FortéBio Bio-layer Interferometry Kinetic Analysis Tutorial

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Outline

- Introduction to Biolayer Interferometry (s. 3-12)
- Kinetic Analysis Basics (s. 13-18)
- Basic Kinetics Data Acquisition (s. 19-35)
- Data Analysis HT (s. 36-63)

ForteBio is a Market Leader in Label-Free Biomolecular Analysis

Octet & BLItz

Bio-Layer Interferometry (BLI)

Pioneer

SPR

Full life-cycle offering for biomolecular interaction analysis

- Label-free assays based on Bio-Layer Interferometry (BLI) and Surface Plasmon Resonance (SPR) platforms
- Instruments, consumables, software, post-sale services (one-on-one training)

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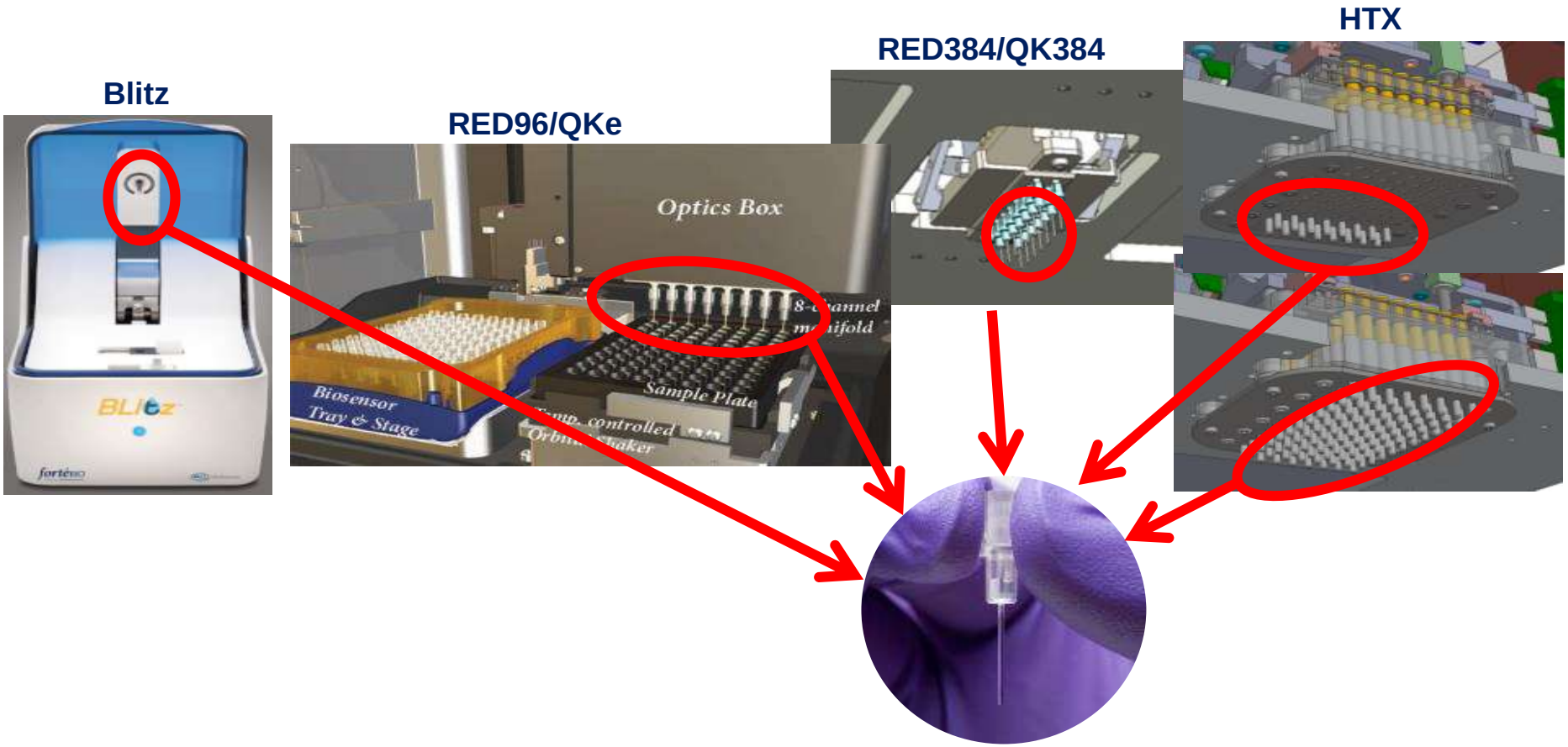
Octet Models





	Octet RED96e	Octet K2	Octet QKe	Octet RED384	Octet HTX
Molecular Weight Range	> 150 Da	> 150 Da	> 5000 Da	> 150 Da	> 150 Da
# Spectrometers	8	2	1	16	16
# Channels per Read	8	2	8	16	1 - 96
Microplate Positions	1	1	1	2	2
Biosensor Reracking	Yes	Yes	Yes	Yes	Yes
Robot Compatible	No	No	No	Yes	Yes
Sample Vessel Formats	96	96	96, 96 HA	96 / 96HA 384 / 384TW	96 / 96HA 384 / 384TW
Minimum Sample Volume	180 µL per well	180 µL per well	180 µL per well	40 µL per well	40 µL per well
Affinity range (approximate)	1 mM to 10 pM	1 mM to 10 pM	0.1 mM to 10 pM	1 mM to 10 pM	1 mM to 10 pM
Sample Usage	Non-destructive and recoverable				
Temperature Control	15 – 40 ° C	4° C above ambient to 40° C			
Analysis time per sample	Up to 12 hrs with evaporation cover	Up to 4 hrs			
21 CFR Part 11 Compliance	Available as option for all systems				

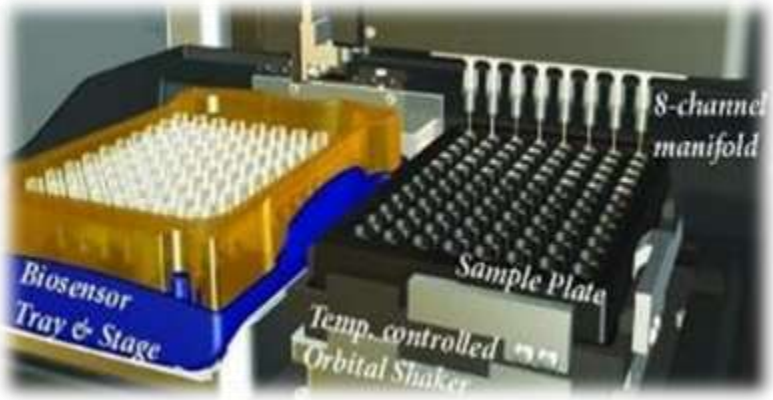
Biosensor-based Technology



Use of biosensors is core to BLI technology

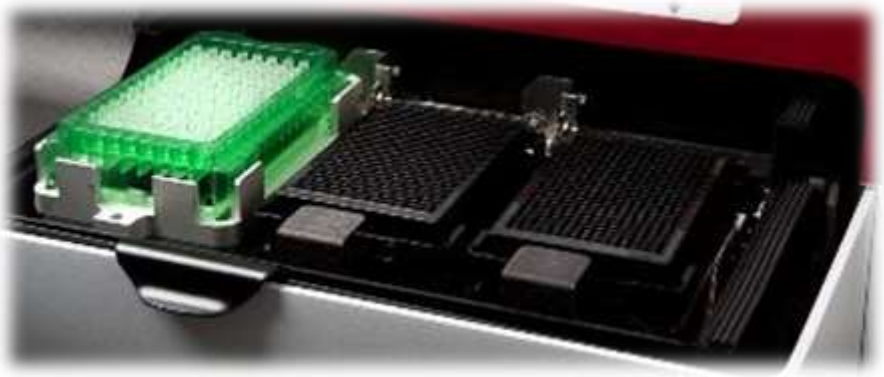
The Octet Design Features

Octet 96/QKe



- Optics box moves the biosensors to samples
- One biosensor tray
- One 96-well sample/reagent plate
- Upto 8 interactions simultaneously in one experiment for **Octet RED96e**

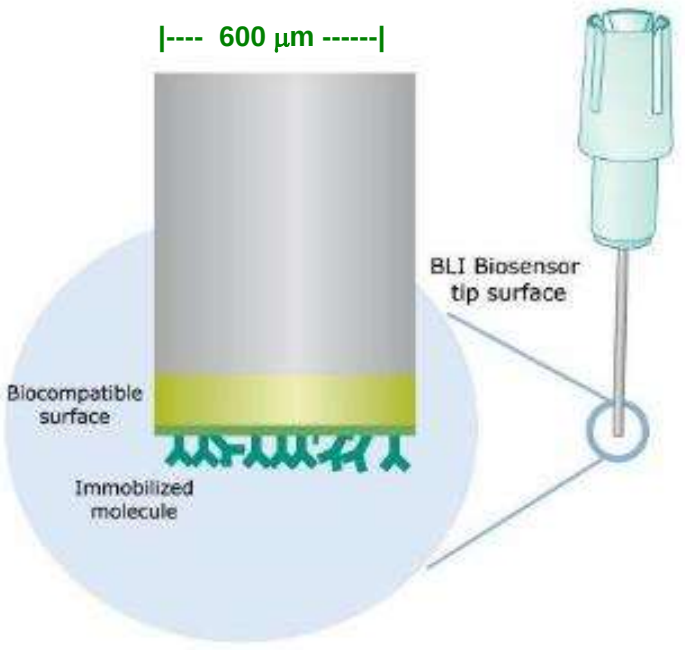
Octet 384/HTX



- Microplate format for samples allows for a large number of interactions to be studied in one experiment.
- Compatible with 2-96-well or 384-well sample plates.
- Upto 16 interactions simultaneously in one experiment for **Octet RED384**
- Upto 96 interactions simultaneously in one experiment for **Octet HTX**

Dip and Read™ Biosensors

The Octet Dip and Read™ Biosensor consists of a fiber optic embedded into a polypropylene hub with a sensor-specific chemistry at the tip



- *Two-dimensional binding surface*
- *Biocompatible Matrix (minimizes non-specific binding)*
- *Uniform*
- *Non-denaturing*

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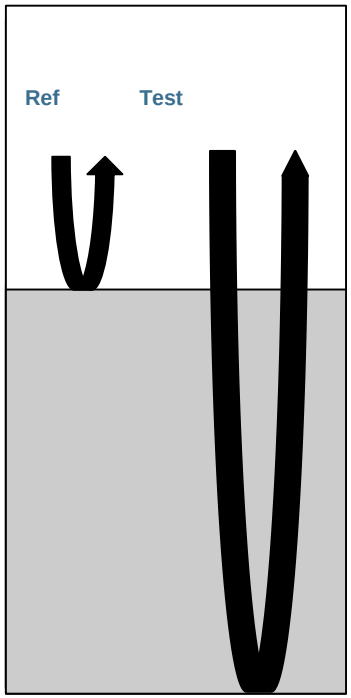
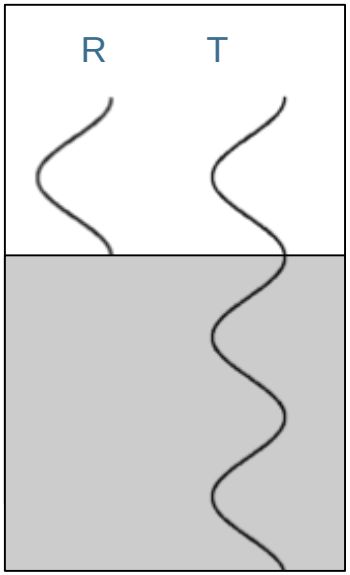
Bio-Layer Interferometry

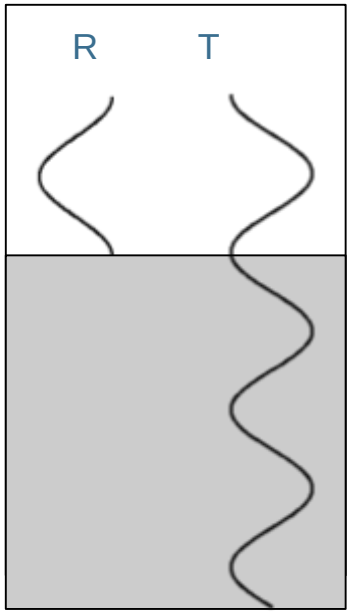
- In BLI, light is directed down an optical fiber (the sensor) toward two interfaces separated by a thin layer at the end of the fiber
- The two reflected beams interfere constructively or destructively at the spectrometer CCD detector array



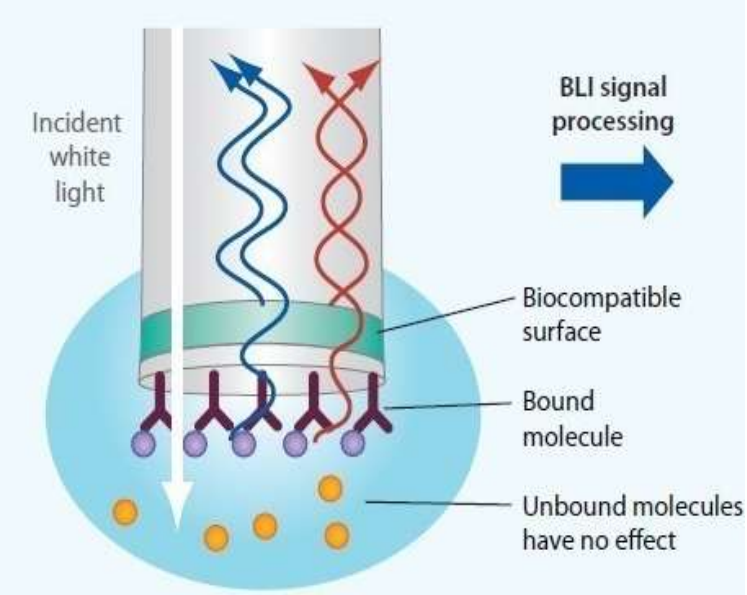
Reflections R & T are in phase
Constructive interference
Strong signal at the spectrometer

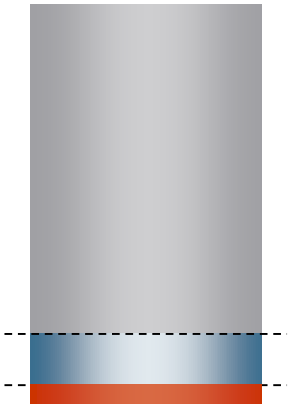


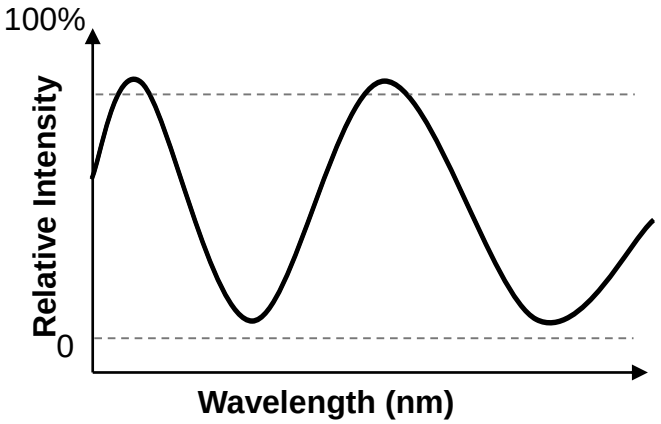
Reflections R & T out of phase
Destructive interference
Weak signal at the spectrometer

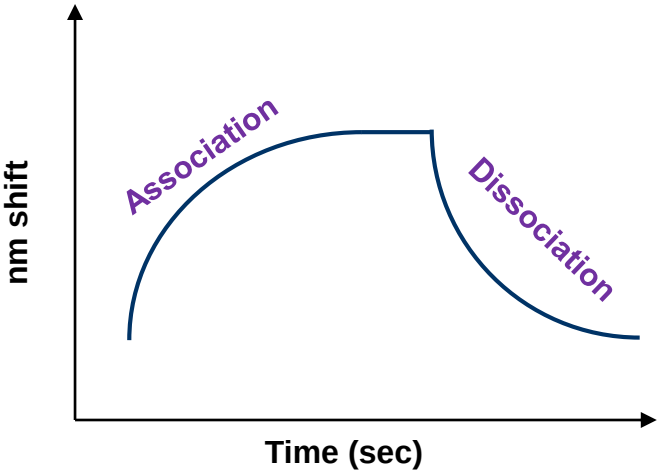


Monitoring nm-shift Against Time

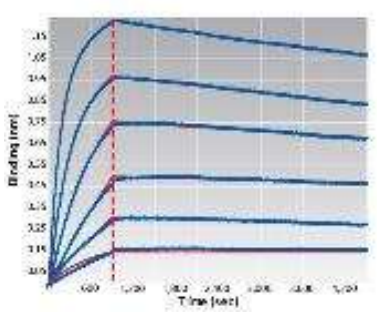






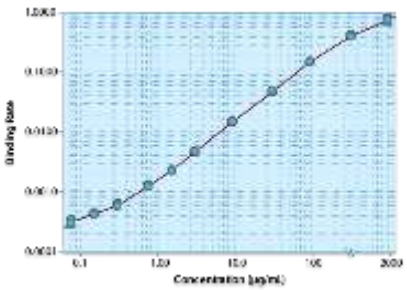


Versatile Applications On ForteBio Label-Free Systems



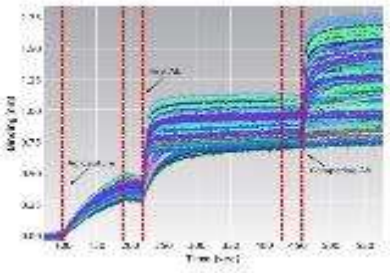
Kinetics

- k_a, k_d, K_D
- Proteins, Abs
- Peptdes, oligos
- Small molecules
- Fragments



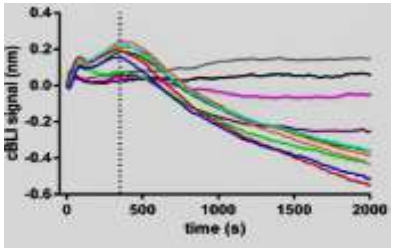
Quantitation

- Direct, 1-step
- Sandwich
- ELISA
- mg/mL – pg/mL



Diversity

- Function testing
- Epitope binning
- Rank ordering
- Isotyping



cBLI

- Cell capture based assays
- Dynamic Mass Redistribution (DMR)
- Toxicity assays

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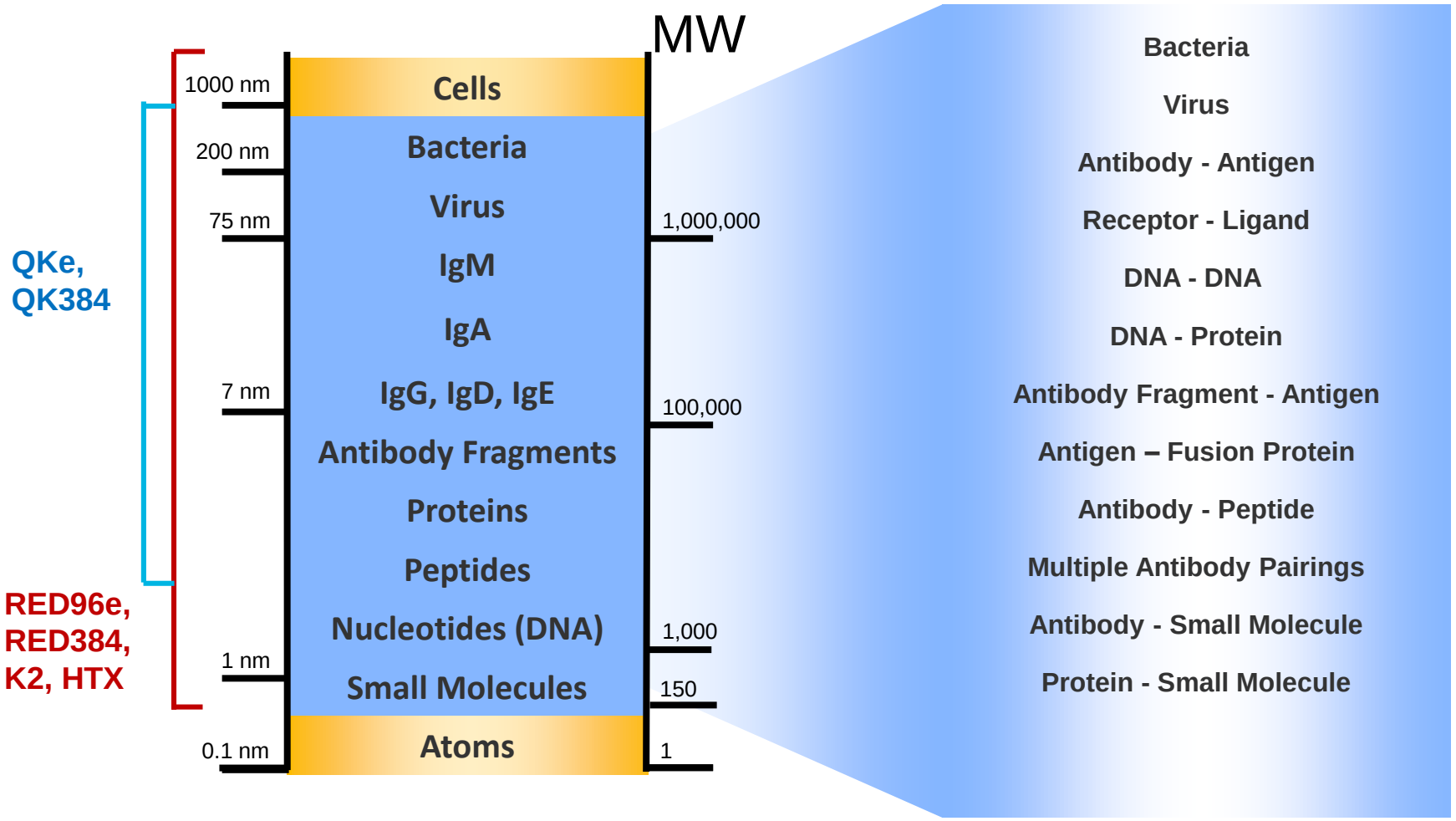
Size Range & Octet Versatility in Interaction Analysis



