

Foreign body response to neuroimplantation: machine learning–assisted quantitative analysis of astrogliosis

Anastasiia A.Melnikova

Anastasiia_Melnikova@outlook.com

Anton A. Egorchev

Alexandr A. Rosin

Leniz F. Nurullin

Nikita S.Lipachev

Daria S. Vedischeva

Dmitry V.Derzhavin

Stepan S. Perepechenov

Ekaterina Sukhodolova

Gleb V.Shabernev

Angelina A.Titova

Ramziya G. Kiyamova

Andrey P.Kiyasov

Dmitry E.Chickrin

Albert V. Aganov

Dmitry V. Samigullin

Irina Yu.Popova

Mikhail Paveliev

paveliev@outlook.com

Research Article

Keywords: brain-computer interface, neuroinflammation, artificial intelligence, glia, astrocyte, gliosis

Posted Date: March 10th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9053707/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: The authors declare no competing interests.

Foreign body response to neuroimplantation: machine learning–assisted quantitative analysis of astrogliosis

Anastasiia A. Melnikova¹, Anton A. Egorchev², Alexandr A. Rosin², Leniz F. Nurullin^{3,4}, Nikita S. Lipachev¹, Daria S. Vedischeva^{1,5}, Dmitry V. Derzhavin², Stepan S. Perepechenov⁵, Ekaterina Sukhodolova^{1,6}, Gleb V. Shabernev², Angelina A. Titova⁵, Ramziya G. Kiyamova⁵, Andrey P. Kiyasov⁵, Dmitry E. Chickrin⁷, Albert V. Aganov¹, Dmitry V. Samigullin^{3,8}, Irina Yu. Popova⁶, Mikhail Paveliev¹

1 - Institute of Physics, Kazan Federal University, Kremlyovskaya 16a, Kazan 420008, Tatarstan, Russia; 2 - Institute of Computational Mathematics and Information Technologies, Kazan Federal University, Kremlyovskaya 35, Kazan 420008, Tatarstan, Russia; 3 - Kazan Institute of Biochemistry and Biophysics, FRC Kazan Scientific Center, Russian Academy of Sciences, P.O. Box 261, 420111 Kazan, Russia; 4 - Kazan State Medical University, Butlerova 49, Kazan 420012, Tatarstan, Russia; 5 - Institute of Fundamental Medicine and Biology, Kazan Federal University, Karl Marx 74, Kazan 420015, Tatarstan, Russia; 6 - Institute of Theoretical and Experimental Biophysics, Russian Academy of Science, Institutskaya 3, Puschino 422290, Moscow oblast, Russia; 7 - Institute of Artificial Intelligence, Robotics and Systems Engineering, Kazan Federal University, Kremlyovskaya 18, 420008 Kazan, Russia; 8 - Department of Sports Engineering, Kazan National Research Technical University Named after A.N. Tupolev-KAI, 10 K. Marx St., 420111 Kazan, Russia;

Correspondence: paveliev@outlook.com (M.P.); AnastasAMelnikova@kpfu.ru (A.M.);

Abstract

Neuroimplants represent an emerging medical technology, offering new therapeutic approaches for severe neurological and psychiatric disorders. One of the key limitations to long-term neuroimplant performance is the foreign body response elicited by intracortical implantation. Among the contributing cell types, astrocytes play a central role in glial scar formation around the implant, which can compromise device functionality. Implantation-induced astrogliosis can be assessed by quantifying immunofluorescence of the astrogliosis marker glial fibrillary acidic protein (GFAP) in brain sections. Here, we used a pipeline-embedded random forest classifier implemented in the LabKit plugin for Fiji software to compare GFAP expression and astrocyte morphology in peri-implant scar versus distant cortical areas. We report an increase in GFAP expression, cell area and astrocytic process length as well as redistribution of GFAP signal along astrocytic processes within scar regions. We compare multiple classifiers producing different astrocyte segmentation outcomes and describe a procedure for generating a robust set of classifiers applicable to samples from repeated and independent experiments. Our results highlight the potential of artificial intelligence–assisted image analysis for automated and quantitative assessment of implantation-induced cellular and tissue responses.

Keywords: brain-computer interface, neuroinflammation, artificial intelligence, glia, astrocyte, gliosis.

1. Introduction

Brain–computer interfaces represent a rapidly developing branch of medical technology with the potential to transform the treatment of severe neurological and psychiatric disorders [1,2]. Invasive interfaces, in particular, provide the spatial and

temporal resolution required for neuroprosthetic applications and high-fidelity neural recording [3,4]. However, implantation of cortical electrodes induces a foreign body response (FBR) in the surrounding brain tissue, ultimately compromising device functionality and long-term stability [5,6].

Multiple cell types contribute to the development of the FBR, including microglia, astrocytes, oligodendrocyte progenitor cells, and vascular-associated cells [7–9]. Among these, astrocytes play a central role in the formation of the peri-implant glial scar, which can mechanically and electrically isolate the electrode from surrounding neurons [10]. During scar formation, astrocytes undergo astrogliosis characterized by changes in gene expression, cytoskeletal organization, and cellular morphology [11,12]. Upregulation of glial fibrillary acidic protein (GFAP) is widely recognized as a hallmark of reactive astrocytes in both foreign body response and diverse CNS pathologies [13,14]. Furthermore, a limited number of studies have reported that quantitative parameters of GFAP-stained astrocyte morphology can serve as markers of the cell functional state and brain tissue pathology outcomes [15,16]. In contrast to purely qualitative assessments, quantitative analysis of astrocyte morphology and GFAP signal provides a set of measurable parameters that reflect astrocyte functional states and tissue pathology. Therefore, quantification of the astrocytic GFAP expression and cell morphology can be viewed as a promising readout of the severity of the FBR caused by neuroimplantation and, more generally, for neuroimplant biocompatibility studies.

Reliable morphometric analysis requires automated and unbiased image processing pipelines that minimize operator-dependent variability. Machine learning-based image segmentation has become a widely adopted strategy for biomedical microscopy analysis over the past two decades [17,18]. Deep learning approaches demonstrate high performance but typically require large annotated datasets and substantial computational expertise, limiting their accessibility for routine laboratory use [19,20]. With respect to astrocytes in brain sections, deep learning-based segmentation tools have not yet become widely adopted although several highly promising pioneering studies were reported [21,22].

Shallow learning approaches provide an attractive alternative for applications with limited ground truth data. Several user-friendly implementations enable efficient cell segmentation with minimal training data and rapid classifier generation [23,24]. In the present study, we took advantage of the LabKit plugin for Fiji, which is based on a random forest classifier and integrates seamlessly into reproducible image analysis pipelines [24,25]. It has been successfully applied to segmentation of diverse microscopy datasets including cultured cells, bacterial images, tissue sections, large light-sheet volumes, and 3D ultrastructural datasets [24,26–28]. We demonstrate the peri-implant scar-specific changes in astrocyte morphology, GFAP expression and subcellular distribution. We further compare several classifiers with various astrocyte segmentation outcomes and propose a procedure for generating robust classifiers applicable to repetitive and independent experiments. We also provide an explicit validation of annotation strategies for shallow learning-based astrocyte segmentation.

2. Results

We first asked whether the recently developed advanced algorithm for the shallow learning-based image segmentation LabKit [24] can be used for segmentation of GFAP-stained astrocytes in histological sections of mouse brain with the implantation-induced scarring. At the 2 month time point after implantation cortical sections exhibited pronounced GFAP-positive scars at the implantation sites (Fig. 1A-D). The GFAP-positive astrocytes were tightly packed within the scar (Fig. 1C-D). In addition to that, some sparse

GFAP-positive astrocytes could be observed in the cortex areas away from the implantation sites.

