Computational high-resolution methods

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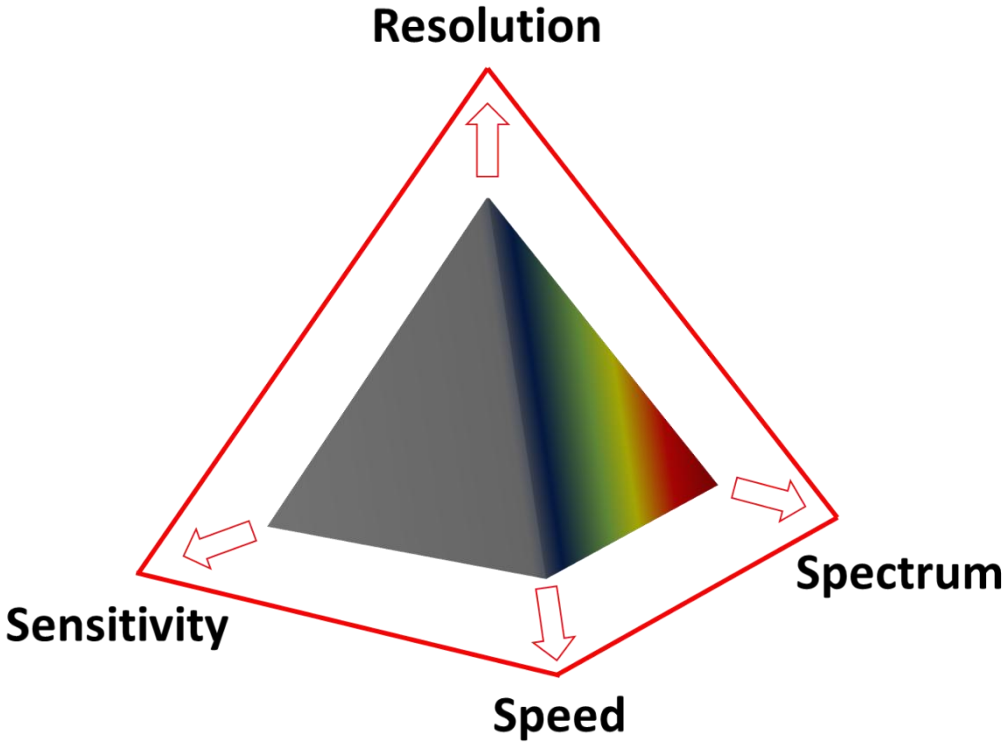
- Super-resolution methods

- Computation and optical methods
  - *Confocal*
    - Pinhole closing
    - Pixel reassignment
    - Optical photon reassignment
  - *SIM*
  - *Fluctuation-based*
    - SOFI
    - SRRF
- Comparison of methods

- Conclusion

Super-resolution methods

- STED
  - *Resolution increase proportional to intensity of the donut beam*
  - *Phototoxicity*
- SMLM
  - *Simple set-up (WF or TIRF microscope)*
  - *High laser intensities, toxic buffers, UV illumination*
  - *Slow acquisition speed*
- SIM
  - *Routinely used for live cell imaging*
  - *Only 2-fold increase in resolution*


Resolution
Spectrum
Speed
Sensitivity

Computational approaches

- Confocal microscopy (laser scanning, spinning disk), WF, TIRF
  - No specialized hardware
  - Lower illumination intensities
  - Faster image acquisition
  - Higher resolution *(from v2 x better to ~ Single Molecule Localization Microscopy)*
  - Often no specialized fluorophores
  - **OVERSAMPLING needed!**

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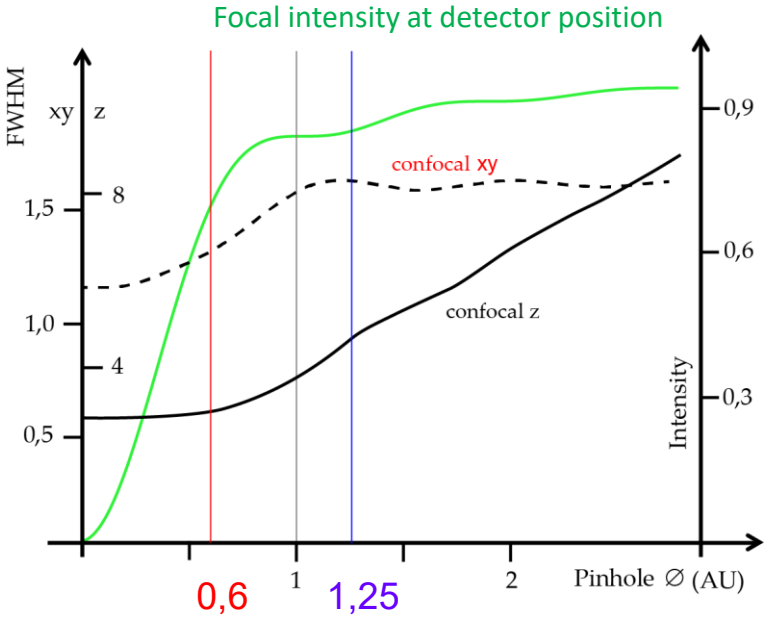
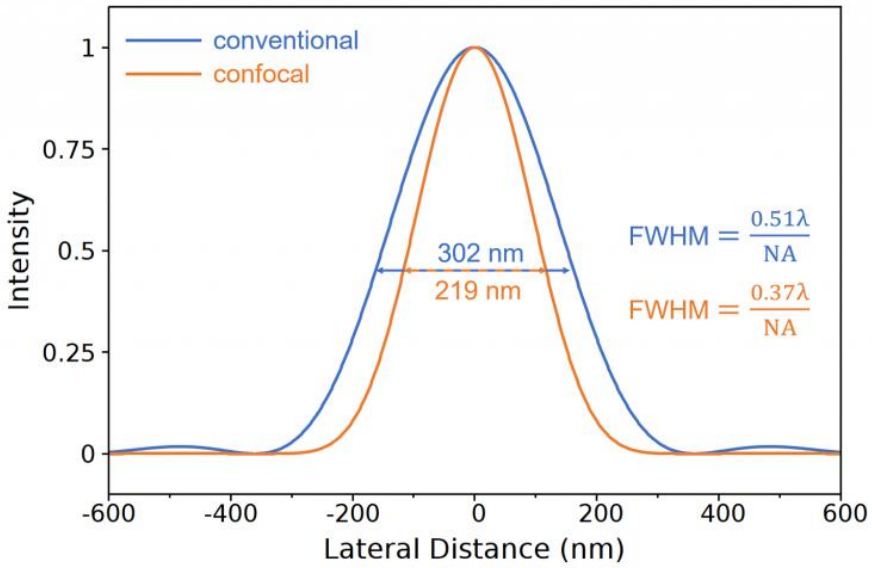
Pinhole closing



Pinhole closing



- LSCM
- Simulation of the effective PSF of a conventional and confocal microscope in the X-Y plane with 532 nm excitation, 100x 0.9 NA objective
- Infinitely small pinhole diameter for ~1.4x improved lateral resolution
- 3D resolution improvement

https://www.edinst.com/blog/pinhole-confocal-microscope/

Lightning

- LSCM
- Closing the pinhole (e.g. down to 0,6 Airy units)
- Adaptive deconvolution

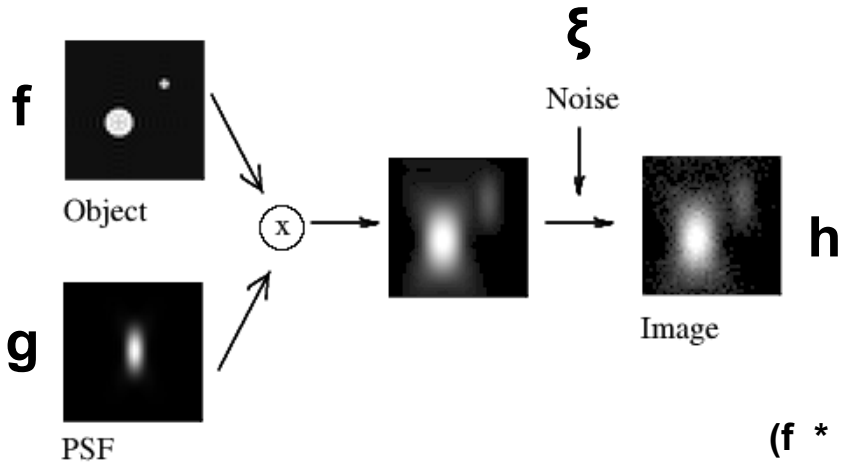
- ~2x improvement of resolution (in 3D)

Leica MICROSYSTEMS

https://www.leica-microsystems.com/science-lab/life-science/obtain-maximum-information-from-your-specimen-with-lightning/