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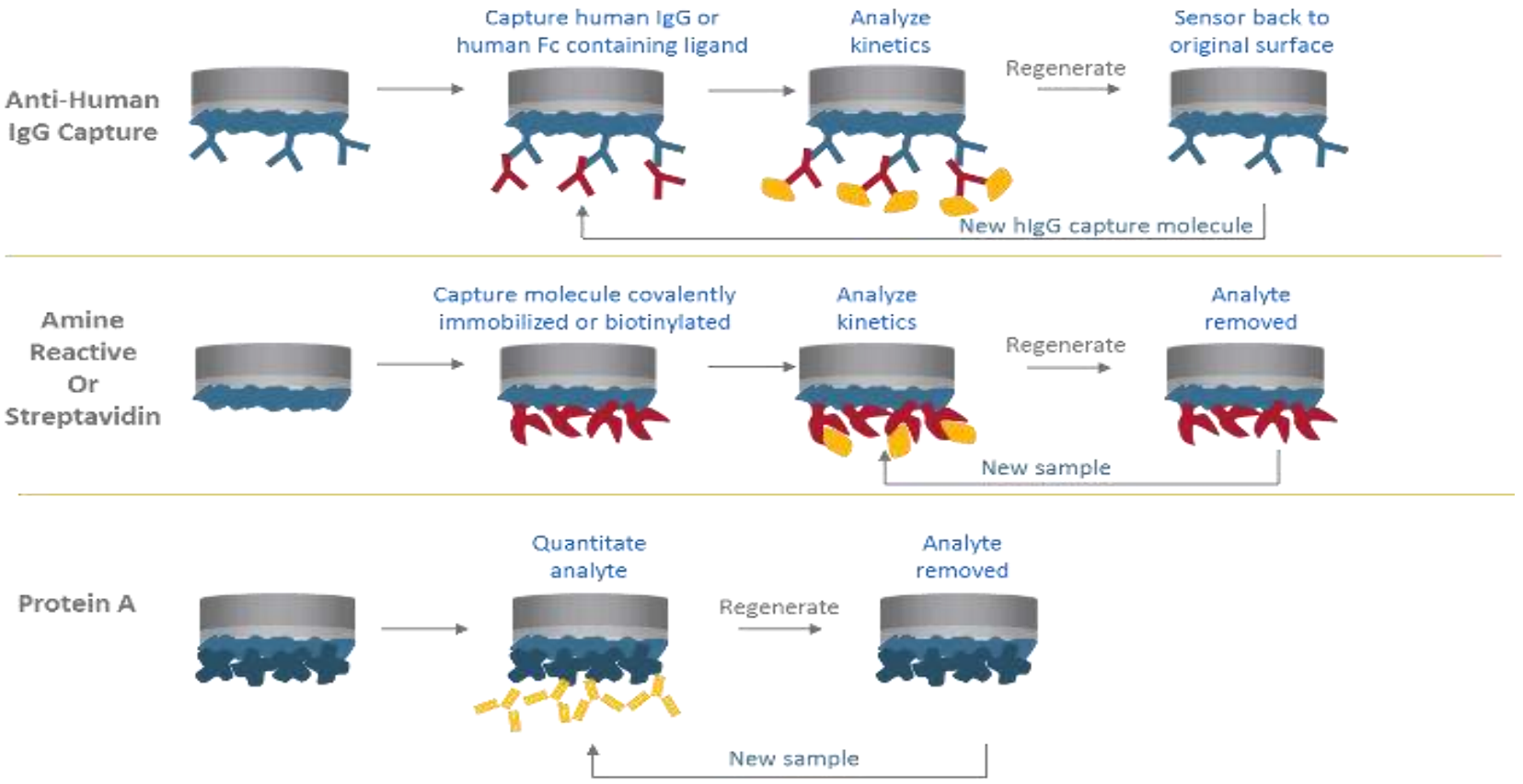
The Art of Biosensor Regeneration



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Kinetic Characterization on the Octet

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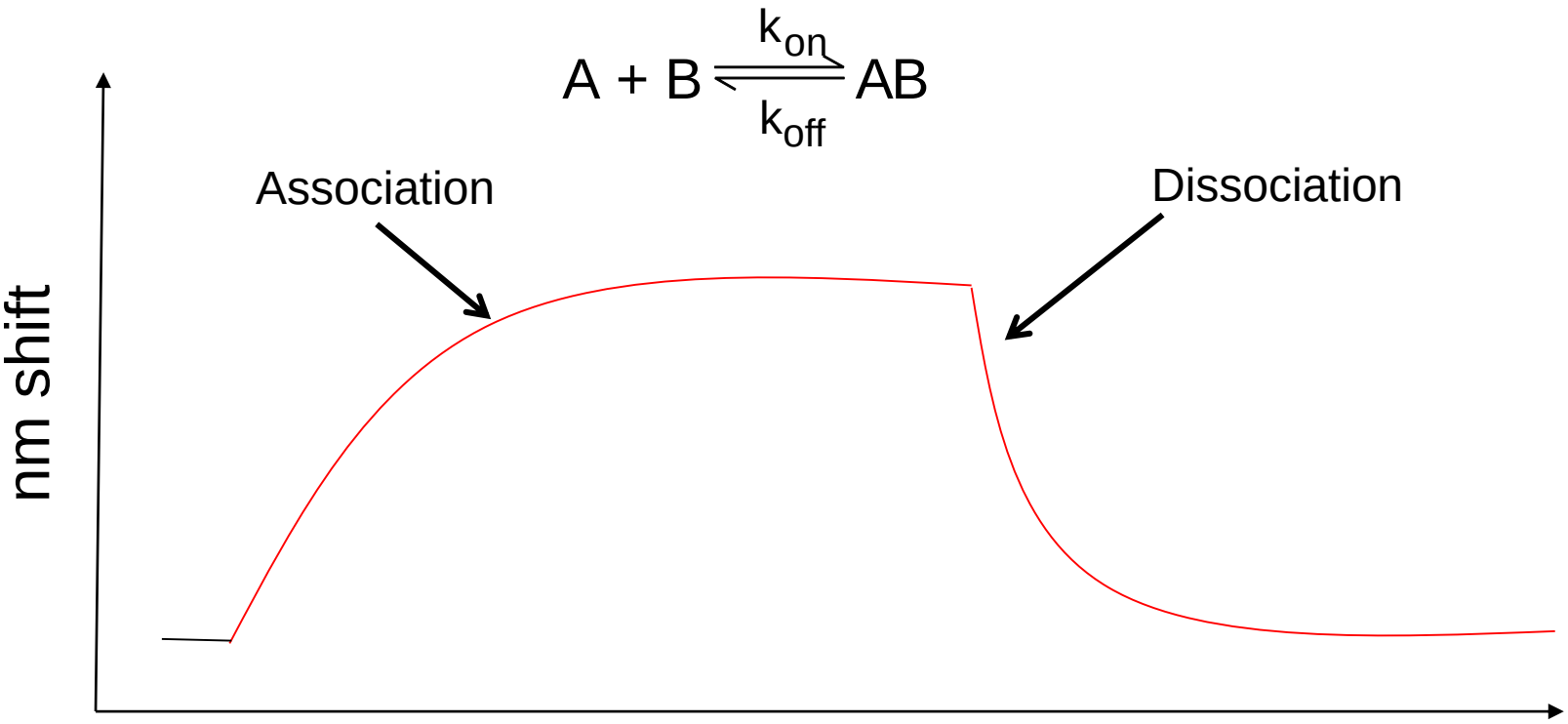
The Ideal Binding Behavior



In a simple 1:1 binding model, the association and dissociation phases are described by a single exponential function

Biosensors for Kinetic Analysis

Biosensor	Application
Antibody-Specific Capture	
• **Anti-Human IgG Fc Capture (AHC)**	**Human IgG Fc region, kinetic analysis**
• Anti-Human IgG Fc Capture (AHQ)	Human IgG Fc region, quantitation
• **Anti-Mouse Fc Capture (AMC)**	**Mouse IgG1, 2a & 2b Fc regions, kinetic analysis**
• Anti-Mouse Fc Capture (AMQ)	Mouse IgG1, 2a & 2b Fc regions, quantitation
• **Anti-Human Fab-CHI (FAB)**	**Fab-CH1 domains of human IgG**
• Protein A (ProA)	Quantitation of various species IgG
• Protein G (ProG)	Quantitation of various species IgG
• Protein L (ProL)	Quantitation of IgG via kappa light chain
Affinity Tag Capture	
• **Streptavidin (SA)**	**Biotinylated ligands**
• **High Precision Streptavidin (SAX)**	**Biotinylated ligands (4% CV loaded SA)**
• **Super Streptavidin (SSA**	**Biotinylated ligands (high-density surface)**
• **Anti-GST (GST)**	**GST-tagged recombinant proteins**
• **Anti-Penta HIS (HIS1)**	**HIS-tagged recombinant proteins**
• Anti-Penta HIS 2nd Gen (HIS2)	HIS-tagged recombinant proteins
• **Ni-NTA (NTA)**	**HIS-tagged recombinant proteins**
Immobilization	
• **Amine Reactive 2nd Gen (AR2G)**	**Covalent coupling to reactive amine groups**
• **Aminopropylsilane (APS)**	**Adsorption to hydrophobic moieties**

Kinetic Biosensors are Highlighted

Differences Between Kinetic and Quant Biosensors

Kinetics Biosensors

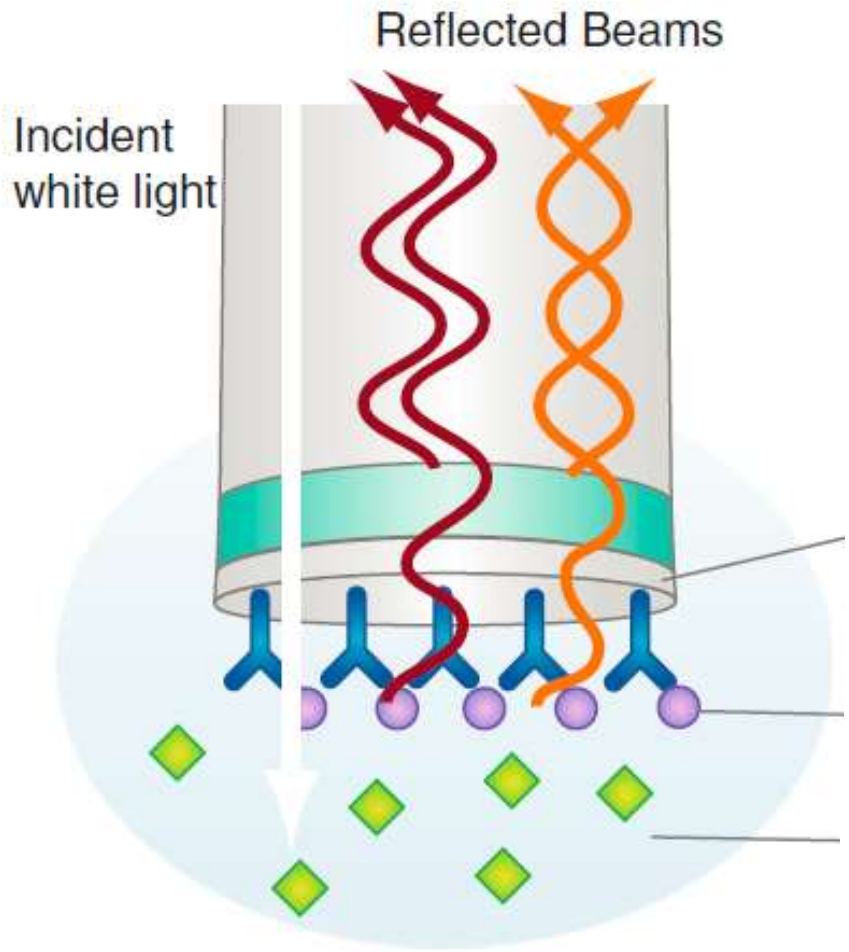
- Minimal Baseline Drift
- Higher Coefficient of Variation (CV)

Quant Biosensors

- Precise – CV must be within a certain range
- Not checked for baseline drift – Short assays, high signal

Different Manufacturing and QC Criteria

Label-free Terminology

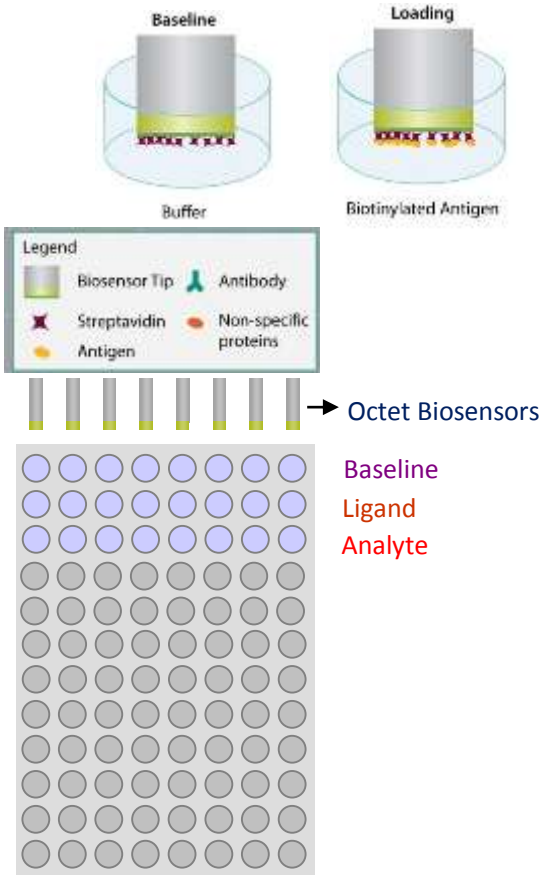


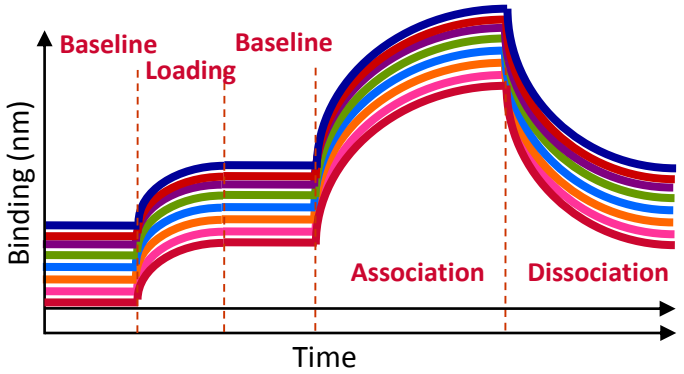
LIGAND - **Immobilized molecule on biocompatible surface (e.g. Biotin-tagged antibody)**

ANALYTE - **binding partner in buffer (e.g. protein, SM, DNA, etc.)**

SAMPLE WELL - **bound/unbound molecules in solution (96-/384-well plate)**

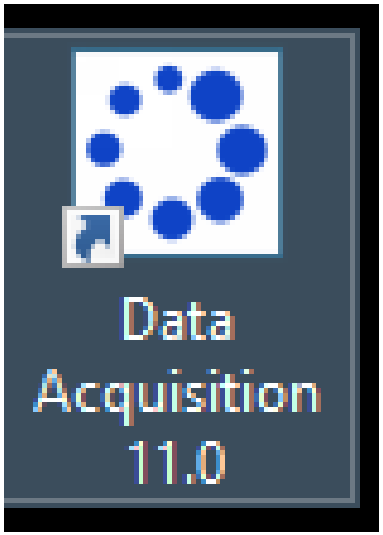
Automated Kinetic Work Flow on the Octet





- 1 to 96 samples can be analysed in parallel
- Measure on rates and off rates, multiple binding models
- Data is displayed in real-time
- Experimental protocols can be customized

Kinetic analysis Workflow with Data Acquisition V11

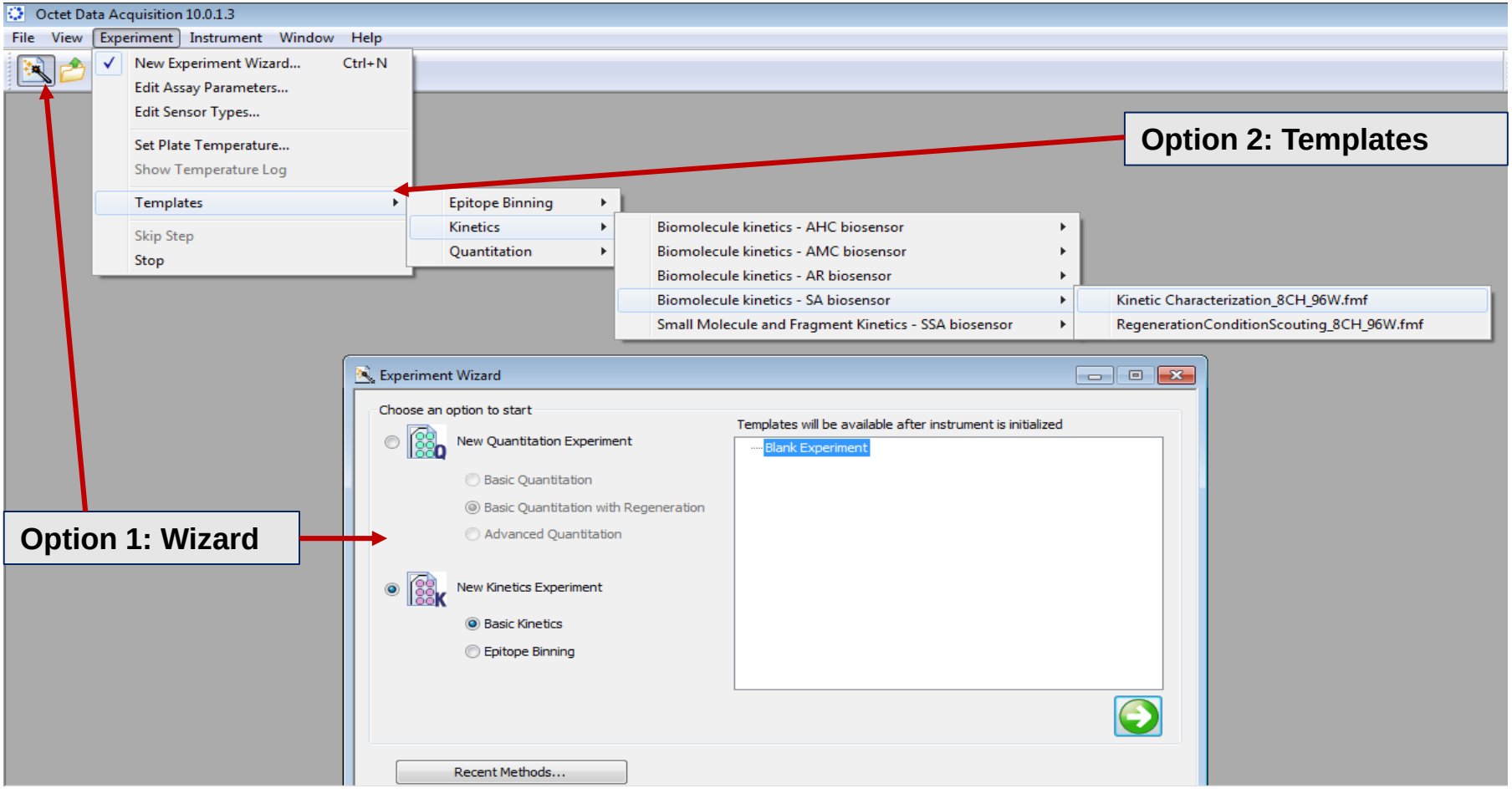


Double click the Data Acquisition 11.0 software icon

to start the program

Setting up Kinetics Assays

- Use either **Experiment Templates** or the **Experiment Wizard** for assay design and optimizations such as pH scouting, regeneration scouting, etc.



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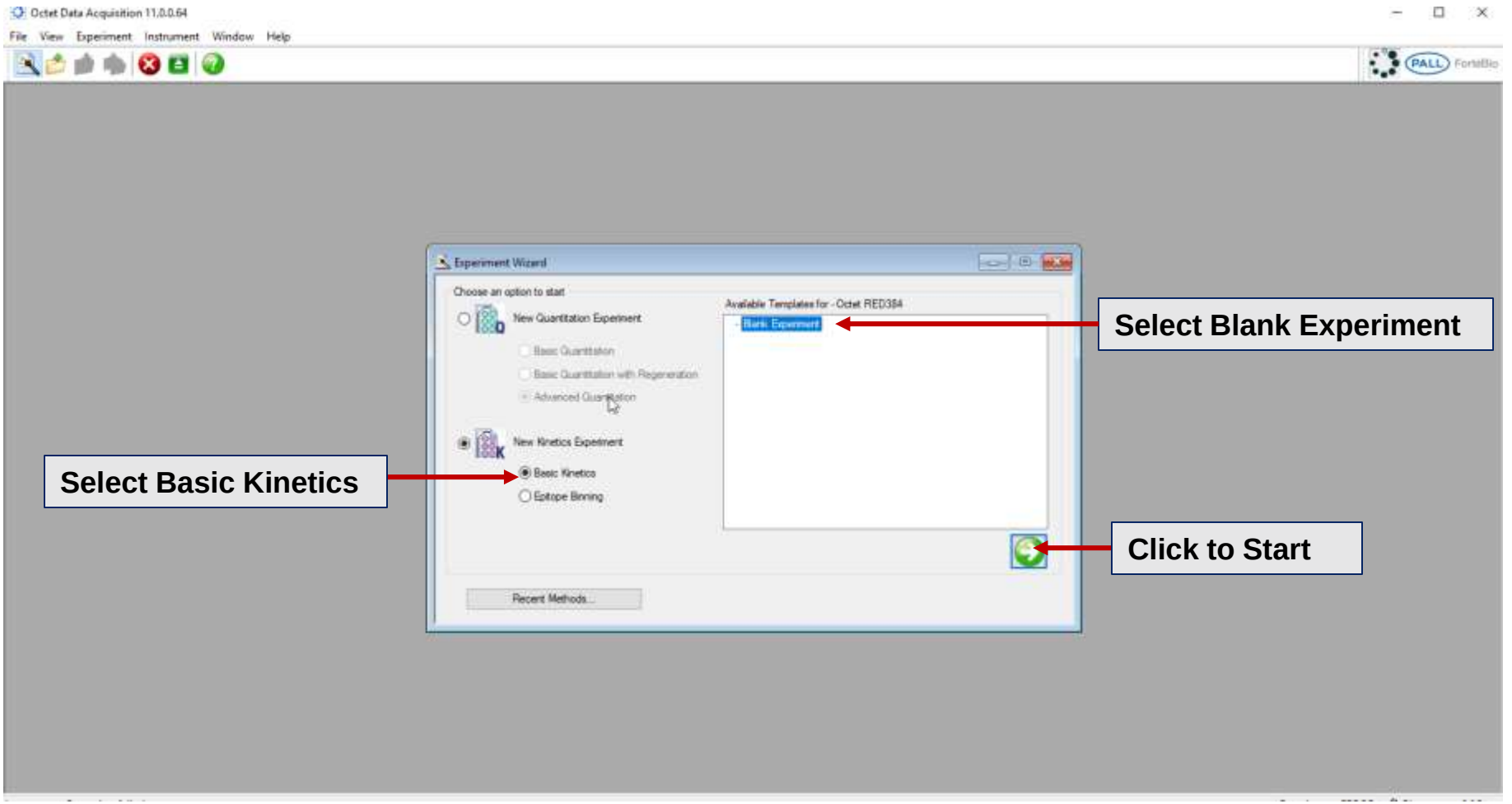
Setting up Kinetics Assays



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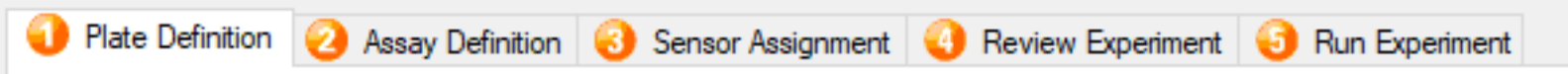
Acquisition Steps for Basic Kinetics

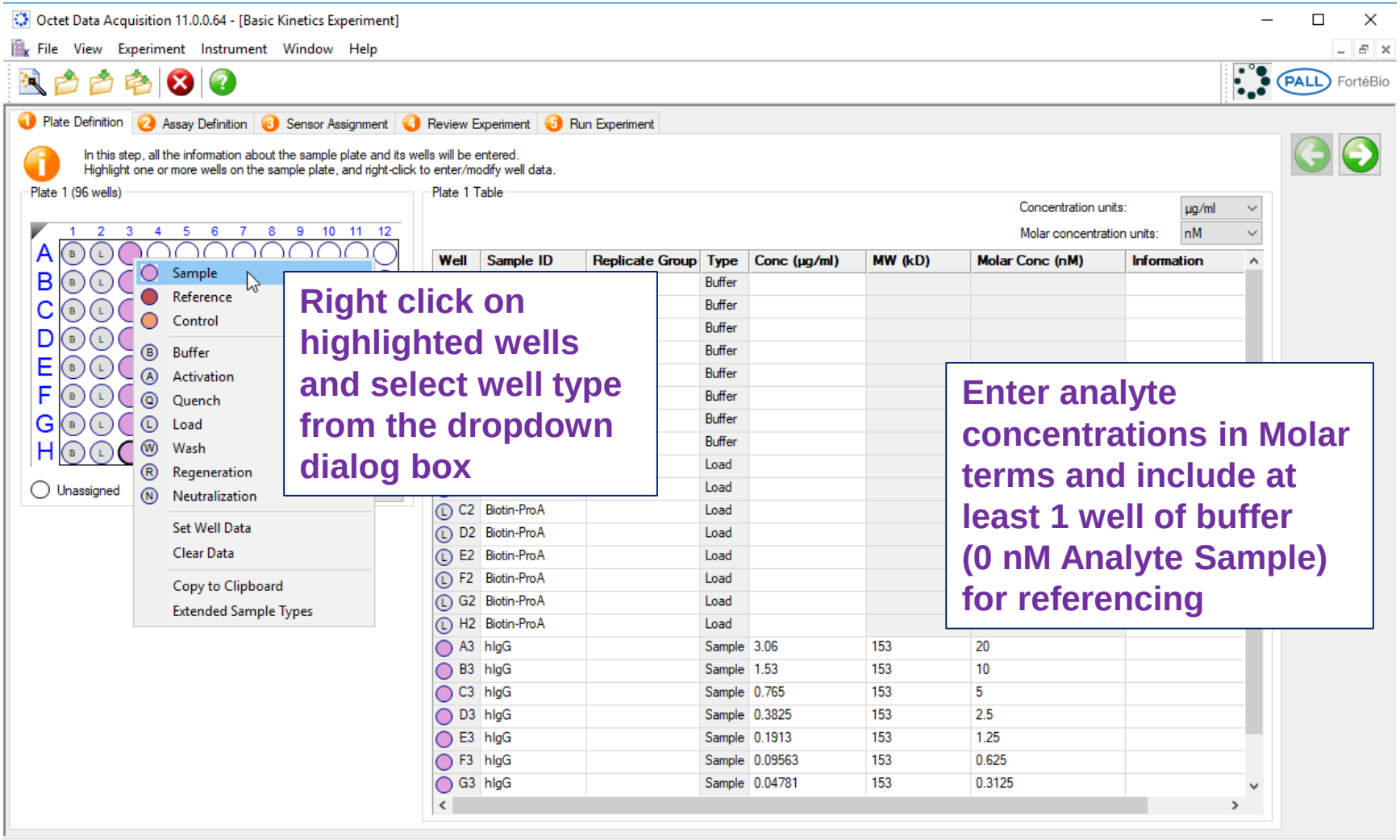
Work from left to right, from Tab 1 to Tab 5.



