**ACEA**

# NovoCyte® Flow Cytometer Operator's Guide

# Prologue

## I.　　Revision History

| Version | Revision Date |
|---------|---------------|
| 1.0 | 2014.04 |
| 1.1 | 2014.07 |
| 1.2 | 2014.11 |
| 1.3 | 2015.04 |
| 2.0 | 2015.12 |
| 2.1 | 2016.08 |
| 2.2 | 2017.05 |
| 2.3 | 2018.08 |

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

ACEA Biosciences, Inc.
6779 Mesa Ridge Road, Suite 100
San Diego, CA 92121
USA

## II.  Contact Information

| | | |
|---|---|---|
| ◣◣◣ | Manufacturer | ACEA Biosciences, Inc.<br>6779 Mesa Ridge Road, Suite 100<br>San Diego, CA 92121<br>USA |
| | China Distributor | ACEA Bio (Hangzhou) Co., Ltd.<br>Building 5, 2# Xiyuan Wu Rd., Xihu District<br>Hangzhou, Zhejiang Province, P. R. China<br>310030 |
| | International Distributors | Please contact ACEA Biosciences, Inc., or visit 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., in the United States and some other countries.

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 405 nm, 488 nm, 561 nm and 640 nm 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 standard configuration, please refer to the Appendix B: NovoCyte Laser and Detection Channel Configuration section in this guide. Customized model or configuration is also available. Please contact ACEA Biosciences Inc. local sales representatives or distributors for customized model request.*

## 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 ① ② ...... | Describes the steps in a procedure that must be performed in the listed order. |
| *Italic Font, gold* | Points to a different chapter in this guide, which should be referred to for better understanding. |
| *Italic Font* | 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, *File → Print* means to select the *Print* function from the *File* 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:
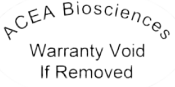
| Symbol | Meaning | Description |
|---|---|---|
| ⚠ (biohazard) | BIOHAZARD | This symbol indicates that certain precautions must be taken when working with potentially infectious biological specimens and materials. |
| ⚠ (laser) | LASER RADIATION | This symbol indicates that the operation could potentially cause exposure to laser radiation. Protective measures should be taken during the operation. |
| ⚠ (warning) | WARNING | This symbol indicates that improper or unsafe operation could result in instrument damage, data loss, and personal injury. |
| ⚠ (shock) | SHOCK HAZARD | This symbol indicates that the operation has a risk of electric shock. |
| ! (important) | IMPORTANT NOTE | This symbol indicates information which is critical to the success of the procedure or use of the product. |
| 🔍 (additional) | 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. |
| SN | 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 | 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. |
| SGS | SGS conformity marking | This symbol indicates conformity with the provisions of relevant directives for SGS 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, NovoSampler or NovoSampler Pro to indicate a potential hazard. Please do not remove these labels. Use appropriate precaution to avoid injury by the indicated hazard. Please refer to *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. |
| CAUTION: MOVING PARTS | MOVING PARTS | This label indicates the moving parts in the instrument. Be careful during the operation. |
| CAUTION: CLASS 1 LASER PRODUCT | CLASS I LASER PRODUCT | This symbol indicates the laser is a Class I product (see *Prologue VIII Safety and Limitations: Laser Product Classification* in this guide for more information.) |
| PINCH POINT KEEP HANDS CLEAR | 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. |
|---|---|---|
| ACEA Biosciences Warranty Void If Removed | | |

# 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 |
| BXXX | 488nm (blue) laser excited fluorescence channels or optical detectors. For example, B530 denotes the 530/30 channel excited by the 488nm laser, and so on. |
| RXXX | 640nm (red) laser excited fluorescence channels or optical detectors. For example, R675 denotes the 675/30 channel excited by the 640nm laser, and so on. |
| VXXX | 405nm (violet) laser excited fluorescence channels or optical detectors. For example, V445 denotes the 445/45 channel excited by the 405nm laser, and so on. |
| YXXX | 561 nm (yellow) laser excited fluorescence channels or optical detectors. For example, Y586 denotes the 586/20 channel excited by the 561 nm laser, and so on. |
| PMT | Photomultiplier Tube |
| PD | Photodiode |
| 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 |
| PE | Phycoerythrin |
| PE-Texas Red® | Phycoerythrin-Texas Red |
| PerCP | Peridinin chlorophyll protein |
| PE-Cy™7 | PE and Cyanine 7 tandem dye |
| APC | Allophycocyanin |
| APC-Cy™7 | APC and Cyanine 7 tandem dye |

| Glossary Abbreviation | Meaning |
|---|---|
| EYFP | Enhanced Yellow Fluorescent Protein |
| PI | Propidium Iodide |
| PE-Cy™5 | Phycoerythrin and Cyanine 5 Tandem Dye |
| PE-Cy™5.5 | Phycoerythrin and Cyanine 5.5 Tandem Dye |
| PerCP-Cy™5.5 | Peridinin Chlorophyll Protein and Cyanine 5.5 Tandem Dye |
| BV711™ | Brilliant Violet 711 |
| 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 |

Texas Red® is a registered trademark of Molecular Probes, Inc.
Cy™ is a trademark of Amersham Biosciences Corp.
BV711™ is a trademark of Sirigen Group, Ltd.

# 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.

► If the power cord becomes frayed, broken, or damaged, please contact ACEA Biosciences. Do not replace with a lower rated cord. If the equipment is been used in a manner which is not specified by ACEA Biosciences, the protection provide by the equipment may be impaired.

## 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, eyeware, 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.*

⚠ *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 you need to move the instrument, be sure to contact ACEA Biosciences professionals. ACEA Biosciences professionals will re-install the instrument in accordance with requirements and ensure the optimal operation of the instrument.*

⚠ *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 and NovoSampler Pro have mechanical movable parts that can pinch or injure your fingers. To prevent accidents when running samples using the NovoSampler and NovoSampler Pro, be sure to close the cover. Only when placing the sample plate onto the NovoSampler or NovoSampler Pro or during routine maintenance, should the cover be opened. Even though the NovoSampler and NovoSampler Pro will automatically stop running when the cover is opened during operation, always confirm that the NovoSampler or NovoSampler Pro 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

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.*

⚠ *Do not use the expired consumables and 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.

*When encountering an issue during operation of NovoCyte flow cytometer, NovoSampler or NovoSampler Pro, it is highly recommended to submit a Technical Support Request by clicking* **Home → Technical Support Request** *in the NovoExpress main window. The Technical Support Request Creation Wizard automatically collects NovoCyte configurations, NovoExpress system logs, current screenshot, current experiment file and other information that helps in the diagnosis and troubleshooting of NovoCyte instrument. User can also attach any other files using this function. Please refer to the NovoExpress® Software Guide for detailed information on this function.*

# 1. Introduction

The following topics are covered in this chapter:

► Overview

► System Components and Installation

► Basic Principle

► Fluidics Station

► NovoCyte Workstation

► NovoSampler and NovoSampler Pro (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 μm ~ 50 μm. 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 and an optional NovoSampler Pro 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.



Fluidics Station    NovoCyte® Instrument    NovoSampler®    NovoCyte® Workstation
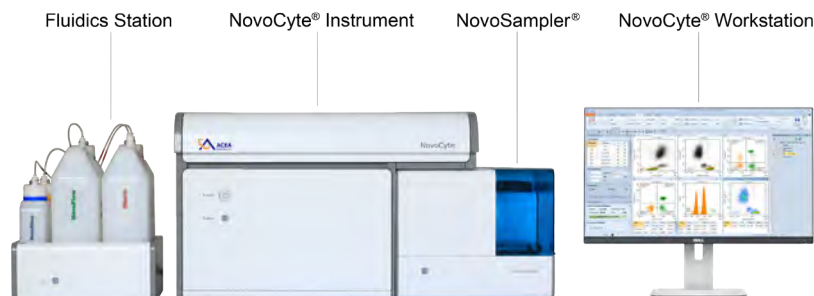
**Figure 1-1 (a) NovoCyte Flow Cytometer with NovoSampler**
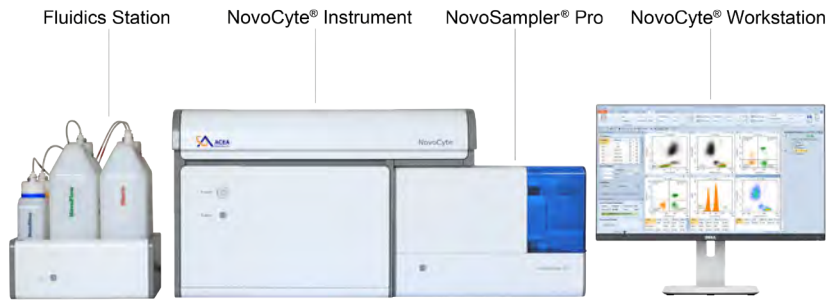
**Figure 1-1 (b) NovoCyte Flow Cytometer with NovoSampler Pro**

*For detailed information about the operation, maintenance, and troubleshooting of the NovoSampler and NovoSampler Pro, refer to NovoSampler® Operator's Guide or NovoSampler® Pro Operator's Guide.*
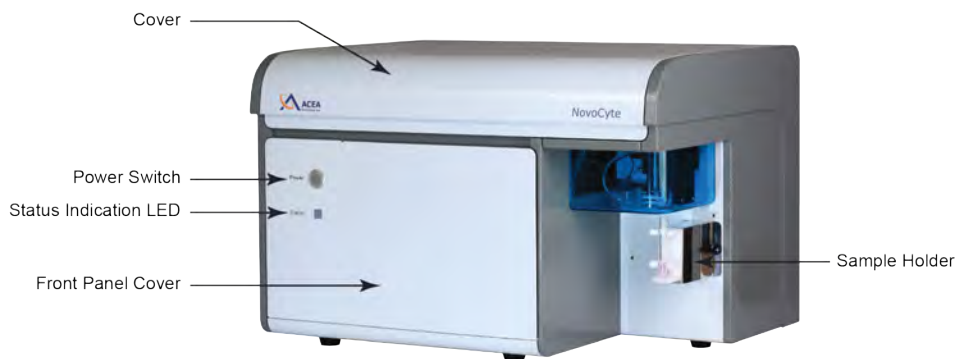


**Figure 1-2 NovoCyte Instrument (Front View)**



**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 and 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.
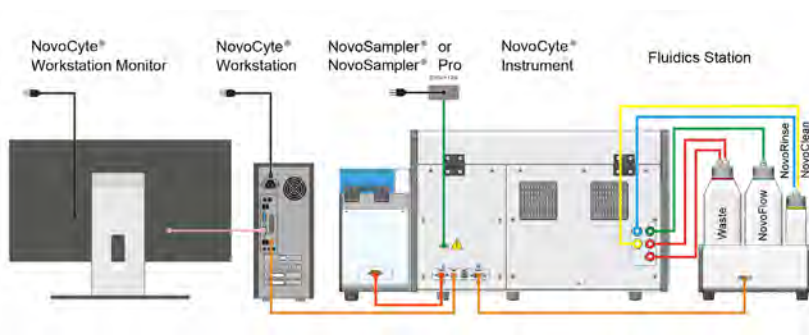
**Figure 1-4 NovoCyte Flow Cytometer System Connection**

### 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 or NovoSampler Pro (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**

⚠️ *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, Novo-Clean, Waste 1, and Waste 2, respectively. ACEA NovoRinse is the rinsing solution and is connected by the blue tubing. ACEA NovoFlow is the sheath fluid and is connected by the green tubing. ACEA NovoClean is the decontamination solution and is connected by the

yellow tubing. Waste 1 and Waste 2 connect to the red tubing.
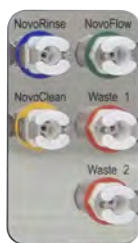


**Figure 1-6 Interface of the Fluidics Port**

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 or NovoSampler Pro (optional)**

Please refer to *NovoSampler® Operator's Guide* or *NovoSampler® Pro Operator's Guide* for the detailed procedure.

⚠ *For specific requirements of the operating conditions, please refer to the Appendix C: NovoCyte 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, or a NovoSampler Pro, it needs to be uninstalled from the instrument. Moving the instrument with the NovoSampler or NovoSampler Pro 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*, *Appendix B: NovoCyte Laser and Detection Channel Configuration* and *Appendix C: NovoCyte 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 up to three lasers for each instrument to meet the needs for different applications. Table 1-1 shows the laser configuration for the standard NovoCyte models and typical fluorochromes that could be detected off different lasers. Customized model other than the standard configuration is available. Please contact ACEA Biosciences Inc. local sales representatives or distributors for customized model request.

| Model Number | | 1000/ 1040/ 1050 | 1000Y | 2000R/ 2060R/ 2070R | 2070V/ 2090V/ 2110V | 2100YB | 3000/ 3005 | 3000VYB | 3000RYB |
|---|---|:---:|:---:|:---:|:---:|:---:|:---:|:---:|:---:|
| **Lasers** | 405 nm | | | | ◆ | | ◆ | ◆ | |
| | 488 nm | ● | | ● | ● | ● | ● | ● | ● |
| | 561 nm | | ■ | | | ■ | | ■ | ■ |
| | 640 nm | | | ▲ | | | ▲ | | ▲ |

**Table 1-1 (a) Laser Configuration for Standard NovoCyte Model**

| Laser Wavelength (nm) | Typical Fluorescent Labels |
|---|---|
| 405 | Pacific Blue™, AmCyan, Pacific Orange™, Qdot® 605, Qdot® 705, Qdot® 655, Qdot® 800, BV711™ |
| 488 | FITC, PE, PI, PE-Texas Red®, PerCP, PerCP-eFluor®710, PerCP-Cy™5.5, EYFP, PE-Cy™5, PE-Cy™7 |
| 561 | PE, PE-Texas Red®, PE-Cy™5, PE-Cy™5.5, PE-Cy™7, PE-Alexa Fluor® 700 |
| 640 | APC, APC-Cy™7, Alexa Fluor® 680, Alexa Fluor® 700 |

**Table 1-1 (b) Laser Wavelength and Detectable Fluorochromes on NovoCyte**

**1**

Pacific Blue™ and Pacific Orange™ are trademarks of Molecular Probes, Inc.

Alexa Fluor® and Texas Red® are registered trademarks of Molecular Probes, Inc.

Qdot® is a registered trademark of Quantum Dot Corporation.

eFluor® is a registered trademark of eBioscience, Inc.

### 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 photodiodes (PD) to detect the forward and side scatter light, and photomultiplier tubes (PMT) to detect the fluorescence signal.

### 1.3.2     Fluidic System

The fluidic system consists of the sample injection probe (SIP), SIP cleaning 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 solutions (i.e. ACEA NovoRinse and ACEA NovoClean) are used to clean and sterilize the fluidic system.

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

The sample injection probe, which is 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 cleaning 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 cleaning function ensures minimal inter-sample carryover rate (<0.1%) and eliminates tedious manual cleaning processes.
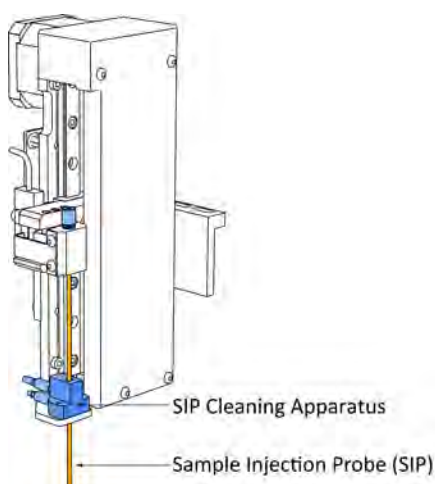
**Figure 1-8 Sample Injection Probe (SIP) and SIP Cleaning Apparatus**

*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. Wear gloves when handling the sample injection probe.*

*If the NovoSampler or NovoSampler Pro is installed, make sure the NovoSampler or NovoSampler Pro 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, which is Slow (14 µL/min), Medium (35 µL/min) and Fast (66 µL/min). The sheath flow rate is fixed at 6.5 mL/min, which corresponds to approximately 7.7 µm, 12.2 µm and 16.8 µm 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 µL/min to 120 µL/min, which corresponds to a core diameter of 4.6 µm to 22.7 µm. Sample flow rate can be easily set in the NovoExpress software.

*To set the flow rate, please refer to Section 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 volumetric absolute counting results.

The NovoCyte aspirates a certain volume of the sample set by the user in the NovoExpress software, plus an additional volume. When the injection volume is less than 100 μL, sample injection can be done by one aspiration. When the injection volume is greater than 100 μL, 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.
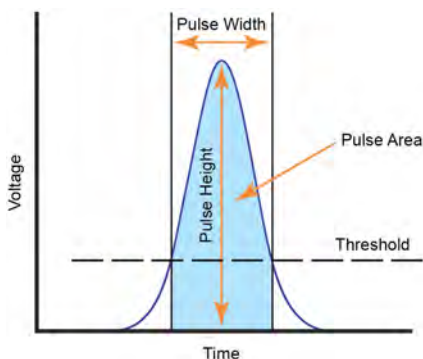


**Figure 1-9 Pulse Measurements**

### 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 shutdown cleaning procedure. |

▶▶▶

| Name | Color | Meaning |
|------|-------|---------|
| 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 occur, follow the instructions in the prompted dialog box, and refer to Section 5 Troubleshooting in this guide for troubleshooting methods.*

## 1.4　Fluidics Station

The NovoCyte flow cytometer comes with one ACEA NovoFlow sheath fluid container (3 L), one ACEA NovoRinse solution container (500 mL), one ACEA NovoClean solution container (500 mL), and one 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).
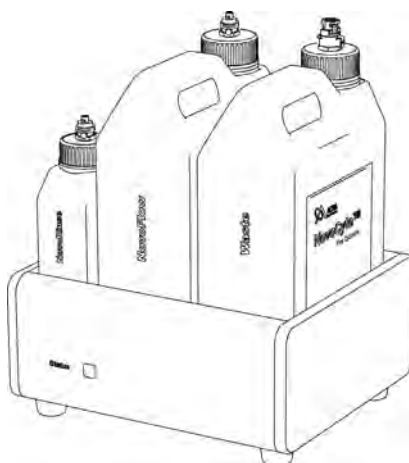


**Figure 1-10 Fluidics Station**

► Sheath fluid: For forming a sheathing layer to hydrodynamically focus the sample stream. Use 1X ACEA NovoFlow (Cat # 875B601) directly as sheath fluid or dilute 100X ACEA NovoFlow (Cat # 871B607) one hundred folds with filtered, deionized water.

