Research article

Tectonic infarct analysis: A computational tool for automated whole-brain infarct analysis from TTC-stained tissue

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ABSTRACT

Background: Infarct volume measured from 2,3,5-triphenyltetrazolium chloride (TTC)-stained brain slices is critical to in vivo stroke models. In this study, we developed an interactive, tunable, software that automatically computes whole-brain infarct metrics from serial TTC-stained brain sections.

Methods: Three rat ischemic stroke cohorts were used in this study (Total n = 91 rats; Cohort 1 n = 21, Cohort 2 n = 40, Cohort 3 n = 30). For each, brains were serially-sliced, stained with TTC and scanned on both anterior and posterior sides. Ground truth annotation and infarct morphometric analysis (e.g., brain-V brain, infarct-V infarct, and non-infarct-V non-infarct volumes) were completed by domain experts. We used Cohort 1 for brain and infarct segmentation model development (n = 3 training cases with 36 slices [18 anterior and posterior faces], n = 18 testing cases with 218 slices [109 anterior and posterior faces]), as well as infarct morphometrics automation. The infarct quantification pipeline and pre-trained model were packaged as a standalone software and applied to Cohort 2, an internal validation dataset. Finally, software and model trainability were tested as a use-case with Cohort 3, a dataset from a separate institute.

Results: Both high segmentation and statistically significant quantification performance (correlation between manual and software) were observed across all datasets. Segmentation performance: Cohort 1 brain accuracy = 0.95/f1-score = 0.90, infarct accuracy = 0.96/f1-score = 0.89; Cohort 2 brain accuracy = 0.97/f1-score = 0.90, infarct accuracy = 0.97/f1-score = 0.80; Cohort 3 brain accuracy = 0.96/f1-score = 0.92, infarct accuracy = 0.95/f1-score = 0.82. Infarct quantification (cohort average): V brain (ρ = 0.87, p < 0.001), V infarct (0.92, p < 0.001), V non-infarct (0.80, p < 0.001), %infarct (0.87, p = 0.001), and infarct:non-infarct ratio (ρ = 0.92, p < 0.001).

Conclusion: Tectonic Infarct Analysis software offers a robust and adaptable approach for rapid TTC-based stroke assessment.

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1. Introduction

Stroke is the second leading cause of death worldwide, and acute ischemic stroke (AIS) accounts for 87% of all cases [1]. In the United States, ~795,000 people have an AIS every year, equating to one stroke every 40 s [1]. Despite advancements in treatment and interventional strategies, such as recombinant tissue plasminogen activator (tPA) [2] or mechanical thrombectomy techniques [3], AIS outcomes remain suboptimal, with high rates of long-term disability and recurrence [4–6]. Towards improving outcomes and secondary prevention, researchers continue to experimentally study the pathobiology and mechanism of AIS [7].

In vivo models of AIS have been extensively used to study stroke biology, develop biomarkers, and test novel therapeutics [8]. The most popular animal model is the middle cerebral artery occlusion (MCAO) suture model in small animals, namely mice and rats [8]. An almost universal cornerstone of any stroke model is the assessment of stroke burden and interventional success by measurement of infarct volume on 2,3,5-Triphenyltetrazolium Chloride (TTC)-stained brain slices [9,10]. TTC-staining, which highlights viable brain tissue red and infarcted tissue pale/unstained, is often performed on serial, coronal brain slices. The infarct regions are then assessed and summed across all slices for calculation of whole-brain stroke morphometrics (e.g., infarct volume). Unfortunately, this is often done manually, which is subjective and prone to inter-observer variability [10,11]. On the other hand, available image analysis platforms for infarct estimation are limited. For example, they can only analyze one slice at a time or do not generalize well to data with low-contrast staining [12,13]. These drawbacks ultimately limit the statistical power of in vivo stroke studies that rely on TTC-staining, as whole-brain infarct calculation in large, powerful datasets can be highly subjective and time-intensive.

