FCAP Array Infinite

User Manual

For Windows™ operating systems

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Limitations

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

FCAP Array software has been written and manufactured by Soft Flow, Ltd.

Software is covered by a one-year policy under which revisions released during the twelve month period from original installation will be provided at no charge. Software is provided as-is and is not warranted to be defect-free. The foregoing is exclusive and in lieu of all other warranties either expressed or implied, including warranty of merchantability, fitness for particular purpose, and non-infringement. Purchaser agrees that SoftFlow, Ltd is not liable under the software warranty or otherwise relating to the software for indirect, consequential, or business damages that can be suffered.

Customers who have questions or problems regarding the FCAP Array software should contact Soft Flow Ltd., at support@softflow.com, see section Technical Assistance.

Contents

PREFACE

The ***FCAP Array Software User Manual*** describes how to install and use this **Flow Cytometric Analysis Program (FCAP) Array** software. The software facilitates experiment design and data analysis for multiplex bead assays. The author assumes you have working knowledge of the basic Microsoft® Windows® operating system and basic laboratory practice and experience regarding multiplex bead assay analysis.

If you are not familiar with the operating system of your computer, refer to the documentation provided with your system and/or google your problem regarding the version of Microsoft® Windows® operating system.

Before using FCAP Array™ software, please review the **ReadMe** file by ***double-clicking*** the FCAP Array ReadMe icon added to **Start > All Programs > FCAP Folder** at installation.

The **ReadMe** file contains important information not covered in this manual.

The *User Manual* explains the use of the software through normal multiplex bead assay data processing. If you have any problems or questions please contact us at: support@softflow.com

ORGANIZATION OF USER MANUAL

This user manual was written according to logic of the **Main windows** of each **Library (Experiments, Bead Library, Plex Templates, User Management, Units)** and the functions that can be used in the **Home tab** of each library, the **Menu** and **Settings tabs** have the functions regardless of which library is selected.

NOTE: Any text that is **blue** and **underlined** is a **hyperlink** to either an **external website** or an **internal link** to a section within this document, click on any of them by using the **(Ctrl + hyperlink)** to go the relevant section or use the Document map of Microsoft Word or Adobe Reader bookmarks to navigate through this user manual or click on the hyperlinks below. The beginning of the user manual starts off with the usual, Copyright and Legal Information, Limitations and then the following:

Thank for using **FCAP Array Infinite** software, because we like to make a difference for the analysis of multiplex bead assays.

TECHNICAL SUPPORT AND POINTS OF CONTACT

Technical Assistance

For technical questions or assistance in solving a problem read the sections of this documentation specific to the operations you are performing.

See Section 9: Troubleshooting

See online Manual on FCAP Array website (http://softflow.com/fcap-array/)

FAQ (http://softflow.com/support/faq/)

Technical support (http://softflow.com/support/technical-support)

Please report any bugs in our software on our generic contact form online at (http://softflow.com/support/contact-us/)

If additional assistance is required contact a Soft Flow Hungary Ltd. technical support representative or your supplier.

When contacting Soft Flow, have the following information available:

- name and version of the software you are using
- windows operating system the software is installed and its configuration
- any error messages
- details of recent system performance

Point of Contact

For FCAP Array software support in North America:

Soft Flow, Inc., Sales Office
5009 Excelsior Blvd. Suite 154
St. Louis Park, MN 55416

Tel: +1 (952) 681-7960 Fax: +1 (952) 303-5178
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TERMS USED AND DEFINITIONS

Terms Used

The following terms are used throughout this manual to indicate specific elements, procedures, or concepts. For additional term definitions, see Glossary.

Item	Specification
Term	Description
bead	An analyte-specific capture particle with distinctive, discrete fluorescence characteristics. Also, an FCAP Array software element that describes the analyte binding and fluorescence characteristics of a bead.
bead assay	A method using beads and reporter antibodies to detect or measure the concentration of analytes in samples.
cluster	A population of events in a bead assay data file. Each cluster corresponds to one bead set, therefore, the events in a cluster have a distinctive, discrete fluorescence characteristic.
experiment	A bead assay. Also, formalizations or file representations of a bead assay, including an FCAP Array software element that defines the **plex(es), standards, controls**, and **test samples** for a bead assay.
Plex	One or more bead set used in a bead assay. Also, an **FCAP Array software element** that specifies a **plex, a standards set, and the bead-cluster assignment**.
replicate(s)	One or multiple identical sample preparations. When more than one replicate of a sample is used, average results are reported.
Sample	A solution containing analytes to be measured by a bead assay. A sample can be a test, a standard, or a control.

Definitions

The following table lists text and keyboard conventions used throughout this guide.

1.1 Table	Text and keyboard conventions
Convention	Use
NOTE	Indicates key information about the software.
TIP	Highlights features or hints that can save time and prevent difficulties.
Italics or Bold	***Bold -Italics*** are used to highlight "actions" by user as in ***Clicking*** or ***Selecting***. Plain **Bold** is used to clarify **GUI elements** and are used to indicate how to start software functions.
>	The **arrow** indicates a menu choice. For example: **Select File > Print** means to ***select*** **Print** from the **File menu**.
Ctrl-X	When used with **key** names, a dash means to press two keys simultane-ously. For example, **Ctrl-S** means to hold down the **Control key** while ***pressing*** the **S** key.

SYSTEM SUMMARY

This chapter specifies system requirements and provides instructions for installing, reinstalling, uninstalling and updating FCAP Array software.

System Requirements and supported Cytometers

Minimum Hardware configuration

Item	Specification
CPU	Intel Pentium® 4 (2 GHz or equivalent)
RAM	512 MB or higher
Video RAM	16 MB or higher
Hard drive	50 MB for installation
Monitor	XGA, 1024 x 768 pixels or higher (1440 x 900 recommended)
Internet connection	Required for license check

Software requirements

Microsoft Windows XP, Vista, or Windows 7 / 8 / 8.1 (32-bit and 64-bit versions)

Validation

FCAP Array software has been validated for use on computers running English (United States) Regional Options with the following operating systems:

- Microsoft Windows XP Professional
- Microsoft Vista
- Microsoft Windows 7 / 8 / 8.1 (32-bit and 64-bit versions)

Compatibility with computers and operating systems using other Regional Options has not been tested.

Supported Cytometers

- BD FACS Instruments
- BD Accuri C6 Instrument
- iCyt
- ec800
- Stratedigm Instruments
- Beckman-Coulter Instruments
- Luminex Instruments
- Attune
- Miltenyi MACSQuant

1

STARTUP AND AUTHENTICATION OF FCAP ARRAY

This section describes the process of starting and authenticating FCAP software.

NOTE: Once the FCAP has been installed the software license needs to be authenticated.

1.1 Installing FCAP

To install FCAP Array software, take the following steps:

- Download FCAP Software from the following link:

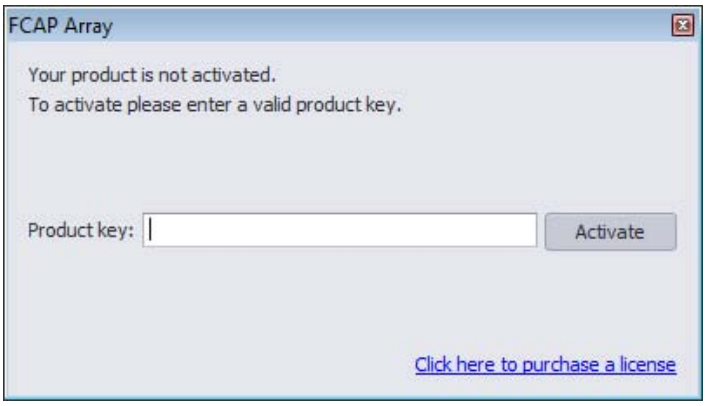
  http://softflow.com/Download/getdownloadlink.html?type=fcapfull2015

- ***Fill*** in your details **Name, Company** and Email address and ***click*** on Get download link button, then on the next page ***click*** on FCAP Array Release Installer link.
- ***Click*** on the **Save File** button.
- Ensure that no other programs are running.
- Locate the **FCAP Array Installer.exe** file and ***double-click*** on the file then ***click*** **Run**.
- ***Click*** **Next** on the installer **Welcome** screen.
- Read the license information, then ***select*** **I Agree** and ***click*** **Next**.
- Change the destination folder for FCAP Array software if necessary and ***click*** **Install**, however we recommend you accept the default location.
- ***Click*** **Finish** to exit the installer and start using FCAP Array software.
- The **Read Me** PDF file should start automatically if the **Read Me** file checkbox was ***ticked***, if not please install Acrobat Reader.
- The **Activation dialog box** will request your **Product Key**, see below.

1.2 Authentication of FCAP Array Software (1st time)

Purchase a subscription for the use of FCAP Array Infinite Software

- Purchase subscription from the following link: http://softflow.com/buyfcap/
- You have received your Product key via email.
- Enter the Product key, then ***click*** the **Activate** button in the Activate dialog box.



1-1. Figure Activation window

NOTE: 1 month, 3 month, 6 month and 1 year subscription is available. Your software will be valid for the duration of your subscription, once expired, please renew when you need again!

Enjoy the software and explore the results of your experiments.

1.3 Updating FCAP

To update FCAP Array software, take the following steps:

- ***Click*** the **Help (?)** icon in the upper right corner of FCAP Array software and click on the **Check for Update** in the drop down menu or go to the following link:

http://softflow.com/downloads/download-fcap-array/

1.4 Uninstalling FCAP

To uninstall FCAP Array software, take the following steps:

Windows XP

- **Select Start > Programs > FCAP Array > Uninstall > Yes > OK.**

OR

- **Select Start > Settings > Control Panel > Add or Remove programs > FCAP Array > Change/Remove > Yes > OK.**

For Windows 7/8

- **Select Start > All Programs > FCAP Array > Uninstall > Yes > OK.**

OR

- **Select Settings > Control Panel > Programs and Features > double-click FCAP Array > Yes > OK.**

1.5 Reinstalling FCAP

To reinstall FCAP Array software, take the following steps:

- Locate the FCAP Array Installer.exe file and double-click on the file then ***click*** **Run**.
- ***Click*** **Next** on the installer **Welcome** screen.
- Read the license information, then ***select*** **I Agree** and ***click*** **Next**.
- Change the destination folder for FCAP Array software if necessary and ***click*** **Install**, however we recommend you accept the default location.
- ***Click*** **Finish** to exit the installer and start using FCAP Array software.

NOTE: If no hardware changes have been made since uninstalling FCAP Array and your product key is still valid you will be greeted with the **Login** Screen.

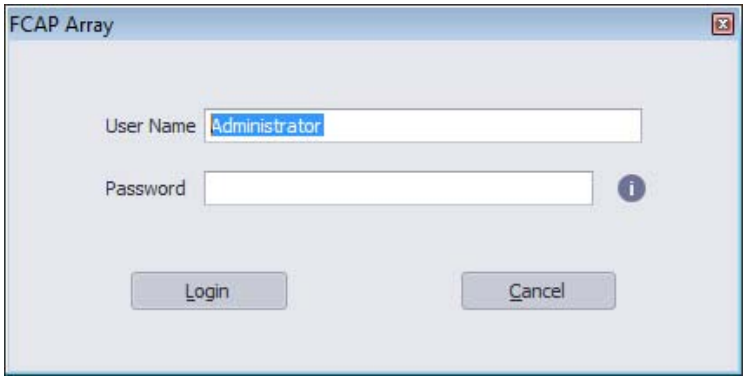
1.6 Login to FCAP

Start using the Software

To ***launch*** FCAP Array software, do either of the following:

- ***Double-click*** the FCAP Array software **shortcut icon** on the desktop
- ***Select*** **Start > All Programs > FCAP Array > FCAP Array**

The FCAP **Login** screen appears, fill in your login credentials or as an **Administrator**; use **welcome** for the **default password** and ***click*** **Login**.



1-2. Figure Login screen

NOTE: Change the "welcome" default password for administrator, if you want to save your data in FCAP Array.

- **Get to know your FCAP Array software features (see Section 4).**
- **Start using FCAP Array software (see Section 5).**
- **Tutorial (Sample Experiment) is provided in (Section 8).**

2

BEAD ASSAY SUMMARY

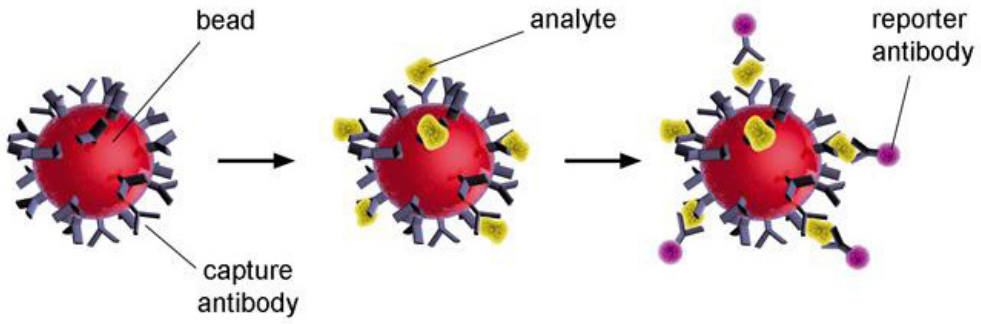
FCAP Array software facilitates the design and data analysis of bead assays. These assays can detect the presence of, or determine concentrations for multiple analytes (example: proteins and peptides) in a particular sample.

In a bead assay, one or more bead populations (beads) with discrete and distinct fluorescence intensities are used to simultaneously detect multiple analytes in a small sample volume.

The beads capture and quantify the soluble analytes by the use of a sandwich assay (**2-1. Figure**).

A particular analyte within the sample binds to a corresponding bead with a given fluorescence characteristic. The bead is coated with capture antibodies specific to that analyte. A reporter antibody (different from the capture antibody) binds to the analyte. The reporter antibody is conjugated with fluorescent molecules (different in color from those used to distinguish the beads). Unbound (excess) reporter antibodies and analytes are eliminated by washing the plate. Samples are acquired with a flow cytometer and acquisition software capable of saving the data in a Flow Cytometry Standard FCS 2.0, 3.0 and 3.1 file formats. **FCAP Array software** analyzes these **FCS files**. The bead populations are identified by a process of clustering, and the beads are assigned to the specific clusters. Analyte-specific binding is measured by the fluorescence intensity of the reporter antibody in a bead population.

2.1 Quantitative Analysis



2-1. Figure Bead sandwich assay

Quantitative analysis determines analyte concentrations based on a set of standards of known concentration.

Multiple analytes can be simultaneously measured with **FCAP Array**, within a single sample and where each analyte corresponds to a unique bead set. The calibration of the standard curve is effortlessly attained by the versatile settings of the following: number of con¬centration levels, number of samples and the standard replicates.

FCAP Array software processes data files from instruments that export standard FCS (versions - 2.0, 3.0 and 3.1) files from processing an experiment; first the software automatically identifies the clusters (to which analytes have been assigned), and then the software proceeds to calculate the median fluorescence intensity (MFI) of the detector antibody for each specific analyte.

The software fits a standard curve to the data from the concentration of the standards. The curve that is used to fit the data is selected from several available mathematical models. The standard curve is used to calculate the concentration values for each of the measured analytes in each sample.

2.2 Qualitative Analysis

Qualitative analysis allows us to detect the presence of a specific analyte in a sample. The presence of multiple analytes can be simultaneously detected with FCAP Array software within a single sample, where each analyte corresponds to a unique bead set. The controls can be either positive, negative, or both.

FCAP Array software reads the **FCS 2.0, 3.0** or **3.1** data files from an instrument used to analyze an experiment, identifies the clusters (to which analytes have been assigned), and then calculates the median fluorescence intensity (MFI) of the detector antibody for each analyte.

The calculated result will be either a positive or negative standard. The sample's result will be positive, if the measured value is larger than the positive standard's value, and the result is negative, if the measured value is less than the negative standard's value.

Comparisons can be made of the sample concentrations with the Control values. The Control definition parameters can be setup by ***clicking*** the **Control Definition** icon in the **Plex Pane.**

3

GET FAMILIAR WITH THE SOFTWARE FEATURES

The main windows of FCAP

To get familiar with FCAP features and while reading this section you should be logged in (and experimenting with the functions of FCAP) or read this section carefully **(see section on Login to FCAP).**

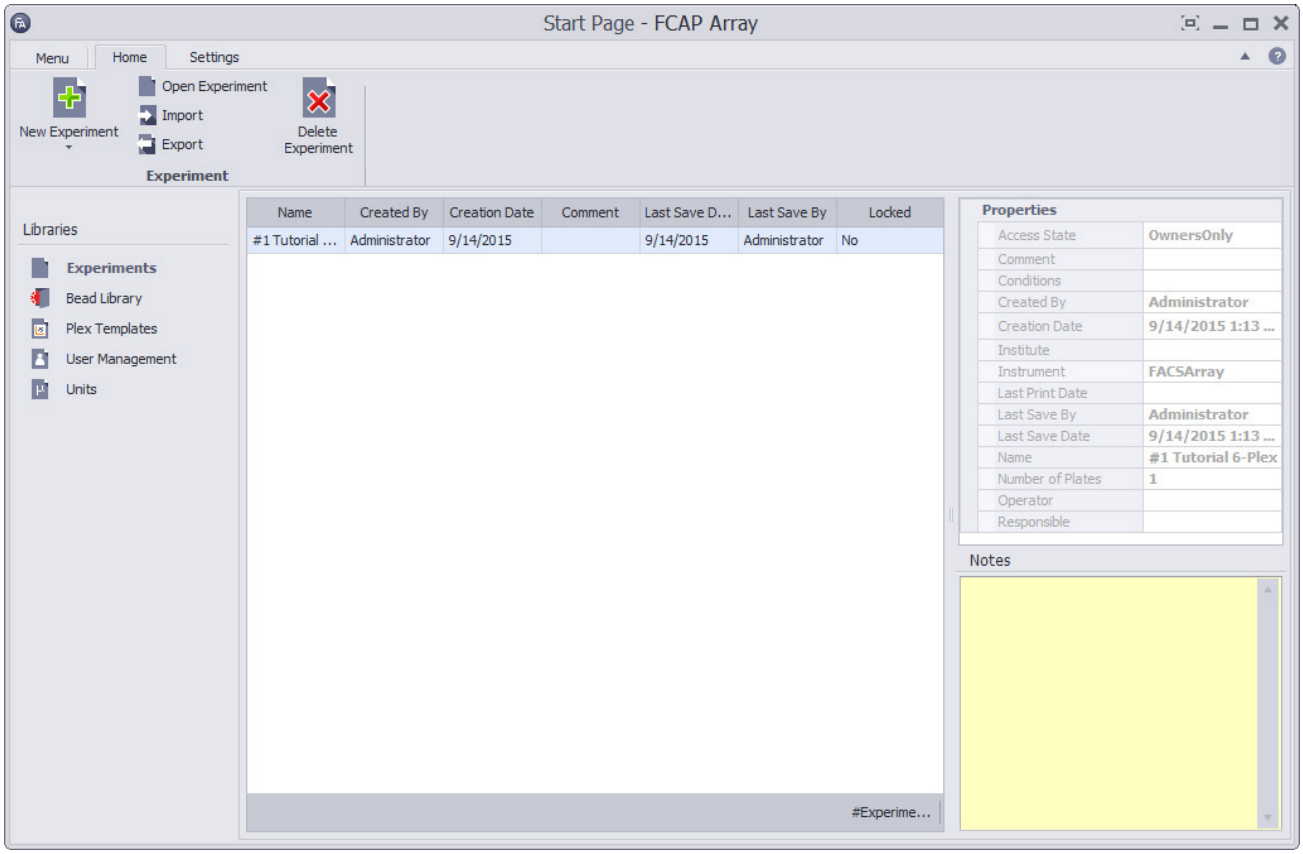
NOTE: The **Menu** and the **Settings tabs** are always available to a user if ***selected***, however the **Home tab's ribbon** is specific to the selected and to the following libraries in the **Library pane (Experiments, Bead Library, Plex Templates, User Management, Units)**

Once you have logged in to FCAP Array the **Main window** with the **Experiment Library** will appear.

To ***launch*** FCAP Array software:

Select Windows ***Start > All Programs > FCAP Array > FCAP Array***

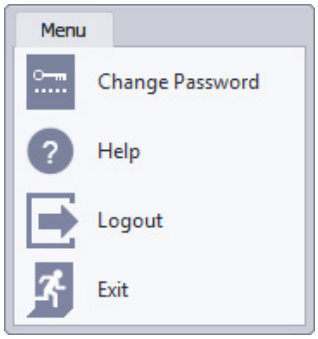
As the software launches, the **Login** screen and then the **Main window** is displayed with the **Experiment Library**.



3-1. Figure Main window with Experiment Library view

3.1 Menu Tab

Clicking on the **Menu** tab rolls down and the following functions are available for the user to ***select*** from **Change Password, Help, Logout, and Exit.**

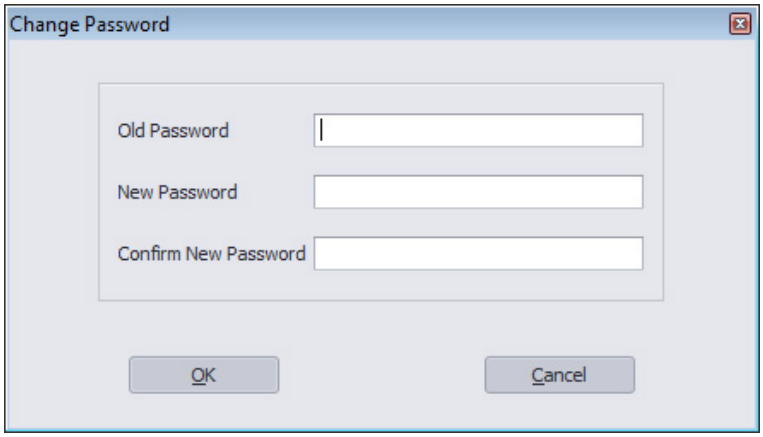


3-2. Figure Main menu button group

3.1.1 Change Password

To change the password of a user, take the following steps:

Select **MENU > Change Password >** ***type*** **Old Password >** ***type*** **New Password >** ***type Confirm*** **New Password >** ***click*** **OK.**



3-3. Figure Change password screen

3.1.2 Help

To be directed to our online **Help**, take the following steps:

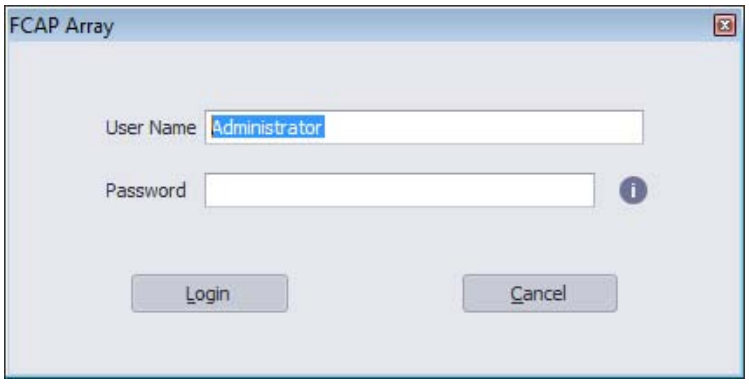
Select **Menu > Help**

3.1.3 Logout

To **Logout**, take the following steps:

***Select* Menu > Logout**

The FCAP **Login** screen appears, ***fill*** in your **login credentials** and ***click*** the **Login** button.



3-4. Figure Login screen

NOTE: This option is used to switch between users and not to exit FCAP Array.

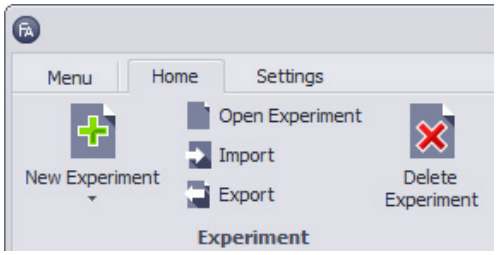
3.1.4 Exit

To shut down and exit FCAP Array, take the following steps:

***Select* Menu > Exit**

NOTE: This option is used only to **exit** FCAP Array

3.2 Home Tab ribbon



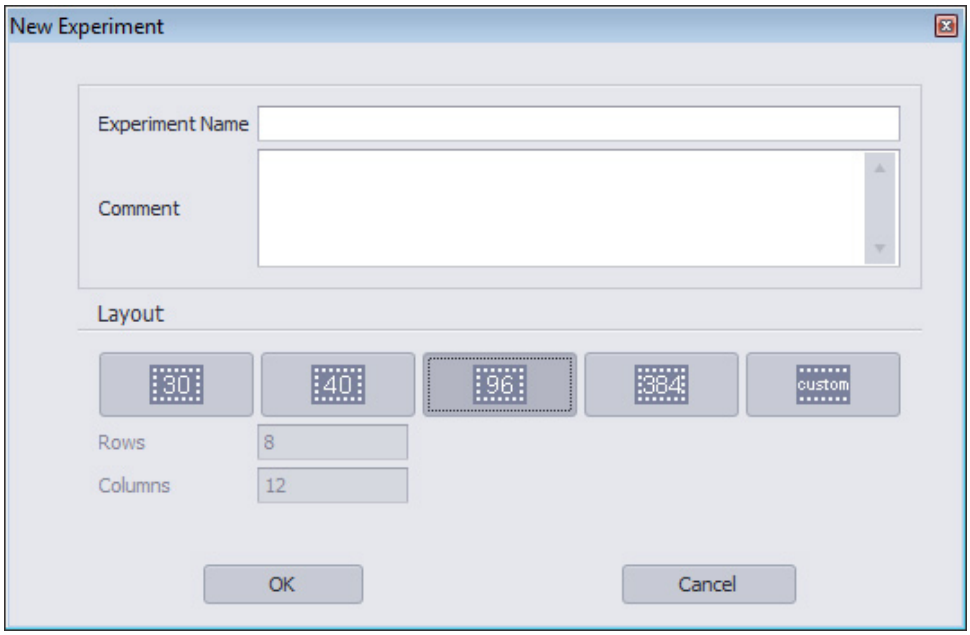
3-5. Figure Experiment button group

The **icons** in the **Home** ribbon allow the user to ***select*** from the following software functions, see below:

3.2.1 New Experiment and Empty Experiment

You can choose from either **New Experiment** or **Empty Experiment** to start a fresh experiment, by ***clicking*** the upper half of the icon - **New Experiment** or by ***clicking*** on the lower half of the icon and ***selecting*** **Empty Experiment**, the dialog box below appears, see the next page.

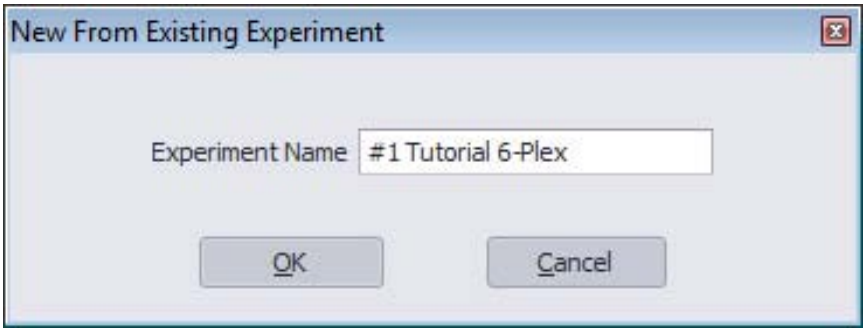
Fill in the details of Experiment and ***select*** your plate size from the available **plate buttons (30, 40, 96, 384 or custom)** and ***click*** on the **OK** button.



3-6. Figure New Experiment screen

3.2.2 From Existing Experiment

To use a previously saved or rather an existing experiment, ***select*** this option and the following dialog box will appear, see below. ***Enter*** the experiment's name and ***click*** **OK**

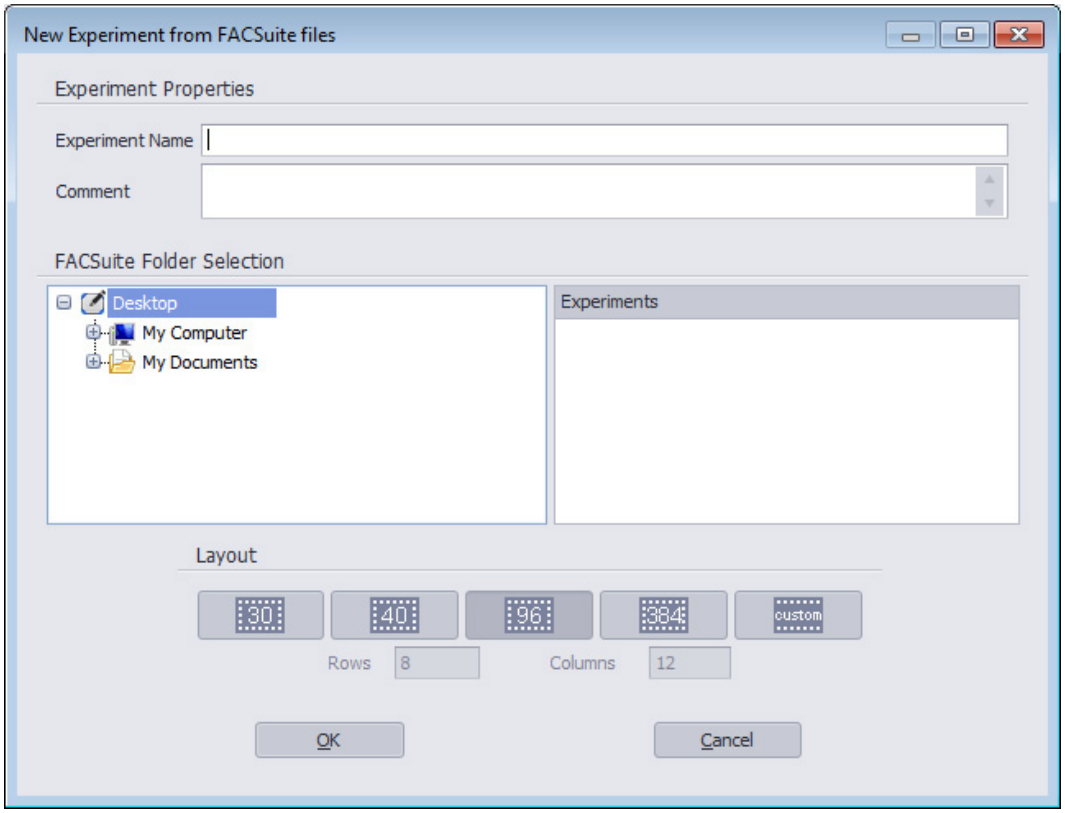


3-7. Figure New From Existing Experiment screen

The plate design layout window will appear and the user can use the previous experiment as a basis for this current experiment, all functions and features are readily available.

