Image Processing in Fluorescence Microscopy and its Utilization in Cell Biology Experiments

Habilitation thesis
(Collection of articles)

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Abstract

Digital image processing is an indispensable part of modern microscopic methods. The microscope image is recorded and computer-processed. Computers are used not only for control of data acquisition, but also for visualization and information retrieval from image data. Many tasks demand the automation of the whole process and the processing of large amount of data.

This work is devoted to digital image processing in fluorescence microscopy and its use in cell biological experiments. The work is conceived as a collection of 26 scientific articles divided into four groups according to areas that they contribute to (1) automation of image acqusition and image analysis, (2) image segmentation, (3) object tracking and (4) applications in the cell nucleus research. All these areas are challenging and raise unsolved problems.

In the field of automation of image acqusition and image analysis, the thesis summarizes several improvements to high-resolution cytometry technique, which was originally developed in our laboratory. Later, our software, named Acquiarium, is introduced. The software is capable of automated image acqusition and analysis of a large number of 3D images of cells.

Modern approaches to image segmentation are formulated as a minimization problem. The first subsection presents several segmentation methods based on deformation of simplex meshes. The next two subsections summarize our contribution to segmentation using level-set methods and graph cuts. In particular, we present three different methods for Chan-Vese functional minimization, interphase chromosomes segmentation using fast marching algorithm, graph-cut segmentation of touching cells and fast algorithm which guarantees topology preservation. Finally, we discuss segmentation using graph cuts in fluorescence microscopy, where we work with large anisotropic 2D and 3D images.

The object tracking section presents a point-based method for cell alignment in live-cell imaging, a method for object tracking based on fast level set methods, a study of the applicability of variational optical flow methods, and finally a nuclear proteins tracking procedure is described.

The last part describes how we solved four practical image processing problems of cell nucleus research. They include: the method of gene localization in the chromatin, the definition of normalized distance map for flat nuclei, the study of HP1 protein association with nucleolei and chromocenters in the cell nucleus and evaluation of spatial distribution of Polycomb

bodies in the cell nucleus.

My contribution to the presented results ranges from 10 % to 100 %. The estimate of my contribution to the particular result is given in the thesis. The average contribution computed over all papers is around 1/3.

Abstrakt

Zpracování digitálního obrazu je nepostradatelnou součástí moderních mikroskopických metod. Obraz z mikroskopu je zaznamenán a počítačově zpracováván. Počítače slouží nejen k řízení procesu pořizování dat, ale i k vizualizaci a získávání informací z obrazových dat. V mnoha úlohách je kladen důraz na automatizaci postupu a zpracování velkého množství dat.

Tato práce se věnuje zpracování obrazu ve fluorescenční mikroskopii a jeho využití v buněčně biologických experimentech. Práce je koncipována jako kolekce 26 vědeckých článků rozdělených do čtyř skupin podle oblasti, ke které přispívají (1) automatizace snímání a analýzy obrazu, (2) segmentace obrazu, (3) sledování objektů a (4) aplikace v oblasti výzkumu buněčného jádra. Všechny uvedené oblasti jsou náročnými a dosud nevyřešenými problémy.

V oblasti automatizace snímání a analýzy obrazu práce shrnuje několik vylepšení techniky cytometrie s vysokým rozlišením, která byla původně vyvinuta v naší laboratoři. Poté je představen náš software Acquiarium na automatizované snímání a analýzu velkého množství 3D obrazů buněk.

Moderní přístupy k segmentaci obrazu jsou formulovány jako minimalizační úloha. Práce prezentuje nejprve několik segmentačních metod založených na deformacích simplexových sítí. Další dvě podkapitoly shrnují naše výsledky v oblasti segmentace pomocí level-set metod a grafových řezů. Zejména jsou představeny tři různé metody minimalizace Chan-Vese funkcionálu, segmentace interfázních chromosomů, segmentace shluků buněk a rychlý algoritmus garantující zachování topologie modelu. Podrobně se věnujeme segmentaci pomocí grafových řezů v podmínkách fluorescenční mikroskopie, tj. jak uchopit velké 2D a 3D anizotropní obrazy.

V oblasti sledování objektů je představena metoda založená na bodech na odstranění globálního pohybu buněk, metoda na sledování objektů na bázi rychlých level set metod, studie použitelnosti metod variačního optického toku a nakonec je popsána kompletní procedura sledování jaderných proteinů.

Poslední část popisuje řešení čtyř praktických problémů z oblasti výzkumu buněčného jádra. Je zařazena metoda lokalizace genů v chromatinu buněčného jádra, definice normalizované distanční mapy pro plochá jádra, studie rozložení HP1 proteinu v buněčném jádře a jeho asociace s chromocentry a jadérky a nakonec hodnocení prostorového rozložení polyComb tělísek v buněčném jádře.

Můj podíl na zařazených výsledcích se pohybuje od 10% do 100%. Odhad mého podílu na výsledcích je uveden u každého článku zvlášť v textu práce. Průměrný podíl přes všechny práce vychází kolem 1/3.

Acknowledgment

First and foremost I would like to thank Michal Kozubek. I am very glad I can work in his CBIA group. I have always admired his extensive knowledge of microscopic techniques and image acquisition. I also warmly thank other colleagues from CBIA. Without them I would never have achieved my results. I am especially grateful that I could work with Petr Matula, Honza Hubený, Martin Maška and Ondra Daněk. Each of them is exceptional in some sense. I always enjoyed to work with them.

A big thanks belongs to Eva Bartová for endless supply of new chalenging tasks. I always look forward to meet her.

Finally, I must thank my wife for having survived the difficult period during writing this thesis and tolerating my overload. Honey, I will check soon why your computer does not work!

Contents

Part I

Commentary

Chapter 1

Introduction

Fluorescence microscopy is one of the key techniques in biomedical research and clinical applications. It is a kind of optical microscopy permitting the observation of cellular components of interest via specific labeling with fluorescence molecules. One can use multiple labels in a specimen, e.g., one label for cell nuclei and another for a specific protein (or proteins) or particular sequences of nucleic acids (sequences of DNA or RNA including individual genes) and study their mutual interactions or investigate the dynamics of complex mechanisms that occur in the cell. Fluorescence microscopy is suitable for observation of fixed as well as living cells.

Optical microscopy is capable of optical sectioning of specimens, which offers a noninvasive, minimally destructive option for obtaining spatial and volumetric information about the structure and function of cells and tissues. The most popular approach to 3-D microscopy is confocal microscopy, which uses point illunination and a pinhole to eliminate the out-of-focus light. Spinning-disk (Nipkow-disk) confocal microscopes use a series of moving pinholes arranged on a spinning disk to shorten the scanning time.

Contemporary fluorescent microscopes consist of controllable motorized parts that together with autofocus capabilities allow for long-term unsupervised acquisitions of the specimen. This results in huge amounts of data produced in the biological experiments making manual analysis of the data sets cumbersome or even impossible. Automation of data processing is therefore necessary.

The usual procedure in fluorescence microscopy experiments consists of the following steps:

Image acquisition: it is the process of capturing an image and digitizing

it into a pixel representation. While the dimension of light detectors ranges from 0 (PMT) to 2 (cameras), the dimensionality of image data ranges from 2 (2D gray-scale image) to 5 (if wavelength and time are considered in addition to spatial dimensions). The difference between these two values must be compensated by scanning.

