MVA2005 IAPR Conference on Machine VIsion Applications, May 16-18, 2005 Tsukuba Science City, Japan
3-15

A New Automated Microtomy Concept for 3D Paper Structure Analysis

Michael Donoser [1,2], Mario Wiltsche [2] and Horst Bischof [1]

[1]Institute for Computer Graphics and Vision
Graz University of Technology
Inffeldgasse 16, 8010 Graz, Austria
michael.donoser@TUGraz.at

[2]Institute for Paper, Pulp and Fiber Technology
Graz University of Technology
Kopernikusgasse 24, 8010 Graz, Austria
mario.wiltsche@TUGraz.at

Abstract

This paper describes a novel prototype for automated digitization of the three-dimensional paper structure. The concept is based on a light microscope which is rigidly attached to a microtome. The microscope can be moved in all three directions of space with high accuracy in order to be able to digitize large samples with high spatial resolution. All components are controlled by a PC interface which enables an automated digitization process. Sample sizes of one square-centimeter are digitized within hours. Two different pre-processing steps, stitching and aligning, are applied to the raw data set to get an accurate 3D representation of the paper structure. Initial experiments on analyzing the created data sets based on image analysis methods prove the applicability of the concept.

1 Introduction

Many physical paper properties are strongly influenced by the spatial distribution of the raw materials in the paper sheet, like fibers, fiber fragments, filler particles and sometimes a coating layer. In-depth knowledge of this spatial microstructure makes fundamental research on the interrelations between paper quality and papermaking process possible. Thus, a detailed analysis of the three-dimensional paper structure is of high industrial interest.

The main challenge is to digitize paper structure with high spatial resolution, sufficient sample sizes and with adequate investment and operating costs. The sample size requirement originates from the heterogeneity of the paper itself, therefore at least one square centimeter should be analyzed. None of the current technologies is able to fulfill these requirements. Therefore a new concept was developed and a prototype was designed and built, which is presented in detail in section 2.

Image analysis algorithms needed for creation of an accurate, digital 3D paper structure representation are described in section 3. The created data set can constitute the basis for many different applications, that derive characteristics of the paper structure. Initial applications, which prove the usefulness of the novel concept are presented in section 4.

2 Novel Approach for Digitization of Paper Structure

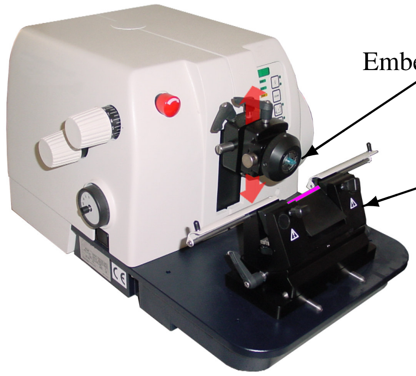

Figure 1. Microtome for cutting embedded paper samples.

The novel concept is based on automated microtomy and serial sectioning combined with light microscopy.

Microtomy is a widespread technique with applications in medicine, biology and material sciences in which uniformly thin sections are cut of embedded specimens for detailed microscopic examination. The sample is put into the specimen clamp in the microtome, see figure 1. When sectioning is started the specimen head moves downwards over the knife and a thin slice is cut off. The cutting movement is completed in the upper limit.

It is necessary to embed the sample before sectioning to increase the stability of the sample and to support internal structures of the specimen during cutting. The choice of the embedding material depends on the specimen type and the desired slice thickness. The hardness of this embedding compound determines the achievable slice thickness. Hard materials like resin allow ultra thin sectioning in the range of 50 nm, whereas with soft embedding media like paraffin wax only thick sections in the range of 10 μm can be obtained [9].