3.2.3 From FACSuite Files

To start an experiment from **FACSuite** files ***click*** on the lower half of the **New Experiment** button and the following window will appear, locate your **FACSuite** files ***enter*** your experiment's name and ***click*** **OK**.



3-8. Figure New Experiment from FACSuite files screen

3.2.4 Open Experiment

If the row of an experiment is highlighted in the **Main window** of the E**xperiments Library**, just ***click*** on the **Open Experiment** button from the **Home settings** tab and your selected experiment's **Layout window** will appear, with **Experiment Pane** and **Plex Pane** active and the **HOME** tab.

3.3 Import

To **Import** an FCAP Experiment into the software:

Select **Home > Import >** ***select*** file to **import.**

NOTE: Imports an experiment of the file extension of f3e from a specified path and the last used path is set as the default for both export and import functions, until another path is chosen or another location is available (e.g. USB drive). The default path for export and import functions is the same.

3.4 Export

To ***export*** an Experiment

Select **Home > Export >** ***select*** location to **export**

NOTE: Exports an experiment of the file extension of f3e to a specified path and the last used path is set as the default for both export and import functions, until another path is chosen or another location is available (e.g. USB drive), it is the default path for both functions.

3.5 Delete Experiment

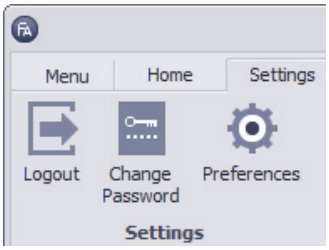
To ***delete*** an experiment, take the following steps:

Select **Home >** ***select*** (the row) of the experiment to delete **>** ***delete*** experiment **>** ***click*** **Yes**

NOTE: Each row in the main experiment list window is an experiment. The selected experiment will be deleted from FCAP's database and will not be recoverable.

3.6 Settings group (Settings Tab)

The **icons** in the **Settings group** ribbon allow the user to ***select*** from the following software functions, the other functions on ribbon of the **Maintenance** group and **Tools** group are explained below:



3-9. Figure Settings button group

3.6.1 Logout

To **Logout**, take the following steps:

Select **Settings > Logout**

NOTE: This option is used to switch between users and not to exit FCAP Array

3.6.2 Change Password

This function of the program is redundant under the **Settings** tab you can also find it under the **Menu** tab also, however if you wish to change your password in the **Settings** tab, do the following as before, see below:

To change the password of a user, take the following steps:

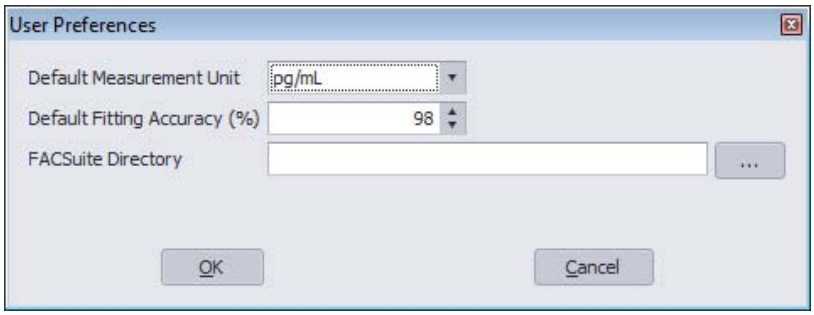
Select **Settings > Change Password**

3.6.3 Preferences (User Preferences)

This button is used to set the following preferences **(Default Measurement Unit, Default Fitting Accuracy, FACSuite Directory)** for each user that has been assigned an account under **User Management** in the **Libraries** pane, see User Management.

To change **User Preferences**, take the following steps:

***Select* Settings > Preferences**



3-10. Figure User Preferences screen

Default Measurement Unit

To change the **Default Measurement Unit**, take the following steps:

***Select* Settings > Preferences > Measurement Unit** from drop down list

NOTE: To add new measurement units to the list - go to the **Units Library**

Default Fitting Accuracy (%)

To set the **Default Fitting Accuracy** percentage, take the following steps:

Select* Settings > Preferences > *type the percentage between 90 -100%.

NOTE: The default fitting accuracy is 98%, the minimum fitting accuracy is 90%.

FACSuite Directory

To set the **FACSuite Directory**, take the following steps:

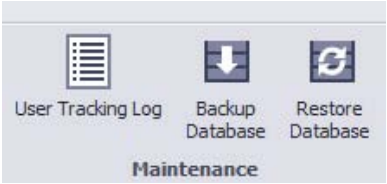
Select* Settings > Preferences > User preferences *window* > *type the path of the **FACS Suite Directory**

OR

Click on the **browse folder button > *select*** the path of the **FACS Suite Directory** from **Browse For Folder** dialog box

NOTE: for further details regarding experiments from **FACSuite (see section 7 using FACSuite)**

3.7 Maintenance group (Settings Tab)



3-11. Figure Maintenance button group

The icons in the **Maintenance** group on the ribbon allow the user to, ***select*** from the following functions: **User Tracking Log, Backup Database, Restore Database**.

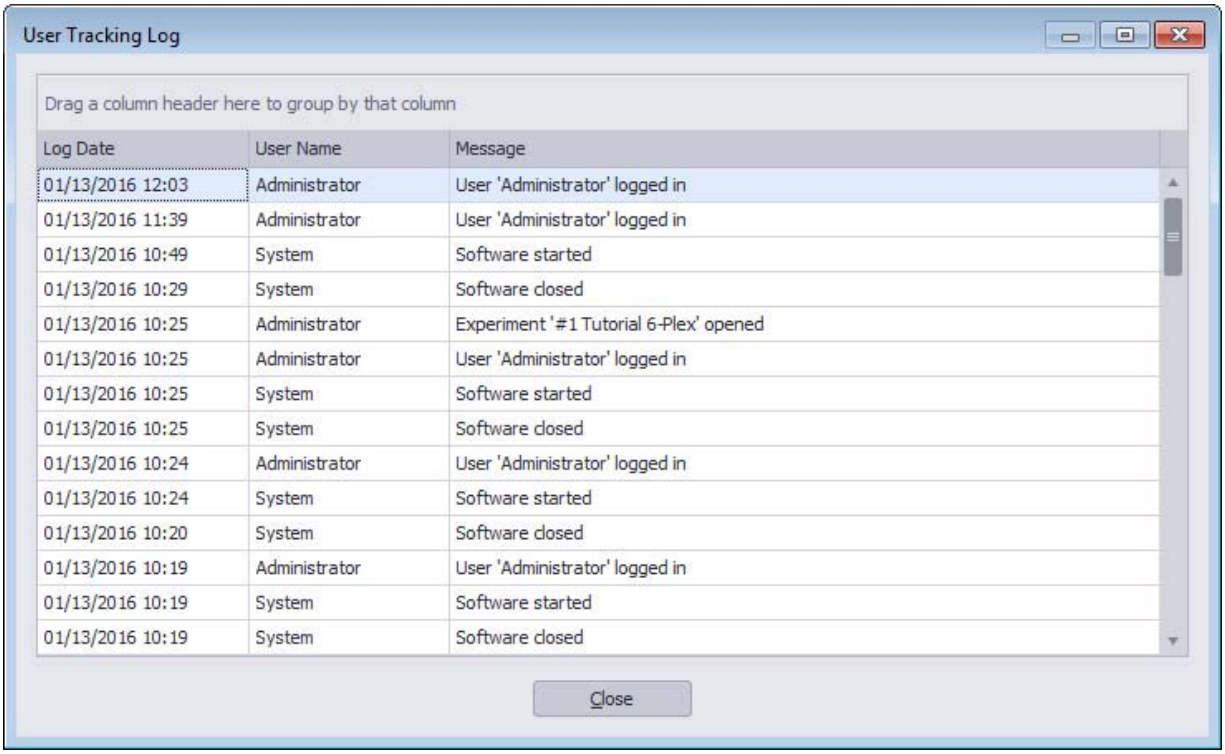
3.7.1 User Tracking Log

To track all users' actions, take the following steps:

***Select* Settings > User Tracking Log**

The **User Tracking Log** window contains the events for all users' actions within the software. The following events are logged: software startup, software close, user login, user logout, open an experiment, close an experiment, create an experiment, save an experiment, delete an experiment, export an experiment, and import an experiment.

The details in the columns include the log date, **User Name** of user and the message of the action taken by the user. Each **column header** can be **filtered** for specific information.



3-12. Figure User Tracking Log screen

3.7.2 Backup Database

Backs up the database of FCAP to a specified path, with the file extension of f3d.

To back up the database of FCAP, take the following steps:

Select **Settings > Backup Database,** ***add*** the path of the backup file in the **Save As** window ***> type*** the file name and ***click*** the **Save** button.

You have a copy of your FCAP database now.

NOTE: Do not forget to back up your FCAP data before reinstalling the operating system of the computer (or before reinstalling FCAP) or deleting a user profile on your operating system.

3.7.3 Restore Database

Restores the database of FCAP from a previously backed up file, the file is of the extension "f3d".

To restore the database of FCAP, take the following steps:

Select **Settings > Restore Database**, and then ***click*** **OK**. Select the backup file from **File Explorer**, and ***click*** on Open. The **Login** screen appears, so ***type*** your user and password

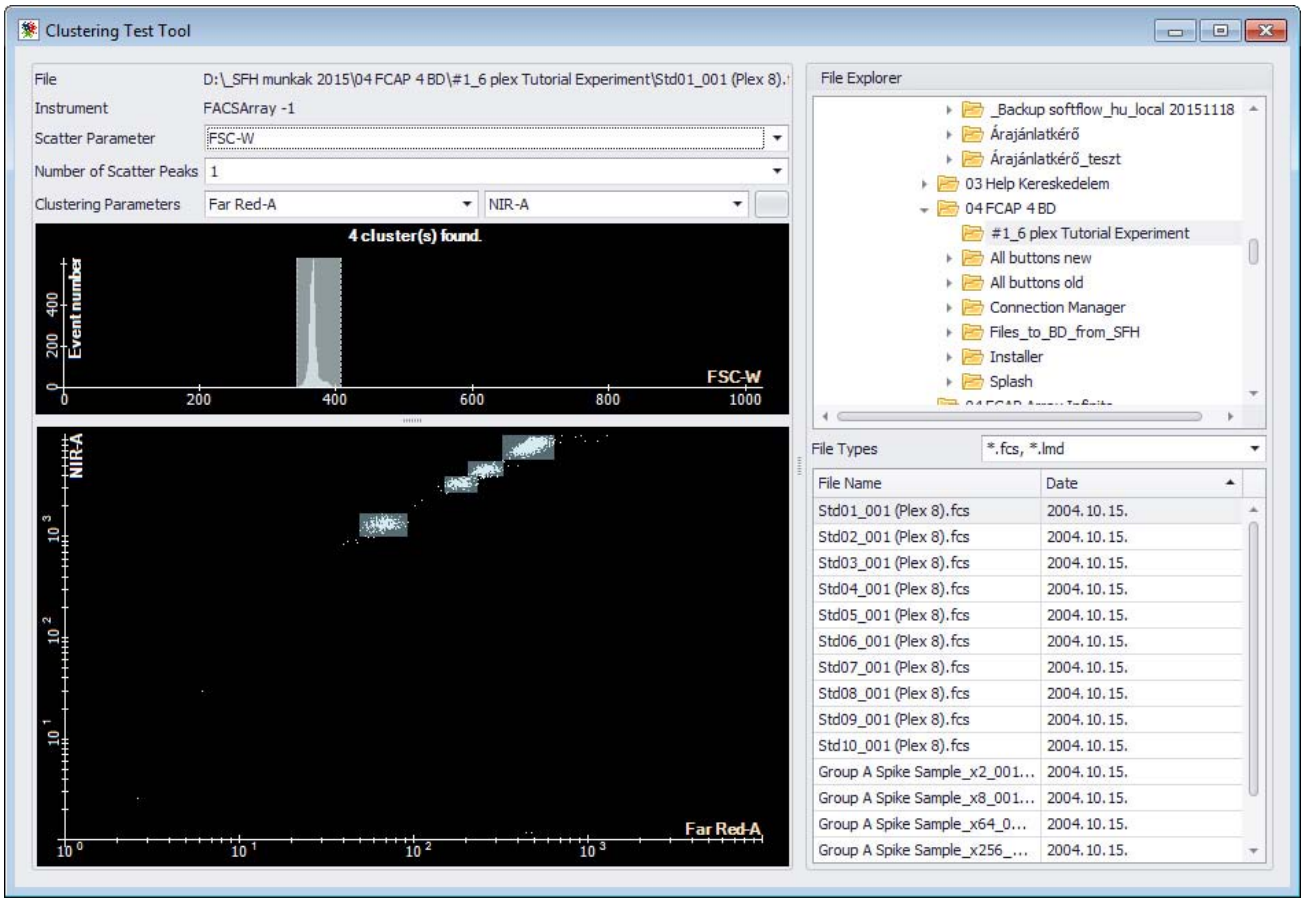
The database of FCAP is now restored.

3.8 Tools group (Settings Tab)

Clustering Tool is a single icon in the **Tools** button group on the ribbon.

3.8.1 Clustering Tool

The **Clustering Tool** is an independent clustering tool within FCAP used to analyze the clustering of bead(s) from any fcs file. It is used to test the automatic clustering process on single fcs files. If it works, the files will be suitable for further analysis with FCAP Array.
If it does not work, you should not create the whole experiment design in FCAP Array to realize the problem.



3-13. Figure Clustering test tool module

a. ***Select*** **Settings > Clustering Tool >** ***select*** file to be analyzed from **File Explorer**

b. ***Select*** **Scatter Parameter** from drop down list > ***select*** or ***type*** **Number of Scatter Peaks**

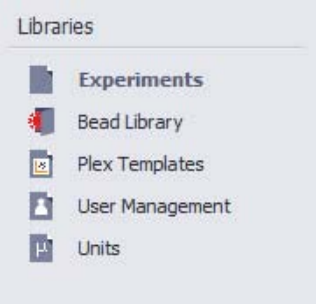
c. ***Select*** the **Clustering Parameter(s)** from the two drop down lists, if the second clustering parameter is not required

d. ***Click*** on the red X button next to the **second clustering parameter's** drop down list to ***cancel*** second parameter, if it is not being used.

3.9 Library Pane

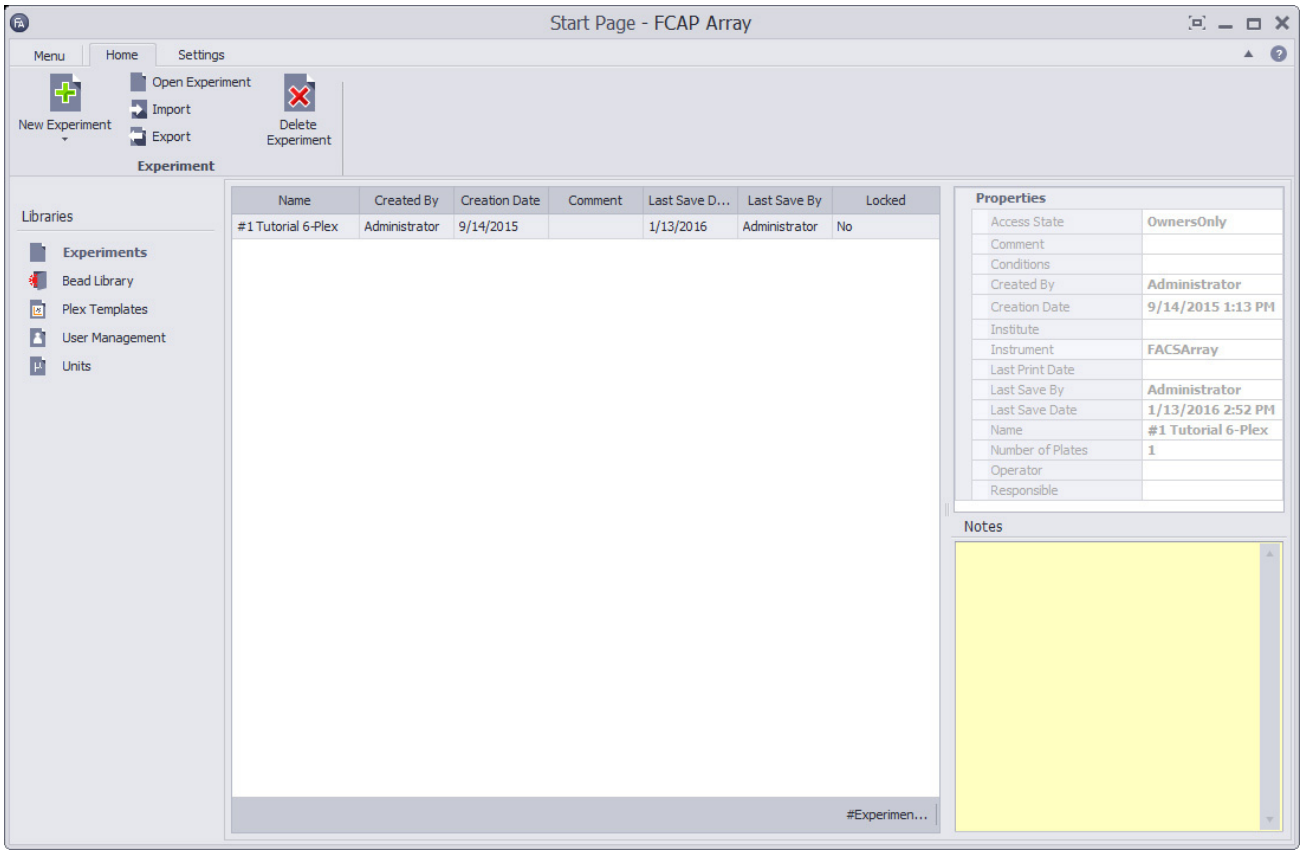
The icons in the **Library pane** allow the user to: ***Select*** from the following **Libraries (Experiments, Bead Library, Plex Templates Library, User Management Library, Units Library)**

3.9.1 Experiments Library



3-14. Figure Libraries pane

The **Experiment Library** contains the list of available experiments in FCAP. The **properties** and **notes panel** on the lower right hand side of the screen lets the user view the details of each individual experiment that have been highlighted in **Experiment Main** window view (list).



3-15. Figure Experiments list

When a highlighted experiment is ***double-clicked*** the design view appears, with the **Home Tab** active, the ribbon contains the followings controls or functions:

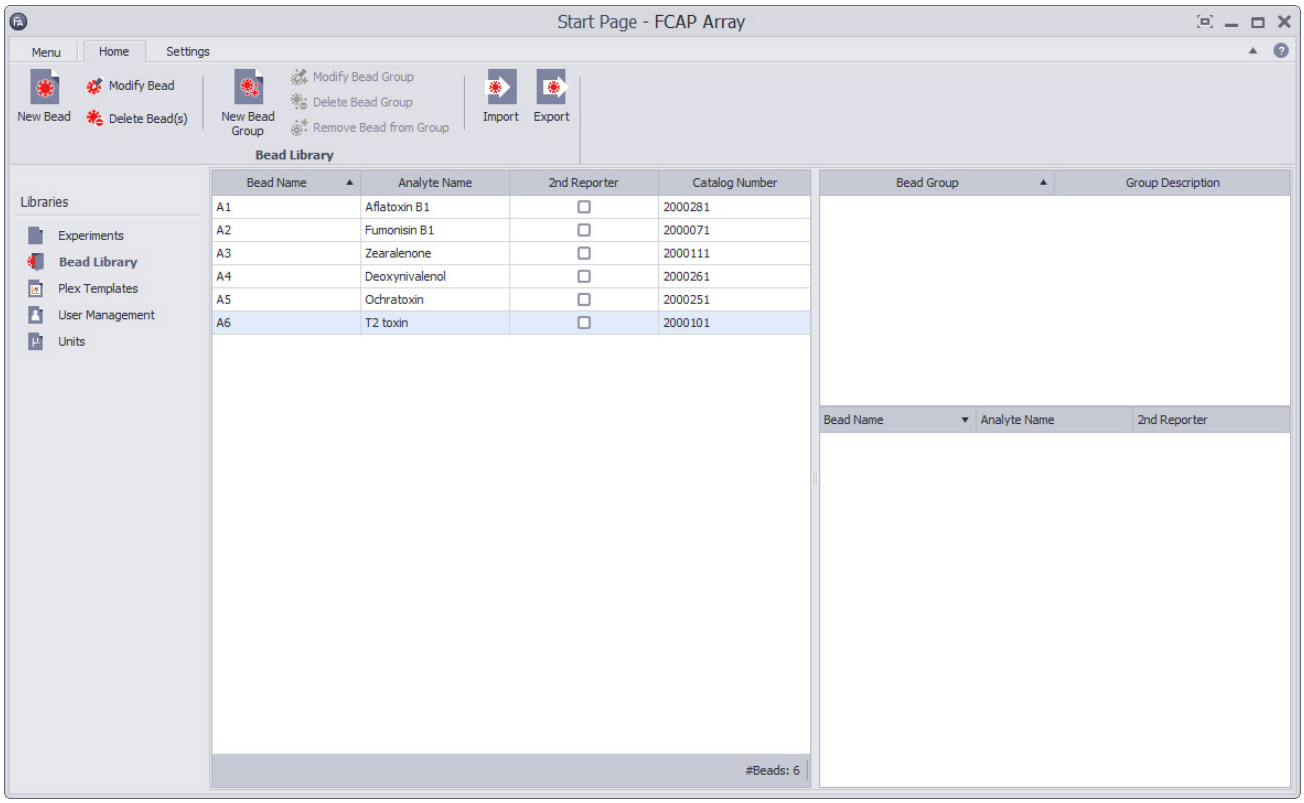
3-16. Figure Ribbon of the Design view

3.9.2 Bead Library

The **Bead library** is where we can define our beads or bead sets, it is possible to import bead(s) or bead groups from an XML file and we can also export them in a XML file. When necessary, we can create bead groups if we have more beads and the grouping makes the bead selection faster. A bead can be assigned to more bead groups.

Once we have our beads defined, we can assign them to our clusters of analyte's under **instrument and settings**. The lower right corner of the screen shows the list of our beads that have been assigned to the group.

Above the list of our beads in the pane we can see our list of **bead**, in the image below there is only one bead group called Fungi, if there was more than one bead group it would be listed under Fungi.

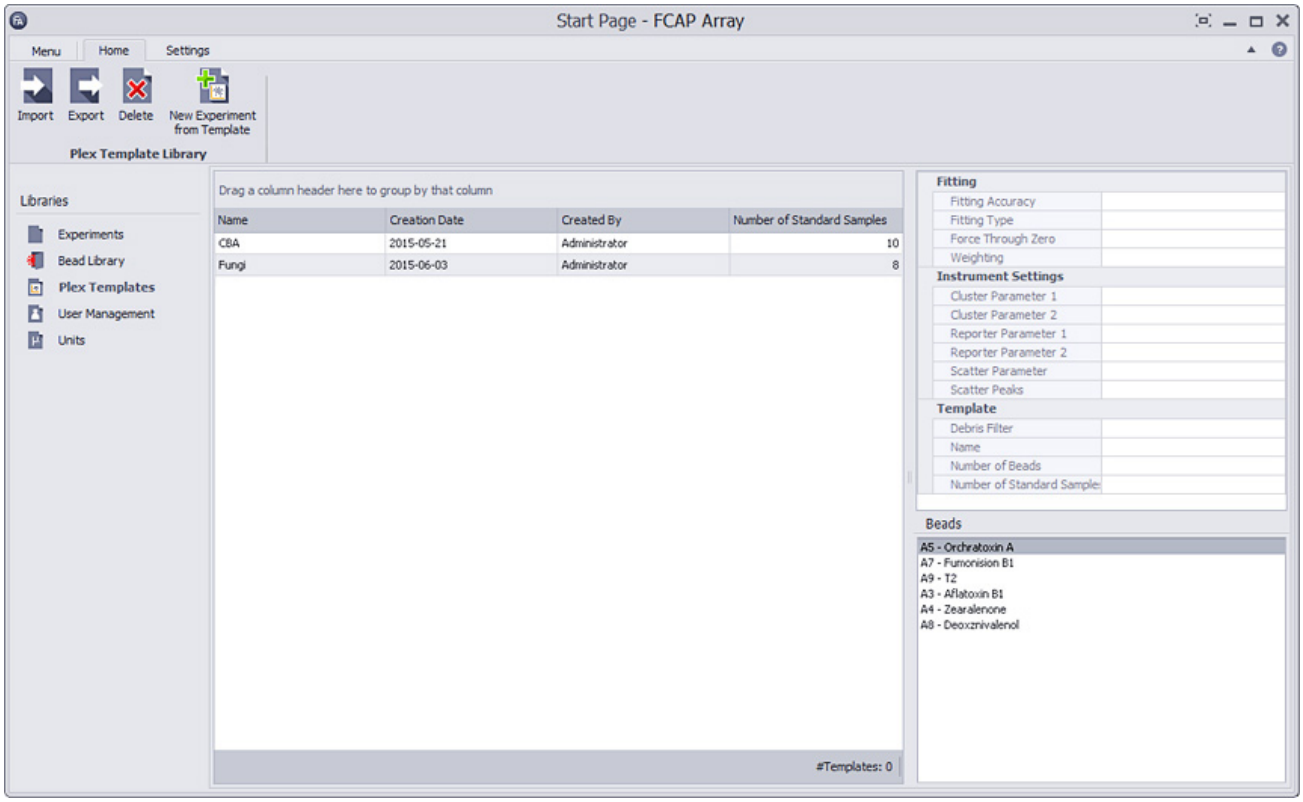


3-17. Figure Bead library view

3.9.3 Plex Templates Library

Plex(es) can be saved in the **Plex Template library**, the saved **Plex template** will contain the following information, see below under the bullet points. Saving a plex is usually used to save time to set up another experiment that has similar settings as the one you have just saved. In the right upper panel you can view the details of you **instrument settings** and other relevant information regarding your experiment. In the lower panel you can view your assigned beads. In the main window you have the list of your saved Plex templates.

- **Standard Samples** - Number, name, concentration, number of replicates
- **Beads and Model** - Selected beads, lot numbers, analysis models
- **Instrument Settings** - Scatter and clustering parameters, cluster positions, bead-cluster
- **Debris Filtering** - Defined debris filtering gates, status of the filtering (active or not)
- **Standards and QC** - Concentrations, measurement units (quantitative model), positive/negative standard selection (qualitative model), MFI threshold values (QC model)
- **Standard Curves** - Axis types (linear/logarithmic), force through zero, weighting type, fitting type, fitting accuracy (%)

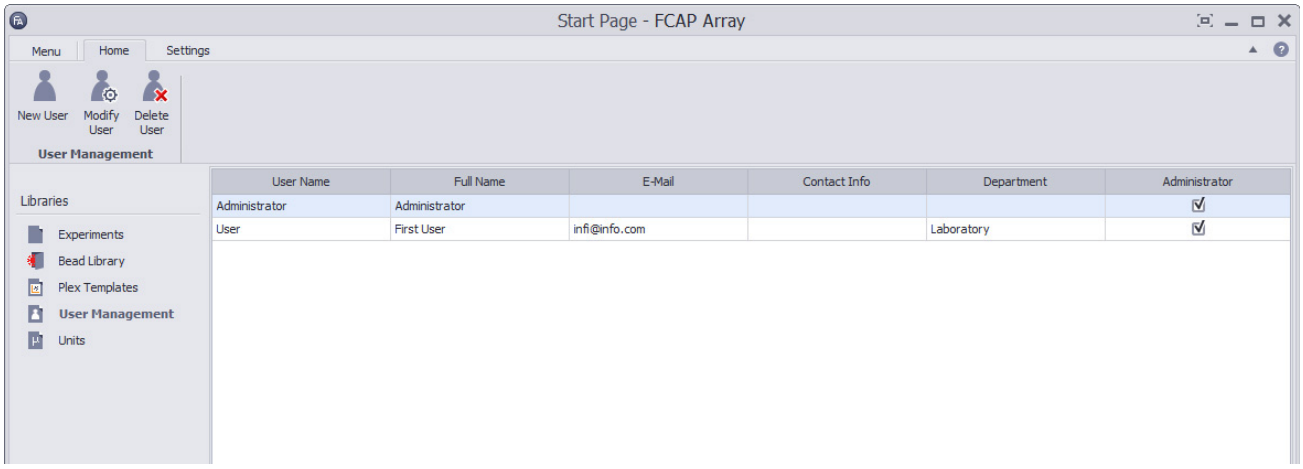


3-18. Figure Plex templates library

3.9.4 User Management Library

The **user management library** is used to setup user accounts for the individuals that will be using FCAP Array software, the Administrator of the lab can setup their colleagues in the program, it is from this list that an Administrator can assign privileges to an experiment after the experiment has been created.

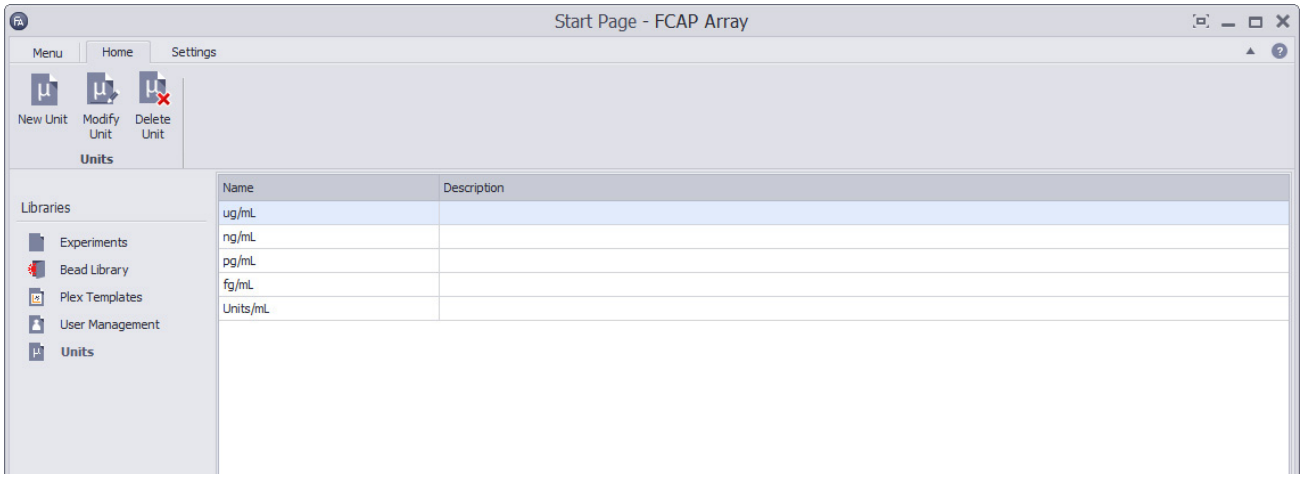
There are three icons, under this user management library **New User, Modify User** and **Delete User**. In the main window all users are listed.