Image correction: instrument- and sample-based aberrations are always present in fluorescence microscopy. The most common are background inhomogenity, dark current, autofluorescence, photobleaching, chromatic aberration and intensity attenuation with depth. It is critical these problems are identified and appropriately corrected.

Image segmentation: the goal of this step is to identify the objects of interest in images. Precise localization of objects is important for furher analysis and measurements. There is no universal method to accomplish this task.

Measurements: knowing the shape and location of objects in the image one can study their mutual relations (e.g., distances, collocalization, association, radial distribution) or their topological and geometrical properties (e.g., volume, area, surface, shape parameters, intensity, number of cells, number of protein sites).

Classification: in some experiments the classification of objects based on the measured parameters is demanded (e.g, determination of positive or negative cases, cell phase, cell phenotype).

Statistical evaluation: different specimens (e.g., healthy vs. pathological and/or treated vs. non-treated) are usually captured. In order to draw statistically significant conclusions about the studied phenomenon a large number of cells (images) is needed to be processed.

This thesis contains the collection of 26 articles that address four challenges of this usual procedure and describes our contribution in the particular area. Firstly, we will deal with automation of image acqusition and image processing in Chapter 2. Then, we will discuss image segmentation problem and present our results in this field, see Chapter 3. The next Chapter 4 concerns object tracking and finally the Chapter 5 presents the solution to specific problems we faced recently in four studies of nuclear architecture in cell nuclei.

Chapter 2

Automation of Image Acquisition And Image Processing In Fluorescence Microscopy

Motorized microscope components and accessories enable the investigator to automate image acquisition. Interconnection of the image acquisition with image analysis leads to powerfull measurement instruments. It should be noted that assembling a fully automated and optimized multi-dimensional imaging system is an extremely complex task. A variety of commercial systems are available at very high cost. Alternatively, costs can be reduced by at least 50 percent by assembling a system from scratch, but this effort requires sufficient expertise and experience in optical microscopy and programming. The primary problem in automatic microscope configuration is the integration of hardware and software components purchased from different sources into a well-coordinated and efficient system.

Michal Kozubek, et.al. [16] developed a completely automated, high-resolution system (high-resolution cytometer, HRCM) capable of analyzing microscope slides with FISH-stained interphase nuclei in two dimensions as well as in three dimensions using a fully motorized epi-fluorescence microscope and a cooled digital CCD camera fully controlled by a high-performance computer which performs both acquisition and related on-line image analysis.

Several improvements to HRCM technique are presented in [15], which is included in the collection. The main contribution is the combination of con-

focal and wide-field modes. The paper discusses also hardware improvements and new image analysis options.

In [18], we compare possible approaches to image acquisition and processing in confocal in vivo microscopy and suggest new alternatives to the previously published methods. Special attention is paid to spinning disk systems. This study shows how to optimize image acquisition process in live cell studies using camera binning feature and how to perform object tracking using a new fast image registration method based on the graph theory.

We have implemented free software [21] for carrying out the common pipeline of many spatial cell studies using fluorescence microscopy. It addresses image capture on spinning disk microscopes, image correction, image segmentation, the quantification and spatial arrangement of segmented objects, volume rendering, and statistical evaluation. The software is designed for the easy processing of a collection of many 3D images.

[15] M Kozubek, S Kozubek, E Lukášová, E Bártová, M Skalníková, Pavel Matula, Petr Matula, P Jirsová, A Cafourková, and I Koutná. Combined confocal and wide-field high-resolution cytometry of fluorescent in situ hybridization-stained cells. *Cytometry*, 45(1):1–12, sep 2001

I participated on the development of the system and contributed to writing the paper. Especially, I worked on image analysis part. (10%)

[18] M Kozubek, Petr Matula, Pavel Matula, and S Kozubek. Automated acquisition and processing of multidimensional image data in confocal in vivo microscopy. *Microscopy Research and Technique*, 64:164–175, 2004

I participated on the development of the image analysis as well as image acquisition part of the system. I contributed to writing the paper. (20%)

[21] Pavel Matula, M Maška, O Daněk, Petr Matula, and M Kozubek. Acquiarium: Free software for the acquisition and analysis of 3D images of cells in fluorescence microscopy. In *IEEE International Symposium on Biomedical Imaging*, pages 1138–1141, 2009

I designed the software and led its development. I wrote the paper. (50%)

Chapter 3

Image Segmentation

Image segmentation is a task of fundamental importance in digital image processing. It is commonly defined as a partitioning of the input image into multiple disjoint regions or segments, each of which typically corresponds to one object. Many approaches to image segmentation exist [38, 31, 10]. The classical methods are thresholding, region growing, split-and-merge, watershed algorithm, and edge-based algorithms. The methods are based on the idea that (1) the segments have similar image properties (region-based) or that (2) the segments are separated by pixels with different image properties (boundary-based).

Powerful and vigorously researched approaches, called active contours or deformable models, are based on energy minimization, where the value of image segmentation is mathematically defined and the segmentation of the lowest energy is searched. The energy usually involves data term (taking region and/or boundary information into account) and smothness term incorporating the information about the shape of regions. The term contour indicates a curve in 2D or a surface in 3D separating the segments.

There are two views on active contour segmentation (1) a contour of minimal energy is searched directly or (2) the contour evolves under the external (data) and internal (smoothness) forces that push the contour in a minimum energy state. There is often a close mathematical connection between these two views, but not all deformational rules imply reasonable and understandable energy minimization problem and some energy minimization problems are practically unsolveable.

One of the best suited segmentation models to fluorescence microscopy is Chan-Vese model [2], originally called active contours without edges. The

two-phase Chan-Vese model aims for partitioning the input image into two regions with a smooth boundary and low intra-region intensity variance. The model has the following continuous formulation. Let $\Omega \subset \mathbb{R}^n$ specify the image domain and let $u : \Omega \to \mathbb{R}$ be a given image function. Further, let $\Omega_1 \subset \Omega$ and $\Omega_2 \subset \Omega$ define a partitioning of the image domain into two possibly disconnected regions with $C = \partial\Omega_1$ being the separating boundary. The Chan-Vese functional is defined as:

$$E_{CV}(C, c_1, c_2) = \lambda_1 \int_{\Omega_1} (u(x) - c_1)^2 dx + \lambda_2 \int_{\Omega_2} (u(x) - c_2)^2 dx + \mu|C|, \quad (3.1)$$

where c_1 and c_2 are unknowns representing the average intensity inside Ω_1 and Ω_2, respectively, $|C|$ denotes the length or surface of C in 2D or 3D, respectively, and λ_1, λ_2 and μ are fixed positive weights. The optimal segmentation (C, c_1, c_2) corresponds to the minimum of (3.1) with the background and foreground regions being given by Ω_1 and Ω_2, respectively.

Our results in the field of energy based segmentation of fluorescence images are divided into three groups:

Simplex meshes The contour is represented by a discrete set of linked points and a deformational rule involving internal and external forces is defined. The main advantage of these approaches is relatively fast computation.

Level set methods The contour is represented implicitly as a zero set of a higher dimensional function. The main advantage is the easy implementation for 3D images.

Graph cuts The energy minimization is formulated as the problem of finding the minimal cut in a graph, which can be solved efficiently by polynomial algorithms. Graph cut framework is popular not only for its computational efficiency but also its numerical robustness, ability to integrate visual cues and contextual information, global optimality of solutions, unrestricted topological properties and applicability to N-D problems.

