A biochemical DNA nanoscope that identifies and localizes over a humdred unique features with nanometer accuracy

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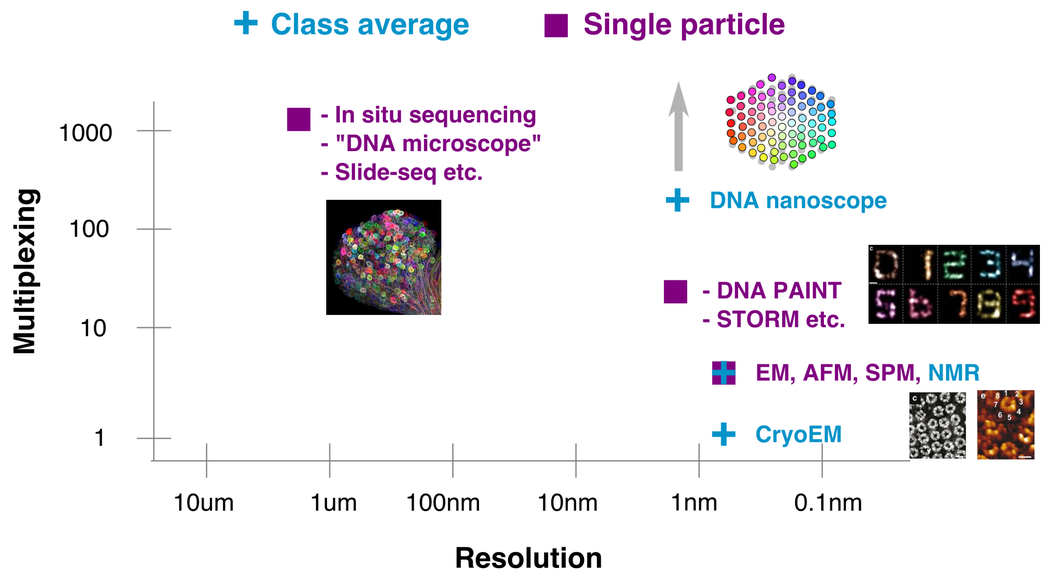
Abstract

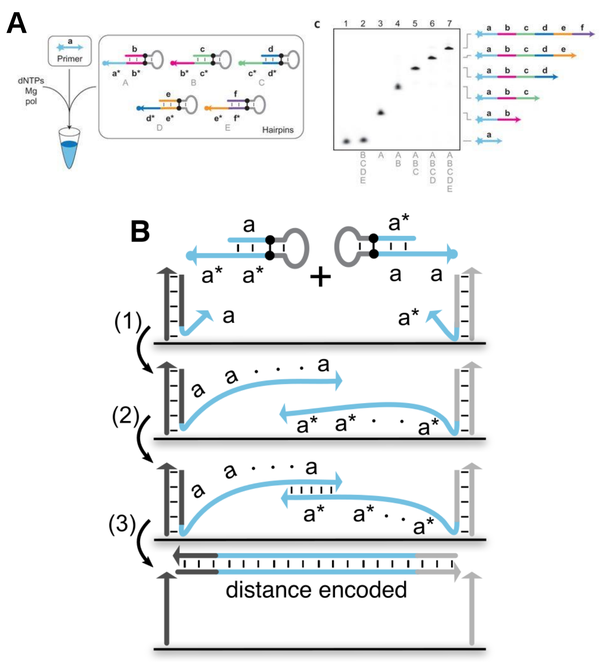
Techniques that can spatially localize molecular-scale features while discriminating between many targets would be highly valued in studying nanoscale processes. Here, we present a biochemical DNA nanoscope, an '*imaging by sequencing*' technique that begins by labeling points with unique barcoded primers. The primers are extended and meet in random pairs, such that the length of the intervening sequence encodes the distance between primers. The records are then identified with massively parallel next-gen sequencing using the MinION (Oxford Nanopore Technologies). Finally, the obtained reads are demultiplexed and the pattern is algorithmically reconstructed by minimizing the total error between measured and reconstructed distances. This enables unique, "full color" identification of every feature on a DNA origami testbed with ~ 2 nm average accuracy (RMS deviation), thus combining state-of-the-art accuracy (e.g, EM, SRM, and AFM) with the ability to label and identify 100 or more targets. We demonstrate the technique on many geometries in an ensemble manner, with many copies of the same origami structure.

