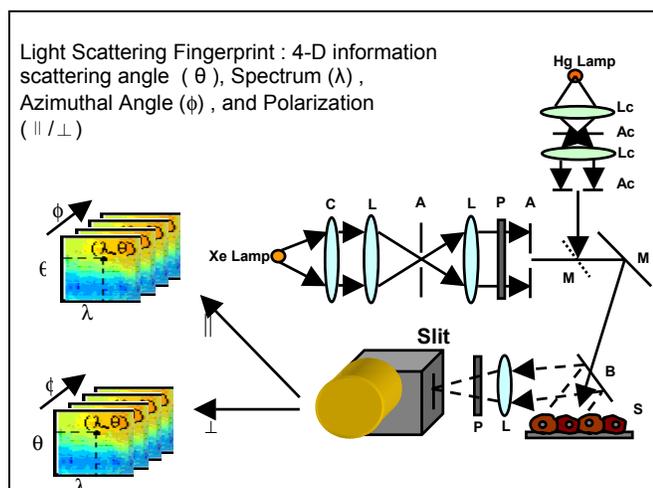


## Assessing cell-material interactions on a novel biodegradable elastomer

Josephine Allen, Yang Liu, Vladimir Turzhitsky, Vadim Backman, and Guillermo Ameer  
Biomedical Engineering Department, Northwestern University, Evanston, IL 60208, USA

### Introduction

The characterization of cellular interactions with a biomaterial surface is important to the development of novel biomaterials. Traditional methods used to characterize processes such as cell adhesion, retention, growth, and phenotypic differentiation on biomaterials are time consuming, destructive, and do not allow for in situ quantitative assessment. The overall goal of this research is to describe the interactions between cells and a newly developed biodegradable elastomeric biomaterial using a technique referred to as 4-D elastic light-scattering fingerprinting (4D-ELF).



**Figure 1. Schematic representation of 4D-ELF instrument**

**Xe**: xenon lamp, **Hg**: mercury lamp, **C**: condenser, **L**: lenses, **A**: apertures, **P**: polarizer, **M**: mirrors, **B**: beam splitter, **SS**: sample stage, **SP**: spectrograph, and **CCD**: CCD camera. **Lc**: calibration lenses, **Ac**: calibration apertures

4D-ELF exploits the fact that the spectral and angular distribution of light scattered by cells is determined by the size and refractive index distribution of the cellular components. 4D-ELF imaging provides quantitative information about histological properties of the cell at the

submicron scale. The 4 dimensions of 4D-ELF are (1) wavelength of light, (2) the scattering angle, (3) azimuthal angle of scattering, and (4) polarization of scattered light. The combination of these measures enables quantitative characterization of the subcellular architecture, which can potentially be correlated with the state of differentiation of the cells. We use 4D-ELF to describe, quantitatively, the interactions between human aortic smooth muscle cells (HASMC) and various substrates including the newly developed biomaterial poly-1,8-octanediol-co-citrate (POC).

### Experimental

**Cell Culture and Synchronization.** HASMCs, were grown at 37°C, 95% relative humidity and 5% CO<sub>2</sub> for 5-7 days on either 25ug/ml laminin or fibronectin coated glass cover slips or the elastomer, POC, to synchronize them into the contractile and proliferative phenotypes respectively. The proliferation index was assessed using the Bromo-2'-deoxy-uridine (BrdU) assay. To confirm SMC synchronization into these phenotypes, cells were probed with phenotype specific antibodies. Immunohistochemistry was performed using antibodies specific for smooth muscle myosin-heavy chain (SM-myosin-HC) and smooth muscle  $\alpha$ -actin.

**Polymer synthesis.** Equimolar amounts of citric acid and 1,8-octanediol to a 250ml three-neck round bottom flask fitted with an inlet and outlet adapter. The mixture was melted under flow of nitrogen gas by stirring at 160° C-165° C in a silicon oil bath, and then the temperature is lowered to 140° C. The mixture is stirred for another hour at 140° C to create the pre-

polymer solution. (Yang et. al. 2004) The pre-polymer was post-polymerized at 80° C for 3 days to create a thin POC film on which to seed the cells.