Lightning

Deconvolution

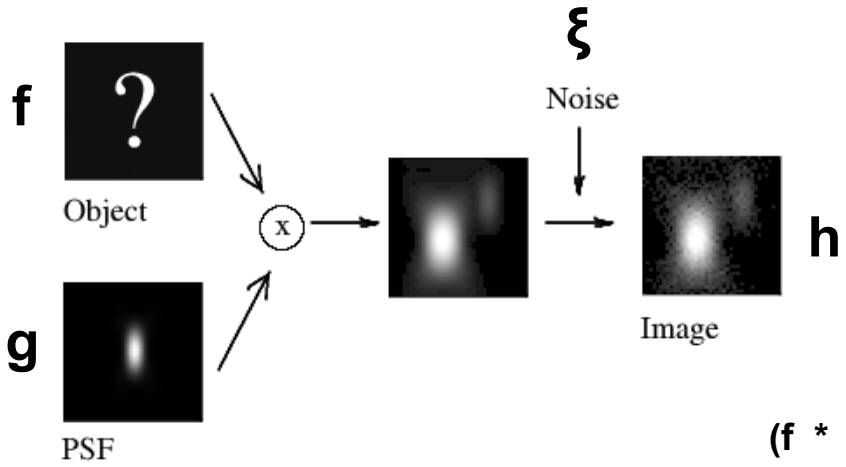


(f * g) + ξ = h

f signal that we wish to recover
g point spread function (PSF)
ξ noise
h recorded signal

Lightning

Deconvolution

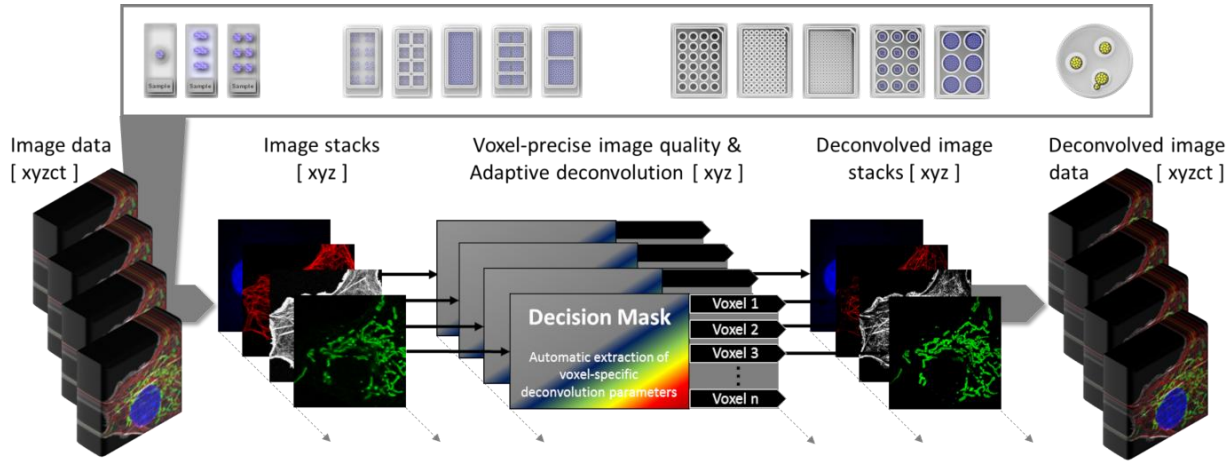


$$(f * g) + \xi = h$$

f signal that we wish to recover
g point spread function (PSF)
ξ noise
h recorded signal

Lightning

- Fully automated information extraction
- Instead of global deconvolution parameters, background and signal-to-noise ratio are extracted *for each voxel / volume segment -> local image quality characteristics*

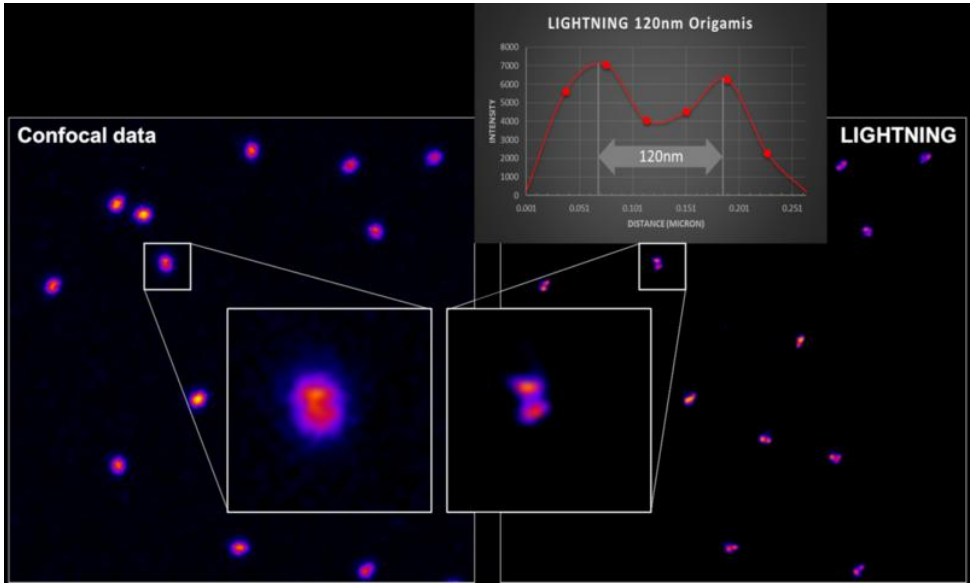


https://www.leica-microsystems.com/science-lab/life-science/obtain-maximum-information-from-your-specimen-with-lightning/

Lightning

- LSCM
- 120 nm lateral resolution, 200 nm axial resolution
- Speed 40 fps (512x512)
- Imaging formats up to 2496x2496 pixels (w. resonant scanner)
- Up to 8 colors simultaneously with STELLARIS microscope

- **Full integration into the online image acquisition**



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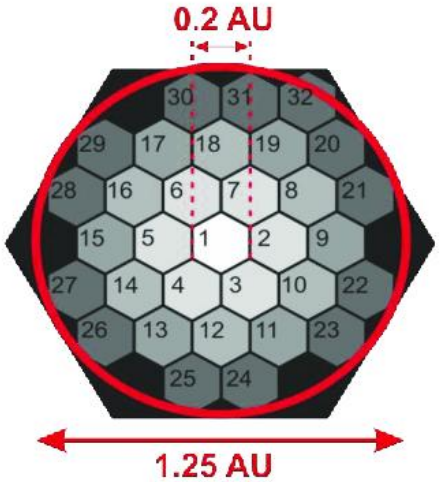
Pixel reassignment

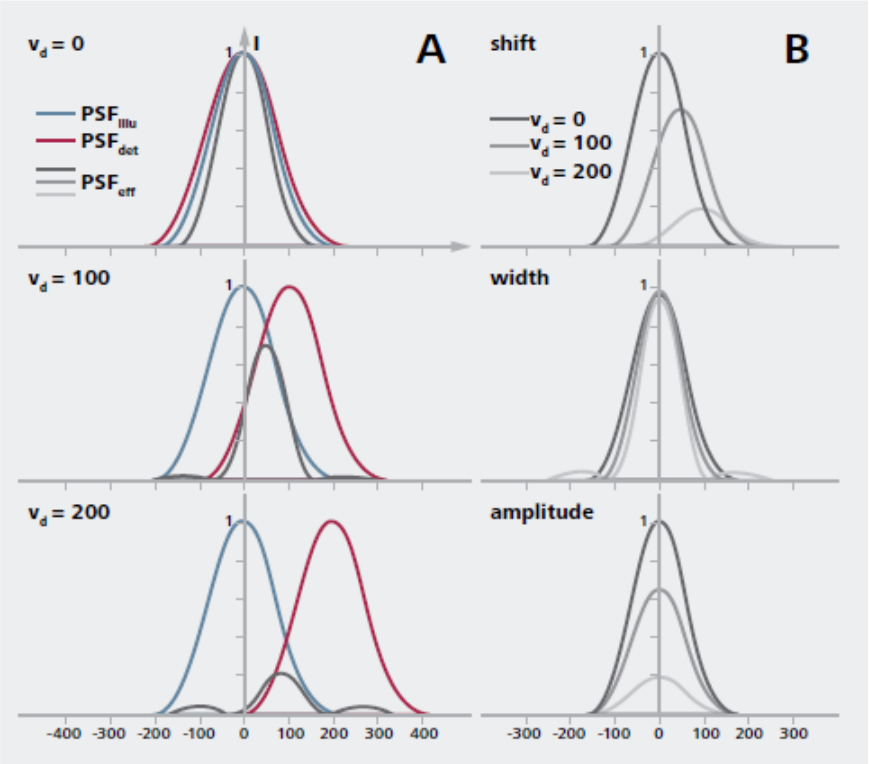
- LSCM
- Confocal illumination
- Area detector in the position of pinhole
  - Weight the contribution from each detector, deconvolution applied to image from each detector element
  - Reconstructed sum image
- ~2x improvement in 3D resolution
  - 100-120 nm x/y
  - 300-350 nm z
- Increase in SNR



Korobchevskaya K., **Exploring the Potential of Airyscan Microscopy for Live Cell Imaging,** Photonics 4(3):41, 2017

Airyscan

- Pixel reassignment:
  - Displacement of detection pinhole from optical axis
  - -> PSF_{eff} has lower amplitude & narrower width
  - -> more precise location of point emitters (laterally)
  - Shift back images from off-axis detectors to optical axis and adding them up
  - Followed by deconvolution
  - Lateral resolution improvement



