
**Time resolved image microscopy.**


**Abstract**

"Time Resolved Image Microscopy" integrates temporally resolved dynamic properties with the spatial resolution of the light microscope. Fast kinetic and luminescence decay parameters are measured simultaneously at every pixel of a CCD image. We are developing time resolved methods that can be applied conveniently and routinely to biological material in the microscope over a large time domain. In addition to the augmented purely spectroscopic and reaction kinetic information, simultaneous spatial and temporal resolution of an image in a microscope provides significant improvement in image contrast, probe identification and differentiation. For instance, the ability to separate phosphorescence and prompt fluorescence furnishes a new parameter, the ratio of delayed to prompt luminescence at every pixel of the picture, emphasizing particular objects. The time resolution makes it possible to recover structures in an image that are concealed by faster decaying intense luminescence. Examples of these procedures for both delayed luminescence and prompt fluorescence will be given, and the instrumentation required for data acquisition and analysis will be discussed. A normal fluorescence microscope is used; phase-locked coordination between the modulation and image recording is used.