Fluidic Sciences

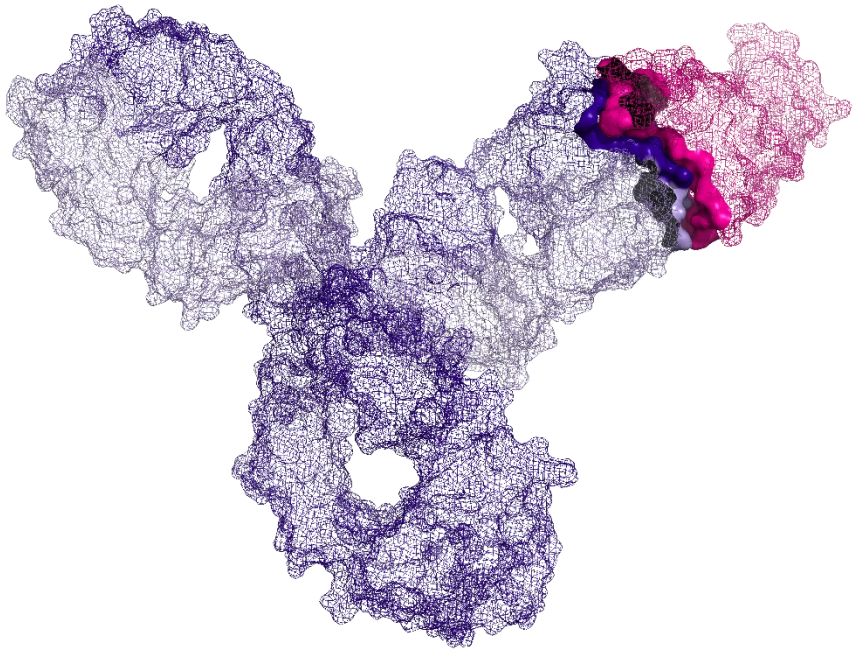
Fluidity Insight

User Guide for Fluidity™ Insight

Instructions for use

SOFTWARE VERSION 2.5.0

Released June 2024



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1. Introduction

Fluidity Insight provides the following features:

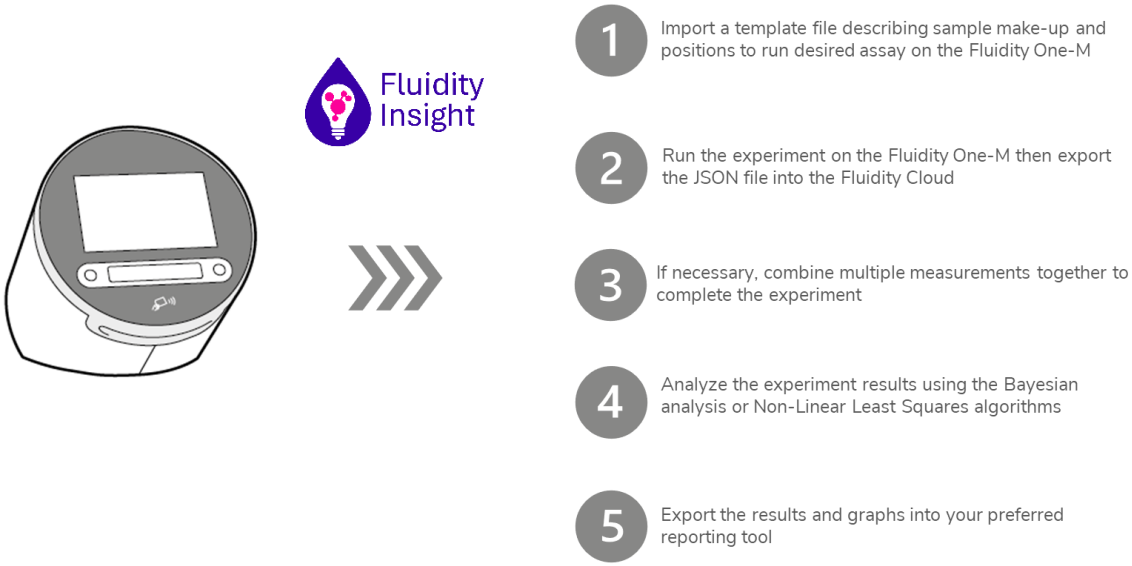
- Analyzing the experiment results using advanced analytical algorithms for offering insights into biomolecular interactions.
- Importing and combining measurements together over multiple runs to form a full set of experimental results.

The Fluidity One-M instrument has three pre-set run modes:

- Size measurements
- K_D determination
- Custom import

The Size measurement and K_D determination pre-set modes guide you quickly through the fixed information required for the measurement to be run locally at the instrument. The third pre-set mode, Custom import, allows complete flexibility on the type of experiment, the parameters of the experiment and the chip-plate layout of species and buffers selected for the run. The Custom import protocol can be conveniently set up remotely using excel templates provided by Fluidic Sciences and downloaded onto the Fluidity One-M in the laboratory when ready to be run.

Standard workflow



For analyses like the SeroAffinity and Concentration (SAffCon) Assay, template tools are available from Fluidic Sciences (techsupport@fluidic.com) that conveniently identify samples to be prepared and their positions on the chip. After the run, results from the Fluidity One-M can be uploaded onto Fluidity Insight for storage and analysis using the built-in library of data analysis tools provided. Once your measurement has been run on the Fluidity One-M, and the corresponding JSON file transferred from the instrument onto Fluidity Insight via a USB flash drive or the data bridge, the results are ready for analysis. Here multiple measurements can be linked together to generate experimental results collected over multiple runs.

On Fluidity Insight, assays can be analyzed using either a Bayesian fit analysis or non-linear least squares analysis. Fluidity Insight will run the analysis and display the results in both tabular and graphical formats.

Other documents

- IFU-001 - User guide for Fluidity One-M
- IFU-002 - Fluidity One-M chip-plate package insert

Glossary

Analysis: Computation of the measurements to produce results.

Bayesian analysis: A method of statistical inference that allows one to combine prior information about a population parameter with evidence from information contained in a sample to guide the statistical inference process.

Components: An automatically selected group of measurements where the species (labeled, unlabeled and/or complex) in each measurement match but concentrations differ.

Custom import: A CSV file describing sample positions and makeup that is used for running the instrument in custom import mode.

Experiment: A group of measurements that are analyzed together.

MAffCon: Membrane protein Affinity and Concentration.

Measurement: A sizing result from a single circuit in a single run.

NeSt: Neuroaffinity and Stoichiometry.

Non-linear Least Squares: (NLLS) is an optimization technique that can be used to build regression models for data sets that contain nonlinear features. Models for such data sets are nonlinear in their coefficients.

