Application of the Flocking Method for Spatial Analysis of Brain Activity in Optogenetics Datasets

Margarita Zaleshina[1][a] and Alexander Zaleshin[2][b]
[1]*Moscow Institute of Physics and Technology, Moscow, Russia*
[2]*Institute of Higher Nervous Activity and Neurophysiology, Moscow, Russia*

Keywords: Brain Imaging, Pattern Recognition, Optogenetics, Mouse Brain.

Abstract: This work introduces a new approach for spatial analysis of assumed dynamics of neuronal activity in mouse brain images obtained by light-sheet fluorescence microscopy methods (LSM). In calculations we used flocking algorithms based on neuronal activity distributions from slice to slice with a time delay that occurs during scanning. We applied GDAL Tools and LF Tools in QGIS for topological processing of multi-page TIFF files with LSM datasets. As a result, we identified localizations of sites with small movements of group neuronal activity passing in the same locations (with retaining localization) from slice to slice. An important advantage of this result is the ability to reveal locations with pronounced neuronal activity in a sequence of several adjacent slices, as well as to identify set of sites with interslice activity.

1 INTRODUCTION

This paper presents a new approach in spatial analysis of optogenetic data using a flocking method.

Optogenetics is a widely used method to study neuronal activity in living organisms at the cellular level. Genetically encoded indicators enable high spatiotemporal resolution optical recording of neuronal dynamics in behaving mice (Patriarchi *et al.*, 2018). These recordings further makes it possible to collect and process brain images, revealing important indicators of activities of sets of neurons in various behavioural tasks, as well as in the study of spontaneous activity.

Optogenetic data are usually presented as a multi-page TIFF file consisting of a set of stitched 2D slices. Specialized programs such as NeuroPG (Avants *et al.*, 2015), MicroMator (Fox *et al.*, 2022) have already been developed to view and analyse data. Moreover, optogenetic image processing is also used in other Platforms for Optogenetic Stimulation and Feedback Control (Kumar and Khammash, 2022).

Optogenetics uses sets of images of registered neuronal activity in the form of high-resolution 2D slices. Typical number of slices in one multi-page TIFF file is from 100 to 1000. Each pixel in these images represents an activity of neurons at a specific point, which is linked to a relative coordinate system. Taking into account the fact that the time of 2D recordings is nonzero, the change in activity from slice to slice can also be used in computational operations to determine the dynamics of activity.

When detecting neuronal activity, the main problem faced by researchers is noises. A noise in images, on the one hand, leads to the detection of a 'false activity' (false positives), but on the other hand, makes it difficult to identify the existing activity (false negatives), lowering the overall recognition quality. In areas with redundant information the influence of noise is higher. As a result, those zones that are in the middle range of activity are of interest for analysis, and allow to distinguish and highlight weak effects.

The main goal of this work is to develop and apply computational methods and tools that help reduce the influence of noise on recognition of neuronal activity and increase the predictability of dynamic optogenetic (neuronal) activity through brain slices. To eliminate the problem of noise in optogenetic images, in this paper we propose a new approach based on the principles of spatial analysis of flock trajectories (flocking method).

[a] https://orcid.org/0000-0001-5273-6579
[b] https://orcid.org/0000-0001-9356-9615

Zaleshina, M. and Zaleshin, A.
Application of the Flocking Method for Spatial Analysis of Brain Activity in Optogenetics Datasets.
DOI: 10.5220/0012154100003595
In *Proceedings of the 15th International Joint Conference on Computational Intelligence (IJCCI 2023)*, pages 471-478
ISBN: 978-989-758-674-3; ISSN: 2184-3236

The flocking method is based on keeping the distances and co-direction of movements of elements in the flock and can be used in analysis of dynamic changes in brain activity. The principles of flocking are already applied in brain imaging analysis (Aranda, Rivera and Ramirez-Manzanares, 2014). In our paper, their scope is expanded to analyse the neuronal activity of fluorescently activated mouse brain cells. The main scheme of our work is presented in Figure 1.

