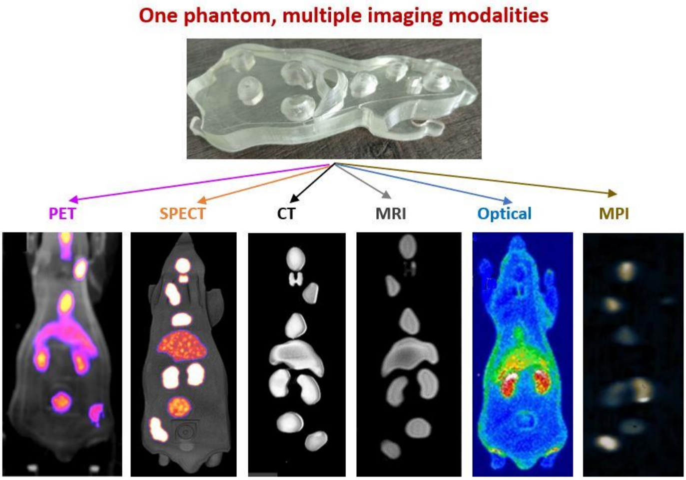

WHITE PAPER

Fillable mouse phantom: A simple tool for fast and ethical assessment and optimization of different imaging protocols.

BIOEMTECH

info@bioemtech.com

Version 1.2, October 2022

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BIOEMTECH

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The BIOEMTECH 'Fillable Mouse/Rat phantom' is considered as a commercial biomedical device. It is not designed or intended for use in diagnosis or classification of any medical complaint or for any other medical use. The user represents and warrants that it will not use or redistribute the product for such purposes. This device is for preclinical research purposes only.

INTRODUCTION

1 Introduction

BIOEMTECH introduced in 2020 the first fillable mouse phantom, initially to address internal needs when testing our desktop imaging systems -the *eyes*-, without using real mice.

Apart from the ethical restrictions, using normal mice was not efficient to evaluate the performance of an imaging system for certain radioisotopes and organs. On the one hand, single isotope administration cannot target organs of interest. On the other hand, the cost of specific radiopharmaceuticals or specific mouse models can be rather high, thus not included in a routine evaluation step. Furthermore, experimental tracers cannot be easily synthesized just to be used for testing purposes. However, using a simple phantom that can be filled just with isotopes, at known concentrations overcomes those restrictions.

We were surprised to see that when our first internal results were presented, many research groups asked if this phantom was commercially available. Consequently, we made some more improvements, which lead to the fillable mouse and rat phantoms and the phantom kits. Today, these phantoms have been adopted by well-known teams all over the world and the first publications are now emerging!

Recently a new fillable mouse phantom was released in an updated version (v.2), containing lungs and thus, facilitating more major organs' imaging for both mice and rats. he lungs-included version is already available while the first preliminary studies are being performed, setting high expectations.

1.1 "Fillable Mouse/Rat phantom" inspiration

BIOEMTECH's 'Fillable Mouse/Rat phantom' was inspired by the fact that tests in clinics are always performed on dedicated phantoms, prior to any examination or application on humans. The same principle could easily be applied to preclinical research too! However, up to now, simple phantoms are used to mimic a mouse. Although these phantoms can be used for evaluating parameters such as spatial resolution and sensitivity, they cannot replicate typical challenging imaging scenarios, such as high concentration in the liver or the bladder and simultaneously low tumor uptake that can make low concentrations non-detectable.

Over time, both us, as well as our collaborators, identified a large number of applications that can benefit from the use of the fillable phantoms. Typical applications include:

- Studying very specific biodistributions without the arbitrariness related to a living animal.
- Reproducible assessment of different imaging protocols, using known concentrations
- Establishment of protocols for less frequently used isotopes or for hard-to-image isotopes
- Assessment of collimator penetration or positron range that deteriorate imaging quality
- Optimization of protocols for multiple isotope imaging and quantification
- Detection of low activities in the presence of high concentration in nearby organs

- Effect of different ratios between organs/tumors on the imaging performance
- Optimization of protocols for multiple simultaneous mouse imaging
- Comparison of different imaging systems at a mouse level
- Evaluation of image reconstruction and correction algorithms
- Quantification of multimodal imaging data.

1.2 “Fillable Mouse/Rat phantom” design

The design of the mouse phantom is based on the «Digimouse» published by Dogdas et al in 2007. It is a model of a 28g mouse extracted from anatomical and cryosection data (**Figure** .

The design of the rat phantom is based on a modification of the same model, in combination with Lőw’s et al. Publication, «Atlas of Animal Anatomy and Histology» (2016) and the verification from a designated veterinarian.

