# Cytochrome C Oxidase Deficiency Detection Using Scanning Electrochemical Microscopy in Living Cells

Shubhneet Thind[1], Vikram Singh[1], Sabine Kuss[1]

[1]Department of Chemistry, University of Manitoba

## INTRODUCTION

- Cytochrome C Oxidase (COX) Deficiency is an inherited disorder characterized by the absence or abnormality of the protein cytochrome c oxidase[1]
- No cure for this disorder, however, the resulting symptoms can be managed, and improved upon only if **EARLY** diagnosis is made
- Currently it's diagnosed through muscle biopsies which are painful, invasive, expensive and time-consuming, leading to a **DELAY** in diagnosis.

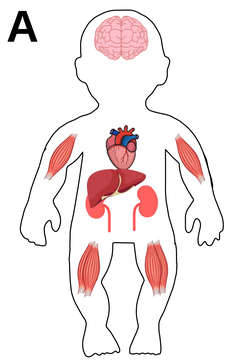


**Figure A.** Areas affected by COX Deficiency

## OBJECTIVE

- Using Scanning Electrochemical Microscopy (SECM), to detect and quantify Cytochrome C Oxidase activity

## METHODOLOGY

- *N, N, N',N'*-tetramethyl-*para*-phenylene-diamine (TMPD) has been used to quantify the COX activity in *Bacillus subtilis* and *E. coli* [3]
- Experiments conducted on HeLa cells, which are cervical cancer cells
- When tip of the microelectrode is polarized at 200mV, the cells are also scanned with a mediator like, Hexaammineruthenium III chloride (RuHex or $[Ru(NH_3)_6]^3$ to see the topography of the cell and detect the influence of the surrounding (acts like control)

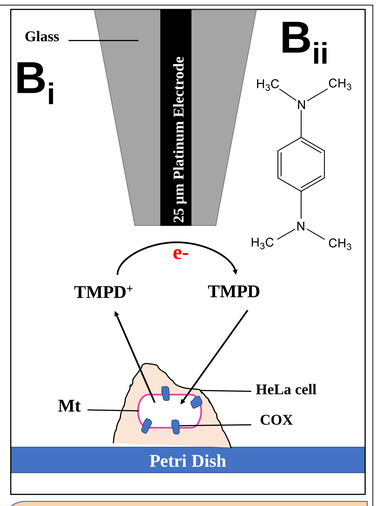


**Figure $B_i$.** SECM methodology. Mitochondria (Mt) with COX in the membrane. **Figure $B_{ii}$.** Molecular structure of TMPD

