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 microscope.

Sean A. McKinney, Alasdair D. J. Freeman, David M. J. Lilley, and Taekjip Ha
Observing spontaneous branch migration of Holliday junctions one step at a time
http://www.pnas.org/content/102/16/5715