Pathology to Enhance Precision Medicine in Oncology: Lessons From Landscape Ecology

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Abstract: A major goal of modern medicine is increasing patient specificity so that the right treatment is administered to the right patient at the right time with the right dose. While current cancer studies have largely focused on identification of genetic or epigenetic properties of tumor cells, emerging evidence has clearly demonstrated substantial genetic heterogeneity between tumors in the same patient and within subclones of a single tumor. Thus, molecular analysis from populations of cells (either a whole tumor or small biopsy of that tumor) is, at best, an incomplete representation of the underlying biology. These observations indicate a significant need to define intratumoral evolutionary dynamics that yield the observed spatial variations in cellular properties. It is generally accepted that genetic heterogeneity among cancer cells is a manifestation of intratumoral evolution, and this is typically viewed as a consequence of random mutations generated by genomic instability within the cancer cells. We suggest that this represents an incomplete view of Darwinian dynamics, which typically are governed by phenotypic variations in response to spatial and temporal heterogeneity in environmental selection forces. We propose that pathologic feature analysis can provide precise information regarding regional variations in environmental selection forces and phenotypic adaptations. These observations can be integrated using quantitative, spatially explicit methods developed in landscape ecology to interrogate heterogenous biological processes in tumors within individual patients. The ability to investigate tumor heterogeneity has been shown to inform physicians regarding critical aspects of cancer progression including invasion, metastasis, drug resistance, and disease relapse.

Key Words: landscape, pathology, ecology, personalized medicine, precision medicine

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Personalized medicine aims to use patient-specific metrics to provide an optimal cancer therapy customized for each individual patient.[1–3] Massive biobanks of patient tissues provide extensive libraries of genetic data that can be evaluated against targeted therapies.[4] However, it is becoming clear that discriminating and cataloging genomic libraries of patient samples falls short, due in part to intratumoral heterogeneity. Personalized cancer treatments will require more than just matching a patient's tumor genomics with that of a central library.

Detailed molecular data from multiple regions in the same tumor reveal striking variations. Distinct populations of tumor cells displaying different biomarkers and gene signatures appear to coexist.[5] This invites a greater understanding of tumor heterogeneity at molecular, cellular, and tissue temporal and spatial scales.[6,7] Unfortunately, current proteomic and genomic methods fail to wholly address heterogeneity. Current techniques rely on single sample that homogenizes into large numbers of undoubtedly variable cells. It is likely that even these "averaged" data will differ from region to region within the same tumor, and certainly between tumor sites in the same patient.[8–10] Batching and averaging information from millions of cells is likely limiting for developing personalized cancer treatments.

We propose extending pathology to identify, classify, and quantify cell to cell, region to region, and tumor to tumor heterogeneities. Such pathology metrics can supplement current efforts toward personalized medicine. We propose analyzing histologic samples by using the theories, tools, and experiences of landscape ecology.

Landscape ecology measures, analyzes, and studies the spatial and temporal heterogeneities of natural ecosystems.[11] Since the pioneering work of Carl Troll in 1939, landscape ecologists have used maps, vegetation, and geologic surveys, photographic images and, most recently, satellite imaging to study the interactions between organisms with their environments. While maps are not the only tools of landscape ecology, these data acquisition methods empower investigators to study spatially explicit biological interactions. Together with information about organisms and the patterns of the organism's environment, investigators can interrogate habitat change, conservation, and other ecological interactions. We propose that many of these same principles and techniques can be developed and applied to create an emerging field of "landscape pathology." While there are many definitions of "landscape," we are using the definition of landscape from Turner,[12] which states that "a landscape is an area that is spatially heterogeneous in at least one factor of interest." Thus, we define landscape pathology as a proposed discipline to apply quantitative, spatially explicit methods from landscape ecology to define the heterogenous biological processes of cancer cells (the "organism") in histologic samples (the "habitat"). Using landscape pathology methods can help investigators gain a more precise understanding of local selection forces and, in turn, adaptations within

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subpopulations of cancer cells in a tumor, which may be clinically important in understanding disease progression, treatment response, and relapse.

