

Scanning Bessel Beam Light Sheet Microscope for Large Clearing Sample and Live Zebrafish Embryo

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We have built a simple light sheet platform with rich features like Bessel beam scanning, dual illumination arms, multiple software compatibility (Micro-manager, Metamorph etc), easily changing to different objective combination and long-range xyz motorized stage (MS-2000, Applied Scientific Instrumentation, USA) that moving sample from the bottom of chamber. In our platform, a pair of galvo mirror is controlled by an Arduino microprocessor that swept laser beams in Y direction upon receiving each camera synchronization signal in each exposure window. Also, galvo mirror in Z direction can cooperate with focus tunable lens (EL-16-40-TC, Optotune, Switzerland) to achieve fast z scanning without moving the objective physically. Gaps between objective and chamber were sealed with durable and flexible membranes that kept the chamber watertight and allow fine adjustment of objective position in different immersion solution. The same flexible membrane is used to seal the chamber bottom and allow the motorized stage moving the sample stage from the bottom. The inner chamber dimension is 24 mm x 25 mm x 50 mm that means the sample can be as large as 12 mm x 12 mm x 25 mm. Our design demonstrates the ability to scan a cleared whole rat brain with tiling mode and long-term monitoring multiple live zebrafish embryos embedded in a large agar block in time-lapse multi-position mode.

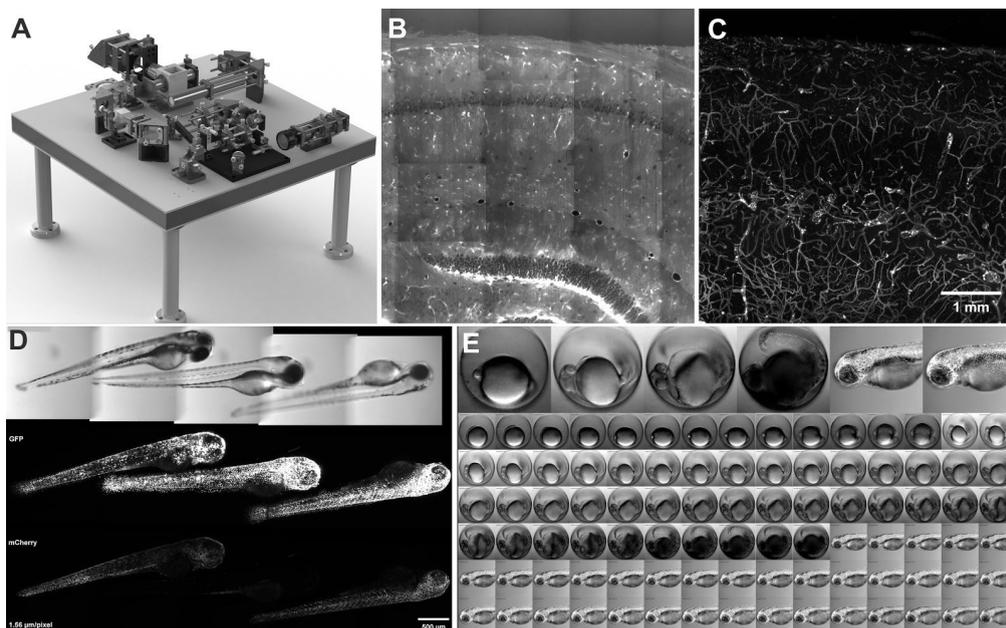


Figure 1. (A) 3D model of Light Sheet platform. (B) Tiling image of GFP signal in expanded hippocampus of Thy-1 tg mice and (C) blood vessel stained with tomato lectin. (D) Multiple zebrafish can be imaged with multi-position mode. (E) Development of zebrafish can be continuously monitored as long as 4 days on same embryo at 20 minutes interval.