From microscopy to nanoscopy via visible light

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The resolution of conventional optical equipment is always restricted by the diffraction limit, and improving on this was previously considered improbable. Optical super-resolution imaging, which has recently experienced rapid growth and attracted increasing global interest, will result in applications in many domains, benefiting fields such as biology, medicine and material research. This review discusses the contributions of different researchers who identified the diffractive barrier and attempted to realize optical super-resolution. This is followed by a personal viewpoint of the development of optical nanoscopy in recent decades and the road towards the next generation of optical nanoscopy.

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INTRODUCTION

Optical microscopy is regarded as one of the most significant tools in the development of science and technology. Since its initial invention in the late sixteen century, the microscope has earned a reputation of enabling the visualization of objects (or fine structures) that are usually invisible to the naked eye, thus shaping various disciplines such as biology, medicine and materials science. The capability of this technique, to sketch the boundary of microstructures, measure surface morphology and localize specified molecule distributions in vivo, has driven modern research. Without optical microscopy, our knowledge of the ‘microworld’ would be severely impaired.

Apparently, the image quality, in particular the resolution, is the core determinant of the performance of a given microscope. It was not until nearly 300 years after its invention, however, that the diffractive nature of light and its potential influence on the resolution was seriously considered. After G. Airy described his ‘Airy disc’ in 1835—a typical diffraction pattern created by the light focused by a lens with a circular aperture—Abbe\(^1\) established himself as the well-recognized pioneer who explicitly described and formalized the diffractive limit for the first time. Although his landmark paper in 1873\(^1\) did not contain even a simple formula, Abbe clearly stated that the resolution of a general microscope was limited to approximately half of the working wavelength modified by the numerical aperture (NA) of the objective lens. Inspired by Abbe, the famous equation that defines the diffractive limit was eventually derived by von Helmholtz\(^2\) and later confirmed experimentally by Stephenson\(^3\) in 1877. The diffractive limit is generally expressed as:

\[
d = \frac{\lambda}{2n \sin \theta} = \frac{\Delta \lambda}{2NA}
\]

where \(\lambda\) is the working wavelength, \(n\) is the refractive index of the medium and \(\theta\) is the half angle over which the objective can gather light from the specimen. \(n\) and \(\sin \theta\) are collectively named the NA of the objective lens. In the axial direction, the resolution is even worse, and Equation (1) should be rewritten as:

\[
d_a = \frac{2\lambda}{NA} \quad (2)
\]

Extensive discussion of the relationship between diffraction and resolution continued after Abbe’s diffractive limit was illustrated. An urgent and practical problem to address, however, was determining an appropriate benchmark for resolution as it was particularly difficult to compare instruments with different point spread functions (PSFs). Of the many diverse resolution criterions that were proposed, the Rayleigh criterion (1874)\(^5\) and the full-width half-maximum (FWHM) method proposed by Houston (1927)\(^6\) gradually became the preferred choices. The Rayleigh criterion can be concisely written as follows: two-point sources are regarded as just resolved when the principal diffraction maximum of one image coincides with the first minimum of the other. In contrast, FWHM define the resolution as the difference between two points of the PSF at which the intensity is equal to the half the maximum PSF value. The FWHM method possesses an additional merit over the Rayleigh criterion in that it is more robust and therefore reliable for microscopes where the intensity distribution (of the focal spot) does not fall to zero, which was common in most practical applications due to background noise, an imperfect polarization state and/or lens aberrations.

Abbe’s work is enormously influential because it not only finds and defines the diffractive limit (mathematically), but also supplies a fundamental guideline for enhancement of the resolution and improvement of sample visualization. By shortening the working wavelength or increasing the NA of the system, the resolution of the microscope can be improved to some extent. Based on this principle, the prototype ultraviolet microscope was constructed by Köhler\(^7\) in 1904, while...
X-rays\(^8,9\) were later introduced into the micro-imaging system. The natural progression of this work was the realization of electron microscopy,\(^6\) where electrons with a critically shorter de Broglie wavelength are used for imaging. Improvements of the NA, on the other hand, began with the first oil immersion lens,\(^11\) which appeared even before Abbe’s period (although the original intention was to correct aberrations). By the 1990s, the technique of filling the objective space with a solid material of high refractive index (for example, the solid immersion lens (SIL)\(^12,13\)) yielded higher magnification and spatial resolution than other commercially available objective lenses. However, both strategies have the following drawbacks: (i) ultraviolet light is not always desirable owing to the high risk of irreversible damage to samples, especially biological cells (the enhanced scattering in the tissue also result in a much smaller penetration depth); and (ii) a high refractive index is always followed by absorption and chromatic dispersion, which has a significant impact on the image quality.

