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Advances in Contrast Enhancement for Optical Coherence Tomography

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***Abstract*- Contrast in optical coherence tomography (OCT) images is often limited, particularly when pathological tissue is morphologically or optically similar to normal tissue. In recent years, there has been increasing interest in developing methods for enhancing OCT contrast. In general, contrast can be enhanced by the administration of passive or targeted exogenous contrast agents, or by exploiting linear and nonlinear techniques for sampling the endogenous molecular composition of tissue. Many exogenous agents, in addition to being targeted to specific cells and tissues, can also serve as multifunctional agents, delivering or facilitating therapy as well as providing enhanced contrast for imaging and localization. This paper and presentation will discuss novel OCT contrast enhancing methods designed to selectively identify tissues of interest.** [*]

I. Introduction

Contrast-enhancing techniques are used in virtually every medical or biological imaging modality including gadolinium-based agents in MRI, iodinated agents in CT, protein microbubbles in ultrasound, fluorescent probes in fluorescent, confocal, and multi-photon microscopy, and tissue staining in light microscopy of histopathology. Despite the application of optical coherence tomography (OCT) to a wide range of medical and surgical specialties in the last decade [1,2], little emphasis has been placed on enhancing the contrast in OCT images to selectively identify cells or tissues of interest. In recent years, groups have begun to investigate methods for selectively enhancing the contrast in OCT images [3,4]. These methods have included the use of hyperosmotic agents [5], exogenous contrast agents such as air-filled microbubbles [6] and engineered microspheres [7], molecular-sensitive pump-probe techniques [8,9], adaptations of near-infrared fluorescent dyes utilizing their absorption rather than their fluorescent properties [10,11], and plasmon-resonant nanoparticles [12,13]. Endogenous molecular-specific contrast enhancement has been possible using spectroscopic OCT methods [14,15] as well as nonlinear optical methods to identify molecular bonds [16-18] and ultrastructural order in biological specimens [19,20]. It is expected that contrast enhancing methods utilizing exogenous agents and endogenous molecules will significantly enhance the diagnostic capabilities and clinical utility of OCT.

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II. Exogenous Contrast Agents

A. Microspheres and liposomes as in vivo scattering agents

Protein-shelled microspheres encapsulating vegetable oil, gases, or potentially drugs; and incorporating gold, melanin, iron-oxides, or carbon nanoparticles in their shells have been investigated as scattering contrast agents for OCT (Fig. 1) [7]. Synthesized using high-intensity ultrasound, these agents are biocompatible and cleared by the hepatic and renal systems. Liposomes, consisting of a lipid bi-layer encapsulating water or aqueous gold colloid, Gd_2O_3, or hematite, have also been 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.

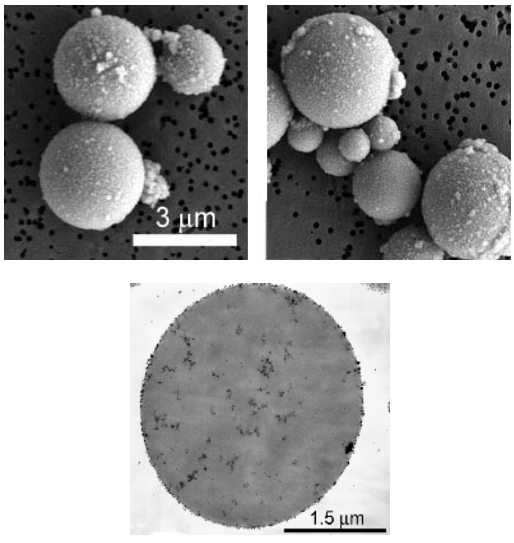

Fig. 1. SEMs and TEM of protein microspheres with embedded scattering nanoparticles to enhance local scattering in OCT images.

