

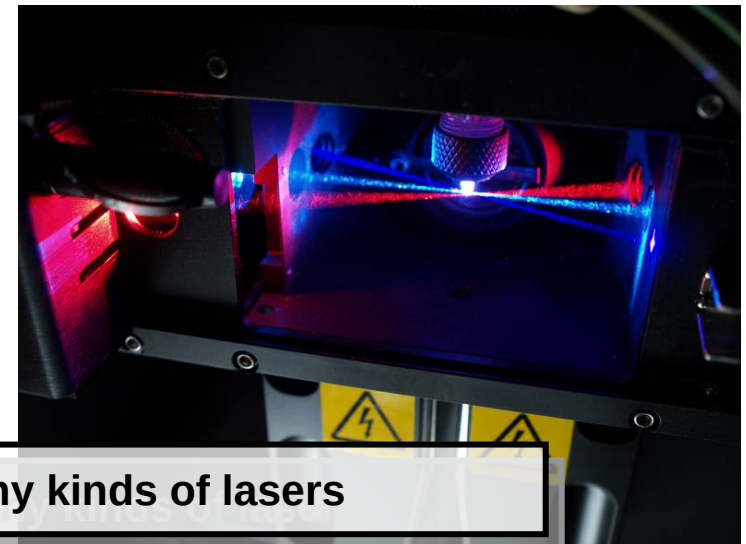
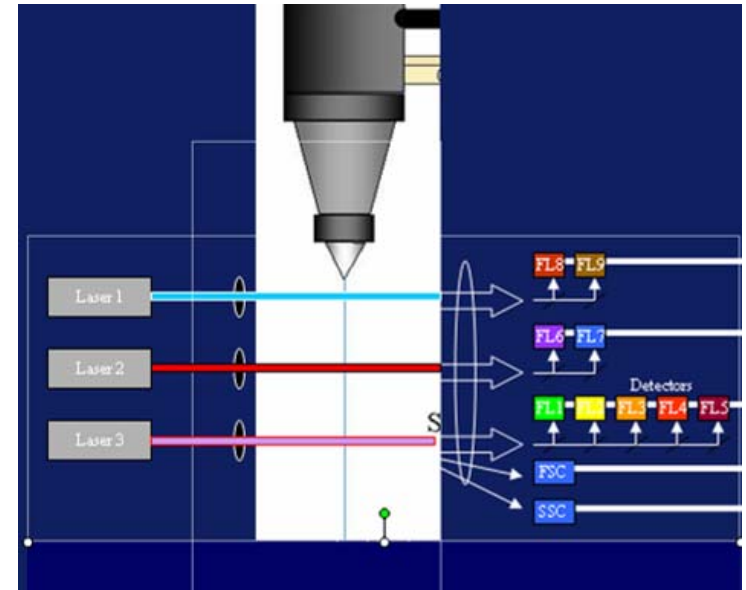
MoFlo™ XDP

The Best Cell Sorter on the Planet

楊人泰 Tim Yang
Marketing Manager
Beckman Coulter Taiwan Inc.

Optic Design

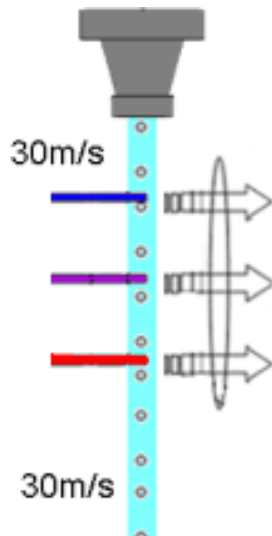
- **Open architecture, unlimited upgradeable**
- **Separated 3 excitation points, capable of :**
 - 488nm, 200mW solid state laser**
 - 635nm, 25mW diode laser**
 - 405nm, 50mW solid state laser**
 - UV, 100mW 355nm solid state
 - water-cooled Argon ion laser
- Laser Engine**
 - **325nm 150mW SS**
 - **561nm 200mW SS**
 - **592nm 200mW SS**
 - **640nm 60mW**
- more.....
- **Up to 8 lasers simultaneously**
- **FSC + SSC + up to 18 Fluorescence**



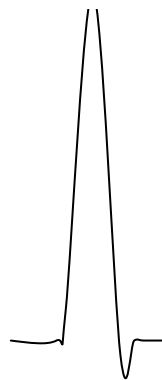
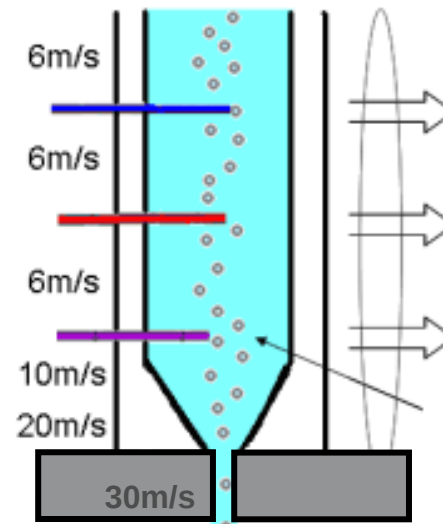
Up to 8 Laser, 18 colors with any kinds of lasers

Jet-in-Air vs. Cuvette system

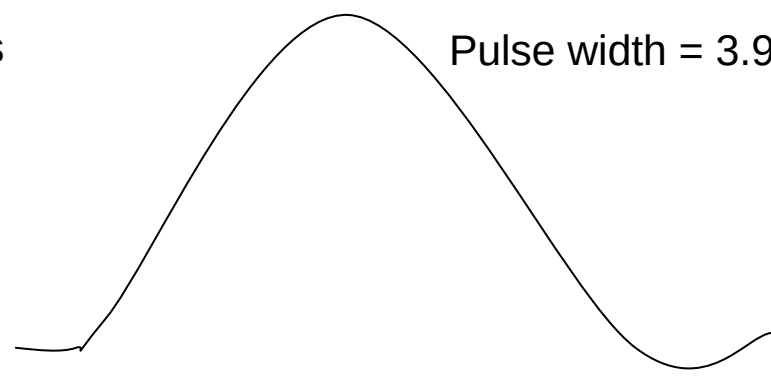
Jet-in-Air System



Cuvette System



Pulse width = 0.82 us

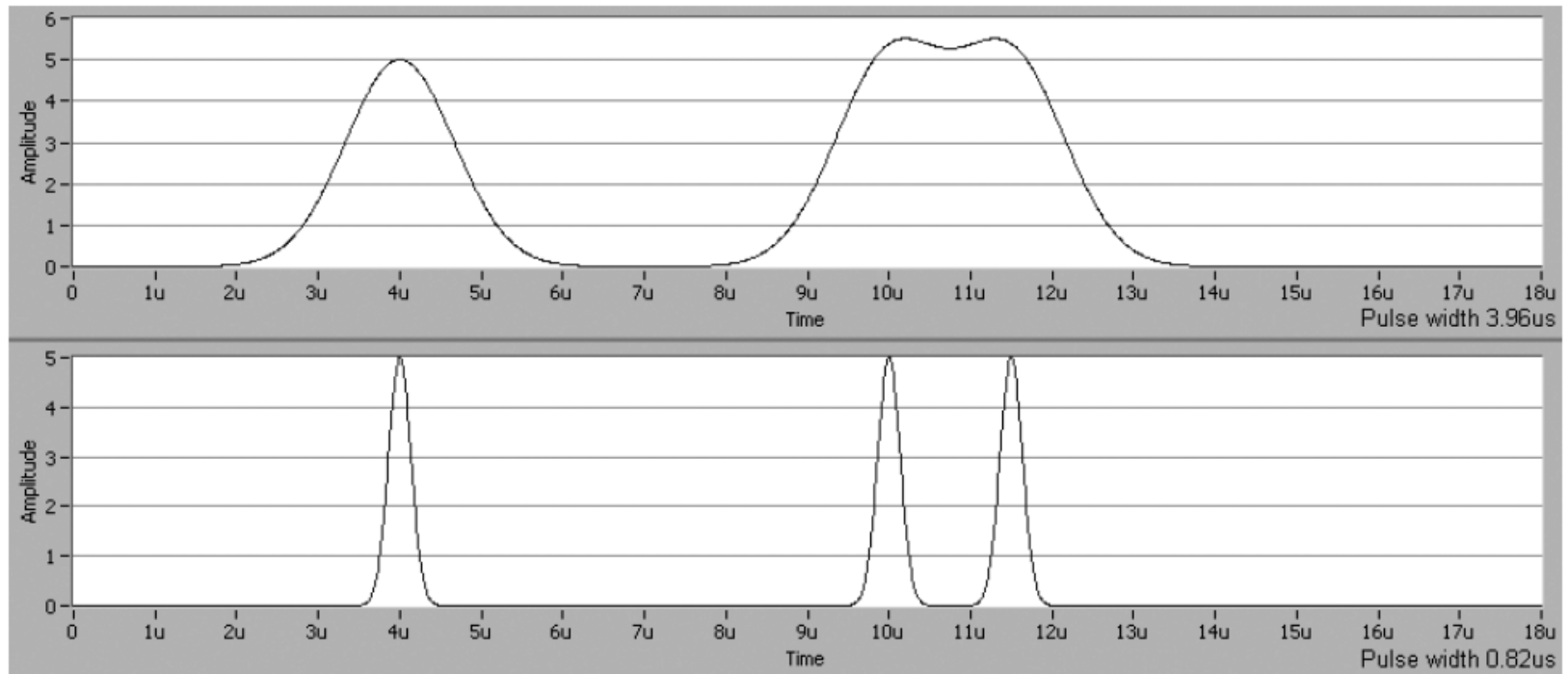


Pulse width = 3.96 us

Jet-in-Air offer faster velocity and shorter pulses than Cuvette

Jet-in-Air vs. Cuvette system

Cuvette System



MoFlo XDP Jet-in-Air System

Shorter pulses allow cells to be analyzed with high accuracy

MoFlo XDP's CytoNozzle



- Patented 'smooth inner surface' CytoNozzle tip **maximize cell viability**
- CytoNozzle comes in a variety of size: **50, 70, 80, 90, 100, 120, 150, 200 and 400 um** for diff. Appl. (Nozzle size must be **3~5 times** larger than sample cells)

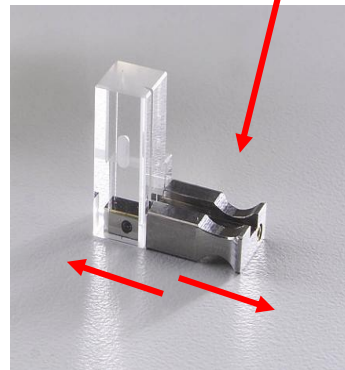
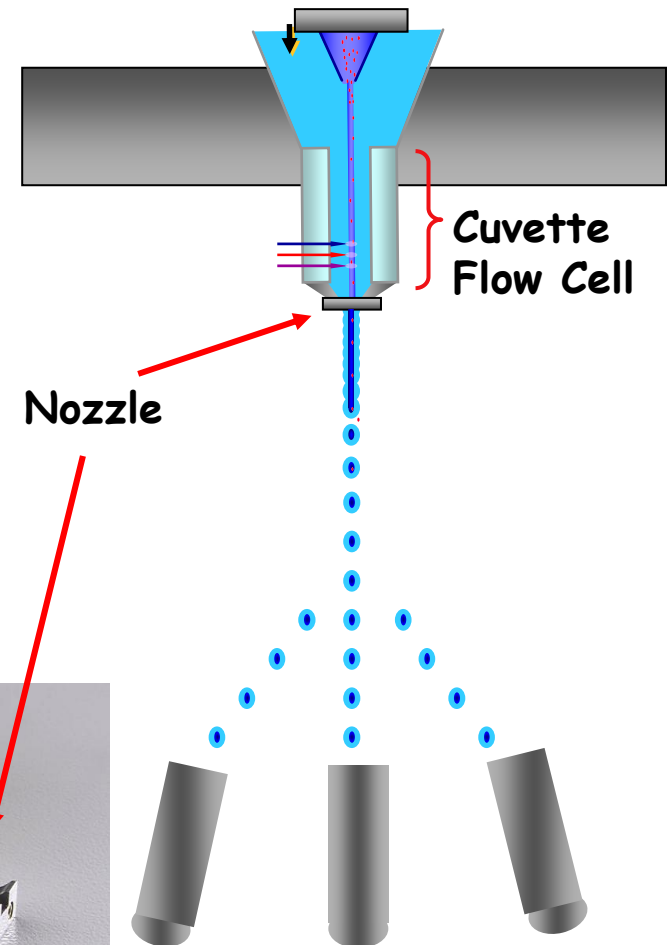
Nozzle size	Applications
50 um	染色體 / 精子 / 細菌 / 病毒顆粒 / 藻類細胞
70-90 um	實驗動物細胞 / 人類淋巴細胞 / 神經細胞
100-130 um	腫瘤細胞
150 um	巨噬細胞等真核生物細胞
200 um	植物細胞 / 花粉 / 大型藻類

