Research activities

Below is the list of the main areas of research of our group. More details can be found in our publications (do not hesitate to ask for PDFs in case you would like to know more).

Automated acquisition of multi-dimensional image data

The number of dimensions to be acquired is usually large nowadays - one can think of up to 5 basic dimensions: x, y, z, Î» (wavelength), t (time). The maximal number of dimensions of the detector is 2 (CCD) but can also be 0 (PMT). Therefore, scanning in up to 5 dimensions is required. Moreover, in lateral dimensions (x, y) two levels of scanning may be required: the low-level scanning used for forming one 2D image of the current field of view (if PMT is used), and the high-level scanning used for the slide scan (typically meander scan of the specimen).

Such a large number of dimensions can be handled only using automated or at least semi-automated scanning. Fortunately, state-of-the-art microscope hardware is motorized and can be driven by means of a computer. If suitable image analysis and auto-focusing software exists, also the high-level lateral scanning can be performed automatically. Thus, the image acquisition process can be completely automated for some applications such as FISH imaging [Kozubek M. et al., 1999 , 2001 , 2004 , Chapter 19 of Alby's book , Chapter in Paolo's book].

Sometimes computers are also employed for the on-line computation of the final image, i.e. the image is created by the computer, not by the microscope, and the live image is observed on the monitor, not in the eyepiece. An example is the case of structured light illumination techniques when several different non-confocal images generated by the microscope are used for the on-line computation of the confocal image [Juskaitis et al., 1996] .

Automated analysis of multi-dimensional image data

The dream of each microscope user is to engage the computer not only in the image acquisition process but also in the image analysis process. Unfortunately, not all applications deal with images that are suitable for the automatic analysis. For example, tissue images are a nightmare for the developers of the image analysis software. Nevertheless, computers can help even with this application - the analysis process is then semi-automatic, the user manually defines cell boundaries, and the computer can do the rest (e.g. compute cell size or other attributes, classify cells, analyze signals inside cells, perform statistical analysis, etc.). In some other applications, the degree of the automation of image analysis can be quite high - for example images of isolated cells are quite easy to analyze automatically [Kozubek M. et al., 1999 , 2001 , 2004].

Image segmentation and object reconstruction

In order to perform measurements on cells and their components it is usually desirable to create a model (mathematical description) of volumetric objects (cells, cell nuclei or chromosomes). This process is called object reconstruction. When dealing with cells (in cytometry) it is advantageous to make use of the a priori knowledge about the size and shape of the studied objects (cells are usually round with a shape close to an ellipsoid). If this knowledge is taken into account, the object reconstruction process is easier. So far we have investigated simplex mesh approach [Matula Pa. & Svoboda, 2001 , 2003a , 2003b , 2003c] and level set / fast marching approach [Hubeny & Matula Pa., 2004 , 2006a , 2006b].

Image registration

Image registration is an indispensable part of many image analysis tasks in fluorescence microscopy [Matula Pe. et al., 2004a]. For instance, different color channels may need mutual alignment or transformation between images of the same objects acquired at different time points need to be found. The latter task is typically solved in time-lapse imaging of live cells [Matula Pe. et al., 2004b, 2006].

Correction of aberrations and artifacts in light microscopy

Unfortunately even the best light microscopes available on the market exhibit monochromatic as well as chromatic aberrations to some extent. In some studies, aberrations of current optics cannot be neglected and a software correction is highly desirable. The most important aberrations are usually chromatic shifts. In order to correct acquired images or image analysis results and compensate for chromatic shifts, at least lateral positional dependence of chromatic shifts is required because the shifts strongly vary with the lateral position in the image: they are negligible in the surroundings of the optical axis and they grow with increasing radial distance from this optical axis. Most severe chromatic shifts are in the corners of the field of view. If the positional dependencies of the chromatic shifts are known it is quite easy to compensate for these shifts [Kozubek M. & Matula Pe., 2000].

Also other artifacts specific to light microscopy (such as crosstalk between fluorochromes caused by the overlap of their spectra) can be compensated by means of appropriate software. Particular care is taken when choosing appropriate fluorochromes - we try to optimize the light throughput and contrast of the system based on our Optic application that performs optimization based on the database of spectral behaviour of light sources, filters, mirrors, fluorochormes as well as light detectors.

Special attention is paid to the suppression of artifacts induced by the specimen preparation such as coverglass thickness, depth below coverglass, immersion and embedding medium refractive index, etc. [Kozubek M., 2001] .

