Welcome to

Basic Analysis of NanoString Gene Expression Data: QC, Normalization, and Differential Expression Testing

Today's Presenter:

- **Ryan S. Friese, Ph.D, Field Applications Scientist for Southern California**

Today's Moderator:

- **Erin K. Brown, Associate Technical Services Scientist, Seattle**

- **This Webinar will be recorded and made available to you**

- **Attendees will be hidden from each other and muted throughout the presentation**

- **For questions, please use the Chat feature to ask your question to the Moderator**
  - If your question will be answered in the course of the presentation, the Moderator will let you know.
  - If your question is pertinent at the time, the Moderator will either communicate to the presenter to address it to the group or answer you directly via chat.
  - The Presenter will open the floor for questions at the end.

Thank you for attending

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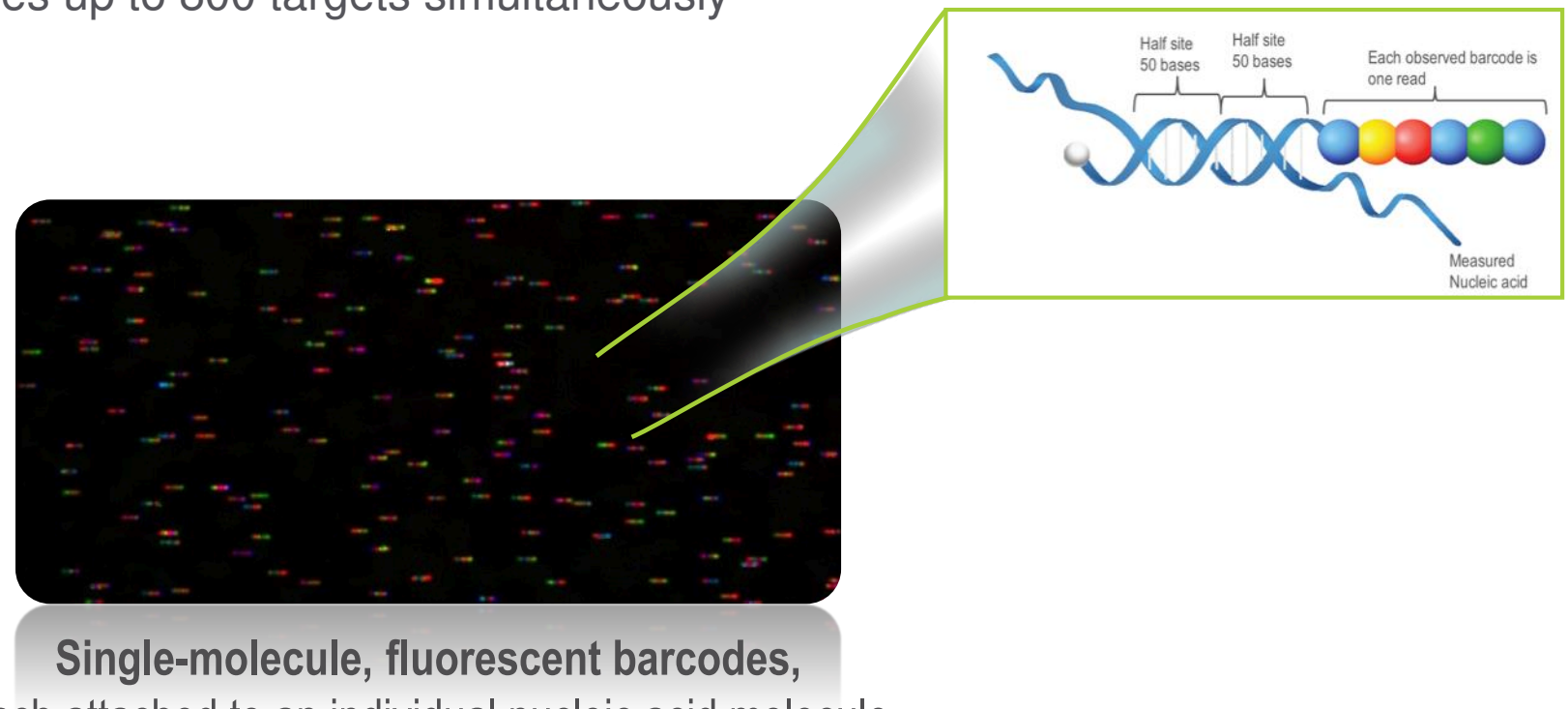
Agenda

1. **Technology overview**
2. **Data import**
3. **Data QC**
4. **Normalization**
5. **Differential Expression**
6. **Visualizations**
   - Heat maps
   - Scatter plots
   - Box plots

