

Analysis of the Circadian Rhythm in the Brain



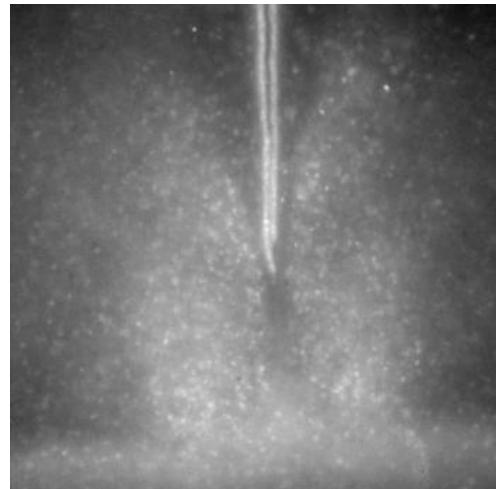
Project for the Quantitative Big Imaging Course – 23rd May 2019
Giovanni Savorana

What & Why?

Suggested project on the course website: <https://www.kaggle.com/kmader/circadian-rhythm-in-the-brain>

What is it?

Transgenic mouse brain where the Circadian clock (biochemical oscillator synchronized with solar time) drives the expression of a fluorescent protein:



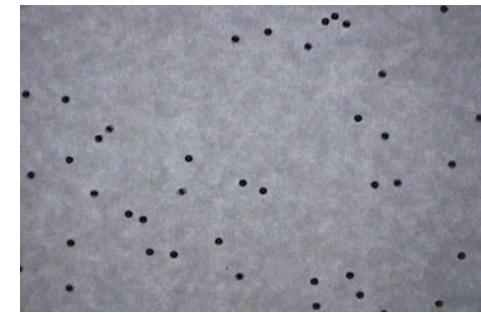
→ Day-Night cycle = Image intensity cycle

Questions:

- Do the cells move during the experiment?
- What is the period of the oscillation?
- Do the cells oscillate in groups?

Why?

Training! Physics:



Biophysics

Methods:

- Tracking
- Analysis of the Intensity over time

Do the Neurons Move?

Tracking the neurons

Implementation of the following steps:

- Feature finding
- Trajectory linking

Tracking the Neurons

Tracking the neurons

Implementation of the following steps:

- Feature finding
- Trajectory linking

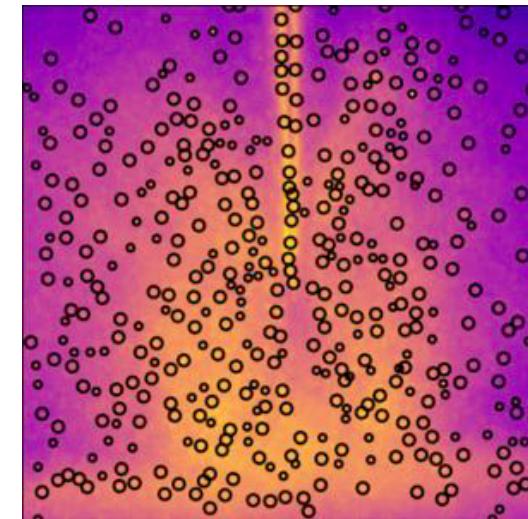
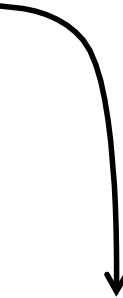


Image enhancement + detection algorithm: `skimage.feature.blob_dog()`

Parameters:

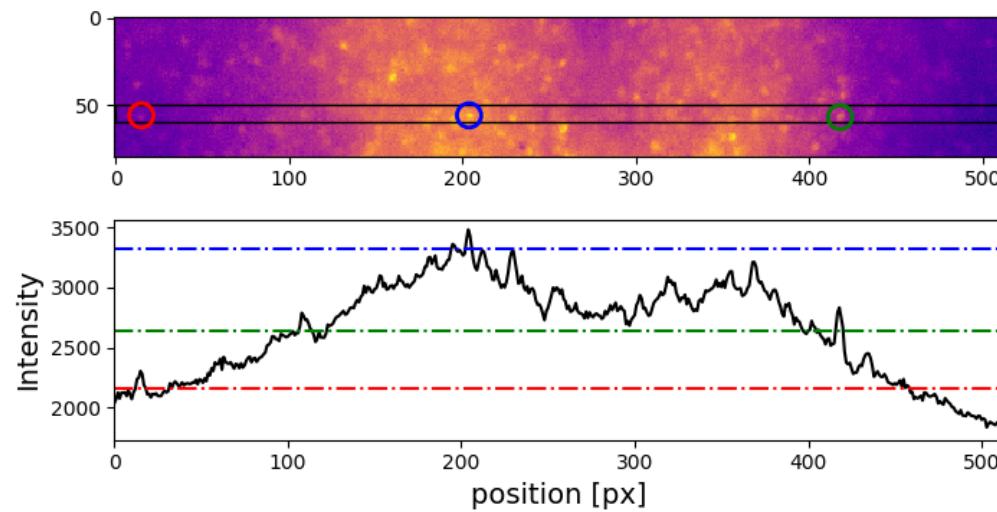
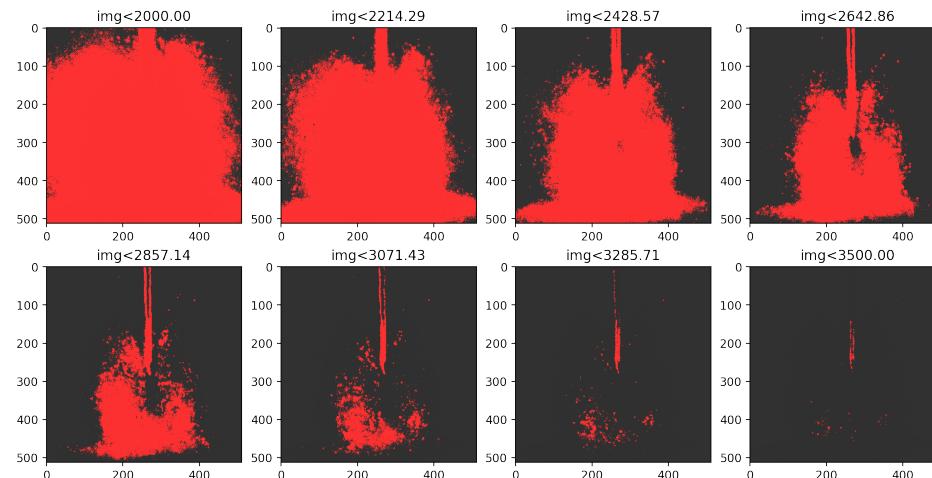
- `minsigma=2.5 [px]` (lower limit for sigma of gaussian kernel) (cell size≈3px)
- `maxsigma=5 [px]` (upper limit for sigma of gaussian kernel) (cell size≈5px)
- `threshold=2` (minimum size of feature)

Neurons Segmentation

I tried to 'help' feature finding by trying to segment cells.

How? Global threshold

Different threshold required!



