**Simple Nanofluidic Device for Single Protein Molecule Identification**

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**Abstract:**
Detection of target protein direct from cell lysate without any prior cleaning procedures has been demonstrated by fused silica nanofluidic channel devices and two-dimensional (2D) photon burst analysis technique. Individual molecule events are scatter plotted in terms of their photon counts and burst width in 2D photon burst analysis. Detection of target protein is achieved by comparing the 2D photon burst analysis of the target sample with that of the control sample. This single molecule technique offers the advantages of high sensitivity, minute sample consumption and reduced processing time over conventional immunological ensemble measurements, such as western blot, immuno-precipitation, etc.

**Summary of Research:**

The nanofluidic device is fabricated on a 500 µm-thick UV grade fused silica wafer using standard photolithographic and etching techniques. A schematic layout of nanofluidic channels is shown in Figure 1. The detection channel has a width of 2 µm and CF₄ plasma is used to dry etch the channel to the depth of 500 nm. A protective surface coating is then spin-coated onto the wafer and injection ports are drilled with high-speed sand-blast tool. After removal of surface coating and a thorough pirahna cleaning, a cover UV grade fused silica wafer of 170 µm is carefully clinched to the substrate wafer using de-ionized water as intermediate. Permanent bonding is achieved by annealing the wafer at 1050°C in air for 5 hours. The target protein, Hemagglutinin (HA) epitope tagged MAX (HA-MAX), in cell lysate was conjugated to polyclonal rabbit anti-HA antibodies and hybridized to the secondary rabbit antibodies with quantum dots (QDs). Sample solution was then loaded into nanochannel and electrokentically driven through the channel at various potentials. QDs were excited by an external laser source while they are passing through the detection volume. Due to the small dimension of the detection volume in nanofluidic channels, photon burst counts strictly from single molecules can be obtained. In addition, because a few quantum dots were hybridized to targeted HA-MAX proteins, they were distinguished from the unbound individual QDs by comparing their two-dimensional photon burst diagrams (photon burst width vs. photon burst counts). From this diagram, the existence of HA-MAX proteins can be detected. The typical photon-burst peaks obtained from these samples from cells with and without HA-MAX are shown in Figure 2a and b respectively. The burst counts for HA-MAX protein is higher than that of the sample without HA-MAX protein.
Figure 1: A schematic layout of nanofluidic channels (scale bar inset 2 μm).

Figure 2: Photon-burst peaks from (a) QD525, (b) QD525 with HA-MAX from cell lysate. On left: the cartoons of QD525 and HA-MAX protein with a few QD525.