Hi Rebecca,

It was great to meet you today. Here is a summary of what we discussed re Cellpose, and some suggestions.

**Using the Cellpose GUI**

The basic workflow is as follows:

* Open Cellpose GUI from command prompt in Anaconda navigator.
* File 🡪 Load Image.
* Set the purple cell size estimate circle to about the size of one cell.
* Use custom model 5 to segment the image.
* Manually correct the segmentation. Ctrl left click deletes a cell mask. Right click to outline a new mask. You can toggle masks and cell outlines on and off to help you to check the segmentation. Be wary of ‘false outlines’ caused by fibres or bits of food on the cells.
* Ctrl S to save a cellpose segmentation, Ctrl N to save the masks as a PNG or TIF (once you are sure that the segmentation is correct).
* Masks will appear blank when opened in the default image viewer. To check them, drag and drop the file into ImageJ. You can download Fiji (ImageJ) here: <https://fiji.sc/>

**Next steps**

I am sending a zip folder via Teams, which includes:

* Model 5.
* training\_data: The raw images and segmentations with which model 5 was trained (I think).
* additional\_training\_data: I found some extra manually segmented shg::GFP images that I had manually corrected, but not used for training models (I think – this was a while ago).
* not\_yet\_segmented: A few shg::GFP images which have not yet been segmented, which could be added to the training data.

I suggest that you check (and correct if necessary) the additional training data and segment the folder of new images to familiarise yourself with Cellpose. You can then have a go at training a new model. As I cannot remember for sure which training data I used for model 5, I recommend training a model from scratch.

(This should generate something better than model 5, as 5 was trained from scratch using some subset of the data I sent).

It is worth reading up on the image enhancement tools in Cellpose 3.

Here is the bioarchive paper for Cellpose 3: <https://www.biorxiv.org/content/10.1101/2024.02.10.579780v1.full>

Cellpose 3 documentation:

<https://cellpose.readthedocs.io/en/latest/>

Cellpose repository:

<https://github.com/MouseLand/cellpose>

Have a think about the questions you want to address.

What time interval will you use in recordings? (Amount of information vs time taken to segment the data).

Which cells do you want to look at? (either side of the midline, or one hemisegment?

Will it help to crop out regions of the image that you do not want to analyse, before segmenting? (ImageJ can help with this).

How will you use the Cellpose labelled images to analyse your data?

**Speeding up model training:**

* Consider using GPU with torch cuda. These two blogs might help:

<https://forum.image.sc/t/cellpose-torch-cuda-version-not-installed-working/77390/6>

<https://github.com/MouseLand/cellpose/issues/916>

* If time is an issue (you might be ok training the model overnight – remember to change settings so your computer does not go to sleep or turn off while training!), you could ask Jochen about using Thor.