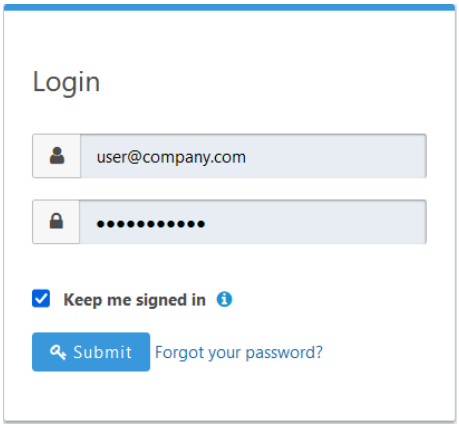
SAffCon: SeroAffinity and Concentration.

2. Account set up

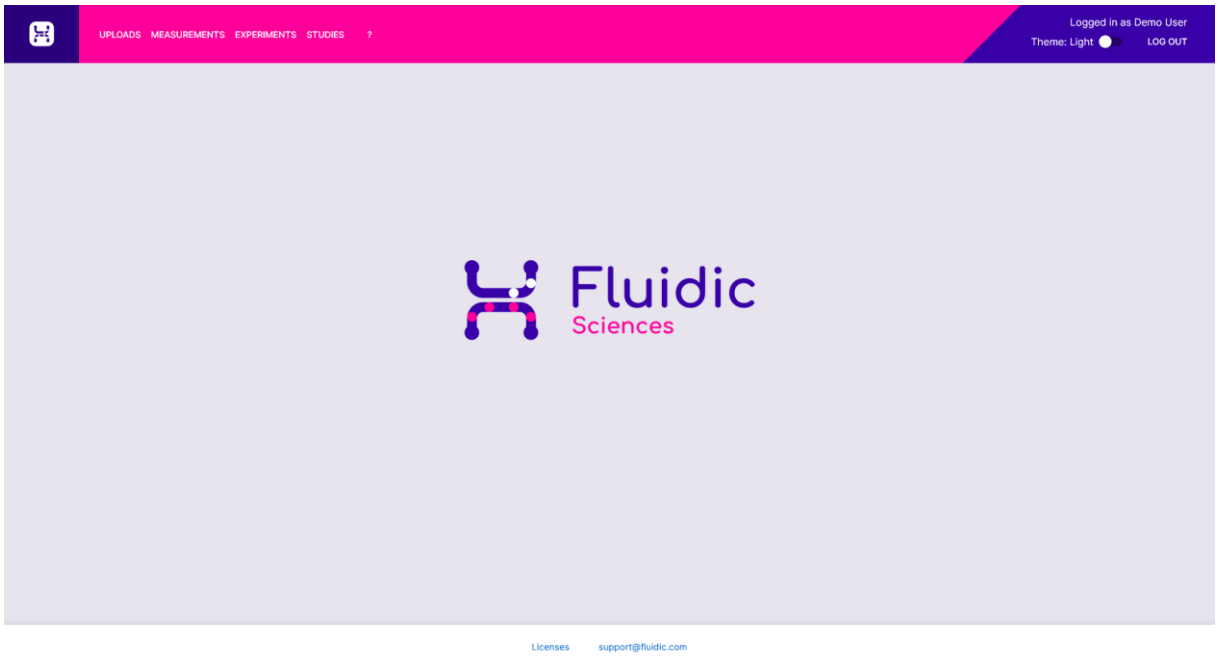
Your Fluidity Insight user account will be created by Fluidic Sciences when you receive your instrument or order a Fluidity Insight subscription. Your user account will be connected to your email address to allow password reset if required. Please contact customer support at techsupport@fluidic.com for support and information.

Logging into Fluidity Insight

1. Navigate to: https://cloud.fluidic.com. You will automatically be directed to the login page.



2. Type Email address and Password.
3. Select *Submit*.



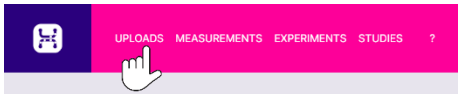
The landing screen will be shown. From here navigate to the UPLOADS tab to upload new data files and MEASUREMENTS tab to view and manage existing data on the platform. The EXPERIMENTS tab allows the setup, running and viewing of analyses, while the STUDIES tab provides tools for visualizing multiple experiments simultaneously. This user guide can be accessed via the question mark icon. A link to email Fluidic Sciences technical support team is provided at the bottom of the page should you need help.

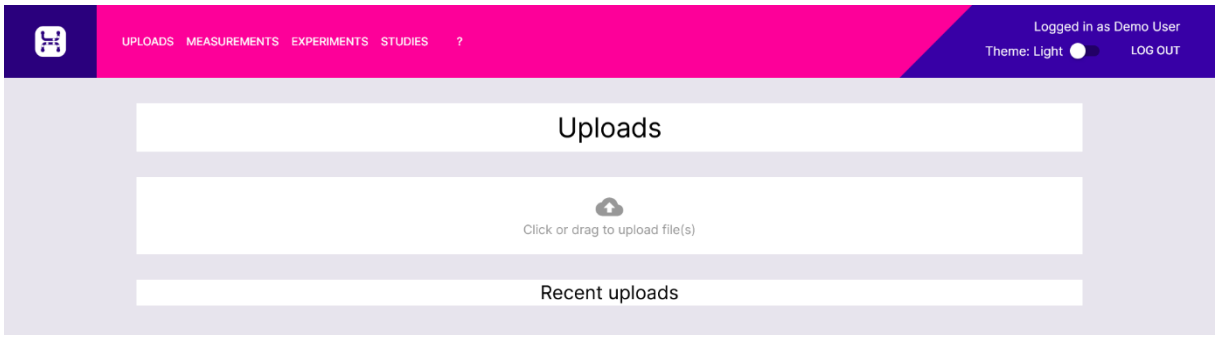
3. Importing measurements

Measurements from Fluidity One-M can be uploaded into Fluidity Insight for analysis.

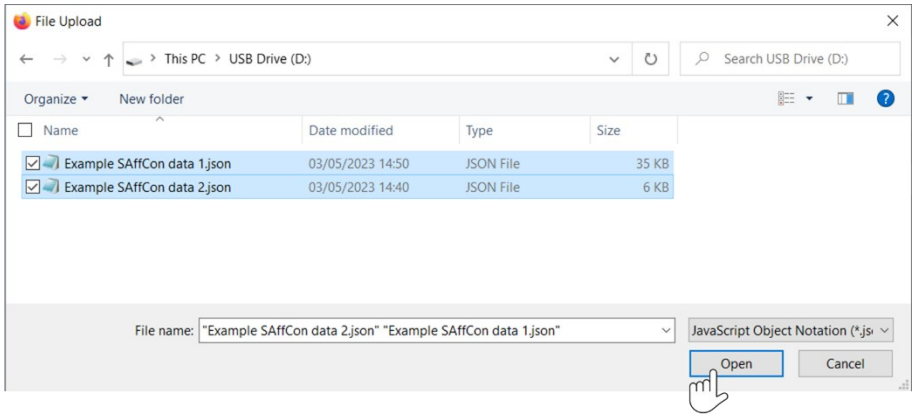
NOTE: A measurement is a sizing result from a single circuit from a Fluidity One-M chip-plate. All the measurements in the JSON file are uploaded to the measurements page. Measurement results on this tab can be combined into an experiment on the Experiments tab (see page 12).

