This sample can be used as a control for training on the Prism or CoSMoS microscopes.

Prepare a 10 µL volume of Holliday Junction Annealing mix at 2:2:1:1 ratio of b:r:h:x (5.0 µM: 5.0 µM: 2.5 µM: 2.5 µM). (NB the strand lettering and labels differ from those in the referenced paper, the 2:2:1:1 ratio is maintained based on the label/attachment on the oligonucleotide – e.g. the Biotin strand is a "1")

b: 23 µM unlabeled DNA = 2.17 µL

x: 24.4 µM Cy3 labeled DNA = 1.03 µL

h: 18 µM biotin labeled DNA = 1.39 µL

r: 26.8µM Cy5 labeled DNA = 1.87 µL

add 2.00 µL 5x Annealing buffer (1x = 50mM Tris pH 7.5, 400mM NaCl)

add 1.54 µL nuclease free water (to final volume of 10 µL)

Heat Anneal in Thermocycler.

Initiate at 95 ºC for 2 min, decrease by 5 ºC every 2 min and then hold at 4 ºC

Final concentration of biotinylated DNA = 2.5 µM.

Serial Dilute down to 50 pM in 1xPBS.

2.5 µM - 50:1 -> 50 nM - 50:1 -> 1nM - 20:1 -> 50pM

Sample can be loaded on prepared slide and observed on miocroscope.

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