The Basic Principle of Airyscanning, Klaus Weisshart, 2014

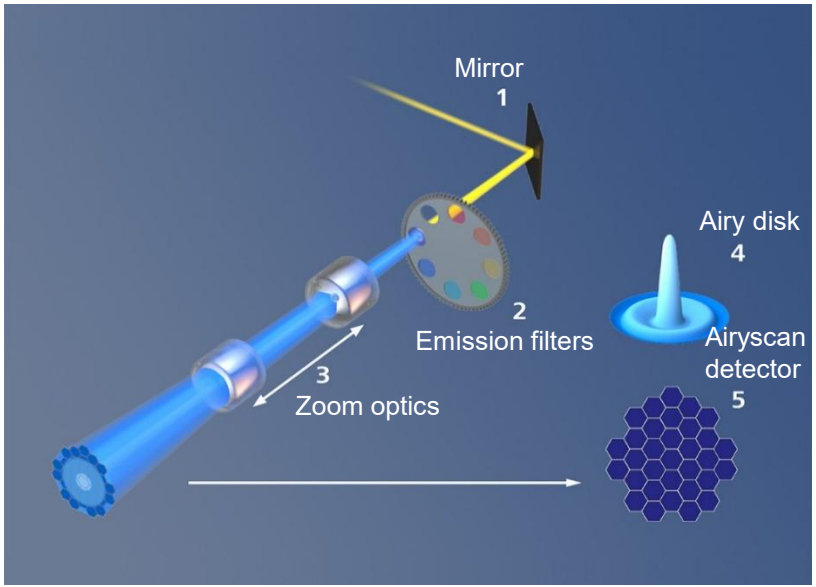
Airyscan

- 32 channel area detector in the position of pinhole
- 1.7x improvement in 3D resolution
  - 120 nm x/y *(down to 90 nm with jDCV)*
  - 350 nm z
- 4-8x increase in SNR
- Up to 36 channels in a single scan

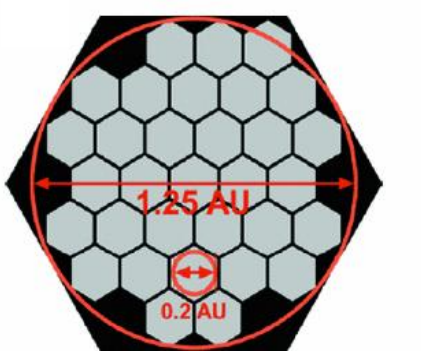
- Airyscan – more efficient way of data analysis:
  - Proper weighing the image of each detector element
  - Linear deconvolution step (Wiener) assign frequencies to their correct location

https://www.zeiss.com/microscopy/us/products/confocal-microscopes/confocal-microscope-with-multiplex-mode-lsm-980-airyscan-2.html

Airyscan



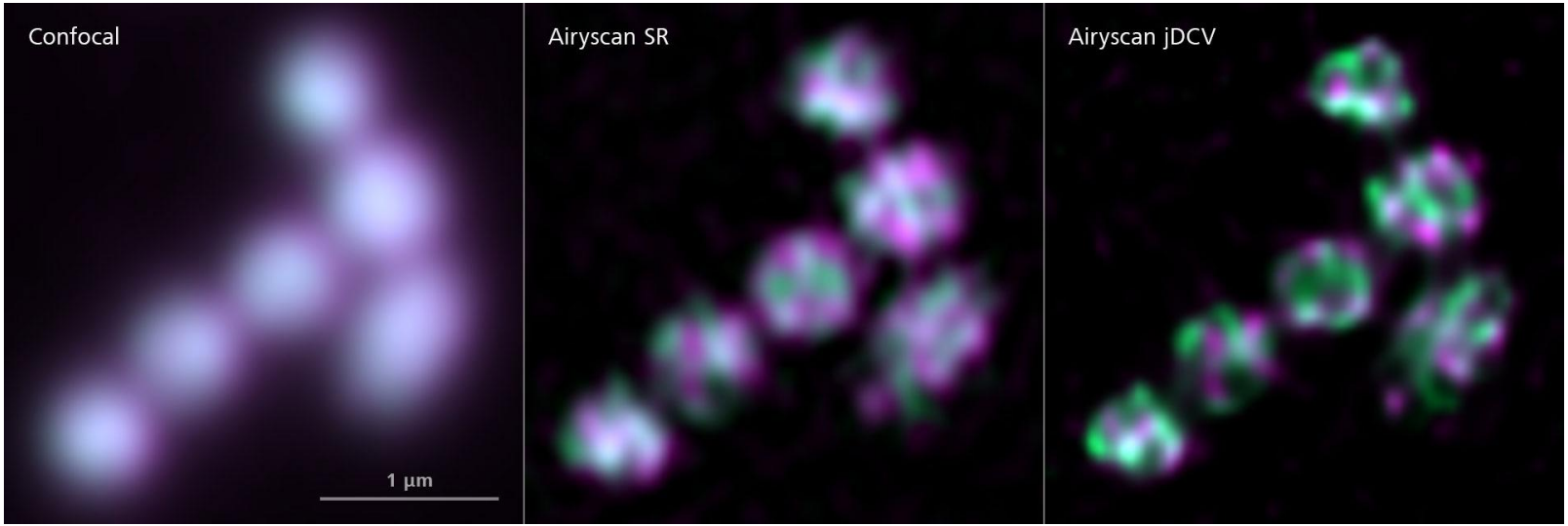
- Airyscan detector
  - at pinhole plane position
  - 32 GaAsP-PMT array
  - capturing 1.25 AU
  - sub-elements 0.2 AU
  - -> Computation required



The Basic Principle of Airyscanning, Klaus Weisshart, 2014

Airyscan

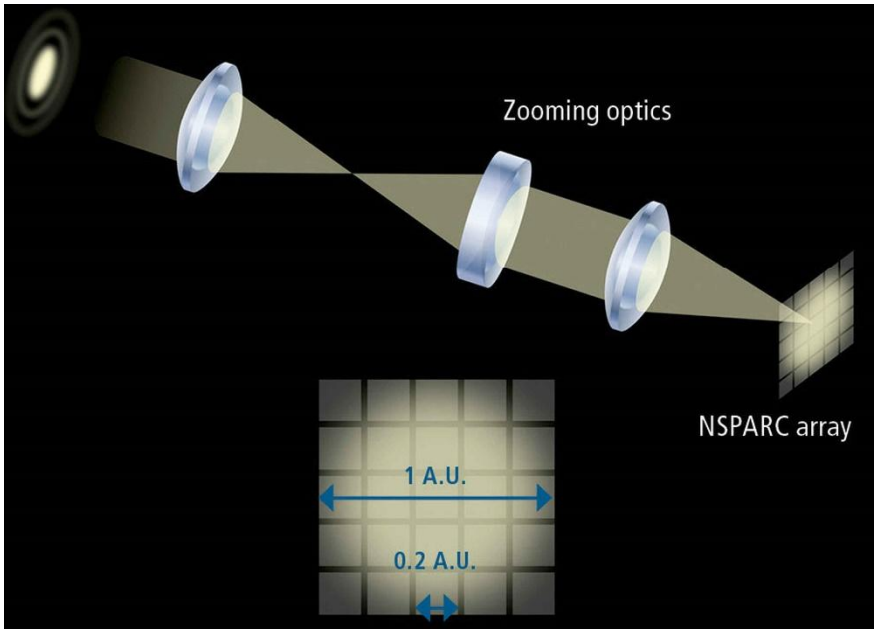
Mitochondria in an Arabidopsis thaliana cell



- Iterative deconvolution (instead of Wiener Filter)
- Joint Deconvolution process of ZEISS Airyscan 2 (joint Richardson–Lucy algorithm)

https://www.zeiss.com/microscopy/us/products/confocal-microscopes/confocal-microscope-with-multiplex-mode-lsm-980-airyscan-2.html

