# Projection neuron activity mapping with the Multimodal Image Registration and Analysis (**MIRA**) platform

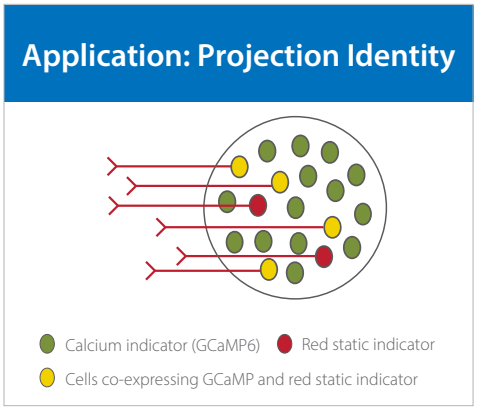


**Figure 1.** Schematic highlighting reagent strategy used for projection identity application. Here, all neurons are targeted with a GCaMP indicator, with a subset of projection-specific cells labeled with a static red indicator.


**Figure 2.** The MIRA confocal adapter with attached Inscopix miniscope (nVista™ or nVoke™ system sold separately).

## Introduction

Inscopix's miniscopes (the nVista™ or nVoke™ system) allow neuroscientists to record and manipulate large-scale calcium dynamics in genetically defined populations of neurons at single-cell resolution in freely behaving animals over months. This is crucial to understanding and investigating the correlative and causal link between neural circuits and behavior. Nevertheless, there remains a critical need to not only record the activity of neural circuit dynamics, but to fundamentally understand the nature of the cells under investigation. We have developed a **Multimodal Image Registration and Analysis** (**MIRA**) platform that fully integrates calcium imaging using miniaturized microscopes with high-resolution laser scanning microscopes (LSMs; either confocal or multiphoton microscopes) using a robust data acquisition and analysis workflow to align and co-register data from both modalities, thus deepening our knowledge of the biological mechanisms underlying brain function and behavior.

## Combining data across two different modalities

The MIRA platform allows scientists to record, align, register, and correlate changes in cell-type specific neural circuit dynamics (using Inscopix miniscopes) with changes in other cell-types/pathology (via LSMs) in the same field of view. The MIRA platform enables a number of applications including co-registration of GCaMP cells and projection specific neurons labeled with a static indicator like tdTomato or mCherry (*Figure 1*). For information on all applications enabled by the MIRA platform, visit our website.

## Materials and supplies for the MIRA platform

The MIRA platform consists of an opto-mechanical adapter (*Figure 2*), calibration slide, Inscopix data acquisition and analysis software. Co-registration of cells can readily be accomplished when the MIRA platform is paired with our implantable integrated lenses with headbars for imaging from the animals in a head-fixed setup. Other materials needed:

- Stereotax apparatus
- Microinjection pump
- An nVista or nVoke system
- LSM (ZEISS LSM 880 or 980 with Airyscan; sold separately)
- GCaMP mice or wildtype mice*
- Static indicator of choice*
- GCaMP indicator of choice*
- Dental cement or adhesive of choice

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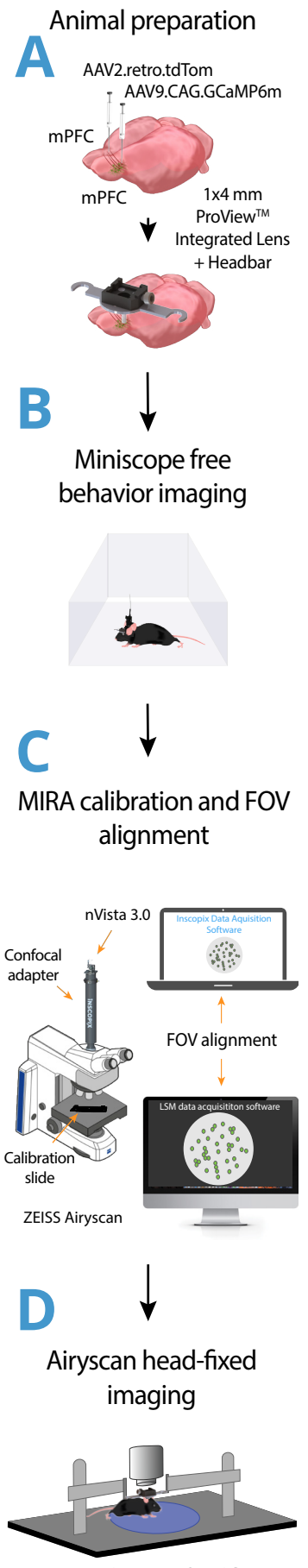


