

Expanding the Capabilities of Benchtop Flow Cytometry





Foreword

Flow cytometry is a powerful, quantitative technique used to count, sort and examine the multiple characteristics of cells. Using this technique, researchers are gaining deeper insights into cell viability, proliferation, apoptosis and cell cycle progression as well as being able to identify cell populations, intracellular and cell surface molecules.

Modern flow cytometers comprising multiple lasers and fluorescence detectors coupled with advances in software analysis and experimental components are enabling the wide-spread use of this technology in fields such as molecular biology, immunology, environmental science and pathology.

Agilent flow cytometers offer scientists excellent capabilities, high-quality data and an easy to use platform. In this eBook, we explore the fundamentals of the technology, the key features of Agilent's newest line of flow cytometers and the benefits provided to researchers using these products for their research.



Content Page

The Fundamentals of
Flow Cytometry _____ **4**

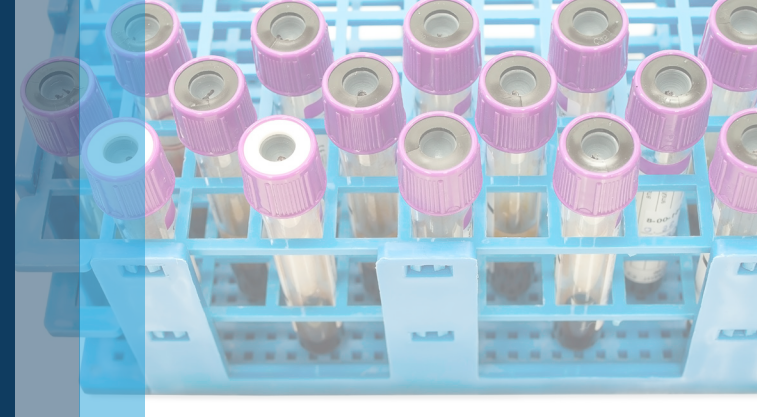
Infographic: What is flow cytometry? _____ **8**

The NovoCyte Range
of Flow Cytometers _____ **9**

Infographic: Choose a Flow Cytometer
that you can trust _____ **14**

Compendium _____ **16**

The Fundamentals of Flow Cytometry



Flow cytometry has become an invaluable tool in both clinical and research settings¹ and this technique provides important information on individual particles in a fluid-suspended population. The instrument focuses particles into a single file using a fluid stream before passing them through a light source where optical and fluorescence readings are detected and recorded. This assay is commonly used to investigate single cells; however, it has been adapted to study microorganisms, nuclei, or even quantitate soluble analytes in solution. The data collected using flow cytometry is then used to analyze the population collectively based on predefined parameters such as cell granularity, size and fluorescence. In addition, this data can be used in real-time to sort particles for further downstream processing.²

Flow cytometry instrumentation has rapidly evolved since its inception; initial instruments were used to measure the electrostatic properties of samples before fluorescence was introduced, however, it is now common for machines to have multiple, and more complex, laser/detector configurations. This allows the detection of more markers simultaneously, increasing time and resource efficiency. Important advances in flow cytometry include the use of more powerful photodetectors and quality lasers.³ Flow cytometers have also been expanded to incorporate mass spectrometry and microscopy, thereby expanding their basic and clinical science application.

A unique feature of flow cytometry is its ability to analyze individual particles in a population and distinguish subpopulations based on defined parameters. This feature is made possible by the speed at which individual particles can be characterized. In some systems, up to 100,000 particles can be analyzed per second, allowing researchers to distinguish rare subgroups within large cell populations rapidly.⁴⁻⁶ Another advantage of flow cytometry is the ability to sort cells according to differences in light scattering, antigen concentration and multiple parameters.⁴ The collected cells remain viable and can be used in further downstream assays, such as *in vitro* cell culture or *in vivo* experimentation.

The components of a flow cytometer

As illustrated in Figure 1, a flow cytometer consists of three components—fluidics, optics, and electronics. The **fluidics** system comprises a particle suspension fo-

cused single file into the center of a rapidly flowing fluid stream (sheath stream). The particles in the stream then pass through a light source, and each event is recorded. The **optics** of the flow cytometer consist of a light source, usually a laser, to illuminate the cells. Particles entering the beam emit scattered and fluorescent light, which is recorded by photo-detectors.⁴ After detection of the photons, the electronics system converts the light into digital signals and these signals are processed through to software where it is interpreted and stored as a standardized format (.fcs) data file.^{4,7}

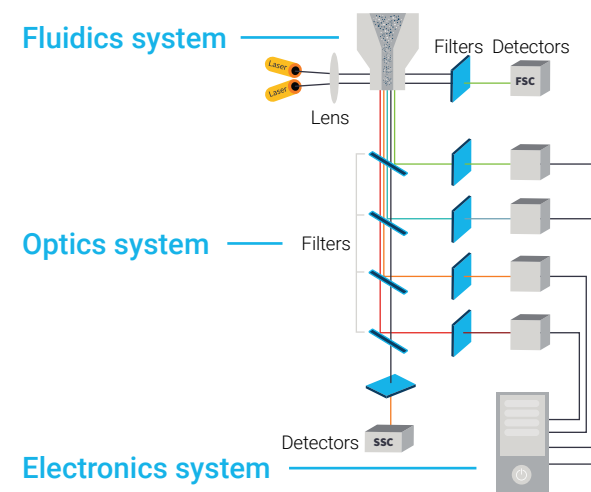


Figure 1. The main components of flow cytometry.