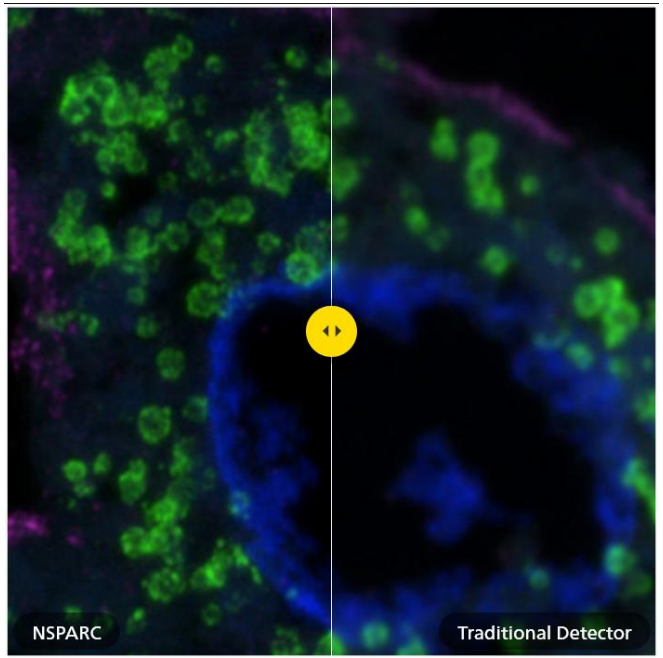
NSPARC



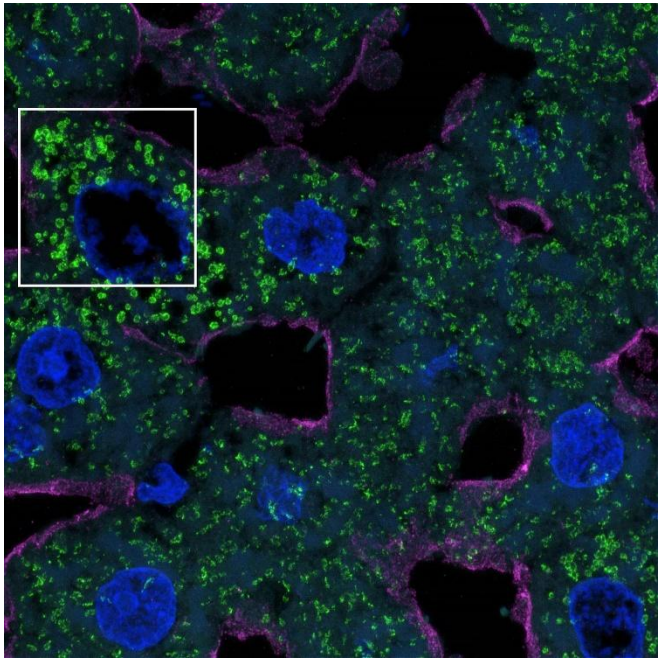
- Nikon Spatial Array Confocal (NSPARC) Detector
- Lateral resolution 100 nm, axial resolution 300 nm
- Equipped with SPPC (Single Pixel Photon Counter) array detector (SPAD array), 5x5
- Improves sensitivity by a factor of 1.3 (compared to AX and AX R confocal microscopes)

https://www.microscope.healthcare.nikon.com/products/confocal-microscopes/ax/ax-ax-r-with-nsparc

NSPARC



Liver peroxisomes



https://www.microscope.healthcare.nikon.com/products/confocal-microscopes/ax/ax-ax-r-with-nsparc

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  - *SIM*
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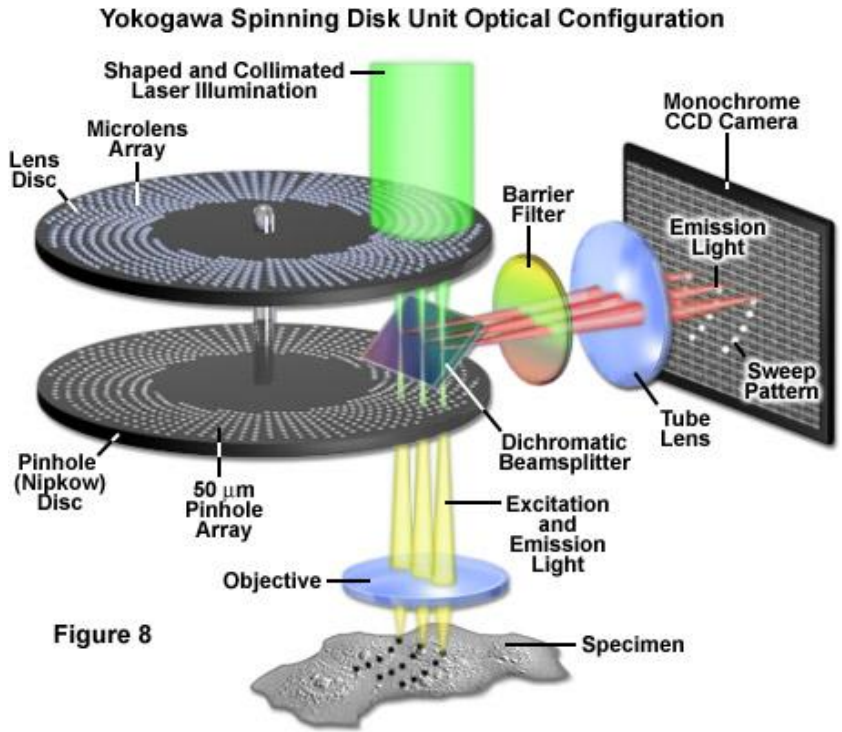
Optical photon reassignment

- Microlens is fitted in the pinhole
- Individual focal points projected onto the pinhole are *optically reassigned* to the center
- Microlens locally contract each emission focus *twofold*
- Increase in brightness and resolution

- Resolution increase ~√2x
- Followed by deconvolution
- ~2x resolution increase in 3D
- **SNR increase**



https://zeiss-campus.magnet.fsu.edu/articles/spinningdisk/introduction.html

Azuma T. and Kei T., Super-resolution spinning disk confocal microscopy using optical photon reassignment, Optics Express 2015, Vol. 23, No. 11

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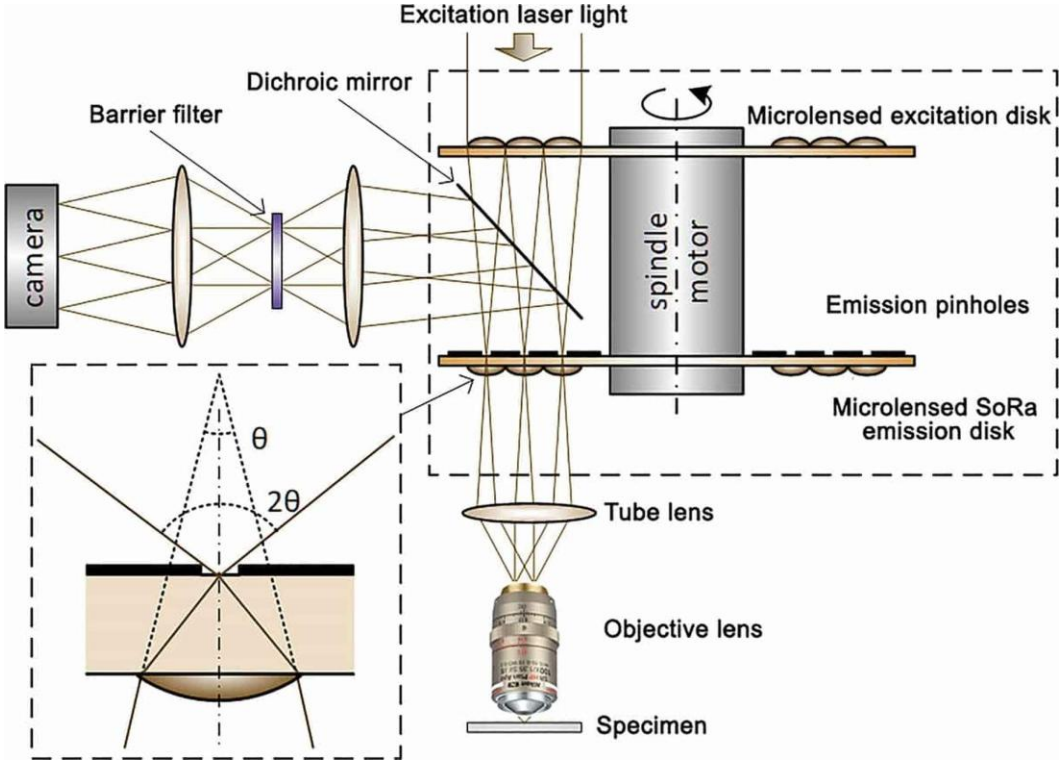

