J REGULARIZATION IMPROVES IMBALANCED MULTICLASS SEGMENTATION

Fidel A. Guerrero Peña1,5∗, Pedro D. Marrero Fernandez1, Paul T. Tarr2,3, Tsang Ing Ren1,
Elliot M. Meyerowitz2,3, Alexandre Cunha4,5∗

1Centro de Informática, Universidade Federal de Pernambuco, Brazil
2Howard Hughes Medical Institute, USA
3Division of Biology and Biological Engineering, 4Center for Data-Driven Discovery,
5Center for Advanced Methods in Biological Image Analysis, California Institute of Technology, USA

ABSTRACT

We propose a new loss formulation to further advance the multi-
class segmentation of cluttered cells under weakly supervised con-
ditions. We improve the separation of touching and immediate cells,
obtaining sharp segmentation boundaries with high adequacy, when
we add Youden’s J statistic regularization term to the cross entropy
loss. This regularization intrinsically supports class imbalance thus
eliminating the necessity of explicitly using weights to balance train-
ing. Simulations demonstrate this capability and show how the reg-
ularization leads to better results by helping advancing the optimization
when cross entropy stalls. We build upon our previous work on
multiclass segmentation by adding yet another training class repre-
senting gaps between adjacent cells. This addition helps the clas-
sifier identify narrow gaps as background and no longer as touch-
ing regions. We present results of our methods for 2D and 3D im-
gages, from bright field to confocal stacks containing different types of
cells, and we show that they accurately segment individual cells
after training with a limited number of annotated images, some of
which are poorly annotated.

Index Terms— Loss modeling, deep learning, instance segmen-
tation, multiclass segmentation, cell segmentation, data imbalance

1. INTRODUCTION

The long-term goal of our work has been the automatic segmenta-
tion of cells found in different modalities of microscope images so
that it can ultimately help in the quantification of biological studies
(see e.g. [1, 2, 3, 4]). The task remains a challenge particularly when
cells are densely packed in clusters exhibiting a range of signals and
when training with a small number of weak annotations (see Fig.1).
Separation of cluttered cells is especially difficult when shared edges
have low contrast and are similar to cell interiors. Weak annotations,
when incomplete and inaccurate, can harm the learning process as
the optimizer might be confused when deciding if annotated and
non-annotated regions with same patterns must be segmented or not.
Our proposed solutions aim to resolve these problems with advances
in loss formulation, class imbalance handling, multiclass classification,
and data augmentation.

We thank financial support from the Brazilian funding agencies FACEPE,
CAPES and CNPq (FAG, PF, TIR), from the Beckman Institute at Caltech to
the Center for Advanced Methods in Biological Image Analysis (AC,FAG),
from the Howard Hughes Medical Institute (PTT,EMM), and thank the IBM
Matching Grants Program for computer donation (AC). ∗Corresponding au-
thors: fagp@cin.ufpe.br, cunha@caltech.edu.

Fig. 1: A poorly annotated test image is segmented with our J regularized
loss, L_JC, using three, J3, and four, J4, semantic classes. We obtain a correct
segmentation when training with an added fourth class representing gaps and
cavities – predicted white patches shown on J4 Probability. The result is supe-
rrior to the annotation, which, unintentionally, missed tracing the right contour
on the round central cell above. The training of our models has been consistently
robust despite the presence of weakly annotated images, which are present in
the total training data. Background (red), cell (green), touching (blue), and gap
(white) are the four classes predicted by our models.

We propose a new deep learning multiclass segmentation
method which classifies pixels into four distinct classes – back-
ground, cell, touching, and gap – by minimizing a loss function that
penalizes both cross entropy and Youden’s J statistic. Pixels and
voxels classified as touching and gap become either cell or back-
ground in a post-processing step, producing a final segmentation
containing a single mask for each individual cell in the image.

We build upon our recent work [1, 2] to further improve multi-
class cell segmentation. The introduction of a fourth class, named
gap, and of a new loss lead to better segmentations where small re-
gions separating nearby cells are now correctly classified as back-
ground regions. Slim cell protrusions are also correctly classified
thanks to the balancing offered by our proposed loss.