Applications of flow cytometry

Flow cytometry is a robust tool that allows for the simultaneous characterization of mixed cell populations. Thus, it is not surprising that it has found widespread use in immunology, molecular biology, bacteriology, virology, cancer biology, and infectious disease monitoring.⁷ Initially, flow cytometry was used to analyze blood cells;

The Fundamentals of Flow Cytometry

however, this was later expanded to cell lines and has been adapted for a range of other cells (such as bacteria, sperm and plankton) and non-cellular material (such as viruses, nuclei, cytokines, DNA fragments and latex beads).^{4,8} Tissue that is capable of being disaggregated, such as lymph nodes and solid tumors, can also be analyzed.^{4,7}

During cell or particle preparation, samples can be made to fluoresce through fluorescent protein expression, staining with fluorescent dyes or immunostaining with fluorescently-conjugated antibodies. With the wide variety of fluorochromes and instrumentation now available, it is possible to devise experiments with more than 30 parameters simultaneously.⁷

Cell counting

Flow cytometry is routinely used for cell counting in research and clinical settings. Complete blood counts form an integral part of human and veterinary diagnostic medicine and quantitative differences in cell abundance can indicate disease.^{8–10} For example, flow cytometry can be used to measure CD4 T cell counts. This technique, known as the CD4 count, provides a reliable indicator of a patient's immunologic status. In the context of HIV, CD4 T cells are selectively targeted and infected with the virus, therefore routine CD4 counts provide valuable information.¹¹

Analysis of cell culture

Flow cytometry is readily used in cell culture to analyze the expression of fluorescent proteins that can monitor gene expression as well as for drug discovery. Furthermore, with the help of fluorescently-conjugated antibodies against phosphorylated molecules, it can be used to investigate cell signaling pathways and it also has applications in cell biology assays.⁷ An exciting application in cell culture has been the increasing use of fluorescent flow cytometry to analyze single-cell expression distribution in bacteria. It is understood that significant heterogeneity in gene expression exists even in isogenic populations of microorganisms that are in homogenous environments. Flow cytometry can therefore be applied to accurately study these single-cell differences that would otherwise be lost using population-level investigations.¹² This application has been taken further by Zhao *et al.* (2020) to

study single-cell RNA expression. The technique uses nucleic acid rolling circle amplification to boost the signal of RNA expression profiles within the cell. They are then labeled with fluorescent detection probes and analyzed, allowing researchers to identify rare RNA events that are masked during homogenous measurements.¹³ Single cell measurements are important to discriminate between bi or multi modal distributions versus a homogenous population where population or well-based measurements would not detect these differences.

Measuring viability

Determining cell viability is a crucial method used in many areas of cell research and diagnostics, and flow cytometry is often preferable to microscopy when conducting these assays. The advantages of flow cytometry-based viability assays when compared to microscopy-based techniques include the ease and speed at which large numbers of cells can be processed, as well as acquiring quantitative measurements.¹⁴ Measuring viability can be coupled with additional markers allowing the ability to characterize which cell types in a sample are still viable. Additionally, multiple markers for viability can be measured simultaneously using the flow cytometer that can provide insights into stages or pathways of cellular death.

Investigating cell proliferation

Cell proliferation is crucial for tissue development and homeostasis – abnormalities often lead to degenerative diseases and cancer.¹⁵ Flow cytometry can investigate proliferation using dye-based (CFSE) and DNA synthesis-based (BrdU) assays, as well as labelling proliferation-related antigens to assess cell division. Dye-based assays, such as CFSE, stain cells through passive absorption and bind covalently to intracellular structures and partitioned equally to the two daughter cells during cell division, indicating proliferative activity.^{4,16} On the other hand, DNA synthesis-based assays incorporate thymidine analogues that can be stained with antibodies.⁴ Flow cytometry-based proliferation assays can be used to evaluate the function of lymphocytes during screening. The gold standard has been a proliferation assay that incorporates radioactive nucleotide [³H]-thymidine; however, this assay is unable to distinguish between T- and B-cell defects. Flow cytometry assays are able to overcome this problem by providing

The Fundamentals of Flow Cytometry

investigators with far more detailed insight with mixed cellular populations.⁷

Phenotyping various cell types

The most common application of flow cytometry is its use in immunophenotyping. The technique is used to distinguish mixed populations using multiple parameters, making it an ideal tool for the characterization of the immune system and the identification of rare cell populations. Fluorescently-conjugated antibodies that target specified cellular antigens (both surface-bound and intracellular) can be used for phenotyping. It is commonplace for immunophenotyping experiments to use between 4-15 colors, or even more. Other immunological assays that can be performed using flow cytometry include antigen-specific responses, intracellular cytokine analysis and apoptosis.⁷

Cell sorting

A valuable application of flow cytometry is the rapid ability to distinguish between differently labeled cells. This can be applied to sort and purify cells into homogeneous populations with the aid of a cell sorter. These sorted populations can then be used for further studies.⁷ Cell sorters are downstream of the optics and detectors; cells are separated into droplets by oscillating the sample stream at high frequency. The droplets then receive a charge before passing through deflection plates that direct the droplets into specific collection vessels. Cells can be sorted into four subpopulations depending on the charge (positive or negative, weak or strong).⁴ Cell sorting is particularly invaluable when enriching for rare cell types. For example, researchers have developed flow cytometry assays that are capable of detecting and isolating a single tumor cell from one million white blood cells.¹⁸

Challenges and limitations

Flow cytometry is not without its limitations; however, researchers continue to address these problems and find solutions as the technology matures. Several challenges and constraints will be discussed below.

