Parameter assisted HE colored tissue image classification

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***Abstract*— The aim of our work was to design and implement a software solution, which supports quantitative histological analysis of hematoxilin eozin (HE) stained colon tissue samples, identify tissue structures – nuclei, glands and epithelium – using image processing methods. Furthermore, based on the result of the histological segmentation, it gives a suggestion for the negative or malignant status of the samples automatically. In this paper we describe the algorithm which builds up mainly by two software components: MorphCheck -our software framework-, which is capable to make effective, morphometric evaluation of high resolution digital tissue images and a modified WND-CHARM (Weighted Neighbor Distance Using Compound Hierarchy of Algorithms Representing Morphology), which is a multi-purpose image classifier. The image classification was performed mainly based on 75+15 pre-defined colon tissue specific parameters, which were measured by MorphCheck, and other 2873 in-built generic image parameters, which were measured by WND-CHARM. We appended WND-CHARM's learning and classification capabilities with our colon tissue specific parameters and with this act we have increased its classification accuracy significantly on HE stained colon tissue sample images.**

I. INTRODUCTION

The quantitative analysis of tissue samples is able to support the histological investigations and contribute to establish the accurate diagnosis. The effectiveness of image analysis methods and computer processing make it possible to collect and analyze many tissue attribution which can support the final decisions of the pathologist. The detection and the investigation of dedicated tissue elements are necessary to classify correctly the pathological aberration. For examples the gland shape, the structure of the epithel and the cell morphology have diagnostic role in colon cases with malignant lesion.

A. Aim of the work

The main aim of our work was to design and implement a digital pathology software solution, which supports scanned hematoxilin eozin (HE) stained colon tissue samples, identify tissue structures – the nuclei, glands and the surface epithelium – using image processing methods, do their quantitative analysis and classify the healthy/malignant samples automatically. MorphCheck - our developed software framework- is capable to do effective, morphometric evaluation of high resolution digital tissue images. Our image classification process applies multi-purpose image parameters and special, pre-defined (75+15) tissue parameter set.

B. The background of our work

After some extensive testing of numerous image classification solutions, we have chosen the WND-CHARM (Weighted Neighbor Distance Using Compound Hierarchy of Algorithms Representing Morphology) algorithm and integrated our architecture with our tissue image parameter set. We have optimized the algorithm and modified the source code.

We have appended its learning and classification capabilities with our tissue parameters successfully and increased its classification accuracy significantly on HE stained colon tissue sample images (Fig. 1).

During our research work a large set of relevant tissue parameters have been identified. We have implemented all the required algorithms to search the relevant tissue structures, found and measured precisely the pre-defined tissue parameters. This tissue parameter was analyzed using the WND-CHARM's image classification process to categorize the input tissue images into healthy (negative) and malignant categories (adenoma, carcinoma). Our aim was to decrease the number of manually evaluated images in everyday work using a classification based automatic pre-filtering solution. Pathologists make a distinction between malignant and normal (or benign) tissue, and further differentiate between malignancy types. If the classification is able to distinguish between normal and malignant tissue images we can offload significant amount of work from pathologists.

In the first section we describe our development targets, give a short state-of-the-art overview. Later on we provide information about our tissue image parameter set and about MorphCheck (our high resolution tissue image analyzer framework).

In the next sections we give an overview what we have modified in WND-CHARM, and we present experimental results demonstrating the efficacy of the introduced tissue parameters in test cases.

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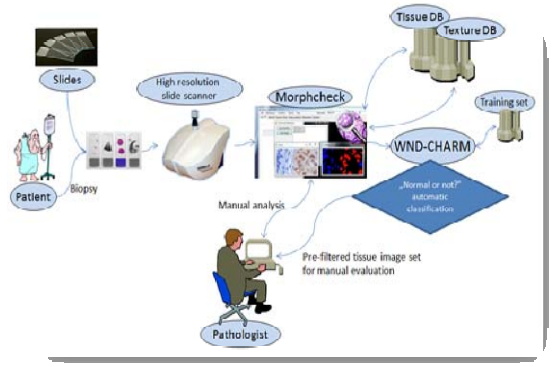

Figure 1. Tissue image processing by MorphCheck and pre-filtering by WND-CHARM

C. State-of-the Art on tissue image analysis

High resolution tissue image analysis and classification is a hot research topic nowadays. Digital microscope vendors such as 3DHistech[1] has developed HistoQuant [2], which is capable to do colorization based segmentation, NuclearQuant [3], which is capable to analyze cell nucleus , and MembraneQuant[4] which can detect and analyze membrane structures between the cellular matrix. Aperio[5] has ePathology software solutions and provides an automatic image analysis platform.

