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DELIVERABLE 2.5

DICOM implementation

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List of Acronyms

ACR	College of Radiology	HCA	Human Cell Atlas	PR	Presentation state
AP	Anatomical pathology	HL7	Health Level Seven	RNA	Ribonucleic acid
CP	Change proposal	HUTER	Human Uterus Cell Atlas Project	smFISH	Single Molecule mRNA Fluorescent in Situ Hybridization
CT	Computed tomography	IHC	Immunohistochemistry	SOP	Service-object Pair
DICOM	Digital Imaging and Communication In Medicine	IHE	Integrating the Healthcare Enterprise	SR	Structured report
DNA	Deoxyribonucleic acid	IOD	Information object definition	TMA	Tissue microarray
DSC	DICOM Standards Committee	MRI	Magnetic resonance imaging	UID	Unique Identifier
FISH	Fluorescent in situ hybridization	NEMA	National Electrical Manufacturers Association	USCAP	United States & Canadian Academy of Pathology
ACR	College of Radiology	nm	Nanometre	WG	Working group
AP	Anatomical pathology	NWIP	New work item proposal	WP2	Work package 2
CP	Change proposal	PACS	Picture Archiving and Communication System	WSI	Whole slide imaging

1. PURPOSE OF THIS DOCUMENT

The purpose of this report is to present the contribution to standards of the HUTER project in terms of imaging. The images handled in research environments, such as HUTER ones, are usually generated in proprietary-closed equipment and software which lack of interoperability among other systems and, consequently, hamper sharing data and results. In this context, the HUTER project proposed to extend the DICOM standard format (the most widely extended IT standard in hospitals for medical imaging) to the research image types developed in HUTER. This report includes the research and work performed that was required to develop a full implementation of the DICOM standard for several HUTER image types (immunohistochemistry, tissue microarray, fluorescence and single molecule fluorescence in situ hybridization or smFISH), for a future implementation in clinical practice.

Another of the objectives of this deliverable was to communicate the research results and the implementation developed for these new image types to the DICOM secretariat (institution which handles the DICOM standard evolutions and modifications). Therefore, the strategy developed to communicate our results to the secretariat, as well as the feedback from the chairs of the working group of the standard have been included.

This report is one of the key deliverables of WP2 “Platform infrastructure” defined in the Grant Agreement. This document has been developed by BAHIA as lead of WP2 and advised and reviewed in close collaboration with DICOM consultants (Fundación Cádiz and Primum mHealth Services) as experts in DICOM secretariat procedures. The work was also reviewed by the rest of the HUTER partners involved in this task (INCLIVA, GRL, UPPSALA, CCHT and UEA).

1.1. Related documents

Documents linked to past actions already delivered:

HUTER_WP2_D2.3_Beta_version_of_data_access_tools

HUTER_WP2_D2.4_Data_access_and_DICOM_visualization_tools

HUTER_WP7_D7.2_Visual_system_and_Digitalisation_software

2. INTRODUCTION

The Human Cell Atlas (HCA) is an international research initiative that aims to create molecular reference maps of all human cells to pool and expand knowledge of the diverse cells found within the human body in order to better understand human health, but also to improve diagnosis, monitoring and treatment of diseases. The 6 European HCA pilot projects aim to characterize single cells or their nuclear components, their interactions and/or spatial location in tissues from one human organ, using state-of-the-art single cell technologies, advanced microscopy techniques, analytical methods and computational tools, and brings together European experts in the respective fields who are joining their efforts to support the creation of the Human Cell Atlas. Among these 6 European pilots, HUTER is applying state-of-art microscopy and cell sequencing technologies to thousands of human uterus cells that generate vast amounts of diverse molecular and cellular data, such as sequencing files and advanced microscopy images. Collectively, all data generated will be the basis of the cellular map of human uterus.

In this context, the insights discovered in the HUTER and other HCA projects must be shared among different research groups and with the scientific community in order to achieve strong validated results. Furthermore, the results of HCA projects promise to improve the human life through a better understanding of human health, improving diagnosis, treatments and so forth. This means that all these state-of-the-art technologies could be adopted in health care systems (hospitals) and other industries such as clinical trials or animal health sector in the future.

In order to facilitate these ambitious goals, data and image formats of the instruments should be as open as possible. Open standards would allow sharing results among the scientific community and to foster the adoption of these technologies in other environments and sectors where the interoperability and compatibility among systems is a must (such as hospitals and industries). However, state-of-the-art research equipment sometimes includes their own non-open output format as default which hampers sharing data and results among researchers even from the same institution due to format incompatibilities. This incompatibility issue coupled with the fact that the scientific sector does not usually have well-established, defined and adopted standard formats and protocols, raise the need of extending an open standard definition for these output data. An open standard would enable data sharing and compatibility not only among HUTER partners but also among different projects that contribute with data to HCA. In this line, HUTER has included the extension and support of the open DICOM standard already proved in the medical image field to the advanced research images as one of its objectives. This standard is well-known by most vendors in the clinical domain (Illumina, Perkin Elmer, Thermo Fisher Scientific, Phillips, Zeiss, Leica Biosystems, etc) that are also the main manufacturers of biomedical research equipment, such as microscopes.

This document includes the definition of the DICOM standard, the institution and working groups in charge of managing its evolution and improvement, and what are the necessary procedures to make changes in the standard. An extensive analysis of 4 types of images generated in HUTER, their support in DICOM before the HUTER and an implementation proposal that includes changes to improve it were also provided.

The strategy for submitting all this work to the DICOM secretariat, the full implementation of HUTER images in DICOM, and feedback received from the standard chairs of the Working Group 26 were also included in this report.

2.1. DICOM introduction

DICOM stands for Digital Imaging and Communications in Medicine. It is the standard for the communication and management of medical imaging information and related data. In the 1980s, it was very difficult for anyone other than manufacturers of computed tomography (CT) or magnetic resonance imaging (MRI) devices to decode the images that the machines generated, or to print them. So, in 1983 the American College of Radiology (ACR) and the National Electrical Manufacturers Association (NEMA) joined forces to form a Standards Committee in order to meet the combined needs of physicists and equipment vendors. First release of DICOM Standard was in 1985 as a result of the Standards Committee. Since that, DICOM has experimented a huge growth and is the current standard in medical imaging. DICOM supports a lot of different image types but the part that will be analysed and implemented in this document is the one related to anatomic pathology (pathology).

The main purpose of DICOM is to provide interoperability between different vendors. Many benefits are derived from the use of interoperability in healthcare environments like¹:

- Individuals can benefit from enhanced quality and safety of treatments received, delivery of healthcare when and where it is required and integrated care plans developed by providers across one or more organisations. Furthermore, interoperability across national borders could facilitate better and more informed emergency care abroad.
- Healthcare professionals can potentially improve the quality and safety of the care they provide through strengthened coordination across the various points of care delivery. This can result in access to timely patient safety information and evidence-based clinical guidelines which in turn supports a better decision-making process.

¹ <https://www.hiqa.ie/sites/default/files/2017-01/Healthcare-Interoperability-Standards.pdf>

- Insurance companies can benefit from the potential cost savings resulting from the reduction in duplicate diagnostic testing, earlier disease diagnosis, a reduction in costs associated with adverse events and general improvements in outcomes for individuals.
- Interoperability standards can benefit the software industry by enabling a single market for digital healthcare, thereby reducing the cost of developing health information systems and opening up competition in the market.
- Efficiency gains brought about by the implementation of healthcare interoperability standards can benefit the provider, individual and insurance providers by facilitating faster access to care, diagnosis and treatment of disease, thereby reducing costs significantly.
- Biomedical research is fostered with the participation of cross-national groups that can collaborate providing larger cohorts of patients, which are essential in the development of new artificial intelligence algorithms.

In pathology, it wasn't until 2005 that a DICOM working group (WG26) was created in order to generate a supplement of the standard to support whole slide microscopic images (WSI) modality. In this field, a supplement is necessary due to the significant differences between pathology images and previous DICOM supported image modalities. These differences will be explained in detail in section 3 . HUTER DICOM RESEARCH paragraph.

Before 2005, because of a lack of a WSI standard, each manufacturer worked independently and in competition with the others, consequently, each had a proprietary approach to connecting their equipment and systems (mainly, digital slide scanners, digital slide managing systems and viewers, and storage systems). Even though Pathology standard supplements (number 122 and 145) were created on 2008 and 2009 respectively, the use of DICOM in pathology is not widely extended due to its recently creation compared with other image modalities widely supported in DICOM (e.g. radiology images).

However, this has changed in the last two years due to the need of creating large networks of pathology services and solutions that has resulted in a demand by end-users to adopt DICOM, and manufacturers are now adopting the DICOM standard also for pathology, and the trend is that in the future all pathology images will be produced in this format in order to provide users with the interoperability that this standard provides.

2.2. DICOM Standards Committee

The DICOM Standards Committee (DSC) consists of its Members and the Secretariat. Membership in the DSC consists of manufacturing companies, independent service organizations, consulting companies, biomedical professional organizations, trade associations, other standards-developing organizations, academic

institutions and government agencies worldwide that have a direct and material interest in the activities of the DSC².

The DSC forms Working Groups (WG) with well-defined responsibilities (e.g., WG-01 Cardiac and Vascular Information and WG-03 Nuclear Medicine). These WG have the responsibility of developing DICOM standard regarding the topic which are responsible. DSC makes an annual review of the scope, duties and memberships of all WG.

A Working Group can also choose to create temporary subgroups to work on specific topics, but they are not reflected in the governance. The work and meetings of such subgroups are considered to be work and meetings of the parent Working Group. As such subgroups are intentionally temporary, when establishing the subgroup, the Working Group should clearly delimit the scope of their tasks to facilitate their expeditious termination.

Currently, there are 34 WGs created. All have a well-defined topic related to medical imaging modalities (WG-19 Dermatology or WG-26 Pathology that will be covered in more detail later), their archive and exchange between systems (WG-33 Data Archive and Management) or information security (WG-14 Security).

Each WG must have a Working Group Secretariat and a secretary who will work closely with the DSC Secretariat to assure proper conduct and documentation of Working Group activities.

The WG that is most closely related to the development of the standard in the field of interest discussed in this document is WG-26, which is responsible for managing the part of the standard corresponding to pathology images, mainly whole slide images.

A previous work on static colour images (pictures taken with a camera adapted to a visible light microscope or in gross imaging) was performed by WG-13 ("Visible Light") for creation and use of visible light colour static images and real-time video.

There are two ways that a company, organization or agency may become a member of a Working Group:

- a) A Member of the DSC may join a Working Group by informing the Secretariat of its desire to participate in the work of the chosen Working Group and designating the name and contact information for its voting representative and, if desired, one or more alternates.
- b) The officers of a Working Group may nominate one or more companies, organizations, agencies or individuals for participation on that Working Group. Such a nomination shall be submitted to the DSC and shall include evidence that the proposed member meets the criteria spelled out in Sections 2.2, above. Such nominations shall be accompanied by information regarding the proposed member's

²<https://www.dicomstandard.org/docs/librariesprovider2/dicomdocuments/wp-content/uploads/2017/11/dicom-policies-and-procedures-2020-04.pdf>

principal representative and alternate(s), if any. The DSC shall vote on such nominations and appoint those companies, organizations, agencies or individuals that are approved.

Each Working Group shall elect a chair or co-chairs. Other officers may be elected by the Working Group. If any member of a Working Group is not represented at two consecutive face-to-face or teleconference meetings of the Working Group, that member will no longer be counted in determining a quorum for face-to-face meetings and telephone conferences. The member will be reinstated to good standing with voting rights and counting toward quorum in the Working Group at the next face-to-face or teleconference meeting they attend.

