Segmentation Methods and Shape Descriptions in Digital Images

Applications in 2D and 3D Microscopy

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Abstract

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Digital image analysis enables creating objective, fast, and reproducible analysis methods of objects or situations that can be imaged.

This thesis contains theoretical work regarding distance transforms for images digitized in elongated grids. Such images are the result of many, mainly 3D, imaging devices. Local weights appropriate for different elongation factors in 2D, as well as in 3D, are presented. Methods adapted to elongated grids save time and computer memory compared to increasing the image size by interpolating to a cubic grid.

A number of segmentation methods for images in specific applications are also included in the thesis. Distance information is used to segment individual pores in paper volume images. This opens the possibility to investigate how the pore network affects the paper quality. Stable and reliable segmentation methods for cell nuclei are necessary to enable studies of tumor morphology, as well as amounts of fluorescence marked substances in individual nuclei. Intensity, gradient magnitude, and shape information is combined in a method to segment cell nuclei in 2D fluorescence and 3D confocal microscopy images of tissue sections. Two match based segmentation methods are also presented. Three types of viral capsids are identified and described based on their radial intensity distribution in transmission electron micrographs of infected cells. This can be used to measure how a potential drug affects the relative amounts of the three capsids, and possibly, the viral maturation pathway. Proteins of a specific kind in transmission electron volume images of a protein solution are identified using a shape based match method. This method reduces the amount of visual inspection needed to identify proteins of interest in the images.

Two representation schemes, developed in order to simplify the analysis of individual proteins in volume images of proteins in solution, are presented. One divides a protein into subparts based on the internal intensity distribution and shape. The other represents the protein by the maximum intensity curve connecting the centers of the subparts of the protein. These representations can serve as tools for collecting information about how flexible a protein in solution is and how it interacts with other proteins or substances. This information is valuable for the pharmaceutical industry, when developing new drugs.

Key words: digital image analysis, volume images, microscopy images, elongated grid, distance transform, segmentation, shape description, grey-level, gradient magnitude, watershed, decomposition

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Papers I – VIII

Papers appended to the thesis

The thesis is based on the following articles. All published papers are reproduced with permission from the publisher.

I   Sintorn I-M., Borgefors G. (2001). Weighted distance transforms in rectangular grids. In Ardizzone, E. and Gesù, V., editors, *Proc. 11th International Conference on Image Analysis and Processing, Palermo, Italy*, pages 322–326. IEEE Computer Society.

II   Sintorn I-M., Borgefors G. (2004). Weighted distance transforms for images digitized in elongated voxel grids. *Pattern Recognition Letters* 25:571–580.

III   Sintorn I-M., Axelsson M., Svensson S., Borgefors G. (2005). Segmentation of individual pores in paper volume images. *Submitted for journal publication*.

IV   Sintorn I-M., Homman-Loudiyi M., Söderberg-Nauclér C., Borgefors G. (2004). A refined circular template matching method for classification of human cytomegalovirus capsids in TEM images. *Computer Methods and Programs in Biomedicine*, 76(2):95–102.

V   Wählby C., Sintorn I-M., Erlandsson F., Borgefors G., Bengtsson E. (2004). Combining intensity, edge, and shape information for 2D and 3D segmentation of cell nuclei in tissue sections, *Journal of Microscopy* 215:67–76.

VI   Sintorn, I-M., Mata, S. (2004). Using grey-level and shape information for decomposing proteins in 3D images. In *Proc. IEEE International Symposium on Biomedical Imaging, Arlington, VA, USA*, pages 800–803. Mira Digital Publishing.

VII Sintorn, I-M., Gedda, M., Mata, S., Svensson S. (2005). Medial grey-level based representation for proteins in volume images. To appear in *Proc. of Iberian Conference on Pattern Recognition and Image Analysis, Estoril, Portugal, Lecture Notes in Computer Science*. Springer-Verlag.

