

Original Article

Role of Microscopy in Forensic Science Research and Investigation

Ketaki Chandiok¹, Prakash Ranjan Mondal^{2*}

¹Senior Research Fellow, Department of Anthropology, University of Delhi, Delhi-110007, India

²Associate Professor, Department of Anthropology, University of Delhi, Delhi-110007, India

(*Corresponding author) email id: *prmondal1@rediffmail.com, ¹chandiokketaki@gmail.com

ABSTRACT

The present review article deals with the utility of microscope in a forensic science and its utility in research and investigation. The review article tries to explain the basic of microscopes ranging from a simple microscope to electron microscope. It further explains the utility of microscopes in a multitude of areas that could ease investigation and research in a forensic science laboratory.

Keywords: Microscope, Research, Investigation, Forensic science, Laboratory, Simple microscope, Electron microscope

INTRODUCTION

A microscope is an optical instrument that uses a combination of lenses for magnifying images of objects. In the recent years, forensic microscopy has occupied a centre stage in forensic science research owing to its enormous utility with its other fellow core science disciplines be it chemistry, physics, botany or zoology in solving criminal cases. From this perspective, it is important to understand the utility of microscopes. The main purpose of a microscope is to magnify the image of a small object. However, after a reasonable magnification is obtained, increased resolution of the object is then desired. For this purpose, microscopes with varied utility are used in forensic science research^[3,15].

Owing to the multidisciplinary nature of forensic science as a subject, it is necessary that the wide utility of it as discussed subsequently should be handled with great care. This is particularly because on a regular basis, evidence available for scrutinising is often available in very diminutive quantities. Therefore, it becomes necessary for a forensic microscopist to be well trained for the utility of microscope in forensic research. The present article with this aim provides an overview of the microscopes that are widely used in forensic science along with the possible utility for the same.

Types of Microscopes Used in Forensic Science Laboratory

There are different types of microscopes used in forensic science laboratory. These are as follows:

1. Stereomicroscope
2. The compound microscope
3. Other Optical Instruments
 - a. Phase contrast microscope
 - b. Fluorescence microscope
 - c. Hot stage microscope
4. Comparison microscope
5. Polarising light microscope
6. Electron microscope
 - a. Transmission electron microscope
 - b. Scanning electron microscope

1. Stereomicroscope

The stereomicroscope is the most basic instrument that is used in forensic science. Stereomicroscope usually consists of two lenses, the objective and the eyepiece. The stereomicroscope has a long working space between

the objective lens and the human eyes. A typical stereomicroscope contains two compound microscopes that are aligned side by side to provide a three dimension image of the object. Human eyes are separated by a distance of approximately 65 mm, due to which each eye perceives an object in a different field. The stereomicroscope transmits twin images to the brain due to which an image in a stereoscopic aspect is perceived by the brain. The magnification range for the stereomicroscope is quite low, ranging from $2.5\times$ to $100\times$. A typical stereomicroscope consists of a light source, sample stage, objective, support and alignment portions and oculars. The type of light used in a stereomicroscope depends upon the sample being viewed. If the object is opaque, reflected light is used, and if it is transparent, transmitted light is used. However, for some samples both reflected and transmitted light is used. In a typical stereomicroscope, the light that enters the objective is divergent light and the one that leaves it is parallel light. This parallel light is then split by a series of prisms, redirecting the light to oculars. In a stereomicroscope, the objective lens is built with the body tube. The utilisation of a typical stereomicroscope does not require any expertise. A stereomicroscope is generally used for critical fundamental analysis of the specimens. It is useful in performing the crucial preliminary examination, which includes manipulation of the evidence for more sophisticated analysis^[15,7].

2. Compound Microscope

The compound microscope like the stereomicroscope has two lenses the objective and the eyepiece, but it offers a greater degree of magnification. The magnification produced by a compound microscope ranges from $2.5\times$ to $1,300\times$. A compound microscope has an additional condenser as well. The light that originates from the illuminator is collimated by the condenser. The light then interacts with the sample and is collected by the objective. The objective refocuses the image and the oculars receive it and focus it on to the eye. There are numerous types of compound microscopes that are available in a forensic science laboratory, which sought their usage under varying circumstances. These include bright field, phase contrast, comparison, hot stage, fluorescence and polarising microscope and others^[15,7].