The Only Direct, Digital Counting Technology

- Novel chemistry invented in Leroy Hood's lab at the Institute for Systems Biology
- Digital counting technology
- Probes up to 800 targets simultaneously



Single-molecule, fluorescent barcodes,
each attached to an individual nucleic acid molecule

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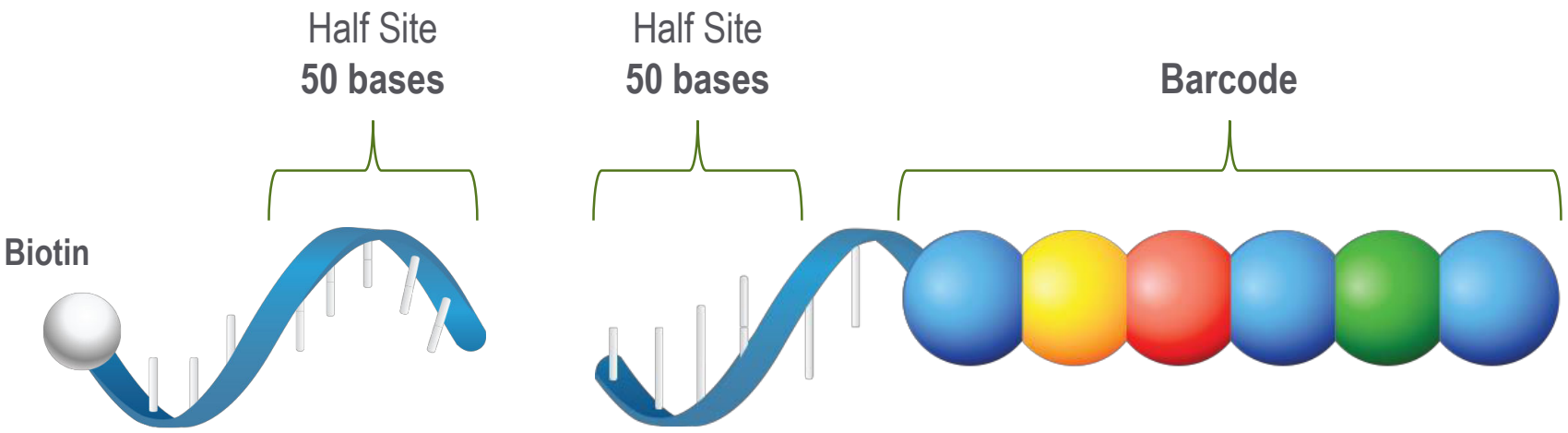
Digital Counting using Barcoded Probes



Barcode	Identity
	XLSA
	FOX5
	PDCD1

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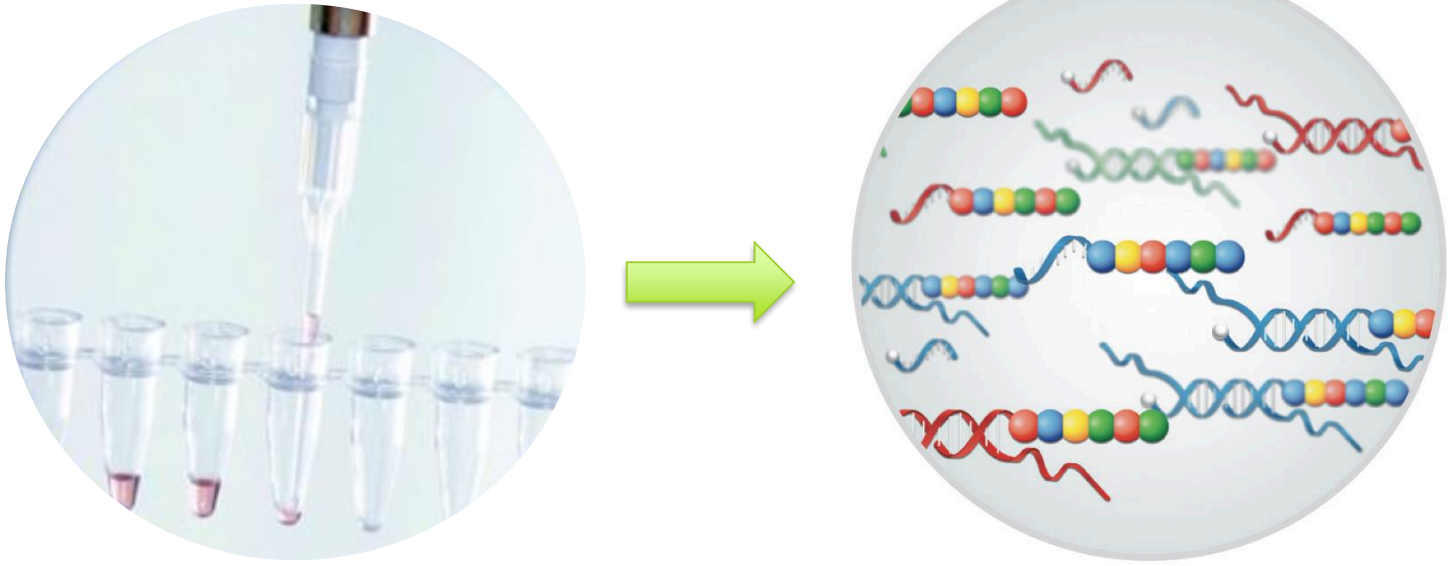
Enzyme-free Digital Counting

