SOP Test 14 - Automated Optokinetic Response Measurements

1 Purpose

Assessing visual performance by triggering eye movements.

2 Scope

This quantified assay can be used to test contrast sensitivity and visual acuity.

3 Safety requirement

4 Associates documents

5 Notes

This behavior works most reliably in larvae older than a 5 dpf. The automated set-up allows for precise quantified assessment of visual performance. This assay is suitable to test motion vision and is not applicable to test color vision.
Recently commercial instruments became available (e.g. VisioTracker from TSE Systems).

6 Quality control

7 Equipment

Computer for measurement, including customized software for camera operation and eye recording and analysis (There is no dedicated separate commercial software available. However, macros can easily be written and used within most image analysis programs e.g., LabView IMAQ software, National Instruments; Image J).
Stimulation Computer, including software for generation of stimulus pattern (e.g., Simple DirectMedia Layer (SDL), Vision Egg) and for control of the stimulus (e.g., LabView IMAQ software version 5.1; National Instruments)
Diffusion screen (cylindrical; translucent wax paper (110 g/m^2) (Rustik; Artoz Papier AG)
Frame grabber card (programmable), for digitization of the video signal (e.g., PCI-1409; National Instruments)
Infrared light source (e.g., KL 1500; Zeiss)
Light projector (digital) (e.g., Proxima 4200)
Microscope (binocular, with a phototube) (e.g., SV8; Zeiss)
Optical bench, including 50-mm and 100-mm convex lenses, iris and optional optical density (OD) filters
SVGA graphics board (e.g., GeForce 4; NVIDIA)
Trigger for video camera, customized with an electronic circuit for on-chip frame integration recording with up to 12.5 frames/second
CCD Video camera (e.g. Sony XT-SC50)

8 Supplies

E3 medium
Methyl cellulose solution (prewarmed to 28°C)
Petri dishes (35-mm and 90-mm diameters)
Pipettes (plastic)

9 Procedure

Setup of the Computer-Based for OKR

1. Connect the DLP projector to the graphic board of the stimulus computer (equipped with software for generation and control of the stimulus pattern) and set up an image-mapping function.

   Since a flat image is projected onto the curved surface of the screen, the program must use a mapping function. We use $x' = R \times \tan (x / R)$, where x' is the horizontal coordinate on the screen, x the position where the ray would hit a flat screen, and R the radius of the screen.

2. Project the light beam via an optical bench consisting of two convex lenses, an iris and an optional OD filter to a cylindrical translucent diffusion screen. Attach the diffusion screen to a 90-mm Petri dish.

   The size of the projection on the screen should cover the entire visual field of the eye as it is exposed to the screen. For five-day-old zebrafish larvae, we use 99° horizontally and 52° vertically.

3. Linearize the DLP projector manually by measuring the light intensity on the screen of different projector settings with a photometer.
4. Illuminate the binocular microscope with infrared light from below.
5. Connect the camera to the binocular microscope and to its trigger.
6. Connect the camera and camera trigger to the grabber card of the measurement computer (equipped with customized software for recording and analyzing images from the video camera).
7. Connect the measurement and stimulus computers to each other.

Measurement of OKR

8. Embed zebrafish larvae in the center of a 35-mm Petri dish filled with prewarmed (28°C) methyl cellulose by using a needle (in pin holder). Embed one larva per dish, dorsal side up.
9. Place a larva under the binocular microscope orthogonally to the light beam (so that only one eye is exposed to the screen), and center the larva in the visual field of the camera.
10. Start the stimulus pattern on the stimulation computer.

    Our standard pattern: velocity is set to 60 pixels per second, one-way stimulus duration to 180 frames per cycle and spatial frequency to 0.6 cycles per degree.

    Depending on the paradigm parameters were changed stepwise within a range of 1 to 100% for contrast, 0.2 to 5 cycles per degree for spatial frequency and 20 to 180 pixels per second for temporal frequency.

12. Record the eye movement with a customized recording software of the measurement computer, and evaluate.

    Instead of taking real-time measurements, the stored movie files can be evaluated after the recording session is over (e.g., using Image J software, available at http://rsb.info.nih.gov/ij/).

10 Supporting Information

Rinner, O, Rick, JM, Neuhauss, SCF (2005). Contrast Sensitivity, Spatial and Temporal Tuning of the Larval Zebrafish Optokinetic Response. *Investigative Optical and Visual Sciences* 46, 137-142

Hodel, C, Neuhauss, SCF (2008). Computer-based Analysis of the Optokinetic Response in Zebrafish Larvae. *Cold Spring Harbor Protocols* 10.1101 prot4961