Review Article

Digital microscopy: A routine mandate in future? A leaf out of Covid-19 pandemic laboratory experience

Huchanahalli Sheshanna Sreeshyla[1], Hegde Usha[1], Priyanka Nitin[1], Sowmya SV[2], Dominic Augustine[3], Vanishri C Haragannavar[3]

[1]Department of Oral Pathology and Microbiology, JSS Dental College and Hospital, A Constituent College of JSS AHER, Mysuru, Karnataka, [2]Department of Oral Pathology and Microbiology, Head of Oral Cancer Research Centre, [3]Department of Oral Pathology and Microbiology, Faculty of Dental Sciences, MS Ramaiah University of Applied Sciences, Bengaluru, India

Abstract

The COVID-19 pandemic has brought out lot of changes among the way people and organisations function. It has also reduced social gatherings and hence social relations considerably, forcing people to adjust to new ways of work and life. An outstanding difference between the current COVID-19 pandemic and previous epidemics or pandemics is the increased availability and use of technology currently, which has been validated by various reports from across the globe. Thus, even with the ensuing pandemic, lockdown and decreased social gatherings, with the technology support we have devised ways to keep in contact with friends, family and work place, so as to continue our lives. Social distancing guidelines and regulations have put pressure on a great many organisations to find new ways of keeping employees and students connected while working remotely. For more deskbound occupations and roles, this can be relatively straightforward, but it is challenging if not impossible for lab-based quality control, research and study. The answer to this is digital remote microscopy which enables sharing of data online, carrying out collaborative work through multi-viewing in real time and facilitates remote training functions.

Keywords: Covid-19, digital microscope, laboratory, microscope, pathology, technology

Address for correspondence: Dr. Usha Hegde, Department of Oral Pathology and Microbiology, JSS Dental College and Hospital, A Constituent College of JSS AHER, Mysuru, Karnataka, India.
E-mail: drushahegde@gmail.com

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INTRODUCTION

Microscopes are invaluable tools based on the phenomenon of magnification. With the help of microscopes, it is possible to view and study very minute objects across varying fields. They are available from small pocket units to full-size models. Way back in the 13th century, the first pair of corrective eyeglasses were invented, and since then there has been manifold development in lens technology, that resulted in the first compound microscope three centuries later. With many inventions and developments, microscopy has emerged as a separate field of research. The advancements in digital technology have been applied to microscopy too, resulting in evolution of digital microscopes (DM). These have become mandate tools during the current pandemic situation.[1] In a regular optical analogue microscope, the specimen to be studied is resized to the required magnification through the various lens system. However, in a digital microscope, the same can be achieved by incorporating the pixel resolutions, software to alter the size of image and adding various

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physical dimensions to the computer and the monitor. By adding a hardware to the existing analogue microscope and connecting it to a computer having a digital image processing software, it is possible to convert it into a digital microscope.[2] Digital microscopes are extremely useful instruments for laboratory testing, but they also have a broad range of quality control and quality assurance applications across different sectors and industries.[3]

With the help of technology, it is possible carry out remote microscopy without any compromise in its capabilities. Concurrence in reports between different forms of digital pathology and conventional light microscopy has been reported. In remote or digital pathology, the virtual images are transmitted to far off locations for opinions and diagnosis.[4] Traditional methods of remote microscopy rely on placing cameras on top of microscopes and offering a digital view of slides. In the simplest form, the eyepieces in a conventional microscope can be equipped with a camera, which can be connected to a computer with appropriate image analysis software and feedback information can be saved.[3] In the modern DM, there is a digital camera which acts as a detector instead of an eyepiece, an image output device and a computer monitor that acts as a display screen. The dependency on the human optic access is eliminated in a DM as an inbuilt LED-source acts a light source unlike that in an optical microscope where it is through the eyepiece from a source outside the microscope.[5]

The credit of developing theoretical knowledge regarding image analysis and its various applications goes primarily to Professor Peter H. Bartels along with few others. They contributed immensely to the development of DM.[6] They used multi-megapixel arrays for recording and merging process so that the viewer could evaluate tissue features by looking at these arrays, what is called presently called as the virtual slide. They also added machine vision and case-based reasoning, the current basis of artificial intelligence.[7] Way back in 1986, the first digital Microscope known as Hirox Co. Ltd. was manufactured in Tokyo. It comprised of a control box and a lens connected to a camera. The computer connection in this DM enabled it to handle enormous digital data that were procured from the digitalized camera lodged within the microscope. Further, a more improved DM that has an in-built unit constituting a monitor and a computer negating the need of an external computer connection was developed in 2005. In 2015, DM with USB connection to the external computer connections were developed. This brought about an increase in the speed and longevity of the computer. It also reduced the computer size and the external cable connections. Addition of an image processing software facilitated the adjustments in image brightness, contrast, magnification and thus produce high-quality images. It is easy to use, has image storage, and added image features such as cropping and adding scale.[5]

