Supplemental Information for:

Integrating Nucleic Acid Sequence-Based Amplification and Microlensing for High-Sensitivity Self-Reporting Detection

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Experimental Procedure

1. Fabrication of microsphere-on-microgel arrays

1.1 Electron-beam patterned microgels

A focused electron beam was used to pattern microgels on silicon substrates following procedures used previously.[1-3] Single-crystal Si substrates were conditioned in piranha solution for 12 h, repeatedly washed in water, and then exposed to an oxygen plasma. Continuous thin films of poly(ethylene glycol) end functionalized with biotin (PEG-B; M_w = 5 kDa; Creative PEGWorks) were spin cast onto these substrates using a 2 wt% of PEG-B dissolved in tetrahydrofuran (THF). A Zeiss Auriga field emission gun (FEG) scanning electron microscope (SEM) with an electrostatic beam-blanking system and a Nanometer Pattern Generation System (NPGS; Nabity) was used for patterning (incident electron energy = 2 keV; beam current ~200 pA). In one set of experiments we patterned arrays of individual microgels by single-point irradiations (point dose = 10 fC) arranged on a hexagonal array with 20 μm spaces between adjacent microgels. In a second format, we patterned arrays of pseudo-continuous microgel pads with a controllable pad diameter from 1 μm to 20 μm using an interpixel spacing of 250 nm. After e-beam exposure, the specimens were repeatedly washed with DI water to remove un-crosslinked PEG leaving an array of microgels or microgel pads patterned in array fashion on the Si surface.

1.2 Microsphere tethering

Microspheres were tethered to the surface-patterned microgels via our previously established method.[4] As-received streptavidin-functionalized polystyrene microspheres (3 μm diameter, 1 w/v% solids, Bangs Laboratories, Inc.) were multiply washed by centrifugation and resuspension in washing/binding buffer (20 mM Tris, pH 7.5; 1 M NaCl; 1 mM EDTA; 0.0005% TritonTMX-100) and re-suspended in washing/binding buffer at 0.25 w/v%. Twenty μL of the suspension was deposited on each e-beam patterned Si substrate at room temperature for 15 min to enable streptavidin binding to the biotinylated PEG microgels and then repeatedly washed with washing/binding buffer.

2. Functionalization of microsphere-on-microgel arrays

We used a NASBA (Nucleic Acid Sequence-Based Amplification), which involves two primers and three enzymes (see Fig. S1), as an amplification platform.[5] We pursued experiments with the primers free in solution (solution-phase NASBA), grafted to the microgel-tethered SA-functionalized PS microspheres (solid-phase NASBA), and combinations of these two. For solid-phase NASBA,[6, 7] the tethered microspheres were exposed to 20 μL of a mixture of 1 μM biotinylated forward primer (solid-phase primer 1, SP1) and 1 μM reverse primer (solid-phase primer 2, SP2) in 0.1×PBS (10 mM Na_3PO_4, 15 mM NaCl, pH 7.4) for 2 h. For experiments without solid-phase NASBA, we blocked excess SA sites on the tethered microspheres with biotin solution (2 mg/mL in 0.1×PBS).

Molecular beacon probes (MBs) were co-tethered on the microgels with microspheres via established methods.[4, 8, 9] Unreacted biotin groups on the PEG-B microgels were first activated

by exposure to 20 μL of SA (200 μg/mL) in 0.1×PBS for 2 h and then sequentially washed using 0.1×PBS containing 0.05% Tween-20, 0.1×PBS, and DI water. The SA-activated microspheres-on-microgel arrays were exposed to 20 μL of 1 μM biotinylated MB solution in 0.1×PBS for 2 h at room temperature. The samples were then washed with 0. 1×PBS twice and with DI water once to remove unreacted MBs. For a MB hybridization experiment without RNA amplification, 20 μL of synthetic (+)DNA target after sequential dilution in hybridization buffer (4 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, pH = 8.0) was deposited on the sample and held there for 2 h followed by extensive washing in hybridization buffer. Twenty μL of hybridization buffer was deposited on the sample as a negative control. The sequences for the primers, MBs, and synthetic DNA targets are all given in Table S1.

