the diagnostic pathology journal DIAGNOSTIC PATHOLOGY

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

How to analyze Structure and Function in Tissue – based Diagnosis?

Klaus Kayser[1*], Stephan Borkenfeld[2], Rita Carvalho[3], Amina Dejounis[4], Gian Kayser[5]

Affiliation:

1 – Institute of Pathology, Charite, Berlin, Germany ;

2 – IAT, Heidelberg, Germany;

3 – Department of Pathology, Central Lisbon Hospital Center, Lisbon, Portugal ;

4 – Institute of Pathology, Batna, Algeria;

5 – Institute of Pathology, University of Freiburg, Freiburg, Germany.

* Corresponding author

E-mail address: Klaus.kayser@charite.de

Abstract

Background: Tissue – based diagnosis (morphological analysis of tissue) judges, measures and interprets morphologic images which have been acquired from human tissue. It translates the findings into a diagnosis or description of biological functions. What are its principle algorithms and theoretical background?

Theory: Pathologists are used to distinguish between structure and function. Biological structures are ordered clusters of material (genes, nuclei, cells, organs, etc.), which remain constant during the period of detection and observation. They are commonly embedded or appear in circumscribed spaces. These spaces are clearly separated from their environment (background).

Functions are forces that act on structures. They modify their appearance, create and delete structures and their spatial relationship. The recognition of both structures and functions is dependent upon the observation time: Material that remains unchanged within the observation period is called structure, its changes between a series of observations a function.

Derivatives: Biological structures and functions should be interpreted in relation to the observation time. Functions can be considered structural gradients of time or of observation periods.

Implementation: The analysis of conventional stained histological slides reflects to a short non changeable observation time, which in reality cannot be repeated at different times on the same tissue.

the diagnostic pathology journal DIAGNOSTIC PATHOLOGY

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

Acquired digital images such as virtual slides (VS) offer the opportunity of simulating different observation times if object features are analyzed that reflect structural changes at different times. The measurement of immune histochemistry intensity levels performed on the same structure can be considered a time series of the binding or antigen – antibody process. The obtained frame of these measurements can be mapped on chemical significant descriptors such as Shannon's and structural entropy, and their entropy flows.

Material and Methods: Histological glass slides taken from a previous biochemical study [1], and displaying with osteoarthritis cartilage of eight patients and of four osteosarcoma patients were incubated with AP labelled polyclonal antibodies against human Gal-1, -2, -3, -4, -7, -8, and -9, which had been obtained by recombinant production and purification.. One snapshot per case was digitalized and the staining intensity was measured in relation to a series of segmentation grey levels (0 – 255). The data were mapped on the principal measures Shannon's and structural entropy as well as on the entropy flow derived from texture analysis.

Results: The mapped functions of entropies display with significant changes between the galectin-7 positive images and their negative control counterparts. The data indicate that the binding capacities of galectin-7 hold a significant function during the development of osteoarthritis cartilage.

Conclusions: Laboratory techniques that simulate time-related series of measurements can be used to describe and interpret biological functions in living organisms at the cellular level.

Keywords: Digital pathology, structure, function, galectin-7, structural entropy, cellular heterogeneity.

Virtual Slides: www.diagnosticpathology.eu/vs/2016_2_106/

Introduction

Medical diagnosis is an established method to forecast and interact on the development of health aberrations [2] [3]. Starting from a broad set of information it finally ends in a digital (yes – no) decision. For example, whether anti-inflammatory drugs should be given to a child or not, whether a cancer should be operated on or not, whether additional therapeutic actions should be undertaken or not [4].

Tissue – based diagnosis which is diagnostic performance on different kinds of human tissue such as cytology, biopsy, aspiration specimens and smears, possesses the highest sensitivity

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

and specificity, or the lowest error rate in human disease detection and classification [5] [6]. Microscopy and tissue analysis at the cellular level contribute to the most frequently applied methods within this spectrum [3].

The technological progress of image digitalisation and information distribution did not pass by tissue – based diagnosis. To the contrast, it influences and modifies pathology laboratories and microscopic diagnosis performance to a high degree, often without being noticed by the involved pathologists [7].

In addition, different technologies also create new pathways in tissue – based diagnosis especially molecular biology and molecular genetics [8, 9]. Both techniques now-a-days play a major role in diagnosis classification and derived applications such as individualized therapy or predictive diagnosis, disease risk evaluation in adults or even in foetal development stages [10].

Most of the implemented molecular and gene or DNA reduplication technologies require sophisticated statistics, image acquisition and interpretation methods to fully detect and interpret the visualized information [11].

Herein we want to discuss and propose a generalized theory and its application of structures and functions in man. The idea allows to simulating the impact of functions on structures and vice versa.

Theory of structure and function

The definition of structure and function is different and specific in different application fields. In mathematics, a structure means a set of defined properties or functions. The relationship between the elements of a set or between different sets has to obey certain conditions and defines the structure [12].

In molecular biology, functions are related to reciprocations between structures that posses and act as information storages. Examples are macromolecules, nuclear acids, proteins, cellular membranes, cells, etc.

Pathologists are interested in these structures that can be visualized at different magnifications and by different methods, most commonly at the cellular level using

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

haematoxylin / eosin (HE) stains. The HE stain permits a clear distinction between different cell types (for example epithelial cells versus fat cells). Histochemistry and derived techniques such as immunohistochemistry (IHC), in situ hybridization (ISH), etc. visualize molecular structures that are involved in reciprocations [3, 6, 10, 13, 14].

All these investigations display with structures. Their functions (reciprocations) have to be detected by different methods such as biochemical assays, tissue incubations, etc. [2, 15, 16].

In surgical pathology or tissue – based diagnosis the analysis of structures means that agglutinations of material have to be evaluated which have lost their biological functions, i.e., which are dead and usually fixed by appropriate dyes. The examination of these structures focuses on causes which have induced remarkable changes, and the forecast of their influence on the living organism.

The general algorithms investigate in numerous already analyzed equivalent structures, the known development of diseases which display with a close relationship to the investigated morphology. The association is commonly intensive and allows precise predictions without knowing the underlying reciprocations.

