Virtual Microscopy in Medical Images: a Survey

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A major objective of the present survey is to provide a comprehensive vision of the most recent methods for the construction of a Virtual Microscopy Viewer (VMV). The Virtual Microscopy (VM) is the microscopy area that provides a realistic digital emulation of a conventional light microscope and the VMV is the software tool that provides such emulation. Construction of a mega-image by stitching a sequential set of microscopic fields of view (FOV) is the first step towards a useful VMV development. Once the mega-image is assembled, this huge amount of generated data should be compressed and stored in hard disk for later recovery. Finally, whether this mega-image is compressed or not, data should be suitably accessed for navigation. The reviewed approaches are herein classified according to their role in VM, that is to say, at the stitching, storage and navigation phases. Main contributions, advantages, and drawbacks of the current navigation methods are presented and discussed, as well the outlook for future research.

Keywords virtual microscopy, mega-images, stitching, image navigation.

1. Introduction

Nowadays, mega-images or images composed of a larger number of pixels than those allowed by conventional capturing devices, are used in very different applications such as satellite, astronomic or medical images [1,2,3], among others. Large images in the Medical domain comprise barely every diagnostic modality because of the amount of information that each generates. However, a very recent field known as virtual microscopy is by far the largest generator of data [4]. Microscopical examination of tissues requires a biological sample is cut into very thin slices which are deposited on glass slides. Once this tissue lies down onto the slide, a complex coloration process highlights the relevant cellular information either by specifically staining nuclei or cytoplasm organelle. Mega-images, understood as whole-slide-images are constructed by a sequential capturing process. They shall be in the very near future a useful tool in most routine microscopical applications. They allow a unique image storage, called virtual slide which makes possible a so far unknown information availability for image retrieval in case of latter studies, medical training, distribution by electronic media, image exchange between pathologists, annotation capabilities and morphometrical measurements [5,6,7,8,9]. Overall, these virtual slides are high resolution images whose visualization requires specialized software: the Virtual Microscopy Viewer (VMV), a specific tool devised for running over images composed of thousands of microscopical FOV. Efficient navigation strategies within such virtual slides should take into account the multiple disk accesses for locating, extracting and processing the requested information. Minimal requirements for this kind of viewer are

- Random access to image information.
- Granularity of information.
- A robust representation of the different magnification levels.
- Adaptable interface to the user needs

Construction of such a VMV implies to solve different kind of problems, whose nature depends on the different involved steps: assembling the mega-image, efficient storage and rapid information availability for navigation. These three general requirements define three complementary processes.

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- **Stitching**. The different microscopical FOV, result of the digitalization process, must be assembled together into a single high resolution image [10], a process which involves registering strategies for finding overlaps between neighbouring FOV.
- **Storage**. Clever storage strategies are needed since the virtual slide demands a huge amount of memory space. For this reason it is required an intensive use of image compression methods [11], but subjected to the restriction that such methods must also allow an efficient access to information when required. Additionally, compression in medical images must be lossless since minimal distortions may lead to a false diagnosis [12].
- **Navigation**. This is the process which permits a user to carry out a microscopical examination of a particular sample as it would be possible using a conventional microscope. This virtual microscope must allow sequential and random translational movements at any of the xy-axes or zooms when moving along the z-axis [13].

