Optical Projection Tomography

Stephen W. Carmichael* and Stephen A. Boppart**[1]
*Mayo Clinic and **University of Illinois at Urbana-Champaign
carmichael.stephen@mayo.edu and
boppart@uiuc.edu

There are many approaches to obtaining high-resolution images and three dimensional volumetric data sets, but all have limitations. Many techniques involve reconstructing volumes of information from sections, either physical sections or optical sections. Recently, James Sharpe, Ulf Ahlgren, Paul Perry, Bill Hill, Allyson Ross, Jacob Hecksher-Sørensen, Richard Baldock, and Duncan Davidson have developed an optical technique that is analogous to computed tomography (CT).[2] Whereas clinical CT involves an X-ray source and detector rotating around the patient, optical projection tomography (OPT) has the specimen rotating within an optical pathway. OPT is based on the principle of projection tomography, as is CT, but rather than using a fan-beam projection of x-rays, OPT uses projections from an image-focusing optical system (a microscope), thereby allowing standard fluorescent imaging to be performed, as well as brightfield imaging.

The specimen (in this case, a mouse embryo) was suspended within a transparent cylinder of agarose gel and mounted in a specially constructed apparatus that rotates through 360° around a single central axis that is perpendicular to the optical axis of a dissecting microscope. Digital images of projection data were recorded at angular steps of 0.9°, producing 400 images over one complete revolution. Virtual sections at arbitrary planes could be reconstructed from the projection data using a back-projection algorithm. OPT sections were remarkably similar to physical sections, demonstrating that OPT microscopy can faithfully map the light-absorbing and scattering properties of tissue as long as they did not vary dramatically within the specimen.

Sharpe *et al.* were interested in extending OPT to visualize the topography of gene expression in the developing mouse. OPT was performed before and after applying a standard *in situ* hybridization protocol to visualize the expression of a specific mRNA. Reconstruction of the specimen data gave a detailed three-dimensional view of the expression pattern. Furthermore, virtual sections from any orientation in the 3D data set not only compared well to results of physically-sectioned specimens, but also displayed the expected hybridization patterns.

OPT was also tested with fluorescent dyes. Sharpe *et al.* adapted fluorescent immunohistochemistry protocols for whole-mount staining of embryos, then placed the specimens on the OPT rotational stage under a dissecting microscope equipped for fluorescence microscopy. Filter sets could be sequentially inserted to gather data at three different wavelengths. Because the embryo could be imaged in exactly the same position for each fluorescent channel, registration of the images was not a problem. Using suitable software, impressive movies were made that can be viewed at http://genex.hgu.mrc.ac.uk/OPT_Microscopy.

Whereas the back-projection algorithm used in this study has been utilized extensively in the medical imaging community for years, the application of this algorithm in optical imaging represents a novel microscopic approach for visualizing morphology and gene expression in the mouse. Because living tissue cannot be cleared to reduce the scattering of light in the same way as fixed tissue, the use of this technique on *in-vivo* specimens may be problematic. Regardless, functional genomics will undoubtedly require new imaging methods such as OPT to understand the complex morphological and functional expression of the wildtype and modified genome.

References:

1 The authors gratefully acknowledge Dr. James Sharpe for reviewing this article.

2 Sharpe, J., U. Ahlgren, P. Perry, B. Hill, A. Ross, J. Hecksher-Sørensen, R. Baldock, and D. Davidson, Optical Projection Tomography as a Tool for 3D Microscopy and Gene Expression Studies, *Science* **296**:541-545, 2002.

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ABOUT THE COVER

by Janina M. Radzikowska
The Foundry Research Institute, Krakow, Poland

Microstructure of austempered ductile iron (ADI) after isothermal heat treatment revealing a graphite nodule in a matrix of lower bainite (colored) and retained austenite (white) revealed by tint etching with a modified Beraha's martensite etch and viewed with polarized light at about 800X. This image won "Second in Class" in the International Metallographic Society, International Metallographic Contest, "Artistic Microscopy" category at the recent M&M-2002 meeting.