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Functional segmentation of dynamic PET studies: Open source implementation and validation of a leader-follower-based algorithm

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A B S T R A C T

We present a novel segmentation algorithm for dynamic PET studies that groups pixels according to the similarity of their time activity curves. *Methods:* Sixteen mice bearing a human tumor cell line xenograft (CH 157MN) were imaged with three different ⁶⁸Ga DOTA peptides (DOTANOC, DOTATATE, DOTATOC) using a small animal PET CT scanner. Regional activities (input function and tumor) were obtained after manual delineation of regions of interest over the image. The algorithm was implemented under the *jClustering* framework and used to extract the same regional activities as in the manual approach. The volume of distribution in the tumor was computed using the Logan linear method. A Kruskal Wallis test was used to investigate significant differences between the manually and automatically obtained volumes of distribution. *Results:* The algorithm successfully segmented all the studies. No significant differences were found for the same tracer across different segmentation methods. Manual delineation revealed significant differences between DOTANOC and the other two tracers (DOTANOC DOTATATE, $p=0.020$; DOTANOC DOTATOC, $p=0.033$). Similar differences were found using the leader follower algorithm. *Conclusion:* An open implementation of a novel segmentation method for dynamic PET studies is presented and validated in rodent studies. It successfully replicated the manual results obtained in small animal studies, thus making it a reliable substitute for this task and, potentially, for other dynamic segmentation procedures.

Keywords: Dynamic PET; Functional segmentation; Kinetic modeling; Clustering; Open Source

1. Introduction

Dynamic nuclear imaging analysis enables quantification of the response of biological tissues more precisely than static analysis in various types of studies, such as those based on myocardial blood flow [21,9] and glucose metabolism [16]. In small animal studies, dynamic imaging based on custom tracers also makes it possible

to assess lung inflammation [30] and specific biological receptor activity [15,39].

The quantification process for these studies requires time activity curves (TACs) to be obtained for the relevant organs or tissues, along with the activity generated in arterial blood (known as the input function [IF]). Continuous blood sampling by arterial catheterization is generally considered the gold standard for estimation of IF [2]. However, the technique is invasive, causes patient discomfort and presents additional difficulties in experiments involving small animals [27,29,34]. IF can be obtained directly from image data in human studies under specific conditions, such as the presence of large blood pools in the field of view [43,5]. In small animal studies, it is also possible to extract the image derived IF (IDIF) from the heart or abdominal aorta and use only one or two blood samples to correct for partial volume and spillover effects [42]. The correct extraction of the IF data using any of

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the previously mentioned techniques is a fundamental step for the adequate quantification of dynamic study results [14,40,44,8].

Typically, extraction of IDIF involves manual delineation of the relevant structures over the image, which is a user dependent and time consuming process [36,41]. During recent years, a number of automatic or semi automatic segmentation approaches have been proposed in the literature [11,13,17 19,22,23,3,6 8]. Several of these methods require a priori knowledge of the number of regions with homogeneous activity into which the image should be partitioned; a classic example is the k means clustering algorithm [10].

However, in most cases, this knowledge does not exist. In the case of PET, it is common to have several regions whose TACs are not homogeneous owing to contamination from a mixture of the activities of surrounding structures. Therefore, it makes sense to design a clustering approach that divides the image into as many regions with similar kinetic behavior as needed using a similarity metric to establish the threshold for creating a new division. This process has been called *functional segmentation* (O.G. [32]) to distinguish it from classic segmentation based solely on morphological or structural considerations. The methods used for the segmentation of dynamic studies differ from those commonly used to segment static PET images. For an extensive review on these methods see [12].

In this paper, we present an open implementation of a clustering algorithm based on a leader follower approach that groups voxels with similar TACs. To do so, we aim to replicate the results obtained in [37] by replacing the manual TAC extraction used in that study with the method presented here. We also include a comparison with the classic k means algorithm. Furthermore, we report the results of applying the algorithm using simulated data. Our study extends and validates a preliminary implementation (J. M. [25]).