3-19. Figure User management library

3.9.5 Units Library

The **Units** library is used to define new measurement units to be used in an experiment. The image below shows the default unit measurements already defined in FCAP Array.



3-20. Figure Units library

4

WORKING WITH AN EXPERIMENT

Defining plexes and setting up instruments

Getting started with experiments in FCAP Array, and understanding the work process used to define a new experiment and complete an analysis:

Starting a **New Experiment** - to start a new experiment in **FCAP**, the user needs to build up an experiment by defining one or more **plexes**, each **plex** may contain one or more **plates** with the **standard samples, test samples and/or control samples**.

A **plex in FCAP software** defines the beads that are used to test for a particular analyte, it is also used to define the **standards and QC**/or **controls** used within in the experiment.

Instrument settings are also defined within the **plex** and **instrument parameters** are used to specify distinct clusters - clustering helps to identify beads of the connecting analyte.

Plexes are defined and can be modified within an experiment and they can be saved in the **Plex Template Library** for later use.

Overview of the procedure for creating an Experiment

Plex design can be started by using data from aquired samples (plate) from an instrument or by importing the previous data (from **existing experiment** or from **FACSuite Files**) of aquired samples after creating and designing the experiments layout.

Take these steps below to complete an experiment.

1. ***Fill*** in the details of the experiment and then ***assign*** users and **security permissions** for the experiment, see Data Sheet icon.

2. Design the **plate** by placing the type of the samples **(test, control, sample, undefined or background)** on the plate layout, see adding samples.

3. **Import** data from **FCS files** and ***assign*** them to the proper **position** of the sample on the **Plate Layout**.

4. Define the lab conditions under which to analyze the **plate** (for beads and analytes).

   - ***Define*** the bead(s)and analyte to be used in the experiment, these are stored in the **Bead Library**
   - Change the analysis model **(quantitative, qualitative and quality control)** in the **Plex** pane **(Beads and Model)** and under the column analyte model ***click*** and change the value of the analysis model. See **Level 1**
   - Set **Instrument settings**, they are the following parameters (the file selected, the type of instrument used for acquirement **of sample, scatter parameter, number of Scatter Peaks, Clustering parameters** (can be two dimensional), and the reporter parameter. NOTE: make sure you assign the beads to the clusters, otherwise automatic clustering will not be initiated. See **Level 1**
   - Set the **Standard's and Quality** control (specify concentrations for each analyte). See **Level 2**
   - Define the controls for the plex (if any are used in the Experiment) – the parameters that can be set or modified **(Sample Name, Nominal CC, and range)** See **Level 2**

5. The remaining software elements in **Plex pane (debris filtering, manual clustering are optional functions)** and to be used to remove debris from the sample and to manually identify clusters if automatic clustering doesn't succeed. See **Level 3**

6. To review your **Standard curves** to see if they match your expectations of your experiment, see **Level 4** for details.

7. **Results per Analyte, results per sample** and **report** icons in the **Plex** pane are used to evaluate **(values, charts and reports)** of either the **results per analyte** or **results per sample.** The **Plex Report** function of **FCAP** is a comprehensive reporting tool of the data contained for the designed Plex of the experiment.

- For details on **Result per Analyte** and/or **Result for Sample** see **Level 5**.
- For details on the **Plex Report** function see **Level 6**

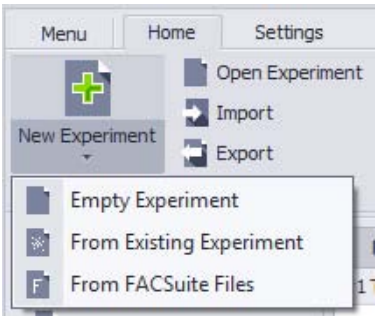
8. The last step is to **lock** the experiment if needed, then **save, export, print** and close the **plex template**. See **Level 7**

4.1 Start a new experiment in FCAP

Select from following options to create a new experiment:

4-1. Figure Option 1

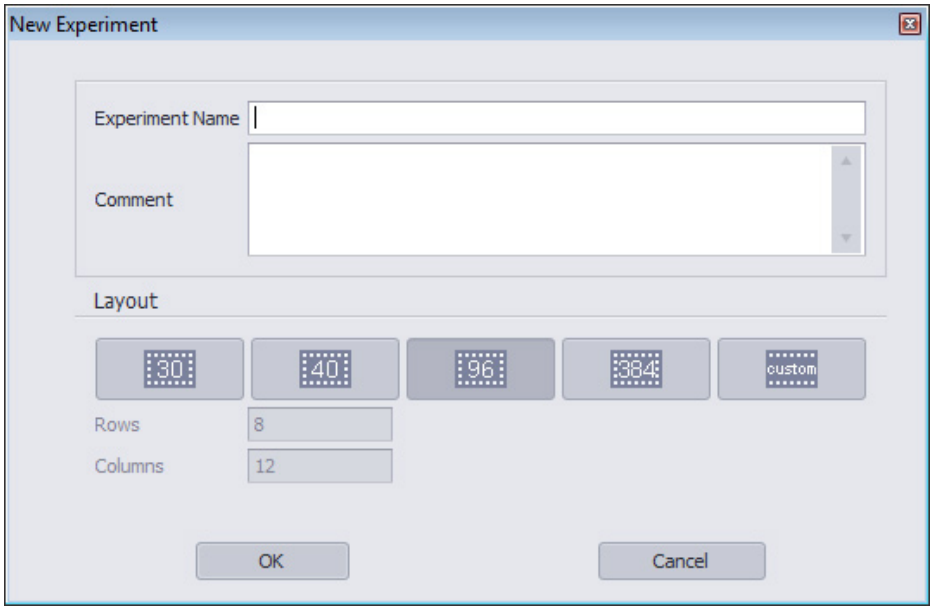
or choose



4-2. Figure Option 2

1. ***Click*** on the upper half of the **New Experiment** button (the **plus sign**) on the **Home** ribbon and the **New Experiment** dialog window will appear, see below figure.

   Fill in the details of Experiment and ***select*** your plate size from the available **plate buttons (30, 40, 96, 384 or custom)** and ***click*** on the **OK** button.



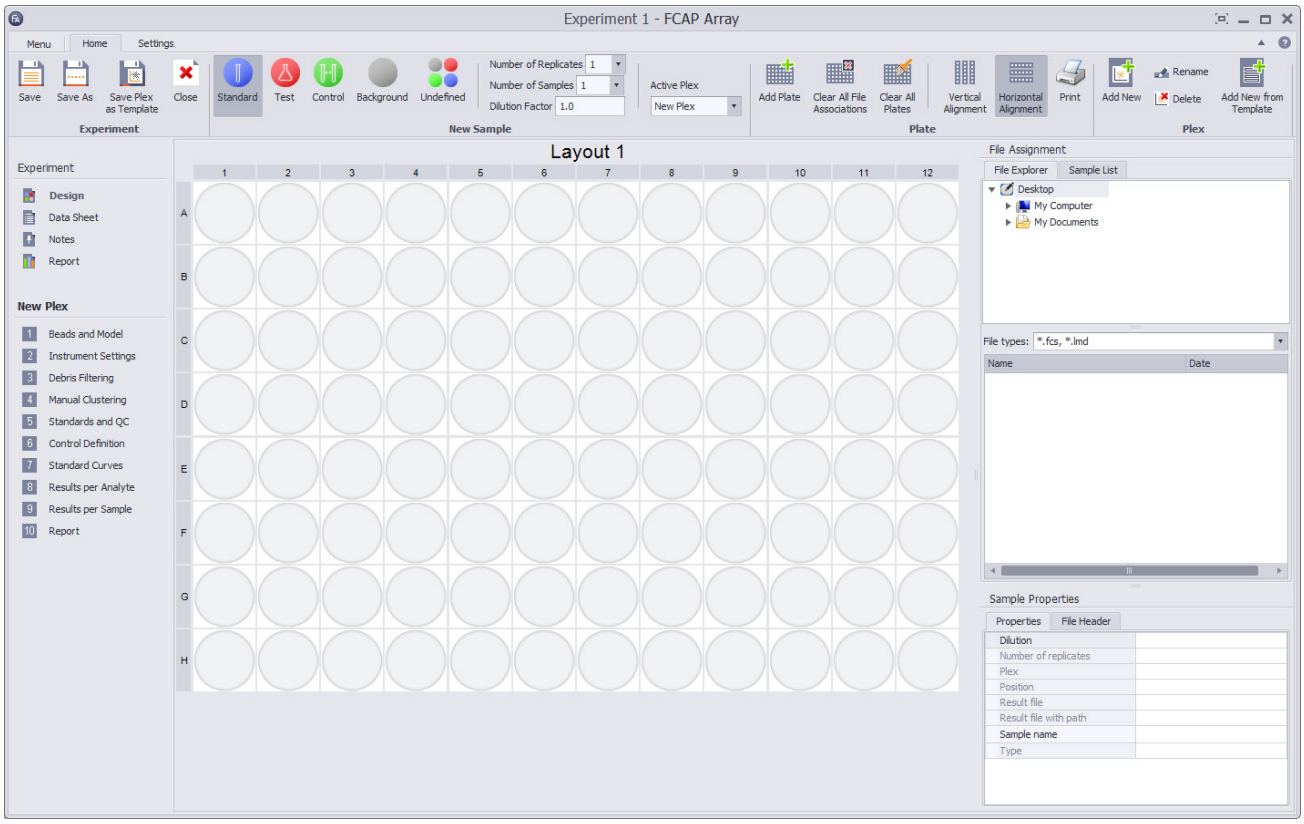
4-3. Figure New Experiment window

OR

2. ***Click*** on the lower half of the **New Experiment** button on the **Home** ribbon and ***select*** from one of the following:

- **Empty Experiment** – ***Select*** this option to create a new experiment, see above **Option 1**
- **From Existing Experiment** –***Select*** this option to use a previously saved experiment.
- **From FACSuite Files** – ***Select*** this option only if you want to use the **FACSuite** workflow. See **Section 7**.

The experiment **Layout** window appears with an empty **plate** (well/tube position) **layout**.

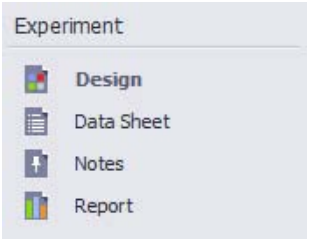


4-4. Figure Experiment design view

A new experiment by default only contains one plate, but multiple plates can be added in an experiment. The functions for working with plates are located on the Plate group ribbon.

4.2 Experiment Pane

The icons in the **Experiment Pane** allow the user to ***select*** from the following functions: **(Design, Data Sheet, Notes and Reports, - see below for details)**



4-5. Figure Experiment pane

4.2.1 Design icon

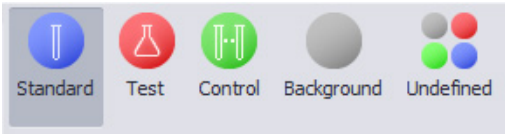
When the design icon is selected and the active plate layout is displayed our work area is empty; it is here where we set up our experiment as in defining our plate layout.

The user can add more plates if necessary. To perform some of the functions of the **Plate** ribbon you can also ***right-click*** on the plate to open the menu. Once the necessary number of plates have been added, then add additional plexes if necessary.

How to setup the design layout for an experiment.

Design a plate by placing one of the 5 different types of **sample type icons (test, control, sample, undefined or background)** the icons should represent the proper type of sample on the plate design layout.

To insert a **sample type**, ***select*** it from the ribbon and ***click*** on the appropriate well position.



4-6. Figure Sample type definition buttons

Setting up the sample layout

This section explains how to set up the sample layout in an experiment.

NOTE: when samples have been acquired manually in tubes, they still need to be arranged on a plate for use in the software.

Working with plates

An experiment always contains one plate as the default setting, but you can have multiple plates in an experiment. The controls for working with plates are located in the **Plate** group on the ribbon.

You can also ***right-click*** on the plate to open a menu to perform some of the functions.

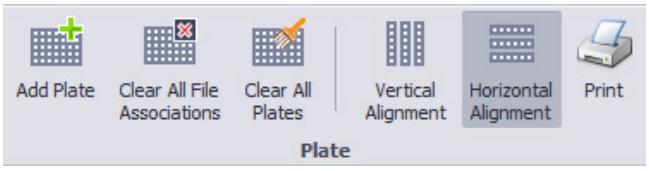
To add a plate:

The new plate is added below the current plate. If you cannot see the plate, scroll down and make sure the new plate was added.

4-7. Figure Add plate button

- Click Add Plate in the Plate group.

Using Plate group icons:



4-8. Figure Plate button group

The direction of alignment for the samples on a plate is by default set to **horizontal**. Make sure the direction you set matches your acquisition order. The selected direction determines the order in which multiple samples or multiple files in **one motion** are assigned. File assignment should match the order and the direction of added samples. The **plate** group also has the icons to **remove, clear** or clear **all plates**.

The selected direction also influences the order of samples in the following screens: **debris filtering, manual clustering, standards and QC, results per analyte, results per sample**, and **report**.

To change the plate alignment:

- ***Click*** the **Vertical Alignment** or **Horizontal Alignment** icons in the **Plate** group. The status button pair determines the orientation of sample and file series on the **Plate Layout** area for 'well – sample' type and 'well – file' associations.

To remove a plate:

- ***Right-click*** a plate and ***select*** **Remove Plate**.
  The selected plate and all samples within the selected plate are removed from the experiment.

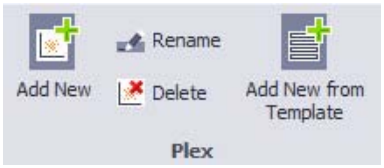
To clear a plate:

- ***Right-click*** a plate and ***select*** **Clear Plate**.
  All samples are removed from the selected plate in the experiment.

To clear all plates:

- ***Right-click*** a plate and ***select*** **Clear All Plates**.
  All samples are removed from all plates in the experiment.

Adding additional Plex(es) to an experiment



4-9. Figure Plex button group

An experiment always contains one plex as the default setting, but FCAP Array allows users to define comparative or summary multi-Plex experiments. By default, a plex named New Plex is added to a new experiment. Saving a plex as a template streamlines your workflow by creating a flexible template which does not have restrictions on required content. Using a saved template reduces manual data entry in following experiments.

The **plex** functions are accessed from the group of buttons on the **plex** ribbon.

To add a new plex:

- ***Click* Add New** in the **Plex** group.
  The **Add New Plex** dialog opens.
- **Enter** a name for the plex and ***click* OK**.

The new plex will be the **active** one in the experiment.

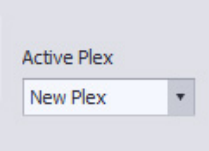
Adding a new plex from a template:

- ***Click* Add New** from Template in the **Plex** group.
  The **Add New Plex** from **Template** dialog opens.
- From the **Plex Template** menu, ***select*** a template from the library.
- In the **New Plex Name** field, **enter** a name, then ***click* OK**.

The new plex will be the active one in the experiment.

Changing the active plex

The ***selected* plex** becomes the active one in the experiment. New samples are added to the **active plex**. Samples belonging to other plexes appear in a shade of pale. Options for the **active plex** are shown in the **plex pane** on the left.



4-10. Figure Plex activation list

- Click on a plex from the Active Plex drop-down list.

Deleting an active plex

- ***Click* Delete** in the **Plex** group.
  The **Confirm plex deletion** dialog opens.
- ***Click* Yes** to confirm.

Saving the active plex as a template

- ***Click* Save Plex as Template** in the **Experiment** group.
  The **Save Plex Template** dialog opens.
- ***Enter*** a name for the plex template in the **Plex Template Name** field and ***click* OK**.

Assigning data to your Plex

Assign data to the design layout by importing the data from **FCS files** to the proper position of the sample in the experiment design layout.

Standards are arranged for the standard curve in alphabetical order by sample name (Std001, Std002, Std003, etc). Make sure that names are assigned in such a way that standards will be plotted in the correct order.

Wells that have been ***assigned*** sample files will have a **floppy disc** icon displayed on the well position.

NOTE: if the acquisitions of samples have been acquired manually in tubes, they still need to be arranged on a plate for use in the software.

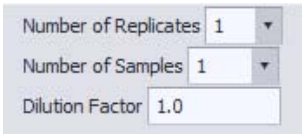
Adding samples – the 3 different ways

There are three different ways to add samples:

Adding samples by designing the layout

This procedure allows you to add samples first and then assign **FCS files** to the selected samples. Use this method when adding samples with replicates.

- ***Select*** one of the four sample types (**Undefined** is not a valid option with this process) from the **New Sample** ribbon by ***clicking*** the icon.
- Define the **number of samples, number of replicates**, and the **dilution factor**.



4-11. Figure Sample definition parameters

- ***Select*** the first well you want to start with and ***click*** on it.
- Depending on your orientation settings, the ***sample type icon*** and the ***number of replicates*** appear on the **plate (number of replicates multiplied by number of samples)**.

NOTE: A new **plate** is automatically added to the experiment if the number of replicates exceeds the available wells.

- Select FCS files from the File Assignment panel and drag & drop them onto the plate.

Adding files to sample types

This procedure allows you to select the sample type and the corresponding **FCS files** from the **File Explorer** window and add them to the plate. Make sure your files are in the correct order in the **File Explorer**, the assignment of files to the plate can be done in one step.

- ***Click*** one of the four sample type icons (except **Undefined**).
- If test samples were diluted, set the **Dilution Factor**.

NOTE: It is not necessary to define dilution factors for standards.

- ***Select* FCS files** from the **File Assignment** panel and **drag & drop** them onto the plate. If your files are in the correct order in the **File Explorer**, you can assign your files all in one motion.

The **5-3. Figure** shows a typical plate layout at this stage.

4-12. Figure Plate layout

Adding undefined samples.

This procedure allows you to add all files to the experiment and then you can define the sample types one by one.

- ***Click*** the **Undefined** sample type icon in the **New Sample** ribbon.
- ***Set*** the **Dilution Factor** if necessary.
- ***Select*** FCS files from the **File Assignment** panel and **drag & drop** them onto the plate. Define the sample types.
- **Select** one of the four colored circles within the well's icon.

To Modify the Layout view for an experiment.

This section describes how to edit and reposition samples that have already been added to a plate.

NOTE: that the well counters for the numbering of the sample name do not reset unless you delete all samples of a given type.

To select a sample:

- ***Click*** on the sample's well.

You can select multiple samples by ***Ctrl + clicking*** the desired wells/tubes or by drawing a rectangle around the desired wells. The background color of the wells indicates the selection. When removing samples, all selected wells become empty.

To move samples:

- ***Select*** one or more samples and then ***drag*** the samples to the new position.

NOTE: Samples cannot be moved to a position on a different plate.

To change the sample type:

- ***Right-click*** the sample you want to change and ***select*** **Change Sample Type**. The changed sample is now undefined.
- Redefine the **sample type** by ***clicking*** the icon (shown in the selected well) of the sample type you wish to assign.

NOTE: To change the sample type, the sample must have a file associated with it.

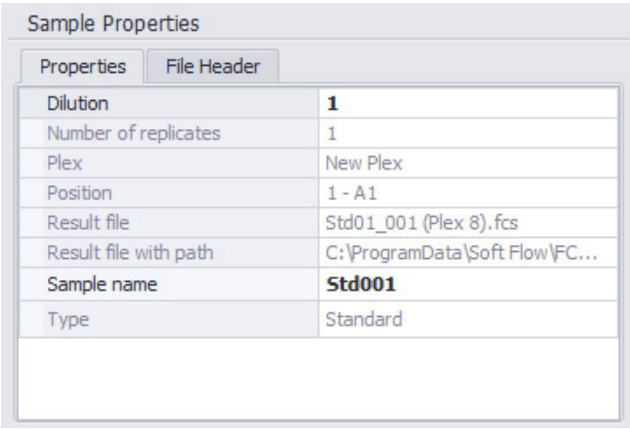
To remove a sample, do one of the following:

- ***Right-click*** the sample you want to remove and ***select*** **Remove Sample**.
- ***Click*** the well, then press the **Delete** key. When removing multiple samples, all selected wells become empty.

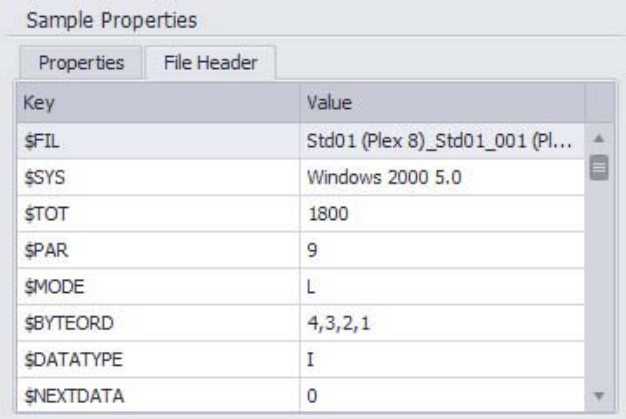
To clear the file association from a sample, do one of the following:

- ***Right-click*** a well and ***select*** **Remove file association.**
- ***Click*** the **Clear All File Associations** icon in the **Plate** group.
- ***Right-click*** a well and ***select*** **Clear all file associations**.

To view sample properties, do one of the following:



4-13. Figure Properties Tab



4-14. Figure File Header Tab

- First ***select*** a file (well position) on the layout
- ***Click*** the **Properties** tab to view data about a selected sample.
- ***Click*** the **File Header** tab to see **FCS file header keywords** for a selected sample.

NOTE: The **File Header** table is empty when no well is selected. The properties tab is also empty when no file is selected. For **FCS file header keywords**, please consult the user guide of your cytometer.

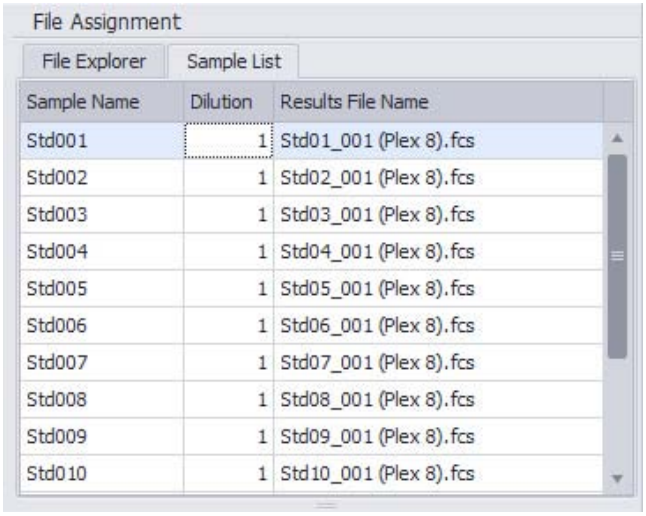
Modifying the sample name and dilution factor

Samples are named automatically with a default setting when they are imported. When using the standard workflow, the prefix for standards is **Std,** for test samples it is **Test**, for controls it is **Control** and for background it is **Background**. You can ***modify*** the name of the sample and its **dilution factor**.

To modify sample information using the Sample List tab:

- ***Click*** the **Sample List** tab on the **File Assignment** panel.

Using the **Sample List** tab enables you to view all the samples at once and to enter data more rapidly.



4-15. Figure Sample list

- ***Select*** a sample by ***clicking*** in the **Sample List**.
- ***Edit*** the name and ***press*** **Enter**.
- ***Select*** a sample by ***clicking*** in the **Dilution** field.
- ***Edit*** the value and ***press*** **Enter**.

NOTE: The user can sort the data in each column by ***clicking*** on the column header.

The next step is to Edit the **details of Experiment data (Experiment Data Sheet icon)** or **create notes for the experiment**. (both are optional and are not required for an experiment to be successful).

4.2.2 Data Sheet icon

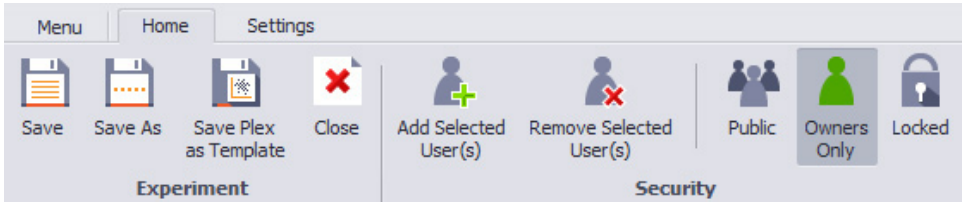
This section describes how to enter information about an experiment in the data sheet, the data sheet icon is located in the experiments library pane. You can also add or remove users from the list of experiment owners to change access rights to the experiment. You can also change the security rights for the experiment.

4-16. Figure Data sheet button

Entering information in the experiment data sheet:

- ***Select*** **Data Sheet** icon in the **Experiment** pane then ***click*** in the fields next to each field type and enter information regarding your experiment.

You can add or remove users from an experiment in the **Experiment Owners** table (**To add user accounts to FCAP**). Users added to the **Experiment Owners** list can ***open, read***, and ***edit*** the shared experiment.



4-17. Figure Data Sheet ribbon

Add a user to an experiment:

- ***Select*** a user name you want to add from the **All Users** list.
- ***Click*** **Add Selected User** from the **Security group** on the ribbon.

The selected user is added to the **Experiment Owners** list.

Remove a user from an experiment:

- ***Select*** the name from the **Experiment Owners** list.
- ***Click*** the **Remove Selected User** icon from the Security group on the ribbon.

NOTE: The selected user is removed from the Experiment Owners list, if there is only one user in the list you will not be able to remove it.

Assign or modify user security rights for an Experiment:

- ***Select*** the **Public** icon - this enables editing of the experiment by all users.
- ***Select*** the **Owners Only** icon - only the respective **owner(s)** and **administrator(s)** may ***edit*** the experiment.
- ***Select*** the **Locked** icon - Prevents users from editing the experiment. ***Click*** the **Locked** icon once more to allow editing or save the experiment with **Save As**, name the ex periment and the selected experiment is then editable.

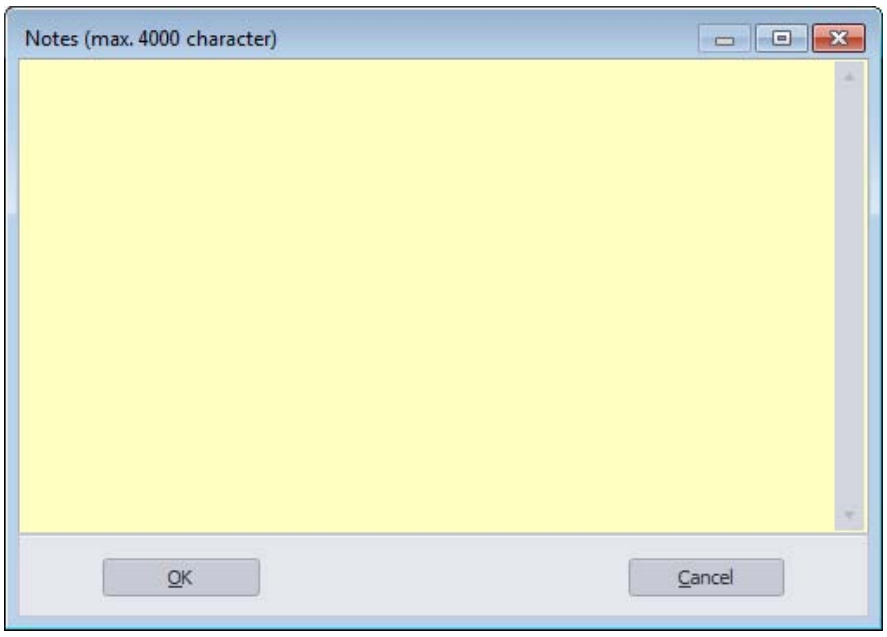
NOTE: a user with administrator rights can ***create, edit*** and view all experiments. If the owner of an experiment needs to add other users to **FCAP**, please visit the **User Management Library**.

4.2.3. Notes icon

4-18. Figure Notes button

Your notes can be viewed when you select an experiment from the experiment library in the lower right corner of your screen.

Adding notes to an experiment:



4-19. Figure Notes screen

- ***Select*** the **Notes** icon in the **Experiment** panel and the **Notes** dialog box opens.
- ***Type*** in your comments or notes and then ***click*** **OK**.

4.2.4 Report icon

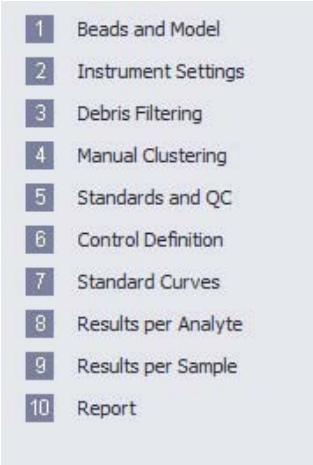
4-20. Figure Data sheet button

An experiment's report is a cumulative report that contains all the data values, charts and graphs for all plexes within an experiment. see **Level 7**

NOTE: The experiment report icon and reports functions can only be used once an experiment has been completed.

4.3 Plex Pane

This section describes plexes, plex templates, and the basic building blocks for creating plex(es) in an experiment and how to complete an experiment.



4-21. Figure Plex pane: the 10 step and views of a plex

An FCAP Array experiment can contain one or more **plexes**. A **plex** is created or modified within an experiment. **Plexes** can be saved to the **plex templates library** to be reused or exported to other users.

To setup a new experiment in FCAP, the user needs to build up an experiment by defining plexes, this section describes the workflow for creating a new experiment.

This same workflow can be used for analyzing data from multiple flow cytometry instruments.

The building blocks of how to complete an experiment are expressed in levels of importance, and which might consist of several steps.

NOTE: Some are optional in certain instances and circumstances.

