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Virtual microscopy and digital cytology: state of the art

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Summary. The paper approaches a new technological scenario relevant for the introduction of the digital cytology (D-CYT) in the health service. A detailed analysis of the state of the art on the status of the introduction of D-CYT in the hospital and more in general in the dispersed territory has been conducted. The analysis was conducted in a form of review and was arranged into two parts: the first part focused on the technological tools needed to carry out a successful service (client server architectures, e-learning, quality assurance issues); the second part focused on issues oriented to help the introduction and evaluation of the technology (specific training in D-CYT, health technology assessment in-routine application, data format standards and picture archiving computerized systems (PACS) implementation, image quality assessment, strategies of navigation, 3D-virtual-reality potentialities). The work enlightens future scenarios of actions relevant for the introduction of the technology.

Key words: telemedicine, digital cytology, virtual-microscopy.

Riassunto *(Microscopia virtuale e citologia digitale: stato dell'arte)*. Il lavoro affronta il nuovo scenario tecnologico relativo all'introduzione della citopatologia digitale nell'ospedale. È stata condotta un'analisi dettagliata sullo stato dell'arte relativo all'introduzione della tecnologia negli ospedali e nei territori disagiati. L'analisi condotta in forma di revisione scientifica è stata organizzata in due parti: la prima parte ha preso in considerazione gli elementi di successo della tecnologia; (le architetture client-server, l'e-learning e la citologia digitale; il controllo di qualità e la citologia digitale); la seconda parte ha preso in considerazione gli elementi di insuccesso e quelli necessari di approfondimento della tecnologia (corsi specifici in citologia digitale, *health technology assessment* e citologia digitale, studi in applicazioni di routine in citologia digitale, standards e PACS in citologia digitale, controllo di qualità nelle immagini e studi sulle strategie di navigazione in citologia digitale, applicazioni della realtà virtuale 3D alla citologia digitale). Da un punto di vista globale il lavoro evidenzia gli scenari futuri di azioni relative all'introduzione della tecnologia.

Parole chiave: telemedicina, citologia digitale, microscopia virtuale.

INTRODUCTION

The virtual microscopy is an alternative solution between the static and dynamic tele-pathology.

Telemedicine applied to pathology (T-P) is considered a valid aid to pathologists [1, 2, 3-13]; in fact it is supposed to allow the remote exchanging of information about a tissue on glass and in particular:

1) the tele-diagnosis;
2) the audit of complex cases, by means of shared virtual desktop;
3) the minimization of resources (more hospitals could share the professionals).

There are two basic methods of T-P. *Static* T-P consists in the capture and digitalization of images selected by a pathologist or pathologist assistant, which are then transmitted through electronic means to a tele-pathologist. *Dynamic* T-P consists in the direct communication between two different centres by using microscopes equipped with a telerobotic system oriented to explore the glass, remotely operated by the tele-pathologist who makes the diagnosis.

As an alternative solution between the two methods, widely increasing today is the diffusion of virtual microscopy (VM) [14, 15]. The latter does not refer to the tele-control of microscopes, whilst the glass is scanned as a whole, producing a "virtual glass", and a pathologist can navigate remotely (via internet) inside this virtual glass or virtual slide in a manner similar to a real microscope [16-18].

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The latter application is not a static T-P function because there is not the exchange of a few snapshots, while what is exchanged is represented by hundreds of digital-images representing the virtual slide.

Moreover it is not a dynamic T-P application because there is not the microscope and the tele-control functions, however the tele-pathologist can navigate inside the virtual slide emulating the manual microscope. Many pathologists consider this approach as dynamic T-P, however there is not a complete agreement about this. Some authors, such as Molnar *et al*. [19], prefer to consider the T-P based on virtual slide a new scenario and thus virtual microscopy represents a new frontier of T-P taking advantages from the previously used static T-P and dynamic T-P [19].

