

# Neuronal Texture Analysis in Murine Model of Down's Syndrome

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**Abstract.** An alteration of neuronal morphology is present in cognitive neurological diseases where learning or memory abilities are affected. The quantification of this alteration and its evolution by the study of microscopic images is essential. However, the use of advanced and automatic image processing techniques is currently very limited, focusing on the analysis of the morphology of isolated neurons. On this article we present a new methodology, based on texture analysis, to characterize the global distribution of different neural patterns in immunofluorescence images of brain tissue sections, where the neurons can be visualized as they are really distributed. We apply the technique to mice brain tissue section dividing them into two classes: Ts1Cje Down's syndrome model and wild type, free of this neurodegenerative disease. Taking into account CA1 region of the hippocampus, we calculate and compare several state of the art texture descriptors that are subsequently classified using machine learning techniques. Achieving a 95% of accuracy, the assumption that texture characterization is relevant to quantify globally morphological alterations in the neurons, seems to be demonstrated.

**Keywords:** Texture analysis · Down's syndrome · Pattern recognition · Machine learning

## 1 Introduction

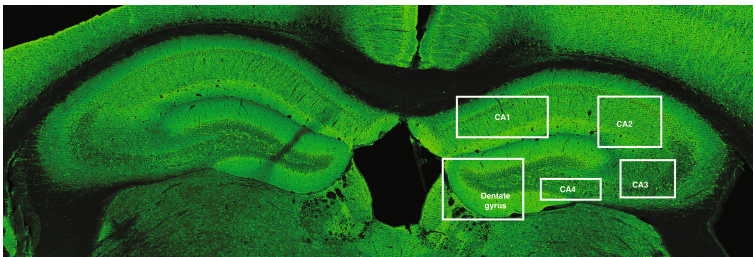
One of the cognitive and neurodegenerative diseases that has awakened most interest in the scientific field is Down syndrome (DS). DS, originated by a trisomy of the human chromosome 21, is the most frequent cause of intellectual genetic disability. Cognitive neurological diseases, where memory and the learning capability is affected, along with neurodegenerative diseases present a generalized neural morphological alteration. This alteration, if correctly quantified, could

provide useful information, specially when assessing the therapeutic potential of drugs aimed at recovery of function and neuronal morphology.

Currently, an objective quantification of the so explained alterations is performed by analyzing the dendritic morphology of isolated neurons. One of the most used techniques to this purpose is the Sholl Analysis [1]. In this method, the dendritic branching pattern that is, the ratio between the number of intersections of the dendrites per unit area and the distance between them and the center of the soma, is calculated. In order to perform this kind of analysis, it is essential to obtain images of isolated neurons. Generally this is achieved by immunofluorescence imaging of low density neuronal cultures, or by studying a kind of tissue staining, Golgi staining for instance, that allows the visualization of just a reduce number of neurons presented in the tissue. This use of neuronal cultures is widespread in the scientific field, although it is an experimental approach to reality. Despite this, in DS study, these staining techniques have been proved to be very useful in the morphogenesis of hippocampal neurons of murine models. As an example, the scientific group led by Dr. Montesinos, director of the Laboratory of Synaptic Local Translation (SLTL) at the University of Seville, has detected morphological differences in dendritic arborization among cultured neurons belonging to Ts1Cje mice, a model of DS, and control mice or wild type (WT), which are free of this neurodegenerative disease [2–6].

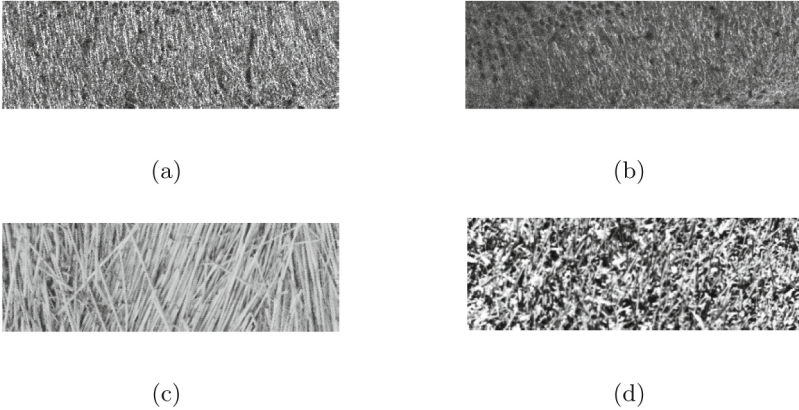
On the other hand, staining of tissue sections allows only the visualization of the dendritic structure of certain neurons, usually 5–10% of the total amount of neurons present in the tissue. The actual mechanism that causes the staining of only these neurons remains unknown compromising the results of the studies based on it.

