A guide to mounting zebrafish larvae for diSPIM imaging

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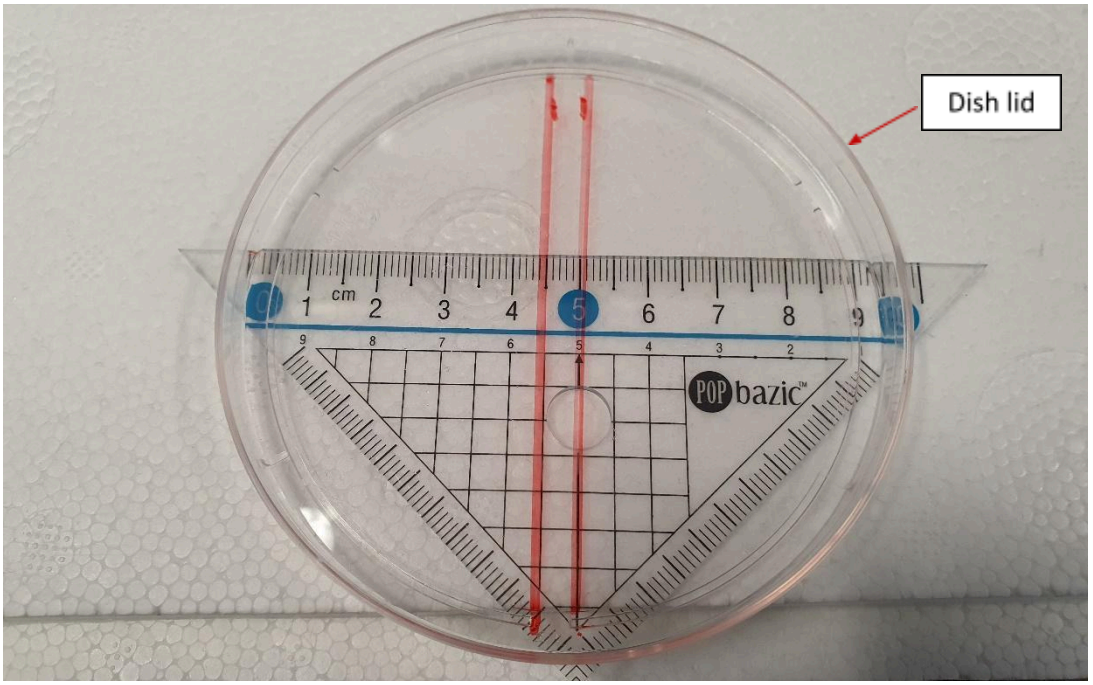
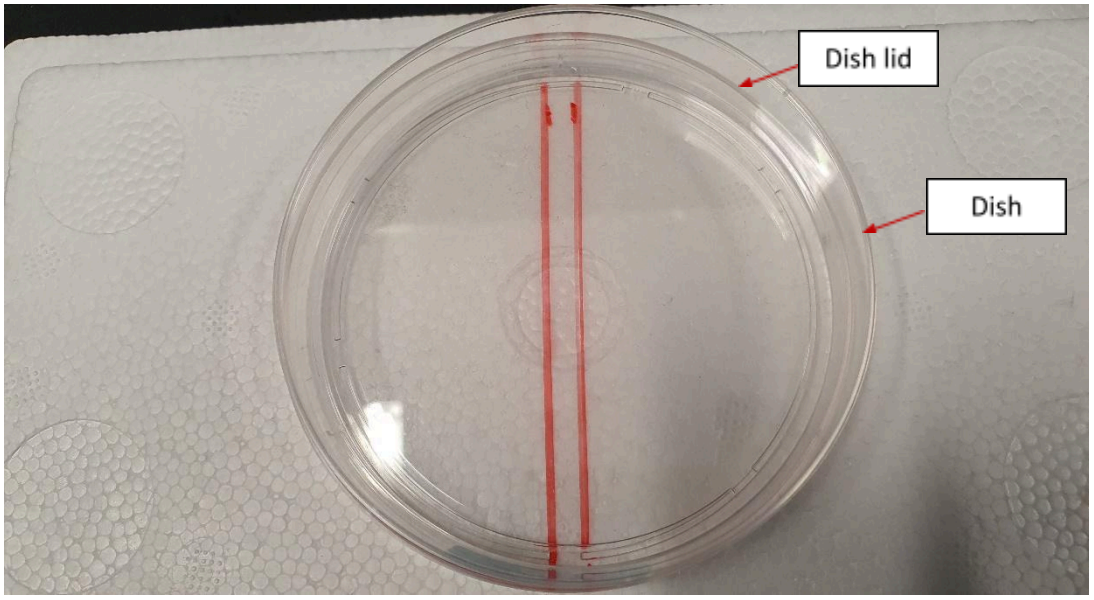
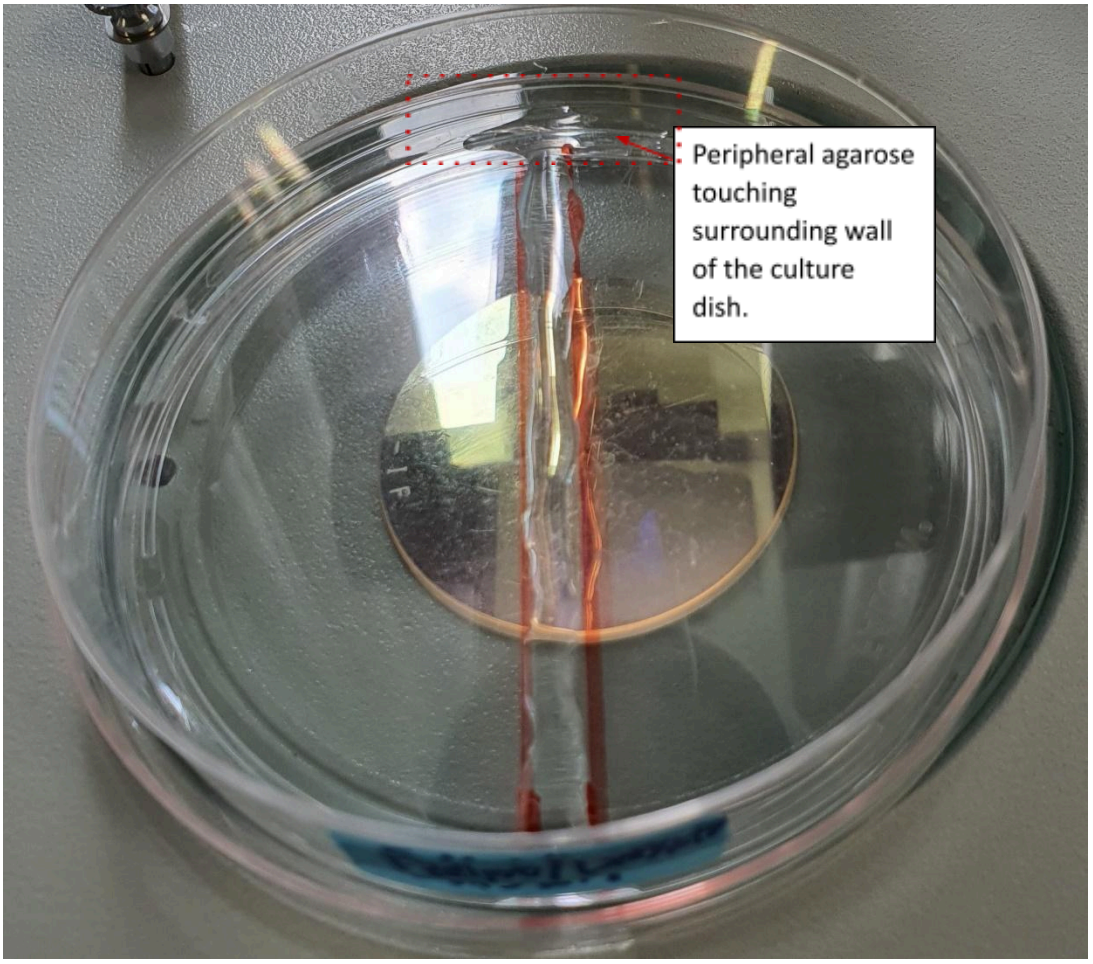
- Prepare aliquots of molten 1% low gelling agarose in water on the day of diSPIM imaging. These aliquots in 1.5 mL microcentrifuge tubes can be kept molten in a 37 °C incubator until zebrafish larvae are ready for mounting.
- We recommend the use of a cell culture dish with specification of 100mm (width) by 20 mm (height) to mount zebrafish larvae. The height of this dish is taller than the standard dishes use for bacterial work (100 mm by 15mm). The advantage of using a taller dish is that water will not overflow from the zebrafish larvae mounted dish after immersion of the 2 water dipping objectives attached to diSPIM.
- Draw 2 parallel lines on the centre of the lid with a gap of 0.5 cm between the lines.