PAST AND PRESENT SCENARIO: TELE-PATHOLOGY

Up to a few years ago the management of the information on the glasses (virtual slide) in tele-pathology applications was principally based on the design and construction of a few identical and expensive platforms with microscope units and software tools for both the display and the tele-control (zooming, moving cutting of pieces of images). In the first applications of these methodologies the latency of information during the transmission caused displacement errors in the positioning of the mechanics of the microscope unit on the glass. For those reasons the need to investigate solutions based on the so called virtual microscopy with virtual navigation on scanned images without using tele-control provided new studies [16-18]. Severe problems were also noticed for example in the remote information exchange. The lack of both a wide-band channels and an *ad hoc* visualization strategy strongly delayed the image transmission. It has to be considered that a single file representing a virtual glass for cytology applications could reach several tens of gigabytes, more than in the case of applications of tele-echography [20]. The design of an appropriate visualization strategy is thus a basic core aspect. Clearly it is not feasible and reasonable to fully manage a single file of several GBs in the WAN.

Today the scenario is completely changed thanks to the introduction of the VM. The principal changes in the world of the information technologies affecting T-P were the following:

1. availability of wide band channels;
2. diffusion of new visualization strategy;
3. availability of new image scanners;
4. availability of free visualization software.

The first point was driven by the diffusion of the information communication technologies (ICT) for internet / intranet / extranet connections. The second point was driven by the pressing request of very large image exchanges by internet. New methodologies today allow the archiving of an image arranged in layers assigned to different magnification factors [15], as for example in Google Earth [15], and allow a remote information exchange using a reasonable wide-band-channel. The third point was driven by the exceptional changes in photonics applied to medicine. Many producers, leaders in photonics, are using their skill to design scanners for virtual microscopy [21]. The fourth point was driven by the diffusion of free web-viewers and by new commercial strategies of the producers of tele-pathology systems [22].

VIRTUAL MICROSCOPY AND DIGITAL CYTOLOGY

In order to face deeply the introduction of the use of digital cytology in the VS we have to make the following basic considerations.

The tele-pathology mainly relates to the world of histology; the histologist navigates the slide without the need to use the focus function. The virtual slide in histology does not require the emulation of the focus function; in histology it is sufficient a flattened vision, in other words the histologist does not need to "smash" in the sample.

When coming to the digital cytology which relates to the world of cytologist, basic problems arise. At first, the cytologist uses a completely different way to navigate from the histologist: cytologist in fact makes a wide use of the focus function. Then, we should consider that the cytologist on the contrary to the histologist, while watching the images uses lateral area of the eye (the same used by the primitives to avoid animals attack); as a consequence, a third aspect arises, *i.e.* the stereo-vision is much more important for the cytologist. The focus function can be emulated by software, by means of specific functions using also interpolation algorithms not opened to the user. This implies that at a defined zoom several focal plans are captured (up to 100) of a sample (depending on the thickness of the sample and the chosen level of magnification). This leads to the generation of very large virtual slides during the digitization, and the focus function at a defined zoom should be emulated by generating different images ready for interpolation; in the VM this functionality is called by manufacturers "Z-stack". This implies the generation of virtual slides many times larger than in the case of pathology [23, 24]. Furthermore the exigencies of the cytologists are different from the ones of the pathologist when interacting with the VM.

METHODOLOGIES

Taking into account the aim of introducing the technology D-CYT in the hospital and more in general in the dispersed territory, a review of strengths and weaknesses was arranged into two parts:

a) the first part focused on the strengths of this technology, which have been individuated in the following issues:

- client server architecures and digital cytology;
- e-learning assisting the diffusion of digital cytology;
- quality assurance and digital cytology.