Opposite to these traditional techniques, we propose studying the dendritic pattern in a global way using advanced techniques of image analysis in immunofluorescence images of histological sections of tissues, where all neurons can be visualized as they are distributed in reality. These images are rich on texture information and posses characteristic architectures that could be studied with pattern recognition methods. An example of an immunofluorescence image of a murine hippocampus section is shown in Fig. 1, in which different parts can be differentiated based only on the texture of each of these zones.



**Fig. 1.** Confocal image of a coronal section of the murine hippocampus region and its representative areas.

The present research has focused on the study of immunofluorescence images of hippocampal sections to study the neuronal texture of control(wild-type) and trisomic Ts1Cje mice. Due to the morphological difference in cultured isolated neurons, it seems direct to infer that there will also be an overall morphological difference on the sections of neuronal tissues. In the particular case of the CA1 region of the hippocampus, these global morphological differences give rise to patterns that are similar to some well-known patterns present in the traditional Brodatz database, a key image database widely used by the pattern recognition community to validate their algorithms. This similarity can be seen in Fig. 2. However, in most of the images analyzed in this research, the difference between the texture of the CA1 region in WT and DS images is not as clear or appreciable at a glance. In order to study the use of texture descriptors as a way to quantify global morphology, we have designed a series of experiments with the goal of automatically classify images of the hippocampal CA1 region into two categories, WT and DS.



**Fig. 2.** Representative images of DS-CA1 region (a), WT-CA1 region (b), Brodatz Straw D15 (c), and Brodatz Grass D9 (d) cases.

## 2 Related Works

Texture is a fundamental parameter in the description of images, as it provides a measure of properties such as smoothness, roughness and regularity [7]. Unfortunately, the amount of research on texture characterization of biological images is limited and usually focused on magnetic resonance and fluorescence microscopy images.

Within texture image research, Haralick texture descriptors [8] are fundamental. They consist of 14 texture features derived from the so-called Gray Level Co-occurrence Matrix (GLCM) matrix. These descriptors have been used for pattern characterization in fluorescence microscopy images of HELA cells [9]

as well as to model the texture of H1-60 cell nuclei [10]. They have also been successfully used in the classification of meningiomas [11], while in [12] where significant changes in mean temporal lobe texture have been detected in patients with Alzheimer’s disease. Recently, other statistical descriptors have been developed improving the performance of GLCM-based descriptors. For instance, in [13] texture characterization is performed on the basis of the gray level zonal-size matrix (GLSZM), with an application to the classification of HEp-2 cells.

Other texture descriptor that has been widely accepted in recent years is the so-called Local Binary Patterns (LBP) introduced in [14] for which there are different variants. In the case of biomedical images, the most interesting are Median Binary Pattern [15] and Local Ternary Patterns [16]. An application of these techniques in combination with other texture-based dispersed scatter descriptors can classify images taken with confocal fluorescence microscopy by fibroid lung cancer [17].

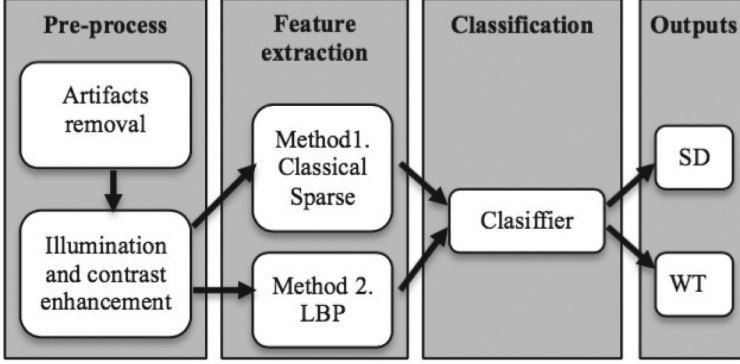
Other texture-based methods for image characterization are Gabor filters [18] and Gauss-Markov models [19]. In [20], texture information using the Gabor filter bank is used for automated segmentation of neurons in high-content scanning or High Content Screening (HCS) images.

