



Foundations of Cancer Therapeutics- Research Technology Workshop 1:

HT Flow Cytometry for drug screening

Margie Moczygemba, Ph.D.

Research Associate Professor

Director, IBT Flow Cytometry Core Facility

Associate Director, IBT Academic Affairs

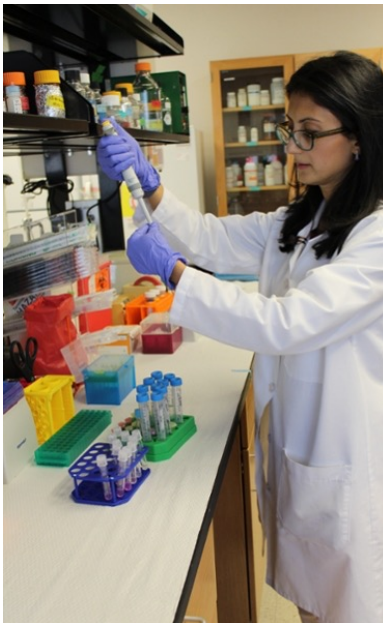
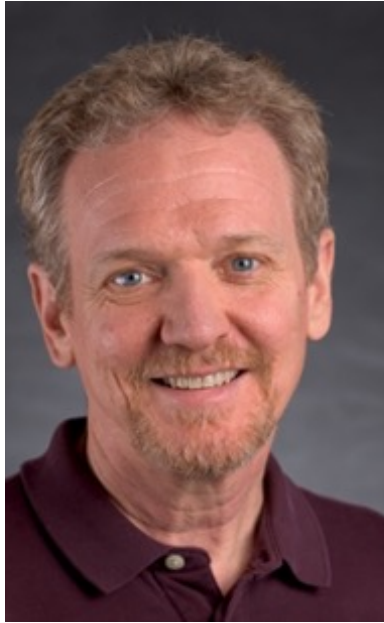
IBT HT Flow Core Facility

Location:

Corner of Holcombe Blvd.
And Pressler

IBT, Texas A&M HSC
Alkek Bldg #711-713





Margie Moczygemba, PhD
PI and Director,
Flow Cytometry

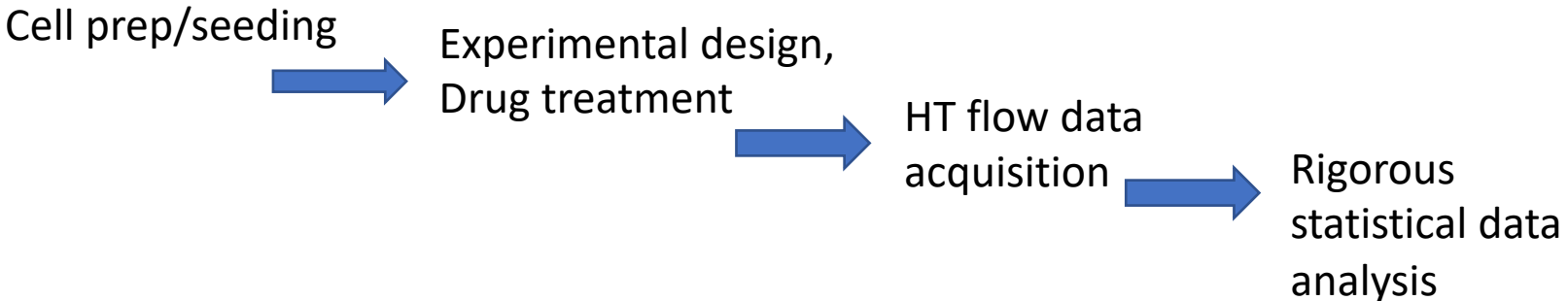
Cliff Stephan, PhD
Co-PI and Director,
HT Drug screening

Mary Sobieski
Senior Research
Associate

Ivy Nguyen
Research Specialist

Sevinj Isgandarova
Flow cytometry
specialist

Reid Powell, PhD
Bioinformatician



Our HT Flow cytometry team

Flow cytometry: a few basics

What is flow cytometry?

Flow cytometry is a powerful **technology** for rapidly and simultaneously **measuring** multiple **cellular characteristics** by optical means.

- Peripheral blood, bone marrow cells
- Cell lines (suspension, adherent (trypsinized))
- Bacteria
- Yeast

Flow cytometry: a few basics

What type of information does flow cytometry provide?

