

# Thesis subject: Deep learning for segmentation of FIB/SEM images for the diagnosis of chronic lymphocytic leukemia

## Advisers and laboratory

The PhD student will conduct his research within the IMAGeS team of the ICube laboratory, University of Strasbourg, on the Illkirch campus site, and will actively collaborate with the IGBMC Integrative Structural Biology team to gain expertise in the field of biology and biological image acquisition processes.

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## Context

The aim of this thesis is the development of robust segmentation tools dedicated to biological imaging for the purpose of quantitative description of the three-dimensional organization of cells. This subject, at the interface of biological imaging (Schultz team, IGBMC) and digital image analysis (IMAGeS team, ICube) is extended through a collaboration with a team of clinicians from Hautepierre Hospital (Dr. L. Mauvois, UMR S-1113) on the morphological study of different populations of normal lymphoid B cells and from patients with chronic lymphocytic leukemia, the objective being to be able to improve the diagnosis of the different forms or stages of the disease.

The morphology of the cells, analyzed by electron microscopy, provides a large amount of information on the size, shape and distribution of cellular components such as nucleus, nuclear pores, nucleolus or chromatin condensation state. In the cytoplasm, the endoplasmic reticulum, the Golgi apparatus, the cytoskeleton or the mitochondria network are visible without difficulty. An atlas of normal and pathological cellular morphology parameters could, in the future, improve the diagnosis or guide researchers studying an unknown disease.

Until recently, this information was essentially descriptive, but the development of new imaging modalities, such as FIB/SEM, opens up the prospect of extracting 3D quantitative information on any cell type under different physiological conditions [Kizilyaprak et al. 2014]. In its most recent version, a focused ion beam (FIB) cuts a thin layer on the surface of the sample, while a scanning electron beam (SEM) images the face of the newly created block. The iteration of these two processes offers the possibility of imaging  $40 \times 40 \mu\text{m}$  areas with an isotropic spatial resolution of 3 nm over a depth of several tens of micrometers. The FIB / SEM technology, with its ability to image whole cells of high spatial resolution, provides images of very large size (of the order of  $2048 \times 1536 \times 500$ , more than 1.5 billion pixels), and containing many complex biological objects (see figure 1).

The segmentation step is necessary to annotate cell compartments and organelles so that quantitative information such as size, distribution, morphology can be extracted from these delimited regions. A human expert can interactively identify and annotate these cellular features, but this task is very time-consuming and requires several days for each cell. In a context of massive data (several tens of cells and several dozens of patients), it is difficult to obtain quantitative and statistically significant information in this way. The automation of this step and the development of robust segmentation methods represent a major challenge.

In this context a difficulty of automatic segmentation is related to the extremely noisy nature of electron microscopy images (low signal-to-noise ratio, low contrast) and to the complex nature of the images related to the many objects present inside the cell. A second difficulty is related to the problematic of semantic segmentation, which consists in recognizing as well as outlining precisely the contours of different classes of objects. Software tools dedicated to automatic image segmentation in electron microscopy