1. Select *UPLOADS* tab.

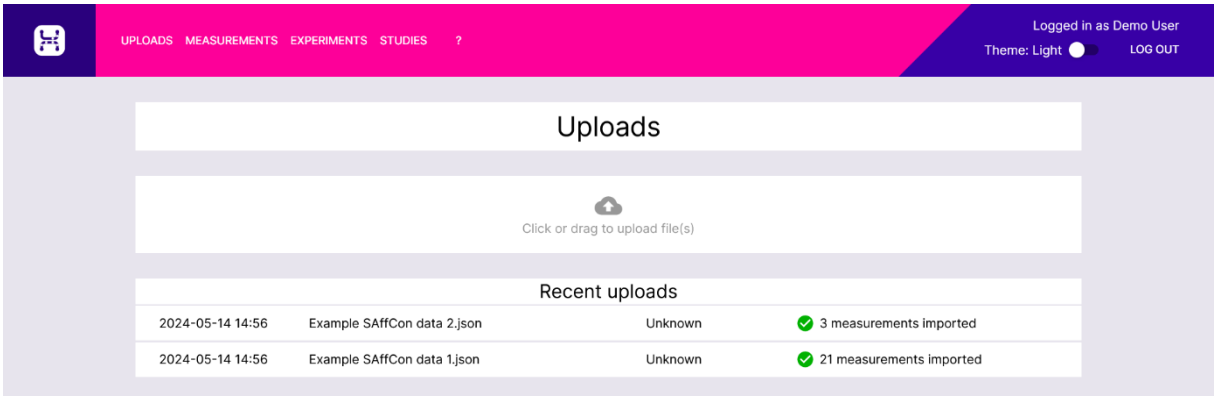




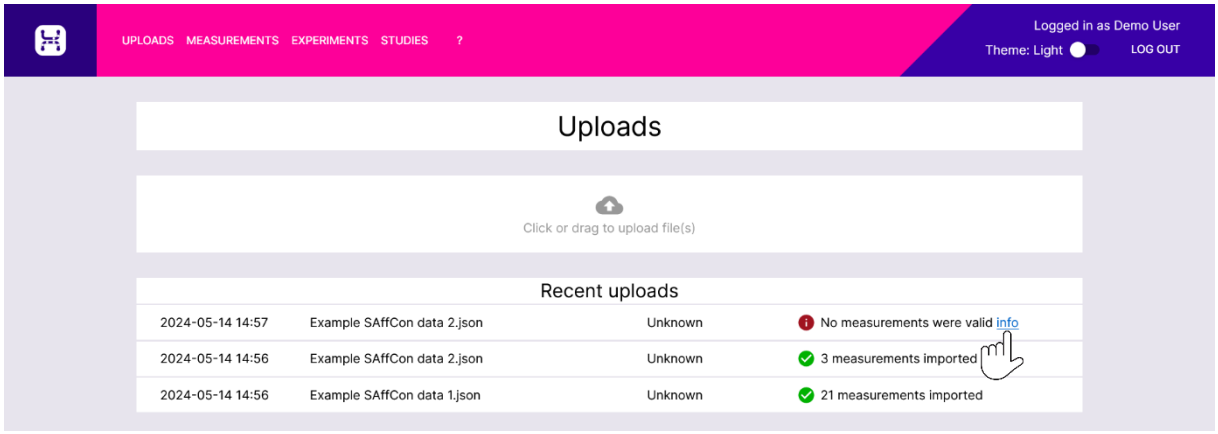
2. Files can be uploaded by dragging and dropping into the upload bar. Alternatively, click on the upload bar and a file dialog will open to select files for upload.



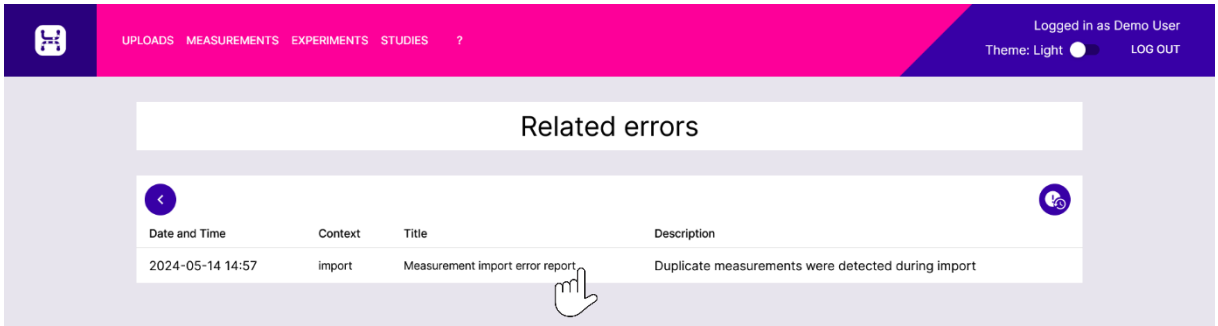
3. Navigate to where the JSON file is stored > select the JSON file(s).
4. Select *Open*.
5. Files will be uploaded and appear in the Recent uploads list. Successful import will be indicated.



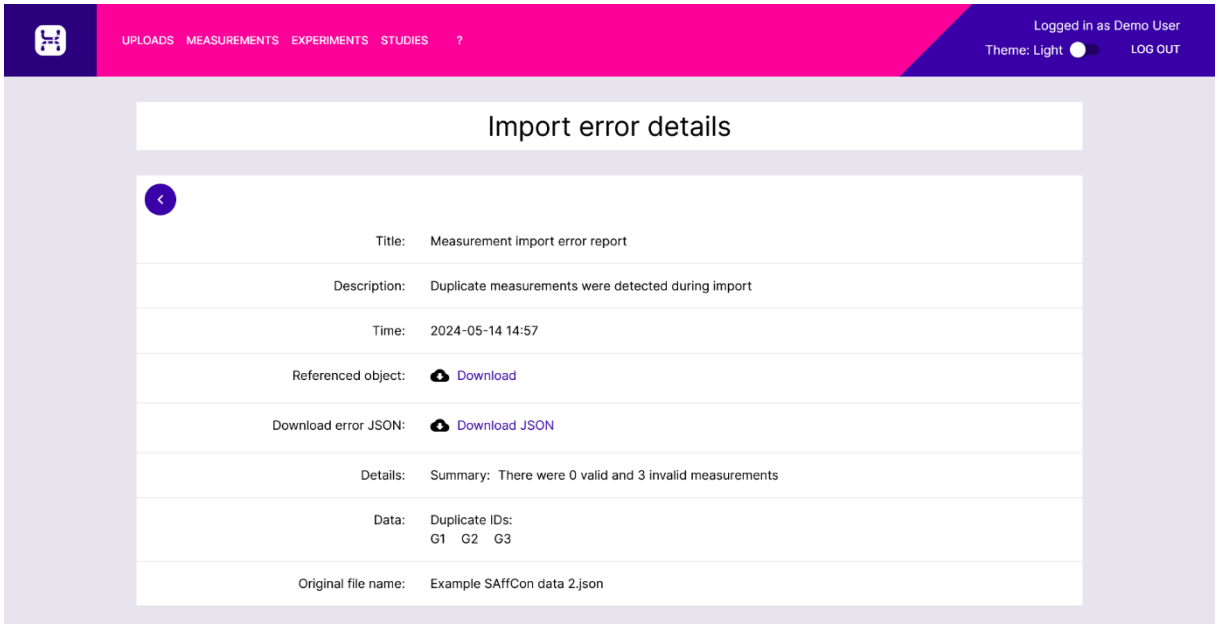
NOTE: Valid measurement data will be imported to the measurements screen and available to be added to an experiment. Measurements that resulted in errors will be automatically excluded and an error report will be generated. If the same file is uploaded more than once this will be recognized and measurements from the initial upload will not be replaced; an upload error report will be generated indicating which measurements were rejected as duplicates.