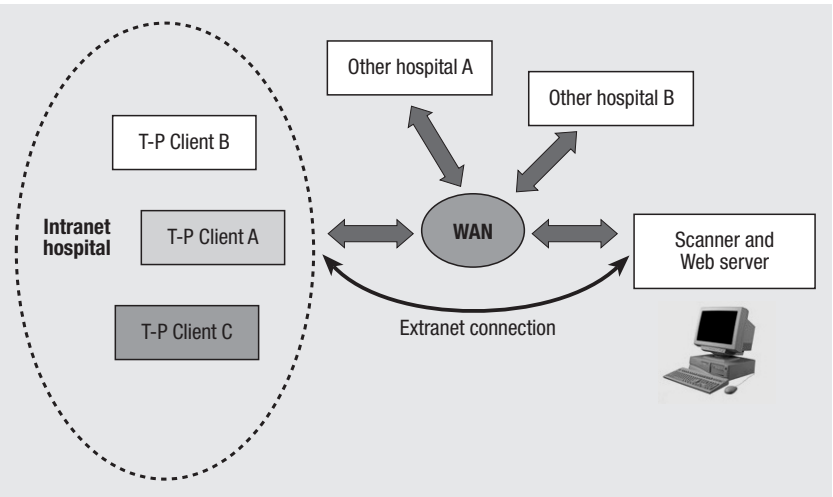

Fig. 1A | *First stage of changes in the D-CYT introduction. The different T-P clients of the hospital can access, via an extranet connection, a scanner for creating virtual slides and a web-server for storing the virtual slides; and can navigate on the virtual glasses with a resident low-cost software (scenario with an external server).*

b) the second part focused on the weaknesses of the technology to be investigated, individuated in the following items:
   - specific courses in digital cytology;
   - health technology assessment and digital cy tology;
   - investigation in routine applications of digital cytology;
   - standards and PACS in digital cytology;
   - image quality and studies on strategies of navigation in digital cytology;
   - applications of the 3d potentialities to the digital cytology.

In all the figures D-CYT represents a novelty with respect to T-P, thus we report at first T-P as a first stage of implementation, while D-CYT represents a wider and ultimately the final step of application.

RESULTS AND DISCUSSION

Strenghts

Client server architecures and digital cytology

In a typical today's architecture there is an on site server with the scanner or alternatively a telepathology third party server (in site or in remote as a Web service such as the Leeds' centre [14] (www.virtualpathology.leeds.ac.uk/index.php) or the Milestone centre (www.onlinetelepatologia.com/contacts.html [15]), for the creation of the virtual glasses and low cost (or free) *Light-client applications* proprietary software tools (PC-client application not asking enough hard disk space, not consuming dynamically PC resources, such as RAM, SWAP etc., simple and user-friendly) for the navigation on the virtual glass, which can be installed in remote clients [25, 22, 15]. This new methodology, especially the one based on a third party server is rapidly spreading and it is becoming the basic aspect of the training for future qualified personnel. Today there are different manufacturers focusing on D-CYT. Different solutions for D-CYT are available and are based on different equipments such as, for a not exhaustive example [25]: ZEISS (www.zeiss.it), NIKON (www.nikon.it), OLYMPUS (www.olympus.it), HAMAMATSU (www.sales.hamamatsu.com),

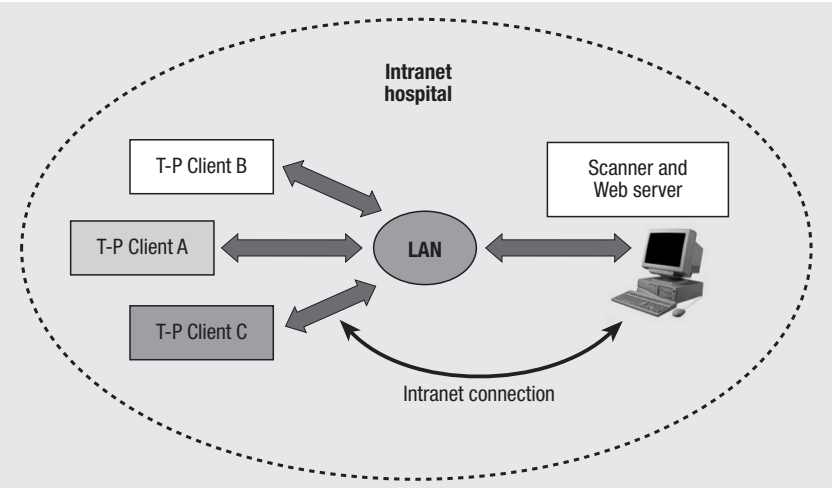

Fig. 1B | *Second stage of changes in the D-CYT introduction. The different T-P clients of the hospital can access, via an intranet connection, an on-site scanner for creating virtual slides and an intranet web-server for storing the virtual slides and can navigate on the virtual glasses with a resident low-cost software (scenario with an internal server: the server is in the hospital).*

