Development of Standards for Calibrated Flow Cytometry

Jacob Beal, *NIST SBSC Flow Cytometry WG*

COMBINE
Salt Lake City
Oct. 2015

NIST Raytheon BBN Technologies

Overview

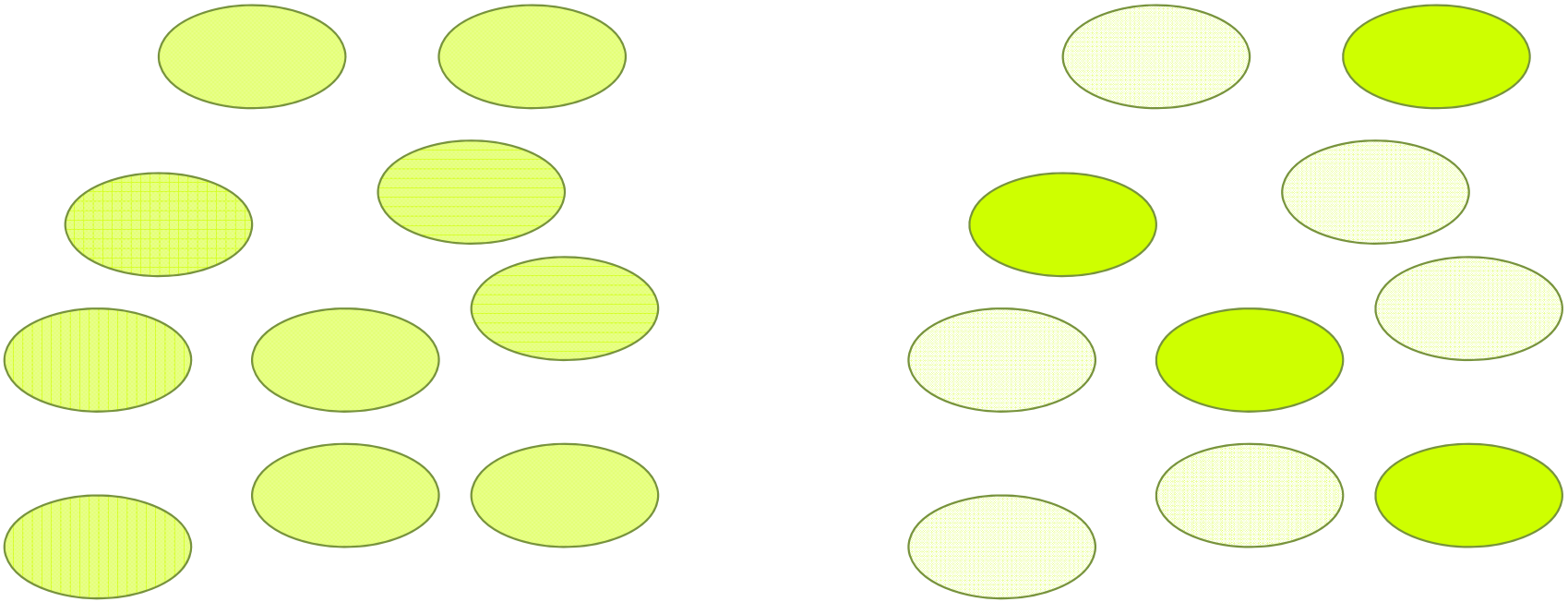
Raytheon
BBN Technologies

- **Need:** absolute unit measurements from large numbers of single cells
- **Technology:** Calibrated Flow Cytometry
- **Process:** NIST SBSC Flow Cytometry WG

Why single cell measurements?

Raytheon
BBN Technologies

Are these groups of cells the same?