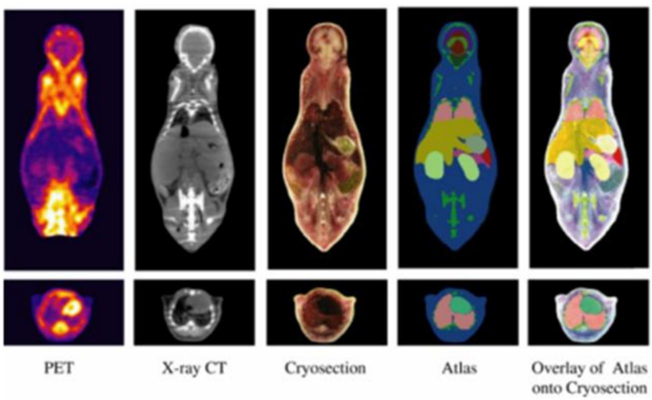

Figure 1. *The fillable mouse phantom is based on Digimouse, published by Dogdas et al in 2007, produced from a combination of real-mouse imaging and anatomical data.*

The external dimensions of both phantoms are close to the publication by the National Electrical Manufacturers Association (NEMA) standard NU 4-2008 for measurements' performance of small animal tomographs. There, mice and rats are depicted by simple cylinders of outer dimensions with 70 mm x 25 mm & 150 mm x 50 mm, respectively.

1.3 Complete product kit

The 'Fillable Mouse/Rat phantom' by BIOEMTECH contains the following parts **(Figure 2)**:

- Combination of your phantoms (mice, rats, or both)
- Filling pipette (x1)
- Pipette tips (x3)
- USB stick (x1)
- User's Manual (x1)
- Transport case (x1)

Figure 2. *The mouse phantom kit, showing all the individual parts that are provided (phantoms, pipette, tips, USB stick).*

2 Phantom Overview

2.1 Design and Volumes (v.1 fillable mouse/rat phantom)

The “Fillable Mouse/Rat phantom“ by BIOEMTECH, is based on the «Digimouse» published by Dogdas et al in 2007 (**Figure** 3), as already mentioned.

A sketch of the phantom designs can be seen in **Figure 3**.

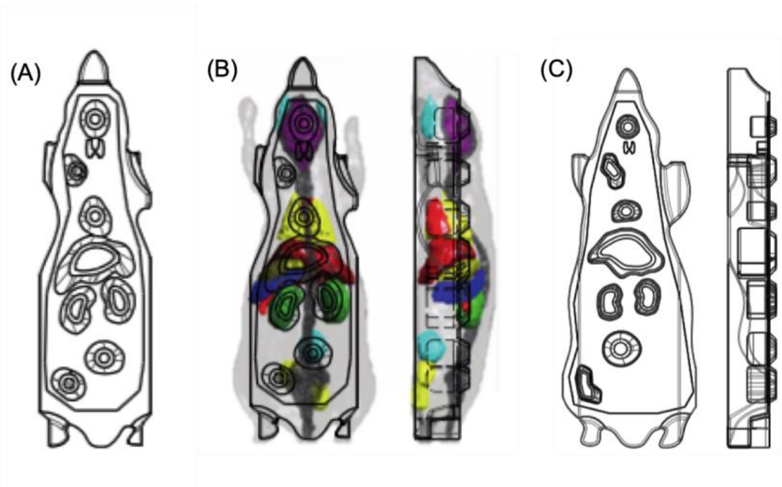

Figure 3. *(A) The v.1 mouse phantom design and all the internal organs (B) Upper and side view of the mouse phantom, superimposed on the Digimouse 3D image and (C) upper and side view of the rat phantom design and organs.*

The material consists of a clear color, photo-polymer Resin, processed in a DLP printer (Digital Light Processing), with 405nm UV light

(Funtodo, Netherlands), compatible to tissue and non-sensitive to magnetic fields. It contains most major organs (brain, thyroid, heart, liver, kidneys, bladder) and two small tumors. All organs/tumors can be easily, separately filled with isotopes or any other agent.

The organ volumes can be found in the following tables **(Table1-2)**:

Organs	Volume (mm^3)
Liver	1164
Heart	277
Left Kidney	352
Right Kidney	352
Brain	169
Thyroid	40
Bladder	294
Tumor (Top)	147
Tumor (Rear)	204

Table 1: *The volumes of organs in the mouse phantom*

Organs	Volume (mm^3)
Liver	4376
Heart	449
Left Kidney	662
Right Kidney	684
Brain	283
Thyroid	41
Bladder	997
Tumor (Top)	467
Tumor (Rear)	586

Table 2: *The volumes of organs in the rat phantom*

2.2 Design and Volumes (v.2)

New ‘Fillable Mouse/Rat phantom’ (v.2), designed following v.1, but with a lungs’ addition, further assists groups dealing with lung applications to have a tool for optimizing their imaging protocols for various pre-clinical imaging applications. The ‘fillable mouse phantom’ v.2 is depicted in **Figure 4**.

