Super-resolution microscopy using single-walled carbon nanotubes

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The past two decades have seen the advent of a series of optical microscopy techniques that allow spatial resolutions higher than those imposed by the Abbe diffraction limit. Acknowledged with the Nobel prize in 2014, these techniques are collectively known as super resolution microscopy (SRM) techniques. The catch was that Abbe's barrier does not prevent finding out the coordinate of a molecule with arbitrary precision if there is no other similar marker molecule within λ/2n distance, λ being the wavelength of light.[1] Thus all these techniques make use of the ability to spatially resolve fluorophores which can be switched between "on" and "off" states within the same diffraction area.[1] Thus SRM mainly finds its roots in progress made in the control and manipulation of the optical properties of single fluorescent molecules.

Super resolution microscopy revolutionized the field of cell biology enabling researchers to visualize cellular structures with nanometric resolution, single molecule sensitivity and in multiple colors. Compared with the visible spectrum, the near infrared (NIR) window (700–1,700 nm) can afford deeper tissue optical imaging with improved signal to background ratio at increased tissue depths due to reduced photon scattering, absorption and tissue autofluorescence interference.[2] But the implementation of super resolution in the NIR range comes with its limitations: higher resolution limit, lack of sensitive and affordable detectors and most importantly, designing controllable fluorescent probes that can absorb and luminesce in the near-infrared. In this context, single walled carbon nanotubes (SWCNTs) offer a class of versatile NIR emitters. SWCNTs combine strong optical resonance in NIR and exceptional luminescence signal stabilities, spectroscopic tunability in the different NIR windows, nanometer diameter, and tunable lengths, allowing morphological adaptability and easy surface functionalization could thus demonstrate promising applications in high resolution bioimaging.[2]

Carbon nanotubes (CNTs) are quasi one-dimensional nanostructures, rolled up in a graphene lattice of sp2 hybridized carbon atoms to form a hollow cylindrical structure with a very high length-to-diameter aspect ratio. When the tubes consist of a single layer of graphene, they are called single-walled carbon nanotubes (SWCNTs) and hold unique physical, chemical and opto-electrical properties compared to double or multi-walled carbon nanotubes. Their absorption spectra lie in the visible (400–750 nm) and NIR-I (750–1000 nm) windows, generating PL emission in the NIR-II (1000–1700 nm) window which makes SWCNTs interesting candidates as

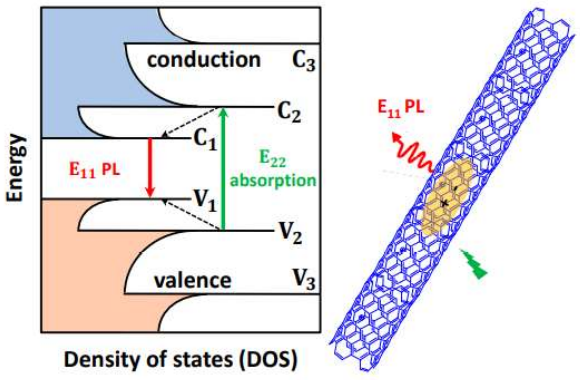

Figure 1: A representative scheme of band gap structure depicting plots of density of states with the respective electronic transitions and photophysical properties of SWCNTs[7]

probes for deep tissue fluorescence imaging. A characteristic feature of one-dimensional crystals is that their distribution of density of states is not a continuous function of energy, but consists of sharp peaks called van hove singularities. Optical transitions occur between the $v_1 - c_1$, $v_2 - c_2$, etc., labelled as E_{11}, E_{22}, etc as shown in figure 1. Optical transitions are rather sharp (~10 meV) and strong making it easy to detect individual nanotubes.[3]

