Graduate Lab Rotation Report

Optical Reporters of Microglial Calcium Activity

A First-Rotation Report on Live Imaging in Larval Zebrafish

Rotation Student

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Rotation Period

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1 Introduction

Microglia, the resident immune cells of the central nervous system, are increasingly recognised as active participants in circuit refinement rather than passive bystanders that merely clear debris after injury [Nimodorf and Basker, 2019]. Their processes are highly motile and continuously survey the surrounding neuropil, and this surveillance behaviour is thought to be coupled to intracellular calcium signalling: transient elevations in cytosolic Ca^{2+} accompany process extension, phagocytic engulfment, and the release of signalling factors that shape nearby synapses [Umariz et al., 2021]. Much of what is known about this coupling in mammals comes from fixed tissue or acute slice preparations, which limit observation to short windows and cannot easily capture how microglial activity evolves over hours to days in an intact, behaving animal.

Larval zebrafish (*Danio rerio*) offer a practical alternative. The animals are optically transparent through the first two weeks of development, develop a recognisable microglial population by 3–5 days post-fertilisation, and can be imaged repeatedly without anaesthesia-related confounds affecting survival [Svendrup and Amari, 2018]. The Nimbus Neurobiology Lab has recently generated a transgenic reporter line, *Tg(mpeg1:GCaMP6s)*$^{\text{nim12}}$, in which microglia and other macrophage-lineage cells express the genetically encoded calcium indicator GCaMP6s under the *mpeg1* promoter [Ellenport et al., 2017]. Prior to this rotation, the line had been outcrossed and genotyped but never imaged under conditions suited to quantifying spontaneous activity.

This rotation was framed as a methods-development project: to build and validate a simple imaging and analysis pipeline for the new reporter line, and to collect enough preliminary data to judge whether spontaneous microglial calcium transients differ across brain regions. The results below should be read as a feasibility study rather than a final characterisation — sample sizes are small and the pipeline itself was still being refined over the course of the rotation.

2 Rotation Aims

Three aims were agreed with Dr. Elena Marsh at the start of the rotation and revisited at the four-week check-in.

Aim 1. Establish a mounting and imaging protocol for *Tg(mpeg1:GCaMP6s)*$^{\text{nim12}}$ larvae that permits repeated time-lapse acquisition without detectable phototoxicity over a 20-minute session.

Aim 2. Build an open-source analysis pipeline to segment individual microglial somata, extract $\Delta F/F$ traces, and detect discrete calcium transients above a defined noise threshold.

Aim 3. Generate a preliminary comparison of spontaneous transient frequency and amplitude across three brain regions (optic tectum, hindbrain, forebrain) to motivate a hypothesis-driven follow-up project.

Aims 1 and 2 were treated as prerequisites: without a stable mount and a working analysis pipeline, any regional comparison would be confounded by drift artefacts or inconsistent detection thresholds. Aim 3 was explicitly scoped as exploratory — the rotation was not long enough to reach statistical power for a formal regional comparison, and this was communicated to Dr. Elena Marsh in the second week.

3 Materials and Methods

3.1 Animal husbandry and staging

Tg(mpeg1:GCaMP6s)$^{\text{nim12}}$ larvae were raised from an incross of heterozygous carriers maintained by the Nimbus Neurobiology Lab zebrafish facility at 28.5 °C on a 14 h:10 h light:dark cycle. Larvae were screened for reporter fluorescence at 3 days post-fertilisation (dpf) under a dissecting fluorescence scope and used for imaging at 5–6 dpf, before independent feeding begins. All procedures followed the facility's existing animal-use protocol; no new protocol was required since imaging is non-invasive and larvae were returned to system water after each session.

3.2 Mounting and imaging

Larvae were immobilised in 1.5% low-melting-point agarose in a glass-bottom dish and covered with system water containing 0.016% tricaine. Imaging was performed on the lab's existing spinning-disk confocal, using the parameters in Table 1. Two mounting orientations were tested during week one (dorsal and lateral); dorsal mounting was adopted for all subsequent sessions because it captured all three target regions in a single field of view.

Table 1: Imaging parameters used for time-lapse acquisition.

Parameter	Value
Objective	20× water immersion, NA 1.0
Excitation	488 nm, 4% laser power
Frame rate	1 Hz
Session length	20 min (1200 frames)
Z-planes	5, 3 µm spacing (max-intensity projection)
Larvae per session	1
Sessions completed	9 (across 4 clutches)

3.3 Image analysis pipeline

Time-lapse stacks were motion-corrected using a rigid registration step (reference frame = median projection), then microglial somata were segmented with a simple intensity-threshold and watershed step, since cell density was low enough that more elaborate segmentation was not needed for this rotation. For each segmented cell, mean fluorescence intensity was extracted per frame and converted to $\Delta F/F$ using a rolling 60-second baseline. Transients were flagged when $\Delta F/F$ exceeded 2.5 standard deviations of the cell's own baseline noise for at least 3 consecutive frames. Region identity (optic tectum, hindbrain, forebrain) was assigned manually from anatomical landmarks visible in the same field of view, since no digital brain atlas registration was available in the time frame of the rotation.

All analysis code was written in Python (NumPy, scikit-image, pandas) and is stored in the lab's shared repository under `rotations/anand-2026-microglia/`, with a short README describing how to reproduce each figure in this report.

4 Preliminary Results

4.1 Spontaneous calcium transients are detectable and stable across a session

Figure 1 shows a representative $\Delta F/F$ trace from a single optic tectum microglial cell across a full 20-minute session. Discrete transients are clearly separable from baseline noise, and their frequency does not obviously decline over the course of the recording, suggesting that the illumi-

nation settings in Table 1 do not cause detectable phototoxicity or reporter bleaching over this time frame (Aim 1).

