Visualization Tool
for
Flow Cytometry Data Standards Project

Evgeny Maksakov
maksakov@cs.ubc.ca

CS533C

Department of Computer Science, UBC
in collaboration with
Terry Fox Laboratory, BC Cancer Agency
(Prof. Ryan Brinkman & Dr. Josef Spidlen)

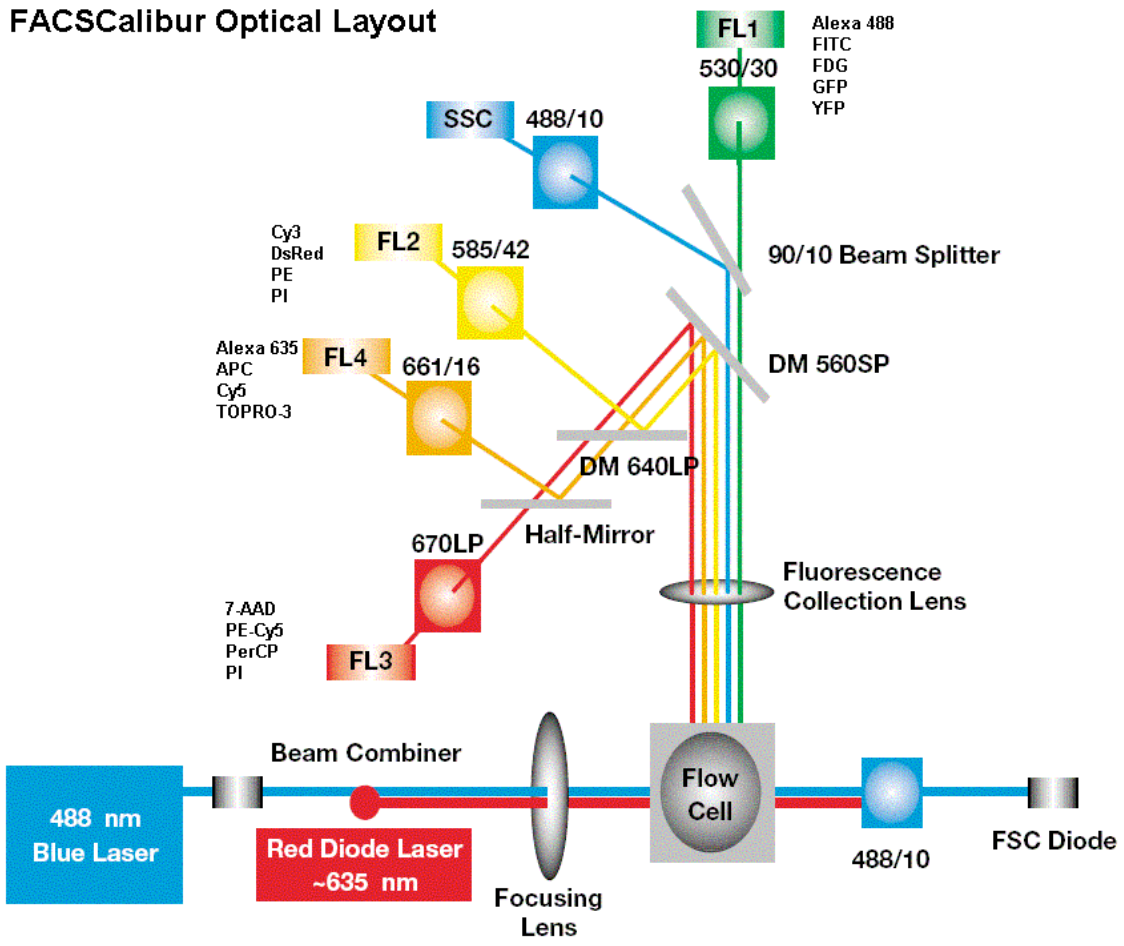
Today

- Flow Cytometry (reminder)
  - Dataset description
- Goals
- Previous work
- FlowCytoVis prototype in details
- Data analysis comparison
  - FlowJo vs FlowCytoVis prototype
- Demo!
- Conclusions and future work

Flow Cytometry (FCM)

