3D Pathology

Developing 3D Digital Pathology with Spectroscopy

DELIVERABLE D5.1
Requirements provided by hospitals with detailed performance expectations

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HISTORY

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V0.1	April 21, 2016	Starting version, template
V0.2	May 12, 2016	New compilation based on input by contributors
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Deliverable review procedure:

- **3 weeks before due date**: deliverable owner sends deliverable – approved by WP leader – to Project Manager.
- **Upfront** PM assigns a co-reviewer from the PMT group to cross check the deliverable
- **1 week before due date**: co-reviewer provides input to deliverable owner.
- **Due date:** deliverable owner sends the final version of the deliverable to PM and co-reviewer.

TABLE OF CONTENTS

1 Executive summary

By defining a '3D digital pathology ecosystem', this project tackles all dimensions and challenges of the 3D image manipulations at the scale of tissue sub-structures (shaped by microscopic and heterogeneous sub-structures). To achieve this major development, the development of 3D digital pathology cannot be foreseen without a good understanding and description of clinical (time, resolution, contrast and project safety) and technical (quantitative analyses, automated alignment of large series of 2D images) requirements.

Having knowledge on the clinical requirements, they will dictate the future requirements on managing multi-dimensional imaging big-data at different levels (efficient and high quality compression methods, high-parallelization for spectral data treatments, data reduction, intensive calculation for 3D data tracing and reconstruction, visualization and interaction tools and equipment and advanced statistical methods for data mining from large 3D image databanks). To facilitate this, the AMC will generate application requirements ensuring seamless clinical implementation of the to-be-developed tooling. Clinical requirements will govern:

- Requirements on temporal 3D sample/tissue parameters (par 4.1)
    - Time indication on 3D sample/tissue preparation for all used modalities
    - Time indication on data acquisition for all used image modalities
- Requirements on spatial sample/tissue image parameters (par 4.2)
    - Resolution, Contrast and staining
- Requirements on 3D visualization and reconstruction accuracy (par 4.3)

Safety of patient information and patient related data for the overall project has to be ensured. Inter and intra institutional tissue and data exchange will be governed by existing and new institutional review board (IRB) approval. Intra and inter institutional logistics is fully supported.
The AMC will also provide IRB approvals which ensure project safety (par 4.4)

2 Glossary

3D	Three-dimensional
H&E:	Hematoxilin and Eosin
MSI:	Mass Spectrometry Imaging
OCT:	Optical Coherence Tomography
BFM:	Bright Field microscopy
Pathology:	Medical specialty which diagnoses disease mostly through the analysis of tissue, and body fluid samples.
Histology:	Study of the microscopic anatomy of tissue using a light microscope or other imaging modality. The ability to visualize or differentially identify biological tissue types and structures is frequently enhanced through the use of histological stains. Histology is an essential tool of biology and medicine.
METC:	Medical Ethical Committee
WSI:	Whole slide image
2D:	Two-dimensional

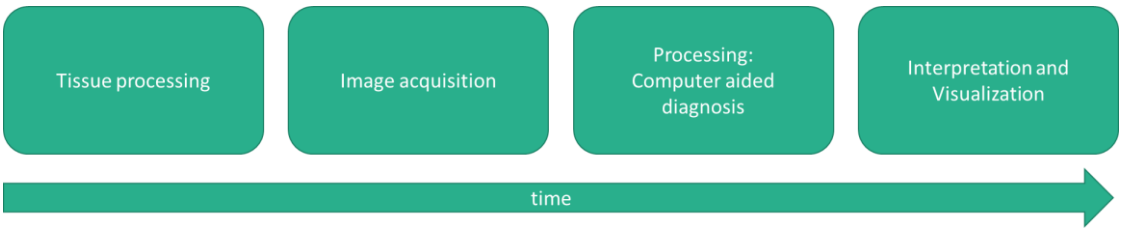
3 General introduction

THE TRADE OFF BETWEEN SPEED AND ACCURACY

Histopathological analysis of most cancers is challenged by time pressure at one hand and difficulty in visually differentiating the subtle structural differences at the other. Due to increased public awareness and the use of screening, an increased number of patients with low and intermediate cancers are diagnosed with a direct effect on the workload of the pathologist. Subsequently, the current diagnostic reproducibility of histopathology is low. Digital Pathology has shown to have a positive effect on workload balancing by improved and easier case distribution. This can lead to more efficiency and specialization at the pathology departments.

