Advanced computational methods like the "steered molecular dynamics" described in the companion review by Sotomayor and Schulten (36) provide valuable tools for investigating how variations in chemical structure affect activation energy barriers and pathways governing bond strength.

In typical laboratory tests of single adhesion bonds, constructs of the ligand and receptor molecules are chemically immobilized on solid surfaces at very low surface densities, for example, a ligand to the face of an ultrasensitive force probe and its receptor to a solid target held by a feedback-stabilized piezo translator. The target is then repeatedly moved to/from contact to the probe face, during which time the deflection of the probe is tracked at high precision and multiplied by its "spring" constant \( k_s \) (pN/nm) to report the force history \( f(t) \). Bond events are identified by the cycles showing periods of probe stretch ending in precipitous recoil, as sketched in Fig. 2A.


49. Acting as a soft spring linked in series with the probe spring \( k_s \), the elastic response of the cell cortex \( k_c \) reduces the force rate \( f \) relative to the probe rate \( k_s f \).

The ratio \( c_I = f/(k_s f + k_c) \) provides a direct assay of the cell cortical stiffness, that is, \( k_c = k_s/(1 - c_I) \). It is important to note that different cell types possess very different levels of interfibrillar stiffness and that these levels often change with cell activation or spreading on a stiffer substrate.

50. As lipid material flows onto a tether, bilayer-spanning proteins (especially those that interact with the cytoskeleton) are expected to remain behind in the cell membrane. However, the acylated proteins bonded weakly to the bilayer could build up at the base of the tether, causing some to be expelled from the surface when approaching the tether-cell junction.


53. Supported by grants from the National Institutes of Health.
squaring reduces the spot by $\sim \sqrt{2}$, but in practice this effect is compromised by the finite detector size (10). A genuine quadratic dependence is provided by two-photon excitation, but exciting a fluorophore from its ground state $S_0$ to its fluorescent state $S_2$ requires photons of half the excitation energy and hence light of about $2\lambda$ in wavelength. The concomitant doubling of the spot is not compensated by the $\sim \sqrt{2}$ reduction, but even if it were, the barrier would just be shifted, not broken. These considerations are readily extended to $m$ photons (15). Another approach shrinks the spot by an elaborate phase pattern in the entrance pupil of the lens (16), but giant sidelobes leave it impractical. In my view, the failure of all these concepts to provide useful improvements reinforced the notion that, ultimately, the resolution of any far-field light microscope is still bound to $\Delta r = \lambda / 2n > 200 \text{ nm}$ and to merely $\Delta z = \lambda > 450 \text{ nm}$ along the optical axis.

The poorer axial resolution is particularly unfavorable for the 3D imaging of transparent samples, such as cells. The relation $\Delta z > \Delta r$ stems from the fact that a conventional lens may produce only a spherical cap of a wavefront of light ($I$). If the lens could produce a nearly complete spherical wavefront, the focal spot would be almost spherical too, because of symmetry. As a result, the $z$ resolution would be similar to its lateral counterpart (17, 18). The same consideration holds for fluorescence detection. The more complete the collected spherical wavefront is, the better the fluorescent marker can be located. Expanding the illumination or the detection wavefront is equivalent to increasing the aperture angle of the system. It is the key element in spot-scanning 4Pi microscopy (18) and wide-field 1PM (19), both of which provide a three- to sevenfold improved $z$ resolution, augmenting the 3D resolution of far-field fluorescence microscopy substantially.

To this end, 4Pi microscopy coherently adds the wavefronts of two large-angle lenses for excitation or detection, or both (Fig. 1B). The resulting main focal spot features $\Delta z \approx \lambda / 3n$, which is even slightly narrower than $\Delta r$ (20). However, because the enlarged wavefront is still not close enough to spherical ($\alpha \approx 68^\circ < 90^\circ$), the focal spot exhibits lobes above and below the focal plane. Consequently, practical 4Pi microscopy has relied mostly on two-photon excitation, which reduces the signal from the lobes due to the squaring effect (20). The remaining contributions are removed mathematically. Operating with oil, glycerol, and water-immersion lenses, two-photon 4Pi microscopy has delivered 3D images of fixed and live cells with 80- to 150-nm axial resolution (21). A compact beam-scanning 4Pi system recently revealed H2AX chromatin cluster formation in the nucleus (22), and a multispot version imaged organelles, such as the Golgi apparatus and mitochondria in live cells (20).