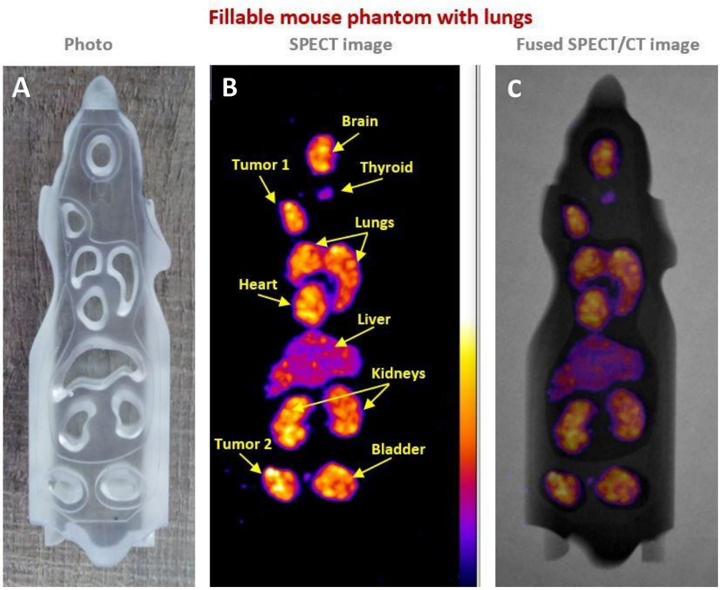

Figure 4 (A) *New fillable mouse phantom (v.2) design and internal organs* ***(B)*** *SPECT imaging by filling the phantom with ^{99m}Tc isotope (C) Fused SPECT/CT imaging by filling the phantom with ^{99m}Tc isotope.*

Organs	Volume (mm^3)
Left Lung	270mm^3
Right Lung	416mm^3

Table 3*: The volumes of the lungs in the v.2 ‘fillable mouse phantom’*

2.3 How to fill and empty the phantom

The ‘Fillable Mouse/Rat phantom’ can be easily filled and emptied by using the provided pipette or any other pipette or syringe. The exact volumes of the organs are given in **Tables 1** and **2**.

Due to the organs’ design and the narrow upper part, spills get avoided and all the procedures of filling and emptying the phantoms are safe.

To clean the phantom, simply empty all the organs with the appropriate pipette or syringe and wash them with pure water and empty them once more. Leave the phantom to decay (if needed) before you use it for another experiment.

<u>Suggestion</u>: **To achieve better phantom maintenance, we propose to always properly clean the phantom, after any application.**

IMAGING APPLICATIONS

3 Imaging examples

The ‘Fillable Mouse/Rat phantom’ by BIOEMTECH can be used in a wide range of applications and experiments. Very interesting applications from different end users are grouped in the following paragraphs.

3.1 Different imaging modalities

Up to now, the fillable mouse phantom has been used for the following imaging modalities **(Figure 5)**:

- SPECT: Single Photon Emission Computed Tomography imaging
- PET: Positron Emission Tomography Imaging
- MRI: Magnetic Resonance Imaging
- CT: Computed Tomography Imaging
- OI: Optical Imaging
- MPI: Magnetic Particle Imaging
- Cherenkov Light Imaging

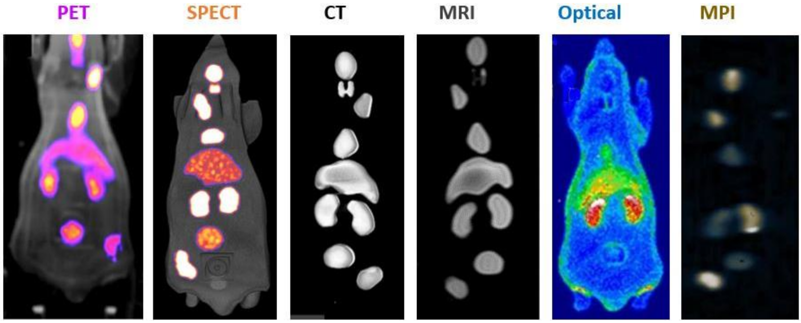

Figure 5. *Different examples of using the fillable mouse phantom for PET, SPECT, CT, MRI, Optical and MPI imaging, by filling the organs with different radioisotopes, dyes or other contrast agents.*

3.1.1 Nuclear medicine

Some first versions of the phantom have been filled in with Tc^{99m} and imaged in BIOEMTECH γ-*eye* **(Figure 6A)** to provide 2D scintigraphic imaging of various organs, to explore quantification as well as resolution limits of the system. Similarly in **Figure 6B** the phantom was filled with FDG and imaged with β-*eye* to provide a 2D PET image. In **Figure 6C**, the fillable mouse phantom was imaged at the 3D SPECT/CT MOLECUBES γ/x-cube and showed the ability of the system to image the two thyroid lobes. On **Figure 6D**, the rat phantom was also imaged with the γ/x-cube. Finally, on **Figure 6E**, the mouse phantom was imaged in a PET/CT.

