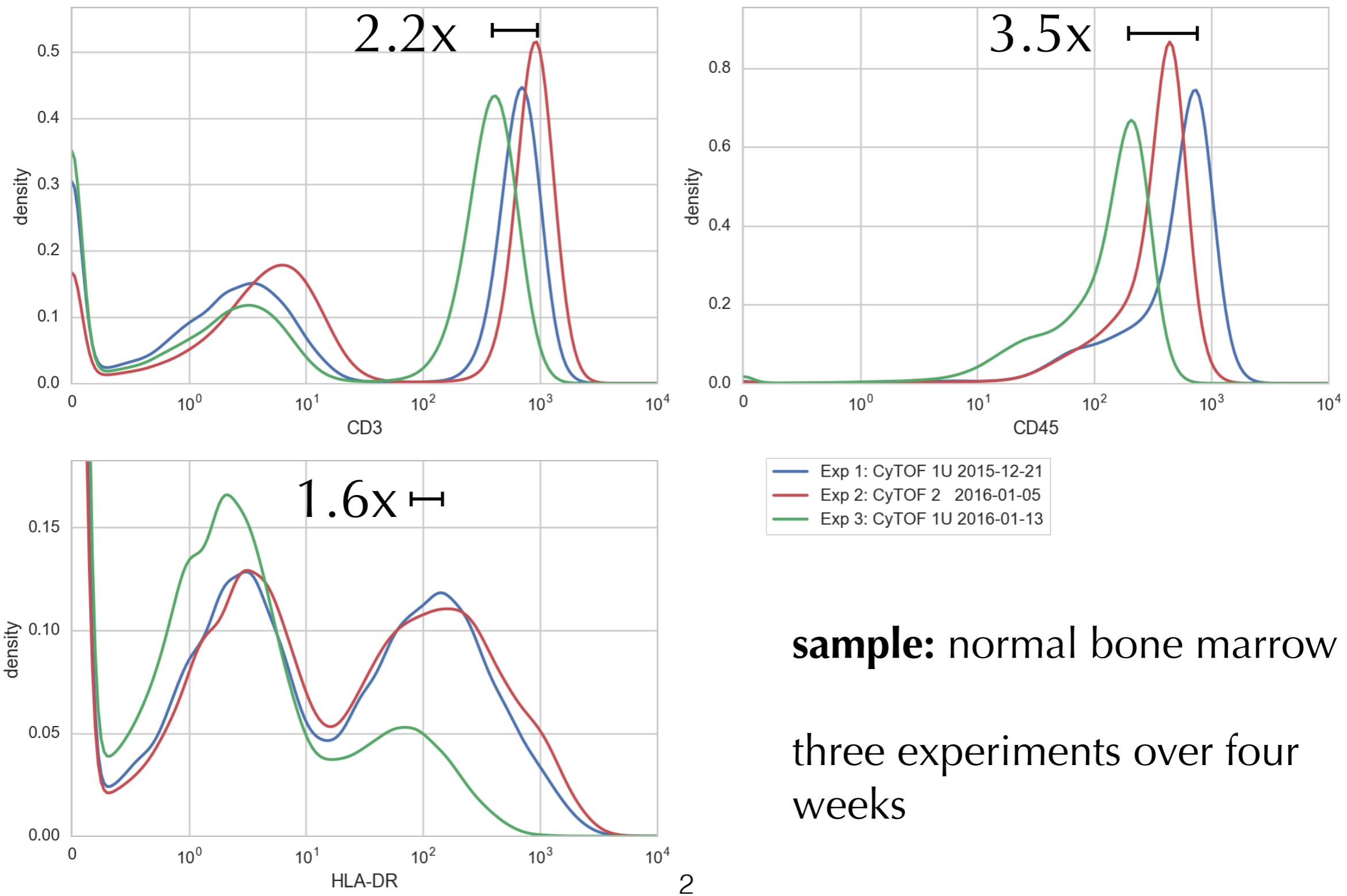


CLEAN Corrects Variations in CyTOF Sample Preparation

Jeffrey M. Hokanson, Ryan McCarthy, Chris Benton, Ahmed AlRawi,
Jeff Sun, Duncan Mak, Jared K. Burks, Michael Andreeff

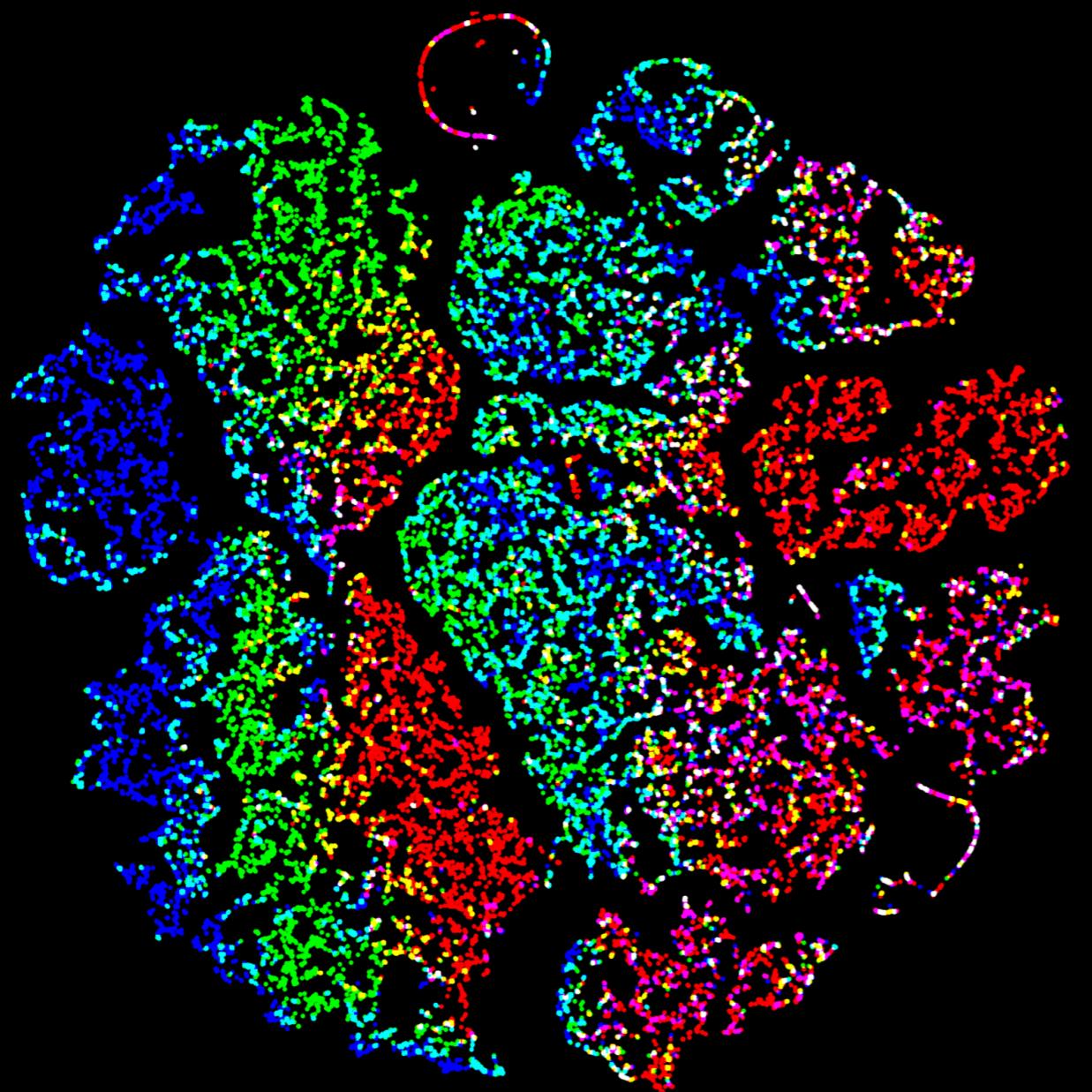
Department of Leukemia, Division of Cancer Medicine
University of Texas MD Anderson Cancer Center

Variability Exists in CyTOF Experiments

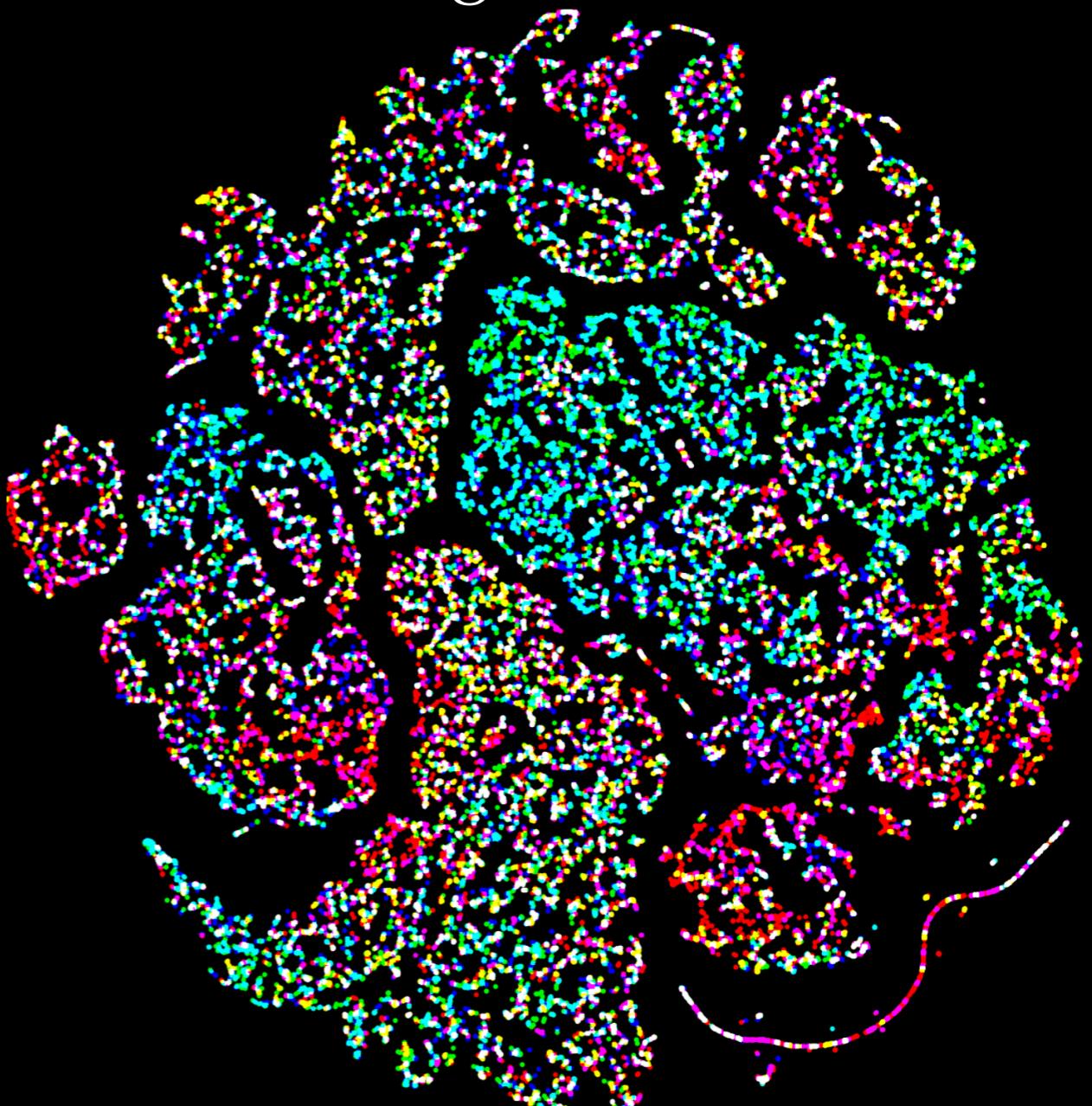


Variability Impacts t-SNE

uncorrected



using CLEAN

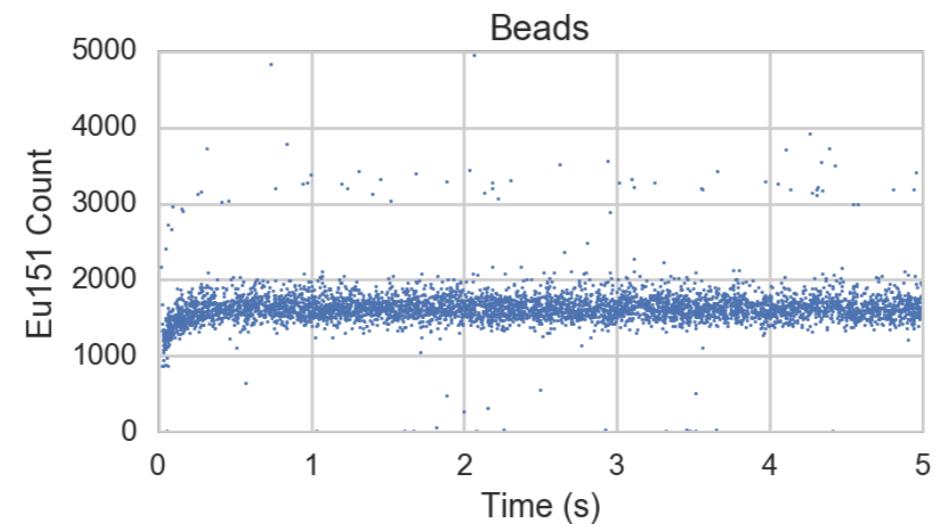


t-SNE using 30,000 cells mapping CD3, CD45, HLA-DR to 2 dimensions

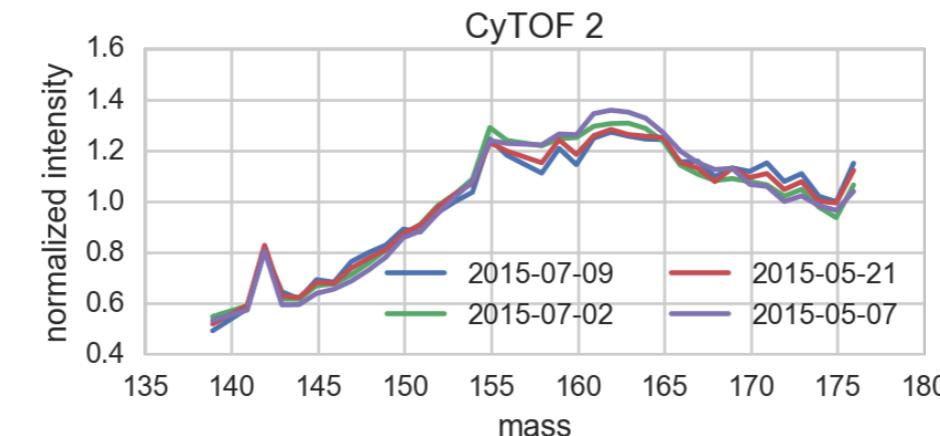
Error in CyTOF Experiments

Source

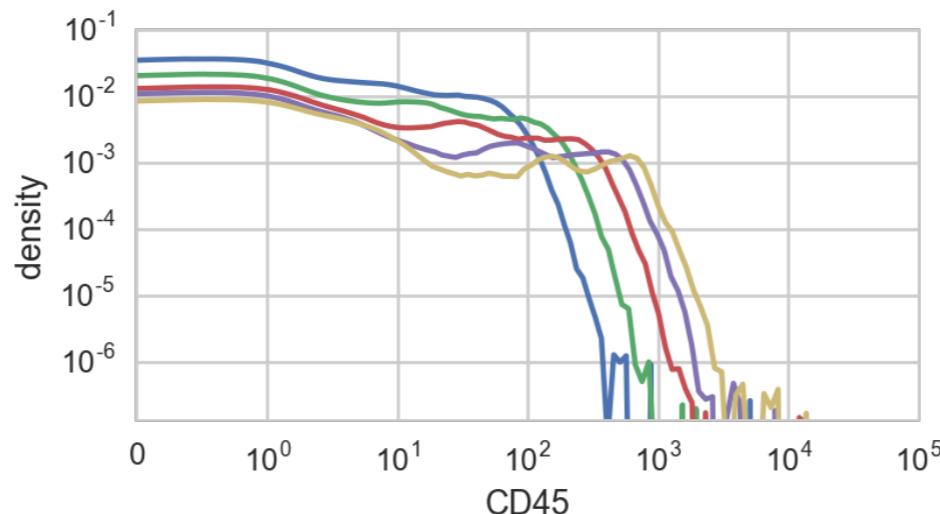
Sensitivity drift
across all channels



Mass-dependent
sensitivity drift



Staining variations



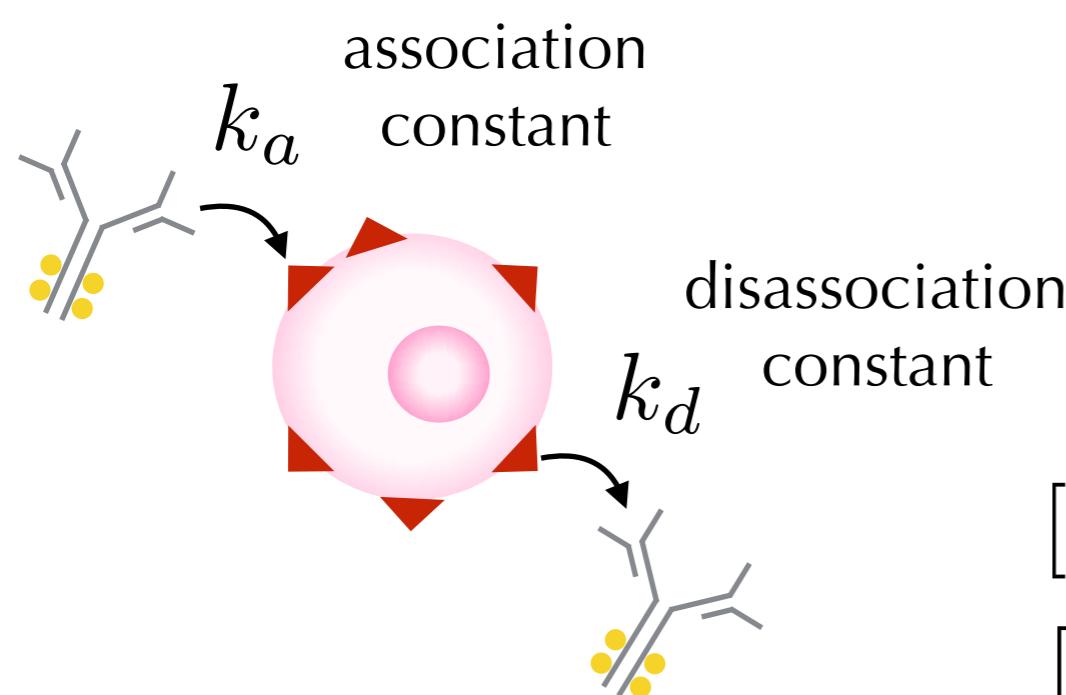
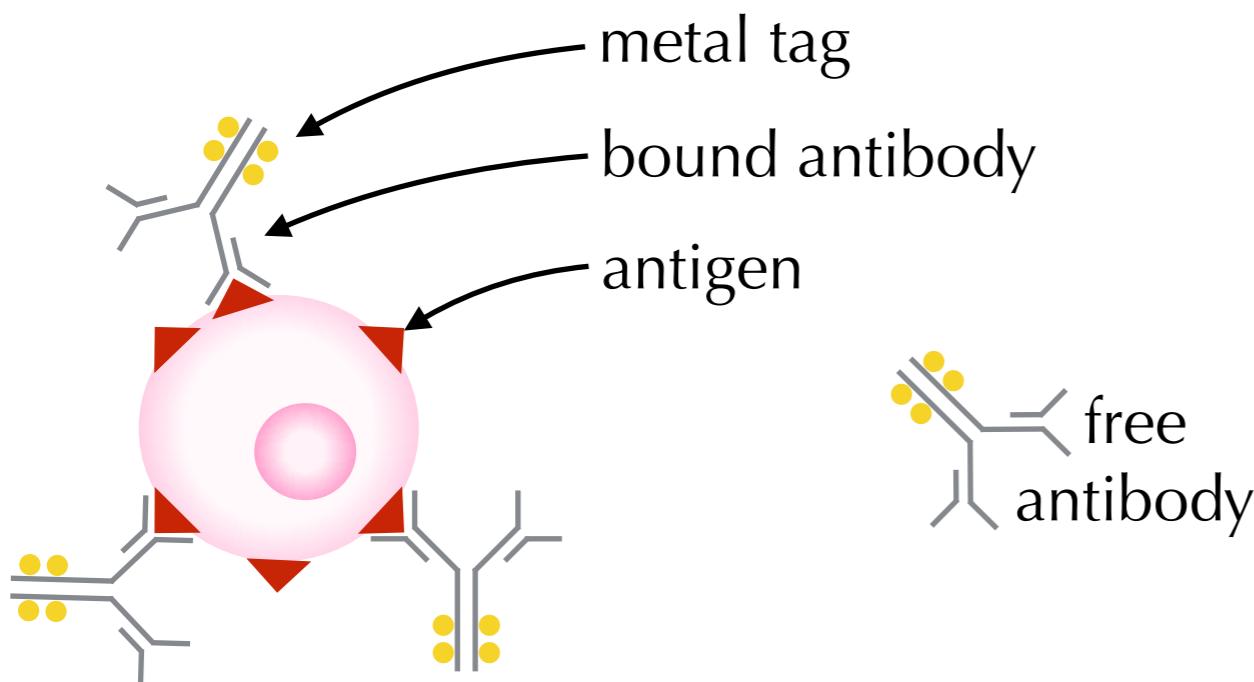
Solution