Level	Building Blocks of an Experiment (workflow)
1	setup of beads, model and instrument settings
2	setup of Standards and QC (Quality Control), control definition *
3	usingDebris filtering, Manual clustering *
4	standard curves **
5	review plex results: results per anaylte, results per sample
6	report for plex
7	completing the experiment

4-1. Table Building blocks of an FCAP Experiment

NOTE: * (optional steps - control definition only required for controlled acquisitions, use debris filtering and/or manual clustering if automatic clustering fails)
** (only for quantitative analysis)

A **plex template** is a data repository for the following types of information, see the table below.

Parameter	Content
Standard Samples	Number, name, concentration, number of replicates. Mandatory.
Beads and Model	Selected beads, lot numbers, analysis models. Mandatory
Instrument Settings	Scatter and clustering parameters, cluster positions, bead-cluster assignment. Mandatory.
Debris Filtering	Defined debris filtering gates, status of the filtering (active or not) Optional.
Manual Clustering	Cluster gates, cluster positions, bead-cluster assignment. Optional.
Standards and QC	Uniformity, concentrations, measurement units (quantitative model), positive/negative standard selection (qualitative model), MFI threshold values (QC model). Mandatory
Standard Curves	Axis types (linear/logarithmic), force through zero, weighting type, fitting type, fitting accuracy (%). Mandatory

4-2. Table Elements of a Plex template

The **plex template** is very flexible, since there are no required fields that are mandatory in order to save a plex as a template.

4.4 Analyzing the Experiment level 1

4.4.1 Beads and Model

This section describes the process of adding beads to a **plex** and ***selecting*** an **analysis model**. If necessary, add beads to the **bead library** for your next experiment.

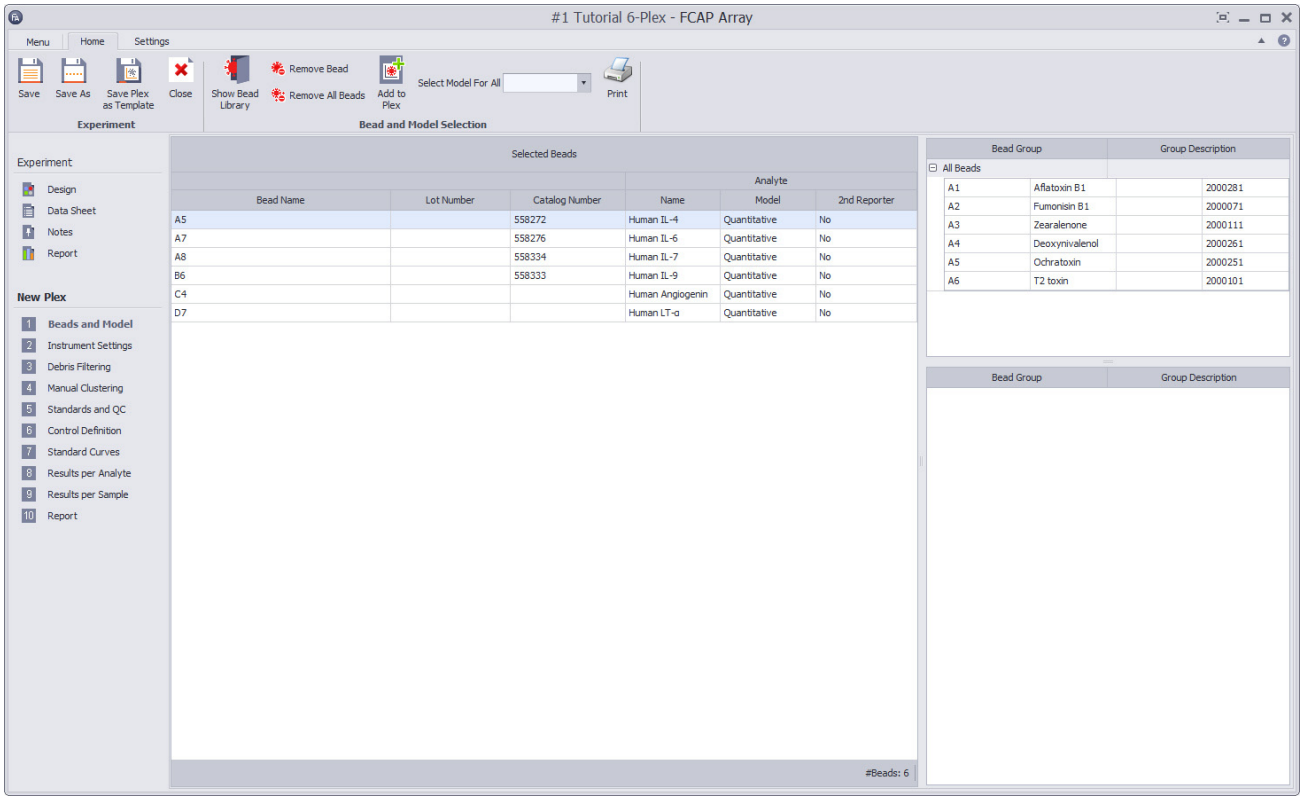
NOTE: When you first install **FCAP Array**, the **bead library** is empty. For **BD CBA reagents**, we recommend adding beads to the bead library by importing the XML file.

See Importing a bead or bead group in the Bead Library group ribbon, see section Bead Library.

How to select beads for a plex:

- ***Click*** **Beads and Model** in the **Plex** navigation panel to setup the beads needed for your plex.

The **selected Beads** panel opens.



4-22. Figure Bead and Model view

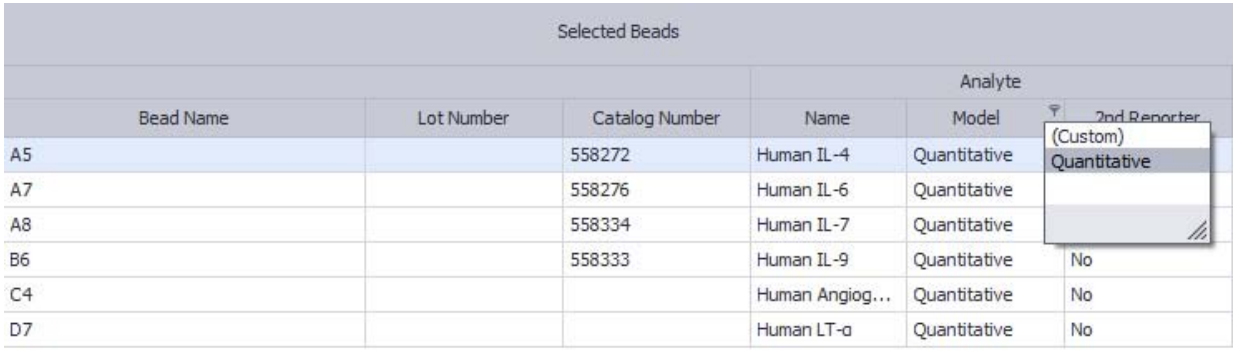
To Select one or more beads from the Library list and do one of the following:

- Drag & drop the beads you need onto the selected Beads window.
- Click Add to Plex in the Bead and Model Selection group on the ribbon.
- If you have a bead group defined in the bead library, you can add all beads of the selected group in one step by adding the group to the plex. Select the group by clicking on its name, then drag & drop onto the Selected Beads window.

Selecting an analysis model:

Each bead has one analyte and a second reporter parameter. is optional. The default analysis model for an analyte is **Quantitative**.

There are three analysis models: (see drop down list in **Model** column)



4-23. Figure Bead model selection

Quantitative analysis determines analyte concentrations based on the known concentration values of a set of standards.

Qualitative analysis determines whether a specific type of analyte exists in a sample based on known controls.

Quality control analysis is used in the case where beads have two reporter parameters, the FCAP compares the MFI value of a sample to a user-defined range or a specific threshold attained from a control.

- ***Select*** the analysis model from the drop down menu in the Model column.

NOTE: This only changes the analysis model for the selected bead.

- ***Select*** analysis model from the **Bead and Model Selection** group > **Select Model For All** list.

NOTE: This changes the analysis model for all beads.

4.4.2 Instrument settings

This section explains how to setup your instrument settings. The process includes ***selecting*** a **sample data file** based on the defined **plex**, and specifying the **scatter, clustering,** and **reporter parameters**.

In order to complete the **instrument settings** and assign clusters, you are required to have a FCS file which was acquired for the experiment and properly assigned to a **sample** on your **plex**.

For the most common instruments, parameter options (as in **scatter, clustering, reporter**) are automatically loaded into the specific parameters (scatter parameter, scatter peaks, the two clustering parameters, reporter parameter 1 or 2 (if available in the file)) based on the information specified in the selected file.

The parameters can also be set manually. Once the parameters have been set or the **FCS file** has been changed, the **scatter histogram** and **plot clustering** automatically refreshes according the information contained in the chosen **FCS file**.

Setup of instrument settings:

- ***Click*** **Instrument Settings** in the **Plex pane**.
- ***Select*** any of the assigned files from the **Selected File** drop down list.

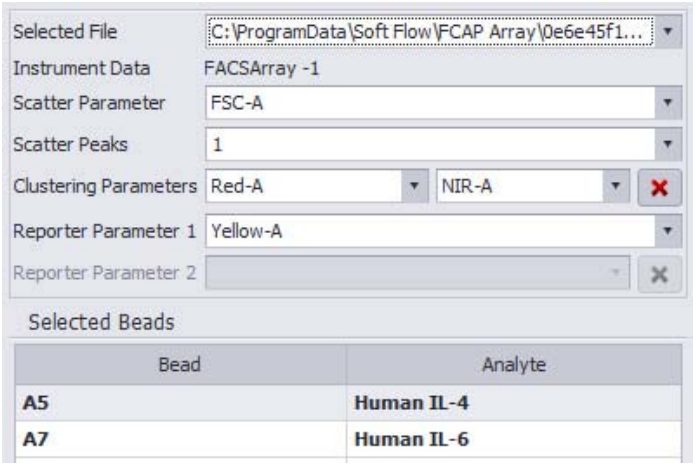
The default **scatter peak** is set to one. Change the value according to the number of different bead used in your assay.

The algorithm searches for the number of **scatter peaks** that you have defined.

NOTE: All **BD CBA assays** use only a single **scatter peak**.

If the algorithm fails to find the specified number of peaks, **FCAP Array** will notify you.

The **clustering parameters** are used for locating beads, suggested clustering parameters can be found in the manual of the reagent.



4-24. Figure Middle section of the Instrument settings view

The clustering algorithm locates the defined clusters. In case the algorithm fails to locate the beads, **FCAP Array** will notify you.

- ***Select*** a parameter from the **Scatter Parameter** menu.
  The **scatter diagram** displays the data.
- ***Select*** a number from the **Scatter Peaks** menu.
- ***Select*** the first and the second optional (if needed) clustering parameter from the **Clustering Parameters** menu depending on the beads fluorescence.

NOTE: For the kits that only use one clustering parameter, the second clustering parameter will be shaded gray.

- ***Select*** a parameter from the Reporter Parameter 1, drop down list for the bead analyte.
- If your plex does contain a **second reporter parameter**, then ***select*** the parameter from the **Reporter Parameter 2** menu.

NOTE: The second reporter parameter can be cleared by ***clicking*** the red **X** icon next to the drop down list.

How to assign beads to Clusters:

This section explains how to assign your plex beads to clusters.

Assign plex beads to the corresponding clusters that are shown on the cluster plot. To make assigning beads easier, ***click*** the **Bead** column header in the **Selected Beads** table and filter it by bead ID.

It is only possible to assign one bead at a time to each cluster.

Assigning beads to Cluster

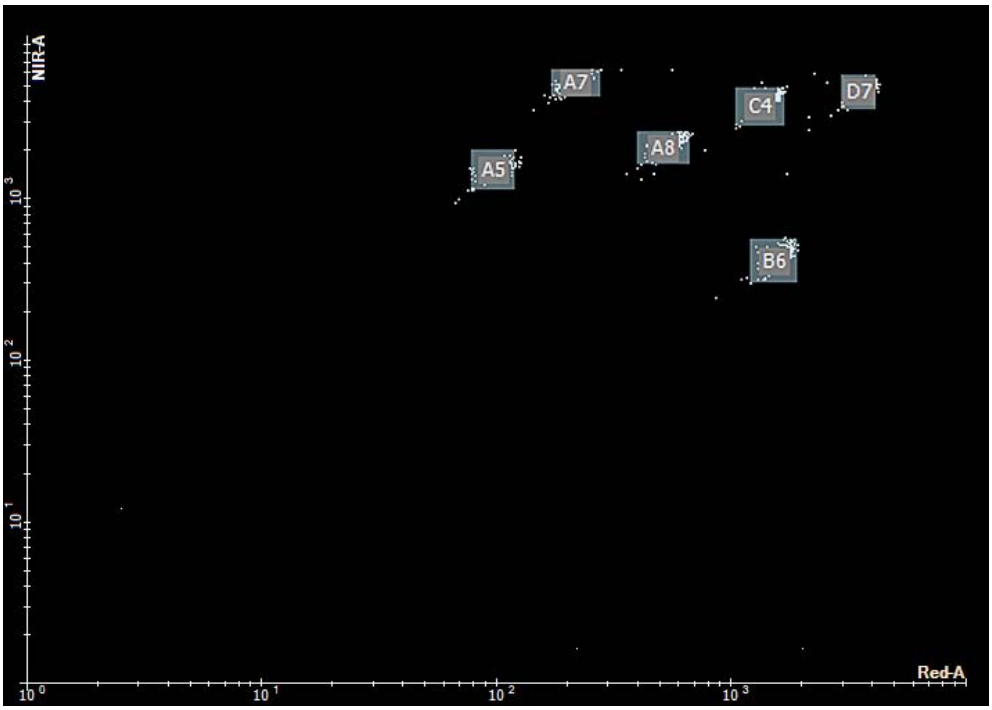
1. ***Select*** the bead you want to assign from the **Selected Beads** table.
   Sort by bead location and assign starting from the top left cluster. Then proceed to assign beads going from the left to right and the top to the bottom.

2. ***Select*** a cluster by doing one of the following:

- ***Double-click*** the cluster that you want to assign the bead to.
- ***Drag & drop*** the selected bead of analyte onto the target cluster.

  The Bead names are displayed on the cluster gates, the Bead row letters turn to bold in the **Selected Beads** table after the successful association.

3. Repeat the process for each cluster.



4-25. Figure clustering plot after beads have been assigned

Zoom in plots

If the cluster structure of the measurement is tight, user has a possibility to zoom in the **Instrument Setting > Clustering Plot** by drawing a rectangle with ***continuous right-click***. The **Instrument Setting > Scatter Plot** has similar zoom function, but the area selection is one dimensional on histograms. **Debris Filtering > Before Filtering & After Filtering** plots have independent zoom.

NOTE: The zoom function become available on **Manual Clustering > Clustering Plot** and **Manual Clustering > Scatter Plot**, when the active file checked for "Use Manual Clustering".

Click the **Reset Zoom** button to reset original plot view.

NOTE: The **Reset Zoom** button appears only on zoomed plots.

Clearing Bead Assignments

To remove bead cluster assignments, choose from the following:

- To clear all bead cluster assignments ***click*** on **Clear Cluster Assignment** on the ribbon.
- To remove an individual bead cluster assignment, ***right-click*** on the selected cluster and ***left-click*** on **Remove Bead Assignment**.

NOTE: If automatic **clustering** is successful and there are no errors; then the **next Level** is to setup **standards and Quality Control (QC)**, however if you need to adjust your gates then go to **Level 3** to find information on how to use **debris filtering** and **the manual clustering** functions.

4.5 Analyzing the Experiment level 2

4.5.1 Standards and QC (Quality Control)

This section describes how to define standards for quantitative, qualitative, and quality analysis.

- ***Click*** on **Standards and QC** from active **plex pane**

The content is shown in correspondence to the selected beads and their analytes' analysis models. When beads in your plex contain both - an **analyte** and **second reporter parameter**, the window is split in two, left and right depending on the order of content.

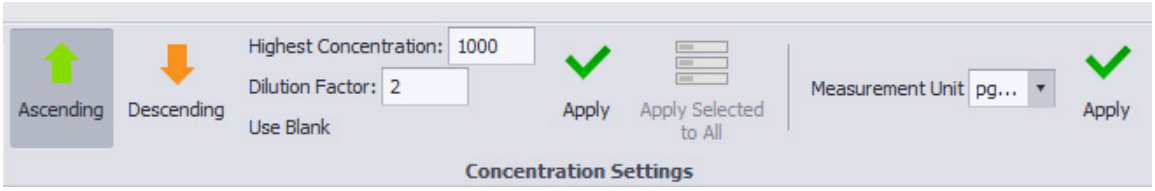
Using Quantitative analysis

When defining standards for Quantitative analysis, you specify the concentration values and the unit of measurement.

You can use uniform concentrations for all analytes (default) or specify individually for each analyte, by ***ticking*** the **checkbox** of **Show individual analytes**.

How to specify Standard Concentration

- ***Select*** **Ascending** or **Descending** by ***clicking*** on the icons to define the order of the standard concentrations.
- ***Fill*** in the **Highest Concentration** and the **Dilution factor** box. ***Check*** **Use blank** if you need zero concentration standard. ***Click*** on a **column header** to make it active and then ***click*** **Apply**.



4-26. Figure Concentration settings button group

FCAP calculates the standard dilution series and fills it in the **Quantitative Analysis** table's concentration column.

Specify uniform Concentrations

- ***Check*** out the Show Individual Analytes check box.
- ***Select*** the only concentration column by clicking **Concentration** column header and then fill **Concentration Settings** as described above.

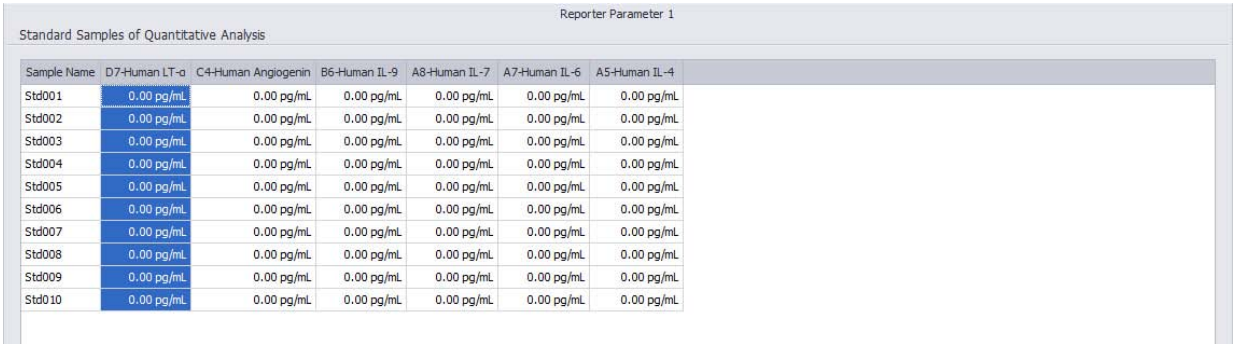
The generated concentrations series will be valid for each analyte.

4-27. Figure Uniform CC series of standards in quantitative analysis

Specify non-uniform Concentrations

Manual edition of the concentration values are also possible by the box values one by one.

NOTE:



4-28. Figure Individual CC series of standards in quantitative analysis

- ***Select*** the **Show Individual Analytes** checkbox.
- ***Click*** in the column header for an individual analyte.
- ***Enter*** a value in the **Highest Concentration** field.
- ***Enter*** a value in the **Dilution Factor** field.
- ***Click*** **Use Blank** if you need a zero concentration standard.
- ***Select*** from the **Measurement Unit** list.
- ***Click*** **Apply**.

NOTE: Unit of measurement is also overwritten. Only the selected analyte concentration is calculated, because the analyte columns are selectable only one by one in **Standard Samples of Quantitative Analysis** table. Repeat this process to define the concentrations for each analyte.

Set the Unit of Measurements

When defining concentrations, the active measurement unit will be valid for your analysis.

- ***Select*** a **Measurement Unit** in the **Concentration Settings** ribbon.
- ***Click*** the **Concentration column** header to highlight it.
- ***Click*** **Apply**.

The selected measurement unit is displayed in the results and report documents.

NOTE: Your results will slightly vary for the standard curves, if you choose to use different measurement units.

It's recommended not to use units that will lead to a concentration of less than 1.

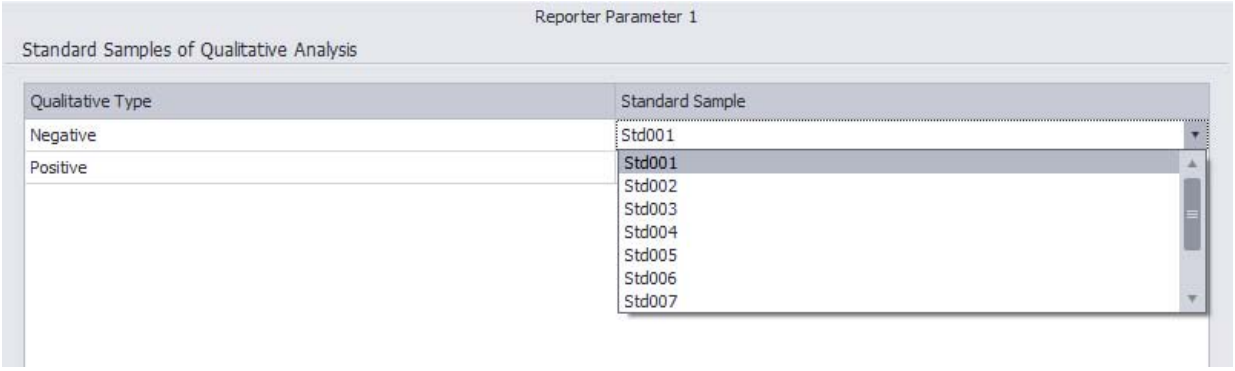
Using Qualitative analysis

You can select both positive and negative standards when you use **qualitative analysis** for all analytes **(default)** or you can individually specify the standards for each analyte.

NOTE: All analytes should have a positive or negative standard in order to attain proper results.

To specify positive and negative standards:

- Select negative standards in the negative row of the qualitative table by selecting a standard sample name from the **Standard Sample** menu.



4-29. Figure Definition of positive and negative standards on Standards and QC view

Using Quality Control

In the case that your selected beads have two reporter parameters, the second parameter's analysis model is set to **Quality Control**.

Quality Control gives you the option to check if **MFI values** are within the specified range.

NOTE: Any anomalies are reported in the both the **Report** and the **Result**.

To set quality control range

The range for all analytes by default are set to the same value.

- ***Click*** on the column header to activate it the **Quality Control Settings** on the Ribbon.
- ***Fill*** in the **NEG MFI Value** and **POS MFI Value** fields.
- ***Click*** **Apply**.

The columns are filled with the minimum and maximum values for all samples. In addition to using the above procedures, you can manually edit each value.

NOTE: Use the same method for each analyte when defining non-uniform values.

Specifying quality control MFI threshold values for all analytes

- ***Click*** a column header. (**Neg MFI Value** or **Pos MFI Value**) to activate the **Quality Control** Settings on the Ribbon.
- Type values into the **Negative Standard's MFI** and **Positive Standard's MFI** fields.
- ***Click* Apply**.

To specify the MFI threshold values for each analyte repeat the above process. If you have a second reporter parameter then to set it up, take the following steps:

- ***Click*** the **Reporter 2** icon and repeat the **above steps**

To setup sample based QC Definition

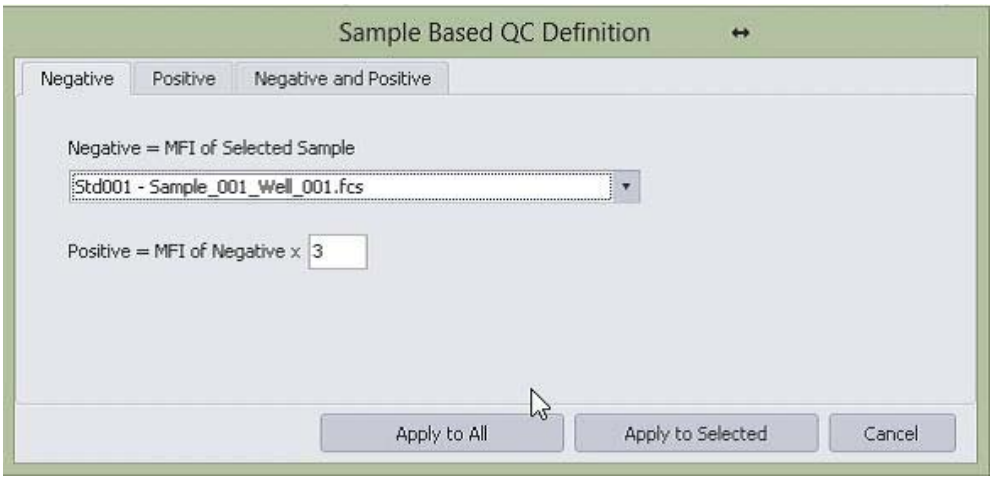
Using the sample based **QC definition** enables the minimum **MFI values** to be derived from the **MFI values** of the negative standard.

You can define your standard as negative, positive, or both a negative and a positive standard by clicking the corresponding tab in the **Sample Based QC Definition** dialog.

The results for your analysis can be found in the **QC Result** column of the statistics table in the report view.

The steps to setup the sample based QC Definition

- Click the **Customize Settings** icon in the **Quality Control Settings** ribbon.
- The **Sample Based QC Definition** dialog opens.
- Select your negative standard from the **Negative=MFI of Selected Sample** menu.
- Specify the multiplier for your negative standard in the **Positive = MFI of Negative x field**.
- The recommended value of the multiplier is 3
- Click **Apply to All**.



4-30. Figure Sample based quality control definition dialog box

To setup the Second Reporter

- Click the **Reporter 2** icon and repeat the above steps.

Verify that you have the correct **Reporter** icon highlighted on the ribbon while performing this procedure, since switching between the two icons will change the focus of the **Quality Control Definition** table. The table shown corresponds to the highlighted icon.

The software provides results for each analyte and reporter. The table below explains the how the results are defined.

Result	Definition
Positive	The MFI of the sample is greater than the (user defined multiplier) times the MFI of the negative standard.
Negative	The MFI of the sample is less than or equal to the MFI of the negative standard.
Indeterminate	The MFI of the sample is greater than the MFI of the negative standard, but less than the (user defined multiplier) times the negative standard.
Error	Calculation error.

To setup sample based QC Definition for an individual analyte

Verify that you have the correct file(s) selected when performing this procedure. Calculations are based on the selected negative standard.

To define sample based QC for an individual analyte:

- ***Select*** the **Show Individual Analytes** checkbox.
- ***Click*** a column header of an individual analyte.
- ***Click*** the **Customize Settings** icon in the **Quality Control Settings** ribbon.
- The **Sample Based QC Definition** dialog opens.
- ***Select*** your negative standard from the Negative = MFI of Selected Sample menu.
- Specify the multiplier for your negative standard in the **Positive=MFI of Negative x field**.
- ***Click*** **Apply** to the Selected

4.5.2 Control Definition

This section explains how you can specify the parameters of the control samples, concentration or qualitative classification, and then use them in your bead assay to verify the accuracy of the measurement and the calculations. Controls added to the plex appear in **quantitative or qualitative** analysis' table, corresponding to the selected analyte model. In this view the values' uniformity matches the selected value **(uniform/non uniform)** defined in the **Standards and Quality Control** window.

To define controls in a quantitative analysis:

- ***Click*** **Control Definition** in the **Plex** navigation panel.
- Specify the concentration of your control samples by ***clicking*** in the **Nominal CC** field and entering the value.

The measurement unit will match the one defined in **Standards and QC**.

- When needed, change the **Acceptance Range** value.

It is set to 20% by default **(Final CC +/- 20%)**. Enter a value between zero and one to change it.

Any out of range values are shown in the results and reports.

To define controls in a qualitative analysis:

- ***Click*** **Control Definition** in the **Plex** navigation panel.
- Define the control samples as negative or positive by ***clicking*** the appropriate sign in the **Negative/Positive** column.

Any out of range values are shown in the results and reports.

4.6 Analyzing the Experiment level 3

4.6.1 Solving Analysis Issues

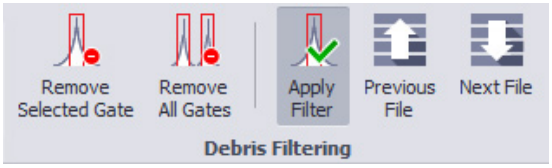
During automatic analysis certain anomalies may arise, this section explains how to use the tools **(Debris Filtering, Manual Clustering)** provided by FCAP Array to prevent or rather resolve the issues regarding these anomalies.

There are two different types of anomalies, which may arise and that cause difficulties the first is the improper positioning of the bead clusters and the second is the excess of debris in the sample being tested. The bead clusters may be too close to each other and the excess of debris will generate improper results calculated by the automatic clustering algorithm. **See section on Debris Filtering** or **See section on Manual Clustering** to solve these issues.

NOTE: if automatic clustering fails it is advisable to start removing the anomalies with the debris filtering function, then see if the automatic clustering algorithm works properly. In the case the algorithm fails to cluster properly use the manual clustering function.

4.6.2 Debris Filtering

This section explains how to use the debris filtering function to filter out debris that affects the automatic clustering algorithm and which can lead to clustering results that are incorrect.