3. Integration of microsphere-on-microgel arrays with NASBA

We used three references strains for experiments involving viral RNA. These were ATCC VR-1469™ (Influenza A/Puerto Rico/8/34 (H1N1)), ATCC VR-1804™ (Influenza B/Florida/4/2006) and ATCC VR-26™ (Human respiratory syncytial virus). The viral isolates were amplified by one additional passage in RhMK cell tubes, and we extracted supernatant to make working stocks of each virus. The total nucleic acid extraction of viral isolates was performed using an EZ1 Advanced XL (Qiagen) with EZ1 Virus Mini Kit v2.0 (REF 955134) according to the manufacturer's instructions.

NASBA amplification of synthetic (-)DNA or viral (-)RNA used a set of commercially available reagent kits (Life Sciences Advanced Technologies). Each NASBA reaction had a total solution volume of 20 μL. The total nucleic acid solution (viral (-)RNA) or the (-)DNA synthetic target solution was heated to 65 °C for 2 min, and 2 μL was then mixed with 10 μL NASBA reaction buffer (Lyophilized Reaction Buffer (LRB) and Reaction Reconstitution Buffer (DRB), Life Sciences), 5 μL NASBA enzyme mix (Lyophilized Enzymes Mix (LEM) and PDGT diluent for LEM (D-PDG), Life Sciences) and 1 μL RNase-free H_2O (Qiagen). For the reactions that involve solution-phase NASBA, the mixture also contained 1 μL of 1 μM forward primer (P1) and 1 μL of 1 μM reverse primer (P2). For solid-phase NASBA performed in the absence of solution primers, 2 μL of RNase-free H_2O was added to the mixture to maintain the same reagent concentration. In all variations (solid-phase, solution-phase, etc.) 20 μL of the NASBA amplification solution with or without (control) the viral RNA or synthetic DNA target was deposited on a patterned silicon sample and left there for 2 h at 41 °C under saturated humidity. The surface was washed twice (10 min) with TE buffer (10 mM Tris−HCl, 1 mM EDTA, pH 7.5) afterwards and then imaged while hydrated.

4. Imaging and data processing

Fluorescence images were collected using a Nikon E1000 upright optical microscope with an X-cite 120 LED light source and a sCMOS Camera (pco.panda). The imaging experiments used a Nikon Plan Apo 40× (NA = 0.95, working distance = 0.14 mm) objective lens when the samples were hydrated and covered by a 0.17 mm glass coverslip. Digital image data were analyzed with Fiji (ImageJ) software.[10, 11] SEM images were taken at 2 kV accelerating voltage with a Zeiss Auriga FEG SEM.

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Table S1

Oligonucleotide Sequences for Amplifying/Detecting Influenza A virus [a, b, c]
(after Moore et al.[12] and Wat et al.[13])

Name	Function	Sequence
SP1	Solid-phase Primer 1	5'BiotinTEG//iSp9/TTTTTTTTTT*AATTCTAATACGACTCACTATAGGG***AGCAGGGTAGATAATCACTC**3'
SP2	Solid-phase Primer 2	5'BiotinTEG//iSp9/TTTTTTTTTT**ATTTCAGTGGCATTCTGGCG**3'
P1	Solution-phase Primer 1	5'*AATTCTAATACGACTCACTATAGGG***AGCAGGGTAGATAATCACTC**3'
P2	Solution-phase Primer 2	5'**ATTTCAGTGGCATTCTGGCG**3'
(-)DNA	(-)DNA mimic of viral (-)RNA	5'**ATTTCAGTGGCATTCTGGCG**TTCTCCATCAGTCTCCATCTGTTCGTAAGATCGTTTGGTGCCTTGAGACGCCATGATTTTGATGTCACTCAGT**GAGTGATTATCTACCCTGCT**3'
(+)DNA synthetic target	(+)DNA mimic of (+)RNA amplicon	5'CGTCT**CAAGGCACCAAACGATCTTA**CGAAC3'
MB	Molecular beacon detection probe	5'Alexa488-CCAAGC**TAAGATCGTTTGGTGCCTTG**GCTTGG-BHQ2-Biotin3'

