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A Hybrid Clustering Method for ROI Delineation in Small Animal Dynamic PET Images: Application to the Automatic Estimation of FDG Input Functions

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Abstract

Tracer kinetic modeling with dynamic Positron Emission Tomography (PET) requires a plasma time-activity curve (PTAC) as an input function. Several image-derived input function (IDIF) methods that rely on drawing the region-of-interest (ROI) in large vascular structures have been proposed to overcome the problems caused by the invasive approach to obtaining the PTAC, especially for small animal studies. However, the manual placement of ROIs for estimating IDIF is subjective and labor-intensive, making it an undesirable and unreliable process. In this paper, we propose a novel hybrid clustering method (HCM) that objectively delineates ROIs in dynamic PET images for the estimation of IDIFs, and demonstrate its application to the mouse PET studies acquired with [¹⁸F]Fluoro-2-deoxy-2-D-glucose (FDG). We begin our HCM using K-means clustering for background removal. We then model the time-activity curves using polynomial regression mixture models in curve clustering for heart structure detection. The hierarchical clustering is finally applied for ROI refinements. The HCM achieved accurate ROI delineation in both computer simulations and experimental mouse studies. In the mouse studies the predicted IDIF had a high correlation with the gold standard, the PTAC derived from the invasive blood samples. The results indicate that the proposed HCM has a great potential in ROI delineation for automatic estimation of IDIF in dynamic FDG-PET studies.

Index Terms

Position Emission Tomography (PET); curve clustering; segmentation; image-derived input function

I. INTRODUCTION

Positron Emission Tomography (PET) is a valuable functional imaging technique, which plays an important role in several areas of medical treatment and preclinical research, such as disease diagnosis, staging, therapy assessment [1] and drug development [2]. Dynamic PET images can be evaluated visually or quantified by tracer kinetic modeling, which uses a tracer plasma time-activity curve (PTAC) as an input function in order to characterize the target tissue time-activity curve (TTAC) for a predefined region of interest (ROI). This characterization is achieved using physiological parameters [3], such as the influx rate and glucose metabolic rate. Although frequent invasive arterial blood sampling is the current gold standard for obtaining PTACs for kinetic quantification, it is harmful to subjects and may expose operators to extra radiation. Invasive blood sampling is also a challenge in preclinical studies with small animals because of the small size of blood vessels and the limited volume of blood. An alternate noninvasive method that eliminates blood sampling has been proposed for simultaneously estimating the input function and physiological parameters from multiple distinct ROIs [4,5]. Without using ROIs, methods based on factor analysis have been developed to extract the input function from the dynamic PET studies for several different tracers [6,7]. Population-based methods [8,9] and image-derived input function (IDIF) methods [10,11] have been introduced to avoid the invasive approach in clinical environments. The IDIF methods, which rely on a sufficiently large vascular structure in the imaging field of view (FOV), are more direct and convenient to apply than population-based methods. Especially for small animal PET imaging, the entire body of the animal is often located in the FOV including the heart which could be assumed to be a large blood pool. In the existing IDIF methods, the PTAC can be obtained by simply placing a ROI on a large blood cavity such as left ventricle of the heart [12,13]. TTACs can be obtained from other ROIs placed on the target tissues for the further quantitative analysis using tracer kinetic modeling. The quality of the ROI delineation depends upon the experience of the operator. This manual approach is subjective, labor-intensive, and time-consuming, and as such semi- or fully-automatic ROI delineation methods that utilize some objective criterion are necessary in order to overcome these disadvantages.

Despite the significant efforts that have gone into attempting to achieve automatic delineation or segmentation in PET images, there are still issues that need to be addressed today. In dynamic PET images, every pixel has a corresponding tracer time-activity curve (TAC) to record the temporal information. Many pixel-clustering methods have successfully used the temporal information for image segmentation by assuming that the TACs of pixels in the same tissue or organ have similar shapes and magnitudes. For example, K-means clustering has been proposed for the segmentation of major organs from dynamic PET images [14]. This method was later extended to 3D segmentation of white and gray matters in the brain by integrating the kinetic features [15]. K-means clustering has also been used to segment PET images of the brain after processed with principle component analysis (PCA) [16]. An unsupervised method, called local means analysis (LMA), has been introduced for tissue segmentation in rodent whole-body dynamic PET image, without guidance from anatomical image [17]. Clustering methods have also been used to obtain noninvasive arterial input functions for human brain studies [18,19] and small animal cardiac images [20]. A clustering method with iterative coordinate descent has been adopted to directly segment dynamic PET images in the projection domain [21], in addition to successfully segmenting reconstructed images automatically. However, these clustering methods handled the TACs or the sinograms as sequences of discrete points in vector form and did not utilize the potentially valuable smoothness or continuous temporal information. Specific statistical techniques based on the regression mixtures framework have been introduced to directly address curve clustering. These techniques could be considered as model-based clustering

[22]. Curve clustering using polynomial regression mixture models (PRMs) is the most computationally efficient method among these statistical techniques.

In this study, we explore a novel hybrid clustering method (HCM) based on curve clustering with PRMs for the automatic delineation of ROIs that will be used to estimate IDIF. We demonstrated the application of our approach to small-animal PET images acquired using the most common tracer [^{18}F]Fluoro-2-deoxy-2-D-glucose (FDG). In our HCM, we first remove the background to obtain a coarse mask of the heart. We then use PRMs to model the set of TACs from the heart mask in order to obtain distinct parameters for curve clustering. This is used to classify the pixels. Finally, we merge the classes and obtain two refined ROIs: the myocardium (ROI_{myo}) and blood cavity (ROI_{b}). The performances of the HCM and K-means clustering method were compared in automatic ROI delineation and evaluated them using a series of computer simulations as well as a set of experimental small animal dynamic FDG-PET studies. In the small animal studies, the ROIs obtained automatically by HCM were applied in the IDIF estimation. We validated the predicted IDIF using the gold standard obtained from the invasive blood samples.

