



# NovoCyte™ Flow Cytometer Operator's Guide





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# Prologue

## I. Revision History

Version	Revision Date	Amendments
1.0	2014.04	Initial Release
1.1	2014.07	Reorganization of the contents
1.2	2014.11	1. Revise “4.1.2 Replace Fluidic System Consumables” in support of NovoExpress Software v1.0.2. 2. Add details in “4.2.5 Fluidic System Cleaning before Long-Term Storage”. 3. Add “4.2.7 Calibrate the Fluidics Station”.
1.3	2015.04	Add description of Storage Gate in “3.3.3 Threshold”

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Questions or comments regarding the contents of this guide can be directed to the address below or to your local ACEA Biosciences representative.

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## II. Contact Information



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International Distributors

Please contact ACEA Biosciences, Inc., or visit [www.aceabio.com](http://www.aceabio.com) for a complete list of international distributors

## III. Trademark

ACEA Biosciences, the ACEA logo, NovoCyte, NovoExpress, NovoSampler, NovoFlow, NovoRinse, and NovoClean, are trademarks of ACEA Biosciences, Inc.

All other company and product names might be trademarks of the respective companies with which they are associated.

## IV. Intended Use

The ACEA NovoCyte™ flow cytometer can be used for qualitative and quantitative measurement of cells and other particles by detecting both biological and physical properties. It can simultaneously detect forward scattered light, side scattered light, and the excitation of multiple fluorescent molecules using the 405nm, 488nm, and 640nm lasers to perform multi-parameter analysis of the cells or particles. Additional laser source may be available as well. Please contact ACEA Biosciences, Inc. for more information.

ACEA NovoCyte™ flow cytometer is for Research Use Only and should not be used for diagnostic or therapeutic procedures.



*The configuration of lasers and fluorescence detection channels in the NovoCyte™ instrument is dependent on the instrument model. For detailed specifications, please refer to the [Appendix C: Technical Specifications](#) section in this guide.*

## V. Using this Guide

This operator's guide describes the operation and maintenance of the ACEA NovoCyte™ flow cytometer. Most of these functions are controlled by the ACEA NovoExpress™ software. For additional information about the ACEA NovoExpress™ software, please refer to [NovoExpress™ Software Guide](#).

ACEA NovoCyte™ Flow Cytometer Instrument Operator's Guide assumes that the user is familiar with the Microsoft Windows® operating system. For questions regarding the Microsoft Windows® operating system, see the operating system's help and support files.

## VI. Conventions

### Text Conventions

To impart information that is consistent and easy-to-read, the following text conventions are used in this guide:

Format	Description
Numbered Listing <b>1</b> <b>2</b> .....	Describes the steps in a procedure that must be performed in the listed order.
<i>Italic font, gold</i>	Points to a different chapter in this guide, which should be referred to for better understanding.
<i>Italic font</i>	Describes buttons, icons or functions when operating the NovoExpress™ software. In addition, important notes and information are also shown in italic font.
→	Indicates the sequence of the menu operation in NovoExpress™ software. For example, <i>File</i> → <i>Print</i> means to select the <i>Print</i> function from the <i>File</i> menu.
Ctrl+X	When used with keyboard characters, + means to press two keys simultaneously. For example, Ctrl + C means to hold down the Control key while pressing the letter C key.

### Symbols

The following table lists the symbols used in this guide:

Symbol	Meaning	Description
	BIOHAZARD	This symbol indicates that certain precautions must be taken when working with potentially infectious biological specimens and materials.
	LASER RADIATION	This symbol indicates that the operation could potentially cause exposure to laser radiation. Protective measures should be taken during the operation.
	WARNING	This symbol indicates that improper or unsafe operation could result in instrument damage, data loss, and personal injury.
	SHOCK HAZARD	This symbol indicates that the operation has a risk of electric shock.
	IMPORTANT NOTE	This symbol indicates information which is critical to the success of the procedure or use of the product.
	ADDITIONAL INFORMATION	This symbol provides additional information about the current topic or procedure.
		Table continues on next page.
		End of a table.

The following symbols appear on NovoCyte™ flow cytometer:

Symbol	Meaning	Description
	Manufacturer	This symbol indicates the device manufacturer.
	Serial number	This symbol indicates the manufacturer's serial number for identification of the device.
	Direct current	This symbol indicates only direct current should be applied to the device.
	CE conformity marking	This symbol indicates conformity with the provisions of relevant directives for CE marking.
	CSA conformity marking	This symbol indicates conformity with the provisions of relevant directives for CSA marking.
	DIRECTIVE 2002/96/EC conformity marking	This symbol indicates conformity with Waste from Electrical and Electronic Equipment (WEEE) directives.
	Consult Instrument Operator's Guide	This symbol indicates the need for the user to consult the Instrument Operator's Guide.
COM		COM communication port
USB		USB communication port
NET		Network communication port

The following precaution labels appear on the ACEA NovoCyte™ flow cytometer, NovoCyte™ fluidics station, or NovoSampler™ to indicate a potential hazard. Please do not remove these labels. Use appropriate precaution to avoid injury by the indicated hazard. Please refer *Prologue VIII. Safety and Limitations* in this guide for more information.

Symbol	Meaning	Description
	BIOHAZARD	This symbol indicates that certain precautions must be taken when working with potentially infectious biological specimens and materials.
	SHOCK HAZARD	This symbol indicates that the operation has a risk of electric shock.
	LASER RADIATION	This symbol indicates that the operation could potentially cause exposure to laser radiation. Protective measures should be taken during the operation.
	MOVING PARTS	This label indicates the moving parts in the instrument. Be careful during the operation.
	CLASS I LASER PRODUCT	This symbol indicates the laser is a Class I product (see <i>Prologue VIII Safety and Limitations: Laser Product Classification</i> in this guide for more information.)
	PINCH HAZARD	This symbol indicates the part may cause potential pinch hazard. Keep hands and fingers away from the part.
	WARRANTY VOID	This symbol indicates that the user is not allowed to remove or disassemble the parts. Doing so will void the warranty on the product.

## VII. Glossary of Abbreviations

The following table lists the meaning of abbreviations used in this guide:

Glossary Abbreviation	Meaning
FSC	Forward Scatter, forward scattered light
SSC	Side Scatter, side scattered light
BL	488nm (blue) laser excited fluorescence channels or optical detectors. BL1 denotes the first channel excited by the 488nm laser, and so on.
RL	640nm (red) laser excited fluorescence channels or optical detectors. RL1 denotes the first channel excited by the 640nm laser, and so on.
VL	405nm (violet) laser excited fluorescence channels or optical detectors. VL1 denotes the first channel excited by the 405nm laser, and so on.
PMT	Photomultiplier Tube
SD	Standard Deviation
CV	Coefficient of Variation
HPCV	Half-Peak Coefficient of Variation
MESF	Molecules of Equivalent Soluble Fluorochrome
MFI	Mean Fluorescence Intensity
SIP	Sample Injection Probe
FCS	Flow Cytometry Standard
CSV	Comma-Separated Values
FITC	Fluorescein isothiocyanate. A typical fluorescent dye of the BL1 channel.
PE	Phycoerythrin. A typical fluorescent dye of the BL2 channel.
PE-Texas Red	Phycoerythrin-Texas Red. A typical fluorescent dye of the BL3 channel.
PerCP	Peridinin chlorophyll protein. A typical fluorescent dye of the BL4 channel.
PE-Cy7	PE and cyanine 7 tandem dye. A typical fluorescent dye of the BL5 channel.
APC	Allophycocyanin. A typical fluorescent dye of the RL1 channel.
APC-Cy7	APC and cyanine 7 tandem dye. A typical fluorescent dye of the RL2 channel.
TEC	Thermoelectric cooling
ADC	Analog-to-Digital Converter
FPGA	Field-programmable gate array
LP	Long Pass. Longpass filter
SP	Short Pass. Shortpass filter
BP	Band Pass. Bandpass filter

## VIII. Safety and Limitations

The NovoCyte™ flow cytometer is limited to be operated by professionals trained by ACEA Biosciences or an authorized representative. The NovoCyte™ flow cytometer incorporates safety measures to protect the operator. Please refer to this guide to operate the instrument in strict accordance with the instructions and in accordance with the provisions of instrument maintenance and repair. Please ensure that the safety information can be found at any time. The instrument may be damaged if it is operated under any conditions that are not described in this guide.

### Electrical Safety



*To prevent electrical shock and instrument damage, please follow the guidelines listed below.*

- ▶ Unless otherwise specified, turn off the power switch and unplug the power cord before servicing the instrument.
- ▶ Use the power adapter supplied by ACEA Biosciences only. Connect the instrument only to an approved power source. If damaged electrical wires, plugs, or cables are detected, promptly contact your ACEA Biosciences representative for replacement.
- ▶ Only use the power outlets that are located at a conveniently accessible location so that the cytometer, workstation, and monitor can be easily plugged to and unplugged from the power outlets.
- ▶ To ensure your safety, the power cord must be inserted in the three-pin socket with a protective ground connection. Please make sure the three-pin socket connects to the ground reliably.
- ▶ If leaks are detected, immediately turn off the power by pressing and holding the power button on the front of NovoCyte™ instrument for about 6-7 seconds. Immediately contact your local ACEA Biosciences representative for support.

### Laser Safety

Laser or laser systems emit intense, coherent electromagnetic radiation that has the potential of causing irreparable damage to human skin and eyes. The main hazard of laser radiation is direct or indirect exposure of the eye to thermal radiation from the visible and near infrared region (325 ~ 1400 nm). Direct eye contact with the laser will burn the cornea, retina, or both injury and may lead to possible blindness.

There are other potentially serious hazards in other spectral regions. Excessive ultraviolet exposure produces an intolerance to light (photophobia) accompanied by redness, a tearing discharge from the mucous membrane lining the inner surface of the eyelid (conjunctiva), shedding of the corneal cell layer surface (exfoliation), and stromal haze. These symptoms are associated with photokeratitis, otherwise known as snow blindness or welder's flash, which results from radiant energy-induced damage to the outer epidermal cell layer of the cornea. These effects can be the result of laser exposure lasting only a fraction of a second.

### Laser Product Classification

Laser hazard levels depend on the wavelength used and the energy content. A numbered system is used to categorize lasers according to different hazard levels. The higher the classification number, the greater the potential hazard. The ACEA NovoCyte™ flow cytometer is a Class I laser product according to IEC/EN 60825-1: 2007. The lasers and the laser energy are fully contained within the cytometer structure and do not require a

special work area except during service procedures. These service procedures, including laser maintenance and repair, can only be carried out by service professionals by qualified ACEA Biosciences representatives.



*In order to avoid the risk of exposure to laser light, be sure to follow the guidelines listed below.*

- ▶ Modification or removal of the optics covers or laser shielding could result in exposure to hazardous laser radiation. To prevent irreparable damage to human skin and eyes, do not remove the optics covers or laser shielding, adjust controls, or attempt to service the cytometer at areas where laser hazard warning signs are attached.
- ▶ Operating a NovoCyte™ flow cytometer under conditions other than those specified in this guide may result in hazardous radiation exposure.
- ▶ Keep all doors and covers closed during operation of the cytometer. When operated under these conditions, the cytometer poses no danger of exposure to hazardous laser radiation.

### **Biosafety**



*All biological specimens and materials in contact with biological specimens are potentially biohazardous. In order to avoid exposure to biohazardous material, be sure to follow the guidelines listed below.*

- ▶ Treat and handle all biological specimens and materials as if they are capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations. Never pipette by mouth.
- ▶ Avoid directly touching the biological specimens and waste. Wear suitable protective clothing, eyewear, and gloves when handling biological specimens and materials. If the biological samples accidentally come into contact with the skin, follow proper work safety procedures and immediately consult a medical professional.
- ▶ Gloves must be worn when manually loading the sample to the sample holder on the cytometer.
- ▶ Reagents, including the sheath fluid, cleaning solution, and rinsing solution, may cause damage to the skin. Be cautious around sheath fluid, cleaning solution, and rinsing solution, and prevent direct contact with hands and clothes. If there has been accidental contact with hands or clothing, immediately rinse with soap and tap water. If it gets into eyes, immediately rinse with plenty of water and consult a medical professional.
- ▶ Sheath fluid, cleaning solution, rinsing solution, and waste may contain materials controlled by contamination regulations and emission standards. Please follow local regulations and guidelines for proper disposal.
- ▶ Expose waste container contents to bleach (10% of total volume) for 30 minutes before disposal. Dispose of waste in accordance with local regulations. Use proper precaution and wear suitable protective clothing, eyewear, and gloves when disposing of waste.
- ▶ Prevent waste overflow by emptying the waste container frequently or whenever the system gives *Waste is Full* warning.
- ▶ Be sure to place the second sample tube only after the rinsing procedure is fully completed for the previous sample. Otherwise, there is possibility that the content from the previous sample may contaminate or dilute the second sample, or even cause the second sample to over flow which may lead to exposure to biohazardous material.

### General Safety

-  When operating the cytometer, keep your hands and clothing away from the cytometer. Do not touch the sample injection probe (SIP) when the cytometer is in operation. Mechanical movable parts within the cytometer can pinch or cause injury to hands or fingers.
-  To avoid burns, do not touch the fan guards on the back of the cytometer. The fan guards may get hot during and after cytometer operation.
-  The data is automatically stored on the workstation's hard drive. But if the workstation hard drive data are deleted or the hard drive is corrupted, the data can be unrecoverable. Please regularly backup the cytometer data to a server or other mobile storage devices.
-  The sample injection probe is a mechanical movable part and may pinch or injure your fingers. To prevent accidents, do not put your hand underneath the sample injection probe during the sample acquisition process. Also, when placing or removing the sample tube, do not put your hand underneath the sample injection probe.
-  The NovoSampler™ has mechanical movable parts that can pinch or injure your fingers. To prevent accidents when running samples using the NovoSampler™, be sure to close the cover of the NovoSampler™. Only when placing the sample plate onto the NovoSampler™ or during routine maintenance, should the cover be opened. Even though the NovoSampler™ will automatically stop running when the cover is opened during operation, always confirm that the NovoSampler™ is fully stopped when opening the cover to load the sample plate or carry out maintenance.
-  Do not place any heavy objects on top of the cytometer. Doing so may cause damage to the instrument, resulting in compromised instrument performance and abnormal data.
-  Regarding the restrictions on the use of reagents, please see the relevant package insert and technical data sheet of the reagents.

### Limitations

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

ACEA Biosciences provides the software and workstation that are intended for running the NovoCyte™ flow cytometer supplied by ACEA Biosciences. Buyers/users are responsible to ensure that all software, transmitted media, and electronic documents are virus free. If the workstation is used with a network connection, buyers/users are responsible for installation and maintenance of up-to-date virus protection software. ACEA Biosciences does not provide warranty with respect to the workstation remaining virus free after installation. ACEA Biosciences is not liable for any claims related to or resulting from buyer/user's failure to install and maintain virus protection.

-  Do not use the instrument including the flow cytometer, workstation, and other parts supplied by ACEA Biosciences in an oxygen-enriched atmosphere.
-  Do not use the instrument including the flow cytometer, workstation, and other parts supplied by ACEA Biosciences with flammable liquid/explosive gas or reagents.

## IX. Technical Support

If there are operating or technical questions, please refer to the sections relating to the instrument operation in this guide. If a problem occurs, refer to the troubleshooting section in this guide for solutions. For additional technical support, please contact your local ACEA Biosciences representative or distributor. When contacting ACEA Biosciences, be sure to provide the following information:

- ▶ The product name, product model and serial number
- ▶ Usage history of the instrument
- ▶ Instrument status information on the status bar from the NovoExpress™ software. Or if there is a warning or error message, please also provide that information.
- ▶ Experiment information conducted on the instrument, if not confidential.
- ▶ Details of recent instrument QC test.

For support within the US, please call 866-308-2232.

For support within China, please call 400-600-1063.

For users in other countries or regions, contact your local ACEA Biosciences representatives or distributors, which may be found at [www.aceabio.com](http://www.aceabio.com).

# 1. Introduction

The following topics are covered in this chapter:

- ▶ Overview
- ▶ System Components and Installation
- ▶ Basic Principle
- ▶ Fluidics Station
- ▶ NovoCyte™ Workstation
- ▶ NovoSampler™ (Optional)
- ▶ System Requirements

## 1.1 Overview

The NovoCyte™ flow cytometer can be used for scientific research, including but not limited to detection and analysis of cell surface antigens, intracellular activity analysis, detection and analysis of apoptosis, DNA content detection, cell cycle analysis of the nucleus, analysis of cell proliferation, and GFP reporter gene detection, etc.

The NovoCyte™ flow cytometer can detect a particle with the size of  $0.2\ \mu\text{m} \sim 50\ \mu\text{m}$ . Besides human cells, it can also be used to analyze:

- ▶ Animal Cells
- ▶ Plant Cells
- ▶ Marine plankton
- ▶ Bacteria
- ▶ Fluorescent microspheres

## 1.2 System Components and Installation

The ACEA NovoCyte™ flow cytometer includes the NovoCyte™ instrument, fluidics station, and NovoCyte™ workstation. In addition, there is an optional NovoSampler™ for automatic collection of multiple samples. Figure 1-1 to 1-3 shows the NovoCyte™ flow cytometer system, the front view, and the back view of the NovoCyte™ instrument respectively.

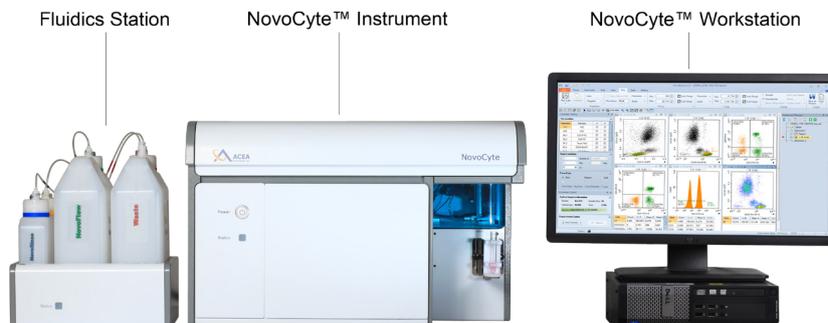


Figure 1-1 NovoCyte™ Flow Cytometer



Figure 1-2 NovoCyte™ Instrument (Front View)



Figure 1-3 NovoCyte™ Instrument (Rear View)

### 1.2.1 Installation

A minimum space of 150 cm (W) × 80 cm (D) × 80 cm (H) is required to install the NovoCyte™ instrument and fluidics station. Make sure there is at least 20 cm distance between the NovoCyte™ instrument to the adjacent object (i.e. walls). Leave at least 10 cm distance between the NovoCyte™ instrument and the fluidics station.

Figure 1-4 illustrates the connection of the NovoCyte™ flow cytometer system.

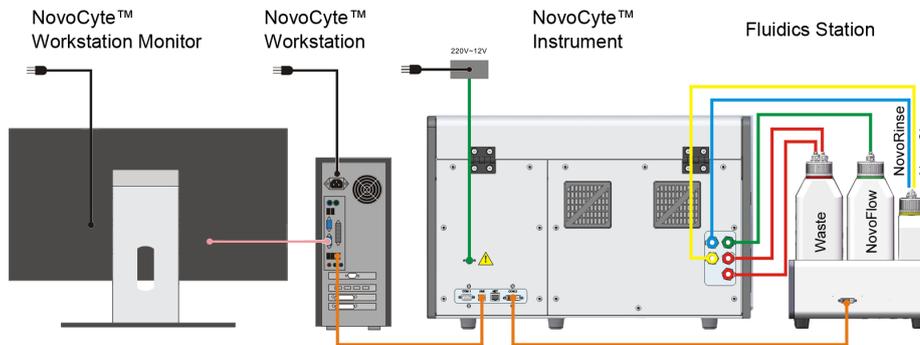


Figure 1-4 NovoCyte™ Flow Cytometer System Connection

#### 1.2.1.1. Connection to the Power Supply

- ▶ Connect the NovoCyte™ instrument to the supplied power adapter (Output: 12VDC) through the power port (refer to Figure 1-3), and then connect the power adapter to a power supply (100-240 VAC, 50-60Hz).

- ▶ Connect the workstation and the monitor to the power supply. For more details, please refer to the instructions from the computer and monitor supplier enclosed in the package.

### 1.2.1.2. NovoCyte™ Flow Cytometer System Connection

Communication ports which connect the NovoCyte™ instrument to the auxiliaries are located at the back of NovoCyte™ instrument and are shown in Figure 1-5. COM 1 port connects to the NovoSampler™ (optional). USB port connects to the NovoCyte™ workstation. COM 2 port connects to the fluidics station through a DVI cable. The NET port is reserved for network connection.



Figure 1-5 NovoCyte™ Instrument Communication Ports

**!** There is a DVI cable port on the workstation monitor as well. In order to prevent incorrect connection of the NovoCyte™ fluidics station to the workstation monitor through DVI cable, the DVI cable port of the monitor is shielded by ACEA Biosciences, Inc. in the factory. Please do not remove the cover and make sure the NovoCyte™ fluidics station is correctly connected to the NovoCyte™ flow cytometer instrument through the COM2 port at the back of the cytometer.

**!** Please make sure the instrument is properly connected to the auxiliaries and reagent containers. Incorrect connections will result in damage to the instrument or cause operation failure.

#### ▶ Connect the instrument to the computer

Use the supplied USB cable to connect the NovoCyte™ flow cytometer to the USB port of the NovoCyte™ workstation. Please see Figure 1-5.

#### ▶ Connect the fluidics station to the instrument

The fluidics station is connected to the COM 2 port of the NovoCyte™ instrument through the supplied DVI cable. Please see Figure 1-5.

The connections for the reagent containers to the NovoCyte™ instrument are shown in Figure 1-6. Five sets of tubing connect reagent containers on the fluidics station to the ports at the back of the instrument. These ports are labeled with NovoRinse, NovoFlow, NovoClean, Waste 1, and Waste 2, respectively. NovoRinse™ is the rinsing solution and is connected by the blue tubing. NovoFlow™ is the sheath fluid and is connected by the green tubing. NovoClean™ is the decontamination solution and is connected by the yellow tubing. Waste 1 and Waste 2 connect to the red tubing.

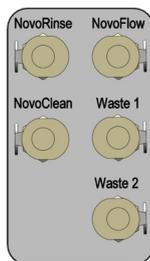


Figure 1-6 Interface of the Fluidics Port

The reagent containers are connected through the same color coded tubing to the ports at the back of the NovoCyte™ instrument, as shown in Figure 1-7.



Figure 1-7 Tubing Connections of the Reagent Containers

► **Connect the Instrument to the NovoSampler™ (optional)**

Please refer to *NovoSampler™ Operator's Guide* for the detailed procedure.

 For specific requirements of the operating conditions, please refer to the *Appendix C: Technical Specifications* section in this guide.

### 1.2.2 Instruction for Lifting and Carrying

- Do not attempt to lift or move the instrument without the assistance of others. Improper lifting can cause painful and permanent back injury. Depending on the weight of the instrument, moving or lifting the instrument may require two or more persons.
- Make sure to unplug the power adapter and disconnect the quick couplers of the instrument reagent containers from the back of the instrument. If the instrument is equipped with a NovoSampler™, it needs to be uninstalled from the instrument. Moving the instrument with the NovoSampler™ installed is not allowed.
- Lift with two hands from the bottom of the instrument with at least one person at each side. Carefully move the instrument to the desired location and gently put it down on a flat laboratory bench. Keep the instrument horizontal during the moving.
- If needed, proper lifting technique and appropriate moving equipment could be used to facilitate moving the instrument.

 To prevent accidents and to ensure the quality of the data, after ACEA Biosciences professionals install the instrument, moving the instrument is not recommended. If any change of performance is observed, please contact your local ACEA Biosciences representative.

## 1.3 Basic Principle

The NovoCyte™ flow cytometer contains the optical system, the fluidic system, and the electronic system. This section describes the basic principle of these subsystems and their components. For more technical details, please refer to *Appendix A: Technical Overview* and *Appendix C: Technical Specifications* sections in this guide.

### 1.3.1 Optical System

The optical system consists of lasers, beam shaping optics, light collection optics, optical filters/mirrors, and optical detectors.

#### Lasers

NovoCyte™ uses low-power solid-state lasers to provide reliable and high performance excitation sources for the flow cytometer. Solid-state lasers have compact size, excellent performance, good stability, long lifetime, and other advantages. The lasers come with a thermoelectric cooling (TEC) system to ensure its extraordinary beam quality. NovoCyte™ offers three lasers to meet the needs for different applications.

