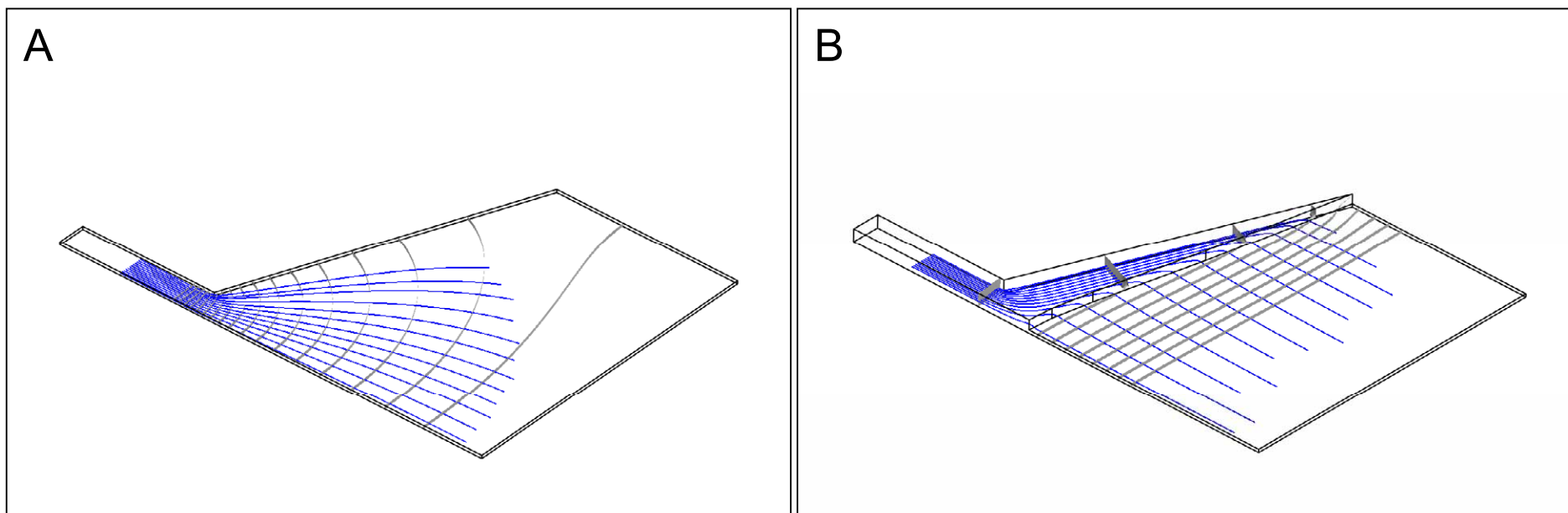


Supplementary figure 1: Temperature measurements for heating block and chip (PDMS insert).