► ACEA NovoRinse solution: For cleaning the fluidic system.

► ACEA NovoClean solution: For cleaning and removing protein deposits, adherent cell debris, etc. in the fluidic system.

When the volume of ACEA NovoFlow fluid, ACEA NovoRinse solution, or ACEA NovoClean solution is less than the set threshold (ACEA NovoFlow 0.9 L, ACEA NovoRinse 150 mL, ACEA 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 | Reagent volume is above the designated threshold and is sufficient to run experiments (ACEA NovoFlow > 0.9 L; ACEA NovoRinse > 150 mL; ACEA NovoClean > 150 mL), and the waste container is not full (< 2.8 L). |
| | Orange | Reagent volume is below the threshold, but is still sufficient to finish the current running experiment. (0.7 L < ACEA NovoFlow < 0.9 L; 100 mL < ACEA NovoRinse < 150 mL; 100 mL < ACEA NovoClean < 150 mL), or the waste container is close to full (2.8 L < waste < 3 L). Please add more reagents to the corresponding container or empty the waste container. |
| | Red | Reagent volume is too low to run experiments (ACEA NovoFlow < 0.7 L; ACEA NovoRinse < 100 mL; ACEA 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**



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

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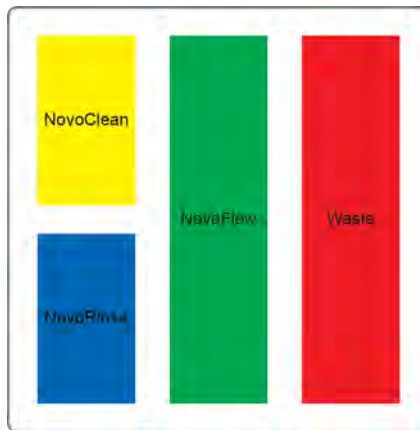
**Figure 1-12 Schematic of the Container Position in the Fluidics Station**

⚠️ *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 brand desktop or laptop with Microsoft Windows® operating system, Microsoft Office, and NovoExpress software preinstalled. For details about the workstation, please refer to the computer supplier's 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 and NovoSampler Pro (Optional)

The NovoSampler (Figure 1-13 (a) ) and the NovoSampler Pro (Figure 1-13 (b) ) are optional accessories of the NovoCyte flow cytometer, which is used for automatic collection of multiple samples from a microtiter plate or from $12 \times 75$ mm tubes using ACEA NovoSampler 24 tube rack.



**Figure 1-13 (a) NovoSampler**

**Figure 1-13 (b) NovoSampler Pro**

The NovoSampler and NovoSampler Pro are compatible with the following plates and tubes:

► 12 × 75 mm tube (with ACEA NovoSampler or NovoSampler Pro 24 tube rack)

► Standard 96-well plates (V-bottom)

► Standard 96-well plates (U-bottom)

► Standard 96-well plates (flat-bottom)

► Standard 48-well plates

► Standard 24-well plates

⚠ *Use only the ACEA manufactured 24 tube rack on the NovoSampler and NovoSampler Pro. 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.*

🔍 *For details about the NovoSampler Pro, please refer to the NovoSampler® Pro 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: NovoCyte 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

ACEA NovoFlow Sheath Fluid

ACEA NovoClean Solution

ACEA NovoRinse Solution

*Please consult the ACEA website or contact your local representative for more detailed ordering information.*

### 1.7.5    QC Particles

ACEA NovoCyte QC Particles

*Please consult the ACEA website or contact your local representative for more detailed ordering information.*

# 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 or NovoSampler Pro (if applicable), and the workstation to the NovoCyte instrument are correct and secure.

Check the ACEA NovoFlow sheath fluid container, the ACEA NovoRinse solution container, and the ACEA 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 300 mL of bleach to the waste container.

### 2.1.2 Add Instrument Reagents

**1** Make sure the instrument is in *Ready* or shut down 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 <span style="color:orange">Section 2.2.5. Priming</span> 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 300 mL of bleach 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 or multiple NovoExpress software is open on the NovoCyte workstation with the NovoCyte flow cytometer connected, only the software opened first 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 NovoExpress softwares 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.*

### 2.1.6    Verify and Modify Instrument Configuration

Each NovoCyte instrument may have more than one compatible optical configuration. It is recommended for users to view and verify the current optical configuration before each experiment.

To view current optical configuration, click *Instrument → Configuration* to open the Instrument Configuration window.

If optical configuration other than the current configuration is needed, follow the instructions below to modify the optical configuration.

**1** Ensure the instrument is properly connected and powered on. Click *Instrument → Configuration* to open the Instrument Configuration window.

**2** Click the *Optical Configuration* panel. Click and select the desired optical configuration as shown in Figure 2-1. Click *OK* to continue.

*By default, only the standard configurations (i.e. recommended by ACEA) will be displayed in this window. If configurations other than the ones listed in this window are needed, follow the instructions in **Step 3**.*
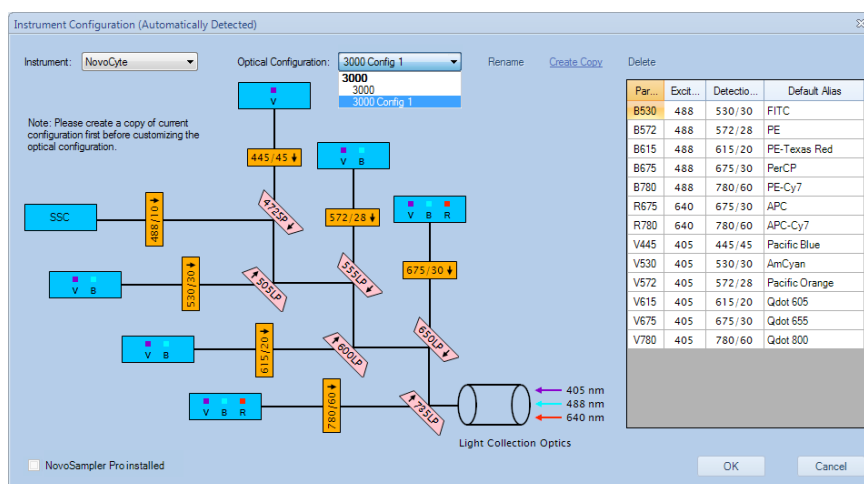


**Figure 2-1 Instrument Configuration Window**

**③** If optical configurations other than the ones listed by the software are needed (i.e. users need create their own customized configuration), the *Customize Optical Configuration* in the *Access Privilege* window for the current user account needs to be enabled first as described in *Section 2.6.2.3* in *NovoExpress® Software Guide*. To make the customized optical configuration, click *Create Copy* in the *Instrument Configuration* window (Figure 2-2) to generate an editable copy of the configuration. Click *Rename* to rename the configuration if needed. Click the bandpass filter or dichroic mirror to be replaced and select the appropriate filter or mirror from the pop-up list. Once all the filters and mirrors desired to be replaced have been edited, click *OK* to continue. A customized optical configuration can also be deleted by clicking *Delete*.

🔍 *Only the user-defined optical configurations can be deleted.*
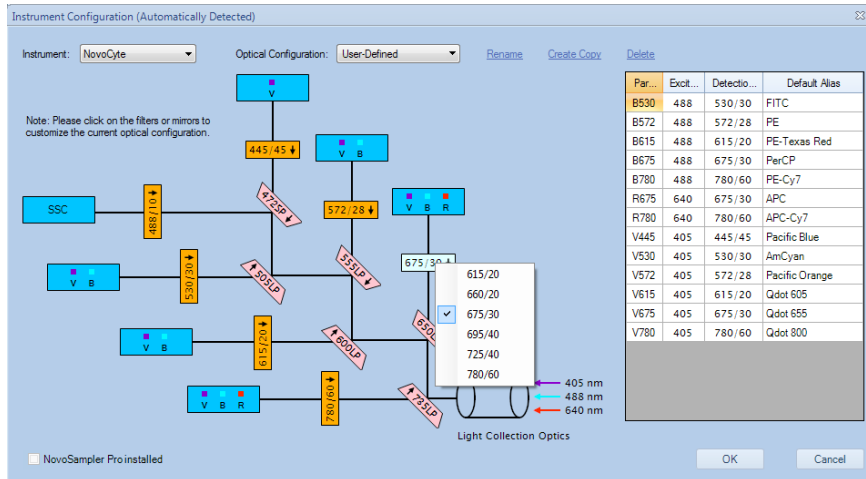


**Figure 2-2 Customize the Instrument Configuration**

🔍 *The software will automatically check the validity of user-defined configuration. The following error message (Figure 2-3) will pop up if the user-defined optical configuration is not valid.*
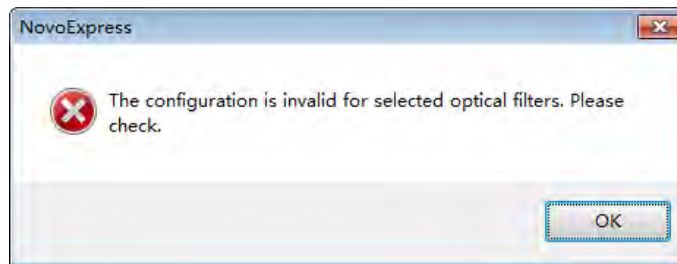


**Figure 2-3 Dialog Box for Instructions on Properly Applying New Optical Configurations**

▶▶▶

**4** Ensure to read the instructions from the prompted window as shown in Figure 2-4. To change the optical filters, press the power button on the front panel of NovoCyte to turn off the instrument first. Insert the NovoCyte key into the keyhole on the left side of the instrument to open the top cover of the instrument.
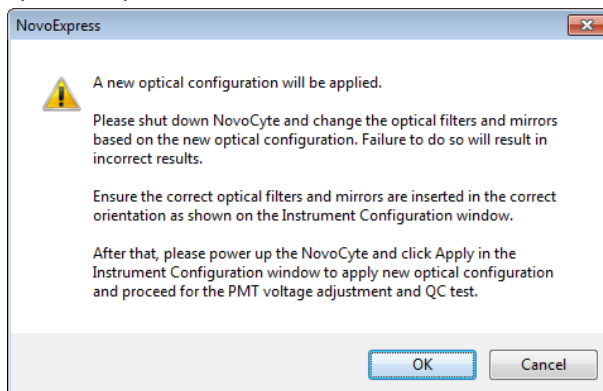


**Figure 2-4 Dialog Box for Instructions on Properly Applying New Optical Configurations**

Remove the two mounting screws and open the cover of the filter module as shown in Figure 2-5.
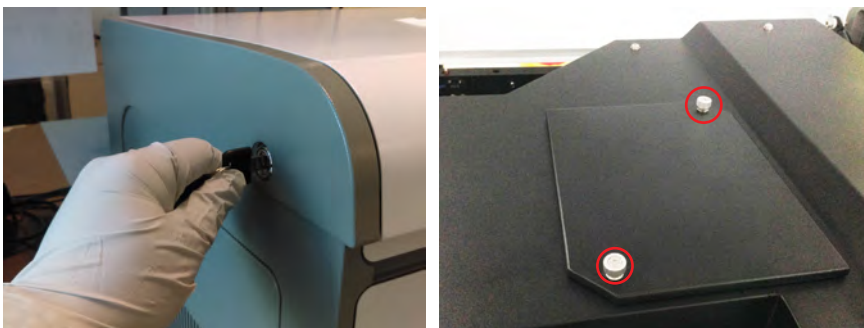


**Figure 2-5 Open the Cover of the Filter Module**

**5** Gently hold the selected optical filter or dichroic mirror and pull it upward to remove it from the slot. Insert the new optical filter or dichroic mirror into the slot as shown in Figure 2-6. Record the position, wavelength and arrow direction for each optical filter and dichroic mirrors. Install the filter module cover by screwing in the two mounting screws. Close the top cover of the instrument. Press the power button on the front panel of NovoCyte to turn on the instrument.

*Ensure all the new optical filters and mirrors are fully inserted in the correct filter slot and in the correct orientation as indicated by the arrows (i.e. the arrow of each optical filter and dichroic mirror should point away from the corresponding PMT detector).*
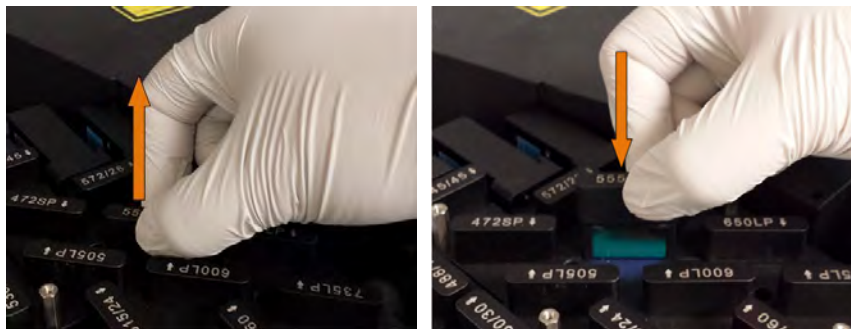


**Figure 2-6 Remove the Old Filter and Insert the New Filter**

**6** Wait until the instrument initialization process is completed. Click *OK* in the prompted window shown in Figure 2- 4 to continue.

**7** Ensure that the recorded optical configuration (i.e. the position, wavelength, and the orientation of each bandpass filter and dichroic mirror) matches the schematic of the selected optical configuration. Click *Apply* to continue.
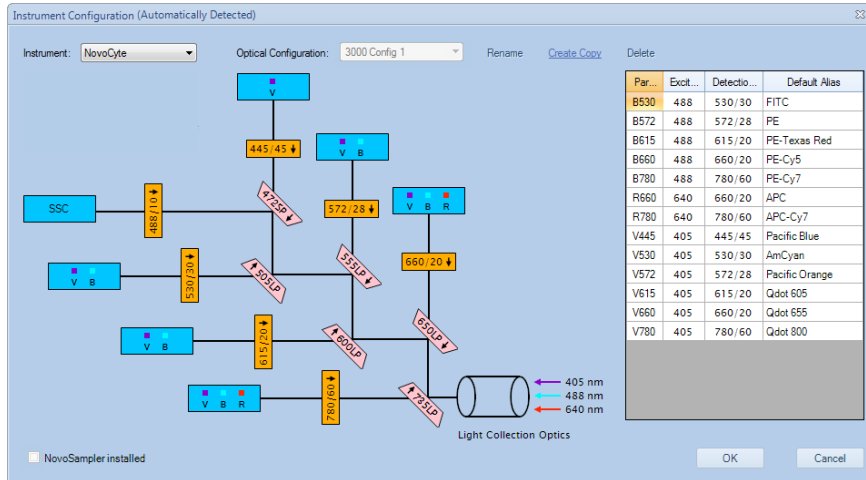


**Figure 2-7 Confirm and Apply the New Optical Configuration**

**8** Click *Restart* in the next window (Figure 2-8) to restart the software to apply the new optical configuration setting.
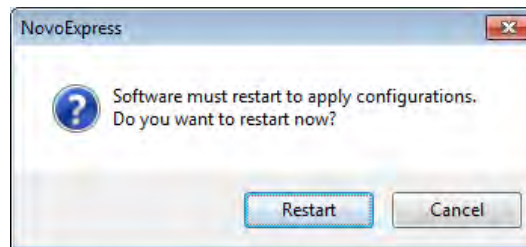


**Figure 2-8 Dialog Box for Restarting the Software**

**9** After NovoExpress is restarted, the following window (Figure 2-9) will appear. Click *QC Test* to continue.
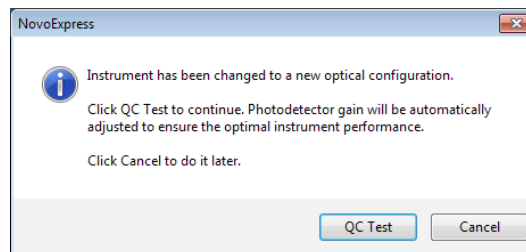


**Figure 2-9 Dialog Box for Performing the QC Test**

▶▶▶

**10** Properly prepare 1 mL ACEA QC Particles sample as described in the *Section 2.3.1: Pre-pare ACEA NovoCyte QC Particles Sample* in this guide. Place the sample tube in the tube holder or NovoSampler (Pro). Fill in the test information in the pop-up window (Figure 2-10). Click *Next* to continue.



