

Cytometry and Fluorescence Activated Cell Sorter (Lecture in English)

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●講義内容：

FACS の基本原理を紹介し、FACS を用いた実験手技については具体例を挙げながら説明します。

Flow cytometer is a device that can semi-quantitatively measure the expression of individual cell molecules. There are two types of devices, an analyzer and a cell sorter. Antibodies recognized by fluorescent dyes are often used to identify the expression of molecules. It has also been attempted to measure fluorescent substances other than labeled antibodies.

* FACS (fluorescence activated cell sorter) is a trademark of Becton, Dickinson and company.

Lecture content:

I will introduce the basic principle of FACS and explain the experimental technique using FACS with concrete examples.



Flow Cytometry Seminar

Nippon Becton Dickinson Company, LTD.
2023

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Today's contents

Introduction to Flow Cytometry (FCM)

1. Basic of FCM
2. Application for FCM



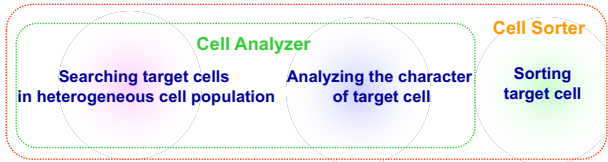
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Flow Cytometry (FCM) is

- Flow Cytometry (FCM) is...

Technique to analyze the character of cells or particle by measuring the scattering light and fluorescence exposed to a laser running in a stream

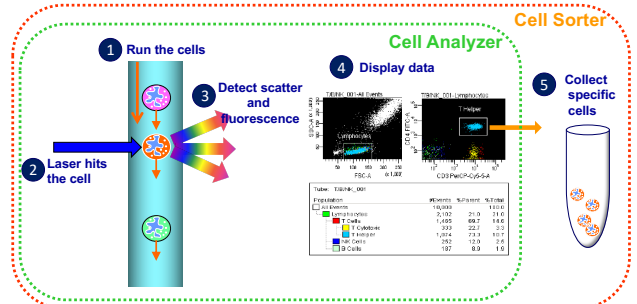


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FCM is

- FCM is...



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History of FCM

- In the late 1960s Leonard Herzenberg, PhD, professor at Stanford University developed Fluorescence Activated Cell Sorter (FACS)
- 1973 Commercialized by US Becton-Dickinson (FACS I)
- New products are released every few years
Improving laser and data processing system, making it possible to analyze more items much faster



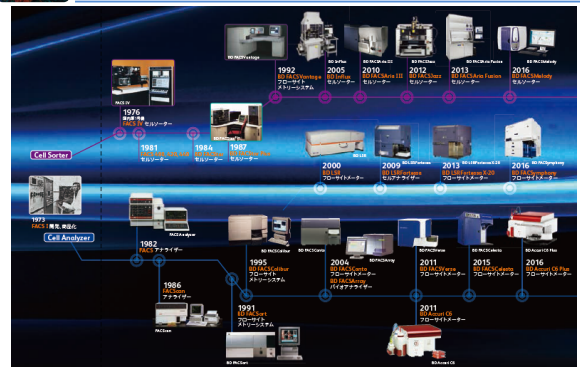
Instrument	Maximum number of laser equipment	Number of fluorescent color	Processing speed (Cells/sec)
Early FACS	1	2	-
Present FACS	6	18	70000



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Changes in BD FACS™ series



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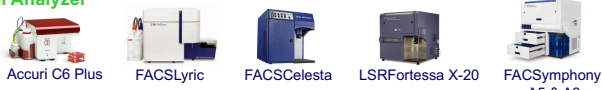


BD FACS™ series

Cell Sorter



Cell Analyzer



More Colors & Hi Performance



Central Research Laboratory Shiga University of Medical Science

Cell Sorter



Cell Analyzer



More Colors & Hi Performance



Character of FCM

FCM

- ✓ Process abundant cell data
- ✓ Multicolor
- ✓ Objectivity
- ✓ Cell sorting

Fluorescence microscope

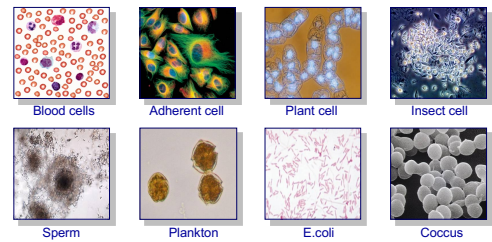
- ✓ Image (3D)
- ✓ Intracellular distribution
- ✓ Time-lapse



