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# 1 Introduction

Neuronal responses from primary visual cortex are collected for different visual stimuli. This work analyses the responses to bring out meaning and patterns from the responses. Pattern recognition is very important in this because it will give an insight to how brain process information.

Following topics are studied in this work

1. Analyzing characteristics of neurons in V1.
  - To map orientation tuning responses of excitatory pyramidal neurons in V1.
  - To determine response reliability at preferred orientation.
  - To determine correlations between neurons to find similarly tuned neurons.

2. Detecting of ‘motifs’ in neuronal responses.

We aim to study how neurons encode the information in V1. Motifs are recurring patterns in the response of a single neuron or a population of neurons. If we take several trials of same stimuli, we try to extract what is invariant in these. The simplest example is getting similar spike rate in every trials for a neuron. This is called reliability of a neuron or a population. A neuron is reliable implies it produces similar responses to a stimuli every time it is presented.

The responses of single neurons are not very correlated; or the time series responses of single neurons are reliable. Looking further, we search for recurring subsequences/ motifs. In a population of neurons, It will be a multidimensional subsequence.

# 2 Experiments

## 1. Experiment 1: Response to sinusoidal grating stimuli.

Virus expressing GCaMP6f was injected into the V1 of mice. Approximately 3 weeks post infection, mice were imaged under a 2-photon microscope while sinusoidal drifting gratings were presented on a computer screen placed 3 inches from the mouse (1 degree of visual space  $\sim 21.3$  pixels on the screen). The stimulus was varied as:

- (a) Sinusoidal drifting gratings at 16 different directions (0:22.5:337.5). Spatial frequency was fixed at 0.03 cycles per degree.
- (b) Each direction was repeated 10 times (i.e. 10 trials per direction). Directions are presented in a randomized order.
- (c) Each direction was presented for 2s and was always preceded by a 4s gray screen. Therefore the total duration of the stimulus is 6s.
- (d) Calcium signals (GCaMP6f in awake mice) were acquired from awake mice at 20 frames per seconds. Thus, sampling rate is 20Hz.

## 2. Experiment 2: Responses to amplitude spectrum modified natural scenes video stimuli.

**Setup** Calcium images were scanned using a Two-photon calcium imaging device on mice injected with GCaMP6f. Firing rates of these cells were then inferred using a fast nonnegative deconvolution algorithm on the Calcium imaging data.

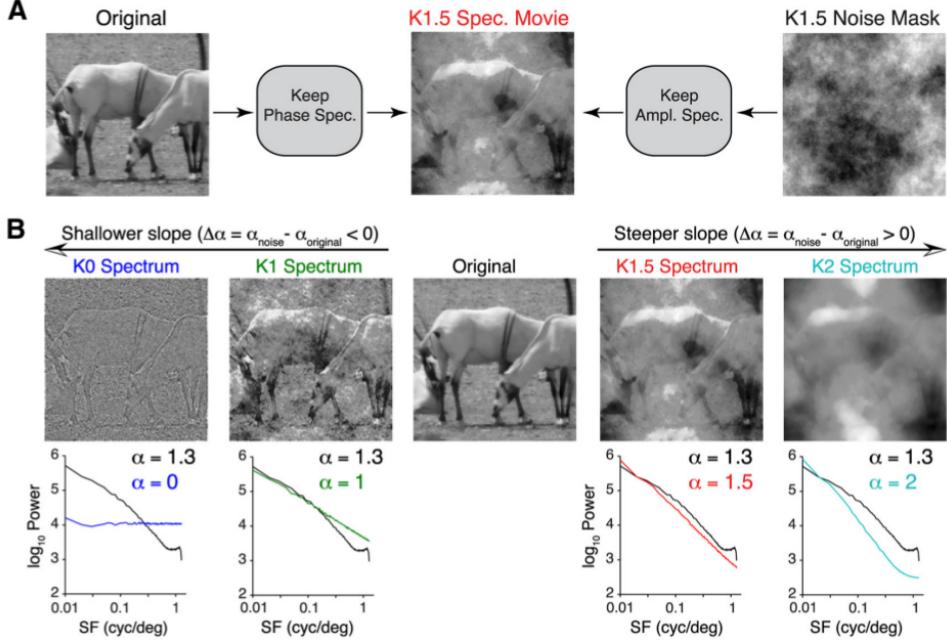


Figure 1: Process of making noise movies

**Visual stimuli-Creation of noise movies** “We developed an algorithm that allowed us to create noise images with a user-defined spectral slope. To do so, we took advantage of the inverse-square law:  $P \sim k^{-\alpha}$ , which translates to a circle with radius  $\alpha$  in two-dimensional Fourier space. Thus, we constructed all noise movies in the Fourier domain. We first defined a matrix of the same size as the original image ( $256 \times 256$  pixels) and then created a noise amplitude spectrum as a 2D circle of radius  $\alpha$ , with  $\alpha$  taking values from 0 (K0 movie) to  $\sqrt{2}$  (K2 movie). This was due to the squared relationship between the amplitude spectrum and the power spectrum. To create the final noise image, we combined this noise amplitude spectrum with a random phase spectrum, where phase values were randomly sampled from the range  $0 - 2\pi$ . The final noise images were visualized by computing its 2D inverse Fourier transform. Each frame of the noise movie was created using a new random seed, and as a result, the raw noise movies had no temporal correlations between frames. Noise-masking procedure. Figure 1 provides a schematic of the noise- masking procedure. First, each frame of a natural movie was decomposed into its Fourier components (phase and amplitude) via a 2D fast Fourier transform implemented in MATLAB. Next, a noise image was created as described above. The phase spectrum of the original movie was then combined with the amplitude spectrum of the noise movie. The resulting image was then inverse Fourier transformed to yield a noise-masked movie frame. This procedure was repeated for all frames. We used a total of five different natural movies, each 4 s in duration, from the van Hat- eren movie database.” [Sur and Rikhye 2015]

### 3 Quantifying the neuron activity

The 120 point time series provide the activity of single cell in response to a particular stimulus in a trial. The activity of cell has to be studied. The first 80 samples are expected to have spikes due to only background activity, as the stimulus was gray during that time. Peaks in Ca concentration denotes the spikes in the neuron. From neuroanatomy, spike rate is a good quantify to measure neuron activity. In Calcium imaging, the amplitudes corresponds to Ca ion concentration. From the neuron models, more the Ca concentration, more is the spike rate.

Following methods are used for quantifying spike rate.

Using various methods, a time sequence  $F_t$  is calculated.  $F_t$  represents the spiking activity of neuron. A relatively large  $F_t$  represents a spike and small corresponds to no-spike. Number of spikes in an interval is what we seek. In the 120 sample data,  $F_t$  of size 120 is found using various methods. Finally, spike rate is calculated by subtracting the background activity from the response.

$$spikeRate = \frac{\sum_{n=81}^{120} F_n}{40} - \frac{\sum_{n=1}^{80} F_n}{80}$$

Various methods for finding  $F_t$  is the first problem statement. Following approaches are made to solve this.

### 3.0.1 Averaging

The Calcium signal amplitudes averaged and the average is believed to be a proxy for the spike rate. From the smoothed data, just taking the mean of last 40 samples (time during which stimuli was presented) provides the estimated spike rate for that trial.