**Figure 2-10 Fill in the Test Information for QC Test**

**11** Ensure all the test information is correct and the sample tube is properly installed on the tube holder. Click *Run* to start (Figure 2-11). The software will automatically adjust the photodetector gain by running the QC Particles. When the adjustment is completed, the software will automatically perform the QC Test (Figure 2-12). Click *Report* when the QC test is completed.
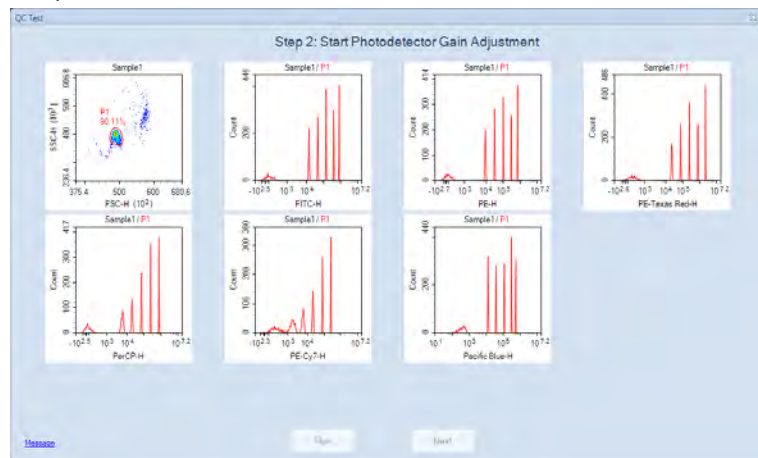


**Figure 2-11 Automatic Photodetector Gain Adjustment**
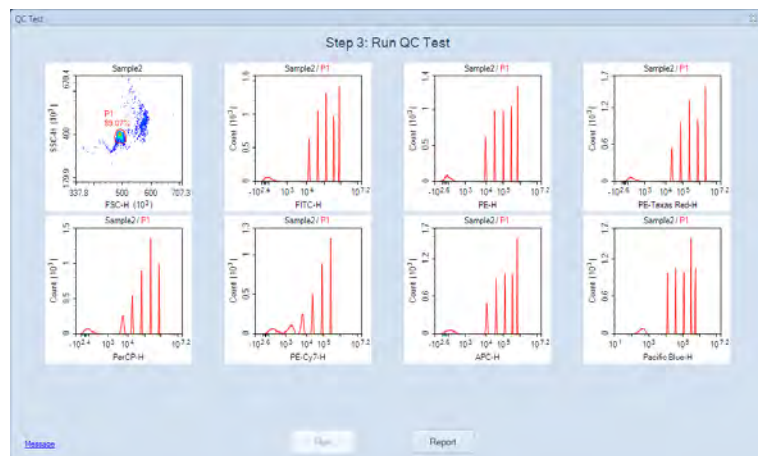


**Figure 2-12 Automatic QC Test**

**12** Ensure the result shows *Pass* for all the channels. Click *Finish* to close the window and complete the optical configuration modification (Figure 2-13).
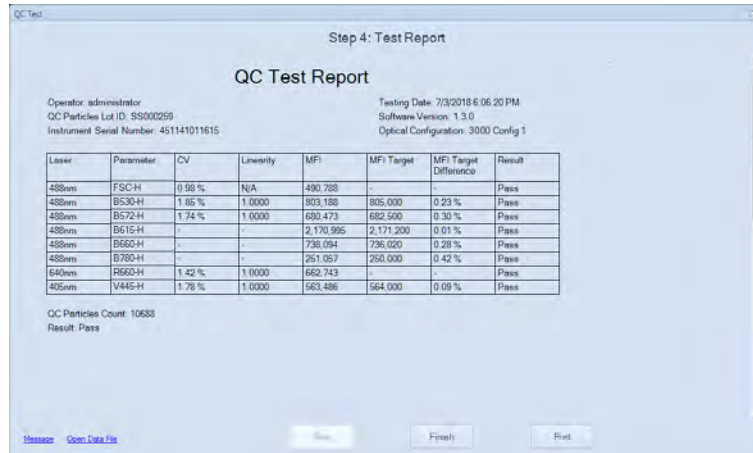


**Figure 2-13 QC Test Report**

*If the result of certain detection channels shows **Acceptable** or **Failed** as below, make sure the optical filters configured in the hardware matches with the selected optical configuration in the software and the QC Particles are properly prepared. Run the QC Particles once again after the correct actions have been taken. Ensure there is at least 300 µL sample remaining in the sample tube. Click **Repeat Test** to repeat the QC test (Figure 2-14). The photodetector gain will be automatically re-adjusted.*

*Please contact ACEA technical support if the QC test failed for three times in a row.*
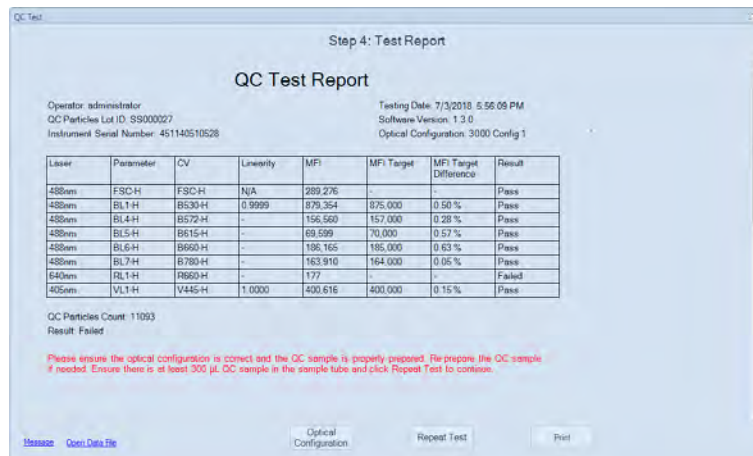


**Figure 2-14 QC Report Indicating Failed QC Test**

## 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 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* icon, and the sample injection tube will aspirate the ethanol to initialize the debubble process.

⚠ *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 ACEA NovoClean solution and ACEA NovoFlow sheath fluid in sequence to flush the entire fluidic system.

### 2.2.3    Rinse

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

The *Rinse* process uses ACEA NovoFlow sheath fluid to flush the entire fluidic system.

### 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 ACEA NovoRinse into the fluidic system, soaks the fluidic tubing for a certain period of time, then flushes the fluidic system with ACEA NovoFlow sheath fluid.

### 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 ACEA NovoRinse solution and ACEA NovoFlow sheath fluid in sequence to flush the entire fluidic system.

### 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* process once a month as a preventative measure.

The *Unclog* process uses ACEA 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.

### 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 cleaning apparatus.

### 2.2.8    Purge

When the NovoCyte flow cytometer need to be shipped or will not be used for longer than a month, use this function to purge the fluidic system. Only accounts with *Purge Instrument* privilege can perform the purge function.

### 2.2.9    Decontamination

When the NovoCyte flow cytometer is known to have bacterial contamination or preventing the occurrence of bacterial contamination is needed, use this function to decontaminate the fluidic system. Only accounts with *Decontaminate Instrument* privilege can perform the decontamination function.

## 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.

*The fluorescence channel configuration varies with different NovoCyte models. Please refer to Appendix B for standard configurations of each model. In the following sections, NovoCyte 3000 default system is used as an example to describe the detailed procedures.*

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. 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 Particles 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 ACEA 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 two drops of ACEA NovoCyte QC Particles (Generation 2, Cat. # 8000004) 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 Particles sample is not intended to be run immediately, store the sample in a 2-6 °C refrigerator away from light. The prepared QC Particles sample should be used within four hours after preparation.

■

*Make sure the QC Particles sample is prepared properly following the above procedure. Properly prepared QC Particles 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). 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 2-15.



**Figure 2-15 QC Test: Step 1 - Fill out Test Information**

**4**  Enter the operator's name in the dialog box, select the NovoCyte QC Particles 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.

**5**  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 2-16. The QC test takes about 2 minutes.
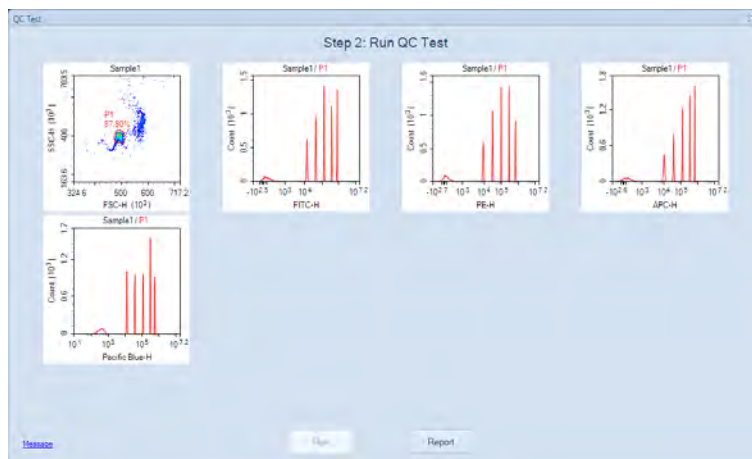


**Figure 2-16 QC Test: Step 2 - Running QC Test**

**6** After the QC test is completed, click the *Report* button to view the test results. Figure 2-17 shows one example of the QC test results.
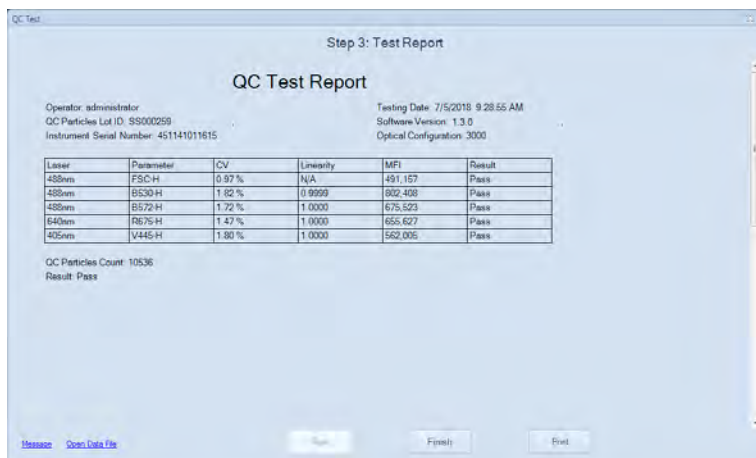


**Figure 2-17 QC Test: Step 3 - QC Test Report**

*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 2-17.*

### 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 2-18.
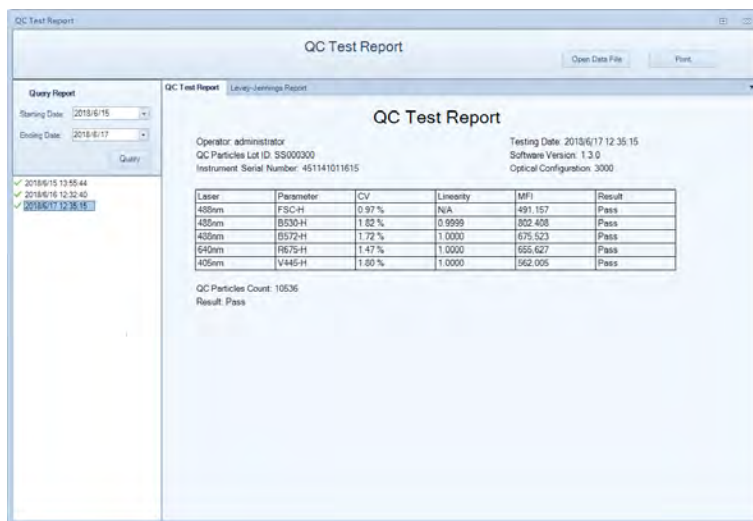


**Figure 2-18 QC Test Report**

Levey-Jennings plots on Mean value and CV (Coefficient of Variance) of the FSC-H and the highest peak of the B530-H, B572-H, R675-H, and V445-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 2-19.
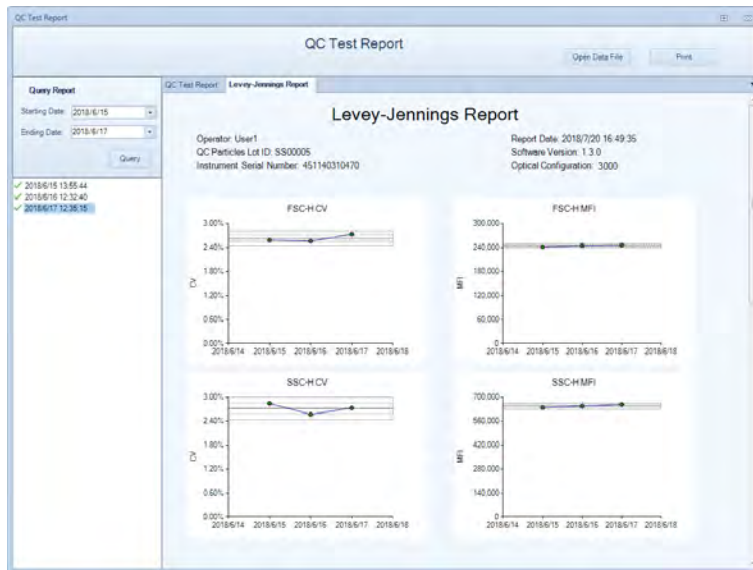
**Figure 2-19 QC Test Levey-Jennings Report**

## 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.

*The NovoCyte can also be automatically shut down at a defined time through the instrument general setting window. Please refer to Section 3.3.8 Setting in NovoExpress® Software Guide for more details about this function.*

**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.

*When shutdown is initiated from NovoExpress software, if no additional function option is selected, the NovoCyte instrument automatically performs the shutdown cleaning process and turns off the power, and no user interference is needed. If additional function option is selected, please refer to NovoCyte® Flow Cytometer Maintenance Guide for more information.*

**2** Turn off the NovoCyte workstation.

**3** Empty the waste container.

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

# 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 FITC/CD8 PE/CD45 PerCP/CD4 APC Reagent (Cat # 8930008) and the ACEA QuadriTest CD3 FITC/CD16+CD56 PE/CD45 PerCP/CD19 APC Reagent (Cat # 8930009) 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.

*The fluorescence channel configuration varies for different NovoCyte model. Please refer to Appendix B for standard configurations of each model. In the following sections, NovoCyte 2060R default system is used as an example to describe the detailed procedures.*

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 FITC/CD8 PE/CD45 PerCP/CD4 APC Reagent and the ACEA QuadriTest CD3 FITC/CD16+CD56 PE/CD45 PerCP/CD19 APC Reagent. The test needs to use both 488nm and 640nm lasers, and collect signals of the Height and Area parameters on FSC, SSC, and the fluorescent channels including B530 (FITC), B572 (PE), B675 (PerCP), and R675 (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.

► 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. Click the *Next Sample* button on *Cytometer Control* panel to create a new sample to start the experiment.

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] 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.
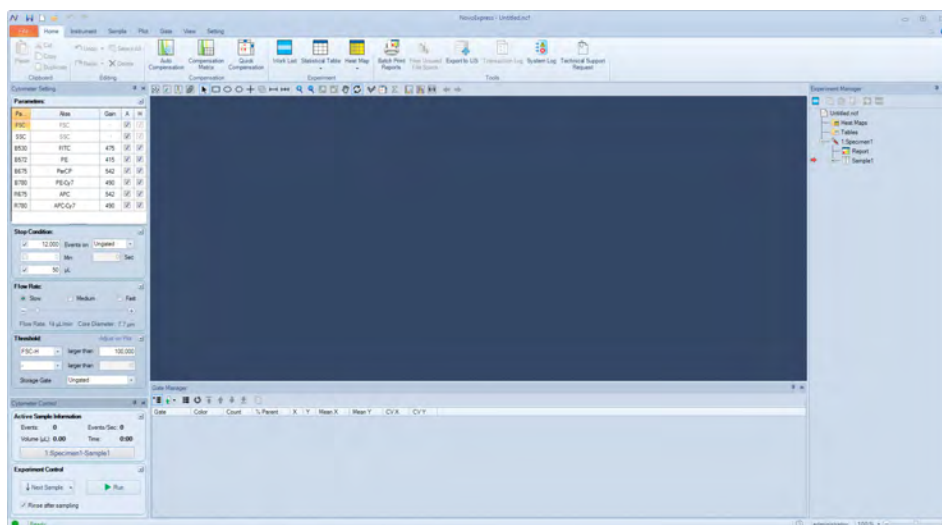


**Figure 3-1 NovoExpress Software Interface**

*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] 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.
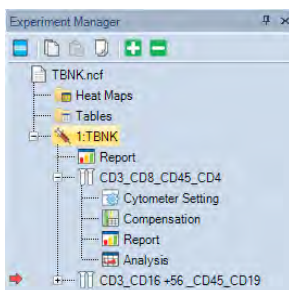


**Figure 3-2 Experiment Manager**

*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, B530, B572, B675, R675 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 B530, B572, B675, and R675 to be CD3 FITC, CD8 PE, CD45 PerCP, and CD4 APC, respectively.
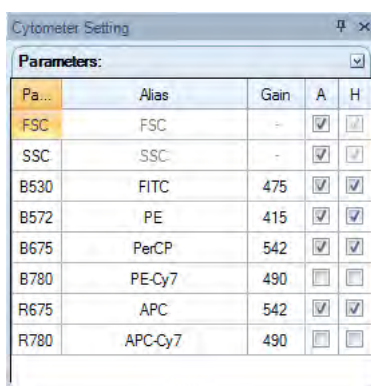


**Figure 3-3 Define Sample Acquisition Parameters**

⚠️ *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 needed in the experiment will unnecessarily 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.*

🔍 *The* **Gain** *column shows photodetector gains of all fluorescent channels. NovoCyte uses fixed photodetector gain by default. Adjustable photodetector gain is available as advanced function. Please refer to the* NovoExpress® Software Guide *for details about photodetector gain adjustment.*

## 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 is 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.
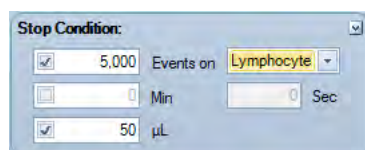
**Figure 3-4 Set Stop Condition**

*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 μL) 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 μL 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 μL/min, 35 μL/min and 66 μL/min, respectively. You can also manually set a desired flow rate within the range of 5 μL/min to 120 μL/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.
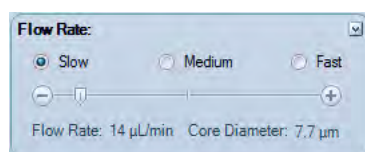
**Figure 3-5 Set the Sample Flow Rate**

### 3.3.3  Threshold

Threshold can be set in the *Cytometer Settings → Threshold* panel or through *Adjust on Plot* (Figure 3-6). The threshold sets a limit so that only the data values that 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 relevant 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) or Fluorescent signal Height at available channels, 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 with signal intensities meeting both thresholds.