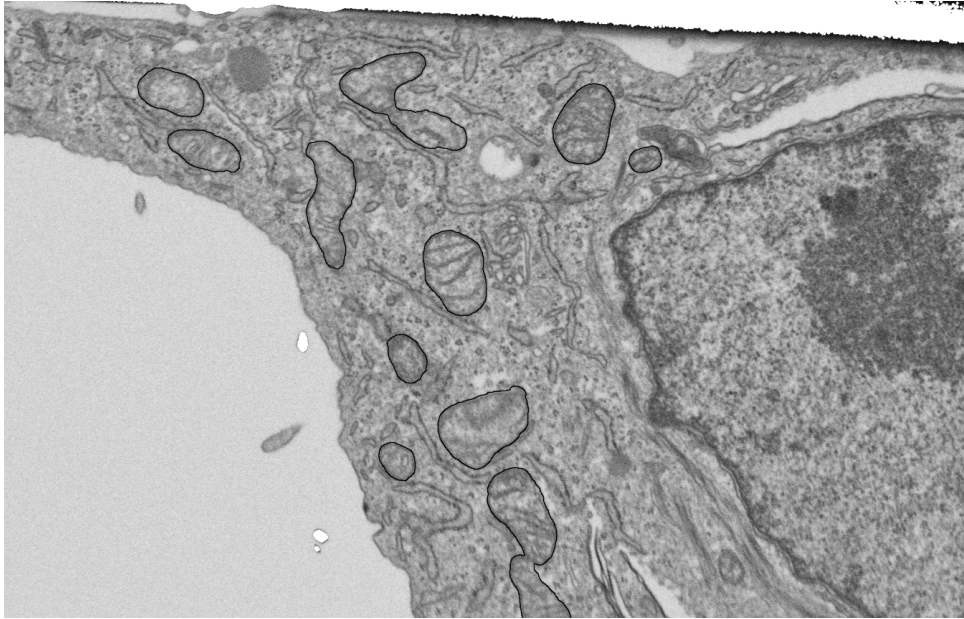


Figure 1: 2D slice of 3D FIB/SEM image. Segmented mitochondria by convolutional network are delimited in black.

have been developed: interactive tools in the Fiji [Schindelin et al. 2012] platform, *active learning* based on graphs requiring an annotated database [Nunez-Iglesias et al. 2014], thresholding techniques after pretreatment of the image [Hoang et al. 2017]. For segmentation of regions, superpixel-based strategies are used to over-segment the image into relevant regions, and then a split and merge strategy is used to group regions [Jones et al. 2015]. Finally, supervised classification techniques are used in an interactive framework to identify regions of interest [Sommer et al. 2011]. These techniques, which require for the most part a minimum of interactions, are not adapted and / or not sufficiently robust for our problem.

Segmentation methods based on convolutional neural networks have recently proved their value in the field of biomedical imaging [Ronneberger et al. 2015]. In the context of electron microscopy, recent work has proposed segmentation approaches using these techniques [Zeng et al. 2017, Haberl et al. 2018].

## Research topic

A first part of this thesis will be devoted to the development of dense segmentation methods based on fully convolutional neural networks adapted to the context of FIB/SEM imaging. The objective will be to segment several types of objects, mitochondria, endoplasmic reticulum, cell membrane, nucleus, based on the work done in the IMAGeS and SDC teams in the field of 3D MRI imaging [Sanchez et al. 2019] and histopathological imaging [Abreu et al. 2019].

In a second step, the objective is to evaluate the usefulness of connected operators [Salembier and Wilkinson 2009] in an image simplification scheme in order to facilitate and/or accelerate the learning of convolutional networks. Connected operators are non-linear operators that have the property of preserving image contours (a contour is either preserved or removed, but can not be shifted). Connected operators are fast to calculate (linear complexity) and allow to preserve the integrity of the information present in the image (in particular the location of the contours) which makes them particularly attractive in the context of image quantification.

The ultimate goal of this work will be to develop a statistical morphological atlas devoted to healthy and pathological cell populations.

## Required skills

- Master's degree in computer science or applied mathematics
- Skills in image processing, machine learning and deep learning
- Programming skills in C++ and Python

