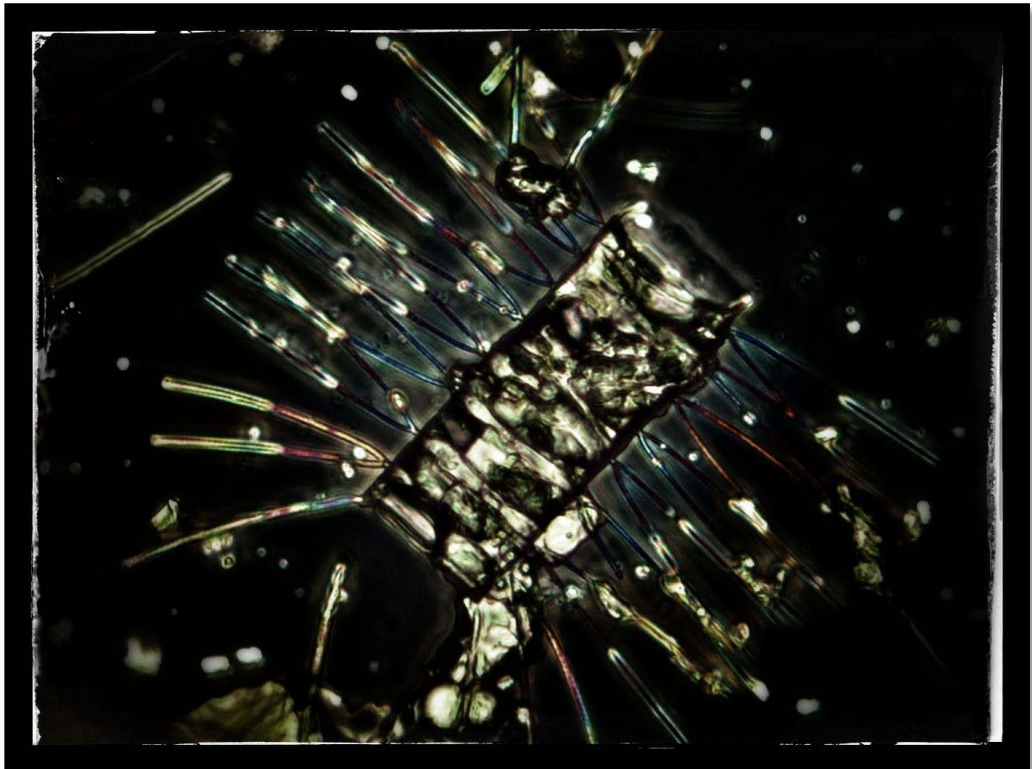


Accelerating phytoplankton phenomics through FTIR spectroscopy



Olivia Sackett

Doctoral Thesis, February 2015

Supervisors:

Professor Peter Ralph, Professor John Beardall, Dr Philip Heraud,

Dr Martina Doblin and Dr Ross Hill

Prediction is difficult, particularly if it involves the future.
— Uncertain...depends on whom you ask.

Certificate of original authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Production Note:

Signature removed prior to publication.

Olivia Sackett

February 15, 2015

Acknowledgements

To John Beardall and Phil Heraud, thank you for a decade of education, encouragement, mentoring, facilitation, relentless reviewing skills and friendship. Learning from you has been an honour and a privilege. I hope to continue to work with you both for many more years.

Thank you to Peter Ralph, Martina Doblin and Ross Hill for making my candidature at the University of Technology, Sydney possible, and for facilitating my doctoral degree. Ross, your enthusiasm, attention to detail and rapid turn-around-time never cease to impress me.

To Katherina Petrou, thanks for your friendship and guidance. You have provided focus, direction, humour, cups of tea and soothing words during tough times. I will miss our many hours in the lab together. Thank you to my fellow PhD candidates (Sutinee Sinutok, Verena Schrameyer, Ying Hong and Noni Dowsett) for all the great times we've had.

Finally, this project would not have been possible without the seemingly limitless support of my family and friends. In particular, my partner Wayne Vest has played a critical role by providing technical, moral, emotional and financial support. Thank you, Wayne, for encouraging and enabling me to follow my dreams. Thanks to Mum and Dad (Na-Na and Gan-Pa) for making education such a high priority in our family, for providing enthusiasm and for helping us to bring up our two beautiful daughters. I would especially like to thank Gan-Pa for cheerfully providing porridge and wheat bix to Merren at 5 am during winter. Thank you to Christine Connelly for endless PhD pep-talks over coffee and chai, and for technical assistance at the Australian Synchrotron.

Thank you all once again, what a fabulous time it's been!

List of Figures

Figure 1-1 Key Research Questions	6
Figure 2-1 Major Southern Ocean habitats and related distribution of diatom species.	10
Figure 2-2 Average second derivative spectra of <i>F. cylindrus</i> , <i>C. simplex</i> and <i>P. subcurvata</i> . ..	16
Figure 2-3 Discrimination of cell spectra by treatment condition.	18
Figure 2-4 Cellular concentrations of macromolecules under treatment conditions.	21
Figure 3-1 Visible images of <i>Chaetoceros</i> spp. (a), <i>F. kerguelensis</i> (b) and <i>E. antarctica</i> (c) cells showing the infrared measurement positions (indicated by cross hairs).....	32
Figure 3-2 Average second derivative cell spectra from four common genera: <i>Chaetoceros</i> , <i>Eucampia</i> , <i>Fragilariopsis</i> and <i>Pseudo-nitzschia</i>	34
Figure 3-3 Taxonomic Classification by PLSDA results for four common genera: <i>Chaetoceros</i> spp, <i>Eucampia antarctica</i> , <i>Fragilariopsis kerguelensis</i> and <i>Pseudo-nitzschia</i> spp.	36
Figure 3-4 Classification of cell spectra by PLSDA with data pooled across stations. PLSDA scores plot (a) shows clustering of cell spectra by taxon.....	37
Figure 3-5 Variations in macromolecular composition for four taxon pooled (a-c) and separately for <i>F. kerguelensis</i> (d-f).	39
Figure 3-6 Average second derivative spectra (a), PLSDA scores plot (b) and PLSDA loading weights plot for <i>Pseudo-nitzschia</i> spp. at stations E-1, E-5 and E4-W.....	40
Figure 4-1 Average second derivative spectra for <i>C. simplex</i> under various temperature and salinity treatments.	53
Figure 4-2 PLSR prediction model for cellular carbon content.	56
Figure 4-3 Productivity (a), carbon (b) and energy (c) content for <i>C. simplex</i> versus salinity and temperature treatments.....	58
Figure 4-4 Carbon productivity versus cellular carbon (a), protein (b) and minimum total energy content (c).	59
Figure 5-1 Key Research Questions (repeated from Chapter 1)	65