The material used for embedding paper samples is epoxy resin or glycol methacrylate. Sample preparation follows standard embedding procedures in preparation of paper samples – see for instance [4, 7, 14]. Different types of molds are used, for instance gelatine capsules with diameter up to 9 mm or histological molds which allow embedding of samples with sizes up to $10 \times 25\ mm^2$. In most cases tungsten carbide knives, glass knives or diamond knives are used for sectioning these embedding materials. The best section quality could be achieved by using diamond knives, they also ensure a stable and long tool life.

Common practice in microtomy is to remove the thin sections from the knife manually and to mount these slices on microscope slides. Due to the fact that sections tend to curl during sectioning the manipulation of each single slice is a very sophisticated and time consuming process which requires a lot of skill and experience. Distortions or even loss of single slices due to cutting and manipulation are unavoidably. These disturbances complicate 3D reconstruction or even make it impossible.

Therefore a new technique was developed in order to eliminate these limitations. The digitization is done on the non distorted surface, hence no manipulation of the slices is required. The configuration basically consists of a light-optical microscope which is rigidly connected to a microtome. The system is equipped with a CCD camera for digital imaging. The microscope is fixed on a stage which can be moved with high accuracy in all three directions of space. To ensure sufficient sample size the cut block surface is digitized by means of a sequence of adjacent images covering the entire region-of-interest.

The microtome, light source, CCD camera and stage are controlled by a PC interface. A software application triggers the microtome, initiates the correct camera movement and acquires the desired sequence of images of the previously selected region-of-interest. Repeatedly, slices are cut off and the surface is scanned, which enables a completely automated digitization of a paper sample without any user-interaction needed.

The resolution in image plane – x-y-plane, see Figure 2 – is dependent on the lens used and can be varied from $0.26\,\mu m$ to $1.29\,\mu m$ with a corresponding field-of-view of $336 \times 259\,\mu m^2$ to $1681 \times 1293\,\mu m^2$. The cut thickness defines the distance between two adjacent slice-images and therewith the resolution in z direction, see Figure 2. The minimal cut thickness is $0.5\,\mu m$, the upper limit depends on the hardness of the embedding material and can be up to $10\,\mu m$.

The digitization of paper a sample with a size of $2\,mm$ in y direction and $1\,mm$ in z direction with a resolution of $0.65\,\mu m$ in y direction and $5\,\mu m$ in z direction takes for instance 105 minutes. Time need for digitization of one square centimeter with such a resolution is less than 12 hours.

3 Creation of Digital 3D Paper Structure Representation

The result of the presented automated microtomy concept is a set of images, showing the region-of-interest of the particular slice area. Before the digital data set can be analyzed in detail two different pre-processing steps – stitching and aligning – have to be applied to the set of acquired images.

The field-of-view covered by a single image does not reach the required sample size. That is why the slice area has to be scanned in a sequence of overlapping, adjacent images, which is realized by the three-dimensionally moveable microscope. Due to algorithmic and performance reasons this set of images has to be connected to a single composite image, showing one particular slice area. This process is referred to as stitching or mosaicing, see section 3.1.

The second challenge are the misalignments between subsequent slices. These displacements have their origin in the limited sample positioning accuracy of the microtome after each cut. To obtain an accurate 3D representation of the paper structure the displacements have to be corrected. This step is referred to as aligning and is described in more detail in section 3.2.

3.1 Stitching

Image stitching has been a central issue for a variety of problems in image analysis. It is a well investigated research area, especially due to the success of digital photography and its application area of creating panoramic views [3]. Therefore, most photo programs already provide some simple stitching functionality. Stitching also has its applications in virtual reality [11], 3D model recovery [12] and texture synthesis [5].

BROWN et al. [2] give a comprehensive overview of different stitching concepts. In general image stitching techniques can be classified into two main groups: the direct and the feature based methods. The direct methods use the entire available pixel information to calculate the ideal image registration, whereas feature-based methods first extract points of interest and estimate transformation to find the best registration.

