computer image analysis in

bioscience

Olsztyn, Poland — 22/24 June 2014

Institute of Animal Reproduction and Food Research

of the Polish Academy of Sciences

COMPUTER IMAGE ANALYSIS IN BIOSCIENCES

Workshops

22 – 24 June, 2014

Olsztyn, Poland

This conference is organized within support of the 7th Framework Programme project entitled "Unlocking the potential of the Institute of Animal Reproduction and Food Research for strengthening integration with the European Research Area and regional development" (FP7-REGPOT-2010-1-264103).

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Dear Colleagues,

On behalf of the Organizing Committee we wish to cordially welcome you at "**Computer Image Analysis in Bio-sciences**" workshops organized by Society for Biology of Reproduction and Institute of Animal Reproduction and Food Research PAS within the REFRESH project entitled "**Unlocking the potential of the Institute of Animal Reproduction and Food Research for strengthening integration with the European Research Area and regional development"** financed from the European Union 7th Framework Programme.

Image analysis has proved to be an objective investigation tool in many areas of biological sciences, from agriculture, crops processing, engineering, to biology and medicine. During the workshops lecturers from leading European scientific units will present the current state of knowledge on diversified methods of image analysis. The communications will start from the basis of digital imaging technique which constitutes an interdisciplinary source of data for researchers of different specialties.

A special attention during the meeting shall be drawn to the current advances in the development of hyperspectral imaging as well as confocal and multiphoton microscopy. The issue of image analysis in engineering and food processing, even in relation to archaeological research, will also make a strong contribution to the thematic scope of the workshops. The final subjects elaborated upon during the meeting will be closely connected to biology (of reproduction as well) and medicine.

In addition to lectures, a crucial part of the workshops will be both practical seminars, providing participants with the opportunity to get acquainted with the practical aspects of image analysis, as well as the poster session.

Let this meeting be an open forum for scientific discussion and exchange of opinions. As the turn of spring and summer in the region of Warmia & Mazury is always the most charming season of the year, I hope that this will be an additional pleasant aspect of your visit in Olsztyn.

I wish you successful deliberations, great atmosphere and enjoyable stay in our city.

Dr. Tomasz Jeliński
Chair of the Organizing Committee

Organizing Committee

Aneta Andronowska
Tomasz Jeliński
Iwona Kieda

Workshops programme

22 June, 2014 (Sunday)

Welcome reception

23 June, 2014 (Monday)

8:30–8:45	Official welcome
8:45–9:30	Artur Zdunek, *Image analysis – general aspects – part I,* Lublin, Poland
9:30–10:00	Da-Wen Sun, *Recent advances in hyperspectral imaging technology – applications for food quality and safety evaluation and inspection*, Dublin, Ireland
10:00–10:30	Wouter Saeys, *Hyperspectral and RGB-D imaging for orchard automation*, Leuven, Belgium
10:30–11:00	Ferenc Firtha, *Complex imaging characterisation of meat marbeling and lean-to-fat ratio*, Budapest, Hungary
11:00–11:30	**Coffee break**
11:30:12:00	Artur Zdunek, *Image analysis – general aspects – part II,* Lublin, Poland
12:00–12:30	Rafał Socha, *Confocal and multiphoton microscopy and imaging*, Warsaw, Poland
12:30–13:00	Piotr Pieczywek, *Parameterisation of plant tissue microstructure by confocal microscopy for Finite Elements Modelling*, Lublin, Poland
13:00–13:45	Krzysztof Borkowski / Tomasz Kasikowski, *Introduction to practical workshops*
13:45–14:45	**Lunch**
14:45–17:45	Practical workshop by OLYMPUS Polska Automatic image analysis – Tomasz Kasikowski, Olympus Polska Image analysis in optical microscopy – Krzysztof Borkowski, Olympus Polska High content screen analysis – Rafał Socha, Olympus Polska
19:30	**Barbeque**

24 June, 2014 (Tuesday)

9:00–9:45	Sofiane Guessasma, *Bridging computational food mechanics with advanced image analysis,* Nantes, France / Cambridge, United Kingdom
9:45–10:15	Mirosław Zarębski, *Analysis of multidimensional confocal and super-resolution images of the cell nucleus*, Kraków, Poland
10:15–10:45	Jakub Włodarczyk, *The extracellular matrix modifier in control of dendritic spine structural plasticity*, Warsaw, Poland
10:45–11:15	Grzegorz Dietrich, *Modern methods of the fish milt evaluation*, Olsztyn, Poland
11:15–11:45	**Coffee break**
11:45–12:30	Poster session
12:30–13:00	Gianfranco Venora, *Image analysis applications to the study of the durum wheat chain*, Caltagirone, Italy
13:00–13:30	Piotr Szczypiński, Łódź, Poland / Piotrz Zapotoczny Olsztyn, Poland, *Computer analysis of food images – case studies*
13:30–14:00	Oscar Grillo, *Biometric characterisation of grapevine seeds by image analysis and comparison with archaeological remains*, Oliena, Italy
14:00–15:00	**Lunch**

