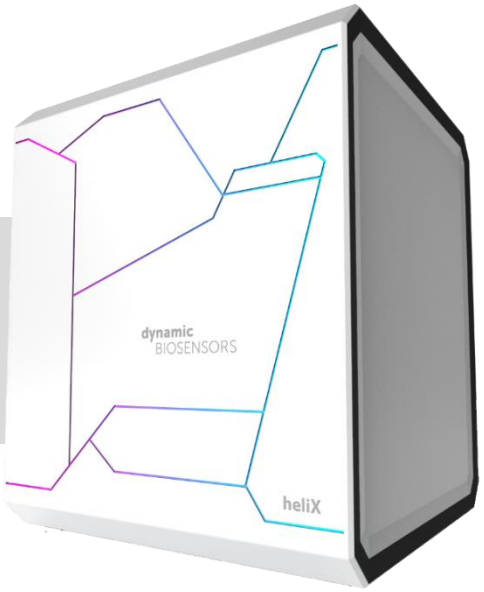
heliX® Guide
Quick Start

dynamic BIOSENSORS



heliX® Quick Start Guide

Protocol for getting started with a **heliX®** device

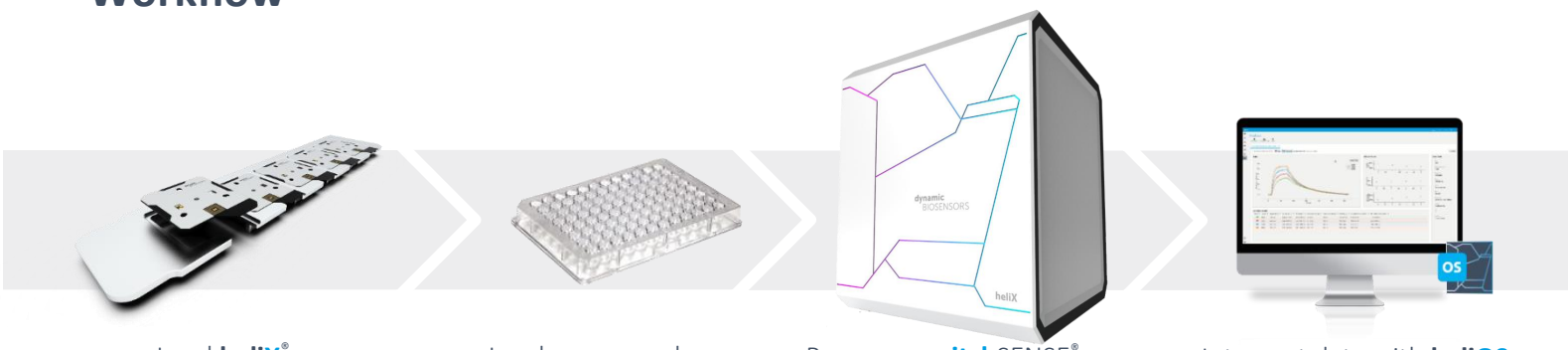
Version 4.1

Key Features

- **heliX®** Instrument Features and General Handling
- **heliX®** Maintenance Workflows
- Adapter Biochip Information and Status Test
- **heliOS** software: Assay Set-up and Data Analysis
- Software Installation Instructions
- Troubleshooting Guide

dynamic BIOSENSORS

Workflow



Load **heliX**® Adapter biochip

Load your samples

Run your **switch**SENSE® measurement

Interpret data with **heliOS** automatic analysis

Contents

About heliX® Quick Start Guide

This guide details the full process of setting up a **switch**SENSE® measurement, covering instrument and biochip handling, sample preparation as well as the basics of data acquisition and analysis.

1 heliX® Instrument Features

The **heliX**® series features a new generation of the **switch**SENSE® technology allowing for high precision characterization, versatile parameter determination and robust microfluidics. **heliX**® devices operate with an embedded control system allowing them to run autonomously without the need for a continuous PC connection and enabling multiple devices to be connected and operated simultaneously. An advanced microfluidic system is designed for rapid solution exchange and contains integrated microvalves enabling a feedback-controlled flow of analyte and buffer from two opposite directions across the sensor. The optical detection system for fluorescence sensing comprises four single photon counters for real-time dual-color detection, E-TCSPC (Electrically-triggered Time Correlated Single Photon Counting) allows for the detection of molecular dynamics with 0.1 µs resolution. Up to five microfluidic biochips can be inserted into the chip compartment and exchanged automatically during use. The sample tray allows the use of individual vials or well plates (96, 384) and is temperature controlled from 4-40°C. Three buffer tubes are present in the buffer compartment with sufficient space for up to three bottles, tube 1 and 2 for running buffers and tube 3 for maintenance buffer (TE40).

modular connection to heliOS network
Autonomous device operation with embedded control system.
Stable data transfer to control PC.

optics
4 single photon fluorescence counters.
Cell imaging.

microfluidics
Advanced fluidics for rapid liquid exchange.
Separate line for in-run washing.

autosampler
384 and 96 well plates.
Temperature controlled sample compartment (4–40 °C).

automatic chip loader
Automatic exchange of 5 chips.
RFID tags for seamless traceability.

temperature control
Sample temperatures on chip range from 15–40 °C.
Measurement in temperature gradient 10 °C / min.

2 heliX® Maintenance

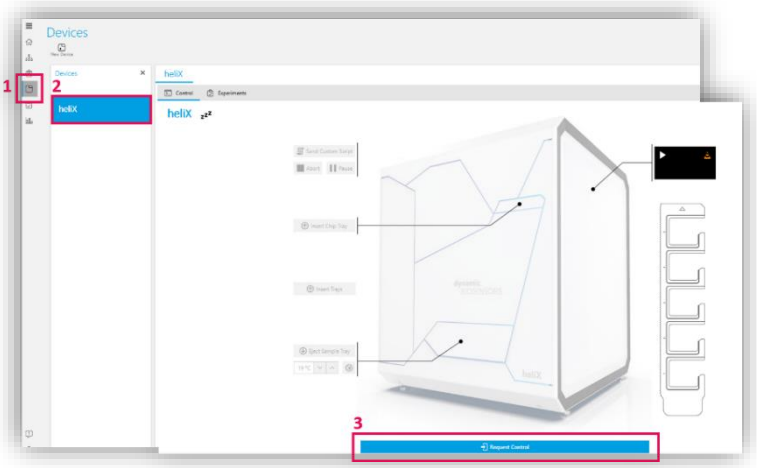
2.1 Connecting to the heliX® Device

Switch on your **heliX®** device using the power button on the bottom left of the instrument and make sure that it is connected to your PC via Ethernet cable. The line color of the device should turn blue when it is initializing/working and green when it is idle. Start the newest **heliOS** software version.

(1) In **heliOS**: open the device overview by clicking the **Devices** icon as shown.

(2) Choose the **heliX®** device which you want to use for this assay to see the control panel of your instrument.

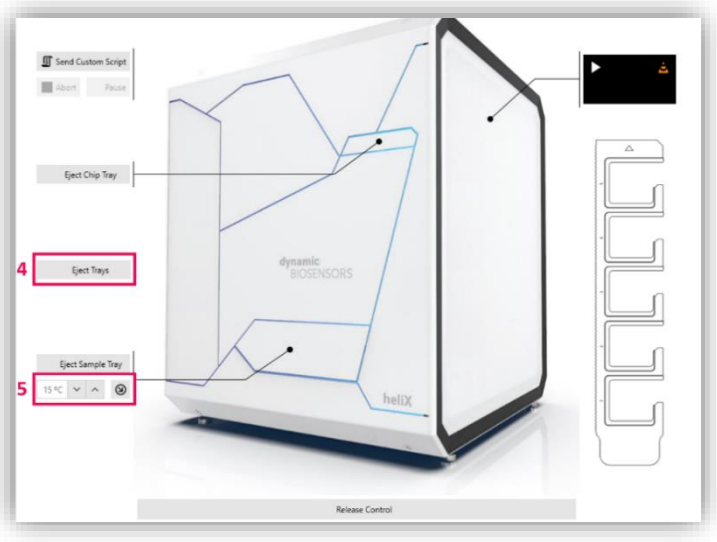
(3) Select **Request Control** and wait until the control connection is established. After a successful connection, the **Request Control** button will turn into a **Release Control** button. Only one computer can control the device at a time.



(4) Select **Eject Trays**. Remove the chip tray from the compartment and place your biochip in any of the five chip positions. Push the tray back into the compartment. Remove the sample tray from the compartment and place a 10 mL vial with DI water (no cap) into position A. Fill the remaining vial or well plate positions according to your assay set-up. Push the tray back into the compartment. Click **Insert Trays**.

Recommendation: Exchange the DI water on a daily basis. All vials have to be equipped with a cap except for the water vial on position A.

Attention: Both trays must be manually inserted slightly over a point of resistance (around 2 cm inside the compartment).



(5) Optional: Set the Sample Tray **Temperature** to the required temperature and click on the arrow to start the temperature control.

2.2 Clean & Sleep Routine

A **Clean & Sleep** routine rinses the fluidic tubing of a **heliX®** device with DI water and subsequently with cleaning solution (70 % ethanol). Finally, all tubes are vented with air. The cleaning procedure is fully automated and takes approximately 38 minutes. During the run, the cleaning solution is ejected into the water bottle. Therefore, the content of the bottle needs to be discarded afterwards. It is recommended to clean the instrument at least once a week or if the instrument will not be used for a longer period of time ("sleep"). After a **Clean & Sleep**, the instrument is set into a sleeping mode and cannot be used until a **Wake Up & Prime** is performed. For both runs, a **heliX® Cleaning Chip** is required.

Set-up of a Clean & Sleep Routine in heliOS

Connect your PC to your **heliX®** device as shown in Section 2.1.

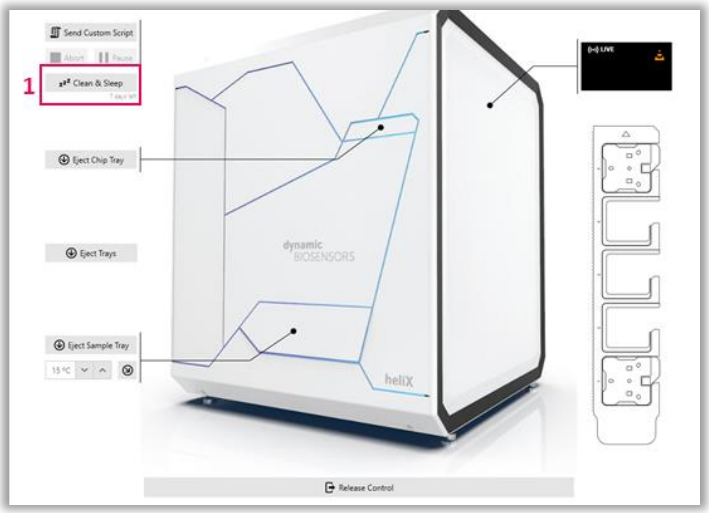
(1) Select **Clean & Sleep** in the **Devices** menu. Below the Clean & Sleep button, the recommended days until the next cleaning procedure are displayed.

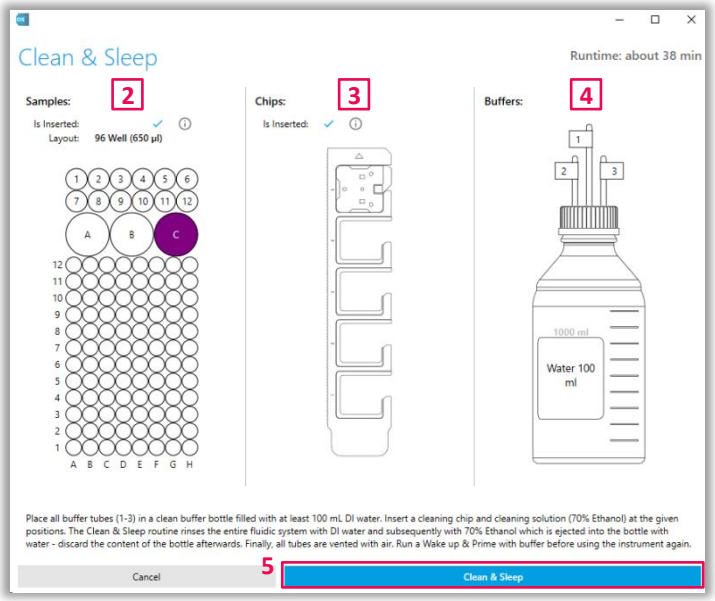
(2) Fill a big glass vial with 10 mL of 70 % ethanol (v/v) and place it at position C in the sample tray as shown in the scheme under **Samples**.

