

Bao-kai WANG, Martina BARBIERO, Qi-ming ZHANG, Min GU, 2019. Super-resolution optical microscope: principle, instrumentation, and application. *Frontiers of Information Technology & Electronic Engineering*, 20(5):608-630. <https://doi.org/10.1631/FITEE.1800449>

# Super-resolution optical microscope: principle, instrumentation, and application

**Key words:** Super-resolution; Imaging; Optical microscope

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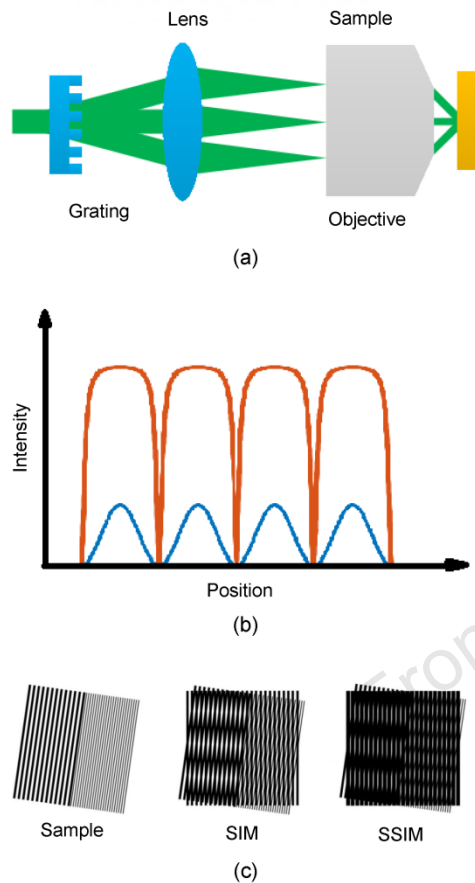


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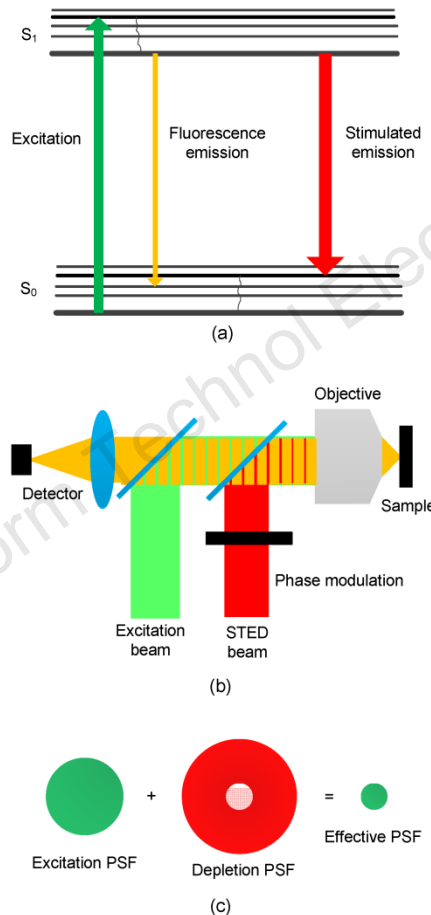
# Introduction

1. Over the past two decades, several fluorescence- and non-fluorescence-based optical microscopes have been developed to break the diffraction limited barrier.
2. In this review, the basic principles implemented in microscopy for super-resolution are described.
3. Furthermore, achievements and instrumentation for super-resolution are presented.
4. In addition to imaging, other applications that use super-resolution optical microscopes are discussed.