II. METHODS

The proposed hybrid clustering method (HCM) for automatic ROI delineation consisted of three major steps: (1) background removal, (2) curve clustering with polynomial regression mixture models (PRMs), and (3) classes merging. The overall approach is depicted in Fig. 1. The processes are described in detail in Section II-A, using an FDG-PET image as an example. We briefly introduce our approach to FDG IDIF estimation in Section II-B.

A. Hybrid Clustering Method

Step1: Background Removal—During this step, the heart was coarsely localized prior to delineating the ROI_{myo} and ROI_{b} . The background that was removed included pixels outside the scanned subject, which contained only noise and reconstruction artifacts, and pixels of tissues with lower activities, such as muscles, fat and skin. In late frames, the pixels depicting the heart had higher time-activities than their surroundings. However, it was possible that the bladder could also have high activities during the late frames. As such, only the thoracic part of the FDG-PET image was processed in this step, in order to exclude the bladder based on prior anatomical knowledge. K-means clustering was used to cluster all the pixels of the truncated thoracic FDG-PET image into several classes based on the different magnitudes of tracer activities in the last frame (~60min post tracer injection). This was achieved by minimizing the cost function given in (1).

$$\theta = \sum_{j=1}^k \sum_{i=1}^n \|A_i^j - C_j\|^2 \quad (1)$$

where, A_i^j is the activity of the i^{th} pixel assumed to be in the j^{th} class, C_j is the center of the j^{th} class, which can be initialized as the equal distance center in dataset. k is the cluster number, and n is the number of pixels in the j^{th} class, which can be updated in each iteration.

The pixels in the class with the highest average activity were denoted as a coarse part of the heart. We then created a binary mask in which these pixels were set to be one; the remaining pixels were set to zero, as they were considered to be part of the background and would therefore be removed. In the next two steps, this heart mask was applied to individual temporal frames of the FDG-PET image in order to obtain the coarse heart localization, which included the entire heart as well as parts of surrounding tissues such as partial lungs and blood vessels.

Step2: Curve Clustering with PRMs—Every pixel in a dynamic PET image has a corresponding TAC, which can be represented as a sequence of discrete activities. This sequence has a length of T . The imaging time points can be considered to be a vector x_i . Let n be the number of pixels in the preliminary heart mask obtained from Step 1. These pixels could then be represented as a set of n TACs $\{y_1, y_2, \dots, y_n\}$. A p^{th} order polynomial regression relationship was assumed between each TAC y_i and the imaging time points x_i with additive Gaussian noise term ε_i , as given by (2).

$$y_i = X_i \beta + \varepsilon_i, \quad \varepsilon_i \sim \mathcal{N}(0, \sigma^2 I) \quad (2)$$

where $y_i \in \mathbb{R}^T$ is the TAC of the i^{th} pixel, β is the vector containing the p regression coefficients, and X_i is the $T \times p$ Vandermonde regression matrix evaluated at the imaging time points $x_i = [x_{i1} \ x_{i2} \ \dots \ x_{iT}]^T$, which is formulated as (3).

$$X_i = \begin{bmatrix} 1 & x_{i1} & x_{i1}^2 & \dots & x_{i1}^p \\ 1 & x_{i2} & x_{i2}^2 & \dots & x_{i2}^p \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ 1 & x_{iT} & x_{iT}^2 & \dots & x_{iT}^p \end{bmatrix} \quad (3)$$

Therefore, the conditional probability density function (PDF) of y_i can be modeled in terms of the regression equation as a normal distribution $\mathcal{N}(y_i | X_i \beta, \sigma^2 I)$. This PDF represents a probabilistic curve model that is associated with a curve cluster. If the set of TACs was separated into K distinct clusters in accordance to the similarity of the physiological kinetics, the density of a TAC y_i could be described as a finite mixture model with a number of component PDFs whose densities were noted by the parameters $\{\beta_k, \sigma_k^2\}$ as $\mathcal{N}(y_i | X_i \beta_k, \sigma_k^2 I)$. In other words, these cluster-dependent PDFs were incorporated into a conditional mixture density given by (4). This resulted in the definition of the PRMs for curve clustering.

$$p(y_i | X_i, \Theta) = \sum_{k=1}^K \alpha_k \mathcal{N}(y_i | X_i \beta_k, \sigma_k^2 I) \quad (4)$$

where α_k denotes the probability that the i^{th} TAC y_i is assigned to cluster k . The mixture model parameters $\Theta = (\beta, \sigma^2, \alpha)$ were estimated by maximizing the log-likelihood function of n observed TACs, as given by (5).

$$L(Y | \Theta) = \sum_{i=1}^n \log p(y_i | X_i, \Theta) \quad (5)$$

An Expectation-Maximization (EM) algorithm was adopted to search for the maximum likelihood estimates of the parameters for the probabilistic curve model. These parameters depended upon unobserved latent variables.

