Analyzing the GATTA-PAINT Nanoruler using DNA PAINT

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Background

DNA PAINT[1-3] Technique:

- DNA Point Accumulation for Imaging of Nanoscale Topography
- Method used to localize single DNA molecules (oligonucleotides with attached fluorophores)
- Shows binding between molecules immobilized on slide surface and molecules in solution

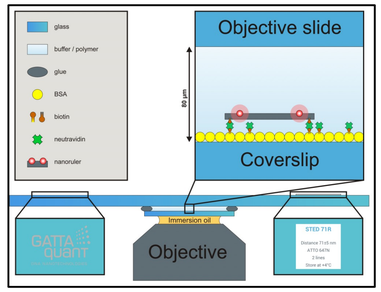


Figure 1: This is a representation of how DNA PAINT works, showcasing the immobilized strands on the surface binding to molecules in solution (GATTA-quant)

Applications in protein sequencing

- Organic receptors can recognize specific amino acid side chains
- Binding rates can indicate which amino acids are present within a peptide bound to an oligonucleotide

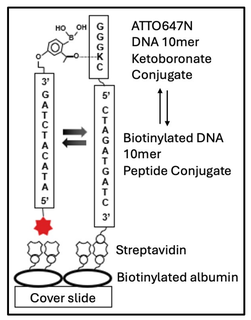


Figure 2: DNA PAINT can have applications in protein sequencing when short oligonucleotides with attached fluorophores and receptor molecules recognize specific amino acids in a surface-adhered protein or peptide.

Validation of previous research

- Testing without a bound peptide
- The GATTA-PAINT Nanoruler kit from GATTA-quant was compared to sample data to determine if successful experiments could be replicated

Data Collection

Slide comparison

- The GATTA-PAINT Nanoruler kit from GATTA-quant was compared to sample data from GATTA-quant
- The sample uses the ATTO-655 red fluorophore

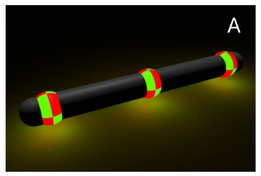
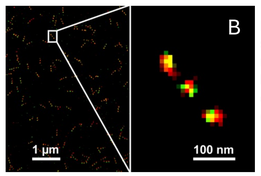


Figure 3:
A) A representation of a molecule in solution in the GATTA-PAINT Nanoruler kit.

Camera and microscope

- The Zyla sCMOS 4.2 camera from Andor was used
- The NIS-elements AR software was used to take videos of the activity



B) The Nanoruler is shown under the microscope as it binds to the surface of the cover slide (GATTA-quant)

Important settings

- Frame rate (70 ms - 3 s)
- Exposure time (20 ms - 1 s)
- Shutter on/off
- Laser intensity (0-100%)

In order to most accurately compare the slide to the sample data, videos were run with settings as close as possible to those used to produce the sample data

- 10 frames per second
- 100 millisecond exposure time,
- 180 x 180 pixel boundary

The experiment revealed problems with the software setup, as having frame intervals and exposure times near 100 ms caused data to be lost. Due to frequent missing frames because of the quick camera speeds, smaller exposure times were necessary to allow for even time intervals between frames.

Analysis Methods

Visualizing the binding activity

- Fiji-ImageJ was used to analyze groups of pixels in the resulting TIFF files to generate a plot describing the binding of the oligonucleotides at specific spots over time

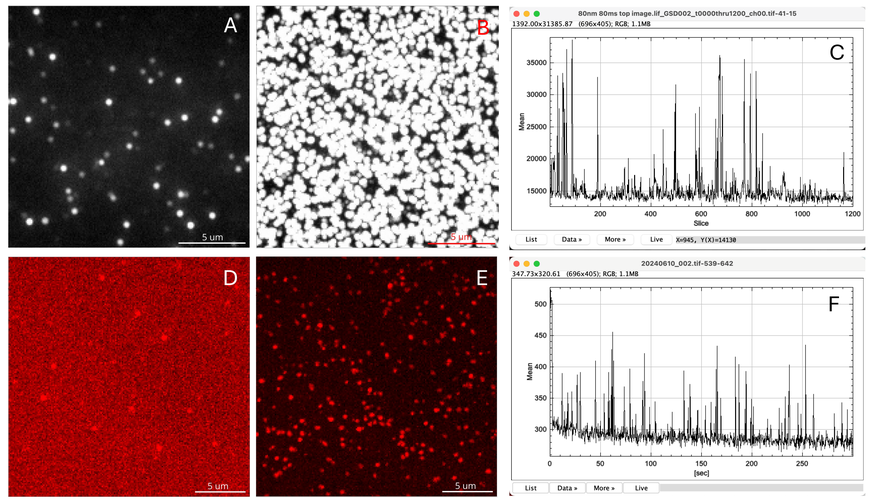


Figure 4: A) A frame from the sample data provided by GATTA-quant. (100 ms frame interval, 100 ms exposure, shutter off). B) A z-projection over the course of the sample video shows the maximum pixel values. C) The activity graph from one of the points in the sample data. D) A frame from data acquired from the GATTA-PAINT Nanoruler kit. (300 ms frame interval, 100 ms exposure, shutter off, 100% laser intensity). E) A z-projection over the course of the sample video shows the maximum pixel value. F) The activity graph from one of the points in the GATTA-PAINT Nanoruler data acquired by us.

