Real-time, automated characterization of algal lipidome and metabolome using Laser-Assisted Rapid Evaporative Ionization Mass Spectrometry

Júlia Balog [1,2], Richard Schäffer[1], Milán Szabó[3,4], Unnikrishnan Kuzhiumparambil[4], Peter Ralph[4], Steven Pringle[5] and Zoltán Takáts[2]
[1]Waters Research Center, Budapest, Hungary; [2]Imperial College, London,United Kingdom, [3]Biological Research Center, Szeged, Hungary; [4]University Technology Sydney, Australia; [5]Waters Corporation, Wilmslow, United Kingdom.

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

INTRODUCTION

- There is a need in the industry for a rapid, high throughput, automatic characterization method in order to speed up genetic engineering and production
- The rapid metabolic phenotyping of different algal species allows the real-time monitoring of different processes within the cells and the detection of molecules produced by the cells
- Laser-Assisted Rapid Evaporative Ionization Mass Spectrometry (LA-REIMS) is a rapid and efficient method for profiling complex biological samples such as tissues, microbial samples or food products without the time consuming sample preparation steps
- In this study, we present an automated, fast and effective way to identify different algae based on their metabolic and lipidomic profile using LA-REIMS and a prototype home-built automated system.

AIMS

- **To evaluate if we could acquire a metabolomic and lipidomic profile from algae using Laser-Assisted REIMS and an automated system for plates and pellets**
- **To identify specific lipids using the same LA-REIMS method**
- **To evaluate if we can spot differences between the lipidomic profiles of algae cultured under environmental stress**

METHODS

Samples

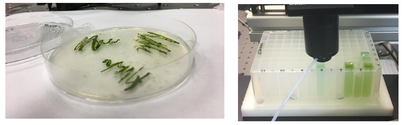
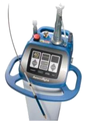


Figure 1. Sample formats: (i) cultured on plates; (ii) concentrated liquid.

- Chlorella sorokiniana - normal media and nitrate negative media pellets
- Nannochloropsis sp. - normal media and nitrate negative media pellets
- Synechocystis PCC6803 in liquid form

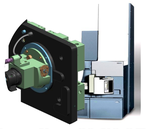
Sampling



Aesculight CO_2 laser
- 10600 nm
- 15W peak power
- Set power: 2W
- Repetition rate: 5Hz
- Pulse width: 10 ms

OPOTEK OPO
- 2940 nm
- 120mW peak power
- Output power: 80 mW
- Repetition rate: 20Hz
- Pulse width: 7 ns

Detection



Waters Xevo G2-XS QToF
- REIMS interface
- Negative ion mode
- Mass range: 50-1200 Da
- Coll. energy:15-40 eV (MSMS)

AUTOMATED LASER-REIMS WELL PLATE READER

- Sample with laser directly from well plate
- Generate aerosol from liquid, pellets and solid samples and transfer generated aerosol into mass spectrometer
- Scan whole well plate automatically including start/stop MS acquisition
- Control movement, laser and acquisition parameters.

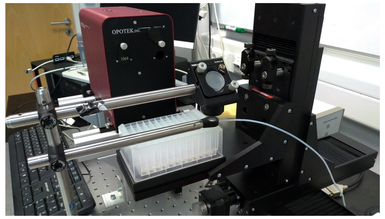
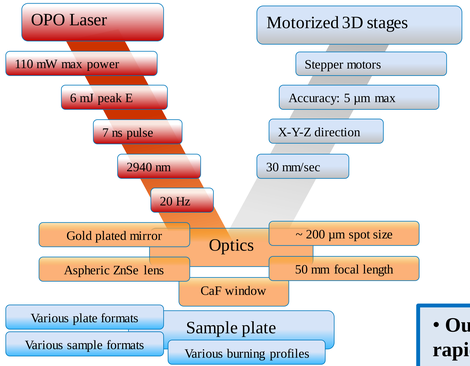


Figure 2. Well plate reader lab prototype.



