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Massively parallel genomic analysis using tunable nanoscale confinement

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Linearly extending long DNA molecules in sub-50 nm nanochannels for genomic analysis, while retaining their structural integrity, is a major technological challenge. We employ "Convex Lens-induced Confinement" (CLiC) microscopy to gently load DNA into nanogrooves from above, overcoming the limitations of side-loading techniques used in direct-bonded nanofluidic devices. In the CLiC technique, the curved surface of a convex lens is used to deform a flexible coverslip above a glass substrate, creating a nanoscale gap that can be tuned during an experiment to load and confine molecules into nanoscale features embedded in the bottom substrate. Since DNA molecules are loaded into the embedded nanotopography from above, CLiC eliminates the need for the high pressures or electric fields required to load DNA into direct-bonded nanofluidic devices. To demonstrate the versatility of CLiC, we confine DNA to a variety of nanostructures (linear, circular, gridded patterns of nano grooves), demonstrating DNA nanochannel-based stretching and denaturation mapping (Berard et al, PNAS 2014). We have successfully extended DNA in as small as 27-nm channels, achieving high stretching (90 percent) that is in good agreement with Odijk deflection theory and demonstrating mapping of genomic features using denaturation analysis. Additionally, we have recently demonstrated single-fluorophore resolution and resolved nick-labeled DNA in these devices, establishing compatibility with a suite of DNA mapping methods in the biotechnology sector.

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