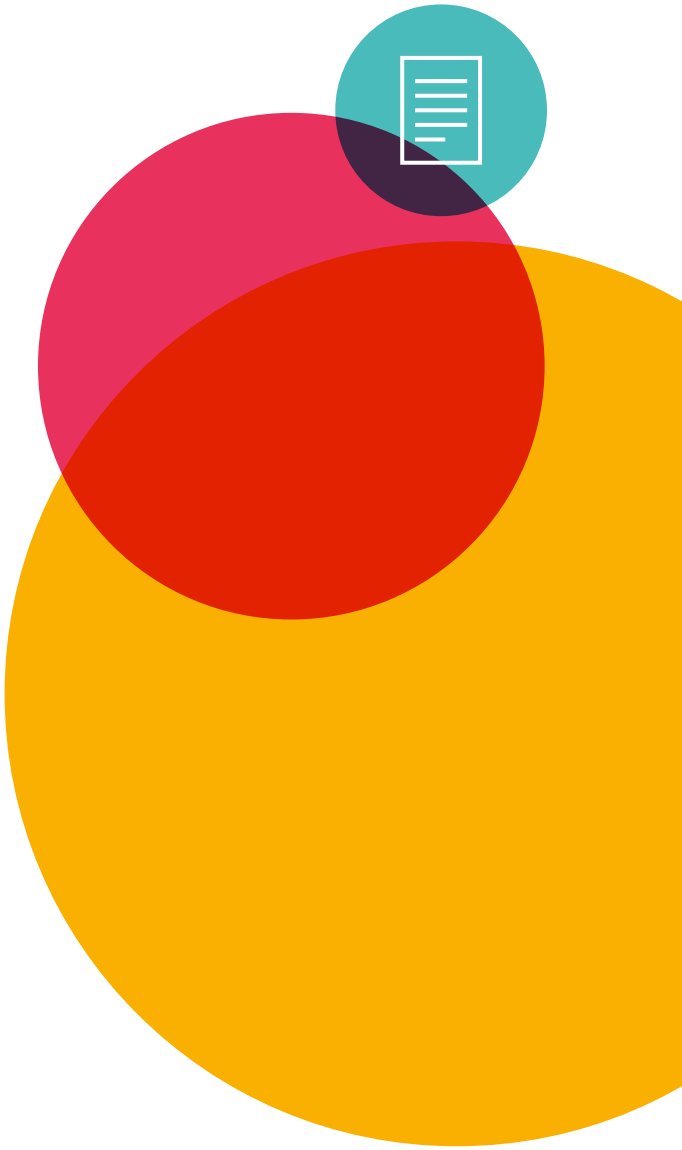
Evalution™

Key features, unique and versatile assay formats



Summary

› A reliable breakthrough technology for robust multiplex assay formats
› Single channel control for flexible user-defined throughput
› Unique microfluidic environment ensures fast reaction kinetics with robust readouts
› Open platform architecture allowing qualitative and quantitative analysis of different molecules: proteins, nucleic acids, small molecules etc.
› Live reaction visualization allows kinetic as well as end-point measurements, powerful reaction optimization tools and reliable reaction artefact management
› Full temperature control of multiple zones along the reaction channel allows unique and sophisticated assay formats
› User-friendly software interface with drag and drop functionality for fast and easy protocol management, real-time graph building and comprehensive data analysis

MyCartis

BCLA-00347 version 1

A reliable breakthrough technology[1] for robust multiplexing assay formats

The disk-shaped microparticles [Figure 1A], 40µm in diameter and 10 µm in height are produced from silicon wafers using most advanced MEMS manufacturing technology. The periphery of the microparticle is unambiguously encoded using a 10-digit binary code formed by the presence or absence of holes. A bright-field image of loaded microparticles allows the robust deciphering of such codes at any time in the protocol. The encoded microparticles act as a solid support for a multitude of possible molecules including antibodies, target proteins, peptides, nucleic acids or other biomolecules. To boost the analytical performance, microparticles are coated with an optical enhancement layer inducing constructive interference of the excitation and emission light for the fluorophores close (bound) to the microparticle surface. Although fully coated, only the fluorescence in the central part of the disc gets analyzed. This central zone of a microparticle provides over 1500 pixels of data to be captured, processed and visualized. The cartridge or assay plate features 16 microfluidic channels where microparticle populations tile in a monolayer arrangement. With a channel volume of 700nl and a forced sample flow of low nl/s for several minutes only, very low sample volumes are required for analysis. The footprint of an assay plate complies with a microplate format compatible with most laboratory automation systems [Figure 1B].