## 4 Estimating orientation selectivity of neuron

Neurons in the striate cortex of primary visual cortex in the brain are of three types. Cells which respond to orientation of a contrasting visual stimuli and which are not sensitive to orientation. Among the orientation selective cells, some cells are also sensitive to motion of the stimuli in a particular direction in addition to orientation. These cells are called complex cells. Cells which are only sensitive to orientation, but not to motion are called simple cells. These cells are pyramidal in structure and have a receptive field in shape of a rectangular slit. This section deals with methods to quantify the selective behavior of these cells from experimental data.

### 4.0.1 Selectivity Index measures - OSI, DSI

OI and DI are used as the normalized measures of “peak to trough” orientation selectivity and direction selectivity. These quantities are defined as:

$$OSI = \frac{(R_{pref\_ori} - R_{orth})}{(R_{pref\_ori} + R_{orth})} \quad (1)$$

$$DSI = \frac{(R_{pref} - R_{null})}{(R_{pref} + R_{null})} \quad (2)$$

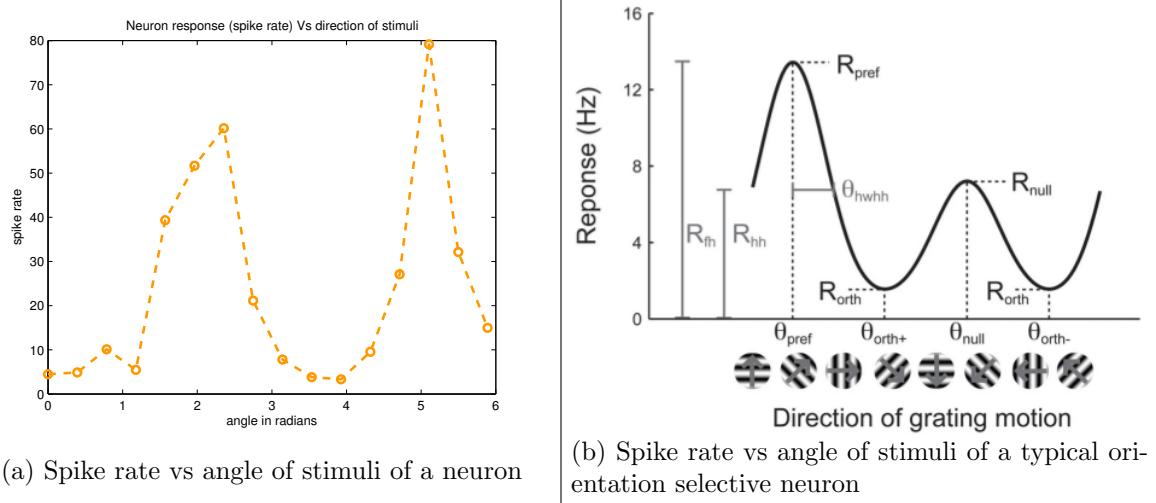
The response at the preferred orientation  $R_{pref\_ori}$  and the response at the preferred direction  $R_{pref}$  can be determined in different ways. In some measures, these are taken to be the best response to one of the stimulus orientations or directions that was explicitly measured; that is, if we measure responses at stimulus directions  $\theta_1, \dots, \theta_n$ , then we choose the response at the best  $\theta_i$ . In other measures, we perform a fit to the tuning curve, and choose the maximum value of the fit as  $R_{pref\_ori}$  or  $R_{pref}$ .

This approach is more sensitive to noise. One of the plot of spike rate vs angle of stimuli of a neuron is shown in Figure 2.

### 4.0.2 Circular variance of direction and orientation

As discussed in the paper, Circular variance is a more robust quantifier of orientation and direction selectivity. A scalar value representing the neuron response is extracted from the 6s long time series for each of the orientations and directions (There are 8 orientations and 16 directions). As we have 10 trials for each class, we will have 160 such values for each neuron.

Figure 2: Comparing response of a neuron Vs angle of stimuli with the expected response



The values are first expressed as vectors in the orientation space. The vector sum of the values provide the ‘preferred orientation’; Magnitude should indicate how much orientation selective the neuron is.

Let  $R(\theta)$  be the response of neuron to angle  $\theta$  and we denote the ‘preferred direction’ as  $\theta_{pref}$ . Normalized vector sum in orientation and direction space is found as.

$$L_{ori} = \frac{\sum_k R(\theta_k) \exp(2\theta_k)}{\sum_k R(\theta_k)} \quad (3)$$

$$L_{dir} = \frac{\sum_k R(\theta_k) \exp(\theta_k)}{\sum_k R(\theta_k)} \quad (4)$$

- Neurons with orientation selectivity are expected to have high  $L_{ori}$ .
- Simple cells are orientation selective, but less direction selective. They are expected to have large  $L_{ori}$  and small  $L_{dir}$ .
- Complex cells are orientation selective as well as direction selective. They are expected to have both large  $L_{ori}$  and large  $L_{dir}$ .

Circular orientation variance and direction variance is given as

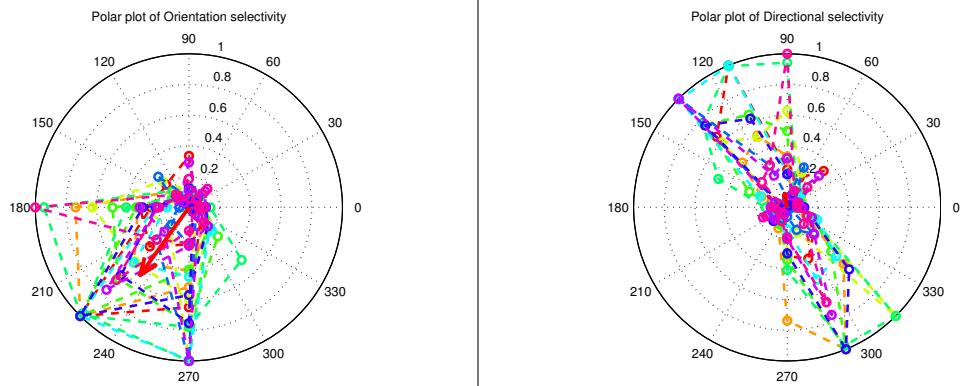
$$CirVar = 1 - |L_{ori}| \quad (5)$$

$$DirCirVar = 1 - |L_{dir}| \quad (6)$$

Following shows plots of orientation and direction selectivity of various kinds of neurons.

1. **Orientation selective simple cell**  
Refer Figure 3
2. **Direction selective complex cell**  
Refer Figure 4
3. **Orientation and direction Un-selective cells**  
Refer Figure 5

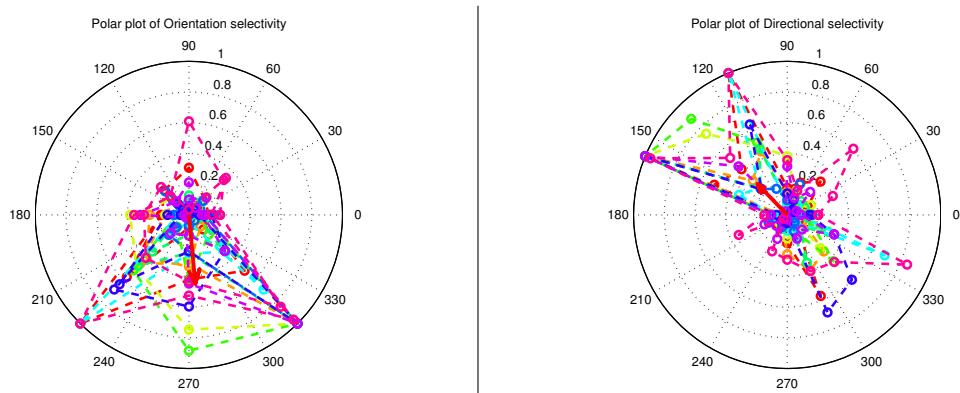
Figure 3: Orientation selective simple cell