List of Tables

Table 2-1 PLSDA Classification by Treatment Summary Statistics	15
Table 2-2 Salinity and Temperature Prediction Summary Statistics	19
Table 3-1 Description of sampling stations and associated biogeochemical characteristics.	28
Table 4-1 PLSR predictive model validation statistics	57
Table 5-1 Key research findings summarized by thesis chapter	65

Abstract

Marine phytoplankton play a driving role in global biogeochemical cycling, providing fuel for marine and terrestrial ecosystems, and removing substantial quantities of CO₂ from the atmosphere. Phytoplankton respond to environmental change by varying their phenotypes, including photophysiology, macromolecular composition and morphology. Southern Ocean phytoplankton are subjected to one of the most extreme habitats on earth, resulting in great phenotypic variation between and within taxonomic groups. Given they provide a significant net sink of atmospheric CO₂ and support the most biologically productive ecosystem on earth, improving our ability to predict the responses of Southern Ocean phytoplankton to environmental change is of global importance. At present, our ability to predict the responses of these critical organisms to environmental change, including climate change, is limited by a bottleneck in the efficiency with which we can characterise phytoplankton phenotypes.

This thesis demonstrates the feasibility of accelerating phytoplankton phenomics using Fourier Transform Infrared (FTIR) microspectroscopy, a powerful yet under-utilised frontier technology. The extensive phenotypic variation shown by Southern Ocean phytoplankton provided excellent scope for demonstrating the power of the microspectroscopic approach. Coupling the microspectroscopic approach with multivariate modeling tools enabled the characterisation of phenotypic plasticity from cell FTIR spectra. When combined with mass spectrometry data, cell FTIR spectra provided accurate estimates of multiple phenotypic parameters including cellular protein, carbon and energy. Of particular value, spectroscopic models were able to accurately estimate rates of carbon production from samples taken at a single time-point, circumventing the need to take measurements over time. Further, the high spatial resolution achievable with microspectroscopy enabled the analysis of individual cells, revealing taxon-specific responses to iron availability within samples taken from a mixed natural Southern Ocean phytoplankton bloom. This work demonstrates that incorporating FTIR microspectroscopy into the phenomics toolbox will improve the efficiency of phenotypic data collection and, in combination with multivariate modeling, will enable the development of powerful, taxon-specific predictive phenomic models.

Table of Contents

1	Introduction.....	1
1.1	Importance of marine phytoplankton.....	1
1.2	Infrared spectroscopy-based phenomics: strengths, prospects	4
2	Phenotypic plasticity of Southern Ocean diatoms: key to success in the sea ice habitat?	7
2.1	Introduction.....	7
2.2	Materials and Methods	11
2.2.1	Culturing	11
2.2.2	FTIR microspectroscopy	11
2.2.3	Multivariate modeling	12
2.2.4	Statistical analyses.....	14
2.3	Results.....	14
2.3.1	Degree of phenotypic plasticity varies between diatom species	14
2.3.2	Source of plasticity: changes in macromolecular composition	15
2.3.3	Predictions of environmental history of cells.....	19
2.3.4	Change in concentration of macromolecules	19
2.4	Discussion	22
2.5	Acknowledgments.....	25
3	Taxon-specific responses of Southern Ocean diatoms to Fe-enrichment revealed by FTIR microspectroscopy	26
3.1	Introduction.....	26
3.2	Materials and Methods	29
3.2.1	Sampling	29
3.2.2	Microspectroscopy	29
3.2.3	Multivariate Modeling.....	30
3.3	Results.....	32
3.3.1	Stations E-1 and E-5 (moderate Fe availability)	32
3.3.2	Stations E4-W and TEW-8 (higher Fe availability).....	32

3.3.3	Multivariate modeling and taxonomic classification	34
3.3.4	Community averages compared to individual taxon.....	37
3.4	Discussion	40
3.5	Acknowledgments.....	44
4	Snapshot prediction of carbon productivity, carbon and protein content in a Southern Ocean diatom using FTIR spectroscopy.....	45
4.1	Introduction.....	45
4.2	Materials and Methods	48
4.2.1	Microalgal culturing and experimental conditions.....	48
4.2.2	FTIR spectroscopy for macromolecular ‘snapshot’ measurements.....	48
4.2.3	Calibration data for predictive models.....	49
4.2.4	Predictive model calibration and validation.....	51
4.2.5	Significance testing	52
4.3	Results.....	52
4.3.1	FTIR spectroscopy for macromolecular ‘snapshot’	52
4.3.2	Predictive model calibration and validation.....	54
4.4	Discussion	60
4.5	Acknowledgements	63
5	Synthesis	64
	References.....	69
	Appendix I: Infrared Band Assignments	88
	Appendix II: Publications resulting from this thesis.....	89