Do algorithms exist that allow such calculations?

Basic conditions: Let us presume that we visualize biological structures microscopically at a time ***ts*** within a certain time period ***to*** that is needed to separate structures from its background. The end of the observation period is then ***ts + to = te***. Obviously, all objects that can be detected within the period ***to*** care structures, and no functions or reciprocations can be detected.

We then theoretically repeat the same analysis after a delay ***td*** on the same sample using the same observation period ***to***. We then can measure active functions that act on the structures if

1. the structures have changed their position within the observation space (background), or
2. they have changed their appearance.

Measurable changes are related to the surface (form factor, shape) and/or internal properties (light absorption, colour, etc).

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

We then repeat the observation in a series of measurements ***ts1, ts2, ts3,…tse,*** and derive a set of functions from the visible changes of structures at the times ***tsi.*** In other words, the functions are related to structural differences between ***ts1*** and ***ts2***, or ***ts2*** and ***ts3***, etc. In mathematical description the functions or reciprocations reflect to the gradient ***F* = Ds/dts.**

Unfortunately, in reality we cannot perform such measurements because the tissue sample under investigation is unique and cannot be reproduced.

What about a virtual (digital) environment?

Hypothesis: How to simulate the gradient ***F*** in the virtual world? We have to find a visual signal that is related to the multiple observation periods, because we then can replace the gradient ***dts*** by a ***tsi*** related intensity of signals ***iti***. The more intensive a signal the older is the associated staining start, which can be replaced by the observation start ***ts*** multiplied by a constant ***c***. Thus, ***F = c*{iti/tsi}***.

Practical considerations: When searching for appropriate potential simulations IHC and lectin histochemistry (LHC) seem to be suitable. Both techniques visualize intensity related chemical properties in terms of antigen-antibody or sugar – protein related binding forces. The term entropy is an adequate descriptor or frame to measure intensity related and other properties of physical – chemical reactions [17] [17, 18].

Entropy properties: Entropy is a fundamental thermo-dynamical state variable which defines the direction of development of closed (and open) thermodynamic systems [17]. Shannon has demonstrated that entropy is a suitable descriptor of disorder in systems that are composed of numerous microstates [19].

Tsallis has extended the entropy to systems that contain a certain rest – entropy, i. e, whose entropies cannot be added in a simple manner [20] [21].

Kayser introduced a morphology – adjusted so – called structural (MST) entropy [17, 22, 23]. This entropy is derived from graph theory applications. It is an eligible descriptor of cellular disorder in tumours and other tissue lesions [24-26]. Shortly, cellular proliferation is assumed to create two identical daughter cells form their mother cell. The daughter cells should display with (nearly) identical properties and (nearly) identical distance under normal conditions [17]. Thus, the combined measurement of distance and feature adjusted entropy properties should

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

be a descriptor of disorder during the cellular proliferation (or apoptosis) process. It can be reproducibly applied to microscopic images and serve for reproducible statistics [17].

In fact, MST entropy and its entropy flow turned out to be of prognostic impact for patients who have developed different cell types of malignancy [27-30].

Observation series and entropy frame: A digital (microscopic) image can be adjusted to a two-dimensional plane which is defined by its grey value levels. All pixels with grey values above and within the plane are defined objects, those below the plane background. The plane is the event space, and the objects are events which serve for the entropy calculations.

A time ordinate is observed when passing through all entropy planes, which obviously reflect to the staining procedure: the more intensive the staining the longer or more active it took place, i.e., the stronger have been the attractive forces. Thus, the intensively stained objects are equivalent to early events (or high attractions) in contrast to their weakly stained counterparts. These visualize weak attractions and/or a late staining start.

These are the prerequisites that serve for time – series measurement simulation of former object – related thermodynamic in vivo properties, which can be observed in reality by changes of the environment (tissue cultures) and disintegration of tissue only.

How can they be visualized and measured?

Image analysis

Image content information: Each microscopic image possesses information which can be assessed when viewing the image. The information is presented in circumscribed two dimensional arrangements of grey values in specific colour spaces (red, green, blue for example). The pathologist interprets these arrangements and associates his knowledge to them. In other words, interpretation or evaluation of a diagnosis is a communication with the image and knowledge of the arrangement of nuclei, membranes, vessels, lymphocytes, etc. Such a judgement can be separated in two components: 1. Information of the image itself, i.e. independent from the pathologists' knowledge, 2. external knowledge, i.e. knowledge of the pathologist. The combination of both results in the. stated diagnosis. It is important to

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

separate these issues, especially, when algorithms are designed that should be applied to different kinds of images [10].

Object, structure and texture: When evaluating image content information, it is useful to clearly define and distinguish between object, structure and texture in analysis of microscopic images. Objects are grey value agglutinations that posses a distinct significance for the viewer. They include biological meaningful events, such as chromosome, nuclei, cells, vessels, etc. They are "composed" of image content information and external knowledge. Structures are spatial iterative objects and correspond to higher order symmetries [10]. Obviously they cannot be defined without external knowledge.

Textures are grey value distributions that correspond to individual grey values of pixels and do not require external information.

The following statements are valid: Textures are prerequisite for objects, and objects prerequisite for structures.

Image primitives are pixel based structures. They correspond to letters in a word and can be composed to pixel structures. A set of such structures is shown in <Figure 1>.