Affordability

Flow cytometers require a capital investment upfront and can have high operating costs. This often makes these instruments unavailable to small laboratories and departments. Once installed, the complete flow cytometer setup is often bulky, requiring a large lab footprint, and the size of the system excludes its application in the field.

Maintenance

Flow cytometers require careful maintenance; otherwise, problems can arise within the various components. Failure to thoroughly clean the fluidics system can result in contamination and cause blockages in the tubing. Considering that flow cytometers are generally restricted to particle sizes ranging between 0.5-30 μm , buildup inside the tubing can quickly cause the pressure to rise and the instrument to clog. There is a particular risk for this during the analysis of larger cells, such as cancer cells. Additionally, sometimes the optics require regular alignment to ensure consistent results between experiments.

Signal complexity

It can be challenging when an experiment involves many parameters, therefore, most flow cytometers are limited to recording a maximum of 20 parameters.¹⁴ However, this can restrict researchers from identifying all the subpopulations within a sample, resulting in a loss of valuable information.

Signal spillover between multiple fluorophores can also affect the data; it occurs when the excitation and emission spectra for fluorophores overlap, resulting in the measurement of signals in both the main and the neighboring channels. This spillover can be corrected using a process called compensation. However, detecting

The Fundamentals of Flow Cytometry

low-abundance proteins in high parameter experiments can become difficult due to a phenomenon known as spillover-spreading error, which occurs as a result of three factors: an increase in the number of fluorophores used, the spillover of the fluorophore into another detector and an increase in the intensity of the fluorophore.¹⁹ In addition, the high volume of data that is generated makes analysis more challenging – this is compounded by the fact that most software available is not user-friendly.¹

Sample preparation

In some instances, the use of enzymes to prepare samples, such as tissue, into single-cell suspensions can alter the antigenic characteristics of the population. Changes to these characteristics can affect the binding of fluorescently conjugated antibodies and therefore impact the results. Additionally, tissue dissociation causes information about cell distribution and tissue architecture to be lost. The differentiation and analysis of subpopulations become complicated when two populations have similar marker expression profiles.

Conclusion

Flow cytometry is an invaluable asset for many laboratories. It provides highly detailed data on individual cells and particles within a population and offers solutions to a wide range of research questions. As with any technique, there are limitations, however, these are not insurmountable. Further developments in this area such as standardization and automation will have a significant impact on throughput and the reliability of analyses.

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What is flow cytometry?

Flow cytometry is a technique that translates light detected by cells into signals that can be analyzed and measured. The figure below shows the three main components of a flow cytometer, as well as a schematic of the light scatter measured by the two optical detectors. Taken together, both measurements allow for some degree of cellular differentiation within a heterogeneous population.¹

Fluidic Sheath

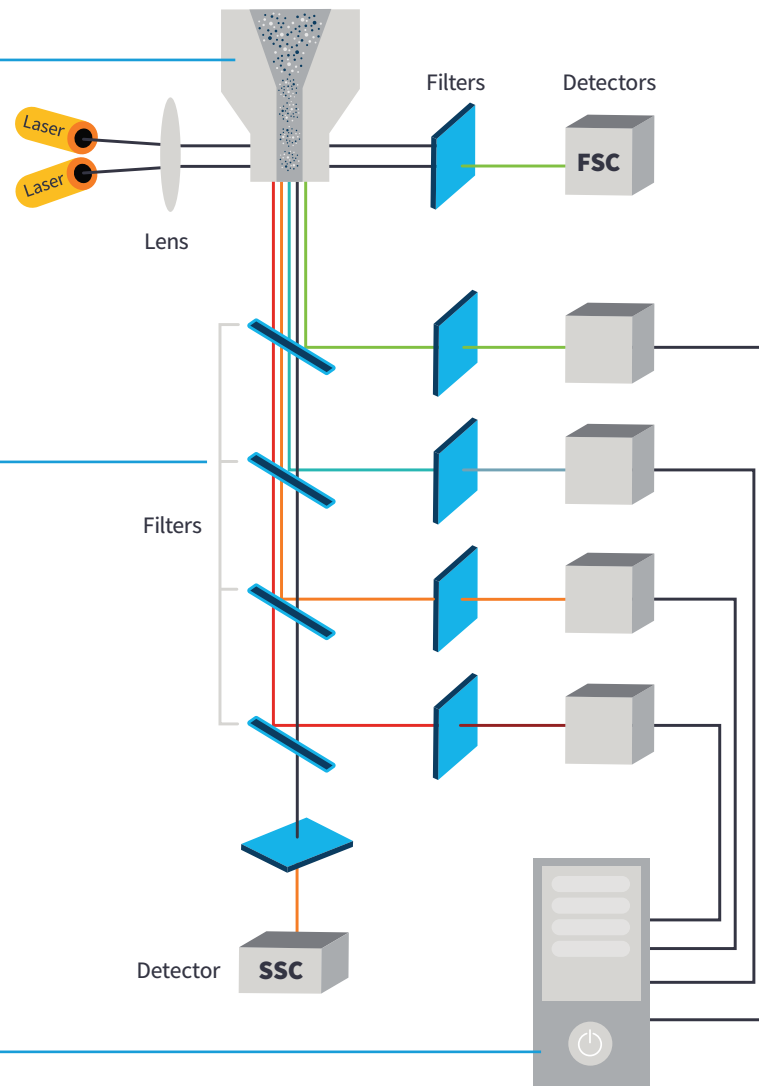
Contains sheath fluid that is pressured through the machine to guide the cell sample past the laser for separate measurement of every single cell.

