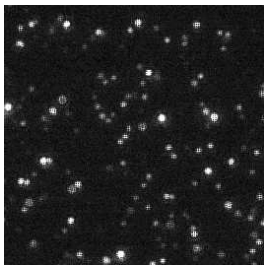
May 08, 2024 Version 1

Imaging single SYTOX Orange molecules on a PLL-coated cover glass V.1



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Manuscript citation:
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bioRxiv 2023.02.07.527479, doi: https://doi.org/10.1101/2023.02.07.527479

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We use this protocol and it's working.

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Abstract

This is a protocol for the preparation of a microscopy sample of single SYTOX Orange molecules on a PLL-coated cover glass. This protocol was used to generate the data shown in **Figure 1a, 1b** and **1c** of the following publication:

- Bruggeman *et al.,* POLCAM: Instant molecular orientation microscopy for the life sciences. bioRxiv 2023.02.07.527479 (Feb **2023**), doi: https://doi.org/10.1101/2023.02.07.527479

Protocol

1h 30m

1 Argon plasma clean cover glass (VWR collection, 631-0124) for 00:30:00 in a plasma cleaner (Expanded Plasma Cleaner, PDC-002, Harrick Plasma). 30m

2 In the meantime:

- Filter phosphate-buffered saline (PBS) using a 0.02 µm syringe filter (6809-1102, Whatman).
- Dilute SYTOX Orange (S11368, Invitrogen) in filtered PBS to a concentration of [M] 1 nanomolar (nM) .

Note

Always use a new aliquot of SYTOX Orange to prepare the 1 nM dilution, as dye doesn't store well at low concentrations.

3 Create a sample well on the cleaned cover glass by sticking a frame-seal slide chamber (9x9 mm, SLF0201, Bio-rad) on the cover glass.

4 Pipet 70 µL of 0.01% PLL (0.01% poly-L-lysine solution, P4707, Sigma-Aldrich) into the well and wait for 00:15:00 . The PLL will coat the surface of the cover glass. 15m

Note

Always use a freshly thawed aliquot of PLL. You can aliquot the PLL and store it in a -20 °C or -80 °C freezer.

5 Use a pipet to remove the excess PLL from the well and immediately replace it with 70 µL of filtered PBS.

Note

It is important to always have liquid on top of the PLL-coated glass and not let it dry out.

6 Use a pipet to remove the excess filtered PBS from the well and immediately replace with 70 µL filtered PBS. Gently pipet up and down in the corners of the well. Repeat this step 2 more times.

7 Use a pipet to remove the excess PBS from the well and immediately replace with 50 µL of [M] 1 nanomolar (nM) SYTOX Orange (S11368, Invitrogen). The SYTOX Orange molecules will stick to the surface of the PLL-coated cover glass.

8 Image the sample straight away and make sure it doesn't dry out during imaging.