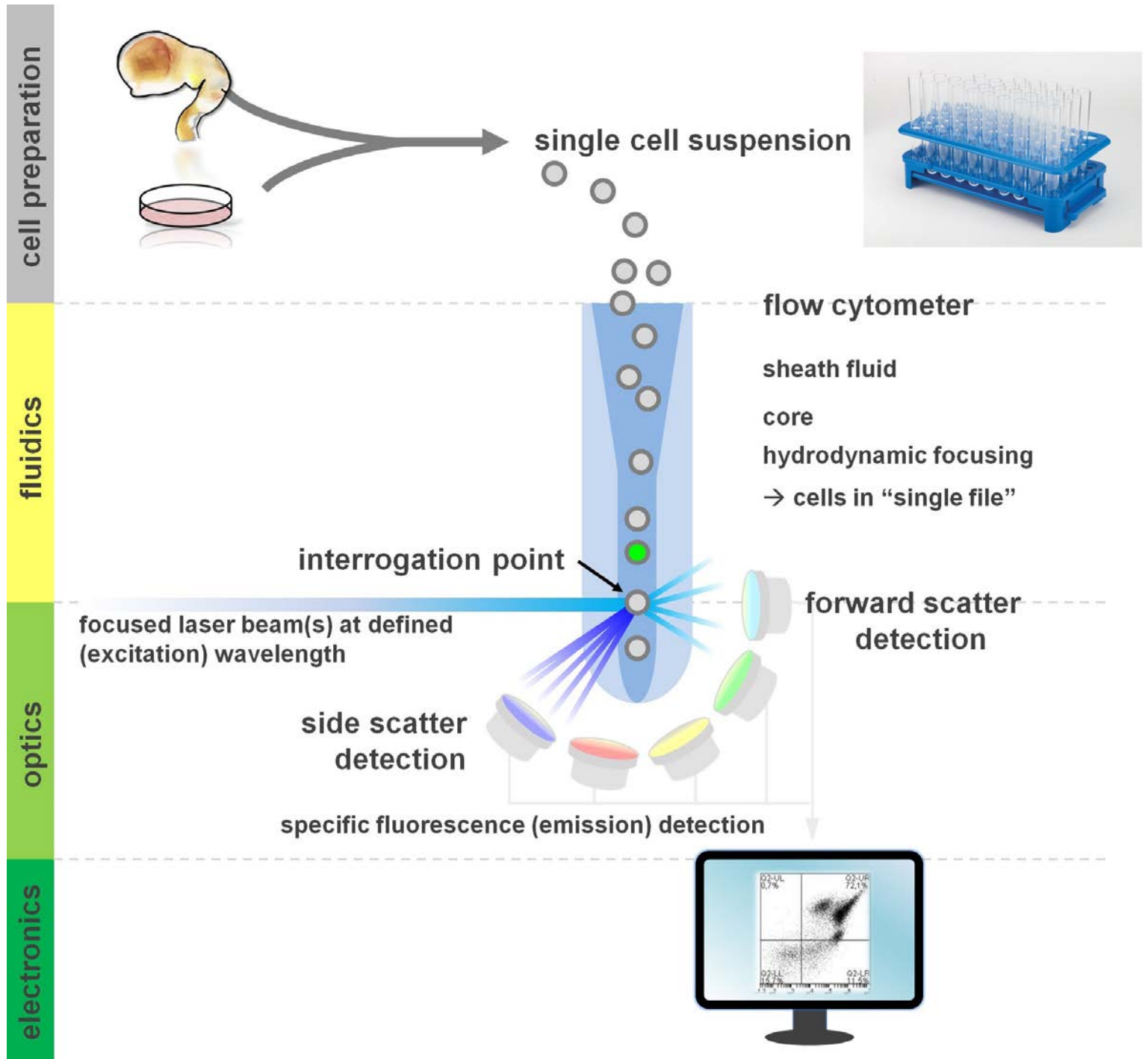
- Relative cell (particle) size
- Intracellular complexity (granularity)
- **Rapidly** measures **multiple characteristics** on large number of cells
- Because single cells are measured, it will reveal **heterogeneity** within a population
- Ability to multiplex allows for the ability to **resolve small sub-populations**
- Offers rich **statistical analysis** on cell populations (FlowJo or FCS Express)