Motivation



There is a trade-off between multiplexing and resolution

Background



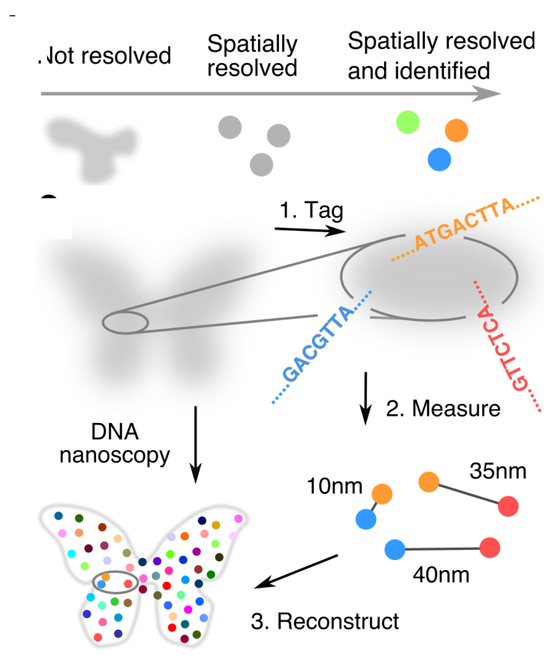
A) The basis of the biochemical DNA nanoscope is the Primer Exchange Reaction (PER) that enables in situ synthesis of templated ssDNA. Short hairpins repeatedly add the stem-encoded sequence with the help of a polymerase and dNTPs.

B) Molecular distance records are formed as follows:

1) Two targets are labeled with DNA handles that have recording primers hybridized to them.
2) The primer takes part in PER reaction adding sequence "a" and "a*" at respective ends.
3) The primer arms meet randomly, hybridize, and are extended on one another.

Distance records are ultimately displaced from the structure and released into solution.

"Imaging by sequencing" by the DNA nanoscope



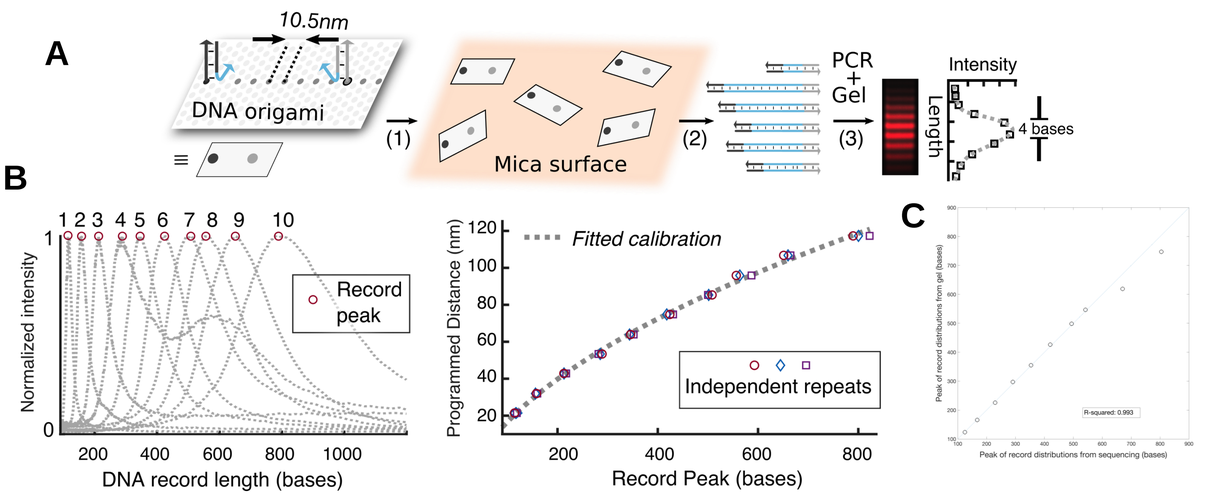
Principle of the "Imaging by sequencing" technique by the DNA nanoscope:

1) Targets are tagged with unique DNA barcoded handles.