**Figure 3.** Schematic of workflow methods for the MIRA platform.

## General experimental workflow (Figure 3; abbreviated protocol)

### A. Animal preparation

1. Obtain the virus constructs, target coordinates, volume and injection rate to label cells of interest.
2. Prepare the animal for survival stereotaxic surgery procedures.
3. Inject the indicators in target brain regions using a micropump injector and suture the skin back and allow the animal to recover.
4. Either in the same surgery or a separate surgery, prepare the animal for a stereotaxic integrated lens installation procedure.
5. Open a craniotomy to gain access to the target brain region with the integrated lens probe with headbars attached.
6. Slowly lower the probe through the craniotomy into the brain until desired depth is reached based on the target coordinates. Aspiration of overlying tissue prior to probe insertion may be necessary.
7. Use adhesive of choice to adhere the integrated lens to the animal's skull.
8. Return the animal to its homecage and administer analgesics (per your institution's guidelines) and allow the animal to recover.

### B. Miniscope free behavior imaging

1. Prepare your miniscope and DAQ box for data acquisition. Ensure there is plenty of storage space for your session.
2. Briefly anesthetize or awake restrain the animal, remove the baseplate cover, and attach the miniscope.
3. Place the animal with attached miniscope in the behavioral arena of choice. Allow the animal to recover briefly from anesthesia or handling, capture a snapshot, and begin experiment.

### C. MIRA calibration and FOV alignment

1. Mount Inscopix confocal adapter on the Airyscan and dock miniscope onto the adapter using the ProView screwdriver.
2. Locate and focus on the fluorescent beads on the calibration slide provided by Inscopix through the Airyscan and capture a snapshot image using Airyscan software.
3. Adjust the miniscope mounting stage on the confocal adapter to bring the calibration slide sample in to focus with the miniscope in-place. Capture a snapshot with miniscope using Inscopix Data Acquisition Software. Once both images are captured, the systems are now parfocal (in the same z-plane of focus).

### D. Airyscan imaging

1. Prepare the animal for head-fixed experiment by securing the headbars into a head-fixed setup.
2. Remove the baseplate cover and place the animal under the Airyscan objective.
3. Locate the free behavior session FOV with miniscope through the confocal adapter.
4. Record a functional data set with the Airyscan.
5. Capture a Z stack with both red and green channels with the Airyscan.

## Data analysis and results

### Imaging contralateral projection neurons

In this study, contralateral projection-specific pyramidal neurons were labeled with tdTomato and all neurons were labeled with GCaMP in a mouse medial prefrontal cortex (mPFC). The activity of GCaMP expressing neurons was recorded during free behavior in an open field arena for 20 minutes. Later, the animal was head-fixed on a running wheel and tdTomato labeled projection neurons and GCaMP expressing neurons were imaged with an Airyscan confocal microscope through the GRIN lens (*Figure 4*).

### Registration of projection neurons

Post data acquisition, cell maps from both modalities were generated using Inscopix Data Processing Software (IDPS) API. The two channel structure images from the confocal microscope were maximally projected to generate 2D images matching the focal depth of the miniscope data. The green (GCaMP) structure images together with recognizable landmarks were used to generate a transformation matrix which was then applied to the cell footprints. This enabled registration of cells across modalities (*Fig. 4B-D*). Our processing software simplifies the co-registration workflow and allows researchers to get to the results quickly.

