WARW-UK

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Platform: Linux

Prerequisites: Matlab 2018a, CUDA-capable GPU (Kepler or newer)

WARW-UK: SUMMARY

Our approach is based on the curvature-enhanced random walker (CERW), which we have described previously [1]. The CERW is designed to segment cells with large protrusions (e.g., filopodia) and deep invaginations (e.g., macropinocytotic cups).

WARW-UK: PREPROCESSING

Preprocessing steps are performed on each image to generate two images, namely the preprocessed image and the seed image. To generate the preprocessed images, the following steps are applied. Images are scaled in z by a factor r_z using cubic interpolation to produce isotropic resolution, and normalized to [0, 1]. A band-pass filter is applied to all images I using two Gaussian filters G_s and G_b, with standard deviations σ_s and σ_b, to generate the image $J = G_s * I - G_b * I$. For images in **Fluo-C3DH-A549-SIM-02**, 2D contrast-limited adaptive histogram equalization (CLAHE) is applied to J with tile width w_{CLAHE} to yield the preprocessed image, while J is taken as the preprocessed image in all other time-lapse sequences analyzed. Seed images are generated from the input images using Phansalkar thresholding [2]. Prior to thresholding, the following preprocessing steps are applied to the input images. The images are scaled in z and normalized as above. A gamma correction of 0.5 is applied to images in **Fluo-C3DH-A549-SIM**. A median filter of radius r_m is applied to each image. Additionally, a mean filter of radius r_μ is applied to all images in **Fluo-C3DH-A549-SIM-02**. Band-pass and CLAHE preprocessing are then applied to all images in the manner described above. Phansalkar thresholding [2] is applied to the resulting images using a radius r_p, with image-dependent parameter values generated as described previously [1]. Background seeds for all images are obtained from the thresholded image by applying a morphological dilation (radius r_D^{BG}), fill, and erosion (radius r_E^{BG}) operators, and inverting the resulting image. In a similar manner, foreground seeds are obtained using dilation (radius r_D^{FG}), fill, and erosion (radius r_D^{FG}). These seeds are added to a second set of seeds in **Fluo-C3DH-A549-02** and **Fluo-C3DH-A549-SIM-02**, obtained by identifying maxima along the local gradient direction as described previously [1].

CALT-US: SEGMENTATION

Random walker segmentation can be modeled as the steady state of a discretisation of the non-linear diffusion system [3, 4]:

$$\frac{\partial v}{\partial t} = \nabla(W \nabla v),$$

with $\frac{\partial v}{\partial n} = 0$ at the volume boundary with normal n, subject to the constraints $v(x,t) = 1$ if x is a foreground seed voxel, and $v(x,t) = 0$ if x is a background seed voxel, and W is the diffusion weighting function, defined discretely between two voxels x and y, as

$$W(x,y) = \exp[-\beta \|I(x) - I(y)\|^2 - \alpha(\|x - y\|^2 - 1)]$$

where $\|\cdot\|$ is the Euclidean norm, $I(x)$ and $I(y)$ are the preprocessed image intensities at x and y respectively, and β and α are fixed parameters. The discretised form of the diffusion system for the point x is

$$\frac{v(x + \Delta t) - v(x)}{\Delta t} = \sum_{y \in N(x)} W(x,y)[v(y,t) - v(x,t)]$$

where $N(x)$ is the 18-connected neighborhood of x, and time step $\Delta t < \max(W)/\,18$ to satisfy the Courant-Friedrichs-Lewy (CFL) condition for numerical stability [5]. The equilibrium values of v are computed using first forward Euler method, with the segmented foreground (in the absence of curvature enhancement) given by voxels with v > 0.5 [4].

The curvature-enhanced random walker is defined by the system

$$\frac{\partial v}{\partial t} = \nabla(W \nabla v) + \kappa \widetilde{H}(v) v(1 - v),$$

subject to the same constraints as above, where κ is a fixed parameter, W is defined as above, and

$$\widetilde{H}(v) = \begin{cases} H(v) & \text{if } H(v)(v - 0.5) > 0, \\ 0 & \text{otherwise} \end{cases}$$

where $H(v) = -\nabla \cdot (\nabla v / \|\nabla v\|)$ is the mean curvature. The time step is taken to be the same as in the random walker, since this value is sufficiently low to provide numerical stability for the combined system. As with the standard random walker, equilibrium values of v are calculated and the segmented foreground is given by voxels with v > 0.5.

The gradients for computing $H(v)$ are approximated in each direction using an extension of the 3D Sobel filter, which is given in the *x*-direction as a smoothing in *y* and *z* by applying the 1D filter (1,4,6,4,1)/16 in both *y*- and *z*-directions, followed by a differencing filter in *x* with radius 3, given by (−1,0,0,0,0,0,1)/6. The formulation for *y*- and *z*-directions are defined similarly. The number of operations involved in this computation are much higher than for the finite differences in the random walker, and therefore increase computation time. This time is reduced in the implementation used here by only computing the curvature every 10 time steps, which had a negligible impact on the resulting segmentation.

The curvature-enhanced random walker segmentation is implemented on a GPU as follows. Initially, the equilibrium values v_1 of the standard random walker system are computed, with initial conditions $v(x,0) = 0.5$ for all x not in either of the seed sets. The equilibrium values v_2 of the curvature-enhanced diffusion system are subsequently computed with initial conditions $v(x,0) = v_1$. Equilibrium in both cases is defined as the point where the mean relative error falls below a threshold ϵ, or if a maximum number of time steps $T_{\max}$ is reached. The segmented object is given by the set of voxels with $v_2 > 0.5$.

WARW-UK: POST-PROCESSING

A large value of the curvature weighting coefficient, κ, was chosen to enable the identification of long and branching filopodia in **Fluo-C3DH-A549-02** and **Fluo-C3DH-A549-SIM-02**. Because the curvature term is independent of the original image, this leads to the filopodia being detected with a wider cross-section than desired. Additionally, a higher curvature weighting can lead to background leaking into the cell through areas of low membrane intensity in some images. Accordingly, the following postprocessing steps are required.

The first step is to apply morphological dilation (radius r_D^{PP}), fill, and erosion (radius r_E^{PP}) to fill any holes inside the cell. The second step is to move the surface of each binary mask to the nearest edge of the original input image. A Gaussian filter with standard deviation σ_e is applied to the input image followed by a Sobel filter to obtain the gradient magnitude. A surface mesh is generated from the binary segmentation mask using Matlab's isosurface function. For each vertex *v* of the mesh, lines of length $r_{\max}$ are drawn along the surface normal from *v* extending to the interior and exterior of the surface. To prevent intersection with another part of the surface, each line is truncated so that the distance to the surface only increases along the line away from *v*. The voxel *u* along both of these lines with the highest gradient magnitude is selected as the new location of the surface vertex. If u is interior to the surface, then all voxels intersecting the line between *v* and *u* are assigned the background value, while the opposite assignment is made if *u* is exterior. The volume generated in this manner is smoothed using a median filter of radius 1.

For all datasets analyzed, a morphological fill operator is applied to all images and each image is reduced to the largest 6-connected component. Finally, all images are rescaled back to the original image dimensions by downsampling in *z* using the scale factor $1/r_z$.

REFERENCES

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