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Basic Principal and General Application of BD FACSCalibur

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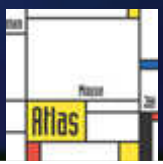
尚博生物科技有限公司

www.cell-bio.com.tw

Glenn Yang

apoptosistw@gmail.com

02-27855860-0953062485



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Out Line

- Principle of FACS:
  Total Concept
- Introduction of BD FACSCalibur:
  Anatomy of FACS System
- FACS as a Research Tool:
  Applications , from Basic to Advance

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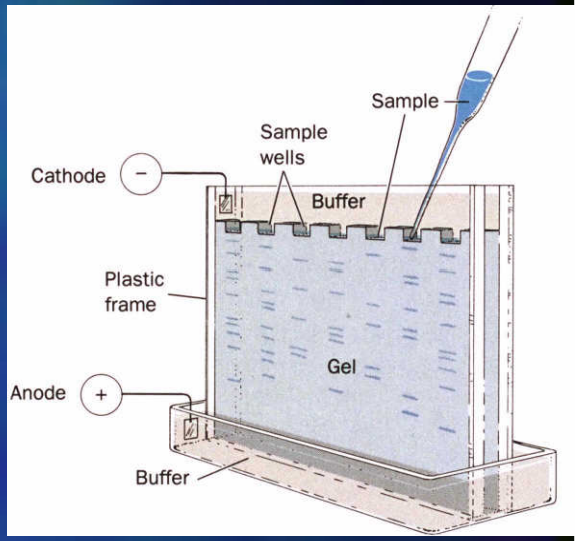
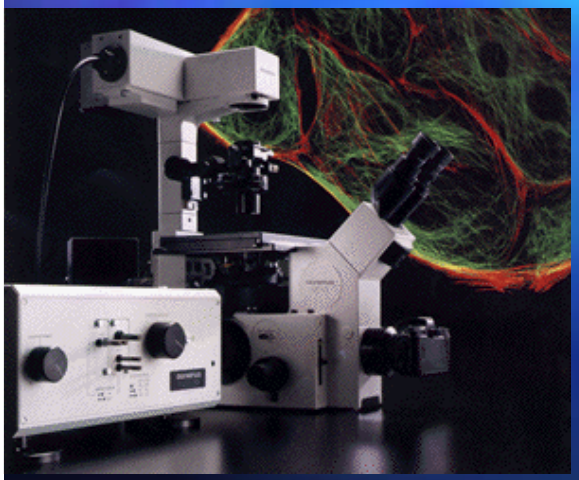
Introduction





CELL ANATOMY!

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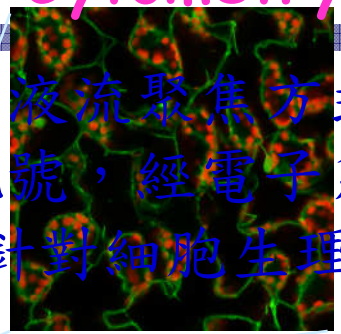
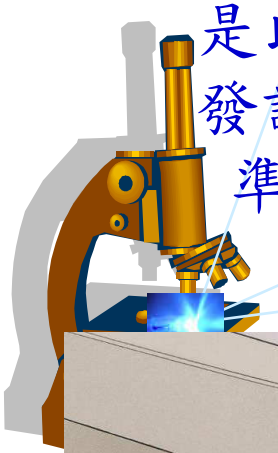
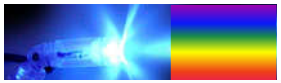
Introduction

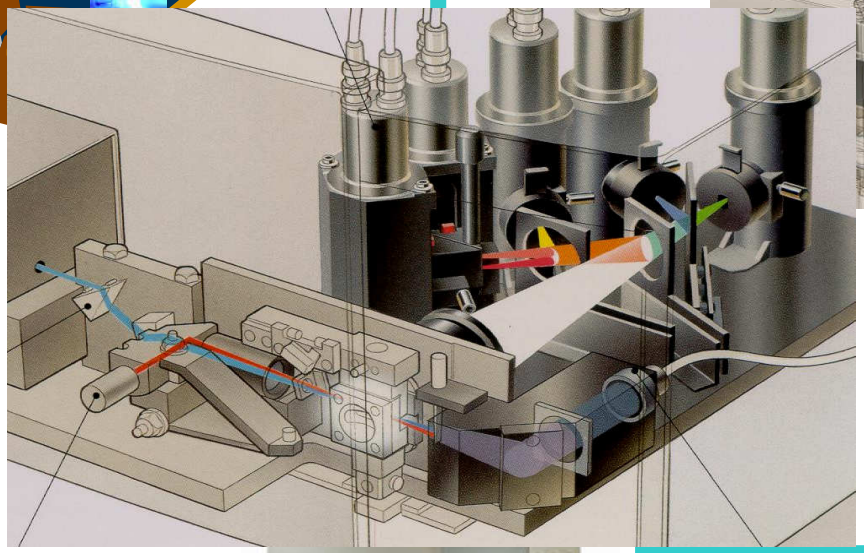
Flow Cytometry，

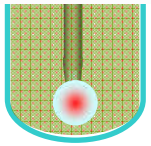


是以液流聚焦方式收集單一細胞或顆粒，藉光學系統激發訊號，經電子系統擷取，輔以電腦系統分析，迅速精準針對細胞生理進行多項指數偵測的一種分析方式！









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Why FACS?

- Simultaneous Multicolor Analysis
- 3,000~100,000 of Total Events to Be Acquired
- High Speed Acquire : 200~20,000 Events Per Second
- High Accuracy
- More Sensitive

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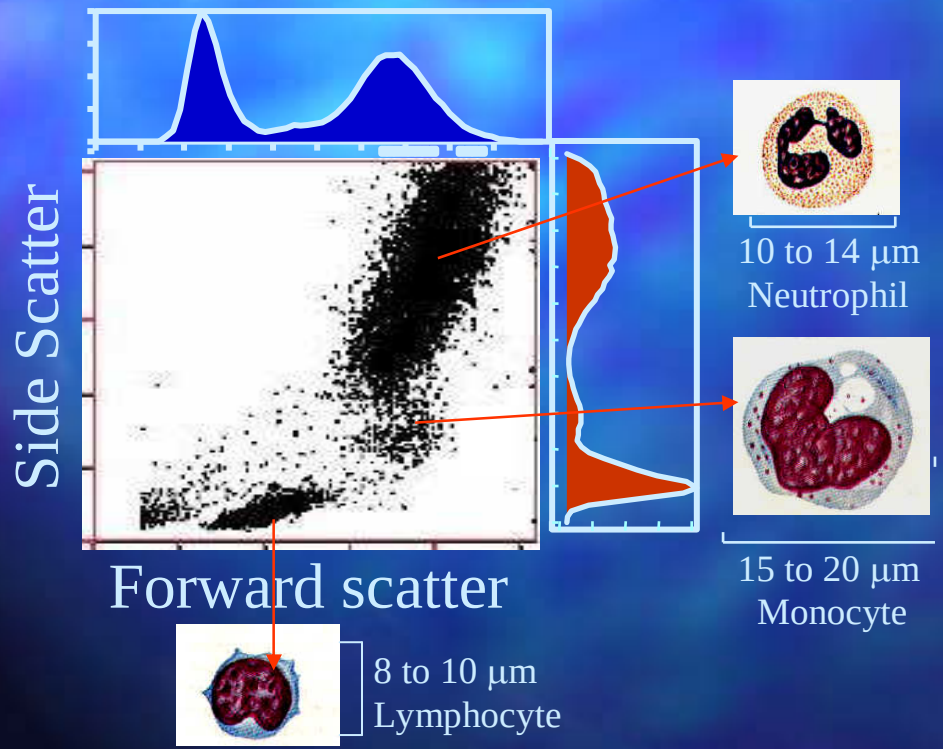
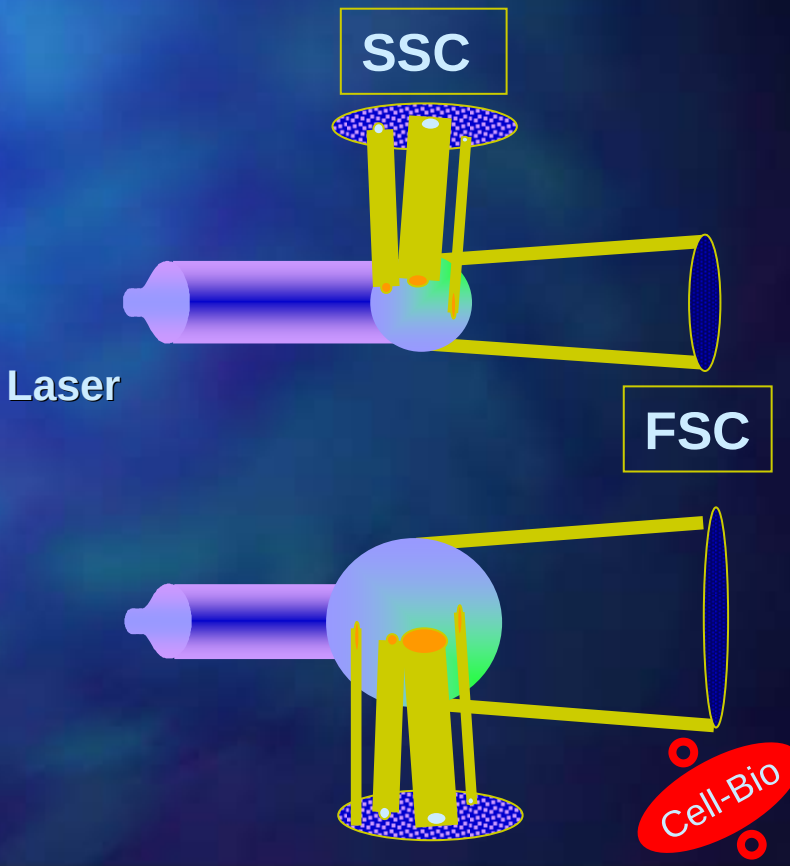
What we got from FACS?

