Voyager: Vol. VIII, No. 1, June 2017, ISSN :(p) 0976-7436 (e) 2455-054x Impact Factor 3.8919 *(ICRJIFR)*
UGC Approved Journal No. 63640

Advances in Microscopy-A Review

Neetu Nimesh*, Deepali Joon, Manoj Nimesh*****
Deptt. of Zoology, RGPG College, Meerut, Dr. B.R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi, ** Shri Guru Tegh Bahadur Khalsa College, University of Delhi, Delhi*

Reference to this paper should be made as follows:

Neetu Nimesh, Deepali Joon, Manoj Nimesh,
" *Advances in Microscopy-A Review*",
Voyager: Vol. VIII, No. 1, June 2017, pp.27- 36
http://anubooks.com/?page_id=2430

Abstract

Biological microscopy has undergone a revolution in last two decades. Light microscopy is a mainstay of modern molecular, cell and developmental biology laboratories and spans applications in basic research, preclinical and recently even clinical domains. Electron microscopy is also growing with various changes. Rapid developments at all levels of microscopy experiments – from improvements in sample labelling, contrast, illumination, resolution, signal detection and data processing have all occurred and there is every reason to expect that these advances will simply continue. Applications for these new imaging methodologies are also growing. Combining these technical advances with robotics has resulted in automated microscopes suitable for screening small molecule libraries for drug discovery and recording cell phenotypes that result from systematic gene knockdown by RNA interference or tissue phenotypes caused by diseases. Present review summarizes the historical developments in the microscopy technique. It also highlights the advancements in the technique and its myriad applications.

Keywords: *Microscopy, Light microscopy, Electron microscopy.*

Introduction

From the post renaissance era of human society to the modern era, the microscope has made a tremendous contribution leading to revolutionary breakthrough in science and technology. It helps in various areas of research such as-cell biology, cancer, systems biology and all other branches of biology. It helps students to study various histological slides. The earliest microscopes used visible light to create images and were little more than magnifying glasses. Today, more sophisticated compound light microscopes are routinely used in microbiology laboratories. The various types of light microscopy include bright-field, dark-field, fluorescence, and phase contrast microscopy. Each method has specific applications and advantages, but the most commonly used in introductory classes and clinical laboratories is bright-field microscopy. In many research applications, electron microscopy is used because of its ability to produce higher quality images of greater magnification. Correlative microscopy which combines the power and advantages of different imaging system, e.g., light, electrons, X-ray, NMR, etc., has become an important tool for biomedical research in recent times. Among all the possible combinations of techniques, light and electron microscopy, have made an especially big step forward and are being implemented in more and more research labs.

History

Optical microscope invention

It is widely believed that Dutch spectacle makers, Zacharias Jansen and his father Hans were responsible for making the first compound microscope in the late 16th century. The microscope consisted of three draw tubes with lenses inserted into the ends of the flanking tubes. The eyepiece lens was bi-convex and the objective lens was plano-convex, a very advanced compound design for this time period. Focusing of this handheld microscope was achieved by sliding the draw tube in or out while observing the sample. The microscope was capable of magnifying images approximately 3 times when fully closed and up to ten times when extended to the maximum. It is believed that Zacharias Jansen's father, Hans, helped him build the first microscope in 1595.

It was Anton van Leeuwenhoek (1632-1723), a Dutch draper and scientist, and one of the pioneers of microscopy who in the late 17th century became the first man to make and use a real microscope. He was the first to see bacteria, yeast, blood cells and many tiny animals swimming about in a drop of water. He was lucky to achieve magnification of about 270x at that time. He is called as the "Father of microscopy". Van Leewenhoek's work was verified and further developed by English scientist Robert Hooke published Micrographia in 1665. Micrographia presents several accounts of Hooke's observations through the use of the

microscope. He looked at all sorts of things (snow, a needle, a razor, etc.) with a primitive compound microscope. He observed the fleas under the microscope and was able to observe the tiny hairs on the fleas' bodies. On the cork he saw pores. Upon examination of the pores, he decided to call them "cells"; however, he did not know he had just discovered plant cells.

In the 1850s, German engineer Carl Zeiss began making refinements to the lenses he used in the microscopes he manufactured. In the 1880s, Zeiss hired glass specialist Otto Schott, who conducted research on optical glass, greatly contributing to the improvement of the optical quality of the microscope. We should also mention Ernst Abbe, who was hired by Zeiss to improve the manufacturing process of optical instruments, Abbe carried out theoretical studies of optical principles, improving the understanding of the optical quality of a microscope (Abbe, 1873; Ford, 1989).