Measurable sample with FCM

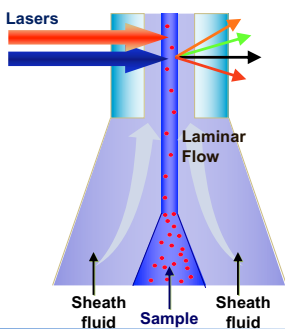
- Single cells in suspension
- Cell size 0.2µm~40µm

Example of measurable sample



Detection of FCM

- Laminar Flow formation / Laser / Detection

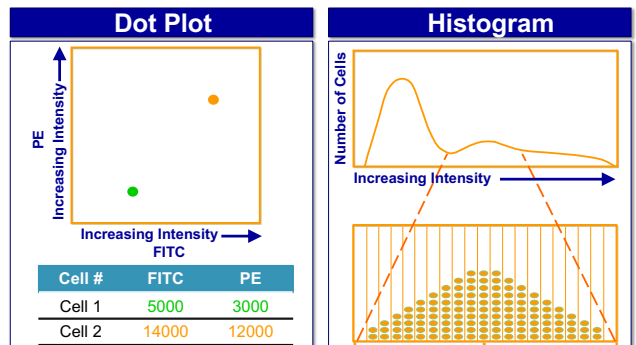


Formation of narrow and laminar flow, enables cells to run one by one and passes through the laser

- Detects scatter and fluorescence
- Process few thousand cells/sec



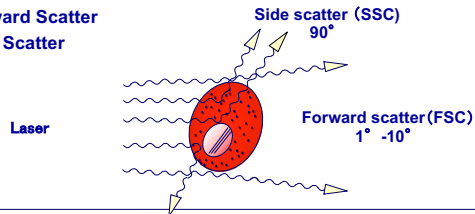
Data Display: Dot Plot and Histogram





Character of scatter light

- Forward Scatter
- Side Scatter



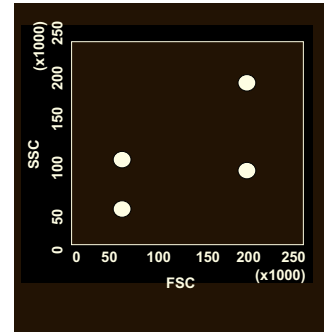
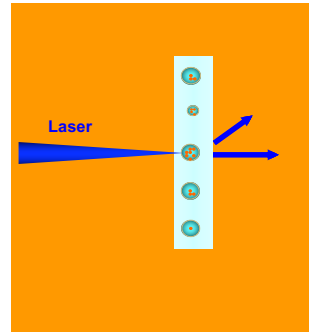
- Forward Scatter (FSC)
Indicates the size of cells, surface area
- Side Scatter (SSC)
Indicates the complexity of internal structure, cellular granule



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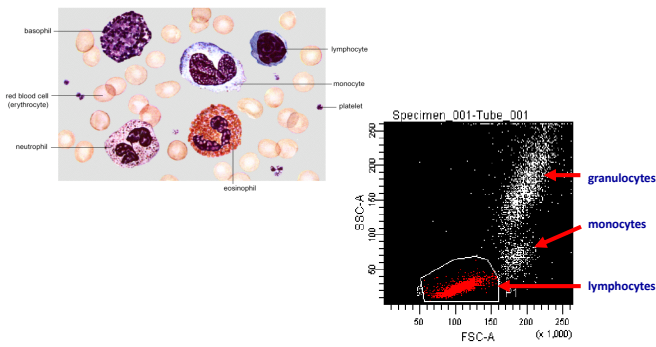
Scatter light signal



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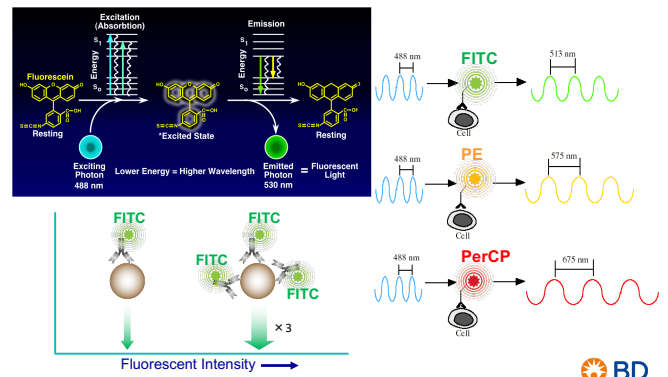
Scatter light analysis of blood cells