Figures 1A and B provide an example of this concept. Here, hematoxylin and eosin–stained histology slides, the standard for primary diagnoses in cancer, may be compared with traditional landscapes commonly viewed as maps or satellite imagery. Regional classifications, spatially explicit analyses, and quantifiable metrics common in ecology can add to the pathologist's clinical toolbox.[13,14] These classifications and analyses will provide pathologists with the ability to quantify morphologic heterogeneity within a tissue section with exquisite precision. This in turn will empower pathologists with the ability to rapidly identify areas of necrosis, high rates of proliferation, high incidences of inflammatory response, regions of high or low nuclear pleomorphism, and similar clinically pertinent morphologic features.

THE CLINICAL PROBLEM

Intratumoral heterogeneity manifests in at least 3 general ways:

(1) *Mixtures of normal and malignant cellular populations within tissue*. Pathologists typically describe this variation in qualitative terms. The pathologists will, for example, recognize benign cells such as fibroblasts, lymphocytic, and epithelial cells. They will also identify inflammation, necrosis, hyperplasia, preneoplastic disease, benign tumors, and malignant cancers.[15]
(2) *Variations in the microenvironment*. Blood flow in tumors typically results in temporal and spatial variations in concentrations of growth factors, substrate, and metabolite concentrations. These in turn manifest as regions of necrosis and variable cell density. Each of these variations selects for the adaptive evolution of local tumor populations which can be spatially evaluated in a quantitative manner including, but not limited to spatial evaluation of clustering populations or proliferative phenotypes.[16]
(3) *Genetic heterogeneity*. Increased mutation rate owing to intracellular properties such as DNA repair or genotoxic environmental factors such as hypoxia appear to continuously generate new mutant cells. Cells carrying these mutations, in turn, proliferate if the phenotypic expression of that mutation confers an increased fitness. An example is the perpetuation of the mutator phenotype.[17]

In current practice, pathologists attempt to overcome this heterogeneity by selecting regions of tissues for genetic analysis that minimizes normal or necrotic cells. This is commonly performed using a slide marker to draw directly on the glass slide in an effort to select regions of high tumor cellularity or conversely, to scratch out large regions that are not of interest including normal margins or necrosis. In this way the technique currently "homogenizes" the samples and averages any downstream information that may be useful in evaluating intratumoral heterogeneity.[17]

We propose that current methods for personalized cancer therapy—treating target lesions as a single heterogenous genetic sample—are not wholly adequate. This is primarily because evolutionary strategies or adaptations often involve several phenotypic changes that in turn can be achieved through an even larger number of genetic pathways. That is, evolution directly acts on cellular phenotypes and not genotypes. In fact, Darwinian dynamics, which can be described as dynamics of systems which drive fitness by natural selection, are manifested in phenotypic changes.[18] Second, clear evidence indicates that extensive genetic heterogeneity exists within cancer cells in the same tumor.[5] Averaging or lumping tumor heterogeneity into single metrics or qualities may mask key aspects of the tumor's progression and state, and unwittingly "throw away" valuable information on the heterogeneity itself and what it indicates.[19] We suggest embracing the information content of spatially explicit considerations of cellular, microenvironmental, and regional heterogeneities. The ability to investigate tumor heterogeneity has been shown to inform physicians regarding critical aspects of cancer progression including invasion, metastasis, drug resistance, and disease relapse.[20–23]

It is important to understand why intratumoral heterogeneity is clinically important. Clinicians have deep experience with disease relapse, changes in treatment effectiveness, and hormone status or other clinically relevant deviations in the greater cancer cell population. We propose that the described changes in the patient's overall disease state are due in significant part to cancer cell populations changing as different subpopulations of cancer cells evolve toward increased fitness by natural selection. Intratumoral heterogenous subpopulations of different cellular phenotypes in a single tumor make these Darwinian dynamics possible, which in turn makes treating cancer a moving target.