Lasers	Wavelength (nm)	Power (mW)	Typical fluorescent labels
Coherent OBIS Solid State	405	50	Pacific Blue™, AmCyan
	488	20	FITC, PE, PI, PE-Texas Red®, PerCP, PerCP-Cy™5.5, PE-Cy™5, PE-Cy™7
	640	40	APC; APC-Cy™7

Table 1-1 Laser powers and detectable fluorochromes on NovoCyte™

#### Beam Shaping Optics

Beam shaping optics shape the laser beam into an elliptical spot of approximately 10 μm × 80 μm and focus the spot at the center of the flow cell.

#### Light Collection Optics

The light collection optics efficiently collect the light emitted by the particles or cells from the center of the flow cell, including forward scatter light, side scatter light, and fluorescence signals.

#### Filters/Dichroic Mirrors

Light collected by the light collection optics is separated by a series of dichroic mirrors and filtered by a bandpass filter in front of each optical detector. NovoCyte™ uses longpass dichroic mirrors and bandpass filters to detect light within a specific range.

#### Optical Detectors

Optical detectors convert light into electrical signals, which are then processed by the electronic system. The NovoCyte™ uses a photodiode (PD) to detect the forward and side scatter light, and a photomultiplier tube (PMT) to detect the fluorescence signal.

### 1.3.2 Fluidic System

The fluidic system consists of the sample injection probe (SIP), SIP wash apparatus, the flow chamber, driving pumps, solenoid valves, and a series of tubings and connectors. The sample is introduced into the flow chamber through the fluidics system and is hydrodynamically focused by the sheath fluid to ensure reliable signal detection. Cleaning and decontamination solution is used to clean and decontaminate the fluidic system.

## Sample Injection Probe (SIP) and SIP Wash Apparatus

The sample injection probe, a hollow stainless steel tube, is used to aspirate the sample from the test tube so that the sample pump can drive the aspirated sample to flow through the flow cell. When running samples, the sample injection probe will move downward from the housing into the test tube, draw a certain volume of the sample, and then retract back to the original position. By default, the SIP wash apparatus (Figure 1-8) will automatically clean the inner wall and the outer wall of the SIP after each sample acquisition. The SIP cleaning waste is collected to the waste container. This automatic SIP wash function ensures minimal inter-sample carryover rate (<0.1%) and eliminate tedious manual cleaning processes.

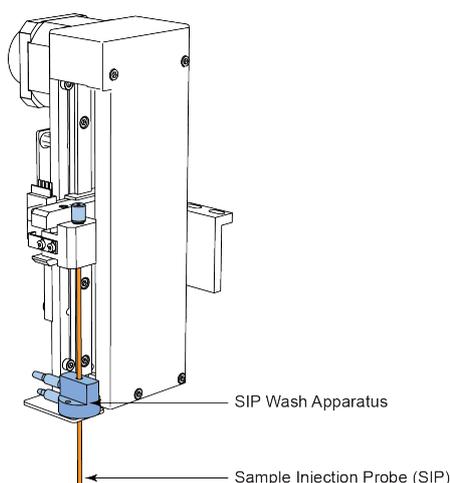


Figure 1-8 Sample Injection Probe (SIP) and SIP Wash Apparatus

-  If you want to increase the sampling speed, you can turn off the automatic cleaning procedure that occurs after each sampling by default. To do so, uncheck the **Rinse after Sampling** box in the **Cytometer Control** panel from the NovoExpress™ software.
-  During sampling, do not obstruct the moving SIP. Do not place your hand on the test tube.
-  The sample injection probe directly contacts biological samples which may be biohazardous. Appropriate personal protective equipment should worn when handling the sample injection probe, in accordance with local safety regulations.
-  If the NovoSampler™ is installed, make sure the NovoSampler™ cover is closed during the process of running samples.

## Flow Cell

When the sample flows into the flow cell, it will be focused by the sheath fluid to ensure that only one cell or particle passes through the laser beam at a time.

## Sample Flow Control

There are three standard sample flow rate settings in the NovoCyte™: Slow (14  $\mu\text{L}/\text{min}$ ), Medium (35  $\mu\text{L}/\text{min}$ ) and Fast (66  $\mu\text{L}/\text{min}$ ). The sheath flow rate is fixed at 6.5 mL/min, which corresponds to approximately 7.7  $\mu\text{m}$ , 12.2  $\mu\text{m}$  and 16.8  $\mu\text{m}$  core diameter under the above mentioned three standard sample flow rates, respectively. The NovoCyte™ also allows continuous adjustment of the sample flow rate from 5  $\mu\text{L}/\text{min}$  to 120  $\mu\text{L}/\text{min}$ , which corresponds to a core diameter of 4.6  $\mu\text{m}$  to 22.7  $\mu\text{m}$ . Sample flow rate can be easily set in the NovoExpress™ software.

-  To set the flow rate, please refer to 3.3 Set Sample Acquisition Conditions in this guide and relevant sections in NovoExpress™ Software Guide.

### Fluidic System Status Monitor and Alarm

The NovoCyte™ uses advanced technology and multiple high-performance pressure sensors to monitor the status of the fluidic system in real time with high reliability. Abnormal working conditions of the fluidic system will be reported with warning information, ensuring data accuracy on every sample collection.

### Sampling Method

The NovoCyte™ uses a high-precision syringe pump to aspirate the sample and drive it into the flow chamber for analysis. The sampling method can precisely control the volume of the sample, thus guarantee accurate absolute counting results.

The NovoCyte™ aspirates a certain volume of the sample set by the user in the NovoExpress™ software, plus an additional 20  $\mu\text{L}$ . When the injection volume is less than 100  $\mu\text{L}$ , sample injection can be done by one aspiration. When the injection volume is greater than 100  $\mu\text{L}$ , multiple aspirations are necessary to reach the user-defined volume.

**!** *The sample injection probe may perform multiple aspirations for one sampling. Do not remove or change the sample tube until the entire sampling process has completed.*

### 1.3.3 Electronic System

When a cell or a particle passes through the optical detection point, a Gaussian-shaped electronic pulse will be generated from the optical detectors. The amplitude of the generated pulse is proportional to the intensity of the optical signal, which indicates the properties of the cell or particle (FSC and SSC) and the amount of the fluorochrome associated with it. Such electronic signals generated by the optical detector are further amplified, filtered and converted into digital signals by the high-speed, high-precision Analog-to-Digital Converter (ADC). The digital signals are processed and analyzed by algorithms programmed with high-performance field-programmable gate array (FPGA) to reveal the flow cytometry characteristics of the cell or particle including FSC, SSC, and fluorescence signals. All the signals with amplitudes higher than the set threshold would be processed by the NovoCyte™ electronic system. Parameters extracted from the pulse signals include Width (W), Height (H) and Area (A), which are illustrated in Figure 1-9.

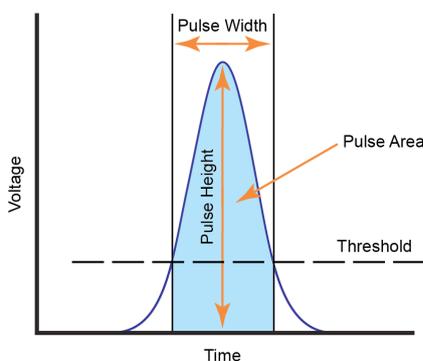


Figure 1-9 Pulse Measurements

### Control Buttons and Indicator LED

The front panel on the NovoCyte™ instrument has a power switch and an LED status indicator. The front panel of the fluidics station also has an LED status indicator. The power switch turns the NovoCyte™ instrument on and off. The LED status indicators change color to give direct visible indication of the instrument status. A detailed description of the status indicator is summarized in Table 1-2.

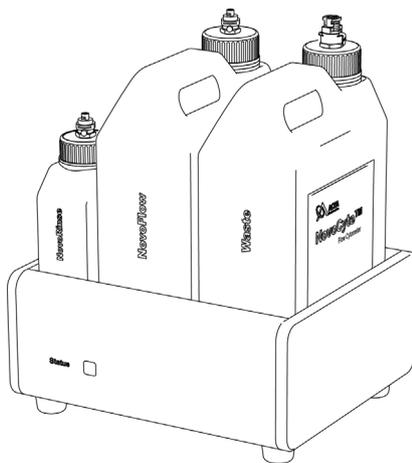
Name	Color	Meaning
The power switch light	Steady green	Instrument is powered on
	Flashing green	Instrument is performing the shut-down cleaning procedure .
LED Status Indicator	Green	Normal status
	Orange	Warning. Click on the status bar in the NovoExpress™ software to review the warning message .
	Red	Errors. Click on the status bar in the NovoExpress™ software to review the error message.

**Table 1-2 Power Switch Light and LED Status Indicator Information**

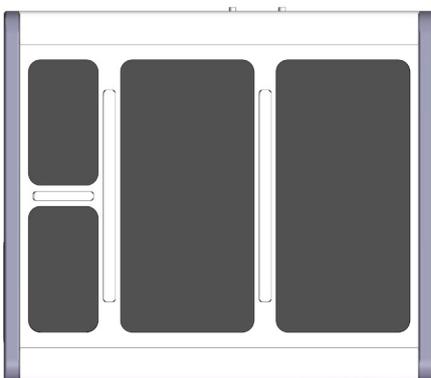
 If warnings or errors encountered, follow the resulting message prompt, and refer to 5 *Troubleshooting* section in this guide for troubleshooting methods.

## 1.4 Fluidics Station

The NovoCyte™ flow cytometer comes with a NovoFlow™ sheath fluid container (3 L), a NovoRinse™ solution container (500 mL), a NovoClean™ solution container (500 mL), and waste container (3 L) (Figure 1-10). The four containers should be placed in the designated positions on the fluidics station. Underneath each container there is a pressure sensor to monitor the remaining liquid volume in real time, allowing the system to give a warning message when the fluid runs low or the waste is close to full (Figure 1-11).



**Figure 1-10 Fluidics Station**



**Figure 1-11 Fluidics Station with Pressure Sensor Underneath Each Reagent Container**

- ▶ NovoFlow™ sheath fluid: for forming a sheathing layer to hydrodynamically focus the sample stream.
- ▶ NovoRinse™ solution: for cleaning the fluidic system.
- ▶ NovoClean™ solution: for cleaning and removing protein deposits, adherent cell debris, etc. in the fluidic system.

When the volume of NovoFlow™ fluid, NovoRinse™ solution, or NovoClean™ solution is less than the set threshold (NovoFlow™ 0.9 L, NovoRinse™ 150 mL, NovoClean™ 150 mL), the NovoExpress™ software will prompt a warning message to remind the user to add the appropriate reagents. Similarly, when the waste volume exceeds a set threshold (2.8 L), the software will give a warning to remind the user to empty the waste container. The colors of the LED status indicator on the front of the fluidics station also give information on the fluid level and are summarized in Table 1-3.

Name	Color	Meaning
Fluidics Station LED Status Indicator	Green	Fluid volume is above the designated threshold and is sufficient to run experiments (NovoFlow™ >0.9 L; NovoRinse™ >150 mL; NovoClean™ > 150 mL), and the waste container is not full (<2.8 L).
	Orange	Fluid volume is below the threshold and is insufficient to run experiments (NovoFlow™ <0.9 L; NovoRinse™ <150 mL; NovoClean™ < 150 mL), or the waste container is close to full (>2.8 L). Please add more reagents to the corresponding container or empty the waste container.
	Red	Fluid volume is too low to run experiments (NovoFlow™ <0.7 L; NovoRinse™ <100 mL; NovoClean™ < 100 mL), or the waste container is full (>3 L). Any operation of the instrument is prohibited until there are enough reagents added or the waste container has been emptied.

**Table 1-3 Fluidics Station LED Status Indicator Information**

**!** When adding reagents and emptying the waste, be sure to clean residual liquid on the outside of the containers, and place the container onto the appropriate position in the fluidics station.

**!** Please ensure that the container position in the fluidics station is correct (see Figure 1-12 and the label on the container). A similar label can be found on the left side of the fluidics station as well. The containers are color coded to match the illustrated position as well.

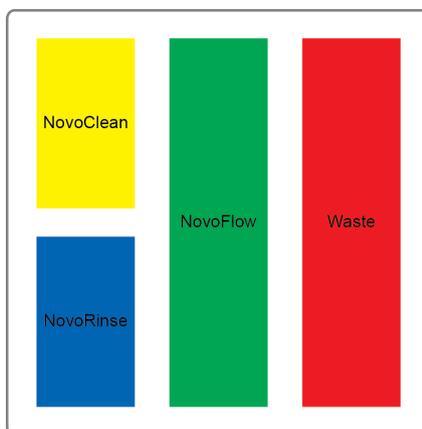


Figure 1-12 Schematic of the Container Position in the Fluidics Station

 Use caution to gently place the containers back into the fluidics station to avoid damaging the pressure sensors.

## 1.5 NovoCyte™ Workstation

The NovoCyte™ flow cytometer is equipped with a Dell or other comparable desktop and preinstalled Microsoft Windows® operating system and NovoExpress™ software. For details about the workstation, please refer to the suppliers' website. For details about the operating system, please refer to the operating system help files. For NovoExpress™ software, please refer to *NovoExpress™ Software Guide*.

## 1.6 NovoSampler™ (Optional)

The NovoSampler™ (Figure 1-13) is an optional accessory of the NovoCyte™ flow cytometer, which is used for automatic collection of multiple samples from a microtiter plate or from 12 × 75 mm tubes using the ACEA NovoSampler™ 24 tube rack.



Figure 1-13 NovoSampler™

The NovoSampler™ is compatible with the following plates and tubes:

- ▶ Standard 96-well plates (V-bottom)
- ▶ Standard 96-well plates (U-bottom)
- ▶ Standard 96-well plates (Flat bottom)

- ▶ Standard 24-well plates
- ▶ Standard 48-well plates
- ▶ 12 × 75 mm tube, with the ACEA NovoCyte™ 24 tube rack

 Use only the ACEA manufactured 24 tube rack on the NovoSampler™. Using any other type of 24 tube rack may cause severe damage to the instrument and void the warranty.

 For details about the NovoSampler™, please refer to the *NovoSampler™ Operator's Guide*.

## 1.7 System Requirements

### 1.7.1 Software

ACEA NovoExpress™ software.

### 1.7.2 Workstation

ACEA NovoCyte™ workstation. For details, refer to *Appendix C: Technical Specifications* in this guide.

### 1.7.3 Compatible Sample Tubes

The NovoCyte™ flow cytometer can be equipped with two types of sample tube holder, one for 12 × 75 mm tubes (standard) and one for 1.5 mL Eppendorf tubes (optional), respectively.

### 1.7.4 Instrument Reagents

NovoFlow™ Sheath Fluid (1X, 10 L) (Cat# 875B601)

NovoFlow™ Sheath Fluid (6X, 10 L) (Cat# 875B608)

NovoClean™ Solution (1X, 500 mL) (Cat# 872B602)

NovoClean™ Solution (5X, 500 mL) (Cat# 872B609)

NovoClean™ Solution (5X, 100 mL) (Cat# 871B609)

NovoRinse™ Solution (1X, 500 mL) (Cat# 872B603)

NovoRinse™ Solution (5X, 500 mL) (Cat# 872B610)

NovoRinse™ Solution (5X, 100 mL) (Cat# 871B610)

### 1.7.5 QC Particles

NovoCyte™ QC particles (Cat# 8000001)

## 2. Instrument Operation

This chapter describes basic operation of the NovoCyte™ flow cytometer, including the following contents:

- ▶ Instrument Start-up
- ▶ Fluidic System Maintenance
- ▶ Instrument Quality Control
- ▶ Instrument Shutdown

### 2.1 Instrument Start-up

Follow the procedures below to start the NovoCyte™ flow cytometer.

#### 2.1.1 Pre-Inspection

Check the power cords of the NovoCyte™ instrument and NovoCyte™ workstation and ensure that they are securely connected to a power outlet. Check that the connection between the fluidics station, the NovoSampler™ (if applicable), and workstation to the NovoCyte™ instrument are correct and secure.

Check the NovoFlow™ sheath fluid container, the NovoRinse™ solution container, and the NovoClean™ solution container to make sure there are enough reagents inside. If the reagent level is below 1/3 capacity on the container, add more reagent to the container. If the waste container is more than 2/3 capacity, empty the waste container, and add 50 mL of NovoClean™ solution to the waste container.

#### 2.1.2 Add Instrument Reagents

- 1 Make sure the instrument is in *Ready* or shutdown status.
- 2 Disconnect the tubing from the corresponding container by gently pressing the metal clip of the quick coupler on the cap.
- 3 Move the container from the fluidics station to a sink. Unscrew the container cap and add more reagents.
- 4 Screw the container cap back onto the container.
- 5 Reconnect the quick coupler on the cap and gently place the container onto the fluidics station. Be careful to prevent any twisting or kinking of the tubing.
- 6 If the instrument is in *Ready* status, click the *Instrument* → *Fluidics Maintenance* → *Priming* on the NovoExpress™ software to prime the fluidic system before running any samples.



For more information on the *Priming* function, please refer to 2.2.5. *Priming* section in this guide.



Please make sure the instrument is in **Ready** or shutdown status before adding any instrument reagents.

-  Be sure to correctly connect the corresponding reagent container to the fluidics port on the rear of the NovoCyte™ instrument. Incorrect connection will cause the instrument malfunction. The tubing and containers are color coded to prevent such errors.
-  The fluidics station contains pressure sensors that may be damaged by spilled liquid. To prevent the risk of sensor damage, do not add reagent while the containers are on the fluidics station.

### 2.1.3 Empty Waste

- 1 Make sure the instrument is in *Ready* or shutdown status.
- 2 Disconnect the tubing from the waste container by gently pressing the metal clip of the quick coupler on the cap.
- 3 Move the container from the fluidics station to a sink. Unscrew the container cap and pour the waste to a tank for disposing of biohazardous materials.
- 4 Add 50 mL of NovoClean™ solution to the waste container.
- 5 Screw the container cap back onto the container.
- 6 Reconnect the quick coupler on the cap and place the container onto the fluidics station. Be careful to prevent any twisting or kinking of the tubing.

 Please make sure the instrument is in **Ready** or shutdown status before emptying the waste container.

  Biological samples may be biohazardous. Appropriate personal protective equipment should be worn when handling the waste, which should be disposed of in accordance with local safety regulations.

### 2.1.4 Power on the NovoCyte™ Instrument

Gently press the power switch on the front panel of the NovoCyte™ flow cytometer to turn on the instrument. The power switch light should turn green, indicating that the instrument has entered the initialization process.

 During the initialization process, the instrument conducts an automatic cleaning process, warms up the lasers, and prepares the instrument to be **Ready** for running samples.

### 2.1.5 Turn on Workstation

Turn on the NovoCyte™ workstation and monitor. When ready, double-click the NovoExpress™ software icon  on the workstation desktop to start. When prompted, select your username and log in. The software should display  *Initializing* on the status bar, which indicates that the instrument is properly connected, and that the instrument is in the initialization process. The initialization process takes several minutes. After completion, the status bar will display  *Ready*, indicating that the instrument is ready to use.

-  If the software displays a black icon  in the status bar, it means the instrument is not properly connected to the workstation. Check the USB cable between the instrument and the workstation and secure the connection. Then restart the NovoExpress™ software.
-  If more than one NovoExpress™ software are open on the NovoCyte™ workstation with NovoCyte™ flow cytometer connected, only the software opened firstly will connect with the NovoCyte™ flow cytometer when it is on. The other opened software will not connect with the NovoCyte™ flow cytometer and the black icon  will be displayed in the status bar. If the NovoCyte™ is turned on but you observed the black icon  in the NovoExpress™ status bar, check if there are multiple software opened at the same time.
-  The NovoExpress™ Software initially includes an **administrator** account. The default password for this account is administrator. The **administrator** login allows for creation of additional users and control of password settings. Please refer to the [NovoExpress™ Software Guide](#) for details.

## 2.2 Fluidic System Maintenance

The NovoCyte™ fluidic system has been designed to minimize the need for human intervention and maintenance and to ensure that the system is stable and reliable. The automatic maintenance processes can be accessed from the NovoExpress™ software under the *Instrument* → *Fluidics Maintenance* menu. Click the appropriate button to start the corresponding maintenance procedure. When a process starts, the NovoExpress™ software will display the process currently in progress in the status bar. After completion of the maintenance process, the status bar displays *Ready* status.

The following fluidics maintenance processes are available through the *Instrument* → *Fluidics Maintenance* menu in the NovoExpress™ software.

### 2.2.1 Debubble

Use the *Debubble* function in the *Fluidics Maintenance* panel if bubbles are suspected to exist in the fluidic system. To use the *Debubble* function, place a test tube containing at least 1 mL of 70% ethanol in the sample holder. Click the *Debubble* function, and the sample injection tube will aspirate the ethanol to initialize the debubble process. The *Debubble* process takes approximately 3 minutes.

 Always use at least 1 mL of 70% ethanol as sample when using the **Debubble** function. Failure to do so will cause instrument malfunction.

### 2.2.2 Cleaning

Use the *Cleaning* function to clean and decontaminate the fluidic system to remove any residual proteins and impurities.

The *Cleaning* process uses NovoClean™ solution and NovoFlow™ sheath fluid in sequence to flush the entire fluidic system. The *Cleaning* process takes approximately 14 minutes.

### 2.2.3 Rinse

Use the *Rinse* function to clean the fluidic system.

The *Rinse* process uses NovoFlow™ sheath fluid to flush the entire fluidic system. The *Rinse* process takes approximately 2 minutes.

### 2.2.4 Extensive Rinse

It is recommended that the users run the *Extensive Rinse* function once a month or as needed to thoroughly clean the fluidic system.

The *Extensive Rinse* process first flushes NovoRinse™ into the fluidic system, soaks the fluidic tubing for a certain period of time, then flushes the fluidic system with NovoFlow™ sheath fluid. The *Extensive Rinse* process takes approximately 12 minutes.

### 2.2.5 Priming

The *priming* function should be used whenever one of the following situations occurs:

- ▶ Non-operation of the instrument for more than two days.
- ▶ Running out of sheath fluid during a sample run, resulting in introduction of bubbles into the fluidic system.
- ▶ After adding reagent into the reagent container.
- ▶ Running out of sample during a sample run resulting in introduction of bubbles into the fluidic system.

The *priming* process uses NovoRinse™ solution and NovoFlow™ sheath fluid in sequence to flush the entire fluidic system. The *priming* process takes approximately 17 minutes.

### 2.2.6 Unclog

When the flow cell is clogged, use the *Unclog* function to clear the clog. In addition, it is recommended to run the *Unclog* function once a month as a preventative measure.

The *Unclog* process uses NovoClean™ solution to flush the flow cell under high pressure to remove the clog, soak the flow cell for a certain period of time, and then flushes with the sheath fluid. The *Unclog* process takes approximately 17 minutes.

### 2.2.7 Backflush

When the sample injection probe is blocked, use the *Backflush* function to clear the blockage.

The *Backflush* process flushes the sample injection probe under high pressure using the sheath fluid in reverse of the normal sample flow direction and the waste is aspirated by the SIP wash apparatus. The *Backflush* process takes approximately 3 minutes.

## 2.3 Instrument Quality Control

The NovoCyte™ Quality Control (QC) procedure monitors the performance of the NovoCyte™ instrument and prevents any tests being conducted under non-optimal instrument conditions. It is recommended to run the QC test on a daily basis to ensure the consistency of the instrument performance.

The NovoExpress™ software QC Test function automatically conducts the QC procedure using NovoCyte™ QC particles. Click *Instrument* → *QC Test* from the NovoExpress™ software to initialize the QC test. The QC Test Report tracks the QC test results over time and can be reviewed through *Instrument* → *QC Test Report* from the NovoExpress™ software.

ACEA NovoCyte™ QC particles are polystyrene microspheres with uniform size (average diameter of 3 µm). The QC particles contain 5 kinds of microspheres with differing fluorescence intensities and one blank microsphere. These fluorescent microspheres are internally stained with fluorescent dye, which can be excited by all the lasers in the NovoCyte™ flow cytometer, and emit 6 fluorescence peaks on BL1, BL2, RL1, and VL1 channels. With known values of Molecules of Equivalent Soluble Fluorochrome (MESF) for each fluorescent peak, the linearity can be calculated.

There are three test results:

▶ **Pass**

Indicating that all the performance parameters meet the requirements.

▶ **Acceptable**

Indicating that the performance parameters are not optimal compared to the manufacturing QC criteria. However, continued use of the instrument is still allowed and the experiment results should not be impacted.

▶ **Fail**

Indicating that the performance parameters do not meet the requirements. Please contact your local ACEA Biosciences representative when the QC test fails.