The latent variable z_i was used to indicate the cluster membership for the TAC y_i . In the E-step, the posterior probability $p(z_i = k | y_i, X_i)$, which indicates the probability of the TAC y_i belonging to the cluster k , was calculated by (6).

$$w_{ik} = p(z_i = k | y_i, X_i) = \frac{\alpha_k \mathcal{N}(X_i \beta_k, \sigma_k^2 I)}{\sum_{k=1}^K \alpha_k \mathcal{N}(X_i \beta_k, \sigma_k^2 I)} \quad (6)$$

In the M-step, the expected value of the complete log-likelihood was maximized with respect to the parameter $\{\beta_k, \sigma_k^2, \alpha_k\}$. This allowed us to calculate updated solutions for mixture model parameters using (7).

$$\begin{cases} \hat{\beta}_k = \left[\sum_{i=1}^n w_{ik} X_i' X_i \right]^{-1} \sum_{i=1}^n w_{ik} X_i' y_i \\ \hat{\sigma}_k^2 = \left(\sum_{i=1}^n w_{ik} \|y_i - X_i \beta_k\|^2 \right) / \left(\sum_{i=1}^n w_{ik} \right) \\ \hat{\alpha}_k = \frac{1}{n} \sum_{i=1}^n w_{ik} \end{cases} \quad (7)$$

After the convergence of the EM algorithm, the polynomial regression function for each cluster could be expressed in terms of the regression coefficients β . The clusters of the TACs were thus defined by obtaining the cluster membership from the model parameters. The n pixels within the heart mask could be divided into K classes, based on their corresponding TACs. During the next step, the refined ROI delineations would be obtained by utilizing these K classes.

Step3: Classes Merging—In dynamic FDG-PET studies, it is assumed that equilibrium will be reached between plasma and free tissue concentration after a sufficiently long time (approximately 30 minutes) post tracer administration. This process is recorded in the late frames. Moreover, the late frames of dynamic FDG-PET images usually have a higher SNR than early frames as they have a relatively longer scanning duration. As a result, it is possible that the images of different tissues could be clearer and more stable in the late frames compared to the early frames. The activity information in the late frames was suitable for refining the ROI delineation.

The heart, which is the indispensable organ for the blood circulation, is primarily composed of cardiac muscle tissue. This tissue forms the ventricles and atriums. The heart can be proposed to be divided into two structures: the myocardium and the blood cavity. The heart mask defined in Step 1 may include some surrounding tissues, such as partial lung tissue and large blood vessels. After considering the properties of dynamic PET images and the characteristics of the heart's anatomy, the classes derived from Step 2 were merged into three major clusters using the activity information from the last three individual frames. This was achieved through hierarchical clustering. The complete linkage clustering algorithm was applied to iteratively merge the two classes with the smallest distance as calculated in (8).

$$d(A_m, A_n) = \|A_m - A_n\|^2 = \sum_{i=1}^3 (A_{mi} - A_{ni})^2 \quad (8)$$

where A_m and A_n represent the average time-activities of the pixels within two distinct classes m and n in the last three individual frames.

The pixels within the heart mask were separated into three distinct clusters: one is for the myocardium; another is for the blood cavity, and a final cluster for the surrounding tissue. The ROI_b was defined to be the cluster whose mean TAC had the highest peak. The ROI_{myo} was defined from the cluster that had a mean TAC with a high tail, i.e. higher myocardium activity was expected in the late frames. ROI_{myo} and ROI_b were then recorded as binary images and the ROI delineation was refined by a morphological opening operation that used a diamond-shaped structuring element with distance of 1 to remove pixel-size artifacts and noise.

The numbers of clusters for these three steps were set as the parameters of the HCM. To achieve optimal performance in ROI delineation, the parameters used in the HCM were chosen empirically according to the properties of the FDG-PET image. The heart, lungs, and muscles were considered as the major organs observed in a thoracic PET image. The muscles often have relatively lower activities in the late frames of dynamic FDG-PET images. As such, they formed a cluster along with the parts outside the scanned subject. For background removal, we therefore set the number of clusters for the K-means clustering in Step 1 to 3. The number of clusters for curve clustering was empirically set to 5 for the detection of small structures. This setting was more stable than having 4 clusters and was less computationally expensive than 6. For Step 3, we set the number of clusters for hierarchical clustering to 3 because the typical properties of FDG concentrations in the heart could lead to the myocardium, the blood cavity and the surrounding tissue being produced as three distinct clusters.

The performance of the proposed HCM for ROI delineation was evaluated by computer simulation studies. We then applied this HCM to delineate ROIs for the automatic estimation of the IDIF in a set of mouse FDG-PET studies.

B. Estimation of Imaged-derived Input Function

The dynamic behavior of FDG in myocardium has been characterized by the three-compartment four-parameter model whose corresponding differential equations are given by (9) [23].

$$\begin{cases} dC_e/dt = k_1 C_p - (k_2 + k_3) C_e + k_4 C_m \\ dC_m/dt = k_3 C_e - k_4 C_m \end{cases} \quad (9)$$

where C_e and C_m are the TACs of FDG and FDG-6-PO₄ in the myocardium, respectively. C_p denotes the FDG PTAC, which is the input function in kinetic modeling. k_1 , k_2 , k_3 , and k_4 are the rate constants of the compartment model.

The TTAC of myocardium measured from ROI_{myo} is a combination of the total activities (the sum of C_e and C_m) in the myocardium and the spillover from blood cavity because PET records the total activities in the tissue. Similarly, the TAC measured from ROI_b contains the PTAC and the spillover from the myocardium. These relationships were described by (10).

$$\begin{cases} \tilde{C}_t(t) = C_t(t) + f_m^b C_p(t) \\ \tilde{C}_b(t) = f_b^m C_t(t) + C_p(t) \end{cases} \quad (10)$$

where $\tilde{C}_t(t)$ is the TTAC of myocardium measured from the ROI_{myo}, and $\tilde{C}_b(t)$ is the TAC measured from the ROI_b. The parameters f_m^b and f_b^m are the mixing coefficients that reflect the spillover effects between the blood cavity and the myocardium.

