Review Article

Small Animal micro-PET imaging: an overview

Khalil, M. PhD

Division of Medical Biophysics, Department of Physics, Faculty of Science, Helwan University.

ABSTRACT:

Molecular imaging has become an interesting and growing clinical practice and valuable research field. It permits one to understand the molecular pathways and biochemical changes associated with disease development. It also allows researchers to interrogate particular expression of key molecules that play essential role in diagnostic or therapeutic processes. Small animals remain instrumental in deciphering many biological aspects of human diseases. The implementation of modern imaging technologies in preclinical models provides excellent tools for investigating intrinsic molecular and biochemical changes associated with a given disease model. The role of micro-positron emission tomography (µPET) in this context is quite unique and has potential to prove its utilities in drug discovery and development. One of the challenges associated with this technology is selection of appropriate animal models, how far it can represent a human disorder, and how the experimental outcome is predictive of future clinical trials. Animal anaesthesia, which if not taken into consideration could have an adverse impact on data interpretation. Animal handling and monitoring protocols are key during preclinical imaging. There are also some questions regarding the performance of the µPET scanner used to acquire the data and how this information can be integrated with other modalities or correlated with histo-pathological finding to reveal valuable and reliable outcome. The applications of µPET imaging are numerous and have been extensively reported in the literature with special focus on cardiology, neurology and oncology.

Corresponding Authors: Khalil, M. **E-mail:** magdy_khalil@hotmail.com.

INTRODUCTION:

Molecular medicine has witnessed a noticeable change since the successful release of gene sequencing and advances in molecular biology and genetics [1, 2]. Medical diagnosis and molecular therapy techniques have been tightly associated with these developments as they provide more insights into the progression of the disease and its response to novel therapeutic approaches. In fact, imaging plays a key role in providing new tools for minimally invasive in vivo characterization of biologically active processes that occur at cellular and molecular levels at different phases of disease development. Single photon emission computed tomography (SPECT) and positron emission tomography (PET) are the two moving arms of nuclear medicine in diagnosing many human disorders and do provide great emphasis on functional status of body organs.

Several PET and SPECT radiotracers are available for a large variety of medical applications, including oncology, cardiology, neurology, inflammatory disorders and others. Some of these tracers are specially designed to target specific molecular pathways and can also be used as surrogate indicators of drug efficacy with great scope for early detection and staging of disease. Development of molecular clinical strategies starting from design and identification of molecular targets and/or contrast agents towards its translation into the clinic involves a series of steps similar to those used in drug discovery and development. During the discovery phase, preclinical studies that comprise the efficacy and safety in animal models are key to their approval for clinical use. Molecular imaging in small animals has become an invaluable part of clinical translation [3]. Moving from the clinic to the bench-side and vice versa, PET imaging plays a significant role in both preclinical and clinical research environments. Advances in transgenic and animal models have allowed researchers to use imaging not only for drug discovery but also for phenol-typing and understanding the pathophysiology of particular diseases. Furthermore, its implementation represents an important refinement in the use of animals for preclinical research, allowing longitudinal studies and enabling a powerful,

Non-invasive and clinically translatable way for assessing anatomical, physiological and functional parameters. Hybrid imaging strategies that combine the features of morphology and function has apparently become standard in clinical practice of clinical oncology using PET/CT imaging systems. This also has become evident in small animal imaging using micro PET (µPET) hybrid imaging techniques. ***Figure 1*** maps the available imaging modalities and their differential capabilities in characterizing morphological and/or biological information starting from organ physiology down to subcellular biochemical processes in addition to genetic and molecular changes.

