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Supplemental Information

Visualizing Calcium Flux in Freely Moving Nematode Embryos

Evan L. Ardiel, Abhishek Kumar, Joseph Marbach, Ryan Christensen, Rishi Gupta, William Duncan, Jonathan S. Daniels, Nico Stuurman, Daniel Colón-Ramos, and Hari Shroff

Figure S1. Micro-manager plugin. Screenshots of the setup **(a)** and acquisition tabs **(b)**. Further documentation on the plugin is available at https://micro-manager.org/wiki/ASIdiSPIM_Plugin and http://dispim.org/software/micro-manager.

Figure S2. Calcium events in muscles of two-fold embryos. Raster plots duplicated from **Fig. 1c** with traces of mean dF/F of the bundle. Open circles denote calcium waves (peaks separated by at least 5 s with dF/F > 1.5), while arrows denote more localized events. Imaging was performed at 2 Hz (volumetric).

Figure S3. Cell counts. Number of *unc-31* expressing cells at various stages of embryogenesis. The 2-fold cell counts are from three different embryos, while the 3-fold cell counts are derived from the embryo described in text. Circles correspond to individual volume counts.

Figure S4. Segmenting nuclei. 5 of 40 slices raw **(a)** or with colored dots denoting local maxima **(b)** or nuclear centers **(c)**. Scale bar: 10μm.

Figure S5. Fluorescein fluorescence control. To ensure that intensity changes in excitation did not cause significant fluctuations in GCaMP, we measured the apparent intensity of a uniform fluorescein dye solution under conditions identical to those in the experiments in **Fig. 2**. **a)** Representative maximum projection

showing the positions at which fluorescein intensity was measured over time. **b)** dF/F traces across 298 frames (positions indicated in **a**).

Figure S6. Pan-neuronal imaging. a) dF/F traces for neuronal nuclei tracked from a mid-3-fold (2 hours after the dataset presented in **Fig. 2**) embryo expressing nuclear-localized GCaMP5K from an *unc-31* promoter. Cell 1 here matches cell 1 in **Fig 2**. **b)** 1/length (as indicated in **Fig. 2b**) and dF/F traces for ventral nerve cord neurons. Note the correlation between the length metric (black) and the top trace. Imaging was performed at 1.4 Hz (volumetric).

Figure S7. Determining measurement regions in AVA datasets. a) Maximum intensity projections of an embryo expressing GCaMP3 from an *nmr-1* promoter. Scale bar: 10μm. **b)** Intensity profile through the stack at crosses shown on left. Peak location defines the slice from which intensity is extracted for each cell. **c)** Whole image dF/F traces for 17 embryos. Arrowheads indicate traces with dF/F events > 0.6 and double arrowheads indicate samples in which cell bodies were tracked. Imaging was performed at 1.4 Hz (volumetric).

Movie S1. Lateral maximum intensity projection of 4 embryos at the 1.75-fold stage expressing GCaMP3 in muscle cells. Panels are synchronized to first twitch (at t=100s). Scale bar: 10μm. Imaging was performed at 2 Hz (volumetric).

Movie S2. Two-fold embryo expressing GCaMP3 in muscle cells. Lateral maximum intensity projection (top) and cross-sectional view (bottom, corresponding to white line in top panel). Scale bar: 10μm. Imaging was performed at 2 Hz (volumetric).

Movie S3. Lateral maximum intensity projection of a three-fold embryo expressing GCaMP3 in muscle cells. Scale bar: 10μm. Imaging was performed at 5 Hz (volumetric).

Movie S4. Three-fold embryo expressing GCaMP3 in muscle cells. Maximum intensity projections before (left) and after (middle) computational untwisting. Yellow line denotes the position of the cross-section shown at upper right. Scale bar: 10μm. Imaging was performed at 2 Hz (volumetric).