VIII Sintorn, I-M., Borgefors, G. (2005). Shape based identification of proteins in volume images. *Submitted for publication.*

The work presented in Papers I and II were developed under close discussions with Gunilla Borgefors while the calculations and implementations (C, and C++) and most of the writing was performed by the author. The method presented in Paper III was developed and written mainly by the author but with help, comments and advice from the coauthors. Adapting existing methods and implementing new ones (C++) were performed by the author. The work in Paper IV was closely discussed with Mohammed Homman-Loudiyi, while the author performed the algorithm development, implementations (Matlab) and most of the writing herself. The work in Paper V was performed in close cooperation with Carolina Wählby. The method development, implementation and writing was split between her and the author. The work presented in Paper VI was performed mainly by the author, regarding method development, adapting existing implementations and creating new ones (C++), and writing the paper. Paper VII was produced in close cooperation with Stina Svensson, and the work with developing the method, implementing it and writing the paper was split mainly between her and the author. The method presented in Paper VIII was mainly performed by the author regarding method development, implementation (C++ and Matlab), and writing the paper.

The C and C++ implementations were either done as stand alone programs or as modules in the image processing platform IMP, developed at the Centre for Image Analysis originally by Bo Nordin and with substantial contributions by Joakim Lindblad.

1 Introduction and objectives

The number of areas using digital images as tools, for evaluation and analysis, is steadily increasing. Constantly improved technology for image generating systems, and cheaper and better computers, constitute the main underlying reasons. This growth, in turn, gives rise to a demand and wish for automatic analysis and extraction of information captured in the images. The idea of using computers for performing quantitative and objective studies of information present in images, has been around for quite some time. The first textbook on the subject was published already in the late sixties by Rosenfeld (1969), and since then, the demand and interest for digital image analysis has been ever increasing.

The work leading to this thesis was performed at the Centre for Image Analysis (CBA) in Uppsala, which is a joint institution between the Swedish University of Agricultural Sciences and Uppsala University. CBA was founded in 1988 and theoretical and application oriented image analysis research has since then been performed in the fields of discrete geometry, medical and biomedical image analysis, forestry and agriculture, and remote sensing. This thesis contains work related to several of the mentioned fields. Theoretical work on digital topology has been performed, regarding distance transforms, continuing the work by Professor Borgefors, supervisor of the author, and work presented in earlier CBA theses by Nyström (1997) assistant supervisor for the author, and Svensson (2001). One application of the theoretical results is the analysis of the structure of paper, related to the thesis of Aronsson (2002). A large part of this thesis is devoted to incorporating both shape and intensity information in methods developed for, and adapted to, specific biomedical applications. Many of these methods have their roots in work on binary shape analysis of 2D and 3D images by CBA researchers Borgefors, Nyström, and Svensson. Both intensity and shape information has also been used in the task of digital cell image analysis, connected to the thesis by Wählby (2003).

Objectives

The main objectives of this thesis have been to adopt established image analysis methods to work directly on elongated digitalization grids, and to incorporate intensity information in distance based shape analysis and representation schemes of 2D and 3D biomedical structures.

About this thesis

This is a thesis in digital image analysis, and the emphasis therefore lies on the developed methods, and not on the different applications. The concepts and methods are, hence, presented as generally as possible, although certain application specific circumstances and problems are discussed to motivate the choice of actions. The images studied in this thesis were all acquired with different microscopy techniques. A brief description of how the different microscopes work is given in Section 2. The author has, however, not acquired any of the images herself, and is not an expert on any of the systems. Section 3 contains image analysis concepts that constitute the foundation of this work. In Section 4, the methods in the appended Papers are

described, together with discussions and ideas for further developments. Finally, conclusions and a summary of ideas for future work are presented in Section 5.

2 Microscopy techniques

The methods presented in this thesis were in most cases developed for either a specific application or a specific type of images. The images were all acquired by experts in the microscopy techniques briefly presented below. For comparative purposes, absorption light microscopy is also described, although no images studied in this thesis were acquired using that technique.