Optics System

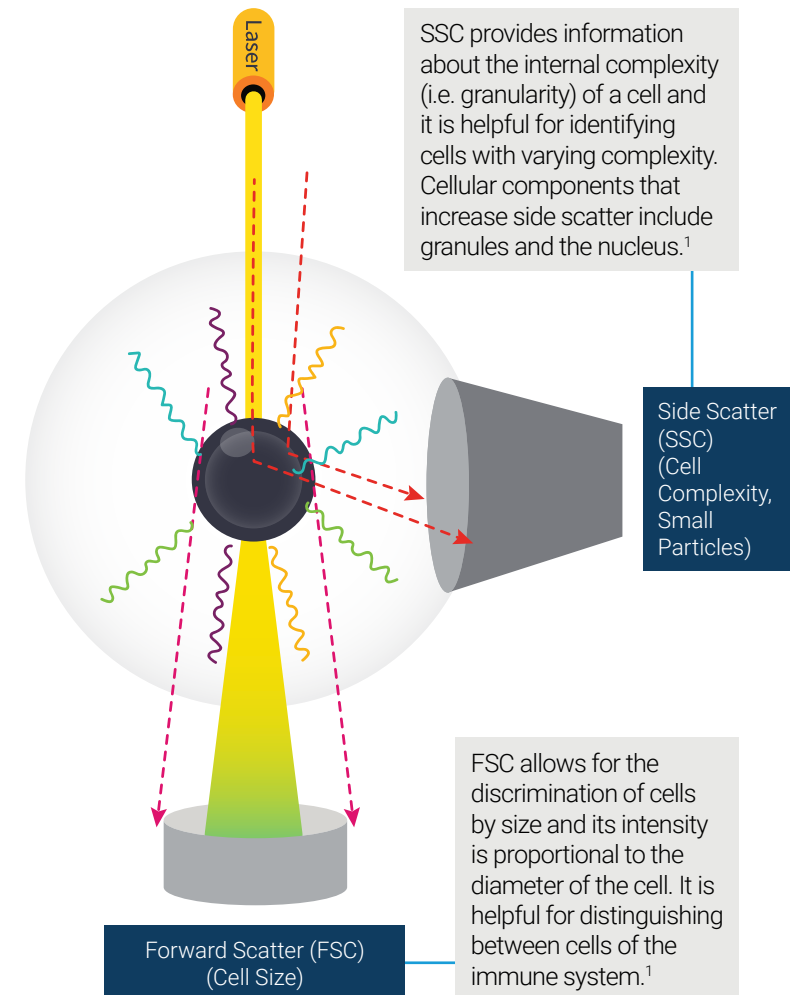
Contains lasers, which emit light to the samples, and collection optics, e.g. photomultiplier tubes (PMTs) that collect signals scattered by the sample.

Electronics Systems

Convert the detected signal to digital parameters that are analyzed using software.



What is forward and side scatter?



1. Taylor I. Forward Scatter vs. Side Scatter. FlowJo

The NovoCyte Range of Flow Cytometers

Introduction

The NovoCyte was Agilent's first-generation high-performance flow cytometer, designed for all levels of users and laboratories. Since its first introduction onto the market in 2014, this budget-friendly and highly reputable instrument has grown to become a trusted device in laboratories worldwide, featured in many highly cited peer-reviewed journals. The key features that make the NovoCyte a success include its ability to measure up to 17 parameters, advanced data analysis capabilities, customizable options, streamlined workflow with quick setup, and the user-friendly NovoExpress software.

Building upon the success of this technology, Agilent has introduced its second-generation instruments – the NovoCyte Advanteon, NovoCyte Quanteon and NovoCyte Penteon – providing researchers with exceptional, high-quality data for a simple flow cytometry experience.

Agilent continues to pioneer development in flow cytometry, providing customers with instruments that are easy to use, have excellent capabilities, and produce publishable data. Here we explore the features of the second-generation NovoCyte systems that are helping researchers to increase productivity and enhance their research.

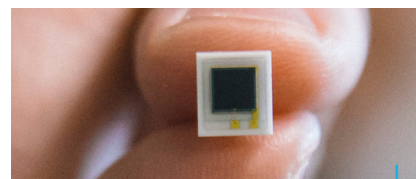
Next generation NovoCyte flow cytometers

The added features built-in to these second-generation instruments include state-of-the-art detectors, silicon photomultipliers, to accommodate an increased number of parameters (up to 32) and lasers (a maximum of five). Even with more parameters, users will continue to receive unrivaled sensitivity and resolution. These instruments have a wide dynamic range (10^7) – so routine detector adjustments are no longer necessary – and various usability enhancements, such as the walk-away operation and smart-design functions. As with the original NovoCyte, these automation-compatible second-generation instruments are supplied with Agilent's NovoExpress software which provides intuitive and robust data acquisition, analysis, and reporting (Figure 1, Table 1).



