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Live, in vivo, imaging (GCaMP6F), Video Processing, and Analysis

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Protocol for GCaMP6F imaging and analysis in gut neurons used in Yoo et al 2021

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Preparation

1 AAV-PHP.S-CAG-GCaMP6F (10^{12}VGs) was delivered systemically to WT C57.

2 3-4 weeks after infection, mice were anesthetized with 2% isoflurane on a heating pad (Kent Scientific, Torrington, CT-DCT15) with plastic sleeve covers (Kent Scientific, Torrington, CT-DCT1520P).

3 The abdominal cavity was surgically opened to expose the intestines.

Imaging

4 The proximal colon was identified, and this portion was placed on top of a stack of 4-6 glass microscopy slides (depending on size of animal) (VWR, Radnor PA-Cat. No. 48300-026).

5 Tissue was secured onto glass slide with a biorthogonal silicon elastomer (Kwik-Sil) (World Precision Instruments, Sarasota, FL-KWIK-SIL), and a glass coverslip.

6 Elastomer stiffens within 1 minute of application and coverslip must be held steadily to ensure a flat imaging surface. Anesthetized mouse is placed under an upright confocal microscope (Zeiss LSM 880).

7 Using a 10X objective, GCaMP6F fluorescence was taken at 5Hz (1 image every 200ms).

8 Periods of movement of tissue and luminal contents is normal during live imaging.

Processing & Analysis

9 Cell tracking was performed in 2D by using TrackMate ImageJ plugin (https://imagej.net/TrackMate) and fluorescence intensity was recorded from cells.

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10 Average background was determined by taking the fluorescence of a region of interest that did not contain a cell, over the duration of the video. Background was subtracted from cell fluorescence intensities.

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