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Advances in live-cell single-particle tracking and dynamic super-resolution imaging

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Resolving the movement of individual molecules in living cells by single particle tracking methods has allowed many molecular behaviors to be deciphered over the past three decades. These methods have increasingly benefited from advances in microscopy of single nano-objects such as fluorescent dye molecules, proteins or nanoparticles as well as tiny absorbing metal nanoparticles. In parallel to these efforts aiming at tracking ever smaller and more photostable nano-objects in living cells, the development of localization-based super-resolution imaging provided means to increase the number of single molecules tracked on a single cell. In this review we will present the most recent advances in the field.

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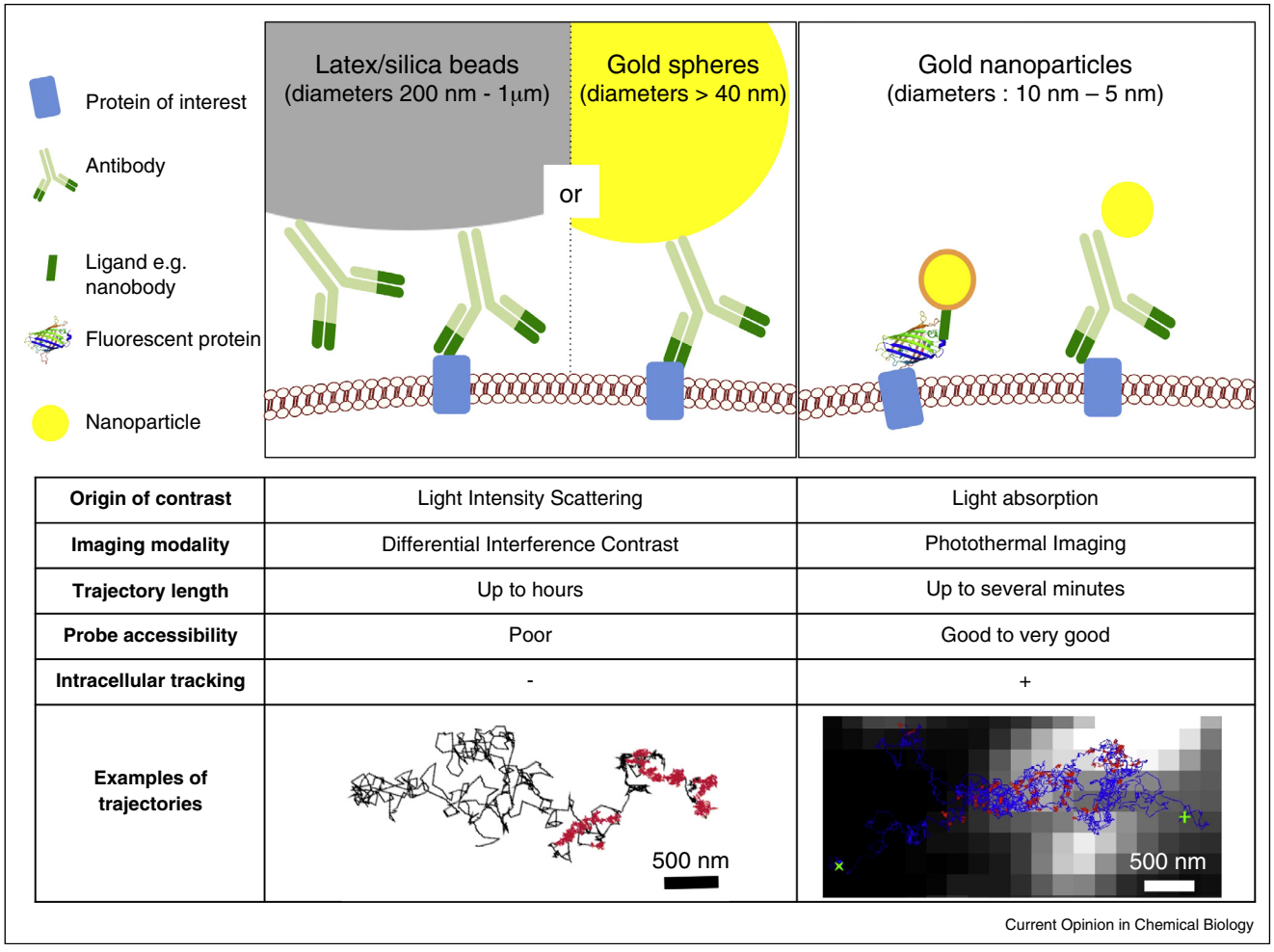
It is widely granted that understanding the complexity of cell functions requires deciphering the dynamic nature of constitutive molecule organizations. The movements of molecules in living cells are however characterized by their wide spatio-temporal diversities, making their study challenging. For instance, membranes exhibit an intrinsic complexity, because they consist of different lipids and a variety of proteins with dynamic and compartmentalized spatial distributions. Membrane molecules display various diffusion modes, frequently interact with each other at specific locations and can trigger signaling processes.

An important development in modern optical microscopy has been the detection of single molecules [1], in varied complex media including living cells [2–6]. Selecting a single molecule at a time allows the elimination of all the implicit averages of conventional optical observations, gaining access to heterogeneity, dynamical fluctuations [7,8], diffusion [9], reorientation [10,11], colocalization, and conformational changes [12] at the molecular level. Single molecule microscopies have changed our approach for conceiving and carrying out experiments on complex molecular systems. Yet, the optical detection of a single molecule remains difficult, because its signal must be extracted from the background arising from billions of other molecules in the focal spot of a microscope. An elegant way to find the needle in the haystack is to attach a ''label'' to the molecule of interest. The ideal label must fulfill contradictory requirements. It should generate an intense optical signal, but at the same time be inert and as small as possible, not to perturb the observed molecule too severely. Finally, single molecule imaging and tracking provides the possibility to locate the molecule position with an accuracy well below the diffraction limit [13], the localization accuracy being mostly limited by the detection signal-to-noise ratio [14].

