Multicut brings automated neurite segmentation closer to human performance

To the Editor: The connectomics community is acquiring volumetric electron microscopy (EM) images of the brain at an unprecedented rate with the aim of mapping out and understanding in detail the physical correlates of information processing in animals. Reliable automatic segmentation is urgently needed for upcoming whole-brain data sets (>100 terabytes (TB) per volume). Manual analysis, despite impressive progress in collaborative annotation[1], will not scale to this massive task. We present an algorithm and software package to segment such data sets with low error rates. The software is made available open source in the **Supplementary Software** and at online repositories, and we also provide precompiled binaries (see **Supplementary Note 1**).

At the ISBI 2012 conference, a challenge for segmenting anisotropic 3D EM images was launched. In this 'blind' challenge, which remains open to new submissions, participants can submit tentative segmentations of the test data set. The organizers then measure the accuracy of the submitted segmentation in terms of Rand error. The latter is a statistic summarizing—for each and every pair of points—how often these points are correctly assigned to the same segment, or to different segments, as dictated by ground truth. The organizers publish the Rand error of a submission without giving away the ground truth segmentation itself, thus ensuring fair comparison and minimal bias[2]. Our algorithm produces the best known result on the ISBI 2012 blind challenge, halving the error of the 2012 winner.

Our pipeline comprises three major steps (**Supplementary Note 2**). First, we apply a cascaded random forest (which needs less training data) or a convolutional neural network (which gives even better accuracy) to predict membrane probabilities. In the neural network, we found skip layers, elastic data augmentation during both training and prediction, and inception-like modules to be critical for performance (**Supplementary Note 3**).
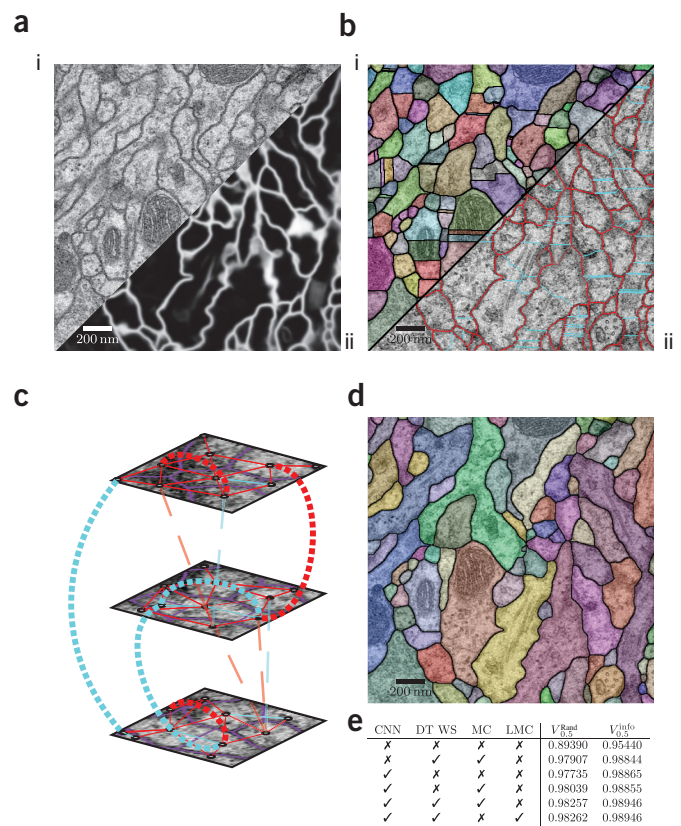
Second, we aggregate pixels into 'superpixels' to coarse grain the problem and to extract higher order region information in a data-dependent fashion. Superpixels should be few (and thus large) to reduce the problem size for the final processing stage; but superpixel boundaries must also form a strict superset of true neurite boundaries. Distance transform watershed superpixels (**Supplementary Note 4**) offered the best trade-off in our experiments, yielding large superpixels that are robust against minor gaps in the boundary probability maps.

Finally, we merge superpixels to tentative neurites while respecting consistency constraints across distances that are larger than a neural network's field of view. Specifically, we solve the (Lifted[3]) Multicut[4] problem, which introduces attractive or repulsive potentials between (nonadjacent) superpixels, and we find the graph partitioning that optimally balances these cues. We always reason in 3D, even for anisotropic data (**Fig. 1** and **Supplementary Notes 5** and **6**). This NP-hard partitioning problem is solved approximately using the fastest known method[5].

Each of these choices is the result of extensive experimentation, and the lesion study summarized in **Figure 1** and in **Supplementary Notes 7** and **8** shows how performance degrades when deviating from these choices.

The same pipeline works well on the anisotropic murine neocortex 'SNEMI3D' data and the isotropic *Drosophila* medulla

CNN	DT WS	MC	LMC	$V_{0.5}^{\mathrm{Rand}}$	$V_{0.5}^{\mathrm{Info}}$
✗	✗	✗	✗	0.89390	0.95440
✗	✓	✓	✗	0.97907	0.98844
✓	✗	✗	✗	0.97735	0.98865
✓	✗	✓	✗	0.98039	0.98855
✓	✓	✓	✗	0.98257	0.98946
✓	✓	✗	✓	0.98262	0.98946

Figure 1 | The automated neurite segmentation pipeline and the influence of its components on performance. (**a**, i) Example of ISBI 2012 data. Membrane probability estimates (**a**, ii) are used to find superpixels (**b**, i). Pairs of these regions are associated with attractive (cyan) or repulsive (red) potentials that are informed by local appearance (**b**, ii). A region adjacency graph is constructed in 3D, even for strongly anisotropic data (**c**). We consider next neighbor interactions (straight lines and straight dashed lines) and longer range interactions (curved lines) for the Lifted Multicut. Solving the (Lifted) Multicut graph partitioning problem yields tentative neurites (**d**). The table (**e**) shows the performance reached on the ISBI 2012 challenge using; from top to bottom: a cascaded Random Forest; the same with distance transform watershed superpixels (DT WS) and multicut (MC); our neural network; the same with standard watershed superpixels and MC; the same with DT WS and MC; and finally the proposed pipeline. Accuracies are measured by scores[2] derived from the Rand index (RI) and the variation of information (VI), and higher scores are better.

