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The concept of entropy in histopathological diagnosis and targeted therapy

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Abstract

Background: Targeted therapy has been developed to apply individual patient – specific drug regimes that are based upon specific bio-chemical intra-cellular pathways and usually related to cellular proliferation or apoptosis.

Entropy Definition and Concept: Entropy is considered to be a physical entity similar to space and time. It quantifies the distribution of events and transport mechanisms in relation to the macrosystem for thermodynamic, chemical and biological processes which are analyzed by statistics, communication and information methods. It amounts the distance of a gross (macro) system from its final stage in heat theory; in biology the entropy flow measures its dynamics (distance from its environment) through the surface of an open macrosystem, for example of a society, an individual, an organ, a gene, cell, and others. A derivative of entropy is the so-called structural entropy that measures the distance of internal (enclosed) microstates in the macrosystem. It can be computed by quantification of internal (vascular) and external (outer) surfaces of solid tumors, and their proliferation.

Entropy and Targeted Therapy: The most frequently investigated pathways include onco and suppressor genes that display pathways with cellular surface receptors such as the epidermal growth factor receptor (EGFR), and others. The presence and signaling intensity of EFGR in a set of malignant tumors can be considered as microstate in a large compartment of the whole cancer (macrosystem). Thus entropy and structural entropy can measure and classify the internal dynamic structure of the investigated cancer and can be associated to the therapy response and patient's survival.

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Results: The concept of entropy and structural entropy has been tested and applied on several cancer cell types including primary lung cancer and pulmonary metastases of colon cancer. The patients' survival rates were closely associated with the corresponding entropies and entropy flows. These tests have been based upon visualization of proliferation (Ki-67). The extension of this entropy concept to be applied in targeted therapy of breast and lung cancer is under investigation.

Conclusion: The concept of conventional and structural entropy is a promising tool in search for understanding biological structures and their related functions, for improved analysis of microstate dynamics in malignant tumors, and can serve for refinement of targeted therapy.

Keywords: Targeted therapy, entropy, entropy flow, liquid biopsy, EGFR.

Introduction

Targeted or individualized therapy is consistent with predictive diagnosis. It includes a series of diagnostic steps that finally define the most appropriate therapy [1-4]. There are several prepositions prior to applying this diagnosis concept. These include the presence / detection of macromolecules that are involved in communication and release of disease specific cellular actions, the participation of the cellular environment, the extension and localization of altered tissues, and finally effects on distant tissues (side effects and alteration of still normal functions) [5-7].

Targeted therapy is mainly applied for patients with malignant diseases such as breast carcinomas, lung carcinomas, or lymphomas [5-7]. In addition, chronic diseases such as diabetes or multiple sclerosis are under investigation [8-12]. Applied for malignant tumors, it commonly combines cytostatic drug regimes with immunotherapy that is based upon patient - specific receptor expressions and their potential blockade [6, 7]. Frequently, malignant tumors release short DNA sequences that can be detected in the peripheral blood and are characteristic for the response / non response of the analyzed lesion [13-19]. These DNA fractions correspond to the exhibition of receptors and binding capacities of the cellular membranes which, for example, regulate functions of the whole cell in terms of proliferation or apoptosis [13-19]. The analysis

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of these DNA fractions is called liquid biopsies. Some of them are already implemented for detection of tumor relapse in colon or breast carcinoma [13-19].

Pathologists should take attention and learn from this new technology:

a) There is no essential association between the common (microscopic) cell type and those tumors that respond or do not respond to the therapy regimes that are recommended by liquid biopsies.

b) In the future, microscopic examination of the lesion (tumor) might not be a prerequisite in order to perform targeted therapy, especially in patients whose tumor localization and extent are known by live imaging (CT, NMR, etc.) and whose biological tumor behavior and response to potential drug regimes can be derived from liquid biopsy data [20-23].

Thus, pathologists have to think about the real biological importance of morphology, and its basic properties.

