

The thing with the Golgi apparatus

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Abstract

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Data processing pipeline

In this chapter I present the work done on processing the rush movies. Several preprocessing steps have been undertaken to improve the quality of the fit, and we present all here. Roughly, we can divide the process in four steps:

1. Segmentation and creation of masks
2. Denoising of movies
3. Calculation of spatial and temporal derivatives
4. The actual fitting

Below we describe each step separately.

3.1 STEP 1: SEGMENTATION

The images obtained from the rush experiments often contain multiple cells. Furthermore, we can also segment the image into roughly three different types: 1) the background, where nothing of interest happens. No cells are present here, 2) the cytoplasm, which is the area where we want to fit our model and 3) the Golgi itself, where we do not necessarily want to fit. Unfortunately, no bright field images were available, making segmentation significantly harder, as no clear cell boundary can be observed. Further complicating the story is the large dynamic range of the movies due to the fluorescence concentrating in the Golgi. The following procedures we present have been developed to deal with these problems. Note that they are empirical methods, i.e. there's no theoretical background as to why they *should* work. However, in practice they do and I haven't found any other method which was able to.

3.1.1 VORONOI DIAGRAM

This method is based on a technique called Voronoi tessellation and doesn't depend on any measure of the intensity. It was developed after noting that since the cargo is spread throughout the ER in the first few frames and as the ER is roughly circumnuclear, we can use this to determine the centre of the cell (roughly). Voronoi tessellation then allows us to divide the frame into areas with just one point per area, i.e. one cell per area (theoretically). More precise, given n coordinates, voronoi tessellation divides the given area into n pieces, where every point in a piece is closest to one coordinate. In practice this means for us that each point in a cell area is closest to its the given cell centre. Figure **ref** shows this. Each calculated cell centre is a red point and the lines depict the borders between each voronoi cell. Assuming the cells don't move too much, they don't cross the cells and thus we apply the voronoi diagram calculated in the first few frames to the entire movie.

3.1.2 INTENSITY

For the fitting however we wish to make a slightly better approach than a voronoi diagram. As stated, we can't find the exact delineation of the cell, but looking at the intensity, we can see an 'area' of interest, separating background from the cell. Since the Golgi is quite bright in the last 200 or so frames, we consider only the intensity for the Golgi, while for the cytoplasm we consider both the intensity and its time derivative. Thus we have two analog but different processes. For the Golgi we do the following:

1. Renormalize the concentration C between 0 and 1.
2. Sum all frames. One then obtains an image such as figure **ref**

$$\sum_{frames} C(x, y, t)$$

3. This image is thresholded, either through an otsu threshold or a manual one, until the mask roughly matches what we want. Note that extreme precision isn't required, since we just want the rough area. This results in figure **ref**

For the cytoplasm we follow the same procedure only now we take the log of sum of the product of the intensity and its time derivative:

$$\log \left(\sum_{frames} C(x, y, t) \cdot \partial_t C(x, y, t) \right)$$

We thus obtain a complete mask for the movie as shown in figure **ref**

3.2 STEP 2 - DENOISING

In order to accurately calculate the derivatives and generally improve the quality of fitting, we wish to denoise and smooth the obtained movies. Denoising and

smoothing is a subject about which many books have been written and there are hundreds of approaches. One oft-used technique is to Fourier transform the signal, cutoff all coefficients above a cutoff frequency and retransform back into the real domain. Next, a Savitzky-Golay filter can be used to finally smooth the result. However, a big issue with all these methods is their non locality. Since our movies have different scales, this is a big problem. Furthermore, they often smooth out sharp peaks. After evaluating several methods, I have settled on a relatively new method presented in **ref**.

The so-called WavinPOD method combines two well-known filtering techniques, known as wavelet filtering and Proper Orthogonal Decomposition. Below we explain each separately. Our explanation is adapted from **ref** and **ref**.

WAVELET FILTER

A wavelet filter is not really the appropriate name, as its more of a transform.

PROPER ORTHOGONAL DECOMPOSITION

Proper orthogonal decomposition is a technique similar to what is known as Principal component Analysis in statistics and falls into the general category of model reduction techniques. It's often used in flow problems to extract coherent structures from turbulent flows. Simply put, in POD we wish to express a function as

WAVINPOD

WavinPOD combines these two techniques in the following way. First, we decompose our problem with a POD transformation. This yields a set of temporal and spatial modes. We select the most energetic modes and wavelet filter these, before transforming them back to the real domain. As shown in **ref**, combining these techniques has an advantage over others.

In our case, we select the number of modes to be used by hand (30 in the case of MANII) and apply a 3-level db4 wavelet. We use a slightly higher than necessary level to increase smoothness. In the figure below we show the result for both a pixel in time and one time snapshot. Note that the result is significantly smoother, but that smaller details have been preserved.

3.3 STEP 3 - DERIVATIVES

Taking spatial and temporal derivatives of these images is not an entirely trivial operation due to the discreteness of the system. More specifically, taking numerical derivatives of data is extremely hard to do properly and becomes even harder in the presence of noise. Next to basic finite difference methods, one can for example use a linear-least-squares fitted polynomial, smoothing spline or a so-called tikhonov-regularizer **ref needed**. Each method comes with its strengths and weaknesses, but one particularly nasty thing for our context is that they don't scale well to higher dimensions and quickly become computationally expensive.