9 sizes of nozzle tip to fit any kind of applications

Cuvette Flow Cell – Coulter ALTRA and BD FACS Aria

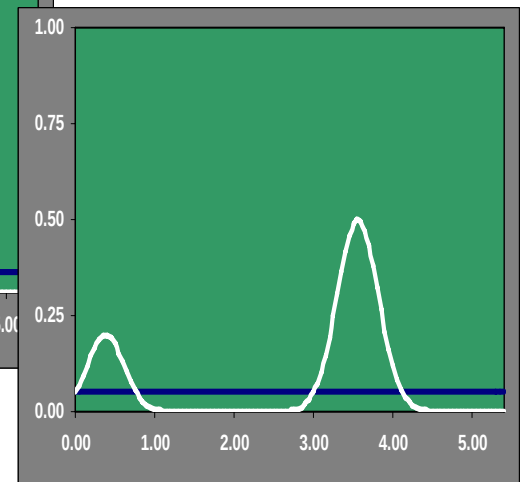
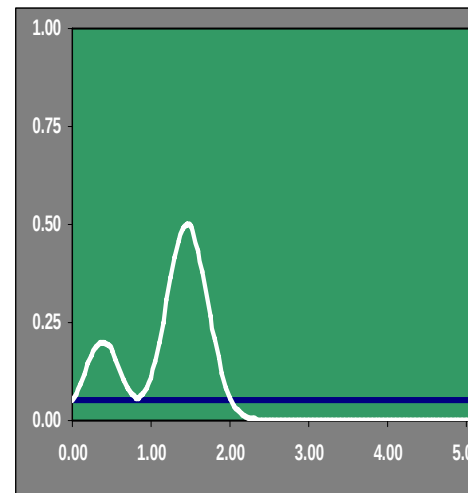
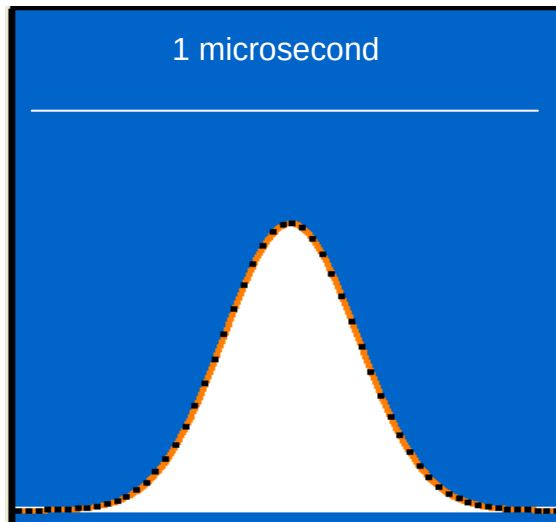
The disadvantage of Cuvette-based sorters :

- **Slower velocity of detected cells**
- **Wider signal pulse**
- **Discontinuous Inner-surface design**
- **Cell Damage**



MoFlo XDP's Electronics：量測速度

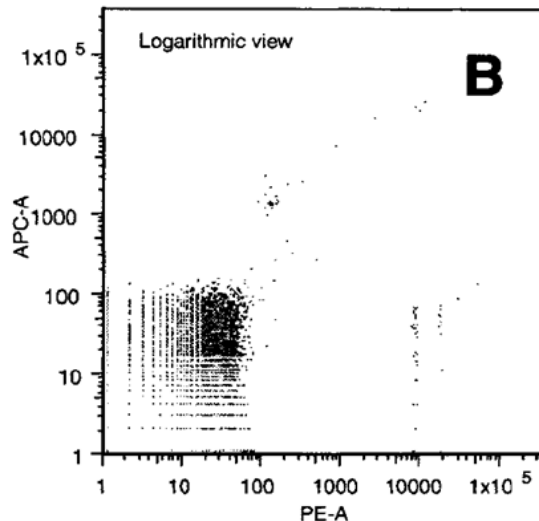
- The fastest signal processing system in the history of Flow
- **100 Megahertz sampling** (量測頻率，每秒量測 100,000,000次)
 - Zero dead time, 不會因為分析速度加快而 Lose data (漏接細胞)
 - 大幅提昇分析速度，每秒可超過 100,000 顆細胞
 - 可精確偵測快速通過的微小顆粒 (<1 um, bacteria, virus)
 - 減少細胞誤判，大幅提高分選時的純度 (Purity) 與產率 (Yield)



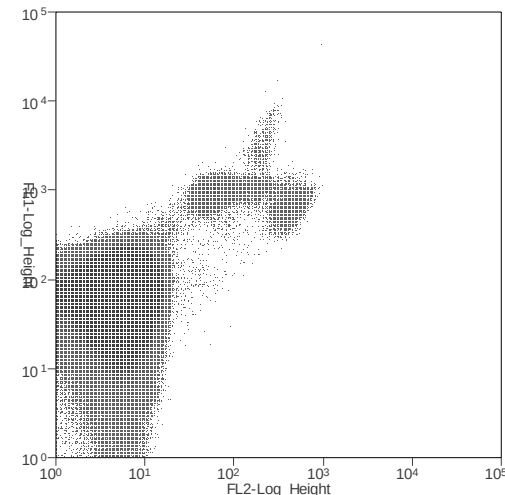
MoFlo XDP offer more than 10 times of sampling speed than other sorter

MoFlo XDP's Electronics : 量測精準度

- Latest ADC technology 提供無與倫比的超高訊號解析度
- 32 bit resolution (vs. FACS Aria 18 bit)
 - 2^{32} (4,294,967,296) channel for pulse measurement
 - No more '**Picket Fence**' on low Log
 - Achieve **5-Log** dynamic range



18 bit data on 5-Log, FACS Aria,
from Practical Flow Cytometry 4th Edition

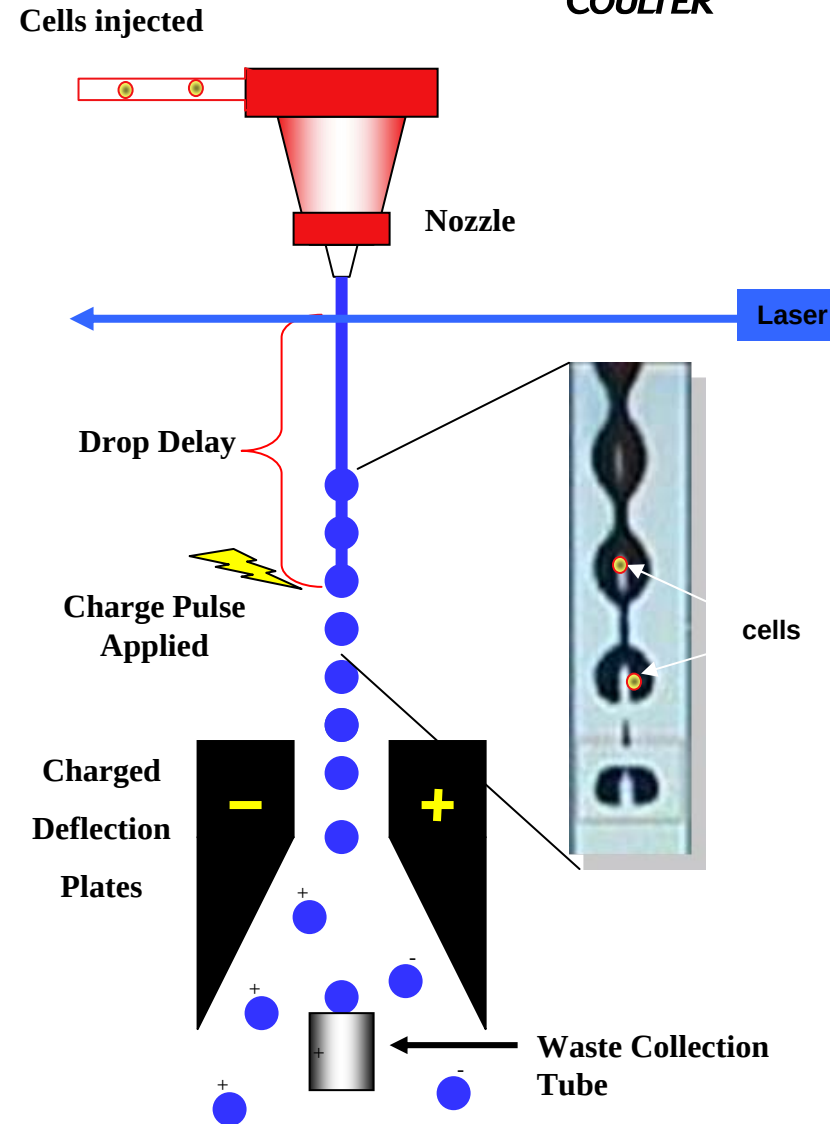


32 bit data on 5-Log, MoFlo XDP

MoFlo XDP offer 4096 times of resolution than BD's FACS Aria II

MoFlo XDP's sorting system

- Standard electronic sorting
- Drop formation frequency: 200 kHz
 - 每秒最多可震出 200,000 顆水珠
 - 為維持分選純度，平均每 3 顆水滴含有 1 顆細胞為最佳狀態
- Sort speed up to 70,000 eps
(20,000/3 ~ 70,000)



MoFlo XDP offer 70,000 cells/sec sorting rate

Sorting Speed limitation based on drop formation

200.000 Hz
50.000 ev/s



200.000 Hz
200.000 ev/s

Sorting Speed limitation based on drop formation

200.000 Hz
50.000 ev/s



200.000 Hz
200.000 ev/s

The most critical issues when sort cells

- **Purity**- What is the Purity of my target cells at all speeds with compensation applied and many parameters selected ?

All provider tell you their sorter could sort with >99% purity!!!!

But.....At what sort speed???

- **Yield**- How many cells do I actually get from the sort ?

How many cell do you lose (Abort) at high speed sorting?

- **Viability** – How many cells are killed or lose function due to the instrument ?

Do you get living cell after sort??

