MATEC Web of Conferences **32**, 04001 (2015)
DOI: 10.1051/matecconf/20153204001

Optomechatronics for Biomedical Optical Imaging: An Overview

Hyungsuck Cho,
Department of Mechanical Engineering
KAIST, Daejeon, Korea

Abstract. The use of optomechatronic technology, particularly in biomedical optical imaging, is becoming pronounced and ever increasing due to its synergistic effect of the integration of optics and mechatronics. The background of this trend is that the biomedical optical imaging for example in-vivo imaging related to retraction of tissues, diagnosis, and surgical operations have a variety of challenges due to complexity in internal structure and properties of biological body and the resulting optical phenomena. This paper addresses the technical issues related to tissue imaging, visualization of interior surfaces of organs, laparoscopic and endoscopic imaging and imaging of neuronal activities and structures. Within such problem domains the paper overviews the states of the art technology focused on how optical components are fused together with those of mechatronics to create the functionalities required for the imaging systems. Future perspective of the optical imaging in biomedical field is presented in short.

1 Introduction

Biomedical optical imaging in this paper refers to a technique for visualization needed for looking the interior of tissue, organ or entire body for clinical analysis and medical intervention. This visualization technology has evolved with the progress of the technology related to clinical procedure and surgical operation: In the case of surgery, 2D television for laparoscopic diagnosis and surgery, a high definition 3D vison in Da Vinci surgical system, guiding image provided by computed tomography, magnetic resonance imaging for surgical planning, and many other imaging modalities used in endoscopic, single port, and natural orifice transluminal endoscopic surgery. Along with these developments, there have been a vast amount of developments in the imaging field needed to carry out in-vivo or ex-vivo clinical analysis. Some examples include those for detection of targets such as cancers or tumors, examining interior surface or deep inside of tissues or organs, visualizing body cavities, and biopsy to remove tissue or a sample of cells. In addition, many developments have been made for guidance of imaging or surgical devices, and manipulating such devices for the enhanced view.

Despite of the complexity involved in the aforementioned tasks, most of the optical imaging works have been done with standard well-structured, time invariant passive optical systems. However, when it comes to looking inside the hollow cavity or the surface of inner organs or imaging of their tissues in a minimally or noninvasive manner, the passive optical system alone is insufficient to achieve high image quality. This may be attributable to the fact that imaging should cope with complex restricted space of body cavity and organ, optical properties of body contents and thick tissue and so on. In order to solve the problems many developments have been made over the past decades as to new imaging modality, optical system design and imaging device maneuverability. Here, came a contribution of optomechatronics, which makes the imaging system active, adjustable and adaptive to the variation of imaging conditions.

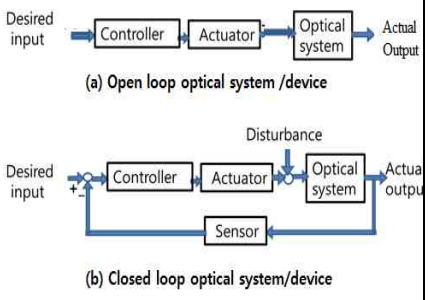
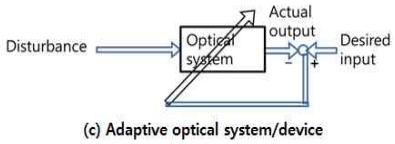
Optomechatronic technology, a multidisciplinary technology, integrates optics and mechatronics synergistically, thus enhancing system performance by creating new functionalities or creating new systems. For example, a simple scanner works in this principle: A mechatronic device interacts with light, diverting light to a desired sequence. Similarly, acousto-optic modulator (AOD) uses the interaction of light with travelling acoustic wave generated by oscillation of crystal materials. More complex systems operated in optomechatronic principle can be found from microscopy systems. An atomic force microscope (AFM) performs the topology measurement by using the interaction of a cantilever tip motion with incident light. Also, various microscopes are integrated with optomechatronic devices such as varifocal lens (VL), AOD, liquid crystal spatial light modulator (LCSLM), deformable mirror (DM) or their combination to improve imaging quality [1]. Table 1 depicts optical systems from the control point of view. The former two cases represent an open loop structure while AFM represents a closed loop system. One more control structure shown in the table is an adaptive control system in which the actuator (e.g. piezo actuator) controls signal is so generated that the optical system (deformable mirror) is adapted to reduce the disturbance (wavefront distortion) coming into the system.

