

Automated flow cytometry for on-line bioprocess monitoring

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Introduction

Process monitoring is crucial in the biopharmaceutical industry, particularly for characterization, optimisation and control of recombinant proteins production. Only limited solutions are presently available for intracellular product formation monitoring.

This study aimed to create a proof-of-concept (POC) automated prototype to sample, dilute, stain and measure via Flow Cytometry (FCM) samples from an *E.Coli* fermentation. The performance of the prototype was then evaluated in terms of accuracy, sensitivity, reproducibility and robustness.

FCM is based on light properties of the cells passing in front of a laser in a single file.

Detection via APD detectors:

- Forward scatter (FSC): information on size.
- Side scatter (SSC): reflects complexity/granularity.
- Fluorescence signal selected through dichroic mirrors and band-pass filters.

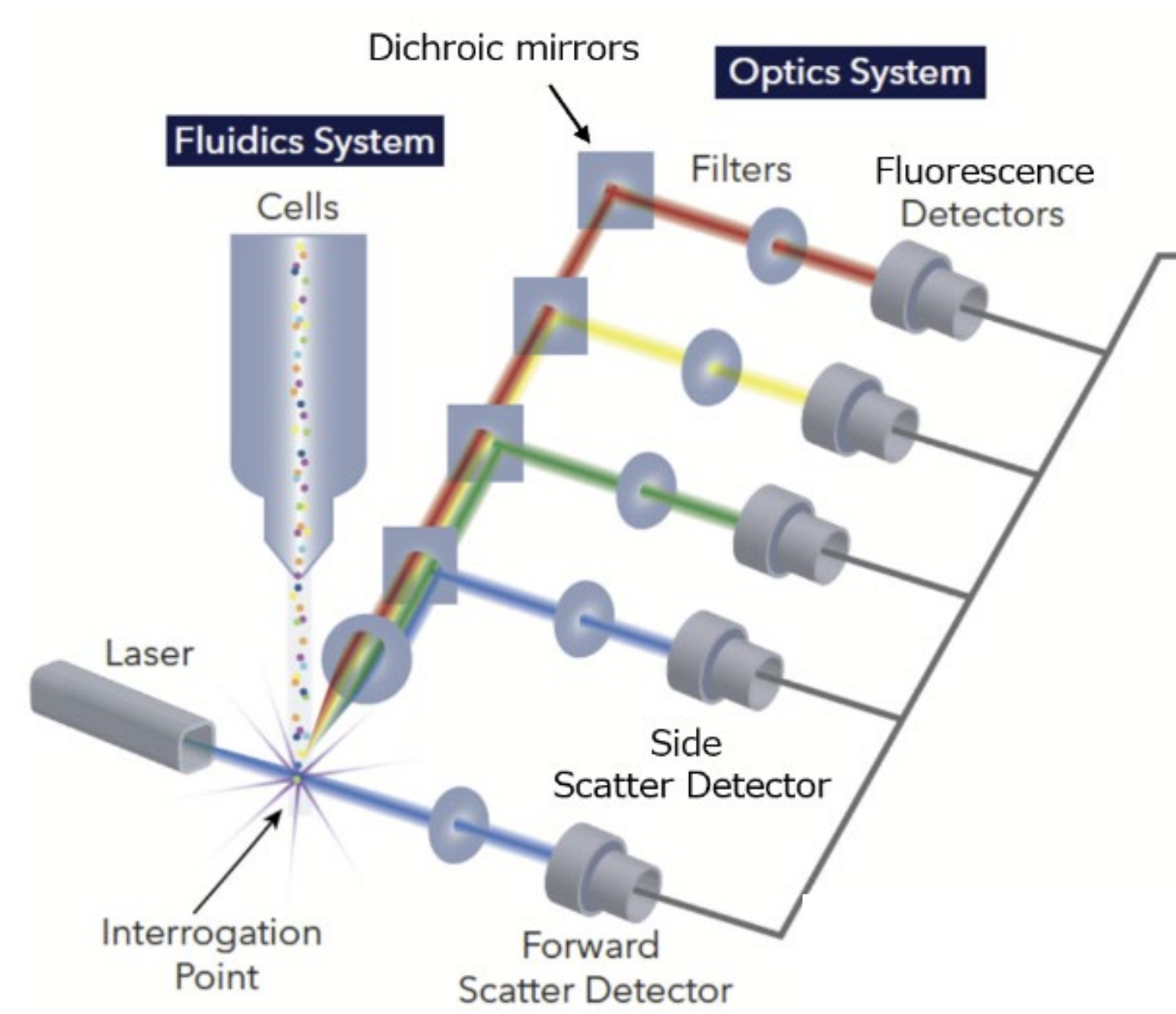


Figure 1. Flow cytometry principle [1]