3.1 Simplex Meshes

The surface of an object can be represented using a *simplex mesh* [9, 8]. Simplex mesh is a structure consisting of vertices and edges. The vertices

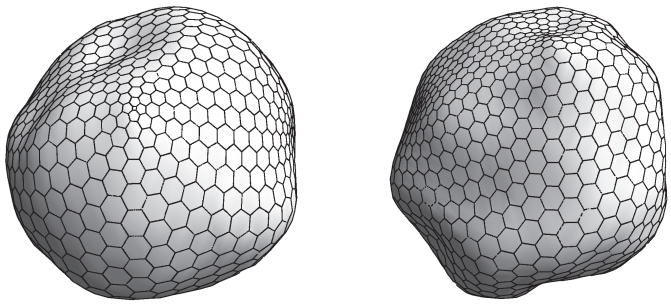

Figure 3.1: Example of simplex meshes

are points in 3D space. Every edge connects two distinct vertices. Each vertex has exactly *three neighbouring vertices* connected via edges (see Fig. 3.1). A simplex mesh is called *star-shaped* (has the shape of a star) if a point exists inside the mesh such that any ray going from the point intersects the mesh only once.

Thanks to the property of three neighbours, the following definitions can be provided. Tangent plane at a vertex is given by its three neighbours. Normal vector at a vertex is equal to the normal vector of the tangent plane. Local shape of the simplex mesh can be controlled by means of a *simplex angle*. The simplex angle at a vertex is related to the local mean curvature of the surface at this vertex and is invariant to translation, rotation and scale transformations [8].

All vertices of a simplex mesh are considered as a physical mass submitted to a Newtonian law of motion including internal and external forces. The low of motion is described by the following discrete formula [9]:

$$P_i^{t+1} = P_i^t + (1-\gamma)(P_i^t - P_i^{t-1}) + \alpha \mathbf{F}_{int} + \beta \mathbf{F}_{ext} \ , \tag{3.2}$$

where P_i^t is a position of i-th vertex in time t. Internal force $\mathbf{F}_{int}$ and external force $\mathbf{F}_{ext}$ are computed at time t and have to be properly defined. Real parameter γ is the damping factor. Real parameters α and β must belong to a given interval to guarantee a stable scheme and their ratio expresses the trade-off between influence of internal and external forces, i.e. between required local shape of the mesh and the closeness of fit.

All forces deforming a star-shaped simplex mesh are acting only along rays called deformational rays. In this way the star-shaped quality is preserved during the deformation process. The general and star-shaped methods differ in the definition of the internal and external force.

We proposed a reconstruction algorithm on the basis of simplex meshes, which is suitable for spherical object reconstruction [22, 19, 23].

[19] Pavel Matula. Effectivity of spherical object reconstruction using star-shaped simplex meshes. In *Proceedings. First International Symposium on 3D Data Processing Visualization and Transmission*, pages 794–799. IEEE Comput. Soc, 2002

I'm the only author of the paper (100%)

[22] Pavel Matula and D Svoboda. Spherical object reconstruction using star-shaped simplex meshes. In *Energy Minimization Methods in Computer Vision*, pages 608–620, 2001

I invented and co-implemented the method and wrote the paper. (80%)

[23] Pavel Matula and D Svoboda. Spherical object reconstruction using simplex meshes from sparse data. In *Discrete Geometry for Computer Imagery*, pages 524–533, 2003

I invented and implemented the method and wrote the paper. (90%)

3.2 Level Sets Methods

Level set methods [30] are very useful numerical technique for tracking interfaces and shapes. The contour $C(t)$ in time t is represented implicitly as a zero level set of a scalar, higher-dimensional function $u(x, y)$, i.e. $C = \{(x, y) : u(x, y) = 0\}$, see Fig. 3.2. This representation has several advantages over the parametric approaches. In particular, it avoids parameterization problems, the topology of the contour is handled inherently, and the extension to higher dimensions is easy and straightforward.

The contour evolution is usually governed by a partial differential equation in the following general form:

$$u_t + F|\nabla u| = 0, \tag{3.3}$$

where F is a speed function determining the motion of the contour. There are three basic types of motion in level set methods: (1) motion in the external velocity field, (2) motion in normal direction, and (3) mean curvature motion, see Fig. 3.3. Each of them needs to have been appropriately implemented. If one is interested in the zero level set only, there is often no need to recompute the function u in the whole image domain during the iterative

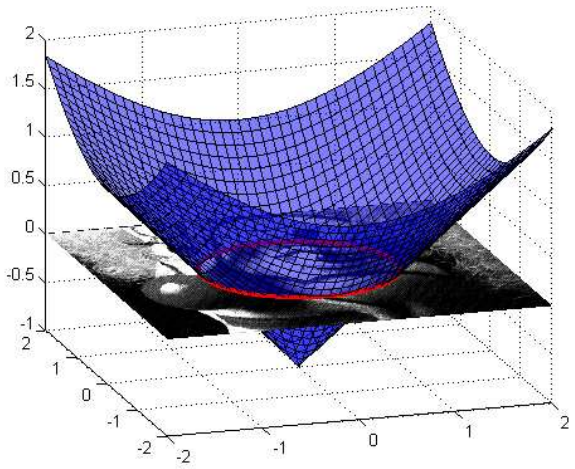

Figure 3.2: The contour (red) is represented as a zero level set of a function $u(x, y)$ (blue) in level set methods.

contour evolution. It is sufficient to update points in a narrow band around the contour or even to track the zero points only.

Moreover, if we consider just unidirectional contour evolution, i.e. $F > 0$ for all points in space and time, then each point in a space is visited by the contour only once. Let $T(x, y)$ be the time at which the contour crosses a given point (x, y) (arrival time). Then (3.3) can be rewritten as

$$\begin{aligned} &1 = F|\nabla T(x, y)|, \\ &T(x_0, y_0) = 0, \quad (x_0, y_0) \in C(0). \end{aligned} \tag{3.4}$$

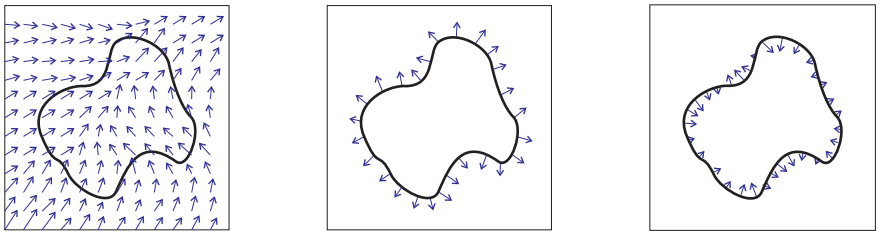

Figure 3.3: Three basic types of motion in level set methods (motion in the external velocity field, motion in normal direction, and mean curvature motion).

The main advantage of this formulation is that a highly effective algorithm exists to solve (3.4). The algorithm is called the fast marching algorithm.

We developed a new method on the basis of fast marching algorithm for interphase chromosome reconstruction [20, 13]. We worked also on fast level set based algorithms. We published fast algorithm for approximate solution of Chan-Vese segmentation model in [26]. Another fast algorithm for solution the same segmentation model is described in [12]. We proposed topology-preserving extension of fast Nilsson and Heyden algorithm in [25]. Finally, more topology-flexible variant of the algorithm [25] is described in [27] and its properties are demonstrated on simultaneous tracking of multiple objects.