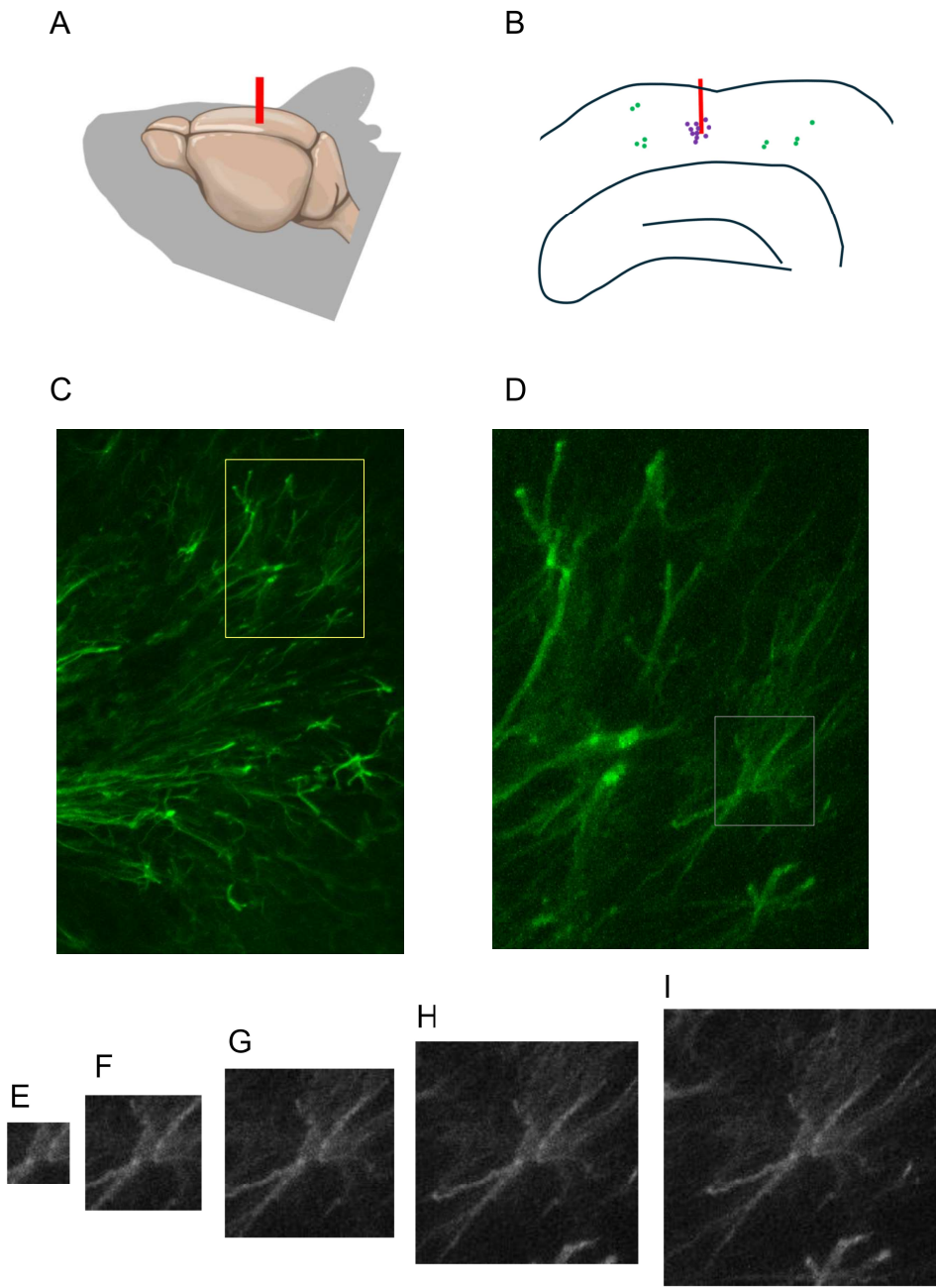


Figure 1. Astrogliosis within foreign body response to neuroimplantation. **(a)** Schematic representation of cortical electrode implantation. **(b)** Scar-resident (violet) and non-resident (green) astrocytes in a mouse brain section. **(c)** GFAP-positive astrocytes within the glial scar at the implantation site. **(d)** An enlarged region of interest corresponding to the yellow rectangle in C. **(e)** A single astrocyte, shown in a white rectangle in D. **(e-i)** Selection of an optimal square size for single cell image analysis.

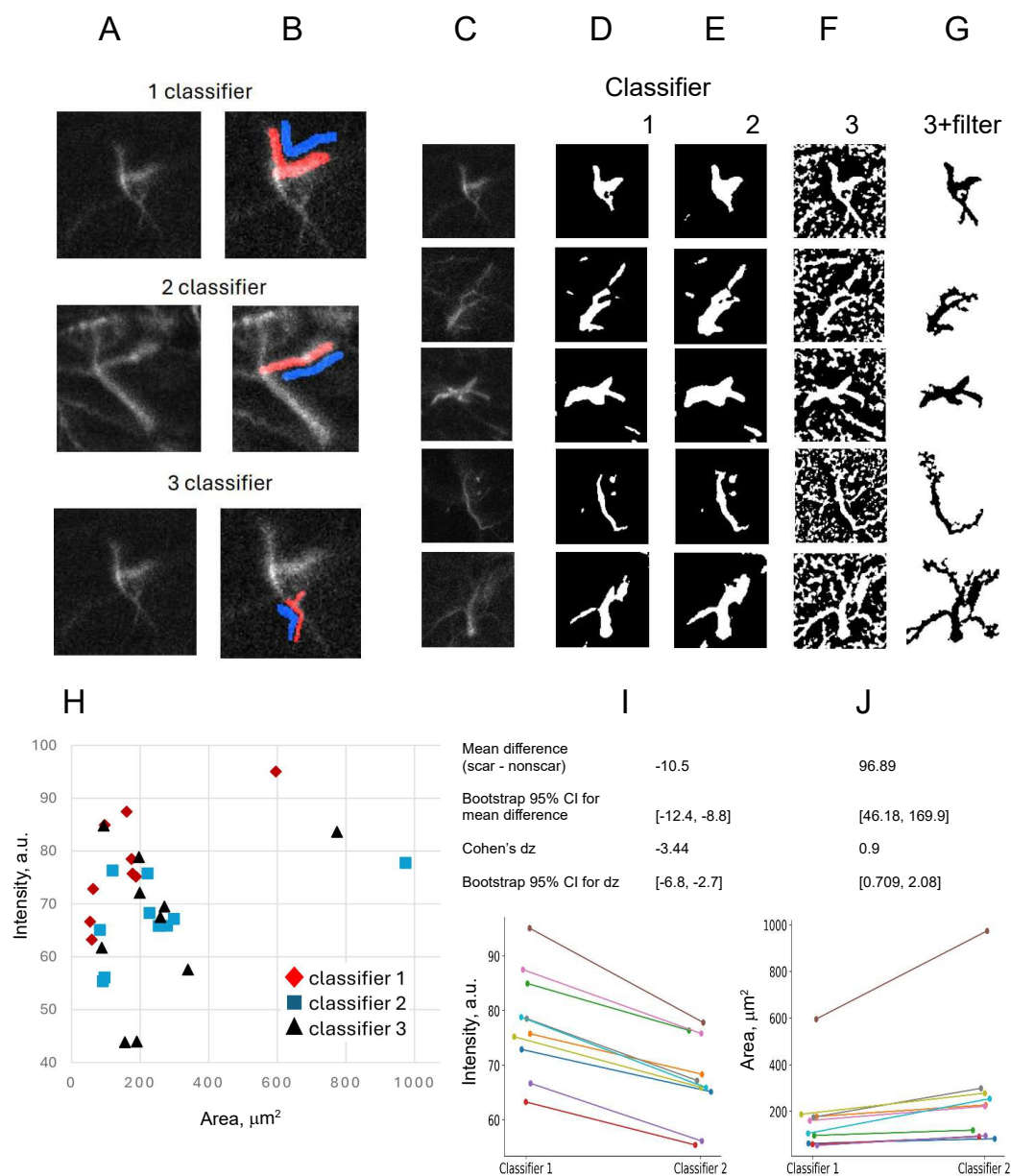


Figure 2. Training and comparison of Labkit classifiers in Fiji software. **(a)** Original cell images used for shallow learning. **(b)** Foreground (red) and background (blue) selection for shallow learning. **(c)** Original images for testing the classifiers. **(d-f)** Masks by classifiers 1-3, respectively. **(g)** Object size threshold-filtered masks for classifier 3. **(h)** Single cell intensity-area scatter plot for 10 cells, classifiers 1-3. **(i)** Classifier 1 produces systematically higher mean GFAP fluorescence intensity results as compared to classifier 2. Data shown for the same 10 cells as in H. **(j)** Classifier 1 produces systematically lower GFAP-positive cell area results as compared to classifier 2.

We then used the semi-automatic procedure for single cell perineuronal net analysis [29] and adjusted it for picking single astrocytes in squares of desired size (Fig. 1E-I).

