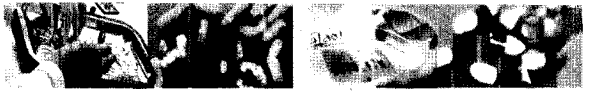

Keynote Lecture I

Intravital Imaging: An Interdisciplinary Approach towards Following Complex Events within Intact Biological Systems

Scott E Fraser

Beckman Institute, California Institute of Technology, USA

Professor Scott E Fraser has a long-standing interest in the imaging and molecular analysis of intact biological systems, and has been active in developing new technologies for novel assays. He has been the Anna L Rosen Professor of Biology and Director of the Biological Imaging Center at the Beckman Institute at the California Institute of Technology since 1991, and the Director of the Caltech Brain Imaging Center since 2002. Before coming to Caltech, he served on the faculty and was the Chair of the Department of Physiology and Biophysics at the University of California, Irvine. Professor Fraser earned his BS with honors in Physics from Harvey Mudd College and his PhD in Biophysics with Distinction from Johns Hopkins University. He has been active in the advanced training of interdisciplinary students and post-doctoral fellows, serving as the co-director of the Marine Biological Lab's Embryology Course (with Professor Marianne Bronner-Fraser) and the co-director of Caltech's Initiative in Computational Molecular Biology (with Professor Michael Roukes). Dr Fraser is involved in many professional societies including the American Association for the Advancement of Science; the Society for Developmental Biology; the Society for Neuroscience; the Biophysical Society; the Society of Photo-Optical Instrumentation Engineers; and the American Society for Cell Biology. He is Editor of the journal Developmental Biology, and serves on the editorial boards for NeuroImage, Molecular Imaging and Development. His teaching has earned recognition: the Silver Beaker Award for Best Medical School Faculty Member, the Kaiser-Permanente Award for Best Medical School Teaching, and the Caltech Graduate Mentoring Award. Dr Fraser was also awarded the McKnight Scholar Award, the Marcus Singer Medal, and was named a Fellow of the American Association for the Advancement of Science and the European Academy of Science. Recent awards include the R&D100 Prize and the NASA Space Act Prize for the invention of new microscope techniques.

The explosion of progress in the fields of cell biology, biochemistry, and molecular biology has offered unprecedented knowledge of the components involved embryonic development. The dramatic progress of these reductionistic approaches poses the challenge of integrating this knowledge into an understanding of developmental mechanics that pattern and construct the embryo. The classic publications in the field of experimental embryology illustrate the power of describing cell behavior (cf. lineages, movements) and perturbing the embryo to test hypotheses of the underlying mechanisms. Advanced imaging techniques offer an important stepping-stone between these disparate approaches, permitting questions about cellular and molecular events to be posed in the most relevant setting of the intact embryo.

Both confocal laser scanning microscopy (CLSM) and two-photon laser scanning microscopy (TPLSM) permit cells to be followed as they migrate in the intact embryo. In vivo imaging of multiple labels should offer the ability to test proposed mechanisms by following different GFP-color variants on multiple molecular species in the same cell. Multispectral approaches involve acquisition of the spectrum of the emitted light from each pixel, followed by decomposition into its component parts by simple mathematics. Fluorochromes as similar as GFP and fluorescein can be separated unambiguously, and even small amounts of FRET can be detected by our approach.

In systems in which light-based imaging is problematic, we are employing microscopic MRI. In MRI, radio frequency energy is used to excite the protons of the water, which generates no toxic by-products. Spatial

resolution is created by imposing gradient magnetic fields on the specimen, thereby making it possible to encode the signals from individual volume elements (voxels) by their resonant frequency and phase. By increasing the magnitudes of the static and gradient magnetic fields, and by improving the electronics, it has become possible to increase the resolution of MRI from the 1mm voxels of a clinical instrument to ~10μm. This approach has the promise of making imaging analyses possible in the systems with limited access to the embryos (e.g. mouse) or in which light scattering renders deep structures invisible (e.g. frog).

Here, MRI microscopy will be used to follow the fates and motions of amphibian gastrulation. Recent studies have defined a set of molecular events involved in the establishment and the function of the Spemann organizer, originally defined by its ability to induce and organize a secondary body axis when grafted to the future ventral side of an embryo. Despite the frog serving as the standard by which results on axis specification in other species are cast, significant open questions remain as to the nature of neural induction in the amphibian. Two opposed pathways of neural induction remain viable: vertical signaling models, in which the mesoderm and endoderm (mesendoderm) involute around the blastopore lip and then induce the overlying ectoderm; planar signaling models argue that the critical inductive interactions take place between mesendoderm and ectoderm in the plane of the embryonic surface before the involution motions of gastrulation. Microscopic magnetic resonance imaging allows us to follow cell movements and contacts in the Spemann organizer region before and during gastrulation. Key events such as vegetal rotation and epiboly bring surface ectoderm and mesendoderm into contact long before the outward signs of gastrulation. A surprising resolution of the debate between vertical and planar signaling models is the finding that the axial mesoderm is internally localized and in vertical contact with much of the future neurectoderm throughout early development. These observations are consistent with the direction of signaling proposed by vertical models and the early timing of the signaling proposed by planar models.