These dilemmas drove the search for other alternatives to satisfy the increasing demand for improved resolution. One inspired idea was to reshape the PSF of the focal spot to decrease the FWHM: the apodization method.\(^13\) Proposed in 1952 by Di Francia,\(^14\) this method had the advantage of sharpening the central maximum of the focal spot at the expense of larger side lobes. This defect made it unsuitable for wide-field optical microscopes, creating artifacts and blurring the image. Nevertheless, it indirectly resulted in the advent of confocal scanning laser microscopy,\(^15\) which was regarded as the most epochal affair in the mid of twentieth century. The introduction of a pinhole into the optical path fundamentally changed the imaging mode of conventional microscopy, where the entire image of the sample could now be obtained either by rastering the sample or by moving a laser beam or pinhole disk. The PSF of the confocal scanning laser microscopy system is given by:\(^16\)

\[
H_{\text{conf}}(t, w) = H_{\text{conv1}}(t, w) \cdot H_{\text{conv2}}(t, w) \otimes P(t, w)
\]

where \(H_{\text{conv1}}\) and \(H_{\text{conv2}}\) are the PSFs of illumination and detection, respectively; \(P\) is the aperture function of the pinhole; and \(t\) and \(w\) are the unit vectors in terms of optical coordinates. If the size of pinhole is infinitely small, Equation (3) can further be simplified as:\(^16\)

\[
H_{\text{conf}}(t, w) = H_{\text{conv1}}(t, w) \cdot H_{\text{conv2}}(t, w)
\]

In this way, the resolution of the confocal microscope can be approximately 1.4 times better than that of conventional microscopy in both the lateral and axial directions.\(^16\) In other words, the light emanating from the out-of-focus plane is blocked by the pinhole, and only the in-focus light can be detected. Hence, the resolution and contrast originally negated by out-of-focus information can be recovered using a confocal microscope. Confocal scanning laser microscopy, combined with fluorescence microscopy developed by Heimstaed\(^7\) and others years before, remains to this day indispensable as a powerful tool to image, localize and identify the target molecules as a powerful tool to image, localize and identify the target molecules, especially biological cells (the enhanced scattering in the tissue also result in a much smaller penetration depth). In other words, the subdiffraction-limited detail can be visualized once the evanescent wave is captured and projected to the far-field. Nevertheless, due to technical limits, an additional quarter century passed before far-field optical super-resolution had become a hot spot for instrumentation research.

In fact, researchers had long been aware of the theoretical basis of breaking the diffraction limit. The presence of evanescent waves was first postulated by Francia\(^14\) in 1942 and revealed experimentally in 1949. Compared with normal propagation waves, an evanescent wave is characterized as a near-field standing wave with an intensity that decays exponentially with distance from the boundary. The wave vector of evanescent waves has the form:

\[
K_e = k_{\parallel} + i k_{\perp}; |K_e|^2 = |k_{\parallel}|^2 + |k_{\perp}|^2 = \left( \frac{2\pi}{\lambda} \right)^2
\]

where \(k_{\parallel}\) and \(k_{\perp}\) are the wave vectors parallel and perpendicular to the boundary, respectively. It follows that the parallel component of the evanescent wave will be larger than for a general propagation wave and corresponds to the information of finer details. In other words, the subdiffraction-limited detail can be visualized once the evanescent wave is captured and projected to the far-field. Nevertheless, due to technical limits, an additional quarter century passed before far-field optical super-resolution was realized.

The first application that triggered the use of evanescent waves in microscopy was near-field scanning optical microscopy (NSOM)\(^25\) in 1972. Using a probe to scatter and collect the evanescent wave in the near-field and recover high spatial frequencies (Figure 1), NSOM could successfully resolve details with dimensions below the diffraction limit. This demonstrated the potential for molecule-scale spatial localization and imaging, and inspired other scientists to develop...
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NSOM was also limited by the size of the aperture (a result of a tradeoff
complex and slows down the imaging speed. The resolution of early
microscopes, the probe-detection method makes the whole system
resolution of the image captured by NSOM is superior to most other

(Far-field/near-field) and detection (propagation/evanescent signals) modes.