This paper addresses the technical issues associated with the imaging tasks and overviews how the optomechatronics are applied to achieve the required imaging goals. The problem statements and states of the

Hyungsuck Cho: hscho@kaist.ac.kr

art technology are presented in short. In the last part of the paper, future prospects are briefly discussed.

Table 1 Optical systems and control modality

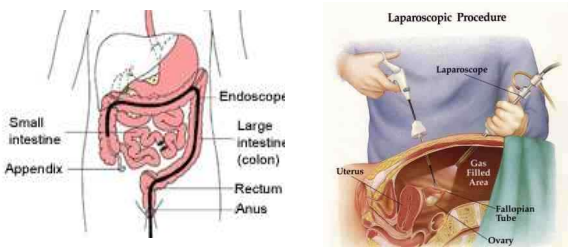
Optical function	Control modality and structure
● Focusing ● Beam shaping ● Beam steering ● Scanning over imaging field ● Beam ● Diffraction ● Zooming ● Scattering ● Filtering	
● Aberration reduction (Wave front control)	

2 Problem Statements and Optomechatronic Considerations

Referring to parts of human body in Figure 1, the fundamental considerations that challenge to the imaging of interest can be classified into the following six issues: (1) purpose of the imaging such as diagnosis, biopsy, surgery and so on (2) location of the spot or area of the imaging target (3) optical system design that satisfies the required imaging objectives (4) delivery of the system to the target area (5) maneuverability of the imaging device to solve the limited field of view and view direction (6) in vivo motion of the tissue or organ which is internally generated disturbance.

The above considerations vary with the site of the human body and thus create body area-specific imaging devices such as colonoscopy examining the colon, the last part of gastrointestinal (GI) tract, gastroscopes used to check the oesophagus and stomach, broncoscopes used to examine the lungs and airways, and so on [2,3]. The figure shows the endoscopic GI tract (a) and abdominal laparoscopic procedure (b) in which imaging or surgical operations are performed in a closed abdominal cavity. In both cases, however, tissue properties differ from area to area, requiring different imaging modalities. Further, in case of GI tract the size and complexity in shape of the hollow cavity yield considerably small field of view (FOV) of the camera whose image is transmitted to the surgeon. This makes it problematic for manipulation of imaging devices.

All of the challenges stated above affect the required image quality. The imaging goal is to attain high quality of image, namely, with faster acquisition speed, higher resolution and sharper contrast. In case of tissue imaging one more factor, depth of imaging is ultimately important for disease diagnosis and clinical analysis. In this tissue imaging the quality factors are, in general, a function of illumination method, type of light source, optical properties of tissue such as scattering, reflectance, transparency or turbidity, and properties of tissue material, and the tissue-light interaction. When light is incident onto the tissue, the interaction between tissue and light occurs in such a way that the intensity of the penetrated light exponentially attenuates as it progresses through the tissue. Figure 2 compares two performance variables of various modalities in tissue imaging [4]. The confocal



(a) GI tract [2] (b) Abdominal cavity [3]

Figure 1 Imaging of various parts of body cavities

microscopy has resolution of 1㎛ relatively very high, whereas depth penetration is ranged around 500㎛. Multiphoton microscope not shown here lies nearly within the same range and OCT has resolution and penetration depth, respectively, are ranged 2-10 ㎛ and 2-3 mm. Other methods such as photoacoustic (ultrasound), CT and MRI have much deeper penetration depth, but their resolution is relative low.

