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EMERGING OPPORTUNITIES AT THE INTERFACE OF PHOTONICS, NANOTECHNOLOGY AND BIOTECHNOLOGY

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This paper presents some examples of emerging opportunities at the interface of photonics, nanotechnology and biotechnology. The examples provided here are in the areas of bioimaging for optical diagnostics and nanomedicine, and they are taken from the work conducted under a multidisciplinary program at our Institute for Lasers, Photonics and Biophotonics.

This is the era for interdisciplinary research with many challenges and opportunities created by the fusion of various disciplines. This paper presents a multidisciplinary program in Biophotonics at our Institute for Lasers, Photonics and Biophotonics that deals with the interface of Photonics, Nanotechnology and Biotechnology [1]. Our Institute involves the participation of three major academic units, College of Arts & Sciences, School of Medicine and Biomedical Research and the School of Engineering and Applied Sciences in a highly interactive environment, thus making it extremely conducive for such multidisciplinary endeavors.

Figure 1 shows the bio:nano:info interface which is capturing the attention of scientific communities, worldwide. It deals with many directions leading to new approaches for optical diagnostics, biosensing and nanomedicine, as well as to technological applications of biomaterials. In our program, the information technology is photonics based, involving optical processes.

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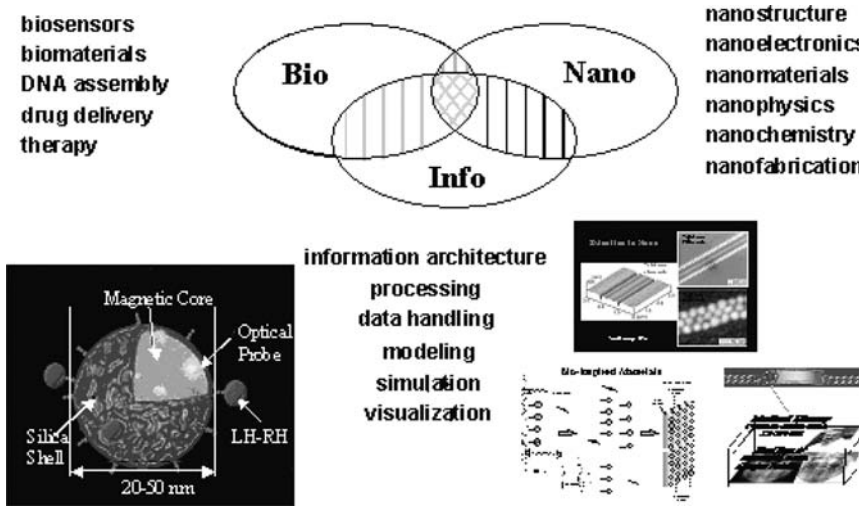

FIGURE 1 The Bio-Nano-Info interface.

In relation to photonics, this paper focuses on one type of photonic processes useful in combination with nanoscience and biotechnology. These are multiphoton processes. An example is two-photon processes, as shown in Figure 2 [1]. We have investigated particularly two of them: (i) a direct two-photon absorption in organics; (ii) the other is step-wise, two-photon

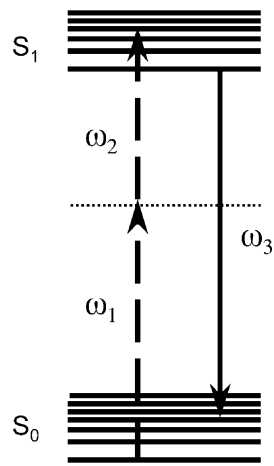

FIGURE 2 Two-Photon induced upconverted fuorescence in dyes.

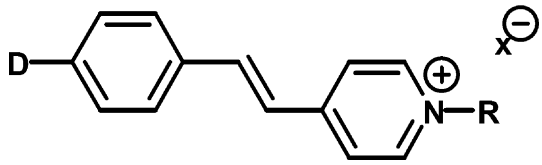

FIGURE 3 Spectrally tunable two-photon chromophore structures.

absorption in rare earth ions. Each can produce an up-converted emission, i.e. the emitted photons are of higher energy than the absorbed photons.