LECTURES

Image analysis – general aspects

Artur Zdunek

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Image analysis is indispensable tool when we need to extract the quantitative data from images, such as objects size, shape, arrangement, surface roughness, etc.. The goal of quantitative image analysis is usually recognizing, measuring and making decision. In the biosciense most often we work with microscopes which nowadays provide digital images with xy special information, coded by the pixel position, whereas the pixel value codes topographic or biochemical data. Depending on a microscope used, features of images are different and usually require adaptation of image analysis protocols. Moreover, an image features depends on the sample studied. Therefore there is no universal image protocol which could be easily applied to any image. Fortunately, today the image analysis protocols are built as algorithms of predefined pixel transformation: point transformations, filters and morphological operators. It makes image analysis very flexible tool for various applications.

This lecture will present bases of image analysis with particular attention on the most practical ones: filters, dilation, erosion, opening, closing, watershead. In the second part, examples of completed algorithms for extraction of quantitative data from various microscopes (CSLM, macroscope and AFM) will be presented.

Recent advances in hyperspectral imaging technology – applications for food quality and safety evaluation and inspection

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Keywords: Computer vision, imaging spectroscopy, multispectral imaging, food quality, food safety

By integrating two mature optical sensing technologies of imaging and spectroscopy, hyperspectral imaging technology can not only provide spatial information, as color imaging systems, but also spectral information for each pixel in an image. With this unique feature, hyperspectral imaging has recently been widely studied and developed for capturing both physical and morphological characteristics such as color, size, shape, and texture, and some intrinsic chemical and molecular information (such as water, fat, and protein) from food products, resulting in many successful applications in the food industry for quality and safety evaluation and control. These applications include meat quality assessment, automated poultry carcass inspection, quality evaluation of fish, bruise detection of apples, quality analysis and grading of citrus fruits, bruise detection of strawberry, visualization of sugar distribution of melons, measuring ripening of tomatoes, defect detection of pickling cucumber, and classification of wheat kernels. In this presentation, basics and theoretical aspects of this technique is first introduced, followed by detailed discussion on some recent typical application examples to illustrate the capability of using this technique in the food industry for sample classification and grading, defect and disease detection, distribution visualization of chemical attributes in chemical images, and evaluations of overall quality and safety of food products.

References:

1. Da-Wen Sun (editor), Computer Vision Technology in the Food and Beverage Industries, Woodhead Publishing Limited, Cambridge, UK, 528 pp., ISBN 978 0 85709 036 2 (2012).
2. Da-Wen Sun (editor), Hyperspectral Imaging for Food Quality Analysis and Control, Academic Press / Elsevier, San Diego, California, USA, 528pp., ISBN 978–0123747532 (2010).
3. Da-Wen Sun (editor), Infrared Spectroscopy for Food Quality Analysis and Control, Academic Press / Elsevier, San Diego, California, USA, 424 pp., ISBN 978–0-12–374136–3 (2009).
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Hyperspectral and RGB-D imaging for orchard automation

Wouter Saeys, Niels Wouters, Tien Thanh Nguyen, Koenraad Vandevoorde, Josse De Baerdemaeker and Bart De Ketelaere

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Keywords: hyperspectral, RGB-D, multispectral, thinning, pear, harvesting, apple

While many agricultural sectors have been largely mechanized, intensive pome fruit production for fresh consumption still requires large amounts of seasonal labor for pruning, thinning and harvesting. This cost for this seasonal labor comprises a significant part of the entire production cost. Moreover, in Western Europe it has become increasingly difficult to find sufficient, good quality seasonal workers. To reduce this demand for seasonal labor, several mechanized solutions for fruit thinning and harvesting have been elaborated. However, as most of these mechanized solutions damage the trees or the fruits, they are not considered to be valuable alternative for manual thinning and harvesting. Therefore, more gentle and more targeted and selective thinning and harvesting methods are preferred. However, one of the bottle-necks in developing such selective machine systems is the detection of the flower buds and fruits on the trees. While image processing is already widely spread in industry, detection of fruits and flower buds in an orchard environment is not straightforward due to the challenges posed by the complex, unstructured and uncontrolled environment. Therefore, the additional information sources have been added to the classical RGB information: spectral information for floral bud detection and depth information for apple fruit detection.

Complex imaging characterisation of meat marbeling and lean-to-fat ratio

(hyperspectral data acquisition algorithms and some promising applications)

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Software: Although the hyperspectral method has several advantages, like remote sensing, segmentation of object and scanning non-homogeneous surface, it also has some disadvantages comparing to a NIR spectrophotometer. Non-isolated environment probably gives lower signal to noise ratio (S/N). After varying the field of view or illumination, the measurement must be recalibrated. Uneven surface necessarily results indefinite geometry (illumination/observation angles). A measurement control sotware will be introduced, that supports easy calibration and ensures high S/N.

