Applying a Modular PET System to Investigate Bioremediation of Subsurface Contamination: A Proof-of-Principle Study

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Abstract. Remediation of subsurface contamination using microbial consortium can be less toxic to the environment and ecosystems than using chemical dispersants or physical measures. But bioremediation involves complex and dynamic processes in a visually opaque medium, which can be difficult to monitor. Positron emission tomography (PET), which reveals the dynamic distribution of labeled molecules, offers a means to probe into the bioremediation process. This study evaluates and verifies the feasibility of employing a freely-rearrangeable modular PET system (BioPET) in combination with a generic tomographic image reconstruction platform, CASToR, for tracking fluid transport, as well as metabolic activities, occurrence, and distribution of subsurface microorganisms. This enables quantitative imaging of bioremediation processes in high resolution with further expanding the flexible modular system and refining the data processing algorithms.

1. Introduction

Remediation of subsurface contamination requires optimization of biological and chemical processes in a visually opaque medium [1]. Cells, pollutants, nutrients and waste products all undergo complex transport processes in the subsurface environment. Positron emission tomography (PET) allows non-intrusively mapping of radionuclide movement and retention in intact soil cores. For example, microorganisms seeded with 2-deoxy-2-[\textsuperscript{18}F]fluoro-D-glucose (\textsuperscript{18}F-FDG), can be readily visualized in repacked sand cores [2]. However, these approaches are hampered by the fixed-ring PET imaging systems generally used in medical fields. Fixed-ring PET imaging systems are configured in predefined geometries and set up in specific orientations, thus confining the applications to subjects of limiting forms and dimensions. Although PET resolution can be improved by shrinking the field of view (FOV) and increasing the solid angle, i.e. by moving the opposing detectors closer, fixed-ring PET systems have no such freedom while imaging smaller regions. Limits on tuning the proprietary software also hinder the development of algorithms for enhancing image quality. In this study, we evaluate the feasibility of applying a custom-built freely-rearrangeable modular PET system, BioPET [3], integrated with
an open-source tomographic reconstruction platform—“Customizable and Advanced Software for Tomographic Reconstruction” (CASToR) [4], for studying bioremediation of subsurface contamination. Using model and field soil cores, BioPET was employed to visualize i) fluid transport, and ii) metabolic activity, microbial distribution, and populations.

2. Materials and methods

2.1. The modular PET system

The custom-built BioPET system used in this study consists of four detector modules as shown in Fig. 1, each with external dimension of $60 \times 60 \times 124$ mm$^3$. The modules can be arranged freely to adapt to different objects and surrounding geometries, and also can be relocated in the course of data acquisition, facilitating the study of objects in various shapes and conditions. Each module consists of Hamamatsu H8500 position sensitive photomultiplier tubes coupled to an array of $37 \times 37$ LYSO:Ce scintillator crystals through a 2.5 mm thick acrylic light-guide. The crystal area is $1.2 \times 1.2$ mm$^2$ and the crystal depth is 13 mm. A 0.1 mm BaSO$_4$ reflector is inserted between adjacent crystals, resulting in a 48 mm$^2$ detection window for each module. The signals from the two crystals at the array edge are merged, giving a $35 \times 35$ crystal map. The LYSO:Ce crystal used in this system is Lu$_{1.9}$Y$_{0.1}$SiO$_5$ with Cerium content of 0.5 mol%. The crystal density is 7.20 g/cm$^3$ and the light output is 29000 photons/MeV. A -20% to +60% energy cut was applied around the calibrated 511 keV peak. Detailed information and characterization of the electronics and the data acquisition architecture can be found in [5, 6, 7]. A 360-degree rotation stage and a 50-cm translation stage are incorporated to allow relative movement between detectors and objects for continuous step-and-shoot acquisition. The modular and translocatable design not only provides flexibility but also reduces capital cost for covering a wider sampling angle. In this study, data acquisition was conducted for both rotational scanner mode and stationary camera mode (below referred to as scanner mode and camera mode, respectively) at different stages of each experiment. In scanner mode, the object was rotated about the z-axis 30 degree at a user-defined frequency.

![BioPET modules](image)

**Figure 1.** A modified Tempe cell placed in the FOV center of the four detector modules, with the face-to-face distance between opposing detectors of 144 mm. The rotation mechanism allows the object to rotate about the z-axis at a user-defined angle and frequency.