The *Storage Gate* is used to filter out events outside the gate. All events above 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 data acquisition, the threshold can be adjusted after clicking the **Adjust on Plot** tab. The threshold will be set to the new value when clicking the desired position on the plot for the subsequent data acquisition.*

🔍 *For a new experiment, it might be difficult to determine how to set the threshold. In this case, conduct a pre-run with threshold adjusted on the plot 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 for the next experiment. 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. Adjust the FSC-H threshold value until the majority of the lysed red blood cells and debris are discarded, but the leukocytes are not affected. Set this threshold in the **Threshold** panel 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 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 µm in cell diameter | 300,000~1,000,000 | Use linear or logarithmic scale for FSC and SSC |
| Cells lines, smaller than 20 µm 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 |

▶▶▶

| Cell Types | FSC-H Threshold | Remark |
|------------|-----------------|--------|
| Platelets | 5,000~10,000 | Use logarithmic scale for FSC and SSC |
| 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 μm | SSC-H 1,000 |
| 0.5 μm | FSC-H 500 |
| 2 μm | FSC-H 50,000 |
| 10 μm | FSC-H 500,000 |
| 15 μm | FSC-H 1,000,000 |
| 30 μm | 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**

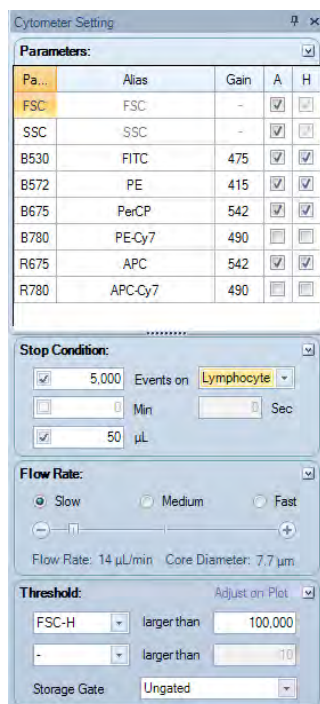After setting the acquisition parameters and acquisition conditions, double-click the *CD3_CD8_CD45_CD4* sample and set it as the Active Sample in the *Experiment Manager*. A red arrow will appear to the left of the sample, indicating it is the Active Sample. At the same time, the sample name in *Cytometer 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 NovoCyte 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. During the data acquisition, the *Restart* button will appear in the *Experiment Control* panel. Clicking this button will delete all collected events, reset the collection time and volume in the *Active Sample Information* panel, and restart the data collection.
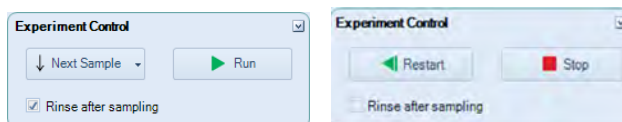


**Figure 3-8 Experiment Control Panel**

⚠️ *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.
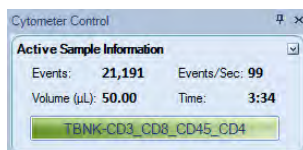


**Figure 3-9 Active Sample Information**

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 NovoCyte 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 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® 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 fluorephore. 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 compensation matrix can be generated either from samples acquired on NovoCyte instrument or from data imported from FCS files. Please refer to *Section 3.5.1.2: Automatic Fluorescence Compensation from Imported FCS Files* in this guide for instructions on generate compensation matrix from imported FCS files. The automatically generated compensation matrix can be applied to the multi-color stained samples to achieve correct fluorescence compensation.

### 3.5.1.1 Standard Automatic Fluorescence Compensation Procedure

The procedure for standard 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*.
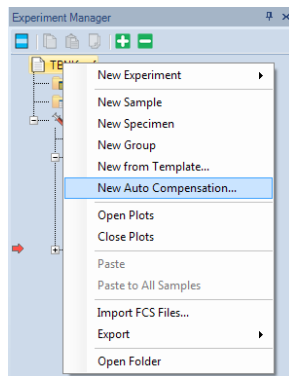


**Figure 3-10 *New Auto Compensation* Menu**
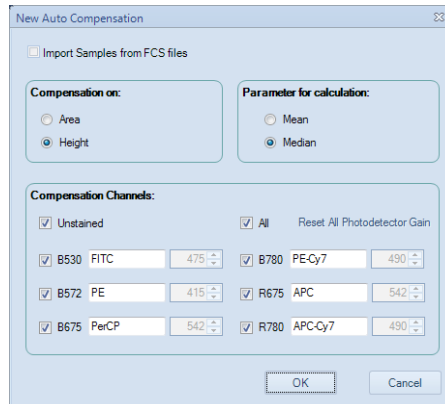


**Figure 3-11 *New Auto Compensation* Window**

If you select the **Unstained** sample in the **Compensation Channels**, 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 ...**.
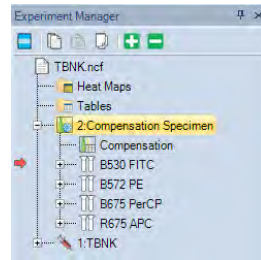
**Figure 3-12 Compensation Specimen and Related Single-Stained Samples**

## 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 20μL 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 recommended to prepare the compensation sample concentration to be $1 \times 10^6 \sim 2 \times 10^6$ /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.
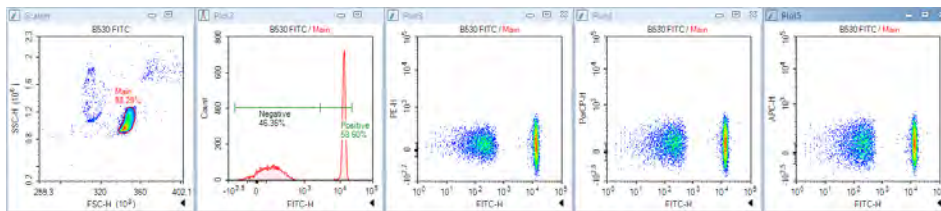


**Figure 3-13 Data Analysis for FITC Single-Stained Sample in Auto Compensation**

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 massage 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*.
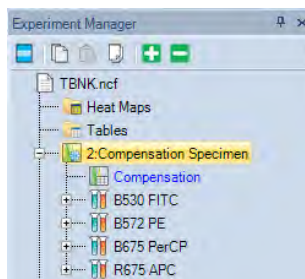


**Figure 3-14 Double-click *Compensation* to View the Compensation Matrix**



**Figure 3-15 Compensation Matrix Generated by Auto Compensation**



**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.*
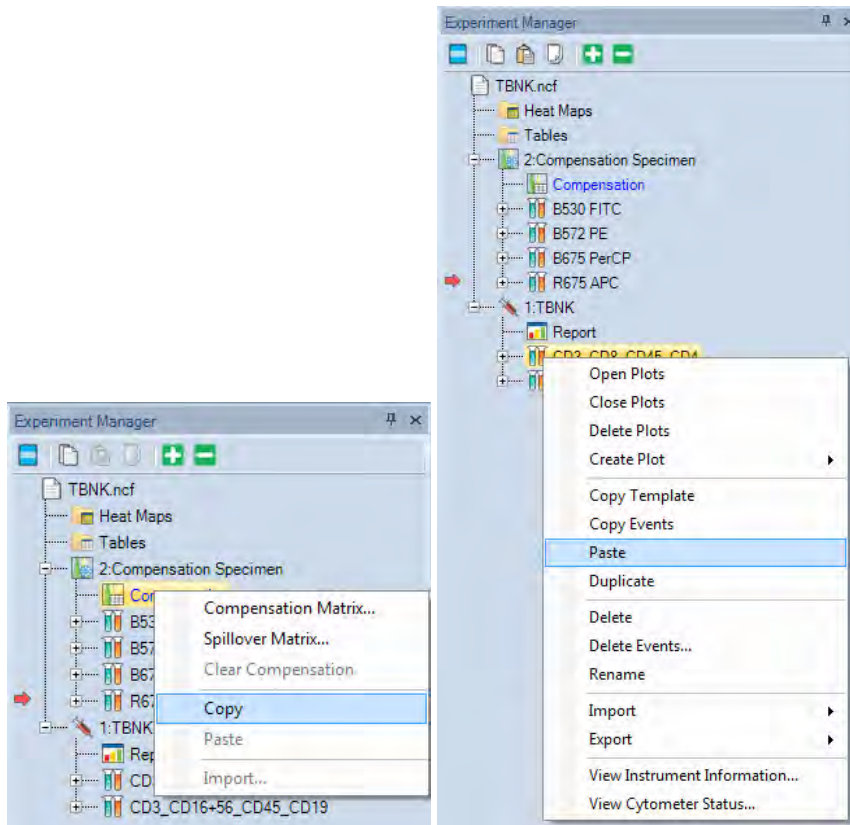


**Figure 3-17 Copy/Paste the Compensation Matrix**

### 3.5.1.2  Automatic Fluorescence Compensation from Imported FCS Files

To perform auto-compensation directly from the imported FCS file:

**1**  Click *Home → Auto Compensation* to open the New Auto Compensation window. Select *Import Samples from FCS files*.

> *This window can also be accessed by right clicking the experiment file and selecting New Auto Compensation in the Experiment Manager window.*
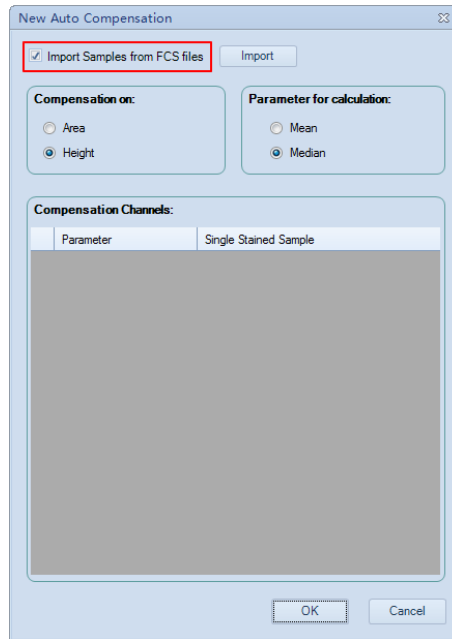


**Figure 3-18 Open the New Autocompensation Window**

**2**  Select the single file or multiple files while holding the *Shift* key in the keyboard. Click *Open* to import the selected file(s) to the software.
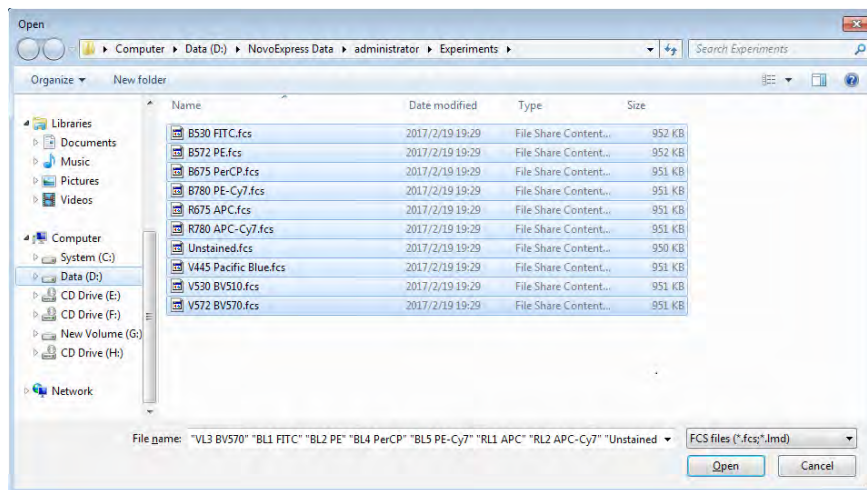


**Figure 3-19 Import Samples from FCS Files**

**3** Ensure all the selected FCS files are successfully imported to the software. Click *OK* to continue.
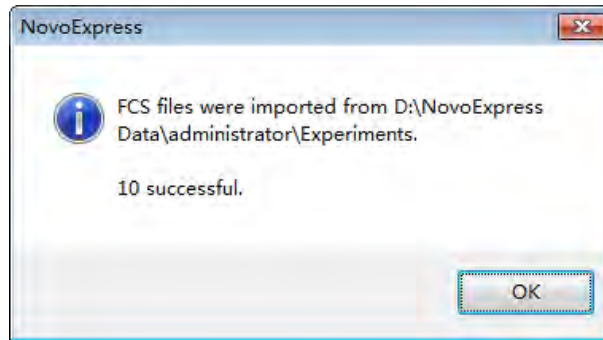


**Figure 3-20 Dialog Box for Successful Importing FCS Files**

**4** Set the compensation parameters in the *New Auto Compensation* window (i.e. *Area* or *Height*, *Median* or *Mean*). Ensure the correct channel parameter is selected for each single stained sample. To select different single stained sample file, click the file name, select the desired file from the pull down menu. If a channel parameter does not need to be included for the compensation, click the blank area in the pull down menu. The check box in front of the corresponding channel parameter will be automatically unselected.

⚠ *The software will automatically associate the imported FCS file with the selected channel parameter based on the keyword of the file name(e.g. B530). Users need to verify and select the correct FCS file for each fluorescence detection channel.*
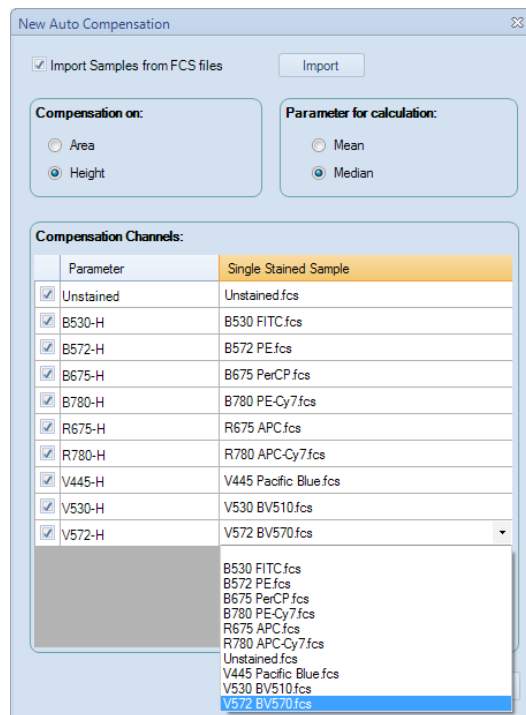


**Figure 3-21 Set the Autocompensation Parameters**

**5** Click *OK* and NovoExpress will automatically calculate the compensation matrix based on the samples imported. Once generated, the compensation matrix can be applied to other experiment samples.

### 3.5.2    Manual Fluorescence Compensation

In addition to automatic fluorescence compensation, users can manually adjust the spill-over 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 3-22). 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 selecting *Spillover Matrix …* to load the compensation editing window.
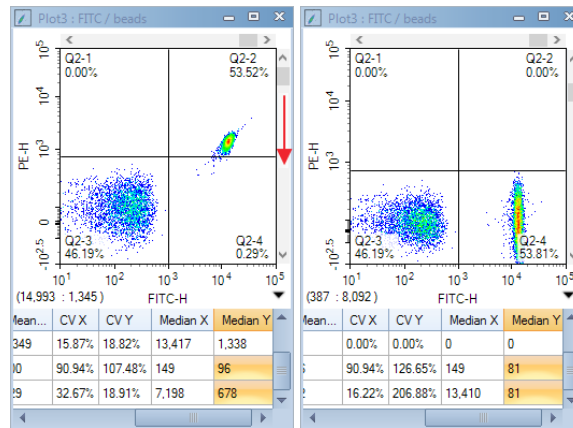
**Figure 3-22 Quick Compensation**

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 3-23. 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 3-24. The spillover matrix for FITC single-stained sample is shown in Figure 3-23.
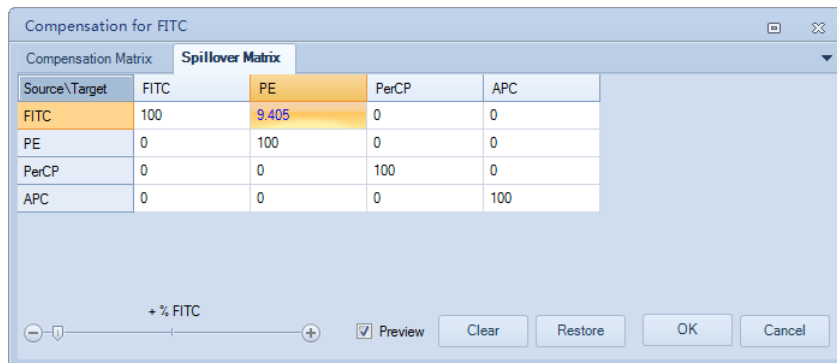


**Figure 3-23 Compensate FITC Spillover into PE Channel in Spillover Matrix**
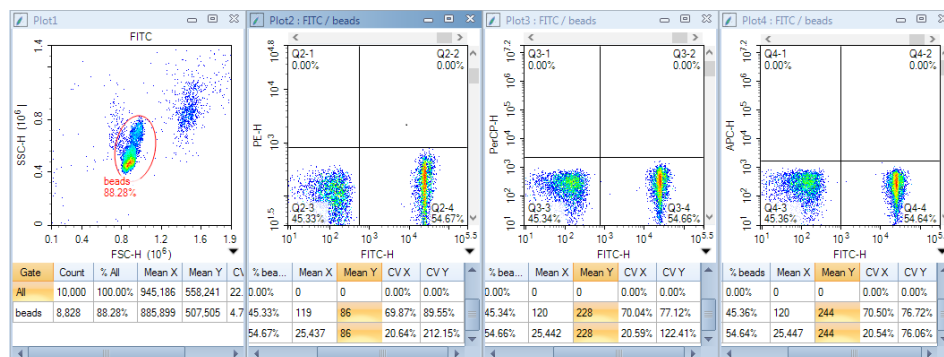


**Figure 3-24 Compensate FITC Spillover into PE, PerCP, and APC Channels**

> 🔍 *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 matrices are shown in Figure 3-25, 3-26, and 3-27, respectively.