EQ-calibration beads

Calibration with
tuning solution

Barcode
CLEAN
(esp. longitudinal
studies)

Antibody Binding Kinetics



Equilibrium Binding



k_a k_d antibody dependent

CyTOF Measures $[\text{AbAg}]$

Equilibrium Equations

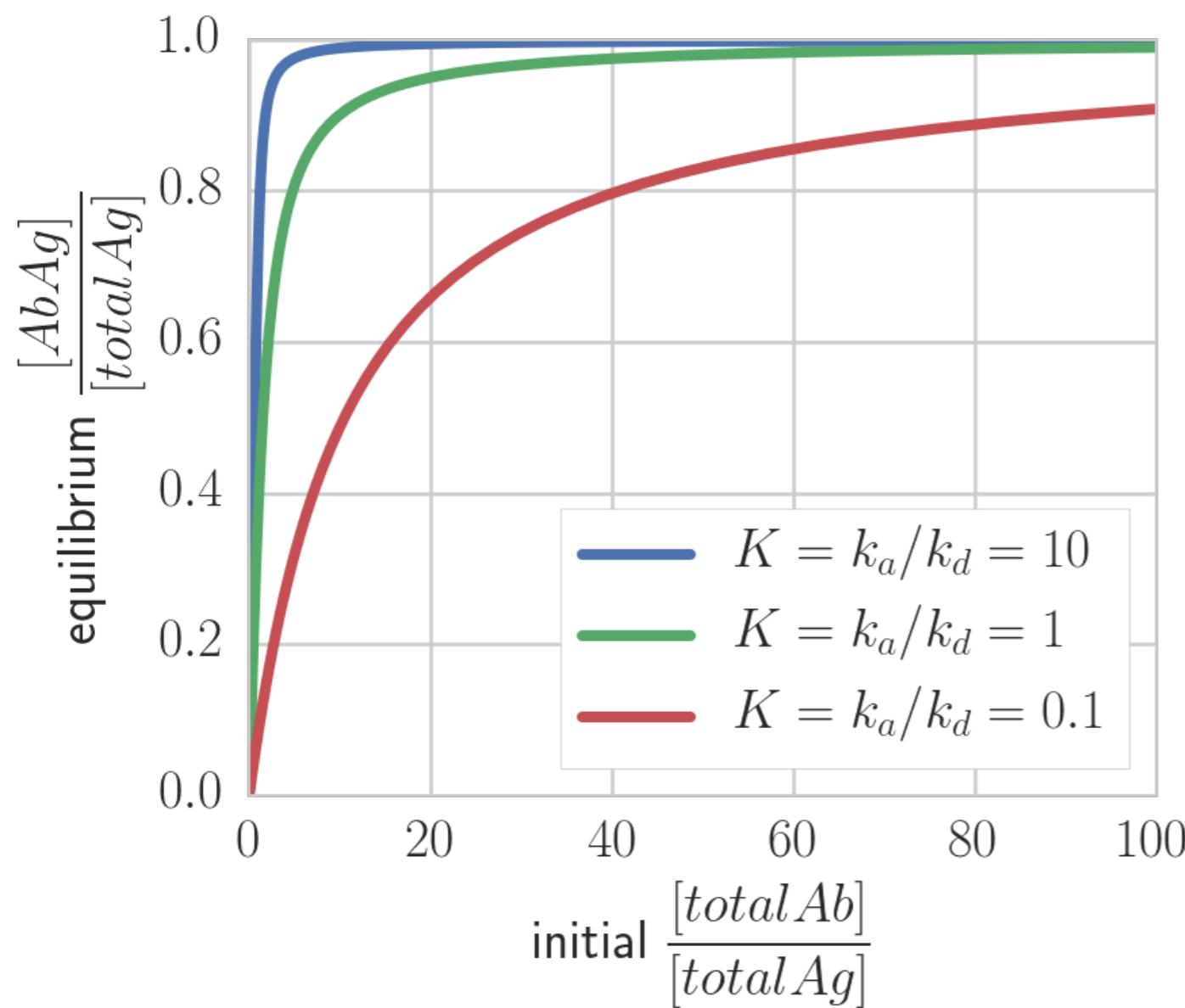
$$0 = k_a[\text{Ab}][\text{Ag}] - k_d[\text{AbAg}]$$

$$[\text{Initial Ab}] = [\text{Ab}] + [\text{AbAg}]$$

$$[\text{Initial Ag}] = [\text{Ag}] + [\text{AbAg}]$$

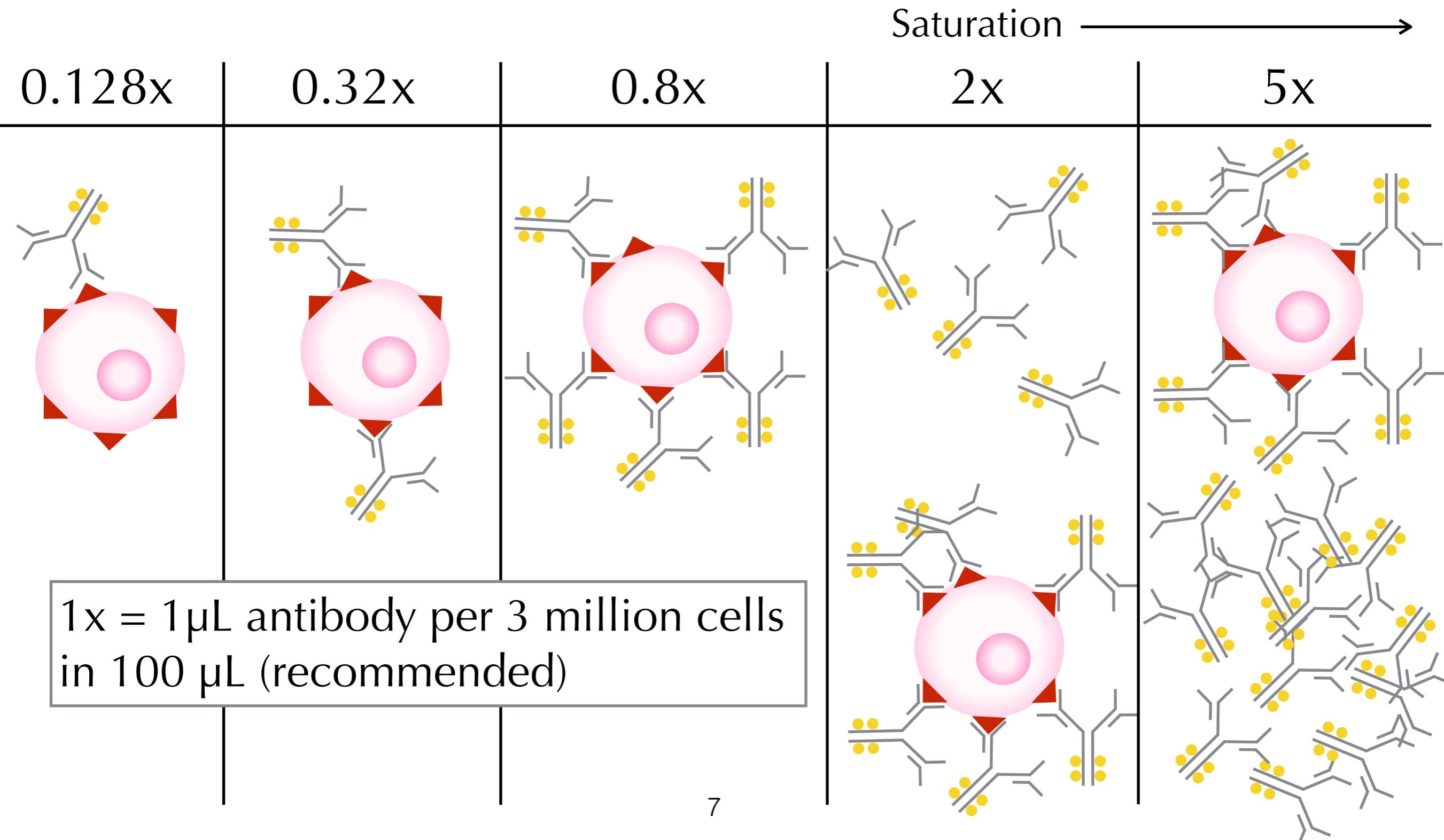
Antibody Binding Kinetics

Binding Curves from Reaction Kinetics

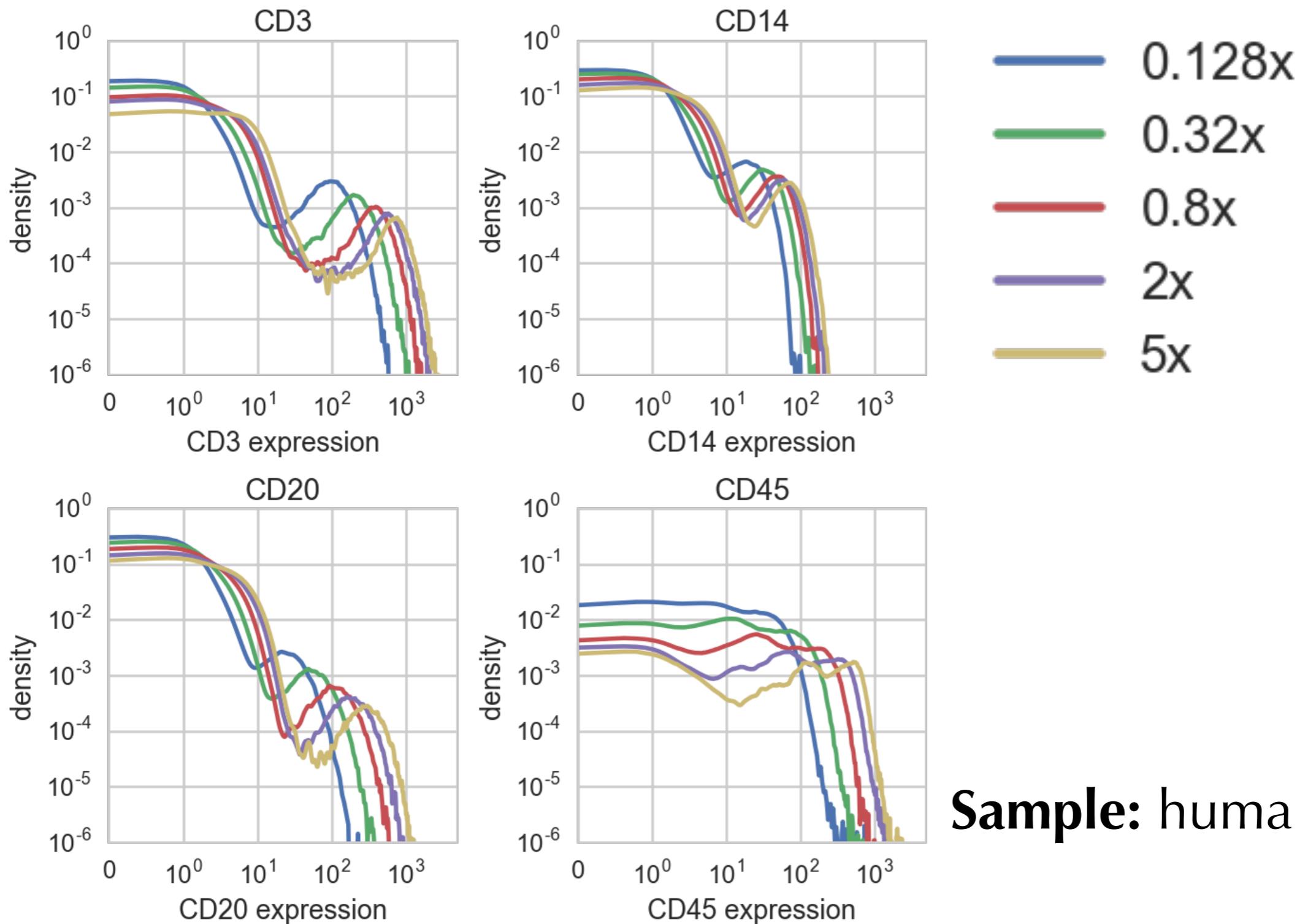


- Saturation: 100% binding
- At saturation: one antibody bound to one antigen
- Without saturation, measured intensities depend on preparation and cell counts