Detectors

- Next generation, solid state SiPM detectors for excellent sensitivity
- Scatter resolution as low as 100nm
- Wide dynamic range of signal detection, dim and bright signals without adjusting settings
- Eliminate the need for routine detector setting adjustments



NovoSampler Q

- Easily accommodate tubes or plates (48, 96, 384, custom)
- Can process 96 well plate in <18min
- Fast electronics, can process up to 100,000 events/sec
- Robotic automation compatible



Fluidics

- Improved design for enhanced fluidic stability
- Syringe pump sample delivery enabling absolute cell counts in every sample
- Push of a button automatic cleaning, rinsing, on/off
- Automatic fluidic level detection and notifications

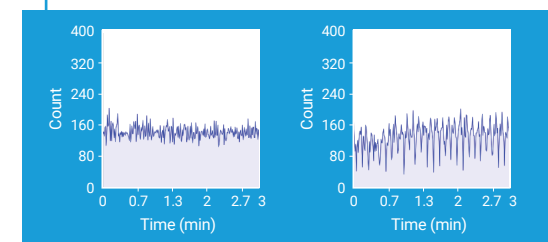


Figure 1: The main features of the new second-generation NovoCyte flow cytometers.

The NovoCyte Range of Flow Cytometers

Table 1: A comparison of the different second-generation NovoCyte flow cytometers.

Flow Cytometer System	Novocyte Advanteon	NovoCyte Quanteon	NovoCyte Penteon
Fluorescence Channels	4-21	16-25	30
Laser Configuration	1-3 laser options with a choice of violet, blue, yellow, and/or red	Violet/Blue/Yellow/Red	UV/Violet/Blue/Yellow/Red
Laser Wavelength(s)	405 nm, 488 nm, 561 nm, 637 nm	405 nm, 488 nm, 561 nm, 637 nm	349 nm, 405 nm, 488 nm, 561 nm, 637 nm
Number of Lasers	1-3 Lasers	4 Lasers	5 Lasers

Expand your capabilities

Sensitivity

The second-generation NovoCyte instruments have received several technical upgrades that enhance their sensitivity. All machines are now equipped with advanced silicon photomultipliers (SiPMs) for signal detection. These represent state-of-the-art photon detection and replace the traditional photomultiplier tubes (PMTs) used in flow cytometry.¹ NovoCyte flow cytometers come fitted with up to 30 independent SiPMs that capture and process signals for each of the fluorescence detection channels.

SiPMs come with several advantages over traditional flow cytometry detectors; they are solid-state detectors, providing durability and a long life span.^{3,4} In addition, no

warm-up time is required for these detectors, and the response time is very fast due to their design.^{5,6} When it comes to signal detection, SiPMs have a high gain capable of detecting single photons and very low concentrations of fluorescently-tagged particles. Detection capabilities are further boosted by high sensitivity and high quantum efficiency, making them ideal for flow cytometry.^{4,7}

In addition to utilizing advanced detector technology, our focus has been directed to enhancing machine optics as well. Scientists are increasingly using flow cytometers to process and analyze submicron particles such as extracellular vesicles, and this is done partially by light scatter, which is dependent on particle size and refractive index. The detection of light scatter of less than 500 nm submicron particles is challenging using traditional instruments.⁸ NovoCyte second-generation cytometers are able to achieve particle resolution down to 100 nm, as they are equipped with optimized scatter detection optics and signal processing electronics.

Furthermore, the high-performance fluidic systems within these second-generation NovoCyte flow cytometers provide steady flow rates to ensure reliable and consistent results. These systems reduce inconsistency and inaccuracy often found in other instruments with large variations when sample flow rates are increased.

Ease of use

Traditional flow cytometers are hindered by long and manual start-up and shutdown processes. To combat this, NovoCyte Advanteon, NovoCyte Quanteon and NovoCyte Penteon have several new features that enhance their usability.

Once the instruments are turned on, short, fluidic protocols automatically rinse and prepare the machines for use. Users can also customize startup/shutdowns and specify a set time for the instrument to undergo a thorough automated shutdown cleaning, removing the need to clean the machine manually at the end of the day.

In labs with many users and ever-changing experiment options, the optics systems automatically detect the configuration based on the optical filters inserted and

The NovoCyte Range of Flow Cytometers

alerts the user of any misplacement. Optical filters are embedded with electronic chips for automatic recognition by the software. The instruments also seamlessly integrate into high-throughput operations with the inclusion of an onboard barcode reader for automatic plate identification.

Conventional workflows require manual inspection of the fluidics levels, however the second-generation NovoCyte instruments include valves and sensors that automatically monitor for clog detection (and recovery), as well as sheath, waste, rinse, and clean fluid levels – users receive alerts when liquid volumes are low, or the waste volume is high. Before a plate/rack run is activated, the fluid consumption is estimated to prevent interrupted sample acquisition during the run and reduce downtime.

Quality control (QC) is at the forefront of these systems, with fully integrated automatic tests that track day-to-day and long-term monitoring. Quick automatic QC checks, comprehensive QC reports, and Levey-Jennings plots, that track long-term performance can be generated to ensure consistent and proper performance monitoring of the instruments. Collectively, these features remove the hassle of manual maintenance and save time, allowing researchers to focus on the experiments.

Accuracy

NovoCyte flow cytometers incorporate novel functions for enhanced accuracy. For example, the NovoCyte line of flow cytometers all are equipped with high-accuracy syringe pumps that control flow rates precisely and enable direct volumetric absolute cell counts during every flow cytometer run.⁹ All instruments make direct cell counts removing the need for expensive reference beads which are used in traditional flow cytometers to allow for counting capabilities. Furthermore, a feedback control system continuously monitors the sheath flow rate to maintain superior fluidic stability and the combination of high-quality lasers, optical filters, and detectors ensure consistent signal detection.

