FULL FIELD HYPERSPECTRAL IMAGING IN FLUORESCECNE MICROSCOPE

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Hyperspectral fluorescence imaging is a powerful method to map multiple fluorophores even with overlapping emission spectra. Commercial fluorescence microscopy systems employ several types of spectral filtering hardware. Novel are thin-film tunable filters with the advantage of improved transmission and higher out-of-band blocking.

The presented TuneCube microscope takes full advantage of the characteristics of these tunable filters making them the crucial element in the newly developed full field fluorescence microscope. TuneCube enables a spectral separation of excitation and emission path without the need for an additional dichroic mirror as well as an easy exchange of the tunable filter in the microscope. This allows the new system to overcome the limitations of fixed filter sets and combines the capability of fluorescence microscopy with hyperspectral imaging in a small footprint.

Spatial resolution and image quality of the TuneCube microscope are characterized by means of a calibration slide, Argo-LM (Argolight), and results are compared with those of a research grade inverse fluorescence microscope.

Hyperspectral imaging is demonstrated with samples of algae *micrasteria* and three types of thin-film tunable filters: 704 nm VersaChrome tunable longpass filter (F35-680 AHF), 704 nm VersaChrome tunable shortpass filter (F35-679 AHF) and VersaChrome HC 697/13 tunable bandpass filter (F35-697 AHF). Spectral resolution of the hyperspectral images has been quantified with samples of quantum dots at slightly varying center emission wavelength @660nm and @690nm and scanning the TuneCube at 1 nm step size.

The criteria illumination inhomogeneity, field distortion, and contrast of the TuneCube microscope turned out to be matched on the same scale as the research grade commercial system. High contrast recording of emission spectra of algae samples *micrasteria* and quantum dots agree with the expected spectra of chlorophyll for all three types of tunable filters. Taking raw data sets without any image correction algorithm yields a spectral resolution of < 3 nm. In conclusion the TuneCube microscope setup proved easy to handle. The tunable filters can be changed quickly without readjustment for fluorescence microscopy and full field hyperspectral imaging capabilities. High throughput emission signals are taken for the full field imaging or regions of interest with one or more fluorophores.

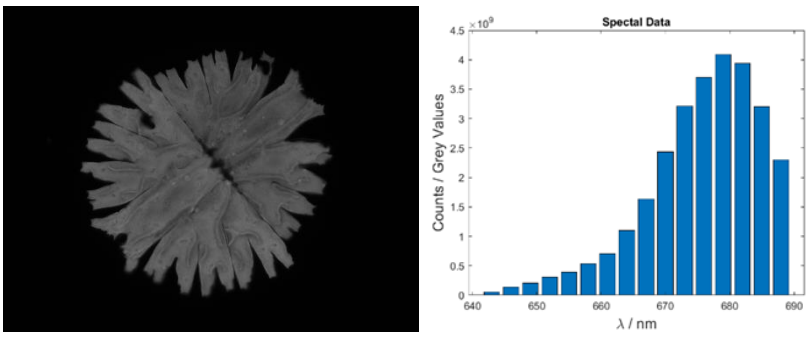

Figure 1: Fluorescence image of micrasteria algae and recorded emission spectra