Computational image analysis and feature engineering pipelines have demonstrated tremendous potential for expediting biomarker analysis in research and clinical practice [14–17,42–48]. In this study we developed and tested a computational pipeline for whole-brain infarct estimation using a dataset of TTC-stained whole brain slices from rats undergoing the MCAO stroke model. We packaged the pipeline into a user-friendly software called Tectonic Infarct Analysis [18], then applied it to two independent datasets of TTC-stained brain slices, one from our institution prepared at a different timepoint to assess batch effect, and one from an outside institution to assess interinstitutional variability. Tectonic’s intuitive functionalities (i.e., real-time calibration, segmentation, and model training) can reduce analytical time and enable larger in vivo stroke studies. Furthermore, interactive segmentation and training options may resolve challenges in infarct segmentation for low quality staining.

2. Methods

2.1. MCAO models

All procedures were approved by the Institutional Animal Care and Use Committee of both the State University of New York at Buffalo as well as the University of Massachusetts Chan Medical School. In all, our entire dataset featured 3 rat stroke cohorts. Two of these cohorts, Cohort 1 and Cohort 2, were generated at our institution and based on the permanent suture MCAO (sMCAO) model. In brief, male Wistar rats (Envigo, Indianapolis, IN) were anesthetized with 100% oxygen and isoflurane using a precision vaporizer and a nose-cone. The surgical approach was modeled after the original publication by Longa et al. [19]. A silicone-tipped intraluminal filament (Doccol, Sharon, MA, USA) was passed through the ligated external carotid artery into the internal carotid artery and advanced to the middle cerebral artery (distance 2.5 cm). The carotid artery was unclamped, and the rat remained on isoflurane while the filament remained in place for 1 h. The filament was then removed, the external carotid artery was tied-off with a silk suture, analgesia was provided via Nocita (Aratana Therapeutics Inc., Leawood, KS) infiltration, and the incision was closed. Rats recovered with supplemental oxygen as needed, after which they were returned to the animal facility where they were singly housed overnight. The next day, rats were euthanized via decapitation under isoflurane anesthesia. The brain was removed and chilled in saline on ice. Serial, coronal slices (2 mm) were collected using a rat brain matrix (Ted Pella, Redding, CA, USA).

An external cohort, Cohort 3, was based on an embolic MCAO (eMCAO) model [20]. Here, Male Wistar rats were anesthetized with isoflurane (5% for induction, 2% for surgery and 1.2% for maintenance). A PE-50 polyethylene tube was inserted in both the femoral artery and vein. The artery was catheterized for continuous monitoring of mean arterial blood pressure and obtaining blood samples to measure pH, PaCO$_2$, PaO$_2$ and blood glucose at baseline and 120 min post embolic stroke. The vein was catheterized for intravenous infusion. Body temperature was monitored continuously with a rectal probe and maintained at 37.0 ± 0.5 °C with a thermostatically controlled heating lamp. Then, embolic stroke was induced in all animals using a previously-described method [20]. Animals showing greater than 65% reduction in the regional cerebral blood flow post middle cerebral artery occlusion as measured by the laser Doppler flowmetry probe at the right primary somatosensory cortex (S1) were enrolled in the study and randomized to the treatment group [21]. The next day, the rats were euthanized via decapitation under isoflurane anesthesia. The brain was removed and kept chilled in saline on ice. A rat brain matrix (Ted Pella, Inc., Redding, CA, USA) was used to perform 1.5 mm coronal slices with carbon steel blades (8 slices per brain).

2.2. Brain TTC staining and imaging for each cohort

We analyzed 91 serially-sliced, TTC-stained brains from 91 MCAO stroke rats across 3 different independent cohorts. Cohort 1 consisted of $n = 21$ male Wistar Rats (average weight = $351 ± 39$ g) that underwent transient MCAO [19]. Data from this cohort included scanned images of serially sectioned (2 mm), TTC-stained brains had 21 paired, 2 mm, anterior and posterior brain slices from the 21 rats (5-6 slices per rat, 127 total brain slices [254 anterior and posterior faces]). A total of 13/21 (62%) of the rats were positive...
for stroke, as indicated by TTC staining. Cohort 2 data consisted of 40 paired anterior and posterior brain scans from the 40 rats (6 slices per rat, 240 total brain slices [480 anterior and posterior faces]). A total of 26/40 (65%) of the rats were positive for stroke, as indicated by TTC staining. Staining and imaging for these 2 cohorts followed the same protocol, as described in detail in the Supplemental Methods.

