## Structure and dynamics of cellular components using fluorescence and X-ray diffraction techniques

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The techniques of Fluorescence Recovery After Photobleaching (FRAP) and Fluorescence Correlation Spectroscopy (FCS) can be employed using the optics of a confocal microscope to examine the organization and dynamics of different components in cellular systems. For these studies the protein of interest is generated as a chimera with the green fluorescent protein (GFP) and expressed in transfected live cells. We have prepared constructs encoding GFP appended to N-terminal fragments of a series of exported malaria parasite proteins, including the major cytoadherence antigen, PfEMP1. We have used FRAP techniques to examine PfEMP1-GFP dynamics in live cells and have found that the chimera exhibits a half-time for fluorescence recovery of a few seconds indicating that it is trafficked to the host cell membrane as a protein complex. These measurements are at the limit of the accessible time domain using FRAP analysis, therefore we have explored the use of FCS as a means of monitoring the rapid motion of GFP-labelled proteins. For FCS measurements, fluctuations in fluorescence intensity are measured as molecules move in and out of a small illuminated region (volume ~0.5 fL). Analysis of the fluctuations as a function of time provides information about the diffusion of the labelled species and has enabled us to measure the diffusion coefficient diffusion of GFP in the cytoplasm of the malaria parasite.

A new Centre of Excellence in Coherent X-ray Science has been established to develop techniques for imaging cellular architecture with greatly increased resolution and to develop methods for determining the structures of membrane protein without the need for crystallisation. The methods employ a highly coherent curved beam and imaging in the far field with iterative Fourier transformation protocols to extract phase image information. Soft X-rays have wavelengths of about 1-10 nm; these wavelengths allow imaging at high spatial resolution and will be used to study the intracellular structures of *P. falciparum*-infected erythrocytes with a resolution of down to 10 nm.