

MU-CZ (4)

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Platform: Windows

Prerequisites: NVIDIA CUDA v11+, Python 3.10+

MU-CZ (4): SUMMARY

Our method uses a novel pipeline based on simulated gravitational force fields for both detection and tracking [1]. By treating every pixel as a point-like body with a mass equal to its brightness, we locate high luminosity objects using Newton's law of gravitation. The resulting detections serve as seed points for a local contrast-based region growing algorithm, which performs the segmentation step. Finally, by examining the gravitational fields of the cells at multiple time points, it is possible to reconstruct tracklets along the temporal axis.

MU-CZ (4): PREPROCESSING

The pipeline utilizes two different preprocessing strategies, henceforth referred to as methods (A) and (B). In both cases, the images are first brightened to increase contrast, then normalized to the $[0, 255]$ range before denoising using a custom implementation of the Kuwahara filter [2]. Method (A) employs a log transform for the enhancement step, whereas method (B) uses contrast limited adaptive histogram equalization (CLAHE) instead.

MU-CZ (4): SEGMENTATION

In every image, cells are detected by locating the significant local minima of the gravitational potential field, i.e., those points within the image that exhibit attraction stronger than a given threshold. In practice, this requires a two-stage approach using gravitational force fields instead of potential, which can be constructed via convolutions with two inverse squared distance kernels to calculate the x and y components of the force vectors at every pixel. The convolution is performed with images enhanced using method (A). To allow for an analytical approach, a continuous force field is approximated from the discrete version with bilinear interpolation between four neighbouring pixels. It is possible to recover all the critical points of the continuous fields by identifying locations where both component of the gravitational force are equal to zero. These points can be further classified as minima, maxima or saddle points using the Jacobian Matrix.

After finding the minima of the potential field, we determine their “strength” by calculating the basins of attraction corresponding to the minima. One way of doing this is recovering the stable manifolds of the system’s saddle points by performing gradient ascent from these critical points in the direction of the Jacobian’s eigenvectors corresponding to negative eigenvalues. In practice, an adaptive integration scheme developed by Fehlberg [3] is used for this operation to ensure accurate results. The resulting curves, which are equivalent to watershed lines, delimit basins of attraction, and can therefore be rasterized to separate pixels belonging to different basins.

Once the local minima and the basins of attraction are extracted, minima with sufficiently large basins are used as seed points for segmentation. First, the images are preprocessed using method (B), then the seed points are placed in a heap data structure and examined in order of decreasing brightness. When a pixel is extracted from the heap, all of its neighbours are either labelled with the same label as the pixel itself and added to the heap or marked as a wall and discarded. Which operation is performed depends on the local contrast between the previously marked pixels and the newly examined one. This algorithm continues until the heap is empty, at which point cell masks are used to relabel the minima and attraction basins for the tracking step.

MU-CZ (4): POST-PROCESSING

Since the detection method is prone to returning false positive detections, it is necessary to filter the cell masks after the initial segmentation step. Fortunately, real cells tend to have well defined shapes and smoother boundaries than false detections, which allows a simple classification strategy. Cells above or below certain size thresholds, or of low contrast can be automatically discarded, and since **Fluo-N2DH-GOWT1** mostly contains round cells, the same can be done with low circularity masks.

To address non-split events, every cell mask is used to perform a distance transform, the h -minima of which serve as the seeds of a watershed transform. This method can easily separate touching cells while leaving even oblong cells intact, though it can sometimes result in erroneous splits.

MU-CZ (4): TRACKING

To associate cells along the temporal axis, the attraction basins extracted during the detection step are relabelled with the finalized cell masks. This results in merged basins that correspond to entire cells as opposed to individual minima, and which can be used to determine what cell a given pixel attracts to in near-constant time. As cells tend to remain within their own future or past gravitational fields between

frames, this allows for an accurate one-to-one matching. In cases where multiple candidates are present, the ones with the highest percentage of overlapping pixels are chosen.

The tracklet construction takes place in three passes: first in reverse-, then forward time, followed by a final linking pass in reverse time. The first two stages attempt to locate missing detections by segmenting image regions near disappearing cells using a probabilistic Chan-Vese model [4]. In cases where a cell only disappears for one frame but is present in both neighbouring frames, a simpler strategy is used. In this latter instance, an interpolation algorithm [5] is applied to the two existing masks to create an approximation of the missing, intermediate state.

Once the tracklets have been constructed, a final postprocessing step is performed to filter out false detections. In order to leverage the temporal information gained from the temporal association stage, a hysteresis thresholding strategy (not unlike the one used in a Canny edge detector) is used. The sizes and the local contrasts of the individual tracklets are examined, and if any cells are below a lower threshold or all cells are below an upper threshold for either property, the entire tracklet is discarded.

REFERENCES

1. Eftimiu NJ, Kozubek M. Gravitational cell detection and tracking in fluorescence microscopy data. arXiv: 2312.03509, 2023.
2. Kyprianidis J, Semmo A, Kang H, Döllner J. Anisotropic Kuwahara filtering with polynomial weighting functions. In *Proceedings of EG UK Theory and Practice of Computer Graphics*, 25-30 (2010).
3. Fehlberg E, Marshall GC. Low-order classical Runge-Kutta formulas with stepsize control and their application to some heat transfer problems. NASA Technical Report (1969).
4. Cremers D, Fluck O, Rousson M, Aharon S. A probabilistic level set formulation for interactive organ segmentation. In *SPIE Medical Imaging*, 65120V (2007).
5. Iwanowski M. Morphological normalized binary object metamorphosis. *Computer Vision and Graphics* **32**, 626-632 (2006).