Several approaches have been used to track individual molecules mainly in the plasma membrane of live cells, with distinct advantages and limitations. The first reported method of Single Particle Tracking (SPT) uses non-fluorescent labels large enough to be detectable by conventional microscopes [15,16] through Rayleigh intensity scattering [17,18]. These labels can be large latex, polystyrene or silica particles of 200 nm to 1 μm diameter, or smaller metal particles [16] (Figure 1). When larger than typ. 40 nm, metal particles can indeed be imaged in optical microscopy by means of various methods, such as imaging at the plasmon frequency with dark field illumination [19], with differential interference contrast and video enhancement [18] or with total internal reflection [20]. SPT permits the tracking of individual molecules for very long times and possibly at very fast imaging rates [21]. SPT revealed for instance, barriers set for diffusion by the cytoskeleton [22], and the diversity of lateral diffusion modes of receptor for neurotransmitters in live neurons [23,24]. However, the size of the labels used in SPT precludes tracking single molecules in confined cellular regions [25•]. Indeed, Rayleigh scattering decreases like the sixth power of particle diameter [26] prohibiting the detection of particles smaller than 40 nm with conventional microscopy techniques mentioned above. This is particularly true if the particles are to be detected in cells or tissues where scattering signals must be discriminated from a strong background therein.

Figure 1



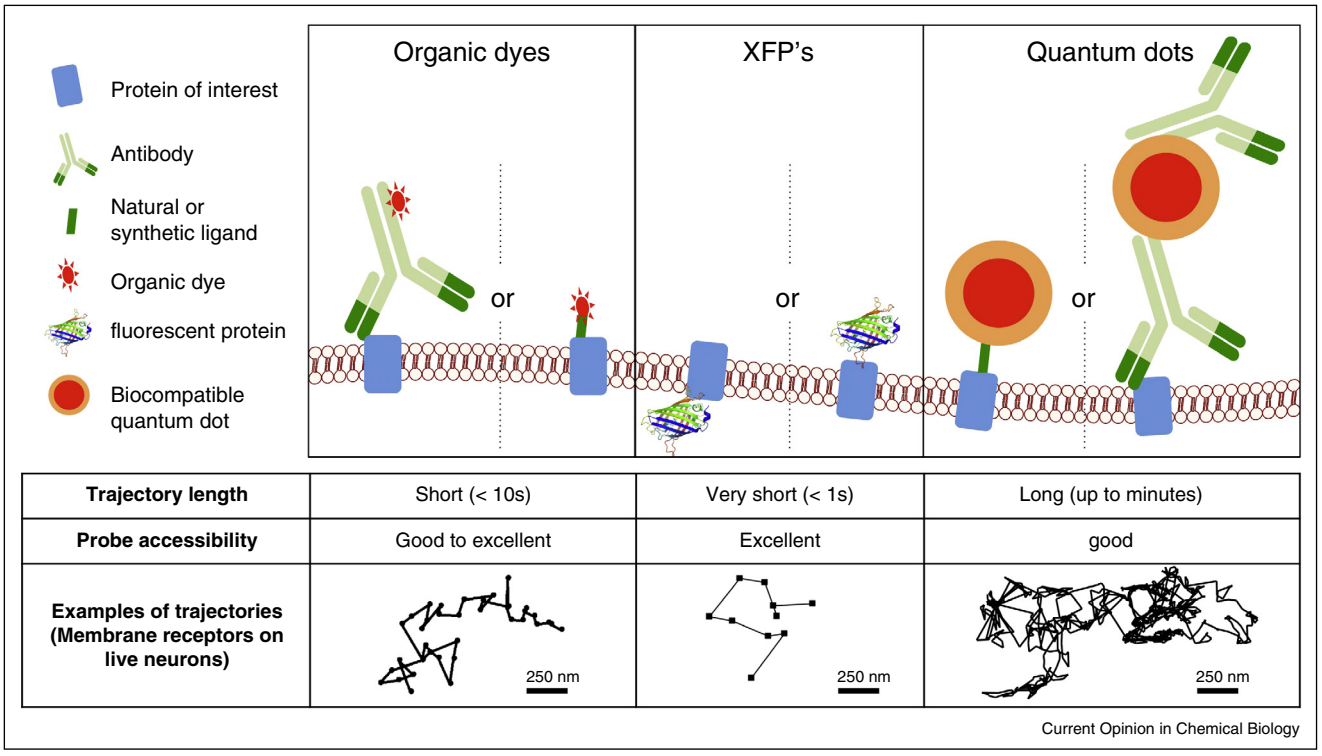
Origin of contrast	Light Intensity Scattering	Light absorption
Imaging modality	Differential Interference Contrast	Photothermal Imaging
Trajectory length	Up to hours	Up to several minutes
Probe accessibility	Poor	Good to very good
Intracellular tracking	-	+
Examples of trajectories		

Principle and comparison of Single Particle Tracking techniques based on non-fluorescent probes. Examples of trajectories are adapted from [23] (left, gephyrin receptors tracked in the plasma membrane of live neurons) and correspond to integrins tracked inside labeled adhesion sites (white signal in background) of live cells (right). In both cases, red portions of the trajectories correspond to reduced diffusion transients of the tracked molecules.

An alternative approach for Single Molecule Tracking (SMT) is based on fluorescence (Figure 2). Single molecule fluorescence detection techniques arose in the 90s following the first demonstration at low temperature [27] and later on the first far-field detection at room temperature [28]. Indeed, by replacing scattering labels used in SPT by fluorescent molecules, SMT was demonstrated in synthetic membranes [29], then in living cells using organic dyes [2,3] or autofluorescent proteins [4]. Organic dyes can be chemically grafted to the molecule under study by several types of natural or synthetic ligands while autofluorescent proteins can be genetically fused to the protein of interest. Ligand-dye complexes can be so small as to neither hamper the diffusion of their host proteins or lipids, nor affect their functions or interactions with partners. The main drawback of fluorescence imaging is photobleaching, which severely limits the imaging times of single molecules. Removing free oxygen by specific scavengers can reduce photobleaching, but this can be a serious obstacle to many experiments in living cells due to the toxicity of the oxygen scavengers. SMT is used to study many different systems in live cells, for instance membrane molecule dynamic organizations [2,30–35,36] or gene expression [37]. Recent noteworthy developments involve tracking single dye in 3D in cell membranes [38] and cell nuclei [39••].