Previous work. Recent modeling of new loss functions for seg-
mentation [1, 5, 6, 7] incorporates a differentiable surrogate over a
known performance measurement. Unfortunately these are not
sufficient to cope with high data imbalance typical when segment-
ing biomedical images. In [8] the authors review regional losses
and propose a contour based loss as an alternative to combat inter-
face. The work of Brosch et al. [9] bears similarities to ours as they
model their loss as a linear combination of sensitivity and specificity
measures. But they use mean square errors instead and recommend
heavily weighting specificity, 95%, in detriment to sensitivity, 5%,
which we believe goes against the importance of equally balancing
both measures. Sudre et al. [10] proposed using the generalized dice
overlap introduced in [11] as a loss function to avert imbalance in
segmentation. Imbalance is achieved by explicitly weighting classes
as in [12] but now inversely proportional to the square number of
pixels. From our experience, this works to isolate cell clusters but it
is not enough to isolate cells in a cluster.

Pixel weights have been adopted as a strategy to balance data
[12, 9] including shape aware weights [2]. While advantageous they
are not sufficient to fully separate packed cells or resolve fine details. Equibatches [7] is yet another balancing strategy for segmentation. It forces training examples from all classes to be present during every training iteration. Multiclass deep learning training for cell segmentation is adopted in [2] for 2D images and in [13] for 3D confocal stacks.

2. METHOD

Notation. The goal of panoptic segmentation is to assign to each pixel or voxel \( p \in \Omega \) of a single channel image \( x: \Omega \subset \mathbb{R}^d \rightarrow \mathbb{R}^+ \) a semantic label, and an instance label when \( p \) belongs to a countable category [14]. For learning a segmentation we are given a training set \( S = \{(x_i, g_i)\} \) where for every image \( x_i \) we know its ground truth segmentation \( g_i \). In general, we have \( g: \Omega \rightarrow \{0, \ldots, m\} \), a mapping where \( g(p) = 0 \) for \( p \) in the background and \( 0 < g(p) < m \) is a unique label for each object in the image. Our task is cast as a semantic segmentation problem by modifying the approach proposed in [1] to transform the instance annotation \( g \) into a semantic ground truth \( h \), generalizing to high dimensions by using a \((2k+1)^d\) neighborhood \( y_k(p), k \geq 1\). Let \( y: \Omega \rightarrow \mathbb{R}^{2k+1} \) be the one hot representation for the \( C \)-classes in the semantic mapping \( h: \Omega \rightarrow \{0, \ldots, C\} \), and \( n_i = \sum_{p \in \Omega} y(p) \) the number of elements of class \( l \). We call \( y \) the bottom hat transform over \( g \) using structuring element \( e \), a hyper-sphere whose size is data dependent. The output of our trained network is a probability map \( z \) such that \( z(p) \approx y(p) \), \( \forall p \). A post-processing similar to the one proposed in [1] is then applied to build a panoptic segmentation \( \hat{g} \) from \( z \).

Gap class. We have previously shown that using three semantic classes, namely image background, cell interior, and touching region, increases the network discriminative power when segmenting cluttered cells [2, 1]. However, misclassified background regions persisted in some cases, see Fig.1. We speculate this is due to losing background information when merging nearby cells in the U-Net contracting path, information which is not fully recovered in the upsampling path. By introducing a new training class representing the gap between nearby cells, the network can now classify the regions separating nearby cells as background. We name this new class, not surprisingly, gap – white pixels shown in separating nearby cells as background. We name this new class, not a semantic label, and an instance label when \( p \) belongs to a countable category [14]. For learning a segmentation we are given a training set \( S = \{(x_i, g_i)\} \) where for every image \( x_i \) we know its ground truth segmentation \( g_i \). In general, we have \( g: \Omega \rightarrow \{0, \ldots, m\} \), a mapping where \( g(p) = 0 \) for \( p \) in the background and \( 0 < g(p) < m \) is a unique label for each object in the image. Our task is cast as a semantic segmentation problem by modifying the approach proposed in [1] to transform the instance annotation \( g \) into a semantic ground truth \( h \), generalizing to high dimensions by using a \((2k+1)^d\) neighborhood \( y_k(p), k \geq 1\). Let \( y: \Omega \rightarrow \mathbb{R}^{2k+1} \) be the one hot representation for the \( C \)-classes in the semantic mapping \( h: \Omega \rightarrow \{0, \ldots, C\} \), and \( n_i = \sum_{p \in \Omega} y(p) \) the number of elements of class \( l \). We call \( y \) the bottom hat transform over \( g \) using structuring element \( e \), a hyper-sphere whose size is data dependent. The output of our trained network is a probability map \( z \) such that \( z(p) \approx y(p) \), \( \forall p \). A post-processing similar to the one proposed in [1] is then applied to build a panoptic segmentation \( \hat{g} \) from \( z \).