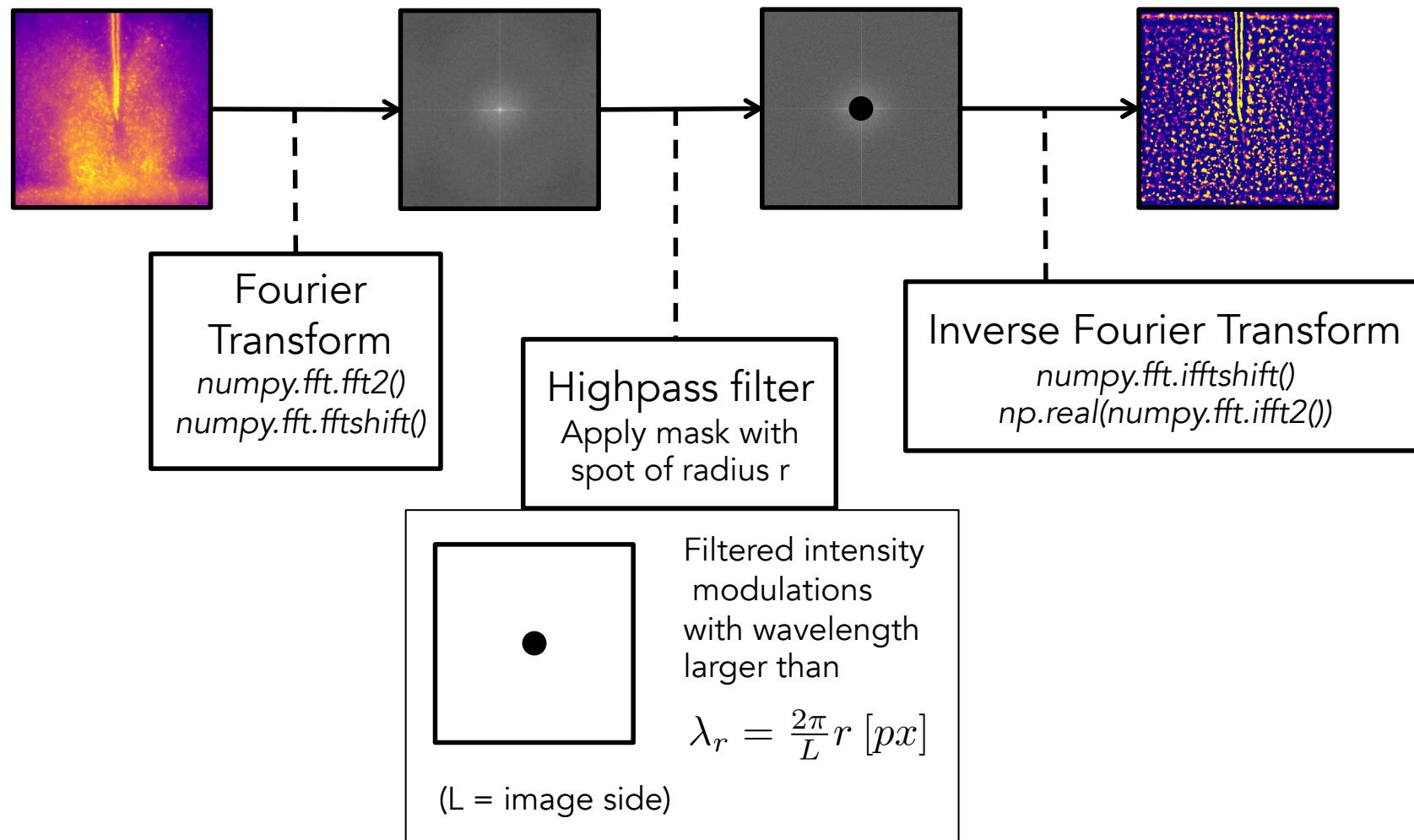
Neurons Segmentation

Problems with neuron segmentation:

- Spatially inhomogeneous intensity
- Intensity of each neuron is varying in time
- low signal-noise ratio

Neurons Segmentation

Highpass filter

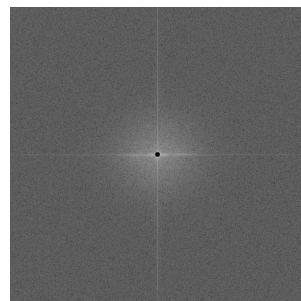


Neurons Segmentation

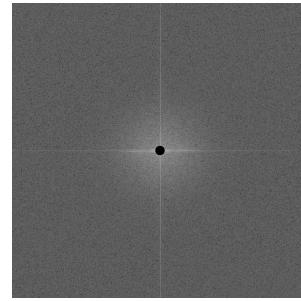
Highpass filter

Highpass Filter for removing modulations on large length-scales

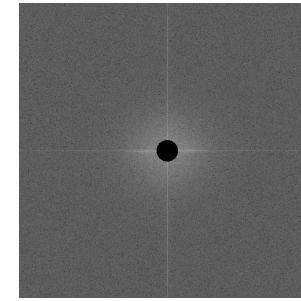
$$\lambda = 64 \text{ [px]}$$



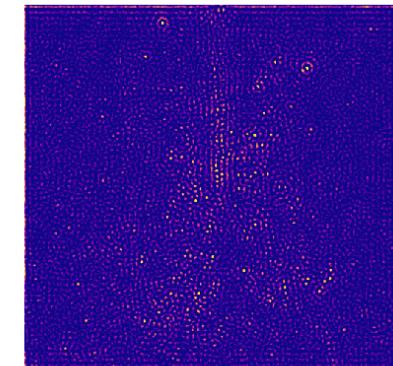
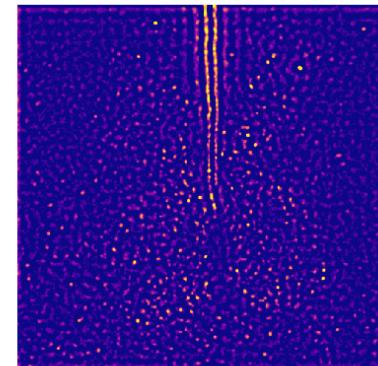
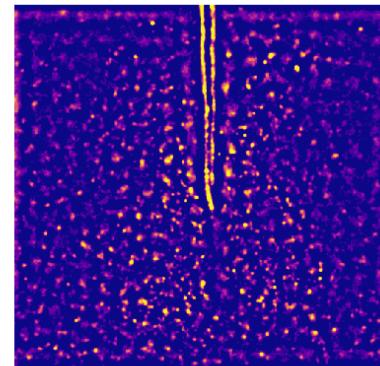
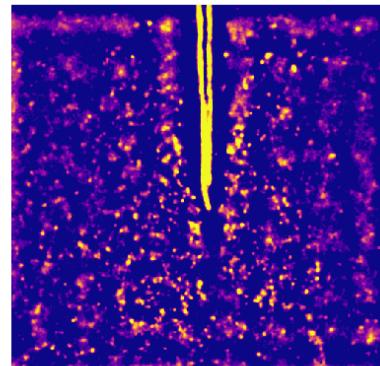
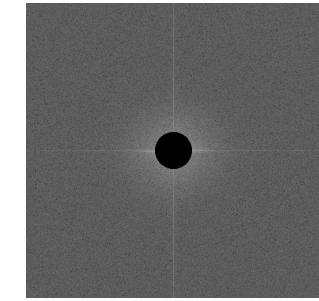
$$\lambda = 32 \text{ [px]}$$