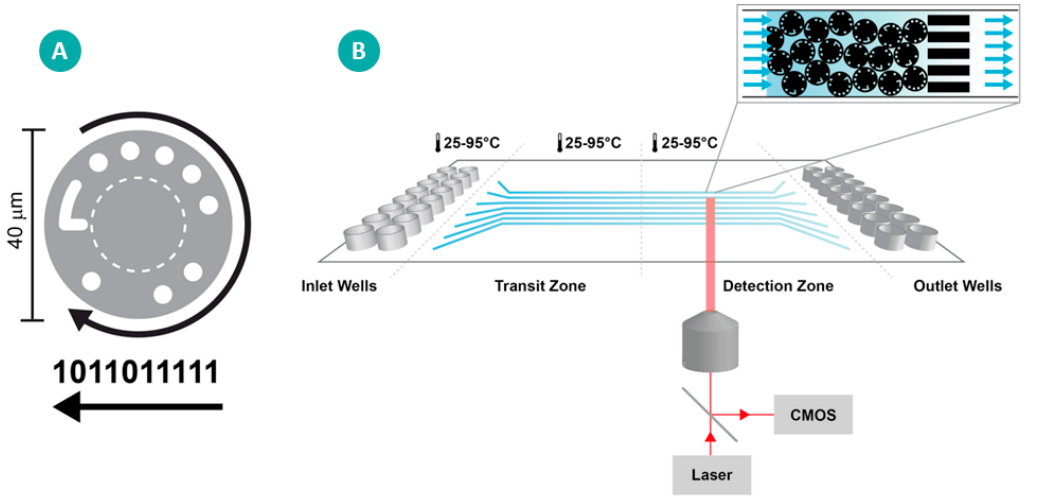


Figure 1: The encoded microparticles (A) fit the microfluidics assay cartridge (B)

(A) The digitally encoded silicon discs with 10 binary bits on the periphery enabling 1024 different codes. An "L"-shaped mark is used to check the orientation of the disc and is also the decoding starting bit. The central zone of this microparticle (dashed lines) provides 1500 pixels of fluorescent data to be captured.

(B) The 96-well plate footprint of the assay plate. The cartridge is composed of 16 independent microfluidic channels. The instrument enables temperature control in 3 zones: the inlet wells, the transit zone and the detection zone.

Single channel control

The inlets and outlets of the microfluidic channels are both pressurized above atmospheric pressure to allow robust microfluidic operation and prevent bubble formation. Fluid flow and flow rate are controlled by a differential pressure applied to each channel individually. This provides the user the advantage of flexible throughput (1-16). Random access to any desired number of channels allows to get fast results cost efficiently without the need to await full or large batches as is the case for many other technologies.

[1] The Evalution™ platform is intended for research use only. Not for use in diagnostic procedures.

Short time to result due to a reaction limited regime

In Evalution™, all assay reactions and measurements are performed in the same reaction chamber of a microchannel. This drastically reduces the assay time and limits the number of manual steps, especially when the assay is run in a co-flow (i.e. simultaneous running of different reagents) workflow [Figure 2].

Many diagnostic technologies suffer from slow binding kinetics as they are mainly driven by diffusion (a mass transfer limited regime). This typically results in long (hours to overnight) incubation and assay times, leading to elevated inter- and intra-assay variability. Not so with Evalution™ where the microfluidic flow guarantees a continuous supply of analytes over the stationary microparticles. In such an environment and at a sufficient flow rate, the maximum binding rate is achieved. Such a condition is also referred to as a reaction limited regime [Figure 3]. The flow rate needed to reach optimal binding kinetics depends on the molecules affinities and capture molecule density. However, it is independent of the analyte concentration.