To our knowledge, the only work in which histological sections of tissue are globally analyzed is [21]. However, in this article, only orientation and anisotropy characteristics are studied by tensor structure analysis, obtaining the same measures that the ones provided by diffusion tensors. On the contrary, our proposal analyses for the first time different texture descriptors, with the objective of obtaining new protocols that allow the objective quantification of the global neuronal morphology.

### 3 Materials and Methods

This section describes the stages of the proposed method. Initially, we detail the collection and selection of hippocampal images. Next, we describe the pre-processing techniques implemented, ending with the description of the different texture descriptors used in this work. The proposed tool has been developed with MATLAB®R2015a software (The MathWorks Inc., Natick, MA). A block diagram of the system is shown in Fig. 3.

**Hippocampal Images Dataset.** The images were taken at the Center for Research, Technology and Innovation of the University of Seville (CITIUS) with a ZEISS LSM 7 DUO spectral confocal spectral scanning microscope. Fifteen hippocampal histological preparations were imaged, with six of them having a coronal section. For the histological preparations, the animals were anesthetized and subsequently perfused with paraformaldehyde to fix the brain tissue. The brains were then sectioned using a vibratome. Finally the slices were subjected to labeling of neuronal somatodendritic structures by immunofluorescence, using specific antibodies against the MAP2 protein. A stack of images composed of twelve focal planes was taken to each preparation. The images obtained were



**Fig. 3.** Block diagram of the proposed method.

in .czi format. These images were converted to .bmp format, a lossless format that facilitates their processing regardless of the operating system. Subsequently, within each hippocampus sample, we selected the focal plane of the stack that had the best contrast and sharpness in the region of interest, and the remaining planes were discarded from the study to avoid correlated data. Also, we discarded images of preparations that were not of sufficient quality.

The final dataset consisted of nine images (one of them with a coronal section), containing a total of ten samples of hippocampus: five of them belonging to the trisomic class, and five others to the wild type. Each initial hippocampus image was converted to gray scale. Then, the CA1 area was manually selected. Finally the selection was rotated in order to visualize the CA1 area horizontally.

### 3.1 Pre-process

In order to obtain the most relevant texture characteristics, it is necessary to determine a protocol for the pre-processing of the images in order to normalize the structural characteristics of the different parts of the brain to be studied. In the case of the CA1 region, it is necessary first to address the elimination of artifacts, where artifacts are understood as the presence of blood vessels in the images. These blood vessels appear as elliptical areas characterized by a low level of gray. The presence of these vessels is not a differentiating characteristic between DS and WT type, and therefore should not influence the characterization of the texture. The solution adopted in our proposal is to detect the vessels thresholding the image and then restore the detected vessels using the algorithm Fast Image Inpainting [22]. This algorithm, uses as a mask of the undesired points the result obtained by the thresholding procedure. Then, values of the pixels belonging to the mask are replaced propagating the grey level information of their neighbours in a direction from the edges of the vessels to their inner part. The pixels values replacement is performed in a way consistent with human perception to avoid blurred patches in the resulting images. Therefore, the inpainted

vessels are substituted in a way that the new gray value and gradient on that location extrapolate the gray value and gradient outside the neighbourhood.

After the removal of artifacts it is necessary to study aspects related to contrast enhancement. The quality of the immunofluorescence images depends not only on the dynamic range of the measuring instrument, but also on the expression and distribution of the marker being evaluated, among other factors. Therefore, the quality of the images under study could vary considerably from one histological sample to another. To improve contrast we have chosen the contrast limited adaptive histogram equalization technique (CLAHE) [23]. Basic histogram equalization (HE) is a process by which pixels' values are mapped in order to obtain an image with the same number of pixels for each gray level. That procedure usually fails when the image content is not homogeneous. To avoid this, improving local contrast and edges definition, adaptive histogram equalization (AHE) performs a basic HE on neighbourhoods of the pixels instead of considering the whole image at once. The amplification of intensity of the amount of contrast enhancement depends on the slope of the cumulative distribution of grey level function, that is, it depends on the value of the histogram at that pixel value. It causes the amplification of noise in homogeneous regions that tries to be overcome by CLAHE. This equalization procedure limits the amount of amplification of AHE to certain levels. An example of application of the pre-processing techniques in one of the study images is shown in Fig. 4.

Finally, in order to obtain a larger sample size for both WT and DS, each region of interest was divided into sixteen non-overlapping 50 square blocks. This procedure is generally adopted by texture analysis techniques.