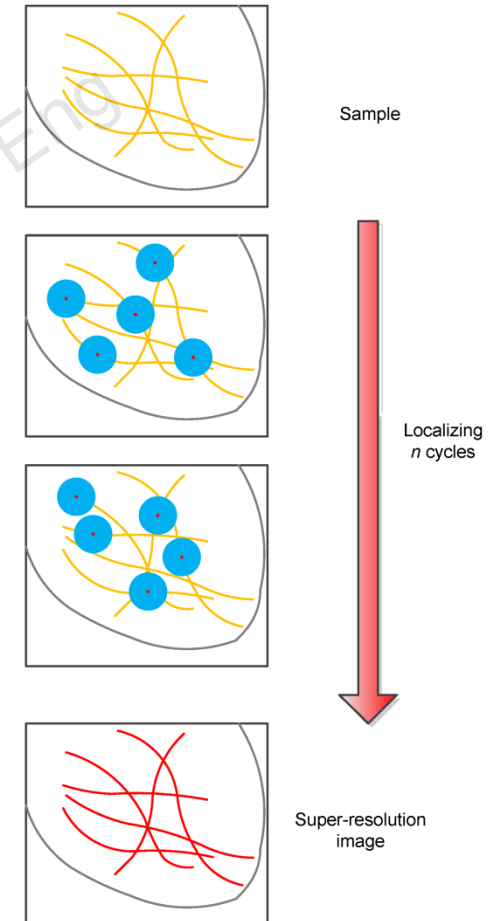
# Principle: fluorescence-based super-resolution



**Fig. 1 Principles of structured-illumination microscopy (SIM) and saturated structured-illumination microscopy (SSIM):** (a) structured illumination generated by a grating; (b) SIM excitation pattern (blue line) and SSIM excitation pattern (red line); (c) imaging the sample with SIM and SSIM

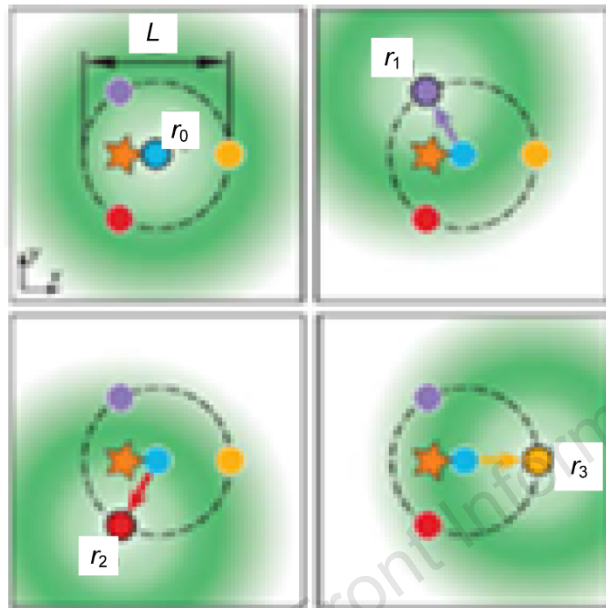


**Fig. 2 Principle of the stimulated emission depletion (STED) microscope:** (a) Jablonski diagram of the process of fluorescence emission and stimulated emission; (b) schematic of an STED microscope; (c) effective point spread function (PSF) generated

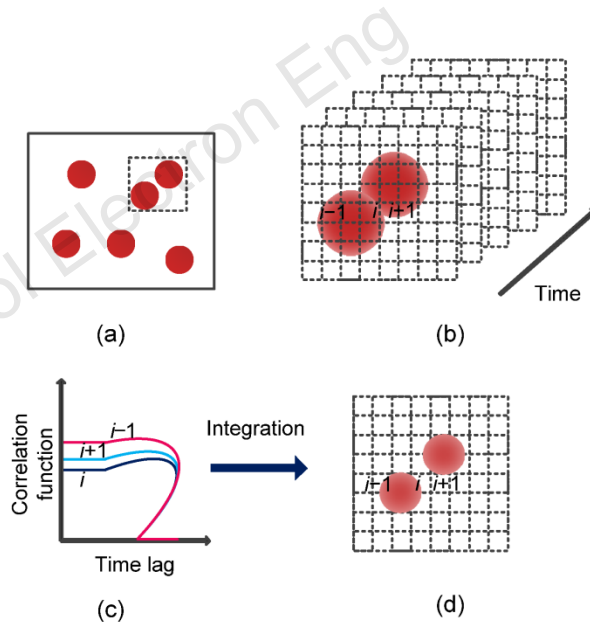


**Fig. 6 Principles of photoactivated localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM), blinking localization, 3B analysis, and ground state depletion microscopy followed by individual molecule return (GSDIM)**

