Extending FluoSens®
to Microfluidic Fluorescence Detection

Feasibility Study for Fluorescent Dye and Particle Measurements in Microfluidic Channels

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Abstract

Microfluidic systems have become indispensable tools in life science research, medical diagnostics and Lab-on-a-Chip technologies. As these platforms continue to shrink in size while increasing in analytical complexity, there is a growing demand for compact fluorescence detection solutions that can be readily integrated into microfluidic devices.

This feasibility study demonstrates the feasibility of extending the FluoSens® fluorescence sensor technology from conventional fluorescence measurements to fluorescence detection within microfluidic channels. **Experimental studies using dissolved fluorescent dyes (HEX and Cy5) and fluorescent polymer beads (Spherotech Nile Red and PAK Blue) confirm that the system is capable of measuring both continuous fluorescence signals and discrete fluorescent events in flowing samples.** The results highlight the potential of FluoSens as a compact fluorescence detector for OEM integration and microfluidic analytical systems.

Introduction

Fluorescence detection is a widely used analytical technique in modern bioanalysis due to its high sensitivity and molecular specificity. Applications such as flow cytometry, cell analysis, molecular diagnostics and Lab-on-a-Chip platforms increasingly rely on fluorescence measurements performed directly within microfluidic channels.

Conventional fluorescence instrumentation often provides excellent analytical performance but is frequently optimized for laboratory benchtop use and standard sample formats such as cuvettes, PCR tubes or microplates. The integration of fluorescence detection into compact microfluidic systems therefore remains an important technological challenge.

The objective of this study was to evaluate the FluoSens fluorescence sensor platform application to fluorescence measurements inside 100 µm microfluidic channels while maintaining reliable signal acquisition under continuous flow conditions.

Experimental Approach

Fluorescence measurements were performed using a dual-channel coaxial FluoSens detector from lumalytix. Samples were pumped through commercially available **75 µm × 150 µm microfluidic channels** while fluorescence signals were continuously recorded.

To increase the efficiency of excitation and emission detection, a **100x microscope objective** lens was implemented, replacing the traditional plano-convex external lens in FluoSens (Figure 1).

Figure 1: FluoSens Go with a 100x microscope objective lens replacing the traditional lens displayed in the background Go.

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This transformation facilitated a **reduction in the light spot diameter from ~800 µm to 150 µm** (Figure 2), allowing the excitation light beam to be fully concentrated into the 150 µm wide microchannel while increasing the light collection angle of the fluorescence emission produced by the dyes as well as the beads.

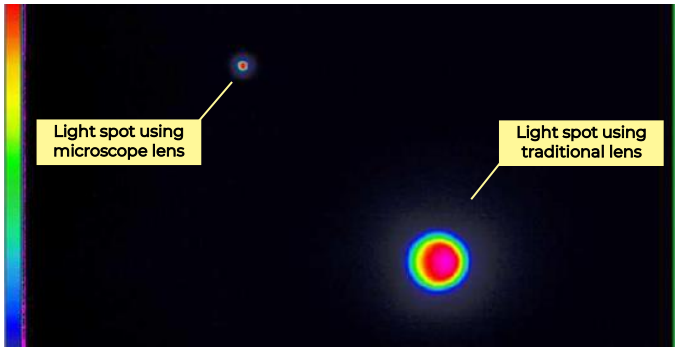

Figure 2: Excitation beam profile (spot) using the traditional plano-convex lens and microscope objective lens in FluoSens.

Two complementary experiments were conducted: Fluorescent dye measurements and fluorescent particle measurements.

- **Fluorescent dye measurements:** Continuous flow measurements using HEX (800 nM) and Cy5 (200 nM) to validate optical alignment, channel-specific fluorescence detection and measurement stability.
- **Fluorescent particle measurements:** Detection of approximately **10 µm fluorescent polymer beads** (Spherotech Nile Red and PAK Blue) suspended in buffer to evaluate the capability of detecting individual fluorescence events under flow conditions.

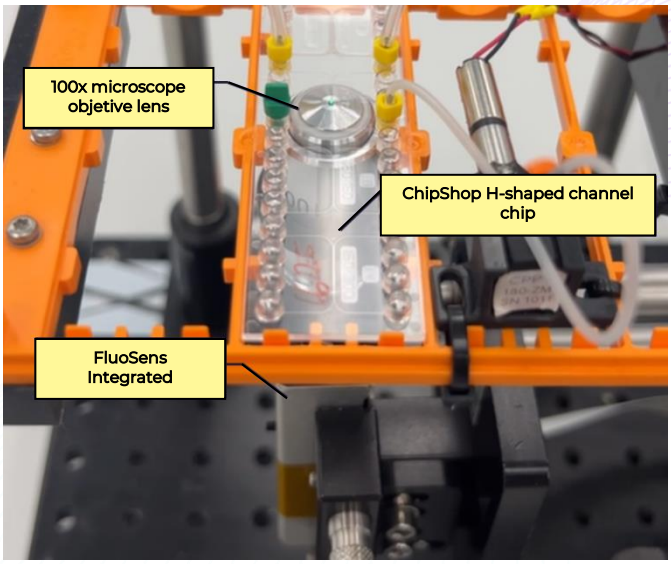

Figure 3: FluoSens microfluidic measurement setup.