By using (10), the model in (9) can be represented by the measured TACs, as shown in (11) [10].

$$\begin{cases} dC_e/dt = -((k_2 + k_3) + k_1 f_b^m) C_e + (k_4 - k_1 f_b^m) C_m + k_1 \tilde{C}_b(t) \\ dC_m/dt = k_3 C_e - k_4 C_m \\ \tilde{C}_t(t) = C_t(t) (1 - f_b^m f_m^b) + f_m^b \tilde{C}_b(t) \end{cases} \quad (11)$$

The input function with spillover correction can then be expressed by (12).

$$C_p(t) = (\tilde{C}_b(t) - f_b^m \tilde{C}_t(t)) / (1 - f_b^m f_m^b) \quad (12)$$

By considering (11) and (12) together, $\tilde{C}_b(t)$ was used as the new input for the represented model with the dual outputs of $\tilde{C}_t(t)$ and $C_p(t)$. There were six unknown parameters in this represented model: four rate constants (k_1, k_2, k_3 , and k_4) and two spillover factors (f_m^b and f_b^m). These parameters were given initial values empirically and were then estimated by fitting the outputs of the model to the measurements of the PET and one blood sample, using the weighted nonlinear least squares method to minimize the objective function given in (13).

$$\Phi(\theta) = \sum_{i=1}^N w_i (\tilde{C}_t^*(t_i) - \tilde{C}_t(t_i))^2 + w_b (C_p(t_s) - p(t_s))^2 \quad (13)$$

where θ is the vector of parameters, which includes the rate constants for the compartment model and the mixing coefficients for the spillover effects. $\tilde{C}_t^*(t_i)$ and $\tilde{C}_t(t_i)$ are the estimated and measured mean time-activities of the myocardium at time t_i (from the i^{th} frame). w_i is the weight that is calculated as the ratio between the duration of the i^{th} frame and $C_t(t_i)$ [24]. $C_p(t_s)$ is the TAC of the spillover corrected input function at time t_s , and $p(t_s)$ is the plasma time-activity of the blood sample at time t_s . w_b is the weight for the blood sample, which is chosen to be a large value (e.g. 3) so that the measurement from this real blood sample is given more weight [11].

The dispersion and delay of the PTAC usually occurs during the circulation of the tracer from heart to either the sampling site or the target tissue. This could potentially induce an estimation error for the IDIF compared with the gold standard, which is obtained by taking the blood samples from a femoral catheter. In this case, the dispersion and delay corrections were applied to the IDIF that was already corrected for spillovers [25]. The corrected IDIF can be expressed by (14).

$$C_{IDIF}(t+\Delta) = C_p(t) \otimes (e^{-t/\tau} / \tau) \quad (14)$$

where $C_{IDIF}(t)$ is the corrected input function based on the spillover corrected IDIF, $C_p(t)$, τ is the dispersion time constant which is set as 1 sec, and Δ is the delay time usually measured in seconds [24], which is set as 2.5 sec.

We applied the automatic estimation of the IDIF on a set of mouse FDG-PET studies. The predicted IDIF was compared with the gold standard, which was the PTAC derived from invasive blood samples.

III. COMPUTER SIMULATION STUDIES

A. Computer Simulation

We simulated a number of dynamic FDG-PET studies to evaluate the performance of the proposed HCM for ROI delineation. The typical FDG TTACs for different thoracic tissues (myocardium, lungs and surrounding muscles) and blood TAC were generated using the Integrated Whole Body Kinetics Experimentation Lab of the virtual experimentation module in the Kinetic Imaging System (KIS) [26], following a routine 60-minute scanning protocol (1×2s, 10×0.4s, 3×5s, 1×30s, 2×60s, 1×120s, 3×180s, and 5×550s) and a 13.5MBq bolus

FDG injection. The parameters of the integrated whole body kinetics experimentation were set to the default values in KIS. The typical TAC of the blood cavity, derived from the blood TAC, included about 50% spillover from the myocardium. The four typical TACs plotted in Fig. 2 were assigned to the corresponding tissue regions in a single slice of the Digimouse phantom [27] for generating the homogeneous image without noise. A slice of the phantom included the heart (the myocardium and an added blood cavity), lungs, and surrounding muscles, as shown in Fig. 3.

The generated noise-free image was forward projected to obtain a dynamic sequence of sinograms. A normally distributed background related to the scanning duration was added to the sinograms in order to simulate the random and scattering events. Poisson noise amplified by a noise level (0.5, 1, and 2) was then added to simulate realistic sinograms. The noisy sinograms were then used to reconstruct the dynamic PET image frame by frame, using filtered back-projection with a Hann filter and a cutoff frequency based on the Nyquist frequency. A realistic PET measurement was then generated by applying the Gaussian blur function to the reconstructed PET image. This allowed us to simulate the influences from partial volume effects and physiological movements.

The proposed HCM was used to delineate the ROI_{myo} and ROI_b in the simulated PET images. Generally, the entire measured TACs, each about 60 minutes in duration, were used in Step 2 of the HCM. This process was noted as HCM with entire TACs (HCM_60). Since the peak of the input function is mainly contained in the TACs of the first minute, partial TACs were considered to be sufficient for the detection of the blood cavity. When the partial TACs of the first minute were used in Step 2, the process was recorded as HCM with the first minute partial TACs (HCM_1). A standard clustering was introduced here in addition to these two HCM. After applying the same process of background removal of the HCM, the measured TACs in the heart mask could be considered as T -dimensional feature vectors. K-means clustering was then used to classify these feature vectors. This method was noted as K-means clustering method (KM) and compared with HCM in automatic ROI delineation.

All three approaches (HCM_1, HCM_60 and KM) were evaluated for the automatic ROI delineation under different noise levels.

