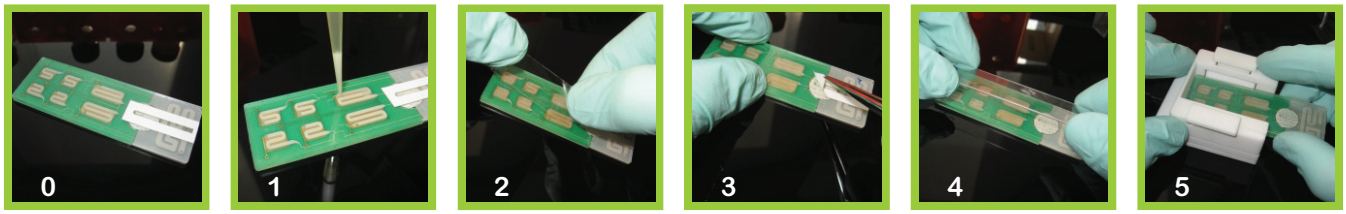
bi.FLOW systems GmbH
biofluidic integration

Detection of CRP with the flex.flow microscope slide

bi.flow systems supplies highly innovative microfluidic solutions for a variety of different applications. In this application note (# 100), the usage of the **flex.flow microscope slide** is described. As an example, the detection of CRP in a microarray format is investigated.

- **Saving reagents** due to small reaction volumes
- CRP assay **in less than 3 minutes** possible
- **Five steps** to run your assay in an automated way



work.flow

INTRODUCTION

Lab-on-chip systems are believed to be one of the mainstream technologies within the next years for applications in medicine and diagnostics, enviromental sciences, pharmacology or food and drug safety. Within this huge market **bi.flow systems** offers such systems with a high degree of integration. Just an electrical current is needed to pump any liquid within a substrate such as a microscope slide. The pumping sequence can be operated via a controler and a software tool which is part of the **flex.flow eval-kit**.
The **flex.flow microscope slide** is one of the first products of **bi.flow systems** and offers the possibility to automate liquid handling steps which would be necessary to perform for a common diagnostic assay.
The flex.flow microscope slide possesses six reservoirs (2 x 40 µL; 4 x 20 µL), which are configurable, and a flow cell (~3 µL) which is defined by a tape which also acts as a bonding and sealing tool for the microarray slide.
To show the handling of the **flex.flow microscope slide** a common diagnostic test for CRP (C-reactive protein) was adapted to this system.

MATERIALS

- **flex.flow mircoscope slide**
- spotted microarray slide (APTES) with
  - carbonate buffer (200 mM, pH 9.6)
  - antiCRP (polyclonal, goat) 1 mg/mL
  - antiHCG-a (polyclonal, goat) 0.5 mg/mL
  - antiMouse (Cy5-labeled, sheep) 5 µg/mL
- milk powder (3 %, PBS, pH 7.4)
- deionized water
- antiCRP (monoclonal, mouse) 1 µg/mL
- antiMouse (Cy5-labeled, sheep) 1 µg/mL

SPOTTING DESIGN

The spots were deposited on a glass microscope slide after surface modifaction with APTES (aminopropyltri-ethoxysilane).
The spotting design was chosen to be a grid of 4x8 spots, each with 2 nL. Within this array, 8 spots are antiCRP, 12 spots are antiMouse (Cy5-labeled) as positive control, 2 spots are antiHCG-a as negative control and four spots are buffer controls (spotting controls).

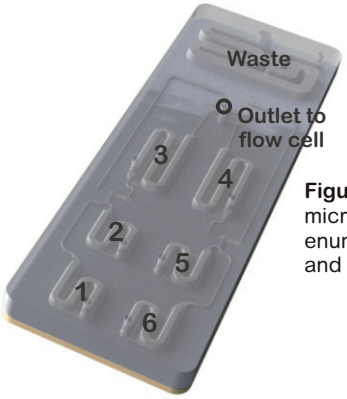
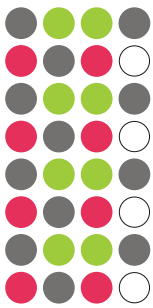


Figure 1. flex.flow microscope slide with enumeration of reagents and sample reservoirs.



antiCRP (polyclonal, goat) 1 mg/mL
antiHCG-a (polyclonal, goat) 0.5 mg/mL
antiMouse (Cy5-labeled, sheep) 5 µg/mL
carbonate buffer (200 mM, pH 9.6)

Figure 2. Scheme of the chosen chip layout.