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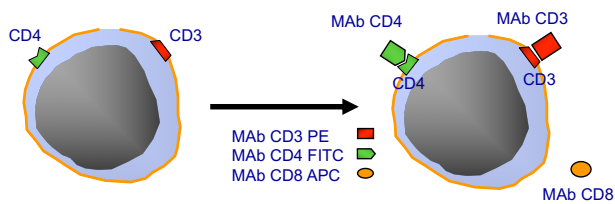
Character of fluorescence



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Fluorescent staining with monoclonal antibody



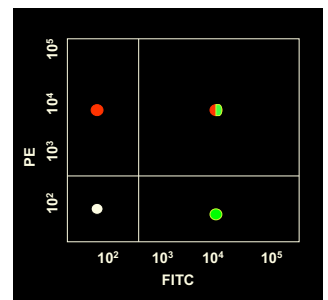
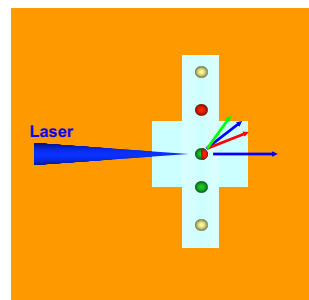
CD # = cluster of differentiation
MAb = monoclonal antibody



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Fluorescent signal

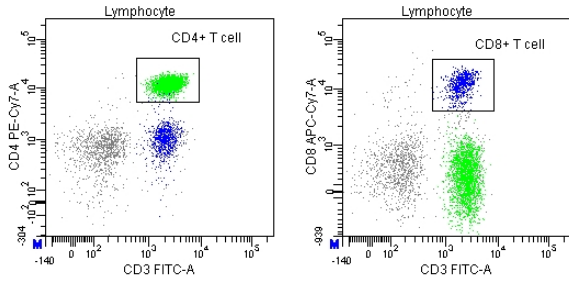


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Fluorescent signal analysis

- Cell surface antigen analysis of lymphocytes

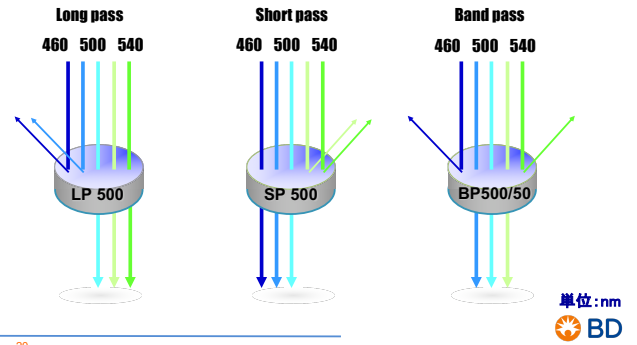


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Mechanism of fluorescence detector

- Filters and mirror to distinguish each fluorochromes

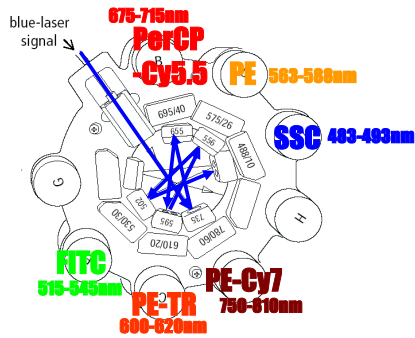


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Fluorescence detector of FCM

- Detector of FACSaria™

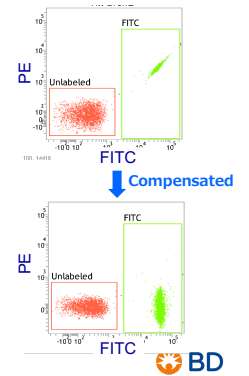
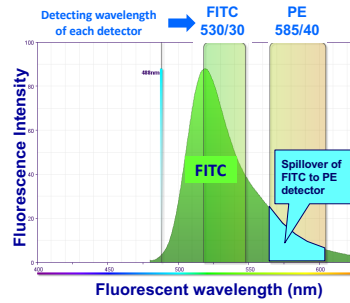