Yet, before improved workload balancing can be achieved, other major time consuming process parameters will have to be dealt with. We have defined them as:

- Tissue processing
- Image acquisition
- Digital processing
- Visualization



Tissue processing is directly related to clinical requirements since this has major implications on quality of the data and the time taken to acquire it. Tissue processing protocols are currently time consuming. Moreover, the different hardware modalities used throughout the project require different tissue preparation protocols. The AMC and UMC are developing a fast protocol which works for all technologies which does not hamper current image quality levels.

Image acquisition is executed in this project by integrating three imaging technologies into one acquisition hardware street, namely Optical Coherence Tomography (OCT), Mass Spectrometry Imaging (MSI) and Bright Field Microscopy (BFM). The two clinical project partners both have vowed to realize lab space to realize this hardware street.

Processing and quantification of the data is an elaborate step before the data can be used by pathologists. Processing the data depends on smart compression techniques. Yet these compression techniques cannot manipulate the raw data in a way that it cannot be used for analysis at a later time point if needed. Used quantification algorithms have to be fast and accurate to help he pathologist in his decision making. The contributing group of pathologists has decided that they will use quantification information as aided diagnosis in the decision making. Within the project these algorithms will be developed and the results will be projected as overlay over the H&E BFM data.

Visualization of the data from all modalities has to be in a fashion that the pathologist can work with it. Within the project, the consortium will test the visualization and interpretation part by creating smart overlays from i.e. MSI over H&E stained data. End results will be visualized on a 3D manipulation and visualization console.

3.1 Contributors

AMC meeting:

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4 Requirements provided by hospitals with detailed performance expectations

4.1 Requirements on temporal 3D sample/tissue parameters

As same-day diagnosis of patients is pursued in this project, the sample preparation, image acquisition and analysis must take place within a day. Within the current pathology workflow, this is not accomplishable due to the time consuming and laborious process of sample preparation.

Current histopathological sample preparation is considered slow and laborious. Therefore, the use of new preparation protocols is being investigated. The choice for the optimal preparation protocol must include the quality of the sample for the investigation using all different image modalities (e.g. bright field microscopy, MSI), as well as the time used for these different examinations.

4.1.1 Time indication on 3D sample/tissue preparation for all used modalities

To achieve same-day diagnosis, the complete sample preparation will be ideally within a six hour time-frame. This includes the process of fixation, sectioning and histopathological staining. With the currently used formalin fixation process, which penetrates the tissue with approximately 1mm/hour, this limits the size of the samples drastically. Therefore, the use of novel sample fixation and preparation protocols is being tested.

Deliverable: Novel preparation protocols

4.1.2 Time indication on data acquisition for all used image modalities

As the tissue preparation will still be laborious, despite the use of new sample preparation protocols, speedy image acquisition and analysis is required. To reduce any unnecessary time loss, the sample will be handled directly at the operation complex. Therefore, a dedicated room will be equipped with the machinery for sample preparation and the different image modalities. For the AMC in Amsterdam, the room will be equipped with machinery for sample preparation (e.g. microtome) and a Whole

Slide Scanner, to digitalize the histopathology coupes, Raman spectroscopy and 3D OCT. At the University of Maastricht, the MSI will be conducted.

4.2 Requirements on spatial sample/tissue image parameters

Currently, histopathological examination of a surgical specimen is done on 2D tissue sections which are examined under a light microscope. No clear guidelines exist for the technical handling of surgical pathological tissue. The College of American Pathologists states that all laboratories must have their own guidelines regarding tissue handling and quality control. There must be daily review of the technical quality of histologic preparations by the pathologists.

Histopathology slides must be of adequate technical quality to be diagnostically useful. Criteria to evaluate include adequate tissue fixation, processing, thickness of sections, absence of interfering tissue folds and tears, good staining techniques and cover slipping.

After surgical removal of the specimen, the tissue must be fixated in order to prevent autolysis and degradation. Tissue fixation is one of the most important determinants of the quality of histological sections. Incomplete fixation can lead to unsatisfactory and poorly reproducible results.

Worldwide, the two most common used techniques for the fixation of surgical tissue are freezing the tissue (used for intraoperative consultation) or formalin fixation (regular diagnostics). Formalin, approximately 4% formaldehyde, is seen as the best fixative for the morphological examination of histological tissue. However, it also has several downsides. Formalin penetrates tissue with only (approximately) 1mm per hour, and other techniques, such as MSI, will have difficulties analyzing tissue fixated this way.

