Infrared active nanoprobes for bio-medical imaging based on inorganic nanocrystals

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ABSTRACT

In this work we will show the results of optical imaging with a different types of inorganic nanocrystals. The list of probes including hydrophilic $NaGdF_4$:Yb, Er, $NaGdF_4$:Eu and PbS nanocrystals all synthesised and modified in our group. We will report on their optical porperties and discuss examples for perspective work for these types of markers.

Keywords: nanocrystals, optical imaging, infrared, lanthanides

1 INTRODUCTION

Optically active inorganic nanocrystals (NCs) (i.e. quantum dots, QDs) are recently widely used in research related to bio-medicine as efficient *in vivo* and *in vitro* optical markers but also as building blocks in bio-sensors. However, even if they characterize with much better optical parameters than proteins or molecular markers their clinical use is still only a future perspective. This is mainly due to three disadvantages characterized these markers: low bio-degrabillity, high toxicity and hydrophobic nature of their surface. While some of these drawbacks can be reduced or even eliminated, the rest need to be accepted as a price of their unique properties.Among these, the most interesting are their optical activity in nearinfrared spectral range (NIR), multi-functionality (simultaneous optical-MRI-CT bio-imaging), possibility of surface chemistry control, high emission quantum yield and reduction of photobleaching and photoblinking.

The special intrest in this field is focused recently on infrared active nanoprobes. Their superior position is based upon the near-infrared (NIR) optical properties. Firstly, NIR photons are less scattered and weakly absored in comparison to light from UV-VIS spectral range. Thus, because the imaging depth is restricted by scattering and absorption, by using the NIR nanoprobes it is possible to see deeper within the biological structure what is crucial for 3D imaging and clinical use. Moreover, within the NIR spectral range the autofluorescence is absent or very small. Therefore the level of nonspecific, background signal is low what signinficantly improves contrast in comparison to imaging in VIS spectral range. This feature is crucial for an acqusition of high resolution data in fluorescence microscopy (FM).

Fluorescence microscopy is an unique tool helping understanding number of fundametal process on the single cells level including both static and dynamic analysis. The quality of such investigations depends on the properties of fluoroscence probes (in terms of emission quantum yield and its stability in time, pH etc.) and performance of imaging systems. In conventional fluorescence microscopy, the lateral resolution is limited by diffraction, which results from the wave nature of light. With the shorten of the excitation wavelength the resolution improves. However, the use of the high energy of UV light is harmful for the living cells and stimulate autofluoroscence. On the other hand, recently developed super-resolution fluorescence microscopy allows for beaking diffraction barrier and imaging with resolution of order of magnitude higher than diffration limit [1].

Nowadays there are three major technics within the super-resolution miscroscopy: stimulated emission depletion microscopy (STED), structured illumination microscopy (SIM), and localisation microscopy (LM) [1]. The first approach is a confocal microscope with a diffraction limited spot which scans over the sample. By shrinking the area from which light is detected super-resolution is achieved [2]. The second technique utilizes patterned illumination that is projected on the sample to down shift high frequency information and allow it to be recorded [3]. The third group of super-resolution techniques is called mainly localization microscopy. Instead of taking a single image a composite image is built up by finding the positions of switching or blinking individual fluorophores within the diffraction limited area [1].

Comparing to STEM and SIM the localisation microscopy techniques do not require sophasticated optics with a complex scanning system. Localization microscopy uses conventional widefield microscope with high-power laser sources and optical probes that switch between two states. Image reconstruction is based on the following procedure. For every frame in image stack, single emitters are localized and fitted with point-spread-function (PSF) model function. The center of the PSF is estimated with a precision of about few tens of nm. Subsequently, accumulated image, obtained from localized positions of single emitters is generated.

The localization accuracy is determined by the number of photons emitted by a molecule, the size of the point spread function, and the background level [4]. Thus, for this

approach the brightness and photostability of the fluorescence probes are of great importance.

2 FLUORESCENT NANOPROBES

The aim of this work is to find out the optimal NCs matrix composition, which guarantees their efficient emission and/or excitation in infrared spectral range. For that purpose we synthesised three types of nanocrystals with use of wet chemistry approach: $NaGdF_4$:Yb, Er (size control from 3 to 100 nm), $NaGdF_4$:Eu (size control from 3 to 20 nm) and PbS/CdS (size control from 2 to 6 nm). Additionaly, we developed ligand exchange protocols and succeed to obtain hydrophilic NCs which are stable in time and pH, and posses NH_2 and/or COOH functional groups at the surface.

In order to choose optimal fluorescence probe, it is crucial to understand optical obstacles for both an excitation and an emitted light for given application. Figure 1 (a) shows the absorption coefficient spectra of pure water, hemoglobin and fat in spectral range from 200 nm to 1650 nm.

