

# Microscopes As An Adjunctive Tool In Endodontic Research

Author

---

## Abstract:

**Background:** Endodontic research aims to improve the understanding, diagnosis, and management of diseases of the pulp and periradicular tissues. Given the complex anatomy of the root canal system and the microscopic nature of many pathological and structural changes, conventional visual methods often fall short in providing adequate detail. Microscopes have therefore emerged as an important adjunctive tool in endodontic research, offering enhanced magnification, illumination, and resolution.

The use of various microscopic techniques has significantly advanced the evaluation of dentin structure, canal morphology, smear layer removal, sealer penetration, microbial biofilms, and material–tissue interactions. Light microscopy, stereomicroscopy, confocal laser scanning microscopy, scanning electron microscopy, and atomic force microscopy each contribute unique insights at different structural and dimensional levels. These tools allow researchers to visualize phenomena that are otherwise undetectable, thereby improving the accuracy, reproducibility, and scientific validity of experimental studies.

Incorporation of microscopes in endodontic research has strengthened the correlation between laboratory findings and clinical outcomes. As a result, microscopy plays a pivotal role in generating evidence-based data, supporting material development, and refining clinical techniques, ultimately contributing to improved endodontic care.

**Key Word:** Microscope, Endodontic research, Root canal system

---

Date of Submission: 02-02-2026

Date of Acceptance: 12-02-2026

---

## I. Introduction

Advances in endodontic research depend on the accurate visualization and analysis of structures and processes occurring at the microscopic level. The complex anatomy of the root canal system, including dentinal tubules, biofilms, and material–tissue interfaces, cannot be adequately assessed using unaided vision or conventional clinical imaging methods. Microscopy has therefore become an essential adjunct in endodontic research, enabling detailed investigation of phenomena beyond the resolution of the human eye.<sup>1</sup>

Microscopic techniques provide enhanced magnification, resolution, and contrast, allowing precise evaluation of dentin morphology, smear layer characteristics, microbial organization, and the adaptation and penetration of endodontic materials.<sup>2,3,4</sup> Various modalities such as light microscopy, stereomicroscopy, fluorescence microscopy, confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM), and atomic force microscopy (AFM) offer complementary qualitative and quantitative insights at different structural levels.<sup>5-11</sup> These techniques facilitate controlled in vitro and ex vivo assessments of surface topography, interface integrity, and three-dimensional spatial relationships within the root canal system.<sup>12-16</sup>

The integration of microscopy into endodontic research has improved methodological rigor, reproducibility, and interpretation of experimental data.<sup>17</sup> By strengthening the correlation between laboratory findings and clinical relevance, microscopic evaluation continues to play a pivotal role in evidence-based advancements in endodontic materials, techniques, and treatment outcomes.

### Simple And Compound Microscope

**Applications:** Used for basic visualization of cells, tissues, and microorganisms; forms the foundation of histological and microbiological studies in dentistry and aids in understanding general morphology and tissue organization.

**Advantages:** Simple to use, cost-effective, widely available, and suitable for routine laboratory examination with adequate magnification for preliminary assessments.

**Disadvantages:** Limited resolution due to the wavelength of visible light, reduced depth of field at higher magnifications, and inability to visualize ultrastructural details.<sup>18-21</sup>

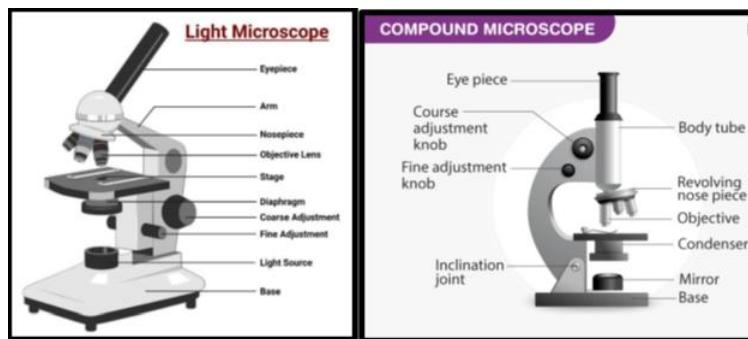


Fig. 1

### Phase Contrast Microscopy

**Applications:** Used to visualize unstained, transparent biological specimens, enabling observation of live cells and intracellular structures.

**Advantages:** Permits examination of living specimens without staining, preserving cellular integrity and enabling dynamic studies.