Fig. 2: Performance of classifiers C1 and C3 [17] measured by Youden’s J, Matthews Correlation Coefficient (MCC), Jaccard, F1 (Dice), Tversky, and Accuracy scores for different imbalance ratios \( \pi \). Youden and MCC are the only ones almost completely invariant to all imbalance ratios.

\[ J = \frac{TPR + TNR - 1}{\pi} \]

Fig. 3: Correlation between values of MCC and J for different imbalance ratios \( \pi \). The linear correlation was measured using Pearson Correlation Coefficient, giving values of 0.92 (\( \pi = 0.01 \)), 0.99 (\( \pi = 0.25 \)), 1.00 (\( \pi = 0.5 \)).

\[ J = \frac{TPR + TNR - 1}{\pi} \]
where $\lambda_{i,k}$ is a pairwise class weight. $\alpha_i$ and $\beta_i,k$ are, respectively, soft definitions for TPR and TNR, where $i$ is considered to be the positive class and $k$ the negative one. These definitions are similar to the ones used for Soft Dice [5] and Tversky [6] loss functions,

$$
\alpha_i = \sum_{p \in \Omega} z_i(p) \cdot \varphi_i(p), \quad \beta_i,k = \sum_{p \in \Omega} (1 - z_i(p)) \cdot \varphi_k(p)
$$

where $\varphi_i(p) = y_i(p)/n_i$. Inserting these values into Eq.2 we obtain

$$
\mathcal{L}_f(y, z) = -\frac{1}{C} \sum_{i=0}^{C-1} \sum_{k=0}^{C-1} \lambda_{i,k} \log \left( \frac{1}{2} + \sum_{p \in \Omega} z_i(p) \cdot \Delta_i,k(p) \right)
$$

with $\Delta_i,k = (\varphi_i - \varphi_k)/2$. We use Eq.3 as a regularizer to cross entropy loss, $\mathcal{L}_{CE}(y, z) = -\frac{1}{|\Omega|} \sum_{i=0}^{C} \sum_{p \in \Omega} y_i(p) \cdot \log z_i(p)$, obtaining our training $JC$ loss $\mathcal{L}_{JC}(y, z) = \mathcal{L}_{CE}(y, z) + \mathcal{L}_f(y, z)$. Of all solutions with equal values of cross entropy, we favor the one that has the highest separation between classes. Note that, contrary to [12, 2], explicit class weights per pixel are not used.

**Simulation.** We simulate the optimization towards the ground truth to show how the $J$ regularization helps cross entropy, CE, reach the optimum result. The target segmentation consists of two touching square cells separated by a one pixel wide notch covering half of a cell side, see Fig.4. Initially, when the solution is far away ($\text{iter} = 1$), CE drives the optimization (large gradients) until it shrinks both cells, at which point ($\text{iter} = 46$) its gradient no longer contributes to advance the segmentation. Around that point, $J$ takes over and its gradient is now driving the optimization and it will do so until the optimum is reached. We slowly increase pixel probabilities to its optimal value until we reach ground truth so to mimic real updates. Plots in Fig.4 show how the combination of cross entropy and Youden’s $J$ statistic work in tandem to achieve the desired result. None would solve the segmentation if considered separately as the vanishing of their gradients would stall the optimization.

**Loss visualization.** We use the approach proposed by Li et al. [20] to help us visualize how our $\mathcal{L}_{JC}$ loss compares to others –

![Image](image.png)

*Fig. 4:* We simulate segmentation towards ground truth by shrinking an initial incorrect segmentation until it shrinks the two target cells (green squares above). This happens while we slowly increase the probabilities of all pixels towards their correct class. During this stage, $\mathcal{L}_{CE}$ drives optimization. Around the shrunk segs point, $L_J$ dominates the gradient descent as $\nabla \mathcal{L}_{CE} \approx 0$ is no longer sufficient to drive the optimization. The ground truth is achieved (one pixel wide notch and touching are identified) thanks to $\nabla \mathcal{L}_J$ which does not vanish until the segmentation is correct. Cross entropy and $J$ statistic work in tandem. They are not sufficient if used separately.