Nanocrystals of semiconductors (quantum dots) have also been used as fluorescent markers [40,41]. They are more photostable than organic dyes and autofluorescent proteins. They however must be passivated, and functionalized by surface layers, because of their high chemical reactivity [42,43]. Functionalized quantum dots have proven to be extremely valuable tools for extended observation in living cells [44] and allow 3D single molecule tracking [45–47], for instance for the study of

Figure 2



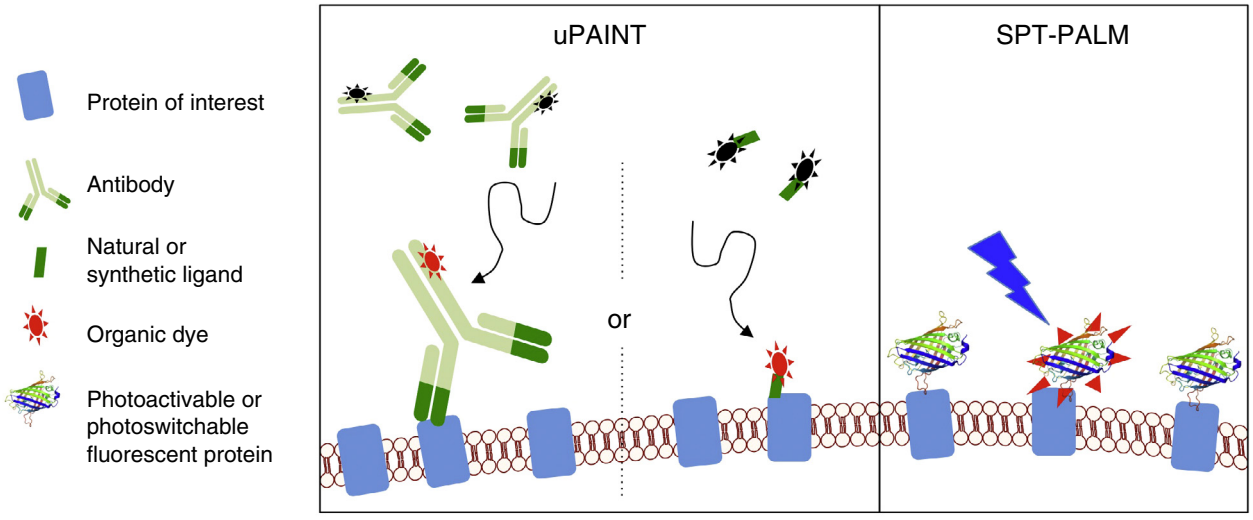
Principle and comparison of Single Molecule Tracking techniques based on fluorescent probes. Examples of trajectories are adapted from [31] (left, glutamate receptors tracked in the plasma membrane of live neurons), from [98] (middle, glutamate receptor fused to a variant of YFP in live cell) and from [59] (right, AMPA receptor tracked in the plasma membrane of live neurons using antibody functionalized quantum dots).

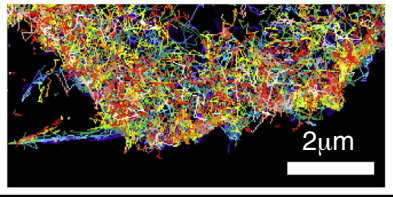
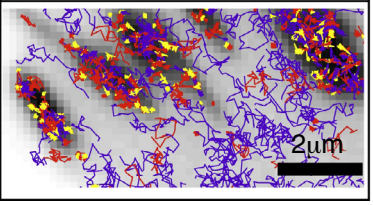
neurotransmitter receptors [48–51] in live neurons and transmembrane proteins in organotypic brain slices [52••], of synaptic adhesion proteins [53] or of dimerization of epidermal growth factor receptors [54•]. However, quantum dot luminescence is subject to blinking [55] at all time scales [56] which renders observations of changes between different lateral diffusion modes of proteins challenging [57,58]. Moreover, once functionalized, quantum dot bulkiness may generate steric hindrance in confined cellular areas which may bias molecule diffusion like in synaptic clefts [59] or prevent access to adhesion sites [60•].

Single molecule methods, allowing the full history of proteins in cells including intermediate states even in highly confined regions to be recorded, would need to combine the advantages of SPT and SMT, namely long observation times and small nanometer-sized labels. An interesting approach consists in developing sensitive optical systems for the detection of the absorption of very small metal nanoparticles (NPs) [61]. Photothermal techniques have proven to be particularly valuable because the signal of NPs can be insensitive to the scattering environment of the cells [62,63]. Photothermal heterodyne imaging (PHI) [64] uses a combination of a time-modulated green beam to heat the NPs and a non-resonant red beam to probe the local variations of the refraction index around the NPs [64,65]. In order to track single 5 nm gold nanoparticles a dedicated tracking strategy was developed [66]. It is based on triangulation acting like a nano-GPS, requiring only three local measurements around the nanoparticle of interest. This triangulation technique, which is able to reach a tracking rate of ~25 Hz is conceptually similar to that developed to track fluorescent beads in 3D by applying orbital movements of a confocal beam around a targeted bead [67]. To fully benefit from the small size of the perfectly photostable gold nanoparticles used in PHI, small and versatile absorbing probes have been developed for live cell imaging [60•,68,69]. As an example, a probe consisting of a 5 nm gold NP functionalized with a GFP-nanobody, a small (2 nm × 4 nm) fragment of camelidae antibody having a high affinity for GFP (Figure 1) allowed imaging and tracking in confined environments. Applications of PHI concern individual integrin tracking in adhesion sites [60•], NP internalization processes [70], intracellular tracking of microtubule end binding [60•] or FGF receptor tracking on the plasma of living cells [71] for instance.