The hypercube gained contains enormous amount of data (~10MB). A simple Matlab algorithm can help to reduce the data. Region of interest (ROI) areas can be selected manually and their average spectra, the 1st and 2nd derivatives are displayed. The effect of indefinite geometry can also be eliminated by normalizing spectra of pixels. The spectra and the derivatives of ROIs can be saved as samples for automatic segmentation (clustering) or as independent parameters of statistical analysis.

Some major, promising application fields of hyperspectral method are shown:

- Invisible properties can be seen. Infection of mushroom can be early detected since the change of spectra spreads on spots. The non-homogeneuity holds the information.
- Remote sensing can be useful on an industrial line but also capable to look through a film covering a product like cheese. The function of some enzymes can be tracking during storage.
- Handycap can be advantage. Since hypercube can be normalized by pixels, in case of uneven surface (tea, coffee, etc.) the hyperspectral system can serve much higher S/N than a NIR spectrophotometer.
- When spatial distribution holds the information: In case of marzipan, the fructose produced by some enzymes cannot distinguished by spectra. Since fructose hydrophil, the shape of spatial distribution can describe the difference from normal Fourier-type distribution.

Complex optical description of marbeling: The marbling of meat is usually marked in SEUROP system by experts. Large group of exclusive pig samples was fed by different lizin-content fodder. Their different tissues were inspected by experts, RGB imaging, hyperspectral imaging and CT/MR imaging. Analytical investigation was also taken. RGB imaging implemented an optimal segmentation and tried to make difference between marbling and lean-to-fat ratio. HSI imaging was involved to really make difference between fat and marbling tissues. CT and MR data were taken to check the efficiency of imaging algorithm, whether it is stable along a tissue.

Confocal and multiphoton microscopy and imaging

Rafal Socha

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The twentieth century was an age of rapid development of microscopy. New developments in science and technology allowed to completely change this area of knowledge and opened new possibilities in imaging techniques. The effects of these changes are also confocal and multiphoton microscopy, which not only increased the possible resolution in optical imaging, but also gave the possibility of a more precise three-dimensional imaging. This presentation recalls the theoretical basis of both techniques and discusses few good practices in imaging.

Parameterisation of plant tissue microstructure by confocal microscopy for Finite Elements Modelling

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Commonly used methods for the study of tissue morphology are based on use of the microscopes and following image processing and analysis. Cross-sections of samples, viewed under microscope provide clear insight into anatomical structure of plant tissues. Observations of plant tissues in microscale reveals complicated structure composed of large numbers of cells and spaces whose shapes, sizes and distribution show high variability, not only between species or varieties, but even within individual plants. This determines a certain texture of the material. Computational simulation with finite element modeling (FEM) has proven to be a valuable tool to study and to predict mechanical properties however one of the important steps there, is to create a physical model of the tested object of highest possible degree of accuracy of real shape reconstruction, but on the other hand, simple enough to allow efficient calculations. Since it was proved that the confocal scanning laser microscope (CSLM) is a useful tool for obtaining images of plant tissue that would be relatively easily segmented, the goal of this research was to identify the method of parameterization which will be used for FEM, and one the other hand, will properly simulate the real structure of plant tissues. Three different methods are tested: vectorization, Voronoi tessellation and ellipse tessellation.

In order to investigate a robustness of the each of the parameterization procedures, potato tuber and carrot parenchyma were chosen as examples. For the each method tested, five geometrical parameters were analyzed: area, perimeter, orientation, elongation and local indicator of spatial association of all individual regions which represent cells.

The reconstruction accuracy of the original tissue microstructure by the each parameterization method was investigated by comparison of geometrical properties of the cells from the segmentation with their virtual equivalents. Linear regression models between reference and modeled parameters were built. The model performance was expressed in term of coefficient of determination (R^2), slope of regression line (which was expected to be close 1) and the root mean square errors for prediction (RMSEP).

Based on the results, Voronoi tessellation was considered as inaccurate in tissue modeling. This method had tendency to increase the size of small cells when they are surrounded

by larger cells and reduction of large cells surrounded by those of smaller area. Moreover, shapes of virtual cells produced by Voronoi tessellation are always convex thus in many cases modeling of intercellular pores in their original form was impossible.

By means of vectorizationprocedure only general shapes of cells are reproduced and a curvature of cell walls is neglected. However, this method has the advantage of ability to create concave shapes. For both, the Voronoi tessellation as well as vectorization, created cells completely fill the space with no additional gaps and possess sharp, angular shapes.

The best overall reconstruction accuracy was obtained for ellipse tessellation. Models created with this method can be considered as representative equivalents of real tissues in terms of cells areas, orientations, perimeters, shapes and spatial arrangement. In contrast to previous methods, ellipse tessellation creates small gaps between corners of adjacent cells and rounds their shapes. Similar to Voronoi tesselation, it has limitations in reconstruction of concave shapes and causes straightening of curved sections of cell walls.