[12] J Hubený and Pavel Matula. Fast and robust segmentation of low contrast biomedical images. In *Visualization, Imaging, and Image Processing*, 2006

I co-invented the method and editted the paper. (15%)

[13] J Hubený, Pavel Matula, Petr Matula, and M Kozubek. Improved 3d reconstruction of interphase chromosomes based on nonlinear diffusion filtering. In *Proceedings of PDE-Based Image Processing and Related Inverse Problems*, pages 163–173. Springer-Verlag Berlin, 2006

I co-invented the method, editted the paper and presented it. (30%)

[20] Pavel Matula, J Hubený, and M Kozubek. Fast marching 3D reconstruction of interphase chromosomes. In *Computer Vision and Mathematical Methods in Medical and Biomedical Image Analysis*, pages 385–394, 2004

I co-invented the method and wrote the paper. (70%)

[25] M Maška and Pavel Matula. A fast level set-like algorithm with topology preserving constraint. In *13th International Conference on Computer Analysis of Images and Patterns*, pages 930–938, 2009

I co-invented the method and editted the paper. (40%)

[26] M Maška, Pavel Matula, O Daněk, and M Kozubek. A fast level set-like algorithm for region-based active contours. In *6th International Symposium on Visual Computing*, pages 387–396, 2010

I co-invented the method and editted the paper. (25%)

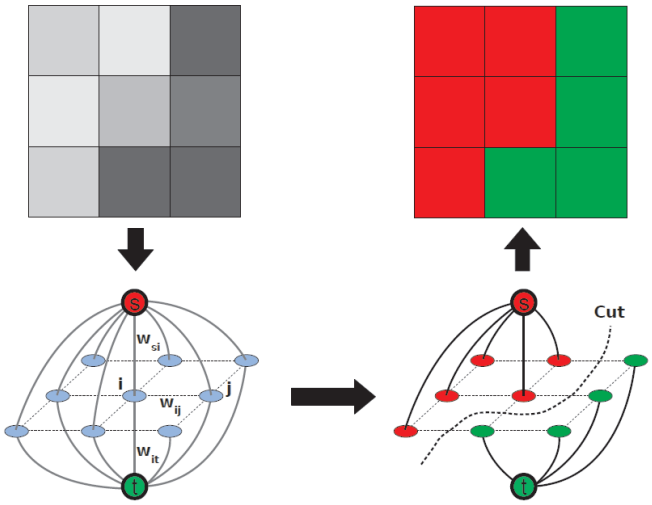

Figure 3.4: A simple example of graph-cut segmentation for a 3×3 image.

3.3 Graph Cuts

"Graph cuts have been used for many computer vision problems including image segmentation. An undirected graph $G = (V, E)$ is defined as a set of nodes (vertices V) and a set of undirected edges (E) that connect these nodes. An example of a graph is shown in Fig. 3.4. Each edge $e \in E$ in the graph is assigned a nonnegative weight (cost) w_e. There are also two special nodes called terminals (s and t). A cut is a subset of edges $C \subset E$ such that the terminals become separated on the induced graph $G(C) = (V, E \setminus C)$. Each cut has a cost which is defined as the sum of the costs of the edges that it severs

$$|C| = \sum_{e \in C} w_e. \tag{3.5}$$

A globally minimum cut on a graph with two terminals can be computed efficiently in low-order polynomial time via standard max-flow or push-relable algorithms from combinatorial optimization." [1].

"Graph cut formalism is well suited for segmentation of images. In fact, it is completely appropriate for n-dimensional volumes. The nodes of the graph can represent pixels (or voxels) and the edges can represent any neighborhood relationship between the pixels. A cut partitions the nodes in the graph. As

illustrated in Figure 3.4, this partitioning corresponds to a segmentation of an underlying image or volume. A minimum cost cut generates a segmentation that is optimal in terms of properties that are built into the edge weights." [1].

We studied how to set the edge weights while processing fluorescence microscopy images that are inherently anisotropic (worse axial resolution in confocal microscopy or unit disagreement in spatial-temporal images). We improved method for Euclidean and Riemannian metric approximation that is embedded in the graph [3, 4]. Our solution is besides being applicable to anisotropic grids also invariant under horizontal and vertical mirroring, has a straightforward generalization from 2D to 3D and has a smaller error compared to the existing approaches. Recently, we have suggested two-stage approach for minimization of Chan-Vese functional that yields smooth boundaries without increasing the computational demands significantly [6].

Graph cut based model for segmentation of touching cell nuclei in fluorescence microscopy images is presented in [7].

[3] O Daněk and Pavel Matula. Graph Cuts and Approximation of the Euclidean Metric on Anisotropic Grids. In *VISAPP International Conference on Computer Vision Theory and Applications*, 2010

I co-invented the method, editted the paper. (40%)

[4] O Daněk and Pavel Matula. An Improved Riemannian Metric Approximation for Graph Cuts. In *Discrete Geometry for Computer Imagery*, pages 71–82. Springer, 2011

I co-invented the method and editted the paper. (30%)

[6] O Daněk, Pavel Matula, M Maška, and M Kozubek. Smooth Chan-Vese Segmentation via Graph Cuts. *Pattern Recognition Letters*, Accepted, 2012

I co-invented the method and editted the paper. (30%)

[7] O Daněk, Pavel Matula, C Ortiz de Solórzano, A Muñoz Barrutia, M Maška, and M Kozubek. Segmentation of Touching Cell Nuclei Using a Two-Stage Graph Cut Model. In *Proceedings of Scandinavian Conference on Image Analysis (SCIA)*, volume 5575, pages 410–419, 2009

I co-invented the method and editted the paper. (10%)

Chapter 4

Tracking in Cell Biology

"The past decade has seen an unprecedented data explosion in biology. It has become evident that in order to take full advantage of the potential wealth of information hidden in the data produced by even a single experiment, visual inspection and manual analysis are no longer adequate. To ensure efficiency, consistency, and completeness in data processing and analysis, computational tools are essential. Of particular importance to many modern live-cell imaging experiments is the ability to automatically track and analyze the motion of objects in time-lapse microscopy images." [28].

"Roughly speaking, time-lapse imaging studies consist of four successive steps: 1) planning of the experiment and acquisition of the image data, 2) preprocessing of the data to correct for systemic as well as random errors and to enhance relevant features, 3) analysis of the data by detecting and tracking the objects relevant to the biological questions underlying the study, and 4) analysis of the resulting trajectories to test predefined hypotheses or detect new phenomena." [29], see Fig. 4.1.

"A frequently studied parameter, especially in particle tracking experiments, is the mean square displacement (MSD). It is a convenient measure to study the diffusion characteristics of the motion of individual particles and also allows to assess the viscoelastic properties of the media in which they move. By definition, the MSD is a function of time lag, and the shape of the MSD-time curve for a given trajectory is indicative of the mode of motion of the corresponding particle." [29].

We developed fast point-based method for the alignment of cells in live cell imaging [24]. This method is the basic building block of tracking procedure described in [34]. The paper [34] addresses all steps defined above. The

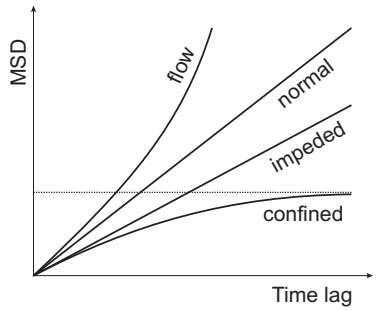

Figure 4.1: Different modes of motion according to MSD curve.