$$\lambda = 16 \text{ [px]}$$



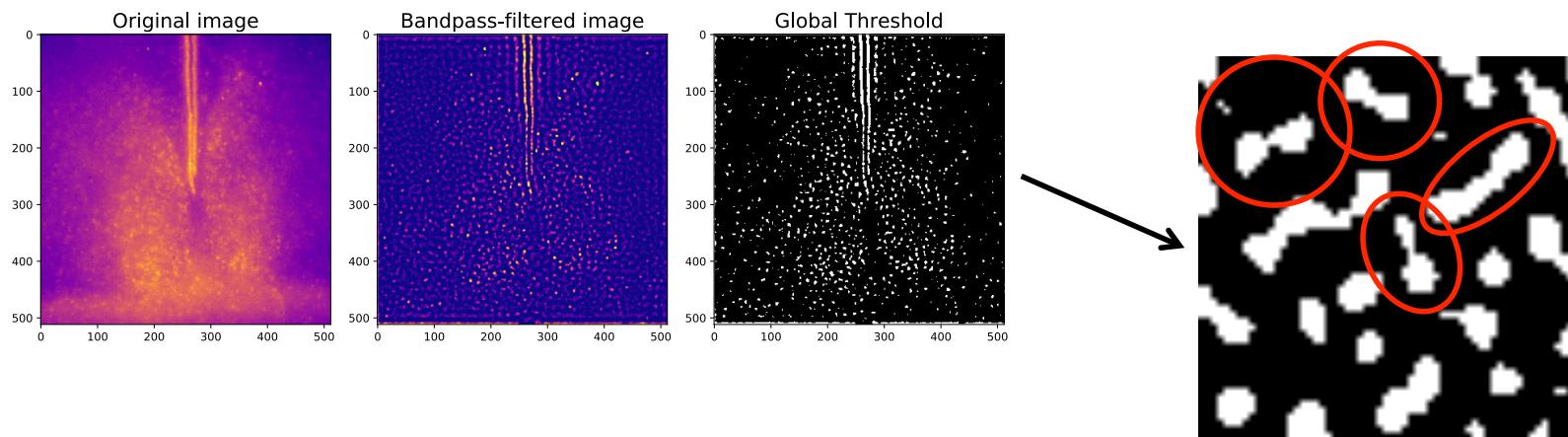
$$\lambda = 8 \text{ [px]}$$



Still large modulations

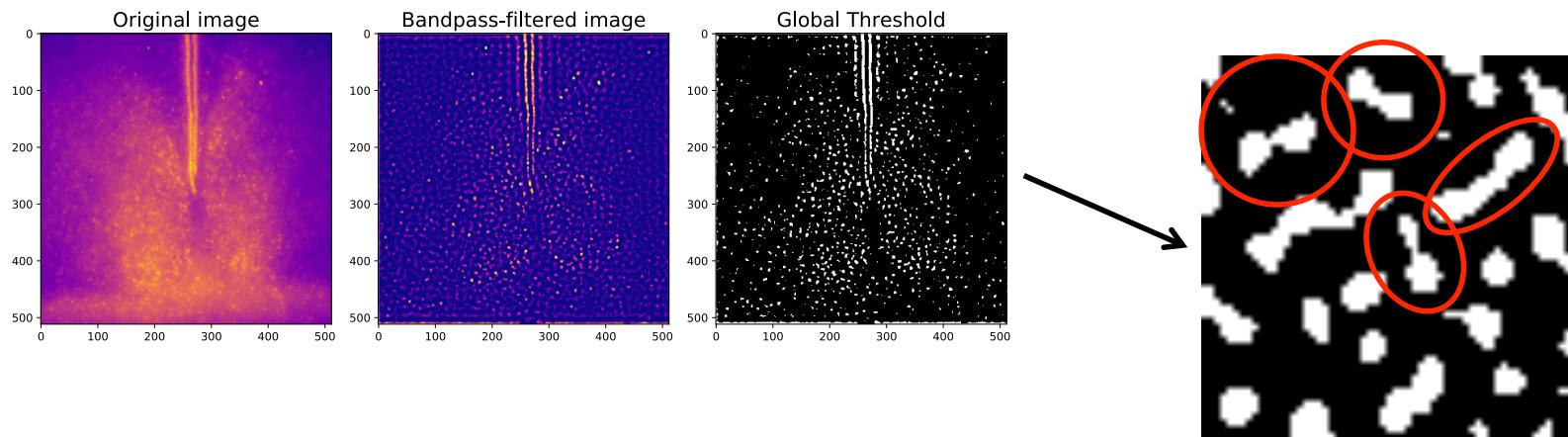
Also spurious
"Edges" enhanced

Neurons Segmentation Highpass filter



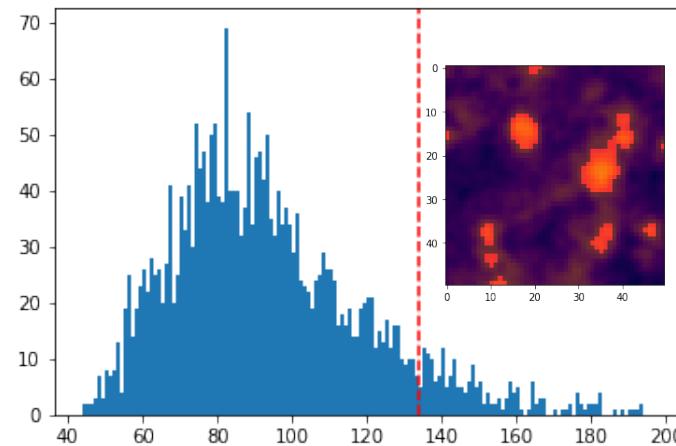
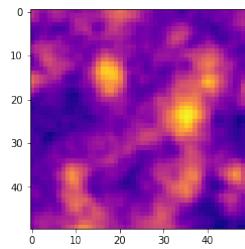
Neurons Segmentation

Local intensity threshold

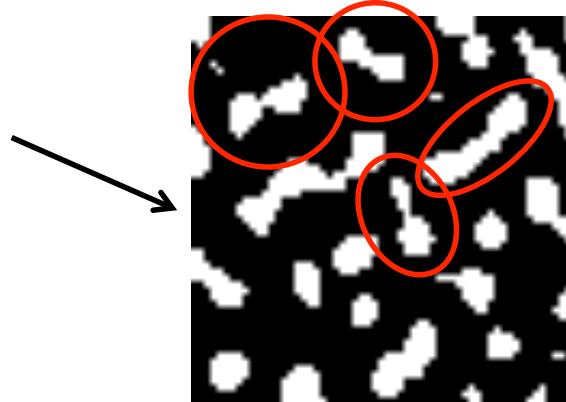
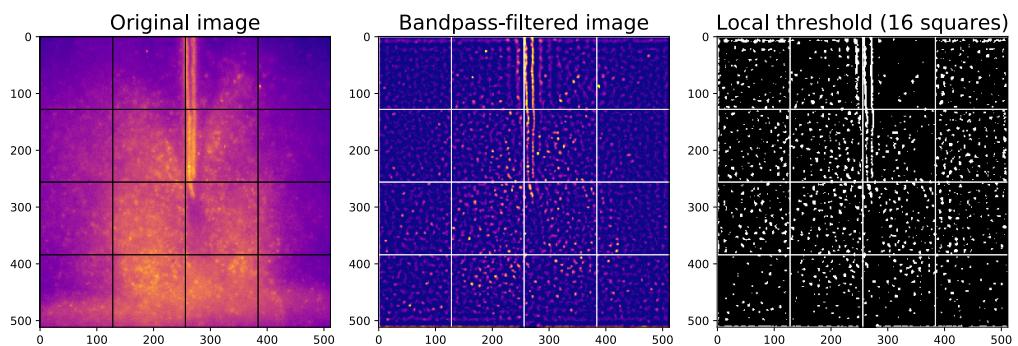
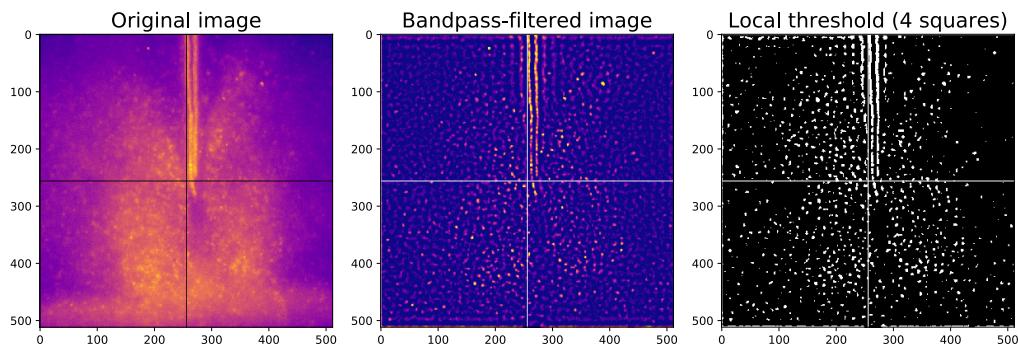
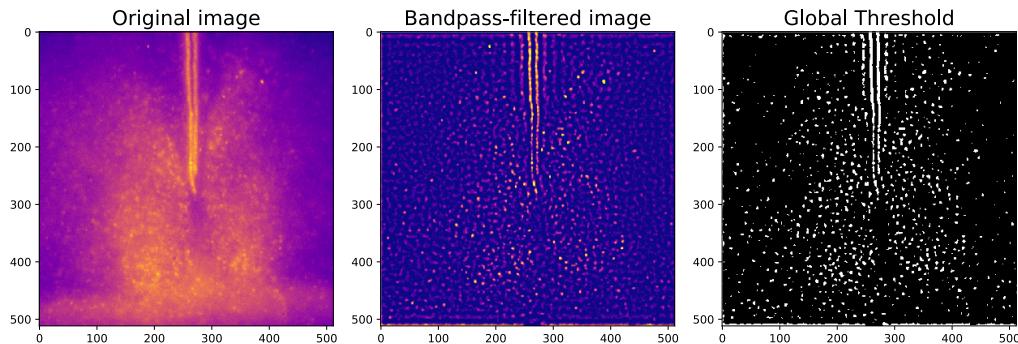