Introduction to practical workshops

Krzysztof Borkowski, Tomasz Kasikowski

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The process of acquisition and analysis of microscopic image can be divided into five steps:
- Acquiring Images
- Image Processing
- Measuring Regions of Interest
- Data Analysis
- Reporting Results

Acquiring images – *arguably, the most important aspect of all.* This step requires proper preparation equipment (microscope, camera, etc.). The main task is to obtain maximum contrast and dynamic range of the image and reduce "noise" and other unwanted artifacts.

Image processing consist in maximizing brightness/contrast, reduce artifacts, separate objects from background and enhance non-visual data (data visible for camera).

Measuring Regions of Interest. This stage consists of some additional steps where we need to determine whether looking for morphometric data or measuring lengths, select appropriate measurements, 'threshold' image to select objects, apply measurement parameters to image.

Data analysis is based on an assessment of figures, imaging or charts created on their basis.

Presentation of the above data in a report is often (but not always) an additional element of the whole process.

Only proper preparation of the whole system and the full knowledge of the various stages of acquisition and image analysis provides reliable results.

Automatic image analysis

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The important part of a microscopic image analysis is counting and the morphological measurement of objects. Since the technology of digital cameras has been widely spread around laboratories typical measurements (distance, surface area of object) are commonly applied. On the other hand very often researchers rely on measurements performed on population not only on single object observed by the digital camera coupled to the microscope. To measure several hundreds of species that are visible in one field of microscope view traditional, manual, methods of measuring are insufficient and may be influenced by various types of errors.

We are going to practically show, using commercially available software, methods to deal with counting and measuring of species shown on typical microscopic pictures. In particular the following features will be presented:

- Counting objects basing on thresholding pixels in regards to theirs color, intensity.
- Filtering thresholded objects basing on their' size or shape factor.
- Classification of thresholded object depending on e.g. their size (one-dimensional classification).
- Two-dimensional classification of objects depending on two parameters (in e.g. size and shape).

Image analysis in optical microscopy

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The process of acquisition and analysis of microscopic image can be divided into five steps described in "***Introduction to practical workshops***".

- Acquiring Images
- Image Processing
- Measuring Regions of Interest
- Data Analysis
- Reporting Results

The main aim of the practical part of the workshop will be appropriate hardware configuration includes not only setting the microscope and camera but also other aspects of the process.

The first step is to select the proper techniques of observation depends on the type of preparation. Since this is also addicted choice of lenses (class, zoom) and other optical components.

A very important step is choosing the right camera. Its resolution, acquisition parameters, the type of photosensitive element and camera/microscope adaptation will affect the quality of the obtained images.

The last but not the least important step is the correct choice of hardware including choosing the right computer, monitor and other components.

Note, however, that at the very beginning we must have well prepared microscope slide.

Bridging computational food mechanics with advanced image analysis

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Mechanics of materials followed a straight path towards food applications. Even if early studies confused strain and structural displacement, mechanical testing of food proved to be unavoidable to achieve objective food quality descriptors. Traditional definitions from sensory perception like firmness, crispness, etc. were explained by some mechanical quantities such as stiffness, strength and stress intensity factors. If all this is well recognised, it is less the case for computational mechanics which seems to be an emerging side effect for some experimentalists. It is the aim of this keynote to shed more light on the potential of computational mechanics in food application. In other research fields and more likely in industry, computational mechanics is a central process in the design of new products. The main motivations for using computation are reflected by cost saving and predictive capabilities. We show that such needs are fulfilled here through the ideas of identification and virtual food design. But from the other end of the subject, painful complexity is sometimes needed to take into account food structure contribution at different scales. Examples based on cereal product texture analysis and chewing process are given to illustrate where exactly complexity is needed. This emphasis on structural complexity is guided by imaging techniques such as X-ray tomography and related advanced analysis. Knowing these techniques is a must to make the bridging with computational food mechanics.

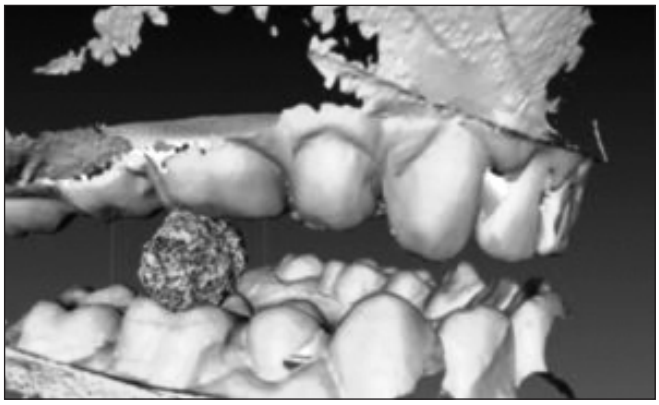

Fig. 1. Example of potential use of computational mechanics in food application: simulation of food chewing process.

