

## **OPTICAL PROJECTION TOMOGRAPHY: A NEW APPROACH FOR 3D MICROSCOPY**

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While CT and MRI have become widespread and routine techniques within hospitals for examining tissues the size of human organs, they display a number of drawbacks for the imaging of much smaller specimens such as vertebrate embryos. As the intended resolution of MRI increases so does the required strength of the magnet used, such that systems designed to image mouse embryos become too expensive for most laboratories. Also, the achievable resolution is not usually sufficient to identify all the tissues or organs within the embryo. Additionally, since they are not optical techniques neither MRI nor CT scanning can image the distributions of commonly-used staining techniques, such as histological stains, standard immunohistochemical protocols, or in-situ hybridisation techniques used to visualise the RNA expression patterns of genes.

At the other end of the size scale is a well-established scanning technology which is indeed optical, but which has not been developed to image anatomy. Confocal laser-scanning microscopy is very efficient at generating clear, 3D images of specimens which have been fluorescently-labelled. However, it is typically used on specimens up to only a few hundred microns in thickness, and usually much less than that. An “imaging gap” has therefore been left between confocal and MRI, and unfortunately for developmental anatomists most vertebrate embryos fall precisely in that gap – too large for confocal imaging, and too small for MRI.

We have developed a new optical imaging technique, OPT microscopy, which fills this gap [1]. Rather than reducing the depth-of-focus as much as possible so as to pinpoint only a precise depth within the tissue (as in confocal microscopy), the OPT scanner tries to maximise its depth-of-focus. The raw data therefore do not explicitly contain information about depth. Instead the technique captures images of the specimen from many different angles and then employs the well-known principle of computed tomography (as used in X-ray CT scanners) to calculate a 3D reconstruction. Since it can image both fluorescent and coloured dyes, it can take advantage of the many cheap staining techniques in routine use in labs throughout the world. This in turn means that it can generate 3D maps of gene activity within intact fixed embryos (or other tissues) using standard laboratory *in-situ* staining protocols. We plan to develop this tool into a high throughput technology which will ultimately allow the mapping of thousands of gene expression patterns in small tissue samples.

[1] Sharpe *et al.* “Optical Projection Tomography as a Tool for 3D Microscopy and Gene Expression Studies” *Science* **296**:541-545 (2002).