List of all the super-resolution nanoscopes and their corresponding resolutions. These techniques can generally be catalogued by their diverse illumination

Figure 2

Schematic of the NSOM. The resolution of the NSOM relies on two key
factors: the aperture size of the probe and the distance between the tip and
the sample. The probe can perform either as the source (for near-field illumination),
as the detector (for collection of the evanescent light scattered by the sample), or
both. NSOM, near-field scanning optical microscopy.

other probe detection techniques such as scanning tunneling microscopy (1982),\textsuperscript{26} and atom force microscopy (1986).\textsuperscript{27} Although the
resolution of the image captured by NSOM is superior to most other
microscopes, the probe-detection method makes the whole system complex and slows down the imaging speed. The resolution of early
NSOM was also limited by the size of the aperture (a result of a tradeoff between the resolution and signal-to-noise ratio), but this problem
was consequently solved by the introduction of a metal tip probe.\textsuperscript{28–30}

Evanescent waves were then used in the TIRF microscope as the illumination light. Although the TIRF microscope does not utilize the
full effectiveness of the evanescent wave for super-resolution, the chief
advantages of this development are a thin optical sectioning and a
lower background noise, both of which (in theory) rely on the decay
of the excitation intensity along the perpendicular direction.

The three decades following the development of NSOM and TIRF
have witnessed explosive advances in optical super-resolution with
visible light. Irrespective of the technical diversity, illumination and
detection are two basic modules that all nanoscopes should have,
thereby providing a simple and clear way to catalog the available
systems (Figure 2). The following sections will center on the discussion
of these solutions and the way in which they overcame the unfavorable
aspects of diffraction.

DETECTION OF THE EVANESCENT WAVE

Amplifying the evanescent waves before they decay to an undetectable
level is the core challenge for evanescent wave detection. In 2000,
Pendry\textsuperscript{31} published his epochal paper predicting a ‘perfect lens’ made
of a slab of material with negative refractive index. Pendry explicitly
derived formulae that led to the conclusion that such a perfect lens had
the power to focus all Fourier components of a two-dimensional (2D)
image (i.e., both propagation and evanescent waves could contribute
to the formation of images). The transition coefficients of the perfect
lens for both s- and p-polarized fields can be written as:

\[
T_s = \frac{2\varepsilon k_z}{\varepsilon k_z + k'_e z + \mu k_z} \exp(i k'_d d)
\]

\[
T_p = \frac{2\mu k_z}{\varepsilon k_z + k'_e z + \varepsilon k_z} \exp(i k'_d d)
\]

where \(d\) is the thickness of the slab; \(\varepsilon\) and \(\mu\) are the dielectric function
and the magnetic permeability, respectively; and \(k_z\) and \(k'_z\) are the wave
vectors of the beam in the vacuum and negative refractive index
material, respectively. A practical dilemma that limits the realization
of the perfect lens is a lack of natural negative refractive index material
for the visible scale. A number of scientists have attempted to create
artificial negative refractive index materials—the ‘metamaterial’, while
others have turned to alternatives. As indicated by Pendry himself, if

Figure 2

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the light is absolutely p-polarized, the dependence of the transition coefficient on \( \mu \) can be eliminated. Hence, it is possible to partially realize the transition by incorporating a p-polarized incident beam and a thin metallic film. This conjecture was experimentally confirmed in 2005 by Zhang and his colleagues, who used a silver superlens (Figure 3a) to successfully achieve subdiffraction-limited optical imaging. The evanescent wave was amplified in the thin metal film, thereby generating a subdiffraction image on the other side (Figure 3e), while the distance between the original object and the image was much larger than the general penetration depth of the evanescent field. Two years later, Zhang’s group further optimized their original implementation and projected the image to the far-field (Figure 3b). This far-field superlens (FSL) combined the original physical model with the theory of frequency shift and enabled a controllable fashion of frequency conversion (Figure 3f). The high frequency component, which carries subdiffraction information corresponding to the evanescent waves, can thus be shifted back to propagation mode. The conversion relationship can be expressed mathematically as:

\[
\mathbf{k} = \mathbf{k}_m \pm m\mathbf{k}_A
\]