Analysis of multidimensional confocal and super-resolution images of the cell nucleus

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Contemporary fluorescence microscopy is not only capable of registering high quality images of biological samples but can also used as a sophisticated tool for quantitative studies and sample manipulation. Currently available microscopy techniques make it possible to obtain numerical data describing cellular and nuclear 3D structure as well as measurements of selected metabolic processes with high resolution and sensitivity. I will present a method of quantitative analysis of spatial (3D) relationships between discrete nuclear events detected by confocal microscopy. As example, I will describe the analysis of spatial patterns of sites of DNA damage signaling (γH2AX foci) and DNA replication (EdU incorporation) in cells subjected to treatments with topoisomerase inhibitors camptothecin (CPT), etoposide (ETP), mitoxantrone (MTX) or hydrogen peroxide (H_2O_2).

There is no simple method to measure the similarity between patterns of foci representing DNA damage and replication. The basic analysis of color channel overlap is not sufficient as it is strongly dependent on parameters of image recording and preparation of the sample. Moreover this approach does not work well in 3D images due to differences in resolution along the optical axis and in the horizontal plane. To deal with this problem we developed an algorithm which reduces the subnuclear foci to points (barycenters or local maxima) and performs the nearest-neighbor distance analysis in 3D confocal images. Such an analysis demonstrates that a high degree of similarity between patterns of replication and ?H2AX foci in cells treated with CPT is coherent with the known mechanism of induction of DSBs by DNA topoisomerase I (topo1) inhibitors at sites of stalling of the moving replication forks. On the other hand, a poor correlation between ?H2AX foci with replication sites as seen

in the case of ETP, MXT and hydrogen peroxide hints at the mechanism of DNA damage unrelated to replication.

Performing measurement of similarities between patterns representing various subcellular foci demonstrates potential new applications of image analysis in multicolor confocal microscopy in studies of various cellular processes and structures confined to small regions.

The extracellular matrix modifier in control of dendritic spine structural plasticity

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Synaptic plasticity is the ability of neurons to change the strength of their synaptic connections according to the demands of the changing environment. The phenomenon underlies such cognitive functions as learning and memory. At the cellular level, the brain plasticity relies on modifications in synaptic connectivity and excitability that are driven by molecular changes in neurons. Synapses undergo dynamic alterations and thus are believed to play the major role in the plasticity. Dendritic spines that harbor excitatory synapses are membranous protrusions along neuronal dendrites through which neurons communicate within neuronal circuit.

Despite numerous molecules proposed to play a role in the brain plasticity, little is known about extracellular matrix (ECM) molecules and extracellular enzymatic activities, including proteolysis that allows for ECM modifications. Recently, the ECM has started to be recognized as a critical factor affecting synapses, as it enshelves them, forming a synaptic element. Matrix metalloproteinase -9 (MMP-9) has been identified as an ECM molecule playing a key role in synaptic plasticity. It has recently emerged as a key molecule in long-term memory and underlying synaptic plasticity (Huntley 2012; Peixoto, et al. 2012; Tsien 2013; Szepesi et al., 2014). Thus, make it a perfect candidate molecule responsible for neuronal circuit remodeling. Despite ongoing research, little is known about MMP-9 activity in modulation of morphology of the dendritic spines and postsynaptic receptors content on the synapse.

We will discuss the data aims to explain the role of MMP-9 in dendritic spine formation and stabilization to define a relationship between structural and functional changes in dendritic spines underlying learning and memory processes.

These studies employ various microscopy based methods including application of FRET based sensors and supperresolution approaches. New approaches will be proposed to enable a quantitative description of MMP-9 effect on structural plasticity.

Modern methods of the fish milt evaluation

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Poor sperm quality can be a limiting factor in artificial spawning in fish farming industry. The novel methods of the fish milt evaluation allow for objective analysis of several sperm parameters crucial for fertilization success. The assessment of fish sperm quality is based on evaluation of sperm motility (percentage, trajectory and velocity of motile sperm), sperm concentration, sperm membrane integrity (viability) or DNA fragmentation. The computer-assisted sperm analysis (CASA) allows an objective evaluation of sperm motility parameters. An accurate calculation of sperm concentration is very important for the control of fish reproduction and scientific experiments, however conventional counting using haemaocytometer is time consuming due to the small size of teleost fish spermatozoa. The introduction of the photometric technique for counting of fish sperm greatly accelerates the estimation of concentration. Viability testing of fish spermatozoa can be performed with the use of microscopic techniques or flow cytometry, or semi-automatic flow cytometry based analyzers as Nucleo-Counter SP-100. Preservation of DNA integrity is essential for protection of sperm quality and can be analyzed using the comet assay.

Image analysis applications to the study of the durum wheat chain

Gianfranco Venora, Oscar Grillo

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In Mediterranean countries and particularly in southern Italy, durum wheat (*Triticum durum* Desf.) is traditionally used for bread and pasta production. Market globalization has involved, also in these regions, the diffusion of bread made with soft wheat flour (*Triticum aestivum* L.) standardizing this product and flattening taste consumers. Dealing with the problems of genetic resources erosion, among its main aims, the *Stazione Consorziale Sperimentale di Granicoltura per la Sicilia* has to maintain and increase the value of old Sicilian durum wheat landraces. Basic points of durum wheat chain, such as the grain raw material producing, its milling and semolina yield and processing in bread and pasta, were the subject of qualitative-quantitative evaluation, using the same image analysis technique.

