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Dr. Manjeeta Mahesh Sinai Dhume
Lecturer (MDS BOND Service), Department of Oral and Maxillofacial pathology, Goa Dental College and Hospital, Goa University, Bambolim, Goa, India

Corresponding Author:
Dr. Manjeeta Mahesh Sinai Dhume
Lecturer (MDS BOND Service), Department of Oral and Maxillofacial pathology, Goa Dental College and Hospital, Goa University, Bambolim, Goa, India

See the unseen: Use of stereomicroscope during grossing of tissues

Dr. Manjeeta Mahesh Sinai Dhume

Abstract
Grossing is one of the most important preanalytical steps in surgical pathology. It refers to visually inspecting and dissecting a surgically removed specimen to prepare the tissues for processing and embedding. This step reveals size, shape, surface and nature of the tissue specimen that act as a bridge between the clinical presentations of the lesion to the microscopic diagnosis by a pathologist. A diagnostic conundrum could result from incorrectly grossing and then improperly embedding the surface of a tiny surgically removed tissue. Hence there is a need to incooperate use of stereomicroscope during routine procedures such as grossing and embedding. Present review reveals the importance of grossing of biopsied tissues along with recent review of literature.

Keywords: Stereomicroscope, grossing, macroscopy, microscopy

Introduction
For the diagnosis of lesions, histopathological examination of the affected area is regarded as the gold standard. To make a precise histological diagnosis, the tissue samples must be examined macroscopically (grossing) [1]. Grossing is regarded as an important but frequently skipped process in histology [2]. Rarely do pathologists receive specimens with enough tissue and pertinent clinical and radiological information [3]. However, having a keen eye for the details and the ability to associate macroscopy with microscopy is a difficult skill for a pathologist to master. It creates a bridge between the pathologist and the patient as well as between the surgeon, the pathologist, and the histotechnologist in close cooperation and harmony [4, 5].
Correct specimen identification and orientation are crucial for microscopic analysis. In order to provide a conclusive diagnosis, an accurate grossing method reduces errors while simultaneously providing useful information about the specimen. But this step's importance is frequently disregarded and forgotten.
Any error in this crucial stage could result in an incorrect diagnosis since incorrect specimen orientation could delay diagnosis or result in a diagnostic conundrum [6].
Imaging techniques including stereomicroscopy, radiographic analysis, and digital photography may be helpful to overcome specimen orientation challenges. Pathologists may benefit from using stereomicroscopy to examine samples because it can provide important information about the type of proliferation present in the sample, including whether it is mucosal or submucosal, papillary, or whether a capsule or epithelium is present. This information may be used to diagnose the sample and correctly orient it [6]. The present review is compilation of all the relevant data regarding function of the stereomicroscope in routine grossing and its contribution to making a conclusive diagnosis.

History about Stereomicroscope
Cherubin d'Orleans (1613-1697) who was a monk created the first pseudo-stereoscopic. He built a little microscope with two distinct objective and eyepieces. English scientist and inventor Charles Wheatstone (1802-1875) is credited with introducing the concept of stereoscopic vision.
1807-1865: John Leonard Riddell is contributed for the development of stereo microscopy. In the Quarterly Journal of Microscopical Science, he reported his findings in his work titled "On the Binocular Microscope".

American biologist and instrument designer Horatio S. Greenough created the stereo microscope as a substitute for the Common Main Objective (CMO) microscope. Horatio S. Greenough's principles are still the foundation of the stereo microscope used today hence he is regarded as forefather of modern stereomicroscopes.

The "Greenough principle"-based stereo microscopes produce extremely high-quality, true stereoscopic images. Bausch & Lomb introduced its Stereo Zoom Greenough design in the late 1950s with a ground-breaking advancement: a stepless magnification (zoom) changer. Modern stereo microscope designs are almost universally based on a zoom system. A stereo microscope with optics based on the telescope or CMO (Common Main Objective) approach was first introduced in 1957 by the American Optical Company. Due to its adaptability and good performance, this type of stereo microscope was soon provided alongside the Greenough type by all manufacturers.

The d'Orleans design had the significant flaw that the right-side picture was projected to the left eyepiece while the left-side image was projected to the right eyepiece. Stereomicroscopy wasn't given enough attention to spur additional research until Sir Charles Wheatstone's dissertation on binocular vision, which was written more than 150 years later [7].

Stereomicroscope/Dissecting microscope/ Low power microscope is an optical microscope variant designed for low magnification observation of a sample, typically using light reflected from the surface of an object rather than transmitted through it.