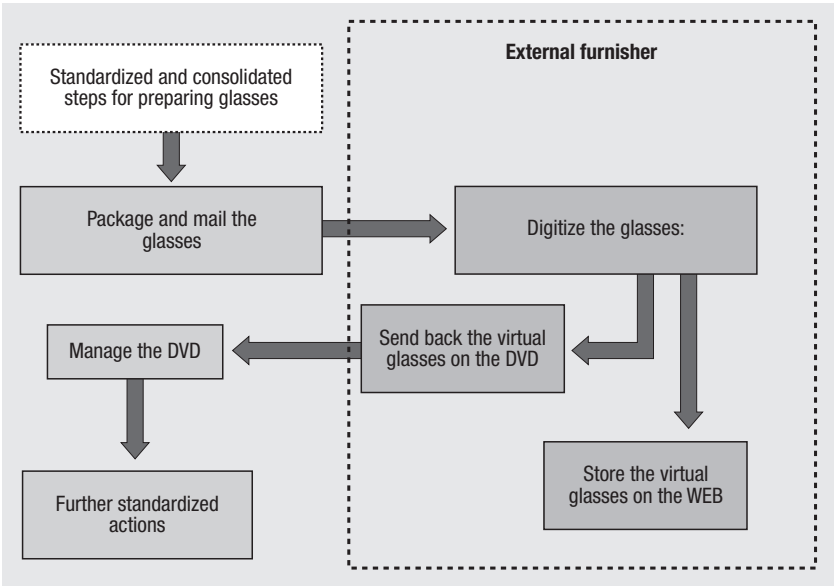

Fig. 2A | *Workflow relevant to the first stage of introduction of D-CYT in the hospital.*

APERIO (www.aperio.com), LEICA (www.leica-microsystems.com). The employment of D-CYT architectures in the hospital using one of the above equipment, implies the realization of:

1a) a remote Web-server such as the Leeds' centre [14] or alternatively; 1b) an on site server with the scanner for the creation of the virtual glasses;

2) a low cost (or free) Light proprietary software tool for the navigation on the virtual glass which can be installed in the D-CYT clients [2, 22] such as the image tool of the Aperio.

The introduction of digital cytology would come in two stages:

- the first stage of progressive introduction of the D-CYT in the hospital is the introduction in the laboratory workflow, of the tools/systems itemized as (1a) and (2). This is allowing a soft introduction of the virtual glass by D-CYT as a support methodology to well standardized procedures based on the actual glass *(see Figures 1A, 2A)*;
- the second stage of progressive inclusion of the D-CYT in the hospital, is the introduction of a dedicated internal scanner for digitalization by using an internet server, which, starting from the glasses, returns virtual glasses. The workflow in the laboratory thus, at this stage, implies the use of the tools itemized as (1b and 2) *(Figures 1B, 2B)*.

E-learning and digital cytology

The virtual slides archived in a server (remote or on-site) allow the tele-consultation and trial potentialities (e-learning). As a basic reference of tele-consultation in the field it can be proposed the solution offered by the web-site of Leeds (www.virtualpathology.leeds.ac.uk/index.php) based on the Aperio technology. One can access to the web for example and see the digital slides relevant to glasses sent for scanning; furthermore here one can find a virtual library of tissues and interesting cases. Without downloading any software, one can navigate by means of the "web viewer" SW, select a region and send the relevant internet path (and not then the file) to a colleague for the discussion and/or second opinion. In a previous paper [25] we have described two pilot studies performed respectively in the course for techni-