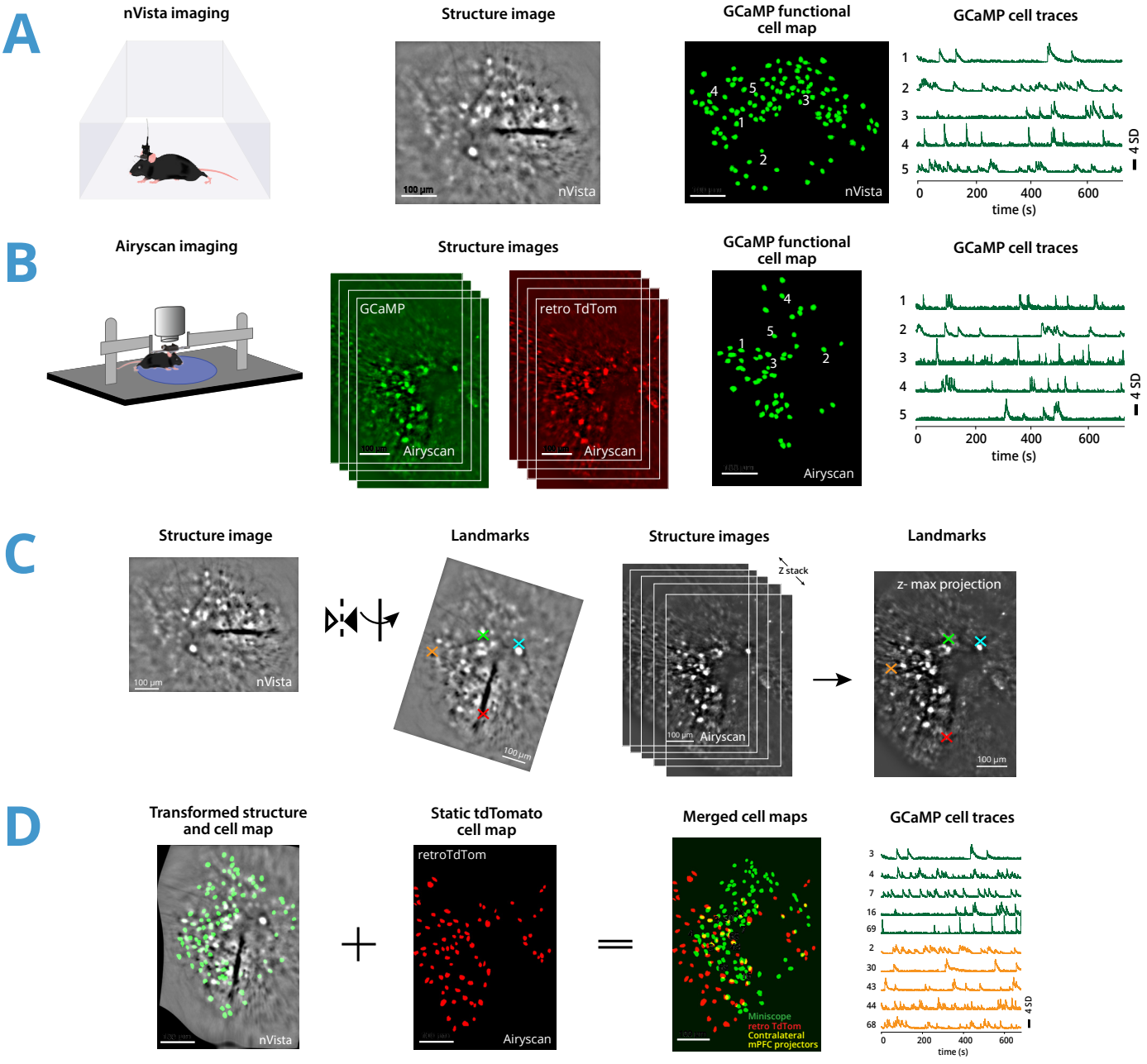

**Figure 4.** **A.** Free behavior imaging of mPFC neurons expressing GCaMP6 with nVista 3.0 generates a structure image and a GCaMP functional cell map with extracted traces. **B.** Head-fixed imaging of mPFC neurons expressing GCaMP6 and tdTomato with Airyscan generates a red and green structure image as well as a GCaMP functional cell map with extracted traces. **C.** Structure image from nVista is rotated and flipped (left) to match the Airyscan structure data in orientation. A maximum projection of Airyscan GCaMP structure data is created to match nVista depth of focus (right). Landmarks similar across both modalities are then selected for generation of a transformation matrix. **D.** Transformation matrix is then applied to nVista structure and its cell map. The transformed nVista GCaMP cell map is overlaid with the static tdTomato cell map from the Airyscan to generate a merged cell map where the yellow overlapping cells are identified to be contralateral projecting neurons along with extracted traces during free behavior.

## Discussion

Understanding the neural circuit mechanisms underlying complex cognition and behavior is of critical importance to investigating brain function in health and disease. The MIRA platform systematically and intuitively bridges together mesoscale neural circuit recordings with high-resolution multichannel imaging to advance our understanding of the brain in health and disease states. MIRA opens up brand new abilities to study neural circuit dynamics in free behavior, including identification of neuronal subtypes and imaging non-neuronal cell types and pathological markers thus expanding the range of applications in basic neuroscience and translational domains.

## References


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