# Principle: fluorescence-based super-resolution (Cont'd)

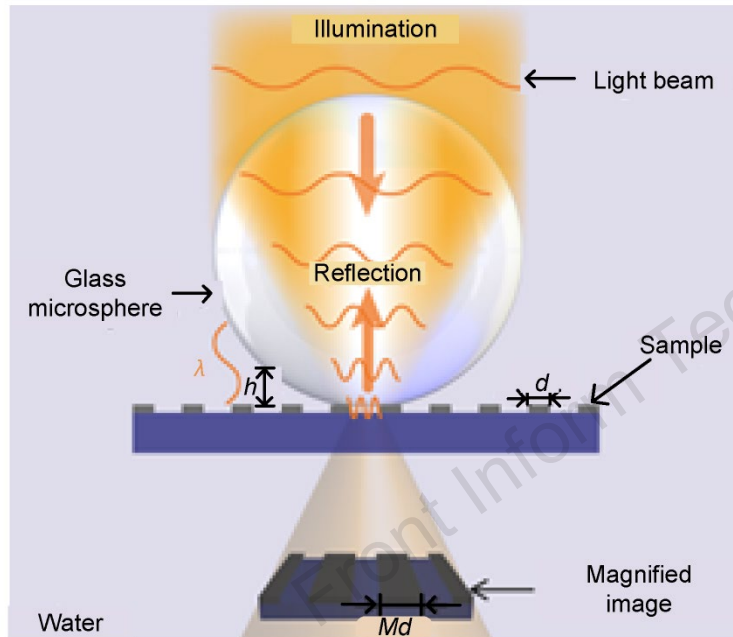


**Fig. 7 Localization of one molecule with four positions**  
The star represents the target emitter. The four points are the central positions of the doughnuts. The fluorescent signals from these four positions are used to localize the target emitter. Reprinted from Balzarotti et al. (2017), Copyright 2017, with permission from the American Association for the Advancement of Science



**Fig. 8 Principle of super-resolution optical fluctuation imaging (SOFI):** (a) wide-field imaging; (b) a movie recording two fluorescent probes in three neighboring pixels; (c) second-order correlation function calculated for each pixel; (d) SOFI intensity value for each pixel

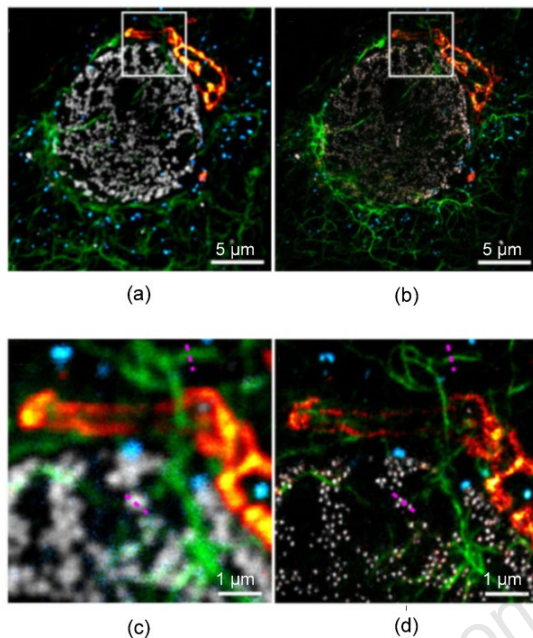
# Principle: non-fluorescence-based super-resolution