**Compensation for PE**

| Compensation Matrix | Spillover Matrix | | | |
|---|---|---|---|---|
| Source\Target | FITC | PE | PerCP | APC |
| FITC | 100 | 0 | 0 | 0 |
| PE | 3.236 | 100 | 32.252 | 0.008 |
| PerCP | 0 | 0 | 100 | 0 |
| APC | 0 | 0 | 0 | 100 |

**Figure 3–25 Spillover Matrix for PE Single-Stained Sample**

**Compensation for PerCP**

| Compensation Matrix | Spillover Matrix | | | |
|---|---|---|---|---|
| Source\Target | FITC | PE | PerCP | APC |
| FITC | 100 | 0 | 0 | 0 |
| PE | 0 | 100 | 0 | 0 |
| PerCP | 0.025 | 0.04 | 100 | 17.78 |
| APC | 0 | 0 | 0 | 100 |

**Figure 3–26 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 3–27 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 3-28). 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 3-29 and Figure 3-30 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 3–28 Incorporate the Compensation Coefficients from Single-Stained Samples into the Spillover Matrix**
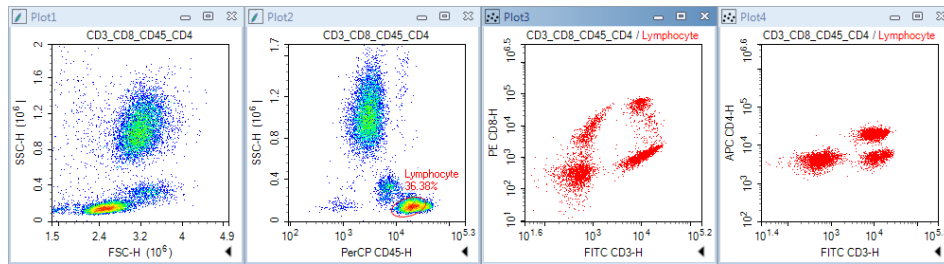
**Figure 3-29 Results of *CD3_CD8_CD45_CD4* Sample: Before Compensation**
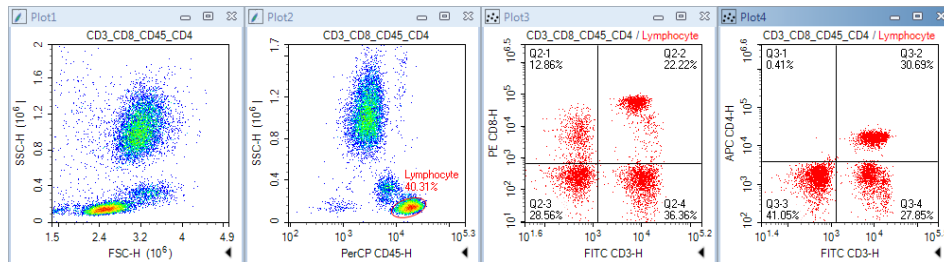


**Figure 3-30 Results of *CD3_CD8_CD45_CD4* Sample: After Compensation**

🔍 *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 bi-range gate ⊢⊢⊣. By clicking the icon in the NovoExpress software data analysis toolbar (Figure 3-31), you can create the corresponding plot and gate.



**Figure 3-31 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 3-32, sample **CD3_CD8_CD45_CD4** is the Active Sample for data analysis, while sample **CD3_CD16+56_CD45_CD19** is currently acquiring data.*
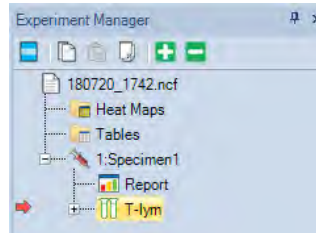


**Figure 3-32 Active Sample in Data Analysis (Red Arrow) and Sample in Data Acquisition (Green Arrow)**

► **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 3-33).
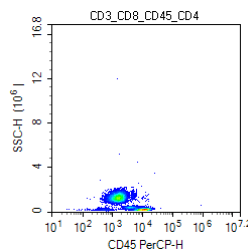


**Figure 3-33 CD45 PerCP-H/SSC-H Density Plot**

The NovoCyte 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 3-34).
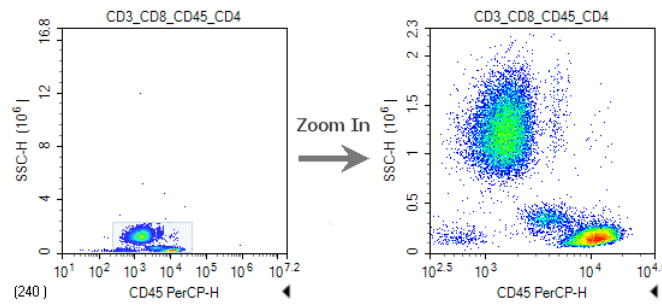
**Figure 3-34 Zooming in the Density Plot**

▶ **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 3-27 and 3-28 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 3-35. Right-click at any place on the statistical table to select additional statistical parameters to be calculated for the data, as shown in Figure 3-36.
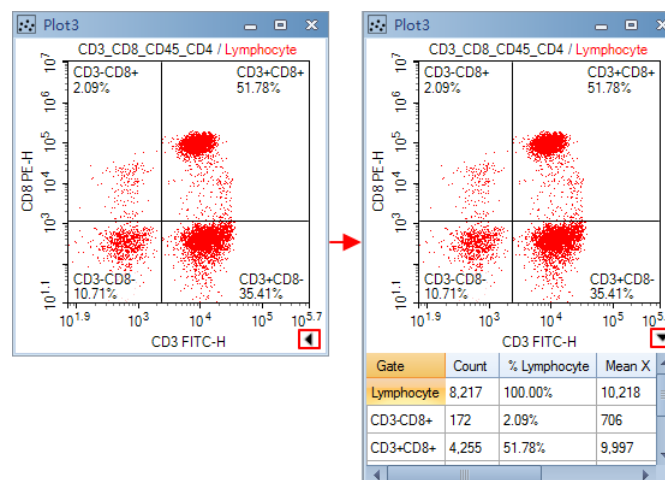


**Figure 3-35 Statistics of the Data**
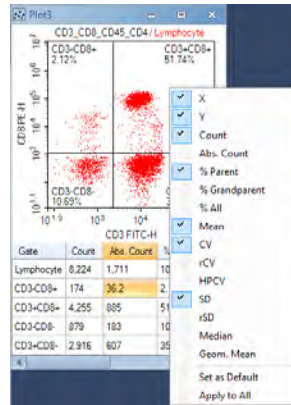
**Figure 3-36 Select other Statistical Parameters**

🔍 *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 3-37. Select *All* to delete all the events from the sample, or only delete events *Outside* or *Inside* a specified gate.
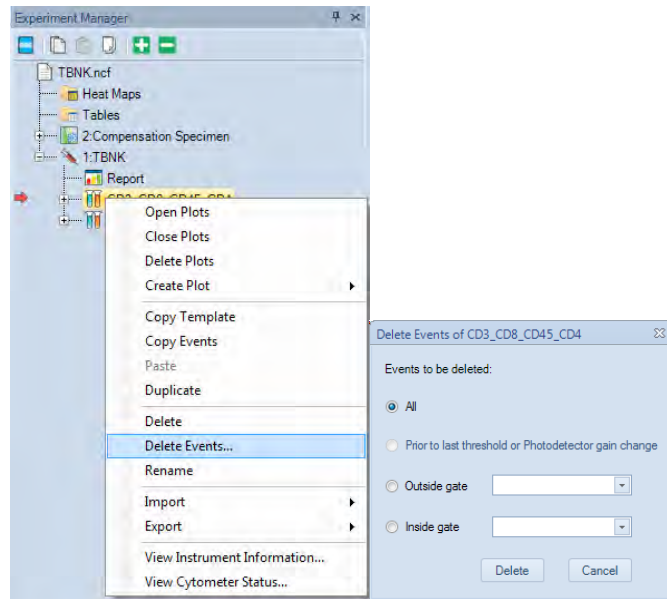
**Figure 3-37 Delete Events**

## 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, FCS 3.1, or CSV file and be analyzed by a third party software. There are two methods to export a file.

► To manually export a file, right-click the specimen or sample name in the *Experiment Manager* and select *Export → Export FCS File …* or *Export → Export CSV File …*, as shown in Figure 3-38. In the prompt window, specify the folder to export the data.

**3**
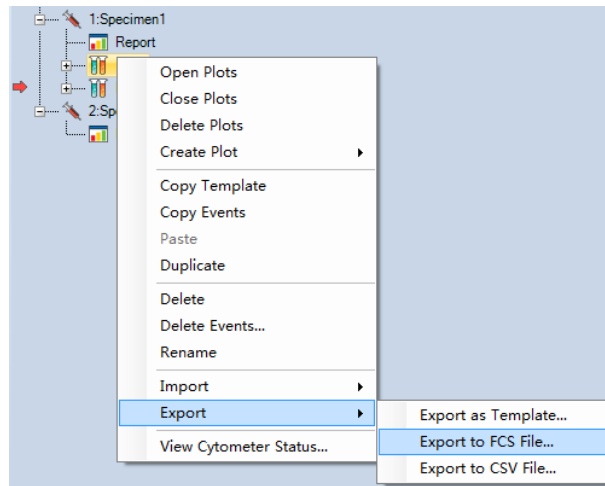


**Figure 3-38 Manually Export Data**

The data can be automatically exported once the data acquisition is completed. To do so, click *Setting → Option → Experiment* in the software main window. Select the check box for *Automatically export samples to FCS/CSV files after data acquisition completed* to enable this function (Figure 3-39). Once enabled, click *Export Setting* to open the *Export Events* window. Export settings, including generated file format (i.e. FCS 3.0, FCS3.1 or CSV), can be defined in this window (Figure 3-40).
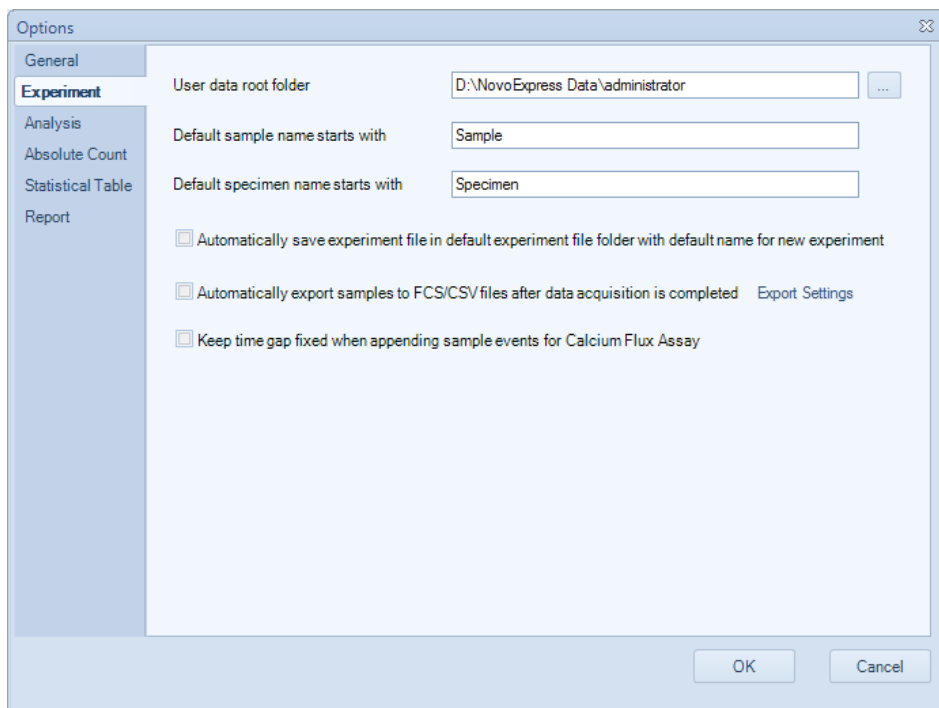


**Figure 3-39 Enable the Automatic Data-Exporting Function**
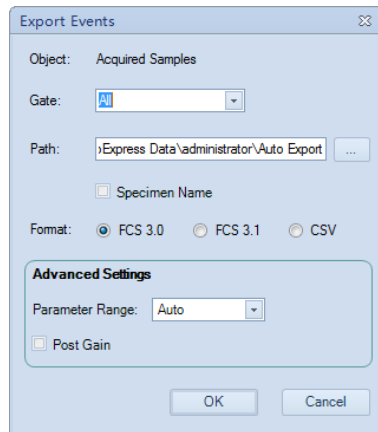
**Figure 3-40 Configure Exporting Settings**

FCS 2.0, FCS 3.0 and FCS 3.1 format files may also be imported into NovoExpress for viewing and analysis. To import the FCS file, create a blank sample or specimen in the *Experiment Manager*, right-click and select *Import → Import FSC File …*, as shown in Figure 3-41.
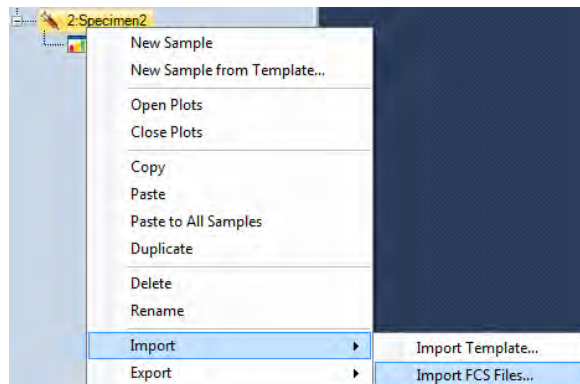


**Figure 3-41 Import FCS File**

*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 the instrument maintenance on a regular basis in order to achieve the optimal performance for the instrument and obtain reliable data. ACEA NovoCyte incorporates automatic procedures for maintenances as much as possible in order to bring conveniences to the users and save them valuable time. Most maintenance procedures will be automatically executed, eliminating the time consuming manual maintenances. This chapter describes the procedures to execute or conduct the maintenance procedures.

*For detailed information about System Maintenance, please refer to the NovoCyte® Flow Cytometer Maintenance Guide.*

*Make sure to use the correct version of NovoCyte® Flow Cytometer Maintenance Guide for reference .Refer to the following list for which version of the NovoCyte® Flow Cytometer Maintenance Guide should be used correspondingly.*
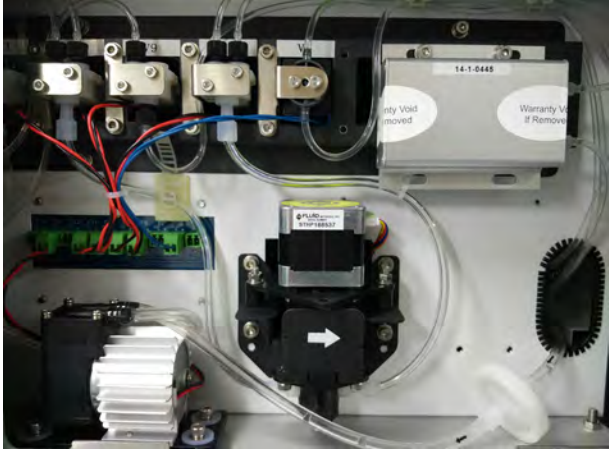
| NovoCyte Flow Cytometer Side Panel | System | Maintenance Guide Version |
|---|---|---|
|  | V1 | NovoCyte® Flow Cytometer Maintenance Guide (V1) |
|  | V2 | NovoCyte® Flow Cytometer Maintenance Guide (V2) |

Table 4-1 Maintenance Guide Version

## 4.1 Scheduled Maintenance

Table 4-2 lists the regular maintenance needs to be done on 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 (ACEA Novo-Flow sheath fluid, ACEA NovoRinse solution, and ACEA 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<br>▶ Clean the sample injection probe (SIP)<br>▶ Clean the SIP cleaning apparatus<br>▶ Conduct *Extensive Rinse*<br>▶ *Unclog* the flow cell<br>▶ Sterilize instrument reagent containers | Every month. |
| Replacing Fluidic System Consumables | Every two months or when prompted by NovoExpress Software. |

**Table 4-2 Scheduled Maintenance and Recommended Frequency**

## 4.2    Unscheduled Maintenance

The following 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. Other components not listed here should also be checked periodically for wear and replacement if necessary.

| Unscheduled Maintenance | When to Perform |
|---|---|
| Remove Bubbles | ► The CV of the data is larger than normal<br>► QC test failed |
| Unclog the Sample Injection Probe (SIP) | ► Much less amount of events are detected than expected<br>► Perform a large amount of tests in one day |
| Clean the External Surface of the Instrument | Keep the external surface of the instrument clean |
| Clean the Sample Injection Probe (SIP) | The sample injection probe's outer surface is dirty |
| Decontamination | When suspecting bacteria contamination in the system |
| Clean Fluidic System before Long-Term Storage | No plan to run the NovoCyte cytometer for more than two weeks |
| Purge Fluidic System before Shipment | The NovoCyte flow cytometer needs to be shipped |
| Calibrate the Fluidics Station | Abnormal warning about the liquid level in the reagent containers is observed |
| Clean the Optical Filters and Mirrors | The optical filters and mirrors get dirty |

**Table 4-3 Unscheduled Maintenance and When to Perform**

# 5. Troubleshooting

This section contains troubleshooting guidelines. Follow the procedures described in this section for troubleshooting methods.