Even if arbitrarily long time single-molecule tracking can be obtained with SPT techniques, the possibility to

Figure 3



Control of fluorescent markers sparsity	Combination of oblique illumination, real time labeling by ligand diffusion and photobleaching	Photo-activation and photobleaching
Trajectory length	Short (< 10s)	Very short (< 1s)
Number of trajectories /cell	>100,000	>100,000
Type of proteins	Endogenous or overexpressed proteins	Overexpressed proteins
Intracellular tracking	-	++
Examples of multiple color-coded single molecule trajectories obtained on a live cell portion.		

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Principle and comparison of localization based super-resolution techniques allowing high-density single molecule tracking. Examples are adapted from [83••] (left, activated EGF receptors tracked in the plasma membrane of live cells) and from [87•] (right, integrins tracked in and out adhesion sites of live cells).

simultaneously study a high number of individual molecules on a single cell is fundamentally limited by the diffraction limit which impose sparse labeling of single molecules to be resolved. Several algorithms have been developed to partially address this limitation [58,72–74]. Importantly, the recent developments of localization-based super-resolution methods opened new perspectives to study a high number of individual molecules on a single cell. Indeed, these methods deliver reconstructed images, which consist in a collection of single emitters localizations imaged successively at low density on a specimen of interest. This is either performed by iteratively activating a small number of photo-activable fluorophores among a dense population of inactivated ones (such as in PALM [75,76], STORM [77] or related techniques [78,79]) or by real-time imaging stochastic molecular interactions (PAINT [80]) like fluorescent ligand binding to receptors (uPAINT [81•]). PALM and STORM inherently require photo-activable or photo-switchable fluorophores which can be either engineered fluorescent proteins (PALM) or emitters immersed in reducing agents to allow the photo-conversion (STORM) while uPAINT requires highly specific fluorescent ligands and is applicable to virtually any fluorophore detectable at the single molecule level. Because they are easily applicable to live cell studies, both uPAINT and the single particle tracking development of PALM (SPT-PALM [82••]) have become key

complimentary methods to allow single molecule tracking at high density (Figure 3). PALM benefits from the versatility of the fluorescent protein labeling toolbox including both intra and extra-cellular markers, while uPAINT, currently limited to extracellular binding on live cells, gives access to endogenous membrane receptors [81•] and allows functional imaging of ligand activated receptors [83••]. SPT-PALM and uPAINT were already applied in many different cellular systems such as neuronal membrane receptor organizations [84,85], adhesion sites [86,87•], intracellular molecular organization of the cytoskeleton [88] or diffusive RNA binding proteins [89], RNA polymerase clustering [90•] or EGF receptor dimerization states [83••]. Interestingly, STORM was recently used for intracellular tracking of membrane receptors using SNAP-tags using live-cell compatible reducing agents [91•]. A related approach to uPAINT uses small fluorescently labeled molecule agents that display reversible binding to sodium ion channel [92]. Interestingly, the specific super-resolution imaging and tracking of receptors dimers activated by their ligand in living cells [83••] was recently demonstrated combining single molecule FRET to dual-color uPAINT.

SPT and SMT have been very active fields of research over more than twenty years, delivering numerous key fundamental insights about the dynamic behavior of biomolecules in live cells. New developments are however still needed to track single molecules in highly confined and complex molecular environments both in live cells and tissues, either to obtain arbitrary long 3D trajectories or a ultra-high number of short trajectories in a small area. We foresee that further technical and conceptual developments in microscopy, chemistry, nanoscience or computer science, will continue to provide breakthroughs in biological science (Box 1).

Box 1 Data analysis

Single particle/molecule positions are first to be identified in each image frame with nm accuracy using for instance centroid determination or Gaussian fitting of the particle optical signal as well as wavelet based algorithms [93]. Their 2D (or 3D) trajectories are then constructed using dedicated algorithms [57,58,73,94,95••]. From the trajectories, the main method for analyzing molecule's modes of diffusion is to construct the mean square displacement (MSD) in order to calculate diffusion coefficient of single particles and further reveal the underlying modes of molecule diffusion mechanism [17]. For instance, a linear function of MSD versus time reveals random diffusion, while deviations from linearity can suggest active transport, confined diffusion, or other physical/chemical molecular interactions that impact diffusion modes. In addition to MSD analysis, which becomes prone to severe statistical imprecisions for short trajectories as often found in SMT, an analysis based on the distribution of the squared displacements has been shown to be particularly efficient [9,31,96]. The distribution of the squared displacements, $P(r^2,\tau)$ corresponds to the probability for molecule starting at the origin to be found within a circle of radius r after a time delay τ. From the shape of $P(r^2,\tau)$ over time delay, distinct diffusion modes corresponding to molecule subpopulations can be studied simultaneously [9,31,96].

Because with SPT-PALM and uPAINT the number of studied single molecule trajectories increased dramatically over conventional SMT approaches specific strategies were developed for data analysis, visualization and interpretation. For instance, in addition to MSD analysis and color-coded molecule trajectories displayed on a 2D image, (see Figure 3), 2D maps of the molecule mobilities are often produced [81•]. These consist in displaying a color-coded image with each image pixel (whose size is chosen by the user, typically 200 nm) the median of the step lengths (corresponding to the distance between two consecutive points of a trajectory) found in this pixel. In addition, combining high-density single-molecule tracking and statistical inference, it was recently shown that it is possible to separately map the 2D diffusion and energy landscapes of membrane proteins across the cell surface at ~100 nm resolution [97].

