Multiphoton Microscopy with Programmable Supercontinuum Pulses

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Abstract

In the field of medicine, histopathology process is undertaken by pathologist. The aim of undertaking histopathology is to examine the existence or the possibility of a disease in the body tissue of a living thing. In addition, the process of histopathology is best practiced on the specimen of body tissue taken during the surgery of a patient. Here, the process involves a series of activities where surgery helps to remove body tissues for examination. After the surgery, the body tissue removed is placed in a fixative place where no external toxicities can contaminate the specimen. This forms the basis for preparation of viewing of the tissue using microscope to examine the disease. However, if the body tissue is large, a pathologist is mandated to take a sample of the tissue and subject it for histopathology. Further, the process of chemical fixation is subjected to the tissue to help remove unwanted chemicals such as alcohol. Water is also removed from the sample through the process of dehydration through the use of a chemical known as xylene. Afterwards, the tissue is cut into slices where a very small slice is used in the examination of the disease. Alternatively, a section of the tissue can be frozen. A device known as microtome is used to slice the section frozen into thin slices. Staining is done on the frozen thin slice. The disadvantage of frozen thin slice is that it requires time to process and it is costly in terms of the equipment needed. The importance of staining is to remove and reveal any presence of cell in the tissue. Finally, the slides are subjected to examination in a microscope by a pathologist. Where cancer was examined using the historical images, then, depending on the report, cancer will be diagnosed. Therefore, in histopathology, the interpretation of historical images after examination is crucial in helping diagnose specific disease. In particular, this paper focuses on the use of Programmable Supercontinuum Pulses. This technique helps in the process of applying multiphoton microscopy. As a result, it helps the technicians to obtain a histopathology which is free of stain.

Keywords

Histopathology, programmable supercontinuum, multiphoton microscopy, stain-free.

Introduction

The use of histopathology in the field of medicine has a long history, and its development and application are critical to the medical practitioners. The process has the backing of clinical medicine and research in the field of medical pathology. Histopathology is relevant in other areas like the crime investigation departments wherein it is used to support research into the causes of deaths. [9] The principle focus of Histopathology is the study of the evidence about the presence or likelihood of the disease being in the sampled patients' body tissues. The pathologist performs surgery to expose the specimen through surgery, autopsy or biopsy and obtain the required slice of tissues for examination purposes. The collected sample from the patient complies with either fixation or frozen section analysis concept but not both. The use of stated investigative methods restrains possibility of tissue damages through putrefaction or autolysis. Analysis of strains with different pigments presents the real diversity on the samples.

The examination of multiphoton microscopy dish requires the application of knowledge acquired through training and experience, as well as exposure of the skilled specialist in drawing the remarks pertaining histopathology findings. Regardless of the level of time required in performing fixation, sectioning and staining procedures ensures scientist has made a great effort in championing the adoption of multiphoton microscopy in conducting diagnosis of infection of the diseased tissues. Intensive application of the multi-photon microscopy technique is great in the field of biological imaging wherein it constitutes the principle and safer option of conducting fluorescence microscopy on living organisms or on tissues of the explants alike. The concept of multi-photon microscopy assesses microscopic calcium transient present in tissues to a depth of 500micro-meters inside the brain of a mouse [1]. Relevant application of the technology may reveal the quantity of blood flowing through capillaries in the form of shadow images of the blood cell. The influence location provides important tools adopted to the original conception analyzing the patient’s conditions while the drastic improvement and innovation in medicine supported the use of supercontinuum pulses in reaching out to the sick people to conduct optimal performance analysis of their health conditions.

Multiphoton Microscopy and Histopathology

The study of Light microscopy represents a significant force in the field of medical biology based on the level of the degree of aptitude it uses to conduct observations on the living cell samples through considerably high-powered microscopy spatial resolutions. The visible light wavelength affects the level of spiral resolution achieved by the instrument adopted in tissue analysis [8]. Research findings developed in the field of medicine concerning the influence of high spiral resolution monitored under light microscopy does not translate to the partial resolution obtained through electron microscopy. The specimen observations made under electron microscopy provide

limited information regarding the cells in question thus attracting limited applications in biological investigations [7].