The NovoCyte™ QC Test procedure includes three steps:

- ▶ Prepare ACEA NovoCyte™ QC particles sample
- ▶ Run NovoCyte™ QC test
- ▶ View QC reports and Levey-Jennings plot

### 2.3.1 Prepare ACEA NovoCyte™ QC Particle Sample

- 1 Label a 12 × 75 mm tube for the QC test. Add 1 mL dilution buffer (0.8 mL PBS and 0.2 mL NovoRinse™ solution) into the tube.
- 2 Take one bottle of ACEA NovoCyte™ QC particles from storage. Invert the bottle a few times by hand to mix the particles thoroughly.
- 3 Add one drop of QC particles into the test tube.
- 4 Vortex the test tube to mix the QC particles thoroughly.

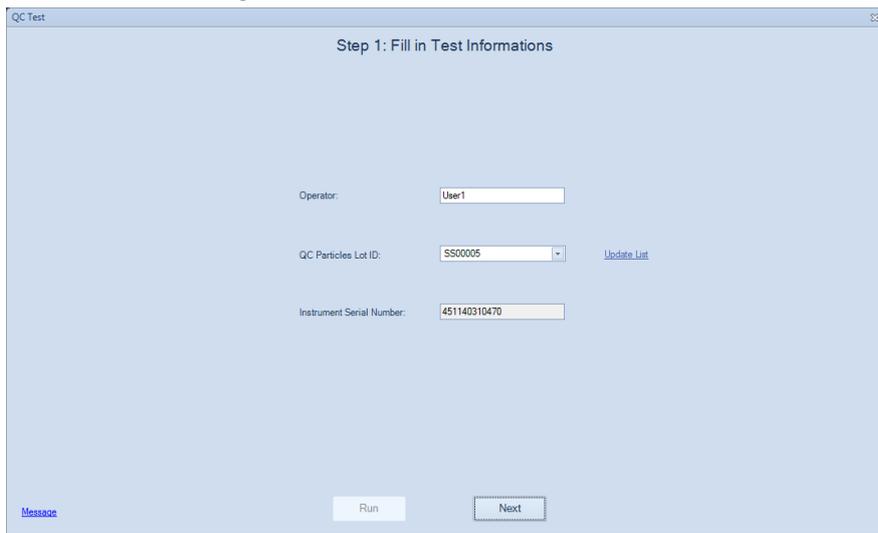


- 5 Place the tube onto the sample holder of the instrument, and start the QC test. If the prepared QC particle sample is not intended to be run immediately, store the sample in a 2-6 °C refrigerator away from light. The prepared QC particle sample should be used within four hours after preparation.

-  Make sure the QC particle sample is prepared properly following the above procedure. Properly prepared QC particle sample would give enough QC particles counts (~10,000 QC particles) during the QC Test.

### 2.3.2 Run NovoCyte™ QC test

- 1 Before running the NovoCyte™ QC test, be sure to download the QC particles Lot File corresponding to the lot number of the QC particles used from the ACEA website ([www.aceabio.com/novocYTE/qc-particles](http://www.aceabio.com/novocYTE/qc-particles)). Save the QC particles Lot File to the *QC\QC Beads* folder in the NovoExpress™ installation directory (the default installation directory for NovoExpress™ software is C:\Program Files (x86)\NovoExpress).
- 2 Make sure the NovoCyte™ instrument is in *Ready* status.
- 3 Click *Instrument* → *QC Test* on the NovoExpress™ software, which will prompt the QC Test window, as shown in Figure 2-1.



The screenshot shows a software window titled "QC Test" with a subtitle "Step 1: Fill in Test Informations". The window contains three input fields: "Operator" with the value "User1", "QC Particles Lot ID" with a dropdown menu showing "SS00005" and an "Update List" link to its right, and "Instrument Serial Number" with the value "451140310470". At the bottom of the window, there are three buttons: "Message" (a link), "Run", and "Next".

Figure 2-1 QC Test: Step 1-Fill out Test Information

- 4 Enter the operator's name in the dialog box, select the NovoCyte™ QC particle lot number from the pull-down list, and click *Next* button. If the pull-down list does not show the lot number of the QC particles you are using, please refer to the first step to download and save the corresponding QC particles Lot File in the correct directory.



- Click the *Run* button. The QC test will start automatically. The test will automatically stop when the desired numbers of particles are collected. The results are shown on the plots dynamically during the QC test as shown in Figure 2-2. The QC test takes about 2 minutes.



Figure 2-2 QC Test: Step 2-Running QC Test

- After the QC test is completed, click the *QC Test Report* button to view the test results. Figure 2-3 shows one example of the QC test results.

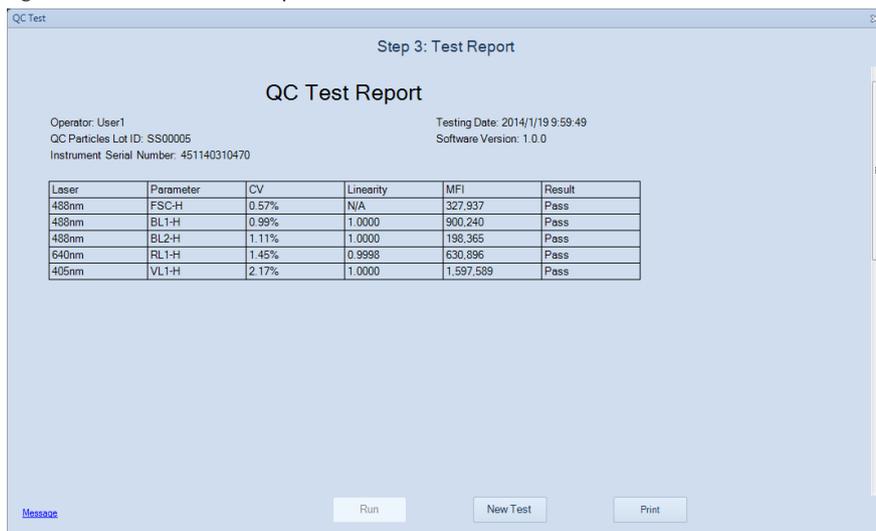


Figure 2-3 QC Test: Step 3-QC Test Report

 The parameters shown in the QC reports depend on the configuration of the *No-voCyte™* instrument and may appear to be different from the example shown in Figure 2-3.

### 2.3.3 View QC Reports and Levey-Jennings Report

The QC test report and Levey-Jennings report can be accessed through *Instrument* → *QC Test Reports* from the NovoExpress™ software, as shown in Figure 2-4.

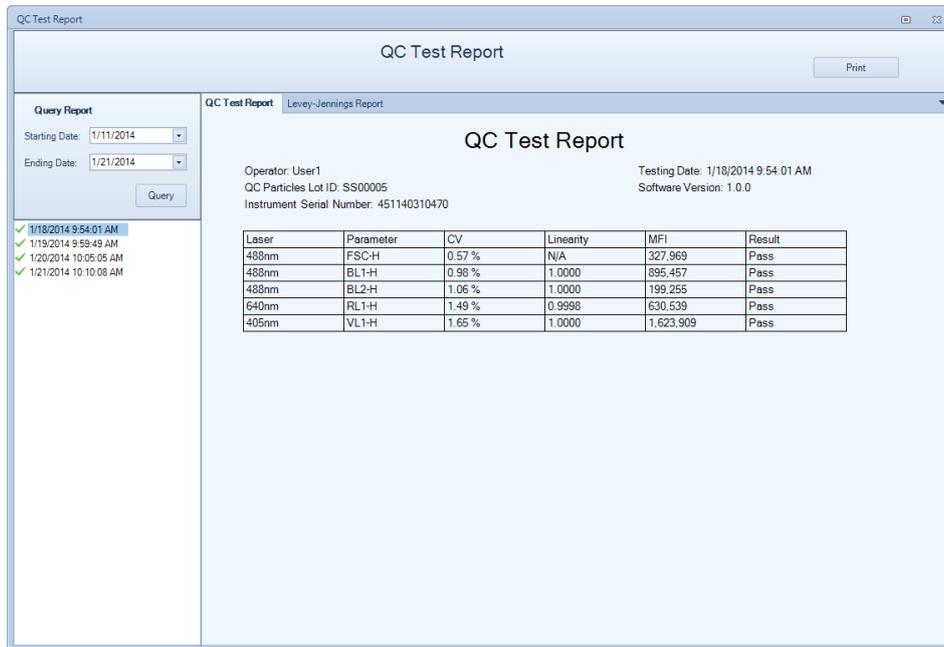


Figure 2-4 QC Test Report

Levey-Jennings plots on Mean value and CV (Coefficient of Variance) of the FSC-H and the highest peak of the BL1-H, BL2-H, RL1-H, and VL1-H can be checked by clicking the *Levey-Jennings Report* tab. Levey-Jennings plots show the stability or any continuous trend of the NovoCyte™ performance over a period of time, as shown in Figure 2-5.



Figure 2-5 QC Test Levey-Jennings Report

## 2.4 Instrument Shutdown

At the end of the day, or when all the tests are completed, please shut down the NovoCyte™ flow cytometer, following the procedure described below.

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### 1 Turn off the NovoCyte™ Instrument

Gently press the power switch on the front panel of the NovoCyte™ instrument, or click the *Instrument* → *Shut Down* icon  on the NovoExpress™ software. During the shutdown process, the power switch light will be flashing green, and the instrument will automatically perform the shutdown cleaning process. After the shutdown process is completed, power is automatically turned off and the power switch light goes out.

 *The NovoCyte™ instrument automatically performs the shutdown cleaning process and turns off the power. No user interference is needed.*

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### 2 Turn off the NovoCyte™ workstation.

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### 3 Empty the waste container.

 *For detailed steps on emptying waste, please refer to 2.1.3 Empty Waste section in this guide.*

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## 3. Running Samples

This chapter describes how to use the NovoExpress™ software to manually collect the samples and analyze the data, using human peripheral blood lymphocyte subsets analysis as an example. This test uses the ACEA QuadriTest™ CD3/CD8/CD45/CD4 Lymphocyte Subsets Detection Kit (Cat # 8730008) and the ACEA QuadriTest™ CD3/CD16 +56/CD45/CD19 Lymphocyte Subsets Detection Kit (Cat # 8730009) to stain human peripheral blood lymphocyte subpopulations. The kit contains two sets of four-color antibodies, which are CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC and CD3 FITC/CD16 +56 PE/CD45 PerCP/CD19 APC respectively. For a detailed description of the reagents and sample preparation process, please refer to the reagent technical data sheet.

This example will create a specimen named *human peripheral blood*, which consists of two tubes of human peripheral blood samples prepared and stained respectively by the ACEA QuadriTest™ CD3/CD8/CD45/CD4 Lymphocyte Subsets Detection Kit and the ACEA QuadriTest™ CD3/CD16 +56/CD45/CD19 Lymphocyte Subsets Detection Kit. The test needs to use both 488nm and 640nm lasers, and collect signals of the Height and Area parameters on FSC, SSC, and the following fluorescent channels: BL1 (FITC), BL2 (PE), BL3 (PerCP), and RL1 (APC). Each sample will be collected at a *Slow* sample flow rate until the events reach 5,000 in the Lymphocyte gate.

This chapter describes the entire sample collection and data analysis processes in the following order

- ▶ Create Experiment
- ▶ Define Sample Acquisition Parameters
- ▶ Set Sample Acquisition Conditions
- ▶ Run Sample
- ▶ Perform Fluorescence Compensation
- ▶ Analyze Data
- ▶ Append Data
- ▶ Delete data
- ▶ Save Data
- ▶ Import and Export Files

### 3.1 Create Experiment

Click the NovoExpress™ software icon on the Desktop or click *Start* → *Program* → *NovoExpress* to start the NovoExpress™ software.

Once the NovoExpress™ software has started, a new NovoCyte experiment file with the default file name of *untitled.ncf* will be created. The extension name of *.ncf* indicates that the file is a NovoCyte experiment file.

Figure 3-1 shows the NovoExpress™ software interface. The status bar indicates that the instrument is properly connected and is *Ready* to use. The Experiment Manager displays *untitled.ncf* as a new experiment file, which contains one specimen with the default name of *Specimen 1*. The *Specimen 1* contains one sample with the default name of *Sample 1*. Click the menu *File* → *Save* or  icon to save the file to the specified folder. When you save, the NovoExpress™ software will name the file according to the date and time when the file is created, with a default format of *yymmdd\_hhmm.ncf*. For example, a *131208\_1035.ncf* file

indicates that the file is generated at 10:35 on December 8, 2013. Users can change the file name and the directory when saving the experiment file. In this example, an experiment file with the name of *TBNK.ncf* will be created.

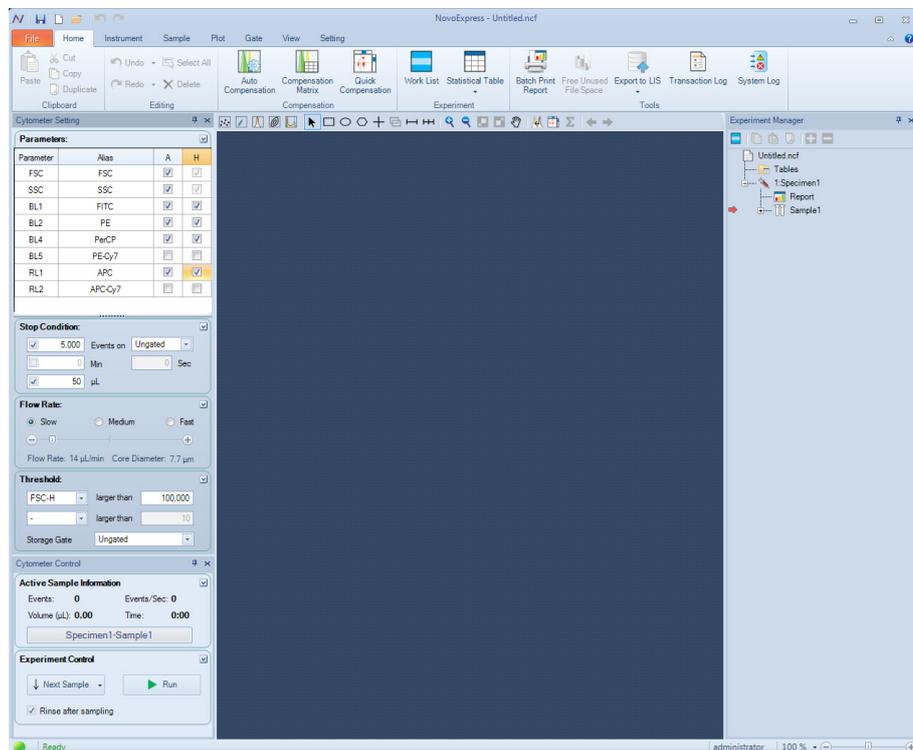


Figure 3-1 NovoExpress™ software interface



A hierarchic tree structure is used in the Experiment Manager for effective organization of the experimental data. You can use **Group**, **Specimen**, and **Sample** to organize your experiments and name them in a systematic way to improve the efficiency and traceability of experimental results. For more information, please refer to the *NovoExpress™ Software Guide*.

Firstly, rename *Specimen 1* as *TBNK* by right-clicking on *Specimen 1*. On the drop-down menu, click *Rename* and type the new name (*TBNK*). In this example, two samples prepared and stained by two reagent kits need to be created under the *TBNK* specimen. To do so, right-click the *TBNK* specimen and select *New Sample* from the drop-down menu. A new sample *Sample 2* will appear. Rename *Sample 1* to *CD3\_CD8\_CD45\_CD4*, and rename *Sample 2* to *CD3\_CD16+56\_CD45\_CD19*. The method of renaming samples is the same as renaming the specimen. Alternatively, you can also rename the sample from the *Active Sample Information* in the *Cytometer Control* window.

Click *File* → *Save* or  icon to save the file in the desired directory. Name the experiment file as *TBNK.ncf*.

After the above operation, the Experiment Manager will appear as shown in Figure 3-2.

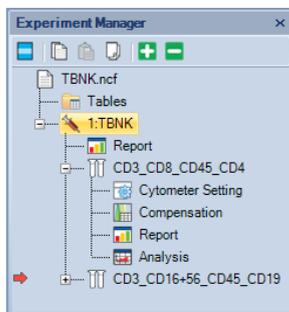
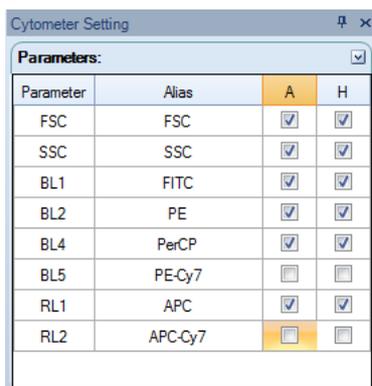


Figure 3-2 Experiment Manager

-  The red arrow on the left of a sample in the Experiment Manager indicates this sample is the **Active Sample**. A sample can be designated as the **Active Sample** by double-clicking the sample in the Experiment Manager.
-  Please refer to the *NovoExpress™ Software Guide* for details about creating Group, Specimen, and Sample.

## 3.2 Define Sample Acquisition Parameters

- 1 Double-click the `CD3_CD8_CD45_CD4` sample to make it the *Active Sample*.
- 2 Set the test parameters in the *Cytometer Setting* → *Parameters* panel (see Figure 3-3). By default, the Height parameters of FSC and SSC signals are always selected. Either Area or Height or both can be selected for fluorescent channels to be used in the experiment. In this example, select FSC, SSC, BL1, BL2, BL4, RL1 Height and Area as detection parameters.
- 3 Click the Alias area in the corresponding fluorescent channels to name the detection channel with the name of the fluorophores. In this example, rename BL1, BL2, BL4, and RL1 to be CD3 FITC, CD8 PE, CD45 PerCP, and CD4 APC, respectively.



Parameter	Alias	A	H
FSC	FSC	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
SSC	SSC	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
BL1	FITC	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
BL2	PE	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
BL4	PerCP	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
BL5	PE-Cy7	<input type="checkbox"/>	<input type="checkbox"/>
RL1	APC	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
RL2	APC-Cy7	<input type="checkbox"/>	<input type="checkbox"/>

Figure 3-3 Define Sample Acquisition Parameters

-  Parameters that are not selected in the **Parameters** setting panel will not be collected in the data, and therefore will not be available for data analysis. However, selecting too many parameters other than those really would be used in the experiment will increase the size of the experiment file, causing decreased data processing speed. Before doing an experiment, the parameters should be set appropriately, in order to avoid incomplete data collection or slow software data processing speed.
-  After the system has started collecting samples, only the **Alias** of the parameters can be edited. Selection or de-selection of parameters is not allowed.
-  The set of parameters available in the **Parameters** panel is dependent on the model of NovoCyte™ flow Cytometer. The software will automatically identify the instrument configuration and detect the available channels to be shown in *Cytometer Setting* → *Parameters* panel.
-  De-selection of parameters that are not used in the experiment will help to reduce the size of the experiment file, and thus improve the data analysis speed.

### 3.3 Set Sample Acquisition Conditions

#### 3.3.1 Stop Condition

The criteria to stop sample acquisition can be set in *Cytometer Setting* → *Stop Condition* panel (Figure 3-4), based on either the number of events to be collected (all events or within a specified gate), a time limit, or a sample volume limit. Multiple stop conditions can be selected as well, when any one of these conditions are met, the instrument will stop collecting samples. The number of events to be collected may be modified during the sample collection process. However, the time or volume stop conditions cannot be modified.

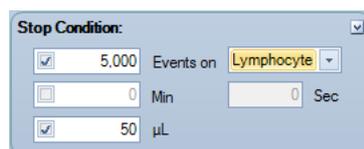


Figure 3-4 Set Stop Condition



For a new experiment, it is difficult to determine when to stop collecting. In this case, first try a small volume as the stop condition (for example, 10  $\mu\text{L}$ ) for a pre-run. The results of the pre-run could serve as a guide to set the stop conditions for the real experiment. In the examples in this guide, 5,000 events within the **Lymphocyte** gate and 50  $\mu\text{L}$  will be used as the stop condition. Details on how to set the **Lymphocyte** gate is described in **Data Analysis** in this chapter.

#### 3.3.2 Flow Rate

Sample flow rate can be set in the *Cytometer Setting* → *Flow Rate* panel. There are three preset flow rates, Slow, Medium, and Fast, corresponding to sample flow rates of 14  $\mu\text{L}/\text{min}$ , 35  $\mu\text{L}/\text{min}$  and 66  $\mu\text{L}/\text{min}$ , respectively. You can also manually set a desired flow rate within the range of 5  $\mu\text{L}/\text{min}$  to 120  $\mu\text{L}/\text{min}$  by adjusting the sliding bar. Different sample flow rates correspond to different core diameters of hydrodynamic focusing. Choosing a proper flow rate is critical to the quality of the data, and a few factors should be considered, such as the size of the cells, the resolution to be achieved, the concentration of the sample, the throughput, etc. In general, it is advisable to choose a core diameter that is similar or slightly larger than the size of the cells. The *Slow* flow rate is always recommended for an experiment that requires high resolution, for example, DNA content analysis. For experiments where the resolution is not so critical, *Medium*, or even *Fast* flow rate can be used in order to increase the throughput, especially when the concentration of the sample is relatively low. In the example in this guide, the *Slow* flow rate will be used.

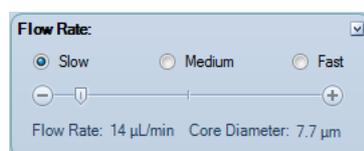


Figure 3-5 Set the Sample Flow Rate

#### 3.3.3 Threshold

Threshold can be set in the *Cytometer Settings* → *Threshold* panel (Figure 3-6). The threshold sets a limit so that only the data values are higher than this limit will be processed and recorded. Appropriate threshold settings improve the efficiency for collection of target particles while excluding debris and background noise, or other particles that are not rel-

evant to the experiment. Two thresholds can be set at the same time. The primary threshold is based on FSC-H (Height of FSC signal) or SSC-H (Height of SSC signal), while the second threshold can be based on any one of the parameters set under the *Cytometer Settings* → *Parameters* panel. When two threshold conditions are set, data will be collected only from particles that are above both thresholds.

The Storage Gate is used to filter out events outside the gate. All events exceed the primary and secondary threshold will be recorded when Storage Gate is set to Ungated. In the example in this guide, the primary threshold is set to FSC-H greater than 100,000, the second threshold is left blank and the Storage Gate is set to Ungated.

Figure 3-6 Set the Threshold

- 

*During sample collection, the threshold cannot be changed. Please set threshold properly before start of the experiment.*
- 

*For a new experiment, it might be difficult to determine how to set the threshold. In this case, conduct a pre-run with a relatively small threshold to determine where the cell population of interest is in the FSC-H/SSC-H plot. The pre-run results should serve as a good guide for setting the proper threshold. In the example in this guide, the distribution of the leukocytes (lymphocytes, monocytes, granulocytes) relative to the lysed red blood cells and debris should be observed. Choose a FSC-H threshold value that would discard the majority of the lysed red blood cells and debris, but does not affect any of the leukocytes. Set this threshold for the real experiment.*
- 

*For new experiments, a pre-run helps to optimize the cytometer settings and determine the most appropriate experimental conditions, including the Stop Condition, Flow Rate, and Threshold.*
- 

*In the example in this guide, setting the FSC-H threshold as 100,000 ensures the correct collection of the leukocyte signals, and effectively discards the most of the impurities. Refer to Table 3-1 as a starting point for setting the threshold for different biological samples. Table 3-2 serves as a reference to determine which threshold value to start from based on microsphere size. Some biological samples may contain a significant amount of impurities or debris. In this case, setting a second threshold with a fluorescent signal helps to improve the quality of the collected data.*

Cell Types	FSC-H Threshold	Remark
Cell lines, larger than 20 $\mu\text{m}$ in cell diameter	300,000~1,000,000	Use linear or logarithmic scale for FSC and SSC
Cells lines, smaller than 20 $\mu\text{m}$ in cell diameter	100,000~300,000	Use linear or logarithmic scale for FSC and SSC
Fixed or un-fixed, freshly isolated cells (leukocytes, spleen cells, thymocytes)	50,000~200,000	Use linear or logarithmic scale for FSC and SSC
Platelets	5,000~10,000	Use logarithmic scale for FSC and SSC



Cell Types	FSC-H Threshold	Remark
Bacteria	1,000~10,000	Use logarithmic scale for FSC and SSC. Recommend to use SSC-H > 5,000 as the second threshold.

Table 3-1 Recommended Threshold for Different Cell Types

Standard Microsphere Diameter	Threshold
0.2 $\mu\text{m}$	SSC-H 1,000
0.5 $\mu\text{m}$	FSC-H 500
2 $\mu\text{m}$	FSC-H 50,000
10 $\mu\text{m}$	FSC-H 500,000
15 $\mu\text{m}$	FSC-H 1,000,000
30 $\mu\text{m}$	FSC-H 2,000,000

Table 3-2 Reference Threshold for Different Size of Standard Microspheres

### 3.4 Run Sample

There are two samples for the example in this guide. Double-click each sample to make it to be the Active Sample (red arrow shown on the left of the sample). Define the collection parameters and set the acquisition conditions for the two samples as shown in Figure 3-7.