Local threshold:

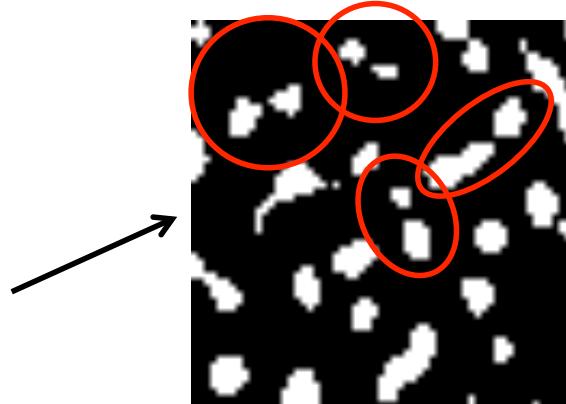
Loop on subimages, calculate the intensity distribution and set threshold 2σ



Neurons Segmentation Local intensity threshold

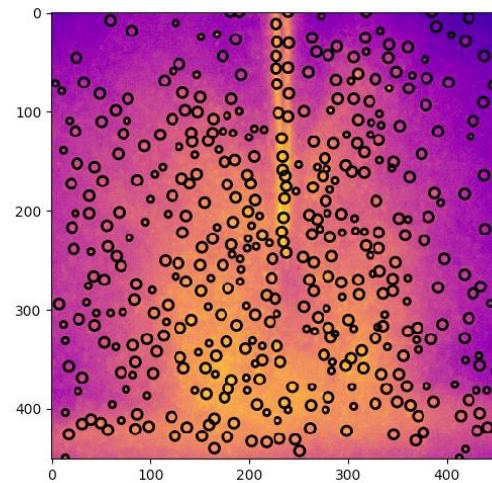


Threshold on smaller squares:
Gaps between bright cells
resolved better

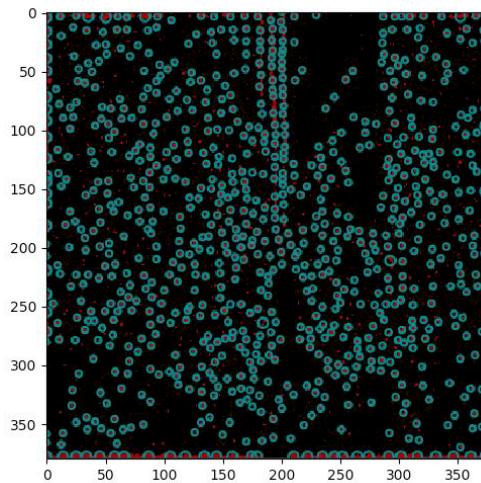


Neurons Segmentation

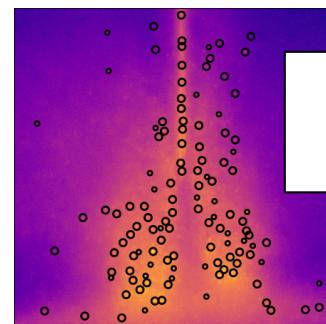
Feature finding
On the original data



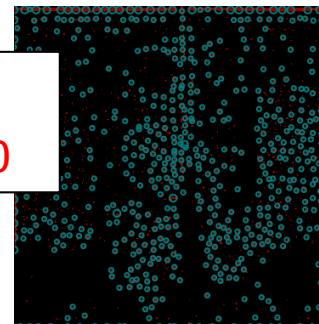
Feature finding after
Filtering + Local Threshold



Many blobs lost
when the intensity
becomes less
homogeneous



Frame
50/1600



Blobs
still recognized!

Tracking the Neurons

Tracking the neurons

Implementation of the following steps:

- Feature finding
- Trajectory linking

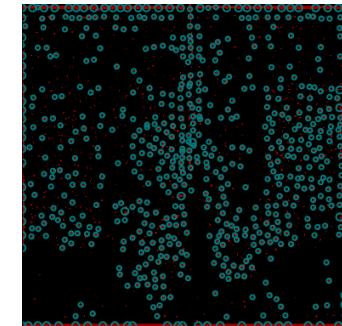
Tracking the Neurons

Trajectory Linking

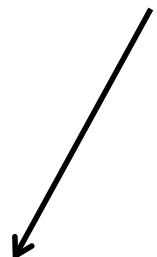
`Skimage.feature.blob_dog()` returns an array storing, for each blob,
- x,y coordinates
- radius
- frame

}

We can store them in a pandas DataFrame labeling the blobs



	x	y	r	frame	blob_label
0	511.0	503.0	5.656854	0	0
1	511.0	486.0	5.656854	0	1
2	511.0	466.0	5.656854	0	2
3	511.0	446.0	5.656854	0	3
4	511.0	425.0	5.656854	0	4



Tracking the Neurons

Trajectory Linking

Look at all possible blob pairs in adjacent frames (n and $n+1$)
and find the nearest neighbour (if 2 blobs in frame n share the same nearest neighbour
in frame $n+1$, keep the pair with smaller distance)

	x	y	r	frame	blob_label	filtered_x_next	filtered_y_next	filtered_r_next	filtered_blob_label_next	filtered_distance
0	511.0	503.0	5.656854	0	0	511.0	503.0	5.656854	850.0	0.0
1	511.0	486.0	5.656854	0	1	511.0	486.0	5.656854	851.0	0.0
2	511.0	466.0	5.656854	0	2	511.0	466.0	5.656854	852.0	0.0
3	511.0	446.0	5.656854	0	3	511.0	445.0	5.656854	853.0	1.0
4	511.0	425.0	5.656854	0	4	511.0	427.0	5.656854	854.0	2.0

We can filter the matches by imposing
a maximum displacement of a blob between two subsequent frames
(Based on data visualization, a maximum displacement of 3 px per frame was chosen)