where \( \mathbf{k}_m \) is the wave vector of the object; \( \mathbf{k}_A = 2\pi/A \); \( m \) is the diffraction order; and \( A \) is the grating period of the FSL. The geometrical structure of the FSL ensures that the transition of diffractive orders other than \( -1 \) will be eliminated so that ‘one-to-one’ conversion—essential for the unambiguous projection of subdiffraction details to the far-field—can be realized. Xiong et al. expanded the super-resolution capability of the FSL to two dimensions in the same year, using a modified, multilayer grating (Figure 3c). Yet the magnification of subdiffraction features into the far-field was still impossible until the hyperlens was demonstrated (Figure 3d). In the hyperlens, a sandwich-like half-cylindrical cavity that will magnify the object while the evanescent waves become propagation waves (a piece of artificial meta-material), is fabricated. In this strongly anisotropic meta-material, the wave vectors of the propagation waves gradually decrease so that subdiffraction information can be detected in air (Figure 3g).

Another option originated from the SIL, where the core device was a transparent dielectric sphere or hemisphere. The nanoscale lens in SIL-type implementation (nSIL) improves the resolution by 25% compared with regular (macroscopic) SIL. By adding an annular aperture to the nSIL, additional optimization of the resolution is achievable as the diffraction lobes are narrowed and subcritical rays are blocked—avoiding aberrations. To further enhance the resolution, in 2011, Wang et al. modified the geometrical shape of the nSIL to be a whole microsphere and illuminated it with white light (Figure 4a). The imaging procedure had changed to a virtual one that was capable of obtaining 50-nm resolution (Figure 4b and 4c). To further optimize the image contrast and expand the viewing field, one could immerse the microsphere in liquid or increase its refractive index. To avoid the potential influence due to the evaporation of the liquid, it would be necessary to change the surface hydrophilicity of the microsphere.

ILLUMINATION WITH AN EVANESCENT WAVE
Apart from collecting the evanescent portion of light scattered or irradiated by fine details, illuminating the sample with an evanescent wave can also lead to optical super-resolution. Compared with the methods mentioned in the previous section, illumination by evanescent light can be converted to a propagation wave by scattering (or other physical mechanisms), so that super-resolution details can be directly detected from the far-field. For metallic samples, one promising route involves surface plasmon polaritons (SPPs). As SPPs are shorter in wavelength than incident light, they will result in a local field enhancement phenomenon along the metal/dielectric interface. The wave vector of SPPs can be written as:

\[
\mathbf{k}_{\text{sp}} = \mathbf{k}_0 \left( \frac{\varepsilon_d \varepsilon_m}{\varepsilon_d + \varepsilon_m} \right)^{1/2} \approx \mathbf{k}_0
\]

where \( \mathbf{k}_0 \) is the wave vector of excited light in a vacuum; and \( \varepsilon_d \) and \( \varepsilon_m \) are the dielectric functions of the dielectric and metal, respectively.

**Figure 3** Schematic of (a) a superlens, (b) an FSL, (c) a 2D FSL and (d) the hyperlens proposed by Zhang’s group. (e–g) Theoretical comparison of the various optical lenses. Although all of these techniques originate from a similar principle, the technical details are actually quite different. In e, the superlens can only magnify the amplitude of evanescent waves. In f, FSL and 2D FSL extend beyond the conventional function by converting the frequencies to the propagation mode, so that super-resolution signals can readily be captured in the far-field. In g, the hyperlens is a piece of artificial meta-material, in which the wave vectors of evanescent waves reduce until they can propagate in free space. Figure reprinted with permission. a, Ref. 32, ©2005 AAAS; b, Ref. 34, ©2007 ACS; c, Ref. 36, ©2007 ACS; d, Ref. 37, ©2007 AAAS. 2D, two-dimensional; FSL, far-field superlens.
The use of SPPs on gold films for optical super-resolution imaging was demonstrated by Smolyaninov et al.,46,47 in 2005. In their studies, the authors deposited a glycerine microdroplet onto a gold film and used its surface boundary to obtain total internal reflection, thereby creating a magnifying parabolic mirror. The sample to be imaged was etched onto the gold film under the droplet, and a magnified image was formed and observed through an ordinary microscope from the far-field. Another SPP-based super-resolution method was proposed by Yuan et al.48 They used an optical vortex beam to excite SPPs and thus generate a structured illumination pattern (SIM). As the wavelength of SPPs is much smaller than that of excitation light, a super-resolution image can thus be generated by measuring the magnified Moiré patterns.