Recent studies have demonstrated the functionalization and targeting of these microspheres in cell culture [21]. Microspheres were functionalized with varying lengths and arrangements of RGD (arginine-glycine-aspartic acid) peptide sequences and mixed with HT29 human colon cancer cells in culture. Functionalized microspheres with terminal RGD sequences produced more effective targeting and localization to the HT29 cells overexpressing the $\alpha_v\beta_3$ integrin receptor, as viewed by fluorescence microscopy of Nile Red dye labeled

microspheres, as shown in Fig 2. Ongoing studies are investigating the targeting and OCT contrast enhancement of these microspheres in carcinogen-induced rat mammary tumor models.

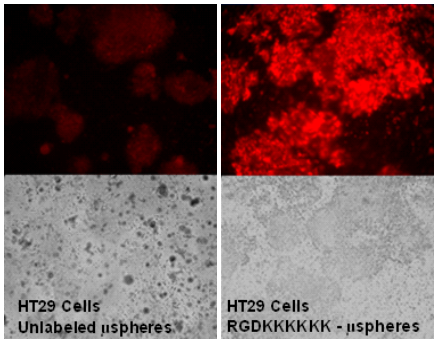

Fig. 2. Targeting of RGD-peptide-labeled protein microspheres to the overexpressed $\alpha_v\beta_3$ integrin receptor on HT29 human colon tumor cells.

B. Magnetomotive detection of ferromagnetic agents

A novel means of detecting exogenous contrast agents is to utilize a magneto-mechanical effect and track magnetic field-induced movement of highly susceptible magnetic particles (Fig. 3) [22,23]. A magnetic field from a solenoid is modulated so that selected alternating axial scan lines of the OCT image correspond to various "pulse-sequences" of 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, and provide enhanced sensitivity of detection by exclusion of the stationary background structures.

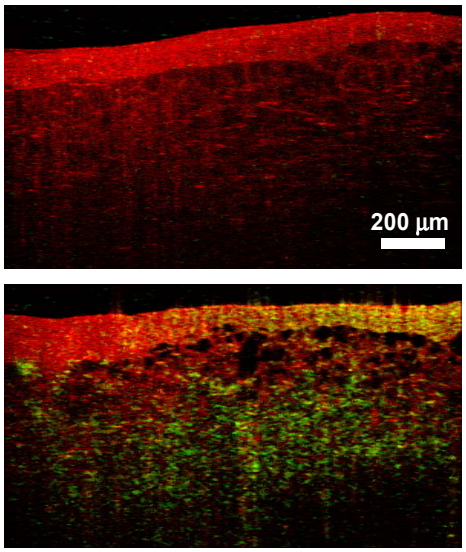

Fig. 3. Magnetomotive contrast enhancement using magnetic particles. Structural (top) and magnetically-modulated (bottom) OCT images of tissue. The magnetic nanoparticles appear in green, distributed throughout the *in vitro* chicken skin tissue.

Magnetomotive OCT (MM-OCT) has been used to track single labeled cells in three-dimensions [22], as well as define regions of magnetic nanoparticle uptake in the living *Xenopus laevis* (tadpole) [23]. MM-OCT has also been combined with Doppler OCT methods to modulate the flow patterns in blood due to the magnetic susceptibility of the iron in heme [24].

C. Near-infrared absorbing dyes

The development of near-infrared (NIR) contrast agents has been fueled by applications in confocal and multiphoton microscopy. However, the use of spectroscopic OCT (SOCT) techniques enables the spatially-resolved detection of absorption changes within the bandwidth of the OCT source. By choosing an appropriately-shaped absorption spectrum with sharp spectral features within the bandwidth of the laser spectrum, the presence of the absorbing dye selectively attenuates particular wavelengths, resulting in recovered spectra that have a shifted spectral centroid. Such a shift can be color-coded and represented in an image-based format. Dyes transported through plant vascular systems can be readily identified with SOCT and correlate strongly with fluorescence images [10].

Related methods have been developed to separate the contributions of absorption and scattering from the attenuated signal, as well as recognize two distinct absorption-mode and scattering-mode spectroscopic OCT methods for assessing the wavelength-dependent changes in the recovered SOCT spectrum [25].