An ideal digital pathology platform for teaching undergraduate and graduate medical student is whole slide imaging (WSI).[8-11] WSI has the advantages of storing teaching materials, case series and isolated cases to improve scholastic teaching. Monitoring of fresh skills acquired such as stain interpretation and assessment of competency through exploration of slides can also be carried out.[10] However, there is a huge upfront cost involved in the establishment of WSI technology and storage requirements associated with it. Therefore, a more plausible solution would be to shift toward dynamic virtual microscopy (DVM) wherein a light microscope with a mounted digital camera, and videoconferencing software to stream a slide image to the learner(s) with a computer, is all that is required.[12]

Principle of digital microscope

The hardware and software tools in a Digital Microscope empower it and make it possible to view the captured image at a distant site. Both still and motion videos can be visualized and is dependent on the software that is used. Further, the images can also be recorded, edited, cropped, labelled and saved. Additionally, the software can measure, magnify and modify the captured images in several ways.[5]

The digital image processor in a digital computer makes the transformation of images into digital forms possible. It can either improve the quality of images for interpretation by humans or alter the images for machine perception.[13,14] By understanding the attributes or traits of the image, recognition and analysis of the digital image is carried out which is based either on the spatial domain (colour, area, perimeter, compactness, circumference, edge direction, strength and contours) or on frequency domain (mean, standard deviation and histogram).[14-21]

A DM can either be used in a simple or normal method. In simple mode, also known as the plug and play mode, there is no use of the software, but a video source will continuously display the objects that are captured in the microscope. In Normal method of testing, the display of the captured image is continuously viewed on a computer monitor which has an installed software. The advantage of the normal method versus the simple method is that it offers a wide range of resolution.[2]

PARTS OF A DIGITAL MICROSCOPE

The digital Microscope is made up of two important parts—the hardware and the software, based on its input and output functioning. The hardware consists of the microscope with a light source—the analogue microscope, camera with its components and computer monitor screen. Here the eyepiece of the traditional microscope is replaced by the camera, where the image from the specimen gets focused. The focused image is displayed on the computer monitor screen. The displayed image will be further stored and processed. The camera drivers and the image processing units constitute the image processing software having organized units. The viewer unit captures the image from the microscope and streams the display of the specimen which is then stored and processed based on the user's preferences. The other units such as brightness adjustment unit, image contrast unit, histogram equalization unit, image scaling unit and the image cropping unit all help in displaying the best images as per requirement.[5]

TYPES OF DIGITAL MICROSCOPY

Different digital microscopes that are both portable and easy to use are available in the market, based on its use and the user needs. DMs are used in varying capacities such as in biomedical to industrial fields and hence require high-quality digital microscope cameras and computer screens. Biological, Fluorescence, Inverted, Metallurgical, Phase, Stereo, Polarizing, Handheld, Portable Digital Microscopes and Digital USB microscopes are the examples of different types of DM.[5]

DIGITAL WORKFLOW

In pathology, interpretation and diagnosis relies heavily on the quality of the stained slides. In digital pathology, there is an added step to the workflow after preparation and staining of the specimen, which is digitizing histology slides.[22] This added step requires additional set up of not only the equipment, but also well-trained personnel. The various quality control measures, regular maintenance of machines and software packages, and adequate information technology (IT) infrastructure are of paramount importance. Further, a proper workstation setup for viewing and analysing digital slides has to be ensured.[23] Once the slides are digitized, any variation in colour calibration from tissue staining to scanning and displaying the colour on a monitor impacts the analysis of the slides. Hence, care should be taken to maintain adequate resolution and colour display and avoid any glitches on the display of image on a computer monitor that would impact the interpretation and eventually the data generated.[21,24] This needs to be addressed chiefly in cases where digital slides from the same study are viewed on many separate monitors.[24] Thus, for viewing of digital slides, it should be made certain that not just the colour display of a monitor, but also the size and resolution of the display image should be appropriate.[25,26] To avoid loss of detail at higher magnification and an overall pixelated view of the image, the resolution of the monitor should not be lower than the WSI resolution. Therefore, a minimum requirement for assessing WSI should be a colour-calibrated monitor with full high definition (1920 × 1080 pixel dimension).[27] This can be made sure by purchasing a precalibrated monitor or adding software solutions to the monitors after the purchase.[28] A minimum internet connectivity speeds of 20 to 100 megabits have been recommended to review the WSI when the computer does not have a local copy of the scan or is not directly linked to the server hosting the images.[27] This is because latency can have an effect on the time needed for focusing a view or changing magnification and moving the slide around.[21,26,29] Another useful feature of digital pathology workstation is the tool used to navigate around a slide, which most commonly is a computer mouse, but more sophisticated tools such as touch screens, Wacom boards and three-dimensional (3D) space navigators are available for more ergonomic, regular use and easier manipulation. The selection of such tool is purely a personal liking. While viewing and evaluating fluorescent scans, a controlled and consistent viewing environment is needed as the viewing angle and surrounding light can influence perception of bright field scanned image.[2]