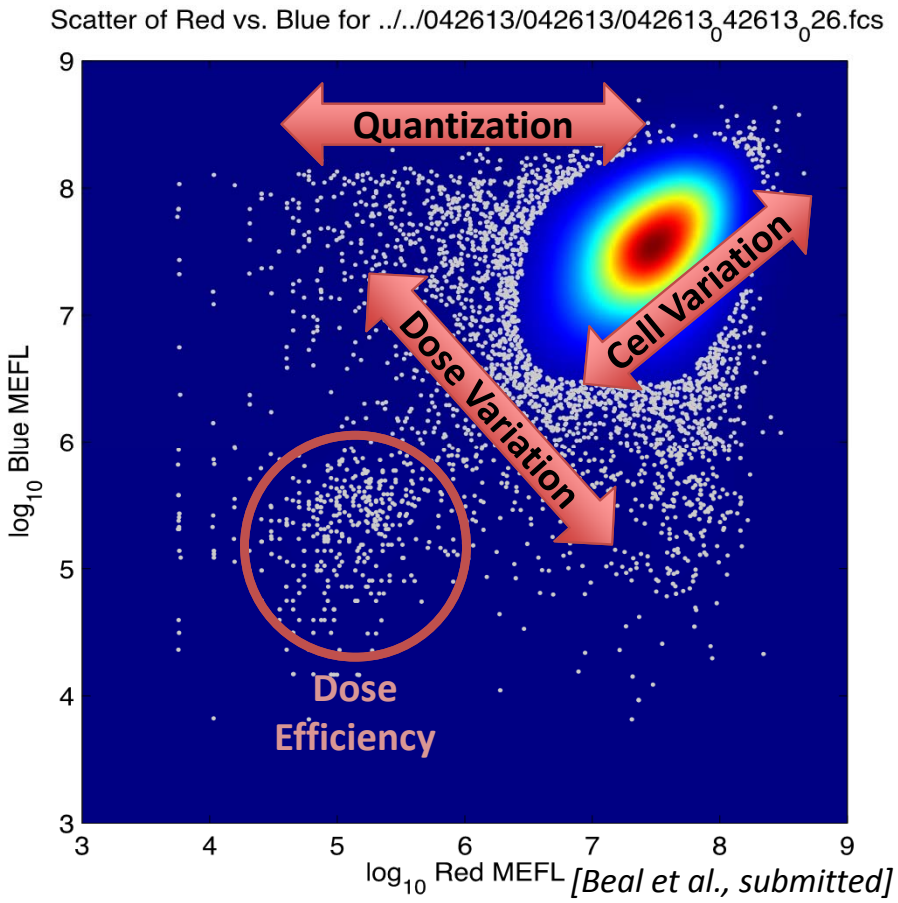


But they've got the same mean fluorescence…?

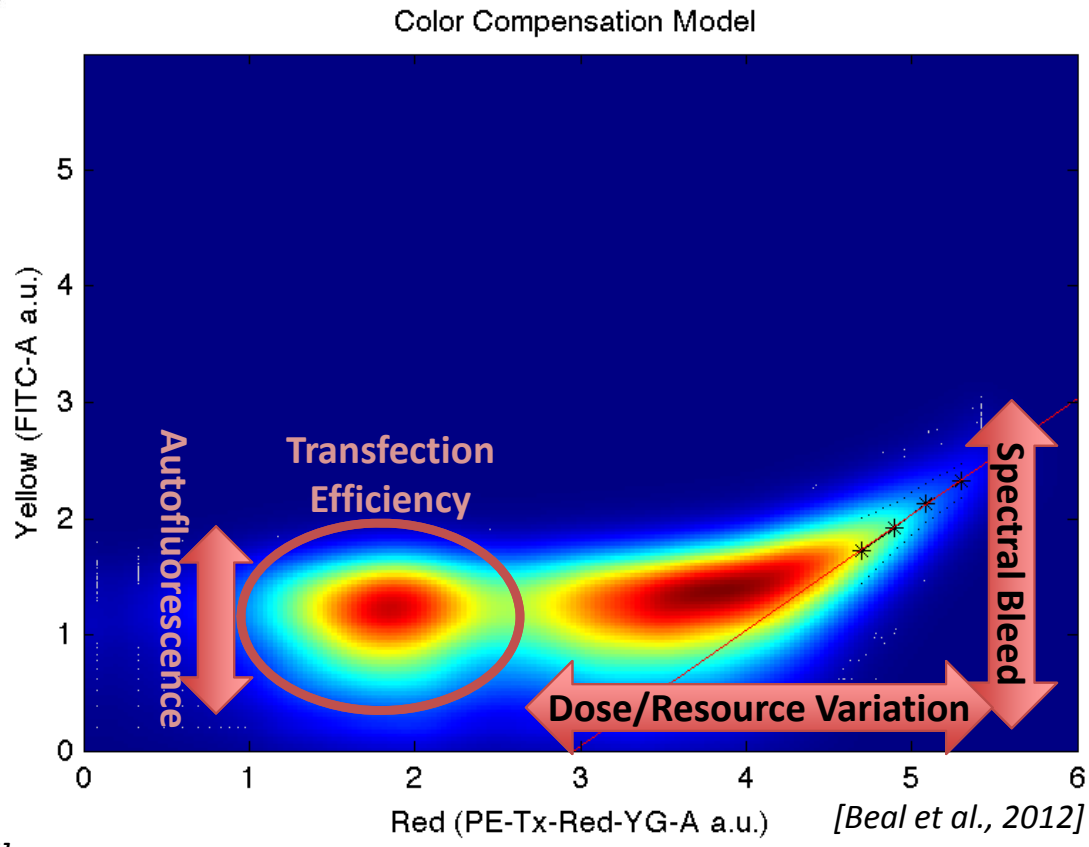
Why large numbers of samples?

