Artificial neural network technologies as a tool to histological preparation analysis

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Abstract. Food fraud has become one of the great internal and external threats nowadays. For effective and objective detection of adulterated products, real-time control systems are used. Microstructural analysis is one of these methods. It allows rapid quality and composition assessment of food’s raw materials and the finished products. Human skills and expertise/involvement in interpreting the results of microstructural analysis is still absolutely necessary, despite the possibility this is subjective. Modern hardware/software systems for histological analysis with neural network technologies will exclude the subjectivity of that result interpretation. For convolutional neural network operating, the “learning with the teacher” model is used. With this training method, a set of data is prepared, which acts as a series of observations, and for which the values of the input and output variables are indicated, such as: histological analysis data > conclusion about the sample composition > adulteration definition. The network learns to establish connections between them. This paper presents the classification parameters for vegetable food component identification as a part of meat raw materials and finished products. A unified database structure has been developed for structuring and summarizing the main microstructural characteristics of histological sections for various types of meat raw materials. The production-rule systems of “If ... then ...else” are given, and vegetable protein components were chosen as an example. Based on these rules, training of convolutional neural network occurs.

1. Introduction

Rapid assessment of the raw materials actually used in a meat product is possible based on the structural microscopy of meat components. Histological analysis is still carried out in laboratories by trained histologists utilizing hardware/software systems for histological studies. However, they do not meet all user requirements. Human involvement is absolutely required in interpreting the results. Therefore, the data obtained can be subjective. Moreover, a lack of skilled histologists prevents application of histological assessment in food enterprises. This disadvantage, together with the frequent non-compliance of those laboratories that do meet the requirements, prevents wide application of the microstructural analytical method. As a result, the assessment can often give unreliable and biased data on the raw materials and finished product composition.

To improve the objectivity of food control, implementation of high-performance intelligent technologies is necessary. Automation of microscopy (in the analysis of histological sections) and processing the results obtained will improve the quality and ergonomics of histologist analysis and will allow a database of the accumulated research experience to be compiled.
Intelligent systems are technical or software systems capable of solving problems that are traditionally considered to be in a specific knowledge area, data of which are stored in the memory of the system. Intelligent system structure includes three main blocks, i.e. the knowledge base, the decision-making mechanism and the intelligent interface[1]. In decision-making technologies, an intelligent system is an information and computing system with intelligent support that solves problems without involvement of a human decision maker.

Currently, artificial neural networks (ANNs) are the most promising algorithms with respect to the full variety of basic principles on which intelligent systems are built. Solutions based on ANNs show the best results in the field of image recognition and classification compared to other existing algorithms. An ANN analyzes information and assesses whether the available data is consistent with the characteristics that it has learned to recognize. The principal difference of such neural network technologies in information processing is that instead of strictly algorithmic, step-by-step data analysis and calculation of program coding, parallel processing of the whole information array and the drawing up of a training set is used.

ANNs are used to identify and classify information in the case of limited, incomplete and non-linear data sources. When developing ANNs, the main goal is to minimize the direct influence of human researchers on the process of solution finding. Thus, the main advantage of an ANN is the potential ability to develop its own solutions. The use of ANNs makes it possible to eliminate the most difficult part of problem solving, i.e. problem characterization and building the mathematical representation, by training the network on experimental data. Thus, it is possible to avoid idealization of the results. ANNs can be classified as shown (Figure 1) [1].

![Figure 1. Classification of artificial neural networks](image)

ANNs are classified as follows:
- by the input information type:
  - analog ANNs – use information in the form of real numbers;
  - binary ANNs – operate with information presented in binary form;
  - graphic ANNs – operate with information presented in the form of images: signs, hieroglyphs, symbols.
- by the training type:
  - learning with a teacher, i.e. the output space of neural network solutions is known;
  - learning without a teacher, i.e. the output space of neural network solutions is formed only on the basis of input effects. Such networks are called self-organizing;
  - reinforcement learning, i.e. a system of penalties and rewards from the program environment.
- by the setting of synapses (weights):
  - with fixed connections (weights are selected immediately based on the problem status while \( \frac{dW}{dt} = 0 \), where W are weights of the network);
• with dynamic connections (synapses are adjusted to the process of learning, so $\frac{dW}{dt} \neq 0$).
  - by the signal transmission time:
    • synchronous, i.e. the transmission time of each connection is either zero or a fixed constant;
    • asynchronous, i.e. the transmission time of each connection between elements is different, but constant.
  - by the connection type:
    • Feedforward networks. All connections are directed strictly from the input neurons to the output ones. Examples of such networks are the Rosenblatt’s perceptron, the multilayer perceptron, the Ward’s networks;
    • Recurrent neural networks. The signal from the output neurons or the neurons of the hidden layer is partially transmitted back to the inputs of the neurons in the input layer (feedback). The Hopfield’s recurrent network “filters” the input data, returning to a steady state and, thus, allows problems of data compression and associative memory build-up to be solved.
    • Radial basis functions. Also known as RBF networks. Radial basis network is characterized by three features: single hidden layer; only neurons of the hidden layer have a non-linear activation function; the synaptic weights of the connections in the input and hidden layers are equal to unity.
    • Self-organizing cards. Competitive neural network without a teacher that solves the problems of visualization and clustering.