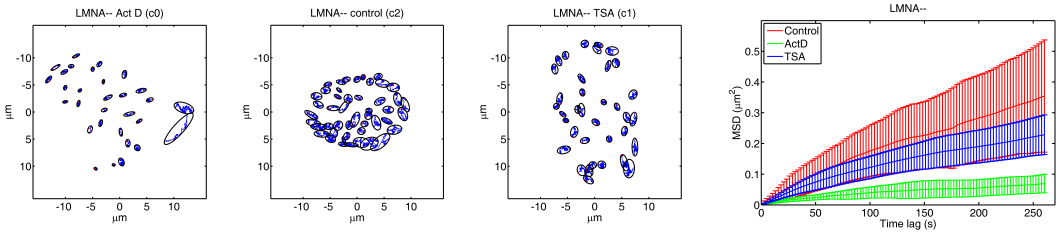

Figure 4.2: Example of average MSD curves for tracking of nuclear protein under three different conditions.

example of MSD curves computed using the procedure is shown in Fig. 4.2.

We also studied variational optical flow methods for motion tracking of fluorescently labeled targets in living cells [14] and developed a fast level set-like algorithm for simultaneous tracking of multiple objects [27].

[14] J Hubený, V Ulman, and Pavel Matula. Estimating large local motion in live-cell imaging using variational optical flow. In *VISAPP International Conference on Computer Vision Theory and Applications*, pages 542–548, 2007

I collaborated on experiment design. (10%)

[24] Petr Matula, Pavel Matula, M Kozubek, and V Dvořák. Fast point-based 3-D alignment of live cells. *IEEE transactions on image processing*, 15(8):2388–96, aug 2006

I collaborated on the method design, editted the paper and worked on evaluation. (20%)

[27] M Maška, Pavel Matula, and M Kozubek. Simultaneous Tracking of Multiple Objects Using Fast Level Set-Like Algorithm. In *Sixth Doctoral Workshop on Math.*

and Eng. Methods in Computer Science (MEMICS10) Selected Papers, pages 69–76, 2010

I co-invented the method and editted the paper. (15%)

[34] L Stixová, E Bártová, Pavel Matula, O Daněk, S Legartová, and S Kozubek. Heterogeneity in the kinetics of nuclear proteins and trajectories of substructures associated with heterochromatin. *Epigenetics & chromatin*, 4(1):5, jan 2011

I designed and performed object tracking algorithm, computed trajectories and MSD graphs, wrote relevant parts of the article. (25%)

Chapter 5

Nuclear Architecture and Chromatin Structure Studies

This chapter comments on four papers, which study the cell nucleus, but they contain significant image processing part. The first paper [33] studies chromatin condensation during granulopoiesis using immunoFISH staining. Image analysis and image acquisition were carried out using Acquiarium software [21]. Segmentation of the nuclei was accomplished using our algorithm [12] minimizing Chan-Vese energy. DNA signals were segmented using morphological EMAX transformation (Extended Maxima) [32]. We developed novel method how to study the location of DNA signal in the cell nucleus with respect to the RNAP II immunofluorescence in regions of the nucleus. RNAP II image channel was the marker of chromatin condensation.

Paper [11] studies association of HP1 protein in cell nucleus with chromocenters and nucleoli. We had to develop image segmentation of nucleus, chromocenters and nucleoli and evaluate the content of HP1 protein (green) in different regions, see Fig. 5.1.

We defined new normalised distance map (called FLR measure) in the paper [36]. The measure is well suited for evaluation of radial distribution of sites in flat nuclei. The difference between standard and our new FLR measure is visualized in Fig. 5.2.

[11] A Harničarová Horáková, E Bártová, G Galiová, R Uhlířová, Pavel Matula, and S Kozubek. SUV39h-independent association of HP1 beta with fibrillarin-positive nucleolar regions. *Chromosoma*, 119(3):227–41, jun 2010

Invention, development and running of image analysis procedure. I

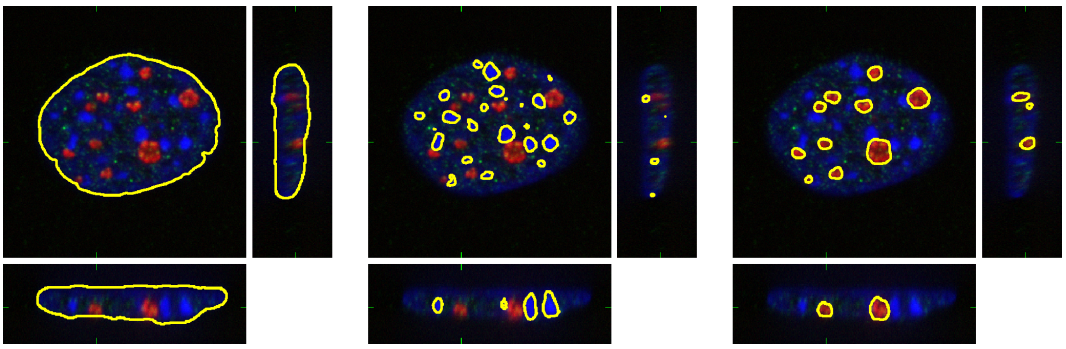

Figure 5.1: Example of segmentation of nucleus, chromocenters and nucleoli in the study of HP1 protein association.

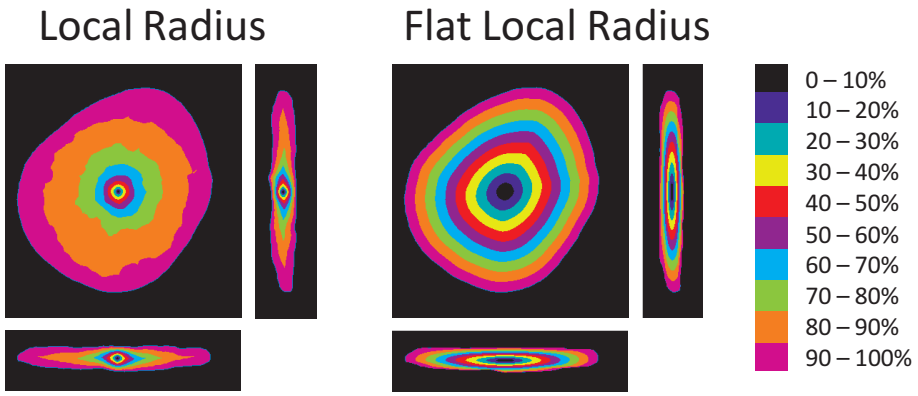

Figure 5.2: Comparison of standard local radius measure vs. our flat local radius measure.

wrote the image analysis part and contributed to the image acquisition part of materials and methods of the paper. (20%)

[33] S Stejskal, I Koutná, Pavel Matula, Z Ručka, O Daněk, M Maška, and M Kozubek. The role of chromatin condensation during granulopoiesis in the regulation of gene cluster expression. *Epigenetics*, 5(8):758–66, 2010

Co-invention and co-development of image analysis procedure. I wrote image acquisition and analysis part of the paper. (20%)

[36] R Uhlířová, A Harničarová Horáková, G Galiová, S Legartová, Pavel Matula, M Fojtová, M Vařecha, J Amrichová, J Vondráček, S Kozubek, and E Bártová. SUV39h- and A-type lamin-dependent telomere nuclear rearrangement. *Journal of cellular biochemistry*, 109(5):915–26, apr 2010

(10%) Invention of normalized distance map. I wrote relevant parts of the paper.

Bibliography

[1] Y Boykov and V Kolmogorov. Computing Geodesics and Minimal Surfaces via Graph Cuts. In *International Conference on Computer Vision (ICCV)*, number November, pages 26–33, 2003.