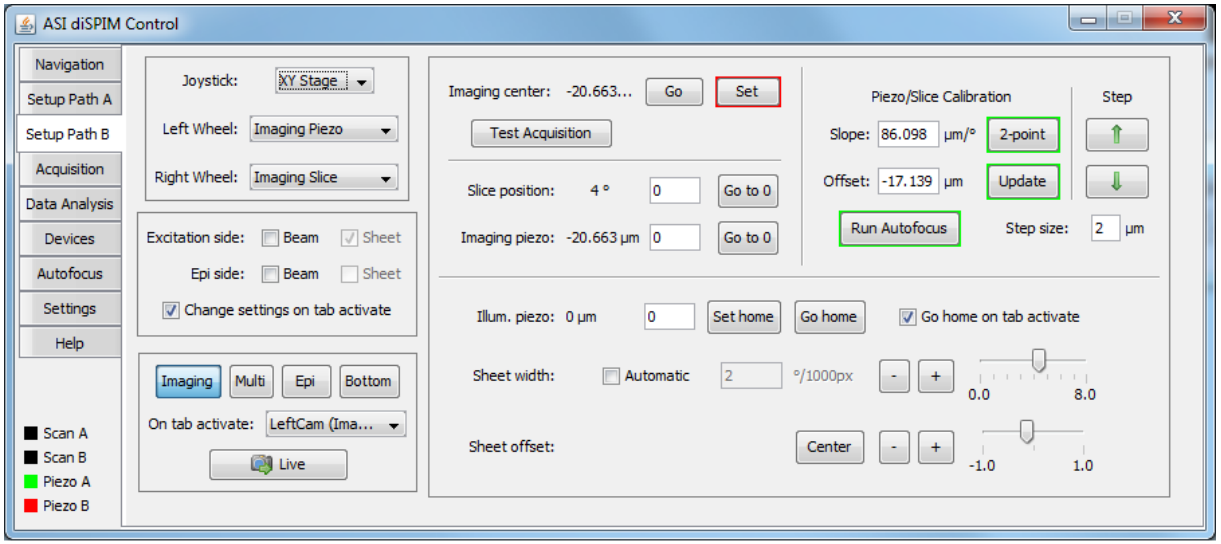
Movie S5. Early three-fold embryo expressing nuclear localized GCaMP5K from an *unc-31* promoter. Lateral maximum intensity projection (left) and corresponding schematic representations of dF/F (right; color and size scale corresponds to dF/F= 0, 1, and 2+). Scale bar: 10μm. Imaging was performed at 1.4 Hz (volumetric).

Movie S6. Mid-stage three-fold embryo expressing nuclear localized GCaMP5K from an *unc-31* promoter. Lateral maximum intensity projection (left) and corresponding schematic representations of dF/F (right; color and size scale corresponds to dF/F= 0, 1, and 2+). Scale bar: 10μm. 1.4 Hz (volumetric).

Movie S7. Maximum intensity projection of a late three-fold embryo expressing GCaMP3 from an *nmr-1* promoter (ImageJ Fire look up table was used for display). Scale bar: 10μm. 1.4 Hz (volumetric).