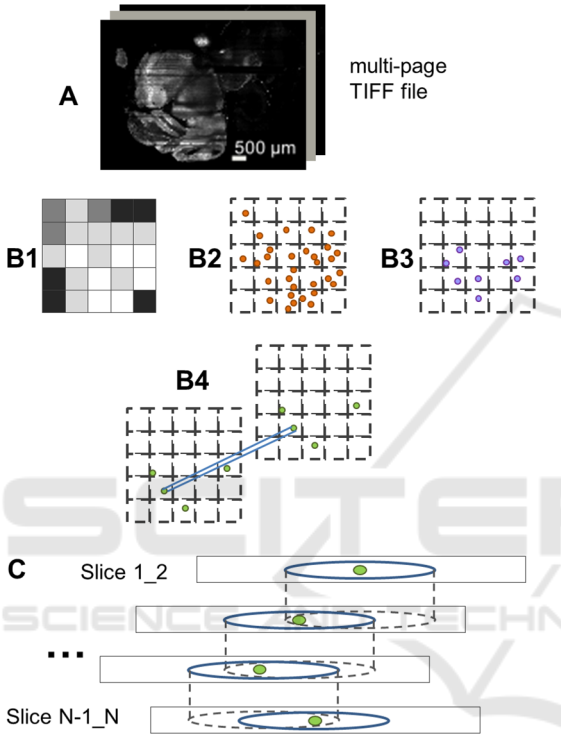

Figure 1: Sources, elementary processed units and tools in the processing of optogenetic images. A. Typical multi-TIFF image. Schematically shows that multi-TIFF image includes slices. Each subsequent slice is recorded with a shift along the brain and with a time delay relative to the previous one. B. Image analysis: B1. Primary image in grayscale mode. B2. Identification of activity points (shown as orange dots) by activity on a pair of neighboring slices. B3. Identification of ensemble locations (shown as lilac dots). B4. Identification of circuit tube between neighboring slices (shown as blue tube), which connect of circuit points (shown as green dots). C. Cross-slice projection between slices 1_2 and N-1_N when identifying circuit locations, taking into account the buffer zones (shown with solid blue lines on the upper slices and dashed lines on the lower slices).

Spatial relationships and neighbourhood in neural networks in fluorescence microscopy datasets enrich the possibilities of processing connectivity. The fact that the activity of neurons is related not to a single element but to a set of elements makes it possible to process data by methods of spatial analysis for flocks, taking into account the joint distribution of 'neuronal ensemble' activity. The presence of a spatiotemporal sweep between slices during scanning makes it possible to take into account the direction of movement of neuronal activity.

The usage of spatial analysis methods made it possible to reveal data from pixels of multi-TIFF images and conduct inter-slice spatiotemporal analysis using flocking method.

2 BACKGROUND

2.1 Brain Imaging Methods and Tools

The purpose of brain imaging analysis is usually to process data on brain structure, neuronal activity and their interrelationships. The possibilities and ways of analyzing the obtained data expand with the development of medical and research equipment used to obtain images of the brain. Thus, as image spatial resolution and the accuracy of localization of individual elements in the image increase, the ability to identify the topology of structures and individual brain areas improves. Also, with appropriate resolution, the level of detail is improved for describing processes in a healthy brain.

The possibility of separating activity of different neuronal populations in the brain tissue was investigated in experiments using various injections to detect activity. Thus, in (Klapoetke *et al.*, 2014) it was shown that two channel rhodopsins can detect two-color neural activation of spiking and downstream synaptic transmission in independent optical excitation of distinct neural populations.

Topology of structures is considered in certain size ranges typical for these structures. Within small areas of interest, an influence of topology from other scales will reduce. Curved surfaces of the brain directly affect the overall measurement of activity of ensembles from different segments. Spatial analysis in the recognition of images of brain tissue images allows individual smaller elements on a curved surface to become similar to linear elements. A formation of dynamically stable ensembles with a self-sustaining configuration can remain in its localization for a prolonged time.

Segmentation, Connectivity

The main task for understanding the functioning of both healthy and damaged brains is segmentation, selection of areas of interest, and identifying

connectivity between individual parts of the brain. Linking experimental results to spatial and temporal reference points is necessary for comparative analysis of multiple heterogeneous data sets of brain structure and activity, obtained from different sources, with different resolutions, and in different coordinate systems. Evaluation of automatic labelling detection is investigated by Papp et al. (Papp *et al.*, 2016), who propose a new workflow for spatial analysis of labelling in microscopic sections.