Additionally, there is no single, optimal procedure that can be used for all types of tissue and image modalities.

As formalin fixation paraffin embedded tissue is currently considered as the favored and most frequently used method, the current and laborious workflow will be described. After fixation, formalin must be washed out and the tissue will be paraffin embedded to facilitate the cutting of the specimen using a microtome. There is a written procedure that indicates the sectioning thickness of paraffin embedded tissue for various tissue types and procedures. Paraffin embedded sections are routinely

sectioned at 4-5 microns. The sections must be cut from sufficient depth in the block to include the entire tissue plane.

Once sections are cut, they are floated on a warm water bath that helps remove wrinkles. Then they are picked up on a glass microscopic slide.

For the staining, the embedding process must be reversed in order to get the paraffin wax out of the tissue and allow water soluble dyes to penetrate the sections. The slides are 'deparaffinized' by running them through xylenes (or substitutes) to alcohols to water. There are no stains that can be done on tissues containing paraffin.

Hematoxylin and eosin is one of the principal stains in histology and is often the golden standard. For hematoxylin and eosin and other routine stains, the patient slide serves as the internal control to ensure adequate staining technique.

The stained section on the slide must be covered with a thin piece of glass to protect the tissue, to provide better optical quality for viewing under the microscope and to preserve the tissue section for years to come. The stained slide must go through the reverse process that it went through from paraffin section to water, then through clearing agents to point at which a permanent resinous substance beneath the glass coverslip can be placed over the section.

The final report will be made by the pathologist and will follow the guideline regarding the resected specimen.

Deliverable: Choice of tissue.

At the pathologists meeting of 15 December 2015 it was decided to use three kinds of tissue: prostate, bladder and esophagus. The reasoning of this choice is a combination of clinical availability, current high variability between different pathologists, and the expertise of the cooperating pathologists.

4.2.1 Resolution and contrast

The routine examination of histological slides is done under a light microscope. Initially the specimen is inspected at a low magnification where the tissue is inspected for structural abnormalities. Higher magnifications are used to inspect individual cells, nuclei and nucleoli. The exact focus of the pathologist depends on the type of tissue and the requirements of the grading of the malignancy (if present).
For example, in prostate biopsies the pathologist focusses on the shape, number, and structural aspects of the prostatic glands. In malignant glands, the basal cell layer of the glands disappears. Where in bladder resection specimen the focus lies on the aspect and thickness of the urothelium, in malignancies of the urothelium, this layer thickens and often papillary lesions are seen.

WSI's are relatively new and it asks for a whole new way of examining. Pathologist are trained to examine slides under a light microscope, therefore it is necessary for the digital image to be of the same quality. Different groups have worked on the standardization of WSI's, but no clear guidelines are set yet. Therefore we must listen carefully to our own pathologists.
In literature, no checklists exist to objectify the quality of a slide.

Deliverable: Quality checklist for the whole slide images.
Together with our pathologist and information we found in literature we made a checklist to score the different aspects[1].

Parameter	Scoring Guidelines			Score
Microscopic Assessment				
Physical quality of section (excludes stain quality)	**2**	**1**	**0**	
Disruption x4	No disruption	Minor disruption	Major disruption, holes, tearing	
Adhesion x4	Completely flat	Minor lifting	Severe lifting	
Cracking (coarse – crazy paving) x4	No large cracks	Some large cracks	Severe large cracks	
Cracking (fine) x40	No cracks	Some fine cracks	Extensive fine cracks	
Section thickness	Uniformly thin	Some variation	Extensive variation	
Quality of tissue preservation	**2**	**1**	**0**	
Nuclear detail (nucleolus, chromatin detail, nuclear envelope, vacuolation, shrinkage or swelling)	Good	Fair	Poor	
Cytoplasmic detail (cohesive, uniformly preserved, texture shown, vacuolation, cell borders defined, swelling or shrinkage)	Good	Fair	Poor	
Special features (kidney – basement membrane definition)	Good	Fair	Poor	
Extracellular components and muscle (collagen, elastin)	Good	Fair	Poor	
Uniformity of preservation (includes zonal fixation)	Uniform across section	Some variation	Extreme variation	
Quality of staining (chemical)	**2**	**1**	**0**	
Uniformity	Completely uniform	Some variation	Extreme variation	
Nuclear stain	Strong and sharp, excellent	Satisfactory	Weak, poor definition, unsatisfactory	
Cytoplasmic stain	Strong and sharp, excellent	Satisfactory	Weak, poor definition, unsatisfactory	
Extracellular components & muscle (collagen, elastin)	Strong and sharp, excellent	Satisfactory	Weak, poor definition, unsatisfactory	

In the pathology meeting our specialized pathologist stated that a magnification of 20x, and if possible 40x, is preferred to make sure all aspects can be examined on the whole slide image. Thus, the whole slide scanner must be able to scan slides with these magnifications with good quality.