2.3. Working Group 26

As is described in the DSC Governance, same duties are delegated in the Work Groups (WG), in the case of the Digital Pathology Images, the WG is the number 26 (WG26).

The Group was created on 2005 and initially led by Dr. Bruce Beckwith, from the Beth Israel Deaconess Health Center (Harvard School of Medicine) in the representation of the American Pathology (CAP). First companies participated was AGFA, General Electrics, Olympus, Toshiba and Zeiss, but suddenly other industry companies like Aperio, Leica and Roche also joined the WG26.

Currently, in WG-26 are a Secretariat for the United States (College of American Pathologists – CAP) and a Secretariat for Europe (Sociedad Española de Informática de la Salud – SEIS).

European WG26's scope is to extend the DICOM Standard to support Pathology images (including cytopathology, surgical pathology, and clinical pathology and autopsy pathology studies). Specific actions are related to:

- Technical standards to facilitate pathology image acquisition, display, transfer and storage. The group will be responsible for formulating components of the DICOM Standard that relate to imaging in the domain of Pathology. The primary focus will be digital formats for clinical imaging, but digital imaging for research applications may also be addressed as appropriate. This would include, for example, conventional imaging, whole slide microscopic imaging, micro-array imaging, flow cytometric “imaging”, and molecular “imaging”. Multi-spectral imaging shall be addressed in the context of how to properly handle the cross- channel dependencies.
- Issues related to specimen and patient identification and workflow integration. The group will address improving the information model for subject identification and workflow integration within DICOM. This is required to account for the specimen-driven nature of the subject in pathology, which differs

from the primarily patient-driven model in radiology. The goal is a common model shared between DICOM and other Electronic Medical Records standards, such as HL7, that will facilitate consistent specimen identification from acquisition through analysis and reporting.

- Development of standards for integrating images and derived information into pathology reports. These include image annotation, templates for common image-based measurements and analyses, and integration of image-based information with textual and coded pathology report information, including structured pathology reports. Some of these activities may overlap with other standardization groups such as IHE and HL7, e.g. HL7 AP SR, and the group is responsible for coordinating such efforts with the respective counterparts in such groups.
- Special technical issues specific to these application domains. These include compression of multi-gigabyte imagery, as well as efficient whole slide microscopic image browsing, microscopic image analysis etc. This could also include methods for correlating clinical images (radiologic PET/CT, endoscopy, etc.) and pathology images as well as potential workflow integrations related to those.

Previously twice a month and, after the COVID-19, one a month, WG26 members are holding regular meetings. An initial agenda is circulated before every meeting and, after each meeting approved minutes are uploaded and publicly available. Medical Imaging Technology Association (MITA), a division of NEMA, serves as the DICOM Secretariat. Their mission is warranting that during the calls and all standard development, no marketing or specific product from specific companies are not the main focus, but only the standard development. The Work Group also encourage to clinical users to participate in these meetings.

WG26 is collaborating with Integrating the Healthcare Enterprise® (IHE) Pathology and Laboratory Medicine (PaLM) on the IHE PaLM draft white paper that can be retrieved from https://wiki.ihe.net/index.php/APW-EDM_White_Paper.

WG26 has published the following supplement and change proposals during the last years:

- Supplement 145 to the DICOM Standard providing for “Whole Slide Microscopic Image IOD and SOP Classes”, formally approved in 2010. Supplement 145 to the DICOM standard introduces an SOP class for WSI
- Supplement 122 to the DICOM Standard providing for “Specimen Module and Revised Pathology SOP Classes”, formally approved in 2008. Supplement 122 to the DICOM standard introduces a new mechanism of pathology specimen identification and revisions to composite Information Object Definitions to use that mechanism.
- Change Proposals 1713, 1740, 1757, 1758, 1759, 1804.

A recurring topic of discussion during several WG26 meetings has been about engaging the pathology community with DICOM and demonstrating the capabilities as well as the benefits of DICOM workflow for anatomic pathology. In this context, the WG26 has decided to perform regular Connectathons. Connectathons is a great opportunity to test the “theoretical Standard” in real life and the output guides us where to focus our future work. In the first showcase, scheduled in 2016 (USCAP 2016), many vendors and research groups participated to highlight the benefits of interoperability and DICOM.

Connectathons try to engage the medical community with the benefits of the DICOM standard. For instance, a vendor neutral pipeline that allows imaging, archival and review using DICOM Standard file format and communications protocols has been demonstrated. Attendees have seen slides scanned, store with their accompanying identification and descriptive metadata, transferred to a central archive and retrieved for display without loss of quality and fidelity.

The Connectathon has these objectives:

- Define a simple acquisition workflow (scanning, archival, retrieval)
- Present the workflow at pre-determined times so that many attendees can visit
- Present the same workflow scenario in every presentation
- Ensure that workflow demonstration does not become a scanner contest
- Host a panel discussion with participating vendors to explain DICOM and interoperability
- Ensure that all vendors involved have a chance to make the presentation

During COVID-19 pandemic, these activities has been adapted to become “virtual Hackathons”, to maintain a collaborative development space for testing of software for the generation, exchange, and use of whole slide image (WSI), including also specific objectives, like annotations in DICOM SR. With this approach, anyone (vendors, academics and individuals) can participate freely and openly.

3. HUTER DICOM RESEARCH

During the development of the HUTER project, it became evident that there was a need of transforming pathology images obtained in a proprietary format into the DICOM standard to make them accessible and interchangeable by any system that supports the DICOM format.

First, research has been conducted on the support that the standard offers to pathology images. These types of images are included in the standard with a new format called Whole Slide Imaging (WSI). WSI images have

an enormous size. Its origin lies in the adoption of microscopic image complete digitization in the field of pathology. These images are obtained from scanning the pathology histopathology and cytopathology slides, and it is the most recent imaging modality used in pathology departments.

Because the magnification needed to view pathology images is extremely high (up to 400X, and sometimes x600), even when tissue specimen original size is about 1 x 3 cm, the size of the images (over 30,000 x 30,000 pixels) obtained is too large to display on a conventional screen. Additionally, colour information, and sometimes multiple planes in Z-axis are also needed. These features lead to this type of images (WSI) to have such an enormous size in disk space. To solve this problem, DICOM WSI images are divided into tiles of a smaller size that can be stored and processed more easily. Also, as the image is acquired at different resolutions to be able to pan and zoom more quickly, several objects corresponding to various levels of resolution are stored. This produces the recognizable pyramidal structure of the WSI images.

Finally, with the purpose that all the levels of the pyramid are correctly referenced and their tiles can be located, the generation of a LOCALIZER object is specified in DICOM, which contains a macroscopic image of the slide. In addition, in the DICOM tags of the LOCALIZER, the location of each one of the tiles of all the previously collected levels is reported relative to the overview image of the LOCALIZER. With this information and using only the LOCALIZER, it is possible to know the relative position of each of the tiles generated for a study.

3.1. DICOM image modalities

Within the HUTER project, the need of converting from proprietary format pathology images acquired using different techniques to DICOM arises. Depending on the acquisition process and the information contained in each format, we can differentiate immunohistochemistry, Tissue MicroArray (TMA), fluorescence, and Single Molecule Fluorescence In Situ Hybridization (smFISH).

DICOM is a comprehensive specification of how to store and exchange image metadata, its structure and associated encoding, for diagnostic and therapeutic images and their associated metadata, as mentioned above.

When generating DICOM pathology images, therefore, the two main sections that need to be standardized are the metadata and the way to organize and encode a very large image, as is the case with pathology images, to make it easily interchangeable and actionable by viewers.

First, to fully inform the metadata of a pathology image, it is necessary to perform an extension of the entity relationship model designed by the standard for a specimen shown in *Figure 1*. In most cases, each image acquired by a scanner will contain one specimen per container (slide), which is the most common in clinical practice. However, in the field of research, the use of techniques that allow grouping up to 1000 specimens in

the same container has been growing, generating the Tissue MicroArray image type that will be defined in greater depth in section 3.1.2 *Tissue MicroArray*. This technique has become one of the most widely used methods to study and validate cancer biomarkers in patient cohorts.

The use of TMA was initially described in supplement 122, where it was stated that each specimen (spot) must have its own ID, and images created for each spot should be assigned to the real patients. It became evident that one image (WSI) can include specimens from multiple patients. It was one of the main reasons why a new Specimen Module was introduced in DICOM.

To solve the changes that TMA needs in comparison with the traditional images of pathology in terms of metadata, the extension of the entity relationship model is carried out. Previously, it was assumed that the container and the specimen were unique, which meant that the relationship was always 1 to 1. Instead, as seen in *Figure 1*, the model has been modified so that the relationship between the container and the specimen is 1 to N, which means that each slide can contain N specimens, enabling TMA images in the standard. This theoretical change in the standard has a practical implication within the implementation of metadata when generating a DICOM Whole Slide Image (WSI).

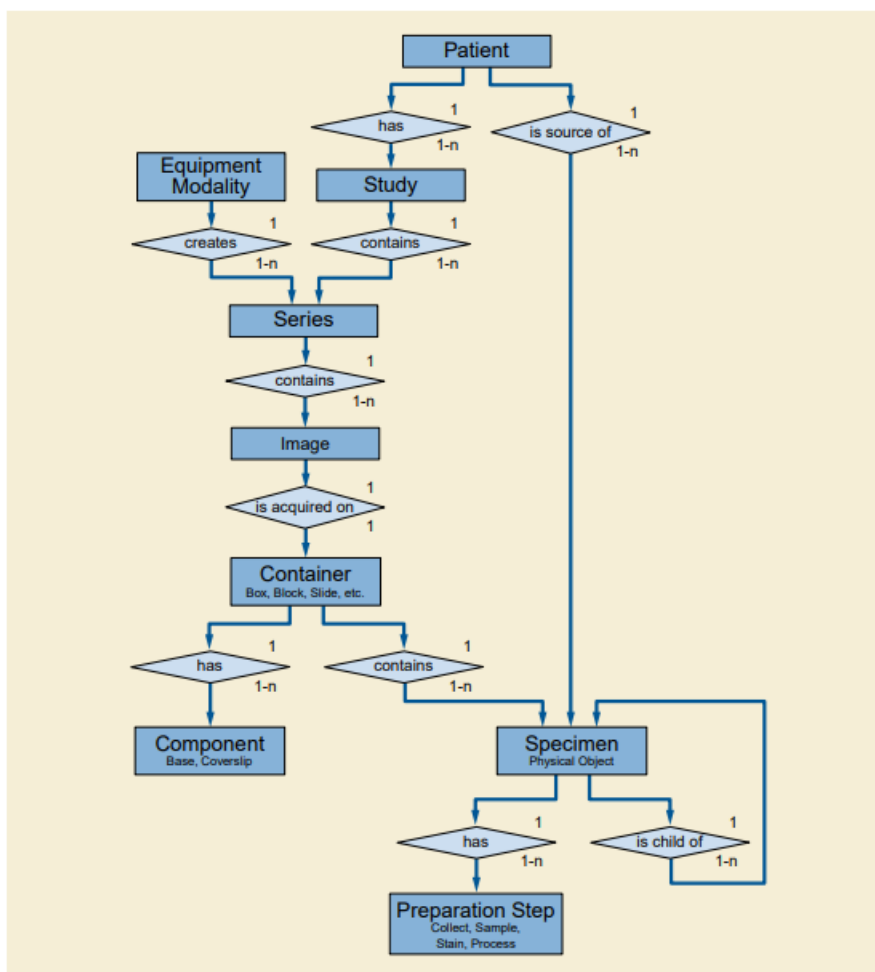


Figure 1: Extension of DICOM Entity Relationship Model for Specimens

(<https://dicom.nema.org/medical/dicom/current/output/pdf/part17.pdf>)

In order to have a quick graphical summary of the main informative modules dealt with by the metadata and the main values that are introduced in each of them, *Figure 2* is shown. From the value legend it can be extracted that the sources of information of each of the modules are different.