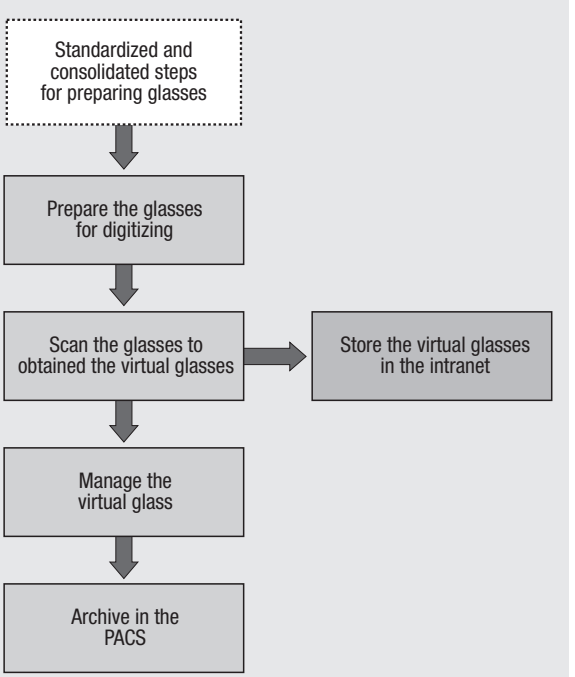

Fig. 2B | *Workflow relevant to the second stage of introduction of the D-CYT in the hospital.*

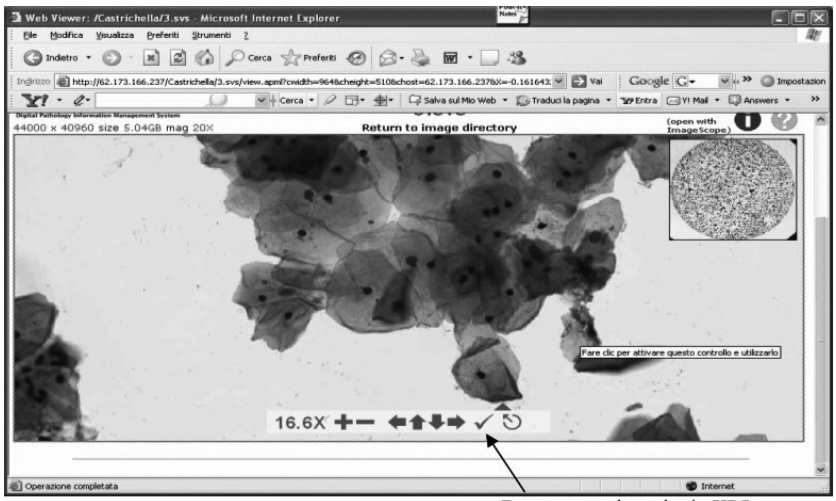

Fig. 3 | *The way to exchange information towards the internet using spectrum web-viewer.*

cians in Biomedical Laboratory and Master Degree based on the Aperio equipments and showing that a simple Microsoft Word table reporting paths (URL) (internet pointers) relevant to regions selected by a teacher could allow a methodology for e-learning in digital cytology *(Figure 3)*.

Quality assurance and digital cytology

The utility of the use of virtual slide as a mean to make quality control has been considered from the experience with robotic tele-microscopy applications, as it has been enlightened by Demichelis *et al.* [26], who showed that the technology permitted a new, computer-based approach to proficiency testing in histopathology and cytology indicating that the use of virtual slides encouraged the diffusion of national quality assurance programmes, which suffered from certain organizational and limitations in logistics. The use of also automated digital systems for future recognition such as PapNET (Neuromedical Systems, Suffern, NY) for computer-assisted cervical/vaginal cytology diagnosis has been considered useful for the quality control [27]. Also other systems such as Tripath or Hologic [27] have been succesfully used in the field of feature recognition and quality control. All these three systems left directly to the machine the decision on the basis of internal algorithms based on the artificial intelligence. The use of virtual microscopy in cytology for quality control and assurance today is no more considered an idea or a dream, while it has been shown to be useful and feasible. Computer tools conjugated with the power of Internet allow in fact the detection and the control of the uniformity of the diagnosis [23]; specific societies, such as the LabQuality (www.labquality.fi) furthermore provides services of quality control/assurance towards the means of virtual microscopy. Important regional health plans are today designed with the consideration that the virtual microscopy plays an important role in the regional screenings, as it has been enlightened by Bondi A [28]. Manufacturers are thus today adapting their instrumentation for the quality control and assurance. As a result of these new trends, manufacturers are focusing on these problems and designing products embedding functionalities typical of the quality assurance.