D. Plasmon-resonant nanorods

Gold nanorods exhibiting surface-plasmon resonances have been developed with sharp narrow absorption bands above 800 nm (Fig. 4) [26]. This is ideal for broadband Ti:Al_2O_3 laser-based OCT systems. The wavelength-dependent attenuation has been observed using SOCT in tissue phantoms by measuring the spectrum of embedded scatterers and observing the shift in wavelengths due to the absorption and scattering by nanorods within the optical pathlength. Recent efforts have focused on the characterization and mapping of the albedo of these agents and the background tissue as an improved method for localization in tissue.

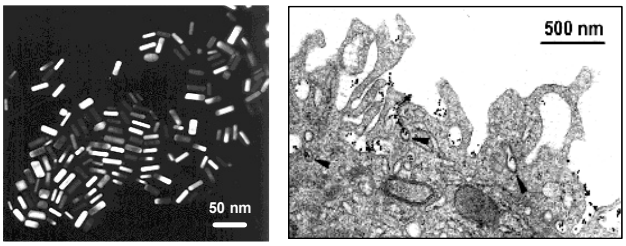

Fig. 4. Plasmon-resonant gold nanorods fabricated for OCT contrast enhancement. By varying the length:width ratio of the nanorods, a narrow absorption spectra can be tuned throughout the near-infrared wavelengths. The TEM image shows collections of nanorods on the cell membrane and within vesicles (arrows).

III. Endogenous Contrast Enhancement

A. Spectroscopic optical coherence tomography

Spectroscopic OCT is a technique by which the wavelength-dependence of the backscattered light is measured and used to reconstruct an image [14]. SOCT can be used to detect endogenous molecules or specific pathological tissues that have wavelength-dependent absorption or scattering. The advantage to this technique is that, in principal, the molecules can be distinguished from background features within the image by measuring localized changes in the scattered spectrum. The use of SOCT to detect highly absorbing exogenous agents or endogenous molecules enables many new potential contrast enhancing mechanisms for OCT.

While absorption-based SOCT methods have been discussed for the detection of near-infrared absorbing dyes, scattering-mode SOCT methods can potentially provide additional information and contrast of endogenous cell and tissue scatterers [27]. The identification and sizing of biological scatterers has been exploited in techniques such as light scattering spectroscopy (LSS) [28], such as the characterization of nuclei size in epithelium as an indicator of dysplastic changes in the tissue [29]. The use of scattering-mode SOCT utilizes similar size-dependent scattering information in a depth-resolved image-based format as shown for tissue and single cells in Fig 5.

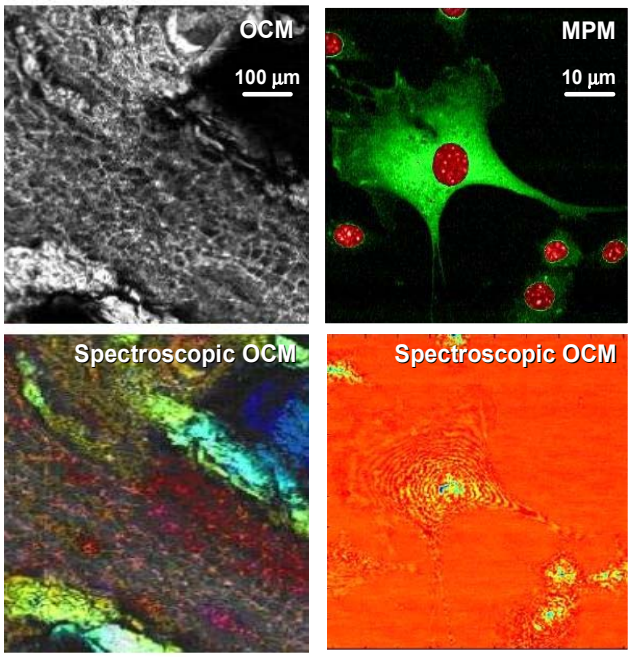

Fig. 5. Scattering-mode spectroscopic optical coherence microscopy (OCM) of rat mammary tissue (left column) and fibroblast cells (right column). Contrast enhancement (color-scale variations) in these images represents varying scatterer sizes. Most evident is the spectroscopic OCM image of single cells, where the color-variation localizes to the nuclei, as confirmed in the multiphoton microscopy (MPM) image.