2.1 Creation of classifiers. We created a few LabKit classifiers on astrocytes within and away from the peri-implantation sites (Fig. 2 A-B) to check how robustly the algorithm would perform on cells of various size, shape and GFAP intensity levels (Fig. 2 C-G). One crucial advantage of the LabKit shallow learning method is that it takes a skilled human researcher less than a minute to create a single classifier that can be then tested on many astrocytes. The classifiers 1 and 2 segmented mostly large GFAP-positive objects corresponding to the astrocytic cells with few or no small peripheral fragments (Fig. 2 C-E). By contrast, classifier 3 segmented large numbers of small objects in the analyzed area (Fig. 2 C, F). We performed extensive trials to find an optimal object threshold size to filter away many small noisy fragments without compromising the main tree of astrocyte processes (Fig. 2G).

2.2 Fiji pipelines with embedded machine learning. The Labkit plugin was designed with implicit capacity for integration in Fiji pipelines and automated processing of big data [24]. To build on those features we assembled two pipelines for semi-automatic and automated processing of the brain section confocal images (Fig. 3). The pipeline 1 (Fig. 3 A) works on raw microscopy images. It starts by manual selection of a cell (region of interest, ROI) center with the PointPicker tool followed by manual selection of the ROI size (Fig. 1 E-I). Multiple cells (ROIs) can be selected within a single pipeline run. The astrocyte-containing ROI is then segmented with a pre-defined Labkit classifier, the resulting mask is filtered for the object size and the minimum operation is applied between the raw ROI and the filtered mask. The cell mean intensity, area, perimeter, area-to-perimeter ratio and circularity (isoperimetric) are written to the output files for further analysis. The set of the quantified parameters can be easily expanded as needed. The pipeline 2 (Fig. 3 B) works on a pre-selected set of ROIs, performs Labkit-based segmentation with subsequent intensity and morphometry analysis in the same way as in the pipeline 1. On average, this approach improves the speed of the image analysis by 5.2 times and reduces profoundly the number of manual operations for the whole procedure.

2.3 Testing the classifiers. The results of astrocyte segmentation performed by those classifiers exhibited consistently higher cell areas and lower mean GFAP intensities for some classifiers as compared to others (Fig. 2 H-J). This result suggests that LabKit classifiers can be created specifically either for the brightest GFAP cytoskeleton area (the cell “core”) or for larger and dimmer areas reflecting more closely the astrocyte area in 2D images and the astrocyte volume in 3D.

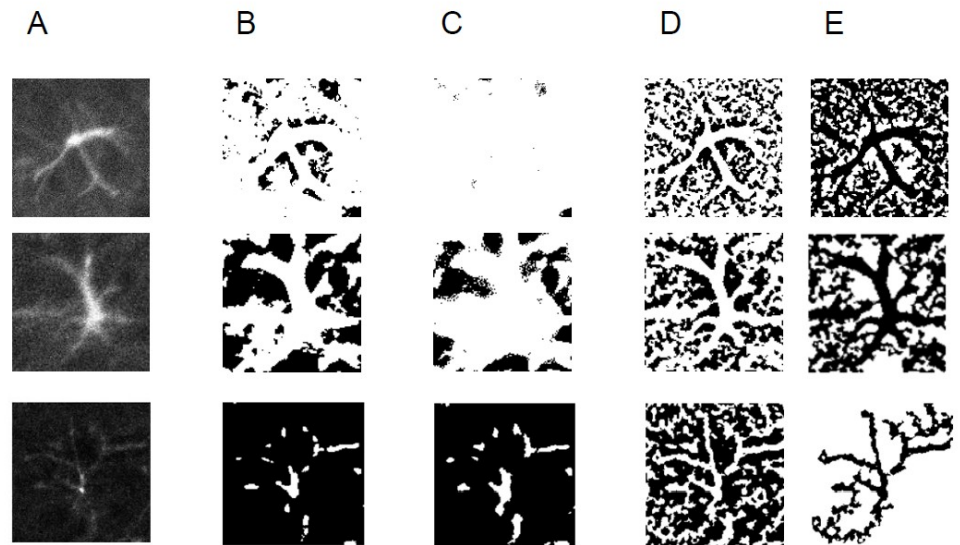


Figure 3. Testing Labkit classifiers on repeated and independent experiments. (a) Original images for 3 astrocytes. (b-d) Applying classifiers 1-3 (shown in Fig.2) to astrocytes from another mouse reveals systematic failure of cell border segmentation. (e) Object size threshold-filtered masks for classifier 3.

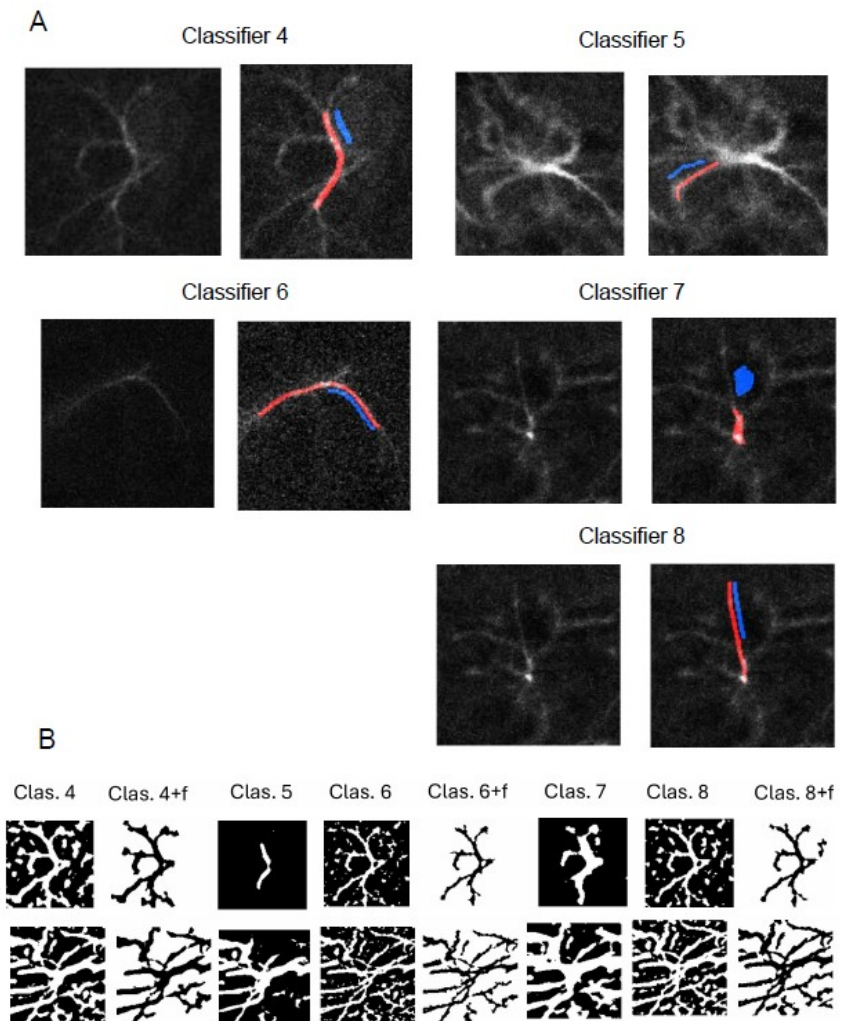


Figure 4. Comparison and selection of Labkit classifiers. **(a)** Five additional classifiers (number 4-8) were trained and tested with different patterns of the foreground (red) and background (blue) selection. **(b)** Comparison of segmentation results showed that classifiers 4, 6, 8 systematically performed more successfully as compared to classifiers 5 and 7 on images from repeated and independent experiments.

Efficient and standardized image processing implies that data from repetitive independent experiments are subjected to exactly the same image analysis procedure. Therefore, it would be crucial that we develop LabKit classifiers equally applicable to astrocyte images from independent experiments. Testing the classifiers 1-3 on confocal images from different mice (3 independent experiments) revealed that some cells were not segmented correctly (Fig. 3). More specifically, classifiers 1 and 2 produced very blurred segmentation masks (Fig. 3A-C), while classifier 3 segmented almost the entire analyzed image as an uninterrupted syncytium of processes (Fig. 3 A, D, E) that does not reflect the true astrocyte morphology previously described by various independent techniques in glial scars [12,30].

