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A Four-Well Dish for High-Resolution Longitudinal Imaging of the Tail and Posterior Trunk of Larval Zebrafish

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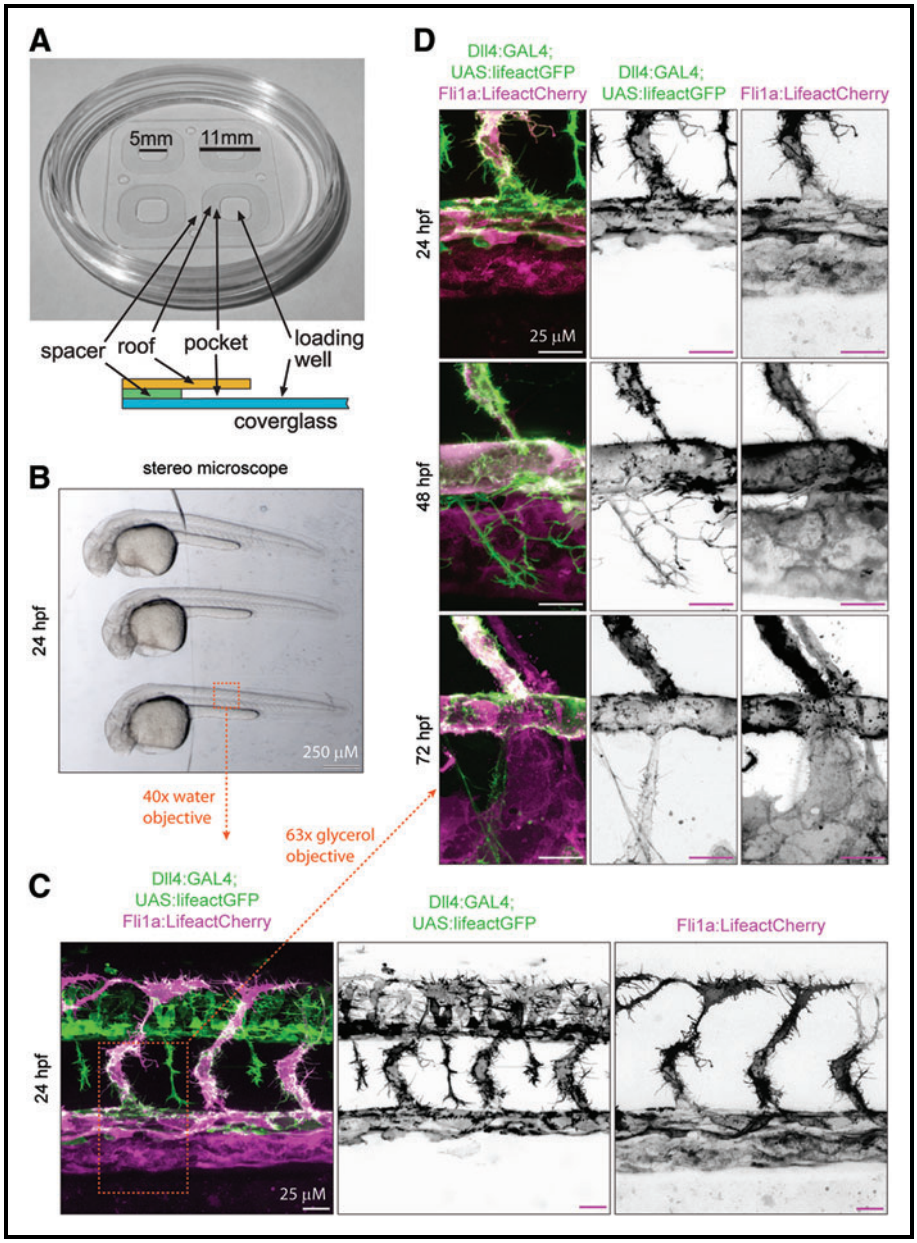

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Abstract

We describe the design, fabrication, and applications of a four-well dish for imaging of the trunk of larval zebrafish. The dish facilitates immobilization of anesthetized zebrafish larvae, with their tails gently pushed against a microscope cover glass, enabling longitudinal imaging at 24–72 h post-fertilization using high-resolution objective lenses.