Increasing Antibody Concentration Increases Measured Expression

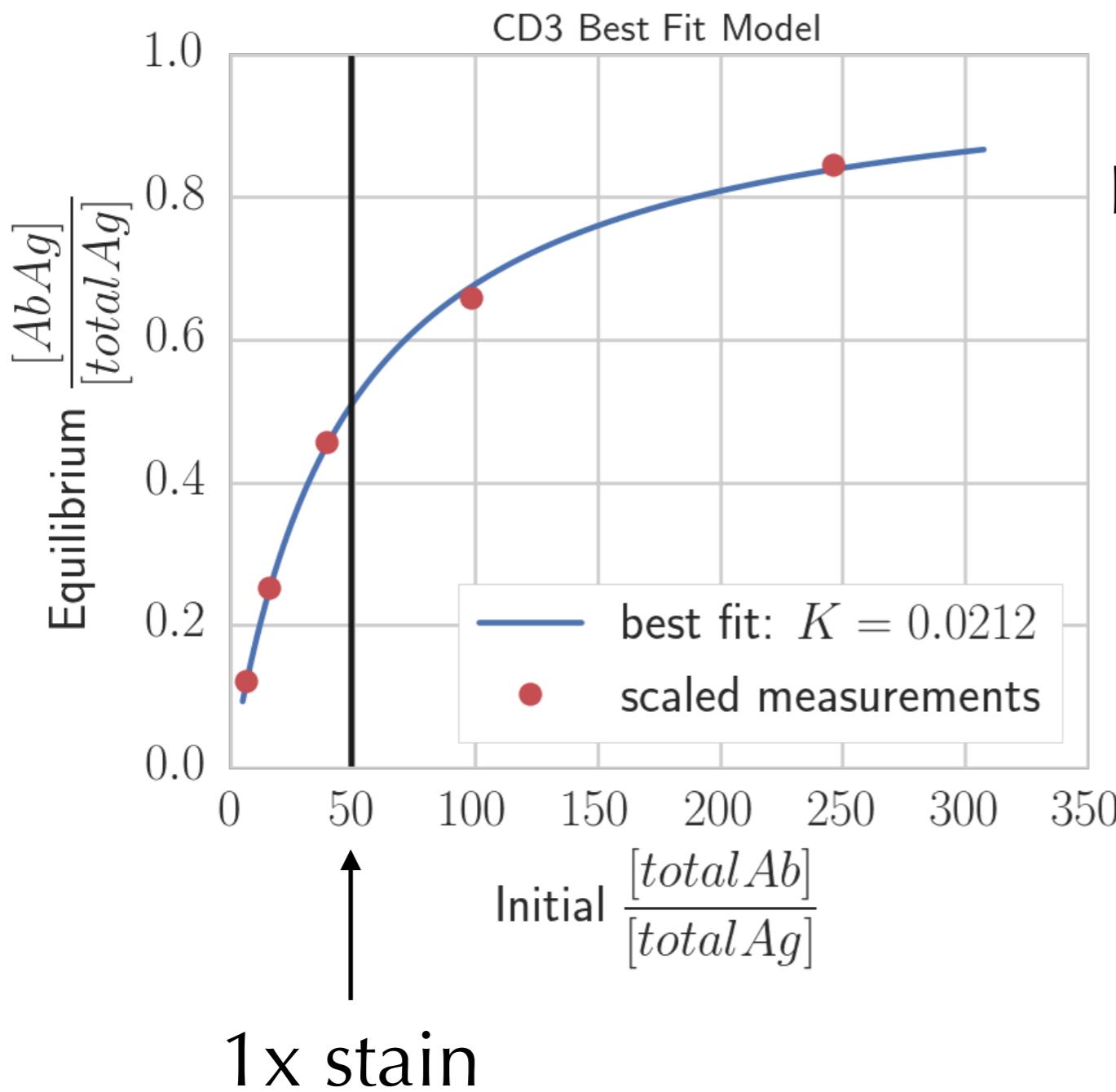


Increasing Antibody Concentration Increases High Population Expression



Sample: human cord blood

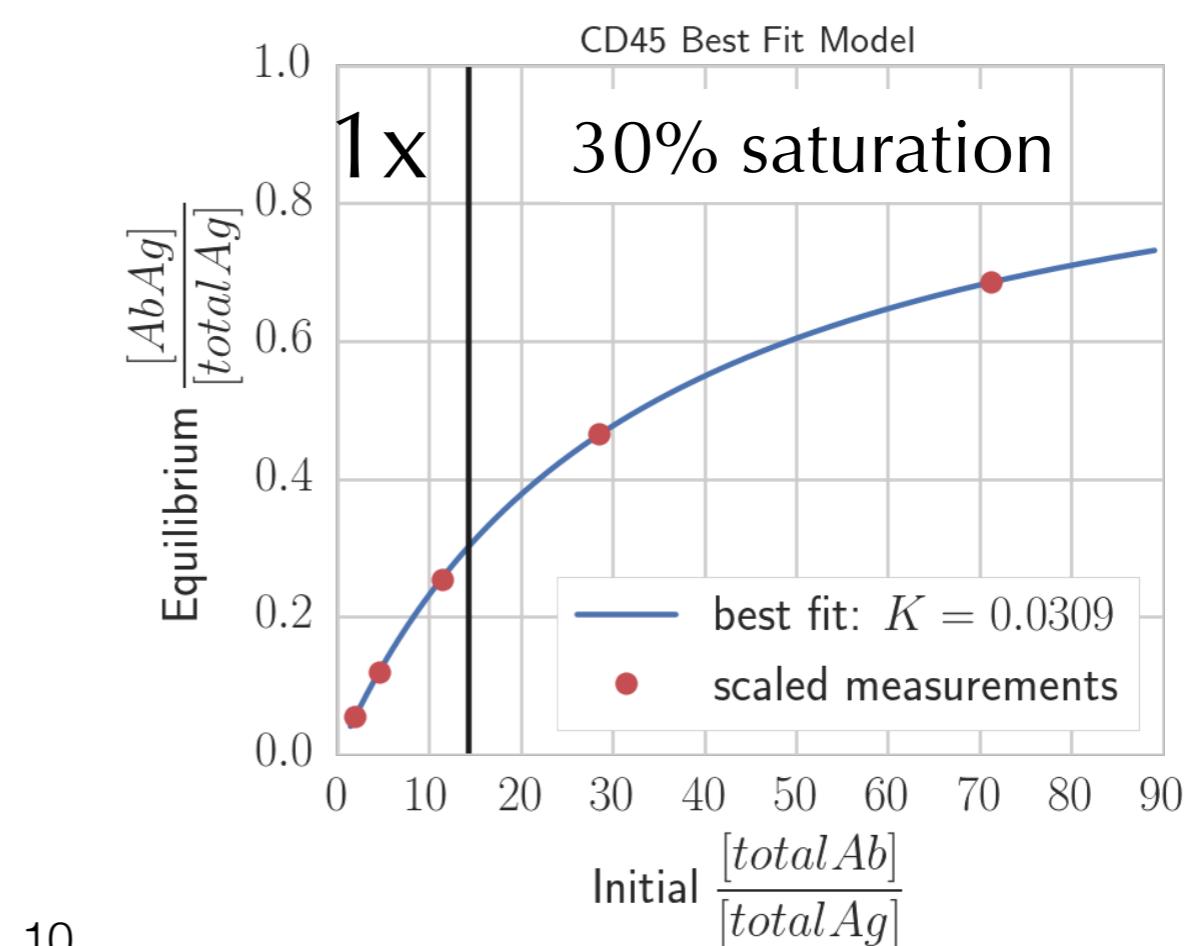
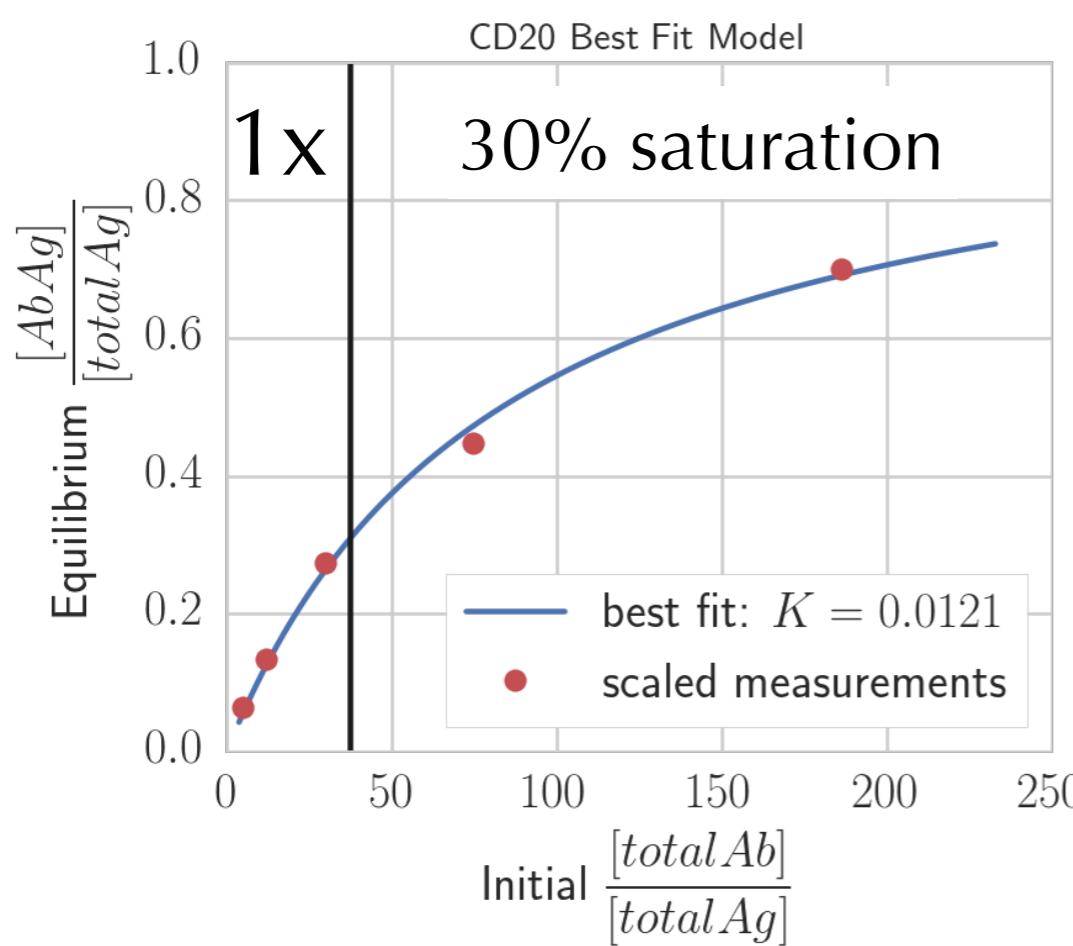
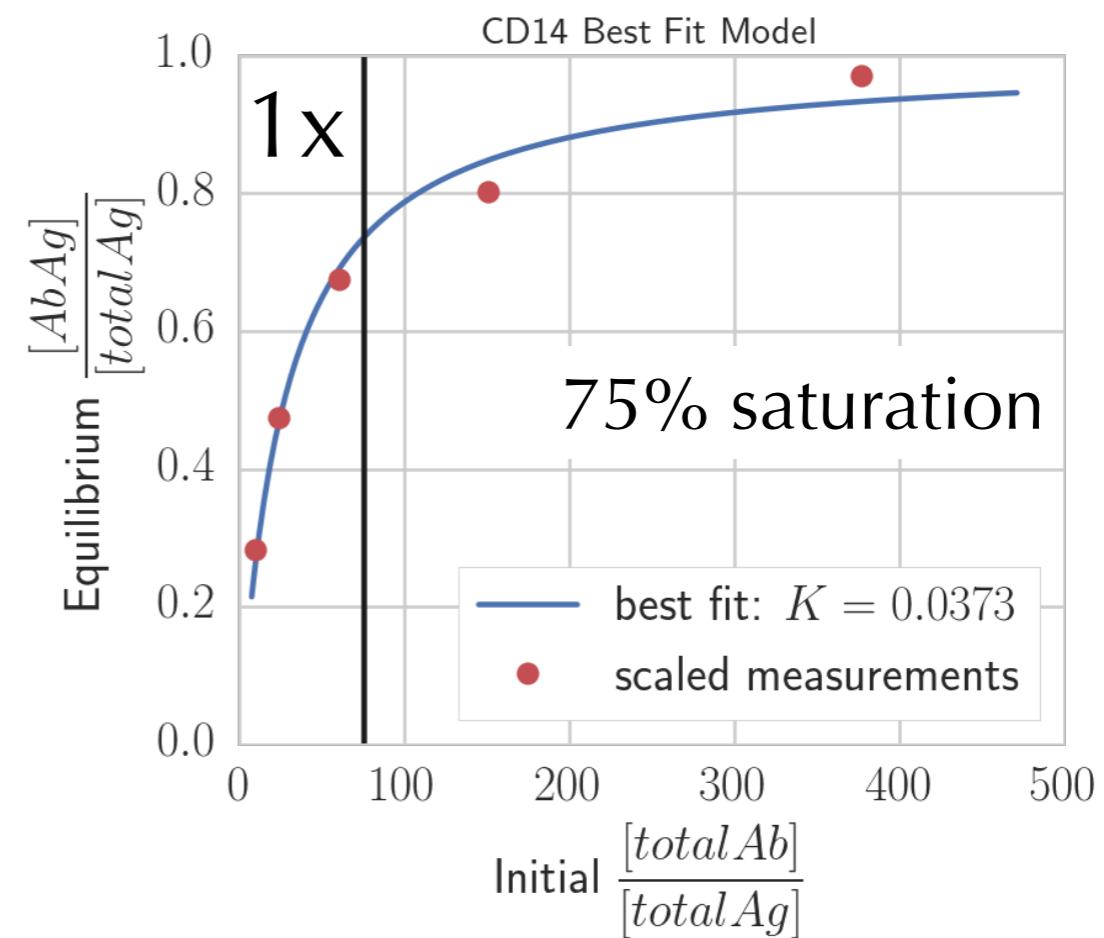
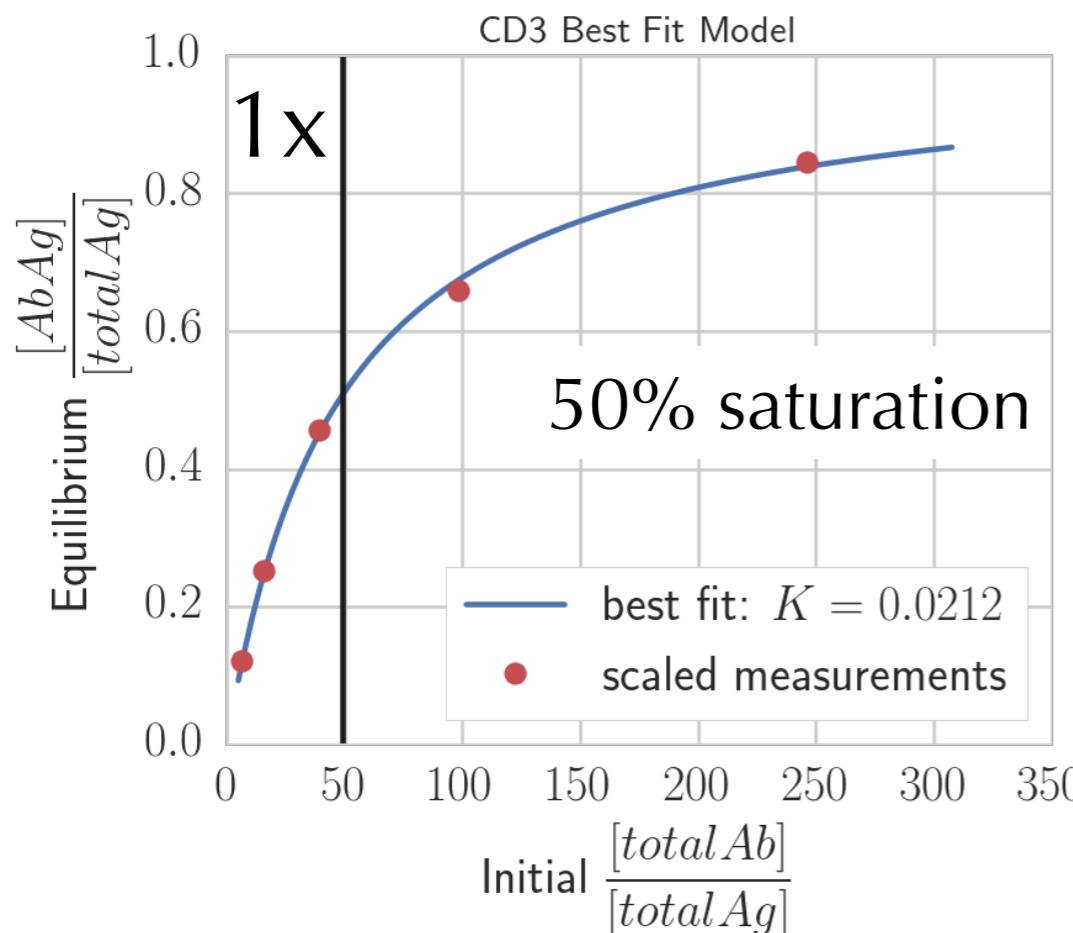
Model Fitting Suggests Experiment is Far From Saturation



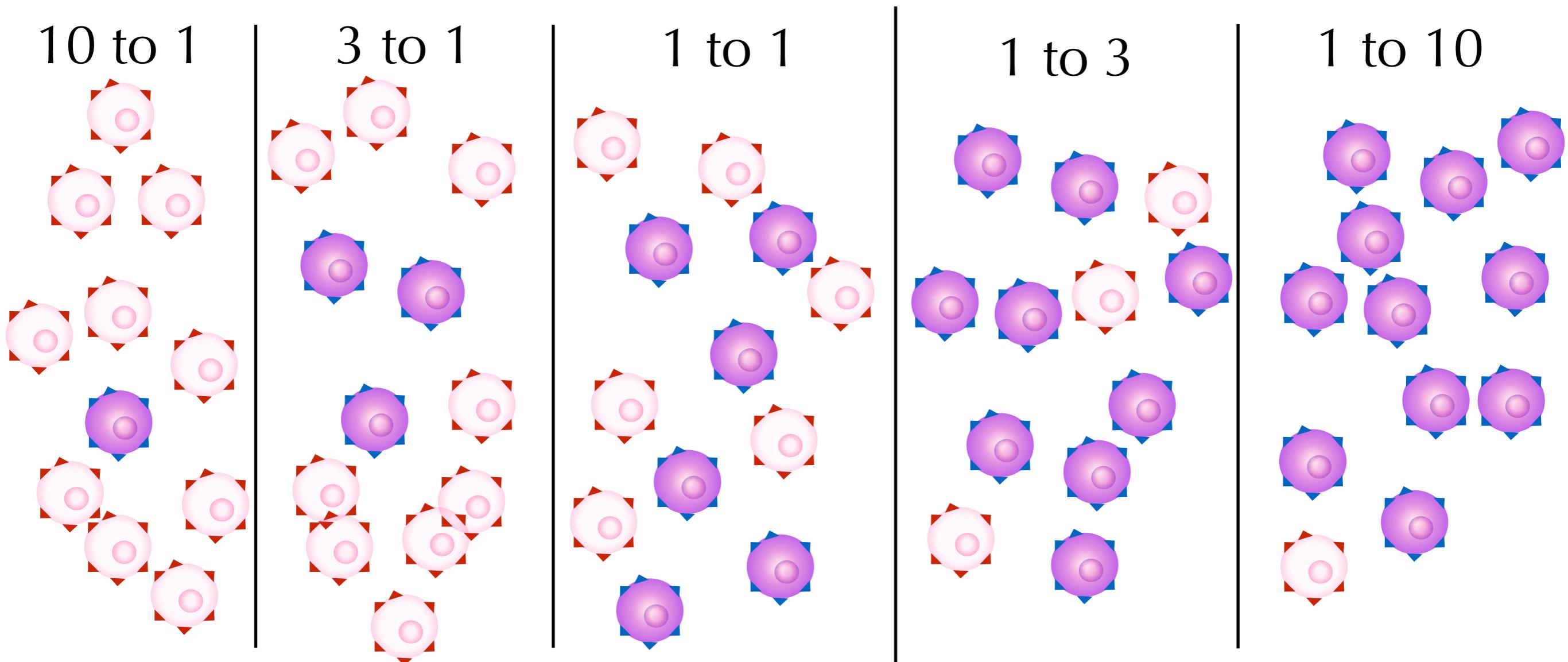
Free parameters:

- antibody-antigen ratio at 1x
- antigens per cell
- antibody rate constant K

Normal staining is at 50% saturation



Without Saturation, Variability Still Exists With Perfect Sample Preparation

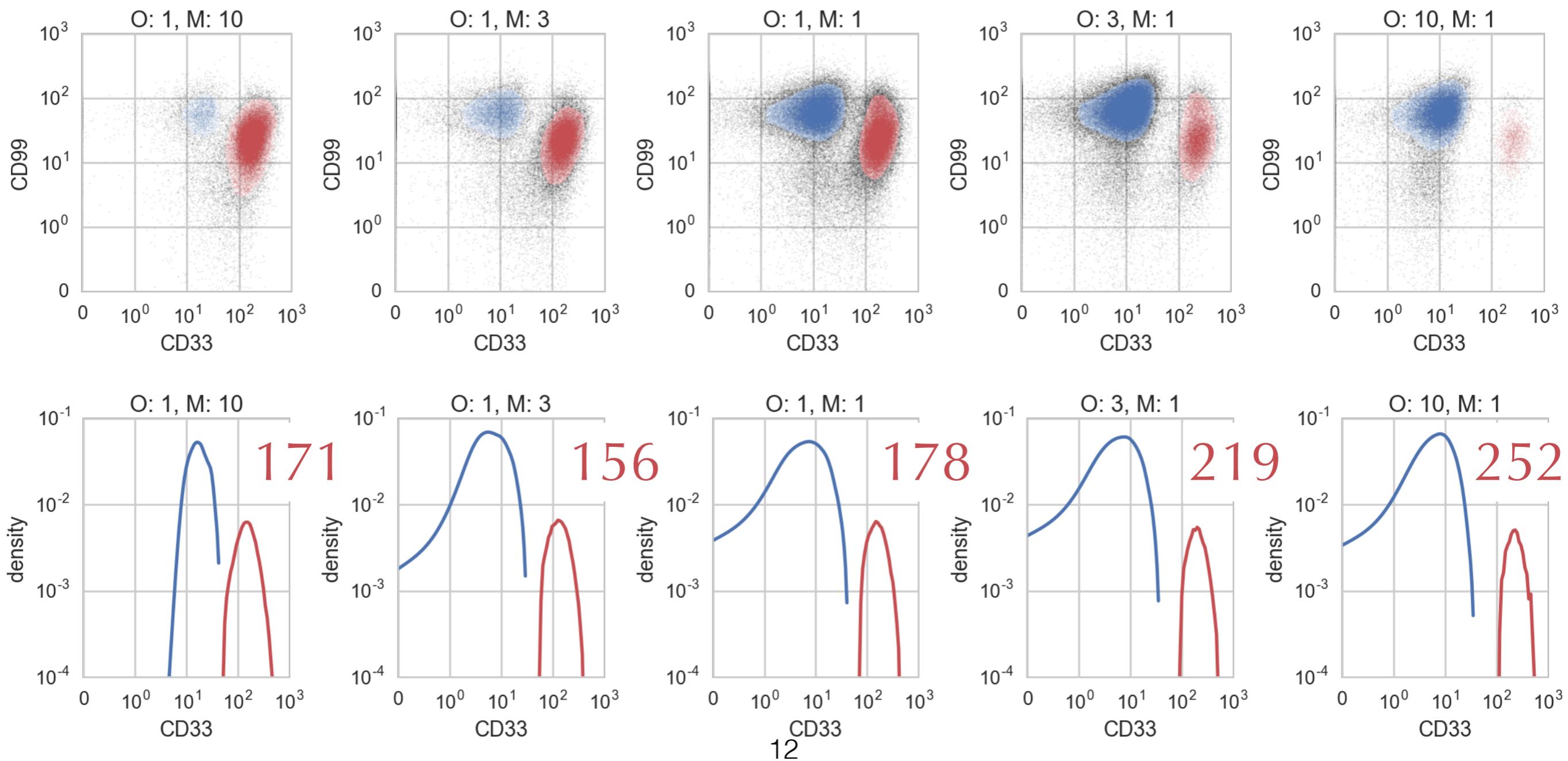


Hypothesis: with less antigen available,
more antibody will bind to each cell

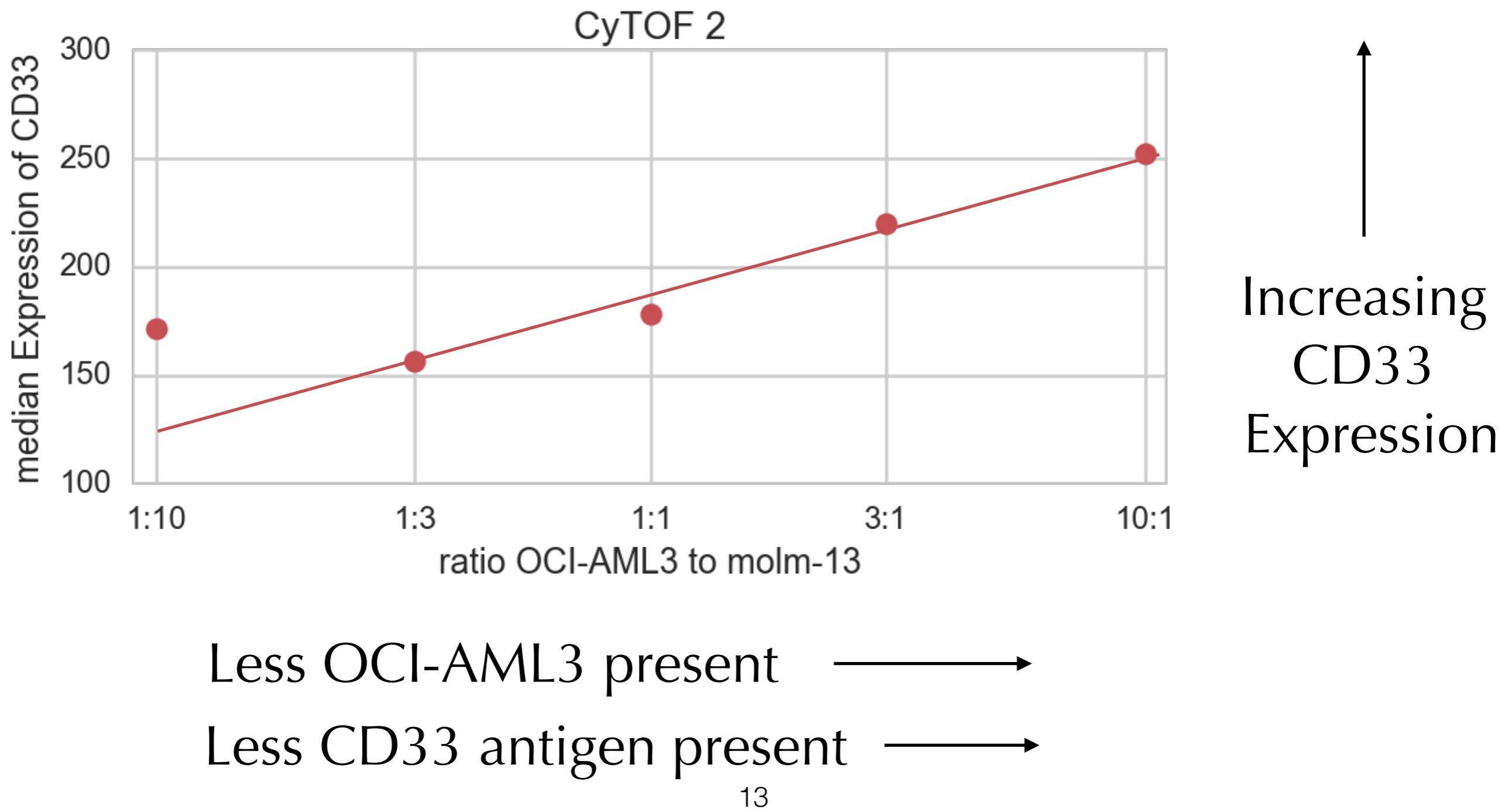
Decreasing Antigen Density Increases Median Expression