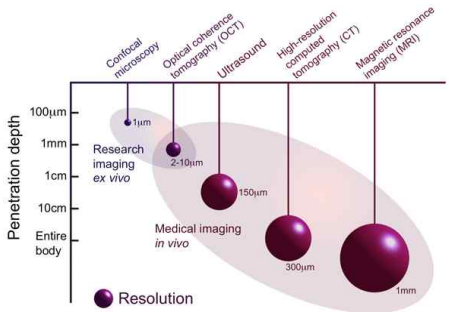


Figure 2 Comparison of modalities for tissue imaging [4]

Table 2 Optomechatronic approach applied to optical imaging

Technical field	Optomechatronic approach	Enabling devices
Diagnosis / treatment		Microscopes Endoscopes
Deeper tissue	Adaptive Optics wave front control	DM, SLM, DMD
Thick tissue	Extending DOF	Varifocal lens Beam shaping SLM
Scattering tissue (Turbid)	Focusing Optical coupler	SLM, Moving mechanism
Interior surface of organ	View angle change	Wedge prisms Rotating endo-tip
Hollow/narrow space	Focal /FOV control	Endomicroscopes Spectrometer
	Multiresolution within FOV	SLM, Scanner mirror
Abdominal/GI tract	In-vivo mobile, Modular robot	Robot platform Self-assembly

Table 2 lists the technical issues and the related optomechatronic solution approaches. As an illustration here it presents the imaging challenges in six different fields. As seen from the table, tissue imaging concerns three different cases of tissues where optomechatronic approach is effectively applied with somehow different concepts to enhance imaging quality. Likewise, the optomechatronic approaches are listed for imaging various parts of biological body. The third column indicates the devices that enable to create such techniques.

This paper groups imaging challenges into four distinct technical fields for the overview: (1) tissue imaging for diagnosis and treatment of diseases, (2) imaging for examination of interior surface of organs and surgical operations, (3) optical imaging stabilization, (4) neuronal structural and activities. Due to a great number of developments in imaging methods of each subject above, only some are reviewed here, focusing those on microscopy, endoscopy, and surgical operations.

3 Optical imaging with optomechatronic principle

A number of microscopes have evolved in a formidable speed in recent years to enhance their ability of visualization. In this section, some of the existing microscopes operated in the optomechatronic principle and the microscopes integrated with mechatronic elements will be examined in short from the perspectives of the operation principle.

3.1Microscope in optomechatronic configuration

Typical example can be found from the optical coherence tomography (OCT). It consists of an interferometer and two scanners that control the scan angle of the beam (femtosecond or broadband superluminescent diode) [5]. Due to this optmechatronic configuration, the time domain together with the spectrum-Fourier domain OCT yields much greater penetration depth through scattering tissue than confocal microscope. Confocal microscope is also operated by optomechatronic principle where a refocusing unit for the objective moves the points of excitation and emission on a specimen to a new plane, two galvanometers scan the x-y planes of the tissue volume, and AOD for laser intensity control and wavelength selection. Another example is the dual axis confocal (DAC) microscope that obtains high resolution axial image from the tissue with deep penetration and employs a post objective scanning [6]. The scanning mirror is placed distal to the two low NA illumination and collection objectives and synchronously scans the two beams from the objectives. The axial scan of the sample is provided by a closed loop controlled micro piezoelectric actuator.

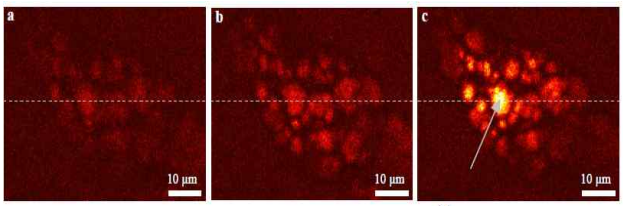
3.2 Microscopes and endoscopes combined with optomechatronic devices: Tissue imaging

A huge number of developments in microscopes and imaging systems have been made from the view point of improving imaging quality. Only some of them are introduced here due to space limitation.