Data from Cohort 3 was generated at different timepoints, with variable TTC-staining and imaging protocols, and thus comprised a diverse dataset to assess robustness and reproducibility against batch-effect and interinstitutional variability. Cohort 3 consisted of $n = 30$ male Wistar rats (average weight $= 307 \pm 27$ g) that underwent permanent, embolic MCAO, of which $n = 15$ rats received tPA and $n = 15$ did not. All 30 rats were positive for stroke, as indicated by TTC staining. Data included single-face (anterior) brain scan images of serially sectioned (1.5 mm), TTC-stained brains from the 30 rats (8 slices per rat, 240 total brain slices [480 anterior and posterior faces]). Staining and imaging were completed as described in detail in the Supplemental Methods.

2.3. Image segmentation pipeline

A series of digital image processing techniques were applied to segment brain slices from scanner image data [22]. For each rat, a scanner image (either anterior or posterior) featured $\sim 6$ brain slices, and image analysis involved segmentation and quantification of every slice in the scanner image. In this study, all rat brains and their respective slices were analyzed; none were omitted. First, the original red-green-blue (RGB) image was converted to the hue-saturation-value (HSV) color space. The saturation channel was then extracted, and a global mean-based threshold was computed and applied to segment tissue from the background. Overlapping slices were then separated through morphological splitting.

Infarct segmentation was achieved through a modified semantic segmentation (see the workflow in Fig. 1) approach using RGB pixel values and a pre-determined filter bank ($n = 4$) applied to the G channel of the original image. Here, the green channel is a component of the RGB image that provides high contrast between red (brain slice) and non-red (infarct) image regions. Further application of image filters to the green channel only increases the contrast between such regions. Given that image analysis was completed in MATLAB, the following kernel sizes are defined by the parameter “Neighborhood” (N). Applied filters quantified image gradient, local maximum (neighborhood $= 3$), and local range (neighborhood $= 3$). This filter bank was selected based on testing a large variety of image analysis and filtering methods and identifying the four that provided the highest contrast between the region of interest (infarct) and background (brain and other regions). A filter bank of size 4 was selected to keep the total number of features per pixel to less than 10. Overall, 7 total features were extracted per pixel. The predictive power of these 7 features was evaluated both qualitatively and qualitatively prior to model development and has been summarized in Supplemental Figure S1. For details on feature ranking and data visualization, please see the Supplemental Methods.

7 classical machine learning (ML) algorithms were compared based on pixel-wise classification performance of infarct ($-1$) from background ($0$). Algorithms included Logistic Regression (LR), Support Vector Machine (SVM), K-nearest neighbor (KNN), and four ensemble methods (Bagged Trees, Boosted Trees, random under sampling (RUS) Boosted Trees, and Subspace KNN). Hyperparameter tuning was completed (Table 1). Classical methods were given preference over modern neural networks since our objective was to design a user-friendly software for benchtop studies that have limited computational resources (CPU only) and data (animal model cohorts are typically small). Training data consisted of manual ground truth annotations of rat image data ($n = 3$ rats [randomly selected], 18 brain slices, $>100,000$ pixels) generated by domain experts. Overall, an ensemble classifier based on boosted decision trees performed best (AUC = 0.99) (Table 2, Supplemental Figure S2). Model parameters included the learning rate (0.1), the minimum leaf size (3), and class weight (prior probability in the pixel dataset). ROCs for all other evaluated classifiers are provided in Supplemental Figure S3.

2.4. Computational estimation of infarct morphometrics

For stroke studies, infarct morphometrics of interest include the volumes of the brain ($V_{\text{brain}}$), infarct ($V_{\text{infarct}}$), and non-infarct
(V_{non-infarct}) regions, as well as the percentage of brain volume featuring infarct (% infarct, Equation (1)) and the infarct:non-infarct volume ratio (Equation (2)). First, infarct, non-infarct, and brain cross-sectional area were computed for anterior and posterior faces of brain slices as the total number of pixels comprising the respective regions (pixels^2). Infarct, non-infarct, and brain cross-sectional areas were then averaged between anterior-posterior faces of each slice (pixels^2) and summed across slices to arrive at estimates of brain, infarct, and non-infarct volume (pixels^3). Volumes were then converted from pixels to millimeters (mm^3) and used to compute:

\[
\% \text{ infarct} = \left( \frac{V_{\text{infarct}}}{V_{\text{brain}}} \times 100\% \right)
\]

\[
\text{infarct : non - infarct ratio} = \left( \frac{V_{\text{infarct}}}{V_{\text{non - infarct}}} \right)
\]

