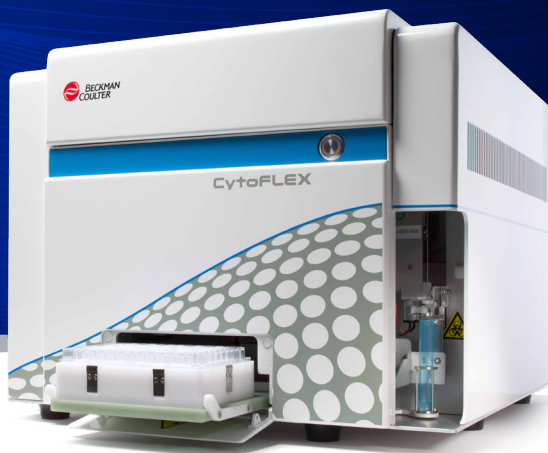# CytoFLEX
QUICK USER GUIDE

EVERY
event matters.

BECKMAN COULTER
Life Sciences

CytoFLEX
QUICK USER GUIDE

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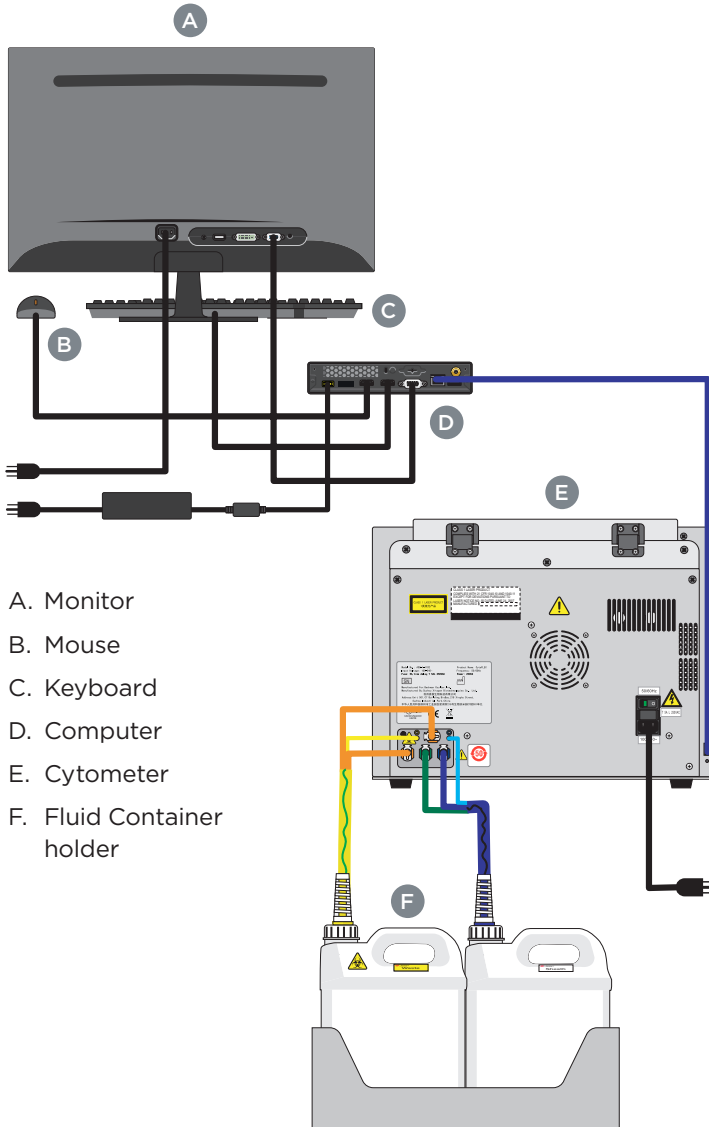
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Instrument
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INSTRUMENT STARTUP