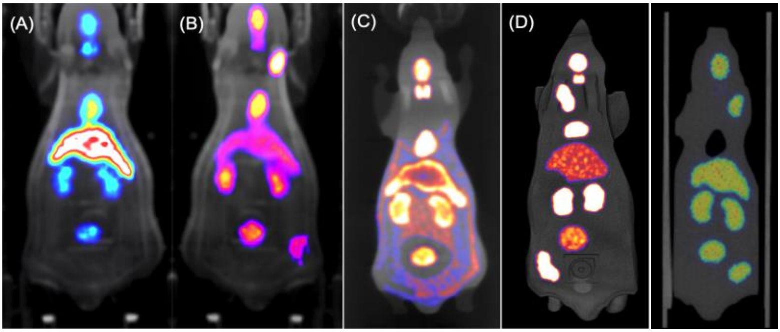

Figure 6. *The "Fillable Mouse/Rat phantom" imaged under different conditions: (**A**) The mouse phantom imaged on γ-eye™ by BIOEMTECH with 35 uCi of Tc-99m, (**B**) The mouse phantom imaged on β-eye™ by BIOEMTECH with 28uCi of 18F-FDG, (**C**) the mouse phantom imaged on γ-CUBE/x-CUBE™ (MOLECUBES) with 35 uCi of Tc-99m & (**D**) The rat phantom imaged on γ-CUBE/x-CUBE™ (MOLECUBES) with 250uCi of Tc-99m. (**E**) The mouse phantom imaged filled in with F-18 and imaged with PET/CT[1].*

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[1] University Hospital Essen, Germany; Pedro Fragoso Costa

3.1.2 Magnetic Resonance Imaging

The material of the fillable is MR compatible and the fillable mouse phantom has been successfully used in MRI under different sequences. In **Figure 7a**, the T2, STIR and PD images acquired in a GE Signaxt 1.5T MRI scanner are shown.

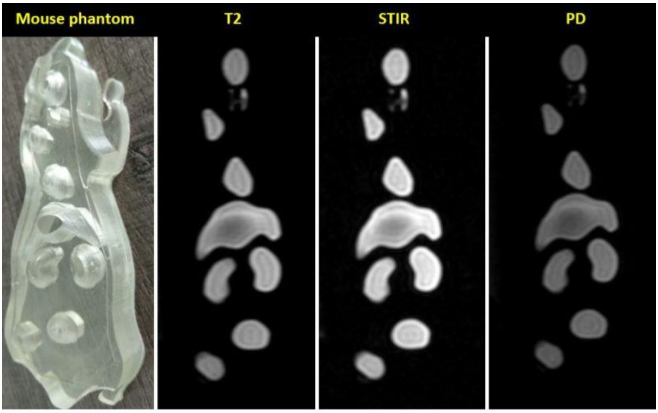


Figure 7a*. The "Fillable Mouse/Rat phantom" imaged under different MRI sequences, on a GE Signa HDxt 1.5T MRI scanner.*

As it can be seen, the phantom is fully magnetic compatible, with no artifacts, thus it can be used for testing MRI contrast agents including magnetic nanoparticles and isotopes inside a PET/MR scanner.

3.1.3 Quantitative MRI

By filling the phantom cavities with different concentrations of Gadolinium based on contrast agent (DOTA), T1 maps could be obtained, using an inversion recovery sequence on a 1T preclinical MRI system (M5, Aspect Imaging Ltd., Hevel Modi'in, Israel). As seen in **Figure 7b**, T1 values, ranging from ~100 ms up to 2400 ms, were determined by exponential fits in every pixel for a total of eleven inversion times and DOTA concentrations in the range of 0.06-2 mmol/l as well as pure water.

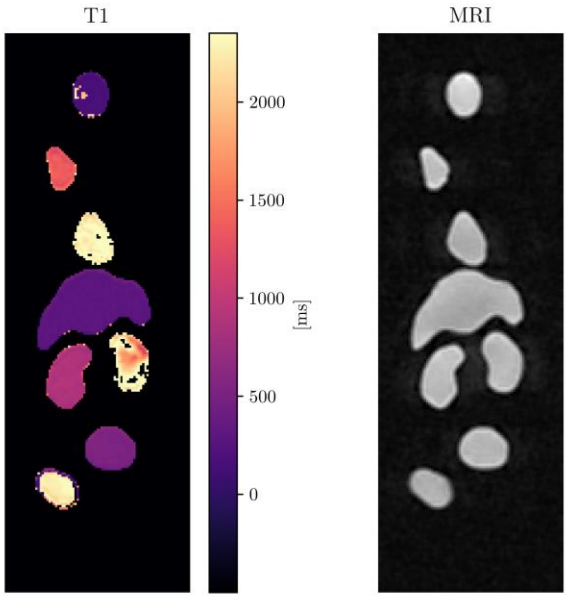


Figure 7b: *Quantitative MRI for the determination of T1 relaxation time of varying concentrations of Gadolinium based contrast agent in water, measured on an Aspect M5 1T preclinical system.*

3.1.4 Computed Tomography

The fillable mouse phantom acts as water in CT imaging and can be filled-in with different contrast agents such as iodine, gold nanoparticles or more. In **Figure 8**, a CT scan has been performed in MOLECUBEs x-cube and the image is fused with the real CT scan of a normal mouse, thus providing a nice anatomical map for the fillable phantom. This DICOM file is available to our end users.