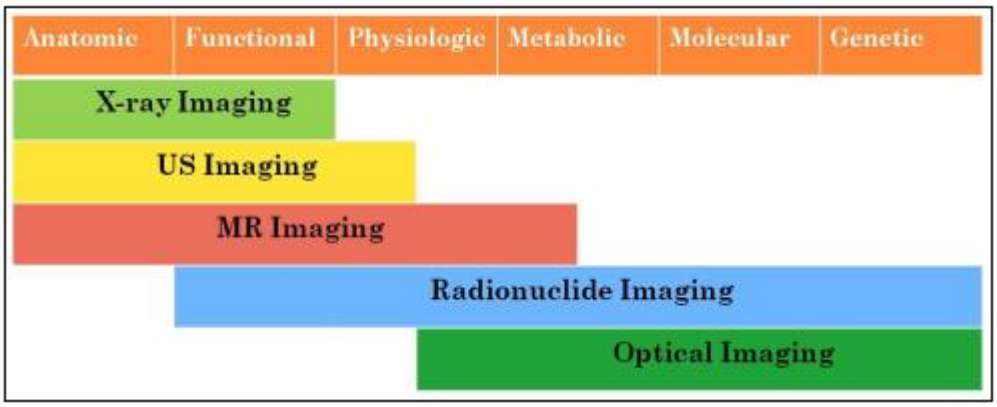

Figure 1. Molecular and morphological imaging matrix.

Due to the above mentioned characteristics of µPET imaging and its capabilities in eliciting more information about disease detection, it has been recognized as one of the most powerful preclinical imaging techniques in many areas of molecular imaging and pharmaceutical industry which is tightly related to drug development and introduction of new treatment strategies into patient health care systems [(4)]. There are several issues to be considered in preclinical µPET imaging. These include many logistical considerations related to the availability and development of µPET radiopharmaceuticals, and imaging systems that provide an adequate spatial and temporal resolution to answer a variety of biological questions. Other important factors such as animal physiology, anaesthetic regime and dietary factors have also their impact on radiotracer uptake and experimental outcome. However, this review has special focus on imaging

Instruments, their performance and associated challenges and future directions.

PET Technology and Instrumentation: Emission radionuclide imaging depends on tracer principles where trace amount of the administered compound is used to map or address a particular target. The accumulation of the tracer within organs and tissues are detected by use of PET scanner. The positron is an anti-particle matter that decays very fast (~10^{-7} sec) through combination with an electron forming a positronium atom. This structure is a hydrogen-like atom configuration and decays with release of two photons emitted in opposite directions almost at 180^{o}. There are a quite significant number of positron emitters that are of particular clinical interest.

PET imaging characteristics: The sensitivity of PET and SPECT probes is very high and can go down to pico-or nano-molar concentrations making them a superior diagnostic approaches over other modalities that have low or inferior diagnostic sensitivity, see ***Figure 2***. PET has extensively been used to provide tracer concentration in units of activity per unit volume (e.g. MBq/ml) and this has enabled researchers to measure tracer kinetics in an absolute quantitative manner applying various kinetic models. This is not the case in SPECT based procedures where their use in the clinic and many animal studies has been limited to qualitative tracer distribution and relative regional uptake.

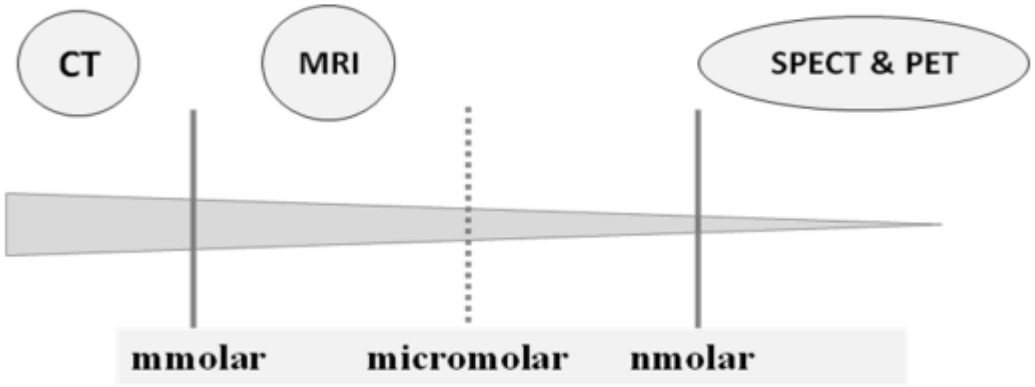

Figure 2. The relative position of diagnostic modalities in detection of molecular probe concentration.

