Calcium Imaging in *Drosophila* Organs

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**Method Article**

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Abstract

Ex vivo calcium imaging in Drosophila opens an expansive amount of research avenues for the study of live signal propagation through complex tissue. Here, we describe how to isolate Drosophila organs of interest, like the developing wing imaginal disc and larval brain, culture them for extended periods, up to 10 hours, and how to image the calcium dynamics occurring within them using genetically encoded biosensors like GCaMP. This protocol will enable the study of complex calcium signaling dynamics, which is conserved throughout biology in such processes as cell differentiation and proliferation, immune reactions and wound healing, and cell-to-cell and organ-to-organ communication, among others. These methods will also allow pharmacological compounds to be tested to observe effects on calcium dynamics with the applications of target identification and therapeutic development.

Introduction

Why is calcium (Ca\(^{2+}\)) such a frequently measured signal in biology? With its innate chemical properties allowing it to be readily bio-reactive and its natural abundance, Ca\(^{2+}\) is a foremost second messenger that biological life utilizes for many, if not most, of its activities. Because of its unique combination of valency, ionic radius, polarizability, hydration energy, and charge density, it can interact with many proteins in various complex and diverse ways. High calcium concentrations are also toxic to cells and will form insoluble phosphate salts with a cell’s main energy source, ATP. This requires maintaining it at low cytosolic basal levels in the nM range. A sophisticated control system for Ca\(^{2+}\) sequestration thus was created and enables calcium to be a complex and highly regulated signaling mediator\(^1\).

Today, Ca\(^{2+}\) has been found to facilitate signaling across numerous cellular processes and does so through several pathways. This includes tissue development, cell fate, cell division, fertilization, metabolism, mobilization, and cognition, among others\(^2–5\). How has it become so diverse? The frequency, amplitude, and spatial/temporal patterning can all influence how Ca\(^{2+}\) messages are conveyed\(^5,6\). Additionally, the Ca\(^{2+}\) “toolkit” is expansive with many components contained within it\(^3,6\). This allows signals to be augmented and shaped in further diverse ways and has allowed Ca\(^{2+}\) to become a key second messenger found in all known biological systems.

To fully comprehend the complexity of calcium (Ca\(^{2+}\)) signaling within and between tissues, the cultivation of live organs is imperative. With the calcium imaging chambers described here, complex Ca\(^{2+}\) signals can be deciphered in real time using explanted micro-organs of Drosophila melanogaster. Drosophila is an excellent model organism to use when studying signaling pathways in developmental biology. This is because multiple phenomena like signal transduction and organ growth can be observed within their thin transparent organs\(^7–11\). Additionally, Drosophila can be used to recapitulate many genetic diseases found in humans, are inexpensive, and have a wide range of genetic tools created to facilitate complex control of protein and genetic expression levels\(^12,13\).
Here, we describe the fabrication and implementation of simple and advanced calcium imaging chambers for use in deciphering complex Ca\(^{2+}\) signals in real time using explanted micro-organs of *Drosophila melanogaster*. To preserve organ viability, an improved culture media using Grace's media supplemented with 20-hydroxyecdysone (20E) is employed\(^8,10,11,14\). These chambers allow for short and extended imaging sessions, which can also be adapted for use with other systems like zebrafish and other 3D tissue cultures. The first and more simple imaging chamber allows for easy setup for capturing rapid responses within the first 2-3 hours of imaging, while the advanced chamber incorporates microfluidics, which facilitates a more controlled environment allowing for long-term imaging (10-12 hours)\(^15-18\). The use of advanced chambers will also allow for the addition and removal of compounds of interest for the detection of Ca\(^{2+}\) stimulation. Ca\(^{2+}\) plays a critical role in developing organs and investigations into its dynamics can now be easily implemented in any laboratory with these devices and imaging techniques.

