Acta Scientific Dental Sciences (ISSN: 2581-4893)

Volume 5 Issue 4 April 2021

Research Article

A New Microscope to Tiny Tumours - A Systematic Review

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Received: February 23, 2021

Published:

Abstract

Background: The authenticity and expertise of various imaging systems have been usually restricted as most of these imaging systems cannot visualize minute tumours. This is the reason certain cancers such as ovarian cancers and breast cancers are only diagnosed in their terminal stages. Moreover, despite landmark advances in varied areas of diagnostic imaging the detection and screening for most of these tumours remain poor. As the world is constantly being woven in the cocoon of technology it thereby becomes imperative to develop and come up with better diagnostic modalities than the ones available to us. Also, with the global cancer burden constantly on the rise, various new imaging modalities come up as new waves in the field of radiology.

Aim of the Study: To assess the significance of imaging systems in the diagnosis of small-sized tumours.

Research Question: Are the current imaging modalities beneficial in viewing and diagnosing small-sized tumours?

Materials and Methods: With the Medline database taken as a source for authenticated scientific research data, articles were selected having undergone Randomized Control Trial. Out of these, articles (studies) were chosen which met the criterion for Systematic Review.

Keywords: Microscope; Tiny Tumours; Radiology

Introduction

It was around (460-370) BC when Hippocrates "Father of Medicine" coined the word "CARCINOS" [1,2], which serves as an etymological origin for the modern-day word "Cancer". History is replete with examples of scientists and researchers who have studied cancer and documented cases which have served for better understanding of this deadly condition, one of whom was British surgeon Percivall Pott [3] who was the first to discover, in 1775 that cancer involving the scrotal region was a common disease among the chimney sweeps. With the advent of the microscope around the 18th century, there was for the very first time an established intersection between the imaging world and the mysterious world of

tumours. The discovery of x rays towards the end of the 20th century served as a turning point, since then the number of available image modalities has quadrupled and today it is even possible to observe cancer cell dynamics. However, there still exist discrepancies in identifying small tumours thereby curbing chances of a better prognosis.

Materials and Methods

Various researches and studies have documented that the use of new optical imaging techniques is both sensitive and specific. With this fact in mind, a literature-based systematic review was carried out to fulfill the aim of the study. Using Cochrane collaboration taken as a source for authenticated scientific research data, about 30 research articles were selected having undergone randomized control trials. Out of these 25 articles were screened and chosen to meet the criterion for systemic review.

Result

As globally the cancer burden is constantly on the rise, it has become imperative to accelerate the development of useful technologies that could clinically prove to be successful in diagnosing the earliest signs of the tumour cells.

The imaging community must expand the frontiers of cancer imaging modalities to introduce new and multidisciplinary approaches that would allow the researches to expertise and find new optical technologies that could prove to be clinically feasible to observe and understand the distinct optical signatures of the precancerous abnormalities.

There is also an urgent need to widen the horizon and start employing new molecular techniques to the imaging world to develop the conventional modalities for overcoming the real-time challenges in these fields.

Not only are the new imaging modalities imperative for screening and diagnosing but it also a haven for the scientific and research community which would benefit from these technologies especially in the fields of cancer research and the understanding of tumour microenvironment.

Also there needs to be improved clinical studies about the field of image-guided interventions especially involving the fields of oncological surgery.

Discussion

Background

Over the years, biomedical imaging has become an integral part of tumour screening and diagnosis. Modern imaging methods have revolutionized early cancer diagnosis and have paved the way for a better and more accurate prognosis. At this given moment, there are six important commercially available imaging modalities, namely X-ray (plain or computed tomography), ultrasound, magnetic resonance scanning (MRI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), and optical imaging. Of the mentioned techniques only CT scan, MRI, SPECT and PET are capable to deliver 3 -dimensional images of the tumour affected areas in the human body [4]. Although these established clinical modalities are present, there still exists a major challenge to detect biological abnormalities of the cell in a complex tissue microenvironment. These limitations range from a relative lack of biocompatible specific probes with excitation and emission in the second infrared optical window to huge expense in developing commercially viable instruments guarantying optimal performance in the second optical window and finally the effective light attenuation in tissue and blood which remains substantial relative to that of visible range.

Now, the root of this problem is that of "scale" [6]. A typical human cell in the body is around 10 μm with a volume of approximately 1 pL. Now, this would mean that every 1 cm of solid tissue would contain approximately 10^9 or 1 billion cells, if we continue this math the total number of cells in the human body would be approximately 10^14 cells. Now if a malignant clone evolves then the clone is directed to a single cell so by logic the clinician needs to diagnose that "one" cell out of 10^14 cells (an inconceivably small number) to detect the tumour. One must also try and understand that the concept of detection and imaging is rather vague and is based on the volume element of the particular imaging modality used (i.e. voxel). At present, the threshold value for detection of a tumour in a human body is a minimum of 10^9 cells, hence with logic while a given individual is being screened for tumour he/she has somewhere between 10 - 10^9 tumour clones in the body (this is an extreme level of uncertainty) unacceptable to the very basics of diagnosis.

