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🌐 spyDNA650 staining with CO2 independent media for live endothelium microscopy

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<https://protocols.io/view/spydna650-staining-with-co2-independent-media-for-b3m7qk9n>



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Staining

- 1 Dilute 1:1000 SPY650 DNA in CO2 independent media(500 uL total volume for 2 slides, 220 uL per slide and incubate in incubator for 20 mins
- 2 Gently remove overnight media from the u-slides and add warmed dye-media mix. Check whether monolayer is preserved. incubate for 2-3 hours

Flow Unit Equilibration

- 3 In 7 mL Co2 independent media, dilute 3.5 μ L SPY650 dye(1:2000 final concentration)
- 4 Add 3 mL media the leftwards syringe, 4 mL on the other
- 5 Set Ibidi Flow units up for equilibration inside microscope.
- 6 Start the heating of the microscope
- 7 Run for 1 hour, at 40 mbar and half the cycling time(about 5 secs), pay close attention to whether neither of the syringes run empty and do the clamp test.

Microscopy

- 8 Quickly bring one of the slides to the microscopy room without any washing step and plug it to the flow unit