**Disadvantages:** Halo artifacts, reduced image quality in thick specimens, and limited applicability for opaque samples.<sup>22,23</sup>

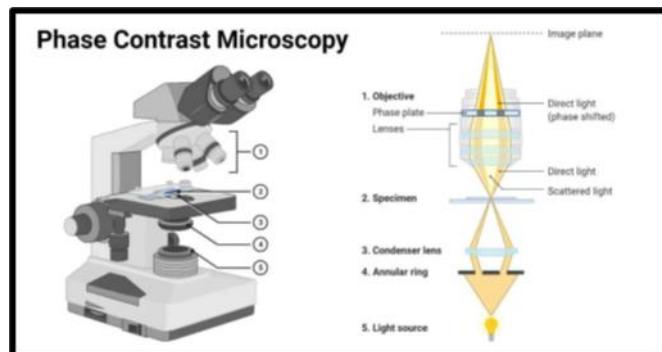


Fig. 2

### Darkfield Microscopy

**Applications:** Useful for visualizing fine structures such as microorganisms and edges that are poorly seen under brightfield illumination.

**Advantages:** Produces high-contrast images against a dark background and improves visibility of thin, unstained specimens.

**Disadvantages:** Low light intensity, difficulty with thick specimens, and increased image noise.<sup>18,19</sup>

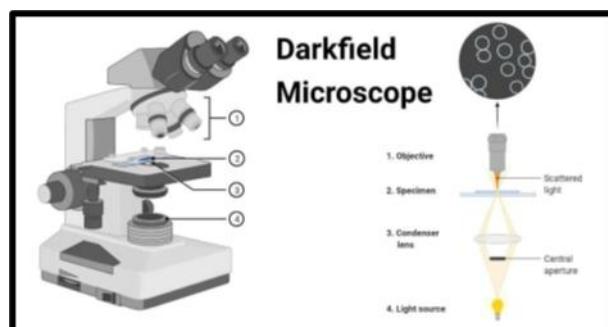


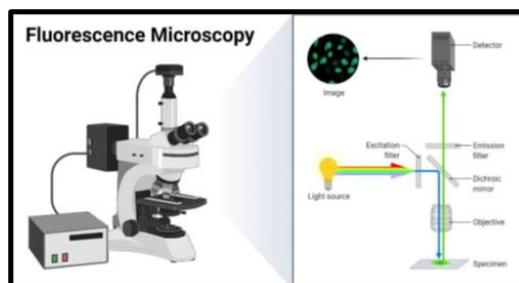
Fig. 3

### **Fluorescence Microscopy**

**Applications:** Used to study microbial biofilms, cellular components, and distribution of fluorophore-labeled materials in dental tissues.

**Advantages:** High sensitivity and specificity with the ability to localize specific structures using fluorescent dyes.

**Disadvantages:** Photobleaching, phototoxicity, and limited penetration depth in thick specimens. <sup>1,16,17,23-29</sup>



**Fig. 4**

### **Confocal Laser Scanning Microscopy (CLSM)**

**Applications:** Extensively used to evaluate sealer penetration, smear layer removal, biofilm architecture, and three-dimensional tissue analysis.

**Advantages:** Optical sectioning, three-dimensional reconstruction, reduced background noise, and ability to examine hydrated specimens.

**Disadvantages:** High cost, limited depth penetration, and dependence on fluorescent labeling. <sup>1,10,15,16,17,24-29</sup>



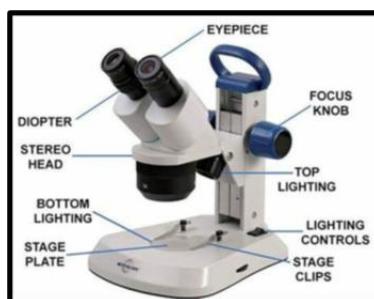
**Fig. 5**

### **Stereomicroscope**

**Applications:** Used for evaluating root canal morphology, apical microleakage, working length determination, fracture analysis, and gross specimen examination.

**Advantages:** Provides three-dimensional visualization with a large depth of field and longer working distance.

**Disadvantages:** Lower magnification and resolution compared with compound microscopes, limiting cellular-level analysis. <sup>23,30-33</sup>



**Fig. 6**

### Scanning Electron Microscope (SEM)

**Applications:** Used to assess dentin surface topography, smear layer removal, sealer–dentin interface, bacterial biofilms, and fracture patterns.

