## Supplementary Table 1

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| **Dataset (DS)** | **DS0001** | **DS0002** | **DS0003** |
| Species | *Drosophila melanogaster* (Meigen)Arthropoda → Insecta → Diptera → Drosophilidae |
| Line | Bloomington #01691 | Bloomington #29724 | Bloomington #24163 |
| Line Genotype | y[1] w[67c23];P{w[+mC]=Ubi-GFP.nls}ID-2;P{Ubi-GFP.nls}ID-3 | w[\*];P{w[+mC]=Tub84B-EGFP.NLS}3 | w[\*];P{w[+mC]=His2Av-EGFP.C}2/SM6a |
| Stock | ~100-200 adults, less than 2 weeks old |
| Stock Medium | Nutri-Fly Bloomington Formulation (66-112, Dominique Dutscher SA, Brumath, France) |
| Stock Conditions | 12:00 h light / 12:00 h darkness at 25°C and 70% relative humidity(DR-36VL, Percival Scientific, Perry, IA, USA) |
| Egg Laying Period | 00:15 h at room temperature (23±1°C) |
| Egg Laying Medium | apple juice (254615, REWE Markt GmbH, Köln, Germany) dishes with 1% (w/v) agarose |
| Pre-imaging Incubation | 00:30 h at room temperature (23±1°C) |
| LSFM Type | mDSLM (monolithic digital scanned laser light sheet-based fluorescence microscope) based on DSLM |
| Laser Lines | 488 nm / 20 mW diode laser (PhoxX 488-20, Omicron Laserprodukte GmbH, Rodgau-Dudenhofen, Germany) |
| Excitation Objective | 2.5× NA 0.06 EC Epiplan-Neofluar objective (422320-9900-000**,** Carl Zeiss, Göttingen, Germany) |
| Emission Objective | 10× NA 0.3 W N-Achroplan objective (420947-9900-000, Carl Zeiss, Göttingen, Germany) |
| Emission Filters | 525/50 single-band bandpass filter (FF03-525/50-25, Semrock/AHF Analysentechnik AG, Tübingen, Germany) |
| Camera | High-resolution CCD (Clara, Andor, Belfast, United Kingdom), 14 bit, 1040×1392 pixel (pitch 6.45 µm) |
| Dataset File Type | TIFF, 16 bit grayscale (planes saved as Z stacks in ZIP-compressed container files, indicated as PL(ZS)) |
| Dechorionation | ~30-60 s in 14% (vol/vol) sodium hypochlorite (425044-250ML, Sigma Adlrich, Taufkirchen, Germany)in autoclaved tap water |
| Mounting Method | Cobweb holder (embryos are glued to a thin agarose film spanning a slotted hole)  |
| Parallel Imaging | With DS0002 and DS0003 | With DS0001 and DS0003 | With DS0001 and DS0002 |
| Mounting Agarose | 1% (w/v) low-melt agarose (6351.2, Carl Roth, Karlsruhe, Germany)in autoclaved tap water |
| Imaging Buffer | autoclaved tap water |
| Imaging Temperature | room temperature (23±1°C) |
| Retrieval | developed to healthy adult,produced fertile progeny | developed to healthy adult,produced fertile progeny | developed to healthy adult,produced fertile progeny |
| Comment | - | - | - |

