Equipment

The equipment of our laboratory can be divided into 4 main categories:

- Automated optical microscopy equipment (so-called high-resolution cytometry instruments)
- Computer hardware equipment (especially servers for data storage and analysis)
- Software equipment (especially image analysis software)
- Laboratory equipment for molecular/cell biology (preparation of specimens for optical microscopy)

High-resolution cytometry (HRCM)

The image data that we analyze are mostly acquired using high-resolution image cytometry (HRCM) technique developed by our group in collaboration with the Institute of Biophysics [Kozubek M. et al. 1999 , 2001 , 2004]. The HRCM technique enables the analysis of large number of cells (comparable to flow cytometry or laser-scanning cytometry) with a high accuracy (comparable to confocal microscopy). The technique is based on the automatic overnight acquisition and analysis of fluorescence-stained microscope slides using a computer-controlled microscope, a computer-controlled low-light camera and special software developed and optimized specifically for this task.

The name of the technique comes from Greek kytos = cell and metrum = measure. These two words form the word cytometry that is used for performing measurements on cells and/or their components. Usually the term cytometry is used when we speak of measuring a large number of cells. There exist two types of cytometry: flow cytometry and image cytometry. In flow cytomerty the cells quickly flow one after another through a thin capillary and 1D intensity profile of each flowing cell is measured. Thus, this technique is fast but provides low quality data. In image cytometry (also called slide-based cytometry) the cells are stationary and "sit" on a microscope slide. This approach allows for better image quality. The word high-resolution in HRCM was appended to denote that we use best optical components (to get best optical resolution) as well as best image detectors in contrary to low-resolution image cytometry (e.g., laser-scanning cytometry developed by Kamentsky et al.).

Present biological applications often require both quality and quantity (because of statistical significance of the results). High quality data can be obtained only using high-resolution image cytometry. Quantity can be addressed using long (overnight) acquisition, which inevitably requires full automation of the acquisition process. The development of such automated instruments has been the main scope of our group from the very beginning (mid 1990s).

So far, our group has developed 4 instruments: one wide-field and 3 combined wide-field and confocal. We use direct-view confocal observations using a spinning Nipkow disk . The confocal mode is required for proper 3-D studies, especially studies of cells in tissues.

All instruments are capable of automatic meander scanning of the microscope slides, automatic focusing of each field of view, automatic acquisition of each image (2-D) or a stack of images using axial scanning (3-D) and automatic on-line or off-line analysis of the acquired images. Besides the images, also lateral stage positions are recorded for each image so that the instrument can re-allocate the objects of interest with high accuracy even after removing the slide from the stage

and placing it back again. This enables to acquire images of the same cells repeatedly after staining another set of targets. In this way, using computer superposition (registration) of all acquired signals, a large number of targets can be visualized simultaneously within the same cell and a complex insight into the spatial arrangement of cellular components can be obtained. Lately, we have also added the possibility of acquiring time-lapse 2D or 3D series of live cells stained with fluorescent proteins.

Presently we have the following HRCM instruments (all controlled by a high-performance computer with our own software):

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- Location: Faculty of Informatics

- Microscope: inverted Zeiss Axiovert 200M

- Confocal unit: Yokogawa CSU-10 with Chroma quad-band dichroic mirror

- Camera (wide-field mode): Photometrics CoolSNAP HQ2 1392x1040 CCD at -30Â°C

- Camera (confocal mode): Andor iXon+ 888 back-illuminated 1024x1024 EMCCD at -80Â°C

- X-Y movement: Prior inverted ProScan H117 stage (stepper motors)

- Z movement: Prior 500Î¼m NanoScanZ piezo + internal stepper motor

- Filter exchange (wide-field mode): internal filter-cube revolver

- Filter exchange (confocal mode): Sutter Lambda 10-3 filter wheels with Chroma filters

- Light source (wide-field mode): HBO 100W mercury lamp

- Light source (confocal mode): Andor Laser Combiner LC-401A with 4 solid state lasers (405/488/561/640nm at 50/75/75/40mW, respectively) + AA AOTF

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- Location: Integrated Laboratories of Biomedical Technologies, ILBIT

- Microscope: inverted Zeiss Axiovert 100S

- Confocal unit: Atto CARV with Chroma quad-band dichroic mirror

- Camera: Princeton Instruments MicroMax 1300-YHS 1300x1030 CCD at -15Â°C

- X-Y movement: Ludl 99S008 stage (stepper motors)

- Z movement: Physik Instrumente piezo PIFOC P-721 and PIFOC P-723

- Filter exchange: Ludl filter wheels with Chroma filters

- Light source: EXFO X-Cite 120W

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- Location: Institute of Biophysics

- Microscope: upright Leica DMRXA

- Confocal unit: Yokogawa CSU-10 with Chroma triple-band dichroic mirror

- Camera: Photometrics CoolSNAP HQ 1392x1040 CCD at -30Â°C

- X-Y movement: Leica DMSTC stage (stepper motors)

- Z movement: Physik Instrumente piezo PIFOC P-725

- Filter exchange (wide-field mode): internal filter-cube revolver

- Filter exchange (confocal mode): Sutter Lambda 10-2 filter wheels with Chroma filters

- Light source (wide-field mode): HBO 100W mercury lamp

- Light source (confocal mode): Coherent Innova 70C Ar/Kr laser (488/568/647nm at 250/150/250mW, respectively) + Brimrose AOTF

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- Location: Institute of Biophysics

- Microscope: inverted Leica DMIRE2

- Confocal unit: none

- Camera: Photometrics Quantix KAF-1400 1316x1035 CCD at -35Â°C

- X-Y movement: Leica CTRMIC stage (stepper motors)

- Z movement: internal stepper motor

- Filter exchange: internal filter-cube revolver

- Light source: HBO 100W mercury lamp

Computer hardware equipment

- File server: gryf.fi.muni.cz (0.9 TB HW RAID)

- Analysis server: alfeios.fi.muni.cz (400 GB SW RAID, 32 GB RAM, Xeon Quad 2.83 GHz)

- Windows server: merops.fi.muni.cz (300 GB HDD, 4 GB RAM, Core 2 Quad 2.4 GHz)

- Web server: sarapis.fi.muni.cz

- Supercomputers: provided by Supercomputing Centre Brno (SCB)

- Network: 100/1000Mbit twist ethernet with gigabit backbone directly connected to high-speed 10Gbit Geant academic network

- Power supply: all computers and microscopes have uninterruptable power supply (UPS connected to an emergency diesel-engine generator)

Software equipment

We use primarily our own image acquisition and image analysis software (see Software development). Nevertheless we have purchased also some commercial packages:

- MATLAB (MathWorks) including all important toolboxes

- Huygens (SVI) with all modules

- Imaris (Bitplane)

- Image-Pro (Media Cybernetics)

- IQ (Andor)

- Some other

Laboratory equipment for molecular/cell biology

Our laboratory has all necessary equipment for the work with cells and preparation of specimens (fluorescence staining) for optical microscopy and microarray analysis. We also have equipment for DNA-probe preparation. Thus, the equipment includes:

- Cooling and freezing units (+4Â°C, -18Â°C, -30Â°C, -80Â°C, -196Â°C)

- Flow-boxes and fume-hoods

- Incubators (37Â°C, 5% CO2)
- Cooled centrifuges (different speeds)

- Water-baths with shakers
- Measurement equipment (scales, Ph-meters, etc.)
- PCR cyclers
- Spectrophotometers
- Electrophoresis equipment

- Cryocuts
- Laboratory glass washers
- Hot air and steam sterilizers
- Water deionization and destillation units
- Some other