4-31. Figure Debris filtering button group

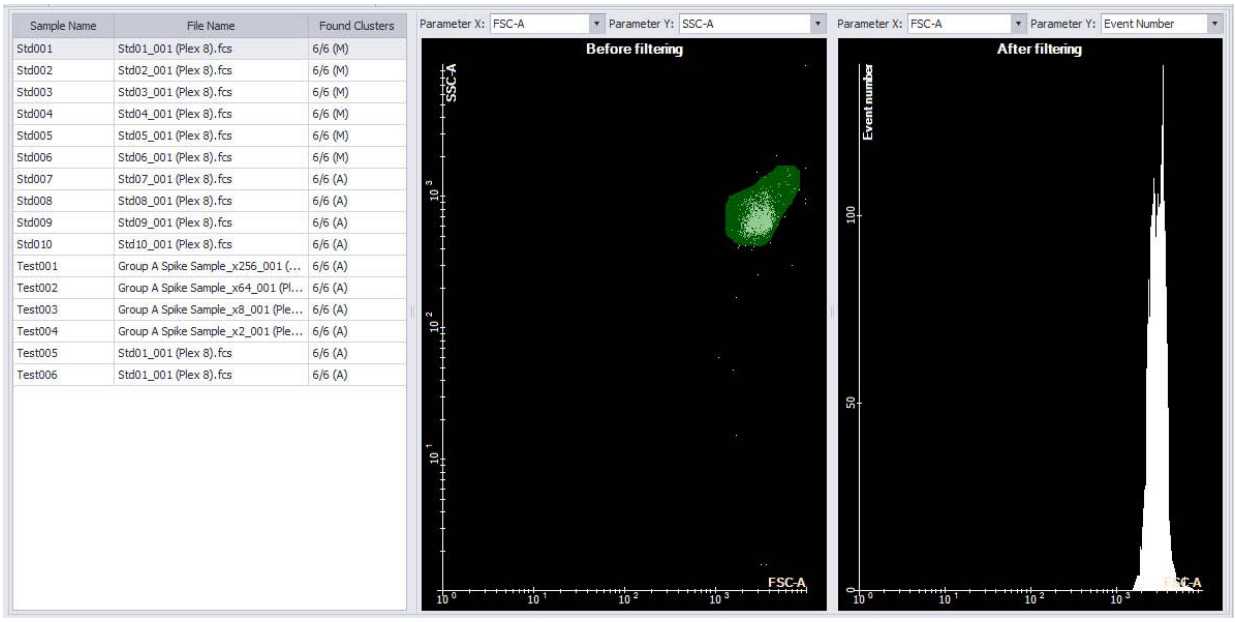
In order to filter out debris from a plot, you need to specify the beads that need to be analyzed on the left plot (**Before Filtering** plot) by drawing a gate around the area. To turn on the filter ***click*** on the **Apply Filter** icon in the **Debris Filtering** ribbon, turns on or off the filter and the results are shown on the right hand side plot.

NOTE: The gate(s) specified apply to all samples and are saved within the plex.

4.6.3 How to use Debris Filtering

- Click Debris Filtering in the Plex pane.

A list of samples and two plots are displayed.



4-32. Figure Debris filtering view

- ***Select*** a sample from the list.
  The histograms are shown according to the set parameters of the selected sample file.
- If needed, change the selected parameters by selecting from the menus above the histograms. You can use different parameters in the two plots.
- On the left histogram, hold down the mouse button and move the mouse around the targeted population to draw your gate. Multiple gates can be specified. The histogram on the right shows the filtered results.
- Instructions to move, resize, or delete the gates. **(Optional)**
  - ***Move*** a gate by ***selecting*** it and ***dragging*** it to another position.
  - ***Resize*** a gate by ***grabbing*** one of the sides.
  - ***Delete*** a gate by ***selecting*** it and clicking the **Remove Selected Gate** icon. You can also press the **Delete** key.

NOTE: The debris filter deletes the assignment of your beads to populations. Therefore they need to be re-assigned with **Instrument Settings** or **Manual Clustering** views.

4.6.4 Manual Clustering

4-33. Figure Manual clustering button group

This section explains how to use the manual clustering function to resolve the problems caused by the automatic clustering algorithm, which was unsuccessful in locating your bead clusters.

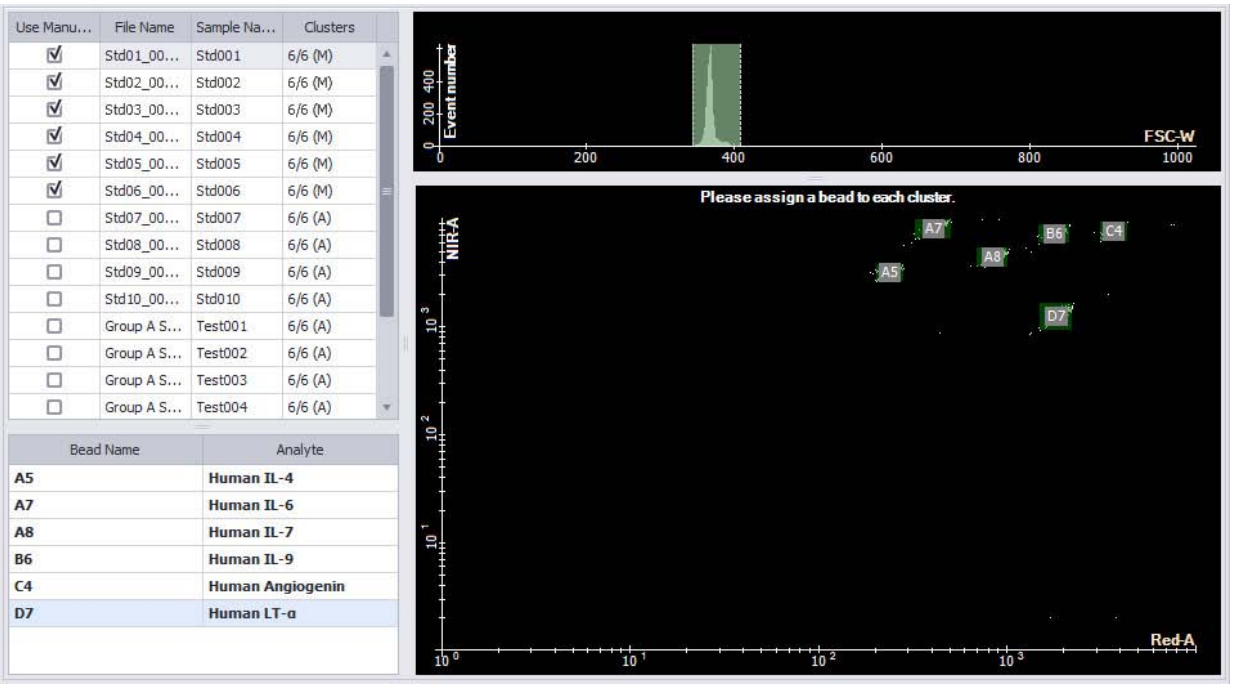
Bead(s) that have been successfully clustered with the Automatic clustering function may

also be modified with this process to promote further accuracy.

The functions located in the **Manual Clustering** ribbon can be used to navigate up or down the sample list and to perform actions on multiple samples all at once.

How to apply manual clustering

- ***Click*** **Manual Clustering** in the Plex navigation panel. The list of the samples open on the left and the two plots open to the right of the list.



4-34. Figure Debris filtering view

- Review the **Clusters** column in the **sample list** table, where you can find out if manual clustering is needed for any of the samples.

NOTE: If a sample row turns red in Manual Clustering > Sample list, the automatic cluster of that sample is failed.

- ***Select*** a sample from the list.
  The ***selected*** data of the sample is shown in the histogram as defined in the **Instrument Settings** view. In the **dot plot** view the data values are plotted as specified by the **clustering parameters** and you can determine which cluster was not located by the algorithm.
- To use **Manual Clustering** – ***click*** the **checkbox Use Manual Clustering** in the row of the sample that you have chosen. Auto clustering will be disabled for that particular sample.
- Specifying your clustering gates – ***click*** on the histogram and ***press*** the mouse button down and slide the sides of the rectangle to the desired position to include the proper population. Instructions to move, resize, or delete the gates. **(Optional)**
  - **Move** a gate by ***selecting*** it and ***dragging*** it to another position.
  - **Resize** a gate by ***grabbing*** one of the sides.

- **Delete** a gate by ***selecting*** it and ***pressing*** the **Delete** key.

- On the **dot plot, *press*** down the left mouse button and move the mouse left or right to move your gate so you can see your population. You can draw multiple gates.
- Use the mouse to draw a new gate around the cluster in the dot plot. Do one of the following:
  - ***Hold*** the mouse button while drawing your freehand gate.
  - Consecutively ***click*** and move your mouse and ***click***, repeat this step for the sides of a polygon gate.
- To resize a **dot plot gate**, ***click*** on the gate, ***hold*** Ctrl + ***drag*** its bottom or right side. **(Optional)**
- After defining all the clusters, ***assign*** beads to them.
  - ***Select*** the bead you want to assign from the **Selected Beads** table.
  - ***Double-click*** the cluster that you want to assign the bead to.

Revert back to auto clustering for selected samples:

- ***Clear*** the **Use Manual Clustering** checkboxes for your selected samples in the list.

Navigation in the sample list

- ***Click*** a row in the **sample list** to show the file's sample data.
- ***Click*** the **Next File** icon to move down the **sample list** and show the next file's sample data.
- ***Click*** the **Previous File** icon to move up the sample list and show the previous file's sample data.

Selecting failed files for manual clustering

- ***Click*** the **Use Manual Clustering** for Failed Files icon.

You can now edit the failed files indicated by red colored text. Adjust scatter parameters, adjust clustering gates, and assign beads.

Selecting all files for manual clustering:

- ***Click*** the **Use Manual Clustering for All Files** icon.

You can now edit all files. Adjust scatter parameters, adjust clustering gates, and assign beads.

Reverting to auto clustering for all files

- ***Click*** the **Use Auto Clustering for All Files** icon.

All parameters are restored to automatic clustering.

Applying manual clustering to selected files

- ***Select*** more than one files from the sample list by clicking the corresponding checkboxes in the **Use Manual Clustering** column.
- ***Click*** on one of the samples selected for manual clustering, to make it active.

- ***Set*** scatter parameter(s), zoom in the plot if necessary, ***draw*** clustering gates, and finally ***assign*** beads. This selected sample will be the template for all the rest of the manually clustered ones.
- ***Click*** the **Apply to All Manual Clustering Files** icon.
- ***Check***, that all the rest of the manually clustered samples have the template structure.
- ***Modify*** the template structure in the rest of the manually clustered samples in the required cases.

All your changes are applied to all selected files.

Clearing the bead – cluster assignments

- ***Select*** a file checked for manual clustering from the sample list by ***clicking*** on it. The sample row becomes highlighted.
- ***Click*** the **Clear Cluster Assignment** icon.

All the bead assignments for the selected file are removed. The scatter parameter and clustering gates remain unchanged.

NOTE: If multiple checkboxes are selected from the sample list, only the sample in the highlighted row is affected when you click the **Clear Cluster Assignment** icon.

Clearing the manual clustering gates

- ***Select*** a file checked for manual clustering from the sample list by ***clicking*** on it. The sample row becomes highlighted.
- ***Click*** the **Clear All Bead Clusters** icon.

All clustering gates are removed with their bead assignments. The scatter parameter remains unchanged.

NOTE: If multiple checkboxes are selected from the sample list, only the sample in the highlighted row is affected, when you click the **Clear All Bead Clusters** icon.

4.7 Analyzing the Experiment level 4

The last remaining FCAP software elements in the **Plex Pane** are used to evaluate (values, charts and reports) of either the **standard curve, results per analyte or results per sample**. The **Reports** function of FCAP is a comprehensive reporting tool of all data contained in the designed **Plex** of the experiment. See below.

4.7.1 Standard Curves

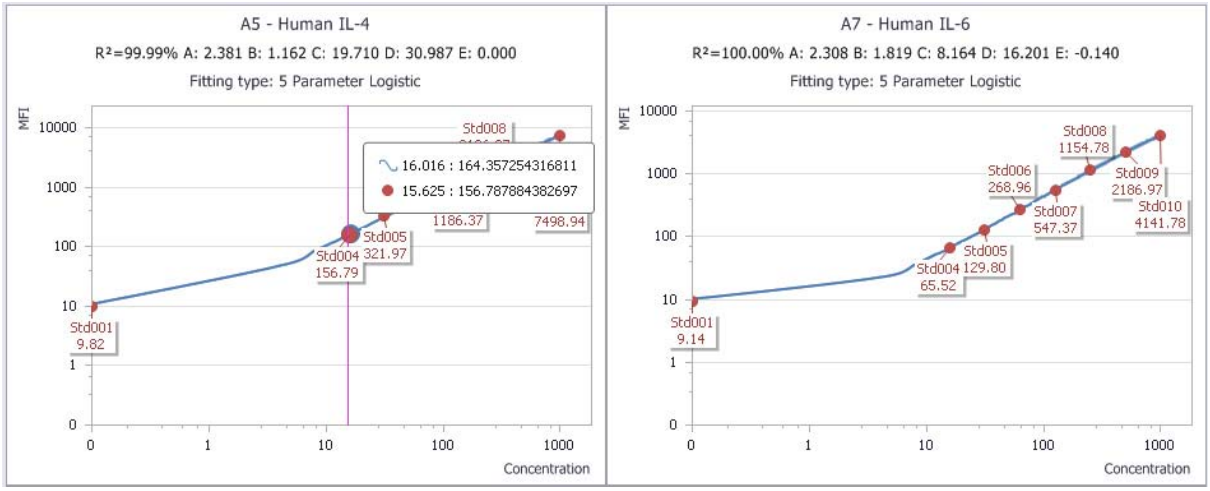
This section explains how to view the standard curves and how to modify the display to optimize the views of the calculated standard curves for an experiment. ***Click*** on **Standard Curves** menu on the left in the active **Plex** pane to display window. The window is separated into two panes, curves on the left and the table of analytes on the right.

The **Standard Cursor** helps to read results directly from the standard curves, making the CC from MFI or the MFI from CC approaching calculations easier.

NOTE: Only the curves for quantitative analysis model of analytes are available.

4.7.2 To view the standard curves:

- ***Click*** **Standard Curves** in the **Plex** pane.
  The following figure shows a portion of a set of standard curves.



4-35. Figure Two standard curves from the Fungi-6 mycotoxin Kit

- If needed, zoom in on the standard curve and statistics for a specific analyte either by selecting an analyte from the Analytes list or by ***double-clicking*** the plot.

The fitted results statistics are displayed below the plot.

NOTE: In experiments containing multiple replicates of standard samples, the result table shows the average values of the sample first, and the rows below contains the values for each replicates.

In the **Standard Curves Options** ribbon the user can modify the parameters of the calculated standard curves by using the displayed features of FCAP Array.

4.7.3 Options for curve settings

The standard curve generation is an important part of the analysis, it is like calibrating your measuring instrument.

The main options to modify standard curve parameters:

- Fitting Type, Weighting and Standard Sample (Ignoring standard points) selection,
- Fitting Accuracy and Force Through Zero setting.

The main options to modify standard curve display:

- Axes Scaling and Chart Color selection,
- Chart Label display and Hide Blank Standard setting.

4-36. Figure Options to create and display the suitable standard curves

4.7.4 Axis scaling

The scales of the X and Y axis are logarithmic by default. You can modify each to linear by ***clicking*** and ***selecting*** "Linear" in the drop-down list of the axes in the **Standard Curve Options** ribbon.

4.7.5 Fitting types

The **Fitting type** specifies the fitting function, which describes your measurement mathematically. In cytometry, this this is a five-parameter logistic function in most cases, so it is set to default in the software. To modify the standard curve type, change the mathematical function in **Standard Curves > Standard Curve Options > Fitting Type** drop-down list. The recalculation of the fitting curves runs automatically, the plots show the refreshed data. If the calculation takes longer, the calculation bar shows the status of the process.

The following **fitting types** are supported: 5 parameter logistic, 4 parameter logistic, exponential sigmoidal, linear, quadratic, cubic, logarithmic, exponential 3 parameter and power 4 parameter.

You can find the equation of each **fitting type** in the drop-down list.

4.7.6 Weighting

FCAP calculates standard curves without weighting by default. You can select a weighting model from the **Weighting** drop-down list in the **Standard Curve Options** ribbon. The available weighting methods are based on equivalent weights **(No Weighting)**, on **Event Numbers** or on **Event Number/CV** values. Under the third option weighting is applied and in this case all standard points are weighted equally.

After changing method, the fittings are recalculated and the curves are redrawn automatically.

NOTE: Fitting accuracy is independent from **Weighting**. It is recommended that you select the **Weighting** method based on your own scientific experience.

4.7.7 Fitting accuracy

If the R square of the fitting algorithm result is lower than the value of the fitting accuracy you receive an error message regarding the related analyte. **Fitting accuracy** is set to 98% by default. **Change the Fitting Accuracy (%)** value in the **Standard Curve Options** ribbon either by entering a new number or using the spin button and then ***press*** Enter.

The minimal value of the R square value is 90%. If the fitting accuracy is lower, the fitted parameters become hidden so as not to be calculated with uncertain results.

NOTE: Fitting accuracy is a user preference, so the default number is customizable for users.

4.7.8 Force through zero

If you have a blank standard sample (0pg/ml) in the **plex**, which degrades the fitting accuracy, you have an option to ignore its data in the fitting calculation. The **Standard Curves > Standard Curve Options > Force Through Zero** status button is enabled by default, click it to disable if necessary.

4.7.9 Save Chart

When reviewing a single curve by clicking to a single bead from **All** beads in **Standard Curves** view > **Beads** grid, the **Save Chart** icon become active. ***Click*** on it to save the active chart to image file in the following formats: **JPEG, PNG** and **BMP**. ***Specify*** the location in the **Save As** file explorer window to save the chart.

4.7.10 Chart color

Select a chart color from the drop-down list in the **Standard Curve Options** ribbon. When saving a curve, the image file will correspond to the selected color.

4.7.11 Chart Labels

Click the **Chart Labels** icon on the **Standard Curve Options** ribbon to toggle the display of standard point descriptors (standard point name and respective MFI value) on the chart.

Inspecting curves, ignoring standard points

When you navigate to the **Standard Curves** view all standard curves are visible.

You can inspect curves and their data one by one. ***Double-click*** on a plot for single view. The statistical results of the standard curve appear below the plot. ***Double-click*** on the plot to go back to the default view. You can also change between plots by ***clicking*** in the beads table.

You can check the standard curves and ignore one or more standard points in case they don't fit correctly in the curves. Go to the detailed view and ***tick*** the **checkbox** in the results table of the standard you want to take out from standard curve. The recalculation runs automatically, the results and the plots reload with the modified setting.

4.8 Analyzing the Experiment level 5

Two types of **Results** views are available, one represents the details per analyte and the other shows per sample.

- ***Click*** on **Results per Analyte** or **per Sample** on the left, in the **Plex pane**.

In the **Results** view, you can inspect statistical analysis, plots, graphs and export data to comma separated, pdf, html files.

Click on each analyte or sample to find listed all the statistical details **(MFI, Event number, CV, SD, Nominal CC, Fitted CC, Final CC)**.

4.8.1 Analysis Results

In the **Results View**, you can inspect analysis statistics and histograms. The analysis statistics contain the **MFI values (median fluorescence intensity)**, the **event number**, the **standard deviation (SD)**, the **coefficient of variation in % (CV%)**, the sample and the **final CC, Quality control** results or **Qualitative** analysis results for each analyte.

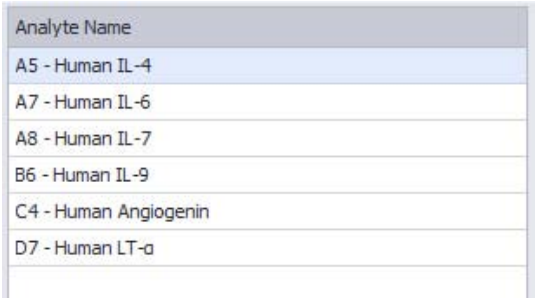
4.8.2 Reviewing the Results

Results are shown in two table views. From the left menu you can choose the view either for samples or for analytes by ***clicking*** on the **tabs** in the header. In the right table you see the details according to the selected row on the left.

Column	Description
Results file	Assigned FCS data file
Event count	Shows the event number
MFI	Shows the median fluorescence intensity
SD	Shows the standard deviation
CV	Coefficient of variation in percentage
Nominal CC	Nominal concentration of the standard or control samples
Fitted CC	Sample concentration calculated from the fitting curves
Final CC	Dilution factor is not applied
QC	Sample concentration calculated from the fitting curve with dilution factor applied
Qualitative	Quality Control analysis result message
	Qualitative analysis result message

4-3. Table Elements of result statistics

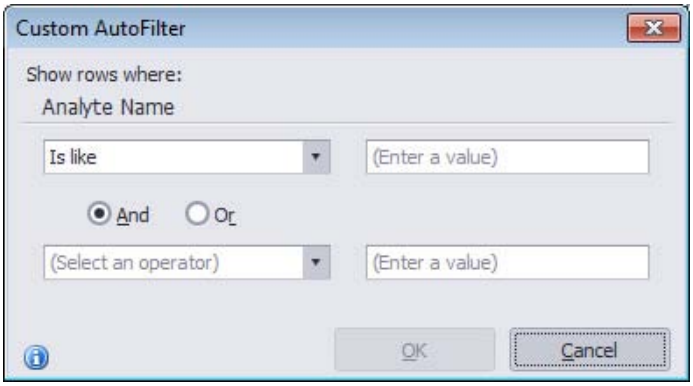
You can organize the order in each column to decreasing or increasing by ***clicking*** the column header. The arrow shows the direction of the order.



4-37. Figure Filtering the results

Filtering is available to customize the data view on both left and right side, by ***clicking*** on the **filter** icon in the column header's right corner. Choose from the options or specify your own parameters by ***clicking*** on **Custom**.

The **Custom filter** dialog appears. ***Fil***l in the details and ***click*** **OK**.



4-38. Figure Custom AutoFilter screen

The filter appears at the bottom of the table. Here you can modify, inactivate or delete the filter.

4.8.3 Customizing Chart

Below the data table, you can also view the results in the graphs. ***Select*** a row in the left table and check the according result chart. If the table contains analytes, you find **result data per sample** in the chart. Furthermore you will see the **result data per analyte** when the table is set to samples.

When **result per analyte** view is active, you have an option to **turn on** or **off** the different **sample types** on the chart.

- ***Click*** on the corresponding icons in the **Results** group on the ribbon. The results are

shown in **MFI values** by default.

In case you want to change the data type, ***click*** in the **Data Type** drop-down list in the **Chart** ribbon and choose from the menu. The menu contains all the listed data types of the results table.

You can also change the chart type by ***clicking*** on the icon in the **Chart** ribbon. Three chart types are available: **Bar Chart, 3D Bar Chart** and **Polar Chart**. If you **select 3D Bar Chart**, after ***clicking*** on the chart you are able to rotate it with your mouse and zoom in/out with your mouse wheel.

NOTE: Charts can be saved in the following formats (file extension: **JPG, BMP, PNG**)

Save your chart by ***clicking*** on the **Save chart** icon in the **Chart** ribbon. The format is set to **JPEG** by default, **BMP** and **PNG** are available as well.

4.8.4 Results per Analyte

4-39. Figure Results per Analyte > Chart & Results button group

The home ribbon of the **Results per Analyte** view offers the following options:

- ***Select*** the chart type by the **Chart Type** roll-down status button containing the following elements:

  Bar Chart: results are shown in a row as hihgt of columns, the chart is in 2D.
  3D Chart: results are shown in a row as hihgt of columns, the chart is in 3D and rotatable among 2 axes.
  Polar Chart: results are shown in individual axes as points, the chart is in 2D.
  Plate Heatmap: results are shown in individual wells, the chart is in 2D, the color is the third dimension, which color represents the result value.

- ***Turn on*** or ***off*** your samples in the chart:
  Standard status button: If the status is ***on***, the standard (blue) samples are shown in the chart, otherwise they are left out of it. The **Test** and **Control** status buttons work the same.

- ***Turn on*** or ***off*** chart labels:
  You can toggle the Chart Labels on or off by the **Chart Labels** status button.

- ***Scroll*** status button:
  When the number of the samples shown in the chart is more than 40, the **on** status of **Scroll *divides*** the results to partitions composed by maximum 20 elements. The off status does not modify the **Results per Analyte Chart**.

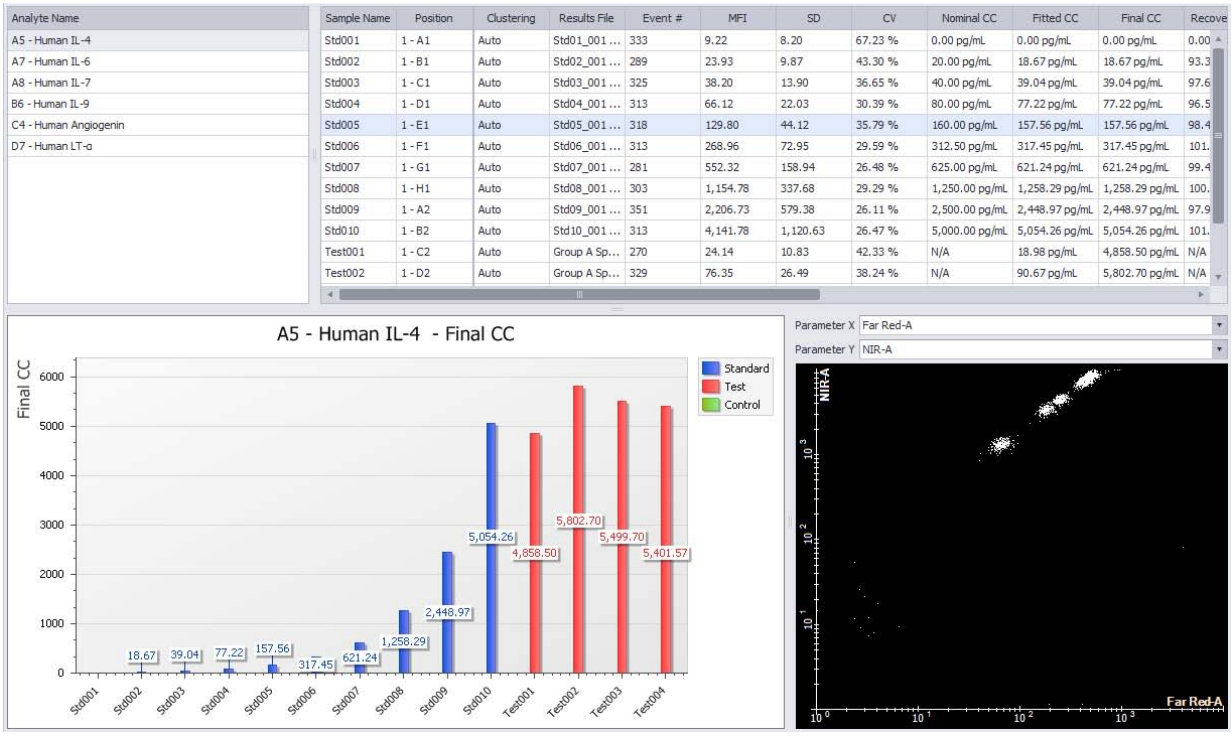
NOTE: Scroll does not modify the **Results per Analyte Chart**, if the number of samples is less than 40 in the **Plex**.

- **Export** roll-down button:
  You can export the **Results per Analyte** result grid in the following file formats by the definition of file location with the help of the **Save As** file explorer window:
  Ecxel (xls), PDF, Rich Text (rtf), HTML and **Comma separated csv.**

- **Save Chart** roll-down button:
  You can save the **Results per Analyte Chart** in the following picture formats by the definition of file location with the help of the **Save As** file explorer window:
  JPEG, BMP and **PNG**.

It also possible to print your results with the **Print** button, by the help of **Preview** view, where you can find the usual print setup functions.

The **Results per Analyte** view shows on the left upper grid the list of the analytes and the selected analyte related sample results in the upper right result list. The **Results per Analyte Chart** is displayed in the left down quarter of the view. The displayed data is depend on the selected analyte in the **Analyte List**; the selected data type in the **Data Type** drop-down list; the selected chart type in the **Chart Type** drop-down list; the status of **Chart Labels, Scroll, Standard, Test** and **Control** status buttons. The **Results per Analyte Clustering Plot** is displayed in the left down quarter of the view. The displayed data is depend on the selected sample in the **Sample List** and the selected parameters in **Parameter X** and **Parameter Y** parameter lists similar to **Instrument Settings**. See **4 40. Figure**.



4-40. Figure Results per Analyte view

4.8.5 Results per Sample

4-41. Figure Results per Sample > Chart & Results button group

The home ribbon of the **Results per Sample** view offers the following options:

- ***Select*** the chart type by the **Chart Type** roll-down status button containing the following elements:
  Bar Chart: results are shown in a row as height of columns, the chart is in 2D.
  3D Chart: results are shown in a row as height of columns, the chart is in 3D and rotatable among 2 axes.
  Polar Chart: results are shown in individual axes as points, the chart is in 2D.

- ***Turn on*** or ***off*** your samples in the chart:
  Standard status button: If the status is on, the standard (blue) samples are shown in the chart, otherwise they are left out of it. The Test and Control status buttons work the same way.

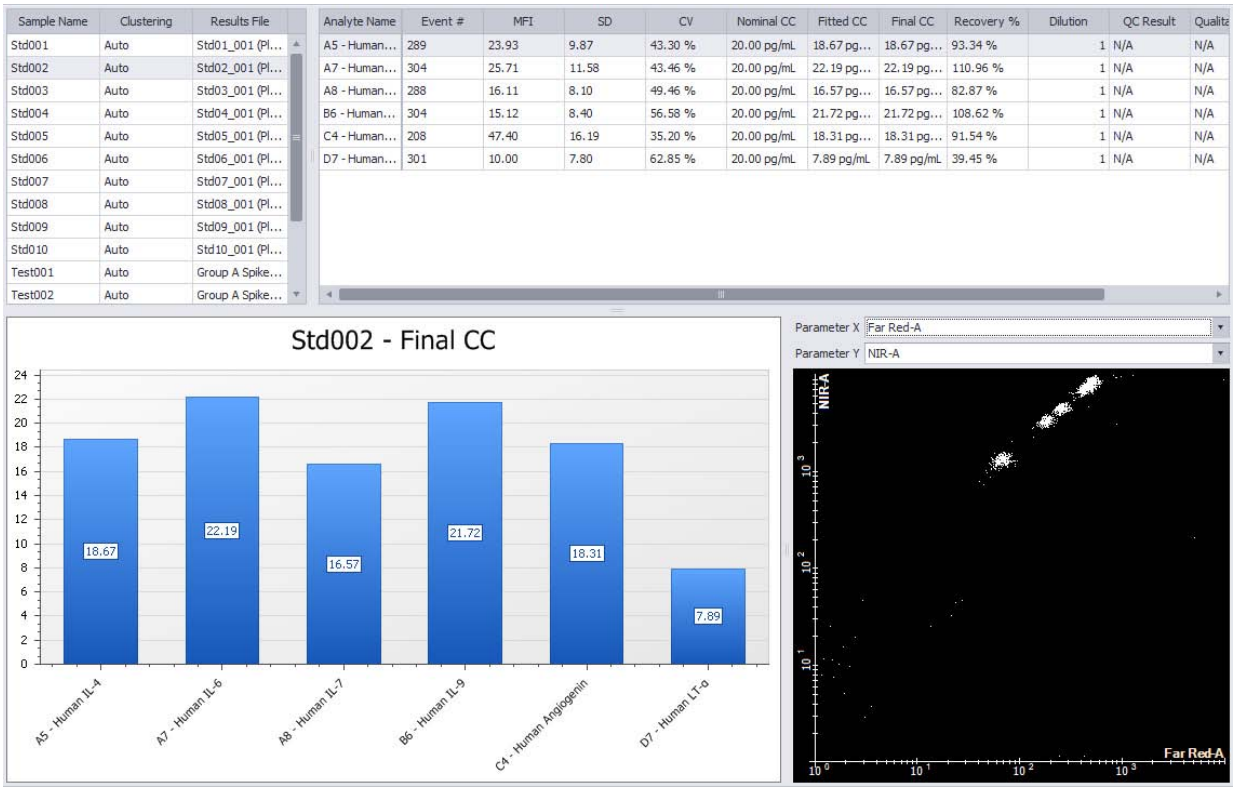
- ***Turn on*** or ***off*** chart labels:
  You can toggle the Chart Labels on or off by the **Chart Labels** status button.

