

Submission ID #: 71516

Scriptwriter Name: Kathyayani Sridharan

Project Page Link: <https://review.jove.com/account/file-uploader?src=21812428>

Title: In Vivo Phenotyping of Dopaminergic Neurodegeneration in Zebrafish Larvae Using Behavioral Analysis and High-Content Imaging

Authors and Affiliations:

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes, all done!**

- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done!**

- 3. Filming location:** Will the filming need to take place in multiple locations? **No**

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 15

Number of Shots: 35

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Kira Yu:** Our research investigates dopaminergic neuron degeneration in Parkinson's disease and screens for novel neuroprotective compounds.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Jeffrey Kim:** Bridging the gap between non-contextual cell cultures and slow rodent models, our zebrafish protocol offers rapid, in vivo compound screening.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.3. **Ethan Chen:** Maintaining identical imaging settings across all larvae is critical, as even subtle variations can distort fluorescence quantification.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Jichen Yang:** Validated hit compounds can advance to dose-response assays, individual larval tracking, and further evaluation in mammalian neurodegeneration models.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Jichen Yang:** Future studies can adapt this platform to other neuron types and disease models by swapping driver and reporter lines.

- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your JoVE article address?

- 1.6. Gha-hyun Jeffrey Kim, Assistant Professor, Department of Clinical Pharmacy Practice, Robert A. Mah Molecular Innovation Center: (authors will present their testimonial statements live)

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What value do you expect this video article to have for other researchers working in your field?

- 1.7. Gha-hyun Jeffrey Kim, Assistant Professor, Department of Clinical Pharmacy Practice, Robert A. Mah Molecular Innovation Center: (authors will present their testimonial statements live)

1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at University of California Irvine

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Baseline Fluorescence Acquisition in Transgenic Zebrafish Larvae

Demonstrator: Kira Yu

- 2.1. To begin, pour tank water containing homozygous transgenic zebrafish embryos through a 400-micrometer mesh filter [1]. Collect [2], and raise them in a 100-millimeter Petri dish containing 30 milliliters of blue egg water, or BEW (B-E-W) [3].
 - 2.1.1. WIDE: Talent pouring tank water through a mesh. ***Videographer:** Please obtain CU shot as well for this shot.*
 - 2.1.2. Talent collecting embryos.
 - 2.1.3. Talent raising embryos in BEW.
- 2.2. At 24 to 36 hours post-fertilization, add 60 microliters of phenylthiourea, or PTU (P-T-U), to the embryos, to achieve a final concentration of 200 micromolar for maintaining larval transparency [1].
 - 2.2.1. Talent adding phenylthiourea to embryos.
- 2.3. At 3 days post-fertilization, use the mCherry channel with fixed microscope settings to screen the larvae, anesthetized with 160 micrograms per milliliter tricaine [1]. ~~Calibrate the exposure time, detector gain, and illumination intensity using a known positive larva to ensure clear, unsaturated dopaminergic fluorescence, and maintain these settings throughout the screening process [2].~~ **Note:** The shot 2.3.2 and corresponding narration were removed at post-shoot stage by the scriptwriter, as it is redundant with 2.4.1.
 - 2.3.1. Talent screening larvae using the mCherry channel.
 - ~~2.3.2. SCREEN: Show the setting of exposure time, detector gain, and illumination intensity using positive control larva in the microscope.~~
- 2.4. Classify larvae as positive if clear mCherry fluorescence is visible in the expected dopaminergic neuron clusters of the ventral diencephalon under fixed screening settings [1]. Discard larvae with no signal above background in this region or with weak, diffuse, or ectopic fluorescence [2].
 - 2.4.1. SCREEN: SCREEN-2.3.2.png. ***Video Editor:** Zoom into the image and emphasize the bright red spots in the labeled region to highlight the neuron clusters.*
 - 2.4.2. Talent removing larvae with no signal above background or abnormal fluorescence.
- 2.5. At 4 days post-fertilization, dispense one anesthetized larva, suspended in 40

microliters of 1.2 percent low-melting-point agarose, into each well of a flat-bottom 96-well plate [1]. Acquire pretreatment baseline fluorescence images, as previously demonstrated [2].

2.5.1. SCOPE: SCREEN-2.5.1.mov. 00:04–00:07

2.5.2. SCOPE: SCREEN-2.5.2.png. *Video Editor: Zoom into the image and emphasize the circled red region.*

3. High-Throughput Plate-Based Drug Screening

Demonstrator: Jichen Yang

3.1. Add treatment solutions directly to the wells [1] as per the plate map [2]. For vehicle control wells, add BEW containing the same final dimethyl sulfoxide concentration used in compound-treated wells [3].