Cancers are complex but not hopelessly so. Tumors can be understood by characterizing and embracing their underlying ecological and evolutionary dynamics, including both spatial and temporal variability. Fortunately, pathologists have been observing and quantifying variations within the morphology of cancer cells for well over a century and are well positioned to visually evaluate heterogeneity across histologic samples.[24] We propose that the challenges are 3-fold: identification of cellular heterogeneity must become (1) regionally explicit, (2) quantitative and reproducible, and (3) high throughput.

Until recently, these challenges would be insurmountable due to the time consuming and subjective manual screening of tumor heterogeneity.[25] However, with the advent of whole-slide imaging technology and recent improvements in pattern recognition software, it is possible to computationally evaluate millions of cells in minutes and hundreds of patients in hours to days.[26] Whole-slide imaging allows high resolution and high throughput image acquisition for every cell within a given tissue sample.[27] There are a number of advantages of these technologies including low costs, high throughputs, quantitative results, and rapid evaluation of tissue samples, which are already routinely produced in every hospital within the United States and the majority of similar centers around the world.[13]

While current imaging technology and methods are necessary, it is not sufficient to visually investigate spatial distribution of cells and microenvironmental properties in patient samples. We propose that the discipline of landscape ecology, with its associated tools and theories, can be used to evaluate the relationship between pattern and process in pathology. While pathology is equipped to identify patterns in tumors, landscape ecology provides the tools to evaluate these patterns and understand the underlying biological relationships.[28–30] Together, quantifiable metrics of digital pathology and landscape ecology can contribute to personalized medicine. Thus,