SPPs-based methods supply the conspicuous capability to image the details beyond the diffraction limit, but their limitation is also very obvious: the sample should be metallic or be coated by a metallic thin film. To get out of this dilemma and expand the application scope, humans also attempt to use other kinds of evanescent (surface) waves. A recent idea to achieve optical super-resolution uses near-field illumination by microfibers49 (Figure 5a) and offers a promising approach to surface tomography imaging without localized field enhancement. In 2013, Hao et al.49 noticed that when a sample with subdiffraction details is illuminated by an external evanescent field, the spatial frequencies are passively shifted to a propagation mode, thereby projecting super-resolution information to the far-field (Figure 5b). This method supplies an effective way to discern simple structures such as gratings or line/dot pairs—which contain a limited number of frequency components—and can result in a resolution of approximately 75-nm (Figure 5c). However, for complex 2D patterns, this passive frequency conversion will result in chaotic images that require the introduction of a series of recovery algorithms (besides the optical means).

**ABSOLUTE FAR-FIELD STRATEGIES**

Evanescent waves are so powerful that they fundamentally break the diffraction limit and have the potential to supply, in theory, infinitely small resolution. However, the confined propagation distance of evanescent waves makes them impractical for the non-invasive detection or illumination of internal details of the sample from the far-field. For biological specimens, which are characterized by high scattering and insulation, this challenge is especially important. As these cell samples are always stained by fluorescent chromophores to emphasize the area of interest before imaging, it would be useful if the natural properties of fluorophores could be applied. In this respect, the discovery of multiple nonlinear processes of fluorescence since the mid-twentieth century, such as fluorescence recovery after photobleaching50 and fluorescence resonance energy transfer,51 have gradually paved the way towards optical super-resolution capability.
All stories began with the invention of two-photon microscopy. Although the initial concept was described by Göppert-Mayer\textsuperscript{52} in 1931 and observed by Kaiser\textsuperscript{53} in 1961, Denk \textit{et al.}\textsuperscript{54} were the first to use this method for imaging in 1990. In two-photon microscopy, excitation and emission are confined to the focal volume, and the fluorescent intensity is proportional to $I$, so that the effective PSF can be shrunk. It should be noted that two-photon microscopy achieves optical super-resolution at the expense of doubling the wavelength of the excited light, therefore, the common excitation spectra lies in the infrared range (700–1000 nm). The actual resolution enhancement rate is very limited. Actually, the chief contribution of two-photon microscopy is that it allows imaging of very deep tissue, using a long excitation wavelength and reducing the sensitivity to scattering associated with excitation confinement. The two-photon microscope always provides clear imaging at 10 times the depth of other microscopes (Figure 6). A higher order of excitation was experimentally realized by Hell \textit{et al.}\textsuperscript{55} in 1996, which further resulted in the invention of three-photon microscope.

Although experimental confirmation of a three-photon absorption process was a remarkable achievement, Hell was remembered for another contribution to microscopy that had begun two years earlier. After its theoretical description in 1994,\textsuperscript{56} the demonstration of stimulated emission depletion microscopy (STED) in 1999\textsuperscript{57} shocked the world. STED restricts fluorescence to the subdiffraction region at known positions by depleting the fluorophores surrounding it (to their ground state). A peak-centered focal spot, nested by a doughnut beam, is scanned over the sample and an image is generated by reading out the signals from a point detector. STED can resolve much more sophisticated details (Figure 7a) than the confocal microscope (Figure 7b), for example, the published resolution record of 2.4 nm was just achieved in 2012.\textsuperscript{58} Dynamic imaging with a frame rate as high as 200 fps has been reported\textsuperscript{59} for this method, and it is also possible to realize multi-color imaging and 3D nanoscale reconstruction.\textsuperscript{60}

