Direct Write Protein Patterns for Multiplexed Cytokine Detection From Live Cells Using Electron Beam Lithography

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Supporting Information

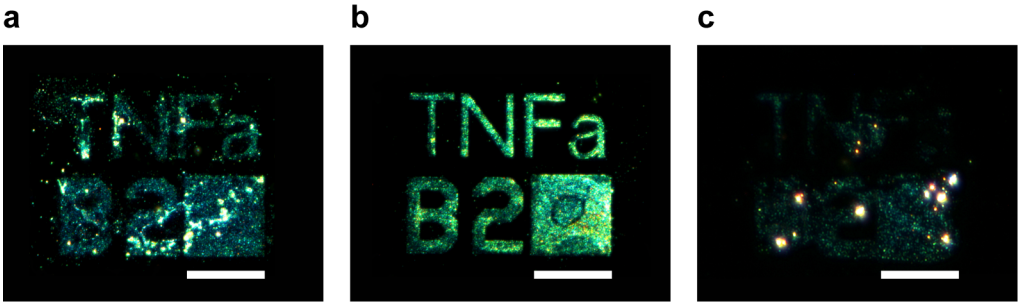

Figure S1. Effect of ascorbic acid on anti-TNFα patterning and immunoassay signals. Anti-TNFα patterned with area dose of 25 μC/cm^2 with 0.5% wt/vol PolyProtek and ascorbic acid concentration of a) 0 mM, b) 1 mM, and c) 2 mM. Scale bars = 35 μm.

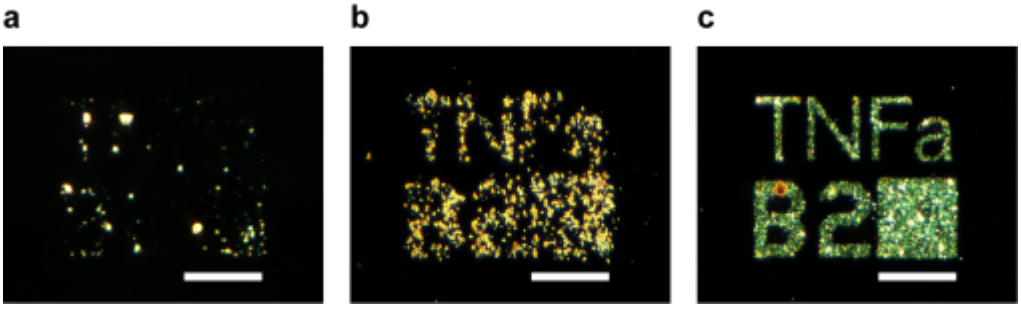

Figure S2. The effect of anti-TNFα spin-coating concentration on immunoassay signals. Concentrations of anti-TNFα used for spin-coating at a) 1 μM, b) 2 μM, and c) 5 μM. Scale bar = 35 μm.

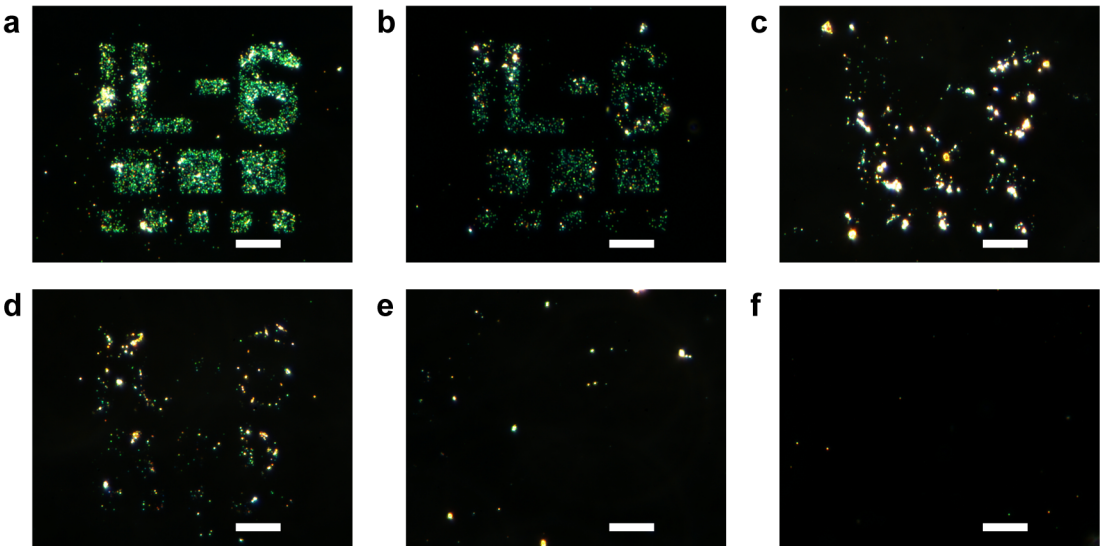

Figure S3. Detection sensitivity of anti-IL-6 patterns to varying concentrations of IL-6 in media: a) 200 ng/mL, b) 50 ng/mL, c) 500 pg/mL, d) 50 pg/mL, e) 5 pg/mL, and f) 0. Scale bars = 20 μm.

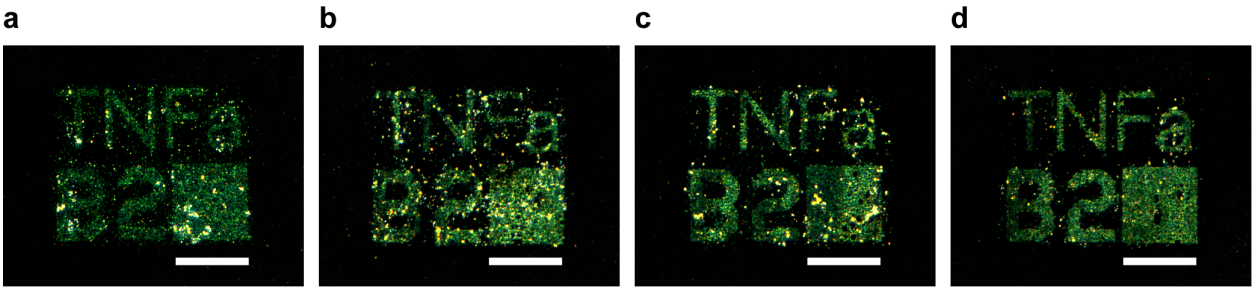

Figure S4. Repeated processing and exposure cycles of anti-TNFα patterned on the same substrate. The same substrate was subjected to four cycles of spin-coating with anti-TNFα antibody, e-beam patterning, and rinsing, and then developing with the immunoassay. Dark field micrographs show the pattern from the a) first cycle, b) second cycle, c) third cycle, and d) fourth cycle. The signal-to-noise of the square in the first cycle was 20.9 and in the fourth cycle 23.4 calculated as (signal from the box – signal of the background) / (stdev of the background). Scale bars = 35 μm.