The NovoSampler Q automatic sample loading system, an optional accessory with all instruments, is equipped with an orbital shaker to maintain all samples

in suspension during sample acquisition for consistent and reproducible results. Uniform cell suspensions guarantee consistent and reproducible results and are particularly important where cell settling impacts outcomes.¹⁰ Importantly, the speed, duration and acceleration of the orbital shaker can be customized for all experiments to allow flexibility depending on sample type. Additional features include:

- Automatically calibrated plates, removing the need for manual alignment
- Versatile and high-throughput loading system that accommodates several sample formats – including tubes, plates, and customizable plates with no hardware changes
- Rapid and high-throughput sample processing (the sampler can read a 96-well plate in less than 20 minutes and a 384-well plate in under 80 minutes)
- Open architecture and developer-ready API to facilitate easy integration into automated laboratories

With these integrations, the second generation NovoCyte flow cytometers provide the user with consistent and reliable data no matter the settings or number of samples.

Streamlined data analysis

NovoExpress software improves versatility and operation by combining sample acquisition and data analysis into one interface. Also, the ability to analyze samples in real-time while sample acquisition happens in the background, and providing batch analysis and reporting, maximizes productivity and efficiency. Additional features include:

- Configurable statistical parameters
- Accurate pre-and post-acquisition compensation
- Publication-ready figures with customizable font, plot scales and legends
- Versatile data presentation for easy communication
- Advanced analysis features including cell cycle, proliferation, bi-variate, and heat map displays

The NovoCyte Range of Flow Cytometers

NovoCyte flow cytometers in action

In this section we explore the various research areas that are benefitting from NovoCyte flow cytometers, such as the successful publications in the fields of oncology, pharmacology and marine biology.

Oncology

NovoCyte Advanteon was employed by Li *et al.* (2021) as part of a series of experiments that investigated Actin-like protein 6A (ACTL6A), a driver of oncogenesis, and its role in esophageal squamous cell carcinoma tissue (ESCC). ACTL6A is a regulatory subunit of the ATP-dependent SWI/SNF chromatin-remodeling complexes. While these complexes are known to be drivers of oncogenesis, little is understood about the function of ACTL6A in ESCC. The NovoCyte Advanteon was used to determine the effect of ACTL6A on distribution in different cell cycle stages and apoptosis. From these experiments it was demonstrated that ACTL6A affects ESCC cell proliferation through cell cycle redistribution as opposed to regulating apoptosis, and through this work, ACTL6A was identified as an alternative therapeutic target for ESCC.¹¹

In the second study focusing on ESCC, a panel of p53 mutants in individual ESCC cell lines were functionally characterized and then assayed for gain-of-function (GoF) oncogenic properties. The TP53 gene, encoding the master tumor suppressor protein p53, is one of the most mutated genes identified across all cancers – the mutation rate is particularly high in ESCC. These mutations can result in GoF oncogenic features. The NovoCyte Quanteon was used in cell-cycle analysis with cells labeled with BrdU. Results of this study identified a novel GoF mechanism that allowed specific p53 mutants to exert oncogenic features and contribute to tumorigenesis.¹²

The NovoCyte Advanteon has also been used in the development of a novel dual-target chimeric antigen receptor (CAR) T cell technology against epithelial ovarian cancer. CAR-T cells are genetically modified T-cells that express CAR fusion proteins. These proteins consist of three domains—antigen recognition,

transmembrane and signal transduction—that direct the T cells toward surface-exposed tumor-associated antigens.¹³ In this study the NovoCyte Advanteon was used not only in the preparation of CAR-T cells, but to measure the viral titer and infection rate (of the lentiviral vector used to transfect the T cells) and to confirm the co-expression of the CARs in the T cells. These isolated dual CAR-T cells were then assessed *in vivo* using a tumor-bearing mice model assay, and demonstrated enhanced killing capacity against OVCAR-3 cells. Based on the success of this clinical research, trials are now being considered.¹⁴

Lastly, making use of the NovoCyte Quanteon, researchers aimed to further understand the molecular mechanism of papillary thyroid cancer (PTC), by identifying immune-related long non-coding RNAs (lncRNAs) in PTC. The NovoCyte Quanteon was used to conduct proliferation and apoptosis assays. Based on these and other assays, researchers were able to identify one lncRNA with the highest association with prognosis.¹⁵ The discovery of this immune-related lncRNA is regarded as the first step to improve the prognosis prediction with PTC.

Marine biology

Dinophyceae are microalgae that are commonly associated with red tides and are hosts for parasites of the genus *Amoebophrya* sp. In this study, researchers aimed to elucidate the relationship between Dinophyceae its parasite. The NovoCyte Advanteon was employed to determine the cell count and membrane permeability of Dinophyceae in the presence and absence of the parasite. The study revealed that Dinophyceae resistant to the parasite have extracellular defenses that benefit the community, including sensitive Dinophyceae. Isolating the anti-parasitic compounds found in these extracellular defenses could be used in the future to mitigate against parasites that affect algal mass cultures.¹⁶

Pharmacology

Flow cytometry has also been applied to investigate the pathogenesis of heart failure. In a study by Lou *et al.* (2021), the authors investigated the effects of Nuanxin capsule,

The NovoCyt Range of Flow Cytometers

a traditional Chinese medicine, on heart failure. Part of the study focused on *in vitro* experiments using Nuanxin capsule and cardiomyocytes. The NovoCyt Quanteon was used to analyze cells from an intracellular reactive oxygen species (ROS) production assay, cell apoptosis assay and mitochondrial membrane potential ($\Delta\Psi_m$) assay. Cell treatment with Nuanxin capsule was found to reduce ROS production, inhibit mitochondrial-mediated apoptosis and enhance $\Delta\Psi_m$ and mitochondrial respiration. This study demonstrated that Nuanxin capsule was capable of improving cardiac function in transverse aortic constriction mice making this traditional Chinese medicine a promising candidate in the management of heart failure.¹⁷

Conclusion

Based on the success of the original NovoCyt flow cytometer, the NovoCyt second-generation instruments build on the original features by incorporating new technologies to enhance sensitivity, provide ease-of-use, hone accuracy and streamline your data analysis. These instruments are already making an impact in the scientific community and contributing to exciting cutting-edge science. High-performance flow cytometers don't have to be complicated, and easy-to-use flow cytometers are allowing researchers of all experience levels to generate the data necessary for the research questions they are asking. Contact us if you think a high-performance, easy to use flow cytometer can benefit your team.