B. Evaluation of the ROI Delineation

The ground truth for each ROI was defined according to the Digimouse phantom. Due to the limited spatial resolution, partial volume effects and the spillover of the PET image, the ROI was usually placed at the core part of the target tissue or organ, where the TACs were representative of the target's theoretical kinetics with the least impacts from local surroundings and physiological movements. The Digimouse phantom was processed with the same Gaussian blur function that was applied to the reconstructed noisy PET image. The ground truth was then obtained by excluding the blurred margin of the target in the phantom while retaining the stable parts.

The automatically delineated ROI_{myo} and ROI_b were compared with the ground truth, and evaluated using their sensitivity, specificity, and Dice Similarity Coefficient (DSC), which were calculated as given in (15) [28]. DSC measures the overlap between the areas of the estimated ROI and the ground truth. The automatically delineated ROI and the ground truth were respectively noted as ROI and GT in the calculations.

$$\begin{cases} \text{Sens} = TP / (TP + FN) \\ \text{Spec} = TN / (TN + FP) \\ \text{DSC} = 2 \times TP / (TP + FN + TP + FP) \end{cases} \quad (15)$$

where $TP = ROI \cap GT$ is the number of true positives, i.e. the count of pixels common to both ROI and GT ; $TN = \overline{ROI} \cap \overline{GT}$ is the number of true negatives, i.e. the count of pixels that were not labeled by neither ROI nor GT ; $FN = \overline{ROI} \cap GT$ is the number of false negatives, i.e. the count of pixels that were not labeled by ROI but were labeled by GT ; and $FP = ROI \cap \overline{GT}$ is false positives, i.e. the count of pixels that were labeled by ROI but were not labeled by GT .

The mean TACs were also derived from the automatically delineated ROI_{myo} and ROI_b . They were compared with the mean TACs obtained from the ground truth by the root mean square (RMS) and correlation coefficient (ρ).

C. Results

Fig. 4 shows the five frames (3s, 6s, 36s, 561s, and 3306s after tracer injection) of the simulated dynamic FDG-PET image under a noise level of 1. The intensity of each pixel in the frames is noted as the magnitude of the time-activity in MBq/ml.

We simulated 100 runs for each noise level in order to compare the performance and reliability of the three proposed approaches (HCM_1, HCM_60 and KM) in ROI definition. As mentioned before, the parameters of the HCM were chosen empirically: the number of clusters for background removal was set to 3, the number of curve clusters was set to 5, and the number of clusters for hierarchical clustering was set to 3. The number of K-means clusters for background removal in KM was the same as HCM, when the number of K-means clusters in KM was set to 3.

Table 1 shows the performance of three approaches in ROI delineation. From our results, it is found that all the ROIs obtained automatically from the three approaches had high sensitivity and specificity (>0.9) when compared with the ground truth. The DSCs of the automatically delineated ROIs from HCM_1 and HCM_60 were around 10% higher than those from KM. The correlation coefficient of the mean TACs were high for HCM (nearly 1.0). The RMS values for HCM were about 2/3 lower than those for KM. The results indicated that the HCM obtained more precise ROI delineation than KM when compared with the ground truth.

Fig. 5 gives examples of ROI definitions obtained by the three approaches (HCM_1, HCM_60 and KM) under a noise level of 2. In each sub-picture of Fig. 5, the automatic ROI_{myo} is sketched by a red solid line along its contour, while its ground truth is highlighted in green. The automatic ROI_b is shown with a blue solid line, while its ground truth is represented in magenta. The result shown in (a) was obtained by HCM_1, whose DSCs were 0.939 and 0.906 for ROI_{myo} and ROI_b , respectively. The RMS values for the estimated mean TACs were about 0.211 and 0.488, while their correlation coefficients were both 0.999. The corresponding DSCs were 0.980 and 0.902 for HCM_60 as shown in (b), and 0.863 and 0.785 for KM as depicted in (c).

During the complete 60 minutes scanning time, the peak that occurred in the first minute post tracer injection reflected the typical feature of the TACs from ROI_b . The tail part that appeared at 30 minutes after tracer injection represented the typical feature of the TACs from ROI_{myo} . HCM_1 used partial TACs of the first minute for curve clustering with PRMs. Thus, the cluster of TACs from ROI_b was accurately defined by HCM_1. HCM_60 used entire TACs for curve clustering and as such the tail part led to the accurate clustering of TACs for ROI_{myo} . As a result, HCM_1 performed better in the delineation of ROI_b than HCM_60, while HCM_60 was better at delineating the ROI_{myo} .

Early frames usually have a lower SNR than late frames as they have a shorter scanning duration. This could potentially impact the results of ROI delineation. Although small artifacts and noise could be removed by morphological processing, the contours of ROIs obtained by HCM_60 were smoother than those derived by HCM_1, as depicted in Fig. 5. The blurred parts were included in the ROIs derived from KM. However, the ground truth has been defined as the core part of the target neglected the blurred margins. Hence, KM had the higher sensitivity but the lower DSC than HCM.

