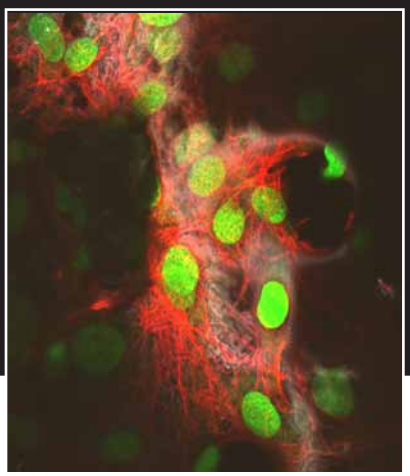
Quantitative Bioimaging

Objective

Our goal is to develop noninvasive optical methods and high affinity probes for quantitative imaging-based characterization of cell state and cell-biomaterial interactions, including molecular signatures of cellular proliferation and differentiation. These methods will lead to increased precision and accuracy in evaluating biomaterials and in determining phenotype homogeneity in expanded cell products. This work will thereby facilitate accelerated development of materials and expanded progenitor cells for use in regenerative medicine.



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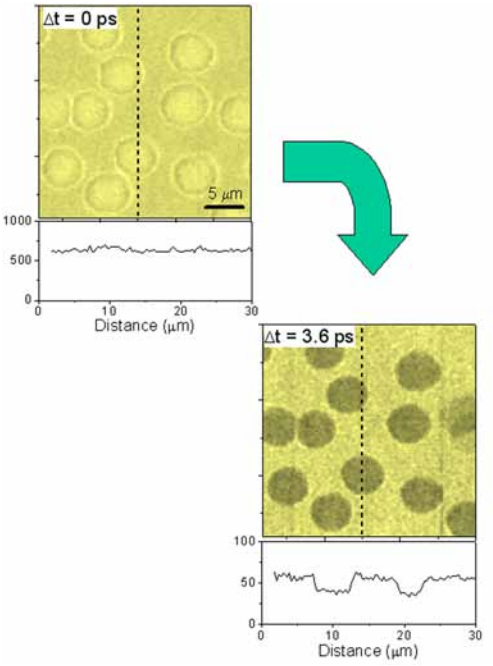


- The Multi Agency Tissue Engineering Science Working Group assessment has estimated the market for tissue engineering based products to be in excess of $3.7 billion dollars. The assessment specifically highlights the need for new tools to characterize the state of complex tissues and the cells within them. The activities in this project address this need.
- We were the first to demonstrate a broadband (3000 cm^{-1}) Coherent anti-Stokes Raman Scattering (CARS) microscope which we are using to identify chemical fingerprints for cell phenotype, differentiation state and disease signatures in clinical specimens.
- The Biomaterials Group is participating as a core member of a NIH P41 Resource Center (the New Jersey Center for Biomaterials), as well as with other Federal Agencies (NIBIB, FDA, DoD), to apply the imaging methods we develop to advance tissue engineering and regenerative medicine.
- The Biomaterials group is collaborating with world leaders in human pathology (Armed Forces Institute for Pathology, University of Nebraska Medical Center) to further benefit medical practice by transferring our imaging technology to them.

Approach

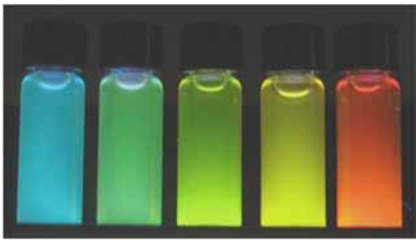
Imaging and image analysis are used ubiquitously in biological and biomedical research, such as regenerative medicine. Imaging is also used widely in medical diagnosis and treatment evaluation. One of the great advantages of imaging is its intuitive information content; much can be learned from just a glance at the data. On the other hand, these data generally contain a very large amount of untapped information. For example, characterization of spatial phenotype heterogeneity in cells on a tissue scaffold is a critical question in tissue engineering, histopathology, and basic systems biology. There is currently no way to obtain such information short of using destructive and very labor intensive approaches.

This and similar problems could be solved in a very straightforward way through imaging with the use of novel contrast mechanisms. We are developing a number of spectroscopic and physical imaging approaches that will allow us to obtain detailed, spatially resolved chemical and dynamic information in an experimentally simple and noninvasive way. We are also developing an optically clear tissue scaffold system that permits imaging deep into its interior, and statistically sound, quantitative algorithms for extracting reliable information from the various imaging modalities we use. The combination of tools we are developing will help researchers solve many longstanding problems in regenerative medicine, medical research, and in basic biology.