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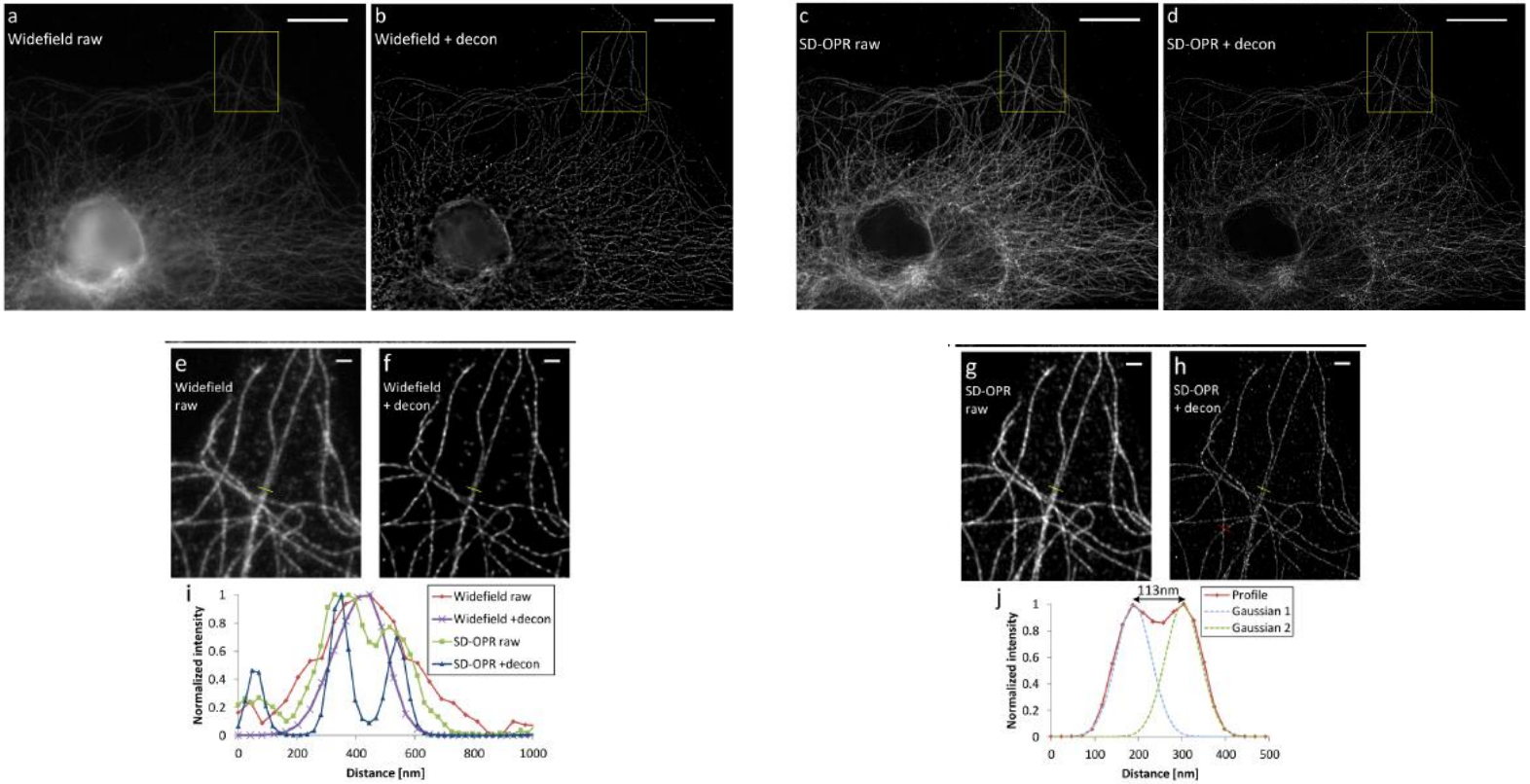


Azuma T. and Kei T., Super-resolution spinning disk confocal microscopy using optical photon reassignment, Optics Express 2015, Vol. 23, No. 11

CSU-W1 SoRa

- Spinning disk SR implementation
  - “Optical photon reassignment”
  - CSU-W1 with SoRa disk (Yokogawa)

  - 120 nm lateral resolution, 300 nm axial resolution
  - Fast live cell imaging (up to 200 frames/s)
  - Less phototoxicity and bleaching in 3D

  - WF, confocal and SR mode

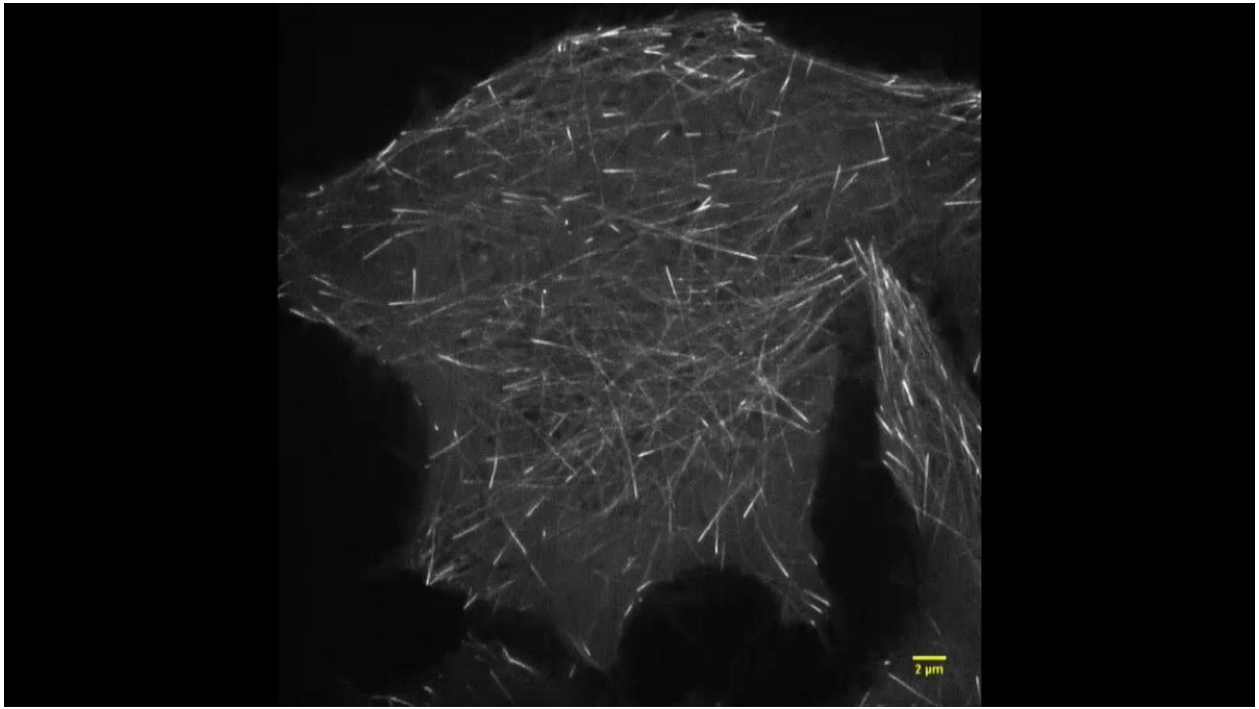
https://www.olympus-lifescience.com/en/landing/ixplore/ixplore-spinsr/

https://www.microscope.healthcare.nikon.com/products/confocal-microscopes/csu-series/csu-w1-sora

CSU-W1 SoRa

IXplore SpinSR

- **Live cell imaging**
- EB3 proteins binding to the top of microtubles extending in HeLa live cells, GFP-labeled
- 500 ms/frame



https://www.olympus-lifescience.com/en/microscopes/inverted/ixplore-spinsr/

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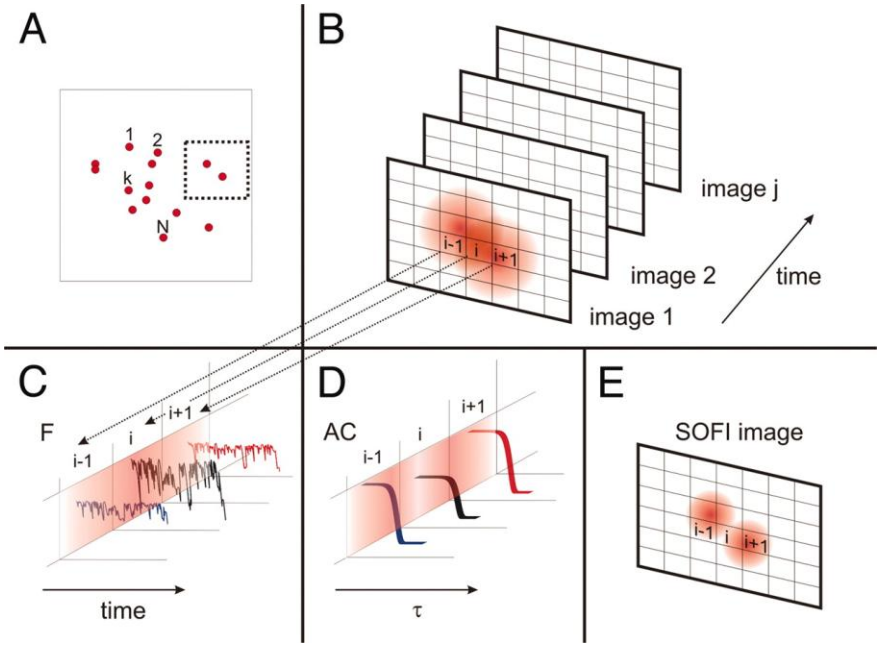
Fluctuation-based methods

- Emitted light of every fluorophore randomly varies over time
- Compatible with most microscopes, do not depend on hardware modifications
- **Typically hundreds of images acquired**
- Resolution enhancement typically – 2-3x (can be higher)

SOFI

- "Super-resolution optical fluctuation imaging"
  - *3D super-resolution method*
  - Based on <u>analysis of temporal fluorescence fluctuations of emitters</u>
    - -> requires multiple image acquisition *(thousands of frames, thus significantly slower)*
  - Allows higher density samples
  - Pixels smaller than diffraction limit