Cell

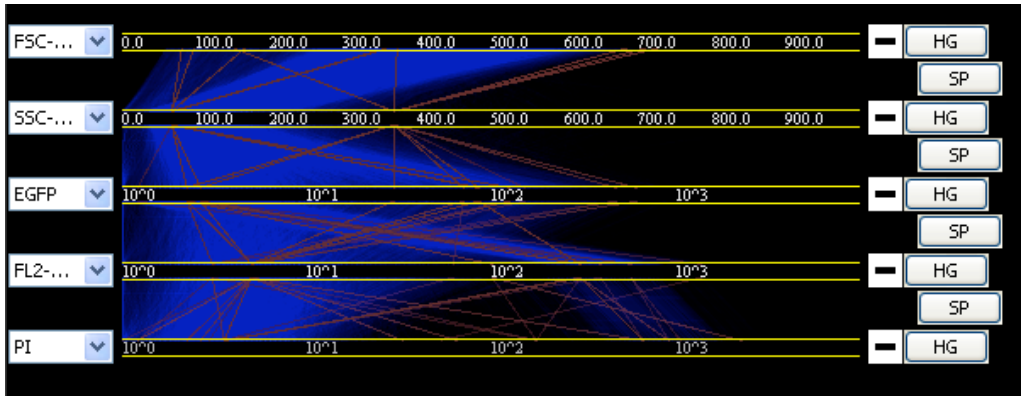
Measure



Dataset Properties

Typically for research at the TFL:

- 100,000+ events
- 5-10 dimensions



Capability:

- 1,000,000 events (cells going through the laser beam) per dataset
- Up to 20 dimensions

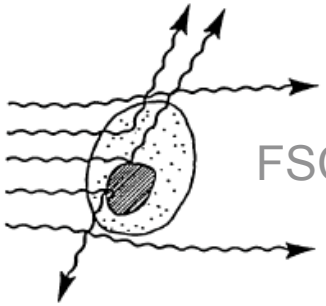
Today demo datasets:

- **20,000 events**
- **5 dimensions**

Dimensions

SSC (Granularity)

Laser



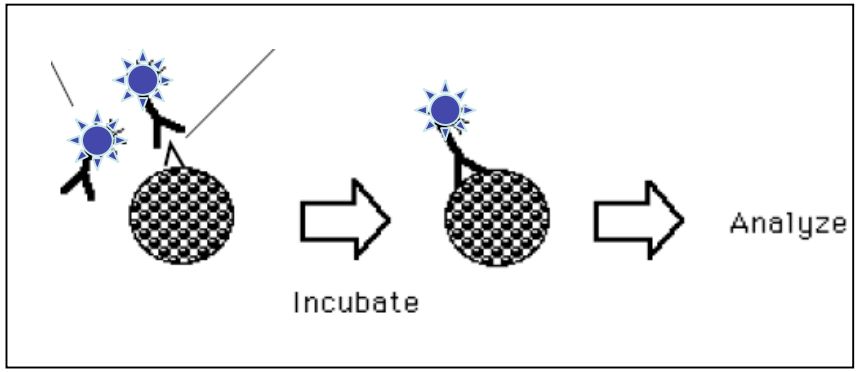
FSC (Size)

PI dye intensity **(measures viability)**

Green Fluorescent Protein intensity
(measures gene expression)

Fluorochrome	Fluoresence Emission Color
Alexa Fluor® 405	Blue
Pacific Blue®	Blue
AmCyan	Green
Alexa Fluor® 488	Green
FITC	Green
PE	Yellow
PE-Texas Red®	Orange
Texas Red®**	Orange
APC*	Red
Alexa Fluor® 647	Red
PE-Cy5*	Red
PerCP	Red
PerCP-Cy5.5	Far Red
Alexa Fluor® 700***	Far Red
PE-Cy7	InfraRed†
APC-Cy7	InfraRed†

16 fluorescence intensities of fluorochromes (used as markers)



Pictures are taken from http://www.upenn.edu/pennnews/photos/, http://www.bdbiosciences.com/image_library/ and flow cytometry manual

Aimed Goals

User requirements (based on user studies):