Issues to deepen

Specific courses in digital cytology

At the end of the complete introduction of the D-CYT in the hospital, new features should be added to the PACS for cytology, similar to those ones daily executed by a radiologist for archiving CT or NMR exams in the PACS for radiology.

The disciplines facing the digital cytology are the following:

1. histology;
2. pathologic anatomy;
3. cytology;
4. informatics (1st; 2nd; 3rd year).

In [25] a revision of courses in the case of the Degree for Technicians in Biomedical Laboratory was proposed. This should have impact at different levels on the study degrees at the university.

Health technology assessment and digital cytology

As an important aspect in the introduction of the new technologies in the hospital, specific studies of health technology assessment (HTA) focused on the new technology for the digital cytology should be performed. The problem to investigate the new technologies is an important aspect in technology assessment (TA). In the previous works [20, 35] a preliminary purpose TA for telemedicine was proposed. This TA was tested on seven products comprehending a dynamic application of T-P developed at the beginning of 2000. The new technologies dedicated to the D-CYT, now available on the *virtual slides*, have radically changed the scenario, increasing the need

to develop a specific HTA centred on the pathologist. Different authors have investigated separately some specific aspects relevant to HTA in T-P based on virtual slides [19, 30, 31]. In [32] some of these authors have reviewed the previous studies facing the single aspects of HTA and proposed a preliminary methodology to approach HTA in T-P. Starting from the outcomes in [32] there is thus the need to widen the approach of the HTA; considering also:

1. further issues relevant to studies of HTA, as from specific experience of mondial networks of HTA such as the EUNETHTA (www.eunethta.net/);
2. integrate specific studies on HTA over the NET, as the focus is the digital cytology is the communication over the WAN/LAN.

Investigation in routine applications of digital cytology

One of the issues to investigate is the use of the digital cytology in routine applications in the hospitals, such as the acceptance of the users and the performance of the technology. We have thus tested the methodology in an application important for impact in the health care system (pap-test). The detailed study on the diagnostic accuracy and time of diagnosis (core aspect for the routine introduction) is reported in the monography [33].

Standards and PACS in digital cytology

The fourth aspect to deepen, fundamental for the introduction of the technology is represented by the digital archiving of the images. The hospital tool used to archive the images is the so called PACS. Digital radiology has reached a high level of standardization, thanks also to the Medical Devices EU Directive Compliance; all the manufacturers use the DICOM standard protocol to exchange information. Unfortunately in tele-cytology there isn't an available standard; all the herein analyzed products have designed a proprietary PACS. Furthermore in digital cytology the very large memory occupancy of the virtual-slide strongly hampers this process as enlightened by Jorma *et al.* [23]. In the companion paper the problems of standardization of the digital cytology versus the PACS standardization was investigated [33].

Image quality and studies on strategies of navigation in digital cytology

As it is well known the cytologist navigates the standard glass in a particular manner: he or she uses the lateral side of the eye, the same used by the primitive men to avoid animals' attack. This represents a great differentiation of the way to inspect the glass between the histologist and the cytologist. This means that the new technology based on a monitor and no more on a microscope is expected to change this way to face the glass of the cytologist. Probably young cytologists will have a new way to explore the glass and the senior-expert cytologist will need severe changes. A core aspect is obviously the image quality not only from a static point of view, but also from a dynamic point of view. In fact the decision to stop a navigation and focus to a detail is a function of both the quality during the navigation and the "in movement". Two are thus the basic questions to investigate:

a) the use of technical solutions to record the "dynamics" of image during the navigation and thus the trajectories/strategies of navigation;
b) the use of automatic tools for the investigation of the image quality during the navigation using the virtual microscope.

Figure 4 shows a possible set-up to record the trials of navigating the virtual glass.