B. Nonlinear interferometric vibrational imaging

We have recently developed a molecular imaging technique called Nonlinear Interferometric Vibrational Imaging (NIVI) [16-18] that utilizes Coherent Anti-Stokes Raman Scattering (CARS) spectroscopy principles for molecular sensitivity and contrast enhancement. The coherent nature of the CARS signal is exploited in a nonlinear interferometric set-up similar to OCT. Incident pump and Stokes beams are split into two arms of an interferometer. A CARS beam or a coherently-related signal is generated in the reference arm, but interference (and hence detection and spatial localization) is produced only if a corresponding CARS signal is generated from the sample. NIVI results have initially produced molecular-sensitive imaging of various molecular species, and novel interferometric-gated heterodyne-detection imaging has enabled background suppression of non-specific four-wave-mixing processes from the molecule-specific resonant signal (Fig. 6).

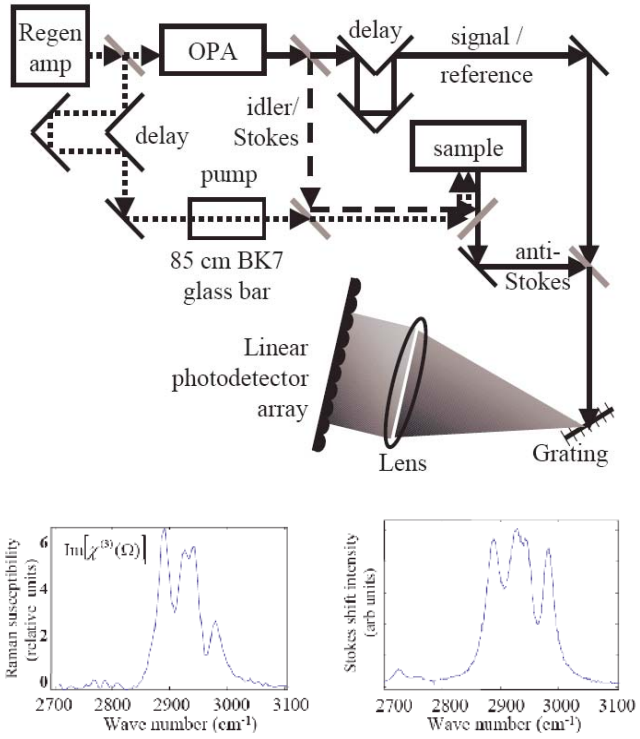

Fig. 6. Nonlinear interferometric vibrational imaging (NIVI). Schematic shows a spectral-domain implementation of NIVI, which permits rapid acquisition of spectral features in the CARS signal, along with separation of the real and imaginary components of the nonlinear susceptibility. Plots show experimental data of the imaginary component of the nonlinear susceptibility of isopropranol, which corresponds to the spontaneous Raman spectra acquired with a commercial spectrometer.

The phase information provided by the NIVI heterodyne detection permits the extraction and separation of the real and imaginary components of the nonlinear susceptibility. The imaginary component, advantageously, corresponds to the spontaneous Raman spectrum of the species [30]. Ongoing developments are focused on NIVI applications in biological specimens and for differentiating and quantifying DNA content in tissue.

C. *Second harmonic generation contrast enhancement*

Using a similar but simplified optical set-up as in NIVI, nonlinear second harmonic generation (SHG) has been used to provide enhanced OCT contrast in structures or regions that exhibit highly ordered molecules or ultrastructure [19,20]. A BBO crystal is used to generate a reference SHG signal which is subsequently interfered with SHG generated in the sample. As CARS, SHG, and many other nonlinear processes exhibit coherent scattering, much investigation remains for utilizing these processes to generate and enhance endogenous molecular contrast in tissue.

IV. CONCLUSIONS

Novel methods for contrast enhancement have been investigated and are being developed for OCT. Future studies will further delineate the advantages and limitations of each of these methods. Molecular OCT imaging methods for endogenous molecules is emerging, and functionalizing the surfaces of exogenous agents will enable targeting to cells and tissues with molecular specificity. Many agents also can be multifunctional, providing a means of delivery drugs to hyperthermia treatments in a highly localized and controlled manner. Together, these contrast-enhancing methods are expected to significantly improve the diagnostic ability and clinical utility of OCT.