(3) Place a **heliX® Cleaning Chip** at position 5 in the chip tray as shown under **Chips**.

(4) Fill a buffer bottle with DI water (min. 100 mL), place it into the buffer compartment of the device and put all tubes (1-3) in the bottle. Close the buffer compartment.

(5) Click on **Clean & Sleep** to start the cleaning procedure. The cleaning run takes approx. 38 minutes and is fully automated. Afterwards, the device is set into a sleeping mode. Discard the content of the water bottle (water/ethanol mixture) before the next run.





2.3 Wake Up & Prime Routine

Before using the instrument after a **Clean & Sleep** has been performed, the **heliX®** device must be primed with buffer. The **Wake Up & Prime** routine rinses the vented fluidic system with running and maintenance buffers and sets the device status to idle again. The procedure takes approximately 6 minutes and a **heliX® Cleaning Chip** is required.

Set-up of a Wake Up & Prime routine in heliOS

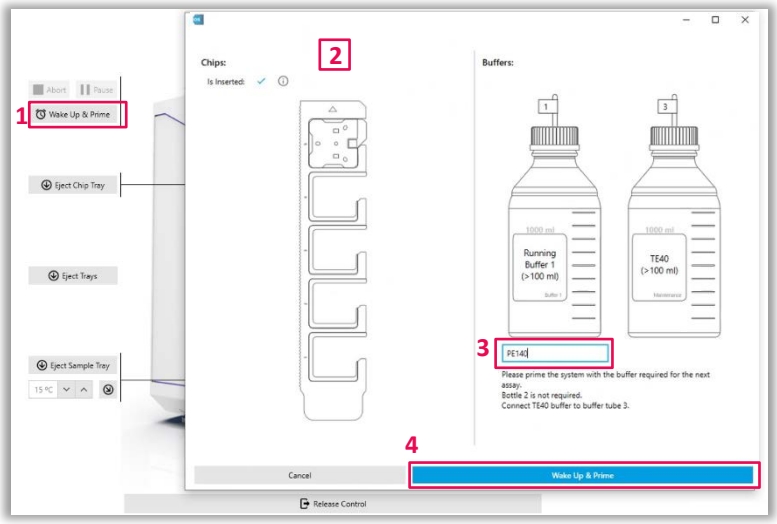
Connect your PC to your **heliX®** device as shown in Section 2.1.

(1) Select **Wake Up & Prime** in the **Devices** menu. This option is only available if a Clean & Sleep has been performed before.

(2) A **heliX® Cleaning Chip** is required at **position 5** in the chip tray as shown under **Chips**.

(3) Connect at least 100 mL **running buffer** to **buffer tube 1**, and enter the name of the buffer (e.g. "PE140"). Connect at least 100 mL **maintenance buffer** (TE40) to **buffer tube 3**. A bottle at buffer tube 2 is not required for this routine.

(4) Click on **Wake Up & Prime** to start the priming procedure. Afterwards, the **heliX®** device is ready to be used.



Tip: The **Cleaning Chip** can remain in the chip tray at position 5 if not all chip positions are required for measurements to facilitate frequent cleaning runs.

Tip: The **Running Buffer 1** can be a standard XE40 or XE140 buffer or a custom buffer. To save time it should be the running buffer required for the next assay.

3 heliX® Adapter Biochips

3.1 Biochip Layout

The sensor surface of an Adapter biochip contains a monolayer of 48-nt ssDNA oligonucleotides (anchor sequences) tethered to a gold surface at the 3' end. The bottom half of the 96-nt long Adapter strand sequence is complementary to the surface tethered anchor sequence (***Adapter strand* 1** to the sequence on **Spot 1** and ***Adapter strand* 2** to the sequence on **Spot 2**) allowing for a selective and exchangeable sensor functionalization. Figure 1 schematically shows the detection spots within the microfluidic channel of a biochip. One DNA nanolever represents a monolayer of nanolevers on each detection spot.

The use of DNA-encoded surface functionalization allows for easy regeneration of the sensor surface and removal of all bound analytes and immobilized ligands by denaturation of the DNA strands. The same chip surface can be used for a variety of biomolecular interactions as the DNA-monolayer can be equipped with almost any ligand molecule.

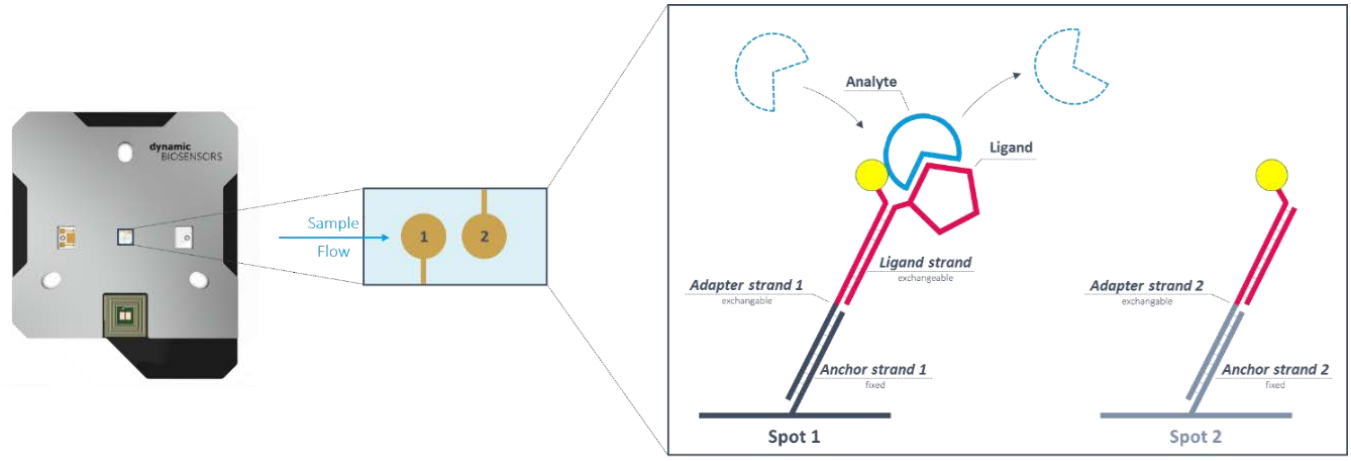


Figure 1 | Overview of a **heliX**® Adapter biochip. The **switch**SENSE® biochip contains one microfluidic flow channel with two detection spots. Each spot comprises a DNA-monolayer with two different anchor sequences (depicted in black and grey, respectively). By hybridization with the respective ***Adapter strands*** the spots can be functionalized with the ligand of interest (here: on Spot 1) or with a ligand-free strand as real-time control (here: on Spot 2).

Fluorescent probe

Adapter strands carry a fluorescent dye at the 3' end for detection. *Dynamic Biosensors GmbH* provides a pair of standard red and green dye (**Ra** and **Ga**), which are optimized for **switch**SENSE® measurements and compatible with most assay set-ups. However, a selection of four additional dyes (**Rb**, **Rc** and **Gb**, **Gc**) are available possessing different chemical properties to optimize the detection readout if necessary. The most sensitive dye can be determined by a quick dye scouting routine (see Section 3.4).

3.2 Adapter Biochip Test

We recommend performing a chip status test before starting an assay to determine the biochip quality. A chip status assay includes a regeneration step to hybridize the DNA surface with blank ***Adapter strands*** (contained in the **Test and standby solution**), a passivation step and a status measurement to obtain fluorescence amplitude signals. The parameters measured in a chip status test are relative fluorescence amplitudes and inflection points (IP) of the DNA monolayer functionalized with blank ***Adapter strands*** carrying a red dye (**Ra**) at standard conditions (25°C, in passivation solution).

Workflow of an Adapter Biochip Test

Regeneration

The regeneration process contains two subsequent steps (as shown schematically in Figure 2):

1. Denaturation of double-stranded DNA nanolevers by a basic regeneration solution; this leaves bare single-stranded DNA anchor strands tethered to the electrode surface.
2. Selective hybridization of ***Adapter 1*** **- Ra - lfs** and ***Adapter 2*** **- Ra - lfs** (the **Test and standby solution**) onto **Spot 1** and **Spot 2**, carrying DNA anchor sequences ***Anchor strand 1*** and ***Anchor strand 2***, respectively.

   Tip: The injection of pre-hybridized ***Adapter strands*** carrying a red fluorophore can be observed in real-time as a step function in the red fluorescence signal.

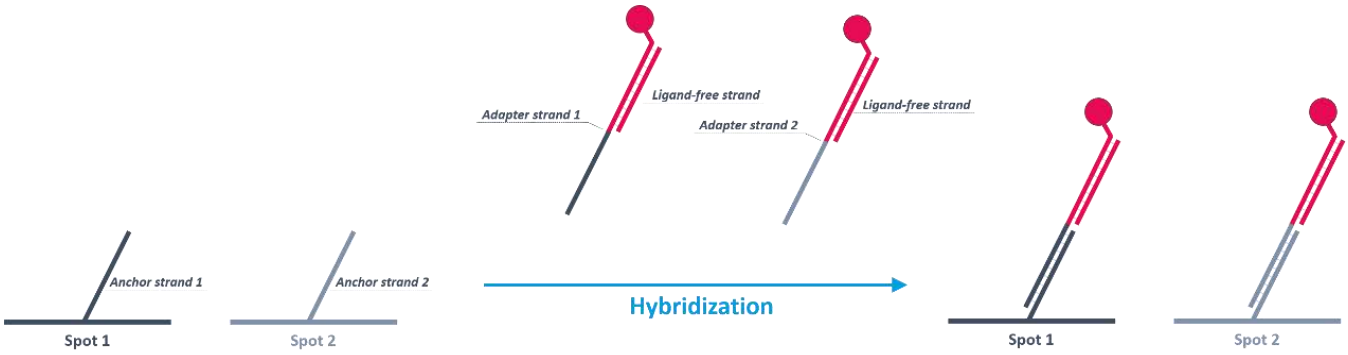


Figure 2 | Schematic overview of a biochip surface functionalization with the Test and standby solution containing ***Adapter strands*** **1** and **2** labeled with fluorophore **Ra** and pre-hybridized with the 48mer Ligand-free strand. **Spot 1** and **Spot 2** are functionalized with the respective ***Adapter strand*** by a specific on-chip hybridization.

Passivation

During the passivation step, the biosensor surface is rinsed with a thiol-containing solution while an alternating potential is applied to actuate the DNA nanolevers. This step creates a monolayer on the chip surface and prevents unspecific surface binding, increases DNA switching amplitudes and prolongs the chip lifetime.

Chip Status

After passivation, the chip is subjected to a two-step status test in passivation solution:

1. **Inflection Point Test:** A voltage calibration is performed by subjecting the DNA surface to a potential gradient starting at -200 mV to completely repel the intrinsically negatively charged nanolevers from the surface and gradually transitioning to a positive potential until the nanolever movement can be sufficiently recorded. For each voltage step, the fluorescence signal is obtained. It represents the orientation of the DNA nanolever relative to the quenching gold surface. This test provides information on the most sensitive switching range in order to efficiently actuate the DNA nanolevers. The readout of this analysis is the inflection point (IP) of the DNA nanolever downward motion (as shown in Figure 3B). This parameter is used for subsequent dynamic response measurements to select the voltage range of the alternating potential. (e.g.: an IP of 300 mV is obtained in the status test. A certain voltage range around this value is selected by the software for efficient DNA switching). A functional chip has inflection points in the range of **0 – 450 mV**.