One of the tools that could be useful for the investigation of the image quality during the navigation is the virtual quality metric (VQM) [34-37]. This tool has been successfully investigated in digital-echography from some of the authors [34-41]. The advantage of the tool is that it is completely objective, but however emulates the subjective perception. *Figure 5* depicts a possible flow for the image quality assessment based on the VQM [38-41]. The videoclip is generated using the process described in *Figure 4*.

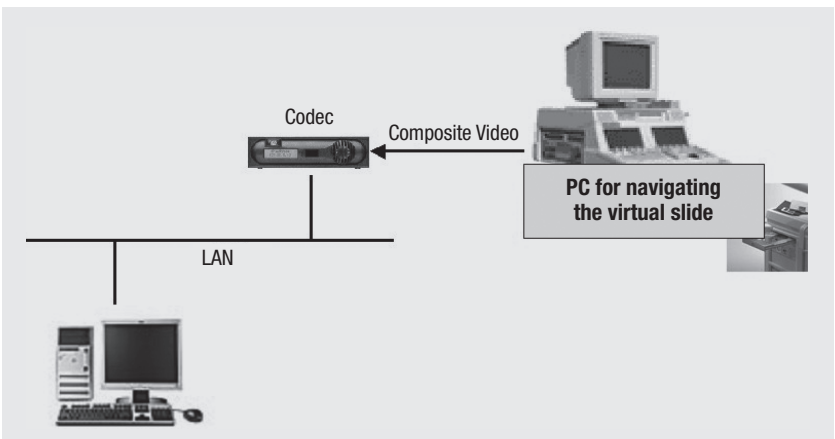

Fig. 4 | *Possible set-up designed to record the trials of navigating the virtual slide.*

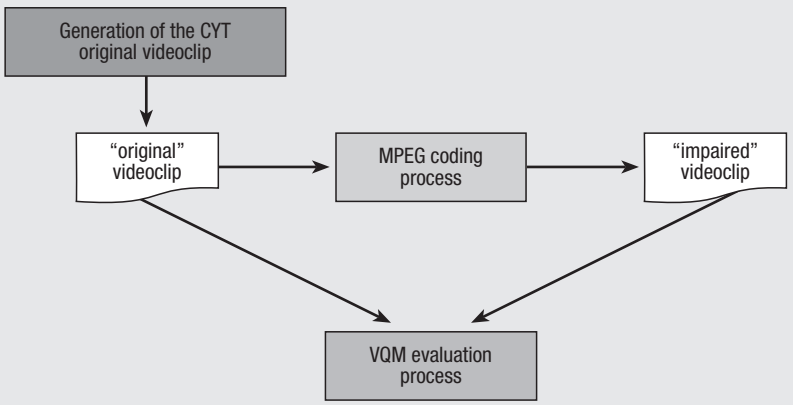

Fig. 5 | *The flow of the video quality assessment process in digital cytology.*

Applications of the 3D potentialities to the digital cytology

Different studies have been proposed to approach the introduction of the potentialities of 3D / holography

techniques in cytology vision [42-47]. However these studies have only dealt with the local diagnosis conducted in stand-alone equipments and have not been conducted over the WAN/LAN in telemedicine applications, apart for cardiovascular purpose (the specific targeted research project Collaborative Holographic Environments for Networked Tasks, COHERENT). The use of these techniques in consideration to the importance of the stereovision of the cytologist could be of aid both to emulate the stereovision thanks to the use of 3D / holography techniques and minimize the data-transferring among remote nodes as a partial solution for the area occupancy caused by the Z-stack.

CONCLUSIONS

Today thanks to the development of the information technology, the diffusion of new visualization strategies and the availability of low cost or free visualization proprietary tools, the scenario of the telepathology has radically changed: the VM offers new promising opportunities oriented to the digital cytology application. This study has reviewed the issues relevant to the promotion of the digital cytology with particular care to the points of success and weakness.

The paper proposes two lines of action relevant to digital cytology application; the two correlated directions are:

- research, proposing to investigators new fields of interest needing strong scientific efforts;
- funding institutional bodies, proposing to the so called "stake-holders" choices and decisions relevant to the destination of funds to be directed towards universities and health care systems for the necessary changes to investigate and/or include the digital cytology in the public administration procedures.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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