Tractography
Modern methods of Brain Imaging analysis apply a transition from selecting areas of interest to tractography techniques that allow visualizing pathways of the brain (white matter tracts) using tractography algorithms. Comparison of tractography algorithms for detecting abnormal structural brain networks presented in (Zhan *et al.*, 2015), influence of pre-processing and comparison of tract selection methods in DTI analysis presented in (Ressel *et al.,* 2018). Sets of images obtained with a fiber-bundle micro probe immersed at different depths inside a fixed brain tissue were processed in (Doronina-Amitonova *et al.,* 2012).

Spatial Analysis of High-Resolution Images
As measurements become more detailed, researchers have an opportunity to monitor not only summary results in the form of connection or tracts but also to identify detailed elements at the cellular level. To do this, analysis algorithms are enhanced. Thomas L. Athey et al. (Athey *et al.,* 2023) presented BrainLine, an open source pipeline that interacts with existing software to provide registration, axon segmentation, soma detection, visualization and analysis of results..

2.2 Artificial Neural Networks for Spatial Processing

Artificial neural networks are frequently used in segmentation of biomedical images. To solve the problem of image processing in differentiated zoom levels of images, mixed sized biomedical image segmentation based on training U-Net (Benedetti, Femminella and Reali, 2022) and DeepLabV3 (Furtado, 2021) are used. Time overlap strategy used in U-Net (Ronneberger, 2017) allows for seamless segmentation of images of arbitrary size, and the missing input data is extrapolated by mirroring. However, U-Net performance can be influenced by many factors, including the size of training dataset, the performance metrics used, the quality of the images and, in particular, specifics of brain functional areas to be segmented.

Despite the development of convolutional neural networks (CNNs) is limited because both efforts required to prepare training dataset, and time spent on data recognition in trained neural networks are still too great. As a result, it is more convenient to solve this type of tasks using spatial analysis methods that allow performing multi-operations and selecting not all objects, but only those that are of interest for further study. These approaches can be employed either individually or in conjunction with CNNs during pre-processing or post-processing stages of data processing. In addition, a well-chosen segmentation labelling algorithm (Lee *et al.*, 2022) helps to optimize work with neural networks.

2.3 Optogenetics in Studying of Neuronal Activity

In 2005, Boyden and Deisseroth published the results of the first optogenetics experiments. Their work (Boyden *et al.*, 2005) reported the ability to control neuronal spiking with a millisecond resolution by expressing a natural occurring membrane localized light-gated ion pump. In further research, Deisseroth explored possibilities of using optogenetics to control brain cells without surgical intervention (Deisseroth, 2010). With the advancement of optogenetics, its experimental applications have spread to all areas of brain activity research. Optogenetic tools are enabling causal assessment of the roles that different sets of neurons play within neural circuits, and are accordingly being used to reveal how different sets of neurons contribute to emergent computational and behavioral functions of the brain (Boyden, 2011).

Currently, optogenetics is actively used to study the neuronal activity of living animals, allowing deep immersion into the brain without destroying its structure. Researchers conduct a variety of optogenetic experiments on mice, including the study of social and feeding behaviour (Jennings *et al.*, 2019), False Memory creation in certain parts of the brain (Ramirez *et al.*, 2013), and, if possible, activating or suppressing the activity of brain cells with a light flash, while affecting the general behaviour of mice (Yang *et al.*, 2021).

Light-sheet microscopy (LSM) was developed to allow for fine optical sectioning of thick biological samples without the need for physical sectioning or clearing, which are both time consuming and detrimental to imaging. The functioning principle of LSM is to illuminate the sample while collecting the fluorescent signal at an angle relative to the illuminated plane. Optogenetic manipulation coupled to light-sheet imaging is a powerful tool to monitor

living samples (Huisken and Stainier, 2009; Maddalena *et al.*, 2023).

2.4 Usage of Flocking Method in Detection of Neuronal Activities

In this paper, we have extended the application of flocking method to the spatial analysis of optogenetic datasets. Flocking is a common behaviour observed in nature, defining the collective behaviour of a large number of interacting individuals with a common aim. Nearby members of a flock should move in approximately the same direction and at the same speed. For studying of collective motion or population dynamics in short trajectories is of-ten applied the flocking method, which based on analysis of joint directions and intersections of trajectories with a time lag. Flock methods analyse a behaviour of multi-sets of similar elements in research on collective behaviour in biology and even in robotics (Vicsek and Zafeiris, 2012; Ban *et al.*, 2021; Papadopoulou *et al.*, 2023). The methods used to calculate a behaviour of animals in a flock can also be extended to model neural networks (Battersby, 2015). Collective motion is also investigated for the analysis of cumulative behaviour of cells (Ascione *et al.*, 2023).

