Theoretical study of laser-based phototherapies’ improvement via upconverting nanoparticles

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Abstract. The introduction of new upconverting nanoparticles (UPCNPs) in the tumor area is being investigated worldwide as a solution for deep tissue theranostics interventions. Moreover, as the development of biophotonics techniques permits bioimaging in nanoscale, both photodynamic and photothermal sensing should be achieved even at cellular level with minimum perturbation, i.e., in absence of any physical contact between cells and sensing units at a single-cell level via optical tweezers. In our work, we discuss the biophotonic upconversion mechanism of nanoparticles’ excitation/emission at cellular level, under laser trapping conditions, via considering laser radiation of NIR (specifically at $\lambda = 808$ nm) for optimal penetration in biological tissues. Moreover, a theoretical simulation model will be presented for evaluation of the electric field distribution in optically trapped particles. Water soluble UPCNPs with maximum absorbance wavelength at $\lambda = 808$ nm and emission at 545 nm and 660 nm will be studied. The photoluminescence of biocompatible UPCNPs could provide a promising powerful tool for PDT single-cell analysis and/or for photothermal enhancement and sensing in an optical tweezers’ platform.

1. Introductory remarks to laser-based deep-tissue theranostics

The clinical translation of coherent light-based theranostics procedures, such as photodiagnosis (Laser Induced Fluorescence – LIF) and phototherapy (e.g. Photothermal Therapy – PTT, Photodynamic Therapy – PDT) has been limited to skin pathologies and tumors which are easily accessible (e.g. through endoscopes). Non-ionizing light delivering to deeper tissues, up to 1 cm, has been limited to wavelength’s emission in the so called “optical window”, spanning approximately in the near-infrared (NIR) range (from 650 to 1200 nm), where no specific tissue chromophores or exogenous photosensitizers absorb or scatter the incident light [1]. Therefore, because of limited transmittance of optical radiation in human body for treating cancer or other pathologies in internal solid organs, the relevant tissues must be approached with the appropriate light source invasively or semi-invasively (e.g. with interstitial, endoscopic, intraoperative, or laparoscopic light delivery systems). Recently, Mallidi et al. [1] highlighted in a comprehensive review the progress made in several strategies for light delivery and nanotechnology-based combinations, to facilitate deep tissue therapy beyond the traditional barriers set by tissue optical properties. Certainly, the light penetration in tissue under consideration is a critical
factor when considering photo-theranostics, an emerging and fast-growing field. Therefore, several efforts were proposed to excite photosensitizers, normally absorbing in the visible range, with NIR radiation (for deep penetration). For example, a PDT procedure has been exploited by performing a two NIR photon absorption process to excite, via a non-linear mechanism, a higher electronic energy level in the visible range [2]. However, it is well known that a nonlinear simultaneous absorption of two photons process is possible when excitation is realized by ultra-short pulsed laser radiation, restricting for example the application of two-photon PDT in vivo and even more in clinical procedures.

2. Perspectives and challenges of proposing upconverting nanoparticles

Recently, a new process entered the scene as a solution for deep tissue theranostics interventions, specifically the use of a unique type of nanomaterials called upconverting nanoparticles (UPCNPs). The term “up conversion” originates from the process of conversion of the absorbed two or more low-energy photons from NIR light into higher-energy visible light emission. This ability to convert NIR light into visible or even ultraviolet, i.e., the photon up conversion, is considered a stepwise process that happens in lanthanide ions doped into an appropriate inorganic host material, while it is not dependent on ultra-short pulsed irradiation as in the case of conventional multiphoton excitation mechanisms. The up-conversion processes, unlike the simultaneous multi-photon absorption process discussed, involves a stepwise multiphoton absorption, which only requires a low-intensity CW laser [3]. The sequential two-photon absorption mechanism of upconverting inorganic nanoparticles implies that UPCNPs emit more efficient photons of higher energy than other technologies, such as those based on quantum dots and organic nanoparticles. The reader should bear in mind that quantum dots are semiconductor nanoparticles, whose laser-induced luminescence wavelength depends on the size and the nature of the semiconductors [3].

Other paragraphs are indented (BodytextIndented style). The introduction of UCNPs in biophotonics and nanophotonics as intra-tissue light sources/emitters, transformed remarkably the fields of cancer and other pathologies diagnosis, therapy, and dosimetry. In the last decade, Wang et al [4] published a review on using UCNP-photosensitizers nanocomplexes as novel theranostics probes and for both in vitro and in vivo PDT cancer cell killing under NIR light. Discussing the perspectives of the applications of UCNPs for PDT, as well as for several other UCNP-based cancer therapeutic approaches, they argue that brighter UCNP need to be synthesized for improving the photodynamic effect. Moreover, before moving to clinical applications, the overall understanding of nanotoxicology of UCNPs should be clarified [4]. Recently, Qiu et al [5] published a review on the use of luminescent UCNPs for photodynamic therapy in cancer treatment, considering also that UCNPs hold the promise for a new generation of optical probes with great potential in biomedical imaging. Apart from several theranostics applications, a new fascinating application of UCNPs was reported last year, namely the injection of photoreceptor-binding UCNPs behind the retinas enabling mammalian eyes to see near-infrared light [6].

