

FACSs & MACS in IMEG

Analysis:

FACS Canto → 2 lasers (488, 633 nm)

Cell separation:

autoMACS

→ magnetic cell separation (time-, economically-efficient)

FACS Vantage Diva

→ 3 lasers (UV, 488, 633 nm)

FACS AriaIIu

→ 3 lasers (violet, 488, 633 nm)

FACS SORP

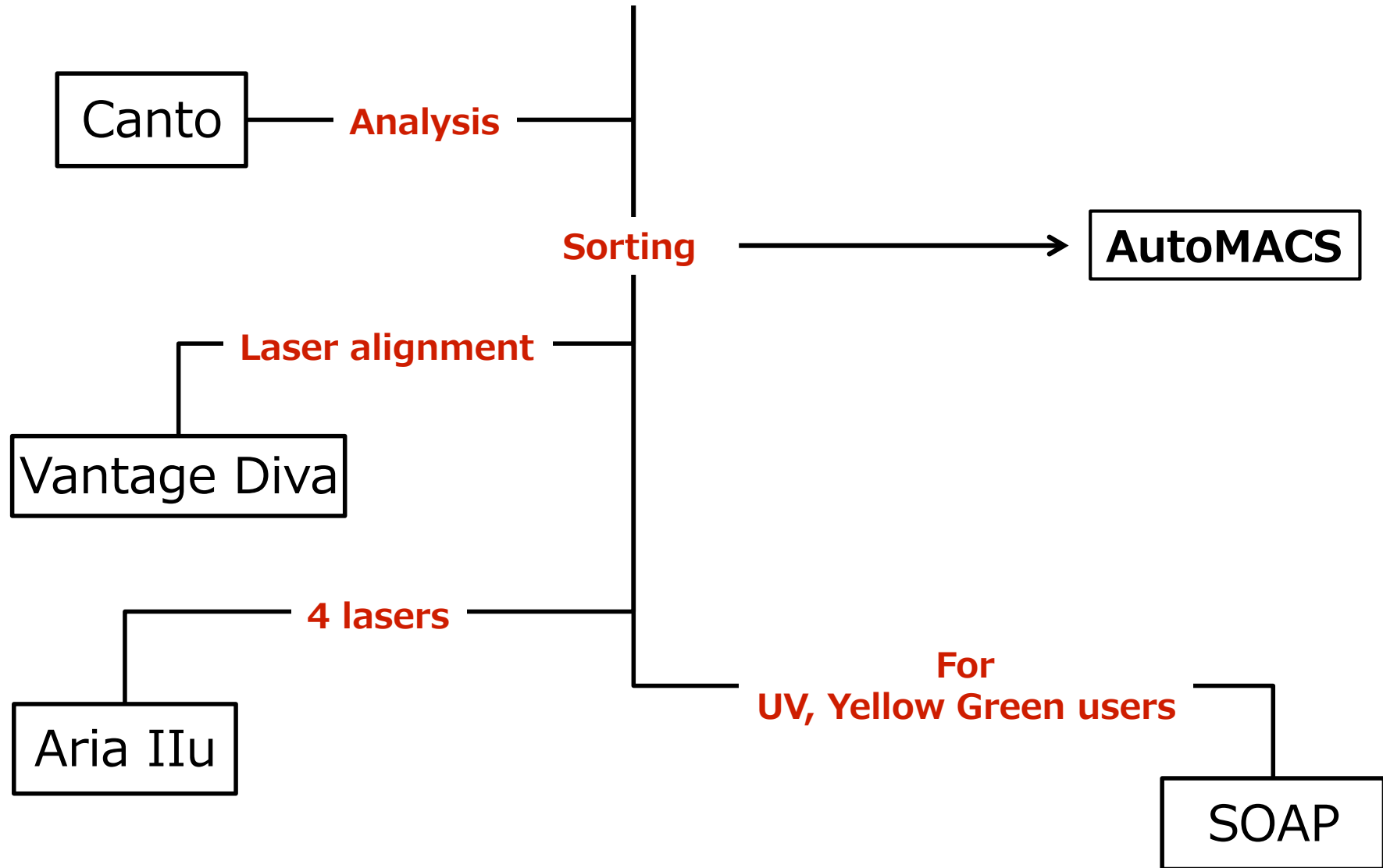
→ 5 lasers (UV, violet, 488, Yellow Green, 633 nm)

List of Fluorescent Dyes

UV	DAPI, Hoechst33342 etc.
violet	AmCyan, Pacific Blue, eFluor450, Pacific Orange etc.
488nm	FITC, PE, PI, PerCP-Cy5.5, PE-Cy7 etc.
Yellow Green	Red Fluorescent Protein (Kusabira Orange etc.), *PE etc.
633nm	APC, APC-Cy7, Alexa 700 etc.

FACSs or MACS ??

Analysis or Sorting?



FACS Canto

Analyser

- **488** nm laser (= Ar laser):

FITC (=EGFP), PE, PI, PerCP-Cy5.5, PE-Cy7 etc.

- **633** nm laser (=HeNe laser):

APC, APC-Cy7, Alexa 700 etc.

FACS Aria IIu

Sorter:

- Violet (= 405nm)

AmCyan, Pacific Blue, Pacific Orange, eFluor450 etc.

- **488** nm laser (= Ar laser):

FITC (=EGFP), PE, PI, PerCP-Cy5.5, PE-Cy7 etc.

- **633** nm laser (=HeNe laser):

APC, APC-Cy7, Alexa 700 etc.