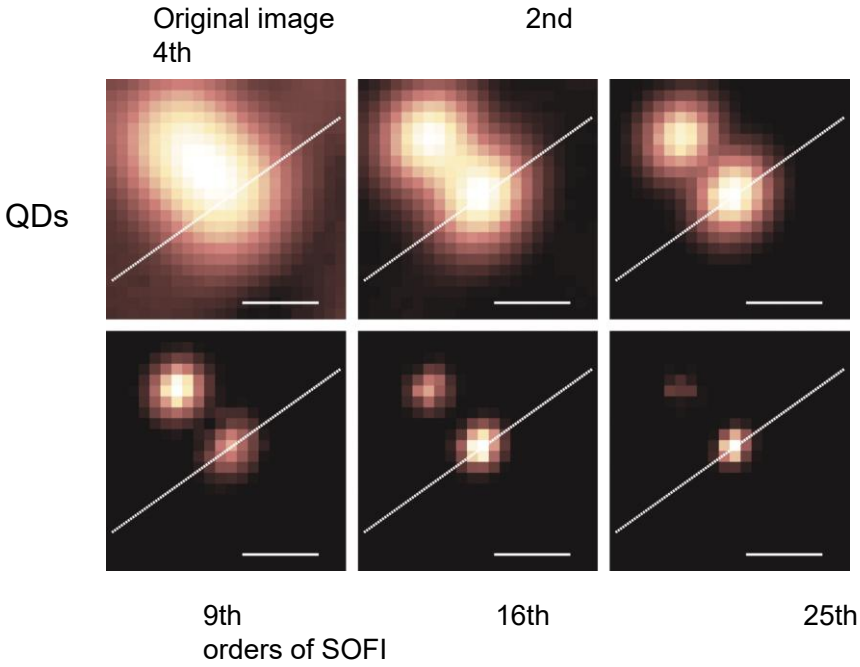
<u>Second-order correlation function</u>

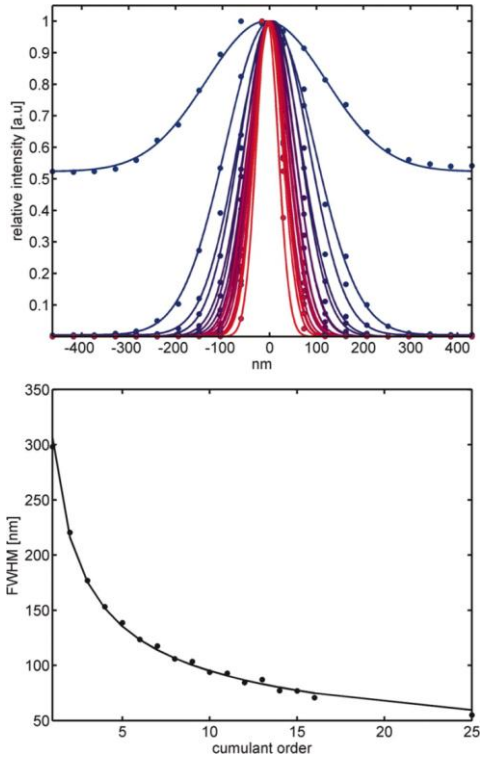
<u>Integral over correlation function</u>

Fast, background-free, 3D super-resolution optical fluctuation imaging (SOFI), Dertinger T. et al., PNAS 2009, 101/52:22287-22292

SOFI

- Increase of resolution by a factor of √n along all 3 dimensions
  - n^{th} order correlation function for *n-1* time lags
  - Computation time and memory requirements ~n^2



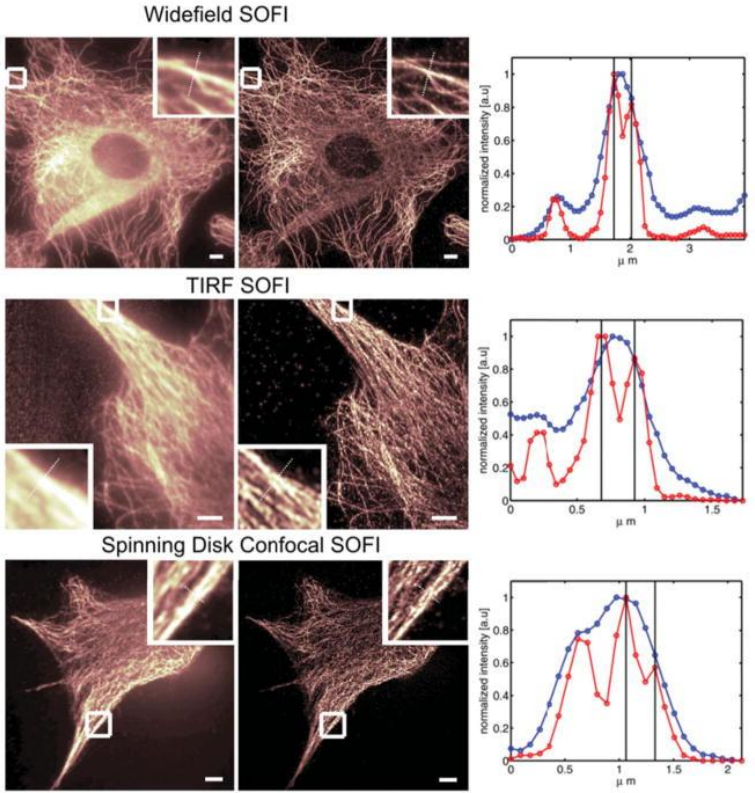


Fast, background-free, 3D super-resolution optical fluctuation imaging (SOFI), Dertinger T. et al., PNAS 2009, 101/52:22287-22292

SOFI

- Increase of resolution by √2 = 1.4 along all 3 dimensions, for 2nd order SOFI
  - *2D resolution of biological samples*

  can be ~80 nm (higher orders of SOFI)
- Works on all imaging platforms



- Tubulin network of NIH 3T3 fibroblasts, infrared QDs (2000 frames)

- The background removal in SOFI images proves especially useful for an optional, <u>subsequent image deconvolution</u>

Advances in superresolution optical fluctuation imaging (SOFI), Dertinger T. et al., Q Rev Biophys 2013, 46(2):210-221

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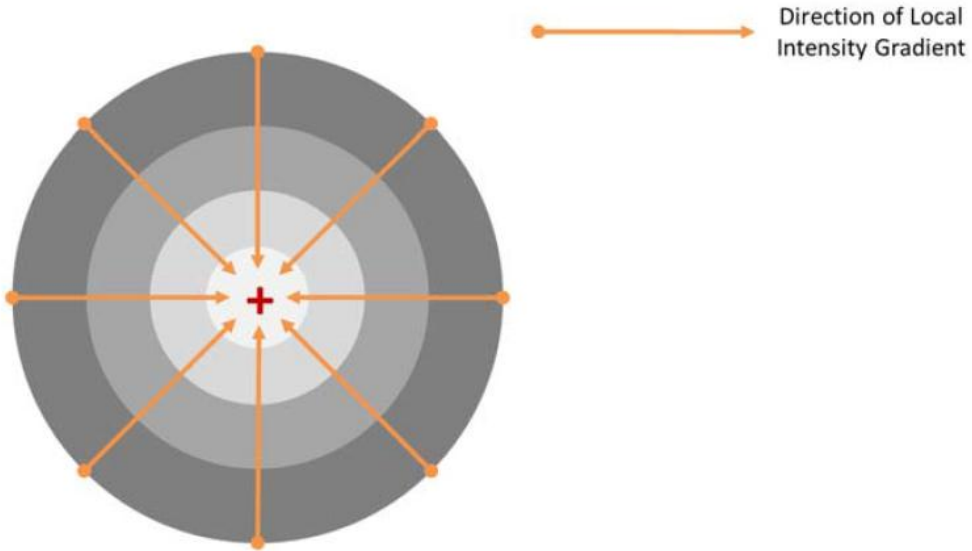
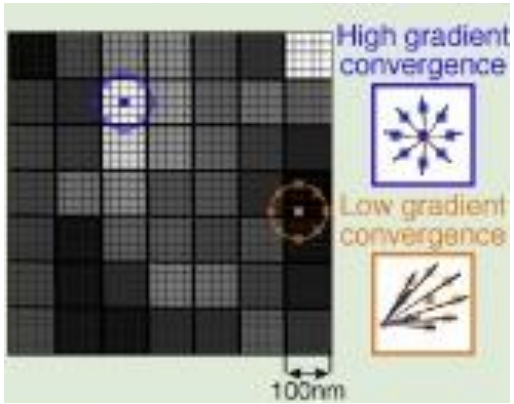
SRRF

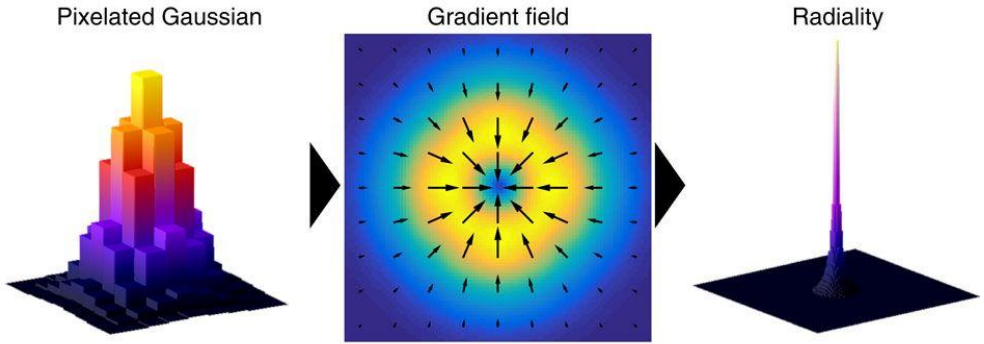
- **“Super-resolution radial fluctuations”**
  - Measures local radial symmetries in the image (intensity gradient vectors are measured for a ring of nearby surrounding pixels)



