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Gate that cell!

Requirement for analyzing Flow Cytometry Data
Reflection on H62 document

Teona Roschupkina

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Outline

- Overview of how data generated
- Poisson distribution for flow
- Variability –accounting for planning
- Gating strategies and their usefulness

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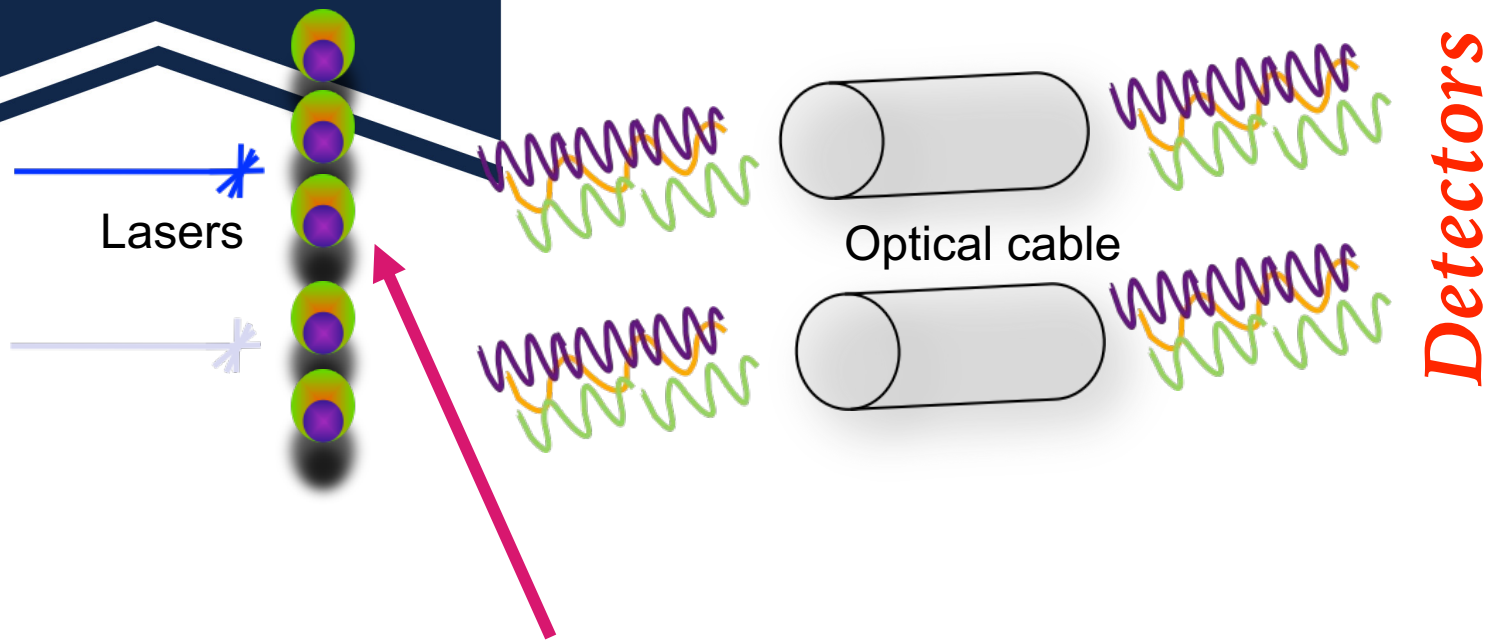
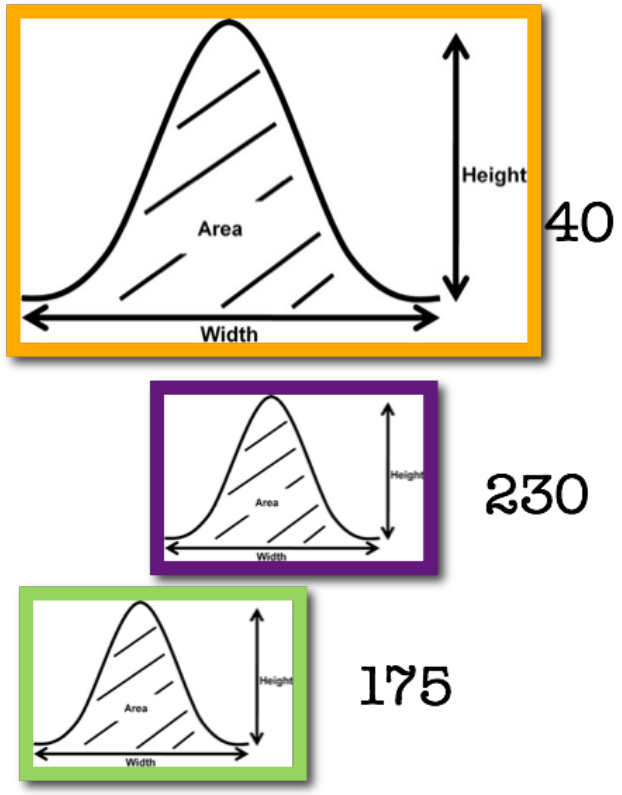
Brief overview of Flow Process

How flow works..





