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Electronic Supplementary Information (ESI)

A mini panel-PET scanner based microfluidic radiobioassay system allowing high throughput imaging of real-time cellular pharmacokinetics

Running Title: High throughput real-time microfluidic radiobioassay

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Conflicts of interest

The authors declare no potential conflicts of interest.

Shielding of the panel-PET based CIMR system

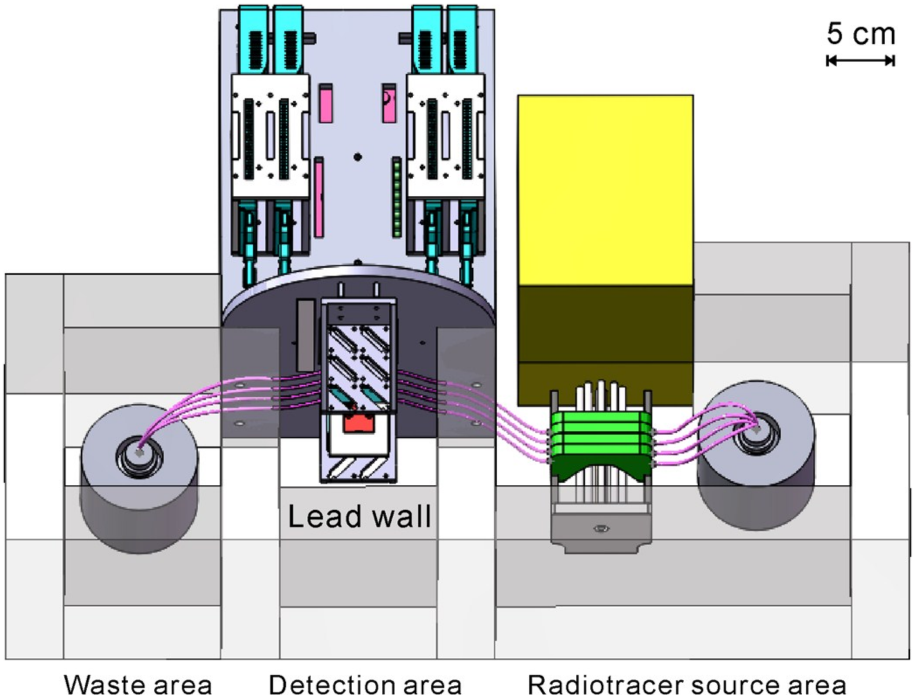

Fig. S1 The shielding of the panel-PET based CIMR system. The shielding walls (transparent) were built with lead bricks. The shielding can be divided into three areas (from right to left): radiotracer source area, detection area, and waste collection area.

Mini panel-PET scanner calibration with a plate source

A plate phantom filled with homogeneous [^{18}F]FDG solution is used for calibration of the mini panel-PET detector whenever a physical setting is changed (i.e., panel-panel spacing, fixation stage distance change). The inner dimension of the plate is 120 mm × 90 mm × 2 mm thickness. The plate phantom was filled with an [^{18}F]FDG water solution of approximately 1.85 MBq (50 μCi). Several drops of red ink were mixed in the [^{18}F]FDG solution for indication if the radiotracer solution is homogeneously distributed within the plate. The plate phantom was filled full with water using an insulin syringe to avoid any air bubbles; then, the plate was sealed with a plastic screw and shaken vigorously approximately 50 times. The plate phantom was then placed on top of the fixation stage (Fig. S2). In the "DH-PET Server" software, after recording the panel-panel spacing, PET acquisition was started until 5 × 10^6 of PET pulse events were reached. The calibration data is stored as an innate file and used as an option for normalization during image reconstruction.