**Reagents**

**Culture Media**

1. Grace's Insect Medium
2. BIS-TRIS
3. Fetal Bovine Serum
4. Penicillin-Streptomycin
5. 20-hydroxyecdysone (*Sigma*: H5142)
6. 0.22 μm Bottle Filters

**Media Preparation**

1. Prepare Grace's Insect Medium with 5 mM BIS-TRIS.
2. Adjust pH to 6.6 – 6.7.
3. Filter sterilize with 0.22 μm bottle filters, twice.
4. Store at 4°C for no more than 1 month or freeze as small aliquots at -20°C (*see* Note 4).
5. On the day of experiment, add Fetal Bovine Serum and Penicillin-Streptomycin for final amounts of 5% and 1%, respectively, as well as 20-hydroxyecdysone for a final concentration of 20 nM.
Solutions

1. Phosphate-buffered saline, PBS (1L): 8 g NaCl, 0.2 g KCl, 1.44 g Na$_2$HPO$_4$, 0.24 g Kh$_2$PO$_4$

Equipment

Materials for Simple Imaging Chambers

1. #1 Glass bottom Petri dishes
2. Millicell Standing Cell Culture Inserts (Sigma: PI8P01250)
3. Blade for cutting
4. Kimwipes
5. Embryo Oil

Materials for Advanced Microfluidic Imaging Chamber

1. Craft Cutter and Software (Silhouette Cameo) (see Note 1)
2. Cutting Mat for Craft cutter (Silhouette) (see Note 2)
3. 3 or 5 mil Thermal Laminating Pouches
4. Rubber Roller
5. 24 x 60 mm #1 cover glass
6. Laminator
7. 6- and 8-mm Furniture Bumpers with adhesive
8. 1- and 2-mm Hole Punches
9. Revolving Punch Pliers
10. Scissors
11. Forceps
12. Double sides tape
13. Polyetheretherketone (PEEK) tubing and syringe adapters (ZEUS)
14. 1 mL Luer lock Syringes
15. Syringe pump (*for small volume control*)

**Material for Dissections**

1. Dissection Microscope
2. A siliconized glass plate (*see Note 3*)
3. Sigmacote for siliconizing plates (*Sigma: SL2*)
4. Two Dissection forceps (*Fine Science Tools Inc.: Dumont #5 Forceps*)
5. 100 μL Micropipette with disposable tips
6. 2 μL Micropipette with disposable tips
7. Four Petri dishes or a 6-well plate for washing larvae

**Procedure**

**Fabrication of Simple Imaging Chamber**

1. Prepare the Millicell Standing Cell Culture Insert by removing the bottom legs with a blade (*see Note 5*).
2. Siliconize the glass bottom Petri dish (*see Note 6*).
3. Your device is now ready for sample loading.

**Fabrication of Advanced Imaging Chamber**

1. If creating a new design, start with hand drawings.
2. Once drawn, create the design on the computer (any software can be used) and surround it with a border (*see Note 7*).
3. Clip a screenshot of the design.
4. Open the Silhouette software.
5. Paste the design into Silhouette.
6. Open the trace panel and trace the design (see Figure 1A).

7. To help size the design, use the grided view (Click on view à show grid).

8. Each square is 1 inch so change the design size by clicking and dragging points.

9. Measure channel width with the line drawing tool (see Figure 1B) (see Note 8).

10. The overall size should be somewhat smaller than a 24 x 60 mm cover glass as this will be the base of the device (see Note 9).

11. Create circles between 1.5 and 2.0 mm in diameter and place them at each entry and exit point.

12. Copy the entire design and paste it nearby.

13. Remove all channels leaving only the entry and exit circles (this will be the top layer of the design).

14. Box your design so that it will be cut out (see example in Figure 2A).

15. When placing your design in the grid, Do Not place it in the top left corner as the Cameo cutter tests its readiness here.

Cutting Design

16. With gloves (to prevent oil transfer from hands), replace the protective cover on the cutting mat with a piece of laminate with the shiny side of the laminate face down (see Note 10).

17. Using a roller, remove any air bubbles from underneath the laminate, fully securing it to the cutting mat.

18. Line the cutting mat up with the blue (line) guide on the CAMEO and hit “Load cut mat”

19. In your design file, click Send.

20. In this menu set the force, speed, material, passes, and blade depth (see Note 11).

21. Connect your computer to the Cameo cutter (see Note 12).

22. Remove anything from the back of the machine as it will use this space to pull the cutting mat through.

23. Make sure the design that you have created in silhouette will be cut on an area where you have placed laminate.

24. The Cameo cutter should indicate it's “Ready” and "TEST" should be selectable.
25. Test the readiness by selecting "TEST".

26. Click Send in the Send window.

27. Once complete hit Unload.

Laminating Design

28. Turn on the laminator and allow it time to warm up (see Note 13).

29. Remove the cut design from the laminate using a pair of tweezers.

30. With small pieces of double-sided tape, assemble the design in the proper layered order with the coverslip on the bottom (see Figure 2B).