For information on why any circuits were rejected, click on the info hyperlink.

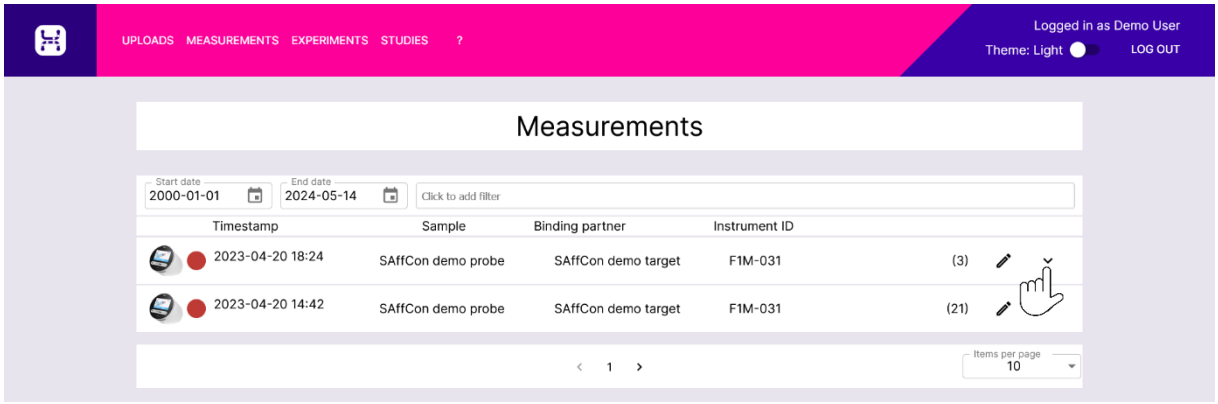


Additional detail such as specific circuits that were rejected and ability to download and check the file concerned are available by clicking on the error information line.

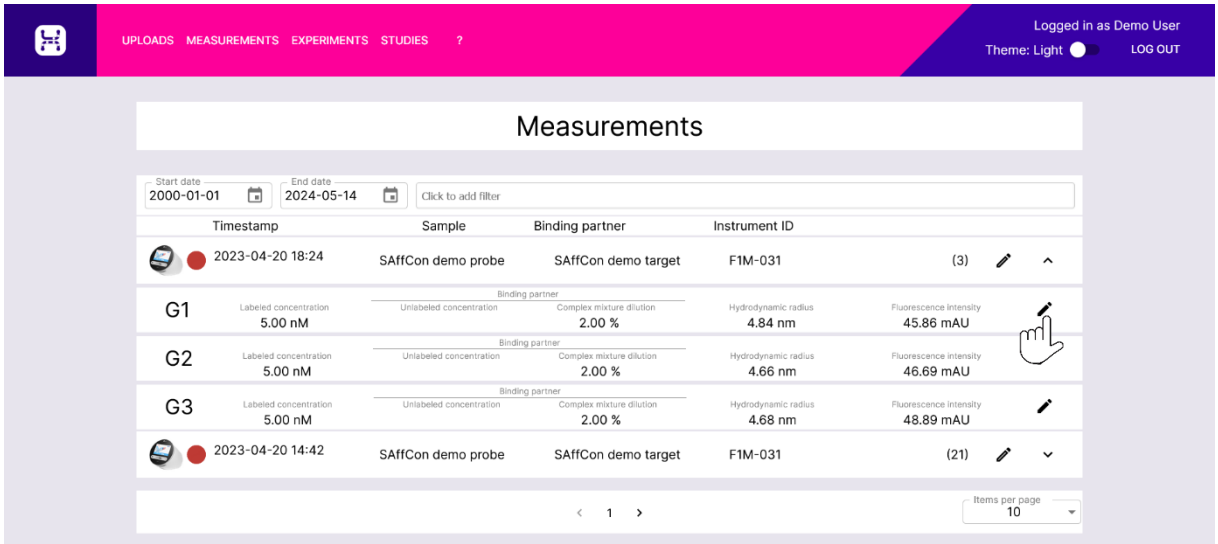

UPLOADS MEASUREMENTS EXPERIMENTS STUDIES ?
Logged in as Demo User
Theme: Light
LOG OUT
Import error details
Title: Measurement import error report
Description: Duplicate measurements were detected during import
Time: 2024-05-14 14:57
Referenced object: Download
Download error JSON: Download JSON
Details: Summary: There were 0 valid and 3 invalid measurements
Data: Duplicate IDs:
G1 G2 G3
Original file name: Example SAffCon data 2.json

4. Viewing and managing uploaded measurements

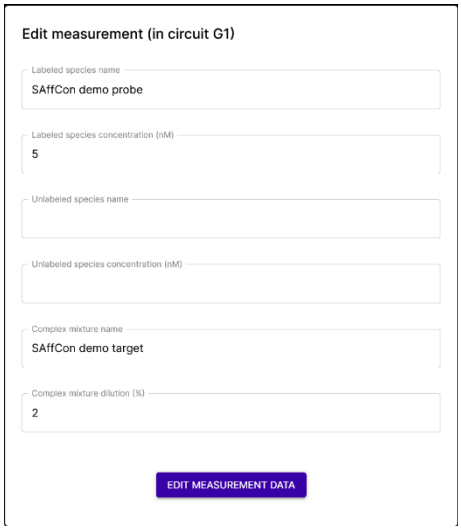
All measurements uploaded to Fluidity Insight are shown on the MEASUREMENTS tab. Measurements are ordered by date of running, with most recently run measurements first. Measurements are automatically grouped by sample name, binding partner name, and date. The color setting of the group (red, green, or dual color) is indicated by the colored circle on the left-hand side. On the right-hand side of each line is the number of individual measurements making up that group.



1. To expand any group and see the detail of the individual measurements in that group click on the down arrow.



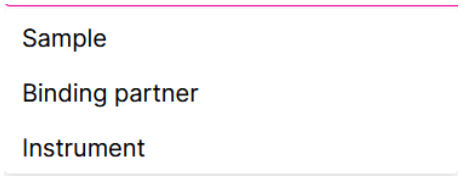
2. If errors were made during template preparation these can be corrected for individual measurements. Click on the pencil icon on the measurement to be corrected and an edit dialog will open.