The choice of the network type (Rosenblatt’s perceptron, Hamming’s network, Kohonen’s network, convolutional neural network, fuzzy multilayer perceptron, etc.) depends on the task. When solving problems of image recognition and classification, a special case of which is a histological section (to determine the microstructure of the sample), convolutional neural networks and multilayer perceptrons are most often used, while Kohonen’s network is more often used to do data categorization [2, 3]. Perceptron (lat.perceptio - perception) is a mathematical or computer model of information perception by the brain (cybernetic brain model) proposed by Frank Rosenblatt in 1957 and first implemented as Mark-1 electronic machine in 1960. Perceptron was one of the first neural network models, and Mark-1 was the first neurocomputer in the world.

The nature of network learning depends on the network type chosen. The “learning with the teacher” model is used to work with the convolutional neural network. Using this training method, a set of data is prepared as a series of observations, with values of the input and output variables indicated (histological analysis data > conclusion on the sample composition > adulteration definition). The network learns to establish connections between them.

The purpose of this work was to determine the main microstructural characteristics (classification parameters) for the identification of vegetable components in meat raw materials and finished products. An information base of the numerical classification parameters was formed, which is necessary for the ANN in automatic mode to learn how to recognize the study materials.

2. Materials and methods
The materials studied were vegetable components of protein and carbohydrate nature, and samples of meat raw materials and finished products (boiled and smoked sausages, delicatessen products). Histological preparations of these studied components were 14 µm thick sections, prepared with a MIKROM-HM525 cryostat (Thermo Scientific), then placed on Menzel-Glaser slides (Thermo Scientific), stained with Ehrlich hematoxylin and 1% aqueous-alcohol eosin solution (BioVitrum), and transferred into glycerin-gelatin according to the standard technique. Histological preparations were studied using an AxioImager A1 light microscope (Carl Zeiss, Germany). Images were obtained using AxioVision 4.7.1.0 image analysis system (Carl Zeiss, Germany).

3. Discussion
Automation of visualization processes is convenient and useful. However, the question remaining is how to implement ANN in histologists’ workflows with the greatest efficiency. We do not want to
repeat the mistakes of the Digital Pathology Association, which were made several years ago when using automatic diagnosis [4]. Statistical analysis of this method implementation in mammography revealed many errors of the first kind (erroneous deviation from the null hypothesis). For example, according to a study by Korean scientists [5], in medical examinations, the number of first-kind errors (i.e. erroneous detection) in computer diagnostics of mammography data was about 70%. This meant that in the diagnosis of healthy mammary glands, non-existent tumors were detected in more than half of the cases. Palazzetti et al. [6] showed that in the diagnosis of breast malignant tumors, in more than half of the cases (250 patients with cancer and 250 healthy women took part in the study), symptoms of the disease were not noticed by specialists. Therefore, we need to be very careful in choosing the ANN architecture and its training method. The main task is to facilitate the work of histological specialists and help them to draw conclusions (on adulteration/not adulteration), and not to hinder their work.

Thus, in the analysis and classification of images, neural networks showed only moderate results, as noted above, until a new ANN architecture, i.e. convolutional neural network (CNN) was developed. Many people associate CNN with computer vision. CNNs achieved great success in image recognition due to the fact that they are arranged like the visual cortex of the brain, i.e. they can focus on a small area and highlight important features in it. CNN operation is usually interpreted as a transition from specific features of the image to more abstract details, and then to even more abstract details, up to highlighting high-level concepts. At the same time, the network is self-adjusting and produces the necessary hierarchy of abstract features (a sequence of feature maps) by filtering out unimportant details and highlighting important ones.

