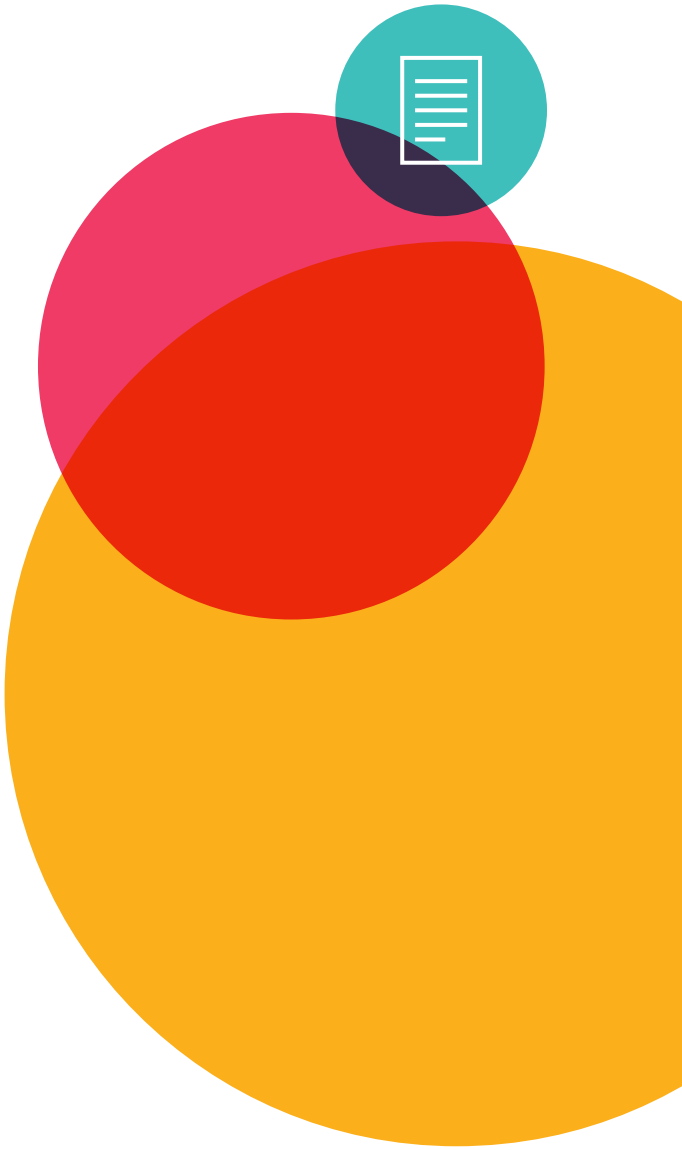
Lightening the black box

by visualizing the reaction



Summary

- Live visualization of binding reactions: a unique feature of Evalution
- Real-time reaction monitoring for rapid quantitation of analytes
- Stepping away from the typical black box measurements for:
  - Kinetic as well as end-point measurements
  - Rational optimization of reaction conditions
  - Detection and correction of reaction anomalies
  - High quality readouts
  - Traceability down to the raw data

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Live visualization of the binding reaction is unique to Evalution[1]

In most diagnostic measuring technologies, assays are developed and biomolecules quantified in a black box environment, i.e. no visual control of the reaction that is occurring is possible. In contrast, Evalution™ allows such visualization of the microparticles at any time, both in bright field and fluorescence mode [Figure 1]. Besides the fact that such setup allows for both kinetic and end-point measurements beside or next to one another, it also enables easy screening of reaction conditions and progress.

