AUTOMATED IMAGE ACQUISITION AND ANALYSIS IN LIGHT MICROSCOPY

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We develop and maintain a number of workflows for automated acquisition and analysis of light microscopy imaging data. Large number of images acquired by high-throughput commercial microscopes requires automation of all steps in analysis protocols. By combining and customizing open-source software tools for image and data processing we create and support robust workflows consisting of data preprocessing, image analysis, quality control and statistical post-processing. Complex acquisitions of images, in which microscope settings need to be changed during the experiment, are automated using Adaptive Feedback Microscopy technology. We developed the *AutoMicTools* package for Fiji which can be used with several commercial microscope brands. The package combines tools to configure robust protocols for Automated Feedback Microscopy and post-acquisition data analysis for microscopy-driven studies of various biological systems.

Key words: high-throughput microscopy; adaptive feedback microscopy; automation; image analysis; light microscopy.

Introduction: Fluorescence microscopy provides a range of powerful methods for studying biological samples at different spatial and temporal scales. Wide range of available microscope types corresponds to the variety of covered applications. Widely used fluorescence imaging techniques include wide field microscopy, confocal point scanning and spinning disk microscopy, super-resolution microscopy and selective plane illumination microscopy also known as lightsheet microscopy. The choice of the respective imaging technique depends on the number of factors including required resolution, optical properties of the sample and acquisition speed in case of studying live specimens. The objects in the fluorescence microscopy images can be further quantified to extract relevant morphological and light intensity parameters. Some modern fluorescence microscopes enable experimenters not only to acquire and quantify images, but also to perform biophysical measurements. Examples of such techniques include Fluorescence Recovery After Photobleaching (FRAP), Fluorescence Correlation Spectroscopy (FCS) and Förster Resonance Energy Transfer (FRET) based techniques. Appropriate analysis and quality control of such measurements allows extracting mobility and interaction parameters of biomolecules in living cells and multicellular systems [1].

Many modern studies involving fluorescence microscopy require a large number of images to be acquired and analysed. For example, systematic evaluation of changes in biological specimens upon multiple treatments requires

imaging and evaluation of a certain number of objects to properly account for biological sample heterogeneity. In the case of hundreds or thousands of investigated conditions such acquisition and analysis of individual images is impossible to perform manually. Therefore automating microscopy experiments and follow up data analysis is essential for using fluorescence microscopy in large-scale biological projects.

In this work different methods for automating fluorescence microscopy experiments and data analysis are considered with a particular focus on protocols developed by the Advanced Light Microscopy Facility of EMBL. Our facility continuously provides access to these techniques for internal and external researchers and constantly works on improving them to enable novel challenging research projects involving fluorescence microscopy.

High-throughput Microscopy (HTM): Over the last couple of decades high-throughput microscopy became a widely used method for systematic evaluation of how molecular perturbations affect biological samples [2]. Typical applications include applying drugs or silencing individual genes and measuring appropriate biological readout for each treatment. In the number of studies expression of each gene in the genome was silenced with appropriate siRNA molecules [3]. The advantages of applying microscopy techniques over biochemical methods for screens include the possibility to measure each individual cell and evaluate cell-to-cell heterogeneity in the sample.

Such large scale studies might require acquiring hundreds of thousands of images containing millions of cells or other objects of interest, therefore each step of sample preparation, image acquisition and analysis has to be automated. In the imaging plates cells exhibiting particular treatments are located in predefined positions. Microscope-controlling software is programmed to sequentially move the sample to positions corresponding to each treatment, automatically focus on the sample and acquire images with predefined settings. Such image acquisition runs without human intervention and can last from several hours to several days.

Analysis of such high throughput data includes image preprocessing, image analysis, quality control, statistical evaluation and data mining. Image pre-processing converts raw images produced by the microscopes and saved in the proprietary microscope formats to the predefined format compatible with follow up analysis steps. We commonly use OME-TIFF as a format to store HTM microscopy data. Converted data are stored with structured file names which contain essential information about imaged sample and couple image files to the database containing all experimental metadata including information about treatment, plate, biological replicate and imaging settings.

Image analysis aims at extracting representative features of individual cells or other imaged objects (e.g. tissues or model organisms). It typically includes

segmentation of cells (identification of their boundaries), identification of cellular organelles or other substructures using images of corresponding fluorescent markers. Consequently either complete cells or specific cellular structures are quantified by measuring morphological parameters such as size and shape or fluorescence intensities. Depending on the purpose of the study image analysis can include tracking of live cells, measuring distribution of fluorescence intensities within the cells, colocalisation analysis of multiple fluorescent markers or other steps. Additional measurements are normally done to evaluate quality of image data (for example, to reject images that were not in focus).