Microscopes like the MRI do not possess similar characteristics like aptitude and resolution necessary in supporting the studies of sub-cellular structures in the tissues of a living organism or the existence of delicate molecular selectivity. Thus, it enhances recognition of the presence of single molecules in the background within the samples destined for investigations like in the case of the light microscope. The study done with different body tissues to establish the performance efficiency of multiphoton microscopy as compared to the standard hematoxylin and eosin concept demonstrated varied information. The performance of a test with MPM to set the presence of lung cancer in new tissues permitted the pathologists to identify the histological subtypes of the tumor and inflammatory cells like lymphocytes and smoker macrophages with ease.[3] The adoption of multiphoton microscopy in the clinical investigation of body tissue disorder enabled the medical practitioners' and doctors to assess samples without waiting for long before getting relevant information about the tissue from the standard fabrics processing methods.

Histopathology Limitations

The principle of conventional histopathology presents significant limitations that hinder its adaptability and spread to other areas that require accurate data retrieved within a limited time interval. Histopathology requires highly trained health personnel with exposure in the on the use of the multiphoton microscopy of about four to six years to achieve the competency and critical thinking necessary while drawing the observatory remarks of the microscopy [2]. The method uses dish stains with the specimen; the process of staining dishes consumes time and prolongs the sample investigation time. The next challenge encountered in adopting the histopathology is the duration needed in sample preparation that span from a minimum of ten hours to several days limits its application in cases where the rapid generation of the result in of critical essence. The method is quite expensive since it in labor intensive and uses expensive machines. The device operates on the principle of truncation or segmentation of data that result in loss of accuracy or clouding of the sample, hence affects the entire outcome of the clinical analysis supported by the method.

Photonic-crystal fiber source

The photonic- crystal fiber contains an array of wavelengths that run along the fiber axis through sized holes created by drawings. The use of photonic crystals in medical research started in the year 1987 that strongly uses the localized light in hesitation processes to monitor spontaneous emission along the band gap. The extent of propagation presents minimal bearing on the direction and polarization attained by the adoption of photonic crystal fiber [10]. The materials possessing the photonic bandgap reflect positive performance on the application of the technique ion

monitoring their performance. Generation of the hole in the in the photonic crystal is through striking capillaries together, drilling or extrusion processes. The two designs of photonic crystal fiber contain air holes in the range of ten to hundred but differ in their physical properties. The solid core type of photonic crystal fiber is quite similar to the conventional fiber that relies on the total internal reflection to guide light rays through low index cladding and high index core.

The solid core photonic fiber relies on the doped core or clouded glass to attaining their functional qualities while implementation of a simple modification of the hole or core shape generates little impact on the fibers' optical characteristics like nonlinearity, dispersion, birefringence and mode shaping. The change in wavelength about variation in light distribution between air and glass facilitates the creation of larger values of dispersion fiber. The existence of different fiber design accommodates endlessly single mode, large pattern area and highly nonlinear optical. The presence of hollow core fibers uses the light guiding mechanism that acts as an empty free mirror without active materials in the center. [5] For such photonic crystal fiber the design accommodates filling of the core with either glass or liquids thus make the tissue gain high application in the development of sensors, high power pulse transmission.

Photonic crystal fiber and Supercontinuum generation

The contributions of photonic crystal fiber in the development of supercontinuum devices enhances the bandwidth analysis. The existence of supercontinuum technology represented a spectral expansion in optical field tackling areas of strong narrow band light impulse propagated through nonlinear media demonstrated by Alfano and Shapiro in 1970. The inclusion of nonlinear optical, procedures widens the scope of intimate performance through cross and self-phase modulation, modulation instability, soliton fission, Ramen scattering, dispersive wave generation, four-wave mixing, and self-steepening. The performance of nonlinear properties depended on the dispersion of the medium used and relied on the selected medium that drastically lowered power requirement and increased supercontinuum generation quality. The presence of photonic crystal fiber reflected feasibility dispersion and nonlinearity that is an excellent medium used in the formation of the supercontinuum by pumping smaller values of pulses closer to zero dispassion. The existence of femtoseconds and picosecond pulses facilitate the process of supercontinuum creation. The use of standard dispersion through the concept of self-phase modulation Raman scattering and four-wave mixing enhances supercontinuum creation. [6]

