

Figure S1

Specimen Fixation

- 4% PFA > 24hrs

Specimen Dehydration (embryo age <e12.0)

- dH₂O 3hrs
- 50%, 70%, 80%, 85%, 90% ethanol 1hr
- 95% ethanol plus* 1hr
- 100% ethanol plus 1hr, overnight

Specimen Dehydration (embryo age e12.0- e13.5)

- dH₂O 3hrs
- 50%, 70%, 80%, 85%, 90% ethanol 1.5hr
- 95% ethanol plus 1.5hr
- 100% ethanol plus 1.5hr, overnight

Infiltration

- JB 4 infiltration solution** : 100% ethanol plus (50:50) 3hrs at 4°C
- JB 4 infiltration solution 3hrs
- Fresh JB 4 infiltration solution overnight at 4°C

Embedding

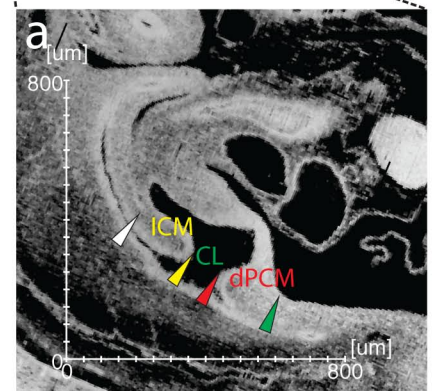
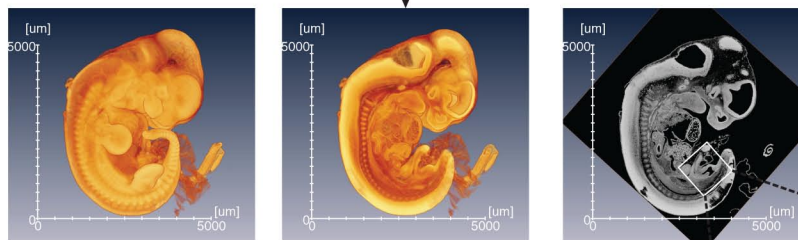
- JB 4 embedding solution*** 24hrs in the mold at 4°C

Episcopic fluorescence image capturing

- Microtome (Leica, RM2265)
- Microscope (Zeiss, Axio Zoom.V16)
- Digital Camera (Hamamatsu Photonics K.K., C11440-10C)

3D Images Visualization

- Photoshop (Adobe, V13.0)
- Amira 5.4.5



*0.275g of eosin B/100ml, 0.055g of acridine orange/ 100ml with ethanol
**0.275g of eosin B/100ml, 0.055g of acridine orange/ 100ml, JB 4 catalyst
1.25/100ml with JB 4 monomer
*** JB 4 accelerator 1.0ml/25ml with JB 4 infiltration solution