The process of obtaining quantitative SPECT data is not an easy task and a number of critical corrections need to be addressed. However, µPET has the same quantitative properties as the parent clinical PET imaging and this can help with some degree of success to have a sort of correlation between human studies and preclinical small animal imaging using candidate PET compounds that have potential translation into the clinic or already undergoing human clinical trials. In SPECT compounds, however, it is easy to perform dual tracer injection/imaging using two different energies. The two energies can be simultaneously acquired and separated into two diagnostic images; a feature that is absent in PET imaging systems. For a review on the potential of micro SPECT (µSPECT) imaging in animal research, the reader is referred to our recent paper [5, 6 and 7].

PET imaging has been founded with inherent tomographic features in system design so that maximum angular coverage can be realized. This permits tomographic dynamic acquisition to be implemented in an easy way especially when the detector has an adequate axial extent to cover the area of interest. Tracer quantification is therefore simpler in PET data processing than with SPECT imaging studies.

The latter has traditionally been applied as sequential multiple 2D projections taken around the object yet with inconsistent temporal data sampling among different views.

Small animal SPECT systems have similar limitations and only few systems can provide a dynamic 3D image acquisition. Regarding cost and availability, SPECT compounds in general outperform PET tracers as they are widely available in nuclear medicine clinics and many of them are already approved for clinical applications.

PET tracers generally needs an on-site cyclotron (some of them not) and well-trained team of chemists, physicists and nuclear physicians are demanded for several tasks including tracer production and synthesis, quality control, scanner operation, image acquisition, reconstruction, analysis and data interpretation.

Overall, SPECT and PET in the preclinical domain can be seen as complementary rather than competitive imaging tools in the broad matrix of molecular imaging. Although there are some trials to use clinical PET scanner in small animal research, dedicated µPET systems are able to provide better system sensitivity and spatial resolution [8].

Clinical PET system might be helpful in some distribution studies or for those experiments with less quantitative interest [9]. ***Figure 3*** depicts the spatial resolution and sensitivity of preclinical and clinical SPECT and PET imaging systems.

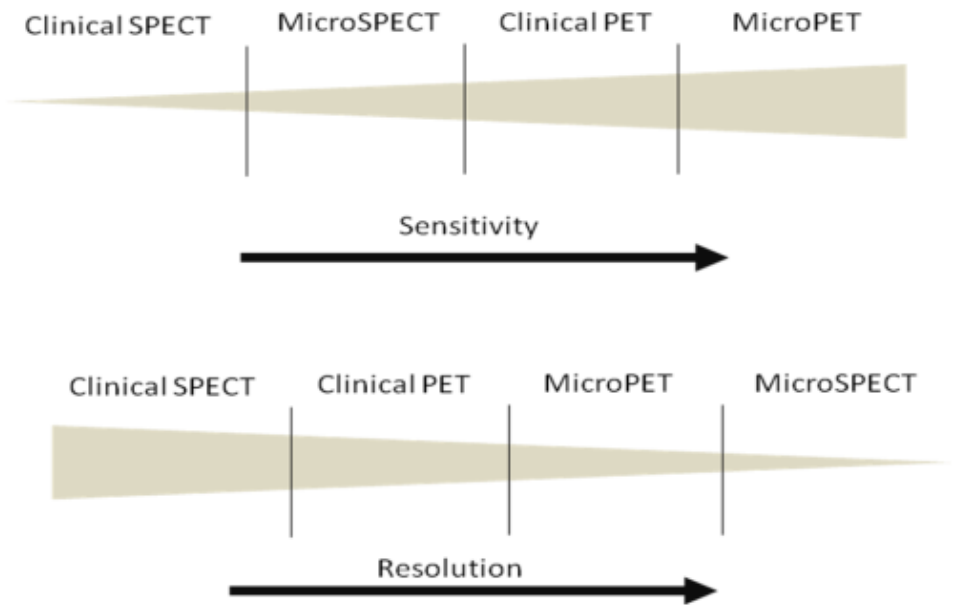

Figure 3. Spatial resolution and detection sensitivity across clinical and preclinical SPECT and PET imaging systems.

