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Fluorescence Pattern Analysis-How To Achieve a Better Quality-Standard?

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Abstract

Quantitative imaging of fluorescent proteins and patterns is accomplished with a variety of techniques, including wide-field, confocal and multiphoton microscopy, ultrafast low-light level digital cameras and multi tracking laser control systems. These microscopic images can be of 2-dimensional or 3-dimensional nature, or even 4-dimensional nature such as videos recording the life cycle of a cell. To make fluorescent pattern analysis feasible in daily practice in cellular and molecular biology as well as in medicine, agriculture or other applications standardization is necessary to obtain authentically and reproducible results. We describe in this article the important steps that are necessary to support standardization.

Short Communication

In the rapidly expanding fields of cellular and molecular biology, fluorescence illumination and observation is becoming one of the techniques of choice to study the localization and dynamics of proteins, organelles, and other cellular compartments, as well as a tracer of intracellular protein trafficking. Quantitative imaging of fluorescent proteins and patterns is accomplished with a variety of techniques, including wide-field, confocal and multiphoton microscopy, ultrafast low-light level digital cameras and multi tracking laser control systems. These microscopic images can be of 2-dimensional or 3-dimensional nature, or even videos recording the life cycle of a cell.

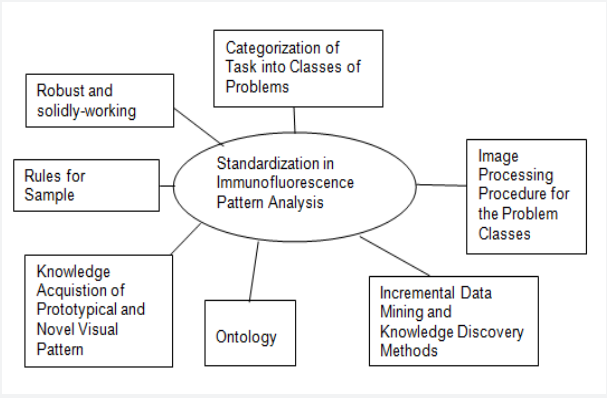


Figure 1: Aspects of standardization in fluorescence pattern analysis.

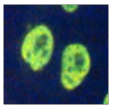
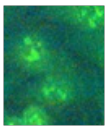
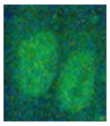
To make fluorescent pattern analysis feasible in daily practice in cellular and molecular biology as well as in medicine, agriculture or other applications standardization is necessary to obtain authentically and reproducible results. Standardization has many aspects (Figure 1). It has to do with sample preparation, imaging techniques, knowledge acquisition, and image interpretation. It is an iterative process and cannot be solved from scratch. It is an interdisciplinary subject that requires input from different disciplines. Some of the aspects of Standardization of Fluorescence Pattern Analysis we will work out throughout this paper.

For research purposes, are usually studied only a few images in order to establish the imaging method. In this case it is feasible to do manually the image-interpretation of the images. The resulting manually obtained image descriptions are given to only a limited and elitare group of researchers for discussion purposes. Over time they will have developed their own image description language that is accepted in this community.

But the human interpretation causes a lot of problems. The interpretation of visual information/pattern requires a lot of experience. Humans are usually good in expressing emotion, giving driving direction that everyone can understand, but to describe what they see in an image so that any person gets the same impression is not a daily task for human and not regularly thought in school. That makes image interpretation difficult. The early research in image interpretation faced exactly on this problem and developed methodologies on visual knowledge

acquisition that made progress in defect classification such as wafer inspection, non-destructive testing or printing process inspection. As a result, ontologies were born for specific image interpretation tasks. The ontology is the basis for the development of an automatically image-interpretation-systems. Standardization requires the knowledge about prototypes of pattern and visual descriptions. Methodically collected image catalogues with showing and naming of examples (Table 1) based on commonly excepted image vocabulary are necessary.

Table 1: Image Catalogue with Visual Description Language sample preparation sollte noch als Block davor.

