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Brain Imaging Technologies - A Mathematical Perspective

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Abstract

The brain is a multilayered and multicompartment system structured for accomplishing robust behaviours which leads to cognitive and physiological functioning of the human system [1]. The total volume of the human brain is 1450cm^3 on an average. It comprises of innumerable neurons and glial cells which make up the building blocks of the brain [2]. Scientists have been trying to understand the brain and unfold it's mysteries for many decades. Inspite of taking avid interest in this field the information gathered does not provide a complete comprehensive perception of the brain [3, 4]. The effort to comprehend the brain has evolved over the years [5]. Machine learning techniques are being widely used to make relevant outcomes for neuro imaging. The advent of imaging technologies has helped to facilitate our understanding of the brain and its complexities [6-8]. Brain imaging technologies provide an unprecedented tool to analyse the changes in the central nervous system (CNS). These technologies have bought with them higher resolution and deeper penetration into the brain thus exposing newer functionalities of it. Exploring the brain to absorb its secrets and unravel its enigma is one of the most promising and rewarding applications of biomedical brain imaging technologies. Brain imaging technologies provide non invasive techniques of viewing the brain and its activities [9]. Doctors and researchers can monitor the brain without having to intrude into it via painful and risk staking neurosurgeries. This is one of the biggest advantages of the emerging and ever evolving brain imaging technologies. Today, a number of these techniques are being put to practical application to save innumerable patients around the globe. The current brain imaging technologies include Functional magnetic resonance imaging (fMRI), Computed tomography (CT), Positron Emission Tomography (PET), Electroencephalography (EEG), Magnetoencephalography (MEG), Near infrared spectroscopy, Two Photon Microscopy and Photoacoustic tomography (PAT). Of these fMRI and PAT are the recent imaging tools of particular interest.

Keywords: *Nanocarriers, RNAi, miRNA, siRNA, Gene delivery, Gene silencing.*

Introduction

Machine Learning and Brain Imaging

Machine learning approaches provide infinite links which collate patterns of brain activation to behaviour of an individual. It is as discrete as it can provide information at the level of an individual-subject. This development has been adorned with the present knowledge of machine learning and algorithms of pattern recognition. Machine learning is an advanced form of regular algorithm. Machine learning involves neural networks which speak about how the complex functions of the human brain are performed [10]. Machine learning algorithm can be divided into two phases a training phase followed by a testing phase. First is the training phase which involves organisation of the randomly stored data in a proper tabular format including all the important attributes. All this data loaded into the algorithm and made to be accustomed with them. Then follows the testing phase where the training data is evaluated and then submitted for further evaluation and classification of data. A classifier is a function which helps categorise several objects into three specific classes [11]. The basic principle of classification in the machine learning task is to provide a rule which will pertain to an observation x assigned to one of the several objects. Here x denotes a vector of N-dimensional neuroimaging data. When considering a simple case there are only two available classes. Classifier can therefore be assigned a decision function $f : R^N \rightarrow \{-1,+1\}$ where x is assigned to one of the classes denoted by -1 and +1 respectively [12, 13]. Linear decision function f matches to a separating hyperplane in which vector w and bias term b are the parameters. Then the label y will be : $y = f(x; w, b) = \text{sgn}(w^T x + b)$. Based on a set of such input-output relations $(x_1,y_1,\ldots x_a,y_a) \varepsilon R^N \{-1,+1\}$. Learning can be formally described as the task of selecting of both the parameter values (w, b) and the decision function $f \varepsilon f$. This will result in classification of x by f. In order to establish the optimal decision function a proper loss function needs to be established. 0/1-

loss function is one of the most commonly used loss functions. Generalization error is a way of determining decision function by minimising the expected risk factors [14].

R [f]= ∫ l(y,f(x)) dP(x,y), where P(x,y) is the unknown distribution.

Another straight forward approach would be to evaluate the risk in an empirical fashion.

$R_{emp}[f] = 1/n \sum_{i=1}^{n} l(y, f(x))$ thus minimize the risk involved around f. Further, computing parameters for linear decision functions, Linear Discriminant Analysis (LDA) and Linear Programming Machines (LPM) can be used. LDA is given as $w = \sum^{-1}(\mu 1 - \mu 2)$ [15]. A more generalised framework of LDA can

be the Fisher's discreminant analysis

Fisher's Discriminant Analysis (FDA)

j (w) = $W^T S_B W / W^T S_W W$ where, S_W is the class scatter, 1 is the class mean, n_i is the total pattern available x_i in a class C_i. In any condition the class distributions cannot be established as discrete values. So the optimality statement of LDA is rather a dependent on the class distribution analysis. So the covariance and mean values need to be estimated from the training data itself. The empirical covariance is used to estimate the covariance matrix as the empirical covariance is unbiased. Neuroimaging data usually contain only a few data points in a high-dimensional data set, thus making empirical estimation imprecise [16]. This is mainly attributed to the reason that the unknown parameters to be estimated are quadratic in nature as compared to the number of dimensions available. This discrepancy results in an error referred as the systematic error. Therefore shrinkage is a reliable correction of the systematic error. Recently a group of scientists established an analytical method for the calculation of shrinkage which was applied to brain imaging data. According to this, the empirical covariance matrix is denoted by an optimal value which depends only on the variance of entries available from different samples. Then the optical parameter γ towards the identity can be calculated as:

$\gamma^* = n/(n-1)^{2*} \sum_{i,j=1}^{d} var_k(z_{ij}(k)) / \sum_{i \neq j} s^2_{ij} + \sum i(sii-v)^2$.

LPMs deal with the parameter values w by following sparse solutions, whereby the value of w becomes zero. This is achieved by putting to use the optimisation of 1-norm from the objective function instead of 2-norm. Optimisation of 2-norm usually generates non-sparse solutions.

Application of Machine learning to Brain Imaging

Both fMRI and EEG/MEG are mostly linear. However, non linear methods can also be easily analysed by adding model classes in the model selection loop. EEG studies involves utilisation of LDA, shrinkage LDA, Fisher sparse and other linear programs. In these cases, the class conditional distributions are converted to Gaussian by proper pre-processing. In all these cases, LDA has been declared as the optimal classifier. fMRI analysis has evolved with the help of machine learning from univariate analysis tool to multivariate analysis. Use of LPMs is supported by the idea that they possess benign abilities whereby the number of input dimensions in x is high while the count of samples is low. This unbalanced situation becomes problematic in fMRI whereby voxels is present in ten thousands while the number of samples very rarely exceeds some hundreds. Statistical machine learning methods are being applied to neuroimaging data analysis. The most important characteristic feature is the ability to model data sets having multi dimensionality. Relating brain images to behavioural or clinical observations can be done by using supervised learning in decoding or encoding settings. The advantage of unsupervised learning is the fact that they unravel the hidden parts of images or locate specific populations in large groups.

NumPy: It is an array based numerical computation for n-dimensional data representation. It takes input and output as array persistence and resultant is a dot product. Mostly used by major scientific Python libraries [17].