REFERENCES

[1] J. S. Schuman, C. A. Puliafito, J. G. Fujimoto, *Optical Coherence Tomography of Ocular Diseases, 2nd ed.*, Slack Inc., 2004.

[2] B. E. Bouma, G. J. Tearney, Eds., *Handbook of Optical Coherence Tomography,* Dekker, New York, NY, 2001.

[3] S. A. Boppart, A. L. Oldenburg, C. Xu, D. L. Marks, "Optical probes and techniques for molecular contrast enhancement in coherence imaging," *J. Biomed. Opt*. vol. 10, pp. 041208-1-14, 2005.

[4] C. Yang, "Molecular contrast optical coherence tomography: a review," *Photochem. Photobiol.* vol. 81, pp. 215-237, 2005.

[5] R. K. Wang and J. B. Elder, "Propylene glycol as a contrasting agent for optical coherence tomography to image gastrointestinal tissues", *Lasers Surg. Med*. vol. 30, pp. 201-208, 2002.

[6] J. K. Barton, J. B. Hoying, and C. J. Sullivan, "Use of microbubbles as an optical coherence tomography contrast agent," *Acad. Radiol*. vol. 9, pp. S52-S55, 2002.

[7] T. M. Lee, A. L. Oldenburg, S. Sitafalwalla, D. L. Marks, W. Luo, F. Jean-Jacques Toublan, K. S. Suslick and S. A. Boppart, "Engineered microsphere contrast agents for optical coherence tomography", *Opt. Lett*. vol. 28, pp. 1546-1548, 2003.

[8] K. D. Rao, M. A. Choma, S. Yazdanfar, A. M. Rollins, and J. A. Izatt, "Molecular contrast in optical coherence tomography by use of a pump-probe technique," *Opt. Lett*. vol. 28, pp. 340-342, 2003.

[9] C. Yang, M. A. Choma, L. E. Lamb, J. D. Simon, J. A. Izatt, "Protein-based molecular contrast optical coherence tomography with phytochrome as the contrast agent," *Opt. Lett.* vol. 29, pp. 1396-1398, 2004.

[10] C. Xu, J. Ye, D. L. Marks, S. A. Boppart, "Near-infrared dyes as contrast-enhancing agents for spectroscopic optical coherence tomography", *Opt. Lett*. vol. 29, pp. 1647-1649, 2004.

[11] C. Yang, L. E. L. McGuckin, J. D. Simon, M. A. Choma, B. E. Applegate, J. A. Izatt, "Spectral triangulation molecular contrast optical coherence tomography with indocyanine green as the contrast agent," *Opt. Lett.* vol. 29, pp. 2016-2018, 2004.

[12] J. Chen, F. Saeki, B. J. Wiley, H. Cang, M. J. Cobb, Z.-Y. Li, L. Au, H. Zhang, M. B. Kimmey, X. Li, Y. Xia, "Gold nancages: bioconjugation and their potential use as optical imaging contrast agents," *Nanoletters* vol. 5, pp. 473-477, 2005.

[13] C. Loo, A. Lin, L. Hirsch, M.-H. Lee, J. Barton, N. Halas, J. West, R. Drezek, "Nanoshell-enabled photonics-based imaging and therapy of cancer," *Tech. Cancer Res. Treat.*, vol. 3, pp. 33-40, 2004.

[14] U. Morgner, W. Drexler, F. X. Kartner, X. D. Li, C. Pitris, E. P. Ippen, J. G. Fujimoto, "Spectroscopic optical coherence tomography", *Opt. Lett*. vol. 25, pp. 111-113, 2000.

[15] B. Hermann, K. K. Bizheva, A. Unterhuber, B. Považay, H. Sattmann, L. Schmetterer, A. F. Fercher, W. Drexler, "Precision of extracting absorption profiles from weakly scattering media with spectroscopic time-domain optical coherence tomography," *Opt. Express* vol. 12, pp. 1677-1688, 2004.