1 The essential parts of the system were built in collaboration with Thomas Jefferson National Accelerator Facility according to our specifications. Further modifications and expansion have been carried out in-house.
2.2. Model and field soil cores

To test if we could successfully label a pure culture as a proof of concept, we constructed cores of sterile sand containing a known concentration of *Pseudomonas* culture (see below). As an application of our methods to a sample from the field, we constructed a microcosm using soil from a diesel-contaminated site in northern Yukon, Canada. All experiments were performed using modified Tempe cells. These cells accept undisturbed soils contained in a 35 mm diameter × 50 mm tall Teflon cylinder, pressurizable to 1 bar. Each cell has one inflow and one outflow with 1 bar ceramic plate filters, and is used to determine water and chemical movement rates through soil, and physical and chemical soil parameters.

2.2.1. Model cores. Cylindrical columns of model cores were made of three layers of silica sand (DS2000/SIL4, SiL Industrial Minerals) contained in the Teflon cylinders. Each layer measured 15 mm in height and 35 mm in diameter to form a total height of 45 mm. Sand was sterilized prior to experimentation. Three types of sand columns were constructed (Fig. 2): The middle layer was (a) infused with 2 ml milliQ water, (b) inoculated with *Pseudomonas* (isolated from environmental soil samples, identified using API strips, bioMérieux) in 2 ml milliQ water, and then autoclaved at 121°C under 110 kPa, and (c) inoculated with *Pseudomonas* in 2 ml milliQ water. The top and bottom layers were composed of dry sand. A ceramic disc and an O-ring were placed between the sand column and the bottom of the Tempe cell to facilitate uniform distribution of fluid in the column and to minimize escape of fine particles.

![Figure 2](image)

**Figure 2.** Three model cores were constructed with three types of middle layer: (a) infused with 2 ml milliQ water, (b) inoculated with *Pseudomonas* in 2 ml milliQ water, and then autoclaved at 121°C under 110 kPa, and (c) inoculated with *Pseudomonas* in 2 ml milliQ water. The middle layers were sandwiched between dry sand layers and each layer was 15 mm thick.

2.2.2. Field soil cores. From the field soil, we created a microcosm: an intact soil core inside a PVC tube, measuring 3.5 cm diameter and 5 cm in height. The microcosm was then placed into a 125 ml amber jar with a biostimulatory solution for 28 days. The biostimulatory solution consisted of nitrogen, phosphate, ammonium iron(III) citrate, and sulfate, and was used to stimulate diesel bioremediation. After 28 days, the soil core was pushed into a Teflon cylinder inside a modified Tempe cell, similar to the model cores.

2.3. Experiments

We evaluated the feasibility of identifying metabolic activity and microbial occurrence using the modular BioPET employing $^{18}$F-FDG as a metabolizable radioactive tracer. An $^{18}$F-H$_2$O solution was used for imaging fluid transport in the soil column. Radioactive tracers were diluted with water and applied to the top of the Tempe cell using a 3/16 inch internal diameter tubing attached to compressed air and adjusted to 1 bar using a gas pressure regulator. The pressure of
1 bar was chosen to minimize disturbance to the soil structure based on preliminary tests. This pressure was applied to the cell top to push the solution through and the cell was continuously imaged in camera mode.

To rule out $^{18}$F-FDG retention in the soil column due to microbial characteristics other than metabolism, we imaged a model sand core dosed with *Pseudomonas* and autoclaved as above (Fig. 2). We compared $^{18}$F-FDG retention concentrations in this autoclaved core (culture control) with concentrations in cores containing 1) only moist sand as a procedural control (Fig. 2 a) and 2) live *Pseudomonas* as a culture treatment (Fig. 2 c).

For the $^{18}$F-FDG experiments with model cores, we injected the Tempe cells with $\sim$16 MBq $^{18}$F-FDG in 42 ml deionized (DI) water, waited $\sim$10 mins, and imaged in scanner mode. Thereafter, $\sim$107 ml DI water was pushed through the cell at 1 bar pressure to wash out residual $^{18}$F-FDG. Finally, the washed core was imaged again in scanner mode.

For the $^{18}$F-FDG experiments with the field soil core, 42 ml of $\sim$13 MBq $^{18}$F-FDG solution was injected into the live (non-autoclaved) core, which was then washed with 145 ml DI water. After autoclaved, the core was treated with 42 ml of $\sim$16 MBq $^{18}$F-FDG solution and then washed with 155 ml DI water.

### 2.4. Data processing

When back-to-back gamma photon pairs were detected, the events were stored in list-mode format containing the information of crystal identification, energy, and time. Images of the camera mode were reconstructed using software developed by Jefferson Lab [5] employing iterative maximum likelihood expectation maximization (MLEM) algorithm [8]. Voxel size 0.5 mm$^3$ and iteration number 50 were used.