2.4 Selection of the classifiers. To overcome the limited applicability of the LabKit shallow learning approach to repetitive and independent experiments we developed an extended panel of the LabKit classifiers producing different astrocyte segmentation results

(Fig. 4). Altogether, we tested 100 cells from 4 mice (6 brain sections, 4 independent experiments) processed with 8 different classifiers. Those extensive trials allowed us to select 3 classifiers (number 4, 6 and 8), robustly and consistently performing astrocyte segmentation in microscopy data from independent experiments.

2.5 Training rules for successful classifiers. Careful comparison of the foreground and background sampling (the red and blue lines in Figs 2, 4) made it possible to suggest some “rules of thumb” for the best classifier creation: 1) the classifiers 4, 6 and 8 were made on cells from outside the scars; 2) the foreground line was traced through the cell body and the longest process of an astrocyte; 3) the foreground line contained both bright and dim pixels; 4) the foreground and the background lines were traced along and next to each other. To further validate this set of rules we created 6 additional classifiers – 3 classifiers following the rules and 3 classifiers trained against the rules. Table 1 presents the percentage of successful segmentation outcomes for those 6 classifiers tested with a dataset of 20 astrocytes from 4 mice. The classifiers following the rules demonstrate consistently higher success rates as compared to the classifiers trained against the rules.

Table 1. Segmentation performance of rule-violating and rule-compliant classifiers.

	Rule-Violating Classifiers			Rule-Compliant Classifiers		
	RV-1	RV-2	RV-3	RC-1	RC-2	RC-3
Analyzed cells	20	20	20	20	20	20
Successful segmentation	8	2	0	17	17	18
Success rate (%)	40	10	0	85	85	90

2.6 GFAP expression and astrocyte morphology as quantitative markers of the foreign body response. After selecting the best classifiers we asked whether the LabKit-assisted segmentation results can reveal specific quantitative difference in the GFAP expression between the scars and distant astrocytes outside the scars. The magnitude of standardized effects was interpreted using conventional benchmarks (small ≈ 0.2 , medium ≈ 0.5 , large ≈ 0.8) originally proposed by Cohen (1988) and discussed for paired designs in Lakens [45]. Across all three classifiers, soma-level morphometric parameters consistently indicated astrocyte hypertrophy in scar regions.

GFAP intensity was increased in scar tissue in all classifiers, with mean paired differences ranging from 22.35 to 30.5 and bootstrap 95% confidence intervals consistently excluding zero. Standardized effect sizes were large to very large (Cohen’s d_z ranging from 2.61 to 8.97), indicating a robust elevation of GFAP signal across animals (Fig. 5A).

Soma area was likewise elevated in scar regions, with mean paired differences ranging from 137.5 to 223.2 μm^2 and bootstrap confidence intervals consistently above zero. Effect sizes were large across classifiers ($d_z = 1.02\text{--}1.51$), supporting pronounced astrocyte hypertrophy (Fig. 5B).

Soma perimeter showed a similar pattern, with mean paired differences between 112.6 and 210 μm and large standardized effect sizes ($d_z = 0.849\text{--}1.09$), consistent with expansion of astrocytic cell contours (Fig. 5C).

The area-to-perimeter ratio was increased in scar regions in all classifiers (mean paired differences 0.0639–0.1373), with large to very large effect sizes ($d_z = 2.67\text{--}7.45$), indicating systematic alteration of astrocyte geometry (Fig. 5D).

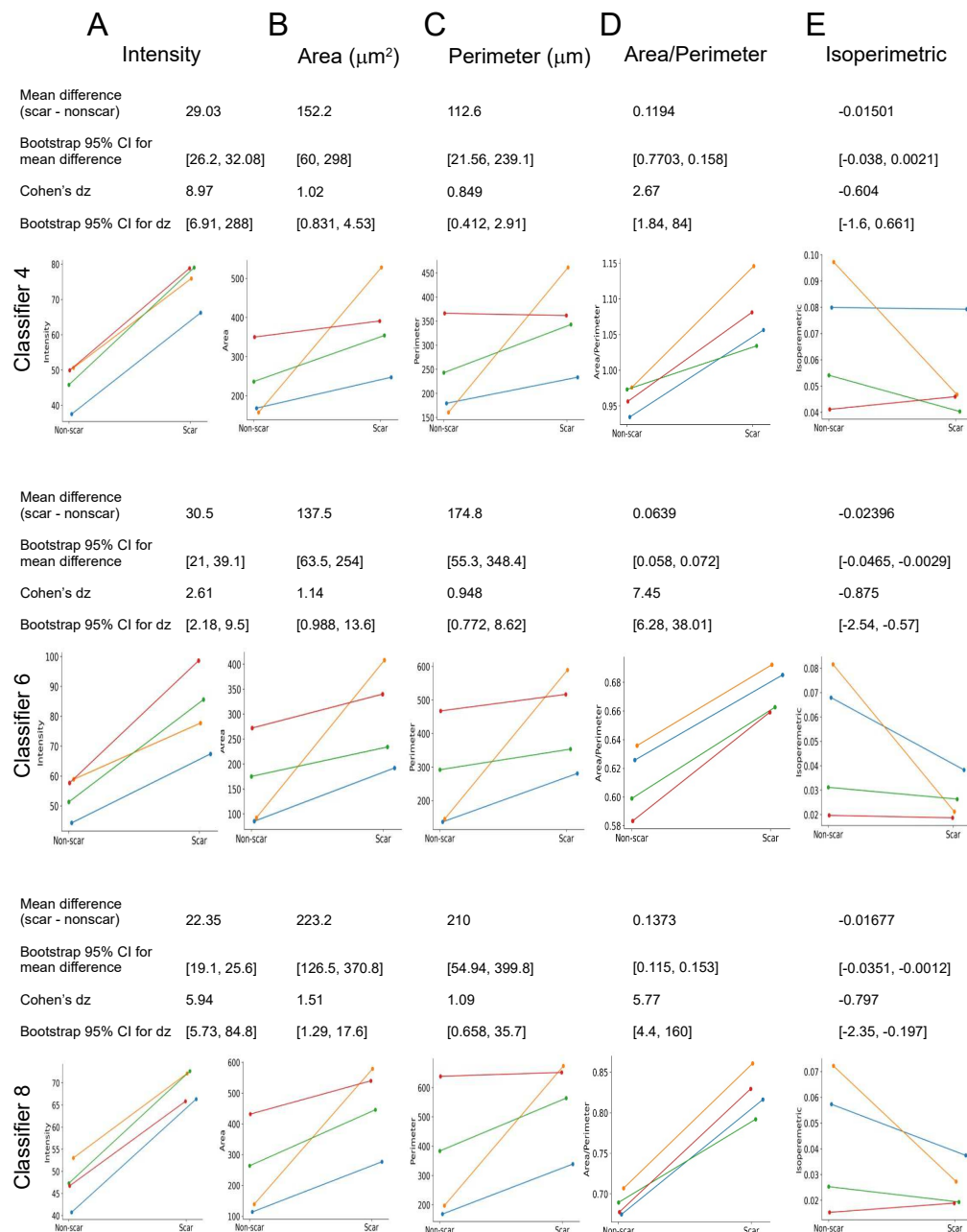
The isoperimetric index, in contrast, showed a modest decrease in scar regions (mean paired differences -0.015 to -0.024). While effect sizes were moderate in magnitude ($d_z = -0.60$ to -0.88), confidence intervals indicated greater variability across classifiers compared to other parameters (Fig. 5E).

Taken together, the three independent classifiers demonstrated strong gross consistency in detecting astrocyte hypertrophy and geometric remodeling in scar regions, despite moderate quantitative variation in specific shape descriptors.

2.7 GFAP expression in the astrocytic processes. Astrocyte processes are a crucial cellular compartment performing highly important physiological functions within the

224
225
226
227
228
229
230
231

blood-brain barrier [31], regulation of synaptic transduction and plasticity [32] and the “palicade” formation with the glial scars [12]. Being a cytoskeleton component GFAP is expected to bear a structural function and to hold a role in shaping the astrocyte process tree. Therefore, GFAP expression in astrocytic processes may be considered as a promising distinct marker of the astrocyte functional state [30]. We first assessed what part of the whole process tree is grasped by the Labkit masks. By comparing total process length in the Labkit masks versus raw images from a random data subset we estimate that Labkit reveals 83% of all processes on average (Fig.6 A-C).