(A solid tumour is known to display "Gompertzian Kinetics" i.e. the mathematical exponential [6-9] function which shows the re-

lationship of the growing tumour to cancer detection and imaging. It begins with the lag phase starting from the single-stage and then slowly enters the log phase which is accompanied by the angiogenesis [7-9] at around 10^5 cell stage followed with the second lag phase up till the death of the patient at around 10^12 cell stage. [The goal of successful imaging should be to detect the tumour ideally before the angiogenic switch]).

Limitations of the currently used imaging techniques

X-ray imaging and CT-scan	Plain films work as a principle of measuring the beam attenuation, however, the main constrain against this principle is that for this technique to effectively function the given exogenous contrast agent must have a high atomic number (exceptionally high to be able to attenuate x-ray beams) At present there are several non -targeted exogenous agents used and are injected at molar concentration. The major problem with this is that these agents become invisible only after a few fold dilutions in the blood [5].
Ultrasound imaging	Imaging via ultrasound uses sound waves anywhere between 1 to 10Hz to image a soft tissue however one must realize that sound waves are prone to scatter at the bone and air interfaces thereby making many parts of the body inaccessible. As a result, the effective imaging depth is reduced to around 10 cms [5].
MRI scan	This imaging modality while functioning uses protons and as these protons are placed in the spin of the magnetic field they align themselves in orientations, now the relative distribution thus achieved is the BOLTZMANN DISTRIBUTION. At 1.5T the small excess in this distribution represents at most, as several parts per million of the total protons present. However one must note that the total proton concentration of the tissue is 80M this means the signal thus arising is only from 80μmol/l of the protons present. Now one can improve the signal to noise ratio by increasing the magnetic strength from 1.5 T to 3 T as the signal to noise ratio runs linear to the field strength one can thereby increase the strength up till 7T which results in 4.7 fold improvements. However one must remember that high field strength introduces other problems such as increased tissue healing. Thereby when contrast agents such as Gadolinium are used it is the effect of the ions of gadolinium on the magnetic resonance relaxation which is being imaged. This effect is only observable at approximately 50μmol/L making targeted agents difficult to develop. [In simple words it would require more than 10^7 Gadolinium ions to improve detectability] [5,9].
SPECT	This imaging modality uses the principle of radioisotope decaying. Now when the radioisotopes decay it releases Gamma rays (i.e. photons) containing energy in random directions. It must be noted that high energy photons cannot be focused using conventional lenses therefore collimators are used to restrict the angle of emitted photons. However typical parallel hold collimators for SPECT scanners have sensitivities of only 0.02% i.e. $1/5000^{th}$ of decay event is measured. (In simple words only 5% of 140keV of Technetium (99m) remains after traveling over 25cms through the body i.e. only $1/100,000^{th}$ of the photons are detected from the cancer site) [5].
PET	PET imaging modality uses positrons (which are antimatter- equal in mass but opposite in charge of electrons). Depending on their energy, positron travels an average distance (or the annihilation distance) before interacting with an electron. Now to be understood here is that the density of the tissues will have a profound effect on the above-mentioned distance (less dense tissues such as the lungs will have an exceedingly higher annihilation distance than others resulting in lower resolution). As the condition progresses the matter or the electron and the antimatter or the positron annihilate to produce 2 antiparallel photons (511 –keV). Now, these photons are detected by the opposing crystals mounted on the stationary ring. As seen before in the SPECT isotope the body tends to attenuate the 511-keV photons with only 10% remaining after passing through 25 cms of solid tissue. On the counts of sensitivity, PET scan detects $1/2000^{th}$ of the photon produced at the cancer site [5,10].

Table 1

To overcome the above-mentioned limitations of the currently existing modalities there are several new imaging modalities which are been experimented and some are being used currently to better diagnose and understand particular tumours and cancer origins. This review aims to understand and study these new techniques and how do they function in a practical environment. Some of the new imaging modalities are discussed below.