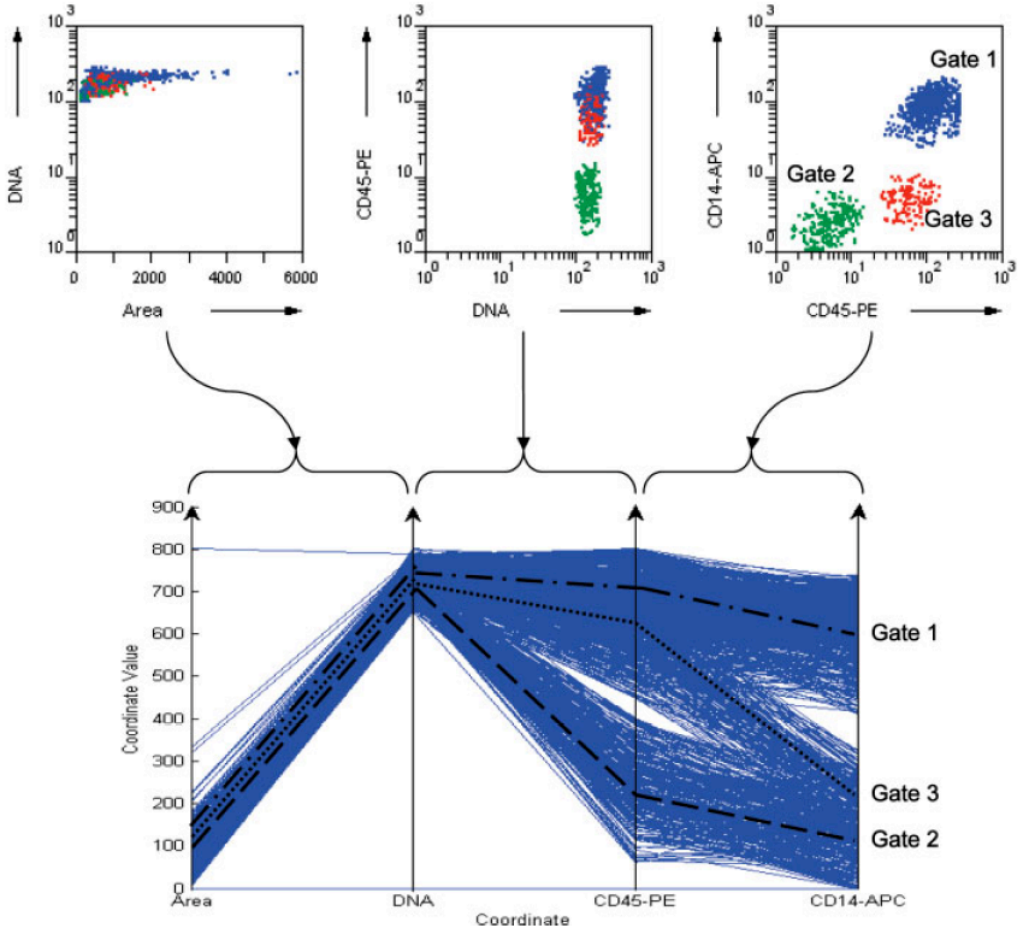
1. See all dimensions at once
2. Improve analysis sequence
3. Leave scatterplots and histograms
4. Gating/Filtering feature
5. Provide better usability than commercial FlowJo

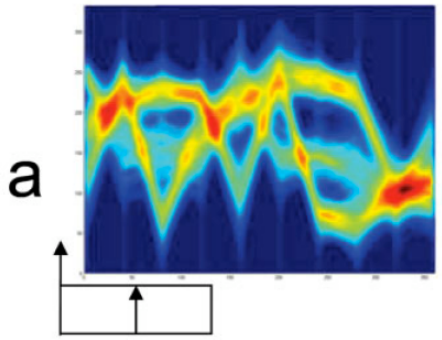
By means of:

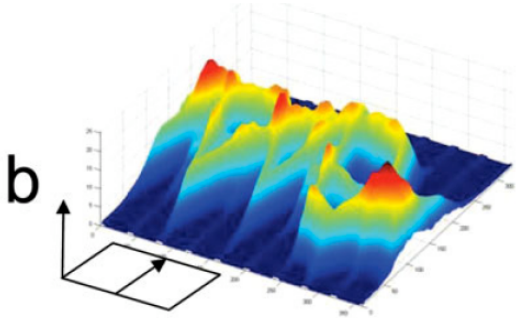
1. Using Parallel Coordinates with Gating/Filtering
2. Implementing data clustering throughout dimensions
3. Include scatterplots and histograms in the interface
4. Make effective, convenient and interactive interface

3D Parallel Coordinate System for FCM

Marc Streit at al. (2006)



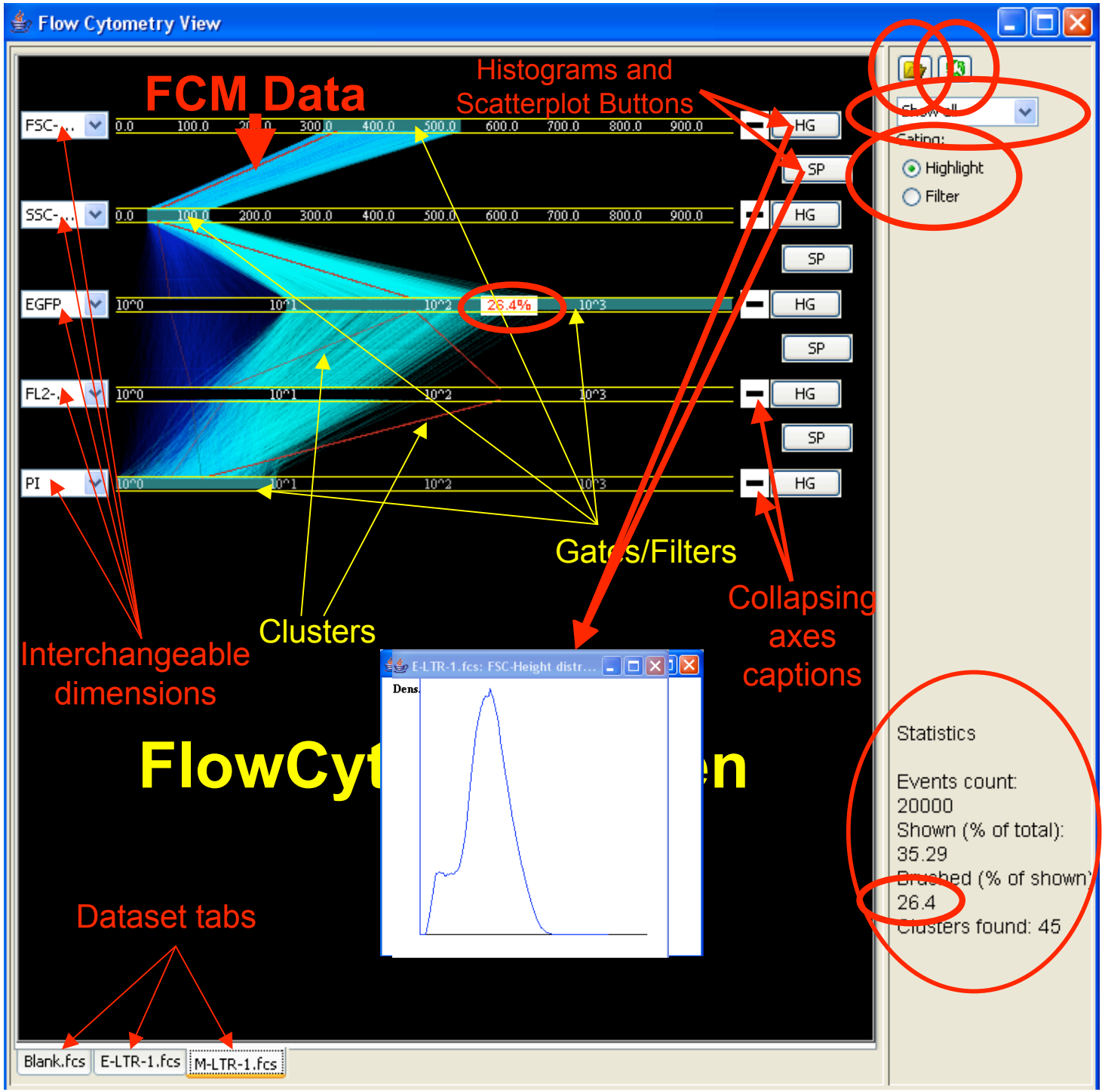




Picture from Marc Streit at al. (2006)