The theoretical resolution of a STED microscope can be expressed as:\textsuperscript{61}

$$\Delta x = \frac{\lambda}{2NA\sqrt{1 + \frac{I_{\text{STED}}}{I_0}}}$$ \hspace{1cm} (9)

where $I_0$ and $I_{\text{STED}}$ are the intensities of the transition saturation and the depletion beam, respectively. It is reasonable to expect improved resolution by increasing the incident intensity of the depletion beam (usually some hundreds of mW); however, this simplified model is based on excitation and depletion beams with appropriate polarizations and temporal alignment\textsuperscript{62,63}—in particular a perfect doughnut focal spot with a close-to-zero-intensity center. Presently, the most popular way to obtain a doughnut focal spot is based on phase modulation under high NA conditions. The strict requirement of the focal spot shape for STED applications has boosted research on the focusing properties of vectorial beams, while corresponding knowledge of focal spot manipulation has, in return, expanded the application of STED principles to other domains such as lithography\textsuperscript{64–67} and optical tweezers.\textsuperscript{68,69} Focal spot manipulation\textsuperscript{70,71} can also partially contribute to reducing the intensity of the depletion beam,\textsuperscript{69} although more straightforward and effective solutions include the ground state depletion, time-gated detection technique (g-STED)\textsuperscript{72,73} (Figure 7c and 7d) and frequency dependent detection using modulated excitation light.\textsuperscript{74}

Although originating from traditional confocal microscopy\textsuperscript{75} (Figure 8a and 8b), STED together with ground state depletion microscopy,\textsuperscript{76,77} saturated pattern excitation microscopy\textsuperscript{78} and dynamic saturation optical microscopy,\textsuperscript{79,80} is always classified as reversible saturable optical transition (RESOLFT) microscopy.\textsuperscript{81} (Figure 8c). The technical details of these methods may differ, but they share a similar foundation: that one of the several energy levels of a fluorophore can be expressed only in a subdiffraction region by a saturation (or switching) effect. In an ideal situation, it is possible to attain infinitely small resolution, whereas the practical performance will be influenced significantly by the noise,\textsuperscript{82} the size of fluorescent molecules, photobleaching and phototoxicity.

While RESOLFT microscopy and its prominent form (STED) were being demonstrated, Gustafsson had committed himself to developing
after the ideal focal plane, so that the axial position can be determined with nanometer accuracy. A similar idea that uses double-helix PSF to create the different arrangement was proposed by Pavani et al. in 2009. There were also some other techniques developed to achieve 3D imaging during the same period, such as biplane, and dual-objective PALM/STORM, and the highest resolution achieved until now is beyond 20 nm in both lateral and axial directions. Last but not least, super-resolution optical fluctuation imaging (SOFI, 2009) relies on higher-order statistical analysis of temporal fluctuations recorded in a movie. The mathematical treatment of SOFI can increase the resolution in all three dimensions by a factor of $\sqrt{N}$, where $N$ is the order of correlation function used in SOFI. By reweighting the PSF mathematically or introducing cross-correlation algorithm, it is possible to achieve a resolution enhancement that is proportional to $N$. Multi-color imaging can also be realized using this method. The main limitation of SOFI is the brightness scaling of the fluorescent image. As it is always necessary to enhance the brightness by increasing the excitation intensity, the fluorophores tend to be photobleached in a very short time, which may in turn influence the resolution.

The nonlinear process of fluorescence supplies the basis for absolute far-field nanoscopy, in which both the illumination and detection waves are propagation waves. However, it is especially important to find a more universal approach to image the non-fluorescent samples. One possible solution is based on explicitly designed microstructures, such as the concentric periodic groove aperture (bull’s eye). The implementation of this structure involved the principle of...
superoscillations,\textsuperscript{105,106} a phenomenon that was originally attributed to Aharonov and was popularized through Berry’s works (2006–2009). Superoscillation occurs when a globally band-limited signal contains local segments that oscillate faster than its fastest Fourier components. It hints at the possibility of transferring subdiffraction information to the far-field if the phenomenon can persist long enough. Since 2007, the superoscillation principle has been successfully applied to shrink the size of the focal spot using a bull’s eye (or similar microstructures).\textsuperscript{107–109} However, it is practically difficult to employ in imaging because a high-intensity halo would simultaneously surround the subdiffraction focal spot. In 2012, Rogers et al.\textsuperscript{110} proposed a method to overcome this barrier by using scanning imaging mode. Their superoscillation lens contained 25 rings and was optimized using the binary particle swarm optimization method,\textsuperscript{111} finally obtaining a lateral resolution of less than λ/6.