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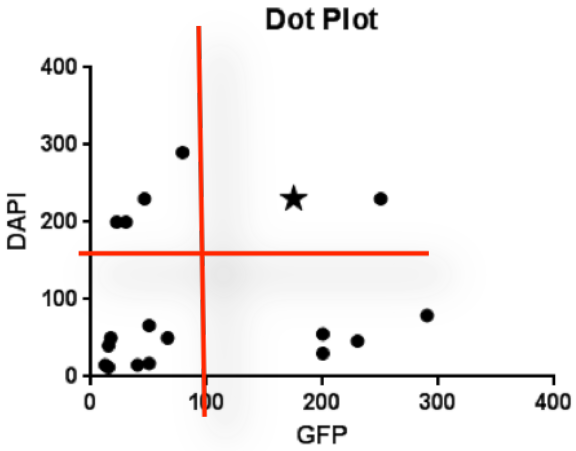
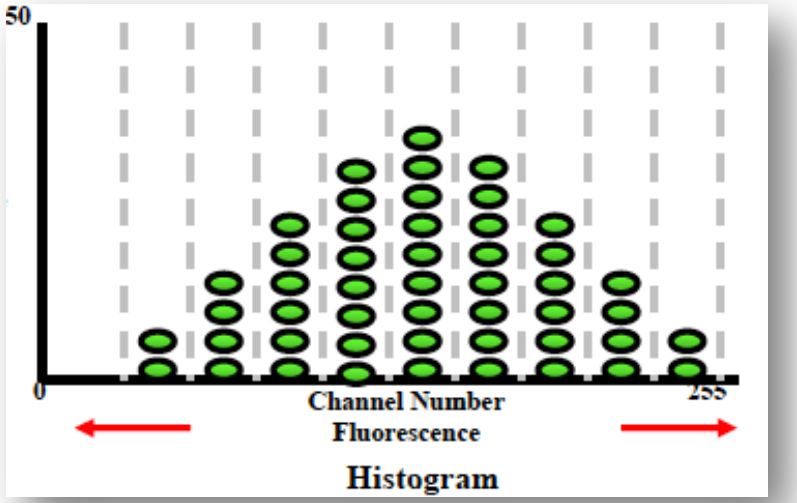
Brief overview of Flow Process

	Violet DAPI	Green GFP	Orange Propidium Iodide
Cell 1	200	30	30
Cell 2	**230**	**175**	**40**
Cell 3	250	50	50
Cell 4	180	190	60
Cell 5	200	46	70