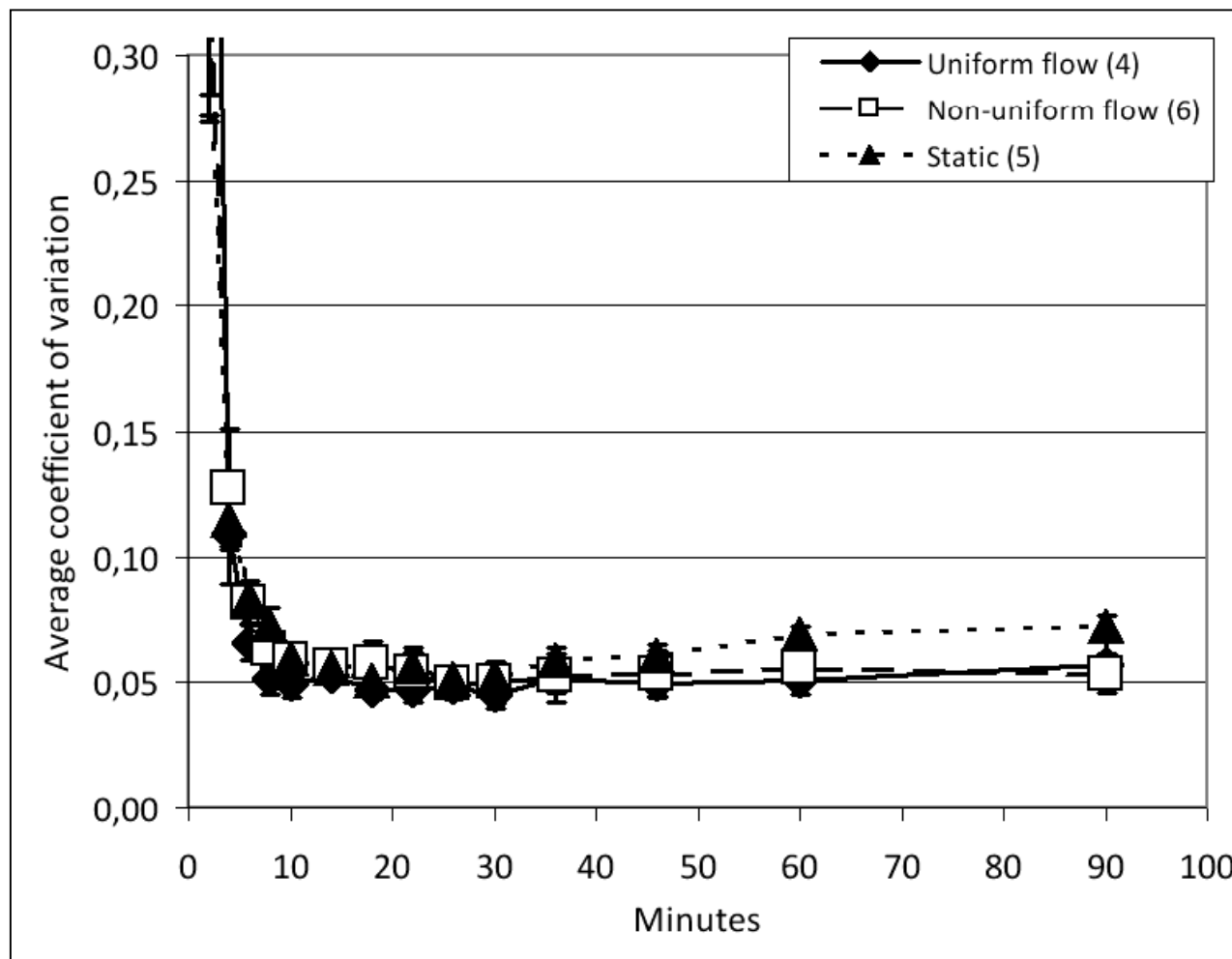
The *HistoFlex* device was maintained at 25 degrees for 10 minutes, heated to 37 degrees for 95 minutes and then cooled to 25 degrees. The heating block overshoots 37 degrees upon heating thereby accelerating heating of the chip (PDMS insert).



