# 3D visualisation of gene expression patterns in early embryos

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We present results from using high resolution episcopic microscopy (HREM) for three dimensional (3D) visualization of gene expression patterns in the context of the anatomy of small and early embryos (early gastrulation to late neurulation) of biologically and biomedically important model organisms. We show results from visualizing mRNA patterns in embryos of the zebrafish, the chick, and the milkweed bug. Our results show that HREM is capable of rapidly generating high resolution digital volume data of whole embryos in 0.54 µm x 0.54 µm x 1.5 µm voxel size. Furthermore they demonstrate, that HREM copes with all embryonic substances (e.g. yolk), which usually complicate 3D visualization of mRNA patterns and tissue information in early embryos.

**Background:** Detailed knowledge of the genetic and epigenetic mechanisms regulating early ontogenetic processes is the basis for researching the evolution of development as well as for researching the etiology of congenital malformations. Such knowledge is gained by systematic comparisons of normal and abnormal embryo anatomy and of normal and abnormal gene expression patterns in embryos. Early embryos are small, but highly complex spatial objects, consisting of various tissues and biological substances. Their three-dimensional (3D) analysis is not trivial. Methods like micro computed tomography (µCT) and micro magnet resonance imaging (µMRI) do not provide sufficient data resolution and do not permit the detection of specifically labeled cells and tissues (1, 2). Optical projection tomography (OPT, 3) is of limited use due to the relatively low resolution of OPT data (voxel size of 5 µm x 5 µm x 5 µm) and the suboptimal image quality, which do not permit detailed histological analysis. Confocal microscopy, which is optimized for the cellular and subcellular level cannot visualize entire embryos in sufficient data quality. Therefore, in most cases, traditional two-dimensional (2D) histological sections are still routinely used for 3D analysis. Methods, developed for generating volume data and 3D models out of histological section series (4), are not in widespread use, because they are time and personnel intensive and suffer from low spatial resolution (section thickness, which is the z-axis of a voxel is greater than 5 µm), or from alignment problems (5).

**Goal:** We aimed at testing the capacities of the recently developed episcopic imaging method High Resolution Episcopic Microscopy (HREM, 6), for visualizing and analyzing 3D mRNA patterns in the context of the gross morphology and tissue architecture of early embryos of the zebra-fish, the chick, and the milkweed bud.

**Material and Methods:** Chick (*Gallus domesticus*), zebrafish (*Danio rerio*), and milkweed bug (*Oncopeltus fasciatus*) embryos, from early gastrulation to late neurulation were harvested at the designated state and underwent whole mount *in situ* hybridization for *mlc2a, krp*, *hunchback*, *QIK*, *Tbx5*, and *Nkx2.5* mRNA. Then the specimens were prepared for HREM sectioning according to standard protocols (7). Volume data of 0.54 µm x 0.54 µm x 1.5 µm to 3 µm x 3 µm x 3 µm voxel size were generated with the HREM procedure (2 to 4 hours data generation time per specimen). The volume data were either analyzed in the software Amira (Mercury Computer systems) by using its virtual resection and volume

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rendering tools, or by creating 3D surface models by using the tools of the label field function of this software.

**Results:** We demonstrate that HREM imaging is capable of generating digital volume data of early chick, zebrafish and milkweed bug embryos, which permit a detailed 3D analysis of mRNA patterns in the context of tissues and organs. After whole mount staining, specimen preparation for HREM, sectioning is of a similar complexity and can be performed in the same time frame as specimen preparation for traditional resin embedding. Digital volume data, consisting of a stack of a few hundred subsequent block face images, which are aligned and do not show section artifacts, can be created within a few hours. In early embryos, the resolution of these data permits the visualization of single cells and tissues in the context of the entire embryo and, at the same time, the highly precise visualization of gene expression patterns (Figure 1). Usually early embryos consist of large amounts of substances, like yolk, which hinder 3D and even 2D imaging. Our results show that these substances do not have negative effects on HREM data quality.

**Conclusion:** Our results demonstrate, that HREM guarantees the rapid creation of highly authentic digital representations of gene expression patterns in early embryos. Thus, HREM is a powerful tool, which recommends itself as routine tool for 3D analysis of gene expression patterns in early embryos.

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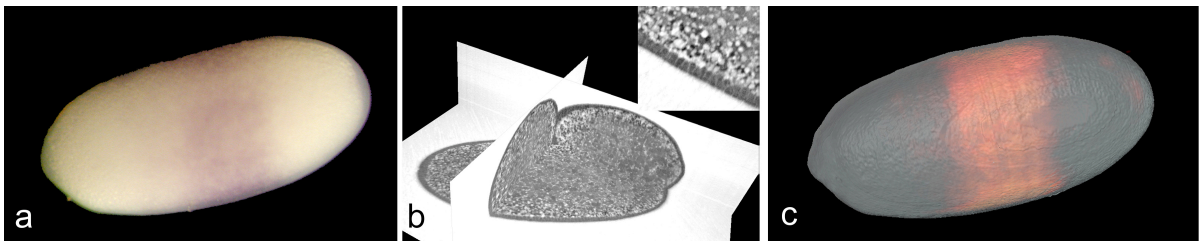


**Figure 1.** Blastoderm stage embryo (36 hours after egg laying) of the milkweed bug *Oncopeltus fasciatus* stained for expression of the gap gene *hunchback* **a.** Image of the embryo prior to resin embedding. Note the bluish stained *hunchback* expression patterns. **b.** HREM volume data set of this embryo. Note the single cells, surrounding the yolk. **c.** Volume rendered virtual 3D model created from the HREM data. Note the gene expression patterns in red.

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