3.2.1 Imaging deeper into tissue

Various microscopes have been integrated with adaptive optical elements [7] for imaging deeper at high resolution by eliminating or reducing the aberration caused by incoming wavefront distortion inside tissue. The use of DM [8, 9] is typical of these techniques and Figure 3 illustrates enhanced deep tissue imaging. Other methods include the use of LCSLM [10], extending DOF of the objective [11], light beam shaping technique [12] in thick tissue, multifocal structured illumination [13] and making incident wavelength longer in two photon and multiphoton microscopy.

In recent years, imaging through scattering media such as deep tissue has been actively researched. The pioneering work in this area used a wave front shaping technique via SLM. The modulator located in front of an opaque sample is so modulated by a feedback control that the modulated light is focused anywhere behind or deep inside the sample [14]. Several demonstrations of imaging deep inside the tissues have been made by means of a variety of feedback signals. For example, a two photon fluorescence signal was used as a feedback signal for focusing, which can represent the amount of light locally excited within the tissue [15]. Imaging of dynamic scattering media was demonstrated by focusing with a DMD [1 6].



(a) (b) (c)

Figure 3 Images of white chicken muscle at a depth of 260 μm for three different cases of aberration corrections: (a) sample (b) system induced (c) system and sample induced [8]

3.2.2 Faster image acquisition

To obtain fast acquisition of image the DOF of the objective has been made tunable to rapidly focus scanning axially throughout thick tissue. And, also, multi-focal microscopy shortens the imaging time of traditional laser scanning microscope by remote focusing [17] which uses the position control of the objective and multipoint scanning by a rotating aperture [18]. Several other methods demonstrated with a multifocal multiphoton microscope include, micro-lens arrays on a rotating disk [19], etalons [20], cascading beam splitters [21], diffractive optical elements [22], and so on. Another approach is to use a tunable acoustic gradient-index lens filled with silicon oil (TAG) [23] as an adaptive optical device.

3.2.3 Imaging with higher resolution

OCT can achieve 1-2 μm axial resolution in tissue by use of broadband light sources, but transverse resolution is low due to low NA of the system's axial resolution requirement. To enhance the transverse resolution OCT is combined with the axial sectioning confocal microscopic method with a coherence gate, called optical coherence microscopy (OCM). High speed OCM with autofocus adjustment was demonstrated for laparoscopic and endoscopic imaging with a miniaturized piezoelectric fiber scanning probe [24]. The multipoint scanning [18], the adaptive TAG [25], multifocal structured illumination [13], and excitation with a Bessel beam [12] were demonstrated to yield high resolution image.

3.2.4 Multifunctional imaging

Typical of multifunctional microscopy is a multispectral imaging microscopy which captures chemical composition as well as morphological image in sample by combining imaging and spectroscopy. The technique obtains the spatial and spectral information in the interested field based upon collection of a complete spectrum at every location of an imaging scene by scanning it visually and in wavelength ranges from the UV to long-wave IR [26]. Here, optomechatronic spectral dispersive devices are used to separate the wavelengths of light, which include tunable gratings, filters such as acousto-optic filter and LC filter, prisms, and interferometers as shown in Figure 4.