Electron microscope invention

In the early 20th century a significant alternative to light microscopy was developed, using electrons rather than light to generate the image. Ernst Ruska started development of the first electron microscope (EM) in 1931 which was the transmission electron microscope (TEM). The transmission electron microscope works on the same principle as an optical microscope but uses electrons in the place of light and electromagnets in the place of glass lenses. Use of electrons instead of light allows a much higher resolution. Development of the transmission electron microscope was quickly followed in 1935 by the development of the scanning electron microscope by Max Knoll. On October 8, 2014, the Nobel Prize in Chemistry was awarded to Eric Betzig, William Moerner and Stefan Hell for "the development of super-resolved fluorescence microscopy," which brings "optical microscopy into the nanodimension" (McMullan,2006).

Advancements in Microscopy

Advances in fluorescence microscopy

The introduction of digital detectors on fluorescence microscopes allowed the storage of direct measurements of the distribution of cellular components. Initially, this was a convenience, as it removed the difficulties of handling and processing film. Most critically, charge-coupled device and photomultiplier tube, when used properly, are linear detectors, so the digitally measured fluorescence signal is, in principle, a faithful measure of the distribution of fluorescence. The introduction of fluorescent protein technology (GFP and its variants) has allowed almost any cellular protein or compartment to be visualized in the living cell or organism. Thus the engineering of cells for fluorescence study and the acquisition of fluorescence localization data is now sophisticated, routine, and at least fairly mature. Fluorescence localization can now be used as an assay. It

is now common to use the localization of a GFP fusion protein to identify a domain required for subcellular localization, identify effectors required for proper localization as well as to study the spatial and temporal dynamics of intracellular proteins and other molecules in live cells. In this age of post-genomic analysis, this immediately suggests the development of genome-wide screens for the localization of all known gene products (Pepperkok et al., 2001; Ni et al., 2001). In addition, the search for new lead compounds for drug development has embraced fluorescence microscopy in screening assays that seek small molecule effectors of subcellular localization, cell-cycle progression, cell migration, etc. (Rosania et al.,1999 ; Haggarty et al.,2000). These types of studies are likely to represent just the beginning of a wave of applications implementing large-scale, high-throughput imaging systems for cellular analysis and drug discovery (Andrews et al., 2002).

But, like all techniques, ûuorescence microscopy is subject to practical physical limitations, the most important of which is resolution. Consequently, most of the recent advances with ûuorescence microscopy have sought to improve image quality by addressing the fundamental problem of image resolution, which is determined by image contrast and the diffraction of light within optical systems (Hell et al., 2003).

Advancement in fluorescence microscopy is two photon imaging which allows imaging of living tissues up to a very high depth by enabling greater excitation light penetration and reduced background emission signal (Helmchen, 2005). A recent development using this technique is called Super penetration multi-photon microscopy, which allows imaging at greater depths than two-photon or multi-photon imaging would by implementing adaptive optics into the system. Pioneered by the Cui Lab at Howard Hughes Medical Center and recently reported by Boston University on focusing light through static and dynamic strongly scattering media. By utilizing adaptive optics, it has allowed the optical wavelength control needed for transformative impacts on deep tissue imaging (Sanderson et al, 2014).

Synchroton – based X ray florescence microscopy (XFM) has found many applications in science particular in biomedical science (Paunesku et al., 2006; Fahrni, 2007; Majumdar et al., 2012). These applications can be classified into two categories. One involves the mapping of naturally existing elements such as in studies of the roles of Fe, Cu and Zn in essential cellular processes or in disease development (Farquharson et al., 2007; Ortega et al., 2007; Leskovjan et al., 2011; Vogt, 2013). The second involves studying how exogenously employed metal-containing reagents such as anticancer drugs are compartmentalized and transported and distributed at the level of tissue ,cell or even subcellular organelles (Corde et al.,2002;Paunesku et al.,2003;Corezzi et al.,2009;Yuan et al.,2013).

Both lines of research have greatly enhanced our understanding of the molecular mechanisms of many cellular processes as well as metal-related disorders, and assisted the development and assessment of targeted therapeutic and diagnostic compounds (Jin et al., 2017).