Other examples are: Definiens's Tissue Studio[6]; Visiopharm's VisiomorphDP™ [7] and TissuemorphDP™, which are providing tissue image analysis and cell population analysis. Targeted users of these software solutions are mostly the professionals of the pathologist community and these software solutions are trying to enable the new era of ePathology/Digital Pathology in an unstoppable way.

More generic software solutions and frameworks are also available, such as the CellProfiler/CellProfilerAnalyst [8], which has a large number of integrated image processing algorithms. It is capable to do generic and effective image analysis and classification based on its existing cell image database. CellProfiler is a modular, open source solutions, it is using a configurable image processing pipeline and also able to work automatically in batch mode.

II. Materials and Methods

A. Tissue structure parameters

The collaboration between John von Neumann Faculty of Informatics, Obuda University and 2nd Department of Internal Medicine, Semmelweis University, Budapest, enabled us to define 75 tissue parameters of the human colonic region. First part of the parameter set targets the nucleus. The stained nucleus can be localized in a cell, which could be a part of the connective tissue, the gland or the surface epithelium.

The second part of the parameter set targets morphological/morphometrical features of the higher tissue structures such as the glands and epithelium. With the whole parameter set we are able to capture in a form of numerical values the most important morphological and morphometrical properties of the tissues in the colonic region. Later, we have defined and appended the core tissue parameter set with another 15 new measurable tissue parameters.

B. Measuring the tissue structure parameter set using MorphCheck

We have extended our generic MorphCheck[9] medical (tissue) image analysis framework with new appropriate algorithms and have defined and implemented image processing workflows in MorphCheck to accurately measure these tissue parameters in the processed images.

MorphCheck software framework is capable to effectively recognize with its extendable algorithm repository large number of tissue structures (such as surface epithelium, gland structures, lamina muscularis, submucosa etc.), and measure their morphological and morphometrical properties. The objective numerical values of the pre-defined tissue parameters enable us to integrate and adapt a generic image classifier software solution, which can do effective tissue image classification automatically based on our parameter set. The two software solutions (MorphCheck and WND-CHARM) have been loosely coupled together.

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The two software solutions (MorphCheck and WND-CHARM) have been easily coupled together. Data communication between the two software solutions is realized in a form of automatic comma separated file exchange. After MorphCheck finished its workflow (ROI selection, tissue structure searching and measurement) the results are forwarded to WND-CHARM for image classification. WND-CHARM put the received tissue image into some pre-defined category and sends back the results of the image classification process (Fig. 2). The pathologist can manually check the images dumped in the malignant (adenoma, carcinoma, etc.) categories for further analysis.

MorphCheck supports specific digital slide image formats and regular image standards (such as Tagged Image File Format /tiff/, Joint Photographic Experts Group /jpg/). It supports various colorization schemes like: HE (Hematoxilin-Eozin), DAB (3,3'-Diaminobenzidin), multi-color FISH. MorphCheck framework contains various texture based algorithms, furthermore intensity and structure based algorithms (such as K-means, region growth, etc.) [12].

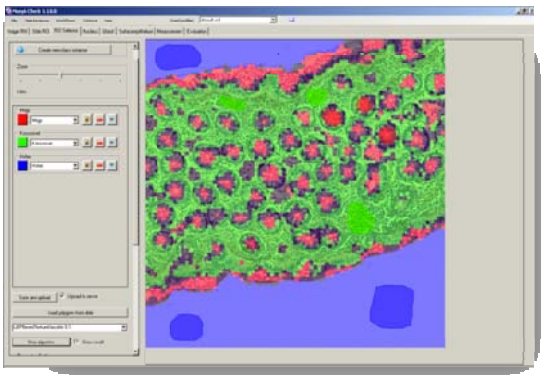

Figure 2. Semi-automatic texture based ROI selection in MorphCheck

MorphCheck provides solutions to process tissue images both automatically or manually. During automatic image processing MorphCheck requires only to define the base directory, where all the images are stored. Definition of the ROI and the image processing workflow is handled automatically. The ROI can be predefined with manual annotation, and texture based automatic ROI selection is also available.

C. *WND-CHARM – a multi-purpose image classifier*

A WND-CHARM [10] is an open source, multi-purpose image classifier. WND-CHARM extracts a large set of (1025 or 2873) image features including polynomial decompositions, high contrast features, pixel statistics, and textures. These features are computed on the raw image, transforms of the image, and second transforms of the image.

The feature values are then used to classify test images into a set of pre-defined image classes. WND-CHARM is using WND-5 as image classifier.

D. *MorphCheck and WND-CHARM integration*

We have used WND-CHARM as core image classifier application, and have combined with some adaptation to our MorphCheck image analysis framework. MorphCheck measures tissue structure parameters (such as: gland diameter/surface, nucleus diameter/surface, etc.) on the tissue images. We will refer to these parameters as MC parameters later on.