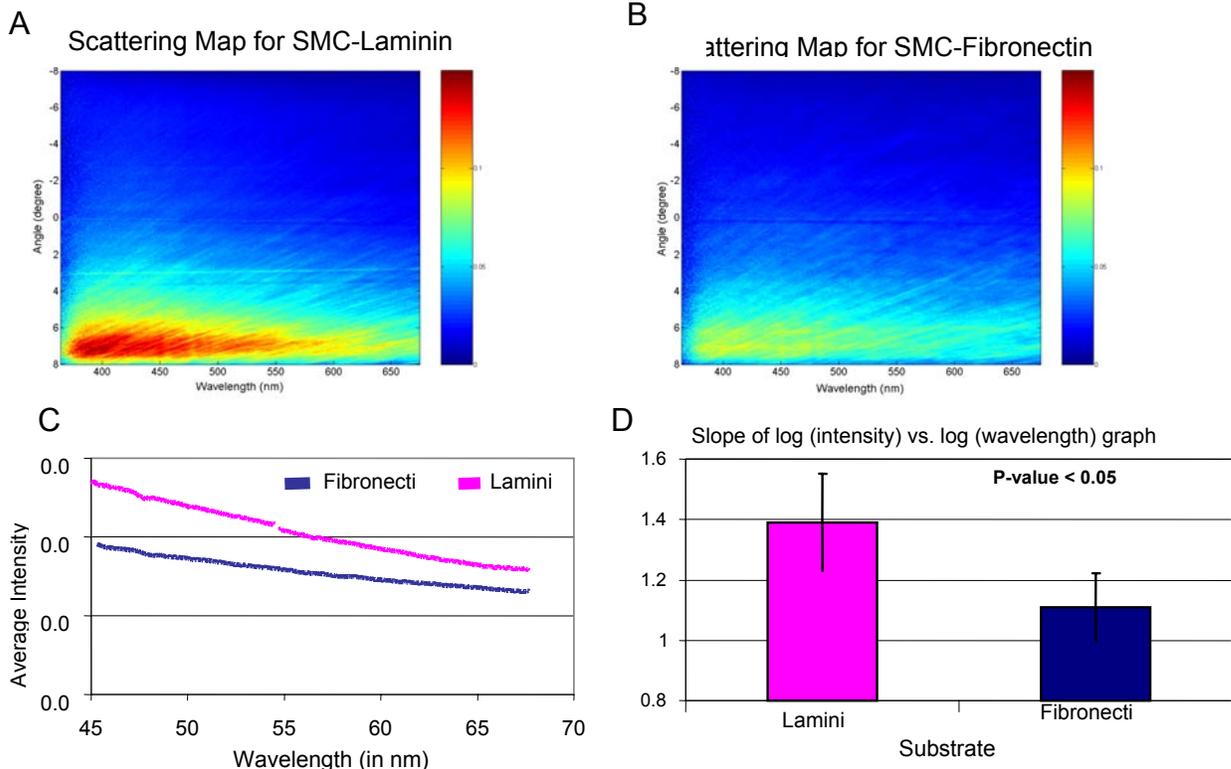
**Spectroscopic Analysis.** A cell site, 1mm in diameter, is illuminated by a xenon lamp. The light scattered by the cells is collected by means of an analyzing polarizer, fourier lens, and the imaging spectrometer coupled to the charge coupled device (CCD). The instrument records a matrix of the distribution of scattered light intensity, where one axis corresponds to the wavelength of light and the other to the angle of scattering.

**Microscopy.** To visualize the morphological differences between the two phenotypes, transmission electron microscopy (TEM) images of the cells in each phenotype were obtained. Primary fixation of the cells was done in 2% paraformaldehyde/ 2% gluteraldehyde. The cells were post-fixed in 2% osmium tetroxide, dehydrated in graded ethanol, and embedded in Epon resin. Sections were placed on 200 mesh copper grids, stained with uranyl acetate, lead citrate, and viewed on a Jeol 1220 TEM at 80 kV.

