Sample preparation for single molecule localisation microscopy and imaging.

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Sep 21, 2022

dx.doi.org/10.17504/protocols.io.3byl4j77olo5/v1

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ABSTRACT

This protocol describes the sample preparation to perform SMLM on midbrain dopaminergic neurons using phalloidin and a DNA aptamer in a combination of STORM and DNA-PAINT.
It also describes the experimental set up on the microscopy with the image acquisition details.

DOI

dx.doi.org/10.17504/protocols.io.3byl4j77olo5/v1

PROTOCOL CITATION

gurvir.virdi 2022. Sample preparation for single molecule localisation microscopy and imaging.. **protocols.io** https://protocols.io/view/sample-preparation-for-single-molecule-localisatio-cgxdtxi6

CREATED

Sep 21, 2022

LAST MODIFIED

Sep 21, 2022

PROTOCOL INTEGER ID

70341

Fixation 22m

1 For single molecule localisation microscopy (SMLM), neurons were grown on glass coated ibidi chambers.

2 Once neurons reached the desired age, they were washed once in PBS

3 They are then fixed for **00:15:00** in 4% paraformaldehyde + 0.1% glutaraldehyde (both from Electron Microscopy Services) in PBS at room temperature. 15m

4 The neurons were then reduced in 0.1% sodium borohydride (Sigma) in PBS for **00:07:00** at room temperature. 7m

Labelling cells 3h 20m

5 To label cells with the DNA-aptamer and phalloidin, after fixation and PBS washes, cells were permeabilised with 0.25% triton X-100 in PBS for **00:10:00** at room temperature 10m

6 The cells were then blocked in blocking solution (0.1% triton X-100, 10% normal goat serum (Abcam), 10% salmon sperm DNA (Thermo Fisher Scientific)) in PBS for **02:00:00** at room temperature 2h

7 The samples were then incubated with 100 nM of the DNA-aptamer made up in the blocking solution at **4 °C** overnight.

> Aptamer binds to beta-sheet rich structures including protein aggregates. The sequence of the aptamer is: GCCTGTGGTGTTGGGGCGGGTGCGTTATACATCTA (ATD Bio).

8 After incubation, cells were washed 1x in PBS

9 They are then incubated with phalloidin-647 (1:400) (Thermo Fisher Scientific) made up in the 1h

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blocking solution for **01:00:00** at room temperature.

10 Cells were then washed 1x in PBS and either imaged, or incubated with DAPI (1:10000) in PBS for **00:10:00** at room temperature followed by 2x PBS washes before imaging. 10m

Labelling aggregates in cell lysate 1h 15m

11 Cells were lysed mechanically in PBS before being centrifuged at **3600 x g, 4°C, 00:05:00** . 5m

12 The supernatant was collected and the protein concentration was quantified using the BCA Protein Assay Kit (Thermo Fisher Scientific).

13 22 x 40 mm, 1 mm thick glass slides, were cleaned with an argon plasma for **01:00:00** before 22x22 gaskets (Bio-Rad) were affixed to the surface to create a well. 1h

14 Cell lysate was diluted 1 in 10 with filtered PBS (0.02 µm) and added to the glass slides

15 100 nM DNA-aptamer was added to the glass slide and incubated with lysate for **00:10:00** . 10m

Aptamer sequence: GCCTGTGGTGTTGGGGCGGGTGCGTTACCACCACCACCACCACCA (ATD Bio)

16 Sample was then washed off with filtered PBS three times before imaging

Microscopy imaging

17 SMLM was performed on a Nanoimager super-resolution microscope (Oxford Nanoimaging Ltd) equipped with an Olympus 1.4 NA 100x oil immersion super apochromatic objective.

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18 To ensure efficient stochastic blinking for STORM (AF647-tagged phalloidin), the samples were incubated with a blinking induction buffer (B cubed, ONI).

19 Separately to this, AD-PAINT was also employed which relies on the addition of an imaging oligonucleotide strand

to the buffer. 1 nM of the imaging strand was added to the B cubed buffer before imaging.

sequence: CCAGATGTAT-CY3B

20 The laser illumination angle was set to 51^{o} for all imaging leading to total internal reflection fluorescence (TIRF).

21 AF647-tagged phalloidin was first imaged for 4000-8000 frames using the 640 nm laser (80% power). After this, 4000-5000 frames at 30% power for the 561 nm laser was used to image and super-resolve the aptamer. Both were recorded at a frame-rate of 50 ms.

22 For imaging aggregates in neuronal lysate using AD-PAINT, 2 nM of the imaging strand was added. Images were acquired on Oxford Nanoimager at 20 frames $s^{-1,}$ for 8000 frames (20% 635 nm laser power, TIRF).

sequence: GGTGGT-ATTO 655

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