Optics associated with stereomicroscope

Various optical terminologies used in relation to stereomicroscope are tabulated as follows [8]: (Table 1)

Table 1: Optics associated with Stereomicroscope

Terminology	Definition	Implication
Working distance	The distance from the bottom of the objective lens to the point of the sample in focus	A larger working distance means a greater distance between the top of the sample and the objective's front lens, allowing for easier handling of the sample during use.
Numerical aperture (NA)	Measure of its ability to gather light and resolve fine specimen detail at a fixed object distance	NA is dependent on the microscope objective and is not affected by eyepiece magnification
Note: Working distance has an inverse relationship to NA and therefore resolution		
Depth of field	The distance between the nearest and furthest points from the objective that is in focus at the same time.	Depth of field is greater at lower magnification
Note: Higher NA results in higher resolution, but usually reduced depth of field.		
Object field	Diameter of the circular area of sample that is visible through the microscope at one time.	Stereomicroscopes typically have large object fields to allow for sample manipulation.
NOTE: Magnification and object field are inversely related, that is, at higher magnifications a smaller area of the sample is visible.		
Depth of perception	Relates to the distance between two points in a specimen so that both remain clear and focused when viewed.	

Principle of Stereomicroscope

Two separate optical channels can be used in a stereo microscope to produce 3D images. Two groups of objectives, often referred to as zoom lenses, in the center are used to split two beams that are modulated by the measured items and form an angle between 12 and 15 degrees. Then, eyepieces picture two beams whose magnification is determined by the separation between the middle lenses. In a stereo microscope, two light beams form an angle rather of being parallel [9].

Illumination for Stereomicroscope

The specimen that will be examined with a dissecting microscope is substantial, opaque, and thick.

To use a stereomicroscope, you need light. Two light sources are commonly used in stereomicroscopes: one from below the sample and one from above. Using ring lamps or spot illumination, incident light is frequently used for solid samples. UV or IR light sources can be used to illuminate samples in order to observe particular traits or reactions. Brightfield, darkfield, phase contrast, differential interference contrast, and confocal illumination techniques are some of the illumination methods [9].

Parts of Stereomicroscope [9]

1. Viewing Head/Body-Houses the optical components in the upper part of the microscope
2. Focus Block-Attaches the microscope head to the stand and focuses the microscope
3. Luminous stand-Stand that supports the microscope and houses any integrated illumination.
4. Eyepieces or Oculars are what you look through at the top of the microscope. Typically, standard eyepieces have a magnifying power of 10x.
5. Working Stage/Stage plate -- is where the specimen to be viewed is placed.
6. Diopter adjustment ring that allows for the possible inconsistencies of our eyesight in one or both eyes.
7. Stage Clips are used when there is no mechanical stage.
8. Top light (Transmitted Illumination) is used to shed light on the specimen. Bottom light (Incident Illumination)-reflected in the eyepiece.
9. Focus control -Only coarse focus controls.

3D visualization of the image

The Porro prism, for instance, is used in many optical viewing devices, including periscopes, binoculars, and monoculars, and is composed of two prisms configured to both invert and reverse an image.

The total internal reflection (TIR) principle underlies Porro prism operation. The two roof surfaces reflect a beam entering perpendicular to the entrance/exit surface so that it exits parallel to itself.

Major differences between Compound and simple microscopes.

Few major differences are as follows in table 2.

Table 2: Difference between Stereomicroscope and Compound microscope

Points of Comparison	Stereomicroscope	Compound microscope
Optical path	Reflected illumination	Single optical path Transmitted through the object
Magnification	Low (2-100x)	High (40-1000x)
Nature	Checks items in which light cannot shine through	Checks ultra-thin pieces of large objects
Working space	Large	Small
Function	Examine solid surfaces	Examine minute things

Ergonomics in relation to stereomicroscope [9].

People generally have a wide range of body types and work habits. As a result, not every user may find the height (eyepieces) of a microscope configured for a certain task with specialized accessories and a given working distance to be adequate. The observer will be forced to bend forward while working if the viewing height is too low, which can cause muscular tension in the neck area. It is recommended to employ a variable binocular tube to account for these height discrepancies. Stereo microscopes with a CMO design, which allow various possibilities to customize the instrument to the user's size or working habits, are the favored option because of the modular product approach.

Types of stereomicroscope

Two types of stereomicroscopes are as follows (Table 3)

Table 3: Two types of Stereomicroscopes

common main objective (CMO) /galilean /telescope design	Greenough design
Presence of single objective with two ocular channels and eyepieces	Produces stereo effect through use of two angled objectives mounted side by side
Superior light gathering capabilities	Inexpensive
Higher resolution	Ideal for lab work

Importance of grossing prior to Histopathological analysis:

Histopathological (H/P) analysis is considered to be the gold standard for diagnosis of any lesion. Macroscopic examination (grossing) of the tissue specimen helps in accurate histopathological diagnosis. But grossing being the most important aspect is still most commonly neglected step during the process of H/P analysis. Identification of the location for optimal embedding is a crucial stage in grossing since improper orientation of the tissue might make a pathologist's job difficult during reporting. Following grossing of tissues if there are difficulties for specimen orientation digital photography along with radiographic examination and use of a stereomicroscope will help in correct orientation of the biopsied tissues.