232
233
234
235
236

Figure 5. Comparison of GFAP intensity and morphometry parameters for scar-resident versus non-resident astrocytes. **(a)** GFAP intensity. **(b)** Cell area. **(c)** Cell perimeter. **(d)** Area-to-perimeter ratio. **(e)** Cell circularity (isoperimetric). Data are shown for three classifiers (Nos. 4, 6, and 8) applied to 100 cells from four independent experiments.

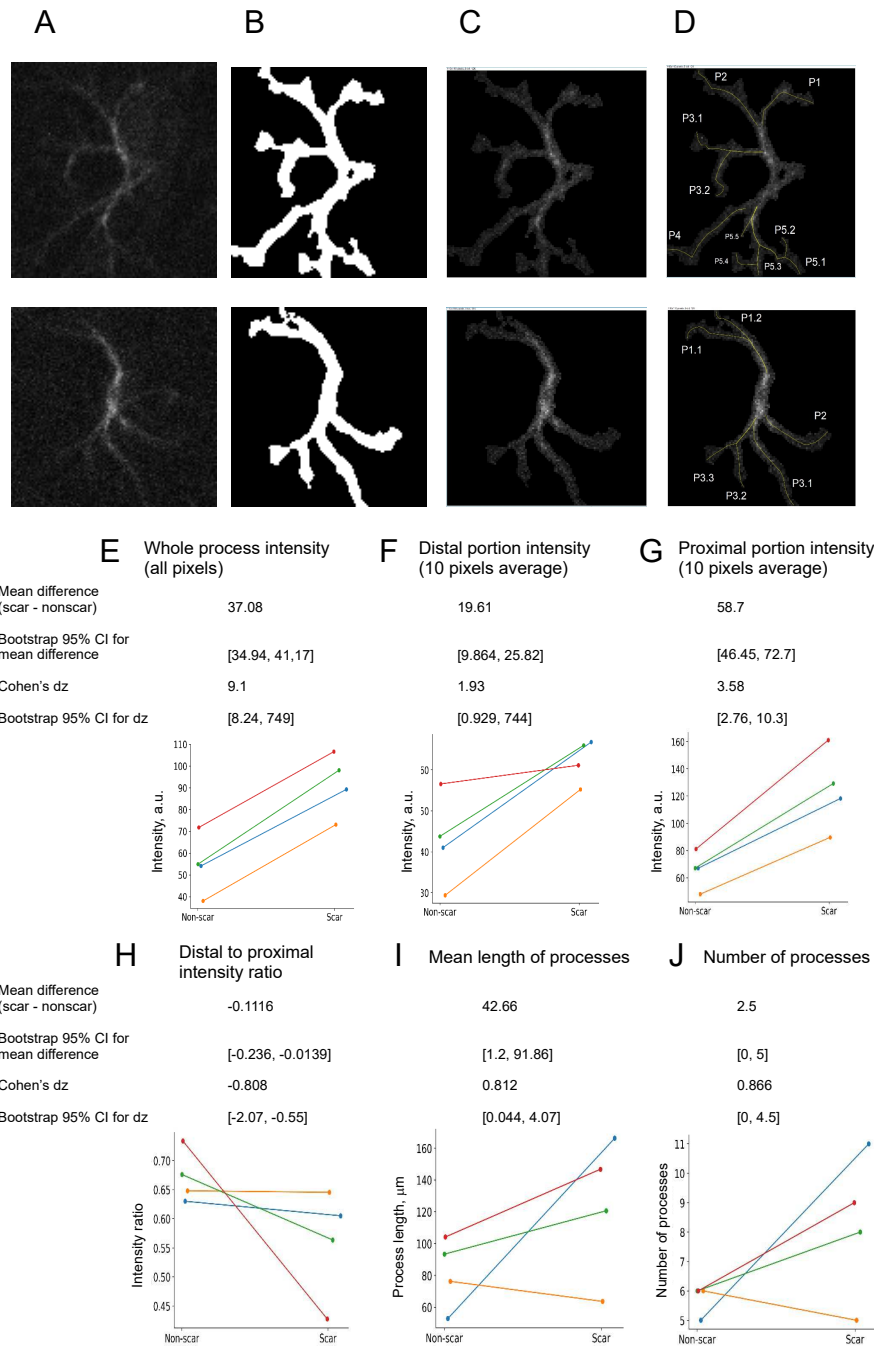


Figure 6. GFAP upregulation and redistribution in the processes of the scar-resident astrocytes based on Labkit-assisted segmentation. **(a).** Original images for 2 astrocytes. **(b)** Masks by the classifier 4. **(c)** Result of the Fiji Image Calculator “minimum intensity” operation applied to images **(a)** and **(b)**. **(d)** Astrocytes with traced processes. **(e-h)** GFAP intensity redistribution upon scarring. **(i)** Process length measurements. **(j)** Number of processes measurements. Data are shown for classifier 4 applied to 100 cells from four independent experiments.

We then used the LabKit-assisted segmentation results to measure GFAP expression specifically in the astrocytic processes. At the level of astrocytic processes, classifier 4 revealed consistent alterations in scar regions.

Whole-process GFAP intensity (all pixels) was markedly increased in scar tissue (mean paired difference = 37.08; bootstrap 95% CI: 34.94–41.17). The standardized effect size was very large (Cohen's $d_z = 9.1$; bootstrap 95% CI: 8.24–749), indicating a highly consistent elevation of signal along astrocytic processes (Fig. 6E).

When spatial compartments were analyzed separately, both distal and proximal portions exhibited increased intensity. Distal process intensity increased with a mean paired difference of 19.61 (bootstrap 95% CI: 9.864–25.82), corresponding to a large effect size ($d_z = 1.93$; bootstrap 95% CI: 0.929–744) (Fig. 6F). Proximal process intensity showed an even larger increase (mean paired difference = 58.7; bootstrap 95% CI: 46.45–72.7), with a very large standardized effect size ($d_z = 3.58$; bootstrap 95% CI: 2.76–10.3) (Fig. 6G).

Consistent with this asymmetric increase, the distal-to-proximal intensity ratio was reduced in scar regions (mean paired difference = -0.1116; bootstrap 95% CI: -0.236 to -0.0139). The effect size was moderate to large ($d_z = -0.808$; bootstrap 95% CI: -2.07 to -0.55), indicating relative proximal enrichment of GFAP signal (Fig. 6H).

Regarding structural parameters, mean process length was increased in scar regions (mean paired difference = 42.66; bootstrap 95% CI: 1.2–91.86), with a large standardized effect size ($d_z = 0.812$; bootstrap 95% CI: 0.044–4.07) (Fig. 6I). The number of processes also tended to increase (mean paired difference = 2.5; bootstrap 95% CI: 0–5), with a large effect size ($d_z = 0.866$; bootstrap 95% CI: 0–4.5), although confidence intervals indicated greater variability (Fig. 6J).

Taken together, process-level analysis demonstrated robust increases in GFAP signal intensity and process elaboration in scar regions, accompanied by relative proximal enrichment of cytoskeletal signal.

3. Discussion

Foreign body response remains a major factor limiting the long-term performance of intracortical neuroimplants [4,33]. Astrocytes are key contributors to this response through formation of a dense glial scar that alters tissue mechanics, electrical properties, and neuron–electrode coupling [5,6]. In the present study, we demonstrate that shallow machine learning–assisted segmentation of GFAP immunofluorescence images provides a practical and quantitative approach for assessing implantation-induced astrogliosis at both whole-cell and process levels. Beyond its specific application to neuroimplantation, the workflow described here may also be useful for quantitative analysis of astrocyte morphology across diverse neuropathological contexts.

Astrogliosis represents a dynamic and graded response. Early stages involve reversible GFAP upregulation and moderate morphological changes, whereas more severe injury can lead to persistent structural remodeling and scar formation [11,13,34]. The increased GFAP expression and morphological differences between scar-resident and distant astrocytes observed in this study are therefore consistent with distinct stages of astrocyte activation.