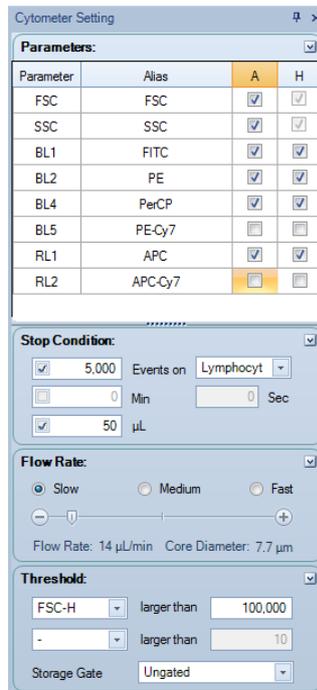


Figure 3-7 Instrument Settings Panel

After setting the acquisition parameters and acquisition conditions, double-click the

CD3\_CD8\_CD45\_CD4 sample and set it as the Active Sample in the Experiment Manager. A red arrow will appear to the left of the sample, indicating it is the Active Sample. At the same time, the sample name in *Cytometer Control* → *Active Sample Information* panel becomes CD3\_CD8\_CD45\_CD4. To begin collecting this sample, place the CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC sample tube into the sample holder on the NovoCyt<sup>™</sup> flow cytometer. Then click the *Run* button in the *Cytometer Control* → *Experiment Control* panel to start sample acquisition (Figure 3-8). At this time, the sample injection probe will move downward to aspirate the sample. After a short period of time for stabilizing the fluidic system, sample acquisition and data collection will start.

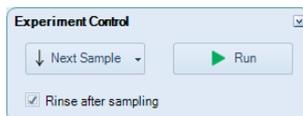


Figure 3-8 Experiment Control Panel

**!** The sample injection probe may perform multiple aspirations for one sampling. Do not remove or change the test tube until the entire sampling process has completed.

During the acquisition, the *Cytometer Control* → *Active Sample Information* panel will show the progress in real-time, as shown in Figure 3-9.



Figure 3-9 Active Sample Information

Once the stop condition is reached, sample acquisition will automatically stop.

**🔍** You can also manually stop the sample acquisition by clicking the **Stop** button in the *Cytometer Control* → *Experiment Control* panel, during the sample acquisition process.

After sample acquisition is completed, the NovoCyt<sup>™</sup> flow cytometer automatically executes the cleaning process for the fluidic system and the sample injection probe. This helps to reduce cross-contamination between different samples. When the cleaning process is completed, repeat the above procedure for sample CD3\_CD16+56\_CD45\_CD19.

**🔍** If you want to increase the sampling speed, you can turn off the automatic cleaning procedure after sampling. To do so, uncheck **Rinse after Sampling** box in the *Cytometer Control* panel from the NovoExpress<sup>™</sup> software as shown in Figure 3-8.

**🔍** Using templates helps to quickly copy the experiment settings or data analysis method of one sample and apply them to the other samples. For detailed information about the template function, please refer to the *NovoExpress<sup>™</sup> Software Guide*. For example, you can quickly copy the CD3\_CD8\_CD45\_CD4 experiment settings to sample CD3\_CD16 +56\_CD45\_CD19 by dragging the *Cytometer Setting* under the CD3\_CD8\_CD45\_CD4 sample node and dropping it to sample CD3\_CD16 +56\_CD45\_CD19 to apply the settings.

**!** During sampling, do not obstruct the moving SIP. Do not place your hand on the sample tube.

**!** Before collecting samples, make sure the tube cap is removed from the sample tube. Failure to do so may cause severe damage of the sample injection probe.

**⚠️** Biological samples may be biohazardous. Appropriate personal protective equipment

should be worn when handling the samples, in accordance with local safety regulations.

## 3.5 Perform Fluorescence Compensation

Fluorescent molecules have their own spectral emission ranges, and it is possible that the emission spectra of two fluorescent molecules are overlapped in a certain wavelength range. This phenomenon is called Spectral Overlap, and will cause one detector designated for detection of one fluorophore to also detect signals from another fluorophore. Fluorescence compensation refers to the process of correcting such spectral overlap, i.e. removing the interference of other fluorescent signals from the desired signals. The NovoExpress™ software offers three fluorescence compensation methods. The user can select the appropriate method according to their needs, which makes fluorescence compensation flexible and convenient.

### 3.5.1 Automatic Fluorescence Compensation

Automatic compensation will calculate the fluorescence compensation matrix by the NovoExpress™ software automatically based on the results of an unstained control sample and a series of single-stained samples. The automatically generated compensation matrix can be applied to the multi-color stained samples to achieve correct fluorescence compensation.

The procedure for automatic compensation is described in the following steps:

- ▶ Prepare samples for fluorescence compensation
- ▶ Set up automatic fluorescence compensation parameters
- ▶ Run samples for automatic fluorescence compensation
- ▶ Automatic calculation of compensation matrix
- ▶ Apply compensation matrix to experimental samples

#### Prepare samples for fluorescence compensation

Prepare a set of single-stained samples (labeled with only one fluorophore for each sample). Single-stained sample can be generated from the same biological sample as the experimental sample, or compensation beads may be used. Compensation beads are microspheres with capturing agent on the surface. The capturing agent on the surface of the compensation beads can react with fluorescent-labeled antibodies used in the experiment. Each single-stained sample should have a negative and a positive population. If there is no negative population in the single-stained sample, an unstained sample has to be prepared for correct automatic compensation.

#### Set up Automatic Fluorescence Compensation Parameters

Click *Home* → *Auto Compensation* icon  or right-click the experiment file name in the Experiment Manager and select *New Auto Compensation ...* (Figure 3-10). The *New Auto Compensation* window will appear (Figure 3-11). In this window, you can set the method and parameters for automatic compensation. Calculation of the compensation matrix can be set based on either the Height or the Area parameter, and based on comparison of the Mean or Median of the positive and negative populations. Select *Compensation Channels* based on the prepared *Unstained* sample and the single-stained samples accordingly.

For the example described in this guide, single-stained compensation beads with FITC,

PE, PerCP and APC will be used for automatic compensation. In *Compensation Channel*, select *FITC*, *PE*, *PerCP*, and *APC*. Select *Compensation on Height*, and select *Median* as *Parameter for Calculation* (Figure 3-11). Click the *OK* button and a new *Compensation Specimen* will appear in the *Experiment Manager* under the experiment file node. This *Compensation Specimen* contains a series of single-stained samples as selected in the *Compensation Channels*.

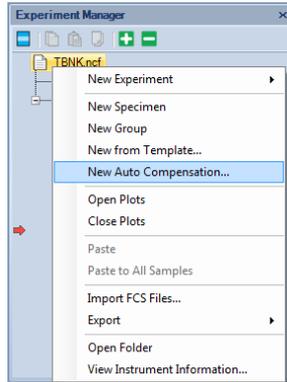


Figure 3-10 New Auto Compensation Menu

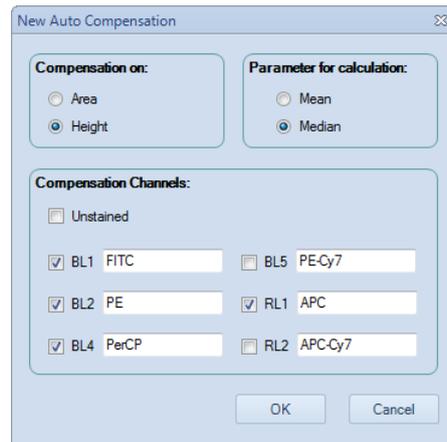


Figure 3-11 New Auto Compensation window

- 
 If you select the *Unstained* sample in the *Compensation Channel*, calculation of the compensation matrix will be based on the unstained sample, instead of the negative population in the single-stained sample.
- 
 The *Compensation Channels* shown in the *New Auto Compensation* window will be all the available channels based on the model of the NovoCyte™ flow cytometer. The NovoExpress™ software automatically obtains such available channels information from the hardware. Correctly select the *Compensation Channels* to ensure the correct automatic compensation results. You can also change the settings by right-clicking the *Compensation Specimen* in the *Experiment Manager* (Figure 3-12) and select *Auto Compensation Setup ...*

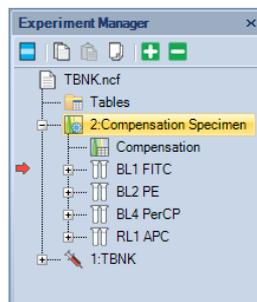


Figure 3-12 Compensation Specimen and Related Single-Stained Samples

## Run Samples for Automatic Fluorescence Compensation

The default setting for acquiring automatic compensation samples are:

- ▶ Parameters: both Height and Area for all the available parameters
- ▶ Stop condition: totally 10,000 events or 20 $\mu$ L sample volume.
- ▶ Sample flow rate: Slow
- ▶ Threshold: FSC-H larger than 100,000.

 In order to ensure normal data acquisition and correct automatic fluorescence compensation, it is recommend to prepare the compensation sample concentration to be  $1 \times 10^6 \sim 5 \times 10^6$  /mL.

 Most samples can be properly collected with the default settings. However, users can also change the settings accordingly.

To collect the data, first double-click the sample to be collected in the Experiment Manager to make it the Active Sample. Then place the corresponding sample tube into the sample holder and click *Run* button. Repeat the procedure until all the compensation samples are collected.

After all the samples are collected, the NovoExpress™ software will automatically generate a FSC/SSC density plot and a histogram for each sample. In the FSC-H/SSC-H density plot, a gate named *Main* will be automatically created for the main compensation beads or cell populations and be applied to the histogram of the fluorescence channel corresponding to each single-stained sample. NovoExpress™ will also set a Bi-Range gate for the histogram to separate the negative (*Negative* gate) and positive (*Positive* gate) populations, as shown in Figure 3-13.

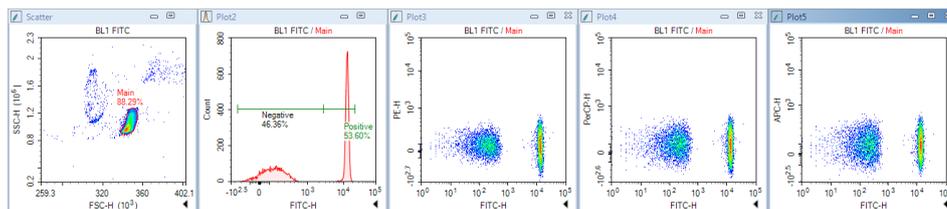


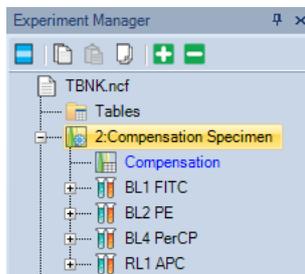
Figure 3-13 Data Analysis for FITC Single-Stained Sample in Auto Compensation

Under normal circumstances, the automatically generated gates are appropriate. However, due to the complexity of the sample, in some cases, the automatic software gating algorithm may not be able to generate the optimal gates. Check the *Main*, *Negative*, and *Positive* gates to see if they are properly set. When necessary, adjust the gates manually to optimize the results.

 If the single-stained sample does not have a negative population, be sure to always include an **Unstained** sample for automatic compensation. Otherwise, the NovoExpress™ software cannot conduct automatic compensation and an error message will be generated.

## Automatic Calculation of Compensation Matrix

The NovoExpress™ software will automatically calculate the compensation matrix based on the signals (Mean or Median) of the negative and positive populations. The font color of *Compensation* under the *Compensation Specimen* node in the Experiment Manager turns blue to indicate the compensation matrix has non-zero values (Figure 3-14). You can then view the Compensation Matrix (Figure 3-15) and Spillover Matrix (Figure 3-16) by double-clicking *Compensation* under the *Compensation Specimen* node in the Experiment Manager.

Figure 3-14 Double-click *Compensation* to view the Compensation Matrix

Compensation Matrix		Spillover Matrix			
Source\Target	FITC	PE	PerCP	APC	
FITC	-100.3048	9.4287	-0.812	0.1475	
PE	3.2388	-100.3128	32.3652	-5.7529	
PerCP	0.0159	0.0244	-100.3089	17.8704	
APC	-0.0003	-0.0004	1.6876	-100.3007	

Figure 3-15 Compensation Matrix Generated by Auto Compensation

Compensation Matrix		Spillover Matrix			
Source\Target	FITC	PE	PerCP	APC	
FITC	100	9.3998	2.2235	0.0041	
PE	3.2341	100	32.2396	0.0132	
PerCP	0.0167	0.0258	100	17.8154	
APC	0	0	1.6824	100	

Figure 3-16 Spillover Matrix Generated by Auto Compensation

### Apply Compensation Matrix to Experimental Samples

There are two ways to apply the automatically generated compensation matrix to the experimental samples. The first method is to *copy* and *paste* the compensation matrix. Right-click *Compensation* under the *Compensation Specimen* node in the Experiment Manager and select *Copy*, as shown in Figure 3-17. Then right-click the experimental sample in the Experiment Manager and select *Paste*. The second method is to treat the compensation matrix as a template. Select *Compensation* under the *Compensation Specimen* node, drag and drop it to the experimental sample to which you want to apply the compensation matrix. For detailed information about the template function, please refer to the *NovoExpress™ Software Guide*.

 You can also use the same method to apply the compensation matrix to a specimen. In this case, the same compensation matrix will be applied to all samples belonging to the specimen.

Once the compensation matrix is applied to the experimental sample, double-check the data after compensation and confirm the correctness of the compensation.

 The compensation matrix generated by automatic compensation cannot be modified.

However, after it is applied to the experimental samples, you can adjust the compensation matrix or spillover matrix if needed.

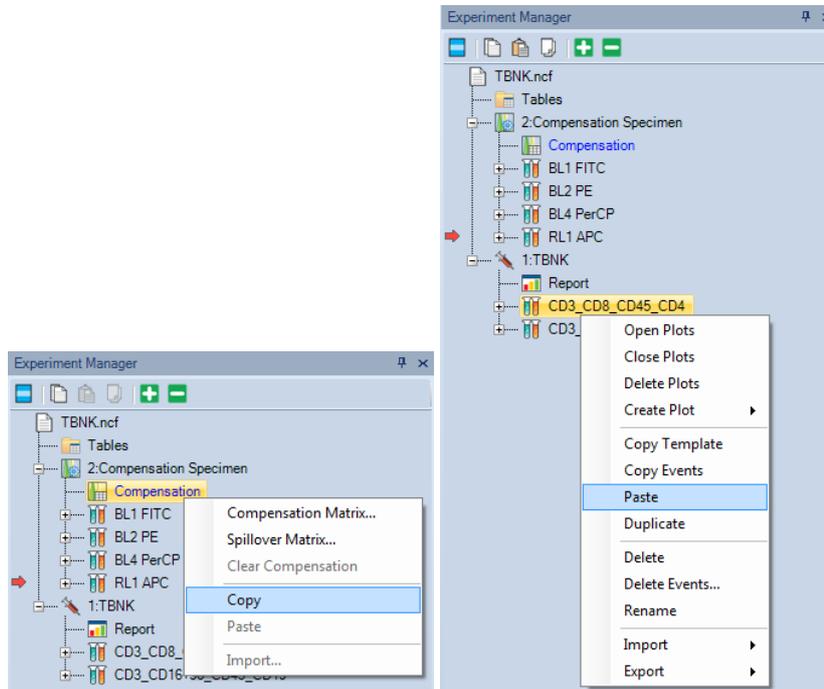


Figure 3-17 Copy/Paste the Compensation Matrix

### 3.5.2 Manual Fluorescence Compensation

In addition to automatic fluorescence compensation, users can manually adjust the spillover matrix for fluorescence compensation. Similarly, a series of unstained and single-stained samples are needed.

Manual compensation could be done using the following procedure:

- ▶ Prepare samples for compensation
- ▶ Run compensation samples
- ▶ Edit the compensation matrix
- ▶ Apply the compensation matrix

#### Prepare Samples for Compensation

Use the same method to prepare the unstained and single-stained samples as described for automatic compensation.

#### Run Compensation Samples

Use the method described in the previous sections to collect the data for all the compensation samples.

#### Edit Compensation Matrix

Create a FSC/SSC density plot and set a gate for the main population on the plot. Then create a density plot with the fluorescence channel of the fluorophore used to stain the sample as the X-axis parameter and each of the other fluorescence channels as the Y-axis parameter. Apply the main population gate to all the fluorescence channel density plots.

For example, in the example in this guide, for the FITC single-stained compensation beads, a gate named *beads* is created for the main beads population on the FSC/SSC plot. Create a FITC/PE, a FITC/PerCP, and a FITC/APC density plot and apply the *beads* gate to all the plots. For the FITC/PE density plot, adjust the spillover matrix coefficient to subtract the PE signal from the FITC signal (i.e. PE - % FITC) to compensate for the FITC spillover into the PE channel. The criterion for correct compensation is to make the Mean or Median value of the FITC positive population and negative population to match each other as closely as possible on the PE channel. The same method and principle apply to compensation of FITC spillover into the PerCP channel on the FITC/PerCP plot and FITC spillover into the APC channel on the FITC/APC plot. Similarly, for the PE, PerCP, and APC single-stained samples, follow the same procedure to subtract the corresponding fluorescence spillover into the other detection channels.

There are three methods to edit the spillover matrix coefficient. One is by clicking the *Quick Compensation* icon  and moving the slide bar on the corresponding fluorescence channel density plot directly (Figure 3-18). Another method is by clicking *Home* → *Compensation Matrix* to load the compensation editing window. The third method is by right-clicking the *Compensation* under the corresponding sample node in the Experiment Manager and select *Spillover Matrix ...* to load the compensation editing window.

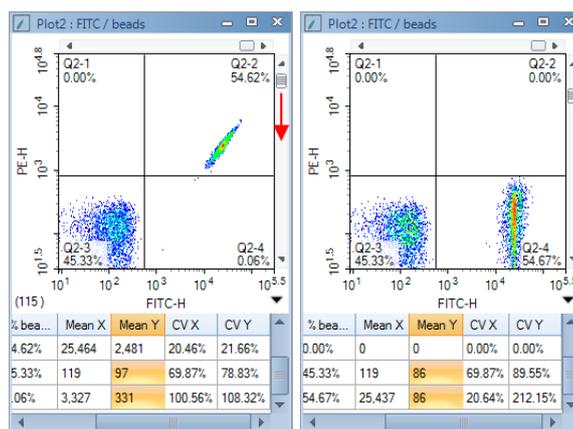


Figure 3-18 Quick Compensation

A quadrant gate helps to judge if the correct compensation has been achieved. For example, in FITC/PE density plot, draw a quadrant plot to divide the plot into four segments. Include the FITC negative population in the bottom-left segment and the FITC positive population in the bottom-right segment. Use the quick compensation sliding bar, or adjust the coefficient in the spillover matrix to conduct the compensation. The correct compensation is achieved when the Mean or Median of the PE signal for the FITC positive population and FITC negative population is the same or is very similar. The coefficient in the spillover matrix directly indicates the percentage needed for the compensation (i.e. PE - % FITC). Click the corresponding coefficient in the spillover matrix, the information will be shown in the bottom-left of the spillover matrix indicating subtraction of a certain percentage of one fluorescence signal (fluorophore in the corresponding row) from the other fluorescence signal (fluorophore in the corresponding column), as shown in Figure 3-19. In the same way, compensate the FITC spillover into the PerCP and APC channels on the FITC/PerCP and FITC/APC density plots. Compensation for FITC signal is then completed and results are shown in Figure 3-20. The spillover matrix for FITC single-stained sample is shown in Figure 3-19.

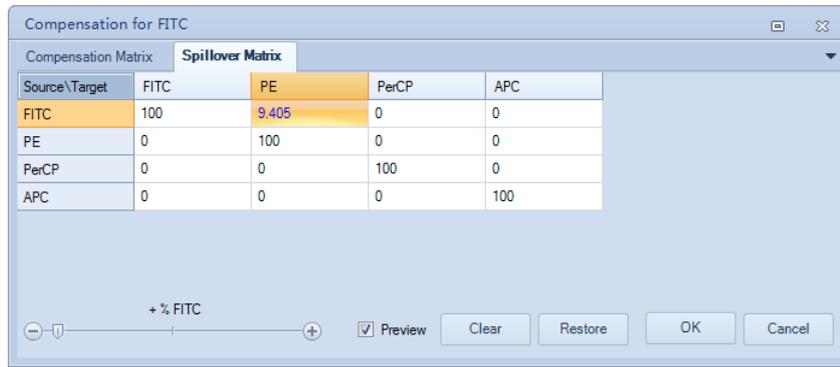


Figure 3-19 Compensate FITC Spillover into PE Channel in Spillover Matrix

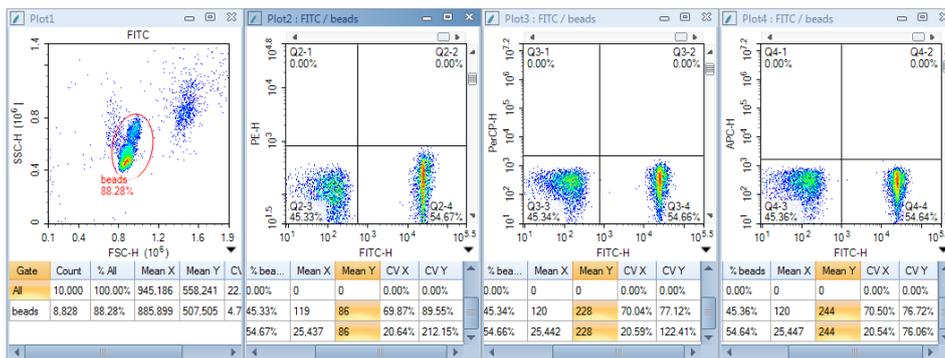


Figure 3-20 Compensate FITC Spillover into PE, PerCP, and APC Channels

Select **Preview** in the compensation window to visualize the compensation results dynamically when adjusting the spillover matrix coefficient. Click the **Expand Statistics** icon in the bottom-right corner of the plot to show the statistical calculation results. Look at the corresponding Mean or Median value to judge if the correct compensation has been achieved.

Repeat the same procedure for the PE, PerCP, and APC single-stained samples and the corresponding spillover matrixes are shown in Figure 3-21, 3-22, and 3-23, respectively.

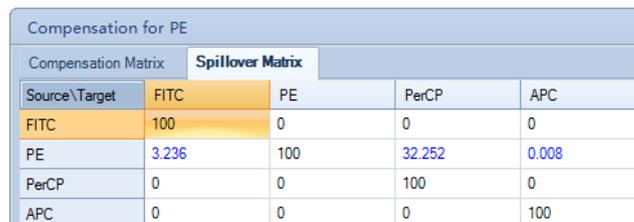


Figure 3-21 Spillover Matrix for PE Single-Stained Sample

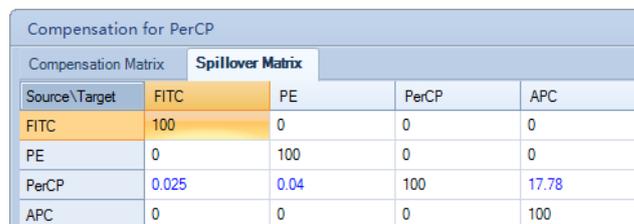


Figure 3-22 Spillover Matrix for PerCP Single-Stained Sample



Compensation for APC				
Compensation Matrix		Spillover Matrix		
Source\Target	FITC	PE	PerCP	APC
FITC	100	0	0	0
PE	0	100	0	0
PerCP	0	0	100	0
APC	0	0	1.694	100

Figure 3-23 Spillover Matrix for APC Single-Stained Sample

### Apply the Compensation Matrix

To use the compensation coefficient generated by the single-stained samples, manually input the corresponding coefficient from each single-stained sample into the compensation matrix of the experimental sample (Figure 3-24). Check to make sure the compensation has been applied to the experimental sample and confirm the correctness of the compensation. This compensation matrix can then be used as a template and may be applied to the other experimental samples. Figure 3-25 and Figure 3-26 shows the results of the CD3\_CD8\_CD45\_CD4 sample before and after the compensation, respectively.