- Morphological Information : *Scatter Light Signal*





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What we got from FACS?

- Varied Biological Information : *Fluorescence Signal*

Laser Excitation wave length	Emission Color	Suitable Fluorochrome			
488	Green	FITC	Alexa Fluor® 488		
	Yellow	PE			
	Red	PE-Texas Red®	PE-Cy5*	PerCP	PerCP-Cy5.5
	Infra Red	PE-Cy7			
633	Red	APC*	Alexa Fluor® 647		
	Infra Red	APC-Cy7			
355	Violet				
	Blue	Alexa Fluor® 405	Pacific Blue®		
405	Blue	Alexa Fluor® 405	Pacific Blue®		

Fluorescence Probes for:
Cell Proliferation , Apoptosis , Signal Transduction , ROS Generation , Ionic Balance , pH Balance , Genomic , Proteomic ……

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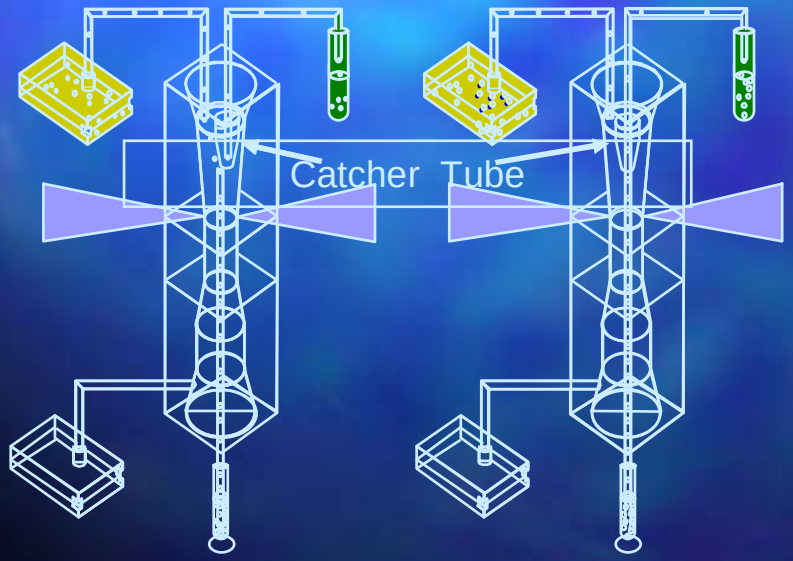
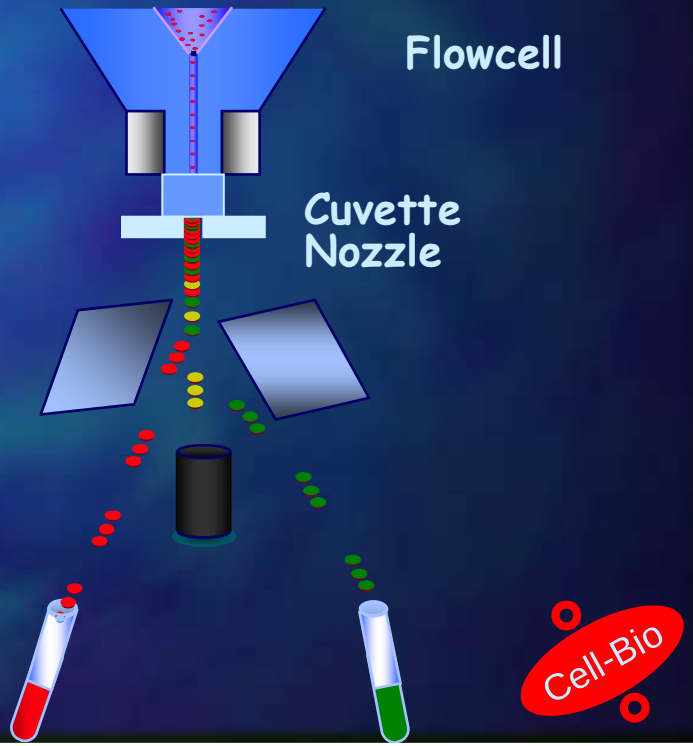
What we got from FACS?

- Not Only Digital Data but Concrete Cell : ***Sorting***
  Collect Interested Cells by Sorting System!





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Anatomy of Flow Cytometer

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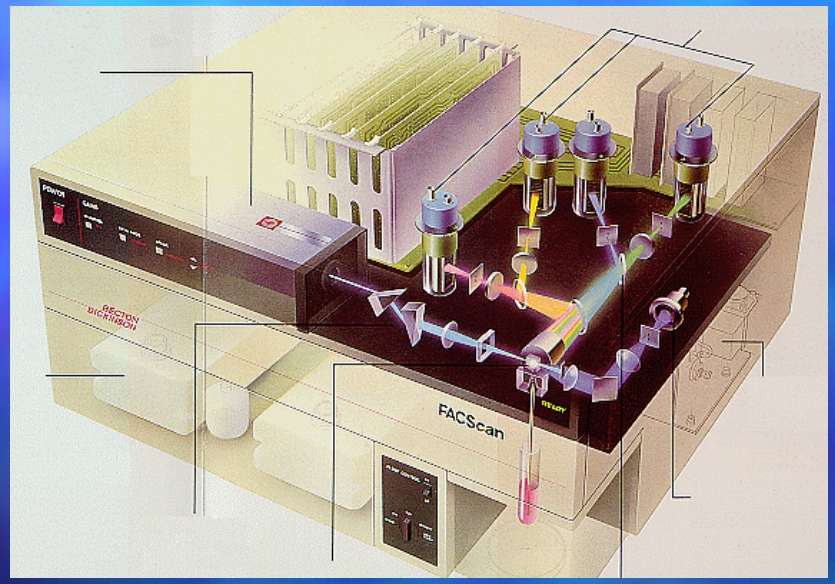
Anatomy of Flow Cytometer



- Fluid System
- Optical System
- Electronic System

(Sorting System)