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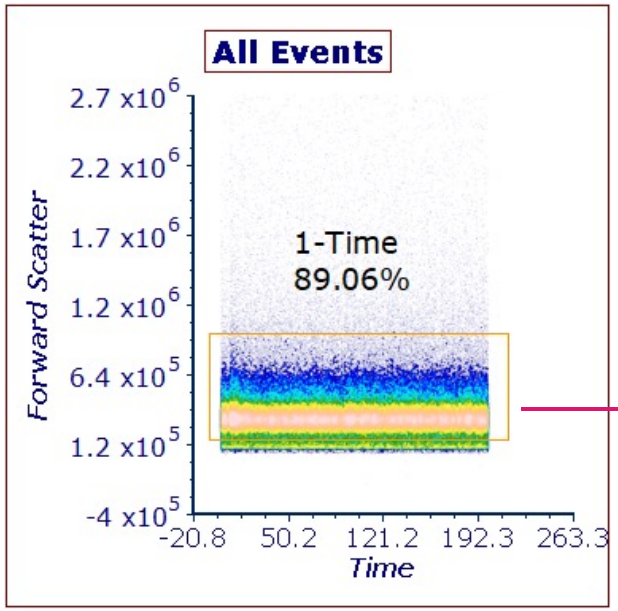
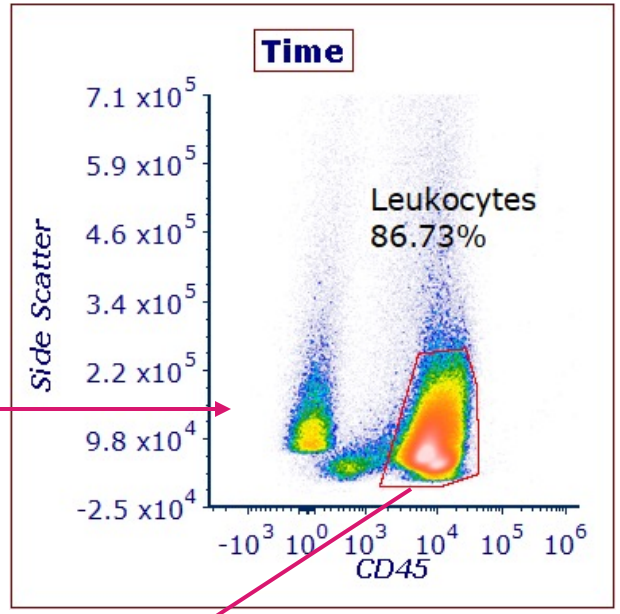
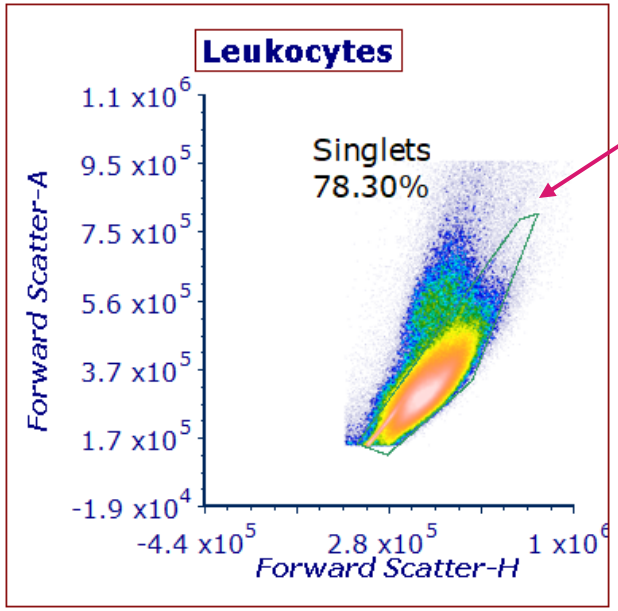
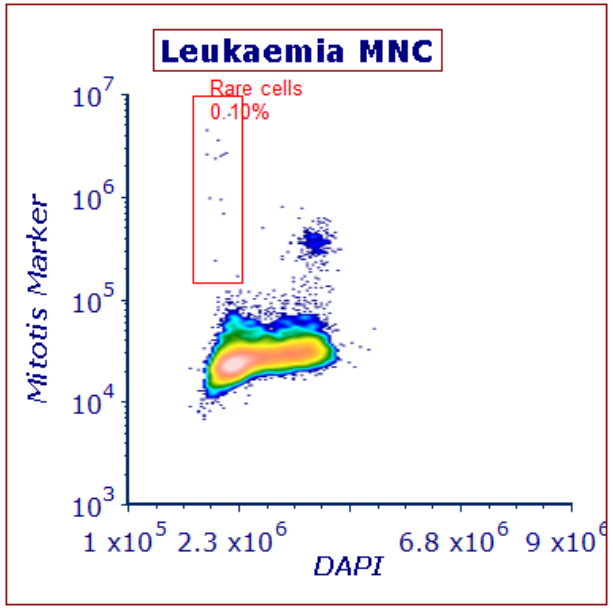
Gating

Gating is used to derive data, but it helps us clean up data.









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Variability enemy of statistics

H62 and FDA references to rare cells

“To define a cell population [to gate it] and allow reproducible enumeration it has been recommended that 20-50 clustered events be present…” H62

Desired CV	1%	2.5%	5%	10%	20%
Required Events for the Population of Interest	10 000	1600	400	100	25
Population of Interest Frequency, %	Total Number of Acquired Events				
10	100 000	16 000	4000	1000	250
1	1 000 000	160 000	40 000	10 000	2500
0.1	10 000 000	1 600 000	400 000	100 000	25 000
0.01	100 000 000	16 000 000	4 000 000	1 000 000	250 000
0.001	1 000 000 000	160 000 000	40 000 000	10 000 000	2 500 000

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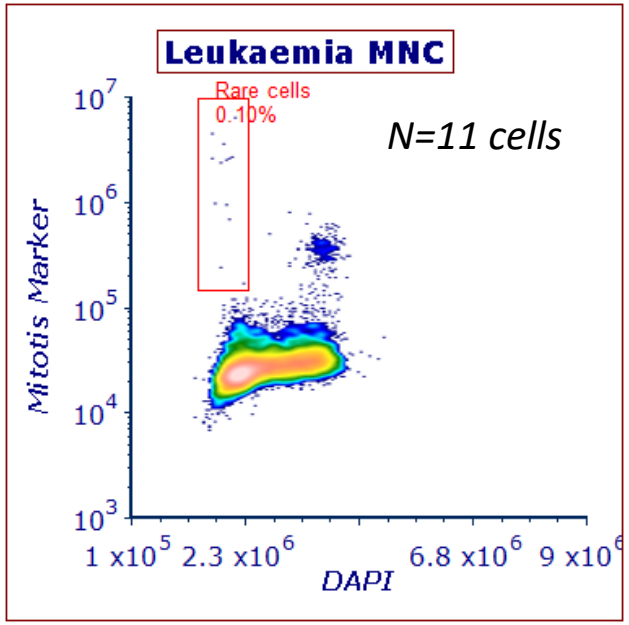
Poisson counting statistics