Acquiring point data

- MATLAB was used to analyze specific points, account for drift, and to find the traces of the activity over time using the gaussian curves fitted to the pixel values.

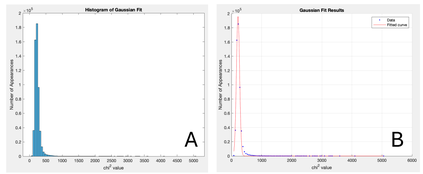


Figure 5: A) The histogram of the chi-squared values for a video of the GATTA-PAINT Nanoruler data. B) The gaussian fit results for the same data, which was used to find traces of the activity points in the video.

- Several different versions of the MATLAB code were used and compared to the data generated by Fiji-ImageJ to determine which method was most effective at analyzing the results.

Determining laser intensity settings

- Due to normalized data and differing laser intensities between our data acquisition and that of GATTA-quant, finding an adequate laser intensity was necessary
- As laser intensity increased, the average pixel value of the activity became increasingly nonlinear

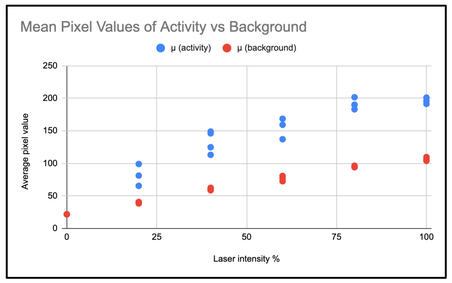


Figure 6: The mean of the background intensities (red) and the mean of signal intensities (blue) at different laser intensities were compared to find if the relationship stopped appearing linear. It was determined that 30% laser intensity would allow for the best comparison.

Results

MATLAB Results

- Several different methods of analysis produced graphs that have similar shapes for the same points

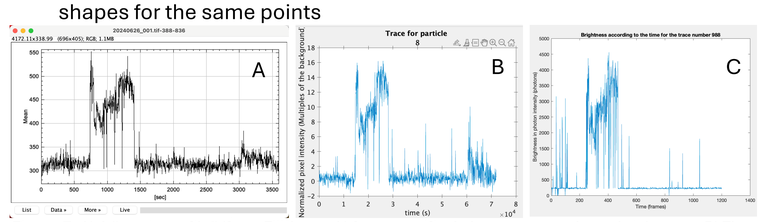
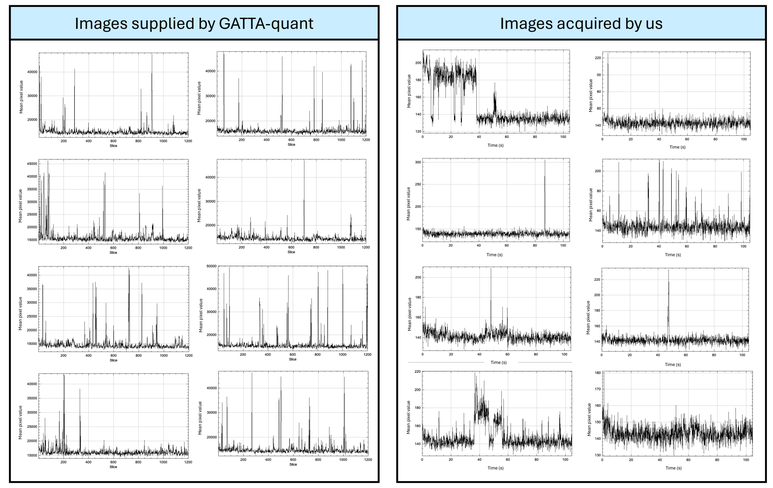


Figure 7: A) A "z-axis profile" (from Fiji-ImageJ) for a specific 4x4 area centered on a bright spot. B) The average pixel values for the same spot generated by the JR code (MATLAB). C) The total intensity of a gaussian fit to the same spot generated by the MDG code (MATLAB).

Binding traces (z-axis profile):

- Many of the traces from the GATTA-PAINT Nanoruler slide resembled the traces from the sample data. However, at a significant number of binding sites the oligonucleotides remained bound for longer than the expected binding time of 700 ms.



Conclusion

Comparable binding times for many activity sites show promising similarities between the sample data and GATTA-PAINT Nanoruler data. However, more research is necessary to validate the data collection methods used to measure the binding rates of these oligonucleotides. In particular, more reliable imaging techniques are necessary to ensure even time intervals between frames, along with faster frame rates with adequate exposure to see the binding activity.

References

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