Compensation for Sample1				
Compensation Matrix		Spillover Matrix		
Source\Target	FITC	PE	PerCP	APC
FITC	100	9.405	0	0
PE	3.236	100	32.252	0.008
PerCP	0.025	0.04	100	17.78
APC	0	0	1.694	100

Figure 3-24 Incorporate the Compensation Coefficients from Single-Stained Samples into the Spillover Matrix

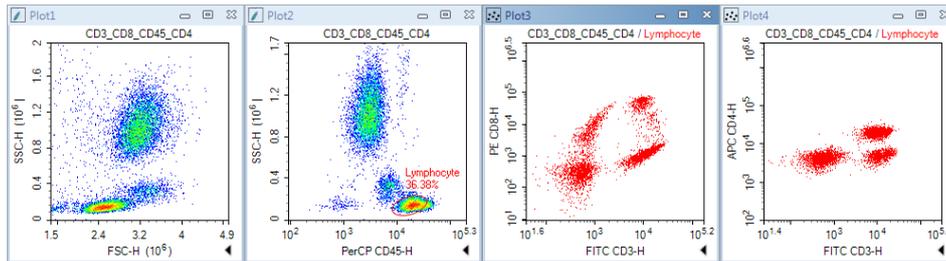


Figure 3-25 Results of CD3\_CD8\_CD45\_CD4 Sample: Before Compensation

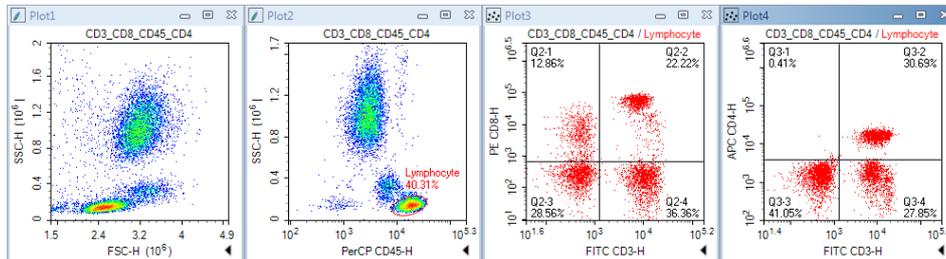


Figure 3-26 Results of CD3\_CD8\_CD45\_CD4 Sample: After Compensation

- Please refer to *NovoExpress™ Software Guide* for details about the compensation functions.
- The fluorescence signal may become negative after compensation, which is problematic to display using a logarithmic scale. To address this issue, the *NovoExpress™* software provides a **Bi-Exponential Transform** for data scaling. Right-click the axes on the density plot and select **Biexponential** as the plotting scale. Please refer to the *NovoExpress™ Software Guide* for details about the biexponential transform.

 The biexponential scale will take more computer resources for plotting and thus may lead to slower refreshing speed for the displayed data, especially when the number of the events is large. When this occurs, using the logarithmic scale may help to improve the data refreshing speed during the compensation process. After the compensation adjustment is done, switch back to biexponential scale to have a better visualization of the data.

### 3.6 Analyze Data

The NovoExpress™ software provides a variety of plots and gates for flow cytometry data analysis. The type of plots includes dot plot , density plot , histogram , contour plot , and cell cycle analysis plot . Gates include rectangular gate , elliptical gate , polygon gate , quadrant gate , logic gate , range gate , and double-range gate . By clicking the icon in the NovoExpress™ software data analysis toolbar (Figure 3-27), you can create the corresponding plot and gate.

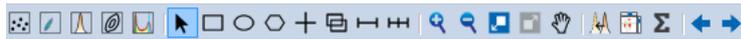


Figure 3-27 NovoExpress™ Software Data Analysis Toolbar

 Please refer to *NovoExpress™ Software Guide* for detailed information about plots and gates.

Data analysis can be conducted during or after the sample acquisition. In this guide, commonly used data analysis methods and procedures will be described using the CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC stained human peripheral blood sample as an example. The general methods apply to the other data analysis as well.

#### ► Set Sample as Active Sample

In the Experiment Manager, double-click the sample to be analyzed to make the sample the Active Sample. A red arrow at the left of the sample indicates this sample is the Active Sample.

 Data analysis can be conducted when the sample acquisition is in process. In this case, a green flashing arrow will show at the left of the sample currently acquiring data. For example, in Figure 3-28, sample **CD3\_CD8\_CD45\_CD4** is the Active Sample for data analysis, while sample **CD3\_CD16+56\_CD45\_CD19** is currently acquiring data.

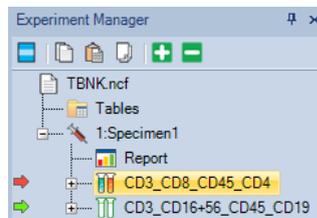


Figure 3-28 Active Sample in Data Analysis (Red Arrow) and Sample in Data Acquisition (Green Arrow)

#### ► Create Plots and Gates

Firstly, we need to look at the CD45 PerCP vs. SSC plot to locate the target cell population, i.e. the lymphocytes, which have relatively large PerCP signal but small SSC signal. In order to create the CD45 PerCP vs. SSC plot, click the dot plot icon  or the density plot icon  from the data analysis toolbar. The sample name will show at the top of the plot. The default parameters for a new dot plot or a new density plot is always FSC Height (FSC-H) as the X-axis parameter and SSC Height (SSC-H) as the Y-axis parameter. In this

example, the X-axis needs to be changed to be *CD45 PerCP-H*. To do so, right-click the *FSC-H* on the plot and select *Height* → *CD45 PerCP-H* from the drop-down list. The X-axis parameter will change to *CD45 PercP-H* accordingly (Figure 3-29).

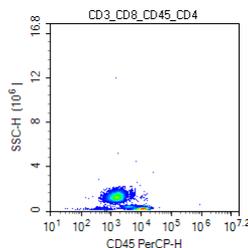


Figure 3-29 CD45 PerCP-H/SSC-H Density Plot

The NovoCyt™ flow cytometer has a very large dynamic range (0 to  $2^{24}=16,777,216$ ), so the data will often only occupy a small area of the plot when using the full-scale axes. Zoom-in to enlarge the data area of interest. To zoom in, click the zoom-in icon  from the data analysis toolbar, or right-click on the selected plot and select *Zoom In* from the drop-down list. Move the mouse across a rectangular area to zoom in the population of interest. Upon release of the mouse button, the range of the plot is automatically enlarged according to the zoom-in operation. You can also *Undo* the *zoom in* operation by clicking the undo icon  or click the zoom-out icon  from the tool bar. Repeat the above steps until the data of interest is properly shown on the plot (Figure 3-30).

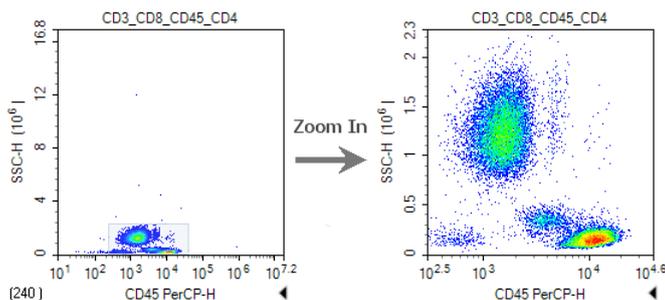


Figure 3-30 Zooming In the Density Plot

#### ► Create a Gate for Lymphocytes

Click the elliptical gate icon , draw an elliptical gate to include the lymphocyte population (large PerCP signal and small SSC signal) on the CD45 PerCP-H/SSC-H density plot. The default name of the gate is E1 (*E* indicates an elliptical gate). Click the E1 gate label, and use the keyboard to change the name of the gate to *Lymphocyte*.

#### ► Create a CD3 FITC-H/CD8 PE-H and a CD3 FITC-H/CD4 APC-H dot plot

Click the dot plot icon  twice to create two new dot plots. Change the X-axis parameter to be CD3 FITC-H and the Y-axis to be CD8 PE-H for the first dot plot. Change the X-axis parameter to be CD3 FITC-H and Y-axis to be CD4 APC-H for the second dot plot. Right-click the name of both plots and select *Lymphocyte* from the list to apply the *Lymphocyte* gate (created in CD45 PerCP-H/SSC-H density plot) to both plots. Observe the change after application of the gate.

#### ► Compensate the Sample

Use the method described in [3.5 Perform Fluorescence Compensation](#) in this guide to apply the compensation matrix created by either automatic compensation or manual compensation to the sample. Figure 3-25 and 3-26 show the data for the *CD3\_CD8\_CD45\_CD4* sample and the *CD3\_CD16 +56\_CD45\_CD19* sample before and after compensation.

### ► Statistics

In the CD3 FITC-H/CD8 PE-H and CD3 FITC-H/CD4 APC-H dot plots, create a quadrant gate to isolate the positive and negative cell populations. Click the small triangle ◀ at the bottom-right of the plot to expand the statistical results of the data, as shown in Figure 3-31. Right-click at any place on the statistical table to select additional statistical parameters to be calculated for the data, as shown in Figure 3-32.

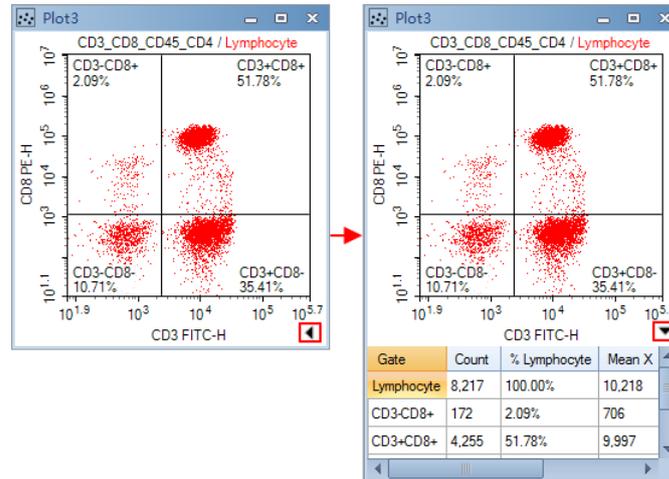


Figure 3-31 Statistics of the Data

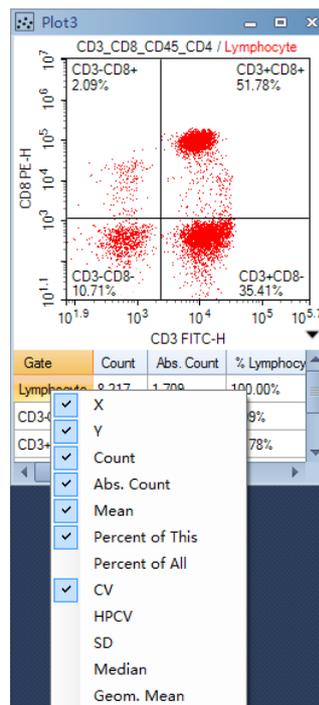


Figure 3-32 Select other Statistical Parameters



For details about statistics, please refer to the *NovoExpress™ Software Guide*.

### 3.7 Append Data

Additional data can be acquired for samples which already include data. Newly acquired data will be appended to the existing data for the sample.

- 1 Select the sample to be the Active Sample. Set the *Stop Condition* for the acquisition.
- 2 Load the sample into the sample holder.
- 3 Click *Run* button in the *Cytometer Control* panel. If the sample already contains data, a dialog box will pop up. Click the *Append* button to add more data to the sample. Click the *Rewrite* button to delete the existing data and re-collect the data.

### 3.8 Delete Data

You can delete all the data or specify a portion of the data to be deleted. Right-click the sample in the Experiment Manager and select *Delete Events*. A window will pop up as shown in Figure 3-33. Select *All* to delete all the events from the sample, or only delete events *Outside* or *Inside* a specified gate.

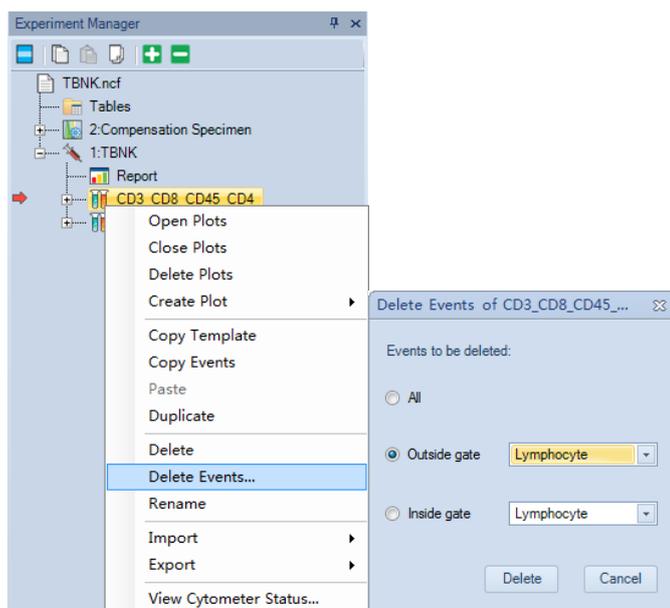


Figure 3-33 Delete Events

### 3.9 Save Data

The NovoExpress™ software automatically saves the data, along with the cytometer settings information, to the experiment file. However, data analysis, including all the plots and gates, fluorescence compensation, created Reports and Statistical Tables, etc., must be saved manually. To save, click *File* → *Save* from the menu or click the Save icon . Otherwise, when closing the NovoExpress™ software, a message will prompt to remind users about saving the data.

### 3.10 Import and Export Files

The .ncf data file includes all the acquired data, information about cytometer settings and data analysis, etc. The data can also be exported as an FCS 3.0 file or CSV file and be analyzed by a third party software.

- ▶ To export a file, right-click the specimen or sample name in the Experiment Manager and select *Export* → *Export FSC File ...* or *Export* → *Export CSV File ...*, as shown in Figure 3-34. In the prompt window, specify the folder to export the data.

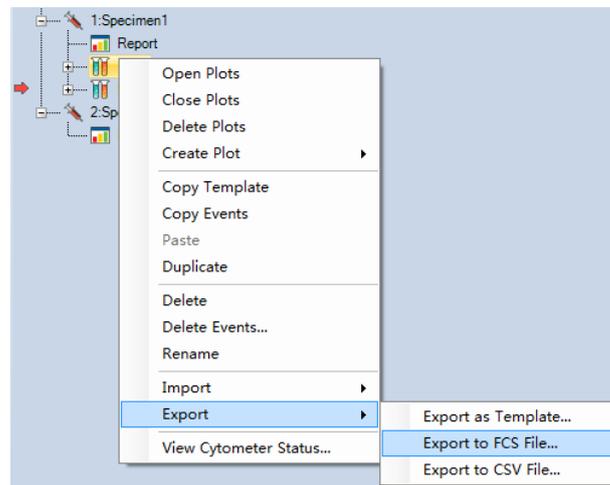


Figure 3-34 Export Data

- ▶ FCS 2.0 and FCS 3.0 format files may be imported into NovoExpress™ for viewing and analysis. Create a blank sample or specimen in the Experiment Manager, right-click and select *Import* → *Import FSC File ...*, as shown in Figure 3-35.

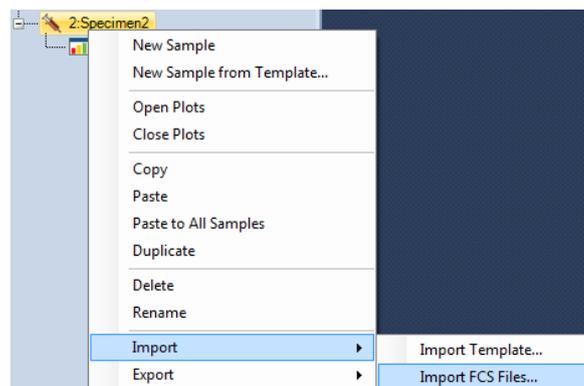


Figure 3-35 Import FCS File

 The NovoExpress™ software provides powerful data analysis functions, such as data analysis templates, batch report, statistical tables, cell cycle analysis, etc. For details about the data analysis function of NovoExpress™ software, please refer to the *NovoExpress™ Software Guide*.

## 4. System Maintenance

It is important to conduct instrument maintenance on a regular basis in order to achieve optimal performance for the instrument and obtain reliable data. ACEA NovoCyte™ incorporates automatic procedures for maintenances as much as possible, eliminating time-consuming manual maintenance. This chapter describes the procedures to execute or conduct the maintenance procedures.

### 4.1 Scheduled Maintenance

Table 4-1 lists the regular maintenance to be done on the NovoCyte™ instrument.

Maintenance	Recommended Maintenance Frequency
Daily Start-up	Daily. Automatic system cleaning during start-up.
Daily Shutdown	Daily. Automatic system cleaning and decontamination during shutdown.
Add instrument reagents (NovoFlow™ sheath fluid, NovoRinse™ solution, and NovoClean™ solution)	As needed. Check the instrument reagent volume in the containers before each experiment and add more reagents when necessary.
Empty waste	As needed. Check waste volume before each experiment and empty waste container when necessary.
Preventive Maintenance <ul style="list-style-type: none"> <li>▶ Clean the sample injection probe (SIP)</li> <li>▶ Clean the SIP wash apparatus</li> <li>▶ Conduct <i>Extensive Rinse</i></li> <li>▶ <i>Unclog</i> the flow cell</li> </ul>	Every month
Replace Fluidic System Consumables <ul style="list-style-type: none"> <li>▶ Replace the sheath fluid in-line filters</li> <li>▶ Replace the waste filter</li> <li>▶ Replace the peristaltic pump tubing</li> <li>▶ Replace the NovoFlow™ sheath fluid inlet filter</li> <li>▶ Replace the NovoRinse™ and NovoClean™ solution inlet filters</li> </ul>	When prompted by NovoExpress Software

**Table 4-1 Scheduled Maintenance and Recommended Frequency**

### 4.1.1 Preventative Maintenance

It is recommended to conduct preventative maintenance once every month to ensure the fluidic system is in good condition.

#### ► Clean the Sample Injection Probe (SIP)

##### Procedure

- 1 Make sure the NovoCyte™ is in *Ready* status;
- 2 Open the top cover of the instrument;
- 3 Soak lint free wipes with NovoClean™ solution (1X) and wipe the sample injection probe back and forth at least five times;
- 4 Soak lint free wipes with DI water and wipe the sample injection probe back and forth at least five times;
- 5 Click *Instrument* → *Fluidics Maintenance* → *Backflush* to flush the sample injection probe;
- 6 Click *Instrument* → *Fluidics Maintenance* → *Priming* to prime the fluidic system.

 *The sample injection probe directly contacts biological samples which may be biohazardous. Appropriate personal protective equipment should be worn when handling the sample injection probe, in accordance with local safety regulations.*

 *The sample injection probe is fragile and may be damaged if handled improperly. When cleaning the sample injection probe, please be careful to avoid deforming or bending it.*

#### ► Clean the SIP Wash Apparatus

##### Procedure

- 1 Make sure the NovoCyte™ is in *Ready* status;
- 2 Open the top cover of the instrument;
- 3 Soak lint free wipes with NovoClean™ solution (1X) and wipe the SIP wash apparatus back and forth at least five times;
- 4 Soak lint free wipes with DI water and wipe the SIP wash apparatus back and forth at least five times;
- 5 Click *Instrument* → *Fluidics Maintenance* → *Backflush* to flush the sample injection probe;
- 6 Click *Instrument* → *Fluidics Maintenance* → *Priming* to prime the fluidic system.

 *The SIP wash apparatus directly contacts biological samples which may be biohazardous. Appropriate personal protective equipment should be worn when handling the sample injection probe, in accordance with local safety regulations.*

 *When cleaning the SIP wash apparatus, handle carefully to avoid damaging it and the sample injection probe.*

### ► Extensive Rinse

When NovoCyte™ is in *Ready* status, click the *Instrument* → *Fluidics Maintenance* → *Extensive Rinse* from NovoExpress™ software and the Extensive Rinse procedure will automatically be executed. The progress of the procedure will be shown in NovoExpress™ status bar.

 For details about the **Extensive Rinse** function, please refer to [2.2.4 Extensive Rinse](#) section in this guide.

### ► Unclog the Flow Cell

When the NovoCyte™ is in *Ready* status, click *Instrument* → *Fluidics Maintenance* → *Unclog* from NovoExpress™ software and the Unclog procedure will automatically be executed. The progress of the procedure will be shown in NovoExpress™ status bar.

 For details about the **Unclog**, please refer to [2.2.6 Unclog](#) section in this guide.

## 4.1.2 Replace Fluidic System Consumables

The NovoCyte™ flow cytometer monitors the accumulated running time of the fluidic system consumables to ensure the consumables are changed in a timely manner for optimal flow cytometry results. When the accumulated running time is reached, the NovoExpress™ software will prompt a message to remind the user to replace the consumables (Figure 4-1).

Click *Replace* to continue. *Close* will cancel the process to allow continued use of the system, however the consumables should be replaced promptly.

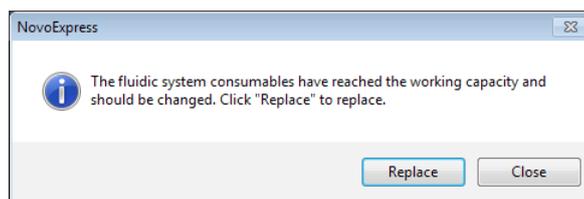


Figure 4-1 NovoExpress™ Software Message for Replacing Fluidic System Consumables

Click the *Replace* button and a prompt will appear (Figure 4-2). The replacement of the peristaltic pump tubing requires the input of the Tubing Calibration Number which is the last three digits of the tubing serial number affixed to the tubing (Figure 4-2). Enter the Tubing Calibration Number in the prompted window and click *Next* to continue

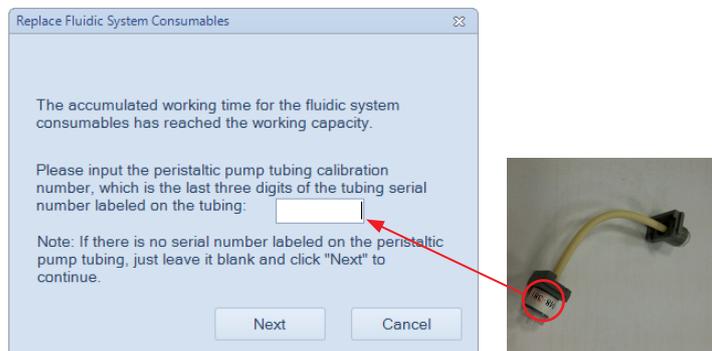


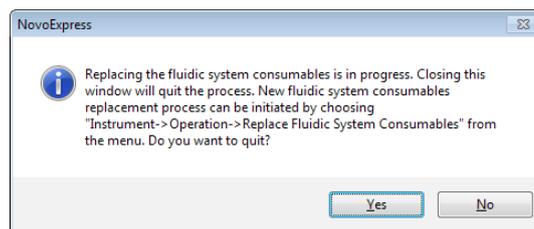
Figure 4-2 NovoExpress™ Software Prompt for Entering the Tubing Calibration Number

-  The fluidic system consumable replacement process can also be initiated at any time manually by clicking the **Instrument → Replace Fluidic System Consumables** icon in the NovoExpress™ software when the instrument is in Ready status.



-  Make sure the **Tubing Calibration Number** is input correctly. An incorrect Tubing Calibration Number will affect the performance of the system, and could lead to system malfunction.

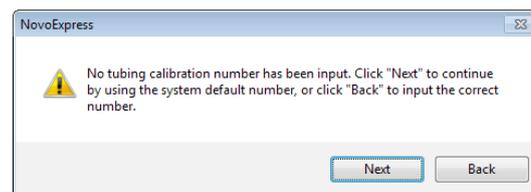
If **Cancel** is selected, the following prompt will appear (Figure 4-3) and the process can be aborted by clicking **Yes**.



**Figure 4-3 NovoExpress™ Software Message for Cancelling Fluidics System Consumable Replacement**

If no Tubing Calibration Number is found on the peristaltic pump tubing, click **Next** to continue and the following prompt will appear (Figure 4-4). The system will use a default value for the Tubing Calibration Number.

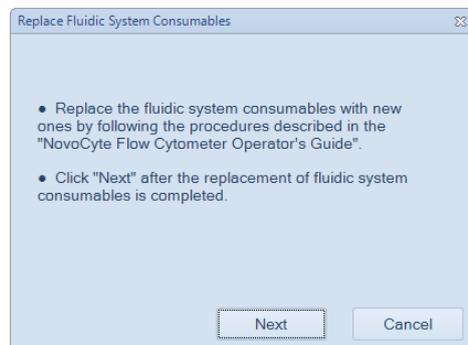
Clicking **Next** will continue the process using the default value.



**Figure 4-4 NovoExpress™ Software Message for No Tubing Calibration Number Entered**

-  It is not recommended to use the default Tubing Calibration Number, if indeed there is a Tubing Calibration Number labeled on the tubing, because it will affect the performance of the system. Click the **Back** button in Figure 4-4 to input the correct Tubing Calibration Number.

Then, NovoExpress™ software will prompt the message shown in Figure 4-5.



