# **Data Set Description**

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# Ca<sup>2+</sup> Imaging Data

Ref. Badel, L., Ohta, K., Tsuchimoto, Y., Kazama, H. Decoding of Context-Dependent Olfactory Behavior in Drosophila Neuron 91(1):155-67, 2016 https://doi.org/10.1016/j.neuron.2016.05.022

#### 1. Contents of the Data Set

This data set contains physiological data on the Ca<sup>2+</sup>response of 37 olfactory glomeruli of the antennal lobe of *Drosophila melanogaster*, in response to the presentation of various of olfactory stimuli *in vivo*. A related data set of behavioral responses to the same stimuli is also available separately. Details on the experimental setup and methods can be found in the published article (ref. above), but the essential points needed to comprehend the nature of the data are repeated here for completeness.

## 2. Data Acquisition

The entire right antennal lobe was imaged *in vivo* with a two-photon laser scanning microscope. A piezo positioner moved the objective lens vertically, allowing the system to cover 33 optical slices separated by 3  $\mu$ m in 595 ms (18 ms/slice). For each odor application trial, images were acquired continuously for  $\sim$  23 s, corresponding to a volume scanning rate of 1.7 Hz. After applying digital zoom, the pixel size of 1.384 x 1.384  $\mu$ m allowed to resolve individual glomeruli measuring on average 10  $\mu$ m in diameter.

The odor delivery system was identical to that used in behavioral experiments. In each experiment, an odor set consisting of 15 pure odors, 6 odor mixtures, or a pair of 2 odors at various concentrations was applied. Each odor in the set was presented for 4 s, with an inter-trial-interval of 1 min between odors. The odor delivery valve opened during imaging frame 9 and closed in frame 16. Each set was presented 4 times, and the order of odor presentation was randomized in each block. The sequence of odor presentation was fixed across experiments of the same type. After collecting odor responses, a high-resolution 3D image of the antennal lobe was acquired for off-line identification of guide-post glomeruli (see below).

### 3. Data preprocessing

To extract fluorescence changes in individual glomeruli in an efficient and objective manner, the GCaMP fluorescence in each glomerulus was exctracted by registering images against a template antennal lobe. To obtain the template, 37 NP225-Gal4-positive glomeruli were delineated in immunostained brains based on nc82 and anti-GFP signals. Delineated glomeruli in different brains were registered to a single (arbitrarily-chosen) reference brain, by optimizing an affine transformation to maximize the cross-correlation between four guidepost glomeruli (DL3, DA2, VM2, and DM5), and applying this transformation to the whole brain. Registered glomeruli were superimposed and thresholded to obtain the template AL, in such a way that each glomerulus in the template covers 50% of the mean volume of the corresponding glomerulus.

At the end of each odor stimulation experiment, all collected images were registered to the template antennal lobe by first delineating the four guidepost glomeruli using the high-resolution image. The affine transformation maximizing the cross-correlation between the four glomeruli and the template was then computed and applied to all images. Finally, the template volume was used to calculate the relative change in fluorescence  $\Delta F/F = (F_t - F_{\rm baseline})/F_{\rm baseline}$  in each glomerulus.

### 4. Data description

The data is reported as the relative change in fluorescence  $\Delta F/F$ , and is organized as a single 5-D matlab array imagingData with dimensions [glomerulus number, time frame, odor number, trial number, brain number]. Glomeruli are labeled 1-37 and identifiable by their standard name listed in the variable glomerulusName. Odors are labeled 1-85 and their identity is listed in the variable odorID. In each brain, each presented odor was applied 4 times, and each of these 4 trials is indexed by the fourth dimension (trial number). A total of 61 brains were imaged in this dataset and are indexed by the fifth dimension.

Because a subset of the whole set of odors was applied to each brain, missing data is encoded as NaN. For example,

```
find(\sim isnan(imagingData(1,1,:,1,1)))
```

returns [1,4,7,8,9,10], showing that brain number 1 was exposed to this set of odors. Similarly,

$$find(\sim isnan(imagingData(1,1,1,1,:)))$$

tells us that brains 1-12 were exposed to odor number 1.