Directly Hybridize Lysate or Total RNA

- Only manual process
- Only 4 pipetting steps per sample required
- No amplification or reverse transcription
- 800 hybridizations in a single tube



solution phase hybridization

(Amplification may be needed for single cell application for low concentrations)

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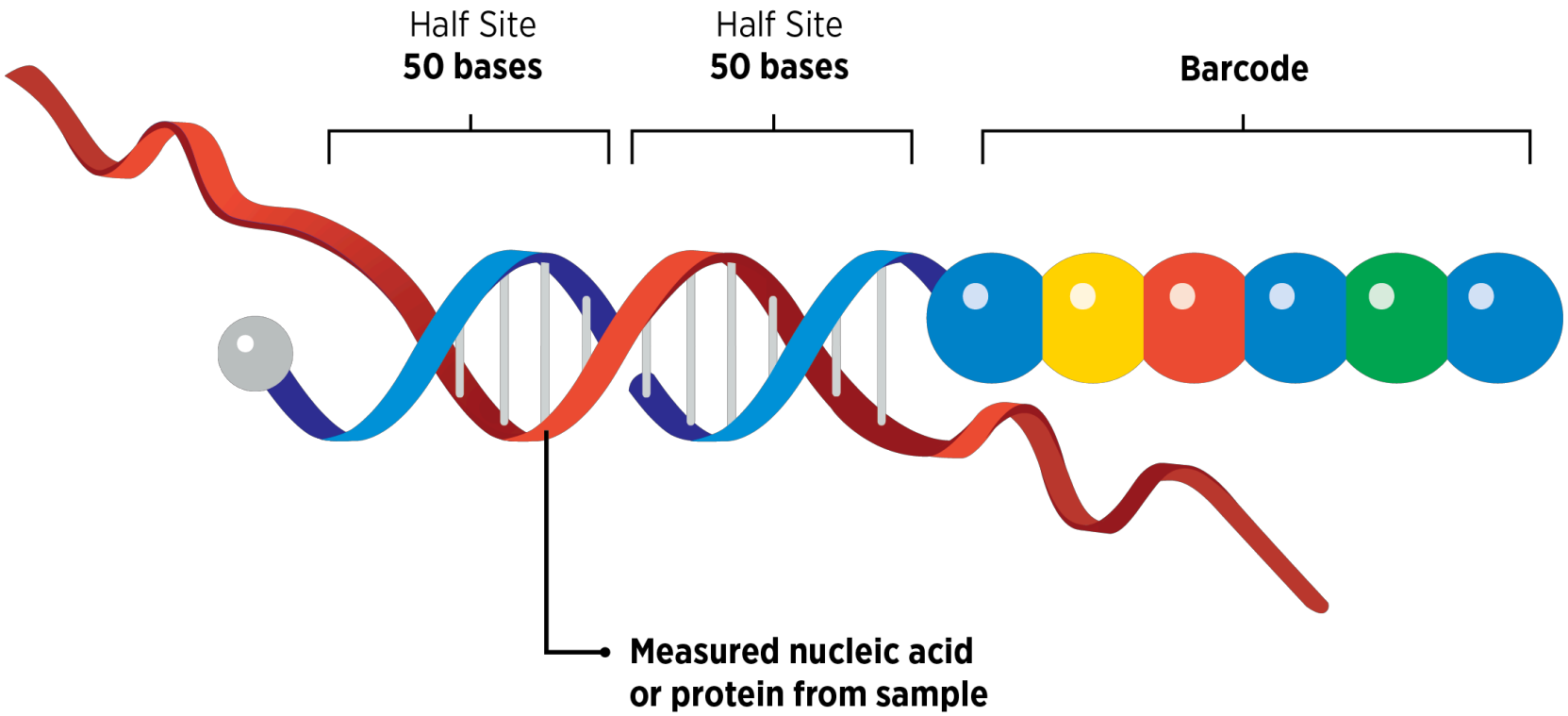
Digital Counting using Barcoded Probes