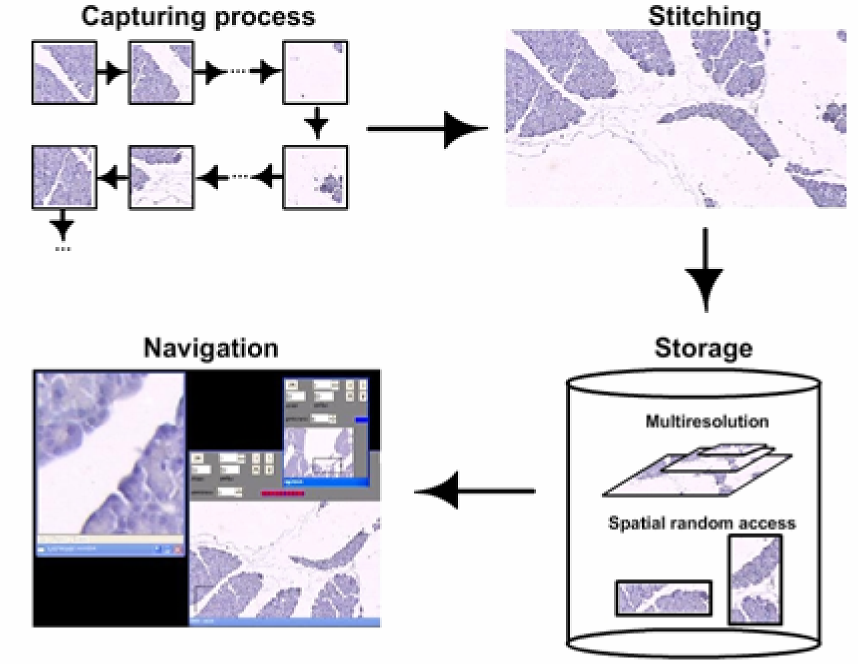

Fig. 1 The whole scheme illustrates the Virtual Microscopy process: First a sequence of microscopical FOV is assembled into a mega-image, which must be stored because of its large size. Finally, navigator must facilitate visualization of different regions of interest at different enlargements and with variable spatial displacements.

In conclusion, virtual microscopy requires a VMV which is able to integrate these three different processes. The main objective of this work is to give a brief introduction to some of the different strategies used in VMVs.

2. Stiching

A complete digitalization of histological slides is reached using a Whole-Slide-Imaging (WSI) device [14]. These systems must be capable of digitizing slides at any magnification and any desired resolution.

The whole system is usually composed of an optical microscopy system, an acquisition system and a software that controls the scan process, as illustrated in Fig. 2.

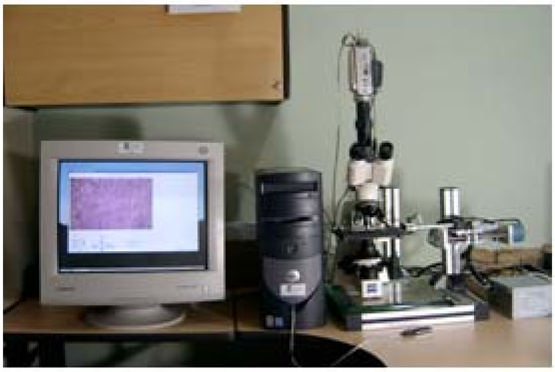

Fig. 2 A Whole-Slide-Imaging is basically composed of a conventional optical system to which a motorized device is somehow adapted for automatic control of the stage. Likewise, a digital imaging system allows capturing the observed microcopical FOV.

The acquisition system is constituted of a digital camera provided with a good charged coupled device (CCD) sensor, a motorized stage which is controlled by some electronic device and a high resolution monitor for visualization of the digitalized FOV. Acquisition of microscopical FOV is usually performed frame per frame, following a particular order in the slide i.e. most cases from upper left to the lower right corners (Figure 1), a frame of coordinates which is usually set on the computer screen for lower magnifications of the microscopical image to be captured. As a general rule, the capturing frame is overlaid with its neighbours in order to avoid possible information losses.

The output of this process is a set of images with overlapping frames, corresponding to the virtual slide. Although the digitalization process is simple, several sources of errors may come out in the process such as variable illumination conditions between different FOV, geometric deformations due to the radial camera distortion and aligning errors because of the microscope stage backlashes [15], resulting in variable seams between neighbouring FOV.

Adequate microscopic virtual reconstruction of a desired part of a biological sample is achieved using image registration and stitching. In the VM context, registration is the process of finding the amount of overlay between two neighbour frames by maximizing a particular similarity measure between them. Two kinds of similarity measures have been used in VM systems:

- **Area based methods.** These measures are based on the similarity of intensities between the two neighbour FOV, using their intersected regions. They are based on low-level image intrinsic properties and therefore they are very sensible to the type of noise.
    - **Sum of squared differences.** Thévenaz et al. have used the sum of squared differences as similarity measure in a VM system [16]. This measure has shown to be appropriate in many applications since it is simple and optimal under controlled conditions i.e. when differences between images are exclusively caused by Gaussian noise [17]. However, inter-image intensity variations are mostly linear in histological applications and constitute the major source of noise [10], together with the unavoidable biological variability and the technical difficulties of any histological procedure.
    - **Correlation.** In routine microscopy, illumination settings are controlled in such a way that most changes regarding intensities between neighbouring FOV can be modelled as linear [18]. Therefore, similarity measures based on correlation such as the normalized cross correlation or phase correlation [19,20], result more robust and become also more general. They are remarkably less sensible to noise than simple measures at the level of pixel differences such as the sum of squared differences and they are also robust to image displacement or rotations produced by microscope stage instabilities.
- **Feature based methods.** These approaches are based on the detection of salient features in the image intersection which can be used in a general manner [21].

- **Corners.** Sun et al. [22] have proposed a method which finds a set of corners in the overlapping region of each field of view, based on the Harris corner detector method [23], which is followed by a match of the corresponding features. In this case the similarity measure is the Euclidean distance between the corresponding features. Although the method is rapid, this is not general or robust since there is not any guarantee for the corners to exist in every microscopical image.

After a similarity measure has been set, the registering phase consists in finding the optimal transformation function which maximizes that similarity between neighbouring FOV. Notice that a large image must be generated by registering hundred or thousand FOV, whereby optimal registration schemes are required. A simple naive registration of one image with its neighbours can result expensive from a computational standpoint because of the number of needed registrations. An efficient strategy consists in registering a couple of neighbouring images, which then form a new image. This new image is then registered against a third consecutive image to construct a new image and so on along the selected row. The process is performed in parallel until rows of images constitute individual images which are then registered to generate the final mega-image. Rankov et al. [24] proposed to start at the centre of the digitalization framework and to follow a spiral-like pattern, under the hypothesis that the image at the centre contains the higher information. Appleton et al. [10] aligned simultaneously rows of FOV, while images associated to each row are firstly stitched into one single image using an efficient dynamic programming algorithm for solving the optimization problem.

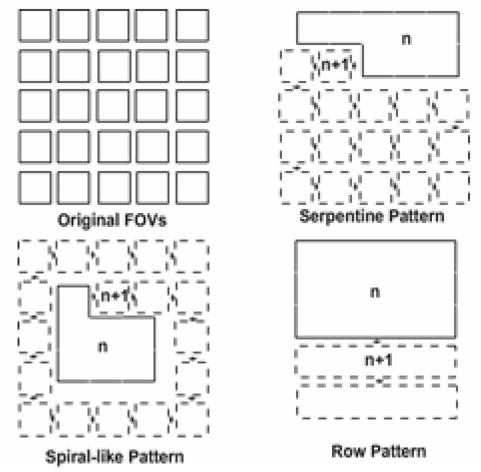

Fig. 3 A sequence of microscopical fields of view is assembled together into a single large image, as illustrated in upper left panel. Different stitching schemes are showed in the other panels: a serpentine pattern in the upper right panel, a spiral-like pattern in the lower left panel and a structural generation for which the large image is constructed by aligning rows of FOV.

Thévenaz [16] developed a method for refining a rough stitching, starting with an initial conventional stitching. During this phase, images are ordered after the intersection surface between them and then aligned following this order.

After an optimal displacement is found, it is quite frequent that visible seams persist between two neighbouring FOV. The stitching process must then correct the seam between two FOV by modifying the intensity values within the boundary of the overlap region. For so doing, Iregui et al. [18] proposed to apply a Gaussian filter on the two neighbouring rows of pixels to smooth them out. Rankov et al. [24] and Thévenaz et al. [16] calculated a weighted bilinear interpolation of the two overlaid images. A pixel value is calculated as an average of its values at both overlaid images, weighted by their distances to their closest image edge.

Correction of the illumination differences in different FOV is achieved in Sun [22] by weighting the intensity values with a second order polynomial which attempts to compensate the intensity differences between every pair of neighbouring images and approximate the corrected illumination to the mean intensity value. Although differences are corrected using this low pass filtering scheme, it is very difficult to ensure that also relevant information could not also be hidden.