Instrument Preparation

1. Ensure all system connections are connected correctly.

A. Monitor

B. Mouse

C. Keyboard

D. Computer

E. Cytometer

F. Fluid Container holder

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INSTRUMENT STARTUP

2. Ensure that all of the tubing and the cables are connected to the instrument according to the color code.
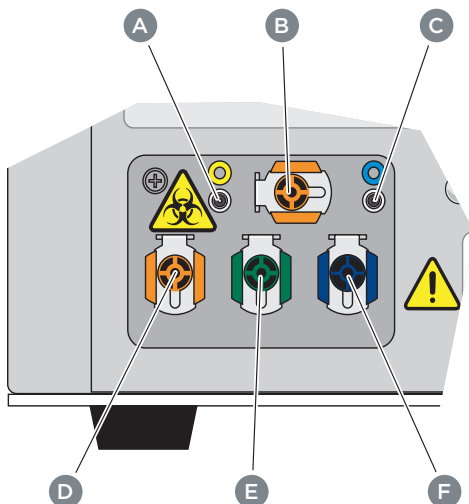
A. **Waste level sense.** Connects to the waste liquid sensor cable.

B. **Flow cell waste out.** Connects to the flow cell waste tubing.

C. **Sheath fluid level sense.** Connects to the sheath fluid sensor cable.

D. **Waste out.** Connects to the waste liquid tubing.

E. **Sheath return.** Connects to the sheath fluid tubing.

F. **Sheath fluid in.** Connects to the sheath fluid tubing.

3. Verify that the USB configuration key has been connected to a USB port.

⚠ CAUTION

Risk of instrument damage. Remove the sheath fluid container from the Fluid Container holder and fill away from the instrument to prevent spills that could damage the instrument circuitry.

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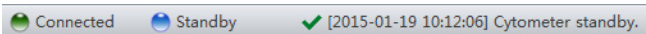
INSTRUMENT STARTUP

4. Remove the sheath fluid container from the Fluid Container holder and fill the sheath fluid container with the supplied sheath fluid.

5. If necessary, remove the right side cover and fill the Deep Clean solution with a mixture of 1 part Contrad 70 and 1 part DI water.

   ⚠ **WARNING**

   Risk of chemical injury from bleach. To avoid contact with the bleach, use barrier protection, including protective eyeware, gloves, and suitable laboratory attire. Refer to the Safety Data Sheet for details about chemical exposure before using the chemical.

6. Empty the waste container if necessary. Add 400 mL of 5 to 6% bleach to the waste container.

Instrument Start Up

1. Turn on the power switch on the back cover of the instrument, located just above the power cable.

2. Log on to the computer and double-click to start **CytExpert**.

   a. Ensure that the **Connected** icon on the Status Bar near the bottom-left side of the display is green.

   b. If the icon is not green, ensure that the instrument USB is securely connected to the computer and restart the computer.

3. Select **System Startup Procedure** in the Cytometer menu, click Initialize and follow the software prompts.

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Quality Control

Procedure for Daily Verification of Alignment and Fluidics

1. Use the filter set recommended by the manufacturer for detecting the appropriate fluorescence parameters (refer to the CytoFLEX Platform IFU).

2. Ensure that the target value file for the specific lot of CytoFLEX Ready to Use Daily QC Fluorospheres has been loaded into the flow cytometer software. Refer to your CytoFLEX Platform IFU for instructions on importing your lot-specific target value file.

3. Vigorously mix the CytoFLEX Ready to Use Daily QC Fluorospheres vial using a vortex mixer for 2-3 seconds. Add:

   a. 10 drops of fluorospheres to a tube if using Semi-Automatic (tube) Sample Injection Mode.

   b. 3-4 drops of fluorospheres to a well for either Standard

   96-well plate or Deep-well plate for the plate loader injection mode.

4. Run the Quality Control (QC) protocol per the instructions in your CytoFLEX Platform Instructions for Use (IFU).

5. Select Start QC/Standardization for the QC/ Standardization Menu. Ensure that the QC bead lot number is selectable in the Lot No. drop-down menu.

   Note: If the lot number is not selectable, then download the lot specific Target File from Beckman.com website by following the steps in Download Target File section.

6. Initialize the system if it has gone into Standby mode , then insert the prepared sample tube into the tube holder. Select correct lot number from the Lot No. drop-down and select Start.