1. Plate Definition – In this tab, all the information about the sample plate and its wells will be entered
2. Assay Definition – In this tab, specific experimental steps are established
3. Sensor Assignment – In this tab, sensors are assigned to samples
4. Review Experiment – In this tab, you can review the steps that make up the experiment
5. Run Experiment – In this tab, you can select where you would like your data saved and name the data file. Run settings may also be changed

Setting up Kinetics Methods

Tab 1: Plate Definition



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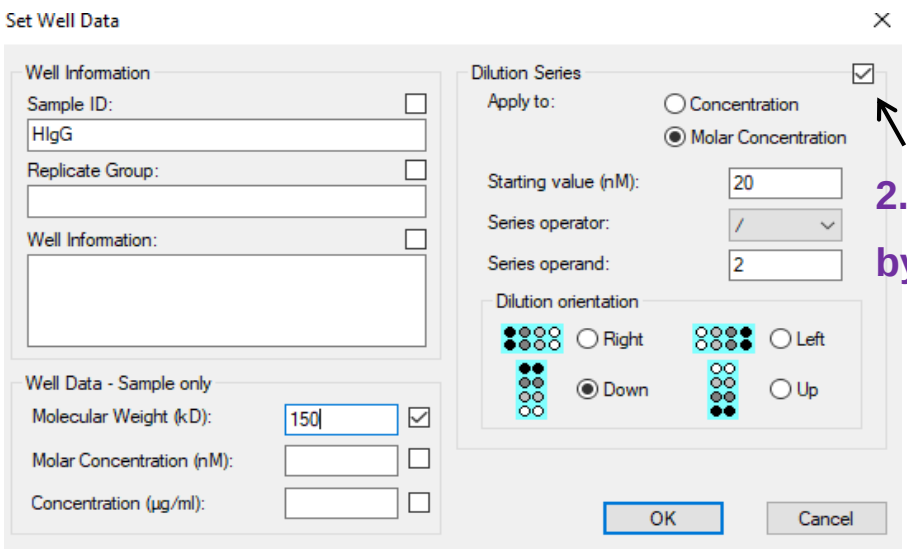
Tab 1: Plate Definition

1. To set up a dilution series, highlight the concentrations of interest, right click, and select Set Well Data

3. Select a starting value, series operator, series operand, and the dilution orientation, and click OK

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)
C1	1x KB		Buffer			
D1	1x KB		Buffer			
E1	1x KB		Buffer			
F1	1x KB		Buffer			
G1	1x KB		Buffer			
H1	1x KB		Buffer			
A2	BPA		Load	6.25		
B2	BPA		Load	6.25		
C2	BPA		Load	6.25		
D2	BPA		Load	6.25		
E2	BPA		Load	6.25		
F2	BPA		Load	6.25		
G2	BPA		Load	6.25		
H2	BPA		Load	6.25		
A3	HIgG		Sample	3	150	20
B3	HIgG		Sample	1.5	150	10
C3	HIgG		Sample	0.75	150	5
D3	HIgG		Sample	0.375	150	2.5
E3	HIgG		Sample	0.1875	150	1.25
F3	HIgG		Sample	0.09375	150	0.625
G3	HIgG		Sample	0.04688	150	0.3125
H3	HIgG		Referen	0	0	0



2. Check the box by dilution series

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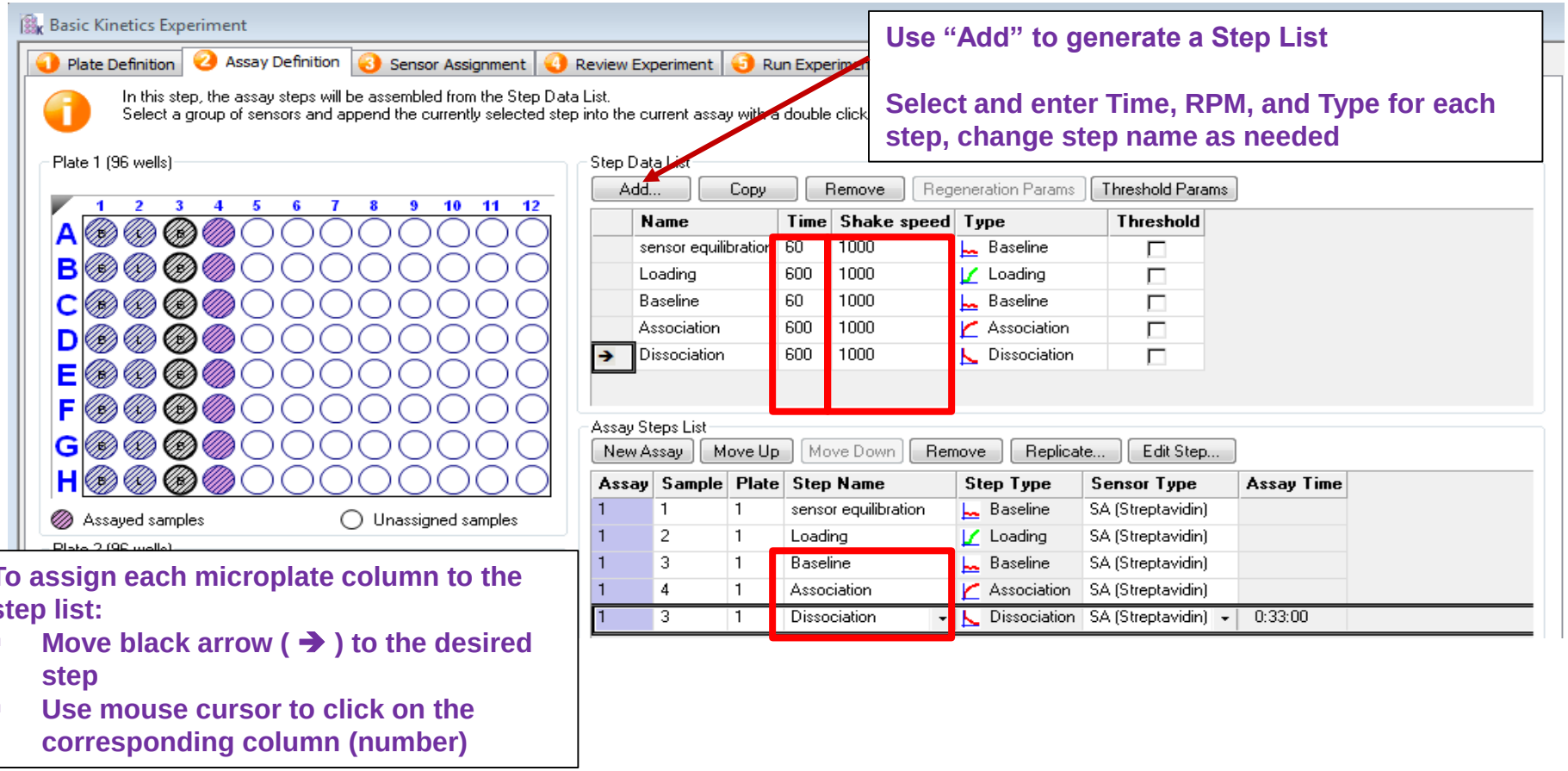
Setting up Kinetics Methods

Tab 2: Assay Definition



Use "Add" to generate a Step List

Select and enter Time, RPM, and Type for each step, change step name as needed

To assign each microplate column to the step list:

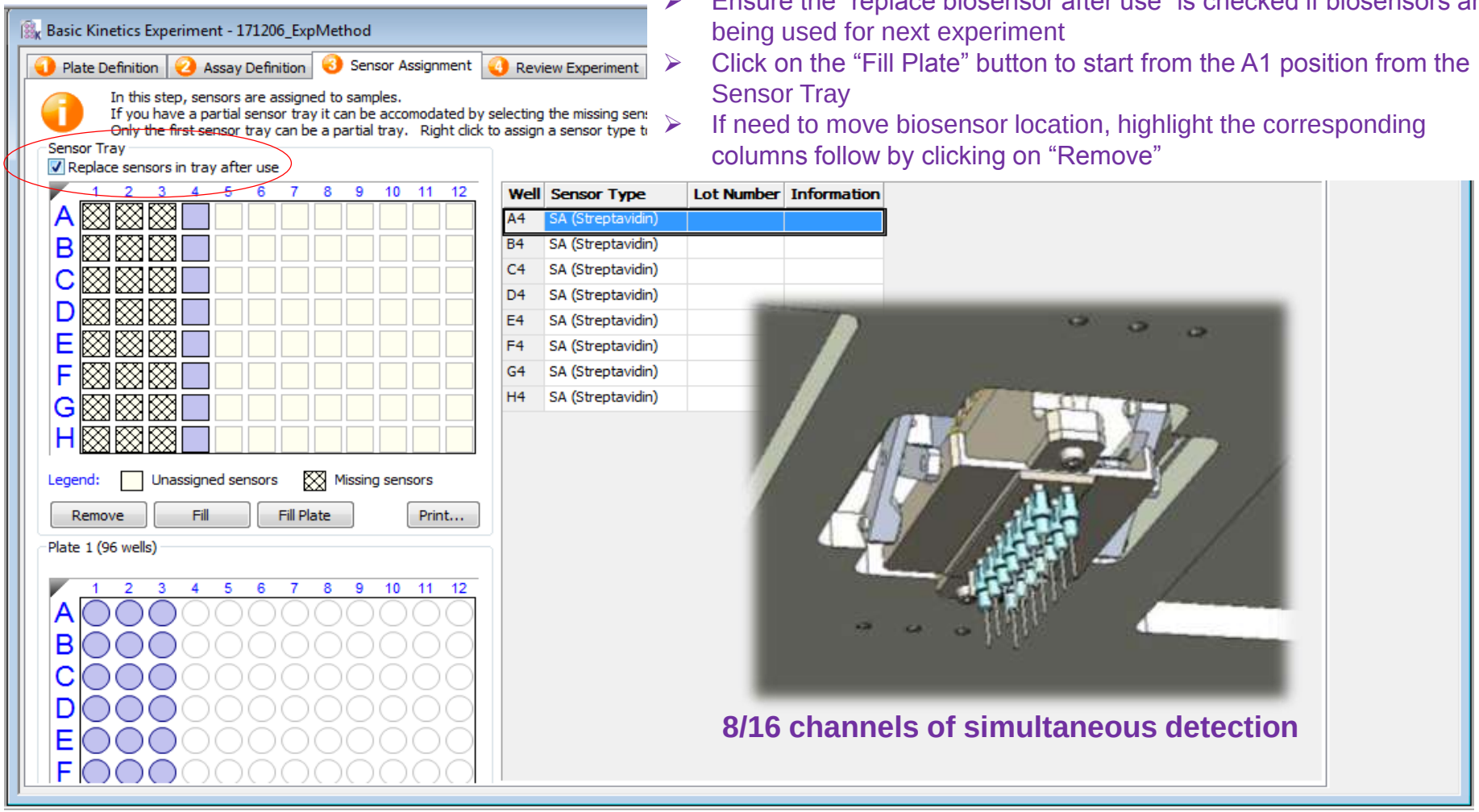
- **Move black arrow (➔) to the desired step**
- **Use mouse cursor to click on the corresponding column (number)**

- **Set Shake speed to 1000 rpm.**
- **Stacking the Baseline, Association, and Dissociation steps consecutively is important!**

Setting up Kinetics Methods

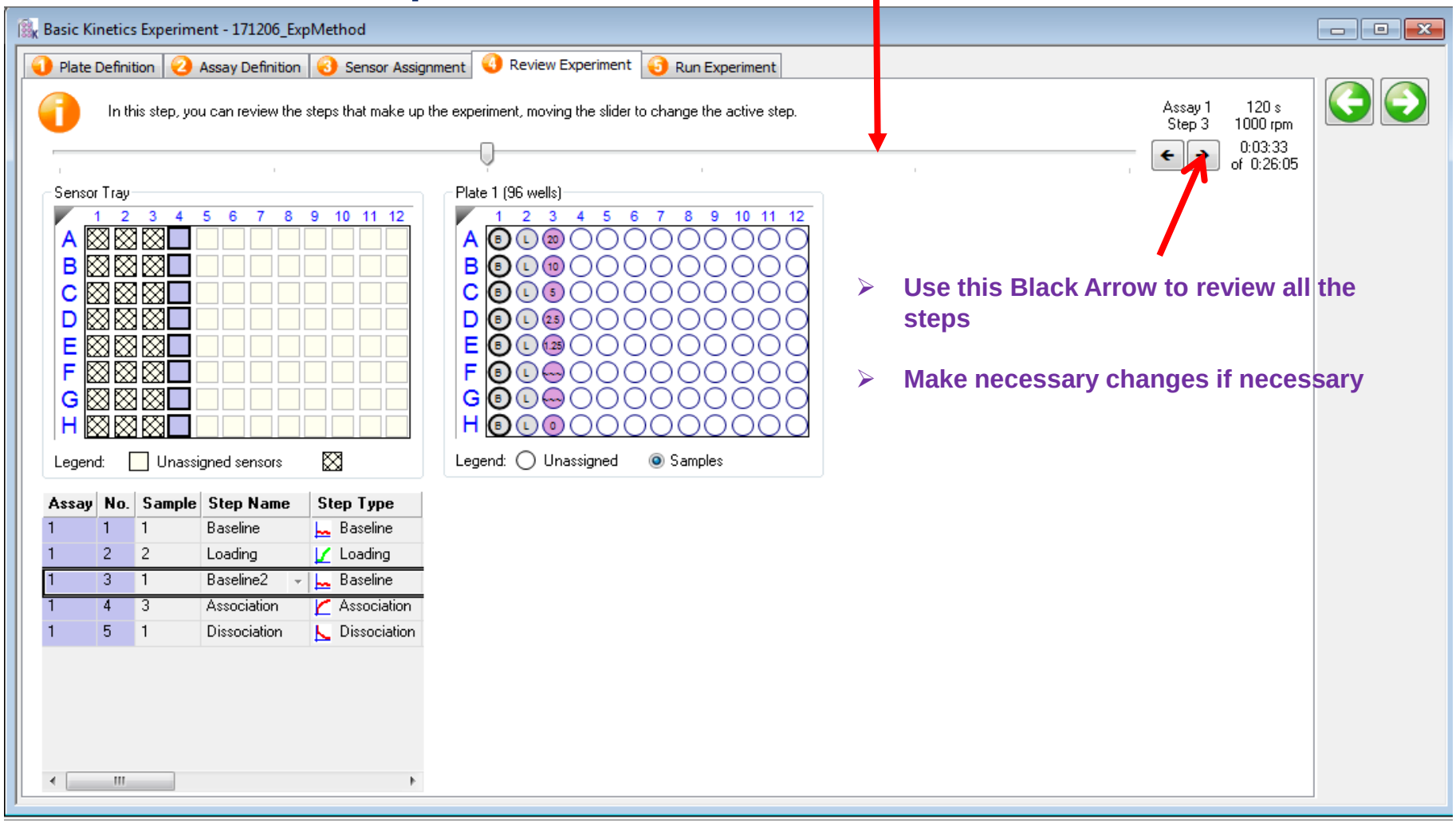
Tab 3: Sensor Assignment

- Always input biosensor lot # for troubleshoot if error happened
- Ensure the “replace biosensor after use” is checked if biosensors are being used for next experiment
- Click on the “Fill Plate” button to start from the A1 position from the Sensor Tray
- If need to move biosensor location, highlight the corresponding columns follow by clicking on “Remove”



8/16 channels of simultaneous detection

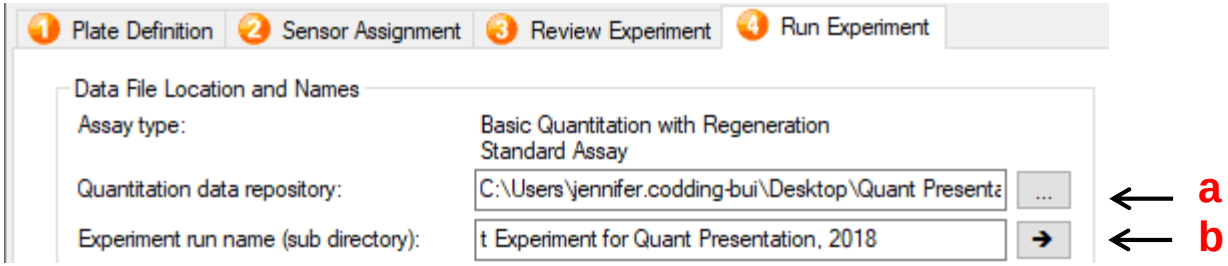
Tab 4: Review of Experiment



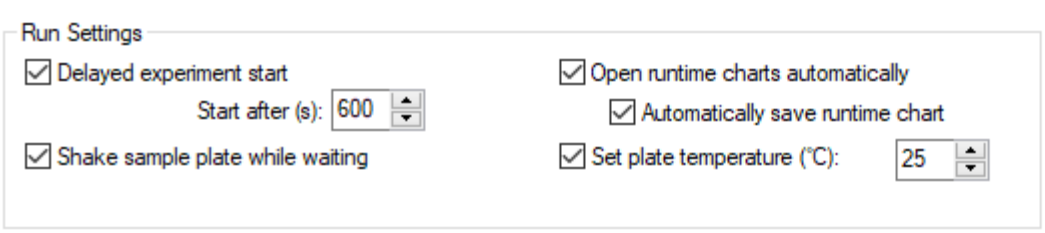
- **Use this Black Arrow to review all the steps**
- **Make necessary changes if necessary**

Tab 5: Run Experiment

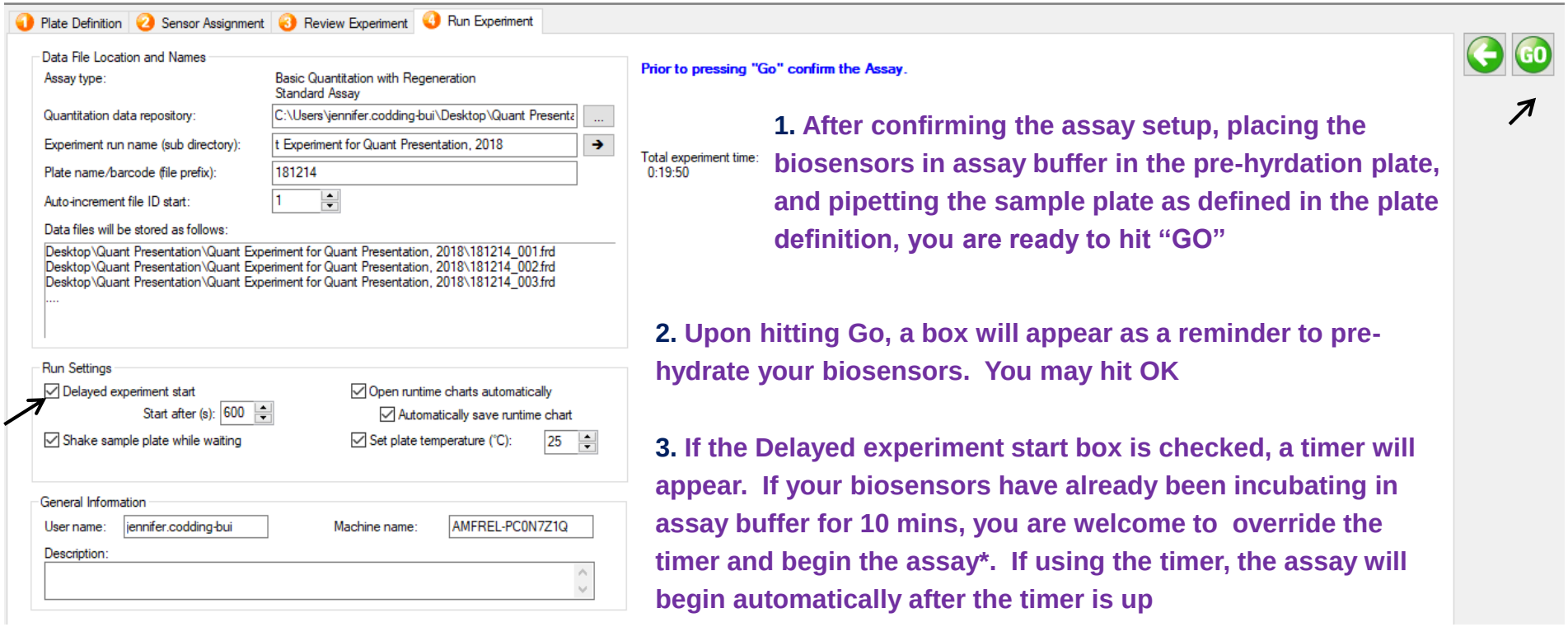
1. Saving an experiment is a two-step process. A. First, click on the box by with the three dots and select the folder where you would like to save the experiment. B. Then, name the experiment in the Experiment run name (sub directory).