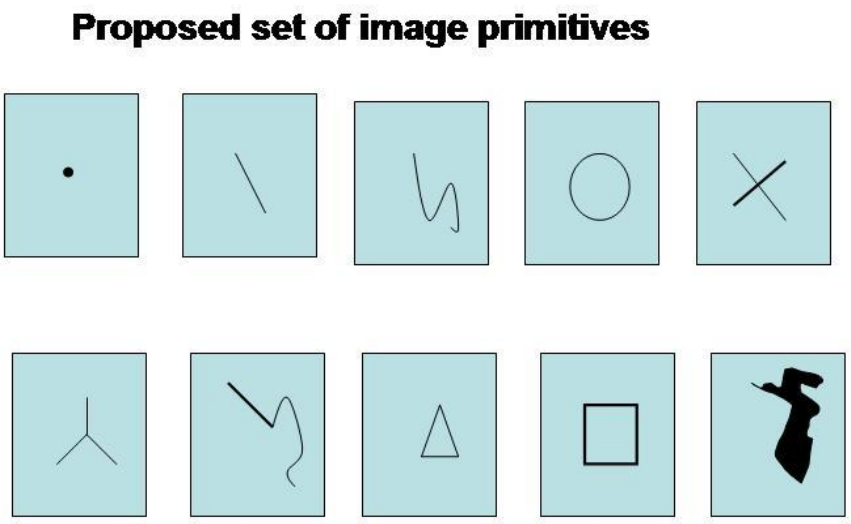

Figure 1: Proposed set of pixel – based texture graph primitives.

the diagnostic pathology journal DIAGNOSTIC PATHOLOGY

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

Implementation

Prerequisites

It might be assumed that all commercially available whole slide scanners acquire standardized and reproducible digital images. This seems, however, not to be realized in reality, and numerous virtual slides display with non-negligible aberrations in terms of illumination, color space and glare effects [31-34]. Thus, prior to applications of automated information detection and extraction the acquired digital images have to be standardized for at least three different parameters:

1. Homogenous illumination or shading. An example is demonstrated in <Figure 2>. Shading correction (vignette) can be performed using the differentiated image or using a standard correctly balanced image [35].

2. Adjustment of the gray value range in all three color spaces for the maximum and mean gray value range. This procedure should be performed after the shading correction, and is needed to balance between different laboratory effect such as thickness of the tissue cut, principal staining intensity (age of the dye), etc. [36, 37].

3. Adjustment of gray value distribution in order to avoid any artificial gray value clusters. They can be induced by non homogenous color sensitivity of the scanner chip [36, 37].

The calculation of the number of potentially useful thresholds in order to separate the object space form the background is a useful step for a) confirming suitable image quality and b) defining mandatory gray value thresholds in relation to the wanted objects [10, 35, 38]. It is less known and less applied in general. The number of statistically useful thresholds (i.e., >95% confidence areas) accounts to three in IHC and conventionally stained images, and to two in one-marker fluorescent images, as shown in <Figure 3>. An IHC image that contains only 1 – 2 detectable thresholds is probably of poor image quality or of inadequate technical origin [10, 35, 38].

The next step addresses to information distribution within the whole VS, or the detection of regions of interest (ROI). Several regions might be present in the image, and all of them can serve for additional analysis. Several different algorithms of ROI detection have been published in the literature [10, 16, 35, 39-42].

the diagnostic pathology journal DIAGNOSTIC PATHOLOGY

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

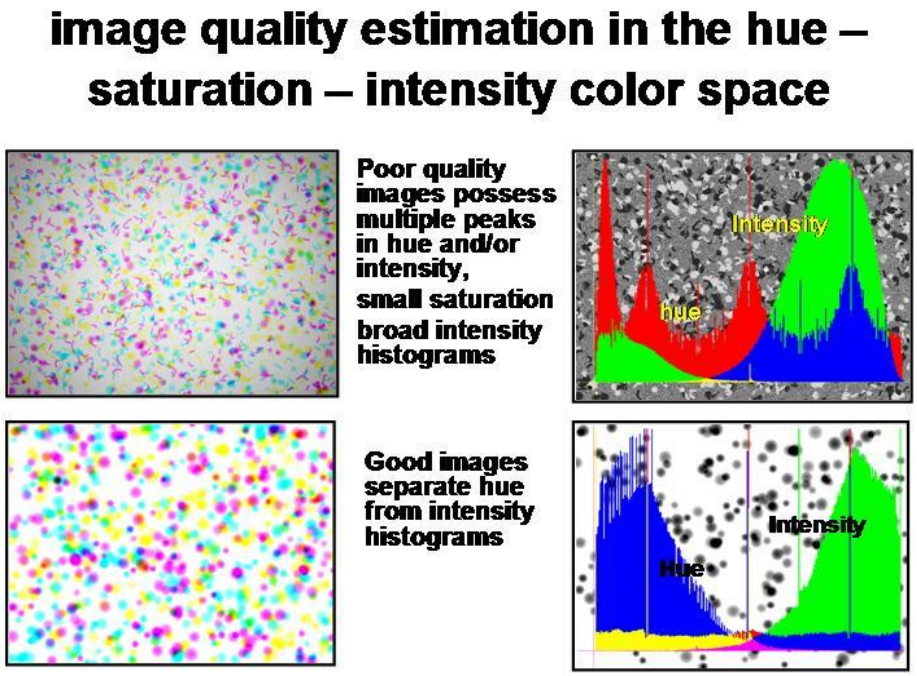

Figure 2: Example of shading influence on image analysis and its potential detection and correction in hue – saturation – intensity (HIS) color space.

The appropriate ROI detection algorithm should be selected in relation of the aim, whether an interactive or an automated diagnosis is addressed to. Interactive diagnosis using ROI with fixed size and frames is to our experience easier and faster to perform than to evaluate diagnoses on ROI of variable sizes [3, 32, 43].

Fixed ROI sizes permit, in addition, the calculation of entropy flow between the different neighboring frames; an option, that cannot be calculated on variable areas due to missing standardization of ROI area (It might be reminded that entropy calculations depend on the size of the selected space) [7, 10].

Variable ROI sizes are appropriate for automated diagnosis in difficult cases. Based upon structure – associated parameters their boundaries separate diagnosis – relevant areas from diagnosis irrelevant areas more distinctly.