Supp Fig 1

a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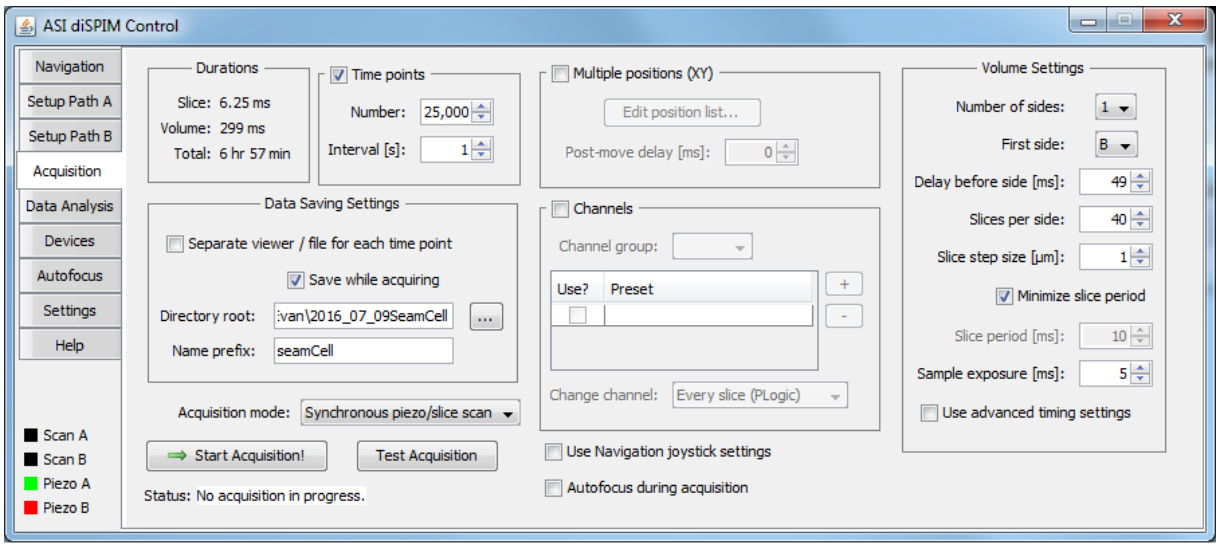
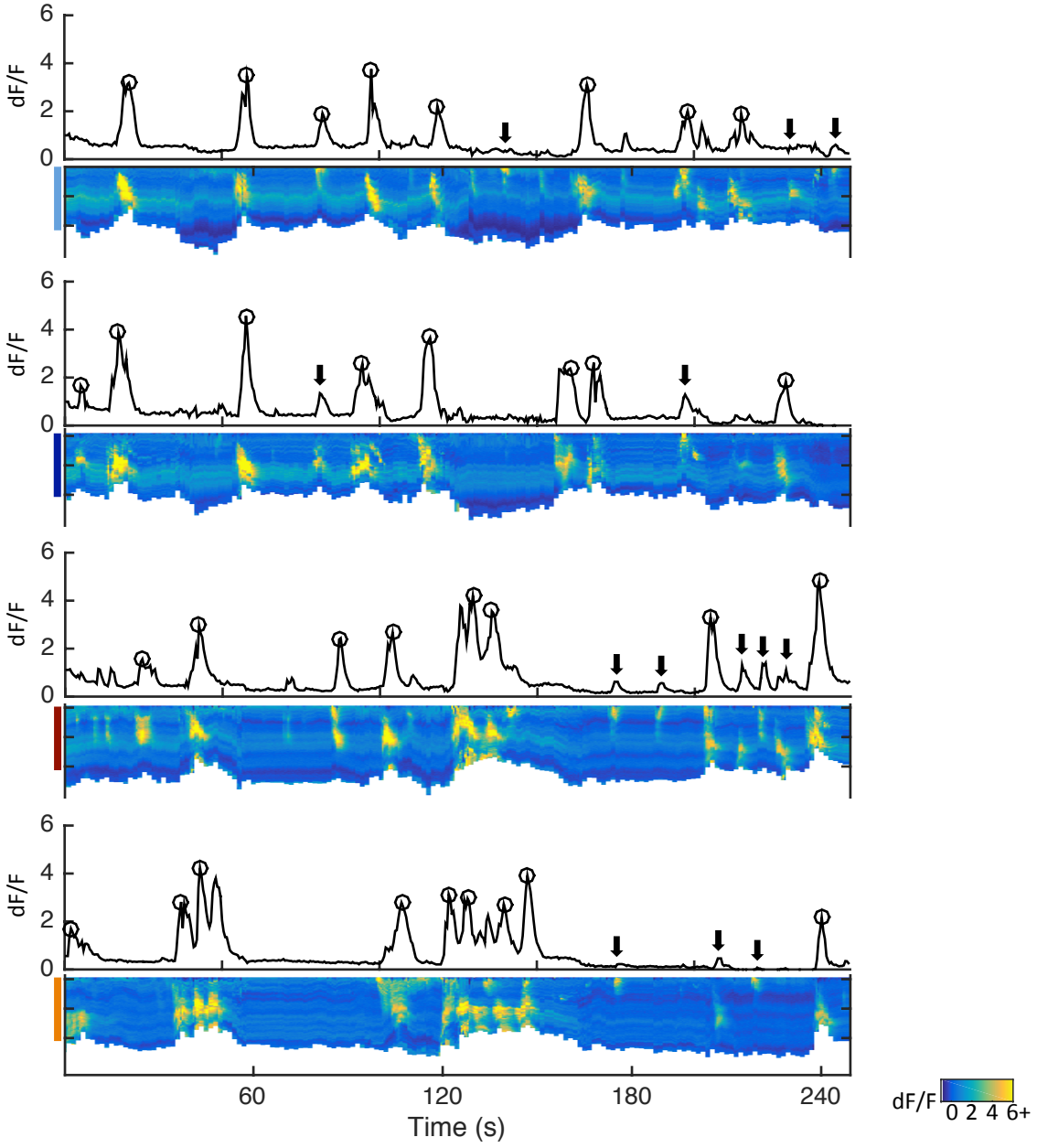
Navigation
Setup Path A
Setup Path B
Acquisition
Data Analysis
Devices
Autofocus
Settings
Help
Scan A
Scan B
Piezo A
Piezo B
Joystick: XY Stage
Left Wheel: Imaging Piezo
Right Wheel: Imaging Slice
Excitation side: Beam Sheet
Epi side: Beam Sheet
Change settings on tab activate
Imaging Multi Epi Bottom
On tab activate: LeftCam (Ima...
Live
Imaging center: -20.663... Go Set
Test Acquisition
Slice position: 4 ° 0 Go to 0
Imaging piezo: -20.663 µm 0 Go to 0
Piezo/Slice Calibration
Slope: 86.098 µm/° 2-point
Offset: -17.139 µm Update
Run Autofocus
Step
Step size: 2 µm
Illum. piezo: 0 µm 0 Set home Go home
Go home on tab activate
Sheet width: Automatic 2 °/1000px
0.0 8.0
Sheet offset: Center
-1.0 1.0