(a) Orientation selectivity plot of an orientation selective simple cell. Each curve shows a trial. Note that the cell is sensitive to an optimal orientation. The red arrow represents the direction and magnitude of orientation selectivity

(b) Directional selectivity plot of an orientation selective simple cell. Each curve shows a trial. Note that the cell is not selective to direction, but is sensitive to orientation. The red arrow represents the direction and magnitude of direction selectivity

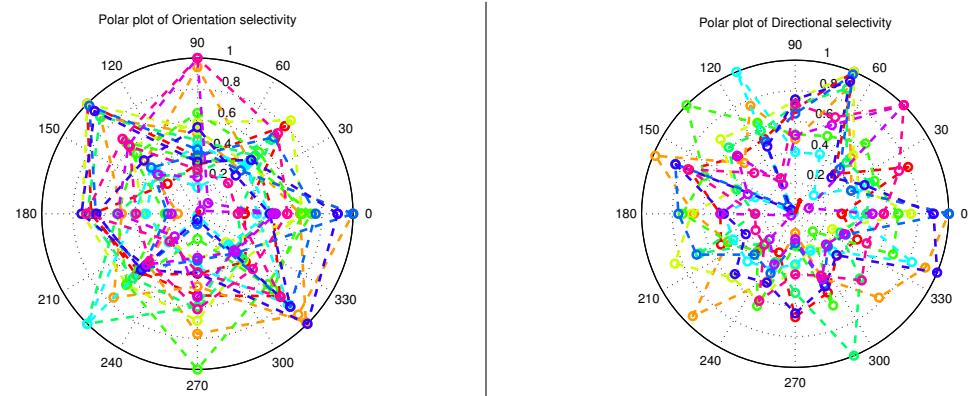
Figure 4: Direction selective complex cell



(a) Orientation selectivity plot of an direction selective complex cell. Each curve shows a trial. Note that the cell is sensitive to an optimal orientation. The red arrow represents the direction and magnitude of orientation selectivity

(b) Directional selectivity plot of an direction selective complex cell. Each curve shows a trial. Note that the cell is selective to direction as well as orientation. The red arrow represents the direction and magnitude of direction selectivity

Figure 5: Orientation and direction Un-selective cells



(a) Orientation selectivity plot of an Orientation and direction Un-selective cell. Each curve shows a trial. Note that the cell is uniformly sensitive to all orientations. The red arrow represents the direction and magnitude of orientation selectivity. Note the small magnitude

(b) Directional selectivity plot of an Orientation and direction Un-selective cell. Each curve shows a trial. Note that the cell is uniformly sensitive to all directions. The red arrow represents the direction and magnitude of direction selectivity. Note the small magnitude

## 5 Modeling the neuron response

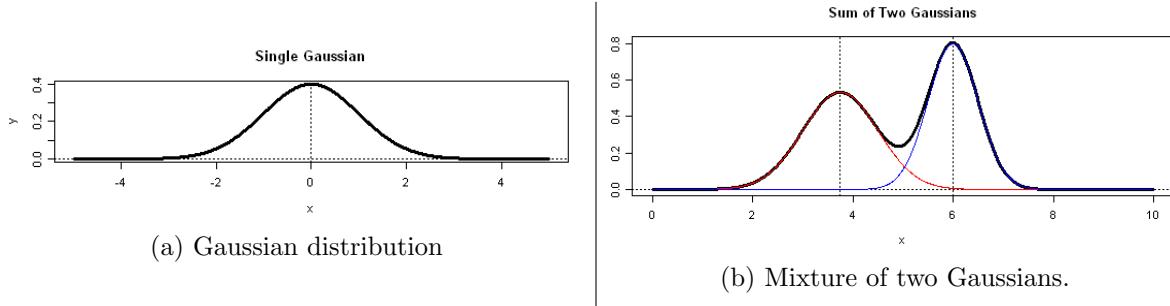
Different measures of orientation selectivity are obtained from the experimental data, aim is to find metrics which represent ‘true’ orientation selectivity. Modeling the response of neuron to various orientations and visualizing is a great way to see if in fact there is a orientation selectivity. If the cell seems selective, further inference can be drawn from models.

In neural data, even though fitting is a good From plotting the spike rate vs theta curve, We can model orientation selectivity of neuron with a Gaussian curve. We can also model the directional selectivity of a orientation/direction selective neuron using mixture of two Gaussian curves. Figure shows 6 a mixture of two Gaussian.

Some Intuitions drawn are

- Fitting is ‘blind’. As we do not know the ‘true’ tuning curve. Though, we can compute the error of fit to test data for judging the model.
- Orientation selective neurons will have distinct peaks in both, or they will have low variance parameter.
- Orientation unselective cells are expected to have uniform response to all angles, thus the Gaussian fit will be poor and the variance parameter will have large values.
- If both peaks in double Gaussian are similar, the cell is not direction specific. It has similar response to both directions, this could be a simple cell.
- For each neuron, the parameters will change. The ‘mean’ parameter will decide the preferred orientation/selectivity. The variance will decide the degree to which they are selective.
- For fitting Gaussian, we have 320 points. the estimation need not be accurate.
- For fitting double Gaussian, we have 160 points. the estimation need not be accurate.

Figure 6: Modeling orientation and directional selectivity using Gaussians



### 5.0.1 Estimating orientation fit

The empirical model for orientation tuning curve is

$$R(\theta) = C + R_p \exp\left\{-\frac{||\theta - \theta_{pref}||^2}{2\sigma^2}\right\}$$

Where parameters  $\theta_{pref}$  and  $R_p$  are to be estimated.  $\sigma$  is the tuning width, which can tell us how much the cell is selective.  $C$  is a constant offset.

Estimation of four parameters are done by minimizing squared sum of error. Sum of squared error is defined as:

$$SSE = \sum_{i=1}^N \left| \left| R(\theta_i) - C - R_p \exp\left\{-\frac{||\theta_i - \theta_{pref}||^2}{2\sigma^2}\right\} \right| \right|^2$$

The optimization is done using gradient descent algorithm.

### 5.0.2 Estimating direction fit

The empirical model for direction tuning curve is

$$R(\theta) = C + R_p \exp\left\{-\frac{||\theta - \theta_{pref}||^2}{2\sigma_1^2}\right\} + R_n \exp\left\{-\frac{||\theta - \theta_{null}||^2}{2\sigma_2^2}\right\}$$

Where parameters  $\theta_{pref}$ ,  $\theta_{null}$ ,  $R_n$  and  $R_p$  are to be estimated. By theory,  $\theta_{pref} = 180 \pm \theta_{null}$ . Here  $\sigma_1$  and  $\sigma_2$  are the tuning widths, which can tell us how much the cell is selective.  $C$  is a constant offset.

**Results** Least squares estimation is used to find the parameters of the model. The curve fitting converged with a ‘good’ guess of initial parameter estimate. One basis of finding direction selective cells will be to analyze the parameters of the best fit. Figure 7 shows some of the fits for complex/simple cells. Figure 8 shows effort to fit the model to a direction/orientation unselective cell.

