Development of a flow cytometer for in-field, multiplexed aquatic microbiota detection

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Introduction

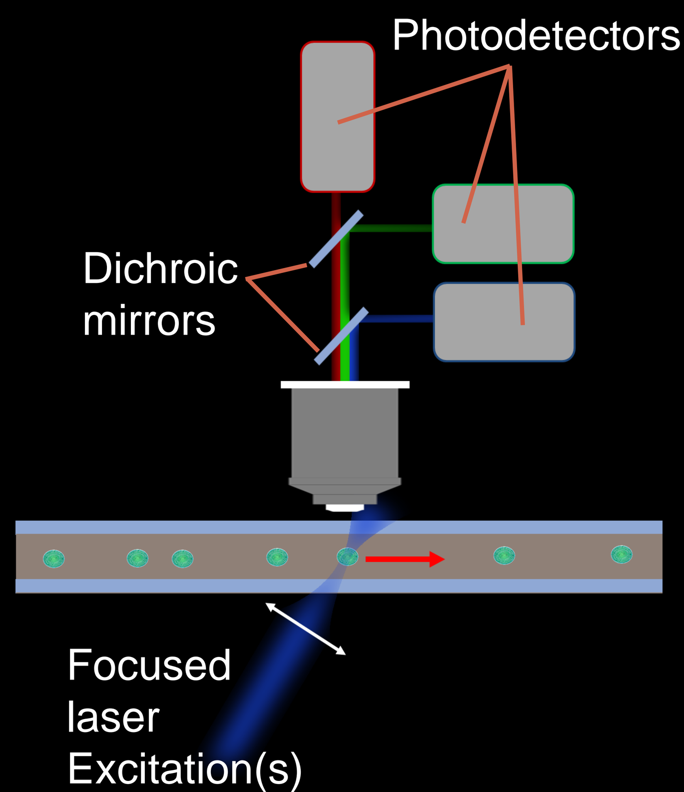
The aquatic microbiota is a key state variable when studying in-land or marine water ecosystems. Microorganisms play a strong role in biogeochemical processes, notably in the oxygen cycle, through respiration and photosynthesis. However, under the effect of climate change, the rapidly changing circumpolar Arctic region is becoming more prone to toxic algal blooms. Thus, for limnologists, oceanographers and biologists studying these remote regions, access to reliable instruments allowing for quick on-site quantification of these autotrophic organisms is becoming all-important.

Flow cytometers have been used for decades to quantify picophytoplanktons, such as cyanobacterias based on their intrinsic photosynthetic pigment autofluorescence. However, in their classical form, these instruments suffer from highly sensitive alignement due to tightly focused lasers and bulkiness mostly due to multiple optical paths and detectors, making them misadapted for in-field use.

We propose here a novel interference spatially modulated flow cytometry approach that allows for multiplexed single detector fluorescence measurement of photosynthetic pigments. This technology holds promise for an alignment-free and compact flow cytometer.

Flow cytometry

A flow cytometer usually consists of a hydrodynamically focused flow of analytes on which one or multiple lasers are focused. Scattering and fluorescence are then seperated on multiple detectors using a set of dichroic mirrors to obtain multiparametric information, such as size, granularity and fluorochrome concentrations on each cell, which in turn allows to distinguish populations.



Advantages

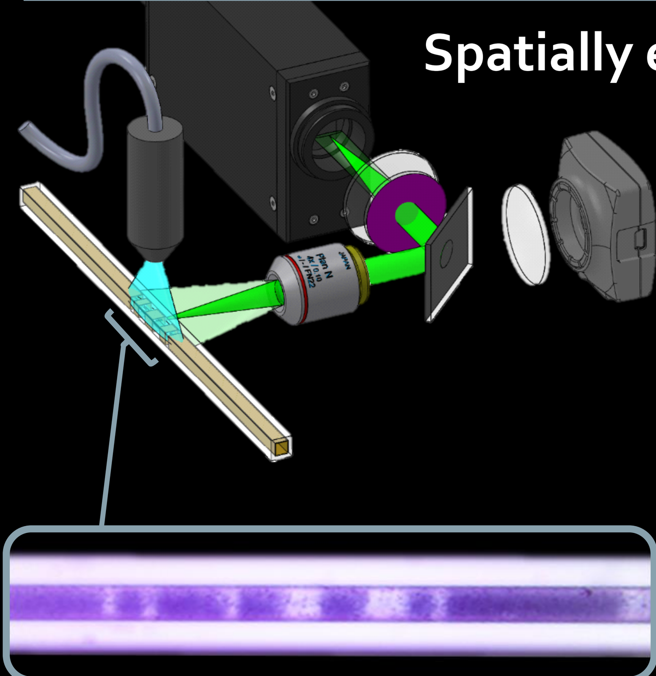
- Fast, high throughput detection
- Multiparametric analysis allows subpopulation identification
- Highly sensitive
- Allows physical cell sorting (FACS)

