Insulin Granule Segmentation in 3-D TEM Beta Cell Tomograms

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To obtain accurate volumetric information from 3-D transmission electron microscopy tomography data of beta cells, we propose an automated segmentation algorithm for insulin granules. Due to the image acquisition procedure, granule segmentation is especially challenging. Typically, insulin granules are described as organelles containing a dense core, surrounded by a halo and an enclosing membrane Fig. 1. Analysis of insulin granules in transmission electron microscopy (TEM) beta cell images is usually done manually. Currently, it is not feasible to analyse large numbers of 3-D TEM beta cell images —as manual segmentation is very time consuming.

3-D electron microscopy tomograms of beta cells are obtained using a similar procedure as for 2-D tomograms. Firstly, the sample has to be fixed using either a chemical or high pressure freezing fixation method. The imaging procedure gives relatively poor contrast and has the greatest effect on granule halos, which is demonstrated in Fig. 1. We tackle the problem of segmenting granules using active contours. We use a region-based active contour segmenting cores and halos. We chose a region-based approach to cope with weak boundaries within the images; the active contour incorporates region variances [3]. For halo segmentation we introduce a novel boundary prior to the Bayesian region-based active contour. To prevent the contour from moving into the core we propose the following; given a level set function ϕ, our boundary prior energy functional is as follows:

$$E_{boun} = -log\left(1 - H\left(\int (C_{core} - H_\varepsilon(\phi))\, C_{core}\, dx\right)\right). \qquad (1)$$

Where C_{core} is the granule core, H is the Heaviside function and H_ε is a smoothed Heaviside function, as shown in [1]. We include our boundary term with the region-based energy term as well as smoothing and signed distance regularisers, to produce the following energy functional:

$$E(\phi) = \sum_{i=1}^{2} \frac{1}{2} \int \left(log(\sigma_i^2) + \left(\frac{(I-\mu_i)^2}{\sigma_i^2} \right) \right) M_i(\phi)\, dx$$
$$+\nu \int |\nabla H_\varepsilon(\phi)|\, dx + E_{boun} + \lambda \int \frac{1}{2} \left(|\nabla\phi| - 1\right)^2 dx. \qquad (2)$$

Where I is the image, $M_1(\phi) = H_\varepsilon(\phi)$, $M_2(\phi) = 1 - H_\varepsilon(\phi)$, ν and λ are constants and $\mu_i(\phi)$ and $\sigma_i^2(\phi)$ are the mean and variances —of the regions inside and outside the contour— respectively.

To help prevent the contour from getting stuck in local minima during its evolution, we utilise the dual active contour framework [4]. The dual energy term is an internal energy, which means it is not influenced by image information. This results in an uniform attraction between the contours. This would be ideal if the granule membrane is always located in the centre of the two contour initialisations, however this is not always the case. In some cases, granules have a membrane which is slightly visible. We introduce a gradient based term to help take advantage of this information and use it to slow the evolution of a contour around the granule membrane:

$$g = \frac{1}{\left(1 + |\nabla G_{\sigma_s} * I|^4 C_{coread\,j}\right)}, \qquad (3)$$
$$C_{coread\,j} = 1 - \frac{G_{\sigma_s} * C_{core}}{max(G_{\sigma_s} * C_{core})}.$$

Where G_{σ_s} is a Gaussian smoothing kernel with standard deviation σ_s. Given two contours initialised outside and inside the granule, with corresponding level set functions ϕ and ψ respectively, we can define our dual energy functional as:

$$E_{dual}(\phi,\psi) = E(\phi) + E(\psi) + \tau g \int \left[H_\varepsilon(\phi) + H_\varepsilon(\psi)\right]^2 dx, \qquad (4)$$

where τ is a weighting constant for the dual term.

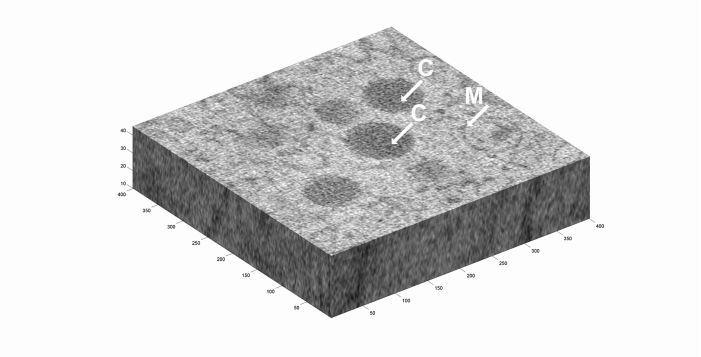

Figure 1: Section of a 3-D TEM data set of beta cell, $401 \times 401 \times 36$ pixels. The dark circles are the granule cores *C*. Note the weak membrane information *M* presented in the image.

Insulin granule segmentation begins with core segmentation, it then uses the results of the core segmentation as a starting point for halo segmentation. We first do 2-D core segmentation on each slice, to get an estimate of the core locations and shape. We utilise the region-based active contour with shape regulariser in [2] to do the core segmentations. The roundness at the vertices is not captured by doing the 2-D segmentation, also it does not produce a smoothly connected core. In order to correct for this and encourage continuity, we extend 2-D dual region-based active contour in to 3-D. Before we do active surface segmentation preprocessing is done to remove incorrect segmentations and connect disjoint cores. The result of this active surface is a smoother, more accurate, core segmentation. We then use the core as a starting point for halo segmentation. We utilise the dual active surface in (4). The core is used as the inner surface and a dilated version of the core is used at the outer surface.

Our proposed method is able to segment granule cores and membranes from 3-D TEM images of beta cells. Our novel active contour, when compared to other state-of-the-art active contours, gives a higher Dice's coefficient when compared to manual segmentations. With this automated method biologists will be able to acquire larger numbers of accurate and unbiased data of insulin granules.

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