Yield and Viability are more critical than purity

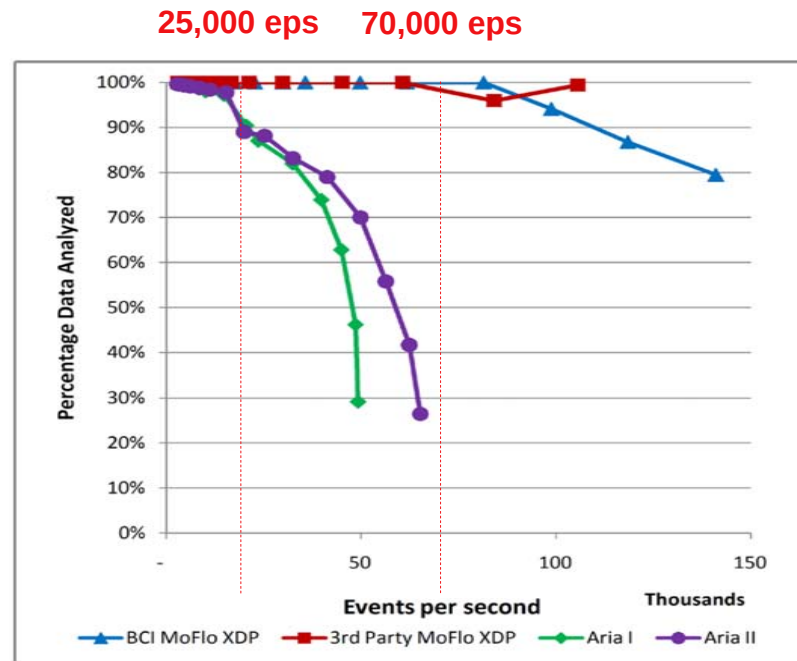
Purity and Yield

Purity

- MoFlo XDP promise the purity is **higher than 99% up to 70,000 eps** !!!

Yield

- MoFlo™ XDP has superior analysis/sort yield at high event rate.



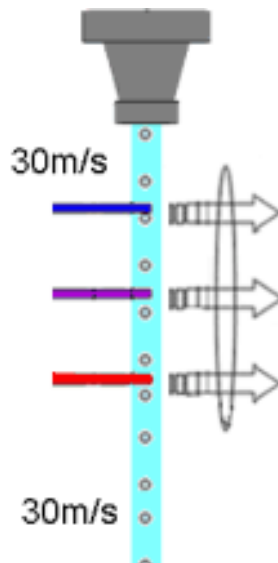
MoFlo XDP offer superior Purity and Yield than FACS Aria II

Sorting viability: Jet-in-Air vs. Cuvette

- Smooth Jet-in-Air 設計大幅降低高速流動對細胞造成的傷害。
- 超高的分選速度（其他機型 4~10 倍的速度），大幅縮短分選時間，並提高細胞活性。

看到，就收的到

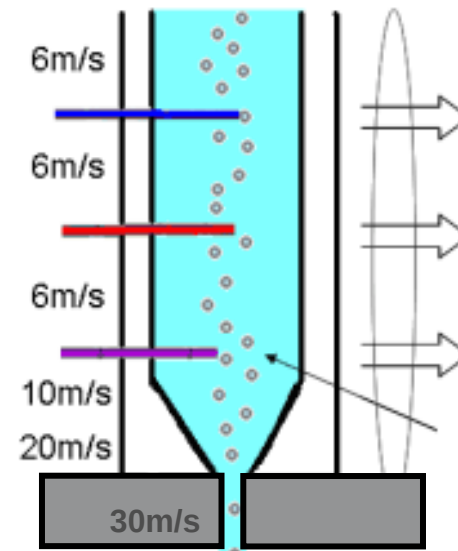
Jet-in-Air System



Viability > 90%

看到，還有可能破掉

Cuvette System

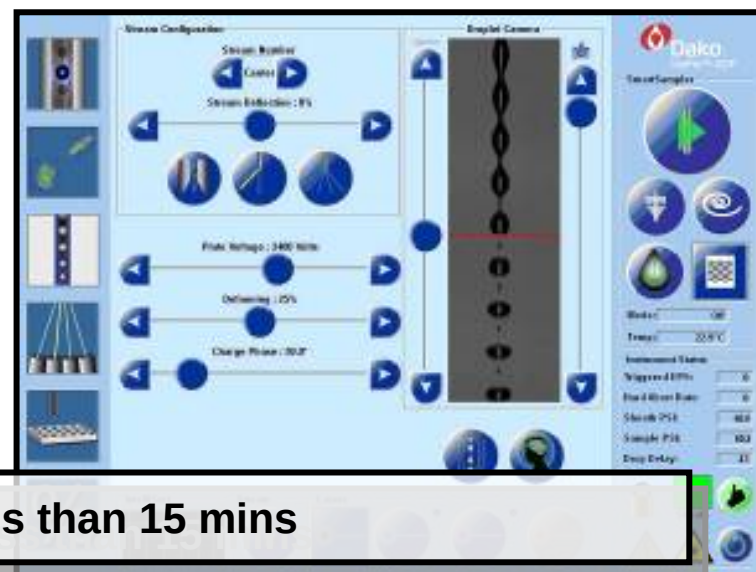


Viability < 50%

MoFlo XDP offer higher viability than Cuvette system

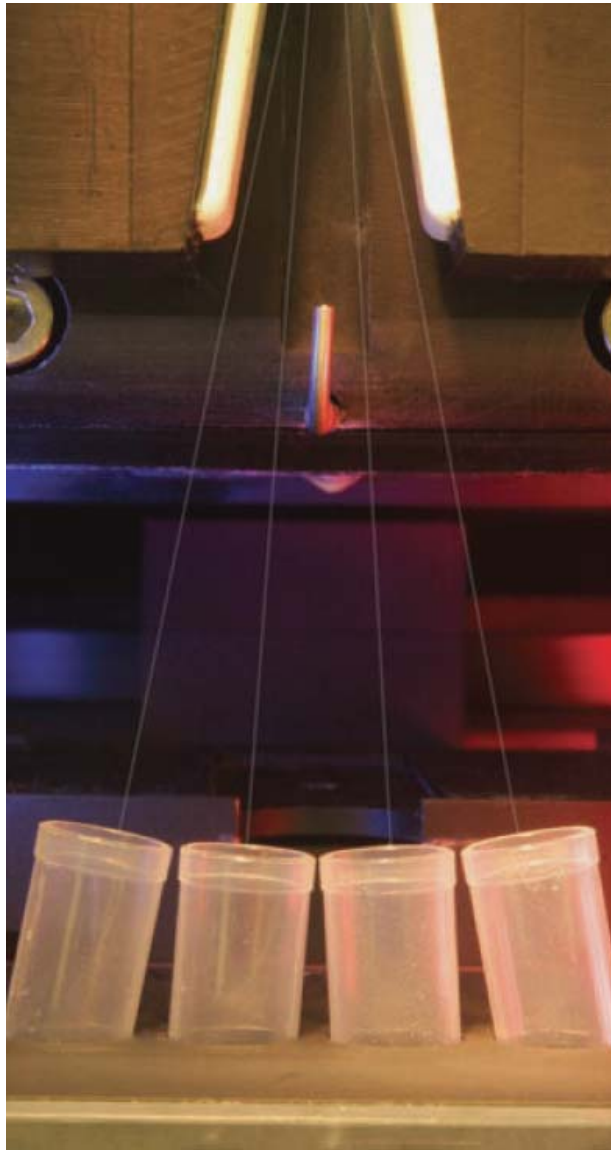
MoFlo™ XDP – Automatic Sort Setup

- IntelliSort II 採用“**One Button touch**”，使用者只需按下啟動設定鈕，系統即可自動設定分選條件。無須使用昂貴的分選校正微球，系統會自動算出最正確的 Drop Delay 進行分選。（不正確的 Drop delay 會造成分選純度大幅下降）
- 流程簡易，**開機後15 分鐘**內即可完成設定，開始進行分選工作。
- Jet-in-air 系統提供最穩定的液流狀況，省卻了不斷找條件的窘況，讓**穩定的分選條件能持續一整天**。（採用 Cuvette 的 Sorter 常因阻塞造成分選條件跑掉，必須進行排塞工作後，重頭調整分選條件）
- 分選工作開始後，IntelliSort II 會持續監控分選狀況，自動微幅調整因外界不穩定因素造成的液流改變，維持穩定且持續的分選工作。

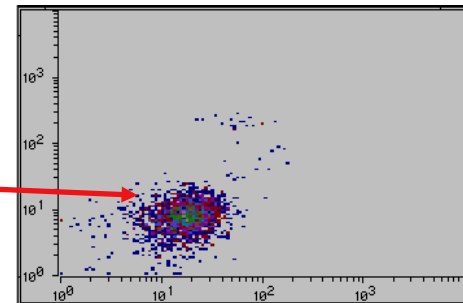
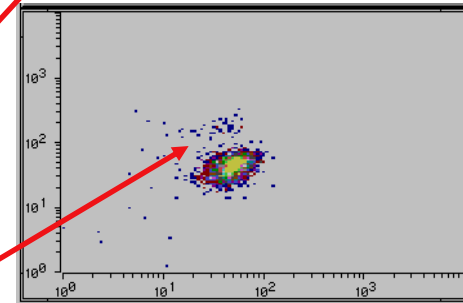
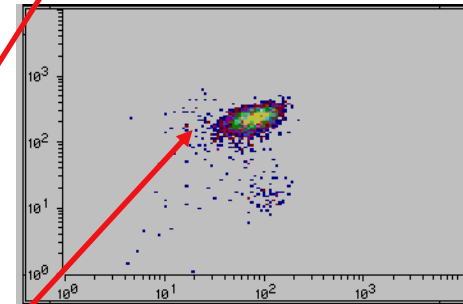
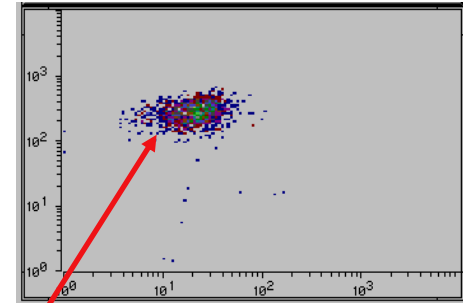
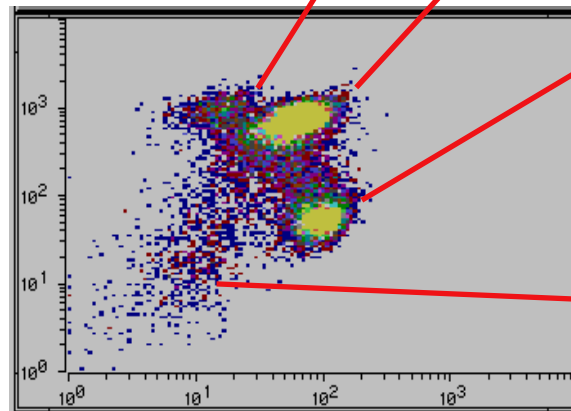


Sorting setup less than 15 mins

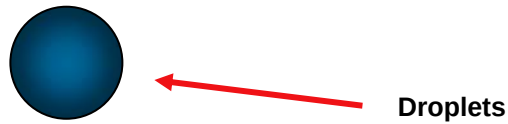
4WAY™ Sorting



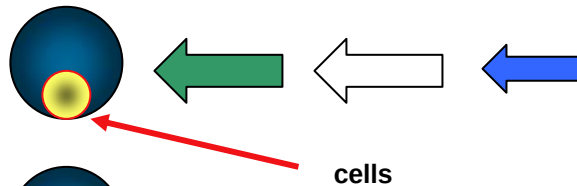
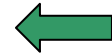
- Sort into four tubes
- Purity can exceed 99.5%
- No performance reductions in purity at any speed



MoFlo XDP offers 3 kinds of Sort Modes



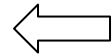
Enrich mode



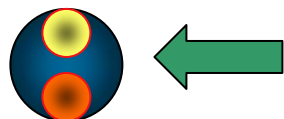
All positive cells are sorted except hard coincidences (cells too close together to discriminate).