For scanner mode, the list-mode data were pre-processed using in-house developed codes, and, together with the corresponding crystal positions and directions, were fed into a modular tomographic image reconstruction platform, CASToR [4]. List-mode image reconstruction, being advantageous in not taking up the storage of empty sinogram bins, was performed. The ordered subset expectation maximization (OSEM) algorithm [9] with subset 16 and iteration number 2 was employed. Before the commencement of iterative reconstruction, the sensitivity image was computed based on all possible detection elements derived from the user-provided crystal map or scanner geometry. The projector employed is Joseph’s method [10], which is a ray-tracer using bi-linear interpolations [11]. While decreasing the image pixel size allows more details to be resolved, it can also reduce the statistics per voxel and thus result in increasing noise. Following the NEMA standard, the pixel size should be no larger than one-fifth of the expected FWHM (full width at half maximum) [12]. A pixel size of 0.4 mm in three axes was chosen based on preliminary analysis of point-source resolution versus pixel size. A 3D Gaussian image convolver of 1.2 mm transaxial FWHM and 1.2 mm axial FWHM—to model a spatially uniform image-based point-spread function [13]—was applied within iterations. 3D convolution with a Gaussian function of radius 0.8 mm was implemented for post-processing.

### 3. Results and discussion

A NEMA NU 4-2008 image quality phantom (Fig. 3 a) [12] filled with $\sim$3 MBq $^{18}$F-FDG was used to verify the modular PET operated in both modes and the data processing methods as described in Sec. 2.4. The phantom comprises five fillable rods with length of 20 mm and diameters of 1, 2, 3, 4, and 5 mm. Averages of the reconstructed image slices covering the central 10 mm length of the rods for scanner mode and camera mode are shown on the right of Fig. 3 b. Fig. 3 b displays profiles of the closed polygons connecting the five local maxima, which were found using the algorithm based on topographical prominence implemented in ImageJ [14]. The activity concentration values were normalized with respect to the peak of 5 mm rod of each mode. Though the activity concentration of the 1 mm rod can be detected in both modes, the scanner...
mode was able to obtain higher signal-to-noise ratios. Also, artifacts and distortion would manifest in the reconstructed images of the camera mode due to limited angular coverage [15].

The modular PET system allowed us to recreate preferential flow paths of $^{18}$F-H$_2$O through the soil column (Fig. 4). The solution initially pooled at the top of the soil surface, though proceeded through the soil profile after $\sim$40 seconds.

![Figure 3](image)

**Figure 3.** (a) NEMA NU 4-2008 image quality phantom [12] comprises five fillable rods with length of 20 mm and diameters of 1, 2, 3, 4, and 5 mm. (b) Profile plots of the closed polygons connecting the five local maxima on the averages of the reconstructed image slices covering the central 10 mm length of the rods. Average images are shown on the right with crosses indicating the five local maxima.

![Figure 4](image)

**Figure 4.** Flow of $^{18}$F-H$_2$O solution in a soil core during 50–90 sec following commencement of 1 bar pressure. Images were reconstructed using consecutive 5-minute list-mode data acquired in camera mode.
The feasibility of imaging microbe colonies and populations was demonstrated in the $^{18}$F-FDG experiments with model and field soil cores. The mean $^{18}$F-FDG activity concentration of each 0.4 mm z-slice indicates that distribution of $^{18}$F-FDG retention after DI water wash could be clearly identified in columns inoculated with *Pseudomonas*, while columns containing autoclaved *Pseudomonas* as well as only milliQ water held little radioactivity throughout the cores (Fig. 5).

Comparison of activity within the field soil core before and after washing and autoclaving indicates that retention of $^{18}$F-FDG was mainly due to microbial metabolism. As shown in Fig. 6, though vertical activity concentration profiles of the original and autoclaved $^{18}$F-FDG-permeated soil were comparable, after the soils were washed, the original soil retained a greater proportion of radioactivity relative to the autoclaved core. The BioPET system appears feasible to probe metabolic activity, as well as to map and relatively quantify the microbe colonies in intact soil cores.

![Figure 5](image-url)

*Figure 5.* Mean activity concentration (in logarithmic scale) at different vertical positions in the three types of model cores (as shown in Fig. 2) after $^{18}$F-FDG permeation and subsequent DI water wash.

4. Conclusion
Here we demonstrate the feasibility of applying the flexibly-rearrangeable modular PET system (BioPET) to investigate bioremediation of subsurface contamination. Operation in camera mode allows us to track fluid transport through the soil core in 4D (3D plus time) with adequate resolutions. Imaging the control model and field soil cores with live bacteria in scanner mode shows that metabolic activities, occurrence, and distribution of subsurface microorganisms can be identified by using metabolizable radioactive tracers.

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Figure 6. Mean activity concentration (in logarithmic scale) at different vertical positions in a field soil core after $^{18}$F-FDG permeation and subsequent DI water wash. The same core was autoclaved and then once again through $^{18}$F-FDG permeation and wash. The small figure shows the 3D volume rendering of live (non-autoclaved) core after washing.

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