2. Change settings of interest, including plate temperature. The Red96e has a temperature range between 15 to 40 °C. Other instruments have heating up to 40 °C but not cooling. For these instruments, the recommendation is to set the temperature from 2 °C above ambient up to 40 °C, to allow the instrument to consistently heat to the recommended temperature.



Tab 5: Run Experiment



1. After confirming the assay setup, placing the biosensors in assay buffer in the pre-hyrdation plate, and pipetting the sample plate as defined in the plate definition, you are ready to hit "GO"

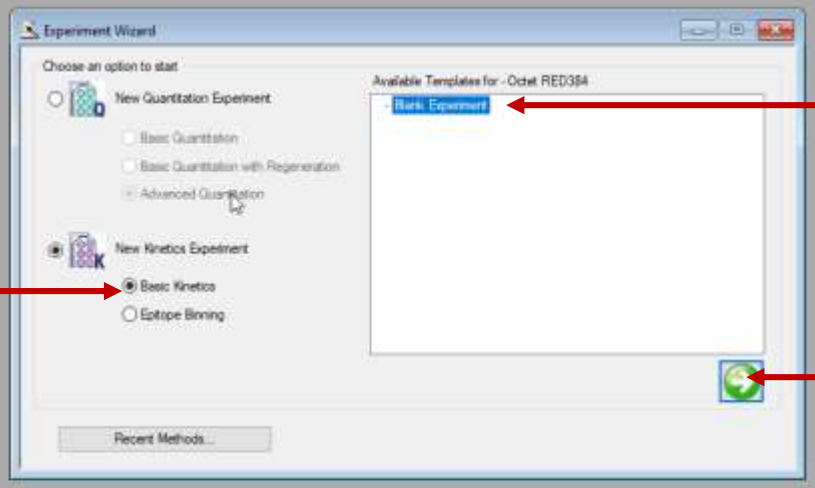
2. Upon hitting Go, a box will appear as a reminder to pre-hydrate your biosensors. You may hit OK

3. If the Delayed experiment start box is checked, a timer will appear. If your biosensors have already been incubating in assay buffer for 10 mins, you are welcome to override the timer and begin the assay*. If using the timer, the assay will begin automatically after the timer is up

***Even if the biosensors have already been properly hydrated at the start of the experiment, ForteBio recommends keeping this box checked and running the timer for 1-2 mins to allow your sample plate to obtain the desired temperature. Shaking the sample plate for a short period of time may also remove air bubbles from solution**

Setting up Kinetics Assays

For 384 and HTX models, click on the Present Button to open the door and the stage will come out.



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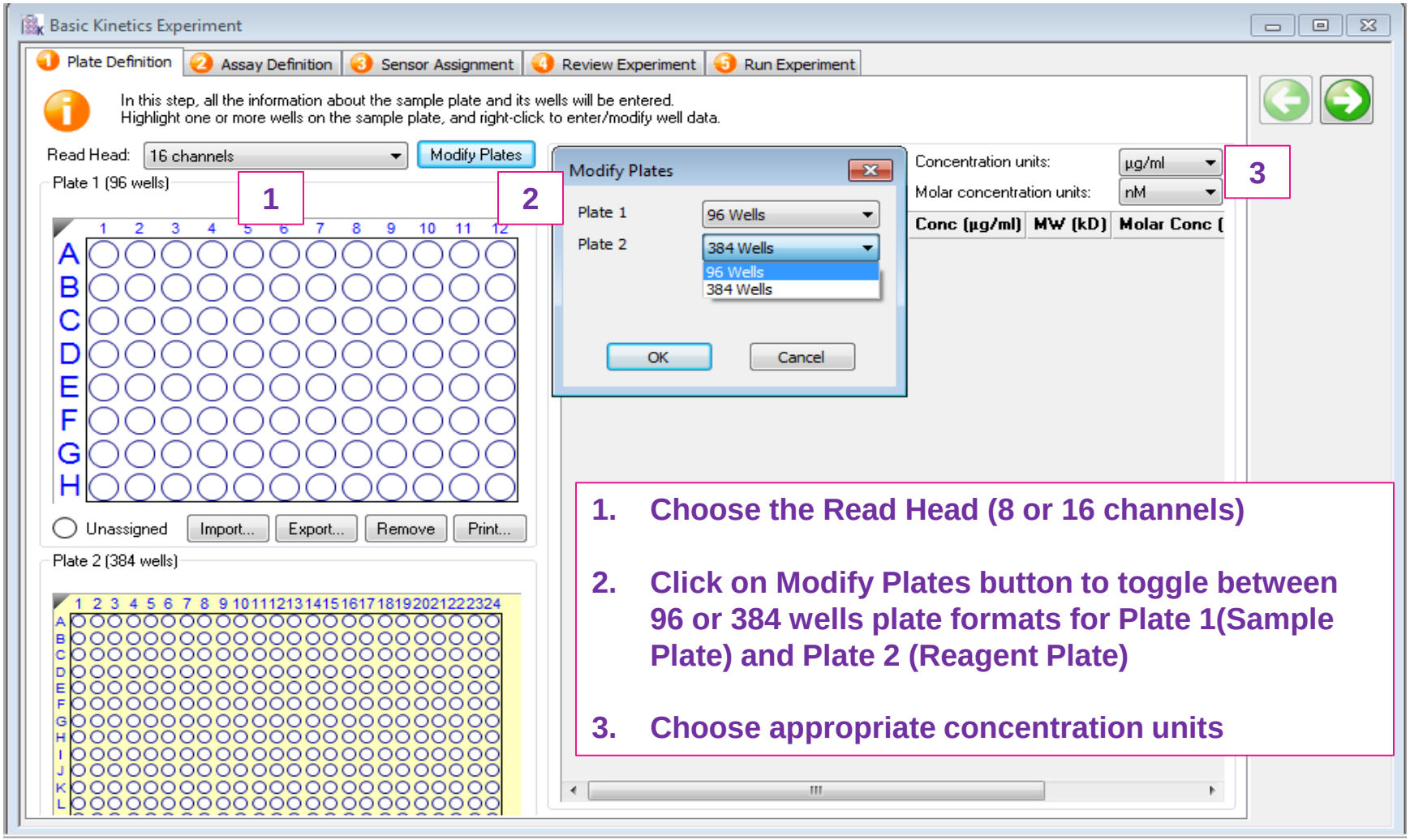
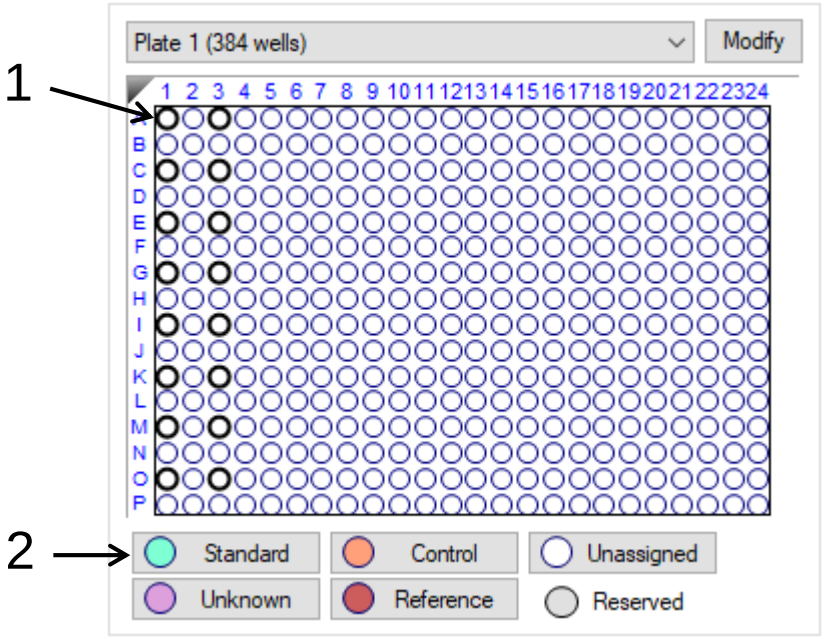
Setting up Kinetics Assays on the High throughput Octet Instruments



Tab 1. Plate Definition (384-well Plate)

1. Hold down the Shift key on the keyboard and click to the upper left most well of choice to highlight the number of “associated” wells
2. Right click on highlighted wells or select from options below plate to select well type



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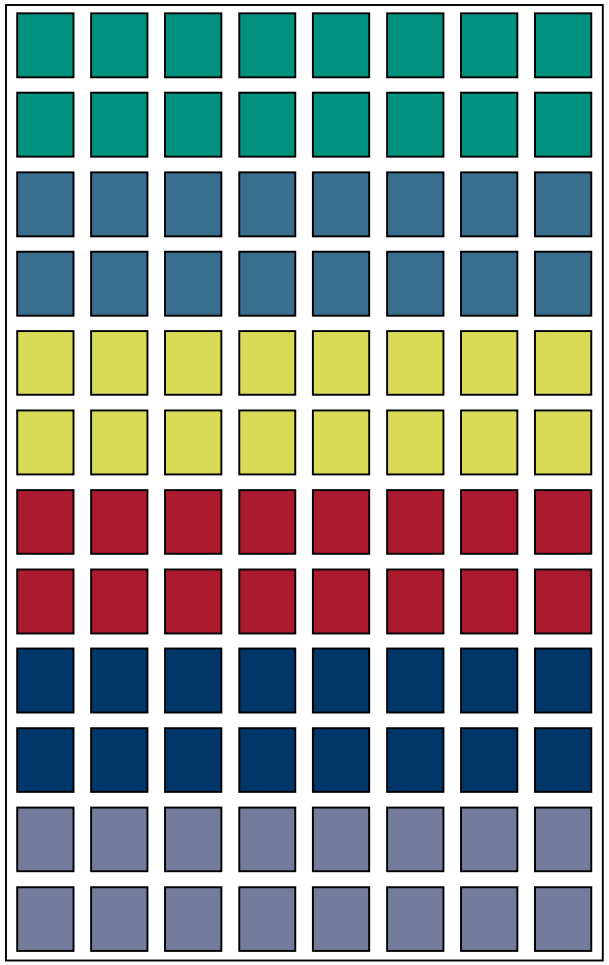
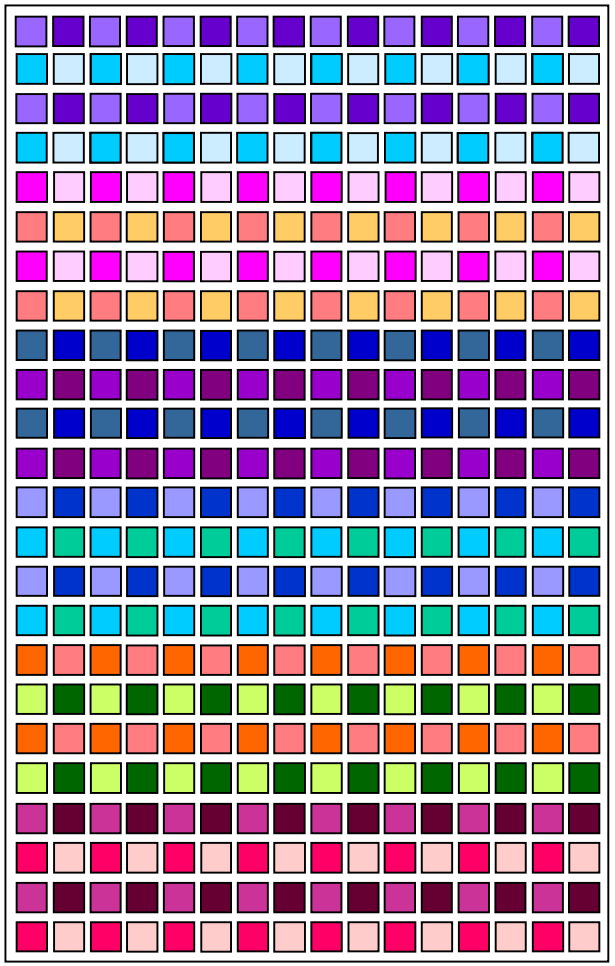
16-Channel Movement on Octet 384 Systems



96 Well Microplate



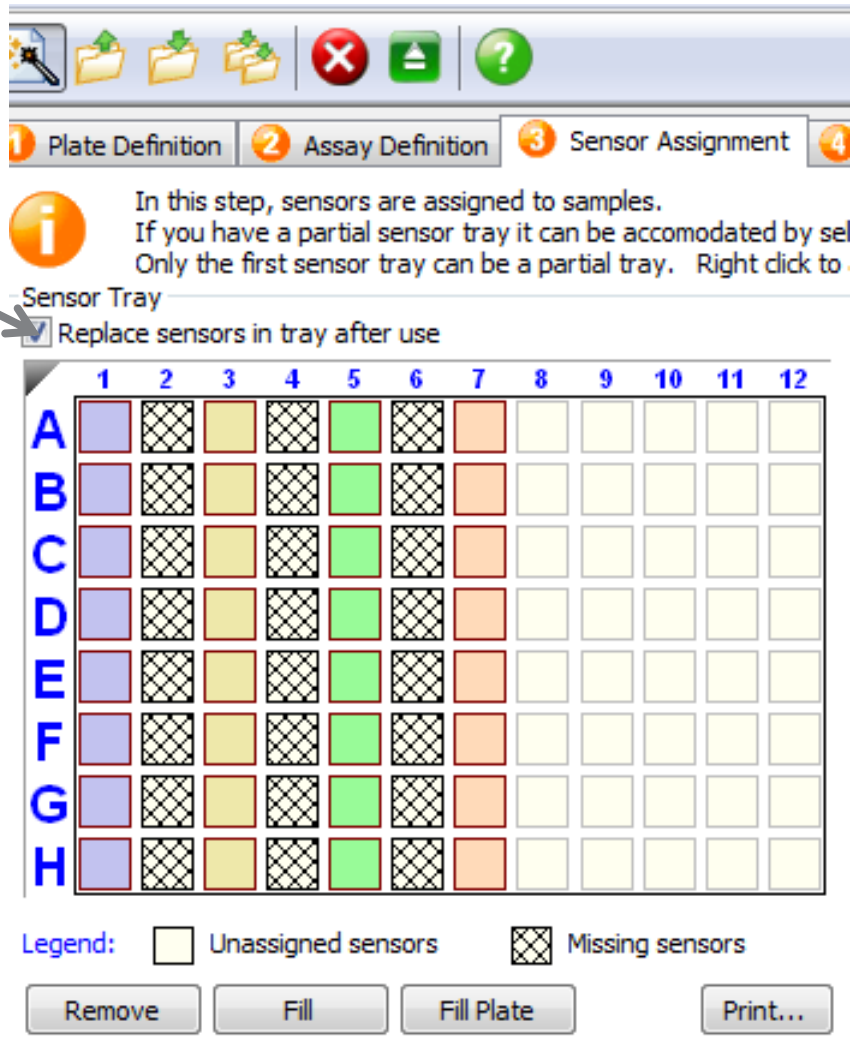
384 Well Microplate

8 Channel Assay “WITH” Re-racking

Important:

- **If the Replace Sensors In Tray After use is Checked, you will have to put biosensors in every other column**

Follow the diagram to load your biosensors



Biosensor Location #1 will be used in this mode with re-racking

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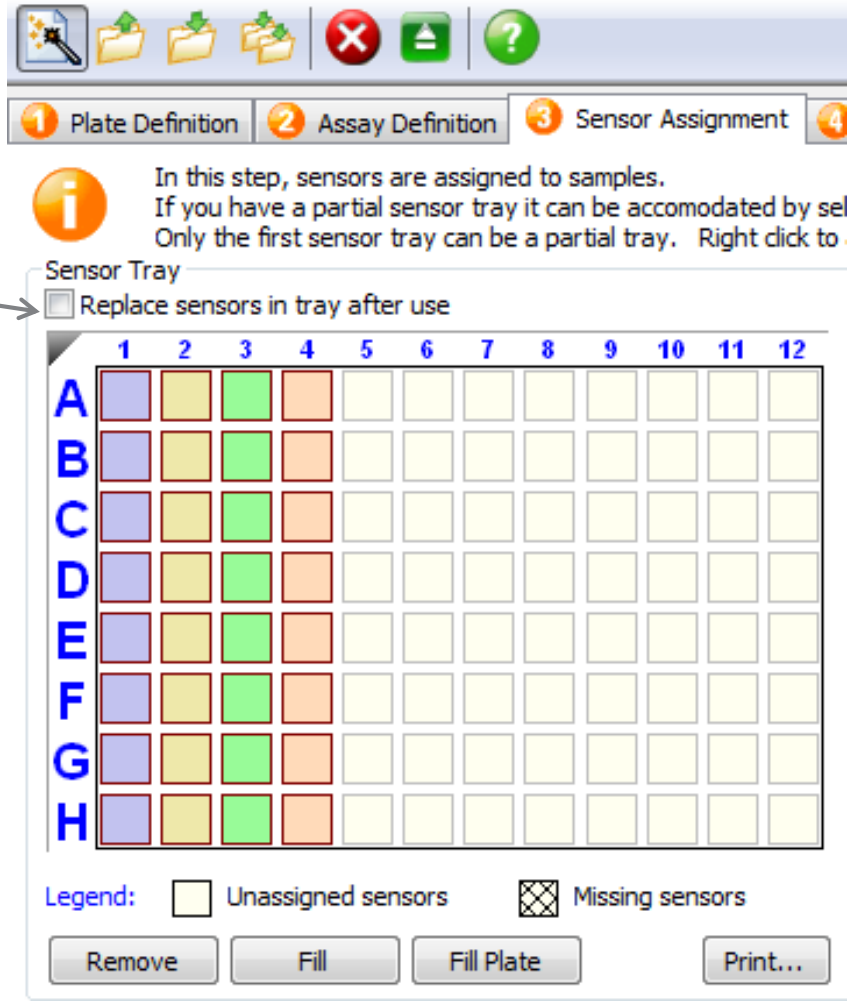
8 Channel Assay “WITHOUT” Re-racking

Important:

- **If you are running 4 different assays in 8 channels mode, then make sure you have the “Replace Sensors in Tray after use” is unchecked (biosensors will be discarded after use)**
- **Then, you can place biosensors in adjacent columns**

Biosensor Location #2 will be used in this mode without re-racking



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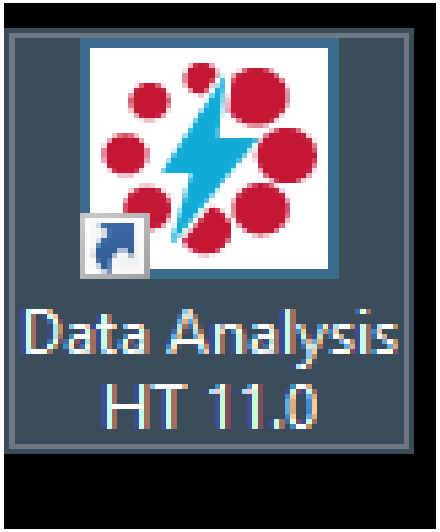
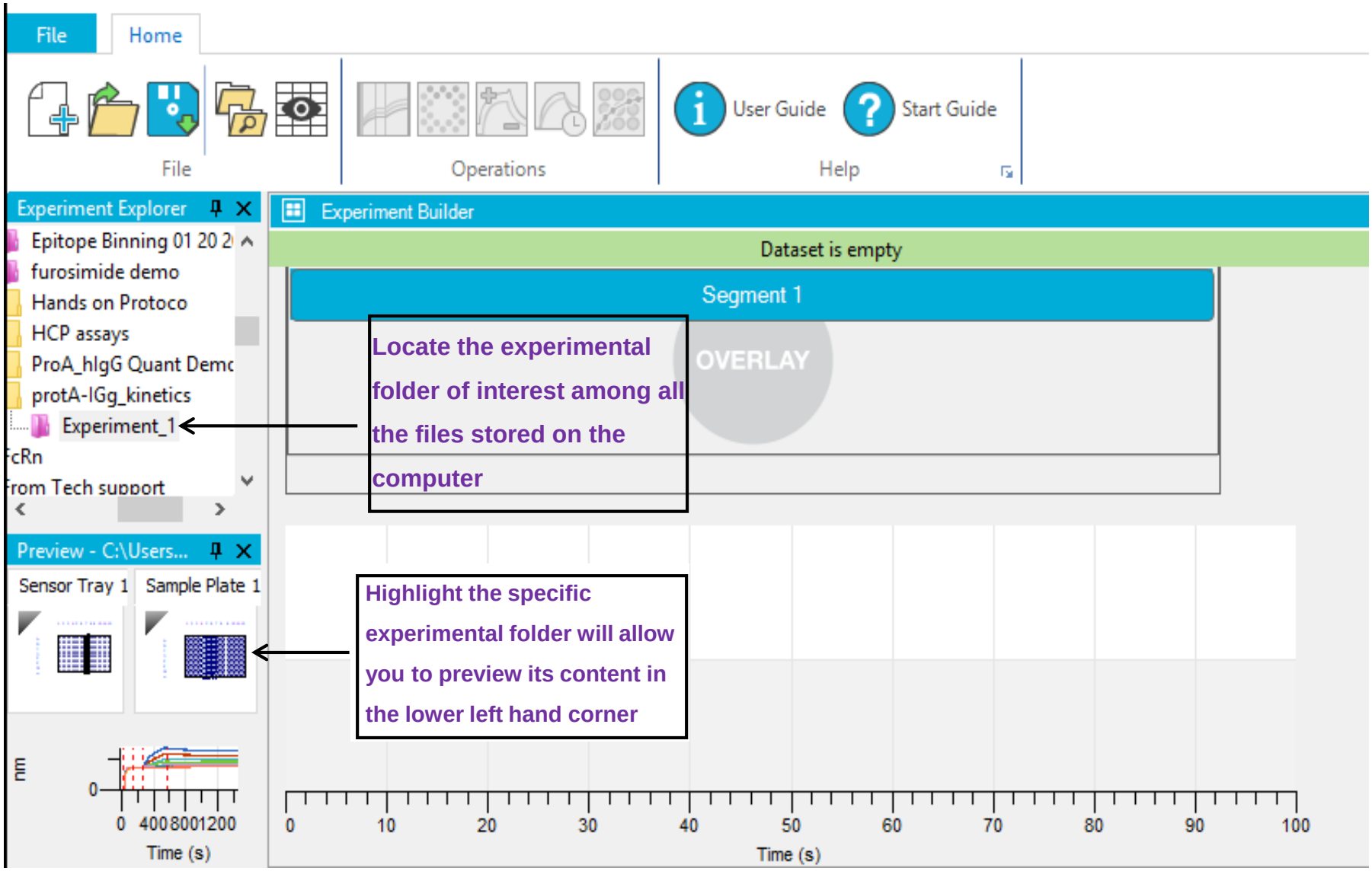
Kinetics Analysis Workflow with Data Analysis HT V11.0