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Requires compensation in multi-color analysis

- Importance of compensation



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Introduction to Flow Cytometry (FCM)

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- Application for FCM

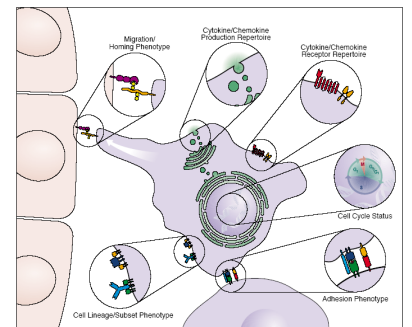


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Applications for FCM

- Cell surface antigen
- Intracellular antigen
- Fluorescent protein
- Cell cycle
- Cell proliferation
- Apoptosis
- Cytokine



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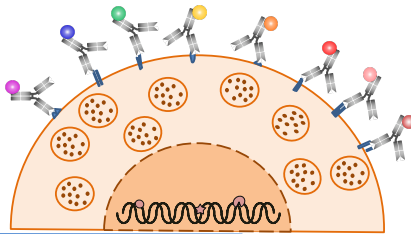


① Analysis of cell surface antigen

Major cell surface antigen

- ✓ Cellular membrane composing protein
- ✓ receptors
- ✓ Adhesion molecules
- ✓ Transporters

Cluster of Differentiation (CD classify)
International classification of monoclonal antibodies used for cell surface antigen

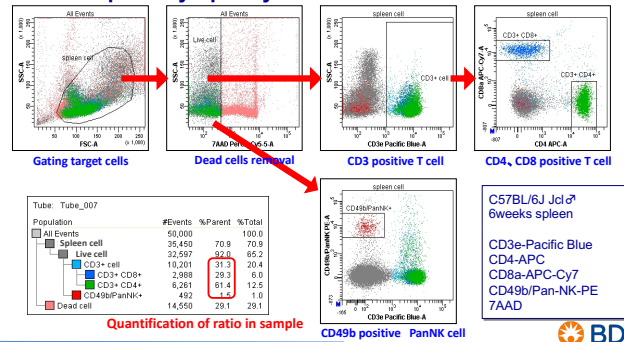


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Analysis of cell surface antigen

• Example of lymphocyte subsets



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BD Lyoplate™ Screening Panels

- Many kinds of antibodies are dispensed in 96 well plate
- Profiling cell surface marker in low cost*
- Antibody is labeled with Alexa Fluor® 647, able to use with GFP expressing cells



*for FACS analysis, 96well plate auto sampler is required

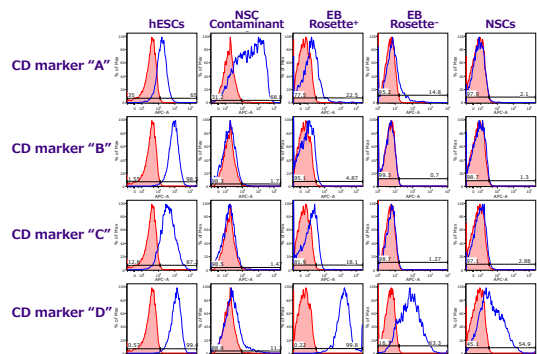
Product	Contents	Size
BD Lyoplate™ Human Cell Surface Marker Screening Panel Cat. No. 560747	<ul style="list-style-type: none"> • 242 CD markers • Isotype controls • Alexa Fluor® 647 second step 	5 tests
BD Lyoplate™ Mouse Cell Surface Marker Screening Panel Cat. No. 562208	<ul style="list-style-type: none"> • 176 CD markers • Isotype controls • Biotin second step • Alexa Fluor® 647 streptavidin third step 	5 tests



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BD Lyoplate™ Screening Panels



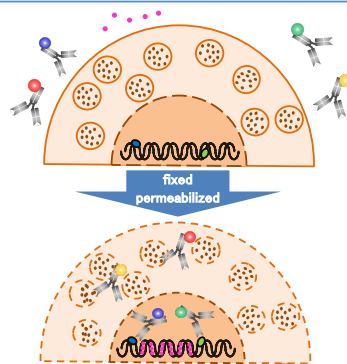
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② Analysis of intracellular antigen