3. Storage

A virtual slide is a high-resolution image, for instance a typical digitalization of a 1 cm^2 glass slide using 20× objective, results in 64 × 64 FOVs [18]. Provided that FOV are digitized to a resolution of 720 × 520 pixels, it is produced a reconstructed image of 45000 × 32000 pixels and 4.3 Gbytes. The demanded high-resolution of the virtual slide leads to several problems:

- **Large storage requirements.** Although the cost of devices for storage is lately falling while their capacity is increasing, the massive application of VM would require an unthinkable amount of storage. A specialized hospital produces between 100.000 - 500.000 histological slides every year [4]. The storage of only a 10% of these preparations, suppose the hospital can count on at least 50 Petabytes [4]. It is then mandatory to incorporate efficient compression techniques into the storage procedures.
- **Lossless compression.** Image compression can solve the problem of efficient storage, but it is important to take into account some particularities of Medical Images. Lossless compression techniques allow exact reconstruction of the original image and avoid annoying distortions introduced by the broadly used loss compression approaches. Overall, in virtual slides of pathology the lossless compression is preferred by several reasons: firstly, it is not easy to reach a consensus about an acceptable quality loss since this is based on exclusively subjective criteria. Second, loss compression can lead to legal disagreements as for instance a controversial diagnosis which could be based on artefacts produced by the compression, or in case of a malpractice suit, if only the compressed version of the image is available [12].

The storage problem for VM has been approached using two different strategies: uni-resolution and Multi-resolution formats.

3.1 Uni-resolution image storage

The virtual slide or mega-image is subdivided into smaller images called tiles [5] and each is stored in a classical format, such as JPEG, TIFF, or RAW [25,26]. All these sub-images or tiles are stored at the original virtual slide resolution. This virtual slide partition facilitates spatial random access to certain regions of the virtual slide. However, generation of an entire virtual slide thumbnail requires both access to every tile and data down sampling. Chang et al. [5] developed a uni-resolution VM system which runs in parallel architectures. This system spends between 1*s* - 8*s* to perform extraction of different WoIs (Window of Interest) at different resolutions. When the requested WoI intersects two or more tiles, the size of the initial tiles becomes definitive, i.e. the largest the selected tile the more time the system spends. Fontelo et al. [6] compared a real microscopy system with a uni-resolution VM system using surgical pathology specimens commonly encountered in a University Hospital. This work shows an 88 % agreement level when comparing both systems. However, this study reports low satisfaction rates when pathologists navigates using low band channels (modem channel – 56 kbps).

3.2 Multi-resolution formats

The multi-resolution formats [27] are characterized by access to different resolutions of the image which is compressed in a unique file.

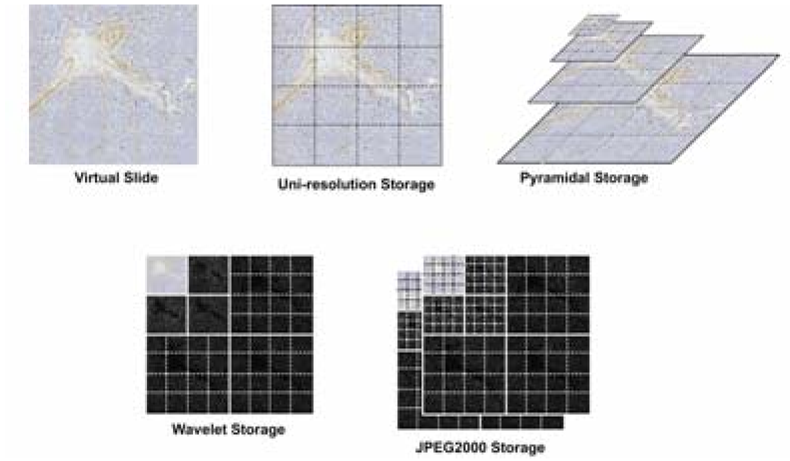

Fig. 4 Different storage strategies from the original virtual slide. Thick-line corresponds to the spatial subdivision into sub-images or tiles. The figure illustrates also different available storage formats. Uni-resolution allows only spatial random access. Pyramidal storage provides spatial random access at several magnifications, but multiple versions of the image are needed to be stored for reconstruction. Storage based on wavelets needs a unique version of the image for spatial random access at various magnifications. Finally, JPEG2000 uses all wavelet advantages and introduces the additional property of progression in quality.