The slice images are stitched with a direct method based on phase correlation [8]. The general requirements on the stitching process can be simplified due to the following two constraints. First, because the images are acquired from a static scene and the movement of the camera is known, the only transformation which has to be considered is a translation. Furthermore the overlap between the images is approximately known, because it is manually set by the user during the automated microtomy process. Another simplification is that the sequence of images is arranged column shaped, hence stitching is only made along one border. Image arrangements in matrix form, which require stitching along two borders, are more difficult to perform, but need not to be considered in this particular case.

The stitching algorithm used can be divided into two main steps: registration and blending.

Registration For the image registration step a method based on phase correlation [8] was chosen. This method is widely used for image registration, especially due to it's exceptional robustness in the presence of noise [6]. Phase correlation uses the frequency domain and exploits the fact that shifts in the spatial domain correspond to linear phase changes in the Fourier domain.

The aim of the image registration step is to calculate a displacement vector $\boldsymbol{v} = [\Delta x\ \Delta y]$ which best matches one image to another. Thus, by assuming that the input of the algorithm are two images named $I_1(x, y)$ and $I_2(x, y)$ the registration problem can be defined as finding those values for Δx and Δy that fulfill equation 1.

$$I_1(x, y) = I_2(x - \Delta x, y - \Delta y) \tag{1}$$

Also the Fourier transforms of the images are then related by

$$\mathcal{F}_2(\omega_x, \omega_y) = e^{-j(\omega_x \Delta x + \omega_y \Delta y)} \mathcal{F}_1(\omega_x, \omega_y). \tag{2}$$

By calculating the phase correlation matrix, one gets

$$\frac{\mathcal{F}_1^*(\omega_x, \omega_y)\mathcal{F}_2(\omega_x, \omega_y)}{|\mathcal{F}_1(\omega_x, \omega_y)\mathcal{F}_2(\omega_x, \omega_y)|} = e^{-j(\omega_x \Delta x + \omega_y \Delta y)}. \tag{3}$$

The inverse Fourier transform of this equation will result in a Dirac pulse δ at the coordinates of the desired translation position.

$$\frac{\mathcal{F}_1^*(\omega_x, \omega_y)\mathcal{F}_2(\omega_x, \omega_y)}{|\mathcal{F}_1(\omega_x, \omega_y)\mathcal{F}_2(\omega_x, \omega_y)|} \xrightarrow{IFT} \delta(x - \Delta x, y - \Delta y) \tag{4}$$

Thus, to finally extract the displacement vector $\boldsymbol{v} = [\Delta x \, \Delta y]$ the maximum value of the result of the Fourier transformation is interpreted as the Dirac location and therefore as the optimal displacement.

REDDY et al. [10] proposed an addition to the phase correlation approach, which considers rotation and scale transformations. But as mentioned before, for the stitching of the slice images only translational transformation has to be considered.

Blending Registration results in a displacement vector, which is used to stitch the images together. One important property of the final composite image should be that the transitions between the stitched images are almost seamless. This is achieved by another image analysis step called blending.

One of the most popular methods for blending, is to use a weighted average of the input images at the overlap region. Such concepts are sometimes referred to as feathering or alpha blending [13]. New intensity values for every pixel in the overlap region are calculated by the equation

$$I(i, j) = \alpha_{ij} I_1(i, j) + (1 - \alpha_{ij}) I_2(i, j), \tag{5}$$

where $\alpha_{ij} \in [0, 1]$ is a weighting parameter and is used to control the blending effect. The values of α_{ij} are set proportionally to the distance to the cutting line. The final result of stitching is a composite image, showing the entire region-of-interest of the slice area after one microtome cut.

3.2 Aligning

Stitching and aligning can be solved by very similar algorithms. The main problem for both is to find the displacement that best registers one image with another. Therefore the phase correlation algorithm, see section 3.1 can also be used for the alignment problem.