Flow cytometry: a few basics

Applications of Flow Cytometry

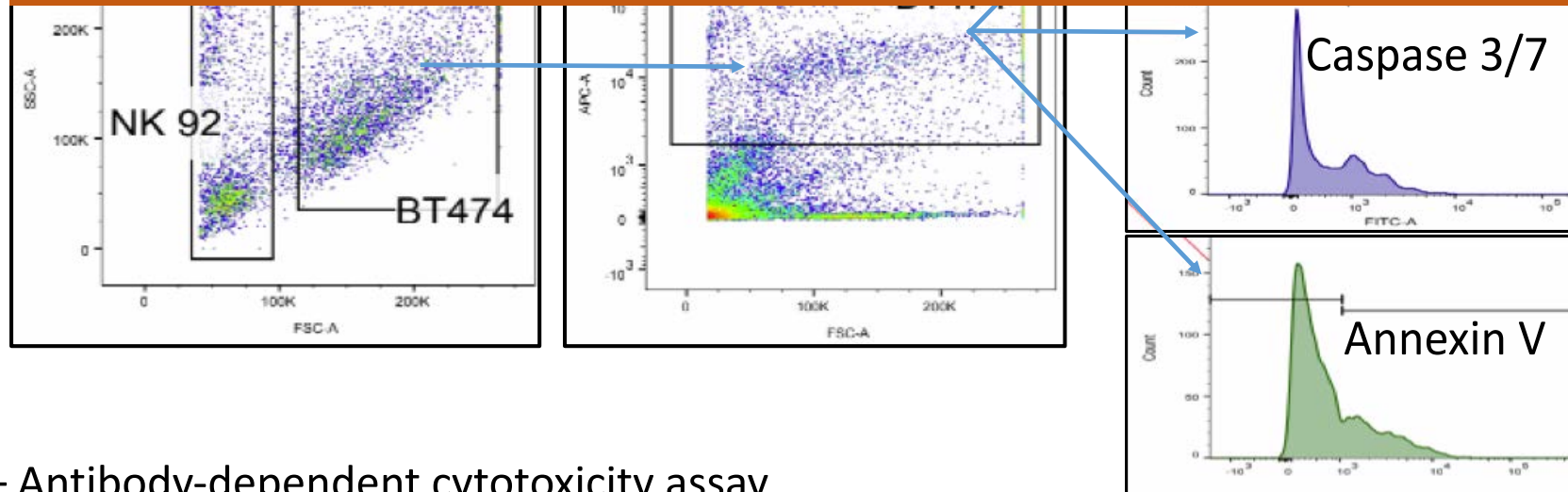
- **Cell sorting**, bulk or single cell cloning (FACS)
- Cell surface antigens (**immunophenotyping**)
- **Apoptosis** (annexin V), **viability** (dyes)
- **Cell proliferation** (CellTrace, BRDU and CFSE)
- **Intracellular cytokine** production
- Intracellular **signalling** (phospho Abs)
- Gene reporter (GFP)
- **Cell cycle**, DNA content

Conventional flow cytometry (low-throughput)



An example of flow cytometry data (low through-put)