The proportion of vitreous kernels in a sample is an internationally recognized specification for determining the value of durum wheat. Vitreous kernels are mostly related to quality, which affects the pasta performance during cooking. Vitreousness and the amount of shrunken kernels are visually assessed during the grading process.

This assessment is subjective and tedious. A machine vision system was developed to determine the percentage of vitreous, starchy, piebald and shrunken kernels, using a trans-illuminated image of one layer of non-singulated kernels (in bulk) acquired by a digital camera. The semola quality was evaluated, by means of black and bran points count. The quality of end products such as pasta, was explored for the presence of black points, which according to Italian legislation, they should not be present. Moreover, durum and common wheat blends and monovarietal durum wheats were studied and their chemical and reological parameters

were recorded. Morphometric measurements were performed by image analysis to assess and monitor the rising kinetic of the different flours, and morph-colorimetric measurements were executed to determine colour, morphology, and texture of bread slices. These and other applications, that should increase in the future, have stimulated the development of the ImageInspector, a marketable prototype portable and time user-friendly.

Computer analysis of food images – case studies

Piotr M. Szczypiński[1], *Piotr Zapotoczny*[2], *Artur Klepaczko*[1]

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Visual inspection is one of the oldest and at the same time reliable food quality assessment methods. However, in an industrial environment it is labor-expensive and arduous for performing employees. With the development of video and image analysis algorithms more often, the human-expert can be replaced by an automatic expert systems. Coherent and optimal methodology for developing such systems is not yet established. Nevertheless each case can be solved individually using unique, tailored algorithms.

The aim of this presentation is to introduce principles of image analysis methods in evaluation of agricultural products properties and quality. The case studies on a qualitative assessment of wheat kernels, barley's variety recognition, a qualitative assessment of potatoes and apples, and the analysis of meat products' composition. The scope of the presented methods will cover image preprocessing, color and texture descriptors computation, objects shape characterization, machine learning and data classification.

Biometric characterisation of grapevine seeds by image analysis and comparison with archaeological remains

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The wine heritage in Sardinia (Italy) consists of 151 cultivars. Considered as local varieties, they are the product of different events, such as direct domestication of wild grape, crosses between local varieties and the adoption of agricultural techniques and cultivars from different ethnic groups that colonized the Island over the centuries. The remaining varieties can be considered as false attributions (synonyms and/or homonyms) due to the existence of different dialects within the same insular region. The grapevine seeds are highly polymorphic and play a crucial role both in taxonomic studies of the genus *Vitis* L. [1], both in the understanding of the processes of domestication and distribution of wild grapes, as suggested by many studies of archaeobotanical character [2]. In archaeobotanical studies, the taxonomic classification of diasporas has usually been done by simple morphological observation and visual comparison with *ex situ* collections of seeds [3], although the use of biometric indices has often proved to be a powerful approach in the taxonomic studies of the genus *Vitis* as well as for the species attribution of archaeological remains [1, 4].

The aim of the project "*Origin, characterization and conservation of autochthonous grapevines of Sardinia*", funded by "*Regione Autonoma della Sardegna*", is to use some of seed morpho-colorimetric parameters and the Fourier Elliptic Descriptors, measured with computer-aided image analysis techniques, to characterize the studied cultivars and implement statistical classifiers able to discriminate among them, and comparing them with archaeological seed lots founded in Sardinia, in order to attempt an historical reconstruction of the origins of viticulture in Sardinia and the processes of domestication of wild grape. Moreover, the project aims to expand the view of knowledge concerning the germination of the genus *Vitis* L., developing germination protocols and obtaining relevant ecological information for the conservation of natural populations and for eventual restoration and populations reinforcement in the study area.

Under this two-year project, specific collaboration agreements will be signed with the Sardinian Germplasm Bank of University of Cagliari, where all the seed lots collected during the project will be stored for *ex situ* conservation, and with the Soprintendenza per

i Beni Archeologici di Cagliari & Oristano and Sassari & Nuoro, which will provide the archaeological seed lots.

The project involves a network of private companies operating in C-N of Sardinia, in particular: Azienda Gostolai S.A.S., Azienda Vitivinicola Ledda and Azienda Vitivinicola Canudu from Oliena (NU), all producers of wines from autochthonous grapes, as *Cannonau di Sardegna* and *Nepente di Oliena*; Azienda Fradiles Vitivinicola s.n.c. from Atzara (NU), winemaker of *Mandrolisai*, valuing one of the most ancient red Sardinian varieties, *Muristellu*; and Azienda Agricola Mulas from Bono (SS), winemaker of another one of the most ancient Sardinian autochthonous varieties, *Arvesiniadu*.