   Note: QC may take several minutes. During QC, the software automatically seeks the CytoFLEX Ready to Use Daily QC Fluorospheres and computes the results. The results are displayed and saved automatically once it is completed.

   • Green icons in the Result section indicate Pass.
   • Red icons in the Result section indicate Fail.

   Note: The minimum flow rate for the Ready to Use Daily QC fluorospheres is 100 events/second.

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QUALITY CONTROL

7. If QC fails, select Daily Clean and Prime from the Cytometer menu, follow the prompts and repeat QC. If QC fails two times in a row on the same day, contact your Beckman Coulter Representative.

8. Select Close QC/Standardization under File menu to exit the QC/Standardization module.

9. Download Target File:
    From Beckman.com home page, navigate to Shop and Products>Reagents>Flow Cytometry Reagents>Fluorospheres and Quality Control. Select CytoFLEX Ready to Use (RTU) Daily QC Fluorospheres product and click Download Target Files.

10. Import Target File:
    While in the QC/Standardization mode select Target Library under the Settings menu and select Import. Navigate to the target value file and select Open and then select Close.

11. In the Search By Product section of the screen   :

    a. Select Research & Discovery from the Market Segment drop-down menu.

    b. Select Flow Cytometry from the Product Line drop-down menu.

    c. Select Instruments from the Product Series drop-down menu.

    d. Select CytoFLEX from the Product drop-down menu.

    e. Select CytoFLEX Ready to Use (RTU) Fluorospheres Target Values from the Software Name drop-down menu.

    f.   Select English from the Language drop-down menu. Select the CytoFLEX Ready to Use (RTU) Daily QC Fluorospheres Target Values for correct lot number of Ready to Use (RTU) QC Fluorospheres and then select Download and save to desired file path or USB drive.

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COMPENSATION

CytExpert software separates compensation and sample acquisition into separate experiments designated .xitc and .xit, respectively. The experimenter can choose which one to execute first.

1. If a new compensation is needed, generate as follows:

    a. Prepare all necessary unstained and single color controls using compensation beads or cells.

    b. Select **New Compensation** in the File menu, name file and click **Save**.
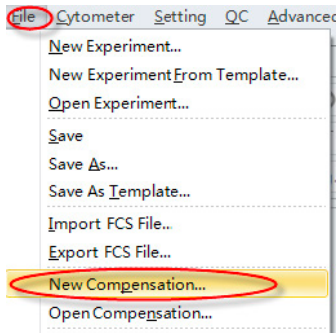
    c. Select the channels requiring compensation and then select **Sample Type** of the prepared control tubes. Unchecked tubes not needed for the experiment. Select **OK**.

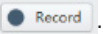   **Note:** Label and Lot No. information can be entered and retained in Compensation Library if desired.

COMPENSATION

CytExpert automatically creates a list of matching empty tubes in the Tube panel as well as all the plots needed to gate the positive and negative populations.
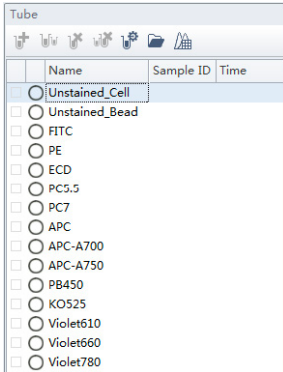
d. Load the corresponding unstained or single color sample fluorospheres or cells and select ▶ Run in the Acquisition panel.

e. If necessary, adjust the scatter gate so that it encloses the desired population. Adjust the slider scale on fluorescence plot so the negative and positive peaks appear in a suitable position. Move the positive and negative gates to enclose the corresponding peaks.

f. Once the gates are in satisfactory locations, select ● Record .

g. Repeat steps d-f for all tubes.

h. After collecting data for all necessary compensation samples, generate the compensation matrix by selecting **Compensation Calculation** from toolbar or settings menu.
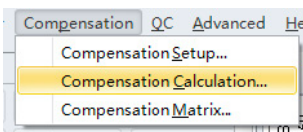
COMPENSATION

i. The Compensation Matrix window appears displaying the compensation values. Select **Save As** to export the matrix as a .comp file and specify file path. Select **Save to Compensation Library** to save the single color compensation values to the compensation library. Select **OK** and select **Close**.

j. To save or overwrite the compensation matrix to the library, **Open** compensation experiment, run compensation controls. Once completed, calculate compensation. Then save the comp library using designated button under the compensation matrix (pop-up). "Save as Comp Matrix", "Save as Comp Library". Choose a name.