Raytheon BBN Technologies

Example: RNA Replicon Cotransfection



Example: Constitutive mKate in HEK293



Quantifying variation components requires per-cell measurements of large populations of cells

Why absolute units?

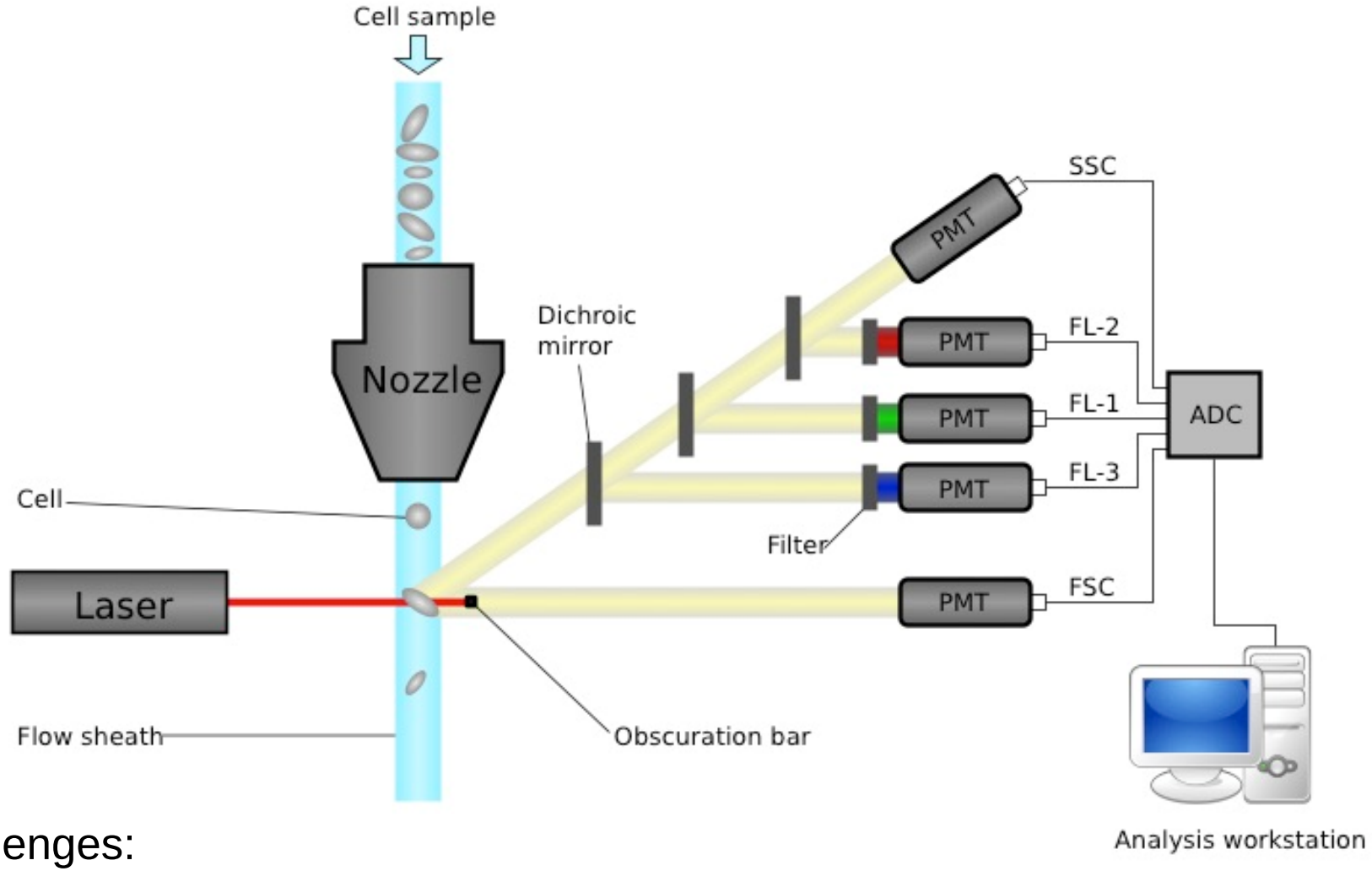
Raytheon
BBN Technologies

- Metrology 101: precision measurement enables:
  - Comparison of results across experiments and labs