The possibility of organizing parallel calculations by using the processing of activity patterns with spatial reference to individual tiles is shown by Marre (Marre *et al.*, 2012); as an extension of this work, the paper (Goldin *et al.*, 2022) shows the possibility of using the CNN model to calculate context dependence for predicting the activity of retinal cells depending on the content of natural images.

In the case of neuronal activity, we are dealing with a set of simultaneously working elements, where behaviour of each of the elements depends on both its neighbours and the environment (Degond, Frouvelle and Merino-Aceituno, 2017; Levis, Pagonabarraga and Liebchen, 2019; Escaff and Delpiano, 2020; Rouzaire and Levis, 2022). Doursat suggested that 'Neuron flocking' must happen in phase space and across a complex network topology' (Doursat, 2013).

'Flocking' behaviour as presented in this work has components comparable to the 'delay activity', which was observed by Miyashita (Miyashita, 1988) and theorized by other researchers (Hamid and Braun, 2019). Specifically, the current work postulates that the activity of a neuron within a set of simultaneously neurons (neuronal network) depends on its neighbours within the neuronal network and, hence, the topology of the network. The formation of mental representations, being based on the temporal statistics of the environment, involves the establishment of stable neural patterns. These patterns of reverberating activity act as attractors within the neural network, enabling efficient encoding and retrieval of information (Hamid and Braun, 2019).

In the paper (Aranda, Rivera and Ramirez-Manzanares, 2014) Aranda et al. have shown that algorithms, based on information about spatial neighbourhood such as tractography methods, as well as the flocking paradigm, can improve a calculation of local tracks. Aranda et al. (Aranda, Rivera and Ramirez-Manzanares, 2014) made an assumption for calculations what 'the flock members are particles walking in white matter for estimating brain structure and connectivity'. The authors applied calculation methods in accordance with Reynolds' rules of flocking behaviour (Reynolds, 1987). This assumption makes it possible to calculate the behaviour of individual sets of elements piece by piece, without using of collective information.

The application of optogenetic scanning made it possible to identify the main components of neuronal activity and various types of activity changes in the same locations over time.

We assume that the topological properties of distribution of individuals in a moving flock are able to represent information about the environment in the same way as it is realized by a network of neurons. In the methodology used in this paper, we further show that considering a set of elementary components of neuronal activity in the form of a flock improves the extraction of meaningful information.

In calculations to study dynamics of neuronal activity, we used the time delay that occurs when moving from slice to slice during scanning using optogenetic methods. An important advantage of using this method is the identification of locations where a pronounced directionality of neuronal activity trajectories can be observed in a sequence of several adjacent slides, as well as the identification of areas of through intersection of activities.

3 METHODS

3.1 Calculations

The proposed flocking method for interslice image analysis allows to identify activity of neural ensembles in the mouse brain, which were obtained using optogenetic technologies.

By applying the flocking principles to the analysis of activity of a set of neurons, it becomes possible to

reduce the influence of noise and replenish the sites with missed activity.

The registered optogenetic highlighting that we considered is caused by neural ensembles. Illuminated elements are presented in the form of pixels of varying degrees of brightness, with an area of 1x1 sq. pixels (10x10 sq. μm). The characteristic size of a single detected ensemble was up to 10x10 sq. pixels. These ranges are typical for cells, ensembles, and agglomerations of cells (Bonsi *et al.*, 2019). All calculations were performed on the basis of the characteristic features of neural circuits, including common intersections and overlapping buffer zones of different track.

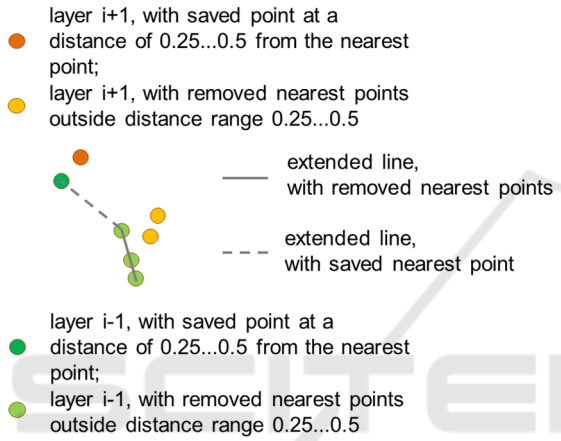

Figure 2: Processing to save or remove intersection points from layers.