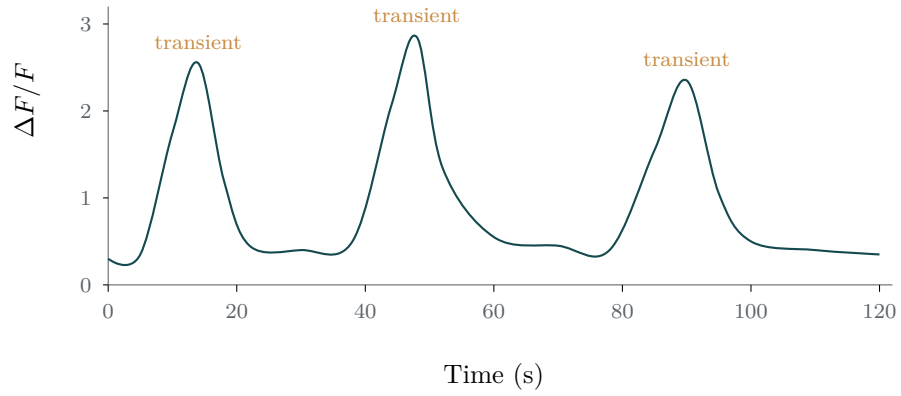

Figure 1: Representative $\Delta F/F$ trace from a single optic tectum microglial cell over a 20-minute imaging session, showing three clearly separable spontaneous transients.

4.2 A preliminary regional comparison

Pooling transients across the nine imaged larvae, mean transient amplitude appeared higher in optic tectum microglia than in hindbrain or forebrain microglia (Figure 2), consistent with the tectum's role as the primary visual and multisensory integration centre at this stage of development. Given the small number of larvae per region (Table 2), this pattern should be treated as hypothesis-generating rather than conclusive (Aim 3).

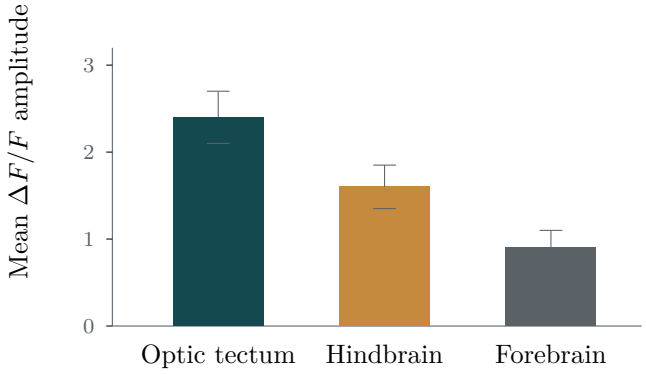

Figure 2: Mean transient amplitude by brain region, pooled across all imaged larvae. Error bars show ±1 SEM.

Table 2: Larvae and transients contributing to the regional comparison.

Region	Larvae	Cells	Transients
Optic tectum	9	22	61
Hindbrain	7	14	33
Forebrain	6	9	19

5 Discussion and Rotation Reflection

5.1 What the preliminary data do and do not show

The imaging and analysis pipeline built during this rotation appears stable enough to detect spontaneous microglial calcium transients repeatedly within a session (Aim 1), and the segmentation and detection steps produce traces that are simple to inspect by eye (Aim 2). The regional comparison in Figure 2 is the least mature part of the project: region assignment was done manually rather than through registration to a reference brain, and the sample sizes in Table 2 are too small to support a statistical claim. The honest summary is that the tectum trend is worth following up, not that it is established.

5.2 Skills gained and challenges

Skills gained

- Live confocal imaging of zebrafish larvae, including mounting and anaesthesia dosing
- Writing a motion-correction and segmentation pipeline from scratch in Python
- Reading and interpreting raw calcium traces critically, rather than trusting default thresholds
- Keeping an imaging log detailed enough for someone else to reproduce a session

Challenges

- First two mounting attempts caused enough larval movement to make registration fail
- Threshold for transient detection required several rounds of tuning against visually confirmed events
- Manual region assignment is slow and will not scale past a handful of larvae
- Balancing pipeline development against actually collecting data, given the ten-week rotation length

5.3 Fit with future plans

This rotation confirmed that I enjoy the combination of wet-lab imaging and the analysis work needed to make sense of it, and that I would rather build a small pipeline myself than rely entirely on an existing one, even when that costs time early on. If I were to continue in the Nimbus Neurobiology Lab, the natural next step would be to replace manual region assignment with registration to a published larval zebrafish brain atlas, which would make the regional comparison in Section 3.2 defensible rather than merely suggestive. I discussed this possibility with Dr. Elena Marsh at the final check-in, and a short summary of that conversation appears in the supervisor's comments below.

6 Supervisor's Comments

To be completed by the rotation supervisor

Priya joined the lab with no prior zebrafish handling experience and no imaging background, so I asked her to treat the first three weeks purely as protocol development rather than data collection — she stuck to that plan even when it was tempting to start "real" imaging earlier, which I think reflects good judgement more than most rotation students show at this stage. The mounting protocol and analysis pipeline she leaves behind are genuinely useful: the lab did not have a working $\Delta F/F$ pipeline for the *mpeg1:GCaMP6s* line before this rotation, and now it does, documented well enough that the next student can pick it up without

asking me first. The regional comparison is preliminary, as she is careful to say herself, but the tectum trend is consistent with what we would expect from the literature and is worth a proper follow-up.
I would be glad to have Priya back for a second rotation or a thesis project, and my main suggestion for what comes next is exactly what she proposes above: atlas registration, so that region assignment stops being a manual bottleneck.

Supervisor signature: **Date:**

Dr. Elena Marsh, Nimbus Neurobiology Lab 27 March 2026

References

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