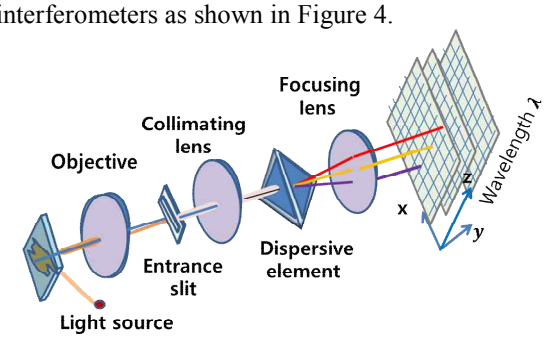


Figure 4 Schematic of a multispectral imaging

3.2.5 Multimodal imaging

In order to improve imaging quality a number of multimodal microscopy were developed, typical of which includes multimodal OCT and fluorescence microscopy, multimodal confocal microscopy, multiphoton microscope and OCT, OCT and spectroscopic technique[27], OCT and OCM [28]and so on. These techniques combine the complementary nature of each modal technique to enhance the imaging performance. Multimodal imaging by a mode switchable method [29] is realized in endoscopic OCT and OCM where an adaptive endoscopic probe can be switched to a desired mode by using tunable optic systems. It comprises a tunable aperture actuated micro-fluidically and two varifocal lenses actuated by micro-electro-fluidics. These are controlled in order to obtain as high DOF extension and transverse resolution as possible.

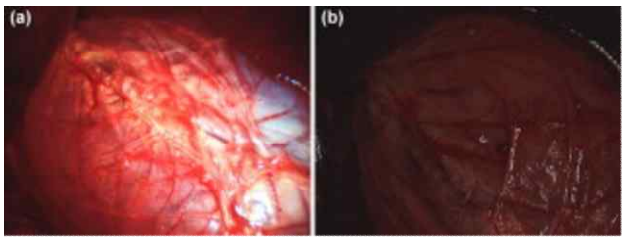
4. Laparoscopic and endoscopic imaging with optomechatronic devices

4.1 Internal body imaging

Various types of minimally invasive (laparoscopic) and non-invasive (endoscopic) imaging were developed for internal body imaging. Particularly, a variety of the microscopes equipped with a robotic endoscope with CCD came out. Typical one is an endoscopic OCT with a miniaturized catheter driven by piezoelectric actuator which in turn manipulates a cantilever fiber [30]. The flexible type to rotate the tip of the endoscope [31] belongs to this type of development. Other efforts are to use a miniaturized fibre raster scanner [32] and a piezo driven-spirally scanning fiber for endoscopes [33]. When cavity spaces do not provide enough room for any device to freely move, a rotating wedge prism imaging system was proposed for the change of view angle [34]. In the body cavities filled with opaque fluid, visibility is completely lost or drastically degraded. With an optical coupler with a clear, soft gel in semi-solid state attached to the distal end of an endoscope or other imaging instruments, visualization can be made possible [35].

4.2 Focusing and zooming

In laparoscopic and endoscopic diagnosis or surgery, most vision systems have a fixed field of view, resolution over the entire field, and fixed view direction. In addition, they lack autofocusing function, possessing very long depth of focus. This constricted vision necessitates accurate manipulation and positioning of a camera port and its maneuvering in various directions inside surgical site. To overcome these, there have been many efforts with a variety of optomechatronic approaches such as adaptive zooming via two tunable fluid lenses [36, 37]. In Figure 5 an image comparison is presented between the adaptive zoom camera and a commercial laparoscope, whose image is taken from a live pig in an operating room under the same LED lightening condition.



(a)With two tunable lens (b) Conventional laparoscope

Figure 5 Comparison of the adaptive continuous zoom camera with a commercial laparoscope [36]

4.3 Foveated imaging with multi-resolution

A foveated imaging has an ability to vary spatial resolution across the image, thus providing a wide field of view while maintaining high resolution in a region of interest. In endoscopes there are needs for clinicians and pathologists to be able to see the suspicious region or detect lesion within the tissue based upon the magnified image of high resolution. Here, two potentially implementable methods are listed: One is to use SLM which changes the region of interest on a millisecond

time rate [38].The other one is to use a dual imaging system in which one imager detects the entire field of view with low resolution, while the other detects a small region of interest with highly magnified resolution by using a scanning mirror [39]. In endoscopes, an imaging device called “the dual view” which is similar to the one was developed, where an image shift mechanism (prism) was used to obtain the zoomed view and wide angle view at a time instead of scanning mirror.