- Kinds of intracellular antigen
 - Cytokine
 - Transcription factor
 - cytoskeleton

Cells fixed and permeabilized makes antibody and reagents possible to access intracellular



- Fixation reagent
 - Ethanol
 - Methanol
 - Paraformaldehyde
- Permeabilization reagent
 - Saponin
 - Triton-X
- Nucleic acid staining reagent
 - PI
 - 7AAD

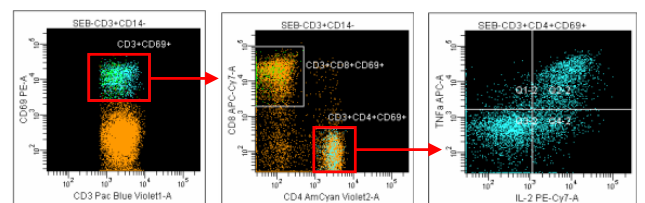


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Cytokine producing cells analysis

- Staining with cell surface antigen, enables phenotype analysis of cytokine producing cells
- Possible to detect cytokine production even from rare cells

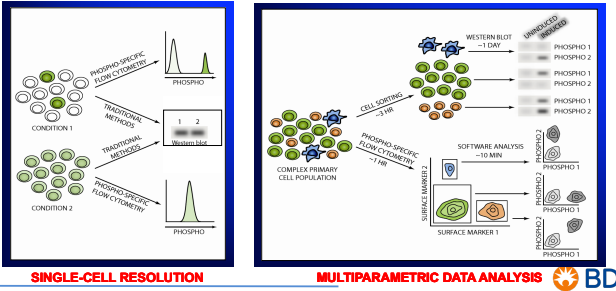


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Protein phosphorylation analysis in single cell

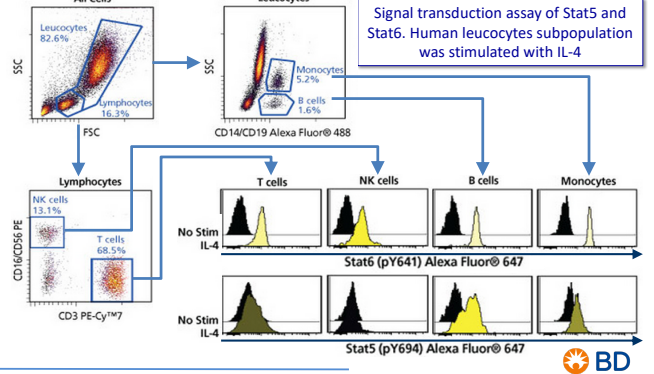
- Able to analyze protein phosphorylation in single cell level
- Combining the staining of cell membrane markers, allows protein phosphorylation analysis of specific cells in heterogeneous cell population
- Easy, short time and less cell sample compared to Western Blot analysis



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Protein phosphorylation analysis in single cell

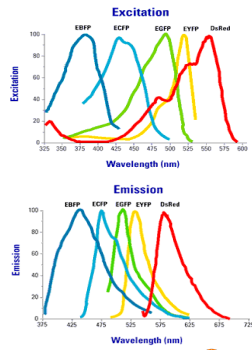


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③ Expression of fluorescent protein

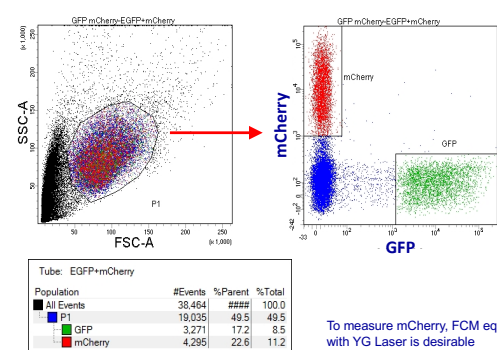
- **Purpose**
 - Verify transgene expression
 - Verify transplanted cells survival
 - Verify interaction between transgene
- **Kinds of fluorescent protein**
 - **GFP series fluorescent protein**
 - GFP, YFP, CFP, RFP, BFP etc.
 - **Fruits series fluorescent protein**
 - mCherry, mPlum, mStrawberry, mBanana etc.
 - **CoralHue series fluorescent protein**
 - Midoriishi-Cyan, Kusabira-Orange, Azami-Green, Keima-Red, Kaede, Doronpa-Green etc.



