Development of an imaging method for in vivo single-cell tracking under high-noise conditions: a proof of principle

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Abstract. This paper reports the development of a positron imaging method for in vivo single-cell tracking under high-noise conditions. Following biological processes spatially and temporally at a single-cell level in a living organism is desirable for inquiring into the relationships between the behaviours and properties of cells. Positron-emitting radionuclides enable detecting and following radioactivity-labelled substances deep inside living organisms. However, positron imaging has several challenges, such as the distribution of high noise in other areas close to the cell under investigation. In this work, an algorithm for locating a cell with millisecond resolution to combat the strong interference of nearby noise is developed. The feasibility of the method is verified by the demonstration of particle tracking and detection of behavioural changes in an environment with the signal-to-noise ratio of 1:9.

1. Introduction

The relationship between single-cell molecular characteristics and in vivo behaviour can play an important role in many critical processes, such as cancer cell metastases, as well as stem cell migration and engraftment. A deepened understanding of the migration process and the factors that affect delivery is needed to detect latent obstacles and develop strategies to improve efficiency and functional outcomes of stem cell implantation. Given that cell populations are heterogeneous and the molecular dynamics amongst cells is inhomogeneous, relating the cell behaviour, biological processes and cell properties at the single-cell level is necessary.

The delivery and fate of implanted stem cells can be assessed by using ex vivo processing at multiple time points. In vitro studies can also shed light on the migration, interactions, proliferation, death and differentiation of cells. However, the properties of cells taken out of their native environment and studied in vitro or ex vivo may be considerably different. Following the biological processes of a single cell spatially and temporally in a living organism is desired to inquire into the behaviour and molecular characteristics of a single cell. Although in vivo single-cell detection has been attempted by using photo-acoustic imaging, bioluminescent

imaging and magnetic resonance imaging, these techniques suffer from inadequate depths and/or limited temporal and spatial resolution. Jung et al. [1] demonstrated a positron emission tomography (PET)-based technique for following a single cell over time in a living mouse. In their study [1], the cells were labelled with nanoparticles carrying ^{68}Ga. A conventional PET image reconstruction technique and a new method requiring a small amount of radioactivity in combination with a spline method were used to derive the cell's trajectory. Cell movement was tracked at the velocity of approximately 50 mm/s. However, the method suffered from the reduction in cell-to-background image contrast within a few hours due to radionuclide efflux from nanoparticles [2].

In addition to radionuclide efflux from cells, radionuclide accumulation in other areas of the organism poses challenges to single-cell tracking by using PET-based techniques. In this study, we aim to develop and demonstrate a positron imaging method for the in-depth tracking of a single cell in vivo under high-noise conditions. Our method can be performed by employing a contemporary PET scanner or a modular PET system as the detector array. This technique intends to contribute to detailing cell biological processes, in vivo cell behaviour and their relationships with molecular characteristics.

2. Background and review of positron imaging methods

The technique developed in this study is based on the idea of positron emission particle tracking (PEPT) [3]. In PET and PEPT, tracers are labelled with positron-emitting radionuclides, such as ^{18}F, ^{68}Ga, and ^{64}Cu. As the radionuclide undergoes beta-plus decay, a positron is emitted and, after travelling a short distance [4,5], undergoes annihilation with an electron. This annihilation event results in two photons of 511 keV each that travel in back-to-back directions with angles close to 180°. By detecting these two photons, the radionuclide can be estimated to be located somewhere along the line connecting the two detection sites. This line is called the line of response (LOR).

In PET, the distribution and concentration of the tracer are 3D-mapped. By contrast, in PEPT, the tracer substances are deliberately confined in a very small volume. Therefore, one of the applications of PEPT can be the acquisition of information on the environment and processes by observing how this tiny amount of radioactive substance (hereafter called 'particle') reacts to its surroundings and environmental conditions. If placed in a process with a complex flow field, such as stirring [6], mixing [7], fluidisation [8] and separation [3], and if the particle properties are representative of the processed matter, flow patterns can be revealed in detail by following the radioactive particle with adequate spatial and temporal resolution.

In conventional PET, 3D tracer maps are reconstructed by processing LORs by using projection-based methods [4]. PEPT methods based on different principles and unlimited by projection have been developed and are undergoing innovation [9–12]. In this work, we developed methods that compare LORs with each other to find the most probable location of the particle centroid. In principle, if the radioactivity distribution is confined in a tiny volume, such as a cell, this volume should be located in the area of the closest distance between two LORs. Thus, the location of the cell can be approximated with a reasonably small amount of LORs because in an ideal case, the location can almost be determined by two LORs. In projection-based PET image reconstruction without time-of-flight information, the whole LOR is taken into account in data processing. However, most sections of the LOR are not the annihilation site. Therefore, the efficient use of LORs is emphasised in the single-cell tracking method presented here. The section of the radioactive decay event is estimated by the LOR's relationships with others at the outset of data processing. Consequently, the positron imaging method for single-cell tracking presented in this article requires a smaller amount of LORs than PET. This condition then allows the enhancement of temporal resolution.