M: molm-13

O: OCI-AML3



Expression in identically prepared samples differs if cell ratios change



Solution: CLEAN

Cell

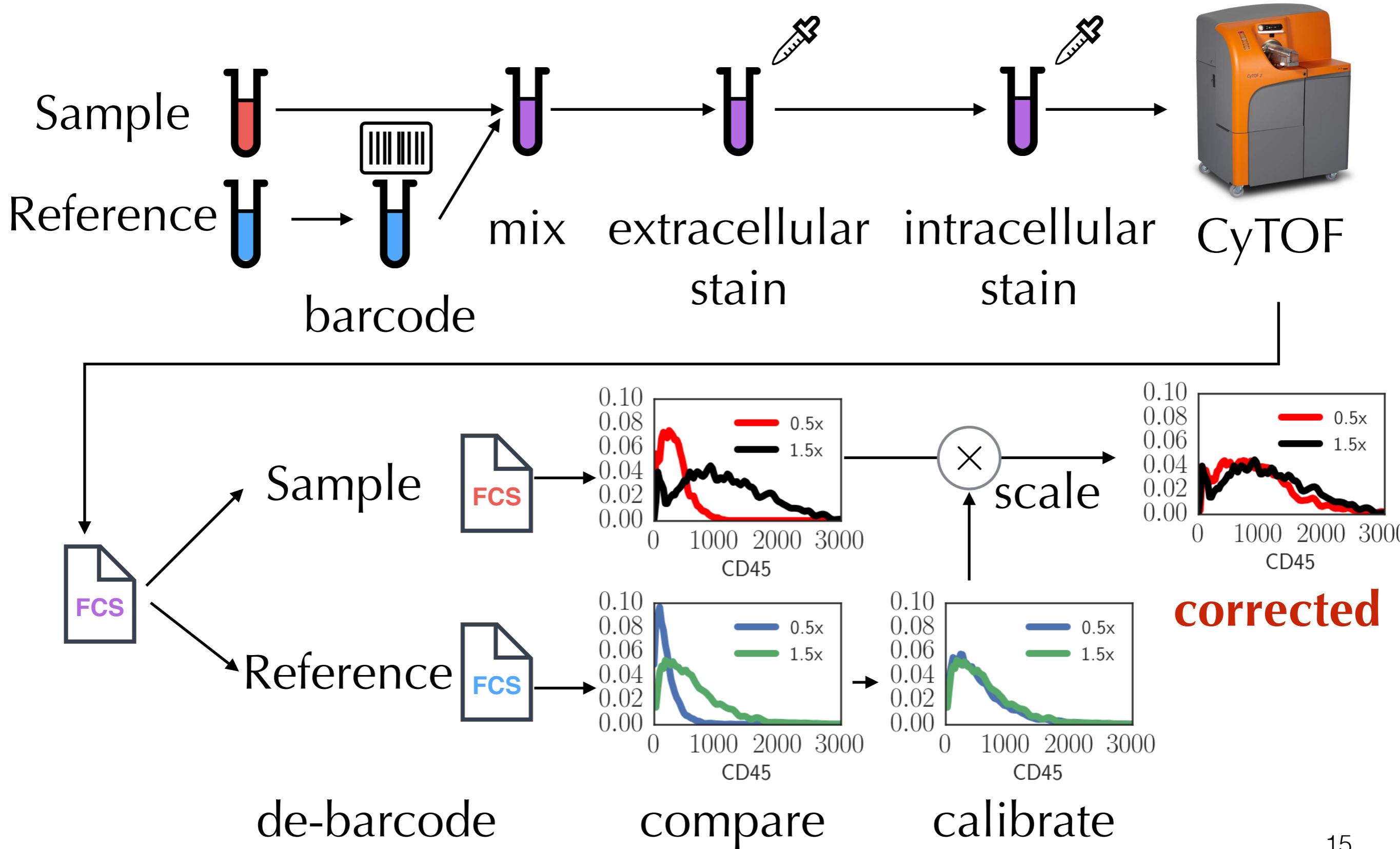
Level

Event

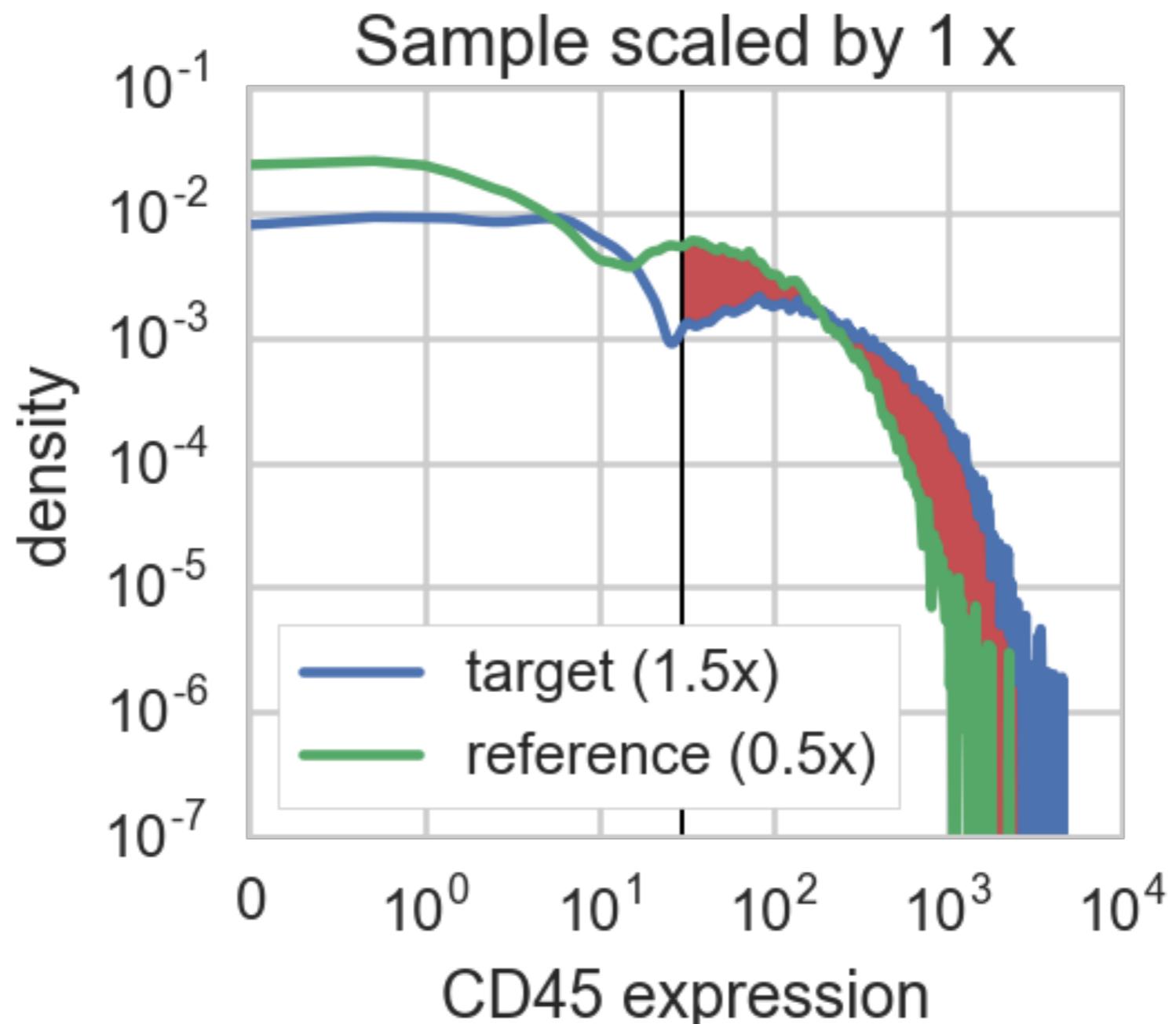
Abnormality

Normalization

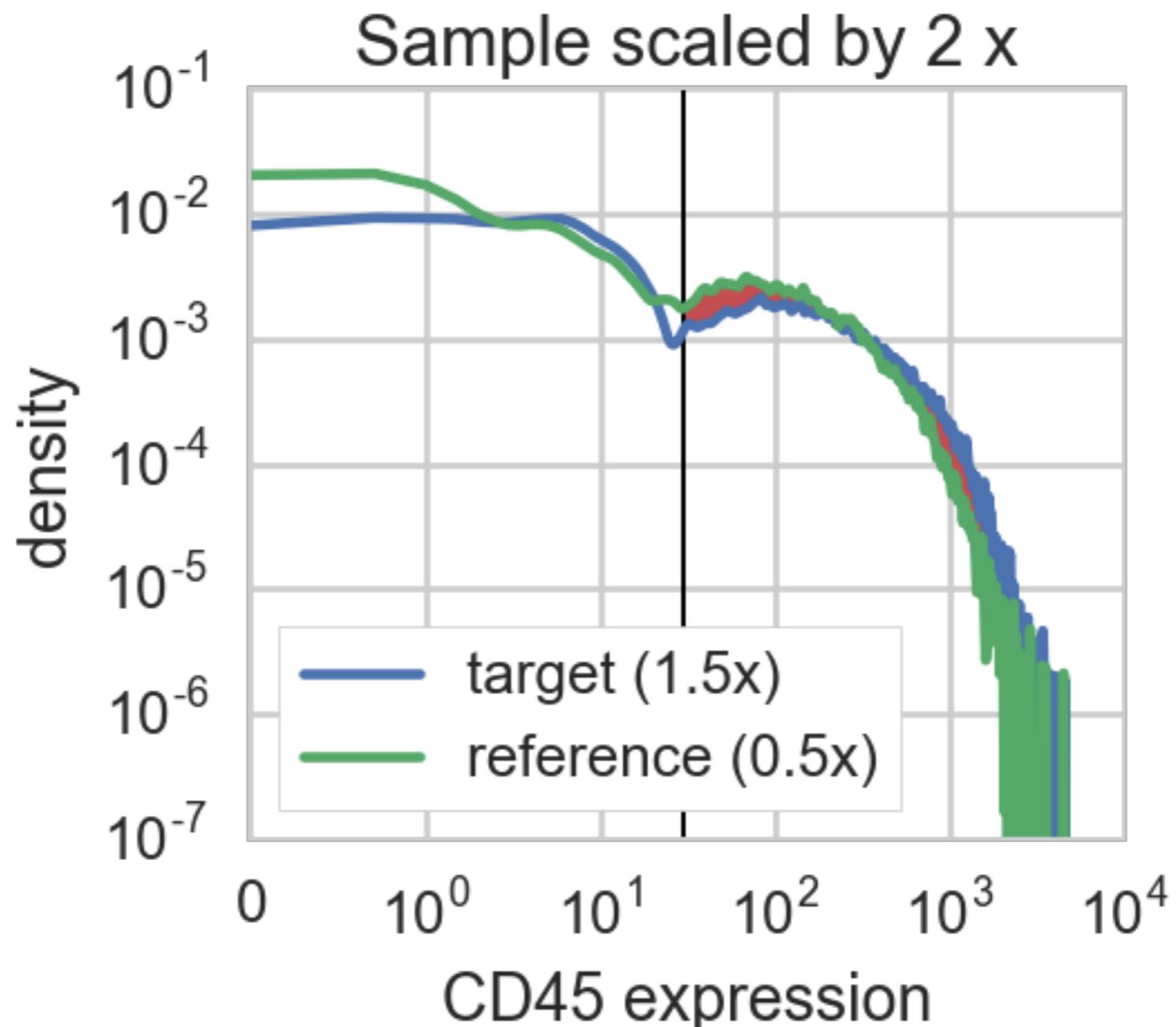
How CLEAN Works



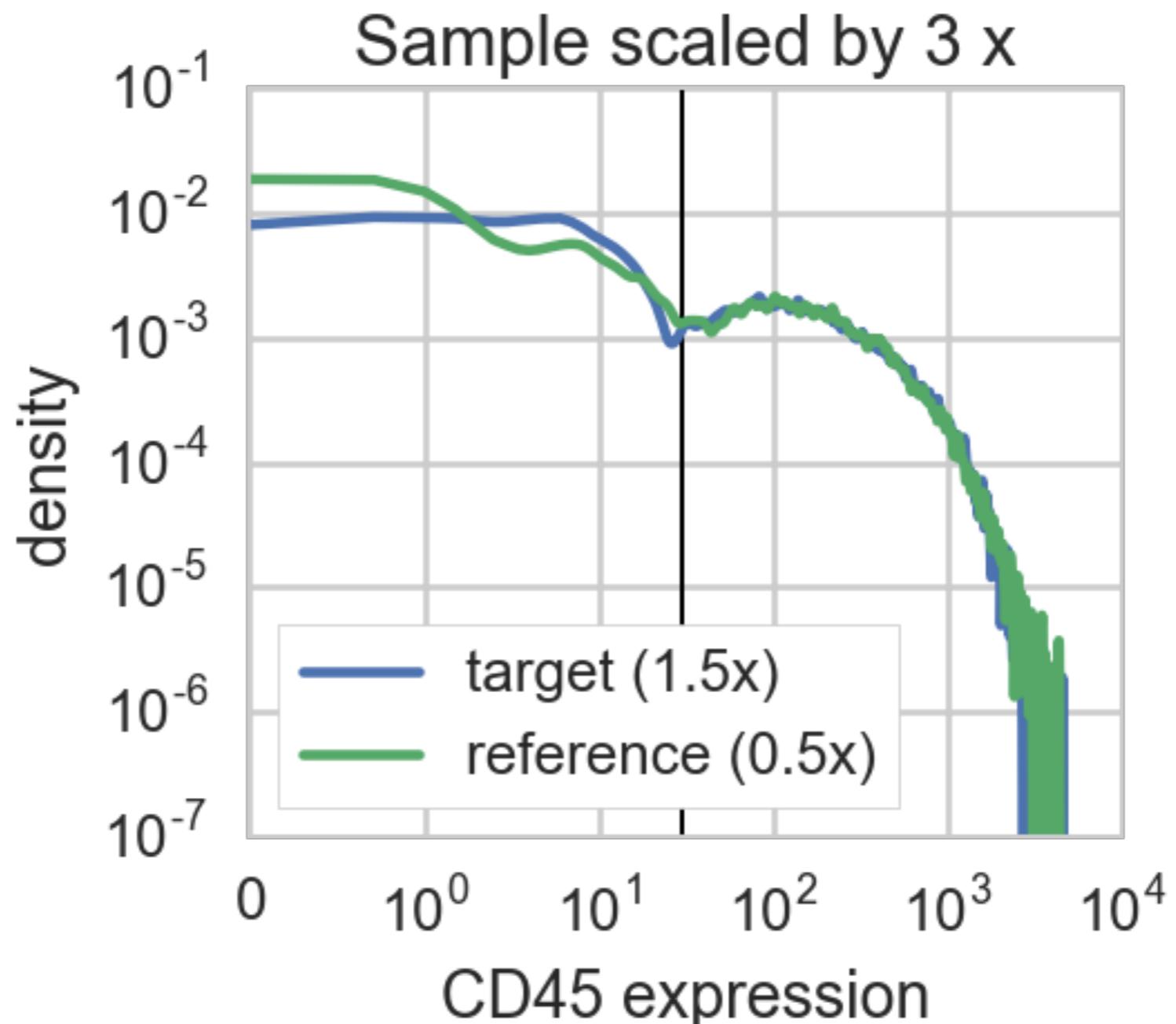
Scaling Step



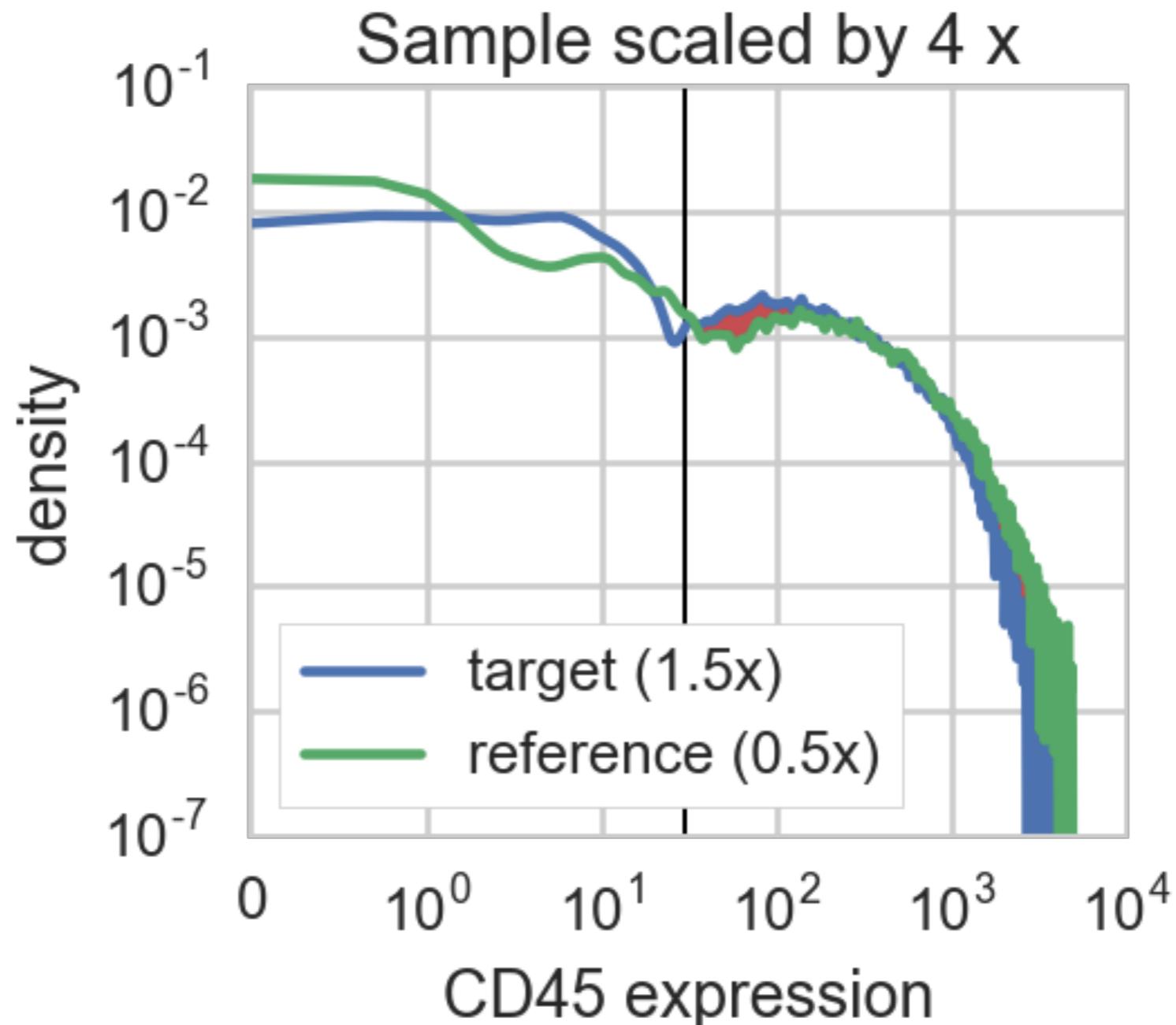
Scaling Step



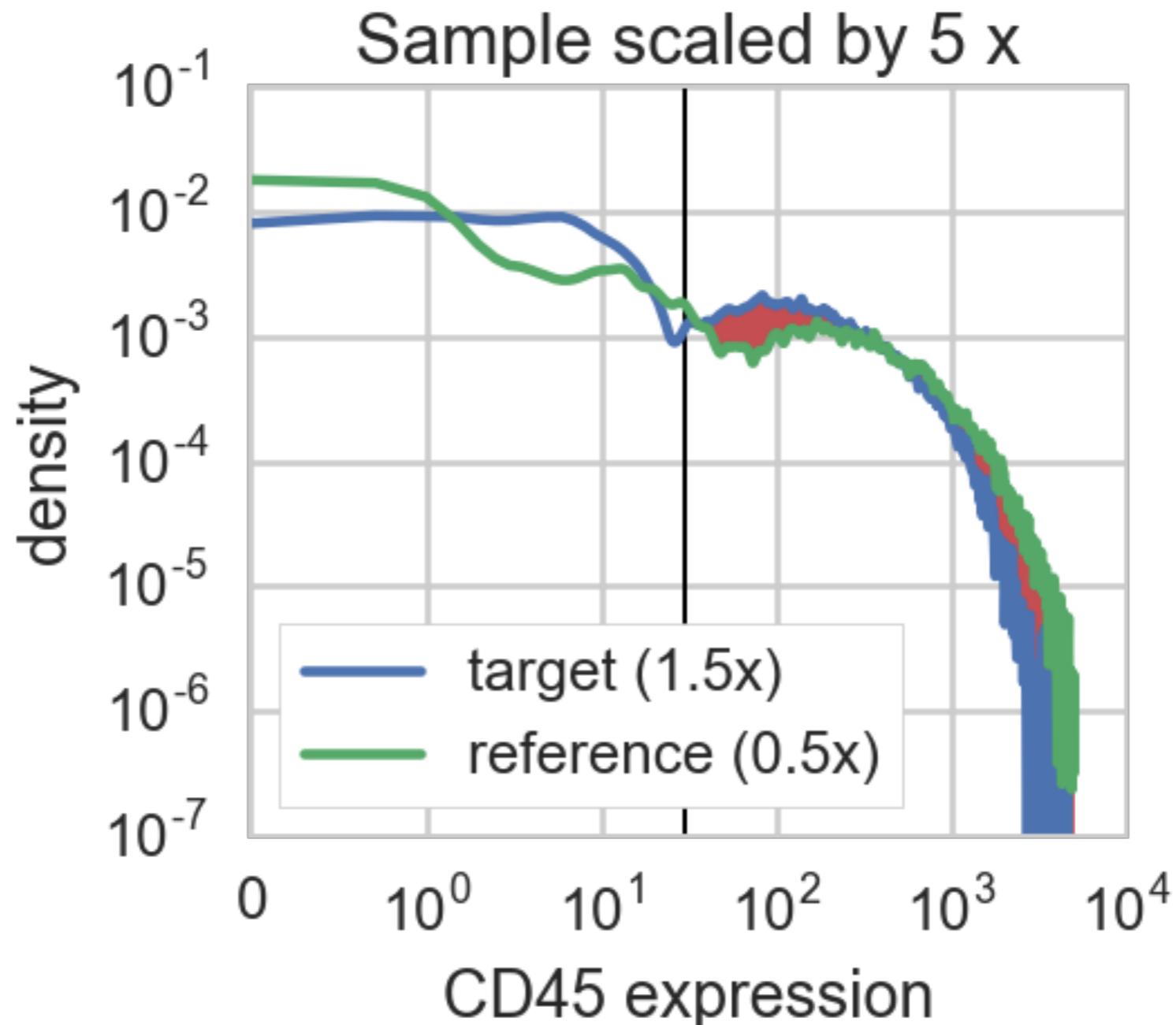
Scaling Step



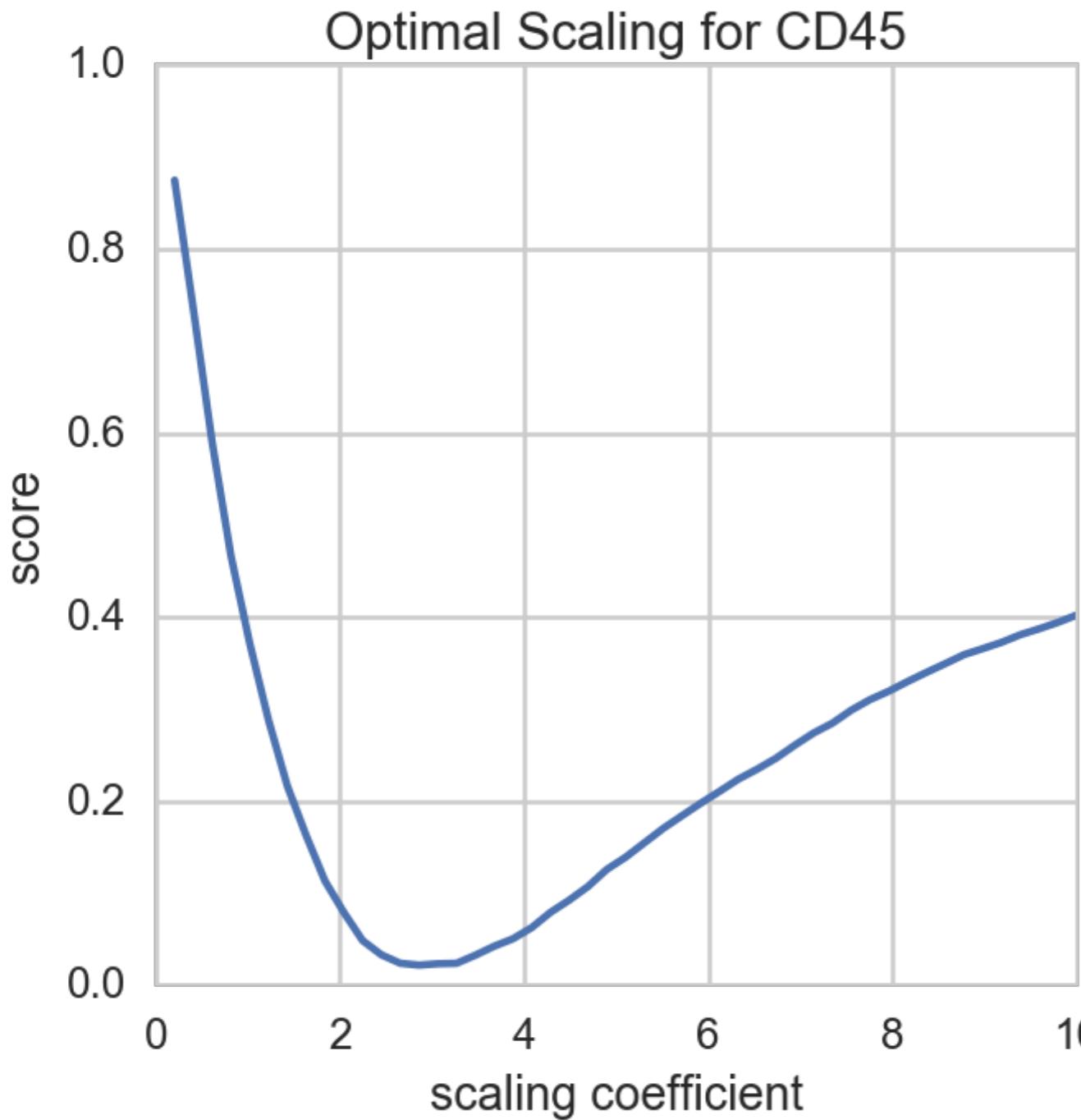
Scaling Step



Scaling Step



Scaling Score is Integral of Mismatch of Densities



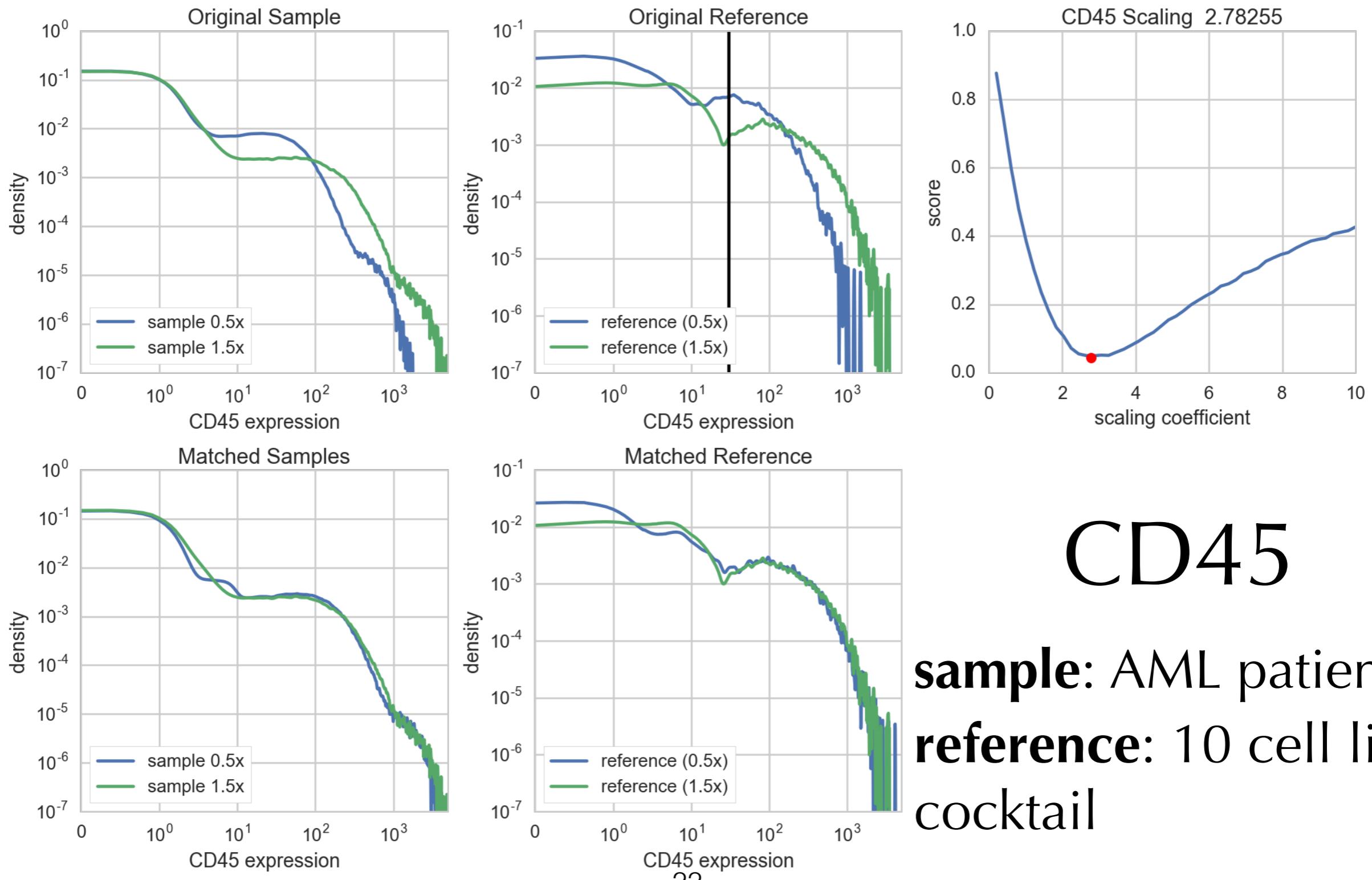
$$\frac{\min_a \int_{x_{\min}}^{\infty} [p(x) - aq(x)]^2 dx}{\int_{x_{\min}}^{\infty} [p(x)]^2 dx}$$

Kernel Density Estimates

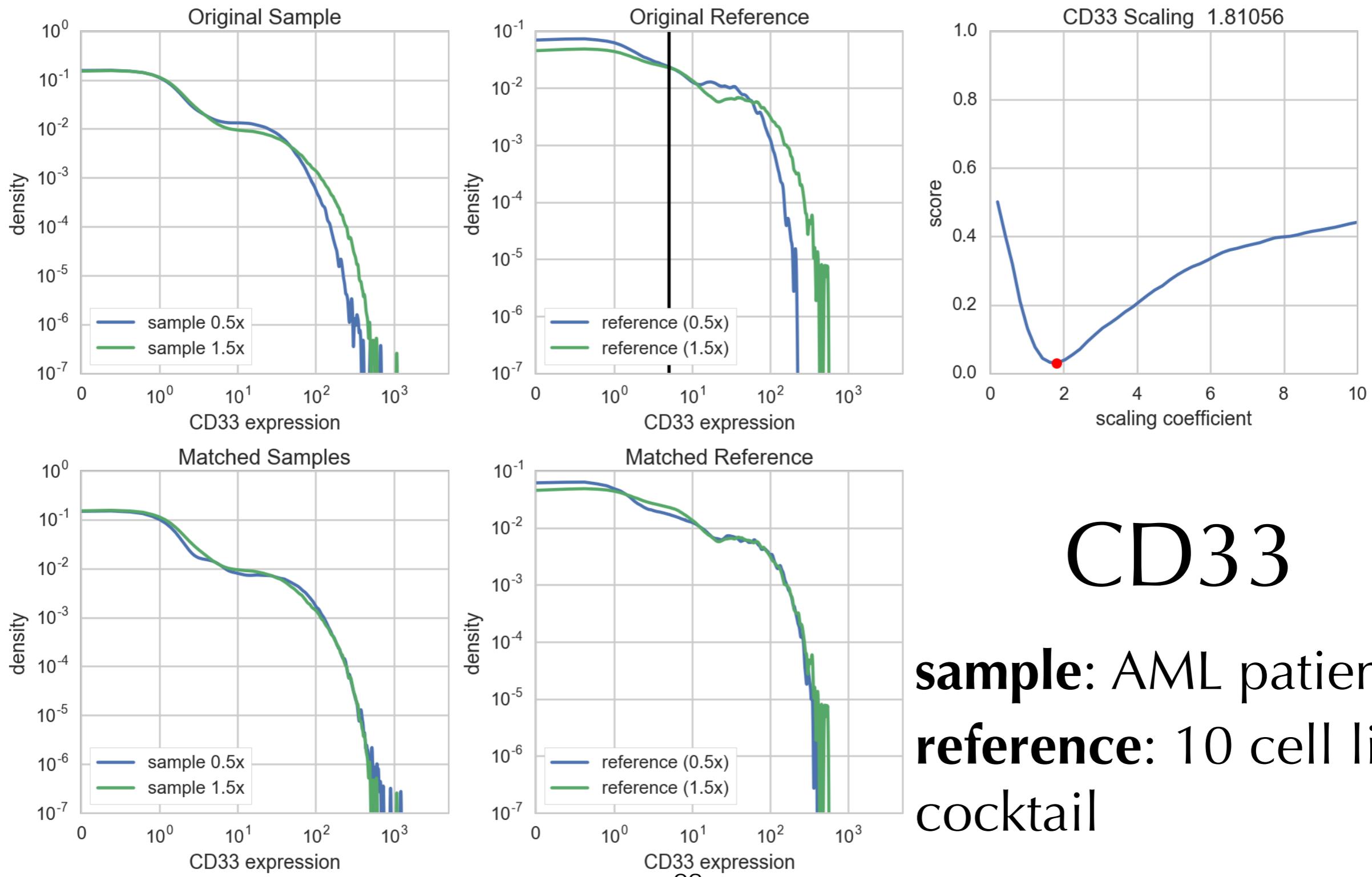
$$p(x) \propto \sum_{k=1}^{n_x} e^{-(x_k - x)^2 / (\alpha + \beta x_k)}$$