2.5. Computational performance evaluation

Manual ground truth annotations and measurements were completed by three domain experts in ImageJ (NIH, Bethesda MD) using the annotation, calibration, and region property tools [23]. Annotation and measurement times using ImageJ software were recorded per rodent brain. Segmentation performance was assessed for brain and infarct regions through pixel-wise comparison of automated segmentations and manual ground truth. Reported performance metrics included pixel-wise accuracy, sensitivity, specificity, precision, recall, and F1-score. Brain, infarct, and non-infarct morphometric estimation was assessed through correlation of computational and ground truth measurements and calculation of percent difference.
2.6. Development of a user-friendly, infarct assessment software

Tectonic Infarct Analysis, an interface for computational image analysis and infarct estimation, was developed using MATLAB’s (Mathworks, Natick MA) App Designer, a platform for deployment of custom MATLAB scripts and functions as interactive software for end-users [24]. The resulting graphical-user-interface (GUI) is a standalone software that does not require MATLAB installation or programming experience. The software estimates infarct volume for each slice and the whole brain, from a single scan of all serial

Fig. 2. High computational performance was observed for data used to develop software (Cohort 1). A-E). Strong and signification correlations were observed between manual ground truth and Software computational estimates. F). Representative brain slice and infarct regions are provided to illustrate similarity between manual and automated segmentations. Brain slice segmentations are depicted in dark blue, while infarct segmentations are depicted in cyan. G-H). High segmentation performance was observed for both brain and infarct segmentation tasks. Abbreviations: CI = confidence interval, GT = ground truth, Comp = computational, ACC = accuracy, SENS = sensitivity, SPEC = specificity, PREC = precision, REC = recall, F1 = F1-score. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
slices, following the standard technique of averaging corresponding anterior and posterior slices. In the event that researchers only collected scans of a single face, the software has a built-in option for single face analysis. The method also includes calibration, is designed for estimation from any number of sections per scan and adapts well to any brain slice thickness. For calibration, end-users may load images with a standard ruler and annotate a 1 cm region, for resolution calculation, to rescale features from pixels to mms. Should end-users know the resolution of their scanned images (from scanner meta-data), they may input it to the text box provided as a part of the Software entitled “Set Scale” and bypass manual calibration. Moreover, infarct segmentation can be completed using one of the trained models described in this paper, or through the software’s training functionality, by which the user can train a custom segmentation model using a subset of their own data. Tectonic is flexible, incorporating (i) more advanced image processing techniques to split in-contact or overlapping brain slices, (ii) a manually-adjustable slider to fine-tune slice segmentations, and (iii) infarct editing functions to refine predicted infarct regions. The software exports all measurements as Microsoft excel files, and outputs all segmentation masks for subsequent image analysis applications. See the Supplemental Materials for software documentation as well as for a description of the use case (Cohort 3) including model deployment.

2.7. Quantification of software ease of use

Here we quantified ease of use as reduced time effort (percent difference) when annotators used the software to segment and quantify infarct regions, as compared to manual effort. More specifically, time for whole brain analysis (segmentation and measurement of all anterior and posterior brain slices) was recorded per rat, and mean analytical time was calculated as the average time across all brain (rats). When software was applied, time was recorded from the loading of the images through to exporting the final segmentation masks and feature file. Similarly, manual annotation and analysis time was recorded from the time images were imported into FIJI ImageJ, through to the point of time at which segmentation masks and region measurements were exported.

2.8. Statistical analysis

All statistical analysis was completed in Python and R. We used an \( \alpha = 0.05 \) to determine statistical significance. Normality of each features’ distribution was assessed by the Anderson-Darling Test Statistic [25], and the corresponding test for equal variance between groups was used (Levene [26], Bartlett [27]). Comparison of manual annotations and software segmentations (infarct and brain) was completed as described above, using standard computational performance evaluation metrics for pixel-wise classification (i.e., sensitivity). Correlation of ground truth and computational infarct morphometrics was completed using Spearman’s Rho (\( \rho \)) [28] to be consistent with prior TTC works. Comparison of mean infarct morphometrics between groups was completed using Student’s t-tests [29] or Mann-Whitney U-tests [30]. Inter-observer agreement among domain experts was assessed using Krippendorff’s Alpha (\( \alpha \)) for 15% of cases (\( n = 3 \)) [31,32].

