Istanbul Medipol University

School of Engineering and Natural Sciences

Graduation Project

2022-2023

PROJECT TITLE
Artificial Intelligence Based Ischemia Detection on NISSL-Body Stained Mouse Brain Sections
PROJECT ADVISOR
Prof. Dr. Mehmet Kemal ÖZDEMİR
TEAM MEMBERS
Muhammed Furkan DAŞDELEN Sezgin ER

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Project Code
Project Title: Artificial Intelligence Based Ischemia Detection on NISSL-Body Stained Mouse Brain Sections
Project Advisor: Prof. Dr. Mehmet Kemal Özdemir
Project Team Members: Muhammed Furkan Daşdelen Sezgin Er
Sponsor Company (if any) : Tubitak 2209 student project

BUDGET (TL)	PROPOSED	CONSENTED
IMU FUNDING	-	-
SPONSOR COMPANY FUNDING	6000 TL	6000 TL
TOTAL	6000 TL	6000 TL

PROJECT PLAN	PROPOSED	CONSENTED
PROJECT PLAN Duration in Weeks	28 weeks	28 Weeks
STARTING DATE		

Istanbul Medipol University

School of Engineering and Natural Sciences

Graduation Project

Project Code	
PROJECT ADVISOR	**DEPARTMENT CHAIR**
Name: Mehmet Kemal Özdemir	**Name:** Mehmet Kemal Özdemir
Contact Information: Tel : E-mail : mkozdemir@medipol.edu.tr	**Contact Information:** Tel : E-mail : mkozdemir@medipol.edu.tr
Signature:	**Signature:**

TEAM MEMBER	**TEAM MEMBER**
Name: Muhammed Furkan Daşdelen	**Name:** Sezgin Er
Contact Information: Tel : 0 553 208 14 28 E-mail : muhammed.dasdelen@std.medipol.edu.tr	**Contact Information:** Tel : 0 507 426 24 98 E-mail : sezgin.er@std.medipol.edu.tr
Signature:	**Signature:**

TEAM MEMBER	**Sponsor Company**
Name:	**Name:**
Contact Information: Tel : E-mail :	**Contact Information:** Tel : E-mail :
Signature:	**Signature:**

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Project Advisor: Mehmet Kemal ÖZDEMİR
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Project Group Title:

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PROJECT OVERVIEW/SUMMARY/ABSTRACT

Stroke remains a predominant cause of mortality across the globe, accounting for numerous fatalities each year and maintaining its position as the second leading cause of death. Consequently, extensive efforts to develop treatments, preventive measures, and therapies for stroke, as well as to investigate its pathology and molecular mechanisms, are crucial. Animal-based studies, particularly those involving mouse stroke models, form a vital part of these endeavors. In these studies, stroke severity is gauged by staining the NISSL body in the brain, followed by calculating the ischemic (damaged) volume in the striatum and cortex and enumerating neurons within these regions. However, these methods are entirely manual, consuming a considerable amount of researchers' time and extending the duration of the investigations. Moreover, the manual nature of these procedures enhances the potential for human error and obstructs standardization.

Although the literature contains applications capable of detecting different regions in histological images in both 2D and 3D, none are capable of segmenting brain regions on damaged brains. Additionally, most algorithms mentioned in the literature function on computer tomography, magnetic resonance, or high-resolution microscopy images. In this project, we aimed to develop a new program capable of swiftly and accurately calculating the size and expansion (edema) of damage inflicted by ischemia after stroke in low-quality scanned damaged mouse brains. We achieved this goal by implementing models designed using state-of-the-art methods. Initially, the position of the section relative to the reference atlas was determined in the isolated ischemic brain sections using YOLOv7 to detect objects from multiple brain images. Subsequently, the boundaries of the striatum and hemisphere were ascertained using a new transformer-based U-net model. This step enabled us to determine the amount of edema present in the damaged hemisphere. Lastly, we used a deep learning approach based on a pix2pix model to identify the ischemic area.

The artificial intelligence software we developed using deep learning models has the potential to significantly assist researchers in their work, providing more accurate and objective results while reducing the time and cost of research. Furthermore, our software contributes to the standardization of stroke models, thus facilitating a more reliable and reproducible research environment.

Keywords: Deep learning, transformer model, cerebral ischemia, NISSL-body staining

1. OBJECTIVE OF THE PROJECT:

The primary objective of this project was to engineer a state-of-the-art workflow utilizing deep learning methodologies to automate traditionally manual analyses in stroke research. We have effectively accelerated the speed and precision of data collection and analysis, leading to more

comprehensive and reliable results in the field. Our specific achievements include the development of an advanced system capable of efficiently isolating the brain from multiple images. This has significantly streamlined our approach to studying brain structures. Additionally, we've automated the process of cerebral and hemisphere edema calculation, a traditionally labor-intensive and time-consuming task. This automation has resulted in an impressive increase in data processing speed and analysis. Furthermore, we've incorporated ischemic core and penumbra measurements through ischemia segmentation. This innovative approach has deepened our understanding of stroke impacts and will undoubtedly assist in the development of more effective treatments and interventions.

2. LITERATURE REVIEW:

Stroke is characterized as a neurological impairment arising from an immediate localized injury to the central nervous system (CNS) due to a vascular incident. A significant portion of strokes occur from ischemia, which is a result of reduced blood flow due to arterial obstruction, although they can also occur from venous or venous sinus blockage or a cerebral artery rupture leading to hemorrhage. Each year, globally, about 9.6 million ischemic and 4.1 million hemorrhagic stroke cases are recorded. Nonetheless, stroke is the second major cause of mortality and the third leading contributor to global disability (Campbell and Khatri, 2020).

Focal cerebral ischemia mouse models are used by researchers to explore the molecular mechanisms and pathophysiology of stroke, and subsequently to devise targeted drugs and therapies to mitigate the effects or progression of stroke. These models are induced by occluding the middle cerebral artery (MCAo), primarily resulting in ischemic cell death in a part of the striatum and the surrounding cortical regions (Carmichael, 2005). Researchers examine the brains of these ischemic mice to gauge the extent of the stroke, taking consecutive sections from these brains to measure both the damaged area and the edema area, indicating the extent of cerebral damage in mice. To determine the ischemic area and detect the area of edema, a standard procedure, cresyl staining, is used (Türeyen et al., 2004). This staining procedure colors neural cells, known as Nissl bodies, making them visible under light microscopy (Alvarez-Buylla et al., 1990). Consequently, brain sections can clearly distinguish between neuron-rich regions like the striatum and cortex, and neuron-sparse areas such as the cingulum, ventricle, and corpus callosum. The damaged areas are then identified by calculating the areas of the striatum and cortex in the stained brain sections (Türeyen et al., 2004). However, the manual implementation of this method extends the research process and increases the researchers' workload.

With the expansion of data sets and computing capabilities, deep learning techniques have emerged as solutions to numerous medical challenges including computer-assisted diagnosis, disease prediction, and image segmentation (Kim et al., 2019). Semantic segmentation, a type of image segmentation, involves assigning a class value to every pixel in an image (Yuan et al., 2021). Convolutional neural network (CNN)-based methodologies have notably enhanced the performance in semantic segmentation recently, achieving high accuracy in segmentation tasks on MRI, CT, X-ray images, and histopathology sections from various organs (Kim et al., 2019). U-Net is one of the most successful neural networks for medical image semantic segmentation.