Advanced confocal microscopy

Conventional microscopy techniques, namely the confocal microscope or deconvolution processes, are resolution limited, ~250 nm, by the diffraction properties of light as developed by Ernst Abbe in 1873. This diffraction limit is appreciably above the size of most multi-protein complexes, which are typically 20–50 nm in diameter. In the mid 2000s, biophysicists moved beyond the diffraction barrier by structuring the illumination pattern and then applying mathematical principles and algorithms to allow a resolution of approximately 100 nm, sufficient to address protein subcellular colocalization questions. This "breaking" of the diffraction barrier, affording resolution beyond 200 nm is termed super resolution microscopy. More recent approaches include single molecule localization (such as Photo Activated Localization Microscopy (PALM)/ Stochastic Optical Reconstruction Microscopy (STORM)) and point spread function engineering (such as Stimulated Emission Depletion (STED) microscopy) (Jalbert et al., 2003).

Coronal sections of the in situ epithelium, Bowman's membrane, stroma, and endothelium can be visualised at a resolution of 1–2 μm. A backscattered light intensity curve allows objective measurements of sublayer thickness and corneal haze to be taken. In vivo confocal microscopy (IVCM) is therefore particularly useful in the areas of infective keratitis, corneal dystrophies, refractive surgery, and contact lens wear, where it aids in differential diagnosis and detection of subtle short and long term changes. Real time endothelial cell assessment can also be performed. Because of their ability to visualise living tissue at cellular levels, IVCM have proved useful additions to the current clinical tools. IVCM is an emerging technology that provides minimally invasive, high resolution, steady-state assessment of the ocular surface at the cellular level. Several challenges still remain but, at present, IVCM may be considered a promising technique for clinical diagnosis and management (Jalbert et al., 2003).

High-throughput (HT) assays, based on multi-well plates traditionally have been used to perform systematic measurements of in vitro binding, enzymatic activity or gene expression. Cell based HT assays generate a single value that represents the sum of the signal from all cells in a well. By combining digital microscopy – wide-ûeld, confocal, multi-photon, FCS, etc. – with a motorised stage, image data can be recorded from multi-well plates or from large format slides that have been printed with arrays with transfectable plasmids or siRNAs (Neumann et al., 2006, Perlman et al., 2004).

Advances In Electron Microscopy

There are many changes in fixation, staining and resolution in electron microscopy applications. It is enriched with the passage of time. CLEM (Correlative light electron microscopy) approach integrates imaging of fluorescent proteins in live cells with the cryo-immunogold technique by modifying previously published CLEM approach on fixed cells at several steps. Briefly, gridded coverslips are coated with Formvar and gelatin, according to an optimized protocol. Cells destined for CLEM are grown on these gridded coverslips, which facilitates tracing back their location in the electron microscope. After live-cell imaging, cells are detached from the coverslip and prepared for electron microscopy. Using this approach, the authors monitored the kinetics and localization at nanometer resolution of the lysosomal membrane protein LAMP-1 in live cells (Van.et .al., 2008).

Electron cryomicroscopy (cryo-EM) yields images of macromolecular assemblies and their components, from which 3D structures can be determined, by using an image processing method commonly known as "single-particle reconstruction." During the past two decades, this technique has become an important tool for 3D structure determination; the result of this study establishes single-particle reconstruction as a high-resolution technique. Cryo –EM structure of a non enveloped virus has revealed a priming mechanism of cell entry by single particle method (Zhang et al., 2008; 2010).

The methods have been optimized for fast freezing and processing of zebrafish embryos for electron microscopy (EM). In the absence of primary chemical fixation, excellent ultra structure, preservation of green fluorescent protein (GFP) fluorescence, immunogold labelling, and electron tomography can be obtained using a single technique involving high-pressure freezing and embedding in Lowicryl resins at low temperature as well as being an important new tool for zebrafish research, the maintenance of GFP fluorescence after fast freezing, freeze substitution, and resin embedding can be used in general for correlative light and EM of biological samples.(Nixon et al.,2009). Advances in electron cryo-microscopy have led to determination of the structure of a mammalian TRP channel, TRPV1, at 3.4Å resolution, breaking the side-chain resolution barrier for membrane proteins without crystallization (Lio et al., 2013). It has also revealed the structure of Dengue Virus at 3.5 Angostorm resolution.(Zhang et al.,2013).The tissue research has been promoted by combined use of correlative light and electron microscopy imaging (Solomonov et al.,2014). Both the techniques have also been applied with chemical tags (Perkovic et al., 2014).

Conventional microscope has given limited information about virus host

interaction. But the development of 3D electron microscopy has provided unprecedented insights into remodelling of intracellular architecture of the host cell. During last several years various 3D-EM methods have been developed such as electron tomography, serial sectioning, serial block face scanning electron microscopy and focus ion beam scanning electron microscopy for dealing with details of virus structure (Brey et al.,2015). The drying, staining and Cryo-TEM (Cryo-Transmission electron microscopy) has significantly contributed in soft matter imaging and supra molecular chemistry (Franken et al., 2017).