[2] T F Chan and L A Vese. Active contours without edges. *IEEE Transactions on image processing*, 10(2):266–277, January 2001.

[3] O Daněk and Pavel Matula. Graph Cuts and Approximation of the Euclidean Metric on Anisotropic Grids. In *VISAPP International Conference on Computer Vision Theory and Applications*, 2010.

[4] O Daněk and Pavel Matula. An Improved Riemannian Metric Approximation for Graph Cuts. In *Discrete Geometry for Computer Imagery*, pages 71–82. Springer, 2011.

[5] O Daněk and Pavel Matula. On Euclidean Metric Approximation via Graph Cuts. In *VISSAP International Conference on Computer Vision Theory and Applications*, 2011.

[6] O Daněk, Pavel Matula, M Maška, and M Kozubek. Smooth Chan-Vese Segmentation via Graph Cuts. *Pattern Recognition Letters*, Accepted, 2012.

[7] O Daněk, Pavel Matula, C Ortiz de Solórzano, A Muñoz Barrutia, M Maška, and M Kozubek. Segmentation of Touching Cell Nuclei Using a Two-Stage Graph Cut Model. In *Proceedings of Scandinavian Conference on Image Analysis (SCIA)*, volume 5575, pages 410–419, 2009.

[8] H. Delingette. Simplex meshes: a general representation for 3D shape reconstruction. Technical Report 2214, INRIA, France, 1994.

[9] H. Delingette. General object reconstruction based on simplex meshes. *International Journal of Computer Vision*, 32(2):111–146, 1999.

[10] R C Gonzales and R E Woods. *Digital Image Processing*. Prentice Hall, Inc., New Jersey, USA, 2002.

[11] A Harničarová Horáková, E Bártová, G Galiová, R Uhlířová, Pavel Matula, and S Kozubek. SUV39h-independent association of HP1 beta with fibrillarin-positive nucleolar regions. *Chromosoma*, 119(3):227–41, jun 2010.

[12] J Hubený and Pavel Matula. Fast and robust segmentation of low contrast biomedical images. In *Visualization, Imaging, and Image Processing*, 2006.

[13] J Hubený, Pavel Matula, Petr Matula, and M Kozubek. Improved 3d reconstruction of interphase chromosomes based on nonlinear diffusion filtering. In *Proceedings of PDE-Based Image Processing and Related Inverse Problems*, pages 163–173. Springer-Verlag Berlin, 2006.

[14] J Hubený, V Ulman, and Pavel Matula. Estimating large local motion in live-cell imaging using variational optical flow. In *VISAPP International Conference on Computer Vision Theory and Applications*, pages 542–548, 2007.

[15] M Kozubek, S Kozubek, E Lukášová, E Bártová, M Skalníková, Pavel Matula, Petr Matula, P Jirsová, A Cafourková, and I Koutná. Combined confocal and wide-field high-resolution cytometry of fluorescent in situ hybridization-stained cells. *Cytometry*, 45(1):1–12, sep 2001.

[16] M Kozubek, S Kozubek, E Lukášová, A Marečková, E Bártová, M Skalníková, and A Jergová. High-resolution cytometry of FISH dots in interphase cell nuclei. *Cytometry*, 36(4):279–93, August 1999.

[17] M Kozubek, Petr Matula, and Pavel Matula. Automated confocal microscopy: the way of achieving both quality and quantity in 3D image cytometry. In *IEEE International Symposium on Biomedical Imaging*, pages 69–72, 2004.

[18] M Kozubek, Petr Matula, Pavel Matula, and S Kozubek. Automated acquisition and processing of multidimensional image data in confocal

in vivo microscopy. *Microscopy Research and Technique*, 64:164–175, 2004.

[19] Pavel Matula. Effectivity of spherical object reconstruction using star-shaped simplex meshes. In *Proceedings. First International Symposium on 3D Data Processing Visualization and Transmission*, pages 794–799. IEEE Comput. Soc, 2002.

[20] Pavel Matula, J Hubený, and M Kozubek. Fast marching 3D reconstruction of interphase chromosomes. In *Computer Vision and Mathematical Methods in Medical and Biomedical Image Analysis*, pages 385–394, 2004.

[21] Pavel Matula, M Maška, O Daněk, Petr Matula, and M Kozubek. Acquiarium: Free software for the acquisition and analysis of 3D images of cells in fluorescence microscopy. In *IEEE International Symposium on Biomedical Imaging*, pages 1138–1141, 2009.

[22] Pavel Matula and D Svoboda. Spherical object reconstruction using star-shaped simplex meshes. In *Energy Minimization Methods in Computer Vision*, pages 608–620, 2001.

[23] Pavel Matula and D Svoboda. Spherical object reconstruction using simplex meshes from sparse data. In *Discrete Geometry for Computer Imagery*, pages 524–533, 2003.

[24] Petr Matula, Pavel Matula, M Kozubek, and V Dvořák. Fast point-based 3-D alignment of live cells. *IEEE transactions on image processing*, 15(8):2388–96, aug 2006.

[25] M Maška and Pavel Matula. A fast level set-like algorithm with topology preserving constraint. In *13th International Conference on Computer Analysis of Images and Patterns*, pages 930–938, 2009.

[26] M Maška, Pavel Matula, O Daněk, and M Kozubek. A fast level set-like algorithm for region-based active contours. In *6th International Symposium on Visual Computing*, pages 387–396, 2010.

[27] M Maška, Pavel Matula, and M Kozubek. Simultaneous Tracking of Multiple Objects Using Fast Level Set-Like Algorithm. In *Sixth*

Doctoral Workshop on Math. and Eng. Methods in Computer Science (MEMICS'10) — Selected Papers, pages 69–76, 2010.

[28] E Meijering, O Dzyubachyk, I Smal, and W A van Cappellen. Tracking in cell and developmental biology. *Seminars in Cell & Developmental Biology*, pages 894–902 ST – Tracking in cell and developmental b, 2009.

[29] E Meijering, I Smal, and O Dzyubachyk. Time-Lapse Imaging. In *Microscope Image Processing*, chapter 15, pages 401–440. 2008.

[30] S Osher and R Fedkiw. *Level Set Methods and Dynamic Implicit Surfaces*. Springer-Verlag, New York, Inc., 2003.

[31] W K Pratt. *Digital Image Processing*. Wiley-Interscience, New York, USA, 2007.

[32] P Soille. *Morphological Image Analysis: Principles and Applications*. Springer-Verlag, Berlin, 2nd editio edition, 2004.

[33] S Stejskal, I Koutná, Pavel Matula, Z Ručka, O Daněk, M Maška, and M Kozubek. The role of chromatin condensation during granulopoiesis in the regulation of gene cluster expression. *Epigenetics*, 5(8):758–66, 2010.

[34] L Stixová, E Bártová, Pavel Matula, O Daněk, S Legartová, and S Kozubek. Heterogeneity in the kinetics of nuclear proteins and trajectories of substructures associated with heterochromatin. *Epigenetics & chromatin*, 4(1):5, jan 2011.

[35] D Svoboda and Pavel Matula. Tissue reconstruction based on deformation of dual simplex meshes. In *Discrete Geometry for Computer Imagery*, pages 514–523, 2003.

[36] R Uhlířová, A Harničarová Horáková, G Galiová, S Legartová, Pavel Matula, M Fojtová, M Vařecha, J Amrichová, J Vondráček, S Kozubek, and E Bártová. SUV39h- and A-type lamin-dependent telomere nuclear rearrangement. *Journal of cellular biochemistry*, 109(5):915–26, apr 2010.