4.4 Enlarging field of view

In case of using micro-laparoscopes or micro endoscopes, they have a very limited field of view due to tiny optics and fiber cables that are designed to be minimized for the minimal invasiveness. In order to overcome the limitation a mosaicking imaging is introduced to enlarge the field of view by performing a spiral scan via a conic structure with a particular surface structure [40].

4.5 Adaptive illumination

In the fibre optic confocal microscope shown in Figure 6 an appropriate set of DMD mirrors sequentially illuminates individual fibre or patterns of multiple fibers [41]. The core idea of the imaging system is that the DMD and the imaging bundle are arranged to be in conjugate image planes, which enables a one-to-many mapping of fibers to DMD pixels.

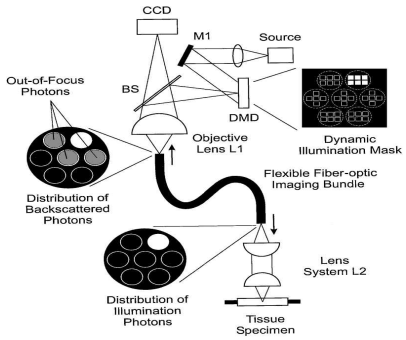


Figure 6 Fiber optic confocal microscope with a DMD-based illumination [41]

In other developments such as diffuse optical tomography [42], two DMDs, one for illumination side and the other for detection side was used to generate the desired illumination and detection patterns, respectively, and the two DMDs together with a spectrophotometer enhance the imaging performance in terms of spatial, spectral and temporal characteristics. Another method includes multifocal structured illumination [13] for multifocal structured illumination microscopy. It uses two core optmechatronic components, a chrome-masked micro-lens array to produce a focal shift between wavelengths and 2D galvanometers used for sample scan.

4.6 Imaging for robotic surgery

In order to mitigate the limitation related to delivery and direct manipulation of the relevant tools from outside the body of the patient several robotic systems have been made in recent years. Modular surgical robotic system known as the assembling reconfigurable endoluminal surgical system has been developed primarily for GI tract and designed to complete assembly processes inside the abdominal cavity [43]. In single port surgery, a robotic end effectors platform is inserted through a trocar into abdomen cavity [44]. This robot is equipped with a stereo vision module to enable triangulation for controlling its structure and the visual feedback for tele-manipulation.

4.7 Optical image stabilization

Image stabilization is of vital importance when imaging is performed on the living biological body where in vivo motion are always present, arising from breathing, peristalsis, licking behavior and heartbeat of living body. To compensate for or nullify its effect a visual feedback technique based upon the control of the objective lens via a piezo driven actuator [45] and a piezo nano-positioner for two photon imaging of neuronal cell shown in Figure 7 [46] have been developed . The nano-positioning utilizes a feedback control system which is based upon optical measurement of the position of the moving tissue via a linear line CCD and the control of the position of the objective via a piezo-nano positioning system to keep constant the distance between the tissue and the objective.

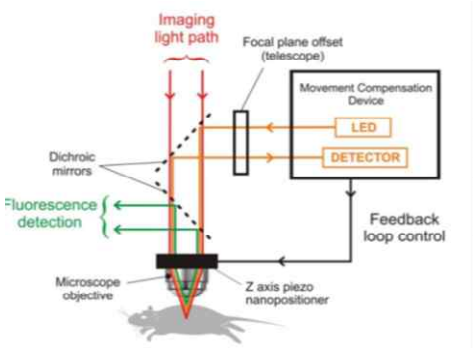


Figure 7. Image Compensation via objective control [46]

The use of a varifocal lens (VL) has been shown to provide focus adjustment to correct the focus shift arising from the licking behaviour of head held mouse [47]. In case when it needs to image large area of organs in vivo, patient motion and irregular tissue surface a varifocal objective lens was added to the confocal endomicroscopy to maintain the same level of imaging depth [48].

