

ANALYTICAL STUDY

Traditional and deep learning-oriented medical and biological image analysis

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ABSTRACT

We investigated various methods for image segmentation and image processing for the segmentation of MRI of human medical data, as well as bioinformatics for the segmentation of brain cell details, in this work. The goal is to demonstrate and bring various mathematical analyses for medical and biological image analysis. We proposed new software and methods for improving the segmentation of biological and medical data. This way, we can find new ways to improve the diagnostic process in medical data and improve results in cell and iron diagnostics. We present the GrabCut algorithm as well as new, improved software for this part, a fuzzy approach and fuzzy processing of tissues, and finally machine-learning techniques with neural networks. We implemented the new software in the C++ programming language for the Grab cut algorithm. Consequently, we present a fuzzy approach to the diagnosis of image data in Matlab. Finally, a deep learning-based approach is used, with a U-Net-based segmentation architecture proposed to measure the various brain cell parameters. We will be able to proceed with data that we were unable to proceed when using other methods. As a result, we improved biological and medical data segmentation to obtain better boundaries and sharper edges on the objects. There is still space to extend these methods to other medical and biological applications (*Tab. 1, Fig. 34, Ref. 46*). Text in PDF www.elis.sk

KEY WORDS: segmentation; image processing; fuzzy segmentation, GrabCut, deep learning.

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Introduction

This contribution deals with the processing of medical and biological data. Mathematical modeling is a very good and strong tool for the analysis and modeling of these data. Consequently, mathematical modeling brings a good analytical, enumeration, and visualization perspective when representing biological and medical data for users. We discuss some methods for medical image analysis. One point brings perspective from discrete mathematics and discrete algorithms, dealing with graph theory, statistics, data analyses, and image processing; the other point brings perspective from machine learning and a fuzzy approach. We focus on the analysis of the techniques while using image processing and segmentation of the objects. Our segmentation process is based on graph cuts, grab cuts, machine learning, and fuzzy techniques. Image segmentation is a crucial stage in image processing that is required almost everywhere if we want to examine the contents of the image. For instance, image segmentation may be required to separate the objects in an indoor image and then analyze each object separately to determine what it is if we want to determine whether a chair or a person is present. Before pattern recognition, feature extraction, and image compression, the image segmentation typically serves as the pre-processing step. The division of an image into various groups is called segmentation. The field of image segmentation using clustering has undergone a wide range of research. The *K*-means clustering al-

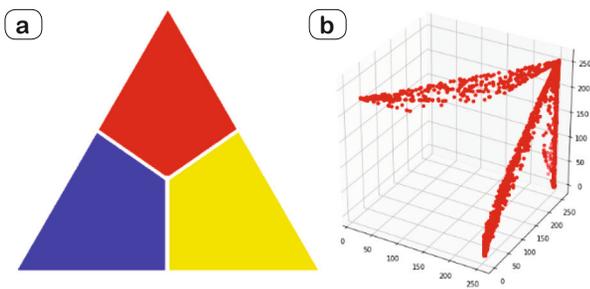


Fig. 1. Plotting an image into three dimensions in Matlab (4): (a) original image; (b) representing the image into three dimensions.

gorithm is one of the most often used techniques out of a variety of techniques. (1)

Medical images are very important in medical diagnostic treatment. Clinical doctors exhibit interest in analyses and descriptions of internal anatomy. In the case of biology, biological images play important roles because biologists are interested in the structure of the cells, edges, membranes, and different objects like atoms, elements, etc. In that case, biologists do analyses, and based on their results, they can predict some illnesses and diseases. In medical image segmentation, medical images do not contain sharp edges. Many times, clinical doctors need to improve their diagnostics and discuss different abnormalities in the images. We can distinguish more types of medical data—MRI, X-ray, CT images, and ultrasound—used in clinical practice. The oldest are the X-ray data, which have been applied in medicine for more than a century. The main principle is that electromagnetic radiation with short wavelengths and high energy has been used. An X-ray, or X-radiation, is a penetrating form of high-energy electromagnetic radiation. Most X-rays have a wavelength ranging from 10 picometers to 10 nanometers, corresponding to frequencies in the range of 30 petahertz to 30 exahertz (31016 Hz to 31019 Hz) and energies in the range of 145 eV to 124 keV. X-ray wavelengths are shorter than those of UV rays and typically longer than those of gamma rays. In many languages, X-ray radiation is referred to as “Roentgen radiation,” after Wilhelm Conrad Roentgen, who discovered it on November 8, 1895. He named it “X-rays” to signify that the type of radiation was unknown. English spellings of X-ray(s) include x-ray(s), x-ray(s), and X-ray(s). The most familiar

use of X-rays is checking for fractures (broken bones), but X-rays are also used in other ways. For example, chest X-rays can spot pneumonia. Mammograms use X-rays to look for breast cancer. CT images are based on different approaches. It is an imaging technique that uses X-rays to create images of internal organs and structures in the body. This produces several parallel slices of each organ by passing X-ray pulses through the body. Computed tomography is commonly referred to as a CT scan. A CT scan is a diagnostic imaging procedure that uses a combination of X-rays and computer technology to produce images of the inside of the body. It shows detailed images of any part of the body, including the bones, muscles, fat, organs, and blood vessels. MRI is magnetic resonance imaging that works based on magnetic characteristics and provides a lot of information about the inner organs, structures, and inner body. This technique has many advantages. It works by creating a series of parallel slices in three dimensions for each organ, with high contrast and resolution between tissue. This method has some drawbacks such as the fact that the data are too large for manual analysis and are influenced by noise. MRI is a type of diagnostic test that can create detailed images of nearly every structure and organ inside the body. MRI uses magnets and radio waves to produce images on a computer. MRI does not use radiation. Images produced by an MRI scan can show organs, bones, muscles, and blood vessels. What is the distinction between an MRI and a CT scan? CT scans take a fast series of X-ray pictures, which are put together to create images of the area that was scanned. An MRI uses strong magnetic fields to take pictures of the inside of the body. CT scans are usually the first choice for imaging. MRIs are useful for certain diseases that a CT scan cannot detect. (2) Bioimaging data (imagining of biological data) have a significant potential for reuse, but unlocking this potential requires systematic archiving of data and metadata in different databases. Data visualization is a fundamental aspect of science. In the context of microscopy-based studies, visualization typically involves presentation of the images themselves. However, data visualization is challenging when microscopy experiments entail imaging of millions of cells, and complex cellular phenotypes are quantified in a high-content manner. Most well-established visualization tools are inappropriate for displaying high-content data, which has driven the development of a new visualization methodology. We deal specially with light and electron microscopy-obtained data. Spectacular advances in light and electron microscopy are rapidly

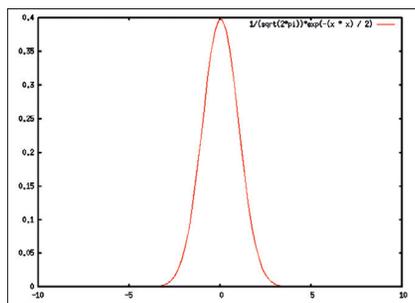


Fig. 2. 1D Gaussian models Clues.

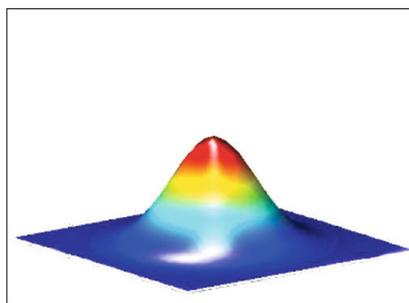


Fig. 3. 2D Gaussian model.

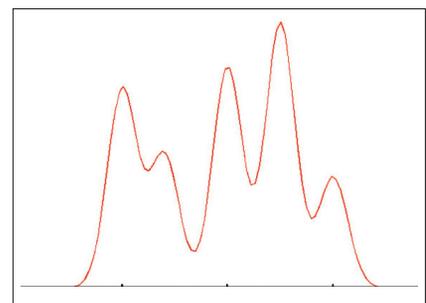


Fig. 4. 3D Gaussian model.

transforming the life sciences. For instance, scientists are now able to image molecular complexes at atomic resolution, follow the fates of individual molecules in a living cell, and image the development of an organism starting from a single fertilized cell. These imaging technologies are generating large amounts of complex data, the interpretation of which often requires sophisticated analyses, as in other ‘omics’ technologies. Moreover, most advanced imaging technologies are expensive, while the biological samples used in the experiments may be unique. To maximize the use of generated data and to realize the full potential of the advances in biological imaging, these datasets need to be good and useful for other analyses and diagnostic processes. Around the world, there are efforts to develop informatic systems for making different types of microscopy data available to the community. Segmentation techniques are also very suitable for processing of different types of biological and microscopy data (3). Medical images are very important in medical diagnostic treatment. Clinical doctors exhibit interest in analyses and descriptions of internal anatomy. In the case of biology, biological images play important roles because biologists are interested in the structure of the cells, edges, membranes, and different objects like atoms, elements, etc. In that case, biologists do analyses, and based on their results, they can predict some illnesses and diseases. In particular, in medical image segmentation, medical images do not contain sharp boundaries. Medical images can be processed and utilized in many ways such as image registration, image segmentation, finding tumors and other pathological tissues, computer-guided surgery, treatment planning, optical character recognition, image recognition, image restoration, blood vessels and brain structure diagnoses, tumor detection and segmentation, functional mapping, blood cell classification, mass detection in mammography, and cardiac image analyses. Some general-purpose algorithms and techniques have been developed for image segmentation. Since there is no common solution to the image segmentation problem, these techniques have to be combined with sphere knowledge for an effective solution to the image segmentation problem in a given domain. The methods can be classified into three main categories: traditional methods, graph theoretical methods, and combination thereof. We provide segmentation techniques on medical and biological datasets for special purposes. In the second section, the theoretical methods and literature reviews define the basic methods for segmentation techniques, and we give an overview of the segmentation methods and our methodology.