The availability of highly efficient two-photon absorbers has broadly impacted biomedical research. An important development for the biological applications is that of two-photon excited emitters covering the entire visible range. Figure 3 shows a simple structure where variation of the electron-donor group, D, and the counter-anion can produce up-converted emission covering a broad spectral range (excitation 800 nm), with the same excitation wavelength 800 nm for a mode-locked Ti-sapphire laser [2]. This can allow for multi-wavelength spectrally resolved bioimaging.

More recently, we reported even very efficient three-photon excited emission which suggests some more exciting possibilities [3]. This has used excitation at 1300 nm where penetration in biological sample is enhanced and the emission produced is in the visible at 553 nm.

Combination of photonics with nanoscience and technology leads to Nanophotonics [4]. We have a multi-institutional Department of Defense University Research Initiative in Nanotechnology grant on Nanophotonics. Nanophotonics deals with light-matter interactions on nanoscale, significantly smaller than the wavelength of light. Examples are near-field optical interactions which form the basis of near-field optical microscopy, and nanoscale optical materials in which optical resonances and excited state dynamics can be controlled by nanoscale manipulation.

The fusion of photonics with biomedical sciences leads to the emerging exciting field of biophotonics [1]. Optical bioimaging is a key area of biophotonics, allowing one to optically probe structures and functions at the cellular and tissue as well as organism levels. We have utilized two-photon confocal microscopy with specially designed two-photon chromophores for bioimaging. When compared with one-photon excitation, two-photon excitation laser scanning microscopy has the following advantages:

- IR excitation produces very little cellular damage, autofluorescence, and greater penetration in tissues
- Two-photon excitation is quadraticly dependent on intensity; thus the up-converted fluorescence is generated only from the focal region

The net result is that 3-D imaging at high degrees of depth and definition can be achieved.

One example of two-photon imaging is in understanding the cellular mechanism of chemotherapy [5]. This work was done in collaboration with Nobel Laurette Andrew Schally who has championed the targetted chemotherapeutic approach. We conjugated chemotherapeutic drugs, with targetting groups, to our two-photon dye for optical tracking of cellular pathways of the chemotherapeutic drug. Two-photon bioimaging, together with localized spectroscopy, showed that the chemotherapeutic agent enters the nucleus. Thus the possible mechanism of chemotherapeutic action is the shutting off of the cell replication process. This two-photon dye:targetting chemotherapeutic agent conjugate was also successfully used to image distribution of cancer at the tissue level. Bioimaging can be used not only to image cells, but also specific organelles and biological structures. We have successfully imaged a cell nucleus caught in the process of division during the cell replication cycle. Spectrally resolved imaging was used to obtain the distribution of DNA and RNA, using the acridine orange dye which emits green when intercalated in the double stranded DNA, as opposed to red emission when interacting with RNA. Again, two-photon excitation was used [6].

Another application of photonics to probe cellular interactions is the use of fluorescence recovery after photobleaching (FRAP) to investigation protein interactions and diffusion in various organelles. In our approach we used a fusion protein consisting of a protein of interest coupled to a two-photon excitable green fluorescent protein. We find different recovery times for different locations in the cell using enhanced green fluorescent protein coupled to a specific protein delta-2 (mutant of FGFR's protein) [7]. Different recovery kinetics observed in the cytoplasm and in the nucleus clearly show differences in their interaction (association) with this specific protein.

We have also been able to use bioimaging of hard tissues, dentinal tubules in teeth. Two-photon excitation of dye doped dental bonding materials using 800 nm, a wavelength that provides greater penetration depth in teeth, enables one to image the dentinal tubules [8].

Application of nanophotonics to biotechnology provides another dimension to biophotonics. One is the use of near-field microscopy for imaging of bacteria and viruses which are much smaller than the wavelength of light. Using near-field microscopy, together with two-photon excitation of the staining fluorophore at 800 nm, we have successfully imaged *P.gingavalis* bacteria.