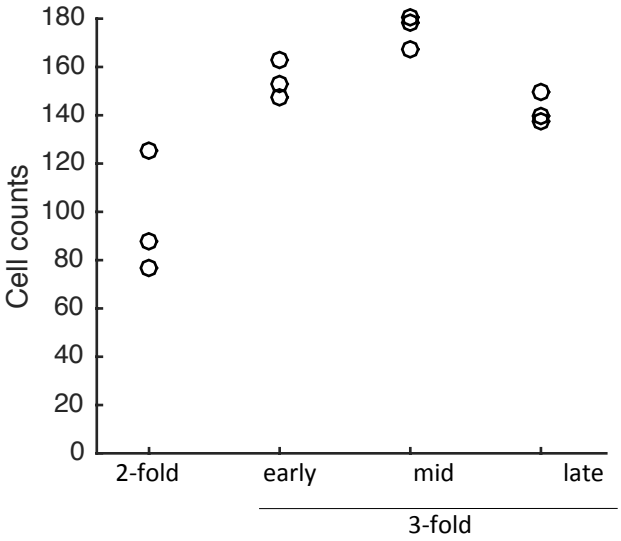
b


ASI diSPIM Control
Navigation
Setup Path A
Setup Path B
Acquisition
Data Analysis
Devices
Autofocus
Settings
Help
Scan A
Scan B
Piezo A
Piezo B
Durations
Slice: 6.25 ms
Volume: 299 ms
Total: 6 hr 57 min
Time points
Number: 25,000
Interval [s]: 1
Multiple positions (XY)
Edit position list...
Post-move delay [ms]: 0
Volume Settings
Number of sides: 1
First side: B
Delay before side [ms]: 49
Slices per side: 40
Slice step size [µm]: 1
Minimize slice period
Slice period [ms]: 10
Sample exposure [ms]: 5
Use advanced timing settings
Data Saving Settings
Separate viewer / file for each time point
Save while acquiring
Directory root: :van\2016_07_09SeamCell
Name prefix: seamCell
Channels
Channel group:
Use? Preset
Change channel: Every slice (PLogic)
Acquisition mode: Synchronous piezo/slice scan
Start Acquisition!
Test Acquisition
Use Navigation joystick settings
Autofocus during acquisition
Status: No acquisition in progress.