5 Imaging of neuronal activities

In neuronal imaging “so called “spatiotemporal resolution” problem has to be overcome, which arises from two fundamental challenges: Electrical impulse of neurons occurs within few msec. And neurons are in nature massively connected but sparsely distributed three dimensional structure in space. Thus, detection of 3D data with high resolution in space and time along with high signal to noise ratio became an ultimate goal.

Many approaches combined with optomechatronic devices such as VL, AOD, DM, and SLM have been introduced in recent years [1]. In confocal microscope, a galvano-mirror based line scan enabled a fast 3D

scanning and AOD based line-scan was shown to yield inertia- free scanning [49]. Other approaches include the axial scanning VL to combine with other scanning modes [50] and the combined use of an AOD to deliver a focused light to the local areas of interest in populated neural network field and DMD to produce a virtual pinhole that admits the fluorescence from the locally excited field [51].

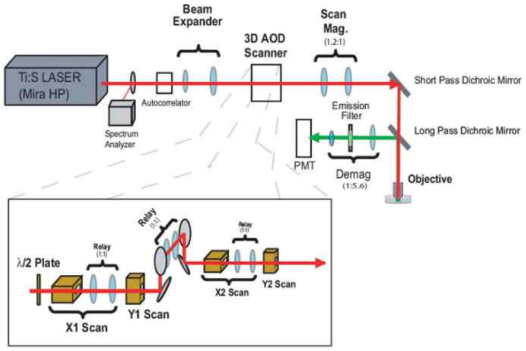


Figure 8 Four AODs for scanning fluorescent light [59]

A laser microscope that can simultaneously image the activities of neurons in 3D space with the aid of a SLM and a phase mask was developed [52]. This method demonstrated a scanless system. Here, the SLM is tuned to provide programmable illumination needed to simultaneously excite all neurons at different focal points while the wavefront coding via the phase mask makes it possible to simultaneously accept light from neurons at all different focal points. Fast axial scanning by changing focal position has been attempted by several methods: adaptive aberration correction with DM [53], extended DOF with a scanning axicon [54, 55], and two photon microscopy with diffractive optical elements (DOE) [56]. For high spatiotemporal resolution, a two photon microscope with simultaneously focusing multiple excitation beams at different positions was developed. This uses the control of femtosecond laser pulse with focus scanning of objective or specimen [57] and multiphoton microscopy combined with four AODs to achieve high speed 3D scanning shown in Figure 8[58].

Future perspective

Remarkable achievements have been made in optical imaging for biomedical field but still many challenges remain to be attacked. So, the future optical imaging will be driven with faster speed toward the imaging goal; imaging with adaptive, miniaturized, reconfigurable systems in noninvasive manner and imaging of dynamic, thick tissues and opaque body contents with higher resolution and faster speed. In this prospect, innovative new technology advances are expected to be accelerated with the benefit of the optomechatronic multidiciplinary approach featured by the aid of micro-nano, sensors and actuators, and control technology. As a result, a variety of novel developments are expected to appear in the microscopy combined with endoscopy in the form of multifunctional, multi modality along with optical combined with non-optical imaging methods.