An algorithm for effective breast cancer CNN-based diagnosis was recently developed at the University of Nijmegen (Netherlands) [7]. Stanford University collected a database of more than 40,000 x-ray images of injured limbs in January 2018. With this database, a neural network was trained and subsequently proved its effectiveness in determining limb injuries comparing to a professional radiologist [8]. Verily Life Sciences (Alphabet Holding) has developed an algorithm that determines the age, gender, and various medical indicators (for example, blood pressure or body mass index) of patients by analyzing their retina [9]. Such a diagnostic method could help in cardiovascular disease prediction. Large databases have contributed to the development of deep learning algorithms, which in such tasks as detection of diabetic retinopathy [10], skin cancer [11], cardiac arrhythmias [12], cerebral hemorrhage [13], pneumonia [14], and hip fractures [15] ensure efficiency at the expert level.

Data on the basis of which ANN builds answers can be of various types and formats: terms describing any situations, numbers or values, graphs, two- or three-dimensional images, etc. Neural network training consists of several stages. Data selection for the network training and their processing is the most difficult step in solving the task. The training data set must meet the criteria of representativeness and consistency.

For detection of the main microstructural characteristics (classification parameters) of identification, we developed a unified database (DB) structure for our histological sections of various meat raw materials and vegetable components present in the composition of meat raw materials and finished products. The identification indicators used were the following [16]: particle shape, size, particle tinctorial characteristics (ability to be stained with histological dyes), soybean shell fragments (for protein components). For example, soybean protein isolate particles (10 to 110 µm size) are stained in pink, soybean protein concentrate particles (30 to 105 µm size) are stained in dark pink to bright red. Starch particles are described as folded strap, or bean, or rounded particle with a dark point in the center. On the other hand, flour particles are orbited and grouped into large aggregates.

The production rules “If..., then...” for identification of vegetable protein components are presented as examples:

If there are eosinophilic structures, and stained substance predominates in the cell complexes, then soybean protein products are present.

If the particles are round, with holes inside, have a homogeneous wall, the shape of donut, dumbbell or flower, then soybean protein isolate is present.
If the particles are cylindrical (longitudinal section) or rounded (transversal section) cells surrounded by a narrow, non-stained area, i.e. cellulose membrane, then soybean protein concentrate is present.

If there is fibrous component, i.e. thin loose fiber bundles and narrow cylindrical cells not stained with common histological dyes, then textured soybean protein product is present.

If there are eosinophilic structures of round shape, in which non-stained starch granules are present, then pea flour is present.

If the particles are round, with holes inside, have a porous wall, the shape of donut, then pea isolate is present.

If there is fibrous component, i.e. thin dense fiber bundles, while there are no non-stained cylindrical cells, then wheat textured product is present.

If the structures are not stained, and they look like narrow cylindrical cells, in which only transparent stacked cell membranes are detected, then soybean shell cells are present.

The database (set of training pairs) will be divided into two unequal parts. Most of the data will be used for training (training samples), the other part will be used for testing the ANN (test samples). Figure 2 shows our ANN training module.

![Figure 2. Artificial neural network training diagram](image)

Training data are used to train the network. Test data are used to calculate the errors of the network. However, test data are never used for network training. A test data error decrease confirms the network generalization process. If the error continues to decrease with the training data and increases with the test data, it means the network has stopped performing a generalization and just “remembers” the training data. This phenomenon is called network retraining or overfitting (overtraining or overfitting) in machine learning and statistics is a phenomenon when the constructed model well explains the examples from the training sample, but does not work well with examples that were not used in training (test sample). This is due to the fact that when developing a model (“in the process of training”), some random patterns are found in the training sample, which are absent in the general data array. In such cases, training is usually stopped. In the process of training, other problems can appear, such as “paralysis” or local minimum of the error surface. It is impossible to predict a particular problem, or to give unambiguous recommendations for their resolution.

4. Conclusion

Objective histological results related to food adulteration are possible only when a skilled histologist with appropriate work experience conducts the assessment. The trained specialist does not just analyze the indicators in accordance with generally accepted criteria, but compares them with those many samples they have already seen and analyzed during their work. The higher the qualifications and the histologist’s experience is, the larger is their “photo library” and the greater is the likelihood that the histologist will correctly evaluate the microstructural characteristics of any sample they examine. Thus, the proper result depends on the knowledge, skills, experience and professional competencies of the histologist. However, the use of modern informational approaches to processing and interpreting information facilitates this decision-making process. The use of neural network
technologies for food composition control is a socially significant procedure. It can significantly speed up the process of adulteration detection in laboratories of both governmental control organizations and food processors. It will definitely increase the objectivity of the results obtained, help to eliminate unfair competition in the food market, ensure product meets consumer expectations as regards food content, and ensure consumer protection.

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