FACS SORP

Sorter:

- **UV** laser:
Hoechst33342, DAPI etc.
- **violet** laser (= 405nm):
AmCyan, Pacific Blue, eFluor450 etc.
- **488** nm laser (= Ar laser):
FITC (=EGFP), PE, PerCP-Cy5.5, PE-Cy7 etc.
- **Yellow Green** (=561nm) laser:
Red Protein (Kusabira Orange etc.), *PE etc.
- **633** nm laser (=HeNe laser):
APC, APC-Cy7, Alexa 700 etc.

Choice of Fluorescent Dye & Antibody

Antibody

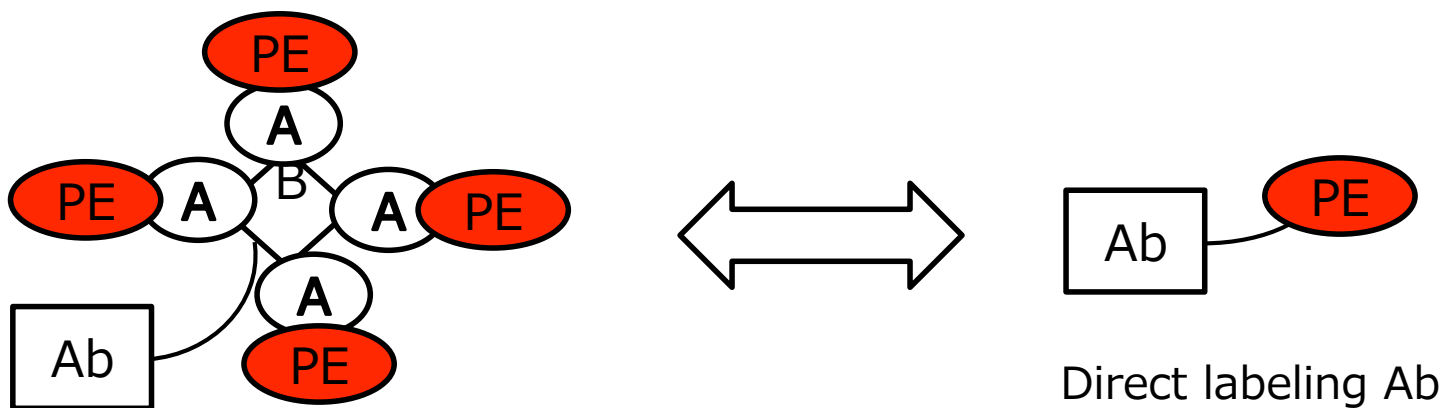
Hybridoma clone:

e.g. CD34 - RAM34 vs MEC14.7

Titration:

Postive signal

Avidin-Biotin Complex (ABC):



Choice of Fluorescent Dye & Antibody

Fluorescent Dye

Brightness:

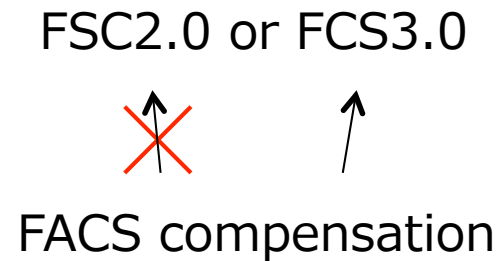
PE (488 nm) = APC (633 nm) > FITC (488 nm) = Pacific Blue (violet)

Tandem dyes:

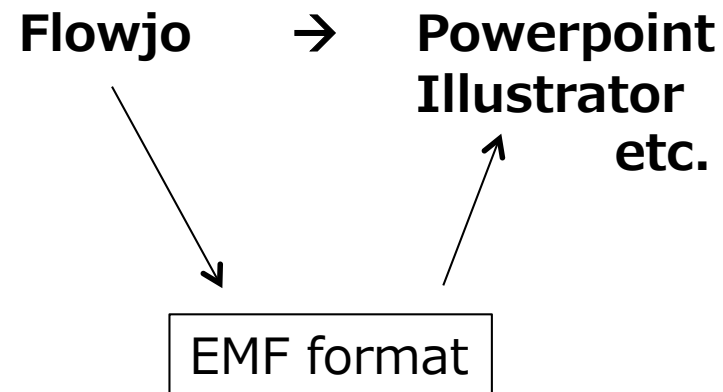
PE-Cy7, APC-Cy7

Software for FACS data

(A)



(B)



JPEG (不可逆压缩 : lossy compression)

EMF (可逆压缩 : lossless compression)

Informative HPs

FACS Dye

- BD Bioscience: BD Fluorescence Spectrum Viewer
(http://www.bdbiosciences.com/research/multicolor/spectrum_viewer/index.jsp)
- eBioscience: A Multi-laser Spectra Viewer
(<http://www.ebioscience.com/resources/fluorplan-spectra-viewer.htm>)
- BioLegend: BioLegend Fluorescence Spectra Analyzer
(<http://www.biolegend.com/spectraanalyzer>)
- Life technologies: Fluorescence SpectraViewer
(<http://www.invitrogen.com/site/us/en/home/support/Research-Tools/Fluorescence-SpectraViewer.html>)

Flow Cytometry

- Beckman Coluter (<http://www.bc-cytometry.com/>)

Flowjo

- The Daily Dongle
(http://flowjo.typepad.com/the_daily_dongle/)
- 日刊ドングル - The Daily Dongle Japan
(http://thedailydonglejapan.blogspot.jp/2012/06/blog-post_21.html) JP

Text book

PRACTICAL FLOW CYTOMETRY

(http://www.bccytometry.com/FCM/practical_flow_cytometry.html)