Thus, while the information of the patient in a hospital would be extracted from the Electronic Medical Record (name, sex, date of birth, ID...), the information about the study, container and specimen generated for the biopsy that has been performed are extracted from the Laboratory Information System, which has been in charge of performing the tissue extraction and the request with associated information (study date, study time, study IDs, series and specimen, description of the container, description of the specimen...).

Lastly, the Series (related to the digital acquisition of the image) and Image modules are reported from the scanner or microscope that reads the image and digitizes it, since the metadata related to the digital acquisition of the image (acquisition date, acquisition time...) and those related to the image (samples per pixel, columns, rows, pixel data...) will be extracted from there.

Figure 2 also shows in section C an approximate proportion of what would be the size of the metadata generated for an image compared to the information that corresponds to the image itself (the pixels stored). Considering the large size of WSI images, it is consistent that metadata occupies a small proportion of DICOM image size compared to pixel information.

Finally, WSI images have significant changes in terms of pixel structuring due to the size limitations imposed by DICOM, the difficult usability of oversized images when processing or viewing them, and the need to view the image in different levels of resolution in the most efficient way possible.

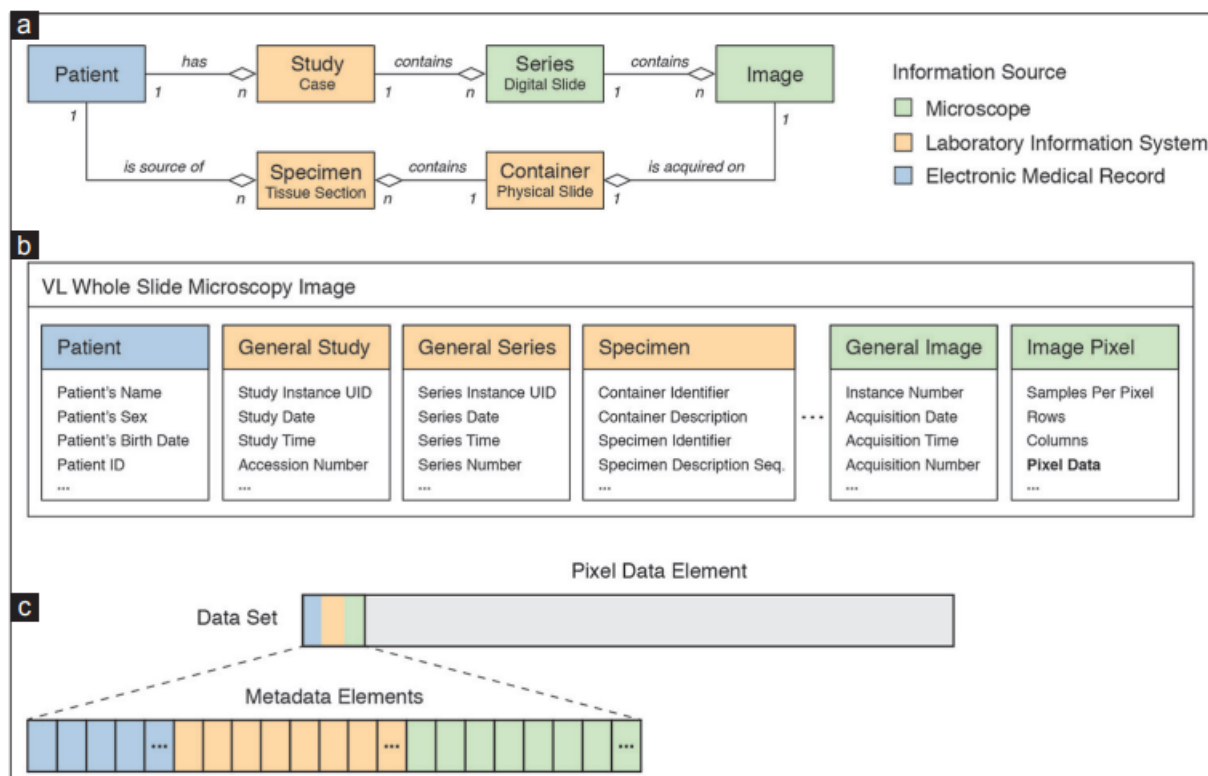


Figure 2: DICOM WSI metadata (Herrmann MD, Clunie DA, Fedorov A, Doyle SW, Pieper S, Klepeis V, et al. Implementing the DICOM standard for digital pathology. *J Pathol Inform* 2018;9:37.)

For WSI images, the generation of a traditional image, such as those used in radiology, is changed by a pyramidal structure with N images at different resolutions, as shown in Figure 3. The goal of this division into N levels is to reduce loading time and power consumption of resources in the viewers of this type of images. Thus, when the display of the WSI image starts, it will be displayed at a low-resolution level, so that the entire image is displayed on the screen. It would be much more expensive to use the base image (with 40x magnification) to show a much lower resolution than this level of the pyramid has, so having N files with different resolutions allows recovering larger or smaller files as required by the region and zoom what you want to show.

Another decisive aspect of the WSI image structure is that within each level there is a multi-frame image. That is, the image of each level is divided into smaller tiles with the aim again of easing its processing to display only certain regions of the image. The use of various levels of resolution and several images would not be useful by itself, since if each level had the image in a single indivisible frame, to display a small part of the base image (x40) it would be necessary for the viewer to retrieve the entire base level and then crop it. This process would not be efficient at all and would not solve the problem of navigation in pathology images.

In contrast, if each level is divided into several tiles of the same size, it is possible to recover a region of the image by loading only a small number of the tiles that make up the level, avoiding loading the rest of the tiles that would not be used and improving the efficiency of the system, the load time and consequently, the usability. The usual organization of the tiles is the one mentioned in *Figure 3* section C, from left to right and from top to bottom. In *Figure 3* section D, the different proportion between the metadata of a level and the information about the pixels depending on the resolution of the image that is observed. The larger the image (higher resolution), the higher the ratio of image to metadata, because most of the memory occupied by the DICOM WSI image is always due to the image.

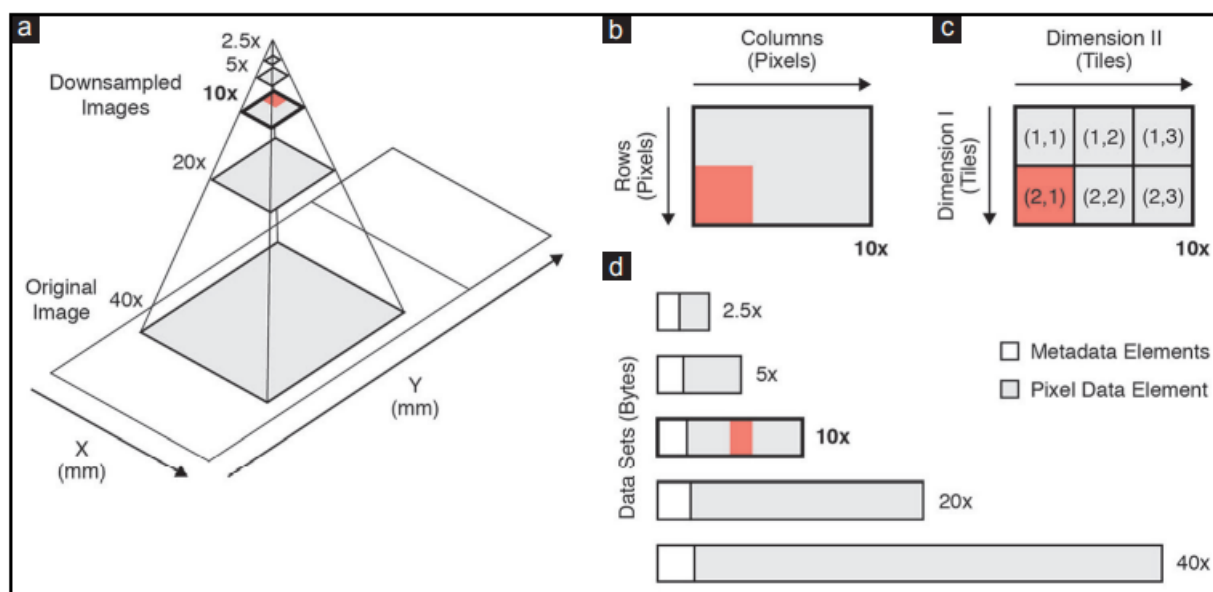


Figure 3: DICOM WSI pyramid structure (Herrmann MD, Clunie DA, Fedorov A, Doyle SW, Pieper S, Klepeis V, et al. Implementing the DICOM standard for digital pathology. J Pathol Inform 2018;9:37.)

Once the structure of the WSI image has been defined to represent its information in the most organized and accessible way (with a pyramidal structure), the need to have a location index of all tiles arises. DICOM provides the ability to implement a new image called LOCALIZER. In this image, a map-style image is shown that contains the entire image that has been generated in the pyramid but in a much smaller size, as a mini-

map. Then, in the metadata of this LOCALIZER, references to all tiles generated previously in the pyramid are included, indicating the position they would have within the image that works as a map. In addition, the UUIDs of each image are included to have a unique reference of which level each of the mentioned tiles corresponds to.

This method of using the LOCALIZER was a method supported by the standard during the HUTER project and was implemented in the generation of the project images. However, the DICOM standard is under continuous revision and during the project it has been seen that WG26 has approved a change to remove the LOCALIZER³ from the standard and add a THUMBNAIL image. The main difference between both images is the absence of references to the tiles that form the pyramid from the THUMBNAIL as it was done in the LOCALIZER.

All modalities are used in a context to obtain the necessary information from the sample that is digitized. Key features of each image type will be briefly explained in this section to justify the main differences between image modalities. An example of haematoxylin-eosin (HE) generated WSI is shown in *Figure 4*.

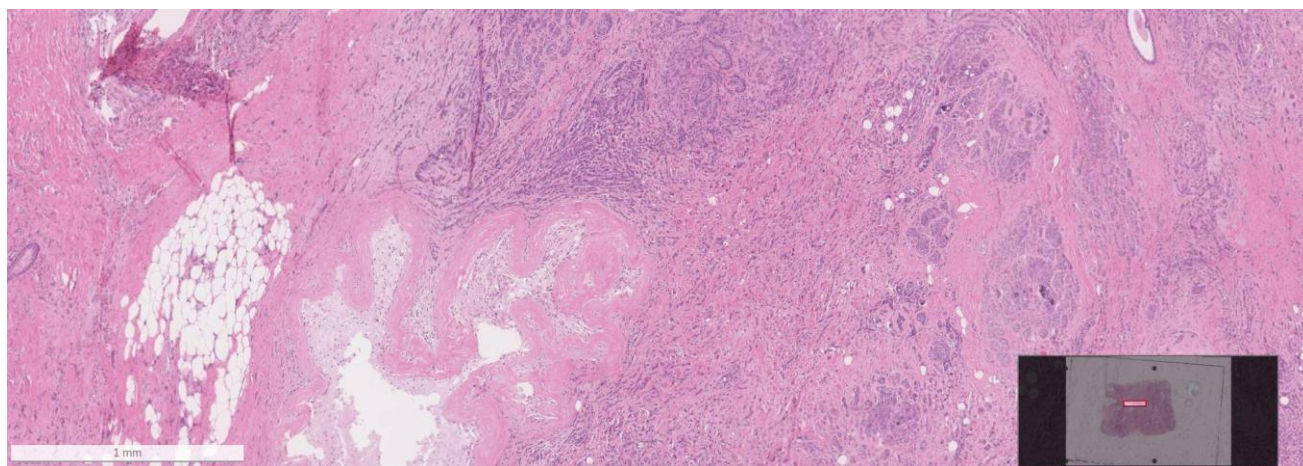


Figure 4: Example of DICOM haematoxylin-eosin image in the HUTER viewer

Immunohistochemistry images are based on immunological staining of tissue samples. Images can be stained in a single colour (older technique) or multi-coloured (newer techniques). It is an important method when making diagnoses in the area of pathology.