Results

Continuous fluorescence measurements

The dye experiments demonstrated stable and reproducible fluorescence measurements within the microfluidic channels for both investigated fluorophores, with an excellent signal-to-background ratio. Distinct signal levels were obtained for HEX and Cy5 in their respective optical channels, confirming correct optical alignment and successful fluorescence detection under continuous flow conditions. The measurements also verified the suitability of the optical setup for subsequent particle detection experiments.

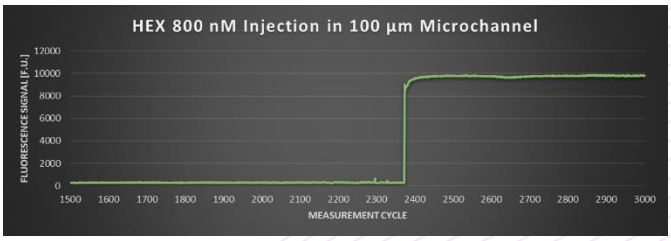

Figure 4: HEX dye measurement in microchannel.

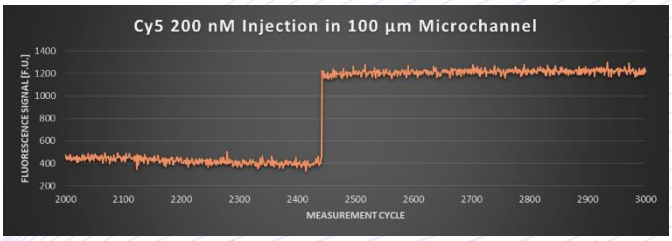

Figure 5: Cy5 dye measurement in microchannel.

Detection of fluorescent particles

Building upon the successful dye measurements, fluorescent bead suspensions were analyzed under identical microfluidic conditions. Individual fluorescence peaks corresponding to particles passing through the detection volume were reproducibly observed for both Nile Red and PAK Blue beads.

The experiments further demonstrated that detector parameters such as LED excitation intensity can be optimized for fluorophores with lower fluorescence emission. While variations in detected peak counts were observed between measurements, as expected for dilute particle suspensions and continuous-flow microfluidic experiments, the fluorescence events remained clearly distinguishable from the background signal throughout the study.

Representative signal traces illustrate both continuous fluorescence measurements and transient fluorescence peaks generated by particles moving through the micro-channel.

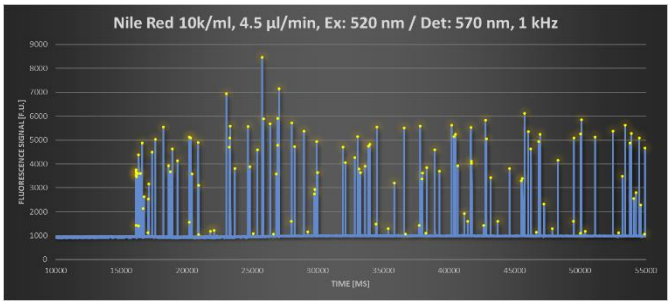

Figure 6: Nile Red fluorescence bead signal in microchannel

The results in Figure 6 demonstrate a test run performed with the 10 µm Nile Red beads in a 10,000 bead/ml concentration, injected into a 75 µm deep x 150 µm wide microchannel of ChipShop's H-shaped channel chip (10001851) using a peristaltic pump running at 4.5 µl/min flow rate.

Implications for Microfluidic Integration

The presented feasibility studies demonstrate that FluoSens can be extended beyond conventional fluorescence measurements to microfluidic applications without requiring fundamental changes to the optical architecture.

Key capabilities demonstrated include:

- Fluorescence detection inside 100 µm microfluidic channels.
- Dual-channel detection of multiple fluorophores.
- Measurement of both dissolved fluorescent dyes and fluorescent particles (diameter: 10 µm)
- Continuous fluorescence monitoring under flow conditions.
- Reduction of the light spot diameter down to 150 µm.
- Sampling rates of up to 1,000 Hz, with the system architecture enabling further increases in sampling rate
- Excellent signal-to-background ratio.
- Compact sensor architecture suitable for system integration.

These characteristics make the platform attractive for integration into Lab-on-a-Chip instruments, microfluidic analytical systems, point-of-care diagnostics and other fluorescence-based OEM solutions.

Conclusion

The performed feasibility studies confirm that the FluoSens fluorescence sensor platform is well suited for fluorescence measurements within microfluidic environments. Successful detection of dissolved fluorophores as well as fluorescent polymer beads demonstrates the versatility of the optical system and its adaptability to flow-based analytical applications.

By combining a compact all-in-one design with customizable dual-channel fluorescence detection, FluoSens offers an attractive sensing platform for developers of next-generation microfluidic instrumentation seeking a flexible and easily integrated fluorescence detection solution.