**Fig. 9 Super-resolution imaging with a dielectric microsphere**

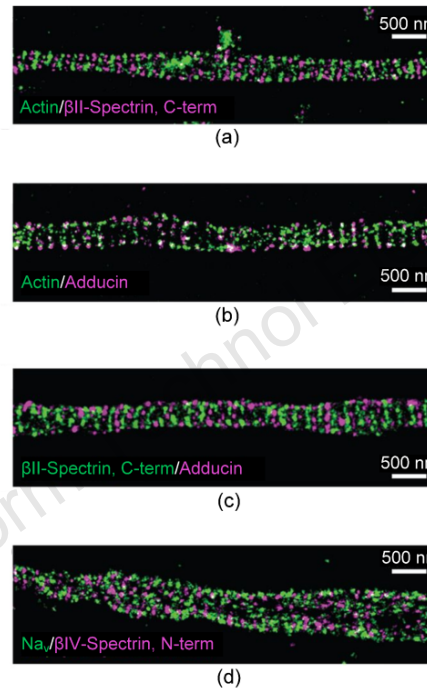
One dielectric microsphere is placed on a grating (line width is  $d$ ) and illuminated from the front. The reflected light from the grating allows detection of a magnified virtual image (magnification factor is  $M$ ). When the distance  $h$  between the microsphere and the grating is small enough (of order of the illumination wavelength  $\lambda$ ), the near-field evanescent wave carrying the high-frequency information begins propagating in the high refractive-index sphere, and is then collected by the microscope objective. Reprinted from Yang et al. (2016), Copyright 2016, with permission from American Chemical Society

# Highlighted achievement: multicolor imaging and live-cell imaging

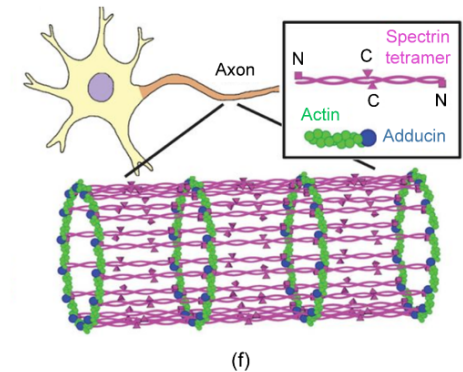
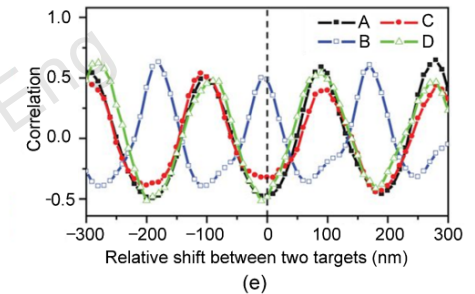


**Fig. 10** Four-color stimulated emission depletion (STED) imaging of a fixed cell: (a) confocal overlay; (b) STED overlay; (c) enlarged view of the region in the white square in (a); (d) enlarged view of the region in the white square in (b)

Excitation wavelength is 612 nm; STED wavelength is 775 nm. The fluorescence is detected by a hyperspectral detection design spanning a total range of 620–750 nm in four channels (blue: peroxisomes–Atto594; green: vimentin–Abberior Star635P; red hot: giantin–KK1441; grey: nuclear pores–CF680R) (Winter et al. (2017), licensed under CC BY 4.0). References to color refer to the online version of this figure



**Fig. 11** Two-color STORM imaging of actin, spectrin, adducin, and sodium channels: (a) two-color STORM image of actin (green) and  $\beta$ II-spectrin (magenta); (b) two-color STORM image of actin (green) and adducin (magenta); (c) two-color STORM image of  $\beta$ II-spectrin (green) and adducin (magenta); (d) two-color STORM image of sodium channels ( $\text{Na}_v$ , green) and  $\beta$ IV-spectrin (magenta); (e) spatial correlations between actin and the  $\beta$ II-spectrin C terminus [(A), black], between actin and adducin [(B), blue], between adducin and the  $\beta$ II-spectrin C terminus [(C), red], and between sodium channels and the  $\beta$ IV-spectrin N terminus [(D), green]; (f) a model for the cortical cytoskeleton in axons  $\beta$ II-spectrin is immunostained against its C-terminal region, which is situated at the center of the spectrin tetramer.  $\beta$ IV-spectrin is immunostained against its N-terminal region, which is situated at the two ends of the spectrin tetramer. Short actin filaments (green), capped by adducin (blue) at one end, form ringlike structures wrapping around the circumference of the axon. Spectrin tetramers (magenta) connect the adjacent actin/adducin rings along the axon, creating a quasi-1D lattice structure with a periodicity of about 180 to 190 nm. Reprinted from Xu et al. (2013), Copyright 2013, with permission from the American Association for the Advancement of Science. References to color refer to the online version of this figure