IV. SMALL ANIMAL STUDIES

A. Dynamic FDG-PET Studies of Mouse

Twelve dynamic FDG-PET studies of mouse were obtained from <http://dragon.nuc.ucla.edu/mqp/index.html>, which is a public domain for mouse FDG-PET image datasets originally collected at the Department of Molecular and Medical Pharmacology at UCLA [29]. The data were acquired from non-fasted C57BL/6 mice with an average weight of 28g. The mice were anesthetized with ~2% isoflurane. A 60 minute dynamic PET scan was performed for each mouse on a microPET Focus 220 scanner after a tail vein bolus injection of approximately 13 MBq FDG. For seven of these twelve studies, the dynamic PET image was reconstructed with a 29-frame imaging protocol: 1×3s, 10×0.5s, 1×2s, 1×4s, 6×5s, 1×10s, 2×30s, 2×120s, 1×180s, 2×600s, and 2×900s frames. Two of the remaining five datasets were conducted with a 26-frame imaging protocol: 1×2s, 10×0.4s, 3×5s, 1×30s, 2×60s, 1×120s, 3×180s, and 5×550s frames. Two other studies followed a 31-frame image protocol: 14×0.5s, 1×2s, 1×4s, 1×6s, 3×10s, 1×60s, 2×120s, 3×180s, and 5×550s. The final study had a 32-frame imaging protocol that was 15×0.4s, 1×2s, 1×4s, 1×6s, 2×15s, 3×30s, 1×60s, 1×120s, 3×180s, and 3×900s. After 60 minute PET imaging, a 10 minute computed tomography (CT) scan was acquired on a MicroCAT II. The CT image was aligned to PET image. The PET image was reconstructed using the filtered back-projection algorithm with CT-based attenuation correction [30]. The dead-time, scatter, and radiation-decay corrections were performed using software provided by the vendor. Around 16 blood samples were manually taken from a femoral catheter during the dynamic PET imaging.

We adopted the proposed HCM and KM to delineate the ROIs for the automatic estimation of the IDIF in the mouse FDG-PET studies. It is possible for the proposed HCM to work on 3D images as it utilizes only temporal information rather than spatial information. The HCM could be adapted to make use of spatial information if it was necessary to cluster contiguous regions. In this study, the HCM and KM were performed independently on each transverse slice in order to reduce the computational complexity. In addition, the HCM_1 was selected for this evaluation, because the simulation study demonstrated that HCM_1 produced comparably accurate ROIs to HCM_60 while having a lower computational cost.

B. Evaluation of the ROI Delineation and IDIF Estimation

ROIs were manually drawn in the myocardium and blood cavity by an experienced operator for the later evaluations. As the blood cavity has a very small size, the ROIs were defined in one transverse slice where there was a clearly observed blood cavity, e.g. left ventricle. The manually drawn ROIs were used as a standard to evaluate the ROIs obtained automatically by HCM or KM from the same transverse slice. In a similar manner to the evaluation of the computer simulation studies, the DSC of each automatic ROI and the correlation coefficient (ρ) of its mean TAC was calculated.

To evaluate the predicted IDIF, we used the PTAC derived from the invasive blood samples using (16) [31] as the gold standard.

$$C_{GS}(t) = B(t)R_{PB}(t) \quad (16)$$

where $C_{GS}(t)$ is the gold standard derived from the whole blood samples $B(t)$. $R_{PB}(t)$ is the FDG concentration ratio of plasma to whole blood, calculated by (17).

$$R_{PB}(t) = 0.38e^{-0.191t} + 1.165 \quad (17)$$

This gold standard (GS) was used to evaluate the predicted IDIF, which was corrected for spillovers, delay and dispersion. The area under the curve (AUC), a commonly used criterion in the evaluation for the estimation of input functions [24,32,33], was calculated by (18). The AUC for the predicted IDIF (AUC_{IDIF}) and the gold standard (AUC_{GS}) were calculated with an integral interval that ranged from 0 to 60 minutes.

$$AUC = \int_0^t C_{IF}(\tau) d\tau \quad (18)$$

where $C_{IF}(\tau)$ is the predicted IDIF or the gold standard input function.

After re-sampling the predicted IDIF to the sampling time points of the blood samples, we used (19) to calculate the ratio of the non-overlapping area and AUC_{GS} . This was used as a supplementary criterion for evaluating the estimation of the IDIF.

$$r = \frac{\sum_{i=1}^N |eIF_i - GS_i| * int_i}{AUC_{GS}} \quad (19)$$

where eIF_i is the re-sampled value from the predicted IDIF at the i^{th} blood sampling time point, GS_i is the i^{th} measured value of the gold standard, and int_i is the i^{th} blood sampling interval.

The influx rate constant (Ki) was estimated for three selected target tissues (brain, myocardium, and muscle). This influx rate constant, which is an appropriate quantitative index for measuring glucose metabolism, can be estimated by the Patlak graphic analysis method [34] using the predicted IDIF or the gold standard as input function. For calculating the Patlak Ki, the mean TTACs for the brain and muscles were obtained using the manually drawn ROIs. The mean TTAC of the myocardium was obtained from the automatic ROI_{myo}. These influx rate constants estimated using the predicted IDIF and the gold standard were compared in pairs for each tissue in the evaluation.

C. Results

The means and standard deviances (SD) of evaluation metrics (the DSC and correlation coefficient (ρ) of the mean TACs) were calculated in order to compare the performance of HCM_1 and KM in automatic ROI delineation. The results are listed in Table 2. The ROIs derived automatically by HCM_1 were highly congruent with the corresponding manually derived ROIs, while KM failed to delineate the ROI_{myo} and ROI_b for most cases in the mouse studies.

The ROIs derived automatically by HCM_1 had a DSC about 0.670 and 0.762 on average for ROI_b and ROI_{myo}, respectively, when compared with the manually defined ROIs across

the 12 mouse studies. The best ROI delineation was obtained using HCM_1, where the values of DSC were 0.747 and 0.871 for ROI_b and ROI_{myo}, respectively. The lowest DSC of the ROI derived automatically by HCM_1 was about 0.419. Much lower mean values with higher SD were observed in the results of the KM method. For direct comparison, the best and worst cases of ROI delineation using HCM_1 and KM are shown in Fig. 6. The correlation coefficient (ρ) was used for the calculation and comparison of the mean TACs for the ROIs derived automatically and manually. For HCM_1, the mean values of ρ were about 0.99 for ROI_{myo} and ROI_b; these were higher than the ρ for the ROIs obtained by KM.

We used the mean TACs of the ROIs obtained automatically by HCM_1 to estimate the IDIF. The predicted IDIF was processed for spillover, dispersion, and delay correction. Fig. 7 shows one example comparison of the predicted IDIF and the gold standard.