A key finding of our work is that LabKit-based random forest classifiers can reliably segment GFAP-positive astrocytes in confocal images of tissue samples without the need for extensive training datasets. This accessibility is particularly important because GFAP immunostaining remains one of the most widely used approaches for evaluating neuroinflammation in a broad range of brain pathologies and neuroimplant biocompatibility. In contrast to deep learning approaches, which require large, carefully curated ground truth datasets and substantial computational expertise, the shallow learning framework implemented in LabKit offers a favorable balance between performance, accessibility, and reproducibility. Classifier training can be performed rapidly by a skilled researcher, making the approach well suited for iterative exploratory analysis and routine laboratory workflows.

Our systematic comparison of multiple classifiers revealed that segmentation outcomes strongly depend on the foreground and background sampling strategy used during training. Some classifiers preferentially captured only the brightest GFAP-positive structures corresponding to the astrocytic “core,” whereas others included extended and dimmer process networks. This variability indicates that different classifiers capture distinct subsets of morphological features within the same cell image dataset, suggesting that classifier design can be tuned to emphasize specific biological aspects of cell morphology. Importantly, by testing classifiers across independent experiments and animals, we identified a subset of classifiers that performed robustly despite inherent variability between experiments. From this analysis, we derived practical “rules of thumb” for classifier generation, including training on astrocytes outside the scar, sampling both bright and dim GFAP regions, and closely juxtaposing foreground and background annotations. Importantly, these guidelines were subsequently validated by generating additional rule-compliant and rule-violating classifiers, demonstrating that classifiers trained according to the proposed annotation strategy consistently achieved higher segmentation success rates. This result indicates that, in shallow learning-based segmentation workflows, the design of the annotation strategy plays a critical role in determining classifier performance. To our knowledge, systematic validation of annotation strategies for shallow learning-based astrocyte segmentation has rarely been addressed explicitly. The resulting training guidelines may therefore facilitate reproducible astrocyte analysis in future studies.

Using the selected robust classifiers, we detected increased GFAP expression in scar-resident astrocytes (Fig. 5). This observation is consistent with multiple qualitative and semi-quantitative reports describing GFAP upregulation as a hallmark of reactive astrogliosis in foreign body response and other CNS injuries [35–37]. Importantly, our approach moves beyond binary or region-based assessments of GFAP intensity and instead enables single-cell-resolved quantitative analysis. Such cell-level resolution is essential for dissecting the heterogeneity of astrocyte responses within and around the glial scar.

Morphometric analysis revealed concordant enlargement of astrocytes in scar regions, including increased cell area and perimeter together with elongation of GFAP-positive processes. These findings are consistent with classical descriptions of reactive astrocyte hypertrophy involving both soma expansion and remodeling of the process arbor [30,31]. While GFAP labeling reflects the cytoskeletal domain rather than the full astrocyte volume, the coordinated increase in soma size and total process length observed here provides a quantitative account of cytoskeletal reorganization accompanying astrocyte activation.

Notably, the circularity shape descriptor exhibited greater variability across classifiers than size-related metrics. This observation indicates that parameters reflecting boundary geometry are more sensitive to segmentation differences and should therefore be interpreted cautiously. At the same time, such variability may help distinguish robust hypertrophic changes from features that depend more strongly on segmentation assumptions.

A particularly novel aspect of this study is the analysis of GFAP expression within astrocytic processes. Astrocyte processes form the primary interface with synapses, vasculature, and other glial elements and contribute critically to synaptic regulation, blood–brain barrier function, and scar organization [31,32]. Notably, the relative distribution of GFAP signal within astrocytic processes appeared shifted toward proximal compartments in scar regions, as indicated by a reduced distal-to-proximal intensity ratio (Fig. 6). To our knowledge, this quantitative pattern has not been explicitly assessed before; however, it is consistent with previously reported qualitative

descriptions of cytoskeletal remodeling and proximal enrichment of intermediate filaments in reactive astrocytes [30,38]. The observed decrease in the distal-to-proximal ratio may reflect increased local mechanical rigidity, redistribution of intermediate filaments toward sites of mechanical load, or formation of a structural “support core” around the implant accompanied by loss of thin distal processes [39,40]. These findings may be particularly relevant for understanding how astrocytes mechanically and biochemically contribute to electrode encapsulation.

The approach described here complements existing high-resolution 3D astrocyte reconstruction techniques, which provide exquisite morphological detail but are labor-intensive, low-throughput, and often rely on transgenic or viral labeling strategies. A recent study by Faust and coauthors implemented Labkit for that kind of high-resolution astrocyte imaging data analysis [41]. By contrast, our method is applicable to conventional GFAP immunostaining and low-resolution imaging, allowing rapid analysis of large numbers of cells and samples. This trade-off favors scalability and translational relevance, especially in studies aiming to compare implant materials, surface modifications, or pharmacological interventions across experimental cohorts.

Several limitations should be considered. GFAP labels only a subset of astrocytic cytoskeletal structures and does not capture the full extent of fine perisynaptic processes. Therefore the metrics reported here may be relevant for quantification of the implant-induced scarring but not for addressing accurately the whole cell morphology. Second, the number of analyzed cells was constrained by the density of GFAP-positive astrocytes outside the scar and by the availability of well-defined scar regions. Nevertheless, the methodological framework described here is readily scalable and could be applied to hundreds or thousands of cells in future studies. Finally, while we focused on GFAP as a canonical marker of astrogliosis, the same workflow could be extended to other astrocytic markers or combined with multiplexed imaging approaches.

In summary, shallow machine learning–assisted image analysis provides a reproducible and accessible framework for quantitative assessment of astrocyte responses to neuroimplantation. By enabling consistent single-cell and subcellular measurements across experiments, this approach supports high-throughput evaluation of foreign body response and has the potential to facilitate the development of strategies to improve neuroimplant biocompatibility. The combination of shallow-learning segmentation and explicit annotation rules may help standardize astrocyte morphometry in studies of neuroinflammation and neuroimplant biocompatibility.

4. Materials and Methods

4.1 Animals. All animal protocols and experimental procedures were performed in accordance with the requirements of Directive 2010/63/EC of the European Union and Order of the Ministry of Health of Russia of 19 June 2003 No. 267 and were approved by the Commission on Biological Safety and Bioethics of ITEB RAS (Pushchino, No. 40/2023 dated February 15, 2023).

4.2 Electrode implantation. Stereotaxic procedures were performed under general isoflurane anesthesia (1–4%). Nichrome electrodes (50 μ m in diameter) were implanted into the primary visual cortex (V1) at the following coordinates: AP 2.8 mm, ML 2.5 mm, DV 0.5 mm.

4.3 Immunohistochemistry. Five weeks after the beginning of the experiment, animals were deeply anesthetized and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed and post-fixed in the same

fixative for storage. Coronal sections (35 μ m thick) were cut using a Leica VT1200 S vibratome (Leica, Germany).

Brain sections were double stained for GFAP (anti-chicken ab4674, Abcam, USA) and perineuronal nets (Wisteria floribunda agglutinin, Vector Laboratories, USA). Staining was performed on free-floating sections. All incubation steps were carried out in 24-well plates. Following sectioning, samples were washed three times in phosphate-buffered saline containing 0.5% Triton X-100 (PBS-T; pH 7.4). Sections were then treated for 1 hour with a blocking solution of 1% bovine serum albumin (BSA; Sigma, USA) in PBS-T. Endogenous biotin was blocked using a Streptavidin/Biotin Blocking Kit (Vector Laboratories, USA) according to the manufacturer's protocol. Subsequently, sections were incubated overnight at 4°C with primary reagents diluted in 10 mM HEPES in PBS (pH 7.4): biotinylated WFA (1:500) and anti-chicken GFAP (1:500). After incubation, samples were washed three times for 5 min each with PBS-T. Sections were then incubated for 30 min with AlexaFluor 633-conjugated streptavidin (Invitrogen; dilution 1:100), followed by three 5-minute washes with PBS-T. That was followed by a 2-hour incubation with goat anti-chicken secondary antibody conjugated to AlexaFluor 488 (A11039; dilution 1:500). Finally, sections were washed three times for 5 minutes each with PBS-T, mounted on glass slides, air-dried, and coverslipped.

4.4 Microscopy. The sections were imaged with laser confocal microscope Leica TCS SP5 (Leica, Germany). Observations were performed using oil immersion lens HC X PL APO CS 20 $\times$ /0.70 IMM UV (Leica Microsystems, Wetzlar, Germany), pixel size 300 nm.