**Advantages:** Very high resolution with excellent depth of field and detailed surface morphology visualization.

**Disadvantages:** Expensive instrumentation, technique-sensitive specimen preparation, vacuum environment, static imaging, and potential artifacts due to dehydration and coating.<sup>21,35-44</sup>

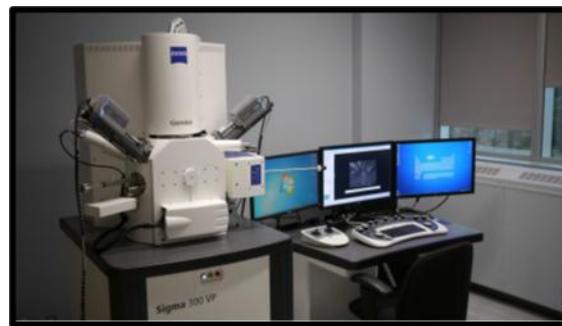


Fig. 7

### Atomic Force Microscope (AFM)

**Applications:** Used to evaluate nanoscale surface topography, mechanical properties of dentin and biomaterials, and biological samples in near-physiological conditions.

**Advantages:** Nanometer-scale resolution, three-dimensional imaging, minimal sample preparation, and ability to study samples in liquid environments.

**Disadvantages:** Small scanning area, time-consuming imaging, high cost, and operator sensitivity.<sup>45-57</sup>

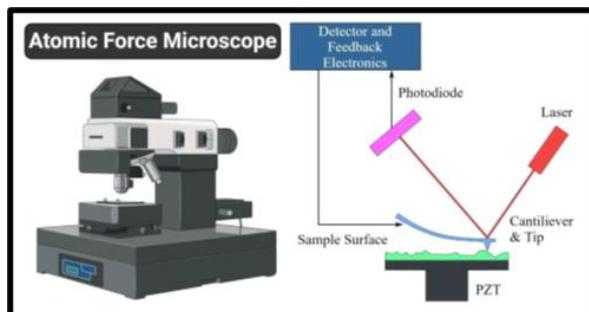


Fig. 8

### Petrographic microscope/polarizing microscope

It can magnify objects between  $\times 4$  and  $\times 100$ . Minerals, ceramics, polymers, urea, fungus, collagen, and amyloid may all be seen using distinct light transmission qualities of material, including crystalline structures.<sup>32,57</sup>

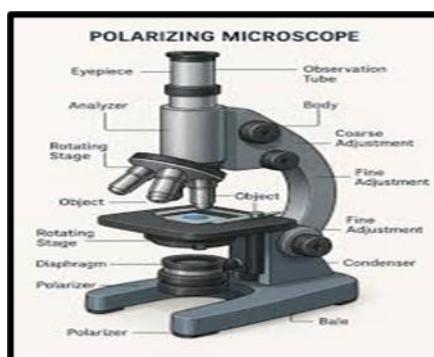
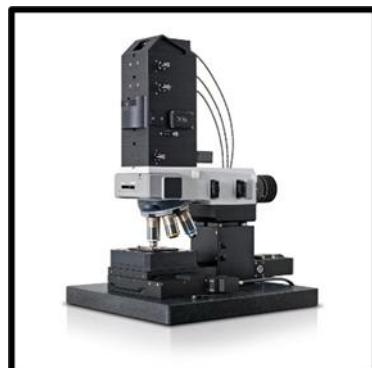


Fig. 9

### Scanning near-field optical microscope

Using the characteristics of evanescent waves, the Scanning near-field optical microscope (SNOM)/near-field scanning optical microscopy microscopic approach for nanostructure analysis overcomes the far-field resolution restriction. Life science and material studies utilize it to detect minuscule surfaces.

**Advantage:** In nanotechnology, nanophotonics, and nano-optics, it is suitable for rapidly and easily imaging optical characteristics of samples. In the SNOM, single-molecule detection is simple. A subwavelength study of dynamic characteristics is also possible. It has a resolution of 70 times greater than an AFM.<sup>58,59</sup>



**Fig. 10**

### II. Conclusion

Microscopy plays a vital role in endodontic research by enabling detailed visualization of the complex microanatomy of the root canal system and material–tissue interactions. Advanced microscopic techniques enhance the accuracy and reproducibility of experimental studies, thereby strengthening the quality of evidence generated. By supporting precise evaluation of biological and material-related parameters, microscopy bridges laboratory research with clinical relevance and continues to contribute significantly to evidence-based advancements in endodontic science.