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GFP / mCherry Expressing cell analysis

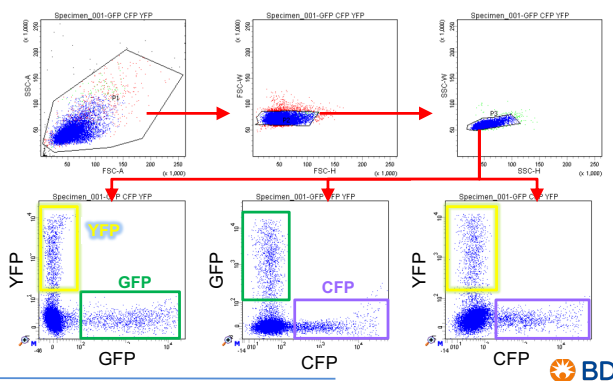


To measure mCherry, FCM equipped with YG Laser is desirable

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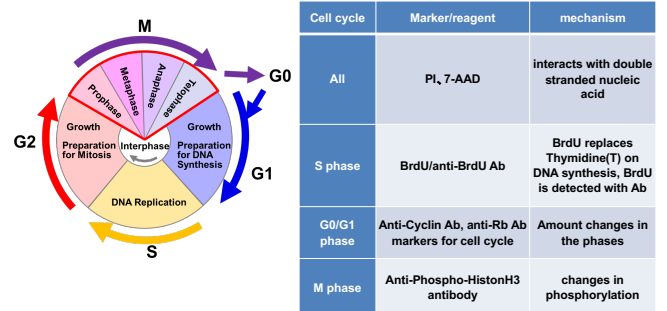
GFP / YFP / CFP Expressing cell analysis



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④ Cell cycle analysis



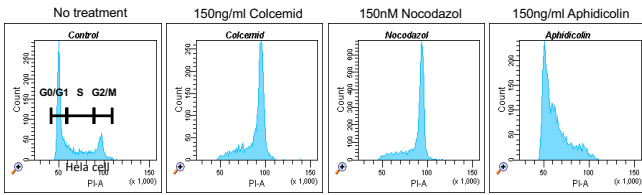
Available to analyze each phases

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④ Cell cycle analysis : PI analysis

- PI staining cell cycle analysis
 - CycleTEST™ PLUS DNA Reagent Kit (Cat#340242)



HeLa cells cultured 48hr in DMEM with 0.5% FBS were treated with reagents. After 16hr culture, cell cycle analysis was performed

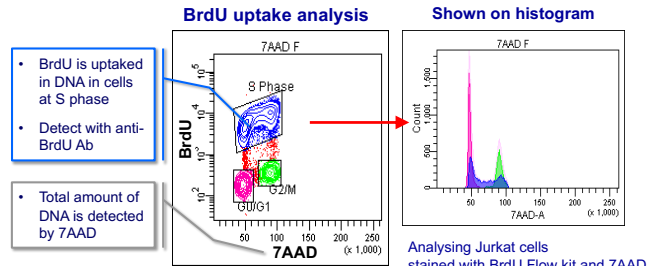


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④ Cell cycle analysis : BrdU / 7AAD analysis

- BrdU detects S phase, 7AAD detects all phases
 - BD Pharmingen™ FITC BrdU Flow Kit (Cat#559619)

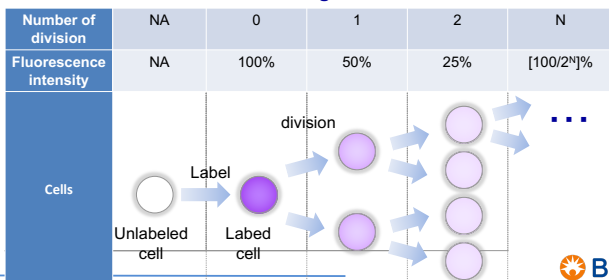


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⑤ Cell proliferation assay

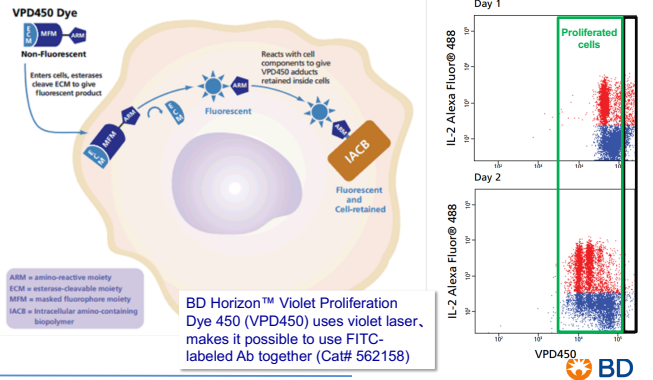
- Label cells with fluorescence which remains in the cells. Cell division is measured by the change of fluorescence intensity.
- Able to stain with makers together