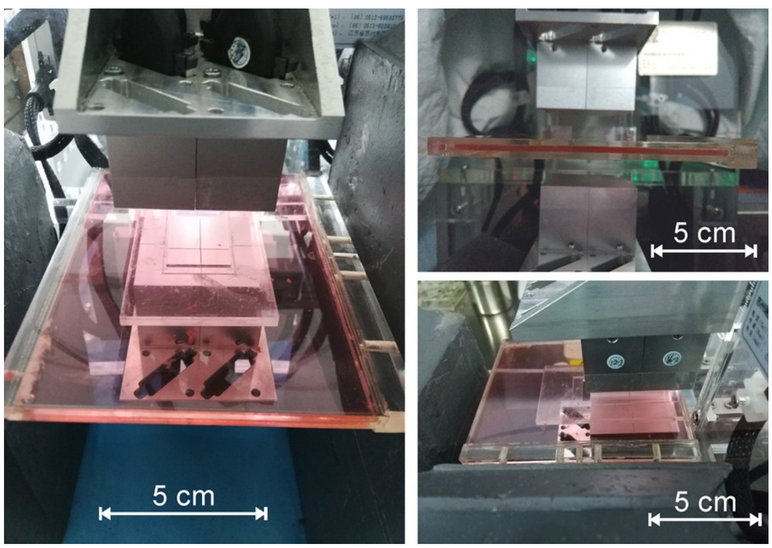

Fig. S2 Photos indication of the calibration set. The plate phantom contained approximately 1.85 MBq [^{18}F]FDG in a water solution with red ink. The red ink help indication of the homogeneity of [^{18}F]FDG solution.

Imaging of a Derenzo phantom

The Derenzo phantom is a plastic cast with six fan-shaped arrays of cylindrical holes indicating 2.4 mm, 2.0 mm, 1.6 mm, 1.2 mm, 1.0 mm, and 0.7 mm in diameter (Fig. S3). The holes are spaced with each other with a diameter distance within each array. The two panels of the LYSO/SiPM detectors were set 6.5 cm apart. The distance between the stage and the lower panel is 30 mm. The energy window was 450–560 KeV; the time window was 3 ns. Before the imaging, all the holes of the Derenzo phantom were filled with the red-ink/water mixture containing [^{18}F]FDG. The phantom was scanned for 5 min. The image was reconstructed with the MLEM algorithm with 50 iterations.

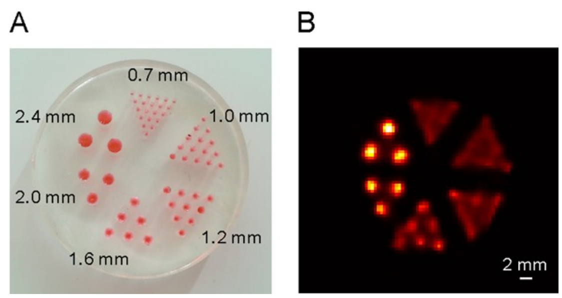

Fig. S3 Imaging of a Derenzo phantom. (A) The Derenzo phantom with dyed [^{18}F]FDG solution; the arrays of different hole sizes are labeled, and the center-to-center spacing is twice the hole diameter in adjacent holes of the same array. (B) A maximum intensity projection (MIP) image of the imaged Derenzo phantom.

Spatial resolution

Quantitative spatial resolution was measured using a point source of [^{18}F]FDG (0.5 μL, 148 KBq) filled in a capillary tube (diameter of 0.8 mm). Thus, the diameter of the point source is controlled to be < 1 mm. The surface of the fixation stage was set midway between the two panels, spaced 3 cm apart. The point source was measured for 10 min. The point source data were reconstructed with the MLEM algorithm with ten iterations. Here, the X and Y axes are parallel to the panel plane; the Z-axis is perpendicular to the panel plane. Only one image slice parallel to the panel plane was selected for the 2D resolution calculation. The full width half maximum (FWHM) of the point-source image (histogram) was determined in the X and Y axes.

