

Ultra-Efficient Plasmonic Nanoparticle Markers for Second Harmonic Imaging Microscopy

Second Harmonic Imaging Microscopy (SHIM) is rapidly becoming an accepted technique in biomedical imaging. A non-linear optical technique, SHIM is similar to the better known and established Two-Photon Excitation Fluorescence (TPEF) microscopy. Both techniques make it possible to create three-dimensional visible light images of structures deep inside tissue that would appear opaque if imaged through other techniques. SHIM differs from TPEF mainly by providing complementary information about the structure and function of cells and tissues not contained in a TPEF signal. This stems from SHIM's unique ability to measure both structural orientation and location of tissue, which generates additional contrast as well as the ability to resolve details beyond the resolving power of other microscopies. SHIM has several other advantages over TPEF such as absence of photobleaching and low phototoxicity. The main drawback with the technique, however, is its low signal level, which curtails its applicability on many occasions.

We propose to develop and test a new class of nanoparticles for use as markers in SHIM. These nanoparticles have the potential to increase SHIM's signal level thousands of times over the current state of the art. They will be small enough to be compatible with biological applications and will be coated and conjugated to bind to specific biological target molecules that can be chosen freely. For the remainder of this proposal, we will refer to the particles as Plasmonically Enhanced Particles for Second Harmonic Imaging (PEPSHI).

The nanoparticles consist of one or more noble metal particles coated by an ionic self-assembled multilayer (ISAM) film. The ISAM film is designed to have a large second-order non-linear optical susceptibility, which means it can efficiently convert light through second harmonic generation (SHG). In this process, two low-energy photons are directly combined into one photon with twice the energy. In other words, light with a long wavelength (say 1000 nm, in the

infrared) is converted into light with half the wavelength (500 nm, green). This is the optical process underlying SHIM.

For their part, the metal nanoparticles are designed with a surface plasmon resonance that concentrates the incident light into a very small volume next to the particle where the ISAM film is deposited. Because SHG is a non-linear process, it becomes more efficient when the light's intensity is increased. The addition of the metal particle can, therefore, dramatically increase the SHG efficiency if it is done properly. The metal + ISAM nanoparticle combination is especially powerful since the ISAM film naturally assembles on the surface of the metal, which is precisely where the light intensity concentration is the greatest. This makes the particles simple to fabricate using standard techniques. A first incarnation of this idea has already delivered an increase in SHG efficiency of 1600 times. After the non-linear optical (NLO) cores of the nanoparticles have been assembled, the final fabrication steps consist of coating them with a protective layer (typically polyethylene glycol – PEG) to render them biocompatible. This layer is conjugated with a specific ligand (such as folic acid) or a monoclonal antibody (such as anti-VEGFR-2 antibody) to promote targeted binding of the particles. The finished PEPShi particles can then be used as an extremely efficient contrast agent for SHIM. Also, some of the incident radiation is lost to heat in the particles, which means that they can be used for hyperthermic treatment of cancerous tumors and other disease states. The switch from imaging to treatment is accomplished by simply increasing the incident laser power by a factor of a few hundred. In other words, the nanoparticles can be used both for diagnosis and treatment of cancer. Because of the power of this synergy, we will pursue this application as the primary test bed for the nanoparticles.

We have identified several target structures expected to possess high values and be amenable to nanofabrication. We will advance the research in several distinct areas aimed at the practical realization and characterization of these structures. These areas are: **(i)** synthesis of noble metal nanoparticles with desired characteristics; **(ii)** perfecting techniques for coating nanoparticles with NLO active ISAM films and for modifying them to obtain desired particle geometries; **(iii)**

characterization of the nanoparticles through hyper-Raleigh scattering, non-linear microscopy, and other techniques; (iv) computer simulation and modeling of nanoparticle NLO performance; (v) developing and perfecting techniques for applying PEG coating onto the particles and for conjugating them; (vi) performing biostability and cytotoxicity assays on the nanoparticles; and (vii) carrying out *in vitro* studies of SHIM imaging and hyperthermia treatment on tissue phantoms and cell cultures.

The proposed research has a high probability of success because of our successful preliminary results and our synergistic skills in fabrication and characterization of optical nanostructures and devices (Robinson), self-assembly techniques, polymers, and surface chemistry (Davis), cell biology and biomedical sciences (Lee), and simulation of electro-magnetic nanostructures (Conway).

Beyond the cancer treatment application, the proposed research will help make SHIM a standard tool in biomedical imaging. SHIM is potentially useful in any imaging application where photobleaching in normal microscopy is a problem, or where imaging deep within tissue is desired. PEPSHI's also have applications as active components in bulk materials and thin films with values significantly higher than in any material that exists today.