Methods

- Samples were taken from an *E.Coli* strain modified to express a product of interest (POI) as intracellular inclusion bodies.
- Biomass quantification was realized with Flow cytometry (Syto62 total DNA staining) and optical density at 600 nm (OD600) measurements.

Prototype design

The prototype created comprised 3 modules (Dilution, FCM measurement and Staining) orchestrated by a NI LabVIEW control application running on a Windows PC.

- Sampling and dilution was managed using two pumps (biomass, D.I. water) with different pump rates (D1, D2).
- A 250 μ L sampling loop mounted on a 6-way valve allowed to switch between dilution and FCM measurement.

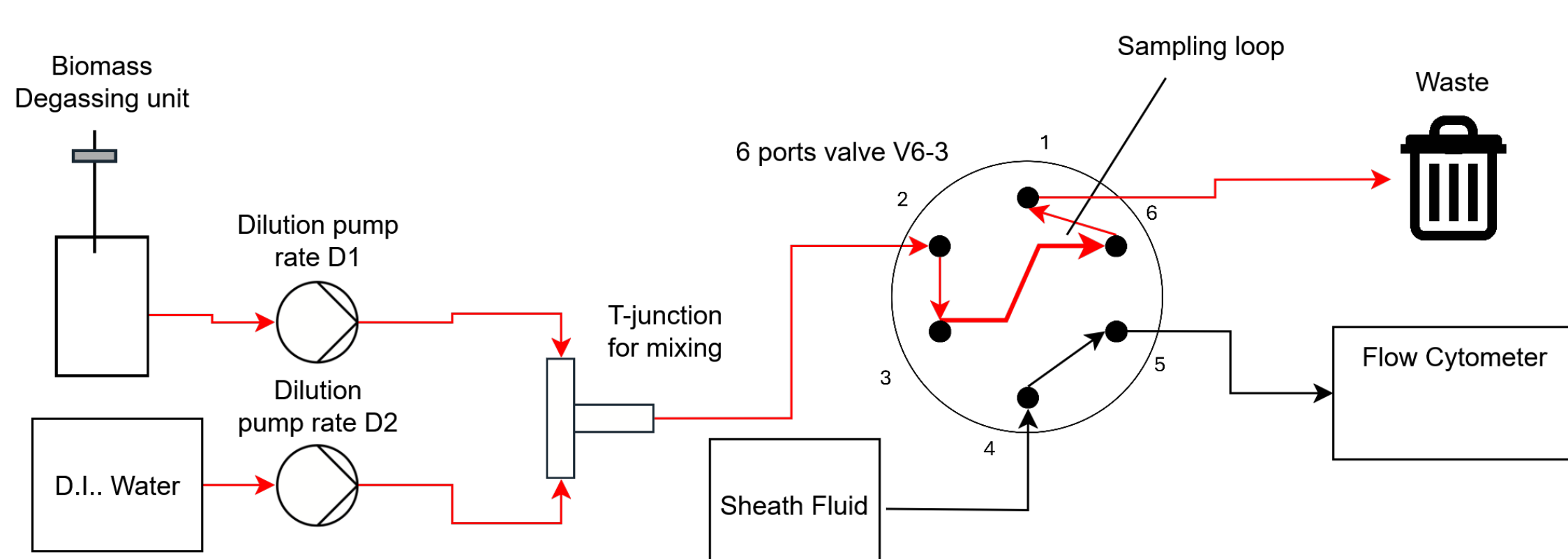


Figure 2. Prototype dilution system diagram

- Sample staining was realized using a microchamber (MC) equipped with a 0.45 μ m cellulose acetate membrane, allowing to retain the cells and perform on-membrane staining and washing.