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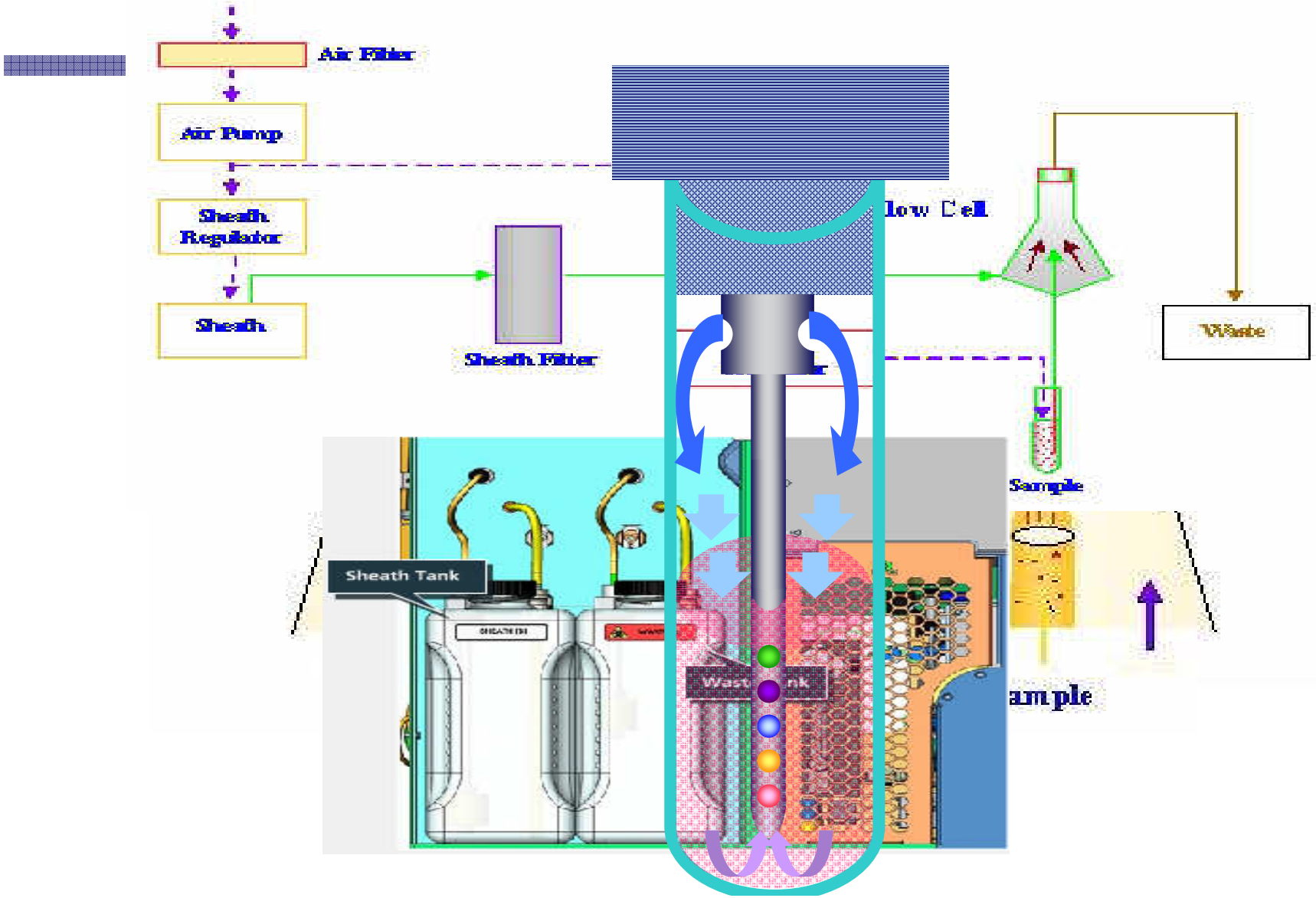
Fluid System



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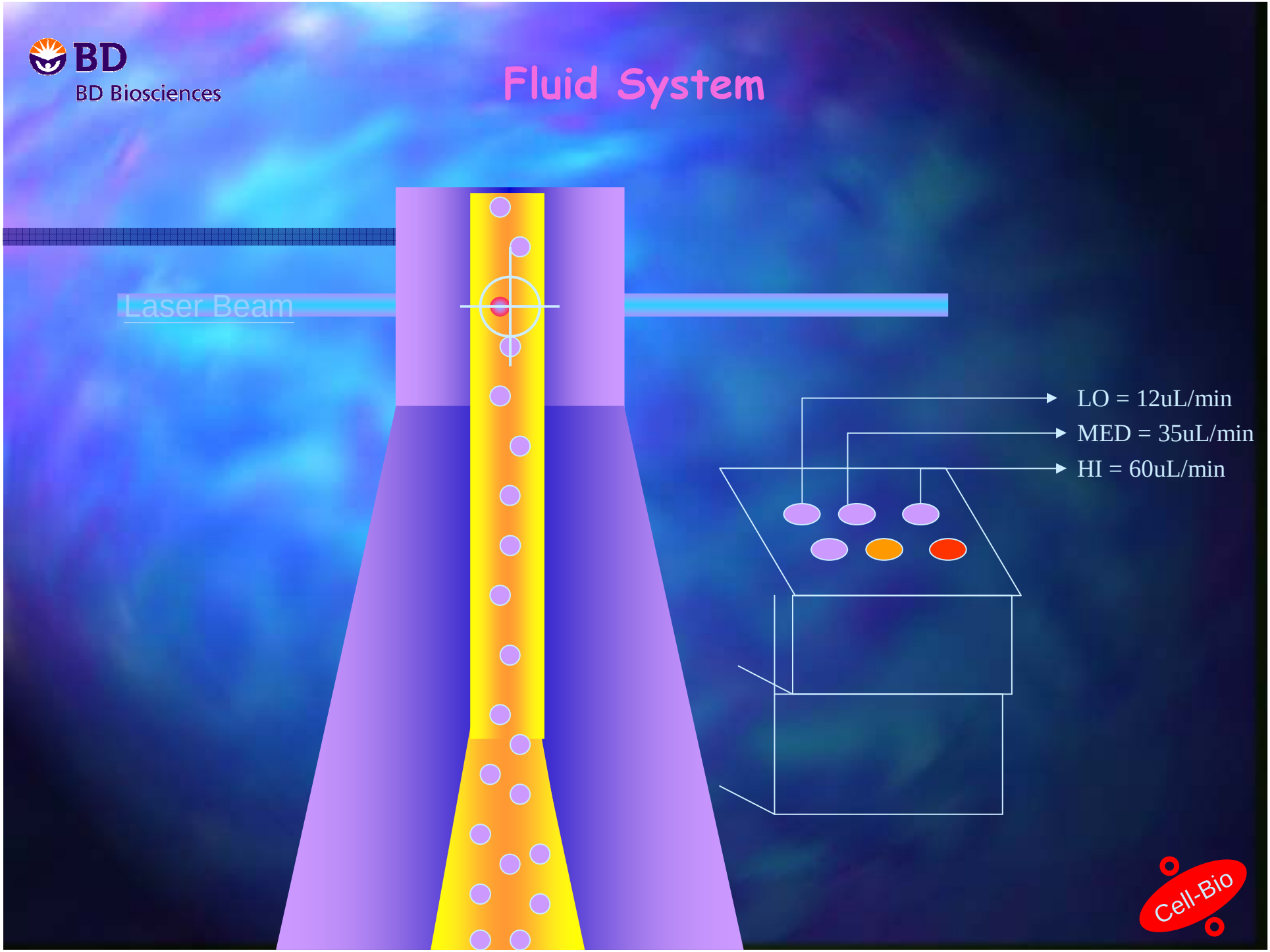
Fluid System



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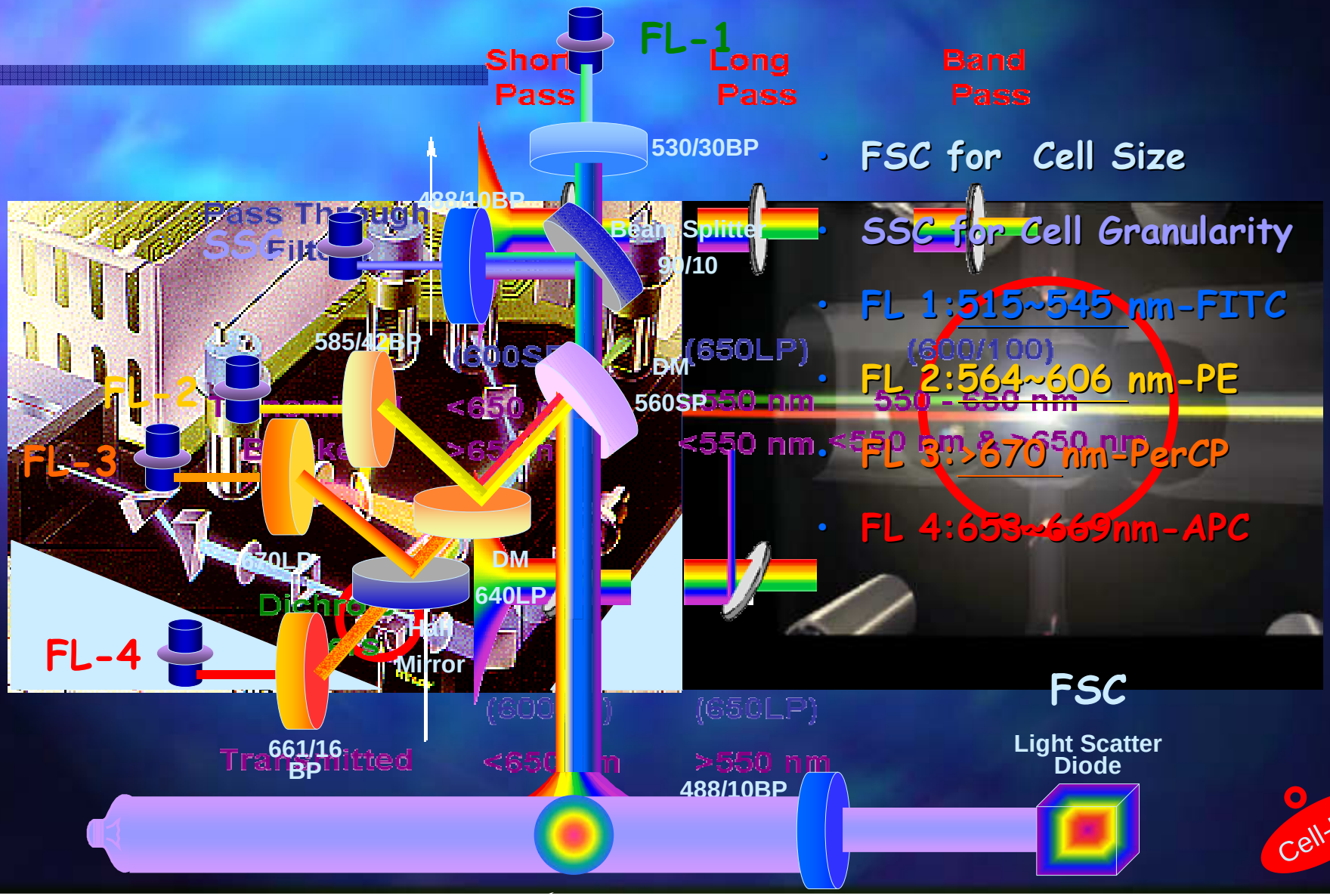
Optical System