In this article we want to analyze the significance of biological structures, and their impact on abnormal functions such as uncontrolled cellular growth or apoptosis. In addition, we want to explain why biological significance of characteristic morphology structures such as squamous carcinomas, solid tumors, etc. does not necessarily predict the outcome of therapeutic (functional) interactions. Furthermore, why can the disturbance of a small part of all body functions which do not affect the main stream of functions such as blood supply or breath destroy all functions (life) of the patient?

To start with: the aim of any therapy is to re-adjust an abnormal function to its original or healthy stage. Therefore, an analysis of definition and interaction between function and structure is the first step to investigate in the above questions.

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Definition and interaction of structure and function

The Oxford English Dictionary (Online ed.). Retrieved 1 October 2015 defines structure an arrangement and organization of interrelated elements in a material object or system, or the object or system so organized [24]. Structures can be arranged at different levels of organization, ranging hierarchically from a lower level, for example cells, to a next higher stage that is built by spatial symmetry of multiple copies of lower level structures (for example gland, vessel, nerve, etc.) [25-29]. This arrangement is called order of structures, and plays a major role in answering the above mentioned questions [25-32].

In image analysis, the basic structure is commonly called object, for example nucleus, membrane, cell, etc. In molecular biology, basic structures include macromolecules that can be arranged at different hierarchic ordered levels. These levels can be analyzed in either a “step-down” procedure, or “step-up” procedure. For example, proteins posses a four level hierarchy (in a step-down procedure), in contrast to microscopic structures that usually start at cellular level and compose different objects to the next higher level (step-up procedure) [32-35].

The mathematical definition of a function is the relationship of a set of inputs and a set of permissible outputs with the property that each input is related to exactly one output *[36].*

An individual input might yield a corresponding individual output, or several outputs. In addition, several inputs might create the same output. For example, f(x)=y, and $y=x^2$ is a function that gives the same output for different inputs. Both the input variables x=2 and x=-2 will result in the output variable y=4. The function f(y)=inverse(y) operates with the reverse property. Both the output variables y=-2 and y=2 will result from the input variable y=4.

Biological structures are subject to be altered by external forces. These alterations include shape, size, division, unification, and movement. The aim of this article is to

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evaluate characteristics of microscopic diagnosis. Therefore, it is useful to restrict our considerations to visual elements only.

Human perception of visual information is limited comparable to its light recognition potency. Light waves below and above certain boundaries cannot be seen as well as those below and above certain intensity.

Human visual perception distinguishes between objects and background. Objects are elements that can be identified and isolated from the background. These objects are usually classified by characteristic properties, for example into epithelial cells, lymphocytes, cancer cells, etc. A reproducible classification requires object features, and, in addition, recognition of higher order structures, which might be present (glands, vessels, etc.) or missing (undifferentiated cancer, ulcerous inflammation, etc.).

Functions can be derived from the spatial arrangement of structures (elements), for example the movement of epithelial cells from the basic layer to the skin surface. Therefore, structural characteristics are associated with their function in principle. One has to know the properties of individual structures (elements) together with those of additional within appropriate time and space limitations “interacting” structures (elements) [30]. In other words, to derive functions from a structure requires “neighborhood” and a metric space. Both are mandatory to define a neighborhood condition which is needed to analyze “interaction” [30].

In histopathology, Voronoi’s neighborhood condition is the most frequently applied algorithm when analyzing structural properties at the 2nd and higher order level (agglutination or clusters of cells, cellular symmetries, diffusion analysis, etc.) [30, 31, 37, 38]. An example is demonstrated in <figure 1>.

When structures and function do interact that closely, are they really different or are they of the same nature in principle?

The detection of a structure (element at basic hierarchic level) may last for a certain time period ***To***. Those elements of the acquired image are called structures that do not

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change in their individual properties within the period ***To*** with the exception of neighborhood (movement).

