ABSTRACT

Cellular signal transduction involves a transport step from the plasma membrane towards the nucleus, during which the signaling molecules are partly deactivated in control loops. This leads to a gradient in the concentration of active signaling molecules. The low number of molecules introduces spatio-temporal fluctuations and the asymmetric cellular architecture further increases the complexity. We propose a technique to represent this pattern in a continuous three-dimensional concentration map. The local concentration is computed and visualized with volume rendering techniques at interactive frame rates and is therefore well-suited for time-dependent data. Our approach allows the transition from the nano-scale of single and discrete signaling proteins to a continuous signal on the cell level. In the application context of this paper, we employ an agent-based Monte Carlo simulation to calculate the actual particle positions depending on reaction and transport parameters in the cell. The applicability of the proposed technique is demonstrated by an investigation of the effects of different transport parameters in Mitogen-activated protein kinase (MAPK) signaling.

Index Terms—Cellular signal transduction, agent-based simulation, protein concentration, visualization, direct volume rendering

1. INTRODUCTION

Cancer is the outcome of over-expressed or misregulated signal transduction pathways in the cell. One of the most important pathways in this context involves the Mitogen-activated protein kinase (MAPK) family [2]. The interaction network and its dynamics can be well described by mass action kinetics and sets of ordinary differential equations (ODE) [1]. However, this approach omits the spatial properties of the cell, especially the distance the activated signaling molecules have to travel between the plasma membrane and the target genes which have to be regulated in the nucleus. In case of MAPK, which is locally activated at the plasma membrane by upstream components of the signaling cascade, the global deactivation in the plasma membrane leads to a gradient in the concentration of active signaling molecules [5]. The signal transduction profile becomes even more complex, if the detailed intracellular architecture, molecular crowding and the intrinsic stochasticity of the discrete signaling molecules is taken into account [10].

While the most detailed approach assembles the signal transduction process from the individual protein with in vivo properties under in vivo conditions, the ODE framework describes the effective behavior and creates a continuous average. In order to explore this transition from the discrete microscopic molecular view to the macroscopic continuous space we have previously developed an agent based Monte Carlo (MC) simulation framework which tracks individual proteins through a realistic virtual cell [3, 6]. Thus, effects like hindered diffusion, transport with motor proteins, and molecular crowding can be explored as well as the influence of non-uniform or symmetric cellular architectures. The resulting effective properties, e.g. the effective diffusion, can be calculated by taking the average of the ensemble [6]. The associated visualization framework provides a virtual microscope whose resolution is only limited by the accuracy of the simulation [3].

In this work, we propose a novel approach to compute three-dimensional concentration maps. The relatively small molecules are difficult to see in the context of the relatively large and crowded cell. Although every molecule is equally important for the signal transduction process, the knowledge of just one molecule position is not sufficient to understand the signaling outcome. A continuous representation is in contrast easier to interpret and more comparable to the results of differential equations.

Accordingly, the visualization framework can be used to calculate local, sub-cellular properties or statistics. Tuning a respective local filter kernel leads to a transition from discrete particles via discontinuous local accumulations of molecules towards a global concentration distribution in the cell, showing how the signal is in fact transported. Direct volume rendering [7] is employed for the visualization on a recent graphic processing unit (GPU), which provides interactive frame rates. The fast calculations make it a useful tool to follow the temporal development of the signal as well as the interactive in-depth exploration of the signal transduction properties. In Fig. 1 the computed concentration map is combined with a virtual cell visualization.

---

**Fig. 1:** Visualization of a virtual cell showing the results of a MAPK simulation. Cell components include: nucleus (white), cytoskeleton (purple lines), receptors (gray) at the cell membrane, phosphatases (yellow), and MAPKp signal proteins (green). The continuous signal concentration is computed from the MAPKp proteins. High concentrations are mapped to red, low to blue.

---

* Martin Falk
† Michael Klann
‡ Matthias Reuss
§ Thomas Ertl

* VISUS – Visualization Research Center, Universität Stuttgart, Germany
† Institute of Biochemical Engineering and Center Systems Biology, Universität Stuttgart, Germany

---

e-mail: {falk|ertl}@vis.uni-stuttgart.de
e-mail: {klann|reuss}@ibvt.uni-stuttgart.de
2. TECHNIQUE

We use a discrete-time, continuous-space, agent-based model to analyze the distribution of e.g. signals in the cell [6]. In the simulation, a virtual cell is set up by defining a spherical plasma membrane as boundary of the cell, inserting a spherical nucleus at a predefined position and a cytoskeleton created by elongated cylinders [3]. If needed, molecular crowding can be introduced by additional spheres which occupy the respective volume. The signal molecules of interest are represented by spheres with the hydrodynamic radius of the physical molecule. In every time step of the simulation, the molecules move according to a random walk, where the step length depends on the diffusion coefficient and the time step. Alternatively the molecules can move with a constant velocity along cytoskeleton cylinders if transport by motor proteins is activated. Particles can react with each other if they are closer than a critical distance as described in [8]. Likewise they can bind to the cytoskeleton for motorized transport if they are close enough. For first order reactions the decay probability within \( \Delta t \) is used to determine the reaction of individual molecules.