**SOME NEW POTENTIAL METHODS**

There are several other methods that have been shown to break the diffraction limit. For example, time-reversal imaging (2007)\textsuperscript{112} is based on the idea that light can propagate and refocus back to the point of the source (either practically or computationally), after it is detected (as if the time had been reversed). To realize this target, it is necessary to make the reflected light interfere with itself in such a way that it can precisely converge towards the source and convert the evanescent waves to propagation waves—through use of a metamaterial—before they decay. One such implementation is Maxwell’s fisheye,\textsuperscript{113} which has a spatially varying refractive index and is expected to cause all light rays emitted from one point to meet at a point exactly opposite it. If all light rays can propagate in this way, one can produce a mirror image of the object with unprecedented resolution. A practical device working on the microwave scale was manufactured by Ma\textsuperscript{114} in 2011; however, it is still an arduous task to realize both ideas using visible light. Whether it is feasible to fabricate a ‘perfect lens’ using materials with a positive refractive index is also arguable.\textsuperscript{115}

In the meantime, van Putten and his colleagues\textsuperscript{116} shared with the world an impressive and innovative idea for optical super-resolution. Before they published their results, all existing approaches had tried to accurately manipulate the propagation of each and every light beam to enhance the resolution of the image. However, van Putten et al. did the opposite. Rather than transferring light clearly to the target, they scattered light in all directions (Figure 10a). This high-index resolution enhancement by scattering (HIRES) benefited from expansion of the maximum cone of light by scattering, so that the NA of the system was larger, thus improving the resolution. The calibration of the phase modulation by the HIRES lens (Figure 10b) should be a priority, thereby enabling the recovery of subdiffraction details from the clutter directly captured by the system. It is notable that this method does not actually break the diffraction limit, although the associated increase of the NA indeed enhances the resolution by a factor of (approximately) three (Figure 10c and 10d). There is still a long way to go to win the battle of finding the perfect imaging approach, but van Putten’s work illustrates that the neat use of the computer instead of finding a new physical mechanism is also a valid route for achieving an improvement in resolution.

**CONCLUSION AND OUTLOOK**

The power that drives the development of optical super-resolution microscopy is that visualizing samples with such unprecedented resolution will yield miraculous discoveries. The applications of these methods have already begun, but the full realization of the initial dream is yet to come. Biologists are still waiting for in vivo 3D super-resolution reconstruction techniques, while material researchers have long been eager to image fine details below the surface. As mentioned in this review, some pioneers have gotten their feet in the door, and numerous state-of-the-art works have been proposed. These works originate from diverse physical mechanisms and have different applications. Although the diffraction barrier has fundamentally been broken, the drawbacks of present techniques are as evident as their merits. Apart from some specific exceptions such as microscope nanoscopy, most other techniques based on the evanescent field lack the capability to reflect color information, and are limited to imaging surfaces. The use of the fluorophors is limited to the phenomena originating from nonlinear effects of fluorescence, which in turn confines their applications. To activate these effects, the incident power of the laser is so strong that it may result in irreversible damage to the biological sample. Although infrared light would have a more moderate impact on cells, the resolution enhancement of 2/3-photon microscopy is counteracted by the comparatively long excitation wavelength. The viewing fields of nearly all super-resolution imaging methods are not sufficiently large. From this point of view, it seems that PALM/STORM and SOFI have some natural advantages, but the drawback, however, is that both approaches require a long time to process the data, which implies that in their present form, they are not reliable for live imaging. We are still waiting for a universal and economical method to obtain sub-10-nm resolution.

Furthermore, how present ideas can be combined to acquire multiple functions will also be a critical part of the process. The intensity distribution alone is too homogeneous to make a complete and rigorous analysis. More other functional information is always expected. While this procedure is in progress in the decades to follow, the introduction of new concepts, such as quantum imaging,\textsuperscript{117–119} and a non-diffractive beam,\textsuperscript{120,121} may inspire the next breakthrough.

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**Figure 10** Super-resolution scattering lens. (a) Principle of light coupling to high transversal \( k \) vectors in a high-index material. (b) Schematic of a HIRES lens that uses light scattering to achieve a high optical resolution. (c) The reference image taken with oil immersion objective lens (NA=1.49). (d) The image of the same area is taken with a scattering lens. Figure reprinted with permission: Ref. 116, ©2011 APS. HIRES, high-index resolution enhancement by scattering; NA, numerical aperture.
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