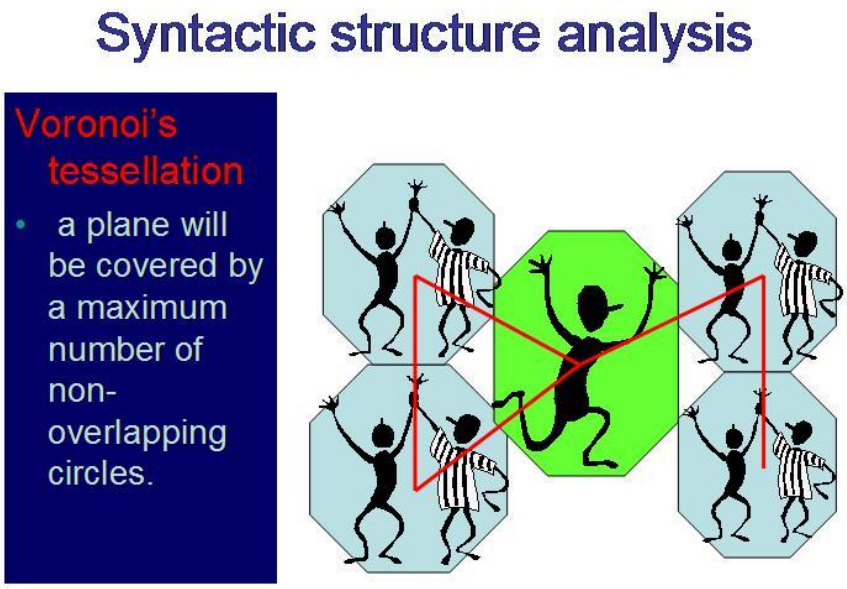

Figure 1 : Example of Voronoi's neighborhood condition.

Functions will be recognized by changes of element properties in relation to the metric space (background), and / or to their neighboring elements (cellular interactions). These functions cannot be seen if the observation period is too short compared to the "velocity" of changes, and all elements remain "frozen" like in a histological glass slide. An observation period that lasts sufficient long to notice structural changes permits the recognition of functions which are modifications of the elements related to the background (shape, size, etc.) or to neighboring elements. They can be analyzed, grouped and classified in a similar manner as structures. Therefore, the analysis of functions of visual elements

a) depends upon the observation period, and

b) is equivalent to interactions between at least two structures of equal or different orders.

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Being the same in principle, but localized in different "backgrounds", the distinction between biological structures and functions is somewhat arbitrary and mainly of practical importance. Any function mandatory requires structures in a hierarchic order, and occurs in relation to the observation period.

In biology, numerous hierarchic structure levels form a set of hierarchic functions which interact between the different structural levels. Therefore, a function without a structure has never been observed in reality, and structures without any function are only seen if the observation period is short when compared to the velocity of structural changes. In other words, life possesses both function and structure. A corpse is a structure without a function. A function without structure has only been mentioned in sayings (see <figure 2>). A more detailed analysis of the situation results in the statements:

a) Structures will appear in any system that possesses one (or several) continuous energy fields which are limited in space within a lower and upper boundary.

b) They can only be directly noticed, if the observation time is long enough.

c) They will create their own boundaries and organize themselves in a hierarchic manner.

d) They posses different entropy compared to their surroundings.

e) They require reversible energy for maintaining themselves.

f) Inner (lower level) structures will decay prior to those at higher levels [30, 31, 37, 38].

Structural entropy

How now to analyze the functions or the relationships between different structural levels? The first step should be to declare the aim or the reason why the analysis should be performed. One aim could be to understand which functions and how do

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they act, followed by the approach to interact with and neutralize destructive functions, i.e., those that destroy equilibrium or involved and non-involved structures. This aim focuses on substances (elements) that trigger or carry out functions, i.e. macromolecules, agglutinations of macromolecules, electro-magnetic fields, virus, genes, etc.

