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# At-line bioprocess monitoring by immunoassay with rotationally controlled serial siphoning and integrated supercritical angle fluorescence optics

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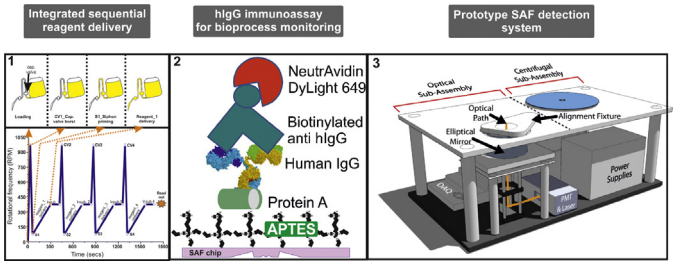
## HIGHLIGHTS

- Sample-to-answer microfluidic lab-on-a-disc device for at-line bioprocess monitoring.
- Centrifugally integrated and automated reagent delivery by serial-siphon valves.
- Supercritical angle fluorescence optics embedded on centrifugal platform.
- Development of fluorescence-linked-immunosorbent assay on human immunoglobulin G.
- Bioprocess samples from industrial reactor determined on the prototype system.

## GRAPHICAL ABSTRACT

A serial siphon based centrifugal microfluidic platform for the quantitative at-line monitoring of human immunoglobulin G (hIgG) in typical industrial bioprocess samples using a prototype optical SAF detection system.

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## ABSTRACT

In this paper we report a centrifugal microfluidic "lab-on-a-disc" system for at-line monitoring of human immunoglobulin G (hIgG) in a typical bioprocess environment. The novelty of this device is the combination of a heterogeneous sandwich immunoassay on a serial siphon-enabled microfluidic disc with automated sequential reagent delivery and surface-confined supercritical angle fluorescence (SAF)-based detection. The device, which is compact, easy-to-use and inexpensive, enables rapid detection of hIgG from a bioprocess sample. This was achieved with, an injection moulded SAF lens that was functionalized with aminopropyltriethoxysilane (APTES) using plasma enhanced chemical vapour deposition (PECVD) for the immobilization of protein A, and a hybrid integration with a microfluidic disc substrate. Advanced flow control, including the time-sequenced release of on-board liquid reagents, was implemented by serial siphoning with ancillary capillary stops. The concentration of surfactant in each assay reagent was optimized to ensure proper functioning of the siphon-based flow control. The entire automated

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microfluidic assay process is completed in less than 30 min. The developed prototype system was used to accurately measure industrial bioprocess samples that contained 10 mg mL$^{-1}$ of hIgG.



## 1. Introduction

Microfluidic lab-on-a-chip [1] and in particular centrifugal lab-on-a-disc systems, continue to be a source of interest in both academia and industry, as they have a high potential to integrate laborious biochemical assay procedures onto a single, automated device with a small footprint and low cost [2–4]. While the emphasis of centrifugal microfluidic sample-to-answer systems is predominantly on clinical diagnostics, the monitoring of bioproduction processes is also an important area that remains relatively unexplored [5,6]. In bioprocess monitoring where product yield is of utmost economic importance, cells are engineered to produce recombinant proteins such as therapeutic antibodies [7]. The monitoring of cells and their products within a complex bioprocess matrix remains an important, demanding, expensive, difficult and challenging task [8]. Analytical techniques such as the use of Raman spectroscopy [6] and biosensors [9] have previously been explored for at-line bioprocess monitoring. However, issues such as sensor failure due to biofouling, inactivation of the sensing probe or non-specific binding have proven to be major obstacles [8]. As with clinical diagnostics, having a rapid, accurate, and easy-to-use test available at the point of sample collection would have tremendous benefit. Thus, the development of an 'at-line', 'sample-to-answer' microfluidic-based assay system for bioprocess monitoring is an important development for the bioprocess industry.

Although the design and fabrication of microfluidic chips is becoming more widespread, the modular integration of these chips with hardware systems, especially signal detection systems, remains a major engineering challenge [3,10–12].