$\mathcal{L}_{BW}$, weighted cross entropy with class balance, and $\mathcal{L}_{W3}$, triplex weight map [1] – around a known optimal point in the optimization space. As shown in Fig.5, our loss has a cone-like shape whose gradients favor a fast descent to the optimum, contrary to the other losses $\mathcal{L}_{BW}$ and $\mathcal{L}_{W3}$ which have near zero gradients all over potentially preventing the optimization to reach the optimum – gradient descent methods are extremely slow to converge in these cases. Although this analysis is based on a visualization that employs dimensionality reduction, our evidences from other experiments suggest this behavior spans the entire optimization space.

**Gap assignment.** We obtain a semantic segmentation from the output probability map $z$ using the Maximum A Posteriori (MAP) decision rule, $\hat{h}(p) = \arg \max_l z_l(p)$. A gap pixel $p$, $\hat{h}(p) = 3$, can be directly classified as a true background pixel or, in case of dubious probabilities, $z_0(p) \approx z_1(p) \approx z_2(p)$, we assign the second most likely class to it. This is equivalent of applying MAP on the first three classes of the output map, $\hat{h}(p) = \arg \max_{l \in \{0,1,2\}} z_l(p)$. An instance segmentation is achieved then by a sequence of labeling operations on each region in the semantic segmentation map [1].

### 3. RESULTS

To facilitate comparing our loss $\mathcal{L}_{JC}$ to losses weighted cross entropy with class balance (BWM), weighted cross entropy with triplex weight map ($W^3$) [1], and cross entropy with dice regularization (DSC) [21] we use all with the same U-Net [12], with initial weights following a normal distribution [22], and all equally initialized by fixing all random seeds. For 3D volumes we used 3D convolutions but maintained the same architecture topology as in 2D [5]. A Watershed post-processing (WT) is also applied to those results showing weak touching separation (see [1] for details). The influence of the gap class over training was also analyzed by comparing $J3$ and $J4$ over a DIC Hela dataset [23], a 3D meristem confocal stack (see Fig.7), and T-Cells from [1]. Zero shot segmentation of Hela cells [24] was obtained by using a model trained over the T-Cells data. We used the optimizer Adam [25] with initial learning rate of $10^{-4}$. Data augmentation included random rotation, mirroring, gamma correction, touching contrast modulation [1], and warping. Precision (P05) and F1 score (RQ) were used for cell detection rates. Segmentation Quality (SQ) and Panoptic Quality were, respectively, used for measuring contour adequacy and instance segmentation quality [14].

**Instance segmentation performance:** Table 1 shows a performance comparison of networks trained with different loss functions. Watershed (WT) post-processing effectively increased the performance of BWM, DSC and $W^3$ when compared with Maximum a Posteriori (MAP). However, the WT method depends on carefully choosing two parameters. Networks trained with the proposed $\mathcal{L}_{JC}$ loss are able to improve instance detection rates using only the
Fig. 6: Segmentation results for Hela cells (A), Hela nuclei (B), T-Cells (C), Arabidopsis thaliana meristematic cells (a YZ-slice of the 3D segmented stack is shown) (D), Drosophila cells (E), and Arabidopsis thaliana sepal cells (z projection) (F) images using networks trained with \( J_3 \) and \( J_4 \) loss functions. Probability maps are shown as RGB images with Background (red), Cell (green), and Touching (blue) classes. For \( J_4 \), the proximity prediction is shown in white. Asterisks (*) indicate zero-shot instance segmentations with networks trained exclusively over T-Cells (C). Colors are to show cell separation. Original images were enhanced to help visualization. Whites arrows and circles are used to indicate some differences between \( J_3 \) and \( J_4 \).