    Quick Tip: To apply compensation retroactively (starting with an experiment first, then applying compensation), open an experiment, run your samples. Right-click on sample, a box appears "Apply Compensation". Next to your tube, there is a clear checkered icon/box (very left), right click on icon to apply compensation matrix. From there, choose your matrix. Then, apply compensation to all tubes.

Creating New Experiments

CREATING NEW EXPERIMENTS

CytExpert software separates color compensation and sample acquisition experiments into separate files designated .xitc and .xit, respectively. The experimenter can choose which one to execute first.

Creating New Experiment (.xit)

1. Select **New Experimen**t in the **File** menu, specify the file path and name the experiment or accept the default naming Exp_YYYMMDD-1 and select **Save**.

2. Select **Set Channel** from the **Settings** menu. Select the desired channel signals in the checkbox. Type in reagent name in the label column if desired.

3. Explore the tool bar buttons to create plots in the experiment workspace. Select the axis name on the plot to change which channel is displayed.

4. To create gates, use the gating control buttons on the toolbar or right click the plot and select the gate type required.

5. Select the heading area of the plot to assign the gates to the required plots.

6. Select the **Population Hierarchy** button from the toolbar to display the population hierarchy. You can change color and names of gates by double clicking the color or name in population hierarchy window.

7. Select the **Statistics** button for the toolbar to display statistics window. Right click the **Statistics** table and select **Statistics Setting** and check desired header, statistics and populations of interest.

8. Select the **Acq. Setting** button on the left side panel to open the **Acq. Setting** window and display cytometer gains and threshold settings. In the **Gain** tab, select **Recommended** in cases where you do not specify your own default parameter gain settings.

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9. Import compensation matrix to apply compensation values to apply experiment if required by selecting **Compensation Matrix** from the **Settings** menu to bring up the compensation matrix table. Select **Import from Library** or **Import** and locate the compensation matrix file (.comp) to import. Select desired option for importing compensation matrix and gain settings. Refer to Importing and Exporting Compensation in Compensation module of the Cytoflex IFU for more information.

Sampling and Collecting Data

1. Place the sample tube in sample tube holder and select **Run** to load the sample. In **Acquisition** panel, select sample flow rate and type in desired acquisition settings for Events to Display, Events to Record, and Time to Record.

2. Adjust gain settings for the different channels in **Gain** tab in the **Acq. Setting** window or use the **Gain Control** button on the toolbar directly on the plot where the data appears during data collection. Raising the gain increases the signal. Lowering the gain decreases the signal. **Note:** Optimize the gain settings according to your own experimental goals. The recommended settings are only for reference.

3. Adjust the threshold settings if required to remove unnecessary signal noise by selecting the **Threshold** tab in the **Acq. Setting** window. Choose the channel for threshold setting and manually enter a value in **Threshold** tab. Alternatively, select the **Threshold** button from the tool bar and move the mouse to desired threshold position in the desired plot.

4. Select **Record** to save the data and wait for the saving process to finish. The sample tube holder returns to the loading position. Add additional tubes to **Tube** list if required, load tubes and record data for each tube until all data required has been collected.

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Identify Statistics to be Exported with the Experiment

Statistics table can be generated by selecting the 'Statistic' icon from the Workspace tab.

1. Once the statistic table pops up, right click on the table to access the menu.

2. Select **Statistics Settings** and here choose the statistics, population, and the header you would like to display.

3. Select **Apply to All Tubes** at the bottom of the **Statistic Setting** window if you want to apply to all the samples. Select **OK** once done.