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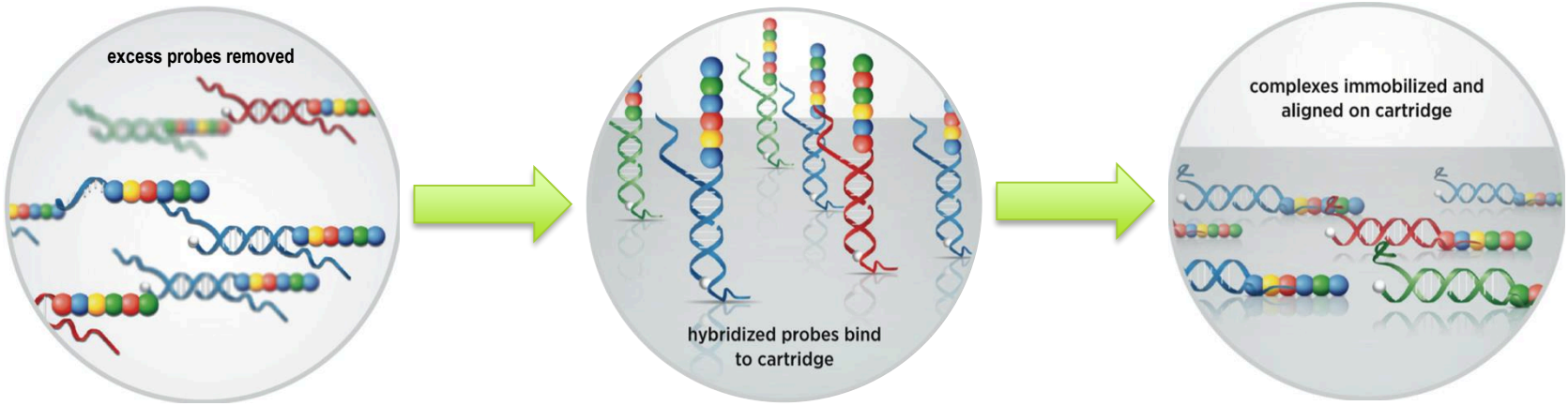
Digital AND Automated: Reproducible and Precise

Automated instrumentation



Purification | Probe binding to slide | Imaging

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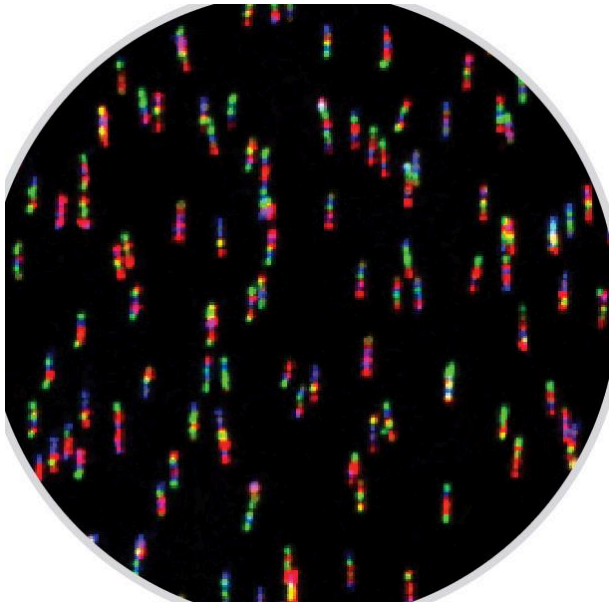
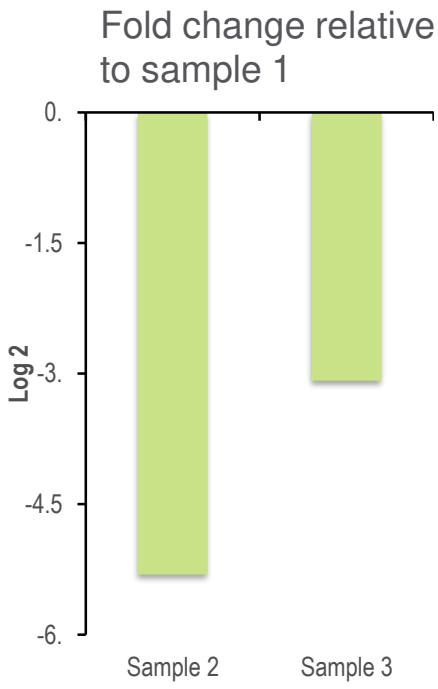
Digital Data: One barcode = one mRNA molecule



