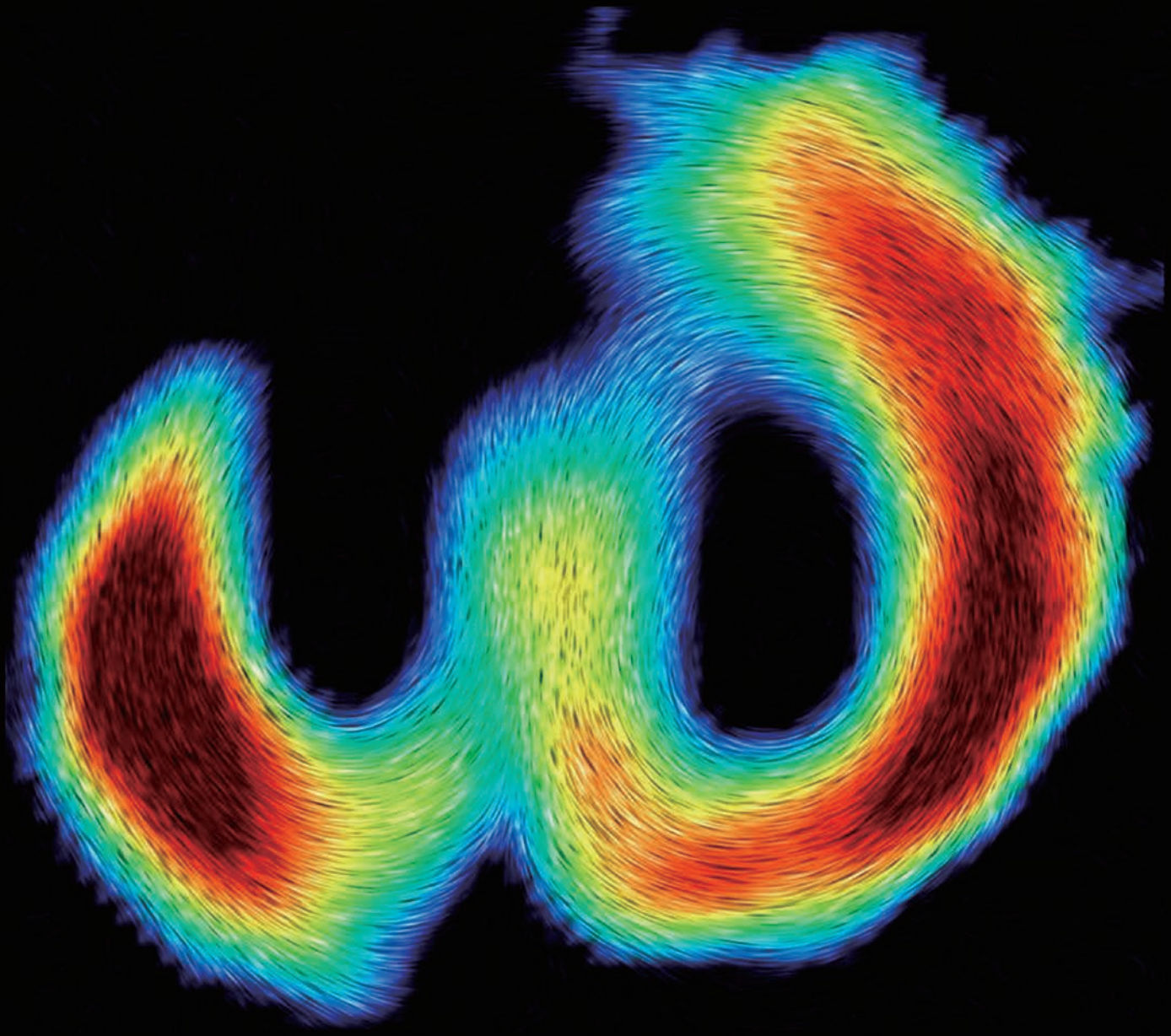
Vol. 90 No. 3 September 2025

CYTOLOGIA

International Journal of Cytogenetics and Cell Biology



Cellular flows during early chick embryo development

Technical Note

Live-imaging with quantitative analysis of cellular flows to visualize left-right asymmetry during early chick embryo development

Rieko Asai[1,2]*, Shubham Sinha[3], Vivek N. Prakash[3,4,5]*, and Takashi Mikawa[1]*