## Results & Discussion

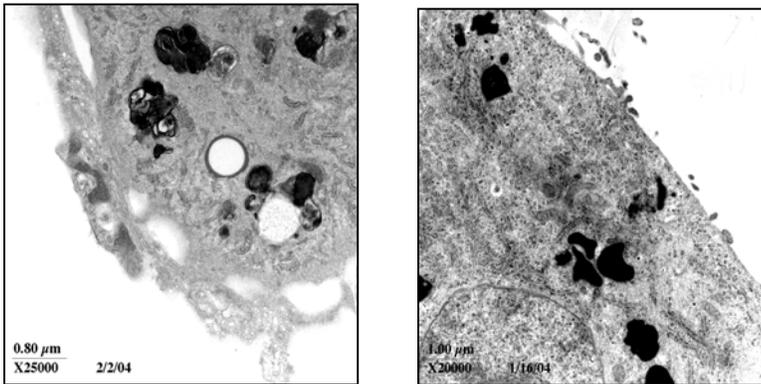
**SMC phenotype synchronization** (data not shown). The cells grown on laminin coated glass cover slips had high expression of the contractile markers, myosin-HC and SM  $\alpha$ -actin as well as a low proliferative index, indicating that they are in the contractile phenotype. In comparison, the cells grown on fibronectin coated cover slips showed low expression of the contractile markers, and a high rate of proliferation, indicating that these cells were in the proliferative phenotype.

**Spectroscopic Evaluation.** The 4D-ELF data indicated that based on the spatial and size distribution of the subcellular organelles, we could quantitatively distinguish between the morphology of the two populations of SMCs.



### **Figure 2. 4D-ELF Data from SMCs grown on POC**

A&B) Spectral fingerprints recorded from SMC grown on laminin and fibronectin coated POC. The color represents the intensity of backscattered light. The differences between these fingerprints indicates significant morphological difference between the two populations of SMCs. C) Graph of the average intensity vs. wavelength. Graphical representation of the spectral fingerprints in A & B above. D) Graph of the slope of log (intensity) vs. log wavelength graph. The data indicate that based on the smaller spectral slope, SMCs grown on fibronectin coated POC have a greater abundance of larger subcellular organelles, as compared to the SMCs grown on laminin coated POC. These data are consistent with the 4D-ELF data obtained for SMCs grown on laminin and fibronectin coated glass cover slips.



**Figure 3. TEM images of SMCs grown on laminin (A) and fibronectin (B) coated glass cover slips**

To elucidate the subcellular morphological characteristics, and correlate the 4D-ELF data with structural information, we obtained transmission electron microscopy (TEM) images. The TEM images

showed that the two phenotypes had very different subcellular morphological characteristics. The cells in the proliferative phenotype had an abundance of “larger” organelles involved in proliferation such as, extensive and enlarged, rough endoplasmic reticulum and golgi stacks. In contrast, the cells in the contractile phenotype had an abundance of organelles involved in contraction, such as, extensive filament networks.

Herein we have shown that 4D-ELF is sensitive enough to detect differences in subcellular characteristics of SMCs grown on different substrates. The spectral analysis of light scattered by living tissues provides information about the internal structures within the cell. This enables us to obtain unique quantitative information about the microarchitecture of living cells and tissues as they interact with various substrates.

### **References**

1. **Backman V. et al.** Detection of preinvasive cancer cells. *Nature* 406: 35-36, 2000.
2. **Hemant, K.R. et al.** Four-Dimensional Elastic Light-Scattering Fingerprints as Preneoplastic Markers in the Rat Model of Colon Carcinogenesis. *Gastroenterology* 2004;126:1071–1081
3. **Worth, N.F. et al.** Vascular smooth muscle cell phenotypic modulation in culture is associated with reorganisation of contractile and cytoskeletal proteins. *Cell Motil Cytoskeleton* 49: 130-145, 2001.
4. **Yang, J. et al.** Novel Citric-acid based biodegradable elastomers for tissue engineering. *Advanced Materials*, 16, No. 6, 2004