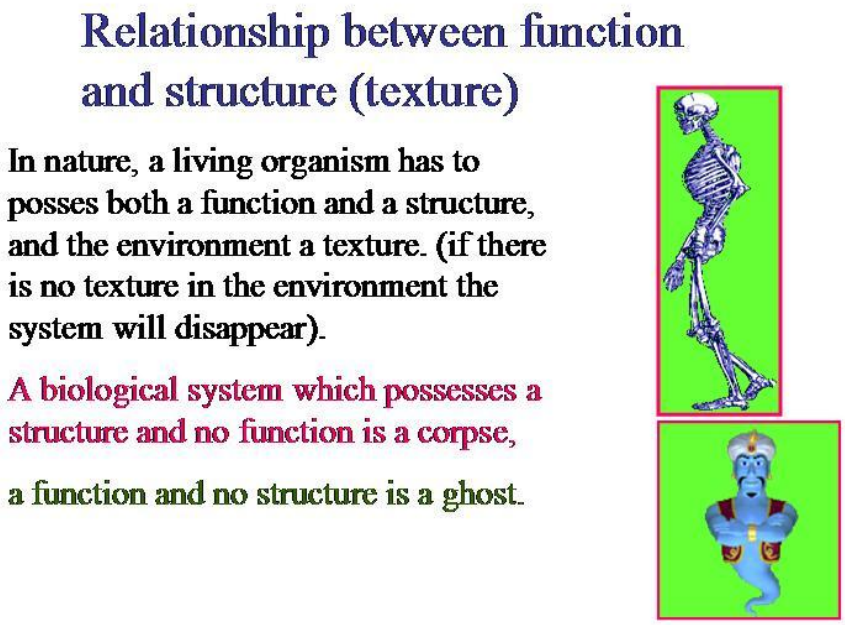

Figure 2 – Relationship between function and structure.

Another approach focuses on investigation and understanding of communication between structures of different element levels [30-33, 39]. In this model functions can be interpreted as mapping of real structures on a virtual space [34, 40]. Such investigations have been successfully applied in understanding, forecasting the future development and construction of new elements in physics, chemistry, biochemistry, genetics, and other natural sciences [34, 40]. It also investigates in principles of self organization and structured chaos [41-50]

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The basic entity for description and construction of heat and energy characteristics is the so-called entropy which is a measure to describe the internal energy stages' nature and development of thermodynamically closed systems [51-54] Shannon applied the thermodynamic laws to information and communication, especially acoustics [55].

Shannon's entropy and its derived entropy flow are useful laws in acoustics and statistics. One assumes a system that is composed of numerous elements at the same hierarchic level and computes their distribution and development [30, 32, 37, 40]. The investigation can be extended to clusters of elements (so-called macrostages) and to different (non symmetry) mathematical models such as Tsallos entropy [56, 57].

The concept of structural entropy which is also called MST (minimum spanning tree) entropy has been specifically developed to work on image analysis algorithms, and to measure heterogeneities of second and higher order structures [28, 30, 31].

The principle algorithm starts with the definition or identification of objects (elements) that might be cells, nuclei, image primitives, genes, vessels or even individual pixels.

The 2nd step is to define and apply an appropriate neighborhood condition. Most frequently in use is Voronoi's / Delaunay's tessellation [58, 59]. It takes the image elements for vertices and calculates connecting edges between the vertices according to the maximum of non overlapping disks which are drawn around the vertices. A different less frequently used neighborhood condition has been published by O`Callaghan who introduced a distant and "shadow" condition of potential neighbors [60].

The 3rd step applies graph theory methods, for example the construction of the minimum spanning tree or trees with different properties, for example weighted, closed, or directed graphs [61]. The vertices associated weights can be defined differently, for example can correspond to staining intensities, cellular shape, nuclear size, binging capacities, and others. In addition edge associated weights can be introduced too, such as length of connected boundaries, staining intensity, shape and

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length of the connecting edge which might be calculated by its minimum gray value difference [61].

The basic formula of this approach can be written:

$E(MST) = 1/N * \Sigma [p^*(mst)* \ln[p^*(mst)]$ (1)

E(MST) = structural entropy, **N** = number of elements, **$p(mst) = SV_{ik} * SE_{ij}$**

SV_{ik} = set of parameters associated with the individual neighboring vertices **i, k**, i.e.