  - Deeper insight into the behavior of devices
  - Effective dissemination of materials and methods
  - Testing and validation of materials and systems
  - Establishment of commercial & industrial services
  - Safety assurance and traceability of responsibility

Many biological measurements are only relative!

How Flow Cytometry Works

Raytheon
BBN Technologies



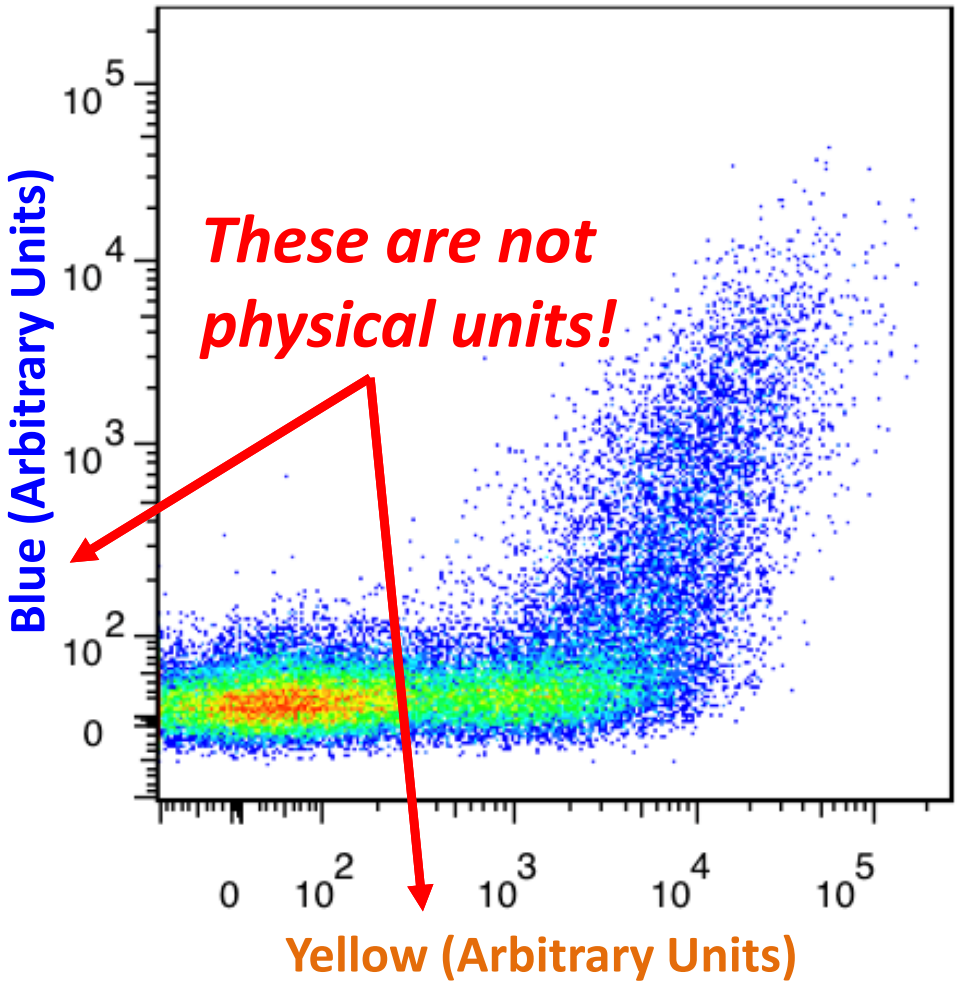
Challenges:

- Autofluorescence
- Variation in measurements
- Spectral overlap
- Time Contamination
- Lots of data points!
- Different protein fluorescence
- Individual cells behave (very) differently

Metrology vs. Flow Cytometry

Raytheon
BBN Technologies

Flow cytometry great for per-cell measurements, but…



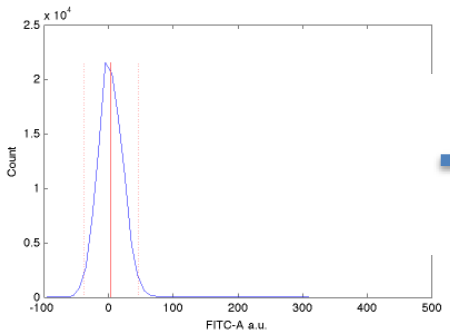
Arbitrary unit output depends on…

- **Instrument brand, configuration**
- **Interference from other colors**
- **Choice of instrument settings**
- **Run-to-run calibration drift**

Fortunately, this can be corrected…

Calibrated Flow Cytometry

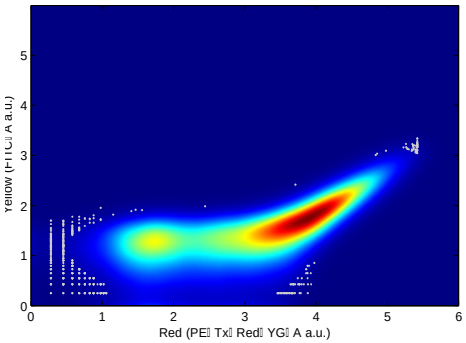
Raytheon BBN Technologies



Negative Control: autofluorescence

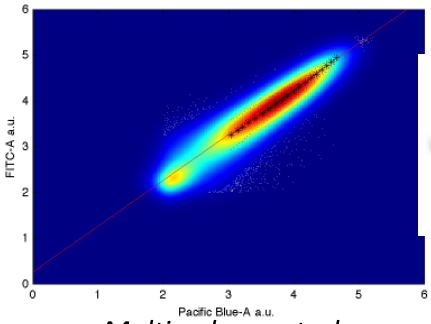
Arbitrary Units (a.u.)

Net a.u.



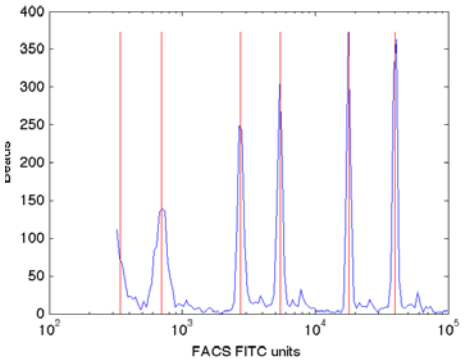
Single positive controls: compensate for spectral overlap

Compensated a.u.



Multi-color control: conversion of non-FITC channels

FITC a.u.



