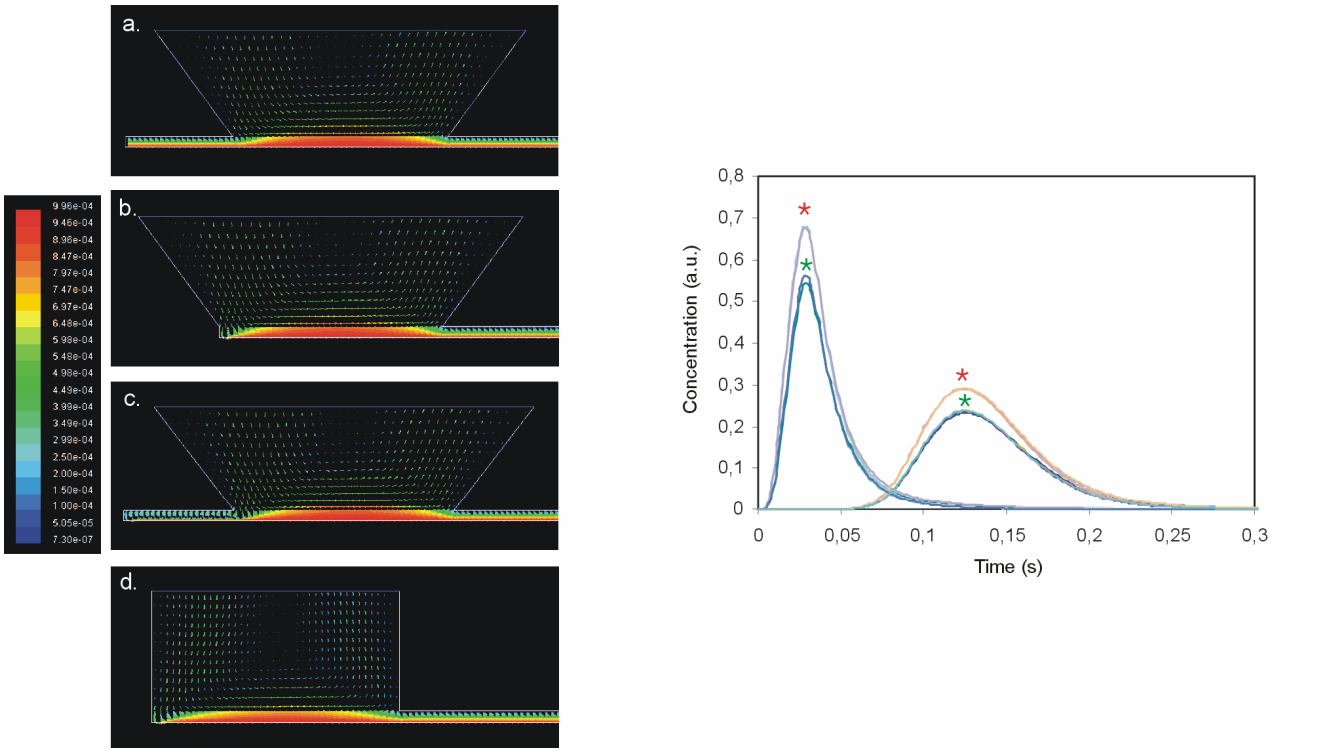

Fig. S1 Left: Flow profiles of different possible injection micro-well configurations. The pyramid-shape micro-well connects either behind the slit to a channel with continuous access to mobile phase (**a.**), is blocked precisely after the slit (**b.**), or connects to a dead-end channel (**c.**). In (**d.**) the injection micro-well has a rectangular design and no extension. Right: Relative dye concentrations for the four different designs **a.** to **d.** at monitor planes situated 10 µm (left hand curves) and 60 µm (right hand curves) downstream of the injector in he separation channel. The curves with the lowest concentrations (*) arise from the assumption that the micro-well was completely replenished with buffer. In the higher concentration curves (*) it was assumed that an analyte layer with the thickness of the channel height remains at the bottom of the micro-well after washing. The curves for all four designs are indistinguishable, independent of whether or not a layer of analyte is assumed to remain in the micro-well.

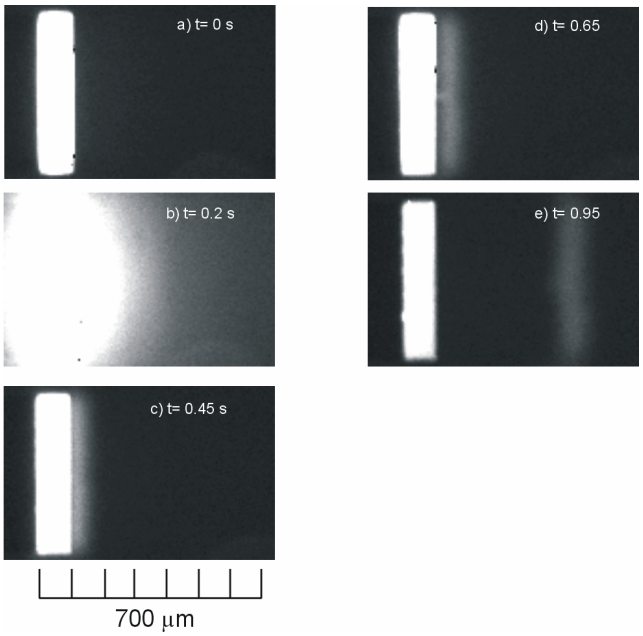

Fig. S2 Different steps in the injection procedure. a) the vacuum system is activated, b) the sample syringe is moved, c) the vacuum system stops while the movable wall is displaced (100 µm, 2 mm.s^{-1}), d) the vacuum is applied again and the well is filled with mobile phase, e) the tracer plug is transported through the channel by displacement of the movable wall (2 mm.s^{-1}).

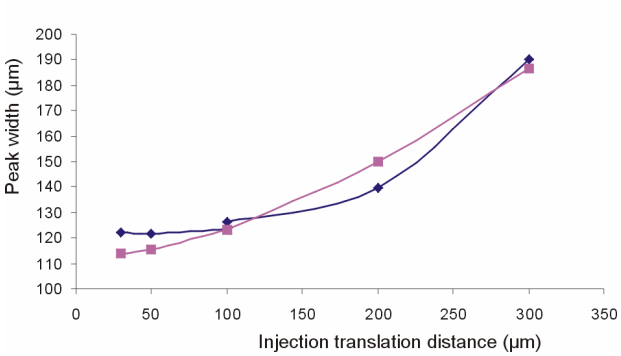

Fig. S3 Peak widths created by different injection translation distances. (—◆—) depicts the experimental values corrected for the peak width gained during movement in the channel after passing position 210 µm. (—■—) represents the theoretical values at position 210 µm. Estimated times for filling and flushing are 0.2 and 0.3 s, respectively.