### References

- [1]. K. R. Spring, Detectors For Fluorescence Microscopy, In A. Periasamy (Ed.), *Methods In Cellular Imaging*, New York: Oxford University Press, 40-52, 2001 \
- [2]. J. Art, Photon Detectors For Confocal Microscopy, In J. B. Pawley (Ed.), *Handbook Of Biological Confocal Microscopy*, New York: Plenum Press, 183-196, 1995.
- [3]. W. B. Amos, Instruments For Fluorescence Imaging, In V. J. Allan (Ed.), *Protein Localization By Fluorescence Microscopy: A Practical Approach*, New York: Oxford University Press, 67-108, 2000.
- [4]. E. Hergert, Detectors: Guideposts On The Road To Selection, *Photonics Design And Applications Handbook*, H110-H113, 2001.
- [5]. W. Piston, G. H. Patterson, And S. M. Knobel, Quantitative Imaging Of The Green Fluorescent Protein (Gfp), In K. F. Sullivan And S. A. Kay (Eds.), *Green Fluorescent Proteins, Methods In Cell Biology*, Volume 58, New York: Academic Press, 31-47, 1999.64. D. R. Carter, *Photomultiplier Handbook: Theory, Design*
- [6]. D. R. Carter, *Photomultiplier Handbook: Theory, Design, Application*, Lancaster, Pennsylvania: Burle Industries, Inc., 1980.
- [7]. J. Art, Photon Detectors For Confocal Microscopy, In J. B. Pawley (Ed.), *Handbook Of Biological Confocal Microscopy*, New York: Plenum Press, 183-196, 1995
- [8]. W. B. Amos, Instruments For Fluorescence Imaging, In V. J. Allan (Ed.), *Protein Localization By Fluorescence Microscopy: A Practical Approach*, New York: Oxford University Press, 67-108,
- [9]. I. C. Chang, *Acousto-Optic Devices And Applications*, In M. Bass, E. W. Van Stryland, D. R. Williams, And W. L. Wolfe, *Optics II: Fundamentals, Techniques, And Design*, New York: McGraw-Hill, 12.1-12.54, 1995.
- [10]. E. S. Wachman, *Acousto-Optic Tunable Filters For Microscopy*, In R. Yuste, F. Lanni, A. Konnerth (Eds.), *Imaging Neurons: A Laboratory Manual*, New York: Cold Spring Harbor Press, 4.1-4.8, 2000.
- [11]. R. D. Shonat, E. S. Wachman, W. Niu, A. P. Koretsky, And D. L. Farkas, Near-Simultaneous Hemoglobin Saturation And Oxygen Tension Maps In Mouse Brain Using An Aotf Microscope, *Biophysical Journal*, 73: 1223-1231, 1997.
- [12]. E. S. Wachman, W. Niu, And D. L. Farkas, Imaging Acousto-Optic Tunable Filter With 0.35-Micrometer Spatial Resolution, *Applied Optics*, 35: 5220-5226, 1996.
- [13]. R. D. Shonat, E. S. Wachman, W. Niu, A. P. Koretsky, And D. L. Farkas, Near-Simultaneous Hemoglobin Saturation And Oxygen Tension Maps In Mouse Brain Using An Aotf Microscope, *Biophysical Journal*, 73: 1223-1231, 1997.
- [14]. Y. Chen, J. D. Mills, And A. Periasamy, Protein Localization In Living Cells And Tissues Using Fret And Flim, *Differentiation*, 71:528-541, 2003
- [15]. E. H. K. Stelzer, Contrast, Resolution, Pixelation, Dynamic Range, And Signal-To-Noise Ratio: Fundamental Limits To Resolution In Fluorescence Light Microscopy, *Journal Of Microscopy*, 189: 15-24, 1997.
- [16]. J. Pawley, Fundamental Limits In Confocal Microscopy, In J. B. Pawley (Ed.), *Handbook Of Biological Confocal Microscopy*, New York: Plenum Press, 19-37, 1995.
- [17]. R. H. Webb And C. K. Dorey, The Pixelated Image, In J. B. Pawley (Ed.), *Handbook Of Biological Confocal Microscopy*, New York: Plenum Press, 55-67, 1995.

[18]. Rayleigh Fl. Investigations In Optics, With Special Reference To The Spectroscope. London, Edinburgh, Dublin Philos Mag J Sci Ser. 1879;5(8):261-74.

[19]. "Dissecting Microscope." Merriam-Webster.Com Dictionary, Merriam-Webster. [Accessed 1 Mar. 2024] Shannon Rr, Ford Bj. Microscope. Encyclopedia Britannica; 2024.