The introduction of new upconverting nanoparticles (UPCNPs) in the tumor area has the potential: (i) to be detected by emitting light of a shorter wavelength than the one they absorb, (ii) to further emit visible light which excites photosensitizers accumulated in tumor, causing damage to the nearby cancer cells by production of reactive oxygen species (ROS), and (iii) to be activated causing additional photothermal destruction of cancer cells [7]. Figure 1 visualize schematically the deeper penetration of NIR laser light and the activation of UPCNPs localized in tumor masses. As far as the photothermal damage of cancer cells is concerned, hyperthermia is defined as the increase of tumor temperature above physiological value (37 °C) up to 42 – 45 °C. The light-induced hyperthermic effect, by means of an external monochromatic light source, is enhanced by the use of tumor-specific nanomaterials that improve targeting and the local heating profile, resulting in cell injury and death via conversion of the strongly absorbed light to thermal energy [8]. As aforementioned, the implementation of diverse applications of UCNPs in NIR bio sensing and/or treating diseases imposes the successful integration of non-toxic biocompatible nanoparticles with biological systems (biomolecules, cells, tissues) accelerating their translation from basic research into pre-clinical and clinical applications. Hence, as
the development of biophotonic techniques permits bioimaging at nanoscale, both photodynamic and photothermal sensing should be achieved even at cellular level with minimum perturbation, i.e., in the absence of any physical contact between cells and sensing units [9] at a single-cell level via optical tweezers.

Figure 1. Artistic representation of the use of upconverting nanoparticles (UCNPs) in photodynamic therapy (PDT). Irradiation with visible light (red beam) is insufficient for the excitation of the photosensitizer molecules (PS), in contrast to irradiation with an infrared beam (IR beam). The IR light, which manages to reach the UCNPs located into the PS region, is converted to light of shorter wavelength, able to excite the PS molecules.

3. Upconverting nanoparticles applications at a single-cell level via optical trapping

Since the first report of optical trapping and manipulation of a living biological cell without damaging it by Ashkin et al in 1987 [10], laser tweezers have gained several applications from basic biophysical research at the single cell or sub-cellular level (e.g. manipulation of single DNA molecules) to biomedical applications (e.g. in vitro fertilization [11–13]), as well as for red blood cell biomechanical analysis [14] and for early detection of cancer, based on changes in viscoelastic properties of cells.

Another interesting cellular level UCNPs application, in optical trapping configuration, is their use for temperature detection originating from the upconversion fluorescence sensitivity to the medium temperature [15]. Rodríguez-Sevilla et al [9] demonstrated that thermal scanning at the cellular level is possible by simultaneous optical trapping and luminescence analysis of a single upconverting fluorescent particle behaving as sensing unit for in vitro studies (luminescence thermometry [9]). In their work, they measured the thermal gradients created in the surroundings of a single cancer cell subjected to a plasmonic mediated photothermal therapy. The Spanish group also reported that, by analyzing experimentally and theoretically the hexagonal unconverted particles’ luminescence in an optical trap, they succeeded in monitoring its orientation during the optically driven rotation as a tool for environmental sensing [16] combining optical trapping with single particle spectroscopy.

In our work, after the first indications of the biophotonic upconversion mechanisms of nanoparticles’ excitation/emission at cellular level, we assessed a theoretical simulation model for evaluation of the electric field distribution in optically trapped particles, via considering laser radiation of NIR (specifically at $\lambda = 808$ nm). Moreover, the computational modelling of optical tweezers involves calculating how light is scattered by a trapped object to calculate forces, torques and other properties of interest.
Figure 2 (a), (b) shows the light scattering of the optical tweezers by the UCNPs at the side-incidence, calculated with the solution of the Maxwell’s equations at wavelength \( \lambda = 808 \text{ nm} \). The COMSOL Radio Frequency Module\textsuperscript{TM} has been used to simulate the electromagnetic field of the optical tweezers on the UCNPs (NaYF\textsubscript{4}:Er\textsuperscript{3+} upconverting nanoparticles) immersing in water for two different geometries: spheroid nanoshells and nanorods. The laser beam was modeled as a radial polarized spherical wave propagating along the \( z \) axis with Gaussian intensity distribution in the transversal sections. The electric field amplitude \( E_0 \) is expressed by [17]:

\[
E_0 = (240nP)^{1/2}W(z)^{-1}\exp(-(x^2 + y^2)W(z)^{-2})\exp[j 2\pi/\lambda(x^2 + y^2 + z^2)],
\]

where, \( W(z) = W_0[1 + (z/z_0)^2]^{1/2} \) is the radius of the cross section of the Gaussian beam, \( W_0 = \lambda/\pi (\tan(sin^{-1}(NA/n))) \) is the waist radius with the Rayleigh range \( z_0 = \pi W_0^2/\lambda \) and with the index of refraction \( n_{1,2} \) of the media. The laser power \( P \) was set to 1.12 W and the numerical aperture of the beam NA = 1.25. The refractive index \( n_2 \) in the UCNPs and \( n_1 \) in the buffer were set to be 1.6 and 1.33 respectively.

![Figure 2](image)

Figure 2. Scattered electromagnetic field distribution on (a) a spheroid nanoshell of 35 nm diameter and 8 nm shell thickness and (b) a nanorod of 35 nm length and 3.5 nm aspect ratio. The laser beam is propagating along the \( z \) axis.

In the case of the trapped nanorod the scattering electric field presents higher values in comparison with the one of the trapped nanoshell. The electric field of the optical trap depends of the geometry of the nanoparticles which consequently affects the optical trapping efficiency.

4. Concluding remarks
The photoluminescence of biocompatible UPCNPs could provide a promising powerful tool for PDT single-cell analysis and/or for photothermal enhancement and sensing in an optical tweezers’ platform.

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