Analyzing the GATTA-PAINT Nanoruler using DNA PAINT

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Introduction

DNA Point Accumulation for Imaging of Nanoscale Topography, or DNA PAINT[1-3], is a method used to precisely localize single DNA molecules on a surface by imaging short fluorescent oligonucleotides in solution that bind to them. An adaptation of this technique is used in a potential method of protein sequencing. This method involves connecting peptides of interest to DNA molecules on the surface and detecting the binding of short fluorescent oligonucleotides conjugated to organic receptors for amino acid side chains in solution. Since this method yields imaging data of considerable complexity, we required a testbed to validate our analysis pipeline. To do this, data from the GATTA-PAINT Nanoruler kit by GATTA-quant Inc., which utilizes the ATTO-655 red fluorophore to visualize the binding of complementary oligonucleotides in solution to oligonucleotides on the surface, was compared to sample data.

Methods

The Zyla sCMOS 4.2 camera from Andor was used to visualize the slide from the purchased GATTA-quant kit. The slide was analyzed by taking videos at different frame rates, exposure times, shutter settings, and laser intensities. 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 (fig. 1 C, D). Additionally, MATLAB was used to analyze specific points, account for drift, and find the traces of the activity over time using the gaussian curves fitted to the pixel values. 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, which had a frame rate of 10 frames per second, a 100 millisecond exposure time, and a 180 x 180 pixel boundary. Due to frequent missing frames because of the quick camera speeds, smaller exposure times were necessary in order to allow for even time intervals between frames. It was determined that a 30% laser intensity was necessary to allow molecule activity to be seen at short exposure times. The ratios between the activity peaks and background intensities were compared.

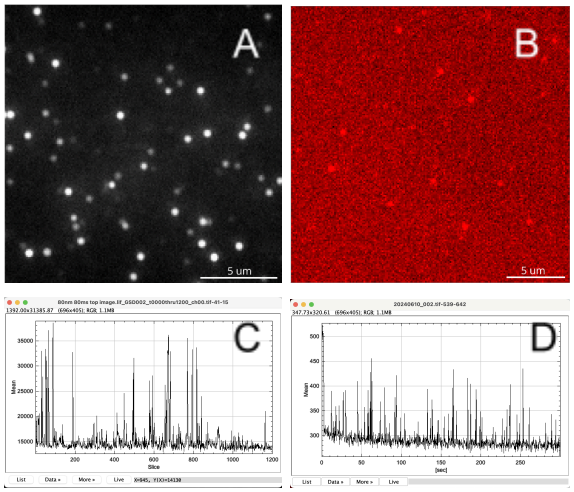

Figure 1: A) One frame from the sample data from GATTA-quant. B) One frame from the GATTA-PAINT Nanoruler slide taken on the Zyla 2.4 Megapixels camera from Andor at a 100 ms exposure. C) Trace data from one binding location for the sample data from GATTA-quant. D) Trace data from one binding location for the GATTA-PAINT Nanoruler slide taken on the Zyla sCMOS 4.2 camera from Andor at a 100 ms exposure.

Results

It was found that many of the traces from the GATTA-PAINT Nanoruler slide resembled the traces from the sample data from GATTA-quant (fig. 1). However, at a significant number of binding sites the oligonucleotides remained bound for longer than the expected binding time of 700 ms. Furthermore, when analyzing the appropriate laser intensity to use, it was found that minor photobleaching of the sample occurred when the same area was imaged repeatedly. The activity to background ratios of the sample data differed from the GATTA-PAINT Nanoruler slide data, which may be attributed to differing laser intensities.

Conclusions

Similar binding rates 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.

References

1. Chatterjee, et al. *Nature Nanotechnology* 12: 920-929 (2017).
2. Johnson-Buck, et al. *Nature Biotechnology.* 33(7): 730-733 (2015).
3. Jungmann, R. et al. *Nano Lett.* 10(11): 4756-4761 (2010).