System Design: There have been several designs for the clinical PET scanner since its introduction in the field. Among those are dual coincidence gamma camera, partial ring and full ring cylindrical design. The latest has received an increased interest due to its geometric configuration that allows an improved detection sensitivity that satisfies resolution requirements. In preclinical µPET scanner, the cylindrical design is the most common approach used by almost all suppliers. A number of designs have been adopted and implemented in the market.

The first one was made of BGO crystal and specifically made to image rodents at the mid of 1990s [10].

The axial field of view was 50 mm while the diameter of the tomography was 115 mm achieving a spatial resolution of 2.3 mm full width at half maximum (FWHM) and 5.6 mm full width at tens maximum (FWTM) at the centre of the scanner.

Many preclinical µPET scanners have originated from academic institutions and released into the market by different vendors.

Detector system

The conventional crystal-photomultiplier assembly has been extensively used in both preclinical and clinical detector designs. There are also different types of scintillation crystals that adopted in preclinical PET scanners. Bismuth germinate oxyorthosilicate (BGO), gadolinium oxyorthosilicate (GSO), and lutetium oxyorthosilicate (LSO) are commonly used scintillator materials.

BGO crystal has been extensively used in clinical scanners and replaced the NaI (Tl) in the early days of PET imaging as it showed better detection efficiency and permitted for manufacturing segmented block structure providing an improvement in system spatial resolution [(13)]. Its non-hygroscopic nature and high stopping power has made it a better alternative to NaI (Tl) crystal in manufacturing efficient PET scanners. Recently, it has also been employed for constructing a bench-top dual-flat panel detector system as a low cost dedicated high-throughput PET scanner [(14, 15)].

LSO-type crystal was found an increasing interest due to its stopping efficiency, high light output and fast response time. It has been implemented in multimodality hybrid imaging systems, time of flight applications, dedicated PET scanners, and of frequent use in preclinical PET systems. Its high light output and non-hygroscopic nature have facilitated the segmentation of LSO crystals to very fine small dimensions. Its incorporation in PET/MRI hybrid systems should be favoured over GSO due to the susceptibility of the later to magnetic fields [(16)]. The GSO crystal has found an interest in parallax error correction by coupling with other crystals such as LSO as demonstrated in depth of interaction section that will be described later. Block detector of pixilated crystal structure coupled to PMT is a commonly used approach in configuring small animal PET scanners. The detector array varies among manufactures so that it can be 8x8, 13x13, or 20x20…etc. The trans-axial field of view also varies among systems and mostly lies in this range 8-20 cm. The axial field of view may be small for some systems (e.g. 3 cm), large in others (e.g. 12.6 cm) or customized based on users' preferences (e.g. 3, 7 or 11 cm).

However, some detector designs were implemented with a proprietary single-continuous crystals (without dead zones) coupled to position sensitive photomultiplier (PSPMT) and PET electronics that allow to correct for depth of interaction by analysing the shape of the detected light [(17)].

The large axial field of view permits one to acquire a whole body PET image and also

facilitate instantaneous observation of tracer uptake across different organs when data are processed with dynamic histogramming. Covering the whole animal in one bed position is also advantageous when image-derived input function is sought for kinetic analysis and compartmental modeling. However, large axial field of view serves to compromise the axial resolution of the imaging system [(18)]. In 1999, the performance of the first µPET system was described and the whole body mouse was reported for 8 bed positions taking 8 min/bed. At the present time, however, systems with large axial field of view can acquire the whole body image in a single bed position with great reduction of imaging time.