Figure 1 (b) shows the photoluminesce (PL) spectra of nanoprobes synthesised in our group. Our main focus within the biomedical application are PbS-based coloidal quantum dots and $NaGdF_4$:Yb^{3+},Er^{3+} (UPC) nanocrystals due to their optical activity in NIR spectral range and multimodality in case of UPC NCs. Quantum dots have their unique optical properties related to a quantum confiment effect, that allows for emission wavelength tunning with the change of their size. Due to this property it is possible to obtain a certain emission spectra within the NIR region (Fig.1 (b)). Another promising candidate for bio-medical optical imaging are UPC nanocrystals, where the excitation wavelength is located within NIR (980 nm) and the emission is in both VIS and NIR spectral region. In this case, in order to obtain a certain wavelength of emission it is necessery to change the kind of lanthanide ions (Eu^{3+}, Tm^{3+}, Er^{3+}) or to play with their relative concentration within th NCs matrix [5, 6].

3 BIO-MEDICAL IMAGING

The nanoprobes active in NIR spectral range seems to be very promissing from the application point of view. Unfortunatelly, the imaging of such probes in practice is not a trivial task due to a lack of comercial systems working at such excitation/emission conditions and high costs of components for custom made setups. In this section we show results obtained with a homemade microscopy systems. The optical setup that we build to image our probes in NIR is suitable for measaurements in broad spectral range from 400 up to 1600 nm for both imaging and emission spectra measurements. On the other hand, the system enabling excitation of probes with a several wavelengths including 532, 660 or 980 nm.

Figure 2 shows an example of fluorescence images of PbS quantum dots uptaken by macrophages. The results, presented in Fig. 2 (a) and (b) shows that the registered emission comes from the active centrers localised mainly in the cell membrane. The center maximum of PbS emission band is ~1250 nm, so within a very efficient optical channel for bio-markes. Other examples of the optical imaging, where our NIR fluorescence probes were used are reported elswhere. This includes the use of $NaGdF_4$:Eu nanocrystals for melanoma cancer cells imaging (600 and 700 nm emission bands) [7] and $NaGdF_4$:Er,Yb nanocrystals for HeLa cancer cells imaging (with 980 nm excitation band) [8].

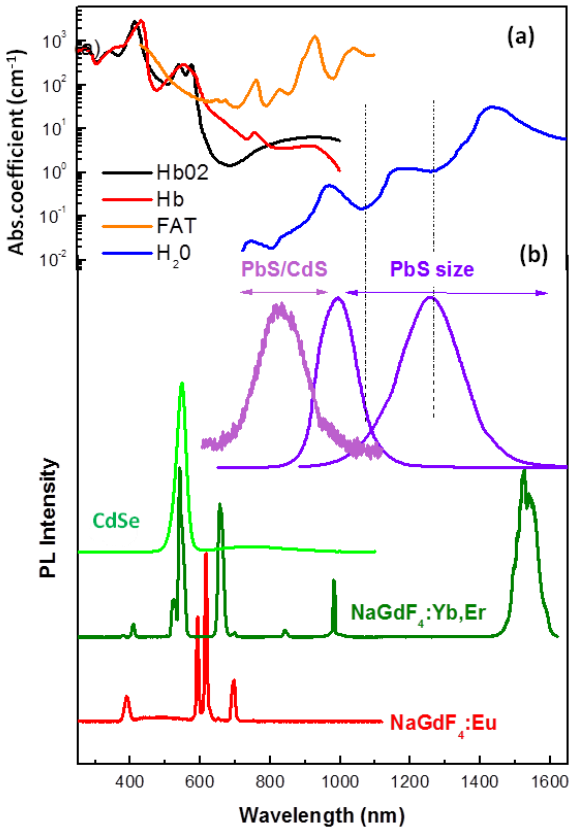

Figure 1: (a) Absorption coefficient spectra of pure water, hemoglobin and fat taken from [9-12] (b) Emission spectra recorded for $NaGdF_4$:Eu^{3+}, $NaGdF_4$:Yb^{3+},Er^{3+} and PbS nanocrystals. In addition PL spectra obtained for CdSe QDs is added for reference.

4 PERSPECTIVES

Optical blinking behavior is related with intermittents of luminesce between on-state, with higher intensity of emitted light and off-state with singnificantly lower intensity. In general, bio-markers with blinking behaviour create more problems that opportunities in advaced imaging techniques like for example in case of optical tracking microscopy. For that purpose, the non-blinking and photostable upconverting nanoparticles are more suitable

from this point of view [13]. However, their emission quantum yield is still very low enabling more advanced studies. For that reason a lot of effort has been paid to solve this issue in case of semiconducting NCs. This problem have been solved already in the case of CdS quantum dots [14] making them an ideal candidate for aformentioned applications.