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Choose a Flow Cytometer that you can trust

Consistent results, reliable performance

Walk-away Automation Simplifies Your Workflow

Continuously monitors fluid levels

A fluidic station will sense low fluid or high waste and eliminates the need for manual inspection. Fluidics consumption is estimated before a plate runs to ensure uninterrupted sample acquisition.

Easy startup and shutdown

Quick startup with automated fluidic rinsing takes only minutes to prepare the instrument for your daily use. The configurable pre-scheduled shutdown thoroughly cleans at a specified time each day to eliminate the hassle of end-of-day manual cleaning with an automatic shutdown cleaning procedure.

Hassle-free fluidics

Electronically monitored valves and sensors allow for automatic clog detection and recovery. A feedback control system continuously manages sheath flow rate to maintain exceptional stability.

Embedded quality control

Quickly run daily QC, automatically generate comprehensive QC reports, and conveniently track performance over time with Levey-Jennings plots. The automatic QC test ensures proper performance monitoring on not only a day-to-day basis, but also over long-term use.



Expanded flexibility with up to 30-color options using up to 5 lasers

High sensitivity and resolution

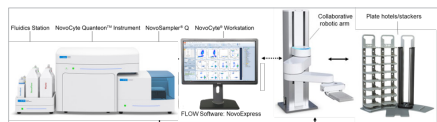
Intuitive and powerful software for data acquisition, analysis, and reporting

Smart-design functionalities and walk-away operation to simplify your workflow

Automation-ready capability for high throughput needs

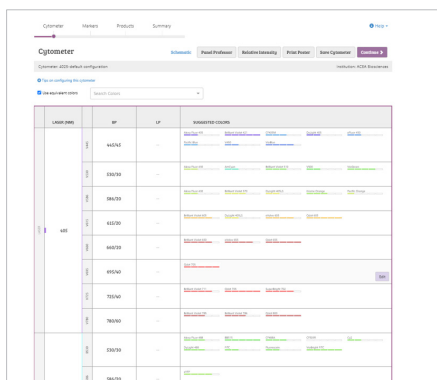
Wide, 7-log dynamic range eliminates the need for routine detector adjustments

Compendium



Robotic Automation

Visit this webpage for resources about the features and benefits of the robotic automation for flow cytometry.



Panel Builder

Visit this webpage to learn more about the various configuration options for the NovoCyte flow cytometers and the benefits of integrating them with the NovoExpress software.



Spectra Viewer

The Novocyte spectra viewer allows users to see the spectra (% Excitation/Emission and wavelength (nm)) of different factors, such as the type of machine and specific fluorophore. Visit this webpage to find out more.



Portfolio

Download this detailed brochure to explore the features and benefits of Agilent's newest range flow cytometers: NovoCyte Penteon, NovoCyte Quanteon, and NovoCyte Advanteon.

Agilent Flow Cytometer Specifications

NovoCyte Penteon (5 Lasers)
NovoCyte Quanteon (4 Lasers)
NovoCyte Advanteon (1-3 Lasers)

A breakthrough in flow cytometry design, the Agilent NovoCyte Penteon, NovoCyte Quanteon, and NovoCyte Advanteon provide up to 30 fluorescence channels to meet the most demanding panels. The detectors have a 7.2 log dynamic range and advanced auto compensation features, alleviating the need to tune each detector. You now have the flexibility to choose from 30 fluorescence channels using one to five lasers, without having to sacrifice the performance in one channel for another.

NovoCyte Penteon
NovoCyte Quanteon
NovoCyte Advanteon
specifications

Lasers	405 nm	488 nm	640 nm	660 nm	785 nm
488/485 nm	●	●	●	●	●
532/485 nm	●	●	●	●	●
568/520 nm	●	●	●	●	●
615/520 nm	●	●	●	●	●
647/520 nm	●	●	●	●	●
680/485 nm	●	●	●	●	●
725/485 nm	●	●	●	●	●
780/485 nm	●	●	●	●	●
NovoCyte Penteon (5 Lasers)	Pick up to 30				
NovoCyte Quanteon (4 Lasers)	Pick up to 30				
NovoCyte Advanteon (1-3 Lasers)	Pick up to 30				

Agilent Flow Cytometer Specifications

Download this resource to learn more about the specifications of each second-generation NovoCyte.

Compendium

Application Note
Immunotherapy
Research


Trusted Answers

Ex Vivo Phenotyping and Potency Monitoring of CD19 CAR T Cells

Using a combined flow cytometry and impedance-based real-time cell analysis workflow

Authors
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Ming Lei, Peifang Ye,
Yan Lu, Xiaoping Ju, Yu Yan,
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Agilent Technologies, Inc.