Conclusion

Rapid developments at all levels of microscopy experiments – from improvements in sample labelling, contrast, illumination, resolution, signal detection and data processing have all occurred and there is every reason to expect that these advances will simply continue. Applications for these new imaging methodologies are also growing. Combining these technical advances with robotics has resulted in automated microscopes suitable for screening small molecule libraries for drug discovery and recording cell phenotypes that result from systematic gene knockdown by RNA interference (Swedlow, 2012).

With the above mentioned examples, it can be concluded that microscopy technique has advanced over the past century and the current revolution in biological microscopy stems from the realization that advances in optics, computational tools and automation make the modern microscope an instrument that can access all scales relevant to modern biology – from individual molecules all the way to whole tissues and organisms and from single snapshots to time-lapse recordings sampling from milliseconds to days.

References

• Abbe E., (1873). Contributions to the theory of the microscope and of microscopic resolution. *Arch f Mikr Anat* **413–420.**

• Brey, I. R., Bartenschlager, R.,(2015).Viral infection at high magnification: 3D Electron microscopy methods to analyze the architecture of infected cells.,**7,6316-6345.**

• Corde, S., Biston, M.C., Elleaume, H. et al. (2002). Lack of cell death enhancement after irradiation with monochromatic synchrotron X rays at the K-shell edge of platinum incorporated in living SQ20B human cells as cis-diammine dichloro platinum *(II).Rad.Res.***158(6),763–770.**

• Corezzi, S., Urbanelli, L., Cloetens, P., Emiliani, C., Helfen, L., Bohic, S., Elisei, F. & Fioretto, D. (2009). Synchrotron-based X-ray fluorescence imaging of human cells labeled with CdSe quantum dots. *Analytic. Biochem.* **388 (1), 33–39.**

• Ford, B.J.,(1989). Antony van Leeuwenhoek—Microscopist and visionary scientist. *J Biol Education* **23: 293–299.**

• Franken, LE., Boekema, E.J., Stuart, M.C.A., (2017). Transmission electron microscopy as a tool for characterization of soft materials: Application and interpretation**.** *Advanced science,* **4, 1600476.**

• Fahrni, C. J., (2007). Biological applications of X-ray fluorescence microscopy: exploring the subcellular topography and speciation of transition metals. *Curr. Opin. Chem. Biol***.11(2),121–127.**

• Farquharson, M.J., Geraki, K., Falkenberg, G., Leek, R. & Harris, A. (2007).The localization and micro-mapping of copper and other trace elements in breast tumours using a synchrotron micro-XRF system. *Appl. Rad. Isotop.* **65 (2),183–188.**

• Hell , S.W. (2003). Toward ûuorescence nanoscopy**.** *Nat Biotechnol* **21: 1347– 1355.**

• Helmchen, F., Denk, W.(2005). Deep tissue two-photon microscopy**.** *Nat Methods* **2: 932–940.**

• Haggarty, S.J., Mayer, T.U., Miyamoto, D.T., Fathi, R, King RW., Mitchison ,T.J., Schreiber, S.L., (2000). Dissecting cellular processes using small molecules: identiûcation of colchicine-like, taxol-like and other small molecules that perturb mitosis. *Chem Biol* **;7:275–286.**

• Jalbert, I., Stapleton, F., Papas, E., Sweeney ,D. F., Coroneo, N., (2003). In Vivo Confocal microscopy of the human cornea: *Br J Opthalmo***:87:225-236**.

• Jin, Q., Paunesku, T., Lai, B., Glebber, S.C.,Chen, S. I., Finney, L., Vine, D., Vogt, S., Woloschak, G., Jacobson, C., (2017).Preserving elemental content in adherent mammalian cells for analysis by synchrotron based X-ray fluorescence microscopy. *Journal of Microscopy,***Vol.265, Issue 1, pp81-93.**

• Leskovjan, A.C., Kretlow, A., Lanzirotti, A., Barrea, R., Vogt, S. & Miller, L.M.(2011).Increased brain iron coincides with early plaque formation in a mouse model of Alzheimer'sdisease. *Neuro Image* **55(1), 32–38**.