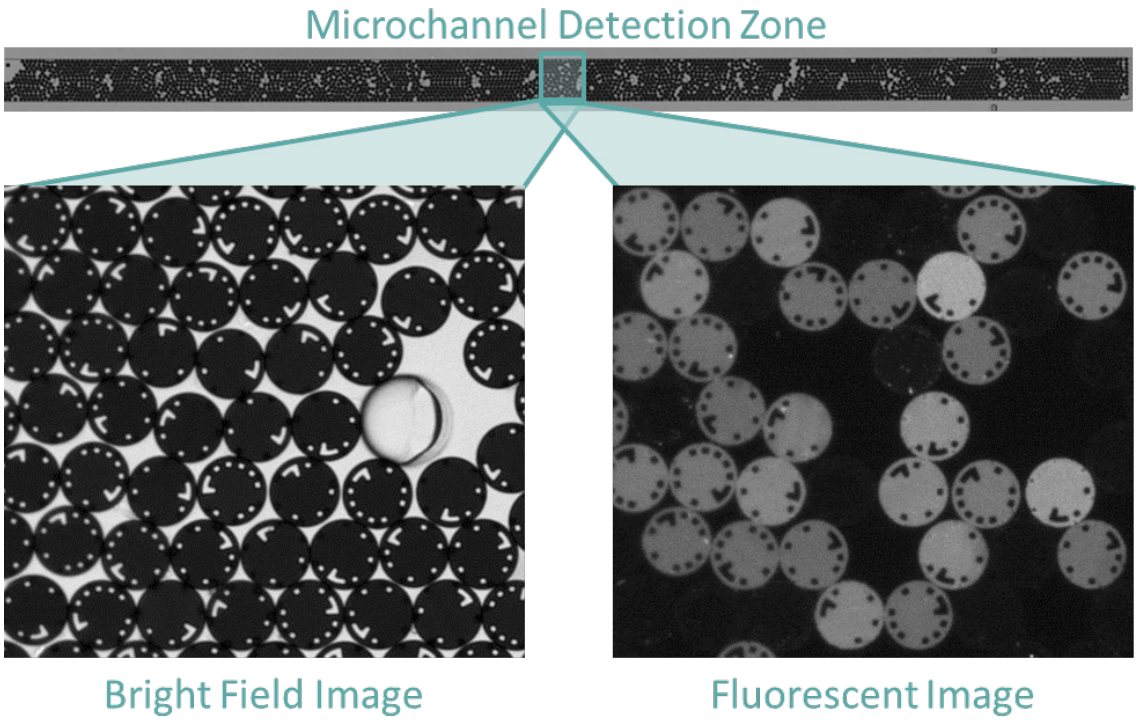

Figure 1: Visualization of the reaction chamber

High resolution visualization of the reaction chamber in bright field (left) and fluorescence mode (right). The transparent circular feature is a supporting post to ensure accurate channel height. The bright field image allows the robust deciphering of a 10-digit binary code formed by holes at the edges of the microparticles, each code representing a specific population for which fluorescent signals are subsequently measured.

[1] Evalution Platform is intended for research use only. Not for use in diagnostic procedures

In-cartridge screening of coupling conditions

Coupling of biomolecules to a solid surface are largely dependent on the physicochemical properties of the biomolecule itself and therefore standard protocols need to be adapted in that regard. For similar groups of molecules, like the IgG antibody family, standard protocols for coupling are available and will work in the vast majority of cases. However, for other proteins or antigens these conditions will need to be optimized. Having a live view on the reaction chamber while coupling is occurring provides considerable advantages in any optimization procedure.

In this process, uncoupled COOH-microparticles are directly loaded in a microchannel of the Evalution cartridge and chemically activated [Figure 2]. Immediately after coupling of the protein of interest under varying conditions per channel (e.g. pH, buffer type and concentration, protein and reagent concentration, temperature...), the process is visualized by performing a functional test.

As part of a multiplex serology test (TN003), coupling conditions for the HIV gp41 protein were optimized using a set of buffers with varying pH [Figure 3]. In general, coupling conditions that provide the best signal-to-noise levels are selected in a black-box setup. However, visualization clearly shows that under certain conditions, severe aggregation can occur both on and off the microparticles even in the absence of anti-gp41 antibodies. In a black-box setup one would probably chose the coupling conditions that provide the highest signals. Clearly in this case this would lead to skewed results in a final serology test for HIV [Figure 4].