**Figure 4-5 NovoExpress™ Software Message for Fluidics Consumables Replacement**

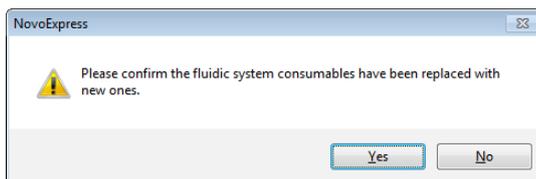
The following fluidic system consumables should be replaced at the same time:

- ▶ NovoFlow™ sheath fluid in-line filter (2pcs) (Cat. No. 2030002)
- ▶ Waste filter (1pcs) (Cat. No. 2030003)
- ▶ Peristaltic pump (sheath pump) tubing (1pcs) (Cat. No. 2030004)
- ▶ NovoFlow™ sheath fluid inlet filter (1pcs) (Cat. No. 2030001)
- ▶ NovoRinse™ solution inlet filter (1pcs) (Cat. No. 2030005)
- ▶ NovoClean™ solution inlet filter (1pcs) (Cat. No. 2030007)

Follow the procedure described below to replace all of the fluidic system consumables.

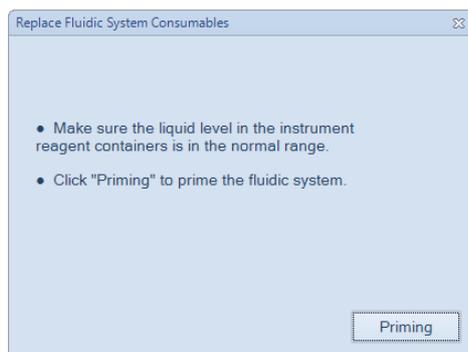
Once all of the fluidic system consumables have been replaced with the new ones, click the *Next* button to continue.

A confirmation window prompt will appear, click *Yes* in the prompted window (Figure 4-6) to inform the system that the consumables have been replaced.



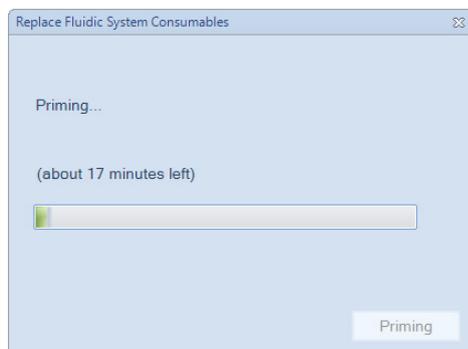
**Figure 4-6 NovoExpress™ Software Message Confirmation for Fluidics Consumables Replaced**

The NovoExpress™ software will prompt a window to remind users to conduct the Priming procedure (Figure 4-7). Ensure there are enough reagents in the NovoFlow™, NovoRinse™, and NovoClean™ containers. If not, add more reagents and click the *Priming* button to start the *Priming* process.



**Figure 4-7 NovoExpress™ Software Message for Priming after Replacing Fluidic System Consumables**

During the Priming process, the following prompt will appear (Figure 4-8). It takes about 17 minutes for the system to complete the priming procedure.



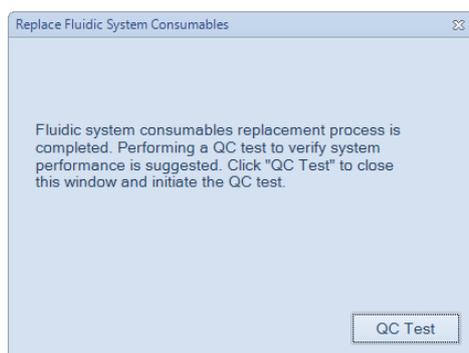
**Figure 4-8 NovoExpress™ Software Message for Priming in Process**

- 

The Priming procedure can also be manually initiated by clicking the **Instrument** → **Fluidics Maintenance** → **Priming** on the NovoExpress™ software when the instrument is in **Ready** status.
- 

For more information on the **Priming** function, please refer to **2.2.5 Priming** section in this guide.

Once the Priming process is complete, the following prompt will appear (Figure 4-9). Prepare the NovoCyte QC sample as described in **Section 2.3.1** in this guide and place it in the tube holder. Click **QC Test** to initiate the QC Test. The QC Test will automatically start. Follow the steps and complete the QC test. A QC test must be conducted each time when fluidic system consumables are replaced in order to check the performance of the system.

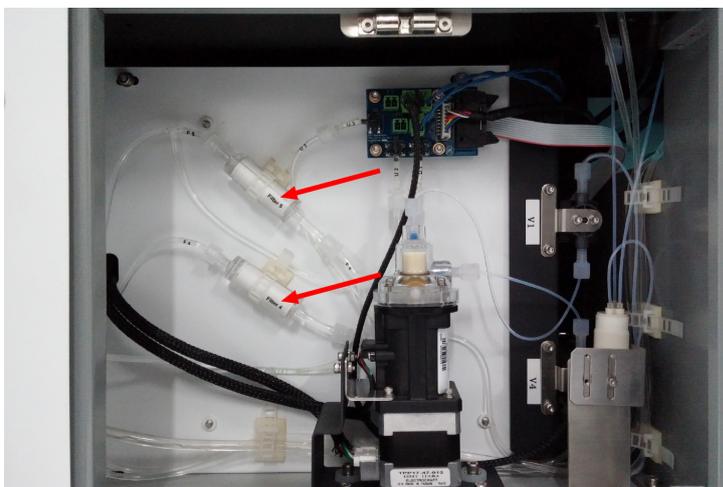


**Figure 4-9 NovoExpress™ Software Message for Initiating the QC Test after Completion of Fluidic System Consumables Replacement**

The following sections describe the detailed procedure for replacing fluidic system consumables. It is highly recommended to replace all the fluidic system consumables at the same time to ensure the optimal system performance. However, each component can also be replaced individually if necessary. Click **Instrument** → **Replace Fluidic System Consumables** in the NovoExpress™ software to initiate the process. Always conduct the **Priming** process after the fluidic system consumables have been replaced as prompted by the NovoExpress™ software or manually conduct the **Priming** process by clicking **Instrument** → **Fluidics Maintenance** → **Priming**.

#### 4.1.2.1. Replace the NovoFlow™ Sheath Fluid in-Line Filter

Open the front panel of the NovoCyte™ instrument, and locate the two sheath fluid in-line filters shown in Figure 4-10.



**Figure 4-10 Sheath Fluid in-Line Filters**

**Procedure**

- 1 Make sure the NovoCyte™ is in *Ready* status;
- 2 Open the front panel of the instrument;
- 3 Unscrew the Luer connectors at two ends of the filter to disconnect the tubings from the filter;
  - ⚠ *Use lint free wipes to gently wipe off the sheath fluid from the tubing, and avoid dripping of the sheath fluid into the instrument.*
- 4 Replace the old filters with new ones and correctly connect the tubing to the filters.
  - ⚠ *Use proper force when tightening the Luer connectors.*
- 5 Click *Instrument* → *Fluidics Maintenance* → *Priming* to prime the fluidic system. Check that the sheath fluid flows through the two filters properly and make sure there is no leakage through the filter-tubing connections.

⚠ *The ends of the sheath fluid in-line filter are different to ensure the in-line filter is connected into the system in the correct direction. Incorrect installation will lead to system malfunction.*

⚠ *When replacing the sheath fluid in-line filter, do not twist or kink the tubing.*

**4.1.2.2. Replace the Waste Filter**

Open the side panel of the NovoCyte™ instrument, and locate the waste filter as shown in Figure 4-11.

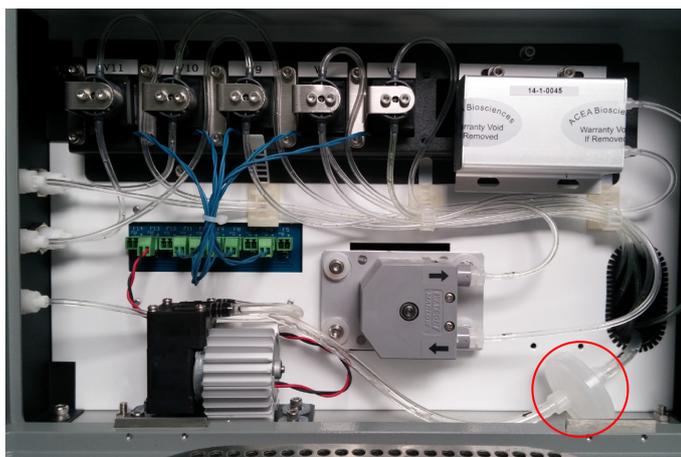


Figure 4-11 Waste Filter

**Procedure**

- 1 Make sure the NovoCyte™ is in *Ready* status;
- 2 Open the side panel of the instrument;
- 3 Unscrew the Luer connectors at two ends of the waste filter to disconnect the tubing from the filter;
  - ⚠ *Use lint free wipes to gently wipe off the fluid from the tubing, and avoid dripping of the fluid into the instrument.*



- 4 Replace the old filter with a new one and correctly connect the tubing to the filters;

 Use proper force when tightening the Luer connectors.

- 5 Click *Instrument* → *Fluidics Maintenance* → *Priming* to prime the fluidic system. Check that the waste flows through the waste filter properly and make sure there is no leakage through the filter-tubing connections.

 Please install the waste filter in the correct direction as indicated by the **inlet** label on the filter and the flow direction arrows labeled on the tubing. Incorrect installation will lead to system malfunction.

 When replacing the waste filter, do not twist or kink the tubing.

  Biological samples may be biohazardous. Appropriate personal protective equipment should be worn when handling waste, in accordance with local safety regulations.

#### 4.1.2.3. Replace the Peristaltic Pump (Sheath Pump) Tubing

 The Peristaltic pump tubing should only be replaced using the **Replace Fluidics Consumables** function described above. The replacement of the peristaltic pump tubing requires the input of the Tubing Calibration Number that is affixed to peristaltic pump tubing. Failure to input the Tubing Calibration Number will affect the performance of the system.

Open the side panel of the NovoCyte™ instrument to access the peristaltic pump as shown in Figure 4-12.

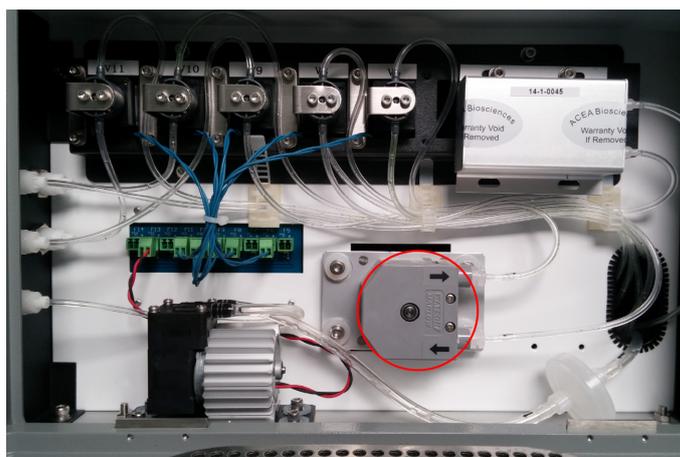


Figure 4-12 Peristaltic Pump

#### Procedure

- 1 Make sure the NovoCyte™ is in *Ready* status;
- 2 Open the side panel of the instrument;
- 3 Unscrew the Luer connectors at two ends of the peristaltic pump tubing;



# 4

- 4 Grab two sides of the pump retainer clip, gently squeeze in and remove the clip from the pump head (Figure 4-13);

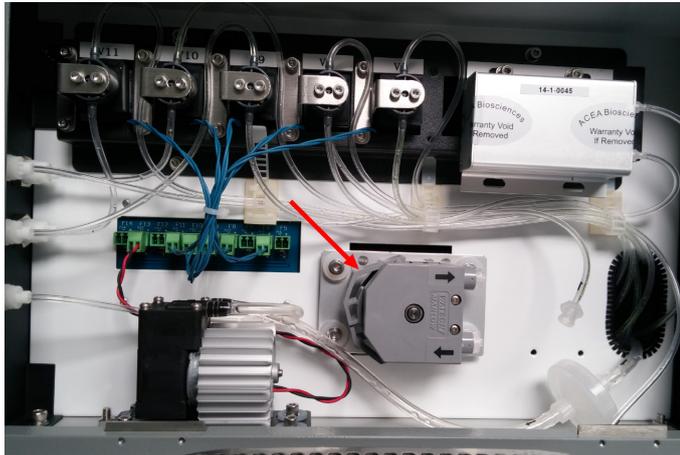


Figure 4-13 Remove the Peristaltic Pump Retainer Clip

- 5 Pull the Luer connectors outward and slide the luer lock fittings off the pump head (Figure 4-14);

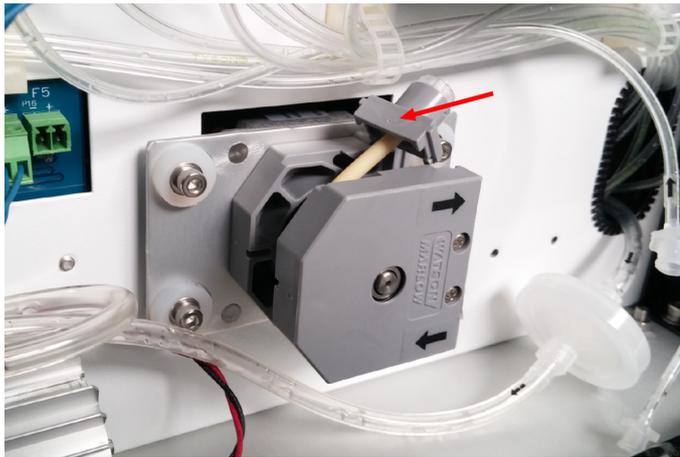


Figure 4-14 Slide Luer Lock Fittings off the Pump Head

- 6 Remove the peristaltic pump tubing and discard according to standard laboratory protocols and regulations;
- 7 Replace with new peristaltic pump tubing by sliding the Luer lock fittings onto the pump head (Figure 4-15);

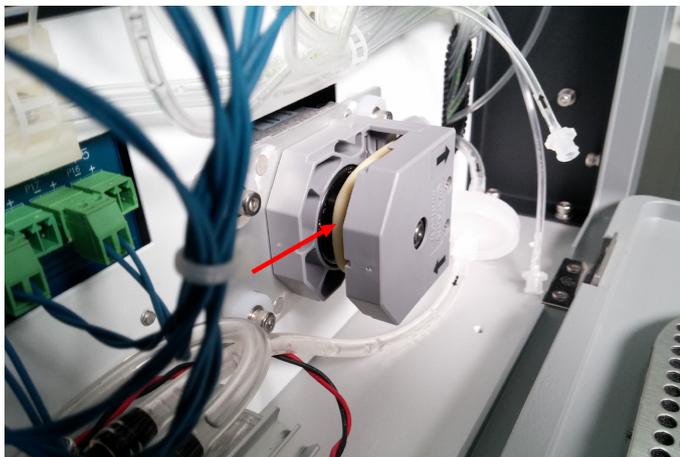


Figure 4-15 Place New Peristaltic Pump Tubing onto the Pump Head



- 8 Snap the pump retainer clip into pump head. Reconnect the Luer connectors of the pump tubing;
  - ⚠ *The arrows labeled on the pump head and the tubing indicate the direction of fluid flow. Connect the tubing to the pump head in the right direction as indicated. Incorrect direction will result in instrument malfunction.*
  - ⚠ *Use proper force to connect the Luer connectors. Ensure the peristaltic pump tubing and the fluidic tubing are not twisted or kinked.*
- 9 Click *Instrument* → *Fluidics Maintenance* → *Priming* to prime the fluidic system. Check that the sheath fluid flows through the peristaltic pump properly and make sure there is no leakage through the pump tubing.

#### 4.1.2.4. Replace the NovoFlow™ Sheath Fluid Inlet Filter

NovoFlow™ sheath fluid inlet filter is placed inside the NovoFlow™ container to filter out micro-particles or impurities in the sheath fluid.

##### Procedure

- 1 Make sure the NovoCyte™ is in *Ready* status;
- 2 Press the metal clip on the quick coupler to disconnect the tubing from the NovoFlow™ sheath fluid container;
- 3 Unscrew the cap and take the tubing-filter assembly out from the container and put it on a clean lint free wipe (Figure 4-16).



Figure 4-16 Change NovoFlow™ Sheath Fluid Inlet Filter

- 4 Replace the old filter with a new one and correctly connect the tubing to the filter;
  - ⚠ *Please use proper force when tightening the Luer connectors.*
  - ⚠ *The ends of the sheath fluid inlet filter are different to ensure the filter is connected into the system in the correct direction. Incorrect installation will lead to system malfunction.*
- 5 Click *Instrument* → *Fluidics Maintenance* → *Priming* to prime the fluidic system.

⚠ *When replacing the sheath fluid inlet filter, do not twist or kink the tubing.*

#### 4.1.2.5. Replace the NovoRinse™ and NovoClean™ Solution Inlet Filter

NovoRinse™ and NovoClean™ solution inlet filters are placed inside the NovoRinse™ and NovoClean™ containers respectively to filter out micro-particles or impurities in the solution.

**Procedure**

- 1 Make sure the NovoCyte™ is in *Ready* status;
- 2 Press the metal clip on the quick coupler to disconnect the tubing from the NovoRinse™ or NovoClean™ container;
- 3 Unscrew the cap and take the tubing-filter assembly out from the container and put it on a clean lint free wipe. Take off the old filter and replace with a new one (Figure 4-17);



**Figure 4-17 Change NovoRinse™ and NovoClean™ Solution Inlet Filter**

- 4 Click *Instrument* → *Fluidics Maintenance* → *Priming* to prime the fluidic system.

## 4.2 Periodic Maintenance

Instrument components should be checked occasionally and maintenance or cleaning should be conducted when necessary. The frequency depends on how often the cytometer is used.

- ▶ Removal of Bubbles
- ▶ Unclog the Sample Injection Probe (SIP)
- ▶ Sterilize Instrument Reagent Containers
- ▶ Clean the Outer Surface of the Instrument
- ▶ Fluidic System Cleaning before Long-Term Storage
- ▶ Purge Fluidic System before Shipment
- ▶ Calibrate the Fluidics Station
- ▶ Clean the Optical Filters and Mirrors

### 4.2.1 Removal of Bubbles

Unusually high variation in data or a QC test failure may indicate bubbles inside the flow chamber. The following procedure should be conducted to remove the bubbles.

When the NovoCyte™ instrument is in *Ready* status, place a tube containing at least 1 mL of 70% medical grade ethanol into the sample holder. Click *Instrument* → *Fluidics Maintenance* → *Debubble* from the NovoExpress™ software to execute the debubble procedure.

 Refer to 2.2.1. *Debubble* section in this guide for more details about removal of bubbles from the flow chamber.

## 4.2.2 Unclog the Sample Injection Probe (SIP)

An unusually low number of events detected may indicate that the SIP is clogged. To solve this issue, when the NovoCyte™ instrument is in *Ready* status, click *Instrument* → *Fluidics Maintenance* → *Backflush* to execute the Backflush procedure to unclog the SIP.

 Refer to 2.2.7. *Backflush* section in this guide for more details about the backflush process.

If the problem persists after running the *Backflush* function, follow the procedure below to manually clear a clog inside the SIP.

### Procedure

- 1 Turn off the power of the instrument;
- 2 Open the top cover of the instrument;
- 3 Unscrew the connector to the SIP and remove the SIP from the instrument (Figure 4-18);

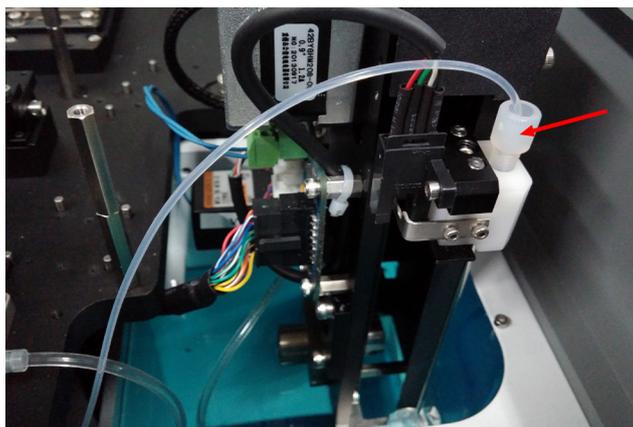


Figure 4-18 Remove the SIP from the Instrument

 *The sample injection probe is fragile and may be damaged if handled improperly. Be careful when handling the sample injection probe to avoid deforming or bending it.*

- 4 Connect the SIP with the 1/16" ID tubing provided in the NovoCyte™ Sample Injection Probe Unclog Kit. Connect the other end of the tubing to a 10 mL syringe filled with 1X NovoClean™ solution (Figure 4-19);



Figure 4-19 Connect the SIP with a 10 mL Syringe



- 5 Insert the other end of the SIP into a beaker containing 1X NovoClean™ solution. Push the plunger of the syringe back and forth for multiple times to flush the SIP with NovoClean™ solution;
- 6 Disconnect the SIP and insert it into a beaker containing NovoClean™ solution and soak for 10 minutes;
- 7 Insert the SIP into a beaker containing NovoFlow™ sheath fluid and soak for 10 minutes;
- 8 Reinstall the SIP into the instrument;
- 9 Power on the instrument. Verify that the SIP is properly installed. If there is fluid leakage during the start-up process, please reinstall the SIP. If leakage persists, contact your local ACEA Biosciences representative for assistance.

#### 4.2.3 Sterilize Instrument Reagent Containers

NovoCyte™ instrument reagents contain constituents which prevent the growth of bacteria. Therefore, under normal operation conditions, there is no need to sterilize the instrument reagent containers. However, if bacterial contamination is suspected, the reagent containers may be sterilized using the following method.

##### Procedure

- 1 Wipe the outer surface of the container using lint free wipes;
- 2 Clean the container using tap water in a sink;
- 3 Fill the container with 10% bleach and soak overnight. Clean the container again with tap water;
- 4 Rinse the container thoroughly with DI water for at least three times and air dry.

#### 4.2.4 Clean the Outer Surface of the Instrument

Keep the outer surface of the instrument clean using the following method.

##### Procedure

- 1 Turn off the power of the instrument and unplug the power cord;
 



*To avoid the risk of electrical shock, make sure to power off the cytometer and unplug the power cord from the power outlet.*
- 2 Gently wipe the surface of the instrument with the lint free wipes or a clean soft cloth soaked with NovoRinse™ solution;
- 3 Wipe the surface again using a dry lint free wipes or a clean soft cloth.

  Biohazardous samples may make contact with the surface of the instrument. When cleaning the surface appropriate personal protective equipment should be worn, and the lint free wipes or cloth should be disposed of in accordance with local safety regulations.

 Do not use isopropyl alcohol (IPA) or ethanol to clean the instrument surface.

#### 4.2.5 Fluidic System Cleaning before Long-Term Storage

If you do not plan to run the NovoCyte™ cytometer for more than two weeks, a thorough cleaning process is recommended to prevent salt crystallization in the fluidic system.

##### Procedure

- 1 When the cytometer is in *Ready* status, replace all the instrument reagents with deionized water;
- 2 Click *Instrument* → *Fluidics Maintenance* → *Priming* from the NovoExpress™ software. Repeat the procedure for two times;
- 3 Press the power switch on the front panel of the NovoCyte™ instrument to initiate the automatic shutdown cleaning process;
- 4 After the shutdown cleaning process is completed, the instrument will power off by itself. The NovoExpress™ software status bar will show *Instrument not Connected* message;
- 5 Empty all the reagent containers and empty the waste. Rinse the containers with tap water thoroughly and air dry.

When re-use the system after long-term storage, follow the procedure below to get it ready.

- 1 Sterilize the reagent containers following the procedure described in [4.2.3 Sterilize Instrument Reagent Containers](#);
- 2 Add the NovoFlow™, NovoRinse™, and NovoClean™ to the corresponding containers;
- 3 Press the power-on button on NovoCyte™ instrument front panel. The instrument starts the automatic start-up cleaning procedure;
- 4 Click *Instrument* → *Fluidics Maintenance* → *Priming* from the NovoExpress™ software. Repeat the procedure for two times;
- 5 The NovoCyte™ instrument is then ready to use.

 If NovoCyte™ instrument has not been used for more than two months, it is recommended to replace the fluidic system consumables before re-use. Refer to [4.1.2 Replace Fluidic System Consumables](#) for detailed procedure.

### 4.2.6 Purge Fluidic System before Shipment

If in any case that the NovoCyte™ flow cytometer needs to be shipped, please follow the following procedure to purge the fluidic system first before packaging and shipment.



The **Purge** function is only available by logging in as **administrator**.

