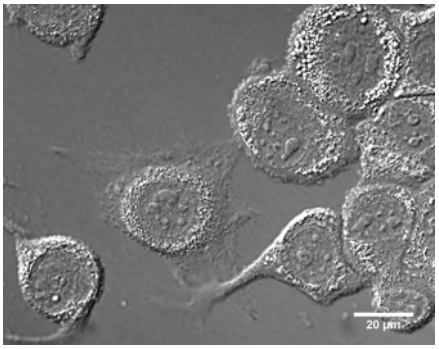
Application of luminescent nanodiamonds to intracelluar imaging

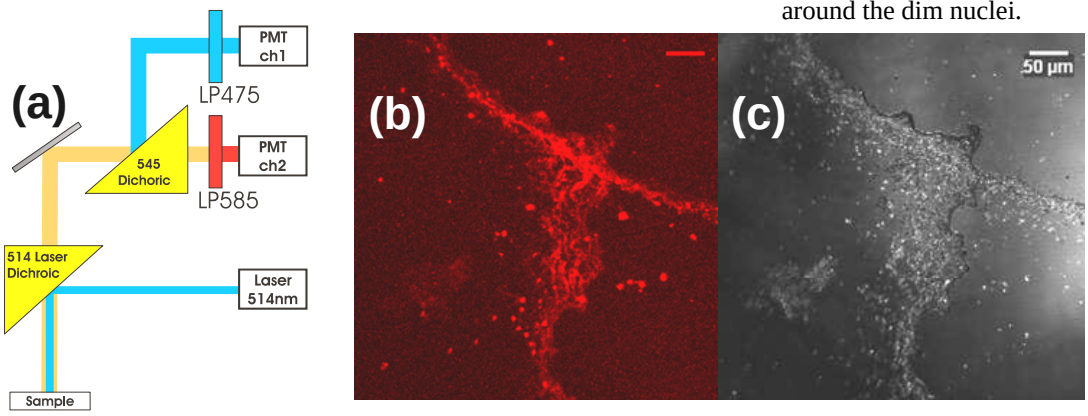
BACKGROUND

Functional intracellular imaging on the molecular level has been enabled by application of optical labels, which tag specific molecular sites to enhance their visibility on the non-specific cellular background. The optical labels can be either ultrabright scatterers, e.g. plasmon nanoparticles, or fluorophores, e.g. fluorescent dyes and quantum dots. Photoinstability, low cross-section, and toxicity of existing optical labels limit the scope of optical imaging, especially in the context of tracking individual molecules in the cells.



DIC image of scattering nanodiamonds transfected through the cells visible as bright rims around the dim nuclei.

An alternative optical label, termed luminescent nanodiamond (LND) features a nitrogen-associated vacancy defect. The luminescence properties of LND, including its lifetime and near-infrared emission band, ensure the LND high visibility on the cell morphological and autofluorescence background. The scattering properties of highly refractile diamond nanocrystals render them visible in the cells (see Figure). LND is extremely photostable. In virtue of their carbon composition, nanodiamonds are biologically compatible, and their bioconjugation is straightforward. We will report results of our collaborative research on production, characterisation of nanodiamonds, and LND-assisted intracellular imaging.



(a) Schematic diagram of the fluorescence microscope. Sample, LNDs spun-coated on a cover slip; LP475, LP585, low-wavelength-pass filters of 475-, 585-nm cutoff wavelength, respectively; ch1, ch2, channels 1, 2 that acquire the reflection, fluorescence signals, shown in (c), (b), respectively.

OUTCOMES

We anticipate the following progress in our research:

- We have developed a LND surface biofunctionalisation protocol, which should allow us to perform specific labeling of cell structures, first, *in vitro*, finally, *in vivo*.
- Efficient background-free optical imaging techniques will be demonstrated and applied to LND-assisted intracellular imaging.
- Biological problems, such as long-time virus tracking in the cell, will be addressed using LNDs making use the new optical labels merits, i.e. photostability, high optical contrast, and biocompatibility.

PROGRESS TO DATE

- A method of production of true nanometer-sized nanodiamonds has been introduced, and we can produce desirable colour centres in the nanodiamonds to render them luminescent.
- We have developed a LND surface biofunctionalisation protocol, which should allow us to perform specific labeling of cell structures.
- Several methods of LNDs transfection to cells have been tested.

THINGS STILL TO DO

- Thorough investigation of LND transfection and anti-aggregation strategy of cell labeling in vivo;
- Demonstration of background-free optical imaging of LNDs on the background of the cell autofluorescence;
- Testing LNDs for tracking of single biological molecules, e.g. proteins, in the cell.

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Background-free optical imaging of biological macromolecules

BACKGROUND

In most cases in optics, imaging resolution is limited to roughly the wavelength of light. At the same time, the optical detection sensitivity of individual particles is theoretically unlimited. Its practical achievement is limited by the signal-to-noise ratio, where the signal represents a number of detected photons from the particle, and noise comes from unwanted photons, termed background. Therefore, the progress in individual particle imaging relies on efficiency of the background suppression. In 80-s, the efficient image processing algorithm was introduced resulting in dramatic improvement of image quality, which permitted biologists to study great amount of details. For example, filamentous cellular structures, microtubules, sized 25-nm in diameter, were routinely imaged (Shotton, 1988). In recent times, imaging of individual 2.5-nm gold particles has been reported (Boyer et al., 2002) pushing detection sensitivity to such a level that optical detection of macromolecules, e.g. individual proteins, may become a reality.