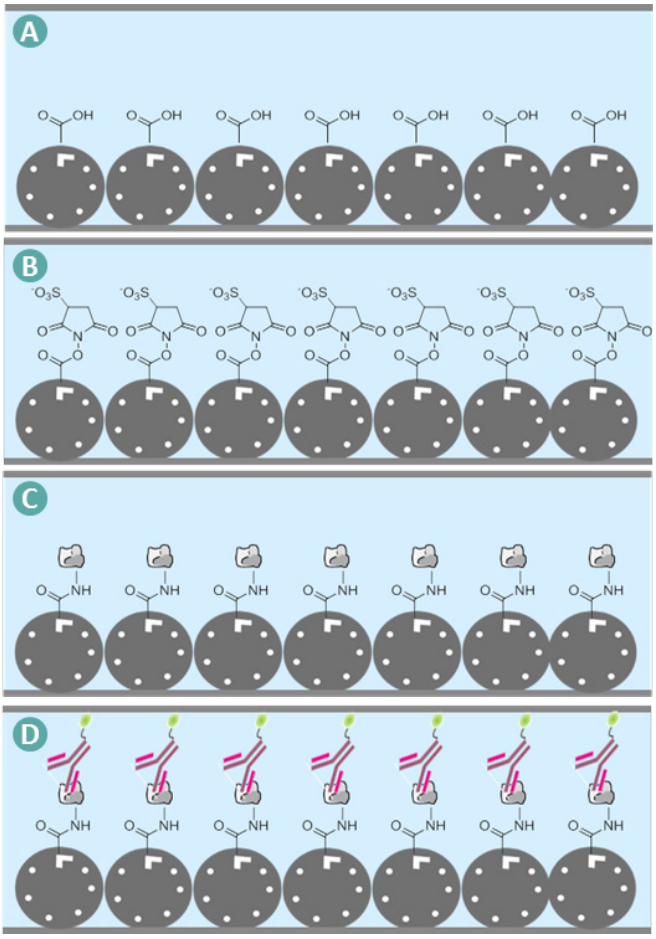

Figure 2: In-flow screening of coupling conditions

Uncoupled COOH-microparticles are loaded into the microchannel of an assay plate (A) and activated using an active flow of EDC/sulfo-NHS (B). The newly formed amine-reactive NHS esters then react with the protein amine groups to form a covalent bond (C). A fluorescently labeled anti-protein antibody then allows visualization of the coupling process (D).

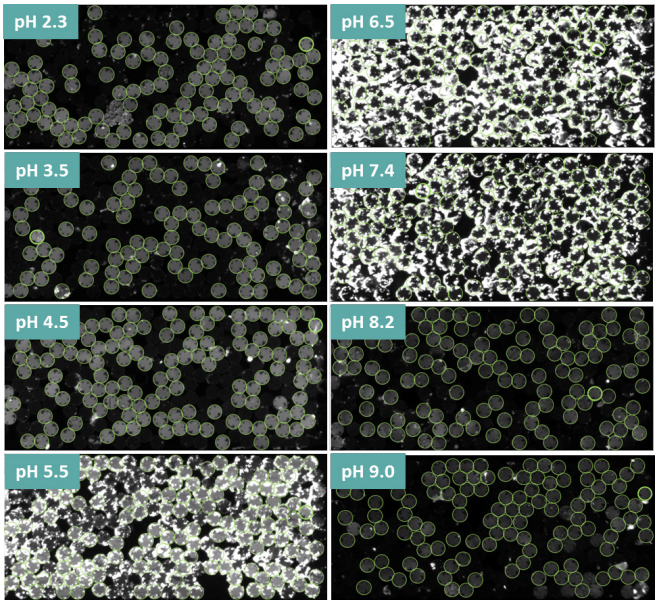

Figure 3: HIV gp41 coupling to microparticles requires the correct buffer choice

Coupling of the HIV gp41 protein to microparticles at varying pH conditions was assessed and visualized as described in Figure 2. Ideally, uniform fluorescent signals are observed on the microparticle with no signals off the surface. Clearly in the example shown here these conditions are not met under all binding conditions. Most ideal coupling conditions for gp41 were found to be at pH values below 5.5 or above 8.2.

