Technical and mathematical problems of microbiological protection of a manned space vehicle and stations

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Abstract: *During longtime space fights and interplanetary missions among numerous outboard risks, the crew faces onboard microbiological intruders as well. There is no way to send biological tests for the analysis to the Earth in such missions, so special onboard system of methods and activities must solve two different problems: sampling different types of bacteria and fungus and perform independent analysis of collected material without professional microbiologist among the crew and any help from the Earth. So we meet an interesting task to create system that would minimize human factor and rely mostly on computing machinery. In order to use pattern recognition method, we need to perform proper tests sampling and prepare them for the machine analysis. Stereoscopy and spectrometry is the only way to achieve our goal. Apart from tests sampling it is necessary to develop modified mathematical model for pattern recognition of bacteria and fungus, which were found during the flight. For that reason we are making mathematical model, describing microbiological samples. Still, we have a lot of work to do but the result of our research could become common use not only in space sector, but also in clinical medicine as well.*

Key Words: *microbiological safety, pattern recognition, machine learning, stereoscopy, spectrometry*

1. INTRODUCTION

Simple earth microorganisms can be a real problem in a long-time space missions. Deep space conditions make negative effect on them and change them badly, providing high rate of gene transmission. Russian scientists come to this conclusion when they studied microbiological samples from "MIR" station, "Foton M2" station and International Space Station. "MIR" and "Foton M2" explorations have shown that microorganisms lose their stable state even in short-term space conditions (12-14 days) not to mention that they face genetic changes too. They don't increase general number of mutations, but some genes may not work properly. At the

same time, the other genes, which were "sleeping" on earth, starts produce "aggressive" ferments. Besides, microorganisms increase their genetic transmission between each other.

Lack of gravitation can be the reason of such changes, because it has great influence on the bacterial bridge, which helps to deliver necessary genes. Hopefully, on Earth microorganisms came to its usual state and we don't see their aggression.

But astronauts do. So, we need to explore microorganisms onboard in order to understand all the changes they face and prevent possible damages to the ship or the crew. The most suitable method is mathematical pattern recognition from 3D photography of the microbiological colony.

Our prime object-S-colony of microorganisms (from word "Smooth"), because such type of colony is typical for the most of the opportunistic bacteria types. First of all we need to find the way of obtaining the samples for the further exploration. For these purposes IMBP together with scientific research Institute of Cosmic Instrument making (Russian Federal Space Agency) made the special device – the experimental device for express diagnostics of microflora-structure which allows incubating colonies of microorganisms in the conditions of real space flight ("Microflora").

It represents the thermostat in the form of a convenient and compact small suitcase with lock and element of control that switches the thermostat between two operating modes – 28°C and 37°C. Petri-dishes are placed in the thermostat in individual cells with a nutrient medium where an incubation takes place.

After we get necessary sample one's must perform multiphoton excitation (MPE) microscopy. Multiphoton excitation (MPE) microscopy is a powerful tool that combines scanning microscopy with multiphoton fluorescence to create high-resolution, three-dimensional images of microscopic samples. MPE is particularly useful in biology because it can be used to probe delicate living cells and tissues without damaging the sample. Although multiphoton excitation has been demonstrated with high-power CW argon and krypton lasers, the laser source of choice for MPE microscopy is an ultrafast Ti: Sapphire laser. When compared to conventional confocal microscopy, MPE microscopy has many advantages: higher axial resolution; greater sample penetration; reduced photo bleaching of marker dyes; increased cell viability.

Multiphoton excitation microscopy is an amalgamation of multiphoton fluorescence and confocal scanning microscopy.

To fully understand MPE microscopy, it is important to have a basic understanding of these two techniques.

2. MULTIPHOTON EXCITATION MICROSCOPY

In traditional fluorescence spectroscopy, a single photon of light is used to excite a molecule from its ground state (S_0) to an upper energy state (S_{1w}), as shown in Fig. 1. Once excited, the molecule then decays to an intermediate energy state $(S_{0(n)})$, giving off a photon of light (fluorescence) that is representative of the difference in energy between those states. The relationships between photon energy (E) frequency (v), and wavelength (X) are given by the equations:

$$E = hv, vX = c, X = hc/E, \tag{1}$$

where h is Planck's constant and c is the speed of light. Since the energy difference between the ground state and the upper energy state $(S_{1(n)} - S_0)$ is greater than the energy difference

between the upper state and the intermediate state $(S_{1(n)} - S_{0(n)})$, it is evident from these equations that the energy of the exciting photon is greater than that of the fluorescing photon, and thus, the wavelength of the exciting photon must be shorter than that of the fluorescing photon.