$$q(x) \propto \sum_{k=1}^{n_y} e^{-(y_k - x)^2 / (\alpha + \beta y_k)}$$

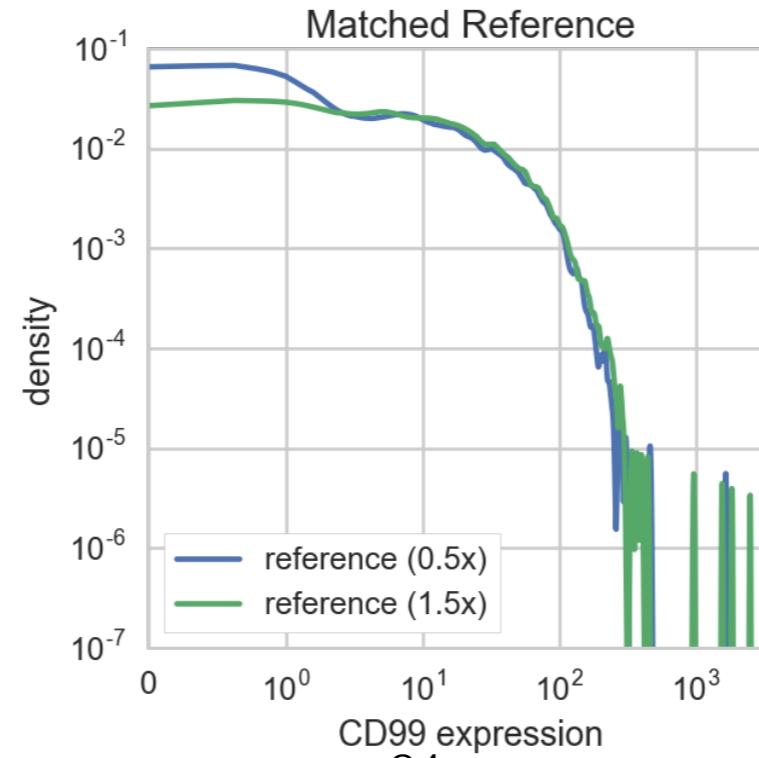
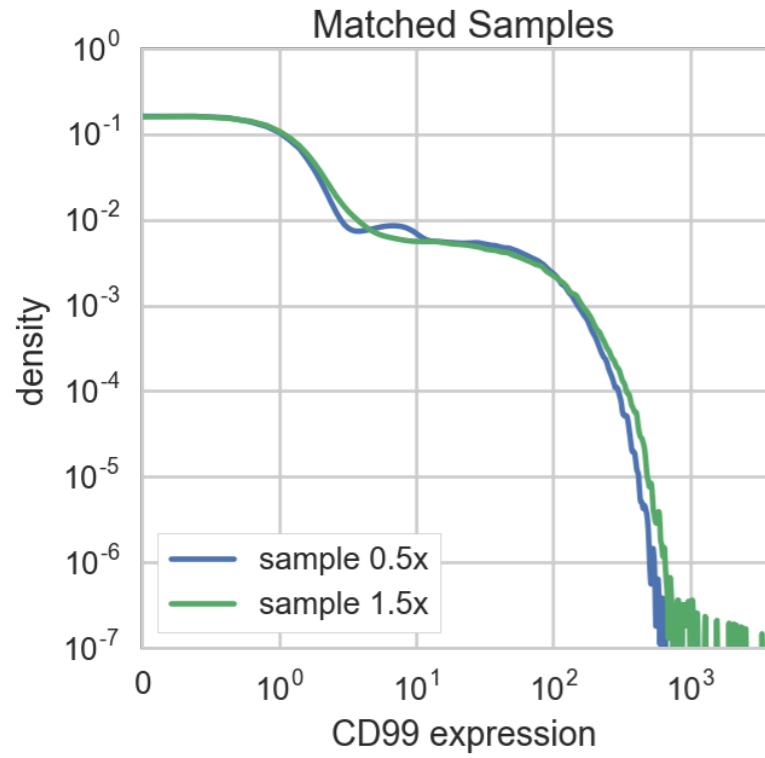
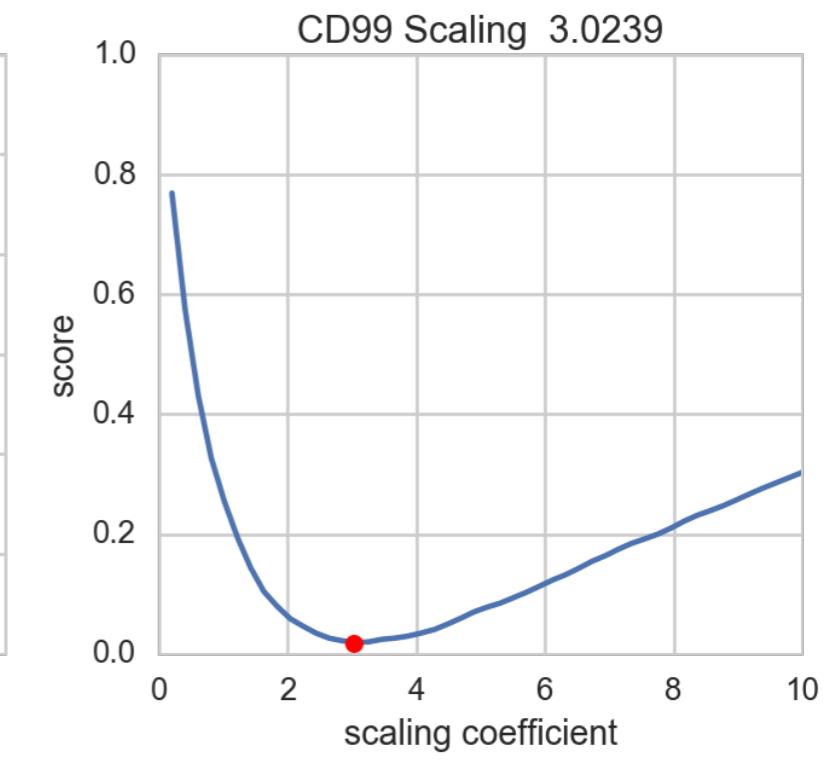
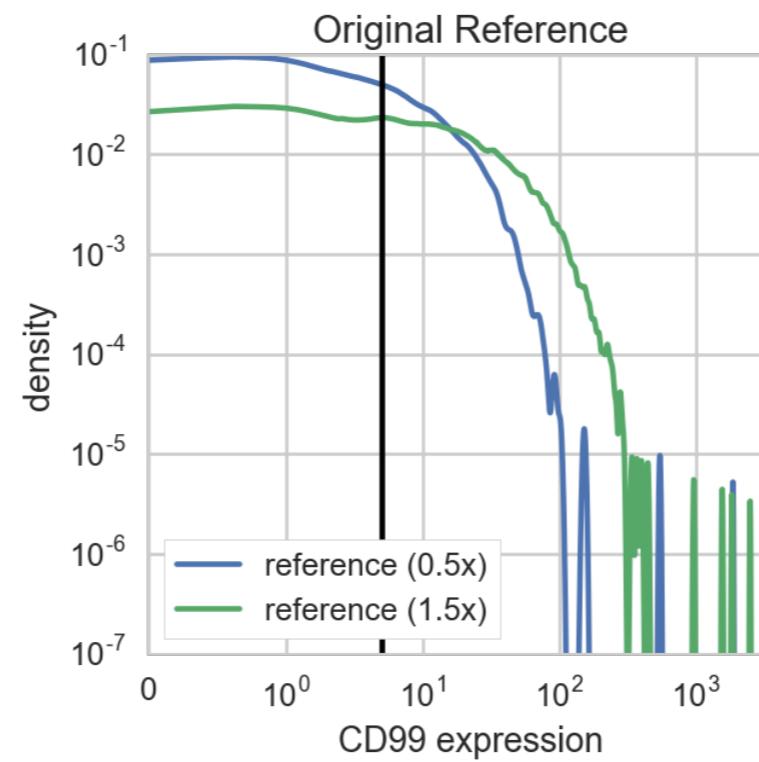
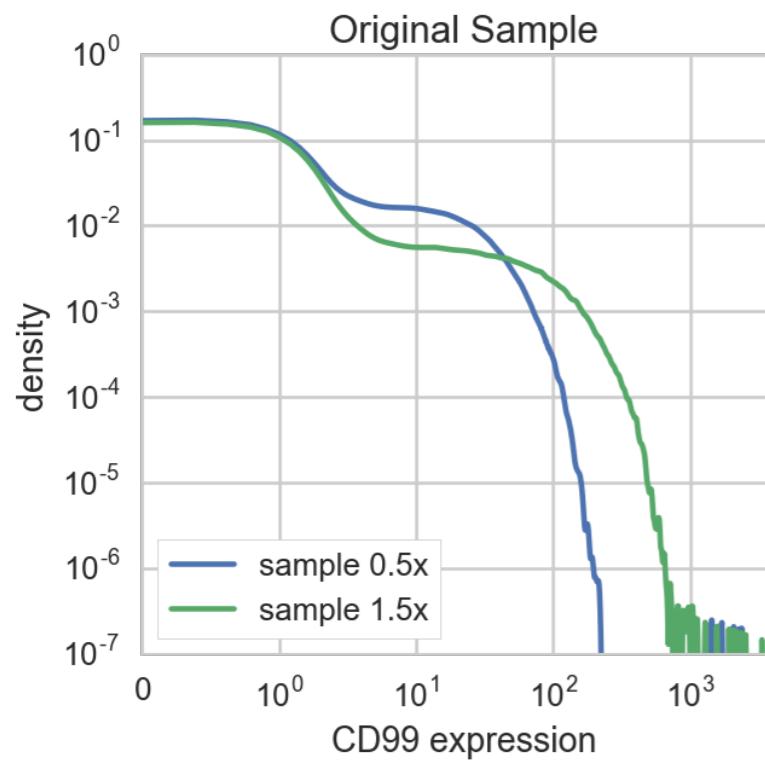
Validating CLEAN with Identical Samples at 0.5x and 1.5x stain



Validating CLEAN with Identical Samples at 0.5x and 1.5x stain



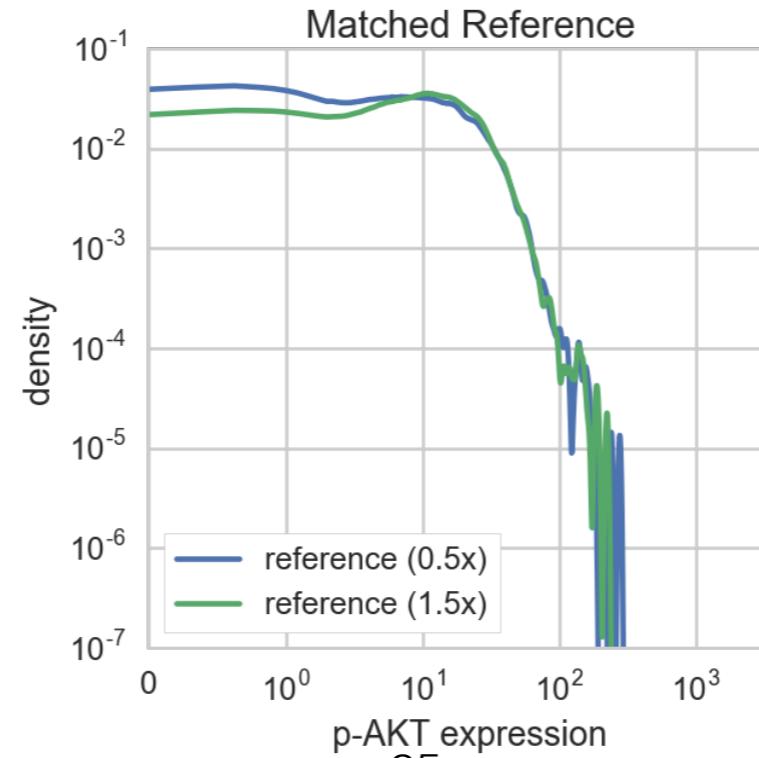
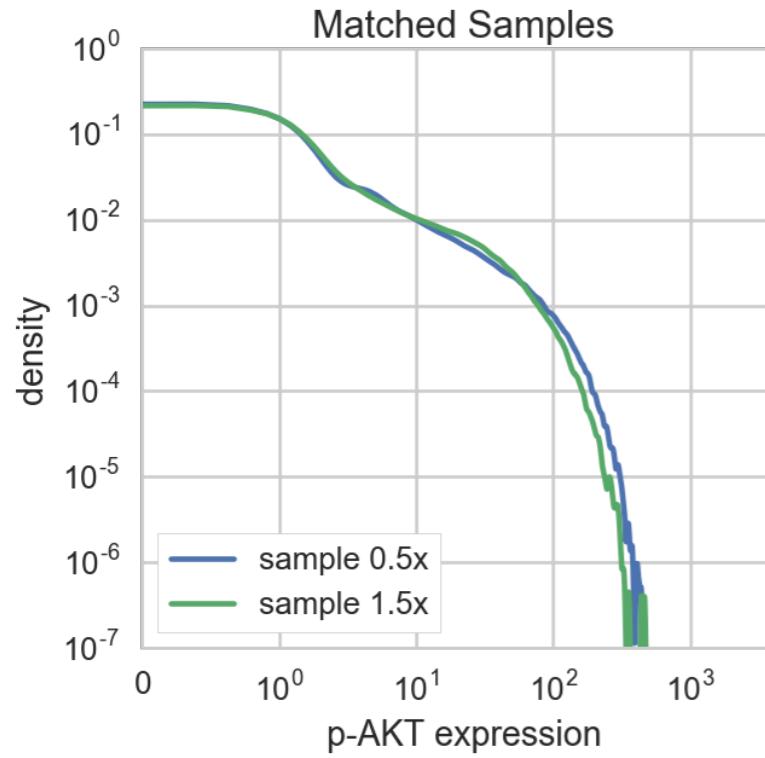
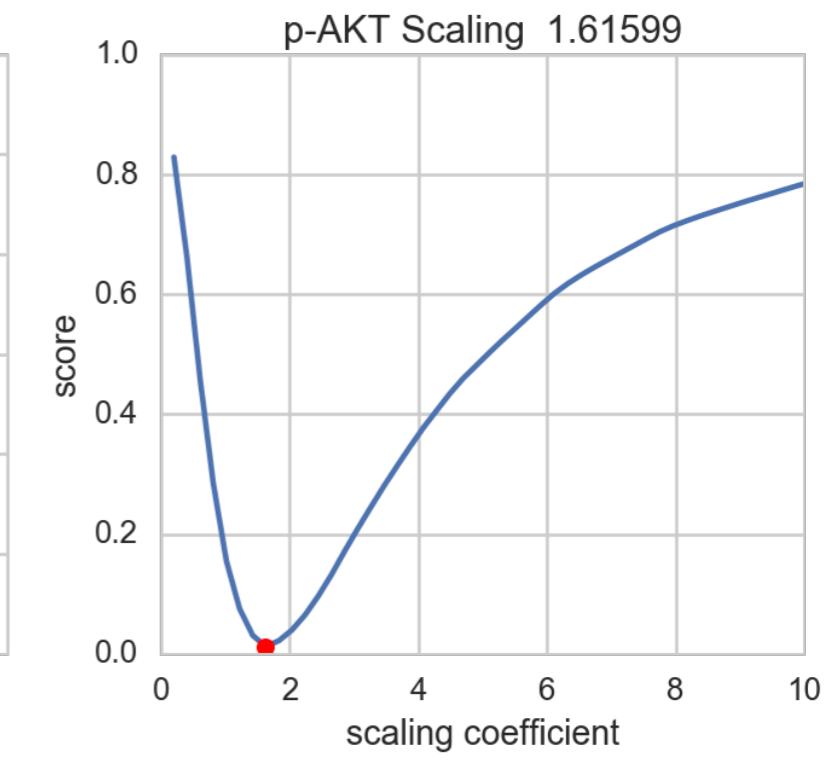
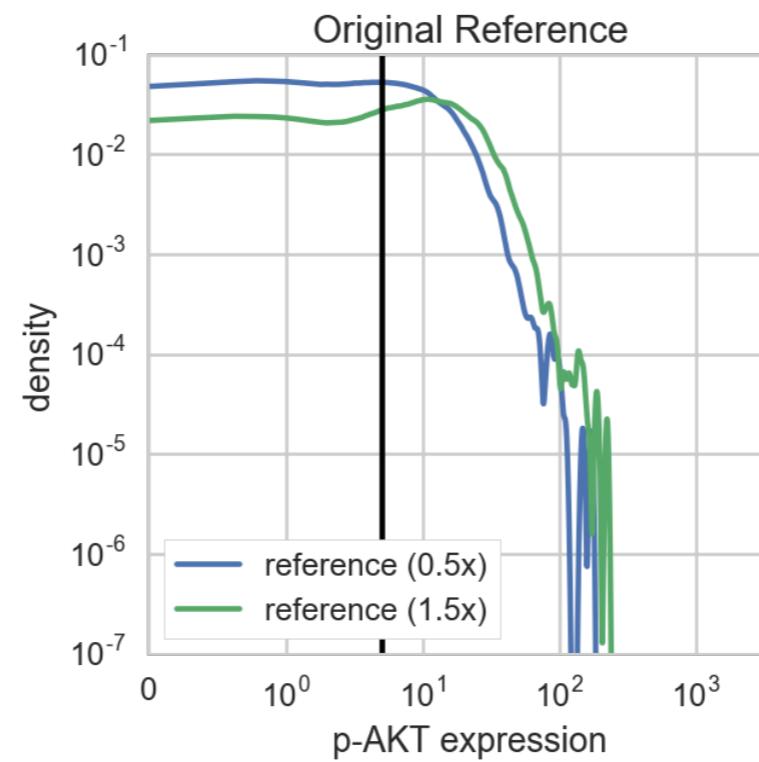
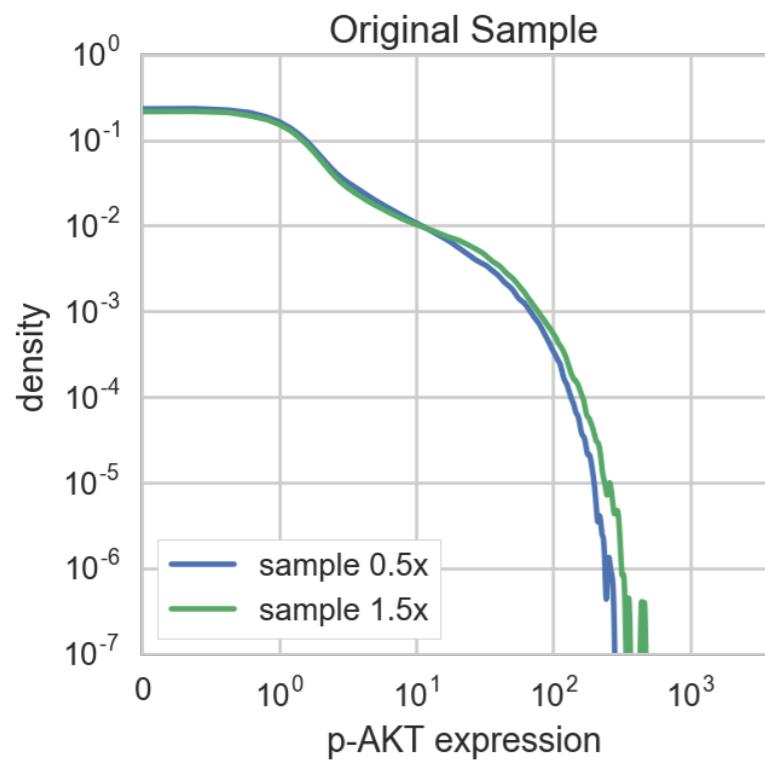
Validating CLEAN with Identical Samples at 0.5x and 1.5x stain