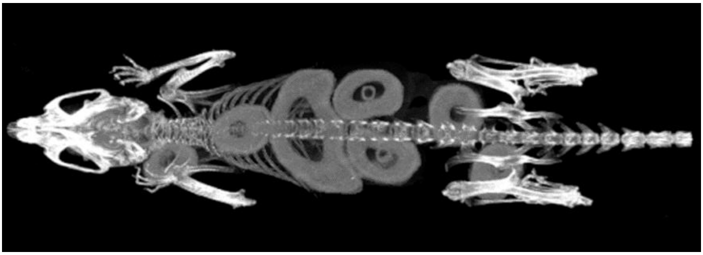


***Figure 8**. The 'Fillable Mouse phantom' CT scan in x-cube and fused with the real CT scan of a normal mouse.*

3.1.5 Optical Imaging

The fillable mouse phantom can be filled in with different dyes for the assessment of different imaging protocols. The phantom material does not mimic the optical properties of tissue and is considered quite transparent both to absorbed and emitted light.

However, it can provide the ground truth when testing either new dyes or new imaging systems and protocols, as it is possible to fill in the organ cavities with different dyes, at known concentrations, while maintaining the mouse shape. Parameters that can be assessed are the lower sensitivity at specific concentrations inside realistic organ volumes; effect of high concentrations at specific organs; detector response at different wavelengths; effect of different exposure times; testing of new experimental dyes without the need to couple them to a tracer; demonstration of imaging systems; training of students or technical staff, etc.

In **Figure 9,** some very interesting results, published by the group of Prof. Bradley Smith at University of Notre Damme, Indiana, are presented. In this study different materials were used for printing the fillable mouse phantom and assess the effect of the material and reflections inside the heart of the phantom when introducing 10μM of ICG and performing excitation at 745nm for 3secs. The results showed clear accumulation of the dye and some reflections in the clear version, which disappear in the black version.

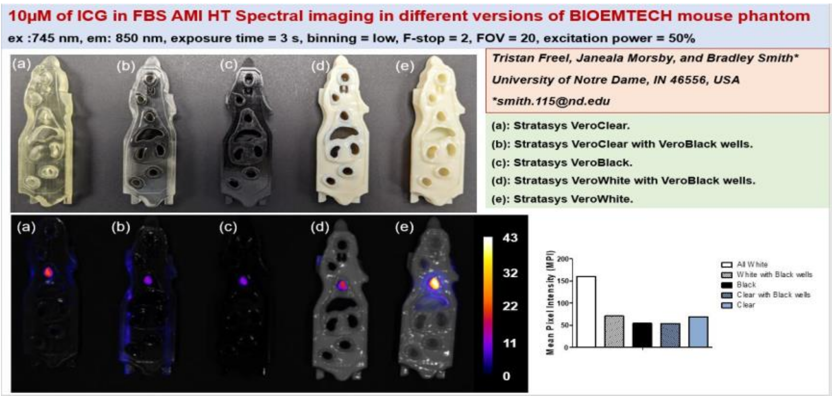
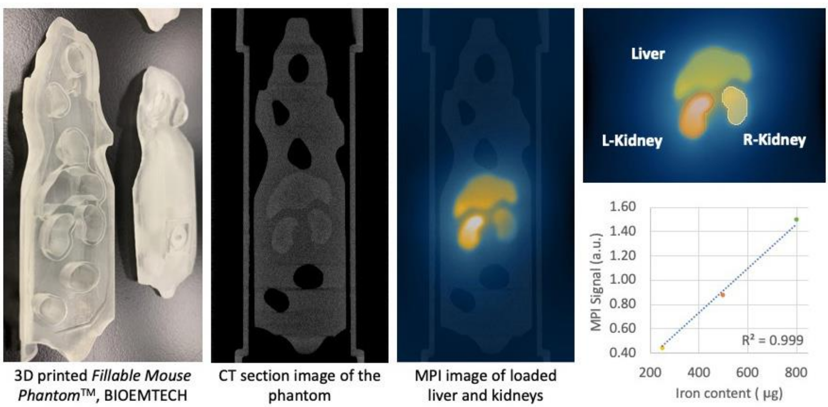


__Figure 9__. Optical imaging of 5 different versions of the fillable mouse phantom, using 10μM of ICG and performing excitation at 745nm for 3secs[2].

[2] University of Notre Damme, Indiana, US; Tristan Freel, Jeneala Morsby, Prof. Bradley Smith

3.1.6 Magnetic Particle Imaging

Magnetic Particle Imaging was conducted with the collaboration of Magnetic Insight. Commercially available Synomag®-D70 nanoparticles (micromod.de, Germany) were loaded into the virtual kidneys and liver of a 3D printed Fillable Mouse Phantom™. The liver was loaded with the same concentration as one of the kidneys and half the concentration of the second kidney. The total iron content of each organ was then estimated using MPI (MOMENTUM™, Magnetic Insight, USA). ROIs were drawn manually as all three organs were identifiable by MPI. Iron concentration was compared against empirical data (previously acquired) to estimate the required AMF and gradient to heat both kidneys by +2.5°C despite their differing MNP concentration while preventing heating in the liver **(Figure 10)**. The sample was then moved to the HYPER™ system (Magnetic Insight, USA) to apply the magnetic heating sequences. Changes in temperature in each kidney as well as the liver were monitored using fiber optic sensors.