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

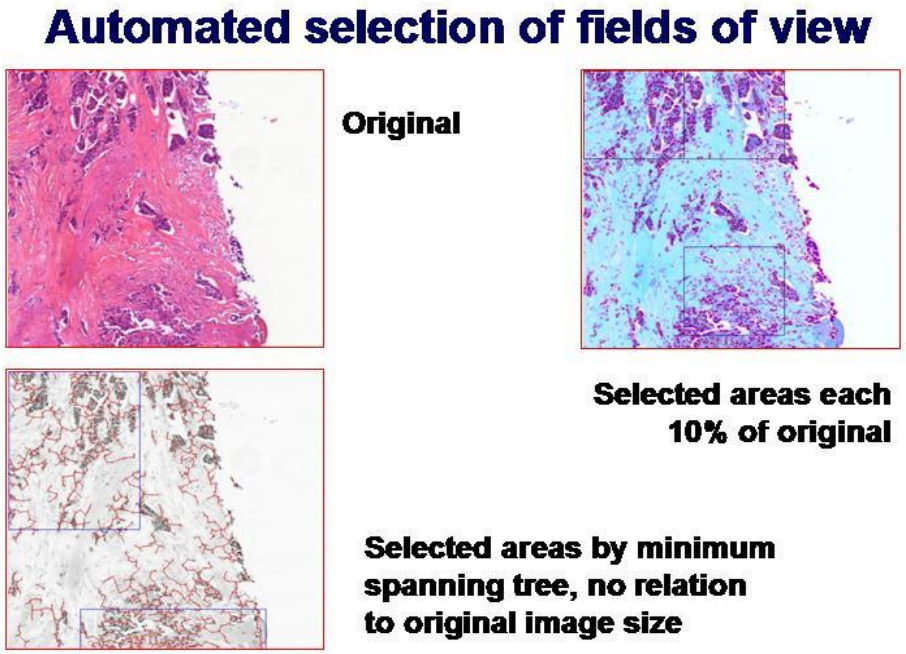

Figure 3: Example of gray value thresholds that are significant and suitable for segmentation.

In case of image comparison approaches fixed ROI areas are a prerequisite in order to use similarity operations between the source and the test ROI [7, 10]. The same statement holds true for pixel based texture approaches. Again, several image transformations can only be performed on square sized images (for example Hough or recursive transformations) [7, 10].

Fixed ROI sizes allow the calculation of entropy differences between neighboring (and all) segments and of entropy flows between the segments. This measure is an extension of cell – based entropies that are calculated between neighboring individual cells. Images of both approaches are shown in <Figure 4>.

The implemented algorithms distinguish between the classic object measurements which include the quantification of nuclei, membranes, and vessels, and the discussed gray value adjusted entropy measurements. The principal algorithm is presented in <Figure 5>. All measurements include the analysis of higher order structures, as described in [2, 7, 10, 16, 17, 35]. These analyses use Voronoi's neighborhood condition and the application of graph theory, namely weighted graphs [44-47].

the diagnostic pathology journal DIAGNOSTIC PATHOLOGY

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

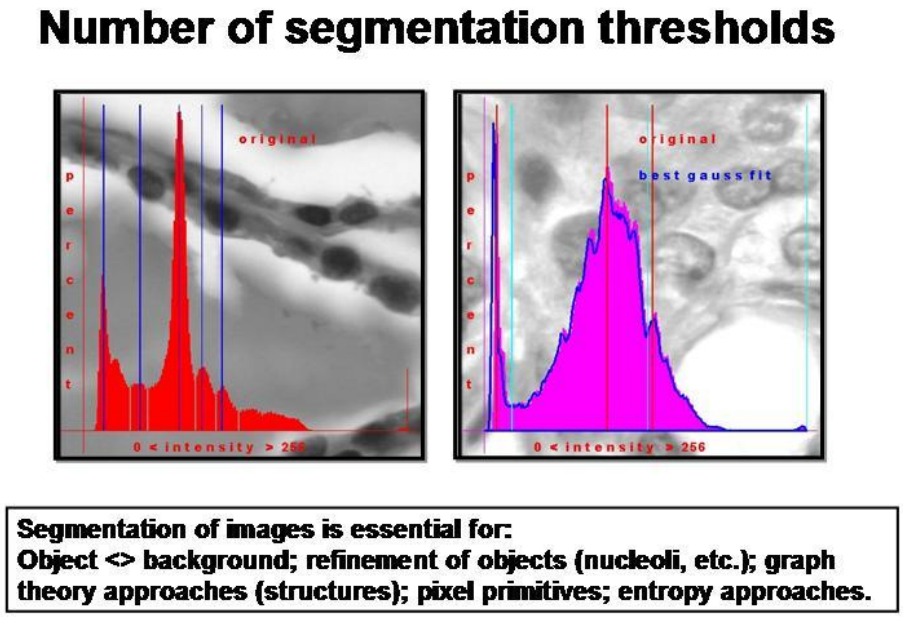

Figure 4: Example of ROI detection by sliding image compartments of fixed size and by graph theory approach.

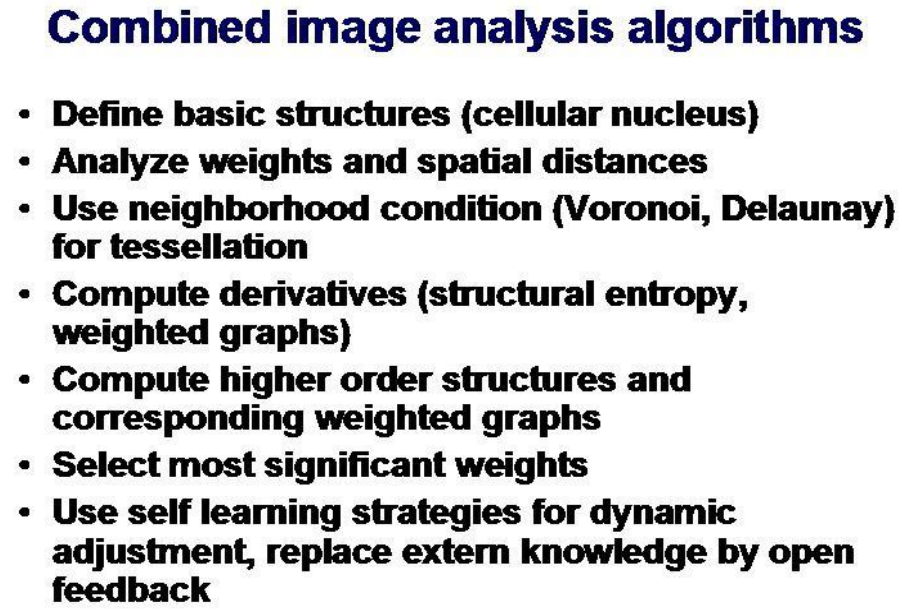

Figure 5: Conventional image analysis strategies to acquire image content information using self learning approaches.

the diagnostic pathology journal DIAGNOSTIC PATHOLOGY

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

In addition to the spatial distance specific features of the included objects serve for weights. These weights are partially dependent upon the underlying staining intensities, and partially on morphologic features such as object size, moments, circumference, and of circumference / area relationship [3, 16, 22, 31, 48].

