In vivo detection of exogenous contrast agents using optical coherence tomography

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Abstract: Contrast enhancement for *in vivo* OCT is investigated using plasmon-resonant gold nanorods, protein microspheres, liposomes, and iron-oxide particles. The spectroscopic, magnetomechanical and scattering properties are explored using *in vivo* rat, mouse and tadpole models.

OCIS codes: (170.4500) Optical coherence tomography; (290.1350) Backscattering; (999.9999) Contrast agents

1. Introduction

We expect that contrast agents will enhance the capabilities of optical coherence tomography (OCT) for noninvasive, micron resolution *in vivo* imaging, ultimately with clinical application. One method is to modify the backscatter to elucidate structures within the OCT image by the use of hyperosmotic agents [1] or micro air bubbles [2]. This could also be achieved by using strongly scattering or absorbing agents at near-infrared wavelengths such as protein microspheres [3] or gold nanoshells [4]. These agents enable molecular imaging by having an affinity for the molecular structure or chemical species one wishes to detect, and can exploit physiological features such as leaky vasculature in tumors. Of course, functionalizing the surface of such agents can achieve molecular sensitivity as with contrast agents in many other biomedical imaging modalities. However, the basic problem of sensitivity, that is, detecting the agents within highly-scattering tissue, remains. We have been exploring a wide variety of novel classes of contrast agents (gold nanorods exhibiting surface-plasmon resonance [5], protein microspheres incorporating nanoparticles into the shell [3] or encapsulating ferrofluids or infrared dye, liposomes encapsulating nanoparticles [6,7], and magnetic particles) and detection methods (spectroscopic and magnetomechanical OCT) in several *in vivo* models (rat, mouse, tadpole) to address this difficulty.

2. Spectroscopic OCT detection of plasmon-resonant nanorods and protein microspheres

Spectroscopic OCT (SOCT) [8] is a technique by which the wavelength-dependence of the backscattered light is measured and composed into an image. This can be used to detect contrast agents that have a wavelength-dependent absorption or scattering, which in practice must be of comparable magnitude to the tissue's inherent wavelength-dependence. The advantage to this technique is that, in principal, the agents can be distinguished from background features within the image by measuring localized changes in the scattered spectrum.

Gold nanorods exhibiting surface-plasmon resonance have been developed which exhibit sharp attenuation above 800nm. This is ideal for broadband Ti:Al_2O_3 laser-based OCT systems. The wavelength-dependent attenuation is observed using SOCT in a cuvette by measuring the spectrum of embedded scatterers and observing the shift to shorter wavelengths due to the absorption by nanorods within the optical pathway to and from the scatterers. A similar and promising observation has been made using protein microspheres (similar to those described below) which encapsulated near-infrared absorbing dye.

3. Liposomes and protein microspheres as *in vivo* scattering agents

Whether using SOCT or standard OCT, it is necessary to demonstrate that the scattering of the agents is directly detectible *in vivo*. Albumin microspheres encapsulating vegetable oil and incorporating gold, melanin, or carbon nanoparticles on their surface have been previously investigated as contrast agents for OCT [3]. Synthesized using high-intensity ultrasound, these agents are biocompatible and cleared by the hepatic and renal systems. The study

reported here made use of a similar class of albumin microsphere containing ferrofluid (colloidal magnetite ~30% w/v in vegetable oil) which typically exhibits similar scattering properties as the previously investigated microspheres. A 250μL solution of these ferrofluidic microspheres (~10^8 spheres/μL) were injected into the tail vein of a anesthetized mouse. The inner intestinal wall was exposed and imaged repetitively over 20 minutes. A region of the OCT image which appears to correspond to an area adjacent to a blood vessel was monitored for scattering changes, as shown in Fig. 1. Results indicate a significant increase in the scattering within this region when compared to the background fatty tissue.