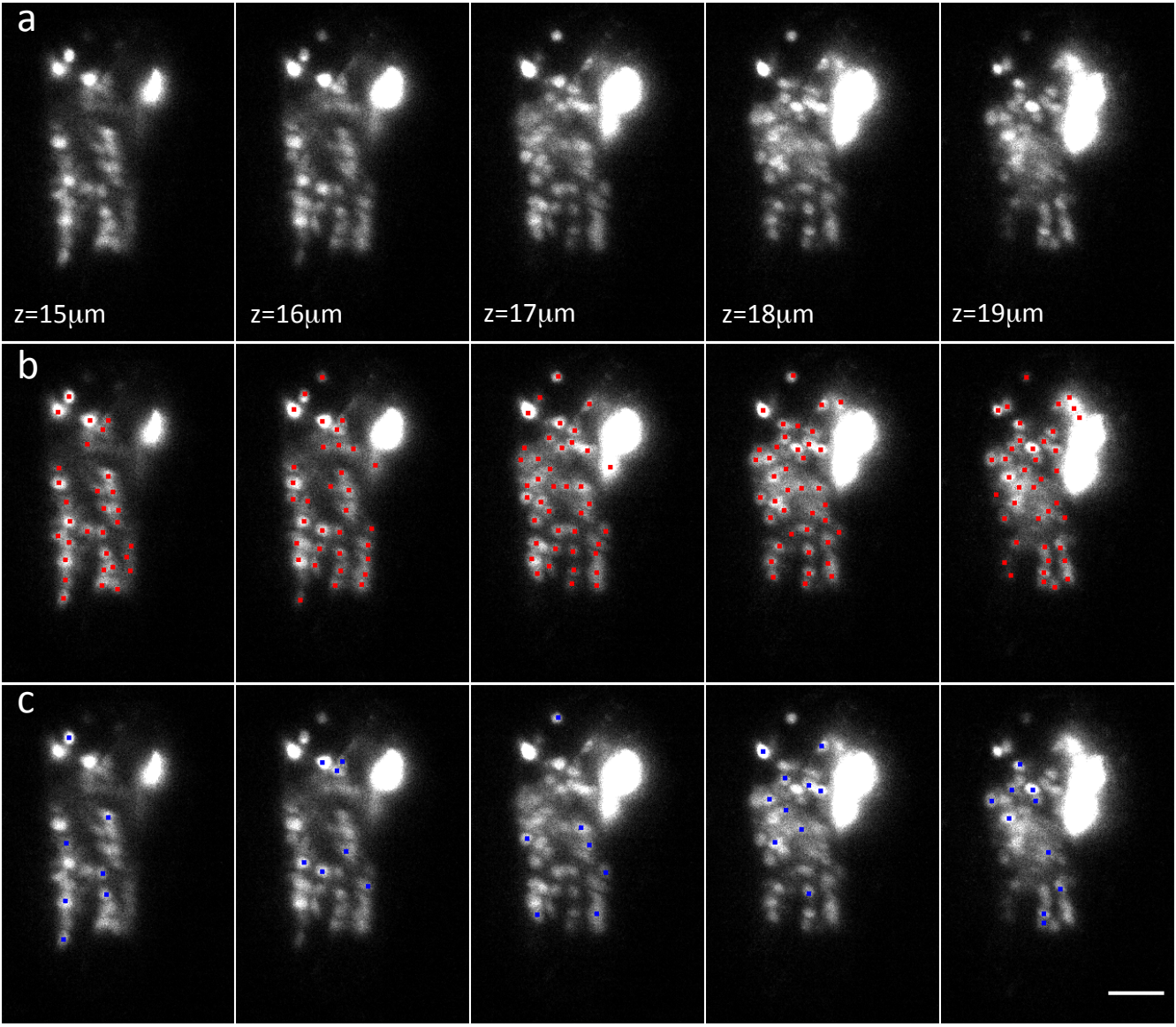
Supp Fig 2



Supp Fig 3

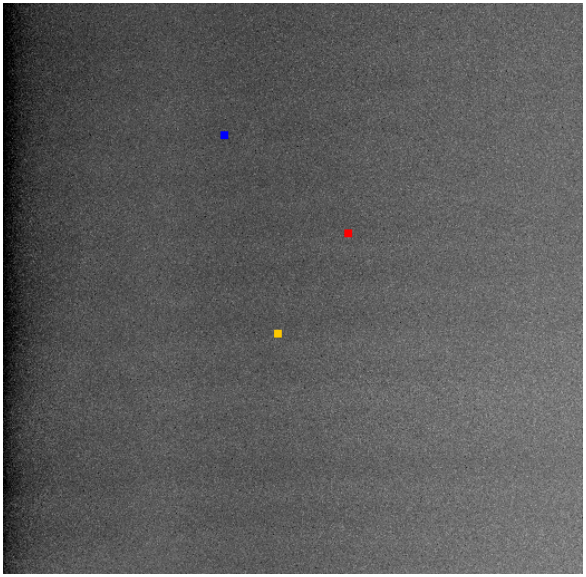

Cell counts
180
160
140
120
100
80
60
40
20
0
2-fold
early
mid
late
3-fold

Supp Fig 4

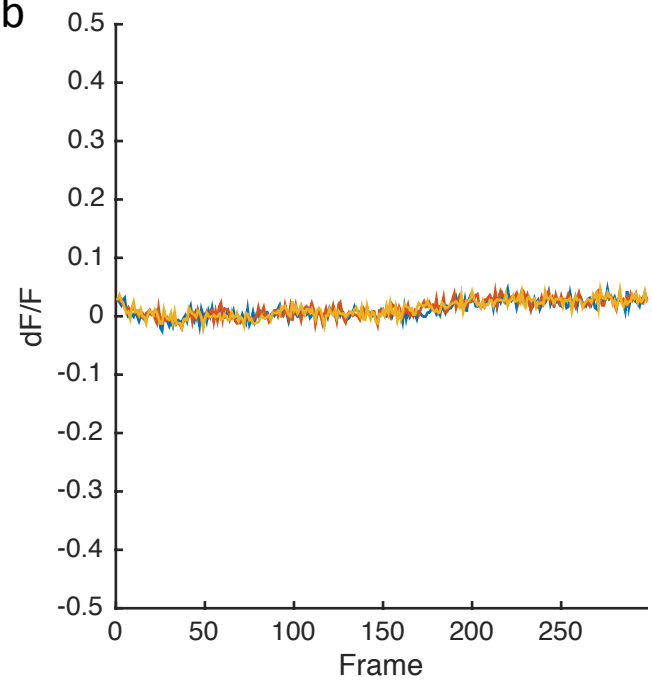


