Carbon dots as subcellular targeting probes for super-resolution imaging

Mahima Unnikrishnan Literature Seminar November 6, 2019

Optical microscopy is, to this day, one of the most widely used visualization techniques. By using fluorescence in conjunction with confocal microscopy, we can obtain higher contrast signals and hence eliminate out-of-focus light better. However, Ernst Abbe in 1873 had come up with a law for the diffraction limit to light microscopy that stated the spatial resolution cannot improve beyond 0.2 micrometers. Electron microscopy can give a much higher resolution but is not specific enough in identifying biological matter where there is not much variation in electron densities between various species. [1] After more than a century of being crippled by this limited resolution, confocal microscopy gained new wind with the advent of super-resolved fluorescence microscopy. The 2014 Nobel Prize in Chemistry was jointly awarded to Eric Betzig, Stefan W. Hell and William E. Moerner for successfully bypassing the diffraction limit of light in optical microscopy. The pathbreaking idea was to spatially resolve the fluorescent molecules with a bright state and a dark state within the same diffraction area over the time period of imaging. This could be achieved by stimulated emission, triplet states, long-lived dark states or photoinduced cis-trans isomerization – all of which can result in "blinking" molecules. In the words of Stefan Hell, "the name of the game is on/off and molecular transitions determine imaging performance". Progress in the field now relies on designing photoswitches that can undergo multiple on-off transitions without deteoration in performance. [2]

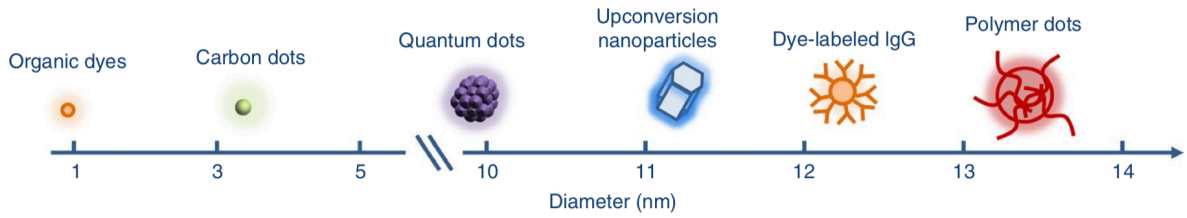

Figure 1. Physical dimensions of dye molecules, carbon dots, quantum dots, upconversion nanoparticles, dye-labeled IgG antibody, and polymer dots used in super- resolution microscopy.[3]

A vast amount of research has gone into developing luminescent nanoparticles with optimal optical properties for super-resolution imaging and single-molecule tracking since then. Semiconductor quantum dots (QDs), upconversion nanocrystals (UCNPs), polymer dots (PDs), fluorescent nanodiamonds (FNDs), carbon- based nanodots (CDs), etc. are a few of these nanoprobes and a comparison of their sizes with respect to organic dye molecules are as depicted in Figure 1. Table I lists a comparison of the same particles with respect to their performance as super-resolution imaging tools. Smaller nanoparticles (ideally <5 nm in diameter) are best for in vivo biological applications because they have more biostability, are less likely to sterically affect the function of their label target and have better chances of cell uptake. Additionally, for super-resolution imaging, size of the fluorescent particles should be smaller than subcellular structures.[3]

Even though QDs have excellent optical properties, small QDs are difficult to synthesize due to poor stability and subpar labeling specificity. Cai et al. developed streptavidin-functionalized small QDs (sQDs) of size 9 nm which were stable up to a month and could access live neuronal synapses.[4] In addition to size limitations though, toxicity is a major drawback of QDs in biological applications. [1] CDs on the other hand have no heavy metal elements in its structure like other semiconductor QDs do.

	QDot[2,3,5,23,25,26,79,80,87-89]	FND[29-31,52]	UCNP[12,13,33,34,37]	PDot[19,20,28,81]	CDot[15,16,18]
Size (super-resolution) (nm)	8–20	35	11.8, 13	13	4.5
Size (tracking) (nm)	15–20	35–45	40	15	
Microscopy resolution (nm)	54 (STED) 85 (SOFI) 67 (3B) 45 (STORM)	29 (STED)	28 (STED) 82 (STED)	142 (SOFI)	25 (STORM) 30 (STED) 184 (SOFI)

Table I. Comparison of the performance of tailored luminescent nanoparticles successfully used in subcellular super-resolution microscopy and/or single-nanoparticle tracking applications. [3]