'Neuroproof' data (**Supplementary Note 7**). This work substantially narrows the accuracy gap between humans and computers for neurite segmentation (**Supplementary Note 9**). We expect this gap to close within the decade, at least for high-quality data, allowing neuroscientists to make the most of the impressive data sets that are currently being acquired.

Data availability statement. Any Supplementary Information and Source Data files are available in the online version of the paper or provided there as references.

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AUTHOR CONTRIBUTIONS
T.B. (lifted multicut, OpenGM), C.P. (multicut), N.R. (neural network), T.P. (superpixels), U.K. (vigra), S.B. and A.K. (ilastik) all wrote the software and ran the experiments. C.P., N.R., S.B. and U.K. bundled the software. D.B., A.C., G.W.K., S.M.P. and L.K.S. contributed to the modeling and proposed features. F.A.H. conceived the project in cooperation with D.B., A.C., G.W.K., S.M.P., L.K.S., U.K. and A.K.; and F.A.H. drafted the paper to which all authors contributed.

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eC-CLEM: flexible multidimensional registration software for correlative microscopies

To the Editor: Correlative light and electron microscopy (CLEM) is becoming increasingly popular within the life sciences. The diversity of light microscopy (LM) and electron microscopy (EM) modalities has led researchers to develop a multitude of CLEM workflows tailored to different scientific investigations[1,2]. Finding the corresponding area between LM and EM images can be facilitated with specific sample holders, finder grids, laser marks or pattern recognition[1]. However, for all these workflows, the accurate association of a fluorescent object with its corresponding ultrastructure from data sets differing in scales by several orders of magnitude remains a universal bottleneck. Although several software solutions to the problem of achieving accurate association have been proposed

(**Supplementary Note 1**), there is currently no available open-access software to achieve high-accuracy localization independent of specific registration fiducials that also offer nonrigid registration both in two and three dimensions (2D and 3D) and semisupervised registration.

We present easy cell-correlative light to electron microscopy (eC-CLEM), a free open-source software implemented as a plugin in the Icy platform[3]. As CLEM applies to a wide variety of data sets, eC-CLEM allows several types of registration, corresponding to different correlative workflows (**Fig. 1 a–c** and **Supplementary Note 2**) in 2D and 3D (or a mix of both dimensions). For example, eC-CLEM can be used to register 3D LM with 2D EM (to reposition a slice in a stack; **Fig. 1a**), or to extract a slice with the correct transformation from an LM stack using artificial (e.g., quantum dots) or natural fiducials (e.g., nuclei, melanosomes, sample defects) (**Fig. 1b**). The software works well with time-lapse, multichannel or multidimensional images; and it offers preliminary image preprocessing for rapid data extraction (**Supplementary Notes 2** and **3**).

Registration can be done rigidly (only scale, rotation and translation are applied), or nonrigidly (nonlinear transformations based on spline interpolation, after an initial rigid transformation; **Fig. 1d**) for 2D or 3D data (**Supplementary Note 4**). All computations are done directly in physical units (nanometers), and evaluation of the registration error is displayed live (**Supplementary Note 5**). With eC-CLEM, we address several challenges of LM and EM micrograph registration in 2D and 3D.

First, we estimate the target registration error (TRE) between a target structure in EM and its corresponding position in LM, in any position of the image (**Supplementary Note 5** and **Supplementary Data 1**). We present the TRE in the form of a heatmap showing the predicted TRE (in nanometers) in every point of the image. This error map can be generated at any moment during the registration process in order to guide the user to add fiducials where needed—for example, next to an area that contains a structure of interest but lacks accuracy in registration.

Our eC-CLEM software also evaluates the need to apply nonrigid registration (warping) to obtain accurate registration. Whilst vitrification techniques allow preservation of biological samples in a near-native state in cryo-electron microscopy (cryo-EM) with limited physical deformations, structural changes and sample deformations can occur at several steps when performing correlative microscopy on material that is not near to native (chemical fixation, embedding, sectioning)[4–6]. Selecting the parameters of the transformation (rigid or nonrigid) may be challenging. Allowing nonrigid transformation can warp the image locally. The warping interpolation can therefore induce warping in neighboring regions where it is not required and lead to false matching. It can also increase the TRE (**Supplementary Note 6** and **Supplementary Data 1**). eC-CLEM analyzes the fiducial localization discrepancy after rigid registration and the predicted registration error, which is a prediction of the error if no deformation occurs. If the discrepancy range is above the predicted registration error for some fiducials, warping registration is suggested to correct for local deformations.

Automated registration is a challenging technical problem, and eC-CLEM offers the 'AutoFinder' option as a major step forward in this area. After markers in LM and EM have been predetected, and without knowing the exact location of the cell of interest, the AutoFinder will screen the image to find the matching position. This option allows finding a cell of interest from LM in EM, or