3.1.1. Talent adding treatment solutions directly to wells

3.1.2. LAB MEDIA: Figure 1C.

3.1.3. CU: Talent adding BEW with dimethyl sulfoxide to vehicle control wells.

3.2. For negative control wells, add metronidazole solution without the candidate compound [1]. For compound-screening wells, add metronidazole and 10 micromolar of the candidate compound together so that larvae are exposed to both during the same 24-hour treatment period [2]. Adjust each well to a final volume of 200 microliters, maintaining identical final dimethyl sulfoxide concentrations across all wells [3].

3.2.1. CU: Talent adding metronidazole solution to control wells.

3.2.2. CU: Talent adding metronidazole and the candidate compound to compound-screening wells.

3.2.3. Talent adjusting each well to a final volume of 200 microliters.

3.3. Incubate plates at 28.5 degrees Celsius under a 14-hour light and 10-hour dark cycle for 24 hours [1].

3.3.1. Talent placing the plate in the incubator.

3.4. At 5 days post-fertilization, acquire post-treatment fluorescence images using the identical acquisition settings used for baseline imaging [1]. Image wells in the same order used during baseline imaging [2].

3.4.1. Talent acquiring post-treatment fluorescence images.

3.4.2. Talent imaging wells in the same order used during baseline imaging.

3.5. Next, calculate the mean integrated density for each treatment group [1], then calculate the fluorescence retention for each group as the ratio of post-treatment to

pre-treatment mean integrated densities [2]. Normalize the fluorescence retention values to the mean vehicle control value corresponding to the same plate [3]. **Note:** A shot has been added to facilitate the division of timestamps for the corresponding narration.

Added shot: SCREEN-3.5.1.mov. 00:09–00:12; 00:15–00:16

3.5.1. SCREEN: SCREEN-3.5.1.mov. 00:17–00:19; 00:27–00:29

3.5.2. SCREEN: SCREEN-3.5.2.mov. 00:17–00:24

3.6. Identify candidate protective compounds as those with fluorescence retention values significantly greater than the metronidazole-only control [1]. Identify hit compounds as those with fluorescence retention ratios significantly greater than the negative control [2].

3.6.1. SCREEN: SCREEN-3.6.1-and-3.6.2.mov. 00:03–00:11

3.6.2. SCREEN: SCREEN-3.6.1-and-3.6.2.mov. 00:16–00:22

3.7. Use Welch's t-test to compare metronidazole-only controls with treatment groups, followed by validation, confirmation, and advanced studies of hit compounds [1].

3.7.1. SCREEN: SCREEN-3.7.1-and-3.7.2.mov. 00:28–00:34. *Video Editor: Please emphasize the “****” symbol on the bar graph and the text “Welch’s t-test of Col: Entering rep” on the left panel*

4. Locomotor Behavioral Assessment

Demonstrator: Ethan Chen

4.1. At 4 days post-fertilization, allocate the transgenic and AB wildtype larvae [1] into 3 treatment groups with at least 10 larvae per group [2].

4.1.1. Talent allocating larvae into 3 treatment groups.

4.1.2. TEXT ON A PLAIN BACKGROUND

Treatment Groups	Components
• Untreated Baseline	BEW only
• Vehicle Control	1% DMSO
• Metronidazole	4.5mM in 1% DMSO/BEW with 200 µM PTU

4.2. Treat larvae for 24 hours at 28.5 degrees Celsius [1]. Use a wide-bore transfer pipette to carefully transfer 5 larvae into each well of a 6-well plate containing 2.5 milliliters of fresh BEW, with minimal carryover volume [2]. Place the 6-well plate on a white LED light pad with uniform diffused illumination maintained at 25 to 28 degrees Celsius, using the same settings for all recordings [3].