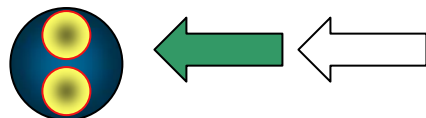
Purify mode



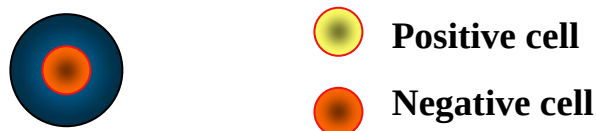
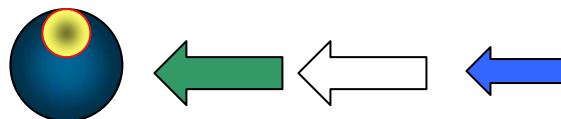
All negative cells are aborted and all positive cells are sorted.



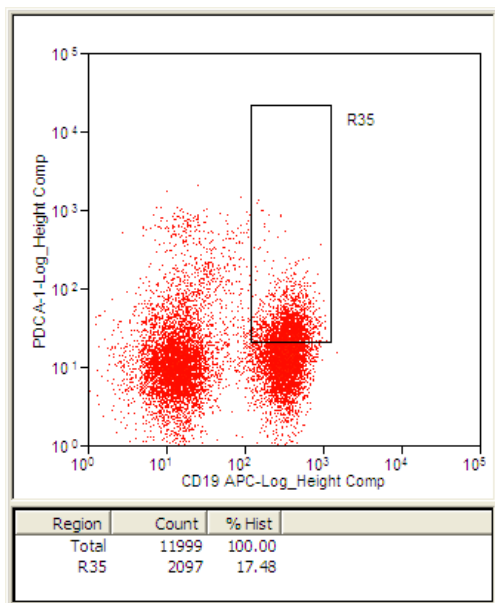
Single mode



All negative cells are aborted and there must be only one positive cell in the drop. This very important mode is used when sorting into 96 well plates.

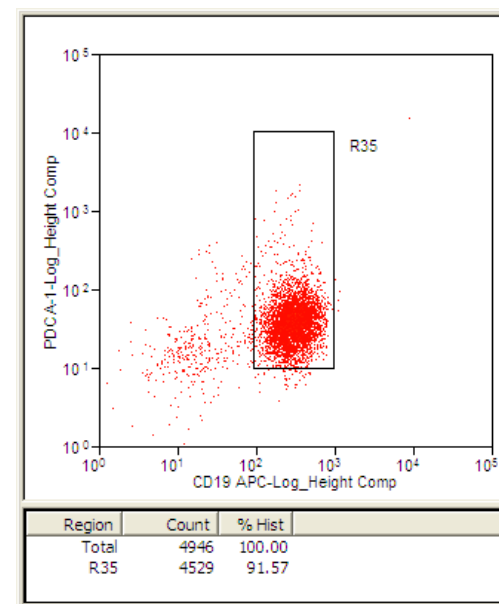


Unique 'Mixed Mode' Sorting

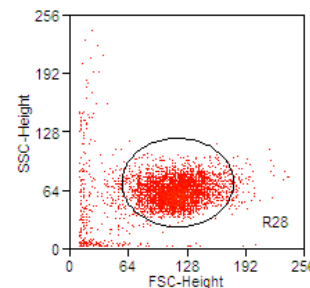
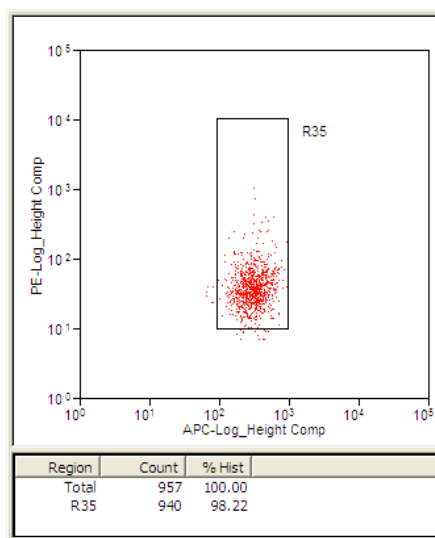


Enrich (right stream)

Sorted at >70,000eps for >2hrs



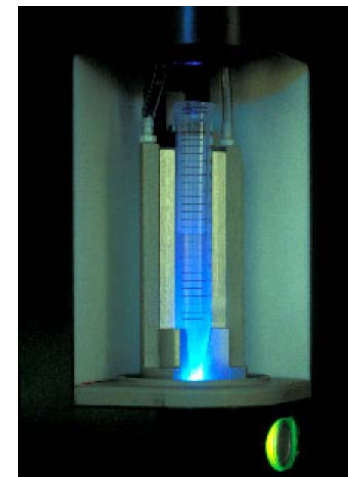
Purify (left stream)



Good viability !

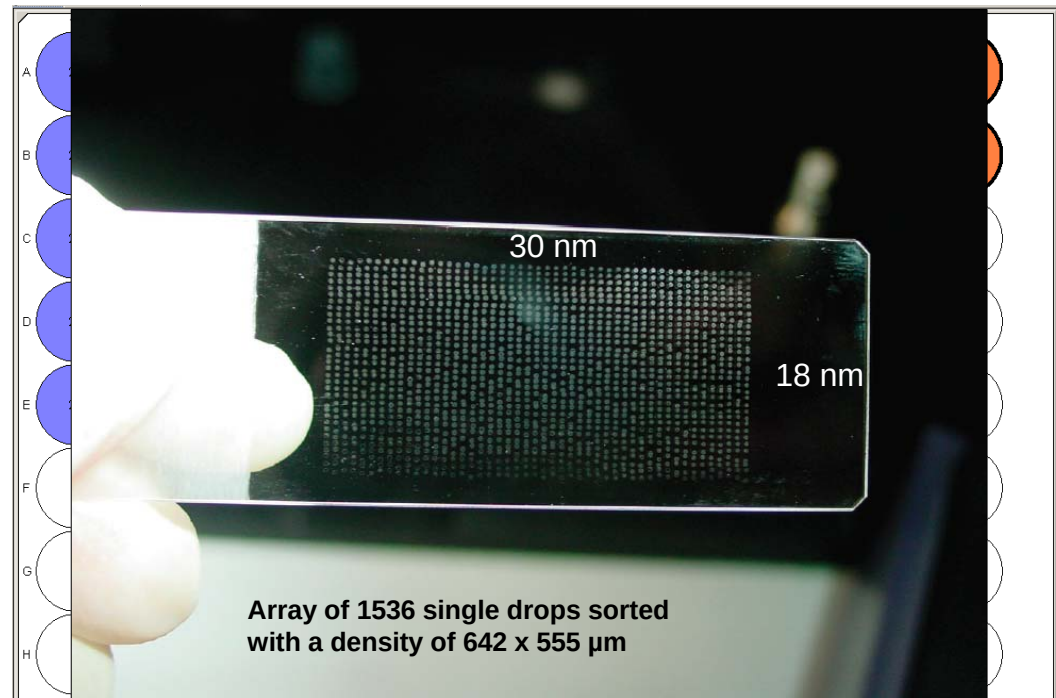
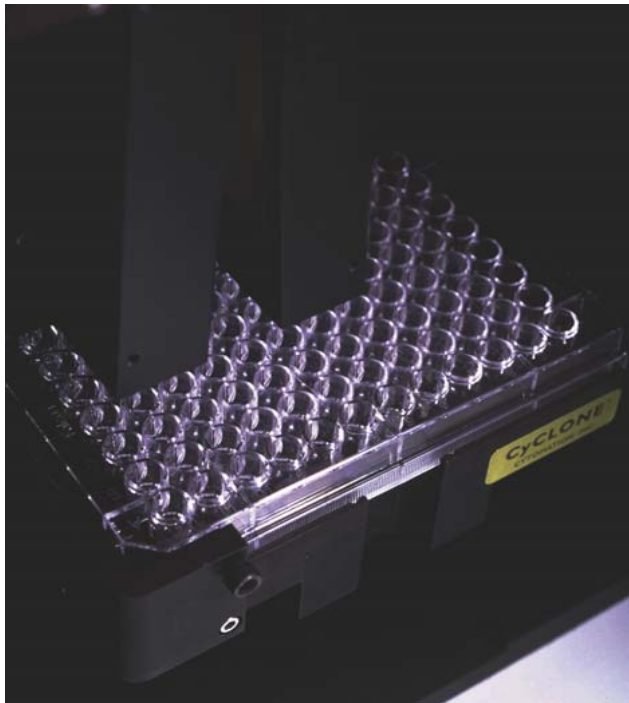
SmartSampler Features

- Wide variety of tube formats: 0.5, 1.5, 5, 14, 15 and 50 mL
- Automatic agitation settings and automatic backflush.
- An air detection feature alerts the user if air enters the sample line, allowing full aspiration of the sample, & preventing air from entering the nozzle.
- Samples can be maintained at temperatures from -4 to 40°C throughout the run with the water bath option.
- Automated cleaning and shutdown
- Environmental safety has been considered by directing depressurized air through a dual HEPA filtration system prior to return to the laboratory

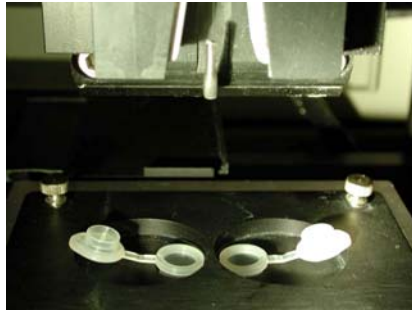


CyCLONE deposition system

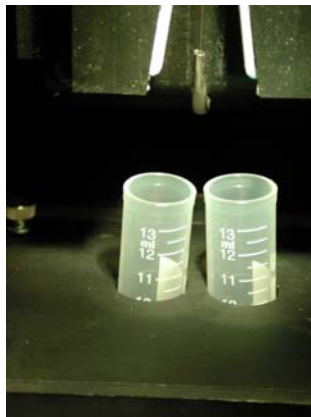
- The CyCLONE™ is a computer driven robotic arm that permits precise, rapid deposition of sorted events into user-defined micro-well plates
- Plates supported: 6, 24, 96, 384, 1536 wells, custom plates and slides
- Single cell deposition for cell cloning protocols with a purity greater than 99.9 %.
- Speed for plates is the fastest in the Flow Cytometry industry
- High accurate movement allow cell deposition into high density cell array



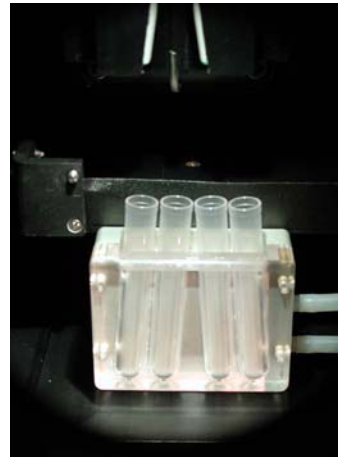
Collection Tubes at MoFlo XDP



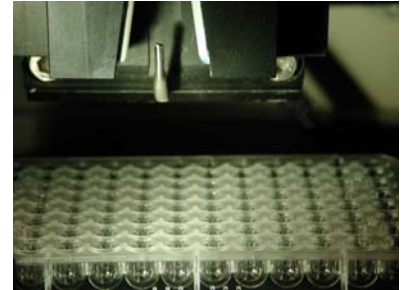
Eppendorf tubes



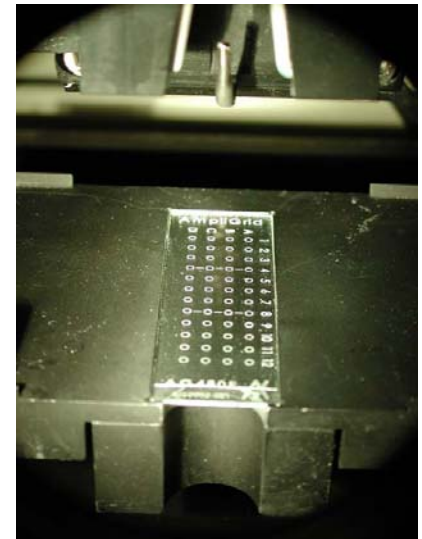
5 ml to 50 ml Falcon tubes



one to four tubes
/populations



multiwell plates
6 – 1536 wells



onto slides or directly
onto filters
or nitrocellulose membranes.

