Using the Virtual Cell Simulation Environment for Extracting Quantitative Parameters from Live Cell Fluorescence Imaging Data

A. E. Cowan*, Y. Li, F. R. Morgan, D. E. Koppel, B. M. Slepchenko, L. M. Loew, and J. Schaff
Center for Cell Analysis and Modeling, University of Connecticut Health Center, 263 Farmington Ave., Farmington, CT 06030
* acowan@nso2.uchc.edu

Introduction

Rapid advances in fluorescence probe and imaging technologies now provide easily accessible tools for biologists to perform highly detailed analysis of molecular interactions in living cells. However it can be difficult to extract accurate parameters from these experiments because of the complex interplay of diffusion-reaction events with the morphology of the cell. As a result, only a small fraction of the available spatiotemporal information is utilized, and in many cases analysis remains at a qualitative level. The Virtual Cell (VCell, http://vcell.org) simulation environment is uniquely suited to analyzing these types of fluorescence imaging experiments because it is designed to solve reaction-diffusion equations within any given geometry [1].

VCell is a problem-solving environment for analysis, modeling, and simulation of cell biological processes within realistic geometries. VCell integrates a growing range of molecular mechanisms, including reaction kinetics, diffusion, flow, membrane transport, lateral membrane diffusion, and electrophysiology. It can associate these mechanisms with geometries derived from experimental microscope images. It is a web-based, client-server system, with more than two thousand worldwide users. The user interface allows experimentalists to input a physiological model in easily understood terms of species, reactions, and compartments. Segmented images derived from experiments are used to create a 2D or 3D geometry that matches the experimental cell for direct comparison. Development of the underlying math description and numerical methods to solve the simulation are transparent, although easily accessible for analysis and editing if desired. Furthermore, VCell can import almost any kind of generic images and proprietary microscopy data to be used within simulations to define arbitrary "fields" as the initial conditions of a simulation. Such fields can specify distributed parameters, initial conditions, or even *x,y,z* components of vector fields corresponding to, for example, cytoskeletal organization. With VCell, a user can predict the spatiotemporal distribution of all molecular species given full information about initial distributions, model parameters, external stimuli, and the cellular geometry.

Many VCell models published by the Center for Cell Analysis and Modeling (CCAM) and others were created to simulate specific experimental situations. Examples include models to analyze cellular effects of uncaging [2], experiments performed using total internal reflection optics [3], Fluorescence Loss in Photobleaching (FLIP) experiments to assess membrane association of the small GTPase rac [4], translocation of a GFP-tagged pleckstrin homology domain sensor for PIP2 and IP3 [5], and Fluorescence Redistribution after Photobleaching (FRAP) studies of tight junction components [6]. The client-server architecture of VCell provides the infrastructure for maintaining models within a VCell database, making models easily accessible for re-use by the investigator or, if published, by the scientific community. This makes it relatively straightforward for investigators to modify existing models and apply them to the geometry of their own experimental cells.

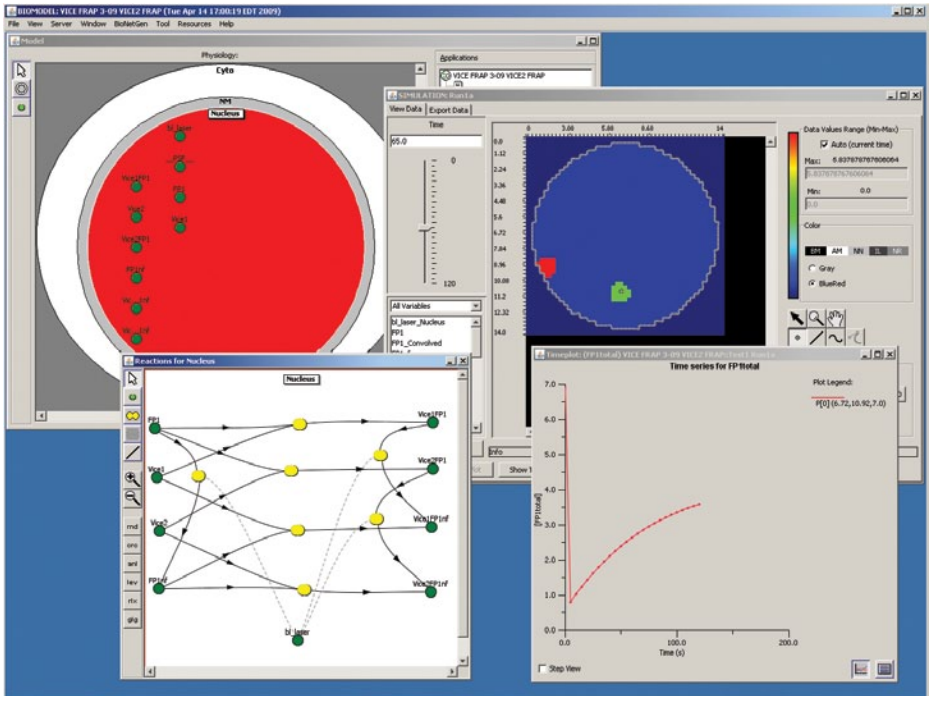

Figure 1: A Virtual Cell model used to simulate photo-bleaching experiments, designed to determine the kinetics of protein binding to specific nuclear domains.