3D Parallel Coordinate Problems

- Does not provide any new information about dataset
- Introduces visual occlusions
- Necessity to rotate to see all data


Flow Cytometry View
FCM Data
Histograms and Scatterplot Buttons
FSC-...
SSC-...
EGFP
FL2-...
PI
HG
SP
26.4%
Gates/Filters
Collapsing axes captions
Interchangeable dimensions
Clusters
E-LTR-1.fcs: FSC-Height distr...
Dens.
Highlight
Filter
Statistics
Events count: 20000
Shown (% of total): 35.29
Brushed (% of shown): 26.4
Clusters found: 45
Dataset tabs
Blank.fcs
E-LTR-1.fcs
M-LTR-1.fcs

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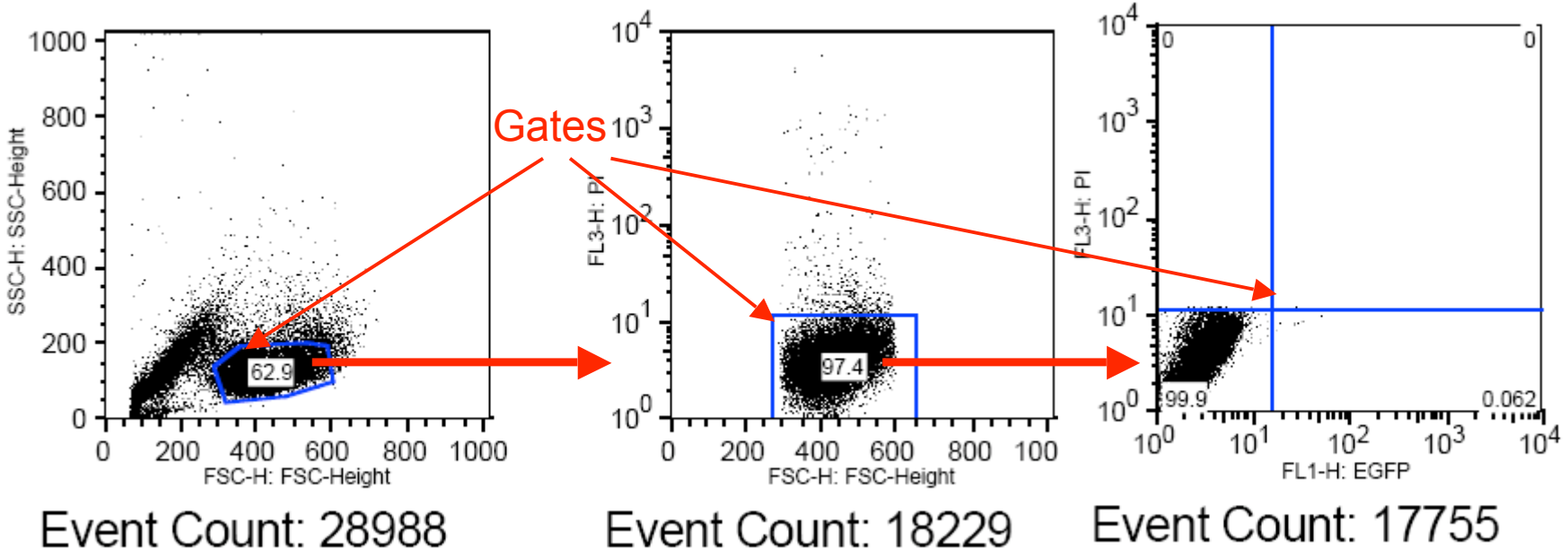
Data Analysis Process (FlowJo)

Negative control

(each scatterplot is a new window)