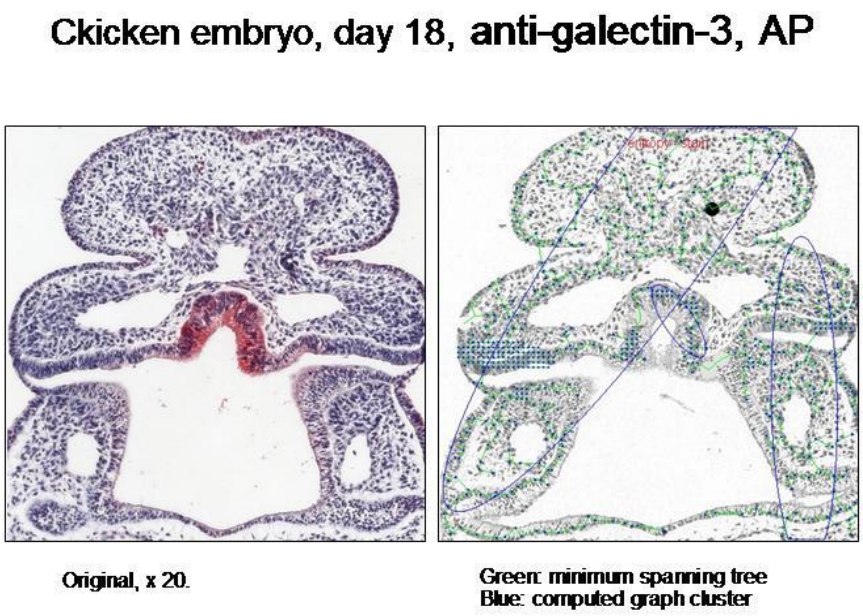

Figure 6: Example of conventional analysis of anti-galectin-7 stained cartilage showing the original image and derived MST graph.

Measurements and Material

Histological slides of eight osteoarthritis cartilages and of four control cases (cartilages of osteosarcoma patients) were incubated with labeled antibodies against a panel of polyclonal antibodies against galectin-1, -2, -3, -4, -7, -8, -9, as described previously by Toegel et al. [1, 49, 50]. The binding capacities were visualized using the DAB technique. Positive and negative controls were performed as usual, i.e. by contemporary performance of know positive cases, and by omission of the primary antibody. The biological significance of the approaches was confirmed by in vitro assays, as described previously [1, 49, 50]. Representative digitized images of the used material as shown in <Figures 6 – 8>.

the diagnostic pathology journal DIAGNOSTIC PATHOLOGY

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

Results

A survey of the obtained measurements is shown in <Table 1>. The differences between positively stained and control cases are highly statistically significant as well as the entropy differences between different gray value levels. In addition, the shape of the MST entropy curves is characteristic for images of highly and wekly degenerated cartilage, see <Figures 7, 8>. All arthritis cases display with characteristic minimum in contrast to the Gaussian – like distribution of control cases <Figures 7, 8>.

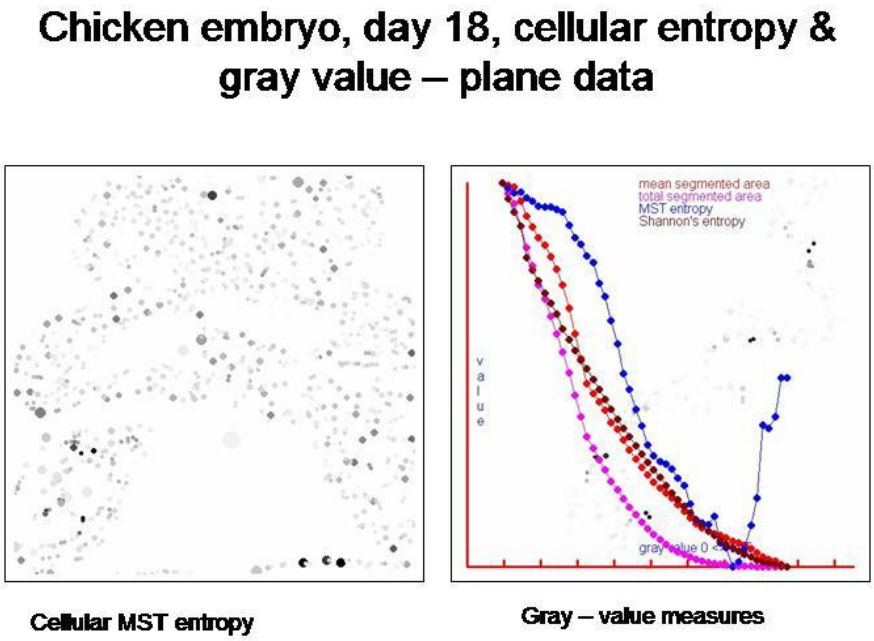

Figure 7: Example of local (cellular) gray value related MTS entropy (left) and intensity – related gradient (right). In contrast to Shannon's entropy and segmented areas the MST entropy characteristically displays with a minimum during its run.

the diagnostic pathology journal DIAGNOSTIC PATHOLOGY

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

Table 1: ***Survey of results****:*

Feature	**Cases**	**Controls**
Number of cases	8	4
Vv stained area	0.11	0.22
Vv stained nuclei	0.24	0.31
No neighboring positive cells	5.17	5.9
MST entropy cell volume	-1.77	-1.73
MST entropy of stained cells	- 0.57	- 0.90
Texture entropy	- 6.57	- 6.55
Original image entropy flow	- 0.11	- 0.13
Texture entropy flow	- 0.09	- 0.24

Explanations:

Vv	Volume fraction
No neighboring positive cells	Number of positively stained neighboring cells according to Voronoi's tessellation
MST entropy cell volume	MST entropy calculated according to cellular size
MST entropy stained cells	MST entropy of positively stained cells
Texture entropy	Shannon's entropy of recursive image texture
Texture entropy flow	Entropy flow of recursive image texture between image compartments of fixed size (10% of image size each)

the diagnostic pathology journal DIAGNOSTIC PATHOLOGY

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

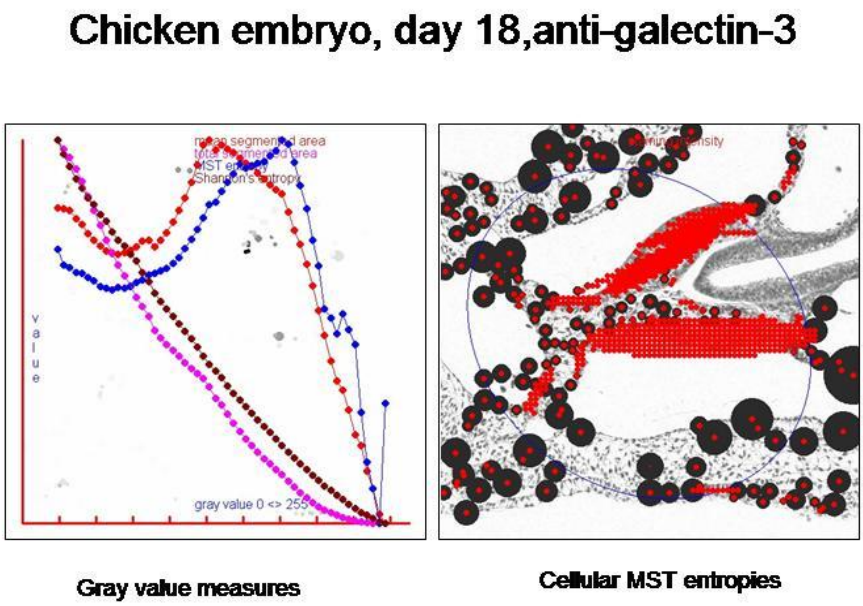

Figure 8: Example of cellular MST entropies (right) and gray value related MST – entropy. MST entropy values of weakly degenerative altered cartilage display with Gaussian like distribution.

Discussion

To our understanding, medicine belongs to the set of natural sciences. They are characterized by general laws that are valid independently from space and time of observation, which does not mean that the laws cannot be influenced by observation, or measurements [3].

Natural sciences basically describe the history of a system and forecast its future, its development, or that of its elements. They describe two different states, namely a state in which its elements stay constant during the observation period, and a state in which appearance, size, or position of the system or its elements alter [19-21].

The elements of the first state are called structures, the changes of the second state functions. Natural sciences such as physics, biology, or chemistry focus on functions occurring in the environment (background) “outside” of man. Examples include the laws of general gravity, relativity, electro-magnetic fields, etc.

We assume that these laws are also valid “inside” the body of man. Indeed, we can visualize internal structures such as liver, heart, vessels, cells, nuclei, and even functions such as heart

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

beats, transportation of macromolecules, etc. However, we also do know, that certain measurements can only performed "once" inside the body, because they destroy contemporary the structures. Examples are surgical interventions, such biopsies, or operations. On the other hand, these interventions are essential if we want to examine the structure of lesions (morphology) [3, 33, 53, 54].

The analysis of structures at a cellular level has been extensively described by Virchow 150 years ago. It is still today the backbone of diagnosis and treatment of fatal diseases such as cancer, progressive atrophy, or autoimmune disorders [3, 33, 53, 54].

By definition, these investigations describe structures, which display with the present state of a disease; however, in order to intervene or to cure the disease we do need to measure properties of functions.

Herein, we describe a general approach how to derive functions from structures. The principal idea is that structure and function are of the same property. They only become different when they are associated with the observation time and period. This association permits the construction and virtual visualization of functions that can be stated upon one measurement only. The measurement has to include different structures that have been formed a different periods and that can distinguish these structures.

Immunohistochemistry and related investigations such as ligandhistochemistry commonly present with different intensities of visualized (stained) structures. Assuming that intensively stained structures needed a longer time for binding (or are equipped with stronger binding forces) we have a model that permits the identification of structures at different measurement times, and, therefore, the analysis of functions.

The term structure and function is not limited to a space – time relation ship. It can be transformed into other frameworks. The terms entropy, entropy flow, and structural (MST) entropy (flow) are important measures in thermodynamics [9, 16, 17, 27, 30, 35, 55] [56]. They describe the development of closed and open systems and the distance from their final state [3, 16, 17]. Therefore, we choose the frame entropy/MTS entropy <> time (staining) intensity in order to analyze functions that cannot be measure otherwise.

The model of binding capacities of galactin-7 binding macromolecules in degenerative altered tissue seems to be suitable for first investigations. In addition, Gabius, Sinowatz and co-

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

workers have demonstrated that these macromolecules play also an important role in the development of fetal organs [57] [58]. Thus, it seems to investigate in a useful approach to test our ideas.

The results clearly indicate that our idea results in data that can describe or even explain functions in biologic development on the basis of measured structures. These measurements have been performed only once and within a short observation period. What are they good for?

The answers include the following statements:

1. The method describes thermodynamic states at a cellular level, for example the entropy distribution in a solid cancer. It is obvious to correlate the insight with the so – called (cancer cell) heterogeneity. Heterogeneity of malignancies is in focus of numerous investigations; however, unfortunately, without any standardized nomenclature until today [11, 17, 26]. Several different elements are in use for this purpose. These include proliferation, cellular (nuclear) appearance, vascularization, genetic aberrations, inflammatory response of host tissue, to name some of them [59].
2. This approach can be easily correlated with distinct molecular or genetic data, such as molecular pathways, gene expressions, or vascular findings.
3. It can be further investigated in search for a general descriptor of birth, development and death of biological systems. These approaches might include thought of orders of biologic structures, breakdown of higher order structures (systems) induced by weak disturbances (functions) of low order structures, enhancement of functions related to structural influences, etc.