Slide image

Gene	Sample 1	Sample 2	Sample 3
SPP1	8,002	201	948
GAPDH	7,452	1,621	1,370
PLA2G2A	6,884	449	948
PDCD1	2,751	915	632
TGFBI	2,096	816	1,054
TIMP1	2,034	473	948
PGK1	1,427	1,420	632
MCL1	1,320	1,374	421
FAT1	1,303	208	948
STAT3	1,270	1,554	1,054
PLG	1,129	7,935	527
XRCC5	1,113	1,854	1,791
COL1A1	1,080	272	1,054
ERBB2	1,028	106	421



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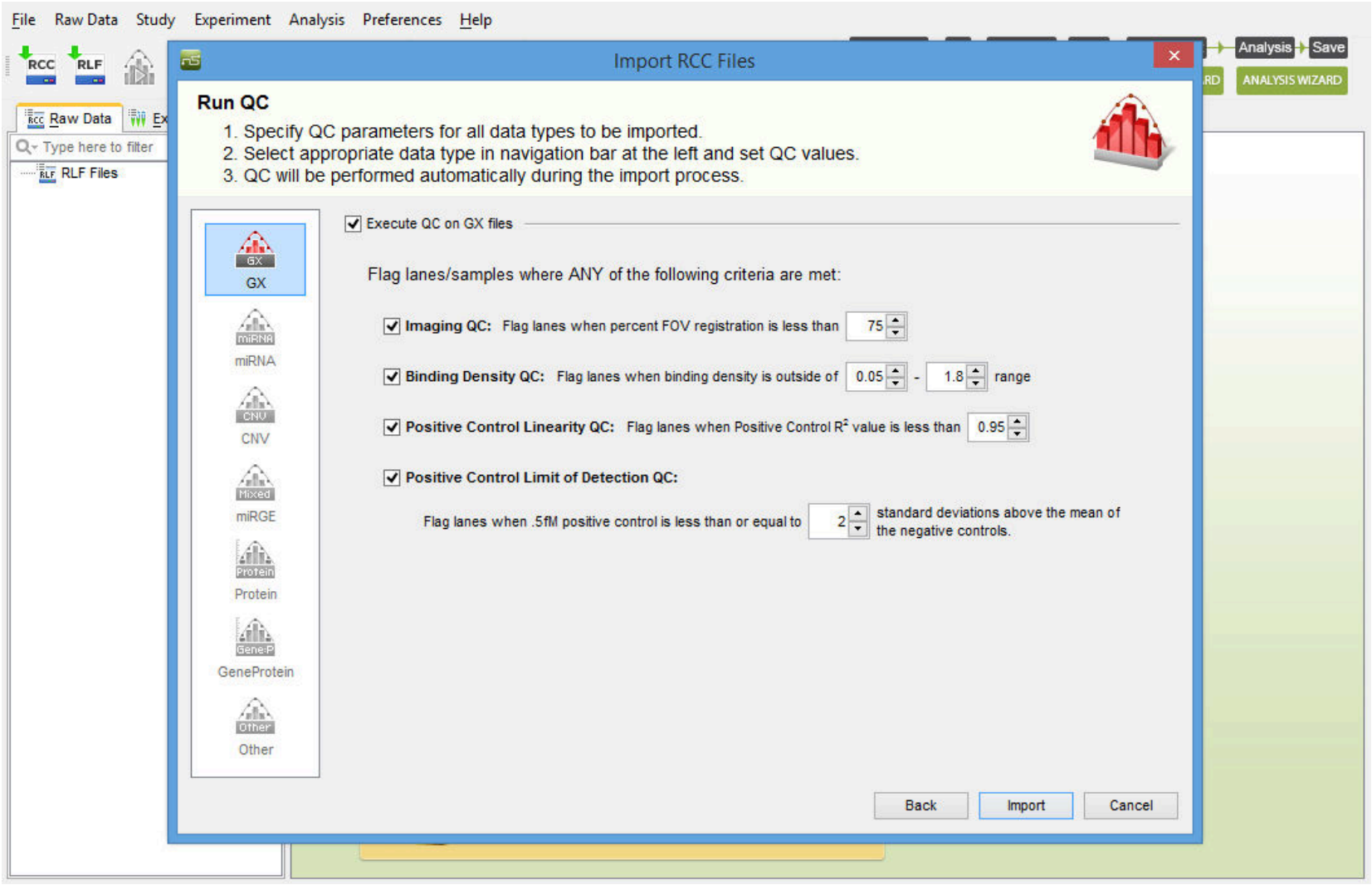
Quality Control Metrics in nSolver™ Software