[a] The bold text indicates sequences that hybridize, and the different styles of underlines indicate complementary sequences or identical sequence.
[b] Italics indicate the T7 promoter.
[c] TEG and iSp9 correspond to triethylene glycol that incorporate between sequence and modifications.

Table S2

Oligonucleotide Sequences for Amplifying/Detecting Influenza B virus [a, b, c]
(after Wat et al.[13])

Name	Function	Sequence
SP1	Solid-phase Primer 1	5'BiotinTEG//iSp9/TTTTTTTTTT*AATTCTAATACGACTCACTATAGGG***CTATTCAACATCTGCGTCCATC**3'
SP2	Solid-phase Primer 2	5'BiotinTEG//iSp9/TTTTTTTTTT**ATYACTTCATAYTGTGGTCTCA**3'
P1	Solution-phase Primer 1	5'*AATTCTAATACGACTCACTATAGGG***CTATTCAACATCTGCGTCCATC**3'
P2	Solution-phase Primer 2	5'**ATYACTTCATAYTGTGGTCTCA**3'
(-)DNA	(-)DNA mimic of viral (-)RNA	5'**ATTACTTCATATTGTGGTCTCA**AATTTTGTTCTTTCCCTTGTCCTTCTAATGCTGTATATGCTTTCCCTTCTTCATCAAGAAAATTCATGTCACTTATTACATAGCAAACCTCTA**GATGGACGCAGATGTTGAATAG3**'
(+)DNA synthetic target	(+)DNA mimic of (+)RNA amplicon	5'AAGCA**TATACAGCATTAGAAGGACAAGG**GAAAG3'
MB	Molecular beacon detection probe	5'Alexa488-CCAAGC**CCTTGTCCTTCTAATGCTGTATA**GCTTGG-BHQ2-Biotin3'

[a] The bold text indicates sequences that hybridize, and the different styles of underlines indicate complementary sequences or identical sequence.
[b] Italics indicate the T7 promoter.
[c] TEG and iSp9 correspond to triethylene glycol that incorporate between sequence and modifications.

Table S3

Oligonucleotide Sequences for Amplifying/Detecting Respiratory Syncytial Virus (RSV) [a, b, c] (after Wat et al.[13])

Name	Function	Sequence
SP1	Solid-phase Primer 1	5'BiotinTEG//iSp9/TTTTTTTTTT*AATTCTAATACGACTCACTATAGGG***GACAGAGGATGGTACTGTGA**3'
SP2	Solid-phase Primer 2	5'BiotinTEG//iSp9/TTTTTTTTTT**CAATGGCTCCTAGAGATGTGA**3'
P1	Solution-phase Primer 1	5' *AATTCTAATACGACTCACTATAGGG***GACAGAGGATGGTACTGTGA**3'
P2	Solution-phase Primer 2	5' **CAATGGCTCCTAGAGATGTGA**3'
(+)DNA synthetic target	(+)DNA mimic of (+)RNA amplicon	5' TTCAA**AAACAG ATGTAAGCAG CTCCG**TTATC3'
MB	Molecular beacon detection probe	5'Alexa488-CCATGC**CGGAGCTGCTTACATCTGTTT**GCATGG-BHQ2-Biotin3'

[a] The bold text indicates sequences that hybridize, and the different styles of underlines indicate complementary sequences or identical sequence.
[b] Italics indicate the T7 promoter.
[c] TEG and iSp9 correspond to triethylene glycol that incorporate between sequence and modifications.