3. Other Optical Instruments

a. Phase Contrast Microscope

The phase contrast microscope is primarily used to distinguish the amplitude (dark and light) differences. Although the human eye has the ability to differentiate the amplitude differences, it cannot observe phase differences. A phase contrast microscope assists in this by converting the phase differences into amplitude differences by using half-silvered rings and disks placed in the optical system of the microscope to bring about phase differences that can be observed and hence photographed^[16,10].

b. Fluorescence Microscope

Fluorescence microscopy is based upon ability of certain objects to emit light of a longer wavelength after they are irradiated with light of shorter wavelength. This fluorescence may originate from the fluorescent tags attached to proteins or other compounds which cause the substance they react with to fluoresce. A typical fluorescence microscope consists of a vertical illuminator that directs light from the mercury burner through a series of lenses and filters onto the specimen and then selects a narrow or wide range of wavelength so as to excite the specimen. The image that is formed through a fluorescence microscope is through the emitted light rays and is well resolved^[12].

c. Hot Stage Microscope

The hot stage microscope is used to observe the behaviour of the specimens as they are exposed to a range of temperature ranging from ambient up to 350°C . In hot stage microscope, the melting temperature is used in the identification of unknown substances^[13].

4. Comparison Microscope

The comparison microscope as the name suggests is used for the comparison of microscopic items simultaneously. Numerous comparisons such as that of bullets, tool marks, hairs and fibres are compared mostly during the final stages of investigations. The comparison microscope derives its importance primarily because of the inability of the human brain in remembering the slight differences between the different items of interest. The human brain can memorise the major differences between two objects,

but for minute differences, comparison microscope holds utility^[7].

5. Polarising Light Microscope

The polarising microscope as the name suggests consists of two polarisers that are inserted below and above specimen. In addition, it also has a rotating stage and a pair of compensators. The compensators are used for providing contrast and to infer the qualitative and quantitative optical properties of the objects under study. A polarised light microscope allows analysis of different objects under plane polarised light, between crossed bars and by convergent polarised light^[7].

6. Electron Microscope

An electron microscope uses electrons as a source of illumination. It has a greater resolving power as compared with light microscope as the wavelength of an electron is shorter than a photon that manifests in greater resolving power.

a. Transmission Electron Microscope

The transmission electron microscope uses an electron beam produced by the tungsten filament cathode (electron source) to produce an image. The electron beam is accelerated by the anode with respect to the cathode, focussed by electrostatic and electromagnetic lenses, and this is then transmitted through the specimen (typically less than 100 nm thick) that is transparent to the electrons and scatters them out of the beam. The light which emerges from the specimen, provides the necessary information about the structure of the specimen is magnified by the objective lens of the microscope^[14].

b. Scanning Electron Microscope

The scanning electron microscope produces image of the specimen by scanning the specimen with an electron beam. When the beam of electron scans the specimen, energy is lost and is converted into alternative forms such as heat, low energy secondary electrons and high energy backscattered electrons, all of which provide the necessary information about the properties of the specimen like topography and composition and others^[2].

Utility of Microscopes in Forensic Science Research and Investigation

The utility of microscopes is forensic research and

investigation is immense ranging from photography, dermatoglyphics, ballistics to biology. In this context, phase contrast microscope is often used for the comparison of mineral grains from sand and soil types, fibres, crystals, cosmetics, crystal material, automotive and architectural paint pigments and others. In contrast to this, the phase contrast microscope is primarily used for the comparison of biological materials like spermatozoa, epithelial cells and others. Fluorescence microscopy though applied to a limited extent in forensic serology is used for the detection of antigen-antibody reaction at cellular level (<http://what-when-how.com/forensic-sciences/microscopy/>). Besides this, it is also based on the comparison of glass fragment samples, wherein based upon the differences in the refractive samples, the type of questioned glass fragment is ascertained^[9].