2. Materials and methods

2.1. Leader follower algorithm: definition and implementation

Let us consider every dynamic voxel in the image $\mathbf{v}=\{v_0, \dots, v_{n-1}\}$ as a vector consisting of the sequence of different activity levels with time ($t=\{0, \dots, n-1\}$); this time vector is the TAC for the corresponding voxel. Let us also consider a cluster $\mathbf{C}$ as a set of voxels whose centroid $\mathbf{c}$ is the average activity for all the voxels in that set.

The classic leader follower formulation uses the following steps [10] to complete the clustering operation:

1. Set a metric and a threshold.
2. Initialize the first cluster with the first voxel in the image.
3. Compute the metric between each voxel and available cluster centroid. If the shortest computed metric is below the threshold, include that voxel in the corresponding cluster. If not, create a new cluster with that voxel.
4. Repeat until all the voxels have been included in a cluster.

The metric of choice is generally the Euclidean distance; however, since this distance may yield quite different values, it is more convenient for this particular algorithm to use a metric for which a known threshold can be set. For instance, it is possible to use the Pearson or Spearman correlation coefficients and the cosine product, which have values bound in $[-1, 1]$ and measure the similarity in shape between two given vectors. For this kind of metric, a voxel is included in a given cluster if the similarity measurement is above the threshold. This algorithm is not limited to searching for connected regions: a cluster can include voxels

from different spatial locations if they have similar kinetic behavior.

In the particular case of dynamic PET studies, two different tissues or organs may have similar TAC shapes but different activity levels. It is therefore interesting to build clusters with voxels that are not only similar in shape but also in amplitude. For this reason, we modified the classic leader follower algorithm by including additional steps to account for amplitude differences without disregarding shape similarity, as follows:

1. Sort all the voxels in the image by comparing the amplitude. The ones with the greatest maximum amplitude are processed first.
2. Set a similarity metric and a threshold. In this case, the cosine metric is used, which is defined as

$$\text{sim}(\mathbf{v}, \mathbf{c}) = \cos \theta = \frac{\mathbf{v} \cdot \mathbf{c}}{\|\mathbf{v}\| \cdot \|\mathbf{c}\|} \quad (1.1)$$

where $\mathbf{v}$ and $\mathbf{c}$ are the voxel TAC vector and the centroid vector for cluster $\mathbf{C}$, respectively. This similarity metric computes the cosine of the angle between two given vectors. Its values are bound in $[-1, 1]$, which allows the user to set a sensible and meaningful threshold.

3. Initialize the first cluster with the first voxel, following the sorted order.
4. Compute the similarity score between each voxel and the available clusters. If the similarity is above the given threshold, include that cluster in a set of candidates, $\mathbf{C}_c$. If no candidates are found, that voxel is used to create a new cluster.
5. Select a cluster $\mathbf{C}_i$ from $\mathbf{C}_c$ such that

$$\arg \min_i D(\mathbf{v}, \mathbf{c}_i) \cdot e^{-\text{sim}(\mathbf{v}, \mathbf{c}_i)} \quad (1.2)$$

where $D(\mathbf{v}, \mathbf{c}_i)$ is the Euclidean distance between the voxel TAC vector $\mathbf{v}$ and the centroid from cluster i . The exponential term (again using the previously described similarity function) penalizes voxels close in amplitude to the centroid but less similar in shape.

6. Include the voxel in the selected cluster $\mathbf{C}_i$.
7. Repeat until all the voxels have been assigned.

Step 1 ensures that the next voxel to analyze is always the one with the largest available amplitude. The algorithm is very dependent on the order in which the voxels are processed for cluster creation, because new clusters are created as needed when none of the existing ones have the desired similarity scores. The initial sorting step optimizes the final result by forcing different clusters to include only those voxels with the greatest amplitude available among all those with a similar shape, thus minimizing the influence of noise and spillover effects.