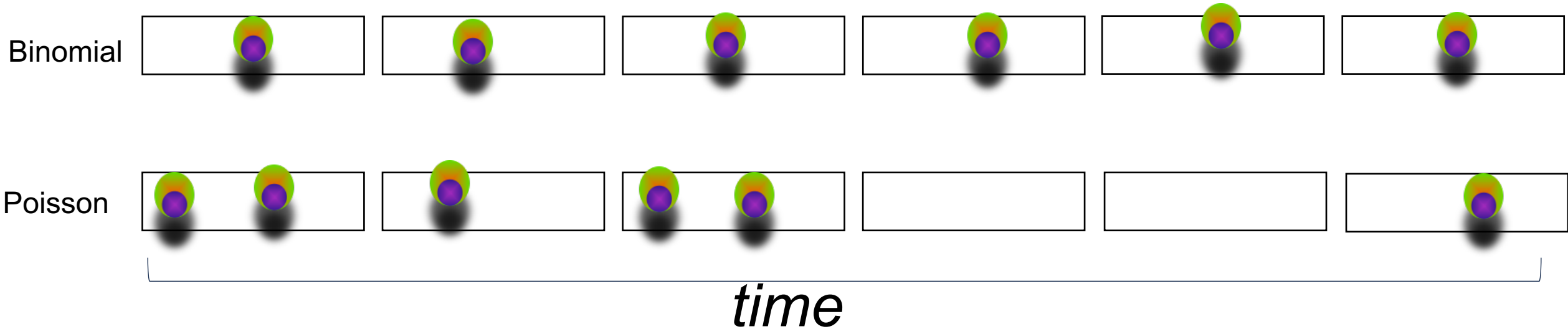
Poisson distribution How many cells we need?!

$$CV, \% = \frac{\sqrt{N}}{N}$$

$$CV = \frac{\sqrt{11}}{11} = 30.2\%$$

$CV_{experiment} = 48.4\%$





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Variability enemy of statistics

Sources of Variability for cells

- Population separation
- Blood Lysis vs. PBMC
  - Debris and Viability
- Analysts
- Instruments
- Temperature
- Centrifugation, xg force
- Type of assay
  - Intracellular
  - PhosphoFlow
- Logistics
- Sample type
- Analysis of data

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Phosphorylated Biomarker MD

- Very low frequency
- Expressed in cell lines
- Mitosis biomarker
- Predictive in number of tumours as progression marker
- Matrix is rare
- Not every patient expresses biomarker of interest
- This was whole blood assay

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H62 Specificity and Gating

"…Once gating strategy is determined the results should be verified against reference or comparative method….."

"…final document should provide how gating was designed to ensure specificity"