References

1. D. Luenberger, F. Voigt, Biophot., April (2015)
2. Children's Hospital of Chicago
3. Washington Center for Reproductive Medicine
4. Introd.to OCT, Obel optical biomedical+ engineering laboratory, Univ. Western Aus.(2015)
5. R.G. Chelliyil, et al., J. Biomed. Opt.**13**, 4(2008)
6. S.Y. Leigh, Y. Chen, et al., Opt. Exp. **5**, 6(2014)
7. M.J. Booth, Light: Sci. & Appl., 3(2014)
8. J. A. Kubby (Ed.);CRC Press (2013)
9. A.J. Wright, et al., Opt. Exp. **15**, 26(2007)
10. T. Shirai, App. Opt. **41**,19(2002)
11. E. J. Botcherby, et al., Opt. Exp.**16**, 26(2014)
12. F.O. Fahrbach, et al., Opt. Exp. **21**, 11(2013)
13 M.Ingaramoa, et al. Proc. Natl. Acad. Sci. (2014)
I4. M. Vellekoop, A.P. Mosk, Opt. Lett. **32**, 16(2007)
15. Katz, et al., Optica **1**, 3(2014)
16. C. Stockbridge, et al., Opt. Exp., **20**, 14(2012)
17. E. E. Hoover, et al., Biomed. Opt. Exp. **2**, 1(2011)
18. M. D. Risi, et al., Photonics, 1(2014)
19. L. Sacconi, et al., Opt. Let. **28**, 20(2003)
20. D. N. Fittinghoff, J. A. Squier, Opt. Let. **25**,16(2000)
21. D. N. Fittinghoff, et al., IEEE Quan Elec. **7**(2001)
22. J. Ou, Y. Shao, et al., Opt. Heal. Sci. **5**, 3(2012)
23 M. Duocastella, et al., J. Biomed. Opt. **17,** 5(2012)
24. A. D. Aguirre, et al., Opt. Exp. **18**, 5(2010)
25. N. Olivier, et al., Opt. Let. **34**, 11(2009)
26. G. Lu, B. Fei, J. Biomed. Opt. **19**, 1(2014)
27. R. Liang (Ed.), Springer (2013)
28. C. H. Lee, et al., J Urol. 187, **2**(2012)
29. M. Choi, et al., Opt. Exp. **21, 2**(2013)
30 T. H. Tsai, et al., Proc. SPIE 7889, 788919(2011)
31. S. D. Schwaitzberg, Seminar **8**, 1(2001)
32 D. R. Rivera, et al., Proc. Natl. Acad. Sci. USA **108,** 43(2011)
33. H. Miller, et al., J. Biomed. Opt. **17**, 2(2012)
34. E. Kobayashi, K. Masamune, et al., MICCAI (2000)
35. Patent US 20150065795 A1 (2015)
36. F. S. Tsai, et al. J. Biomed. Opt. **15, 3**(2010)
37. S. F. Tsai, UCSD, Ph.D. Dissert. (2010)
38. G. Curatu, U. Cent. Florida, Ph.D. Dissert. (2009)
39. Y. Qin, et al., Biomed. Opt. Exp. **5,** 4(2014)
40. M. S. Erden, B. Rosa, et al., *IEEE ICRA* (2013)
41. P. M. Lane, et al., Opt. Let. **25**, 24(2000)
42. Q. Pian, et al., 39th AN Bioeng. Conf., (2013)
43. K. Harada, et al., Robotica **28**, 2(2010)
44. S. R. Platt,et al ,IEEE Tran. Bimed. Eng. **56**, 6(2009)
45. S. Lee, et al., Robotics, IEEE Tran. **24**, 1(2008)
46. S. Laffray, S. Pagès, et al., PlosOne **6**, 5(2011)
47. L. J. Chen, et al., Physiol. **591**, 19 (2013)
48. M. Kim, et al., Biomed. Opt. Exp. **5**, 12(2014)
49. Y. G. Li, L. Liu, et al., Opt. Lett. **35**, 3(2010)
50. B. F. Grewe, et al., Biomed. Opt. Exp. **2,** 7(2011)
51. V. Bansal, et al., J. Biomed. Opt. 11, 3(2006)
52. V. Nikolenko, et al., Front. Neur. Cir. **2**(2008)
53. N. Ji, T.R. Sato, E. Betzig, PNAS USA **109**, 1(2011)
54. P. Dufour, et al., Appl. Opt. **45**, **36** (2006)
55. G. Thériault, et al., Opt. Exp. **21**, 8(2013)
56. B.O. Watson, et al., Front. Neur. Cir. (2009)
57 G. H. Zhu, et al., Opt. Exp. **21**, 18(2013)
58. G. D. Reddy, et al., Nat. Neurosci. **11**, 6(2008)