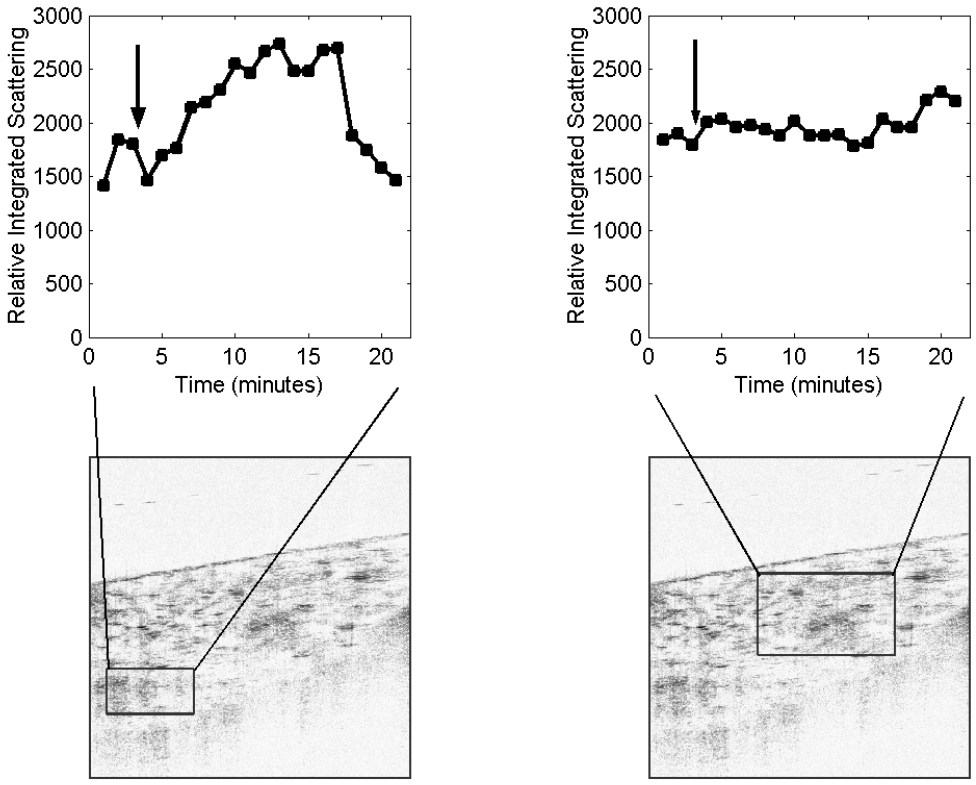

Fig. 1. Inner wall of mouse intestine during tail vein injection of protein microsphere contrast agents. Arrows indicate time of injection. Left: Relative OCT signal (backscattering) vs. time, integrated over a region adjacent to a blood-vessel (bottom). Right: Relative OCT signal from fatty tissue area vs. time. The OCT image dimensions are 1mm by 1mm.

Liposomes [6,7], consisting of a lipid bilayer encapsulating water or aqueous gold colloid, Gd_2O_3, or hematite, were also investigated as potential *in vivo* contrast agents for OCT. The liposomes are synthesized from DSPC (1,2-distearoyl-*sn*-glycero-3-phosphocholine) by extrusion. Liposomes 1μm in diameter were easily detected with OCT in tissue phantoms, and preliminary studies in an *in vivo* rat breast tumor model indicate that a solution of liposomes is detectible within the vasculature following a tail vein injection.

4. Magnetomechanical detection of ferromagnetic agents

Another means of detecting contrast agents is by utilizing a magnetomechanical effect, that is, magnetic field-induced movement of highly susceptible magnetic particles. Power to a solenoid is modulated so that alternating axial scan lines of the OCT image correspond to the magnetic field being on or off. Changes in the scattering between pairs of lines which would otherwise be identical are differenced to look for magnetic-specific motion. One advantage is that this technique may, in principal, be less dependent on the relative scattering of the contrast agent itself, if the agent can induce morphological changes to the surrounding scattering tissue. It also has the advantage of specificity of detection by the exclusion of the stationary background structures.

In vivo tadpoles (*Xenopus laevis*) were anaesthetized with 0.2% v/v benzocaine and subsequently imaged with OCT. The tadpole displayed in Fig. 2 was passively exposed to a powder suspension of micron-sized magnetite for 24 hours before imaging. It was discovered that, although stationary non-magnetic scattering objects typically exhibit no magnetic background, *in vivo* there is constantly slight motion (possibly due to cardiac and respiratory function), which is displayed as a slight magnetic background, independent of whether the magnetic field

was modulating or completely off. However, the tadpoles exposed to magnetic contrast agents consistently showed a large increase in the magnetic signal when the external magnetic field was applied, in comparison to the control. Ferrofluid-filled microspheres as described above have also been imaged within this animal model with positive results.

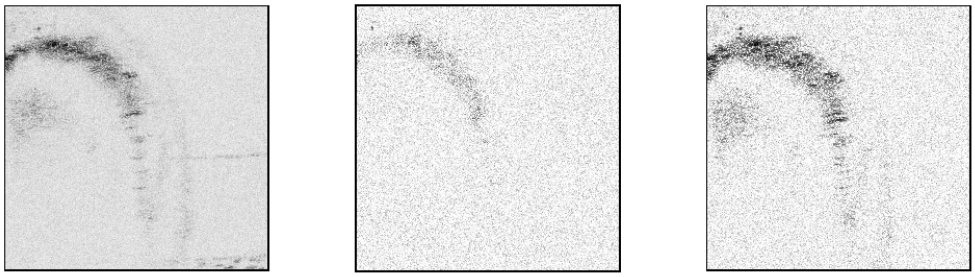

Fig. 2: Structural & magnetomechanical OCT images of the dorsal side of an *in vivo Xenopus laevis* exposed to magnetite powder. Left: Structural OCT image. Center: Magnemagnetical OCT image with the magnetic field off. Right: Magnetomechanical OCT image with the magnetic field alternating on/off between consecutive axial scans. Image dimensions are 1mm by 1mm. Pixel values for magnetomechanical images were evaluated by differencing the OCT signal between adjacent scan lines (difference between scattering with field on vs. off).

5. Conclusions

In conclusion, several novel classes of contrast agents have been investigated as potential *in vivo* contrast agents for OCT. Albumin microspheres have demonstrated significant scattering for *in vivo* detection in the vasculature post-injection, and liposomes also show similar promise. Magnetic detection in tadpoles has been demonstrated with magnetite in both powder form and encapsulated as a ferrofluid in microspheres.

Future studies will further delineate the advantages of agents with a specific dimension or chemical makeup, with the general aim of increasing the overall backscattering efficiency, optimizing the wavelength-dependent absorption or scattering signature within the laser bandwidth for SOCT, and increasing the magnetic susceptibility for magnetomechanical imaging. Surface functionalization will aid in molecular imaging with OCT, and conversely OCT can play a role in non-invasively evaluating the effectiveness of functionalized agents.

6. References

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