DOLPHIN [Detection of Optically Luminescent Probe Using Hyperspectral and Diffuse imaging in near Infrared] - developed by the MIT

On March 7th, 2019 MIT's News Office published a paper that mentioned the advent of a new imaging system for detecting tumours as small as a couple of hundred cells deep within the body. Their researches mentioned that their system relied on near-infrared light to track a 0.1 mm fluorescent probe via the digestive tract of the living mouse thereby detecting signal through the tissue depth of 8 cms. (Now, this was a landmark in the field of imaging modalities as to date no biomedical imaging system could trade-off between resolution and depth of imaging -deeper than 3 cms into the tissue). The researchers hoped to adapt this imaging technique to detect tumours such as ovarian cancer and do so in an absolute non-invasive way and without using any form of radioactive labelling (most of which aren't detected until the final stages) [11,12]. The researches proposed the idea of using near-infrared light with a wavelength ranging from (900 - 1700 nms) which is considered best for biomedical tissue imaging as light with longer wavelength does not scatter much when it strikes an object, this idea allows the light waves to penetrate deeper into the tissues. Further, the researchers also proposed to use the concept of Hyperspectral Imaging to enable simultaneous imaging in multiple wavelengths.

What is hyper spectral imaging?

A good way to define or understand a material is to understand how light interacts with it! This particular imaging technique uses an image spectrometer (to split light into spectrum) and a hyper spectral camera to measure and collect information in relation to different spectra's. Now the collected spectral information is used to form an image or a Target (note each image pixel of this target has complete information of the spectra). To make things more simpler take for instance one has an image clicked from a digital camera, the camera would shoot the target only in three related spectra's i.e. Red, Green and Blue this is to match our human vision [the rest of the colours we see in the image are only a combination to these three colours]. Now if one takes hypothetically the spectral data provided by this picture and compile it into a book one will only get information about 3 pages i.e. red, green and blue. Now in a hyper spectral camera the camera will collect information and shoot the target with tens to hundreds of narrow wavelength [generally 220 different types of wavelengths are used]. So now when one compiles the hypothetical book for this data I get 220 (minimum) pages of information. Thereby we can choose any part of the information and research or question it to understand the Target.

Once the information is obtained from the Hyperspectral scan the researchers can then analyze the data using specific algorithms which would allow them to identify the location of the probe and on analyzing light from a narrow wavelength band in the near-infrared spectrum the researches can also determine the depth of the fluorescent probe. To demonstrate the effectiveness of this system the scientists were able to successfully track down a 0.1 mm cluster of fluorescently labelled nanoparticle in the digestive tract of the living mouse after it swallowed it. Now, this would mean that in practical instances the imaging system could track down non-invasively a 0.1mm sized tumour i.e. nothing but only a cluster of few hundreds of cells (remember the angiogenic switch state mentioned above).

Figure 1: Optical setup for the Dolphin system.
Image source: Scientific Report: - Deep Tissue optical imaging of Near cellular sized features Published on 7th March 2019.

For the first time, a fluorescent imaging system has approached high penetration depth while maintaining a standard trade-off in terms of its resolution. Now this kind of system could be combined with any fluorescent probe which emits light in the near-infrared spectrum. In the practical world, the researchers are trying to detect similar results from ovarian cancer staging processes, as ovarian cancer is one of the few which is detected only in its later stages as the tumour size remains extremely small and most of its signs remain nondescript.

Optical molecular imaging for tumour detection, image-guided surgery, and research for tumour microenvironment

According to the National Cancer Registry, the cancer burden in India alone is 1.16 million cases with more than 784,000 deaths currently [13]. As molecular biology and cytology are evolving every day it has become imperative for us to understand the extremely complicated mechanism of cancer and study its nuances which include cancer cell invasion and metastasis within the tumour microenvironment. To understand these concepts we have to understand what optical imaging means. Bioluminescent imaging using luminescent enzymes such as luciferase and fluorescence imaging using fluorescent proteins and dyes are called optical imaging.

[Later in the paper shall we in detail study each of the concepts, however, before that, we must acquaint ourselves with how does molecular optical imaging associate with tissue microenvironment] [14].

Luciferase derived from Firefly and Renilla have been widely used in reporter assays to study the target genes during the cell culture (Cells which show stability in expressing luciferase proteins as a result of the introduction of the luciferase genes produce bioluminescence after attaching itself with substrates such as D-luciferin or Coelenterazine this can be measured using CCD cameras) using this same logic when cancer cells carrying luciferase are transplanted into the mice are injected along with substrate coelenterazine the movement of the cancer cell can be visualized in the living animal [15-17].

Signal transduction or cancer cell signaling can be observed using a promoter-reporter using the luciferase gene.

Cancer cells + (Firefly derived) Luciferase+TGF-B PROMOTER substrate D-luciferin is injected into the mice the TGF signaling can be obtained from the cancer cells [15,16].

Next factor to be considered is the spatial resolution; now in cases of fluorescent imaging the spatial and temporal resolution is quite high as a result not only will the movement of the cancer cells in the blood vessels easily analyzed but also the complex metabolic metastasis processing such as the intravasation of the cancer cells in metastatic conditions be finally understood.