31. Make sure to have all adhesive, matte, sides facing inwards, away from the laminator (see Note 14).

32. Once assembled, run the cut design attached to the coverslip through the laminator (see Note 15).

33. Perforate furniture bumpers (see Note 16).
   a. Punch holes in larger bumpers with the revolving punch pliers.
   b. Using biopsy punches, create holes in the smaller bumpers.

34. Remove cut material.

35. Place the perforated furniture bumpers over the exit and entry holes (see Note 17).

36. Your device is now complete and ready for sample loading (see Figure 2B).

37. Assemble tubing and syringe with the Polyetheretherketone (PEEK) tubing adapter.

38. Then begin by cleaning the tubing that will be used to supply media to the microfluidic device by flushing the tubing with 70% Isopropyl Alcohol (IPA) and air, alternating between them.

39. Do this three times ending with air and adding solutions to the syringe using a 1 mL pipette by removing the plunger of the syringe.

40. Rinse a final time with PBS or Grace’s media.

41. Place aside and retrieve for final assembly.
**Dissection of Drosophila Tissue:**

1. Obtain wandering larvae that contain a genetic background with a calcium biosensor such as a form of GCaMP (*see Note 18*).

2. Prepare the dissection microscope by shifting the working area to the black surface as this will help make the larva more visible and dissection easier.

3. Place a siliconized black glass dissection plate over the microscope stage.

4. Prepare four small Petri dishes with 2 containing PBS, one with 70% isopropyl alcohol (IPA), and one with a small amount of deionized water (DI) (*see Note 19*).

5. Retrieve larvae using blunt forceps and rinse in first dish of PBS, making sure to remove any debris.

6. Transfer to 70% IPA and let sit for ~3 minutes, swirling occasionally.

7. After sterilizing in 70% IPA, rinse in second dish of PBS.

8. Then place larvae in the final dish with DI water until ready to dissect (*see Note 20*).

9. Many larvae can be “stored” like this at a time so repeat steps 5-8 as many times as needed.

10. Clean the dissection plate with a Kimwipe and 70% IPA.

11. Place a drop of Grace’s Medium (~30μL) onto the glass plate (*see Note 21*).

12. Using the blunt tweezers, place a larva in the drop.

13. Under the microscope, locate the posterior and using the blunt tweezers grab the larva around the middle (closer to the posterior) (*see Note 22*).

14. With the sharp tweezers, grab the larva next to the blunt ones and tear the posterior part away. The posterior half can be discarded (into an adjacent “garbage” drop, for example).

15. Now grab the torn end with the blunt tweezers to stabilize the larva so that the sharp tweezers can be inserted into the anterior end (*see Note 23*).

16. With the blunt tweezers, invert the larva by pushing it onto the sharp tweezers.

17. This will expose the larva tissue which contains the imaginal discs.

18. Remove the sharp tweezers after the larva has been inverted.

19. To locate the discs, begin to remove some of the tissue like the gut and fat.

20. After some tissue removal, locate desired tissues like the developing wing imaginal discs.
21. These can be found by first finding the trachea as this is a bright white line of tissue that runs along the entire larva, is easy to identify, and has the wing imaginal discs attached to it at the anterior end (along with the thoracic leg and haltere discs).

22. If targeting the brain, it is found at the anterior end of the larvae, surrounded by additional developing tissue like the eye discs.

23. When desired tissue is identified, isolate it while paying attention not to stretch, scratch, or tear it.

24. Once isolated, wash tissues by transferring them through a series of drops using a 2 μL Micropipette.

25. Tissues can be stored in a drop of medium until all dissections are complete (see **Note 24**).

**Preparation of Imaging Chambers for Sample Loading**

**Simple Imaging Chamber**

1. Add 20 μL of Grace's medium to the center of the glass bottom Petri dish.

2. Transfer dissected and isolated tissues to the drop of medium using a 2 μL Micropipette.

3. Orient the tissue as required for the microscope you will be imaging with (see **Note 25**).

4. Place the prepped Millicell Standing Cell Culture Insert (phantom legs down) over the tissue, paying attention to not use lateral movements, which can disrupt the orientation of tissue (see **Note 26**).

5. Add 100-150 μL of Grace's medium to the top of the Millicell Standing Cell Culture Insert.

6. Place enough (100-150 μL) embryo oil over the Grace's medium to completely cover it.

7. Place a rolled-up Kimwipe around the inner walls of the Petri dish and wet it with 400 μL of PBS or surround the outside of the imaging chamber with Embryo oil (100-150 μL) (see **Note 27**).