A major application of nanophotonics to biomedical research is the use of nanoparticle platform. Table 1 shows some of the advantages of the nanoparticle platform for bioimaging and drug delivery. Our general

TABLE 1 Advantages of Nanoparticle Platform

- Mild preparative conditions
- Non-immunogenic
- Biocompatibility
- Resistant to microbial attack
- Protect doped biomolocules
- Optically transparent

approach is the use of a nanoclinic platform which is a mobile nanocapsule packaged with various diagnostics (two-photon dye, magnetic nanoparticles, drug delivery system, etc.) and surface functionalized with carrier groups to target specific cancer cells [9]. We have achieved visualization of cancer distribution, effective delivery of drugs, as well as external activation of therapy using these nanoparticle platforms. We have used the organically modified silica (ORMOSIL) nanoparticles to package a hydrophobic photodynamic therapeutic (PDT) drug for more effective distribution in biological fluids. We have established this effectiveness by cellular and tissue imaging as well as by localized spectroscopy that the hydrophobic PDT drug encapsulated in nanoparticles is more effective in distributing among cancer cells and tumor locations in tissues [10].

A major mechanism in PDT is the generation of singlet oxygen by light activation of the photosensitizer PDT drug. The question is whether the PDT drug encapsulated in the nanoparticles can generate singlet oxygen. We proved it by looking at phosphorescence at $\sim$1280 nm from the singlet oxygen. A control experiment on cell lysing shows that the hydrophobic HPPH-ormosil nanoparticles are effective in producing the PDT action [10].

Another area of application is gene therapy, where we have used the ormosil nanoparticles as non-viral vectors for DNA delivery. This approach used an ormosil composition which provided surface binding sites for DNA [11]. We used fluorescence resonance energy transfer (FRET) between the dye encapsulated within the nanoparticle and the dye intercalated in the DNA to show that DNA is actually attached to the nanoparticle surface, as FRET has a very strong distance (R^{-6}) dependence. Two-photon bioimaging with a DNA specific ethedium bromide was used to show that DNA is delivered inside the cell and also delivered to the nucleus as it stains the cell and the nucleus only when the dye is attached to DNA.

Another application of nanoparticles is the use of nanophosphors for bioimaging and photodynamic therapy [12]. These nanophosphors are yttria nanocrystals containing rare-earth which convert a CW 974 nm radiation to blue green or red depending on the nanostructure. The challenge in this case was to produce efficient up-converting emitters with small enough size for cell penetration. We succeeded in this goal and also made

TABLE 2 Future Directions in Biophotonics

- Nanomedicine
- Nanotechnology for photodynamic therapy
- Non-invasive *in vivo* imaging/diagnostic technology
- Nano-biophotonic probes (nanofluorophores)
- Multidimensional/spectral imaging technology
- Optical tracking of cellular processes in real time
- Multiphoton (three-photon) imaging
- Optical technology in proteomics and genomics
- Optical probing for single molecule biophysics
- Interfacing biophotonics with bioInformatics and bioengineering

surface functionalized nanoparticles by putting a silica shell around it. Advantage of this up-converter, as opposed to two-photon organic dye, is that a low-cost CW laser can be used to produce up-converted emission. In contrast, a two-photon organic dye requires a short pulse laser with high peak power. We have utilized these nanoparticles to demonstrate up-conversion PDT, in which the up-conversion by these nanoparticles produces activation of the photodynamic active photosensitizer drugs. Photoactivation of PDT drugs produced by up-converted emission from the nanoparticles was established by HPPH fluorescence derived from energy transfer from the nanoparticle green and red emitters. The blue emitter, energetically too far off from the absorption of the PDT dye, does not produce energy transfer.

To conclude, I believe that there are numerous multidisciplinary opportunities both for fundamental understanding of cellular processes, as well as for applications in imaging, sensing and new modalities of light guided and light-activated therapy. Table 2 lists some of these opportunities.

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