INSTRUCTION SHEET

Immobilization on BSA-biotin-neutravidin surface

Immobilization in a *ibidi µ-Slide 8 Well Glass Bottom* chamber*

i) Wash one of the eight *µ-Slide* chambers three times with 500 µL of PBS.

ii) Incubate the *µ-Slide* chamber with 200 µL of BSA-biotin solution (1 mg/mL in PBS) for 5 min.

iii) Remove the BSA-biotin solution and wash it three times with 500 µL of PBS. Make sure not to scratch with the pipette tip on the surface. Choose one corner of the *µ-Slide* chamber and always pipette at this position.

iv) Incubate the *µ-Slide* chamber with 200 µL of neutravidin solution (1 mg/mL in PBS) for 5 min.

v) Remove the neutravidin solution and wash the chamber three times with 500 µL of 1x PBS supplemented with 10 mM magnesium chloride (so-called immobilization buffer: 1x IB).

vi) Dilute 1–10 µL of the DNA origami solution with 200 µL 1x IB. Incubate the *µ-Slide* chamber with this solution for 5 min. The optimal dilution factor to reach a dense surface for imaging mainly depends on the starting concentration of the DNA origami filtrate (this can vary between a concentration of 1-10 nM). This concentration depends on several factors such as purification type, DNA origami structure, initial scaffold concentration and age of the sample. Final concentration of the diluted DNA nanostructures should be in the mid pM range. To monitor the density of the structures on the surface we recommend to start with 1-3 µl and subsequently to increase the concentration if necessary. The ideal average density of DNA origami structures on the surface is ~1/µm².

vii) If the density is too low, return to Step vi) and use an adequate volume of DNA origami solution to achieve the desired surface density.

viii) If the desired density of DNA origami structures is achieved, wash the chamber three times with 500 µL of 1x IB.

ix) Fill the chamber with the respective imaging buffer matching the requirements of the intended measurement (take care that all buffers contain 5—20 mM magnesium chloride).

* The immobilization in flow chambers works analogues to the *µ-Slide* chambers using your required/ adjusted volumes (for instance 40 µL chamber volume)

DO YOU NEED ASSISTANCE?

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