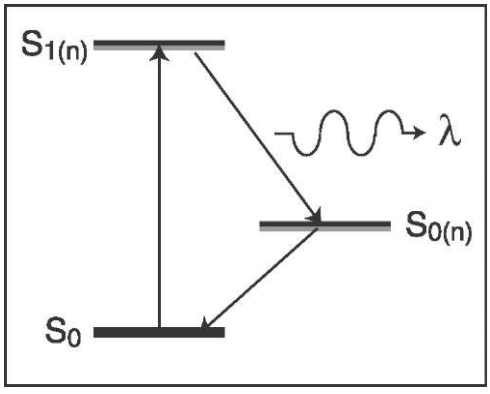

Fig. 1 – Simplified three-level energy diagram

Although the interaction probability is greatest for single-photon absorption, if two or more lower energy (longer wavelength) photons arrive simultaneously, there is some probability that they can excite the molecule as long as:

$$(E_1 - E_0) = hc, (1/l1 + 1/l2 \ldots + 1/ln) \quad (2)$$

where $1/l1 + 1/l2 \ldots + 1/ln$ are the wavelengths of individual photons.

This is demonstrated in Fig. 2, where $5\ eV$ electronic transition in a serotonin molecule can be excited by a single $250\ nm$ photon (deep ultraviolet), two $500\ nm$ photons (green), or three $750\ nm$ photons (near-infrared).

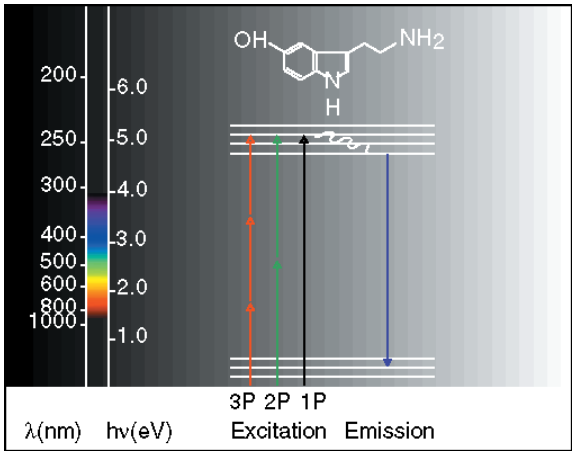

Fig. 2 – Multiphoton absorption in a seratonin molecule

In fact, according to Winfried Denk, who co-invented multiphoton microscopy, "The use of such short pulses and small duty cycles is, in fact, essential to permit image acquisition in a reasonable time while using 'biologically tolerable' power levels".

Another advantage of multiphoton absorption is illustrated in Fig. 3. With single-photon absorption, when a laser is focused to a point within a sample, the sample may, because of the large probability of single-photon absorption, fluoresce throughout the entire beam path. Using

multiphoton absorption, induced fluorescence occurs only at, or near, the focal point of the beam.

Since the position of the focal point can be precisely determined, multiphoton fluorescence can yield a great deal of information about specific points below the sample surface.

Furthermore, longer wavelengths, particularly the near infrared, penetrate deeper in biological materials and are not scattered as much as shorter wavelengths.

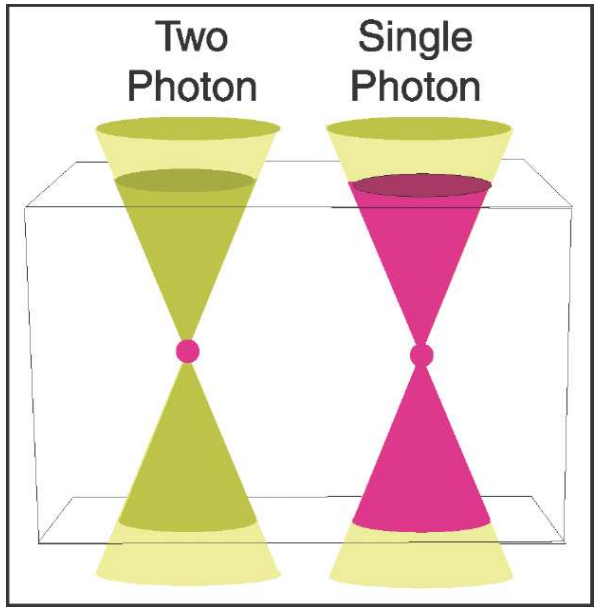

Fig. 3 – Pink volume illustrates two-photon and single-photon fluorescence induced by a focused laser beam (Modified image from: Center for Biomedical Imaging Technology, "Two-photon microscopy").