In 2008 researches from Ehime University Graduate School of Medicine, Japan with Dr. Sakaue Sawano developed "Fucci". Now, this system helps to visualize the progression of the cell cycle in real-time. Fucci is an advanced imaging technique that takes the advantage of the fact, that the cell cycle-related proteins is strictly controlled by the ubiquitin-proteasome system during cell proliferation. Using this knowledge one can know and understand the effect of medications on the cell cycle to help counterfeit the cycle and related tumours.

Currently available imaging modalities do never standalone with the idea of the surgical workflow; they often behave as a standalone test conducted in isolation from oncological surgery. As we understand the molecular and genetic underpinnings of cancer it has now become easier to incorporate the studies to develop suitable treatment plans.

Let's study the reporter probes and various other techniques which fall under the spectrum of optical imaging

Fluorescent reporter: In cases of fluorescence imaging excitation light is required to excite a given fluorophore to generate a signal in form of light at a specific wavelength that needs to be detected to the image. There are many fluorophores known to us however each of them has a unique property [18].

Fluoro dexo glucose positron emission tomography: FDG-PET is a molecular imaging technique for tumour detection with broad clinical use. FDG is an analog of glucose that is internalized and finds its way into the metabolically active tissues including the tumour cells. It has been used to image a variety of cancers and tumours such as melanoma, lymphoma, head and neck cancers, etc [19-21].

Contrast-enhanced fluorescent imaging of cancer: The contrast in cases of imaging especially for *in-vivo* imaging can be improved via increasing the signal through minimizing the process of scattering and absorption by the biomolecules or can also be done via avoiding the autofluorescence spectral window [22-24]. Biological molecules containing chromophores are responsible for the absorption of photons and then these photons are used as energy sources which can then be transferred as heat. Melanin, hemoglobin, amino acids containing groups such as (tryptophan, tyrosine and phenylalanine) or vitamins such as (retinol, riboflavin, and reduced NADH) are some of the common chromophores which can be used for the process. Water and 70% of the human tissues show absorption mainly in the infrared region. Besides scattering and

Reporter	Obtained From	Excitation wavelength	Emission wavelength	Uses
Green Fluorescent Protein	*Aequorea victoria* (Jellyfish)	470 nm	510 nm	Used to study the subcellular processes such as gene localization and expression and protein localization.
Ds RED	Dicosoma Coral	560 nm	580 nm	The stability of this protein allows it to be a viable option for cellular assays, animal work models, and tumour detection.
Near Infrared fluorophores	Based on derivatives from nir dyes	600 nm	700 - 900 nm	The most common example of the protein currently being used is Cyanine (Cy5 and higher). Tissue absorption, scattering, and autofluorescence are lower in NIR fluorophores.
Bioluminescent	*Photinus pyralis* (American Firefly)			Upon reaction with a substrate luciferin in the presence Of ATP and oxygen; light is produced. Luciferase encoding genes have been extensively used by scientists as reporter genes for protein expression.

Table 2

absorption, the endogenous chromophores also contribute to autofluorescence however the magnitude is lower than the externally introduced fluorophores [22-25].

Non targeted small molecular dyes: At present, there are 2 clinically available NIR dyes, they are Methylene blue and ICG. Methylene blue has been used for macroscopic visualization in cases of parathyroid surgery when used in high doses (100 mg/kg). A major advantage is that methylene blue becomes a NIR dye at 700 nm in cases of sufficient dilution i.e. 0.25 - 2 mg/kg (however it hasn't been yet approved as a NIR dye). In many cases, methylene blue is also used to successfully visualize fibrous tumours of the pancreas, parathyroid adenomas and paragangliomas. On the other hand, one can also use ICG (the only approved NIR small- biomolecular dye for surgery). Another agent 5-aminolevulinic acid or 5-ALA has been clinically approved for cancer diagnosis and cancer photodynamic therapy. In some cases, both ICG and 5-ALA exhibit a high background signal and to prevent this from happening Zwitterion NIR Dyes have been developed (showing better signal over noise ratio in comparison to the conventional NIR fluorophores [26-31].

Nanoparticulate agents: Self-illuminating Nanoparticulate agents such as Quantum Dots and single-walled Carbon Nanotubes have been used time and again for tumour detection and image-guided surgery.

Biomolecule-dye conjugates: In many cases the tumours have shown to represent several surface receptors, and the known benefit for targeted imaging design is its high specificity and affinity offered in the interaction of the receptor and the ligand. The prolonged circulation of the antibodies may also improve the accumulation of the imaging agents in the tumours as a result several antibody-dye conjugates have been developed for the same. Factors such as the Epidermal Growth Factor Receptor is frequently expressed in many types of cancers such as glioma, head and neck cancer, liver cancer, lung cancer, and ovarian cancer, and overexpression of EGFR is associated with metastasis and poor prognosis. Therefore, antibodies against the external domain have been developed to target and inhibit EGFR via preventing its association against ligands. With the presence of monoclonal antibodies such as cetuximab and panitumumab these antibodies can be used as carriers for imaging agents (EG: Cetuximab-Cy5.5 conjugate) [32-42].