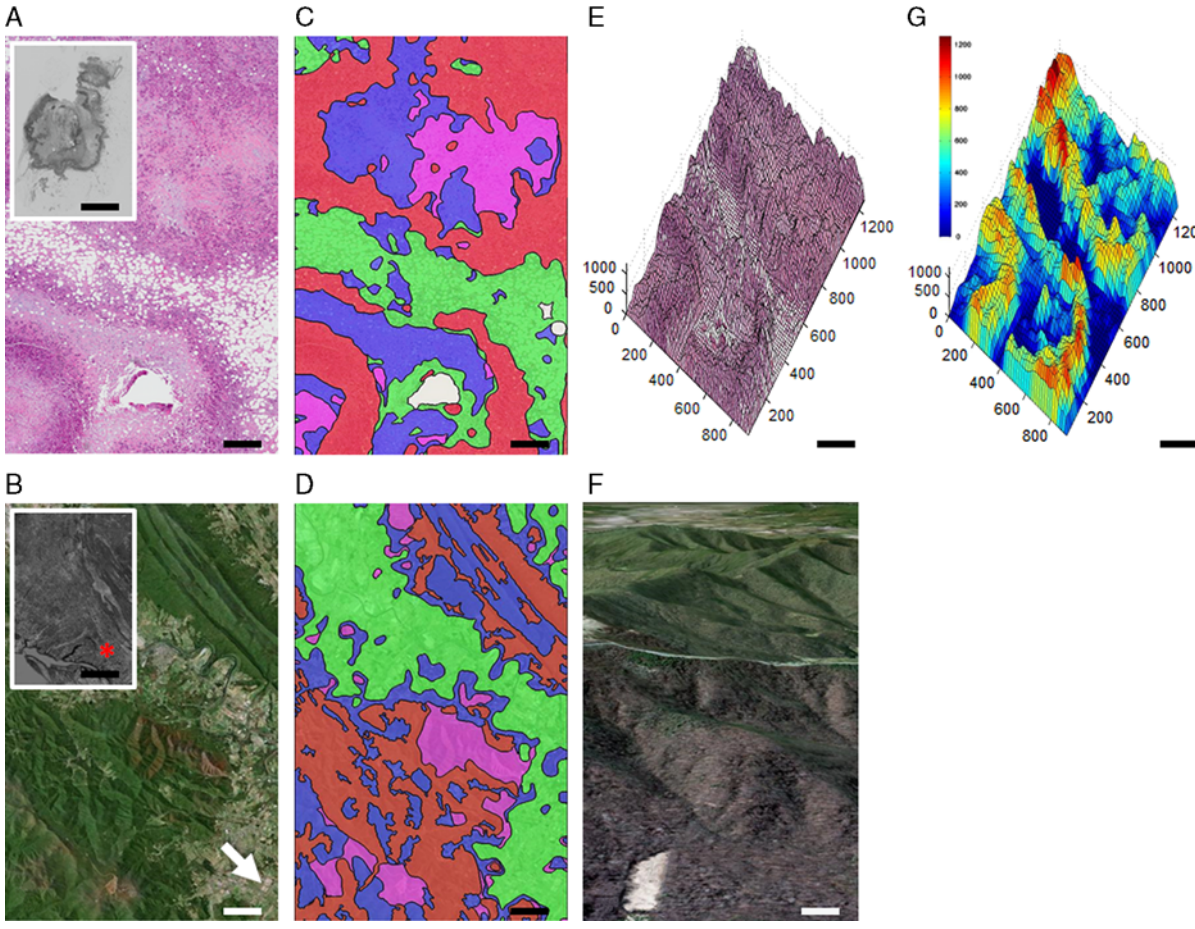

FIGURE 1. Hematoxylin and eosin (H&E)-stained histology slides may be compared with traditional landscapes. (A) A standard histologic H&E image of an invasive tumor compared with (B) a standard satellite image of the Blue Ridge Mountain range in Shenandoah, Virginia. (C) Regional tissue classification of viable tumor (red), mild necrosis (purple), necrosis (blue), and other nontarget tissue (green); (D) compared with random forest classification in variant habitats which seems to correlate most with increasing elevation from green to blue to red to purple. (E) Feature data are displayed in the *Z* dimension to create a topographical map of the H&E relative to the morphologic feature being evaluated (red: blue color layer ratio); (F) 3D topographic representation of the Shenandoah habitat site demonstrating heterogenous elevation. (G) Topographical heatmap is used to rapidly identify regions with like feature characteristics for this H&E image. Top scale bars (A, C, E) represent 300 μm and the top insert map scale bar is 3 mm; bottom scale bars (B, D, F) represent 3000 m and the bottom insert scale bar is 100 km. Credit Google Earth for all satellite imagery. The white arrow indicates North. The red asterisk is the location of Washington, DC.

"landscape pathology" has the potential to provide new information regarding patients' intratumoral heterogeneity.

BENEFITS OF LANDSCAPE ECOLOGY IN PATHOLOGY

Landscape ecologists and pathologists work on a spectrum of scales which spans from 10^{6} to 10^{-6} m, yet the substrates on which both groups work is identical. In modern studies, these are most frequently digital images, whether from a satellite or a microscope lens. Whole-slide microscope images are the maps with which landscape pathologists will work. Distinctive heterogenous regions can be identified along with their relative contribution to the total tumor volume as well as their interactions with each other. This is done by using segmentation and classification methods[14] to identify distinct regions in the tumor and to allow examination of their boundaries.

The first consideration for segmentation and classification in tumors may be the criteria with which regions will be identified. In landscape ecology, "patches of the land cover region" can be wetlands, clusters of trees, or any relatively homogenous area of interest. Habitat patches are identified in a number of different ways including reflectance data, time series of vegetation activity estimated from the reflectance data, estimates of surface roughness/vertical structure from RADAR or LiDAR sensors, or surface temperature from thermal remote sensing, to name a few.[31–33] These variables, which a landscape ecologist may use, might then be chosen to classify habitat using a number of pixel-based or object-based methods.[34,35] Pixel-based and object-based classification methods are also common in the current standards of digital pathology analysis.[36,37] In fact, pathologists now have a plethora of commercially available image analysis tools to segment objects (ie, delineate patches) and classify spatially explicit regions (ie, identify habitats) by examining vascular density, relative

cell viability, and necrosis or immunohistochemical evidence of regional oxygen concentration such as HIF-1α expression.[38]