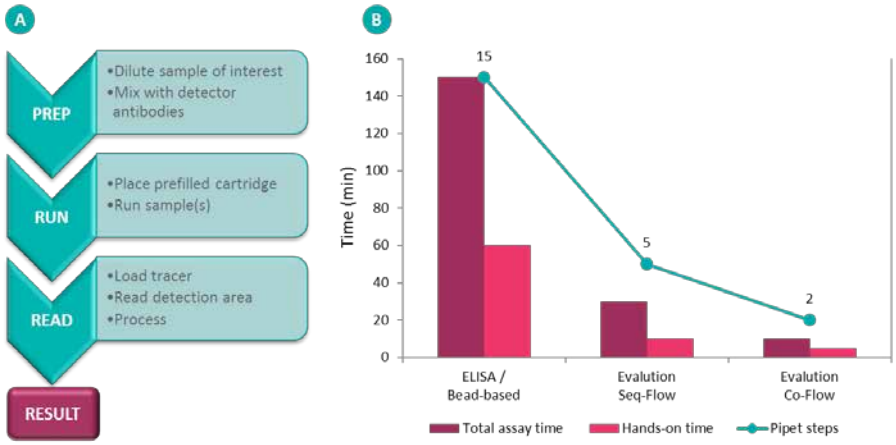


Figure 2: Evalution™ in general and co-flow in particular lead to reduced assay times

(A) The optimal co-flow workflow limits the amount of manual steps and reduces total assay times.
(B) Reduced assay times in Evalution™ as demonstrated for a serological anti HIV p24 assay. This assay can be run in sequential-flow (i.e. no pre-mixing of reagents) or co-flow (i.e. with pre-mixing of reagents) mode.

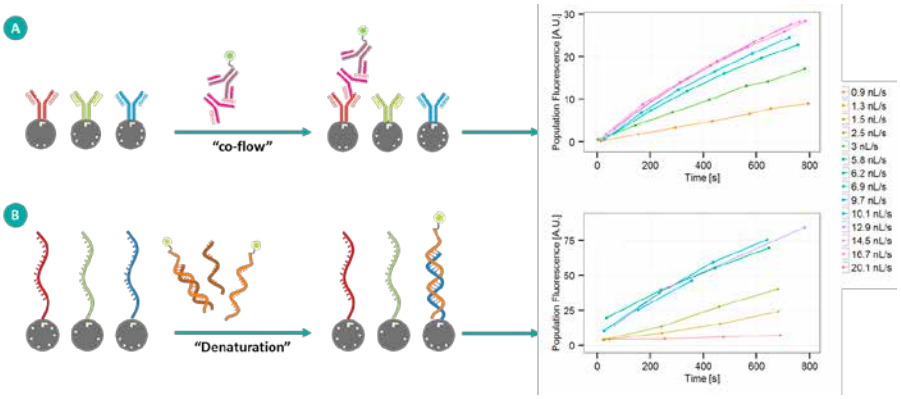


Figure 3: The reaction limited regime

Fluorescence signals in function of time for different flow rates were assessed in a typical co-flow immune assay (A) and a DNA hybridization assay with in-flow denaturation (B). For both assays, when a certain flow rate threshold is past, the kinetic curves overlap.

Operating above this threshold implies a reaction limited regime where the binding rate is maximal and independent of flow rate. Below the threshold, reactions become slower and they show an undesired dependency with flow rate.

Solid platform robustness

A prerequisite to produce reliable data is a low system variability including variation on the instrument output itself and coupled microparticle production and storage. This was assessed by generating an assay that minimizes variation coming from a biological origin [Figure 4]. High intra- and inter-batch reproducibility was demonstrated as well as no influence of microparticle storage at -20°C on the final outcome. The total system variability of 3.8% was calculated by merging all datapoints (n=80).