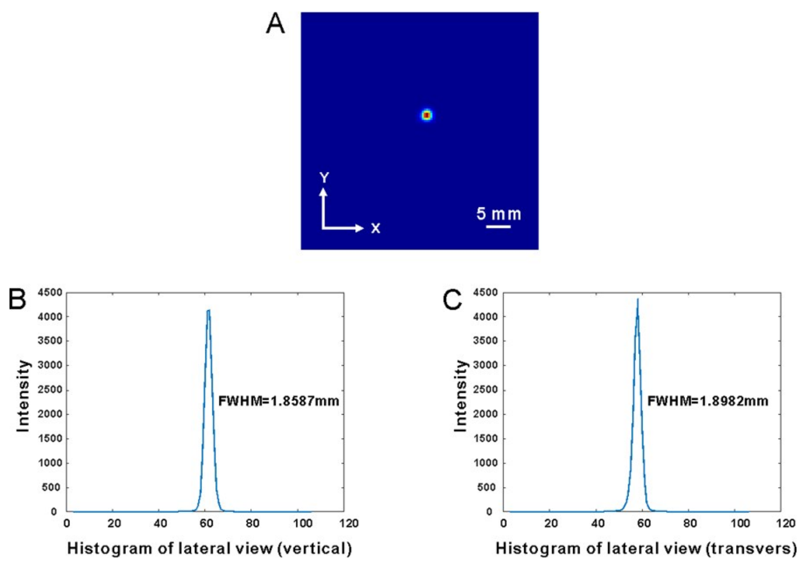

Fig. S4 Quantification of the spatial resolution. Scanning of an [^{18}F]FDG point source (A) and the full width half maximum (FWHM) of the point source image (B&C). The lower left figure is the histogram of the vertical view; the lower right is the histogram of the transversal view. The FWHM is 1.8587 mm (axial) and 1.8982 mm (transverse).

From the panel PET image of the Derenzo phantom (Fig. S3), the array of 1.6 mm diameter can be distinguished at naked eyes. From the [^{18}F]FDG point-source imaging results (Fig. S4), the full width half maximum (FWHM) of the point source was 1.8587 mm for the vertical histogram and 1.8982 mm for the transverse histogram.

Energy resolution and time resolution

The PET signal was digitized by a multi-voltage threshold (MVT) method. The energy resolution and time resolution were tested with a source of [^{18}F]FDG (50 µL, 1.85 MBq) filled in a plastic bowl shape container (maximum inner diameter is 6 mm, ibidi GmbH). The container was placed in the middle of the detector panels. The detector panels were spaced 4 cm apart. The energy window was 450–650 KeV; the time window was 3 ns. The detection thresholds of the 4-level multivoltage threshold digitizer were set as 10-120-230-330 mV.

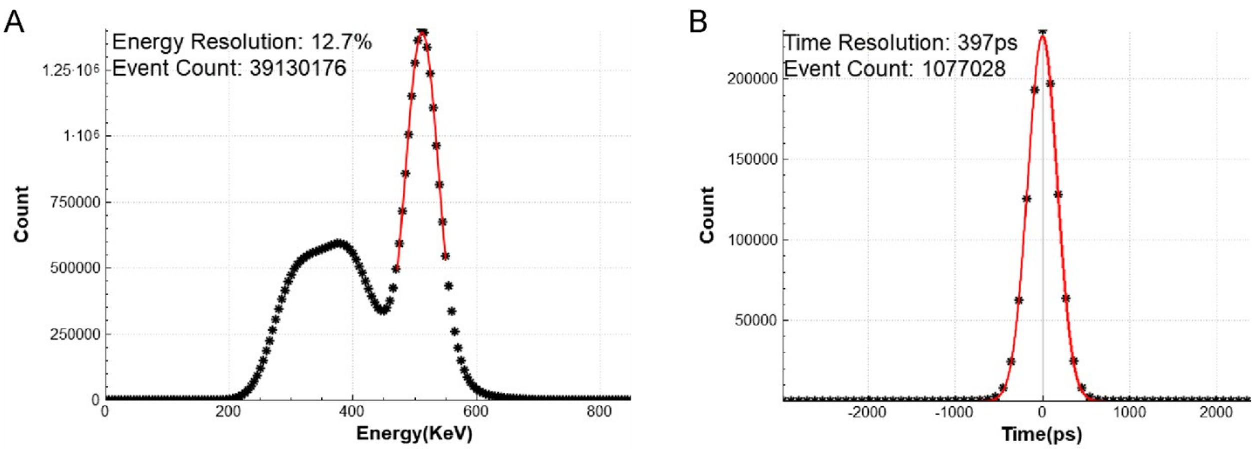

Fig. S5 The energy resolution (A) and time resolution (B) of the mini panel-PET detector for 511 KeV gamma rays ([^{18}F]FDG source). The PET signal was digitized by a multi-voltage threshold (MVT) method. The data was sampled by a 4-level multivoltage threshold digitizer with the thresholds set at 10-120-230-330 mV, separately.

The energy resolution and timing resolution were tested under four thresholds: 10 mv, 120 mV, 230 mV, and 330 mV using the MVT method. The space between the two detection panels was 4 cm. Under this setting, the tested results of the energy resolution (Fig. S5A) and timing resolution (Fig. S5B) were 12.7% at 511 keV and 397 ps, respectively.