K Carmel Shobhita *et al* conducted a study on 50 cases which were in accordance to inclusion and exclusion criteria's of the study. Study samples were categorized into solid tumors, cystic and surface lesions. Diagnosis of 21/50 surface cases was made possible due to in cooperation of use of stereomicroscope during the grossing of the biopsied tissues Naked eye was not able to make a proper differentiation between epithelium and connective tissue while use of stereomicroscope showed allowed for clear-cut demarcation between outer epithelium and underlying connective tissue. Similar findings are also noted in solid and cystic lesions [10].

Another study was conducted by Shah *et al* on 5 cases to evaluate the efficacy of stereomicroscope in routine grossing and its role in arriving at a final diagnosis. They found that stereomicroscopic images aided in recording obscure details of specimen, correct orientation of the specimen and in the final diagnosis [11].

There are numerous case reports that have been published in which the stereomicroscope is used and has aided in accurate diagnosis. Rifana Nasrin *et al* [12] published a case report in 2022 of an oral mucocele in with the use of the stereomicroscopy; it was possible to appreciate the macroscopic features that were seen in the histopathological slide. This included the loose connective tissue with the shadow of blood vessels, the prominent presence of mucous acini at the periphery of the lesion and the obvious separation of mucinous content with surrounding tissue layers. These above details were difficult to record while grossing with only naked eyes.

Another preanalytic study on macroscopic examination of biopsy specimens by Jacob M *et al* [13] in which thirty soft tissue specimens were grossed by three observers with and also without the stereomicroscope. Following this questionnaire using a 5 point Likert scale with options ranging from 1 (very good) to 5 (very poor) was used to indicate the level of satisfaction in utilisation, operation and preference of stereomicroscope in gross examination over the conventional method (unaided eye) in pre-analytic phase of tissue processing. Results of the three observers concluded that use of stereomicroscope could give better details of the biopsied tissue when compared with the conventional method of macroscopic examination.

Abhisikta Chakrabarty *et al* [14] conducted analysis of biopsied tissues while grossing and also after processing of the tissues. He concluded that the routine use of a stereomicroscope in laboratory procedures not only improves surface topographic analysis of biopsy specimens but also serves as an efficient method of embedding the tissues for additional histopathological analysis.

Applications of stereomicroscope in other fields of dentistry

In case of endodontics it is used most commonly in order to measure the depth of micro leakage of endodontic filling material and to inspect for cracks, root caries, fracture and resorption defects and root canal anomalies [15].

In prosthodontics in order to check marginal infidelity of metal ceramic and all full ceramic crowns and also to check bound strength failure mechanism between zirconia and luting cement.

In orthodontics in order to place brackets for the anterior tooth especially in areas of esthetic importance.

Limitations of stereomicroscope

Major drawback of using a stereomicroscope is in relationship to the working distance between the lens and the specimen. Magnification has an inverse correlation with working distance and field of view. With the increase in the magnification being used, there will be a decrease in the working distance and field of view and vice versa. So, it is important to select a microscope with magnification settings that will provide a sufficiently large field of view to observe the sample. Along with this the working distance should be large enough to accommodate the sample between the lens and the base along with achieving the focus at the required level of magnification.

The two types of stereomicroscopes have their own drawbacks. In case of Common Main Objective (CMO) model since it practically removes any image tilt in the focal plane, it may create an optical anomaly-known as perspective distortion - that makes the examined image appear to be elevated in the center.

In case of the Greenough model and effect known as the keystone effect is observed. There is a minor tilt in the focal plane because of the two lenses seeing the same image at different angles. Due to the fact that the lenses are not entirely parallel, the outer region of the image in the field of view may become slightly over focused or under-focused. As a result, only the central parts of the image are properly focused at identical magnifications.

Conclusion

The grossing of tissue samples obtained by the pathology lab is a crucial step in the diagnosis. A thorough grossing and examination of the pathology specimen can reveal numerous hints that help with the ultimate diagnosis. The ability to observe the specimen's sliced surface through a stereomicroscope gives us access to additional knowledge that may help with both accurate diagnosis and specimen orientation. For both incisional and excisional biopsies, a stereomicroscope can be employed as a supplement to the naked eye grossing.

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