- FSC for Cell Size
- SSC for Cell Granularity
- FL 1:515~545 nm-FITC
- FL 2:564~606 nm-PE
- FL 3:>670 nm-PerCP
- FL 4:653~669nm-APC

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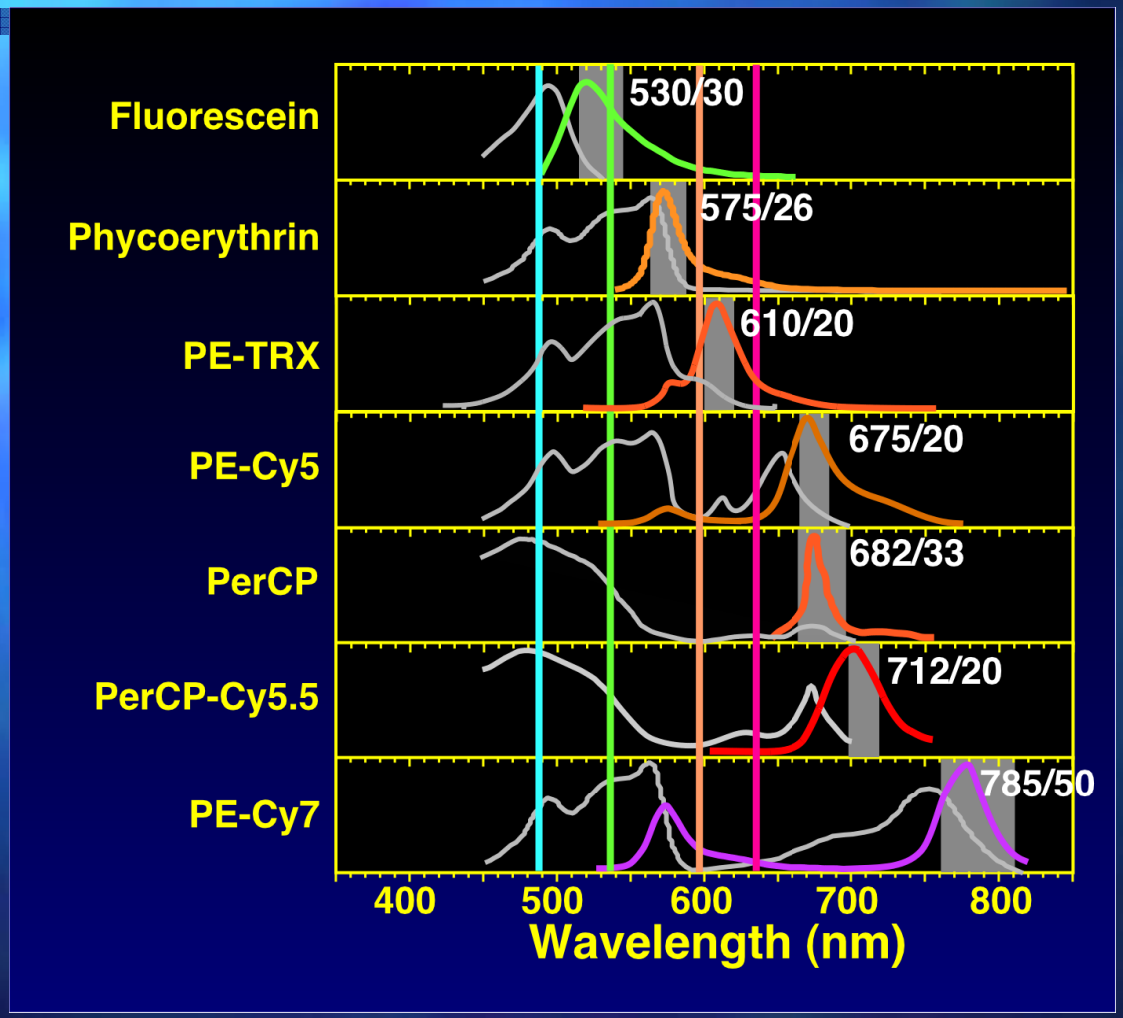
Optical System



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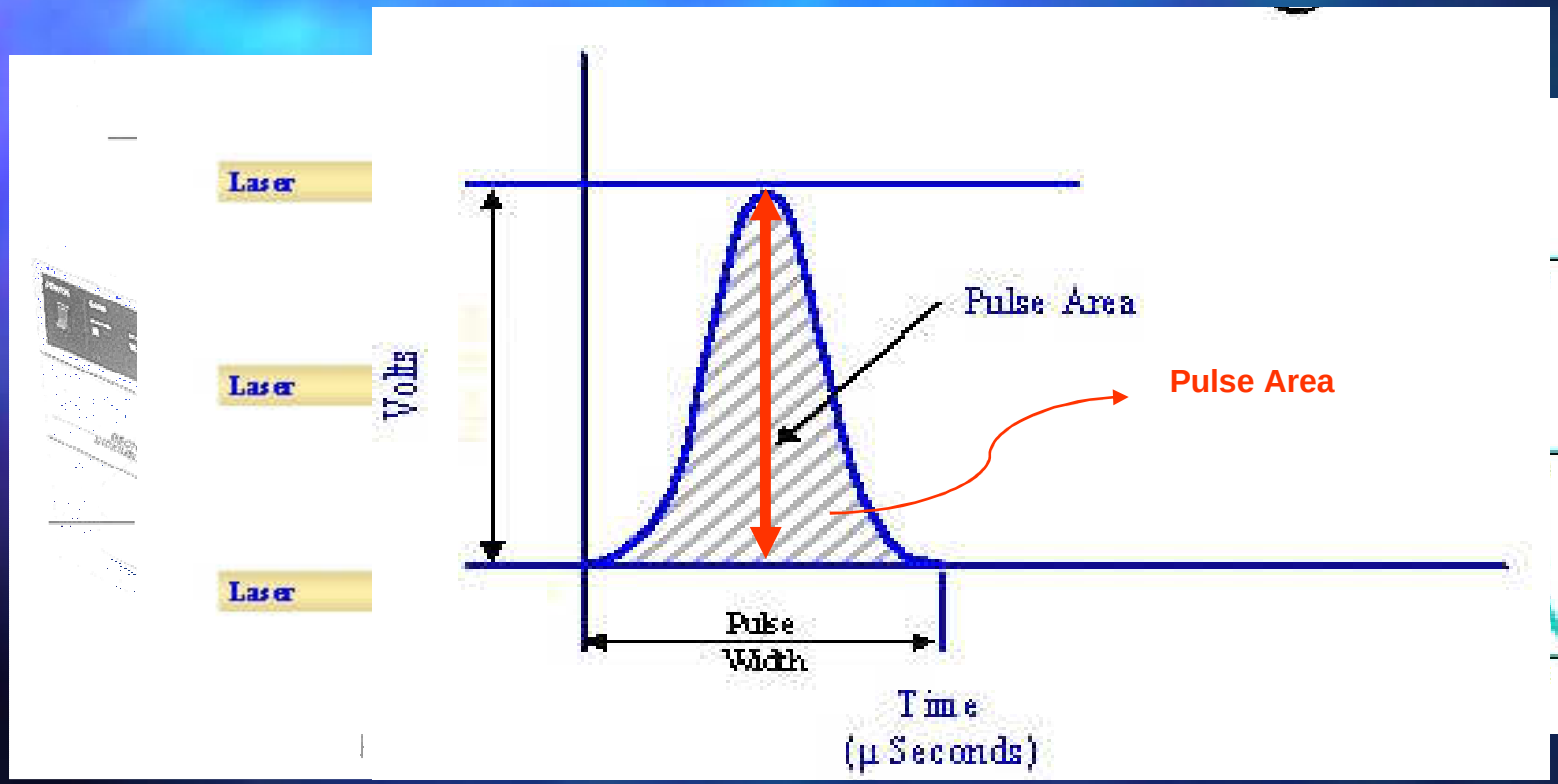
Electronic System