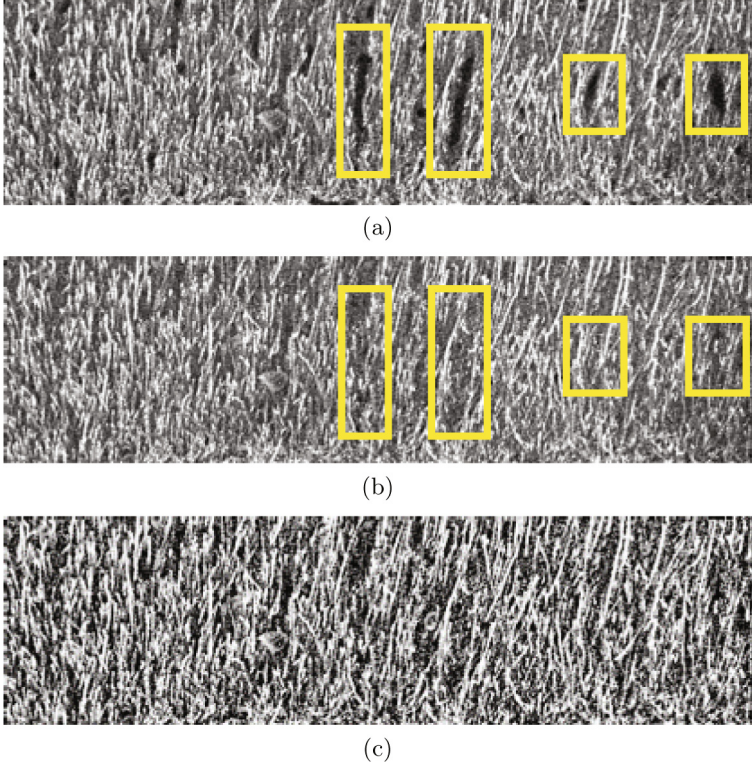
### 3.2 Feature Extraction Procedure

In this work we have evaluated two groups of texture descriptors. Firstly, we use a set of descriptors that combines classic ones that have been proven to be efficient in the past and that are highly validated along with other more recent ones that exploit the sparseness nature of the texture features (method 1). Secondly we use Local Binary Patterns (method 2), descriptors with high impact in state of the art techniques.

**Method 1. Classical and Sparse Descriptors.** Due to the great robustness and proved performance for image classification, we have decided to use classic texture descriptors along with a new set of features that try to take advantage of the sparsity property of the texture for the characterization of the regions of interest. The resulting feature vector is therefore formed by:

1. First order statistical descriptors: mean, standard deviation, asymmetry or skewness coefficient, kurtosis and entropy.
2. The particularization of the fractal dimension called the Hausdorff dimension [24].
3. Haralick texture descriptors [8] from the co-occurrence matrix.
4. Mean and variance of the sparse texture vector described in [25, 26] using a Gaussian mixture of five components.





**Fig. 4.** (a) Original region of interest where Larger blood vessels are highlighted in yellow, (b) image obtained after applying the algorithm of Fast Image Inpainting and (c) image obtained after applying the CLAHE algorithm for contrast enhancement. (Color figure online)

**Method 2. Local Binary Patterns.** Because of their low computational complexity and their discriminative power, LBP-based texture methods have become very popular in recent years [27]. Among all of the existing LBP variants, a circular and rotation-invariant code [14] was selected, with a neighbourhood of eight pixels and a radius equal to one. For each block, thirty-six LBP codes were obtained. The characteristic vector was formed by the histogram of these thirty-six codes.

### 3.3 Training and Classification

Once the feature vector is constructed for each block of the region of interest, we proceeded to perform a binary classification step. For each method the following classifiers were used:

1. Support Vector Machine (SVM) [28]: in its versions of linear, quadratic, cubic and Gaussian kernel).

2. K-Nearest Neighbor (KNN) [29]: in their versions of cosine and cubic distance.
3. Complex Tree (a single decision tree).
4. Bagged Tree [30].
5. Random Forests [31].

We have used an external validation method to assess the quality of all tested classifiers. For that, we selected for each type of image, DS and WT, 3 complete CA1 regions, that are in total 48 blocks, for the training stage of the classifiers, and 2 CA1 regions, that is 32 blocks, for the test stage. This is mainly due to the fact that in texture images there may be a high correlation between areas of the same image, so if we do not use complete CA1 regions, we could get false results.