3D printed *Fillable Mouse Phantom*™, BIOEMTECH

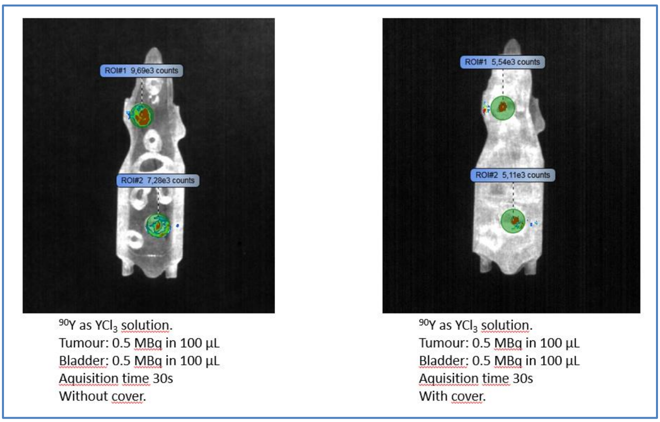
CT section image of the phantom

MPI image of loaded liver and kidneys

Figure 10. *(From Left to Right) The empty Fillable Mouse Phantom™ was loaded with Synomag-D 70 nm in the liver (10% vol/vol), the left kidney (10% vol/vol) and the right kidney (20% vol/vol). The CT image shows different contrast between loaded and unloaded wells but no observable changes between loading concentrations. The MPI images show the difference in concentration between the two kidneys. The total signal from manually drawn ROIs is plotted against the actual iron content (µg), showing a linear relationship between the MPI signal and the iron content[3].*

3.1.7 Cherenkov Luminescence Imaging

Cherenkov Luminescence Imaging was conducted with the collaboration of Dr. Urszula Karczmarczyk from POLATOM (Poland). ^{90}Y as YCl_2 solution was used and different activities were placed in the Tumor and Bladder, to provide the ground truth of the optical singal from ^{90}Y. Acquisition time was set to 30sec and in **Figure 11** the results with and without the top cover are shown.



[3] Magnetic Insight Inc; Brice Tiret, Donna Fong, Matthias Weber, Nicolas Carvou, Patrick Goodwill

Figure 11. *(From Left to Right) The empty Fillable Mouse Phantom™ was loaded with 90Y with activities 0.5MBq in a tumore and bladder cavity. Data were acquired for 30 minutes*[4].

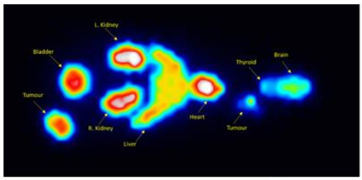
3.2 Optimization of imaging protocols imaging modalities

The phantom has been used for assessing the quantification of imaging systems, under different conditions.

3.2.1 Quantification studies

In **Figure 12,** the fillable mouse phantom was filled in with In-111 solution and known activities were used in all phantom organs. Imaging was performed with BIOEMTECH γ-*eye*. Then the measured activity from the image was compared against the reference injected activities to evaluate the accuracy of image quantification.

Scintigraphic image of BIOEMTECH's mouse phantom filled with In111 with 775uCi total activity



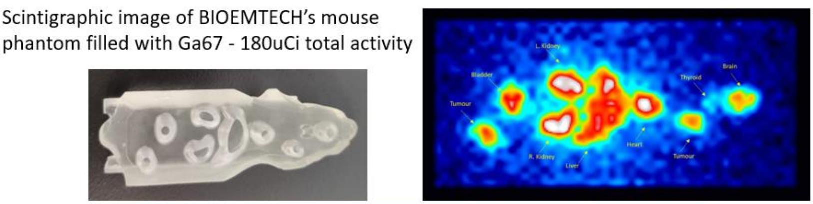
Organ	Injected Activity (uCi)	Measured Counts / min	Injected Activity / Organ	Measured Counts / Organ
Liver	218	6899,8	28,8%	30%
R. Kidney	108	3330,6	14,3%	14,5%
L. Kidney	112	3351,7	14,8%	14,6%
Heart	88	2753,0	11,6%	11,9%
Thyroid	20	419,2	2,6%	1,8%
Brain	52	1498,1	6,9%	6,5%
Bladder	88,7	2653,2	11,7%	11,6%
Tumour Bottom	70	2047,6	9,2%	8,9%
Tumour Up	22,1	574,4	2,9%	2,5%

[4] Dr. Urszula Karczmarczyk from POLATOM, Poland.

Figure 12. Comparison between In-111 activity imaged in BIOEMTECH γ-eye against reference known concentrations, injected in all major phantom organs.