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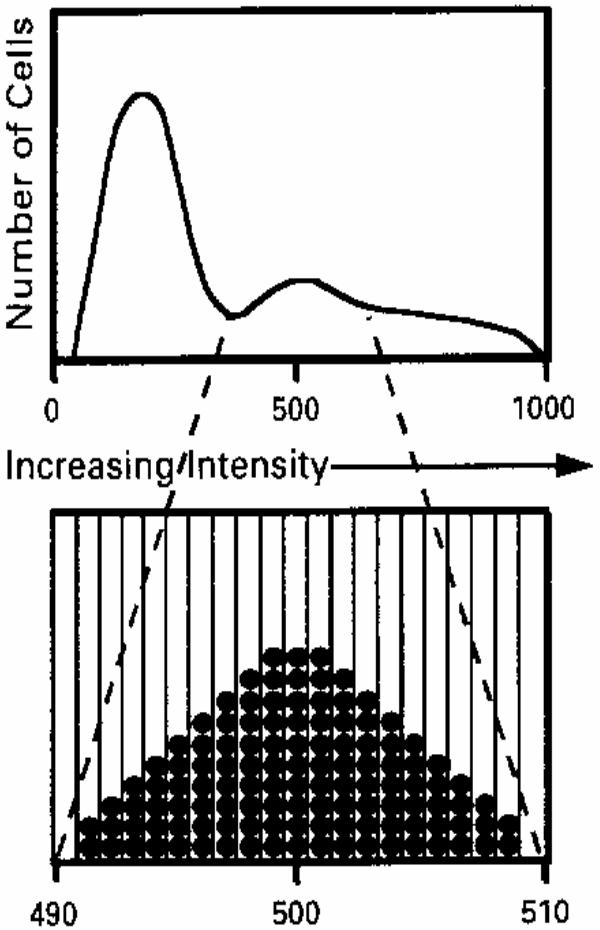
Data File

List Mode

List-Mode

	FSC	SSC	84
Event 1	30	60	6
Event 2	100	160	2
Event 3	300	650	1
			85
Event 1	30	60	6
Event 2	100	160	2
Event 10000	300	650	1

Histogram



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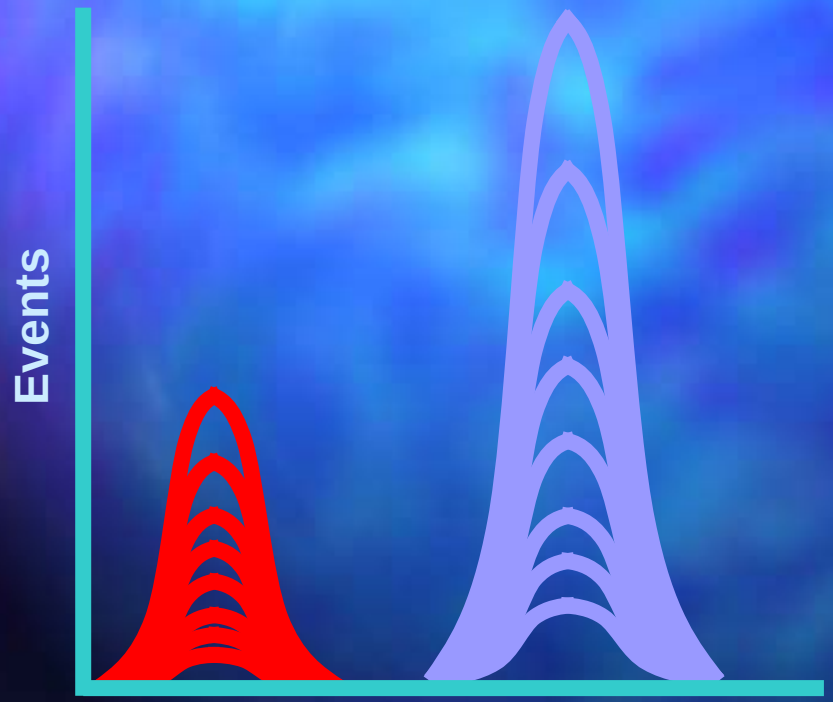
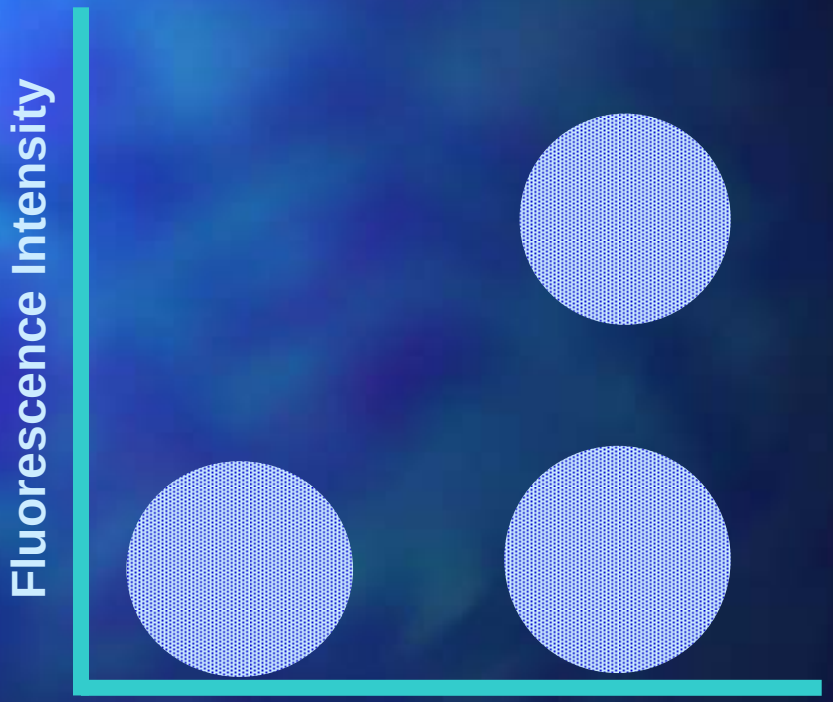
Signal Production

Histogram



Dot Plot



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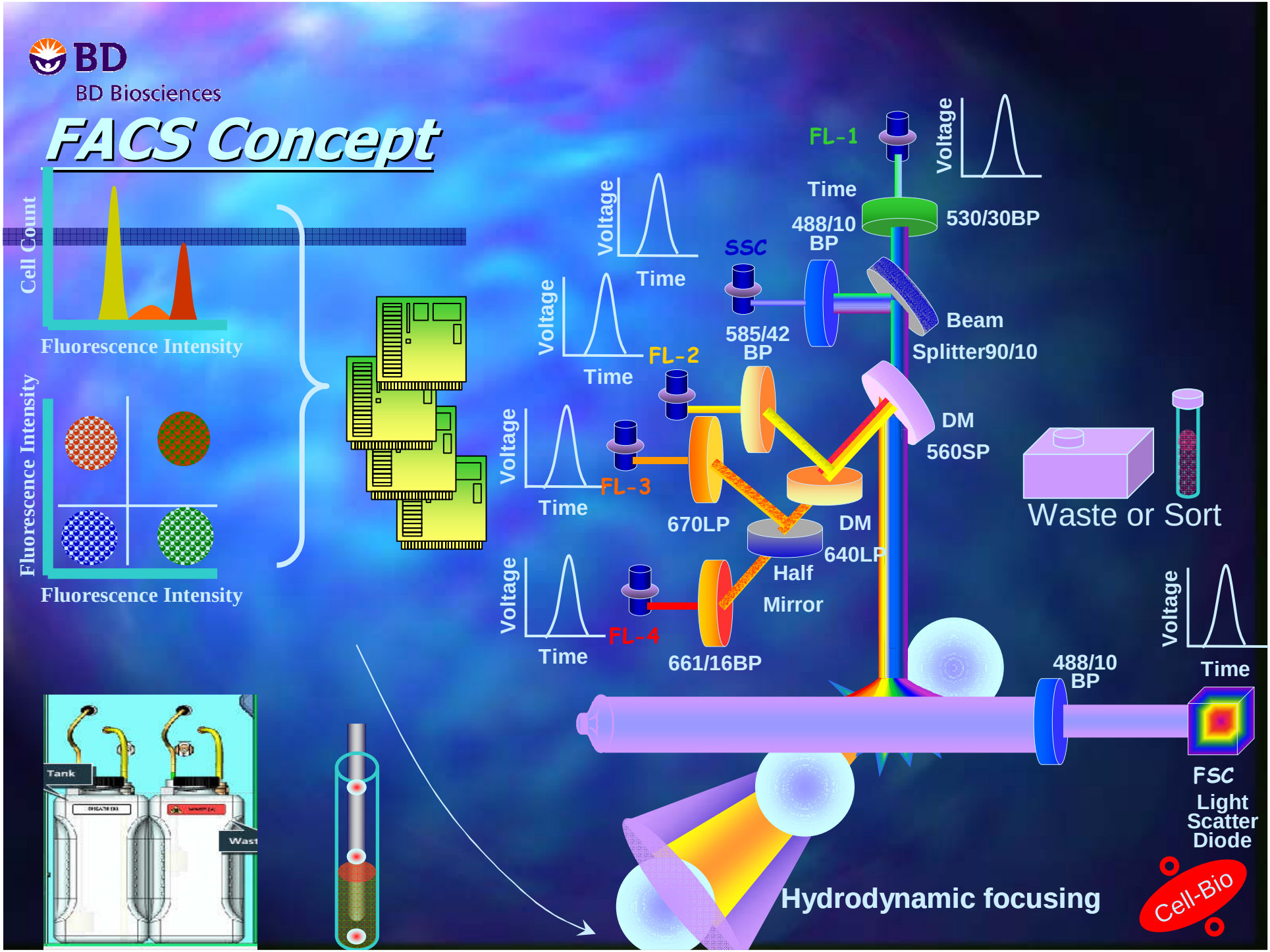
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FACS Concept
Cell Count
Fluorescence Intensity
Fluorescence Intensity
Fluorescence Intensity
Voltage
Time
FL-1
530/30BP
488/10
BP
SSC
585/42
BP
Beam
Splitter90/10
FL-2
DM
560SP
FL-3
670LP
DM
640LP
Half
Mirror
FL-4
661/16BP
Waste or Sort
488/10
BP
FSC
Light
Scatter
Diode
Tank
Hydrodynamic focusing
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Applications