Calibration beads: Convert FITC a.u. to MEFL

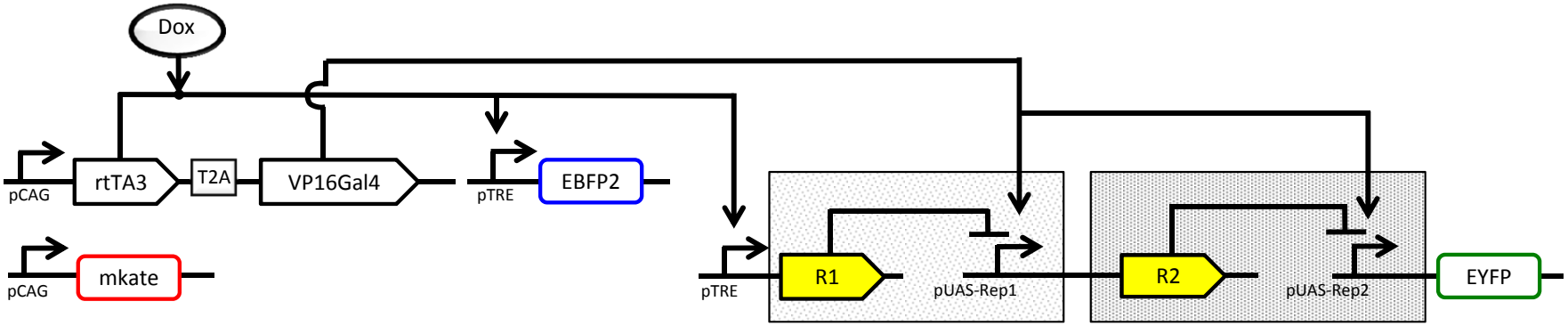
Calibrated MEFL

[Roederer, 2002; Wang et al., 2008; NIST/ISAC, 2012; Beal et al., 2012; Kiani et al., 2014; Beal et al., 2014; Davidsohn et al, 2014; Beal 2015]

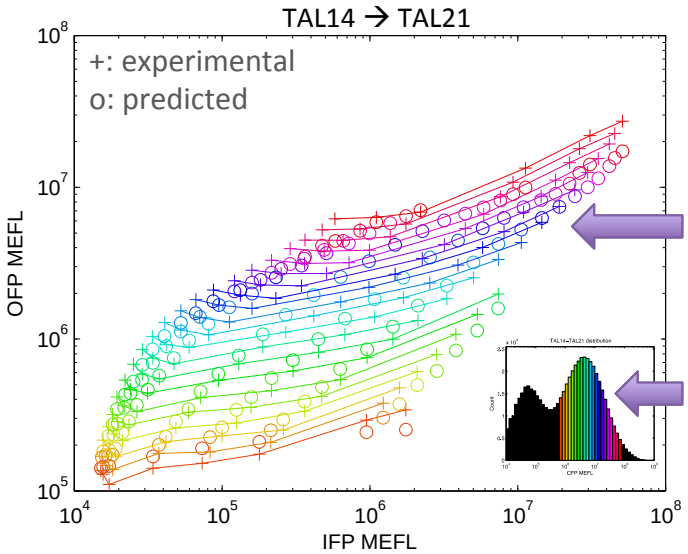
Example: Predicting Repressor Cascades

Raytheon BBN Technologies

Precision dose-response measurement allows high-precision prediction with quantitative models



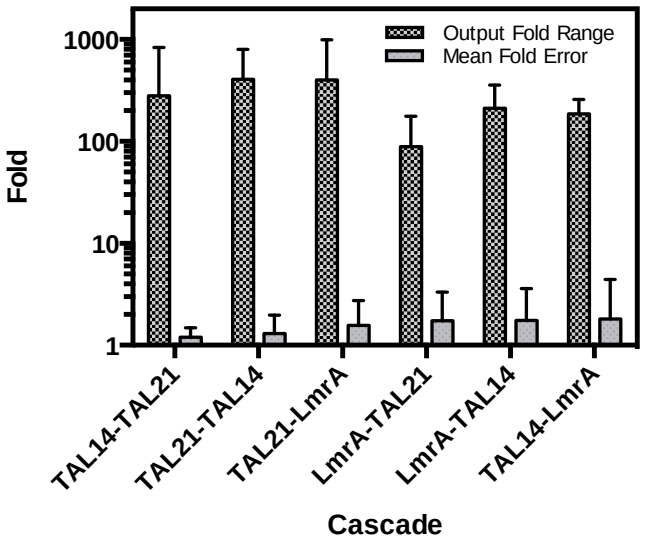
Prediction of Repressor Cascade



Each line is a dose/response curve for a different relative number of circuit copies.