U-Net and similar networks, with their encoder and decoder parts and intermediate skip connections, have shown remarkable success in medical image segmentation. U-Net has demonstrated further success in medical segmentation when paired with transform-based models (Cao et al., 2021).

Existing literature has documented studies on segmentation and identification of brain regions in cresyl-stained mouse brain sections (11,12), but these studies have been performed on healthy tissues and high-resolution microscopy images. Until now, studies aiming to identify ischemic areas have primarily relied on CT or MRI images (13,14). In this study, our goal was to objectively determine the location of the brain section by aligning scanned, low-resolution, damaged mouse brains with a reference atlas, and then for the first time in the literature, using semi-supervised learning to identify the striatum and hemisphere areas on damaged brain sections. Additionally, we aimed to devise an automatic system to detect the damaged area in these brains for the first time. We intend to develop deep learning models utilizing transformer-based U-Net tools. Thus, developed a transformer-based U-Net model studied on histopathological mouse brain images.

3. ORIGINALITY:

Prior investigations primarily employed high-resolution MRI and microscopic imagery for similar evaluations. Nonetheless, this project has innovatively automated the procedure for low-resolution scanned brain images, which are frequently used in stroke research. By contrast, this work serves to bridge a gap in the existing literature, providing a solution not dependent on high-resolution imaging modalities. Moreover, this is the first initiative to develop an all-inclusive workflow encapsulating every necessary analysis. This workflow offers a unified and streamlined methodology, thus enhancing the overall efficiency and effectiveness of the process. As a whole, this project constitutes a significant leap forward in the field, carrying considerable potential to enhance stroke research.

4. EXPERIMENTS/METHODS:

In this project, seven work packages have been employed to automate manual analyses in ischemia studies using deep learning-based methods:

Work Package 1 - Data Collection and Creation of Data Set
Work Package 2 - Detection and Preprocessing of Brains
Work Package 3 - Partial Annotation of Data for Striatum Region and Hemispheres
Work Package 4 - Determination of Brain Section Position by Alignment with Mouse Brain Reference Atlas
Work Package 5 - Determination of Striatum and Hemisphere Segmentation with Semi-supervised Learning
Work Package 6 - Determination of Ischemic Center and Penumbra Segmentation with Deep Learning
Work Package 7 - Detection of Ischemic Area and Brain Edema

In each Work Package, the method that was followed in accordance with the purpose was explained, and the algorithms that were to be used were written in detail. In summary, in this project, the automatic determination of the ischemic area, striatum edema, and hemisphere edema from ischemic brains was targeted. For this purpose, the images of each brain were exported from the input images, the position of the brain section was determined according to the bregma, segmentation was performed for the position-determined brain image for the ischemic area, striatum, and hemisphere, and then the ischemic area and brain edema analyses were performed using this segmentation information (Figure 2.1).

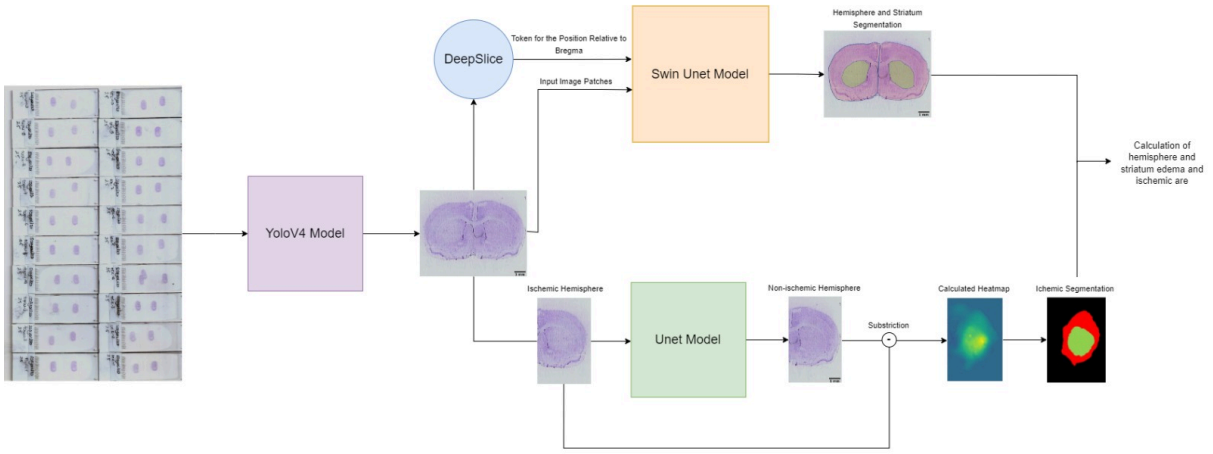

Figure2.1. Flow chart of experimental design and work packages.

Work Package 1 - Data Collection and Creation of Data Set:
1000 coronal ischemic mouse brain sections stained with Cresyl Violet and NISSL were obtained from Istanbul Medipol University SABITA Brain Research Laboratory. The brain sections were taken from various positions according to the bregma. Thus, measurement of the damage area with the model we created could be easily done after the middle cerebral artery damage model was formed in mice. The previously stained samples had been photographed with a scanner. We measured the outcome of this work package by the number of the brain slice images and 1000 images were counted as successful.

Work Package 2 - Detection and Preprocessing of Brains:
Since there were more than one brain section in a scan (see: Attachments, Attachments), the photos were marked with bounding boxes that determined the boundaries of the brains, and an object detection model (YOLOv7) trained with these marks enabled the export of each brain section (Figure 2.2). YOLOv7 (Bochkovskiy et al.) was a preferred object detection model because it performed more efficiently and successfully on the MS COCO object detection data set compared to other object detection models. The exported brains were then subjected to normalization for contrast for further analysis. In this step, the IoU (Intersect over Union), dice coefficient, and localization distance methods were used to test that the brains had been properly isolated. Mean IoU greater than 0.7 was counted as successful as described later in detail.

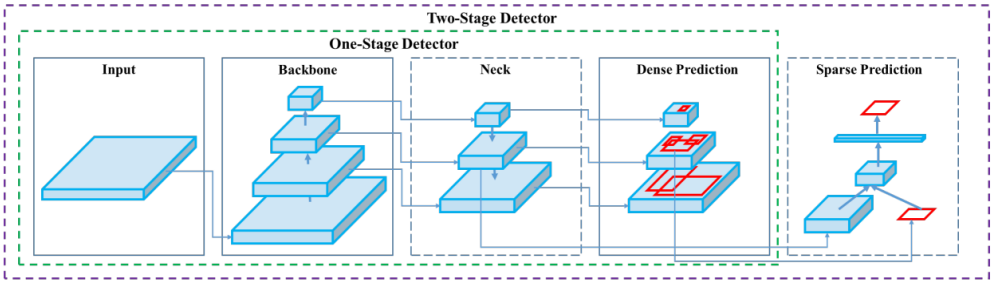

Figure.2.2. The representative design of YoloV4 (Bochkovskiy et.al.)

Work Package 3 - Partial Annotation of Data for Striatum Region and Hemispheres:
30% (300 brain sections) of the obtained data were labeled by expert researchers in the field under the supervision of the striatum region and hemisphere region. The areas of these regions were important in ischemia studies, so with this labeling, the areas of these regions could be determined with the segmentation model that was trained afterwards. The number of successfully labeled brain slices was used to measure the output of this work package.