[37] G Šustáčková, S Kozubek, L Stixová, S Legartová, Pavel Matula, D Orlova, and E Bártová. Acetylation-dependent nuclear arrangement

and recruitment of BMI1 protein to UV-damaged chromatin. *Journal of cellular physiology*, 227(5):1838–50, may 2012.

[38] Q Wu, F A Merchant, and K R Castleman. *Microscope Image Processing*. Academic Press, 2008.

Part II

Collection of Articles

This part contains copies of journal and conference papers which I co-authored and are related to the thesis. The context of all papers and a description of the main results are provided in the previous part.

Automation of Image Acquisition And Image Processing In Fluorescence Microscopy

Journal article [15]

M Kozubek, S Kozubek, E Lukášová, E Bártová, M Skalníková, Pavel Matula, Petr Matula, P Jirsová, A Cafourková, and I Koutná. Combined confocal and wide-field high-resolution cytometry of fluorescent in situ hybridization-stained cells. *Cytometry*, 45(1):1–12, sep 2001

pdfs/2001_Kozubek_Cytometry.pdf

pdfs/2001_Kozubek_Cytometry.pdf

pdfs/2001_Kozubek_Cytometry.pdf

pdfs/2001_Kozubek_Cytometry.pdf

pdfs/2001_Kozubek_Cytometry.pdf

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pdfs/2001_Kozubek_Cytometry.pdf

pdfs/2001_Kozubek_Cytometry.pdf

pdfs/2001_Kozubek_Cytometry.pdf

Journal paper [18]

M Kozubek, Petr Matula, Pavel Matula, and S Kozubek. Automated acquisition and processing of multidimensional image data in confocal in vivo microscopy. *Microscopy Research and Technique*, 64:164–175, 2004

pdfs/2004_Kozubek_MRT.pdf

pdfs/2004_Kozubek_MRT.pdf

pdfs/2004_Kozubek_MRT.pdf

pdfs/2004_Kozubek_MRT.pdf

pdfs/2004_Kozubek_MRT.pdf

pdfs/2004_Kozubek_MRT.pdf

pdfs/2004_Kozubek_MRT.pdf

pdfs/2004_Kozubek_MRT.pdf

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pdfs/2004_Kozubek_MRT.pdf

pdfs/2004_Kozubek_MRT.pdf

pdfs/2004_Kozubek_MRT.pdf

Conference paper [21]

Pavel Matula, M Maška, O Daněk, Petr Matula, and M Kozubek. Acquiarium: Free software for the acquisition and analysis of 3D images of cells in fluorescence microscopy. In *IEEE International Symposium on Biomedical Imaging*, pages 1138–1141, 2009

pdfs/2009_Matula_ISBI.pdf

pdfs/2009_Matula_ISBI.pdf

pdfs/2009_Matula_ISBI.pdf

pdfs/2009_Matula_ISBI.pdf

Image Segmentation using Simplex Meshes

Conference paper [22]

Pavel Matula and D Svoboda. Spherical object reconstruction using star-shaped simplex meshes. In *Energy Minimization Methods in Computer Vision*, pages 608–620, 2001

pdfs/2001_Matula_EMMCV_Spherical.pdf

pdfs/2001_Matula_EMMCV_Spherical.pdf

pdfs/2001_Matula_EMMCV_Spherical.pdf

pdfs/2001_Matula_EMMCV_Spherical.pdf

pdfs/2001_Matula_EMMCV_Spherical.pdf

pdfs/2001_Matula_EMMCV_Spherical.pdf

pdfs/2001_Matula_EMMCV_Spherical.pdf

pdfs/2001_Matula_EMMCV_Spherical.pdf

pdfs/2001_Matula_EMMCV_Spherical.pdf

pdfs/2001_Matula_EMMCV_Spherical.pdf

pdfs/2001_Matula_EMMCV_Spherical.pdf

pdfs/2001_Matula_EMMCV_Spherical.pdf

pdfs/2001_Matula_EMMCV_Spherical.pdf

Conference paper [19]

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pdfs/2002_Matula_3DPVT_Effectivity.pdf

pdfs/2002_Matula_3DPVT_Effectivity.pdf

pdfs/2002_Matula_3DPVT_Effectivity.pdf

pdfs/2002_Matula_3DPVT_Effectivity.pdf

pdfs/2002_Matula_3DPVT_Effectivity.pdf

pdfs/2002_Matula_3DPVT_Effectivity.pdf

Conference paper [23]

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pdfs/2003_Matula_DGCI_SparseData.pdf

pdfs/2003_Matula_DGCI_SparseData.pdf

pdfs/2003_Matula_DGCI_SparseData.pdf

pdfs/2003_Matula_DGCI_SparseData.pdf

pdfs/2003_Matula_DGCI_SparseData.pdf

pdfs/2003_Matula_DGCI_SparseData.pdf

pdfs/2003_Matula_DGCI_SparseData.pdf

pdfs/2003_Matula_DGCI_SparseData.pdf

pdfs/2003_Matula_DGCI_SparseData.pdf

pdfs/2003_Matula_DGCI_SparseData.pdf

Image Segmentation using Level Sets Methods

Conference paper [20]

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pdfs/2004_Matula_CVAMIA_Fast.pdf

pdfs/2004_Matula_CVAMIA_Fast.pdf

pdfs/2004_Matula_CVAMIA_Fast.pdf

pdfs/2004_Matula_CVAMIA_Fast.pdf

pdfs/2004_Matula_CVAMIA_Fast.pdf

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pdfs/2004_Matula_CVAMIA_Fast.pdf

pdfs/2004_Matula_CVAMIA_Fast.pdf

pdfs/2004_Matula_CVAMIA_Fast.pdf

Conference paper [13]

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pdfs/2006_Hubeny_IPonPDE_Improved.pdf

pdfs/2006_Hubeny_IPonPDE_Improved.pdf

pdfs/2006_Hubeny_IPonPDE_Improved.pdf

pdfs/2006_Hubeny_IPonPDE_Improved.pdf

pdfs/2006_Hubeny_IPonPDE_Improved.pdf

pdfs/2006_Hubeny_IPonPDE_Improved.pdf

pdfs/2006_Hubeny_IPonPDE_Improved.pdf

pdfs/2006_Hubeny_IPonPDE_Improved.pdf

pdfs/2006_Hubeny_IPonPDE_Improved.pdf

pdfs/2006_Hubeny_IPonPDE_Improved.pdf

Conference paper [25]

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pdfs/2009_Maska_ICCAIP_AFast.pdf

pdfs/2009_Maska_ICCAIP_AFast.pdf

pdfs/2009_Maska_ICCAIP_AFast.pdf

pdfs/2009_Maska_ICCAIP_AFast.pdf

pdfs/2009_Maska_ICCAIP_AFast.pdf

pdfs/2009_Maska_ICCAIP_AFast.pdf

pdfs/2009_Maska_ICCAIP_AFast.pdf

pdfs/2009_Maska_ICCAIP_AFast.pdf

pdfs/2009_Maska_ICCAIP_AFast.pdf

Conference paper [12]