We present the segmented data from the Grab Cut, Fuzzy, and Neural Networks methods in the section Results. We developed new software to detect and classify tumors, inflammation, abnormalities in medical images, cysts, and edema in MRI data using the GrabCut techniques. This would make it easier to detect the edges of objects on the image, improve the diagnostics of various strengths, and better distinguish some specific cases where visual and radiology diagnostics are not perfect, clear, and unique when seen by different people. We illustrate the use of a fuzzy approach to segmentation of biological cells as well as that of neural network approach. For brain cell segmentation, we have proposed a U-net-based architecture segmentation approach, where we have

used MobileNetV2. where we have used a pretrained model named MobileNetV2, where we calculated the area, diameters, and solidities of segmented objects and cells. In the second and third parts, we look at biological data in form of samples of rats’ brain tissue. They contain cells in the process of apoptosis, necrosis, or in hybrid stage. This type of research is linked to Alzheimer’s disease and some cases of brain death in children. But the research is done on the brain tissues of rats because, under special conditions, the structure is similar to that of the human brain at different stages of age. Our aim is to detect all types of cells on the provided biological samples. Biologists do not have good tools for this kind of segmentation. We show our own implementation and optimization. Finally, in the section Conclusion, we discuss medical and biological applications, summarize our approach and discuss open questions. The practical results of the proposed algorithm are encouraging.

Theoretical methodology and literature review

Image segmentation is a division of a digital image into several separate areas, each having a set of super pixels (groups of pixels) and other related pixels. Image segmentation aims to transform an image’s representation into something more useful and understandable. Image segmentation is frequently used to identify objects and boundaries in images (such as lines, curves, etc.). Image segmentation, in more exact terms, is the process of giving each pixel in the image a label so that pixels with the same label have specific properties. If we use autonomous vehicles as an example, they require sensory input equipment like cameras, radar, and lasers to understand their surroundings and build digital maps. Without object recognition, which requires picture classification and segmentation in and of itself, autonomous driving is not even conceivable. Other instances come from the healthcare sector, where, even with current technological developments, cancer can be lethal if not detected at an early stage. Millions of lives could be saved by swiftly identifying any malignant cells. Using image classification algorithms, it is possible to determine the severity of cancer based on the form of malignant cells. In a similar way, numerous image segmentation algorithms and techniques have been created over time using domain-specific knowledge to suc-

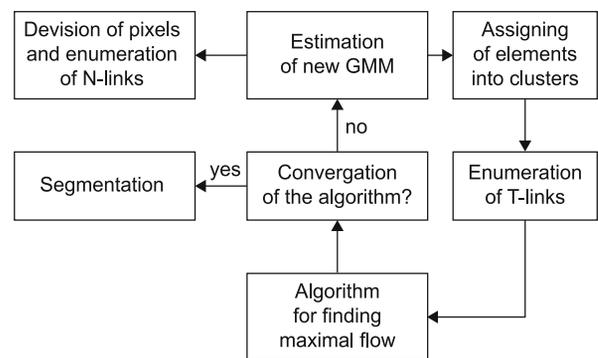


Fig. 5. The iterated scheme of the Grab cut algorithm.

cessfully address segmentation issues in relevant application fields such as medical imaging, object detection, iris recognition, video surveillance, machine vision, and many more. Let us use the Python Matplotlib module to plot an image in three dimensions (Fig. 1).

Using image classification algorithms, it is possible to determine the severity of cancer based on the form of malignant cells. In a similar way, numerous image segmentation algorithms and techniques have been created over time using domain-specific knowledge to successfully address segmentation issues in relevant application fields, such as medical imaging, object detection, iris recognition, video surveillance, machine vision, and many more. Let us use the Python Matplotlib module to plot an image in three dimensions. The plot clearly shows that the data points are forming groups; some areas of the graph are denser than others, which corresponds to the dominance of particular colors in the image. The process of image segmentation Picture segmentation is the process of breaking up an image into groups of pixel regions that are each represented by a mask or labeled image. You can process only the key portions of an image by segmenting it rather than processing the whole thing. Searching for rapid changes in pixel values, which often denote the edges defining a region, is a common strategy. Observing patterns of similarity within an image's regions is another prevalent strategy. This method is followed by some approaches, such as region-expansion, clustering, and thresholding. To successfully address segmentation issues in certain application domains, a range of additional methods for performing picture segmentation have been developed over time. We will survey the most important and widely different segmentation techniques. A survey of different techniques is reported by Basavapraad and Ravindra (5). Basavaprasad et al present methods based on discrete mathematics and graph theory (6), while Saban presents interactive image segmentation (7), Ravindra et al (8), and Brown et al in (9). Cuang et al (10) discuss the Bayesian approach to digital matting, as do Kass et al (11) who discussed active contour models. Gross and Yelen (12) presented the basic knowledge of graph theory needed for image processing. Brain tumor analyses are described by Sharma et al (13). Saraswat et al describe other techniques, such as using “ k ” to mean algorithm (14). Jeevirha and Priya discuss ultrasound images (15); automated

segmentation techniques for disease detection are discussed in another work (16). Modern techniques such as data mining, deep learning, and using them in medicine are visible in many studies (16, 17, 18). Deep learning techniques (19–22) are replacing the handcrafted-based approaches (23, 24) since it has been observed that the performance gain is much greater with deep-learning techniques (25). Varhan et al discovered gradient techniques and a fuzzy approach to image analysis (26), while Haifficker and Tizoosh discovered the fuzzy method (26). Orchard and Bouman (27) present color quantization of data images, which is explained in detail. Khan and Ravi (28) bring a comparative study on image segmentation techniques. Photoshop (29) is open-source software that is available online for free as Adobe Photoshop. A review on shortest path algorithms written by the authors Magzhan and Jani (27) Some known methods: Thresholding is the simplest image processing method; see (5) and (29). When segmenting an image, cluster methods are typically center-oriented; we have given centers and the elements are assigned to the clusters. In the case of image segmentation, the elements are pixels, and the centers are defined as intensities or RGB vectors. “Fuzzy” cluster methods: Thus, in classical cluster methods, the intersection of two clusters is either an empty set or an entire cluster. In “fuzzy” clustering methods, we speak of the so-called “soft segmentation.” This means that elements can belong to multiple clusters at the same time. As a result, clusters in this method are not disjoint, and their intersection can be any. We mention as well as other methods: the histogram method (5, 30), region-growing methods (see Khan and Ravi (30)), magic wands (see Khan and Ravi), Edge detectors: Edge detectors are used to find the boundary of an object; their principle is to search adjacent pixels in the image that have too different an intensity color. Discrete mathematical methods: Very widespread methods include methods based on image representation using graphs and different methods from graph theory. Graph theory methods: Ford and Fulkerson (33, 34) discuss maximal flow in the network. Its connection into image analyses is possible to find in (34–36) by Boykov and Jolly, and Rother et al (30). Methods based on finding the maximum flow and small section are all discrete methods. With these methods, we convert the pixels of the image to the network (graph) in which we try to find maximum

flow and minimum cut. Each pixel represents one point of the graph and in addition we will add two more points (source) and (mouth). Capacity on the edges we calculate based on the intensity / color of each pixel and find the maximum flux using some algorithm (for example the Ford-Fulkerson algorithm). After finding maximum flow, we find out which pixels are still reachable from the source and the minimum we separate them by slice, which gives a segmented object. Mixed methods: techniques that combine traditional and graph methods to improve segmentation results. GrabCut: It is an image segmentation method based on initial clustering using Gaussian blend

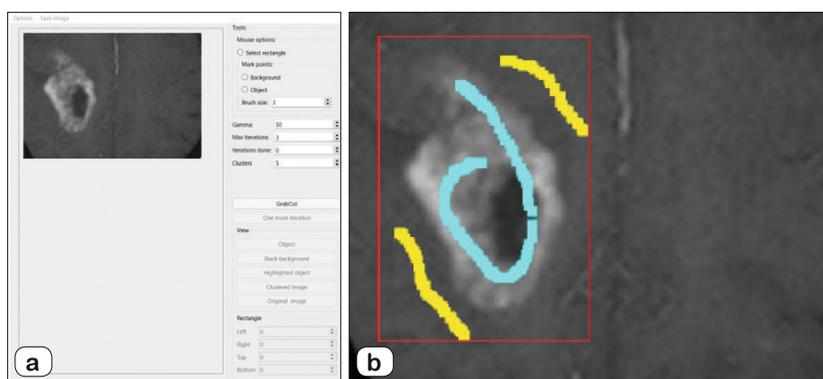


Fig. 6. Users' environments: (a) Including and uploading data into the software (b) The choosing of the rectangle, object and background area.

models and a modified Graph Cut method(Bassavaprasad and Ravindra (5) and other et al (37, 38).

Cluster analysis

It deals with the issue of dividing data into finite numbers of clusters. Clusters are subsets of a whole set of data where one cluster belongs to the elements of the set, which are in some way close, and by unifying all the clusters we get again the whole original set of elements. It follows that everyone must belong to (at least) one cluster, Rother et al (36) discuss GrabCut. Xu and Tian (37) give a survey of clustering algorithms. We distinguish Hierarchical, Non-hierarchical, and Center -based Clustering, Center - based clustering. For this type of clustering, often called k-means, we have a final one where we assign the number of centers and elements based on which center, they are closest to. The term center in image segmentation usually does not mean coordinates in Euclidean space, but for example the color intensity or the vector or the color vector in some other color space. If we look again at the threshold so method in the middle e.g. in, so similar results we could achieve with a simple a two-element model, where we could consider, for example, the intensity of white as the mid-points color and black intensity. Then we would assign the pixel to white or black cluster, depending on which of the centers is its intensity closer, i.e. by calculating the function: in. Although this method seems to be relatively simple, its optimization tends to be a problem. The first is to assign elements to the currently selected centers. The second is to calculate new centers as the mean value of the elements of individual clusters. These steps are repeated until the algorithm converges, i.e. until the model stops changing significantly for further iterations. However, in some cases, a completely random selection of centers may not give ideal results, or the algorithm needs many iterations to get the right result. For this reason, there are also algorithms for selecting the starting centers. Grab cut methods: Mixed models: Are probabilistic models that divide the data into finite ones the number of clusters based on the probability of belonging to one data point to a given cluster, based on the assumption that the data come from the final one number of probability distributions, by Lindsay (38). Huang and Chau (39), Reynolds (40) they all discuss details about mixture models and their applications. Thus, the density function of the whole model will be for each point x of the set M on which we

created a mixed model with K components, defined as where is a function of cluster density and is the ratio of the size of the cluster to the whole model, where applicable: Gaussian mixed models: In the Gaussian mixed model (hereinafter referred to as), we consider Gaussian clusters (normal) distributions with the mean and variance. Each contains a finite number of K clusters, which can be of different sizes. This type of mixed models is also used in image segmentation, such as the GrabCut method. Justin et al (41) explains a good way for implementation of the GrabCut algorithm. Fuzzy K means algorithm: Fuzzy clustering is a form of clustering in which each data point can belong to more than one cluster. Deep learning-based image segmentation as presented by Laskar et al (42–44) where lung segmentation from chest X- ray images and the study on image analysis was portrayed. Gaussian mixed models: 1D Gaussian models Clues are used to build up region models (Fig. 2). One model for foreground and one for background. Region models determine weights between pixel and Source and Sink nodes. The 2 D Gaussian model is presented in Figure 3, and 3D is presented in Figure 4.

