Keller et al. (2008); figure 1

laser scanner

vertically scanned
“light sheet”

detection lens

f-theta lens
tube lens

illumination lens

sample
Keller et al. (2008); figure 2

(a) Animal view at 100 min, 64-cell stage.
(b) Animal view at 280 min, early blastula.
(c) Animal view at 550 min, 80% epiboly.
(d) Animal view at 1440 min, late somitogenesis.
(e) DSLM data showing blastomere nuclei, eye somites, and somites at t = 0 min (interphase) and t = 8 min (anaphase).
Keller et al. (2008); figure 4

(a) up to cycle 9
32 - 256 cells

cycles 10 to 13
512 - 4,000 cells

beyond cycle 13
> 4,000 cells

Asynchronous “patches”

No peripheral divisions

Symmetry break at 3 hpf

Radial wave (r-wave)

Future body axis

75 ± 3°

Peripheral wave (p-wave)

Future body axis

60 ± 3°

(b) Future body axis

100°

P-wave axes

(n = 25)

(c) Symmetry breaks

① Cell divisions
② Cell density
③ Morphology

Nuclei count

time (min)

Ventral ROI

Dorsal ROI

Lateral ROI

Future body axis

ROI definition (animal view)
Keller et al. (2008); figure 5

ventral slice | dorsal slice

384 min

411 min

455 min

494 min

615 min
Keller et al. (2008); figure 6

**lateral view**
- **deposition**
  - hypoblast
- **epiboly**

**dorsal view**
- **shield**

5.8-7.5 hpf
- **stretching**
  - blastoderm

7.5-10 hpf
- **zippering**

10-12.5 hpf
Keller et al. (2008); supplementary figure 4

Paralleled segmentation pipeline operates on EMBL cluster and KIT cluster

- **clusterNuclei**: 3D image segmentation
- **clusterCollect**: fusion of segmentation sections
- **clusterFilter**: morphological filtering, anisotropy filtering
- **clusterCorrelate**: temporal domain filtering, spatial position correlation
- **clusterRecovery**: recovery of false negatives

**DSLM**
- microscopy data
- **createID**: primary image database, image pre-processing
- **deconvolveTL**: 3D image deconvolution, rigid body transformation
- **trackNuclei**: user-guided cell tracking
- **pickNuclei**: user-guided population statistics

**normalizeTL**: laser intensity corrections, data set visualization
- **correlateTL**: 3D drift compensation
- **sliceData**: visualization of embryo parts, tracking of domains
- **trackNuclei**: user-guided cell tracking

**clusterStatistics**: embryo *in toto* population statistics

**clusterCombine**: *in toto* fusion of multi-view data
- **clusterMaps**: nuclear density/migration maps
- **clusterDivisions**: detection of cell divisions
- **analyzeDivisions**: analyze division patterns, determine polarity of divisions

**clusterReduce**: digital embryo slicing, visualization of tissues/organs
- **clusterTracking**: single-cell tracking, tissue and organ lineaging
- **clusterRendering**: digital embryo visualization

**clusterField**: migration vector fields
- **clusterMovement**: long-term *in toto* tracking

Green: automated software modules
Red: user-guided applications
Black: output data

Workstation-based: 4 CPUs
High-end file server: 1,000 CPUs
Low-cost 10TB NAS: 1,000 CPUs
Keller et al. (2008); supplementary figure 5

- a: raw DSLM data
- b: gamma corrected
- c: Gauss convolved
- d: Lucy-Richardson deconvolved
- e: 7x zoom
- f: Core segmentation
- g: Gauss convolved
- h: Core segmentation

- i: blastoderm 422'
- j: blastoderm 695'
- k: notochord 1100'
- l: eye vesicle 1100'

raw DSLM data gamma corrected
Gauss convolved Core segmentation Gauss convolved Core segmentation
blastoderm 422' blastoderm 695' notochord 1100' eye vesicle 1100'
Keller et al. (2008); supplementary figure 6

(a) Graph showing the nuclei count over time (min). The graph indicates early division rate as $18.2 \pm 0.3$ cells min$^{-1}$ and late division rate as $30.5 \pm 0.4$ cells min$^{-1}$. The convergence point is also marked.

(b) Bar chart showing cell division statistics from 100-400 mpf. The division angle alpha ($\alpha$) and division angle beta ($\beta$) are represented with bars indicating the count of cell divisions at different angles.
Keller et al. (2008); supplementary figure 7

- Chromatin domain size: 186 ± 16 µm³, 8.6% variability
- Nuclear movements:
  - Late epiboly: 1.12 ± 0.08 µm min⁻¹, 7.1% variability
  - Early somitogenesis: 0.57 ± 0.04 µm min⁻¹, 7.0% variability
Keller et al. (2008); supplementary figure 8

First symmetry break 3 hpf

Quasi-synchronous divisions

"Slow" peripheral waves

\( v_p \)

\( v_r \)

\( \Delta t \)
Keller et al. (2008); supplementary figure 9

**a** ventral hemisphere

**b** dorsal hemisphere

**c**

- **t** subscripts 1 and 2
- Cells migrating vegetally
- Internalizing cells

**d**

- Internalizing cells
- Time (min)
- Dorsal hemisphere (55%)
- Ventral hemisphere (45%)
- Synchronized embolic wave 1,550 cells (34%)

**e**

- Internalization asynchrony (min)
- Distance between internalizing cells (µm)
- Dorsal hemisphere
- Ventral hemisphere
- Simulation of involution