3. Results

3.1. Model segmentation and quantification performance

Software and preliminary segmentation models were developed using data from the Cohort 1. \( N = 3 \) rat cases (18 brain slices, >100,000 pixels) were used for training, and the remaining \( n = 18 \) rat cases (109 brain slices) were used for testing. Before comparing computational performance with ground truth, we first evaluated the agreement of manual annotation among domain experts. Interrater agreement and reliability assessment showed that domain experts were in agreement and that manual annotations were reliable (Krippendorff’s \( \alpha = 0.548 \)). As shown in Fig. 2A–E, the automated infarct estimates in testing were strongly and significantly correlated with manual ground truths. Brain, infarct, and non-infarct volumes all had significant correlation coefficients over \( \rho = 0.70; \rho = 0.91 \) (95% confidence interval [CI] = 0.90–0.91, \( p < 0.001 \)), \( \rho = 0.97 \) (CI = 0.96–0.97, \( p < 0.001 \)), and \( \rho = 0.71 \) (CI = 0.70–0.72, \( p < 0.001 \)), respectively. Percent infarct and infarct:non-infarct ratio also had significant correlation coefficients over \( \rho = 0.80; \rho = 0.81 \) (CI = 0.81–0.82, \( p < 0.001 \)) and \( \rho = 0.97 \) (CI = 0.96–0.97, \( p < 0.001 \)), respectively.

Qualitative and quantitative assessment of detected brain slice and infarct regions in the testing data confirmed that computational and manual morphometric estimates were based on the same image regions. As shown in Fig. 2F, brain slice segmentations and infarct region segmentations were similar to ground truth annotations. For brain slice segmentation, some false-positive pixels occurred at the edge of overlapping brain slices and required morphological splitting. In some of the infarct segmentation, false-positive pixels corresponding to image regions where infarct and ventricle were closely apposed were noted. Quantitative assessment of brain infarct segmentation showed high performance in pixel-wise classification tasks. Brain slice segmentation had an accuracy of 0.95, sensitivity = 0.98, specificity = 0.95, precision = 0.83, recall = 0.98, and F1-score = 0.90 (Fig. 1G). Similarly, infarct segmentation had an accuracy = 0.96, sensitivity = 0.83, specificity = 0.99, precision = 0.97, recall = 0.83, and F1-score = 0.89 (Fig. 2H). Percent difference from ground truth was found to be –5.4 ± 0.9 for brain volume and 3.5 ± 0.3 for infarct volume, suggesting that, on average, the software underestimated brain volume (by 5.4%) and overestimated infarct volume (by 3.5%).

3.2. Tectonic image analysis: a stand-alone software for TTC staining analysis

The described infarct estimation pipeline was deployed as a standalone software: Tectonic Infarct Analysis (Fig. 3) (see Supplemental Video). A programmatic overview of the software’s computational pipeline has also been provided in Fig. 4. The software, a
GUI as shown in Fig. 2, and its documentation (see Supplemental Material) will be made publicly available as an open-source computational tool. In addition to Tectonic’s computational performance, ease of usability was quantified as the reduced time effort upon software application for the test dataset \( n = 18 \) brains, 109 brain slices). Mean analytical time across all cases was 26.38 ± 18 min for manual and 1.07 ± 0.11 min for automated analysis. Automation reduced time effort by 95%, with a mean decrease of 25.32 min \((p < 0.001)\).

3.3. Segmentation and quantification performance in an independent dataset

Cohort 2 was then used as an internal validation dataset to evaluate software and model robustness against batch effect. The pre-trained model based on MCAO-1, an earlier timepoint, was deployed to analyze the \( n = 40 \) rat stroke cases in Cohort 2. As shown in Fig. 5A-E, automated infarct estimation again exhibited high performance in this dataset, with strong and significant correlations to manual ground truths. Brain, infarct, and non-infarct volumes all had significant correlation coefficients over \( \rho = 0.82; \rho = 0.83 \) (CI = 0.82–0.83, \( p < 0.001 \)), \( \rho = 0.97 \) (CI = 0.97–0.98, \( p < 0.001 \)), and \( \rho = 0.82 \) (CI = 0.82–0.83, \( p < 0.001 \)), respectively. Percent infarct and infarct:non-infarct ratio also had significant correlation coefficients both being \( \rho = 0.97 \) (CI = 0.96–0.97, \( p < 0.001 \)).