System linearity test

Six samples of [^{18}F]FDG source (100 μL) forming a concentration gradient were manually prepared by a sample and dilution method: 0.2 MBq, 0.4 MBq, 0.6 MBq, 0.8 MBq, 1.0 MBq, and 1.2 MBq. Each of the 100 μL sources was filled in a plastic bowl shape container (ibidi GmbH). The container was placed at the center of the panel PET (the panel-panel distance was 6.5 cm) and measured for 240 s. All the measurements were decay-corrected. After the measurement, the coincident events were counted as a function of source radioactivity and plotted; the linear regression function was fitted for assessing the linearity of measurements.

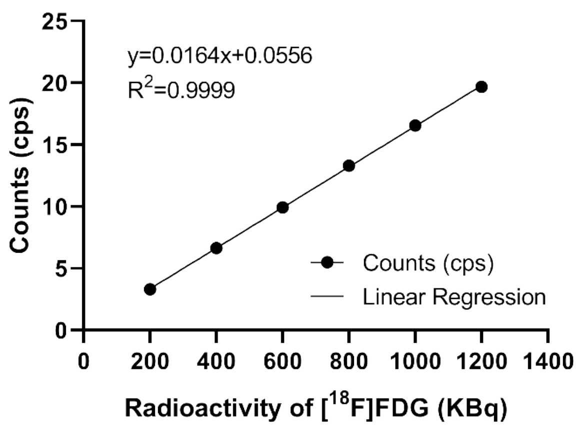

Fig. S6 Linearity test of the mini panel-PET. The dots are the counts (cps) corresponding to the diluted radioactivity. The line is the linear regression of linearity for the measured data (R^2=0.9999). The selected radioactivity of the [^{18}F]FDG is typically in the range of radioactivity that used for measuring cellular pharmacokinetics. It was tested with the source to the upper panel distance of 3.5 cm and the lower panel distance of 3 cm.

The system linearity was tested with the source to the upper panel distance of 3.5 cm and the lower panel distance of 3 cm. From the results shown in Fig. S6, the coincidence event count of the detection system was linear with the radioactivity of the source. The linearity of the panel PET was excellent, with radioactivity in the range of 0.2 MBq – 1.2 MBq (R^2 = 0.9999). This range of the radioactivity is typically used for measuring the cellular pharmacokinetics with the CIMR technique. The radiotracer activity concentration used for CIMR measurement is typically in the range of 1 MBq/ml – 5 MBq/ml (for [^{18}F]FDG), which was tested to be able to acquire sufficient signals while not expose too much dose to cells. This range could be extended for other types of radiotracers as needed. The centered microfluidic chamber (where cells are located) contains only

30 μl liquids. Given the 30 μL volume for calculation, four chambers, and the radioactivity concentration of 1 MBq/ml – 5 MBq/ml, the minimal radioactivity is 0.12 MBq; the maximum radioactivity is 0.6 MBq.

System sensitivity test

The system sensitivity was tested under two different panel-panel distances: the distance between the panels set at 3 cm and the radioactive source in the middle of the FOV; the radioactive source to the upper panel distance of 3.5 cm and the lower panel distance of 3 cm. The energy window was 350–650 KeV; the time window was 3 ns. A point source of [^{18}F]FDG (148 KBq) placed at the center of the stage and measured for 10 min. The system sensitivity was calculated with the following formula:

$$S_A = \frac{C_A - C_{BKG}}{0.967 \times C_{CAL}} \times 100\%$$

C_A is the measured total coincident events within the testing time T, C_{BKG} is the coincident events measured without radioactivity within the testing time T (background of the environment), C_{CAL} is the total coincident events of the [^{18}F]FDG point source within the testing time T. 0.967 is the branching ratio for fluorine-18.

The system sensitivity was 1.81%, with the source to the upper panel distance of 3.5 cm and the lower panel distance of 3 cm. The sensitivity of the system was 5.99%, with the source to each panel distance of 1.5 cm. The different panel-panel distance setting leads to the difference in the sensitivity. The sensitivity decreased with the increase of the panel-panel distance. The difference in sensitivity was mainly caused by how many coincident events can be captured under the detection settings of the geometry.