The signaling model presented here includes the essence of the MAPK signal transduction cascade. We include a plasma membrane bound receptor complex representing the upstream part of the cascade and the MAPK as a mobile component that transports the signal to the nucleus. The MAPK is activated (phosphorylated) at the receptor complex and then diffuses into the cell. In the cytoplasm, the activated MAPK is attacked by phosphatases which deactivate (dephosphorylate) it. The dephosphorylation reaction is modeled as a first order reaction – the phosphatases are only implicitly included.

The concentration or protein density can be approximated by computing local densities. The cell is subdivided into volume elements (voxels) and for each voxel the number of enclosed proteins is determined. Dividing this number by the voxel volume yields the local density. Due to the spatial fluctuations of the discrete and stochastic protein distribution the local density exhibits spikes of high concentrations as well as empty regions around the spikes. At higher particle concentrations or larger voxels the fluctuations become less pronounced. However, the number of particles is determined by the simulation or experimental data. Likewise larger voxels would lead to a reduced spatial resolution. In order to obtain smooth concentration profiles, we therefore employ a Gaussian-like filter kernel.

In order to generate such a concentration map, the cell is subdivided into voxels with a three-dimensional uniform grid. This volume is then updated slice by slice. Each slice has a thickness of one voxel and the density contribution of all proteins has to be computed for all voxels in this slice. The contribution of one protein is obtained by drawing its footprint at the protein location. The footprint is scaled so that it covers the support of the used filter kernel. Each voxel of the currently processed slice, which is covered by this footprint, is updated by evaluating the density function of the kernel with respect to protein and voxel location. Proteins which are too far away from the current slice can be discarded as they do not contribute to the density. The contributions of all proteins are accumulated in each voxel. After computing all slices we obtain a three-dimensional scalar field containing the protein concentrations of the simulated cell.

We use volume rendering techniques to visualize this scalar field [7]. Here, a ray is cast from the virtual camera through every pixel of the window. Each ray is sampled at equally spaced positions, where a look-up in the density volume is performed. A transfer function is used to map density values to color and opacity. The result is a semi-transparent visualization of the protein concentration. Additionally, the shape of the signaling front can be extracted by means of an isosurface. This isosurface is determined and rendered during volume rendering. If two subsequent samples along the ray lie on different sides of the isosurface, the intersection between ray and isosurface is obtained by linear interpolation. The isosurface is shaded and blended with the volume rendering. Spatial perception is improved by means of illumination. In the final step, the volume visualization is combined with the previously rendered cell components [3] to further enhance perception (Fig. 1).

When the rendered density volume is examined from the outside it appears smoothed and exhibits only small changes in the gradient due to the Gaussian-like filter kernel. The concentration distribution is hidden inside, hence clip planes are essential for investigation. Clip planes, which can be positioned arbitrarily, allow the extraction of cellular slabs or cutting the cell to reveal cross-sections.

Current off-the-shelf graphics processing units offer an highly parallelized architecture which is perfectly suited for the tasks of computing the concentration volume and the volume rendering. The computation of the volume can be parallelized easily as each voxel is updated independently. Ray casting on the GPU, e.g. [9, 4], allows for an interactive visualization of the protein concentrations. C++ and OpenGL with GLSL shaders are used for our GPU-based implementation.

3. RESULTS

MAPK signaling molecules are phosphorylated by the activated receptor complex at the plasma membrane and transported into the nucleus. Both, in the cytosol and in the nucleus they can be deactivated by phosphatases. The cytoskeleton is originating just left of the nucleus and radially extends towards the plasma membrane (compare Fig. 3 left). This leads to an overall asymmetric cell, and the asymmetry increases if the nucleus is not placed in the center of the cell. The cytoskeleton provides a track for motor proteins to actively and directly transport the activated MAPK molecules into the cell. We compared undirected diffusion, which is obstructed by the cytoskeleton filaments with transport by motor proteins.

Fig. 3 shows the results for both transportation modes. Diffusion is depicted in the first row and the transport by motor proteins in the second row. In the left image signaling proteins are represented by small spheres (geometric visualization). The exact positions of the signaling molecules are obvious. Spots with high density emerge when the concentration map, computed with a small filter kernel, is visualized. As the filter radius is increased, the density distribution becomes more continuous. Transport by diffusion gives a more uniform distribution than the motorized transport, where the molecules cluster around the cytoskeleton on which they are transported and thus highlight it. The graphs on the far right show the average radial concentration profile. While the local fluctuations can be used to calculate the statistic properties of the concentration profile, the detailed spatial information is lost. Obviously, the radial concentration profile only makes sense in a spherical symmetric cell.