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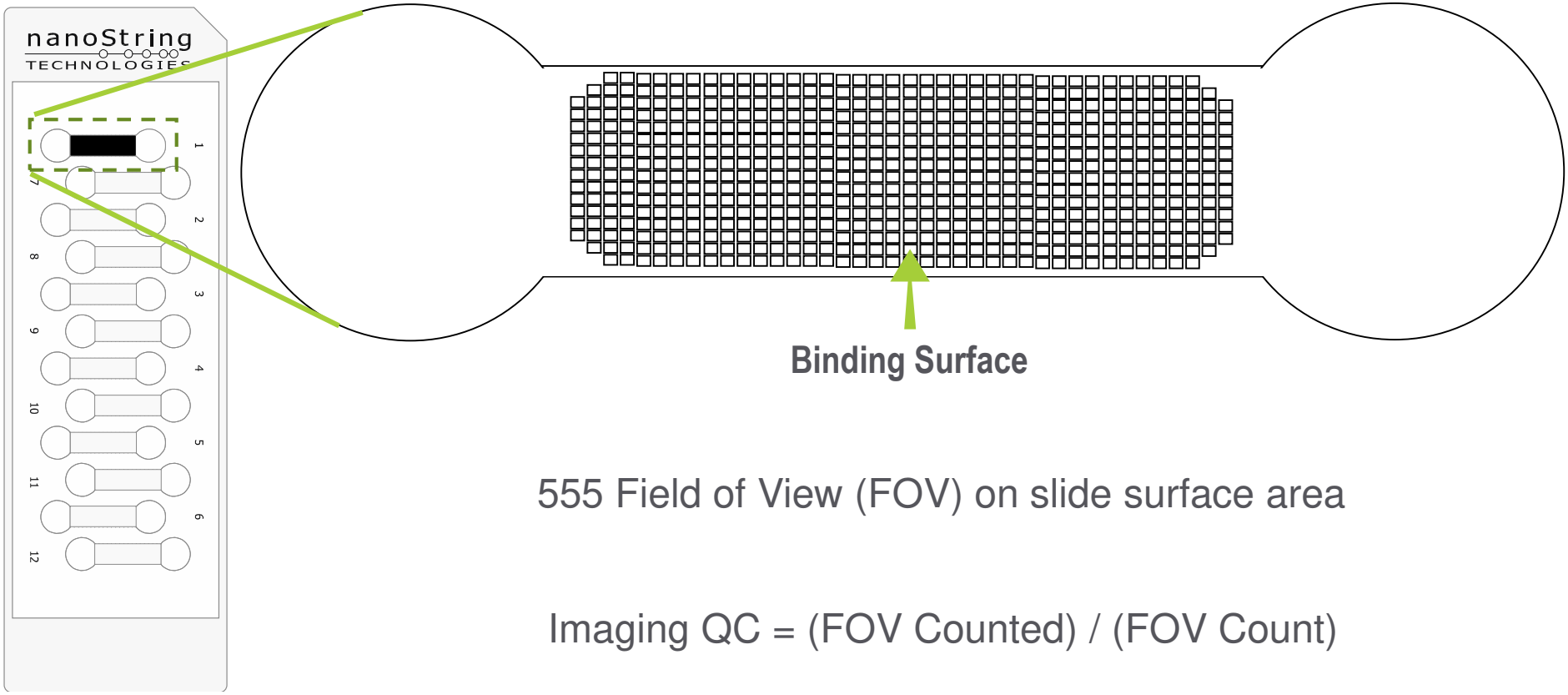
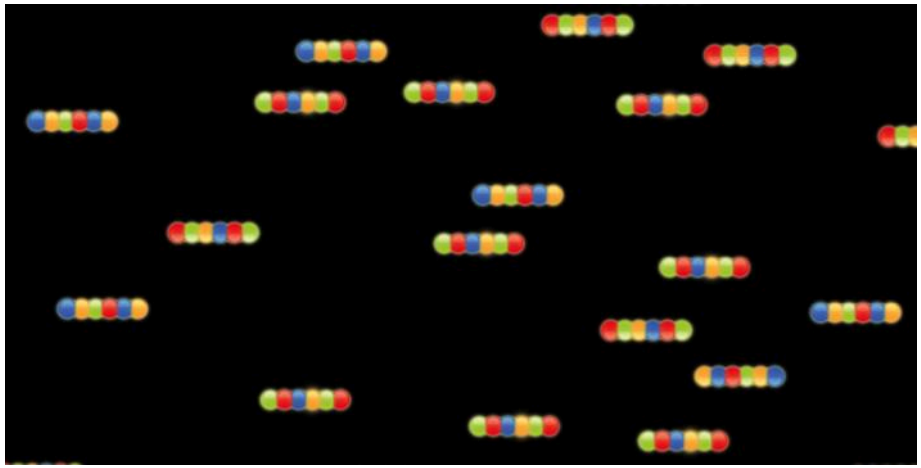
Imaging QC



555 Field of View (FOV) on slide surface area

Imaging QC = (FOV Counted) / (FOV Count)

Binding Density QC



Binding Density = Number of fluorescent spots per square micron

High density indicates that barcodes could be too close to each other for the imaging to successfully resolve them.