Deliverable: Use of staining.

If a pathologist encounters difficulties in differentiating the tissue, immune histochemical stains can be applied on the tissue. For example, to differentiate between benign or malignant prostate tissue p63 (marker of the basal cell layer) can be used.

Since **H&E** is the most commonly used staining, we will be using this stain initially. If required, we can expand by using other stains.

Deliverable: Differentiation of different tissue.

In current practice, a reserved number of slides is made for each tissue specimen. In a study by Buesa, it was shown that the number of slides per block ranged from 1.0 to 6,7, with an average of 1,5.[2] This means that a lot of the tissue will not be taken into account.

In the 3D pathology project we want to present more slides per case to the pathologist. In order not to flood the pathologist with extra work we are working on a semi-automatic analysis system to aid the pathologist. Therefore the pathologists mentioned during the pathologists meeting that they would like guidance between **benign**, **low grade** and **high grade** tissue.

4.3 Requirements on 3D visualization and reconstruction accuracy

As stated before, current diagnostics are done on 2D images whereas the abnormalities that are studied are 3D in nature. The pathologists mentioned that the orientation in 2D slides often is very difficult, especially when resection borders must be taken into account. Therefore, 3D images could aid the pathologist enormously.

To create a 3D image out of histologic slides, the slides must be stacked very precisely. The registration of the stacks is a difficult task because of deformation of the tissue and the absence of alignment markers throughout the tissue.
Deformation occurs during the sectioning and fixation of the tissue and is an important factor to take into account when reconstructing a 3D image. Therefore we want to study this deformation, to see when this occurs, and where (cytoplasm/nucleus).

Deliverable: Markers used for registration
In healthy tissue structures are can often be seen throughout several slides and can often be considered as an alignment marker. In malignancies however, there is structural loss and therefore difficult to stack the slides. We are going to use markers (hair/ cannulas) that can be seen not only in the X and Y direction, but also in the Z direction and in different modalities (e.g. MSI).

4.4 METC Communications/regulations

In the 3D Pathology project we are going use patients material to prove the additional value of a fast, digital, quantitative, spectroscopic and multimodal 3D pathology analysis system. In order to do this, we are going to use resection material from patients diagnosed with prostate cancer, bladder cancer or esophageal cancer.

Deliverable: METC (Medical Ethical Committee) approval.
A letter is send to the METC to explain the setup of our project. We are going to use patient material that need to be resected according to the treatment guidelines. We will not be interfering with patients, therefore it was not clear if METC approval was needed. The letter below states that METC approval is **not needed**.

To whom it may concern,

Referring to our letter of March 9, 2016 (reference number W16_088 # 16.108) we are pleased to confirm that the Medical Research Involving Human Subjects Act (WMO) does not apply to the above mentioned study and that an official approval of this study by our committee is not required.

Yours sincerely,
on behalf of the Medical Ethics Review Committee of the Academic Medical Center,

Mrs. T. Groenveld
secretary

Future perspectives may be expanding from resection material to biopsy tissue. New METC approval will then be needed.

Normally the pathologist or pathology resident performs the gross examination of the specimen. If other individuals assist in gross examination, the extent of their activities and the nature of supervision must be defined in a documented protocol. This must be taken into account if we create our 3D Pathology street on the operation complex.

Surgical pathology records and materials must be retained for an appropriate period. Therefore the 'Commissie Kwaliteit en Beroeps Uitoefening' (2010) has set standards on the minimum storing period. For histology, slides (glass or digital), must be kept for thirty years. If the slides are fully digital, it is not necessary to keep the actual slides.

No clear rules are set concerning the exchange of data to other centers.

1. Rolls G, Farmer N, Tarbet F. Assessing the Quality of Tissue Processing and the Performance of Peloris using the Leica Microsystems Scoring System Living up to Life Assessing the Quality of Tissue Processing and the Performance of Peloris using the Leica Microsystems Scoring System.
2. Buesa RJ(. Productivity standards for histology laboratories. 2010.