3. Experimental

3.1. Tracer particle and setup for particle flow in a high-noise environment

As a proof of principle, an ice particle composed of ^{18}F aqueous solution was prepared to model a cell labelled with positron-emitting isotopes. This approach is a straightforward method for creating radioactive particles, and the radioactivity and size of the particle can be tuned easily. An ice particle of approximately 5.5 mm was used as the particle to be tracked.

Figure 1 shows the experimental setup for particle flow in a high-noise environment. The ice particle was released to fall freely in cold liquid with temperatures below 0 ℃. An inclined ice surface was placed under the release site of the ice particle to model the behavioural changes of the cell in a biological process. The particle was tracked during its free fall in liquid, collision with the ice surface and movement along the ice surface in the environment with a massive amount of positron emission events originating from other sources.

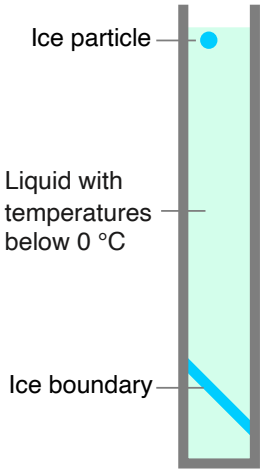

Figure 1. Setup to create the ice particle flow in a high-noise environment. The ice particle was released to fall freely and an inclined ice surface was placed under the release site. Several similar ice particles were placed in the same container to create intensive noise.

An environment with radioactivity much higher than that of a single particle was created to verify the algorithm that was developed to track a single cell under high-noise conditions. Several similar ice particles were placed in the same container where the target particle would move through. Figure 2 (a) shows the LORs generated by a tracer particle and a background source with considerably higher radioactivity. The LORs were drawn between the two detector locations of each photon pair.

3.2. Detector array

A PET scanner (Siemens Biograph Vision 600) was used as the detector array for photon-pair detection. A total of 60800 lutetium oxyorthosilicate (LSO) crystals were arranged to enclose a cylindrical detection space with the radius of 410 mm and axial length of 263 mm, where 760 crystals were in the tangential direction and 80 crystals were in the axial direction. Each crystal had the dimensions of 3.2 mm $\times$ 3.2 mm $\times$ 20 mm. Each 5 $\times$ 5 array of crystal was optically coupled to a 4 $\times$ 4 array of silicon photomultipliers (SiPMs). The coincidence processing unit compared the times of photon arrivals. If the arrival times were within 4.73 ns, the unit determined that the two events formed a coincidence. Additional characteristics of the scanner, such as energy window and electronics details, can be found in the references (see, e.g., [13]).

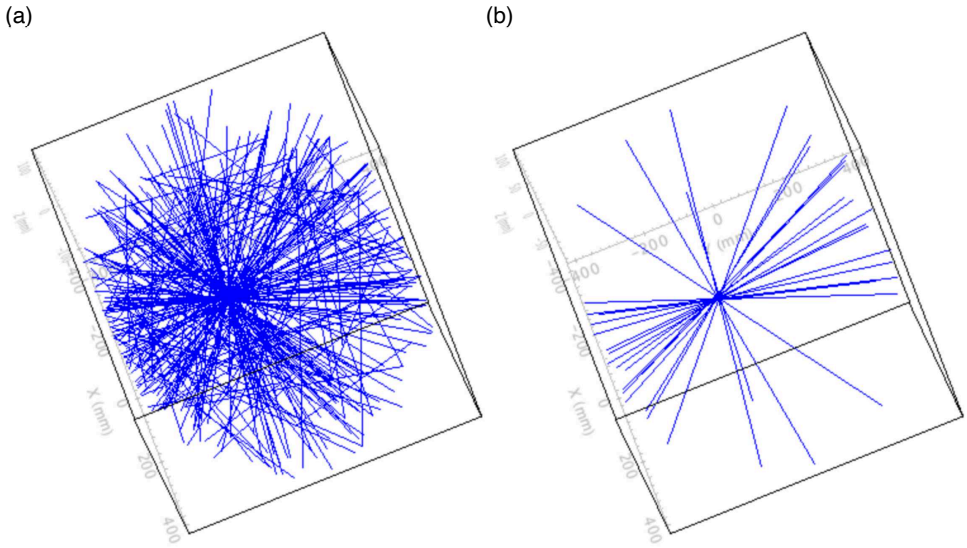

Figure 2. Setup to test the algorithm with an ice particle passing through an environment with high radioactivity. In the instance of the data shown, the edge-to-edge distance between the ice particle and a strong noise source is approximately 13 mm, and the signal-to-noise ratio in the field of view is approximately 1:8. (a) LORs within a period of 0.06 ms. (b) Remaining LORs indicating the location of the ice particle after the data were processed by the developed algorithm.