[16] D. L. Marks, S. A. Boppart, "Nonlinear interferometric vibrational imaging," *Phys. Rev. Lett*. vol. 92, pp. 123905-1-4, 2004.

[17] C. Vinegoni, J. S. Bredfeldt, D. L. Marks, S. A. Boppart, "Nonlinear optical contrast enhancement for optical coherence tomography," *Optics Express*, vol. 12, pp. 331-341, 2004.

[18] J. S. Bredfeldt, C. Vinegoni, D. L. Marks, S. A. Boppart, "Molecularly sensitive optical coherence tomography," *Opt. Lett*. vol. 30, pp. 495-497, 2005.

[19] Y. Jiang, I. V. Tomov, Y. M. Wang, Z. P. Chen, "High-resolution second-harmonic optical coherence tomography of collagen in rat-tail tendon," *Appl. Phys. Lett.* vol. 86, pp. 133901-1-3, 2005.

[20] M. V. Sarunic, B. E. Applegate, J. A. Izatt, "Spectral domain second harmonic optical coherence tomography," *Opt. Lett.* vol. 30, pp. 2391-2393, 2005.

[21] F. Jean-Jacques Toublan, S. A. Boppart, K. S. Suslick, "Tumor targeting by surface modified microspheres", *J. Am. Chem. Soc.* vol. 128, pp 3472-3473, 2006.

[22] A. L. Oldenburg, J. R. Gunther, S. A. Boppart, "Imaging magnetically labeled cells with magnetomotive optical coherence tomography", *Opt. Lett*. vol. 30, pp. 747-749, 2005.

[23] A. L. Oldenburg, F. Jean-Jacques Toublan, K. S. Suslick, A. Wei, S. A. Boppart, "Magnetomotive contrast for *in vivo* optical coherence tomography", *Opt. Express* vol. 13, pp. 6597-6614, 2005.

[24] J. Kim, J. Oh, T. E. Milner, J. S. Nelson, "Hemoglobin contrast in magnetomotive optical Doppler tomography," *Opt. Lett.* vol. 31, pp. 778-780, 2006.

[25] C. Xu, D. L. Marks, M. N. Do, S. A. Boppart, "Separation of absorption and scattering profiles in spectroscopic optical coherence tomography using a least-squares algorithm," *Opt. Express* vol. 12, pp. 4790-4803, 2004.

[26] A. Wei, S. A. Boppart, "Plasmon-resonant nanorods as multifunctional contrast agents for optical coherence tomography," National Institutes of Health, Grant 1 R01 EB001777-01, 2003.

[27] C. Xu, C. Vinegoni, T. S. Ralston, W. Luo, W. Tan, S. A. Boppart, "Spectroscopic spectral-domain optical coherence microscopy," *Opt. Lett*. vol. 31, pp. 1079-1081, 2006

[28] A. Wax, J. W. Pyhtila, R. N. Graf, R. Nines, C. W. Boone, R. R. Dasari, M. S. Feld, V. E. Steele, G. D. Stoner, "Prospective grading of neoplastic change in rat esophagus epithelium using angle-resolved low-coherence interferometry," *J. Biomed. Opt*. vol. 10, pp. 051604-1-10, 2005.

[29] V. Backman, M. B. Wallace, L. T. Perelman, J. T. Arendt, R. Gurjar, M. G. Muller, Q. Zhang, G. Zonios, E. Kline, J. A. McGilligan, S. Shapshay, T. Valdez, K. Badizadegan, J. M. Crawford, M. Fitzmaurice, S. Kabani, H. S. Levin, M. Seiler, R. R. Dasari, I. Itzkan, J. Van Dam, M. S. Feld, "Detection of preinvasive cancer cells," *Nature* vol. 406, pp. 35-36, 2000.

[30] G. W. Jones, D. L. Marks, C. Vinegoni, S. A. Boppart, "High-spectral-resolution coherent anti-Stokes Raman scattering with interferometrically detected broadband chirped pulses," *Opt. Lett.* Vol. 31, pp. 1543-1545, 2006.