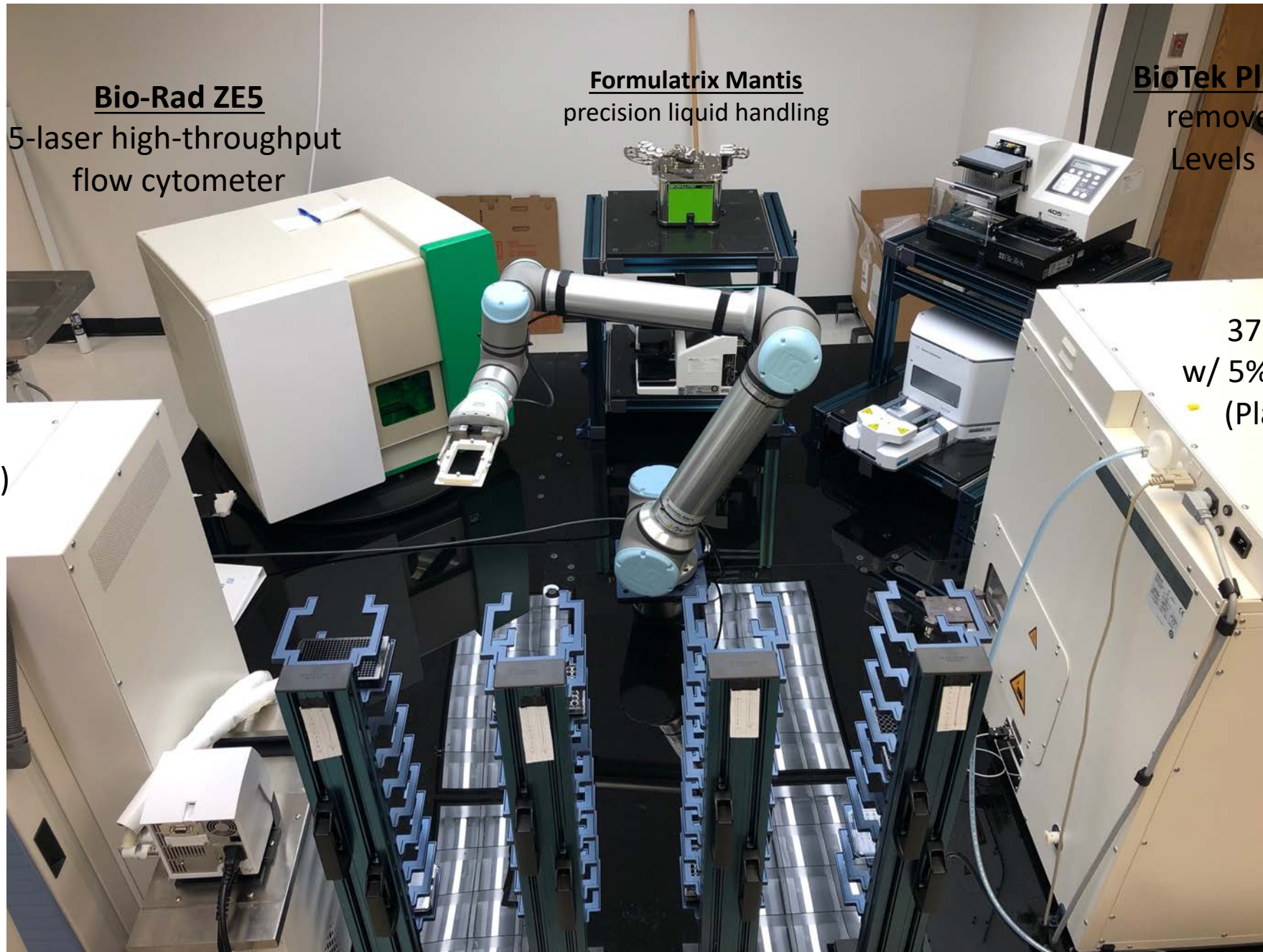
Problem: Too slow for HT drug screening





Solution: High-throughput
flow cytometry

The GCC-HtFCP Integrated Flow Cytometry Platform



Bio-Rad ZE5

5-laser high-throughput
flow cytometer

Formulatrix Mantis

precision liquid handling

BioTek Plate washer

removes liquids
Levels volumes

4°C Cytomat
(Cool storage)

37 °C Cytomat
w/ 5% CO₂ + 95% Hu
(Plate Storage)