$SV_{ik} = [(N-1) * \delta (v_i - v_k) / \Sigma (\delta (v_i - v_k)]^{*2}$

$SE_{ij} = [(N-1) * \delta (e_{ij}) / \Sigma (\delta (e_{ij})]^{*2}$

$\delta (v_i - v_k)$, $\delta (e_{ij})$ = distance of weights between nearest vertices **v_i, v_j,** or connecting edges **e_{ij}**.

An example of calculated structural entropy values is shown in <figure 3>. Several investigations report that the calculated MST entropies significantly correlate with the survival of lung cancer or breast cancer patients, of embryo stages in the development of human lungs and chicken kidneys as well as occurrence of intrapulmonary metastases and the patients' survival [62-65]. An example of the development of fetal human is depicted in <figure 4> [66].

The derivative of entropy calculations for non-closed or open systems is the entropy flow [67]. This approach is even more interesting as biological systems are thermodynamically open.

Approaches how to calculate the MST entropy flow consider a system that creates entropy in its inner space and removes entropy through its surface by an "entropy machine", [65, 68-70]. Such a situation can be formulated [30, 31, 65, 68-70]:

$E(1Ti) = E(1,T0) + dE(12,dT)$. **(2)**

E(1,Ti) is the energy of system **1** at time **Ti**,

E(1,T0) is the energy of system **1** at time **T0**

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dE(12,dT) is the difference of energy passing from system **2** to system **1** within the time **dT.**

The difference of entropy passing from system **E1** to system **E2** is equivalent to the produced heat and the surface of the system under consideration.

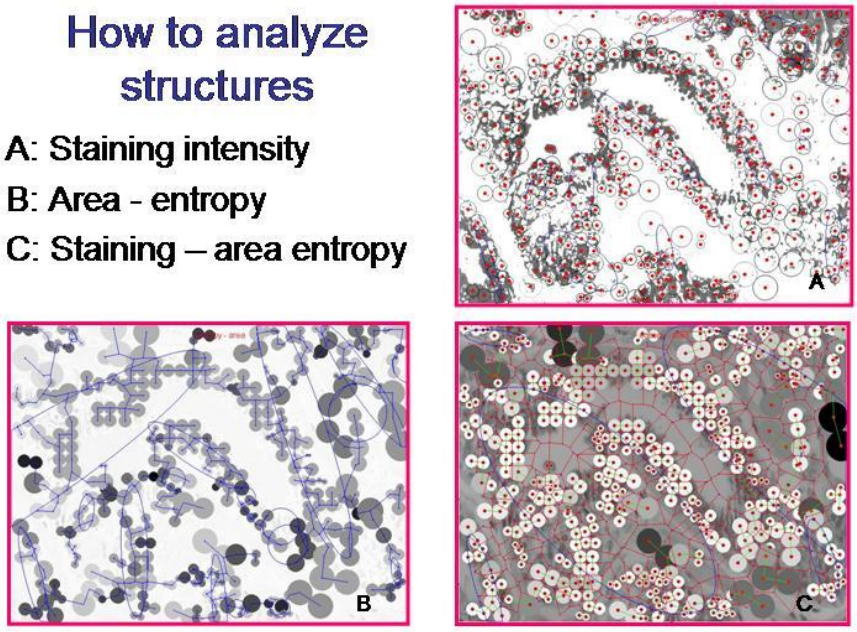

Figure 3: Application of MST entropy derived from different gray value thresholds.

Solid malignancies are an appropriate model to calculate entropy and entropy flows [30, 31, 65, 68-72]. A close relationship between the patients' survival and amount of entropy flow has been reported by [64, 65, 73, 74]. The authors estimated the outer surface of the malignancies by CT measure of the tumor mass or by serial cuts of the resection specimens. The inner surface or vascular density was added. The heat production was set into relation of the density of proliferating (MIB positive) tumor cells [30, 31].