Role of a pathologist in digital microscopy workflow

For the digital slides to be successfully analysed, it is very important to have good quality histopathologic slides. The role of a pathologist cannot be emphasized enough as he/she is uniquely qualified with sound knowledge of all aspects involved in the process affecting a good physical slide preparation. Hence, the inputs given by the pathologist are vital at all stages starting from processing a fresh tissue specimen till the preparation of a slide as in laboratory pathology. The pathologist's role in digital pathology extends further in ensuring good quality scan of the images without any scanning artefacts or poorly focussed scans or improper stitching lines and use of appropriate parameters and design of data capture so that the final data interpretation and reporting would be apt. In case a non-pathologist generates the data, a pathologist must give approval on its quality and his/her expertise in interpreting the data.[30] An obvious advantage of digital slides and WSI consultation is the vast exposure

and opinions that can be obtained by many pathologists, thus enhancing the pathology resources. In addition, WSI is also used for live online discussions and regular telepathology meetings featured by research organizations. In doing so, the organisations should be mindful of the IT policies all concerned institutions so as to not only ensure a well-integrated and secure data, but also to enable institutions' IT teams to workout solutions on practical hurdles faced.[31]

Need of digital microscopy

The laboratory, scientific and academic communities have had to adapt rapidly to the new realities of life and work conditions during Covid-19. The experiences with the new working conditions have led to realisations that some of these changes may well be permanent, as they demonstrate new efficient and cost-effective ways of working. Collaboration is a vital element to scientific research, investigation and testing, since there is not only the need for accessing equipment outside the lab, but also the need for sharing its capabilities with others, remotely, and in real time. Working from home needs lot of co-ordination and networking among the users so that the findings may be compared and analysed and digital microscope is proving invaluable in this regard.[3] The shortcomings in this enduring phase of pandemic have awakened pathologists to move from the age-old approach in examining glass slides with conventional light microscopy to the use of more suitable and need of the hour application of digital microscopy.[32] Thus, the technology such as the DM is enabling the continuity of this kind of close collaborative work, alongside technical benefits.

ADVANTAGES OF DIGITAL MICROSCOPY

The use of digital microscopy has many advantages. The DM enhances viewing of images simultaneously in real time with everyone having access to the same information. This real-time team approach wherein multiple pairs of eyes are looking at the images helps us to eliminate errors. Obviously, these collaborative, diagnostic and training advantages now also extend to remote working with remote microscopy and to train the trainees, enabling a more dynamic, fluid atmosphere for collaboration and discussion. Since DM does not need physical transport of samples to other locations for further analysis, meetings and report findings are readily available at the comfort of one's home or workplace. The sample results and the meetings can be recorded visually where necessary and any issues arising from inspecting a sample can be addressed much more quickly on this online platform. During the Covid-19 pandemic, examination of the samples and collaborative works via DM is still possible as safe social distance can be maintained. As the sample can be viewed instantly on a monitor display, it steers clears the person of spending extended time staring closely into an eyepiece. Where live viewing is not possible, the DM can also capture and save images to its internal memory, a USB stick or a network drive, without having to be connected to a PC for live viewing. From an ergonomic perspective, DMs are better for posture than optical microscopes as viewing on a screen is more comfortable and better for one's posture.[3] Many DMs offer software that allows storage of data for later use, with few DMs offering the feature of multiple user profile storage. This is very useful and beneficial as every user can select and log into their own profile and start working immediately with few or no adjustments to the microscope working station. Since the various parameters are encoded and can be recalled at the touch of a button, the repetitive workflows and reporting become easier than the traditional microscope where in the setting, adjusting and viewing the sample through the eyepieces take some time. This feature of the DM particularly aids the inexperienced users in particular to obtain images of a sample quickly and easily, thus benefitting both novices to microscopy as well as microscopy experts.[33] DM has become a reliable trustworthy tool used worldwide in medical practice and research and are now the standard procedures.[34]

