

Supporting Information:

Materials and Methods

In a typical experiment for DNA hybridization detection, microfluidic chip having a dielectrophoretic (DEP) trap is fabricated by growing microelectrodes (200-nm Au on 30-nm Cr) by standard photolithography on a glass slide¹ (Kimble, 76 mm x 26 mm and 1 mm thick). Microfluidic channels with dual inlets are fabricated with a negative photosensitive acrylic resin based photoresist, JSR THB-126N, to experience simultaneous flow for both beads and target DNA (channel dimension of height x width x length are 10 μm x 1 mm x 1 cm respectively). Using JSR photoresist for thermal bonding has many advantages, including good bonding strength, good flatness and good coverage. We aligned the top and bottom slides in the microscopy and fixed and clipped them using by the C-clamps. The clipped chip are kept in the oven for thermal bonding at 200 °C with 20 min. Carboxylated silica nanocolloid (500 \pm 10 nm in diameter) modified with species-specific amine functionalized oligonucleotide probe sequence² (26-mer) using carbodiimide chemistry³ and is used as probes to monitor the presence of target green crab DNA sequence in the sample. In order to simplify the detection platform, fluorescently labeled target single stranded green crab DNA of 300 bases is prepared by amplifying the target DNA with asymmetric PCR using one of the primers in excess. To accomplish DNA hybridization, target DNA sample and the probe functionalized silica nanocolloids are diluted with 0.08X PBS to obtain a conductivity of 1 mS/cm. Initially, the functionalized nanocolloids are injected into the microchannel with a flow rate of 2.0 $\mu\text{L}/\text{min}$ (linear velocity ~2.0 mm/sec) under the applied AC electric field of 20 V_{pp} at a frequency of 10 MHz. The solution conductivity of 1 mS/cm is selected so that the voltage and frequency can be tuned in such a way to obtain a close packing of beads with high throughput near the cusp shape

region of the electrode where the applied field is maximum. On packing the beads in the trapping region of $5000 \mu\text{m}^3 \pm 20\%$, the guiding electrode is turned on and bead flow is turned off to avoid excess packing of beads in the detection region. The target green crab DNA solution is then passed through the packed bead chamber with a flow rate of $1 \mu\text{L}/\text{min}$ and turned off the guiding electrode pair. The applied AC voltage of trapping electrodes is changed to 17 V_{pp} and scanned at different frequencies starting from 100 kHz to 10 MHz to monitor the rate of hybridization with frequency. The fluorescence intensity of the hybridized bead region is measured by the image software, *Image pro plus 6.3*. The reference intensity is taken before passing the target DNA and is considered as a baseline. The image is captured with CCD camera (QImagin Retiga-EXC) in every one second to observe the change in fluorescence intensity during hybridization. The whole experiment has been done in a dark room to avoid any photo bleaching from normal or white light.

References:

1. I. F. Cheng, V. E. Froude, Y. Zhu, H.-C. Chang and H. C. Chang, *Lab Chip*.2009, **9**, 3193-3201.
2. The probe sequence used to detect target green crab species is 5'-TTAGGGATTTCTCT TTACATTAGC-3'.
3. C. A. Marquette and L. J. Blum, *Top. Curr. Chem.*2006, **261**, 113-129.