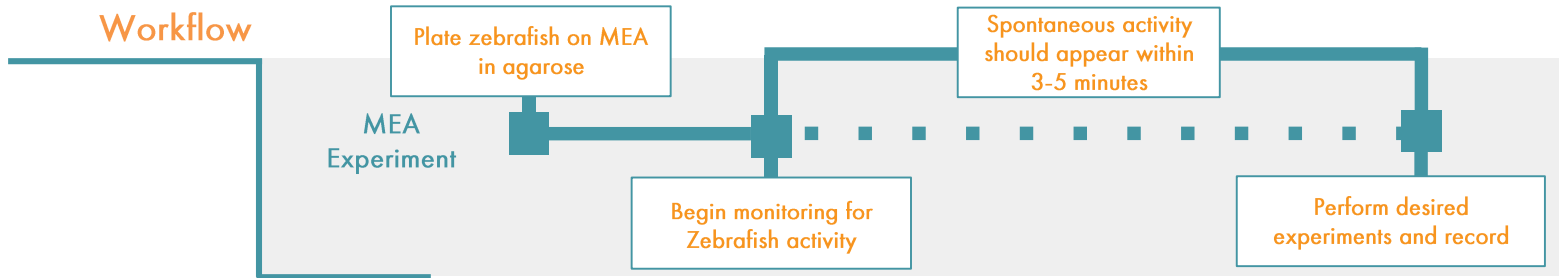


Application Protocol

Zebrafish



Preparing the MEA Plate

1. Dissolve 1.5% Low Melt Agarose in E3 Media in a small bottle and boil.
Note: E3 media is composed of 4.96 mM NaCl, 174.38 mM KCl, 333.39 mM CaCl₂, and 162.29 mM MgSO₄ × 7H₂O.
2. Add a 100 uL drop of agarose to one well in MEA plate as control well.
3. Drop agarose on petri dish and allow to cool between 45 sec to 1 min, check temperature by touching bottom of dish or hovering finger over agarose.

Tip

Agarose is kept in heating block to keep in solution with cap on to prevent evaporation.

Culturing and Plating Zebrafish

4. Once agarose is cooled enough but has not started to solidify, quickly drop larvae into agarose preventing addition of solution.
5. Suck up larvae in agarose and drop into well, fill agarose to lip of electrode area.
6. Invert larvae and gently push to bottom of well, flattening body and head on top of electrodes and holding larvae down until agarose has solidified.

Note: Proper orientation of the larva can be useful to best achieve array coverage of the desired feature, e.g. head, spinal cord, etc.

7. Once agarose has solidified, start recording immediately.
8. Spontaneous recordings are taken over a ten-minute period, heater set to 30 °C.
9. Spontaneous activity will appear after roughly 3 to 5 minutes, may have to do with seal.
10. After recording, place plate back under microscope and record electrodes that touch only the head region.

Note: Discard recording if larvae has moved/rotated.

11. Remove larvae with squirt water bottle into cull bucket that has ice so larvae are immediately sacrificed.
12. Analyze data on specific electrodes, including spike rate, burst activity, and network activity.

Tip

It's critical to not immerse larvae in hot agarose to prevent death and/or activation of TRP channels.

Tip

Use eyelash tool or forceps to mount larvae.

Tip

Critical to have head completely flat on electrodes very quickly.

Recording Activity Notes:

The Maestro is pre-set to a standard 37°C, yet this is higher than the typical temperature used for Zebrafish (28.5°C). The environment control panel in AxIS can be used to lower the temperature or turn off the heating plate altogether. For Maestro Edge and Pro systems, the door can be left open to help lower the ambient temperature. After the organism is plated, the neural real-time configuration is the preferred setting for acquiring data.



With regards to zebrafish, previous users have attempted to paralyze the organism to prevent it from moving using alpha bungarotoxin, a neuromuscular junction inhibitor that prevents skeletal muscle contraction via inhibition of acetylcholine receptors.

Drop Placement

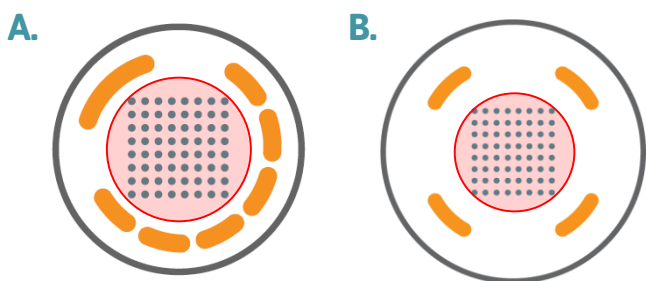


Figure 1: Drop Placement Diagram

The layouts above represent the bottom surfaces of wells in (A) a 6-well MEA with 2.1 x 2.1 mm recording area and (B) a 12-well CytoView MEA with 1.1 x 1.1 mm recording area. The number of electrodes per well and recording area size is different across the plate formats, however the drop placement is the same, with the drop (red circle) centered on the recording electrodes and staying within the ground electrodes.

Visualization of Typical Zebrafish Results

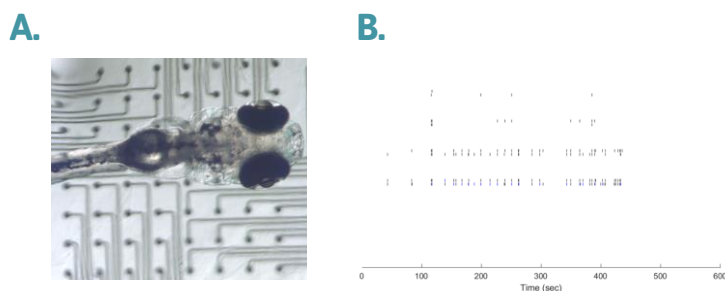


Figure 2: Zebrafish Morphology and Activity

A) Zebrafish on a CytoView MEA 12-well plate. B) Representative well-wide raster plot illustrating spikes generated by the zebrafish head.

Required Materials

Consumables

Item	Vendor	Catalog #
Axion MEA (6-Well)	Axion BioSystems	M384-MEA-6B/W
Low Melt Agarose	Lonza	50081
E3 Media	Various	Various
Fintip 1000 Wide sterile	Thermo Fisher	9405163

Equipment

Item	Vendor
Maestro Pro or Edge MEA System	Axion BioSystems
AxIS Navigator	Axion BioSystems
37°C Water Bath	Various
Cell Culture Incubator	Various
Heating Block	Various
Biological Safety Cabinet	Various
Eyelash tool	Various
Light Microscope	Various

We would like to acknowledge and thank Danielle Tomasello, Ph.D. and Hazel Sive, Ph.D. at the Whitehead Institute in Cambridge Massachusetts, for developing this protocol and sharing the data associated with it.