The Tissue MicroArray images used in HUTER correspond to immunohistochemistry images that are grouped in an array of samples, being able to acquire many samples in a single image, unlike simple immunohistochemistry images that only contain one sample.

On the other hand, fluorescence images are based on the acquisition at different wavelengths of the intensities emitted by the image after using one or multiple fluorophores (or channels). Therefore, these images do not show the image itself, but express the amount of light that each part of the sample emits at

³ <https://www.dicomstandard.org/news-dir/current/docs/cpack112/cp2102.pdf>

different wavelengths, and pseudocolour is usually applied to each channel (e.g. blue for DAPI filter). The two main types of fluorescent images that are very popular in pathology are direct immunofluorescence (very useful in kidney pathology and dermatopathology) and fluorescence in situ hybridization (FISH), a molecular cytogenetic technique that uses fluorescent probes to localize the presence or absence of specific DNA sequences in cells. Multiple Z-planes scanning is optional in fluorescent WSI.

More recently, single molecule fluorescence in situ hybridization (smFISH) has been shown to become a powerful technique to study gene expression in single cells due to its ability to detect and count individual RNA molecules. Complementary to deep sequencing-based methods, smFISH provides information about the cell-to-cell variation in transcript abundance and the subcellular localization of a given RNA.

In this project, smFISH images are additionally acquired on a confocal microscope. This feature makes the generated images have several Z planes, a property that will differentiate smFISH images from images such as fluorescence.

3.1.1. Immunohistochemistry

Immunohistochemistry (IHC) is an auxiliary method used by pathologists for routine diagnoses as well as in basic research to explore biomarkers, since IHC allows confirming or ruling out the expression of the target molecule in that environment.

The goal of IHC imaging is to detect the presence of a specific antigen, usually a protein. Its operating principle is based on the use of antibodies that bind specifically to the substance to be identified. The binding of the antibody to the antigen triggers a reaction that ultimately results in a change in coloration of the area that is later detected in the image.

The immunohistochemistry images obtained in the HUTER project do not have any peculiarity with respect to the generic definition that is applied to WSI images in the DICOM standard. Therefore, its structure and metadata follow the principles defined in DICOM image modalities.

Therefore, applying the pyramid definition to the DICOM image and generating the corresponding metadata (that will be explained in detail in DICOM), the immunohistochemistry DICOM image is created. Within the HUTER project we have also developed a DICOM viewer capable of supporting all the WSI image modalities present in the project. As a result, one of the generated immunohistochemistry image examples is shown in Figure 5.

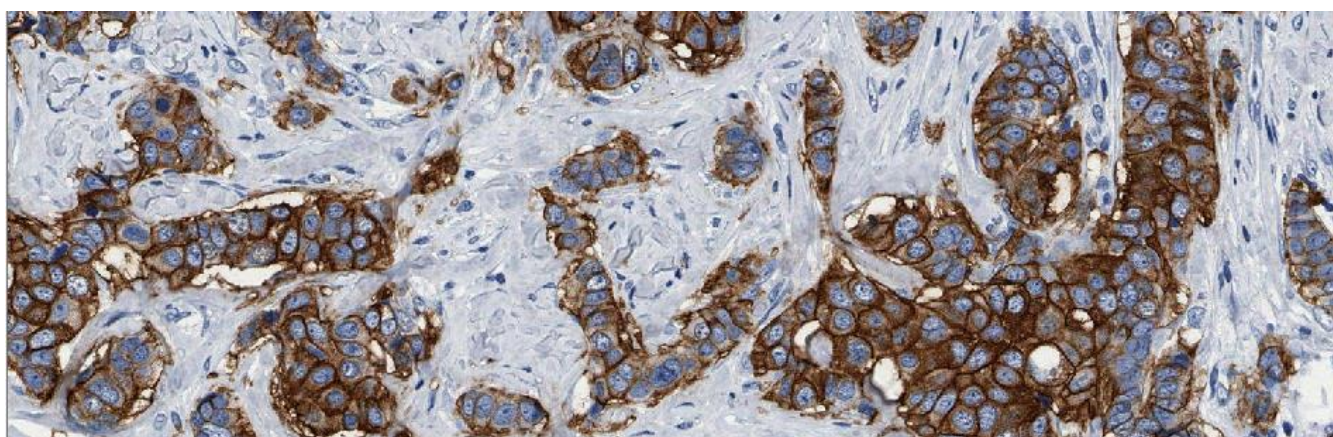


Figure 5: Example of DICOM immunohistochemistry image

3.1.2. Tissue MicroArray

Tissue microarrays (TMAs) make possible to group more than 1,000 samples in a single slide, and to be used for the analysis of numerous and interesting markers of diagnostic, prognostic or therapeutic decision aid and for selection of molecular studies. An example of a small size TMA image is shown in *Figure 6*. Normally, TMAs are used in research due to the easiness for acquiring many samples in a single image to carry out studies on certain biomarkers or tissues and their possible variations.

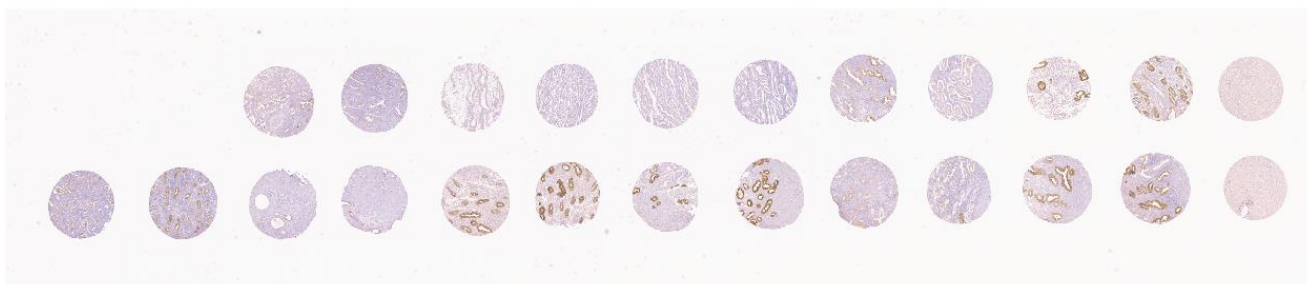


Figure 6: Example of TMA image from HUTER project

TMA brightfield samples usually contain simple HE, immunohistochemistry or other special stains images so this imaging modality could be considered an HE or IHC composed image (see Immunohistochemistry). However, compositing multiple samples into a single image also leads to changes about the compositing that must be done at the metadata level at the DICOM image level. This is due to the schema discussed above in *Figure 1* that indicates the relationship between an image, container, and specimen. The diagram shows that the presence of N specimens is now possible in a single DICOM image, as is the case with the TMA.

3.1.2.1. Multi-specimen image

As discussed above, TMA images are characterized by the presence of multiple specimens. To be in accordance with part 17 of the DICOM standard⁴ :

If there is more than one specimen in a container, there must be a mechanism to identify and locate each specimen. When there is more than one specimen in a container, the Module allows various approaches to specify their locations. The Specimen Localization Content Item Sequence (0040,0620), through its associated TID 8004 “Specimen Localization”, allows the specimen to be localized by a distance in three dimensions from a reference point on the container, by a textual description of a location or physical Attribute such as a coloured ink, or by its location as shown in a referenced image of the container. The referenced image may use an overlay, burned-in annotation, or an associated Presentation State SOP Instance to specify the location of the specimen.

Therefore, for the image to conform to the standard, it is not only enough to show the N specimens in the image, but also establish a mechanism for locating each specimen within the image. This mechanism is established according to the standard by TID 8004 Specimen Localization, which consists of a table with the possible implementations of the specimen location mechanism.

As shown in the table and its description in *Figure 7*, there are 6 possible implementations to include information about the location of the specimen.

In theory, established positions in the table show the significance order of options, with 1 being the higher priority and 8 the lower priority. Depending on the information that is initially available from the image, it will be possible to implement some location methods or others. In the case of HUTER TMA implementation, row 7 option has been used, by creating presentation States that locate each specimen. The following section *Presentation States* details the option chosen and the reasons for choosing it.

⁴ <https://dicom.nema.org/medical/dicom/current/output/pdf/part17.pdf>

Table TID 8004. Specimen Localization

	VT	Concept Name	VM	Req Type	Condition	Value Set Constraint
1	TEXT	DT (111708, DCM, "Position Frame of Reference")	1	U		
2	TEXT	DT (111718, DCM, "Location of Specimen")	1	U		
3	NUMERIC	DT (111719, DCM, "Location of Specimen X offset")	1	U		
4	NUMERIC	DT (111720, DCM, "Location of Specimen Y offset")	1	U		
5	NUMERIC	DT (111721, DCM, "Location of Specimen Z offset")	1	U		
6	IMAGE	DT (111718, DCM, "Location of Specimen")	1	U		
7	COMPOSITE	DT (111718, DCM, "Location of Specimen")	1	U		Presentation State SOP Instance reference
8	TEXT	DT (111723, DCM, "Visual Marking of Specimen")	1	U		

Content Item Descriptions

Row 1	Description of coordinate system and origin reference point used for localizing the Specimen. The value "CURRENT IMAGE" identifies the frame of reference as the pixel space of the Image SOP Instance in which this Content Item occurs.
Row 2	Description of specimen location, either in absolute terms or relative to the Position Frame Reference of Row 1
Rows 3-5	Location of specimen (nominal center) relative to the Position Frame Reference of Row 1. The Content Items include the units of measurement (e.g., mm). If Row 1 value is "CURRENT IMAGE", measurement shall be from the top left hand corner of the Pixel Data of the SOP Instance, using units of {{pixel}}, UCUM, "Pixels").
Row 6	Reference to image of container localizing the specimen; may include referenced Presentation State object
Row 7	Reference to Presentation State object for this SOP Instance, with annotations localizing the specimen
Row 8	Description of visual distinguishing identifiers, e.g., ink, or a particular shape of the specimen

Figure 7: TID 8004 possible values.⁵

3.1.2.2. Presentation States

Presentation State (PR) is a special type of DICOM object that contain information about how another DICOM image should be displayed. PRs may contain labelling or positioning information, windowing values, zoom values, scrolling values, rotations, or any display modification of the original image that is defined within the DICOM standard.

A significant point of this type of objects is that they do not contain an image, since they are designed to be associated with another DICOM object that contains the image to be complemented. The use of PRs allows the image to which they are associated to be displayed with the modifications indicated by the PRs. This provides the advantage that the original status of the image can always be restored by removing the PRs. In addition, it is possible to store PRs in the PACS as they are DICOM objects.

In the case of TMA images, the use of Presentation States is used to generate annotations on the image. In addition, PRs also have the advantage that they can be associated with several images of the same series, which in this case would allow a specimen to be referenced at different resolution levels with a single PR.

⁵ <https://dicom.nema.org/medical/dicom/current/output/pdf/part16.pdf> TID 8004

The use of PRs, therefore, becomes useful to visualize TMA images and mark the positions of all specimens contained in the image. To view DICOM images, a DICOM viewer has been developed in the HUTER project that allows browsing WSI images with the formats mentioned in this document (immunohistochemistry, TMA, fluorescence and smFISH). In the case of the TMA, localization of specimens made by PRs is observed in the viewer in *Figure 8*.