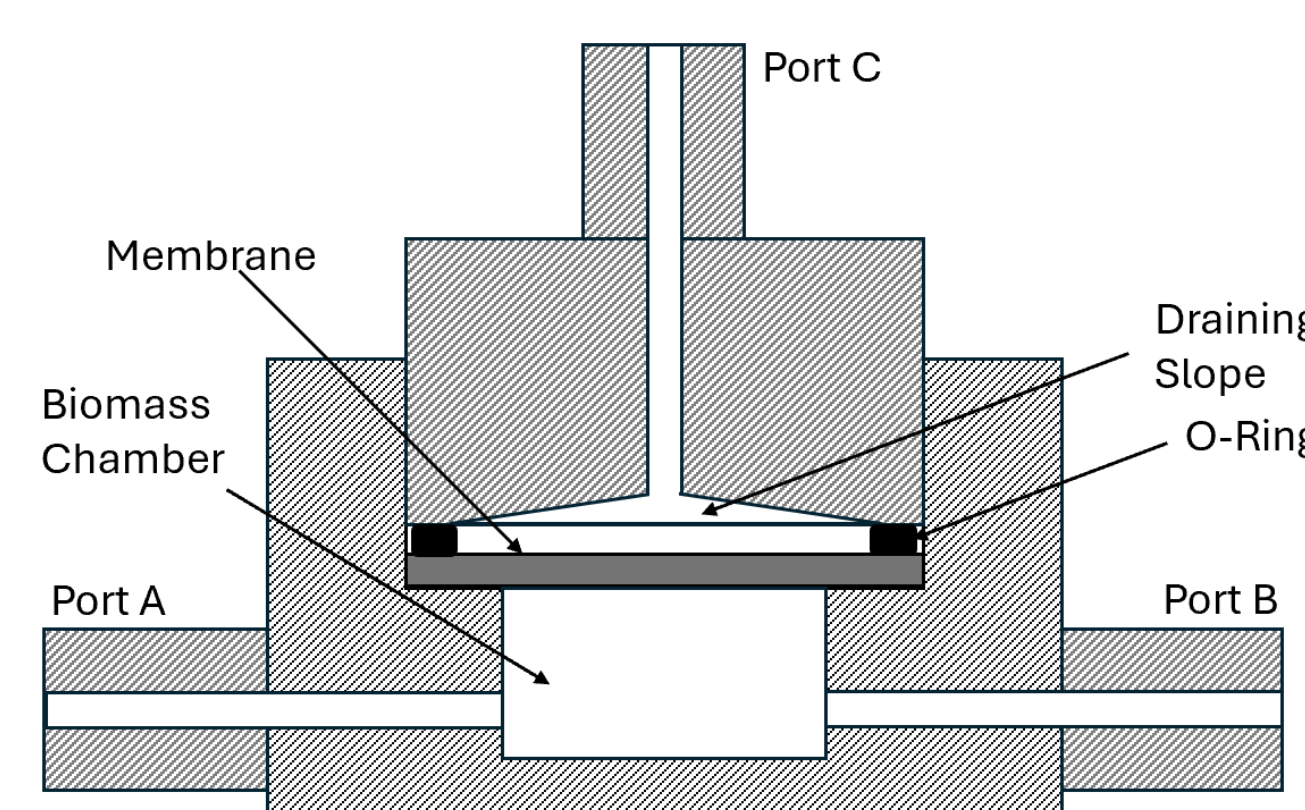


Figure 3. Microchamber concept

Results and discussion

FCM measurement module performance:

- **Accuracy:** Density measured via the FCM module for a known OD600 calibration curve. A strong linear correlation was found in the OD600 0.004-0.525 range, defining the prototype analytical range.