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⑤ Cell proliferation assay: VPD450 assay



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⑥ Apoptosis analysis

- Many ways to analyze apoptosis

測定対象	Feature Measured	Assays	Key Features
フォスファチジルセリンの露出 (Phosphatidylserine Exposure)	Plasma Membrane Alterations (Phosphatidylserine Exposure)	Annexin binding assay <ul style="list-style-type: none"> Single conjugates Annexin V kits 	<ul style="list-style-type: none"> Detects early apoptosis markers Quick and easy Flow cytometry or immunofluorescence application
ミトコンドリアの変化	Mitochondrial Changes	BD MitoScreen Kit	Fast, easy, single cell resolution by flow cytometry or fluorescent microscopy
Caspaseの活性化	Caspase Activation	Caspase Activity Assay Kits and Reagents	Quick and easy, uses spectrofluorometry
		Active Caspase-3 immunoassays	ELISA, flow cytometry, or Western blot
DNAの断片化	DNA Fragmentation	<ul style="list-style-type: none"> APO-BidU TUNEL Assay APO-DIRECT TUNEL Assay 	Works with adherent cells, single cell resolution in conjunction with cell cycle analysis by flow cytometry

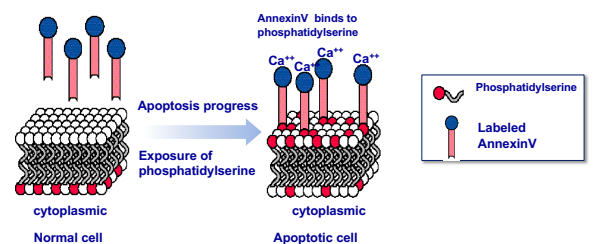


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Apoptosis analysis: AnnexinV detection

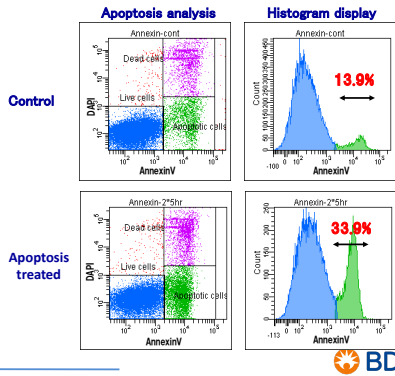
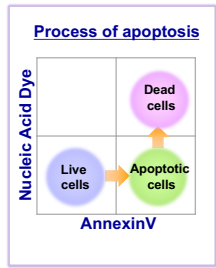
- Phosphatidylserine is exposed to the extracellular side of cell membrane in the early stage of apoptosis
- AnnexinV binds specific to phosphatidylserine



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Apoptosis analysis: AnnexinV detection

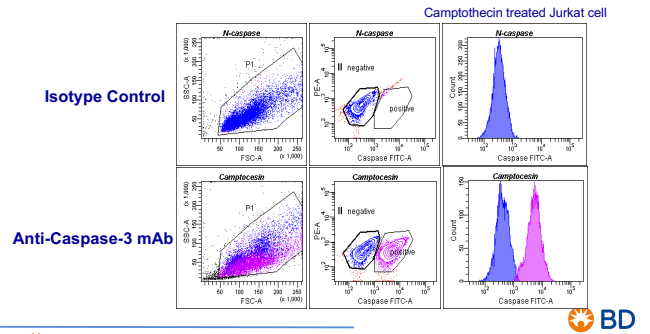


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Apoptosis analysis: Caspase-3 measurement

- Detects activated Caspase-3 with specific antibody

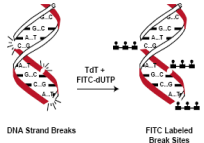


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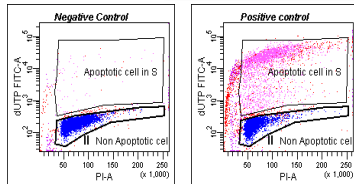