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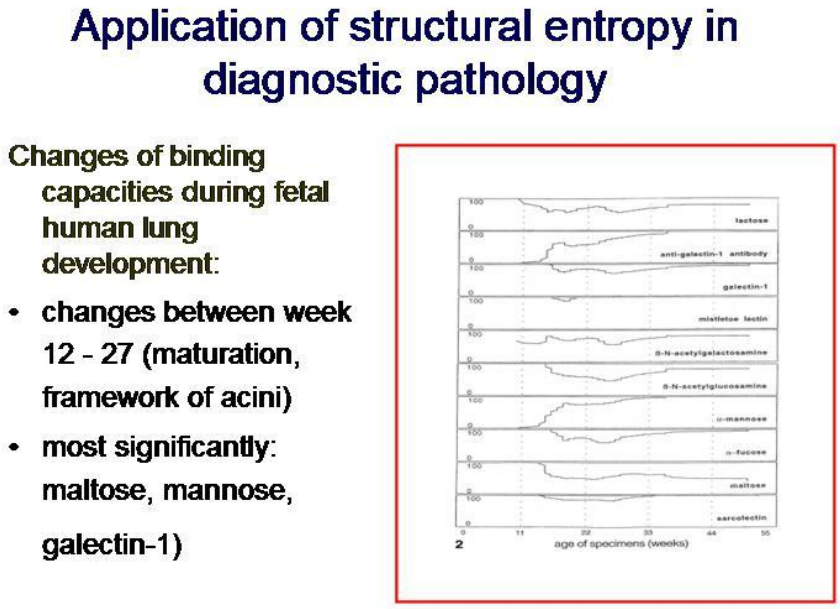

Figure 4: Fetal human development.

A second approach to evaluate MST entropy flow divides the tissue space into neighboring segments and measures each segment's entropy. The obtained differences obviously correspond to an entropy flow from one section into its neighbors. Such systems try to minimize the entropy flow in accordance to the basic laws of thermodynamics [67]. High entropy flows indicate stable structures in contrast to those with minimum flows which are diminishing in their environment (background) [42, 67, 75]. They seem to be appropriate tools to open new insights in the clinical significance of intra-cellular pathways, for example epidermal growth factor receptor (EGFR) and involved onco / receptor genes in carcinomas of various organs [76, 77].

Structural entropy and gray value thresholds

Pixels can fulfill all properties of elements for statistical analysis, in whole agreement with objects [76, 77]. They form the minimum of a measurable area in a digitized image. Their spatial gray value distribution is called texture, and should be

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distinguished from objects and object associated structures because they require external knowledge and definition [29].

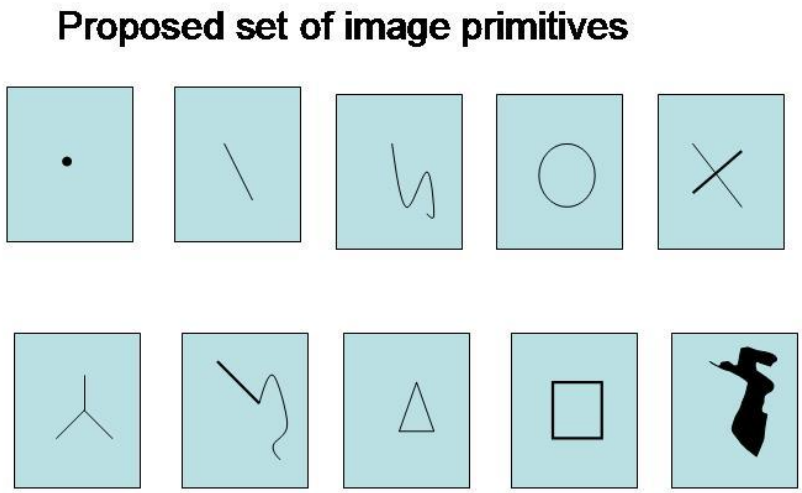

Figure 5: Image primitives.