The optical microscopes are replaced by digital imaging specially in fields such as construction of three-dimensional tissue models and *in vivo* imaging, giving impetus to these contemporary trends.[35] The newer trend is in analysing data on-the-go anywhere, without the physical presence. This is possible because the current scanners can create digital image of the entire slide and viewed virtually on devices such as laptops, tablets and smartphones.[36] DM can be used as a stand-alone instrument for collaborative microscopy or for live streaming where in the image can be seen on a PC or laptop with the easy plug and play USB cable. This livestream can simply be screen shared through proprietary video conferencing software, such as Microsoft Teams or Zoom to name a few, for that instant remote microscopy experience.[3] For effective clinical practice and learning purposes, the digital imaging allows the reanalysis of old slides and aiding in confirming or disproving diagnoses.[35] The archives of pathology can be made stronger and more robust by replacing the box containing glass slides by DM and virtual microscopy. This creates an extensive library consisting of numerous different cases. Further by sharing the image of a single scanned slide, numerous students having access either in the same room or remotely can benefit immensely.[34]

CHALLENGES ASSOCIATED WITH DIGITAL MICROSCOPY

Although a digital microscope is very much desired, especially in the present pandemic scenario and studies have reinforced its reliability with the results from optical microscopy and digital virtual microscopy not differing significantly, there are few obvious challenges that come with it.[37] The high cost of setting a digital microscopy, especially in underdeveloped countries, is one of the major drawbacks limiting its use to larger extent. Adding to this cost is the particularly large file size of digital and virtual slides that require superior and more expensive computers for flawless relaying and execution over the internet.[38] However, with increased usage and more stakeholders in DM, the costs have reduced and continue to rapidly reduce.[37] DM and remote working will see success if the planning and allocation of resources are right from the outset, keeping in mind individual's requirement rather than blindly following somebody else's setup. The mandatory minimum requirement for DM would be 1 power cable and connection to a PC or a viewing screen along with the microscope, since there are no eyepieces. To get the maximum benefit of the remote microscopy equipment, it is necessary to have a good IT infrastructure with the right communications network. Sometimes even though the images seen via DM are the same, problems such as difference in the field of view can be encountered. This would very much depend on the type of digital camera or the monitors used. Another drawback is the difference seen in depth perception when the sample is observed through the binocular eyepieces of stereomicroscope. This depth perception cannot be achieved directly with a 2D image of a digital microscope. Finally, there are a variety of choices of components available for a DM, such as choice of objectives, stands or lighting systems. One should choose them wisely depending on the application and functionalities to serve specific need and purposes.[33] Hence, a thorough understanding of all aspects of DM is of paramount importance before venturing into it.

PROSPECTS OF DIGITAL MICROSCOPY

The way forward with the pandemic is digitalisation. The success of digitalisation of laboratories very much depends on understanding the workflow, data flow and optimally recognises the bottlenecks in the current practices. A laboratory map that includes the different procedures, employees, workflow and routines followed will help in identifying the critical bottlenecks and thus plan solutions to overcome the difficulties. This will eventually increase efficiency and productivity of the laboratory. One should understand the necessity, feasibility, finances incurred and both the short- and long-term benefits of digital transformation before undertaking it. Digital culture is a very important part of digital transformation, which means implementing current values to the digital concept in a step-by-step progressive manner. Digital culture definitely provides great vision and values for the laboratory. However, unless the employer convincingly handles the employees' resistance to the changes that is completely natural, normal and expected, favourable outcomes of digitalisation cannot be achieved. Another aspect of digital transformation is having an external partner to provide different digital tools. In this regard, one should carefully choose the vendors and be clear about work processes, cybersecurity standards and access rights, so as to have smooth hassle-free work experience.[39]

CONCLUSION

The Covid-19 pandemic has been an eye opener in many ways. Although technology and digitalisation are not new, the pandemic has ensured that their utilisation is done with forceful vigour and necessity, opening the pathologists to digital microscopy world. The world is moving to digital era, and laboratories will need to digitalise. Digital transformation is a complex process and does not have to happen all at once. A step-by-step planned strategy will increase the value of the laboratory. It is essential to map the processes and data flows to ensure a more successful digital transformation. Investment in digitalization will not only pay off in the long run, but will also experience short-term savings.

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