Naturally each of the two problems has its own specific requirements. For stitching the main focus lies on achieving a very smooth transition between the stitched images. In the aligning process the registration is more difficult, because the scene changes from cut to cut, but again only translational transformations have to be considered and in addition the blending step can be neglected. Aligning is realized by including the upper border of the embedding mold at the top of each slice image. The contour of the border of the capsule does not change significantly from cut to cut. Thus, the correct alignment can be calculated based on this contour.

4 Potential Applications

After aligning and stitching a digital 3D representation of the paper sample is obtained. Figure 2 shows a small cut-out of a digitized 3D paper structure.

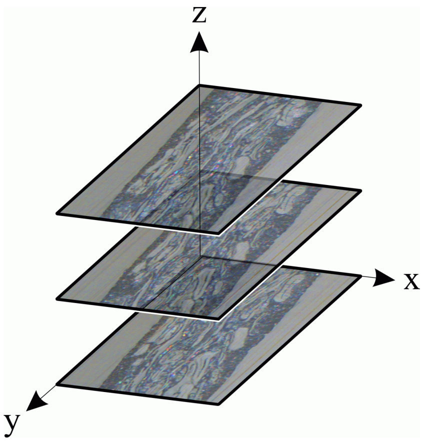

Figure 2. Cut-out of 3D paper structure representation.

4.1 Coating Layer Analysis

As a first, possible application the coating layer formation of paper samples was analyzed. Coating is the application of pigments, polymers or other materials to the surface of a paper sheet in order to improve paper properties for subsequent processing steps, as for instance printing. Coating increases smoothness due to filling cavities and covering basepaper surface. Uniformity of coating layer formation influences print quality directly and therefore is an important quality parameter.

Generally, the coating layer can be identified in the slice images as homogeneously colored areas on each side of the paper, see Figure 2. Therefore a 3D color segmentation algorithm, based on comparing multivariate RGB color distributions with the Bhattacharyya distance [1], was applied. The approach itself is a three-dimensional concept, meaning that for the detection of the coating layer in one slice image, also data from the neighboring slices is incorporated. Thus, the algorithm delivers both, a detailed and robust coating layer segmentation. The generated coating layer thickness values can be visualized three-dimensionally, see Figure 3.

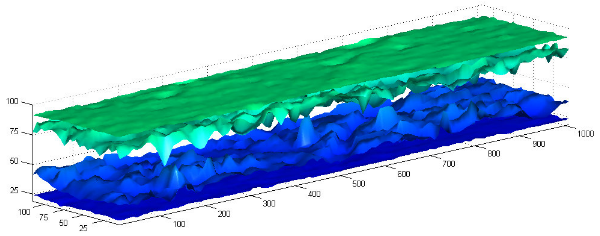

Figure 3. 3D visualization of coating layer [μm].

A volume-mass-balance was made for an initial validation of the coating segmentation concept. With an estimated density of the coating layer of $2.2\,{}^{\mathrm{cm}^3}\!/_{\mathrm{g}}$ and a known amount of applied coating material $20\,{}^{\mathrm{g}}\!/_{\mathrm{m}^2}$ on each side the average coating thickness can be calculated by

$$\frac{20\,{}^{\mathrm{g}}\!/_{\mathrm{m}^2}}{2.2\,{}^{\mathrm{cm}^3}\!/_{\mathrm{g}}} = 9.09\,\mu m.$$

This value corresponds with the value determined by the segmentation process of $9.35\,\mu m$ on the bottom side and $9.56\,\mu m$ on the top side.

In addition the overall paper thickness was measured with the DIN EN 20534 standard, which resulted in a thickness of $62\,\mu m$. Also this value corresponds to the segmentation result of $62.3\,\mu m$.

4.2 Fiber Network Analysis

Current research focuses on the analysis of the 3D fiber network, based on the digital paper structure data. Due to the complex three-dimensional network of fibers enhanced image analysis methods are required. The possibility of accessing single fibers, and determining their position and orientation within the paper sample, will e. g. allow the 3D analysis of fiber orientation, calculation of spatial mass-distribution or determination of bonding areas between fibers. Image analysis methods for fiber network analysis are currently under developement. An initial segmented fiber can be seen in Figure 4.