2.1 Light microscopy

In a common light microscope, the visible light that is not absorbed by the sample creates the magnified image. An illustration is shown in Figure 1 (left). Light from a light source is focused on the sample using a glass lens. The light that passes through the sample is magnified and focused on the detector using two lenses. The resolution limit in a light microscope is $0.2\mu m$, (Alberts et al., 1994), i.e., half the wavelength of blue light, which is the visible light with the shortest wavelength.

2.2 Fluorescence microscopy

In fluorescence microscopy, light emitted from fluorescent molecules in the sample is imaged. A fluorescent molecule absorbs light of certain wavelengths, and then emits light of longer wavelengths (longer wavelengths have less energy). Specific structures can be marked with fluorescent molecules, or sometimes a structure is fluorescent by nature. As this technique also uses light to magnify an object, the resolution limit is $0.2\mu m$, the same as in light microscopy. A dichroic, or beam splitting mirror, reflects light below a certain wavelength while longer wavelengths are transmitted. This is used in a fluorescence microscope, together with two filters, to ensure that only absorption wavelengths for the fluorescent marker to be imaged hit the sample, and at the same time only emitted wavelengths from that marker hit the detector. In Figure 1 (middle), a schematic representation of a fluorescence microscope is seen. Light from the light source of the absorption wavelengths is let through a filter, reflected in the dichroic mirror, and focused onto the sample. When the light reaches the fluorescent molecules they begin to emit light. Light from the sample is transmitted through the dichroic mirror, filtered to ensure that only light of certain wavelengths is present, and focused onto a detector where the image is formed.

2.3 Confocal microscopy

Just as in fluorescence microscopy, light emitted from fluorescent molecules is imaged with confocal microscopy. The technique is similar to ordinary fluorescence microscopy, but with the addition that a laser light source in combination with a blocking pinhole allows for imaging a specific spot of the sample at a time, see Figure 1 (right). The light source needs to be a well focused laser to make sure that the light, which is reflected in the dichroic mirror, is focused onto a small spot in the sample. Light emitted from this spot is transmitted through the dichroic mirror and
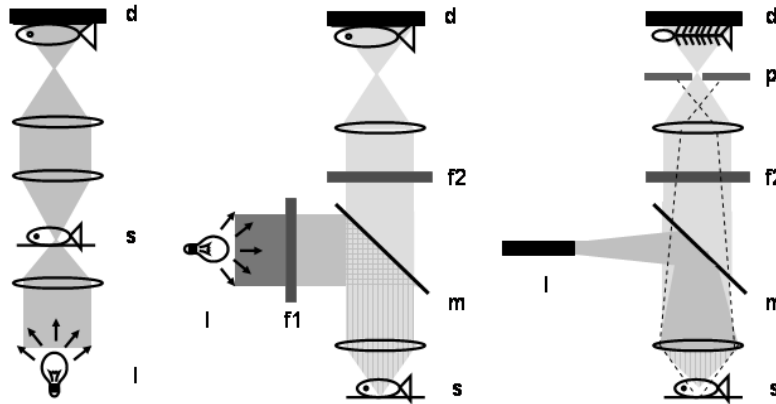
Figure 1: Simplified drawings of a light microscope (left), a fluorescence microscope (middle), and a confocal microscope (right). All three microscopes have a light source (l), a specimen (s), and a detector (d). The fluorescence microscope also has two filters (f1, f2) and a dichroic mirror (m) that control which wavelengths of the light that reach the specimen and the detector. The confocal microscope has a filter (f2) and a pinhole (p), in front of the detector, to ensure that light from out of focus objects (dashed lines) and light of undesired wavelengths do not reach the detector.