- **Export** roll-down button:
  You can export the **Results per Sample** result grid in the following file formats by the definition of file location with the help of the **Save As** file explorer window:
  Ecxel (xls), PDF, Rich Text (rtf), HTML and **Comma separated csv.**

- **Save Chart** roll-down button:
  You can save the **Results per Sample Chart** in the following picture formats by the definition of file location with the help of the **Save As** file explorer window:
  JPEG, BMP and **PNG**.

It also possible to ***print*** your results with the **Print** button, by the help of **Preview** view, where you can find the usual print setup functions.

The **Results per Sample** view shows on the upper left grid the list of the samples and the selected sample related analyte results in the upper right result list. The **Results per Sample Chart** is displayed in the lower left quarter of the view. The displayed data is depend on the selected sample in the **Sample List**; the selected data type in the **Data Type** drop-down list; the selected chart type in the **Chart Type** drop-down list; the status of **Chart Labels**, **Standard, Test** and **Control** status buttons. The **Results per Sample Clustering Plot** is displayed in the lower left quarter of the view. The displayed data depends on the selected sample in the **Sample List** and the selected parameters in **Parameter X** and **Parameter Y** parameter lists similar to **Instrument Settings**. See **Figure X-Y**.



4-42. Figure Results per Sample view

4.9 Analyzing the Experiment level 6

4.9.1 Plex Report Document

The **Plex Report** contains all the main details of the selected Plex. Report Options are the following:

Plex Definition group:

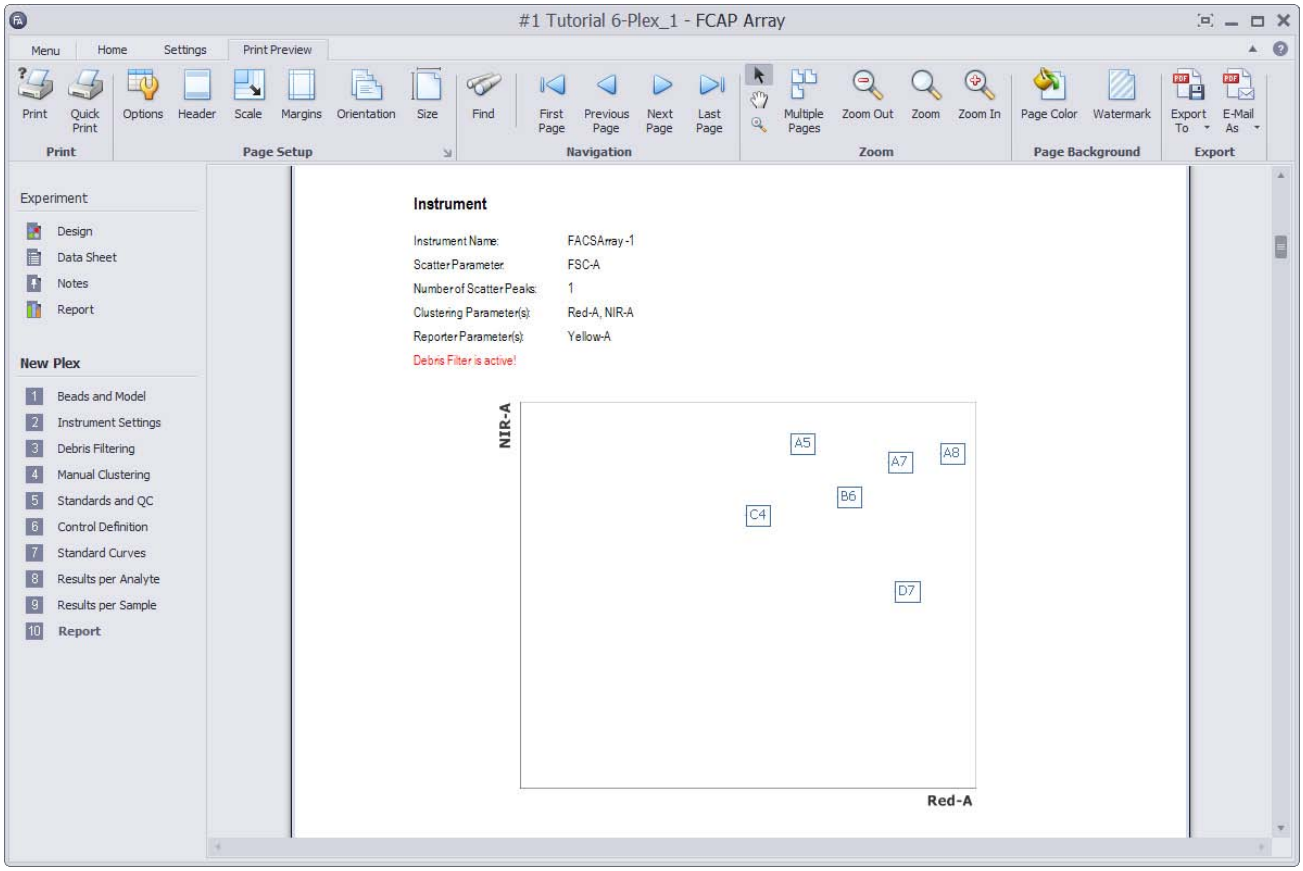
Report Cover, Plex Component Report, Instrument Setting Report, Visual Plate Report, Control Definition Report, File Assignment Report

Standards group:
Standard Definition Report, Standard Curves Report, Standard Curve Tables

Results group:
Sample Statistics Report, Sample Quantitative Report, Sample Qualitative Report, Sample Charts, Analyte Statistics Report, Analyte Quantitative Report, Analyte Qualitative Report, Analyte Charts.

NOTE: Report Options can find by ***clicking*** **Page Setup > Options** on **Report view**.

Clicking on **Plex > Report** view is automatically generated and it opens the plex report document. If report generation takes longer, **Process Bar** appears and shows the status of the generation.



4-43. Figure Plex Report

Each **Plex** has an individual **Report**.

4.9.2 Completing an Experiment

The last step is to review your results and reports if you haven't done it yet, lock the experiment if needed, then save, and close the plex template. For details see below **Level 7.**

5
FINALIZE THE EXPERINMENT

Configuring reports

5.1 Analyzing the Experiment level 7

The final result is the Experiment Report in FCAP Array, which is composed by the Plex Reports. Each Plex Report is generated in predefined but customizable segments. These Reports are simplified and designed for further use.

5.1.1 Working with Report Documents

With FCAP Array you can choose to export and print the report. It is also possible to edit the header of the report. Find below the options for both **Plex** and **Experiment** reports.

Two types of report documents are available in FCAP Array: **Plex Report** and **Experiment Report**. You can view, export and print both. The **Report document** contains all the defined data for the analysis and the results.

Plex report and **Experiment report** can be exported in the following formats (file extension: **XLS, PDF, RTF, HTML, comma separated (CSV), MHT, TXT, & image files (jpg, bmp, png)** and can also be sent via email.

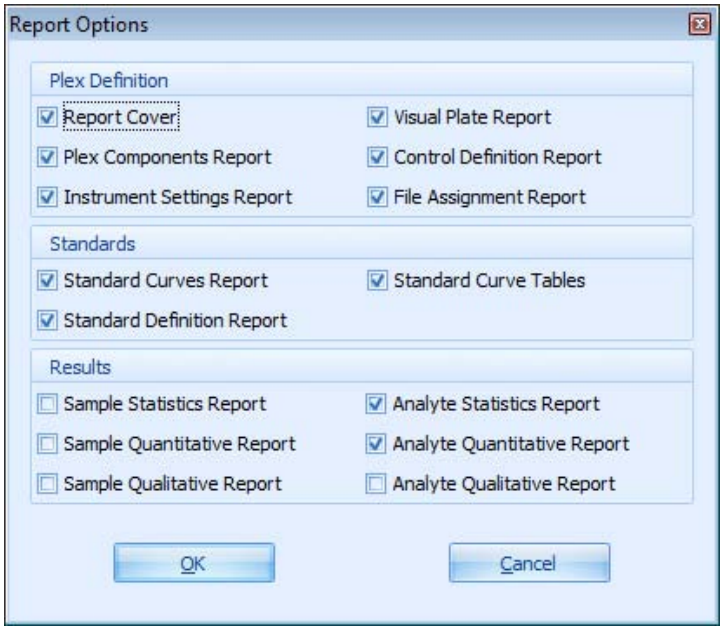
5.1.2 Experiment Report Document

The **Experiment report** contains all the **Plex** reports merged in a single document.

- Navigate to the **Experiment** pane and ***select*** the **Report** menu to open it.

5.1.3 Report Options

Report option shows the elements, that the Plex Report is composed of. It is customizable by ***clicking*** **Report view > Page Setup > Options** button. Figure below shows the default setting of the **Plex Report**.



5-1. Figure Report Options screen

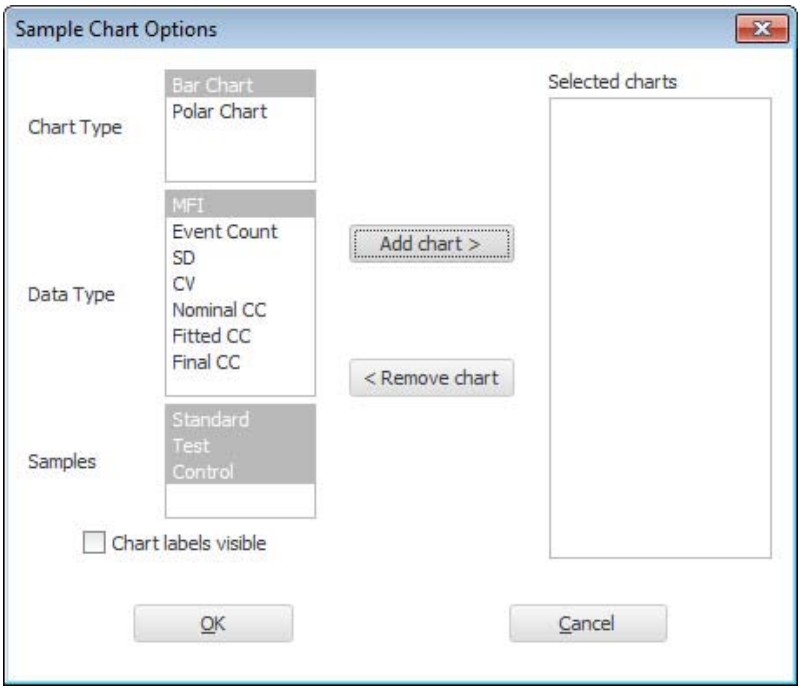
Check on or ***off*** report elements you need or not need in the Plex Report document, then ***click*** **OK** to generate the new Plex Report. Progress bar appears, if more time is needed for generating the document.

NOTE: If **Sample Charts** or **Analyte Charts** element was selected, more setting is necessary.

Sample chart options

If you need a chart on your Plex Report, you have to ***define*** it in the Sample Chart Options screen, and ***add*** it to the Plex Report.

- ***Click*** **Report > Options**.
- ***Check*** **Sample Charts** and ***open*** the **Sample Chart Options** screen.
- ***Select*** **Chart, Data** and **Sample Type** from the appropriate lists.
- Not to forget ***setting*** the **Chart label visibility**.
- ***Click*** **Add chart >**.
- Your chart appears in the **Selected charts** list.
- Add more charts to the **Selected charts** list.
- ***Delete*** a false chart from the **Plex Report** by < **Remove chart**.

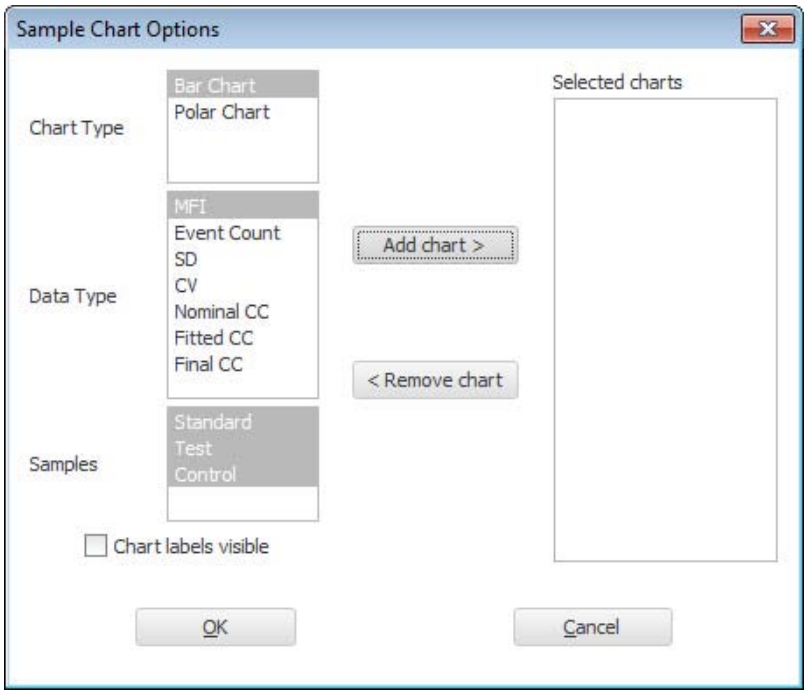


5-2. Figure Sample Chart Options screen

Analyte chart options

If you need a chart on your Plex Report, you have to define it in the Sample Chart Options screen, and add it to the Plex Report.

- ***Click*** **Report > Options**.
- ***Check*** **Analyte Charts** and ***open*** the **Analyte Chart Options** screen.
- ***Select*** **Chart, Data** and **Sample Type** from the appropriate lists.
- Not to forget ***setting*** the **Chart label visibility**.
- ***Click*** **Add chart >**.
- Your chart appears in the **Selected charts** list.
- Add more charts to the **Selected charts** list.
- ***Delete*** a false chart from the **Plex Report** by < **Remove chart**.



5-3. Figure Analyte Chart Options screen

5.1.4 Exporting and Printing

The user can export the completed experiment with file extension **f3e** to another location on the computer if needed or to be used as a backup, before making any changes to hardware configuration or before reinstalling FCAP Array.

Exporting

Result tables can be exported in different file formats such as **MS Excel (xls), PDF, RTF, HTML, CSV**. The file format is set to **xls** by default, ***click*** on the arrow of the **Export** icon to change it.

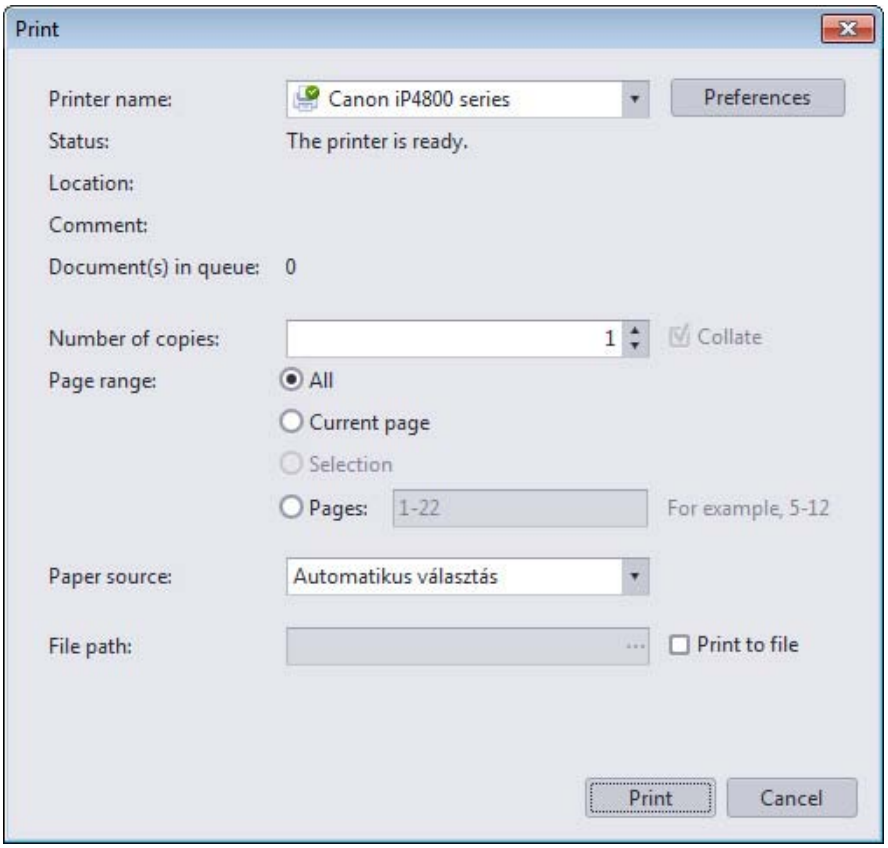
- ***Click*** on **Export** icon in the **Results** ribbon. The **Save as** dialog appears. ***Select*** a folder to **save** the file and ***click*** **Save**.

Sending via Email

- ***Click*** **E-mail As** icon in the **Results** ribbon. > the drop down list appears > Select the file format of the report to send email in e.g. **PDF, RTF, XLS, CVS, TXT**, or one of the image file types > ***Fill*** in the details for the file type > ***Click*** **OK**.

Printing

- ***Click*** on **Print** icon in the **Results** ribbon. The **Print** screen appears. Here you can edit the printer and page setup. ***Click*** on the **Print** icon to print your file.



5-4. Figure Print screen

5.1.5 Closing an Experiment

This section explains the process of finishing an experiment. The user can stop or finish working during any phase in the analysis of an experiment. There are several options to complete an experiment the functions are available from any window or view of the experiment.

If you are working with a locked experiment, saving is not an option and modifications are also disabled.

Saving an experiment

- ***Click*** the Save icon in the **Experiment** group on the ribbon.

FCAP Array saves any or all changes to the experiment, since last saved.

Saving an experiment as

To be able use a locked experiment, save a copy of it and the new copy of the locked experiment will be editable and your modifications can be saved. With the use of the **Save As** function, FCAP Array keeps the original experiment unmodified and saves you a copy with the all the changes and modification of your experiment.

To use save experiment as:

- ***Click*** the **Save As** icon in the **Experiment** group.
  The **Save As** dialog opens.
- ***Enter*** a name for the experiment.
- ***Click*** **Save**.

Saving a plex as a template:

You can reuse a saved plex template for your next experiment and streamline your workflow.

The information under the following will be saved to your new Plex (**Bead and Model, Instrument settings, Debris Filtering, Standard and QC, and Control Definition** – if any control samples in the experiment).

What a plex template does not save is the following: position of the tubes/wells of your standards, your tested samples or the file associations of either.

To save a plex as a template:

- ***Click*** the **Save Plex as Template** icon in the **Experiment** group.
  The **Save As** dialog opens.
- ***Enter*** a name for the plex.
- ***Click*** **Save**.

To close an experiment:

- ***Click*** the **Close** icon in the Experiment group, if the experiment has not been saved since making changes the **SAVE** dialog box will appear and then ***click*** **SAVE**.

The experiment is closed automatically. The program returns to the main window displaying the **experiment library**.

NOTE: In the case that the experiment is no longer needed it can be deleted from the software's database, however it will not be recoverable.

This section concludes the process of conducting and finishing an experiment in FCAP Array software.

6

USING EXPERIMENTS FROM FACSUITE ®

6.1 BD FACSuite workflow overview

This section explains the process for creating an experiment from **BD FACSuite FCS** files. The following steps are to be taken for a typical procedure for processing FCS files from **BD FACSuite** software.

- Starting the software, see Login to FCAP
- Creating a new experiment from BD FACSuite files, see From FACSuite Files
- Evaluating the experiment, see Level 4
- Viewing analysis results, see Level 5
- Viewing reports, see Level 7

NOTE: files of experiments from **BD FACSuite** and **FCAP Array** software are interchangeable between the programs

6.1.1 Information concerning CBA-specific keywords

To use the **BD FACSuite** workflow most efficiently, it is important that CBA-specific keywords are added to the experiment while it is being run in **BD FACSuite** software. **See the Guide for Using BD FACSuite Software with BD Cytometric Bead Array Products to learn how to perform this setup.**

Go to bdbiosciences.com for more BD CBA information.

Keyword	Description
CBA Plex Name	Identifies the specific plex from the FCAP Array software plex library. If a match is found in the plex library, the FCS files are automatically associated with that plex.
CBA Type	Identifies whether the tube or well contains a standard, sample, or control. FCAP Array software lists analysis results in the following order: standards, samples, controls.
CBA Standard ID	• ID of the standard contained in the tube or well. The value can also be Pos or Neg to identify positive and negative for qualitative BD CBA assays. • Standards will be arranged for the standard curve in alphabetical order by the value of the CBA Standard ID keyword. Assign this keyword appropriately to ensure that standards are plotted in the correct order on the standard curve. Use leading zeros in the CBA Standard ID and CBA Control ID keywords. This ensures that your files can always be sorted in order (01, 02, 03 as opposed to 1, 2, 3).
CBA Control ID	• ID of the control contained in the tube or well. The value can also be Pos or Neg. • Controls will be arranged in alphabetical order by the value of the CBA Control ID keyword.

Keyword	Description
CBA Dilution	• Used to specify the dilution of the sample. The default dilution value is 1.00. • Note that the dilution factor can be entered as a keyword or it can also be entered into FCAP Array software.

6 1. Table CBA keywords

6.1.2 Creating a new experiment from BD FACSuite files

This section explains how to create a new experiment using FCS files created in **BD FACSuite** software.

NOTE: That you cannot create a plex using the standard workflow and then try to use that plex in the **BD FACSuite** workflow. The keywords in the **BD FACSuite FCS** files are used to generate the plex(es) for the **BD FACSuite** workflow.

6.1.3 To create a new experiment from FCS files created in BD FACSuite software:

- ***Click*** the **New Experiment** button in the **Home** tab and ***select*** **New Experiment** > From **FACSuite Files**.
- The **New Experiment** from **FACSuite** files dialog opens.
- Navigate to the directory containing the appropriate **FCS files**.

The **FACSuite Experiment** folder defined in **Preferences** is selected as default for the **FACSuite Experiment** file selection browser.

The list of experiments in the right panel is the list of all unique **$PROJ** keyword values extracted from all **FCS files** contained in the folder. The new experiment will be populated with all the **FCS files** that share the selected **$PROJ** value.

- ***Select*** the experiment name in the **Experiments** field to use the same name for the FCAP Array experiment.

A custom name can also be entered into the **Experiment Name** field. ***Enter*** a comment, if needed.

- ***Click*** **OK**.

The new experiment opens with the layout as defined by the keywords in the **BD FACSuite FCS** files.

6.1.4 Evaluating the experiment

This section explains the process of evaluating the experiment that was created after opening the **BD FACSuite FCS** files.

6.1.5 Generating experiment results

FCAP Array automatically generates **plexes, samples, plates**, and their **layout**, based on the information contained in the keywords in the **BD FACSuite FCS** files. **FCAP Array** creates a number of **plexes** to match those defined in with the keywords. If the automatic analysis runs successfully based on the information, the experiment results are generated without defining any additional settings.

6.1.6 To verify or add information:

Check the positions and the types of samples in the **experiment design** view by ***clicking*** a well to see if it matches your corresponding **FACSuite FCS** file (**Sample Properties** panel).

NOTE: During the evaluation of the created experiment, FCAP Array offers the option to ***select*** and ***add*** more **samples, files**, or **plexes** to it. The properties can be modified of previously added files.

Solving problems in the cases where there are missing or undefined **plex** name(s), the user needs to define the pertaining information. To resolve these types of issues, see **Working with plexes** or **Troubleshooting.**

7

TUTORIAL (SAMPLE EXPERIMENT)

This section presents a tutorial, and in particular an experiment based upon the Fungi-Plex™ kit from SoftFlow Hungary Ltd., and we are the only developers of **FCAP Array Software** in the world.

7.1 Experiment background

Fungi-Plex™ is a flow cytometric analytical assay that is based on a microbead analysis, and which was developed specifically for the qualitative and quantitative detection of the following mycotoxins: Aflatoxin B1, Zearalenone, Ochratoxin A, Fumonisin B1, Deoxynivalenol and T2-toxin. They contaminate both our food and feed from grains (respectively – humans and animals).

Fungi-Plex™ is a particle based multiplexed competitive immunoassay. The Fungi-Plex™ system uses the sensitivity of the amplified fluorescence detection by flow cytometry to measure the mycotoxins in the sample.

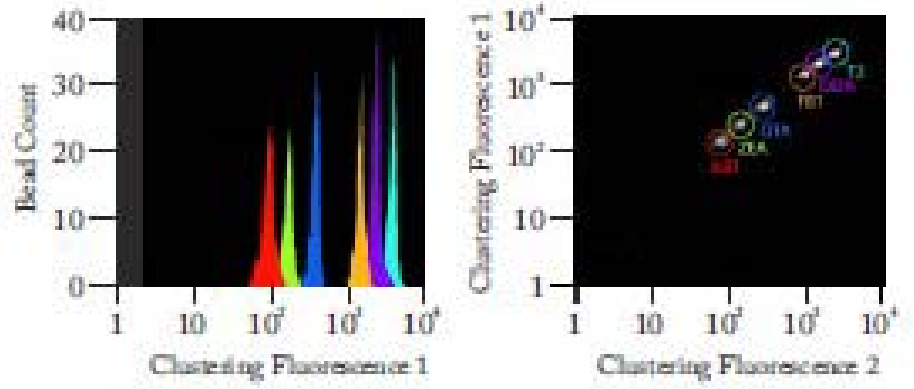
Each bead provides a binding surface for a specific mycotoxin (e.g. T2) and is analogous to an individually coated well in a microtiter ELISA plate. The assay is based on the competition of the mycotoxin molecules (as an antigen) and the mycotoxin-coupled phycoerythrin (PE) macromolecules.

NOTE: Mycotoxins are toxic agents produced by fungi, and they may pose as a health risk accumulated in both, animal feed and the food consumed by humans.

The instrument settings from the Fungi TDS for the BD FACS Array™ flow cytometer instrument:

- Clustering parameters are **Far-Red, NIR** > 640nm
- Reporter/detection parameter is **Yellow** – PE emission maximum ~ 575nm

7.1.1 How to read our beads



Bead Cluster	Specificity
A9 (Brightest)	T2-toxin
A8	Deoxynivalenol
A7	Fumonisin B1
A5	Ochratoxin A
A4	Zearalenone
A3 (Dimmest)	Aflatoxin B1

7-1. Figure Clustering data of Fungi-Plex 6 Kit

7.1.2 Summary of what we need to do to complete the experiment

- Acquisitioning of standards and our test samples with a flow cytometric instrument or device.
- Once we have our FCS files to attain our data for the standards and our sample test data by the specific instrument that we used for our measurements.
- Export or save the resulting FCS files, on the computer from where we can use these files in FCAP Array software.
- Design our Plex and assign our FCS file (standard and test samples) to the proper positions on our plate.
- Set our Beads and Analysis model.
- Set our instrument settings from the information provided in the documentation of the Fungi Kit.
- Set the concentration values for your standards. The standards should be setup in the order of the lowest concentration (Standard Diluent Buffer, 0 ng/ml) to the highest concentration.
- Filter our debris from our experiment (recommended process) to attain better results and if necessary initiate manual clustering (specify our desired gates to locate our beads) if the automatic clustering algorithm fails.
- Check standard curves for accuracy (re-acquisition if results are not satisfactory).
- Evaluate our experiment for result per analyte or result per sample and view our plex report. In this particular experiment, since there is only one plex assigned to our ex periment the plex report and experiment report are nearly the same.
- If we wish to use this plex for another experiment, then save the plex to the plex tem plate, otherwise view (evaluate), export, print our results and save the experiment (in general with computers – it's always recommended to save several times, during any process).

The above mentioned steps are required to conduct our analysis in FCAP Array software.