## References

- [Abreu et al. 2019] Abreu, A., Franchet, C., Frenois, F.-X., Brousset, P., Girard, J.P., Deneffe, P., Naegel, B., Wemmert, C. Ensemble of neural networks for high endothelial venules detection in Meca-79 immunohistochemistry images International Symposium on Biomedical Imaging (ISBI), 2019
- [Birchfield 2018] Birchfield S. Image processing and analysis CENGAGE Publisher
- [Bosch et al. 2015] Bosch, C., Martínez, A., Masachs, N., Teixeira, C.M., Fernaud, I., Ulloa, F., Pérez-Martínez, E., Lois, C., Comella, J.X., DeFelipe, J., Merchán-Pérez, A., Soriano, E. FIB/SEM technology and high-throughput 3D reconstruction of dendritic spines and synapses in GFP-labeled adult-generated neurons. *Front. Neuroanat.* 9, 60, 2015
- [De Winter et al. 2009] De Winter, D.A.M., Schneijdenberg, C.T.W.M., Lebbink, M.N., Lich, B., Verkleij, A.J., Drury, M.R., Humbel, B.M. Tomography of insulating biological and geological materials using focused ion beam (FIB) sectioning and low-kV BSE imaging. *J. Microsc.* 233, 372–383, 2009
- [Farabet et al. 2013] Farabet C., Couprie C., Najman, L. LeCun, Y. Learning Hierarchical Features for Scene Labeling. *IEEE Transactions on Pattern Analysis and Machine Intelligence.*35(8), pp. 1915-1929, 2013
- [Haberl et al. 2018] Matthias G. Haberl, Christopher Churas, Lucas Tindall, Daniela Boassa, Sébastien Phan, Eric A. Bushong, Matthew Madany, Raffi Akay, Thomas J. Deerinck, Steven T. Peltier, Mark H. Ellisman CDeep3M—Plug-and-Play cloud-based deep learning for image segmentation *Nature Methods* 15, 677–680, 2018
- [Hoang et al. 2017] Hoang T.V., Kizilyaprak C., Spehner D., Humbel B.M. and Schultz P. Automatic Segmentation of High Pressure Frozen and Freeze-Substituted Mouse Retina Nuclei from FIB-SEM Tomograms *J. Struct. Biol.* 197(2):123-134, 2017.
- [Jia et al. 2014] Jia Y., Shelhamer E., Donahue J. Karayev S. and Long J. Girshick, R., Guadarrama S. Darrell T. Caffe: Convolutional Architecture for Fast Feature Embedding arXiv:1408.5093, 2014
- [Jones et al. 2015] Jones, C., Liu, T., Cohan, N. W., Ellisman, M. Tasdizen, T. Efficient semi-automatic 3D segmentation for neuron tracing in electron microscopy images *Journal of neuroscience methods* 246, 13-21, 2015.
- [Kizilyaprak et al. 2014] Kizilyaprak, C., Bittermann, A. G., Daraspe, J., Humbel, B. M. FIB-SEM tomography in biology. *Methods in molecular biology* 1117, 541-558, 2014.
- [Martinez-Sanchez et al. 2014] Martinez-Sanchez, A., Garcia, I., Asano, S., Lucic, V., Fernandez, J. J. Robust membrane detection based on tensor voting for electron tomography *Journal of structural biology* 186, 49-61, 2014.
- [Merchán-Pérez et al. 2009] Merchán-Pérez, A., Rodriguez, J.-R., Alonso-Nanclares, L., Schertel, A., Defelipe, J. Counting Synapses Using FIB/SEM Microscopy: A True Revolution for Ultrastructural Volume Reconstruction. *Front. Neuroanat.* 3, 18., 2009
- [Najman and Talbot 2010] Najman L., Talbot H. Mathematical Morphology: from theory to applications. Najman Laurent and Talbot Hugues ed. ISTE-Wiley, ISBN : 978-1-84821-215-2, 2010
- [Nunez-Iglesias et al. 2014] Nunez-Iglesias, J., Kennedy, R., Plaza, S. M., Chakraborty, A., Katz, W. T. Graph-based active learning of agglomeration (GALA): a Python library to segment 2D and 3D neuroimages *Frontiers in neuroinformatics* 8, 34, 2014
- [Romera-Paredes and Torr 2016] Romera-Paredes, B., and Torr, P.H.S. Recurrent Instance Segmentation ECCV 2016 LNCS: 312–329, 2016
- [Ronneberger et al. 2015] Ronneberger O., Fischer P., Brox T. U-Net: Convolutional Networks for Biomedical Image Segmentation Navab N., Hornegger J., Wells W., Frangi A. (eds) Medical Image Computing and Computer-Assisted Intervention – MICCAI 2015. Lecture Notes in Computer Science, vol 9351. Springer, Cham, 2015
- [Salembier and Wilkinson 2009] Salembier P. and Wilkinson, M. H. F. Connected Operators: A review of region-based morphological image processing techniques *IEEE signal processing magazine* 26(6):136–157
- [Sanches et al. 2019] Sanches, P. Meyer, C., Vigon, V., Naegel, B. Cerebrovascular network segmentation of MRA images with deep learning International Symposium on Biomedical Imaging (ISBI), 2019
- [Schindelin et al. 2012] Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J. Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A. Fiji: an open-source platform for biological-image analysis *Nature methods* 9, 676-682, 2012.
- [Sommer et al. 2011] Sommer, C., Straehle, C., Kothe, U., Hamprecht, F. A. ilastik: Interactive Learning and Segmentation Toolkit International Symposium on Biomedical Imaging, 230-233, 2011.
- [Zeng et al. 2017] Tao Zeng, Bian Wu, Shuiwang Ji DeepEM3D: approaching human-level performance on 3D anisotropic EM image segmentation *Bioinformatics*, Volume 33, Issue 16, Pages 2555–2562, 2017