Pyramidal formats. In these formats, the high resolution image is subdivided into spatial tiles which will be used for generating the whole image at different resolutions. After an initial tiling, image versions of multiple resolutions (different levels of enlargement) are obtained from each tile and each is stored in JPEG or TIFF formats, for lossy or lossless compression, respectively. FlashPix [28] is the typical example, this format provides spatial random access to the image data using the tile as the information unit and multiple zoom levels, including the thumbnail. However, storage requirements are very high since multiple versions of the high-resolution image are independently stored into the same file. This type of information management is not optimal in cases in which the requested region overlaps four tiles and therefore the four tiles must be decompressed. In this kind of applications, a compromise must be reached between the size of the tile and the amount of available information. Fred et al. [29] evaluated the utility of the VM as a complementary learning tool during a Cancer Workshop using pyramidal formats. Results show that VM is most effective when compared with traditional microscopy, but this study fails to present storage results. Mikula et al. [30] developed a VM system for display of high-resolution brain maps and atlases. The store strategy supports up to 35 terabytes of information using a standard lossless JPEG compression.

Wavelet based formats. Zhang et al. [31,32] used the Haar wavelet [33] for efficiently storing large resolution images. The proposed algorithm starts by separately processing each component of the RGB image and is as follows

1. Computes the average of 2 × 2 non-overlapping blocks
2. Differences between these averages and the original 2 × 2 blocks is calculated and stored
3. A new image with every calculated average is constructed
4. Repeat steps 1-2 on the new image of step 2 until the size of the low resolution version achieves a desired dimension.
5. Store the last low resolution version in a separated file.

The average values correspond to a low frequency version of the image while the differences keep information of details, that is to say, image high frequencies. Every frequency sub-band is stored in a separate file. Spatial random access is reached by splitting the larger high frequency files into several files, each corresponding to spatial blocks of wavelet coefficients. After the image is decorrelated using this wavelet transformation, every file is codified using a Huffman coder. Lossless compression is achieved using integer arithmetic. The Haar transform allows multiple enlargement versions of the mega-image from a unique representation. Besides, provided that wavelets are local operators, the use of the spatial sub-division of the coefficients guarantees a spatial random access. Yet this approach is suitable for many VM applications, a great limitation is that there is no a progressive quality representation which then results in a major drawback for image transmission and seamless navigations.

JPEG2000 (J2K). J2K [34,35] is a compression standard developed by the Joint Photographic Expert Group and is based on the Discrete Wavelet Transform (DWT) and the Embedded Block Coding with Optimised Truncation (EBCOT) [36]. The compression algorithm works in different complementary steps as shown in figure 5.

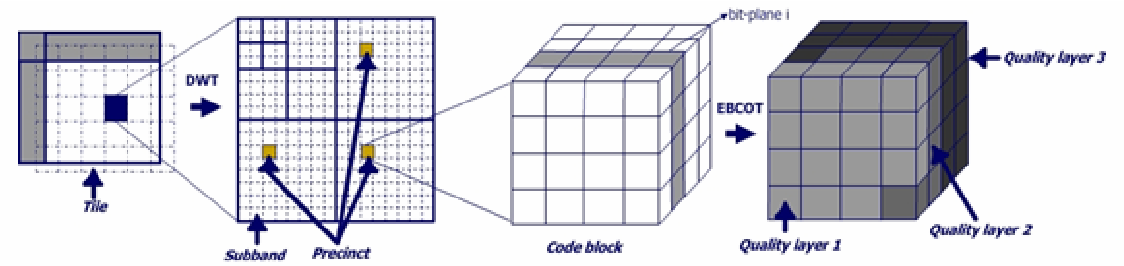

Fig. 5 Structure of J2K: The RGB image is decorrelated into its luminance and chrominance channels. Then each is decomposed into frequency bands, using a reversible Daubechies 5-3 wavelet. Finally, the embedded block coding with optimised truncation (EBCOT) optimally encodes data, allowing granular access by quality layers.