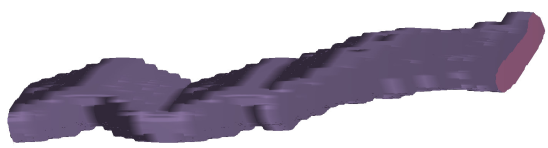

Figure 4. Cut-out of segmented fiber ($200\,\mu m$ length).

5 Summary and Outlook

The presented concept of automated microtomy is a new approach to digitize 3D paper structure in a fast way with high resolution. Sample sizes in the scale of one square centimeter can be digitized within hours. In addition the system allows resolutions of up to $0.26\,\mu m \times 0.26\,\mu m \times 0.5\,\mu m$. This configuration opens a wide range of future research activities. In a first step image analysis methods were used to create detailed data about the spatial coating layer formation. Initial experiments based on industrial paper samples revealed the potential of this method.

Acknowledgements

The authors gratefully acknowledge financial support from Austrian Research Promotion Agency Ltd. (FFG) and from Mondi Business Paper Austria, Mondi Packaging Frantschach, M-Real Hallein, Norske Skog Bruck, Sappi Gratkorn, SCA Graphic Laakirchen, UPM Steyrermühl and Voith Paper.

References

[1] A. Bhattacharyya. On a measure of divergence between two statistical populations defined by their probability distributions. *Bulletin of the Calcutta Mathematical Society*, 35: 99–110, 1943.

[2] L. G. Brown. A survey of image registration techniques. *ACM Computing Surveys*, 24(4):325–376, 1992.

[3] D. Capel and A. Zisserman. Automated mosaicing with super-resolution zoom. *Proc. IEEE Conf. on Computer Vision and Pattern Recognition*, pages 885–891, 1998.

[4] A.R. Dickson. The quantitative microscopic analysis of paper cross-sections: sample preparation effects. *Appita Journal*, 53(5):362–366, 2000.

[5] A. A. Efros and W. T. Freeman. Image quilting for texture synthesis and transfer. *Proceedings of SIGGRAPH*, pages 341–346, 2001.

[6] H. Foroosh, J.B. Zerubia, and M. Berthod. Extension of phase correlation to subpixel registration. *IP*, 11(3):188–200, March 2002.

[7] D.L. Gibbon, R.T. Gray, and G.C. Simon. Stereological and chemical analysis of paper cross-sections. In *Tappi Coatings & Papermakers Conference*, pages 435–454. Tappi Press, Atlanta, 1998.

[8] C.D. Kuglin and D.C. Hines. The phase correlation image alignment method. *Proc. IEEE Int. Conf. Cybernet. Society*, pages 163–165, 1975.

[9] G.H. Michler and W. Lebek. *Ultramikrotomie in der Materialforschung*. Carl Hanser Verlag, Muńchen, 2004.

[10] B. S. Reddy and B. N. Chatterji. An fft-based technique for translation rotation, and scale-invariant image registration. *IEEE Transactions on Image Processing*, Vol. 5(No. 8):1266–1271, 1996.

[11] R. Szeliski. Video mosaics for virtual environments. *IEEE Comput. Graphics Appl.*, 16(2):22–30, 1996.

[12] R. Szeliski and J. Coughlan. Hierarchical spline based image-registration. *Proc. IEEE Conf. on Computer Vision and Pattern Recognition*, pages 194–201, 1994.

[13] M. Uyttendaele, A. Eden, and R. Szeliski. Eliminating ghosting and exposure artifacts in image mosaics. *Conf. on Computer Vision and Pattern Recognition*, II:509516, 2001.

[14] G.J. Williams and J.G. Drummond. Preparation of large sections for the microscopical study of paper structure. *Journal of Pulp and Paper Science*, 26(5):188–193, 2000.