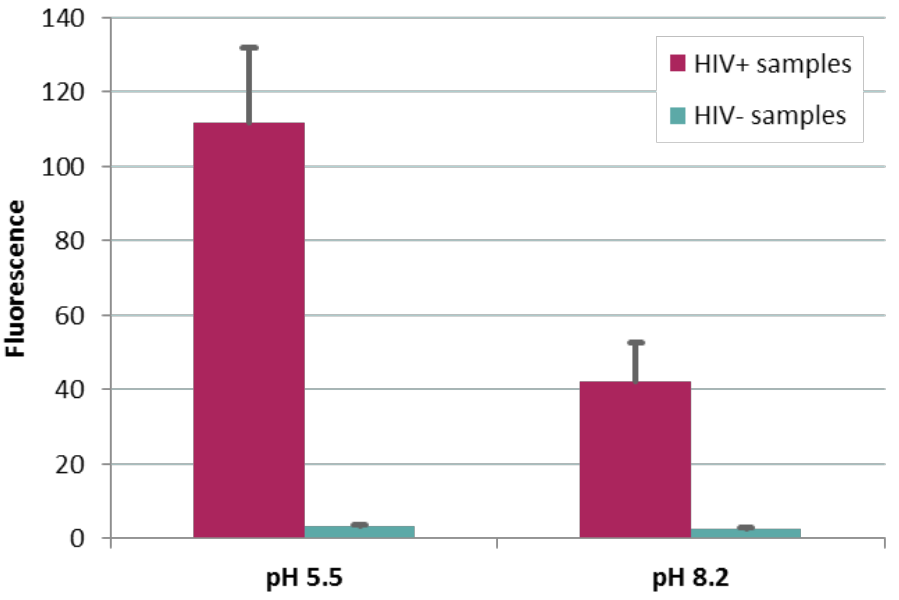

Figure 4: HIV serology testing

Microparticles were coated with the HIV gp41 protein under different pH conditions and loaded in different microchannels. Next, HIV+ and HIV- blood samples were tested for the presence of anti-gp41 antibodies under identical assay conditions for both microparticle preparations. As expected, in the absence of anti-gp41 antibodies, no fluorescent signal is generated. However, when such antibodies are present in the sample, the microparticles coupled under bad pH conditions (pH 5.5) generate a signal almost 3 times higher than the microparticles coupled under good conditions (pH 8.2). Clearly this is an overestimation of the antibody abundance due to aggregated antigen. Such technicality would never get noticed using a black box measurement.

Visualizing the reaction

While running and processing specific samples, certain phenomena can occur that hamper the final reading. Such phenomena can be triggered by the sample itself or by the nature or state of the reagents used. Sample precipitation and reagent aggregation [Figure 5] are just two examples of things that can go wrong. In a black box measurement this does not get noticed, again resulting in skewed readings. Using the Evalution visualization tool, these phenomena are easily detected and proper action can be taken by excluding entire or partial microparticles from the analysis. This can be done manually or automatically by making use of specific exclusion criteria (e.g. fluorescence distribution on the surface of the microparticle) and algorithms built in the software.

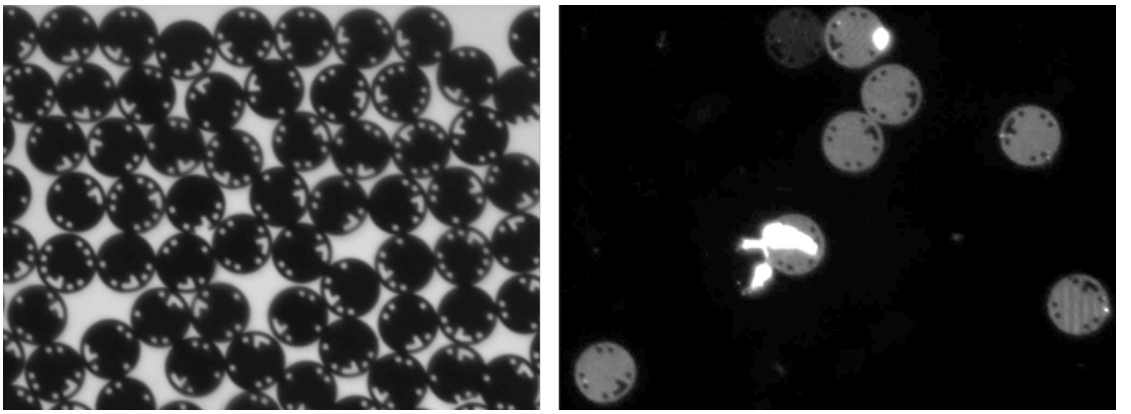

Figure 5: Aggregation of reagents

A multiplex cytokine assay ran under specific conditions resulted in aggregation of detector reagents. This unwanted phenomenon can clearly be detected using the Evalution live visualization tool. Exclusion of full or partial microparticles from the fluorescence aggregation process is possible in this case, leading to trustworthy data if sufficient unhampered microparticles remain. In a black box setting, this would lead to an overestimation of the cytokine concentration.

MyCartis at a glance

The big revolution in healthcare today is that we are finally realizing that we are all equal, but not identical. MyCartis is convinced that the future of healthcare lies in personalization. Our goal is to deliver innovative solutions for fast and cost effective identification of a patient's signature. By building bridges between research and medical practices we are increasing the level of health and the quality of life for everyone. We dedicate ourselves to improve healthcare for future generations.

Evalution™ at a glance

› Powerful tool for assay development and biomarker analysis
› Broad range of applications
› Single platform, different analytes
› Simple workflows with minimal handling
› High sensitivity and broad dynamic range for robust measurements
› Competitive cost per data point

MyCartis (Swiss Branch)
EPFL Innovation Park - G
1015 Lausanne
Switzerland
Phone: +41 21 694 04 30

MyCartis NV
Technologiepark 4
9052 Zwijnaarde
Belgium
Phone: +32 9 241 11 64

www.mycartis.net

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