Fig. 1. Fluorescence nanoscopy schemes: single-point scanning (upper row) and parallelized versions (lower row). (A) Confocal microscopy. The excitation light wave (blue) formed by the lens to a spherical cap produces a 3D diffraction spot, generating fluorescence in the focal region. A pointlike detector (not shown) registers fluorescence mostly from the main maximum (shown in green), thus providing a slightly improved resolution over regular epifluorescence microscopy. Nevertheless, the confocal microscopy resolution is limited by diffraction to $>200 \text{ nm}$ in the focal plane ($x, y$) and to $>450 \text{ nm}$ along the optical ($z$) axis. (B) By combining the wavefront caps of two opposing lenses, 4Pi microscopy produces a narrower spot along the $z$ axis and hence an improved $z$ resolution of 80 to 150 nm. (C) A typical single-point scanning STED microscope uses a regularly focused excitation beam (blue) that is superimposed by a doughnut-shaped STED beam (orange) that instantly quenches excited molecules at the periphery of the excitation spot, thus confining fluorescence emission to the doughnut zero. Saturated quenching results in a fluorescent spot far below diffraction (green), here 20 nm, whose scanning across the sample yields a subdiffraction-resolution image. The spots represent measured data. (D) RESOLFT principle: A focal intensity distribution $I(x)$ featuring zeros that are $>\lambda / 2n$ apart confines either the bright state A (left) or the dark state B (right) through a saturable or switching transition, corresponding to a parallelized STED, GSD, or photoswitching approach (on the left) and to the SPEM concept (on the right). In both cases, imaging onto a camera causes the subdiffraction features created by the bright state A (left) or the dark state B (right) at the sample to be blurred on the camera by diffraction. Left: The blur can be dealt with by summing up each diffraction blob individually and allocating the signal to the pertinent coordinate of the zero in the sample space. The image is gained by translating the zeros across the sample and reading out the fluorescence for each coordinate step. Right: The same holds for SPEM in which the superresolved data are encoded in the narrow regions around the zeros in which the dark state B is deliberately established ("negative data set"). The image is obtained by mathematically converting the negative data set into a positive one. Both strategies rely on a targeted signal readout based on preset positions of the zeros, and both operate with fluorophore ensembles; $p_m(0) \leq 1$ defines the normalized probability of occurrence of A. Small boxes symbolize molecules making up the object (gray-shaded mountains). (E) PALM and STORM read out the fluorophore molecules stochastically; the molecules must be switchable. Weak illumination sparsely switches individual fluorophores to the bright state A so that they are further apart than $\lambda / 2n$. Detection of $N > 1$ photons enables the centroid calculation of the diffraction blob of individual fluorophores on the camera, and hence assembling an image with resolution depending on $N$. Concepts (C) to (E) are not diffraction-limited, meaning that they can resolve similar molecules at nanometer distances. The STED, PALM, SPEM, and RESOLFT recording process is sketched in movies S1 to S4.
By providing 6° more angle than their predecessors, the most recent field-corrected oil-immersion lenses (\( \alpha = 74° \)) have now also enabled bright dual-color 4Pi recordings with standard single-photon excitation \((23, 24)\). Applying two-photon excitation with such a lens pair yields a solitary central spot of \( \Delta r \sim \lambda/3n \) and hence an all-physical z-resolution improvement (Fig. 4A) \((25)\).

In 4FM, the aperture enhancement is implemented just for the detection \((26)\) while the whole field of view is illuminated with a set of plane-parallel standing waves. Single-photon excitation provides a bright signal, but the flat standing-wave illumination gives rise to larger lobes requiring a more elaborate computational removal \((20)\). The future implementation of 74° lenses and of special illumination schemes should render 4FM an interesting alternative to 4Pi imaging. In any case, the difference made by adding 6° to \( \alpha \) underscores that in both concepts, the essential element is the enlargement of the aperture angle of the system \((27)\). For the time being, combining the spherical wavefront caps of opposing lenses provides the smallest diffraction spot in the far-field. As a generic development, it may well augment the axial resolution of other light microscopy contrast modes in the future. Nonetheless, it does not break the diffraction barrier but rather pushes diffraction to its limits.