GrabCut model

Image segmentation is the division of a digital image into a number of separate areas, each having a set of super pixels (groups of pixels) and other related pixels. Image segmentation aims to transform an image’s representation into something more useful and understandable. Image segmentation is frequently used to identify objects and boundaries in images (such as lines, curves, etc.). Image segmentation, in more exact terms, is the process of giving each pixel in an image a label so that pixels with the same label have specific A Gaussian mixture model is the weighted sum of several Gaussians. Encapsulate the concept of a color. Each gaussian is centered around a common color in the image. A GMM has several common colors. This plug has five Gaussian pixels in the foreground and five in the background. Gaussian mixture models and GraphCut bring together a mixed, iterative method called the GrabCut method. Input is a rectangle around the object, and we select seed pixels. We distinguish between object pixels and background pixels. Some pixels are unknown. We need to know the value of the binary variable. Let us refer to denote as an object and 0 as a background. In Gaussian models, we define object

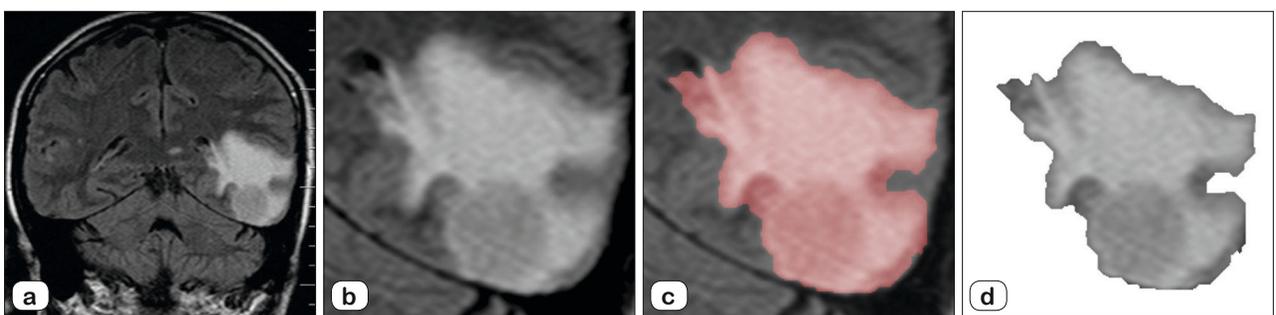


Fig. 7. Brain MRI of 39YO man with lung cancer metastasis. (a)Original data (b) part of the image; (c)segmented metastasis in red color; (d) segmented metastasis.

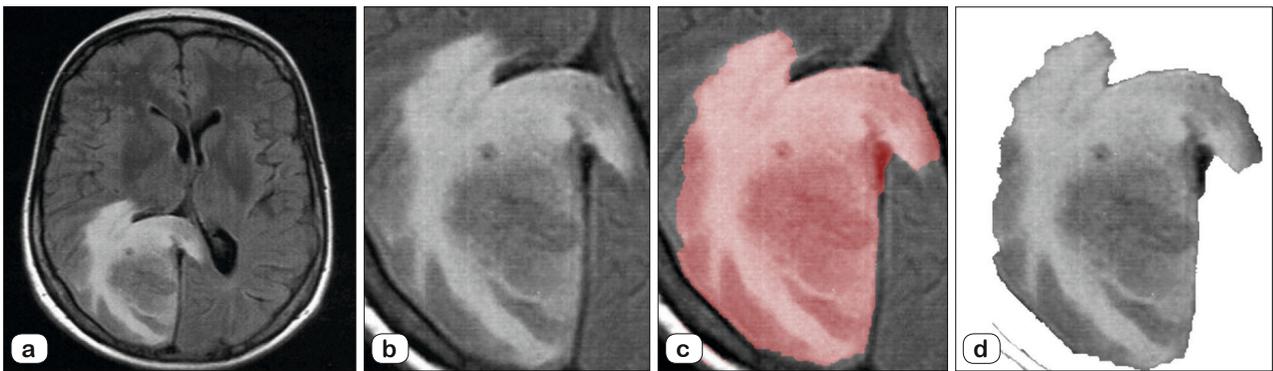


Fig. 8. Skull MRI (t2 flair) of a brain metastasis with accompanying edema. (a)original data (b)selected part of the image; (c)segmented edem in red color; (d)segmented edem.

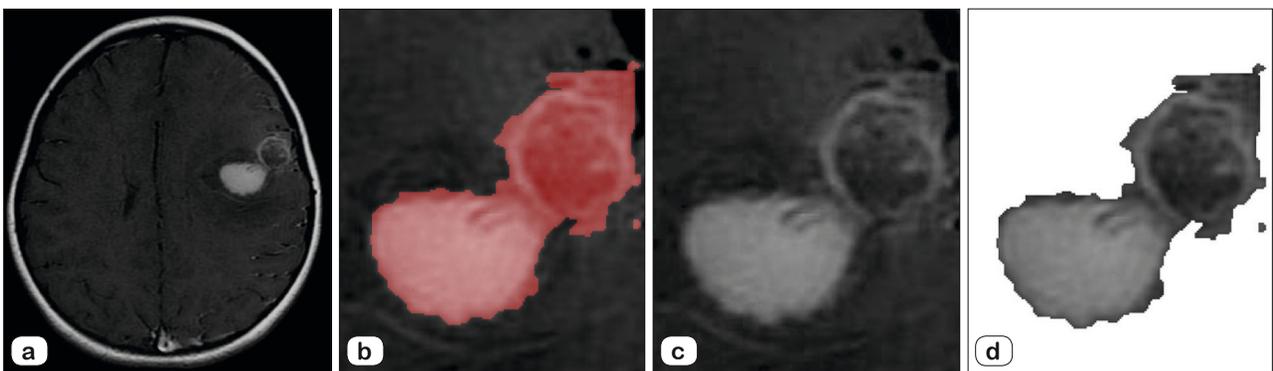


Fig. 9. The tumor presented in Supratentorial central PNET in a 5-year-old patient. (a)Original data (b)selected part of the image; (c)segmented tumor in red color; (d) segmented tumor.

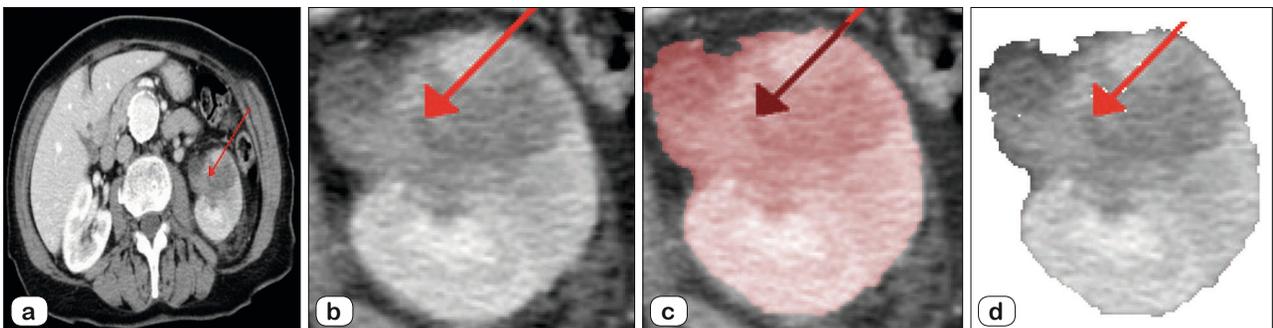


Fig. 10. A kidney tumor that is bleeding as seen on CT. (a)original data (b) selected object on image; (c) Segmented tumor in red color; (d) Segmented tumor.

and background pixels, as well as the outside and inside of the rectangle. All steps in the GrabCut method follow the procedure (Fig. 5). 1. Estimation of Gaussian mixture models using k -means; 2. Initial clustering using k -means; 3. Weighting constant of the component $\pi(k)$: the ratio of the size of the cluster to the whole set. Starting the cluster division process with k clusters.

Graph and capacities

Once the characteristics of Gaussian mixture models' components are known, we can create the graph and calculate the capaci-

ties between nodes. Two additional nodes called source and sink are added, which represent the object and background. After we find the maximal flow, all pixels that are still reachable from the source are considered to be part of the object.

Capacity of N -links: After obtaining the parameters of both *GMMs*, we can create a graph.

Capacity on the N -line between pixels p and q we calculate as: $N(p, q) = \delta(\alpha_p, \alpha_q)$.

Capacity of T -lines: We connect each pixel P to the source and the mouth using a T -line, with the capacity c on these arrows will be calculated as follows:

If $P \in T_B : S(P).c = 0, T(P).c = \lambda$.

If $P \in T_F : S(P).c = \lambda, T(P).c = 0$,

where λ in equations and is the largest of the capacities of all N -links, thus ensuring that these T -lines will not be cut off.

If $P \in T_U : S(P).c = -(\sum_{i=1}^K \pi(i)\phi(P, \mu(i), \Sigma(i)))$,

where $i = 1, \dots, K$ are clusters GMM of background.

Finally, we define

$T(P).c = -(\sum_{i=1}^K \pi(i)\phi(P, \mu(i), \Sigma(i)))$, where $i = 1, \dots, K$

are cluster GMM of background.