Apoptosis analysis: Apo-Direct to detect DNA damage

- Detects DNA damage in apoptotic cells



- TdT (deoxynucleotidyl transferase) and FITC labeled dUTP will bind to the damaged 3'-hydroxyl end of dUTP in fixed cell sample
- FITC labeled dUTP will bind depending on the amount of DNA damage
- Based on TUNEL methods



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⑦ Cytokine measurement

- Able to measure the cytokine concentration using FCM

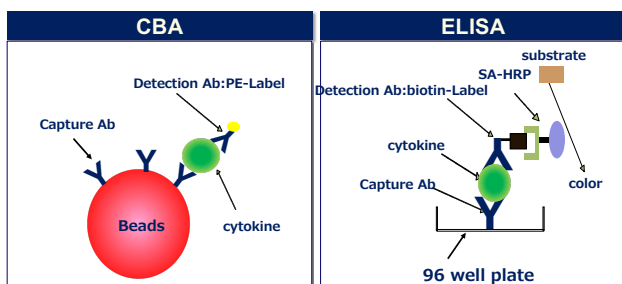
method	object	measurement	instrument
ELISA (OptEIA)	Cytokine concentration in solution	Antibody and enzyme reaction in 96 well plate to color	Plate reader
Intracellular Cytokine	Cells producing cytokine	Stain cytokine with Ab within cells	FCM
ELISPOT	Cells secreting cytokine	Antibody and enzyme reaction in 96 well plate to color	Microscope
Cytometric Beads Array (CBA)	Cytokine concentration in solution	Use special beads to measure cytokine	FCM

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Cytometric Beads Array (BD™ CBA)

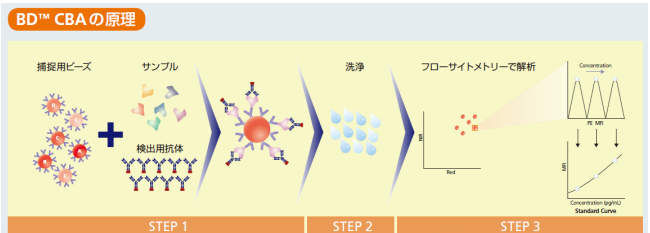
- BD™ CBA is a reagent to measure multiple cytokines in a single tube using FCM.



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Cytometric Beads Array (BD™ CBA)



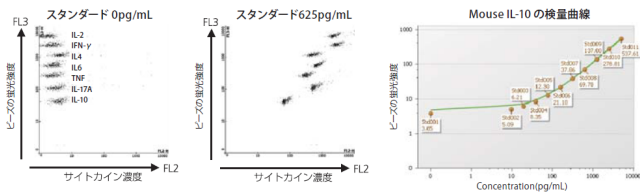
	BD™ CBA	ELISA
Number of items to measure	Up to 30/sample	1/sample
procedure	Wash 1	Wash more than 10
Sample amount	50uL	About 100uL
Format	From single tube	96 well plate
Instrument	flowcytometer	Plate reader

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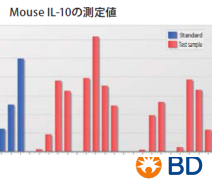


Cytometric Beads Array (BD™ CBA)



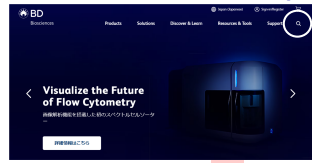
Able to measure multiple cytokine at the same time. Calculates the concentration by using standard curve. Concentration from pg, even fg with enhanced sensitivity systems.

*needs special software for data analysis
Cat#652099 FCAP Array™ v3.0software



Search for BD product

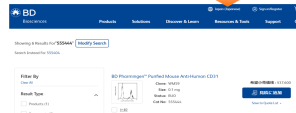
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- Available to download datasheet
- Prices are shown, useful for ordering

Guide for multicolor analysis, posters, protocol, etc., are available to download



Contact details

Flow cytometry operator training

Training takes place at our lab in Tokyo or at your lab.
Please contact for details.

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E-mail : customer_training@bd.com

For question about instruments and reagents

Please contact application support
for any questions about our instruments and reagents

Phone : 0120-4890-77 (weekdays 9:00~17:00)
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Questions?