Drawbacks

- Critical and complex alignment
- Mostly bulky and heavy instruments
- Expensive
- Requires regular calibration and maintenance
- Requires highly qualified operator

Ref: https://flowcytometry.weebly.com/advantages--disadvantages.html, December 2017

Spatially encoded cytometry



Typically, in spatially encoded flow cytometry, the fluorescence emission of an analyte, such as a phytoplancton, is modulated by placing a mask with known features between the particle and the detector. The same caracteristics can be found in the time signal.

Advantages

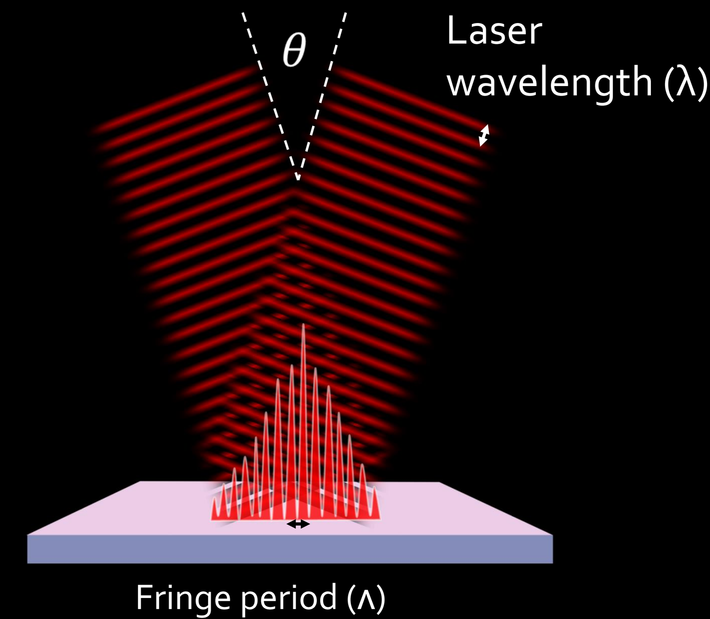
- Enhanced signal-to-noise ratio
- Multiple simultaneous particle detection
- Reduced alignment

Drawbacks

- Complex and expensive capillary fabrication
- Imperfect modulation contrast

Boudreau et al., US Patent 20140356937 Patterned Capillary Device and Process for Fabricating Thereof , **2014**

Two beam interference



$$\Lambda = \frac{\lambda}{2\sin(\theta/2)}$$

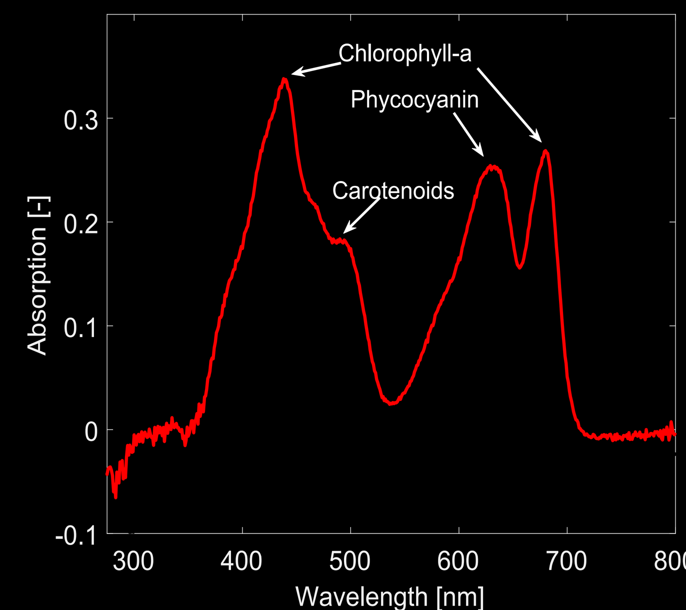
As originally demonstrated by Young's double slit experiment, two mutually coherent light beams, upon crossing each other at an angle, will produce a set of bright and dark fringes through a physical process called **interference.**

The fringe period (Λ) is proportional to the wavelength of the source.