The whole GrabCut algorithm consists of repeating steps until the algorithm converges for maximum flow (Fig. 5). If in the two consecutive iterations, the maximum flow has not changed, or has changed only by less than the value of the pre-specified tolerance,

the algorithm is terminated, and a minimum cut is made for the maximum flow from the last iteration. It is also advisable to select the maximum number of iterations in case the algorithm did not converge. The algorithm is described in more detail in the following pseudocode. We implemented the new software in C++ in Qt library. User environment looks like (Fig. 6):

GMM clustering in Grab cut method: Cluster parameters k are also used to be written with a single symbol θ_k for simplicity entries:

$$\theta_k = \{\pi(k), \mu(k), \sigma^2(k) \text{ or } \Sigma(k), k = 1 \dots K\}$$

where K is the number of all clusters. For each cluster i and element x we can then calculate a multidimensional function density of normal distribution in the following form:

$$\phi(x, \mu(i), \Sigma(i)) = \frac{1}{(2\pi)^{\frac{n}{2}} \sqrt{\det \Sigma(i)}} e^{(-1/2[x-\mu(i)]^T \Sigma(i)^{-1}(x-\mu(i)))}$$

where x is the n -dimensional vector and n is the dimension of the elements.

Estimation of GMM parameters

In practice, for example, in image segmentation, cluster parameters are usually not known and cannot be trivially calculated. Suitable parameters are therefore used to obtain them through estimation and subsequent optimization. One of the most common methods for estimation of GMM parameters in image segmentation is center-oriented clustering data using the k -means algorithm. The mean value, covariance matrix, and weight constant for clusters estimated using the k -means method can then be calculated using the values of the elements assigned to the cluster using the k -means method. Subsequently, we can assign elements to clusters based on probability. To initialize and re-evaluate the GMM, it is often used to use a statistical method called "Expectation Maximization" to find the most plausible estimate. In this method, the user initially

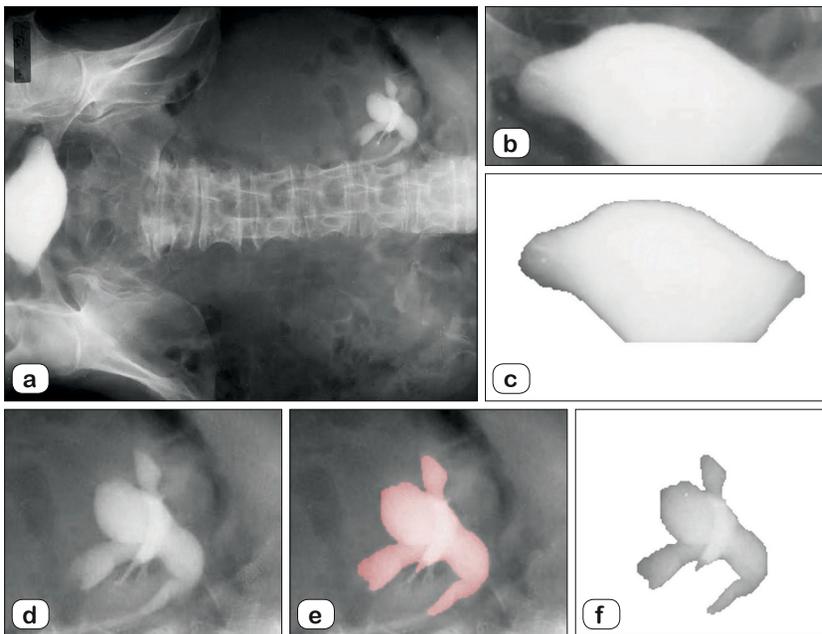


Fig. 11. Expansive formation with soft tissue density in the RD. The tilting of the kidney determines a bending of the ureteropelvic junction. (a) Original data (b) Segmented object (c) Segmented object (d) Segmented object (e) Selected object-soft tissue (f) Segmented object.

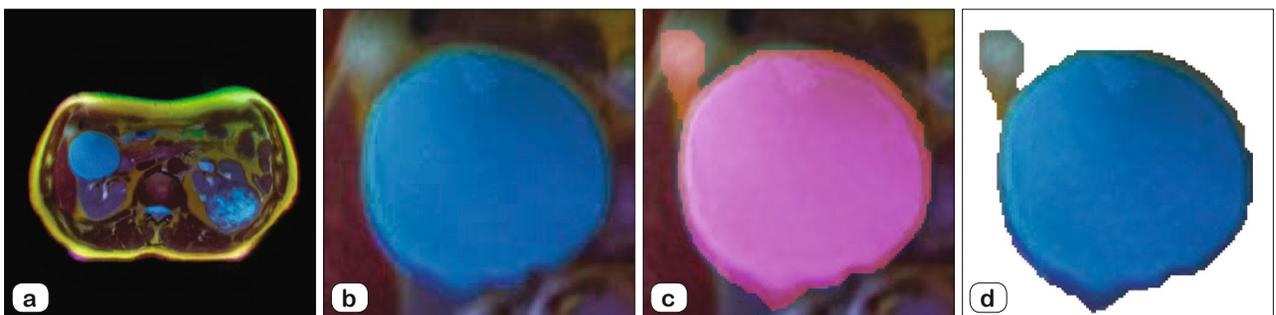


Fig. 12. Patient with Kidney tumor T1W DEcho in Red channel, T2W SSFSE in Green channel, T2W FS PROPRTr in Blue channel. (a) Original data (b) Segmented object (c) Segmented object (d) Segmented object

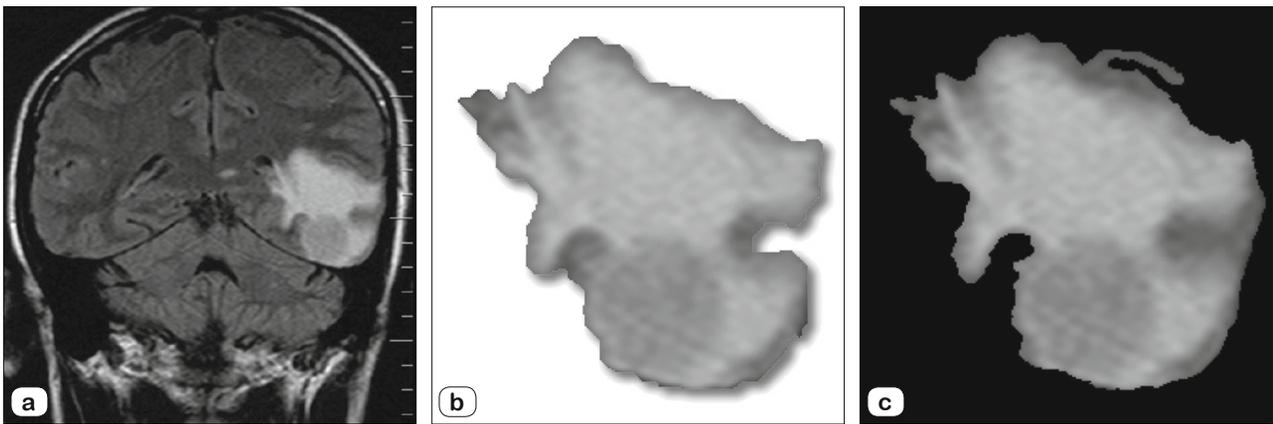


Fig. 13. The tumor presented in Supratentorial central PNET in a 5-year-old patient. (a) Original data (b) segmented tumor by our optimized method; (c) segmented tumor by the method of graph cut using Matlab.

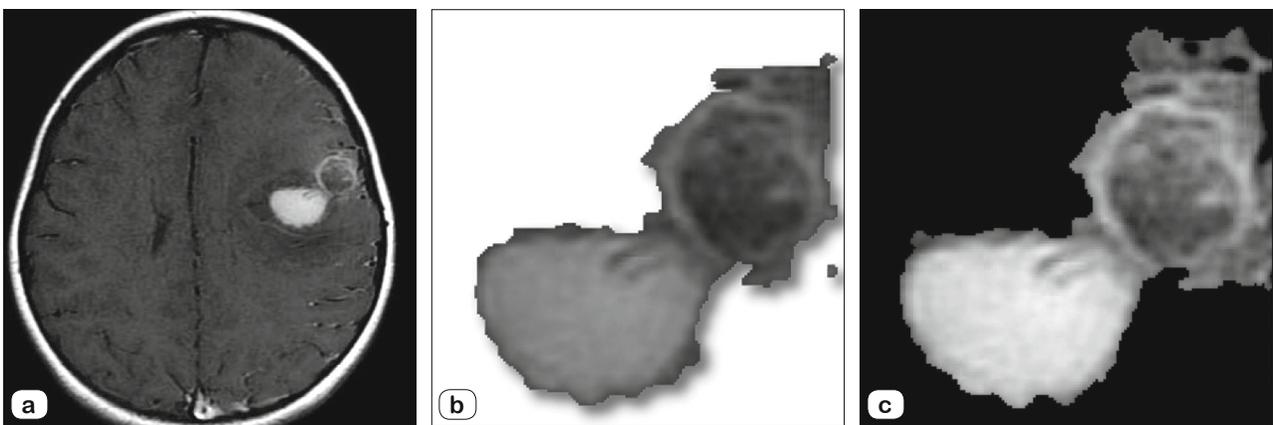


Fig. 14. The tumor presented in Supratentorial central PNET in a 5-year-old patient. (a) Original data (b) segmented tumor by our optimized method; (c) segmented tumor by the method of graph cut using Matlab.

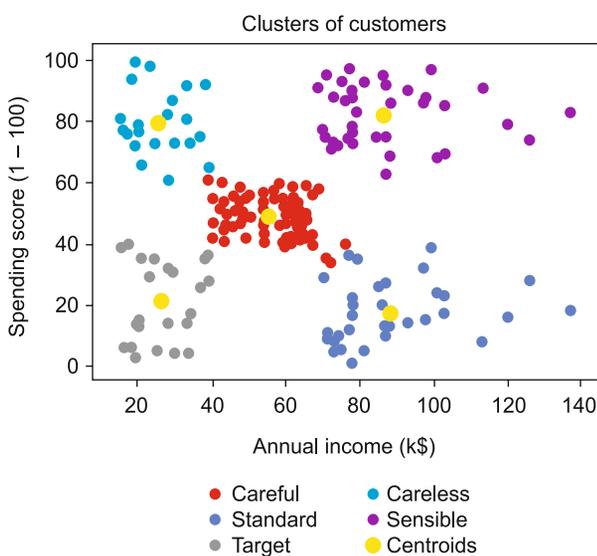


Fig. 15. Presenting of data analyses: clusters of customers.

selects distributions with random parameters and then iteratively optimizes them.

