Paired-image database between two microscopes - a novel approach to Deep Learning Microscopy.

1. Research objective

Optical microscopy is one of the most popular techniques in cell biology research to visualize and manipulate microscopic size objects. It allows to observe, record, and elucidate cellular and subcellular molecular mechanisms, governing cell behavior in normal and pathological states. This leads to the development of new, and improved, therapies for various diseases. Therefore, there is a need for high-resolution, high-throughput, inexpensive imaging techniques, which can visualize details of biological systems at the highest possible resolution.

One of the branches of high-resolution microscopy, called ***deep learning microscopy*** **(DLM),** which combines AI/machine learning technology and high/superresolution microscopy, is being developed at present. The first publication appeared in 2017[1] where authors demonstrated that software-based image enhancement of low-quality microscopy images is possible. Since it does not require additional hardware resources, DLM is a low costs, high efficiency method. Unfortunately, thus far, it was shown to be only an auxiliary technique requiring to have a set of high-quality – low-quality parallel pairs of images from the same microscope, therefore still requiring having a *high-end* costly setup by a research group.

The goal of this project is to demonstrate a proof of a concept that **deep learning based image enhancement** can be done with datasets collected from **different microscopy stations**. We plan to collect parallel sets of images using two different fluorescent microscopes: a confocal and a regular wide-field one. Using deep learning methods, we plan to train a neutral network, and then, **enhance 3D wide-field images to match the confocal ones in resolution**. The crucial step in this project will be to create the aforementioned **parallel images database**, that will serve as a training/test data set in the further stages of the project.

2. Significance of the project

Optical resolution of a conventional microscope is limited due to light diffraction within an experimental setup, resulting in widening of an image of a luminescent point-source. Thus, the smallest possible distance to differentiate two objects from each other is around 250 nm. In comparison, large proteins are about 5 times smaller[2]. The super-resolution microscopy techniques, such as STED, STORM and PALM, allow to overcome the resolution limit of a conventional optical microscopy even by 10-fold[3]. However, these systems are usually

extremely expensive and the alternative solution to cross the cost barrier is to employ **software-based image enhancement**.

To underscore the importance of deep learning methods in biological sciences, "Nature Methods" in 2019 featured a special collection entitled "Deep Learning in Microscopy". Moreover, DLM caught attention of several world class microscopist, including Klaus Hahn (UNC Chapel Hill)[4], William E. Moerner (Stanford University)[5,6], or Hari Shroff (NIBIB NIH)[7]. However, due to its novelty, DLM is still burdened with relatively low robustness[5] but it can be a very useful tool in bio-imaging[8] with further developments. Thus far, deep learning algorithms were able to restore resolution of several-fold undersampled images[1,9], decreasing acquisition time and boosting the temporal resolution of live imaging[4,7]. Moreover, it provides a platform for cross-modality. In its first applications, DLM was used to improve images taken with low-numerical-aperture objectives to reach quality of high-numerical-aperture ones[1,9]. Furthermore, it was able to virtually re-focus wide-field images to match optical sectioning of confocal methods[10] and to improve diffraction-limited methods to achieve the super-resolution level[11]. Finally, deep learning methods applied to non-fluorescence brightfield images predicted fluorescently-staining-based subcellular structures organization[12]. However, to our best knowledge, no one has yet demonstrated the application of DLM for two separate microscopy setups.

This work will set a foundation for an inter-institutional network of microscopy labs, with *central* labs providing high resolution training images and samples. The same samples will be scanned on low-cost microscopes in *satellite* labs to create a parallel images database, that will be used for algorithm training and for image enhancement in these labs. This will allow smaller groups to achieve better imaging and to access imaging techniques that are unavailable for them. It will also increase imaging and analysis of microscopy images throughput. This is one of the main goals of the new LAB, "Jagiellonian Center of Biomedical Imaging", recently set in the frame of DigiWorld program.

3. Work plan

Part I: Optimization and calibration of a correlation microscopy system.

Realization: The correlation microscopy system will be rented from an outside company (such as *Correscopy*) and set on the designated microscopes. Then the correlation between samples between microscopes will be checked.

Part II: Database image acquisition

Realization: Cells, fixed and stained with fluorescent probes for specific cellular structures, like cytoskeleton or adhesion structures, will be imagined twice using a wide-field and a confocal microscope. Application of correlation microscopy will assure precise sample positioning that maintains corresponding imaging area between these microscopes.

Part III: Software-based images micro-correlation and alignment

Realization: The collected images will differ in pixel and image sizes and might also have some micro-shifts. Moreover, they will have to be repositioned in respect to each other in axial direction. Currently, there are available several algorithms allowing to perform this kind of corrections. For the purpose of this project, they will have to be checked for the best solutions and pipelined.

Table 1. Project timeline.

Month / Part	1	2	3	4	5	6	7	8	9	10	11	12
Part I												
Part II												
Part III												

4. Methods of research

Cell culture

Cells from immortalized cell lines will be cultured according to the distributor recommendations. Cells will be plated on microscope cover glasses and allowed to spread before experiment. Our group is certified to work with Genetically Modified Microorganisms e.g. cell lines.

Sample preparation

Cells will be fixed and stained for desired subcellular structures according to experimental recommendations from available literature or manufacturers' recommendations.

Correlation microscopy

To position and correlate images between microscopes we plan to rent a commercially available system.

Wide-field fluorescence microscopy

Wide-field fluorescence microscopy will be performed using a Zeiss Axio Observer.Z1 microscope equipped with 40x 1.3 NA and 63x 1.43 NA oil objectives, a fluorescence halogen lamp, and a high-quality camera (Hamamatsu ORCA).

Confocal fluorescence microscopy

Confocal fluorescence microscopy will be performed using a Zeiss Axio Observer.Z1 microscope equipped with a 40x 1.4 NA oil objective, and a Zeiss 710 LSM confocal module.

Database preparation

Paired images will be superimposed. Micro-translations and rotations will be corrected, through development of an appropriate software pipeline using image-analysis-supporting programming environments such as ImageJ, Matlab, or Mathematica.

Mechanobiology Group has all equipment and expertise necessary to successfully perform planned research.

5. Literature

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