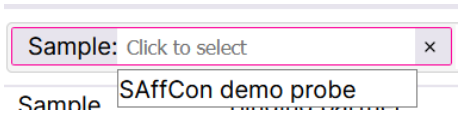
3. Make any necessary changes and click *EDIT MEASUREMENT DATA* to save the changes and return to the measurement home screen. Only numeric values can be entered in the concentration fields, and at least one concentration field must be filled.



4. Measurements in the list can be filtered by date or sample details. Adjust the start or end date to restrict the measurements displayed. To filter by sample properties, click in the filter cell.



5. A pop-up will show the options available for filtering. Select the relevant property to enter it into the filter bar. Click on *select* next to the filter property to bring up an auto-populated list of options.

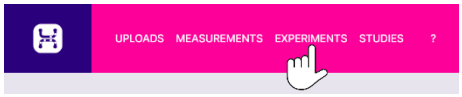


6. Select the desired value to view only measurements with that value in the relevant field.
7. Multiple filters can be applied simultaneously. Only measurements meeting all of the filter requirements will be displayed.
8. To clear a filter, click the cross next to that filter.

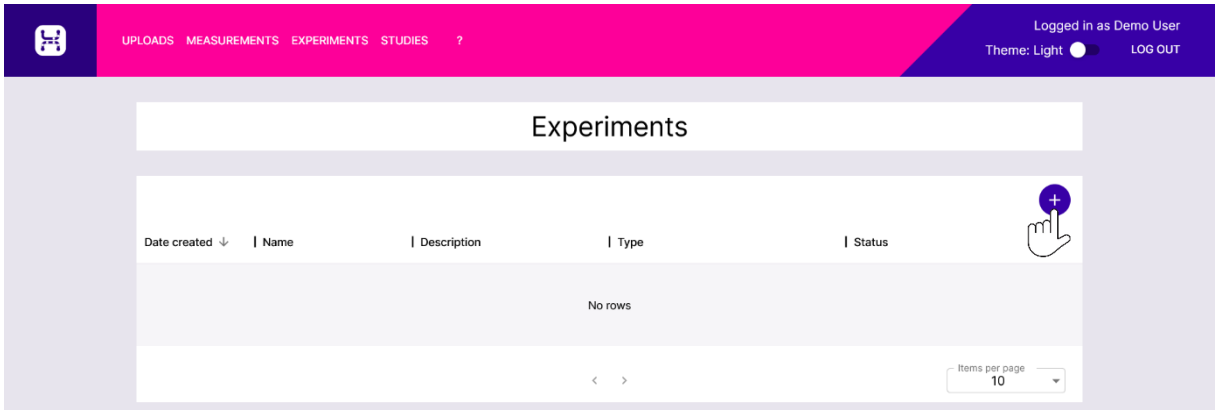
5. Creating and analyzing a new experiment

An experiment is a group of measurements that will be analyzed together, for example as a SAffCon assay.

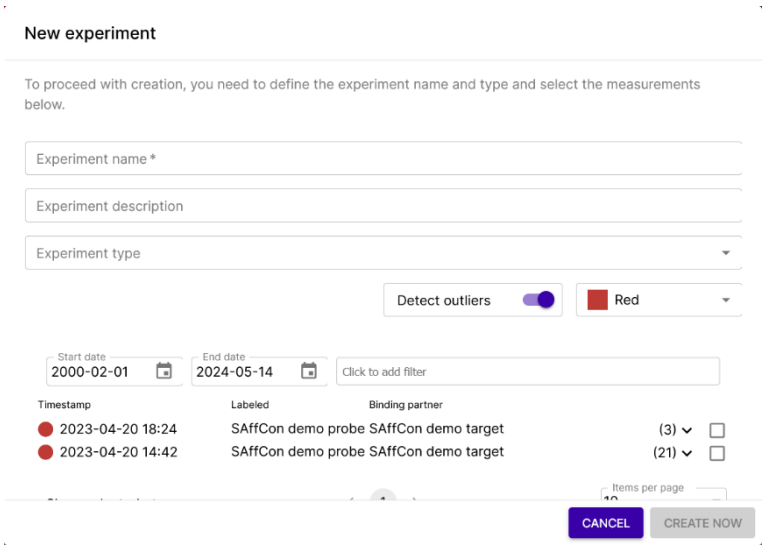
Measurements are collected from the data available on the measurements tab. Measurements can belong to multiple experiments.