Building such multistep image analysis pipelines requires special computational tools. To make our protocols be available to the scientific community, we create image analysis protocols in the open-source image analysis programs such as *KNIME* and *CellProfiler* [4]. These tools have an advantage of a user friendly interface, large number of available image analysis methods, possibility to combine individual steps into pipelines targeting particular project goals and to visualise image analysis results.

Image analysis pipelines produce large numeric tables coupling information about treatments and other metadata to quantification results. These tables have to be further processed to derive biological conclusions. Data post-processing always includes normalisation of measurements to the internal control to reduce effect of systematic experimental errors, statistical analysis to find treatments which result in significant differences of experimental readouts and evaluation of data quality. To perform the essential post-processing methods we develop and maintain the open source tool *shinyHTM* (https://github.com/embl-cba/shinyHTM). In addition to the features listed above it can plot distributions of quantified parameters and automatically open images corresponding to particular data points. This control step is needed to visually check correspondence between imaged objects and quantified results. In case systematic errors are identified, the image analysis pipeline is modified accordingly and data get re-processed.

Finally data mining routines are applied which depend on the particular research question and out of the scope of this paper.

Adaptive Feedback Microscopy: Classical HTM is the method of choice for automated microscopy when the positions of the imaged objects and required imaging settings are known before the start of image acquisition. In many cases, however, such decisions have to be made during imaging. Examples include:

High resolution imaging of specific rare object phenotypes, e.g. mitotic cells in cell culture samples. Such experiments are, in particular, performed on confocal and other microscopes which are capable of switching between

different imaging modalities. Very often experimenter has to compromise between image resolution, size of the field of view, sensitivity and imaging speed, because improving one of these characteristics would lead to degradation of others. Therefore, low resolution image needs to be first acquired to identify target objects and then this object has to be imaged at high resolution. This also applies to live specimens, in which phenotypes of interest might exist only temporarily. Imaging such object will require, in addition, fast decision making and fast switching between low-zoom and high-zoom imaging modalities.

Imaging of moving objects over an extended period of time. If these objects move out of the field of view, the position of the microscope stage has to be adjusted accordingly to image the object correctly on the later time point.

Performing FRAP, FCS and certain types of FRET experiments automatically. After identifying the cell of interest, these measurements require definition of points or regions where measurements have to be performed depending on the purpose of the study.

To perform these and other types of advanced fluorescent microscopy experiments in a high-throughput manner, they need to be automated using Adaptive Feedback Microscopy technology [5,6]. In these techniques low-zoom images are first acquired in predefined positions and automatically transferred to the image analysis program. Specially designed image processing procedures identify cells, organelles or other structures of interest and transfer these data back to the microscope software, which triggers the respective high content microscopy imaging or measurement modality.

We developed the *AutoMicTools* package for Fiji combining tools to configure robust protocols for Automated Feedback Microscopy and post-acquisition data analysis. Customisable workflow procedures trigger execution of image analysis functions. Information about all acquired images, identified regions and decisions made by the system is stored in a special data structure that is exported from the software as a text table. Our visualisation module imports this table and provides a GUI to efficiently navigate through the acquired datasets and show segmentation results as overlays on acquired images. The data is also used by post-acquisition analysis procedures to import raw images and link quantifications to corresponding image and ROI files.

Summary: Development and maintenance of automated techniques for acquiring and analysing light microscopy images is essential for systematic biological research. By applying and customising open-source software tools we support workflows for automated experiments on the facility microscopes and processing acquired data.

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ЦИФРОВАЯ ИНТЕЛЛЕКТУАЛЬНАЯ СРЕДА ФАКУЛЬТЕТА

Ю. И. Воротницкий, К. В. Козадаев, Е. И. Козлова, И. А. Шалатонин, Е. А. Головатая, А. М. Соболь

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Предложена концепция открытой цифровой интеллектуальной среды факультета, при интеграции в которую студент сможет создавать свою уникальную базу знаний и навыков в самых современных областях информационных технологий. Представлена общая схема аппаратной части цифровой платформы, в структуре платформы выделены следующие основные системы: телекоммуникационная, идентификации, отображения информации, хранения и обработки данных, интернет вещей и управление технологическим оборудованием, видеонаблюдения и распознавания образов.

Ключевые слова: цифровая интеллектуальная среда; интернет вещей; видеонаблюдение; идентификация; обработка данных; управление.

Процессы цифровой трансформации системы государственного управления, экономики, социальной сферы Республики Беларусь, расширение сферы деятельности резидентов Парка высоких технологий в направлении наукоемких производств предъявляют новые качественные требования к высококвалифицированным кадрам с высшим образованием [1]. Вместе с тем, рост количества инновационных компаний, приток зарубежных инвестиций, ориентация на развитие продуктовой модели производства, стремительное развитие существующих и появление новых цифровых технологий обуславливают возрастающие потребности в численности специалистов, готовых к практической деятельности в области информационно-коммуникационных технологий [2, 3].