In agglutinate, a clear distinction between structure and function is mandatory to understand and investigate in the future of pathology, despite both of them are of the same nature in principle.

Acknowledgement: The financial support of the Verein zur Förderung des biologisch – technologischen Fortschritts in der Medizin e.V. is gratefully acknowledged.

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

References:

1. Toegel, S., et al., *Human osteoarthritic knee cartilage: fingerprinting of adhesion/growth-regulatory galectins in vitro and in situ indicates differential upregulation in severe degeneration.* Histochem Cell Biol. **142**(4): p. 373-88.

2. Kayser, G., et al., *Towards an automated morphological classification of histological images of common lung carcinomas.* Elec J Pathol Histol, 2002. **8**: p. 022-03.

3. Kayser , K., B. Molnar, and R.S. Weinstein, *Virtual Microscopy Fundamentals - Applications - Perspectives of Electronic Tissue - based Diagnosis*. 2006, Berlin: VSV Interdisciplinary Medical Publishing.

4. Randell, R., et al., *Diagnosis of major cancer resection specimens with virtual slides: impact of a novel digital pathology workstation.* Hum Pathol. **45**(10): p. 2101-6.

5. Patel HD, J.M., Pierorazio PM, Sozio SM, Sharma R, Iyoha E, Bass EB, Allaf ME., *Diagnostic Accuracy and Risks of Biopsy in the Diagnosis of a Renal Mass Suspicious for Localized Renal Cell Carcinoma: Systematic Review of the Literature.* J Urol., 2016. **18**(doi: 10.1016/j.juro.2015.11.029. [Epub ahead of print]).

6. Bilous, M., et al., *Predicting the HER2 status of breast cancer from basic histopathology data: an analysis of 1500 breast cancers as part of the HER2000 International Study.* Breast, 2003. **12**(2): p. 92-8.

7. Kayser, K., *Quantification of virtual slides: Approaches to analysis of content-based image information.* J Pathol Inform. **2**: p. 2.

8. Aitken, S.J., et al., *Quantitative analysis of changes in ER, PR and HER2 expression in primary breast cancer and paired nodal metastases.* Ann Oncol. **21**(6): p. 1254-61.

9. Kayser, G., et al., *Stromal CD4/CD25 positive T-cells are a strong and independent prognostic factor in non-small cell lung cancer patients, especially with adenocarcinomas.* Lung Cancer. **76**(3): p. 445-51.

10. Kayser, K., et al., *Texture and object related image analysis in microscopic images.* Diagnostic Pathology, 2015. **1**(14).

11. Zito, C.I., et al., *Direct resequencing of the complete ERBB2 coding sequence reveals an absence of activating mutations in ERBB2 amplified breast cancer.* Genes Chromosomes Cancer, 2008. **47**(7): p. 633-8.

12. Kumar V, P.S., Bayda S, Corona G, Toffoli G, Rizzolio F., *DNA Nanotechnology for Cancer Therapy.* Theranostics, 2016. **6**(5): p. 710-25.

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

13. Kayser, K., et al., *Digitized pathology: theory and experiences in automated tissue-based virtual diagnosis.* Rom J Morphol Embryol, 2006. **47**(1): p. 21-8.

14. Schmitt, F., *HER2+ breast cancer: how to evaluate?* Adv Ther, 2009. **26 Suppl 1**: p. S1-8.

15. Racoceanu, D. and P. Belhomme, *Breakthrough technologies in digital pathology.* Comput Med Imaging Graph. **42**: p. 1.

16. Kayser, K., et al., *Texture- and object-related automated information analysis in histological still images of various organs.* Anal Quant Cytol Histol, 2008. **30**(6): p. 323-35.

17. Kayser, K. and H.J. Gabius, *The application of thermodynamic principles to histochemical and morphometric tissue research: principles and practical outline with focus on the glycosciences.* Cell Tissue Res, 1999. **296**(3): p. 443-55.

18. Kayser, K., et al., *To be at the right place at the right time.* Diagn Pathol. **6**: p. 2-9.

19. Claude Elwood Shannon, W.W., *The Mathematical Theory of Communication*. 1963, Chicago: University of Illinois Press

20. Tsallis, C. and A.M. Souza, *Constructing a statistical mechanics for Beck-Cohen superstatistics.* Phys Rev E Stat Nonlin Soft Matter Phys, 2003. **67**(2 Pt 2): p. 026106.

21. Weinstein, Y.S., S. Lloyd, and C. Tsallis, *Border between regular and chaotic quantum dynamics.* Phys Rev Lett, 2002. **89**(21): p. 214101.

22. Kayser, K. and H. Hoffgen, *Pattern recognition in histopathology by orders of textures.* Med Inform (Lond), 1984. **9**(1): p. 55-9.

23. Kayser, K. and W. Schlegel, *Pattern recognition in histo-pathology: basic considerations.* Methods Inf Med, 1982. **21**(1): p. 15-22.

24. Szoke, T., et al., *Prognostic significance of endogenous adhesion/growth-regulatory lectins in lung cancer.* Oncology, 2005. **69**(2): p. 167-74.

25. Zink, S., et al., *Survival, disease-free interval, and associated tumor features in patients with colon/rectal carcinomas and their resected intra-pulmonary metastases.* Eur J Cardiothorac Surg, 2001. **19**(6): p. 908-13.

26. Kayser, K., et al., *Pulmonary metastases of breast carcinomas: ligandohistochemical, nuclear, and structural analysis of primary and metastatic tumors with emphasis on period of occurrence of metastases and survival.* J Surg Oncol, 1998. **69**(3): p. 137-46.

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

27. Hagemeyer, O., H.J. Gabius, and K. Kayser, *Paraganglioma of the lung--developed after exposure to nuclear radiation by the Tschernobyl atomic reactor accident?* Respiration, 1994. **61**(4): p. 236-9.

28. Kayser, K., et al., *Atherosclerosis-associated changes in the carbohydrate-binding capacities of smooth muscle cells of various human arteries.* Zentralbl Pathol, 1993. **139**(4-5): p. 307-12.