In addition to this, the increasing utility of electron microscope has increased in forensic microscopy. Scanning electron microscope also finds utility in analysis of bullet marks^[6]. It is also used in forensic odontology for the study of teeth^[4] and in forensic anthropology for the study of sharp trauma force^[1]. In addition, scanning electron microscope also document utility in forensic entomology^[8]. This is because certain species of insects are known to be associated with human cadavers. Scanning electron microscope is identifying eggs of such insects. A recent study by^[11] documented the utility of scanning electron microscope in the investigation of bite marks on food stuffs.

Reference^[5] has highlighted the utility of atomic force microscopy in the examination of line crossings in forensic documentation. Although optical microscopes and scanning electron microscope find utility in such examinations, the benefits that a researcher reaps from atomic force microscope are noteworthy. One of its major advantage is the non-destructiveness of the spots of interest that are not altered by scanning. Besides this, it is also cheap as compared with the classical scanning electron microscope.

ACKNOWLEDGEMENT

The authors would like to thank the Department of Anthropology for providing us an opportunity for the present study.

REFERENCES

- [1] Houck MM, Bowen RB. An argument for light microscopy – a review of forensic microscopy for trace evidence analysis. *Forensic Science Review* 2005;17(1):1–5.
- [2] Wilson LJ, Wheeler BP. Optical microscopy in forensic science. *Encyclopedia of Analytical Chemistry* 2009 DOI: 10.1002/9780470027318.a9101
- [3] Kreindler RJ. *Stereomicroscopy Third Edition*, 2012 <http://www.microscopy-uk.org.uk/mag/rtsep12/jk-stereo3.pdf>.
- [4] Zernike F. Phase contrast, a new method for the microscopic observation of transparent objects. *Physica* 1942;9(7):686–98.
- [5] Richards OW. Phase microscopy 1954–56. *Science*. 1956;124(3226):810–4.
- [6] Spring KR, Davidson MW. Introduction to fluorescence microscopy. *Nikon Microscopy U*; 2004. <http://www.microscopyu.com/articles/fluorescence/fluorescence-intro.html>.
- [7] Vitez IM, Newman AW, Davidovich M, Kiesnowski C. The evolution of hot-stage microscopy to aid solid-state characterizations of pharmaceutical solids. *Thermochimica Acta* 1998;324(1):187–96.
- [8] Williams DB, Carter CB. *The transmission electron microscope*. US: Springer; 1996.
- [9] Cheney B. Introduction to scanning electron microscope, (part of a Senior Project in the Materials Engineering Department at San Jose State University) http://www.sjsu.edu/people/anastasia.micheals/courses/MatE143/s1/SEM_GUIDE.pdf.
- [10] Murphy DB, Oldfield R, Schwartz S, Davidson MW. Introduction to Phase Contrast Microscopy *Nikon Microscopy U*, 2017. <http://www.microscopyu.com/articles/phasecontrast/phasemicroscopy.html>.
- [11] Katterwe H, Goebel R, Grooss KD. The comparison scanning electron microscope within the field of forensic science. *Scanning Electron Microscopy 1982*; (Pt 2):499–504.
- [12] Jakobsen J, Holmen L, Fredebo L, Sejrsen B. Scanning electron microscopy, a useful tool in forensic dental work. *Journal of Forensic Odonto-Stomatology* 1995;13(2):36–40.
- [13] Bartelink EJ, Wiersema JM, Demaree RS. Quantitative analysis of sharp force trauma: an application of scanning electron microscopy in forensic anthropology. *Journal of Forensic Science* 2001;46(6):1288–93.
- [14] Mendonça PM, dos Santos-Mallet JR, de Mello RP, Gomes L, de Carvalho Queiroz MM. Identification of fly eggs using scanning electron microscopy for forensic investigations. *Micron* 2008;39(7):802–7.
- [15] Solheim T, Leidal TI. Scanning electron microscopy in the investigation of bite marks in foodstuffs. *Forensic Science* 1975;6(3):205–15.
- [16] Kasas S, Khanmy-Vital A, Dietler G. Examination of line crossings by atomic force microscopy. *Forensic Science International* 2001;119(3):290–8.