Work Package 4 - Determination of Brain Section Position by Alignment with Mouse Brain Reference Atlas:
To successfully align the mouse brains with the reference atlas, the ischemic area (left hemisphere of the brain) was removed from the brain sections, and the image of the right hemisphere was combined with the mirror image of the right hemisphere. The resulting images were then aligned with the Allen Mouse Brain Atlas using the DeepSlice tool (Carey et al.). This determined the locations and positions of the brain sections according to the bregma in the brain. The images of the brain sections differed in different brain regions, so the area of the striatum differed in different brain regions. Therefore, the location information obtained from here was used for training of subsequent models. Correlation with the real location in the brain was used to measure output. A correlation greater than 0.95 was counted as successful in this work package.

Work Package 5 - Determination of Striatum and Hemisphere Segmentation with Semi-supervised Learning:
Visual transformer models were models based on transformer models that worked by dividing the image into parts and were particularly successful when there was a large amount of data, especially in recent years. Newly developed visual transformer models performed better than advanced Convolutional Neural Networks (CNNs) such as U-NET in object detection and segmentation problems even with a small amount of data set. For example, Swin-Unet was a model that combined Swin transformer blocks in a structure similar to Unet (hierarchical piece merging and skip connection) and performed better than Unet-based models in many segmentation problems (Cao et al.) (Figure 2.3). The Swin-Unet model used the shifted windows strategy instead of multi-head self-attention in its Swin transformer blocks, so it had a lower computational workload and could be trained faster. Therefore, we used the Swin-Unet model in the segmentation of the striatum and hemisphere. The sizes of the striatum in the brain sections differed depending on the position of the section according to the bregma, so when training the Swin-Unet model, tokens specifying the position of the brain sections were given

as input to the model along with the parts of the input images. We aimed to make it easier for the model to be trained in this way. Since labeling the striatum and hemispheres took a long time and the training problem of the model was not a very difficult problem, the model was pre-trained on unlabeled data (%70 - 700 brain sections). In pre-training, contrastive learning was used to determine the image-related features of the model. In this learning method, the aim was for the backbone of the model to output the same result independently of the augmentation applied to the input images, and the loss function of the model was determined accordingly. The weights of the model pre-trained in this way were saved and then trained for segmentation with %30 labeled data (300 images). The performance of the model was evaluated using the IoU (Intersection-over-Union), dice coefficient, precision, and recall metrics. Mean IoU greater than 0.7 was counted as successful.

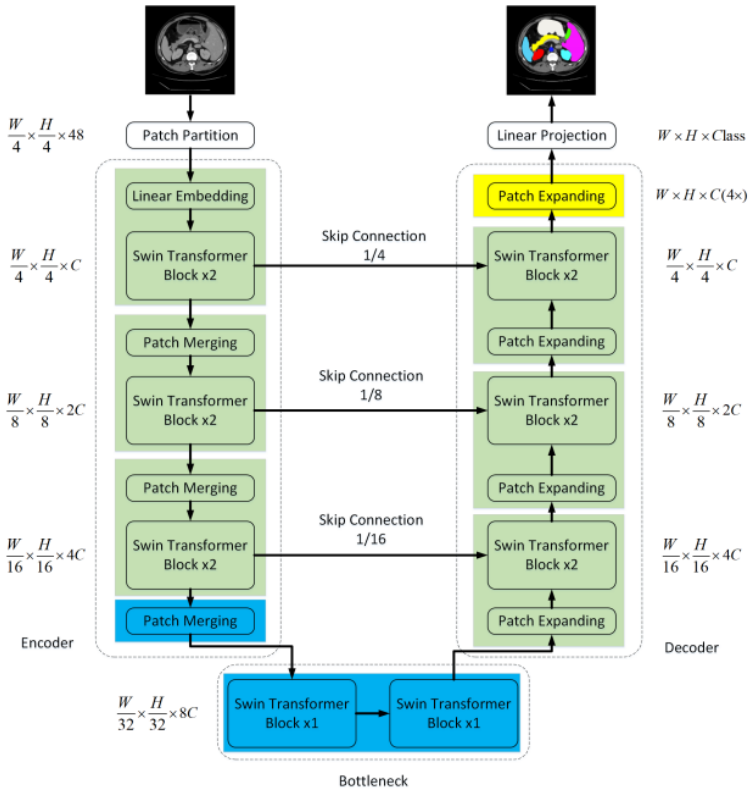

Figure2.3. The design of Swin-Unet. (Cao et.al.)

Work Package 6 - Determination of Ischemic Area Segmentation using Deep Learning: A model based on the Pix2Pix framework, which is essentially an adapted U-Net (Ronneberger et al.) architecture used in a generative adversarial network (GAN) setting, was trained to determine the segmentation of the ischemic center and penumbra (Figure 2.4). This Pix2Pix model was a conditional GAN that aimed to learn a mapping from an input image to an output image.

For this purpose, healthy hemispheres were mirrored and some random noise added on images to create fake ischemia. As output, original healthy hemispheres given. The model trained to transform from fake ischemic images to real healthy hemispheres. So, the model were expected

to learns images pixel by pixel and fill the ischemic areas accordingly. Then, the trained model was used to generate the non-ischemic hemisphere from the real ischemic hemisphere. Subtracting the image of the ischemic hemisphere from the generated image of the non-ischemic hemisphere produced a probability map showing the ischemic area. The ischemic area segmentation was determined using this probability map.

The Pix2Pix model was particularly suitable for this task as it excels at image-to-image translation tasks where both the input and output are images. The success of the model was evaluated based on pixel accuracy and L1 loss, which were suitable metrics considering the task at hand. Pixel accuracy greater than 0.90 was counted as successful. This Pix2Pix-based approach helped leverage the strengths of GANs in capturing complex image distributions, thus improving the results of ischemic area segmentation.

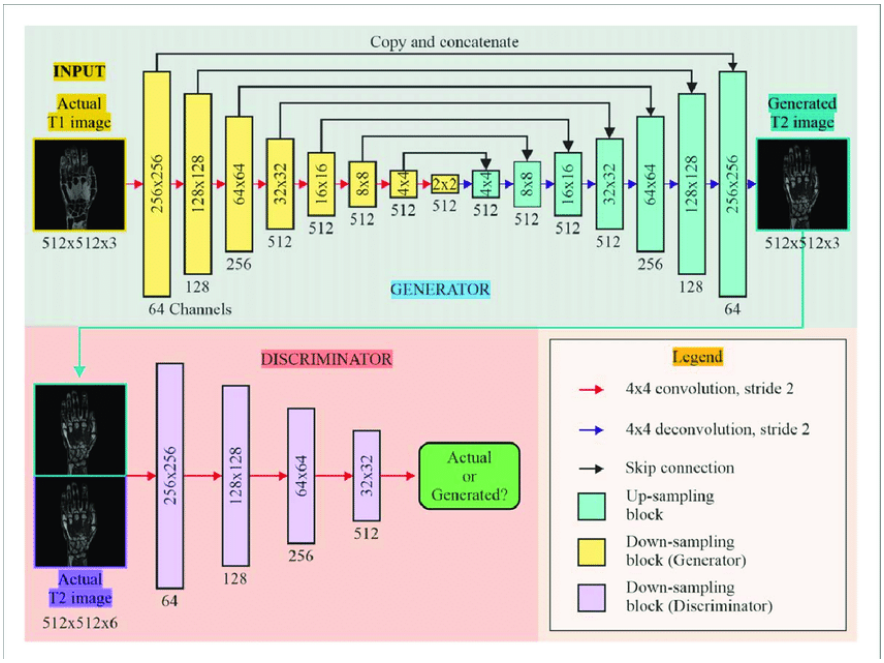

Figure2.4. The design of Pix2pix. (Isola et.al.)