As demonstrated in Figures 1C and D, histologic pattern recognition utilizes random forest classification to identify color and texture variation in both image types through a commercially available image analysis platform (Definiens, Munich, Germany). By teaching the algorithm to recognize homogenous regions, a computer can reliably identify "patches" in a robust and repeatable way. Individual physical or molecular features derived from the images can be used to catalog morphologically homogenous cell populations and relationships between cells or regions and establish testable hypotheses to further interrogate processes of the system.[39] Similar to the patch-matrix (also sometimes referred to as patch mosaic) paradigm in landscape ecology practices, histologic pattern recognition also creates and thus defines a border between tissue regions.[40] Of course, alternatives in landscape ecology to evaluate spatial heterogeneity such as gradient paradigms are also used.[41]

Qualitatively, pathologists could then begin to evaluate the regions of interest with these or other classification methods of patches. With quantitative image analysis and statistical methods, pathologists can quantify precise metrics of the regions.[42] Initially these metrics might include area, intensity, roundness, and other physical features.[43] However, pathology can learn from landscape ecology that patches can form mosaics.[44] They can demonstrate edge effects and they can be clustered.[45,46] These features of tissue architecture can also be quantitatively measured and used to evaluate the disease at a mesoscopic scale (200 μm to 2 mm). For example, the architecture of prostate glands and their orientation have been shown to be informative of patient prognosis[47] and retained architecture in breast cancer tissues has been shown to inhibit malignant progression.[48]

Multiple regions or patches typically exist in a landscape. We expect that similar variations will be found in most clinical cancers using the tools for landscape pathology.[49,50] In turn, this will allow the intratumoral heterogeneity to be characterized, quantified, and ultimately compared. A way in which the heterogeneity may be characterized can include habitats of vascular regions[51–53] or regions of increased lymphocytic response.[54] Each quantitative method cited includes clinically meaningful prognostic or predictive value.

LANDSCAPE ECOLOGY APPLICATIONS IN PATHOLOGY

Evaluation of the consequences of having multiple subpopulations of cells in specific patterns in a tumor will require learning from landscape ecology, which focuses on the feedback loops between patterns and processes. Patterns observed in pathology include points of blood vessels or ectopic lymph nodes, expression levels of biomarkers such as Her2Nue, or regions of high proliferation. Each of these patterns has diagnostic and prognostic value to the pathologist. Here we describe 4 analyses and their utility to pathology.

The first is point patterns, which consist of point locations distributed in 2-dimensional space. Landscape ecologists would characterize these as random, regular, or clustered. In pathology, point pattern analysis would allow quantification of the spatial distribution of some cellular or tissue feature (ie, nuclei) in the tumor. Metrics such as Ripley's K function can be used to compare one region of the tumor to another.[55,56] Specifically, Ripley's K is a statistical metric for quantifying deviations from spatial randomness and has been used in mammography as a classification method.[57] Point patterns in a histologic section have been shown to be important at the histologic scale in interrogating aspects of the environment including lymphocytic invasion, cancer-associated fibroblastic localization, or the distance from vasculature to cancer cell populations.[58,59] This information could help oncologists most carefully predict response to specific therapies.

Second, regional variations in necrosis, ectopic lymph nodes, or other intratumoral features can be described as values of the number of cells, size of necrotic regions, or distribution of lymphocytes in space. In this example, measures of spatial autocorrelation, such as Moran's I,[60,61] can reveal the scale and degree of dependency among observations. This is, for example, important to be useful to quantify dispersal (migration) of specific cell populations by evaluating the pH of the microenvironment.[62] Specifically, the location of cancer cells can be quantified to better understand if cancer cells are moving together in regions in which acid-mediated invasion provides spaces of increased selective advantage or if invasion is more correlated with random Brownian motion.