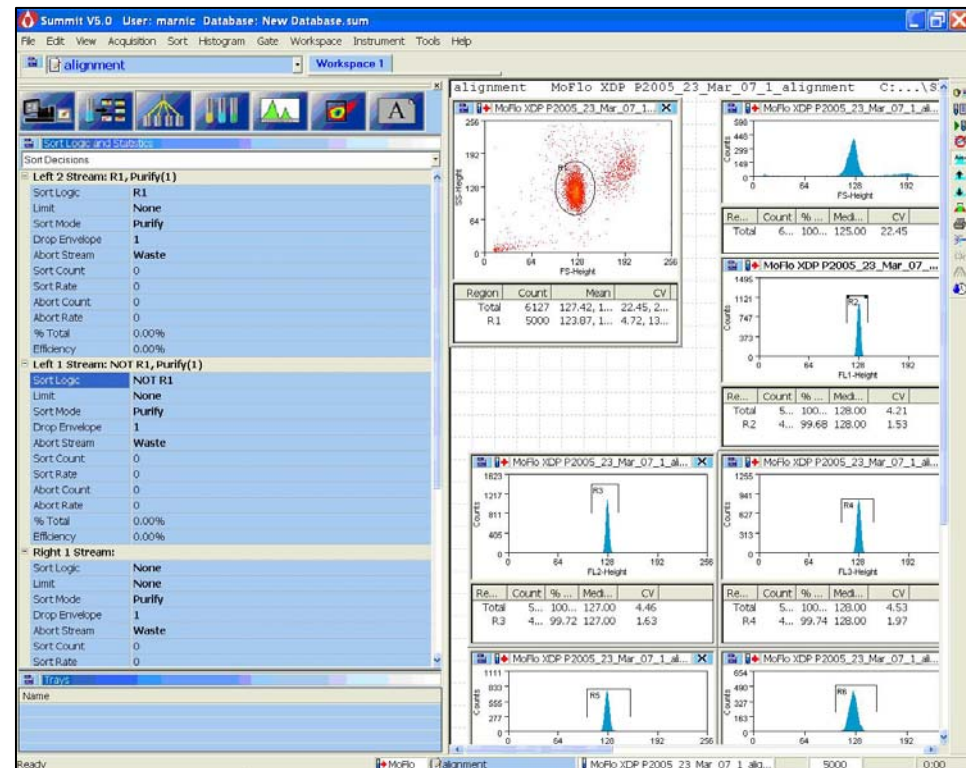
- The volume of one sorted drop is 1.4 nl (70 μ m, 100kHz, 60psi). 10⁶ cells result in 1.4 ml.
- Give 1-2 ml serum into the collection tube or whatever your cells will be happiest in !

MoFlo XDP aXcess Control Panel

- New user interface using touch screen display

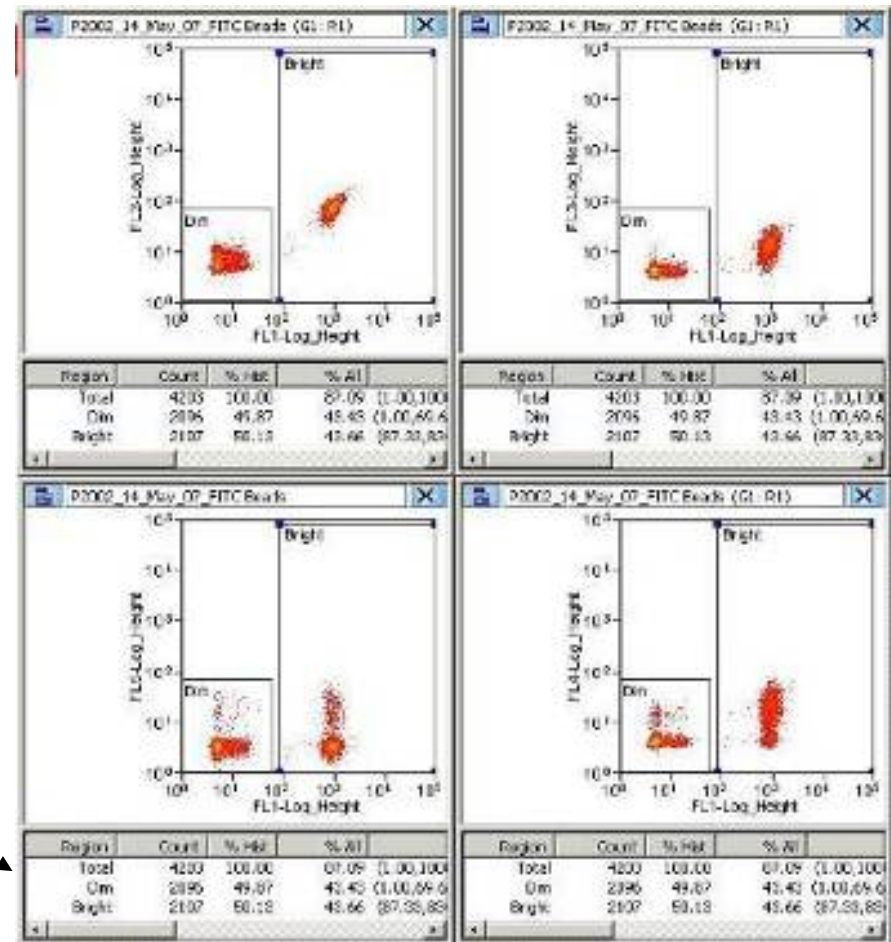


- Windows XP platform
- **Free Summit offline**
- Full control of all instrument function
- Powerful data analysis both during and after acquisition
- Application / sorting protocol
 - Layout
 - Parameter names
 - Parameter type
 - Triggers, Voltages, Gains
 - Compensation settings
 - Sorting condition



Summit Software

- Boolean gates
- **Overlays**
- **Subtraction**
- Multiple file analysis
- **Zoom**
- Statistics
- Smoothing / Contours
- Colours / Fonts
- Exportable
- **Read / Write all FCS formats**



何以 MoFlo XDP 可進行超高速 / 超高效能分選？

- 細胞流得快：JIA design, up to 100psi sheath pressure
- 訊號測得快：100 MHz sampling (每秒量測1億次)
- 訊號測得準：32 bit ADC (以刻度 2^{32} 格的量尺進行量測)
- 可形成最多的分選液珠：up to 200 kHz (每秒20萬個)
- 細胞活的好：Smooth Jet-in Air CytoNozzle



Why we need high speed analysis / sorting?

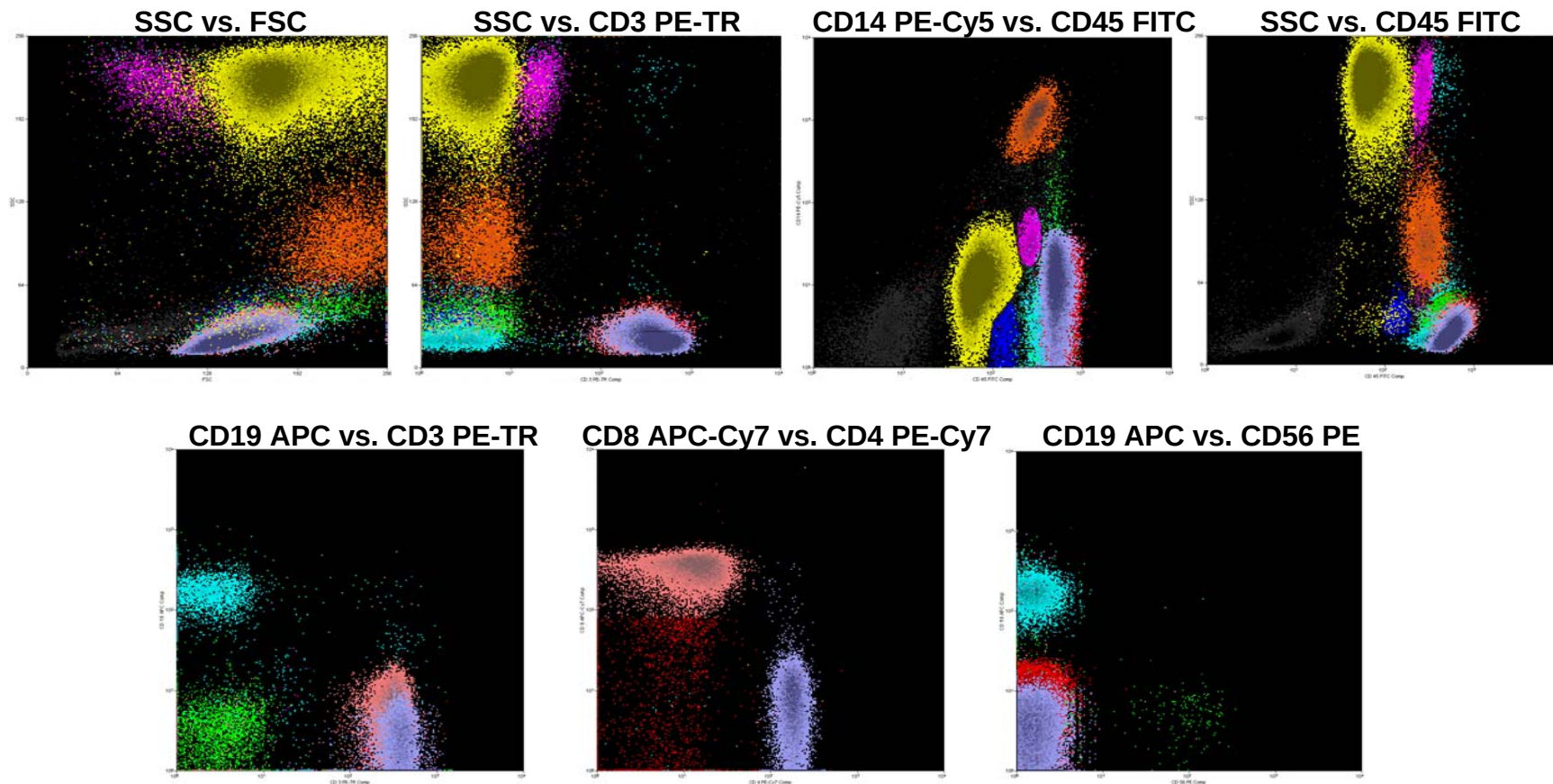
- Minimize the acquisition/sorting time
- Detection/sorting of rare population
 - Antigen specific T cell (tetramer analysis)
 - Side population of stem cell
 - whole blood immunotyping (no lysis)
 - Discriminate cancer cell from normal cells
 - Detection of CEC / CEP (Circulation endothelial cell / Circulation Epithelial Progenitor cell) on blood
 -
- Multicolor immunophenotyping
 - $200 / (0.2)^5 = 625,000$

MoFlo XDP Applications

- Normal Flow applications
- Multicolor immunophenotyping
- High speed analysis
- Rare event detection
- Small Particle analysis
- Large particle analysis
- Plant biology
- Chromosome karyotyping and sorting
- X/Y sperm sorting
- More.....