Accomplishments

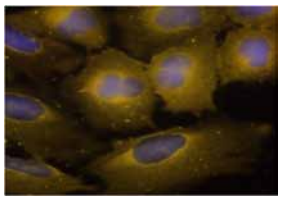
Biomarker Probes

We have developed facile methods to synthesize aptamer-derivatized quantum dots for use as imaging probes for disease signatures. The methods are scalable and cover the visible spectrum affording opportunities for multiplexed detection of 3-5 biomarkers.



QD probes of varying size

We are using these probes to detect cell differentiation, proliferation and stemness in vitro and in clinical specimens. In addition, we are using these probes to identify cofactors in thyroid cancer lesions.



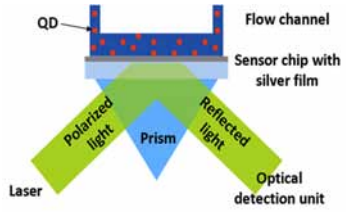
Cells tagged with QD probes for telomerase, a cancer marker

We are developing automated microscopy methods for collection, handling and statistical interpretation of multichannel fluorescence data. This compliments our probe development efforts, and affords opportunities to establish correlations on highly complex data sets and clinical pathology specimens.

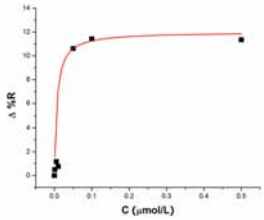
SPR Imaging

Using surface plasmon resonance (SPR) imaging we seek to measure the adsorption potential of engineered quantum dots on surfaces and interfaces. This is also important for measuring the binding constants of our biomarker probes to their specific targets, and in understanding the interaction with membranes and other biological substrates.

In this work, we specifically focused on the interaction of model nanoparticles with a



collagen layer in a controlled microfluidic environment. We investigated the utility of Langmuir adsorption measurements for characterizing quantum dot-substrate interactions.



Binding curves for quantum dots adsorption to collagen layer in the microchannels

D %Rmax ≈ 12, KL ≈ 150.8 mM^{-1}

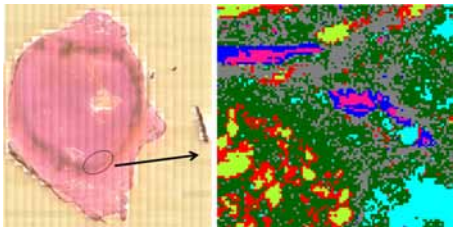
A D qmax = 0.70° results in a surface coverage of ≈ 26 % for the quantum dots on the collagen layer at pH 7.4 and 25 °C.

This will allow us to reliably determine the chemical identity of surfaces (e.g. in phase separated polymer blend scaffolds) by simple fluorescence microscopy.

CARS Microscopy

We first demonstrated broadband Coherent anti-Stokes Raman scattering (CARS) microscopy, and continue to develop this powerful microscopy. This method uses three input light pulses to read out vibrational susceptibility of a sample, and possesses the inherent chemical sensitivity required to spatially map cell phenotype noninvasively.

As initially demonstrated, this microscopy had sufficient specificity and sensitivity to rapidly acquire a 3-dimensional chemical map of materials, such as polymer blends. However, an intrinsic nonresonant background limited sensitivity to weak signals emanating from biological systems. Over the past four years we have developed improved signal generation methods, as well as signal background reduction and analysis methods that have helped to improve sensitivity and specificity of this method sufficiently that it can be applied successfully to biological systems. Below is a biopsy of a cancerous thyroid, chemically mapped using broadband CARS microscopy. The pseudocolored image shows clear differences in cancerous and noncancerous regions of the tissue.



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Publications

Aamer KA, Stafford CM, Richter LJ, Kohn J and Becker ML *Thin Film Elastic Modulus of Degradable Tyrosine-Derived Polycarbonate Biomaterials and Their Blends* Macromolecules, 42(4) 1212-1218 (2009)

Roy MD, Stanley SJ, Amis EJ and Becker ML *High Specificity Hydroxyapatite Binding Motif Identified via Phage Display* Advanced Materials, 20(10) 1830-1836 (2008)

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