

Imaging Gold Nanorod Diffusion in Mucus Using Polarization Sensitive OCT

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Abstract: We demonstrate using PS-OCT to sense changes in the diffusion rate of gold nanorods (GNRs) in actively transporting pulmonary mucus in normal and disease-like states. This novel approach may lead to advances in monitoring pathogenesis and treatment dosimetry in real-time.

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1. Introduction

Muco-ciliary clearance (MCC) is critical in protecting against airborne infection. In diseases like cystic fibrosis and COPD, mucus layers become dehydrated, thicker and more viscous, resulting in loss of MCC and less protection against infections. We have previously shown that OCT provides excellent visualization of the respiratory epithelium and real-time monitoring of MCC *in vitro* [1]. Here we propose the use of diffusive gold nanorods (GNRs) to probe the viscosity of mucus while simultaneously monitoring MCC, which will enable new capabilities for monitoring pathogenesis and exploring methods of drug delivery.

This builds upon our previous work where the rotational diffusion of GNRs was measured via dynamic light scattering (DLS) on a PS-OCT platform; the diffusion rates were shown to be consistent with the viscosities of glycerol-water for varying mixture ratios [2]. GNRs are favorable as biological probes due to their biocompatibility and optical tunability. PEGylation of GNRs minimizes muco-adherence, allowing them to penetrate deeply into mucus. GNR light scattering was maximized by tuning their aspect ratio such that the longitudinal surface plasmon resonance (LSPR) is matched to the central wavelength of our OCT system [3]. Also, light scattered from GNRs is highly polarized due to their optical anisotropy, resulting in a high cross-polarized signal which allows for discrimination of GNRs from the dominantly co-polarized signals from mucus.

Unlike molecular fluids such as glycerol-water in our previous study, mucus is a complex fluid comprised of mucin proteins. As mucus becomes dehydrated, the spacing between mucin strands decreases, slowing the diffusion of GNRs within the matrix. Thus, we sense changes in the complex rheological properties of mucus resulting from dehydration by measuring the diffusion of GNRs. Importantly, we also show that the rate of light scattering fluctuations from GNR diffusion is fast relative to that arising from MCC, allowing us to perform measurements in an actively transporting system *in vitro*. This demonstrates the potential for GNRs to characterize mucus *in vivo*.

2. Methods

A polarization-sensitive, spectral-domain OCT system as previously described [2] was used to image PEGylated GNRs diffusing into mucus. Briefly, the system consists of a Ti:Sapphire laser ($\lambda=800$ nm, $\Delta\lambda=125$ nm, Griffin, KMLabs, Inc.) with 3×12 μm resolution (axial \times transverse) in air. The sample was illuminated with 3.5 mW, linearly polarized light. The signal scattered from the sample arm of the interferometer was then separated into co- and cross-polarized signals (HH and HV, respectively) and directed into a custom spectrometer where they were sampled by a linescan camera (Piranha, Dalsa, Inc.) at a rate of 25 kHz.

The translational diffusion coefficient, D_T , of GNRs was obtained using heterodyne dynamic light scattering theory [4] and the known polarization anisotropy of GNRs at LSPR [2]. GNRs were distributed into mucus with a nominal a concentration of ~ 100 GNRs per coherence volume to avoid GNR-GNR collisions over the time scale of the measurement. M-mode (depth vs. time) scans were obtained by collecting 12000 A-lines over 480 ms. The normalized autocorrelations of the co- (g_{HH}^1) and cross-polarized (g_{HV}^1) signals are given below:

$$g_{HH}^1(\tau) = \frac{5}{9} e^{-q^2 D_T \tau} + \frac{4}{9} e^{-6 D_R \tau} e^{-q^2 D_T \tau}, \quad (1)$$

$$g_{HV}^1(\tau) = e^{-6 D_R \tau} e^{-q^2 D_T \tau}, \quad (2)$$

where D_R is the rotational diffusion coefficient, D_T is the translational diffusion coefficient, and $q=4\pi n/\lambda$. These relationships were then combined to obtain an isotropic normalized autocorrelation (g_{ISO}^1):

$$g_{ISO}^1(\tau) = \frac{9}{5}g_{HH}^1(\tau) - \frac{4}{5}g_{HV}^1(\tau) \approx e^{-q^2 D_T \tau}, \quad (3)$$

from which D_T was obtained independently of D_R .

In vitro studies were conducted by depositing mucus at three different solid concentrations (1.5, 2.5, and 3.5%) premixed with GNRs onto an actively-transporting air-liquid interface (ALI) culture of primary, human bronchi-epithelial (hBE) cells. Both M-mode and B-mode scans were obtained for each solid concentration.

3. Results

Fig. 1 (left) shows B- and M-mode OCT images of mucus containing GNRs during transport on an ALI culture. Although the GNRs and membrane are the dominant light scatterers, the hBE cell layer is evident by a region void of green (HV) speckle, indicating that it is impermeable to GNRs. Rapid intensity fluctuation due to GNR diffusion is observed in the M-mode image compared to the temporally constant intensity of the membrane.

Fig. 2 (right) shows D_T vs. solid concentration for both stationary (experimentally fitted trend line) and transporting mucus, illustrating the potential for accurate mucus characterization during active MCC, as would be the case when imaging *in vivo*. Furthermore, this method is capable of characterizing disease-like mucus up to 3.5%, similar to that found in COPD and cystic fibrosis.

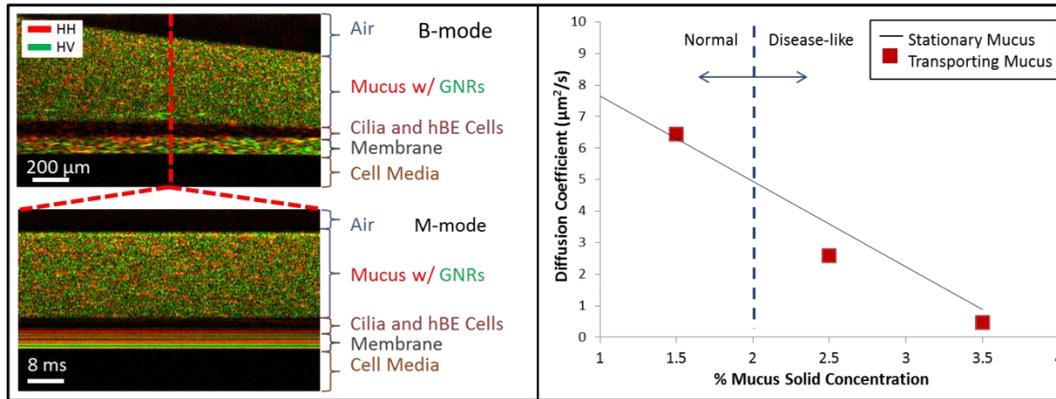


Figure 1. Left. Overlaid co- (red) and cross- (green) polarized B- and M-mode images of mucus on an ALI culture during transport (1.5% solid concentration). **Right.** Translational diffusion coefficients of GNRs in mucus at three solid concentrations during transport on an ALI culture.

4. Conclusion

PS-OCT is well suited to depth resolve the diffusion of GNRs in mucus. Furthermore, their size, biocompatibility, and optical tunability make GNRs favorable as sensors to detect changes in mucus. We have demonstrated that applying dynamic light scattering theory to diffusing GNRs allows for the characterization of healthy and disease-like mucus *in vitro*. This will translate to future efforts in characterizing mucus in patients for diagnosis and real-time monitoring of mucus-thinning treatments.

5. References

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