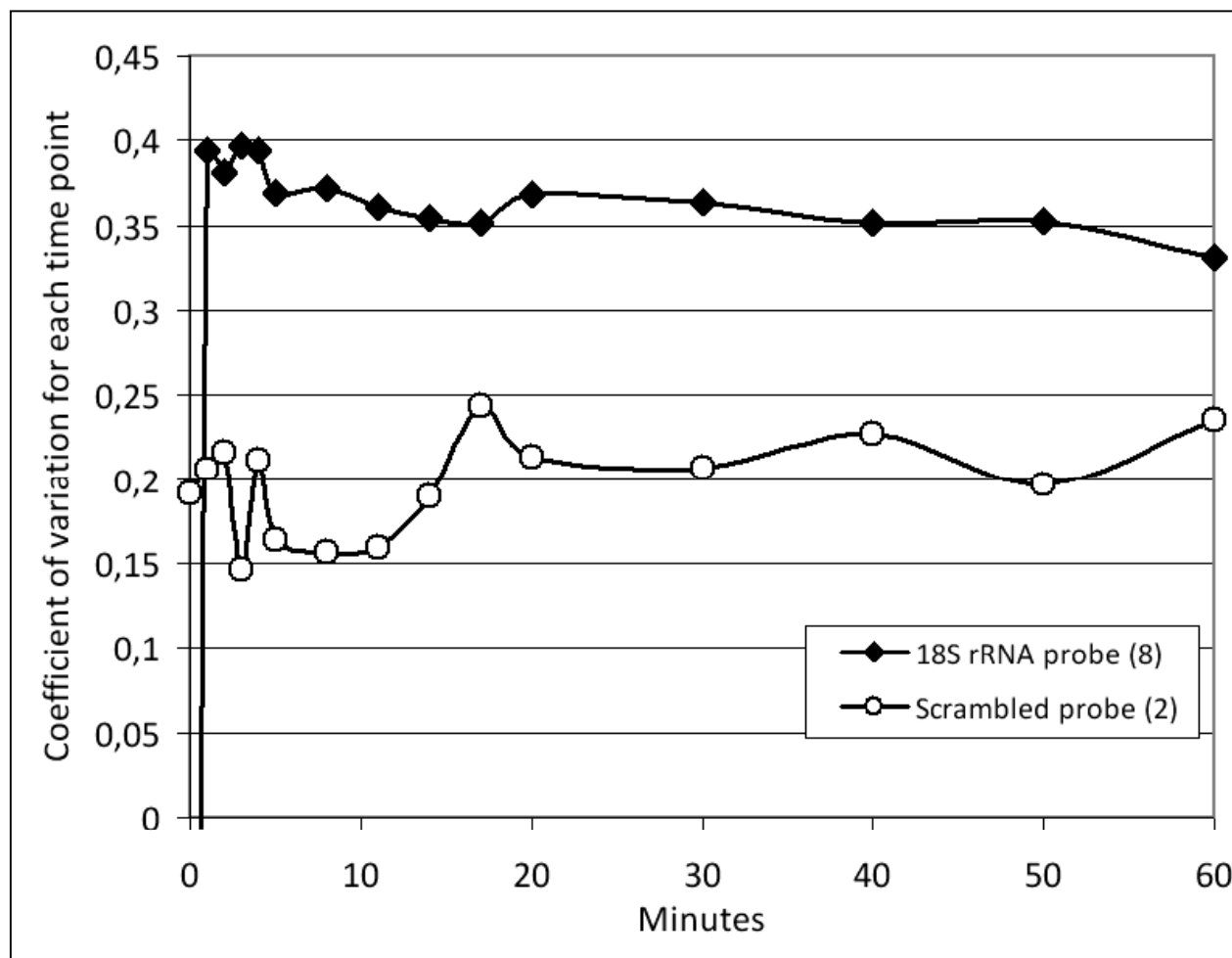
Supplementary figure 2: Numerical simulation of the flow profiles upon changing reagents in the reaction chamber.

One quarter of the reaction chamber is shown.

- a) Flat chamber topology in which the top corner of the chamber is poorly accessed by incoming reagents
- b) Uniform flow chamber topology.

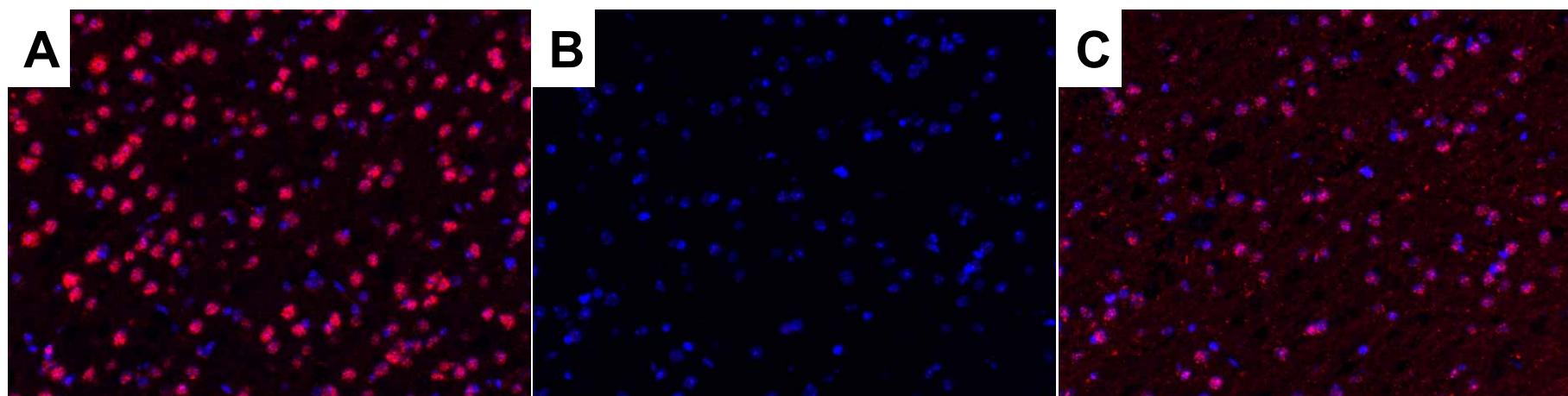


Supplementary figure 3. Microarray - spot to spot variation for 100 μm expansion area.
Parenthesis show number of experimental repeats.



