DOCTORAL THESIS

A light sheet based fluorescence imaging flow cytometer for phytoplankton analysis

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Abstract

Monitoring phytoplankton species composition and their abundance are routine tasks in marine ecological research and environmental monitoring. As phytoplankton populations are highly heterogeneous in terms of size, morphology, and most significantly, their abundance can change drastically in a very short time, it is extremely difficult to quantify and monitor them and there are demands on the instrumentation.

Conventional optical microscopy and flow cytometry are the main tools to enumerate and identify phytoplankton, but they have a compromise between spatial information and acquisition speed. While imaging flow cytometry has the potential to integrate the benefit of high spatial resolution from optical microscopy and the advantage of high throughput from flow cytometry, two intrinsic blur sources, motion blur and out-of-focus blur, prevent imaging flow cytometers from obtaining high spatial resolution images with high throughput.

To address these limitations, in this work, a novel light sheet based fluorescence imaging flow cytometer has been proposed, constructed, and tested for phytoplankton analysis. Both 2D and 3D imaging mode of the light sheet based fluorescence imaging flow cytometer have been investigated.

In the 2D imaging mode, the instrument can screen untreated costal water samples at a volumetric throughput up to 1 ml/min. The instrument demonstrated shows a high immunity to motion blur, and all-in-focus fluorescence images are captured with a lateral resolution of 0.75 ± 0.06 μm for a wide size range ~ 1 μm to ~ 200 μm that includes pico-, nano- and microphytoplankton. This is made possible by suppressing the out-of-focus blur using thin light sheet illumination and image deconvolution, and by precluding the motion blur with a unique flow configuration. With these abilities, the instrument demonstrated has high potential as a practical field instrument for monitoring phytoplankton.

In the 3D imaging mode, the instrument can scan a large number of phytoplankton cells in a short time with spatial resolution as achieved by light sheet microscopy. The lateral resolution is 0.81 ± 0.07 μm, and axial resolution in terms of FWHM of the axial scattering PSF is 1.42 ± 0.15 μm. The volumetric throughput of the instrument is 0.5 μl/min. This is benefitted from the improvement that 3D images can be acquired without the need of sample immobilization, in contrast to existing 3D imaging approaches, such as confocal fluorescence microscopy.

Preliminary results from untreated coastal water samples and cultured samples show promising potentials of the instrument for phytoplankton monitoring and scientific research.

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