## 4 Results

The results of classification obtained with the different classifiers for each developed method are shown in Table 1. The metric used to verify the method studied is the accuracy in the DS or WT classification. The “Classification Learner” tool of Matlab 2015 software was used for the simulations.

**Table 1.** Results of the classification in terms of accuracy (%). The classifier with the best result has been highlighted in bold for each method.

Classifier	Method 1	Method 2
	Accuracy (%)	Accuracy (%)
SVM (lineal kernel)	<b>96.88</b>	92.18
SVM (quadratic kernel)	93.75	90.62
SVM (cubic kernel)	93.75	89.06
SVM (gaussian kernel)	92.19	90.62
k-NN (cosine distance)	75.00	<b>95.62</b>
k-NN (cubic distance)	93.75	89.06
Complex Tree	76.56	76.56
Bagged Tree	85.94	95.31
Random Forest	89.06	93.75

Among all of the possible combinations, the best result, 96.88%, is obtained with method 1 (classical and dispersed texture descriptors) using SVM in its linear kernel version. In the case of method 2, which uses the LBP texture descriptors, the best result, obtained with k-NN cosine, is slightly lower, 95.62%, but still constitutes a high success rate.



## 5 Discussion

The main objective of this research was to verify the use of texture descriptors to discern neuronal morphological differences globally in sections of brain tissue between murine models of Down syndrome and control mouse subjects. For this, an experiment has been carried out where an image of the region of interest in DS or WT is classified based only on texture descriptors using several recent machine learning techniques. Two sets of different texture descriptors have been used, obtaining high percentages of success with both of the methods studied. From the results obtained it can be inferred that there is a texture pattern that characterizes the CA1 region of the hippocampus in murine models of DS and WT. In addition, the texture of the CA1 hippocampal region of DS mice is altered in relation to the texture of the same region in control subjects. This alteration was foreseeable due to the dendritic morphological differences observed in neurons isolated in culture in previous studies carried out in the Laboratory of Synaptic Local Translation of the University of Seville. However, having been able to identify the type of mouse to which a particular image belongs, based solely on the texture opens a new paradigm of investigation that can be useful for the study of many cognitive and neurodegenerative diseases.

Despite the good results obtained, we believe that it is necessary to improve some aspects of this research. In the first place, it would be necessary to have a larger image base to be able to study the complete CA1 region, without having to divide it into smaller pieces. We are currently working in collaboration with the SLTL of the University of Seville to perform a new imaging in new histological preparations. Secondly, it would be interesting that the selection and trimming of the ROI (the CA1 region) within each image was done completely automatically. In this way, we would eliminate a task that is tedious and usually consumes a lot of time to the specialist.

Finally, in order to develop a more complete investigation, we will analyze other areas of the brain. By quantifying and characterizing the distribution of different neuronal patterns in different areas of the nervous system, it will be possible to extract relevant conclusions at the pre-clinical level about the effect of certain compounds on the recovery of global neuronal morphology, and thus contribute to the progress in knowledge scientist of numerous neurodegenerative diseases.

## 6 Conclusions

In this research we have proposed a new paradigm of methodology in the investigation of neurodegenerative diseases, through the study of dendritic structures in complete tissues instead of studying isolated neurons. We can consider that the work developed has had more than positive results considering that the approach of the problem was a new line of research totally unknown to date. One of the key points in obtaining these results is the application of a pre-processing stage that addresses the elimination of blood vessels as well as the contrast enhancement. The final solution described is the result of an exhaustive study of different

alternatives until finding the most appropriate solution to the immunofluorescence images of hippocampus. All parameters of the algorithms of this stage are fixed and do not need to be adapted to each of the images, which makes the procedure completely automatic. On the other hand, we have made a careful selection of texture parameters that compound the resulting feature vector for each of the two methods studied. In the case of the first method, classic texture descriptors were complemented by dispersed descriptors of more recent creation, and in this way it has been possible to reach excellent results. In the same way, the LBP descriptor chosen is invariant under rotations something relevant in the case of our images due to the fact that rotation of the texture pattern should not be considered as a distinctive feature to distinguish between DS and WT. The results obtained with this set of descriptors have also been very satisfactory. Finally, we have made a complete comparison between different classifiers for the two vectors of characteristics calculated. The best-performing classifiers are those based on Vector Support Machine for the first method and K-NN Cosine and Bagged Tree for the second method. We would also like to highlight the reliability of decision tree-based classifiers such as Random Forest.

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