2) Measure pairwise distances between many targets by sequencing the distance records using the MinION (ONT).

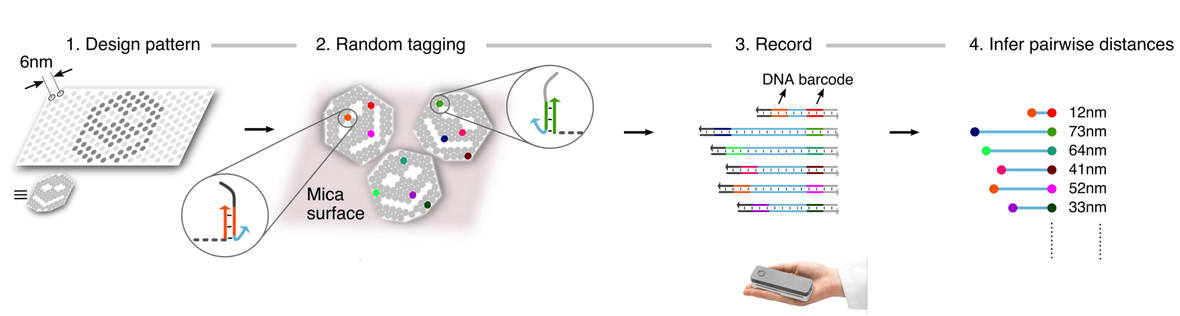
3) Integrate the sequences into a molecular resolution map to reconstruct the image.

Calibration



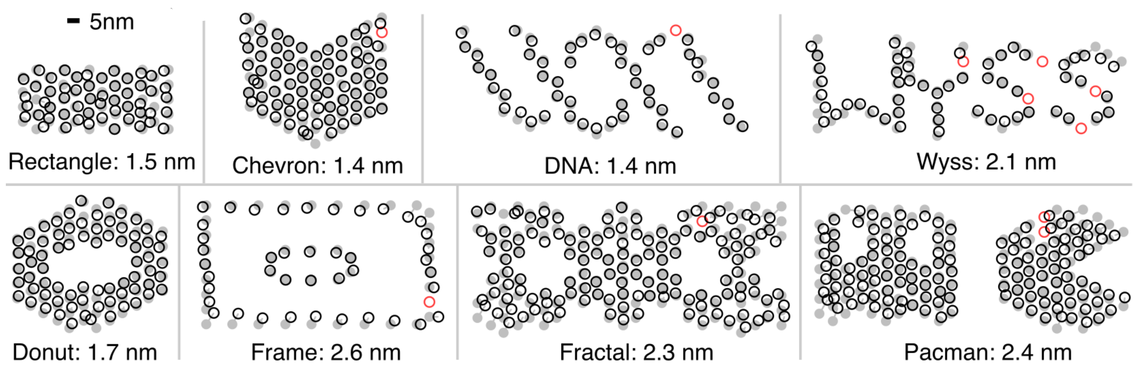
A) Distance records are generated between different points on a DNA origami test-bed, which give a distribution pattern when run on a gel.
B) Gel profiles of various distances can be fit into a calibration function.
C) Distances measured by sequencing correlate well with those measured by gel.

Principle of tagging points on a DNA origami



1) A pattern is chosen on a DNA origami.
2) A random subset of points is tagged with barcoded primers.
3) The identity and distance of the targets are encoded within the records that are sequenced using the MinION (ONT).
4) Pair-wise distance measurements are made and the image is reconstructed using an algorithm.

Reconstructions of images



Many different patterns reconstructed with high accuracy using the DNA nanoscope. Numbers noted are the RMS (average) reconstruction error.

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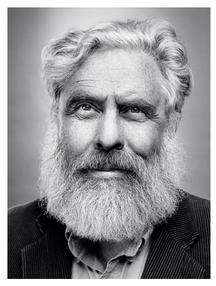
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