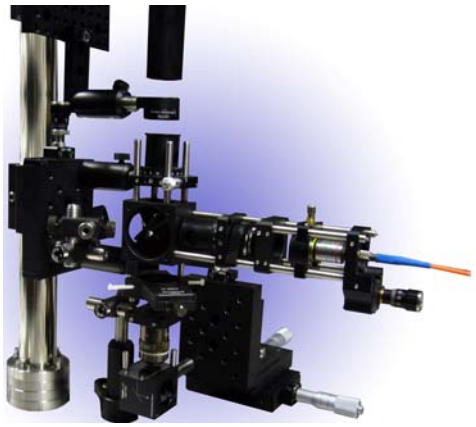

Figure 1. Reflection-mode DIC imaging t

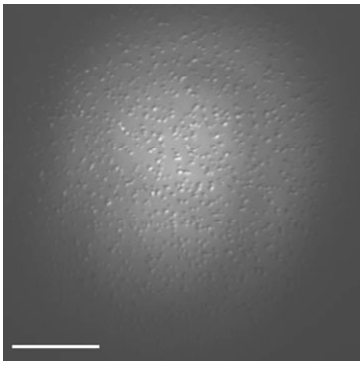

Figure 2. Reflection-mode DIC image of individual 55-nm diamond crystals. Scale bar, 10 μm.

This project aims to create a novel optical imaging system suitable for background-free imaging of nanoparticles and macromolecules. Our longer-term dream is to study conformational dynamics of proteins using this imaging system, where conformational changes should be manifested by variation of the protein image brightness.

OUTCOMES

- Design and build an ultra-sensitive imaging system to achieve background-free imaging of non-stained nanoparticles and macromolecules.
- A new tool to detect individual biological nanostructures, including single viruses. A new insight into the protein conformation problem may become a reality.

PROGRESS TO DATE

- We have carried out preliminary research into the imaging of individual nanoparticles. A reflection-mode differential interference contrast imaging system (DIC) has been built in house, as shown in Figure 1. 55-nm diamond nanoparticles, previously reported in our earlier work (Colpin et al., 2006), have been successfully imaged by using this system.

THINGS STILL TO DO

- Several steps of refinement of the existing DIC system and advanced image processing to suppress the background;

- Theoretical modeling of image formation of nanoparticles of various shapes;
- Demonstration of ultra-sensitive imaging of nanoparticles and macromolecules.

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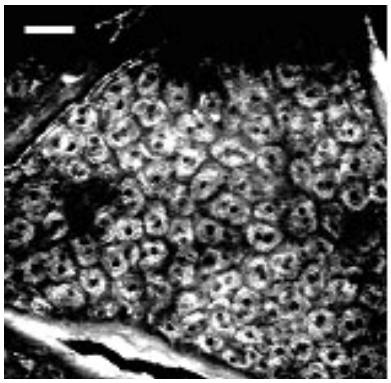
Application of multiphoton microscopy to skin imaging

BACKGROUND

Multiphoton microscopy is emerging as a new imaging modality, which enables *in vivo* imaging of biological matter on the subcellular level (Masters et al., 1997). As confocal microscopy, it permits "optical sectioning" through the specimen. At the same time, ultrashort pulses from the excitation light source stimulate 2-photon absorption and 2nd-harmonic generation processes, which enabled visualisation of the inter- and intra-cellular architecture.

In this project, we aim to advance the commercial imaging system (worth $400k) "DermaInspect" (JenI Physics) towards 3-photon absorption imaging. This will enable us to access absorption bands of numerous molecules in the ultraviolet (UV) spectral range, such as proteins and drugs. Imaging of these biologically relevant UV fluorophores will open exciting opportunities for biology and medical researchers to study *in vivo* drug delivery though skin, inflammatory processes in cells, cell calcification, and many other applications of 3-photon microscopy.



Top image: "DermaInspect" system (box on the left of the optical table) with a pulsed femtosecond laser (box on the right of the optical table), and with a bedside.
Bottom image: Epidermis layer of live skin. Note clearly resolved cells and cell nuclei. Scale bar, 20 μm.
(Courtesy of Prof König, JenLab, Inc.)

The addition of fluorescent life-time imaging capability to the "DermaInspect" system permits further differentiation of various molecular species and organelles in the cells. For example, melanin, NADH, collagen, and exogenous chromophores are clearly discriminated.

OUTCOMES

- Design and build an advanced three-photon imaging system suitable to study drug/nanoparticle delivery through skin and environmental pollution effects on skin.

PROGRESS TO DATE

- It is early days of the project. We have carried out preliminary investigations on imaging of fluorophore diffusion in skin.
- We have commenced study of nanoparticle penetration through skin versus various conditions of nanoparticle surface and skin.

THINGS STILL TO DO

- Set up and test the material dispersion compensation system to optimize the three-photon absorption process in biological specimens;
- Carry out extensive study of imaging of skin samples *in vitro* and *in vivo* to identify and characterize skin endogenous fluorophores that are excited via three-photon absorption process;

- Apply three-photon microscopy to imaging of drug and nanoparticle penetration through skin.

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