2. **Relative Amplitude:** DNA nanolevers are actuated by an alternating voltage in solution and switching amplitudes (relative amplitude in %) are acquired for both electrodes. Positive potentials attract the DNA nanolevers towards the quenching gold surface and result in a low fluorescence signal of the end-tethered fluorophore. Negative potentials repel the DNA nanolevers from the quenching gold surface and result in a high fluorescence signal. This information is used to define chip functionality (as shown in Figure 3A). A functional chip has **Relative Amplitudes of > 40 %**.

To check the chip status values, select "**Status**" as the analysis type in the automatic analysis wizard (see Section 5.1). An exemplary chip status test is depicted in Figure 3 showing fluorescence data of a biochip functionalized with **Test and standby solution**.

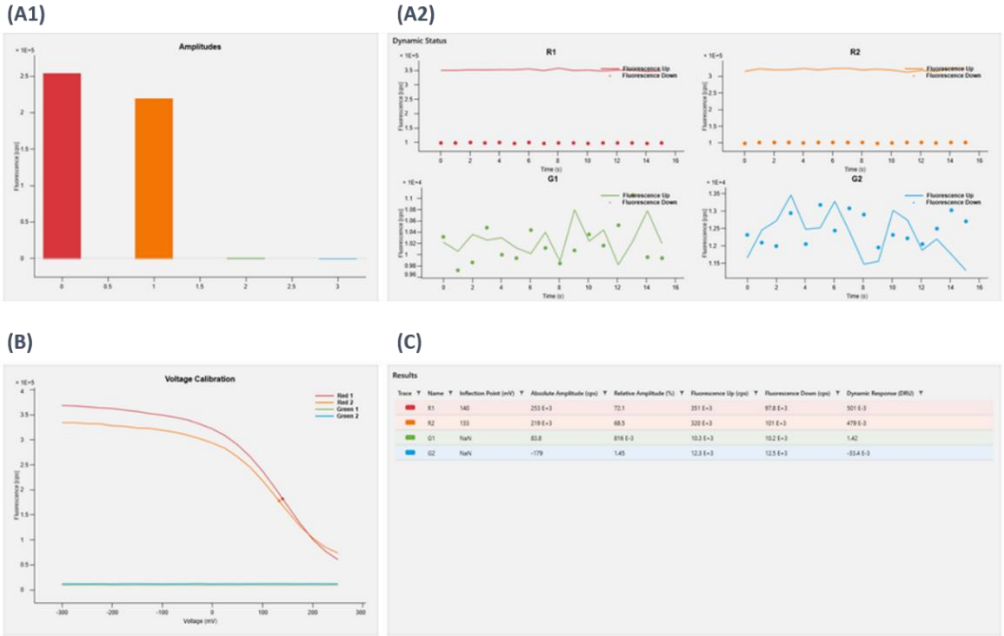


Figure 3 | Exemplary chip status test. As the biochip surface is functionalized with **Adapter strands** carrying a red dye, only red fluorescence signals (R1 and R2) are evaluated, green fluorescence signals (G1 and G2) are inconsequential. (A1): Absolute fluorescence amplitudes of **Spot 1** (red) and **Spot 2** (orange) are depicted as bar diagram. (A2): Real-time fluorescence up and down signal of **Spot 1** and **Spot 2**. (B): Voltage calibration curve for **Spot 1** and **Spot 2**. DNA nanolevers are gradually attracted to the quenching gold electrode as more positive potentials are applied resulting in a decrease of fluorescence intensity. Readout of this analysis is the inflection point (IP) of the curve, representing the sensitivity of the DNA nanolever movement to the applied voltage. (C): Overview of the acquired chip status data. Important parameters to define a functional chip are Inflection Points and Relative Amplitudes (%).

Table 1 | Status parameters of a functional Adapter biochip.

Chip Status Parameter	Value
Inflection Point	0 - 450 mV
Relative Amplitude	> 40 %

Table 2 | Required samples for a chip test run.

Reagent	Vial	Required Volume	Order No.
DI water	Large	10 mL	-
1x Passivation solution	Large	10 mL	SOL-PAS-1-5
Regeneration solution	Small	> 40 µL	SOL-REG-1-5, HK-REG-1*
Test and standby solution	Small	> 35 µL	TS-0

* HK-REG-1 additionally includes heliX® EDTA solution which is not necessary anymore for methods and assays released with heliOS 2022.1 or newer. Older versions of heliOS (1.7.4 and older) and deprecated methods still need the heliX® EDTA solution for the surface functionalization. We recommend to only use new methods that carry a green “verified” tag.

Set-up of an Adapter Biochip Test in heliOS

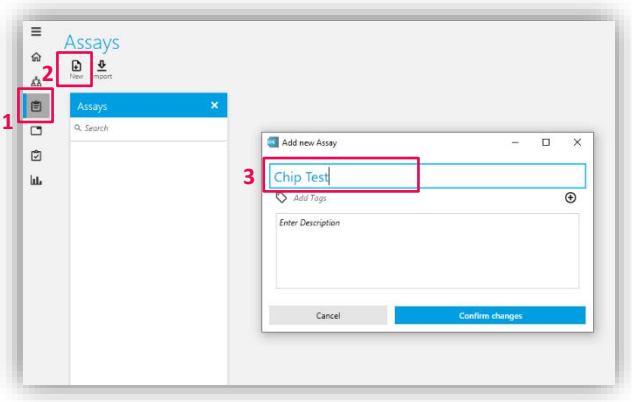
Set up your **heliX®** device as shown in Section 2.

(1) Open **Assays** by clicking the icon as shown.

(2) Select **New** to create a new assay workflow.

(3) Rename the new assay workflow (here: “Chip Test”) and **Confirm Changes**.

Tip: Type in your Chip-ID as assay name to be able to link your chip test data with the respective biochip.

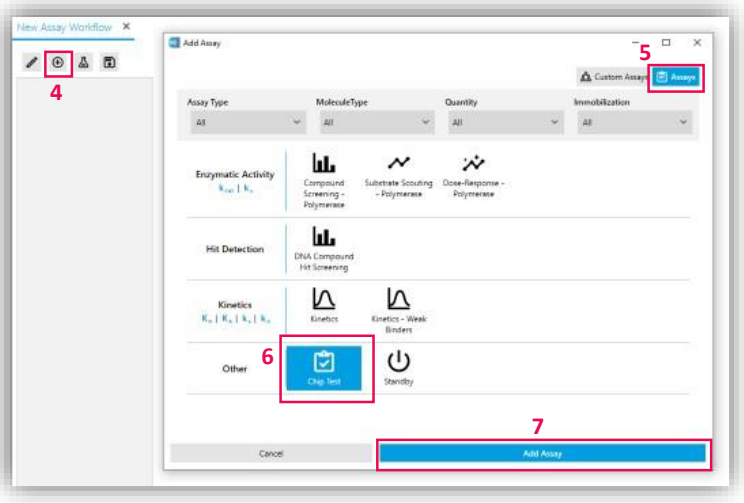


(4) Add a new Assay by clicking the "**+**" icon.

(5) Go to **Assays**.

(6) Choose **Chip Test**.

(7) Confirm by clicking **Add Assay**. The default chip test assay opens automatically.

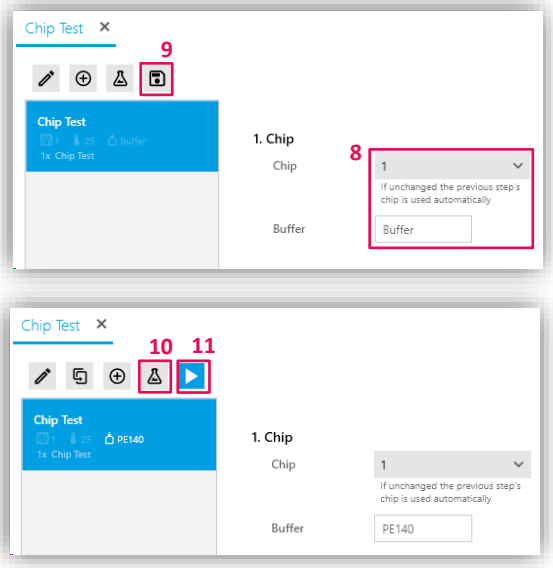


(8) Select the respective chip position by opening the **Chip** drop-down menu (default: position 1). Enter the name of the running buffer in the **Buffer** text field below (e.g. "PE140", default: "Buffer").

(9) **Save** your assay.

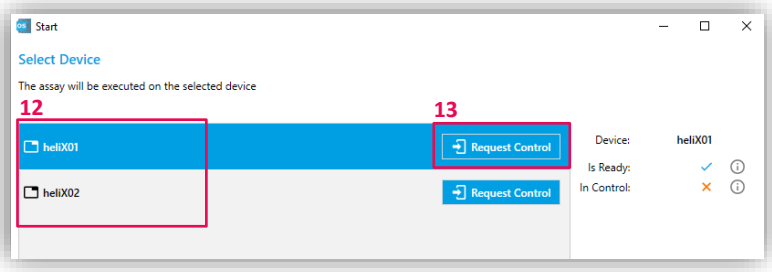
(10) Optional: Click the **Sample tray** button to preview the sample and buffer positions.

(11) Click the **Run** button. The assay start wizard opens.



(12) Select a **heliX**® device from the list.

(13) Use **Request Control** to connect to the device. Click **Next** in the bottom right.



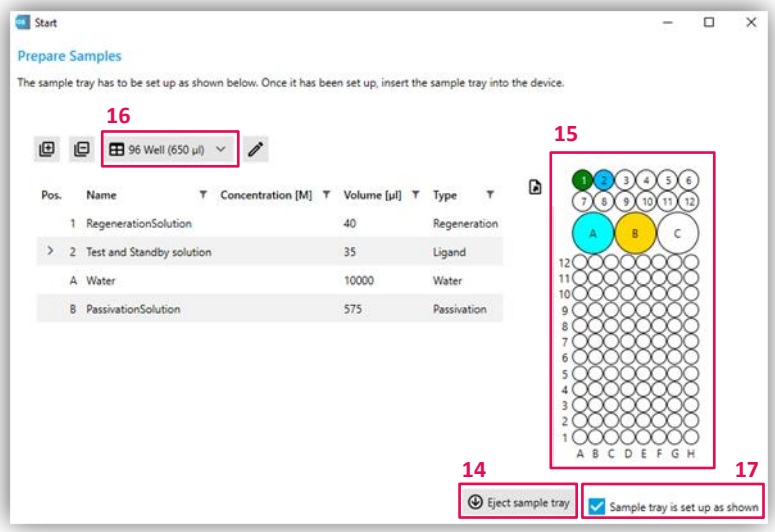
(14) In **Prepare Samples** use **Eject sample tray** for loading the device with samples.

(15) Fill in the respective samples into the depicted positions.

(16) **Tip:** Change the plate layout if required (default: 96 well plate with max. 650 µl per well).

Tip: Sample positions can be manually changed in the sample tray overview if required.

(17) **Insert sample tray**, confirm and click **Next**.