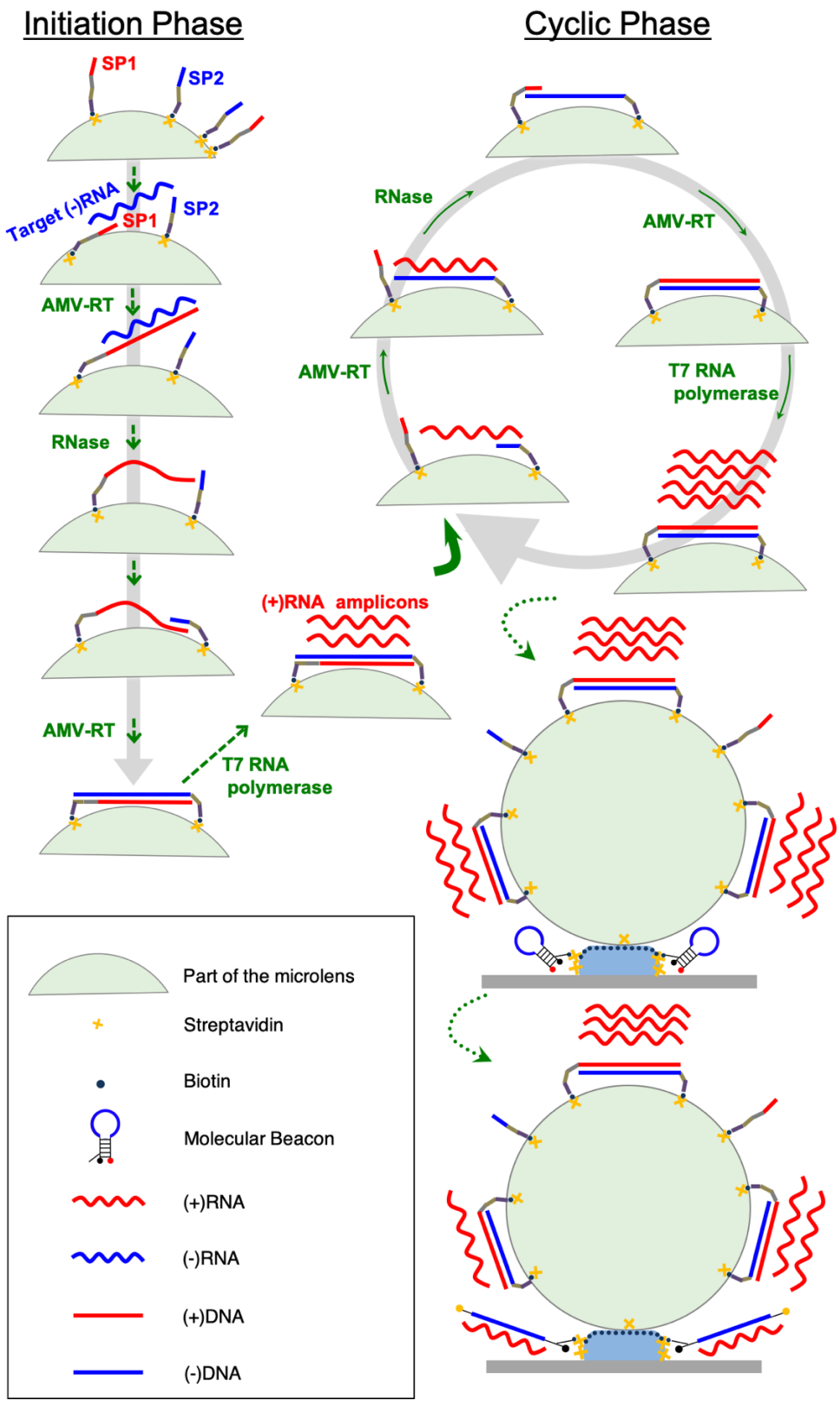

Figure S1: Schematic illustration of solid-phase nucleic acid sequence-based amplification (SP NASBA) on microsphere-on-microgel array.