Nevertheless, there are other alternative approaches that proved a better performance over the conventional crystal-photodetector system. Using semiconductor detectors such as Cadmium Zinc-Telluride (CZT) was found to improve spatial resolution, energy resolution, compactness and room temperature operation [(19)]. Another advantage provided by CZT-type detectors is the absence of cutting, treatment, and assembling of tiny sized-crystal structure. It can be manufactured using standard semiconductor technology including packing, hybridization and MOSFET electronics [(20)]. Technical features such as the need for robust electrode contacts, slower timing characteristics and lower detection efficiency are drawbacks of CZT detectors. There are also a variety of the photosensors used to read out the light emitted from the scintillation crystals. PMT has been extensively used in both preclinical and clinical SPECT and PET scanners. PSPMT with multi-anode configuration and channels up to 256 have been used in preclinical PET systems due to its resolution capability and compact size in comparison to the conventional PMT. Avalanche photodiode (APD) is a semiconductor photodetector that found an increasing interest due to magnetic field insensitivity, compact structure and detector elements can be produced in very small dimensions enabling an improvement in spatial resolution. However, temperature variation is a drawback of the APD operation. APD has been utilized in preclinical PET/MRI to provide simultaneous multimodality imaging protocols. It has also been implemented in clinical and preclinical PET/MRI systems [(21)]. The Geiger-mode APD or silicon photomultiplier (SiPM) is a densely packed matrix of small APDs. This type of photosensors has gained some interest because of its compact size, high photo-detection efficiency and gain.

Its insensitivity to high magnetic field makes it a good candidate for hybrid PET/MRI scanners. Unlike APD, it can be produced in a standard complementary metal–oxide–semiconductor process, enabling a reduction of manufacturing costs. The interest of SiPM is mostly paid toward small detector systems given the fact that current versions of the SiPM are small in size that can fit the requirements of high resolution small animal or miniaturized imaging systems [(22)]. A number of reports have recently been released demonstrating the utility of SiPM in detector design of PET scanners, while other investigators showed a complete system setup based on SiPM technology using dual-layer LGSO (for DOI correction) or a single LGSO/SiPM PET system in prototype configuration [(23)].

System Sensitivity

Detection sensitivity and spatial resolution are two intrinsic performance measures that greatly affect the quality of PET reconstructed images. The former is a matter of detector configuration and solid angle coverage (geometric efficiency) together with detector material, dimension and thickness of the crystal packing fraction (intrinsic efficiency) in addition to other factors including timing and energy settings [(24)].

Due to its compact and smaller diameter, count efficiency of preclinical PET systems is higher than clinical counterparts. There are some approaches reported to improve intrinsic efficiency of the μPET scanner such as the use of monolithic crystals, stacking semiconductor detector slabs, or using tapered crystal arrays. These methods allow one to reduce the crystal pitch and inter-modules spacing [(25)].

Using different detector geometries has also been investigated and a box-shaped configuration using CZT was shown to provide a significant improvement in detection efficiency [(26)].

High temporal resolution to detect fast dynamic biological processes requires imaging systems with high detection sensitivity. Dynamic imaging is required in many small animal experiments and sufficient count collection is desired to satisfy the statistical requirement for high signal to noise ratio and better quantitative measurements.

System Resolution

Intrinsic spatial resolution of PET scanners is influenced by many factors some of them related to positron decays such as accolinearity and positron range, or instrumental such as crystal size, optical reflection, inter-crystal interaction,

scattering effects and position encoding. Reconstruction algorithm including an appropriate modelling for system point spread function is also an important parameter that was shown to play a substantial role in the resolution properties of the reconstructed PET images. Accollinearity is due to the fact that the two annihilation photons are not exactly emitted as 180 degree but with variation of 0.5^{o} FWHM due to residual momentum and kinetic energy. Its effect is more pronounced in clinical PET scanners with large bores than small animal systems.