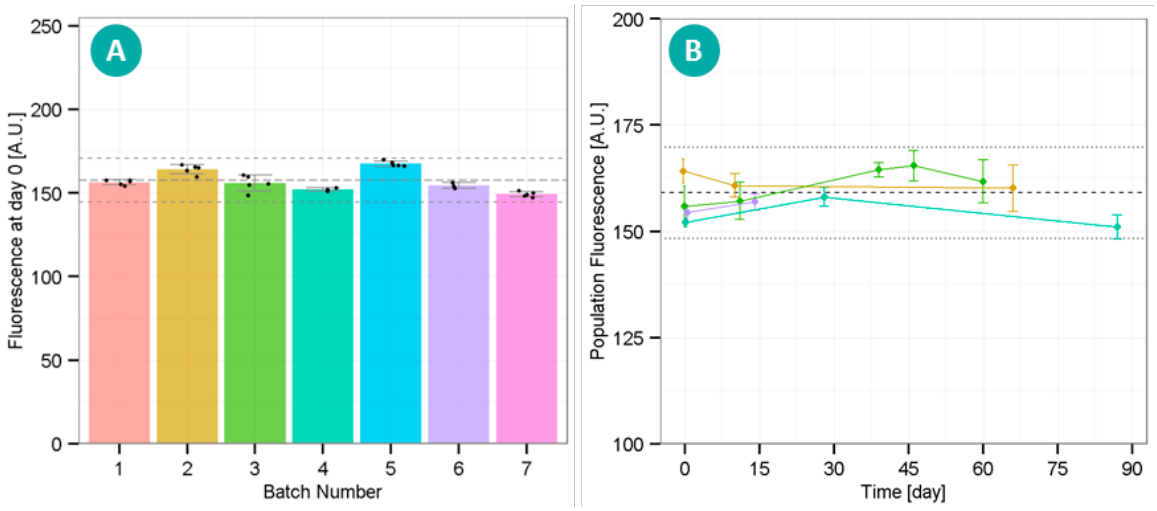


Figure 4: Platform robustness

A mouse IgG was coupled on microparticles and directly detected by a fluorophore labeled anti-mouse IgG without the need for a sandwiched antigen. Different batches of microparticles were produced at different days by different operators using different reagent batches and assays were run on multiple instruments using different assay plates.

(A) Five assay repeats were performed on each batch showing high intra- and inter-batch repeatability (mean coefficients of variation (CV) of 1.37% and 4.5% respectively).
(B) Batches of antibody coupled particles were tested after storage at -20°C for different days showing no significant impact on fluorescence (CV across all batches and storage times is 2.9%).

One platform, multiple targets

Each channel can accommodate a total of 3,000 particles in the 12mm long detection zone. A bootstrap approach is applied on multiple datasets in order to estimate the standard error and coefficient of variation of the population's fluorescence for different population's sizes (one population of microparticles has the same code and capture molecule attached). To obtain a stringent precision criterion of 5%CV on the population's fluorescence, the number of required particles is between 10 and 25, which would allow a multiplex of 120 to 300 targets. As long as a fluorescent signal can be generated from the reaction, this target can be of any nature: nucleic acids, proteins, small molecules etc. And as each channel can be controlled individually, one assay plate can contain reactions targeting molecules of different nature for parallel testing of protein signatures and mutation analysis for instance.

Live visualization

Three main functions are associated to the benchtop Evalution™ instrument: particle imaging, fluid actuation and temperature control. The optical system contains a high sensitivity CMOS camera of which the 10x long working objective is mounted on an automated x-y-z stage for scanning individual microchannel detection zones in real time or at endpoint. Microparticle decoding in bright field and assay quantitation in fluorescent mode are done consecutively to allow optimal image overlaying. Digital pictures can be taken at any time of the reaction, enabling unmet quantitative and qualitative applications. Using this feature, Evalution™ steps away from the typical black box end point measurements by also enabling kinetic measurements. Besides, phenomena like aggregation during protein coupling [Figure 5] or binding which typically lead to skewed results can be easily excluded [TN002].