#### Procedure

- 1 Click *Instrument* → *Fluidics Maintenance* → *Purge* from the NovoExpress™ software;
- 2 NovoExpress™ software will prompt message to guide you to conduct the purging procedure (Figure 4-20). Follow the instructions in each step and click *OK* to move to the next step until completion.

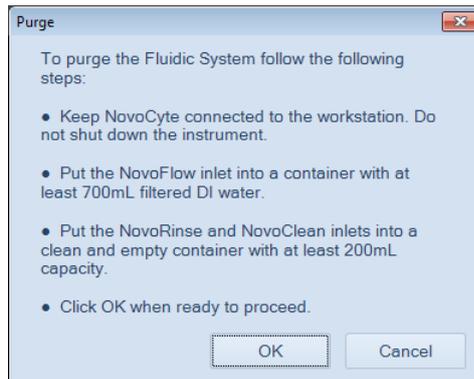


Figure 4-20 NovoExpress™ Dialogue Box to Guide the *Purge* Procedure

- 3 Power off the instrument. Unplug the power supply and accessories.
- 4 Empty all the reagent containers and empty the waste. Rinse the containers with tap water thoroughly and air dry;
- 5 Package the instrument properly for shipment.



### 4.2.7 Calibrate the Fluidics Station

Fluidics station is calibrated at the factory and should be ready to use when connected to NovoCyte™ instrument after installation. However, in some cases there is need to recalibrate the fluidics station after shipment and movement. When abnormal warning about the liquid level in the reagent containers is observed, follow the procedures below to recalibrate the fluidics station.

 **Calibrate Fluidics Station** function only available when login as administrator.

#### Procedure

- 1 Click *Instrument* → *Operation* → *Calibrate Fluidics Station* in the NovoExpress™ software.

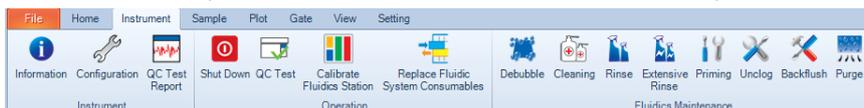


Figure 4-21 NovoExpress™ Software *Instrument* Menu

- 2 Follow the instruction in the software, remove the NovoFlow™, NovoRinse™, NovoClean™, and waste containers from the fluidics station and click *OK* button (Figure 4-22).

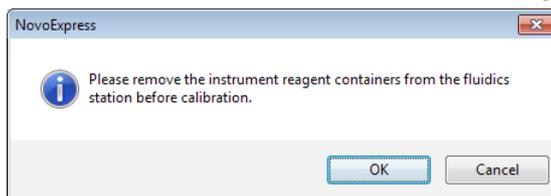


Figure 4-22 Message in NovoExpress™ Software when Performing Fluidics Station Calibration

- 3 The software will prompt message indicating *Fluidics Station Calibration Succeed*. Follow the instruction to place the NovoFlow™, NovoRinse™, NovoClean™, and waste containers back into the fluidics station and click *OK* button (Figure 4-23).

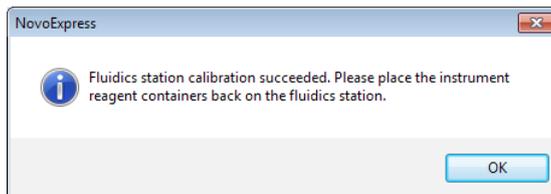


Figure 4-23 NovoExpress™ Software Prompted Message indicating Fluidics station Calibration is successful

### 4.2.8 Clean the Optical Filters and Mirrors

In normal operation, the optical filters and mirrors are tightly enclosed inside the optical fixtures and should remain clean. However, the optical filters and mirrors may acquire dust or debris if unplugged from the slot for rearrangement or replacement. The following method describes how to clean the optical filters or dichroic mirrors. Keeping the optical filters and mirrors surface clean is very important for data quality.

 *When cleaning or replacing optical filters or dichroic mirrors, handle with care to avoid scratching the surface and to prevent the filters from falling out of the holder. Use cotton swabs, optical lens paper, and analytical grade ethanol in a dropper bottle to clean the optical filters. Acetone should not be used for cleaning the optical filters. Always wear laboratory gloves when handling the optical filters.*

#### Materials

- ▶ Analytical grade ethanol
- ▶ Compressed air or a bulb blower
- ▶ Lint-free cotton swabs
- ▶ Lint-free wipe or lens cleaning tissue

#### Procedure

- 1 Blow off the contaminants  
Many contaminants are just loosely attached to the surface of the optical filters and can be blown off. Wear clean laboratory gloves and hold the filter firmly in one hand. Always use a gentle to moderate air flow and maintain an oblique angle. Slowly move the air stream over the filter surface at an angle to gently blow off the contaminants. Repeat this process for the other side of the filter.
- 2 Wrap a triangular-shaped lint-free wipe or lens cleaning tissue around a lint-free cotton swab. Moisten and seal the end with a few drops of alcohol.
- 3 Holding the cotton swab in a horizontal position, gently clean the surface of the filter with a continuous motion at a constant speed.
- 4 Allow the solvent to evaporate. Use a room light to inspect the filter to ensure it is clean. If contamination remains, start with a new wipe and swab and repeat steps 2-3 again.
- 5 Repeat steps 1-4 for the other side of the filter.
- 6 Insert the cleaned filter into the filter slot and push it all the way in.

 *Always blow the contaminants off the filters before using the wipe or swab to clean the filter surface. Failure to do so may scratch the filter surface.*

 *Do not reuse the wipes and swabs. Always use new wipes and swabs for every new cleaning attempt.*

## 5. Troubleshooting

This section contains troubleshooting guidelines. Follow the procedures described in this section for troubleshooting methods. If problems still exist after performing troubleshooting, or there are any problems not listed here, please contact ACEA Biosciences technical support.

 For technical support, please refer to the *Prologue IX: Technical Support* section in this guide.

 Refer to the *NovoExpress™ Software Guide* for related issues with the software.

 Refer to the *NovoSampler™ Operator's Guide* for NovoSampler™ troubleshooting.

Observation	Possible Causes	Recommended Solutions
Software warning: Instrument cover is opened	Instrument cover is open or not tightly closed	Check the instrument cover to make sure it is closed properly.
Software warning: <i>Collision of sample injection probe</i>	Obstacles blocked the moving sample injection probe and caused a collision	Clear the obstacles which blocked the moving sample injection probe. Click the <i>OK</i> button in the prompted dialog box or wait 10 seconds for automatic error handling. Then the instrument will move the sample injection probe to the home position. This procedure will last about 3 minutes.
Zero or low number of events detected when the instrument is normally running	Threshold is set too high	Lower the threshold setting and re-collect the sample.
	Sample concentration is too low	Re-prepare the sample to an appropriate concentration, or use appropriate method to concentrate the sample.
	Sample did not mix well	Mix the sample well to suspend the cells.
	Sample injection probe is clogged	Execute the <i>Backflush</i> function from the NovoExpress™ Software. If the event rate is still erratic, clean the sample injection probe by following the procedure described in <i>4.2.2 Unclog the Sample Injection Probe (SIP)</i> in this guide.
	Flow cell is clogged	Execute the <i>Unclog</i> function from the NovoExpress™ Software. Refer to <i>2.2.6 Unclog</i> in this guide for the detailed procedure.
Sheath fluid container is not correctly connected to the instrument and the sheath fluid does not flow through the tubing	<ul style="list-style-type: none"> <li>▶ Check the quick coupling connectors to ensure the sheath fluid container is correctly connected to the instrument.</li> <li>▶ Turn off the instrument and wait until the shutdown automatic cleaning process is completed.</li> <li>▶ Turn on the instrument again.</li> <li>▶ When the instrument is in <i>Ready</i> status, test the sample again.</li> <li>▶ If the event rate is still erratic, replace the fluidic system consumables following the procedure described in <i>4.1.2 Replace Fluidic System Consumables</i> in this guide.</li> </ul>	



5

Observation	Possible Causes	Recommended Solutions
	Run out of sheath fluid	Refill the sheath fluid container following the procedure described in <a href="#">2.1.2 Add Instrument Reagents</a> in this guide.
	Sampling fluidic tubing is leaking	<ul style="list-style-type: none"> <li>▶ Turn off the power of the instrument.</li> <li>▶ Open the front panel and check for signs of fluid leakage.</li> <li>▶ If fluid leakage is observed, take appropriate protective measures to clean leaked fluid with absorbent paper.</li> <li>▶ Contact ACEA Biosciences technical support for service.</li> </ul>
High event rate	The threshold is set too low	Increase the threshold setting and re-collect the sample.
	Sample concentration is too high	Re-prepare the sample to appropriate concentration, or dilute the sample.
	Sample flow rate is too high	Reduce the sample flow rate.
Instrument QC test fails	QC particle expired or not stored appropriately	Verify the QC particles are still within the expiration date. Verify the QC particles have been stored appropriately. Prepare new QC particles and mix them well to run the QC test again.
	Flow cell is dirty	Execute the <i>Extensive Rinse</i> from the NovoExpress™ Software to clean the flow cell. Refer to <a href="#">2.2.4 Extensive Rinse</a> in this guide for detailed procedure.
	Air bubbles exist in the flow chamber	Execute <i>Debubble</i> from NovoExpress™ Software. Refer to <a href="#">2.2.1 Debubble</a> in this guide for detailed procedure.
	Optical alignment is off	Please contact ACEA Biosciences technical support for service.
Leakage from the sample injection probe	Flow cell is clogged	Execute the <i>Unclog</i> procedure from the NovoExpress™ Software to clean the flow cell. Refer to <a href="#">2.2.6 Unclog</a> in this guide for detailed procedure.
Sample tube does not fit the sample tube holder	Wrong type of sample tube is used	Use 12 × 75mm flow tube or 1.5mL Eppendorf tube. Refer to <a href="#">1.7.3 Compatible Sample Tubes</a> for more information.
NovoExpress™ displays <i>Instrument not connected</i> even though the instrument is powered on	Workstation is in hibernation causing the interruption of communication between the NovoCyte™ instrument and workstation	Restart NovoExpress™ Software.
	Connection between the NovoCyte™ instrument and workstation is disconnected	Reconnect the USB cable between the instrument and the workstation.



Observation	Possible Causes	Recommended Solutions
	More than one NovoExpress™ instances are running.	Only run one instance of NovoExpress™ software.
Software warning: ... <i>liquid level sensor failure</i>	Liquid level sensor in the fluidics station is damaged	Please contact ACEA Biosciences technical support for service.
Software warning: <i>Fluidics station is not connected</i>	Cable between the fluidics station and the NovoCyte™ instrument is loose or malfunctioning	<ul style="list-style-type: none"> <li>▶ Turn off the power of the instrument.</li> <li>▶ Reconnect the cable between the fluidics station and the NovoCyte™ instrument.</li> <li>▶ If the problem still exists, please contact ACEA Biosciences technical support for service.</li> </ul>
Fluid leakage observed from the instrument	Valve failure or tubing connection failure	<ul style="list-style-type: none"> <li>▶ Turn off the power of the instrument.</li> <li>▶ Take appropriate measures to clean the leaked fluid.</li> <li>▶ Contact ACEA Biosciences technical support for service.</li> </ul>
High CV	Air bubbles exist in the flow chamber	Execute the <i>Debubble</i> procedure from NovoExpress™ Software. Refer to <a href="#">2.2.1 Debubble</a> in this guide for detailed procedure.
	Blockage in sampling tubing	Execute <i>Cleaning</i> from NovoExpress™ Software. Refer to <a href="#">2.2.2 Cleaning</a> in this guide for detailed procedure.
	Sheath in-line filter clogging	Replace the fluidic system consumables following the procedure described in <a href="#">4.1.2 Replace Fluidic System Consumables</a> in this guide.
	Poor sample preparation	Repeat sample preparation according to the protocols.
	The sample flow rate is set too high	Lower the sample flow rate setting
	Optical alignment is off	Please contact ACEA Biosciences technical support for service.
Excessive debris in display	Sheath in-line filter is dirty	Replace the fluidic system consumables following the procedure described in <a href="#">4.1.2 Replace Fluidic System Consumables</a> in this guide.
	The threshold is set too low	Increase the threshold setting and re-collect the sample.
	Fluidic system tubing is dirty	Execute <i>Rinse</i> from NovoExpress™ Software. Refer to <a href="#">2.2.3 Rinse</a> in this guide for detailed procedure.
Software warning: <i>NovoClean runs low</i>	NovoClean™ solution is below the pre-set volume limit	Refill the NovoClean™ solution container following the procedure described in <a href="#">2.1.2 Add Instrument Reagents</a> in this guide.



Observation	Possible Causes	Recommended Solutions
Software warning: <i>Run out of NovoClean</i>	NovoClean™ solution is too low to continue to run any samples	Refill the NovoClean™ solution container following the procedure described in <a href="#">2.1.2 Add Instrument Reagents</a> in this guide.
Software warning: <i>Novo-Rinse runs low</i>	NovoRinse™ solution is below the pre-set volume limit	Refill the NovoRinse™ solution container following the procedure described in <a href="#">2.1.2 Add Instrument Reagents</a> in this guide.
Software warning: <i>Run out of NovoRinse</i>	NovoRinse™ solution is too low to continue to run any samples	Refill the NovoRinse™ solution container following the procedure described in <a href="#">2.1.2 Add Instrument Reagents</a> in this guide.
Software warning: <i>Novo-Flow runs low</i>	NovoFlow™ solution is below the pre-set volume limit	Refill the NovoFlow™ solution container following the procedure described in <a href="#">2.1.2 Add Instrument Reagents</a> in this guide.
Software warning: <i>Run out of NovoFlow</i>	NovoFlow™ solution is too low to continue to run any samples	Refill the NovoFlow™ solution container following the procedure described in <a href="#">2.1.2 Add Instrument Reagents</a> in this guide.
Software warning: <i>Waste container is close to full</i>	Waste is above the pre-set volume limit	Empty the waste container following the procedure described in <a href="#">2.1.3 Empty Waste</a> in this guide.
Software warning: <i>Waste container is full</i>	Waste container is too full to continue to run any samples	Empty the waste container following the procedure described in <a href="#">2.1.3 Empty Waste</a> in this guide.
Software warning: <i>Pressure is out of limit</i>	Flow cell is clogged	Execute the <i>Unclog</i> procedure from Novo-Express™ Software to clean the flow cell. Refer to <a href="#">2.2.6 Unclog</a> in this guide for detailed procedure.
	Peristaltic pump is not running	Open the side panel of the NovoCyte™ instrument and check if the peristaltic pump is running normally. If not, please contact ACEA Biosciences technical support for service.
	Sheath in-line filter is clogged	Replace the fluidic system consumables following the procedure described in <a href="#">4.1.2 Replace Fluidic System Consumables</a> in this guide.
	Waste container is not correctly connected to the instrument	Check the quick coupling connectors to ensure the waste container is correctly connected to the instrument.
Software warning: ... <i>laser self test error</i>	Specified laser is not functioning properly	The instrument will automatically reset the laser and run a laser self test. It takes approximately 5 to 10 minutes. If the problem persists, please contact ACEA Biosciences technical support for service.
Software warning: ... <i>laser is not connected</i>	Specified laser is not detected	Restart the instrument. If the problem persists, please contact ACEA Biosciences technical support for service.
Software warning: ... <i>laser does not emit</i>	No laser light emission from specified laser	Restart the instrument. If the problem persists, please contact ACEA Biosciences technical support for service.



Observation	Possible Causes	Recommended Solutions
Software warning: <i>Firmware configuration error</i>	System Error	Restart the instrument. If the problem persists, please contact ACEA Biosciences technical support for service.
Software warning: <i>System voltage is out of range</i>	System Error	Please contact ACEA Biosciences technical support for service.
Software warning: <i>System electric current is out of range</i>	System Error	Please contact ACEA Biosciences technical support for service.
Software warning: <i>Communication error</i>	USB cable between the NovoCyte™ instrument and workstation is loose or not connected properly	Reconnect the USB cable between the NovoCyte™ instrument and the workstation. Restart the instrument and NovoExpress™ software. If the problem persists, please contact ACEA Biosciences technical support for service.

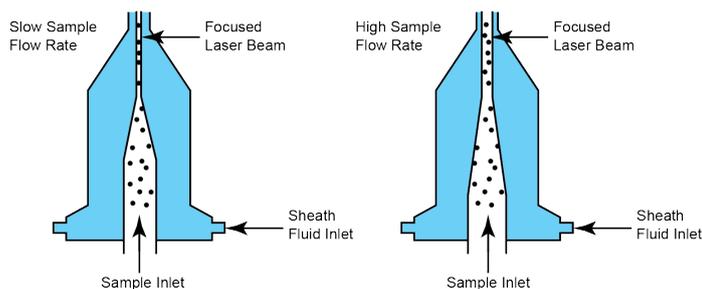
## Appendix A Technical Overview

This appendix contains a technical overview of the following topics:

- ▶ Fluidic System
- ▶ Optical System
- ▶ Electronic System

### Fluidic System

NovoCyte™ flow cytometer uses a peristaltic pump to drive the sheath fluid at a constant flow rate of 6.5 mL/min. A high precision syringe pump is used to drive the sample. During sampling, a certain volume of the sample is first aspirated by the syringe pump from the sample tube through sample injection probe (100  $\mu$ L per stroke at the default condition). Then the syringe pump will push the aspirated sample into the flow cell to be analyzed. The sample is hydrodynamically focused by the sheath fluid to form a small stream inside the flow cell. The diameter of the focused sample stream (called *core diameter*) is determined by the ratio between the sample flow rate and the sheath flow rate. When the sheath flow rate remains constant, the lower the sample flow rate, the smaller the core diameter, as illustrated in Figure A-1.



**Figure A-1 Hydrodynamic Focusing and Core Diameter under Different Sample Flow Rates**

Having at most one cell or particle moving through a laser beam at a given time is critical for flow cytometry. This is achieved by the hydrodynamic focusing process. Proper operation of fluidic components is critical for particles to intercept the laser beam properly. Always ensure that the fluidic system is free of air bubbles, cell debris, and other contaminants. Choosing the appropriate sample flow rate for your experiment is important for obtaining good quality data.

- ▶ A higher sample flow rate is generally used for qualitative measurements such as immunophenotyping. The data is less resolved but is acquired more quickly.
- ▶ A lower flow rate is generally used in applications where greater resolution and quantitative measurements are critical, such as DNA content analysis.

### Optical System

The optical system consists of lasers, optical lenses, optical filters, and detectors. Lasers illuminate the cells or particles in the sample. Optical lenses shape the laser beam into

a spot with appropriate shape and size (usually an elliptical spot of a few microns in the short axis and tens of microns in the long axis). Optical lenses are used to collect the light emitted from the cells or particles when they pass through the laser beam. Optical filters direct the light collected by the optical lenses to the appropriate detectors. When the stained cells or other particles pass through the focused laser beam, scattered light and fluorescence signals will be generated. Such light is collected by the light collecting optics and converted by the optical detectors into electronic signals.

### Light Scatter

When a cell or particle passes through a focused laser beam, light is scattered in all directions (Figure A-2). Light that scatters axial to the laser beam is called forward scatter (FSC). Light that scatters perpendicular to the laser beam is called side scatter (SSC). FSC and SSC are related to certain physical properties of cells:

- ▶ FSC - indicates relative size of the cells or particles
- ▶ SSC – indicates the internal complexity or granularity of the cells or particles

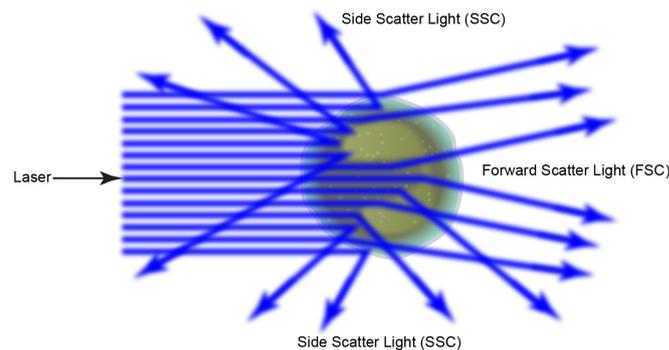


Figure A-2 Forward Scatter (FSC) and Side Scatter (SSC)

### Fluorescence

When a cell or particle stained with fluorochrome-conjugated antibodies or other fluorescent labels passes through the focused laser beam, the fluorochrome or fluorescent labels (in general called *dyes* in this guide) can absorb photons (energy) and be excited to a higher energy state. This higher energy state is not stable so that the dyes will return to their ground state shortly, accompanied by the release of energy. Most energy is released in the form of emitted light, whereas a very small portion of the energy is released in the form of heat. The light emission is known as fluorescence.

Fluorescence is always a longer wavelength (lower-energy photon) compared to the excitation wavelength. The difference between the excitation maximum and the emission maximum is known as the Stokes shift. Some fluorechromes such as FITC have a smaller Stokes shift, absorbing blue light (488nm) and emitting green light (530nm), while other fluorechromes such as PerCP exhibit a larger Stokes shift, absorbing blue light (488nm) and emitting red light (675nm).

The emission spectra for some commonly used fluorechromes are shown in Figure A-3.

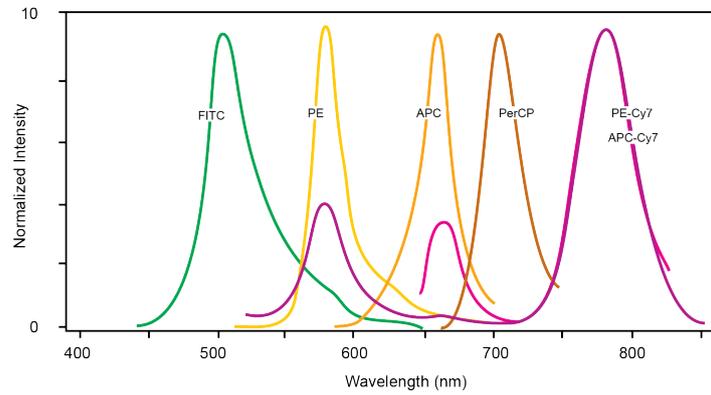


Figure A-3 Emission Spectra of Commonly Used Fluorochromes

### Optical Filters

Optical filters regulate the spectral distribution of light scatter and fluorescence directed to the optical detectors. When photons encounter an optical filter, they are either transmitted, absorbed, or reflected.

In flow cytometry, usually a series of dichroic mirrors are used to separate the collected light at a specific wavelength. A bandpass filter is used in front of each optical detector to only allow the light in a particular range to be detected by the detector. Commonly, the name of the bandpass filter is used interchangeably with fluorescence channel to specify the detection wavelength range. For example, FITC is routinely detected by a 530/30 bandpass filter. Therefore, 530/30 is referred as a FITC fluorescence detection channel.

### Dichroic Mirrors

Also called dichroic beam splitters, dichroic mirrors allow part of the light to be transmitted and part of the light is reflected. Incident light typically enters the dichroic mirror at an angle of 45°. The transmitted light travels in the same direction as the incident light ( $\lambda_2$ ), whereas the reflected light is reflected in a 90° angle relative to the incident light ( $\lambda_1$ ) (Figure A-4). There are two types of dichroic mirrors: longpass dichroic mirrors and shortpass dichroic mirrors. Longpass dichroic mirrors allow light with longer wavelengths to be transmitted, where shortpass dichroic mirrors allow light with shorter wavelengths to be transmitted. Dichroic mirrors are usually named by the cutoff wavelength. For example, a 605nm longpass dichroic mirror allows light with wavelength longer than 605nm to be transmitted, and light with wavelength shorter than 605nm is reflected.

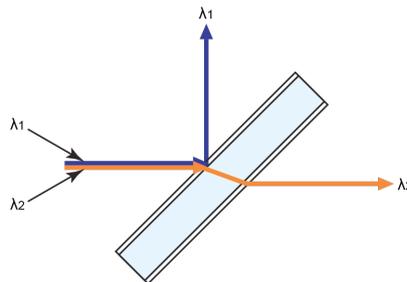


Figure A-4 Dichroic Mirror

### Filters

Filters are usually placed at a 90° angle relative to the incident light and regulate the incident light. Filters can be divided into longpass filters, shortpass filters, and bandpass filters.

### Longpass Filters

Longpass (LP) filters pass wavelength longer than the cutoff wavelength. For example, a 505nm LP filter allows wavelengths longer than 505nm to pass through it and either absorbs or reflects wavelengths shorter than 505nm (Figure A-5).