It can be determined using this relation 0.0022 x detector diameter. On the other hand, positron range varies with the maximum kinetic energy of the positron emitter. Oxygen-15, Rb-82 and Ga-68 are among those tracers where positron range represents a problem in the final reconstructed PET images.

Dealing with positron range by modeling its effect in iterative reconstruction was found influential in improving the spatial resolution of the reconstructed PET images. Further improvement can be done using on-the-fly Monte Carlo based model positron range and the implementation of residual correction matrix in maximum a posterior reconstruction (MAP) [(27)]. Another factor that influences positron range is the medium of interaction. Effect of positron range is higher in soft tissues than in dense tissues such as bone [(28)]. Manufacturing very small crystal size at the sub-millimeter range is challenging and necessitates special treatments in addition to cost requirements [(29)].

This also has its impact on reducing the light output especially if the crystals need to be thicker to improve the detection efficiency. Increasing the crystal thickness has some drawbacks which are the cost and depth of interaction (DOI) errors in

Addition to reduction of energy and timing resolution.

High light output serves to enhance the position encoding, energy and temporal characteristics of the scanner. Not like Scintillation-PMT assembly, the use of semiconductors as detector material does not require cutting, surface treatment, and assembling as in pixilated detector design. Spatial resolution can be controlled through the design and pattern of detector electrodes.

The use of CZT as the PET detector element has found an interest among some investigators to develop imaging system of high spatial resolution [(30)].

Depth of interaction

DOI is another critical resolution element that serves to degrade the resolution

uniformity especially at radial offsets from the centre field of view. This phenomenon is particularly important in preclinical μPET scanners due to the fact that the curvature of the detector ring is more convex than clinical imaging systems. Therefore the likelihood that two coincident photons hit the crystals at oblique angles and at varying depths is relatively high especially as we move further toward the object edge. A variety of approaches have been followed to correct for this problem. It can be classified into two major categories; either to build a scanner with 3D positioning capability or to modify the conventional design that measures the signal in x and y directions to record the third dimension; which is the z coordinate of the event. Here are some of the approaches used to correct DOI spatial resolution errors [(25)]:

1. Using CZT detector with 3D position capability or LSO crystal coupled in parallel to position sensitive APD (PSAPD) can help to mitigate DOI error by accurate determination of event position or interaction [(31)].

2. Other methods developed were to add extra crystal-photo sensor component to the detector design. Using two or more scintillation layers with individual photo detectors would allow a chance to correct for DOI [(32)]. An increased number of readout circuitry and development complexities are a drawback of this design

3. Using two photo detectors at both sides of the scintillator material was a different way to utilize the difference in the amount of light received by each photo sensor to account for proper positioning of photon interaction ([33)]. In this approach, the ratio of the amplitude of the total energy signal measured by the dual-ended two photo detectors (e.g. PSAPD) is used to account for DOI. However, the dependence on signal differences between the two photo sensors, their stability and calibration pose some difficulties using this technique [(34)]. A four-layer depth of interaction (DOI) detector was also reported; it contains five detector units axially lined up per layer board. Each of the detector units consists of a LYSO scintillator array finely segmented (1.2 mm) and an 8 × 8 array of multi-pixel photon counters (MPPCs) [(34)].

4. On the other hand and instead of using two photo detectors, some other groups have used two different scintillation materials (i.e. phoswich design) where DOI determination is made by pulse shape discrimination capabilities utilizing the difference in decay time between the two crystals. The small-animal scanner Xplore (GE Healthcare) uses LYSO/GSO stacked together, and the difference in their

5. scintillation decay time is used to identify the DOI by analysing the energy signal using pulse shape discrimination [35]. This approach suffers from limited decay time differences; intrinsic time fluctuation, multiple interactions and light loss at crystals interface [35].

6. Other methods that rely on software algorithms that characterize and analyse the distribution of the detected signal from monolithic detector system have also been developed [36]. Regardless of calibration issues, this approach provide multiple number of benefits such as reduction of photon loss and increased detection efficiency; it also avoids cutting and fine crystal segmentation.