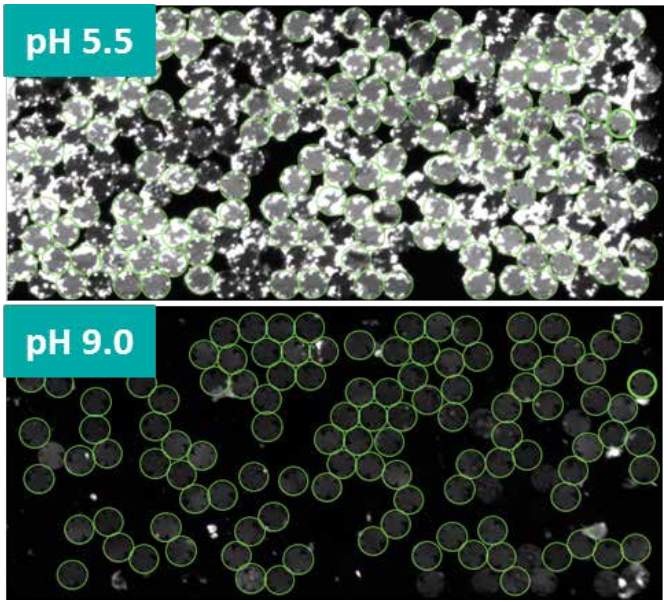


Figure 5: In-cartridge screening of coupling conditions

Protein coupling success is visualized using a fluorescently labeled anti-protein antibody upon coupling under different pH conditions. Coupling at a pH of 5.5 clearly leads to a poor coupling quality because of protein aggregation on and off the microparticles. Higher pH conditions provide homogenous protein coupling as desired. Coupling conditions are dependent on the physicochemical properties of the protein of interest.

Expanding the dynamic range through kinetic measurements

Within a sample, analytes are typically present at largely different concentrations. In a multiplex assay format, this may cause serious problems as the analyte working range might exceed the analytical dynamic range making it impossible to measure all analytes in the same run. Tackling this problem by measuring different dilutions of a sample is not only tedious and sample consuming; it also defeats the purpose of multiplexing itself. When using an endpoint measurement, the detector dynamic range determines the assay dynamic range. Because of its unique setup, Evalution™ also allows kinetic measurements, i.e. fluorescent readings of the same microparticles at different times during the reaction. MyCartis has shown that an unknown analyte concentration can be reliably determined based on the initial rate of the signal build-up [Figure 6]. As such, the assay dynamic range is no longer determined exclusively by the sensor dynamic range.