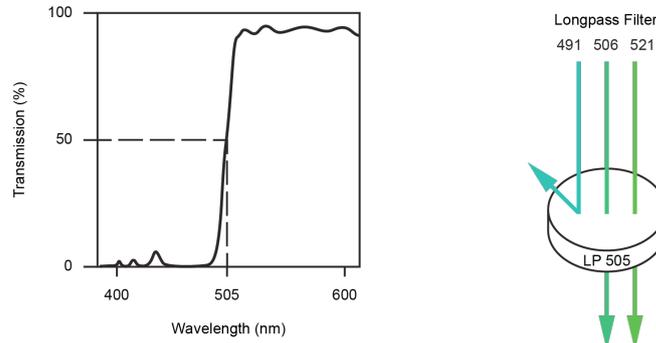


Figure A-5 505nm Longpass Filter

### Shortpass Filters

Shortpass filters (SP) pass light with a shorter wavelength than the cutoff wavelength. For example, a 472nm shortpass filter allows wavelengths shorter than 472nm to pass through it and either absorbs or reflects wavelengths longer than 472nm (Figure A-6).

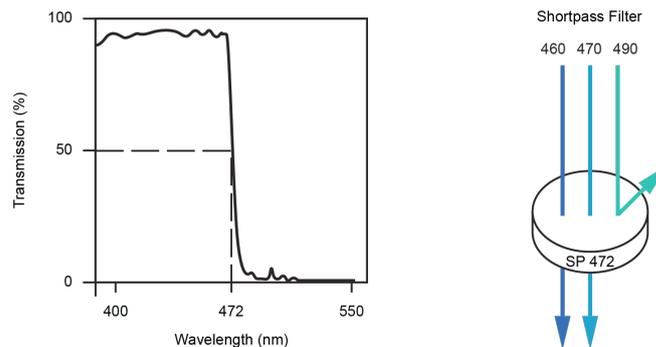


Figure A-6 472nm Shortpass Filter

### Bandpass filters

A bandpass (BP) filter transmits a relatively narrow range or band of light. Bandpass filters are typically designated by two numbers. The first number indicates the center wavelength and the second number refers to the range of the light wavelength that is passed through. For example, a 530/30 BP filter transmits light that is centered at 530nm and has a total of bandwidth of 30nm. Therefore, this filter transmits light between 515nm and 545nm (Figure A-7).

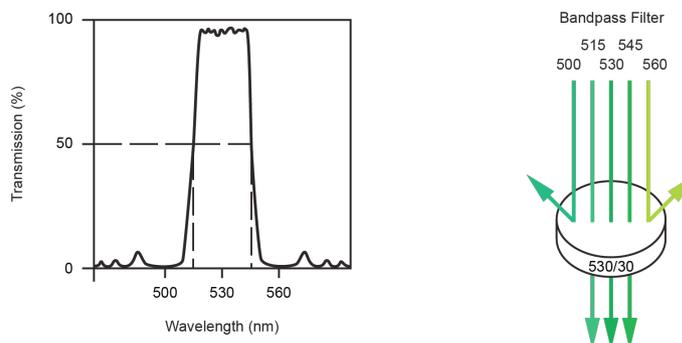


Figure A-7 530/30 Bandpass Filter

The ACEA NovoCyte™ flow cytometer uses longpass and shortpass dichroic mirrors and bandpass filters for light collection. Figure A-8 shows the diagram of the NovoCyte™ optical bench. Table A-1 lists the dichroic mirrors and bandpass filters used in the NovoCyte™ instrument and recommended fluorochromes for each detector.

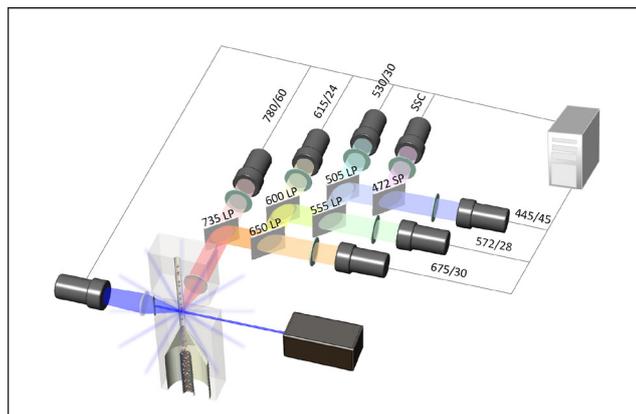


Figure A-8 NovoCyte™ Optical System Schematics

Excitation Laser	Detector	Dichroic Mirror	Bandpass Filter	Fluorochrome
488 nm	Side Scatter	-	488/10	
	BL1	505 LP	530/30	FITC
	BL2	555 LP	572/28	PE
	BL3	600 LP	615/24	PE-Texas Red™
	BL4	650 LP	675/30	PerCP
	BL5	735 LP	780/60	PE-Cy7
640 nm	RL1	650 LP	675/30	APC
	RL2	735 LP	780/60	APC-Cy7
405 nm	VL1	472 SP	445/45	Pacific Blue
	VL2	505 LP	530/30	AmCyan
	VL3	555 LP	572/28	Pacific Orange
	VL4	600 LP	615/24	Qdot 605
	VL5	650 LP	675/30	Qdot 655
	VL6	735 LP	780/60	Qdot 800

Table A-1 Default Dichroic Mirrors and Bandpass Filters

### Change the Optical Filters

NovoCyte™ flow cytometer allows the user to change or re-configure the optical filters according to their needs. Please contact your local ACEA Biosciences representative regarding any specific filters or dichroic mirrors.

When changing the filters, pull out the original filter from the slot and insert the new filter in the same direction as indicated by the labels by the side of the slot (Figure A-9).

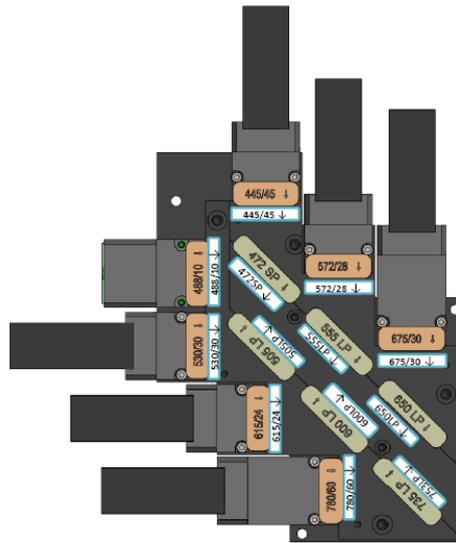


Figure A-9 Orientation of the Dichroic Mirrors and Bandpass Filters

**⚠** Avoid contamination of the optical filters. Always wear clean laboratory gloves and handle the filters carefully. Avoid touching or scratching the filter surface.

### Optical Detectors

Detectors convert the light signal into an electrical signal. In the NovoCyte™ flow cytometer, a photodiode (PD) is used to detect the FSC and SSC signals. A photomultiplier tube (PMT) is used to detect the fluorescence signals.

### Fluorescence Compensation Theory

Fluorochromes emit light over a range of wavelengths (Figure A-3). Optical filters are used to limit the range of wavelength measured by a given detector. However, when two or more fluorochromes are used in the same test, the overlap in wavelength ranges often makes it impossible to isolate light from a given fluorochrome from another fluorochrome using optical filters. As a result, light emitted from one fluorochrome will appear in a detector intended for detecting the other fluorochrome (Figure A-10). This is known as fluorescence spillover. Spillover can be corrected mathematically by using a method called compensation.

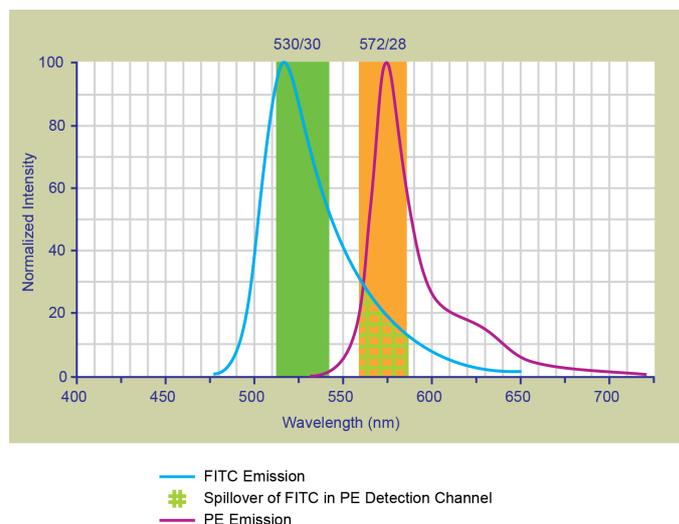
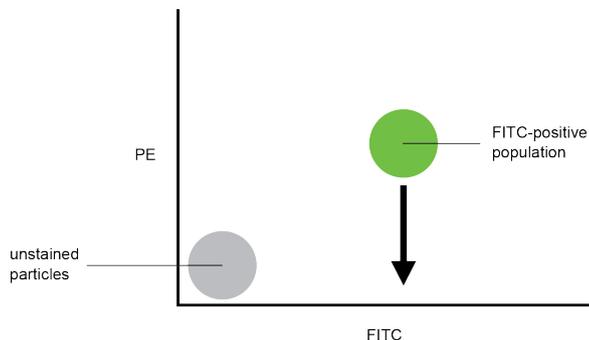
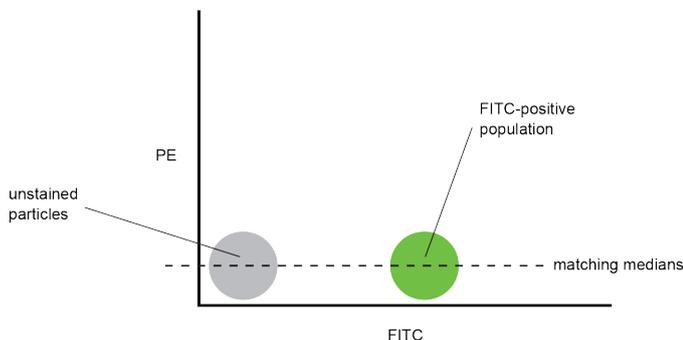


Figure A-10 Spillover from the FITC Fluorochrome to the PE Detector

For example, Figure A-10 shows FITC emission appears primarily in the FITC detector, but a small portion of its fluorescence spills over into the PE detector. The spillover is corrected by compensation, as indicated by the arrow in Figure A-11. The purpose of the compensation is to move the FITC-positive population downwards until it has the same Mean or Median value of PE signal as the unstained population (Figure A-12).

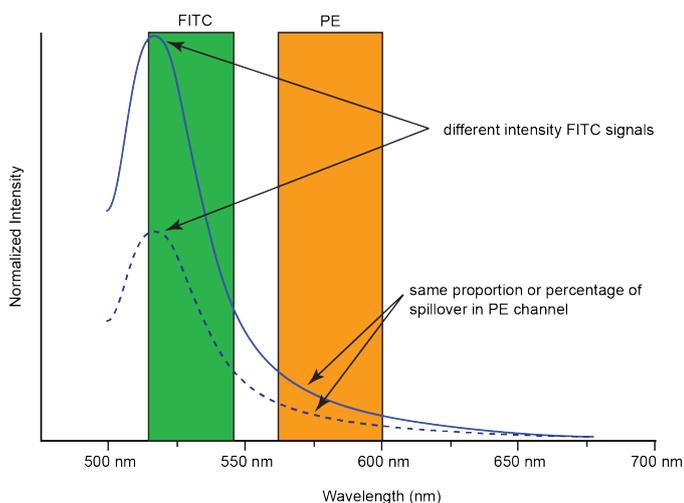


**Figure A-11 Theoretical Display of FITC vs. PE without Compensation**



**Figure A-12 FITC Spillover Optimally Compensated out of the PE Signal**

Once fluorescence compensation has been set using compensation control samples, such compensation settings remain valid for any subsequent sample, because compensation subtracts a percentage of the fluorescence signal, independent of the overall fluorescence intensity. Figure A-13 illustrates this principle. Although the signals differ in intensity, the percentage of the FITC spillover into the PE detector remains constant.



**Figure A-13 Spillover of Two FITC Signals of Different Intensities into PE Channels**

The NovoExpress™ software provides flexible fluorescence compensation functions. Users can modify the compensation matrix manually or use the automatic compensation function. Fluorescence compensation can be conducted during or after sample acquisition.



For more information on how to conduct fluorescence compensation, please refer to 3.5 *Perform Fluorescence Compensation* section in this guide and *NovoExpress™ Software Guide* for details.

## Electronic System

As cells or other particles pass through a focused laser beam, they scatter the laser light and can emit fluorescence. Because the laser beam is focused on a small spot and particles move rapidly through the flow cell, the scatter or fluorescence emission has a very brief duration, usually only a few microseconds. This brief emission of light is converted into an electrical signal (a pulse shown in Figure A-14) by the optical detectors (PD for scattered light and PMT for fluorescent light).

- 1 A pulse begins when a particle enters the laser beam. At this point, both the beam intensity and signal intensity are low.
- 2 The pulse reaches a maximum intensity or height when the particle reaches the middle of the beam, where the beam and signal intensity are the brightest. The peak intensity, or height of the pulse, is measured at this point.
- 3 As the particle leaves the beam, the pulse trails off below the threshold.

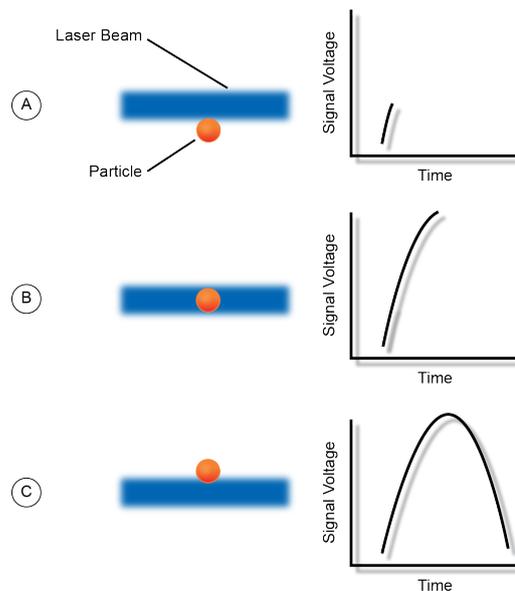
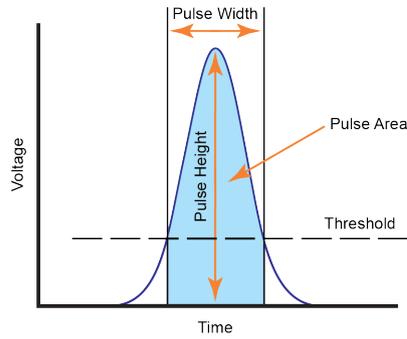


Figure A-14 Illustration of Pulse Generation

## Pulse Measurements

The electronic system measures pulses by three characteristics: Height, Area, and Width, as shown in Figure A-15.



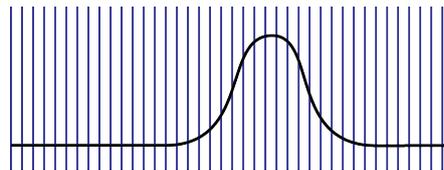
**Figure A-15 Pulse Measurements**

- ▶ Pulse Height is the maximum digitized intensity measured for the pulse.
- ▶ Pulse Area is an integration of the digitized measures of the pulse over time.
- ▶ Pulse Width is a time period within which the pulse measurement is above the threshold.

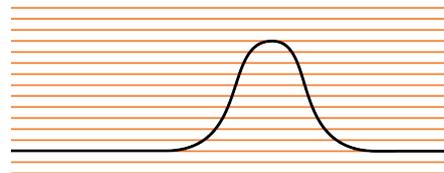
ACEA NovoCyte™ calculates the Height and Area for FSC, SSC, and every selected fluorescence channel. Width is based on the FSC signal. One effective event has one Width parameter.

### Pulse Digitization

The ACEA NovoCyte™ electronic system digitizes the pulse produced by the detector. The digitized data is stored in memory and further processed to calculate the pulse Height, Area, and Width. A high-accuracy high-speed 24-bit analog-to-digital convertor is used to process the pulse in a speed of 80 million times per second. Each pulse signal is divided into 16,777,216 (2<sup>24</sup>) levels (Figure A-16). The Height, Area, and Width parameters of all available channels for each processed cell or particle are then transferred to the NovoCyte™ workstation for further display and processing by the NovoExpress™ software.



Signal Sampling Rate at 80M per second



24 bits (16,777,216) Digitization

**Figure A-16 Pulse Digitalization**

## Appendix B Accessories and Consumables

To order spare parts and consumables for NovoCyte™ flow cytometer:

- ▶ Within US, call 1-866-308-2232 or visit [www.aceabio.com](http://www.aceabio.com).
- ▶ Within China, call 400-600-1063 or visit [www.aceabio.com.cn](http://www.aceabio.com.cn).
- ▶ Users in other countries or regions, please contact your local ACEA Biosciences distributors.

For a complete list of distributors, please visit [www.aceabio.com](http://www.aceabio.com) (USA), [www.aceabio.com.cn](http://www.aceabio.com.cn) (China).

### NovoCyte™ Flow Cytometer

Flow Cytometer	Catalog No.
NovoCyte™ 1000	2010001
NovoCyte™ 2000	2010002
NovoCyte™ 2060	2010004
NovoCyte™ 3000	2010011



*Includes the NovoCyte™ flow cytometer with one year standard warranty and complimentary starter kit (10L 1X NovoFlow™ sheath fluid, 500 mL 1X NovoRinse™ solution, 500 mL 1X NovoClean™ solution, and 1 bottle of NovoCyte™ QC Particles).*

### Accessories and Replacement Parts

Item	Catalog No.
<b>Fluidic System Consumables and Kits</b>	
NovoFlow™ Sheath Fluid Inlet Filter	2030001
NovoFlow™ Sheath Fluid In-Line Filter	2030002
Waste Filter	2030003
Peristaltic Pump Tubing	2030004
NovoRinse™ Solution Inlet Filter	2030005
NovoClean™ Solution Inlet Filter	2030007
NovoCyte™ 1 Year Fluidic System Maintenance Kit (including 6 NovoFlow™ sheath fluid inlet filter, 12 NovoFlow™ sheath fluid in-line filter, 6 waste filters, 6 peristaltic pump tubing, 6 NovoRinse™ solution inlet filter, and 6 NovoClean™ solution inlet filter)	2030006
NovoCyte™ 6 Months Fluidic System Maintenance Kit (including 3 NovoFlow™ sheath fluid inlet filter, 6 NovoFlow™ sheath fluid in-line filter, 3 waste filter, 3 peristaltic pump tubing, 3 NovoRinse™ solution inlet filters, and 3 NovoClean™ solution inlet filter)	2030008



Item	Catalog No.
<b>Accessories</b>	
Spigot for NovoFlow™ Sheath Fluid Refill	2020002
NovoCyte™ Fluidics Station	2020003
NovoFlow™ Sheath Fluid Container (3L)	2020004
Waste Container (3L)	2020005
NovoRinse™ Solution Container (500 mL)	2020006
NovoClean™ Solution Container (500 mL)	2020007
Fluidic Container Quick Coupler and Tubing Set	2020008
<b>Sample Holder</b>	
12 x 75 mm Tube Holder	2020010
Eppendorf Tube Holder	2020011
<b>Workstations and Cables</b>	
NovoCyte™ Workstation (NovoExpress™ pre-installed)	2020015
NovoCyte™ Workstation Monitor	2020016
NovoCyte™ Instrument Power Adapter	2020017
NovoCyte™ Instrument Power Cord Set	2020044
USB Cable (NovoCyte™ Instrument to Workstation)	2020021
DVI Cable (NovoCyte™ Instrument to Fluidics Station)	2020022
<b>Software</b>	
NovoExpress™ Software, Single License	1010013
<b>Auto-sampling</b>	
NovoSampler™	2020001
NovoSampler™ Connection Cable	2020038
NovoSampler™ 24 Tube Rack	2020014

## Reagents and Kits

Item	Catalog No.
NovoCyte™ QC Particles (2mL; 50 tests)	8000001
SPHERO™ 8 Peaks Rainbow Calibration Particles	8000002
SPHERO™ 1 Peak Ultra Rainbow Fluorescent Particles	8000003
NovoCyte™ Flow Cytometer Starter Kit (including NovoFlow™ sheath fluid 1X 10L, NovoRinse™ solution 1X 500mL, NovoClean™ solution 1X 500mL, NovoCyte™ QC Particles 2mL 50 tests)	8100001

Item	Catalog No.
NovoFlow™ Sheath Fluid (1X, 10 L)	875B601
NovoFlow™ Sheath Fluid (6X, 10 L)	875B608
NovoClean™ Solution (1X, 500 mL)	872B602
NovoClean™ Solution (5X, 500 mL)	872B609
NovoClean™ Solution (5X, 100 mL)	871B609
NovoRinse™ Solution (1X, 500 mL)	872B603
NovoRinse™ Solution (5X, 500 mL)	872B610
NovoRinse™ Solution (5X, 100 mL)	871B610

## Appendix C Technical Specifications

### Lasers

Manufacturer	Wavelength	Model	Power
Coherent	405nm	OBIS	50mW
	488nm	OBIS	20mW
	640nm	OBIS	40mW

	NovoCyte Platform									
	Model	1000			2060		3000			
	Lasers (nm)	488	488	640	488	640	405	488	640	
Detectors	445/45 nm						●			
	530/30 nm	●	●		●		●	●		
	572/28 nm	●	●		●		●	●		
	615/24 nm						●	●		
	675/30 nm	●	●	●	●	●	●	●	●	
	780/60 nm				●	●	●	●	●	
Optics	Laser Configuration	Spatially separated beams with 10 x 80 μm elliptical spots								
	Optical Alignment Procedure	Fixed, no operator alignment required								
	Flow Cell	170 x 290 μm rectangular quartz flow cell								
	FSC/SSC Resolution	FSC: 0.5 μm; SSC: 0.2 μm								
	Cell Size	0.2 - 50 μm								
	Fluorescence Threshold Sensitivity	FITC < 75 MESF; PE < 50 MESF								
	Fluorescence Resolution	< 3% CV for CEN								
Filters	User Exchangeable									
Fluidics	Sample Acquisition Rate	35,000 events/second								
	Volumetric Absolute Count Precision	< 5%								
	Sample Flow Rate	5-120 μL/min								
	Sheath Flow Rate	6.5 mL/min								
	Sample Aspiration Volume	10 - 100 μL								



	Fluid Container Capacity	3 L sheath, 3 L waste, 500 mL cleaning, 500 mL decontamination
	Carryover	< 0.1%
	Fluidics Maintenance	Automated startup, cleaning, decontamination and shutdown
Data Management	Parameters	Height and Area for FSC, SSC and all Fluorescent Channels, Width and Time
	Dynamic Range	24 bit; 7.2 decades logarithmic scale; no need for PMT voltage adjustment
	Compensation	Full inter-beam matrix, during or post acquisition
	Output Data Files	FCS 3.0; CSV; Batch Reports
	Computer Operating System	Microsoft Windows® 7 Professional (64 bit)
	Software	ACEA NovoExpress™
Sampling	Manual Sample Loading	12 X 75 mm tube (12 X 75 mm tube holder) Eppendorf tube (Eppendorf tube holder)
	Automatic Sample Loading	Optional - compatible with 12 x 75 mm tube, 24-well and 96-well microtiter plates
Operating Conditions	Instrument Dimension (W X D X H)	23.6 × 17.7 × 15.4 in. (60 × 45 × 39 cm)
	Instrument Weight	86.0 lb (39 kg)
	Power Requirements	100/115/230 VAC, 50-60 Hz
	Environment Requirements	Operating Temperature: 15-32 °C Relative Humidity: 80% maximum

### Fluidics Station Specifications

Dimension (W × D × H)	10.0 × 11.4 × 16.5 in. (25 × 29 × 42 cm)
Weight	13.2 lb (6 kg)
NovoFlow™ sheath fluid container	3 L
NovoClean™ solution container	500 mL
NovoRinse™ solution container	500 mL
Waste container	3 L

### NovoSampler™ Specifications

Dimension (W × D × H)	12.2 × 10.2 × 11.0 in. (31 × 26 × 28 cm)
Weight	6.6 lb (3 kg)
Compatibility	Compatible with standard 96 well plates (flat bottom, U-bottom, V-bottom), standard 24 well plates, and NovoCyte™ 24 tube rack for 12 × 75 mm tubes

## **Appendix D      Limited Product Warranty**

ACEA Biosciences warrants that all standard components of its NovoCyte™ flow cytometer will be free of defects in materials and workmanship for a period of one (1) year after the date of installation, and the software will be free of substantial programming errors or defects when properly installed. ACEA Biosciences will repair or replace, at its discretion, all defective components during this warranty period. ACEA Biosciences reserves the right to use new, repaired, or refurbished instruments or components for warranty service agreement replacements. Information on warranty conditions is specified in the sales contract. Contact ACEA Biosciences representative for further information.

Any unauthorized modification of the instrument invalidates the guarantee and service contract





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