We have loosely coupled the two software solutions together, and have created an automatic image classifier with batch mode support.

We have included our MC tissue parameters into WND-CHARM's feature set. Before any integration we have evaluated the classification effectiveness and performance of WND-CHARM with its iicbu-2008 [11] training image set.

Later on, we have used HE colored tissue images to evaluate the classification procedure. During our study we have also checked the improvement differences between WND-CHARM versions.

E. *Software modifications*

We have implemented measurement algorithms for each MC parameters in MorphCheck. We have created workflows for automatic ROI selection and ROI processing. We have extended the storage capabilities of the software to hold the newly measured and derived MC tissue parameters. MorphCheck provides the measured MC tissue parameter values and the high resolution tissue image as input for WND-CHARM. We have integrated the MC parameters into WND-CHARM's feature set. Finally, the modified WND-CHARM application was able to use 75 MC tissue parameters for training and classification as well.

III. TISSUE IMAGE CLASSIFICATION MEASUREMENTS

A. *Workflow of the measurements*

The performance of the modified (tissue specific) WND-CHARM was evaluated using several datasets.

Firstly, we have measured the basic classification effectiveness of the WND-CHARM. We have tested various versions of the software. We have tested the windows compatible version (it was released in 2009[13], and many Linux based versions (v1.30; v1.31; v1.32b, r369, r57) with the test image set of the application. We have compared their accuracy and have checked how the initial configuration parameters influence the classification results.

Finally, we have used v1.30.227 as the baseline application for our measurements. We have used fully, manually pre-annotated high resolution colon tissue images. The annotation was done manually by professional pathologists from 2nd Department of Internal Medicine, Semmelweis University, Budapest.

Measurement I.

- Annotated training set: 30 tissue images, 3 classes (10 healthy, 20 malignant /10 adenoma, 10 carcinoma/).
- Annotated input image set for classification:16 tissue images (10 healthy, 4 adenoma, 2 carcinoma).

Used classifiers

- WND-CHARM = wnd-charm v1.30.227 with 1025 internal parameter
- WND-CHARM_MC = wnd-charm v1.30.227 with 1025 internal parameter + 75 tissue parameters measured by MorphCheck.

Measurement II.

- Annotated training set: 48 tissue images, 6 classes (8 healthy, 8 healthy_noep, 8 adenoma, 8 adenoma_noep 8 carcinoma, 8 carcinoma_noep).
- Annotated input image set for classification:45 tissue images (14 healthy, 31 malignant /18 adenoma, 13 carcinoma).

Used classifiers

In these measurements we have increased the number of generic features during the classification procedure.

- WND-CHARM_x = wnd-charm v1.30.227 with 2873 internal parameters.

- WND-CHARM_x_MC = wnd-charm v1.30.227 with 2873 internal parameters + 75 tissue parameters measured by MorphCheck.

B. *Measurement results*

Measurement I.

- WND-CHARM: Malignant/healthy classification accuracy was 50% at malignant images (from 6 malignant 3 was missed) and 90% was at healthy images (from 10 healthy 1 was missed). Overall classification accuracy was 75 %.
- WND-CHARM_MC: Malignant/healthy classification accuracy was 66% at malignant images (from 6 malignant 3 was missed) and 100% was at healthy images (from 10 healthy 0 was missed). Overall classification accuracy was 87 %.

WND-CHARM vs. WND-CHARM_MC

WND-CHARM_MC (extended with the tissue structure specific MC parameters) outperforms significantly (Table I) the native version. To create a usable classification procedure for colonic tissue images based on automatic pre-filtering solution we have to sort out successfully every malignant tissue image from the image set. If a healthy image marked falsely malignant, it is not a critical problem, because it will be processed manually by the pathologists.

We have introduced a cut-off number which defines a tolerance level of the classification certainty. Below this certain numerical value the image marked automatically as malignant and forwarded to manual evaluation. In this measurement the cut-off value can be as low as 53%. This confidence level provides 100% accuracy at healthy category (means healthy category contains only healthy images).

Measurement II.

In this measurement we have almost doubled the used images, introduced new image classes, and enlarged the generic feature set during classification (1025→2873 parameters)

- WND-CHARM_x: Malignant/healthy classification accuracy was 77% at malignant images (from 31 malignant 7 was missed) and 50% was at healthy images (from 14 healthy 7 was missed). Overall classification accuracy was 68 %.
- WND-CHARM_x_MC: Malignant/healthy classification accuracy was 93% at malignant images (from 31 malignant 2 was missed) and 71% was at healthy images (from 14 healthy 4 was missed). Overall classification accuracy was 80%.