Grab Cut techniques – results

AIM: Grab cut algorithm, application in medical data: image processing of biological and medical data focused on Graph Theory (application in segmentation of images), Clusters analyses and Mixed models (application in segmentation of images). We use Probability, Statistic, Image processing and Image segmentation. Used methods are GraphCut, GrabCut, Machine learning (convolution neural network) Diagnostics using MRI. The problem of determining the sharp border Appropriate image segmentation methods is being sought to distinguish them from the rest of the brain. In some facilities, this is still done manually using the “golden approach,” which involves using simple measuring tools (with poor accuracy). The main outcome is new, specialized software in GrabCut for C++ and Qt that uses graph cuts and grab cuts methods. This software is made to meet the specific requirements of medical clinics and biologists. Analyses of medical images: application for the detection of a brain tumor We developed

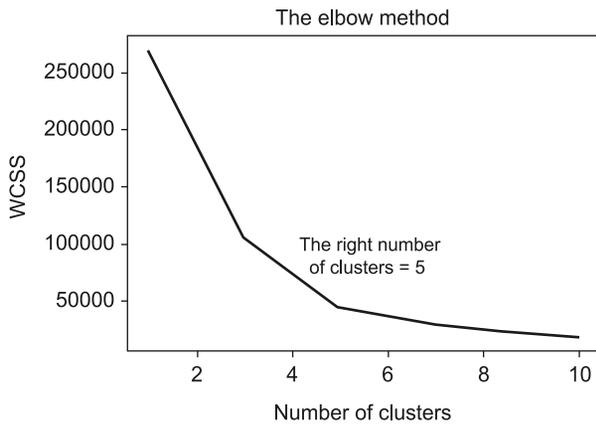


Fig. 16. The illustration of Elbow Method.

new software to detect and classify tumors, inflammatory lesions, anomalies in medical images, cysts, and edema. This would make it easier to detect the edges of objects on the image, improve diagnostics of various strengths, and better distinguish some specific cases where visual and radiology diagnostics are not perfect, clear, and unique by different people. They, like the doctors, should have a better idea of its area than just its width measurements.

Clue marking: Clues are used to build up region models. One foreground model and one for the background. Region models determine weights between pixels and the Source and Sink nodes. In the following Figures 7–13 we present original data as well as segmented medical objects with better, sharper boundaries.

Dataset: We proceed free available MRI data from “Wikimedia commons”, listed under free licencing wiki commons “Creative Commons”.

On the Figure 14 we did comparison of the segmentation of data data getted by Matlab by extending the results of graphcut mentioned by segmnetation of images using Graph cut methodwith our improved optimized implemented techniques - our

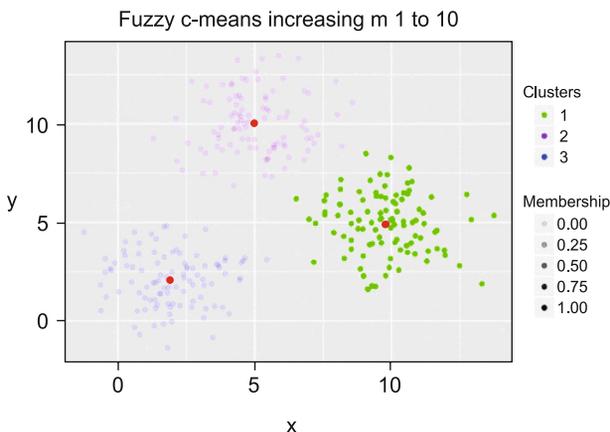


Fig. 17. Illustration of Fuzzy C-means increasing m from 1 till 100.

Improved Grab Cut technique. After discussion with real clinic we can prove, our segmnetation process is getting more realistic results and getting more precisely boundary.

Fuzzy approach for proceeding of medical data

We introduce very important approach into segmentation techniques: fuzzy approach. So in this subsection we will look at a fuzzy method for reading an image and clustering different regions of the image. But first, let us discuss: *K*-Means technique for clustering; Merge *K*-Means clustering Algorithm with Image Segmentation and Canny Edge detection. We continue with *K*-Means clustering, one of the clustering-based methods for image segmentation and also, we compare with fuzzy *C* means clustering.

K-Means technique for clustering

Unsupervised clustering methods are similar to classification algorithms but differ in their underlying assumptions. In clustering, you are attempting to locate some segments or clusters in your data without knowing what you are looking for. When you apply clustering algorithms to your 11 dataset, unexpected structures, clusters, and groups that you would not have anticipated may appear. The interest area is separated from the background using the unsupervised *K*-Means clustering approach. The given data is clustered or divided into *K*-clusters or sections based on the *K*-centroids. When dealing with unlabeled data, the method is employed (i.e. data without defined categories or groups). Finding particular groups based on data similarity with the number of groups represented by *K* is the objective.

Based on their income and spending score, shoppers at a mall have been divided into 5 groups in the Figure 15. Each cluster’s centroid is shown as a yellow dot. The sum of squared distances between all points and the cluster centre must be kept to a minimum when using *K*-Means clustering. Let us define

$$J = \sum_{j=1}^k \sum_{i=1}^n \|x_i^{(j)} - c_j\|^2$$

where *J* is the objective function, *K* is the number of clusters, *N* is the number of cases, *X* presents case *I*, *C* corresponds to the centroid for cluster *j* and

$\|x_i^{(j)} - c_j\|^2$ is the distance function

K-Means algorithm steps:

1. *K* is the number of clusters to choose.
2. Choose randomly *K* points and the centroids (not necessarily from your dataset).
3. Each data point should be matched to the *K* nearest centroid clusters.
4. Calculate and position each cluster’s new centroid.
5. Each data point should be reassigned to the new nearest centroid. Step 4 should be followed if any reassignments were made; otherwise, the model is complete. There is a parameter

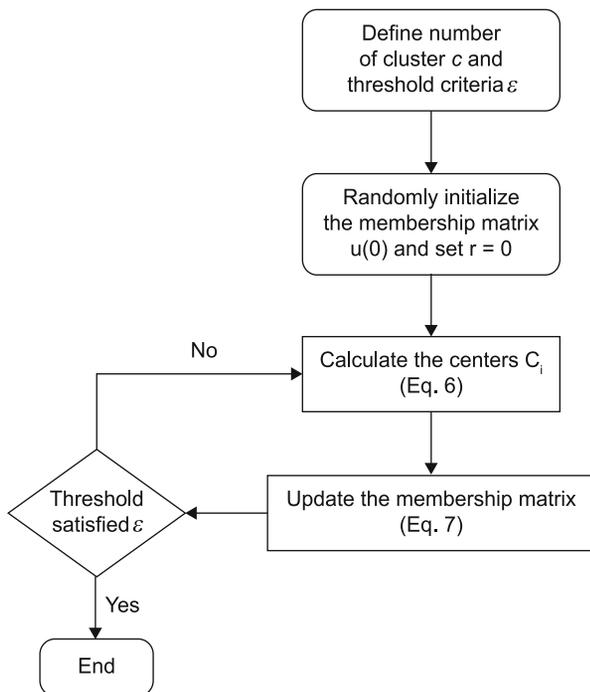


Fig. 18. Comparison of hard and soft clustering data.

known as K that sets the number of clusters to detect for a particular class of clustering algorithms (namely K -Means, K -medoids, and expectation-maximization method). Hierarchical Clustering completely solves the problem. Other algorithms, such as DBSCAN and OPTICS, do not require the declaration of this parameter. The right choice of K when discussing K -Means is frequently confusing, with interpretations varying based on the size and shape of the distribution of points in a data collection and the user’s preferred clustering resolution. Additionally, if each data point is treated as its own cluster, increasing K will always result in a reduction of error, to the extreme situation of zero error (i.e, when K equals the number of data points, n). Therefore, it makes sense that the ideal selection of K will strike a balance between the most data compression utilising a single cluster and the maximum accuracy achieved by allocating each data point to a separate cluster. If a suitable value for K cannot be deduced from knowledge of the characteristics of the data set, it must be determined in some other way. The Elbow Method is one of many types of strategies available for making this choice.

Elbow approach

To create clusters in such a way that the total intra-cluster variation, or in other words, the total within-cluster sum of square (WCSS), is minimised, is the fundamental notion behind partitioning methods like K -Means clustering (Fig. 16). We want the total WCSS to be as minimal as feasible because it represents how compact the clustering is.

Elbow technique examines the relationship between the total WCSS and the number of clusters: In order to prevent the total WCSS from being significantly improved by adding another cluster, one should select a number of clusters. How to select K the ideal number of clusters:(Elbow Technique). Calculate K -Means clustering for a range of K values between 1 and 10 clusters. Calculate the sum of all within-cluster squares for each K . (WCSS). Plot the WCSS curve against the K -fold increase in clusters. The ideal number of clusters is typically determined by where the bend (knee) in the plot occurs. Plot the WCSS curve vs the quantity of clusters K .

Canny edge detection

Canny edge detection is an image processing technique that minimises noise while detecting edges in an image.

1. Five phases make up the canny edge detection algorithm.
2. Calculating gradients.
3. Insufficient suppression.
4. Two thresholds.
5. Hysteretic tracking of the edge.

For edge detection, OpenCV offers the cv2.Canny (image, threshold 1, threshold 2) function. Our input image is the first argument. Our minimum and maximum thresholds are the second and third arguments, respectively. The Canny algorithm is used by the function to identify edges in the input picture (an 8-bit input image) and mark them on the edges of the output map. For the edge linking, the value with the lowest value between thresholds 1 and 2 is used. To locate the initial segments of strong edges, the greatest value is employed. In a few decades, there will be millions of robots in the globe, changing the way we go about our daily lives as a result of developments in image processing, machine learning, artificial intelligence, and related technologies. The list of practical uses for these developments is infinite and includes spoken instructions, anticipating government information needs, language translation, object recognition and tracking, medical condition diagnosis, surgery, reprogramming of human DNA flaws, autonomous cars, and many more.

