

README in vivo

This folder contains all the light sheet movies and cell tracking files that were used to produce the *in vivo* data in our paper:

Cell-autonomous timing drives the vertebrate segmentation clock's wave pattern
(Rohde & Bercowsky-Rama et al., 2024 eLife)

DOI: <https://doi.org/10.7554/eLife.93764>

Annotated analysis and the associated output files from these movies and cell tracking can be found in: https://github.com/EPFL-TOP/WSC_NotebooksPaper

For guidance beyond that given in the methods in eLife, please consult:

<https://doi.org/10.1101/2023.06.01.543221>

Timelapse Movies

We provide the following files for each timelapse of an embryo:

- **OME/TIF** raw data
- **max projection**
- Time registered **HDF5** and associated **XML** files that include a record of transformations performed on the raw data.

We imaged and tracked cells in three different types of transgenic embryos at 15ss. All movies and tracking files are grouped in a folder according to the specific transgenes carried:

- *Tg(her1-YFP;h2b-mCherry)* in folder:
20191213_Tg_her1YFP_h2bmCherry
- *Tg(her1-YFP; mesp-ba-mKate2a)* in folder:
20190511_Tg_her1YFP_mespmKate2
- *Tg(esp-ba-mKate2a;h2a-gfp)* in folder:
20191031_140601_11_Tg_mespbamKate2a_h2agfp

Two embryos were imaged simultaneously for each of the three genotypes on a Viventis LS1 dual-illumination light sheet microscope. Embryos were designated:

- **Position 1**
- **Position 2**

Detailed Metadata from the imaging acquisition is included as JSON files in the folder:

- **Settings**

In brief, stacks of YFP, GFP, mCherry or mKate2 (150 planes, 1.5 μm spacing) were acquired at each position every 90 seconds:

20191213_Tg_her1YFP_h2bmCherry

Image has the following dimensions (XYZC): 2048, 2048, 150, 2
There are 299 frames in total.
Pixel Size: x = 0.347 micron, y = 0.347 micron z = 1.5 micron
There are 150 Z-slices in total

20190511_Tg_her1YFP_mespmKate2

Image has the following dimensions (XYZC): 2048, 2048, 150, 3
There are 119 frames in total.

Pixel Size: x = 0.347 micron, y = 0.347 micron z = 1.5 micron
There are 150 Z-slices in total

20191031_140601_11_Tg_mespbamKate2a_h2agfp

Image has the following dimensions (XYZC): 2048, 2048, 150, 2
There are 239 frames in total.

Pixel Size: x = 0.347 micron, y = 0.347 micron z = 1.5 micron
There are 150 Z-slices in total

Cell Tracking

We provide Mastodon cell tracking files accompanying the timelapse movies in the three main transgene folders described above. Mastodon files require the timelapse movie (HDF5 and XML). Cells are annotated with Tags in the Mastodon TrackScheme that can be viewed by coloring. Mastodon files included are:

Folder **20191213_Tg_her1YFP_h2bmCherry**:

- **Position2_PSMd_20191213_checked_LR.mastodon**
Tracks of PSM4 cells tagged as “PSMdtoSom” that are used as the representative embryo in eLife Figure 1G. Other tracks are tagged by “PSMdtoSomDiv” (PSM4 cells that divide while tracked) or “TBtoSom” (cells tracked from the TB to somite).
- **Position1-PSMd_20191213_LR_checked.mastodon**
Tracks of PSM4 cells tagged as “PSMdtoSom” (eLife Figure 1 data). Other tracks are tagged by “PSMdtoSomDiv” (PSM4 that divides) or “TBtoSom” (cells tracked from the TB to somite).
- **Position2_PSMd_20191213_Som1BT_LR.mastodon**
Contains cells backtracked from Somite 1 to capture the last peak of Her1-YFP (eLife Figure 2). Tagged as “SOM1”.
- **Position1-TB_20191213-LRjune.mastodon**
- **Position2-TB_to_TB_PSMd_SOM_LRCHKD_20191213.mastodon**
Contains cells backtracked from TB to TB, PSM4 to TB or Somite to TB (Position2 used for eLife Figure 4E-H). Tagged accordingly in the TrackScheme.

Folder **20190511_Tg_her1YFP_mespmKate2**

- **20190511_135308_12-Position2_checked_LR_HtoL.mastodon**
- **20190511_135308_12-Position1-checked_ABR_HtoL.mastodon**
Contains cells backtracked from Mesp-mKate2 expression through the Her1-YFP last peak (eLife Figure 2 – supplement figure 1D).

Folder **20191031_140601_11_Tg_mespbamKate2a_h2agfp**

- **Position1_20191031_S-1_LR.mastodon**
- **Position2_20191031_LR_tagged.mastodon**
Contains cells backtracked from Somite 1 to capture Mesp-mKate2 onset (eLife Figure 2).