

## ***Raman Microspectroscopy for Discrimination of Bacterial Pathogens Causing Acute Otitis Media***

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Otitis media (OM), an inflammatory disease of the middle ear, is the most frequent cause of physician visits and prescription of antibiotics for children<sup>1</sup>. Current methods to diagnose acute otitis media (AOM), which is caused by a bacterial infection, rely on varying symptoms that overlap with another form of OM that is rarely caused by pathogens. Thus, there is a clinical need to rapidly identify specific bacteria which cause AOM to enable accurate diagnose and proper antibiotic prescription. Our goal is to accurately identify bacteria that cause AOM based on their biochemical fingerprint using Raman spectroscopy (RS). Raman bands of biochemical features specific to microbial cells have been identified<sup>2</sup>. For example, RS has been used to distinguish Gram-positive from Gram-negative bacteria based on specific biochemical components, such as peptidoglycan<sup>3</sup>. We used a Renishaw inVia confocal Raman microscope at 785 nm to characterize the Raman signatures of the most common bacteria that cause AOM, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae*. In addition, human ear fluid from de-identified patients undergoing tympanostomy, a procedure used to treat recurrent cases of AOM, was collected and examined using RS. Preliminary results were able to identify characteristic Raman spectral features for each type of bacteria causing AOM *in vitro*. Furthermore, a Raman peak intensity ratio between 1450  $\text{cm}^{-1}$  and 1045  $\text{cm}^{-1}$  was important in determining the type of bacteria. Raman signatures from microbial colonies of ear fluid samples were also analyzed and compared to the three main otopathogens that cause AOM. Based on our findings, RS has the potential to accurately diagnose AOM and provide physicians with the information needed to prescribe the correct antibiotic.

### References

- [1] J.O. Klein, *Vaccine* 19, S2-S8 (2001).
- [2] K. Maquelin, C. Kirschner, L.-P. Choo-Smith, N. van den Braak, H.Ph. Endtz, D. Naumann, G.J. Puppels, *J. Microbiol. Methods*. 51, 255-271 (2002).
- [3] F.S. de Siqueira e Oliveira, H.E. Giana, L. Silveira, Jr., *J. Biomed. Opt.* 17, 107004 (2012).