Fuzzy C means clustering algorithm-results

Multidimensional data can be clustered using fuzzy logic principles, which rate each point’s membership in each cluster centre from 0 to 100 percent. Comparing this to conventional hard-thresh-

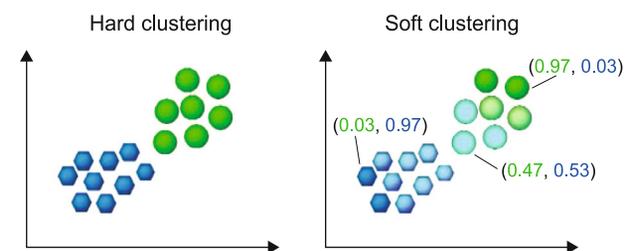


Fig. 19. Diagram of Fuzzy C - means algorithm.

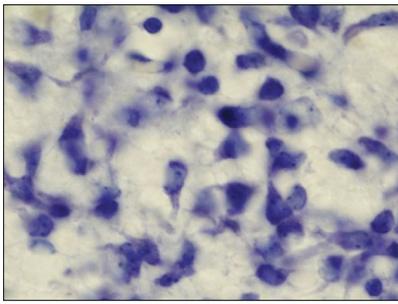


Fig. 20. Original biological data 1.

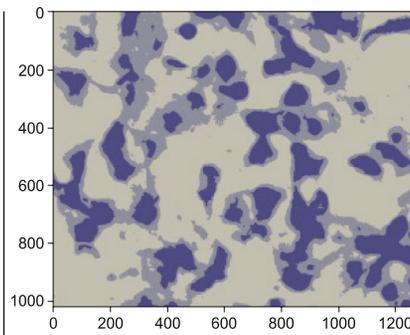


Fig. 21. Results of data 1.

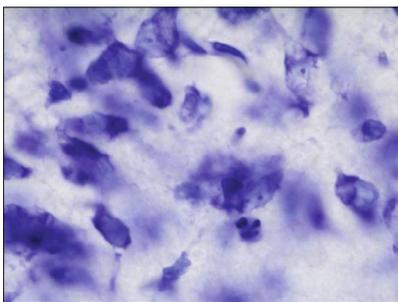


Fig. 22. Original biological data.

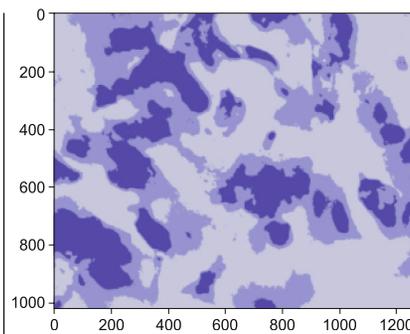


Fig. 23. Segmented biological data.

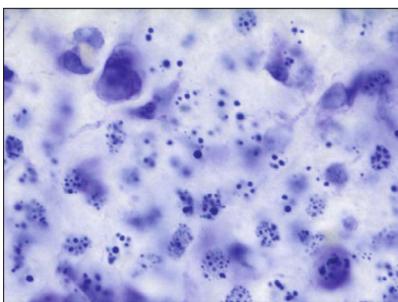


Fig. 24. Original biological data 3.

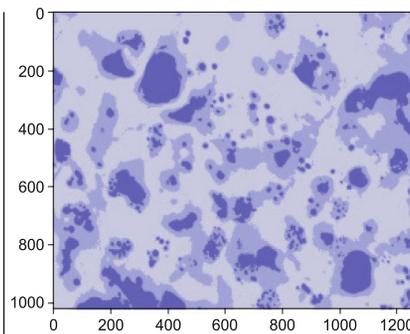


Fig. 25. Segmented biological data 3.

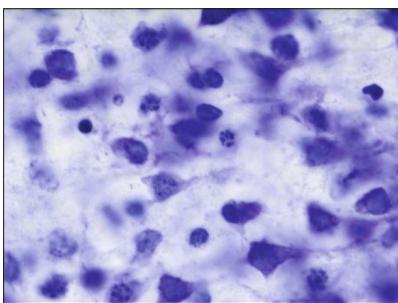


Fig. 26. Original biological data 4.

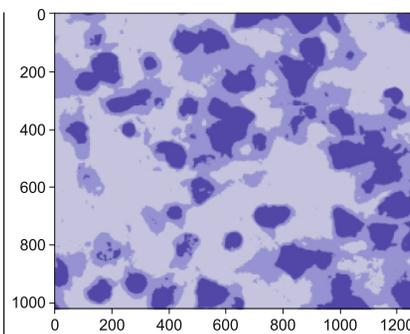


Fig. 27. Segmented biological data.

old clustering, where each point is given a clear, precise label, can be quite effective. Based on the distance between the cluster centre and the data point, this algorithm determines each data point's membership in relation to each cluster centre. The data is more likely to belong to a specific cluster centre the closer it is to the cluster centre. It should be obvious that adding the membership of each data point should equal one. It is an approach for unsupervised clustering that enables us to create a fuzzy division out of data. The approach is dependent on a parameter m that represents how fuzzy the result is. The classes will become muddled for high values of m , and all items will tend to belong to all clusters. The parameter m affects how the optimization problem is solved. In other words, different m choices will likely result in distinct partitions. A gift illustrating the impact of the m selection determined by the fuzzy C -means is provided below (Fig. 17).

K-means versus fuzzy C-means

Let's compare these two powerful algorithms to better understand where the fuzzy C -means method fits (Fig. 18). When using fuzzy clustering, each point has a chance of being a member of each cluster, as opposed to being a member of just one cluster, as is the case with classical k -means. Each point in the fuzzy mean clustering process is assigned a weight that is related to a specific cluster. As a result, a point is not so much "in a cluster" as it is "associated" with it, with the association's strength depending on how far it is from the cluster's center. Speed: Fuzzy means that it will typically run more slowly than K signifies because it is actually working harder. Every cluster evaluates every point, and each assessment involves a larger number of operations. K -Means must only calculate distance, whereas fuzzy C -means must also weight inverse distance. It is critical to understand that fuzzy C -means is a subset of C -means in which the probability function is 1 for the data point closest to the centroid and 0 for all other cases.

Steps in fuzzy C-means

The fuzzy C -means process flow is listed below: Assume that there are k fixed clusters.

Initialization: Calculate the likelihood that each data point x_i belongs to a specific cluster k using a random initialization of the k -means k and the formula $P(\text{point } x_i \text{ has label } k | x_i, k)$. Iteration (Fig. 19): Recalculate the cluster centroid as the weighted centroid based on the likelihood that each data point in x_i will be a member

$$\mu_k(n+1) = \frac{\sum_{x_i \in k} x_i * P(\mu_k | x_i)^b}{\sum_{x_i \in k} P(\mu_k | x_i)^b}$$

Metrics for cluster evaluation

For Fuzzy C-Means, the majority of the metrics employed to assess the clusters under other clustering methods are applicable. Despite the fact that these techniques depend on the expert's field of study, we have listed some common metrics for assessing the resulting clusters below:

1. Study of the clusters' homogeneity after formation.
2. Fuzzy C-Means was used to create the clusters, which must be homogeneous and distinct from other clusters.
3. Analysis of Coefficient of Variation for each cluster.
4. Pearson Cluster quality can be validated using correlation.
5. Precision, recall, and f-score can also be taken into account if we know ground truth cluster values. Some statistical measures for assessing your clusters are the Elbow Method and Silhouette.
6. Entropy-based methods.

Cons and Pros

Pros gives the best result for a set of overlapping data and performs significantly better than the k -means method. Data points are assigned membership to each cluster centre, as opposed to k -means, where they must solely belong to one cluster centre. As a result, data points may belong to more than one cluster centre. **Cons is defined as** a specification of the number of clusters beforehand. A lower value of yields a better outcome, but at the cost of more iterations. Euclidean distance calculations might not accurately account for underlying factors. The choice of the first cluster centre and/or the starting membership value affects how well the FCM algorithm performs.

Results

Fuzzy approach-results:

Analysis of images by using fuzzy K- means clustering algorithm

We describe results of proceeding biological data got by fuzzy approach. We present some of parameters and also image results. Other segmented results of biological issues in the Figures 20–27 follow similar process of coding in Matlab. On the Figure 28 we see edge detection. Dataset: On data is illustrated the process of Apoptosis is an ATP-dependent process, lacking ATP in the cell for completion apoptosis (e.g. when mitochondria are damaged) this process degenerates into necrosis and is marked with as necrosis or continuum. Our aim to detect all objects, all cells. We are not focused on special categories of special stage of cells dying, just on improving segmentation techniques if this kind of data. Selected

coronal brain of rat pups' sections were stained with Cresol violet or Fluor-Jade B to visualize neurons in process of neurodegeneration. The aim is to select and segments all types of cells and neurons. Biological Data are down and get under ethic committee. All procedures involving the animals were performed in compliance with the Principles of Laboratory Animal Care issued by the Ethical Committee of the Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences and by the State Veterinary and Food Administration of Slovakia.