This algorithm was implemented under the *jClustering* framework (José María [26]) for the ImageJ platform [35] and has been released under a free software license (GNU GPL); specific implementation details can be found in (José María [26]). The source code, along with binary downloads, can be obtained at https://github.com/HGGM_LIM/jclustering/blob/master/src/main/java/jclustering/techniques/LeaderFollower.java.

2.2. Experimental protocol

Sixteen male nude NU/NU mice were used in this study. The animals were purchased from Charles River Laboratories (Spain), maintained at a constant temperature ($24 \pm 0.5^\circ\text{C}$) under a 12 h light/dark cycle and permitted free access to commercial rodent laboratory chow and water. All experimental animal procedures were conducted in conformity with European Communities

Council Directive 2010/63/EU and approved by the Ethics Committee for Animal Experimentation of our institution.

$1.5 \cdot 10^6$ CH 157MN human tumor cells was injected in both mouse flanks. Imaging was performed 7–21 days after tumor cell inoculation using a small animal PET/CT scanner (ARGUS PET/CT, SEDECAL, Madrid). Scans were obtained under anesthesia with isoflurane (3% induction and 1.5% maintenance in 100% O₂). Three different ⁶⁸Ga DOTA peptides (referred to in this paper as DOTANOC, DOTATATE and DOTATOC) were injected into the tail vein (mean activity 11.47 MBq, range 9.25–20.35 MBq). Selection of the tracer, day and mouse was randomized. A total of 24 images were acquired, since a mouse could be injected with more than one tracer on more than one day.

2.3. Image acquisition and manual segmentation

Tracer injections were followed by a 90 min dynamic PET study. Dynamic data were reformatted into 112 time frames (20 s × 10 s, 10 s × 30 s and 82 s × 60 s). Frames corresponding to the last 60 minutes of the study were summed to create a static image. Images were reconstructed using a 2D OSEM (Ordered Subset Expectation Maximization) algorithm to provide images with 1.45 mm full width half maximum (FWHM) resolution (voxel size $0.3875 \times 0.3875 \times 0.7750$ mm³). The energy window was 400–700 keV. Decay, dead time and random corrections were applied.

CT images were acquired using the PET/CT scanner described above with standard parameters for tumor imaging: 320 mA, 45 kV, 360 projections, 8 shots and 200 µm of resolution. CT images were reconstructed using a Feldkamp algorithm to obtain an isotropic voxel size of 0.125 mm [1]. These anatomical images did not require any registration with their corresponding PET scans thanks to the intrinsic alignment of the PET/CT device.

CT images were used to manually draw the regions of interest (ROI) for the tumors. The IF was obtained by manually delineating the descending aorta in the static PET image. Manual segmentations were performed by two operators with more than 5 years' experience and revised by a senior operator with more than 10 years' experience.

2.4. Automatic functional segmentation process

2.4.1. Algorithm presented

Prior to segmentation, the image volume was masked to eliminate voxels outside the mouse body using a simple thresholding method.

The proposed leader-follower algorithm was applied to the resulting masked volume using different similarity thresholds, as explained in step 2 of the algorithm workflow (selected values are 0.4, 0.5, 0.6, 0.7 and 0.8). The centroids for the selected clusters were the TACs to be used as input for the Logan analysis. A successful segmentation is defined as that which enables clear differentiation of at least the IF and the tumor tissue from the surrounding organs. Clusters were selected by visual identification.

2.4.2. *k* means

For comparison purposes, the classical *k* means algorithm was also used to segment the studies and obtain the TACs for the relevant tissues. *k* means was selected due to its status as a baseline algorithm and for its availability in a wide range of software libraries. For the comparison to be as uniform as possible, the fixed number of clusters was chosen according to the average number of clusters generated by the leader-follower method across all studies when the threshold was set at 0.6.

2.5. Quantification

In order to contrast both methods (automatic and manual), we directly compared the post-processed volumes of distribution after the kinetic analysis and not the TACs obtained, because TACs that are apparently similar may yield very different volume of distribution values. The volume of distribution was obtained for both the manual and the computer-assisted segmentations using the Logan plot [20]. The model was resolved using the traceR kinetic modeling library ([24]). *t** time was automatically chosen so that a < 10% error was obtained for the linear regression. This criterion is the same as that applied in other kinetic analysis tools, such as PMOD [4].