8. You are ready to transfer the chamber to the microscope (see **Note 28**).

**Advanced Microfluidic Imaging Chamber**

1. Retrieve a microfluidic device (see **Note 29**).

2. Partially fill the larger exit channel (large bumper) with Grace's media.

3. Place the desired tissue in the larger opening using a 2 μL Micropipette.
4. Use a wire to push the tissue down and slightly into the channel, orienting it properly (see Note 25).

5. With a 20 μL Micropipette, slowly suck the wing disc down into the channel by pipetting volume up from the entrance channel (small bumper) until it has reached a desired location in the design (see Note 30).

6. Once the microfluidic device has been prepared, retrieve the cleaned tubing

7. With a new syringe (and attached needle), suck up ~1 mL of Grace's media and remove as many air bubbles as you can in the syringe by taping the tip and expelling some media.

8. Remove the needle and replace the syringe that is attached to the tubing with this new one loaded with media (see Note 31).

9. Wash about 0.1 – 0.2 mL of media through the tubing, ensuring all air is removed.

10. With the syringe and microfluidic device prepared, mount the syringe into the syringe pump, which should be placed next to the microscope.

11. Using the menu on the syringe pump, push out a small drop of media and then insert the tubing into the entrance of the entrance channel (small bumper) on the microfluidic device.

12. Set the flow rate on the syringe-pump (see Note 32).

13. Place the imaging chamber onto the microscope and begin imaging.

**Live Calcium Imaging of Drosophila Tissue**

It is recommended to utilize a spinning disc confocal microscope for improved acquisition speed with conserved resolution. The chosen interval and light exposure levels are essential for proper image acquisition and downstream analysis. This will largely depend on the signaling pathway that will be observed and biosensors being used (see Note 18). Generally, for long-term imaging (8+ hours) intervals of 5-6 min or larger are recommended to avoid damage to cellular tissue. Shorter intervals of 10-30 seconds can be used but will limit the length of imaging to about 2 hours before the tissue's health starts to become impaired. Additionally, laser power is recommended to be maintained at the lowest detectable setting as is allowed by the system. This will also help prevent tissue damage.

It is also important to understand that the working distance of spinning confocal microscopes are limited to boost resolution. Therefore, the imaging plan chosen is significant when acquiring data. Either a single z-slice should be imaged, or small Z-stacks taken. Larger Z-stacks can be acquired with larger step sizes; however, this is not recommended as the resolution of the z-axis will be severely limited. The imaging
plan should be chosen based on the dynamics to be observed and the tissue oriented appropriately (see Note 25).

**Calcium Analysis**

Following the imaging of calcium dynamics, is a workflow of image analysis around the acquired fluorescent signals. This usually involves denoising, motion correction if drift is present, segmentation and identification of regions of interest (ROI) (cells experiencing calcium spikes), and signal extraction. This is then ultimately followed by the analysis of the calcium transients and their interpretation. Many platforms have been created for this over a variety of cell types\textsuperscript{19–24}. Here we utilize and demonstrate analysis with CaImAn—an open-source library that can be used in either MATLAB or Python for calcium image analysis\textsuperscript{19}. We have adapted here for use in the developing wing imaginal disc instead of its original use in neurons (Figure 3). This tool takes time-lapse videos of fluorescent capture (Figure 3A) and begins by correcting for motion, which is followed by ROI identification (Figure 3B), and finally data extraction (Figure 3C).

Modifications will likely be required and depends on the platform chosen. Key parameters that must be accounted for in the analysis include the interval rate of capture and cell size, along with the dynamics of the calcium indicator. When selecting an image analysis method or platform, one must also factor into account the amount of prior coding experience needed. Many of the platforms, like CaImAn, allow for the specification of the system to sample needs but can require an advanced understanding of computer languages. Others, like EZcalcium, include a graphical user interface and can be navigated by those with little coding experience\textsuperscript{20}. In conclusion, the post-acquisition analysis workflow involves several decisions to quantify and interpret the results. Several open-access image analysis methods are available. However, regardless of the chosen platform, customization and understanding of coding languages may be necessary, although user-friendly options are available. Thus, selecting the appropriate platform requires consideration of specific experimental needs and the level of coding expertise available.

**Troubleshooting**

Notes:

1. Silhouette Cameo will come with a cutting mat; however, it is recommended to have replacements for unavoidable wear and tear.