Supplementary figure 4. Development of hybridization signal using on-line monitoring in the *HistoFlex*

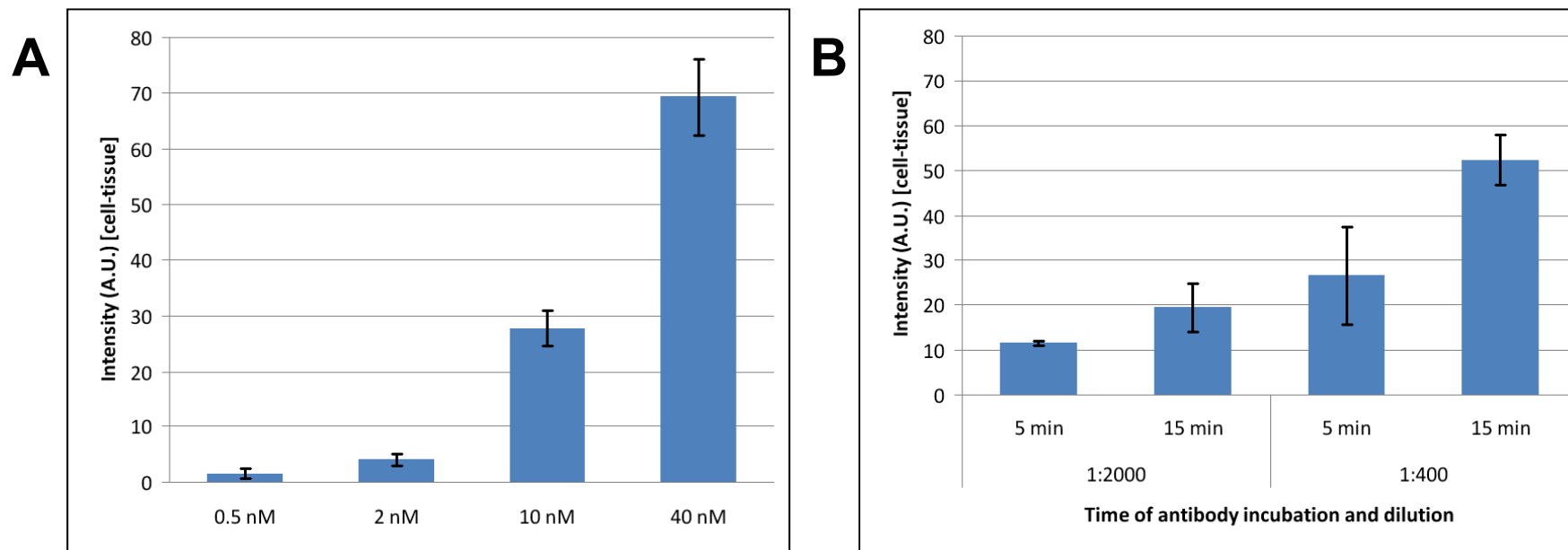
Slide to slide variation for direct ISH to 18S rRNA. Parenthesis show number of experimental repeats.



Supplementary figure 5: MicroRNA-130a hybridization.