passes through a pinhole before it hits the detector. This pinhole blocks most of the light emitted from out of focus parts of the sample. The focal spot can scan the sample in x- and y-direction using a set of mirrors, and the sample can be moved in the z-direction. The confocal technique thereby allows for imaging of 3D structures, illustrated with the fish bone in Figure 1 (right). If a sampling corresponding to the best possible resolution is chosen for a specific setup, the resulting volume image will have elongated voxels, as light from out of focus objects limits the resolution in the z-direction more than in the x- and y-directions. It is very difficult to give any numbers for how thick objects that can be imaged with a confocal microscope as it depends on the sample itself as well as on the microscope setup. The amount of light reaching the detector decreases with depth, as emitted light from deeper into the sample will be absorbed and reflected by the sample on top of it and less light will, hence, reach the detector than from the top part of the sample. An example, to give a feeling for the order of the size it is possible to image, is that a good $50\mu m$ cell tissue section with a resolution of approximately $0.2\mu m$ in the x- and y-direction and $0.3\mu m$ in the z-direction can be achieved.

2.4 Transmission electron microscopy (TEM)

A transmission electron microscope, see Figure 2 (left) for a schematic drawing, images the amount of electrons that passes through the sample at different positions. The denser the material is in a spot of the sample, the more the electrons are

scattered and less electrons will, hence, be transmitted through that spot. This is illustrated with the dense fish bone appearing in the resulting image in Figure 2 (left). Air molecules also scatter electrons and therefore the imaging takes place in vacuum. Electrons are focused on the sample by an electromagnetic lens and the electrons that are transmitted through the sample are magnified and projected onto a detector using two more electromagnetic lenses. The resolution limit in an electron microscope for biological samples is about $2nm$, (Alberts et al., 1994).

Electron tomography, or 3D imaging of an object using electron microscopy, can be performed by reconstruction from a series of images of the sample, acquired at different tilt angles. Sidec^{TM} Electron Tomography (SET), (SET webpage, 2004), uses the common reconstruction method filtered back projection, in combination with a refinement method called COMET, (Skoglund et al., 1996). This makes it possible to use a lower dose of electrons, which in turn, allows for acquisition of more images in a tilt series, without destroying the sample. With the combination of low dose electron microscopy and a refinement method, SET can reconstruct individual molecules down to a resolution of approximately $2nm$ (SET webpage, 2004).

2.5   Scanning electron microscopy (SEM)

In scanning electron microscopy (SEM), see Figure 2 (right), scattered electrons are detected instead of transmitted electrons. In backscatter mode, the electrons scattered back from the top part of the object are detected. The electron beam in a scanning electron microscope is focused by an electromagnetic lens and is bent using scan coils or a beam deflector to scan over the sample. At each spot of the sample, the backscattered electrons are detected and converted to an intensity value reflecting the density of the spot. Hence, a SEM image is a 2D image of the top part of the sample. The resolution limit for biological samples in SEM is about $10nm$, (Alberts et al., 1994). 3D SEM images can be produced as stacks of 2D images, each acquired using SEM, with a thin slice of the sample cut off between successive image acquisitions. The resolution in the x- and y-direction is usually higher than the thickness of the slices, and, hence, 3D images with elongated, box-like, voxels are the result.
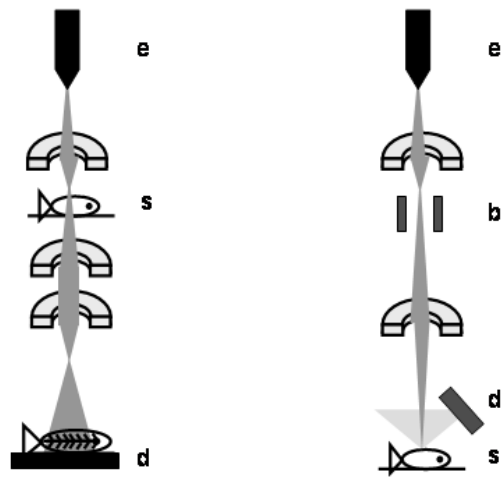
Figure 2: Simplified drawings of a transmission electron microscope (left), and a scanning electron microscope (right). Both microscopes have an electron gun (e), a specimen (s), and a detector (d). The scanning electron microscope also has a beam deflector (b).