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## **Figure Legends**

Fig. 1 (A) Schematic of the combined microevaporator/rotary SMD microdevice. The control layer (lighter grey) shows the <sup>20</sup> evaporation membrane, rotary pump, and isolation valves. Target accumulation is accomplished by solvent removal from the fluidic layer (black, inlet labeled i.) through the pervaporation membrane (inlet labeled ii.). Following target accumulation the concentrated sample plug is transferred to the SMD-Rotary

- <sup>25</sup> Chamber for probe hybridization and detection; probes and hybridization buffer are introduced through separate inlets (labeled iii.). (B) Side view of the operating microevaporator, prior to sample transfer into the detection chamber. Solvent removal through the pervaporation membrane must be <sup>30</sup> compensated by convection from the sample reservoir, while
- actuation of the accumulation valve enables target collection at the dead end.

Fig. 2 (A and B) Photo- and fluorescence micrographs of the accumulation zone just prior to the closed accumulation valve at time 0 after loading the evaporator coil with 500 nM fluorescently labeled DNA sequences. (C) Fluorescence micrograph showing target accumulation after 6 hours of evaporation in the 1000 mm membrane pervaporator with 20 PSI nitrogen pressure and at room temperature. (D)

- <sup>40</sup> Photomicrograph of the SMD-rotary chamber just prior to sample injection with valves bisecting the chamber into analyte (left three-quarters) and probe/buffer (right one-quarter) compartments. (E) Accumulated model target from figure 2C is injected into the rotary chamber along with DI water in the probe/buffer section (E) Mixing of the content shoung in figure
- <sup>45</sup> probe/buffer section. (F) Mixing of the contents shown in figure 2E for 1 second using the rotary pump at 10 Hz, mixing was complete within 5 seconds (data not shown).

Fig. 3 (A, B, and C) Bulk evaporation rates versus evaporation pressure (A), microdevice temperature (B), and evaporation membrane length (C). Pressure data was taken using a 1000 mm membrane at room temperature. Temperature data was taken using a 1000 mm membrane at 25 PSI, while evaporation length data was taken at room temperature and 25 PSI. (D) Time trace of the measured fluorescent burst duration of tetraspec beads at the

start of the evaporation channel at two different evaporation pressures (25 and 5 PSI). Large fluctuations at low pressure are due to evaporation membrane vibration upon initiation of nitrogen flow. Points for A, B, and C are mean evaporation rates from a single device after three separate two hour
measurements ± standard error.

Fig. 4 Calibration curve of fluorescence burst counts versus target concentration loaded into the SMD-rotary chamber<sup>23</sup> without evaporation-based accumulation (10 pM molecular beacon concentration). The solid line represents the average <sup>65</sup> number of fluorescent bursts from the no target control (dotted

so number of fluorescent bursts from the no target control (dotted line equals one standard deviation from an average of four measurements).

**Fig. 5** Number of fluorescent bursts detected versus hybridization time (5 pM targets, 10 pM probe) within the device. <sup>70</sup> Hybridization time follows a 15 second mixing period using the rotary pump and a 5 second incubation at 80°C.

**Fig. 6** (**A and B**) Raw fluorescence burst traces from the recirculating SMD chamber <sup>23</sup>(100 Hz pump frequency) after 20 hours of target enrichment and probe hybridization with no target <sup>75</sup> control (A) and 50 aM target (B) samples. (**C**) Number of fluorescent bursts detected versus loading concentration after 20 hours of evaporation within the 1000 mm membrane device (10 pM probe, room temperature, 25 PSI), along with no target controls.