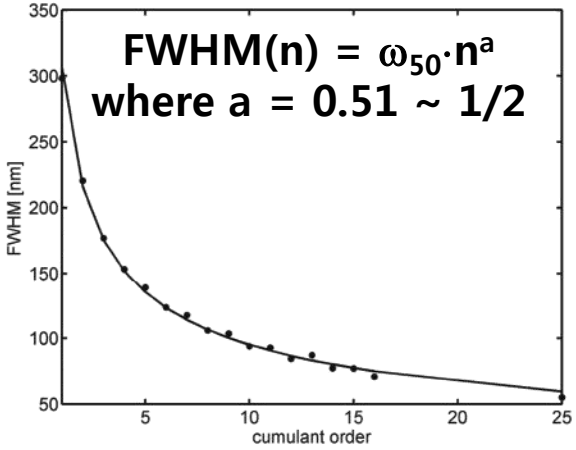


FWHM : 55nm

Cross-section of
Original Image

Profiles & Fits





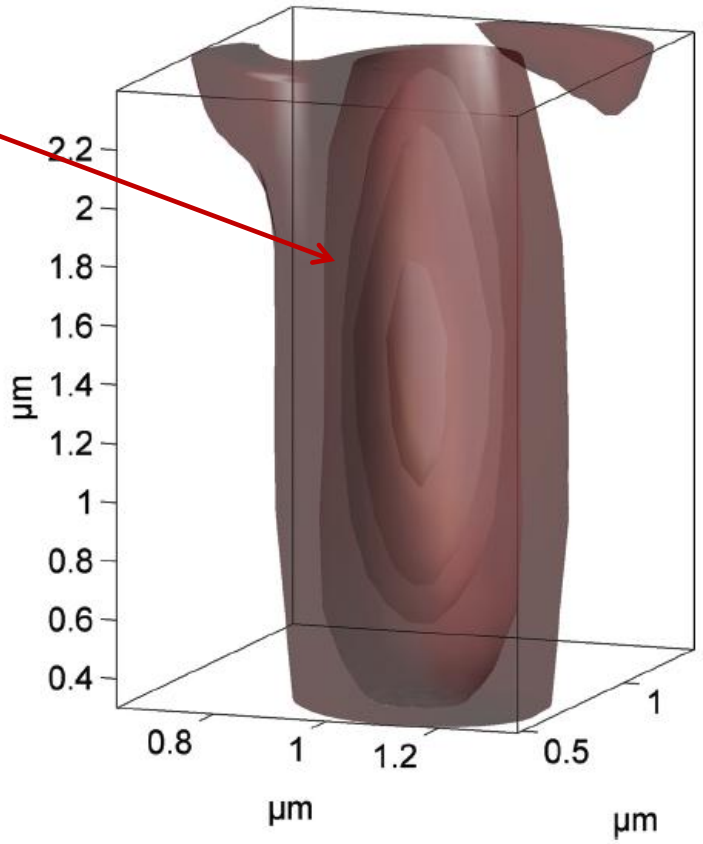
FWHM versus Correlation Order

Signal to Background Ratio (S/B)
2,000 frames (100 ms per frame)

Results 2 – Point Spread Function from Single Quantum Dot

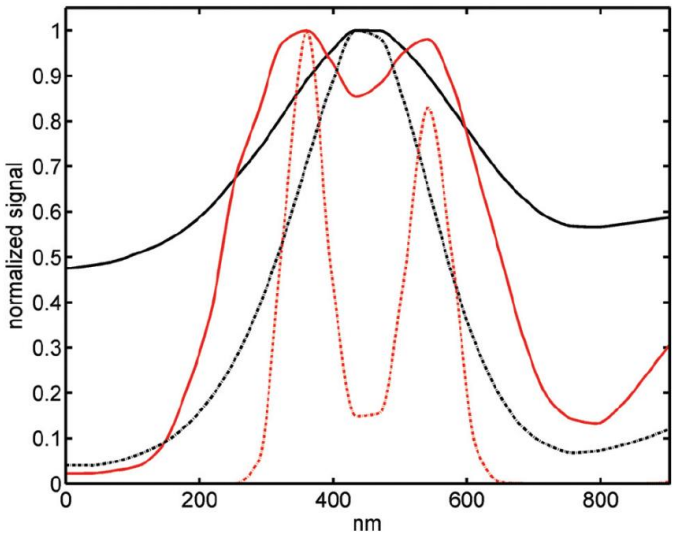
Original, 2nd, 4th, 9th and 16th order Image

$1/e^2$ iso-surfaces



300nm spacing scan image
4,000 frames (75 ms per frame)