## RESULTS

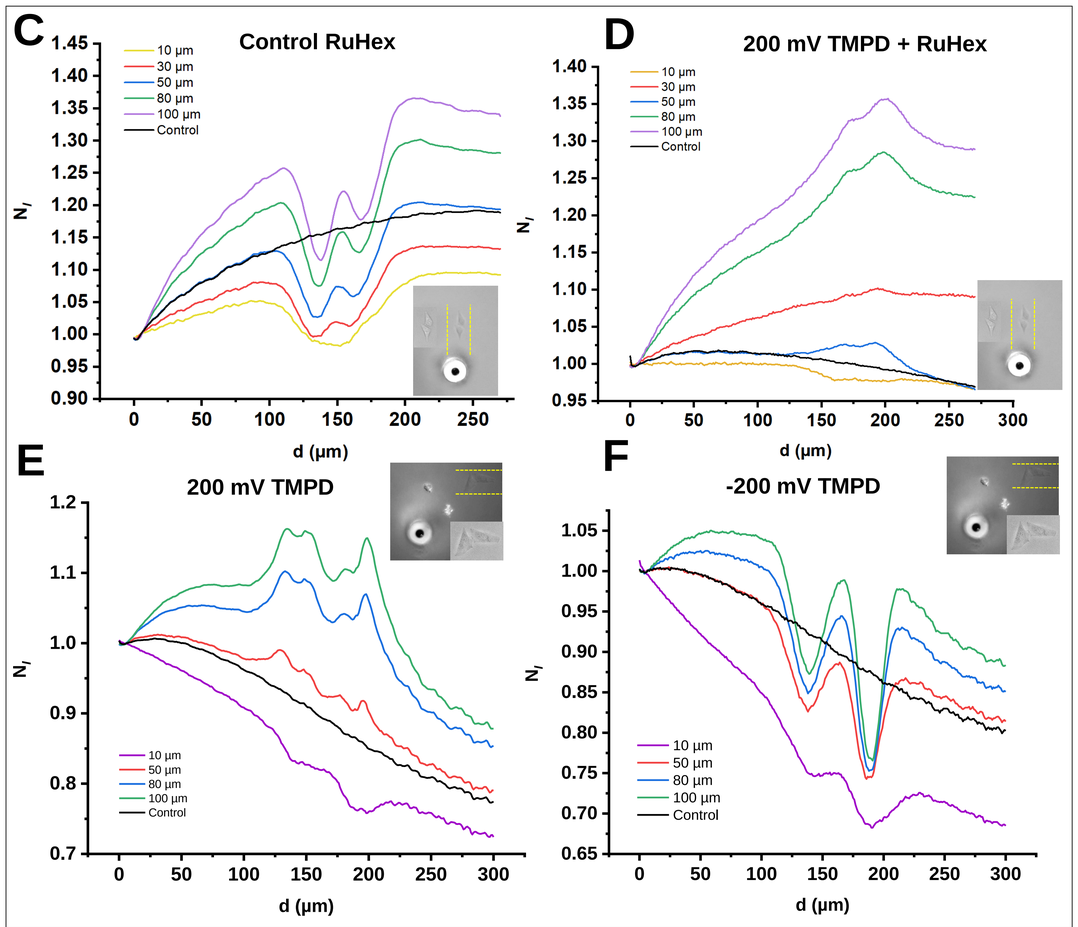


**Figure C.** Line scans in 1mM RuHex on HeLa cells to measure topography **Figure D.** Line scans in 1mM RuHex + 1mM TMPD at 200 mV to measure Cytochrome C reductase activity (TMPD → $TMPD^{+\bullet}$) **Figure E and F.** Line scans in 1mM TMPD to measure Reductase (TMPD → $TMPD^{+\bullet}$) and Oxidase activity ($TMPD^{+\bullet}$ → TMPD) , respectively.

## REFERENCES

[1] *Cytochrome C Oxidase Deficiency*. (n.d.). NORD: National Organization for Rare Disorders. https://rarediseases.org/rare-diseases/cytochrome-c-oxidase-deficiency/

[2] *Cytochrome C Oxidase Deficiency*. (n.d.). NORD: National Organization for Rare Disorders. https://rarediseases.org/rare-diseases/cytochrome-c-oxidase-deficiency/

[3] Kuss, S., Tanner, E. E. L., Ordovas-Montanes, M., & Compton, R. G. (2017). Electrochemical recognition and quantification of cytochrome c expression in Bacillus subtilis and aerobe/ anaerobe Escherichia coli using N,N,N′,N′-tetramethyl-para-phenylene-diamine (TMPD). Chemical Science. https://doi.org/10.1039/C7SC03498A

## DISCUSSION

- By applying reduction potential of -200 mV at the microelectrode, $TMPD^{+\bullet}$, generated by the cell's Cytochrome C **Oxidase**, is converted to TMPD
- By applying oxidation potential of 200 mV at the microelectrode, TMPD, generated by the cell's Cytochrome C **Reductase**, is converted to $TMPD^{+\bullet}$
- These behaviours are measured as a change in current and are therefore quantified

## CONCLUSION

- By using SECM, a signal is observed when oxidation and reduction potentials are applied (therefore possible detection of Cytochrome C reductase and oxidase activity respectively)

## FUTURE DIRECTIONS

- Testing Control and Cytochrome C Oxidase deficient fibroblast cell lines from patients, specifically SCO1 and TACO1 fibroblast cell lines
- Development of a biosensor which would allow for quick detection of COX deficiency

## ACKNOWLEDGEMENTS

**Dr. Eric Shoubridge, McGill University for providing fibroblast cell lines**