SRRF

- **“Super-resolution radial fluctuations”**
  - Measures local radial symmetries in the image (intensity gradient vectors are measured for a ring of nearby surrounding pixels)
  - Magnifies each pixel into subpixels





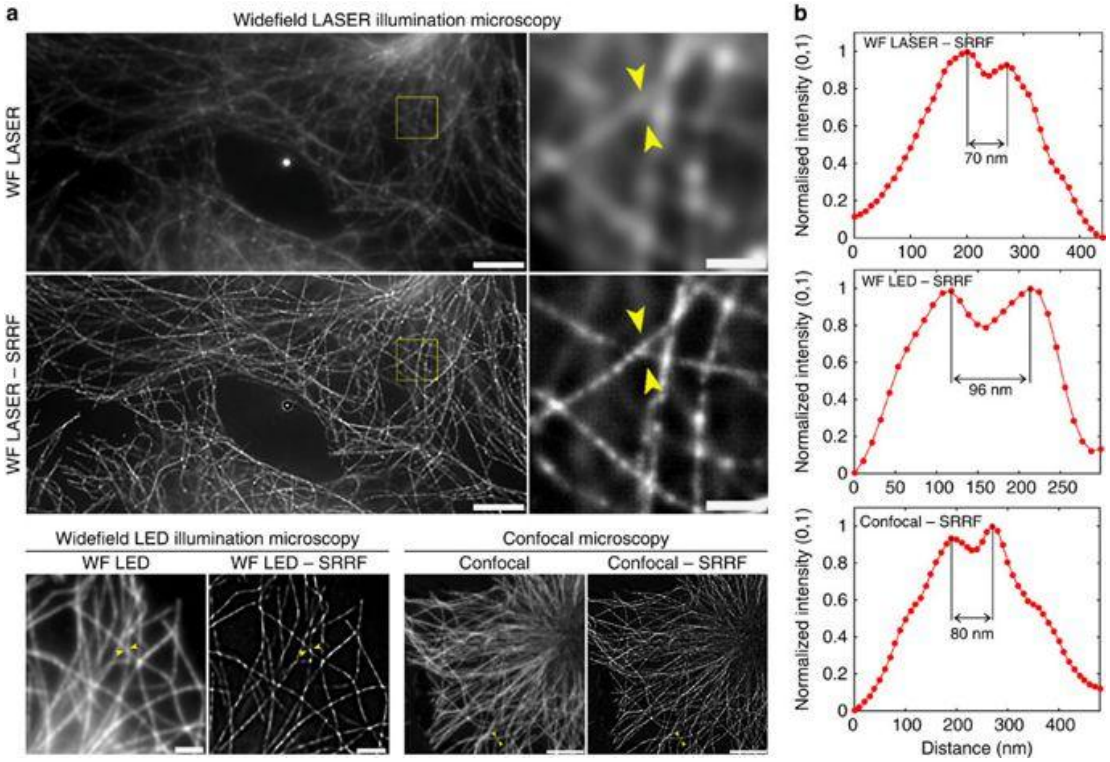
  - Radiality over time can be analysed to extract further information on the underlying positions of the fluorophores

    –> requires multiple images (50-100 frames)

SRRF: Universal live-cell super-resolution microscopy, Culley S. et al., Int J Biochem Cell Biol. 2018, 101:74-79

SRRF

Fixed sample,
Alexa Fluor 647 -
microtubules



Peak-to-peak
separations

Fast live-cell conventional fluorophore nanoscopy with ImageJ through super-resolution radial fluctuations, Gustafsson N. et al., Nature communications 2016, 7:12471

SRRF-Stream

- SRRF implementation on spinning disk microscope
  - 2-6 – fold lateral resolution improvement (50-150 nm)
  - Low excitation intensities (mW-W/cm^2)
  - Conventional fluorophores

  - *Real time computations*

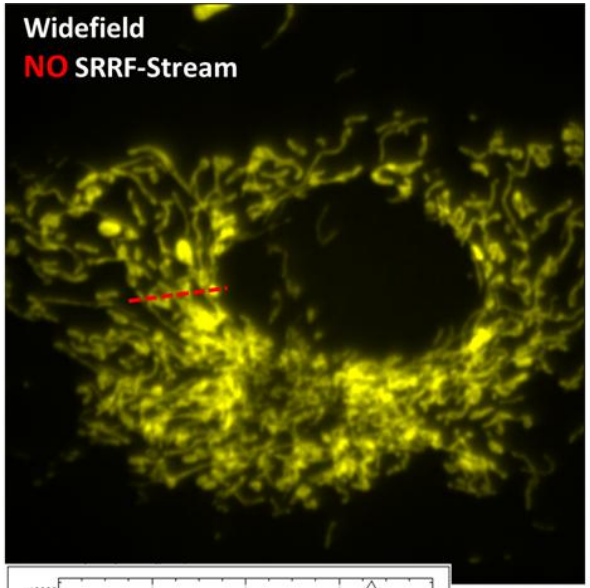
    30x faster than NanoJ-SRRF (ImageJ)

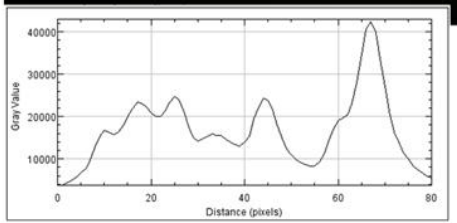
ANDOR
an Oxford Instruments company

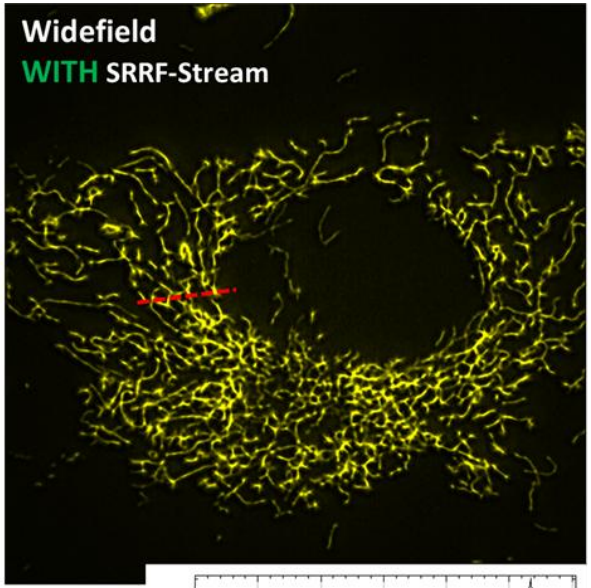
http://www.andor.com/microscopy-systems/dragonfly