2. The Silhouette software can be found at Silhouette Software Downloads (https://www.silhouetteamerica.com/software). Laminating Pouches, laminate rollers, laminators,
furniture bumpers, biopsy punches, and revolving punch pliers can be purchased from many craft stores or on Amazon.

3. Alternatively, this can be a siliconized plastic plate like the bottom of a petri dish, however, it will not perform as well.

4. Check for precipitates or small floating particles throughout storage time as this will indicate when the medium has expired and/or been contaminated. Usually, 100 mL of Grace's media is made up at a time to reduce waste. 4 g Grace's Insect Medium with 0.1 g BIS-TRIS in Milli Q water. 20-hydroxyecdysone is prepared fresh weekly and stored as a 1000x stock solution in ethanol at -20°C. Other organ culture media are available and will lead to various levels of stimulation of calcium. This includes WM1, with FEX, which results in the formation of intercellular calcium waves²,¹⁶,¹⁷.

5. Completely remove any leg material, making sure that the surface is smooth. This will ensure a nice seal around the edges.

6. Though not required, it is recommended that Petri dishes are siliconized before each use as this ensures that drops of media bead well.

7. Using 6-point thick lines is recommended for initial creation. Leave space in one of the corners where tape can be placed when assembling. It may be beneficial to place tape in two corners if the design is large. This will prevent the layers from shifting when being laminated.

8. Channels should never be greater than 1 mm. When larger than this, the top and bottom layers will bend and touch each other in the channel when assembled.

9. A glass coverslip is used as the bottom layer of designs for imaging purposes but if this is ever not required, laminate can also act as a bottom layer.

10. Pieces of laminate come as pouches, but you only need a single piece, so separate them. Placing the shiny side of the laminate face down on the cutting mat has been found to work best.

11. Force: 25, Speed: 4, Material: Sticker paper-clear, Passes: 1, and Blade Depth: 5 are used for most designs, however, these settings are adjustable depending on the thickness of the laminate you are cutting.

12. Hard connection (plug the Cameo cutter into your computer), External memory (copy files onto a flash drive and plug into the Cameo cutter), or Bluetooth (depends on the Cameo model).

13. For any device with 4 or more layers, use the 5-mil setting otherwise use 3-mil (1-mil is short for a thousand of an inch). Material thickness determines channel thickness, so stack materials if thicker channels are required. Remove anything from the back of the machine as anything placed in the laminator will come out of the back (depending on the model).
14. Do not overlap tape throughout layers as this will make the device too thick and will delaminate. **Make sure to place the adhesive, matte, side down so that it won’t stick to the laminator.** Only the shiny side should ever be facing the laminator. If the adhesive matte side faces up towards the laminator, then it will stick, and that part of the laminator will no longer be usable.

15. It is recommended to run the design through the laminator two times to ensure complete lamination. This will prevent any leaks.

16. Hole sizes on bumpers depend on the desired use. Larger holes on entry bumpers can be used to create a reservoir of solution, whereas smaller holes will allow for tight connections with tubing.

17. For exit channels use the large bumpers (8 mm) and for the entry channels use the small bumpers (6 mm).

18. Calmodulin-based genetically encoded fluorescent calcium indicator (GCaMP and derivatives) has been developed across numerous generations to improve its ability to quickly detect Ca\(^{2+}\) with low and high affinity binding for dynamic observation. There are three variants of GCaMP that are each fast to rise in detecting and fluorescing when in the presence of Ca\(^{2+}\) but have unique decay times. GCaMPs is sensitive and has a slow (s) decay (BDSC #’s 92593, 92594, 92595), GCaMPm has a medium (m) decay (BDSC #’s 92590, 92591, 92592) and GCaMPf has a fast (f) decay (BDSC #’s 92587, 92588, 92589)\(^{25}\). We recommend using the GCaMPf variant as it is the most dynamic and will accurately depict rapid Ca\(^{2+}\) signaling events. However, each variant has its use and should be used accordingly. There are multiple GCaMPf fly lines as the GCaMP has developed rapidly. Currently, the latest is GCaMP8, which is available at Bloomington Drosophila Stocks Center in each of its variants (s, m, and f).

19. Make sure to use solutions at room temperature to minimize stress on the larvae.

20. The DI water is simply used to facilitate easy transport of larvae into the final dish. This way, larvae will not stick to the forceps.