Double click the Data Analysis HT 11.0 software icon

to start the program

Locating Data File



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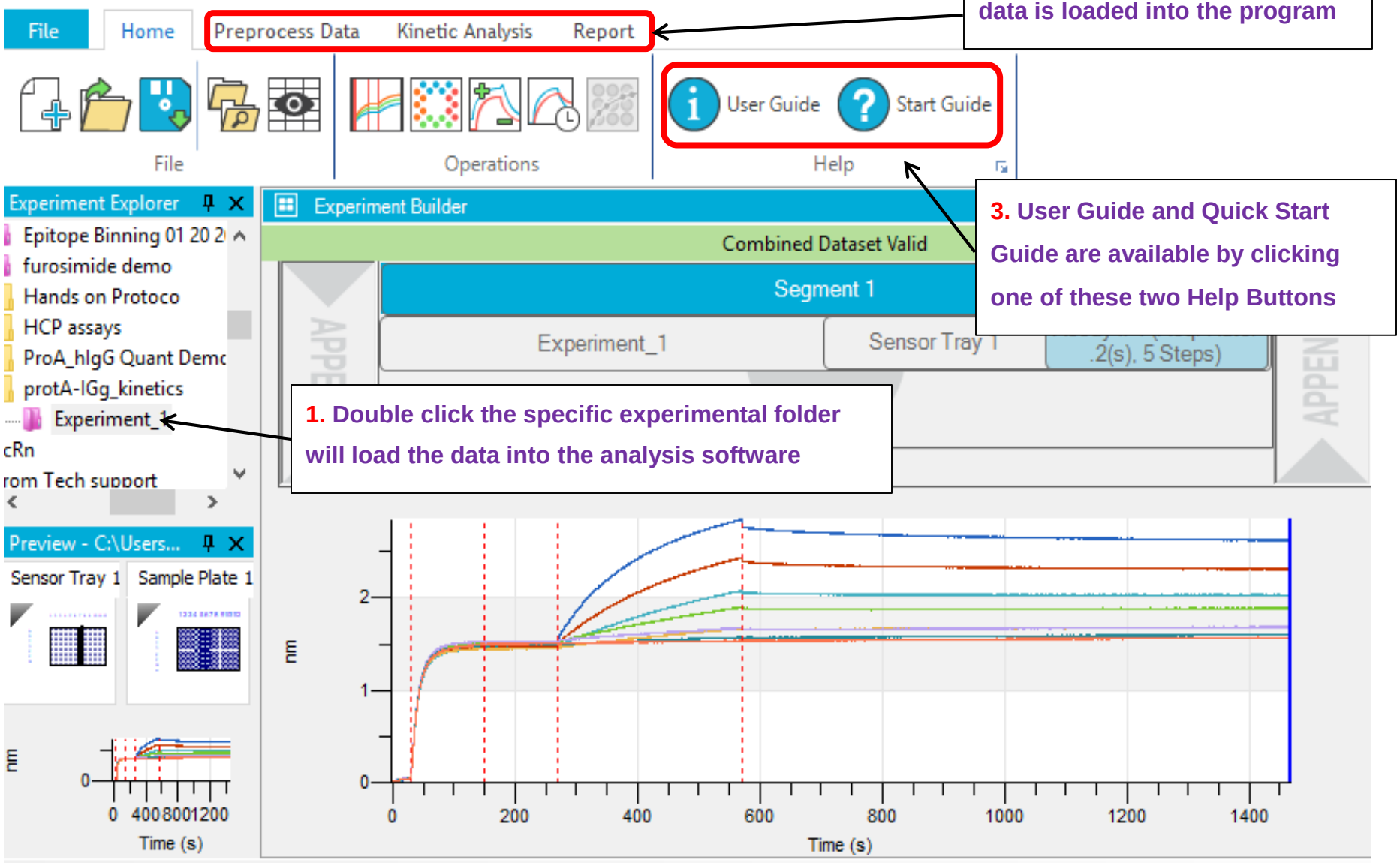
Loading Data File



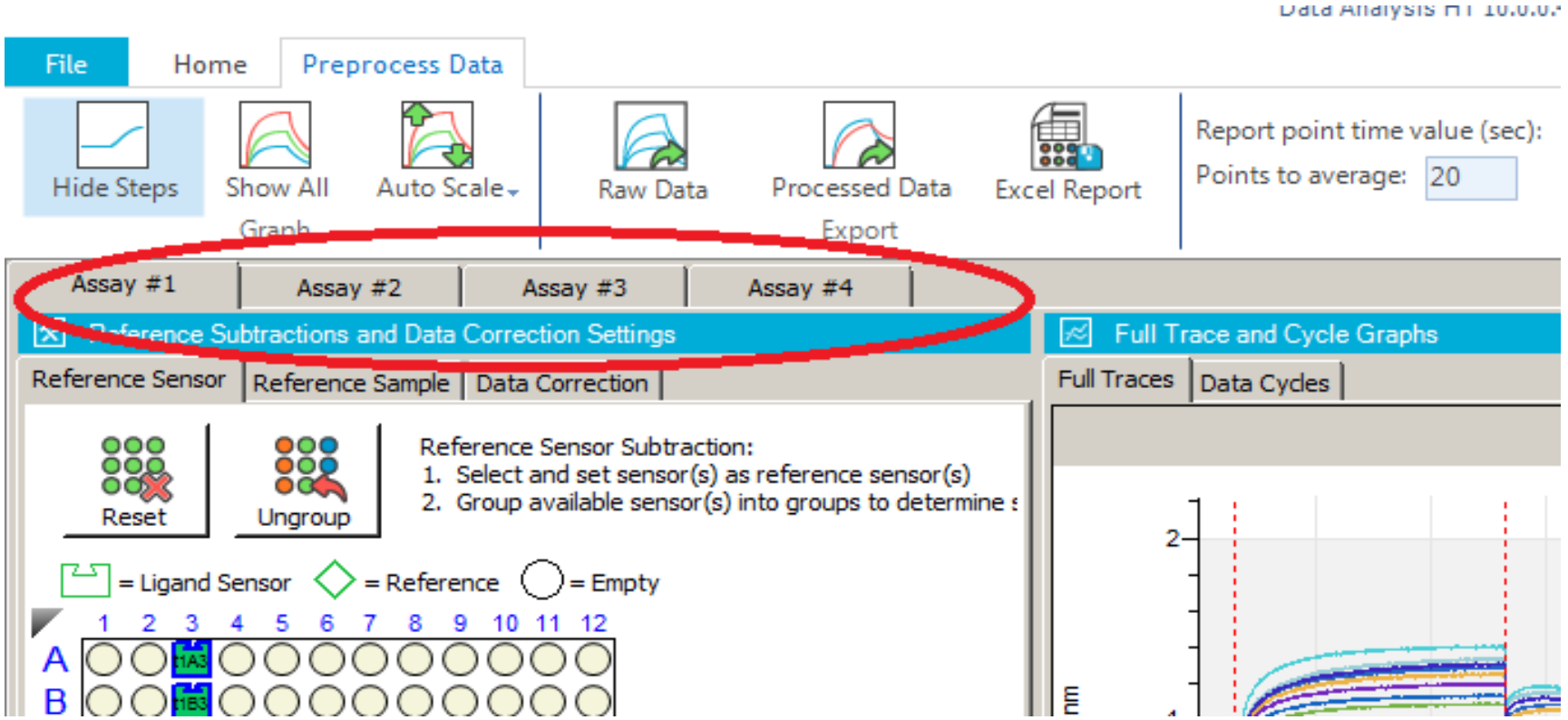
2. Additional tabs appear after data is loaded into the program

3. User Guide and Quick Start Guide are available by clicking one of these two Help Buttons

1. Double click the specific experimental folder will load the data into the analysis software

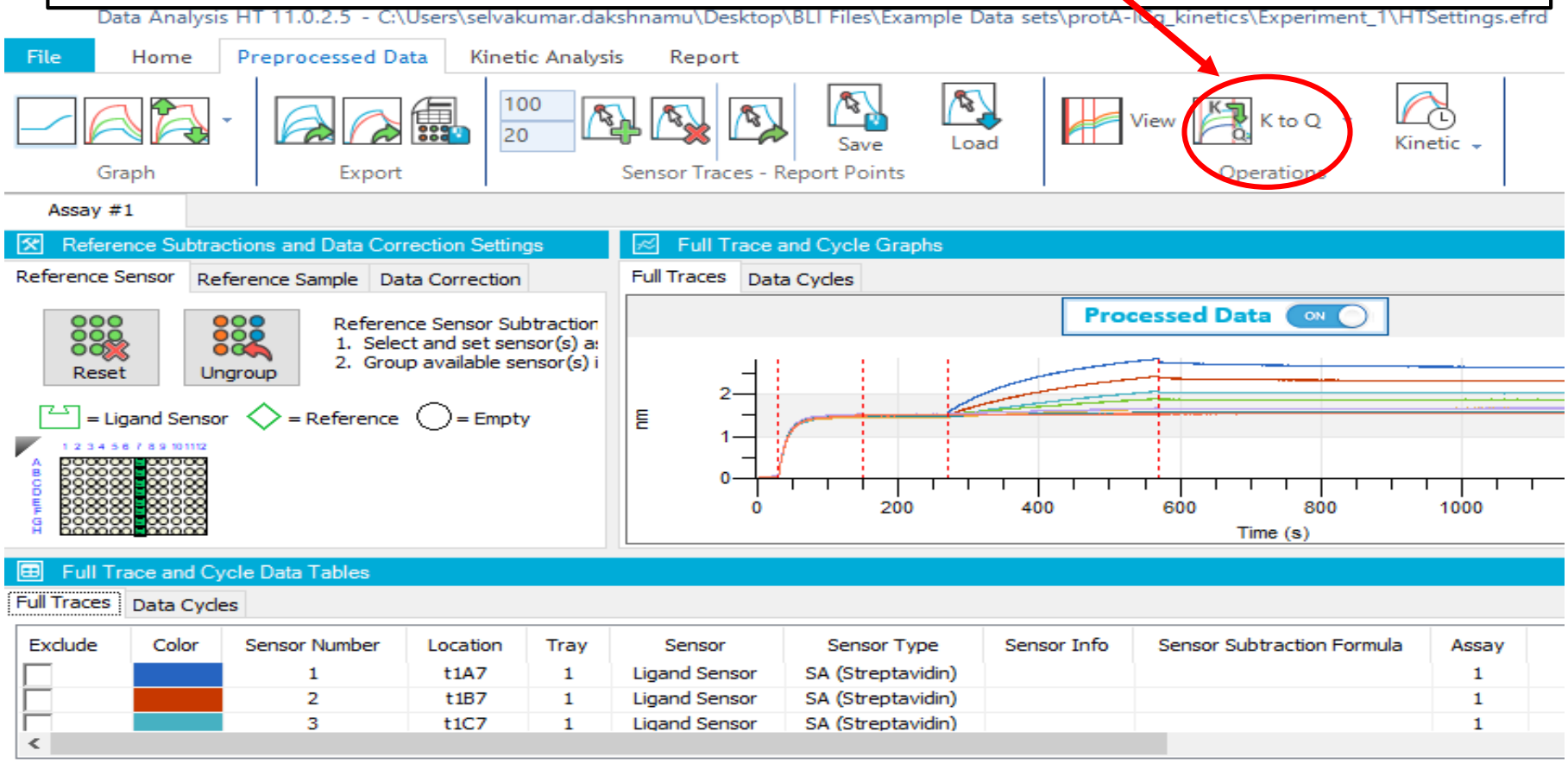
HT Kinetics Analysis
Preprocessing Kinetics Data Sets

- **Idea is same as before (subtracting references and making other data corrections), but with much more flexibility**
- **Now you can see the baseline after subtraction**
- **Experiments with multiple assays will show up as separate assays as before, but will combine in final data table/fitting graph.**



In preprocess data tab there is a new K to Q option. Using this new feature you were able to quantify any of the kinetic steps by selecting the appropriate step list. For example if you load unknown amount of ligand by using this option you should be able to quantify the amount of loading concentration.

However, now if you would be able to quantitate multiple step, you can select quantitate step type means that will analyte all the loading steps in different experiment.



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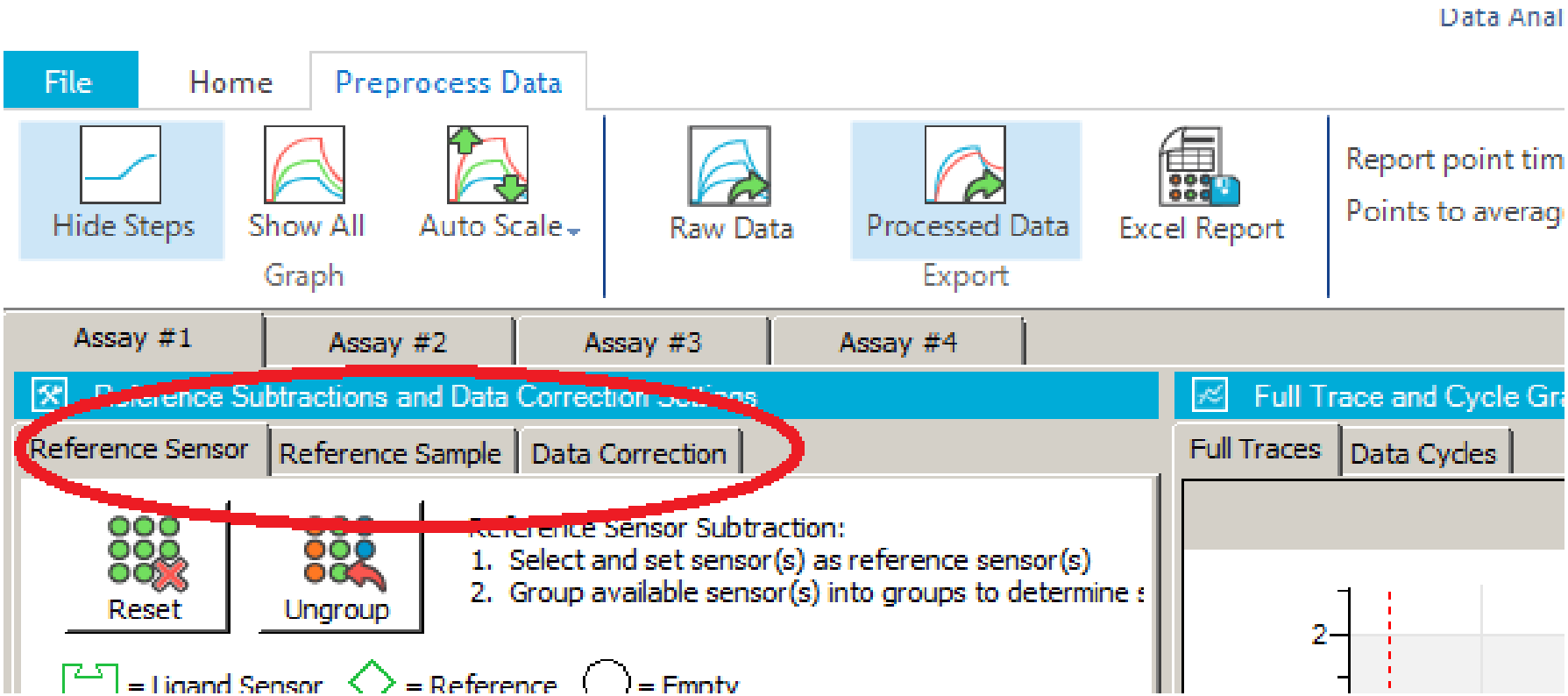
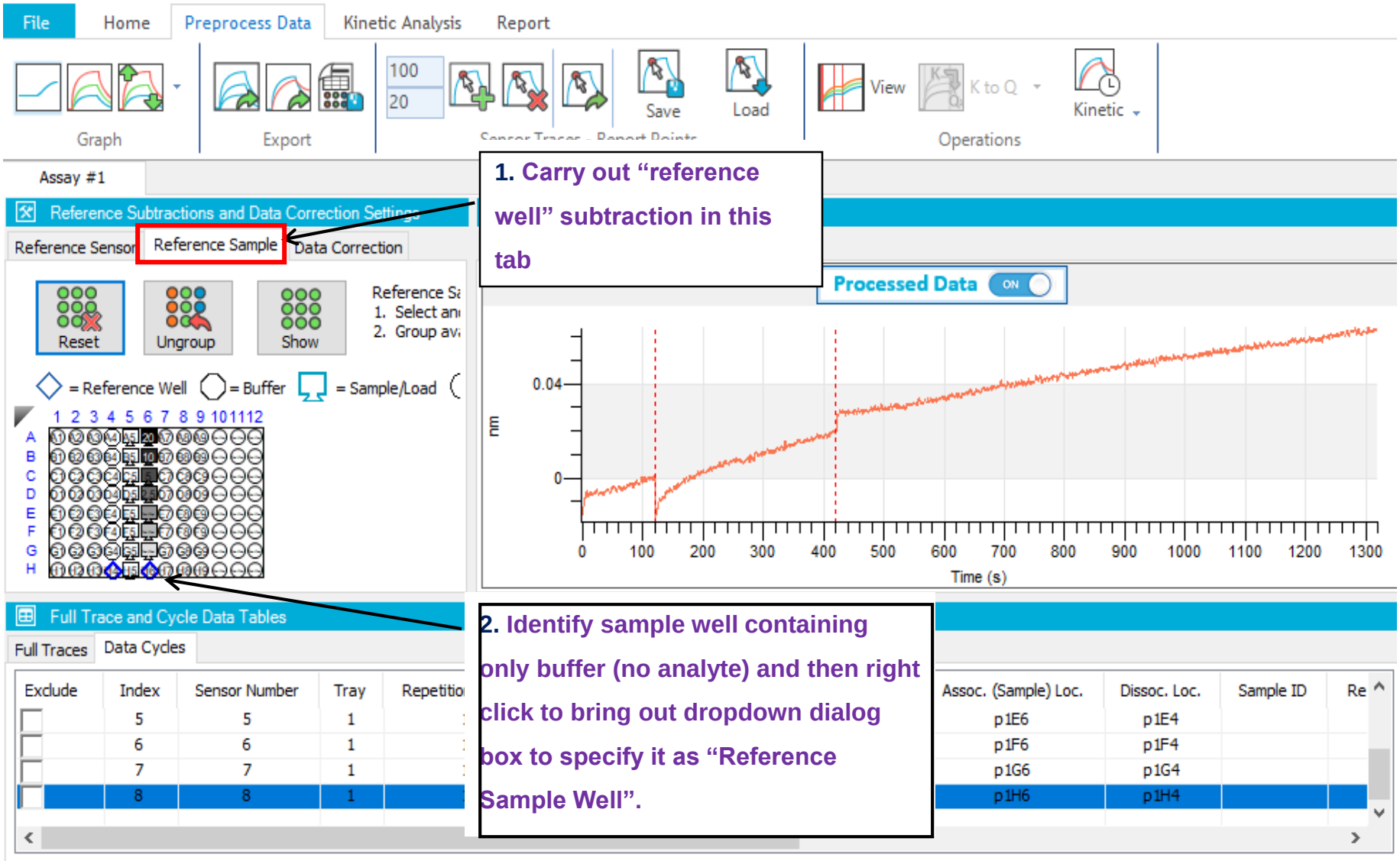
HT Kinetics Analysis Reference Tabs

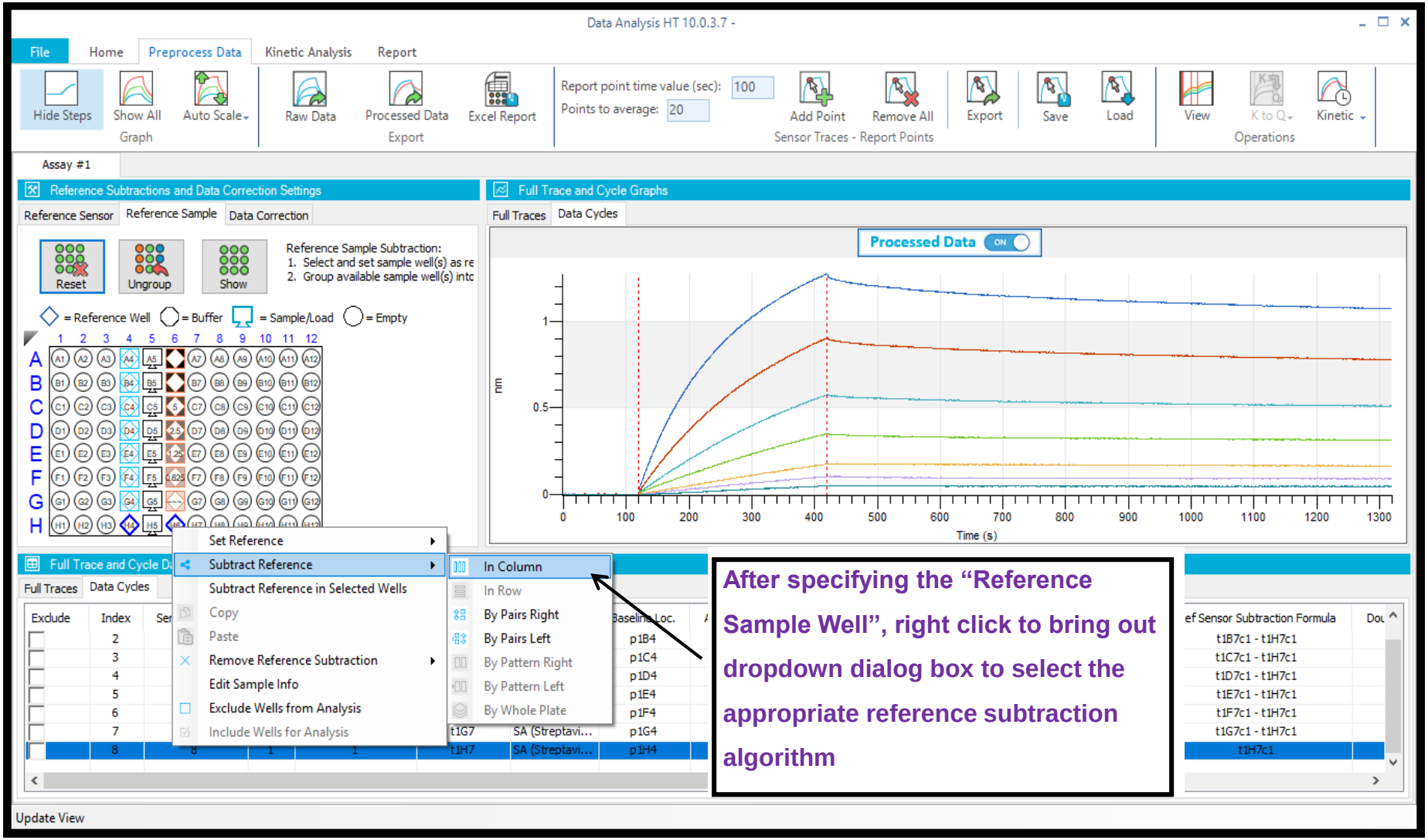


- **Reference Sensors, Ref Wells/Samples and Data Corrections options have been separated into sub-tabs**
- **Edit sample or sensor information, Exclude samples or sensors**
- **This is a new look to the interface**