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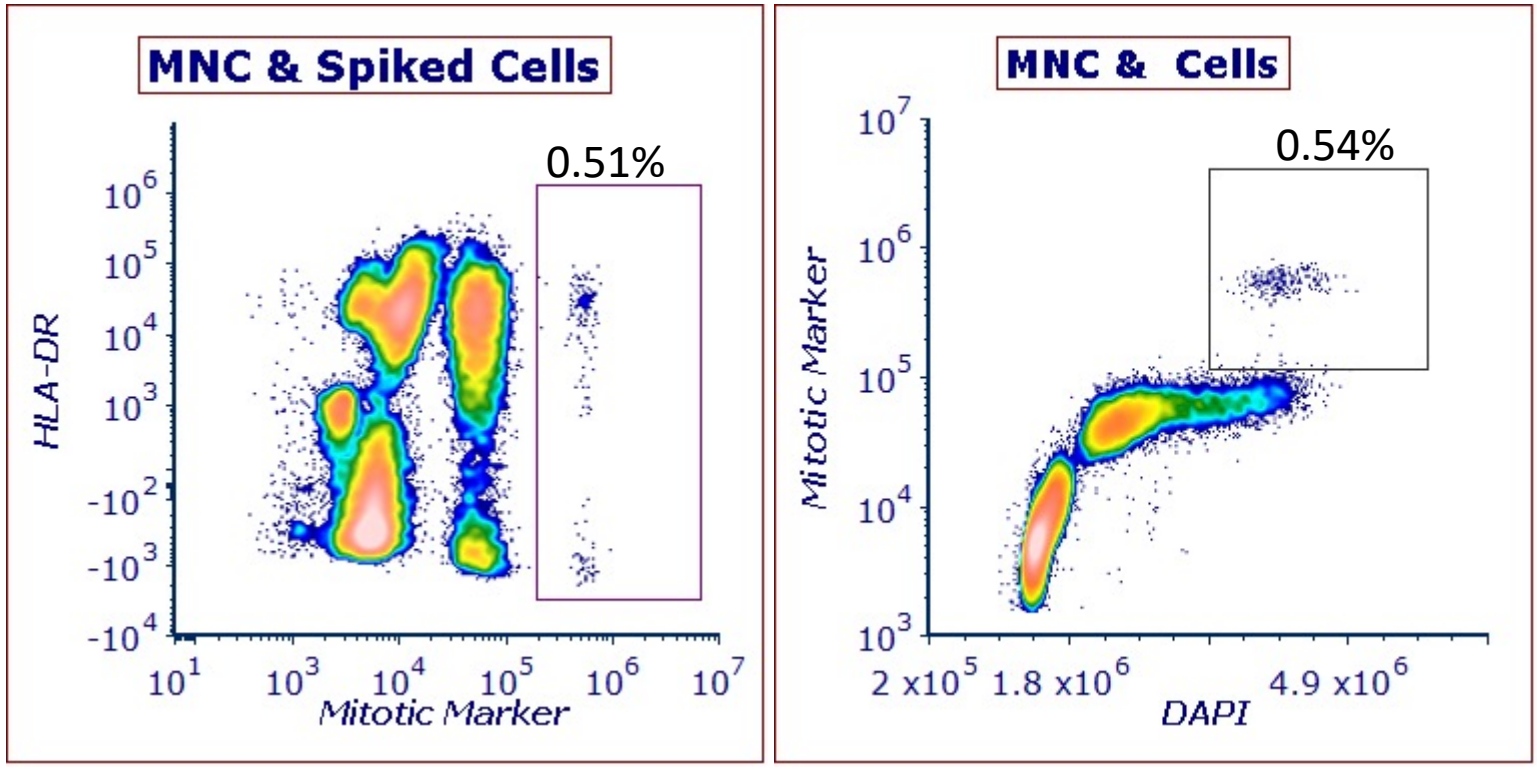
Spiked healthy individuals



0.177% of total
CV=4.04

0.18% of total
CV=2.71

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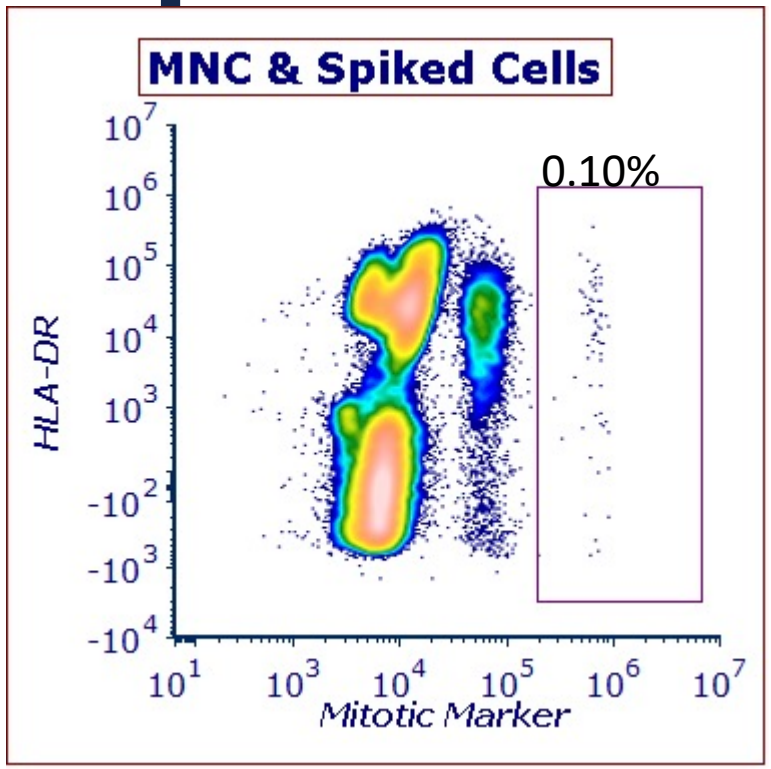
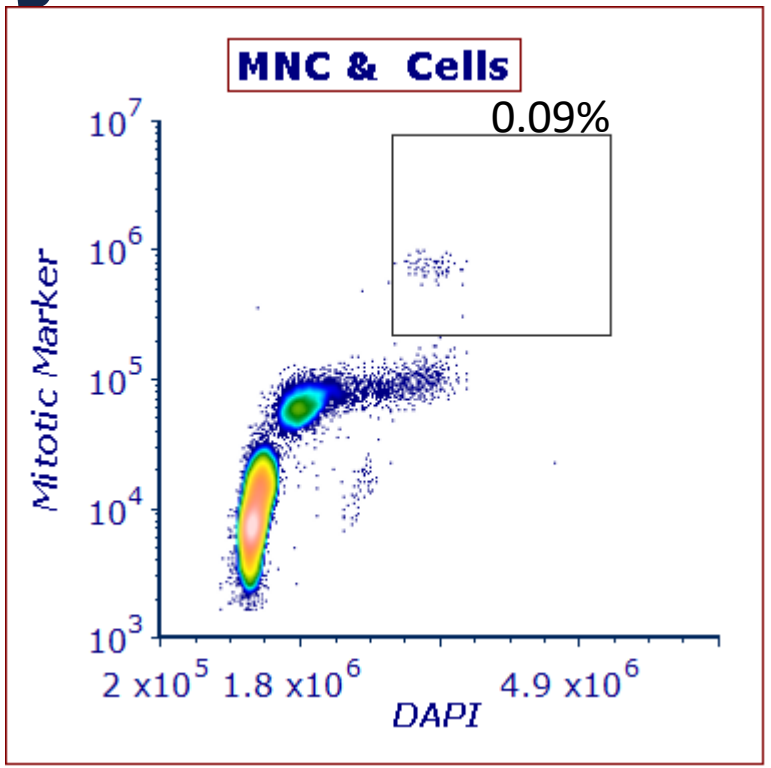
Spiked healthy individuals