3. LASER SCANNING CONFOCAL MICROSCOPY

Laser scanning confocal microscopy (LSCM, also referred to as CSLM, confocal scanning laser microscopy) has been established as a valuable tool for obtaining high resolution images and three dimensional reconstructions of a variety of biological specimens.

The basic operation of a confocal laser microscope is shown in Fig. 4. A beam of laser light (usually from an argon or krypton ion laser) is focused onto a fluorescent specimen by a microscope objective lens.

The fluorescent energy from the sample is then collected through the same microscope objective and recorded by a photodetector.

The optical system is designed so that the laser's focal point in the sample is imaged exactly on the face of the photodetector (i.e., confocal).

By its nature then, any fluorescence emanating from the point of laser focus will be focused on the photodetector, and any fluorescence emanating from points other than the point of laser focus will be out of focus at the photodetector.

Thus, by inserting a small aperture in front of the photodetector, the gathered fluorescence can be limited to a region very close to the point of focus of the laser. The smaller the aperture is, the higher the resolution will be.

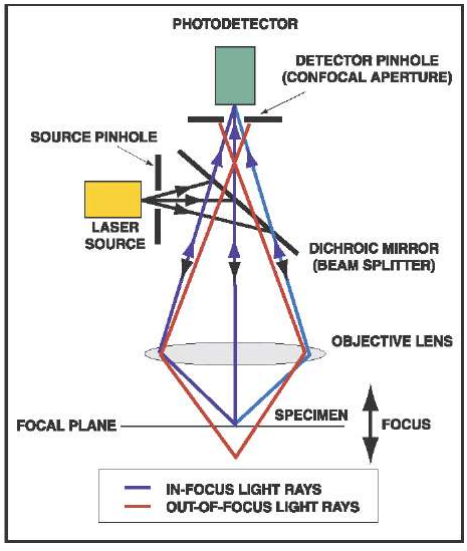

Fig. 4 – Simplified optics of a confocal laser microscope (Lance Ladic, "Simplified optics of a LSCM")

In LCSM, the focal point of the laser spot is stepped across the sample in a raster $(x - y)$ pattern, always maintaining the confocal nature of the image at the detector. Fluorescence information is accumulated on a point-by-point basis with a digital processing system, and a fluorescent cross section of the sample at the focal plane is obtained. By stepping the focus vertically (z), multiple slices can be used to build up a full three dimensional image. With non-opaque samples, the interior structure can be clearly seen. By scanning in the $(x - z)$ direction, a vertical cross section can be obtained.

When working with biological samples, serious problems can occur with normal confocal fluorescence microscopy. One problem is photo bleaching of the fluorescent label (fluorophore). In many cases, researchers are interested in observing living specimens, often at several stages during development.

Because the small confocal aperture blocks most of the light emitted by the tissue, including light coming from the plane of focus, the exciting laser must be very bright to allow an adequate signal-to-noise ratio. This bright light causes fluorescent dyes to fade within minutes of continuous scanning. Thus the fluorescence signal weakens as subsequent scans are made, either to produce a three dimensional image or to observe a single slice at several time points. Phototoxicity is another problem. Many fluorescent dye molecules generate cytotoxins like singlet oxygen or free radicals, and one must limit the scanning time or light intensity to keep the specimen alive.

Multiphoton microscopy solves the problems of LSCM: improving the signal-to-noise ratio by eliminating fluorescence except at the focal point of the laser, and reducing or eliminating photo bleaching and phototoxicity by using low average power. There are two main differences between multiphoton and confocal microscopy:

- the source is an ultrafast laser (usually Ti: Sapphire) with very high peak power but low average power;
- the confocal aperture is unnecessary, because all of the fluorescent light originates from the laser focus spot.

The differences between multiphoton microscopy and confocal microscopy are shown in Fig. 5.