[20]. Ford Bj. Single Lens: The Story Of The Simple Microscope Hardcover. Harpercollins; 1985.

[21]. Rochow, T.G., Rochow, E.G. (1978). Scanning Electron Microscopy. In: An Introduction To Microscopy By Means Of Light, Electrons, X-Rays, Or Ultrasound. Springer, Boston, Ma.

[22]. Parija Sc. Textbook Of Microbiology & Immunology. 2edn. India: Elsevier India; 2012.

[23]. Lichtman Jw, Conchello Ja. Fluorescence Microscopy. Nature Methods 2005;2:910-9.

[24]. Y . Chen, J. D. Mills, And A. Periasamy, Protein Localization In Living Cells And Tissues Using Fret And Flim, Differentiation, 71: 528-541, 2003.

[25]. H. Wallrabe And A. Periasamy, Imaging Protein Molecules Using Fret And Flim Microscopy, Curr. Opin. Biotech., 16: 19-27, 2005.

[26]. R. N. Day, A. Periasamy, And F. Schaufele, Fluorescence Resonance Energy Transfer Microscopy Of Localized Protein Interactions In The Living Cell Nucleus, Methods, 25: 4-18, 2001.

[27]. G. H. Patterson And J. Lippincott-Schwartz, A Photoactivatable Gfp For Selective Photolabeling Of Proteins And Cells, Science, 297: 1873-1877, 2002.

[28]. V. V. Verkhusha And K. A. Lukyanov, The Molecular Properties And Applications Of Anthozoa Fluorescent Proteins And Chromoproteins, Nature Biotechnology, 22: 289-296, 2004.

[29]. R. Ando, H. Hama, M. Yamamoto-Hino, H. Mizuno, And A. Miyawaki, An Optical Marker Based On The Uv-Induced Green-To-Red Photoconversion Of A Fluorescent Protein, Proc. Natl. Acad. Sci. Usa, 99: 12651-12656, 2002.

[30]. Ippel E, Derose J, Goegegel D, Schué A, Müller C. Key Factors To Consider When Selecting A Stereo Microscope: Choose The Right Stereo Microscope For Your Application. Science Lab [Leica Microsystems]; 2012.

[31]. Shobhita Kc, Shyam N, Kumar Gk, Narayen V, Priyanka M, Shravani R, Et Al. Stereomicroscope As An Aid In Grossing And Histopathological Diagnosis: A Prospective Study. J Oral Maxillofac Pathol. 2020;24(3):459-65.

[32]. Shah Pu, Mane Dr, Angadi Pv, Hallikerimath Sr, Kale Ad. Efficacy Of Stereomicroscope As An Aid To Histopathological Diagnosis. J Oral Maxillofac Pathol 2014;18:356-60.

[33]. Swarupa Ch, Sajjan Gs, Sashi Kanth Y. An In Vitro Stereomicroscopic Comparative Evaluation Of A Combination Of Apex Locator And Endodontic Motor With An Integrated Endodontic Motor. J Conserv Dent 2013;16:458-61.

[34]. Saghiri Ma, Asgar K, Lotfi M, Saghiri Am, Neelakantan P Et Al. Back-Scattered And Secondary Electron Images Of Scanning Electron Microscopy In Dentistry: A New Method For Surface Analysis. Acta Odontol Scand 2012;(18):1-7

[35]. Coraça-Huber Dc, Fille M, Hausdorfer J, Pfaller K, Nogler M. Staphylococcus Aureus Biofilm Formation And Antibiotic Susceptibility Tests On Polystyrene And Metal Surfaces. J Appl Microbiol 2012; 112(6):1235-43.

[36]. Li Hf, Wang Yb, Zheng Yf, Lin Jp. Osteoblast Response On Ti- And Zr-Based Bulk Metallic Glass Surfaces After Sand Blasting Modification. J Biomed Mater Res B Appl Biomater. 2012; 18:1721-8.

[37]. Rosslenbroich Sb, Raschke Mj, Kreis C, Hans-Tholema N, Uekoetter A, Reichelt R Et Al. Daptomycin: Local Application In Implant-Associated Infection And Complicated Osteomyelitis. Scientificworldjournal 2012; 18:1-9.