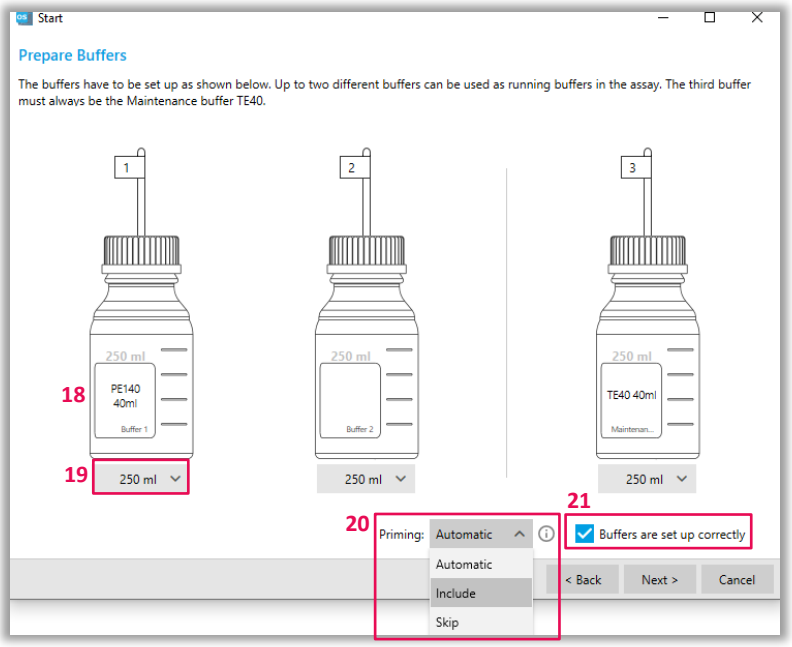


(18) In **Prepare Buffers** the required bottle sizes, buffers and volumes are shown. Connect the required running buffer(s) to the tubes 1 and/or 2 and the maintenance buffer to tube 3.

(19) **Tip:** The suggested bottle type can be changed manually (250–1000 mL, custom) if required.

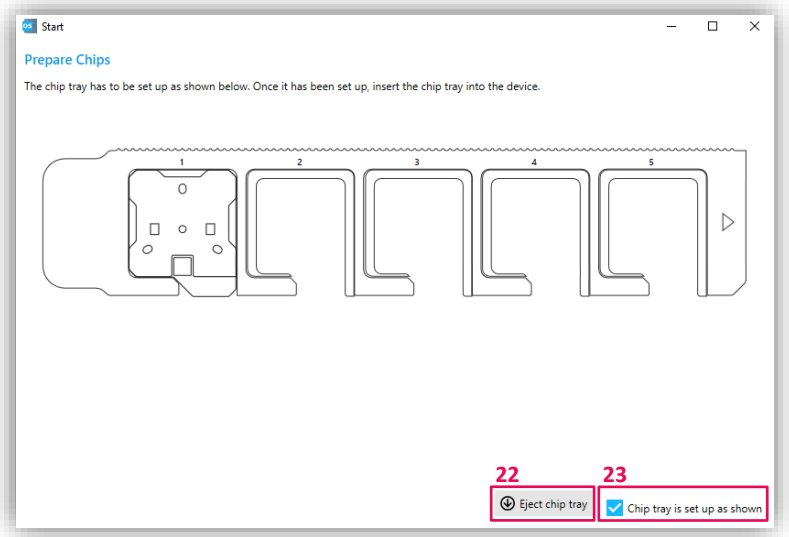
(20) The **initial priming** step before the first assay can be controlled by the dropdown-menu next to **Priming**. Choose one of the following options:
Automatic: The system remembers the last used buffer. If the buffer (name entered in the assay input field) stays the same priming is automatically skipped. If a different buffer is entered in the assay the priming will be automatically performed.
Include: Use this option if you want to force a priming, or if you are unsure about the state of the fluidics system (**recommended here**).
Skip: Select this option from the dropdown-menu if you want to skip the priming.

(21) Confirm and click **Next**.



(22) Use **Eject chip tray** to load the device with the required chip(s).

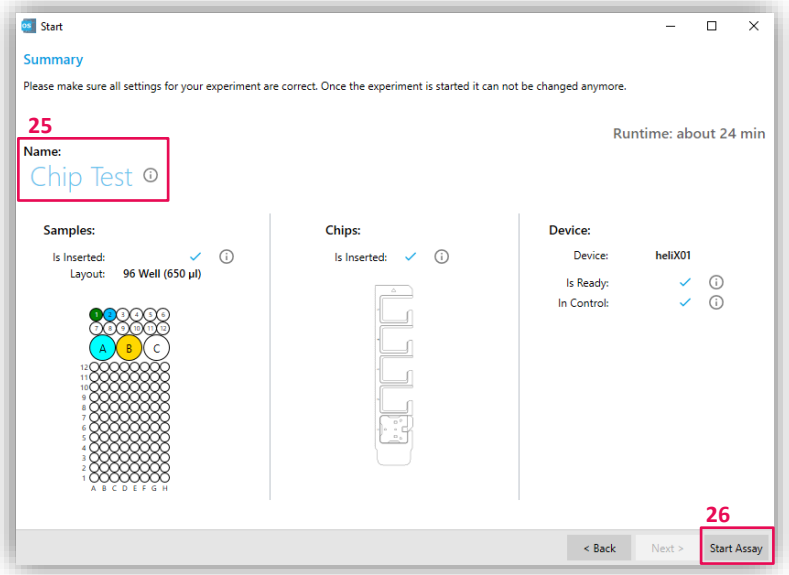
(23) **Insert chip tray**, confirm and click **Next**.



(24) Make sure that all settings are correct.

(25) **Tip:** Change the name of the experiment if required.

(26) Click **Start Assay** to start the chip test run. The run will take around 16 minutes.



3.3 Surface Functionalization

Nucleic acid sequence as ligand

The 48-nt ***Ligand strand*** can be elongated at the 5' end with any DNA/RNA sequence of interest. For optimal signal readout, short sequences (< 50-nt) are recommended as an overhang. The overhang can be designed single- or double-stranded. For surface functionalization, the elongated ***Ligand strand*** is pre-hybridized with the top half of the ***Adapter strand* 1** leaving the bottom half of the ***Adapter strand* 1** free for on-chip hybridization (see section 0).

Ligand strand sequence:

5'-ATC AGT ACT TGT CAA CAC GAG CAG CCC GTA TAT TCT CCT ACA GCA CTA-3'

Protein as ligand

The ***Ligand strand*** can be crosslinked with a protein of interest via amine- or thiol-coupling using a coupling kit provided by *Dynamic Biosensors GmbH*. Purification of the conjugation product can be performed using the **pro**FIRE® or any other purification system. A step-by-step explanation of the conjugation workflow is available. For surface functionalization,

the protein-***Ligand strand*** complex is pre-hybridized with the top half of ***Adapter strand* 1** leaving the bottom half of the ***Adapter strand* 1** free for on-chip hybridization (see section 0).

Alternatively, ready-to-use capture kits can be used for surface functionalization. Capture of the Ligand molecule then takes place after the functionalization step on the chip surface. His-, Biotin-, Twin-Strept-tag-, Fc- and GFP-**Capture Kits** are available.

Adapter Strand Preparation – MIX&RUN

This section provides a step-by-step guide how to pre-mix the Adapter strands for a correct surface functionalization. The ligand molecule is attached to the ***Ligand strand*** either by elongation during nucleic acid synthesis or conjugation (e.g. protein).

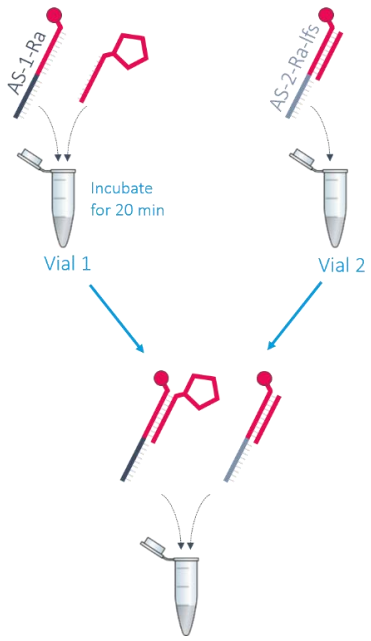
In-solution hybridization of adapter and ligand strands:

1) Mix ***Adapter strand 1* - Ra** (400 nM) and conjugated ***Ligand strand*** (500 nM) at a 1:1 ratio (v/v)

2) Incubate at 25°C at 600 rpm in the dark for 20 minutes to ensure complete hybridization.

3) Mix solution of step 2) and ***Adapter strand 2* - Ra - lfs** (200 nM) at a 1:1 ratio (v/v)

Solution is ready to use for biochip functionalization.



Example

Required volume for three functionalizations (as suggested in **heliOS**): **100 µL**

Vial 1		Vial 2
***Adapter strand 1* - Ra** (400 nM)	Conjugated ***Ligand strand*** (500 nM)	***Adapter strand 2* - Ra - lfs** (200 nM)
25 µL	25 µL	50 µL

After incubation time, mix vial 1 and vial 2 to obtain 100 µL of ready-to-use DNA solution.

Tip: Create your own custom **heliX®** biochip to meet your experimental needs using the **heliX® MIX&RUN** app. The **heliX® MIX&RUN** app determines the volumes of your ligand(s) and Adapter-dye strands in order to create a biochip surface with your desired ligand densities.

3.4 Dye Scouting Information

The two standard fluorescent probes for **switch**SENSE® measurements are the red and green dye **Ra** and **Ga**, respectively. Fluorescence proximity sensing is based on the change in the local environment of the dye upon binding of an analyte to the ligand, which in turn results in a change of the fluorescence signal. This effect depends on the chemical nature of the dye and the interaction partner. Therefore, a different dye may yield a higher signal response depending on the type of interaction.

Dye scouting enables to screen for the most sensitive fluorophore for the respective application. Three red and three green fluorophores with different chemical properties are available for dye scouting. The dyes differ in net charge and hydrophobicity as depicted below (Figure 4).

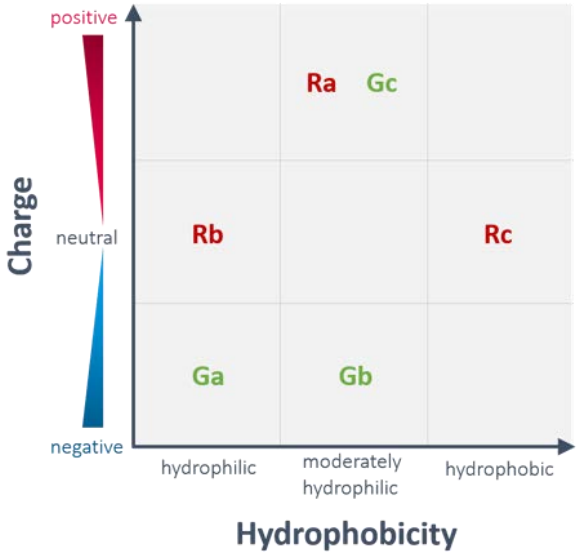


Figure 4 | Overview of the **switch**SENSE® dye properties. Six fluorophores - three red dyes (**Ra**, **Rb**, **Rc**) and three green dyes (**Ga**, **Gb**, **Gc**) - are available. Dye scouting is compatible with the **heliX**® Adapter biochip. Depending on the interaction partner, different fluorescent probes may obtain different signal responses. Dye scouting allows to quickly screen for maximum signal amplitudes.