2.6. Statistical analysis

Paired Wilcoxon rank-sum tests were used to determine whether the segmentations provided by the algorithm yielded results that were significantly different from the manual ones within the same tracer. No multiple comparison correction was used for these tests.

The differences in the volume of distribution for the different tracers within the same segmentation method, including manual delineation, were also tested. Differences between tracers were compared using a Kruskal-Wallis test, which was followed by a pairwise Wilcoxon rank test with the Bonferroni correction for multiple comparisons. Results were considered significant for $p < 0.05$.

The statistical analysis was performed using the statistical package R v3.0.0 (R [31]).

2.7. Simulated data

The validation procedure based on simulated data can be found in [Supplementary material File 1](#).

3. Results

[Fig. 1](#) shows several static frames from the acquired data. The algorithm presented was successful in separating the desired regions when the threshold values were 0.6, 0.7 or 0.8 ([Fig. 2](#)). Segmentation was not successful in some of the studies when the lowest threshold values were used (0.4 and 0.5), and no statistical results are reported here for these values. [Fig. 3](#) shows examples of the TACs obtained using the algorithm along with the manual results.

The total computation time taken by each algorithm and the final number of clusters created depending on the threshold are shown in [Table 1](#).

The results for the statistical analysis are summarized in [Fig. 4](#) and [Table 2](#). No significant differences in volume of distribution were found within the same tracer between the manual method and the computer-assisted method. The statistical analysis revealed significant differences (Kruskal-Wallis p value = 0.0108) between DOTANOC and DOTATATE ($p = 0.020$) and DOTANOC and DOTATOC ($p = 0.033$) for the manual segmentation method; similar differences were found for all values obtained with the algorithm for which statistical results are reported (Kruskal-Wallis p values for each threshold {0.6, 0.7 and 0.8} = 0.0019, 0.0023 and 0.0013; see [Fig. 4](#) for pairwise p values).

The segmentation with *k* means ($k = 150$) yielded higher volume of distribution values than those obtained either manually or using the algorithm (DOTANOC = 0.873 ± 0.09 ; DOTATATE = 0.975 ± 0.07 ; DOTATOC = 0.987 ± 0.11). All these values were different to the manual ones ($p < 0.05$); furthermore, the difference between the

Fig. 1. Three frames from two different mice (left and right) acquired for this study.

tracers DOTANOC and DOTATOC, found manually and with the leader follower algorithm, was not statistically significant. More results from the k mean segmentation can be found in [Supplementary Material File 2](#).

4. Discussion

In this paper, we present a novel segmentation algorithm based on a leader follower approach that, instead of setting a final number of clusters, creates as many clusters as needed depending on a similarity threshold set by the user. In our tests, the algorithm successfully extracted the TACs for the relevant tissues or regions when the threshold was above a certain critical value, and the results were comparable with those obtained in [37]. Images with lower injected radioactivity, which tend to be more affected by noise, were separated into more clusters for the same threshold value. This approach ensures the basic assumption of kinetic analysis, namely, homogeneous kinetic behavior of the voxels assigned to a particular region [28,38]. The algorithm has been implemented using an open clustering framework (José María [26]) and is also available under an open source license (GNU GPL).

Our algorithm addresses one of the weaknesses of the k means algorithm, in which the user needs to run the algorithm several times before reaching an optimal solution because of the random initialization step [45]. From this perspective, our algorithm ensures the reproducibility of the segmentation results and

provides a result that does not change for each run. It has to be noted that the result might not be the optimal one, even in the manual case. Our rationale is that the comparison against manual segmentation helps to test the algorithm in a real world environment. This validation approach shows that the algorithm is a viable substitute for manual tasks. This finding is also supported by the results of applying this algorithm to a set of simulated data (see [Supplementary material File 1](#)), which shows excellent results in recovering the original TACs at least as well as manual segmentation.

