## Segmentation (1)

Fiduciary marker, neuron segmentation (Ilastik; Fig 1)

Pan-nuclear segmentation (StarryNite; Fig 2)

Relevant features are segmented.

Generate internal coordinate systems (2)

Center and align to X-axis (Fig 3)

Embryo center of mass and AP-axis identified.

Identify head (Fig 3)

Unrotate developmental rotations (CPD; Fig 4)

Embryo internal coordinate system is established.

Temporal alignment (least squares; Fig 2) Scale test to reference (moments of inertia; Fig 3) Estimate initial DV – LR angle (Fig 3) Map early time point to reference (CPD; Fig 3) Map all time points to reference using summed rotations (CPD; Fig 3) Test embryo is roughly aligned to reference. 3D fine correction, map all time points to corresponding reference time points (CPD) Test embryo is precisely aligned to reference.

Map test coordinates to reference (3)