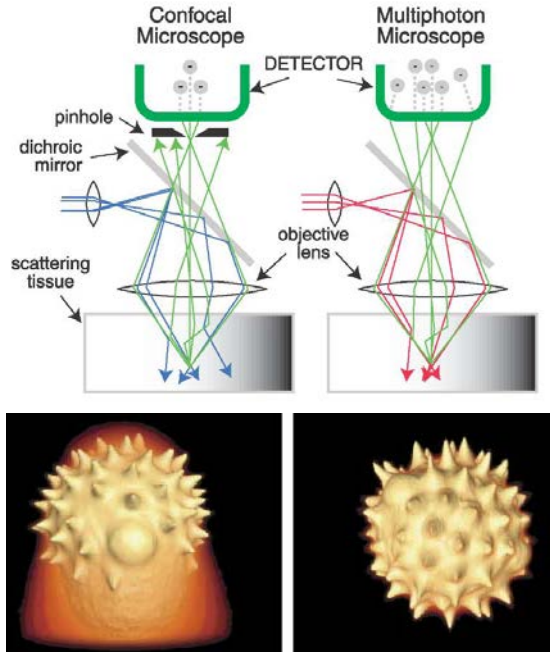

Fig. 5 – Comparison of a confocal and multiphoton microscope (Microcosm, Inc., "Multiphoton fluorescence microscopy")

In the confocal case, fluorescence occurs throughout the sample and must be blocked by the pinhole aperture. This not only eliminates the fluorescence away from the focal point, but also the scattered (diffusing) fluorescence from the focal point. Only the ballistic (straight line) fluorescence is detected. In the multiphoton case, both the ballistic and the diffusing photons are collected.

Furthermore, since the excitation wavelength has a longer wavelength, less excitation light is lost to scattering.

Unfortunately, we still can't deliver electronic microscope onboard the spaceship, besides that – such method propose long measurements, but we can't afford it, so we need to find something new.

As was mentioned above, an important benefit of Multiphoton microscopy is the improved axial resolution brought about by the nonlinear processes involved. In two-photon processes, the excitation cross section is proportional to the square of the laser intensity. Furthermore, the intensity of a Gaussian beam decreases roughly as the square of the distance from the peak.

Consequently, the cross section for two-photon fluorescence is inversely proportional to the fourth power of the distance from the focal point of the laser beam. Use of three-photon excitation can enhance the $z-axis$ resolution even more, as demonstrated in Fig. 6. In this example, the focal point of an ultrafast Ti: Sapphire laser was moved from a cover glass into a fluorescing film (the laser was operating at $900\ nm$).

In curve a, an ultraviolet transition ($300\ nm$) in BBO/toluene was probed by three-photon excitation. In curve b, a blue transition ($450\ nm$) in rhodamine 6G was probed by two-photon excitation.

The smaller cross section and greater nonlinearity of the 3-photon transition significantly increases the $z-axis$ resolution (Microcosm, Inc., "Multiphoton fluorescence microscopy").

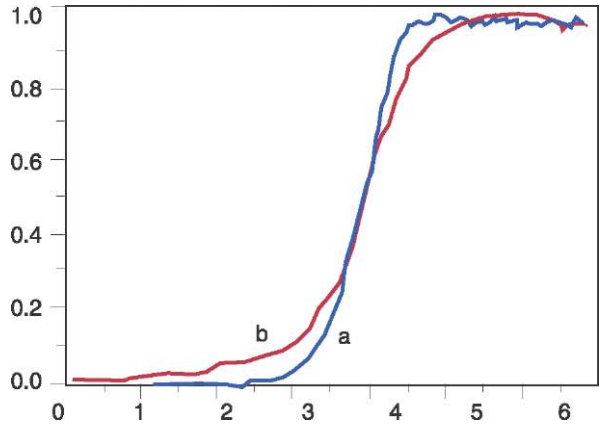

Fig. 6 – Resolution along the z-axis for two-photon and three-photon excitation

4. THE CONCEPT AND METHODOLOGY OF PATTERN RECOGNITION

The task of image identification of microorganisms is often "the first step", preparatory treatment in the process of solving problems of "superior level" (for example recognition of microorganisms). The task of detecting microorganisms on the image is quite simple for the trained human vision, however at attempt to generate automatic microorganisms detection system one has to encounter the following complications: varying appearance of different microorganisms; potential existence of individual characteristics, that often depends on growing conditions, essentially complicates automatic detection; shooting conditions (illumination, camera color balance, image distortion, introduced by system's optics, image quality) considerably affect images of microorganisms.

