Rapid evaporative ionisation mass spectrometry (REIMS) for biomarker discovery and mechanistic studies of *Pseudomonas aeruginosa* stress responses

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BACKGROUND

- To establish infection in the CF lung, *Pseudomonas aeruginosa* must adapt to overcome a variety of stresses.
- These stresses include antimicrobial oxidants such as **hypochlorous acid** (HOCl) and **hypothiocyanous acid** (HOSCN) that are generated by the innate immune system.[1]
- Little is known about how *P. aeruginosa* protects against oxidative stress in either the early or the chronic stages of infection.[2]
- Rapid evaporative ionisation mass spectrometry (REIMS) (Figure 1) is a novel analytical technique with two key characteristics:
  - **Ultra high-throughput analysis.**
  - **No sample preparation required.**[3]
- The ability to quickly analyse a large number of replicates directly from culture suggests REIMS may be a highly efficient technique for **biomarker discovery** and **mechanistic studies**.

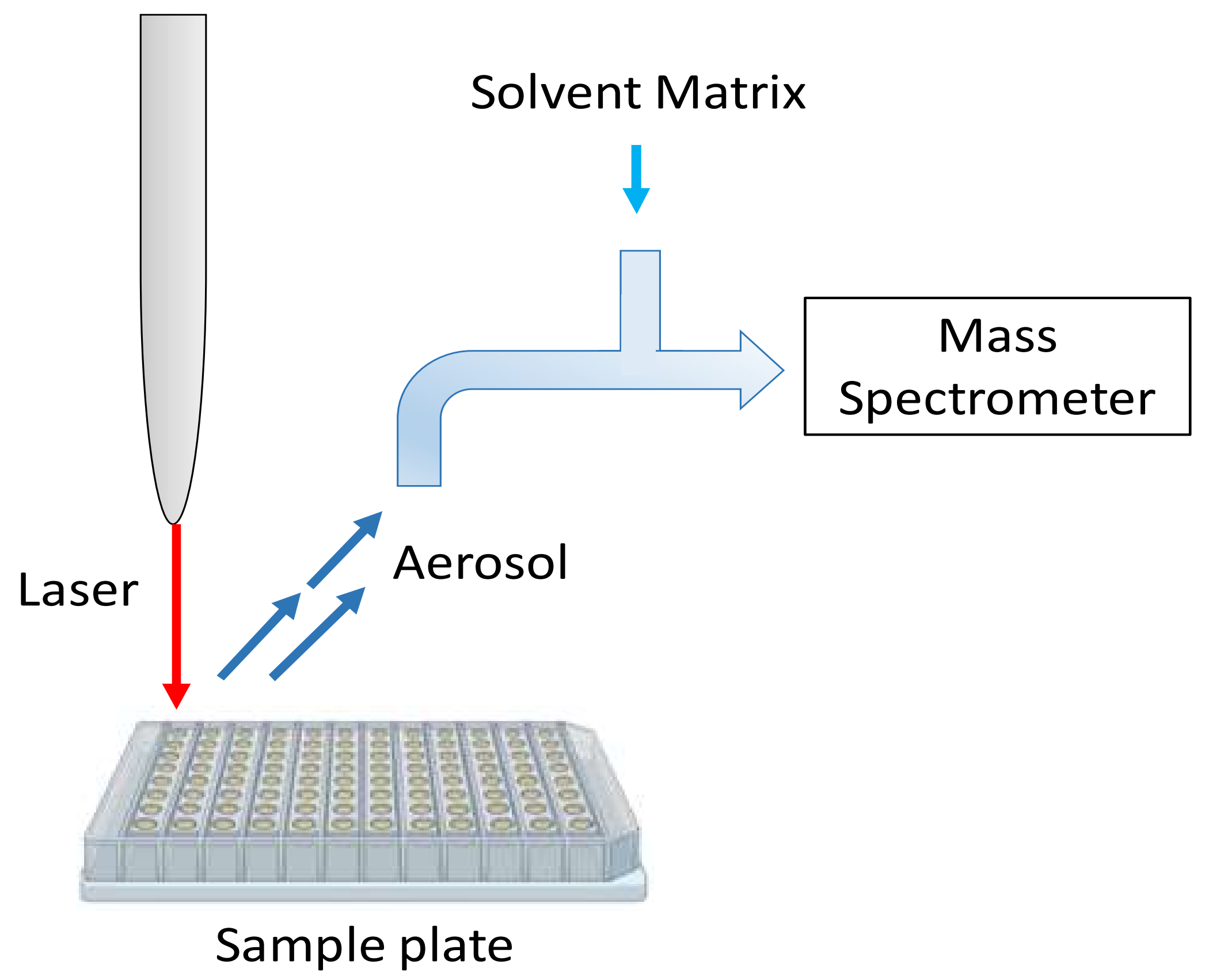


Figure 1: Simplified schematic depiction of the REIMS set-up

- This project will investigate this application of REIMS through the analysis of *P. aeruginosa* samples exposed to HOCl and HOSCN.

AIMS AND OBJECTIVES

- **Can REIMS discriminate between untreated *P. aeruginosa* samples and *P. aeruginosa* samples exposed to HOCl/HOSCN stress?**