4.5 Image analysis. The FIJI software was used for image analysis. Astrocyte processes were traced with the Line tracer tool. Pixel intensity values along the traced processes were recorded. Average intensity values were calculated for 1) the whole process, 2) the most distal 10 pixels, 3) the most proximal 10 pixels.

4.6 Statistical analysis. Paired differences were quantified using estimation statistics, reporting mean paired differences with bootstrap confidence intervals rather than relying solely on null-hypothesis significance testing [42,43].

Standardized paired effect sizes (Cohen's d_z) were calculated to characterize the magnitude of within-animal differences [44,45].

Funding: The study was supported by the Russian Science Foundation, project number 24-75-00123. (<https://rscf.ru/en/project/24-75-00123/>.)

Institutional Review Board Statement: The study was conducted in accordance with the requirements of Directive 2010/63/EC of the European Union and Order of the Ministry of Health of Russia of 19 June 2003 No. 267 and were approved by the Commission on Biological Safety and Bioethics of ITEB RAS (Pushchino, No. 40/2023 dated February 15, 2023).

Acknowledgments: Part of the confocal microscopy investigations have been carried out using the equipment of Distributed Spectral-Analytical Center of Shared Facilities for Study of Structure, Composition and Properties of Substances and Materials of FRC Kazan Scientific Center of RAS. LFN and DVS work is supported by the government assignment for FRC Kazan Scientific Center of RAS.

Conflicts of Interest: The authors declare no conflicts of interest.

- 446 1. Eisenstein, M. Seven Technologies to Watch in 2024. *Nature* 2024, 625, 844–848, doi:10.1038/d41586-024-00173-x.
- 447 2. Willett, F.R.; Kunz, E.M.; Fan, C.; Avansino, D.T.; Wilson, G.H.; Choi, E.Y.; Kamdar, F.; Glasser, M.F.; Hochberg, L.R.; Druck-
448 mann, S.; et al. A High-Performance Speech Neuroprosthesis. *Nature* 2023, 620, 1031–1036, doi:10.1038/s41586-023-06377-x.
- 449 3. Gao, W.; Yan, Z.; Zhou, H.; Xie, Y.; Wang, H.; Yang, J.; Yu, J.; Ni, C.; Liu, P.; Xie, M.; et al. Revolutionizing Brain–computer
450 Interfaces: Overcoming Biocompatibility Challenges in Implantable Neural Interfaces. *J Nanobiotechnol* 2025, 23, 498, doi:10.1186/
451 s12951-025-03573-x.
- 452 4. Wu, X.; Ye, Y.; Sun, M.; Mei, Y.; Ji, B.; Wang, M.; Song, E. Recent Progress of Soft and Bioactive Materials in Flexible Bioelec-
453 tronics. *Cyborg Bionic Syst* 2025, 6, 0192, doi:10.34133/cbsystems.0192.
- 454 5. Kozai, T.D.Y.; Jaquins-Gerstl, A.S.; Vazquez, A.L.; Michael, A.C.; Cui, X.T. Brain Tissue Responses to Neural Implants Impact
455 Signal Sensitivity and Intervention Strategies. *ACS Chem. Neurosci.* 2015, 6, 48–67, doi:10.1021/cn500256e.
- 456 6. Paveliev, M.; Melnikova, A.; Egorchev, A.A.; Parpura, V.; Aganov, A.V. Neuroimplants and the Glial Scar: What Makes the
457 Brain–Computer Link Work? *Journal of Neurochemistry* 2025, 169, e70203, doi:10.1111/jnc.70203.
- 458 7. Polikov, V.S.; Tresco, P.A.; Reichert, W.M. Response of Brain Tissue to Chronically Implanted Neural Electrodes. *Journal of*
459 *Neuroscience Methods* 2005, 148, 1–18, doi:10.1016/j.jneumeth.2005.08.015.
- 460 8. Wellman, S.M.; Li, L.; Yaxiaer, Y.; McNamara, I.; Kozai, T.D.Y. Revealing Spatial and Temporal Patterns of Cell Death, Glial
461 Proliferation, and Blood-Brain Barrier Dysfunction Around Implanted Intracortical Neural Interfaces. *Front. Neurosci.* 2019, 13,
462 493, doi:10.3389/fnins.2019.00493.
- 463 9. Wellman, S.M.; Forrest, A.M.; Douglas, M.M.; Subbaraman, A.; Zhang, G.; Kozai, T.D.Y. Dynamic Changes in the Structure
464 and Function of Brain Mural Cells around Chronically Implanted Microelectrodes. *Biomaterials* 2025, 315, 122963, doi:10.1016/j.bio-
465 materials.2024.122963.
- 466 10. Savva, S.P.; Li, F.; Lam, S.; Wellman, S.M.; Stieger, K.C.; Chen, K.; Eles, J.R.; Kozai, T.D.Y. In Vivo Spatiotemporal Dynamics
467 of Astrocyte Reactivity Following Neural Electrode Implantation. *Biomaterials* 2022, 289, 121784, doi:10.1016/
468 j.biomaterials.2022.121784.
- 469 11. Sofroniew, M.V. Molecular Dissection of Reactive Astrogliosis and Glial Scar Formation. *Trends Neurosci* 2009, 32, 638–647,
470 doi:10.1016/j.tins.2009.08.002.
- 471 12. Cieri, M.B.; Ramos, A.J. Astrocytes, Reactive Astrogliosis, and Glial Scar Formation in Traumatic Brain Injury. *Neural Regen-*
472 *eration Research* 2025, 20, 973–989, doi:10.4103/NRR.NRR-D-23-02091.
- 473 13. Escartin, C.; Galea, E.; Lakatos, A.; O’Callaghan, J.P.; Petzold, G.C.; Serrano-Pozo, A.; Steinhäuser, C.; Volterra, A.;
474 Carmignoto, G.; Agarwal, A.; et al. Reactive Astrocyte Nomenclature, Definitions, and Future Directions. *Nat Neurosci* 2021, 24,
475 312–325, doi:10.1038/s41593-020-00783-4.
- 476 14. Verkhratsky, A.; Li, B.; Scuderi, C.; Parpura, V. Principles of Astroglipathology. In *Astrocytes in Psychiatric Disorders*; Li,
477 B., Parpura, V., Verkhratsky, A., Scuderi, C., Eds.; *Advances in Neurobiology*; Springer International Publishing: Cham, 2021; Vol.
478 26, pp. 55–73 ISBN 978-3-030-77374-8.
- 479 15. Kulkarni, P.M.; Barton, E.; Savelonas, M.; Padmanabhan, R.; Lu, Y.; Trett, K.; Shain, W.; Leasure, J.L.; Roysam, B. Quantita-
480 tive 3-D Analysis of GFAP Labeled Astrocytes from Fluorescence Confocal Images. *Journal of Neuroscience Methods* 2015, 246, 38–
481 51, doi:10.1016/j.jneumeth.2015.02.014.
- 482 16. Zhang, A.Y.; Elias, E.; Manners, M.T. Sex-Dependent Astrocyte Reactivity: Unveiling Chronic Stress-Induced Morphological
483 Changes across Multiple Brain Regions. *Neurobiology of Disease* 2024, 200, 106610, doi:10.1016/j.nbd.2024.106610.
- 484 17. Krikid, F.; Rositi, H.; Vacavant, A. State-of-the-Art Deep Learning Methods for Microscopic Image Segmentation: Applica-
485 tions to Cells, Nuclei, and Tissues. *J. Imaging* 2024, 10, 311, doi:10.3390/jimaging10120311.
- 486 18. Yang, R.; Xue, G.; Wang, Z.; Cai, Y.; Yang, W.; Que, J.; Tan, R.; Sun, H.; Wang, P.; Xu, Z.; et al. Systematic Evaluation of Com-
487 putational Methods for Cell Segmentation. *Briefings in Bioinformatics* 2026, 27, bbag066, doi:10.1093/bib/bbag066.
- 488 19. Moen, E.; Bannon, D.; Kudo, T.; Graf, W.; Covert, M.; Van Valen, D. Deep Learning for Cellular Image Analysis. *Nat Meth-*
489 *ods* 2019, 16, 1233–1246, doi:10.1038/s41592-019-0403-1.

20. Paveliev, M.; Egorchev, A.A.; Musin, F.; Lipachev, N.; Melnikova, A.; Gimadutdinov, R.M.; Kashipov, A.R.; Molotkov, D.; Chickrin, D.E.; Aganov, A.V. Perineuronal Net Microscopy: From Brain Pathology to Artificial Intelligence. *IJMS* 2024, 25, 4227, doi:10.3390/ijms25084227.