The contribution of these companies would impact not only on the scientific achievements, but also on the studied territory and desirably on the same companies profits, spreading their brands beyond the regional or national boundaries.

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POSTERS

An algorithm for quantitative analysis of spatial association between discrete nuclear events represented by microfoci in multicolor 3D confocal microscopy

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BACKGROUND.

Multicolor 3D microscopy images of various subnuclear microfoci, representing replication sites, DNA damage response regions or other nuclear phenomena provide vast amounts of information about localisation of these processes. However, co-occurrence of different processes cannot be quantified by colocalisation analysis of microfoci. In order to assess potential correlations between two processes represented by microfoci quantitative analysis of mutual distances between their centres in 3D is required.

GOAL.

This work was focused on constructing an algorithm for quantitative analysis of spatial relations between two classes of discrete events represented by large numbers of microfoci in three-dimensional images of cell nuclei.

METHODS.

Newly replicatedDNA was labelled using incorporation of a precursor (EdU) and click chemistry. Microfoci of phosphorylation of histone H2A.X and 53BP1 were labelled by immuno-fluorescence. 3D confocal fluorescence images were deconvolved prior to further analysis.

RESULTS.

An algorithm was constructed and applied in analysis of a relationship between DNA damage signalling and repair (H2AX histone phosphorylation, 53BP1 recruitment), and DNA replication, in nuclei of cells treated with topoisomerase inhibitor camptothecin or hydrogen peroxide. Relationship between spatial distributions of these two groups was quantified using distributions (histograms) of nearest-neighbor (nn) distances. Populations

of correlated and uncorrelated signals were isolated using histogram thresholding. Analysis of spatial distributions of γH2AX and 53BP1 foci demonstrate the existence of a population of γH2AX signals associated or located afar of 53BP1 signals. The nn distance calculation was supplemented with analysis of cumulative distribution of all possible distances (Ripley's K functions) between signals of the same and different kinds. We found an expected, statistically significant spatial correlation between DNA replication and damage induced with topoisomerase I inhibitor camptothecin, but a very low correlation in cells subjected to oxidative stress. This approach to analysis of spatial association of two nuclear events is expected to be suitable for investigations of a relationship between any other types of cellular events represented by small foci, in multicolor patterns found in standard and super-resolution 3D confocal images.

Analysis of changes in color of buckwheat seeds during the production of groats

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Buckwheat seeds before and after roasting were analyzed in order to determine whether the manufacturing process influences the change in their color. Color measurement was made by digital image analysis using the RGB and HSI model and the results were expressed as the minimum, maximum and mean value of the individual attributes. In each sample 150 grains were analyzed. Color analysis showed that the best parameters for differentiating the changes under the technological process were: component B – blue attribute in the RGB system and S – saturation in HSI system. The minimum, maximum and mean value of the component B did not significantly change under dehulling process, but significantly reduced after roasting. The decrease of about 50% of mean value of unroasted groats was caused by the blackening of seeds. The attribute S increased from the mean value of 52.2 to 148.6 under dehulling process and to 171.8 under roasting. The results showed that the technological process used in mills caused the change in color groats and a good tool for the assessment of these changes is digital image analysis.

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Stability of biopolymer-candelilla wax emulsions – a comparative study

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The emulsions with different candelilla wax (CW) concentrations (0.1–0.4% w/w) were prepared in the presence (1% w/w) of different biopolymers: carboxymethylcellulose (CMC), oxidized potato starch (OPS), soy protein isolate (SPI), and gelatin (GEL). The emulsion stabilizing properties of biopolymers were evaluated by measuring emulsion activity index (EAI), emulsion stability index (ESI), and emulsion particle size. The test results showed that, for all obtained emulsions, stability decreased together with the time of storage. GEL emulsions proved to be very stable, exhibiting only slightly changed ESI values after 48h storage (ESI_{48h}≈91–94%). SPI emulsions also had high ESI values (ESI_{48h}≈90%); however, only at higher CnW concentrations. Polysaccharides gave less stable emulsions (ESI_{48h} reached values of 4–13% and 7–24% for CMC and OPS, respectively). The largest wax particles, viewed by SEM images, were observed in CMC emulsion (5–20 μm). In other emulsions, the diameter of wax globules was much smaller and ranged from <1 μm (protein-based emulsions) to about 1–2 μm (OPS-based emulsion). A good negative association was found between particle size of wax emulsion and emulsion stability, i.e. the lower particle size, the higher ESI.

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Methods of water sensitive paper analysis overcoming limitations of the spray quality assessment based solely on the coverage area

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Water sensitive paper was developed to assess the coverage of the area with liquid in many applications. It has been particularly popular amongst researchers and farmers to visualize and evaluate spray distribution and deposition in the field experiments. The work shows that assessment based solely on the coverage area can be insufficient and misleading. Spraying quality can be also evaluated visually, but this approach is always influenced by human subjectivity. Considering this, the work proposes to implement methods of digital image analysis helping to overcome these limitations and giving additional information concerning the spraying quality. Proposed methods have been developed on the two following assumptions. The first is that they should be easily modifiable and efficient, what predestines them for implementation in real time systems, especially those based on programmable logic devices, like FPGAs and CPLDs. The second assumption is that the outcome of analysis should be given as numerical data, providing material for the quantitative comparison and interpretation. The work proposes several indicators that can be used to evaluate the spraying quality in reproducible and objective manner. It also shows few examples of using them to evaluate and differentiate data from field experiments.