Work Package 7 - Detection of Ischemic Area and Brain Edema:
The detection of ischemic area and striatum and hemispheric edema was frequently used in stroke research. Using the segmentation information obtained from the segmentation models trained in Work Package 5 and Work Package 6, the aim was to determine the ischemic area in the ischemic hemisphere and the striatum and hemispheric area in both hemispheres. After the striatum and hemispheric area were determined in both hemispheres, the ischemic edema was determined by comparing the striatum and hemispheric area of the ischemic hemisphere (hemisphere with ischemic edema) to the healthy hemisphere. We measured the output of this work package by comparing the results with manually detected ischemic area and brain edema.

5. PROJECT TARGETS AND SUCCESS CRITERIA:

In this project, we aimed to identify the edema area and damage area in brain slices of mice induced with ischemia model and stained with NISSL. To achieve this goal, we successfully completed the following steps:

1. Object detection: Brain images scanned multiple times were isolated one by one using an object detection algorithm. This approach saved researchers from having to cut and measure each brain separately.
2. Determination of the position of the brain slice: We determined exactly from which position of the mouse brain the brain slices, one hemisphere damaged and the other healthy, were taken. This method eliminated potential different interpretations among researchers and provided a more objective evaluation.
3. Striatum and hemisphere detection in the brain was carried out, and the amount of edema in the damaged region, an important parameter in ischemia studies, was calculated.
4. Deep learning-based ischemia detection was carried out in order to measure the size of the damage occurring in the damaged hemisphere. In addition, the center of ischemia and penumbra in the ischemic area were also detected (regions affected around the center of ischemia). This method allowed for the analysis of these areas that researchers cannot distinguish visually to be done by a computer.

With this software we developed, we aimed to enable researchers working on ischemia in animals to obtain faster and more accurate results. In addition, the model flow we set up created a new schema for deep learning processing of other histological and medical images of brain ischemia.

As for our overall success criteria, we achieved the following conditions:

Work Package 1 and 2 - Data Collection and Isolation of Brains:

- We began by amassing a collection of 150 images, each of which contained multiple brains. By employing the YOLOv7 object detection model, we managed to isolate these brains, ending up with around 6000 individual instances. We achieved 0.98 mean average precision 0.5 (mAP50) value in the detection of brain images. Following this, we meticulously filtered through this dataset to exclude any brains that did not display signs of ischemia. As a result of this careful selection process, we were left with a refined collection of 3000 brain images that all featured signs of ischemia. Further experiments were carried out by using those 3000 brain images.

Work Package 3 and 4 – Annotation of Data and Alignment of Brain Slices

- 300 ischemic images were annotated by two different experts for hemisphere and striatum segmentation. Then, images were aligned to their original position in the brain by using DeepSlice. Their position were further confirmed by visualizing the brains in QuickNII.

Work Package 5 - Determination of Striatum and Hemisphere Segmentation with Semi-supervised Learning

- We achieved following values by using Swin-Unet for segmentation task:

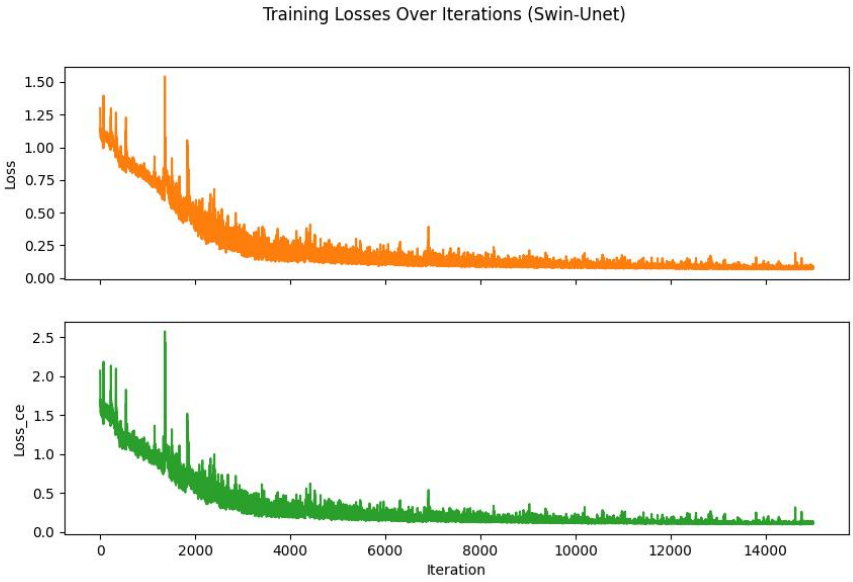

Figure 3. Training loss graph of swin-unet

- Upon completing the training phase with a dataset of 300 images, we proceeded to apply our developed model on a larger set of 3000 ischemic brain images for the purpose of segmentation. Utilizing the segmentation data, we were able to identify and demarcate the hemisphere and striatal areas of these brains. Consequently, this enabled us to calculate the amount of edema, a key factor in stroke assessment, present in each brain.

Work Package 6 - Determination of Ischemic Area Segmentation using Deep Learning

- The model underwent training using simulated ischemic brain images, and impressively, it achieved a pixel accuracy of 90%.

Work package	Target	Measurable outcome	Contribution to overall success (%)
WP1	Image collection and dataset formation	Collection of 1000 brain images	10
WP2	Brain isolation from multiple brain objects and pre-processing	Isolation of brain images into separate files (IoU>0.7)	10
WP3	Labeling of striatum and hemispheres	Successful labeling of striatum and hemispheres by specialists	15
WP4	Image registration to allen mouse brain atlas by using DeepSlice to healthy hemispheres and determination of brain slice position	>95% correlation of real brain positions with mirrored healthy hemisphere brain positions	5

WP5	Semi-supervised learning by using u-net based transformer models for detection of striatum and hemispheres	>0.7 IoU value of detected striatum	25
WP6	Detection of ischemic core and penumbra by using u-net based models	Pixel accuracy of detected ischemic area > 90%	25
WP7	Calculation of ischemic area and edema	No significant difference between the model outputs and the measurements done by a specialists	10
			Total: 100

6. RISKS AND B PLANS:

Work Package #	Risk	B-Plan
WP 1-7	We could not access to server for computational source for a while	We prepared our models and data until we reach the cluster. However, this preparation process resulted in a significant expenditure of time, leading to some delay in our progress
WP 2	Brains cannot be isolated from multiple brain images	We first tried Diffusion based object detection algorithm. But due to high computational source need and data, we used Yolov7.
WP4	Not able to determine brain positions according to reference atlas	We successfully aligned brain images to their position. However, determining their position did not improve the Swin-unet model training accuracy. So, we decided to exclude extra token coming from their position while training the model.
WP6	Not able to differentiate core and penumbra on ischemic area	Calculation on total ischemic area