- **Can REIMS be used to identify specific biomarkers of HOCl/HOSCN stress?**
- **Can REIMS be used to provide mechanistic insight into the oxidative stress protection systems employed by *P. aeruginosa*?**

METHODS

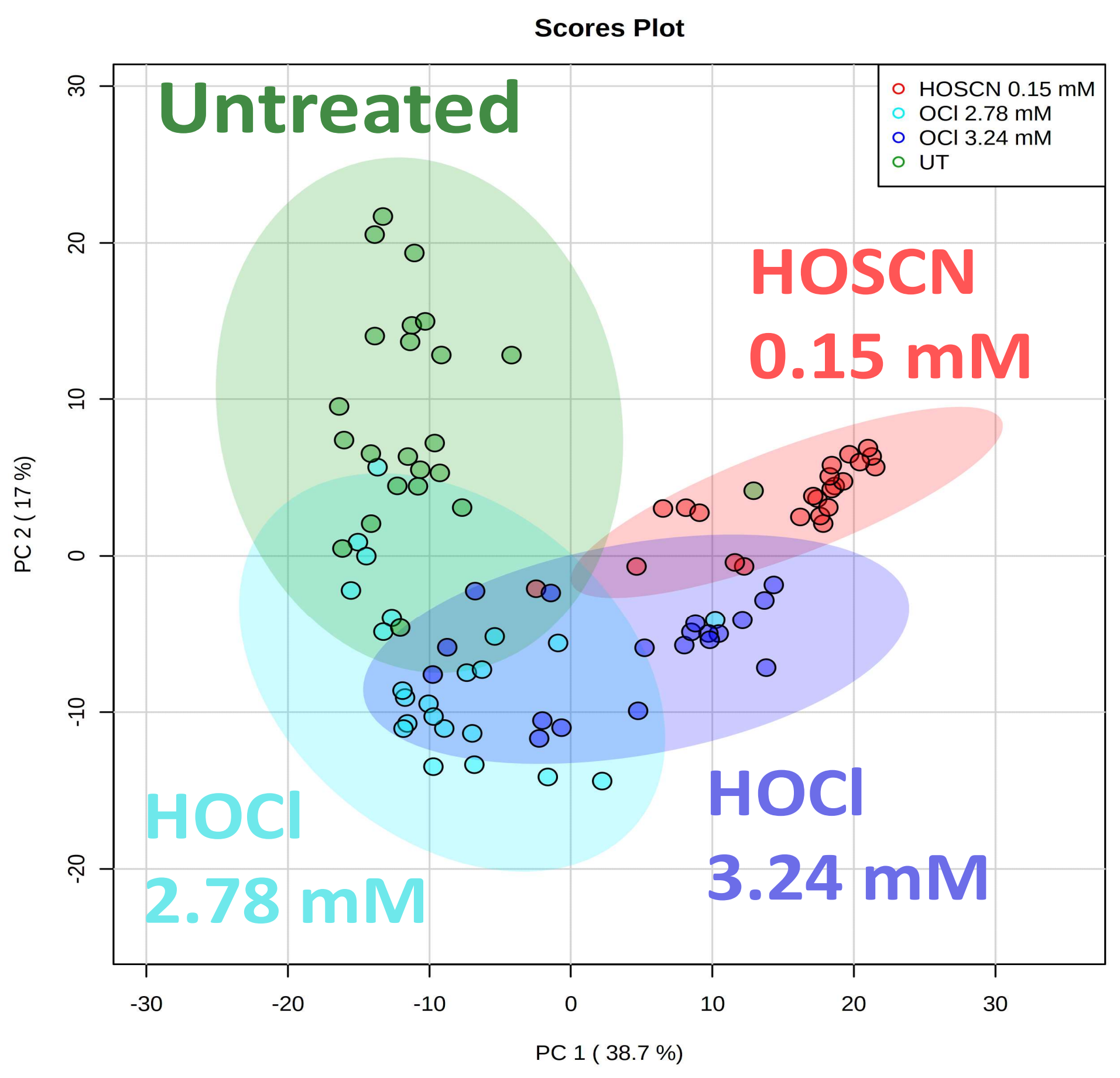
- *P. aeruginosa* samples grown in a 96 well-plate format, untreated or exposed to varying concentrations of oxidant.
- At mid-log growth phase, samples washed, pelleted and the supernatant removed.
- REIMS analysis performed via laser-mediated evaporation of the bacterial pellets.
- One mass spectra generated per pellet – 20 mins to analyse one 96 well-plate.
- The resulting mass spectra processed using peak-picking software and statistical analysis performed using the MetaboAnalyst platform.