Let us segment a complete digitized image from its maximum value to its minimum gray value the step by step by the gray value **gt**. All pixels that fulfill the gray value condition

p(g) ≤ gt are assigned to the background, and all pixel fulfilling the condition **p(g) > gt** are considered image elements. The obtained elements form a statistical population which can be measured for MST entropy, entropy flow, Shannon's entropy etc. [29]. In addition, so – called image primitives can be derived as exemplarily demonstrated in <figure 5>, which again can be considered elements of a (different) statistical population and undergo the same calculations.

The result will be a gray – value associated graph that indicated at which gray value maximum and minimum entropies are apparent <figure 6>. These data indicate the

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significance (stability) of the analyzed structures if the gray value intensity is associated with specific oncogene visualization techniques (antibodies).

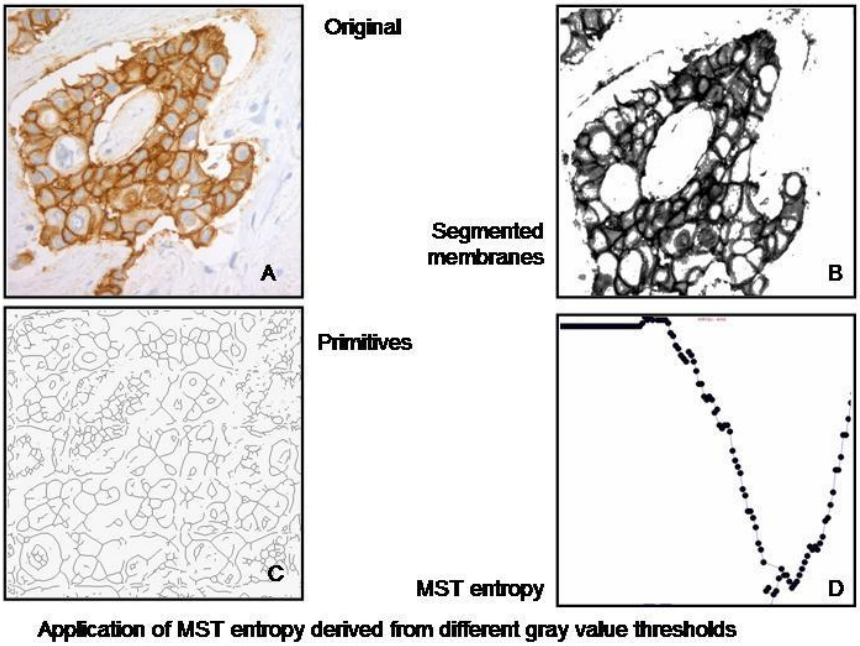

Figure 6: Gray value intensity evaluation.

A different approach includes the introduction of micro- and macrostages which can be defined as follows: A microstage is the scored staining intensity ***I*** of HER-2-New investigated membrane (0, 1, 2, 3) and the number ***N*** of connecting membranes (1, 2, 3, 4). The macrostages consist of ***{I * N}.*** Shannon's and MST entropies can be computed and associated with the total score. This procedure is easy to apply and can serve for automated and reproducible clinical application. An example of immunohistochemistry (IHC) visualization technique is given in <figure 7>, and that of corresponding fluorescent in situ hybridization (FISH) in <figure 8>. The obtained results differ slightly according to the different investigations; however, both indicate a successful HER2-NEW related treatment.

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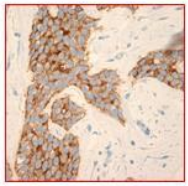
Results: IHC

Microstages: Σ tumor cells: 320
Macrostages: 16; Intensity stain & neighbors
M (0+) = 22, M(1+)=66, M(2+)=214, M(3+)=38
Entropy total : 2.25; maximum: 2.72

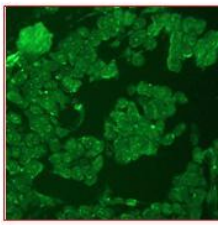


Neighbor	N	none	1	2	3	Entropy
M	0+	6 0.07	16 0.15	0	0	0.22
M	1+	14 0.14	20 0.17	12 0.12	20 0.17	0.61
M	2+	6 0.07	50 0.29	90 0.36	68 0.33	1.05
M	3+	12 0.12	20 0.17	6 0.07	0	0.37
Entropy	2.25	0.41	0. 78	0.55	0.50	2.25

Figure 7: Example of immunohistochemistry (IHC) visualization technique.