SciPy: It encompasses a variety of domains including algebra, processing signals and optimization. It operates on ndarrays by opting for higher level mathematical functions. SciPy ensures efficient data management and high performance by linking to compiled libraries like BLAS, Arpack, MKL. Both NumPy and SciPy provide a perfect numerical computing process which are the elementary bits used in general algorithms [18].

Matplotlib: It mainly focuses on figure processing and offers a multitude of formats to choose from for the purpose of publication [19].

Nibabel: It is used to assess the data in neuroimaging file format. Scikit-learn is a very modern machine learning application in neuroimaging. It contains a huge repertoire of both supervised and unsupervised algorithms based on statistical learning. The application of Scikit-learn provides a very strong stool to image the brain. Scikit-learn is a machine learning library which is written in Python. The Scikit-learn tool is highly efficient in producing state-of-the-art algorithms which are accessible by all and also reusable. It also offers easy prototyping adopted from Python. The application of Scikit-learn is unusually broad and the applications to neuroimaging are too wide. In Scikit-learn data input is in the form of two dimensional arrays comprising of size samples and features. The methods adopted are discrete and depend on their roles: the role of estimators is to fit models from data, the role of predictors is to make predictions on newly archived data and the role of transformers is to convert data from one mode of representation to the other mode. Tuning of hyperparameters can be done using GridSearchCV estimator.

Processing of neuroimaging data requires extensive processing. The process to remove artifacts from neuroimage to ensure enhanced efficiency of the algorithm is called Signal Cleaning. It includes removal of singular trends from a series of voxels reffered to as detrending. Next includes the harmonization of the time series to 1, a process called normalization. Removal of varying frequency signals is done by a method called frequency filtering. One major advantage of machine learning is the conversion of brain scan images into two dimensional data. The final steps involve data visualisation and tracing of objects within the image using Support Vector Machines.

The term machine learning involves an umbrella term which comprises of a set of topics dealing with the generation and evaluation of algorithms which help in recognition of patterns, categorisation, and prediction, all these features are based on models which are derived from pre- existing data. Since its conception, in all the years machine learning has helped to understand biology in leaps and continues to do so. It is hoped in the coming years it will contribute to enhance the understanding of biological complexities in brain. The ability to create a connection between cognitive biology and its neural circuits are the ultimate aim of cognitive neurosciences. The information provided by the EEG or the fMRI are mostly incomplete or merged with a lot of unwanted data [20]. Mapping neural circuits is tremendously beneficial in the field of neurobiology. Application of it to humans shall open up a galore of possibilities in cognitive neurobiology and disease diagnosis [21]. Machine learning plays a pivotal role in this accomplishment as by employing encoding and decoding of signals they can assume fine algorithmic results [22]. However, the disadvantage is that it cannot sufficiently unravel the detailed mechanistic brain functions. Keeping this in mind statistical machine learning owns its due importance in data analysis. Further the hindrances which occur while the processing of machine learning of neuroimages is actually in the methods employed. The high variance nature of the methods adopted and the multi dimensional nature of the statistical models give rise to more variability in the outcome. Nevertheless, machine learning has definitely established increasingly expressive and robust neuroimaging technology.

Machine Learning and Different Brain Imaging Technologies

Electroencephalography (EEG)

EEG measures the electrical activity of the brain by means of electrodes placed on the scalp. The electrical activity detected by EEG is on a millisecond level. Electrical signals from every neuron are collected and the resulting traces are known as an electroencephalogram (EEG) [23]. Therefore, it provides high temporal resolution in brain image computing.

Magnetoencephalography (MEG)

MEG is an imaging technique which measures the magnetic fields produced by electrical activity in the brain. MEG provides mill second time resolution and also allows for real time tracking of brain activity [24]. They work together with innumerable superconducting quantum interference devices, SQUIDS, which receive signals generated by the coherent action of cortical neurons. MEG measures involve recording of the evoked responses, regulating cortical rhythms, properties of neuronal activity and their connectivity.

Positron Emission Tomography (PET)

PET maps functional processes in the brain by use of trace amounts of short-lived radioactive materials. Brain activity is associated with high radioactivity [25]. When there occurs radioactive decay due to brain functional activity, a positron is emitted by the decay, this is then detected by the detector.

Computed Tomography (CT)

CT scan helps constructing the brain image based on the differential absorption of X-rays by the tissue the X-ray beam passes through. Bone and hard tissue absorb maximum X-ray followed by soft tissue [26]. Least amount of X-ray absorption is displayed by air and water. Hence the image produced by a CT scan depicts the gross structure of the brain but does not provide a high resolution detailed image of it.

Functional Magnetic Resonance Imaging (fMRI)

fMRI is a technique which maps brain activity at the macroscopic level. It works by monitoring the difference in levels of blood oxygenation and flow of blood which occurs due to neuronal activity. An active brain area denotes more activity in that particular region which requires an enriched supply of oxygen [27]. The oxygen supply is replenished by the increased blood flow to the active area. fMRI scans are used to produce brain activation maps which provide information on the parts of the brain involved in particular mental processes. These include complex cognitive functions, such as language and vocabulary, emotion, decision-making, memory and cognition. However fMRI cannot achieve spatial resolution at the level of a single neuron.

Multiphoton Microscopy (MPM)

Two-photon (2P) microscopy and multiphoton microscopy allows detailed and direct examination of neuroactivities at the microscopic level [28]. The measurements include parameters like action potential firing, the release of signaling molecules, and extra-cellular pumping of ions [29]. However 2P microscopy cannot penetrate through an intact skull to provide an image. It involves the simultaneous interaction of more than two low energy photons. These photons interact with a fluorescent molecule which further involves an electronic transition which is equivalent to the absorption of a single photon bearing energy almost two times as that of a single photon. The resultant emission from the excited fluorophore comprises of a single photon. With MPM minimally invasive microscopy can be performed. The MPM provides a spatial resolution of 1μm which is higher than that of MRI or PET by two magnitudes. MPM allows differential imaging of the cellular and the subcellular regions without the disadvantage of slow image acquisition.

Photoacoustic Tomography (PAT)

PAT is a state-of-the-art imaging modality which bridges the gap between the macroscopic brain activity rhythms observed in

humans and the microscopic activity details observed in small animal models. PAT is based on the photoacoustic (PA) effects. The process is initiated by the optical absorption by molecules in the tissue and final ultrasonic emission which is through thermoelastic expansion. The utilisation of both the optical excitation and ultrasonic detection provides PAT with two advantages over the other imaging technologies, (i) The PA signals thus produced is highly sensitive to the contrast produced by the brain tissue's rich optical absorption. These results in imaging functional and metabolic aspects of the brain (ii) acoustic waves are more resistant to the phenomenon of scattering as compared to light. This provides PAT with the advantage of high spatial resolution images of the deeper tissues.

EEG and MEG

Electroencephalography or a window to the brain, was discovered a century ago. It is a brain mapping and imaging technique which measures electrical activities of the brain by the electrodes placed on the scalp [30]. It provides information about spatio-temporal functioning of the brain [31]. Over the years EEG has evolved as the analysis of temporal waveforms at particular channels, aggregates of post synaptic currents, amplitudes and latency of peaks and troughs or grapho elements in disease stages to a comprehensive analysis of the brain's electric field [32]. The number of electrodes placed on the scalp may vary between 64-256 [33, 34]. The more the electrical signals corresponds to more neural communication which in turn means increased brain activity [35]. The brain activities measured by EEG are of two types: spontaneous and event-related activities. Spontaneous activities refer to brain responses that occur without any stimulus [36].