Tracking the Neurons

Trajectory Linking

	x	y	r	frame	blob_label	filtered_x_next	filtered_y_next	filtered_r_next	filtered_blob_label_next	filtered_distance
0	511.0	503.0	5.656854	0	0	511.0	503.0	5.656854	850.0	0.0
1	511.0	486.0	5.656854	0	1	511.0	486.0	5.656854	851.0	0.0
2	511.0	466.0	5.656854	0	2	511.0	466.0	5.656854	852.0	0.0
3	511.0	446.0	5.656854	0	3	511.0	445.0	5.656854	853.0	1.0
850	511.0	503.0	5.656854	1	850	511.0	504.0	5.656854	1694.0	1.0
851	511.0	486.0	5.656854	1	851	511.0	486.0	5.656854	1695.0	0.0
852	511.0	466.0	5.656854	1	852	511.0	465.0	5.656854	1696.0	1.0
853	511.0	445.0	5.656854	1	853	511.0	444.0	5.656854	1697.0	1.0

Tracking the Neurons

Trajectory Linking

	x	y	r	frame	blob_label	filtered_x_next	filtered_y_next	filtered_r_next	filtered_blob_label_next	filtered_distance
0	511.0	503.0	5.656854	0	0	511.0	503.0	5.656854	850.0	0.0
1	511.0	486.0	5.656854	0	1	511.0	486.0	5.656854	851.0	0.0
2	511.0	466.0	5.656854	0	2	511.0	466.0	5.656854	852.0	0.0
3	511.0	446.0	5.656854	0	3	511.0	445.0	5.656854	853.0	1.0
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Tracking the Neurons

Trajectory Linking

	x	y	r	frame	blob_label	filtered_x_next	filtered_y_next	filtered_r_next	filtered_blob_label_next	filtered_distance
0	511.0	503.0	5.656854	0	0	511.0	503.0	5.656854	850.0	0.0
1	511.0	486.0	5.656854	0	1	511.0	486.0	5.656854	851.0	0.0
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3	511.0	446.0	5.656854	0	3	511.0	445.0	5.656854	853.0	1.0
850	511.0	503.0	5.656854	1	0	511.0	504.0	5.656854	1694.0	1.0
851	511.0	486.0	5.656854	1	851	511.0	486.0	5.656854	1695.0	0.0
852	511.0	466.0	5.656854	1	852	511.0	465.0	5.656854	1696.0	1.0
853	511.0	445.0	5.656854	1	853	511.0	444.0	5.656854	1697.0	1.0

Tracking the Neurons Trajectory Linking

	x	y	r	frame	blob_label	filtered_x_next	filtered_y_next	filtered_r_next	filtered_blob_label_next	filtered_distance
0	511.0	503.0	5.656854	0	0	511.0	503.0	5.656854	850.0	0.0
1	511.0	486.0	5.656854	0	1	511.0	486.0	5.656854	851.0	0.0
2	511.0	466.0	5.656854	0	2	511.0	466.0	5.656854	852.0	0.0
3	511.0	446.0	5.656854	0	3	511.0	445.0	5.656854	853.0	1.0
850	511.0	503.0	5.656854	1	0	511.0	504.0	5.656854	1694.0	1.0
851	511.0	486.0	5.656854	1	851	511.0	486.0	5.656854	1695.0	0.0
852	511.0	466.0	5.656854	1	852	511.0	465.0	5.656854	1696.0	1.0
853	511.0	445.0	5.656854	1	853	511.0	444.0	5.656854	1697.0	1.0

And so on...

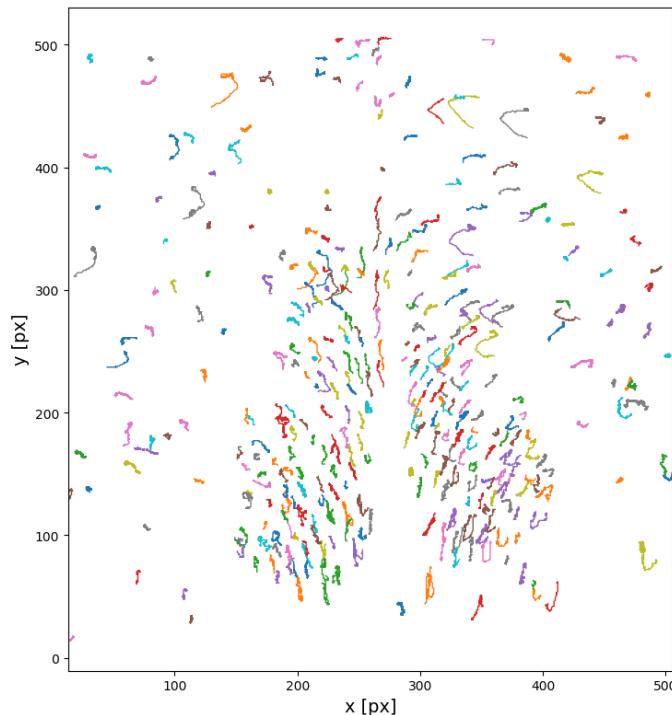
Tracking the Neurons

Trajectory Linking

In this way we obtain cell trajectories

A threshold on the minimum number of frames is needed to reject 'false' trajectories:

```
df.groupby('blob_label').filter(lambda x : x['blob_label'].count()>min_traj)
```

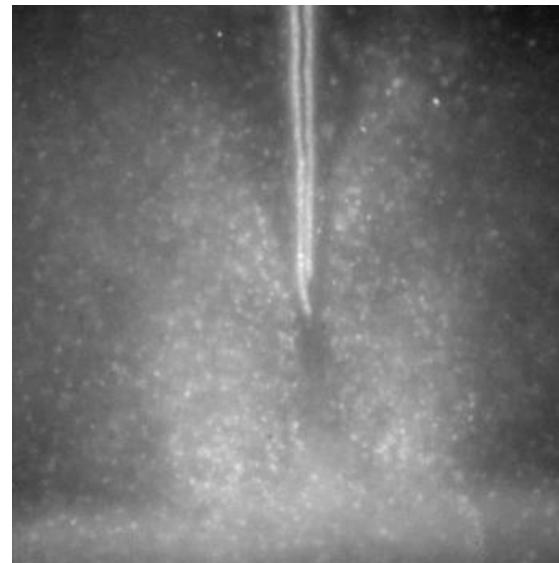
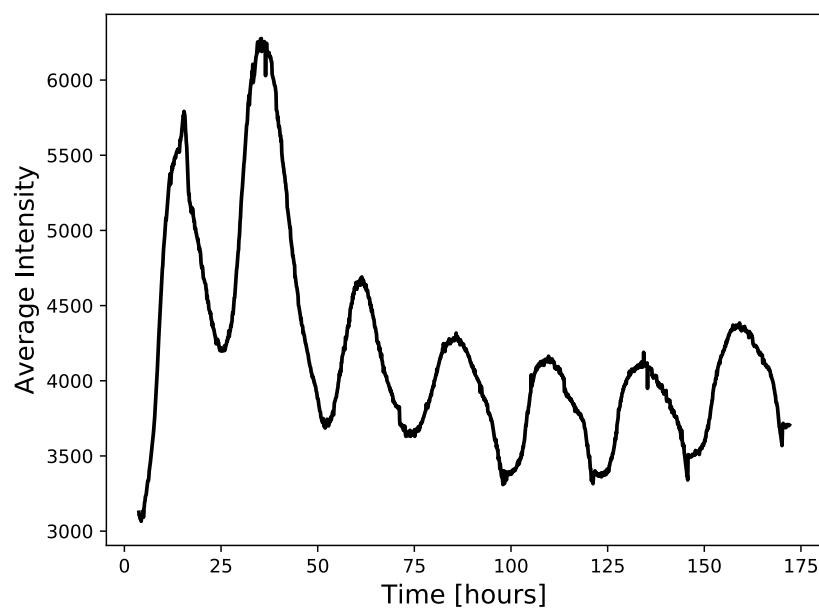


→ Trajectories:
Neurons move!

