

CONFOCAL RAMAN IMAGING OF CYTOCHROME B_{558} IN SINGLE CELLS

Henk-Jan van Manen,¹ N. Uzunbajakava,¹ R. van Bruggen,² D. Roos,² and C. Otto¹

¹University of Twente, Faculty of Science & Technology, Biophysical Engineering Group,
P.O. Box 217, 7500 AE Enschede, The Netherlands

²Sanquin Research, Department of Experimental Immunohematology, and Landsteiner
Laboratory, Academic Medical Centre, University of Amsterdam, Plesmanlaan 125,
1066 CX Amsterdam, The Netherlands
E-mail: h.w.j.vanmanen@tnw.utwente.nl

KEY WORDS: Raman imaging, resonance, confocal, cytochrome b_{558} , granulocytes

Phagocytes are crucial in the innate immune response against microorganisms that invade the human body. These cells generate superoxide from oxygen using the enzyme system **NADPH oxidase**. All the catalytically active parts of NADPH oxidase reside in the flavohemoprotein **cytochrome b_{558}** (cyt b_{558}), which is located in the membranes of secondary granules and secretory vesicles in resting neutrophils.

Due to the presence of heme groups, the characterization of cyt b_{558} in neutrophils has been facilitated by resonance Raman (RR) spectroscopy. In our group, we have studied the activation of NADPH oxidase by both soluble and particulate activators by recording high-quality RR spectra from the cytoplasm of living neutrophils [1]. Our latest advance has been to visualize the intracellular distribution of cyt b_{558} in single neutrophils by scanning over a cell while gathering RR spectra [2]. Upon phagocytosis, part of the intracellular cyt b_{558} pool translocates to the phagosomal area in neutrophils. This result is consistent with studies using more commonly applied techniques such as immunocytochemistry combined with electron or fluorescence microscopy. In contrast to these methods, our Raman microscopic technique is a label-free, chemical/vibrational imaging method that requires minimal sample preparation and disturbance.

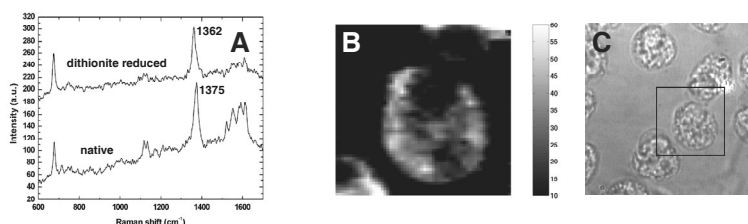


Figure 1. A) Resonance Raman spectra from the cytoplasm of fixed neutrophils. B) RR image of cytochrome b_{558} in a resting, fixed neutrophil. C) Corresponding white-light transmission image.

REFERENCES

- [1] N. M. Sijtsema; A. G. J. Tibbe; G. M. J. Segers-Nolten; A. J. Verhoeven; R. S. Weening; J. Greve, and C. Otto, "Intracellular reactions in single human granulocytes upon phorbol myristate acetate activation using confocal Raman microspectroscopy", *Biophys. J.*, **78**, 2606-2613 (2000).
- [2] H.-J. van Manen; N. Uzunbajakava; R. van Bruggen; D. Roos, and C. Otto, "Resonance Raman imaging of the NADPH oxidase subunit cytochrome b_{558} in single neutrophilic granulocytes", *J. Am. Chem. Soc.*, **125**, 12112-12113 (2003).