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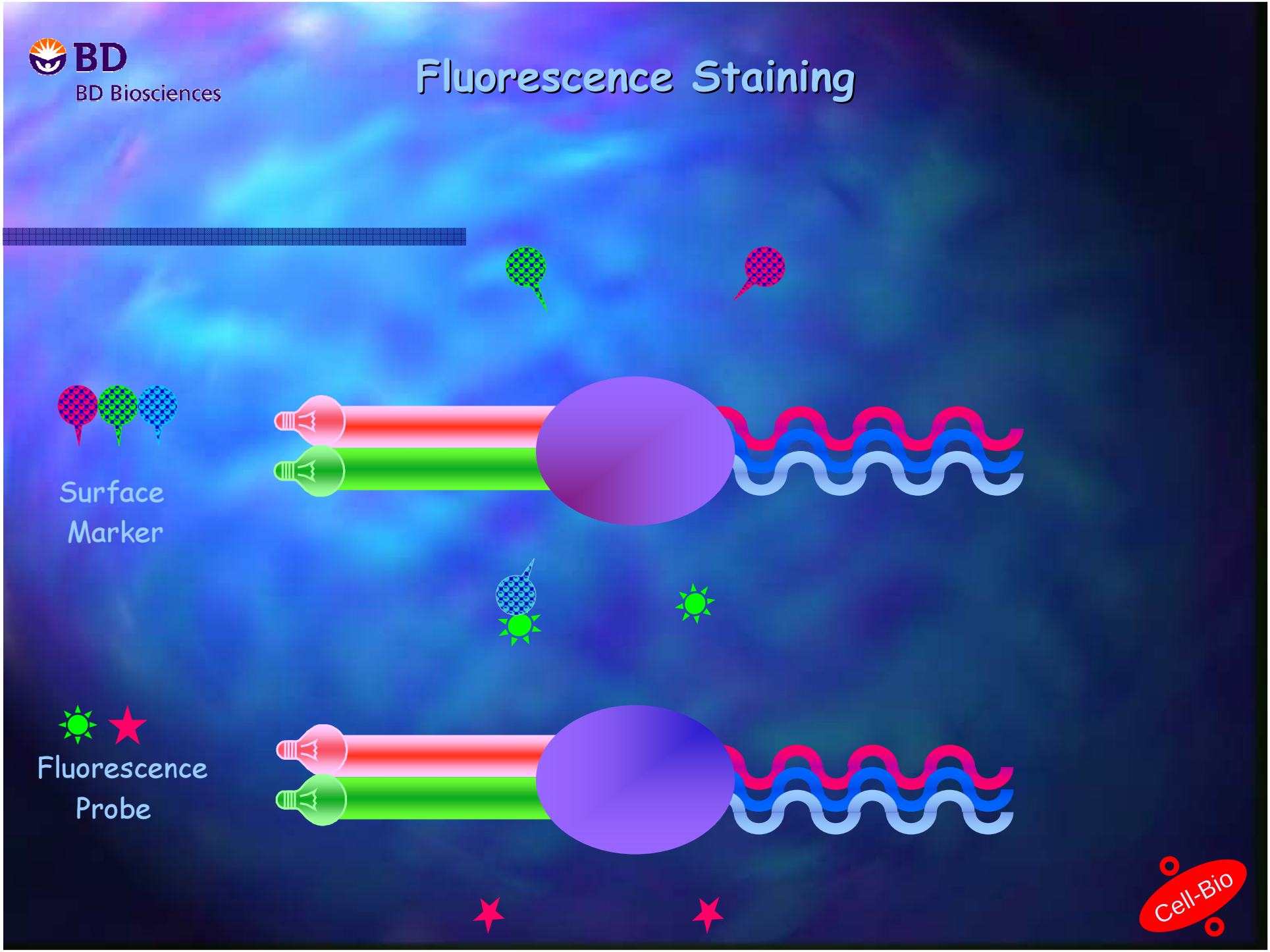
Fluorescence Staining



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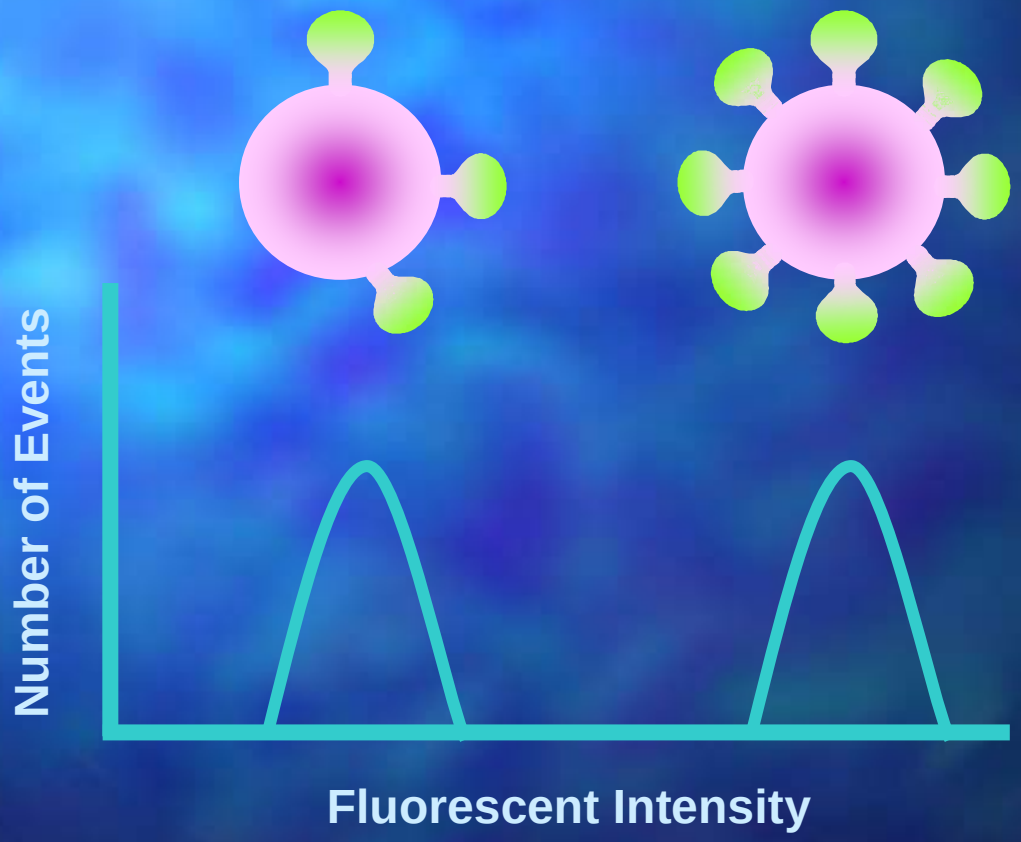
Fluorescence Staining



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測量參數	螢光染劑	吸光波長(nm)	螢光波長(nm)
標示抗體用染劑	Fluorescein, FITC	490	520
	Phycoerythrin-R, PE	480	578
	Peridinin-chlorophyll, PerCP	490	677
	PerCP-Cy5.5	490	680
	Cy-Chrome	495	670
	PE-Cyanine 5	480	670
	Allophycocyanine, APC	**650**	**660**
	APC-Cy7	**650**	**770**
核酸含量分析	Ethidium Bromide	510	595
	Propidium Iodide	536	617
	Acridine Orange	480 (+ DNA) 450 (+ RNA)	520 650
	Thiazole Orange	509	533
細胞存活率	Propidium Iodide	536	617
	7-Amino Actinomycin D	545	647
	YOYO-1, YO-PRO-1	491	509
	Ethidium Monoazide	493	620
	TOTO-3, TO-PRO-3	**642**	**661**
報導基因	GFP S65A, S65C	475	506
	GFP S65L, S65T	486	510
細胞膜電位	DiO-C6(3)	485	525
粒腺體膜電位	Rhodamine 123	485	546
細胞內 pH 值	BCECF-AM	488	Ratio 520/620
	SNARF1-AM	514	Ratio 587/640
細胞內鈣濃度	Fluo3-AM	506	528
	Calcium Green-1	488	530
	Fura Red	488	660
H2O2 sensitive	Dihydrorhodamine 123	505	534
	DCFH-DA	505	535
O2- radical sensitive	Hydroethidine	518	605
Esterase sensitive	Fluorescein -DA	495	525