Qualitative and quantitative assessment of detected brain slice and infarct regions in the validation data confirmed that computational and manual morphometric estimates were based on the same image regions. As shown in Fig. 5F, brain slice segmentations and infarct region segmentations were similar to ground truth annotations. Quantitative assessment of brain infarct segmentation showed high performance in pixel-wise classification tasks. Brain slice segmentation had an accuracy = 0.97, sensitivity = 0.97, specificity = 1.00, precision = 0.84, recall = 0.97, and F1-score = 0.90 (Fig. 5G). Similarly, infarct segmentation had an accuracy = 0.97, sensitivity = 1.00, specificity = 0.78, precision = 0.76, recall = 1.00, and F1-score = 0.80 (Fig. 5H). Percent difference from ground truth was found to be 2.3 ± 5.7 for brain volume and 4.4 ± 19.51 for infarct volume, suggesting that, on average, the software overestimated brain volume (by 2.3%) and underestimated infarct volume (by 4.4%).

Mean analytical time across all cases was 20.23 ± 7.64 min for manual and 1.24 ± 0.28 min for automated analysis. Automation reduced time effort by 94%, with a mean decrease of 19.0 min \((p < 0.001)\).
Fig. 4. Programmatic overview of Tectonic Infarct Analysis’ computational pipeline. Analysis is completed as follows. The pre-trained segmentation model and new image data are loaded into the software. Following user (i) indication of whether image data includes anterior and posterior faces of brain slices, and (ii) pixel/mm calibration (from scanner metadata or annotation functionality), brain and infarct segmentations are completed. Upon segmentation, software functionalities may be used to automatically split overlapping brain slices and adjust both infarct and brain slice boundaries. If segmentations are deemed sufficient, stroke infarct morphometrics are computed and written to the local directory as a Microsoft excel file along with binary images of segmentations.
3.4. Segmentation and quantification performance in an external dataset

Cohort 3 was treated as a “use-case” to evaluate Tectonic Infarct Analysis’ adaptability to an interinstitutional data featuring a different section thickness, staining protocol, and scanner. As in the two internal datasets, correlations between automated and ground truth estimates for the external dataset were strongly and significantly correlated (Fig. 6A–E). Brain, infarct, and non-infarct volumes
all had significant correlation coefficients over $\rho = 0.80$; $\rho = 0.86$ (CI = 0.86–0.87, $p < 0.001$), $\rho = 0.83$ (CI = 0.82–0.83, $p < 0.001$), $\rho = 0.83$ (CI = 0.82–0.83, $p < 0.001$), respectively. Percent infarct and infarct: non-infarct ratio also had significant correlation coefficients over $\rho = 0.80$; $\rho = 0.84$ (CI = 0.84–0.85, $p < 0.001$) and $\rho = 0.84$ (CI = 0.83–0.84, $p < 0.001$), respectively.

Qualitatively, the automated and manual brain slices segmentations were indistinguishable. Infarct segmentations were comparable to ground truth, with some false-positive pixels corresponding to the boundaries between ventricle and infarct. In these instances, most of the ventricle region was correctly excluded and the few pixels lying along the boundary between regions were labeled differently by

Fig. 6. Tectonic Infarct Analysis software demonstrates robustness when applied to an external dataset (Cohort 3). A-E). Strong and significant correlations were observed between manual ground truth and Software computational estimates. F). Representative brain slice and infarct regions are provided to illustrate similarity between manual and automated segmentations. Brain slice segmentations are depicted in dark blue, while infarct segmentations are depicted in cyan. G-H). High segmentation performance was observed for both brain and infarct segmentation tasks. Abbreviations: CI = confidence interval, GT = ground truth, Comp = computational, ACC = accuracy, SENS = sensitivity, SPEC = specificity, PREC = precision, REC = recall, F1 = F1-score. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
the algorithm and manual annotators. Quantitative assessment of brain infarct segmentation showed high pixel-wise classification performance. Brain slice segmentation had an accuracy = 0.96, sensitivity = 0.99, specificity = 0.96, precision = 0.94, recall = 0.98, and F1-score = 0.92 (Fig. 6G). Infarct segmentation had an accuracy = 0.95, sensitivity = 0.96, specificity = 0.94, precision = 0.73, recall = 0.96, and F1-score = 0.82 (Fig. 6H). Percent difference from ground truth was found to be 0.5 ± 0.05 for brain volume and −4.9 ± 0.2 for infarct volume, suggesting that for the validation dataset, the software overestimated brain volume (by 0.5%) and underestimated infarct volume (by 4.9%).