0.03% of total
CV=15.71% of
Blast gate



0.03% of Blast gate
CV=8.32%

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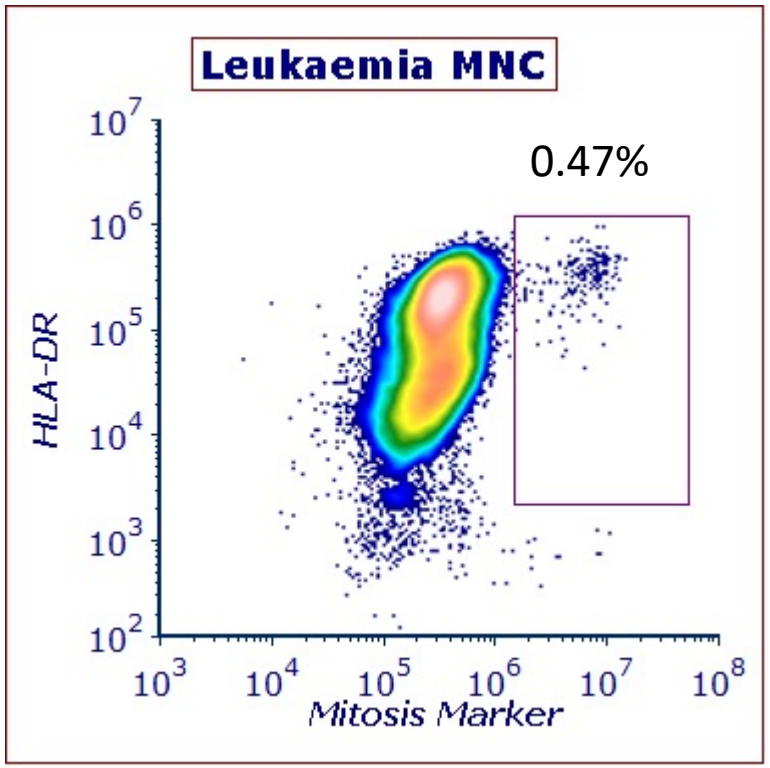
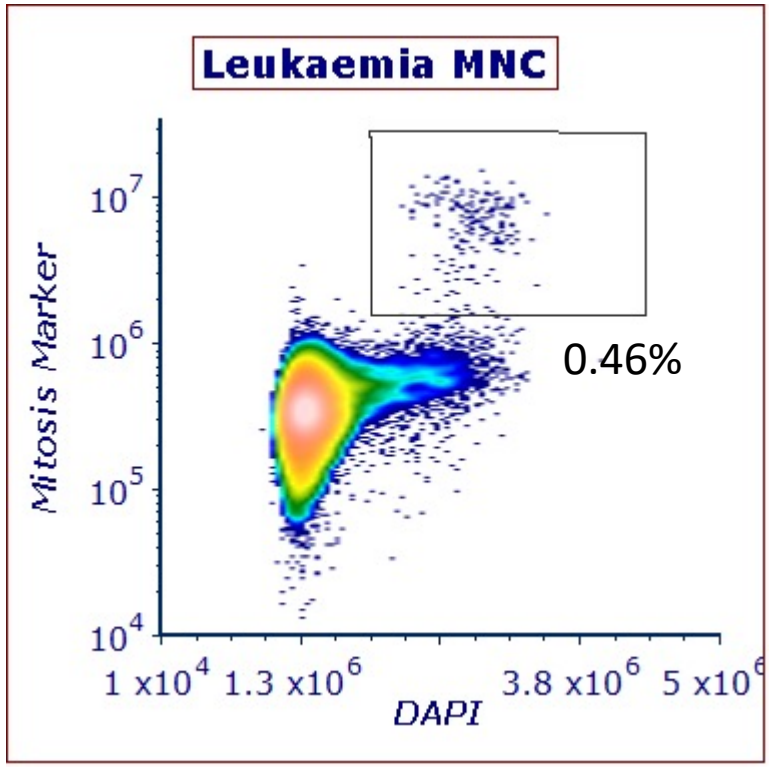
Disease Matrix