These metrics may also be useful in predicting response to specific treatments such as hypoxia-activated prodrugs or predicting prognosis of the patient's own immune response.[54] Specifically, the spatial correlation of cancer cell populations is useful in understanding the overall heterogenous organization of cancer cells. That is to say, cancer is not randomly oriented or unorganized, but rather is a function of selective Darwinian dynamics of an adaptive landscape which may be measured and investigated. Furthermore, quantification of spatial relationships among tumor cell clusters of networked populations in relation to these environmental responses can be used to reveal prognostic indicators such as increased nuclear pleomorphism in regions of increased vascularity.[63,64]

Thirdly, distance measures are frequently used tools to collect quantitative data in histologic images.[65] For example, number of interactions with neighbors in a nearest neighbor analysis to discern morphologic or other similarities between nearby individuals can provide information about the size and connectivity of pockets of cells with distinct morphologies. The spatial identification of vessels and ectopic lymph nodes may have near-term clinical implications as key pathologic findings and progression projections.[66]

Finally, it is likely that landscape pathology, as in landscape ecology, will require mathematical models for simplifying and interrogating complex ecological and evolutionary systems.[67] Ecologists and pathologists alike use models to expand testing beyond the time, expense, and often feasibility of experimental designs.[68,69] Mathematical models have also had great impact in cancer research in recent years.[70–72] Spatial models in particular have a clear role in interrogating pathologic samples.[73] One such model not yet translated to medicine deals with ecological niche modeling.[74,75] Here niches are defined as the geographic areas necessary for a species to survive. This approach uses matching of individual traits in a species to available resources on the landscape. Furthermore, the niche may limit the distribution of invasive species to a particular region of the landscape.[76] These models are often also referred to as bioclimatic envelop models and species distribution models. Here we draw striking parallels to cancer, which grows asymmetrically due to what has been proposed as localized niches of heterogenous microenvironmental resources.[53,77] Also interesting is the direct link of the

ecological niche model to spatial distribution of resources within regions of a system.[78,79] These models have been highly successful in predicting invasive species spread into new regions of the globe.[80] These concepts may be mapped in landscape pathology images in 3 dimensions as a topographical representation of an histologic image where the third dimension is a measure of multiplexed features of tissues or cells including glucose levels, pH, physical space, and oxygen concentrations much like a number of variables including elevation, rainfall, food sources, and other considerations might be used in niche models (Figs. 1G, H).

SUMMARY AND FUTURE DIRECTIONS

In summary, we propose that pathologists have the opportunity to define the Darwinian dynamics within cancers through application of methods and principles of landscape ecology. Using automated image analysis techniques much more precise information can be investigated regarding intratumoral heterogeneity. Furthermore, spatial heterogeneity in these tissue "habitats" can be measured and used to define both prognosis and optimal therapeutic strategies. The latter will require transition from targeted therapies based on genomic analysis of small tumor samples to environmentally and phenotypically defined targets based on comprehensive knowledge of the spatial variations throughout the tumor. This is facilitated by automated, high throughput image analysis technologies to identify variations in the physical or molecular metrics of cells and environmental properties.

This approach will require quantitative, reproducible, and comprehensive analysis of cancer as an ecological system. Evaluation of these data as prognostic and predictive biomarkers will require significant effort. However, we propose that this approach to understanding and quantifying intratumoral heterogeneity is necessary to achieve current goals of personalized cancer therapy.[35]