The temporal development of the average radial concentration profile is shown in Fig. 2. The difference between diffusion and motor protein transport is evident. In case of diffusion the signal is attenuated due to the deactivation. In case of motor proteins the directed motion into the cell center is fast enough to overcompensate the deactivation and leads to a perinuclear accumulation. The volume of the spherical shell just around the nucleus is much smaller than the volume of the spherical shell at the plasma membrane, where the activated molecules originated. Even though many
molecules are deactivated, the remaining ones arrive in a much smaller volume, which leads to a higher concentration. Fig. 4 depicts the spatial development of the signaling front in four steps. Again, the effect of the cytoskeleton is apparent: the signal concentrates at the center of the cytoskeleton. The graph in Fig. 4 shows the accumulation of signal proteins in the nucleus. The peak in case of motor transport comes from the accumulation of active signaling molecules in the nucleus. They do not recycle quick enough for a further round of activation at the plasma membrane. The steady state is subsequently reached at a lower level of active MAPK molecules in the nucleus. In the case of diffusion, a much lower signal arrives at the nucleus. Spatial properties, like e.g. a shifted nucleus increase the signal by only 9% compared to the centered nucleus.

200 seconds of the MAPK simulation with approximately 30,000 signaling proteins took about 2 hours on one core of an Intel Core2 Quad Q6700 with 2 GB RAM. Measurements of the rendering performance were conducted on a Windows PC with a Intel Core2 with 2.4 GHz, 2 GB RAM, and a NVIDIA GeForce 280 GTX with 1 GB. The resolution of the density volume was set to 256 × 256 × 256. We measured between 10 and 30 frames per second for the computation and visualization of the concentration map depending on the size of the filter kernel.

4. DISCUSSION

It is a challenging task to extract all information from three-dimensional data. Projections into two-dimensional static images and even more one dimensional profiles abandon information. The human perception is however not able to grasp all details from a static image. Cross-sections (see Fig. 5) are a good way to gain insights, especially in a asymmetric data set. To fully access the information, it is crucial to be able to explore the simulated data in an interactive manner (see accompanying movie at http://www.vis.uni-stuttgart.de/~falkmn/signalfront). This applies in particular to time-dependent data. Only the combination of the detailed views and average properties advance the understanding of three-dimensional data.

In this work, we have presented an interactive GPU-based technique to compute and visualize cellular signal concentrations from agent-based Monte Carlo simulations. Filtering is used to perform the transition of discrete particle locations into a contiguous concentration map. The volume rendering of this map was combined with existing cellular visualization to improve spatial perception. The signaling pathway of MAPK was used to study the effects of diffusive transport and transport by motor proteins. As our approach is independent of the underlying model or the scale of the particle distribution, it could also be applied to analyze e.g. drug concentration in a solid tumor [3].

Interesting future directions include the investigation of vesicular transport of the receptor and improving the cellular model. The first step will be to apply a more realistic cellular architecture and non-spherical cells. Skeletal data reconstructed from cells could be used to replace the current regular cytoskeleton. Refining our MAPK model to explicitly contain all species of the signaling cascade would allow a comparison with experimental data or images. The visualization should then be enhanced to handle more than one concentration map when studying the differences between different signal states.

5. ACKNOWLEDGMENTS

M.F., M.K., and M.R. would like to thank the state of Baden-Württemberg and the Center Systems Biology in Stuttgart for the funding. The authors M.F. and T.E. would like to thank the German Research Foundation (DFG) for financial support of the project within the Cluster of Excellence in Simulation Technology (EXC 310/1) at the University of Stuttgart. Special thanks to Alexei Lapin for the valuable discussions.

6. REFERENCES

Fig. 3: Comparison of the signal transportation by diffusion (first row) and by motor proteins (second row). Adjusting the size of the filter kernel allows the transition from a discrete visualization (left) to a more contiguous one (center left and right). Low signal concentration is mapped to blue and higher concentration to red. On the far right, the signal distribution is shown as 1D concentration profile where spatial effects are not included.

Fig. 4: Development of the signaling front in four time steps of a MAPK simulation. The graph on the right shows the number of signal proteins which reach the nucleus over time. Additional to the transport with motor proteins, results for diffusion-only transport are shown.

Fig. 5: Different cross sections of the same time step from a MAPK simulation with motorized transport. The high concentration near the nucleus illustrates the asymmetric nature of the simulation and the importance of considering spatial effects. The uniform-like distribution in the $y - z$ plane is caused by a rotational symmetry of the skeletal filaments. The cytoskeleton was arranged in the same way as in Fig. 3.