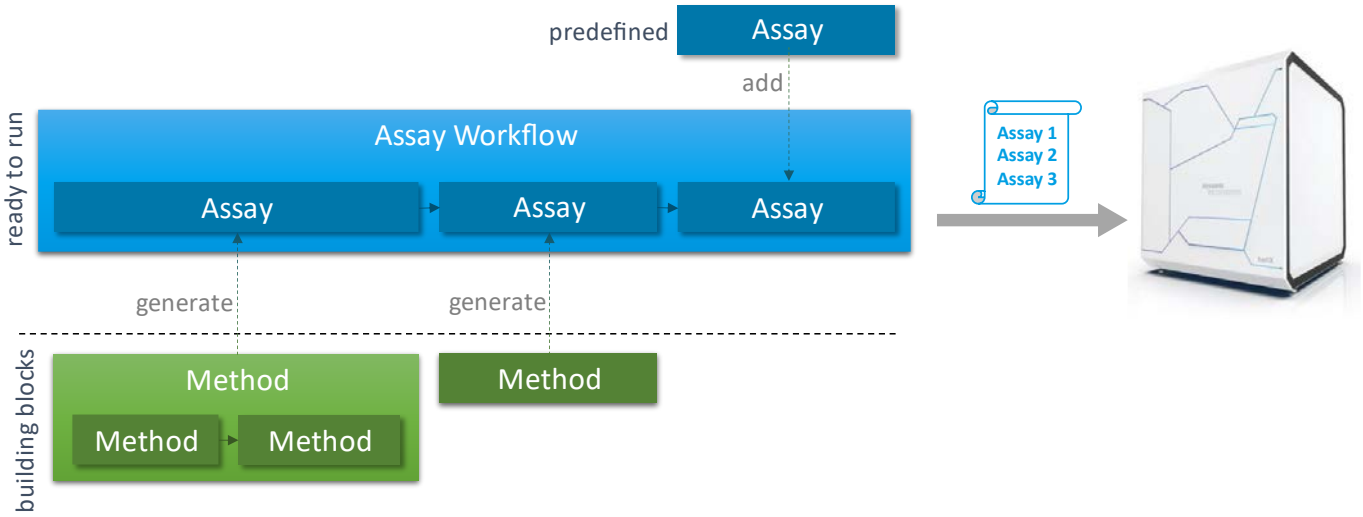
4 heliOS Assay Workflow Set-up

The Assay section in **heliOS** allows for an easy and flexible assay generation. Several predefined **Assays** optimized for different types of molecular interactions are available in **heliOS**. Additionally, the user is free to design any customized method by combining building blocks. This set-up is ideal for an experienced user and enables the generation of **Custom Assays** with an individual and broader choice of parameters.

In short, when setting up an assay two options are available:

1. Using already predefined **Assays** as described in chapter 4.1 and
2. Setting up a customized assay composed of custom **Methods** as described in chapter 4.2.

An **Assay Workflow** is defined as a sequential arrangement of **Assays** (either predefined **Assays** or custom **Assays**) that are sent to the **heliX**®.



4.1 Predefined Assays

This section provides information about how to use the predefined **Assay** user interface in **heliOS**.

To set up *an **Assay Workflow** using predefined **Assays** perform the following steps:*

(1) *Click* on **Assays** in the main **heliOS** page.

(2) Choose the icon **New**, the **New Assay Workflow** window will open.

(3) Delete the **New Assay Workflow** text and replace it by your **Assay Workflow** name.

(4) After entering the name, confirm the changes and an empty **Assay Workflow** opens.

Recommendation: Use the nomenclature "initials_experiment name_date" as assay name.

(5) Click the "**+**" symbol at the top left of the page to add an **Assay**.

(6) A new window will open and predefined **Assays** will be displayed per default. Choose the required **Assay** and add it to the **Assay Workflow**.

Multiple predefined **Assays** can be queued in one **Assay Workflow** by adding more **Assays**. This is helpful for example when testing multiple different analytes as each **Assay** will be shown as such during the data analysis. The **Assays** will appear on the left side of the **Assay Workflow** set-up window and will be performed from top to bottom during the measurement run. An **Assay** can be removed from the workflow by selecting it and pressing the delete key. Additionally, **Assays** can be duplicated via context menu. As default setting for an added **Assay**, the chip position of the previous **Assay** is applied. However, different chip positions can be used during the same measurement run if required. The chip position will be changed automatically. All required chips have to be loaded into the chip tray before starting the measurement. (These steps are explained in more detail in section 3.2, where a **Chip Test** is selected as predefined **Assay**).

An exemplary **Assay**: Kinetics is shown in Figure 5.

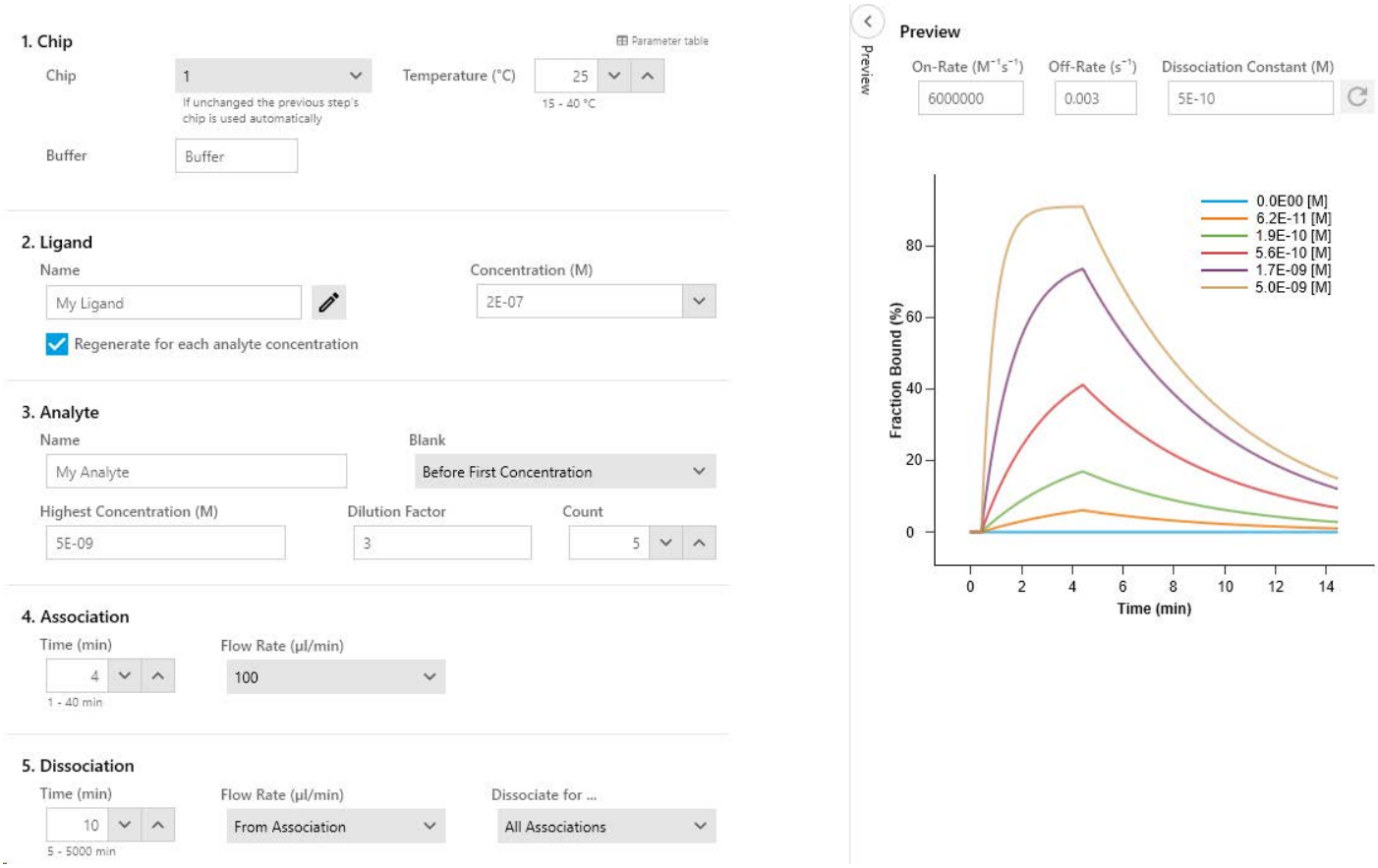


Figure 5 | **Assay**: Kinetics in **heliOS**. This predefined assay set-up can be customized by adjusting chip position, chip temperature, running buffer, ligand and analyte information and defining assay parameters, such as concentration count, dilution factor, flow rate and duration of association and dissociation. A graph on the right side shows simulated results based on the set rate constants and assay parameters.

Table 3 | Tips for setting up a kinetics assay.

<table>
<tr><th>Input Field</th><th>Information</th></tr>
<tr><td>Chip</td><td>The chip position does not influence the measurement. Choose any available position in the chip tray to place your biochip and select a temperature between 15 and 40°C (default 25°C). The chip temperature is only applied during the main measurement. During all other steps (e.g. functionalization, wash steps, capture reactions) the temperature is automatically set to 25°C to guarantee optimal preparation results. Specify the name of your running buffer in the input field Buffer. You can use an optional second running buffer for a different assay in the same assay workflow. In case of two running buffers, a priming step is added automatically between two assays whenever running buffers are switched.</td></tr>
<tr><td>Ligand</td><td>The Kinetics Assay is optimized for conjugated ligands only (i.e. for a capture approach use a manually generated Assay instead). Type in your conjugated ligand name.
Ligand concentration refers to the concentration of the ligand-DNA complex. In most cases, this is a pre-hybridized Adapter strand 1 with the conjugated ligand strand mixed with pre-hybridized Adapter strand 2 with the ligand-free strand (e.g. AS-1-Ra with conjugated ligand strand mixed with AS-2-Ra-lfs). Standard concentration in the final solution are 100 nM each.

The functionalization time represents the duration in which the complementary Adapter strand sequences are incubated in the microfluidic flow channel to hybridize onto the biosensor surface. The default hybridization time of 200 s can be used for most assay set-ups. For lower ligand concentrations, longer times are recommended. Functionalization times can only be adjusted when “Advanced Options” are visible. Recommended times in respect to ligand concentrations are:
<table>
<tr><th>Funct. time</th><th>Ligand conc.</th></tr>
<tr><td>200 s</td><td>≥ 100 nM</td></tr>
<tr><td>400 s</td><td>50 nM</td></tr>
<tr><td>900 s</td><td>< 50 nM</td></tr>
</table>
For complete dissociations, the surface does not need to be regenerated after each analyte concentration and the ligand can remain on the sensor surface. For incomplete dissociations, a surface regeneration is highly recommended.</td></tr>
<tr><td>Analyte</td><td>Type in your analyte name and define the highest concentration for your binding assay. If multiple concentrations should be measured, increase the concentration count accordingly. The dilution factor can be adjusted depending on the required concentration range. If unsure, use a high dilution factor and a low concentration range to screen for the most sensitive concentration range.</td></tr>
<tr><td>Association</td><td>Define your association time and flow rate. A standard kinetics assay allows to select association times of 1 - 200 min at a flow rate of 20 - 500 µL/min. If unsure, use a flow rate in the range of 100 µL/min.</td></tr>
<tr><td>Dissociation</td><td>Define your dissociation time and flow rate. The flow rate can either be linked to the association flow rate or adjusted individually. For long dissociations (e.g. > 1h) it is recommended to dissociate the last concentration only and regenerate the surface between analyte concentrations instead.</td></tr>
</table>

Click the **Run** button to start your **Assay Workflow**. A wizard guides you through the process of starting the experiment.

- ✓ Select your **heliX®** device.
- ✓ Confirm the sample set-up by ticking the box **Sample tray is set up as shown**.
- ✓ Confirm the buffer set-up by ticking the box **Buffers are set up correctly**. Choose, whether an initial priming step should be added before the first assay (**Automatic**, **Include, Skip**. If unsure select **Include**).
- ✓ Confirm the chip position in the chip tray
- ✓ Re-check the sample tray, chip position and device status and click **Start Assay**

  Tip: The **Name** of the experiment can be modified if required.

The measurement can be observed in real-time. Once the **Assay Workflow** is started, the device runs independently from the PC. Hence, the measurement will continue even in the case of a connection loss between the PC and the **heliX®** device.

4.2 Custom Assays

This section provides information on how to work with customized **Assays** in **heliOS**, when the desired assay set-up is not available as a predefined **Assay** in **heliOS**.

A custom **Assay** is generated from individually composed **Methods** which can be placed like building blocks one after the other. These **Methods** equip the more advanced user with flexibility and allow for a broader choice of parameter needed for example during assay development.

To set up an **Assay Workflow** using customized **Assays** perform the following steps:

(1) Click on **Assays** in the main **heliOS** page,

(2) Choose the icon **New**, the **New Assay Workflow** window will open.

(3) Delete the **New Assay Workflow** text and replace it by your **Assay Workflow** name.

(4) After entering the name, confirm the changes and an empty **Assay Workflow** opens.

Recommendation: Use the nomenclature “initials_experiment name_date” as assay name.

(5) Click the “**+**” symbol at the top left of the page to add an Assay.