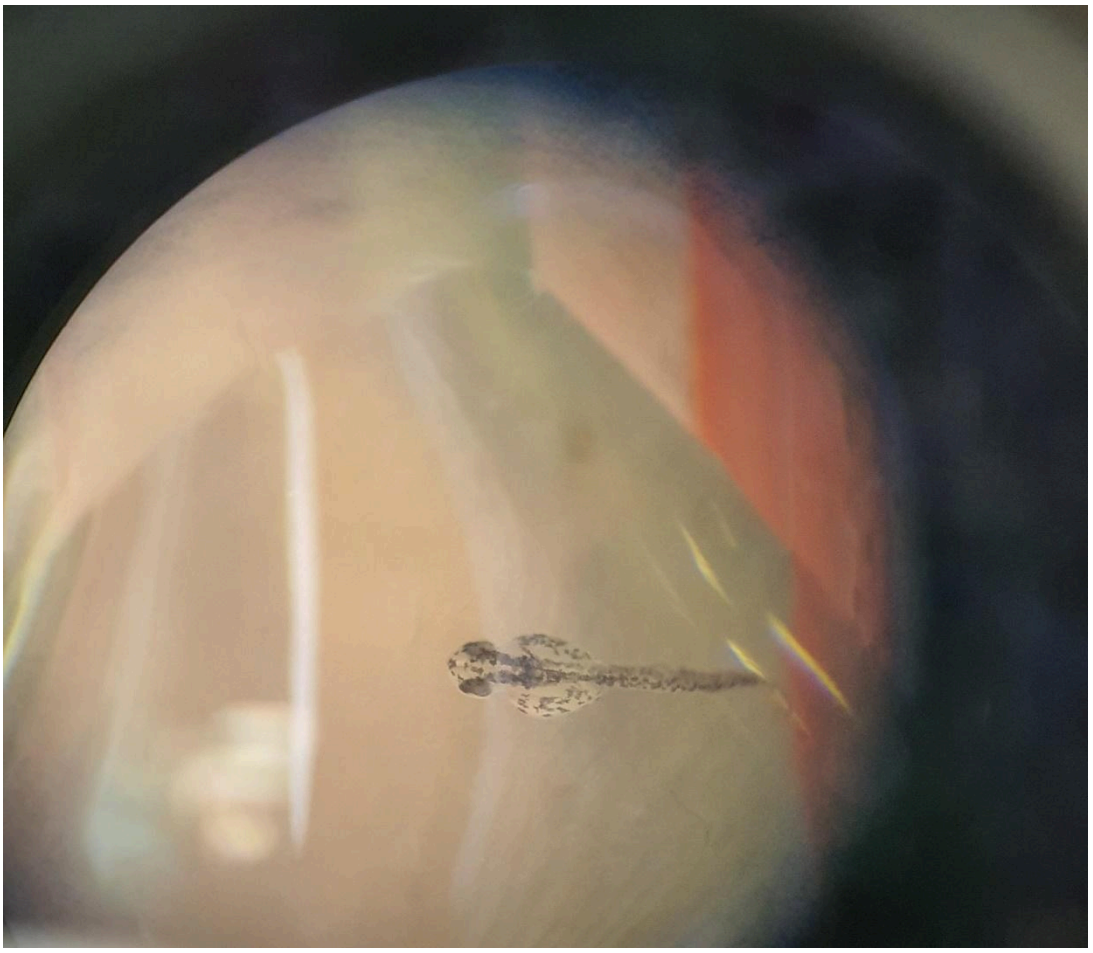
- Placed the marked lid beneath the dish as shown.