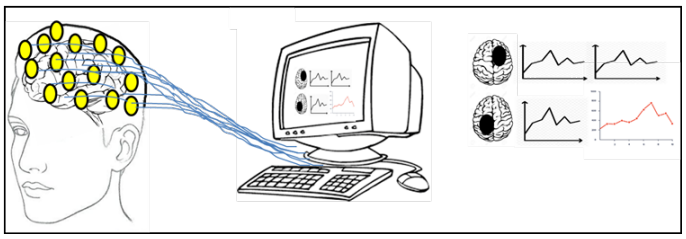

Figure 1: Schematic representation of EEG

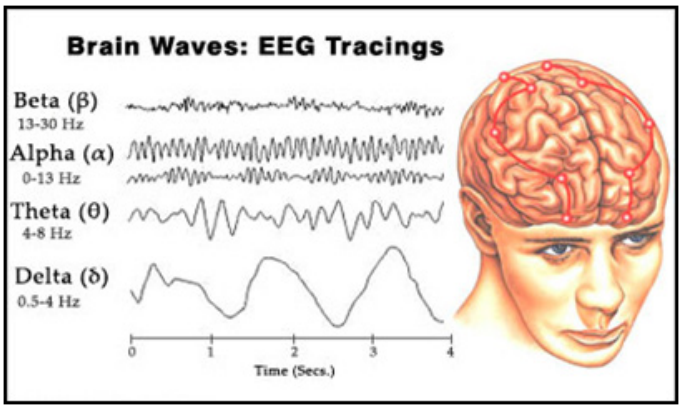

Figure 2: Representation of brain waves generated using EEG (Adapted from www.medindia.net)

These neuronal responses generally do not have any physical or behavioural manifestations. Event related activities or potentials on the other hand are low amplitude potentials influenced by specific stimulus or thoughts [37]. It is used in the diagnosis of seizures, drowsiness or other states of psychological problems that cause overt work or less activity in specific regions of the brain. Spatial analyses data generated by the EEG are mostly unambiguous and definite. MEG on the other hand measures brain activity by detecting magnetic fields produced in the brain. The disadvantage of EEG signals is the fact that they record impulses at the surface of the brain hence it becomes difficult to decipher whether the signal generated was from an area near the brain surface or from deeper parts of the brain [38].

The qualities which make EEG data reliable are: high temporal resolution, cost effective and easy to handle technique. Even performing high density EEG recordings is easy and fast. It gives a detailed analysis of the spatio-temporal dynamics of large-scale brain networks in practical life conditions [39]. Further it does not require to expose the patient to any harmful radiation. All these benefits taken together make EEG a powerful brain imaging and mapping device available even for the poor. EEG combined with fMRI and other imaging techniques surely offers the best brain images [40].

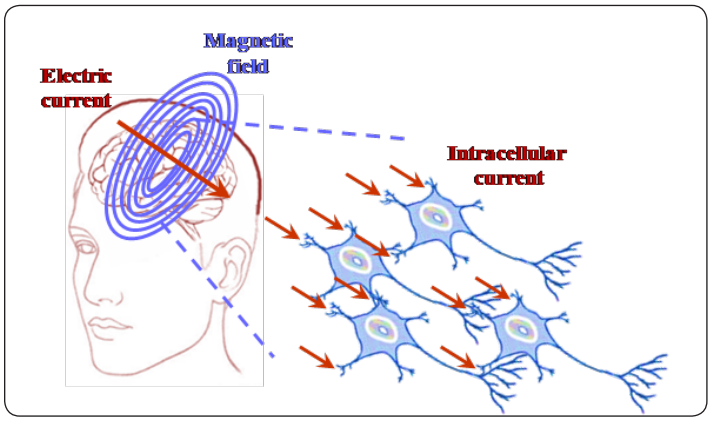

Figure 3: Representation of brain waves generated using MEG

Positron Emission Tomography (PET)

PET scan a gamma imaging technique, when applied to the brain mainly has two functions, first to measure the brain metabolism and secondly to detect the presence of radio labelled chemical agents in the brain [40]. Exogenous radioactive chemicals are also metabolically active, emissions radiated from these chemicals are picked up by PET [25]. The positrons annihilate when they collide with electrons present in the tissue. The resulting released pair of photons triggers signals [42].

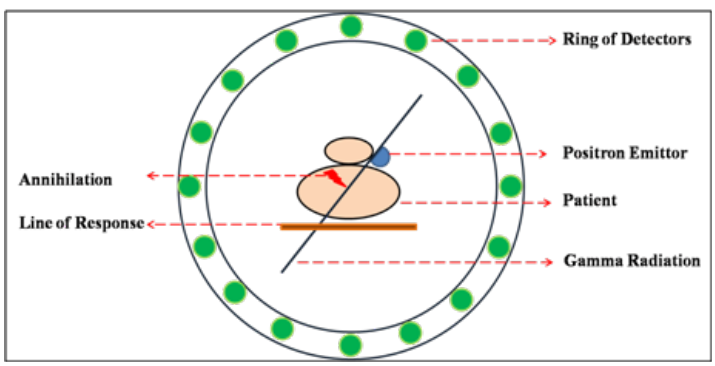

Figure 4: Schematic representation of PET image analysis

The data generated from PET are then processed in the computer to produce multi dimensional images depicting the spatial distribution of the chemical throughout the brain [43]. This data helps construct maps of biological processes which help clinicians in their interpretation of patient's health. Cyclotrons are used to produce positrons emitting radioisotopes which label the chemicals used to image the brain. The injected chemical referred to as radiotracer is injected in the blood stream of the patient which finds its way up to the brain. PET images can depict oxygen and glucose metabolism in living brain tissues. Brain activity in various regions of the brain can be easily deduced from these PET images.

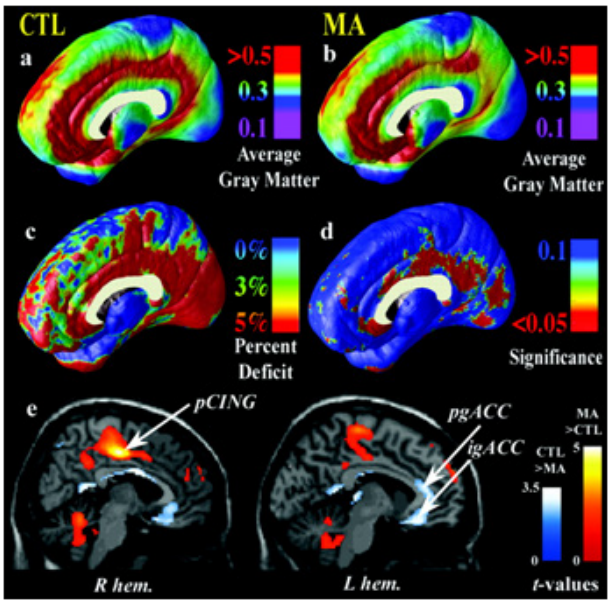

Figure 5: Brain imaging using PET scan. Showing gray matter differences in Methamphetamine addicted patients compared with control (CTL) Reprinted with permission from [43].