In Cohort 3, half of the rats received TPA, while the others received vehicle control. We therefore used software-computed infarct morphometrics to compare the tPA-treated and untreated rats (Fig. 7A–E). We found that TPA administration significantly reduced infarct volume: vehicle = 269.2 mm$^3$ and tPA = 237.0 mm$^3$ ($p = 0.03$). Percent brain infarcted (vehicle = 19.0%, tPA = 16.3%, $p = 0.07$) and infarct:non-infarct ratio (vehicle = 0.26, tPA = 0.22, $p = 0.07$) were also decreased in tPA-treated rats, although differences were not statistically significant. Between treatment groups, brain volumes (mm$^3$) (vehicle = 1457.1, tPA = 1456.4, $p = 0.75$) were nearly identical. Non-infarct volume (mm$^3$) (vehicle = 1187.9, tPA = 1219, $p = 0.24$) was greater in the tPA-treated group, albeit not significantly. Quantitative differences between groups were also appreciable in image data (Fig. 7F). For brain slices collected at equal depths it is clear that the infarct cross-sectional area is significantly reduced in tPA-treated rats.

4. Discussion

In this study we present, to our knowledge, the first stand-alone, trainable software for automated whole-infarct volume estimation from TTC-stained brains. Image segmentation and infarct estimation pipelines at the core of the software were rigorously assessed by comparing software estimates against manual ground truths. Software and pre-trained model evaluation were completed in testing for a preliminary, internal dataset (Cohort 1) as well as in validation for a secondary internal dataset (Cohort 2) prepared at a later date. In

![Fig. 7](image-url) Use Case in Cohort 3: tPA significantly reduced infarct volume in stroke. Stroke infarct morphometrics were compared between tPA-treated and Vehicle groups. Validated, computational estimates were used in statistical analysis. A). Brain volumes were comparable between groups. B). Infarct volumes were significantly reduced in tPA-treated rats. C). Non-infarct volume was higher in tPA-treated rats, indicating preservation of tissue integrity. D). Infarct: Non-infarct ratio was lower in tPA-treated rats, indicating preservation of tissue integrity. E). Percent infarcted tissue was lower in tPA-treated rats, indicating preservation of tissue integrity. F). Representative brain slices from tPA-treated and Vehicle rats are provided. Vertically paired slices were extracted from equal brain depth to illustrate reduced infarct volume in tPA-treated rats. Abbreviations: % = percentage, Veh. = vehicle.
both cases, high segmentation and quantification performance was observed. The model trained using a subset of Cohort 1 performed well when applied to data from Cohort 2. Moreover, software usability and model trainability were assessed through analysis of an external dataset (Cohort 3) from a separate institution featuring different sample preparation, staining, and imaging methods. Overall ease of usability was demonstrated as reduced time effort with automated analysis as compared to manual approaches.

Prior to the discovery of TTC as an ischemic indicator, histology and light microscopy were a costly standard [33, 34]. TTC-staining provided a much more feasible approach to infarct identification than H&E in the study of ischemic tissues [33–35]. The earliest TTC quantification methods demonstrated accurate region quantification but required time-intensive and subjective manual annotations, as well as expensive camera equipment [33, 34, 36–38]. Later methods, enabled by scanner technology, explored simple image analysis techniques (e.g., RGB channel splitting and thresholding) to segment infarct and brain regions [12, 13]. While these methods achieved semi-automation and reduced time-effort, they were sensitive to stain variation and performed poorly on low contrast images. Furthermore, most methods analyzed single slices at a time [12, 13], introducing bias and requiring users to externally compute whole brain morphometrics. Those methods, designed with an adjustable segmentation threshold, required considerable manual supervision, restricted imaging parameters (e.g., black backgrounds required) [13], produced un-editable regions, and were unable to export endpoint data (e.g., percent infarct) or segmentations in user-friendly formats [13, 39]. Computed features displayed in figure windows were robust against stain variation and poor contrast. Additional software functions enabled ease-of-use. Infarct editing tools allowed for refinement of predicted infarct regions with real-time display of updated region boundaries. Calibration functionalities provided for conversion of volume estimates to interpretable units (e.g., from pixels to mm$^3$) for data export. Simultaneous analysis of serial brain sections captured in the same scan significantly reduced analysis time and provides for whole-brain infarct estimation. Finally, data export options were able to output both binary image segmentation maps (e.g., brain slice and infarct masks) and full feature files in .xlsx format to local operating systems. A total of five infarct morphometrics were recorded for both the whole brain and individual cross-sections.