*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.*

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

*Refer to NovoCyte® Flow Cytometer Maintenance Guide for maintenance of the NovoCyte instrument.*

Table 5-1 provides guidance on troubleshooting the NovoCyte instrument, NovoSampler and NovoSampler Pro. Table 5-2 lists the message IDs and the associated software prompted messages.

*Please contact ACEA Technical Support if issues are not resolved after performing the recommended solutions or if there are any issues which are not listed in these two tables.*

*Investigate possible causes and perform the recommended solutions in the given order.*

| Observation | Possible Causes | Recommended Solutions |
|---|---|---|
| Instrument QC test fails | QC Particles are expired or not stored properly | Prepare a new tube of QC Particles and mix them well to run the QC test again. |
| | Flow cell is dirty | Perform the maintenance functions from the NovoExpress software: *Unclog, Priming, Extensive Rinse*, and *Extensive Clean*. |
| | Air bubbles exist in the flow chamber | Execute Debubble and Priming function in NovoExpress Software. |
| Zero or low number of events detected | Threshold is set too high | Lower the threshold setting and re-collect the sample. |
| | Sample concentration is too low | Redo sample preparation using an appropriate method to concentrate the sample. |
| | Sample did not mix well | Mix the sample well to suspend the cells. |
| | NovoFlow container is empty | Refill the NovoFlow container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in this guide. |
| | NovoFlow container is not correctly connected to the instrument and the NovoFlow solution does not flow through the tubing | ► Reconnect the NovoFlow quick coupling connectors to ensure the NovoFlow container is correctly connected to the instrument.<br>► Execute *Prime* function from the NovoExpress Software. |

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| Observation | Possible Causes | Recommended Solutions |
|---|---|---|
| Zero or low number of events detected | Sample Injection Probe is clogged | ► Execute the Backflush function from the NovoExpress Software.<br><br>► Follow the procedures described in *Section 2.2 Unclog the Sample Injection Probe (SIP)* in *NovoCyte® Maintenance Guide* to unclog the sample injection probe. |
| | Flow cell is clogged | Execute the *Unclog* function from the NovoExpress Software. |
| | Sample tubing is leaking | Clean the leaked fluid with wipes and properly reconnect the sample tubing. |
| | Air bubbles exist in the Sampling Pump | Execute *Debubble* and *Priming* function from NovoExpress Software. |
| High CV | Air bubbles exist in the flow chamber | Execute the *Debubble* and *Priming* function from NovoExpress Software. |
| | Sampling tubing is clogged | Perform maintenance functions from NovoExpress software: *Unclog, Priming, Extensive clean, Extensive rinse*. |
| | Sheath in-line filter is clogged | Replace the sheath in-line filter following the procedures described in *Section 1.2 Replacing Fluidic System Consumables* in *NovoCyte® Maintenance Guide*. |
| | Improper sample preparation | Re-prepare the sample properly. |
| | The sample flow rate is set too high | Lower the sample flow rate setting in the NovoExpress software. |
| Excessive debris in display | Sheath in-line filter is dirty | Replace the sheath in-line filter following the procedures described in *Section 1.2 Replacing Fluidic System Consumables* in NovoCyte® Maintenance Guide. |
| | The threshold is set too low | Increase the threshold setting and re-collect the sample. |
| | Fluidic system tubing is dirty | ► Execute *Rinse* function from NovoExpress Software.<br><br>► Execute *Decontamination* function from NovoExpress Software. |
| 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. |

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| Observation | Possible Causes | Recommended Solutions |
|---|---|---|
| NovoExpress displays *Instrument not connected* even though the instrument is powered up | Workstation is in hibernation causing the interruption of communication between the NovoCyte instrument and workstation | Restart NovoExpress Software. |
| | Improper connection between the NovoCyte instrument and workstation | Reconnect the USB cable between the instrument and the workstation. |
| | More than one NovoExpress instances are running. | Only run one instance of NovoExpress software. |
| Unstable Signal or gap in the data plot | Air bubbles exist in the flow chamber | Execute the Debubble and Priming function from NovoExpress Software. |
| Sample tube does not fit the sample tube holder | Wrong type of sample tube is used | Use 12 × 75 mm flow tube or 1.5 mL Eppendorf tube. |
| Sample Injection Probe is not properly aligned with the wells or tubes | Plate or tube rack is not loaded correctly. | Load the plate or tube rack with the correct orientation. Make sure the plate or tube rack is fully seated onto the sample tray properly. |
| | NovoSampler (Pro) is not calibrated. | Click *Instrument → NovoSampler Pro → Calibrate* in the NovoExpress Software to re-calibrate the NovoSampler (Pro). |
| Leakage from the SIP | Waste tubing is deformed | ▶ Reconnect the waste tubing to ensure it is properly connected.<br>▶ Move the instrument forward as needed to ensure sufficient space between the instrument and wall. Refer to *Section 1.2.1 Installation* in this guide for more information on properly installing NovoCyte instrument. |
| | Waste filter is clogged | Replace the waste filter following the procedures described in *Section 1.2 Replacing Fluidic System Consumables* in *NovoCyte® Maintenance Guide*. |
| Leakage from Sampling Pump | Incorrect tubing connection to the sampling pump | Reconnect the tubing to the sampling pump. |
| Leakage from other components of the instrument | Incorrect pump or valve or tubing connection or bad sheath in-line filter | ▶ Turn off the NovoCyte instrument.<br>▶ Clean the leaked fluid.<br>▶ Replace the sheath in-line filter following the procedures described in *Section 1.2 Replacing Fluidic System Consumables* in *NovoCyte® Maintenance Guide*.<br>▶ Contact ACEA technical support for service. |

**Table 5-1 NovoCyte Instrument Troubleshooting Guide**

**5**

| Message IDs | Software Messages | Possible Causes | Recommended Solutions |
|---|---|---|---|
| 0x0001 | *Collision of SIP* | The movement of the SIP is blocked by some obstacles | Locate and clear the obstacles. Click the OK button in the prompted dialog box or wait 10 seconds for automatic error handling. The instrument will move the SIP to the home position. This procedure will take about 3 minutes. |
| | | Incorrect plate is selected in plate manager | Select the correct plate in the plate manager window. |
| | | Incorrect positioning of plate in NovoSampler (Pro) | ▶ Position the plate on the shaker correctly. Ensure the plate is seated flat on the stage inside the clamps.<br>▶ Re-calibrate NovoSampler Pro. |
| | | Dirty SIP or SIP wash apparatus | Clean the SIP or SIP wash apparatus following the procedures described in *Section 1.1 Preventative Maintenance* in *NovoCyte® Flow Cytometer Operator's Guide*. |
| 0x0002 | *Running out of NovoFlow* | NovoFlow solution is not sufficient to continue to run any samples | Refill the NovoFlow container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in this guide. |
| | | Fluidic station is not working properly | Replace the fluidic station. |
| 0x0003 | *Running out of NovoRinse* | NovoRinse solution is not sufficient to continue to run any samples | Refill the NovoRinse container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in this guide. |
| | | Fluidic station is not working properly | Replace the fluidic station. |
| 0x0004 | *Running out of NovoClean* | NovoClean solution is not sufficient to continue to run any samples | Refill the NovoClean container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in this guide. |
| | | Fluidic station is not working properly | Replace the fluidic station. |
| 0x0005 | *Waste container is full* | Waste container is too full to continue to run any samples | Empty the waste container following the procedures described in *Section 2.1.3 Empty Waste* in *NovoCyte® Flow Cytometer Operator's Guide*. |
| | | Fluidic station is not working properly | Replace the fluidic station. |
| 0x0006 | *System voltage is out of range* | System Error | Restart the NovoCyte instrument. |

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| Message IDs | Software Messages | Possible Causes | Recommended Solutions |
|---|---|---|---|
| *0x0007* | *System electric current is out of range* | System Error | Restart the NovoCyte instrument. |
| *0x0008* | *Firmware configuration error* | System Error | Restart the NovoCyte instrument. |
| *0x0009 0x000A 0x000B* | *…… laser self test error* | 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. |
| *0x000C 0x000D 0x000E* | *….laser is not connected* | Specified laser is not detected | Restart the NovoCyte instrument. |
| *0x000F* | *NovoSampler (Pro) communication lost* | The cable between the NovoSampler (Pro) and NovoCyte Flow Cytometer is not securely connected | Reconnect the cable between the NovoSampler (Pro) and NovoCyte Flow Cytometer. |
| | | NovoSampler (Pro) is not communicating with NovoCyte | Restart NovoCyte instrument. |
| *0x0010* | *NovoSampler (Pro) has not been calibrated* | NovoSampler (Pro) is newly installed or re-connected. | Follow the prompted instructions to calibrate the NovoSampler (Pro). |
| *0x0011* | *NovoSampler (Pro) calibration failed…* | NovoSampler (Pro) is not installed properly | Re-install and calibrate the NovoSampler (Pro) following the procedures described in *Section 1.2 Installation* in *NovoSampler® Operator's Guide* or *NovoSampler® Pro Operator's Guide*. |
| | | The NovoSampler (Pro) cover is opened during calibration. | Close the NovoSampler (Pro) cover and redo the calibration. |
| *0x0012* | *The movement of plate is out of range* | The movement of the orbital shaker is blocked. | ▶ Check the path of the orbital shaker to make sure there are no objects blocking the movement. Clear the block if there is any. ▶ Click *Instrument → NovoSampler (Pro) → Calibrate* in NovoExpress Software to re-calibrate the NovoSampler (Pro). |
| | | NovoSampler (Pro) is not installed properly | Re-install and calibrate the NovoSampler (Pro) following the procedures described in *Section 1.2 Installation* in *NovoSampler® Operator's Guide* or *NovoSampler® Pro Operator's Guide*. |

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| Message IDs | Software Messages | Possible Causes | Recommended Solutions |
|---|---|---|---|
| 0x0013 | *Cover of NovoSampler (Pro) is opened during moving plate* | The cover of NovoSampler (Pro) is opened during moving plate | Close the NovoSampler (Pro) cover. NovoSampler (Pro) will automatically reset and be ready for operation. |
| 0x0014 | *Pressure is out of limit* | 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. |
| | | Sheath in-line filter is clogged | Replace the sheath in-line filter following the procedures described in *Section 1.2 Replacing Fluidic System Consumables* in *NovoCyte® Maintenance Guide*. |
| | | Flow cell is clogged | Conduct *Unclog* from NovoExpress. |
| 0x0015 | *NovoSampler (Pro) firmware error* | NovoSampler (Pro) firmware is not working properly | Re-install or upgrade the NovoSampler (Pro) firmware. |
| 0x0016 0x0017 0x0018 | *...... laser does not emit* | Specified laser is not connected properly or laser is not working properly | ► Reconnect the laser cable properly. <br> ► Restart the instrument. |
| 0x0019 0x001A 0x001B | *...... laser communication error* | Interference or bad connection or bad laser | ► Reconnect the laser properly. <br> ► Restart the instrument. |
| 0x001C | *Sample injection probe reset failed* | Bad connection or optocoupler is damaged | Click the *OK* button in the prompted dialog box or wait 10 seconds for automatically error handling. Restart the instrument. |
| 0x001D | *Sampling Pump reset failed* | Bad connection or optocoupler is damaged | Restart the instrument. |
| 0x001E | *The SIP module needs to be upgraded to support NovoSampler Pro* | SIP module is not compatible with connected NovoSampler Pro | Contact ACEA technical support for service. |
| 0x0020 | *System initialization is paused* | Liquid level in the reagent containers is not within normal range when NovoCyte is powered up. | ► Make sure that the instrument reagent containers are placed correctly and the liquid level in the containers is within the normal range. <br> ► Click *OK* on the prompted dialog to continue system initialization. |
| 0x0021 | *Sheath fluid pump reset failed* | Bad connection or optocoupler is damaged | Restart the instrument. |

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| Message IDs | Software Messages | Possible Causes | Recommended Solutions |
|---|---|---|---|
| *0x0022* | *Resetting NovoSampler Pro to zero position failed* | NovoSampler Pro is not working properly. | Click *Reset*. Restart the NovoSampler Pro. |
| *0x0023* | *Resetting NovoSampler Pro to home position failed* | NovoSampler Pro is not working properly. | Click *Reset*. Restart the NovoSampler Pro. |
| *0x0100* | *Instrument cover opened* | Instrument cover is opened or not tightly closed | Close the instrument cover. |
| *0x0101* | *NovoFlow running low* | NovoFlow solution is below the pre-set volume limit | Refill the NovoFlow container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in this guide. |
| | | Fluidic station is not working properly | Replace the fluidic station. |
| *0x0102* | *NovoRinse running low* | NovoRinse solution is below the pre-set volume limit | Refill the NovoRinse container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in this guide. |
| | | Fluidic station is not working properly | Replace fluidic station. |
| *0x0103* | *NovoClean running low* | NovoClean solution is below the pre-set volume limit | Refill the NovoClean container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in this guide. |
| | | Fluidic station is not working properly | Replace fluidic station. |
| *0x0104* | *Waste container is close to full* | Waste is above the pre-set volume limit | Empty the waste container following the procedures described in *Section 2.1.3 Empty Waste* in this guide. |
| | | Fluidic station is not working properly | Replace fluidic station. |
| *0x0105* | *Cover of NovoSampler (Pro) is opened* | Cover of NovoSampler (Pro) is opened | Close the cover. |
| *0x0106* | *NovoSampler (Pro) is disconnected when powered up* | The NovoSampler (Pro) is disconnected when powered up | ► Shut down NovoCyte Flow Cytometer.<br>► Reconnect the cable of the NovoSampler (Pro) to NovoCyte flow cytometer.<br>► Turn on NovoCyte and follow the prompts to calibrate the NovoSampler (Pro). |

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| Message IDs | Software Messages | Possible Causes | Recommended Solutions |
|---|---|---|---|
| *0x0109* | *Fluidics station is not connected* | Cable between the fluidics station and the NovoCyte instrument is not properly connected | ► Turn off the instrument.<br>► Reconnect the cable between the fluidics station and the NovoCyte instrument.<br>► Power up the instrument. |
| *0x010A*<br>*0x010B*<br>*0x010C*<br>*0x010D* | *… liquid level sensor failure* | Specified liquid level sensor in the fluidics station is not working properly | ► Reconnect the fluidics station cable.<br>► Restart the NovoCyte instrument.<br>► Replace the fluidics station. |
| *0x010F* | *Sheath filter is clogged. Please replace the sheath filter and run the Priming procedure* | Sheath in-line filter is clogged | Replace the sheath in-line filter following the procedures described in *Section 1.2 Replacing Fluidic System Consumables* in this guide. |
| *0x0110* | *Recovering collision error* | Plate stops at an incorrect position | No action is needed. Instrument will automatically recover the error. |
| *0x0111* | *Orbital shaker homing position reset failure* | Orbital shaker of NovoSampler Pro is not working properly | No action is needed. The NovoSampler Pro can be used normally. |
| | *Communication error (code: xx, xx). Please restart NovoCyte and NovoExpress.* | USB cable between the NovoCyte instrument and workstation is not connected properly | ► Reconnect the USB cable between the NovoCyte instrument and the workstation.<br>► Restart the instrument and NovoExpress software. |

**Table 5-2 NovoExpress Prompted Error Messages and Message IDs**

# 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 sheath 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 the sample injection probe (100 μL 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.
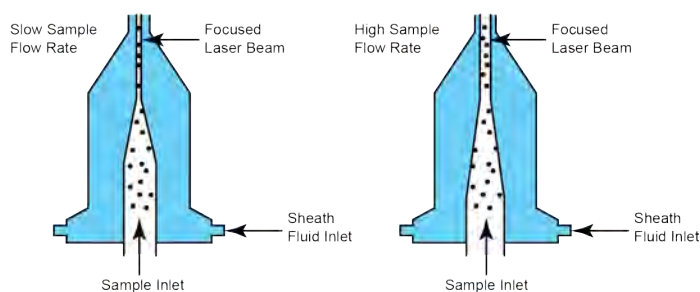
**Figure A-1 Hydrodynamic Focusing and Core Diameter under Different Sample Flow Rates**

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
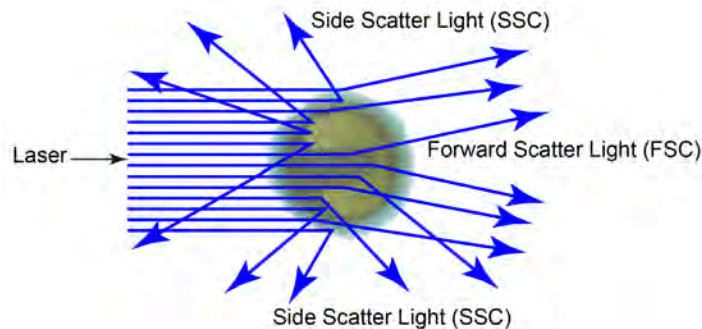


**Figure A-2 Forward Scatter (FSC) and Side Scatter (SSC)**

## 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 (488 nm) and emitting green light (530 nm), while other fluorochromes such as PerCP exhibit a larger Stokes shift, absorbing blue light (488 nm) and emitting red light (675 nm).