CD99

sample: AML patient
reference: 10 cell line cocktail

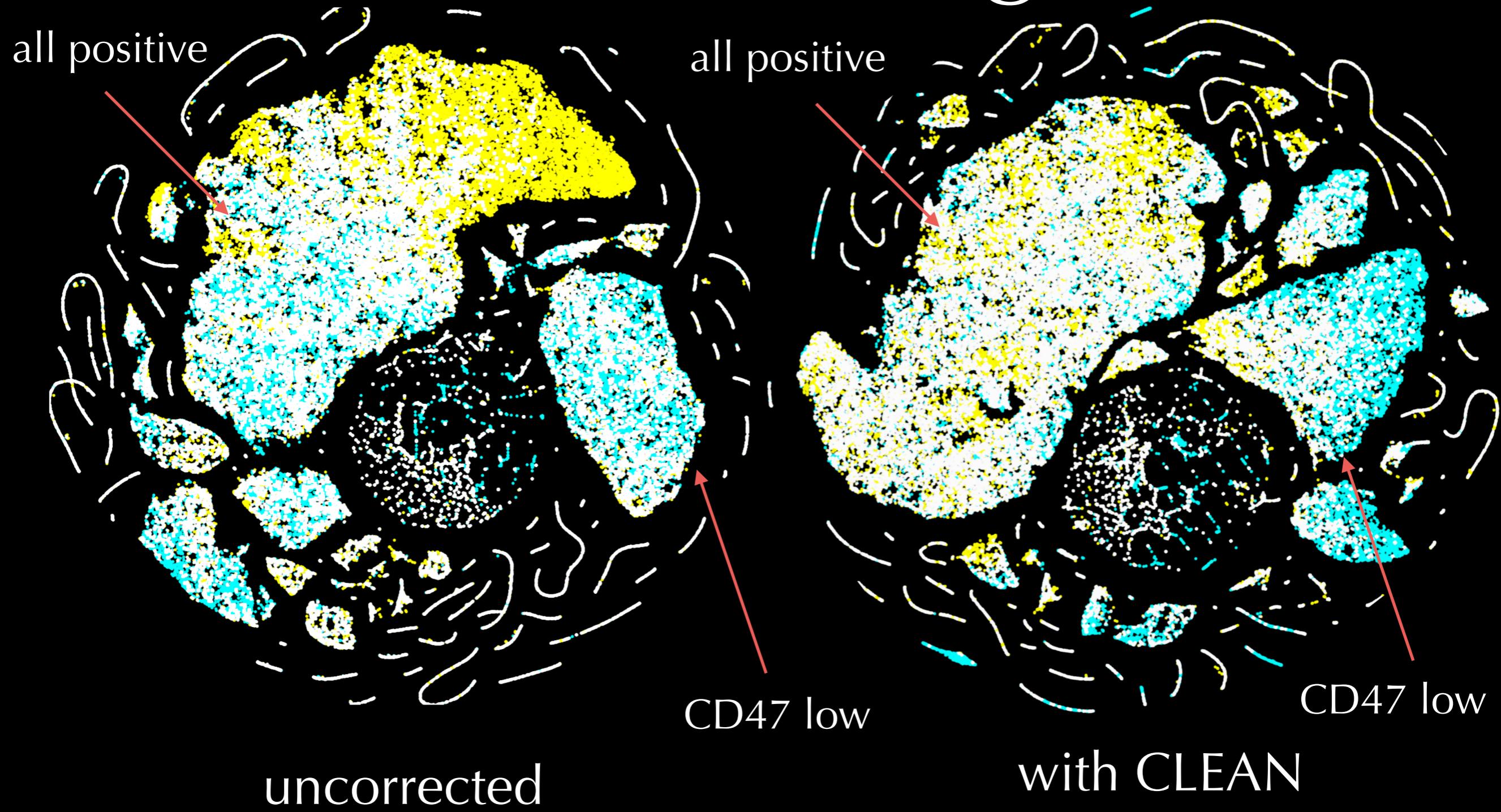
Validating CLEAN with Identical Samples at 0.5x and 1.5x stain



p-AKT

sample: AML patient
reference: 10 cell line cocktail

CLEAN Yields Better Embeddings



CLEAN-ing up CyTOF

CLEAN corrects mean expression changes due to:

- Staining variations
- Changing cell frequencies, e.g., MRD in leukemia

Future Work:

- Standardization of controls
- Application to ongoing clinical trials

Acknowledgements

Funding

- University of Texas System Proteomics Core Facility Network
- NCI Cancer Center Support Grant P30 CA016672
- NCI P01CA49639
- Paul and Mary Haas Chair in Genetics