First reported in 2004, CDs are fluorescent carbon nanomaterial with at least one dimension less than 10 nm in size. Their general makeup consists of sp^2 or sp^3 carbon and oxygen or nitrogen-based groups or polymeric aggregations. CDs can be prepared either by bottom-up methods from small organic molecules or polymer precursors via dehydration and carbonization; or by bottom-up methods such as hydrothermal, solvothermal, microwave-assisted hydrothermal/solvothermal, etc. Their photoluminescence (PL) mechanism has been widely debated. There are four PL mechanisms that have been confirmed so far : the quantum confinement effect or conjugated π-domains (originating from the carbon core) ; the surface state, which is determined by hybridization of the carbon backbone and connected chemical groups ; the molecule state (dependent on the fluorescent molecules doped to the surface or interior of the NPs) ; and the crosslink-enhanced emission effect. [5,6] CDs were first used for in vivo imaging by Yang *et al.* where they performed cytotoxicity studies on PEGylated carbon dots and found that these dots were generally nontoxic when injected into mice. [7]

Certain biocompatible CDs also exhibit emission blinking and were found to be suitable for super-resolution optical fluctuation bioimaging (SOFI). [8] A type of 4.5-nm CD has been developed with burst- like blinking behavior, and its use has been demonstrated for imaging microtubules immunostained with primary antibodies and CD-labeled secondary antibodies. The performance of the dots with that of Cy3, Cy5, Alexa Fluor 647, and CdSe/ZnS QDs 605 were first compared for imaging. CDs have more brightness than that of Cy3, is photostable for longer than 30 mins, and have a very low duty cycle. Following these promising conclusions, the authors achieved a resolution of 25 nm for stochastic optical reconstruction microscopy (STORM) with CD-stained microtubules in a cell. [9] In another study, as-synthesized malic acid carbon dots (MACDs) are also found to possess photoblinking properties that are superior compared to those of traditional organic dyes like Atto488 and TAMRA. A newly developed C_{18} reverse-phased silica gel column chromatography was used to purify the MACDs. Cell viability tests demonstrated in fixed and live trout gill epithelial cells (Figure 2) showed that these CDs are suitable for super-resolution fluorescence localization microscopy and that they are taken up easily by the cells upon incubation. [10]

A promising fluorescent nanoprobe for super-resolution microscopy, CDs need to be investigated further. Some of the big challenges where CDs are concerned are the uncertain nature of the chemical groups on the surface of these nanoparticles which arises from our lack of control over the synthetic procedures and incomplete knowledge of their PL mechanism which will require more vigorous characterization techniques. Mass production of high-quality CDs are also a work in progress. Having said that, their high biocompatibility, extremely small size and low-cost precursors render them luminescent nanoparticles to watch out for in the field of in vivo bioimaging.

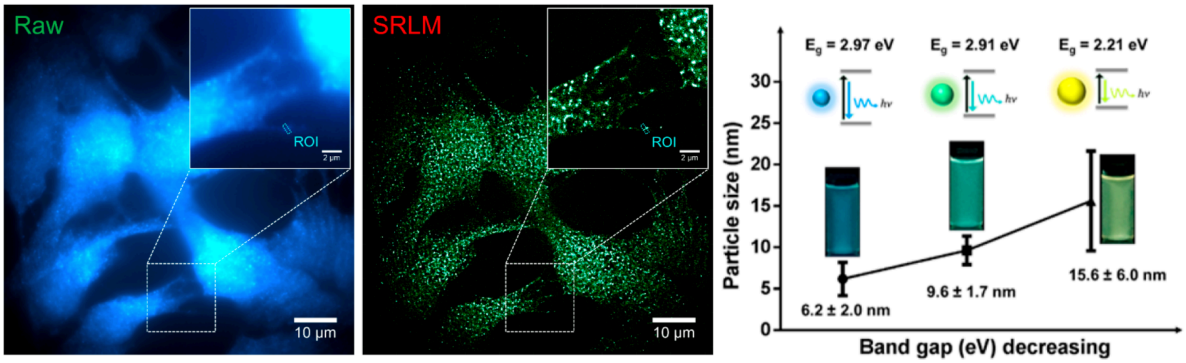

Figure 2. Super-resolution localization microscopy (SRLM) of MACDs in fixed trout epithelial gill cells. [10]

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