The following operations were performed with each of the multi-page TIFF files:

Split Multi-Page TIFF Files into Distinct Slices in TIF Format

Image Pre-Processing and Interpolation

(a) Create contour lines of intensity (in the form of isolines, the applied parameter is 25 pixels) for each of the distinct slices.

(b) Apply LF Tools Extend lines plugin to a set of contours in each of the distinct slices and creating extended lines of contours at their start and/or end points, 100 μm in length.

Flocks Identifying

(c) Create intersection points of extended lines from neighboring slices.

(d) Search for intersection points of extended lines from neighboring slices that are located at a distance in the range of 0.25-0.5 pixel (Figure 2).

(e) Remove all intersection points from the previous item that are present in more than one on the same extended line.

(f) Search for remaining intersection points from three neighboring slices, which (points) are not more than 0.25 pixel away from each other.

(g) Search for all intersection points from the previous item that are more than one on the same extended line.
This operation reveals either a long marginal chain (more than 10 pixels in length) in several neighboring slices or the movement of a large object (10x10 pixels). The spread of intensively of these identified objects occurs over areas of distinct slices.

(h) Remove all intersection points from 3(d) item that are more than one on the same extended line.

(i) Search for remaining intersection points from the previous item.
As a result, small movements (movement within the identified localization, 10x10 pixels) of small objects (3x3 pixels) are revealed.

Plotting of Flock Trajectories

(j) Splice of intersection points from 3(g) item (defining the localization of small movement) into a sequence corresponding to the sequence of transitions from slice to slice, if the intersection points from 3(g) item are not more than 10 pixels away from each other.

Table 1: Spatial data processing applications.

Plugin	Description
Extracts contour lines https://docs.qgis.org/3.28/en/docs/user_manual/processing_algs/gdal/rasterextraction.html	Generate a vector contour from the input raster by joining points with the same parameters. Extracts contour lines from any GDAL-supported elevation raster.
Nearest neighbour analysis https://docs.qgis.org/3.16/en/docs/user_manual/processing_algs/qgis/vectoranalysis.html#qgisnearestneighbouranalysis	Performs nearest neighbour analysis for a point layer. The output presents how data are distributed (clustered, randomly or distributed).
LF Tools https://github.com/LEOXINGU/lftools/wiki/LF-Tools-for-QGIS	Tools for cartographic production, surveying, digital image processing and spatial analysis (Extended lines)

The parameters used for the calculations were established by selecting and optimizing the number of intersection points connecting lines from two different neighboring slices, taking into account the Nearest neighbor analysis. An extended line is

constructed according to the distance between the cells.

3.2 Applications for Spatial Analysis

In our work we processed optogenetic mouse brain images using Open Source Geographic Information System QGIS v.3.

Applications and special plug-ins (see Table 1) were used for spatial analysis of data both within single slices and between sets of closely spaced slices.

4 EXPERIMENTS AND RESULTS

4.1 Datasets

Our work considered optogenetic datasets on 23 mice. Datasets were presented as multi-page TIFF files, which were exported to QGIS (http://qgis.org).

Recognition of multipoint activity and spatial analysis of the distribution of neuronal activities according to fluorescence microscopy datasets was performed based on data packages published in an open repository (https://ebrains.eu). As source material, we used fluorescence microscopy datasets:

Set 1 (see Table 2): We used whole-brain datasets (Silvestri *et al.*, 2019) from transgenic animals with different interneuron populations (PV, SST and VIP positive cells) which are labelled with fluorescent proteins. These datasets were obtained from 11 mice *(*male animals, on post-natal day 56). The data was represented in 48 multi-page TIFF files. Each multi-page TIFF included 160 - 288 slices with dorsal or ventral projections of the mouse brain. The data resolution is 10.4x10.4x10 μm.

Set 2 (see Table 2): We used whole-brain datasets (Silvestri, Di Giovanna and Mazzamuto, 2020) obtained using LSM in combination with tissue clearing. These datasets were obtained from 12 mice *(*male animals, on post-natal day 56). The data was represented in 14 multi-page TIFF files. Each multi-page TIFF file included 800 slices with dorsal or ventral projections of the mice brain. The data resolution is 10x10x10 μm.

