Abstract

Background
In recent years *in vivo* microscopy studies have revealed that the nucleus and its components are more dynamic than previously assumed. A widely used technique in these studies is fluorescence recovery after photobleaching (FRAP) which allows characterizing the dynamic behavior of proteins *in vivo*. Besides a qualitative interpretation of the data, quantitative FRAP procedures allow measuring the dynamic properties of proteins, namely diffusion and binding to chromatin. Unfortunately, the accuracy of these estimates is uncertain, since no benchmark exists.

Results
This present study improved FRAP methodology in three areas. First, the establishment of benchmark measurements was begun by comparing three recent studies on transcription factor binding to DNA. The published binding estimates for each protein were significantly different. When comparing the FRAP procedures it was found that that this difference was due to previously unidentified incorrect assumptions. Corrected procedures were developed and when applied to the three proteins consistent estimates were obtained. This showed not only that the errors identified were corrected, but also suggested that these proteins have a similar mode of interaction with chromatin. Second, existing FRAP models were extended to account for inhomogeneous distributions of binding sites by combining different modeling approaches. These models were successfully applied to analyze the binding behavior of two nuclear proteins in more detail. Third, given that quantitative FRAP procedures might still be flawed, the qualitative FRAP approach was improved by developing novel tools to analyze and visualize data. The value of these improved tools was demonstrated in two publications.

Conclusions
This study illustrated vividly that *in vivo* binding measurements are not trivial and when done improperly can lead to erroneous biological conclusions. Improved methods for FRAP were presented and it was demonstrated how the combination of different experimental and modeling approaches can be utilized to better understand the dynamic behavior of nuclear proteins. Continuing this combined approach in future studies will improve the accuracy and reliability of these methods and ultimately help to understand protein function in the complex nuclear network.

Keywords
FRAP, protein dynamics, live cells, chromatin binding, modeling