Supp Fig 5

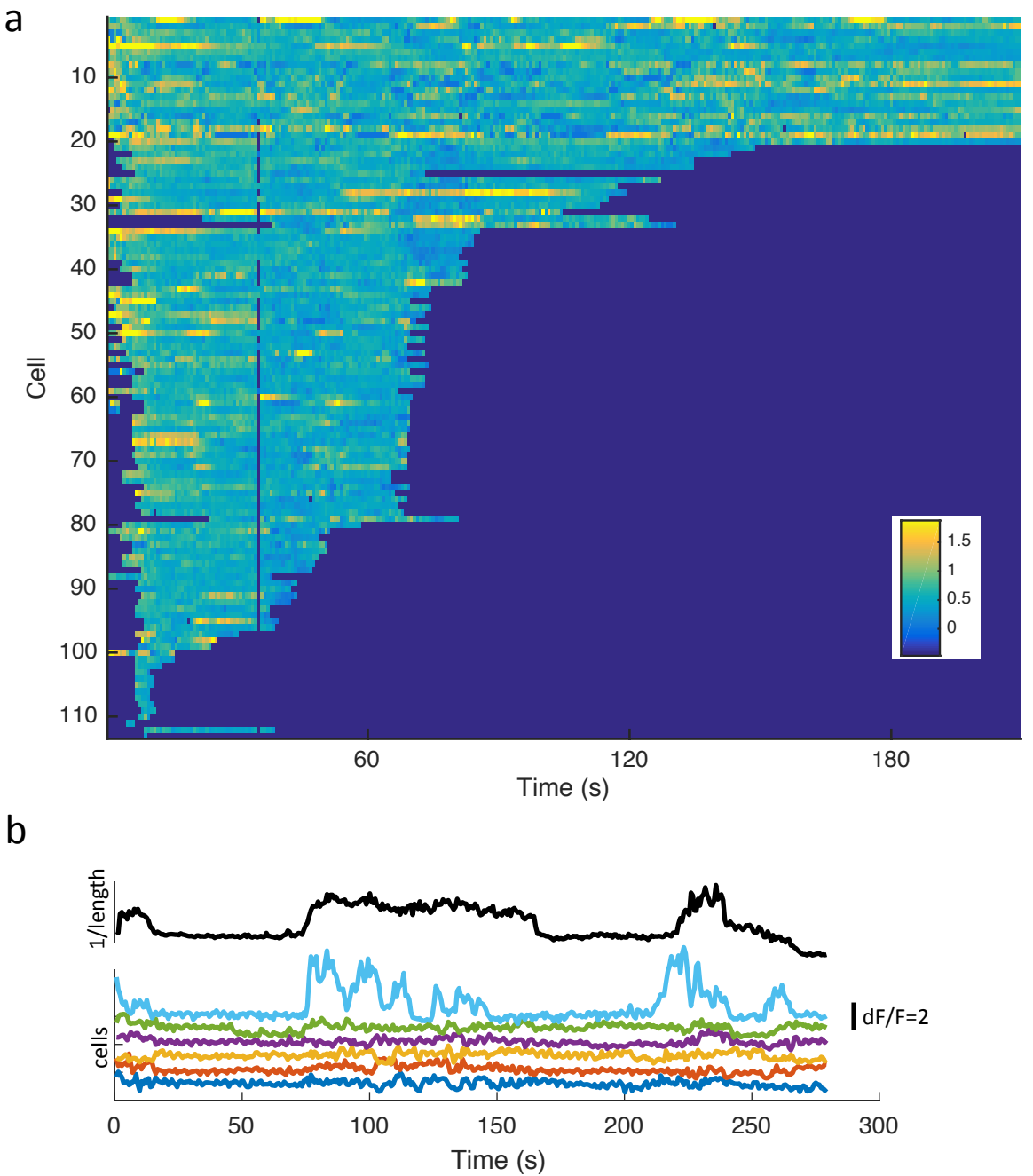
a



b



Supp Fig 6


a
Cell
10
20
30
40
50
60
70
80
90
100
110
60
120
180
Time (s)
1.5
1
0.5
0
b
1/length
cells
dF/F=2
0
50
100
150
200
250
300
Time (s)

Supp Fig 7