[1] Cardiovascular Research Institute, University of California, San Francisco, San Francisco, California 94158, USA
[2] Kumamoto University, IRCMS, Kumamoto, 860–0811, Japan
[3] Department of Physics, University of Miami, Coral Gables, Florida 33146, USA
[4] Department of Biology, University of Miami, Coral Gables, Florida 33146, USA
[5] Department of Marine Biology and Ecology, University of Miami, Miami, Florida 33149, USA

Received July 16, 2025; accepted July 18, 2025

Large-scale cellular flows during gastrulation represent an evolutionarily conserved phenomenon across the animal kingdom. During this process, bilaterian embryos morphologically initiate the left-right (LR) symmetric body plan along the midline. Avians have served as an excellent model for studying gastrulation in amniotes (*e.g.*, birds and mammals), owing to their accessibility and the morphological similarity of their gastrula to those of mammals. In a chick gastrula, vortex-like cellular flows, termed the 'polonaise movements', bilaterally occurs along the midline (Gräper 1929). It has been believed that bilateral cellular flows exhibit LR symmetry; however, our latest study quantified the cellular flows by combining high-resolution live-imaging with a fluid dynamics-based quantitative analysis method called Particle Image Velocimetry (PIV), and clearly identified LR asymmetry in the cellular flow patterns of the chick embryo (Asai *et al.* 2024, 2025).

The cover image generated by Shubham Sinha, Drs. Santhan Chandragiri and Vivek Prakash, which was originally from our latest study (Asai *et al.* 2025) illustrates the LR asymmetry in bilateral cellular flows during primitive streak formation in a chick embryo. Cell speed is color-coded in the image, with red representing areas of maximum speed and blue representing areas of minimal speed. The lines or streaks represent the velocity vector field, and were calculated using the line integral convolution (LIC) method of flow visualization. Since imaging was performed dorsally using an inverted microscope, the right side of the image corresponds to the right side of the embryo. Thus, this speed heatmap indicates that the area and the speed of the cell movements in the right side was larger compared to the left side of the embryo. Here, we briefly describe the materials and methods underlying this image. An unincubated chick embryo, known as an "embryonic disc" or "blastoderm", was isolated from the vitelline membrane and yolk in a Tyrode's solution and transfected with fluorescent-conjugated control morpholino by electroporation. The fluorescently tagged embryo was then transferred onto a new vitelline membrane, which had been stretched over a glass ring placed on albumin in a 35-mm dish. The dish containing the *ex ovo* cultured embryo was placed on the stage of an inverted microscope (Nikon Ti inverted fluorescent microscope with CSU-W1 scanner), which was enclosed in a dark hood equipped with a heating system maintained at 37°C. Live-imaging of the embryo was performed for approximately 10 h prior to and during primitive streak formation. The recorded sequence of images was post-processed using PIV (PIVlab package in MATLAB) to quantify the velocity and vorticity of the cellular flows.

Despite the presence of bilateral cellular flows, the underlying mechanisms of their LR-asymmetry remain unclear. Classical methods of *in situ* hybridization for laterality genes (*e.g.*, *Lefty*, *Shh*, and *Nodal*) did not detect LR-asymmetric expression during the same developmental stages as the cellular flows (Asai *et al.* 2025). Future state-of-the-art analyses may help elucidate the mechanisms of LR asymmetry in the cellular flows and their contribution to LR asymmetric body patterning.

References

Gräper, L. 1929. Die Primitiventwicklung des Hühnchens nach stereokinematographischen Untersuchungen, kontrolliert durch vitale Farbmarkierung und verglichen mit der Entwicklung anderer Wirbeltiere. Dev. Genes Evol. **116**: 382–429.

Asai, R., Prakash, V. N., Sinha, S., Prakash, M., and Mikawa, T. 2024. Coupling and uncoupling of midline morphogenesis and cell flow in amniote gastrulation. eLife **12**: RP89948.

Asai, R., Sinha, S., Prakash, V. N., and Mikawa, T. 2025. Bilateral cellular flows display asymmetry prior to left–right organizer formation in amniote gastrulation. Proc. Natl. Acad. Sci. U.S.A. **122**: e2414860122.

* Corresponding author, e-mail: rasai@kumamoto-u.ac.jp; vprakash@miami.edu; takashi.mikawa@ucsf.edu
DOI: 10.1508/cytologia.90.145