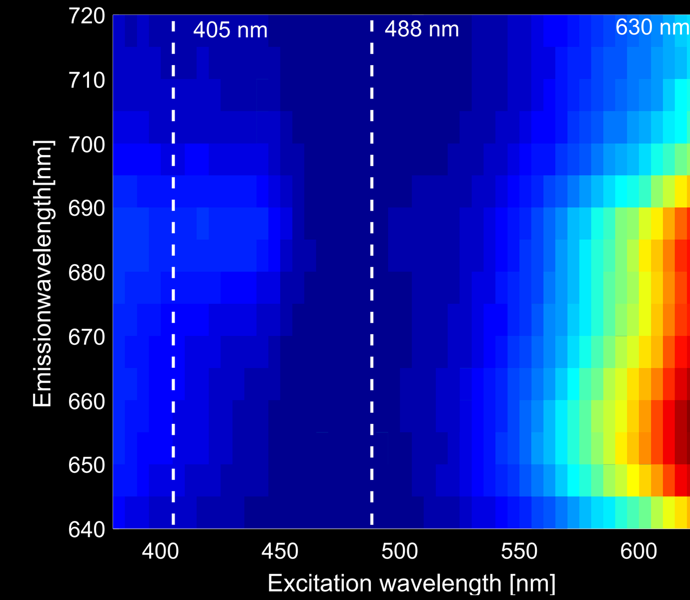
The interference spatially modulated flow cytometry concept exploits this property of light.

Spectroscopic caracterization of cyanobacterias

Absorption spectra



3D fluorescence spectra



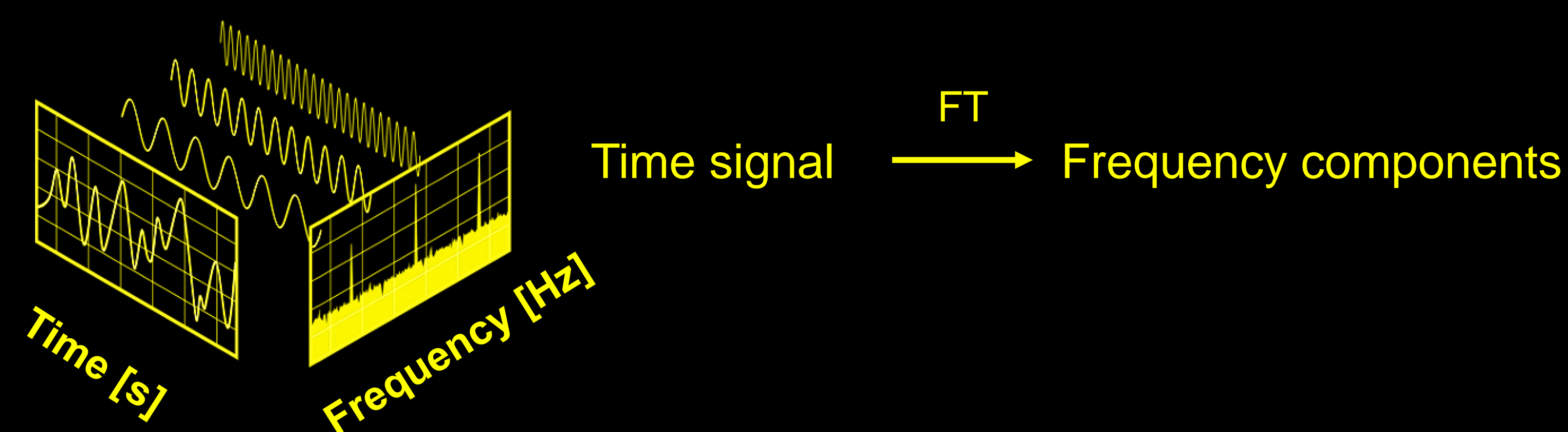
Absorption maximas:
- **Chlorophyll-a** : 440 nm, 680 nm
- **Phycocyanin** : 630 nm