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Moerner WE, Orrit M: **Illuminating single molecules in condensed matter**. *Science* 1999, **283**:1670-1676.
2. Sako Y, Minoghchi S, Yanagida T: **Single-molecule imaging of EGFR signalling on the surface of living cells**. *Nat Cell Biol* 2000, **2**:168-172.
3. Schutz GJ, Kada G, Pastushenko VP, Schindler H: **Properties of lipid microdomains in a muscle cell membrane visualized by single molecule microscopy**. *EMBO J* 2000, **19**:892-901.
4. Harms GS, Cognet L, Lommerse PH, Blab GA, Kahr H, Gamsjager R, Spaink HP, Soldatov NM, Romanin C, Schmidt T: **Single-molecule imaging of l-type Ca^{2+} channels in live cells**. *Biophys J* 2001, **81**:2639-2646.
5. Iino R, Koyama I, Kusumi A: **Single molecule imaging of green fluorescent proteins in living cells: E-cadherin forms oligomers on the free cell surface**. *Biophys J* 2001, **80**: 2667-2677.
6. Seisenberger G, Ried MU, Endress T, Buning H, Hallek M, Brauchle C: **Real-time single-molecule imaging of the infection pathway of an adeno-associated virus**. *Science* 2001, **294**:1929-1932.
7. Vanden Bout DA, Yip W-T, Hu D, Fu D-K, Swager TM, Barbara PF: **Discrete intensity jumps and intramolecular electronic energy transfer in the spectroscopy of single conjugated polymer molecules**. *Science* 1997, **277**:1074-1077.
8. Lu HP, Xun L, Xie XS: **Single-molecule enzymatic dynamics**. *Science* 1998, **282**:1877-1882.
9. Schuetz GJ, Schindler H, Schmidt T: **Single-molecule microscopy on model membranes reveals anomalous diffusion**. *Biophys J* 1997, **73**:1073-1080.
10. Ha T, Enderle T, Chemla DS, Selvin PR, Weiss S: **Single molecule dynamics studied by polarization modulation**. *Phys Rev Lett* 1996, **77**:3979-3982.
11. Lacoste TD, Michalet X, Pinaud F, Chemla DS, Alivisatos AP, Weiss S: **Ultrahigh-resolution multicolor colocalization of**

single fluorescent probes. *Proc Natl Acad Sci U S A* 2000, **97**:9461-9466.

12. Zhuang X, Bartley LE, Babcock HP, Russell R, Ha T, Herschlag D, Chu S: **A single-molecule study of RNA catalysis and folding**. *Science* 2000, **288**:2048-2051.

13. Thompson RE, Larson DR, Webb WW: **Precise nanometer localization analysis for individual fluorescent probes**. *Biophys J* 2002, **82**:2775-2783.

14. Bobroff N: **Position measurements with a resolution and noise-limited instrument**. *Rev Sci Instrum* 1986, **57**:1152-1157.

15. Geerts H, De Brabander M, Nuydens R, Geuens S, Moeremans M, De Mey J, Hollenbeck P: **Nanovid tracking: a new automatic method for the study of mobility in living cells based on colloidal gold and video microscopy**. *Biophys J* 1987, **52**: 775-782.

16. Sheetz MP, Turney S, Qian H, Elson EL: **Nanometre-level analysis demonstrates that lipid flow does not drive membrane glycoprotein movements**. *Nature* 1989, **340**: 284-288.

17. Saxton MJ, Jacobson K: **Single-particle tracking: applications to membrane dynamics**. *Annu Rev Biophys Biomol Struct* 1997, **26**:373-399.

18. Gelles J, Schnapp BJ, Sheetz MP: **Tracking kinesin-driven movements with nanometre-scale precision**. *Nature* 1988, **331**:450-453.

19. Schultz S, Smith DR, Mock JJ, Schultz DA: **Single-target molecule detection with nonbleaching multicolor optical immunolabels**. *Proc Natl Acad Sci U S A* 2000, **97**:996-1001.

20. Sönnichsen C, Geier S, Hecker NE, Von Plessen G, Feldmann J, Ditlbacher H, Lamprecht B, Krenn JR, Aussenegg FR, Chan VZH *et al.*: **Spectroscopy of single metallic nanoparticles using total internal reflection microscopy**. *Appl Phys Lett* 2000, **77**:2949.

21. Fujiwara T, Ritchie K, Murakoshi H, Jacobson K, Kusumi A: **Phospholipids undergo hop diffusion in compartmentalized cell membrane**. *J Cell Biol* 2002, **157**:1071-1081.

22. Kusumi A, Sako Y: **Cell surface organization by the membrane skeleton**. *Curr Opin Cell Biol* 1996, **8**:566-574.

23. Meier J, Vannier C, Serge A, Triller A, Choquet D: **Fast and reversible trapping of surface glycine receptors by gephyrin**. *Nat Neurosci* 2001, **4**:253-260.

24. Borgdorff AJ, Choquet D: **Regulation of AMPA receptor lateral movements**. *Nature* 2002, **417**:649-653.

25. • Gu Y, Wang G, Fang N: **Simultaneous single-particle superlocalization and rotational tracking**. *ACS Nano* 2013, **7**:1658-1665.
Highlights the need for labeling with small particles to track single molecules in crowded environments.

26. Mie G: **Beiträge zur Optik trüber Medien, speziell kolloidaler Metallösungen**. *Ann Phys* 1908, **330**:377-445.

27. Orrit M, Bernard J: **Single pentacene molecules detected by fluorescence excitation in a P-terphenyl crystal**. *Phys Rev Lett* 1990, **65**:2716.

28. Nie S, Chiu DT, Zare RN: **Probing individual molecules with confocal fluorescence microscopy**. *Science* 1994, **266**: 1018-1021.

29. Schmidt T, Schuetz GJ, Baumgartner W, Gruber HJ, Schindler H: **Imaging of single molecule diffusion**. *Proc Natl Acad Sci U S A* 1996, **93**:2926-2929.

30. Vrljic M, Nishimura SY, Brasselet S, Moerner WE, McConnell HM: **Translational diffusion of individual Class II MHC membrane proteins in cells**. *Biophys J* 2002, **83**:2681-2692.