Advantages of using high-throughput flow cytometry

1. Scalability – Can analyze hundreds to thousands of samples in HT mode

2. Speed – can analyze small volumes at fast rate; get data in minutes to hours vs days

3. Miniaturization - use less reagents to get same answer (saves money)

4. Affordability – cost effective

5. High content platform – can get lots of data, especially if multiplex

Broad library screen: Cytotoxicity assay heat map (Draq7) Cell Line #1

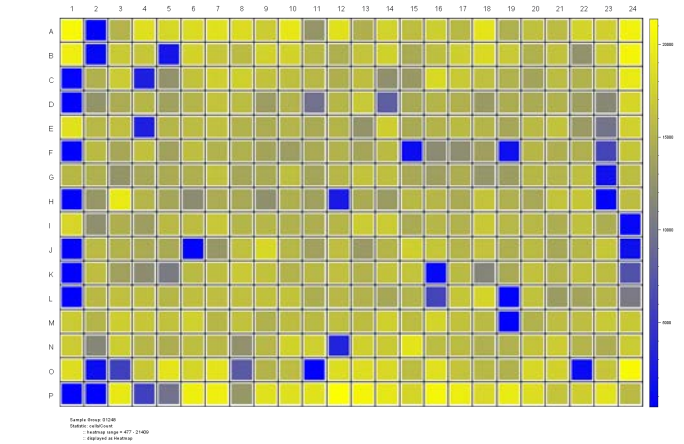
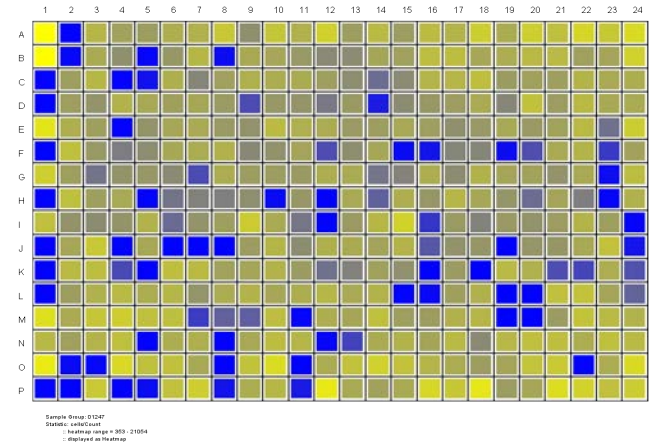
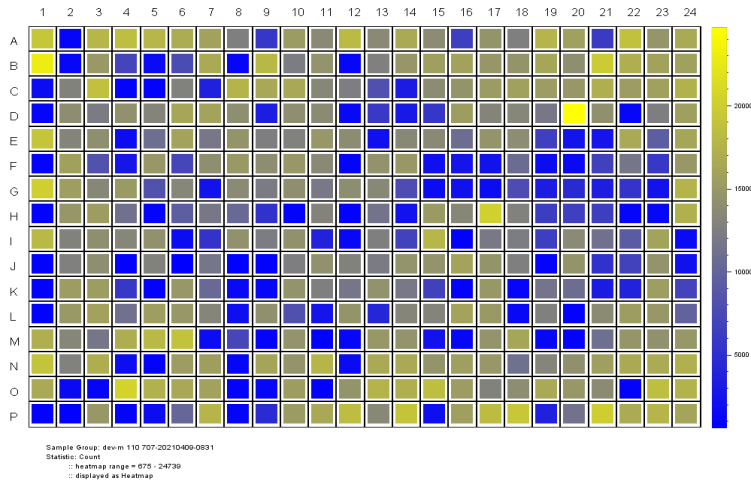
Dose [conc]:

1 μ M

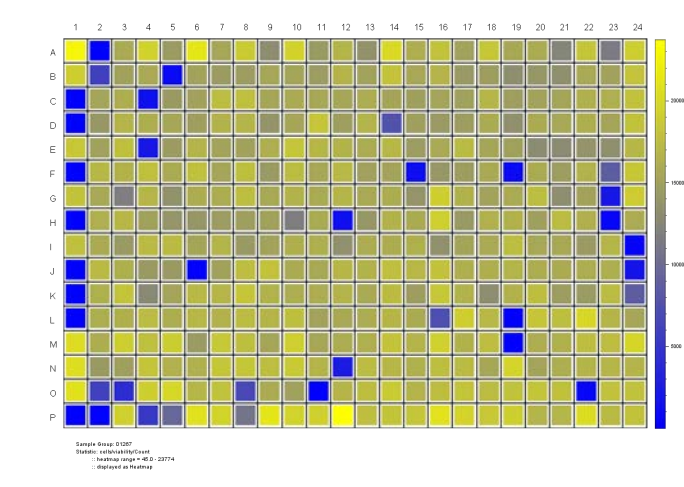
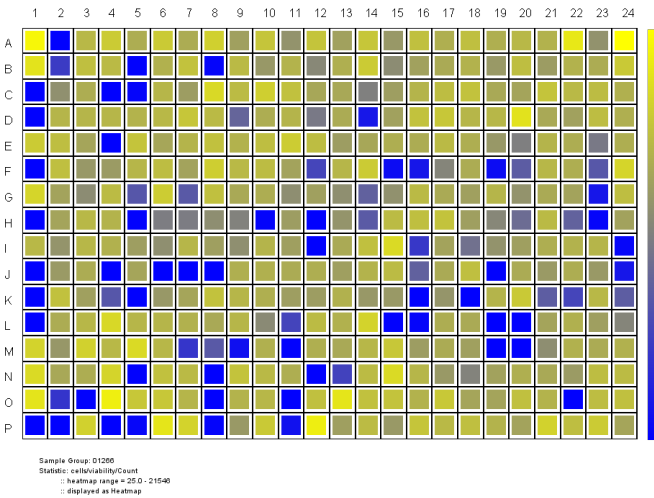
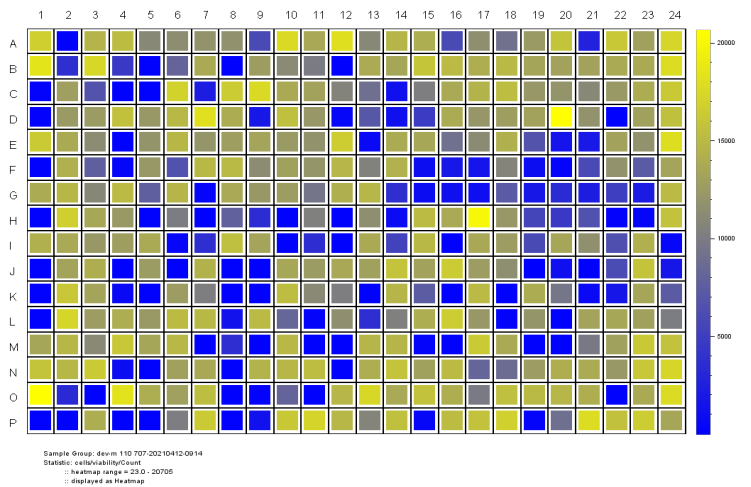
0.1 μ M

