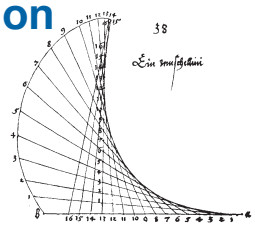
Steps Towards Microscopic Simulations of Skin Penetration

E. Sontak[1], A. Holmes[2], M. Pastore[2], H. Studier[2], E. Abd[3], J. Grice[3],
M. Roberts[2,3], J. Brandner[4], M. Heisig[1], A. Nägel[1], G. Wittum[1]
1) Goethe-Center for Scientific Computing, Goethe Universität Frankfurt am Main, Germany
2) School of Pharmacy & Medical Sciences, University of South Australia, Adelaide, Australia
3) Translational Research Institute, The University of Queensland, Brisbane, Australia
4) Klinik und Poliklinik für Dermatologie und Venerologie, Universitätsklinikum Hamburg-Eppendorf, Germany



Objective

Microscopic simulation is a well-established tool to study permeation processes in-silico [1]. At the same time, imaging data allows new insights into morphology and structural mechanisms with a high level of detail. Thus, these aspects are expected to be intertwined strongly in the forthcoming generation of modelling tools that are currently under development. This study suggests processing pipeline from microscopy images to a virtual, three-dimensional tissue sample. This reconstruction can be used for further analysis as well as for simulations.

Methods

The steps in the pipeline are as follows:

1. **Obtaining microscopy images** from a 101x101x37 μm^3 sample with a resolution of 5x5x1 pixels/μm. Sample was human female abdominal skin, and co-stained with nile-red and acriflavin.
2. **Processing image stacks** in several steps using [2, 3]: (a) Illumination correction (cf. Fig. 1), followed by (b) Contrast limited adaptive histogram equalization (CLAHE, [4]), and (c) anisotropic diffusion filtering.

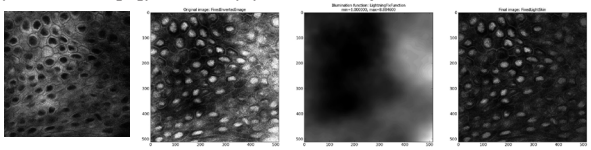


Figure 1: The raw image (left) is inverted and an illumination function is computed. This yields images with high contract an equal level of brightness for the whole image stack.

3. **Detecting of cell nuclei** using the TrackMate plugin in [2]. Each nucleus is represented by an ellipsoid, that is described by its center an by the three principal axes.

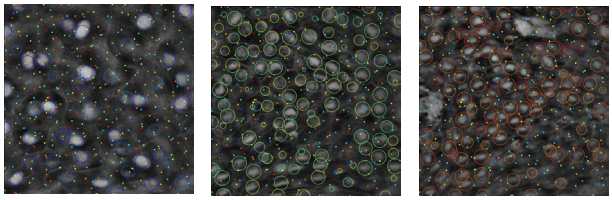


Figure 8: Distribution of detected nuclei centers, with color-coded depth information (samples images from top, middle, and bottom of stack).

4. **Reconstructing cell membranes** based on Voronoi diagrams. Cell nuclei are collapsed to their centre of gravity, before Voronoi cells are generated [5]. The surface mesh is then extended to a volume mesh with tetrahedra [6].

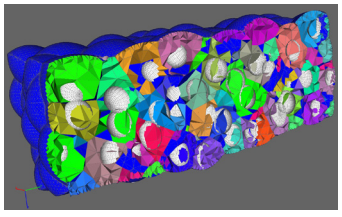


Figure 3: Reconstructed nuclei (white ellipsoids) shown in the mesh given by Voronoi cells (various colors).

Results

- Successfully tracked ~600 nuclei in the sample (some duplicates and some false-positives) with very good agreement of positions (cf. Fig. 2).
- Direct recovery of membranes (similar to nucllei) yields incomplete data (cf. Fig. 4b). Voronoi diagrams provide a reasonable match and can be used as a substitute.