Further improvement of the quality of an image degraded by an optical system can beachieved using image restoration techniques. Presently we perform studies of the positional dependence of point spread function (PSF) in order to improve deconvolution process.

Multiple view imaging using micro-axial tomography

In some studies it may be advantageous to acquire the same object several times from different points of view and to use the computer to merge individual views (or analysis results obtained from individual views) into one image (or into single analysis result). This approach is useful, for example, for improving optical resolution (especially in the axial direction). A typical example is micro axial tomography. In this technique, objects of interest (cells or cell nuclei) are placed onto a thin glass fiber that is rotated along its own axis perpendicularly to the optical axis. In this way each object is acquired at different observation angles during its rotation. In our laboratory, we have automated the acquisition in micro-axial tomography [Kozubek M. et al., 2002] as well as feature-based image registration of multiple-view images [Matula Pe. et al., 2003] .

More details can be found on the pages of our Heidelberg colleagues.

Optical flow in live cell imagingÂ

The progress in staining of living cells together with advances in confocal microscopy devices has allowed detailed studies of the behavior of intracellular components including the structures inside the cell nucleus. The typical number of investigated cells in one study varies from tens to hundreds in order to achieve a reasonable statistical significance of the results. One gets time-lapse series of two or three dimensional images as an output from the microscope. The manual analysis of such large data sets is very inconvenient and annoying. This is especially true for 3D series. Moreover, there is no guarantee on the accuracy of the results. Therefore, there is a natural demand for computer vision methods to help with the analysis of time-lapse image series. Estimation or correction of global as well as local motion belongs among the main tasks in this field.

We have implemented and tested several optical flow methods, which are available in our software libraries. If you are

interested in our results, please continue with this link.

Generation of synthetic image datasets

Image cytometry still faces the problem of the quality of cell image analysis results. Degradations caused by cell preparation, optics, and electronics considerably affect most 2D and 3D cell image data acquired using optical microscopy. That is why image processing algorithms applied to these data typically offer imprecise and unreliable results. As the ground truth for given image data is not available in most experiments, the outputs of different image analysis methods can be neither verified nor compared to each other. Some papers solve this problem partially with estimates of ground truth by experts in the field (biologists or physicians). However, in many cases, such a ground truth estimate is very subjective and strongly varies between different experts.

To overcome these difficulties, we have created a toolbox [Svoboda et al., 2007, 2009, 2011] that can generate 3D digital phantoms of specific cellular components along with their corresponding images degraded by specific optics and electronics. The user can then apply image analysis methods to such simulated image data. The analysis results (such as segmentation or measurement results) can be compared with ground truth derived from input object digital phantoms (or measurements on them). In this way, image analysis methods can be compared with each other and their quality (based on the difference from ground truth) can be computed. We have also evaluated the plausibility of the synthetic images, measured by their similarity to real image data. We have tested several similarity criteria such as visual comparison, intensity histograms, central moments, frequency analysis, entropy, and 3D Haralick features. The results indicate a high degree of similarity between real and simulated image data.

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Currently, we have been developing the grouth truth generator of time-lapse series [Svoboda et al., 2012]. It is based on the optical flow approach which guarantees the smoothness of movement of synthetically generated objects.

Applications in molecular/cell biology

The above-mentioned automated microscopy and image analysis techniques are being applied in biological research held in our laboratory as well as collaborating biological laboratories. We perform 4 basic types of fluorescent staining of cell material:

- Fluorescence in situ hybridization (FISH) for the visualization of DNA sequences (whole chromosomes, individual genes, telomere or centromere regions)

- Immuno-fluorescence staining for the visualization of proteins (specific antigen cites)

- Fluorescent proteins (such as GFP) for the visualization of selected processes in live cells

- Fluorescent dyes used for the visualization of gene or protein expression level in microarray experiments

Analysis tools for all 4 types are being developed in our group. Specific applications that use one or several of these 4 stainig techniques include:

- Studies of the spatial structure of chromatin as well as function-structure relationship in human genome

- Changes of this structure and/or function during cell cycle, differentiation and between healthy and cancer cells

- Studies of the function, dynamics and interactions of selected proteins in live cells

- Comparison of gene and/or protein expression profiles of blood sample of healthy people versus blood sample of a patient using microarrays

- Finding new biomarkers which make difference between normal healthy cell and cancer cell