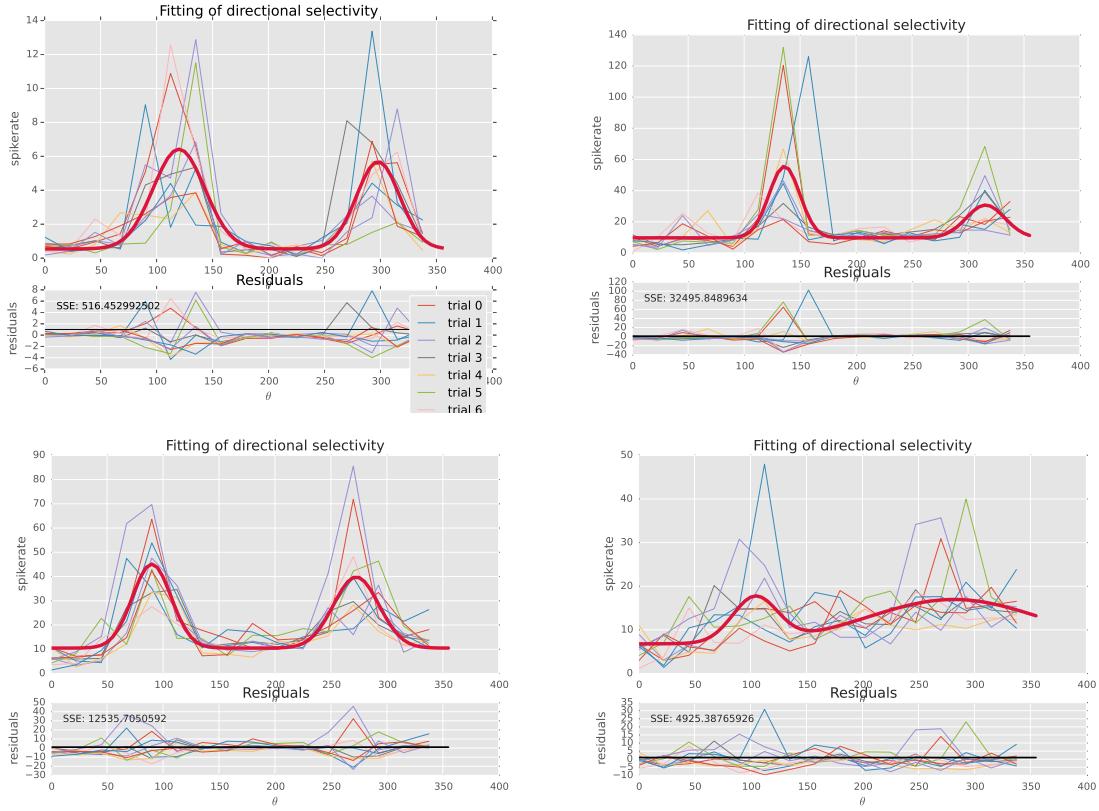


Figure 7: Fit of directional selectivity of complex cell (probably). The red curves show the fit and other curves are measured data

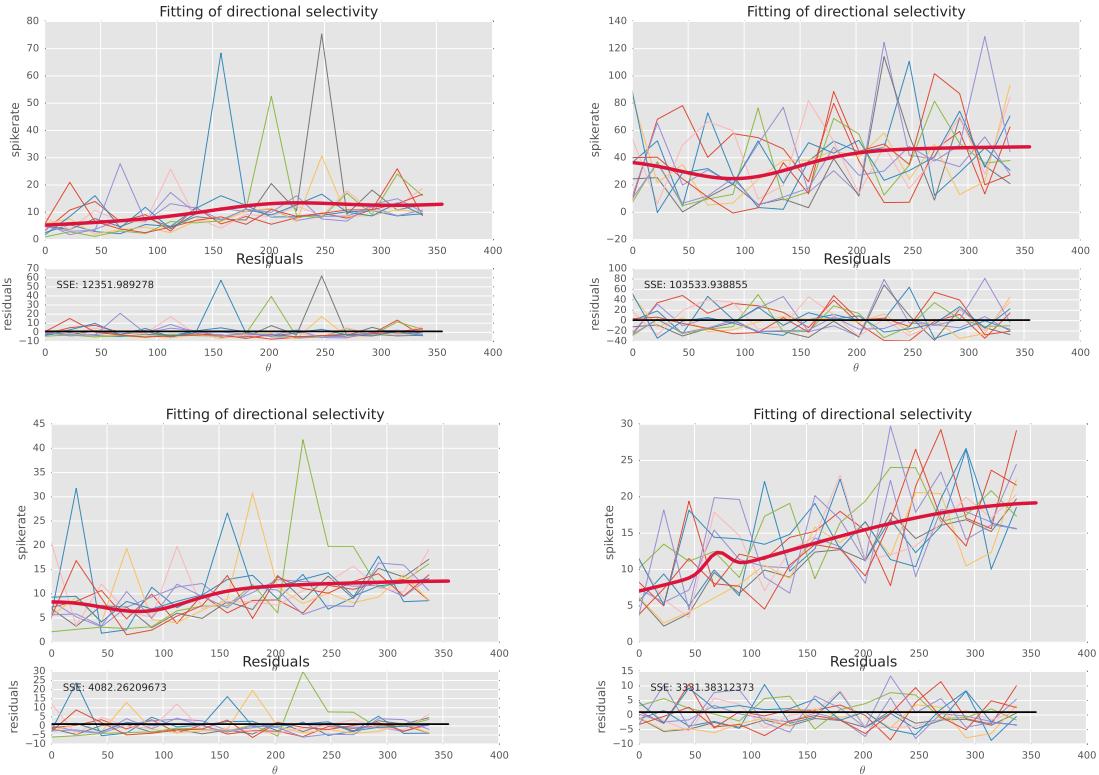


Figure 8: Fit of directional selectivity of unselective. The red curves show the fit and other curves are measured data. Note the large residuals

## 6 Classification of simple and complex cells

The properties that distinguish the three types of cells are discussed before. This problem statement deals with using machine learning tools for making a classifier for these three classes of cells.

The main challenge here is that we do not have the ground truth data for the data, which force us into category of unsupervised learning methods. This also makes it hard to evaluate the performance of the classification algorithm. We make an effort to classify cells based on some features extracted from the time series data. The feature selection process and the intuition behind it are discussed below.

- OSI : This is a measure of orientation selectivity, as an individual feature this may not be a strong classifier as the measure is not very robust, but along with other features, this could contribute towards classification.
- DSI : This is a measure of directional selectivity, as an individual feature this may not be a strong classifier as the measure is not very robust, but along with other features, this could contribute towards classification.
- $L_{ori}$  : This is more robust than OSI, and is expected to be a better feature.
- $L_{dir}$  : This is more robust than DSI, and is expected to be a better feature.

Following unsupervised learning techniques are used for the classification.

### 6.0.1 k-means clustering

We hope that three classes of cells will form three clusters in the feature space. We run the algorithm with 3 clusters and features selected above.

As we said, analyzing efficiency of classification is hard without ground truth. Here, what we use to analyze is this:

- Find the global mean
- Find the sum of squared distance of each point in feature space to global mean. denoted by  $total\_ss$ .
- Find the sum of squared distance of each centroids to the global mean multiplied by number points in the cluster. denoted by  $between\_ss$ .
- If there is no well clustering, the centroids will be close to the global mean, and  $between\_ss$  will be small compared to  $total\_ss$ .
- If  $clusterEfficiency = between\_ss/total\_ss$  is high, the clustering is good and forms distinct clusters.

Table 1 shows the clustering efficiency in each mice.