Upper limit of binding density is conservative.

A QC flag doesn't necessarily mean assay failure!

Positive and Negative Controls

Internal controls based on ERCC sequences.

Spiked-in to every sample. Contained in probe reagent tubes.

Positive controls

- In vitro transcribed RNA spike-ins
- Linear dilution series, six concentrations
- 0.125, 0.5, 2, 8, 32,128 fM

Negative controls

- No target RNA is spiked in
- Used to establish background or limit of detection (LOD)

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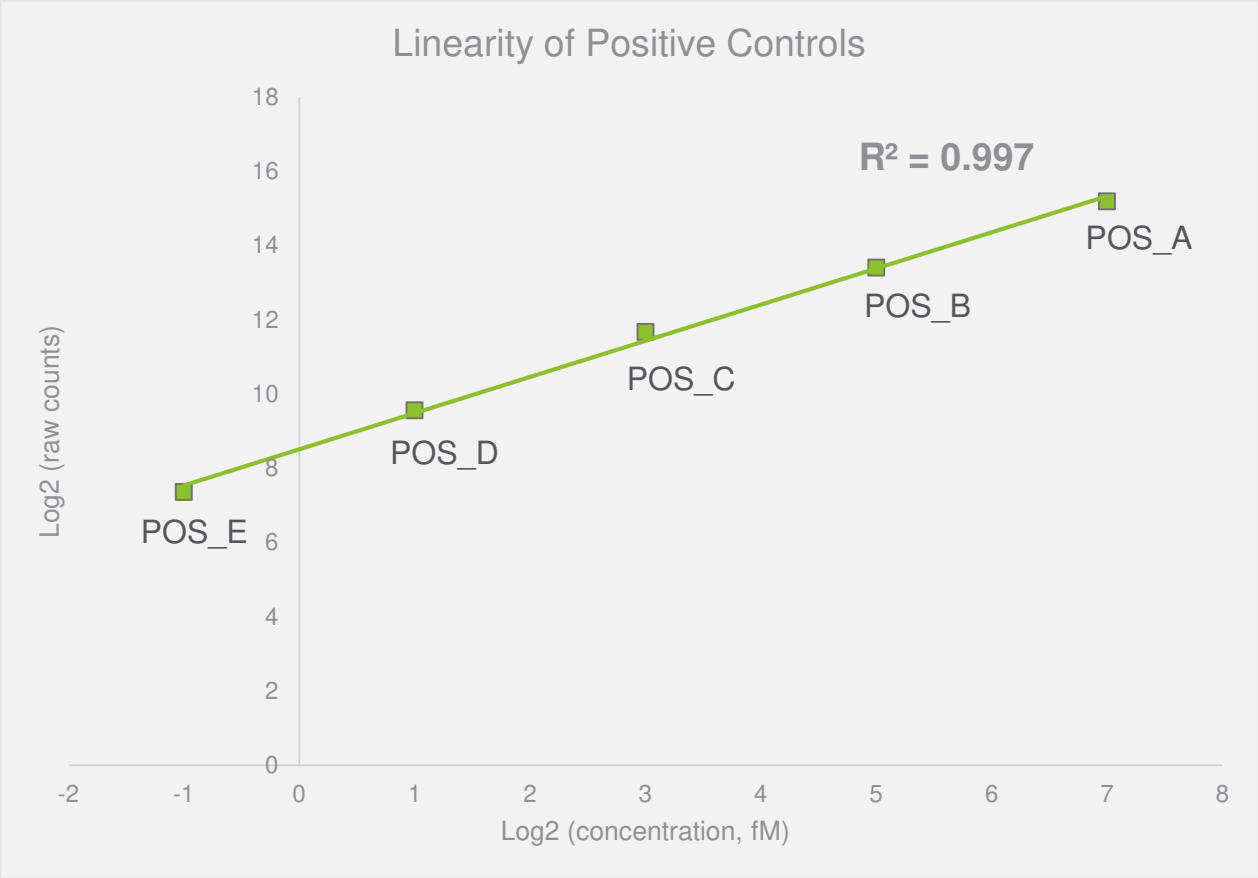
Positive Control Linearity QC



POS Control	Concentration (fM)	Raw Counts
POS_A	128	37206
POS_B	32	10829
POS_C	8	3259
POS_D	2	755
POS_E	0.5	165
POS_F	0.125	79

Note: As POS_F is considered to be below limit of detection, POS_F is not included in the linearity check.