The existing microorganism detection algorithms can be divided into two wide categories. The methods which are using personal experience in recognition of microorganisms are refer to the first category and trying to formalize and make an algorithm to this experience, which can be used for further development of the mathematical model of such recognition. The second category relies on toolkit of pattern recognition and considers detection problem of microorganisms as a special case of pattern recognition. It is easy enough to collect a set of simple and obvious properties of the microorganism's image. Based on these properties, it is possible to develop an algorithm of recognition of the microorganisms, checking their presence on microorganism's image. In this paper we set up key parameters of recognition from the point of view of development of a mathematical model of a microorganism's recognition for medical and biologic control of human's habitat, including in an extreme situation of manned space flight and conditions.

Human brain manages the task of detecting the microorganisms on the images more than successfully. It would be natural to try to determine and to use the principles, by which the brain with the solution of the problem of recognition is guided. Among the methods, which make this attempt, it is possible to isolate two directions: the methods of recognition "from top to bottom" based beyond the knowledge and the methods of recognition "from below – upward" based beyond the special features.

Recognition "from top to bottom" indicates the construction of a certain collection of rules, which it must answer the fragment of image, in order to be the acknowledged microorganism. This collection of rules is the attempt to formalize empirical knowledge about how microorganism on the images precisely appears and how is guided man with decision

making microorganism it sees or not. It is sufficiently easy to construct the collection of the simple and obvious properties of the image of microorganism. Relying on these properties, it is possible to construct algorithm checking their presence on the fragment of image. Down the same family of procedures it is possible to also carry recognition with the aid of the templates, assigned by developer. The detection of microorganisms with the aid of the template consists before the checking by each of the regions image down the correspondence to the assigned template.

Recognition "from below – upward" uses invariant properties of the images of microorganisms, relying on the assumption that once of men can effortlessly recognize microorganism on the image independent of its orientation, lighting environment and specific features, then must exist some signs of the presence of microorganisms on images, invariant relative to conditions surveys. The algorithm of the work of the methods of recognition "from below – upward" can be briefly described as follows: the detection of elements and special features, which are characteristic for the image of microorganism; analysis of the discovered special features, arbitration about quantity and arrangement of microorganisms. Investigating live objects we face their main ability – change forms and properties. So we must carefully study all the possibilities of their form in order to make proper recognition.

Edges – the sharp passages of brightness. They usually correspond to the boundaries of objects on the image. This property also is used before a number of the works, which consider edge on the image as the signs of the potential presence of microorganisms.

Brightness. Regions the images, which correspond to microorganisms, are often darker than their nourishing environment. After using this observation, the number of researchers uses algorithms of detection and underlining of the regions of the local minimums of brightness, considering them as potential microorganisms. Before some works is made the attempt to use the specific diagrams of the interrelations of brightness's, characteristic for some microorganisms.

Color. Despite the fact that brightness is usually basic information source before many tasks of machine sight, color (because of the additional information about the nuance of object) is the more powerful means of recognition and discrimination of objects on the image. As showed experiments, the color of different microorganisms occupies the sufficiently small limited subregion of color space, even with the examination of the colors of the microorganisms of different classes.

Characteristic form of microorganisms. On the basis of the fact that the processes of the recognition of the visual means of the high level before the brain precedes the certain low-level organization of visual information, were proposed several operators, which emphasize regions image, as far as the possessing properties, characteristic for the microorganisms. By such, for example, as symmetry. The result of applying such operators is the collection of points on the image, with the high probability relating down the microorganisms. Another close version of recognition – use of the rigid or deformed templates for detecting the microorganisms.

Are after on the image isolated the regions, which possess properties, characteristic for the microorganisms, is produced their complex checking for the development of the regions, which are actually been microorganisms. The essence of this checking depends on the nature of the utilized signs, and also beyond strategy selected researchers. For example, if as the signs come out the potential features of microorganisms, discovered with the aid of the analysis of map it is boundary, then the analysis of their mutual arrangement for the purpose of definition will be checking, they can form the colonies of microorganisms. If is used also recognition on the color, then can be added the additional condition that to be examined as the potential

microorganisms there will be only regions close ones before the color down the nuance of microorganisms. Checking the relationship of the discovered signs of microorganisms can be based down: a certain empirical algorithm [1], to the statistics of the mutual arrangement of signs, assembled on the images of microorganisms [2], the simulation of processes, proceeding before the human brain during the recognition of visual means, the application of the rigid or deformed templates and so forth.