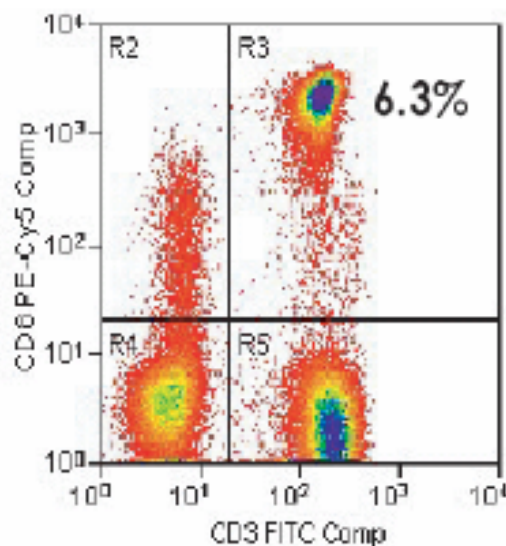
Multi-color immunophenotyping

7 colors: CD45-FITC / CD54-PE / CD3-PE-TR / CD14-PC5 / CD4-PC7
CD19-APC / CD8-APC-Cy7



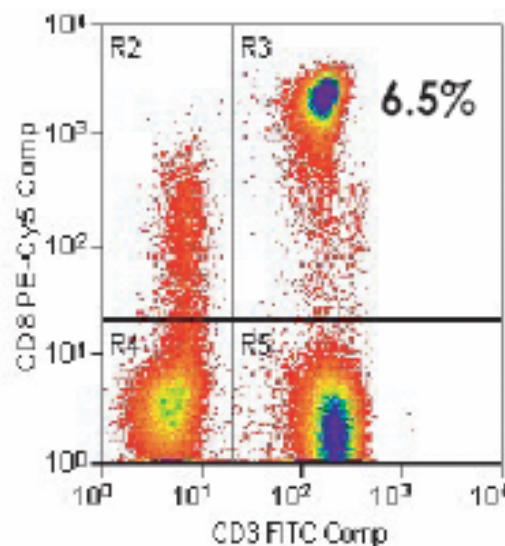
High speed analysis

1 x 10⁶ cells/ml
500,000 events
8 min 18 sec



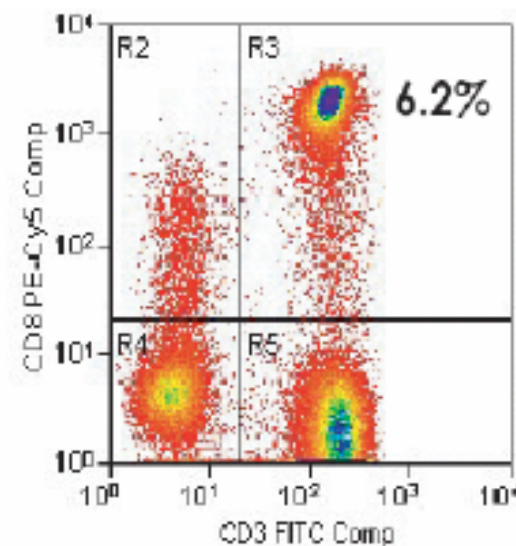
1,000 events per sec

5 x 10⁶ cells/ml
500,000 events
1 min 38 sec



5,000 events per sec

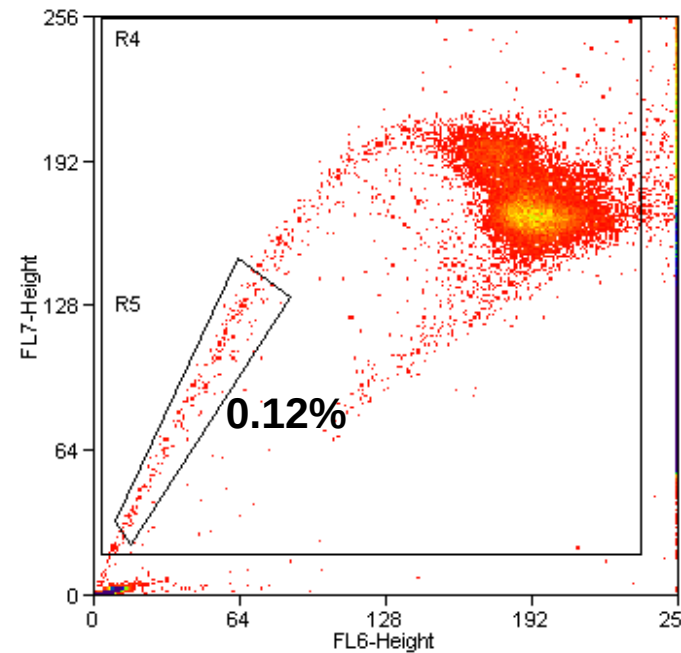
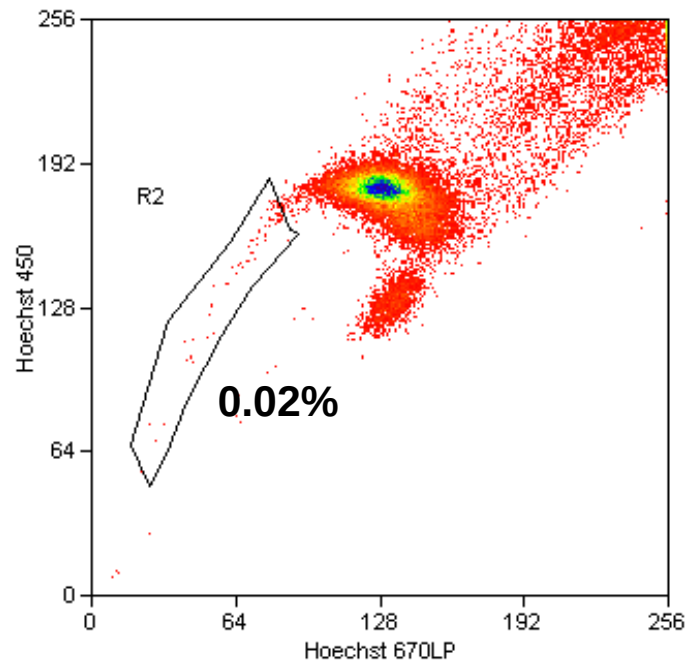
50 x 10⁶ cells/ml
500,000 events
10 seconds



50,000 events per sec

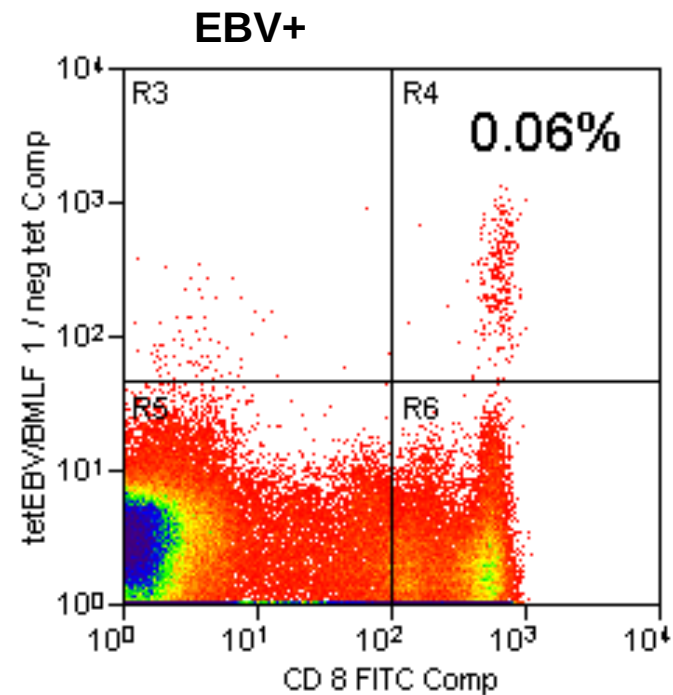
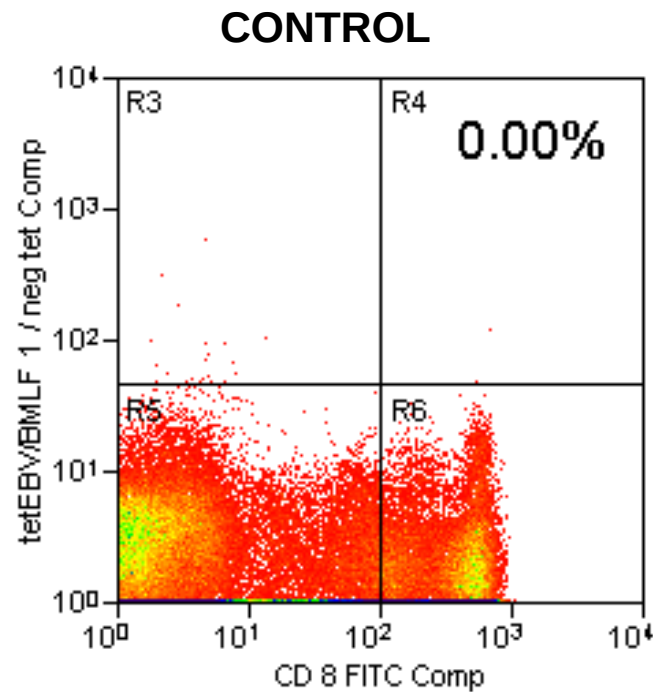
Rare population: Side Population (SP) of Stem Cell

The method of measuring the SP (side population) of stem cells from mouse bone marrow



Rare population: antigen specific T cell

High speed detection of Antigen (Epstein-Barr-Virus) specific tetramers from whole blood (less than 0.1% total blood cells), with very low background.