(6) A new window will open and the overview about predefined **Assays** will be displayed per default. Go to the **Custom Assays** tab. Select a **Method** from the list which you want to use for generating an **Assay**, and add it to the **Assay Workflow**.

Multiple **Assays** can be queued in one **Assay Workflow** by adding more **Assays**. This is helpful for example when testing multiple different analytes as each **Assay** will be shown as such during the data analysis.

If you click on your **Assay** within the **Assay Workflow**, a parameter table will be displayed so that you can enter the parameters you need. Figure 6 shows an assay set-up based on a custom **Assay**.

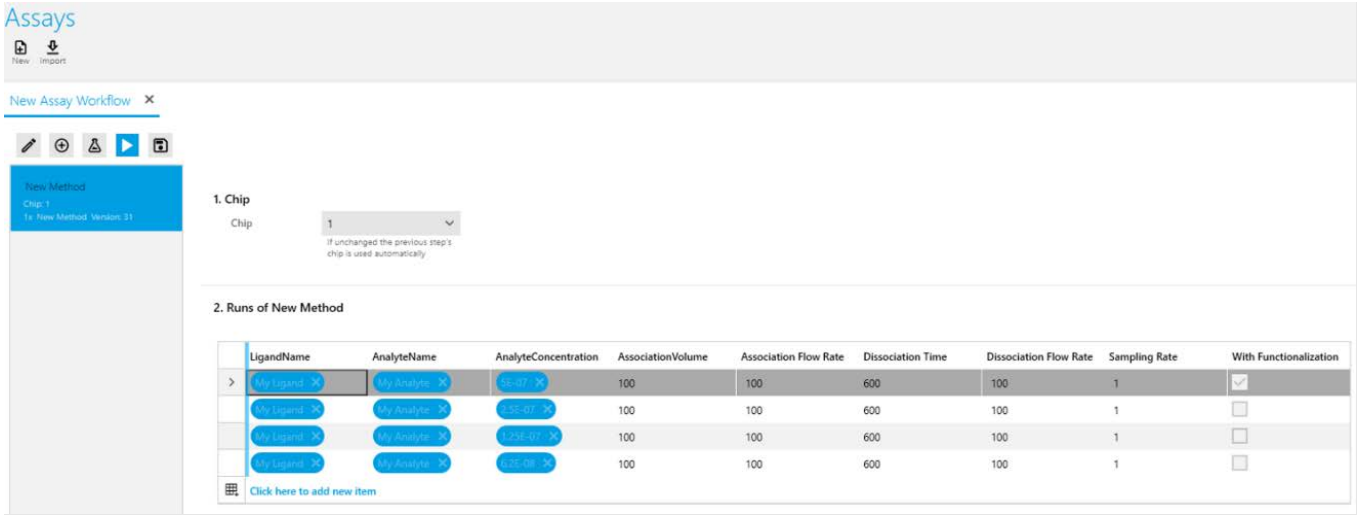


Figure 6 | Example **heliOS** assay based on a custom **Assay**. Each column represents a parameter, in this case: Ligand name, Analyte name, Analyte concentration, Association flow rate, Dissociation time, Dissociation flow rate, Sampling rate, and optional "With functionalization". Each row represents one measurement run - rows can be added simply by clicking on the last free row. Parameters of a previous row can be copied by "Copy&Paste" or manually adjusted if necessary. In this example, the assay loop is subsequently performed four times (four rows) with increasing analyte concentrations (third column). Surface functionalization is only selected for the first loop and is deselected for all other loops (last column). Hence, in this case the ligand remains on the surface throughout the complete set of measurements.

Click the **Run** button to start your assay. A wizard guides you through the process of starting the measurement.

- ✓ Select your **heliX®** device.
- ✓ Confirm the sample set-up by ticking the box **Sample tray is set up as shown**.
- ✓ Confirm the buffer set-up by ticking the box **Buffers are set up correctly**. Choose, whether an initial priming step should be added before the first assay (**Automatic**, **Include, Skip**. If unsure select **Include**).
- ✓ Confirm the chip position in the chip tray
- ✓ Re-check the sample tray, chip position and device status and click **Start Assay**

  Tip: The **Name** of the experiment can be modified if required.

The measurement can be observed in real-time. Once the **Assay Workflow** is sent to the instrument, the device runs independently from the PC. Hence, the measurement will continue even in the case of a connection loss between the PC and the **heliX®** device.

4.3 Custom Methods

Individual building blocks, called **Methods**, can be placed one after the other to create a new, individually composed **Method**. Once saved, this composed method can be used directly to generate a customized **Assay** or can be further used as building block for other, individually composed **Methods**. By using this way of nesting, a high level of flexibility and parameter accessibility is gained for all types of sophisticated assays. For each composed **Method**, parameters can be defined individually. This allows to select which parameters should remain constant and which should be displayed later on in the assay parameter table to be adaptable in the final **Assay**.

The **Methods** list contains **Method scripts** (📄) and **composed Methods** (🖧). A **Method script** is the smallest unit of a **Method**. It reflects a single, functional unit (a wash step, an association step etc.) which needs to be combined with

other **Methods** to generate a fully functional **Assay**. Since **Method scripts** contain script language which enables full device control, they cannot be opened upon double-clicking, but rather used as building blocks for composed methods.

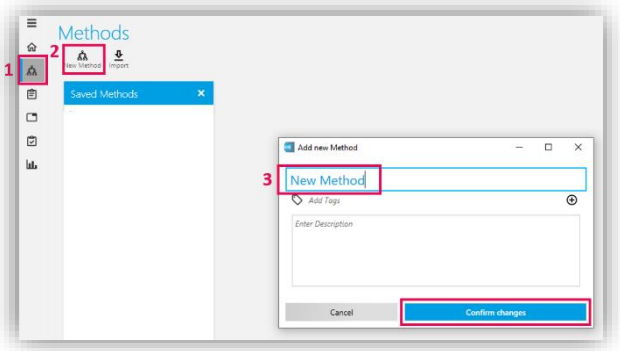
Methods that contain other **Methods** are called **composed Methods**. You can combine **Method scripts** and **composed Methods** into your own (composed) **Method** with parameters of your choice. In order to prepare your own **Method** please follow these steps:

(1) Open **Methods** by clicking the icon as shown.

(2) Select **New Method**.

(3) Rename the method (here: "New Method") and **Confirm Changes**.

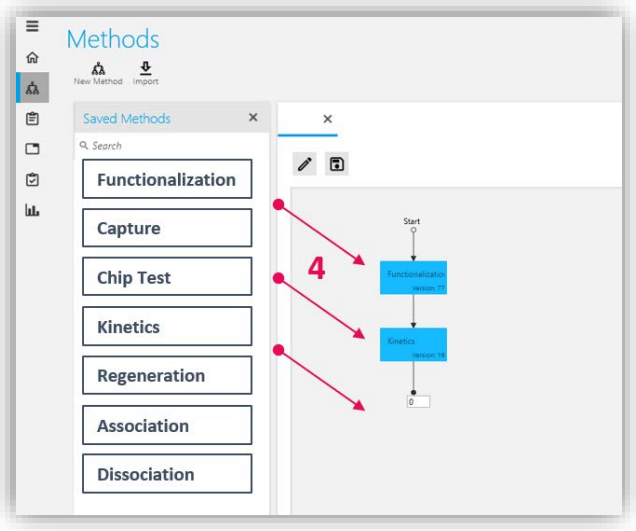
Tip: Make sure the **Method** has a descriptive name to be able to find it again at a later stage.



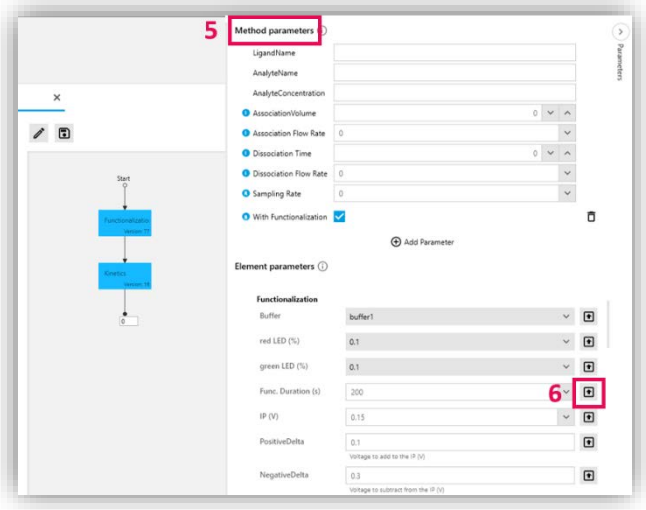
(4) A blank flow diagram opens comprising a start symbol and a line in the middle of the window. Add **Methods** to the flow diagram by "Drag&Drop" from the menu on the left side. There are several **Method scripts** and **composed Methods** to choose from to generate your own **Method**. **Save** your **Method** once all required building blocks are transferred to the flow diagram.

Tip: Method building blocks can be removed from the diagram by selecting and clicking the delete key.

Tip: Each of the **Method** building blocks can be set as "optional" allowing to run the same **Method** with and without the respective block. For this option right-click on the block in the flow diagram, select "**Make optional**" and then "**Add Parameter".** This option will appear as exposed parameter in the final assay.



(5) For each added **Method** building block, a list of **Method parameters** appears on the right side of the window.



(6) Select every parameter, you want to be adjustable in your assay and visible in the final assay parameter table by clicking the arrow button on the right of the parameter.

(7) A window opens, where the name of the parameter, and optional description and default settings can be defined (screenshot is not shown for this step). Once your customized **Method** is saved, this parameter will be shown in the final assay parameter table.

Tip: Default settings can be used for most parameters. Avoid selecting too many parameters to ensure easier assay set-up at a later stage, define every parameter here which won't be adapted in the final assay (e.g. LED power, voltages, …) and select only parameters which are frequently adapted (e.g. ligand and analyte name, analyte concentration, flow rates,..).

Tip: Composed Methods found in the list are marked with a green "verified" tag – these are protected from editing to guarantee the quality. To edit a **Method**, you need to duplicate it, rename it, and remove the "verified" tag.

Tip: If you choose a custom **Method** and you need to select more parameters for the final parameter table, double click on the Method in the Saved **Methods** list and click the button duplicate. Give the duplicated **Method** a new name, click save and expose the parameters which you need.

(8) Your own **Method** will appear on the **Methods** list and is ready to be used as custom **Assay** for a **New Assay Workflow** as described in the Section 4.2.

5 heliOS Data Analysis

The Analysis section in **heliOS** allows to open, analyze, plot and export measurement data from one or more experiments. **heliOS** provides two of data analysis tools: The **automated** and the **manual analysis**. Both tools are described in the following sections.

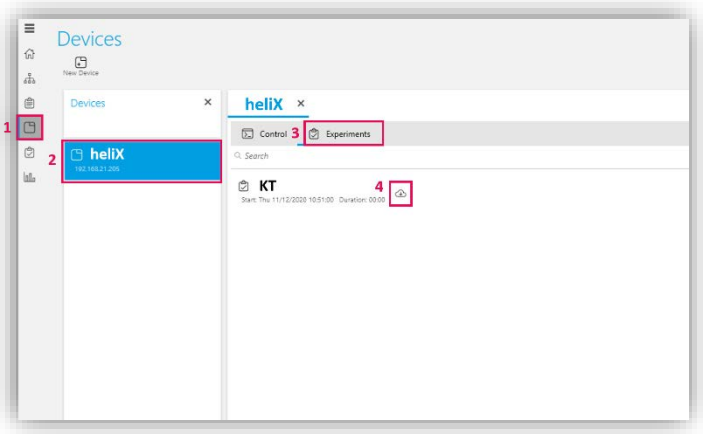
5.1 heliOS Automated Data Analysis

(1) Open **Devices** by clicking the icon as shown.

(2) Select your **heliX**® device.