The IDIF estimation was evaluated by comparing AUC_{IDIF} with AUC_{GS}. The ratio between the non-overlapping area and AUC_{GS} was calculated for the predicted IDIF. The Patlak Ki estimated using the predicted IDIF was compared with that derived using the gold standard for each selected target tissue.

Linear regression was performed to evaluate the results of the predicted IDIF. In Fig. 8 (a), the AUC_{IDIF} is plotted against the AUC_{GS} for all 12 mouse studies. The slope of linear regression was 0.990 and R^2 was 0.974. The percentage of the absolute error between AUC_{IDIF} and AUC_{GS} was $4.4\% \pm 3.0\%$ on average. The Wilcoxon signed-rank test did not detect significant difference ($P > 0.05$) between AUC_{IDIF} and AUC_{GS}. Furthermore, the average ratio between the non-overlapping area and AUC_{GS} across the 12 mouse studies was 0.18 ± 0.04 . The plots of the linear regression of the Patlak Ki are shown in Fig. 8 (b) for the brain, (c) for the myocardium, and (d) for the muscles. The slope were all close to 1 with high R^2 (> 0.9). The percentage of the absolute error of the Patlak Ki was $5.3\% \pm 4.9\%$ when considering all the results of three target tissues as a group. According to the Wilcoxon signed-rank test, the Patlak Ki estimated by the predicted IDIF was not significantly different ($P > 0.05$) from that obtained by the gold standard for each target tissue.

V. DISCUSSION

We showed that the proposed hybrid clustering method (HCM) was effective in delineating ROIs for the myocardium and the blood cavity in the heart, using computer simulations and actual small animal studies. In general PET studies, the pixels preferred for forming the ROI are the pixels within the region that are further from the border of the target organ, tissue or structure. When obtaining the theoretical kinetics, these pixels are less likely to be affected by the spillover and physiological movements that affect the pixels near the border. Therefore it is necessary for the automatic ROI delineation to also exclude the blurred borders in the forming the ROIs. Furthermore, the limited spatial resolution and relatively higher noise of PET images usually hamper ROI definition in small structures, such as the blood cavity in the heart. HCM has demonstrated that it possesses an advantage over K-means clustering for these cases in simulated and experimental FDG-PET studies. The success of the HCM has a critical dependency on curve clustering with PRMs as it is the key step of the HCM. It contributes to the extraction of the curve-specific memberships for clustering. In this step, the curve clustering employs a polynomial fit to restrain noise fluctuations and reduce the impacts to clustering. On the other hand, curve clustering methods that represent the TACs by probabilistic curve models can be considered as curve modeling techniques that naturally allow for curves of variable lengths and missing measurements. As a particular type of modeling technique, curve clustering with PRMs has proven to be more efficient in dealing with curves with missing measurements than non-curve-based techniques, such as Gaussian mixtures models [22]. In dynamic PET imaging,

the time-activities of the pixels in early frames may be recorded as negative values because of high noise. These negative activities can be considered as missing measurements for the TACs. Hence, curve clustering with PRMs is quite appropriate for clustering the TACs of dynamic PET images.

We compared the results of the application of partial TACs from first minute to that used entire TACs. One obvious effect of using partial TACs for curve clustering over entire TACs was a reduction in the computational expense, due to a smaller dataset being considered. The results of the computer simulation studies indicated that using partial TACs allowed us to obtain ROIs that were comparable to those obtained by using entire TACs. In addition, the use of partial TACs allowed us to derive more precise delineation of ROI_b.

The HCM with partial TACs (HCM_1) and KM were applied to automatically delineate ROIs in mouse FDG-PET studies. The TTAC of the myocardium is similar to the TAC of the blood cavity because the myocardium has an ample blood supply for the delivery of oxygen and nutrients. Their similarity of the shape and magnitude could be further increased by the partial volume effects and spillovers between these two regions. These factors could be potential reasons for the failure of the KM in correctly delineating ROIs in most of the mouse studies. The proposed HCM modeled the TACs using PRMs for curve clustering. This allowed the use of all the kinetic information available in the TACs. Under the HCM, the pixels whose corresponding TACs were curve-specific members of the blood TACs cluster could be counted as part of ROI_b. The ROI_b derived automatically by HCM included several structures that contained a large amount of blood, in addition to the left ventricle where the manual ROI_b was placed. This is the reason for the low DSC observed in some cases. The ROIs of blood cavity could also have been placed in these blood-filled structures, which include regions such as the right ventricle and the nearby aorta, for extraction of the reasonable input function [35,36].

A noninvasive methodology for obtaining an input function is still an attractive topic in the quantitative analysis of PET images. IDIFs are well established and have been improved with the addition spillover correction, allowing for the derivation of more accurate PTACs [10,13,37]. There has recently been an increase in the number of small animal studies using microPET-CT, mainly due to the advent of booming biological techniques. It is still necessary to develop IDIFs for these studies. However, most proposed methods still continue to rely upon the manual placement of ROIs within the heart [24,38]. Our proposed HCM was explored for the fully automatic estimation of the input function. In this study, the well-established IDIF method with spillover, dispersion, and delay corrections was employed for the estimation of an IDIF using automatically derived ROIs. The results demonstrated that this technique was successful in deriving an IDIF that was comparable to the gold standard obtained from invasive blood samples.

The manual initialization of the IDIF parameters is a drawback of our method as it is largely dependent on the experience of the person setting the values. We could improve the accuracy of our estimation by initializing our IDIF parameters from the mean and SD of invasive blood samples taken from a small sample population with Bayesian parameter constraints [24].