PET scan offered the advantage of superior quality images and high resolution even before fMRI became more widespread [44, 45]. PET scan images have helped in performing radiosurgery and stereotactic surgeries. PET scan is also used for preliminary diagnosis of brain disorders like AD, PD and multiple sclerosis [46-48]. It is mainly involved with identification and treatment of the pathogenesis of the diseases rather than the diagnosis. It can give information about brain tumours, strokes and neuro damage as in traumatic brain injury disorders [49]. The disadvantage with PET images is the fact that the radioactivity of the radio chemicals used decays very fast and so the process of imaging can also not be prolonged for longer observations. In recent times PET is used in conjunction with CT scan and MRI to provide detailed and definitive information about the brain [50].

Computerised Tomography (CT)

The term computed tomography was derived from three words, computer, tomo (meaning to cut) and graph (meaning pictures) [51]. A computerised tomography scan uses ionising radiations or X-rays coupled with an array of electronic detectors to elaborate the structure of the brain by analysing a pattern of densities from the sliced or cut sections of the brain tissue [52]. It involves further detailing like blood perfusion. The X-ray beam rotates around the object situated in the scanner. Rotation is the choice of action as it allows multiple projection beams to pass through the object. The X-ray beam passes through the subject and a beam corresponding to each unit measurement is called a ray. A view is a set of measurements accomplished during the translation of the beam through the subject [53].

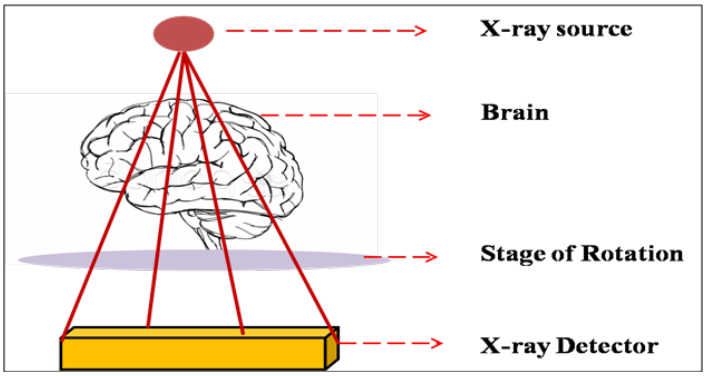

Figure 6: Schematic representation of brain CT scan

The first generation CT referred to as Hounsfield's Mark I scanner could measure a total of transmission of 160 rays per view [55]. Over years of improvement today the scanner has attained a speed of 750. This completes one view. After completion of the first view the tube detector is rotated around the subject by 1° to capture the second view. The Mark I scanner would complete the process by taking 180 views over 180°. The scanner today collects over 1000 views over 360° for a complete view. Data collection is obtained by a single narrow beam with a sodium Iodide scintillation detector. This complete arrangement is referred to as first generation CT. The objective of the CT scan image construction is to determine complete and detailed analysis of attenuation that occurs in each matrix. The calculated attenuation values are then represented as gray scores on a two dimensional level. Reconstruction of image by the CT scanner can be accomplished by following the equation : $X_i = u_1+u_2+u_3+.....+u_n$ where $X_i = -\ln (N_i/N_o)$, N_i is the transmitted x-ray intensity measured by the detector. N_o is the x-ray intensity entering the subject (patient) for the particular ray and $u_1 = w_1\mu_1$ is the attenuation in each view. Similarly, sum of all attenuation values measured at all possible angles is obtained [55].

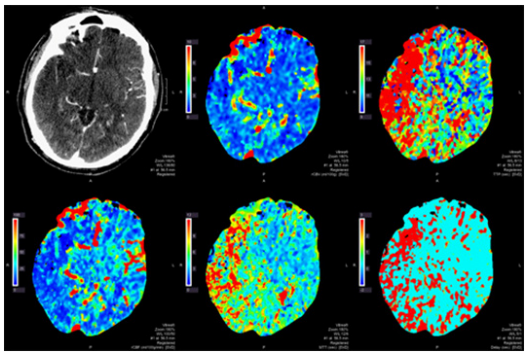

Figure 7: Representative CT brain images to study the effect of stroke. Red denotes areas of stroke while blue denotes brain blood flow affected due to stroke (Reprinted from Cedars Sinai website).

The four unknown's ui to u4 can be obtained by solving all the equations simultaneously. However the advanced version includes a computationally efficient approach. This algorithm is referred to as algebraic reconstruction technique (ART). It is achieved by taking measurements from each view and comparing attenuation values with respect to the values obtained from the first view. Adjustments are made accordingly and finally these adjustments are divided equally along the rays. The process continues to generate several estimates adjusted on the basis of the last estimate. Since the attenuation values predicted by the last obtained estimate matches with most of the previous measurements the final image thus constructed is reffered to as the true image. One limitation faced by the ART images was that it lacked image quality as the transmitted x-ray intensities were low. This would lead to noise referred to as quantum mottle, which is rather unavoidable during detector measurements. However, since ART does not achieve 100% exactness with measurement values as these values contain a degree of error. Backprojection is one innovation which helps a better reconstruction of image. Back projection is the division of the sum of the attenuated measurements along the path followed by the ray. Back projection is much efficient and resolves the problem faced by ART in image construction. Difference in attenuation of the X-rays among the surrounding tissues generates a contrasting image X-ray image. The attenuation of X-rays is proportional to the brighter appearance of the image [56]. The higher the attenuation of the X-rays the brighter the image appears. Air causes least attenuation of X-rays and hence appears black whereas, bone and calcified structures appear white on an X-ray plate [57]. The images produced are not of very high resolution but provide considerably reliable data and analysis of the brain condition [58]. Images produced are two dimensional. A CT scan reveals those parts of the brain which are underdeveloped, sites of brain injury, or regions impacted due to stroke, trauma, infections or lesions [59, 60].

FMRI

Functional magnetic resonance imaging (fMRI) maps brain activity by measuring the small changes in blood flow that are caused due to brain activity [61]. It can show specifically which part of the brain is functioning in response to the task performed by the patient. Not that the MR signals are directly sensitive to the brain activity, in fact, changes in neural activity cause changes in blood flow which is in turn reflected in changes in the MR signals [62]. Data from fMRI can be used to examine brain's functional activity, evaluate damage to the brain due to stroke or injury, to aid in diagnostic and treatment strategy. fMRI is able to detect anomalies in the brain activity which cannot be detected by any other imaging technique [63]. The main principle behind MRI is the fact that all atoms and molecules contain protons [64, 65].