1. Select *EXPERIMENTS* tab.

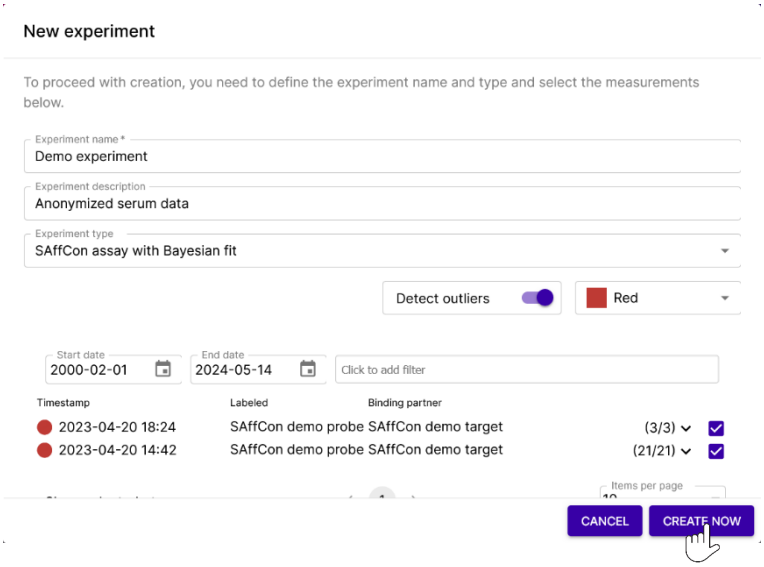


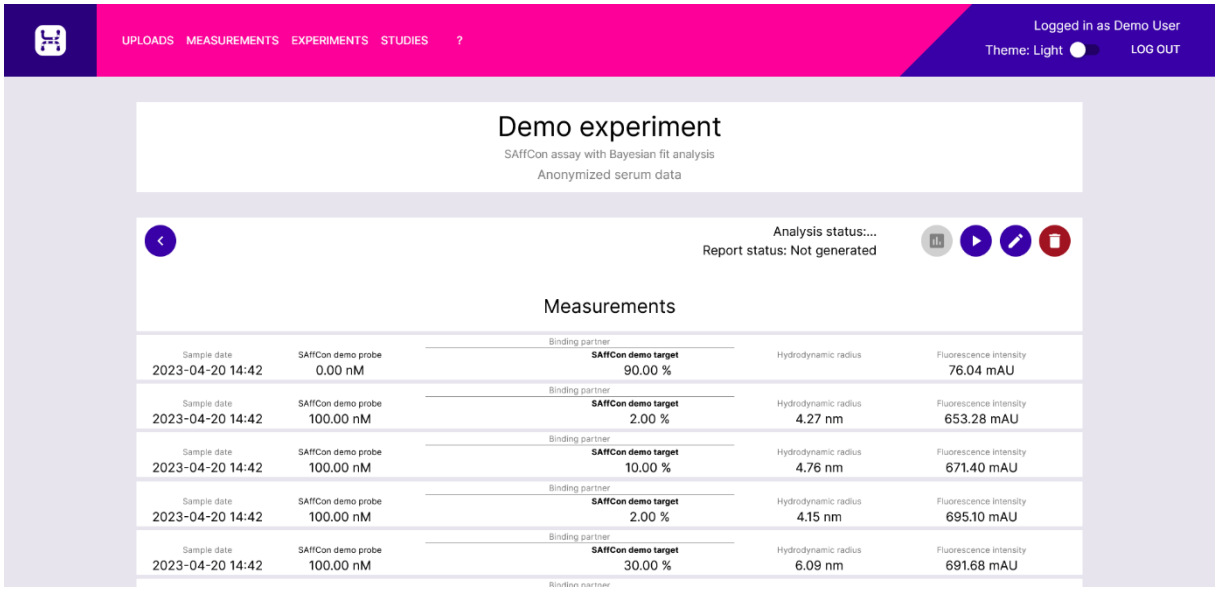
2. Select the + icon to create a new experiment.



3. Enter an Experiment name, and description if desired. Select assay type (**Binary binding assay**, **SAffCon assay**, ***MAffCon assay***, *or* ***NeSt assay***) and fitting approach (***Bayesian*** *or* ***Non-linear least squares***) as appropriate. Finally, select the measurements to include in the analysis. The measurement list can be filtered and viewed as described above in Section 3. For MAffCon assays the number of cells and total volume used to produce the membrane protein library are also required inputs.

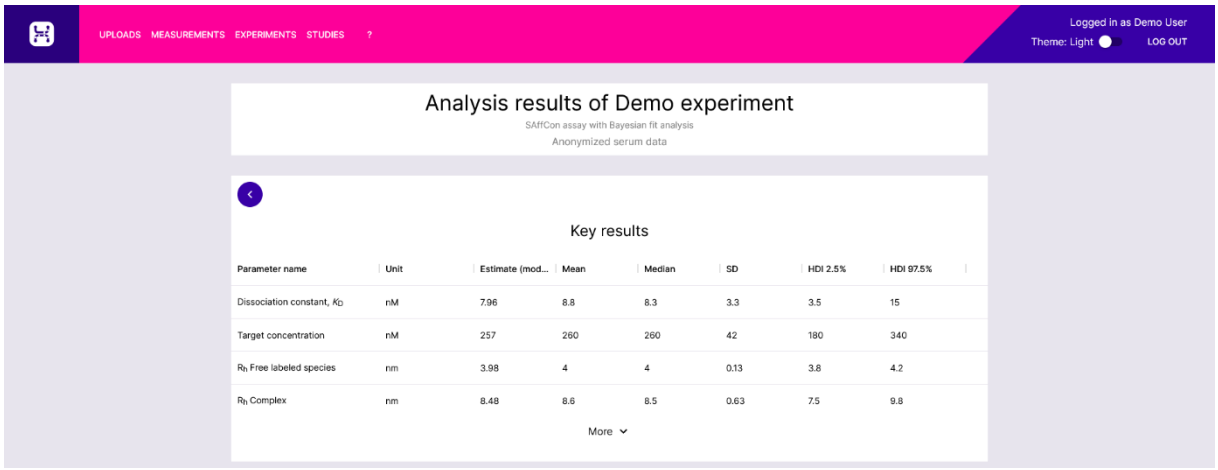
4. If automatic detection of outliers is not required then turn this function off using the toggle switch. Outlier detection checks all datapoints and flags points failing any of the following criteria for exclusion:
    - Physically impossible results: negative diffusion, greater than 100% diffusion, negative hydrodynamic radius, negative total intensity after background correction, or undetermined hydrodynamic radius
    - Points with diffusion more than five times the standard deviation of the other replicate points
    - Points deviating by more than two times the standard deviation of the whole replicate set from the mean of the replicate set
    - Points with an absolute diffused fraction more than 5% from the mean of the replicate
5. The color selection function is for use with dual color measurements to allow selection of the desired color set from the group. For single color measurements it has no effect.
6. When ready, select *CREATE NOW*.



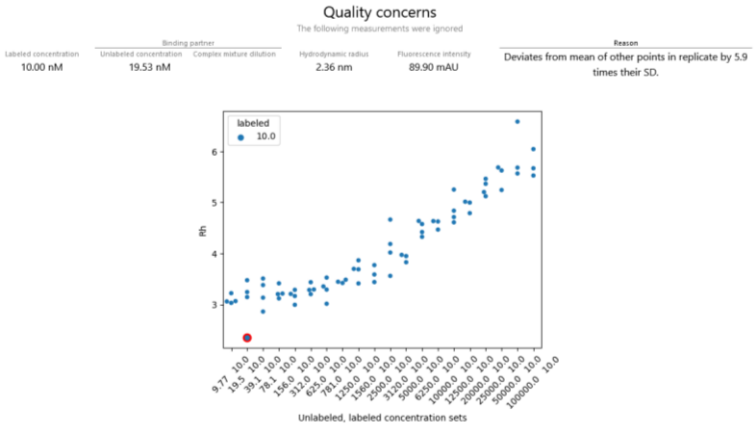


7. An experiment overview screen will be displayed. This lists the measurements composing the experiment along with control options on the top right. When the experiment is complete a results data package can be downloaded by clicking the Download link beside Report status. The data package contains a pdf summary of the results, png images of all output plots, fit parameters in json format and a csv file detailing the input data, including background-corrected sizes. If the report is not autogenerated click Generate for manual creation.

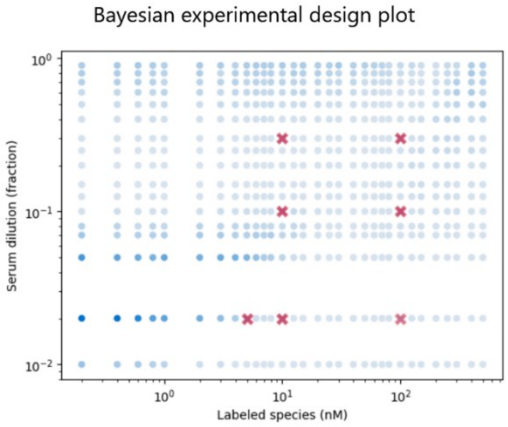
Symbol:	Description:
	The view results button is greyed out until the analysis has been run.
	When complete the view results button will be activated. Click this button to access the results.
	Click the play button to run the analysis. Analysis status will update to Created and then to Running while the experiment is analyzed. When complete the status will update to Completed. If fitting was unsuccessful the status will state Failed. In case of failure contact techsupport@fluidic.com for help with diagnosis. **NOTE**: Due to the computational complexity of Bayesian fitting, the analysis will take approximately three minutes to complete; multiple fitting analyses can be started simultaneously and they will be queued and then run sequentially in the order started. NLLS fitting is significantly faster.
	Select the pencil icon to edit the experiment details. This will re-open the experiment setup screen (step 2-3 above) for editing and updating.
	Click the rubbish bin icon to delete the experiment. A confirmation dialog will appear, and if confirmed the experiment will be deleted. The measurements making up the experiment will remain on Fluidity Insight.



