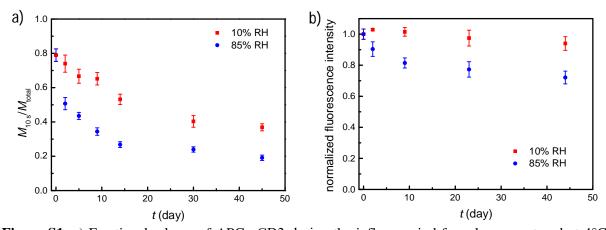
## Temperature switch cytometry – releasing antibody on demand from inkjet-printed gelatin for on-chip immunostaining

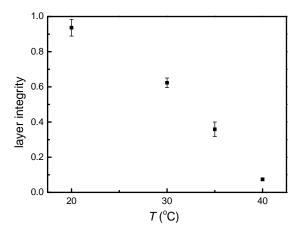
Xichen Zhang, † Dorothee Wasserberg, † Christian Breukers, † Leon W.M.M. Terstappen, † Markus Beck†, \*

<sup>†</sup>Medical Cell Biophysics Group, MIRA Institute for Biomedical Engineering and Technical Medicine, Faculty of Science and Technology, University of Twente, The Netherlands

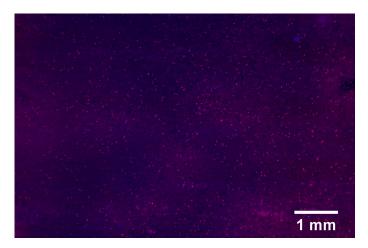
\*Corresponding author: Markus Beck E-mail: <u>m.beck@utwente.nl</u>



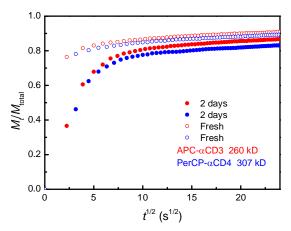
**Figure S1.** a) Fractional release of APC- $\alpha$ CD3 during the inflow period from layers matured at 4°C under 85% relative humidity (RH) (blue circles) and 10% RH (red squares) for varying storage periods. b) Normalized fluorescence intensity (APC- $\alpha$ CD3 readout) of dry layers matured at 4°C under 85% RH (blue circles) and 10% RH (red squares) for varying storage periods, respectively. Data points represent mean ± standard deviation (n=3).



**Figure S2.** The thickness ratio of layers, which are subject to initial maturation at 4°C under 85% RH for 2 days and follow-up maturation at 4°C under 10% RH for 6 weeks, before and after incubation in PBS at different temperatures for 3 min. Data points represent mean  $\pm$  standard deviation (n=3).



**Figure S3.** A complete overlay image (including both APC/red and PerCP/blue excitation) of matured gelatin/antibody layers (subject to 2-day initial and 6-week follow-up maturation) after 40 min incubation with blood. Randomly distributed stained cells are shown. Double positive cells are CD4<sup>+</sup> T-lymphocytes, seen as purple dots.



**Figure S4.** Kinetics of APC- $\alpha$ CD3 (red) and PerCP- $\alpha$ CD4 (blue) release from freshly printed layers (open circles) and from layers subject to 2-day maturation at 4°C under 85% RH (solid circles). The differences between the release kinetics of the two antibodies are negligible.