31. Tardin C, Cognet L, Bats C, Lounis B, Choquet D: **Direct imaging of lateral movements of AMPA receptors inside synapses**. *EMBO J* 2003, **22**:4656-4665.

32. Nakada C, Ritchie K, Oba Y, Nakamura M, Hotta Y, Iino R, Kasai RS, Yamaguchi K, Fujiwara T, Kusumi A: **Accumulation of anchored proteins forms membrane diffusion barriers during neuronal polarization**. *Nat Cell Biol* 2003, **5**:626-632.

33. Lommerse PH, Blab GA, Cognet L, Harms GS, Snaar-Jagalska BE, Spaink HP, Schmidt T: **Single-molecule imaging of the H-ras membrane-anchor reveals domains in the cytoplasmic leaflet of the cell membrane**. *Biophys J* 2004, **86**:609-616.

34. Groc L, Heine M, Cognet L, Brickley K, Stephenson FA, Lounis B, Choquet D: **Differential activity-dependent regulation of the lateral mobilities of AMPA and NMDA receptors**. *Nat Neurosci* 2004, **7**:695-696.

35. Uyemura T, Takagi H, Yanagida T, Sako Y: **Single-molecule analysis of epidermal growth factor signaling that leads to ultrasensitive calcium response**. *Biophys J* 2005, **88**: 3720-3730.

36. Kasai RS, Suzuki KGN, Prossnitz ER, Koyama-Honda I, Nakada C, Fujiwara TK, Kusumi A: **Full characterization of GPCR monomer–dimer dynamic equilibrium by single molecule imaging**. *J Cell Biol* 2011, **192**:463-480.

37. Yu J, Xiao J, Ren X, Lao K, Xie XS: **Probing gene expression in live cells, one protein molecule at a time**. *Science* 2006, **311**:1600-1603.

38. Li Y, Hu Y, Cang H: **Light sheet microscopy for tracking single molecules on the apical surface of living cells**. *J Phys Chem B* 2013, **117**:15503-15511.

39. •• Abrahamsson S, Chen J, Hajj B, Stallinga S, Katsov AY, Wisniewski J, Mizuguchi G, Soule P, Mueller F, Dugast Darzacq C *et al.*: **Fast multicolor 3D imaging using aberration-corrected multifocus microscopy**. *Nat Methods* 2013, **10**:60-63.
Intracellular single molecule tracking in 3D with an speed optimized detection scheme.

40. Dubertret B, Skourides P, Norris DJ, Noireaux V, Brivanlou AH, Libchaber A: **In vivo imaging of quantum dots encapsulated in phospholipid micelles**. *Science* 2002, **298**:1759-1762.

41. Wu X, Liu H, Liu J, Haley KN, Treadway JA, Larson JP, Ge N, Peale F, Bruchez MP: **Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots**. *Nat Biotechnol* 2003, **21**:41-46.

42. Medintz IL, Uyeda HT, Goldman ER, Mattoussi H: **Quantum dot bioconjugates for imaging, labelling and sensing**. *Nat Mater* 2005, **4**:435-446.

43. Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, Sundaresan G, Wu AM, Gambhir SS, Weiss S: **Quantum dots for live cells, in vivo imaging, and diagnostics**. *Science* 2005, **307**:538-544.

44. Pinaud F, Clarke S, Sittner A, Dahan M: **Probing cellular events, one quantum dot at a time**. *Nat Methods* 2010, **7**:275-285.

45. Holtzer L, Meckel T, Schmidt T: **Nanometric three-dimensional tracking of individual quantum dots in cells**. *Appl Phys Lett* 2007, **90**:053902.

46. Ram S, Prabhat P, Chao J, Ward ES, Ober RJ: **High accuracy 3D quantum dot tracking with multifocal plane microscopy for the study of fast intracellular dynamics in live cells**. *Biophys J* 2008, **95**:6025-6043.

47. Liu SL, Li J, Zhang ZL, Wang ZG, Tian ZQ, Wang GP, Pang DW: **Fast and high-accuracy localization for three-dimensional single-particle tracking**. *Sci Rep* 2013, **3**:2462.

48. Dahan M, Levi S, Luccardini C, Rostaing P, Riveau B, Triller A: **Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking**. *Science* 2003, **302**:442-445.

49. Groc L, Heine M, Cousins SL, Stephenson FA, Lounis B, Cognet L, Choquet D: **NMDA receptor surface mobility depends on NR2A-2B subunits**. *Proc Natl Acad Sci U S A* 2006, **103**: 18769-18774.

50. Heine M, Groc L, Frischknecht R, Beique J-C, Lounis B, Rumbaugh G, Huganir RL, Cognet L, Choquet D: **Surface mobility of postsynaptic AMPARs tunes synaptic transmission**. *Science* 2008, **320**:201-205.

51. Ehrensperger MV, Hanus C, Vannier C, Triller A, Dahan M: **Multiple association states between glycine receptors and gephyrin identified by SPT analysis**. *Biophys J* 2007, **92**:3706-3718.

52. •• Biermann B, Sokoll S, Klueva J, Missler M, Wiegert JS, Sibarita JB, Heine M: **Imaging of molecular surface dynamics in brain slices using single-particle tracking**. *Nat Commun* 2014, **5**:3024.
Single particle tracking of lipids and transmembrane proteins in organotypic brain slices using quantum dots.

53. Liu DS, Phipps WS, Loh KH, Howarth M, Ting AY: **Quantum dot targeting with lipoic acid ligase and HaloTag for single-molecule imaging on living cells**. *ACS Nano* 2012, **6**:11080-11087.

54. • Chung I, Akita R, Vandlen R, Toomre D, Schlessinger J, Mellman I: **Spatial control of EGF receptor activation by reversible dimerization on living cells**. *Nature* 2010, **464**:783-787.
Single particle tracking of dimerized EGFRs using quantum dots.