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Basic Applications-1

Immune Staining

FITC, PE, PerCP, APC.CY7

- Immune Monitoring
- Leukemia/Lymphoma Identification
- Stem Cell Analysis
- Dendritic Cell Analysis
- Platelet Function Analysis
- AIDS Monitoring
- Transplant Monitoring
- Other …….etc

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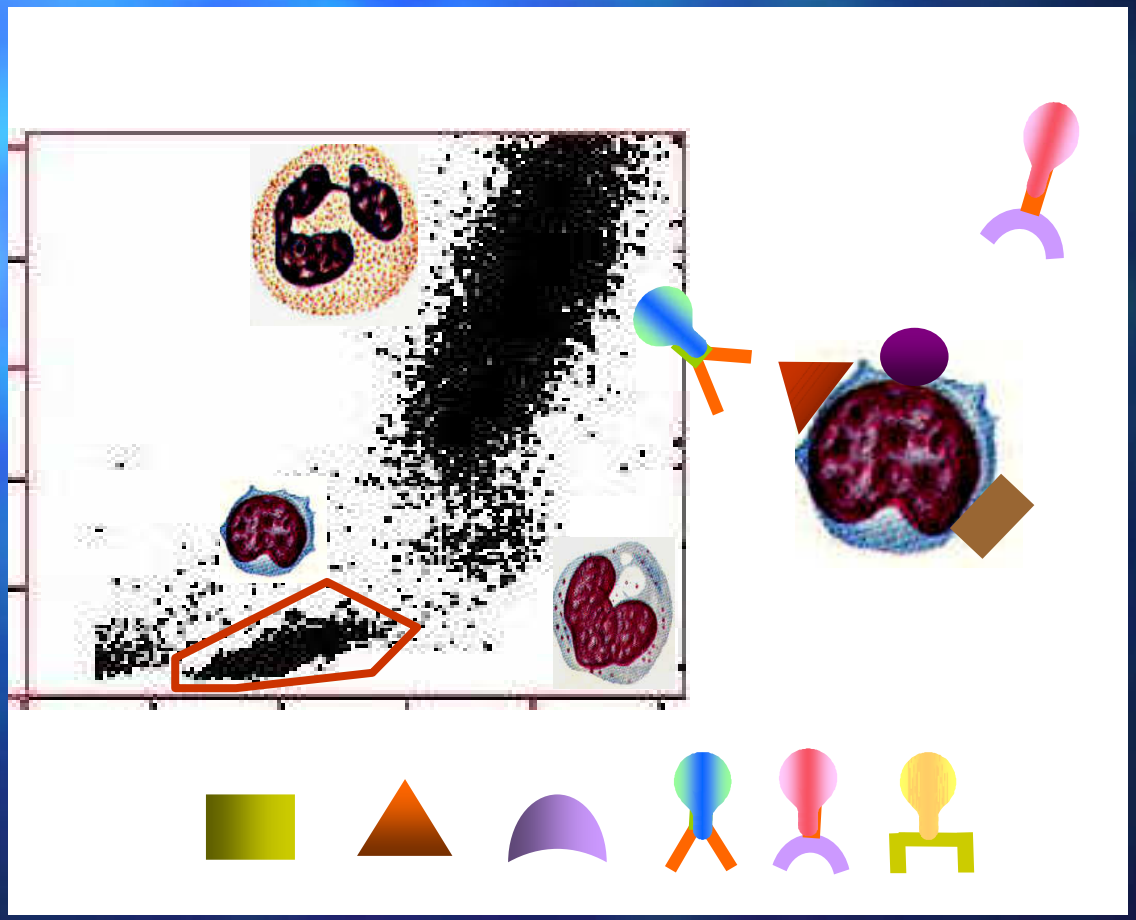

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Basic Applications-2

DNA or RNA

Propidium Iodie, EtBr. AO.

–Reticulocyte Counting
– Ploidy Analysis
(Diploidy, Hyper-diploidy, Hypo-diploidy…..)
(DNA Index, Proliferative Index)
– Cell Cycle Analysis
(G0/G1, S, G2/M) (CyclinB, D Analysis)
– Apoptotic and Necrotic Cell Detection
– Live and Dead Cells Counting (Viability testing)

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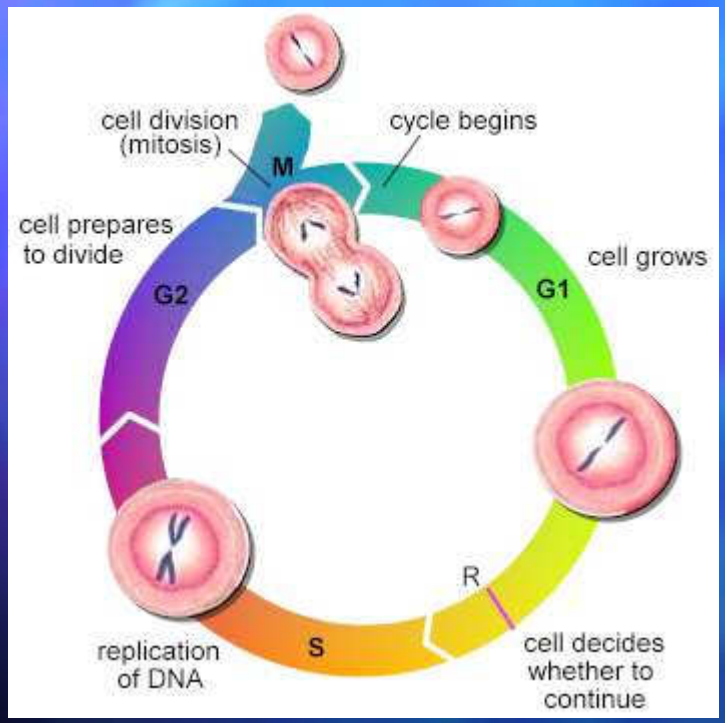
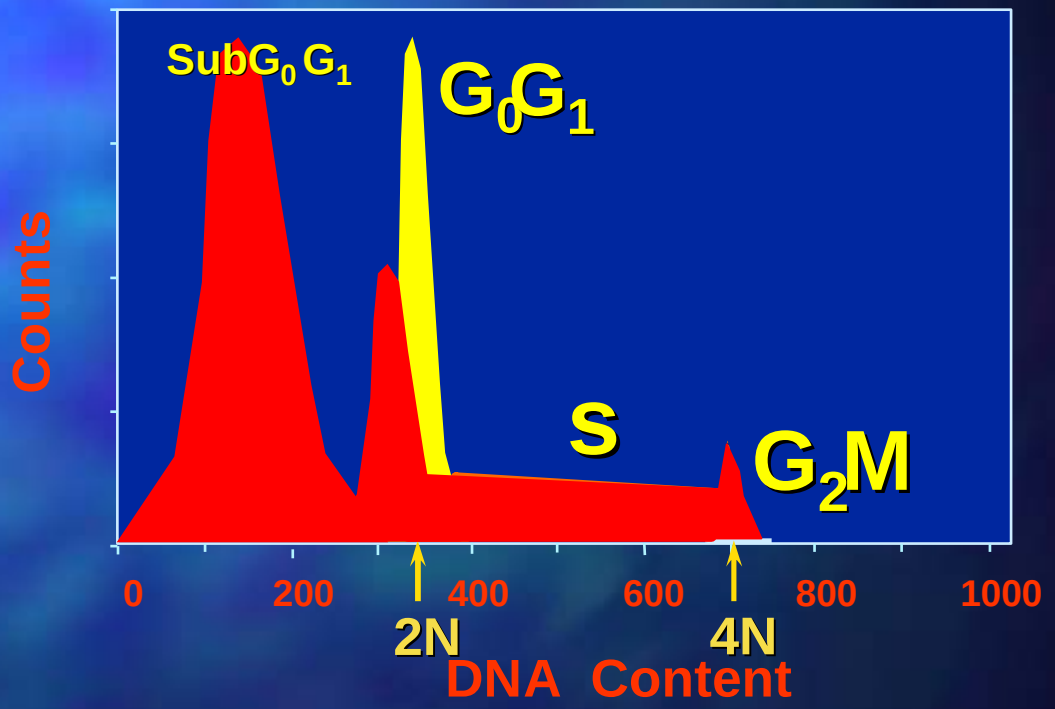
DNA Analysis