TABLE I. WND-CHARM VS. WND-CHARM_MC CLASSIFICATION ACCURACY COMPARISON

	WND-CHARM	WND-CHARM_MC
Malignant tissue image classification accuracy	50%	66%
Healthy tissue image classification accuracy	90%	100%
Overall classification accuracy	75%	87%

TABLE II. WND-CHARM_X VS. WND-CHARM_X_MC CLASSIFICATION ACCURACY COMPARISON

	WND-CHARM_x	WND-CHARM_x_MC
Malignant tissue image classification accuracy	77%	93%
Healthy tissue image classification accuracy	50%	71%
Overall classification accuracy	68%	86%

WND-CHARM_x vs. WND-CHARM_x_MC

WND-CHARM_x_MC (2873 internal parameters extended with the tissue structure specific MC parameters) outperforms significantly (Table II.) the native version. In this measurement the cut-off value can be as low as 61%. This confidence level provides 100% accuracy at healthy category (means healthy category contains only healthy images, which can be automatically pre-filtered and marked as healthy).

IV. CONCLUSION

We have designed and implemented a software solution, which supports scanned HE (Hematoxilin Eozin) stained colon tissue samples, identify tissue structures – the nuclei, the glands and the surface epithelium – using image processing methods.

After the quantitative analysis of the tissue slides healthy/malignant samples can be categorized automatically. In this paper we have described our software solution which builds up mainly by two software component: MorphCheck tissue analyzer and a modified WND-CHARM multi-purpose image classifier. We have defined 75+15 pre-defined colon tissue specific parameters, which are measured by MorphCheck.

We have successfully appended WND-CHARM's learning and classification capabilities with our colon tissue specific parameters and with this act we have increased its classification accuracy significantly on HE stained colon tissue sample images. We have provided measurement results about the accuracy of the classification process.

According to our measurements, our colonic tissue parameter set increases the classification accuracy significantly. The classification accuracy of the proposed software solution is high, however not 100%. To do reliable healthy tissue image pre-filtering we have introduced a cut-off level at 0.61, which can assure that we filter out strictly only healthy tissue samples from the data set. The image processing and classification cause significant amount of time in our proposed solution.

As future work we are planning to speed-up MorphCheck's and WND-CHARM's image processing and classification capabilities and to further enlarge our annotated tissue image database. We are also planning to further expand the tissue parameter set to let decrease the cut-off level of the classification phase. Our proposed solution is able to pre-filter healthy colonic tissue images effectively; thus, decrease the amount of manually processed tissue image slides, and can lower the overall cost of such evaluations.

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REFERENCES

[1] 3DHistech - http://www.3DHistech.com [acc. 09.10.2012]

[2] HistoQuant - http://www.3dhistech.com/HistoQuant_module [acc. 09.10.2012]

[3] NuclearQuant - http://www.3dhistech.com/nuclearquant_module [acc. 09.10.2012]

[4] MembraneQuant- http://www.3dhistech.com/membranequant_module [acc. 09.10.2012]

[5] Aperio - http://www.aperio.com [acc. 09.01.2013]

[6] Definiens - http://www.definiens.com/ [acc. 09.01.2013]

[7] Visiopharm - http://www.visiopharm.com/ [acc. 15.12.2012]

[8] Martha S. Vokes, Anne E. Carpenter; Using CellProfiler for Automatic Identification and Measurement of Biological Objects in Images ; Curr. Protoc. Mol. Biol. 82:14.17.1-14.17.12. C! 2008 by John Wiley & Sons, Inc.

[9] MorphCheck-http://biotechweb.nik.uni-obuda.hu/web/hu/morphcheck [acc. 09.01.2013]

[10] Lior Shamir, Nikita Orlov, D Mark Eckley, Tomasz Macura1, Josiah Johnston, Ilya G Goldberg; Wndchrm – an open source utility for biological image analysis; Source Code for Biology and Medicine 2008, 3:13 doi:10.1186/1751-0473-3-13

[11] Nikita Orlov, Lior Shamir, Tomasz Macura, Josiah Johnston, D. Mark Eckley, Ilya G. Goldberg ;WND-CHARM: Multi-purpose image classification using compound image transforms; Pattern Recognit Lett. 2008 January; 29(11): 1684–1693. PMC2573471

[12] S. Szénási, Z. Vámossy, M. Kozlovszky, "Evaluation and comparison of cell nuclei detection algorithms", 16th International Conference on Intelligent Engineering Systems (INES), Lisbon, July. 2012, pp. 469 - 475, ISBN 978-1-4673-2694-0

[13] http://ome.grc.nia.nih.gov/wnd-charm/ [acc. 09.10.2012]