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| **Dataset (DS)** | **DS0001** | **DS0002** | **DS0003** |
| Dataset Size | 16.1 Gigabyte (TIFF) | 14.4 Gigabyte (TIFF) | 22.8 Gigabyte (TIFF) |
| Figures | Figure 3 | Figure 3 | Figure 3 |
| Supplementary Videos | Supplementary Movie 1 | Supplementary Movie 1 | Supplementary Movie 1 |
| Dataset DOI (Zenodo) |  |  |  |
| **Time Points (TP)** | **143 (TP0001-TP0143)** | **143 (TP0001-TP0143)** | **143 (TP0001-TP0143)** |
| TP Interval | 00:10 h | 00:10 h | 00:10 h |
| Total Time (TP×TP Interval) | 23:40 h | 23:40 h | 23:40 h |
| **Directions (DR)** | **4 (DR0001-DR0004)** | **4 (DR0001-DR0004)** | **4 (DR0001-DR0004)** |
| DR Orientations | 0°, 90°, 180°, 270° | 0°, 90°, 180°, 270° | 0°, 90°, 180°, 270° |
| **Channels (CH)** | **1 (CH0001)** | **1 (CH0001)** | **1 (CH0001)** |
| CH0001 Excitation | 488 nm | 488 nm | 488 nm |
| CH0001 Power | 135 µW (close to the embryo) | 135 µW (close to the embryo) | 135 µW (close to the embryo) |
| CH0001 Exposure Time | 50 ms | 50 ms | 50 ms |
| CH0001 Emission Filter | 525/50 single-band bandpass filter | 525/50 single-band bandpass filter | 525/50 single-band bandpass filter |
| **Planes (PL)** | **85 (PL0001-PL0085)** | **85 (PL0001-PL0085)** | **85 (PL0001-PL0085)** |
| Z Spacing | 2.58 µm | 2.58 µm | 2.58 µm |
| Z Distance (PL×Z Spacing) | 219.3 µm | 219.3 µm | 219.3 µm |
| **X-Dimensions (XD)** | **500 pixels (cropped)** | **500 pixels (cropped)** | **500 pixels (cropped)** |
| X Spacing | 0.645 µm | 0.645 µm | 0.645 µm |
| X Length (XD×X Spacing) | 322.5 µm | 322.5 µm | 322.5 µm |
| **Y-Dimensions (YD)** | **900 pixels (cropped)** | **900 pixels (cropped)** | **900 pixels (cropped)** |
| Y Spacing | 0.645 µm | 0.645 µm | 0.645 µm |
| Y Length (YD×Y Spacing) | 580.5 µm | 580.5 µm | 580.5 µm |
| Data Access | 10.5281/zenodo.3932189 | 10.5281/zenodo.3932193 | 10.5281/zenodo.3932195 |

## Supplementary Table 1

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| **Dataset (DS)** | **DS0004** | **DS0005** | **DS0006** |
| Species | *Tribolium castaneum* (Herbst)Arthropoda → Insecta → Coleoptera → Tenebrionidae |
| Line | AGOC{Zen1’#O(LA)-mEmerald}#1 subline | AGOC{Zen1’#O(LA)-mEmerald}#2 subline | AGOC{Zen1’#O(LA)-mEmerald}#2 subline |
| Line Genotype | one insert(mC/mC) homozygous | one insert(mC/mC) homozygous | one insert (mC/mC) homozygous |
| Stock | ~200-300 adults, less than 3 month old |
| Stock Medium | full grain wheat flour (113061006, Demeter, Darmstadt, Germany)supplemented with 5% (wt/wt) inactive dry yeast(62-106, Flystuff, San Diego, CA, USA) |
| Stock Conditions | 12:00 h light / 12:00 h darkness at 25°C and 70% relative humidity(DR-36VL, Percival Scientific, Perry, IA, USA) |
| Egg Laying Period | 01:00 h at room temperature (23±1°C) |
| Egg Laying Medium | 405 fine wheat flour (113061036, Demeter, Darmstadt, Germany)supplemented with 5% (wt/wt) inactive dry yeast (62-106, Flystuff, San Diego, CA, USA) |
| Pre-imaging Incubation | 15:00 h at 25°C and 70% relative humidity in darkness, 01:00 h at room temperature (23±1°C) |
| LSFM Type | mDSLM (monolithic digital scanned laser light sheet-based fluorescence microscope) based on DSLM |
| Laser Lines | 488 nm / 20 mW diode laser (PhoxX 488-20, Omicron Laserprodukte GmbH, Rodgau-Dudenhofen, Germany) |
| Excitation Objective | 2.5× NA 0.06 EC Epiplan-Neofluar objective (422320-9900-000**,** Carl Zeiss, Göttingen, Germany) |
| Emission Objective | 10× NA 0.3 W N-Achroplan objective (420947-9900-000, Carl Zeiss, Göttingen, Germany) |
| Emission Filters | 525/50 single-band bandpass filter (FF03-525/50-25, Semrock/AHF Analysentechnik AG, Tübingen, Germany) |
| Camera | High-resolution CCD (Clara, Andor, Belfast, United Kingdom), 14 bit, 1040×1392 pixel (pitch 6.45 µm) |
| Dataset File Type | TIFF, 16 bit grayscale (planes saved as Z stacks in ZIP-compressed container files, indicated as PL(ZS)) |
| Dechorionation | ~30-60 s in 14% (vol/vol) sodium hypochlorite (425044-250ML, Sigma Adlrich, Taufkirchen, Germany)in autoclaved tap water |
| Mounting Method | Cobweb holder (embryos are glued to a thin agarose film spanning a slotted hole)  |
| Parallel Imaging | With DS0005 and DS0006 | With DS0004 and DS0006 | With DS0004 and DS0005 |
| Mounting Agarose | 1% (w/v) low-melt agarose (6351.2, Carl Roth, Karlsruhe, Germany)in autoclaved tap water |
| Imaging Buffer | autoclaved tap water |
| Imaging Temperature | room temperature (23±1°C) |
| Retrieval | developed to healthy adult,produced fertile progeny | developed to healthy adult,produced fertile progeny | developed to healthy adult,produced fertile progeny |
| Comment | - | - | - |