- 4.2.1. Talent placing larvae under the defined treatment conditions.
- 4.2.2. Talent transferring 5 larvae into each well of a 6-well plate containing fresh BEW.
- 4.2.3. Talent placing the 6-well plate on the white LED light pad.
- 4.3. Illuminate the plate from both below and above using diffused light sources to minimize shadows, glare, and reflections [1]. Use a stable support, such as a tripod, to mount a high-resolution camera in a fixed top-down orientation above the plate [2]. Place a ruler adjacent to the plate within the field of view for spatial calibration [3]. Ensure that the camera is perpendicular to the plate surface to minimize parallax distortion before recording locomotor activity [4]. Confirm nitroreductase-dependent effects, if metronidazole reduces locomotion in transgenic larvae but not in AB wild-type larvae [5].
 - 4.3.1. Talent illuminating the plate with diffused light from below and above.
 - 4.3.2. Talent mounting the high-resolution camera above the plate.
 - 4.3.3. Talent placing a ruler adjacent to the plate.
 - 4.3.4. Talent aligning the camera perpendicular to the plate surface.
 - 4.3.5. SCREEN: SCREEN-4.1.3.png. Video Editor: Please emphasize the bars corresponding to labels “MTZ” and “Control” together, followed by emphasizing the wells corresponding to “Control” and “MTZ” labels.

Results

5. Results

5.1. Representative fluorescence images of the dopaminergic neurons of transgenic zebrafish larvae showed a dose-dependent reduction in signal intensity after 24-hour treatment with varying concentrations of metronidazole, indicating successful chemogenetic ablation of nitroreductase-expressing neurons [1].

5.1.1. LAB MEDIA: Figure 2. *Video Editor: Please emphasize the reducing fluorescence intensity starting from Control panel through 9 mM MTZ panel.*

5.2. Locomotor behavioral assessment, based on total distance traveled over 60 minutes, revealed that vehicle-control larvae covered a mean distance of 2,100 to 2,200 centimeters [1]. The larvae treated with 3 millimolar metronidazole showed an 8-percent reduction [2], followed by a 32 percent reduction in the 4.5 millimolar treatment group [3]. A 68-percent reduction was observed in the 9-millimolar treatment group, indicating dose-dependent dopaminergic neuron loss and locomotor impairment [4].

5.2.1. LAB MEDIA: Figure 4A. *Video Editor: Please emphasize the heights and Y-axis values corresponding to light gray bars in all 3 panels.*

5.2.2. LAB MEDIA: Figure 4A. *Video Editor: Please emphasize the reduction in height of the black bar corresponding to the gray bar in the first panel. Please add the text 8% decrease below the text “=0.035”*

5.2.3. LAB MEDIA: Figure 4A. *Video Editor: Please emphasize the reduction in height of the black bar corresponding to the gray bar in the second panel. Please add the text 32% decrease below the text “=0.004”*

5.2.4. LAB MEDIA: Figure 4A. *Video Editor: Please emphasize the reduction in height of the black bar corresponding to the gray bar in the third panel. Please add the text 68% decrease below the text “<0.0001”*

5.3. Linear regression analysis across all treatment groups demonstrated concordance between relative fluorescence intensity and locomotor distance, confirming that locomotor impairment is proportional to the degree of dopaminergic neuron loss [1].

5.3.1. LAB MEDIA: Figure 4C. *Video Editor: Please emphasize the text “ $R^2 = 0.704$, $p < 0.0001$ ” and the dots falling in the linear range of the graph.*

Pronunciation Guide:

