**14. Cy5 Standard Curve for Relating Intensity to DNA Concentration**

The Cy5 standard curve is essential for binding affinity experiments where the experimental intensity of DNA solubilized in the binding chamber is converted to a concentration. By fitting known concentrations to various intensities, one should be able to convert any experimental intensity back to a concentration.

**Preparing Fluorescent DNA**

1. Extend a random DNA oligo with Alexafluor-647 as outlined in Section 1 with 3x volume
2. Dilute klenow extended product to 5 uM with 1x PBS
3. Serial dilute in PBS to achieve the following concentrations:

* 2.5 uM
* 1.25 uM
* 0.625 uM
* 0.3135 uM
* 0.15625 uM
* 0.0753125 uM

**Imaging Device**

1. Prepare a MITOMI device as outlined in Section 5, but stop after the first 30 minute BSA wash
2. Wash out BSA with 1x PBS for 10 minutes and stop the flow
3. Image device using the Cy5 channel with 1x1 binning for 10 ms using the imaging protocol listed in Section 7
4. Repeat Step 5-6 while iteratively increasing the concentration of klenow product flowing through the device

**Image Analysis**

1. Stitch images as outlined in Section 12
2. Run images through Genepix
3. Partition background subtracted data into a sample concentration vs intensity format and fit with a line. y=mx+b where m is the conversion unit that translates intensity to concetration
4. When applying conversion factor to datasets, correct for binning and exposure time differences