Emission maximas
- **Chlorophyll-a** : 690 nm
- **Phycocyanin** : 650 nm

Phycocyanin and chlorophyll-a have partially overlapping emission spectrums which would require complex time gating in classical cytometry. However, the interference spatially modulated flow cytometry technique allows to measure both their fluorescence at different temporal frequencies.

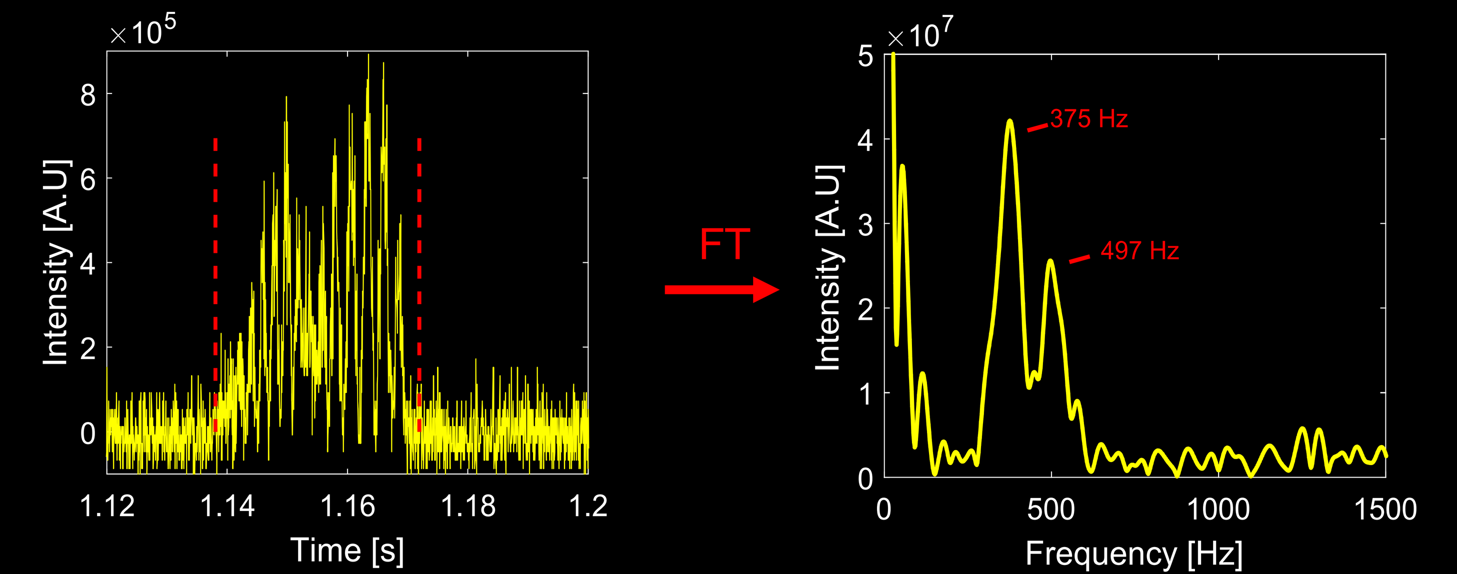
Signal processing

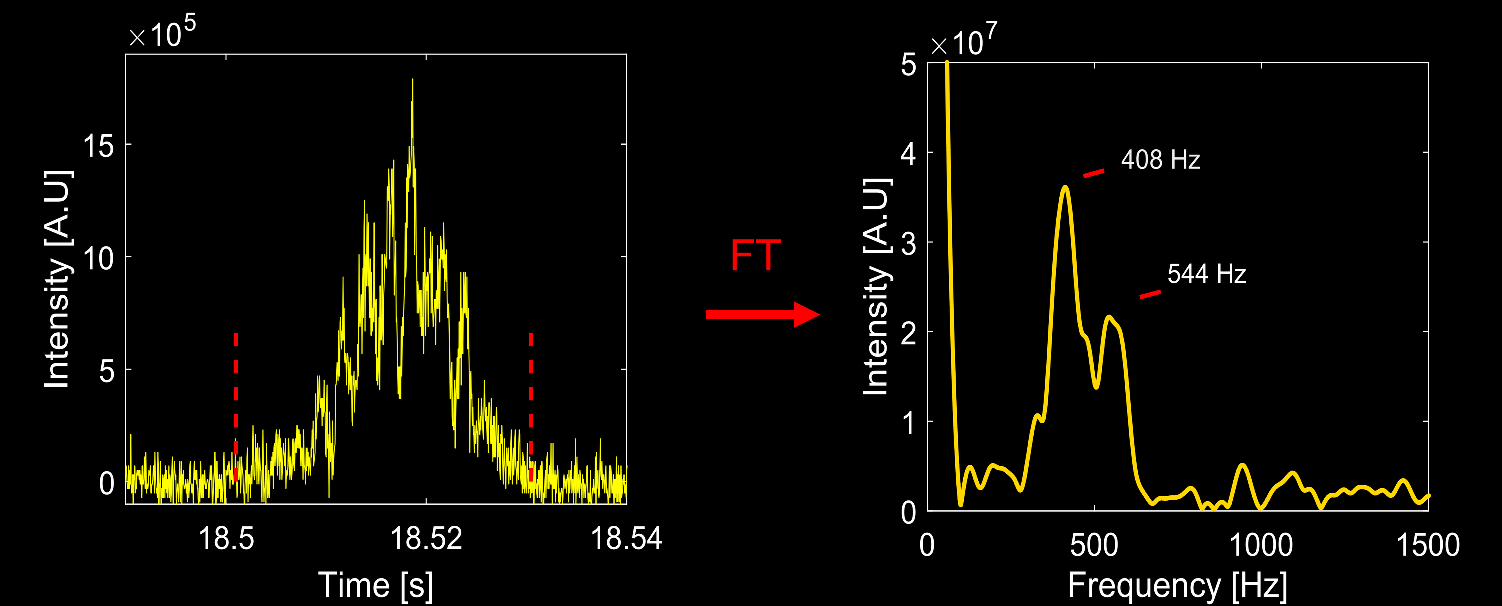
Spatially encoded flow cytometry allows to apply different signal processing techniques such as cross-correlation to enhance known time signal features. In this case, the periodic nature of the interference modulation makes the **Fourier transform (FT)** especially effective. The Fourier transform is a mathematical operation that decomposes a time signal into its different frequency components.



Cytometric measurements

Two excitation configurations were tested:





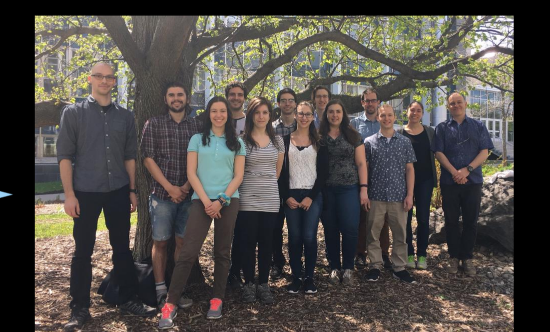
The results shown here present the time signal and the Fourier transform of detection events obtained when cyanobacterias are flowed into the cytometer. In both cases, chlorophyll-*a* and phycocyanin fluorescence, corresponding respectively to the higher and lower frequency peaks can be distinguished. We also note the constant ratio between the frequency maximas which allows for easy identification and validation of these peaks.

Conclusions and perspectives

In conclusion, we demonstrated here an excitation scheme for flow cytometry that allows for multiplexed measurements of photosynthetic pigment fluorescence. We believe that this concept can be extended with more sources, for example to incorporate phycoerythrin detection. Future works in this project will include the production of a miniaturized, field-ready device through opto-fluidic integration.

Acknowledgements

INNOVATION.CA
CRSNG NSERC
Fonds de recherche Nature et technologies Québec
Sentinel North
Professor Boudreau's research group



- Professor Warwick F. Vincent and his group