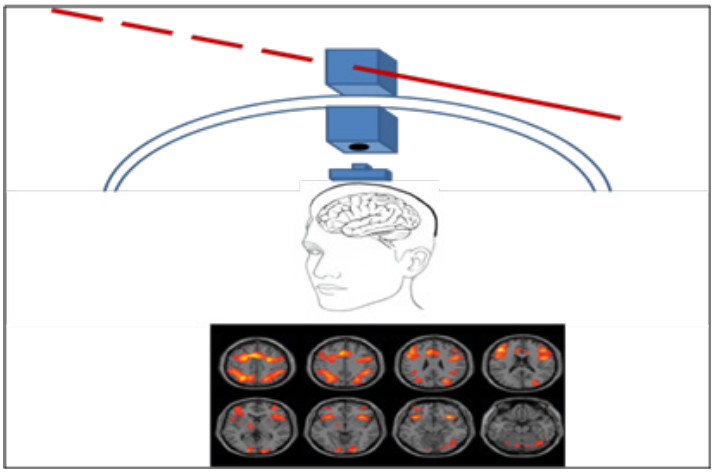

Figure 8: Schematic representation of fMRI

These account for magnetic resonance emitting wave signals which can be detected. NIRS is an optical technique which measures the level of blood oxygenation in the brain. A beam from the infra red part of the spectrum is focussed through the skull. The emerging light shows some degree of attenuation which is dependent on the level of blood oxygenation which in turn is directly proportional to the amount of brain activity [66]. The more the brain activity the more will be the oxygenation of blood. NIRS therefore provides an indirect measure of brain activity. Blood which carries oxygen, oxyhaemoglobin is present around the brain. Brain activity uses up this oxygen from blood causing it to covert to de-oxyhaemoglobin. Oxyhaemoglobin and de-oxyhaemoglobin have different magnetic properties due to the presence and absence of haemoglobin. Oxygenated blood has a slightly stronger signal as compared to the de-oxygenated blood. fMRI detects these sites of usage of oxygen in the brain and that is referred to as site of brain activity.

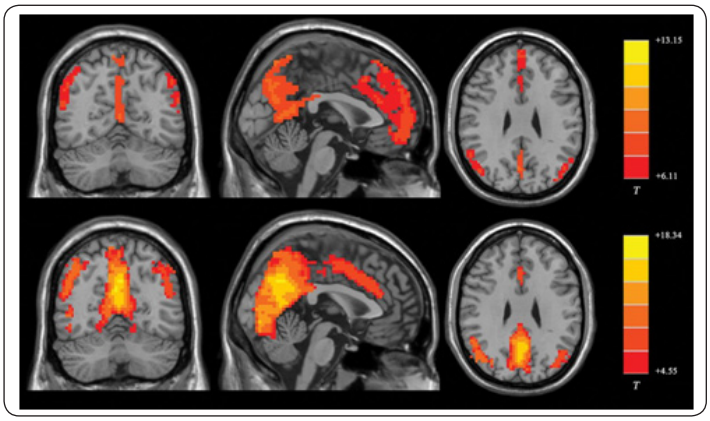

Figure 9: Representative fMRI images. Reprinted with permission from: (Lv et al. 2018)

Of advantage is the fact that different molecules possess different magnetic resonance and can be tracked to measure brain activity. Interestingly, neural activity causes higher blood flow than the changes that occur due to oxygen metabolism. This causes more blood flow and oxygenation when brain activity increases. This phenomenon is referred to as blood oxygenation level dependent (BOLD) effect [68].

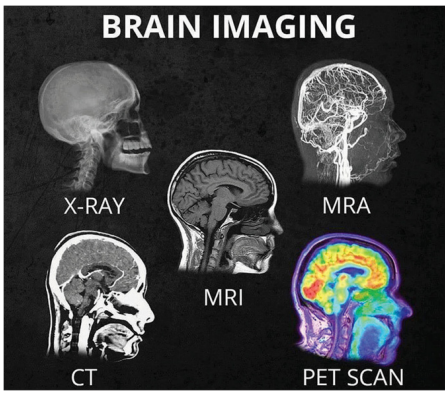

Figure 10: Figure depicting comparison of brain images with X-Ray, CT, MRA Magnetic Resonance angiography, MRI and MRI. (Reprinted with permission from: San Diego brain injury foundation)

These serve as the basis for fMRI. The complete image is generated by a cumulative monitoring of wave frequencies differences between active and inactive states in the brain. The image generated is of high resolution and good contrast between tissues. The fMRI detections are therefore a qualitative method as it depends on the complex changes that occur due to blood flow and metabolism of oxygen [69]. Despite the imposing applications in functional brain imaging fMRI has its own disadvantages. (i) Functional brain imaging studies can establish the regions involved in performing a particular task, however it does not have the capability to establish whether that particular region is necessary for that task (ii) laboratory settings for the performance of a task does not match with the real life practical conditions which exist under whose influence the task is actually performed. This creates a discrepancy in the functional analysis of the brain imaging taking place thereof (iii) human decision making behaviour is much influenced by several parameters, it is difficult to establish whether functional imaging technique is enough to assess this fundamental challenge. In this respect forward and reverse inferences should be avoided (iv) Lastly performing an fMRI on a patient is risky if the patient is claustrophobic. Even patients with implanted medical devices are at a risk of device failure under fMRI exposure. To overcome these issues is the next big challenge in the field of fMRI brain imaging. Achieving better temporal and spatial resolutions and the use of better contrasting mechanisms could be of advantage in the improvement of this technique. The software parameters should be best arranged to get reliable statistical data.

Arterial spin labelling (ASL) is a method used to directly detect changes in the blood flow [70]. ASL detects magnetic resonance signals arising from the arterial blood before it is distributed to different regions of the brain. By comparing MR signals from two different regions of the brain which have utilised the oxygenated blood differentially, the static signals arising from the hydrogen nuclei is subtracted and this results in signal differences from the delivered arterial blood to remain which are then depicted as an image. A combination of ASL and BOLD imaging techniques is being developed. This technology will provide a better quantitative analysis of brain function and oxygen metabolism changes [71]. This synergistic technology may act as the next generation of fMRI methods and hold immense potential in brain imaging technology [72].

MPM

Multiphoton Microscopy (MPM) is considered to be the best method for imaging live cells and intact tissues which can be imaged from the molecular level upto the whole organismic level [73, 74]. MPM is further suited to perform brain imaging with minimal invasion. Imaging can also be done over a prolonged period of time [75]. This makes it possible to image dynamic biological processes over a period of days or weeks [76]. As such inherently complex biological processes can be explained well by the vast imaging data made available by MPM [76]. MPM provides an additional advantage of imaging live tissues by improving the depth of penetration and so helps in reducing further photodamage. MPM can achieve imaging depths ranging from 500µm to 1mm without any compromise in image quality [77]. Deeper structures in the brain situated at distances relatively far away from the lens can be imaged by regulating the Gradient index (GRIN) which is able to resolve the optical sections located deeper in the brain [78, 79]. This is achieved by the use of near infrared (NIR) lasers of time length of the order femto seconds to generate non linear signals in the visible range [80]. NIR excitation increases the capability of imaging at deeper depths into a sample by using a light scattering system which is proportional to the fourth power of the excitation wavelength. Multiphoton excitation (MPE) is based on the principle that the transition energy of two or more photons used is enough to cause the fluorophores to excite from the ground state to the excited state and arrive at the sample simultaneously [81]. The probes could be of two types. Exogenous probes like Hoechst or Alexa fluors or endogenous molecules which include NADPH or other proteins can serve to generate fluorescent signals. MPM follows the second harmonic generation trend whereby two or more photons simultaneously transfer their energy to a single photon bearing half the wavelength. Some of the key features in MPM imaging are: (i) it eliminates out-of-focus fluorescence and produces sharper images by limiting the fluorescence excitation to the focus on the microscope objective (ii) an additional advantage is the availability of a huge variety of fluorophores to choose from for both structural and functional imaging (iii) multiple measurements can be taken simultaneously