Class	Image	Description
Fine Speckled		Smooth and uniform fluorescence of the nuclei; Nuclei sometimes dark; Chromosomes fluoresced weak up to extreme intensive
Fine dotted (speckled) nuclei fluorescence		Dense fine speckled fluorescence Background diffuse fluorescent
Homogeneous Nuclear		A uniform diffuse fluorescence of the entire nucleus of interphase cells. The surrounding cytoplasm is negative.

An automatic image interpretation system would allow producing results that are reproducible and objective. Reproducible since the system creates from the same image always the same output as long as the automatic image processing procedures work without any system failure nonetheless if the image is processed today or ten days later. A human might not able to do that! His daily performance heavily influences the results. He might interpret an image differently today than he did yesterday and as long as he does not calculate features from the image by automatic image processing procedures his measurements are qualitative and not quantitative. He cannot give objective results rather his decisions are subjective. One expert´s answer might be different from the answer of another expert. Therefore, an automatic image interpretation system will always be a big step towards standardization of the desired image inspection tasks.

However to build such a system is difficult since not only a human has problems to describe the visual content it is also difficult to develop automatic image processing procedures that can map the numerical representation of an image to the desired visual description.

The problem related to the automatic processing of multimedia content resulted in MPG-3 standard that grouped conventional image processing methods to visual symbolic low-level terms that should allow a user to pick the right image processing method in order to obtain the desired information he wants to extract and describe for image retrieval or other purposes.

In defect classification, medical image interpretation, non-destructive testing as well as in fluorescent pattern analysis the visual terms are usually more complex and cannot be described by a single visual symbolic low-level term. To give you an example: How to describe fuzzy margin of an object by low-level terms? This is a term very often appearing in medical image interpretation across applications as well as in wafer inspection. The recent developments in multimedia processing are therefore not sufficient for many new arising visual image interpretation tasks and we need to further develop new methods.

After an image method has passed research and goes into industrial applications then they should usually work on-line in a process. There is a growing use of these techniques in industry for pharmacological aspects or diagnostic purposes in medicine and agriculture. The huge amount of data created in these processes cannot anymore be handled manually. They require automatic image interpretation system. These image interpretation systems should allow to interpret these images automatically, and also to detect automatically new knowledge to study the cellular and molecular processes.

A necessity for good image interpretation is images with good image quality. Protocols for sample preparation as well as robust and solidly-working imaging devices are important to ensure that the objects in the image get imaged with high contrast and constant brightness. This is another step towards Standardization in Fluorescent Pattern Analysis.

Although there has been achieved a lot in that direction it is not standard that the same image quality can be ensured over the whole process. Researcher in the image processing field are therefore more and more engaged with the development of methods for assessing the image quality for different imaging techniques for application in medicine, chemistry and biology that allow to select the best image during the imaging process.

In general we can describe automatic image interpretation as the process of mapping the numerical representation of an

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image into a logical representation such as suitable for image description (Figure 2). An image interpretation system must be able to extract symbolic features from the pixels of an image (e.g., irregular structure inside the object, colocalization of mitochondria, sharp margin). This is a complex process; the image passes through several general processing steps until the final symbolic description is obtained. These include image preprocessing, image segmentation, image analysis, and image interpretation (Figure 2). Interdisciplinary knowledge from image processing, syntactical and statistical pattern-recognition and artificial intelligence is required to build such systems. The primitive (low- level) image features will be extracted at the lowest level of an image interpretation system. Therefore, the image matrix acquired by the image acquisition component must first undergo image pre-processing to remove noise, restore distortions, undergo smoothing, and sharpen object contours. In the next step, objects of interest are distinguished from background and uninteresting objects, which are removed from the image matrix.

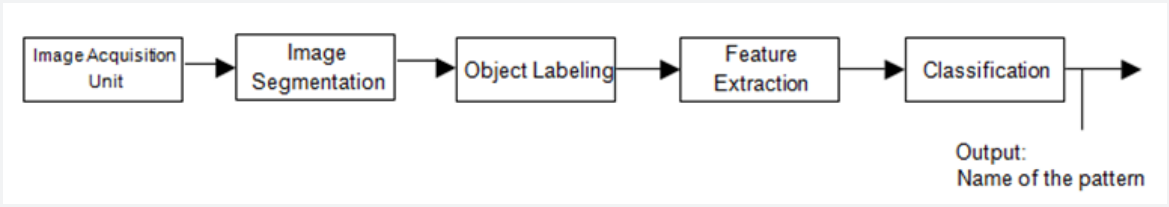

Figure 2: Architecture of the system.