# Instrumentation

**Table 1 Specifications of microscopes based on structured-illumination microscopy (SIM)**

| Company | Product   | Lateral resolution (nm) | Axial resolution (nm) | Acquisition speed                          | Camera | Number of colors |
|---------|-----------|-------------------------|-----------------------|--------------------------------------------|--------|------------------|
| Nikon   | N-SIM     | 85–115                  | 300                   | 0.6–1.0 s/frame<br>(1–2 s for calculation) | EMCCD  | 5                |
| Zeiss   | ELYRA S.1 | 120                     | 300                   | 1.5–1.6 s/frame                            | EMCCD  | 2                |
| Olympus | SpinSR10  | 120                     | X                     | 5 ms/frame                                 | CMOS   | 2                |

**Table 2 Specifications of microscopes based on stimulated emission depletion (STED)**

| Company  | Product       | Lateral resolution (nm) | Axial resolution (nm) | STED wavelength (nm) | Excitation wavelength (nm) | Number of colors |
|----------|---------------|-------------------------|-----------------------|----------------------|----------------------------|------------------|
| Leica    | Leica TCS SP8 | 30–80                   | 130                   | 592, 660, 775        | Up to 8 wavelengths        | 5                |
| Abberior | 775 STED      | 20–30                   | Confocal              | 775                  | 594, 640                   | 2                |
| Abberior | 595 STED      | 25–40                   | Confocal              | 595                  | 488, 518                   | 2                |
| Abberior | Easy3D STED   | 75–100                  | 75–100                | 775                  | 594, 640                   | 3                |

# Instrumentation

**Table 3 Specifications of microscopes based on stochastic optical reconstruction microscopy (STORM), photoactivated localization microscopy (PALM), and ground state depletion microscopy followed by individual molecule return (GSDIM)**

| Company | Product         | Lateral resolution (nm) | Axial resolution (nm) | Maximum field of view ( $\mu\text{m}$ ) | Acquisition speed                                                     | Camera | Number of colors |
|---------|-----------------|-------------------------|-----------------------|-----------------------------------------|-----------------------------------------------------------------------|--------|------------------|
| Bruker  | Vutara 352      | 20                      | 50                    | 40×40                                   | Up to 3000 frames/s                                                   | sCMOS  | 2                |
| ONI     | Nanoimager      | 20                      | 50                    | 50×80                                   | 100 frames/s for full frame and 5 kHz with frame height cropped to 2% | sCMOS  | 2                |
| Nikon   | N-STORM 5.0     | 20                      | 50                    | 80×80                                   | Up to 500 Hz                                                          | sCMOS  | 3                |
| Zeiss   | ELYRA P.1       | 20                      | 50–80                 | 81.1×81.1                               | 30 frames/s for full frame and >100 frames/s in sub-array mode        | EMCCD  | 3                |
| Leica   | Leica SR GSD 3D | 20                      | 50                    | 40×40                                   | Over 1000 frames/s                                                    | sCMOS  | 3                |

**Table 4 Specifications of microscopes based on reversible saturable optically linear fluorescence transitions (RESOLFT)**

| Company  | Product                  | Lateral resolution (nm) | Optical system | Doughnut number | Number of colors |
|----------|--------------------------|-------------------------|----------------|-----------------|------------------|
| Abberior | RESOLFT                  | <70                     | Point scanning | 1               | 2                |
| Abberior | 2-color RESOLFT parallel | <80                     | Wide-field     | >100 000        | 2                |

# Other applications

1. Three-dimensional sub-diffraction optical laser lithography
2. Magnetic imaging

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# Summary

1. We reviewed the principles of various super-resolution microscopes, including fluorescence- and non-fluorescence-based methods.
2. We also present the status of instrumentation of super-resolution microscopes and compare the specifications of commercial products.
3. Inspired by super-resolution methods in STED microscopy, three SPIN methods were developed and enabled 3D nanofabrication with application in optical data storage. Super-resolution optical magnetic imaging is also very promising as a new tool to understand biological processes.