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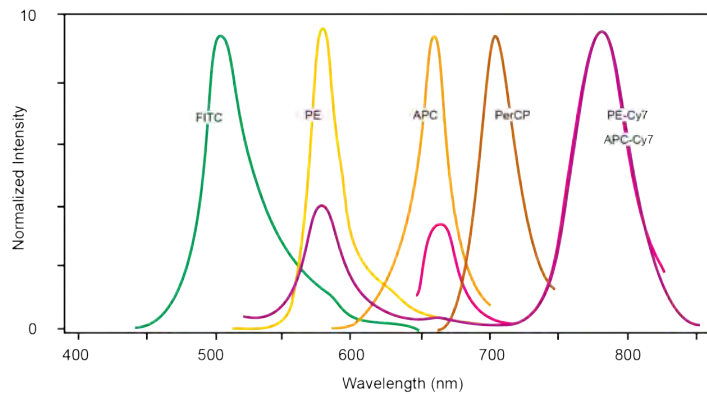


**Figure A-3 Emission Spectra of Commonly Used Fluorochromes**

## 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, whereas shortpass dichroic mirrors allow light with shorter wavelengths to be transmitted. Dichroic mirrors are usually named by the cutoff wavelength. For example, a 605 nm longpass dichroic mirror allows light with wavelength longer than 605 nm to be transmitted, and light with wavelength shorter than 605 nm is reflected.
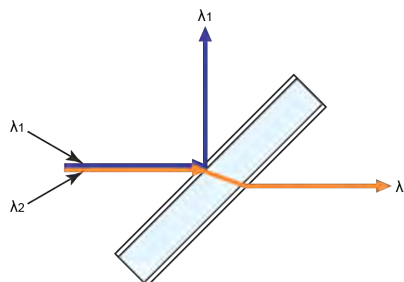


**Figure A-4 Dichroic Mirror**

## 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 505 nm LP filter allows wavelengths longer than 505 nm to pass through it and either absorbs or reflects wavelengths shorter than 505 nm (Figure A-5).
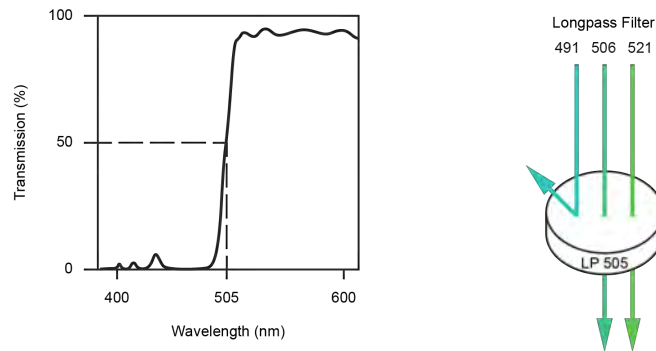


**Figure A–5 505 nm Longpass Filter**

## Shortpass Filters

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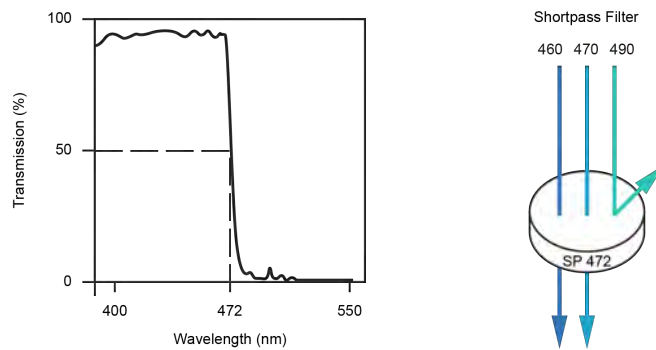


**Figure A-6 472 nm Shortpass Filter**

## 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 530 nm and has a total of bandwidth of 30 nm. Therefore, this filter transmits light between 515 nm and 545 nm (Figure A-7).

**Figure A-7 530/30 nm Bandpass Filter**

The ACEA NovoCyte flow cytometer uses longpass and shortpass dichroic mirrors and bandpass filters for light collection.

Refer to *Appendix B* for details of the optical bench schematics and dichroic mirrors and bandpass filters used for different NovoCyte models.

### 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 (Figure A-8).



**Figure A-8 (a) Orientation of the Dichroic Mirrors and Bandpass Filters (NovoCyte 3000 Default)**

**Figure A-8 (b) Orientation of the Dichroic Mirrors and Bandpass Filters (NovoCyte 3000VYB Default)**
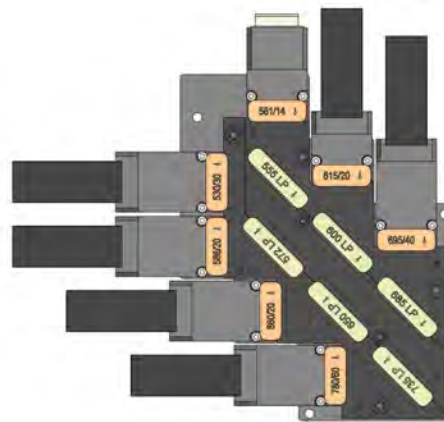


**Figure A-8 (c) Orientation of the Dichroic Mirrors and Bandpass Filters
(NovoCyte 3000RYB Default and 2100YB Default)**

⚠️ *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, photodiodes (PD) are used to detect the FSC and SSC signals. A photomultiplier tubes (PMT) are 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-9). This is known as

fluorescence spillover. Spillover can be corrected mathematically by using a method called compensation.
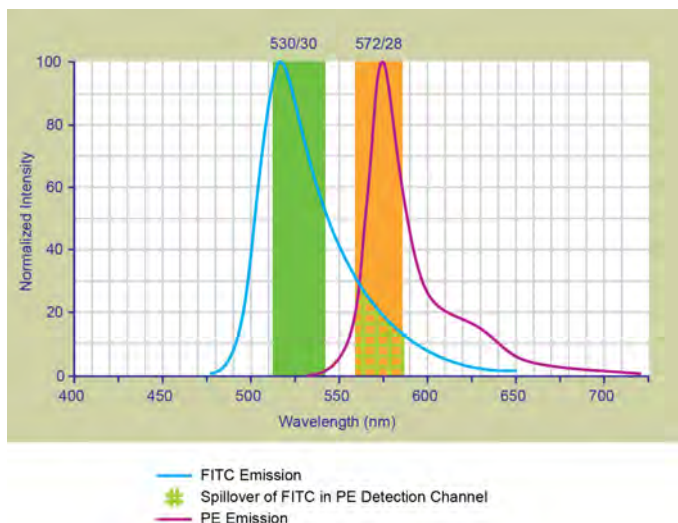


**Figure A-9 Spillover from the FITC Fluorochrome to the PE Detector**

For example, Figure A-9 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-10. 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-11).
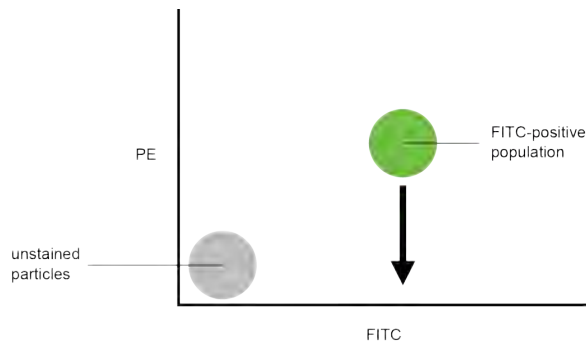


**Figure A-10 Theoretical Display of FITC vs. PE without Compensation**
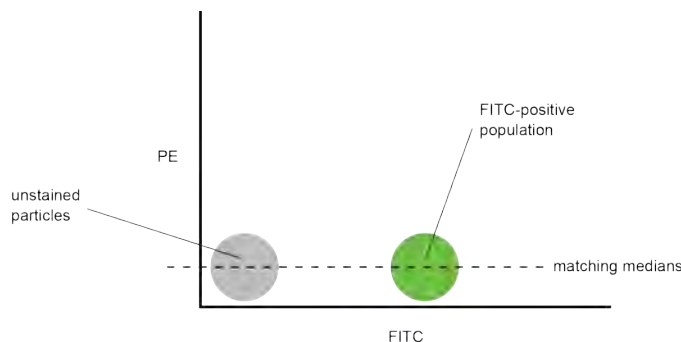


**Figure A-11 FITC Spillover Optimally Compensated out of the PE Signal**

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-12 illustrates this principle. Although the signals differ in intensity, the percentage of the FITC spillover into the PE detector remains constant.
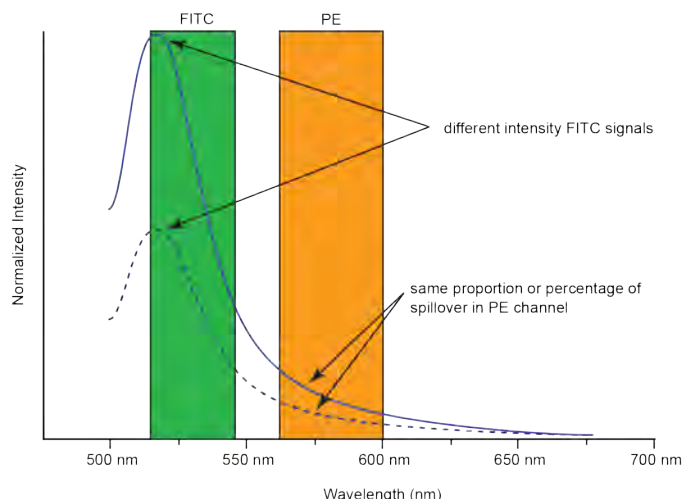
**Figure A-12 Spillover of Two FITC Signals of Different Intensities into PE Channels**

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-13) 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.
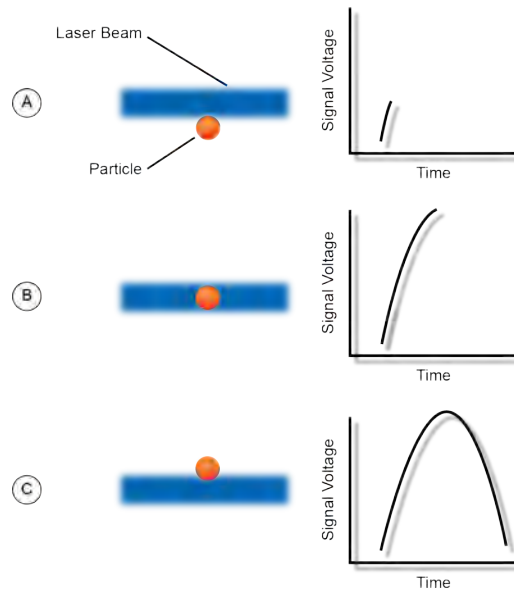
**Figure A-13 Illustration of Pulse Generation**

## Pulse Measurements

The electronic system measures pulses by three characteristics: Height, Area, and Width, as shown in Figure A-14.
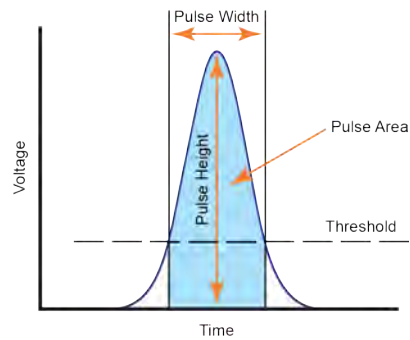


**Figure A-14 Pulse Measurements**

► 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 ($2^{24}$) levels (Figure A-15). 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.
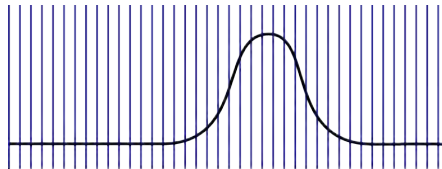
Signal Sampling Rate at 80M per second
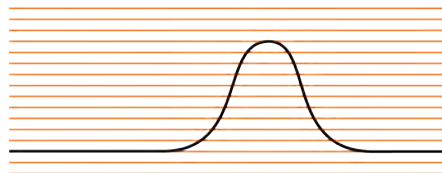
24 bits (16,777,216) Digitization

**Figure A-15 Pulse Digitization**

# Appendix B  NovoCyte Laser and Detection Channel Configuration

## Lasers Configuration

| Model Number | | 1000/ 1040/ 1050 | 1000Y | 2000R/ 2060R/ 2070R | 2070V/ 2090V/ 2110V | 2100YB | 3000/ 3005 | 3000VYB | 3000RYB |
|---|---|---|---|---|---|---|---|---|---|
| Lasers | 405 nm | | | | ◆ | | ◆ | ◆ | |
| | 488 nm | ● | | ● | ● | ● | ● | ● | ● |
| | 561 nm | | ■ | | | ■ | | ■ | ■ |
| | 640 nm | | | ▲ | | | ▲ | | ▲ |

## Detection Channel Configuration

| Detection Channel | | 445/45 | 530/30 | 572/28 | 586/20 | 615/20 | 660/20 | 675/30 | 695/40 | 725/40 | 780/60 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Models and Configurations | 1000-Default | | ● | ● | | | | ● | | | |
| | 1000-Config 1 | | ● | ● | | | ● | | | | |
| | 1000-Config 2 | | ● | ● | | | | | ● | | |
| | 1000Y | | | | ■ | ■ | ■ | | ■ | | ■ |
| | 1040-Default | | ● | ● | | | | ● | | | ● |
| | 1040-Config 1 | | ● | ● | | ● | | | | | ● |
| | 1040-Config 2 | | ● | ● | | | ● | | | | ● |
| | 1040-Config 3 | | ● | ● | | | | | ● | | ● |
| | 1050-Default | | ● | ● | | ■ | ◆ | ● | ▲ | | ■ |
| | 1050-Config 1 | | ● | | | | | | | | |
| | 1050-Config 2 | | ● | | | | | | | | |
| | 1050-Config 3 | | ● | | | | | | | | |
| | 2000R-Default | | ● | | | | | ●▲ | | | |

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Detection Channel Configuration

| Detection Channel | 445/45 | 530/30 | 572/28 | 586/20 | 615/20 | 660/20 | 675/30 | 695/40 | 725/40 | 780/60 |
|---|---|---|---|---|---|---|---|---|---|---|
| 2000R-Config 1 | | ● | | | | ●▲ | | | | |
| 2060R-Default | | ● | | | | | ●▲ | | | ●▲ |
| 2060R-Config 1 | | ● | | | | ●▲ | | | | ●▲ |
| 2060R-Config 2 | | ● | | | | ●▲ | | ●▲ | | ●▲ |
| 2060R-Config 3 | | ● | | | | ●▲ | | | ●▲ | ●▲ |
| 2070R-Default | | ● | | | | ●▲ | | | | ●▲ |
| 2070R-Config 1 | | ● | | | | ●▲ | | | ●▲ | ●▲ |
| 2070R-Config 2 | | ● | | | | ●▲ | | ●▲ | | ●▲ |
| 2070V-Default | ◆ | ◆● | ◆● | | | | | ◆● | | |
| 2070V-Config 1 | ◆ | ◆● | ◆● | | | ◆● | | | | |
| 2090V-Default | ◆ | ◆● | ◆● | | | | | ◆● | | |
| 2090V-Config 1 | ◆ | ◆● | ◆● | | | ◆● | | | | |
| 2090V-Config 2 | ◆ | ◆● | ◆● | | ◆● | | | | | |
| 2090V-Config 3 | ◆ | ◆● | ◆● | | | ◆● | | ◆● | | ◆● |
| 2110V-Default | ◆ | ◆● | ◆● | | | ◆● | | ◆● | | ◆● |
| 2110V-Config 1 | ◆ | ◆● | ◆● | | ◆● | | | ◆● | | ◆● |
| 2110V-Config 2 | ◆ | ◆● | ◆● | | ◆● | ◆● | | | | ◆● |
| 2100YB-Default | | ● | | ●■ | ●■ | ●■ | | ●■ | | ●■ |
| 3000-Default | ◆ | ◆● | ◆● | | ◆● | | ◆●▲ | | | ◆●▲ |
| 3000-Config 1 | ◆ | ◆● | ◆● | | ◆● | ◆●▲ | | | | ◆●▲ |
| 3000VYB-Default | ◆ | ◆● | | ◆●■ | ◆●■ | ◆●■ | | | | ◆■ |
| 3000VYB-Config 1 | ◆ | ◆● | | ◆●■ | | ◆●■ | | ◆●■ | | ◆■ |
| 3000VYB-Config 2 | ◆ | ◆● | | ◆●■ | ◆●■ | | | | | ◆■ |
| 3000RYB-Default | | ● | | ●■ | ●■ | ●■▲ | | ●■▲ | | ■▲ |

*(Row label group along left edge: Models and Configurations)*

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| Detection Channel | | 445/45 | 530/30 | 572/28 | 586/20 | 615/20 | 660/20 | 675/30 | 695/40 | 725/40 | 780/60 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Models and Configurations** | 3000RYB-Config 1 | | ● | | ●■ | ●■ | ●■▲ | | | ●■▲ | ■▲ |
| | 3000RYB-Config 2 | | ● | | ●■ | | ●■▲ | | ●■▲ | ●■▲ | ■▲ |
| | 3005-Default | ◆ | ●◆ | ●◆ | | | ●◆▲ | | | ●◆▲ | ●◆▲ |
| | 3005-Config 1 | ◆ | ●◆ | ●◆ | | | ●◆▲ | | ●◆▲ | | ●◆▲ |
| | 3005-Config 2 | ◆ | ●◆ | | | | ●◆▲ | | ●◆▲ | ●◆▲ | ●◆▲ |