POS Control Linearity QC: R^2 of concentration vs raw counts

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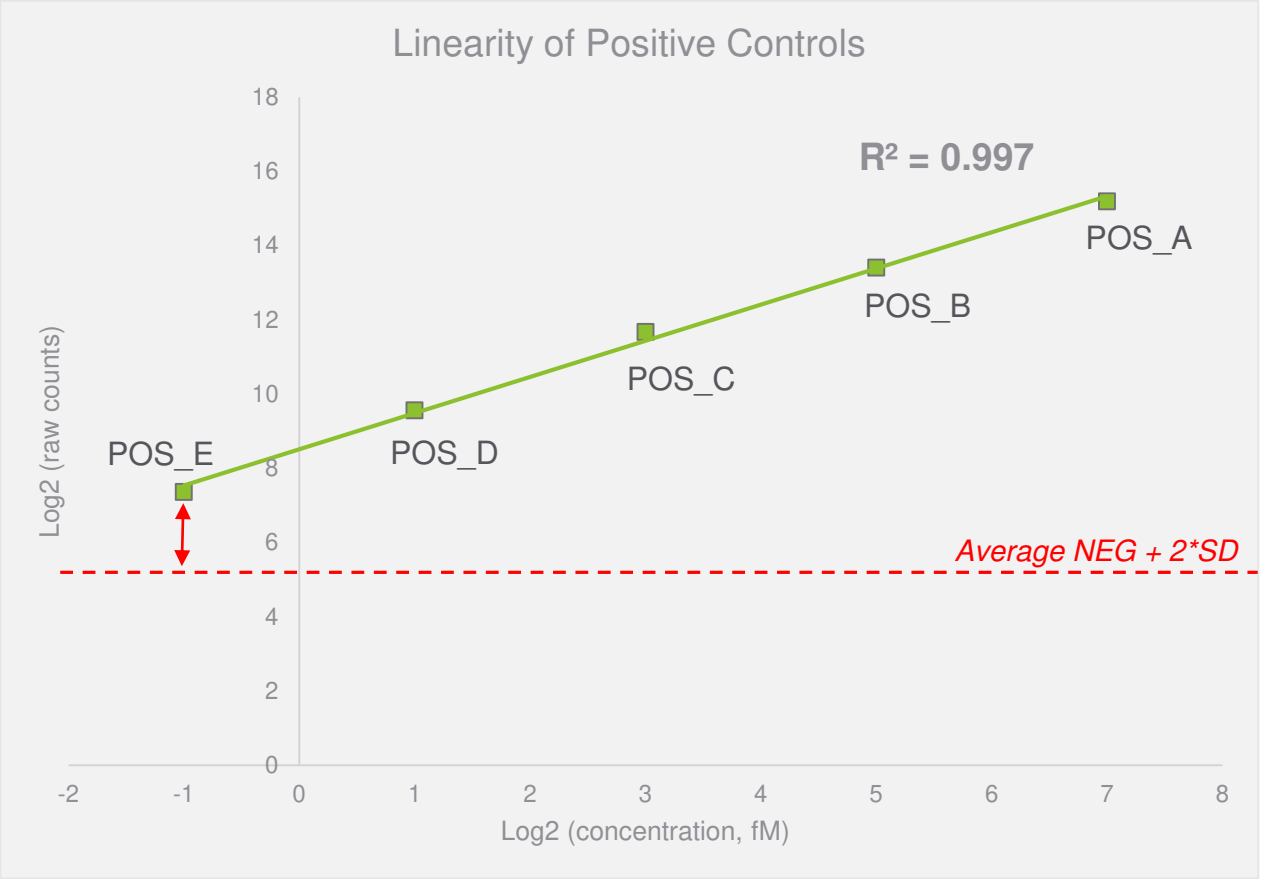
Limit of Detection QC



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POS_A	128	37206
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POS_D	2	755
POS_E	0.5	165
POS_F	0.125	79

NEG Control	Raw Counts
NEG_A	21
NEG_B	11
NEG_C	5
NEG_D	5
NEG_E	15
NEG_F	19
NEG_G	8
NEG_H	3

Limit of Detection QC: *POS_E > Average of NEG controls + 2*SD*

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NanoString nSolver Webinar Series

- **For additional support, please contact NanoString Support at:**
  - support@nanostring.com
  - (888) 358-6266
- **Join us for our next nSolver webinars:**
  - nSolver Basics webinar in August.
  - nSolver Advanced Analysis in September.
- **Slides, sample data, and a recording will be made available after the webinar is complete.**
- **Thank you for attending!**

Thank You

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