RESULTS

Method optimisation

- As a novel technique, initially a wide range of experimental conditions were tested.
- The most effective protocol was determined to be growth in a 96 well format, harvesting at mid-log growth phase and washing one time with dH_2O prior to analysis.

PCA analysis demonstrates clear discrimination between both untreated and treated samples and between oxidants.



- Samples exposed to two HOCl concentrations and one HOSCN concentration.
- 24 replicates per class.
- PCA score plot (Figure 2) shows both separation between untreated and treated samples and between HOCl and HOSCN treated samples.

Figure 2: PCA scores plot. Shaded areas represent 95% confidence interval.

PLS-DA modelling further confirms discriminative ability of REIMS.

- PLS-DA model implemented to further explain separation seen in PCA plot.
- Score plot generated based on the validated PLS-DA model (Q^2 of 0.7588).
- Variables most able to explain separation identified.

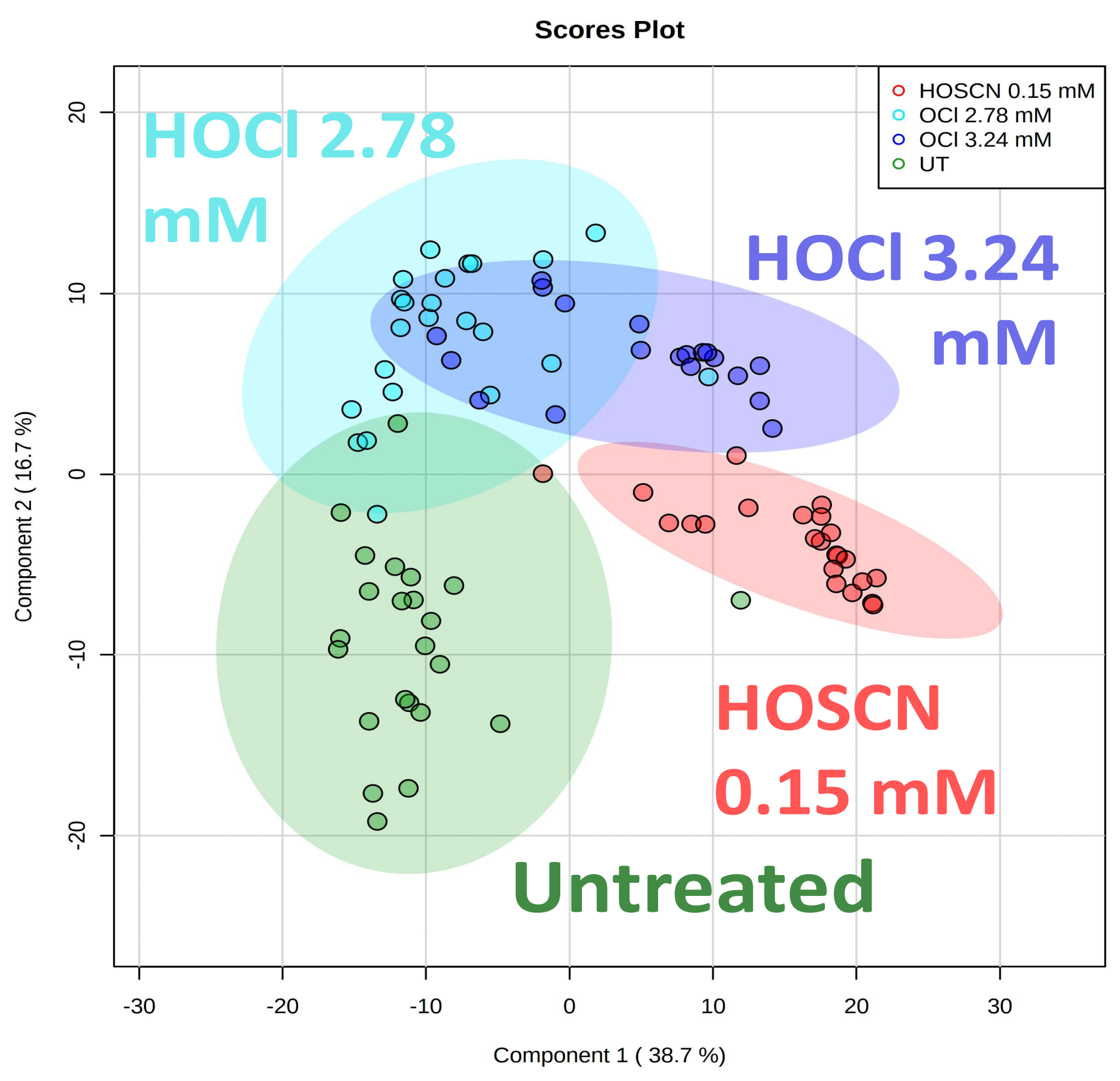


Figure 3: PLS-DA scores plot. Shaded areas represent 95% confidence interval.

Random forest algorithm able to accurately classify samples as untreated or treated.