Figure 8: TMA image in the DICOM viewer

The image shows how each of the specimens is surrounded by a superimposed PR that locates the associated specimen. In addition, the ID of the specimen associated with that sample is indicated inside the PR, so that the identification is unique. The use of the Presentation State tags and their implementation will be discussed in detail in section 4.2.2 *Presentation States*.

In this case, PRs that have been generated are associated by means of a 1:1 relationship to the specimens in the image and, in addition, each PR refers to a specimen at all levels of resolution of the pyramid. This is possible due to the characteristics of the PRs, which, on the one hand, allow them to be associated with N images of the same series and, on the other hand, can resize the PR from the viewer depending on the resolution level of the image, so that it is always correctly pointing out the location of the specimen.

3.1.3. Fluorescence

Fluorescence microscopy images are obtained using the principle of fluorescence on the sample of interest. The specimen is illuminated under the microscope with light of one or more specific wavelengths, which will be absorbed by the fluorophores present in the sample. Typically, these fluorophores are artificially introduced into the sample to act as markers. In other words, it is intended that these molecules will be directed to certain areas of the sample (for example the cell nucleus) so that they are later visualized with the wavelength that corresponds to each fluorophore. The most widely used fluorescence microscope in life sciences is the epifluorescence microscope like the one shown in Figure 9. The excitation and emission light follow the same

path through the objective. However, the dichroic mirror acts as a wavelength filter, in such a way that it reflects the wavelength corresponding to the excitation light and allows the wavelength of the emission light to pass, which reaches the ocular to be captured by the detector and the image is generated.

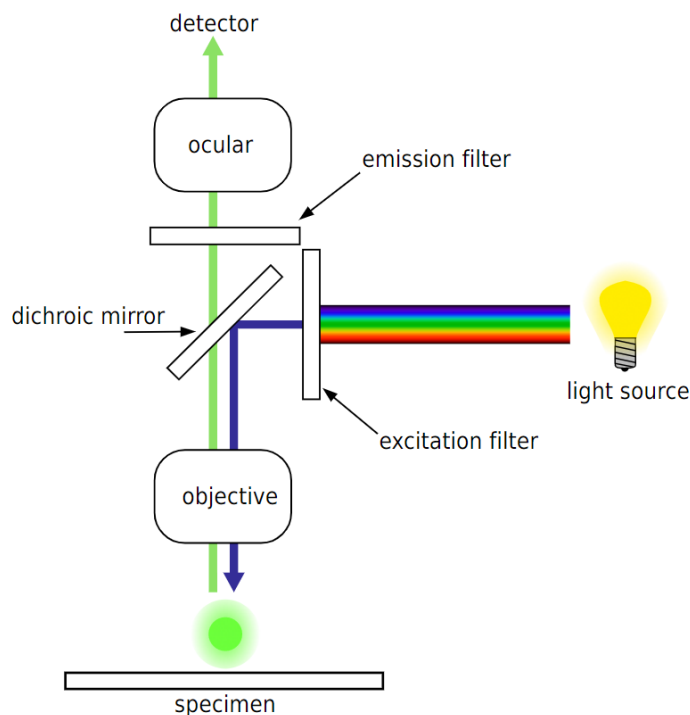


Figure 9: epifluorescence microscope schema.⁶

In the case of HUTER project, fluorescence images that have been acquired also present several different wavelengths (also called channels), which means that different parts of the sample can be viewed at the same time, since each wavelength will mark with greater affinity an area according to the properties of the fluorophore.

This type of images will imply a new subtype of DICOM WSI images in the project due to its differential characteristics. The main features are the presence of N channels per image and how this will be addressed both in the definition of the image and the associated metadata to correctly report the characteristics of each of the channels.

Fluorescent techniques (both immunofluorescence and FISH) can also be applied to TMA. In DICOM, this requires a combination of the approach described for TMA and fluorescence.

⁶ Image source: https://en.wikipedia.org/wiki/Fluorescence_microscope#/media/File:FluorescenceFilters_2008-09-28.svg

3.1.3.1. Multichannel image

The most characteristic feature of the project's fluorescence images is the presence of N channels (1 per acquired wavelength). This is due to the use of N different fluorophores, which allows different information to be acquired from each of the channels. Despite the advantages of a multichannel image, there are problems regarding its definition in the DICOM standard that must be resolved. One of these problems is that this image modality is defined within the standard but its use in DICOM format has not been yet established like other images (e.g., immunohistochemistry).

Reviewing the DICOM standard searching support for an N-channel WSI image, Supplement 145⁷ indicates that it is possible to include multiple Z planes and channels in a pyramid image as shown in Figure 10.

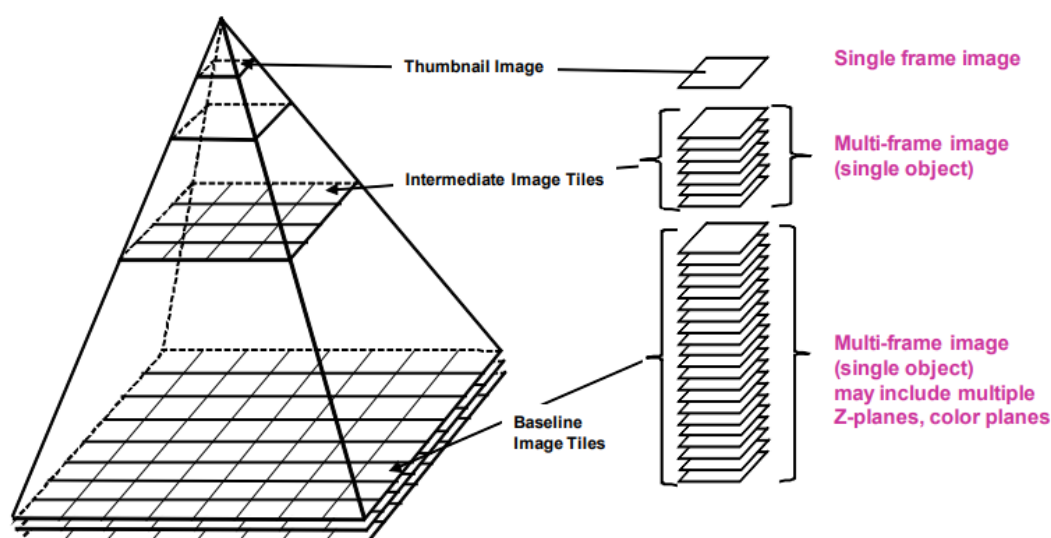


Figure 10: WSI pyramid with multiple channels.⁷

As established in Supplement 145:

Multi-spectral images may have a single frequency band encoded in each frame with up to 16-bit pixel depth; such images will be identified as monochrome, although the image object may include many coextensive frames representing a tile in different spectral bands. The colour mapping of each frame is conveyed through a description of the optical path.

Therefore, multichannel images will be encoded with monochrome frames corresponding to individual channels that can then be superimposed. That is, for a 3-channel image, 3 monochrome frames would be generated for each position of the pyramid, which would then be superimposed with different colours to generate the final image in the viewer.

⁷ <https://www.dicomstandard.org/News-dir/ftsups/docs/sups/sup145.pdf>

Following this standard proposal, it has been decided to generate N layers per level (for the N image channels), which are then superimposed on the viewer with the different colours of each one. Furthermore, all the layers are referenced in a single LOCALIZER that contains the image information in all its channels. An example of a fluorescence image with 3 channels generated in the project is the one shown in *Figure 11*.

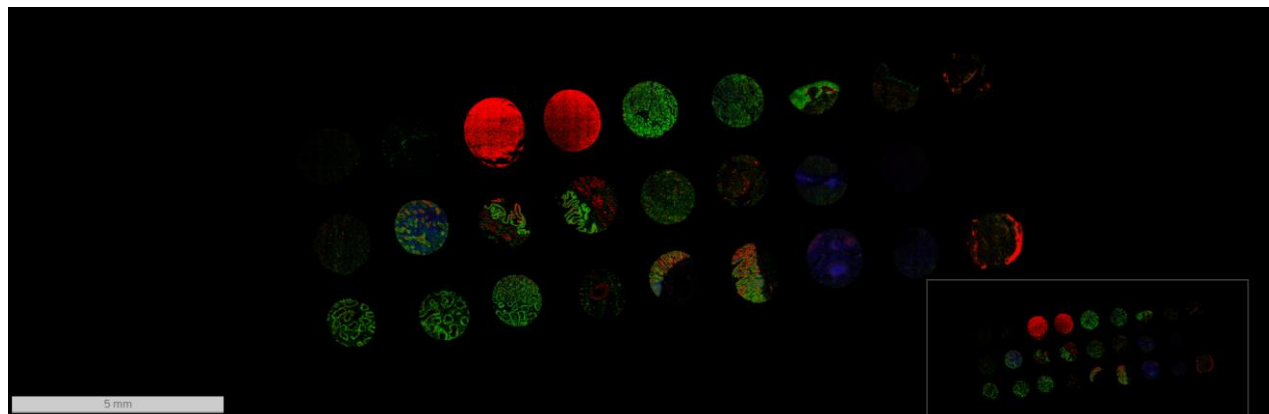


Figure 11: Example of fluorescence image in HUTER DICOM viewer

3.1.3.2. Metadata support

Appearance of new features in fluorescence images also affects DICOM's ability to store this information in its metadata. For this modality, the main differences with respect to a standard WSI image will reside in the ability to describe the acquired channels and their properties.

The main changes to carry over to DICOM are the excitation and emission wavelengths and the name of the channel that will contain information about the fluorophore associated with that wavelength. This information will be treated in more detail about its implementation in section 4.3.2 Metadata support.

3.1.4. smFISH

Fluorescent in situ hybridization (FISH) is a technique that uses fluorescent DNA or RNA fragments that bind only certain parts of nucleic acids with high complementarity. It was developed in the 1980s to detect the presence of certain DNA sequences on chromosomes. This technique has been improved and specialized over the years, appearing different more specific modalities from it.

One of the most prominent modalities is single molecule fluorescence in situ hybridization (smFISH). It is a very powerful technique for studying gene expression in individual cells since, as its name suggests, it allows the detection of individual RNA molecules. Binding of a single fluorescent dye to a nucleic acid molecule would produce too weak a signal to allow individual RNA molecules to be distinguished. However, in the smFISH technique, multiple fluorescent dyes are used that, when bound, produce a stronger signal and improve the signal-to-noise ratio, allowing the differentiation of individual RNA molecules.

Regarding the characteristics of these images in the HUTER project, they are multichannel images with several Z planes.

First, it is a multichannel image because the fluorescence obtained from different fluorophores are superimposed, since several different RNA molecules are detected inside the cells.

Second, the images have multiple Z planes because they are acquired on a confocal microscope. These microscopes allow multiple 2D images of the sample to be obtained at different depths.

Comparing smFISH images with fluorescence images, it is observed that both share the characteristic of having multiple channels. However, a characteristic of the smFISH images of this project is the presence of multiple Z planes.

Therefore, it is necessary to check if the DICOM standard also allows the generation of images that combine more than one channel and more than one Z plane. Returning to Figure 10, it is indicated that the DICOM WSI image pyramids can contain images with more than one Z plane and more than one channel. This implies that it is possible to generate the smFISH images that we acquired in this project in DICOM format.

3.1.4.1. Multichannel image

As mentioned for the fluorescence images, the approach taken to express the N channels of the smFISH image will be the same in terms of the pyramid structure.

The only significant difference is in the metadata acquired in each modality. In the case of smFISH images, it would be interesting to be able to report, apart from the excitation and emission wavelength of each channel and the fluorophore used, the gene that is shown in each of the channels. In this way, the greater amount of clinical information of the image would be reflected in the DICOM metadata.

Figure 12 shows an example of a smFISH image in DICOM format generated in the HUTER project and containing 4 channels. As can be seen in the Channel Management section, the channel names contain the names of the fluorophores, which give information about which structure is being stained.