After having found the objects of interest in an image, we can then describe the objects using primitive image features. Depending on the particular objects and focus of interest, these features can be lines, edges, ribbon, etc. A geometric object such as a block will be described, for example, by lines and edges. Typically, these low-level features have to be mapped to high-level features. A symbolic feature such as fuzzy margin will be a function of several low- level features. Lines and edges will be grouped together by perceptual criteria such as collinearity and continuity in order to describe a block.

Image classification is usually referred to as the mapping of features to predefined classes. Sometimes image interpretation requires only image classification. However, image classification is frequently only a first step of image interpretation. Low-level features or part of the object description are used to classify the object into different object classes in order to reduce the complexity of the search space. The image interpretation component identifies an object by finding the object that it belongs to (among the models of the object class). This is done by matching the symbolic description of the object in the scene to the model of the object stored in the knowledge base. When processing an image using an image interpretation system, an image's content is transformed into multiple representations that reflect different abstraction levels. This incrementally removes unnecessary detail from the image. The highest abstraction level will be reached after grouping the image's features. It is a product of mapping the image pixels contained in the image matrix into a logical structure. This higher level representation ensures that the image interpretation process will not be affected by noise appearing during image acquisition, and it also provides an understanding of the image's content. A bottom-up control structure is shown for the generic system in Figure 2. This control structure allows no feedback to preceding processing components if the result of the outcome of the current component is unsatisfactory. A mixture of bottom-up and top-down control would allow the outcome of a component to be refined by returning to previous component.

Assuming the prototypical pattern or scenes are known as standard then it is possible to develop the necessary image processing algorithm as a standard for analysing fluorescent paper. In order to do that in a more systematic way, a categorization of the tasks in the application area of Fluorescent Pattern Analysis is necessary. The observation of prototypical pattern or scenes empirically done by a human is usually a time consuming process. Much more preferable would it be to discover the prototypical appearance of pattern automatically.

In many high-content analysis project in drug discovery are therefore recently calculated a lot of image features based on conventional image processing algorithm from fluorescent images. These features are more or less the features on which the MEP3-standard is based on. They are not specially constructed to describe the visual appearances of the objects in microscopic cell fluorescent images. The experts usually try to summarize these features by descriptive statistics or simple classifier to discover some knowledge from the data. The problem with the described feature description based on the low-level features still exists here. Besides that, the large amounts of features are hardly to overlook by the statistical data summarization methods.

New methods on data mining are necessary that can automatically discover the final information needed for the respective process. In general, we need to identify groups of objects or events or map an image description into the final decision (bacteria..., mitochondria). New clustering methods based on conceptual clustering, incremental classification method based on decision tree induction, case-based reasoning and prototype- based classification have been developed so far for this task.

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As shown above, Standardization has to do with the standardized preparation of the right probe, with the establishment of robust and stable imaging devices as well as with knowledge acquisition according to the established visual knowledge elicitation methodology. An accepted and understood ontology is necessary to describe the visual content in order to be able share the knowledge and as basis for building an automatic image interpretation system. The image interpretation system should have application-oriented image pre-processing, image segmentation and interpretation methods that allow adapting the system to different application in the class of application. To understand what the class of application is we need some categorization of different application.

We have established a new forum to discuss this task in more detail and show new research results to the community. The forum is running under the umbrella of the community of Mass Data Analysis of Images and Signals with Applications in Medicine, r/g/b Biotechnology, Food Industries and Dietetics, Biometry and Security, Agriculture, Drug Discover, and System Biology (www.mda-signals.de). The aim of our new forum should be to establish a forum of experts and practioners where we can work on these topics and make progress toward Standardization in Fluorescence Pattern Analysis.

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