Subpopulation identified by color on inset mKate histogram

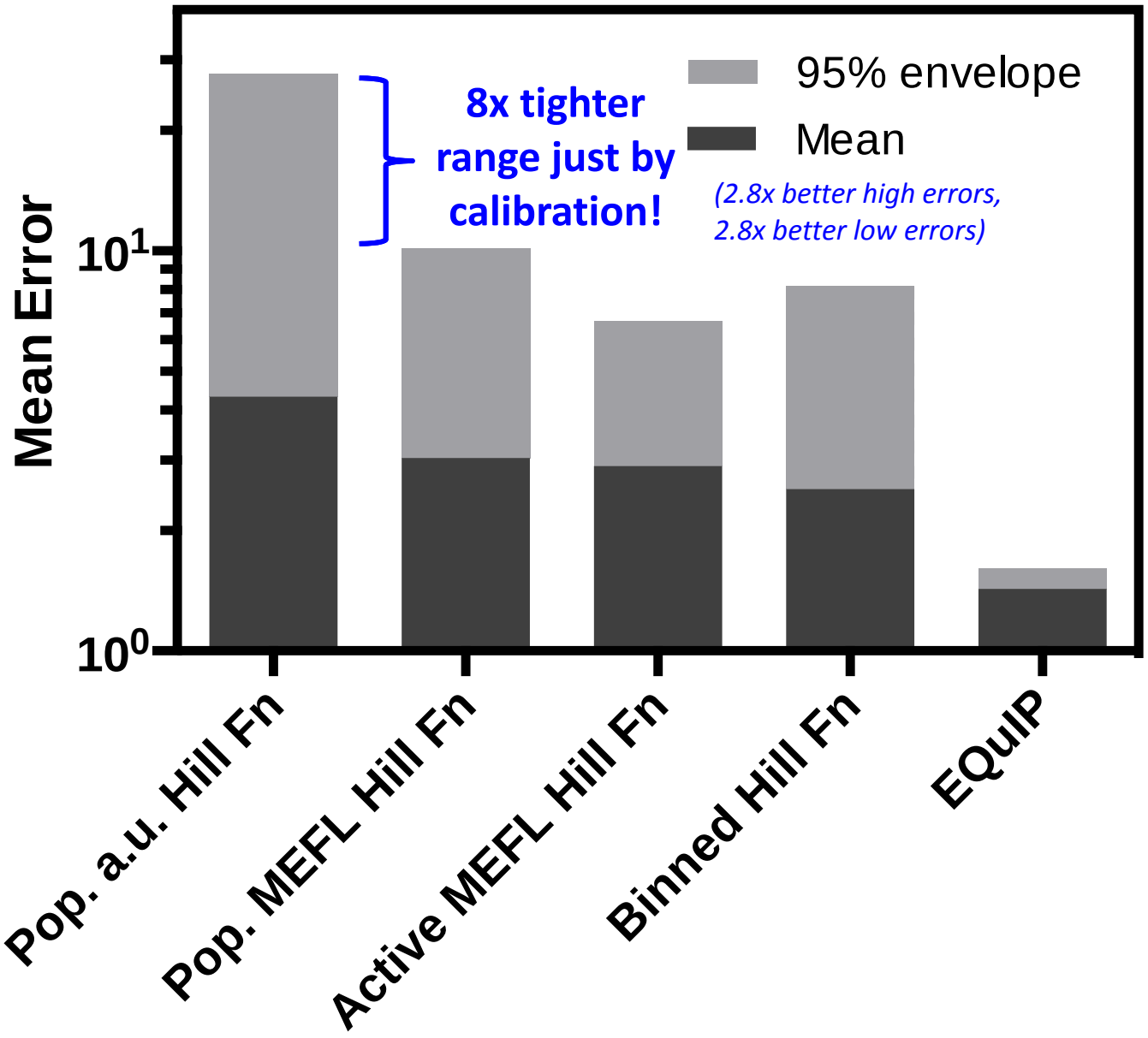
Range vs. Error for 6 Cascades



[Davidsohn et al., 2015]

How much does calibration matter?

Raytheon
BBN Technologies



[Davidsohn et al., 2015]

NIST Synthetic Biology Standards Consortium

Raytheon BBN Technologies

- Flow cytometry working group:
  - Initial goal: two standards documents
    - Minimum Standard for Flow Cytometry Calibration Information *what needs to be done*
    - Methods for Calibrated Flow Cytometry *a way to do it*
  - Process & Timeline:
    - Currently: drafting in progress
    - Nov 3: NIST SBSC meeting in San Francisco
    - Dec ‘15: Dissemination for critique & improvement
    - Feb ‘16: Version 1 ready for publication process
    - Onward: calibrate FSC, SSC, interlab studies

Join this open group: https://groups.google.com/forum/#!forum/sbsc-flow-cytometry