Results: FISH

Microstages: Σ tumor cells: 120
Macrostages: 16 (No Fish & neighbors)
N(0+) = 8, N(1+)=16, N(2+)=46, N(3+)=50
Entropy total: 2.26, maximum 2.77



Neighbor	N	none	1	2	3	Entropy
FISH	0	4 0.11	4 0.11	0	0	0.22
FISH	1	3 0.09	4 0.11	9 0.19	0	0.39
FISH	2	2 0.07	6 0.15	18 0.28	20 0.29	0.79
FISH	3	4 0.11	20 0.29	18 0.28	8 0.18	0.86
Entropy	2.26	0.38	0.66	0.75	0.47	2.26

Figure 8: Example of fluorescent in situ hybridization (FISH) visualization technique.

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Discussion

Research of cancer cell properties and cancer cell interaction with normal cells and serum of the patient indicates that small aberrations of these properties induce significant influences on the fate of patients, such as recovery, survival or death [13, 16, 17, 21]. At the first glance it seems to be a miracle that such a minimum aberrations in small cellular compartment of the body without any disturbance of mandatory energy supply (vascular system) is able to determine the patient's life.

None of the basic vital functions seems to be involved which could be responsible for the patient's fatal outcome. Obviously we have to neglect all theories that are founded on quantitative measures because space, location, interaction with vital functions, and intensity of the fatal cause are minimal.

One possible answer is the described concept of hierarchically ordered structures which is exemplarily demonstrated in this short excurse. Consistency of structures and the needed free energy are calculated in terms of (MST) entropy. Structures of the same level are usually multiple identical copies. They are arranged in clusters which separate elements with different properties from each other. These clusters build elements of the next level, which again form elements of the next level, and so on.

Frequently, these clusters break down in accordance with noise, external influences, or internal damage. A breakdown of these clusters can usually be repaired by different clusters of the same level in terms of self organization, i.e., under the same conditions, when they have been born originally. The whole system remains stable.

If the break down cannot be repaired neighboring clusters will break and destroy the elements of the next level. The action induces a life threatening snowball effect, or a feed forward mechanism that cannot be kept under control, even if all elements that are not "neighbors" of the breaking clusters will remain untouched. In other word the macro system (body) cannot function any more although most of the other internal systems remain untouched and can survive for a certain time.

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This short excuse demonstrates the "power" of structures and derived parameters, such as entropy.

Entropy is a basic descriptor of nature next to energy, space and time [41-43, 57, 78]. It is a tool for image interpretation and to "extract" image content information [29, 40, 76]. The addition of external information can create algorithms that can be applied to forecast the development of closed and open visualized biological systems under various conditions [40, 69].

In tissue – based diagnosis its application is promising for

a) automated scoring of evaluated intra- and extra – cellular pathways

b) forecasting the stability of the system (prognosis)

c) creation of a general theory of structures in relation to the associated functions.

The "tool" entropy can be applied in different or interdisciplinary problems [56]. For example, Bridgewater et al have demonstrated that traffic problems in the internet can be successfully analyzed by entropy approaches [79]. It can also be extended to a general theory from the mathematical and physical point of view [57].

When analyzing structures and their association to the period of observation the MST entropy might be a useful term to describe functions and their interaction with associated structures, i.e. how significant functions can be distinguished from non important ones if only the associated structures can be analyzed. This question still remains one of the important issues of tissue – based diagnosis, despite all biochemical, genetic, or other subcellular investigations.

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