Quantitative analysis of DNA damage induced by DNA topoisomerase I and II inhibitors

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DNA topoisomerase I and II inhibitors are a class of drugs used in anti-cancer therapy. Topoisomerases relax DNA supercoiling by transient cleavage of DNA strand in transestrification reaction. The mechanism of action of topoisomerase I and II inhibitors involves preventing the enzyme from religation of the phosphodiester bond in DNA, and subsequent inhibition of enzyme activity. This action can induce DNA damage. In order to study the specificity of topoisomerase inhibitors towards replicating DNA, multicolor three-dimensional confocal imaging and computer image analysis were used. A549 adenocarcinoma cells were treated with camptothecin (topoisomerase I inhibitor), etoposide or mitoxantrone (topoisomerase II inhibitors). Algorithms calculating positions of the fluorescence intensity maxima representing: replication sites, DNA damage signaling sites (γH2AX) or DNA double strand breaks were constructed. Quantitative and spatial analysis [1] of these sites in the cell nuclei revealed a strong correlation between the number and positions of the detected DNA damage sites induced by camptothecin and the number and positions of active replication sites. These dependencies were much less pronounced in the case of treatment with mitoxantrone or etoposide, especially at the end of S-phase of cell cycle.

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Microstructure and textural properties of acid-casein processed cheese analogues with whey protein preparations

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The effect of different whey protein preparations (WPC35, WCP65 and WPC85) on the microstructure and textural properties of processed cheese analogues obtained on the basis of acid casein and anhydrous milk fat was examined. Hardness of processed cheese analogues increased significantly with increased casein concentration. The addition of whey protein concentrates (WPC35 and WPC 65) to processed cheese analogues produced harder cheeses than cheese analogues obtained solely from AC.Cheese analogues with the addition of 3 % WPC35 exhibited the highest hardness.Processed cheese analogues obtained with addition of whey concentrates were much more cohesive than cheese analogues obtained solely from acid casein, but their cohesiveness decreased with increased concentration of whey products. Microstructure of processed cheese and processed cheese analogues is dictated by the nature of the protein matrix and extend of protein aggregation. Processed cheese analogues obtained with 1 % of WPCs addition exhibited fine stranded network whereas cheese analogues with 3 % of WPCs addition presented particulate, much more unhomogenous network. This structure was probably responsible for decreased cohesiveness.

Use of GIS tools In the analysis of land use on the bases of black and white aerial photos

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Black and white aerial photos are a valuable source of information abort natural environment. Existing GIS software enables fast quantitative picture analysis in the form of raster layers. It is estimated by regularity in distribution of particular pixels. A histogram is used for this purpose. Moreover, GIS software enables a visualization of raster layer. In black and white photos it is a standard color palette. A grey hue depends on the intensity of radiation reflection from the objects in the landscape. It is a also possible to assign colors to particular values of raster cells.

For the analysis two tiles (A and B) of black and white aerial photo with diversified land use was used. Both tiles had the same area (31.36 ha) and resolution (1 m^2). The photo was taken in July 1969 and presenting the area around Bytryny (aprox. 25 km south of Olsztyn). The analysis of raster layers (A and B) was made in TNT mips free 2014 software. In A layer mainly arable lands and grasslands. Main statistical values for this layer are: minimum: 57, maximum: 240, mean: 184,99, first mode: 205 (2332 pixels), second mode: 163 (1037 pixels). In B layer, mainly forest were present. Main statistical values for this layer are: minimum: 6, maximum: 225, mean: 91,03, mode: 45 (758 pixels).

Application of computer vision system in assessing the quality of pork meat with varying intramuscular fat content

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Keywords: Computer image, fat tissue, pork meat, Fourier analysis

The objective of this study was to determine the possibility of using computer image analysis (CIA) for estimating fat content as a pork meat quality factor. Obtained in industrial conditions pork loin and pork neck was used as a research material. PH, fat and protein content in muscle samples were determined. Colour was determined instrumentally using CIE Lab measuring system. Muscles cross-section images were obtained with the use of digital camera (QImaging, Micro Publisher 5.0 RTV) at a resolution of 2560x1920 pixels. Image Pro Plus 7.0 (Media Cybernetics) application was used for image analysis and data processing (white fields fractions represented fat and connective tissue). Statistically significant correlation was achieved between the percentage of white fields fraction obtained with CIA method, and content of fat and connective tissue obtained with near infrared spectroscopy (NIRS).

The research showed that computer analysis (CIA) can be used in assessing the quality of pork meat with varying intramuscular fat content.

Organizers

Partner

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