Loss function Post P05 RQ SQ PQ
\( BWM \) MAP 0.6756 0.5580 0.8674 0.4858
\( DSC \) MAP 0.9028 0.7674 0.9011 0.6923
\( W^3 \) MAP 0.7384 0.6305 0.8721 0.5513
\( BWM \) WT 0.8193 0.8405 0.8831 0.7437
\( DSC \) WT 0.8726 0.8269 0.8925 0.7390
\( W^3 \) WT 0.9028 0.8775 0.8995 0.7896
\( J_3 \) (Ours) MAP 0.9127 0.9069 0.8733 0.7921
\( J_4 \) (Ours) MAP 0.9334 0.9353 0.8689 0.8132

Table 1: Performance comparison of networks trained over Weighted Cross Entropy with class Balance (BWM), Cross Entropy with Dice regularization (DSC) [21], Weighted Cross Entropy with Triplex weight map (\( W^3 \)) [1], and \( L_{JC} \) over three, \( J_3 \), and four, \( J_4 \), classes.

Fig. 7: \( J_4 \) 3D segmentation of Arabidopsis thaliana meristems. Original and enhanced versions (left column) of a portion of a shoot apical meristem image stack and their respective segmentations (two views on the middle and right columns). Due to space limitation we show only results for this portion which has been previously carefully segmented using the watershed with markers technique, which we consider as an approximate ground truth. Enhancing the signal quality improves segmentation, as shown for those undersegmented regions of the noisy stack manually marked with black circles. Our trained network can process large, 1024x1024x508, meristem stacks in under 9 minutes using 2 Nvidia K80 GPU cards (31 minutes using a single card). Visualizations were prepared using ImageJ 3D Viewer plugin [26].

4. CONCLUSIONS

We proposed \( JC \) loss, a Youden’s \( J \) statistic regularization to the bare cross entropy loss. We build upon our previous work and introduced a new pixel/voxel class we call gap which improves classification and contour adequacy. The approach improved 2D and 3D instance segmentation of highly cluttered cells even after training with weak annotations. Landscape analysis and performance evaluation with different loss functions suggest our new loss is superior to segment cluttered cells. In future work we plan to optimize the proposed pairwise loss to be linear in the number of classes and extensively compare our methods using benchmarks.
5. REFERENCES

[1] Fidel A Guerrero-Peña, Pedro D Marrero Fernandez, Tsang Ing Ren, and Alexandre Cunha, “A weakly supervised method for instance segmentation of biological cells,” in Medical Image Learning with Less Labels and Imperfect Data, MICCAI Workshop, pp. 216–224. Springer, 2019.

[2] Fidel A Guerrero-Peña, Pedro D Marrero Fernandez, Tsang Ing Ren, Mary Yui, Ellen Rothenberg, and Alexandre Cunha, “Multiclass Weighted Loss for Instance Segmentation of Cluttered Cells,” in 2018 IEEE ICIP. IEEE, 2018, pp. 2451–2455.

[3] Alexandre Cunha, Paul T Tarr, Adrienne HK Roeder, Alphan Altinok, Eric Mjolsness, and Elliot M Meyerowitz, “Computational analysis of live cell images of the Arabidopsis thaliana plant,” in Methods in Cell Biology, vol. 110, pp. 285–323. Elsevier, 2012.

[4] Alexandre Cunha, Adrienne HK Roeder, and Elliot M Meyerowitz, “Segmenting the sepal and shoot apical meristem of Arabidopsis thaliana,” in IEEE EMBS International Conference, 2010, pp. 5338–5342.

[5] Fausto Milletari, Nassir Navab, and Seyed-Ahmad Ahmadi, “V-Net: Fully Convolutional Neural Networks for Volumetric Medical Image Segmentation,” in 2016 Fourth International Conference on 3D Vision (3DV). IEEE, 2016, pp. 565–571.

[6] Seyed Sadegh Mohseni Salehi, Deniz Erdogmus, and Ali Ghilipour, “Tversky Loss Function for Image Segmentation Using 3D Fully Convolutional Deep Networks,” in International Workshop on Machine Learning in Medical Imaging. Springer, 2017, pp. 379–387.

[7] Maxim Berman, Amal Rannen Triki, and Matthew B Blaschko, “The Lovász-Softmax Loss: A Tractable Surrogate for the Optimization of the Intersection-Over-Union Measure in Neural Networks,” in Proceedings of IEEE CVPR, 2018, pp. 4413–4421.

[8] Hoel Kervadec, Jihene Bouchiba, Christian Desrosiers, Eric Granger, Jose Dolz, and Ismail Ben Ayed, “Boundary Loss for Highly Unbalanced Segmentation,” in Proceedings of The 2nd International Conference on Medical Imaging with Deep Learning, 2019, vol. 102, pp. 285–296.