Results 3 – Resolution Enhancement



Original Image

2nd order Image

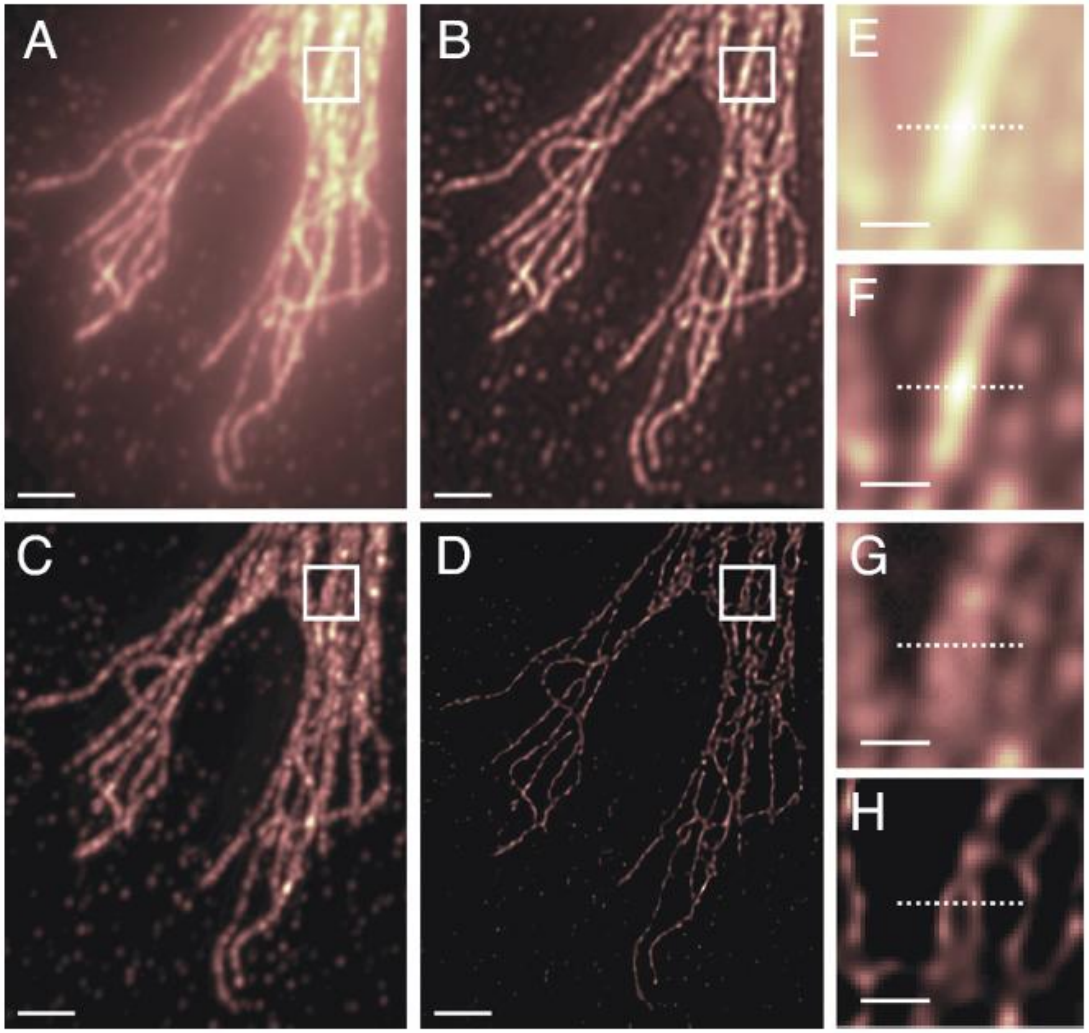
Cross-section of Original Image

Cross-section of 2nd order Image

Results 4 – SOFI Images of Cells (α-tubulin network of a 3T3 fibroblast cell)