21. Multiple drops can be placed along the dissection plate so that dissections can be conducted in rapid order.

22. The larva will be breathing through its posterior spiracles so they will naturally orient this side to be at or slightly above the surface. Lightly grab and slowly squeeze to prevent it from popping the stomach and releasing all its contents. This will prevent clouding and contamination of solution.

23. The anterior end is the head of the larva and will look like a mouth trying to eat things. Insert the tweezers into the mouth.

24. It is recommended to dissect 2-3 specimens to ensure a viable specimen is retrieved for live imaging. Practice is key here.
25. Tissue orientation is important and will depend on the type of microscope you are using, upright or inverted microscope. The general rule of thumb is to orient a tissue so that the brightest part of the tissue is facing the objective. This way, light will not “bleed through” from below, impairing resolution while imaging. For example, the developing wing imaginal disc is oriented with the Peripodial cells facing the objective as they tend to have the brightest signal.

26. This can be difficult, and practice will be required. Tissue orientation will be inevitably disturbed, so add multiple samples to the chamber in the hopes that at least one has the proper orientation.

27. Embryo oil and/or wetted Kimwipe will prevent evaporation. Embryo oil is recommended.

28. An even simpler imaging chamber can be created by only using a glass bottom petri dish with a larger drop of Grace's medium (100 - 200\(\mu\)L). This is only recommended for short imaging sessions of 30 minutes or less as evaporation will begin to affect accurate data acquisition.

29. Devices can be cleaned and reused; however, this is not recommended. If cleaning the device, flush the channel with 70% IPA to remove any dust and air bubbles. Follow this with at least three flushes of water. Make sure not to introduce any air bubbles. Then flush with Grace's media to remove any water and any remaining IPA. Again, make sure not to introduce any air bubbles.

30. It's helpful to add some Grace's media to the 20 \(\mu\)L Micropipette (not the full volume) so that you can pipette up and down, pushing and pulling the tissue into the channel. Make sure not to remove all the solution from the other side otherwise you will suck air in, and bubbles will impair imaging. It isn't the end of the world if you get an air bubble, if it is not next to the tissue it won't impair the imaging. Also, it is smart to place the tissue a little past the desired location because when you add the tubing, the insertion of it will push the tissue back just a little bit. This will take practice.

31. A new syringe is used for each experiment. Replace them at the beginning of experiments but leave them attached to the tubing at the end. This is a good way not to lose any pieces.

32. Usually, a flow rate of \(~2 \mu\)L/hr. is sufficient but can and should be adjusted based on the design of the imaging chamber.

**Time Taken**

The duration of experiments will vary and depend on the imaging chamber employed and the frequency at which calcium levels are measured. Using the simple chamber will allow approximately 2 hours of imaging before tissue viability declines due to nutrient depletion. This can be extended to 8-10 hours by utilizing the advanced imaging chamber, which continuously replenishes media, thus extending the tissue life span. Tissue deterioration will also be affected by the intensity of image acquisition. More frequent imaging (every 2-3 seconds) accelerates tissue damage compared to less frequent acquisition (every 5-10 minutes). The desired observation of calcium dynamics should guide the choice of imaging setup.
Anticipated Results

References


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**Figures**

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**Figure 1**
Figure 1: Tracing designs in Silhouette software. (A) In the “Trace Panel” choose “Select Trace Area.” Click and drag over the design (the yellow highlight will become the design). To create the trace, select “Trace” or “Trace Outer Edge.” (B) To measure channel widths, use the “Line Tools” selection and draw a line that spans the length of the channel. The length (and width) of this line will be displayed.

Figure 2: Example of advanced microfluidic imaging chamber. (A) Silhouette outline of chamber design, (B) which consists of one glass layer and two laminate layers. The top layer (left) consists of inlets and outlets, while the middle layer contains the channels. At the bottom of the imaging chamber is a glass cover slip (24 x 60 mm #1). The fully assembled device (right) is sealed from the bottom with the glass slide and from the top, the in/outlet channels. Bumpers then allow for easy access and the addition of solution and tissue.
Figure 3

Figure 3: Calcium analysis using CalmAn on a developing wing imaginal disc treated with 1 mM Yoda1. (A) Temporal color-code of timelapse series over 30 minutes of imaging with an interval of 3 seconds. (B) Segmentation mask and ROI identification by CalmAn following motion correction. (C) Calcium dynamics extracted and presented as frequency of oscillation.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Yoda160Start5sInt30minW2.tif