Subtracting Reference Sample

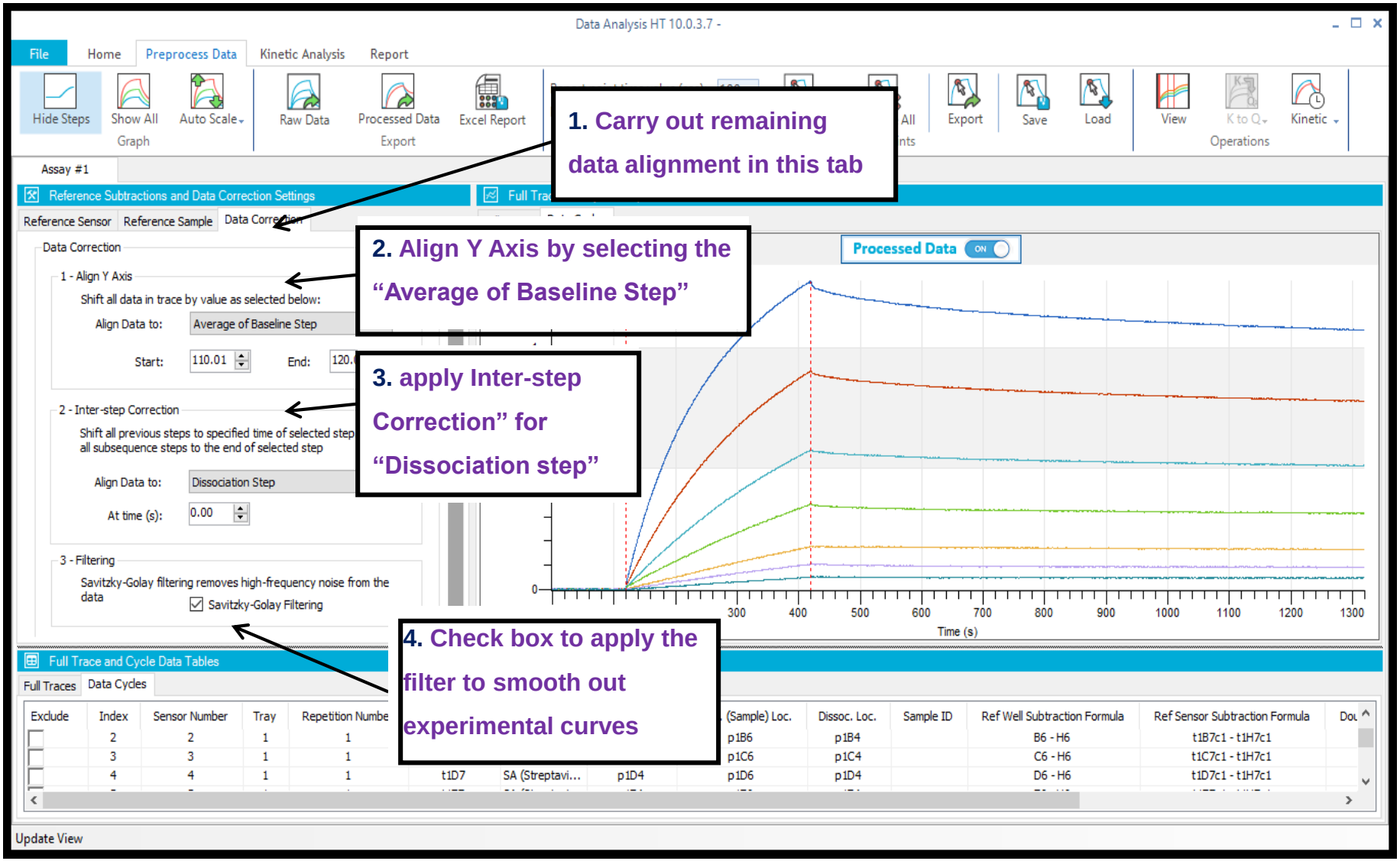


Subtracting Reference Sample

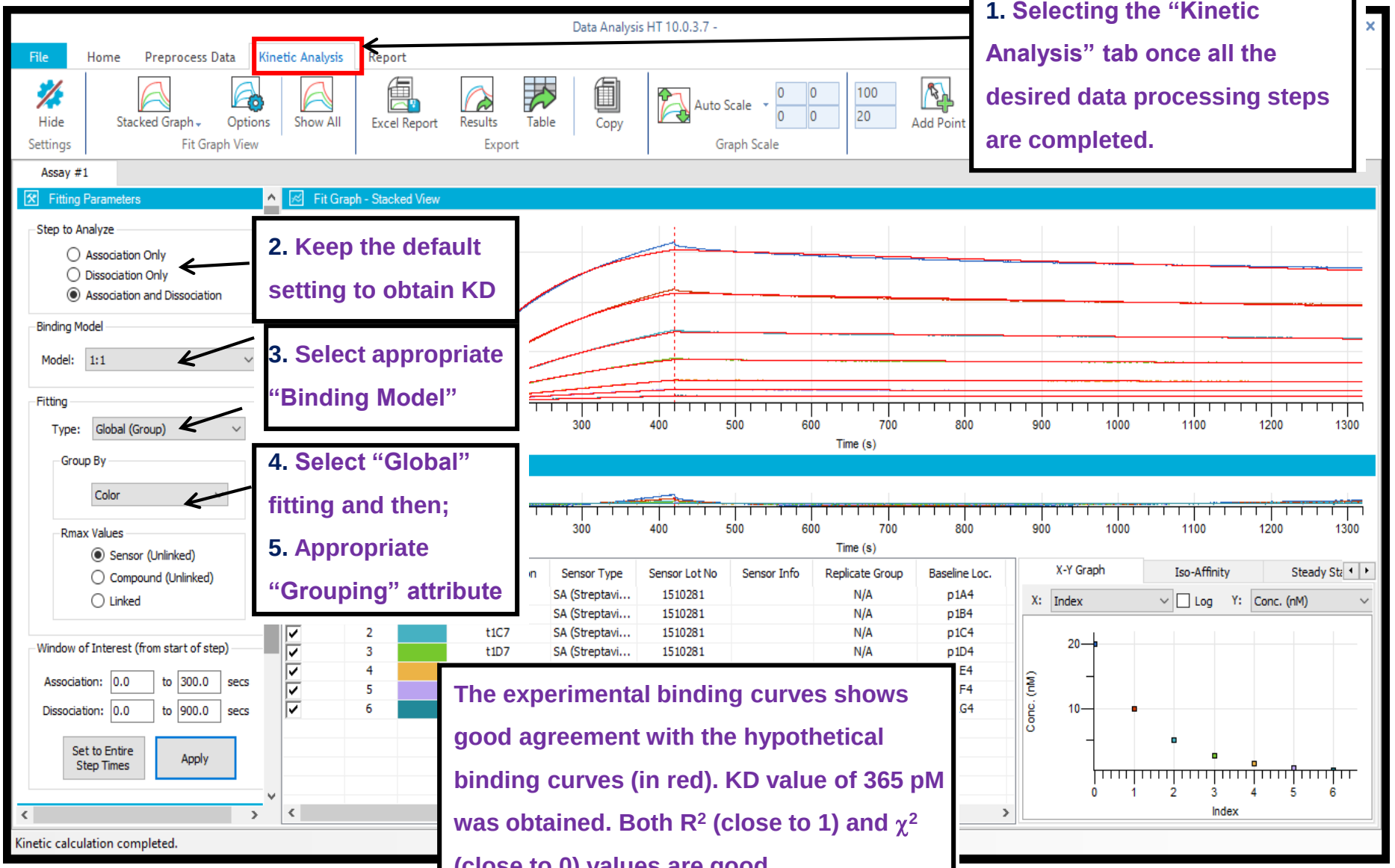


After specifying the "Reference Sample Well", right click to bring out dropdown dialog box to select the appropriate reference subtraction algorithm

Data Correction



Fitting Parameters



Double Reference on Kinetic Analysis

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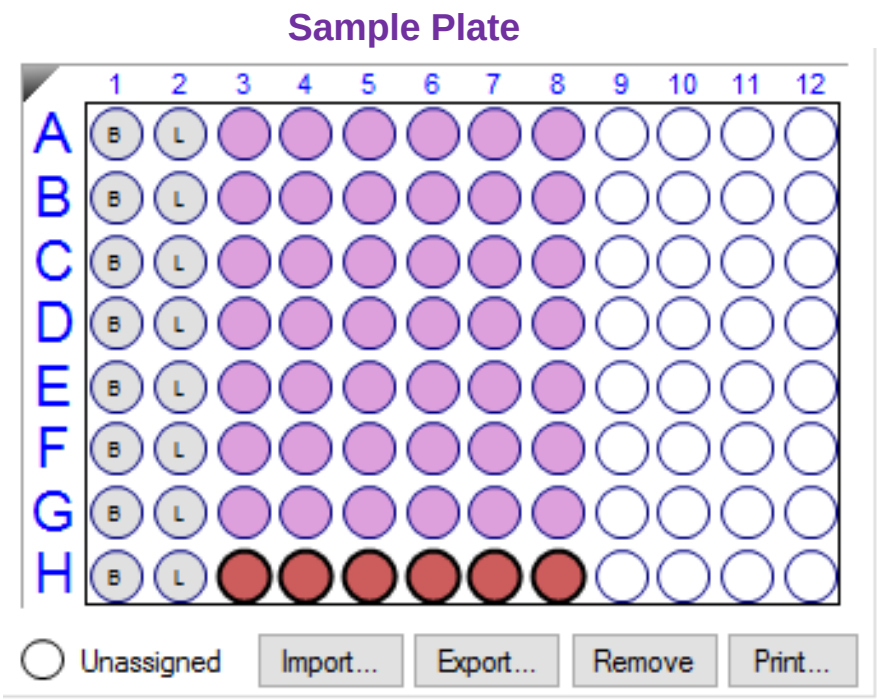
Refence Sensor vs. Reference Well

- Reference WELLS are wells in the sample plate containing assay buffer only (no analyte)
- The sensor dipped into them DOES have ligand loaded on it
- Their purpose is to subtract any drift in the BLI signal
  --- Drift in the signal is usually caused by certain buffer components, like Tween or DMSO
- Usually there is one reference well per column of samples
- Reference wells should **ALWAYS** be included in kinetics assays



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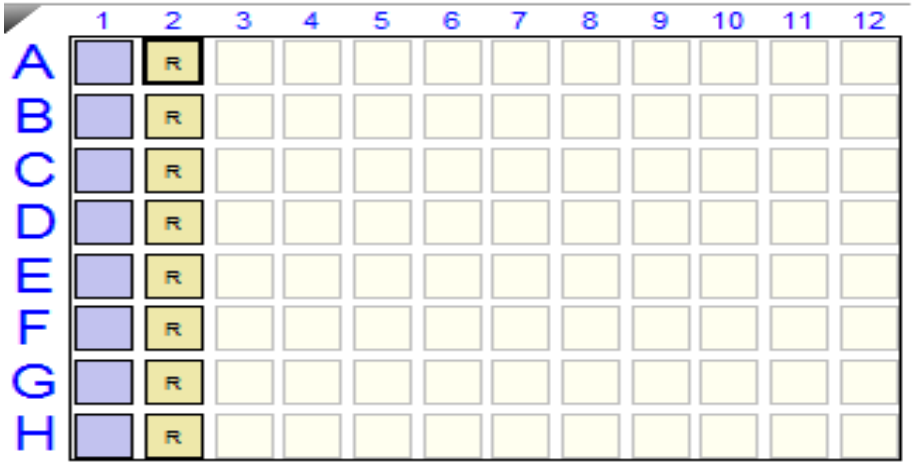
Refence Sensor vs. Reference Well

- Reference SENSORS are a second complete set of sensors that are NOT loaded with ligand (or are loaded with a control ligand that does not bind to the analyte)

- The reference sensors are dipped into all the same samples as the sensors with the ligand loaded on them
  --- Note that when using reference sensors, you MUST replicate your assay steps exactly with the reference sensor
  --- The only allowable difference is that reference sensors can be “loaded” in buffer or a different ligand well

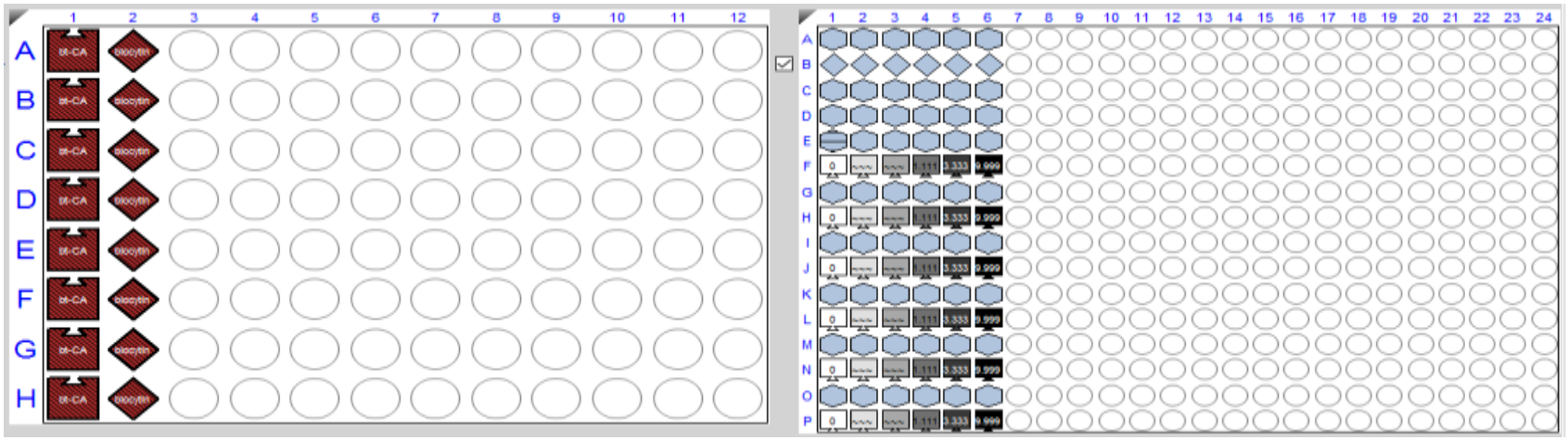
- Refence sensors are used to subtract NSB, and therefore are not always used

Sensor Tray

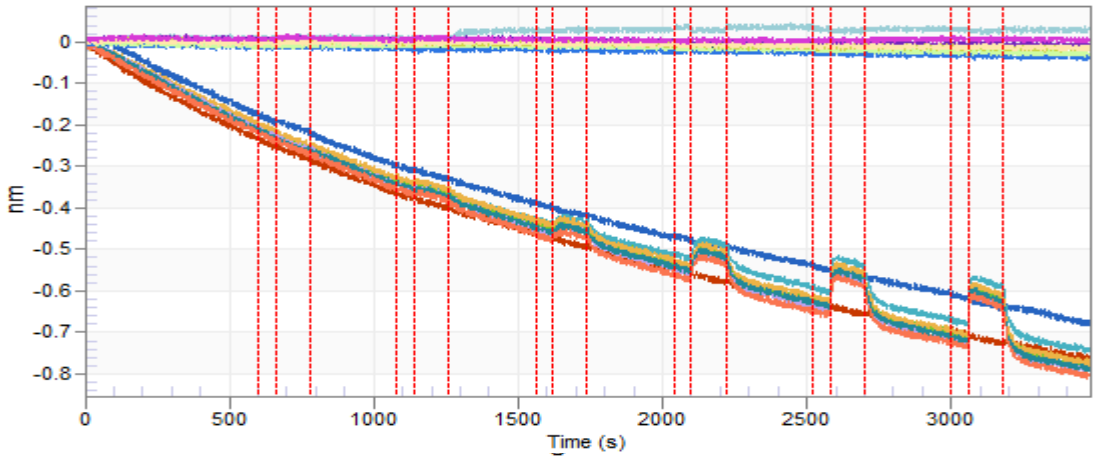


Assay	No.	Sample	Step Name	Step Type	Sensor Type	Assay Time	Comment
1	1	1	Baseline	Baseline	SA (Streptavidin)		
1	2	2	Loading	Loading	SA (Streptavidin)		
1	3	1	Baseline	Baseline	SA (Streptavidin)		
1	4	3	Association	Association	SA (Streptavidin)		
1	5	1	Dissociation	Dissociation	SA (Streptavidin)	0:25:40	
2	1	1	Baseline	Baseline	SA (Streptavidin)		
2	2	1	Loading	Loading	SA (Streptavidin)		
2	3	1	Baseline	Baseline	SA (Streptavidin)		
2	4	3	Association	Association	SA (Streptavidin)		
2	5	1	Dissociation	Dissociation	SA (Streptavidin)	0:25:40	