We showed that the algorithm was stable with respect to the TACs obtained after applying the algorithm, we confirmed that the algorithm is stable: the variation of the threshold value does not cause significant changes in the volume of distribution for each tracer (Fig. 5), since the differences between the tracers and the sign of those differences are maintained. As this threshold value is increased, the variability of the volume of distribution decreases. We hypothesize that this is due to the reduction in the region size obtained when the similarity threshold is increased (Fig. 2(b)). Therefore, TAC heterogeneity decreases owing to partial volume effects or noise, as the area included in the cluster is concentrated on the center of the region, where voxels are less mixed with activity from surrounding organs and tissues [23]. This effect is seen clearly in Fig. 2(c), where the tumor region is surrounded by smaller clusters that are heavily affected by spillover. Those small clusters are not included in the segmented tissue because their TACs do not conform to the similarity or distance constraints

Fig. 2. (a) Clustering results for one of the mouse studies analyzed here (threshold 0.6, three different views for the same plane: axial, coronal, sagittal). The regions belonging to the input function and the tumor were selected and are the only ones shown here; the kidneys were also added to the final composition to show that the algorithm is able to segment different organs. (b) Effects of increasing the threshold value in the definition of the input function region (same region as in Fig. 2(a)). As expected, as the threshold is increased, the cluster tends to concentrate in the center of the region, while the edges, which are heavily affected by spillover, form their own clusters; those clusters can be discarded. (c) Detail of the tumor region affected by spillover from surrounding tissues. The window/level settings of the image have been adjusted to clearly visualize the tumor region; surrounding clusters are shown in white, although they do not necessarily form a single connected region.

Fig. 3. Example of TACs obtained with the automatic method and the manual method. The input function comparison figure (left) clearly shows that the automatic input function presents smaller noise levels than the manually obtained one. Tumor TACs remain very similar, with virtually no differences between them.

Table 1

Total computation time (s) and number of clusters formed for each threshold. Values are shown as mean $\pm$ standard deviation (median). Values are not rounded even though fractional clusters are not possible; this is a purely numerical result. When a high number of clusters are formed, many contain a very small amount of voxels owing to noise; given that the user interface presents the clusters by size (largest first), the relevant ones will always be among the first to be inspected by the user.

	Threshold 0.6	Threshold 0.7	Threshold 0.8
Time (s)	18.1 $\pm$ 4.8 (17.8)	26.3 $\pm$ 18.2 (22.2)	67.6 $\pm$ 92.9 (34.61)
# of clusters	149.5 $\pm$ 143.9 (102.5)	611.2 $\pm$ 820.8 (297)	2255.9 $\pm$ 2476.1 (1375.5)

imposed by the algorithm. This same effect also applies to TACs heavily affected by noise: in low activity studies, noise can distort the activity signal to the point of making the algorithm create a new cluster for the regions with the lowest activities.

While the manually segmented regions and the ones obtained using the algorithm tended to overlap (Fig. 5), they are not identical. Some of the segmented tumors show internal heterogeneity, that is, they are often divided into high uptake and low uptake

regions, probably because of necrosis. As the tumor ROIs were drawn manually over the CT image, where they appear homogeneous, the functional differences observed were not taken into account, and in some cases, necrotic tissue, which is usually present in the center of the tumor region, was included. This higher sensitivity of the algorithm may account for the slightly higher overall volume of distribution (Table 2), as the tumor TAC was extracted using only the high uptake areas. This manual

segmentation error is shared among the different tracers, and its overall effect can be disregarded if the final objective is to evaluate which tracer has the greatest uptake in the tumor tissue. However, it is important to consider when the quantification measurements need a precise result that accounts faithfully for the volume of distribution in the active tumor; in this respect, the same regions of interest could have been drawn using the summed PET image. Furthermore, it is common practice to obtain the IF from a very small region to minimize the influence of spillover. In that respect, when the segmentation process aims to extract the TAC for each tissue, region overlap is not as useful for purposes of comparison as the results of the kinetic analysis.