By processing using CLARITY-TDE method (Chung *et al.*, 2013; Costantini *et al.*, 2015) images have been partially cleaned up.

Allen Mouse Common Coordinate Framework (Wang *et al.*, 2020) served in our work as a frame of data reference to spatial coordinates.

Table 2: Source material.

	Set 1	Set 2
Number of mice	11 mice	12 mice
Gender and age of animals	male animals, post-natal day 56	male animals, post-natal day 56
Parvalbumin-positive interneurons parvalbumin (PV)	4 Animals	5 Animals
Somatostatin-positive interneurons somatostatin (SST)	3 Animals	3 Animals
VIP-positive interneurons vasoactive-intestinal peptide (VIP)	4 Animals	4 Animals
Number of multi-page TIFF files (several files per mouse)	48 multi-page TIFF files	14 multi-page TIFF files
Tissue clearing method	CLARITY/ TDE	CLARITY/ TDE
Resolution	10.4x10.4x 10 μm	10x10x10 μm
Number of slices in one multi-page TIFF file	about 288 slices	800 slices
Size of one slice	about 1200x1500 pixels	1140x1500 pixels

4.2 Results

Spatial Processing in QGIS for Identifying Tiles With Activity Locations (see Section 3.1)

Output: set of tiled rasters with activity locations, which contain the ID-numbers of individual slices

The values obtained as a result of our work after spatial processing in QGIS:

- mean number of localization sites in neighboring slices, averaged over multi-page TIFF files, is 124;
- the percentage of sites with identified ‘small movement’ (movement within the identified localization, 10x10 sq. pixels) to the total number of identified localizations, averaged over all multi-page TIFF files, is 73.4%.

As a result of spatial processing in QGIS, localizations of sites of (10x10 sq. pixels) with ‘flocks’ were identified, based on the intersection points of extended lines in these localizations Figure 3 (Figure 3).

Further, only tiles with the identification of activity locations will be processed. This is much less than the full data from multi-page TIFF sets, and therefore the volume of processed materials is reduced by 10 or more times.

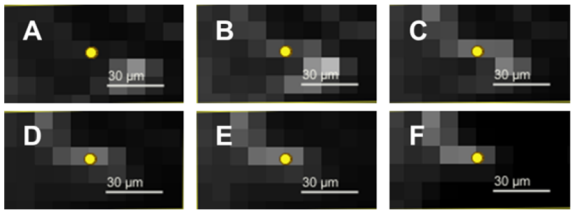

Figure 3: Slice-by-slice activity near the identified localization (marked by yellow dots). Scale bar: 30 µm.

The identified sites were further used as segmentation labelling for training U-Net and DeepLabV3Plus neural networks.

Post-processing of Tiles with Identified Activity Locations Using Convolutional Neural Networks for Image Segmentation

Output: set of activity coordinates inside of tiles which contain the ID-numbers of individual slices and tiles. The finished results can be uploaded to JSON files.

After training used our segmentation labelling, U-Net showed next results in Precision, Recall, and F1-score (F1-score = 2 * Precision * Recall / (Precision + Recall): 81.4%, 76.2%, and 78.7%, respectively; and DeepLabV3Plus showed next results in Precision, Recall, and F1-score: 76.4%, 79.4%, and 77.9% respectively.

Further Using of Final Results

Final results can be used in external applications, both for calculating the trajectories of activity movements, and for constructing tractograms inside 3D multi-page TIFF files.

5 CONCLUSION

In our work, we applied the flocking method to analysis of spontaneous brain activity. We selected different cell groups and determined areas occupied by ensembles of cell groups in mouse brain. When performing computational experiments, we analyzed the interslice propagation of neuronal activity for sets of mouse brain images.

In summary, the contributions of this work are as follows:

- We performed a spatial analysis of mouse brain optogenetic images using the flocking method.
- We have shown that using the flocking method, it is possible to detect more accurately both areas and tracks of neuronal activity, identifying the connectivity of extended areas of activity
- We were able to identify localizations of sites with small movements of group activity (stably localized flickering of activity with small movements).

In the future, the flocking method can be used not only in processing of optogenetic images but also in the analysis of other tracks, including the analysis of data obtained by diffusion-weighted magnetic resonance imaging (DW MRI) + High Angular Resolution Diffusion Imaging (HARDI).