Finally, our proposed HCM only needs to rely on the temporal information available in dynamic PET images for ROI delineation. Therefore the HCM can be applied to other organs by defining an ROI in large vascular structures, such as the carotid artery in the brain, as well as the hepatic arteries and the portal vein in the liver. The HCM could also be practically and reliably used for ROI delineation in PET images using other tracers.

VI. CONCLUSION

A novel hybrid clustering method (HCM) has been explored for the delineation of ROIs in dynamic PET images. These automatically derived ROIs were used for the estimation of image-derived input function for small animal FDG-PET studies. The proposed HCM consisted of three steps: K-means clustering for background removal; curve clustering with PRMs for the detection of classes; and hierarchical clustering for merging classes. We demonstrated the high accuracy of our ROI definition in both computer simulations and small animal studies. In the small animal FDG-PET studies, the ROIs derived by the HCM were used for the automatic estimation of an IDIF with one blood sample. The predicted IDIF, which was corrected for spillover, dispersion, and delay, had a high correlation with the gold standard, as calculated by the AUC and Patlak Ki. We believe our HCM has great potential for automatic ROI delineation in small animal dynamic FDG-PET studies and may extend for the general dynamic PET studies using other tracers.

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Fig. 1.
Flowchart of the proposed HCM for ROI delineation

Fig. 2.
The typical FDG TACs for the major thoracic tissues (myocardium, blood cavity, lungs and surrounding muscles) and blood TAC in 60 min.

Fig. 3.

A transverse slice of the Digimouse phantom. An extra blood cavity was added inside the heart position, while the rest part of the heart was considered to be the myocardium.

Fig. 4. Five frames of the simulated dynamic FDG-PET image under noise level of 1. The mid-scan time points are (a) 3s, (b) 6s, (c) 36s, (d) 561s, and (e) 3306s.

Fig. 5.

The delineations of the automatic ROI_{myo} and ROI_b obtained by (a) HCM_1, (b) HCM_60, and (c) KM. The contours of the automatic ROI_{myo} and ROI_b are sketched by red and blue solid line, while the ground truths are respectively highlighted in green and magenta on the Digimouse phantom.

Fig. 6.

Illustrations of the best and worst cases of ROI delineation among the 12 mice studies. The top row shows the best case (m17332) of ROI delineation using HCM_1 and the results from KM for comparison. The bottom row depicts the worst case (m19019) using HCM_1 and the results from KM for comparison. (a) and (d) show the original PET image frames at about 6s, 60s and ~3600s post injection. (b) and (e) exhibit the ROIs derived automatically from HCM_1 compared with the manual ROIs. (c) and (f) display the results from KM. In (f), KM failed to get ROI_{myo}. In (b), (c), (e) and (f), the left sub-figure shows the results of automatic ROI delineation compared with the ground truth for myocardium, while the right sub-figure shows the same for the blood cavity. The contours of the automatically derived ROIs are sketched using a red solid line, while the manually defined ROIs are colored in green.

Fig. 7.
The plot of the predicted IDIF for one mouse (m17332) compared with gold standard obtained from invasive blood sampling. The mean TAC directly from ROI_b without any correction is also depicted.

Fig. 8. Comparison of (a) the AUC of the predicted IDIF and the gold standard (GS), (b) the Patlak Ki of the brain, (c) the Patlak Ki of the myocardium, (d) the Patlak Ki of the muscles. The results of linear regression are shown in the legends.

TABLE 1

The mean values of the evaluation metrics*

Method	ROI#	Myocardium					Blood Cavity				
	noise level	DSC	Sens.	Spec.	ρ	RMS	DSC	Sens.	Spec.	ρ	RMS
HCM_1	0.5	0.936	0.913	0.999	0.999	0.264	0.894	1.000	0.998	1.000	0.615
	1	0.934	0.913	0.999	0.998	0.278	0.894	1.000	0.998	1.000	0.626
	2	0.930	0.914	0.998	0.998	0.293	0.893	1.000	0.998	1.000	0.625
HCM_60	0.5	0.950	0.948	0.999	0.999	0.192	0.889	1.000	0.998	1.000	0.649
	1	0.949	0.949	0.998	0.999	0.196	0.889	1.000	0.998	1.000	0.651
	2	0.948	0.949	0.998	1.000	0.193	0.883	1.000	0.998	1.000	0.697
KM	0.5	0.837	0.990	0.999	0.994	1.061	0.787	1.000	0.996	0.995	1.723
	1	0.848	1.000	0.989	0.995	0.735	0.791	1.000	0.996	0.999	1.640
	2	0.830	0.980	0.989	0.995	1.383	0.781	1.000	0.995	0.990	1.848

* The evaluation metrics included Dice Similarity Coefficient (DSC), Sensitivity (Sens.), Specificity (Spec.), Root Mean Square (RMS), and the Correlation Coefficient (ρ). They were used to compare the automatic ROIs obtained by HCM_1, HCM_60 and KM across three noise levels.

TABLE 2

The mean and standard deviance (sd) of the evaluation metrics for the rois derived by hcm_1 and km^{*}

Method	ROI#	Myocardium		Blood Cavity	
	Metrics	DSC	ρ	DSC	ρ
HCM_1	Mean	0.762	0.991	0.670	0.990
	SD	0.075	0.009	0.115	0.008
KM [†]	Mean	0.269	0.465	0.362	0.786
	SD	0.321	0.491	0.358	0.225

^{*} The evaluation metrics included Dice Similarity Coefficient (DSC), and Correlation Coefficient (ρ). They were used to compare the automatic ROIs obtained by HCM_1 and KM across 12 mice studies.

[†] For KM, the DSC and ρ of failed cases were recorded as 0 in the statistical computations. There were 6 cases failed to get ROI_{myo} across 12 mice studies. In addition, other 3 cases lost the ROI_b.