FluoReader—LED-based Fluorescence Reader with Configurable Color Channels for Flow Cytometry Applications

P. Cristofolini, G. Orawez, Z. Halvorsen, S. Cattaneo

Fluorescence measurements are widely used for measuring chemical or physical properties of cells and particles in solution. CSEM is developing a configurable, cost-effective and sensitive fluorescence reader for flow cytometry applications and fluorescent readout of microfluidic assays.

Fluorescence (FL) is the spontaneous emission of light by a substance that has absorbed (in most cases) light of higher frequency. Many minerals, molecules, proteins, DNA and living organisms fluoresce under ultraviolet illumination. In life sciences, fluorescence staining is a much-used technique to visualize tissue structure and metabolic or physiological processes with high specificity and spatial resolution. For this purpose, fluorescent marker molecules are introduced into cells and accumulate in specific cell compartments due to physiological or osmotic transport, or due to the unique chemical binding properties of the fluorescent stain.

Stained cell are often used in flow cytometry (FCM), a powerful technique to screen tens of thousands of cells per second for specific chemical or physical properties, like the presence of a specific protein, metabolic or physiological state and reproductive activity. FCM is widely used in biotechnology, cancer research, genetic engineering and water quality monitoring. It relies on a simple working principle: cells suspended in water are aligned in a single row and flow through a focused light beam that excites fluorescence in the cells. The faint fluorescence light is collected, filtered in different spectral regions and measured electronically. These fluorescence signals are often combined with scattering signals and analyzed in multi-dimensional plots, where cells with different properties separate into clusters whose size can be used for statistical analysis of the cell population.

A typical flow cytometer is benchtop-sized, and its detection system requires several sets of expensive components: high power lasers with special emission wavelengths, matching spectral filters with very sharp edges and extremely sensitive photon detectors with large gain (photomultiplier tubes). In the project FluoReader a standalone fluorescence reader is being developed with two excitation sources (cyan and orange) and three detection channels (Figure 1):

- green: SYBR green stain for discriminating biological cells for abiotic particles
- red: DiBAC stain for bacterial metabolic activity
- NIR: propidium iodide stain for bacterial viability

The measurement configuration was chosen to be in reflection (Figure 1), so that samples can be excited and measured from the same side. This does not limit the sample options to a transparent measurement cell, so that the readout from non-transparent surfaces becomes possible. The reader has exchangeable excitation and detection modules that can be fitted with laser diodes or LEDs. The use of high-power LEDs allows to easily customize the excitation wavelength to the measured stain at a very reasonable cost. For detection, multi-pixel photon counters (MPPCs, arrays of silicon photomultipliers) are used instead of expensive conventional photomultiplier tubes to count single fluorescence photons one-by-one. The housing measures 174 x 122 x 56 mm and is fully 3D printed, with alignment features for the optics and a 3D positioning stage for microfluidic chips. Special care has been taken to reduce stray light, especially for the LEDs, due to their large emission angle. For the laser diode option, a 25 mm diameter eccentric mount was developed, which allows seamless positioning of the laser diode in xyz in a range of ±1 mm for exact alignment with the optical axis of the reader. Electronic readout is carried out on an oscilloscope interfaced with MATLAB. At a later stage the oscilloscope will be replaced with a dedicated microcontroller that processes the data onboard.

The fluorescent reader can be used to measure fluorescence properties of static and in-line samples. It is ideally suited for the fluorescent readout of microfluidic chips and in-line flow cytometry. It is planned to benchmark the reader in single cell detection against commercial FCM systems and to use it for fluorescent readout of the in-house developed Inca slide assays.

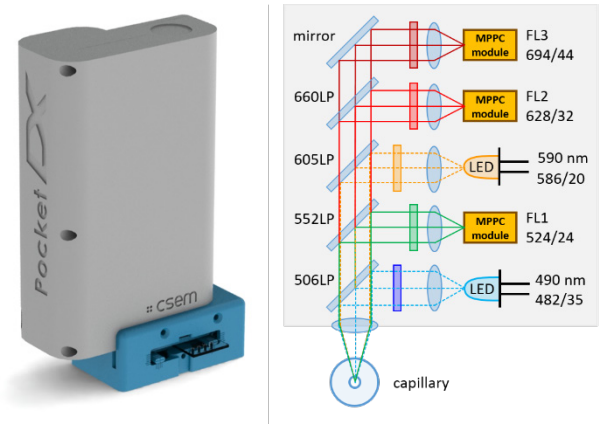

Figure 1: (left) Rendered view of the reader with microfluidic slide holder; (right) Optical sketch with spectral properties of filters and light sources.

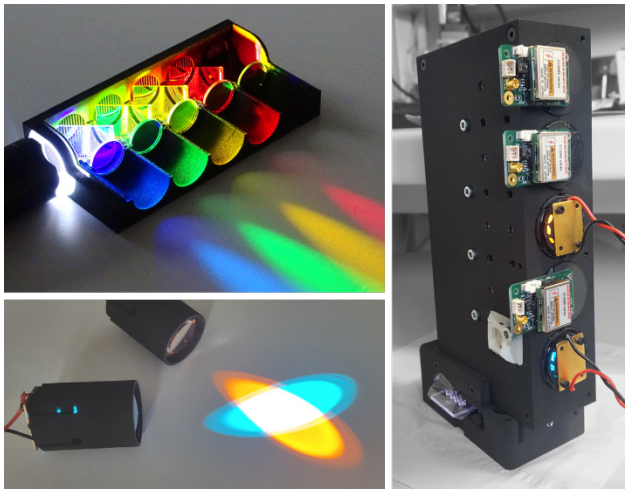

Figure 2: (top left) Optical setup with mounted spectral filters separating the light for the respective emission/detection channels; (bottom left) LED modules with cyan and orange light; (right) Reader equipped with light sources and detectors.