0.01 μ M

• RUN1



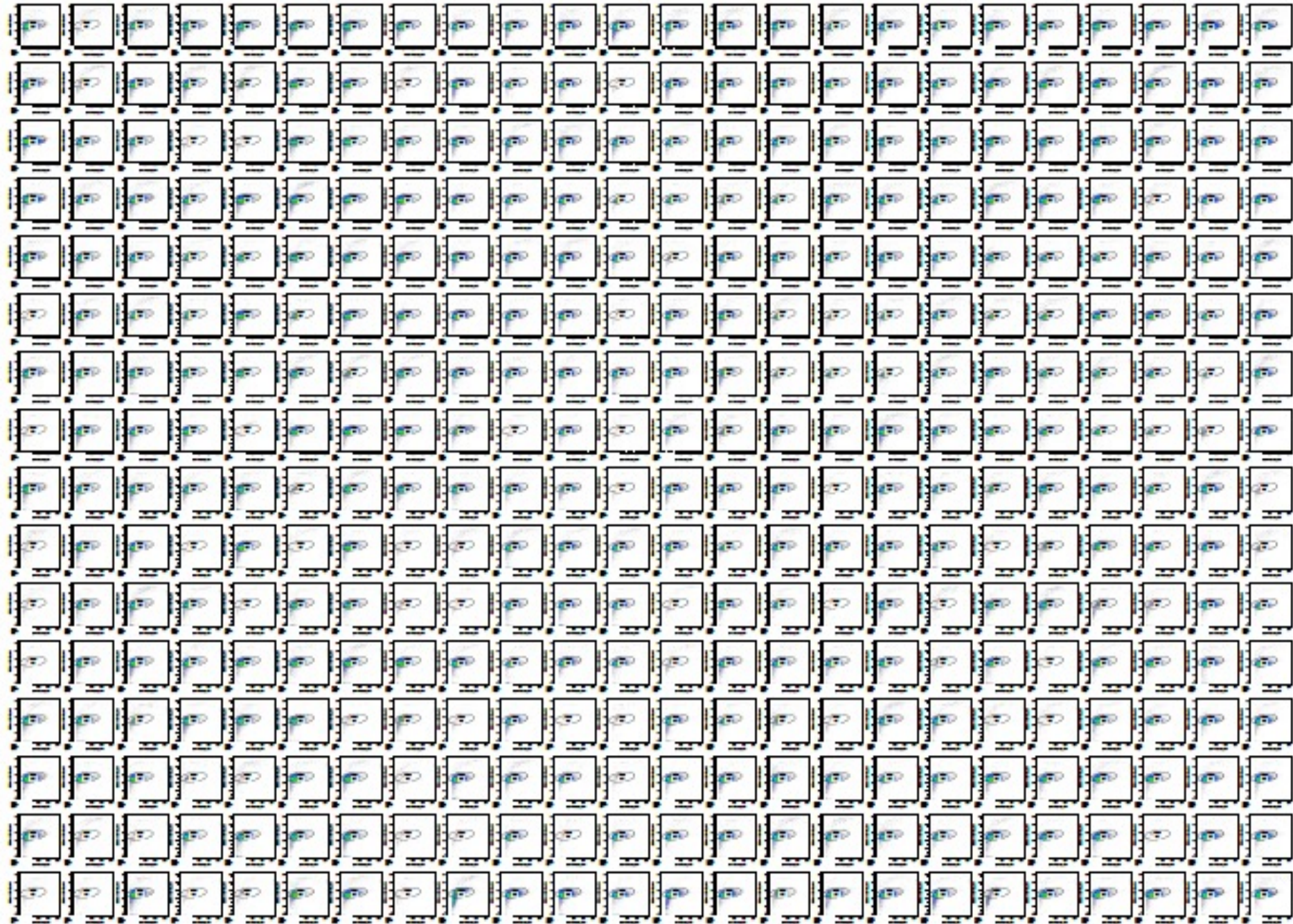
• RUN2



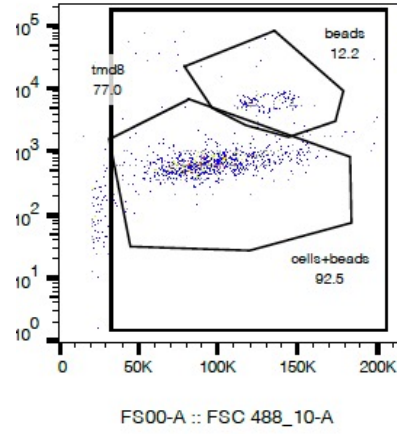
 Dead cells

 Live cells

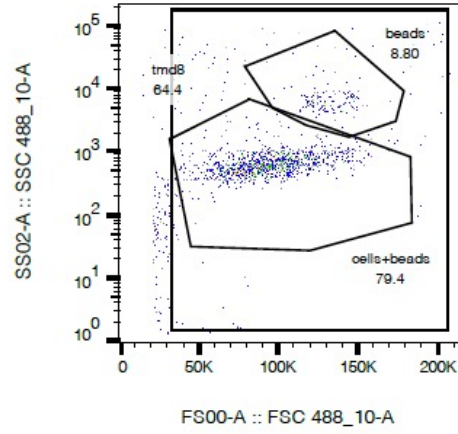
Gating cell
population in
384-well
plate