J Hubený and Pavel Matula. Fast and robust segmentation of low contrast biomedical images. In *Visualization, Imaging, and Image Processing*, 2006

pdfs/2006_Hubeny_VIIP_Fast.pdf

pdfs/2006_Hubeny_VIIP_Fast.pdf

pdfs/2006_Hubeny_VIIP_Fast.pdf

pdfs/2006_Hubeny_VIIP_Fast.pdf

pdfs/2006_Hubeny_VIIP_Fast.pdf

pdfs/2006_Hubeny_VIIP_Fast.pdf

pdfs/2006_Hubeny_VIIP_Fast.pdf

pdfs/2006_Hubeny_VIIP_Fast.pdf

Conference paper [26]

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pdfs/2010_Maska_VC_AFast.pdf

pdfs/2010_Maska_VC_AFast.pdf

pdfs/2010_Maska_VC_AFast.pdf

pdfs/2010_Maska_VC_AFast.pdf

pdfs/2010_Maska_VC_AFast.pdf

pdfs/2010_Maska_VC_AFast.pdf

pdfs/2010_Maska_VC_AFast.pdf

pdfs/2010_Maska_VC_AFast.pdf

pdfs/2010_Maska_VC_AFast.pdf

pdfs/2010_Maska_VC_AFast.pdf

Image Segmentation using Graph Cuts

Conference paper [3]

O Daněk and Pavel Matula. Graph Cuts and Approximation of the Euclidean Metric on Anisotropic Grids. In *VISAPP International Conference on Computer Vision Theory and Applications*, 2010

pdfs/2010_Danek_VISAPP_Graph.pdf

pdfs/2010_Danek_VISAPP_Graph.pdf

pdfs/2010_Danek_VISAPP_Graph.pdf

pdfs/2010_Danek_VISAPP_Graph.pdf

pdfs/2010_Danek_VISAPP_Graph.pdf

pdfs/2010_Danek_VISAPP_Graph.pdf

Conference paper [4]

O Daněk and Pavel Matula. An Improved Riemannian Metric Approximation for Graph Cuts. In *Discrete Geometry for Computer Imagery*, pages 71–82. Springer, 2011

pdfs/2011_Danek_DGCI_Riemann.pdf

pdfs/2011_Danek_DGCI_Riemann.pdf

pdfs/2011_Danek_DGCI_Riemann.pdf

pdfs/2011_Danek_DGCI_Riemann.pdf

pdfs/2011_Danek_DGCI_Riemann.pdf

pdfs/2011_Danek_DGCI_Riemann.pdf

pdfs/2011_Danek_DGCI_Riemann.pdf

pdfs/2011_Danek_DGCI_Riemann.pdf

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pdfs/2011_Danek_DGCI_Riemann.pdf

pdfs/2011_Danek_DGCI_Riemann.pdf

pdfs/2011_Danek_DGCI_Riemann.pdf

Conference paper [7]

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pdfs/2009_Danek_SCIA_Segm.pdf

pdfs/2009_Danek_SCIA_Segm.pdf

pdfs/2009_Danek_SCIA_Segm.pdf

pdfs/2009_Danek_SCIA_Segm.pdf

pdfs/2009_Danek_SCIA_Segm.pdf

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pdfs/2009_Danek_SCIA_Segm.pdf

pdfs/2009_Danek_SCIA_Segm.pdf

pdfs/2009_Danek_SCIA_Segm.pdf

pdfs/2009_Danek_SCIA_Segm.pdf

Journal article [6]

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pdfs/2012_Danek_PATREC.pdf

pdfs/2012_Danek_PATREC.pdf

pdfs/2012_Danek_PATREC.pdf

pdfs/2012_Danek_PATREC.pdf

pdfs/2012_Danek_PATREC.pdf

pdfs/2012_Danek_PATREC.pdf

Tracking in Cell Biology

Journal article [24]

Petr Matula, Pavel Matula, M Kozubek, and V Dvořák. Fast point-based 3-D alignment of live cells. *IEEE transactions on image processing*, 15(8):2388–96, aug 2006

pdfs/2006_Matula_TIP.pdf

pdfs/2006_Matula_TIP.pdf

pdfs/2006_Matula_TIP.pdf

pdfs/2006_Matula_TIP.pdf

pdfs/2006_Matula_TIP.pdf

pdfs/2006_Matula_TIP.pdf

pdfs/2006_Matula_TIP.pdf

pdfs/2006_Matula_TIP.pdf

pdfs/2006_Matula_TIP.pdf

Conference paper [14]

J Hubený, V Ulman, and Pavel Matula. Estimating large local motion in live-cell imaging using variational optical flow. In *VISAPP International Conference on Computer Vision Theory and Applications*, pages 542–548, 2007

pdfs/2007_Hubeny_VISAPP_Estimating.pdf

pdfs/2007_Hubeny_VISAPP_Estimating.pdf

pdfs/2007_Hubeny_VISAPP_Estimating.pdf

pdfs/2007_Hubeny_VISAPP_Estimating.pdf

pdfs/2007_Hubeny_VISAPP_Estimating.pdf

pdfs/2007_Hubeny_VISAPP_Estimating.pdf

pdfs/2007_Hubeny_VISAPP_Estimating.pdf

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pdfs/2011_Maska_MEMICS_Simultaneous.pdf

pdfs/2011_Maska_MEMICS_Simultaneous.pdf

pdfs/2011_Maska_MEMICS_Simultaneous.pdf

pdfs/2011_Maska_MEMICS_Simultaneous.pdf

pdfs/2011_Maska_MEMICS_Simultaneous.pdf

pdfs/2011_Maska_MEMICS_Simultaneous.pdf

pdfs/2011_Maska_MEMICS_Simultaneous.pdf

pdfs/2011_Maska_MEMICS_Simultaneous.pdf

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pdfs/2011_Stixova_EaC.pdf

pdfs/2011_Stixova_EaC.pdf

pdfs/2011_Stixova_EaC.pdf

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pdfs/2011_Stixova_EaC.pdf

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pdfs/2011_Stixova_EaC.pdf

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pdfs/2011_Stixova_EaC.pdf

Nuclear Architecture and Chromatin Structure Studies

Journal article [33]

S Stejskal, I Koutná, Pavel Matula, Z Ručka, O Daněk, M Maška, and M Kozubek. The role of chromatin condensation during granulopoiesis in the regulation of gene cluster expression. *Epigenetics*, 5(8):758–66, 2010

pdfs/2010_Stejskal_Epigenetics.pdf

pdfs/2010_Stejskal_Epigenetics.pdf

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pdfs/2010_Stejskal_Epigenetics.pdf

Journal article [36]

R Uhlířová, A Harničarová Horáková, G Galiová, S Legartová, Pavel Matula, M Fojtová, M Vařecha, J Amrichová, J Vondráček, S Kozubek, and E Bártová. SUV39h- and A-type lamin-dependent telomere nuclear rearrangement. *Journal of cellular biochemistry*, 109(5):915–26, apr 2010

pdfs/2010_Uhlirova_JCBioch.pdf

pdfs/2010_Uhlirova_JCBioch.pdf

pdfs/2010_Uhlirova_JCBioch.pdf

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pdfs/2010_Uhlirova_JCBioch.pdf

pdfs/2010_Uhlirova_JCBioch.pdf

Journal article [11]

A Harničarová Horáková, E Bártová, G Galiová, R Uhlířová, Pavel Matula, and S Kozubek. SUV39h-independent association of HP1 beta with fibrillarin-positive nucleolar regions. *Chromosoma*, 119(3):227–41, jun 2010

pdfs/2010_Harnicarova_Chromosoma.pdf

pdfs/2010_Harnicarova_Chromosoma.pdf

pdfs/2010_Harnicarova_Chromosoma.pdf

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