Another issue related to discretization is the size of the grid w.r.t. the size of the features. To see this, we plot a 2D-gaussian with $\sigma = 1$ in figure **ref**.

As expected, the derivative is normal to the isolines of the object. Now consider the discretized version of the object. Taking the naive spatial derivative w.r.t. to each direction means only considering a single row or column of and taking the derivative in that direction. Figure **ref** shows the result of this operation. An artifact is clearly visible: instead of a nice uniform derivative, we see a 'cross'. This effect is a cause of the discretization grid being too large for some smaller, often bright, objects.

To remedy this, one can for example artificially upscale the grid, interpolate the values inbetween, and take the derivatives from this grid. This is not ideal however, since the upscaling requires a large amount of memory and is computationally expensive. Another solution which is common in image processing is applying a

kernel operator. The advantage of a kernel operator is that it is extremely computationally cheap, as it involves convolving the original picture with a differentiation kernel. The differentiation kernel is an approximate version of a finite difference scheme. We use and show here the Sobel filter, which is the most commonly used one.

In a simple finite central difference scheme, we set

$$\frac{dx}{dt} \approx \frac{x_{i+1} - x_{i-1}}{2h}$$

where h is the distance between two points. In terms of a kernel operator, this would look like (the h drops out as the distance in terms of pixels is 1):

$$\frac{1}{2} \cdot \begin{bmatrix} 1 & 0 & -1 \end{bmatrix}$$

And applying it by convoluting it to a matrix gives the x-derivative:

$$\partial_x A \approx A * \begin{bmatrix} 1 & 0 & -1 \end{bmatrix}$$

and analogous for the y-direction. However, as we've seen, looking at just a single row introduces cross-like artifacts. To remedy this, we wish to include diagonal pixels as well. However, the distance between the diagonal pixels and the center pixel is not 1 but $\sqrt{2}$ and furthermore we need to decompose it into \hat{x} and \hat{y} , introducing another factor $\sqrt{2}$. Thus, one obtains the classic 3×3 Sobel filter **ref**:

$$\mathbf{G}_x = \frac{1}{8} \cdot \begin{bmatrix} 1 & 0 & -1 \\ 2 & 0 & -2 \\ 1 & 0 & -1 \end{bmatrix} \quad \mathbf{G}_y = \frac{1}{8} \cdot \begin{bmatrix} 1 & 2 & 1 \\ 0 & 0 & 0 \\ -1 & -2 & -1 \end{bmatrix}$$

Although not extremely accurate, the Sobel filter seems to do the tricks for us. Several other versions such as Scharr or Prewitt exist, offering several benefits such as rotational symmetry, but we have not pursued these. They just change the

coefficients. Although we have shown a 3×3 filter here, the filter can take into account higher order schemes such as a 5×5 or 7×7 . The major benefit of the spatial derivatives as a convolution operator is its computational efficiency: convolutional operations are performed parallel and are extremely fast.

For the time derivative, we apply a second order accurate central derivative scheme, while for the spatial derivatives (both first and second order) we apply the 5×5 Sobel filter. We analyze these in the next chapter

3.4 STEP 4 - FITTING

Now that we have gathered all our data we can use it to fit. We use a simple least squares method.

4

Results data analysis

Here we present the results from our analysis on the RUSH experiments. We only show the results of MANII because this is the only thing we studied.

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4.1 ANALYSIS OF TIME DERIVATIVES

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4.2 ANALYSIS OF FIT

4.2.1 DIFFUSION

4.2.2 ADVECTION

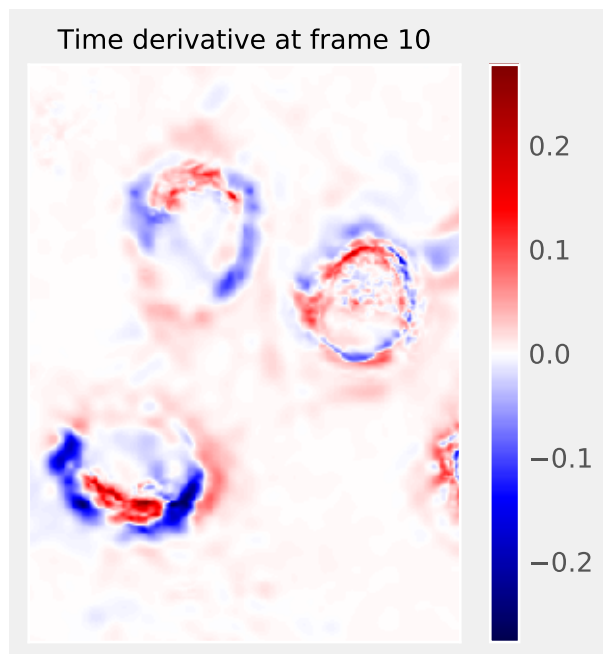


Figure 4.1.1: Wolla zieke subscript.

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Conclusion

Appendix 1: Some extra stuff

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References