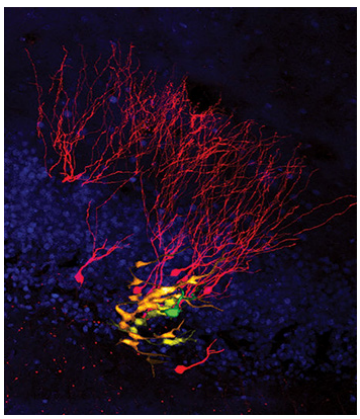

Figure 11: MPM image of a single neural stem cell (Reprinted with permission from: Sebastian Jessberger, University of Zurich)

due to the fact that a single excitation wavelength can be used to excite

different fluorophores (iv) Apart from the extrinsic signals, available intrinsic signals arising from autofluorophores, can also be used for imaging cellular metabolic processes [82]. However MPM suffers with a disadvantage in which the tissue area in close proximity with the lens may get injured and hence develop immune response or suffer mechanical damage [83]. MPM imaging is used to image the brain through a thin skull or through the cranial windows. These allow time kinetic imaging of the brain which is essential for pharmacological treatments and studying chronic brain diseases. Though MPM offers high resolution in vivo imaging, yet however the fact that the skull needs either to be thinned or a part of it removed to obtain brain imaging restricts its applications in the clinical fields.

Applications of MPM

MPM has been used to study the dynamic deposition of A in the brain in Alzheimer's Disease (AD). Amyloid plaques and neurofibrillary tangles are the main pathognomonic features of AD [84, 85]. Imaging of the plaques has been possible using MPM. Derivatives of thioflavin-T and congo red have been synthesised which can cross the bbb and label the plaques [86, 87].

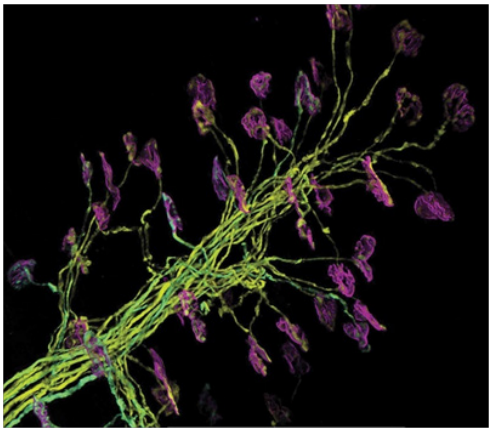

Figure12: MPM image of motor neurons of 9 day old mouse (Reprinted with permission from: Sebastian Jessberger, University of Zurich)

By utilising these engineered derivatives alongwith high signal to noise ratio and huge resolution capacity MPM is able to detect and image individual plaques in the brain. Another way of labelling the amyloid plaques in the brain is by the usage of fluorescent antibodies targeted against it [88]. MPM has also been used to study oxidative stress by imaging the reactive oxygen species associated with the amyloid plaques. MPM has also revealed a lot about amyloid angiopathy whereby MPM determined the structural damage and functional disruptions caused to the affected vessels as a result of Amyloid deposition in the brain [89, 90]. MPM has also been used to study the dynamics of the dendritic spines of the hippocampal neurons. This was achieved by two-photon imaging of the CA1 pyramidal neurons which were fluorescently labelled [91]. MPM has also made it possible to study the plasticity of the dendritic spines in vivo. The high resolution ability allows MPM to study cell metabolism, synaptic signalling mechanisms, spine morphology and dentritic abnormalities associated with brain diseases. MPM with its classical neuroimaging technologies provides the additional advantage of imaging subcellular structures and neuropathological features which were otherwise too tiny to be detected. This sets the possibility for high end critical evaluation of these structures for improved diagnosis and treatment of brain disorders. Power densities on the order of MW/cm^2 are required for signal generation. This power density generated is reached at the focal plane of the objective lens. Optical sectioning, which refers to the confinement of the signal to the focal plane reduces any damage to the photo arising due to signals from above and below the plane of focus. Less sample damage is obtained by using lasers belonging to the femtosecond range which possess high peak power, essential for maintaining low average power. It follows the equation:

$$I_{2photon\ signal} \alpha I^2_{laser} \alpha (P_{avg} / F_{rep} \tau)^2$$

Where intensity can be increased with a constant repetition rate (F_{rep}), and a reduction in pulse width or alternately by increasing the average power (P_{avg}). This equation therefore depicts that shorter pulses are more advantageous in imaging living samples. Creating images from the molecular level upto the whole organism requires complete information of the depth of the image to be able to reconstruct a three dimensional display of the given sample. Information on depth can be obtained by optical sectioning, by adjusting the microscopic focus further into the sample.

Challenges and Future Prospects of MPM

MPM offers imaging a sample with several fluorophores. This causes two disadvantages: from the economic point of view it can be very expensive as the cost of usage of several femtosecond lasers can be exorbitant which ultimately limits the use to a single laser. Further, this technique often requires fine tuning of the laser to adjust the wavelength such that the fluorophores can be excited simultaneously. Another way to scan a sample sequentially can include tuning of the laser to the optical excitation wavelength of each fluorophore though this can cause some photo damage and compromise the imaging speed. To overcome these issues a laser with short pulse width and high bandwidth can be used. A recent collaboration between Thorlabs and IdestaQE have designed Octavius-2P, a highly engineered Ti:Sapphire turn-key laser of 10fs for multiphoton applications[92]. The advantage of using a 10fs laser is the fact that it possesses high peak power and low average power which is required for deep tissue imaging with minimum photo damage. It is also accompanied with a broad bandwidth laser pulse which is absolutely perfect for the excitation of multiple fluorophores simultaneously [93].The broadband laser pulses increase the strength of the signal generated by the fluorophores without requiring tuning the laser at the exact excitation peak. Further, it is noted that the two photons required to generate the non linear signal do not require being of the same wavelength. This results in complete utilisation of the entire spectrum of the laser pulse towards excitation of the

fluorophore. If the width of a laser pulse is reduced it leads to an increased propensity in the broadening of the temporal pulse due to dispersion. This necessitates the design of a microscope with pre-compensation abilities and minimized dispersion. The NIR excitation path does play a role in minimising dispersion, additionally, an optional dispersion compensation module (DCOMP-BCU) can be added to the existing microscopic system [94]. The DCOMP-BCU relies on a new chirped mirror technology which compensate for the arising dispersions, which is mapped with the microscope requiring no further tuning.