REFERENCES

Aranda, R., Rivera, M. and Ramirez-Manzanares, A. (2014) 'A flocking based method for brain tractography', *Medical Image Analysis*, 18(3), pp. 515–530. doi: 10.1016/j.media.2014.01.009.

Ascione, F. *et al.* (2023) 'Collective rotational motion of freely expanding T84 epithelial cell colonies', *Journal of The Royal Society Interface*, 20(199):20220719.

Athey, T. *et al.* (2023) 'BrainLine: An Open Pipeline for Connectivity Analysis of Heterogeneous Whole-Brain Fluorescence Volumes'. doi: 10.1101/2023.02.28.530429.

Avants, B. W. *et al.* (2015) 'NeuroPG: open source software for optical pattern generation and data acquisition', *Frontiers in Neuroengineering*, 8.

Ban, Z. *et al.* (2021) 'Self-Organised Collision-Free Flocking Mechanism in Heterogeneous Robot Swarms', *Mobile Networks and Applications*, 26(6), pp. 2461–2471. doi: 10.1007/s11036-021-01785-7.

Battersby, S. (2015) 'News Feature: The cells that flock together.', *Proceedings of the National Academy of Sciences of the United States of America*. United States, 112(26), pp. 7883–5.

Benedetti, P., Femminella, M. and Reali, G. (2022) 'Mixed-Sized Biomedical Image Segmentation Based on U-Net Architectures', *Applied Sciences*, 13(1), p. 329. doi: 10.3390/app13010329.

Bonsi, P. *et al.* (2019) 'RGS9-2 rescues dopamine D2 receptor levels and signaling in DYT1 dystonia mouse models.', *EMBO molecular medicine*, 11(1).

Boyden, E. S. *et al.* (2005) 'Millisecond-timescale, genetically targeted optical control of neural activity.', *Nature neuroscience*, 8(9), pp. 1263–8.

Boyden, E. S. (2011) 'A history of optogenetics: the development of tools for controlling brain circuits with light.', *F1000 biology reports*. England, 3, p. 11.

Chung, K. *et al.* (2013) 'Structural and molecular interrogation of intact biological systems.', *Nature*. England, 497(7449), pp. 332–7.

Costantini, I. *et al.* (2015) 'A versatile clearing agent for multi-modal brain imaging.', *Scientific reports*, 5(1), p. 9808. doi: 10.1038/srep09808.

Degond, P., Frouvelle, A. and Merino-Aceituno, S. (2017) 'A new flocking model through body attitude