High Concordance!!!

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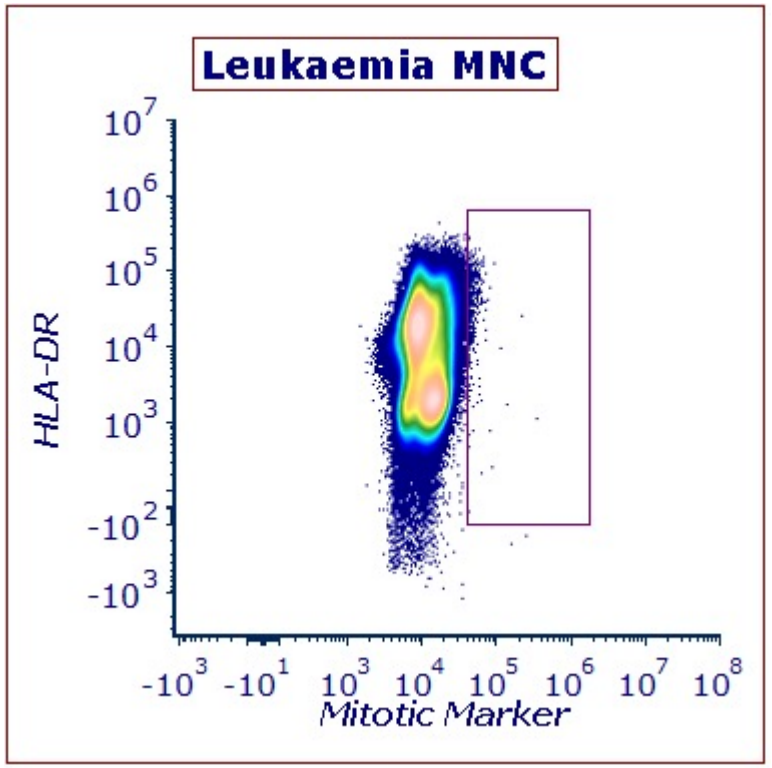
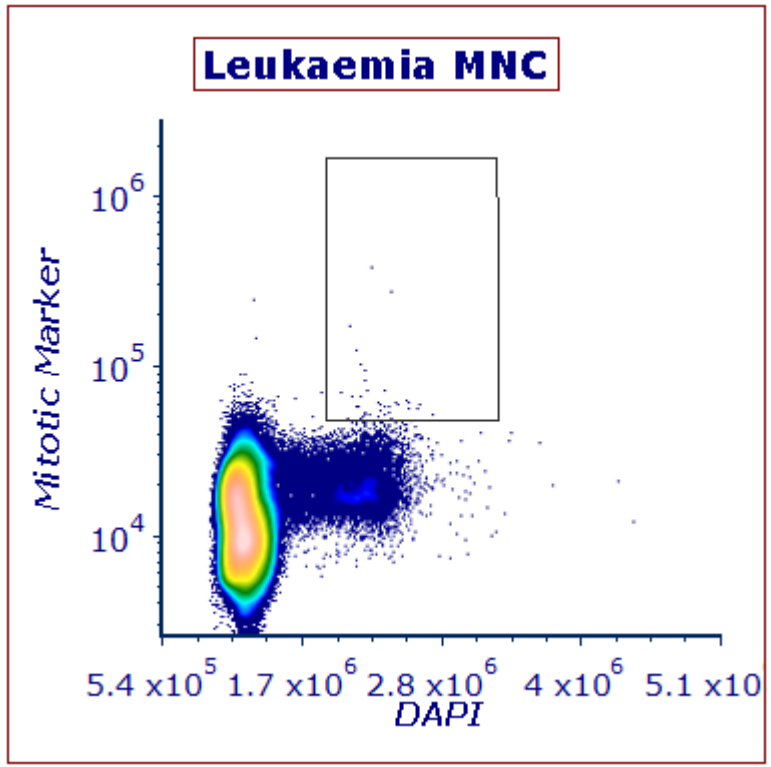
Disease Matrix