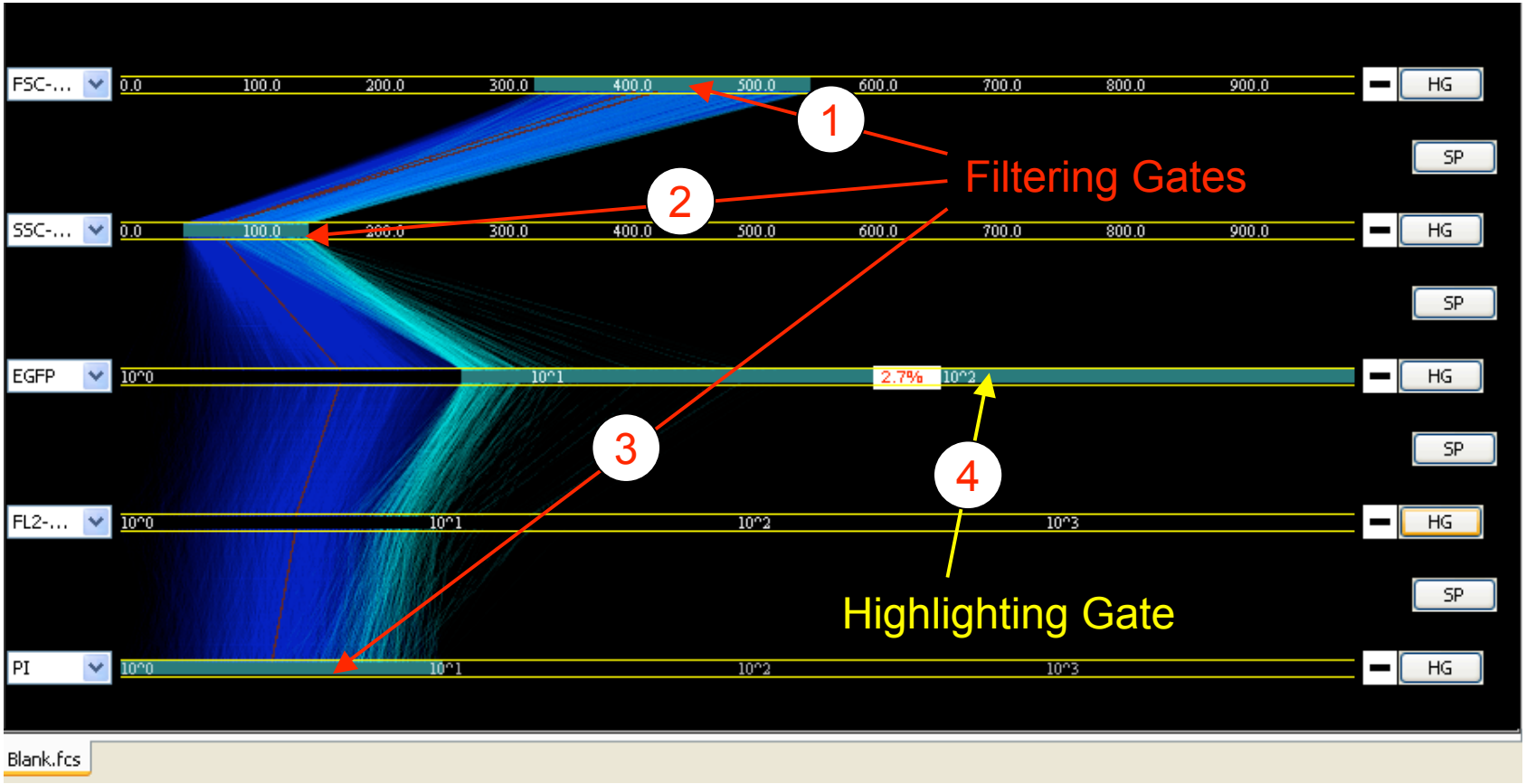
Event Count is a total number of cells passed through the laser beam

Important note: sequence of actions is the same all the time for negative control!