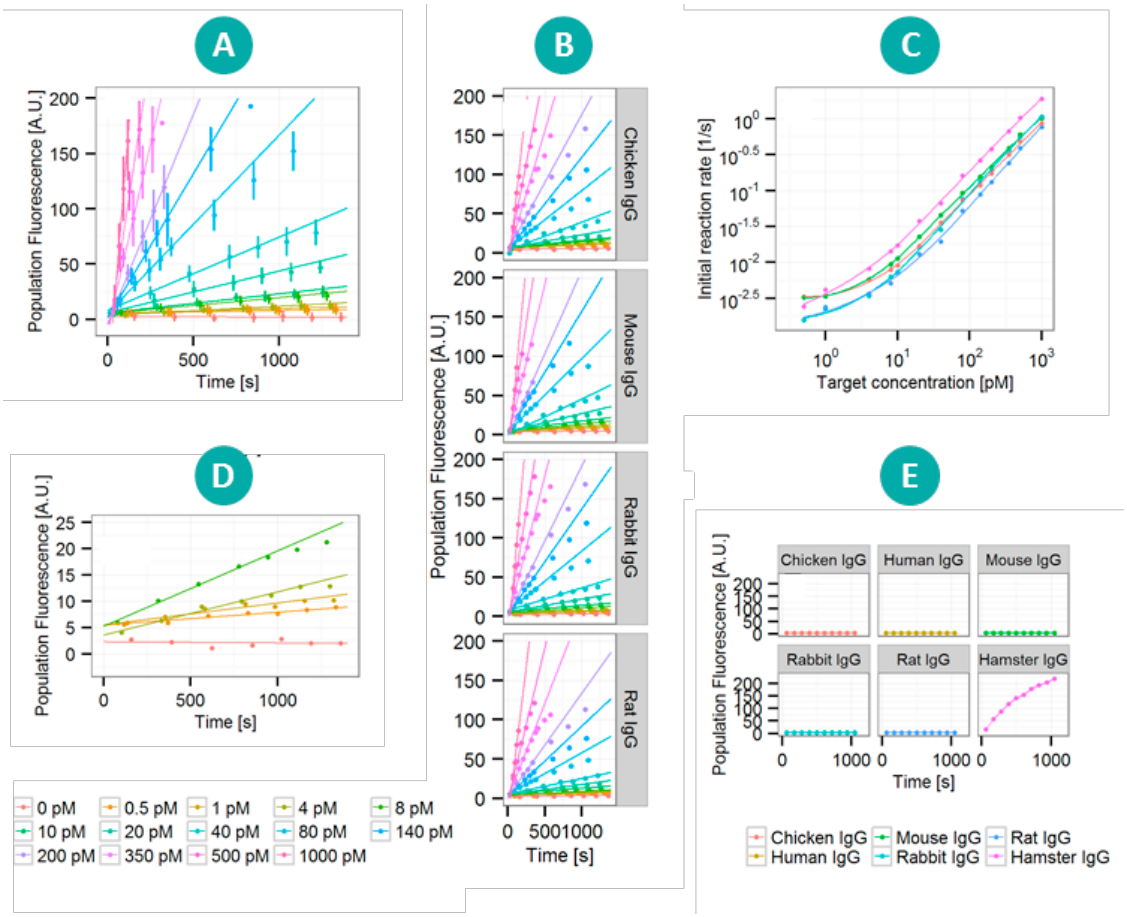


Figure 6: Increased dynamic range through measurement of the initial binding rate

A 5-plex Evalution™ assay was developed by coupling IgG molecules of different origin

(A) Hamster,

(B) Chicken, Mouse, Rabit and Rat) to differently encoded microparticles. A mixture containing 14 different concentrations of Goat antibodies specific for the different IgG origins together with a fluorescently labeled anti-goat antibody were then co-flowed and measured at different timepoints. The co-flow approach generates little to no cross-reactivity (E).

(C) A kinetic calibration curves for analyte quantitation based on the initial binding rates (slope of the linear fit of the first 5 data points).

(D) For some assays, a binding time of 1000 seconds is required to measure low concentrations in an endpoint format. For others (A), signal saturation has been reached at that time point making it impossible to be measured together in such format, stressing the advantage of the kinetic approach.

Independent temperature control in 3 assay plate zones

The temperature in three independent zones of the cartridge (the inlet wells, the fluid transit zone and the reaction/detection zone) is precisely and independently controlled between 25°C and 95°C by Peltier elements [Figure 1B]. Having full temperature control throughout the assay also provides a reliable environment to minimize assay variability. Controlled in-flow denaturation during an assay and on-the-fly control of reaction conditions during assay development [Figure 7] are just some applications enabled by this unique Evalution™ feature.