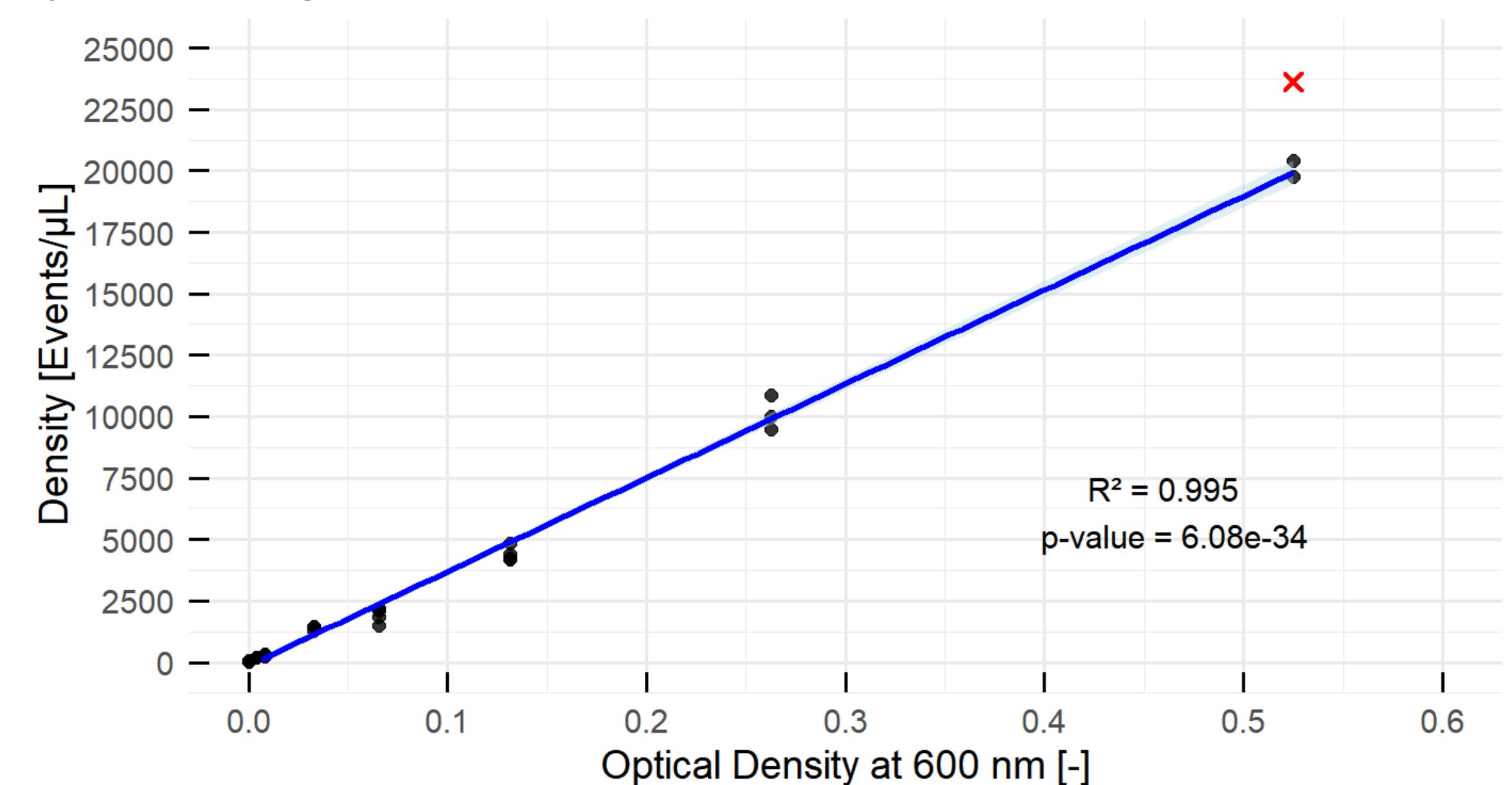


Figure 4. Density measured versus OD600

- **Sensitivity:** Limit of detection (LOD: Mean + 3.33*SD) was found to be 85.5 Events/ μ L, with a measured limit of quantification (LOQ) of 187 $\pm$ 7 Events/ μ L.
- **Repeatability:** Analytical range average variation coefficient (CV) was 8 $\pm$ 5%.

Staining module performance:

- Biomass samples were stained using Syto62 using the MC and measured via FCM. Compared to a conventional Syto62 staining, a higher mean fluorescence intensity was attained for the MC-stained samples.