Rare target detection

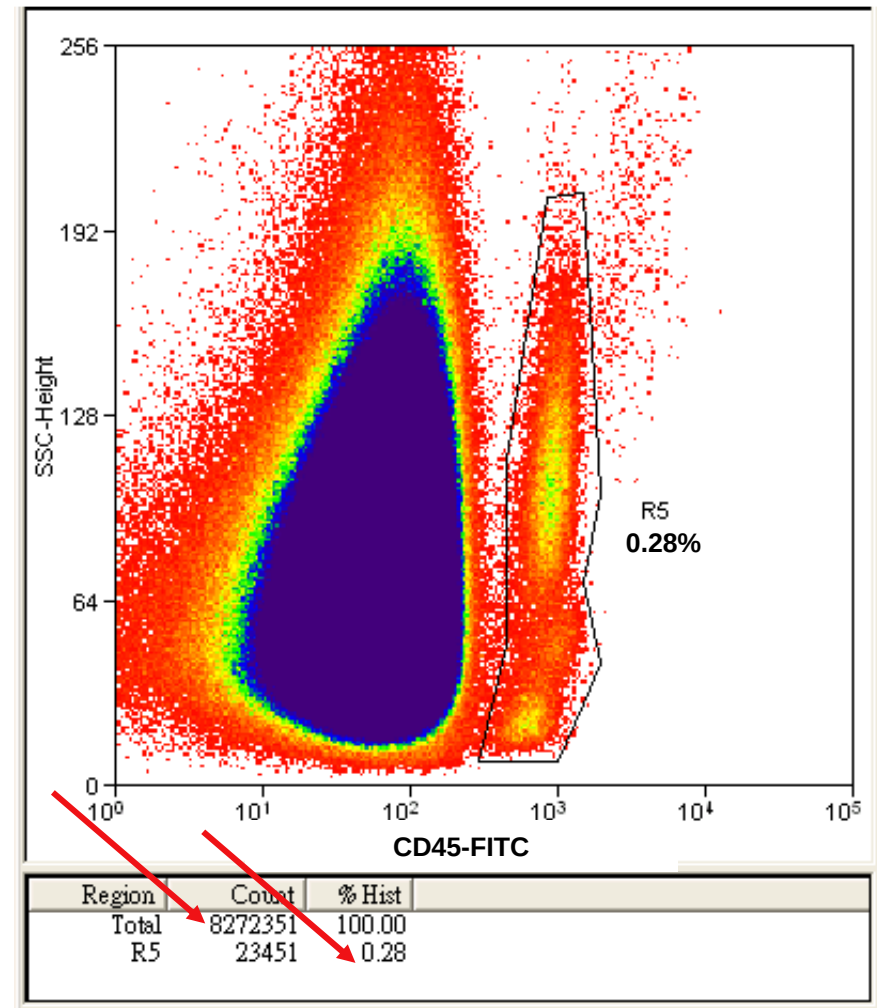
Whole Blood stained with CD45-FITC

No Lysing

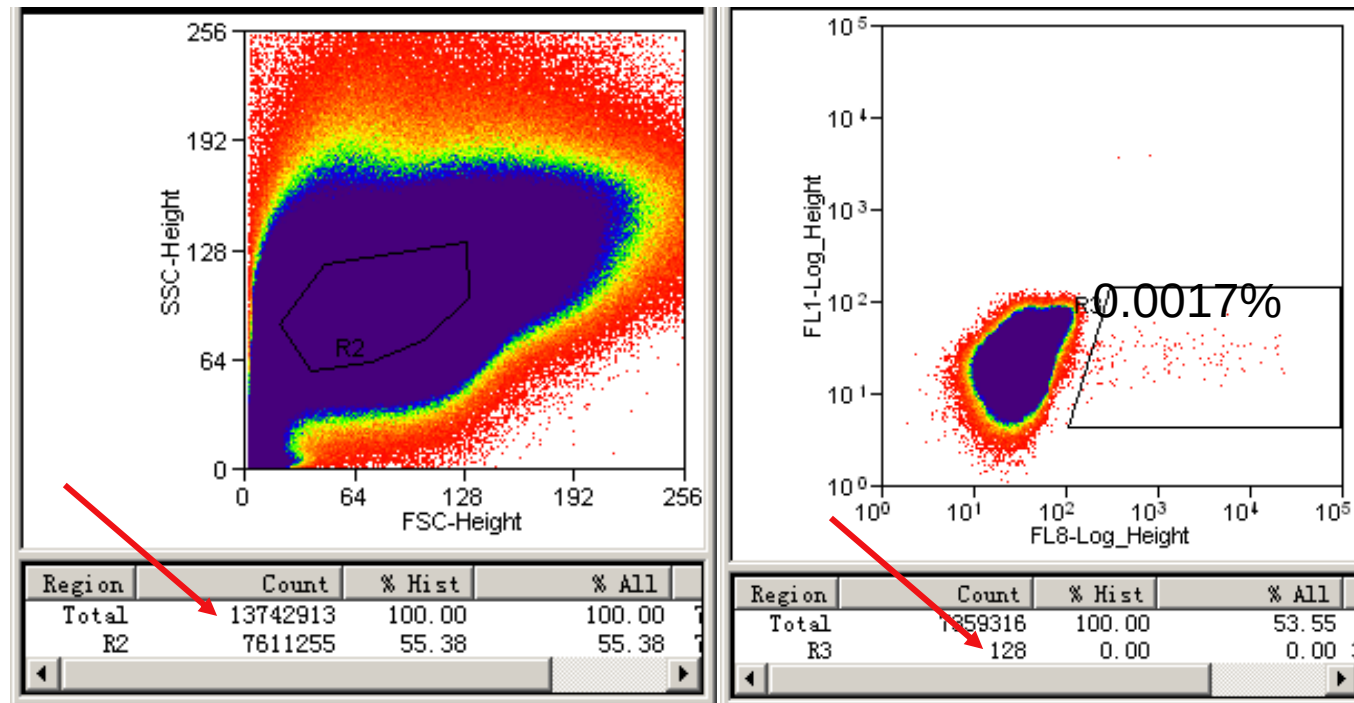
Total Event: 8,272,351

Elapsed time: 1m:24s

Average rate: 98,480 events/sec



Rare target detection



超低含量的肝癌細胞系 HuH7 幹細胞分析與分選。

分析之細胞數量超過 1300 萬個細胞，CD133 陽性細胞含量低於 1/10 萬（0.0017%），共分選出 128 個目標細胞，用已注射至裸鼠皮下進行成瘤觀察。

CyClone application: single cell PCR

Single cell typing by on-chip Low Volume PCR (LV-PCR)

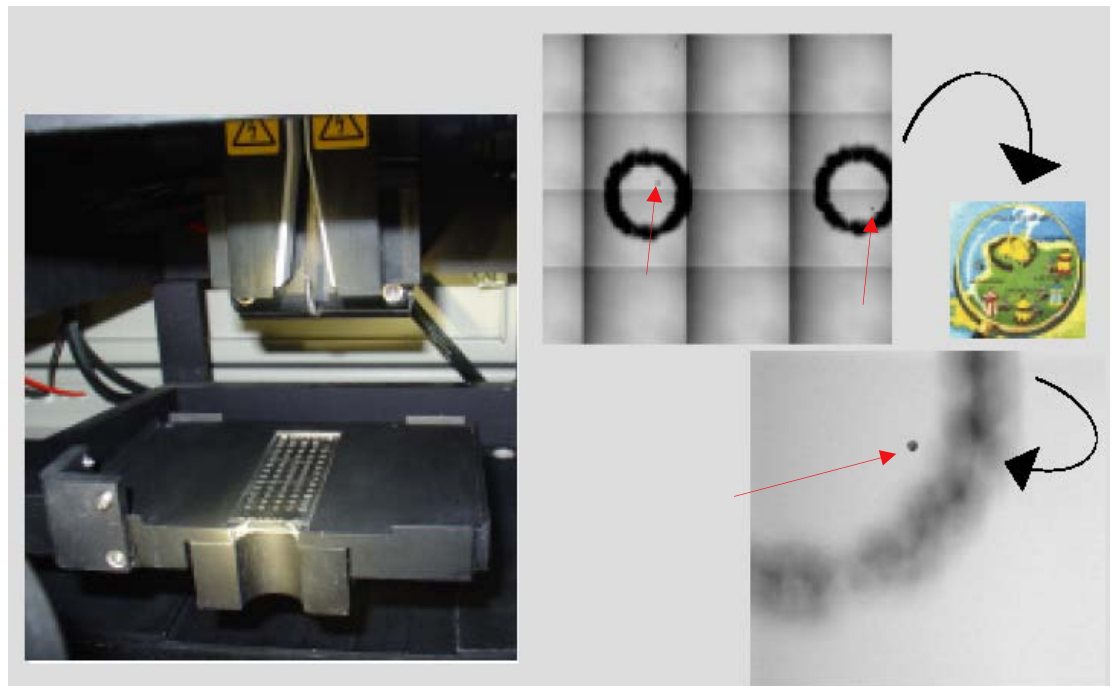
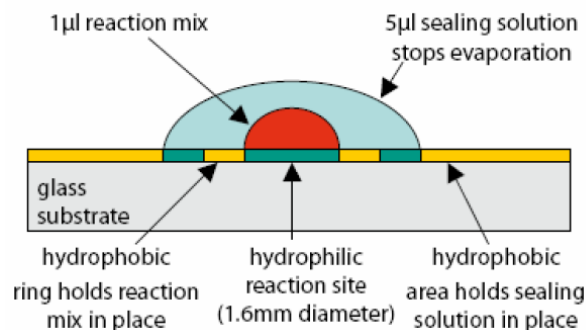
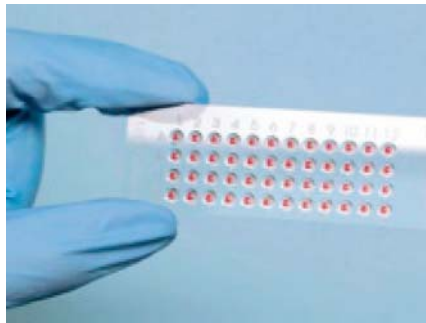
C. Proff[#], W. Mann^b, J.W. Ellwart^c, M.A. Rothschild^a, P.M. Schneider^a

^aInstitute of Legal Medicine, University of Cologne, Germany

^bAdvatix AG, Brunnthal, Germany

^cGSF-National Research Center for Environment and Health, Munich, Germany

[#] present address: Institut für Blutgruppenforschung LGC GmbH, Cologne, Germany



Small particle detection: Chromosome Sorting

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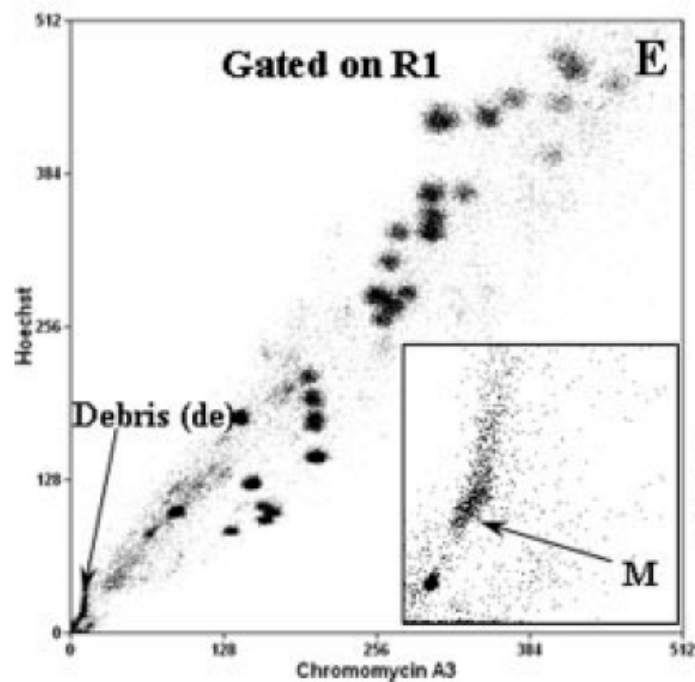
Cytometry Part A 71A:410–413 (2007)

Flow Analysis and Sorting of Microchromosomes (<3 Mb)

Bee L. Ng,* Fengtang Yang, and Nigel P. Carter

The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, United Kingdom

Received 13 November 2006; Revision Received 24 January 2007; Accepted 29 January 2007