Stimuli responsive nanoprobes for tumour detection and image guided surgery

Imaging of tumour using raster scan optoacoustic mesoscopy

Neo-angiogenesis is considered to be a cornerstone for understanding tumour progression [43]. As the number of tumour cells grows so does the requirements of these cells increase. The

Probes/ Sensors/ Agents	Uses
Enzyme Activated Probes	There are certain imaging probes that are activated in presence of proteases such as matrix metalloproteinase, lysosome hydrolases, and cathepsins (all of these are important constituents of the tissue microenvironment). E.g. Copolymer of poly-L-lysine and methoxy-polyethylene glycol succinate conjugated with Cy5.5 (dev. by Weissleder and his colleagues) [4].
pH Sensitive Probes	Otto Warburg had stated that cancer cells have an unusual rate of glycolysis followed via lactic acid fermentation compared to oxidation of pyruvate in normal cells, as a result of this continuous metabolic indifference there exists a huge amount of lactic acid in tumour microenvironment [4]. An ideal probe must be non-fluorescent in blood or intestinal fluid i.e. pH=7.4 and remain highly fluorescent in the acidic environment that is the tumour environment.
Hypoxia Responsive Nanomaterials	Dev. by Zheng and his colleagues a poly N vinylpyrrolidone conjugated with Iridium (iii) was developed and it showed an increase in the phosphorescent intensity with even a slight decrease in oxygen saturation levels [4].

Table 3

absence of enough oxygen in the microenvironment of these cells creates hypoxic spots, these hypoxic regions act as a stimulating factor for the new angiogenetic networks so with the growth of the tumour the rate of angiogenesis also increases. Now as angiogenic factors are continuously being released, the development of mature blood vessels ceases. On the application of *in vivo* imaging one can understand the dynamic processes involved in cancer development and treatment techniques. In today's time, angiogenesis is studied based on indirect mechanisms such as the degree of contrast enhancement post the contrast agent has been administered [44-50]. Scientists and researchers from Institute for Biological and Medical Imaging, Neuherberg, Germany used Raster Scan Optoacoustic Mesoscopy (RSOM) to study the performance of the instrument to reveal the angiogenic network supporting melanoma growth *in vivo*, at 50 MHz and 100 MHz via several millimeters of tumour depth.

On studying the performances of the two approaches the researchers concluded that: The images obtained via 100 MHz had a better and improved quality of small newly formed blood vessels. However, the images taken with 50 MHz produced better imaging of larger structures with lower frequency as a result of which more and better vasculature (ones > 50 micrometer) could be seen. Moreover, the smaller angular coverage of the 50 MHz detector performed better when it came to imaging the oblique blood vasculatures. The researchers also suggested that the combined approach of both the detectors could assist the clinician in obtaining a better picture of the angiogenesis. Application of RSOM proved successful in visualizing the small structures in respect to the higher frequencies, this was beneficial to understand the newly forming networks of the blood vessels [43].

Figure 2: Comparison of the imaging performance achieved by RSOM100 and RSOM50 imaging the vasculature of a melanoma tumour.

Image source: Pushing the Optical Imaging Limits of Cancer with Multi-Frequency-Band Raster-Scan Opto acoustic Mesoscopy (RSOM).

(The amplitude of the signal generated is directly proportional to the size of the object via which the signals have been generated. Moreover, one must also understand that the high frequencies have a lower signal-to-noise ratio than the lower frequencies. At the same time, the attenuation of the higher frequencies is more than that of lower frequencies thus if all the frequencies could be simultaneously constructed the higher frequencies would be masked by

the lower ones. Moreover, via processing the higher frequencies separately it has been found possible to improve the visibility for small structures) [43-45].

Thereby when we study the obtained data we can better understand the tumour growth, blood vessel networking, physiology, metastasis, and its impact on various therapeutic approaches. RSOM in- specific is a breakthrough in understanding the therapies that impact on the tumour vasculature.

Imaging tumours via second harmonic generation microscopy

Second-harmonic generation microscopy is a nonlinear process in which two photons with similar frequencies are taken and are made to interact with a nonlinear material to generate a new photon twice the energy of the initial proton (twice the frequency with half the wavelength) thereby conserving the coherence of excitation. The major significance of this technique lies in probing the collagen architecture and determining how it's altered from any other normal tissue.

(Collagen remodeling occurs in the cases of Epithelial cancers, E.g. alterations which occur in form of collagen/fibrillar morphology- in general, the pro-collagen molecules are covalently linked together to form fibers which are around 500 nm in diameter).