For the first time, we present here a new system for the quantitation and monitoring of human immunoglobulin G (hIgG) by uniquely integrating a number of different technologies, namely–fluidic (centrifugal microfluidics) [13], immunoassay, optical (supercritical angle fluorescence) [10], and chemical (plasma enhanced chemical vapour deposition) [14] elements. Although these technologies (i.e. serial siphon valving [13], hIgG immunoassay [15], SAF collection technique [10] and APTES-functionalized Zeonor® [14,16]) have been reported separately; we for the first time demonstrate their integration for the fully automated quantification of hIgG in typical bioprocess samples.

In this work, we first outline the operational principle of the centrifugal, serial siphon controlled platform. Next, the custom benchtop hardware system for SAF detection is highlighted. Finally, the hIgG immunoassays performed on the opto-fluidically integrated lab-on-a-disc platform and the read-out on our custom-built benchtop SAF instrument are presented.

## 2. Operational principle

### 2.1. Centrifugal flow control by serial siphoning

Centrifugal microfluidic platforms have significantly advanced over recent decades. A variety of laboratory unit operations (LUOs) have been demonstrated on lab-on-a-disc technology, that includes: valving, metering, mixing, dilution, and particle handling [2,17,18]. Furthermore, centrifugal microfluidics has been shown to improve 'flow-through' assays, especially for surface-based assays, by decreasing total assay time, while producing results comparable to standard instrumentation but with increased assay sensitivity [2,19,20]. Fig. 1I shows the exploded assembly drawing of the multi-layered, centrifugal "lab-on-a-disc" platform used in this work which will be outlined later in Section 3.1; a quarter segment of the disc with the microfluidic layout is represented in Fig. 1II. Fig. 1III illustrates a cross-sectional view of the fluidic flow over the assay spot through a micro-structured 3D via, and Fig. 1IV shows the several components of the hIgG sandwich immunoassay.

A common valving mechanism on centrifugal platforms is siphoning [13,17,21,22] where a liquid reservoir is connected to a hydrophilic outlet. This siphon channel starts at the outer end of this reservoir and bends inwards towards a crest point which is located closer to the centre of rotation than the (original) liquid level in the reservoir. Due to the hydrostatic equilibrium, the difference in filling levels of the reservoir and the inbound ("rising") segment of the siphon diminishes at sufficiently high spin rates. Yet, when the spinning is slowed down, capillary action takes over to drive the liquid meniscus past the crest point and below the liquid level in the reservoir. At this stage, centrifugation leads to a depletion of the reservoir (Fig. 2A).

Unique to centrifugal platforms, siphoning can be used to stop flow at elevated spin rates and facilitate capillary flow, i.e. open the "gate", at reduced pressures (i.e. low spin rates). No external, physical actuation mechanism (e.g., heating for wax melting) is required for the valves to function. Another advantage of siphon-based valving, is its tolerance to small spin-speed variations.

However, a particular challenge on centrifugal platforms is the implementation of the rotationally controlled, sequential release of multiple on-board liquids (i.e. sample and reagents). This problem was solved by placing a series of siphons next to each reagent reservoir. The number of windings in these serial siphons establishes the order of release in the assay. By using a series of cycles with slow and fast spinning rates, the reagents were consecutively released into a common flow (i.e. assay) channel. A fully assembled disc showing the serial siphons and the loading ports for the assay reagents is given in Fig. 1II.

In order to prevent capillary action from driving the liquid menisci through all the subsequent siphons, a (low pressure threshold) capillary stop, represented by a sudden geometrical expansion, was introduced in the inbound segment of each hydrophilic siphon channel (Fig. 2A).

After loading all liquids into their respective reservoirs at rest, the liquids protrude into their respective siphon outlets until they reach the (first) expansion. The spin rate is then increased to burst this first tier of capillary stops and thus let both the liquid levels in the reservoir and the rising parts of the outlet equilibrate. The disc is then decelerated until capillary action takes the menisci past their crest points and down the falling flanks. The liquids then either run against the next capillary stop at the entry of the subsequent siphon or they are released into the common outlet (Fig. 2A).

### 2.2. Supercritical angle fluorescence (SAF)

Amongst the many assay detection methods available, fluorescence-based methods provide sensitive and specific detection while not requiring physical contact with the device [12], which is an advantage for the rotational lab-on-a-disc platform. The chip comprises a focussing lens for excitation with a laser beam at 635 nm and a spherical ring lens structure at the bottom. The SAF emission is transmitted through the spherical structures at the bottom of the chip, thus avoiding total internal reflection (TIR) and is redirected by an elliptical mirror towards a