• Liao, M., Cao, E., Julius, D., Cheng ,Y.,(2013). Structure of the TRPV1 ion channel etermined by electron cryo-microscopy. *Nature,* **504: 107–112PubMed CentralPubMed CrossRef Google Scholar**

• Majumdar, S., Peralta-Videa, J.R., Castillo-Michel, H., Hong, J., Rico, C.M. & Gardea-Torresdey, J.L. (2012): Applications of synchrotron µ-XRF to study the distribution of b biologically important elements in different environmental matrices: : a review. *Analytic.Chim.Acta.***755, 1–16**.

• McMullan, D., (2006).Scanning electron microscopy **1928–1965. Scanning 17:175CrossRef**

• Neumann, B., Held, M., Liebel, U., Erûe, H., (2006). High-throughput RNAi screening by time-lapse imaging of live human cells. *Nat Methods* **3: 385–90.**

• Nixon, S.J. (2009) . A single method for cryofixation and correlative light, electron microscopy and tomography of zebrafish embryos**.** Traffic **10, 131–136**.

• Ni, L., Snyder, M., (2001). A genomic study of the bipolar bud site selection pattern in Saccharomyces cerevisiae. *Mol Biol Cell,* **12:2147– 2170.**

• ***Ortega, R., Dev'es, G., & Carmona, A., (2009)*** Bio-metals imaging and speciation in cells using proton and synchrotron radiation X-ray microspectroscopy.*J. R. Soc. Interf.* **6,S649–S658**.

• Perkovic, M. (2014) Correlative light- and electron microscopy with chemical tags. J. Struct. Biol. **186, 205–213.**

• Paunesku, T., Vogt, S., Maser, J., Lai, B. & Woloschak, G. (2006): X-ray fluorescence microprobe imaging in biology and medicine**.** *J. Cell. Biochem.***99(6),1489–1502.**

• Paunesku, T., Rajh, T., Wiederrecht, G.et.al. (2003).Biology of TiO2 oligonucleotide nanocomposites.*Nat.Mater.***2(5),343–346.**

• Pepperkok ,R, Simpson, J.C., Wiemann, S.,(2001). Being in the right location at the right time. *Genome Biol ;***2:1024.1–.4.**

• Perlman, Z.E., Slack, M.D., Feng, Y., Mitchison, TJ.,(2004).Multidimensional drug proûling by automated microscopy. *Science* **306: 1194–8**.

• Rosania, G.R., Merlie, J. J.R., Gray, N., Chang ,Y.T., Schultz, P.G., Heald, R.,(1999). A cyclin-dependent kinase inhibitor inducing cancer cell differentiation: biochemical identiûcation using Xenopus egg extracts**.** *Proc Natl Acad Sci USA* **96:4797–4802.**

• Sanderson MJ, Smith I, Parker I, Bootman MD.(2014). Fluorescence microscopy. *Cold Spring Harb Protoc.* **(10):pdb top071795.**

• Solomonov, I., (2014). Introduction of correlative light and airSEM microscopy imaging for tissue research under ambient conditions. *Sci. Rep.* **4, 5987**.

• Swedlow, J.R., (2012). Innovation in biological microscopy: current status and future directions. *Bioessays;* **34 , (5):333-340**.

• Vogt ,S. & Ralle, M. (2013).Opportunities in multidimensional trace metal imaging: taking copper-associated disease research to the next level. *Analytic.Bioanalytic.Chem***. 405(6),1809–1820.**

• Van, R.C., Oorschot, V., & Klumperman, J., (2008). Correlative light-electron microscopy (CLEM) combining live-cell imaging and immunolabeling of ultrathin cryosections. ***Nat. Methods* 5, 973–980**

• Yuan, Y., Chen, S., Paunesku, T., (2013). Epidermal growth factor receptor targeted nuclear delivery and high-resolution whole cell X yimaging of Fe_2O_3 and TiO_2 nanoparticles in cancer cells**.** *ACS Nano* **7(12),10502–10517**.

• Zhang, X., Jin, L., Fang, Q., Hui, W.H., Zhou, Z.H.,(2010). 3.3 Å cryo-EM structure of a nonenveloped virus reveals a priming mechanism for cell entry. *Cell,* **141: 472–482** **PubMedCentralPubMedCrossRefGoogle Scholar**

• Zhang, X, Ge, P, Yu ,X, Brannan, J.M., Bi G., Zhang, Q, Schein, S, Zhou, Z.H.,(2013). Cryo-EM structure of the mature dengue virus at 3.5-Å resolution**.** *Nat Struct Mol Biol,* **20: 105–110 PubMedCentralPubMedCrossRefGoogle Scholar**