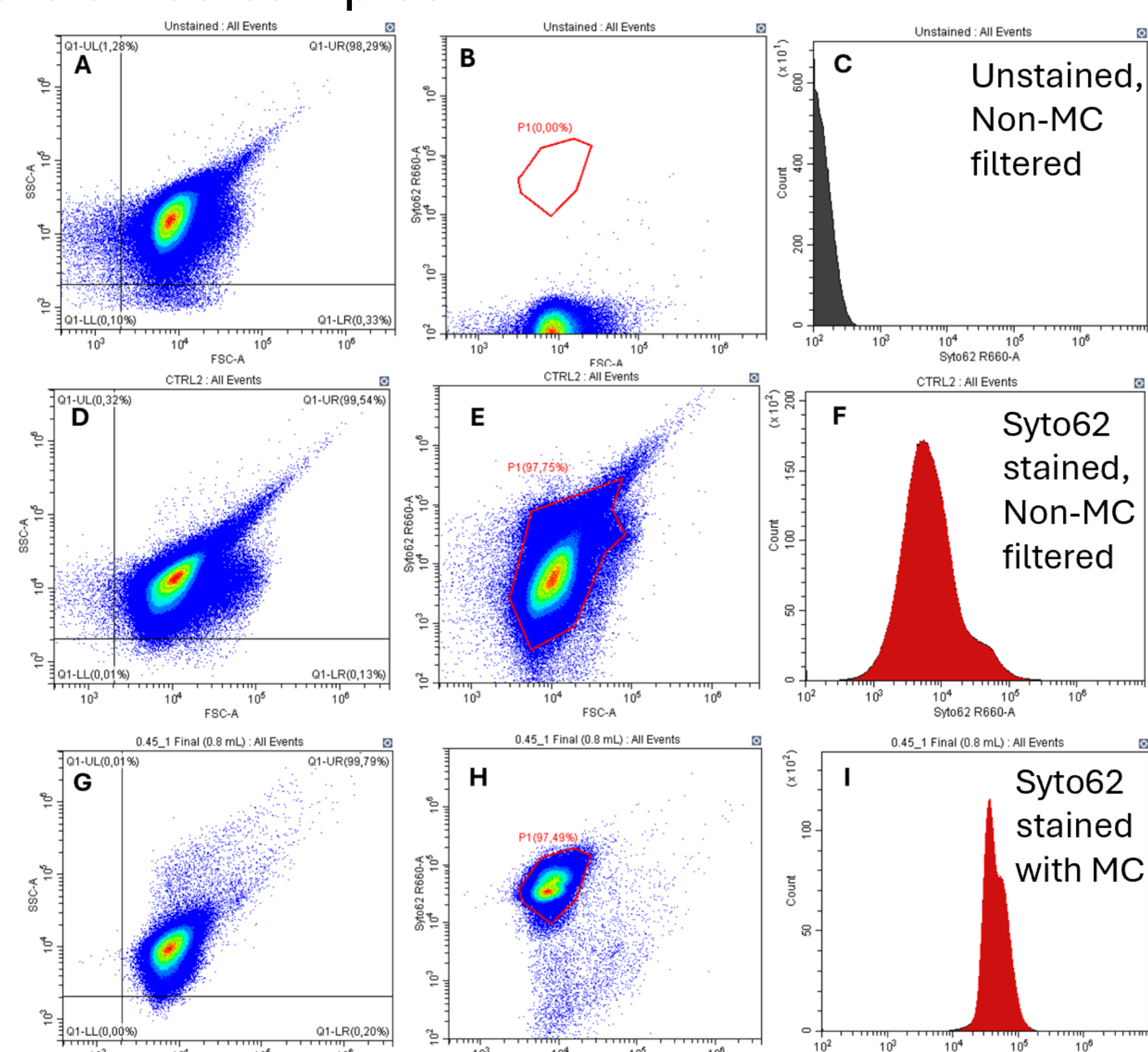


Figure 3. FCM measurements for MC-stained samples versus unstained and control stained

Conclusion

POC prototype operated reliably in manual and automated modes, producing experimental data for all modules.

Prototype demonstrated higher accuracy at low cell density, an LOD of $\approx$ 85 Events/ μ L, and < 10% CV for the analytical range. Staining results demonstrated feasibility of microchamber staining and application to biomass quantification and growth monitoring.

References

[1] Funakoshi and R&D Systems (biotechnie), 'Multi-Color Flow Cytometry Kit (R&D Systems): How Flow Cytometers Work'. [Online]. Available: <https://www.funakoshi.co.jp/contents/71721>