Large particle sorting: *C. elegans*

BMC Biology 2006, 4:22

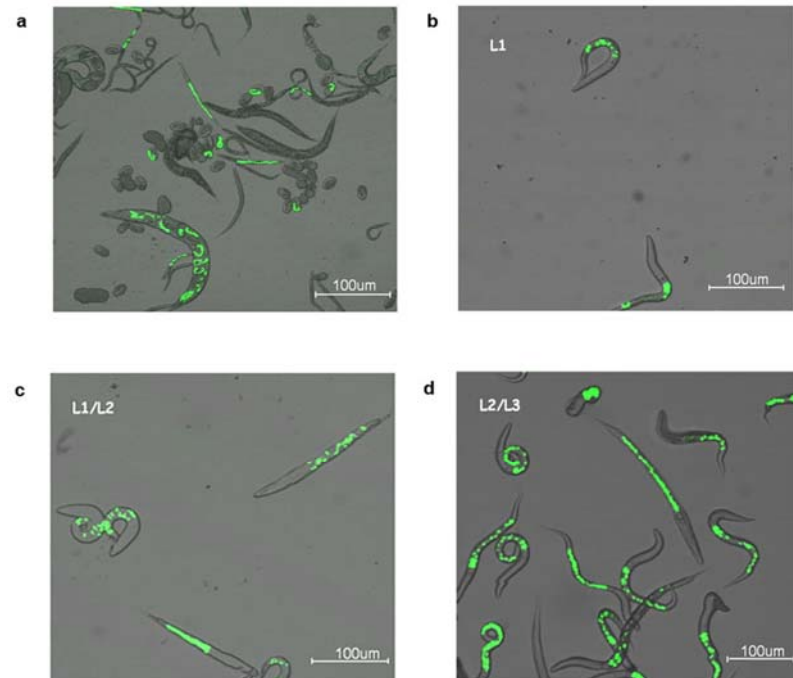
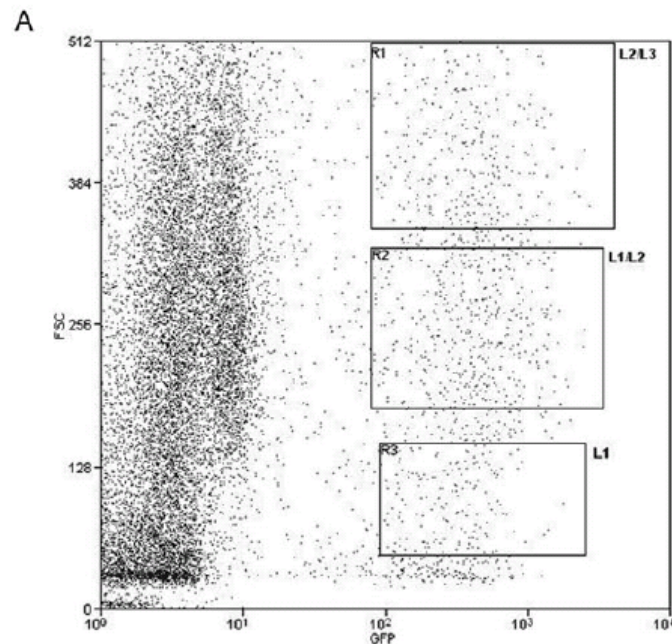
<http://www.biomedcentral.com/1741-7007/4/22>

Research article

Open Access

Expression of mammalian GPCRs in *C. elegans* generates novel behavioural responses to human ligands

Michelle S Teng¹, Martijn PJ Dekkers², Bee Ling Ng¹, Suzanne Rademakers¹, Gert Jansen², Andrew G Fraser¹ and John McCafferty^{*1}



Phosphorylation-Dependent Regulation of T-Cell Activation by PAG/Cbp, a Lipid Raft-Associated Transmembrane Adaptor

CD1d-Restricted NKT Cells Express a Chemokine Receptor Profile Indicative of Th1-Type Inflammatory Homing Cells¹

A

PB CD4⁺
NKT (○)

PB DN
NKT (○)

Dec CD4⁺
NKT (○)

Ratio (Indo-1)

Time

SLC MIP-1 β GRO- α RANTES

TARC

I-309 ITAC

SDF-1 α

GRO- α MIP-1 β SDF-1 α

SLC TARC

ITAC

SDF-1 α

GRO- α

SLC ITAC

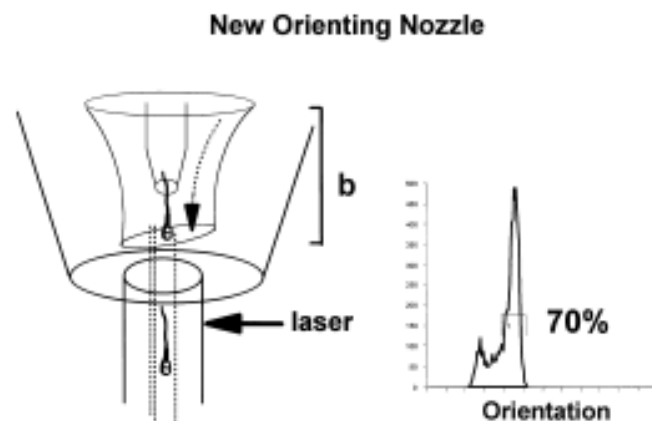
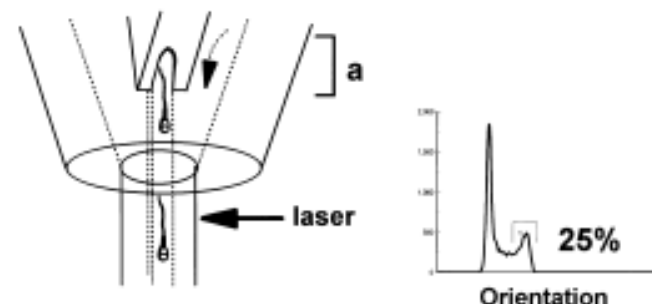
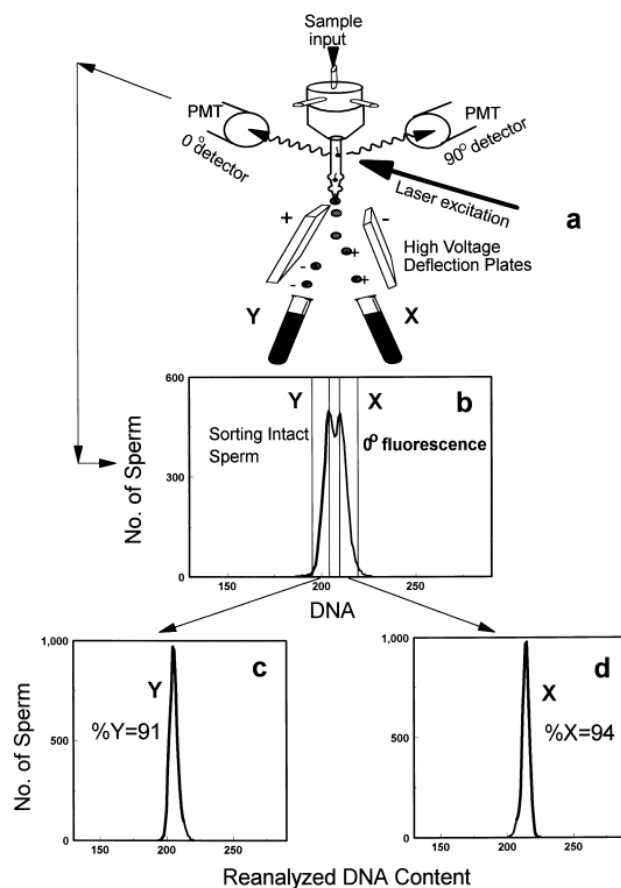
MIP-1 β TARC

Sperm sex selection

The Beltsville Sperm Sexing Technology: High-Speed Sperm Sorting Gives Improved Sperm Output for In Vitro Fertilization and AI

Lawrence A. Johnson, Glenn R. Welch and Wim Rens

J Anim Sci 1999. 77:213-220.



Beckman Coulter Inc.:

- The Pioneers of Flow Cytometry
- An Expert in Cell Analysis

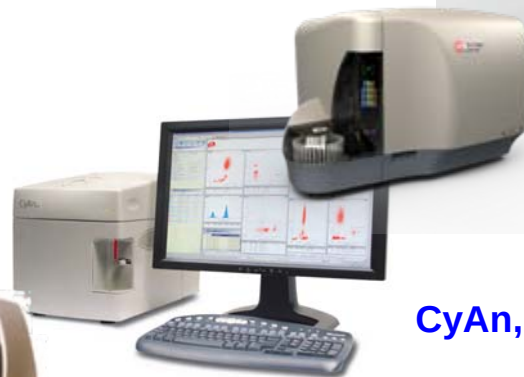
Astrios, 49 color high end cell sorter



MoFlo, 18 color high end cell sorter



Gallios, 10 color Flow Analyzer



CyAn, 7/9 color Flow Analyzer



FC500, 5 color Flow Analyzer



Quanta SC, 3 colors, Versatile Flow Analyzer



ViCell, Cell viability analyzer

MoFlo after-service of Beckman Coulter Taiwan :

1. MoFlo 全球裝機數量已逾 600 台，亞太地區超過100台。
2. Beckman Coulter 在台灣超過120 台流式細胞儀裝機及培訓經驗（100台Analyzer、18台Sorter、3 台 MoFlo XDP）。
3. 6 位專職維修工程師，3 位專職產品專員。
4. 強調 on site training，依使用者需求，協助完成實驗。
5. On-line tools and MoFlo User's Club: CoulterFlow.com



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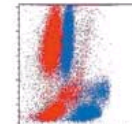
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Sunday 17 January, 2010

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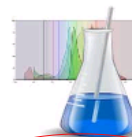
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Kaluza™

» On-line Tools



Experiment design
Spectra viewer

MoFlo™ XDP –



Sorting Performance

Drop formation: 200,000 drops/sec

Sorting Speed: >70,000 cells/sec

Purity: >99%

Yield: >90%

Maintain high cell viability

4Way™ sorting

CyClone deposition system

Analysis Performance

Analysis speed: >100,000 cells/sec

32 bit (2^{32}) digital resolution

>5-Log dynamic range

1,000,000,000 event/LMD

Excitation & Emission

3 Excitation Line (standard)

FSC + SSC + up to 18 FLs

FL sensitivity: FITC<150 MESF, PE<100 MESF

18 x 18 (full matrix) auto- or manual compensation

Ease of Operation

aXcess Control Panel (touch screen)

Sorting setup time< 15 min

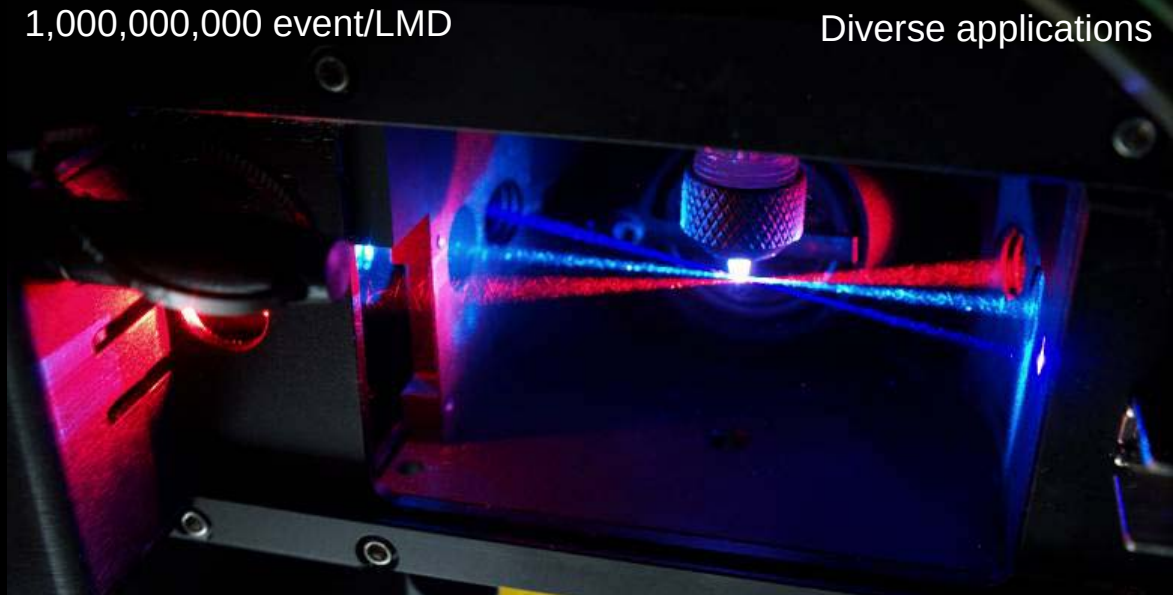
Summit Software v5.2

Flexibility

Air or water-cool laser

50~400 um CytoNozzle

Diverse applications



Questions?