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| --- | --- | --- | --- |
| **Dataset (DS)** | **DS0001** | **DS0002** | **DS0003** |
| Dataset Size | 57.1 Gigabyte (TIFF) | 60.0 Gigabyte (TIFF) | 49.6 Gigabyte (TIFF) |
| Figures | Figure 4 | Figure 4 | Figure 4 |
| Supplementary Videos | Supplementary Movie 2 | Supplementary Movie 2 | Supplementary Movie 2 |
| Dataset DOI (Zenodo) |  |  |  |
| **Time Points (TP)** | **237 (TP0001-TP0237)** | **237 (TP0001-TP0237)** | **237 (TP0001-TP0237)** |
| TP Interval | 00:30 h | 00:30 h | 00:30 h |
| Total Time (TP×TP Interval) | 118:00 h | 118:00 h | 118:00 h |
| **Directions (DR)** | **4 (DR0001-DR0004)** | **4 (DR0001-DR0004)** | **4 (DR0001-DR0004)** |
| DR Orientations | 0°, 90°, 180°, 270° | 0°, 90°, 180°, 270° | 0°, 90°, 180°, 270° |
| **Channels (CH)** | **1 (CH0001)** | **1 (CH0001)** | **1 (CH0001)** |
| CH0001 Excitation | 488 nm | 488 nm | 488 nm |
| CH0001 Power | 135 µW (close to the embryo) | 135 µW (close to the embryo) | 135 µW (close to the embryo) |
| CH0001 Exposure Time | 50 ms | 50 ms | 50 ms |
| CH0001 Emission Filter | 525/50 single-band bandpass filter | 525/50 single-band bandpass filter | 525/50 single-band bandpass filter |
| **Planes (PL)** | **150 (PL0001-PL0150)** | **150 (PL0001-PL0150)** | **150 (PL0001-PL0100)** |
| Z Spacing | 2.58 µm | 2.58 µm | 2.58 µm |
| Z Distance (PL×Z Spacing) | 387.0 µm | 387.0 µm | 387.0 µm |
| **X-Dimensions (XD)** | **600 pixels (cropped)** | **600 pixels (cropped)** | **600 pixels (cropped)** |
| X Spacing | 0.645 µm | 0.645 µm | 0.645 µm |
| X Length (XD×X Spacing) | 387.0 µm | 387.0 µm | 387.0 µm |
| **Y-Dimensions (YD)** | **1000 pixels (cropped)** | **1000 pixels (cropped)** | **1000 pixels (cropped)** |
| Y Spacing | 0.645 µm | 0.645 µm | 0.645 µm |
| Y Length (YD×Y Spacing) | 645.0 µm | 645.0 µm | 645.0 µm |
| Data Access | 10.5281/zenodo.3932197 | 10.5281/zenodo.3932199 | 10.5281/zenodo.3932201 |

All datasets can be downloaded at www.physikalischebiologie.de/bugcube.