MPM is therefore an efficient technology for imaging live and intact tissues. It has given a rapid boost to research in brain imaging and a huge impetus to the commercially available imaging systems. The Thorlabs Multiphoton Microscope combined with the Idesta Octavius-2P represents a well engineered flexi microscopic system towards the latest and most advanced technologies in brain imaging. It has achieved desirable capabilities by the concurrent amalgamation of current and next-generation technologies in imaging the brain.

PAT

The average width of the human cortex is about 3mm, but the thickness of each of the six layers of the cortex is in the range of micrometre hundreds [95]. PAT has the ability to image the brain across a varied range of thickness [96]. The spatial resolution of PAT can be regulated at both optical and acoustic levels while the depth of imaging domain adjusts automatically [97]. PAT qualifies as a high resolution imaging application surcompassing a wide range of scale length [96]. The ratio between image depth and spatial resolution in PAT is approximately 200. PAT can generally be classified on the basis of units of imaging depth of optical transport mean free path (OTMFP) which ranges from 1mm in the muscles to 0.6mm in the brain. Specifically PAT can be classified into three categories based on image depth of the target. The three groups are (quasi)ballistic, quasidiffusive and diffusive PAT. The imaging depth for the three groups (quasi)ballistic < quasidiffusive < diffusive in terms of TMFP are 1 TMFP < 1-10 TMFP < 10 or more TMFP, while that in terms of mm are .6mm < 0.6 to 6mm < 6mm or more respectively. This exemplifies the expanse of optical scattering tolerance of PAT which is a crucial parameter in high-resolution optical imaging.

(Quasi)ballistic PA Brain Imaging: The typical PAT application in the ballistic regime is optical-resolution photoacoustic microscopy (OR-PAM) [98]. The structure of an OR-PAM includes an objective lens which focuses the excitation laser beam to an optical diffraction-limited spot. The resultant PA signals are detected by an ultrasonic transducer characteristically of a single-element. This ultrasonic transducer is usually aligned with the objective lens confocally for achieving optimum detection sensitivity. The optical focussing is generally 10 times more regulated than acoustic focussing, hence lateral resolution of OR-PAM is mainly determined by its optical focussing. The axial resolution of OR-PAM is measured by the detection bandwidth of the ultrasonic transducer. As the acoustic attenuation that occurs is frequency dependent, the detection bandwith of the ultrasonic transducer is usually matched with acoustic path length. Lateral resolution of OR-PAM ranges from 220nm to 5µm, while it's imaging depths range from 100µm to 1.2mm [99].

Applications of OR-PAM

Prefect cortical hemodynamics as an additional platform to understand underlying neural activities. High quality resolution images of mouse cortical vasculature can be obtained using haemoglobin in the red blood cells as the working endogenous contrast [100].

Imaging a single neuron is also possible using exogenous or endogenous contrast. OR-PAM when used with appropriate and meticulously selected contrast agents can be used to study physiological activities of a single neuron, which include propagation of action potential, release of neuro transmitters, and synaptic connections and communications between them [101].

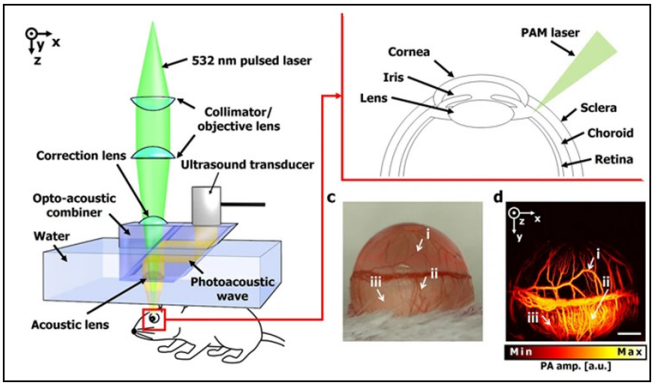

Figure 13: Schematic representation of OR-PAM (Reprinted with permission from[102])

Quasidiffusive PA Brain Imaging: It mainly includes targets having image depths more than 1 TMFP but less than 10 TMFPs. This range of imaging depth makes it an ideal imaging technology for whole-brain imaging of small animals. The core principles used in quasidiffusive PAT are almost similar to those used in (quasi)ballistic PAT. The typical PAT application in the ballistic regime is acoustic-resolution photoacoustic microscopy (AR-PAM) [103]. The spatial resolution achieved by the AR-PAT is approximately 50µm when a 50MHz ultra transducer is used [104, 105]. Hence it can be classified as one of a microscopic modality. Since it can break the diffusion limit of the optical domain it is also considered as a "quasidiffusive" modality. Both the OR-PAM and the AR-PAM follow the same focussing principle, however, the AR-PAM tunes the laser focus at a range wider than the ultrasonic focal spot, such that the entire area of the ultrasonic focal zone is illuminated well.

Applications of AR-PAM

AR-PAM was engineered for imaging mouse cortical vessels by keeping both the scalp and the skull intact. A focused ultrasonic transducer of 20MHz and a 90% bandwidth has helped AR-PAM achieve a lateral resolution of 70µm an imaging depth of more than 3.6mm into the mouse brain.

Diffusive PA Brain Imaging: The typical PAT application in

the diffusive regime is photoacoustic computed tomography (PACT). PACT uses an array of transducers to detect simultaneous signals from a bigger region of interest which can be exploded with a larger laser beam. A high resolution PACT image is then contstructed by the use of an inverse algorithm [106]. This inverse algorithm is a method triangulation of sources available from time-resolved acoustic signals. The advantage of imaging in PACT is that depending on the target organ the transducer array in PACT can be constructed to produce varied and desired shapes. Further PACT is the best suited imaging technique for deep brain imaging. A combination of near-infrared (NIR) optical excitation and low frequency ultrasonic detection help it to achieve deep penetration of the organ of interest to produce high resolution images. PACT has been successful in imaging brain structures 8mm beneath the scalp. By using a variant technology called thermoacoustic tomography brain images with a resolution of 4mm can be produced. High resolution and image depth availability makes PACT a promising technology for impeccable human brain imaging.

Applications of PACT

A PACT system has been used to an imaging depth of 7cm in tissue with the machine operating at 8 MHz, lateral resolution of 720µm and an axial resolution of 400µm. PAT has been successful in producing 2mm thick brain images of rhesus monkeys. PACT shows promise for non invasive brain imaging.

Molecular Imaging of the Brain using PA

Molecular imaging is a high end technology which enables the viewer to visualise both cellular functions at the molecular level [107]. Recently a lot of effort has been vested to develop PAT imaging technology which is accompanied with better sensitivity, high detection, optimization of contrast agents and better spectral mixing. Research community has now come up with a new technology referred to as the multispectral optoacoustic tomography for PA molecular imaging [108]. The difference between PA molecular imaging with that of traditional PA imaging is that in PA molecular imaging probes are conjugated with the particular targeting molecule and further imaged for the particular pathway. However brain imaging has always been a challenge more, so because of the presence of the blood-brain barrier (bbb) which poses an additional problem for PA brain imaging. Ideal PA molecular brain imaging requires the movement of the contrast agents across the bbb, which is a difficult feat to achieve. Several measures have been taken to overcome this hurdle, these include physically or chemically unravelling the BBB, direct delivery into the cerebro-spinal fluid, and intrinsic carrier mediated active transport. Of these active transport of agents is by far the most safest procedure for example 2-NBDG, a glucose analog, can be transported across the BBB via glucose transport protein GLUT. The only disadvantage is the fact that only a very small number of molecules can be actively transported across the bbb. Nanoparticles could also be a transporting agent of choice here. However, nanoparticles used for this purpose need to be specifically engineered with very small size >10nm and favourable peak absorption wavelength. The endeavour for an ideal nanoparticle for PAT imaging to cross the bbb is still on.