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Advance Applications

Cell Function Assay

- 細胞膜電位: Di-O-C6
- 粒腺体膜電位: Rhodamine 123, JC-1
- 細胞內PH 值: SNARF
- 細胞內鈣流動: Fluo-3, Fluo-4, Fura Red.
- H2O2: DHR 123, DCFH-DA
- $O2^{-}$ 自由基: Hydroethidine (HE)
- Sorting

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流式細胞技術於醫事技術上之應用

- 臨床微生物檢測工具之研發
- 監測疾病病程與治療預後狀況
- 敗血症之研發
- 輸血醫學相關研究
- 抗藥性研究
- 感染症相關研究
- 免疫功能相關研究
- 凝血疾病相關研究
- DNA分析與腫瘤診斷
- 定量分析工具之研發
- 血腫疾病研究
- 癌症相關研究

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流式細胞技術於生物技術開發之應用

- 單珠抗體研發與螢光標識抗體之生產
- 癌症診斷試劑研發
- 老化研究〈細胞凋亡、自由基等〉
- 免疫功能與相關疾病診斷，治療與監控工具之開發
- 細胞生物學研究〔細胞毒性，氧化代謝，訊息傳導等〕
- 商用檢測試劑組開發
- 新藥開發
- 造血幹細胞分化研究
- 遺傳探針之研發
- 病原特定性T細胞偵測工具之開發
- 細菌基因鑑定
- 轉染工具-報導基因之螢光蛋白開發

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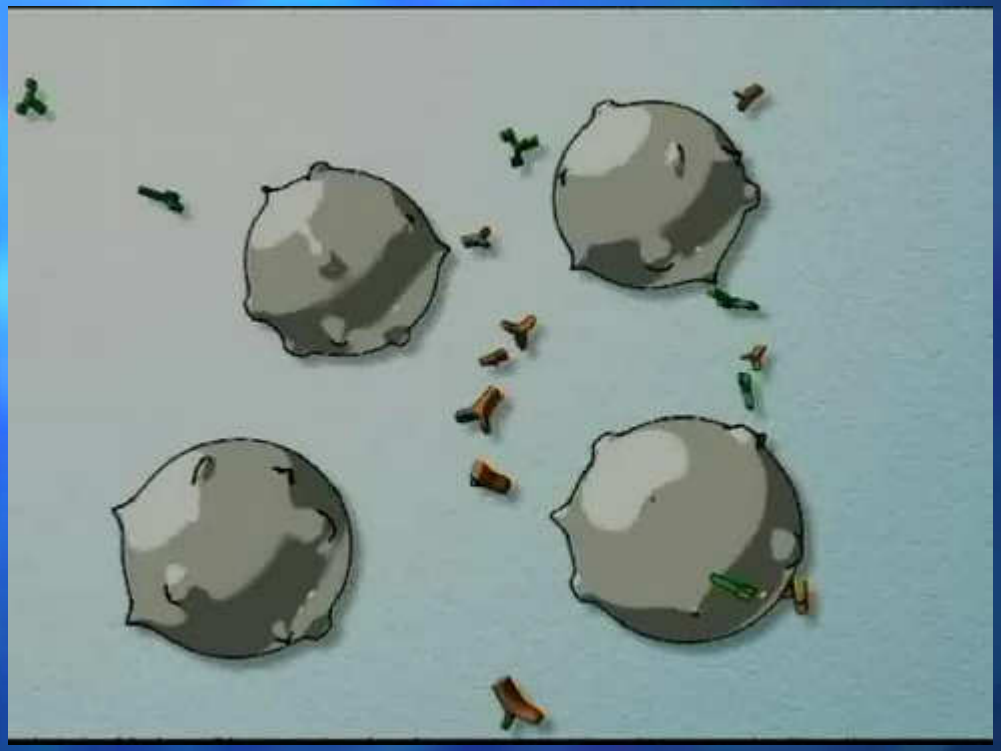

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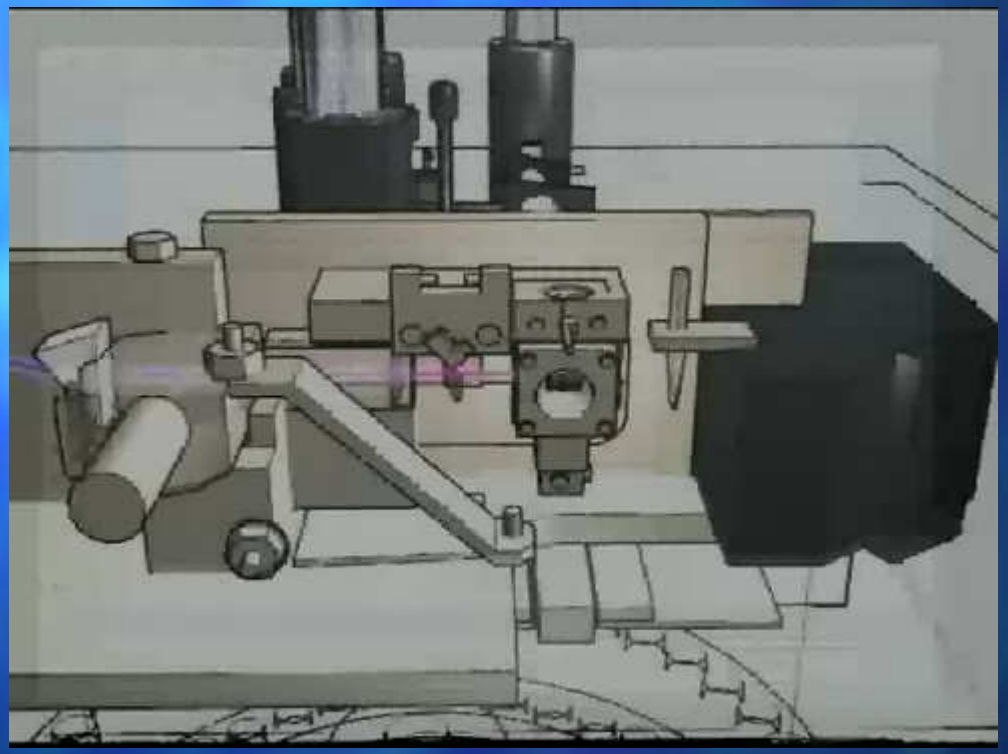

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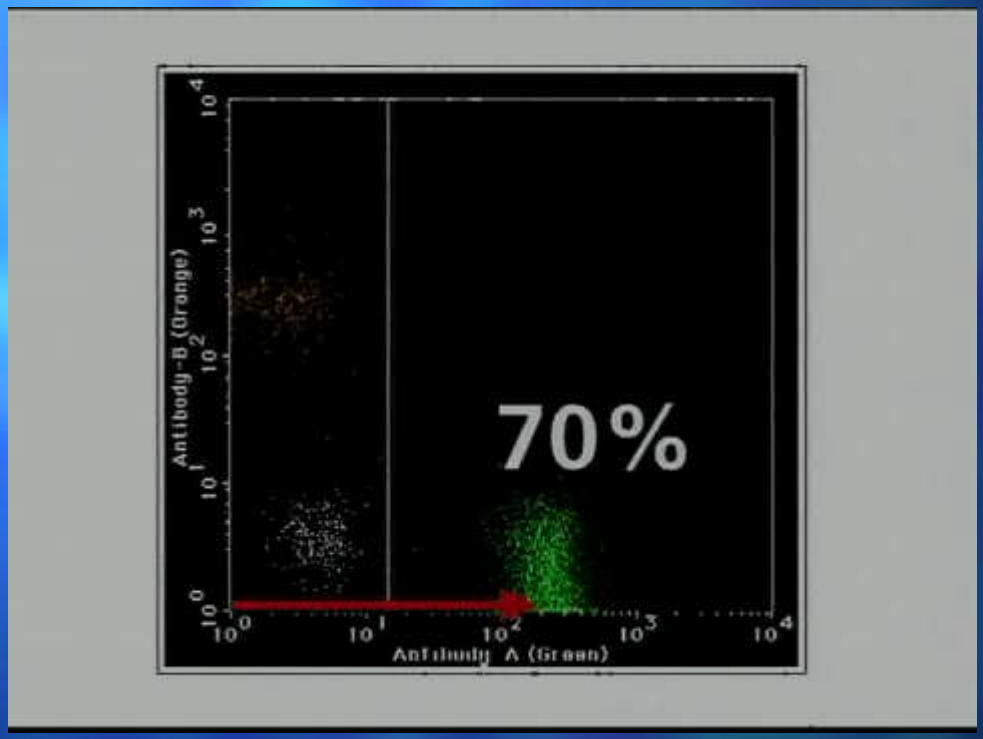

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Any Question?

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www.cell-bio.com.tw

產品專員:楊利君

apoptosistw@gmail.com

02-27855860　　0953062485

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