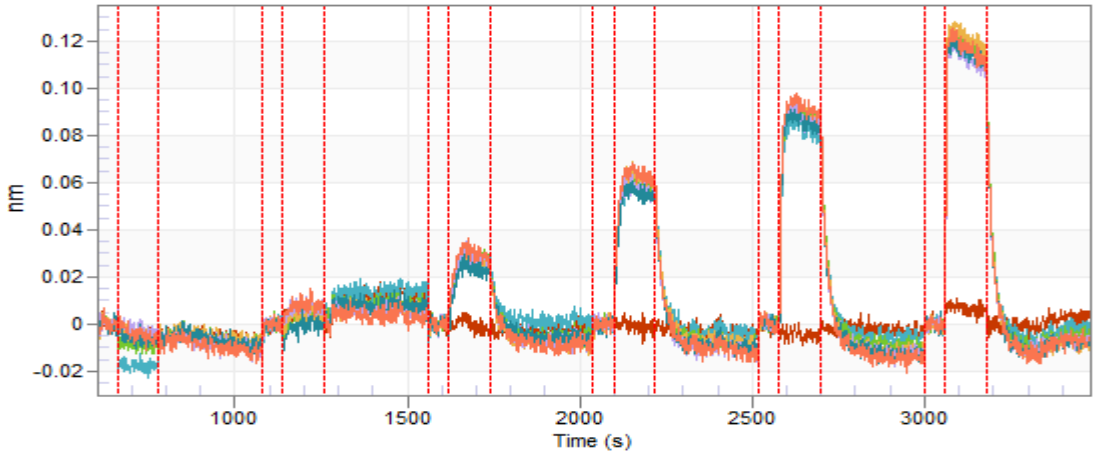
Double Referencing



Double Referencing

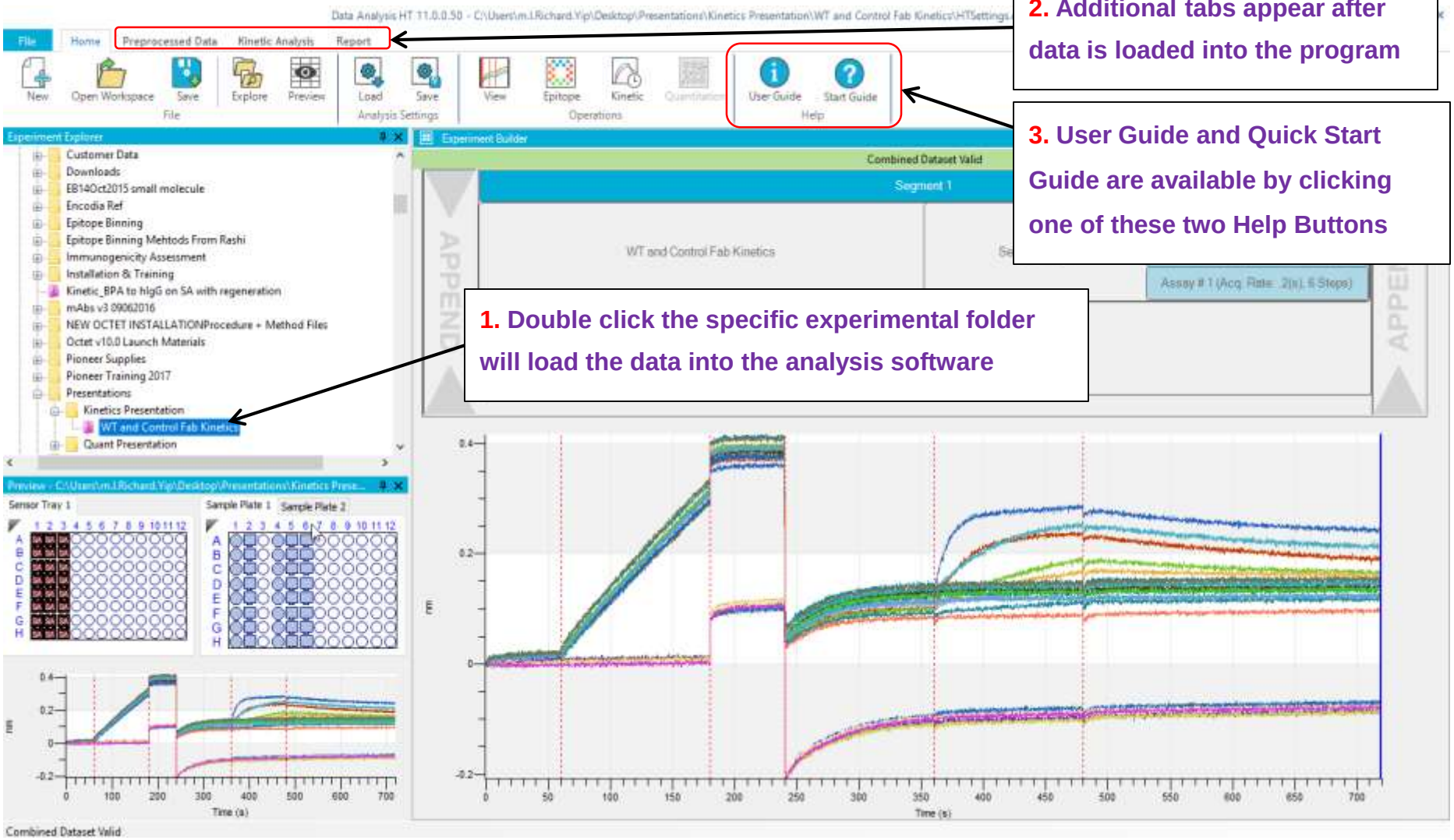


Raw Data

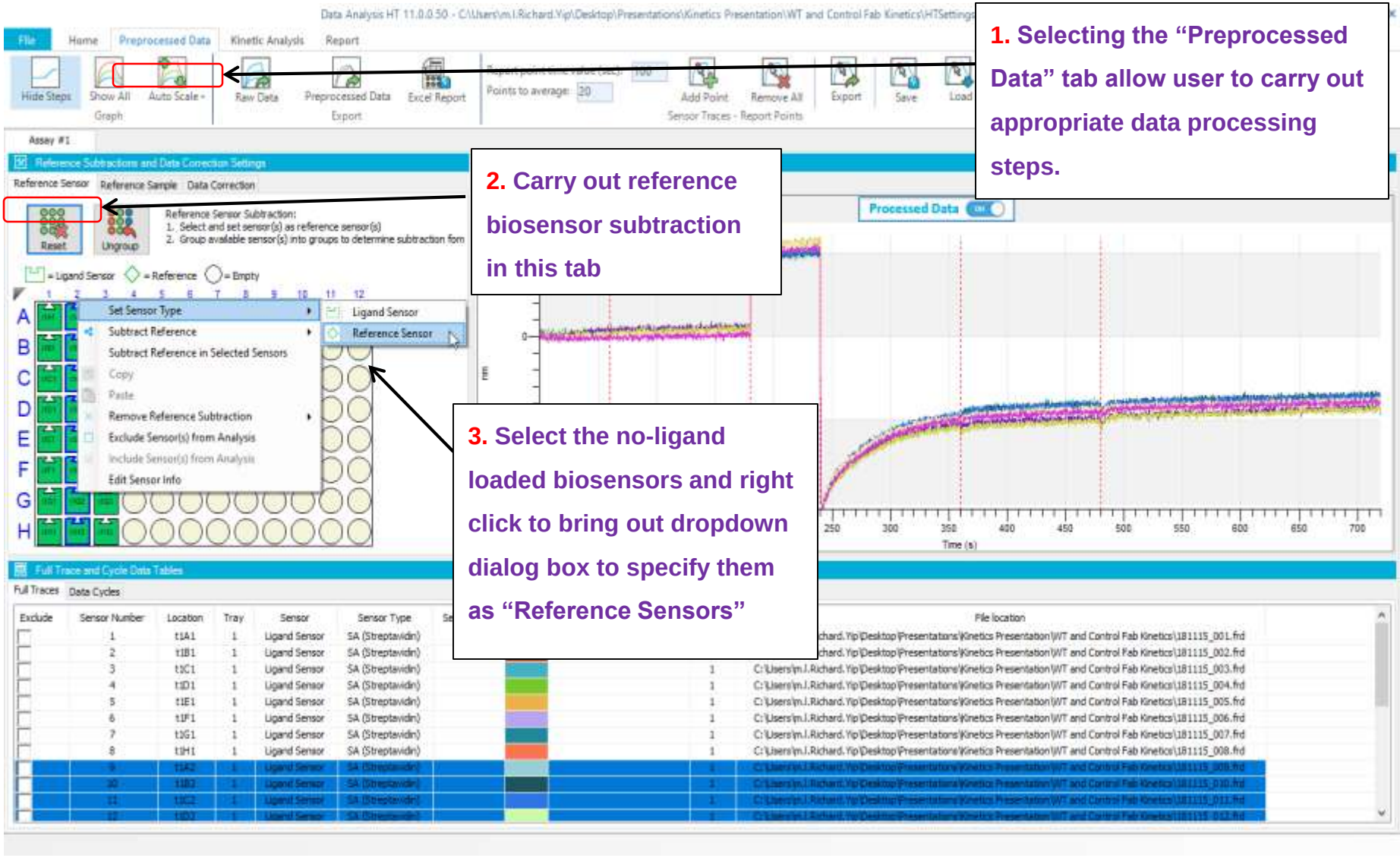


After Double Referencing

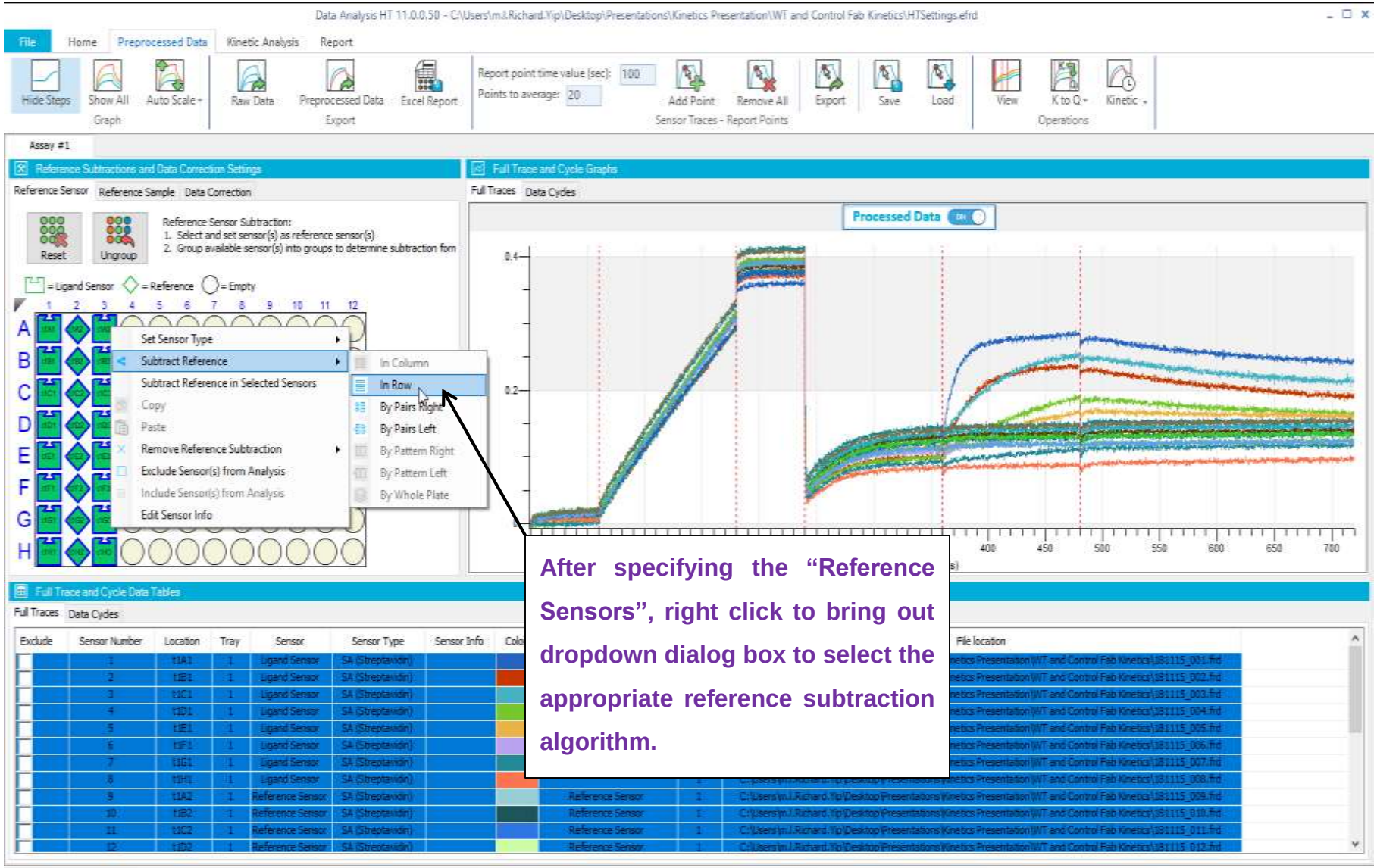
Loading Data into the Analysis Software



Reference Sensor Subtraction

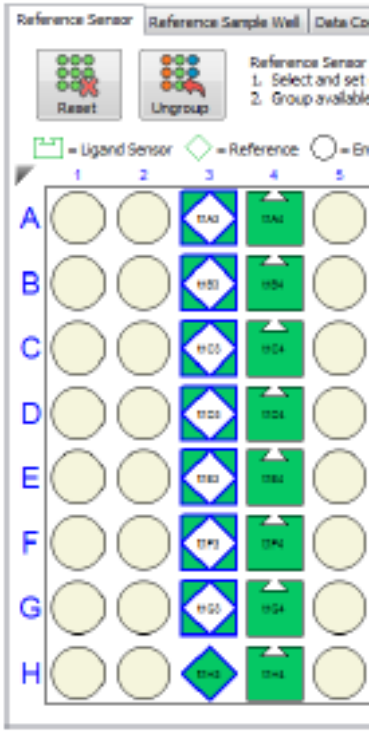


Reference Sensor Subtraction

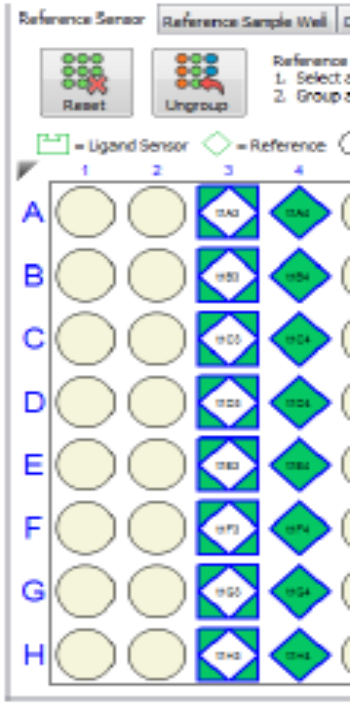


HT Kinetics Analysis Increased Flexibility in Reference Subtraction

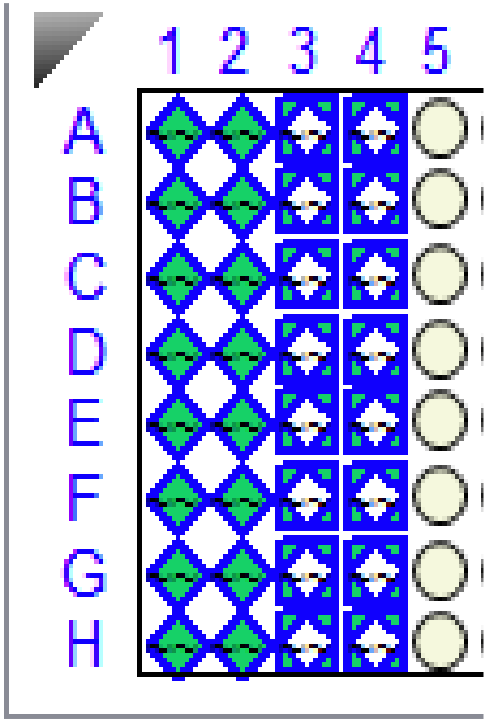
By Column



By Row



By Pattern



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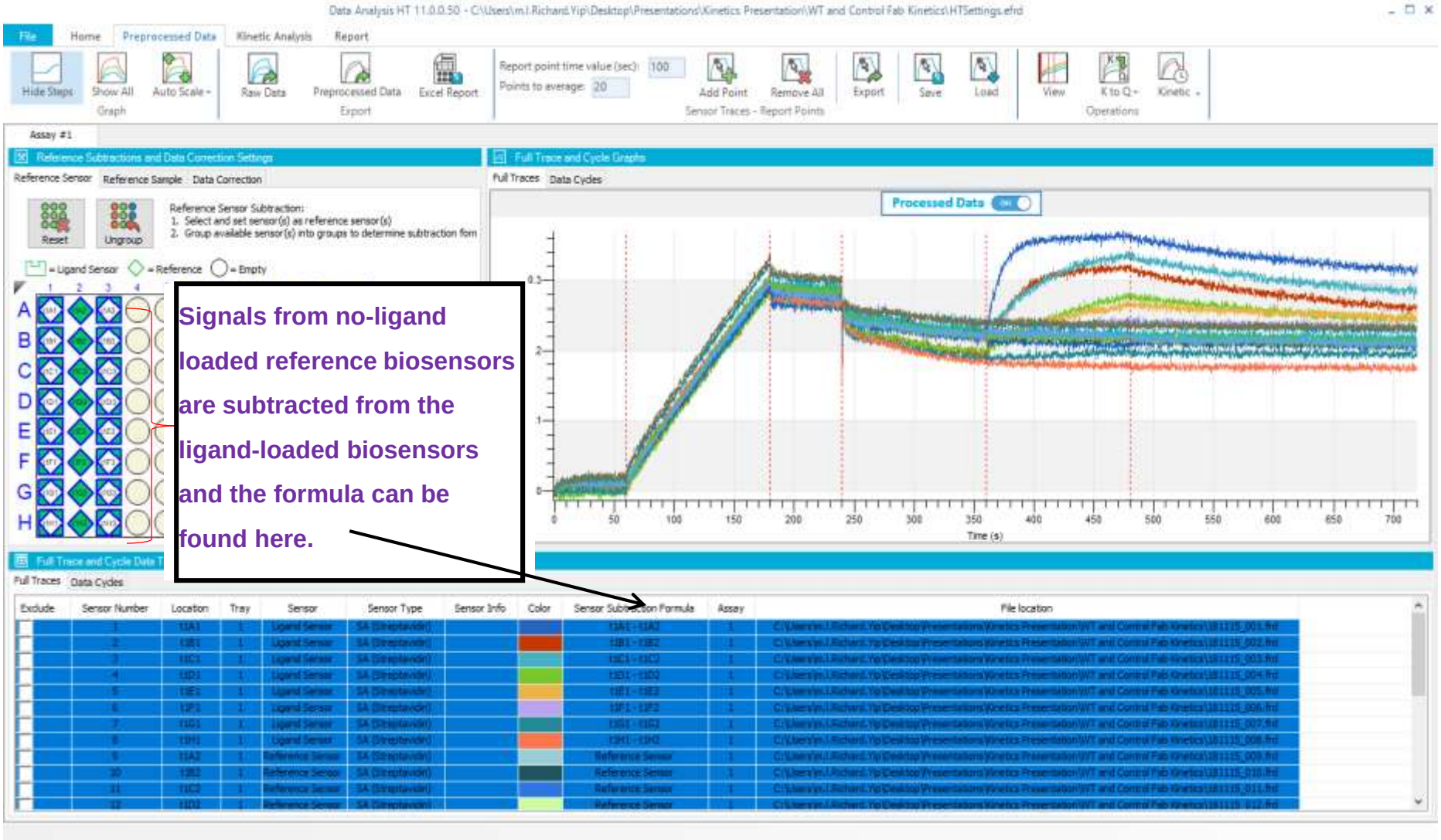
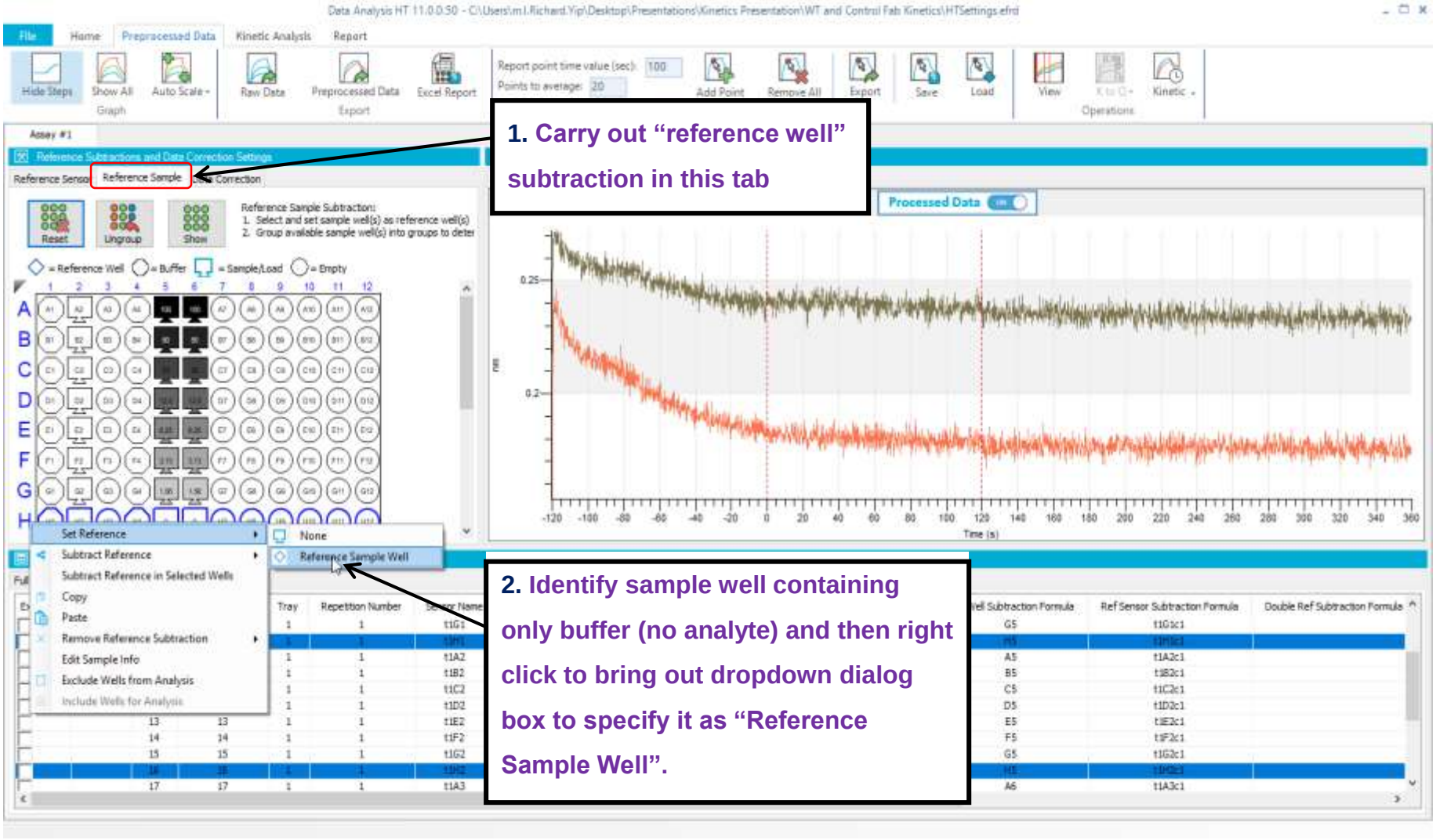
Double Referencing



Subtracting Reference Well



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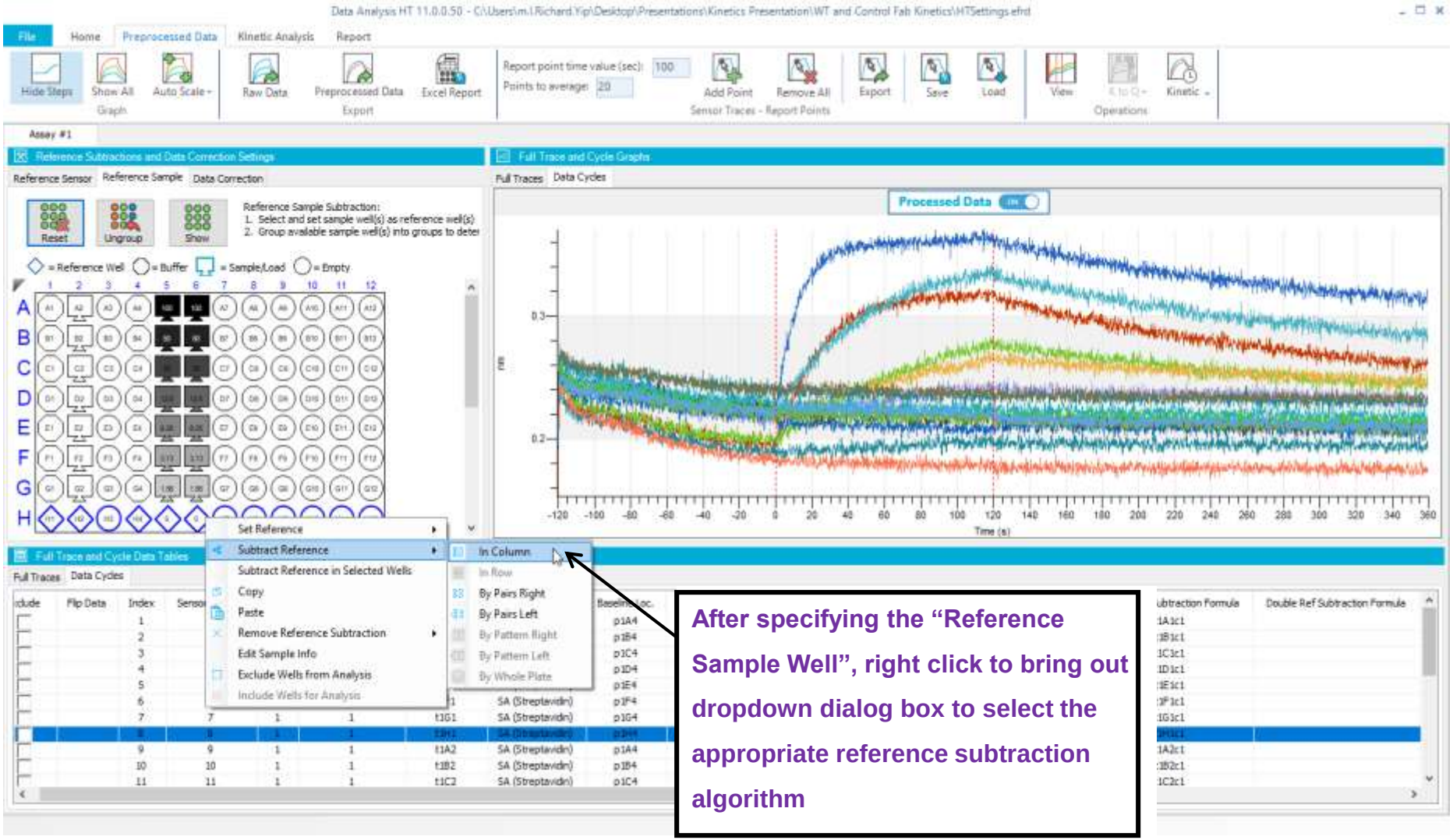
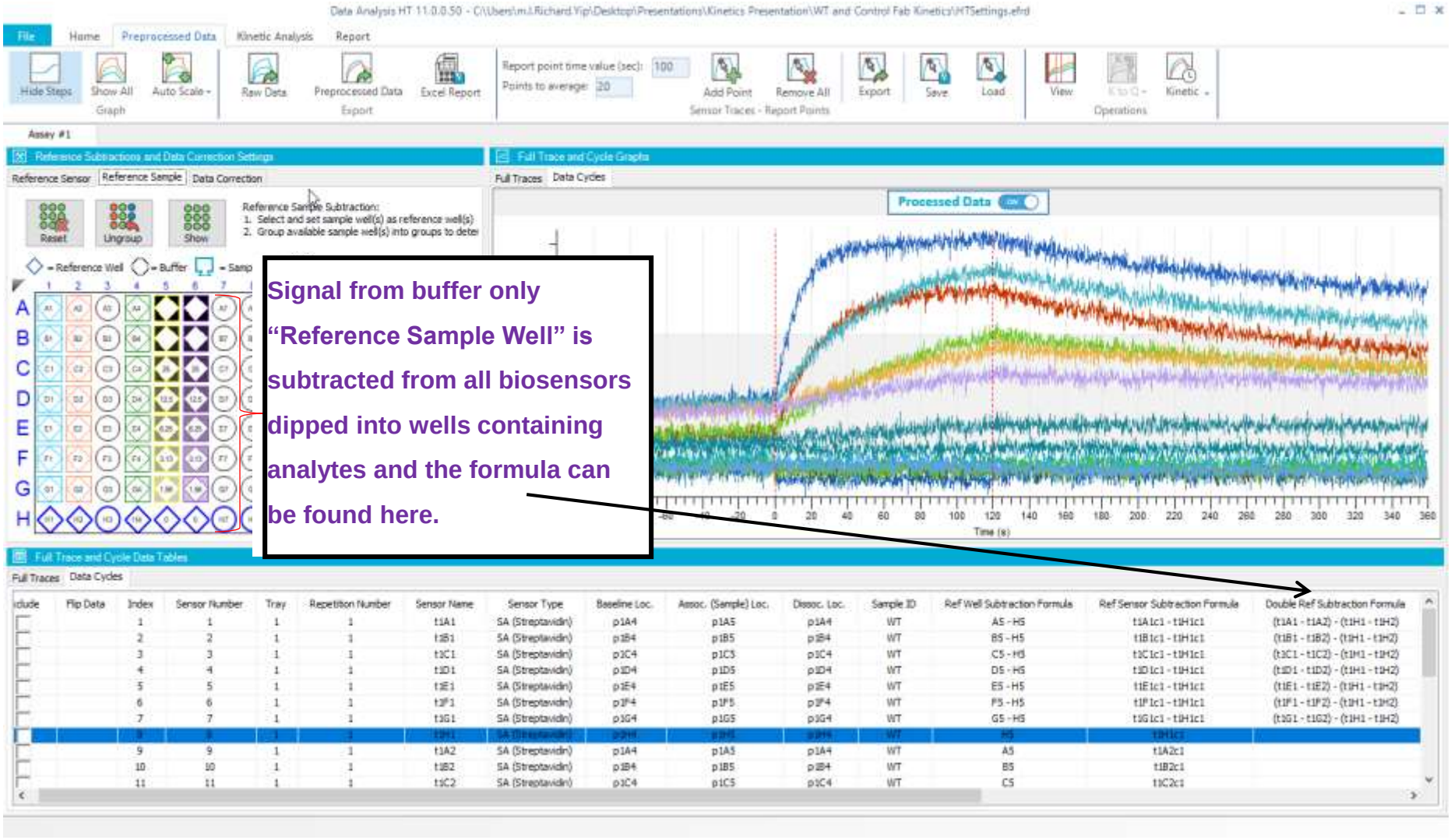
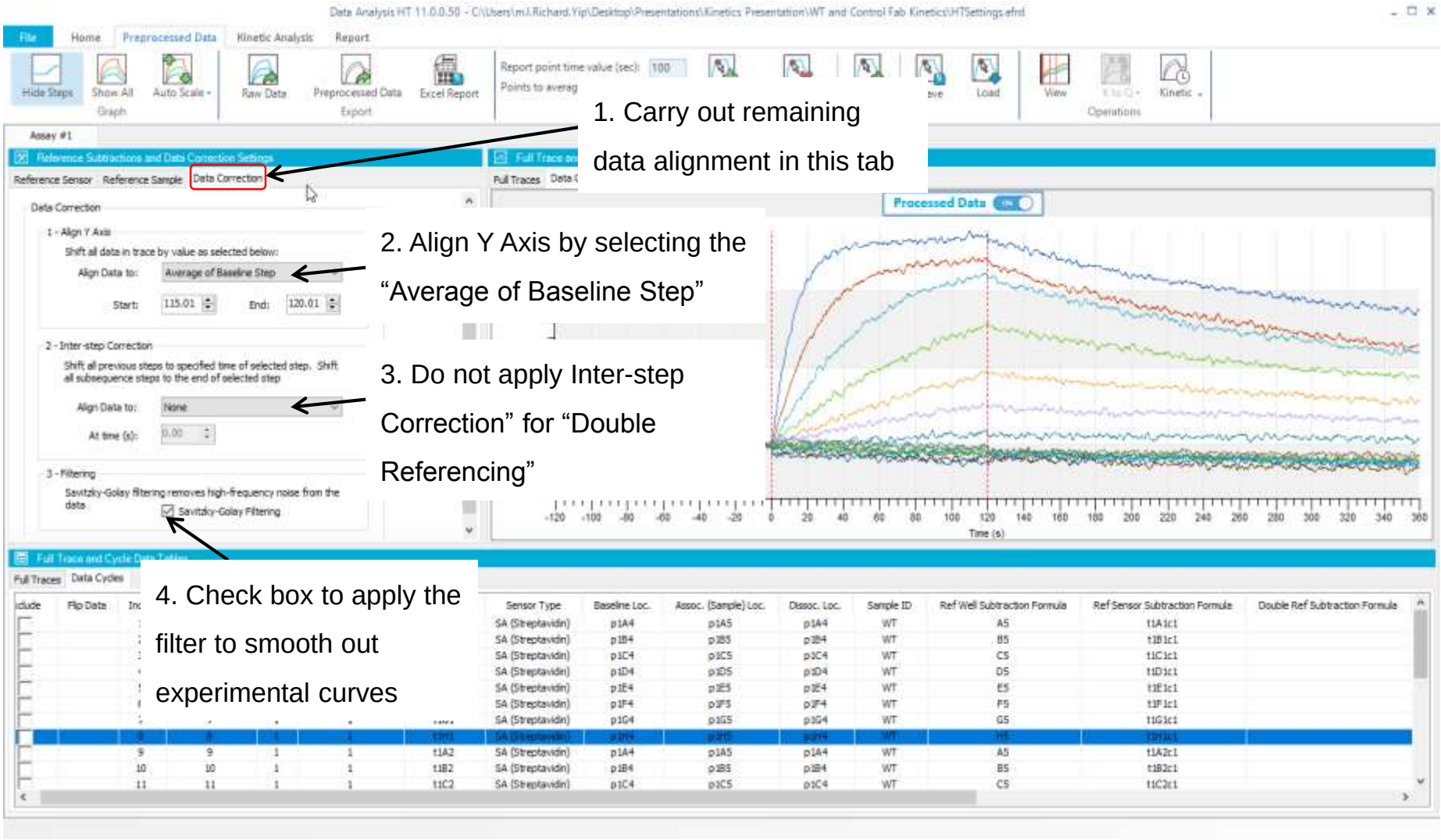
Subtracting Reference Well