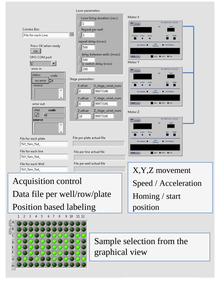


Figure 3. Labview based control software.

M.S. is supported by the grants of the Hungarian Academy of Sciences, MTA Premium Postdoctoral Research Program (PREMIUM-2017-38) and the Hungarian National Research, Development and Innovation Office (FK 128977)

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS

RESULTS

Comparison of lasers and algal species

10600 nm CO_2 and 2940 nm OPO lasers were used on concentrated media, pellet and plate

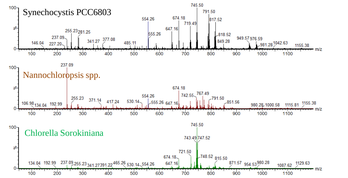


Fig. 4. Full spectra of 3 different algae species sampled from plates using a CO_2 laser.

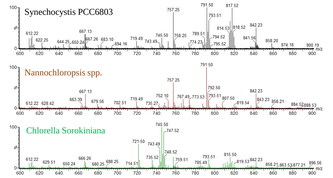


Fig. 5. Spectra in the phospholipid range of 3 different algae species sampled from plates using a 2940 nm OPO laser.

- Both lasers provide sufficient lipid signal
- Species identification is 99.7% correct using all methods in one model
- CO_2 is suggested when using algae cultured on plates
- 2940 nm OPO laser is suggested when working on concentrated liquid samples
- Small metabolites, free fatty acids and phospholipids can be observed in the signal

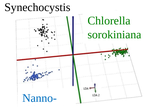


Fig. 6. 3D pseudo LDA plot on samples acquired with all methods.

	Chlorella sorokiniana	Nannochloropsis spp.	Synechocystis PCC6803	Total
Chlorella sorokiniana	137	0	0	137
Nannochloropsis spp.	0	119	0	12
Synechocystis PCC6803	0	1	79	80
Total	137	120	79	329

Table. 1. Confusion matrix of samples acquired with all methods on species level.

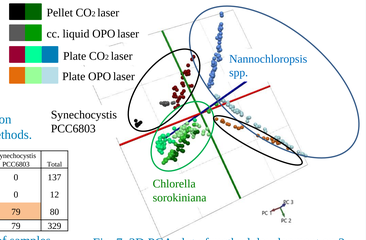


Fig. 7. 3D PCA plot of method development on 3 species, 2 lasers and 3 different sample types.

Effect of environmental perturbations

Nitrogen stress by reducing nitrate in the media (pellets)

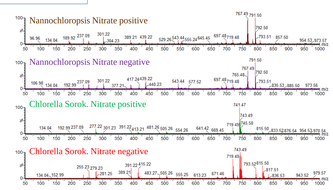


Fig. 9. Full spectra of algae showing environmental effects.

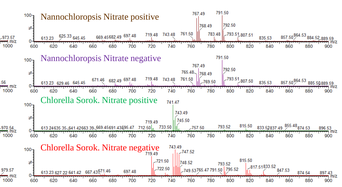


Fig. 10. Spectra in the m/z 600 to 900 (phospholipid) range.

	Chlorella nitrate neg	Chlorella nitrate pos	Nannochloropsis nitrate neg	Nannochloropsis nitrate pos	Total
Chlorella nitrate neg	24	0	0	0	24
Chlorella nitrate pos	0	26	0	0	26
Nannochloropsis nitrate neg	0	0	22	0	22
Nannochloropsis nitrate pos	0	0	0	18	18
Total	24	26	22	18	90

Table. 3. Confusion matrix showing the effect of Nitrogen perturbations.