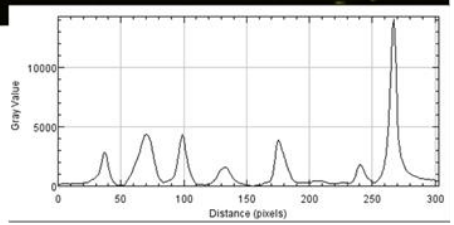
SRRF-Stream

BPAE cell,
100 frames





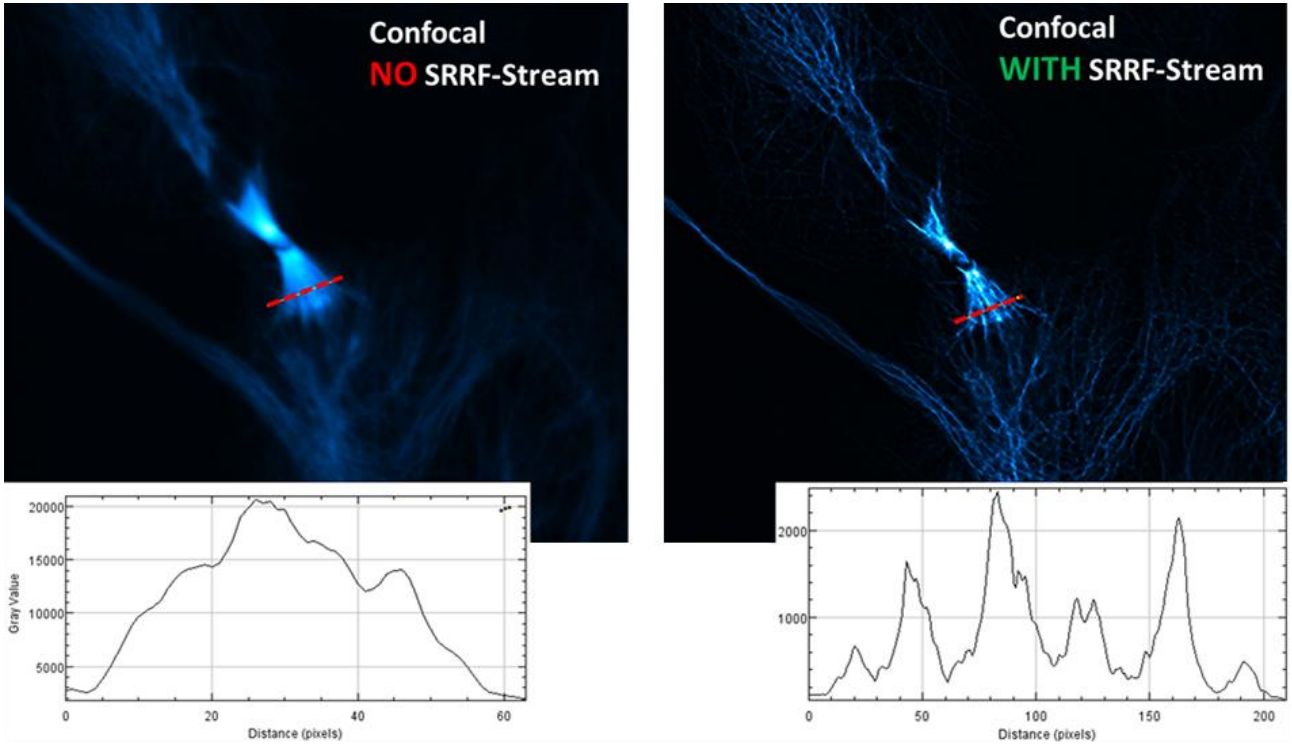




http://www.andor.com/microscopy-systems/dragonfly

SRRF-Stream

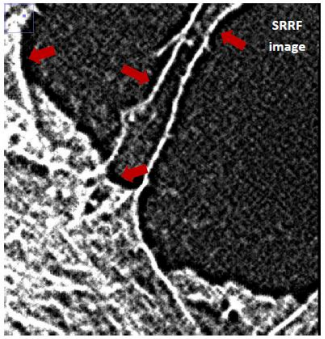
U2OS cells, tubulin,
100 frames



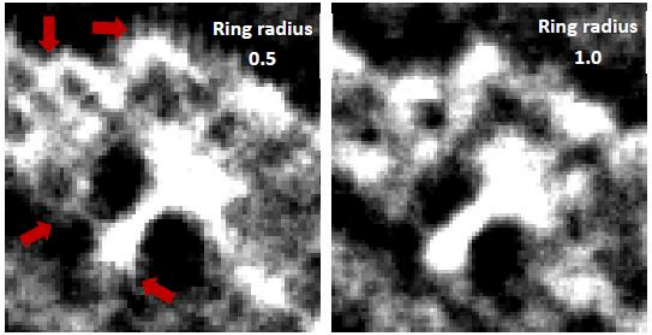
http://www.andor.com/microscopy-systems/dragonfly

Artifacts

- SRRF
  - ‘Shadow region’

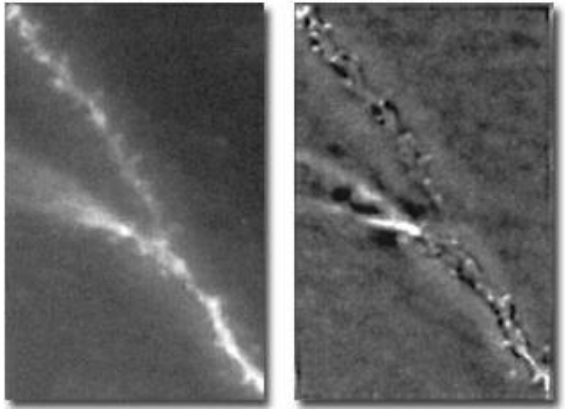


  - ‘Star-shaped’ (caused by under-sampling)



- Deconvolution
  - ‘Ringing’

(caused by discontinuities in signal or inappropriate PSF)



http://www.andor.com/microscopy-systems/dragonfly, https://svi.nl/RingingArtifacts

Artifacts

- How to avoid them
  - Examine the raw data
  - Start with default parameters
  - Compare the raw data with the computed image –> QC (e.g. NanoJ-SQUIRREL)
  - Use different algorithms or settings

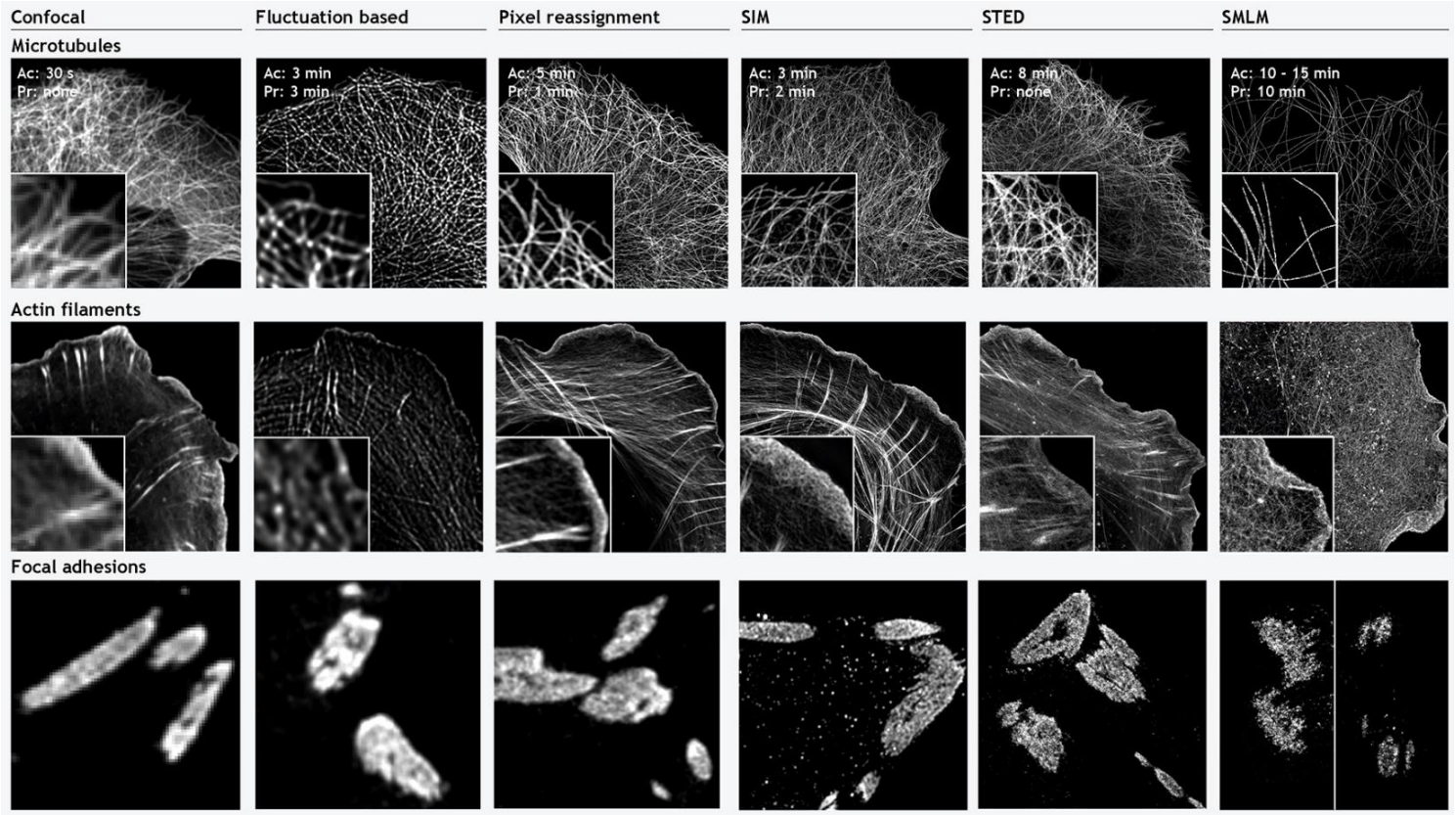
- How to compensate
  - Use image correction when available (imaging artifacts)
  - Filter-out artifacts

https://github.com/superresolusian/NanoJ-SQUIRREL

Comparison of methods

Method	Lateral resolution (at 488 nm)	Axial resolution at 488 nm)	Speed (512x512)	Platform
Lightning	120 nm	200 nm	40 fps	Confocal (LSCM)
AiryScan	120 nm	350 nm	4.7 fps	Confocal (LSCM)
NSPARC	100 nm	300 nm	10 fps	Confocal (LSCM)
IXplore SpinSR	120 nm	300 nm	200 fps	Confocal (spinning disk)
CSU-W1 SoRa	120 nm	300 nm	200 fps	Confocal (spinning disk)
SOFI	80-120 nm, higher possilble	300 nm, higher possible	~100 frames per 1 image	Any platform
SRRF-Stream	100 nm, higher possible	500 nm	~10 frames per 1 image	Any platform

Comparison of methods



https://journals.biologists.com/jcs/article/133/11/jcs240713/224727/The-cell-biologist-s-guide-to-super-resolution

Thank you for your attention!