55. Nirmal M, Dabbousi BO, Bawendi MG, Macklin JJ, Trautman JK, Harris TD, Brus LE: **Fluorescence intermittency in single cadmium selenide nanocrystals**. *Nature* 1996, **383**:802-804.

56. Kuno M, Fromm DP, Hamann HF, Gallagher A, Nesbitt DJ: **Nonexponential "blinking" kinetics of single CdSe quantum dots: a universal power law behavior**. *J Chem Phys* 2000, **112**:3117-3120.

57. Bonneau S, Dahan M, Cohen LD: **Single quantum dot tracking based on perceptual grouping using minimal paths in a spatiotemporal volume**. *IEEE Trans Image Process* 2005, **14**:1384-1395.

58. Jaqaman K, Loerke D, Mettlen M, Kuwata H, Grinstein S, Schmid SL, Danuser G: **Robust single-particle tracking in live-cell time-lapse sequences**. *Nat Methods* 2008, **5**:695-702.

59. Groc L, Lafourcade M, Heine M, Renner M, Racine V, Sibarita JB, Lounis B, Choquet D, Cognet L: **Surface trafficking of neurotransmitter receptor: comparison between single-molecule/quantum dot strategies**. *J Neurosci* 2007, **27**:12433-12437.

60. • Leduc C, Si S, Gautier J, Soto-Ribeiro M, Wehrle-Haller B, Gautreau A, Giannone G, Cognet L, Lounis B: **A highly specific gold nanoprobe for live-cell single-molecule imaging**. *Nano Lett* 2013, **13**:1489-1494.
Intracellular single particle tracking in live cells using small 5 nm gold nanoparticles.

61. Van Dijk MA, Tchebotareva AL, Orrit M, Lippitz M, Berciaud S, Lasne D, Cognet L, Lounis B: **Absorption and scattering microscopy of single metal nanoparticles**. *Phys Chem Chem Phys* 2006, **8**:3486.

62. Boyer D, Tamarat P, Maali A, Lounis B, Orrit M: **Photothermal imaging of nanometer-sized metal particles among scatterers**. *Science* 2002, **297**:1160-1163.

63. Cognet L, Tardin C, Boyer D, Choquet D, Tamarat P, Lounis B: **Single metallic nanoparticle imaging for protein detection in cells**. *Proc Natl Acad Sci U S A* 2003, **100**:11350-11355.

64. Berciaud S, Cognet L, Blab GA, Lounis B: **Photothermal heterodyne imaging of individual nonfluorescent nanoclusters and nanocrystals**. *Phys Rev Lett* 2004, **93**:257402.

65. Berciaud S, Lasne D, Blab GA, Cognet L, Lounis B: **Photothermal heterodyne imaging of individual metallic nanoparticles: theory versus experiments**. *Phys Rev B* 2006, **73**:045424.

66. Lasne D, Blab GA, Berciaud S, Heine M, Groc L, Choquet D, Cognet L, Lounis B: **Single Nanoparticle Photothermal Tracking (SNaPT) of 5-nm gold beads in live cells**. *Biophys J* 2006, **91**:4598.

67. Levi V, Ruan Q, Gratton E: **3-D particle tracking in a two-photon microscope: application to the study of molecular dynamics in cells**. *Biophys J* 2005, **88**:2919-2928.

68. Levy R, Thanh NT, Doty RC, Hussain I, Nichols RJ, Schiffrin DJ, Brust M, Fernig DG: **Rational and combinatorial design of peptide capping ligands for gold nanoparticles**. *J Am Chem Soc* 2004, **126**:10076-10084.

69. Duchesne L, Gentili D, Comes-Franchini M, Fernig DG: **Robust ligand shells for biological applications of gold nanoparticles**. *Langmuir* 2008, **24**:13572-13580.

70. Leduc C, Jung JM, Carney RP, Stellacci F, Lounis B: **Direct investigation of intracellular presence of gold nanoparticles via photothermal heterodyne imaging**. *ACS Nano* 2011, **5**:2587-2592.

71. Duchesne L, Octeau V, Bearon RN, Beckett A, Prior IA, Lounis B, Fernig DG: **Transport of fibroblast growth factor 2 in the pericellular matrix is controlled by the spatial distribution of its binding sites in heparan sulfate**. *PLoS Biol* 2012, **10**:e1001361.

72. Semrau S, Schmidt T: **Particle image correlation spectroscopy (PICS): retrieving nanometer-scale correlations from high-density single-molecule position data**. *Biophys J* 2007, **92**:613-621.

73. Serge A, Bertaux N, Rigneault H, Marguet D: **Dynamic multiple-target tracing to probe spatiotemporal cartography of cell membranes**. *Nat Methods* 2008, **5**:687-694.

74. Arnspang EC, Schwartzentruber J, Clausen MP, Wiseman PW, Lagerholm BC: **Bridging the gap between single molecule and ensemble methods for measuring lateral dynamics in the plasma membrane**. *PLoS ONE* 2013, **8**:e78096.

75. Betzig E, Patterson GH, Sougrat R, Lindwasser OW, Olenych S, Bonifacino JS, Davidson MW, Lippincott-Schwartz J, Hess HF: **Imaging intracellular fluorescent proteins at nanometer resolution**. *Science* 2006, **313**:1642-1645.

76. Hess ST, Girirajan TP, Mason MD: **Ultra-high resolution imaging by fluorescence photoactivation localization microscopy**. *Biophys J* 2006, **91**:4258-4272.

77. Rust MJ, Bates M, Zhuang XW: **Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM)**. *Nat Methods* 2006, **3**:793-795.

78. Heilemann M, van de Linde S, Schüttpelz M, Kasper R, Seefeldt B, Mukherjee A, Tinnefeld P, Sauer M: **Subdiffraction-resolution fluorescence imaging with conventional fluorescent probes**. *Angew Chem Int Ed* 2008, **47**:6172-6176.