0.1% of blasts
CV=0%



0.01% of blasts
CV=0%

Conclusion

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- Plan should contain descriptive element of gating: Clustered events, Pattern
- Gating strategy comes first!
- Effective gating strategy for biomarker and disease
- Inclusion of further means to resolve population may improve specificity and sensitivity

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Thank you for your attention

Any further questions?

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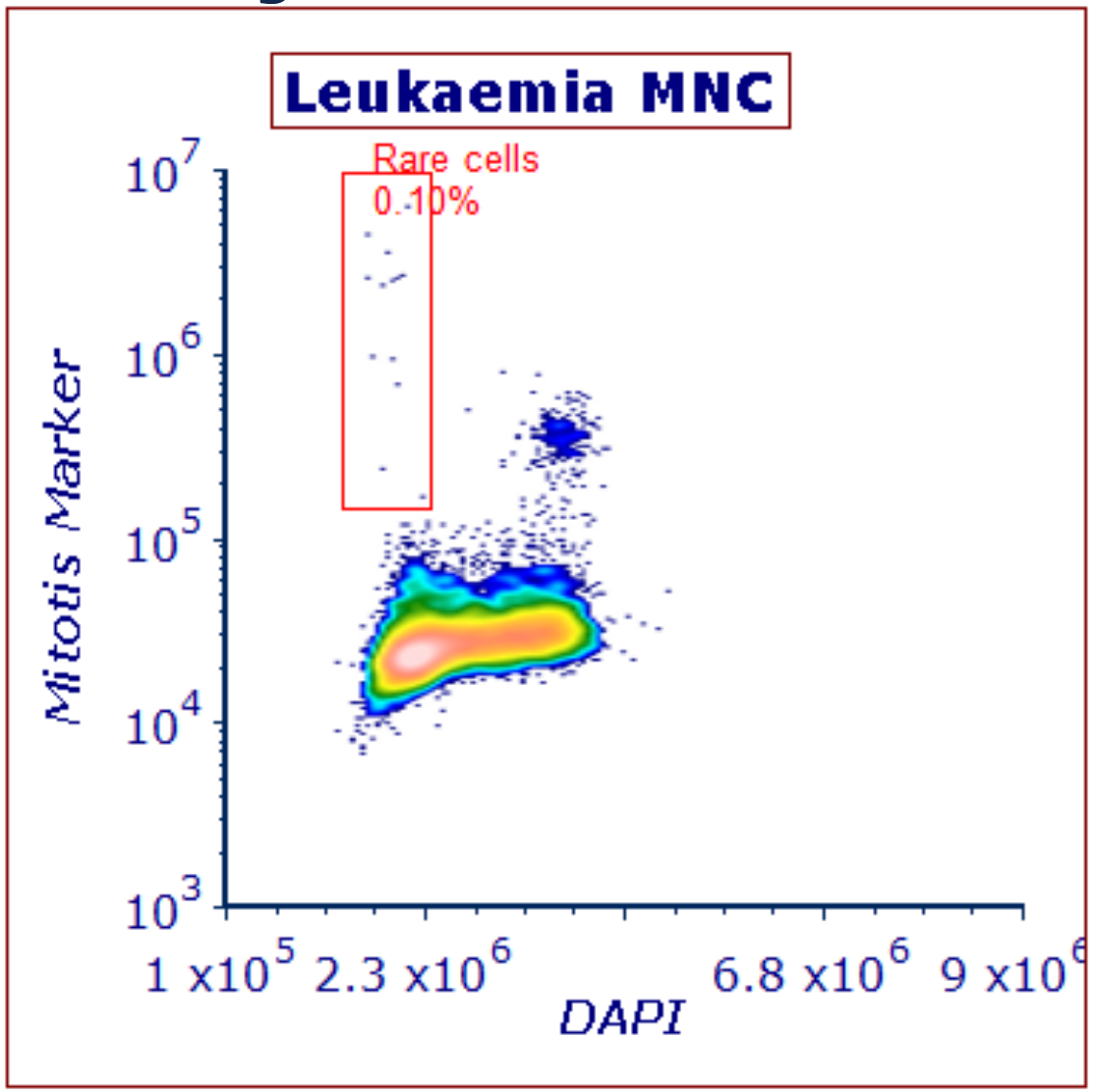
Additional Slide for question

Q: Were there any cases with Mitotic Marker expressed aberrantly?