3.3. Single-cell tracking algorithm

The goal of this work is to develop a single-cell tracking algorithm that is in principle different from the projection-based tomography image reconstruction. While the fundamental aim of these algorithms is to find the most probable particle location by evaluating the relations between LORs, their implementation can be of great variety. For instance, the relationships between two LORs can be evaluated on the basis of the existence of a cutpoint (cross point) in each 2D plane and further evaluated by comparing their cutpoint with others' cutpoints. If this cutpoint falls very far from most of the other cutpoints, the likelihood of an annihilation event occurring around this cutpoint is determined as low. By running this kind of evaluation iteratively, the most likely location can be determined [3, 14]. The evolutions of the algorithms, such as fitting the density of cutpoints with peak shape functions for single and multiple particle tracking and weighting the likelihood of a cutpoint on the basis of the particle location found in the previous time step, have been demonstrated to be useful under different conditions [12, 15].

In this work, the algorithm was redeveloped to evaluate the relationships between LORs directly in 3D. The algorithm was designed to allow users to define region(s) of interest/noninterest with a relevant time point or period. A filter was then applied in accordance with time- and space-specific prior information, if available, to reduce the influences of LORs that were likely generated by the nonrelevant background. The filter was designed to evolve in accordance with the result of every time step. In this work, an ice particle was set to pass by an area with considerably higher radioactivity to test the capability of the algorithm. Figure 2 (a) shows all the LORs within a period of 0.06 ms and (b) shows the remaining LORs, indicating the location of the ice particle, after processing by the algorithm. In the situation shown by Figure 2, the edge-to-edge distance between the ice particle and an intensive noise source was approximately 13 mm, and the signal-to-noise ratio was approximately 1:8.

4. Results and discussion

Figure 3 shows the pathline of an ice particle freely falling in liquid with a temperature between 0 ℃ and -20 ℃, colliding with an inclined ice surface and continuing to move along the ice surface. The temporal resolution of 4 ms was chosen for processing the LORs (i.e. the location of the particle was obtained every 4 ms) because the particle was moving at a speed below 0.3 mm/ms. Properties, such as velocity component, speed (as shown in Figure 3 (b)), acceleration and kinetic energy can be derived from the ms positions of the particle. The colour scale bar in Figure 3 (a) indicates the elapsed time in ms and that in (b) indicates the speed in m/s.

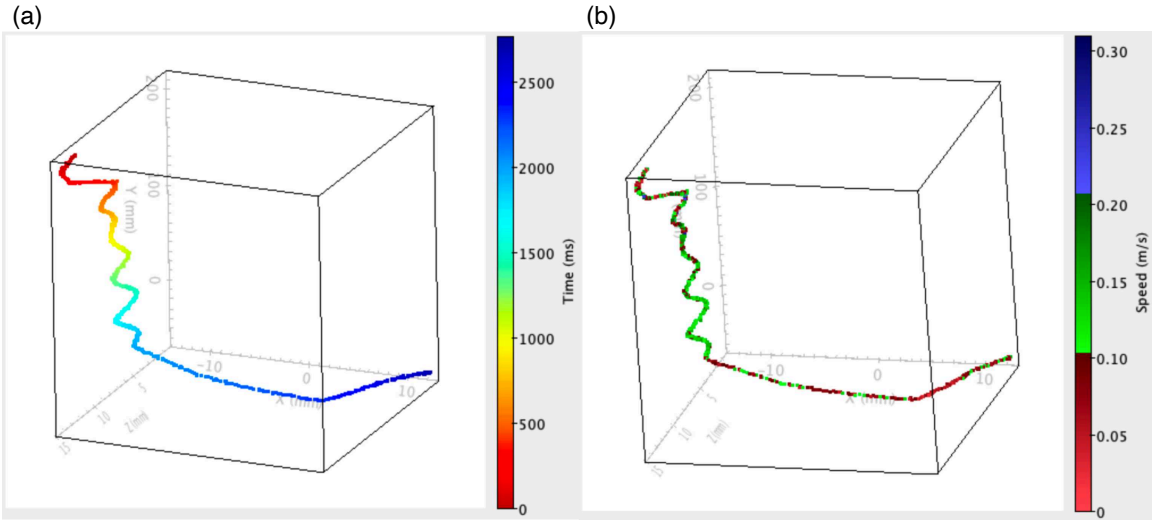

Figure 3. Pathline of an ice particle freely falling in liquid with a temperature between 0 ℃ and -20 ℃, colliding with an inclined ice surface and continuing to move along the ice surface. The colour scale bar in (a) indicates the elapsed time in millisecond and that in (b) indicates the speed in m/s.

During this tracking period, the average signal-to-background ratio was approximately 1:9 to demonstrate the capability of the single-cell tracking method under high-noise conditions. Figure 3 (b) illustrates that the behavioural changes (here the speed changes) in time and space due to interactions with the environment can be clearly seen by using the single-cell tracking method. The particle speed dropped abruptly as the particle hit the ice surface.

5. Conclusions

In this work, a positron imaging method for tracking a single cell in vivo under high-noise conditions was developed. A setup of ice particle flow and ice-ice collision in a high-noise environment was utilised to test the method. The capability of the method to follow a particle and unveil its behavioural changes at the signal-to-noise ratio of 1:9 was successfully demonstrated.

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