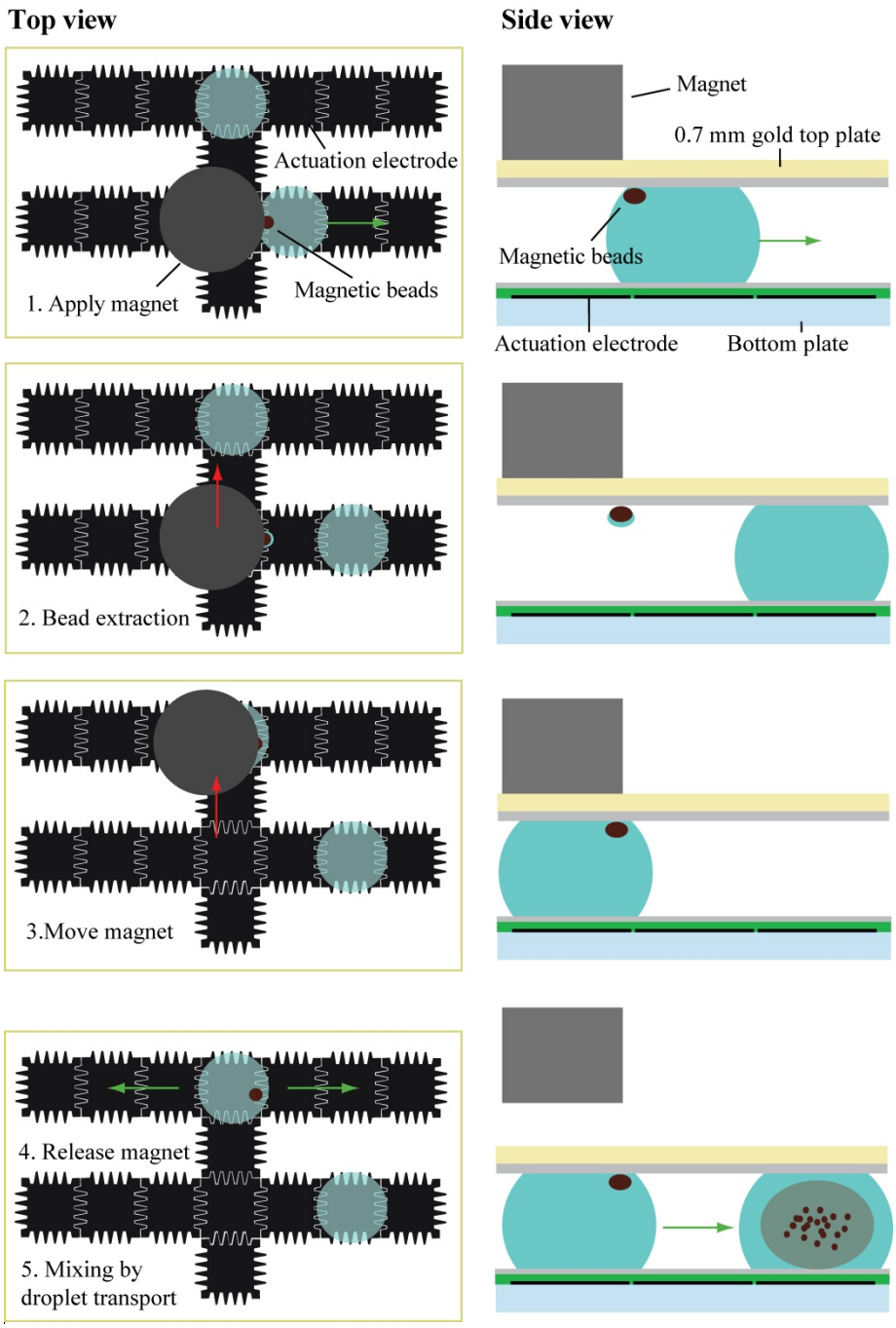
Supplementary information

Supplementary note 1: Digital quantification of C2CA products on 2 dimensional slide surfaces