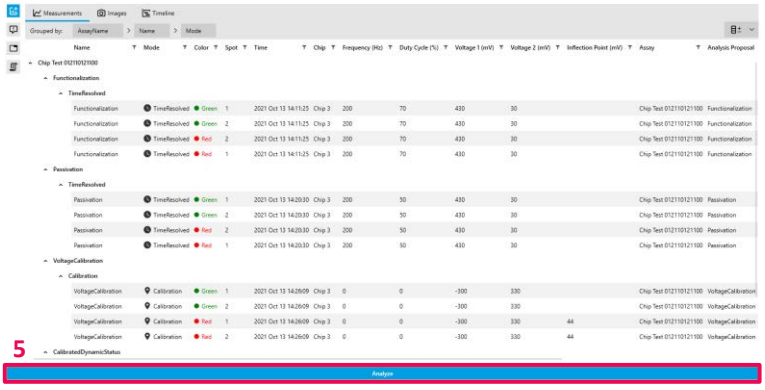
(3) Open the tab **Experiments** to show all experiments performed on this device.

(4) Download the acquired dataset by clicking the cloud icon. Once the download is completed, the cloud icon disappears. Double-click on the dataset to open it.



(5) The dataset is opened automatically under the tab **Measurements**. Click on **Analyze** on the bottom of the window.

Tip: Click on the tab **Timeline** in the top row (next to **Measurements**) to see all measurements, events and data traces of the entire experiment.



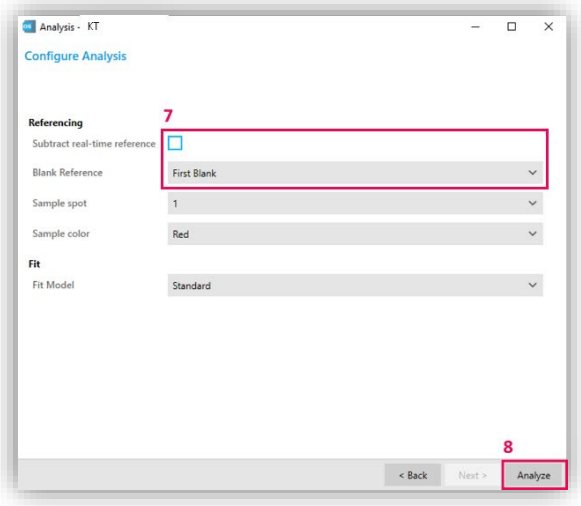
(6) A pop-up window appears showing all performed assays in this measurement run: Select the assay you want to analyze and click **Next**. Then select **1:1 kinetics** and click **Next** (screenshot is not shown for this step).

Tip: Here, the chip status can be viewed by clicking **Status**.

(7) A pop-up window appears allowing to configure the analysis: Select **Subtract real-time reference** if required; choose a **blank reference** or select **None**; choose your sample spot, sample color, and fit model.

Tip: A more detailed explanation is provided in the table below.

(8) Select **Analyze** to start an automated analysis (Figure 7).



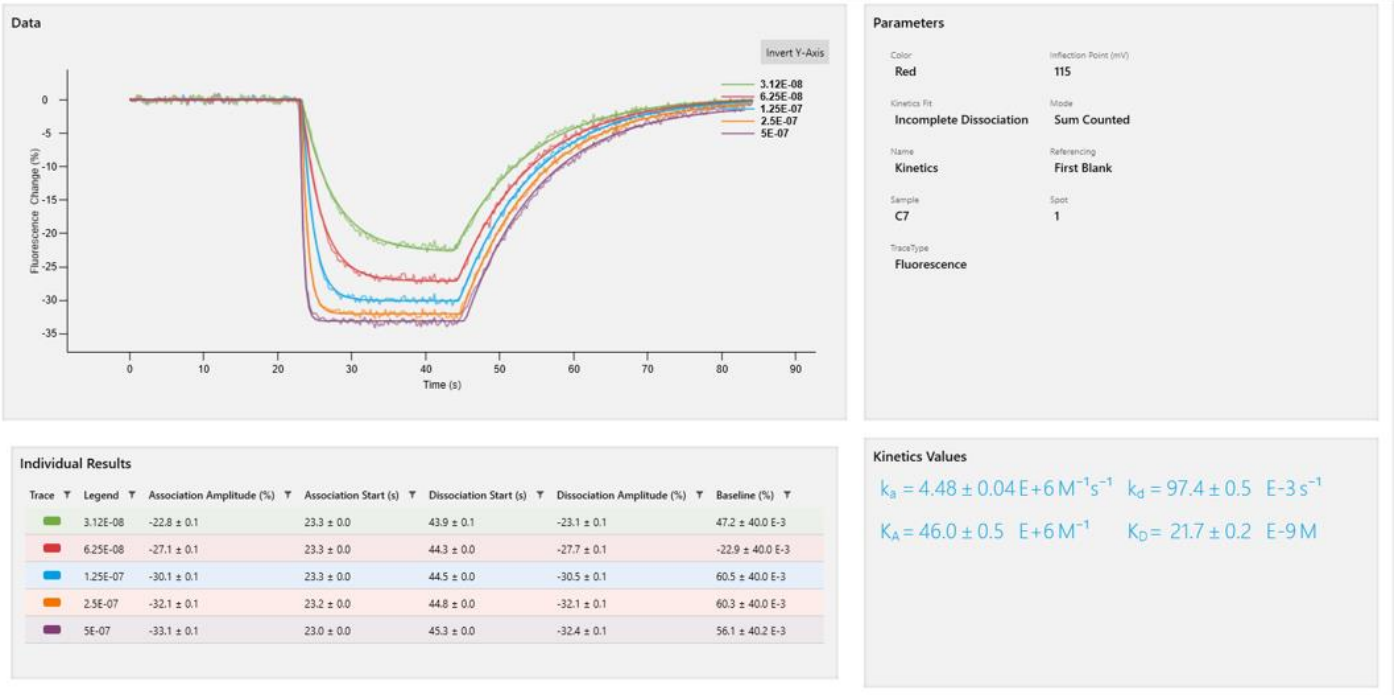


Figure 7 | Exemplary kinetics data using automated analysis. The data is automatically referenced with the blank injection signal. A global mono-exponential fit for association and dissociation is applied. Calculated values for the on-rate (k_a), off-rate (k_d) and the resulting dissociation constant (K_D) are depicted on the bottom right side.

Table 4 | Guide to choose parameters for an automated analysis.

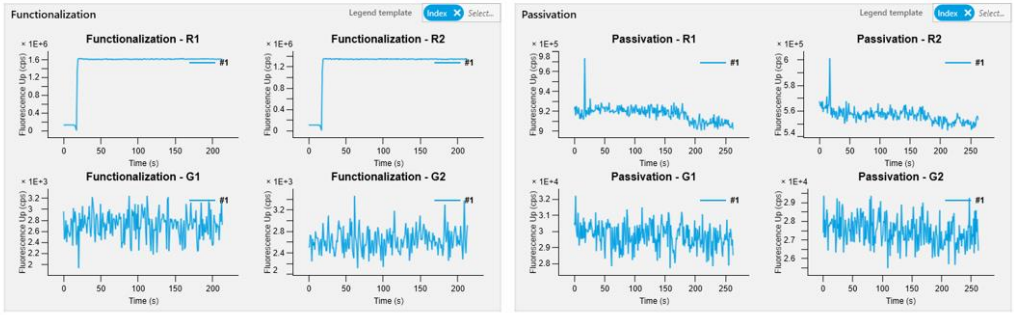
Parameter	Purpose
Real-time reference	Real-time reference refers to the acquired data on the reference electrode during the measurement. In most cases, subtracting the real-time reference is recommended if one is interested in referencing with the signal of the ligand-free electrode.
Blank reference	Blank reference refers to the acquired data during a separate blank injection. Depending on the assay, the blank can be either in the beginning of a concentration series and/or at the end. If both blanks are performed, the user can choose any one of them or an average of both for blank referencing.
Sample spot	Define whether the ligand of interest was immobilized on **Spot 1** (default) or on **Spot 2**.
Sample color	Define whether red (default) or green fluorescence intensities should be analyzed.
Fit model	Define your required Fit model. A global mono-exponential fit will be implemented. Optionally, the dissociation can be set to incomplete (supporting different association and dissociation amplitudes) or to independent (supporting analysis of different interaction partners in one run).

heliOS Data Overview

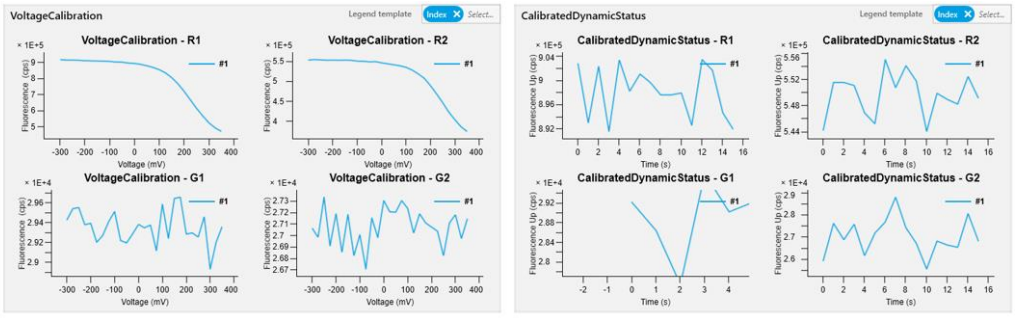
The data overview tool in **heliOS** shows all raw data traces of the selected measurement. In this overview, raw signals are depicted for all four detection channels (red and green fluorescence detection of **Spot 1** and **Spot 2**) for Functionalization, Passivation, Chip Status and Kinetics. This enables a quick overview of all acquired signals for this measurement and allows for direct observation of specific and non-specific signals, of chip quality and comparison between red and green data traces without the need for an advanced analysis (such as normalization, referencing, fitting).

To display your data in the overview format, perform steps (1) - (6) of section 5.1. In step (6) select **Data overview** and click **Next** and **Analyze**. The data overview opens automatically, as shown in Figure 8.

Functionalization & Passivation



Chip Status: Voltage Calibration & Dynamic Status



Kinetics

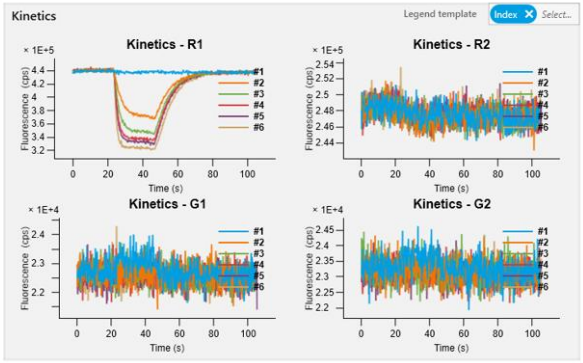


Figure 8 | Example of a data overview in **heliOS**. Raw data traces for Functionalization, Passivation, Voltage Calibration, Dynamic Status, and Kinetics are depicted. Each measurement contains four simultaneously acquired raw data traces (red and green signal readout for **Spot 1** and **Spot 2**, respectively). In this case, the biochip was functionalized with **AS-1-Ra** pre-hybridized with a ligand strand and **AS-2-Ra** pre-hybridized with a ligand-free strand. During functionalization, a step signal is obtained in the red fluorescence trace (R1 and R2) upon injection of the labeled Adapter strand solution. The Voltage Calibration signal shows the downward signal in red of functionalized DNA nanolevers. In Kinetics, multiple traces are shown representing an analyte concentration series (in this case a **Kinetics Test assay** was performed). As only **Spot 1** carries the ligand of interest, binding is only observable in "**Kinetics - R1**".

5.2 heliOS Manual Data Analysis

For standard data analysis the automated data analysis tool described in chapter 5.1 can be used. If the analysis of individual data traces, different fit or normalization operations are required, an advanced manual data analysis is available in **heliOS**.

