**“The beetle amnion and serosa functionally interact as apposed epithelia”**

**Supplementary file 2**

**Acquisition parameters for featured mDSLM light sheet experiments.**

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| --- | --- | --- | --- | --- |
| **Dataset** | **Amnion x Heart** | **Serosa x Heart** | **Amnion** | **Serosa** |
| Microscope | mDSLM (EMBLEM, Germany) | | | |
| Excitation objective | 2.5x NA 0.06 EC Plan-Neofluar (Carl Zeiss, Germany) | | | |
| Detection objective | 10x NA 0.3 W N-achroplan (Carl Zeiss, Germany) | | | |
| Illumination laser | 488 nm diode laser, 20mW max. pulsed output power  (PhoxX 488-20, Omicron GmbH, Germany) | | | |
| Emission filter | 525/45 bandpass (525/45 Brightline HC Semrock, USA). | | | |
| Camera | 1392x1040 CCD, pixel size 6.45 x 6.45µm  (Andor Clara, Oxford Instruments, UK) | | | |
| GEKU line | Amnion (HC079) x Heart (G04609) | Serosa (G12424) x Heart (G04609) | Amnion (HC079) (homozygous) | Serosa (G12424) (homozygous) |
| Dataset unique identifier | TM12162330 | TM26171836 | TM03151712 | TM08150418 |
| Date acquired | 01/12/2014 | 26/11/2014 | 03/06/2015 | 08/06/2015 |
| Agarose (%) | 1.4 | 1.0 | 1.4 | 1.4 |
| Acquisition temperature (°C) | 19.5 ± 1 | 19.5 ± 1 | 30.0 ± 0.1 | 30.0 ± 0.1 |
| Laser power close to embryo (µW) | 0.87 | 1.24 | 0.75 | 0.86 |
| Exposure time (ms) | 55 | 55 | 55 | 55 |
| Z-spacing (µm) | 2.58 | 2.58 | 2.58 | 2.58 |
| Number of slices per time point | 68 | 78 | 90 | 101 |
| Z-Stack depth (µm) | 175 | 200 | 230 | 260 |
| Total number of time points | 4320 | 4320 | 1304 | 1265 |
| Time between time points (s) | 20 | 20 | 120 | 120 |
| Total duration of TL (hh:mm) | 24:00 | 24:00 | 43:28 | 42:10 |
| Presented in | Fig. 4G; Figure 4-figure supplement 1A; Movie S3 | Figure 4-figure supplement 1B; Movie S3 | Fig. 4D1-D2; Figure 2-figure supplement 1E1-E5 | Fig. 4E1-E2; Figure 2-figure supplement 1D1-D5 |
| Hatched?\* (yes/no) | yes | yes | yes | yes |

\*After time lapse imaging, the agarose core containing the embryo was moved to 1x PBS at room temperature and inspected at daily intervals until hatching was confirmed.