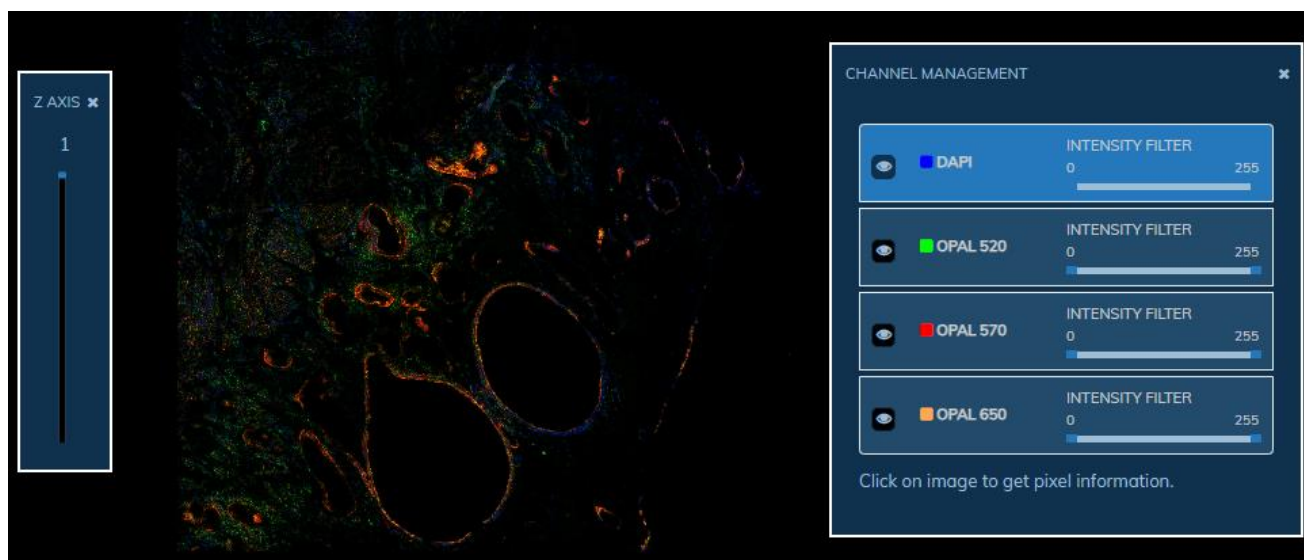


Figure 12: Example of smFISH image acquired during HUTER project

3.1.4.2. Multi-Z-plane image

The differential point of the smFISH images in this project is the presence of more than one Z plane. To generate DICOM images with more than one Z plane, it is necessary to generate different levels of the WSI pyramid corresponding to all the Z planes and then include the Z coordinate of each one of the levels so that they are ordered according to that value on the Z axis. For more details regarding the implementation in DICOM go to the section 4.4.1 Multi-Z-plane image.

The result, as seen in Figure 12, is a DICOM image that, if interpreted correctly by the viewer, allows you to navigate in its resolution like all WSI images and as a differential aspect, it is possible to navigate between the different depths of the Z plane with the selector that has enabled in our viewer (Z Axis).

3.2. DICOM lack of definition

During the investigation of DICOM standard and its implementation in all DICOM imaging modalities of the HUTER project, some shortcomings have been seen that could be interesting to address in future updates of the standard.

The main points of conflict are in the assignment of certain metadata of images with multiple channels (fluorescence and smFISH). The name of the channel does not have any specific tag in DICOM, therefore, within the HUTER implementation, a tag for generic use (0048,0107) Optical Path Description has been adapted to include the name of the channel, while the standard does not add an explicit support for this field.

Another problem identified is related to the required mechanism to indicate the fluorophores used in the fluorescence images since none of the existing tags supports this functionality. In any case, there is a change proposal that is in progress and seems to address this problem in "CP-2082 Add acquisition codes and template

for fluorescence and immunohistochemistry in microscopy". Until this modification is approved, the standard does not provide support for this data that would be useful to include in the DICOM image for the end user.

4. DICOM IMPLEMENTATION

Once the principles that govern the DICOM standard are shown in section 3 HUTER DICOM RESEARCH and the different image modalities that will be converted to DICOM format in this project, it is necessary to move on to the practical part of how to generate the image and what metadata to use in each case.

Therefore, in this section the full implementation of each image format will be explained in detail with their similarities and differences. As a base implementation, the immunohistochemistry image could be taken, since it is the closest format to the real implementation among manufacturers (some are already capable of generating this type of images in DICOM format) and the one that presents the least conflicts with the current standard.

All modalities present differential characteristics that, although in some cases they are indicated as supported by the standard, have never been implemented or do not have any examples available as a guide, as is the case of the use of N channels, N Z planes, the presence of N specimens in an image and their location in the metadata. All these points will be treated in depth in each of the imaging modalities that make use of them.

For the general implementation, the format to follow for the image is the one mentioned in Figure 3 with levels of different resolution organized in a pyramid and all of them referenced and localized tile by tile in the LOCALIZER, which will contain a global image of low resolution.

As for the metadata, these are divided into several modules in the standard. There are modules that are common to all DICOM images of any modality and that have practically no variations such as the Patient module, the Study module...

However, there are modules that can have different interpretations depending on the imaging modality on which they are applied, as in the case of the specimen module that does not have the same interpretation in a pathology image as in other modalities in which in each image there is only a specimen always.

There are also modules that only exist in one imaging modality, such as the Whole Slide Microscopy Series module that is only present for WSI DICOM images.

At this point, emphasis will be placed on the most significant modules for WSI images, which are those that have important changes or are unique to this type of image. Modules such as Patient are common to all types of images and do not change between them, so it is not necessary to delve into them either.

The modules to analyse are:

1. Patient

Patient module is mandatory and is used to inform attributes to describe and identify the patient subject of the study. Some of the most significant metadata of this module is (0010,0010) Patient's Name, (0010,0020) Patient ID, (0010,0040) Patient's Sex and (0010,0030) Patient's Birth Date. In case the image is to be anonymized and no patient data appears, there is also the possibility using the metadata (0012,0062) Patient Identity Removed with YES value and setting the de-identification method in the tag (0012,0063) De-Identification Method. There are many other data that can be covered about patients but they do not apply to our use cases in the project.

2. General Study

General Study module specifies attributes that allow identifying and describing the study that has been performed on the Patient. Among the most significant metadata of this module are (0020,000D) StudyInstanceUID which corresponds to a unique study identifier, (0020,0010) StudyID which is a study identifier that does not have to be unique and is generated by the user requesting the study. (0008,0050) Accession Number is an identifier generated by the request to generate the image in the System. Finally, some descriptive metadata of the study are (0008,0020) Study Date, (0008,0030) Study Time, (0008,1030) Study Description about the date, time and description of the study performed.

3. Patient Study

In the Patient Study module, attributes that correspond to information about the patient at the time the study was performed are defined. Thus, for example, in the Patient module the date of birth was collected and in this module the patient's age at the time of the study (0010,1010) Patient's Age or other data such as height, weight... which are common data to any imaging modality.

4. General Series

General Series module stores the metadata that identifies and describes general information about the series acquired in the study. The most important data of this module is the (0020,000E) Series Instance UID which is a unique identifier of the series. (0020,0011) Series Number identifies the series internally to the study. Finally, some descriptive metadata of the series are (0008,0021) Series Date, (0008,0031) Series Time, (0008,103F) Series Description about the date, time and description of the series made.

5. Whole Slide Microscopy Series

Whole Slide Microscopy Series module is a complement to the General Series module that specializes the use of certain metadata according to the needs of WSI images. This module limits the use of (0008,0060) Modality with the SM value corresponding to the Slide Microscopy modality and the use of the (0008,1111) Referenced Performed Procedure Step Sequence, which contains a reference to the related instance with the procedure

step (0008,1555) Referenced SOP Instance UID with the reference to the SOP Instance UID of the current instance and (0008,1150) Referenced SOP Class UID that contains the referenced SOP Class.

6. Frame of Reference

Frame of Reference module contains the necessary attributes to identify a reference frame that ensures the spatial relationship of the images in the same series. It contains the metadata (0020,0052) Frame of Reference UID that indicates the selected reference frame by means of an identifier and (0020,1040) Position Reference Indicator that, being an image divided into slides, must contain the value "Slide Corner" used as reference.

7. General Equipment

General Equipment module contains information that identifies and describes the equipment used to produce all instances belonging to the acquired series. This module uses the metadata (0008,0070) Manufacturer that informs who is the producer of the image, (0008,0081) Institution Name, (0008,1010) Institution Address, (0018,1020) Software Versions that contain information about of which institution the image is produced, its address or the version of software used.

8. General Image

General Image module contains metadata that informs and describes the image within the series that has been acquired. The metadata for this module is made with generic information about the image such as (0008,0008) Image Type containing the type of image based on its acquisition, (0008,0022) Acquisition Date, (0008,0023) Content Date containing dates when the image was acquired. Other parameters such as (0020,0013) Instance Number identify the instance with a number to differentiate it from the rest of the instances in the series.

9. Image Pixel

Image Pixel module contains metadata related to the technical properties of the image. The number of columns and rows of the image is indicated in (0028,0010) Rows and (0028,0011) Columns. Other important metadata of the module are (0028,0100) Bits Allocated which contains the number of bits reserved per pixel of the image or (0028,0101) Bits Stored which indicates the number of bits stored per pixel of the image. Finally, the most important tag of the module and of the image is the one that contains this module (7FE0,0010) Pixel Data. This tag contains the pixels that make up the image.

10. Acquisition Context

Acquisition Context module contains metadata about the conditions under which images were acquired. It presents a description field (0040,0556) Acquisition Context Description and a Sequence field that allows to identify acquisition conditions through multiple items (0040,0555) Acquisition Context Sequence.

11. Multi-frame Functional Groups

Multi-frame Functional Groups module contains metadata that is common to all image frames such as (0008,0023) Content Date or (0008,0033) Content Time indicating the date and time the image acquisition started. It also contains two important element sequences: (5200,9229) Shared Functional Groups Sequence and (5200,9230) Per-Frame Functional Groups Sequence. Shared Functional Groups Sequence contains elements that are common to all frames, such as the Optical Path that indicates the ID of the channel used in the image and its properties, or the Specimen reference that refers to which Specimen all frames belong to. The values that Per-Frame Functional Groups Sequence can contain are the same as in the previous case, but the difference is that in this case each item of the sequence is applied to a single frame, which allows different values of Optical Path, Specimen ... to be assigned to each of the frames of an image.

12. Multi-frame Dimension

Multi-frame Dimension module contains metadata that indicates a pointer to where to find the image dimensions or what metadata will manage them. It is a significant module in WSI image since it also allows to indicate the way in which the image is sampled (TILED_FULL or TILED_SPARSE). This value is passed in the (0020,9311) Dimension Organization Type tag. For WSI images, two ways of sampling the image into tiles are possible. The first corresponds to sampling all the tiles of the image even if it has blank holes in the middle. This procedure is called TILED_FULL in DICOM. The other sampling method that DICOM supports is to sample only the tiles that have information (not empty) and store them indicating their position to facilitate reconstruction. This type of procedure is called TILED_SPARSE in DICOM.

13. Specimen

Specimen module is a very important module for WSI images because these types of images are based on the presence of 1 or more specimens. Therefore, this module mainly collects information about the identification of the image specimens and their container. The most important tags are (0040,0512) Container Identifier, (0040,051A) Container Description that contain the identifier and description of the image container. (0040,0520) Container Component Sequence contains the description of all the elements that make up the container. (0040,0560) Specimen Description Sequence is the main component of this module. It stores the identifier of each of the specimens (0040,0551) Specimen Identifier and (0040,0554) Specimen UID, the description of the specimen (0040,0600) Specimen Short Description and (0040,0602) Specimen Detailed Description. It may also be important to use the (0040,0620) Specimen Localization Content Item Sequence to locate the specimens within the image. Its use will be better detailed in section 4.2 Tissue MicroArray.