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flex.flow microscope slide - HANDLING

To run a diagnostic assay in a fully automated way the **flex.flow mircoscope slide** has to be prepared in 5 steps (see **work.flow**).

Step 1: Filling the reservoirs with reagents, washing solutions and sample with a standard pipette tip.
Step 2: Sealing of the filling holes with a tape.
Step 3: Detachment of protection layer of flow-cell tape.
Step 4: Attachment of the microarray slide.
Step 5: Insertion of the **flex.flow mircoscope slide** into the clamp for software-based pumping.

EXPERIMENTAL SET-UP

The adapted diagnostic test is a sandwich immunoassay with an immbolized antiCRP antibody and different immobilized control antibodies. To detect the bound CRP a monoclonal antiCRP(mouse) is used followed by an antiMouse, Cy-5 labeled antibody as marker.
For the experimental set-up the pumping sequence and the allocation of the different reagents, washing buffers and the sample has to be chosen.

The different reagents were inserted as follows:

Reservoir 1:	CRP sample
Reservoir 2:	antiMouse (Cy5-labeled, sheep)
Reservoir 3:	milk powder (3%, PBS)
Reservoir 4:	deionized water
Reservoir 5:	milk powder (3%, PBS)
Reservoir 6:	antiCRP (monoclonal, mouse)

Pumping Sequence:

Step 1:	Reservoir 3	**10 s**
Step 2:	Reservoir 1	**30 s**
Step 3:	Reservoir 5	**30 s**
Step 4:	Reservoir 6	**30 s**
Step 5:	Reservoir 3	**20 s**
Step 6:	Reservoir 2	**20 s**
Step 7:	Reservoir 4	**30 s**
overall:		**170 seconds**

DATA ANALYSIS

After the pumping sequence, the microarray slide can easily be removed from the **flex.flow mircoscope slide** and evaluated in common microarray scanner.

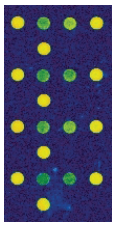

Figure 3. Examplary fluorescence image of 1 µg/mL CRP obtained after automated assay performance.

As in the normal work flow, the data can be quantified via the spot intensity in comparison to the background.

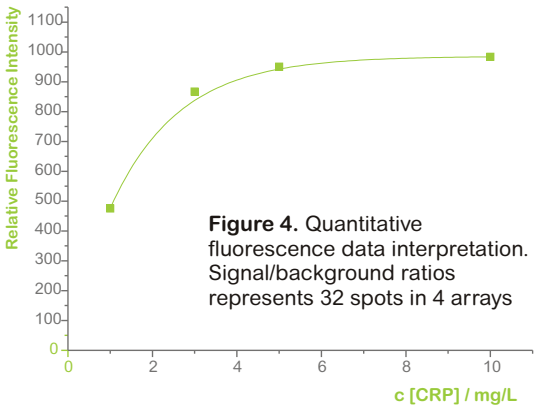

Figure 4. Quantitative fluorescence data interpretation. Signal/background ratios represents 32 spots in 4 arrays

CONCLUSION

In this application note, the performance and handling of a common diagnostic test using the **flex.flow mircoscope slide** was evaluated. It could be shown that with a minium of reagents, an assay can be performed in less than 3 minutes.
The detected analytical range matched with the clincal relevant range for CRP.
To conlude, the **flex.flow microscope slide** opens the possibility to evaluate sandwich assays in an automated way.

ORDERING INFORMATION

PRODUCT	DESCRIPTION	ORDER NO.
flex.flow microscope slides	Quant.: 1, 10, 50, 100 slides	1101-1
flex.flow eval-kit	Clamp, Software, Electronic control, 10 slides	1501-1

In addition **bi.flow systems** offers customized microarray slides with different possible surfaces and layouts. Please contact us: info@biflow-systems.com and receive your quote today.
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