Abstract
The development of cell-mediated immunotherapies has revolutionized cancer research as well as the study of the immune system. One of the most promising types of cell therapy involves the genetic engineering of novel chimeric antigen receptor (CAR) T cells to target cancer cells. To efficiently determine optimal CAR construction, researchers must develop a robust screening protocol to identify the ideal parent T cell populations and evaluate their cell killing potency. Here, we describe a workflow using a combination of the Agilent xCELLigence RTCA analyzer and Agilent NovoCyte Quanteon flow cytometer to thoroughly evaluate and characterize CAR T cells.

[Ex vivo Phenotyping and Potency Monitoring of CD19 CAR T Cells](#)

Download this application note to explore the workflows available for the evaluation and characterization of T cells.

Application Note
Cell Analysis


Trusted Answers

Tumor Cell Killing by T Cells

Quantifying the impact of a CD19-BiTE using real-time cell analysis, flow cytometry, and multiplex immunoassay

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Abstract
The ability to quantitatively correlate T cell biomarker expression or secretion with target cell killing is critical in tumor immunology studies. This application note demonstrates a workflow using the Agilent xCELLigence real-time cell analysis (RTCA) instrument with an Agilent NovoCyte flow cytometer to study both cytotoxic T lymphocyte (CTL)-mediated destruction of tumor cells and the corresponding secretion of cytokines and cytolytic proteins by CTLs. Bispecific T cell engagers (BiTEs) enhance the ability of CTLs to specifically recognize and eliminate tumors. This enhancement effect was evaluated using three distinct assays. Target cell death was monitored using an Agilent RTCA biosensor assay and a flow cytometry-based cytotoxicity assay, while secretion of cytokines and cytolytic proteins was quantified in a bead-based multiplex flow cytometry assay.

[Tumor Cell Killing by T Cells](#)

Download this application note to learn how Agilent's solutions are being used to study the cytotoxic T lymphocyte (CTL)-mediated destruction of tumor cells and the corresponding secretion of cytokines and cytolytic proteins by CTLs.

Application Note
Cell Analysis


Trusted Answers

Comprehensive 18-Color Pan-Leukocyte Flow Cytometry Analysis for Immune Surveillance

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Abstract
This application note describes the use of an 18-color immunophenotyping stain to examine leukocyte subsets in human peripheral blood mononuclear cells (PBMCs), using the Agilent NovoCyte Quanteon flow cytometer. This method simultaneously identifies and quantifies monocytes, B cells, plasmablasts, CD4+ and CD8+ T cells, regulatory T cells, $\gamma\delta$ T cells, NK T cells, NK cells, and dendritic cells. NK and T cell status are indicators of a proper immune response to a vaccine, therefore NK and T cell activation and differentiation statuses were evaluated to obtain a deeper understanding of immune status.

[Comprehensive 18-Color Pan-Leukocyte Flow Cytometry Analysis for Immune Surveillance](#)

Download this application note to explore the benefit of using the Agilent NovoCyte Quanteon flow cytometer to examine leukocyte subsets in human peripheral blood mononuclear cells (PBMCs).

Application Note
Cell Analysis


Trusted Answers

Sentinel Panel Design of 16-Color, 28-Markers for Immunophenotyping Peripheral Human Whole Blood

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Abstract
Flow cytometry is a powerful tool to analyze highly heterogeneous cells such as those in blood. However, when using traditional panel design, the number of markers that can be analyzed is limited to the number of available channels on the instrument and available fluorochromes. By using a sentinel panel design, in which antibodies can use shared fluorophores when they have unique cellular expression, a 16-color panel using 28 different antibodies can be designed for the three-laser Agilent NovoCyte Quanteon flow cytometer. The immunophenotyping panel presented in this application note comprehensively assesses the immune system in only a small amount of sample, covering 40 distinct cell populations in human peripheral blood including all major immune cell subsets and hematopoietic stem cells (HSCs).

[Sentinel Panel Design of 16-Color, 28 Markers for Immunophenotyping Peripheral Human Whole Blood](#)

Download this application note to learn how Agilent's solutions are enabling enhanced immunophenotyping.

Application Note
Cell Analysis


Trusted Answers

Detection of Small Particles by Flow Cytometry

Analysis of small particle beads and extracellular vesicles

Authors
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Abstract
This application note describes the ability of the Agilent NovoCyte Quanteon flow cytometer to resolve small particles, including cell-derived extracellular vesicles (EVs) and beads. The NovoCyte Quanteon, equipped with advanced detectors, provides high-sensitivity side scatter (SSC) and fluorescence detection to give superior results to those of traditional flow cytometers.

[Detection of Small Particles by Flow Cytometry](#)

Download this application note to discover how the Agilent NovoCyte Quanteon is enhancing the detection of small particles.

Application Note
Cell Analysis


Trusted Answers

Generational Analysis of T Cell Proliferation using Cell Tracking Dyes

Authors
Lauren Jachymowicz, Ming Lei,
Peifang Ye, Garret Guenther,
and Nancy Li

Abstract
Cell proliferation analysis is essential when monitoring cell growth and differentiation, and tracking cellular responses to various stimuli. Generational proliferation was monitored in peripheral blood mononuclear cells (PBMCs) using cell tracking dyes, with automated flow cytometric analysis with Agilent NovoExpress software. The results demonstrate the utility of using flow cytometry for simultaneous assessment of proliferation with respect to T cell subtype as well as the value of automated analysis for ensuring the consistency of results.

[Generational Analysis of T Cell Proliferation using Cell Tracking Dyes](#)

Download this application note to learn how uses the Agilent NovoExpress software is enhancing the flow cytometric analysis of T cell proliferation.

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Agilent Technologies, Inc. 2019

Published in the USA, November 1, 2019