Keywords: zebrafish, imaging, vascular development

ZEBRAFISH HAS BEEN WIDELY USED as a lower vertebrate development model. The tail and posterior trunk of larval zebrafish are particularly accessible for high-resolution imaging in the sagittal plane, because of their small thickness, which is within the range of the working distances (WD) of high numerical aperture (NA) immersion objective lenses (typically $\leq$200 μm). Nevertheless, when a larva is mounted on a substrate horizontally for sagittal plane imaging, because of the large diameter of the yolk sac (from >600 to $\sim$420 μm for 24–72 hpf larvae),[1] the trunk and a large part of the tail usually end up lifted above the substrate, making them not fully accessible to imaging with high-NA microscope objectives. The imaging of the trunk and tail is further complicated by their tilt with respect to the horizontal plane of the substrate. For example, as a result of the tilt, major blood vessels are spread along the optical axis of the microscope (z-direction) and cannot be readily captured in a single imaging plane.

Several mounting techniques and devices have been proposed to facilitate imaging of larval zebrafish. Encapsulation in plastic and glass capillaries addresses the problem of the refractive index mismatch for 360° imaging of axially rotated larvae,[2] including longitudinal imaging with light sheet microscopy.[3] Molds with different shapes, micromilled,[4,5] or solid printed,[6,7] have been used to cast imaging microwells in agarose gel[5,7] and silicone,[6] facilitating optimal orientation of the larvae.[4–7] However, none of the proposed techniques enables full optical access with short WD objectives to the tail and posterior trunk.

This experimental problem is addressed by the proposed easy-to-use and simple-to-build four-well dish, which is based on a 50 mm WillCo dish with a 40 mm #1.5 cover glass bottom.[8] Each well of the dish is a 11 mm square with a 5 mm square opening (both with rounded corners) in the middle and 3 mm wide margins, in which an $\sim$250 μm thick clear silicone elastomer roof is lifted above the cover glass substrate, forming an $\sim$120 μm tall pocket (Fig. 1A), where zebrafish larva tails are inserted (Fig. 1B). The making of the dish is described in detail in Supplementary Figure S1 (Supplementary Data are available online at www.liebertpub.com/zeb). An anesthetized zebrafish larva in an E3 medium supplemented with 1× tricaine methanesulfonate (0.15 mg/mL; used as an anesthetic) is loaded into the opening of a well and gently pushed, tail first, into the pocket under the roof (Fig. 1B). When under the roof, the tail and posterior trunk are both within the WD of the common high-NA oil and water immersion (WI) objectives (Fig. 1C, D). A single well can be readily loaded with up to 12 larvae.

Next, the well is filled with 0.4% low melting point agarose dissolved in the E3 medium supplemented with 1× tricaine methanesulfonate (0.15 mg/mL; used as an anesthetic). The agarose is left to cool and solidify. Before imaging, a small amount of E3 with 1× tricaine is added to prevent drying of the gel. For imaging using an upright microscope, the dish is fully filled with E3 containing 1× tricane and the lid is sealed

FIG. 1. **(A)** Photograph of the multiwell dish and schematics of the vertical cross-section of a well. **(B)** Micrograph of three 24 hpf zebrafish embryo in a well. **(C, D)** Sagittal plane images of a *Tg(Dll4:Gal4; UAS:lifeactGFP; Fli1a:LifeactCherry)* zebrafish embryo showing the trunk vasculature with all vascular endothelial cells in *red* and all arterial cells in *green*. Images were taken with a confocal microscope using a 40×/1.2 water immersion objective **(C)** or 63×/1.3 glycerol immersion objective **(D)** at the indicated time points. Color images available online at www.liebertpub.com/zeb

using vacuum grease. The dish enables imaging of ~2 mm long posterior parts (trunks and tails) of 24 hpf larvae (Fig. 1C) and longitudinal imaging of 24–72 hpf larvae (Fig. 1D) with high-NA objectives (WI 40×/1.2 and glycerol immersion 63×/1.3) under a confocal microscope.

Low elastic modulus (~2 MPa) of the silicone roof minimizes the chance of injury of the larvae. In addition, because of its relatively small thickness, the silicone roof is sufficiently flexible to partly accommodate the wedge-like shape of the trunk, facilitating immobilization of the larvae and near-horizontal orientation of their posterior trunks and tails. The flexibility of the roof also minimizes the distance between the posterior of trunk and the coverglass, and facilitates continued immobilization despite substantial growth of the trunk and shrinkage of the yolk sac between 24 and 72 hpf (Fig. 1D). The height of the pocket can be readily reduced to adopt the dish for smaller larvae, and both the height of the pocket and the width of the chamber margins can be increased to make the dish suitable for zebrafish at later developmental stages. The technique enables mounting of many embryos in the same orientation and in the same z-plane, potentially enabling high-throughput imaging and screening for genetically or chemically induced phenotypes.

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Disclosure Statement

No competing financial interests exist.

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