- Lay a cushion of 1% low gelling agarose along the 0.5 cm gap such that it formed a continuous column, do ensure that the molten agarose does not overflow outside the boundaries of the 2 red parallel lines. The 0.5cm agarose column ensure that mounted embryos will be illuminated by either light sheet and the agarose cushion width is not so broad such that it will be crushed by the 2 water dipping objectives during z direction movements of the objectives.
- To ensure that the agarose column doesn't dislodge upon addition of water during diSPIM imaging, make sure the edges of the agarose touch the periphery of the dish as shown below.



- Mount zebrafish larvae on top of the agarose cushion using 1% low gelling agarose with tissue of imaging interest facing the upright water dipping objectives. Low gelling agarose containing a final concentration of 1x buffered MS22 is used to mount zebrafish larvae, this minimize embryo movement during image acquisition. A mounted zebrafish larvae prepared for dorsal brain imaging is shown below as a guide. Note that the agarose never crossed the border demarcated by the red line. Pigmented zebrafish larvae is used for ease of visualization. Only optically transparent larvae e.g. zebrafish cultured in egg water with PTU are used for diSPIM imaging.



- Add water to mounted zebrafish larvae after 15minutes, adjust the water level such that it covers the agarose. The dish of mounted zebrafish larvae is now ready for diSPIM imaging.