- There is a significant effect of nitrate reduction on the algal lipidome
- Specific lipids can be identied behind these differences
- Phospholipids become more saturated with the reduction of nitrate

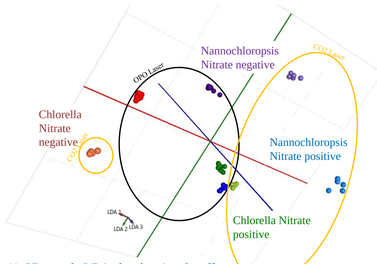


Fig. 11. 3D pseudo LDA plot showing the effect of nitrate reduction and use of different lasers.

Identification of specific lipids

Using exact mass measurements and MSMS

- Specific lipids could be identified with LA-REIMS method
- There are multiple lipids with odd fatty acid chains – (We used media doped with antibiotics to ensure there is no bacterial infection)

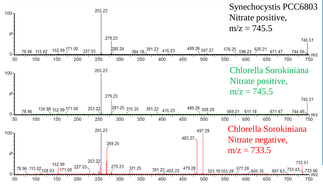


Fig. 8. MSMS spectra of different species.

Measured mass	Theoretical mass	Species	Ion	Found in, abundant in
697.482	697.4814	PA(18:1/18:2) PE(16:1/18:1)	M-H M-NH4	Nanno
719.4863	719.4869	PG(16:1/16:0)	M-H	Chlorella, Synecho, Nanno
721.5018	721.5025	PG(16:0/16:0)	M-H	Chlorella, Synecho
733.504	733.5025	PG(17:1/16:0)	M-H	Chlorella
735.5172	735.5182	PG(16:0/17:0)	M-H	Chlorella
741.472	741.4712	PG(18:3/16:1)	M-H	Chlorella
743.4875	743.4869	PG(16:0/18:3)	M-H	Chlorella, Synecho
745.5025	745.5025	PG(16:0/18:2)	M-H	Chlorella, Synecho
747.5167	747.5181	PG(16:0/18:1)	M-H	Chlorella, Synecho
759.5161	759.5182	PG(17:0/18:2)	M-H	Chlorella, Nanno
765.477	765.4712	PG(18:3/18:3)	M-H	Nanno
767.489	767.4869	PG(18:3/18:2)	M-H	Nanno
787.5128	787.549	PG(18:1/19:1)	M-H	Synecho, Nanno
791.5008	791.4985	SQDG(16:0/16:1)	M-H	Synecho, Nanno
793.5125	793.5141	SQDG(16:0/16:0)	M-H	Synecho, Chlorella, Nanno
833.5182	833.5186	PI(16:0/18:2)	M-H	Chlorella
847.5268	847.5342	PI(17:0/18:2)	M-H	Chlorella

Table. 2. Phospholipids identified with LA-REIMS using MSMS and exact mass measurements.

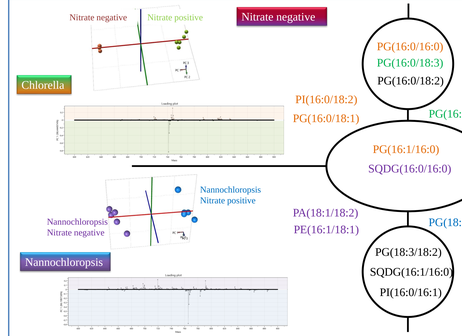


Fig. 12. Differences due to environmental perturbations. 3D PCA and adjacent loading plot of PC1 showing the differences due to Nitrogen stress, lipids identified with MSMS underlying the differences, Box plots of two selected lipids.

CONCLUSIONS

- **Our novel automated well plate reader can be used for the rapid identification of algae in ~11 minutes/96 well plate**
- **We can generate a complex lipidomic profile within seconds from a sample**
- **Identifying specific lipids with LA-REIMS method is feasible using exact mass and MSMS measurements**
- **A POC study on the effect of Nitrogen enviromental stress has showed that there are multiple changes in the lipidome – with the reduction of nitrate in the media, the saturation of the fatty acid side chains increases**