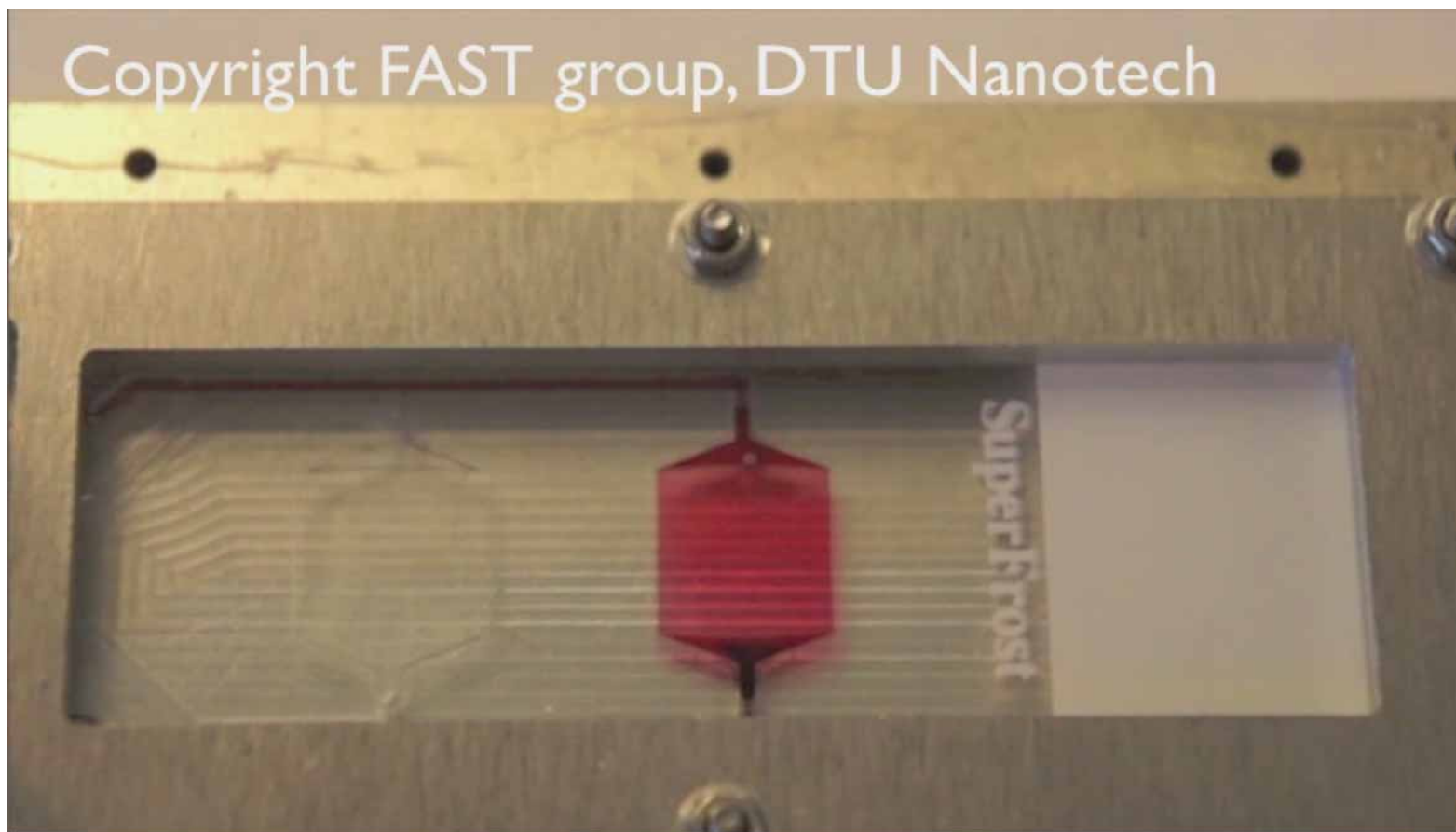
Hybridization step was performed in the *HistoFlex* and the rest of protocol using conventional techniques similar to figure 5.

- a) Flow based hybridization, imaged at 250 ms exposure time
- b) Static hybridization, imaged at 250 ms exposure time
- c) Static hybridization (identical to B), imaged at 1000 ms exposure time.



Supplementary figure 6. Adapting ISH assay for the *HistoFlex* device

- MicroRNA-138 *in situ* detection in the *HistoFlex* using short protocol similar to figure 6 with varying probe concentrations.
- MicroRNA-138 *in situ* detection in the *HistoFlex* using short protocol with varying concentrations and incubation times of antibody. Antibody has an anti-FITC region that recognizes the FITC hapten on the miR-138 probe and a HRP enzyme.



Supplementary video 1: Switching reagents in uniform flow chamber.

Chamber is filled with red dye which is replaced by blue dye and then red dye. Video is in real-time.