As you work through this tutorial you'll learn key FCAP Array soft¬ware features, and use FCS files provided with this user manual to perform data analysis. The tutorial starts with software launch instructions. The main content is in two sections:

- Designing a New Experiment in section **Design icon.**
- Data Analysis of bead assay, see **Level 5.**

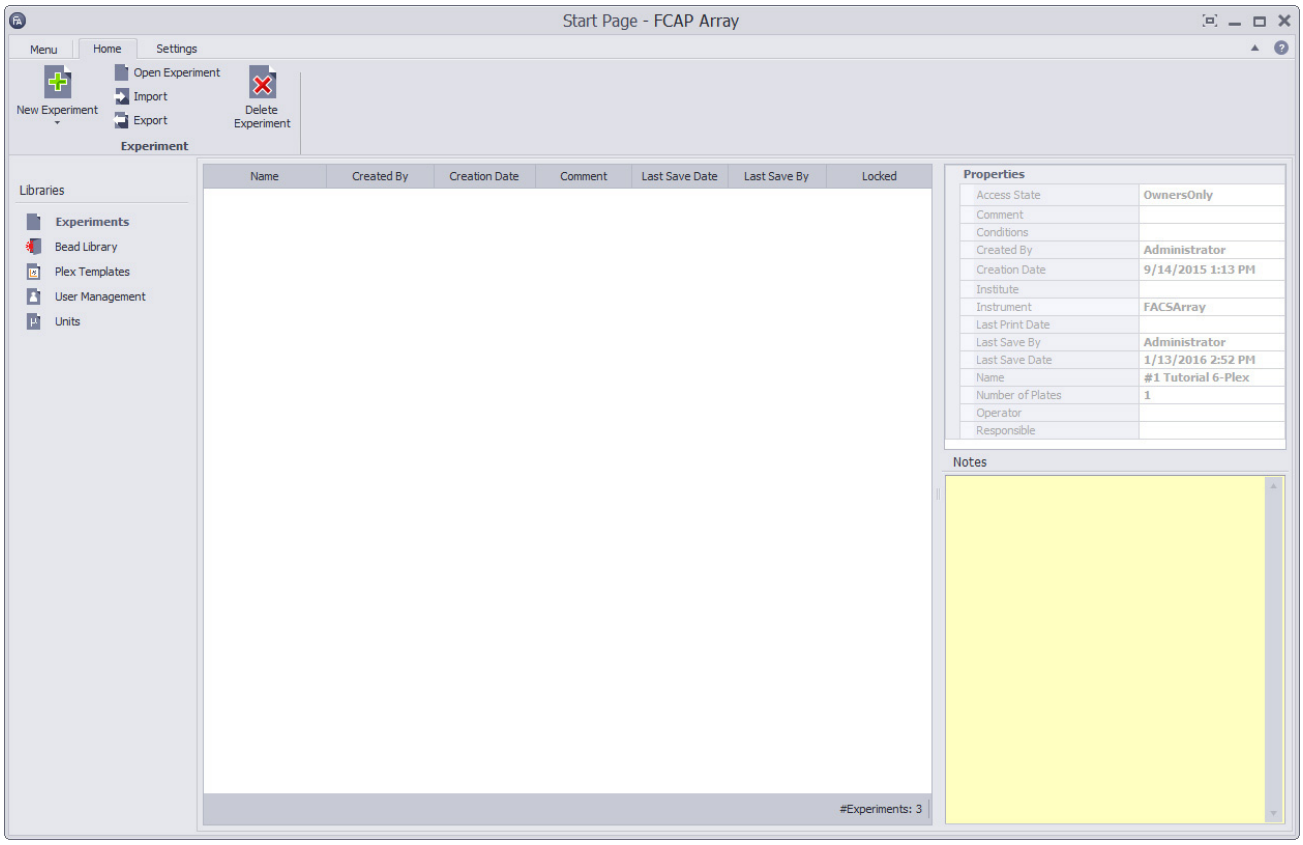
7.2 Getting Started Tutorial

So let's get started with our experiment!

7.2.1 Start FCAP Array

To start FCAP Array software, take the following steps:

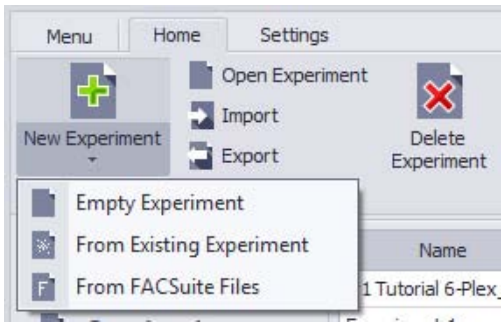
Select **Start > All Programs > FCAP Array > FCAP Array** or ***double-click*** the **FCAP Array** icon on your desktop. As the software starts up, the **Login** screen (***enter*** your login credentials) and then the **Main** window appears with the **Experiment Library** view.



7-2. Figure Main window > The empty Experiment Library

7.2.2 Creating a New Experiment

To create your **New Experiment *click*** on the lower half of the **New Experiment** icon and select **Empty Experiment** or ***click*** on the **green plus sign** on the **Home** tab.



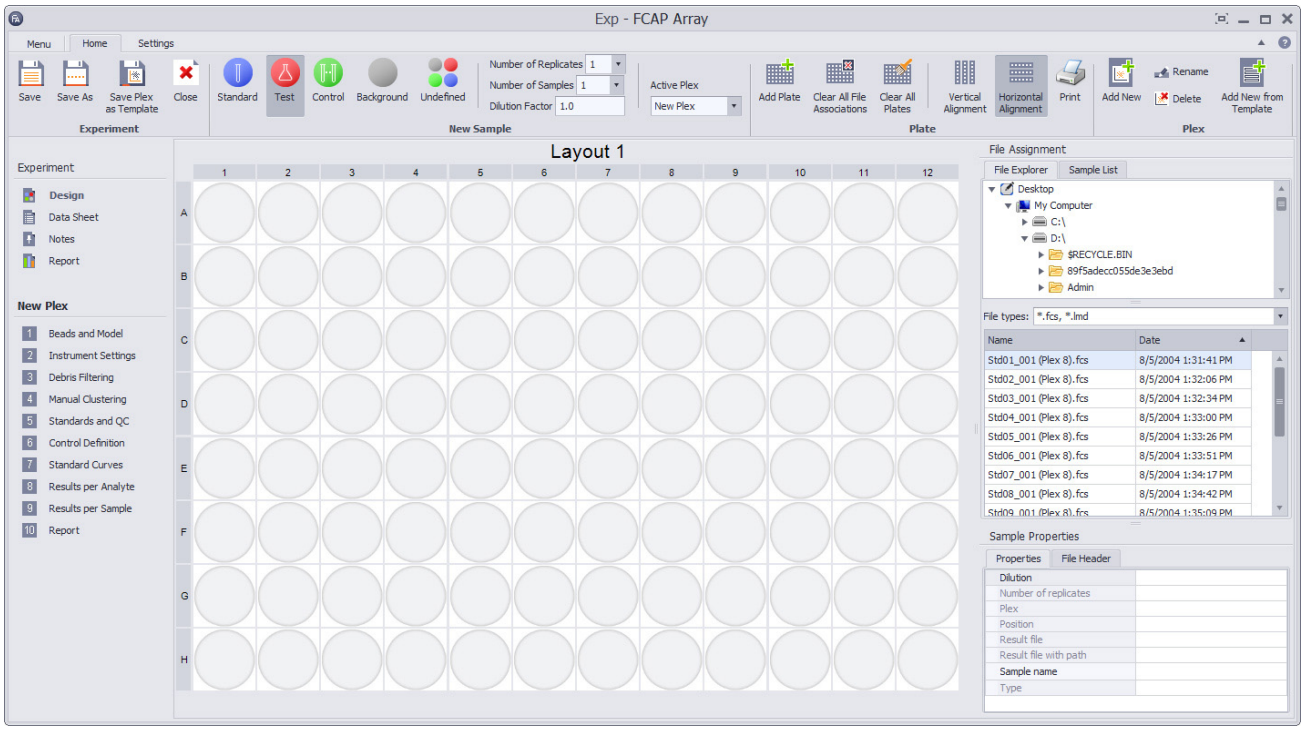
7-3. Figure Starting the "FCAP Tutorial 1" experiment

The New Experiment dialog is displayed.

- ***Type*** the name of the **New Experiment** "FCAP Tutorial 1" and optionally your comment (8-element duplicated standard series with 4 test samples).
- ***Select*** the 96 plate size by checking the **96** status button.
- ***Click* OK**, the **Design** view of the Fungi-6 experiment appears.

7.2.3 Designing the Layout

The FCS data files of this tutorial contain four test samples and two replicates of each standard point. We have 16 standard points that have to be placed on our layout and four test samples. So in total we have 20 FCS files saved to Fungi-6 Tutorial folder on which we will start our experiment.

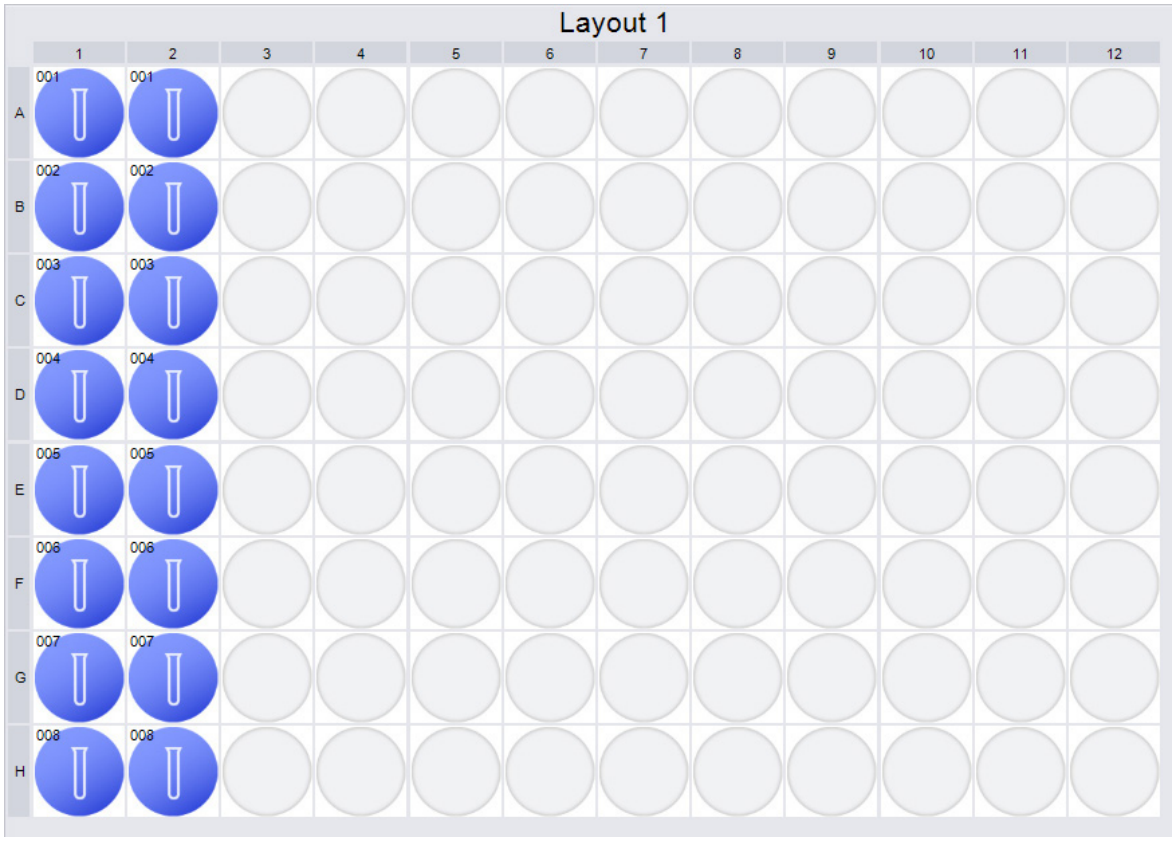


7-4. Figure Empty Layout of the "FCAP Tutorial 1" experiment

Adding standard types to the wells

- First ***click*** on the blue **Standard** icon in the **New Sample** group to add standard samples to your experiment.
- Make sure **Horizontal Alignment** status button is selected in the **Plate** menu button group. In order for us to lay down our standards set the **Number of Replicates = 2** and **Number of Samples = 1** in the **New Sample** button group, then ***click*** our first row of standards onto our layout starting with position A1 and our standards will appear in positions **A1** and **A2**, then proceed down to **B1**, **C1** until position **H1**.

Your **Layout** should look like **7 5. Figure**.

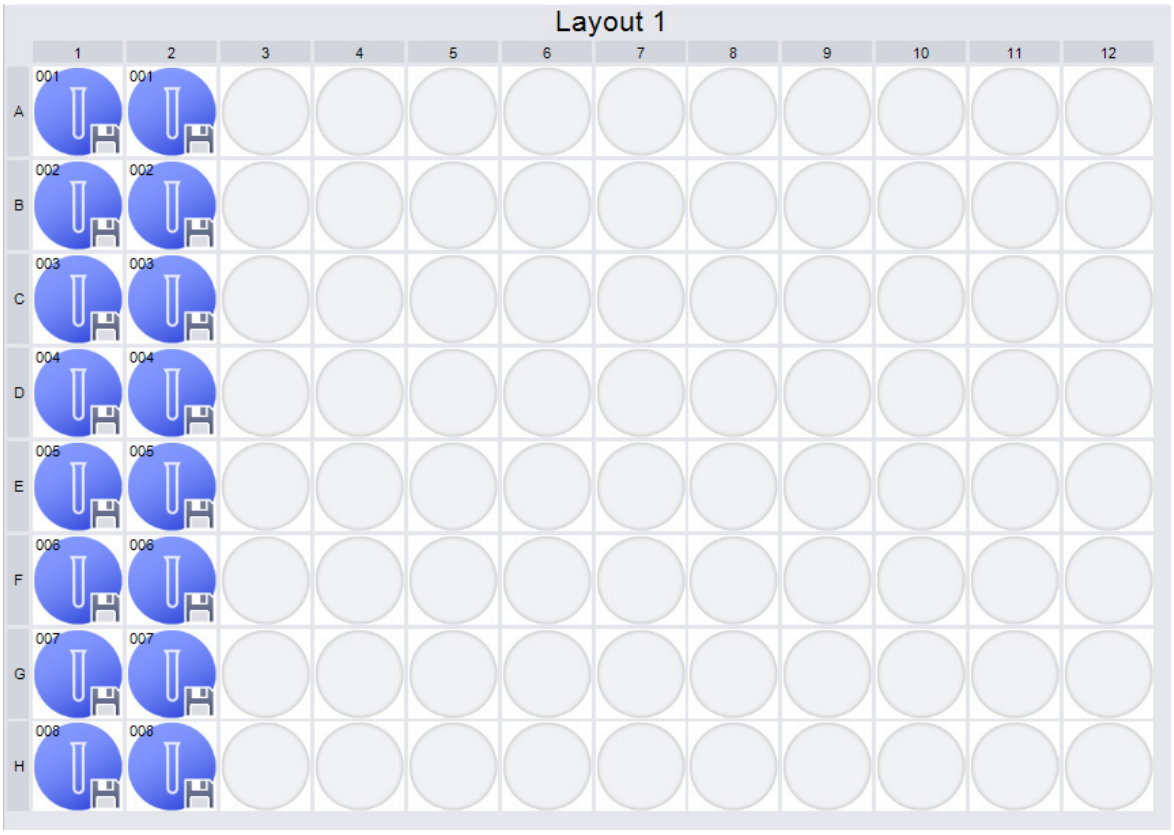


7-5. Figure Experiment Layout, after standard definition

Assign standard wells with standard FCS files

- Now navigate to the folder or location that your FCS files are located at, in the left pane of the **File Explorer** window. Our files are named (Standard 0_a.fcs through Standard 7_a.fcs) for our first set of standards and our second set is (Standard 0_b.fcs through Standard 7_b.fcs).
- ***Select*** all of them by holding down the **SHIFT** Key and ***clicking*** Standard 0_a.fcs then **scroll** down and ***click*** on Standard 7_b.fcs. All the files are selected now and highlighted.
- Change the alignment to vertical (**Vertical Alignment** status button) and then ***assign*** the selected files by ***clicking*** on the position of **A1**.

Your **Layout** now should look like **7 6. Figure**. The **floppy disc image** indicating that the files are assigned to the wells.



7-6. Figure Experiment Layout, after file assignment of standards

NOTE: Check the file assignment propriety by ***clicking*** on each **standard** well and in the connected **Sample Properties** grid verify that the position matches the appropriate file.

Adding test samples to the experiment

- Now make sure **Vertical Alignment** is selected in the **Plate** group, set the **Number of Replicates = 1** , **Number of Samples = 1** , ***click*** on the **Test** icon and then ***click*** on positions **A3, B3, C3, D3** to lay down our **Test** samples to the layout.
  Otherwise set **Test, Vertical Alignment**, the **Number of Replicates = 1**, the **Number of Samples = 4** and click on position **A3**.
- ***Select*** all of test samples (Sample 1.fcs through Sample 4.fcs) by holding down the **SHIFT** key and ***clicking*** Sample 1.fcs then **scroll** down and ***click*** on Sample 4.fcs, all the files are selected now and highlighted.
- ***Assign*** the selected files by ***clicking*** on the position of **A3**.

Other method is to set **Test, Vertical Alignment**, and ***click*** on position **A3** after ***selecting*** the 4 test sample FCS files.

Your Layout now should look like **Figure 7.7**.

7-7. Figure Experiment Layout, after test sample definition

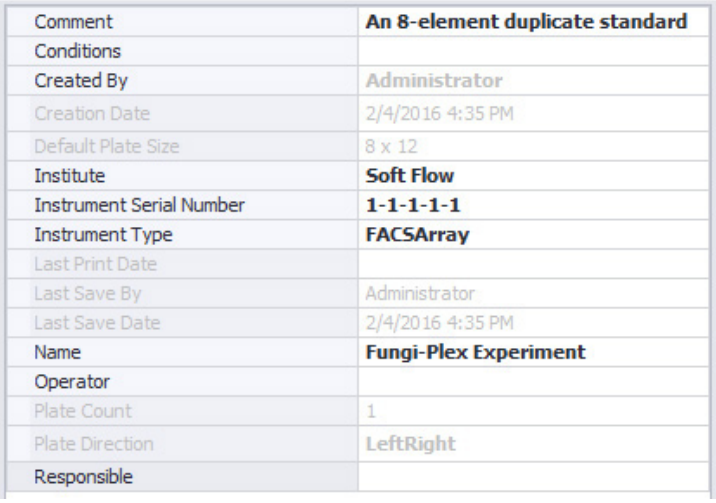
7.2.4 Designing the Plex

Rename the plex from the default "New Plex" to "Fungi-6" by **Plex** button group > **Rename**. The tutorial is a single plex experiment, so no further layout design and plex definition is needed.

7.2.5 Entering Experiment Data

By ***clicking*** on the **Data Sheet** icon on the left in the Experiment pane, the **Data Sheet** view appears.

Fill all the details for your experiment shown by **7 8. Figure**.



Comment	**An 8-element duplicate standard**
Conditions	
Created By	Administrator
Creation Date	2/4/2016 4:35 PM
Default Plate Size	8 x 12
Institute	**Soft Flow**
Instrument Serial Number	**1-1-1-1-1**
Instrument Type	**FACSArray**
Last Print Date	
Last Save By	Administrator
Last Save Date	2/4/2016 4:35 PM
Name	**Fungi-Plex Experiment**
Operator	
Plate Count	1
Plate Direction	LeftRight
Responsible	

7-8. Figure Data Sheet of the "FCAP Tutorial 1" experiment

7.2.6 Beads and Model

In this section, you will enter new beads in the bead library and add them to your **Plex**. While you are at the library, you also learn how to create a group for your new beads.

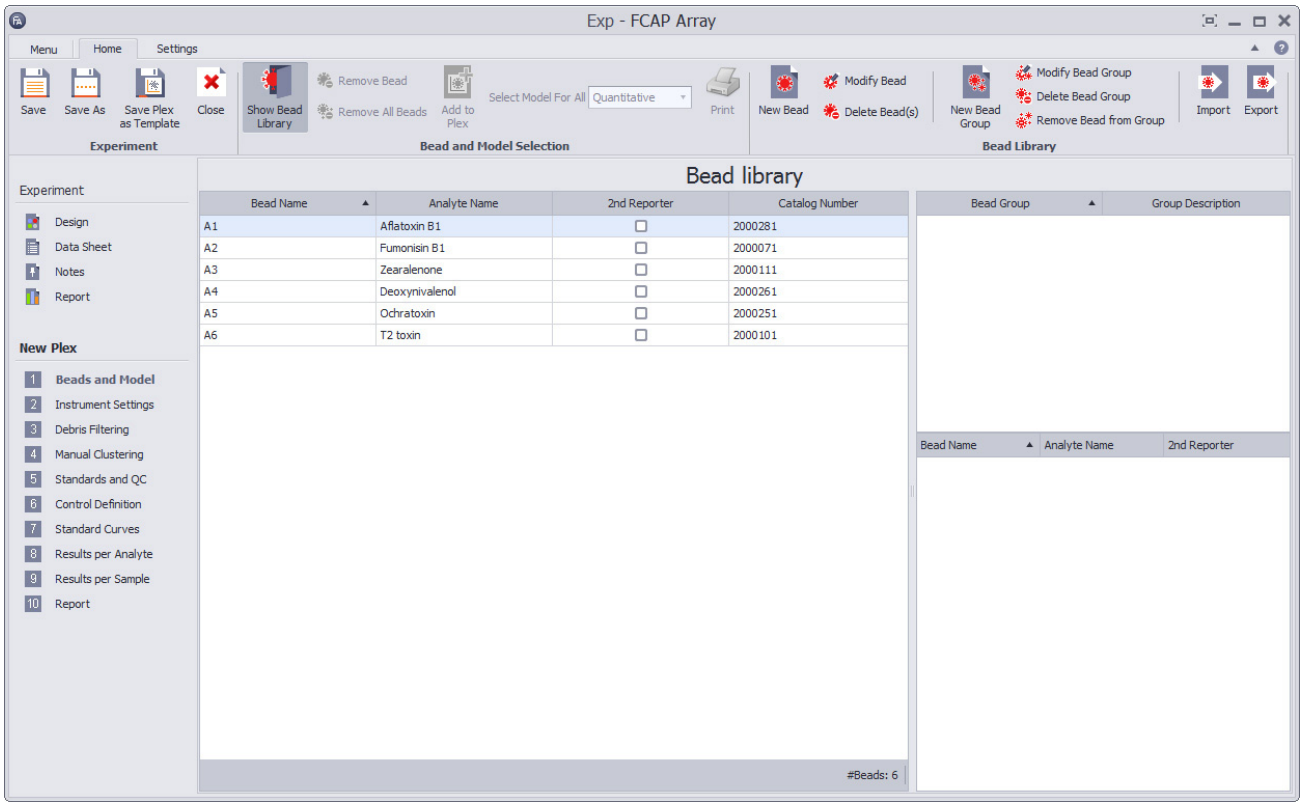
- ***Click* Beads and Model** on the left in the **plex pane**. The **Selected beads** table appears with the **Bead groups** on the right.
- ***Click* Show Bead Library** icon in the **Bead and Model** view.
- **Bead Library** view appears.
- ***Click* New Bead** in the **Bead Library** group.
- **New Bead** dialog appears.
- For each specific bead, specify the **Bead Name**, the **Analyte**, enter the **Catalog number** "SFH-503106" and ***do not select* Second Reporter Parameter** then ***click* OK**.
- Make sure you have added the following beads to the **Bead Library**.

Bead ID	Analyte
A3	Aflatoxin B1
A4	Zearalenone
A5	Ochratoxin A
A7	Fumonisin B1
A8	Deoxynivalenol
A9	T2

7-1. Table Beads

- Add a new group of beads, click on **New Bead Group** in the **Bead Library** group.
- ***Enter*** the group name "Fungibeads-6" and the description "Food safety".
- ***Click*** **OK** to save.
- ***Select*** all the beads **(Ctrl+A)**, ***drag & drop*** them to the "Fungibeads-6" bead group.

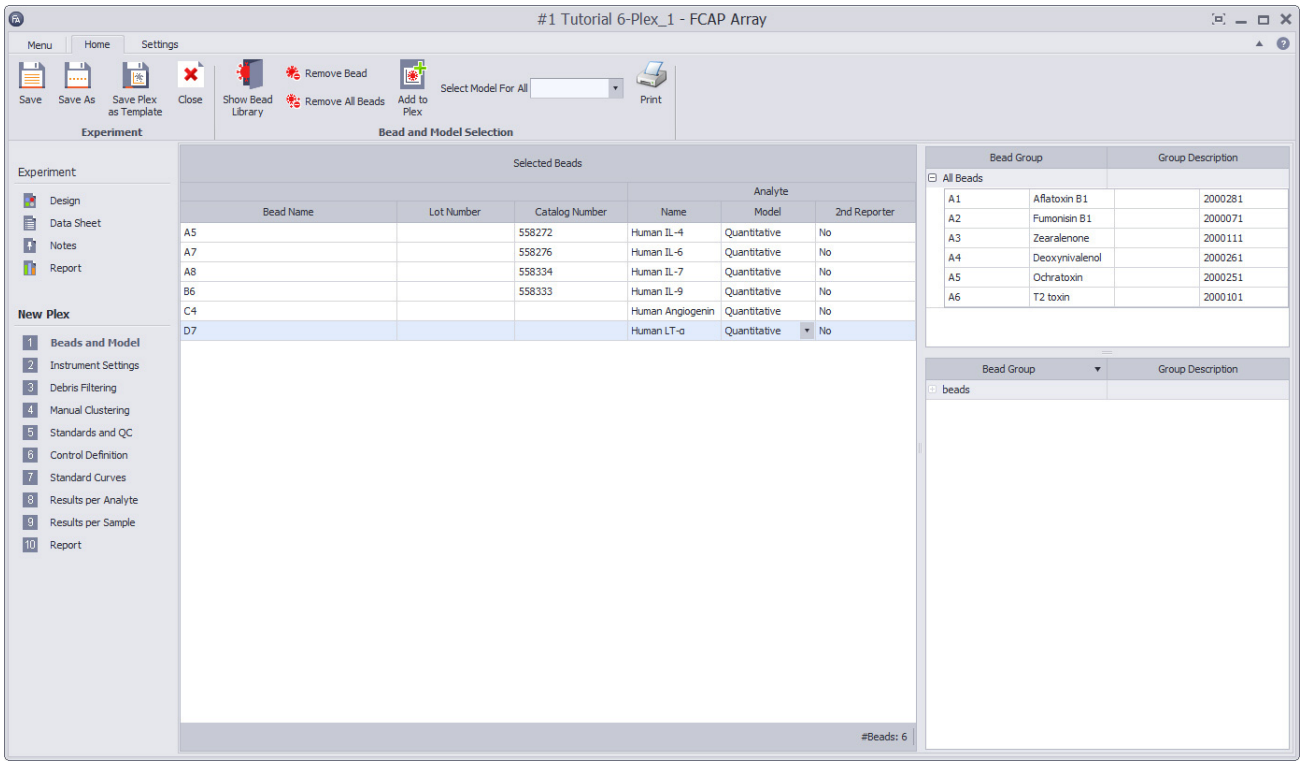
NOTE: Please download our "Fungibeads-6" bead library in XML format and import by doing the following: **Select Beads and Model > Show Bead Library** in **Bead and Model Selection** group > **Import** in **Bead Library** group > ***select*** the downloaded XML file and ***click*** **Import**.



7-9. Figure Bead list of the "FCAP Tutorial 1" experiment

- ***Click*** on **Show Bead Library** in **Bead and Model Selection** button group once more to close the **Bead library** view.

You can add Beads to your plex by ***dragging & dropping*** the "Fungibeads-6" bead group onto the **Selected Beads** list.

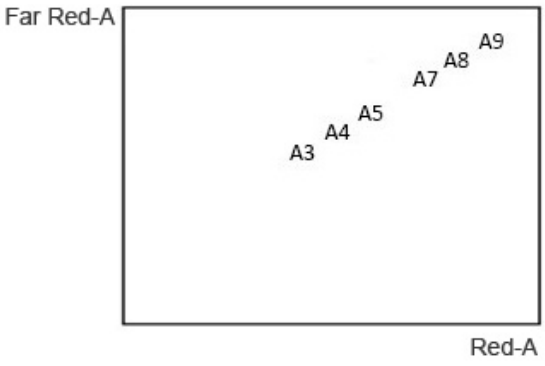


7-10. Figure The "Fungibeads-6" bead group

Keep the analysis model of all beads as the default setting (quantitative), and the LOT numbers empty

7.2.7 Instrument Settings

Under **Instrument Settings > Selected file** list you load an FCS data file from your experiment and identify the key bead assay parameters. In this case it should look like the image below.



7-11. Figure Bead cluster positions of the "FCAP Tutorial 1" experiment

- ***Select*** "standard 4_a.fcs" data file from **Selected File** drop-down list.
- ***Select*** "SSC-W" from **Scatter Parameter** list.
- Leave 1 as number of **Scatter Peaks**.
- ***Select*** "Far Red-A" from the first **Clustering Parameters** list and "NIR-A" from the second. Verify that no failure message indicates 6 clusters were found.
- ***Select*** "Yellow-A" from the **Reporter Parameter 1** list.

NOTE: If the automatic clustering method was successful, usually no additional manual correction needed. We do not need the functions of the **Manual Clustering** view in this tutorial.

7.2.8 Debris filtering

Go to **Debris filtering** view.

Set **Parameter X** axis to "FSC-A" and **Parameter Y** axis to "SSC-A" of the **Before filtering** plot. ***Draw*** a filtering gate in the **Before filtering** plot.

Set **Parameter X** axis to "FSC-A" and **Parameter Y** axis to "SSC-A" of the **After filtering** plot and see the remaining scatter events.

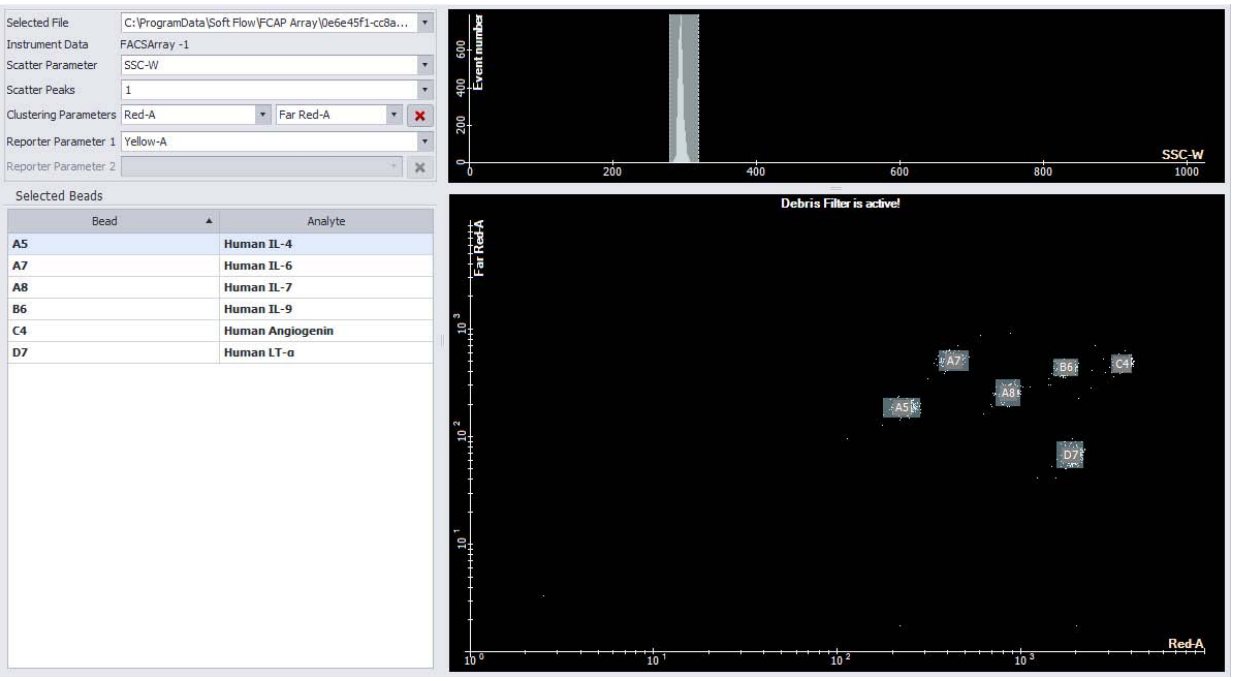
Step through all the fcs files by **Previous File** and **Next File** buttons in the **Sample** list to make sure, your gate does not filter out bead events from the further analysis. Otherwise ***fix*** the debris filter gate and continue the process.