4. If you would like to export the statistics to a csv table, right click on the table and select **Export All Samples to CSV File**. Choose the directory and save the file.

5. You can open this file in Excel and furthermore save as an .xls file.

Data Analysis

1. Open an existing experiment (.xit)

   File Menu > Select FCS File, Open. Imported files now have a green circle.

2. Choose the analysis icon on left side of screen

3. Use plotting tools as needed to complete the analysis. Multi-data or overlay plots (pull-down menu). Select the diamond tool to fill overlays, or drag and drop files.

Refer to user manual instructions for data analysis for more detailed instructions for use.

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Remote Support

BeckmanConnect Remote Support Software

BeckmanConnect enables remote diagnostics and troubleshooting for maximum uptime & fewer workflow disruption. Firewall-friendly BeckmanConnect software includes policy configurations designed to limit connections only to Beckman Coulter who are trained to protect your data privacy and security. It is easy to set up, and is offered free to all CytoFLEX customers.

Installation Steps

1. Navigate to **beckman.com/beckmanconnect** and click the "Register" button.

2. Complete & Submit the online Enrollment Form.

3. Customer receives copy of completed Enrollment Form and Installer via Email.

4. Customer launches Installer, and completes the onscreen installation instructions.

For further clarification or support, please contact your Beckman Coulter sales or service representative, or email **connect@beckman.com**.

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Daily Shutdown

DAILY SHUTDOWN

Daily Instrument Shutdown

Instrument shutdown is performed using a wizard and following all the software prompts.

1. From the 'Cytometer' menu select 'Daily Clean' and follow the cleaning prompts.

2. First add a tube of FlowClean solution (2ml) followed by a tube of deionized water. NO BLEACH. Wait for system to finish.

3. Once completed, select close.

4. Exit software. Turn off cytometer.

Maintenance and Cleaning

For instruments under service contracts, PMs (Preventative Maintenance) are scheduled by the customer. Please call Technical Support at **800.369.0333** to schedule the PM.

Daily Cleaning, Changing Out Sample Line

Daily cleaning steps can vary depending on what application you are using your cytometer to research. Below is a general cleaning guideline that should work for most uses. If you need support on applications like small particle, please reach out to Applications Support.

Daily Clean

Daily Clean should be performed during instrument startup and instrument shutdown to clean the sample line. After sampling an excessively large sample or a sample that can easily clog the sample probe, it is recommended to perform the Daily Clean procedure. Daily Clean can also be used to remove residual sample from previous tubes.

1. Open the CytExpert software and confirm that the instrument is connected and that it has already been initialized.

2. Select Daily Clean in the Cytometer menu.

3. Add 2 mL of FlowClean solution to an unused sample tube.

4. Add 3 mL of DI water to an unused sample tube.

5. Insert the sample tube with 2 mL of FlowClean solution into the sample holder and select Run. **NOTE:** The default cleaning time is 3 minutes.

6. Remove the Flow Clean tube.

7. Insert the sample tube with 3 mL of DI water into the sample holder and select Run to perform the second step of the cleaning process. NOTE: The default cleaning time is 5 minutes.

8. After the process has been completed, remove the sample tube and close the Daily Clean Window.

MAINTENANCE AND CLEANING

Daily Clean (with Plate Loader)

Daily Clean should be performed during instrument startup and instrument shutdown to clean the sample line. After sampling an excessively large sample or a sample that can easily clog the sample probe, it is recommended to perform the Daily Clean procedure. Daily Clean can also be used to remove residual sample from previous tubes.

1. Open the CytExpert software and confirm that the instrument is connected and that it has already been initialized. Refer to Logging Into the Software in CHAPTER 3, Daily Startup.

2. Select Daily Clean in the Cytometer menu. The Daily Clean window appears. The plate loader automatically ejects the plate holder stage.