7. WORK TIME PLAN OF THE PROJECT:

Work Plan of the Project (*)

Work Package #	Work Package Name	Done by Whom	Time Interval (..-.. Months)
WP 1	Image collection and dataset formation	MFD	0-1. months
WP 2	Brain isolation from multiple brain objects and pre-processing	MFD, SE	1-2. months
WP 3	Labeling of striatum and hemispheres	MFD, MSK	2-3. months
WP 4	Image registration to allen mouse brain atlas by using DeepSlice to healthy hemispheres and determination of brain slice position	MFD, SE	3-4. months
WP 5	Semi-supervised learning by using u-net based transformer models for detection of striatum and hemispheres	MFD, SE	4-5. months
WP 6	Detection of ischemic core and penumbra by using pix2pix model	SE	5-6. months
WP 7	Calculation of ischemic area and edema	MFD, SE	6-7. months

- MFD: Muhammed Furkan Daşdelen
- SE: Sezgin Er
- MSK: Mehmed Serhed Kuzu

We used the DeepSlice algorithm to align the brain slices in our study. Following this, we performed two separate experiments for the training of the Swin-Unet model. In the first experiment, we incorporated the position information of each brain slice into the background as noise. Conversely, in the second experiment, no additional information was supplied. The model's accuracy indicated no significant difference between the two experiments, leading us to the decision of excluding brain alignment from the process.

However, to aid segmentation, we created a total of seven distinct classes. For slices which included the striatum, the following segmentation categories were used:

- Ischemic hemisphere,
- Ischemic striatum,
- Non-ischemic hemisphere,
- Non-ischemic striatum.

For slices that did not include the striatum region, we utilized the following segmentation classes:

- Ipsilateral (ipsi) hemisphere,
- Contralateral (contra) hemisphere.

By employing different hemisphere classes for various positions of slices, we effectively reinforced our model's ability to learn and recognize different brain positions.

8. DEMO PLAN:

For our demonstration, we plan to provide a side-by-side comparison of manual and automated processes in assessing ischemic areas and edema on brain slices.

To illustrate this, we will begin by selecting a researcher who will manually analyze a set of brain slices, while simultaneously, our model will undertake the same task on a randomly chosen dataset. The idea behind this is to visually display the process of how traditional, manual calculations of ischemic areas and edema are performed, while also showing how our model operates in real-time.

As the researcher delves into the meticulous process of identifying and measuring ischemic areas and edema, our model will be scanning, analyzing, and outputting its findings on the selected data simultaneously. Viewers will be able to observe the ease and efficiency with which our model accomplishes these tasks, presenting the results clearly and concisely.

Our key aim for this demonstration is to showcase not only the functionality of our model and the outputs it generates but also to highlight how it substantially reduces the burden on researchers. We want to underline the stark contrast between the time and effort consumed in manual analysis versus the convenience and speed of using our automated model. Ultimately, we hope to convey how the application of our model can significantly streamline research processes, making it a valuable tool for those studying ischemia.

9. FINANCIAL EVALUATION:

We successfully secured funding for our project through TUBITAK 2209-a, receiving a grant of 6000 TL (Table 7). A significant advantage of our project is that we were able to conduct our model training using the advanced computing clusters provided by Medipol University, eliminating the need for additional computational expenses. With this funding, we will develop a user-friendly web-based interface that enables researchers to conveniently upload scanned brain slices and receive automated analysis results in the future with the results of our project. This interface will greatly enhance the efficiency and accessibility of our trained models, facilitating advanced brain research and analysis in a streamlined manner.

***Table 1**. Actual Budget in TL (what you spent indeed)*

	ITEMS				
	PEOPLE	MACHINE-INSTRUMENT*	MATERIALS*	SERVICE	TRAVEL
IMU FUND	0	0	0	0	0
SPONSOR COMPANY FUND	0	0	6000	0	0
TOTAL	0	0	6000	0	0

10. RESULTS:

A) Object Detection with Yolov7

Figure 4 displays the successful outcomes of our Yolov7 model in addressing the brain detection problem outlined in WP2. Our model achieved high accuracy in detecting brains, as evidenced by the results. By utilizing the detected brain coordinates, we were able to crop individual brain slices, as shown in Figure 5. These cropped slices provide isolated images for further analysis.

