

## **BIU Generic Lightsheet Microscope Protocol**

### **Notes:**

1. This is a generic protocol; you may (and likely will) have to optimise it for different tissue types, source species, size of sample, age of sample at fixation, time in fix.
2. For permeabilisation, washing, antibody staining etc. BUT NOT dehydration and clearing, samples may benefit from being placed on a roller mixer\*
3. All timings depend on tissue size and type.

### **Protocol**

1. Fix tissue – 4% PFA in PBS, (pieces of tissue should be no larger than 10 mm x 10 mm). 2 hours at 4°C, wash out as much blood as possible before fixing to reduce autofluorescence.
2. Wash 3x PBS.
3. Permeabilise tissue – 2% Triton-X 100 in PBS, 2 hours at room temperature (time depends on sample size and type).
4. Wash 3x PBS.
5. Block – 2% BSA in PBS, 2 hours at room temperature (time depends on sample size and type).
6. Primary antibody – overnight at 4°C, dilute antibody in 2% BSA in PBS (dilution needs to be determined).
7. Wash 3x PBS.
8. Secondary antibody – 2 hours at room temperature, dilute antibody in PBS (dilution needs to be determined).
9. Wash 3x PBS. Repeat steps 6 to 9 as necessary for other antibodies
10. OPTIONAL - incubate with FITC-lectin – 2 hours at room temperature, use a 1 in 20 dilution of 1mg/ml lectin solution in PBS. Then wash 3x PBS.
11. OPTIONAL - stain nuclei - 60 minutes at room temperature, use DAPI at 1 in 500 dilution of 1mg/ml stock. Then wash 3x PBS.
12. Dehydrate in methanol series

- i. 50% methanol in water x1
- ii. 70% methanol in water x2
- iii. 80 % methanol in water x2
- iv. 95% methanol in water x2
- v. 100% methanol x 2 (it might be worth keeping molecular sieve in the stock)

Incubation time depends on sample size – try 3-4 hours per step.

During incubation prevent exposure to light. DON'T USE A ROLLER MIXER for dehydration and clearing steps. You can use gentle rotation.

13. Place tissue in dichloromethane (DCM), leave until tissue sinks to the bottom of the vial.
14. Clear in DBE – 100% DBE x 3 Incubation time depends on sample size – try 2 hours per step. During incubation prevent exposure to light.
15. Store at 4 °C, protect from light.