The method of main components [3] adapts for reduction in the dimensionality of the space of signs, without leading down the essential loss of the in formativeness of the training collection of objects. The application of a method of main components down the collection of the vectors of linear space Rn, makes it possible to pass to this basis of space, that the basic dispersion of collection will be directed along several first axes of basis, called *the principal* $axis$ (or main components). Thus, the basic changeability of the vectors of training collection is several main components, and appears the possibility, after rejecting those remaining (less essential), to pass to the space of substantially smaller dimensionality.

Stretched down obtained thus principal axes subspace of dimensionality m, n is optimum among all spaces of dimensionality m in the sense that in the best way (with the smallest error) describe the training collection of images. Before the application to the task of detecting of microorganisms, PCA is commonly used as follows. After the calculation of the principal axes of the training collection of the images of microorganisms, the vector of the signs of test image is projected down the subspace, formed by principal axes. Two values are calculated: distance from the projection of test vector to the average vector of training collection – Distance in of feature of space, and distance from the test vector to its projection beside the subspace of main components – Distance of from of feature of space. On the basis of these distances will be decided on the belonging of test image for the sake of the class of the images of microorganisms [4].

Factor analysis as many methods of the analysis of multidimensional data, rests beyond the hypothesis about the fact that the observed variables are indirect of the manifestation of the relatively small number of certain concealed factors. FA, thus, is this the totality of models and methods of those oriented down development and analysis of the concealed (latent) dependences between the observed variables. Before the context of the tasks of recognition, the observed variables are usually the signs of objects. Factor analysis can be considered as the generalization of the method of main components. Purpose of FA before the context of the task of detecting the microorganisms – to obtain the model of the image of microorganisms (with the visible number of parameters), with the aid of which it is possible to conduct the estimation of the proximity of test image to the image of microorganisms [5].

5. PROBLEM OF THE COLLECTION OF MEASURES FOR TRAINING THE CLASSIFIERS

The methods, which use PCA and FA require for training the classifier only of collection of the positive cases of recognition (images of microorganisms), by them are not required counterexamples (images without the microorganisms). Methods described below do require also the counterexamples, what does raise one additional problem – how to find the representative collection of images “not microorganism” for the successful training of classifier? Before the work is proposed the solution of this problem as far as the method of self-adjusting – it consists before the gradual forming of the collection of counterexamples, about the results of the conducted tests. Against the first step for training the classifier it is used the small training collection of image – counterexamples. Then is produced testing on

certain random class from the base of data of images. All images, in the course of test erroneously identified as microorganism, are added down the collection of counterexamples and training is repeated [6], [7].

PCA and factor analysis are the powerful and convenient methods of obtaining the subspace for the effective idea of the class of objects in many instances; however, they are not compulsorily optimum tools for the simulation of the variety of the images of microorganisms. Attempt to construct the model, which consists besides several clusters of the images of microorganisms and "not microorganisms", after modelling each of them with the aid of the multidimensional normal distribution density it was made beside. Considering black and white images as far as the size of 19x19 of pixels as vector before the 361 – measured space, the collection of the clusters, formed by the images of microorganisms was found and "not microorganisms". Distances to these clusters were transferred to the neuron network (multilayer perceptron), that decided on the presence of microorganism on the image.

Linear discriminant analysis, in contrast to PCA and FA is not set as its goal to find the subspace of smaller dimensionality, which in the best way describes the collection of training images. Its task – of finding projection before the space, before which the difference between different by the classes of objects is maximum. This requirement is formulated as obtaining maximally compact clusters, which correspond to different classes, removed for a maximally possible distance. With the aid of LDA it is possible to obtain the subspace of small dimensionality, before which the clusters of the images of microorganisms and "not microorganisms" intersect minimally. To produce classification before this space it is considerably simpler [5].