Now, SHG microscopic technique has been used in several studies involving *ex-vivo* imaging however it is seen that the same technology has the potential to yield greater knowledge in intravital studies as they permit dynamic imaging of tumour dynamics. Today there is a number of papers and scientists who are constantly using this technique to describe the pathogenesis of breast and ovarian tumours.

[The earliest example of this technique was done in 1992 using the dorsal flap technique was limited to mimic breast tumours as the mammary environment remains absent in the dorsal skin].

Chemiluminescent imaging method to detect cathepsin-B in tumour cells

Cathepsin B is associated with the cysteine protease family; it is heavily expressed in the number of invasive cancer cells and also in increased malignancy conditions. Moreover it also a known focal adhesive for the aggressive cancer conditions, where it promotes degradation of the extra cellular matrix via breakdown of key building blocks such as laminin, fibronectin, and type IV collagen. Traditionally there are several methods to monitor the levels of cathepsin and other proteases such as Traditional Molecular Biology Techniques, radiotracers, and fluorogenic substrates. For the first time the researches from the Department of Chemistry; Sothern Methodist University (L.S Ryan and Prof. A.R. Lippert) used the chemiluminescence technique to observe the presence of Cathepsin-B.

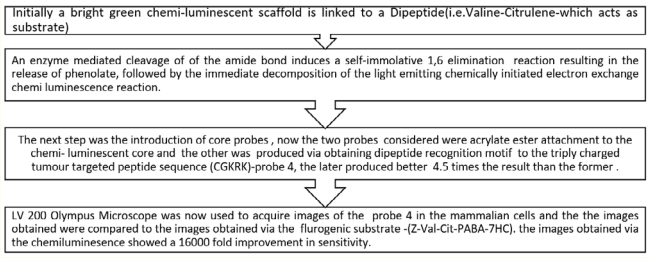

Figure 3

Quantification tumour cell colonies by portable optical coherence tomography

In most cases to study tumour development or to evaluate the extent of malignant cell pathogenesis it is important to conduct animal experiments however due to the discontinuous parts of the scientific world with the inhumane treatment of the animals at testing centers, living cell culture microbiology is a perfect alternative for the same. Not only this method of histopathological examination superior but also is of high biological relevance. Researchers of Chang Gung University, Taiwan, and Chang Gung Medical College, Taiwan published a paper on 2nd January 2019 to explain the importance of optical coherence technology in understanding the growth within the tumour cell colonies histo-pathologically.

FDA regulatory pathways for device approval

For any forms of imaging devices, the regulatory process by the FDA is regulated by the Centre for Devices and Radiological Health (CDRH). The entire process begins with a meeting(pre-optional) which serves as a pre-submission meeting with the FDA post a Q certificate request to the agency has been done.

This meeting serves as a platform for the individual sponsors where they discuss the planned Investigational Device Exemption (IDE)or marketing application and clearance submission is done.

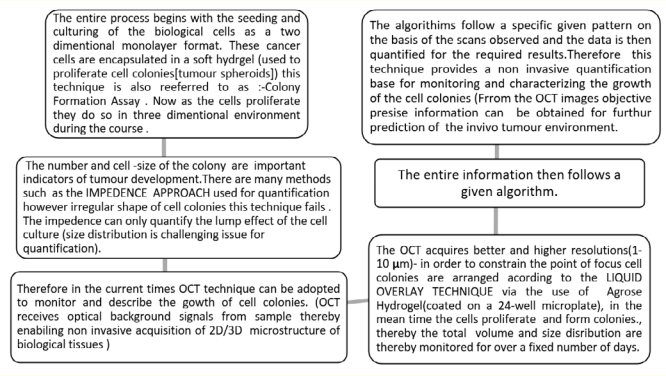

Figure 4

Figure 5

(When the goal is anatomic such as locating the lymph node or blood vessels, the FDA typically views optical imaging systems as low risk or nonsignificant risk (NSR)- thereby IDE is not required). Once there has been a clearance of safety and efficacy, a sponsor can thereby submit a 501(k) application required for market clearance (only when the substantial equivalence to a predicate device is claimed).

Optical imaging systems used as diagnostic, prognostic, or treatment suited purposes are considered as significant risk devices (SR) regardless of the disease severity. If the device is used for any investigational human study, the FDA requires sponsors to file an IDE application. For the use of any combination drug and the device products, an Investigational New Drug application needs to be filled in place of IDE, if the Centre for Drug Evaluation Research is assigned. Post this the response then presents the appropriate NSR or Sr designation in front of the local Institutional Review Board (IRB). Once the IRB and IDE are on board sponsor is free to proceed with the investigation for the same. Finally, a Pre Market Approval or PMA needs to be applied for market approval for class iii medical devices (evidence must clearly state the user intent for the same).