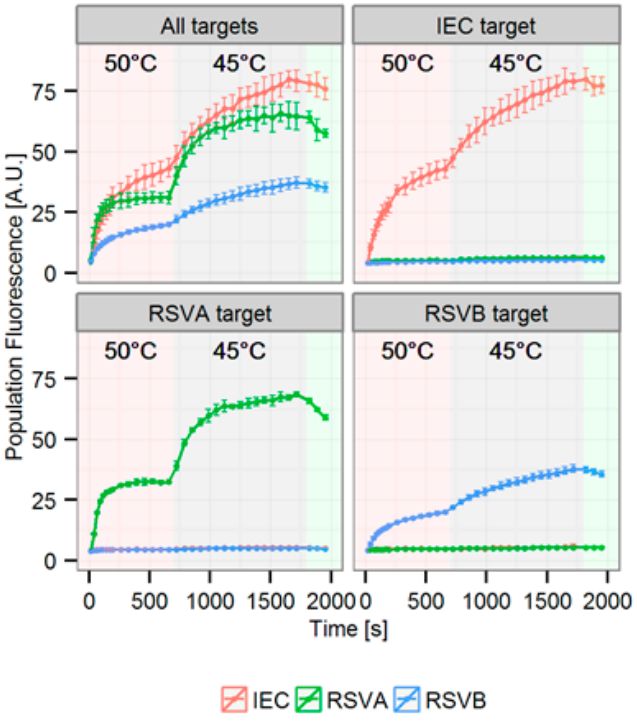


Figure 7: Dynamic control of the hybridization environment

An assay setup with in-flow denaturation as described in figure 3B is used. Target sequences for Respiratory Syncytial Virus (RSV) subtypes RSVA, RSVB and IEC (an internal control) were adapted from literature and coupled to differentially encoded microparticles. Parallel channels were used to measure either all targets flowed simultaneously or the individual ones. Fluorescent measurements in all channels were very similar indicating minimal cross-hybridization. Differences in reaction rates for the three targets at 50°C are identified: for RSVA the equilibrium is reached at 700 seconds, not for the others. For the assay developer, such information is of high importance. Lowering the temperature to 45°C induces a shift in equilibrium with increased binding affinity for all targets but without significantly increased off-target hybridization. The protocol was finalized with a wash step at 45°C highlighting the possibility to also measure dissociation constants in Evalution™.

Built-in software for assay optimization, assay running and data processing

Together with the Evalution™ hardware a user-friendly interface to control all features of the instrument provides both the assay developer and the assay user all tools necessary. Easy drag and drop functionalities, on-the-fly adjustments for fast assay optimization, image capturing and processing, real-time generation of graphical outputs as well as comprehensive and powerful data analysis tools are just some of the features provided [Figure 8].

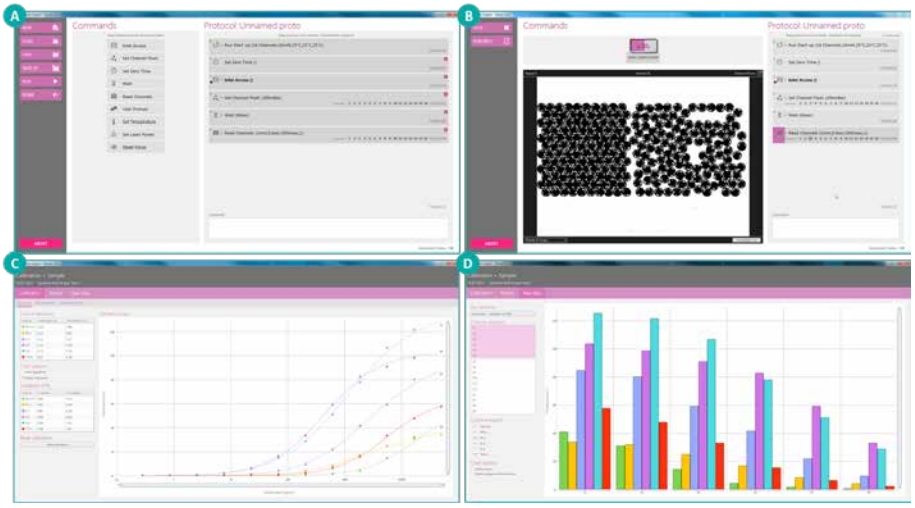


Figure 8: User-friendly software

(A) Drag and drop functionalities for fast and easy protocol management.
(B) Live visualization and control of the ongoing reaction and protocol.
(C+D) Extensive data analysis tools.

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

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