Complex and dynamic tissue microenvironments play critical roles in health and disease – examples include tumors, stem-cell niches, brain tissue surrounding neuroprosthetic devices, retinal tissue, cancer stem-cell niches, and immune system components. Progress in these areas is much too slow compared to the need. Knowledge is pieced together painstakingly from large numbers of experiments, each of which collects a small amount of information. Much of the knowledge still remains qualitative. There is a compelling need to accelerate progress towards a quantitative understanding. In this talk, I will describe strategies based on multi-dimensional optical microscopy and computational image analysis.

Modern optical microscopy has progressed dramatically, and is poised to transform the manner in which biological microenvironments are studied in the laboratory and diagnosed in the clinic. Fluorescence multiplexing & multi-spectral microscopy enables structural and functional entities to be imaged simultaneously in their relative spatial context. Multi-photon time-lapse imaging with multiple fluorescent proteins makes it possible to record dynamic processes in living specimens in the form of 5-dimensional movies (3-D space, time, spectra). Each 5-D movie is far more informative than numerous lower-dimensional images, since it directly reveals biological processes in their full spatial habitat and temporal order. These technologies can revolutionize the study of complex biological systems by yielding discoveries at an accelerated pace, providing quantitative measurements of complex phenomena, setting the stage for a much-needed system-level understanding.

The primary barrier preventing 5-D imaging driven biological research is the fact that the size & complexity of 5-D datasets exceeds human analysis ability. There is a need for automated systems to map the tissue anatomy, quantify structural associations, identify critical events, and map their anatomic locations and timing, identify and quantify spatial and temporal dependencies, produce meaningful summaries of multivariate measurement data, compare 4-D/5-D datasets for hypothesis testing, exploratory studies, and predictive modeling.

The NIH-funded FARSIGHT project is based on 4 key ideas: (i) ‘divide and conquer’ approaches to tackle the classical challenges of automated image segmentation, parameter tuning, and object tracking; (ii) efficient pattern analysis aided inspection & wholesale editing of automated segmentation results; and (iii) use of graph theory to represent spatio-temporal associations; and (iv) algorithmic graph data mining methods to summarize and analyze associations. These ideas form the basis for an open source toolkit. To illustrate these ideas, I will use examples from neuroscience, breast histopathology, immunology, and retinal stem-cell biology. Finally, I will describe my view of the future of associative image analysis, and relate them to great challenges and ‘holy grails’ of bioscience.