Fully funded PhD scholarship
Diatom Recording Using Metabarcoding (DRUM)

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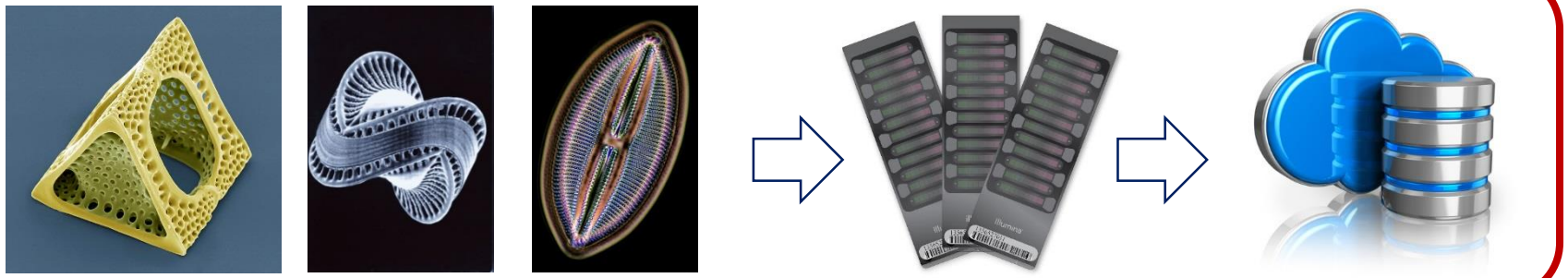

Diatom surveys inform decision-making on ecological status in rivers and lakes. Currently light microscopy and a standardised metabarcoding technique are used. You will develop novel Next Generation Sequencing approaches which will improve our understanding of the ecological impact of water quality.

You will benefit from being exposed to both Newcastle University and Fera research environments and will receive training in next generation sequencing, statistics, bioinformatics, and transferable skills such as design of research project, analytics, scientific writing and presentation.

Start date / duration
September 2018 / 3 years.

Application closing date
Saturday 31st March 2018.

Interviews will take place during April/May 2018.

Diatom Recording Using Metabarcoding (DRUM)

Microscopical surveys of abundance and species composition of freshwater diatoms in environmental samples are a well-established tool for ecological assessment in rivers and lakes. This project will develop second generation standardised cost-effective diatom survey techniques based on DNA barcoding. Recently Fera scientists successfully tested the usage of *rbcL* metabarcoding for diatom surveys within the Environment Agency project 'A DNA based diatom metabarcoding approach for Water Framework Directive classification of rivers'. We will further develop and extend this approach by measuring diatom DNA and RNA concentration in the environmental samples using a combination of metabarcoding and next generation sequencing of the chloroplast *rbcL* and *rbcS* genes encoding the key CO_2-fixing enzyme, Rubisco. We will investigate if environmental DNA and RNA concentrations could be used as proxies to estimate photosynthetic productivity as well as precise species composition of different diatom species and communities.

The work programme will include, but not be limited to: (a) Improvements to species identification by investigating the requirement for operational taxonomic unit (OTU) clustering. At present, Environment Agency only accesses about half the NGS reads by matching to known sequences. This is a major problem with the existing method and one of the key areas this PhD will address. We will develop an approach which makes use of the unassigned reads. The raw NGS sequences can group very similar species together into one OTU. It may be possible to move from a computational power-saving OTU based analysis pipeline to one where each individual NGS sequence is analysed instead. This can be investigated by comparing datasets with and without OTU clustering, alongside a comparison between the current pipeline's BLAST identification of sequences versus machine learning classification systems. Environmental Agency in the UK as well as its colleagues in Europe are interested in this approach, and we will receive a full access to the extensive diatom barcoding dataset created for Agency by Fera.

(b) Complementary to the objective (a) we will expand the existing full length *rbcL* diatom barcode database that currently contains only 176 species or less than 10% of the diatom species that have been described from the UK. We will also create full length *rbcS* diatom barcode database to test if this shorter but more polymorphic "sister" gene can be used as a complementary barcode.

(c) Testing accuracy of metabarcoding approach using prepared samples with known number of diatom cells. The contributions made by *rbcL* reads from diatoms of different cell volumes as well as variable chloroplast numbers are thought to be among major unassessed factors. Flow cytometry facilities at Newcastle University will be used to calculate cell numbers in monocultures of different diatom species, and monocultures with known cell numbers will be mixed to create a range of test samples. DNA from the test samples will be isolated and processed to assess accuracy of the metabarcoding approach to estimate correct species composition.

(d) NGS enabled DNA metabarcoding 'mirrors' existing techniques such as light microscopy. We propose to take it a step further and to develop second generation methods that move beyond simplistic metrics and unlock the huge potential of NGS to evaluate ecosystem function in ways that can enhance assessments and, thereby, regulation and management (Sagarin et al. 2009). This project will test if species absolute or relative photosynthetic productivity could be assessed using combination of NGS enabled RNA metabarcoding. While being one of the major photosynthetic producers globally, diatom species show significant diversity of both photosynthetic capabilities linked to several factors including differences in Rubisco kinetics and facultative heterotrophy. Traditional surveys only assess number of cells, missing the fact that they could be photosynthetically active or inactive. NGS enabled RNA metabarcoding of the Rubisco encoding *rbcL* gene may give an estimate of how photosynthetically active particular species are in a particular sample. Further, RNA-based estimates of active Rubisco will be linked with known diatom Rubisco kinetics to estimate the photosynthetic contribution of particular species.

Training provided. The student will benefit from being exposed to both Newcastle University and Fera research environments and will receive training in molecular biology methods, including next generation sequencing, as well as in statistical and bioinformatics methods. The student will also learn the basics of diatom isolation and culturing as well as their morphology, physiology and ecology. Vigorous attention will be given to transferable skills such as design of research project, analytical skills, scientific writing and presentation. The student will be given the opportunity to present her/his findings at national and international meetings. The student will be encouraged to liaise with other Newcastle University and Fera PhD students to organise workshops and seminar series.