29. Kayser, K., et al., *Correlation of expression of binding sites for synthetic blood group A-, B- and H-trisaccharides and for sarcolectin with survival of patients with bronchial carcinoma.* Eur J Cancer, 1994. **30A**(5): p. 653-7.

30. Kayser, K., et al., *Expression of lectin, interleukin-2 and histopathologic blood group binding sites in prostate cancer and its correlation with integrated optical density and syntactic structure analysis.* Anal Quant Cytol Histol, 1995. **17**(2): p. 135-42.

31. Moles Lopez, X., et al., *An automated blur detection method for histological whole slide imaging.* PLoS One. **8**(12): p. e82710.

32. Oger, M., et al., *Automated region of interest retrieval and classification using spectral analysis.* Diagn Pathol, 2008. **3 Suppl 1**: p. S17.

33. Park, S., et al., *The history of pathology informatics: A global perspective.* J Pathol Inform. **4**: p. 7.

34. Yagi, Y., et al., *The First Congress of the International Academy of Digital Pathology: digital pathology comes of age.* Anal Cell Pathol (Amst). **35**(1): p. 1-2.

35. Kayser, K., et al., *AI (artificial intelligence) in histopathology--from image analysis to automated diagnosis.* Folia Histochem Cytobiol, 2009. **47**(3): p. 355-61.

36. Kayser, K., et al., *How to measure diagnosis-associated information in virtual slides.* Diagn Pathol. **6 Suppl 1**: p. S9.

37. Kayser, K., et al., *Interactive and automated application of virtual microscopy.* Diagn Pathol. **6 Suppl 1**: p. S10.

38. Kayser, K., et al., *History and structures of telecommunication in pathology, focusing on open access platforms.* Diagn Pathol. **6**: p. 110.

39. Al-Janabi, S., et al., *Whole slide images for primary diagnostics of urinary system pathology: a feasibility study.* J Renal Inj Prev. **3**(4): p. 91-6.

40. Gray, A., et al., *Quantification of histochemical stains using whole slide imaging: development of a method and demonstration of its usefulness in laboratory quality control.* J Clin Pathol. **68**(3): p. 192-9.

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

41. Greenbaum, A., et al., *Wide-field computational imaging of pathology slides using lens-free on-chip microscopy.* Sci Transl Med. **6**(267): p. 267ra175.

42. Kayser, K., S. Borkenfeld, and G. Kayser, *How to introduce virtual microscopy (VM) in routine diagnostic pathology: constraints, ideas, and solutions.* Anal Cell Pathol (Amst). **35**(1): p. 3-10.

43. Wright, A.I., H.I. Grabsch, and D.E. Treanor, *RandomSpot: A web-based tool for systematic random sampling of virtual slides.* J Pathol Inform. **6**: p. 8.

44. Lu, S.Y., *A tree-to-tree distance and its application to cluster analysis.* IEEE Trans Pattern Analysis and Machine Intelligence, 1979. **PAMI-1**: p. 219-234.

45. Lu, S.Y. and K.S. Fu, *A syntactic approach to texture analysis.* Computer Graphics Image Processing, 1978. **7**: p. 303 - 330.

46. Prewitt, J.M.S. and S.C. Wu. *An application of pattern recognition to epithelial tissues*. in *Computer Applications in Medical Care*. 1978: IEEE Computer Society.

47. Sanfeliu, A., K.S. Fu, and J.M.S. Prewitt. *An application of a distance measure between graphs to the analysis of muscle tissue patterns.* in *Workshop on Structural and Syntactic Pattern recognition*. 1981. Saratoga Springs, New York.

48. Kayser, K., et al., *New developments in digital pathology: from telepathology to virtual pathology laboratory.* Stud Health Technol Inform, 2004. **105**: p. 61-9.

49. Toegel, S., et al., *Glycophenotyping of osteoarthritic cartilage and chondrocytes by RT-qPCR, mass spectrometry, histochemistry with plant/human lectins and lectin localization with a glycoprotein.* Arthritis Res Ther. **15**(5): p. R147.

50. Toegel, S., et al., *Lectin binding patterns reflect the phenotypic status of in vitro chondrocyte models.* In Vitro Cell Dev Biol Anim, 2009. **45**(7): p. 351-60.

51. André S, K.H., Kayser K, Murphy PV, Gabius HJ., *Merging carbohydrate chemistry with lectin histochemistry to study inhibition of lectin binding by glycoclusters in the natural tissue context.* Histochem Cell Biol., 2016. **145**(2): p. 185-99.

52. Corredor, G., E. Romero, and M. Iregui, *An adaptable navigation strategy for Virtual Microscopy from mobile platforms.* J Biomed Inform.

53. Khalbuss, W.E., J. Cuda, and I.C. Cucoranu, *Screening and dotting virtual slides: A new challenge for cytotechnologists.* Cytojournal. **10**: p. 22.

54. Virchow, R., *Cellular Pathologie.* Virchows. Arch. Path. Anat., 1855. **8**: p. 3-39.

Klaus Kayser et al, diagnostic pathology 2016, 2:106
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-2:106

55. Kayser, G., et al., *Poor outcome in primary non-small cell lung cancers is predicted by transketolase TKTL1 expression.* Pathology. **43**(7): p. 719-24.

56. Werle, B., et al., *Cathepsin B in infiltrated lymph nodes is of prognostic significance for patients with nonsmall cell lung carcinoma.* Cancer, 2000. **89**(11): p. 2282-91.

57. HJ., G., *The magic of the sugar code.* Trends Biochem Sci., 2015. **40**(7): p. 341.

58. Kaltner H, S.T., Manning JC, Raschta AS, André S, Sinowatz F, Gabius HJ., *Network monitoring of adhesion/growth-regulatory galectins: localization of the five canonical chicken proteins in embryonic and maturing bone and cartilage and their introduction as histochemical tools.* Anat Rec (Hoboken). 2015. **298**(12): p. 2051-70.

59. Badea P, P.A., Moldovan L, Zarnescu O., *Structural heterogenity of intraluminal content of the prostate: a histochemical and ultrastructural study.* Microsc Microanal., 2015. **21**(2): p. 368-76.