7.2.9 Assigning Beads to Clusters

Go back to the **Instrument setting** view. You assign beads to corresponding clusters in the dot plot. If your beads have standard Bead ID location information, the ID codes indicate relative cluster positions as shown in Figure *-* Fungi Plex-™ Bead ID location codes see image above.

TIP To simplify assignment, first click on the Bead column header in the Cluster Assignment view to sort by Bead Name. Then assign the clusters left to right in successive rows, top to bottom.

a) ***Click*** the first row of the bead list to select the bead ID = A3.
b) In the dot-plot, ***double-click*** the down left cluster (labeled A3 in Figure *-*) to assign the selected bead to that cluster. The bead ID appears on the plot in the middle of cluster.
c) As in step b, assign the remaining beads to the corresponding clusters. The correct completed assignment is shown in Figure 7.11.



7-12. Figure Bead – cluster assignment of the "FCAP Tutorial 1" experiment

NOTE: Correct assignment of beads to clusters is mandatory for proper analysis.

TIP You can assign beads to clusters by dragging a bead row to a cluster

7.2.10 Standard definition

For a **Quantitative** assay, the concentration of each standard must be specified using the **Standards and QC** view.

In the **Concentration Settings** group, set up your **Standard Dilution** series by the followings:

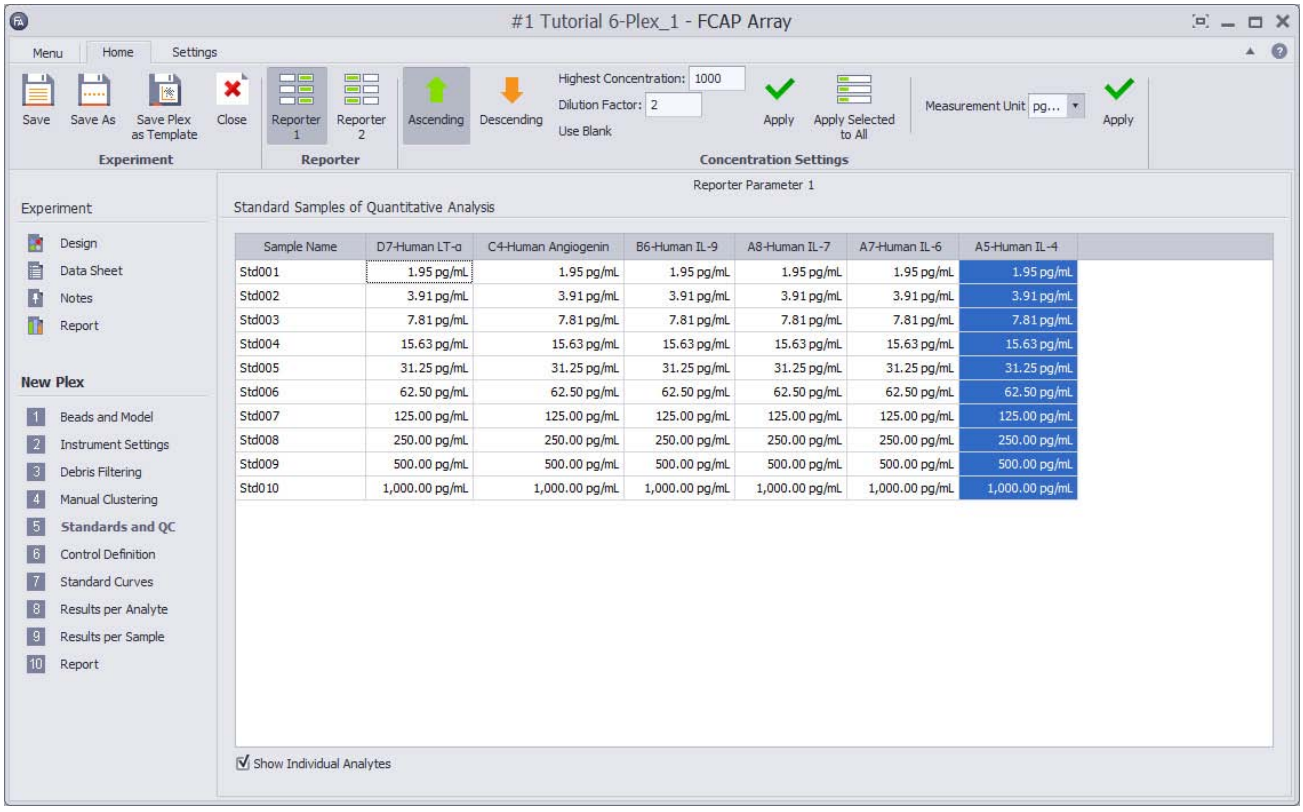
- ***Click*** on **Standards and QC** view in the **Fungi-6** plex pane.
- ***Select*** "pg/mL" as the concentration from the **Measurement Unit** drop-down list.
- ***Select*** **Ascending** calculation method.
- ***Check*** in the **Use Blank** button to have zero pg/mL as the lowest standard concentration.
- ***Check*** in the **Show Individual Analytes** box to unfold individual standard series.
- ***Enter*** to the **Highest concentration** (640) and the ***Dilution factor*** (2.0) for the "A3" bead.

Bead ID	Toxin	Concentration maximum (pg/mL)
A3	Aflatoxin B1	640
A4	Zearelenone	16000
A5	Ochratoxin A	640
A7	Fumonision B1	80000
A8	Deoxynivalenol	100000
A9	T2	32000

7-2. Table Concentrations

- ***Click*** in the "A3" column header to make it active and then ***click*** **Apply**.
- ***Repeat*** the previous two steps 5 more times according to concentration maximums found in **Table 7-2** to generate all standard series of the **Fungi-6** plex shown in **Figure 7-13**.

TIP *Define* pg/mL as a concentration in the **Units Measurement library** on **Main** view > **Libraries** pane if it is missing from **Experiment > Standard and QC** view > **Concentration Settings** button group > **Measurement Unit** drop-down list.



7 13. Figure Standard series of the "FCAP Tutorial 1" experiment

7.2.11 Controls Definition

In this tutorial we **do not have** Controls, so we skip this step.

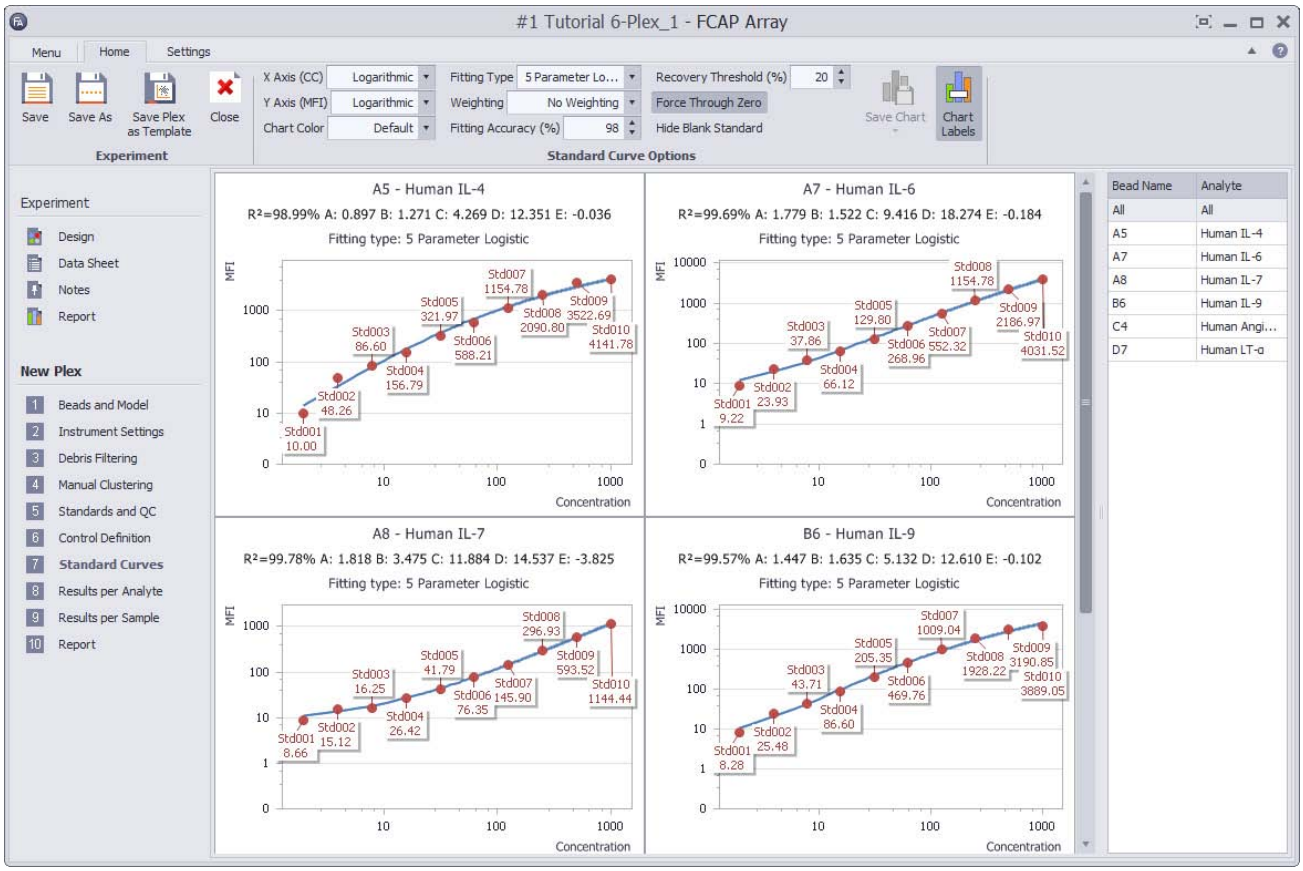
The default **Standard Curve Options** are:

- **X Axis (CC)**: log;
- **Y Axis (MFI)**: log;
- **Chart Color**: Default;
- **Fitting Type**: 5 Parameter Logistic;
- **Weighting**: No Weighting;
- **Fitting Accuracy**: 98% (It is a user preference.);
- **Recovery Threshold (%)**: 20;
- **Force through zero**: in;
- **Hide Blank Standard**: out.

Change **Y Axis (MFI)**: to "lin" to get better-looking curves shown by Figure **7-14**.

Select "A7" bead from the right bead curve selection list to see the individual curve fit with the analysis result for the "Fumonision B1" analyte.

Read from the fitted curve that the MFI is nearly 578.5 for the 3200 pg/mL concentration by the **Fitting Cursor**.



7 14. Figure Standard curves of the "FCAP Tutorial 1" experiment

7.2.12 Reviewing the Results

Results per Analyte

Click on **Results per Analyte** view.

Select "Bar Chart" from **Chart Type**.

Select "A5" from the **Analyte** list.

Select "Final CC" from **Chart group > Data type**.

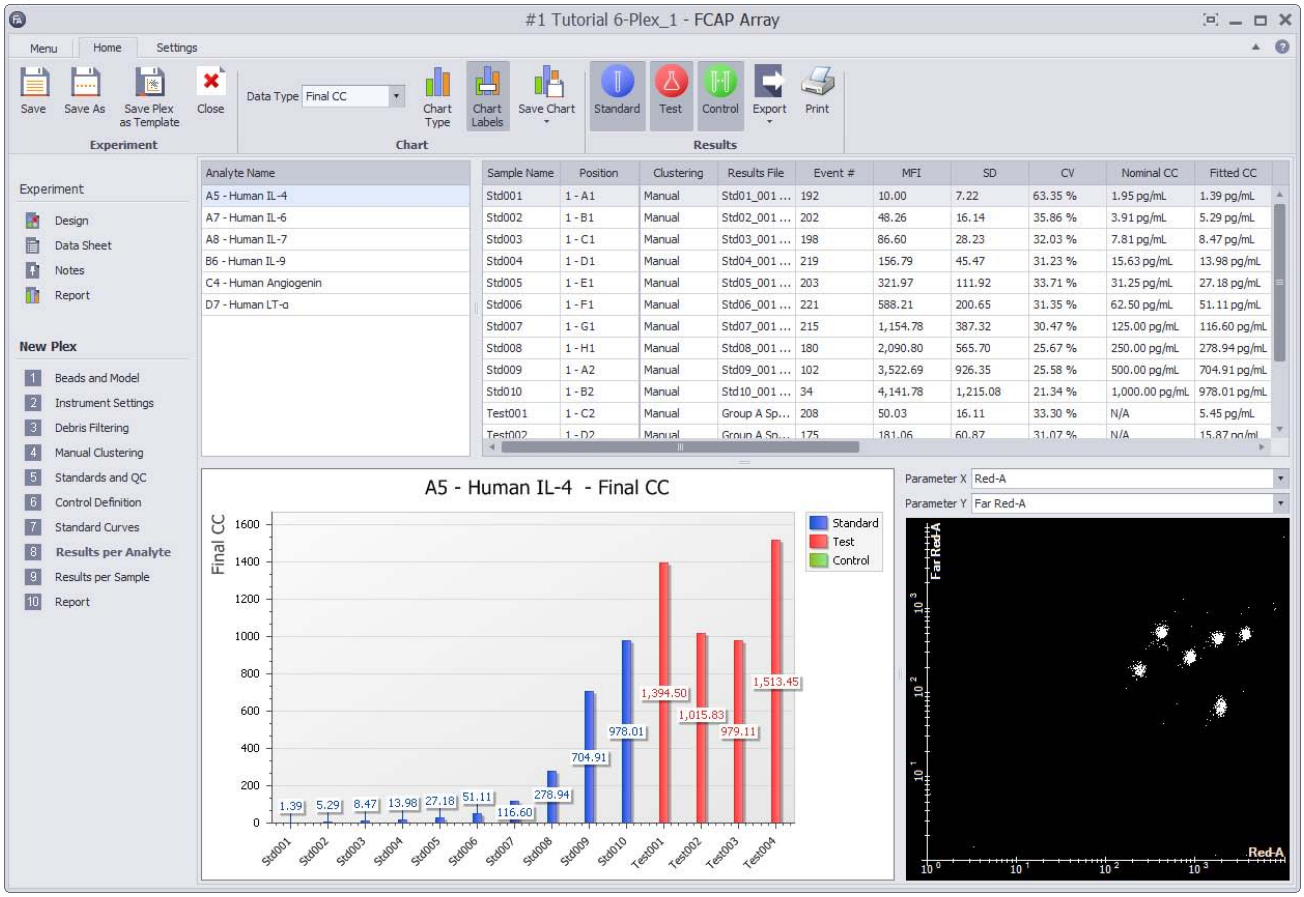
Click out **Results** group > **Standards** status button.

See, that the Ochratoxin concentration of the 4 samples was nearly the same.

Select Std004 replicate one. The down-right clustering plot become active and shows filtered events with last used or default parameter settings.

Select "A4" from the **Analyte** list.

See, that the Zearelenone content of the **Test Sample 1 & 2** is much higher than **Test Sample 3 & 4**.



7 15. Figure Results per Analyte view of the "FCAP Tutorial 1" experiment

Results per Sample

Click on **Results per Sample** view.

Keep "Bar Chart" selected in **Chart Type**.

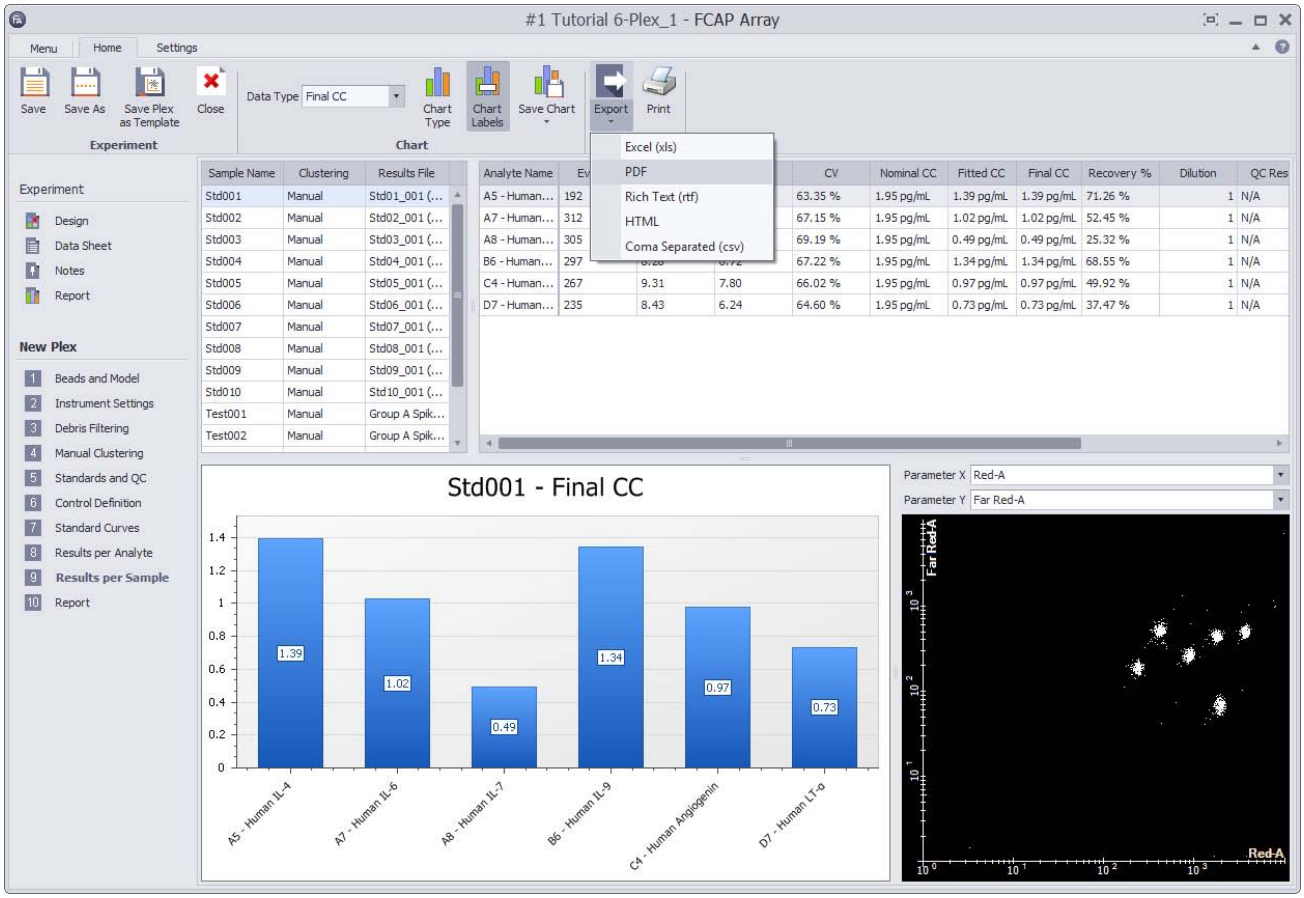
Select "Test004" (Sample 4.fcs) from the **Sample** list.

The down-right clustering plot become active and shows filtered events with last used or default parameter settings.

See, that the Deoxynivalenol content of the sample is much higher than the other toxins. Save your chart by ***clicking*** on **Save Chart** icon in the **Chart** group then ***select*** **JPEG** for picture type.

Set the file location in the **file explorer**, ***name*** the picture "SampleResult_Test004".

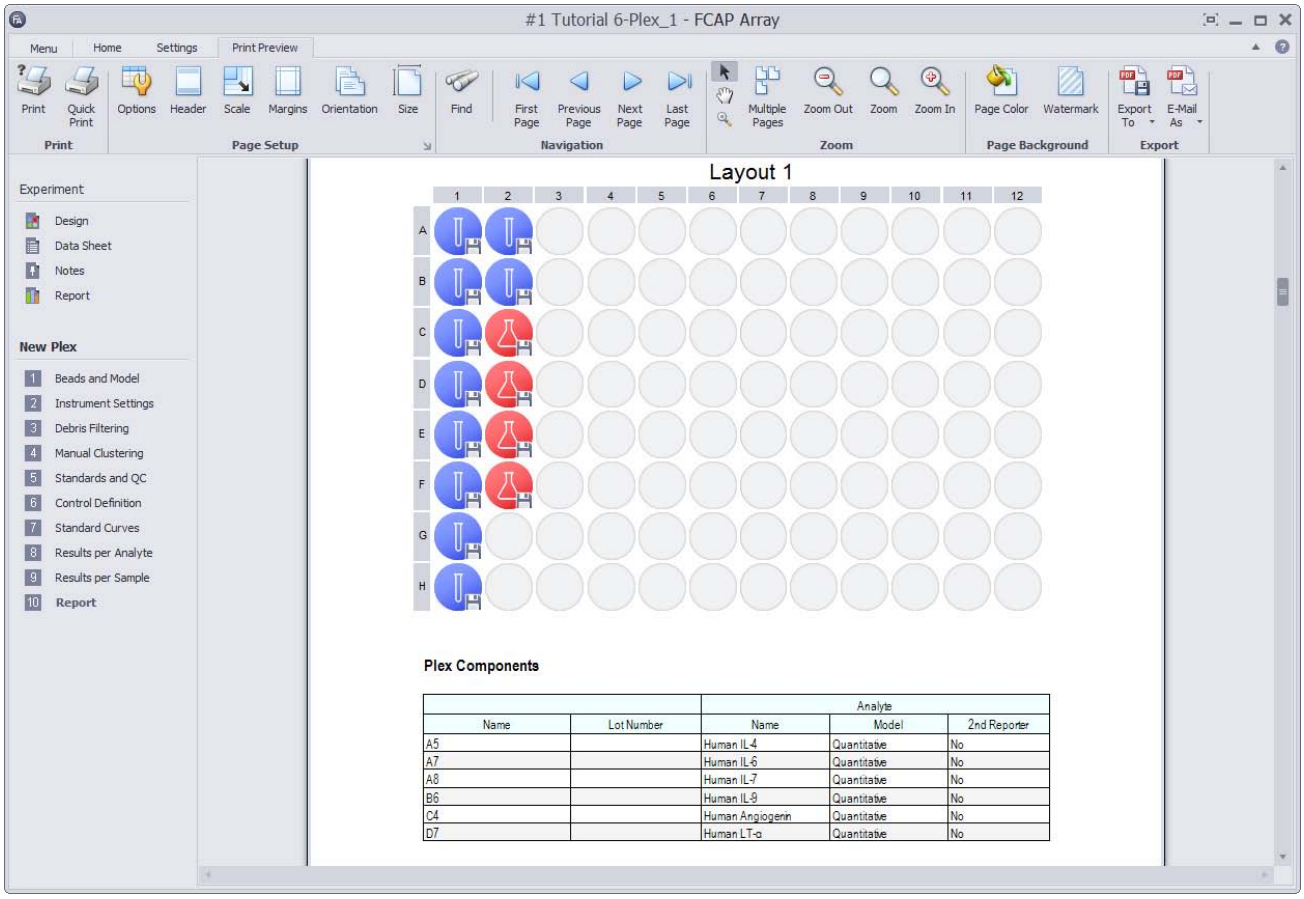
Click **Results** group > **Export** drop down button group > **PDF**, than ***select*** the path in the file explorer to save "Fungi-6_result_samples.pdf" file and to export Results per Sample data.



7 16. Figure Saving the "Fungi-6_result_samples.pdf"

7.2.13 Reviewing the Report

In the **Fungi-6 > Report** view, you can see the generated report document of the current plex.



7 17. Figure Plex Report of the "FCAP Tutorial 1" experiment

Go to the **Page Setup** group and ***click*** on Header.

The **Report Header Settings** dialog appears.

Modify the **Title** text to "Fungi-6 Report".

Fill **Header line 1-3** with your company or institute data.

Brows and ***select*** company or institute logo. - Optional

Click **OK** to confirm changes on your report header.

Click **Options** in **Page Setup** group.

The **Report Options** dialog appears.

Select **Report Cover, Visual Plate Report** and **Instrument Settings Report** from the **Plex Definition** group.

Select **Standard Curves Report** from the **Standards** group.

Select **Sample Quantitative Report** from the **Results** group.

Click **OK** to confirm the report option selection.

Wait until (the progress bar reaches 100%) the modified plex report is generated.

Select **Export > Export to > PDF file**.

The **PDF Export Options** dialog appears.

Click in **Convert Images in Jpeg, select Images quality**: to **highest**, ***check*** in **compressed** then ***click*** **OK**.

The **File Explorer** dialog appears.

Enter the name "Fungi-6 Report.pdf" and select the path of the plex report file.

Click Save to save plex report.

NOTE The **Plex** and the **Experiment Report** is the same for a single plex experiment in practice.

8

TROUBLESHOOTING

This section explains the some tips to help you troubleshoot problems with FCAP Array software.

If you have any problems with using FCAP Array, you can find help included in the software or call our Technical Assistance. The **Help** menu is located on the right side of the ribbon or under the **Menu** Tab.

Calculation error messages

Message	Description
NaN	Acronym for not a number. Can be the result of zero divided by zero or no calculation done.
negative infinity	Negative number divided by zero.
positive infinity	Positive number divided by zero.

FCAP Array does not start

Possible causes	Recommended solutions
Too many software instances are running on the PC	Close unnecessary applications.
Invalid Hardware ID	Hardware configuration has changed, since the installation of FCAP, remove any external devices USB, SD Card, make sure you using the same port for your internet connection. If you replaced your hard drive or any major hardware items, call technical support for a new product key.
Missing program files	Reinstall the software.
Subscription has expired	Please renew your subscription

User cannot log in

Possible causes	Recommended solutions
User name is incorrect	Check the caps lock key. Re-type your user name.
Forgot user name	Contact your software administrator.
Password is incorrect	Check the caps lock key. Re-type your password.
Forgot password	Your software administrator can reset your password.

User unable to open or edit data

Possible causes	Recommended solutions
Database is corrupted	Restore a database backup or delete the database file and FCAP Array will regenerate the database at the next run.

User Manual does not open from Help Menu

Possible causes	Recommended solutions
User manual file was not installed with the software	Re-run the FCAP Array installer and select **Repair.**

Experiment cannot be edited

Possible causes	Recommended solutions
Experiment is locked	Make a copy of the experiment by click the **Save As** icon in the **Experiment** group.

New bead(s) cannot be added to the bead library

Possible causes	Recommended solutions
Bead name and analyte already exist in the bead library	Make corrections to the bead identifiers.

Unexpected analysis results

Possible causes	Recommended solutions
Re-using saved fitting curves	Calculate new standard curves for every experiment.
Software extrapolation leads to inaccurate values	Single-color and dual-color beads require different instrument setups.
Single-color and dual-color beads are in the same experiment	Correct the experiment layout, redo the file assignment, and re-analyze.
Experiment sample layout does not match actual sample layout	Correct the information.
Incorrect bead information in the bead library	Import the bead list from an XML file to avoid entry errors.
Incorrect file assignment	Review the acquisition sample order. Correct the file assignment and re-analyze.
Too much debris in one or more samples	Use **Debris Filtering** to define gated data that excludes debris.

Unable to fit standard curves

Possible causes	Recommended solutions
One or more standard samples failed	Remove bad data points in the **Standard Curves** window and re-analyze. Acquire another standard sample series.
Standards are fitted out of sequence	Correct the file assignment in the **Experiment Design** view.
Choice of fitting curve does not model the standard sample data	Choose another fitting curve. **4 Parameter** and **5 Parameter** Logistic curves are recommended for most beads.
Fitting accuracy is too high	Lower the **fitting accuracy** value in the **Standard Curves** window.
Sample preparation error	Prepare and acquire a new standard sample set.
Experiment design does not match the actual layout	Correct the experiment layout, redo the file assignment, and re-analyze.

Cannot analyze data file

Possible causes	Recommended solutions
Corrupt or incorrect FCS file format	Use FCS 2.0, 3.0 or 3.1 list-mode data files only. Re-export list mode data files from the instrument system in FCS 2.0, 3.0 or 3.1

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GLOSSARY

The following terms are defined as they are used in **FCAP Array** software and this user manual.

Term	Description
bead	An analyte-specific capture particle with distinctive, discrete fluorescence characteristics
Bead	An FCAP Array software construct that specifies the analyte binding and fluorescence characteristics of a bead.
bead assay	A method of using beads and reporter antibodies to detect or measure the concentration of analytes in a sample.
Bead group	A subset of the Beads in the Bead library.
Bead library	A structure used to organize the Beads available for use, consisting of the Bead list and the Bead groups.
Bead list	All Beads contained in the Bead library.
CC	Calculated concentration of analyte in a sample.
cluster	A population of events in a bead assay data file. Each cluster corresponds to a bead, therefore, the events in a cluster have a distinctive, discrete fluorescence characteristic.
clustering	The process of determining the clusters (bead populations) in a data file.
clustering parameters	The parameters used to measure the distinctive fluorescence characteristics of beads.
control sample	A sample of known concentration (or fluorescence intensity) added to a bead assay to verify the accuracy of the calculations.
Debris filtering	Debris and other components in data files can complicate the analysis. Filtering gate is defined by the user on one of experiment's files. The program automatically applies the gate to all the files of the experiment.
experiment	A bead assay. Also, formalizations or file representations of a bead assay other that the FCAP Array software Experiment document.
Experiment	An FCAP Array software construct that defines the Plex(es), standards, controls and test samples for a bead assay.
FCS data file	The Flow Cytometric Standard for recording the data from a cytometry acquisition. FCAP Array software requires data files that conform to the FCS 2.0, 3.0 or 3.1 specifications.
Fitting Accuracy	The regression coefficient obtained from a curve fit, defined in the range 0 to 1 (0% to 100%), with 1 being a perfect fit. An FCAP Array software preference allows the user to specify a minimum acceptable fitting accuracy in the range 90% to 100%.
Manual clustering	If the automatic clustering algorithm fails, it can be performed manually using the manual clustering tool.
MFI	The median fluorescence intensity measured from a population (cluster).
Negative control sample (NS)	Negative sample for a qualitative analysis.
Plex	An FCAP Array software construct that defines a plex and its bead characteristics.
Plex template library	A structure used to organize Plexes available for use.