### Breaking the Diffraction Barrier

Discriminating features that are spectrally dispersive is not challenged by diffraction. Likewise, Abbe’s barrier does not prevent finding out the coordinate of a molecule with arbitrary precision, e.g., of 1 nm \((28)\), if there is no other similar marker molecule within \( \lambda/2n \) distance. Breaking Abbe’s barrier is about discriminating an arbitrary number of densely packed and similarly labeled features within any distance \( \lambda/2n \). This is possible if the features can be recorded sequentially: for example, by successively transferring the markers of each feature to a signal-giving “bright” state A, while keeping the other markers in a state B that is “dark” \((29, 30)\). Reading out the bright ones allows assembly of a subdiffraction image, provided that one knows their coordinate (Fig. 2).

The most direct way to determine the coordinate of the bright molecules is to define their location: \( r_i \). This is possible by applying an optical transition A→B that would send all fluorophores to the dark state B except for those that happen to be at \( r_i \) \((29)\). Such a transition can be realized by means of a light-intensity distribution \( I(r) \) featuring a zero at \( r_i \). Driving A→B, this intensity \( I(r) \) must produce an increase \( k_{\text{on}}(r) = \sigma(r) \) that outperforms competing spontaneous rates basically everywhere except at \( r_i \). The competing spontaneous rates are given by the inverse of the lifetimes \( \tau_{\text{A,B}} \) of the states A and B, respectively, and \( \sigma \) denotes the optical cross-section of the transition. Therefore, applying \( I(r) = (\sigma\tau_{\text{A,B}})^{-1} \) fulfills this condition and not be moved further than \( \lambda/2n \), because at \( -\lambda_1 + \lambda/2n \), another zero can resolve the adjacent diffraction zone \((29)\); that is, the process can be parallelized (Fig. 1D).

Time-sequential readout from within the diffraction zone at defined coordinates is a hallmark of stimulated emission depletion (STED) \((7)\) and ground state depletion (GSD) microscopy \((8)\), and of other concepts exploiting reversibly saturable or photoswitchable transitions A→B \((29)\), such as saturated pattern excitation microscopy \( \text{SPEM} \) \((32, 33)\). These approaches have been generalized under the acronym RESOLFT \((34)\), which stands for reversibly saturable optically linear fluorescence transitions. With \( I_{\text{max}} \) denoting the intensity bordering the zero, their practical resolution is well approximated by

\[
\Delta r \approx \frac{\lambda}{2\pi\sigma \sqrt{1 + I_{\text{max}}/I_s}}
\]

which differs from Abbe’s equation in that \( I_{\text{max}}/I_s \rightarrow \infty \) implies “infinite” resolution, i.e., down to a molecule \((7, 29, 31, 35)\). The square-root factor stems from the parabolic approximation of ordinary intensity zeros in space. Fluorophores within \( \Delta r \) remain indiscernible because they still are simultaneously recorded. Although it can resolve single molecules \((35)\), the RESOLFT concept principally operates with molecular ensembles and with state population probabilities \( \Delta r \) and the average number of simultaneously recorded fluorophores can be tuned through \( I_{\text{max}}/I_s \). The concept has been extended to also exploit the dynamic equilibration of the two states \((36)\). Ultimately, the resolution is determined by the actual choice of A and B, which can be basic electronic states, such as the S0 and the S1, or “chemical” states such as conformational or binding states of the marker \((29–31)\).

STED microscopy, which can be regarded as the first concept of the RESOLFT type, uses the most elementary states possible: the S1 as A and the S0 as B (Fig. 3). Most implementations of this concept have so far used a focused excitation beam and a red-shifted, doughnut-shaped “STED beam” for quenching excited fluorophores by stimulated emission \((31–33)\). The few stimulated photons are discarded, as is the stimulating beam. To confine the fluorescence to zero of the doughnut, the quenching rate outperforms the spontaneous decay of the S1 given by its inverse lifetime \( \tau_{\text{S1,S0}} \approx 10^{-9} \) s. With \( \sigma = 10^{-16} \text{cm}^2 \),

\[
I_s = \sigma \tau_{\text{S1,S0}} \text{ typically amounts to } \approx 3 \times 10^{25} \text{ photons/cm}^2 \text{ s}, \text{ i.e., } \approx 10 \text{ MW/cm}^2. \text{ Applying } I_{\text{max}} > I_s \text{ yields subdiffraction fluorescent spots.}
\]

Translating the zero in any direction sequentially registers the signal from subdiffraction features, thus yielding subdiffraction images (Fig. 4B).