Programmable Supercontinuum pulses

The programmable supercontinuum pulses represent the current technological innovation that relies on the concept of multiphoton microscopy in conducting tissue analysis and performs histopathology in a very short time. The machine uses the ultrafast laser technique to accomplish investigation on the sampled tissue with the assistance of experienced pathologist. The

fundamental features of the device often bypass the need for ultrafast laser training requiring system realignment. The technological approach offers a user-friendly platform and optical sources that created current microstructures that operate contrary to the standard procedures of histopathology [5].

Operation

The operation of Programmable Supercontinuum Pulses concept adopted new photonic variables in place of discrete optical alignment configuration applicable in gathering co-localized label-free images produced by endogenous contrast from the three-photon and two-photon auto-florescence, third harmonic generation and coherent anti-Stokes Raman scattering [2]. The new invention uses programmable light producing customized chemical differences from both transient absorption and documented modalities in mixing wave platform and stimulated emissions. The presence of light source in the sample analysis serves as a programmable variable within elaborate excitation and detection chart [6]. The system can probe specific endogenous substances through simple programs that target different samples of interest apart from the biological models that represent fluctuating chemical alignment and intrusive background.

Characteristics and capabilities

The principle of virtual histo-chemistry retrieved through manipulation of light has more benefits compared to immunihisto-chemistry. The prominent advantages of the model relied on the increased quantities of little marks that may generate images on the tissue pigment, thus exploits the information reflected by every pixel that covered significant information regarding the existing co-relationship between the samples [4]. The comprehensive approach observed in the use of the Programmable Supercontinuum Pulse technique depended on the little contrast involving the implementation of virtual histo-chemistry.

Advantaged of programmable supercontinuum pulse technique

The process provided reliable data as it uses the chemical treatment of the samples before making observations, the chemicals dictates sampled tissues subject to subsequent image based histopathological interpretation of the findings [2]. The chemical treatment involved embedding, fixation staining and sectioning of the specimen for the test inducing distortion of artifacts and loss of biological components through deterioration of the chemical composition.

The technology limited the level of patient waiting time for the test result. The process involves active mean of conducting biological tests, thus reducing possible time span in the system linked to financial, economic burden incurred in medication. Other than the traditional models that require more days, the current technology of histological –histochemical uses experimental procedures

that are cost efficient and faster regarding data processing for the selected specimen. The adaptability of the process is to other techniques improves the performance of the systems in support of cellular and molecular examination of fresh tissue and selected biological samples tested for analyzing performance and status of the cells [4]. The use of Programmable Supercontinuum Pulses techniques in multiphoton microscopy system improves the performance of the scheme in comparison to the conventional histo-technology [1]. The technology has eliminated the paradigm of time-consuming processes and accommodates lesser alignment to generate explicit images of tissues taken for analysis [6]. Visualization of the biological activities taking place in the system, quantification will subject the middle class to operate efficiently. The concept may classify the biological group of the body cells and that of the pathogens.

The technology provides the multiple issues relating to procedure analyses cellular metabolism and other related cases linked to ill health. Regardless of the cost incurred, the use of the programmable supercontinuum pulse technique accelerates the possible performance of the system. The technology performs poorly about evaluating the existence of pathogens in the bone marrows though with the assistance of surgeon specimen may originate from another section of the body that link the potential infection from one location to the next [8].

Conclusion

The sophistication of technology and need to reduce cost and time in hospitalization require the adoption of robust processes in data mining, the formative use of must be in a position to provide reliable data at the least time possible. The existence of sub-pulses provides significant data that support any temporary solutions, the difference aid instrument design is important, therefore the adoption of the operating systems that support the growth and development of systemic authority provide better performance for the system operating under the presiding cognitions. The use of the programmable supercontinuum pulse technique that enables the technicians to achieve clear and stain free histopathology of fresh thick or thin specimens in a very short time. This new technology would reduce the cost of histopathology, reduce the time to analyze the sample, and the amount of training a technician has to go through.

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