Together, these functionalities produced a software that achieved advanced performance in both brain and infarct segmentation, as well as infarct volume estimation, for two internal datasets (testing and validation) and an external dataset (use-case and software demonstration). We demonstrated computational performance through comparison of automated and manual infarct feature calculations. Strong and significant correlations were observed for all five features in both datasets ($\rho \geq 0.0.83$, $p < 0.001$). We further studied pixel-wise classification accuracy for brain and infarct regions, and calculated percent difference for brain and infarct volumes. In our first dataset (Cohort 1) used for software development and model pre-training, brain and infarct detection accuracy were 95% and 96%, respectively. When pre-trained models were deployed for analysis of an internal validation dataset (Cohort 2), brain and infarct detection accuracy were both 97%. In the external “use-case” dataset (Cohort 3), the model developed using Tectonic Infarct Analysis’ training functionality also exhibited high brain and infarct detection accuracy (96% and 95%, respectively). Across all three datasets the percent difference between computational and ground truth measurements were $\sim 6.5\%$ or less. We believe that the use of computation provided for infarct quantification with improved precision. This may be evidenced by the fact that the software facilitated identification of a subtle, but significant reduction in infarct volume ($p = 0.03$) upon tPA administration in our use case study (MCAO-3).

This study has several limitations. First, the software can only evaluate TTC staining, but not other popular ischemic indicators like Nissl staining [41]. Engineering and validation of additional infarct features for histological stains is within the scope of our future work. Second, the total number of rats used to develop Tectonic Infarct Analysis was small ($n = 21$ with $n = 127$ brain slices). However, incorporation of model training as a software functionality, and the demonstrated computational performance on an internal validation dataset featuring a greater number of ($n = 40$) rats ($n = 240$ brain slices) suggests that the deployed software is robust to batch effect and can handle common challenges in TTC quantification. Lastly, only five infarct morphometrics are output: brain volume, infarct volume, non-infarct volume, percent infarct, and infarct: non-infarct ratio. Given accurate and precise isolation of the infarct regions, additional image processing techniques may be used to extract other infarct features of biological significance (e.g., duration of ischemia [35]) including textural metrics.

5. Summary

In this study we developed and validated Tectonic Infarct Analysis, a software tool for TTC-staining analysis. The software contributes novel and intuitive functionalities, such as trainable segmentation, to increase the accessibility and feasibility of whole-brain infarct estimation for future studies. The deployed GUI is a standalone software, completely independent of proprietary programs (e.g., MATLAB), that does not require any coding experience on behalf of the end-user. Here, use of Tectonic Infarct Analysis Software facilitated rapid assessment of three rat stroke cohorts through objective and precise measurement of stroke infarct morphometrics. Brain and infarct quantification performance among the three cohorts demonstrated high correlation with ground truth measurements and annotations. Software use also significantly reduced time effort on behalf of the end user. Through a use case, we demonstrated
how our computational approach to TTC-staining analysis can determine successful outcome in rats treated with tPA. Ultimately, we hope that this tool will greatly contribute to in vivo stroke studies.

Author contribution statement

Briana A Santo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Shiau-Sing K Ciecierska, S. Mostafa Mousavi Janbeh Sarayi, TaJania D Jenkins, Ammad A Baig and Andre Monteiro: Performed the experiments.
Carmon Koenigsknecht, Donald Pionessa Jr., Liza Gutierrez, Robert M King and Matthew Gounis: Performed the experiments; Contributed reagents, materials, analysis tools or data.
Adnan H Siddiqui: Contributed reagents, materials, analysis tools or data.
Vincent M Tutino: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interest’s statement


Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e14837.

References
