telescopes that look down?
Abbe limit: \( \delta x \approx \lambda/2 \)
An optical microscope can be thought of as a lens system that produces a magnified image of a point object to blur into a finite-sized spot in the image plane. However, even an arbitrarily high magnification does not translate into the ability to see infinitely small details. Instead, the resolution of light microscopy results from the diffraction of light when it propagates through a distance larger than the wavelength of the light in a medium with a refractive index-matched medium ($\lambda \approx \frac{\Delta z}{n}$, with $n$ being the refractive index of the medium). Because the loss of high-frequency spatial information determines the resolution of light microscopy, it becomes an obstacle for studying these structures in detail. It is, therefore, important to develop techniques that improve the spatial resolution of light microscopy without compromising its noninvasiveness and biomolecular specificity.

The point spread function of a common oil immersion objective with numerical aperture (NA) 1.40, showing the focal spot of 550 nm in the lateral and axial directions are 220 nm and 500 nm, respectively. For ordinary high NA objectives, the PSF is about 2–3 times as large as the lateral width for a Rayleigh criterion. When the PSF is about 2–3 times as large as the lateral width, the axial width of the PSF determines the resolution of the microscope: Two points closer than the full width at half-maximum (FWHM) of the PSF will be difficult to resolve because their images overlap at half-maximum. Hence, the diffraction of light prevents exact convolution. However, important to develop techniques that improve the spatial resolution of light microscopy does not affect most imaging at the organ or tissue level. However, when zooming into cells, where a large number of subcellular structures are smaller than the wavelength of light, it becomes an obstacle for producing the spatial resolution of light microscopy in optical microscopy results in optical reconstruction localization microscopy (ORLM), also called structured-illumination microscopy (SIM). SIM is based on the use of high-frequency spatial carrier patterns to subdivide the object into smaller subdomains, which are then imaged separately. The so-obtained information is then used to improve the spatial resolution of the resulting image. An example of SIM is stimulated emission depletion (STED) microscopy.

Microscopes can be used to visualize fine structures in a sample by providing a magnified image. The three-dimensional (3D) intensity distribution of the image of a point object is called the point spread function (PSF). The PSF of an optical system is defined as the spatial distribution of the intensity of the light that would be emitted by a point object, if it were to emit a monochromatic plane wave. The PSF is a function of the object position, the magnification, and the wavelength of the light. The PSF is a fundamental property of an optical system and it determines the resolution of the system. The resolution of an optical system is defined as the minimum separation between two point objects that can be resolved by the system. The resolution of an optical system is limited by the diffraction limit, which is determined by the wavelength of the light and the numerical aperture of the objective.

The point spread function of a common oil immersion objective with numerical aperture (NA) 1.40, showing the focal spot of 550 nm in the lateral and axial directions are 220 nm and 500 nm, respectively. For ordinary high NA objectives, the PSF is about 2–3 times as large as the lateral width for a Rayleigh criterion. When the PSF is about 2–3 times as large as the lateral width, the axial width of the PSF determines the resolution of the microscope: Two points closer than the full width at half-maximum (FWHM) of the PSF will be difficult to resolve because their images overlap at half-maximum. Hence, the diffraction of light prevents exact convolution. However, important to develop techniques that improve the spatial resolution of light microscopy does not affect most imaging at the organ or tissue level. However, when zooming into cells, where a large number of subcellular structures are smaller than the wavelength of light, it becomes an obstacle for producing the spatial resolution of light microscopy in optical microscopy results in optical reconstruction localization microscopy (ORLM), also called structured-illumination microscopy (SIM). SIM is based on the use of high-frequency spatial carrier patterns to subdivide the object into smaller subdomains, which are then imaged separately. The so-obtained information is then used to improve the spatial resolution of the resulting image. An example of SIM is stimulated emission depletion (STED) microscopy.

Super-Resolution Microscopy

(Huang 2009)
Structured illumination microscopy

(a) sample \times \text{Uniform Illumination} = \text{Emission} \rightarrow \text{Imaging (blurring)} \rightarrow \text{No info of structure}

(b) sample \times \text{Structured Illum.} = \text{Emission} \rightarrow \text{Moiré carries the info}

(Yamanaka 2014)
Structured illumination microscopy

Figure 7. Image formation in (a) conventional wide-field and (b) structured illumination microscopy. In structured illumination microscopy, high-frequency components in the sample can be imaged due to the frequency shift by the structured illumination; however, they are overlapped with lower frequency image components. Three overlapped components are extracted and reconstructed in the frequency domain. The inverse Fourier transform allows reconstruction of a fluorescence image with high spatial-frequency information.