Figure:
Trajectories more than 50 frames long and starting in the first 150

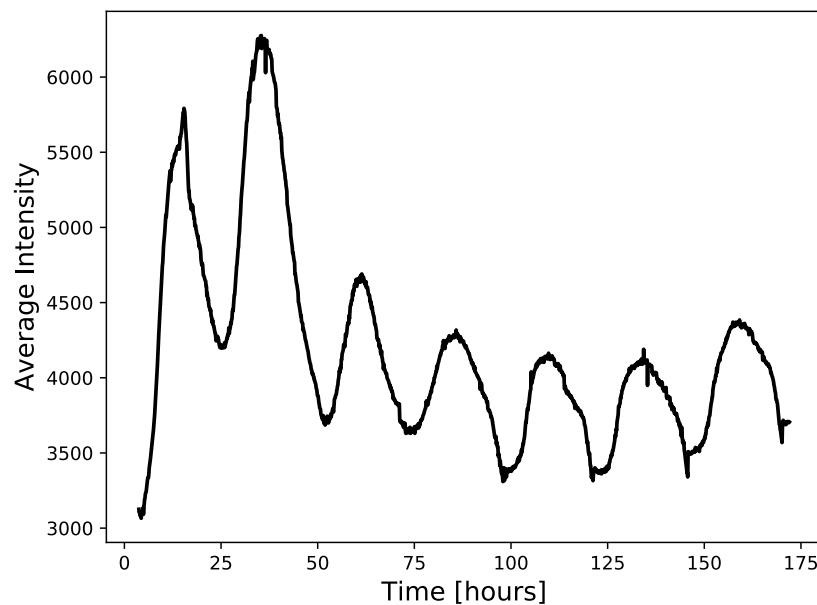
What is the Period of the Oscillations?

Average Intensity – Whole Image

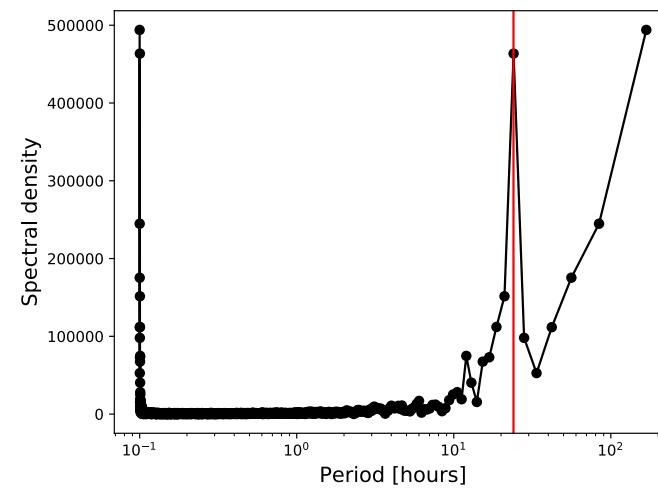


What is the Period of the Oscillations?

Average Intensity – Whole Image



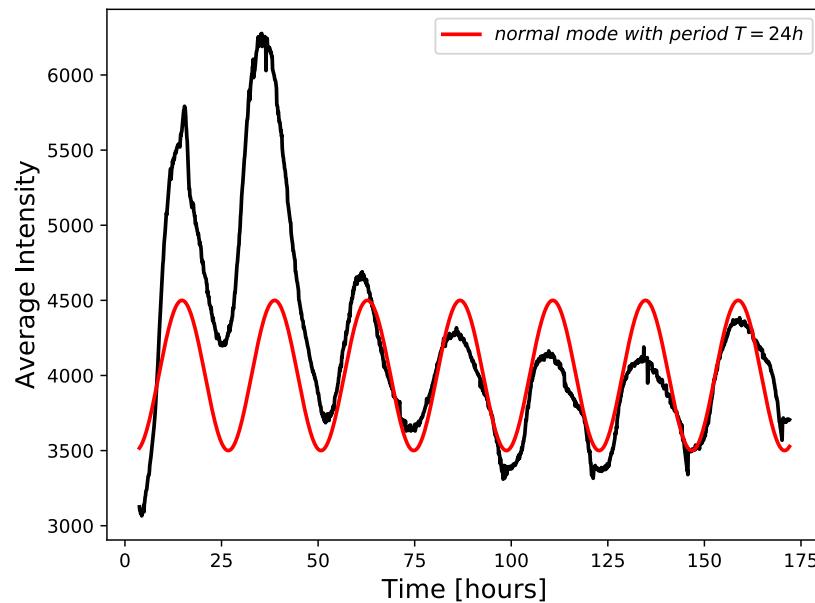
Peak at $T = 24$ h



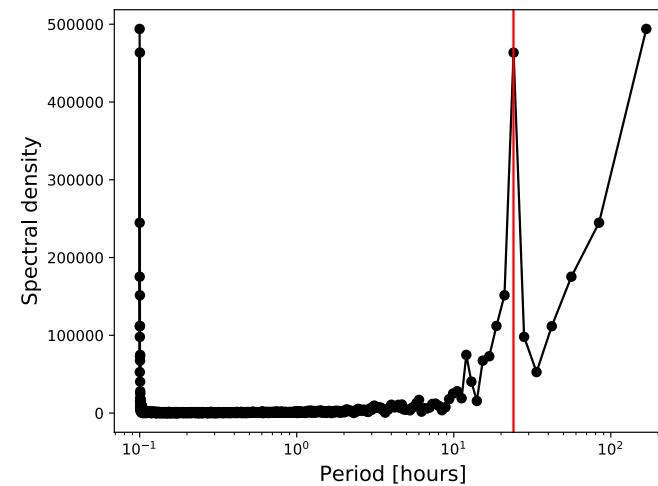
Fourier
Transform
`numpy.fft.fft()`

What is the Period of the Oscillations?