Comparison of the positron detector and the mini panel-PET

Some physical properties of the positron detector and the mini panel-PET are compared in Table S1. The mini panel-PET has an increased FOV over the positron detector at the expense of spatial resolution (ca. 177 μm vs. ca. 1.9 mm). The sensitivity of the positron detector (contact detection) is also significantly higher than that of the mini panel-PET. However, as the sensitivity of positron detector varies with detection distance (depth-dependent sensitivity),[1] and the sensitivity of panel PET can also be changed according to the panel-panel distances. Therefore, the comparison of sensitivity between the two detectors is dependent on concrete settings.

Table S1 Comparison of the positron detector and the mini panel-PET

	Positron detector (Silicon pixel detector)	Mini panel-PET
Spatial resolution	177.1±4.1 μm (without energy weighting) 155.5±3.1 μm (with energy weighting)[2]	1.8587 mm × 1.8982 mm
The total field of view (FOV) in 2D	14 mm × 14 mm	50 mm × 50 mm
Energy resolution	Not available	12.7% at 511 keV
Time resolution	Not available	397 ps
Sensitivity	0.35 cps/Bq (35% sensitivity) in Timepix mode[2] (measured in direct contact of the detector surface)	5.99% at an energy window of 350–650 KeV and a time window of 3 ns (measured with the panel-panel spacing of 3 cm)

CIMR system on a cart

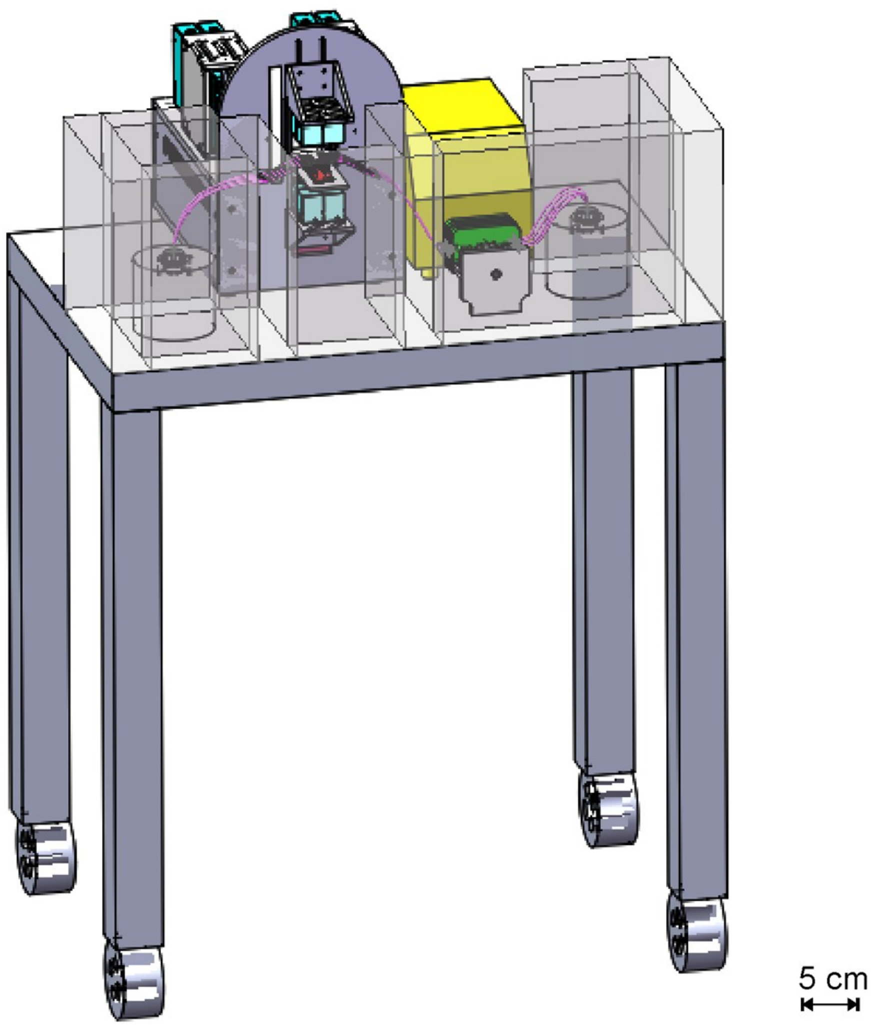

Fig. S7 The CIMR system, including the shielding, on a moveable cart. The system can be moved to free space and connected to a laptop for the measurement.

Reference

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2. Q. Wang, J. Tous, Z. Liu, S. I. Ziegler and K. Shi, *Journal of Instrumentation*, 2014, **9**, C05067.