14. Whole Slide Microscopy Image

Whole Slide Microscopy Image module complements the General Image module described above with metadata that is only suitable for WSI-type images, such as (0028,0008) Number of Frames or (0048,0010) Specimen Label in Image indicating the number of frames of the image and if the slide label has been captured in the image. Otherwise, it contains general information about the image in the same way as the General Image module. They have common tags that are only implemented once to avoid unnecessary repetition.

15. Optical Path

Optical Path module is one of the most significant of this type of WSI images, together with the Specimen module, due to the novelties it includes. That is, information about the channels captured in the image, such as the wavelength in which the image is acquired, identifier, description, magnification... All this information is grouped around (0048,0105) Optical Path Sequence with one element for each channel that contains the image. Then, the tag (0048,0302) Number of Optical Paths is used to indicate the number of items present in the previous sequence.

16. Multi-Resolution Navigation (Retired in 2021D version)

Multi-Resolution Navigation module is only present on WSI images. However, as of version 2021D its use has been withdrawn due to the disappearance of the LOCALIZER that was the one that used it. The change was made after a Change Proposal CP-2102⁸ was approved. However, as this modification was made after the implementation of the DICOM standard in HUTER, it has continued to be used to generate the LOCALIZER image. In this module, the frame-by-frame metadata of its relative position within the LOCALIZER image, the Optical Path to which the frame belongs, the instance or level of the pyramid to which the frame belongs, is stored in the sequence (0048,0200) Referenced Image Navigation Sequence and the offset in the Z plane that the frame has. With the information of all the frames contained in the LOCALIZER, it would be possible to navigate through all the levels of the pyramid by accessing only the mentioned metadata.

17. SOP Common

SOP Common module contains metadata that makes it easy to identify the instances associated with the image. In the case of images that affect the project, each level of the WSI pyramid will be contained in a single instance, so this relationship will be 1 to 1. The most significant metadata contained in this module is (0008,0016) SOP Class UID and (0008,0018) SOP Instance UID.

⁸ <https://www.dicomstandard.org/news-dir/current/docs/cpack112/cp2102.pdf>

4.1. Immunohistochemistry

In the case of immunohistochemistry images, there are no significant changes in the implementation of the standard. As mentioned, these images are fully supported by the standard and it is not necessary to make adaptations to be able to implement this type of images. The only part that may be specific to this type of imaging or further developed is the description of sample preparation. In immunohistochemistry images, sample preparation steps and stains used can be important for the interpretation of the image and therefore their presence in the metadata is necessary.

To do this, multiple nested sequences from the Specimen module are used. Inside (0040,0610) Specimen Preparation Sequence is included all preparation information for all specimens in the image. This information is divided into N steps that will be collected in another sequence (0040,0612) Specimen Preparation Step Content Item Sequence. Within this sequence, DICOM allows you to add data of all supported types such as text, number, references to other DICOM objects... In addition, another point that gives great power to the DICOM standard is that in this sequence and in other similar ones it is possible to enter values that reference standard medical vocabularies such as SNOMED and LOINC.

With all these possibilities, the explanation of how the immunohistochemistry specimen has been composed will be complete and useful for end users.

4.2. Tissue MicroArray

In the case of TMA images, main modifications that their implementation would require with respect to a base image such as immunohistochemistry have been commented on the document (see section 3.1.2 Tissue MicroArray). In its implementation, changes are highlighted because it is a multi-specimen image and the need to implement a new DICOM object called Presentation State in order to meet the specimen location requirements indicated by the standard.

4.2.1. Multi-specimen image

With this option of using Presentation State to reference specimen location, it must be defined the implementation of the whole Specimen Module for TMA. The only part that differs for conventional immunohistochemistry images is the Specimen Description Sequence. So, for the rest of the Specimen Module, the same implementation as previously defined for immunohistochemistry images can be used.

For TMA, Specimen Description Sequence must contain one item for each specimen that contains the image. In each item, an individual spot is completely identified and located. First, Specimen ID and Specimen UID must be specified.

Other tags, as Issuer of The Specimen Identifier Sequence, Specimen Type Code Sequence, Specimen Short Description, Specimen Detailed Description or Specimen Preparation Sequence should be implemented as specified for single immunohistochemistry pathology images. The only difference in these tags with single immunohistochemistry images is the number of items, since for TMA is mandatory to create an item for each specimen as said before. The important part of this sequence is to specify the Specimen Localization Content Item Sequence. This tag is type 1C and it is required if multiple specimens are included in the image (the case of TMA).

To create items in Specimen Localization Content Item Sequence, Value Type must be COMPOSITE to reference the Presentation State associated. It must be specified also the Referenced SOP Sequence indicating the SOP instance value of the Presentation State associated with each spot and each pyramid layer. Thus, Presentation State objects surrounding each specimen must be created before creating the Specimen Localization Content Item Sequence.

4.2.2. Presentation States

Presentation State (PR) is defined in the DICOM standard as an object capable of storing display information about a referenced image. In this case, it allows to indicate on the pyramidal image where the specimens are located and include information such as the Specimen ID and Specimen description. Therefore, the PR objects complements the immunohistochemistry images, adding location information without modifying the original image, so that it would be possible to modify PRs to indicate new values without affecting the pyramidal image.

These PRs will be referenced from each of the levels of the pyramid generated in the image. On the other hand, each of the levels of the pyramid must also be referenced in the PRs.

A point to highlight about the functionality of PRs when working as locators of the spots is that the coordinates of the PRs are not affected by the size of the image on which they act, so that both the base level of the pyramid and the top level will use the same PR position for the same specimen. In addition, the possibility of being editable without affecting the original image allows that from a DICOM viewer they can be moved to the liking of the user who is viewing the image or even edit the identifier or description of the PRs.

Going further into the technical part of the implementation, the principal information at the DICOM tag level have been the following:

The PR object contains the UID of all DICOM objects it references (all levels of the TMA pyramid). For this, the Referenced Series Sequence (0008,1115) is used. Within this sequence, the Referenced Image Sequence (0008,1140) is implemented, which is the one that contains the UIDs of each of the referenced levels. These values are collected in N items (as many as DICOM objects of the TMA pyramid are referenced) with the Referenced SOP Class UID (0008,1150) and Referenced SOP Instance UID (0008,1155) tags.

To include the necessary information in the annotations that the PR objects allow, the Graphic Annotation Sequence (0070,0001) is used and within it the Text Object Sequence (0070,0008), in which the Tracking ID (0062,0020) tags are included to indicate the ID of the annotation to be entered (TITLE for the annotation identifier and DESCRIPTION for the description). Then in the Unformatted Text Value tag (0070,0006) the value of each annotation is included.

The Graphic Annotation Sequence tag (0070,0001) not only contains information about the text annotations, but also contains the information about the coordinates and shape of the PR in the Graphic Object Sequence (0070,0009). Within it, the shape of the PR is defined in Graphic Type (0070,0023), which in this case will be CIRCULAR because it is the shape that best adapts to the spots. On the other hand, the position of the PR is defined in the Graphic Data tag (0070,0022) with the coordinates that determine its position. Finally and also within this sequence, it is possible to determine the colour and transparency of the PR in the Fill Style Sequence (0070,0233).

4.3. Fluorescence

In fluorescence images, the main novelty with respect to the base implementation of WSI images is the presence of N channels in a single image. To solve this point, it is necessary to see the organization of the pyramid for these cases and new metadata that arise from the use of fluorescence in terms of channel acquisition.

4.3.1. Multichannel image

For the implementation of multiple channels in a single image, a DICOM object has been created per resolution level and channel, so that each object represents a single channel. Then, from the LOCALIZER all levels and channels are referenced so as not to lose traceability that WSI images have in the LOCALIZER. As for DICOM metadata that supports the use of multiple channels, all are found within the sequence (0048,0105) Optical Path Sequence. Since only one channel is included in each DICOM object, the implementation of the Optical Path Sequence within each level of the pyramid is the same as that discussed in the base implementation 4 DICOM .

However, from the LOCALIZER it is necessary to reference all the Optical Paths of the levels present in the WSI pyramid. Inside the sequence (5200,9230) Per Frame Functional Groups Sequence of the LOCALIZER, the sequence (0048,0207) Optical Path Identification Sequence must be included and inform the field Optical Path Identifier with the ID of the Optical Path employed in each referenced frame.

4.3.2. Metadata support

Within the support for new metadata required by fluorescence images, we can highlight those that arise from the new acquisition technique used in this modality. Therefore, those related to the wavelength of each channel, the name of each channel or the fluorophores used are the main novelties.

To collect the wavelength, the tag (0022,0055) Illumination Wavelength will be used, which collects the value of the wavelength used to illuminate the sample in this channel in nm.

The channel name does not have any specific tag in the Optical Path Sequence, so a generic use tag (0048,0107) Optical Path Description has been adapted to include the channel name in it.

Problems have also been found to be able to indicate the fluorophores used since none of the existing tags supports this functionality. In any case, there is a change proposal that is in progress and seems to address this problem in "CP-2082 Add acquisition codes and template for fluorescence and immunohistochemistry in microscopy".

4.4. smFISH

For the multichannel issue and metadata support, the proposal is the same as in the fluorescence images. Only secondary differences like genes information can be included in addition to the metadata needed in fluorescence images.

The main difference of this type of images in the HUTER project when adapting them to the DICOM format is their multidimensionality both at the channel level and in Z planes. This feature makes important to address the way in which multiple Z-planes will be represented in a pyramid.

4.4.1. Multi-Z-plane image

Implementing more than one Z plane in a DICOM image requires an approach like the one followed for using more than one channel in the image. To do this, N planes must be created, each one representing a Z plane. The tags used in each individual level do not vary, except for the use of (0040,074A) Z Offset In Slide Coordinate System that allows indicating the offset of the selected level in the Z plane with respect to the chosen coordinate system. The use of different values in this tag will cause the depth of each of the Z planes of the image to be different.

Generating the LOCALIZER image, the main change is how to reference all the Z Coordinates from the different Z planes that are included in the LOCALIZER. The reference is in the Referenced Image Navigation Sequence (0048,0200). Items of this sequence contains information about all frames of the pyramid and its relative location in the LOCALIZER. In this sequence, tag (0040,074A) Z Offset In Slide Coordinate System must be correctly informed with the correct Z position in the coordinate system of this Z plane.

5. MANAGEMENT OF PROPOSALS

The HUTER project aims not only to develop research and an implementation of the DICOM standard to HUTER image types (previously detailed in sections 3 and 4), but also to transfer this knowledge to DICOM secretariat. In this way, this work can contribute to foster the development of DICOM and the support for these kinds of advanced image types generated in HUTER. In this section, the mechanisms to submit information to improve the standard, the final strategy followed, and the feedback received from the DICOM secretariat will be explained.

5.1. Management of WG26 for new proposals

DICOM standard is not a one-shot document, but it's evolving with the new use or cases appeared in the image in health. Indeed, two or three times a year, a new version of the standard is published.

DSC has defined a standard procedure to add or correct aspects of the standard in the Policies and Procedures for the DICOM Standard Committee (Figure 13).

There are two main types of proposals: a **Change Proposal (CP)** and a **New Work Item Proposal (NWIP)**.

What is a CP? A CP is a small document to describe and address an issue like:

- Ambiguities - the Standard is not precise and/or leads to multiple interpretations.
- Omissions - necessary text is missing from the Standard.
- Inconsistencies - two or more sections of the Standard conflict.
- Clarifications - the Standard is correct but additional text would facilitate interpretation.
- Errata - normative or informative text in the Standard contains an error.