Subject	Cluster efficiency (%)
Mouse A	78.8155
Mouse B	72.3963
Mouse C	72.293
Mouse D	78.8882
Mouse E	76.4573

Table 1: Cluster efficiency of various subjects

## 7 Study of correlation

The activity of a population of cells was recorded. We would like to study the functional similarities of different neurons in a mouse. We aim to find out similarly tuned neurons. We could study the effects of receptive fields by this hopefully.

Similarly tuned cells are expected to have similar tuning curves (The double Gaussian we modeled before). We can study the pairwise correlation of tuning curves as first approach. Estimating correlation of the tuning curve may not be close to accurate as there are only 160 samples per neuron. The Figure 9 shows heat map of correlations between tuning curves for different mice. To see the sanity of the results, we could plot tuning curves of neurons with high correlation.

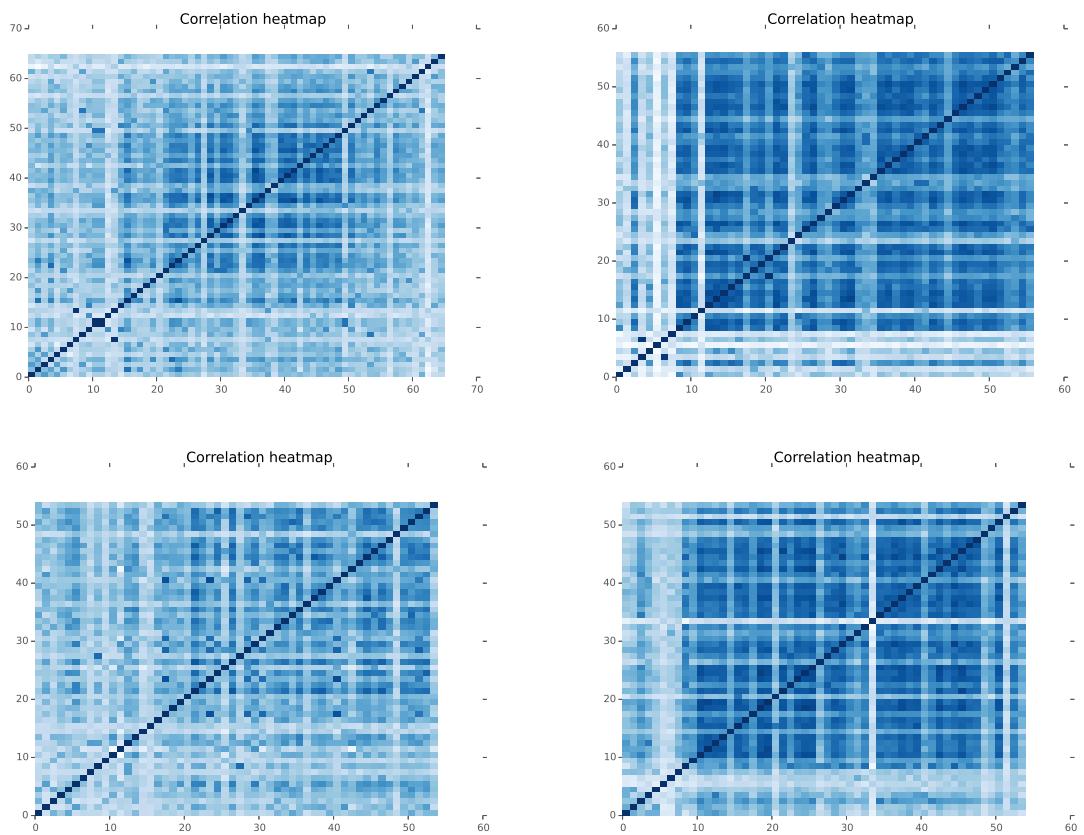


Figure 9: Correlation of tuning curves for different mice

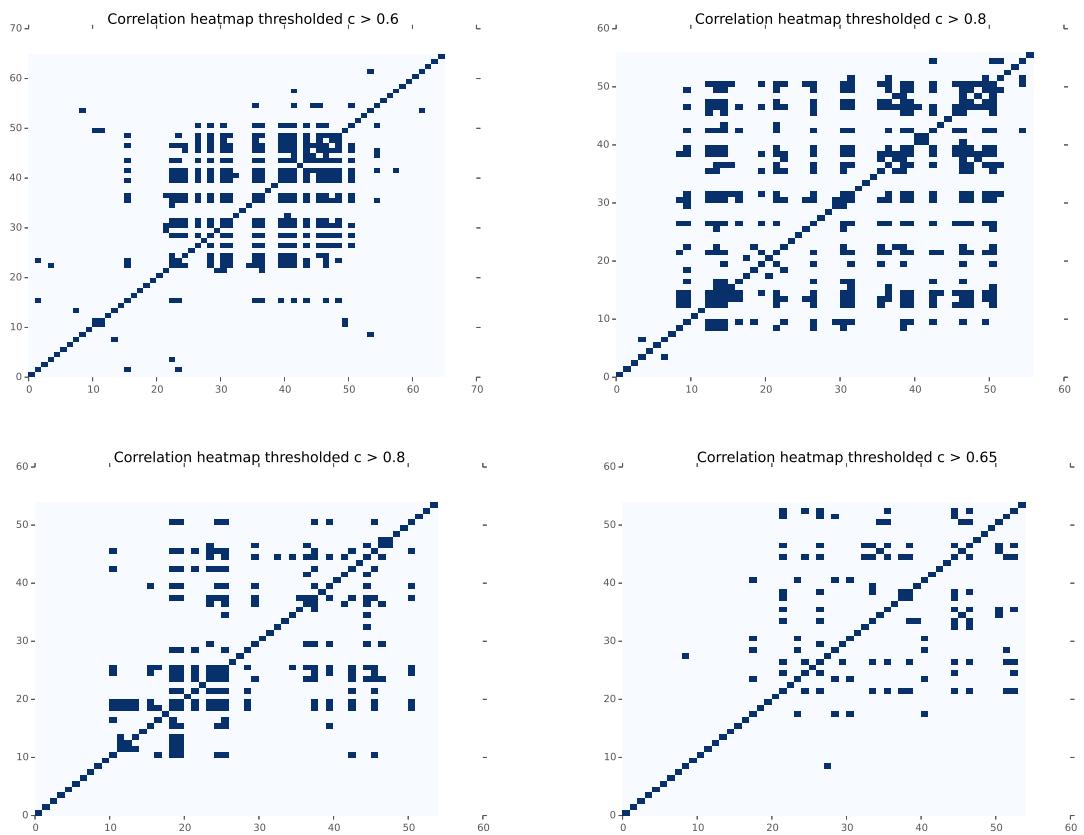


Figure 10: Correlation of tuning curves thresholded for different mice

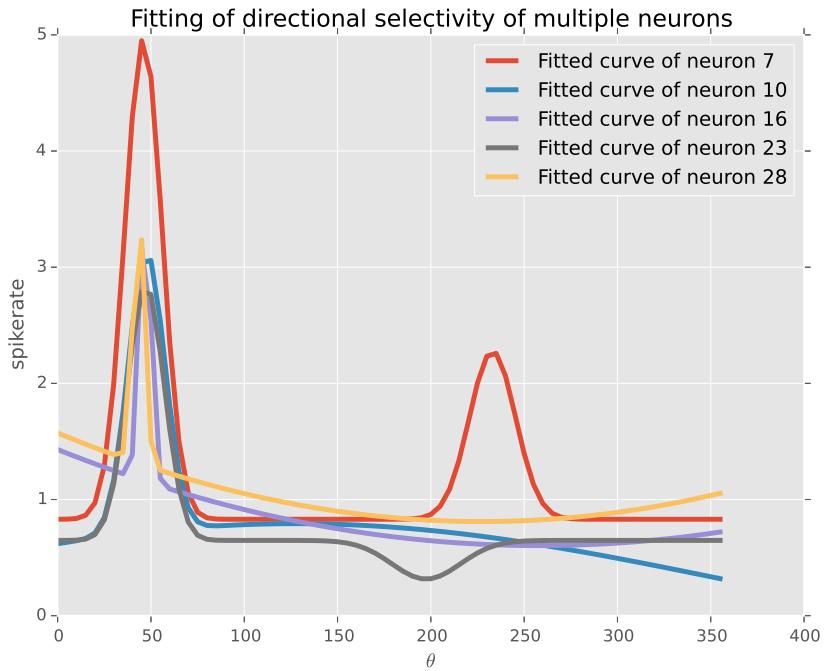


Figure 11: Showing correlated neurons are similarly tuned. correlation > 0.7

## 8 Study of principal components

It is known that there are three kinds of cells in V1 - simple, complex and orientation insensitive cells. Are there any correlation within these neurons? Is there redundancy in the code? We do an eigen analysis on the responses of all neurons in a mice to find how many uncorrelated dimensions are required to capture most of the variance.

Here we take number of neurons as feature dimension. The responses are averages across trials to form each point in the feature space. Every such time series responses were stacked to form the rows. Then PCA is done on the data to find number of components to variance explained ratio. Figure 12a shows the result for a mouse towards a sinusoidal grating stimuli.

It is evident that original basis contain correlation/have features that doesn't contribute to variance in data. We project the original data to a smaller Eigen basis which capture most of the variance. Two studies are possible with this:

1. Recreate the initial data from subset of principal components- Check how many principal components it takes for reasonable reconstruction.
2. Do Further analysis like Orientation selectivity analysis with these data in Eigen space.

### 8.1 Reconstruction

We study the effect of number of principal components on information capacity on Figure 12b. As we increase principal components, the error should decrease. And finally as the number components equal the original feature dimension, reconstruction error should vanish.

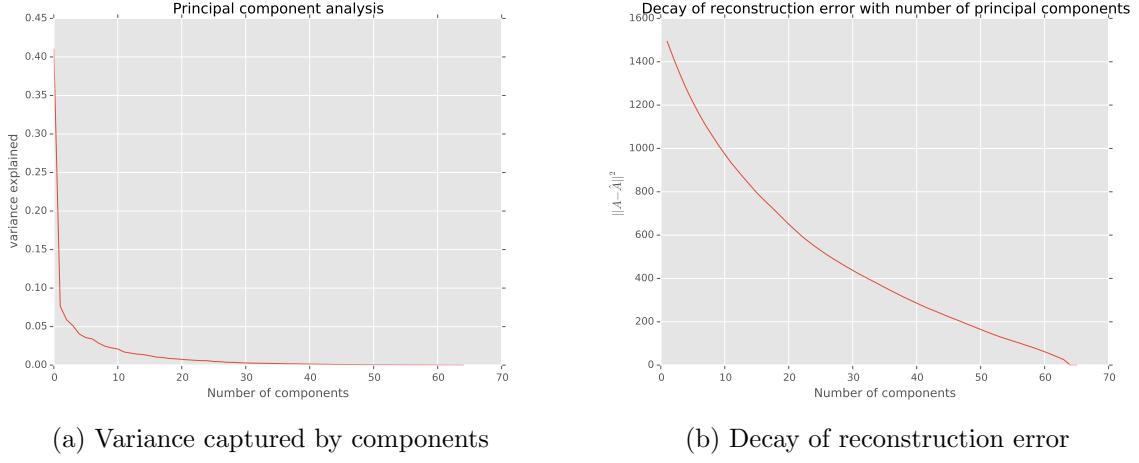


Figure 12: Principal Components Analysis.

## 9 Study of Reliability

Here we analyze how reliable are the time series responses produced by a neuron to various trials of same stimuli. We would like to bring out a metric for quantifying reliability of a neuron. Also we analyze the orientation selectivity properties of these ‘reliable’ neurons.

Plots of responses to various trials of same stimuli are given in Figure . The responses are not similar/ have less correlation. This demands further study to find what is conserved between trials. To quantify a neurons reliability, we use following reliability measure. Response reliability to movie A ( $R_A$ ) is calculated using the following equation:

$$R_A = \frac{2}{T^2 - T} \sum_{i=1}^T \sum_{j=i+1}^T \rho(f_{i,A}, f_{j,A})$$

where  $f_{i,A}$  is the response of neuron to  $i^{th}$  trial of movie A and  $\rho$  is the Pearson correlation.

From figure 14 is seen that the above measure is very low ( $< 0.4$ ) for most of the neurons. Which implies that the time series response is not the invariant structure we are looking for.

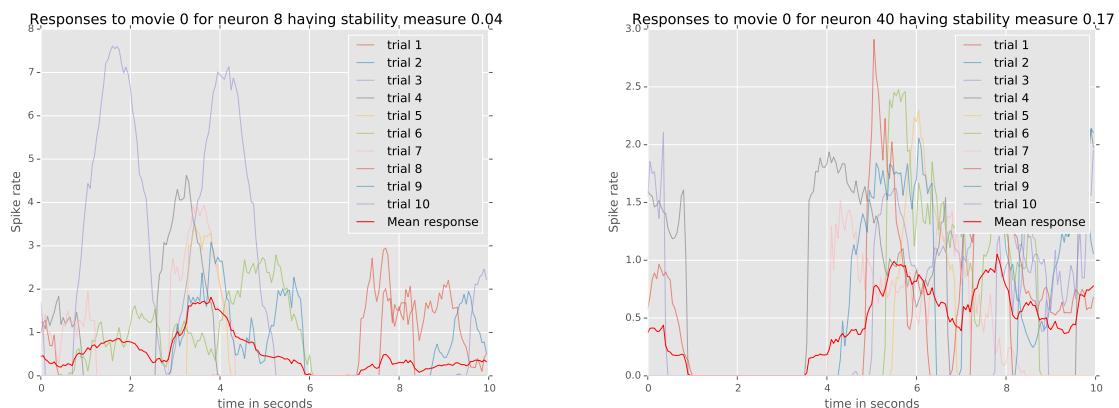


Figure 13: Responses of two neurons to various trials of same stimuli.

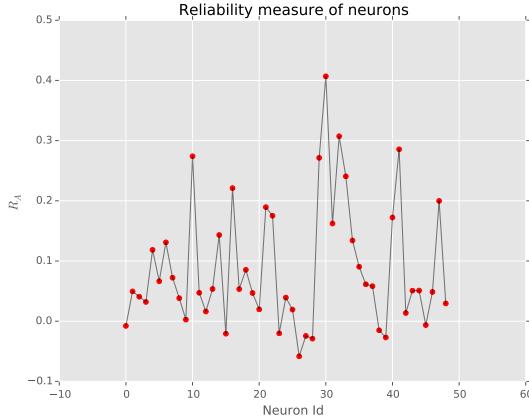


Figure 14: Reliability measure  $R_A$  for different neurons

## 10 Searching for subsequences

If the whole sequences are not reliable, could there be subsequences that are reliable? These motifs can repeat within response of the same neuron, or in a different trial, or in a different neuron. In this section, we search for such segments using ACVF.