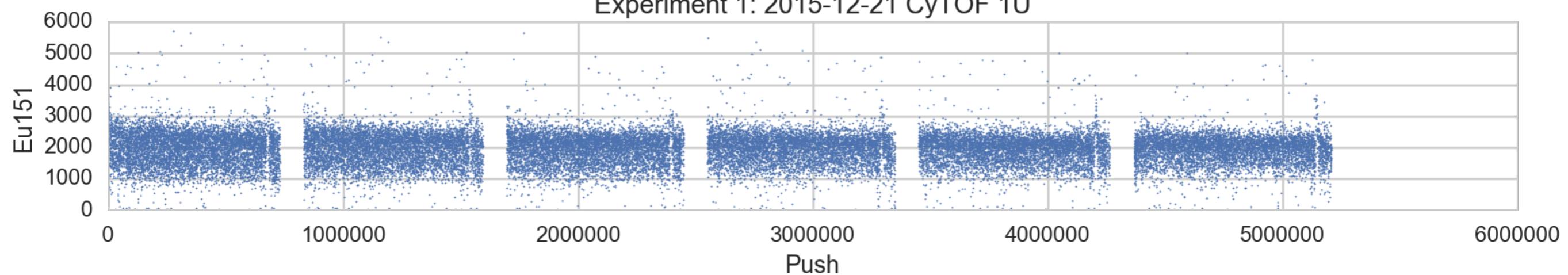
Experimental Data

- Lina Han
- Jeff Sun
- Ahmed AlRawi

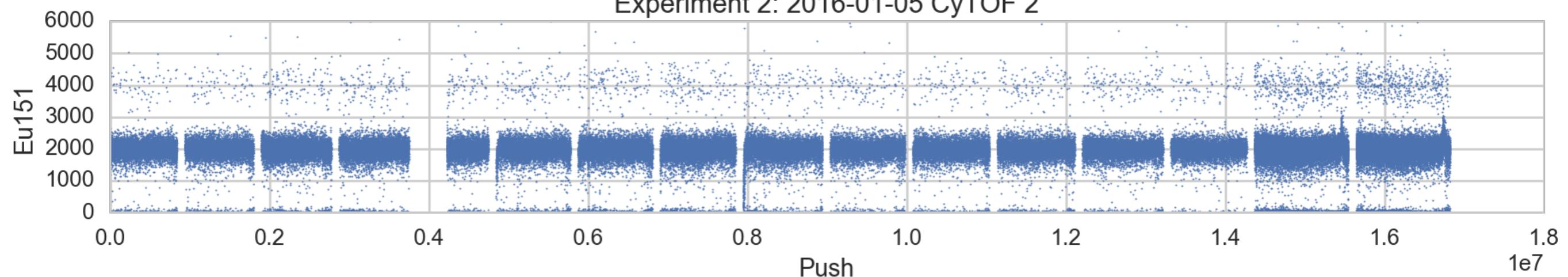
Additional Material

Bead Normalization

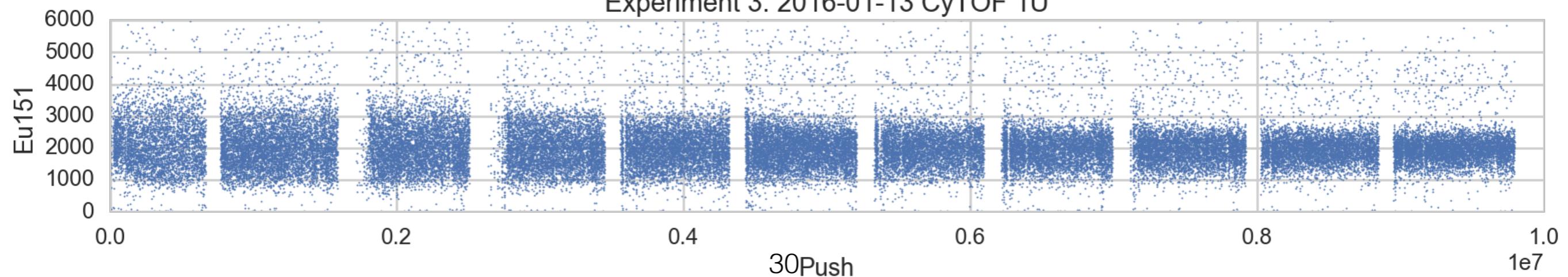
Experiment 1: 2015-12-21 CyTOF 1U



Experiment 2: 2016-01-05 CyTOF 2

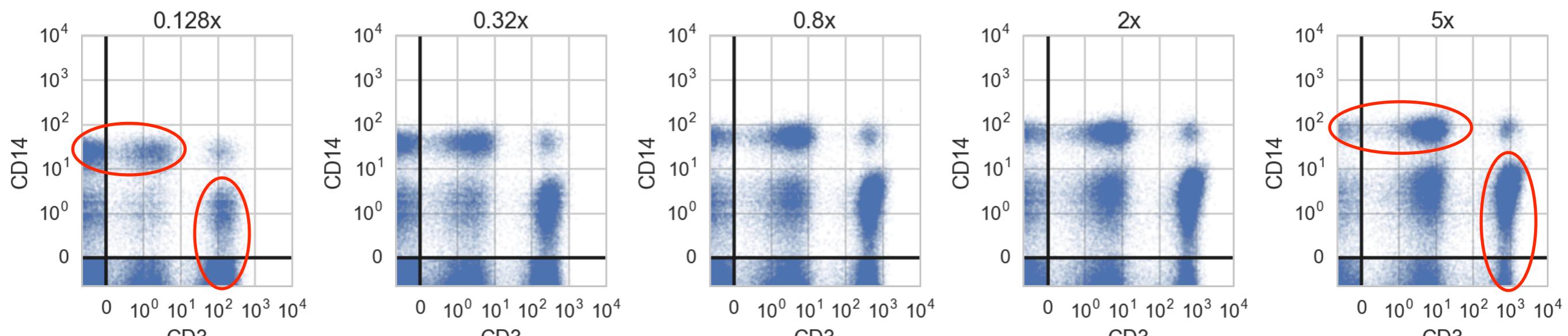


Experiment 3: 2016-01-13 CyTOF 1U



Increasing Stain Increases Background

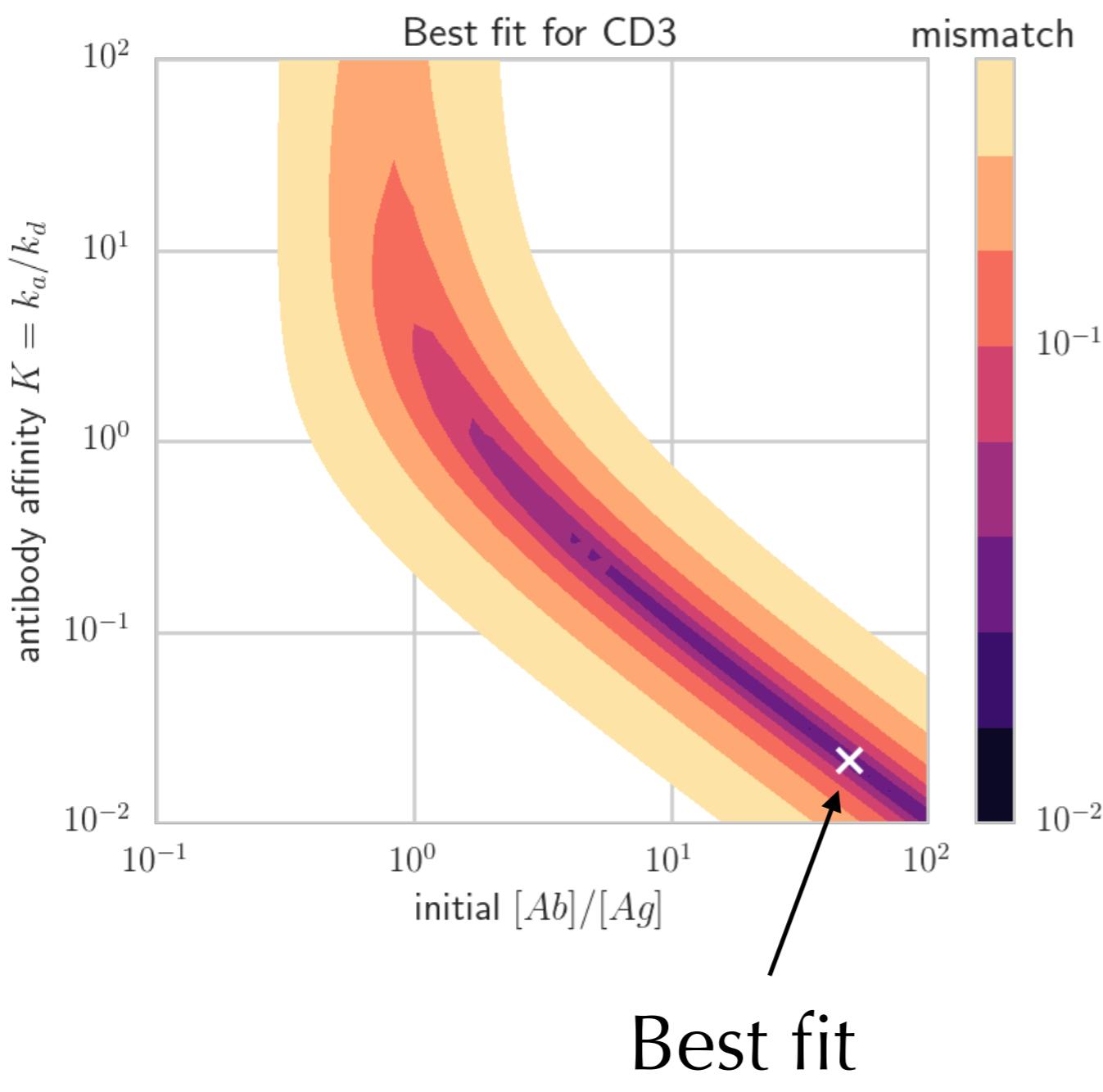
Increasing Stain



Background ~2 counts

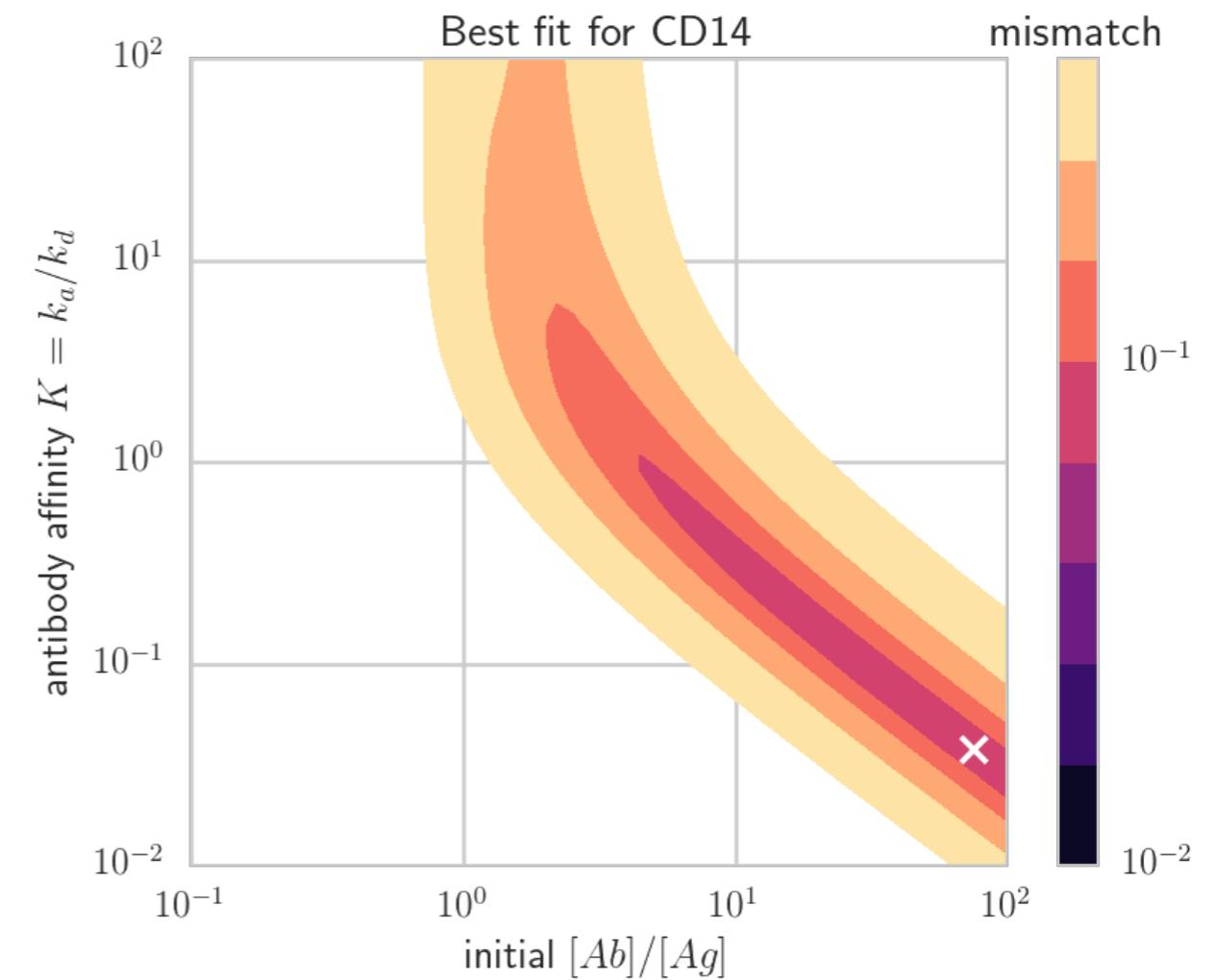
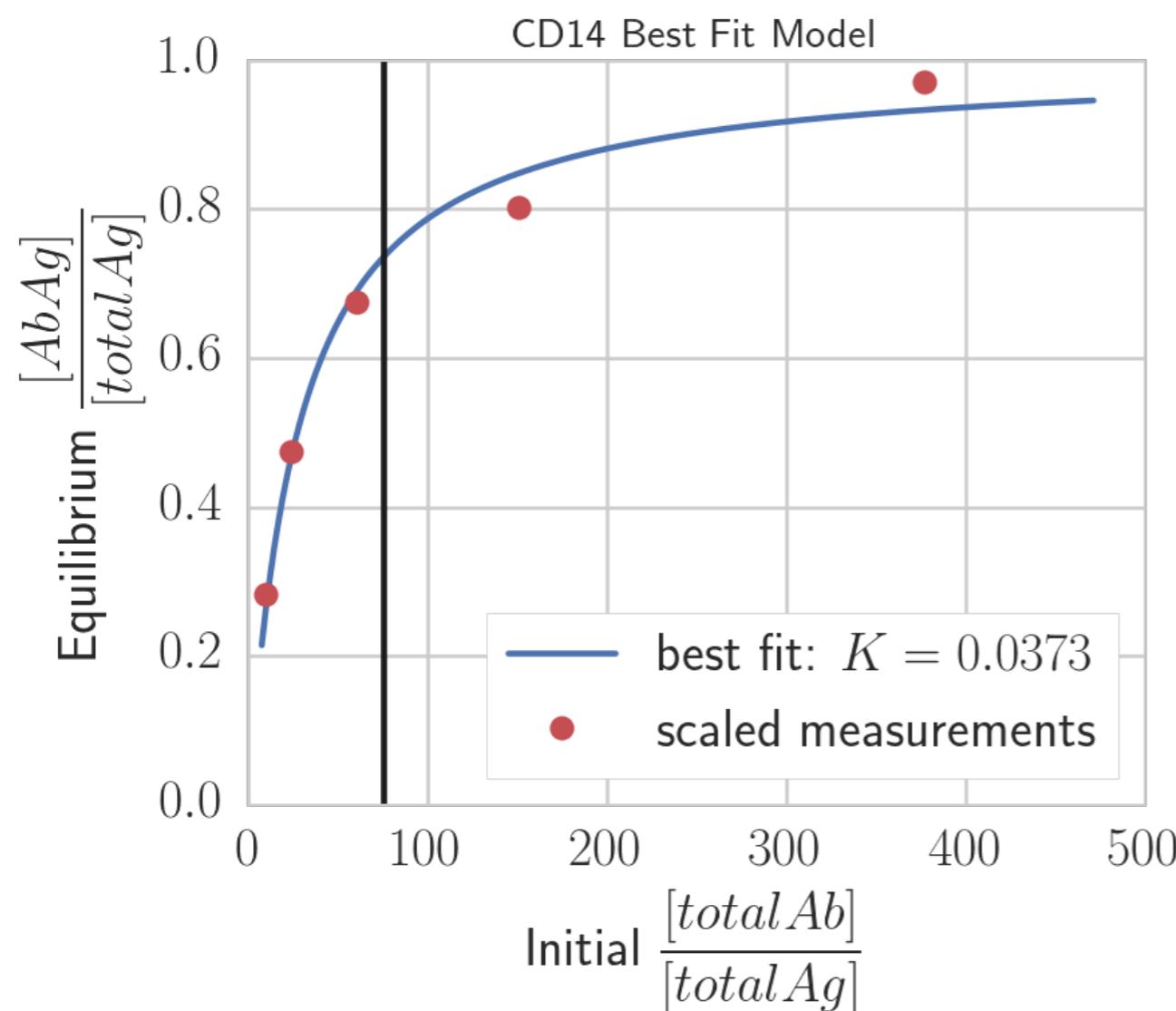
Background ~10 counts

Parameter Estimates Uncertain



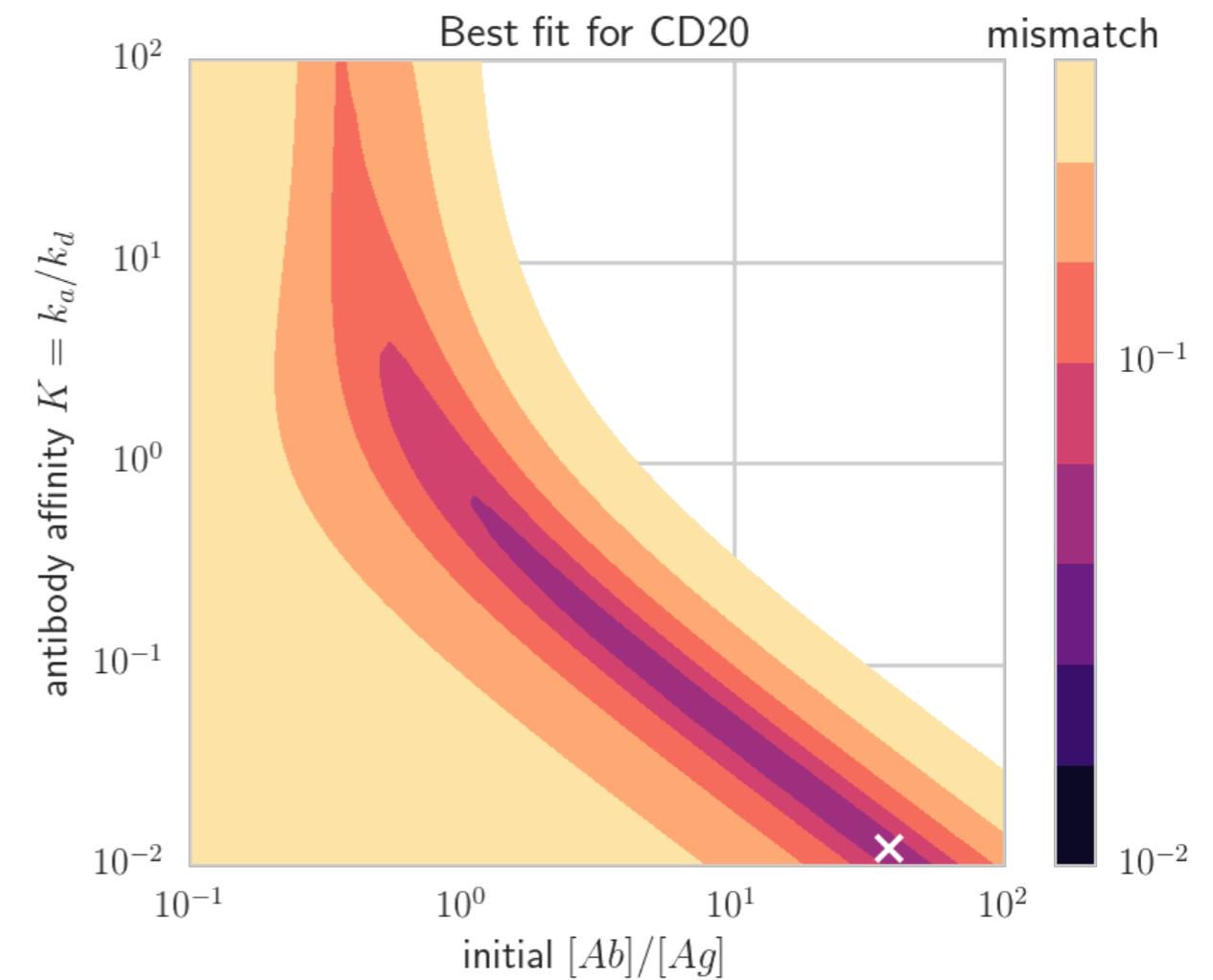
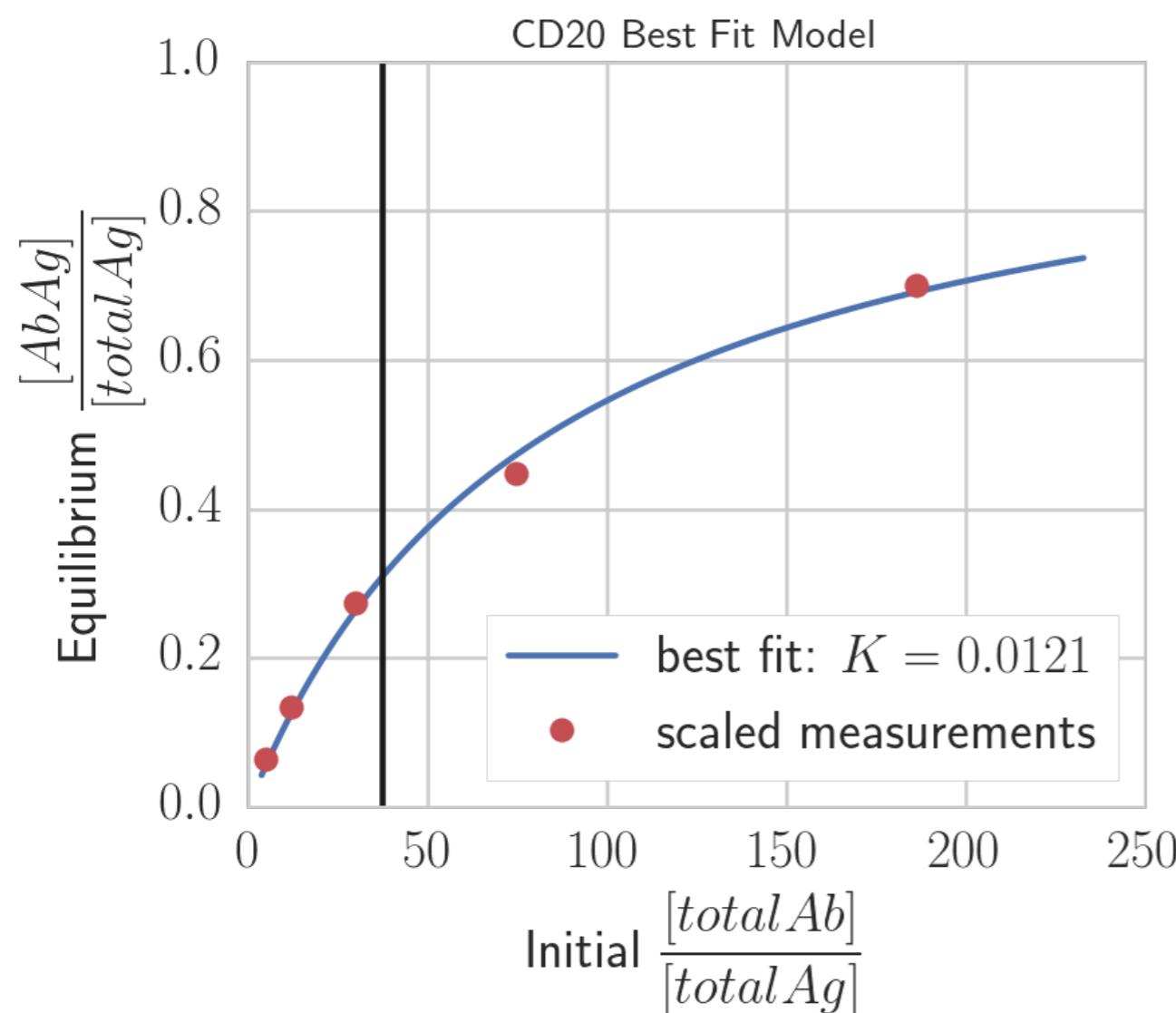
- Mismatch for a given affinity K and initial antibody-antigen ratio
- Large valley of small mismatch shows large uncertainty in fit
- Increased staining would remove uncertainty but risks damage to the CyTOF

CD14 Binding Estimate



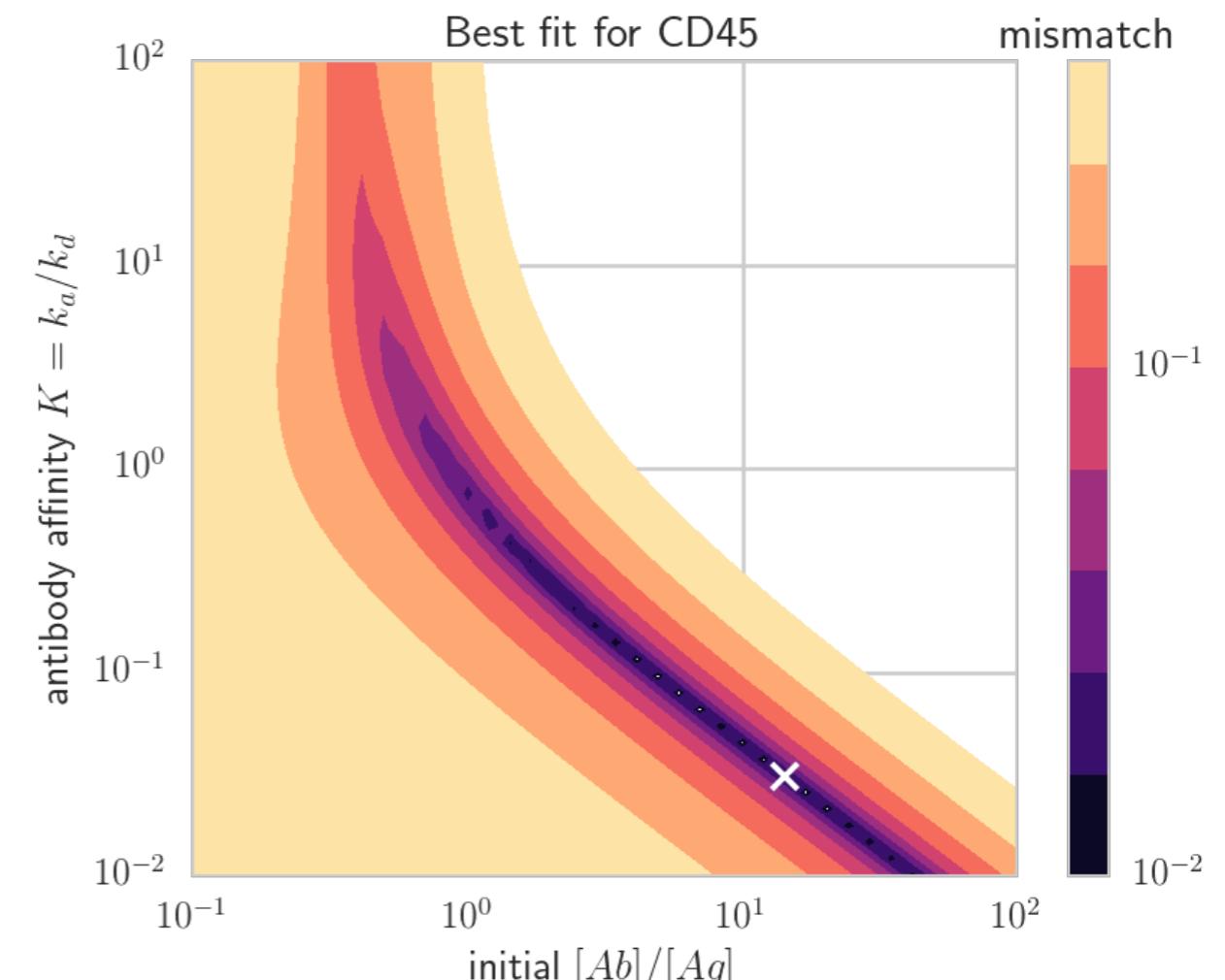
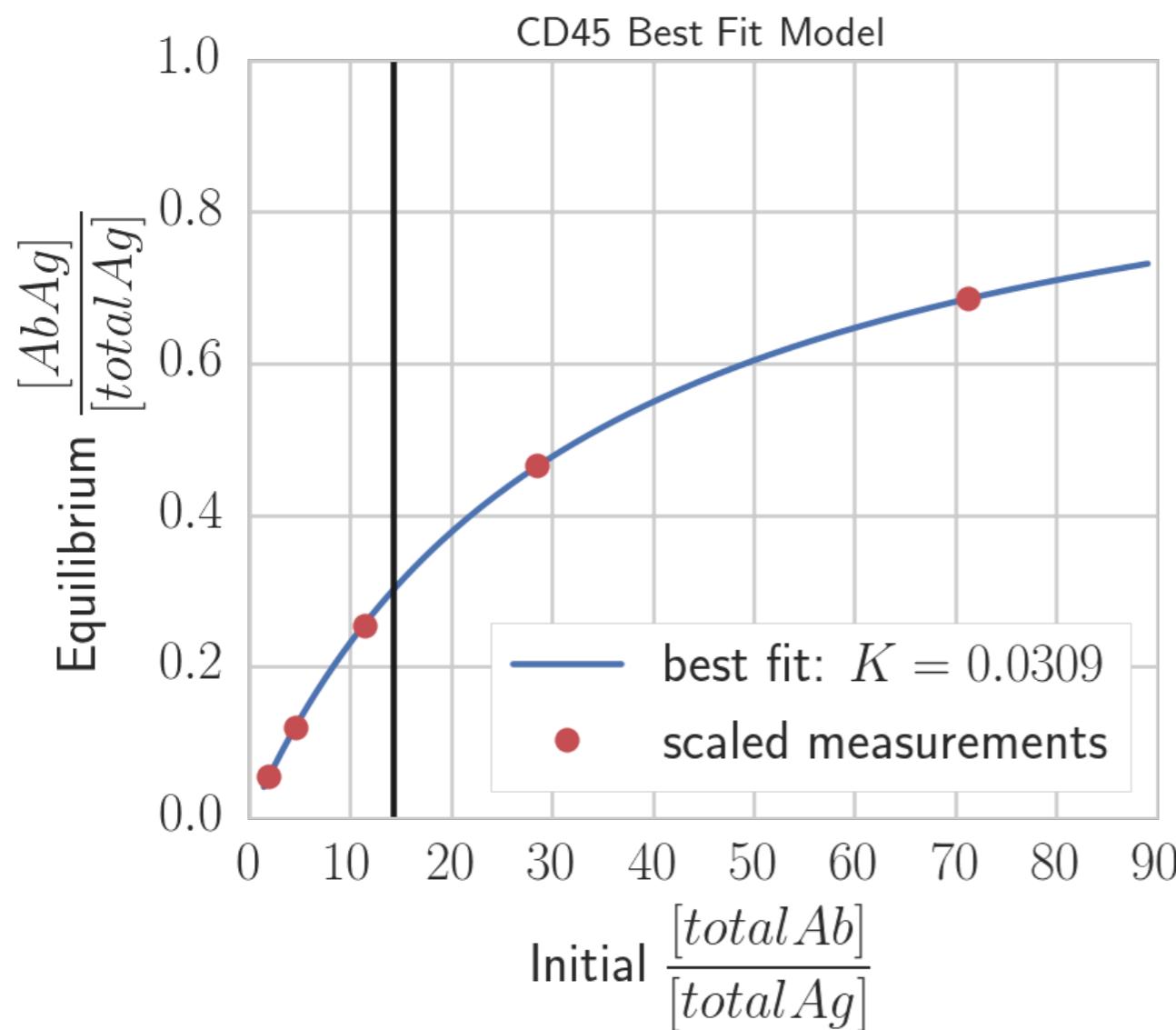
normal staining = 75% saturation

CD20 Binding Estimate



normal staining = 30% saturation

CD45 Binding Estimate



normal staining = 30% saturation

Estimate CD3 Binding Fit