Average Intensity – Whole Image



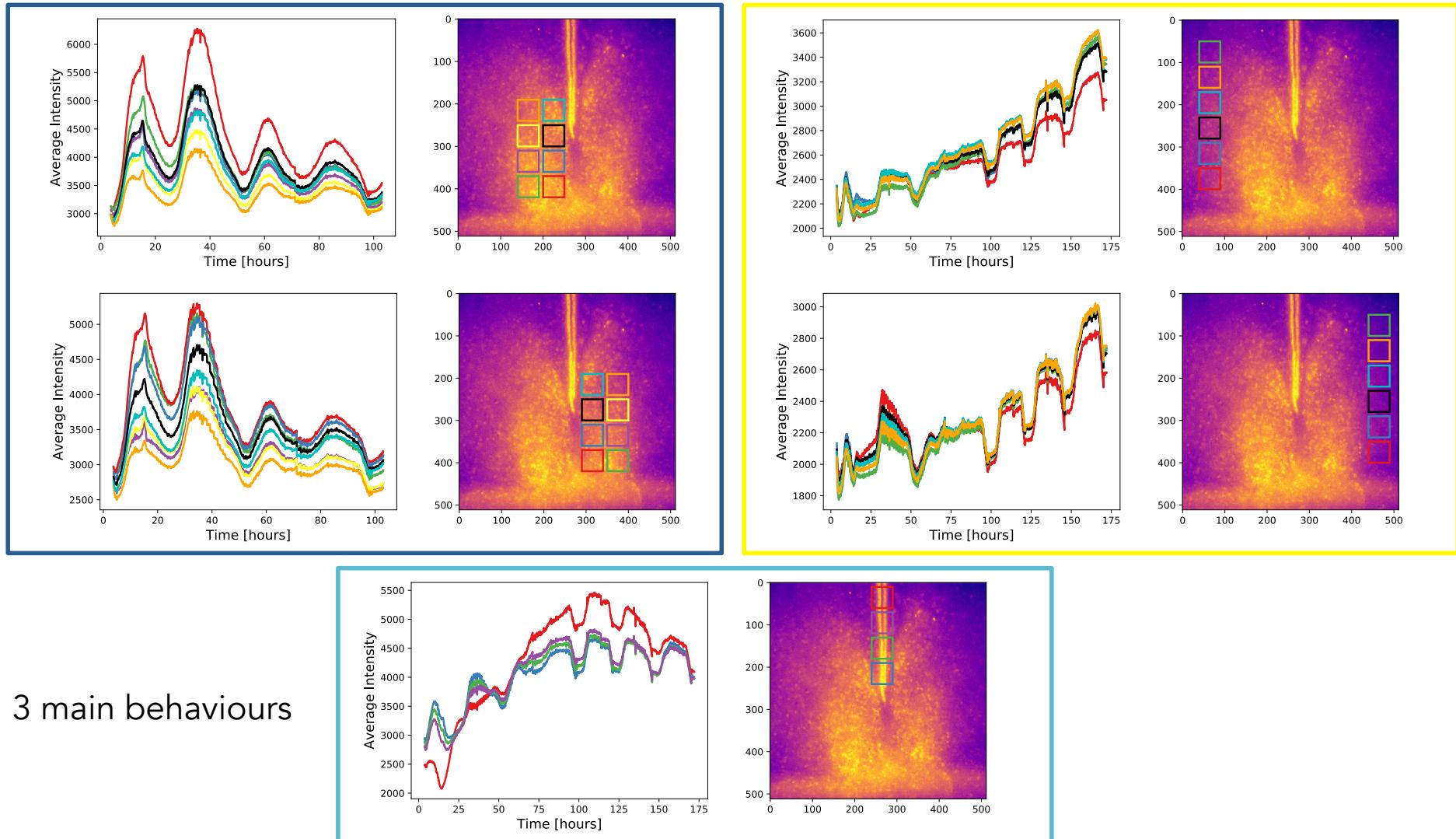
Peak at $T = 24\text{ h}$



Fourier
Transform
`numpy.fft.fft()`

Higher frame rate and longer sampling time would allow a more precise estimate

Do the cells oscillate in groups?



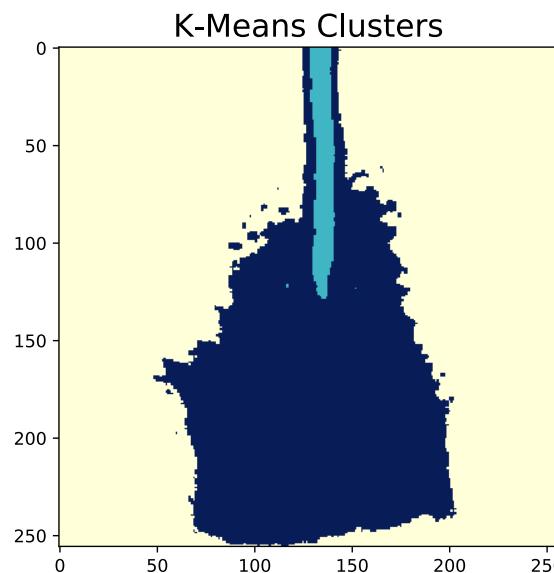
Do the cells oscillate in groups?

Can we precisely identify the boundaries of these groups ?

Yes!

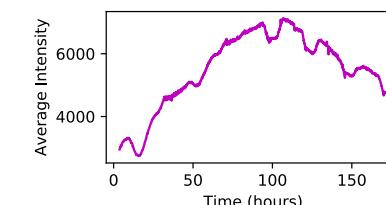
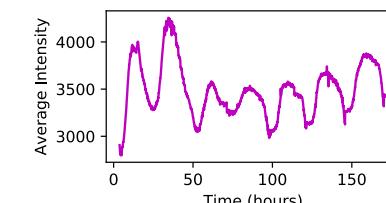
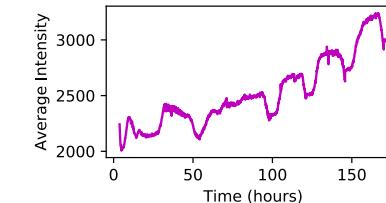
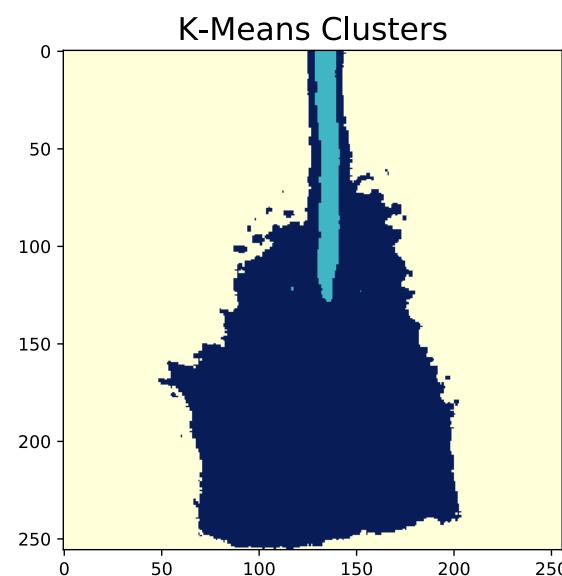
K-Means clustering
on the temporal evolution of
pixels
=
Group pixels oscillating
“in a similar way”

Qualitatively we see 3 main behaviours:
I tried with 3 clusters



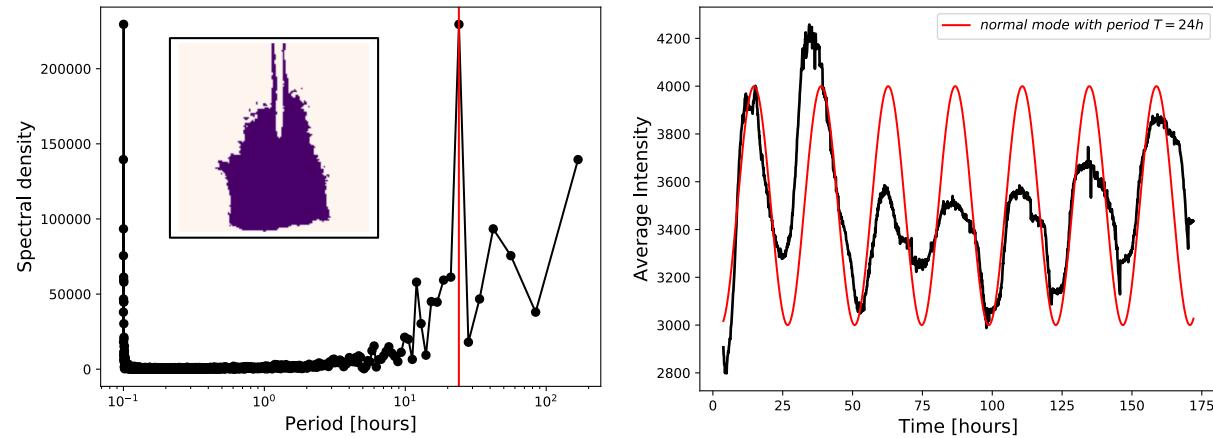
Do the cells oscillate in groups?