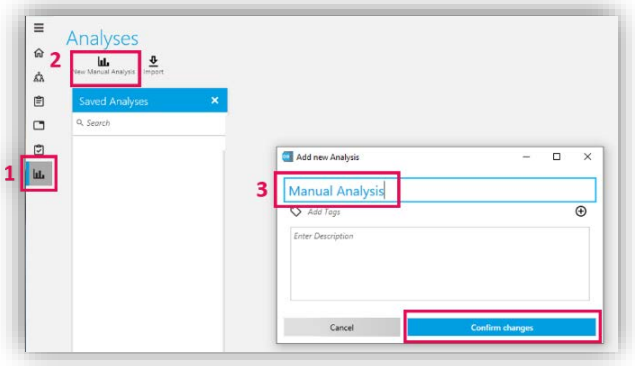
For a manual data analysis, perform the following steps:

(1) Open **Analysis** by clicking the icon as shown.

(2) Select **New Manual Analysis**.

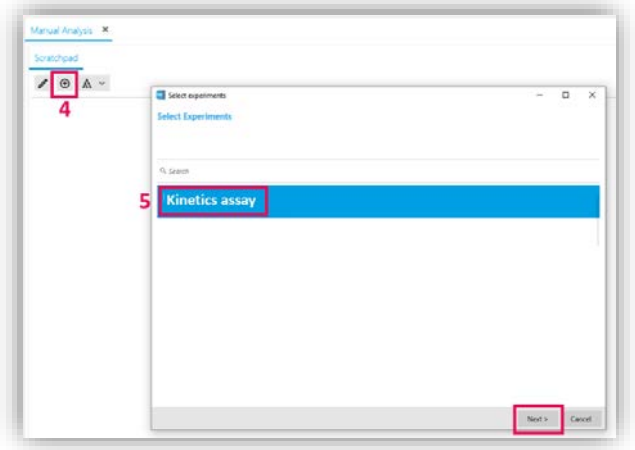
(3) Rename the analysis (here: "Manual Analysis") and **Confirm Changes**.

Tip: Make sure the analysis has a descriptive name to be able to find it again at a later stage.



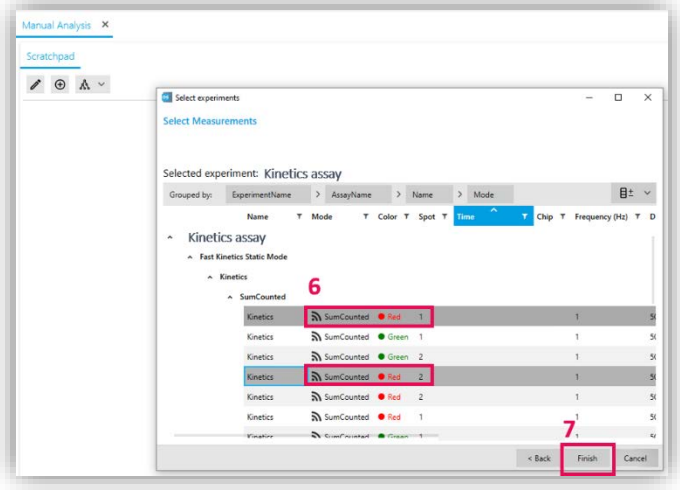
(4) Add a new manual analysis by clicking the "**+**" icon.

(5) Select your assay (here: "Kinetics Assay") and click **Next**.



(6) Individual data files of the selected measurement are listed and grouped into individual measurement blocks. Select the data you want to analyze. For each measurement trace, four data sets are listed (red and green signal readout for each of the two measurement spots).

Tip: Data file names do not necessarily contain the sample name or concentration but are listed as "SumCounted" in the case of a static mode kinetics assay. However, data sets are sorted by time from top to bottom. Hence, the correct set of data can be selected depending on the order of measurement (as set in the assay set-up). In the shown example, the red signal traces of **Spot 1** and **Spot 2** are selected for the first measurement.



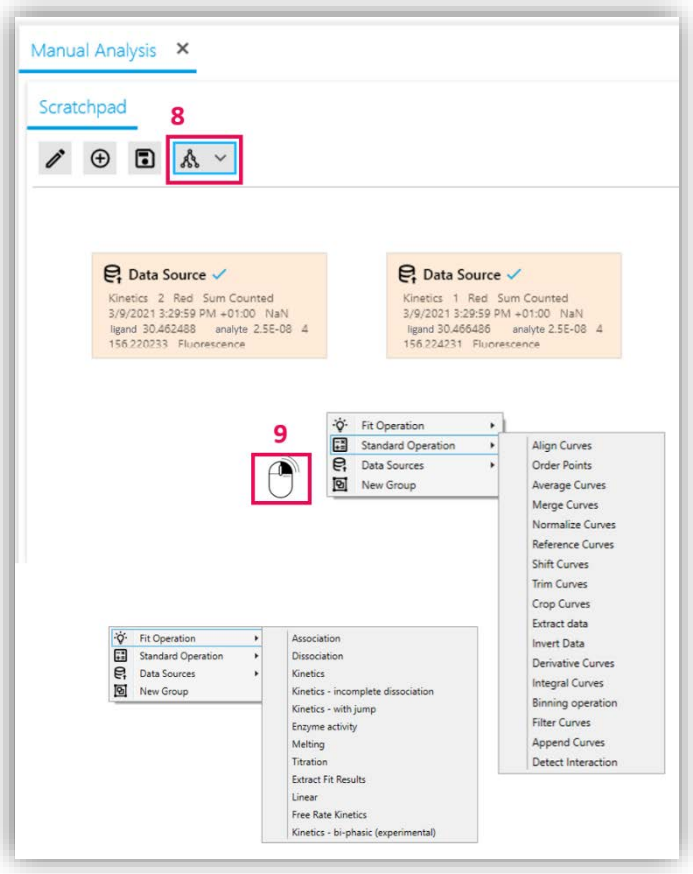
Tip: Multiple data traces can be selected by pressing the "shift" key during selection.

(7) Once all relevant data traces are selected, click **Finish**.

(8) Individual data files are depicted as yellow boxes containing details on ligand and analyte name as well as analyte concentration. Click on the **Layout Graph** icon as shown and pick any layout to view all individual data files.

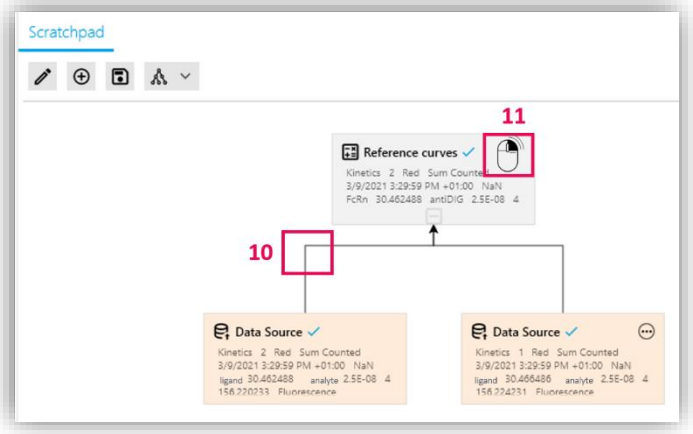
(9) Right-click anywhere within the scratchpad to open possible operations. Select the operations you would like to perform to treat the data. Commonly used standard operations are **Normalize Curves** and **Reference Curves**. Commonly used fit operations are **Kinetics** or individual **Association** or **Dissociation**.

Tip: Perform a standard operation first (e.g. normalization) followed by a fit operation.



(10) Once an operation is selected, it appears as a grey box in the scratchpad. Connect the yellow data file boxes to the grey operation box as shown here.

(11) Right-click within the grey operation box and select **create plot** to visualize the result of the operation. This step can be performed for any operation accordingly.



6 Software Installation Instructions

In order to receive the newest features and bug fixes, please always use the newest software version. The firmware of your **heliX®** device must always be up to date for a maximum level of functionality and compatibility with **heliOS**. We recommend to first update your **heliX®** device followed by your **heliOS** version.

6.1 heliX® Firmware Update

(1) Download the newest version of the **heliX®** firmware from *www.dynamic-biosensors.com/helios-download*.
(2) Ensure that the **heliX®** device is running, and install the **heliX®** firmware by running the installer (.exe file) on the measurement computer connected to it. After approx. 10 minutes the **heliX®** will be restarted and is ready to use.

6.2 Installation of heliOS

Please note: heliOS requires the **Microsoft .NET 6** Runtime on your operating system. Please go to *https://dotnet.microsoft.com/download/dotnet/current/runtime* and select one of the download options under **Run desktop apps**. If your PC is running on a 64 bit Windows system, select **Download x64**.

(1) Download the newest version of **heliOS** from *www.dynamic-biosensors.com/helios-download*.
(2) Run the **heliOS** installer (.msi file) and follow the instructions.
(3) The first time you open **heliOS** you need to enter your license key (Settings > License).
(4) Copy and paste the license key into the intended input field and confirm.

Now **heliOS** is ready to use and can be connected to your **heliX®** device.

Tip: The easiest way to **backup** all measurement data is to create a backup of the **PostgreSQL database** on your measurement PC.

7 heliX® Troubleshooting Guide

Table 5 | Guide for general device and handling issues.

Issue	Solution
A control connection to the device cannot be established.	Close **heliOS** and switch off the device. Restart the device and wait 5 min until the system has booted. Open **heliOS** and try reconnecting.
A measurement is stopped due to an error.	Check the error message icon for more information and hints what caused the issue. If the error cannot be resolved, please contact the support. (support@dynamic-biosensors.com)
Device is out of sync.	Close and open the device in the device view of **heliOS**.
heliOS tells me that I am already in control of the instrument, but this is not correct.	Close **heliOS** and open the task manager of your PC. Close all **heliOS** background instances. Open **heliOS** and try reconnecting.
A banner appears on the top of the software stating "Device connection is lost. Changes are not saved".	Please restart the **heliOS** software.
Chip/autosampler tray cannot be inserted	Check if trays are pushed correctly into the device. Eject the respective tray in **heliOS**. Once ejected, push the tray around 2 cm into the designated compartment. Try inserting the tray in **heliOS** again.
Chip/autosampler tray cannot be ejected	Make sure you are connected to the device and in control. 1. Check if the command was properly sent and the device tried to eject the tray/s. Repeat the ejection once more. 2. If the error remains, please restart the device by pressing the power button to turn off the device, wait for around 10 seconds and press the power button again to start the device.
What defines a good chip status test?	A functional chip has inflection points in the range of 0 – 450 mV and relative amplitudes of > 40 %.
When should I change my biochip?	When chip parameters of a chip status test are not within the required range (see above), it is recommended to change the biochip.
How do I handle the biochip correctly?	- Handle the biochip with gloves only. - Dry the used flow channel (compressed air or nitrogen line). - When not in use, store dry in original plastic bag in the fridge with the channel openings facing the desiccant bag. - DNA nanolevers should be stored double-stranded without attached ligand.

Table 6 | Guide for Assay related questions.

Question	Solution
There are air bubble spikes visible during a measurement.	Check if all buffer bottles / sample vials contain enough fluid. Prime the instrument with your running buffer.
How can I use different buffer solutions in one assay workflow?	Specify the running buffer in the respective assay in your assay workflow (one running buffer type per assay). Maximum two different running buffers can be used. The software detects automatically if the buffer type has changed between two assays and includes a priming where it is necessary.
How long can the solutions: DI water/ passivation/ regeneration/ EDTA solution be stored in the device?	**DI Water** should be exchanged daily. **Passivation Solution** should be exchanged at least once per week. **Regeneration Solution** is stable up to 2-3 weeks if there is no turbidity or precipitation. Generally, all solutions should be examined before each measurement to check the remaining volume and to check for potential turbidity or precipitation. In such a case exchange the solution immediately.
How much running buffer is required for my assay?	If the software does not indicate the amount of required buffer, a good quick estimation is to calculate the amount of buffer required for dissociation measurements. (e.g. dissociation time: 60 min. Flow rate: 500 µL/min. → 30 mL running buffer is required for each measurement run). A minimum volume of 100 mL is required per buffer compartment.

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