3.2.2 Evaluation of collimator at higher energies

In **Figure 13,** the fillable mouse phantom was filled in with Ga-67 solution and known activities were used in all phantom organs. Imaging was performed with BIOEMTECH γ-*eye* and acquisition window at 300keV. The aim of the study was to assess if a tungsten collimator was capable of preserving image quantification, even if a portion of the high energies penetrated through it. The results showed that both the shape of the organs, as well as quantification is preserved.



Organ	Injected Activity (uCi)	Measured Counts / min	Injected Activity / Organ	Measured Counts / Organ
Liver	63,3	335,7	32,7%	32,3%
R. Kidney	26,2	139,2	13,5%	13,4%
L. Kidney	30,6	142,9	15,8%	13,7%
Heart	17	104,7	8,8%	10,0%
Thyroid	3,2	19,8	1,7%	1,9%
Brain	12,6	70,9	6,5%	6,8%
Bladder	18	102,9	9,3%	9,9%
Tumour Bottom	11,9	69,4	6,2%	6,7%
Tumour Up	10,4	53,9	5,4%	5,2%

***Figure 13**. Comparison between Ga-67 @300keV activity imaged in BIOEMTECH γ-eye against reference known concentrations, injected in all major phantom organs, to evaluate if the collimator used can image high activities.*

3.2.3 Low activity scans

In **Figure 14** we performed a mouse phantom scan, using Ga-67 and the 90keV peak, with total activity of 28.5uCi. A scan with γ-*eye* was performed for only 5 minutes and the activity in each organ ranged from 1.5uCi on the upper tumor to 7.9uCi to the liver. As it can be seen, accumulation in all organs is shown, despite the short scan duration and low activity. Thus, it has been possible to confirm that the system can be used for realistic mouse scans, at this activity level and scanning times.

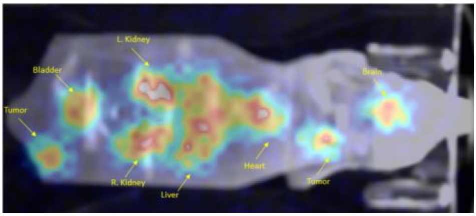


Figure 14. *A scan of the mouse phantom using BIOEMTECH γ-eye and Ga-67 @ 90keV. Total activity in the phantom was 28.5uCi and scanning time 5 min. Activity in the organs was: Liver: 7.9uCi; Bladder: 3.4uCi; Upper Tumor: 1.5uCi; Right Kidney: 4uCi; Left Kidney: 4uCi; Brain: 2.1uCi; Heart: 3.2uCi; Bottom Tumor: 2.4uCi.*

3.3 Assessment of correction algorithms

The phantom provides reference data for the evaluation and optimization of different correction algorithms so that they can be assessed under realistic mouse conditions.

3.3.1 Scatter correction for high energy isotopes

One of the challenges in high-energy isotopes is the contribution from higher energy peaks that are difficult to be excluded only by the collimator. This is the case in Pb203/212, which are very promising alpha emitters. Optimizing imaging protocols by using standard point or capillary sources is not an efficient approach, as these sources are rather ideal and cannot mimic the distributions of a tracer in a mouse or the different contribution from adjacent organs.

We used the fillable mouse phantom to tune γ-*eye* for imaging both isotopes, select the proper energy windows and develop the proper scatter correction techniques. In **Figure 15,** the fillable mouse phantom has been filled in with Pb-212 and Pb-203 solutions. As it can be seen on the left side, the initial images, although centered at the main photopeak windows are very poor and especially for Pb-212 no structures can be observed. However, by implementing proper scatter correction algorithms, it has been possible to resolve all organs and define the proper protocols for imaging real mice.

It is also important to take into consideration that the phantom is filled only with Pb212/203 or any clinically available Pb-labelled radiopharmaceutical and the user can select which organs/tumors to fill in, and at which concentrations. If the protocols had to be optimized by using real mice, it would be necessary to use the specific experimental tracer, without knowing a priori if it can target the tumor and at which concentration.

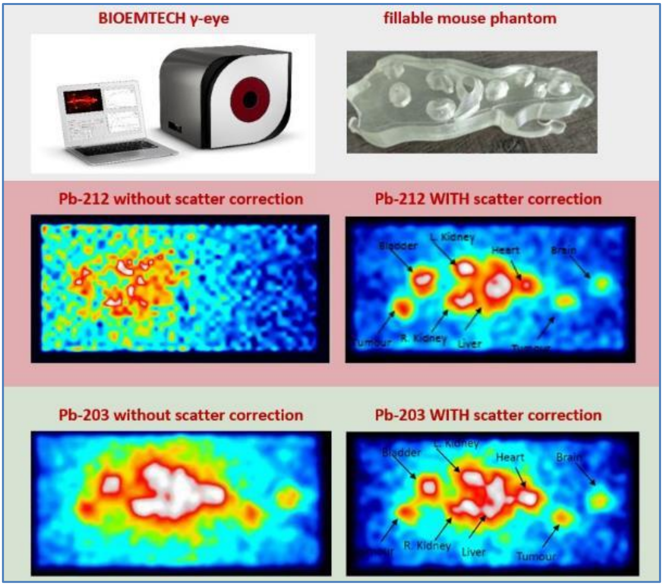


Figure 15. *Use of the fillable mouse phantom to optimize a scatter correction algorithm in γ-eye for imaging Pb212/203*[5]*.*

4 Contributors

For this White Paper, we had the pleasure to use data obtained by excellent research groups, who used the fillable mouse and rat phantoms at different applications. The groups are cited inside the White Paper and all contributors are collected here.

