Single-molecule detection of DNA hybridization under optical condensation

([1]*Graduate School of Engineering Science, Osaka University*, [2]*Research Institute for Light-induced Acceleration System (RILACS), Osaka Metropolitan University*, [3]*Graduate School of Engineering, Osaka Metropolitan University*, [4]*Graduate School of Science, Osaka Metropolitan University*) ○ Bhagya Lakshmi Sankaramangalam Balachandran,[1] Shuichi Toyouchi,[2] Syoji Ito,[1,2] Shiho Tokonami,[2,3] Takuya Iida[2,4]

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DNA hybridization studies are utilized in understanding biological processes, developing tools for early-stage medical diagnosis, DNA sequencing etc. The hybridization process and underlying kinetics are studied using single-molecule (SM) fluorescence microscopy, opto-plasmonic sensing etc. A majority of these studies involve the diffusion of target DNA towards probe DNA immobilized on solid substrates followed by complementary base pairing leading to transient or permanent hybridization. Here we utilize optical condensation (OC), a process that gathers and traps small molecules under the photothermal effect induced by near-infrared (NIR) laser excitation,[1] to increase the efficiency of hybridization and drastically reduce the time for detection. The authenticity of DNA hybridization under OC is confirmed by SM fluorescence microscopy.

The sample was prepared by sandwiching DNA aqueous solution with two cover glasses using a cellophane spacer; one of the cover glasses was sputtered with gold thin film followed by immobilization of the probe DNA using Au-S linkage chemistry. A complementary stranded target DNA tagged with Alexa 532 dye was introduced into the sample solution and the hybridization was observed using SM fluorescence microscope under 532 nm CW laser excitation. A simultaneous NIR (1064nm) excitation initiated the OC of the DNA molecules at the focal point, accelerating the hybridization of the target and the probe DNA. As shown in the figure, this OC process was successfully tracked at the single molecule sensitivity.

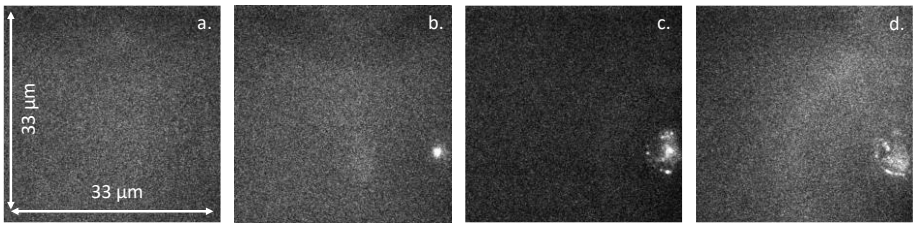

Figure: SM fluorescence images under 532 nm excitation showing OC of target DNA molecules tagged with Alexa 532 onto the substrate with probe-DNA immobilized on it. a) Before NIR laser excitation, b) just after NIR laser ON, c) under NIR excitation, and d) just after NIR laser OFF. The events from (a-d) show accelerated diffusion of the target DNA allowing their hybridization with probe DNA even after the removal of NIR excitation.

1) T.Iida et al. Sci. Rep. **2016**, *6*, 37768.