Conclusion

Since the past few decades, our understanding of human diseases and our approach towards treating them has completely changed and this has only been possible due to the constant development in basic molecular techniques. The fact that we have successfully been able to minimize the effects of several diseases and conditions can only be understood because of our constant efforts in diagnosing them as accurately as possible. It has been centuries since cancer drew its fangs on us humans and everyday countless of individuals succumb to this deadly plague, however, the beacon of hope that exists here is the proper investigation of these tumours in their earliest stages, this is only possible if we keep evolving our existing techniques for the same which would be able to successfully guide and image the areas conditioned by the tumours. In this paper, we have tried our best to introduce some new techniques which although are mostly in their nascent stages, however have the potential to armor us against these deadly tumours. Thereby allowing the clinicians to accurately stage, locate and diagnose these tumours and somewhere shortly with all these advances by our side, we would finally be able to win our battle against this deadly monster.

Bibliography

1. Early History of Cancer Defining cancer.
2. "The History of Cancer. Institut Jules Bordet (Association Hospitalière de Bruxelles - Centre des Tumeurs de ULB). Retrieved 2010-11-19.

3. Marilyn Yalom. "A history of the breast". New York: Alfred A. Knopf (1997).

4. Chensu Wang., *et al*. "Optical molecular imaging for tumour detection and image-guided surgery".

5. John V Frangioni. "New Technologies for Human Cancer Imaging".

6. Norton L., *et al*. "Predicting the course of Gompertzian growth". *Nature* 264 (1976): 542-545.

7. Thariat J., *et al*. "Epidermal growth factor receptor protein detection in head and neck cancer patients: a many-faceted picture". *Clinical Cancer Research* 18.5 (2012): 1313-1322.

8. Naumov GN., *et al*. "Role of angiogenesis in human tumour dormancy: Animal models of the angiogenic switch". *Cell Cycle* 5 (2006): 1779-1787.

9. Hoult DI and Phil D. "Sensitivity and power deposition in a high-field imaging experiment". *Journal of Magnetic Resonance Imaging* 12 (2000): 46-67.

10. Hofmann M. "From scinti-mammography and metabolic imaging to receptor targeted PET-new principles of breast cancer detection". *Medical Physics* 21 (2006): 11.

11. Xiangnan Dang., *et al*. "Deep-tissue optical imaging of near cellular-sized features".

12. New optical imaging system could be deployed to find tiny tumours Near-infrared technology pinpoints fluorescent probes deep within living tissue; may be used to detect cancer earlier. Anne Trafton | MIT News Office (2019).

13. 12% rise in India's cancer burden predicted (2020).

14. Takeshi Imamura., et al. *In vivo* optical imaging of cancer cell function and tumour Microenvironment.

15. Katsuno Y., *et al*. "Bone morphogenetic protein signaling enhances invasion and bone metastasis of breast cancer cells through Smad pathway". *Oncogene* 27 (2008): 6322-6333.

16. Hara-Miyauchi C., *et al*. "Bioluminescent system for dynamic imaging of cell and animal behavior". *Biochemical and Biophysical Research Communications* 419 (2012): 188-193.

17. Rathbun CM and Prescher JA. "Biochemistry. Bioluminescent probes for imaging biology beyond the culture dish". *Biochemistry* 56 (2017): 5178-5184.

18. Garry Choy., *et al.* "Current Advances in Molecular Imaging: Noninvasive *In Vivo* Bioluminescent and Fluorescent Optical Imaging in Cancer Research (2003).

19. Gambhir SS. "Molecular imaging of cancer with positron emission tomography". *Nature Reviews Cancer* 2.9 (2002): 683-693.

20. Quon A and Gambhir SS. "FDG-PET and beyond: molecular breast cancer imaging". *Journal of Clinical Oncology* 23.8 (2005): 1664-1673.

21. Juweid ME and Cheson BD. "Positron-emission tomography and assessment of cancer therapy". *The New England Journal of Medicine* 354.5 (2006): 496-507.

22. Büning-Pfaue H. "Analysis of water in food by near infrared spectroscopy". *Food Chemistry* 82.1 (2003) 107-115.

23. Hong G., *et al*. "Near-infrared fluorophores for biomedical imaging". *Nature Biomedical Engineering* 1 (2017): 0010.

24. Zipfel WR., *et al*. "Live tissue intrinsic emission microscopy using multiphoton-excited native fluorescence and second harmonic generation". *Proceedings of the National Academy of Sciences of the United States of America* 100.12 (2003): 7075-7080.

25. Xu C., *et al*. "Multiphoton fluorescence excitation: new spectral windows for biological nonlinear microscopy". *Proceedings of the National Academy of Sciences of the United States of America* 93.20 (1996): 10763-10768.