On the other hand, there are however imaging techniques that take advantage of single emitters blinking behaviour. For example, the localisation microscopy has the requirement for the probe emission to be well separated in time. Blinking fluorophores has been studied intensely within recent years and scientists discovered certain demands for appropriate blinking nanoprobe.

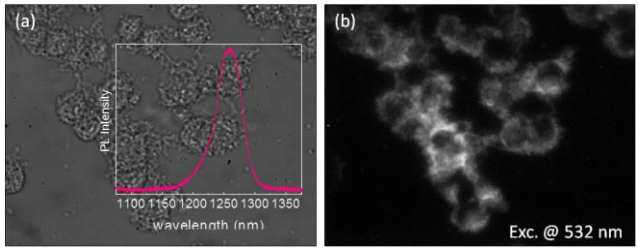

Figure 2: (a) Optical image and (b) photoluminescence image of macrophages labeled with PbS nanocrystals. Inset: emission spectrum measured for cells shown in Fig. 2 (b).

These are for example: ability to produce bright events, not to long on-states, completely dark off-state, resistanse to bleaching and the most important high blinking dynamic [15].

These demands indicates the need of a high degree control over fluorophore behaviour. Till now, there are few tested groups of materials like for example photoactivatable proteins [16], conjugated dye [17] or organic dyes such as Alexa647 [18]. All of fluorophores mentioned above show the bleaching and/or blinking phenomena what limit an emission quantum yield.

There are few analysis standards that take the advantage of blinking phenomena. One of the example is 3B analysis [19]. The output of the calculation procedure is a probability map showing the likelihood of a fluorophore being present in certain area. As arising from a number of fluorophores under going blinking and bleaching the data is modelled and leads to spatial resolution of 50 nm, though the analysis requires a minimum of several hours computational time.

In order to make the analysis technique more efficient the super-resolution optical fluctuation imaging (SOFI) has been developed [20]. It is based on the analysys of the pixel intensity fluctuation and calculation of the autocorellation function. This method essentially sharpens the PSF by raising it to the power of order of autocorrelation function and rejects uncorrelated background signal. SOFI is fast to compute, and can work with a range of blinking fluorophores including quantum dots, organic fluorophores (when they are induced to blink) and fluorescent proteins, giving a resolution as low as 50 nm [21].

So far, there is no reports on superresolution techniques using infrared optical markers. To test the potential of our nanoprobes for such imaging we measured the blinking behaviour of one of our infrared active probes. Figure 3 shows our investigation of emission character for single PbS/CdS nanocrystal, which reveal the blinking and bleaching behavior. Panel (a) shows the photoluminesces and absorbance spectra of PbS nanocrystals which were grown in our laboratory. Below, at the panel (b) fluorescence image from 2D camera recorded with integration time of 015 ms shows the spatial distribution of single coloidal PbS quantum dots. deposited on the glass substrate. Blinking behavior of a single nanocrystal (labeled in the image with a red circle) was recorded and are presented at the panel (c).

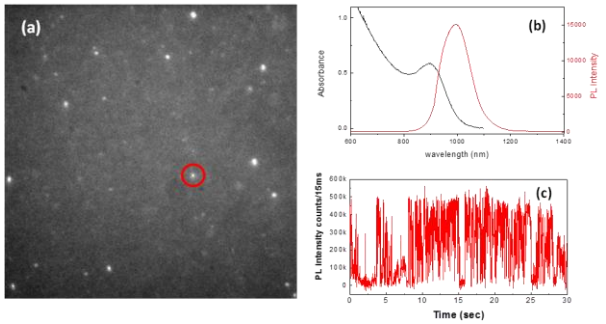

Figure 3: (a) Image of the PbS quantum dots showing separately deposited nanocrystals on the microscopic slide. (b) The absorption and emission spectra of PbS nanocrystals. (c) Blinking intensity trace recorder for single PbS NCs. The NC for which the trace was recorded is marked in a red circle at panel (a).

5 CONCLUSIONS

Quantum dots in comparison with other groups of bio-markers have many advantages like strong resistance to photobleaching, continuous absorption spectrum covering broad spectrum range, narrow emission bands, large effective Stokes shifts, long fluorescence lifetimes and excellent multiphoton emission and easy possibility to tune emission spectra with the change of the quantum dots size [22]. Because of the biological optical window, the NIR region is the very attractive in context of improving imagining and deaper investigation of living cells. It makes NIR emiting PbS quantum dots an ideal candidate for bioimaging applications. We believed that PbS NCs are also a promising probe for blinking based super-resolution imaging techniques in NIR spectral range.

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