Developments in Hybrid µPET devices:

The last decade has witnessed a noticeable revolution in hybrid imaging, not only on the clinical level but its application has been extended also to the preclinical arena. µPET scanners have been coupled with morphological imaging modalities such as CT and MRI to improve the localization capability and also to utilize the other imaging features of these techniques. CT can provide attenuation correction and also superior image quality in bone imaging. It has also great advantage to provide angiographic details and micro-vessel identification with contrast agents. Numerous applications of µCT in small animal imaging have been reported and can be reviewed elsewhere [37, 38]. Imaging technologies using single or hybrid imaging techniques have recently been reviewed by our group for a number of preclinical musculoskeletal applications [39].MRI, on the other hand, has a better soft tissue contrast and can provide important complementary information not only on the anatomical level but also it can reveal invaluable metabolic or functional information of the examined tissues. Therefore, an integrated hybrid µPET/MR scanner are expected to provide a broad range of imaging options that enable researchers to simultaneously acquire functional information using PET together with high resolution capabilities of tissue morphology. The functional properties of MRI sequences would also enrich the diagnostic process by looking at different aspects of biological process in an ultidimensional/multipara metric fashion.

PET/CT scanners have been designed in a side-by-side configuration due to the counting and signal detection differences between the two methods. This is to a large extent similar to clinical PET/CT scanners. The interest of researchers towards simultaneous PET/MR was in great part due to the lengthy acquisition times posed by MRI sequences [40].

This is of particular interest in small animal imaging to minimize time duration of animal anesthesia that could confound the experimental outcomes as discussed later.

A key element that served the incorporation of PET inserts inside the MRI magnet was the development of avalanche photodiodes (APD) that substantially showed less magnetic interference in comparison to photomultiplier tubes (PMT). The later was used in early designs where the PMT was placed at a far distance from the magnetic field using optical fibers for light transmission [(42)].

Once the limitations between PET and MRI compartments are tackled in a simultaneous integrated scanner, several opportunities for different imaging protocols can be implemented utilizing the useful characteristics of both techniques. MR sequences such as T1- or T-2 weighted, echo planar, functional as well as magnetic resonance spectroscopy can be implemented along with PET data acquisition [(43)]. Nowadays, one can see the imaging modalities µPET; µSPECT and µCT are combined and incorporated into a single instrument known as trimodality imaging scanners. Inveon™ (Siemens Medical Solution, Inc) is a trimodality imaging scanner that provides the three aforementioned modalities and enables the user to apply more than one or two imaging techniques in the same imaging workflow. Triumph® (Gamma Medica, Inc) is also trimodality small animal scanner that contains the three imaging modalities using the same system. MiLabs has recently released the Vector (Versatile Emission Computed Tomography, or VECTor); a scanner that developed by researchers at the Delft University of Technology in the Netherlands and the company Molecular Imaging Labs and enables the researcher to acquire SPECT and PET data in a simultaneous manner using specialized collimator technology [(44)], ***Figure 4.***

Figure 4. (a) Micro-PET from MILabs that acquires simultaneous SPECT and PET data using a patented pinhole collimator technology. The system has also an option for CT making it a three in one molecular imaging device, (b) Nano Scan PET/CT from Mediso Company. First sub-half mm^3 PET volumetric resolution. 12 cm PET transaxial FOV @ 9% absolute sensitivity. It has Monte-Carlo DOI estimation and PSF modelling.