Deep learning technique-theoretical models and results

A machine learning-based segmentation technique is required to get good performance to deal with biological images. Through semantic segmentation, regions of interest can be identified for cell assessment. Clinicians can use segmentation results to identify abnormal cell and improve therapy planning. The creation of high-quality labelled and annotated datasets is a critical part of achieving the algorithmic goal of automated medical image segmentation. It is often difficult to collect clean annotations for cell segmentation at pixel level. To overcome the pixel level segmentation approach, semantic segmentation is one of the most important steps in the computer-aided diagnosis process. In this work, we propose a semantic segmentation framework which is based on the U-net architecture. This framework consists of an encoder-decoder network and a skip connection. The encoder-decoder networks share a common characteristic of combining coarse-grained, shallow, low-level semantic features of the encoder sub-network with fine-grained features of the decoder sub-network. A machine learning-based segmentation technique is required to get good performance to deal with biological images. Through semantic segmentation, regions of interest can be identified for cell assessment. Clinicians can use segmentation results to identify abnormal cell and improve therapy planning. The creation of high-quality labelled and annotated datasets is a critical part of achieving the algorithmic goal of automated medical image segmentation. It is often difficult to collect clean annotations for cell segmentation at pixel level. We use and present MobileNetV2 as Pre-train for Unet. MobileNetV2 is an architecture that is optimized for mobile devices. MobileNetV2 has less parameters, due to which it is

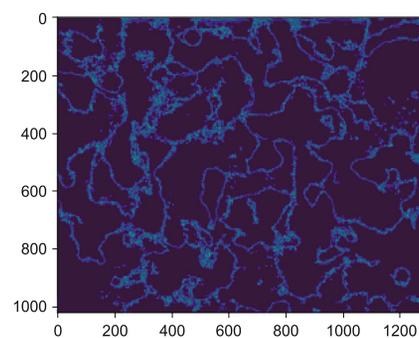


Fig. 28. Edge detection: biological data.

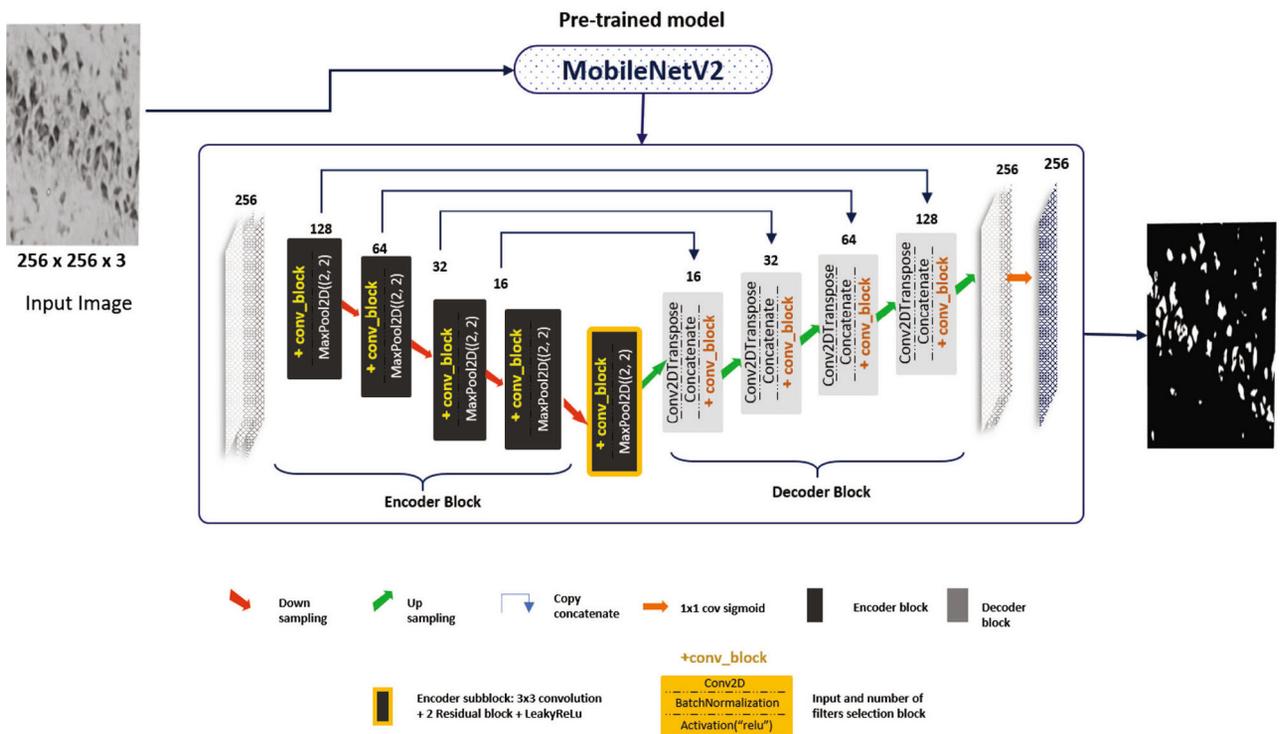


Fig. 29. Proposed U-Net based architecture for segmentation of brain cells.

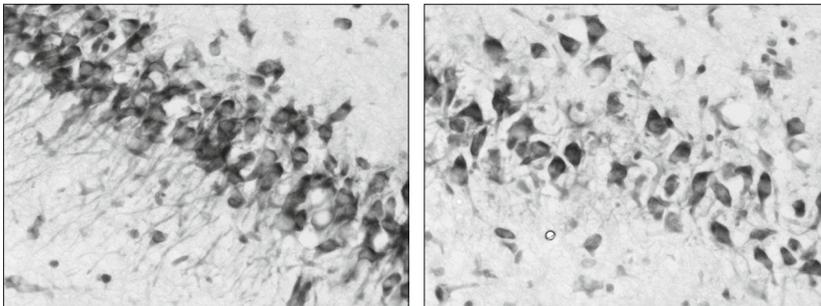


Fig. 30. Sample image of brain cells.

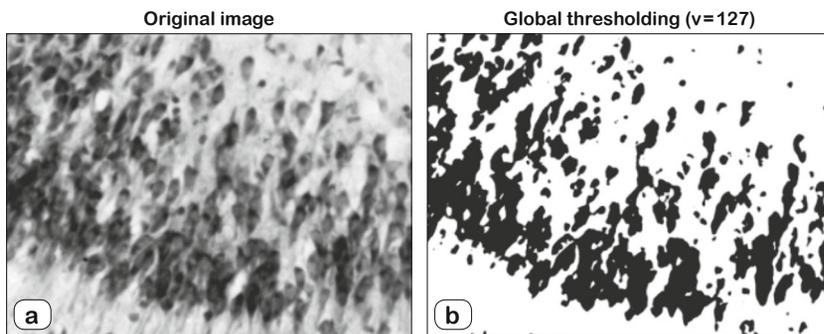


Fig. 31. After binarization using Otsu’s method the original image is shown, (a)Original data, (b)Thresholding processing of data.

easy to train. Using a pre-trained encoder helps the model to converge much faster in comparison to the non-pre-trained model. A pre-trained encoder helps the model to achieve high performance as compared to a **non-pre-trained** model. The proposed deep meaning-based architecture is shown in Figure 29. Sample image of brain cells is shown in Figure 30. In Figure 31 the binarized images are shown. The training curve of U-net segmentation is presented in Figure 32. In Figure 33 the test image and corresponding predicted output is shown with ground truth and without ground truth.

Dataset

On data is illustrated the process of Apoptosis is an ATP-dependent process, lacking ATP in the cell for completion apoptosis (e.g. when mitochondria are damaged) this process degenerates into necrosis and is marked with as apoptosis, necrosis, or continuum. Our aim to detect all objects, all cells. We are not focused on special categories of special stage of cells dying, just on improving segmentation techniques if this kind of data. Selected coronal brain of rat

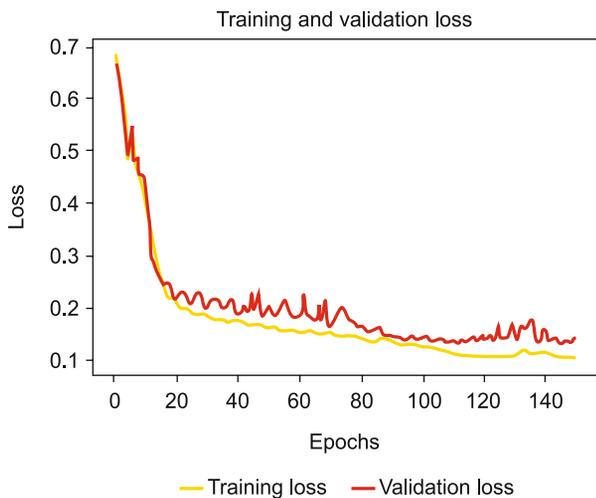


Fig. 32. Training curve of U-net segmentation architecture.

pups sections were stained with Cresol violet or Fluor-Jade B to visualize neurons in process of neurodegeneration. The aim is to select and segments all types of cells and neurons. Data are own and get under ethic committee.

The original data looks like on the Figure 30.

The procedure is as follows:

First step: Original image to masking image using thresholding image processing technique.

Second step: Prepare original image folder and masking folder for Unet process.

Third step: Train semantic segmentation Unet model.

Output shown below predicted test image and completely different image which are no in original or mask image folder.

In Figure 34, we used component labeling to extract statistical information about a region of interest and conduct structural analysis. Here, we represent three different statistical information of segmented cells such as area, diameter, and solidity. In Table 1 the values of different evaluation metric are presented.

Termination: Continue iterating until convergence or up to the user-specified maximum number of iterations (the iteration may be trapped at some local maxima or minima).