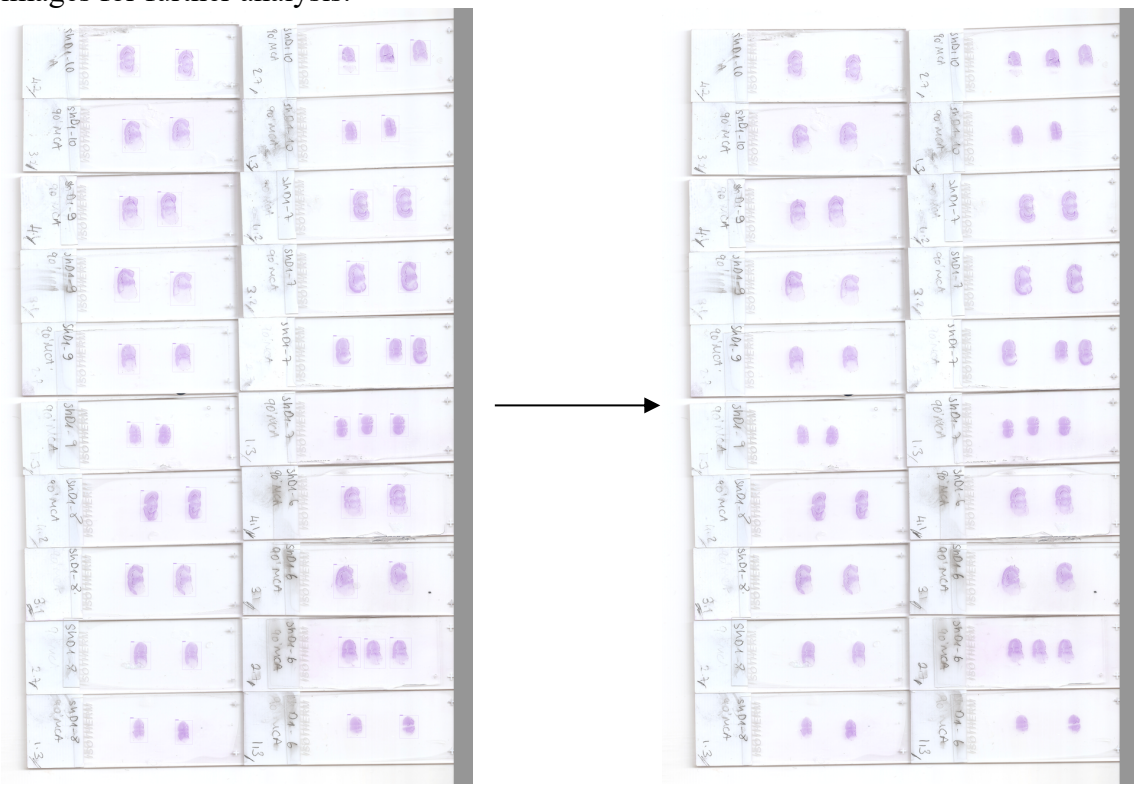

Figure 4. Detection of brains with Yolov7

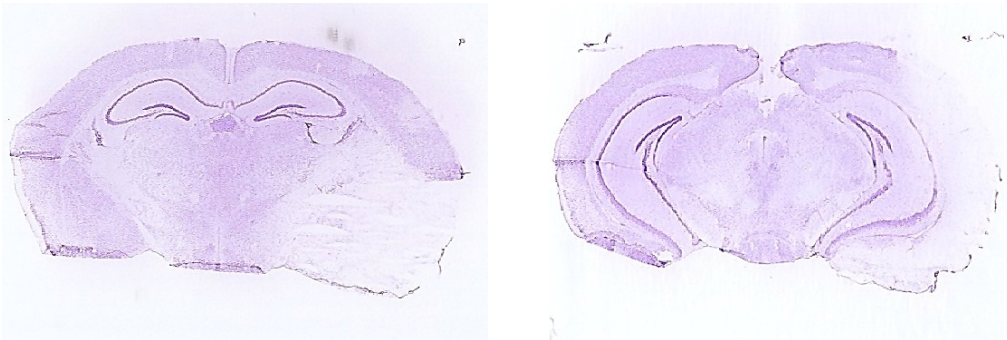

Figure 5. Isolated brain images

Figure 6 presents performance metrics evaluating the accuracy of our detection model. These metrics include box accuracy, objectness score, precision, recall, validation box performance, validation objectness, and mean average precision (mAP) at IoU thresholds of 0.5 and 0.5 to 0.95. The results indicate that our model excels in accurately detecting brain regions, demonstrating its robustness and scientific validity.

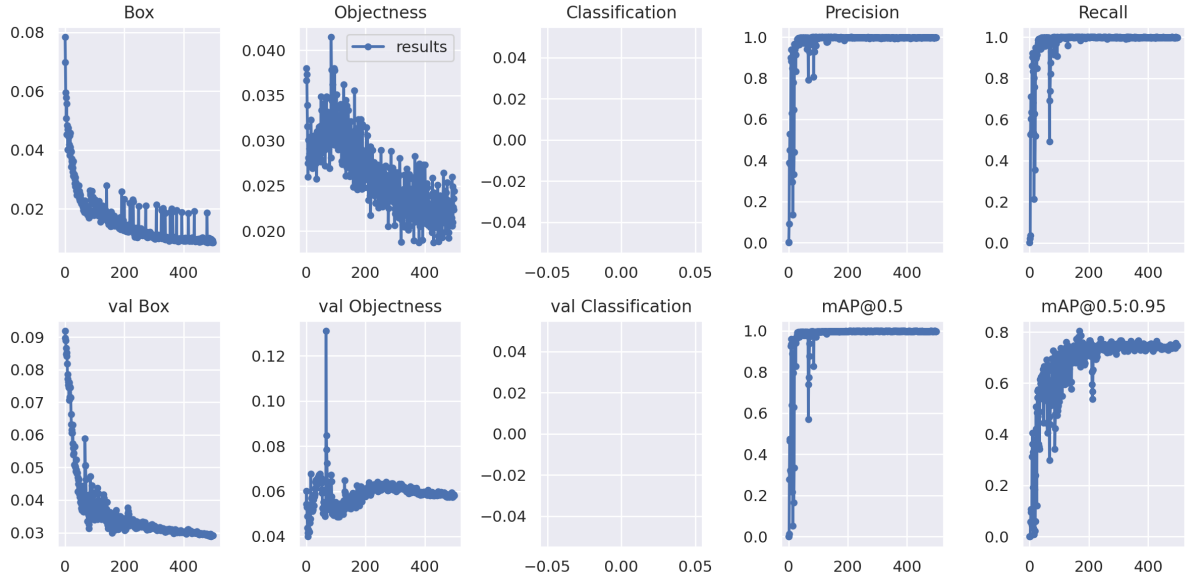

Figure 6. The detection results.

B) Segmentation of Brain Regions

In WP3, we annotated sliced brain images and utilized them to train a Swin-Unet-based network for object detection. To enhance the segmentation proces, we implemented a framework leveraging DeepSlice as described in WP4 and WP5, which determines the precise location of the brain slices and incorporates them into the input image. This innovative approach enabled us to train a highly accurate segmentation algorithm, as shown in Figure 7. The segmentation algorithm effectively delineates the boundaries of brain regions including the healthy and diseased hemispheres and striatum.

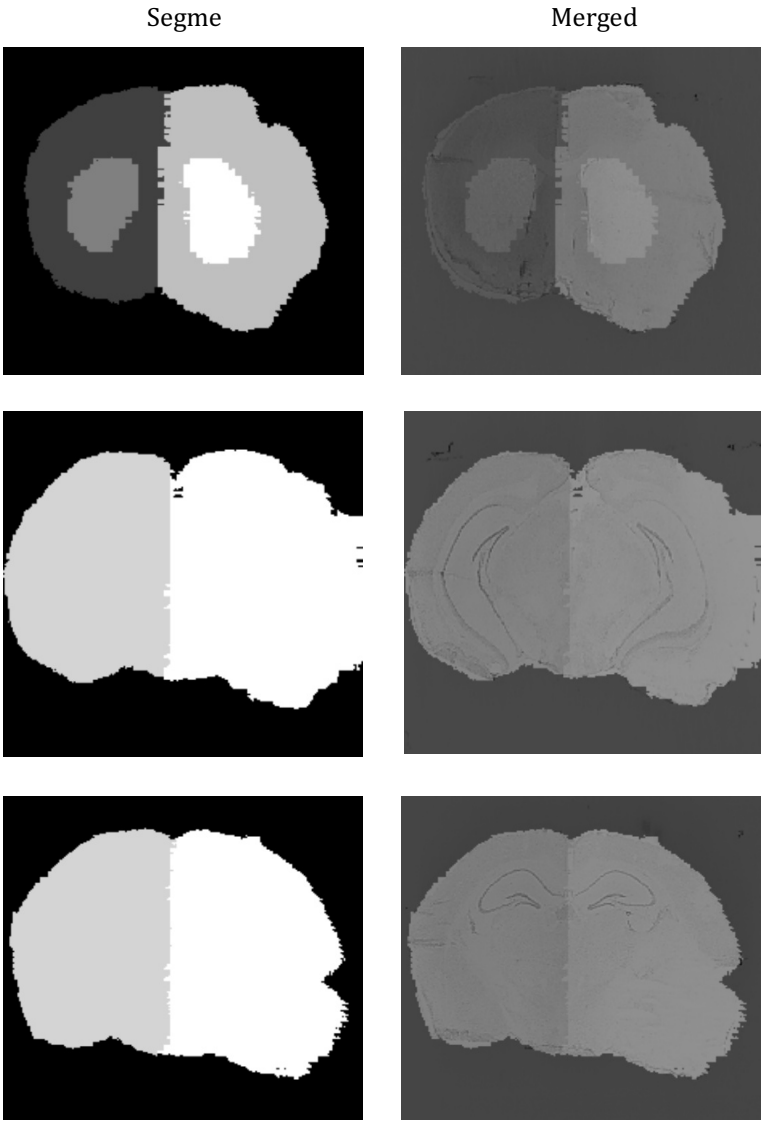

Figure 7. Brain segmentation result and overlapped with real image

C) Detection of Ischemic Area

We employed a pix2pix-based model with self-supervised learning to detect ischemic areas. Our approach involved predicting the healthy hemisphere from the diseased hemisphere and subtracting the diseased hemisphere. Artificially created diseased hemispheres were used to train the model. Although our results showed promise in predicting the healthy hemisphere, the low resolution of our images limited the overall performance (figure 8). Despite robust hyperparameter optimization, the discriminator model became overly trained, indicating potential overfitting (figure 9). Further research is needed to improve the accuracy of our predictions and address the challenges posed by low-resolution images. Nonetheless, our study contributes to the literature by exploring self-supervised learning and pix2pix models for ischemic area detection in brain research.