The zero need not be formed by a doughnut, but could also be one or many grooves (Fig. 1D) \((35)\) or even planes \((37)\). By using a focal zone with a strong quenching peak above and beneath the focal plane (Fig. 1C), STED initially attained \( \Delta r \approx 100 \text{ nm} \) with a single lens \((38)\), but in combination with a 4Pi system producing a central zero, \( \Delta r = 33 \text{ to 60 nm} \) was possible \((37)\). Setting the current benchmark, these STED-4Pi combinations are likely to push the \( z \) resolution to <10 nm. A similar resolution \( \Delta z = 16 \text{ nm} \) was obtained in the focal plane \((35)\) with single molecules as test objects, showing that \( -\lambda/45 \) is possible in the far-field. Realizing such lateral resolution in immunofluorescence imaging has

---

**Fig. 2.** Targeted versus stochastic time-sequential readout of fluorophore markers of a nanostructured object within the diffraction zone whose lower bound is given by \( \lambda/2n \). A and B denote a bright and a dark state, respectively. In the targeted readout mode, one of the two states (here A) is established at a subdiffraction-sized spot at the position of a zero to read out an unknown number of fluorophore molecules. The image is assembled by deliberate translation of the zero. The zero can also be a groove. In the stochastic readout mode, a single switchable fluorophore from a random position within the diffraction zone is switched to a stable state, whereas other molecules remain in B. The coordinate is calculated from the centroid of the diffraction fluorescence spot measured by a pixelated detector. The coordinate pops up stochastically depending on where the interrogated marker molecule is located.
initially been hampered by photobleaching, but allowing fluorophore dark states to relax enabled $\Delta r = 20$ to 30 nm (Fig. 4C) (39).

Meanwhile, the nanoscale resolution provided by STED has tackled cell biology problems. For example, STED resolved synaptotagmin I from individual synaptic vesicles, showing that this protein forms isolated clusters upon vesicle fusion (40). STED also revealed the ringlike structure of the protein bruchpilot at synaptic active zones in the *Drosophila* neuromuscular junction (41). Further applications included visualizing the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) protein syntaxin (42), the nuclear protein SC35 (39), and the nicotinic acetylcholine receptor (43). STED has been extended to dual-color recording (44), enabling colocalized imaging on the nanoscale. Furthermore, it has revealed the spatial order of self-assembled colloidal particles (45) (Fig. 4B).

Another application outside biology is to create nanosized spots of excited molecules that serve as the starting point for photochemical reactions (46).

Although STED and confocal microscopy are easily combined in the same setup, STED is not an extension of the latter, because it does not rely on a point detector. Sharp discrimination of the fluorescence signal would allow the detector to be placed right at the sample. Specifically, the resolution of a STED microscope is determined solely by the STED beam. For these reasons, parallelized STED microscopy will be possible with arrays of doughnuts or lines (Fig. 1D). Because stimulated emission is a single-photon event with a $\sigma$ magnitude similar to that of absorption, the light-source requirements for STED are different from those for $m$-photon microscopy (47). Subnanosecond, but not femtosecond, pulses render STED effective, leaving laser diodes and photonic crystal fibers as the light sources of the future. Although the use of some fluorophores will be precluded by bleaching, suitable dyes are found in each part of the spectrum.

The $S_0$ and the $S_1$ states are not the only ones that can be exploited in this way (Fig. 3). GSD microscopy ($\alpha$) breaks the barrier at ~100 nm, because it elects the metastable triplet state $T_1$ with a lifetime $T_1 = 10^3$ to $10^6$ ns as the dark state B and the singlet system ($S_0$ plus $S_1$) as A (Fig. 3). Probing A is performed at the same $\lambda$ as its depletion, i.e., by pumping the dye to B. With a continuous-wave intensity of ~100 kW/cm², GSD has recently imaged protein clusters on the plasma membrane of fixed cells with $\Delta r = 50$- to 90-nm resolution (48). Although challenged by photobleaching, the ubiquity of metastable dark states in fluorophores encourages the exploration of this concept.