Metrics for cluster evaluation

Discussion and conclusion

In the first part we presented new created software for image processing and segmentation using GrabCut techniques. We segmented different types of medical objects in the MRI tissues. We improved the segmentation to get sharper borders of the objects. We focused on the processing of MRI data. The aim is to improve questionable better diagnostic of medical pathological objects visible on MRI data. We proceed and segmented medical human objects like cysts, different tumors, blood edema. This diagnostic is important, because many times clinical doctors in the praxes can see by eyes objects on MRI data, but nor always they can define clearly precisely and sharply borders, boundaries, shadows, and so on. Diagnostic process is then not completely perfect and depends

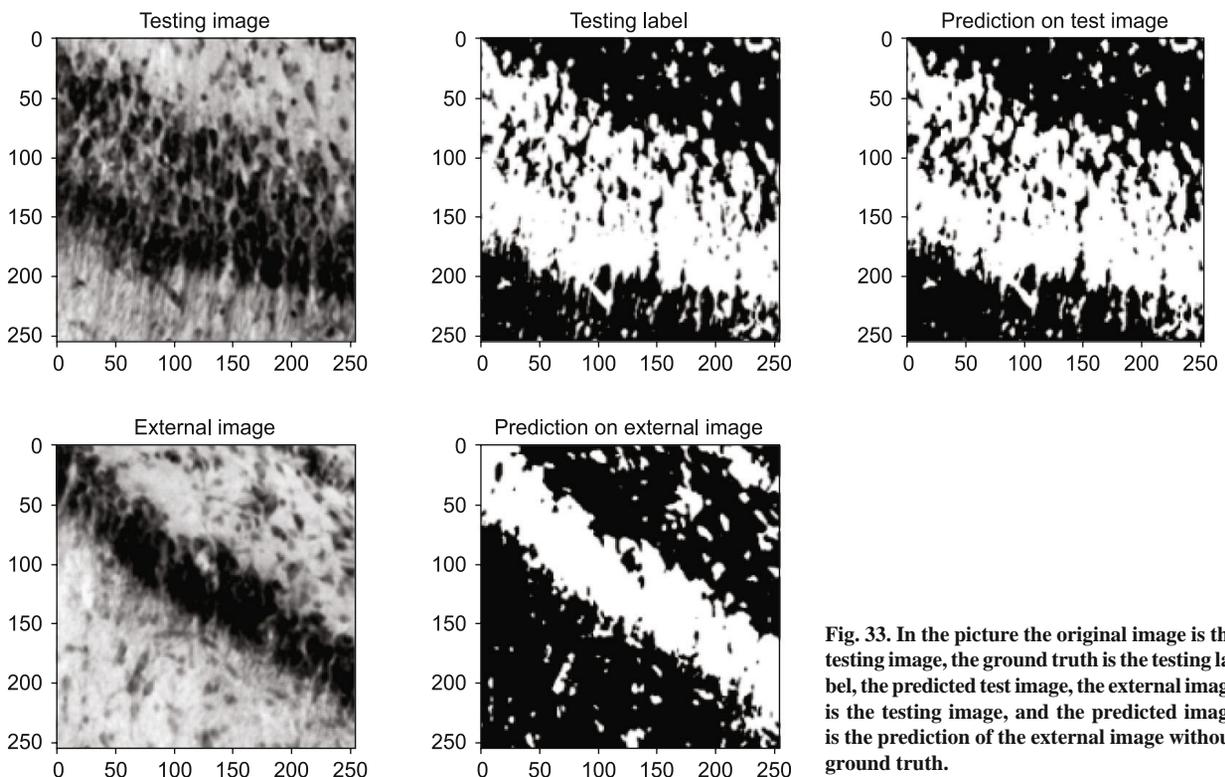


Fig. 33. In the picture the original image is the testing image, the ground truth is the testing label, the predicted test image, the external image is the testing image, and the predicted image is the prediction of the external image without ground truth.

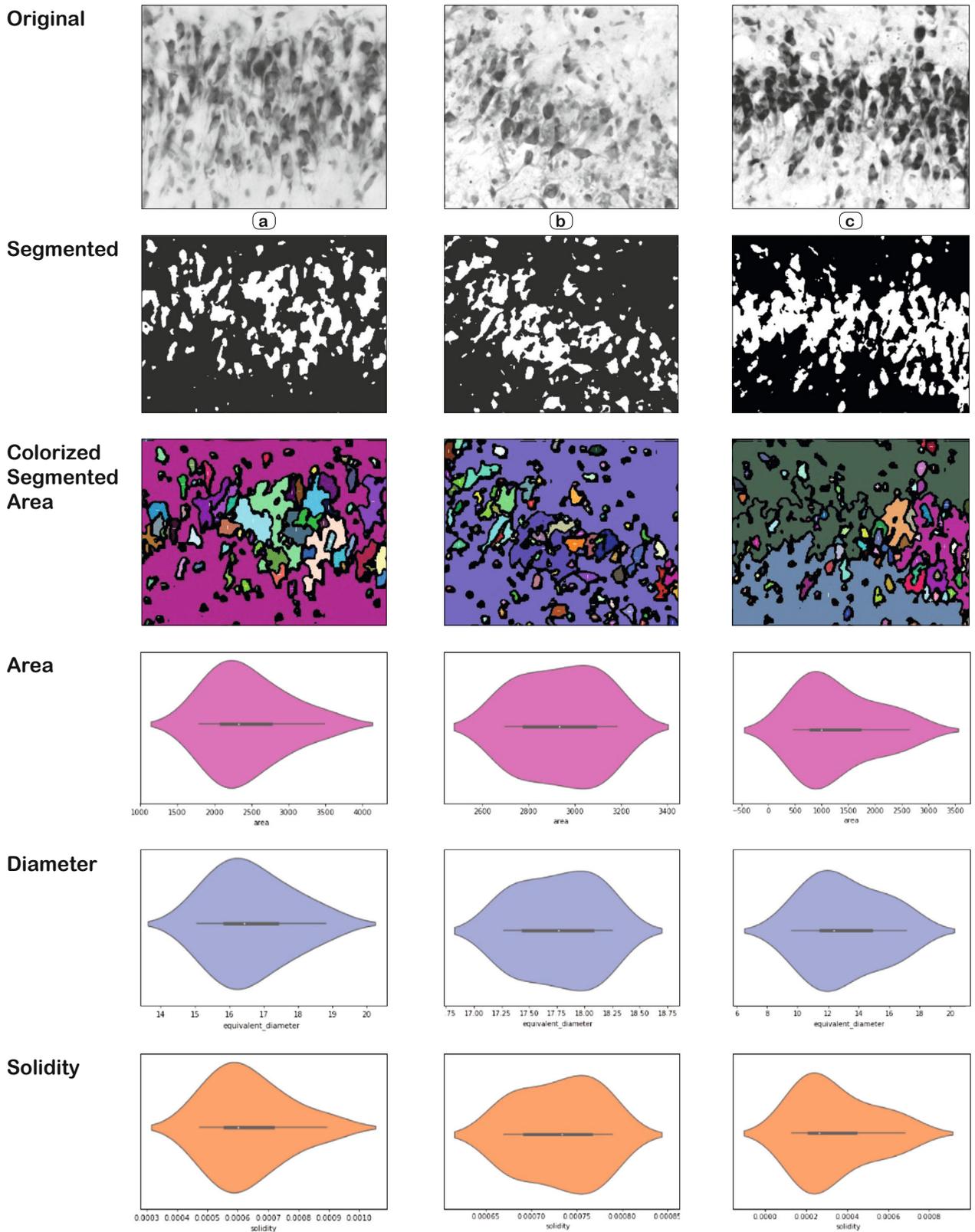


Fig. 34. With Violin plot, find the minimum, maximum, and average areas, diameters, and solidities of segmented cells.

Tab. 1. Using connected components labeling (45) find the minimum, maximum, and average pixels area, diameter, and solidity cover for single cell.

Sample Name	Area	Diameter	Solidity
(a)	Min: 1200	Min: 14.00	Min: 0.00030
	Avg: 2200	Avg: 16.00	Avg: 0.00060
	Max: 4400	Max: 20.0	Max: 0.00010
(b)	Min: 2400	Min: 16.85	Min: 0.00055
	Avg: 3120	Avg: 18.00	Avg: 0.00077
	Max: 3350	Max: 18.70	Max: 0.00080
(c)	Min: 1200	Min: 7.00	Min: 0.00000
	Avg: 2200	Avg: 12.00	Avg: 0.00020
	Max: 4400	Max: 19.70	Max: 0.00080

on the personal experiences of the clinical doctors. GrabCut techniques improve segmentation of the medical objects, brings better and software diagnostic of the borders of the objects. Grab Cut technique improves diagnostic of different kind of questionable medical finding, and detection of the medical pathologies on MRI and different types of medical data. Specially on blood edema and tumors (concretely brain tumors) the boundaries of the tumors are not always sharp. Because of this, when it comes to real operation of the brain, clinical doctors have question, when the tumor ends and how to lead a surgery cut. GrabCut technique improves this diagnostic a lot. In the future we want to apply this technique to different types of questionable diagnostic on different type of medical questions presented by image techniques, MRI, CT, Roentgen, like blood edema, different types of inflammatory of tissues, cysts, and tumors. There is also a question to extend this technique into 3D space and brings to medical doctors a 3D visualization of the tumors.

In the second part we presented fuzzy approach into processing of biological data of cells, and elements in the biological tissues. Specially we processed data set of the brain cells, under the process of their death. Data set contains the healthy cells, the cells in the process of dying and completely death and destroyed cells. In biology there is still a problem in identification of this damage cells, and as we can see the fuzzy approach can bring and segment damage cells. After discussion with biologist we improved the identification of the damage cells, even death cells, because in real paxes, this process is still done manually.

In the third part we proceed again the biologist data of the brain of rats. Again, the process of dying of cells is observing. The biologist put the question? Is there a possible processing of the damaged cells done automatically? On big data sets? As we mentioned before, this diagnoses of the cells, broken cells, was long time done manually, or just with basic segmentation techniques as thresholds. But thresholds techniques did not provide enough good numerical results when it was compared visible with real data. Therefore, we tried to bring and developed better techniques for diagnostic and segmentation of cells in data. These include normal, hybrid and death cells. With the deep / learning techniques we were able to select almost all damaged cells, even bring numerical results about their properties: the area, diameter, solidity of the cells is found out which was shown by Violin plot. In the future we can improve the program and focused also on defining which cells are healthy, which are in the middle stage of dying and which are already

death. But first, we supposed developed and bring segmentation technique suitable for the best detection of the cells on the tissues.

Using deep learning-based segmentation approach i.e., U-net Based segmentation the segmentation of brain cells for both using ground truth and without using ground truth is presented here. For this we prepared ground truth of hundred images. Also, the area, diameter, solidity of the cells is found out which was shown by Violin plot.

In the future we want to proceed and develop GrabCut technique into 3D space. In fuzzy approach we want to extend the processing of biological data for damaged cells also for different part of human organs, not just special parts of the brain tissues. The aim idea is applied in the third part, in deep learning models, which appears as very strong tools for processing, and defining difficult object, in our case damage cells, in different type of brain cells and brain and tissues.

In the future, we will use newly developed efficient deep learning models to get the prominent cell regions to get more accurate area, diameter, solidity etc, parameters for the cells. We can conclude that the proposed processing medical data help in diagnostic of pathological objects and their different types of structures. The numerical evaluation of segmented objects in biological segments (whole and disintegrated cells) also impart in better diagnosis results.

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