◆ 405 nm Laser     ● 488 nm Laser     ■ 561 nm Laser     ▲ 640 nm Laser

# NovoCyte Violet/Blue/Red System (NovoCyte VBR System)

NovoCyte VBR system (i.e. NovoCyte 1000, 1040, 1050, 2000R, 2060R, 2070R, 2070V, 2090V, 2110V, 3000 and 3005 system) use 488 nm laser for FSC and SSC detection. Figure B-1 shows the schematics of the optical bench for NovoCyte 3000 default system. Novo-Cyte 1000, 1040, 1050, 2000R, 2060R, 2070R, 2070V, 2090V, 2110V and 3005 systems have similar optical schematics as 3000 default configuration, with difference on certain lasers, fluorescence detection channels, bandpass filters or dichroic mirrors for each model. The optical configuration of NovoCyte VBR system can be modified to alternative optical configurations by replacing certain bandpass filters and dichroic mirrors. Refer to *Laser Configuration* and *Detection Channel Configuration* above for the differences of each standard optical configuration. Refer to *Section 2.1.6: Verify and Modify Instrument Configuration* in this guide for details on modifying the instrument optical configurations.
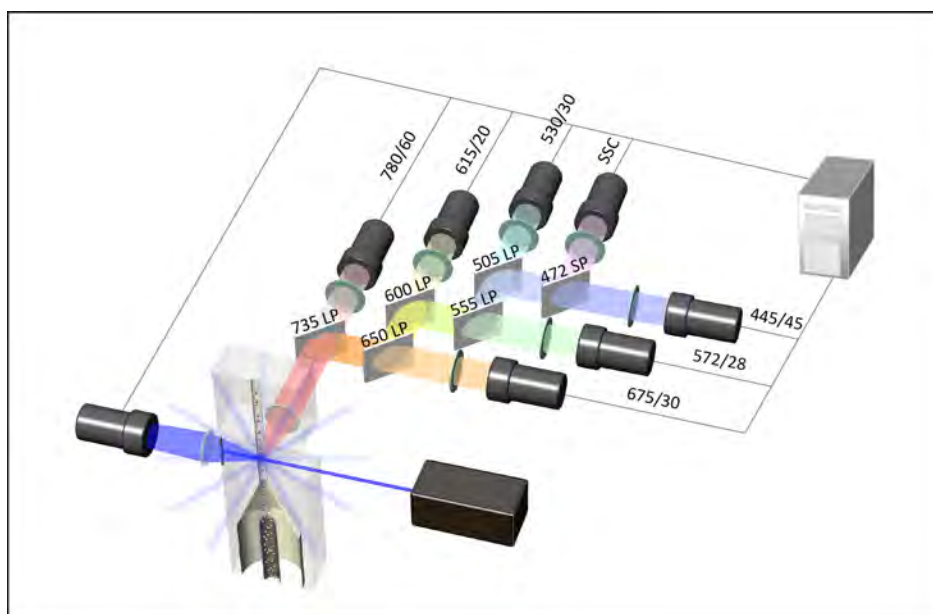


**Figure B-1 NovoCyte 3000 Default Optical System Schematics**

Table B-1 lists the dichroic mirrors and bandpass filters used in the NovoCyte 3000 default configuration and the recommended fluorochromes for each detector.

*NovoCyte Violet/Yellow/Blue System (NovoCyte VYB System)*

| Excitation Laser | Detector | Dichroic Mirror | Bandpass Filter* | Fluorochrome |
|---|---|---|---|---|
| 488 nm | Forward Scatter (FSC) | – | 488/10 | – |
| | Side Scatter (SSC) | – | 488/10 | – |
| | B530 | 505 LP | 530/30 | FITC |
| | B572 | 555 LP | 572/28 | PE |
| | B615 | 600 LP | 615/20 | PE-Texas Red® |
| | B675 | 650 LP | 675/30 | PerCP |
| | B780 | 735 LP | 780/60 | PE-Cy™7 |
| 640 nm | R675 | 650 LP | 675/30 | APC |
| | R780 | 735 LP | 780/60 | APC-Cy™7 |
| 405 nm | V445 | 472 SP | 445/45 | Pacific Blue™ |
| | V530 | 505 LP | 530/30 | AmCyan |
| | V572 | 555 LP | 572/28 | Pacific Orange™ |
| | V615 | 600 LP | 615/20 | Qdot® 605 |
| | V675 | 650 LP | 675/30 | Qdot® 655 |
| | V780 | 735 LP | 780/60 | Qdot® 800 |

*The bandpass filters and dichroic mirrors can be changed by the users to other bandpass filters and dichroic mirrors for alternative optical configuration. However, the users may need to perform necessary validation to ensure the data quality. Please refer to *Section 2.1.6: Verify and Modify Instrument Configuration* in this guide for details.

**Table B-1 NovoCyte 3000 Default Dichroic Mirrors and Bandpass Filters**

## NovoCyte Violet/Yellow/Blue System (NovoCyte VYB System)

NovoCyte VYB system (i.e. NovoCyte 3000VYB system) uses 561 nm laser for FSC and SSC detection. Figure B-2 shows the schematics of the optical bench for NovoCyte 3000VYB default configuration. The optical configuration of NovoCyte VYB system can be modified to alternative optical configurations by replacing certain bandpass filters and dichroic mirrors. Refer to *Laser Configuration* and *Detection Channel Configuration* above for the differences of each standard optical configuration. Refer to *Section 2.1.6: Verify and Modify Instrument Configuration* in this guide for details on modifying the instrument optical configurations.

**Figure B-2 NovoCyte 3000VYB Default Optical System Schematics**

Table B-2 lists the dichroic mirrors and bandpass filters used in the NovoCyte 3000VYB default configuration and the recommended fluorochromes for each detector.

| Excitation Laser | Detector | Dichroic Mirror | Bandpass Filter* | Fluorochrome |
|---|---|---|---|---|
| 561 nm | Forward Scatter (FSC) | – | 561/14 | – |
| | Side Scatter (SSC) | 555LP | 561/14 | – |
| | Y586 | 572LP | 586/20 | PE |
| | Y615 | 600LP | 615/20 | PE-Texas Red® |
| | Y660 | 650LP | 660/20 | PE-Cy™5 |
| | Y780 | 735LP | 780/60 | PE-Cy™7 |
| 488 nm | B530 | 495LP | 530/30 | FITC |
| | B615 | 600LP | 615/20 | PI |
| | B660 | 650LP | 660/20 | PerCP |
| 405 nm | V445 | – | 445/45 | Pacific Blue™ |
| | V530 | 495LP | 530/30 | AmCyan |
| | V586 | 572LP | 586/20 | Pacific Orange™ |
| | V615 | 600LP | 615/20 | Qdot® 605 |
| | V660 | 650LP | 660/20 | Qdot® 655 |
| | V780 | 735LP | 780/60 | Qdot® 800 |

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\* The bandpass filters and dichroic mirrors can be changed by the users to other bandpass filters and dichroic mirrors for alternative optical configuration. However, the users may need to perform necessary validation to ensure the data quality. Please refer to *Section 2.1.6: Verify and Modify Instrument Configuration* in this guide for details.

**Table B-2 NovoCyte 3000VYB Default Dichroic Mirrors and Bandpass Filters**

## NovoCyte Red/Yellow/Blue System (NovoCyte RYB System)

NovoCyte RYB system (i.e. NovoCyte 1000Y, 2100YB and 3000RYB system) use 561 nm laser for FSC and SSC detection. Figure B-3 shows the schematics of the optical bench for NovoCyte 3000RYB default configuration. NovoCyte 1000Y and 2100YB have similar optical system schematics as the 3000RYB default configuration, with difference on certain lasers, optical detectors, bandpass filters, and dichroic mirrors. The optical configuration of NovoCyte RYB system can be modified to alternative optical configurations by replacing certain bandpass filters and dichroic mirrors. Refer to *Laser Configuration* and *Detection Channel Configuration* above for the differences of each standard optical configuration. Refer to *Section 2.1.6: Verify and Modify Instrument Configuration* in this guide for details on modifying the instrument optical configurations.
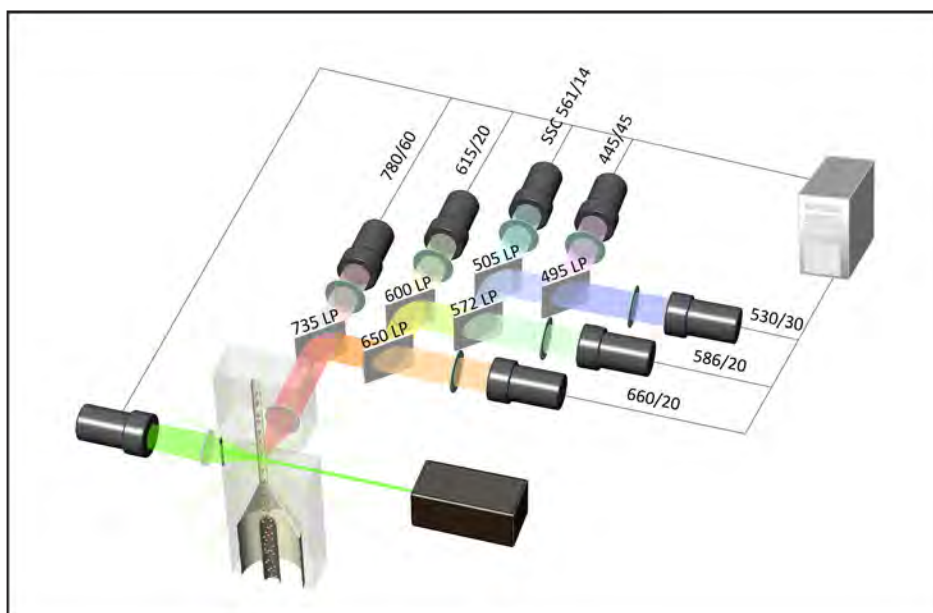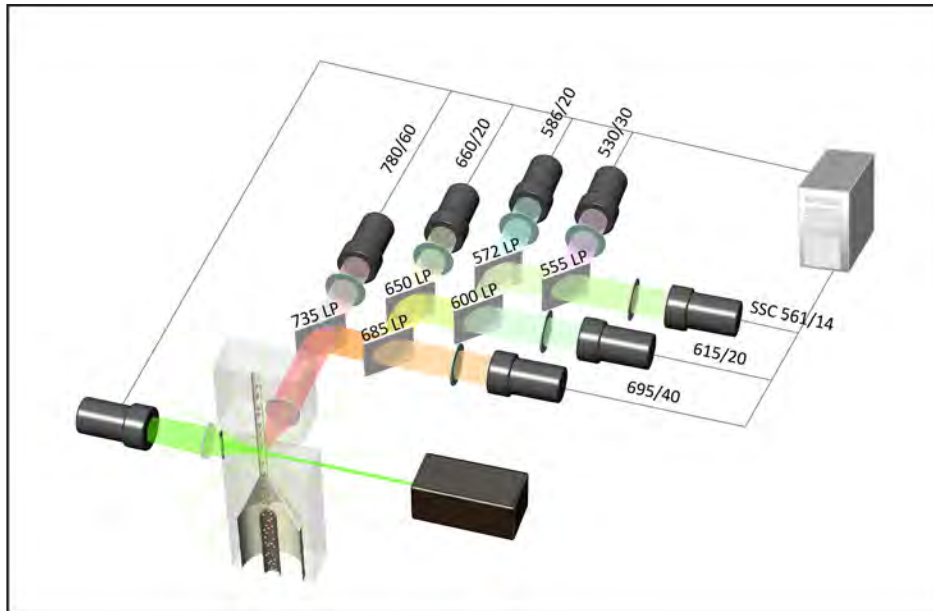


**Figure B-3 NovoCyte 3000RYB Default Optical System Schematics**

Table B-3 lists the dichroic mirrors and bandpass filters used in the NovoCyte 3000RYB default configuration and the recommended fluorochromes for each detector.

| Excitation Laser | Detector | Dichroic Mirror | Bandpass Filter* | Fluorochrome |
|---|---|---|---|---|
| 561 nm | Forward Scatter (FSC) | – | 561/14 | – |
| | Side Scatter (SSC) | 555LP | 561/14 | – |
| | Y586 | 572LP | 586/20 | PE |
| | Y615 | 600LP | 615/20 | PE-Texas Red® |
| | Y660 | 650LP | 660/20 | PE-Cy™5 |
| | Y780 | 735LP | 780/60 | PE-Cy™7 |
| | Y695 | 685LP | 695/40 | PE-Cy™5.5 |

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*NovoCyte Red/Yellow/Blue System (NovoCyte RYB System)*

| Excitation Laser | Detector | Dichroic Mirror | Bandpass Filter* | Fluorochrome |
|---|---|---|---|---|
| 488 nm | B530 | – | 530/30 | FITC |
| | B586 | 572LP | 586/20 | EYFP |
| | B615 | 600LP | 615/20 | PI |
| | B660 | 650LP | 660/20 | PerCP |
| | B695 | 685LP | 695/40 | PerCP-Cy™5.5 |
| 640 nm | R660 | 650LP | 660/20 | APC |
| | R780 | 735LP | 780/60 | APC-Cy™7 |
| | R695 | 685LP | 695/40 | Alexa Fluor® 680 |

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* The bandpass filters and dichroic mirrors can be changed by the users to other bandpass filters and dichroic mirrors for alternative optical configuration. However, the users may need to perform necessary validation to ensure the data quality. Please refer to *Section 2.1.6: Verify and Modify Instrument Configuration* in this guide for details.

**Table B-3 NovoCyte 3000RYB Default Dichroic Mirrors and Bandpass Filters**

# Appendix C NovoCyte Technical Specifications

| | | |
|---|---|---|
| **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 |
| | Scatter Resolution | 0.2 µm |
| | Cell Size | 0.2 - 50 µm |
| | Fluorescence Threshold Sensitivity | FITC < 75 MESF; PE < 50 MESF; APC < 20 MESF |
| | Fluorescence Resolution | < 3% CV for CEN |
| | Filters | User Exchangeable |
| **Fluidics** | Sample Acquisition Rate | 35,000 events/second |
| | Volumetric Absolute Count Precision | Syringe Pump; CV < 5% |
| | Sample Flow Rate | 5-120 µL/min |
| | Sheath Flow Rate | 6.5 mL/min |
| | Sample Aspiration Volume | 10 µL - 5 mL |
| | 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 Acquisition** | 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 photodetector gain adjustment |
| | Photodetector Gain Control | Fixed photodetector gain as a default, adjustable photodetector gain as an advanced function. |
| | Compensation | Automatic compensation, manual compensation, visual compensation tools available for pre/post/ live acquisitions |
| | Output Data Formats | FCS 3.1; NovoExpress (.ncf), PDF reports, bitmap graphics, vector graphic, CSV |
| | Workstation | Dell or other compatible models, 1 TB with 23.8" LCD monitor |

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| | | |
|---|---|---|
| Data Acquisition | Computer Operating System | Microsoft Windows® and Microsoft Office® |
| | Software | ACEA NovoExpress® |
| Sampling | Manual Sample Loading | 12 X 75 mm tube, 1.5 mL Eppendorf tube |
| | Automatic Sample Loading | Optional - compatible with 12 x 75 mm tube, 1.5 & 2 mL tubes, "bullet" tubes in 96-pos. racks, 24 well, 48 well and 96 well microtiter plates |
| Operating Conditions | Instrument Dimensions (W × D × H) | 23.6 × 17.7 × 15.4 in (60 × 45 × 39 cm) |
| | Instrument Weight | 86 lb (39 kg) |
| | Required Space for Installation | Width: > 59.1 in ( > 150 cm) Depth: > 31.5 in ( > 80 cm) Height:> 31.5 in ( > 80 cm) |
| | Power Requirements | Input: 12 VDC Power: 90 VA |
| | Audible Noise | < 60 dBA at 1.0 m |
| | Operation Requirements | Temperature: 15-32°C Relative Humidity: 80 % maximum Atmosphere Range: 76-106 kPa |
| | Transport and Storage Requirements | Temperature: 1-40°C; Relative Humidity: 80% maximum Atmosphere Range: 76-106 kPa |

*Specification and performance claims would be based on certain specific experiment conditions.*

*The Fluorescence Threshold Sensitivity values are applicable to NovoCyte 1000, 1040, 1050, 2000R, 2060R, 2070R, 2070V, 2090V, 2110V, 3000 and 3005 instruments.*

## Power Adapter

| Input | 100-240 VAC, 47-63 Hz, 1.2-0.5 A |
|---|---|
| Output | 12 VDC, 8.33 A maximum |

## Workstation Specifications

| Computer | Manufacturer:  Dell or other suppliers Rated Voltage:  AC 100-240 VAC, 50/60 Hz |
|---|---|
| Software | NovoExpress Software |

## Fluidics Station Specifications

| Dimensions (with reagents containers) (W × D × H) | 9.8 × 11.4 × 16.5 in (25 × 29 × 42 cm) |
|---|---|
| Weight | 13.2 lb (6 kg) |
| ACEA NovoFlow sheath fluid container | 3 L |
| ACEA NovoClean solution container | 500 mL |
| ACEA NovoRinse solution container | 500 mL |
| Waste container | 3 L |

## NovoSampler Specifications

| Dimensions (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 and 48-well plates, and ACEA NovoSampler 24 tube rack for 12 × 75 mm tubes |

## NovoSampler Pro Specifications

| Dimensions (W × D × H) | 14.6 × 10.2 × 11.0 in (37 × 26 × 28 cm) |
|---|---|
| Weight | 19.8 lb (9 kg) |
| Compatibility | Compatible with standard 96-well plates (flat-bottom, U-bottom, V-bottom), standard 24-well and 48-well plates, and ACEA NovoSampler Pro 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.

**ID**150014