Functional analysis of the brain using PAT

PAT has been used for functional brain imaging on multiple aspects. PAT provides information on brain oxygenation levels. PAT is sensitive to both oxygenated and deoxygenated haemoglobin. Hence it can differentiate between increased blood oxygenation and decreased blood perfusion. Blood oxygenation is a positive indicator of increased neural activity. Another important indicator of brain activity is brain metabolism [109]. Faster consumption of glucose and oxygen indicate strong neural impulses and action potential. Hence tracking these elements can provide information on brain metabolism. PAT is able to measure changes in glucose and oxygen levels with high resolution and at a low cost. Full ring-array PACT system has been adopted to study resting-state connectivity of the brain. Several researchers have applied PACT to study the intricacies of brain injury and function using small animal models [110].

Challenges and Future Prospects of PAT

With much higher resolution and multifaceted functional analysis capability PAT is surely the most exciting brain imaging option today [111, 112]. Clinical translation of PAT to image the human brain is an exhilarating direction in the field of brain imaging. It promises to provide a better understanding of the neural processes and cognitive abilities of the brain [113]. The main obstacle in PAT human brain imaging is the fact that the human skull is the thickest (7 to 11mm) as compared to other animal models. This skull is therefore capable of distorting signals and absorbing and scattering light thus interfering with the imaging technology [114]. The severe aberration of the acoustic signals caused by the skull can significantly deteriorate the quality of the constructed image [115]. Research community is applying engineering improvements to overcome this problem [116]. In order to optimize the delivery of the incident light a novel photon cycler has been developed. This photon cycler recycles the photons back scattered by the skull thus reducing the loss of efficiency and intensity of the incident light. These photons are recycled back to the brain where they are available to generate PA signals. Concerted efforts are also being made to improve the distortions of the acoustic signals. This can be achieved by combining ultrasonic computed tomography with PACT for correcting the disparity in the speed of sound (SOS) within the field of interest. This holds promise in improving the image quality. High scalability and functional imaging ability of PAT will provide a whole new experience of looking into the brain when in resting condition or in a disorder.

Technology	Principle	Advantages	Disadvantages
EEG	Electrodes are used to detect brain activity. These electrodes are placed on the head of the patients. These electrodes then record the electrical charge generated by the neurons when they are transferring information in the brain.	Requires no surgery or invasive technique. Safe for the patient. Provides valuable information about sleep research in humans.	The result is only very qualitative. It does not provide a true detailed analysis of brain function. Since the electrodes are placed on the head, the exact neurons to be detected are untraceable, the procedure involves assessing several other unaccountablde neurons.
MEG	Electrodes are used to detect brain activity. The electrodes are placed on the head of the patients. These electrodes then record the changes in the magnetic field generated by the neurons when they are transferring information in the brain.	Requires no surgery or invasive technique. Safe for the patient. Provides valuable information about sleep research in humans.	The result is only very qualitative. It does not provide a true detailed analysis of brain function. Since the electrodes are placed on the head, the exact neurons to be detected are untraceable, the procedure involves assessing several other unaccountablde neurons.
PET	Radioactive glucose is fed to the patient. This radioactive glucose when in the blood stream reaches the brain. Once in the brain, it is metabolised for energy release. The utilisation of the radioactive glucose releases gamma rays which are then detected by the radiation detectors. The signals generated by the detectors are then processed into colourful image showing regions of brain activity.	It can record signals generated from an active brain in real time manner. It can be beneficial in detecting changes in the brain activity at an early stage of a disease. Hence it is more sensitive than MRI or CT scans.	It is expensive. The intake of the radioactive material may be harmful for some patients. The results are not absolutely precise. The colour generated in computerised image does not give a very detailed picture of the brain activity.
CT	A continuous source of X ray beam is passed through the head. The images are captured on a sensitive X ray plate. These series of images creates a structural image of the brain. Computes signals received from X - ray technology	Clearly depicts structural damage to the brain.	Does not have any implication on brain function. The impact of exposure to X ray can be of concern to the patient. Cost is Reasonable.
fMRI	Works on the principle that active areas of the brain require more oxygen. On the cellular level it means neuronal activity in specific regions of the brain implicate more blood supply to that region of the brain. The images can provide ideas about the specific region of the brain which the patient uses for performing a task. The resultant image is a three dimensional brain structure which is computed by analysing both radio and electrical signals.	The data produced is far more specific than that obtained from the PET. Resolution of image is high. The resultant image provides information about both anatomical and functional aspects of the brain.	The focus of the data is mainly on localised area of the brain. This procedure does not take into consideration the actual dispersed nature of the neuronal population.

MPM	Multiphoton microscopy, relies on the principle of simultaneous absorption of two or more photons by a fluorophore. It allows real-time observation cells and molecules while they are still intact within tissues.	With MPM it is possible to image dynamic biological processes over a period of days or weeks. MPM provides an additional advantage of imaging live tissues by improving the depth of penetration and so helps in reducing further photodamage. MPM can provide superior quality of resulting image. MPM can provide exceptionally large penetration with equally less phototoxicity.	It is highly expensive. The problem of simultaneous activation of the various wavelengths used persists.
PAT	PAT provides information on brain oxygenation levels. PAT is sensitive to both oxygenated and deoxygenated haemoglobin. Hence it can differentiate between increased blood oxygenation and decreased blood perfusion. Blood oxygenation is a positive indicator of increased neural activity. Another important indicator of brain activity is brain metabolism [110]. Faster consumption of glucose and oxygen indicate strong neural impulses and action potential. Hence tracking these elements can provide information on brain metabolism. PAT is able to measure changes in glucose and oxygen levels with high resolution and at a low cost.	High scalability and functional imaging ability of PAT provide a whole new experience of looking into the brain when in resting condition or in a disorder.	The skull acts as a physical hindrance in PAT imaging. It is capable of distorting signals and absorbing and scattering light thus interfering with the imaging technology

Conclusion

Brain imaging technologies not just help in viewing the brain at a multiscalar level but also create a better understanding of it for the development of improved diagnostics and therapeutics of brain diseases. Several technologies have been developed so far to accomplish the mission of brain imaging. Each developed technique is a better and an advanced version of the previous technology. PAT is the recent brain imaging technology which promises to bring in breakthrough data in the forthcoming years. Considering PAT's highly scalable spatial resolution, huge imaging speed, wide range of penetration depth, and improved functionality, it acts as a promising tool for fundamental neurophysiological research and applications in clinical neurology.

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