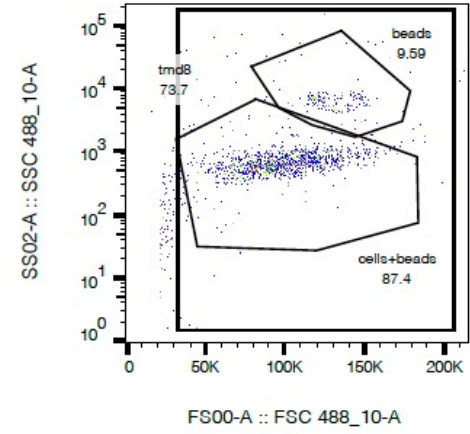
Zoom in
Gating: 384-
well plate



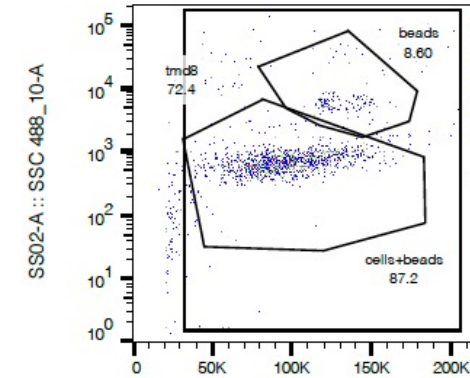
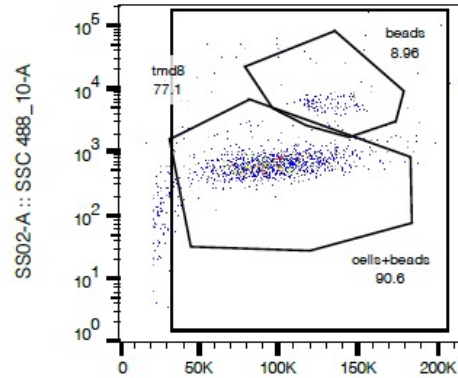
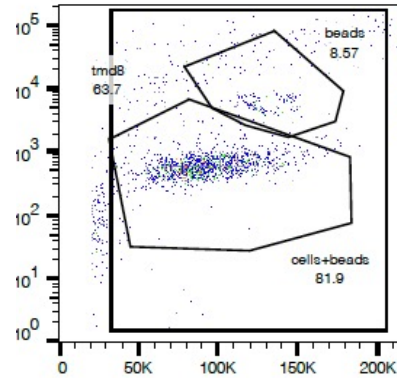
A4.fcs
Ungated
1233