Previous to compression there is a pre-processing phase, which prepares the image for transformation. Firstly, the image is split into rectangular tiles or image regions, particularly useful for memory management. The sample values are then level shifted to make its value symmetric around zero and simplify implementation issues. After a level-shifting on the image values, each tile usually in RGB format is decorrelated into luminance and chrominance components (YUV) by means of a reversible or irreversible colour transform. Afterwards, a Discrete Wavelet Transform (DWT) decomposes the input signal into frequency bands called subbands (see Figure 5), providing the multi-resolution image representation. The bank of filters L and H splits the signals into two levels of resolutions, each with low and high frequencies. Two filters can be chosen for transformation: either the Daubechies 9-7 which is adopted for lossy compression or the reversible Daubechies 5-3 for lossless compression. After an optional quantisation, the next step is the entropy coding of each tile with the Embedded Block Coding with Optimised Truncation (EBCOT).

The DWT coefficients of each subband are subdivided spatially into small blocks called code-blocks. Each code-block is composed of bit-planes ordered by significance levels, obtained from the coefficient binary representation; these bit-planes are encoded in significance, magnitude and clean up coding passes, with an arithmetic encoder which provides the final compression: the so-called MQ coder [34]. The coding passes provide several truncation points of the bit-stream. The encoded code-blocks of each tile-component are then assigned to different quality layers by using a Post-Compression Rate-Distortion Optimisation (PSRD-opt [36]). In order to obtain a codestream with embedded information about resolution, region and quality layer, the algorithm introduces the precinct and packet concepts. A precinct can be defined as a block grouping from different sub-bands with the same resolution that is mapped to a specific region of the image (see Figure 5). A precinct from a specific tile, component, quality layer and resolution that appears in codestream units, is called a packet, which is finally the basic unit of the J2K codestream. The J2K structure is shown in Figure 4. Finally, the reconstruction problem can be defined in terms of packets. Respecting the constraints imposed by the standard, these packets are puzzle pieces that are dynamically used to attend navigation requests.

Wildermoth et al. [37] proposed a VM system with an extended depth of field, which for the registering phase uses a normalised cross correlation similarity measurement and J2K for storing. However, the least information units for decompression are the tiles, which correspond to the primary

J2K image partition that could not be optimal for navigation since they need to be large enough for avoiding distortion effects at low bit rates [35]. This study fails to exploit the minimal coding unit allowed by the standard: the precinct [38].

Iregui et al. [18] developed a VM system which allows progression by quality and uses strategies such as cache and pre-fetching for accelerating navigation. The storage uses J2K algorithm in mode lossless, while extraction time amounts to 500 ms in average for real pathology navigations, using the precinct as the least information unit. Additionally, this study reports that a correct tuning of the compressor parameters reduces the size of the file up to a 20 %.

4. Image Server

The image server is the software responsible for efficiently accessing the image from the storage data. Since the ultimate goal of the VM is to emulate a real microscope, a necessary condition is that the system guarantees low latency response times. .

Transmission over low band channels. In VM it is reasonable a storage of the virtual slide in a high capacity server for virtual slide access from light clients, using low band channels. Simulation of a real microscope in this scenario requires a full dynamic interaction between clients and server. A usual solution consists in packing at the server side progressive incremental quality versions of the image which are sequentially fetched to the client for display [18]. However, it is crucial to devise strategies for efficient generation of these multiple versions which should be incrementally handled.

Saltz [4] proposed a general image server for multidimensional datasets that runs in parallel architectures. Each image pixel is associated with a point in a multidimensional space. As mentioned before (section 3), image data are subdivided into tiles which are distributed across different machines for compression. In this system, a usual query is actually a range query i.e. multidimensional box. A particular region of interest is either a tile so that the system returns this tile, or a region touching multiple tiles, case in which all these tiles are returned within the same query. A rapid retrieval of tiles is reached using an index file, which contains information about geometrical bounds of the tiles and machine addresses. However, after any tile is available the server must still perform additional operations such as decompression, clipping and sub-sampling. Finally, after the query has been attended data must also be assembled together and sent back to the client.

The proposed architecture permits a parallel access to the disk, remarkably reducing the whole processing time, in function of the number of machines. Its main drawback comes also from this fact, most health institutions or real pathology laboratories could very hardly count on dedicated parallel architectures for such task.

Gómez et al. [39] show that J2K defines a new image space with five dimensions: colour component, two positions, resolution and quality. This representation permits to directly obtain the required image versions in VM. This work also proposed a use of an index for faster localization of the minimal storage units.