coordination', *Mathematical Models and Methods in Applied Sciences*, 27(06), pp. 1005–1049.
Deisseroth, K. (2010) 'Controlling the Brain with Light', *Scientific American*. United States, 303(5), pp. 48–55.
Doronina-Amitonova, L. V *et al.* (2012) 'Multicolor in vivo brain imaging with a microscope-coupled fiber-bundle microprobe', *Applied Physics Letters*, 101(23), p. 233702. doi: 10.1063/1.4767386.
Doursat, R. (2013) 'Bridging the mind-brain gap by morphogenetic 'neuron flocking': The dynamic self-organization of neural activity into mental shapes', *AAAI Fall Symposium - Technical Report*, FS-13-02, pp. 16–21.
Escaff, D. and Delpiano, R. (2020) 'Flocking transition within the framework of Kuramoto paradigm for synchronization: Clustering and the role of the range of interaction', *Chaos: An Interdisciplinary Journal of Nonlinear Science*, 30(8), p. 083137.
Fox, Z. *et al.* (2022) 'Enabling reactive microscopy with MicroMator', *Nature Communications*, 13, p. 2199.
Furtado, P. (2021) 'Testing Segmentation Popular Loss and Variations in Three Multiclass Medical Imaging Problems', *Journal of Imaging*, 7(2), p. 16.
Goldin, M. A. *et al.* (2022) 'Context-dependent selectivity to natural images in the retina', *Nature Communications*, 13(1), p. 5556.
Hamid, O. H. and Braun, J. (2019) 'Reinforcement Learning and Attractor Neural Network Models of Associative Learning', in Sabourin, C. et al. (eds) *Computational Intelligence: 9th International Joint Conference, IJCCI 2017 Funchal-Madeira, Portugal, November 1-3, 2017 Revised Selected Papers*. Cham: Springer International Publishing, pp. 327–349.
Huisken, J. and Stainier, D. Y. R. (2009) 'Selective plane illumination microscopy techniques in developmental biology', *Development*, 136(12), pp. 1963–1975.
Jennings, J. H. *et al.* (2019) 'Interacting neural ensembles in orbitofrontal cortex for social and feeding behaviour', *Nature*, 565(7741), pp. 645–649.
Klapoetke, N. C. *et al.* (2014) 'Independent optical excitation of distinct neural populations', *Nature Methods*, 11(3), pp. 338–346.
Kumar, S. and Khammash, M. (2022) 'Platforms for Optogenetic Stimulation and Feedback Control', *Frontiers in Bioengineering and Biotechnology*, 10, p. 918917. doi: 10.3389/fbioe.2022.918917.
Lee, Jonghyeok *et al.* (2022) 'A pixel-level coarse-to-fine image segmentation labelling algorithm', *Scientific Reports*, 12(1), p. 8672.
Levis, D., Pagonabarraga, I. and Liebchen, B. (2019) 'Activity induced synchronization: Mutual flocking and chiral self-sorting', *Physical Review Research*. American Physical Society, 1(2), p. 023026.
Maddalena, L. *et al.* (2023) 'Optogenetics and Light-Sheet Microscopy', in, pp. 231–261.
Marre, O. *et al.* (2012) 'Mapping a Complete Neural Population in the Retina', *The Journal of Neuroscience*. United States, 32(43), pp. 14859–14873. doi: 10.1523/JNEUROSCI.0723-12.2012.
Miyashita, Y. (1988) 'Neuronal correlate of visual associative long-term memory in the primate temporal cortex', *Nature*, 335(6193), pp. 817–820.
Papadopoulou, M. *et al.* (2023) 'Dynamics of collective motion across time and species', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 378(1874). doi: 10.1098/rstb.2022.0068.
Papp, E. A. *et al.* (2016) 'Brain-Wide Mapping of Axonal Connections: Workflow for Automated Detection and Spatial Analysis of Labeling in Microscopic Sections', *Frontiers in Neuroinformatics*, 10.
Patriarchi, T. *et al.* (2018) 'Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors.', *Science (New York, N.Y.)*. United States, 360(6396). doi: 10.1126/science.aat4422.
Ramirez, S. *et al.* (2013) 'Creating a false memory in the hippocampus.', *Science (New York, N.Y.)*, 341(6144), pp. 387–91. doi: 10.1126/science.1239073.
Ressel, V. *et al.* (2018) 'Comparison of DTI analysis methods for clinical research: influence of pre-processing and tract selection methods', *European Radiology Experimental*, 2(1), p. 33.
Reynolds, C. W. (1987) 'Flocks, herds and schools: A distributed behavioral model', *ACM SIGGRAPH Computer Graphics*, 21(4), pp. 25–34.
Ronneberger, O. (2017) 'Invited Talk: U-Net Convolutional Networks for Biomedical Image Segmentation', in Maier-Hein geb. Fritzsche, K. H. et al. (eds) *Bildverarbeitung für die Medizin 2017*. Springer Berlin Heidelberg, pp. 3–3.
Rouzaire, Y. and Levis, D. (2022) 'Dynamics of topological defects in the noisy Kuramoto model in two dimensions', *Frontiers in Physics*, 10, p. 976515.
Silvestri, L. *et al.* (2019) 'Whole brain images of selected neuronal types'. Human Brain Project Neuroinformatics Platform. doi: 10.25493/68S1-9R1.
Silvestri, L., Di Giovanna, A. P. and Mazzamuto, G. (2020) 'Whole-brain images of different neuronal markers'. Human Brain Project Neuroinformatics Platform. doi: 10.25493/A0XN-XC1.
Vicsek, T. and Zafeiris, A. (2012) 'Collective motion', *Physics Reports*, 517(3–4), pp. 71–140.
Wang, Q. *et al.* (2020) 'The Allen Mouse Brain Common Coordinate Framework: A 3D Reference Atlas', *Cell*, 181(4), pp. 936-953.e20.
Yang, Y. *et al.* (2021) 'Wireless multilateral devices for optogenetic studies of individual and social behaviors', *Nature Neuroscience*, 24(7), pp. 1035–1045. doi: 10.1038/s41593-021-00849-x.
Zhan, L. *et al.* (2015) 'Comparison of nine tractography algorithms for detecting abnormal structural brain networks in Alzheimer's disease.', *Frontiers in aging neuroscience*, 7(APR), p. 48.