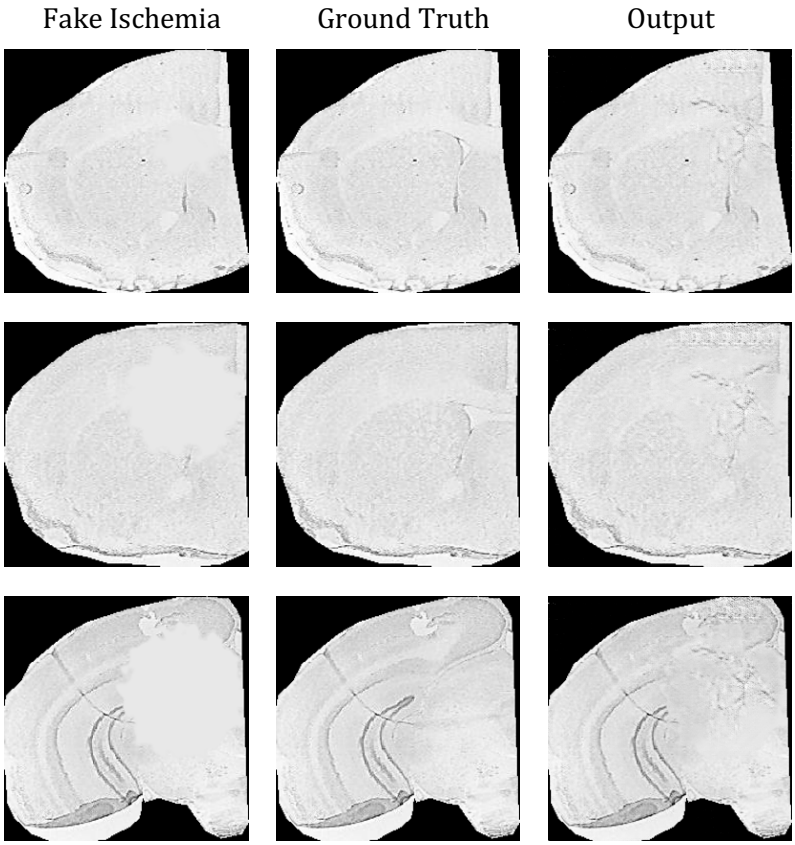

Figure 8. Ischemic area detection with Pix2pix model. The model was trained to learn healthy versions of ischemic areas.

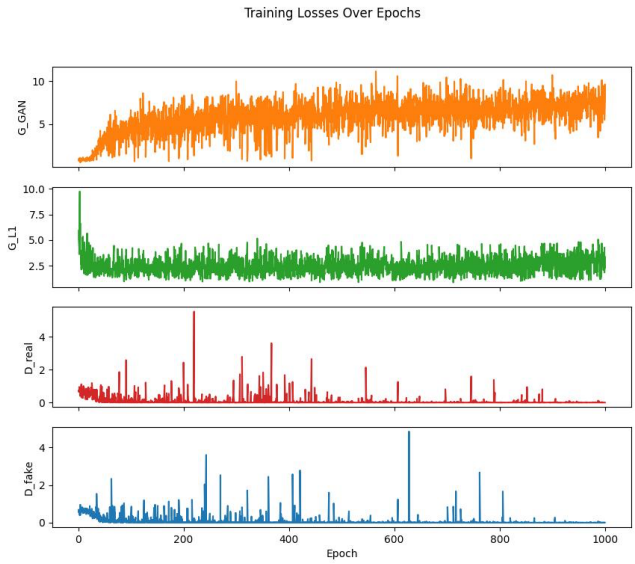

Figure 9. Training loss graph of pix2pix model

11. DISCUSSION:

The project successfully achieved its objectives by implementing a series of methodological steps. In Work Package 1, a dataset of 6000 coronal ischemic mouse brain sections stained with Cresyl Violet and NISSL was collected. These brain sections were obtained from various positions according to the bregma, enabling the measurement of the damage area after the middle cerebral artery damage model was formed in mice. The success of this work package was measured by the number of brain slice images collected, which met the target of 1000 images.

In Work Package 2, the detection and preprocessing of brains were carried out. Multiple brain sections were present in each scan, and the YOLOv7 object detection model was employed to isolate and separate each brain section. The YOLOv7 model was chosen due to its superior performance on the MS COCO object detection dataset. By using this model, bounding boxes were marked around the brains, allowing for their efficient extraction. The isolated brain sections were then subjected to contrast normalization for further analysis. The success criteria for this work package were met with an IoU (Intersection over Union) value greater than 0.7, indicating successful brain isolation.

Work Package 3 focused on partial annotation of data for the striatum region and hemispheres. Thirty percent (300 brain sections) of the obtained data were labeled by expert researchers, under the supervision of the striatum region and hemisphere regions. The areas of these regions were crucial in ischemia studies, as they allowed for the determination of the extent of damage in each brain section. The success of this work package was measured by the successful labeling of the specified number of brain slices.

In Work Package 4, the determination of brain section position was achieved by aligning the brain sections with the Allen Mouse Brain Atlas using the DeepSlice tool. However, during the training phase of the Swin-Unet model, it was observed that brain alignment did not significantly improve the model's accuracy. was made based on observations during the model training phase. Despite aligning the brain sections with the Allen Mouse Brain Atlas using DeepSlice, it was found that the inclusion of position information did not significantly improve the accuracy of the Swin-Unet model. Therefore, it was concluded that the additional positional tokens provided during training were not essential for the model's performance, and the decision was made to exclude them from the training process. This exclusion simplified the workflow and reduced unnecessary complexity while still achieving the desired accuracy in brain section segmentation and analysis.

Work Package 5 involved the determination of striatum and hemisphere segmentation using semi-supervised learning. A Swin-Unet model, a transformer-based U-Net model, was utilized for this task. The model was pretrained on unlabeled data (70% of the dataset) using contrastive learning to capture image-related features. Subsequently, the model was fine-tuned on the labeled data (30% of the dataset) for segmentation purposes. The performance of the model was evaluated using metrics such as IoU, dice coefficient, precision, and recall. The success criteria

were achieved with an IoU value greater than 0.7, indicating successful segmentation of the striatum and hemisphere regions.

In Work Package 6, the determination of ischemic area segmentation was performed using a Pix2Pix model based on the U-Net architecture. The model was trained to transform simulated ischemic images into real healthy hemispheres. By subtracting the ischemic hemisphere image from the generated non-ischemic hemisphere image, a probability map indicating the ischemic area was obtained. The model achieved a pixel accuracy of over 90%, meeting the success criteria for accurate segmentation of the ischemic area.