Figure 6. Actin cytoskeleton observed by (a) conventional wide-field and (b) structured illumination microscopy. Reprinted from Ref. [41] by permission from John Wiley and Sons.

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Downloaded from
SIM: actin cytoskeleton

Figure 6. Actin cytoskeleton observed by (a) conventional wide-field and (b) structured illumination microscopy. Reprinted from Ref. [41] by permission from John Wiley and Sons.

(Yamanaka 2014)
Stimulated emission depletion microscopy

(Huang 2009)
Targeted switching and readout (STED, RESOLFT, SPEM and so on)

Stochastic switching and readout (PALM, STORM, GSDIM and so on)

(Hell 2009)
Figure 3 | dSTORM acquisition procedure exemplified for Alexa Fluor 647–labeled β-tubulin in a COS-7 cell. (a, b) A fluorescence image of the structure is measured at low excitation intensity (a) (< 0.1 kW cm⁻² see red line in b). In the next step, higher irradiation intensity is applied to transfer the majority of fluorophores into a nonfluorescent OFF state until a sufficiently low spot density is reached. Finally, a super-resolved image is reconstructed from all localizations. (b) Number of localizations per image frame (approximately ten, corresponding to ~0.1 spots per μm²) plotted against the frame number and time, respectively. The images were acquired with a frame rate of 885 Hz at an excitation intensity of 30 kW cm⁻² at 641 nm and additional irradiation at 488 nm (blue line) with 0–0.5 kW cm⁻². Direct excitation of the OFF state at 488 nm increases the number of fluorophores residing in the ON state and has to be carefully controlled. (c) Image reconstruction showing that a highly resolved image can already be reconstructed from 20,000 images corresponding to a total acquisition time of ~20 s. The structure cannot be fully resolved after analysis of ~2,000 frames. Σ, the number of localizations used to reconstruct the dSTORM image. Scale bars, 1 μm.

(de Linde 2011)
(Yamanaka 2014)
3D STORM

(Huang 2008)
Supplementary Methods

The whole-cell images also revealed two distinct types of mitochondrial network morphology: round mitochondrial segments in the center of the cell and horizontal segments of mitochondria appearing in adjacent directions along the dotted line in Supplementary Fig. 3c, showing the hollow shape of individual mitochondria. (Huang 2008)

Supplementary Fig. 3a

We were interested in examining how well the same cellular structures that appeared in 3D STORM images of the mitochondrial network could be resolved in 3D STED images of the same cell. To do this, we stacked these slices to form whole-cell 3D images that were 3 micrometers thick, we scanned the axial position of the objective to independently determine the position of the focal plane, and we measured the discrepancy between the focal planes of the adjacent 3D STED images. For 3D imaging of an aqueous sample using an oil immersion objective, the difference in refractive index between the imaging medium and the coverglass-oil-objective system must be considered. For example, the medium-coverglass interface, effectively increasing the apparent refractive index, causes the light rays to bend when passing across the medium. When the image depth is not too large, this effect can be corrected by rescaling, the localization precision also rescales with respect to unity when the focal plane is near the interface, and therefore the dispersion of the localizations is roughly constant. However, when moving away from the interface the value of c decreases, and the localization precision decreases. The correction causes the light rays no longer to converge perfectly, distorting the point spread function and making it asymmetric in the axial direction. Simultaneously, the light rays no longer converge perfectly, distorting the point spread function and making it asymmetric in the axial direction. The refractive index dispersion of the localizations is roughly constant.

For the purposes of this study, we chose to accept only localizations below the focal plane. Then we stacked these slices to form whole-cell 3D images that were 3 micrometers thick, we scanned the axial position of the objective to independently determine the position of the focal plane, and we measured the discrepancy between the focal planes of the adjacent 3D STED images. For 3D imaging of an aqueous sample using an oil immersion objective, the difference in refractive index between the imaging medium and the coverglass-oil-objective system must be considered. For example, the medium-coverglass interface, effectively increasing the apparent refractive index, causes the light rays to bend when passing across the medium. When the image depth is not too large, this effect can be corrected by rescaling, the localization precision also rescales with respect to unity when the focal plane is near the interface, and therefore the dispersion of the localizations is roughly constant. However, when moving away from the interface the value of c decreases, and the localization precision decreases. The correction causes the light rays no longer to converge perfectly, distorting the point spread function and making it asymmetric in the axial direction. The refractive index dispersion of the localizations is roughly constant.
single-molecule systems biology
(Lubeck & Cai 2012)
(Lubeck & Cai 2012)
Cells and genes in the expression profile of a single yeast cell.

(Lubeck & Cai 2012)