The purpose of training most classifiers to minimize error classification in training set (called empirical risk). In contrast, using the reference vectors it is possible to construct a classifier minimizes the upper estimation error classification (including for unknown objects not included in the training set). Possibility of linear separation of such difficult classes, as microorganisms and "not microorganisms" is rather poor. However, classification using support vector machine allows the use of nuclear functions for implicit projection vectors-signs of the potentially much higher dimension (even higher than image dimension), in which classes can be linearly separated. Implicit projection using nuclear functions does not complicate the calculations so that we can successfully use the linear classifier for linearly inseparable classes [8], [9].

Neuron networks have been successfully adapted for the solution of many problems of recognition. By the merit of use neuron networks for the solution of the problem of detecting the microorganisms is the possibility of obtaining of classifier, well simulating complex function distribution of the images of microorganisms [10], [11], [12]. However, drawback is the need for thorough and tedious tuning to neuron networks for obtaining the satisfactory result of classification.

With the aid of Active appearance models (that can be transferred as "active models of exterior view") it is possible to simulate the images of the objects, subjected both down rigid (rigid) and nonrigid (non-rigid) deformation. Rigid deformation – any deformation, which can be presented in the form to the composition of transfer, turning and scaling. AAM consists of the set of the parameters, part from which controls the form of object, rest assign its texture. The parameters of model are selected automatically, on the basis of the most characteristic deformations of form and changes in the texture, which are present before the training collection of the images of object. The active model of the exterior view of microorganisms assigns changes in the form of microorganisms and its characteristic features, and also possible changes in the texture of microorganisms. For the solution of the problem of detecting the

microorganisms on the image, is made the attempt to find the parameters (arrangement, form and texture) of AAM, which assign image closest down that observed [13], [14]. The degree of the proximity of the exterior view of model before the optimum configuration to the observed image gives the possibility to estimate we see we microorganism or not. As the conditions, which influence the selection of the method of solution of problem, it is possible to transfer the following:

- assumed variety of the microorganisms: the limited collection of microorganisms, limitation down the possible type of microorganisms, the absence of limitations;
- colored or black and white image;
- scale of microorganisms, permission and the quality of image (noisiness, compression ratio);
- assumed quantity microorganisms, which are present on the image: it is known, approximately known, it is unknown;
- lighting environment: the fixed known, approximately known, any;
- background: the fixed, contrasting single-tone, low-contrast, unknown;
- the idea of synthesis of automatic recognition are the means by which describe and separate the class of images.

Specifying a class listing of images included in its composition, the process involves the implementation of the automatic recognition of images by comparison with a standard. The set of images belonging to one class, remembers recognition system. Upon presentation of system unfamiliar (new) images, it consistently compares them with stored in its memory. Pattern recognition system classifies a new image to the class to which belonged in memory of the image, which coincided with the new [15]. For example, if the memory of the recognition system introduced letters of various type cases, the approach based on the transfer of the class members, enables us to identify the corresponding letters, but only in cases where their images are not distorted by the noise associated with smearing or poor application of paint, porous paper, etc. Undoubtedly, this is a simple method, but it allows you to build low-cost recognition system, which in some application areas it is to cope with their problems [16]. The method of transferring members of the class is working satisfactorily, if the sample images are close to ideal.

Specifying a class with properties that are common to all its constituent members provides for the implementation of the automatic recognition through the provision of such features and work with them. The main assumption in this method is that the images that belong to the same class, have certain common properties or attributes that reflect the similarity of these images. These common properties can be, inter alia, to enter into the memory of the recognition system. When the system is presented unclassified image, then released a set of describing its features, the latter sometimes coded, and then compares them with the traits embodied in the memory of the recognition system. In this case, the last credits presented for recognition of images in class with a system of signs, such signs of this image. So, using this method the main task is to allocate a number of general properties of the finite sample images, which sought class membership, is known.

6. CONCLUSIONS

It is obvious that this concept is recognition in many ways superior to recognition of the principle of transfer of class members. To memorize the signs of class requires much less memory than the storage of all objects within the class. Because the symptoms that characterize the class as a whole have the invariance principle of matching features allow

variation characteristics of individual images. The procedure for comparison with the standard, on the other hand, does not allow significant variation characteristics of individual images. If all the features that determine the class can be found on the available sample images, the process of recognition is simply a comparison of features. Extremely difficult, but if not impossible at all, as mentioned above, to find a class full set of distinguishing characteristics. Consequently, the appeal to this principle recognition is often associated with the need to develop methods for selecting the features that are in some sense optimal.

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