PET/Optical hybrid devices were also in mind of active researchers to merge the characteristics of both worlds. PET has its high penetration capability, accurate quantitative assessment, and wide variety of labelling options. Optical methods, on the other hand, provide a high throughput, low cost, and high sensitive functional information together with possibility of acquiring the data over longer time periods. Recently, µPET has been conjugated with optical camera using 3D fluorescence conical mirror insert placed inside µPET II system enabling researchers to acquire PET/optical data in a simultaneous manner [(45)]. Multimodality fusion probes are interesting approach and constructed where two or more reported genes give rise to a single transcript and single polypeptide [(46)]. This multifunctional biomarker strategy permits greater opportunity to examine a multiple number of biological processes that happen simultaneously and thus a multimodality imaging devices that enables a concurrent spatial and temporal imaging would be highly desirable. A state-of-the-art imaging regime has been used in combining between small animal PET and small animal micro-CT in ***Figure 5.***

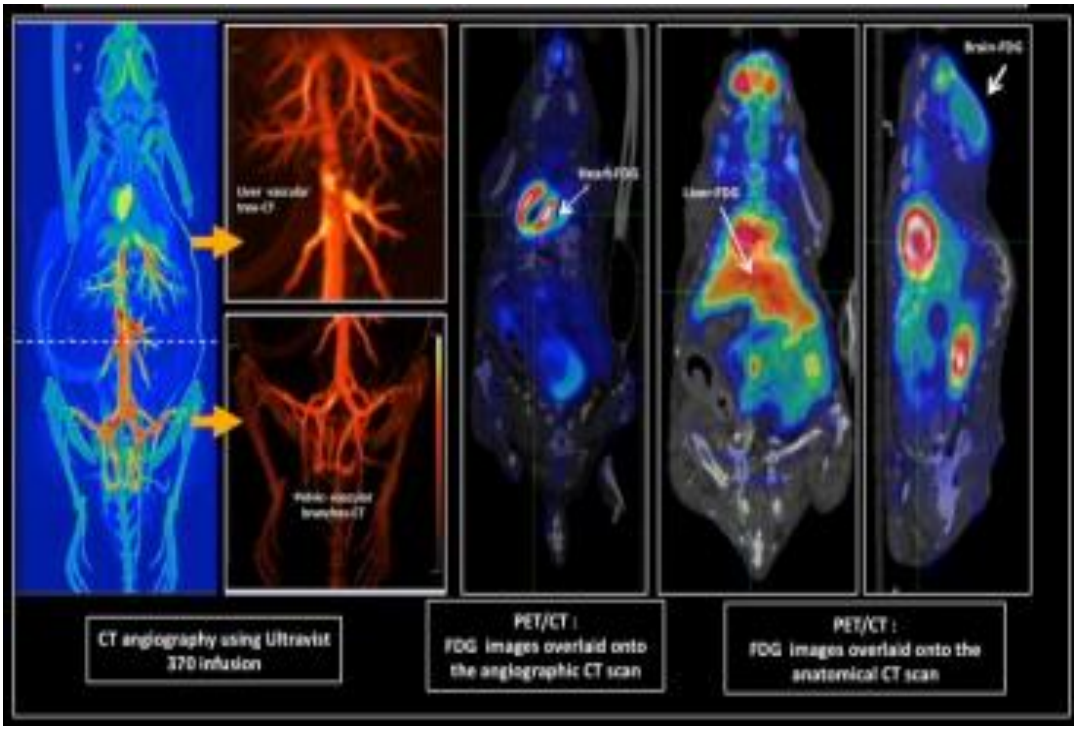

Figure 5. Multipara metric image using CT contrast enhanced vascular phase co-registered with animal F18-FDG PET imaging in a single imaging session. The image provides a one-stop-shop imaging regime in extracting valuable metabolic and morphological information.

CONCLUSION:

Small animal micro-PET imaging is a substantial tool in molecular medicine and will be able to contribute significantly in developing new drugs and formulating new therapeutic regimens. It will also be an important role in basic molecular biology research and discovering key molecular processes in health and disease. Technological aspects are the motivating aspects that potentially could enhance the characteristic performance of this micro-scale imaging devices. There is still a wide room for the micro-PET to prove usefulness in many areas of disease detection and new drug developments and therefore multi-disciplinary efforts are highly encouraged and demanded.

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