21. Bonato, J.; Curreli, S.; Romanzi, S.; Panzeri, S.; Fellin, T. ASTRA: A Deep Learning Algorithm for Fast Semantic Segmentation of Large-Scale Astrocytic Networks 2023.

22. Huang, Y.; Kruyer, A.; Syed, S.; Kayasandik, C.B.; Papadakis, M.; Labate, D. Automated Detection of GFAP-Labeled Astrocytes in Micrographs Using YOLOv5. *Sci Rep* 2022, 12, 22263, doi:10.1038/s41598-022-26698-7.

23. Berg, S.; Kutra, D.; Kroeger, T.; Straehle, C.N.; Kausler, B.X.; Haubold, C.; Schiegg, M.; Ales, J.; Beier, T.; Rudy, M.; et al. Ilastik: Interactive Machine Learning for (Bio)Image Analysis. *Nat Methods* 2019, 16, 1226–1232, doi:10.1038/s41592-019-0582-9.

24. Arzt, M.; Deschamps, J.; Schmied, C.; Pietzsch, T.; Schmidt, D.; Tomancak, P.; Haase, R.; Jug, F. LABKIT: Labeling and Segmentation Toolkit for Big Image Data. *Front. Comput. Sci.* 2022, 4, 777728, doi:https://doi.org/10.3389/fcomp.2022.777728.

25. Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; et al. Fiji: An Open-Source Platform for Biological-Image Analysis. *Nat Methods* 2012, 9, 676–682, doi:10.1038/nmeth.2019.

26. Schroeder, A.B.; Dobson, E.T.A.; Rueden, C.T.; Tomancak, P.; Jug, F.; Eliceiri, K.W. The IMAGEJ Ecosystem: Open-source Software for Image Visualization, Processing, and Analysis. *Protein Science* 2021, 30, 234–249, doi:10.1002/pro.3993.

27. Patai, R.; Patel, K.; Csik, B.; Gulej, R.; Nagaraja, R.Y.; Nagy, D.; Chandragiri, S.S.; Shanmugarama, S.; Kordestan, K.V.; Nagykaldi, M.; et al. Aging, Mitochondrial Dysfunction, and Cerebral Microhemorrhages: A Preclinical Evaluation of SS-31 (Elamipretide) and Development of a High-Throughput Machine Learning-Driven Imaging Pipeline for Cerebromicrovascular Protection Therapeutic Screening. *GeroScience* 2025, 47, 4871–4887, doi:10.1007/s11357-025-01634-5.

28. Paveliev, M.; Melnikova, A.; Samigullin, D.V.; Egorchev, A.A.; Titova, A.A.; Kiyasov, A.P.; Popova, I.Yu.; Parpura, V.; Aganov, A.V. Second Harmonic Generation for Brain Imaging: Pathology-Related Studies. *Biophys Rev* 2025, 17, 1563–1582, doi:10.1007/s12551-025-01370-2.

29. Lipachev, N.; Arnst, N.; Melnikova, A.; Jääliñoja, H.; Kochneva, A.; Zhigalov, A.; Kuleshkaya, N.; Aganov, A.V.; Mavlikeev, M.; Rauvala, H.; et al. Quantitative Changes in Perineuronal Nets in Development and Posttraumatic Condition. *J Mol Hist* 2019, 50, 203–216, doi:10.1007/s10735-019-09818-y.

30. Sun, D.; Jakobs, T.C. Structural Remodeling of Astrocytes in the Injured CNS. *Neuroscientist* 2012, 18, 567–588, doi:10.1177/1073858411423441.

31. Díaz-Castro, B.; Robel, S.; Mishra, A. Astrocyte Endfeet in Brain Function and Pathology: Open Questions. *Annu. Rev. Neurosci.* 2023, 46, 101–121, doi:10.1146/annurev-neuro-091922-031205.

32. Liu, X.; Ying, J.; Wang, X.; Zheng, Q.; Zhao, T.; Yoon, S.; Yu, W.; Yang, D.; Fang, Y.; Hua, F. Astrocytes in Neural Circuits: Key Factors in Synaptic Regulation and Potential Targets for Neurodevelopmental Disorders. *Front. Mol. Neurosci.* 2021, 14, 729273, doi:10.3389/fnmol.2021.729273.

33. Yan, Z.; Gao, W.; Duan, Y.; Zhou, H.; Ni, C.; Huang, L.; Ye, Z. Bidirectional Mechanisms and Emerging Strategies for Implantable Bioelectronic Interfaces. *Bioactive Materials* 2025, 52, 634–667, doi:10.1016/j.bioactmat.2025.06.014.

34. Parpura, V.; Heneka, M.T.; Montana, V.; Oliet, S.H.R.; Schousboe, A.; Haydon, P.G.; Stout, R.F.; Spray, D.C.; Reichenbach, A.; Pannicke, T.; et al. Glial Cells in (Patho)Physiology. *Journal of Neurochemistry* 2012, 121, 4–27, doi:10.1111/j.1471-4159.2012.07664.x.

35. Luijckink, L.; Rodriguez, M.; Machaalani, R. Quantifying GFAP Immunohistochemistry in the Brain – Introduction of the Reactivity Score (R-Score) and How It Compares to Other Methodologies. *Journal of Neuroscience Methods* 2024, 402, 110025, doi:10.1016/j.jneumeth.2023.110025.

36. Yu, G.; Zhang, Y.; Ning, B. Reactive Astrocytes in Central Nervous System Injury: Subgroup and Potential Therapy. *Front Cell Neurosci* 2021, 15, 792764, doi:10.3389/fncel.2021.792764.

37. Moulson, A.J.; Squair, J.W.; Franklin, R.J.M.; Tetzlaff, W.; Assinck, P. Diversity of Reactive Astroglia in CNS Pathology: Heterogeneity or Plasticity? *Front. Cell. Neurosci.* 2021, 15, 703810, doi:10.3389/fncel.2021.703810.

38. Sofroniew, M.V.; Vinters, H.V. Astrocytes: Biology and Pathology. *Acta Neuropathol* 2010, 119, 7–35, doi:10.1007/s00401-009-0619-8.

536 39. Wilhelmsson, U.; Li, L.; Pekna, M.; Berthold, C.-H.; Blom, S.; Eliasson, C.; Renner, O.; Bushong, E.; Ellisman, M.; Morgan,
537 T.E.; et al. Absence of Glial Fibrillary Acidic Protein and Vimentin Prevents Hypertrophy of Astrocytic Processes and Improves
538 Post-Traumatic Regeneration. *J Neurosci* 2004, 24, 5016–5021, doi:10.1523/JNEUROSCI.0820-04.2004.

539 40. Burda, J.E.; Sofroniew, M.V. Reactive Gliosis and the Multicellular Response to CNS Damage and Disease. *Neuron* 2014, 81,
540 229–248, doi:10.1016/j.neuron.2013.12.034.

541 41. Faust, T.E.; Lee, Y.-H.; O'Connor, C.D.; Boyle, M.A.; Gunner, G.; Durán-Laforet, V.; Ferrari, L.L.; Murphy, R.E.; Badimon, A.;
542 Sakers, K.; et al. Microglia-Astrocyte Crosstalk Regulates Synapse Remodeling via Wnt Signaling. *Cell* 2025, 188, 5212-5230.e21,
543 doi:10.1016/j.cell.2025.08.023.

544 42. Efron, B.; Tibshirani, R.J. *An Introduction to the Bootstrap*; Springer US: Boston, MA, 1993; ISBN 978-0-412-04231-7.

545 43. Ho, J.; Tumkaya, T.; Aryal, S.; Choi, H.; Claridge-Chang, A. Moving beyond P Values: Data Analysis with Estimation Graph-
546 ics. *Nat Methods* 2019, 16, 565–566, doi:10.1038/s41592-019-0470-3.

547 44. Cohen, J. *Statistical Power Analysis for the Behavioral Sciences*; 0 ed.; Routledge, 1988; ISBN 978-1-134-74270-7.

548 45. Lakens, D. Calculating and Reporting Effect Sizes to Facilitate Cumulative Science: A Practical Primer for t-Tests and
549 ANOVAs. *Front. Psychol.* 2013, 4, doi:10.3389/fpsyg.2013.00863.

550