[9] Tom Brosch, Youngjin Yoo, Lisa YW Tang, David KB Li, Anthony Traboulsee, and Roger Tam, “Deep convolutional encoder networks for multiple sclerosis lesion segmentation,” in MICCAI 2015. Springer, 2015, pp. 3–11.

[10] Carole H Sudre, Wenqi Li, Tom Vercauteren, Sebastien Ourselin, and M Jorge Cardoso, “Generalised dice overlap as a deep learning loss function for highly unbalanced segmentations,” in Deep Learning in Medical Image Analysis and Multimodal Learning for Clinical Decision Support, pp. 240–248. Springer, 2017.

[11] William R Crum, Oscar Camara, and Derek LG Hill, “Generalized overlap measures for evaluation and validation in medical image analysis,” IEEE Transactions on Medical Imaging, vol. 25, no. 11, pp. 1451–1461, 2006.

[12] Olaf Ronneberger, Philipp Fischer, and Thomas Brox, “U-Net: Convolutional Networks for Biomedical Image Segmentation,” in 2015 MICCAI. Springer, 2015, pp. 234–241.

[13] Dennis Eschweiler, Thiago V Spina, Rohan C Choudhury, Elliot Meyerowitz, Alexandre Cunha, and Johannes Stegmaier, “CNN-based preprocessing to optimize watershed-based cell segmentation in 3D confocal microscopy images,” in 2019 IEEE ISBI. IEEE, 2019, pp. 223–227.

[14] Alexander Kirillov, Kaiming He, Ross Girshick, Carsten Rother, and Piotr Dollár, “Panoptic Segmentation,” in Proceedings of IEEE CVPR, 2019, pp. 9404–9413.

[15] William J Youden, “Index for Rating Diagnostic Tests,” Cancer, vol. 3, no. 1, pp. 32–35, 1950.

[16] Guogen Shan, “Improved Confidence Intervals for the Youden Index,” PloS One, vol. 10, no. 7, pp. e0127272, 2015.

[17] Sabri Boughorbel, Fethi Jarray, and Mohammed El-Anbari, “Optimal Classifier for Imbalanced Data Using Matthews Correlation Coefficient Metric,” PloS One, vol. 12, no. 6, pp. e0177678, 2017.

[18] Ian Goodfellow, Yoshua Bengio, and Aaron Courville, “Deep Learning,” MIT Press, 2016.

[19] Brian W Matthews, “Comparison of the Predicted and Observed Secondary Structure of T4 Phage Lysozyme,” Biochimica et Biophysica Acta (BBA)-Protein Structure, vol. 405, no. 2, pp. 442–451, 1975.

[20] Hao Li, Zheng Xu, Gavin Taylor, Christoph Studer, and Tom Goldstein, “Visualizing the Loss Landscape of Neural Nets,” in Advances in Neural Information Processing Systems, 2018, pp. 6389–6399.

[21] Fabian Isensee, Jens Petersen, Andre Klein, David Zimmerer, Paul F Jaeger, Simon Kohl, Jakob Wasserthal, Gregor Koehler, Tobias Norajitra, Sebastian Wirkert, et al., “nnU-Net: Self-adapting Framework for U-Net-Based Medical Image Segmentation,” in Bildverarbeitung für die Medizin, pp. 22–22. Springer, 2019.

[22] Xavier Glorot and Yoshua Bengio, “Understanding the Difficulty of Training Deep Feedforward Neural Networks,” in Proceedings of the International Conference on Artificial Intelligence and Statistics (AISTATS), 2010, pp. 249–256.

[23] “ISBI Cell Tracking Challenge: http://celltrackingchallenge.net/2d-datasets/,” 2019, Accessed on 10.07.2019.

[24] Vebjorn Ljosa, Katherine L Sokolnicki, and Anne E Carpen- ter, “Annotated High-throughput Microscopy Image Sets for Validation,” Nature Methods, vol. 9, no. 7, pp. 637–637, 2012.

[25] Diederik P Kingma and Jimmy Ba, “Adam: A Method for Stochastic Optimization,” arXiv:1412.6980, 2014.

[26] Benjamin Schmid, Johannes Schindelin, Albert Cardona, Mark Longair, and Martin Heisenberg, “A high-level 3D visualization API for Java and ImageJ,” BMC Bioinformatics, vol. 11, no. 1, pp. 274, 2010.