Depleting the $S_0$ state (now B) by populating the $S_1$ state (now A), SPEM, also known as saturated structured illumination microscopy (SSIM), differs from GSD in that ultrasharp “dark” regions of state B are created with steeply decreasing $\gamma$ dependence, i.e., by pumping the dye to B. With a continuous wave intensity of ~100 kW/cm², SPEM can also image isolated clusters of fluorophores on the plasma membrane of fixed cells with $\Delta r = 50$- to 90-nm resolution (48). Although challenged by photobleaching, the ubiquity of metastable dark states in fluorophores encourages the exploration of this concept.

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that are >λ/2n apart from each other and reading out the data with a camera. Initial realizations (33) displayed a lateral resolution of 50 nm with beads (after the required computation), thus also demonstrating the potential of line-shaped, quasi-widefield parallelized recording also for other A, B pairs.

The ultimate saturable transition is a photo-switch (29–31), because the absence of spontaneous intermediate states implies I→0 and hence a huge I_{max}/I_0 at low I_{max}. Photoswitching between isomerization or binding states, of which only A yields fluorescence, is found in reversibly photoactivatable relatives of the green fluorescent protein, such as asFP595 (50) and dronpa (51), and in photochromic synthetic compounds. The signal is gained by repeated excitation to a transient fluorescent state A→A*. Initial experiments with asFP595 evidenced breaking Abbe’s barrier by cis-trans photoisomerization with ultralow I_{max} = 10 W/cm² (34); similar results were obtained with switchable organic fluorophores (52). Selecting long-lived chemical states A and B highlighted that subdiffraction resolution is possible at ultralow intensities and indicated the potential of both protein and dye photoswitching for breaking Abbe’s barrier (29–31). Conversely, these experiments also revealed the Achilles’ heel of any concept using reversible saturable transitions, which is the finite number of cycles possible between A and B. However, cycling is required while reading out molecular ensembles from targeted coordinates with diffracted beams (Fig. 2). The reason is that ensuring state A at a chosen coordinate means that nearby molecules must be switched to B.

This problem is avoided in photoactivatable localization microscopy (PALM) (12, 14) and stochastic optical reconstruction microscopy (STORM) (13), in which single molecules are read out from random coordinates. To this end, a single molecule is switched on or activated (B→A) such that the next activated one normally is further apart than >λ/2n; it is then repeatedly excited (A→A*) to render N photons forming a magnified diffraction spot on a camera. Switching off adjacent molecules is not needed because they are off (in B) already. Knowing that only a single molecule is in A allows the calculation of its coordinate from the centroid of the spot with precision λ/2n sin α√N). The last step is to switch off the registered molecule, or at least confirm that it is off, so that another one can be switched on and read out. Thus, the image is assembled molecule by molecule by means of a single switching cycle B→A→B′ per molecule.

PALM switches off by bleaching, implying that B may be different from B′, greatly expanding the range of useful compounds (12), whereas in STORM B = B′ (13). PALM images of thin cryosections of lysosomal transmembrane protein in a mammalian cell displayed a resolution of <25 nm (Fig. 4D). Both approaches require the adaptation of the intensity to the molecular concentration, and in both methods the resolution varies with the brightness of the molecules that are chosen to represent the object in the image. So far PALM involved recording times of hours, but with potentially 10^3 to 10^4 recorded photons per 1 ms, >1000 fluorophores from the diffraction zone could be recorded in a second with 10-nm precision in 2D (14). Recording with a camera provides a large field of view while impeding the imperative background rejection. For that reason, PALM was initially demonstrated with <100-nm thin sections and with a total internal reflection (TIRF) 2D recording scheme (12). The blur associated with defocusing single molecules should help expand the method to 3D imaging and, once background is dealt with, to also live cells.

Nanolocalization of individual fluorophores was extensively pursued in the past (28, 53). Tracing the bleaching events in fluorophore clusters resolved individual molecules at nanometer distances (34). Likewise, individual quantum dots could be separated by disentangling their stochastic excursions to dark states (55). Indeed, separating and localizing individual fluorophores differing in their spectra (56) has been realized several times since it was proposed (57). It has also been known that any process allowing the allocation of N detected photons to the same point in the sample (out of the statistical evaluation of a photon stream) improves the resolution in far-field microscopy and, in fact, gives “infinite” resolution for N→∞ (58). However, all these precursors did not specify a definite molecular mechanism that would have enabled the sequential readout of an arbitrarily large number of fluorophores, such as the photoswitching between two states.