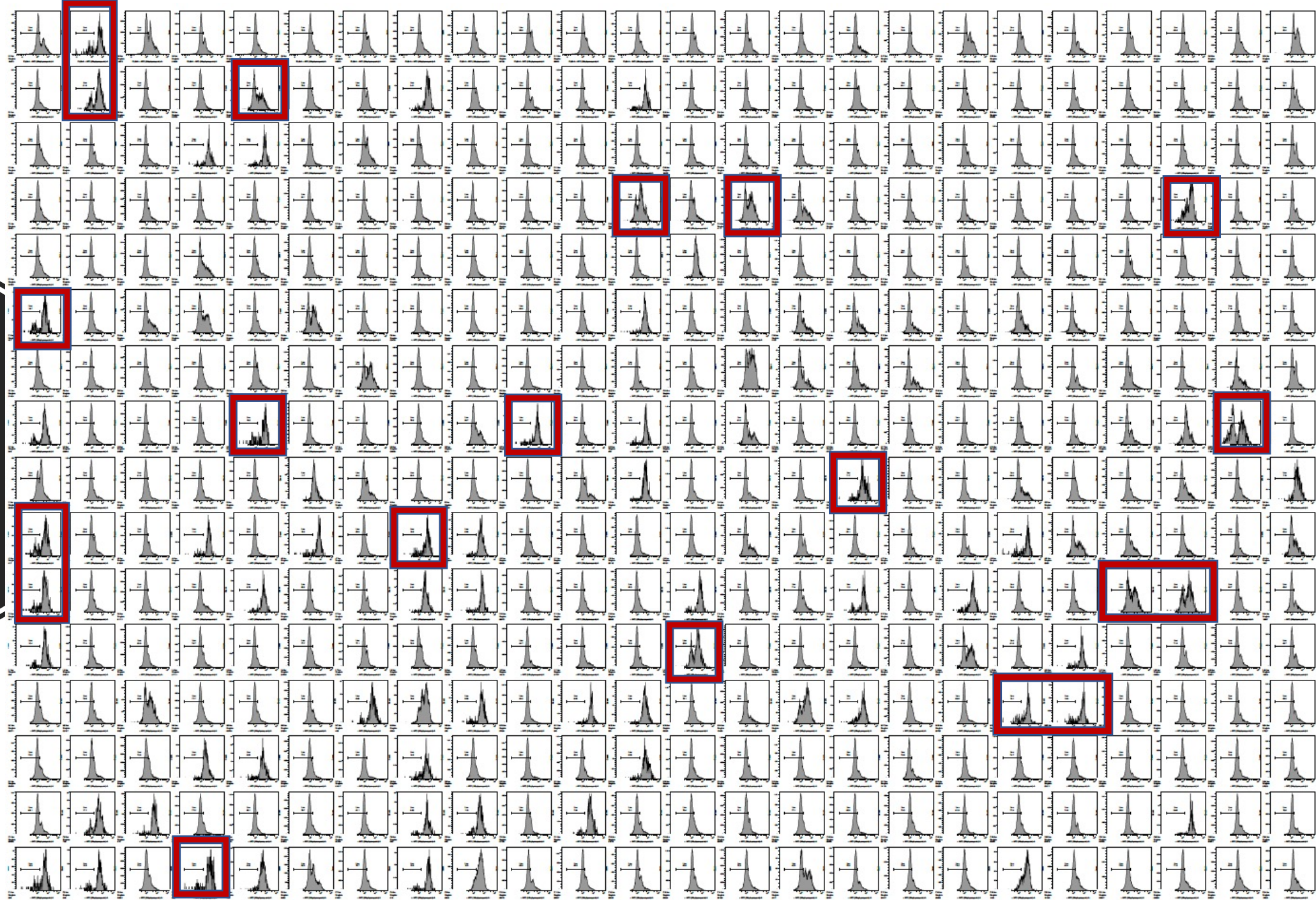
A5.fcs
Ungated
1613



A6.fcs
Ungated
1502



384 well
viability
analysis



Zoom in 384-
well viability
analysis



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Thank you!