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

**INTRODUCTION**
- 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.

**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.
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**METHODS**
- **Methylation**
  - Chlorella sorokiniana - normal media and nutrient negative media pellets
  - Nannochloropsis spp. - normal media and nutrient negative media pellets
  - Synechocystis PCC6803 - liquid form
- **Synthetic PCOM** in lipid form

**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 MS spectrometer
- Scan whole well plate automatically including every MS acquisition
- Control movement, laser and acquisition parameters

**RESULTS**
- Effect of environmental perturbations
  - Nitrogen stress by reducing nitrate in the media (pellets)
- Specific lipids could be identified with LA-REIMS method.
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**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 environmental stress has shown 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.

**Figures**
- Figure 1: Typical 3D pseudo LDA plot showing the effect of Nitrogen perturbations.
- Figure 2: Well plate reader lab prototype.
- Figure 3: Confusion matrix showing the effect of Nitrogen perturbations.
- Figure 4: Full spectra of 3 different algae species sampled from PG(18:3/18:2)
- Figure 5: Spectra for the phospholipid range of 3 different algae species sampled from PG(16:0/18:2)
- Figure 6: 3D pseudo LDA plot on species level.
- Figure 7: Differences due to environmental perturbations. 3D PCA and adjacent loading plot of PC1 showing the differences due to Nitrogen perturbations.
- Figure 8: Differences due to environmental perturbations. 3D PCA and adjacent loading plot of PC1 showing the differences due to Nitrogen perturbations.
- Figure 9: Full spectra of algae showing Chlorella Nitrate negative, Chlorella Nitrate positive, Annochloropsis Nitrate negative, Chlorella Sorok. Nitrate negative.
- Figure 10: 10600 nm OPo lasers were used on concentrated media, pellet and plate.
- Figure 11: 10600 nm OPo lasers were used on concentrated media, pellet and plate.

**Tables**
- Table 1. Confusion matrix showing the effect of Nitrogen perturbations.

**Output data**
- Phospholipids become more saturated with the reduction of nitrate.
- Specific lipids could be identified with LA-REIMS method.
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- Specific lipids could be identified with LA-REIMS method.

**Proprietary Information**
- Support services, 5th layer
- Analytical, 5th layer
- Accessories, 5th layer
- VARIOUS plate formats
- VARIOUS sample formats
- VARIOUS burning profiles

**Automation**
- Rapid evaporative ionization mass spectrometry (REIMS) and an automated system for plates and pellets.
- Various plate formats, various sample formats, various burning profiles.