Intensity oscillations inside clusters:
The 3 main behaviours are well captured by the k-means clusters

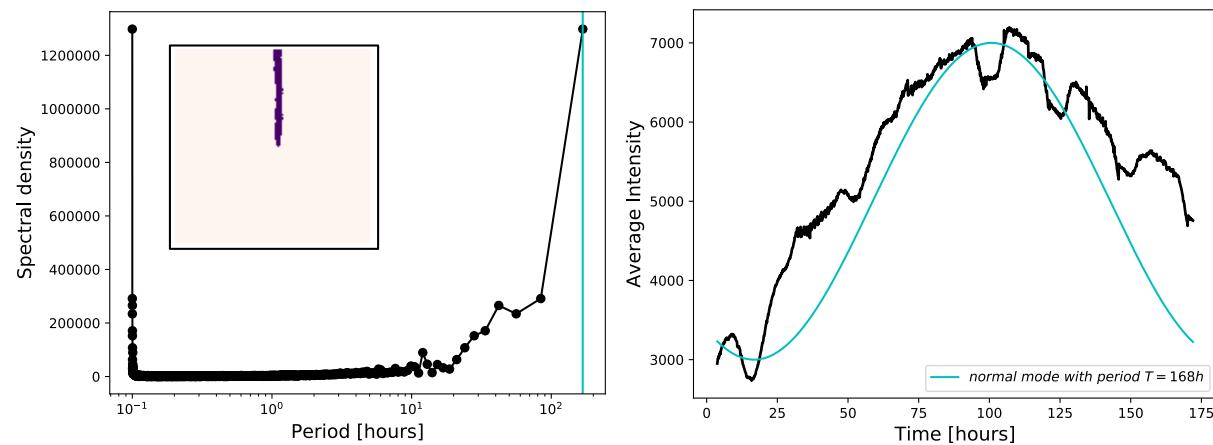


Do the cells oscillate in groups?

Main mode:
24 h period



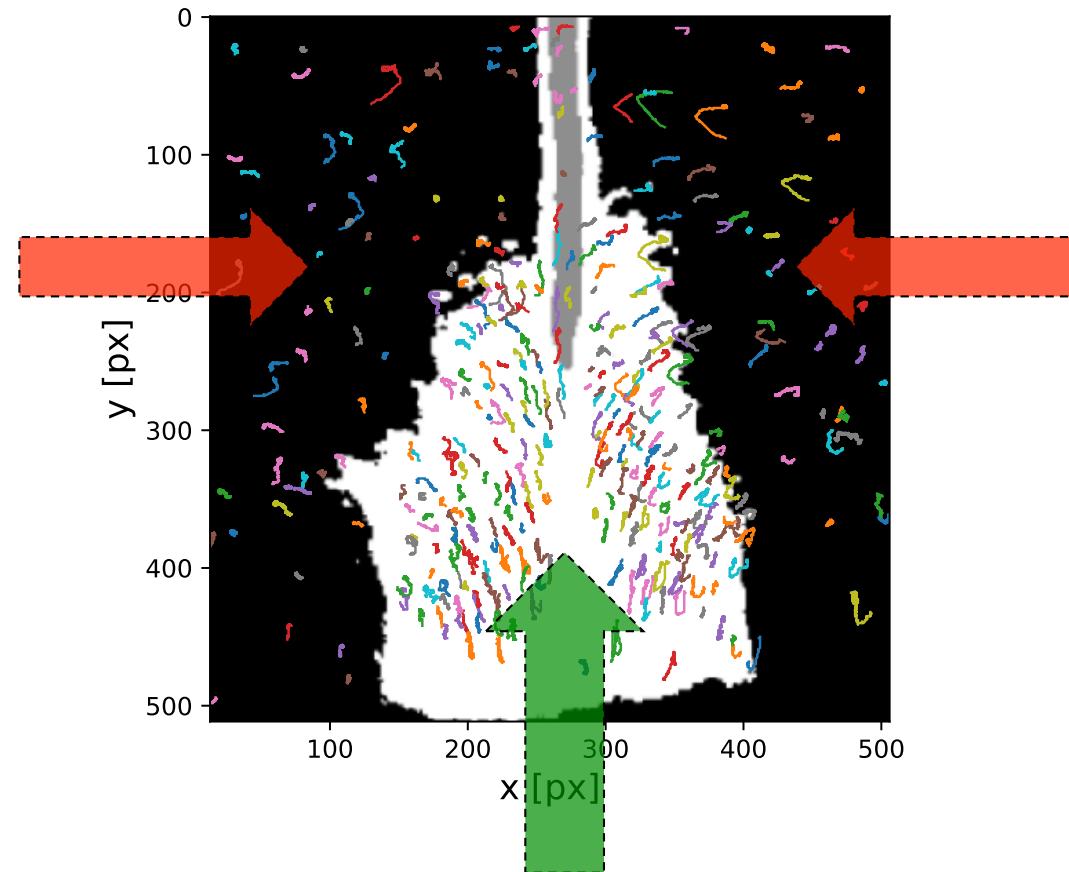
Main mode
168 h period
(at the limit of the sampling time)



The third cluster has a period much higher than the sampling time

Motion/Intensity Correlation?

Is there a correlation between motion and intensity cluster?
K-means on trajectories (clusters based on MSD)

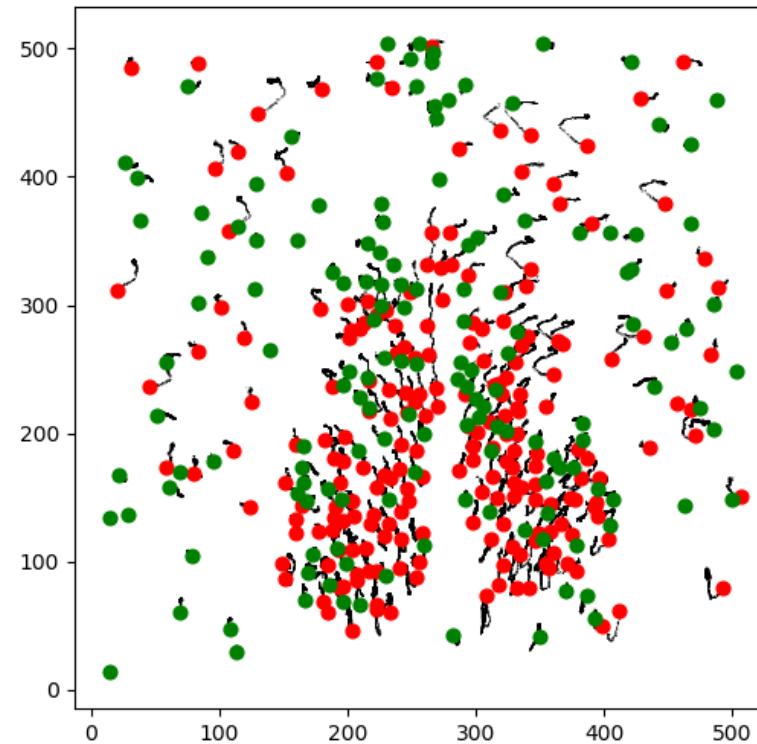


Motion/Intensity Correlation?

Is there a correlation between motion and intensity cluster?
K-means on trajectories (clusters based on MSD)

2 Clusters

Also tried with
 Δx and Δy



None of these seems to be a good quantity
for capturing the observed different behaviour

Summing Up:

Questions:

- Do the cells move during the experiment? → Approach: Tracking
- What is the period of the oscillation? → Approach: Fourier Transform
- Do the cells oscillate in groups? → Approach: K-Means clustering