The comparison against k means showed that the present algorithm was more precise when separating the different regions, even though the number of clusters chosen for this comparison was reasonably high (150). Several factors may account for these changes, although we hypothesize that this might be due to the region growing approach (starting from high amplitude seed points) used by the algorithm, which is better suited for this particular application. In any case, the region growing approach proves that the leader follower modification proposed is not simply an inverse k means algorithm that substitutes a fixed number of clusters with a fixed similarity threshold.

Fig. 4. Volumes of distribution for the various segmentation procedures (manual and automatic with different thresholds). p values within the same procedure are adjusted for multiple comparisons using the Bonferroni correction.

Table 2

Volume of distribution values per tracer and segmentation method; p values are greater than the significance level (0.05) for the observed differences. V_t values are shown as mean $\pm$ standard deviation.

	Manual	Threshold 0.6	Threshold 0.7	Threshold 0.8
DOTANOC	0.45 $\pm$ 0.06	0.44 $\pm$ 0.07	0.46 $\pm$ 0.06	0.47 $\pm$ 0.05
DOTATATE	0.66 $\pm$ 0.16	0.72 $\pm$ 0.14	0.71 $\pm$ 0.12	0.71 $\pm$ 0.09
DOTATOC	0.63 $\pm$ 0.14	0.68 $\pm$ 0.15	0.69 $\pm$ 0.12	0.65 $\pm$ 0.10

Our study has a number of limitations. The algorithm does not include any partial volume or spillover correction logic, such as the geometric transfer matrix algorithm (Olivier G [33]); however, this is not the primary purpose of the algorithm, which is intended to replicate the results obtained from manual segmentation. Since our small animal studies did not use blood sampling, the volume of distribution values are potentially biased. However, the validation process has been used to show that both the manual and the computer assisted segmentation approaches yield TACs that lead to equivalent results. In addition, although for this particular application we found no differences in the threshold values used to segment the image, a successful segmentation may not have held true for other applications or for other quantification analyses.

The similarity threshold may take arbitrary values and, even if its mathematical significance is clear, the practical result is subject to a process of trial and error, as the final number of clusters depends on several factors, including image noise. In any case, as the algorithm is fast, different values can be tested until the relevant structures can be discerned visually. Table 2 shows the computation times and the number of clusters created. A high threshold value (i.e. 0.8) over segments the data, but most of the clusters created are only a few pixels in size and can be ruled out as noise. Furthermore, as the algorithm presents the results so that larger clusters (i.e., those including more voxels) are shown first, it is easy to locate the relevant organs in the user interface. Since higher values also cause the final result to stay closer to the center of the region (Fig. 2(b)), a balance needs to be achieved: larger threshold values imply more concentrated clusters, but also more fragmentation.

Finally, although we validated the algorithm using the cosine similarity metric, other similarity metrics, such as the Pearson correlation score or the Spearman rank correlation coefficient, have also been implemented and yielded similar results in preliminary tests, albeit with higher computation times.

5. Conclusion

We present an open implementation of a segmentation algorithm for dynamic PET studies based on grouping voxels with similar TACs. The algorithm can be used to successfully replicate results obtained from manual segmentations performed in small animal studies using ^{68}Ga DOTA peptides, thus making it a reliable substitute for this task and, potentially, for other dynamic segmentation procedures. This leader follower based algorithm is fast, has a publicly available source code and can be used directly from the ImageJ image analysis and processing platform.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

Fig. 5. Sagittal slice of the tumor region: automatic segmentation revealed two regions that differ in their kinetics, namely, an area of necrotic tissue (low uptake) surrounded by an area of high-uptake tissue. Left: average image obtained by adding the last image frames in order to enhance tumor tissue. Center: automatic segmentation for the necrotic tissue (red) and high-uptake tumor tissue (white); threshold = 0.6. Right: manual ROI (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.combiomed.2015.12.012>.

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