1. *University Hospital Essen, Germany; Pedro Fragoso Costa*
2. *Magnetic Insight Inc; Brice Tiret, Donna Fong, Matthias Weber, Nicolas Carvou, Patrick Goodwill*
3. *University of Notre Damme, Indiana, US; Tristan Freel, Jeneala Morsby, Prof. Bradley Smith*

[5] RadioMedix Inc, Texas, US; Izabela Tworowska, Leo Flores

4. *POLATOM, Poland, Dr. Urszula Karczmarczyk.*
5. *RadioMedix Inc, Texas, US; Izabela Tworowska, Leo Flores*

5 About BIOEMTECH

BIOEMTECH is located at the Technological Park of Demokritos Research Center, in Athens Greece. Having a strong academic background, the company provides unique, high-quality products and services in the field of drug research and biotechnology.

5.1 BIOEMTECH products: *eyes*

BIOEMTECH specializes in the design and construction of desktop, in small animal imaging systems for pre-clinical, in pharma, biotechnology, and medical research; Our trademark '*eye*' refers to compact and desktop devices, which transform lab desks into in-vivo imaging labs, allowing easy and real-time *in-vivo* dynamic screening of radiolabeled compounds, providing unique information for imaging PET and SPECT isotopes, as well as fluorophores (**Figure 16**).

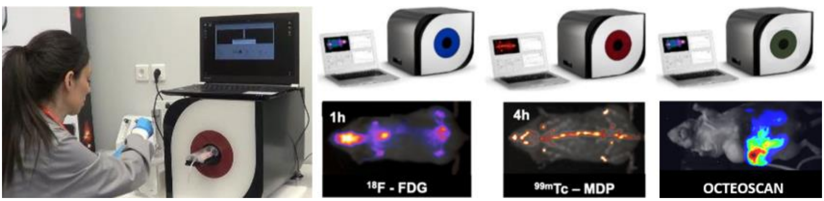


Figure 16*: (Left) The γ-eye™ system in use, (Right) Top to bottom: β-eye™, γ-eye™, φ-eye™ systems, indicative images from the eyes with ^{18}F-FDG, ^{99m}Tc and Octeoscan respectively.*

5.2 BIOEMTECH CRO services

BIOEMTECH Laboratories offer a full preclinical platform, from *in vitro* studies to *in vivo* imaging. We provide small animal imaging services, for advanced experiments on multi-scale level for both diagnostic and therapeutic protocols, in our unique, fully equipped, and licensed laboratories (**Figure 17**) that include:

- *In vitro* lab for cell studies
- Animal facility (mice & rats)
- Radiochemistry lab
- Imaging facility (microCT/SPECT/PET)

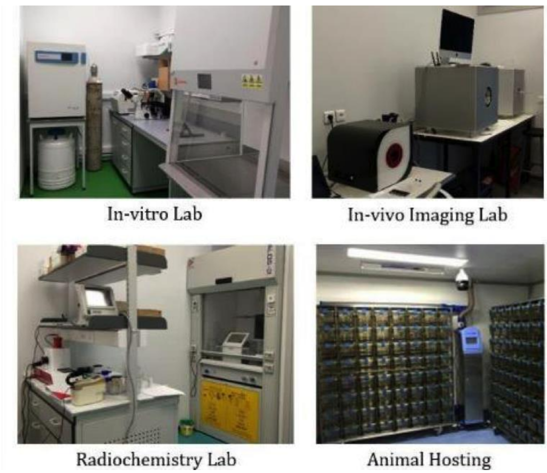


Figure 17*: BIOEMTECH Laboratories: in vitro, radiochemistry, animal hosting and imaging labs, door-to-door.*

All studies are in full accordance with the 3Rs principle (animal reduction, replacement and refinement) and all international standards, while our personnel is specially trained on the undertaken activities (FELASA & radioactivity accreditation).

5.3 Fillable mouse phantom

BIOEMTECH introduced the first generation of fillable mouse and rat phantoms, designed for nuclear medicine (PET/SPECT) applications, but already successfully tested for X-ray (CT), Magnetic Resonance Imaging (MRI), Optical Imaging (OI) and Magnetic Particle Imaging (MPI) (**Figure 18**).

Figure 18: *Fillable mouse phantom kit and indicative images from PET SPECT, CT, MRI, Optical and MPI imaging tests*

6 Contact Us

For more info do not hesitate to contact us.

BIOEMTECH

Lefkippos Attica Technology Park N.C.S.R. Demokritos, Athens, Greece

+30210 6548192

info@bioemtech.com

sales@bioemtech.com

www.bioemtech.com