For the scope of this section, ‘template’ neuron is the neuron from which we take a subsequence to check for its other occurrences in the response of a ‘target’ neuron. When template and target is same neuron, we study repeated occurrences of a subsequence in a neuron’s response. Here, the search space is exponential in length of response. So before arriving at a method to reduce the search space, we analyze ACF with manually set template subsequence. Trial averaged responses of two neurons- template and target are taken, then a subset of former is extracted by frame ending and frame width. This is compared with target sequence as a function of lag. The results for this study with a subsequence starting from beginning of signal and of various widths are in Figure 15 for same neuron and figure 16 for different neurons.

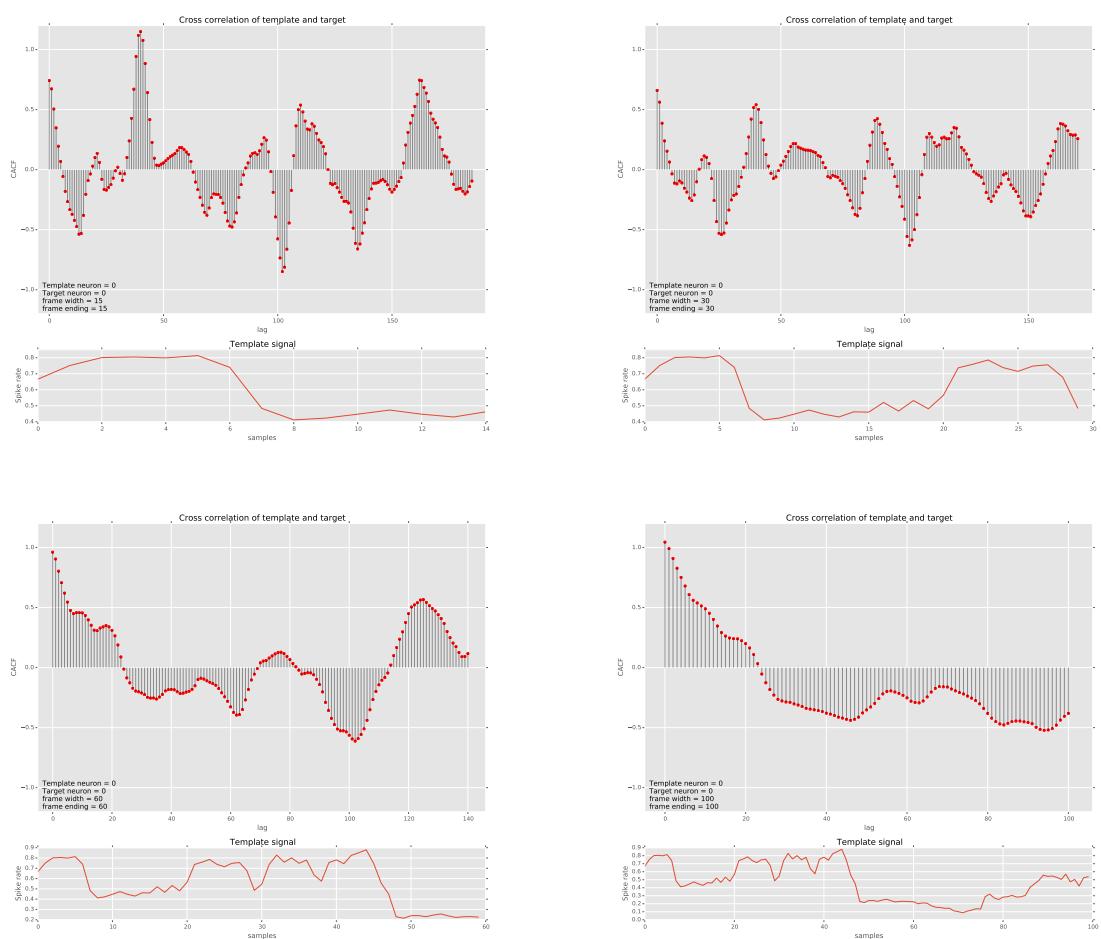


Figure 15: ACF plots for template and target neurons as same.

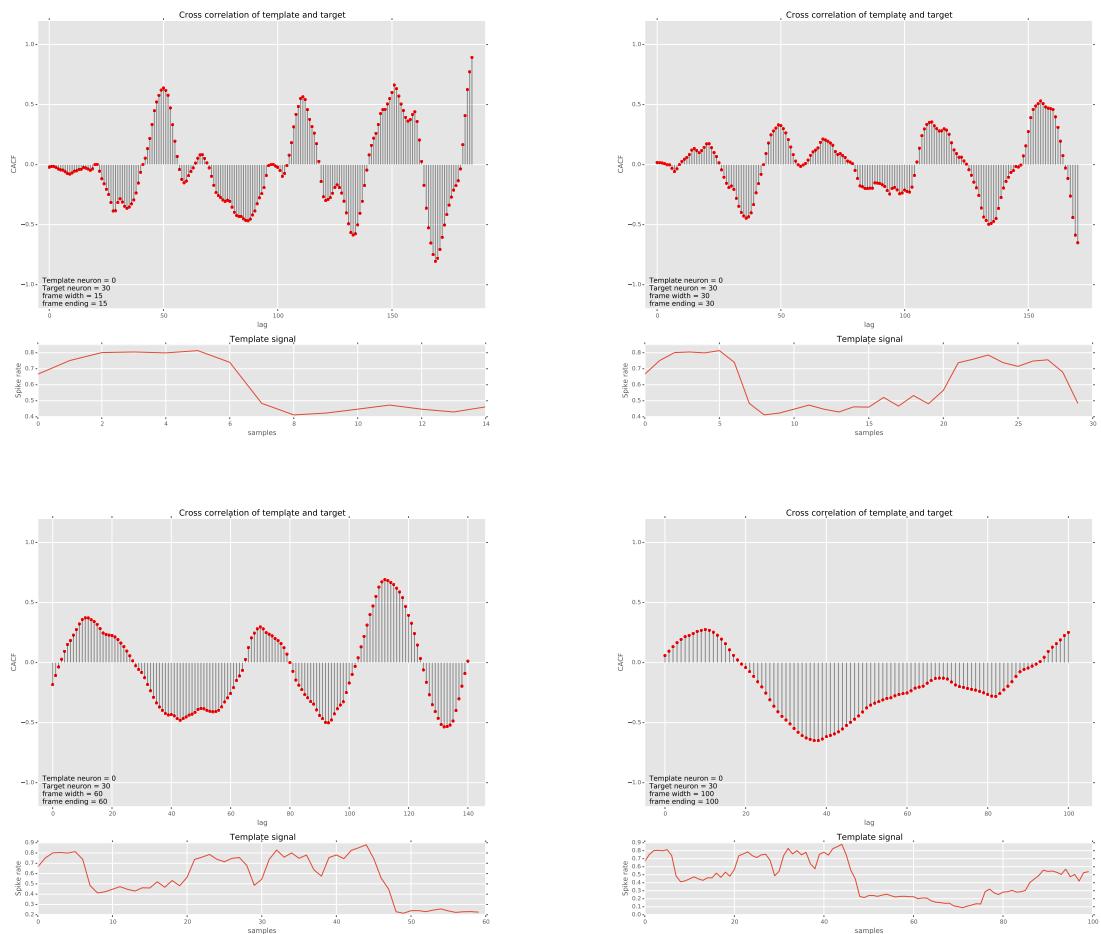


Figure 16: ACF plots for template and target neurons as different .

## 10.1 ACF Gram

In the previous study, we fixed the beginning of the frame in template and changed the width of frame. Here we will slide the frame also to create a temporal dimension too. Heatmaps of the ACF gram - a time vs lag vs ACVF plot are given in figure 17 for same neuron and figure 18 for different neurons.