As described in the section "Digital quantification of C2CA products" in the experimental section 6 areas on the microscope slide were imaged with 20x objective. One imaged area corresponds to approximately 0,5mm^2. Hence, 6 areas contain approximately 3mm^2. The C2CA products are spread out over a total area 225mm^2 on the microscope slide which means that a total area was imaged of 3mm^2/225mm^2 = 1.3%. To be able to count every RCA product (after one round of RCA) the entire 225mm^2 area had to be imaged. C2CA makes it possible to circumvent that by generating more copies of the initial molecule. The time of the first round of RCA in the C2CA assay determines the amount of products generated after C2CA (with increasing time more padlock copies are generated that after digestion and re-ligation will give rise to a product). During 20min in the first RCA step approximately 300 copies of the padlock probe are generated which in turn make 300 countable products after the second round of RCA in the C2CA assay. That means that 1 detected molecule is amplified to 300 countable C2CA products in total. Theoretically, to be able to detect only 1 initial molecule 1/300 = 0.33% of the total area has to be imaged to be able to detect 1 of the 300 C2CA product which result from 1 initial molecule.

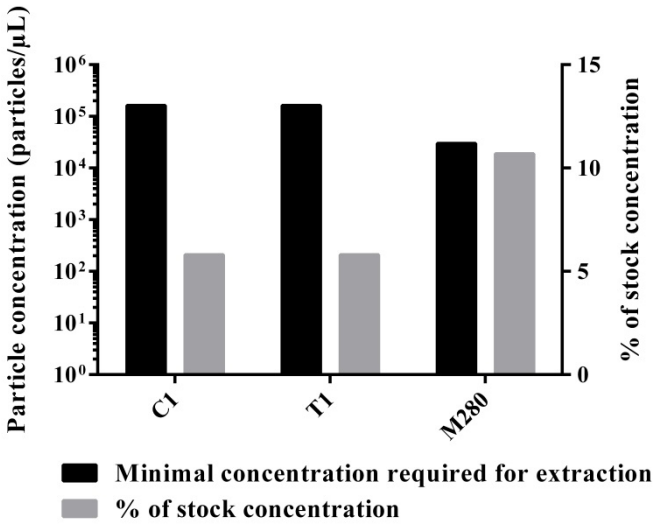
The process of imaging 6 random areas takes relatively little time. After the droplet containing C2CA products is pipetted onto the microscope slide and covered with a cover slip the distance between cover slip and slide is approximately 20 micrometer which makes the diffusion to the slide surface relatively fast. Once the diffusing C2CA products reach the positively charged slide surface they immediately bind to it. After ~5 min most/all C2CA products have bound to the surface. Manually imaging 6 areas takes approximately 2 minutes. Image analysis takes approximately 2 minutes. Conclusively, only approximately 10 extra minutes have to be spent for analysis.

Supplementary figures



Supplementary figure 1: Extraction and transportation protocol for superparamagnetic particles on the DMF chip. A permanent magnet is applied onto the top of a 0.7 mm thick top plate with gold as conductive layer. A droplet with superparamagnetic particles is transported under the magnet. The particles form a pellet that gets extracted from the droplet when the droplet moves to the neighboring electrode. By moving the magnet the extracted particle pellet is moved to another droplet where the

magnet is released and the particles mixed in the new droplet by transportation between neighboring electrodes as indicated by green arrows.



Supplementary Figure 2: Total amount of magnetic particles needed for complete particle extraction. 1 µm sized C1/T1 dynabeads in comparison with 2.8 µm sized M280 dynabeads.

Supplementary movies

The supplementary movies are provided in a zip-compressed folder. The following movies are provided:

Supplementary movie 1: Extraction, transfer and mixing of superparamagnetic particles on the DMF chip

Supplementary movie 2: On-chip pipetting of reagent droplets

Supplementary movie 3: Incomplete magnetic particle extraction with a 1.1 mm thick top plate

Supplementary movie 4: Complete extraction with a 0.7 mm thick top plate and the magnet edge positioned on the electrode border

Supplementary movie 5: Incomplete extraction with a 0.7 mm thick top plate and the magnet edge positioned in the electrode middle