26. Van Der Vorst JR., *et al*. "Near-infrared fluorescence imaging of a solitary fibrous tumour of the pancreas using methylene blue". *World Journal of Gastrointestinal Surgery* 4.7 (2012) 180-184.

27. Tummers QR., *et al*. "Intraoperative near-infrared fluorescence imaging of a paraganglioma using methylene blue: a case report". *International Journal of Surgery Case Reports* 6 (2015): 150-153.

28. Vorst JR., *et al*. "Intraoperative near-infrared fluorescence imaging of parathyroid adenomas with use of low-dose methylene blue". *Head Neck* 36.6 (2014): 853-858.

29. Gioux S., *et al*. "Image-guided surgery using invisible near-infrared light: fundamentals of clinical translation". *Molecular Imaging* 9.5 (2010): 7290.

30. Mieog JSD., *et al*. "Toward optimization of imaging system and lymphatic tracer for near-infrared fluorescent sentinel lymph node mapping in breast cancer". *Annals of Surgical Oncology* 18.9 (2011): 2483-2491.

31. Schaafsma BE., *et al*. "The clinical use of indocyanine green as a near-infrared".

32. Antaris AL., *et al*. "A small-molecule dye for NIR-II imaging". *Nature Materials* 15.2 (2016): 235-242.

33. Nicholson R., *et al*. "EGFR and cancer prognosis". *European Journal of Cancer* 37 (2001): 9-15.

34. Fan Z., *et al*. "Blockade of epidermal growth factor receptor function by bivalent and monovalent fragments of 225 anti-epidermal growth factor receptor monoclonal antibodies". *Cancer Research* 53.18 (1993): 4322-4328.

35. Rosenthal EL., *et al*. "Use of fluorescent labeled anti–epidermal growth factor receptor antibody to image head and neck squamous cell carcinoma xenografts". *Molecular Cancer Therapeutics* 6.4 (2007): 1230-1238.

36. Gleysteen JP., *et al*. "Fluorescent labeled anti-EGFR antibody for identification of regional and distant metastasis in a preclinical xenograft model". *Head Neck* 30.6 (2008): 782-789.

37. Heath CH., *et al*. "Use of panitumumab-IRDye800 to image microscopic head and neck cancer in an orthotopic surgical model". *Annals of Surgical Oncology* 19.12 (2012): 3879-3887.

38. Becker A., *et al*. "Receptor-targeted optical imaging of tumours with near-infrared fluorescent ligands". *Nature Biotechnology* 19.4 (2001): 327-331.

39. Ke S., *et al*. "Near-infrared optical imaging of epidermal growth factor receptor in breast cancer xenografts". *Cancer Research* 63.22 (2003): 7870-7875.

40. Sandoval RM., *et al*. "Uptake and trafficking of fluorescent conjugates of folic acid in intact kidney determined using intravital two-photon microscopy". *American Journal of Physiology-Cell Physiology* 287.2 (2004): C517-C526.

41. Veiseh M., *et al*. "Tumour paint: a chlorotoxin: Cy5. 5 bioconjugate for intraoperative visualization of cancer foci". *Cancer Research* 67.14 (2007): 6882-6888.

42. Van Dam GM., *et al*. "Intraoperative tumour-specific fluorescence imaging in ovarian cancer by folate receptor-[alpha] targeting: first in-human results". *Nature Medicine* 17.10 (2011): 1315-1319.

43. Murad Omar., *et al*. "Pushing the Optical Imaging Limits of Cancer with Multi-Frequency- Band Raster-Scan Optoacoustic Mesoscopy (RSOM)".

44. Hanahan D and Weinberg RA. "Hallmarks of cancer: the next generation". *Cell* 144 (2011): 646-674.

45. Carmeliet P and Jain RK. "Angiogenesis in cancer and other diseases". *Nature* 407 (2000): 249-257.

46. Lammers T., *et al*. "Drug targeting to tumours: principles, pitfalls and (pre-) clinical progress". *Journal of Controlled Release* 161 (2012): 175-187.

47. Maeda H. "Macromolecular therapeutics in cancer treatment: the EPR effect and beyond". *Journal of Controlled Release* 164 (2012): 138-144.

48. Gambhir SS. "Molecular imaging of cancer with positron emission tomography". *Nature Reviews Cancer* 2 (2002): 683-693.

49. Charles-Edwards EM. "Diffusion-weighted magnetic resonance imaging and its application to cancer". *Cancer Imaging* 6 (2006): 135.

50. Ale A., *et al*. "FMT-XCT: *in vivo* animal studies with hybrid fluorescence molecular tomography-X-ray computed tomography". *Nature Methods* 9 (2012): 615-620.