Images show results for for different manually fixed window widths. The following inferences can be taken:

- For small window length ( $\approx 15$ ), the template is small. Such a small template does not satisfy as a motif. Also as the samples are less, estimate of sample correlation will be poor.
- For window lengths  $\approx 30$  we see patterns. These shows that there are repeating subsequences in the response.
- The repeating patterns seem to be reappearing periodically in lag.
- For window widths  $\approx 60$ , there are two subsequences that match. Above this, Only match is for lag 0.
- There are common subsequences across different neurons.

## 10.2 [\*Future work] Longest common subsequences detection

In previous study, we fixed the window widths and saw that there are in fact repeating patterns. Here we try to find set of common subsequences in neurons. Also, till now we used Pearson correlation to compare two signals. We look into other ways of comparing two responses too.

**RLCS** It is an improved version of the LCS to avoid some problems occurring in global alignment matching.

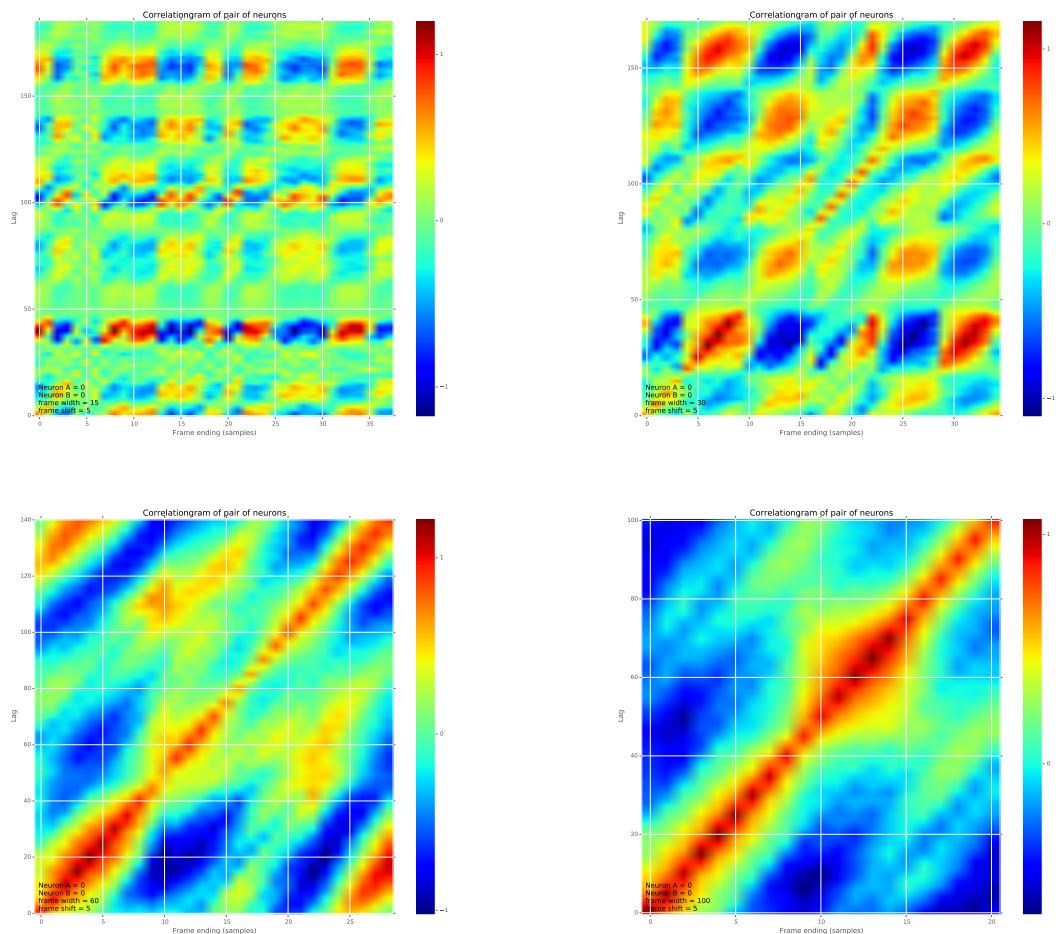


Figure 17: ACFgram for template and target neurons as same.

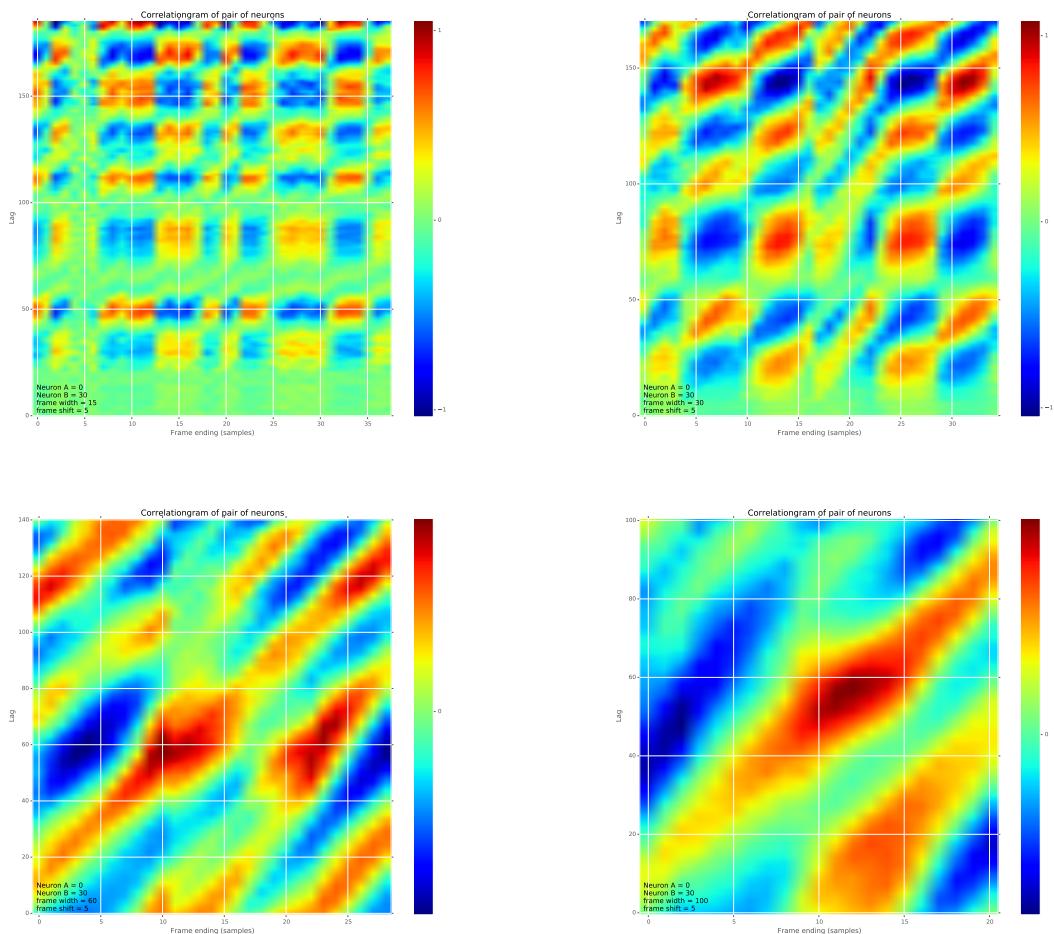


Figure 18: ACFgram for template and target neurons as different .