SoRA microscopy protocol for imaging oligomers in human brain tissue

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We use this protocol and it's working

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ABSTRACT

This protocol gives a step by step guide to imaging oligomers in human brain tissue using a spinning disk confocal microscope. The microscope used for this protocol was a commercial set up produced by 3i, however the techniques described can be used as general guidelines with alternative microscopes.

GUIDELINES

- It is important to use a high NA objective lens for these experiments.

MATERIALS

- Pre-prepared human brain tissue samples (see step 1).
- Spinning disk confocal microscope and associated control PC, software etc.
- High NA 100x objective lens.
- Immersion oil (type will depended on microscope and objective lenses being used).
- Laser power meter and slide sensor head (the one used for this protocol was produced by ThorLabs).

SAFETY WARNINGS

- Spinning disk confocal microscopes typically use high powered lasers which when used incorrectly can cause severe eye damage. Please make sure to abide by any manufacturer and local institution laser safety rules.

BEFORE START INSTRUCTIONS

- Before starting prepare tissue samples as per the protocol referenced in section 1.

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Sample preparation and handling

1 Prepare samples in advance of imaging according to protocol: Single-molecule Immunofluorescence Tissue Staining Protocol for Oligomer Imaging V. by Rebecca Andrews.

2 Stores samples in fridge at 4 °C . Only remove samples from the fridge one at a time for the duration of imaging. If only imaging each sample for short periods, or if the fridge is a long distance from the microscope keep samples on On ice .

3 In order to reduce photobleaching of the samples (and ensure best results), expose samples to the minimum possible amount of laser light to image effectively.

Microscope switch on and set up

4 Switch on and set up the microscope Please note: the specific instructions in the sub-steps below apply to 3i commercial spinning disk confocal microscope (inverted configuration Zeiss microscope body) with the associated SlideBook software, however they can be adapted for another set up as appropriate.

4.1 Switch on the the microscope hardware, including the laser launcher, cameras, mswitcher, microscope body, white light source and all accompanying power and control units. Wait for camera initialising lights to stop flashing.

4.2 Switch on the microscope control PC. Once on, load the microscope control software (in this

case SlideBook).

4.3 On the laser control unit switch the interlock key to on and use the software to switch on each laser individually and check they are lasing as expected.

5 Check the laser power output. This has two purposes; to ensure the lasers are functioning correctly (power is not decreasing over time), and to enable the accurate calculation of the laser power density exposure of the tissue.

5.1 Put a drop of immersion oil onto the 100x objective lens (or whichever lens is being used for imaging).

5.2 Place the slide laser power meter head onto the microscope stage, ensuring the sensor is facing towards the objective lens.

5.3 Switch on the bright field mode and make the white light spot as small as possible. Move the microscope stage in x-y so that the light spot is positioned in the centre of the cross hairs on the back of the power meter head. (This will ensure the objective touches the centre of the sensor giving the most accurate results).

5.4 Carefully raise the objective lens in (move upwards in z), until it is nearly touching the sensor.

5.5 Switch on the laser power meter and ensure it is set up for the appropriate wavelength and power range.

5.6 Use the microscope software to switch on a laser. The power meter should begin to read a value. Gently move the objective so that immersion oil makes contact with the sensor. It is possible to tell that contact has been made as there will be a large step increase in the

measured power output. (If you are still unsure you can switch off the laser and remove the sensor. If contact has been made there will be an oil spot on the sensor. You may need to re-position the sensor if you do this).

5.7 Individually switch on and off the lasers being used for imaging and record their output power. Ensure that the power meter is configured correctly each time.

5.8 Gently retract the objective lens from the sensor before removing. Clean the sensor head of immersion oil before putting away.

5.9 It is best practise to also repeat this laser power meter reading at the end of imaging to ensure that the power remained consistent throughout imaging.

Imaging process

6 Mount the sample and focus the microscope in preparation for imaging.

6.1 Before loading samples onto the microscope check that the objective lens is clean and retracted away from the stage. (It is fine to reuse immersion oil used with the laser power sensor if confident the sensor if clean and dust free).

6.2 Put a drop of immersion oil onto the 100x objective lens (or whichever lens is being used for imaging).

6.3 Place the sample on the microscope stage, coverslip towards the objective lens. (If performing large scans, it is advisable to make sure that the sample is resting on just the slide at all four corners, or just the coverslip at four corners, to give the flattest possible surface).

6.4 Gently raise the objective lens, until the immersion oil makes contact with the coverslip.

6.5 Switch on the brightfield mode and adjust the white light power so that it is not saturating the camera. Gently move the objective lens up and down until the tissue is in focus.

7 Quick staining check before main imaging.

7.1 Move the objective to a place on the sample in which you do not want to image in detail. Check the focus using brightfield mode.

7.2 Switch on the laser in the oligomer channel (typically 568nm), in a PD sample it should be possible to see bright puncta across the image.

7.3 If using cell markers alongside the oligomer marker, check the addittional channels. Cell markers are typically significantly brighter than oligomers. The laser power may need adjusting down to ensure the camera is not saturated and to minimise possible channel crosstalk.

8 Run an imaging scan. These scans consist of a 3x3 FoV scan, with 25 0.5um steps in z. These parameters can be adjusted to fit any desired grid size and depth of field of view (depending on camera detector size and sample thickness).

8.1 First move the objective to the desired point on the tissue. Switch on the brightfield mode and use the white light to check the brain tissue is in focus.

8.2 In SlideBook (microscope software) save a new slide file (you will be automatically prompted to do this if you open the capture window).

8.3 In Slidebook go to the focus window, select the z tab, click ‘clear all’ to wipe the previous focus positions.

8.4 Switch on the oligomer channel laser and use the 1um up and down arrows to move the focal plane through the sample. In the focus window, in the z tab, set the top and bottom focus positions with a distance of 12um (easiest way to do this is to find the top of the sample, click set top, click the 1um down arrow 12 times then set the bottom position).

8.5 Once set click the FOV position right arrow once (bottom left of the focus window) - this moves the objective so that the position where the z positions were set is no longer part of the final image, minimising the impact of photobleaching on results.

8.6 Set the image extents to 3x3 and click the **top left corner** (make sure it is top left so you do not re-image where you focussed).

8.7 In the capture window ensure that all the necessary settings are selected - montage, 3D scan (using top and bottom positions, 25 slices of 0.5um depth and total range of 12um), and that the correct laser and cameras are selected e.g. 561nm confocal.

8.8 At the bottom of the capture window name the slide item. Click start and wait for the capture to complete - should take approximately 90 seconds.

8.9 Repeat the steps in section 8 as many times as desired, making sure to move to a new position each time.

8.10 Please note that if imaging a much larger area, e.g. a 30x30 FoVs scan, it is advisable to set the focus and z steps in the centre of the imaging region as this will increase the number of in focus steps over the tissue (due to variability in tissue flatness). In large scans such as this, where there are 900 positions, the impact of photobleaching in one position will have minimal impact on the over all results.