8. Results are presented on a single page. Key values are tabulated at the top of the page with graphical presentations below. Images can be saved by right clicking and selecting *Save image as...*



9. If outlier exclusion was enabled during setup any excluded points and the reason for their exclusion will be displayed below the results table, with a plot showing all data and highlighting the excluded points.

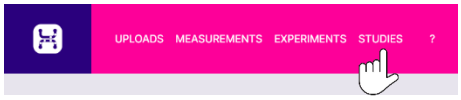


10. If Bayesian fitting was selected for the analysis, at the bottom of the page the Bayesian Experimental Design plot is shown. This provides guidance on the next experiments that should be run to most efficiently improve the data fit given the data obtained already. The darkest blue points are the most information rich and should be targeted for the next experimental conditions to be run. To do so, setup a new custom template and run the experiment on the Fluidity One-M. The red crosses indicate experimental conditions that are already included for the analysis.

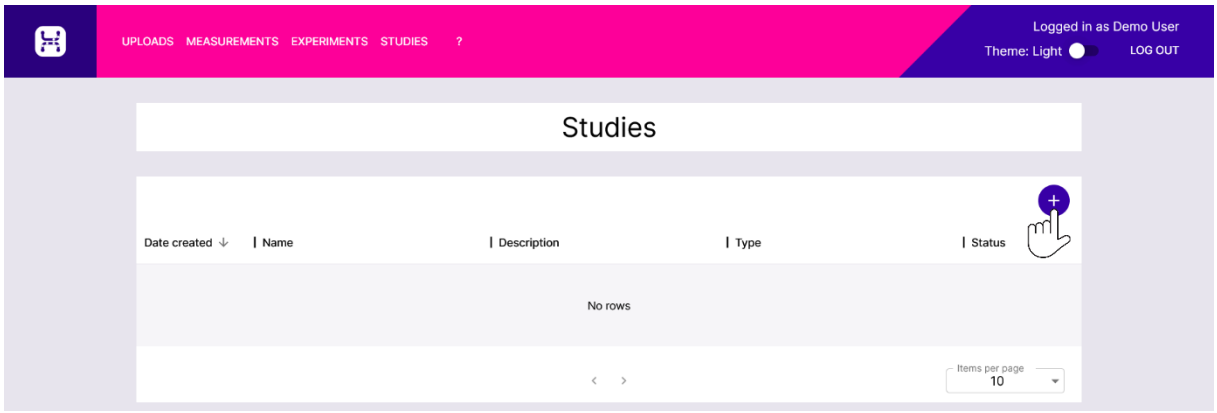
NOTE: If any measurements in a completed experiment analysis are edited, or if measurements are added or removed from an experiment then the experiment is reset and must be run again to account for the changes made.

6. Combining experiments to form studies

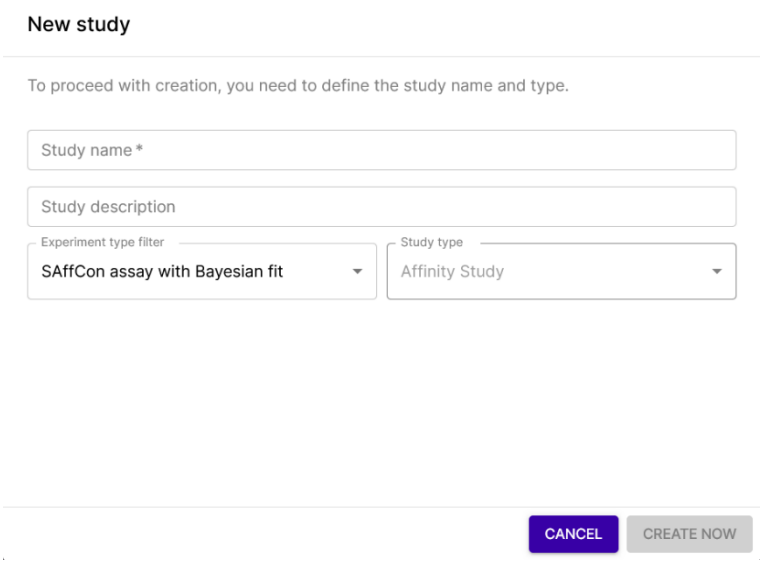
A study is a group of experiments that are compared to understand the differences between samples or cohorts.



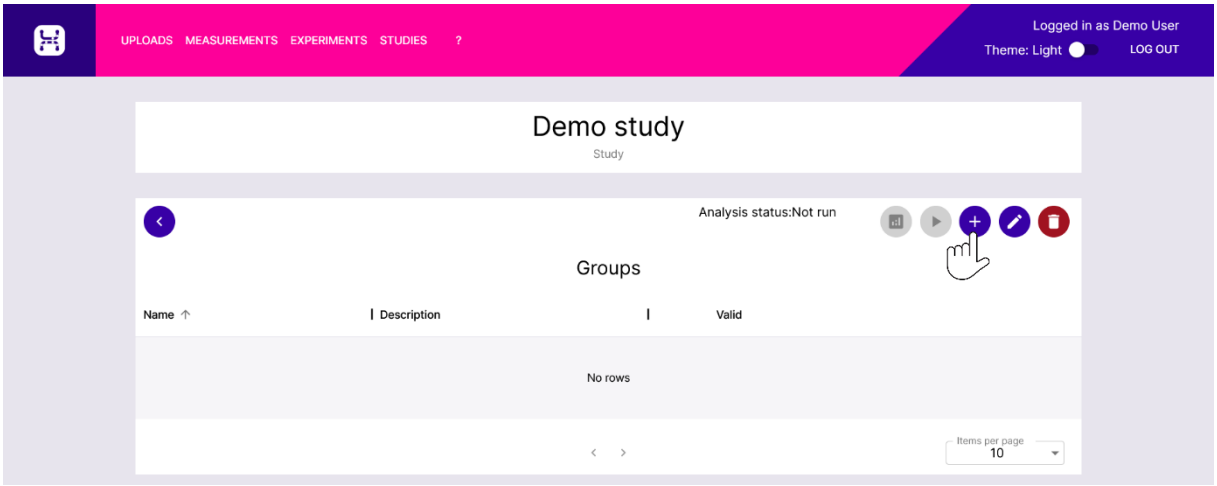
1. Select *STUDIES* tab.