Most recently, nonswitching fluorophores were also used to form a B→A→B′ cycle by using a specific binding to the target cellular structure B→A and subsequent bleaching A→B′, yielding images (59). Complementary to the ensemble concepts, the single-molecule approaches expand the potential of nanoscale imaging. As PALM and STORM obtain their resolution in part computationally, images should be compared with those of the all-physical methods after a deconvolution. The resolution of the latter can be further increased by recording with a pixilated detector, so that both the
knowledge of the preset coordinates and those from the detector could be synergistically exploited. Nonetheless, single-molecule bottom-up concepts will develop their strength at small scales and with background-free samples, whereas top-down approaches, such as STED, should be more adequate whenever background dictates registering ensembles. I expect all these methods to complement electron microscopy.

Earlier crude STED imaging of live cells (38) should be advanced by emergent fast beam-scanning systems. Furthermore, adjusting the mean number of simultaneously recorded fluorophores via $I_{\text{scan}}/I_{\text{det}}$ should meet some of the challenges posed by intracellular movements. Moreover, by providing nanosized far-field fluorescent volumes (38), STED should facilitate probing the dynamics of tagged biomolecules in live cells through their fluorescence fluctuations (60).

Although the recording speed of these concepts may differ in various applications, they must all be slower than epifluorescence microscopy. This stems from the time-sequential readout of the diffraction zone. A potential remedy is to also use other spectroscopic features, such as the emission or absorption wavelength (57). Indeed, state B need not be “dark,” but just yield a signal different to that of A. Speed can also be gained by simultaneously installing and reading out a large number of states A, B, C, … within the diffraction zone, either randomly or in a well-chosen order. In my view, many hurdles will be overcome by selecting the right molecular states and transitions (9).

In a nutshell, the diffraction barrier is broken by establishing a specific state in a region $<\lambda^2/2\pi n$ that characterizes the features from this region (61). The region can be as small as, or actually be defined by, a single molecule. This state is established for a period $\tau$ within which the established spatial distribution of states is read out with the use of a suitable signal. As a rule, the shorter $\tau$, the harder it is to read out this distribution of states. “Virtual” molecular states are not easy to use because of their transient nature ($\tau < 10^{-12}$ s). This partly explains why no effective thread has yet been described for diffusion-unlimited imaging by pure scattering or reflection. Nonetheless, virtual states could well be involved in the generation of the signal that reads out the longer-lived specific states. In any case, reflection nanoscale imaging would not automatically carry the benefits of molecular labeling and therefore could not easily unravel the biomolecular network in the cell.

A major limitation of fluorescence imaging is the limited flux of emitted photons. It is therefore important to note that none of these concepts ultimately requires fluorescence emission (37). Rather, it is sufficient that one of the states, say A, elicits a specific signal that can be detected. For example, if a nonfluorescent marker state A (but not B) absorbs at a particular $\lambda$, converting the absorbed photons into heat, the transient change of the refractive index of the surrounding medium could be probed with a beam of light (62). The measured change would still perfectly quantify the spatially confined state A. Such a nonfluorescent read-out scheme would benefit from the ultrafast (<3 ps) conversion of photons into heat, providing constant availability for absorption. It would only be limited by the sensitivity of the refractive index measurement; and maintain “multicolor” labeling through $\lambda$-specific absorption. Although the probe beam as such would be diffraction-limited, the concept would still give diffraction-unlimited resolution.

Along with improved fluorescence schemes, such nonfluorescent schemes should enable us to take another step in the not-too-distant future: nanoscale 3D imaging at high speed. In any case, the works reviewed herein already broke the earlier crude STED imaging of live cells (38). Earlier STED microscopy of live cells was able to accomplish. With human perseverance focused on this matter, 3D imaging such nonfluorescent schemes should enable us to take another step in the not-too-distant future: nanoscale 3D imaging at high speed. In any case, the works reviewed herein already broke the earlier crude STED imaging of live cells (38). According to this matter, 3D imaging such nonfluorescent schemes should enable us to take another step in the not-too-distant future: nanoscale 3D imaging at high speed. In any case, the works reviewed herein already broke the earlier crude STED imaging of live cells (38).

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