An ideal probe for SRM should have controllable photoswitching and blinking properties to match the super-resolution method for which the reporter is intended.[2] Blinking photoluminescent CNTs have been observed as early as in 2008, in acidic environments or through charge transfer near surfaces, which allowed achieving super-resolution imaging of CNTs emission sites.[4] However, photoblinking was observed without control of the blinking rate or efficiency. Recently, Godin et al. generated the first milestone for the extension of PALM in the NIR through the generation of photoswitchable SWCNTs and provided a proof-of-concept for SMLM using such nanotubes.[5] They presented a hybrid nanomaterial made of (10,2) SWCNTs covalently functionalized with spiropyran–merocyanine (SP-MC), used to control the emission of SWCNTs and making them photoswitchable. SP-MC molecules were covalently attached to the SWCNTs by means of a nitrene-based cycloaddition reaction, obtaining fully conjugated SP-SWCNT hybrids through unique functionalization. Upon illumination with an ultraviolet (UV) light, loss of around 50% of the signal of the SP-SWCNTs at the ensemble level took place within seconds. When the UV lamp was taken away, the PL was recovered after a few tens of seconds. They took advantage of the induced blinking to apply an SMLM strategy (akin to Photo-activated localization microscopy) and generate super-resolved images of the nanotubes. Blinking sites were fitted by two-dimensional Gaussian distributions, and the centroid was extracted with subwavelength precision of < 22 nm. Combined with simulations, they provides the means to create photoinduced blinking CNTs having arbitrary dynamics by varying the density of functionalization or illumination.

Moreover, the elongated high-aspect-ratio structure of SWCNTs poses an additional challenge on super-resolution techniques as the size of fluorescent particles should be smaller than subcellular structures.[2] Unfortunately, the intrinsic NIR PL is known to be quenched in usCNTs because of their small size compared to the exciton diffusion length (<100 nm). Using sp^3 defects, excitons can be prevented from reaching nanotube ends by local trapping, hence resulting in bright fluorescent ultrashort CNTs. It has been shown that covalent sp^3 functionalization of SWCNT is accompanied with a red-shifted E_{11}* emission from trapped excitons compared to the nanotube's original excitonic E_{11} transition in the pristine SWCNTs. To directly observe this mechanism, Danne et al. developed a super-resolution imaging methodology which can resolve the different emission sites with <25 nm spatial resolution on individual nanotubes.[6] Fluorescent ultrashort nanotubes can constitute a milestone for biological imaging where ultrasmall, bright photostable emitters in the near-infrared biological window are vividly required.

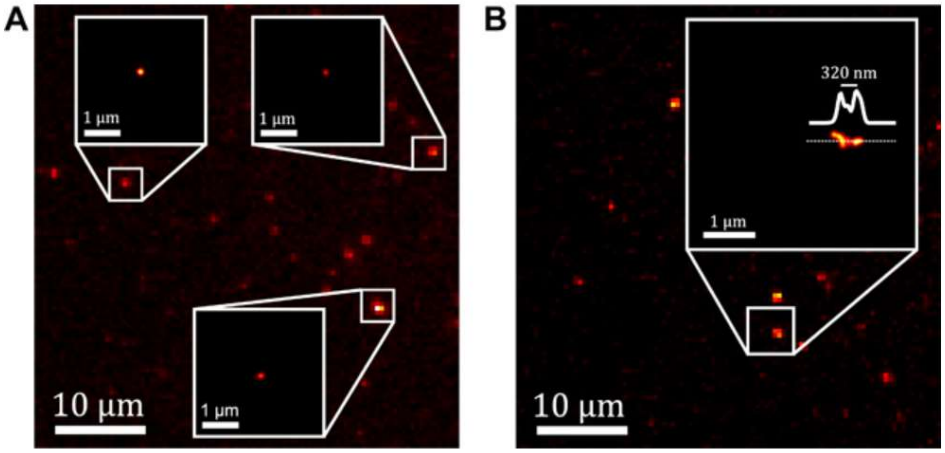

Figure 2: Super-localization and super-resolution imaging of single CNTs using photocontrolled luminescence intermittency[5]

Although a very versatile fluorescent nanoprobe, SWCNTs do suffer from shortcomings. Their relative insolubility requires the nanotubes to be surface functionalized before they can be used for bioimaging. Also, even ultrashort carbon nanotubes have a length of 40 nm which is still a significant size for SRM which considers point emitters. Thus, further improvements in nano-engineering are required to improve the efficiency of SWCNTs for super resolution imaging.

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