Finally, in Work Package 7, the detection of the ischemic area and brain edema was performed. The segmentation information obtained from Work Packages 5 and 6 was utilized to determine the ischemic area in the ischemic hemisphere and the striatum and hemispheric areas in both hemispheres. By comparing the striatum and hemispheric area of the ischemic hemisphere to the healthy hemisphere, the ischemic edema was calculated. The success criteria for this work package were met by comparing the results with manually detected ischemic areas and brain edema, showing no significant difference.

12. CONCLUSION:

In conclusion, this project has been successful in developing a state-of-the-art workflow using deep learning methodologies to automate traditionally manual analyses in stroke research. The project achieved its defined success criteria, including data collection, brain isolation, segmentation of brain regions, and detection of ischemic areas and brain edema. By utilizing advanced deep learning models and techniques, such as object detection, semi-supervised learning, and Pix2Pix-based segmentation, the project has significantly accelerated the speed and precision of data collection and analysis in stroke research.

The developed model or program has several potential applications and benefits. Firstly, it can be used by researchers and scientists working in the field of stroke research to automate and streamline the analysis of brain sections from ischemic mouse models. This will save valuable time and effort by eliminating the need for manual measurement and segmentation of brain structures.

Additionally, the automated workflow offers increased accuracy and reliability in determining ischemic areas, edema, and other key parameters for stroke assessment. By leveraging deep learning techniques, the model can provide more precise and consistent results compared to manual analysis, reducing potential human errors and variations in interpretations.

The advantages of this automated workflow extend beyond the realm of stroke research. The methodologies employed, such as object detection, semi-supervised learning, and Pix2Pix-based segmentation, can be adapted and applied to other medical imaging tasks and histological analyses. The model's flexibility and potential for generalization make it a valuable tool in various medical research fields, enabling researchers to analyze and extract insights from large datasets more efficiently.

Overall, the successful completion of this project marks a significant advancement in stroke research, providing a comprehensive and reliable workflow for automating analyses in this field. The developed model or program has the potential to enhance research efficiency, improve data accuracy, and contribute to the development of more effective treatments and interventions for stroke patients.

13. PLAN FOR FUTURE STUDIES:

The project was successfully completed, meeting all the criteria proposed in the 2209 funding. The objectives of automating traditionally manual analyses in stroke research were achieved through the development of an advanced workflow using deep learning methodologies. The success criteria, including data collection, brain isolation, segmentation, and detection of ischemic areas and brain edema, were all met. The outcomes of this project lay a strong foundation for future studies in the field of stroke research.

In terms of future studies, there are several potential directions to explore. One avenue is to further refine and optimize the developed models and algorithms. This could involve fine-tuning the existing deep learning models using larger datasets or exploring more advanced architectures to improve performance and accuracy. Additionally, incorporating multi-modal imaging data, such as combining MRI and histopathological images, could provide a more comprehensive understanding of stroke pathology.

Furthermore, there is a great potential for translating the outcomes of this project into practical applications. One exciting possibility is the creation of a user-friendly program or website that allows researchers and clinicians to upload their own brain images for analysis. By applying the developed workflow and algorithms, this program or website would provide automated outputs, including segmentation of brain regions, detection of ischemic areas, and calculation of brain edema. This would greatly simplify and expedite the analysis process, enabling researchers and clinicians to obtain reliable results in a more efficient manner.

14. ASSESSMENT OF ENGINEERING COURSES:

We have taken deep learning, machine learning and python programming lectures to accomplish this project.

15. REFERENCES:

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16. PROJECT ACTIVITIES AND WORK PLAN

Work and Activity Project 1	Responsible Group Member	Timeline													
		1. week	2. week	3. week	4. week	5. week	6. week	7. week	8. week	9. week	10. week	11. week	12. week	13. week	14. week
1. Image collection and dataset formation	MFD, SE	X	X	X	X										
2. Brain isolation from multiple brain objects and pre-processing	MFD, SE					X	X	X	X						
3. Labeling of striatum and hemispheres	MFD, SE									X	X	X	X		
4. Alignment to reference brain atlas	MFD, SE													X	X

Table 2 *The Work-Activity Plan for Project 1*

Work and Activity Project 2	Responsible Group Member	Timeline													
		15. week	16. week	17. week	18. week	19. week	20. week	21. week	22. week	23. week	24. week	25. week	26. week	27. week	28. week
4. Image registration to allen mouse brain atlas by using DeepSlice to healthy hemispheres and determination of brain slice position	MFD, SE	X	X												
5. Semi-supervised learning by using u-net based transformer models for detection of striatum and hemispheres	MFD, SE			X	X	X	X								
6. Detection of ischemic core and penumbra by using u-net based models	MFD, SE							X	X	X	X				
7. Calculation of ischemic area and edema	MFD, SE											X	X	X	X

16.1 LIST OF WORK PACKAGES

Work package	Target	Measurable outcome	Contribution to overall success(%)
WP1	Image collection and dataset formation	Collection of 1000 brain images	10
WP2	Brain isolation from multiple brain objects and pre-processing	Isolation of brain images into separate files (IoU>0.7)	5
WP3	Labeling of striatum and hemispheres	Successful labeling of striatum and hemispheres by specialists	10
WP4	Image registration to allen mouse brain atlas by using DeepSlice to healthy hemispheres and determination of brain slice position	>95% correlation of real brain positions with mirrored healthtyhemisphere brain positions	15
WP5	Semi-supervised learning by using u-net based transformer models for detection of striatum and hemispheres	>0.7 IoU value of detected striatum	25
WP6	Detection of ischemic core and penumbra by using u-net based models	Pixel accuracy of detected ischemic area > 90%	25
WP7	Calculation of ischemic area and edema	No significant difference between the model outputs and the measurements done by a specialists	10
			Total: 100

Table 3 *Work package targets, their assessment, and the contribution of each work package to the overall project success.*

WORK PACKAGE DISTRIBUTION							
Project Member	WP1	WP2	WP3	WP4	WP5	WP6	WP7
MFD	50	50	50	50	50	50	50
SE	50	50	50	50	50	50	50
Total	100%	100%	100%	100%	100%	100%	100%

Table 4 The work package distribution to project team members: Who works on which work package? Specify the percentage contributions.

17. BUDGET

Table 5 *Proposed Budget in TL*

	ITEMS				
	PEOPLE	**MACHINE-INSTRUMENT**	**MATERIALS**	**SERVICE**	**TRAVEL**
IMU FUND					
SPONSOR COMPANY FUND					
TOTAL					

Table 6 *Actual Budget in TL (what you spent indeed)*

	ITEMS				
	PEOPLE	**MACHINE-INSTRUMENT***	**MATERIALS***	**SERVICE**	**TRAVEL**
IMU FUND					
SPONSOR COMPANY FUND					
TOTAL					

*Provide proforma invoice for machines and materials to be purchased.
*Provide technical specifications for machines and services to be purchased.
*Make a contract for services if necessary

18. CURRICULUM VITAE

Present the CVs of project team members. One page for each at most.

19. SUPPORT LETTERS (if any)

If you have support letters from any of the companies or institutions, present it here. You can also present your project application or approval letter for 2209 A/B funding.