Data Analysis Process (FlowCytoVis)

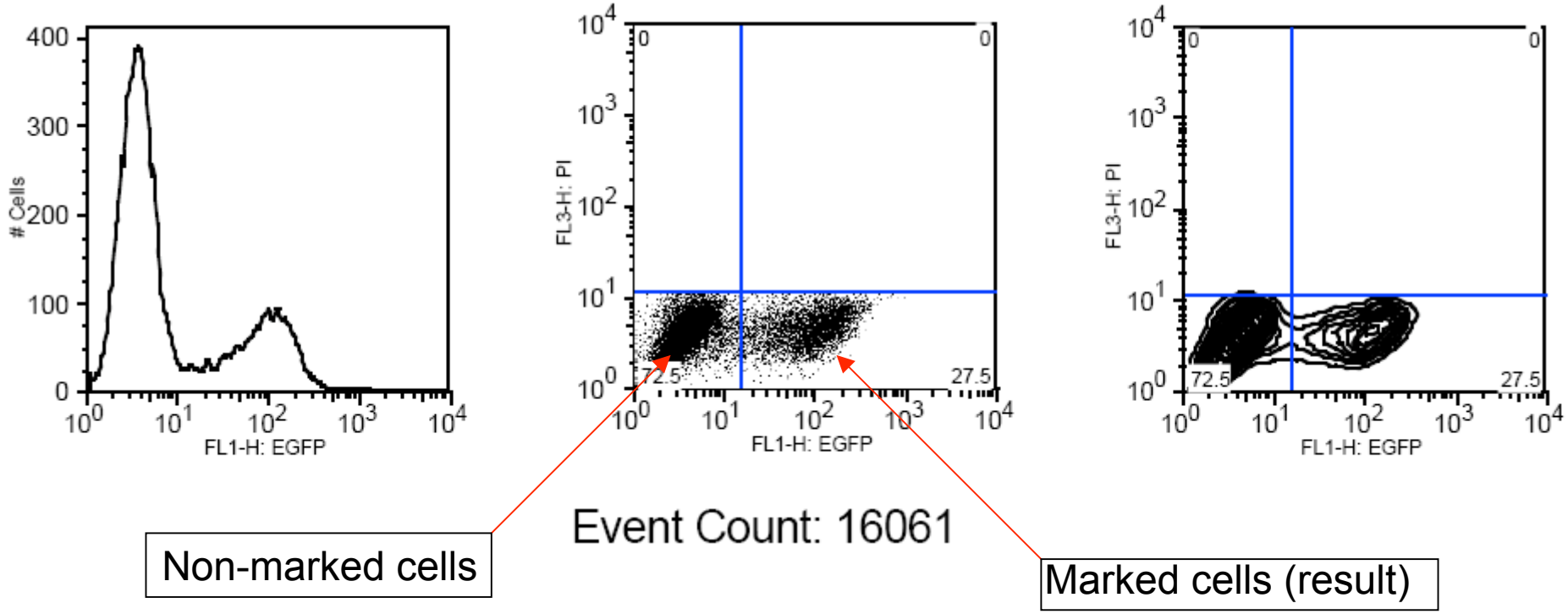
Negative control

(everything happens in one window)



Data Analysis Process (FlowJo)

