# Cellular v. Tissue Motion during Embryogenesis

Charles D. Little, Brenda J. Rongish, András Czirók, Evan A. Zamir, and Cheng Cui

## I. Purpose

The long-range goal of our studies is to understand the forces that shape the embryos of warm-blooded animals. The conceptual framework underlying our studies is that by using computational time-lapse imaging we can record and measure BOTH individual cellular trajectories and long-range tissue motion in a live embryo. Tissue motion analysis is based on measuring the passive displacements of endogenous extracellular matrix (ECM) fibers [1]. By subtracting the tissue displacements (ECM motion) from the total cellular displacements it is possible to calculate the residual, or actual, motion of individual cells [2]. Thus, our approach yields a measurement of relative motion, if any, between embryonic cells and the ECM scaffold upon which such cells locomote [3]. We examine embryos during, arguably, the most critical stages of embryogenesis — gastrulation and formation of the vertebral axis [2. 3].

## II. Results

To visualize biological motion we use two-color scanning time-lapse microscopy [4]. This fluorescence-based technique records the motion of tagged cells and their surrounding ECM fibers. The resulting data are then subjected to computational analysis. To conduct non-biased analyses of relative cellular and ECM motion we employ particle image velocimetry (PIV) derived from the time-lapse recordings [5]. Using the PIV-determined incremental displacement field, we "seed" an image with an array of evenly spaced "virtual material particles" (VMP), and then calculate the spatial (eulerian) trajectories for each set of particle coordinates. The main results show: 1) The displacements of VMP depicting ECM or cellular motion are large (hundreds of micrometers) and generally directed towards the midline or vertebral axis of the embryo. 2) The classical vortex-like movements near the vertebrate "organizer" are clearly visible regardless of whether the observer is examining individual cellular trajectories, VMP trajectories, or time-projected ECM immuno-fluorescence patterns [2, 3]. The data allow an observer to compare the VMP trajectory patterns and the corresponding cellular and ECM displacement maps at any arbitrary region of the embryo, and at multiple time points. The results demonstrate that trajectories for both individual cells and the corresponding ECM-VMP are approximately equivalent — with respect to direction and total cumulative distance traveled. Thus, the time-lapse data reveal that the motion of the ECM is very similar to the cellular motion pattern as demonstrated by both the VMP displacements, and the actual excursions of the fluorescent ECM fibrils. While there is evidence for "independent" or autonomous cellular motility, most of what is commonly referred to as "cell migration" is, in fact, due to passive displacement due to the motion of the surrounding ECM scaffold. The tissue driven cellular displacements are substantial, i.e., motion occurs across tissue-level length scales (millimeters). These results have profound implications for our understanding of vertebral axis formation and gastrulation, and will influence how future investigators study embryonic morphogenetic movements and tissue patterning — in particular the contributions of cell-autonomous migration versus composite tissue motion (cells+ECM). Further, all of these critical motion patterns are reported to be regulated by cellular responsiveness to extracellular chemotactic morphogen gradients. However, our data show that the extracellular milieu is characterized by constant motion — therefore any morphogen gradient, proposed to drive cellular "migration" is itself in motion. It will be incumbent upon investigators who advocate the importance of morphogen gradients to explain how such guidance mechanisms operate in a constantly moving environment.

## III. Conclusion

We conclude that models describing early embryonic cellular guidance mechanisms must take into account extracellular matrix motion.

This work was funded by NIHLB R01 068855 (CDL); NIH-NCRR P20 RR016475 (BJR); AHA Grant 0535245N (AC). The studies were conducted at the Department of Anatomy and Cell Biology, University of Kansas Medical Center. E-mail: clittle@kumc.edu

## References

[1] Czirok, A., Rongish, B.J., and Little, C.D. Extracellular matrix dynamics during vertebrate axis formation. Dev. Biol, 268,1:111-122, 2004.

[2] Zamir, E. A., Czirók, A., Cui, C., Little, C. D., and Rongish, B. J., 2006, "Mesodermal cell displacements during avian gastrulation are due to both individual cell-autonomous and convective tissue movements," Proc Natl Acad Sci U S A, 103(52), pp. 19806-19811.

[3] Zamir, E.A., Rongish, B.J and Little, C.D., The ECM moves during primitive streak formation—computation of ECM versus cellular motion. PLoS, Biology; 6(10): 2163-2171, 2008

[4] Czirók, A., Rupp, P., Rongish, B., and Little, C., 2002, "Multifield 3-D scanning light microscopy of early embryogenesis," Journal of Microscopy, 206, pp. 209-217.

[5] Zamir, E., Czirók, A., Rongish, B., and Little, C., 2005, "A digital image-based method for computational tissue fate mapping during early avian morphogenesis.," Ann Biomed Eng, 33(6), pp. 854-865.