3. Follow the on screen software prompts and select the desired wells for cleaning agent and deionized water.

   IMPORTANT: You must select at least one cleaning solution well and one water well.

   a. Select the desired wells for the cleaning agent and select Set As Cleaning Agent Well.

   b. Select the desired wells for the deionized water and select Set As Deionized Water Well. **NOTE:** To deselect water wells, select the desired well and select Set As Empty Well.

   c. Select the Turn off cytometer after daily clean checkbox to automatically shutdown the cytometer after Daily Clean is finished. (CytoFLEX LX Only)

4. Select Start to start the cleaning procedure. The message Please confirm that the correct plate is placed properly and press OK appears. Select OK.

5. Select Close.

HELPFUL TIP: If you don't use your CytoFLEX every day consider filling the cleaning solution bottle with a smaller amount of Contrad 70.

Consumables
and Reagents

Where to Re-order Consumables and Reagents

Your single- and multi-color reagents, sheath, replacement parts and more can be found on **beckman.com**.

Recommended for You

Cell Cycle Kit

Optimized kit for assessing cell cycle status.

DURAClone IM Phenotyping Basic

8-color, 8-monoclonal antibody reagent that allows the identification of common extracellular markers of different subpopulations of lymphocytes, present in whole blood specimens.

CytoFLEX Sheath Fluid

A nonionic, non-fluorescent, and azide-free sheath fluid for use on CytoFLEX flow cytometers.

Introducing Cytobank

Cytobank provides an established cloud-based platform that accelerates research productivity by enabling you to analyze and visualize multiple complex single-cell data sets efficiently and effectively.

No-Risk 30 Day Trial

Go online and sign up for a risk free 30 day trial at **premium.cytobank.org**.

RESOURCES

Common Links and Phone Numbers

Beckman Coulter Life Sciences website
beckman.com

CytoFLEX User Guide Online
becls.co/startyourcytoflex

Reagents
beckman.com/reagents/coulter-flow-cytometry

Tech Support
beckman.com/contact-us (search by country)

Customer Service North America
800.742.2345

Service Repair and Installation North America
800.369.0333

Tech Docs
beckman.com/techdocs

About CytoFLEX

CytoFLEX consists of three main components: **cytometer, fluid containers and the workstation.**

1. **Cytometer** creates and collects fluorescent signals utilizing these different components:

    OPTICAL COMPONENTS: Optical Bench includes <u>lasers, optical beam combiner and integrated optical flow cell assembly</u>. The optical bench cover is equipped with a <u>laser interlock</u> that turns the lasers off unless the cover is tightly covered.

    WAVELENGTH DIVISION MULTIPLEXER: each WDM is a unique detector array that corresponds to a different laser. It contains the <u>optical filters and detectors</u> for capturing fluorescence or scatter generated from a laser source. Optical fibers transmit emitted fluorescence specific to laser path. It is necessary to ensure that the filter and software settings match for each channel. Color of ring indicates laser. Optical filter mounts are labeled with the corresponding laser and bandpass information; dot = laser, band = color

    FLUIDICS MODULE: located on right side of instrument. User accessible. Contains pumps, valves and tubing, plus sheath filters and deep clean solution bottle. Alarm warns user of problems with fluid container capacity or with performance of certain operations. <u>Deep Clean solution (50% Contrad-70, peristaltic pump and deep clean solution</u> hold and transfer cleaning solution to the flow cell. Sheath filter is 0.2µm.

2. **Fluid containers** accommodate sheath fluid and waste liquids as required for the operation of the instrument

3. **Workstation** displays and manipulates the contents of the workstation and displays data generated from the cytometer. The monitors and computer peripherals display and manipulate the contents of the workstation.

Choose Beckman Coulter Life Sciences for Benchmark Expertise and Innovation

For over 80 years Beckman Coulter Life Sciences has driven innovation. We remain committed to shaping flow cytometry technology to fit seamlessly into your lab's workflow and to provide an optimal user experience. When you choose one of our solutions, you receive a high level of expertise, innovation, and quality assurance.

Contact your local Beckman Coulter Life Sciences sales representative.

beckman.com

For Beckman Coulter's worldwide office locations and phone numbers, please visit "Contact Us" at **beckman.com**

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