A deconvolved

Original Image



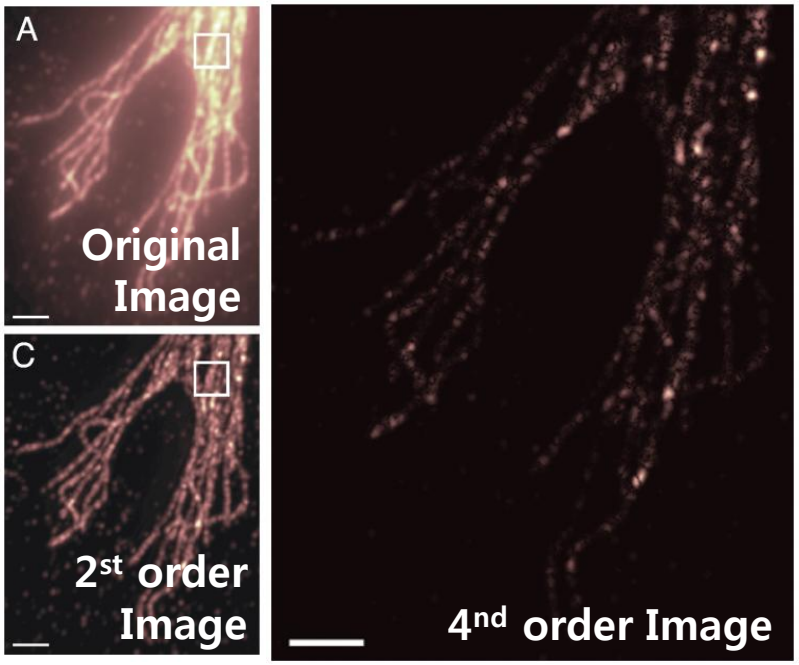
2nd order Image

C deconvolved

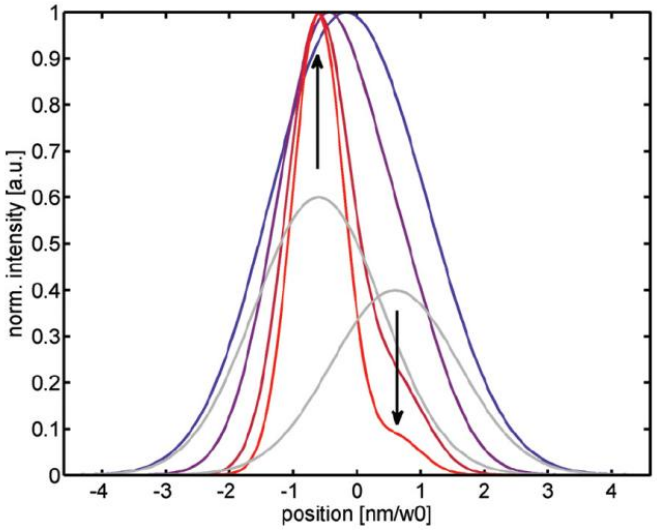
QD625 Labeled
3,000 frames (100 ms per frame)

Limitation 1. The Brightness Scaling of the Images

n-fold larger brightness will appear 2^n times brighter in n^{th} order Image
-> very high Dynamic Range
-> Masking effect of dim emitters in proximity to bright emitters



Blue to red : cumulant order 1,2,4,6
Gray : individual diffraction limit



Blinking rate : 10% of Frame rate

Limitation 2. Limited measurement time

Theoretically, SOFI eliminate any kinds of noise

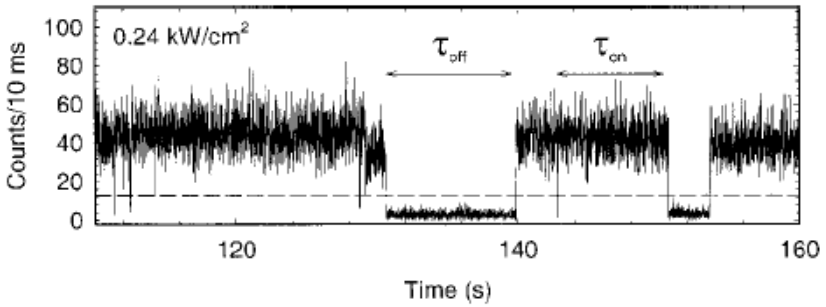
Acquisition times are limited
(by photobleaching …)

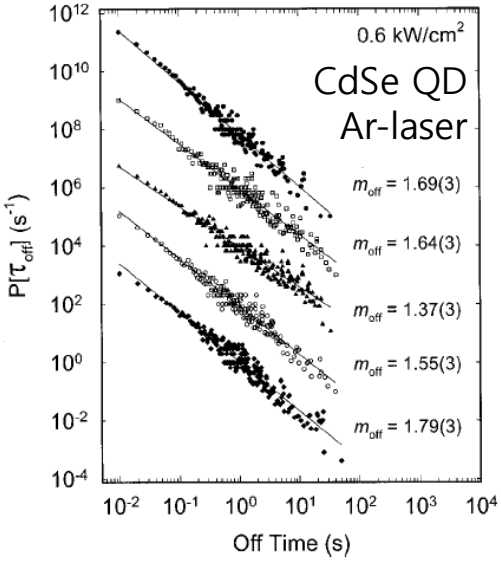
Potential to super-resolution imaging at high frame rates

QDs remain in the on state for a few seconds leading to very different brightness values in image

-> Need to uniform and fast blinking emitter for SOFI at high frame rates

Kuno *et al.*, J. Chem. Phys (2001)
Power law on/off distribution





Conclusion

statistical order) while simultaneously enhancing the image contrast. We argue that no other super-resolution microscopy technique can compete with the simplicity of the SOFI approach and its undemanding requirements with regard to fluorescent labels, optics, and other hardware. The experimental procedure essentially amounts to taking a movie of a fluctuating signal.

ground reduction. Last, SOFI is not limited to blinking between fluorescent on and off states. Any (even nonfluorescent) fluctuating objects, such as rotating dipoles, or blinking of celestial objects, such as binary stars, could be imaged and superresolved by SOFI.