1. Transgenic

Pronunciation link: <https://www.merriam-webster.com/dictionary/transgenic>

IPA: /trænz'dʒenɪk/

Phonetic Spelling: tranz·JEN·ik

2. Zebrafish

Pronunciation link: <https://www.merriam-webster.com/dictionary/zebrafish>

IPA: /'zi:brəˌfɪʃ/, /'zebrəˌfɪʃ/

Phonetic Spelling: ZEE·bruh·fish; ZEH·bruh·fish

3. Homozygous

Pronunciation link: <https://www.merriam-webster.com/dictionary/homozygous>

IPA: /ˌhoʊməˈzaɪɡəs/

Phonetic Spelling: hoh·muh·ZYE·guhs

4. Embryos

Pronunciation link: <https://www.merriam-webster.com/dictionary/embryo>

IPA: /'embriəʊ/

Phonetic Spelling: EM·bree·oh

5. Micrometer

Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>

IPA: /maɪˈkrɑːmɪtər/

Phonetic Spelling: my·KRAH·muh·ter

6. Petri

Pronunciation link: <https://www.merriam-webster.com/dictionary/Petri%20dish>

IPA: /'pi:tri/

Phonetic Spelling: PEE·tree

7. Phenylthiourea

Pronunciation link: <https://www.howtopronounce.com/phenylthiourea>

IPA: /ˌfɛnəlˌθaɪoʊjʊˈri:ə/

Phonetic Spelling: FEN·uhl·thy·oh·yoo·REE·uh

8. Micromolar

Pronunciation link: <https://www.merriam-webster.com/dictionary/micromolar>

IPA: /ˌmaɪkroʊˈmoʊlər/

Phonetic Spelling: MY·kroh·MOH·ler

9. Larval

Pronunciation link: <https://www.merriam-webster.com/dictionary/larval>

IPA: /'lɑ:rval/

Phonetic Spelling: LAR·vuhl

10. mCherry

Pronunciation link: <https://www.howtopronounce.com/mcherry>

IPA: /ɛmˈtʃəri/

Phonetic Spelling: em·CHAIR·ee

11. Anesthetized

Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>

IPA: /əˈnɛsθəˌtaɪzd/

Phonetic Spelling: uh·NES·thuh·tyzd

12. Tricaine
Pronunciation link: <https://www.howtopronounce.com/tricaine>
IPA: /'traɪkeɪn/
Phonetic Spelling: TRY·kayn
13. Dopaminergic
Pronunciation link: <https://www.howtopronounce.com/dopaminergic>
IPA: /dɒs,pæmə'nɜːdʒɪk/
Phonetic Spelling: doh·PAM·uh·NER·jik
14. Fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊː'resəns/
Phonetic Spelling: flor·ESS·uhns
15. Diencephalon
Pronunciation link: <https://www.merriam-webster.com/dictionary/diencephalon>
IPA: /,daɪɛn'sɛfə,lɑːn/
Phonetic Spelling: dye·en·SEF·uh·lon
16. Ectopic
Pronunciation link: <https://www.merriam-webster.com/dictionary/ectopic>
IPA: /ɛk'tɑːpɪk/
Phonetic Spelling: ek·TOP·ik
17. Agarose
Pronunciation link: <https://www.merriam-webster.com/dictionary/agarose>
IPA: /'ægə,roʊs/
Phonetic Spelling: AG·uh·rohs
18. Pretreatment
Pronunciation link: <https://www.merriam-webster.com/dictionary/pretreatment>
IPA: /,pri:'tri:tment/
Phonetic Spelling: pree·TREET·muhnt
19. Dimethyl
Pronunciation link: <https://www.merriam-webster.com/dictionary/dimethyl>
IPA: /daɪ'mɛθəl/
Phonetic Spelling: dye·METH·uhl
20. Sulfoxide
Pronunciation link: <https://www.merriam-webster.com/dictionary/sulfoxide>
IPA: /sʌl'fɑːksaɪd/
Phonetic Spelling: sul·FOK·side
21. Metronidazole
Pronunciation link: <https://www.merriam-webster.com/dictionary/metronidazole>
IPA: /,metrə'nɪdə,zoʊl/
Phonetic Spelling: met·ruh·NID·uh·zohl
22. Incubate
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubate>

IPA: /'ɪŋkjə,bert/

Phonetic Spelling: ING·kyuh·bayt

23. Integrated

Pronunciation link: <https://www.merriam-webster.com/dictionary/integrated>

IPA: /'ɪntə,greɪtɪd/

Phonetic Spelling: IN·tuh·gray·tid

24. Normalize

Pronunciation link: <https://www.merriam-webster.com/dictionary/normalize>

IPA: /'nɔːrmə,lɑɪz/

Phonetic Spelling: NOR·muh·lyze

25. Welch's

Pronunciation link: <https://www.merriam-webster.com/dictionary/Welch>

IPA: /wɛltʃ/

Phonetic Spelling: welch

26. Locomotor

Pronunciation link: <https://www.merriam-webster.com/dictionary/locomotor>

IPA: /,ləʊkə'moʊtər/

Phonetic Spelling: loh·kuh·MOH·ter

27. Wildtype

Pronunciation link: <https://www.merriam-webster.com/dictionary/wild%20type>

IPA: /'waɪld,taɪp/

Phonetic Spelling: WYLD·type

28. Diffused

Pronunciation link: <https://www.merriam-webster.com/dictionary/diffuse>

IPA: /dɪ'fjuːzd/

Phonetic Spelling: dih·FYOOZD

29. Parallax

Pronunciation link: <https://www.merriam-webster.com/dictionary/parallax>

IPA: /'pærə,læks/

Phonetic Spelling: PAIR·uh·laks

30. Distortion

Pronunciation link: <https://www.merriam-webster.com/dictionary/distortion>

IPA: /dɪ'stɔːrʃən/

Phonetic Spelling: dih·STOR·shuhn

31. Nitroreductase

Pronunciation link: <https://www.howtopronounce.com/nitroreductase>

IPA: /,naɪtroʊrɪ'dʌktɪs/

Phonetic Spelling: NYE·troh·ree·DUK·tays