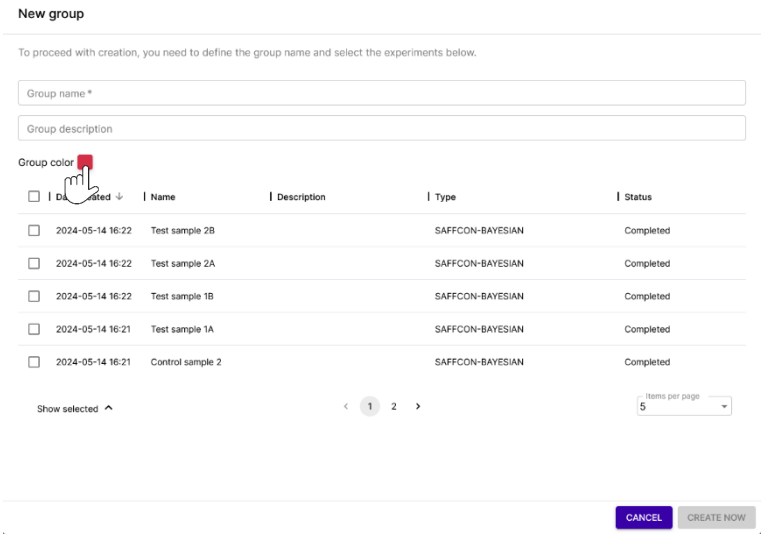
2. Select the + icon to create a new study.



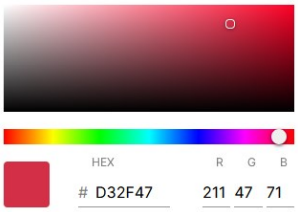
3. Enter a study name and description if desired. Select the study type and when ready, select *CREATE NOW*.



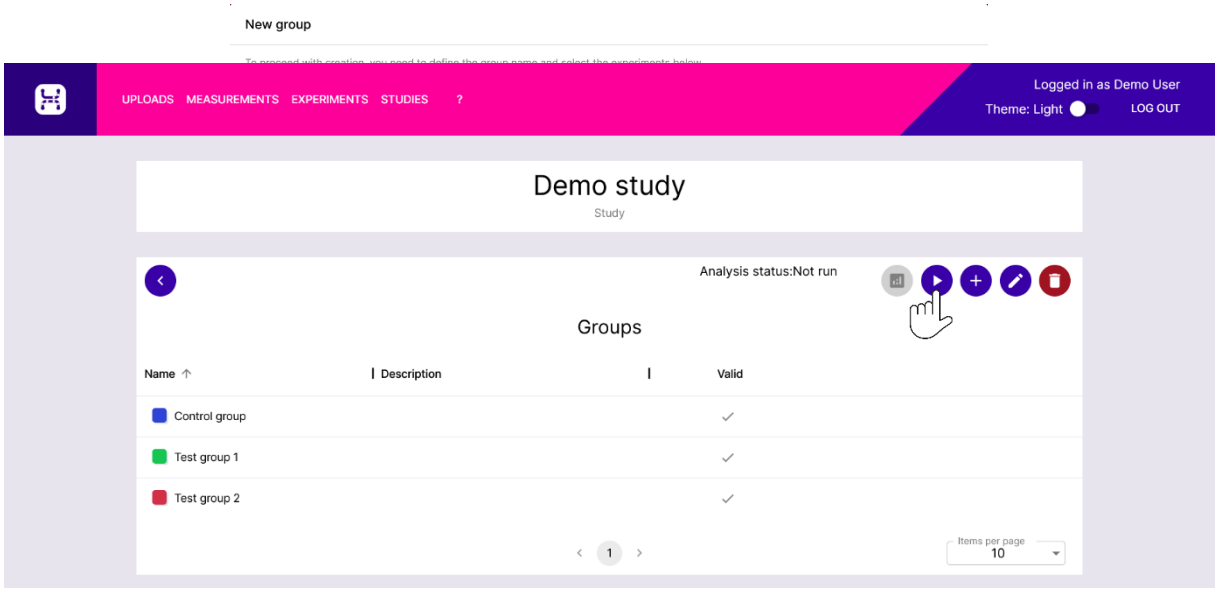
4. A group setup screen will be displayed. All members of a group will be plotted in the same color and typically would represent a particular treatment group. To create a group select the + icon. Note the icon symbols have the same meaning as the experiment icons (p15) applied to groups rather than experiments.



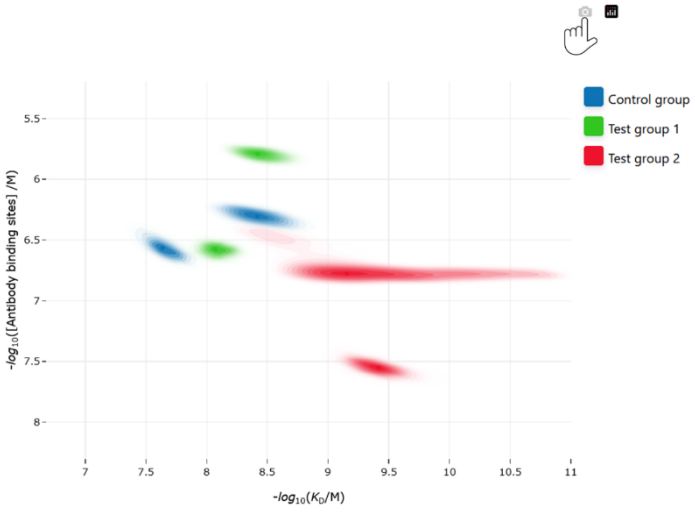
5. Input a name for the group and a description if desired. Select the experiments to include in that group by checking the box on the left of the relevant lines. The color to be used for plotting the group is initially randomly selected and can be changed by clicking on the group color indicator box.



6. To change the color use the sliders to change the hue and transparency, and click in the brightness-saturation plot to pick a color. Specific colors can be chosen by entering either hex or RGB values. Colors can also be adjusted later when the plot is drawn.
7. When the group is complete, click *CREATE NOW*.



8. Repeat steps 4-6 for each different group.
9. When complete, a collection of groups will be displayed. If everything is correctly assigned press the play button to generate the study plot.
10. Analysis status will change to Created and processing will begin. The processing is computationally intensive and will take a few minutes to complete. The processing time increases as more experiments are included.
11. When processing finishes the Analysis status will update to Completed and the View analysis button will become active. Press this button to access results. Note that plotting can also take a few minutes to complete.



12. The colors on the plot can be adjusted by clicking on the color indicator boxes in the legend. The plot can be downloaded in png format by clicking on the camera icon.

NOTE: If any experiments in a completed study are edited or voided then the study is reset and must be run again to account for the changes made.

About us

It’s not just the proteins that make life, it's the interactions among them. Here at Fluidic Sciences, we make protein interaction analysis easy and robust by developing transformative in-solution technologies and accessible instruments that help scientists quickly and accurately understand how proteins truly interact.

For more information about us, please visit our website www.fluidic.com.