What is a NWIP? A NWIP is a proposal of a new SOP Class or a large change that affect many parts of the DICOM standard. The result of a NWIP is a supplement.

How is possible to submit CP? CPs may be submitted to the DICOM Secretariat by following the CP Templated published by de WG-06. Anyone who identifies an error in the Standard or a need for clarification of the Standard is invited to submit a CP, even if is not member of the NEMA or any DICOM WG. However, a very deep knowledge of DICOM standard and the stated of the art is needed. So, CP that comes from WGs members has more impact.

All the CPs will be reviewed and processed by DICOM WG-6. CPs are assigned to a member of WG-06; subsequent participation by the submitter is welcome but not required. Progress of CPs may be found in the

minutes of WG-06. Eventually CPs are collected into a CPack and distributed in a ballot to members of the DICOM Standards Committee. Approved Final Text CPs are incorporated into the next edition of the Standard.

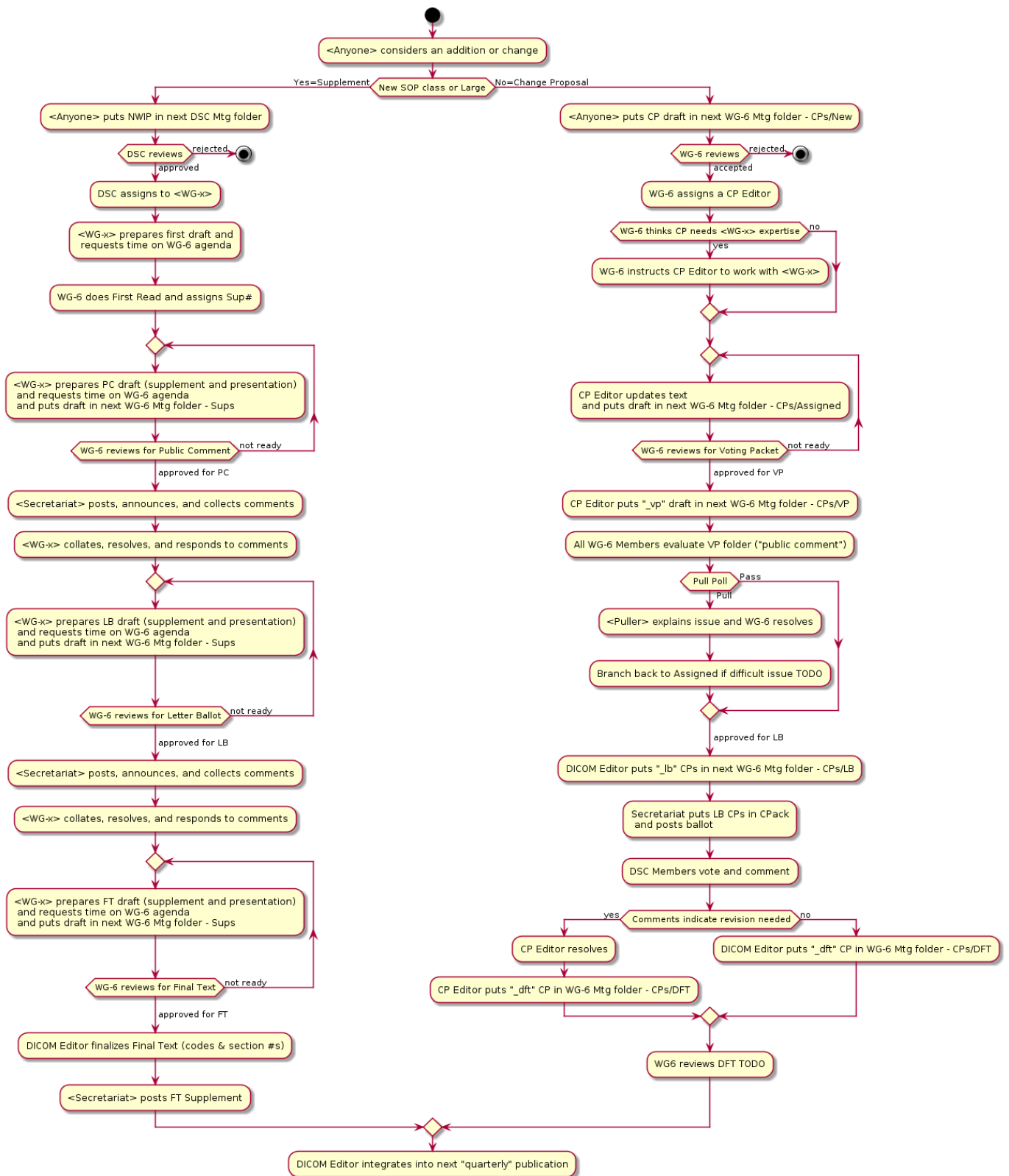


Figure 13: Procedure to propose changes or additions in the DICOM standard

How is possible to propose a NWIP? NWIP may be submitted to the DICOM Secretariat with the Work Item Proposal. The proposals are indicated by a WG member and will be reviewed for approval by the DICOM Standards Committee (DSC). Proposals must be received at least 2 weeks before the DSC meeting to be put on the agenda. Proposal authors should plan to attend and present their proposal or arrange for another presenter. Supplement editors should become familiar with “How to Prepare a Supplement to the DICOM Standard.”

The result of a NWIP is a Supplement of the DICOM standard. It's true that there is a nine-month minimum time for a supplement, but the experience said that, for a supplement redaction and presentation is needed:

- Three-and-one-half years of working time,
- 19 face-to-face meetings (12 in Europe and seven in the USA),
- 13 teleconferences,
- 24 different participants,
- Six invited guests,
- Two editors,
- 49 versions of the supplement,
- Five NEMA and four NNI / NEN secretaries,
- Two chairmen,
- More than 3000 e-mails and
- A multitude of Euros for travel expenses.

As working in a NWIP is far away from the HUTER objectives, the decision was to contact WG-26 member as explained in the following section 5.2.

5.2. Strategy developed for HUTER proposal

As was previously detailed in the previous section 5.1, there are many requirements which hinder that HUTER performs a CP or NWIP. The most critical impact is the timeframe of the HUTER project, which is restricted to 2.5 years, being the average time required for a NWIP (3.5 years) and which is also beyond of HUTER project aims. On the other hand, being a full active WG member is also required for the presentation of NWIP and CP. As explained the process to become a Full Active Member of the DICOM Secretariat is complex, takes considerable time and it is out of the scope of the HUTER project.

Considering the time and the restrictions for the submission of Change Proposal (CP) and New Work Item Proposal (NWIP) imposed by the DICOM Secretariat, BAHIA SOFTWARE cannot submit and finalize a Change Proposal within the HUTER timeframe.

However, in order to foster the development of a CP with the HUTER results, **we decided to contact WG-26 members to provide the DICOM research, analysis, implementation performed in HUTER as a contribution for a potential CP that they can consider.** For such a purpose, several emails were interchanged with the WG26 members group to explain HUTER objectives and development, getting their feedback and posing open questions about the CP of the DICOM standard. We have been in contact with David Clunie (author of DICOM standard), Markus Daniel Herrmann (WG26 chairman) Mikael Wintel (WG26 co-chairman for Europe) and Kevin Schap (WG26 secretary) were contacted about the HUTER research and results.

We have received feedback from different members of the WG-26. **David Clunie** contributed with the following states regarding our research:

- *There is definitely uncertainty about TMAs, which WG 26 should clarify.*
- *TID 8004 might help but there is still the question of who is the (single, pseudo) Patient for TMAs, etc.*
- *Presentation States should not be used*
- *LOCALIZERS have been retired and should not be used*
- *TMA and multiple channel (fluorescence) issues are separable issues*
- *Absorption and excitation wavelength are already in the Optical Path Module, albeit perhaps not clearly identified for those purposes*
- *Specimen prep and optical path need to be linked for fluorescence and IHC in general (see CP 2082⁹ work in progress).*
- *Considering splitting the channel information into separate instances for Z channels (Markus Daniel Herrmann also agreed).*

Markus Daniel Herrman (WG26 chairman) suggested the following ideas:

- *Storing each optical path and Z-plane in separate DICOM image would be a good idea.*
- *It facilitates data management, especially when dealing with multiple resolution levels.*
- *Eases map optical paths to specimen preparation steps.*
- *A Connectathon for multi-channel whole slide imaging should be performed to test these ideas.*
- *These ideas would be worth bringing this up for discussion at WG-26.*

⁹ ftp://medical.nema.org/medical/dicom/cp/cp2028_01_Extensiveness.xml

After the feedback interchanged, an interest was creating in the WG26 about the result of HUTER research about Z-plane and multifuorescent images. These results are aligned with what the WG26 is doing in terms of the CP2082. CP2028 is an example how DICOM standard can change across the years and how research project like HUTER can contribute to this change. Looking the result of the project and the interaction with WG26, HUTER outcome would be useful starting point for another Change Proposal from WG-26.

In summary,

- The HUTER project was present in the WG26 recurrent meeting discussion on February 15th as suggested by the chairman. The proposal has received great attention and it was considered as a real candidate for a Change Proposal.
- The HUTER proposal cannot be presented as a change proposal, because the process needs in average more than 3 years of discussions with the members of the DICOM Secretariat
- The HUTER proposal should follow the process of the CP2082, a successful experience of how research around the DICOM standard is finally translated and implemented to become worldwide adopted by the DICOM community and vendors.
- Therefore, the HUTER proposal will follow the example of the CP2028.
- In order to continue with that process, the Chairman of the WG26 proposed to BAHIA SOFTWARE researchers to participate in the next Connectathon for multi-channel WSI to be organized by the DICOM Secretariat. The Next Connectathon will be organized by the European Society of Digital and Integrative Pathology meeting (ECDP), and it will be held on June 15-18 on Berlin.

6. CONCLUSIONS AND FUTURE RESEARCH LINES

In conclusion, the use of the DICOM standard in pathology is a line in development with great potential to implement or speed up interoperability in digital pathology systems in hospitals. The use of DICOM images in this field would allow digital pathology images to be stored, viewed and consulted with specialists from other centres using any tool that works with the DICOM standard for WSI.

However, during the research carried out on the standard in the HUTER project, it has been found that the development of this part of the standard is at a much earlier stage in terms of pathology images than images such as radiology in which it is fully established and widely used.

The lack of implementation of the DICOM WSI image standard in practice suggests that the future lines of research could be in the Connectathons that are being developed annually by the WG26 DICOM. In them, different companies related to this image modality implement a use case of image generation, storage and visualization of the images generated in WSI format. These meetings are very useful to find points of

improvement in the standard since discrepancies appear between different implementations that sometimes cause communication problems between systems and may lead to reviewing certain points of the system.

In fact, chairmen of the WG-26 of DICOM recommends BAHIA SOFTWARE researchers to participate in Connectathon to show this implementation. They also discussed some of the features of the HUTER implementation in their meetings and highlighted the importance of developing the Z-plane of fluorescent images in DICOM among others (detailed in section 5.2). Therefore, the HUTER research and implementation has fostered, at least, the work in the DICOM standard this line of WSI.

In addition, the fact that more and more companies are involved in this type of event will lead to the future involvement in clinical use of DICOM WSI images once this part of the standard reaches enough interest level.

On the other hand, the work carried out in the HUTER project has uncovered that although immunohistochemistry images are supported in DICOM, there are other WSI imaging modalities that are not fully contemplated in the standard. A point of improvement for the future would be to focus on this type of images with multiple channels, Z planes or both so that all modalities are optimized in the standard with the aim of facilitating their use by manufacturers of pathology scanners. Expanding and optimizing the standard for all WSI imaging modalities could lead to greater implementation of the standard in practice.