Subtracting Reference Well



Data Correction



Fitting Parameters

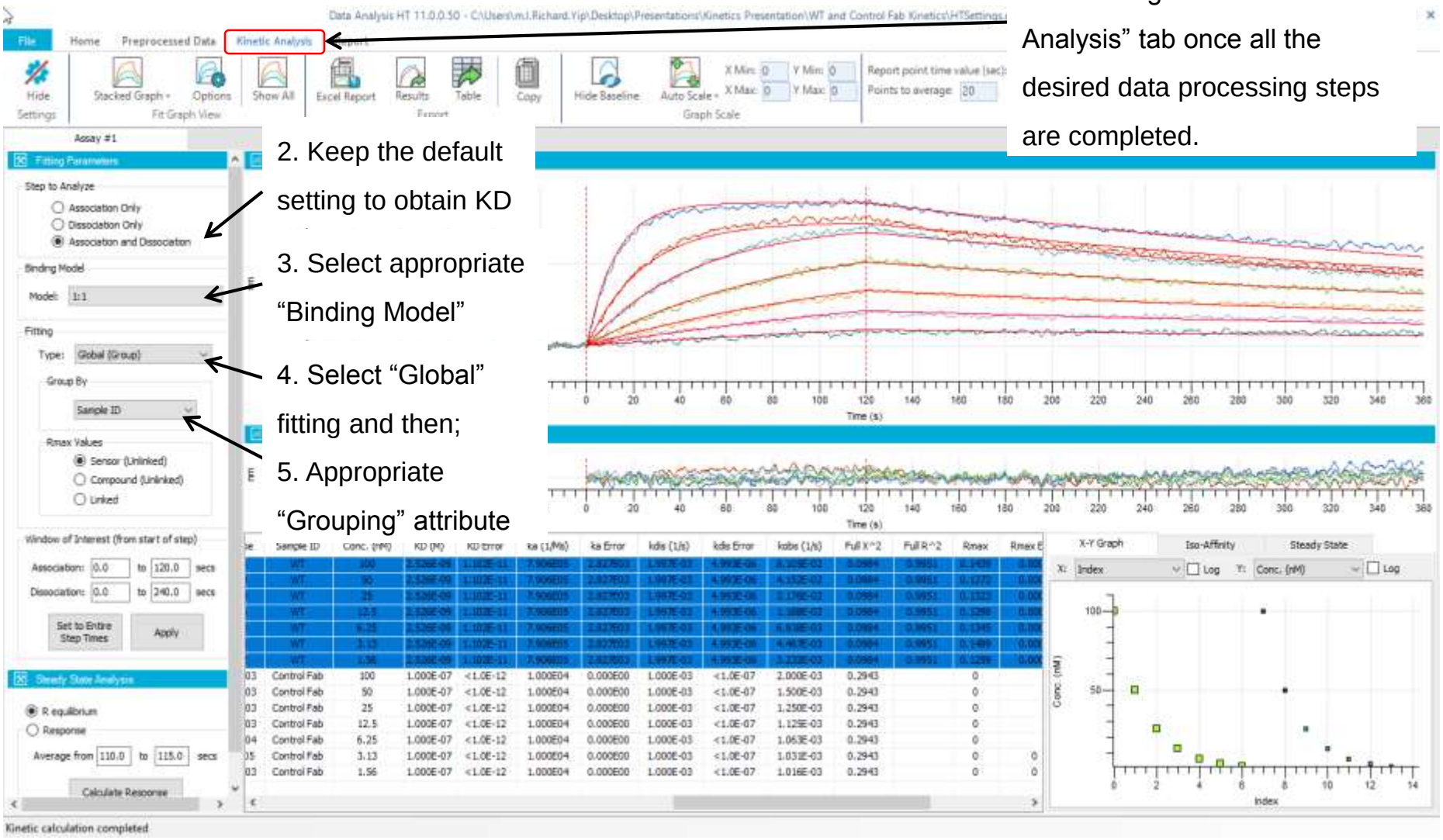
1. Selecting the “Kinetic Analysis” tab once all the desired data processing steps are completed.

2. Keep the default setting to obtain KD

3. Select appropriate “Binding Model”

4. Select “Global” fitting and then;

5. Appropriate “Grouping” attribute



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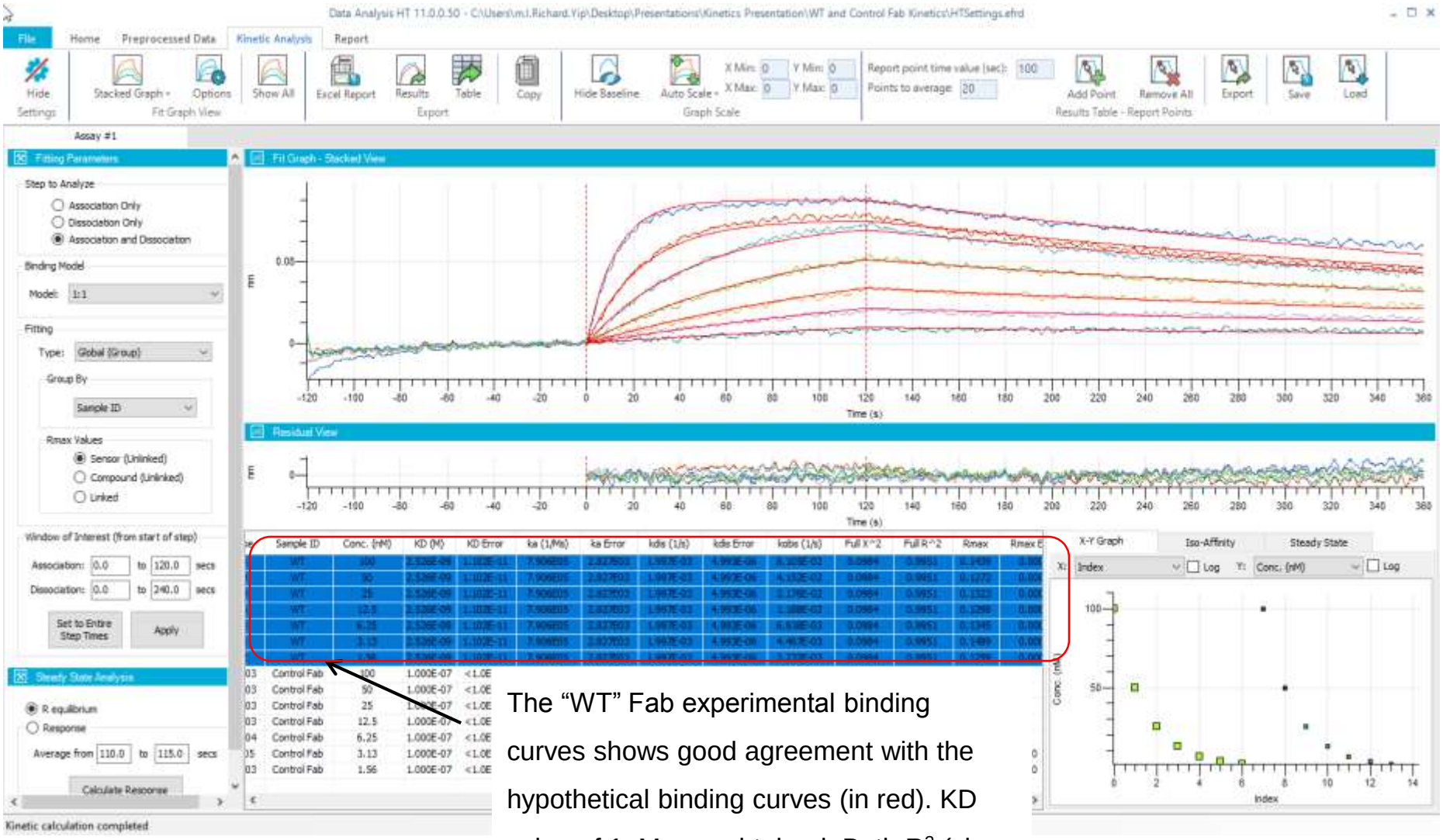
Kinetic Analysis



The “WT” Fab experimental binding curves shows good agreement with the hypothetical binding curves (in red). KD value of 1nM was obtained. Both R^2 (close to 1) and χ^2 (close to 0) values are good.

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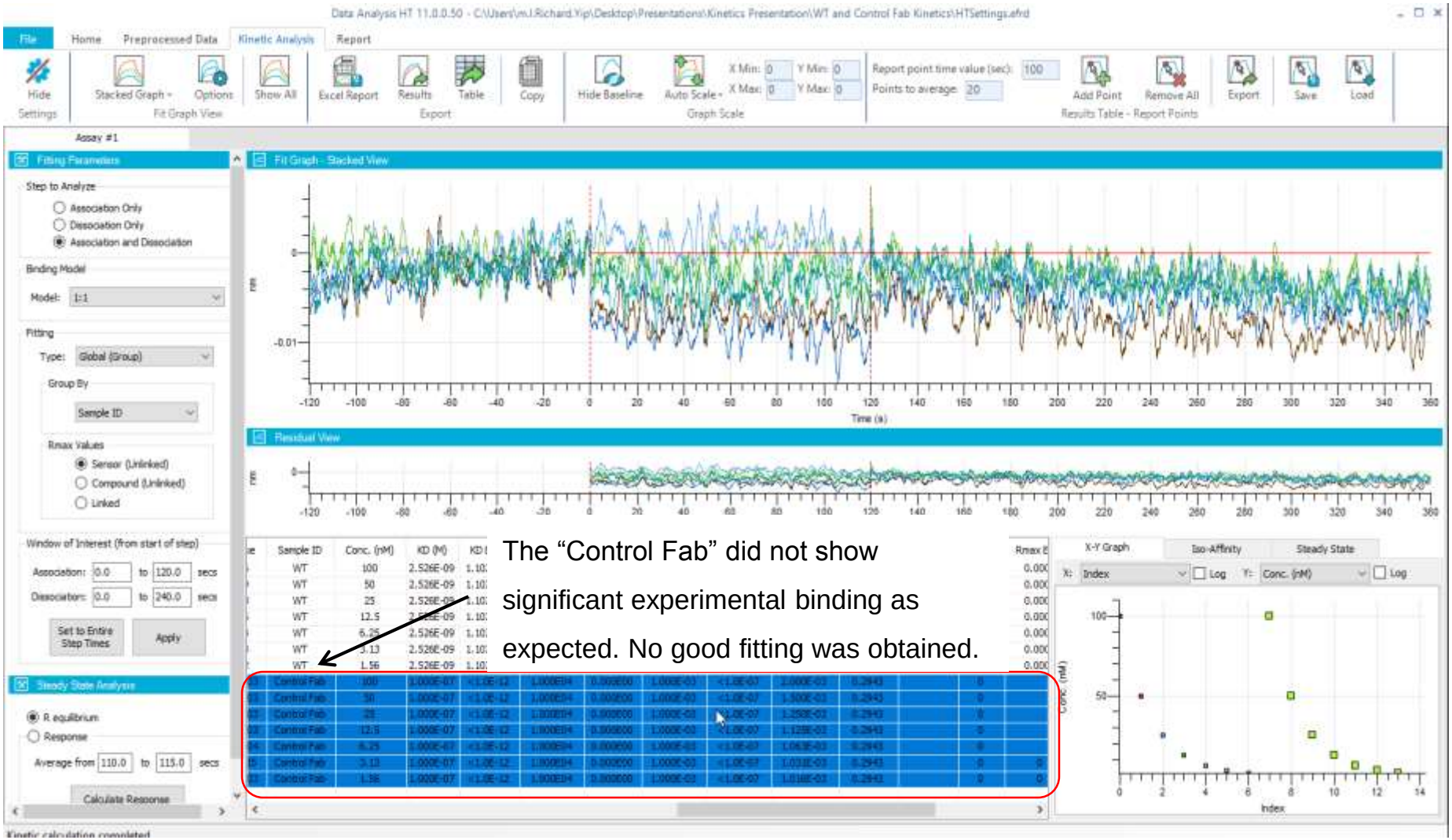
Kinetic Analysis



The “Control Fab” did not show significant experimental binding as expected. No good fitting was obtained.

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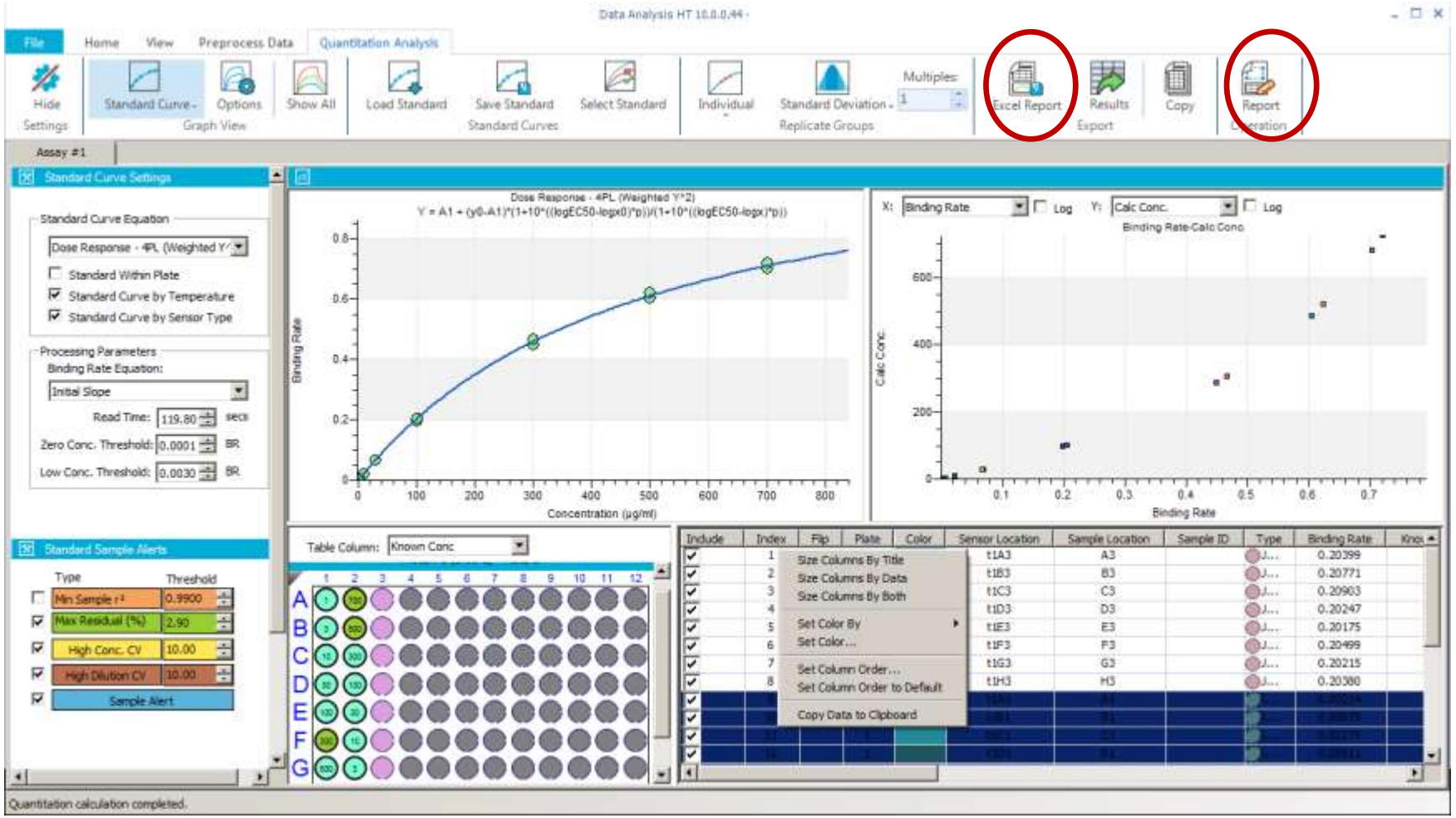
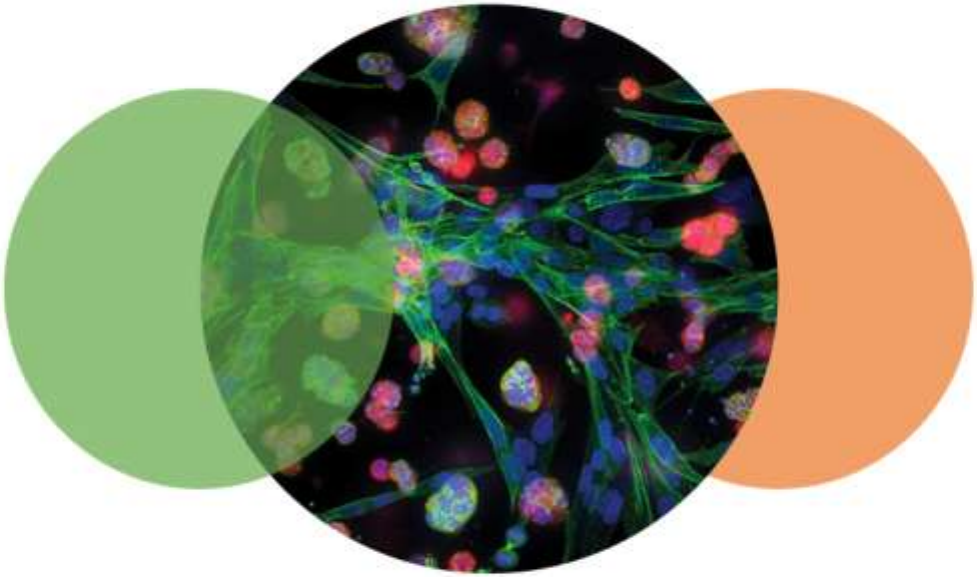
HT Quantitation/Kinetics Analysis Excel or PDF Report



Can make traditional excel report or generate a customized PDF report



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