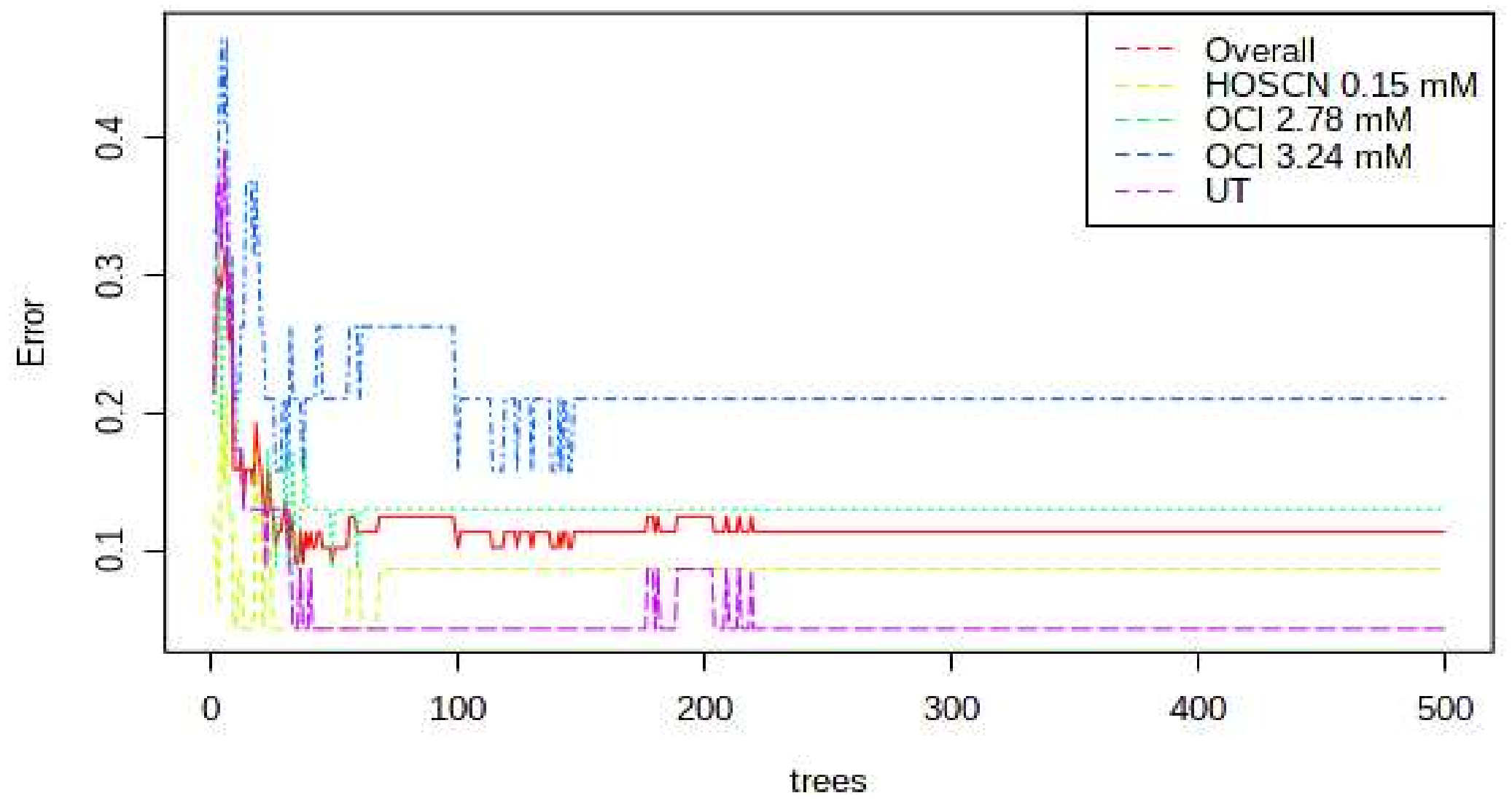


Figure 4: Cumulative error rates by Random Forest classification. The overall error rate is shown as a red line

- Untreated samples successfully classified with a class error of 0.04.
- HOSCN distinguished from HOCl with a class error of 0.09.
- Model less able to distinguish between HOCl concentrations with class error of 0.13

REIMS can rapidly and accurately detect difference between untreated PA14 samples and those treated with HOCl or HOSCN with no sample preparation required.

FUTURE WORK

- **Key metabolites** highlighted from PLS-DA and ANOVA that best explain class separation will be **identified**.
- These metabolites may constitute **biomarkers of specific oxidative stress** and/or provide **mechanistic insight** into the action of HOCl/HOSCN on *P.aeruginosa*.
- The assay will be extended to encompass **other chemical species relevant to the CF lung environment**.

REFERENCES

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[3] - Kailasa, S. K., Koduru, J. R., Park, T. J., Wu, H. F., & Lin, Y. C. (2019) Progress of electrospray ionization and rapid evaporative ionization mass spectrometric techniques for the broad-range identification of microorganisms. *Analyst*, **144**, 1073-1103.