Looking for result



Event Count: 16061

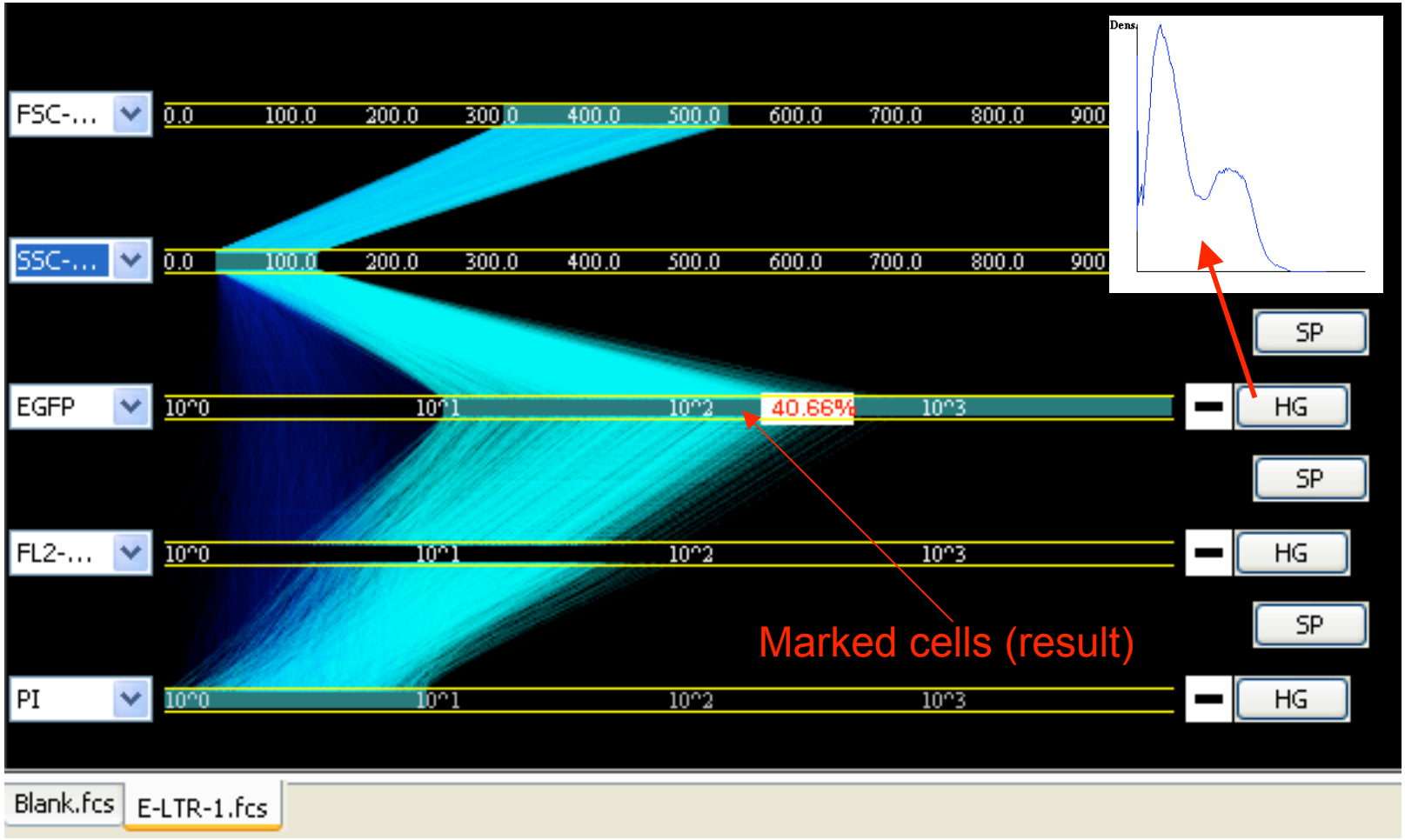
Non-marked cells

Marked cells (result)

Important note: Same gates as in neg. control apply automatically on the positive set!

Data Analysis Process (FlowCytoVis)

Looking for result



Important note: Gates apply automatically on the positive set here too!

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Demo

Implementation details:

- Java2D + Swing
- CFCS library for reading .fcs (FCM datasets) format

Strengths and Weaknesses of the FlowCytoVis

+ Can provide insights into the data
+ Convenient (less clicks to get the same result)
+ Interactive
+ Allows intuitive multidimensional filtering
+ Visually appealing

- Slow picture rendering relatively to Scatterplots
- At the moment does not provide full functionality that FlowJo provides.

Conclusions

- The FlowCytoVis proved to be a relevant solution for the Flow Cytometry data visualization and was accepted with enthusiasm
- Parallel Coordinates (PC) view is a nice addition to canonical Scatter Plots for Flow Cytometry
- Clustering works very well together with PC and can save some rendering time
- Clustering needs refinement and improvement
- Improving speed is vital for PC

Future Work

- Implement all the functionality still missing
- Integrate existing clustering made for the Flow Cytometry Data Standards Project into the FlowCytoVis
- Improve rendering speed for parallel coordinates

Acknowledgements

- Dr. Tamara Munzner
- Dr. Ryan Brinkman
- Dr. Josef Spidlen
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- Irina Maksakova
- Other TFL Members

Questions…