7th FerryBox Workshop

Flow-through PSICAM – Detecting changes in phytoplankton based on autonomous hyperspectral absorption measurements

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Part 1
MOTIVATION, BACKGROUND AND TECHNICAL DEVELOPMENT
High quality monitoring of the marine environment benefits from autonomous, high frequency measurements.

Autonomous devices like FerryBox operating on VOS further reduce monitoring effort.

Phytoplankton mostly estimated by fluorescence:
- Mostly bulk biomass
- Limited taxonomical information
- Variable due to physiological condition of the cells
- Frequent calibrations necessary

**Absorption** as a more stable alternative/addition?
Introduction: The integrating cavity approach

Obstacles in absorption measurements:

- Particles scatter light
  - Additional light loss
  - Correction necessary

- Low amount of absorbing material
  - Concentration necessary
  - Manual handling
  - Long cuvettes

**Aim:** Adapting the PSICAM approach for flow-through operation
Current status of development

The Hyperspectral Absorption Sensor (HyAbS)

- LabView-based software
- Automated operation
  - By time schedule
  - Only refill of necessary liquids
- Stand-alone or connectable to FerryBox
Absorption spectra

Shape: Influenced by pigments present

Indicator for composition

$\text{a}_{\text{pigm}} 676 \text{ nm}: \text{ Indicator of } \text{biomass}$

($\text{a}_{\text{pigm}} 676 \text{ nm} = \text{a}_p 676 \text{ nm} - \text{a}_p 700 \text{ nm}$)
Part 2

APPLICATION AND DATA EVALUATION
Field test in the Norwegian Sea

- Different water masses (coast + fjord regions)
- Continuous automated operation over 19 days
- No major technical problems
- One spectrum per minute (approx. 20000)
- Control measurements by conventional PSICAM
- Continuous in situ fluorescence measurements
Data examples

Partially, spectra were distorted

Potential reason: Light measurements biased by air bubbles in the cavity

High number of data requires automated quality check

73 % of data are compliant to these criteria
Biomass estimation

- Good accordance between continuous and manually obtained data
- No light induced variations
Group determination: Approach

Sample spectrum

Database of derivative spectra

Similarity Index
(Millie et al. 1994)

- 0.80
- 0.74
- 0.64
- 0.69
- 0.89

e等
Group determination: Test with cultures

140 spectra of algal cultures
(85 species, 16 spectral groups)

Mathematical creation of artificial communities
- All possible combinations
- One dominant spectrum, one background spectrum
- Different proportions (90:10, 80:20, 70:30, 60:40, 50:50)

Result:
Reference library of approx. 80000 mixed spectra

Test sample dataset
Test reference dataset

Evaluation:
Identification of dominant group in samples by comparison with references
Group determination: Results of lab test

<table>
<thead>
<tr>
<th>Dominating group (60 %)</th>
<th>Recognized correctly [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diatoms</td>
<td>87</td>
</tr>
<tr>
<td>Chlorophyte Type I</td>
<td>95</td>
</tr>
<tr>
<td>Chlorophyte Type II</td>
<td>59</td>
</tr>
<tr>
<td>Chlorophyte Type III</td>
<td>43</td>
</tr>
<tr>
<td>Chrysophyte</td>
<td>91</td>
</tr>
<tr>
<td>Cryptophyte Type II</td>
<td>94</td>
</tr>
<tr>
<td>Cyanobacteria Type I (bluegreen)</td>
<td>93</td>
</tr>
<tr>
<td>Cyanobacteria Type II (brown)</td>
<td>96</td>
</tr>
<tr>
<td>Cyanobacteria Type III (Prochlorococcus)</td>
<td>96</td>
</tr>
<tr>
<td>Dinophyte</td>
<td>56</td>
</tr>
<tr>
<td>Haptophyte</td>
<td>38</td>
</tr>
</tbody>
</table>

- Good identification of
  - Crysophytes
  - Cryptophytes
  - various groups of cyanobacteria

- Chlorophytes were often confused with each other, but not with other groups

- Summarizing chlorophytes in one group?

- Difficult identification of diatoms, dinophytes and haptophytes

- Summarizing in one group?
• Only occurrence of „difficult groups“
• **But:** Difference between the two fjords visible
• Results reliable?
• SI field samples < SI culture samples
• Limited number of culture spectra in database
• Light conditions different

Inclusion of field spectra in database required
Conclusions

- Progress regarding continuous automated hyperspectral absorption measurements
- Emphasis has to be put on avoiding air in the system
- Applied criteria for quality check allow a reasonable filtering of the data
- Phytoplankton identification algorithm shows successful detection of groups with distinctive pigments
- Differences between regions also in field data visible
- Further validation of results necessary (microscopy, pigment data)
- Further improvement of identification by supplementing the database with field spectra

Thank you for your attention
Group determination: Test with cultures

<table>
<thead>
<tr>
<th>Group dominating the sample</th>
<th>Dominant group was recognized as [% of samples]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillariophyte</td>
<td></td>
</tr>
<tr>
<td>Chlorophyte I</td>
<td></td>
</tr>
<tr>
<td>Chlorophyte II</td>
<td></td>
</tr>
<tr>
<td>Chlorophyte III</td>
<td></td>
</tr>
<tr>
<td>Chrysophyte</td>
<td></td>
</tr>
<tr>
<td>Cryptophyte II</td>
<td></td>
</tr>
<tr>
<td>Cyanobacteria I (Bluegreen)</td>
<td></td>
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<tr>
<td>Cyanobacteria II (Brown)</td>
<td></td>
</tr>
<tr>
<td>Cyanobacteria III (Prochlorococcus)</td>
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</tr>
<tr>
<td>Dinophyce</td>
<td></td>
</tr>
<tr>
<td>Haptophyte</td>
<td></td>
</tr>
</tbody>
</table>

- **Good identification of**
  - Cryptophytes
  - Various groups of cyanobacteria
- **Chlorophytes were often confused with each other, but not with other groups**
- **Summarizing chlorophytes in one group?**
- **Difficult identification of bacillariophytes, dinophytes and haptophytes**
- **Summarizing in one group?**
Quantifying differences in spectral shape

1.) Enhancement of spectral features

Sample spectra

4th derivative

2.) Calculation of similarity

Similarity Index (Millie et al. 1994)
Range: 0 to 1

Matrix of Similarity Indices between samples

<table>
<thead>
<tr>
<th></th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.95</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.95</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Sample 3</td>
<td>0.89</td>
<td>0.92</td>
<td></td>
</tr>
</tbody>
</table>

3.) Arrangement of stations according to their similarity
Example for conventional PSICAM data

- Regional differences in spectral shape are visible
Biomass estimation

- Diagram showing the estimation of biomass over the period from 10/Jul to 30/Jul, with data points for Sognefjord and Trondheimfjord.
- Scatter plots showing the relationship between absorption coefficient at 676 nm (a_pig) and fluorescence for HyAbS, PSICAM, and fluorescence sensor.

Mathematical equations:

- For HyAbS: $y = 1.1533x + 0.0018$, $R^2 = 0.898$, $n=47$
- For PSICAM: $y = 0.0168x + 0.0021$, $R^2 = 0.901$, $n=63$
In progress: Solid Standard Calibration

Problem: In integrating cavities, optical path length is a function of cavity-reflectivity

- Reflectivity can be calculated using a dye with known absorption
- Requires cleaning and regular dye supply

Solution: Creating a known absorption for reflectivity calculation using a solid standard
Biomass estimation

\[ a_{\text{pigm}} \text{ 676 nm is a reasonable proxy for chl-a} \]