79. Shtengel G, Galbraith JA, Galbraith CG, Lippincott-Schwartz J, Gillette JM, Manley S, Sougrat R, Waterman CM, Kanchanawong P, Davidson MW *et al.*: **Interferometric fluorescent super-resolution microscopy resolves 3D cellular ultrastructure**. *Proc Natl Acad Sci U S A* 2009, **106**:3125-3130.

80. Sharonov A, Hochstrasser RM: **Wide-field subdiffraction imaging by accumulated binding of diffusing probes**. *PNAS* 2006, **103**:18911-18916.

81. • Giannone G, Hosy E, Levet F, Constals A, Schulze K, Sobolevsky AI, Rosconi MP, Gouaux E, Tampé R, Choquet D *et al.*: **Dynamic superresolution imaging of endogenous proteins on living cells at ultra-high density**. *Biophys J* 2010, **99**:1303-1310.
Single molecule tracking of endogneous receptors at high density with super-resolution imaging.

82. •• Manley S, Gillette JM, Patterson GH, Shroff H, Hess HF, Betzig E, Lippincott-Schwartz J: **High-density mapping of single-molecule trajectories with photoactivated localization microscopy**. *Nat Methods* 2008, **5**:155.
Single molecule tracking at high density with super-resolution imaging using PALM.

83. •• Winckler P, Lartigue L, Giannone G, De Giorgi F, Ichas F, Sibarita JB, Lounis B, Cognet L: **Identification and super-resolution imaging of ligand-activated receptor dimers in live cells**. *Sci Rep* 2013, **3**:2387.
Single molecule tracking of ligand activated dimers of membrane receptors at high density with super-resolution imaging using single molecule FRET.

84. Nair D, Hosy E, Petersen JD, Constals A, Giannone G, Choquet D, Sibarita JB: **Super-resolution imaging reveals that AMPA receptors inside synapses are dynamically organized in nanodomains regulated by PSD95**. *J Neurosci* 2013, **33**:13204-13224.

85. Specht CG, Izeddin I, Rodriguez PC, El Beheiry M, Rostaing P, Darzacq X, Dahan M, Triller A: **Quantitative nanoscopy of**

inhibitory synapses: counting gephyrin molecules and receptor binding sites. *Neuron* 2013, **79**:308-321.

86. Shroff H, Galbraith CG, Galbraith JA, Betzig E: **Live-cell photoactivated localization microscopy of nanoscale adhesion dynamics**. *Nat Methods* 2008, **5**:417-423.

87. • Rossier O, Octeau V, Sibarita J-B, Leduc C, Tessier B, Nair D, Gatterdam V, Destaing O, Albigès-Rizo C, Tampé R *et al.*: **Integrins β1 and β3 exhibit distinct dynamic nanoscale organizations inside focal adhesions**. *Nat Cell Biol* 2012, **14**:1057-1067.
A combination of SMT and dynamic super-resolution imaging techniques to reveal the nanoscale organization of integrins in confined areas.

88. Niu L, Yu J: **Investigating intracellular dynamics of FtsZ cytoskeleton with photoactivation single-molecule tracking**. *Biophys J* 2008, **95**:2009-2016.

89. Persson F, Linden M, Unoson C, Elf J: **Extracting intracellular diffusive states and transition rates from single-molecule tracking data**. *Nat Methods* 2013, **10**:265-269.

90. • Cisse II, Izeddin I, Causse SZ, Boudarene L, Senecal A, Muresan L, Dugast-Darzacq C, Hajj B, Dahan M, Darzacq X: **Real-time dynamics of RNA polymerase II clustering in live human cells**. *Science* 2013, **341**:664-667.
A combination of SMT and dynamic super-resolution imaging techniques to reveal the molecular clustering in cell nuclei.

91. • Benke A, Olivier N, Gunzenhauser J, Manley S: **Multicolor single molecule tracking of stochastically active synthetic dyes**. *Nano Lett* 2012, **12**:2619-2624.
Intracellular tracking of single molecules using SNAPtags and STORM using live-cell compatible reducing agents.

92. Ondrus AE, Lee HL, Iwanaga S, Parsons WH, Andresen BM, Moerner WE, Du Bois J: **Fluorescent saxitoxins for live cell imaging of single voltage-gated sodium ion channels beyond the optical diffraction limit**. *Chem Biol* 2012, **19**:902-912.

93. Izeddin I, Boulanger J, Racine V, Specht CG, Kechkar A, Nair D, Triller A, Choquet D, Dahan M, Sibarita JB: **Wavelet analysis for single molecule localization microscopy**. *Opt Express* 2012, **20**:2081-2095.

94. Racine V, Hertzog A, Jouanneau J, Salamero J, Kervrann C, Sibarita JB: **Multiple-target tracking of 3D fluorescent objects based on simulated annealing**. *2006 3rd IEEE International Symposium on Biomedical Imaging: Macro to Nano, Vols 1–3*. 2006:1020-1023.

95. •• Chenouard N, Smal I, de Chaumont F, Maska M, Sbalzarini IF, Gong Y, Cardinale J, Carthel C, Coraluppi S, Winter M *et al.*: **Objective comparison of particle tracking methods**. *Nat Methods* 2014, **11**:281-289.
An initiative gathering different laboratories to compare single particle tracking algorithms.

96. Renner ML, Cognet L, Lounis B, Triller A, Choquet D: **The excitatory postsynaptic density is a size exclusion diffusion environment**. *Neuropharmacology* 2009, **56**:30-36.

97. Masson JB, Dionne P, Salvatico C, Renner M, Specht CG, Triller A, Dahan M: **Mapping the energy and diffusion landscapes of membrane proteins at the cell surface using high-density single-molecule imaging and Bayesian inference: application to the multiscale dynamics of glycine eceptors in the neuronal membrane**. *Biophys J* 2014, **106**:74-83.

98. Cognet L, Coussen F, Choquet D, Lounis B: **Fluorescence microscopy of single autofluorescent proteins for cellular biology**. *C R Phys* 2002, **3**:645-656.