[38]. Gu Y-X, Du J, Zhao J-M, Si M-S, Mo J-J, Lai H-C. Characterization And Preosteoblastic Behavior Of Hydroxyapatite-Deposited Nanotube Surface Of Titanium Prepared By Anodiza-Tion Coupled With Alternative Immersion Method. J Biomed Mater Res B Appl Biomater. 2012; 30:1-9.

[39]. Yu X, Ning C, Li J, Huang S, Guo Y, Deng F. In Vivo Evaluation Of Novel Amine-Terminated Nanopore Ti Surfaces. J Biomed Mater Res A. 2012 Jul 13; 1-8

[40]. Wang W, Tao R, Tong Z, Ding Y, Kuang R, Zhai S Et Al. Effect Of A Novel Antimicrobial Peptide Chrysophsin-1 On Oral Pathogens And Streptococcus Mutans Biofilms. Peptides 2012;33(2):212-9.

[41]. Agrawal Vs, Kapoor S. An In Vitro Scanning Electron Microscopic Study Comparing The Efficacy Of Passive Ultrasonic And Syringe Irrigation Methods Using Sodium Hypochlorite In Removal Of Debris From The Root Canal System. J Ir Dent Assoc. 2012;58(3):156-61.

[42]. Kuga Mc, Campos Ea, Faria-Júnior Nb, Só Mv, Sinohara Al. Efficacy Of Niti Rotary Instruments In Removing Calcium Hydroxide Dressing Residues From Root Canal Walls. Braz Oral Res 2012;26(1):19-23..

[43]. Souza Sfc, Franci C, Bombana Ac, Kenshima S, Barroso Lc, D'agostino Lc Et Al. Qualitative Sem/Eds Analysis Of Microleakage And Apical Gap Formation Of Adhesive Root-Filling Materials. J Appl Oral Sci 2012; 20(3):329-34

[44]. Fotiadis D, Scheuring S, Müller Sa, Engel A, Müller Dj. Imaging And Manipulation Of Biological Structures With The Afm. Micron. 2002;33:385-97.

[45]. Nano Science Instruments [Internet]. Usa: Atomic Force Microscopy And Nanoprobe; 2002. The Institute [Updated In June 2010].

[46]. Sokolov I. Atomic Force Microscopy In Cancer Cell Research [Internet]. Cancer Nanotechnology; 2007

[47]. Hersam Mc, Chung Yw. Detecting Elusive Surface Atoms With Atomic Force Microscopy. Proc Natl Acad Sci U S A. 2003;100:12531-2.

[48]. Binnig G, Rohrer H. Scanning Tunnelling Microscopy-From Birth To Adolescence. Rev Mod Phys. 1987;59:615-25

[49]. Nano Science Instruments [Internet]. Usa: Atomic Force Microscopy And Nanoprobe; 2002.

[50]. Wiesendanger R. Scanning Probe Microscope And Spectroscopy [Internet]. Cambridge University Press, Cambridge; 1994

[51]. Piner Rd, Zhu J, Xu F, Hong S, Mirkin Ca. "Dip- Pen" Nanolithography. Science. 1999;283:661 3.

[52]. Xia Y, Whitesides Gm. Extending Microcontact Printing As A Microlithographic Technique Langmuir.

[53]. Pelling Ae, Sehati S, Gralla Eb, Valentine Js, Gimzewski Jk. Local Nanomechanical Motion Of The Cell Wall Of Saccharomyces Cerevisiae. Science. 2004;305:1147-50

[54]. Hörber Jk, Miles MJ. Scanning Probe Evolution In Biology. Science. 2003;302:1002-5.

[55]. Greenleaf Wj, Woodside Mt, Block Sm. High Resolution, Single-Molecule Measurements Of Biomolecular Motion. Annu Rev Biophys Biomol Struct. 2007;36:171-90.

[56]. Charras Gt, Horton Ma. Single Cell Mechanotransduction And Its Modulation Analyzed By Atomic Force Microscope Indentation. Biophys J. 2002;82:2970-81

[57]. Heydarian H, Yazdanfar P, Zarif A, Rashidian B. Near Field Differential Interference Contrast Microscopy. Sci Rep 2020;10:9644

[58]. Ushiki T. Scanning Probe Microscopy And Its Biomedical Application From The Historical Viewpoint. Microscopy 2019;68:127-21.

[59]. Thibierge S, Nechushtan A, Sprinzak D, Gileadi O, Behar V, Zik O, Et Al. Scanning Electron Microscopy Of Cells And Tissues Under Fully Hydrated Conditions. Proc Natl Acad Sci U S A 2004;101:3346-51.