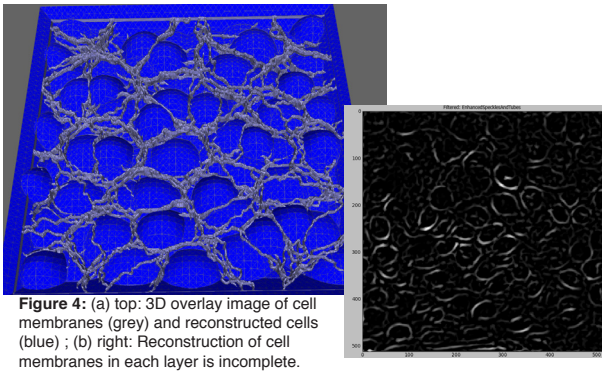


Figure 4: (a) top: 3D overlay image of cell membranes (grey) and reconstructed cells (blue) ; (b) right: Reconstruction of cell membranes in each layer is incomplete.

- Analysing the z-stack layer by layer yields depth-dependent information the number of cells (per layer), the volume fraction occupied by nuclei, the average diameter per nucleus, and its deviation from a sphere (cf. Fig. 5).

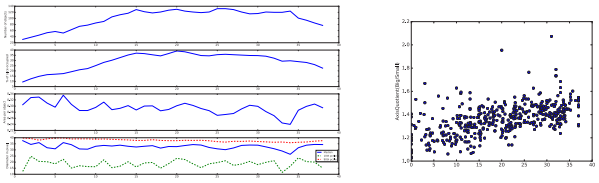


Figure 5: Individual statistics for each image in the z-stack (left). The nuclei can be represented by ellipsoids with a ratio of the principle axes slighly larger that 1 (right).

- As a last step, the obtained meshes can also be used for simulations. The integration of a proper sub-scale model (based on [8]) is an ongoing topic of our research.

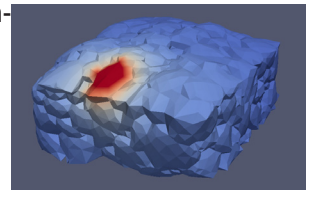


Figure 6: Visualization of a diffusion process on a reconstructed geometry. (computations with UG4 [7]).

Summary

- Presented a pipeline for processing microscopy data that allows recovering information on the distribution of nuclei and, with some limitations, also on the cell membranes (via Voronoi diagrams).
- Can be used for automated data analysis (Fig. 5).
- Goal: Extension to modelling and simulation of transdermal transport on a cellular level (Fig. 6).

References:

1. NAEGEL A., et al.: *Detailed modeling of skin penetration - an overview*, ADDR 2013, 65(2):191-207.
2. SCHINDELIN, J. et al.: *Fiji - an open source platform for biological image analysis*, Nature Methods 2012, 7: 676-682.
3. CARPENTER, A. et al.: *CellProfiler: Image analysis software for identifying and quantifying cell pheontypes*, Genome Biology 2006, 7(10): pp.100.
4. ZUIDERVELD K, *Contrast limited adaptive histogram equalization*, Graphics gems IV, Academic Press Professional, Inc., 1994, pp. 474–485.
5. BARBER, B.C.: *The Quickhull algorithm for convex hulls*, ACM TOMS 1996, 22(4): 469-483.
6. REITER S., WITTUM G.: ProMesh - Grid Generation for Scientific Computing, http://www.promesh3d.com
7. VOGEL A., et al.: *UG 4: A Novel Flexible Software System for Simulating PDE Based Models on High Performance Computers*, Comput. Vis. Sci. 2013, 16:165-179.
8. NITSCHE, J.M., KASTING G.B., *A Microscopic Multiphase Diffusion Model of Viable Epidermis Permeability*, Biophysical Journal 2013, 104(10): 2307-2320

Acknowledgements: This project was funded in part by FDA grant U01FD005232-01. The views expressed in this presentation do not reflect the official policies of the U.S. Food and Drug Administration or the U.S. Department of Health and Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government.

Goethe-Center for Scientific Computing