5. Navigator

Even in the larger monitor screens it is impossible a complete display of the virtual slide since its resolution is much higher than typical resolutions supported by conventional display devices, below 2000 × 2000 pixels. In consequence, it is essential a design of methods for efficient access to the image data regarding the different dimensions of the problem: spatial displacements and enlargement representations.

A Graphic User Interface (GUI) in a VM system should emulate a microscopical examination performed by an expert in a real microscope. In general, this design should exploit the importance of low magnification for exploration and high enlargements for navigation. Typically, the navigator consists of two windows: a thumbnail version of the mega-image, in which a re-sizable window is displaced and used to define a particular WoI. Several approaches have been introduced in VM systems. A rough approximation has been to bring together this WoI into the whole system by a second re-sizable window with the original resolution [4]. Iregui et al. [18] proposed the use of a second window for displaying intermediate resolutions, while higher resolutions are displayed in a third window. This WoI can be configured by size, resolution and quality [5,18]. The expert can then request a rectangular region of interest at any resolution and any quality while the WoI can be displaced for exploration.

Mikula [40] proposed an interface for faster visualization of stacks of virtual slides by pyramidal resolution representations which are stored using a quadtree structure [41]. Quadrants are related by their parent tile. This method displays with higher resolution the quadtree nodes whose pixels are closest to the screen.

6. Acceleration of navigation

Spatial locations, decompression and visualization unavoidably introduce considerable response delays, which make impossible interactive and fluid navigations [14]. Strategies such as the cache or the prefetching have been developed for decreasing the latency times and therefore to permit a fluid navigation. Cache is a rapid access to a space of memory in which it is stored the portions of the image that shall be visited in the future [42]. Prefetching is the anticipated uploading of those parts of the mega-image to which the navigation will be addressed in the future [43]. Those techniques have shown to highly improve navigation times [44].

Spatial cache [18] is a reserved part of memory, which is set to store visited pixels. When a WoI is requested, the algorithm calculates the intersection between what is stored in the cache and what the WoI is demanding, and this intersection is displayed directly from the cache. In zoom-in operations, Catalayeuk [5] used information of the thumbnail for a temporal display while the rest of data are being loaded. Iregui et al. [18] used the multi-resolution nature of the DWT while the cache was dynamically constructed by storing the wavelet coefficients of lower resolutions, which had been already visited. This strategy is a soft cache [45], which maintains image wavelets coefficients of low resolution versions in memory for reusing them to construct high resolution versions of the image [45]. Using these two strategies, navigation velocities grows up to a 30 %.

7. Future Works

VM is an incipient area with multiple open problems and a great variety of applications. So far the state-of-the-art technology has allowed development of useful prototypes with some critical limitations, which have restricted a broader use. Future navigators would require faster registering methods for handling the huge amount of data generated from each particular application. These navigators also require flexible storage methodologies with optimal compromise between a quantity of stored data and image processing for reconstructing requested pieces of images. Acceleration of the navigation turns out to be a critical factor in seamless navigation either when images are locally stored or must be remotely accessed from a local client.

VM can not be a real option until it is fully reliable, efficient and easy to use when accessing sets of mega-images. Main open problems which need new approaches are a more flexible access to image data, efficient indexing of data for rapid and opportune retrieval, compression strategies able to adapt to this kind of applications and seamless navigation methods. J2K has introduced a preliminary step towards novel navigation proposals; the paradigm has been changed into a fast, easy and optimal access to the image data rather than simple compression policies. New techniques must easily allow adaptation of interfaces for the use of experts and analysis of the image contents for selective compression or selection of a particular multi-dimensional representation which can be set after a matching pursuit [46] assessment. These new approaches must also include decompression parameters which must permit integral tunings for increasing benefit of information packets from quality and utility standpoints. Furthermore, in cases in which VM is used in server-client systems, it is fundamental to count on optimal strategies for managing information when the band channel is narrow as it will always be when one considers the amount of data that may be generated. Cache and prefetching have shown to be efficient strategies for accelerating navigations in VM, but more sophisticated methods are nevertheless needed for reaching real time performances in those navigations.

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