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Imaging single AF647 molecules immobilised in PVA on a cover glass

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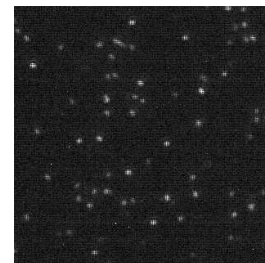
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Manuscript citation:

POLCAM: Instant molecular orientation microscopy for the life sciences

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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 Alexa Fluor 647 molecules immobilised in PVA polymer on a cover glass. This protocol was used to generate the data shown in **Figure 2i and 1j** 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

- 1 Prepare a 1% poly(vinyl) alcohol (PVA) solution:
 - 1.1 Filter  100 mL Milli-Q water using a syringe filter (0.02 µm syringe filter, Whatman, 6809-1102).
 - 1.2 Dissolve  1 g of solid PVA into  100 mL of filtered milli-Q water in a glass beaker.
 - 1.3 Place the beaker on a stirring hot plate and heat the solution to  90 °C under continuous stirring for  00:30:00 or until the PVA is completely dissolved. 30m
 - 1.4 Let the solution cool. Once cooled, filter it using a syringe filter (0.02 µm syringe filter, Whatman, 6809-1102). You can store the filtered 1% PVA solution at  4 °C for later use.

Note

The filter tends to clog if you try to filter too large a volume of 1% PVA in one go. If this happens, filter in separate smaller batches, replacing the filter each time for a new one.

- 2 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
- 3 Dilute Alexa Fluor 647 (AF647) to  500 picomolar (pM) in the filtered 1% PVA solution.
- 4 Place a clean cover glass into a spincoater (WS-650Mz-23NPPB, Laurell) and spin-cast  10 µL of the AF647 solution onto the cover glass for  00:00:45 at  3000 rpm . 45s

Note

To avoid forgetting which side of the cover glass the sample was put on, you can use a marker to place a small dot in the corner of the cover glass before spin coating.



- 5 *Optional:* Seal the sample by sticking it to a standard glass microscope slide (631-1550, VWR) using a double-sided frame-seal slide chamber sticker (9×9 mm, SLF0201, Bio-rad). Make sure the coated side of the cover glass is facing the microscope slide.