Although VCell is a powerful general-purpose simulation tool, light-weight toolboxes oprtimized for specific routine data analyses could be of help. We are therefore developing a suite of application-specific tools based on VCell technology to facilitate data analysis. This project, the "Virtual Microscopy Suite," will contain a series of applications, each directed at a single analysis mission, and they will be experiment-centric rather than model-centric. These tools will require that we develop new algorithms for directly comparing and optimizing simulations

 doi: 10.1017/S1551929509991039

https://doi.org/10.1017/S1551929509991039

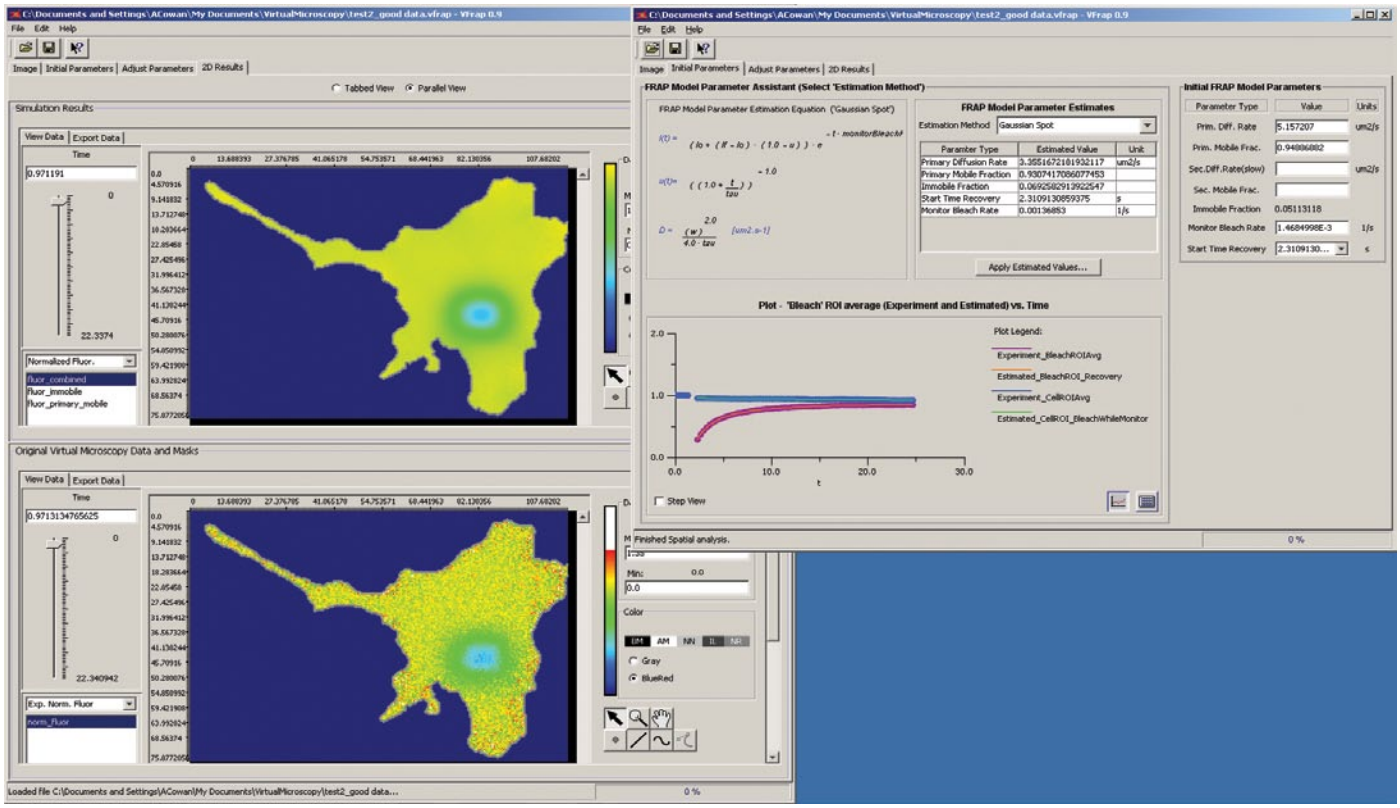

Figure 2: Comparison of the results from a simulation of a fluorescence photobleaching experiment (top cell) to the actual experimental data (bottom cell), using the VirtualFRAP tool.

against the original experimental data. The first of these tools is VirtualFRAP, a downloadable executable designed to analyze FRAP experiments (http://vcell.org/vfrap). It includes optimization algorithms for partial differential equation models and the ability to use experimentally derived heterogeneous molecular distributions as initial conditions for simulation and parameter fitting. Currently, VirtualFRAP can be used to analyze two-dimensional FRAP experiments of whole cells to fit diffusion coefficients and fractional recovery for either one or two diffusing components of cytosolic (soluble) proteins. We are continuing to develop VirtualFRAP and to create additional components of the Virtual Microscopy Suite. On the list for future development are tools for the analysis of other photochemistry-based microscope manipulations, such as FLIP, uncaging, or photoactivation experiments, as well as for the analysis of biosensors, probe translocation, and Fluorescence Resonance Energy Transfer (FRET) experiments [7]. MT

References

[1] B M Slepchenko et al., *Trends Cell Biol* 13 (2003) 570-76.
[2] P Roy et al., *J Cell Biol.* 153 (2001) 1035-48.
[3] M Holt et al., *Curr Biol* 14 (2004) 173-83.
[4] K Moissoglu et al., *Mol Biol Cell* 17 (2006) 2770-79.
[5] C Xu, J Watras, and L M Loew. *J. Cell Biology* 161 (2003) 779-91.
[6] L Shen et al., *J Cell Biol* 181 (2008) 683-95.
[7] Virtual Cell is developed by the National Resource for Cell Analysis and Modeling, supported by NIH P41RR13186; Virtual Frap is supported by U54RR022232.

https://doi.org/10.1017/S1551929509991039 Published online by Cambridge University Press