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REFERENCES

1. Howland RH. Where are we today with personalized medicine? *J Psychosoc Nurs Ment Health Serv*. 2012;15:1–3.
2. Maitland ML, DiRienzo A, Ratain MJ. Interpreting disparate responses to cancer therapy: the role of human population genetics. *J Clin Oncol*. 2006;24:2151–2157.
3. Mirnezami R, Nicholson J, Darzi A. Preparing for precision medicine. *N Engl J Med*. 2012;366:489–491.
4. Malinowski MJ. Could biobanking be a means to include health care have-nots in the genomics revolution. DePaul J. *Health Care L*. 2005;9:1005.
5. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med*. 2012;366:883–892.
6. Faratian D, Christiansen J, Gustavson M, et al. Heterogeneity mapping of protein expression in tumors using quantitative immunofluorescence. *J Vis Exp*. 2011;56:e3334.
7. Powathil GG, Gordon KE, Hill LA. Modelling the effects of cell-cycle heterogeneity on the response of a solid tumour to chemotherapy: biological insights from a hybrid multiscale cellular automaton model. *J Theor Biol*. 2012;308:1–19.
8. Askensten UG, von Rosen AK, Nilsson RS, et al. Intratumoral variations in DNA distribution patterns in mammary adenocarcinomas. *Cytometry*. 1989;10:326–333.
9. Rabes HM, Carl P, Meister P, et al. Analysis of proliferative compartments in human tumors. I. Renal adenocarcinoma. *Cancer*. 1979;44:799–813.
10. Raychaudhuri M, Schuster T, Buchner T, et al. Intratumoral heterogeneity of microRNA expression in breast cancer. *J Mol Diagn*. 2012;14:376–384.
11. Turner MG. Landscape ecology: the effect of pattern on process. *Ann Rev Ecol Syst*. 1989;20:171–197.
12. Turner MG. *Landscape Ecology in Theory and Practice: Pattern and Process*. New York, New York: Springer Science & Business Media; 2001.
13. Park S, Pantanowitz L, Parwani AV. Digital imaging in pathology. *Clin Lab Med*. 2012;32:557–584.
14. Sexton M, Jones DB, Maloney ME. Histologic pattern analysis of basal cell carcinoma: study of a series of 1039 consecutive neoplasms. *J Am Acad Dermatol*. 1990;23(: 1118–1126.
15. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol*. 2010;17(: 1471–1474.
16. Potten CS, Chwalinski S, Swindell R, et al. The spatial organization of the hierarchical proliferative cells of the crypts of the small intestine into clusters of 'synchronized' cells. *Cell Prolif*. 1982;15(: 351–370.
17. Blin N, Stafford DW. A general method for isolation of high molecular weight DNA from eukaryotes. *Nucleic Acids Res*. 1976;3(: 2303–2308.
18. Yuan Y, Failmezger H, Rueda OM, et al. Quantitative image analysis of cellular heterogeneity in breast tumors complements genomic profiling. *Sci Transl Med*. 2012;4:157ra143.
19. Merlo LM, Pepper JW, Reid BJ, et al. Cancer as an evolutionary and ecological process. *Nat Rev Cancer*. 2006;6: 924–935.
20. Dexter DL, Leith JT. Tumor heterogeneity and drug resistance. *J Clin Oncol*. 1986;4:244–257.
21. Fidler IJ. Tumor heterogeneity and the biology of cancer invasion and metastasis. *Cancer Res*. 1978;38:2651–2660.
22. Heppner GH, Miller BE. Tumor heterogeneity: biological implications and therapeutic consequences. *Cancer Metastasis Rev*. 1983;2:5–23.
23. Marusyk A, Polyak K. Tumor heterogeneity: causes and consequences. *Biochim Biophys Acta*. 2010;1805:105–117.
24. Wilks S. The Pathology of Cancer. *British Medical Journal*. 1874;1:573.
25. Campbell LL, Polyak K. Breast tumor heterogeneity: cancer stem cells or clonal evolution? *Cell Cycle*. 2007;6:2332–2338.
26. Chantrain CF, DeClerck YA, Groshen S, et al. Computerized quantification of tissue vascularization using high-resolution slide scanning of whole tumor sections. *J Histochem Cytochem*. 2003;51:151–158.
27. Romer DJ, Yearsley KH, Ayers LW. Using a modified standard microscope to generate virtual slides. *Anat Rec B New Anat*. 2003;272:91–97.
28. Addicott JF, Aho JM, Antolin MF, et al. Ecological neighborhoods: scaling environmental patterns. *Oikos*. 1987; 49:340–346.
29. Diaz G, Romero E. Micro-structural tissue analysis for automatic histopathological image annotation. *Microsc Res Tech*. 2012;75:343–358.
30. Tischendorf L. Can landscape indices predict ecological processes consistently? *Landsc Ecol*. 2001;16:235–254.
31. Kerr JT, Ostrovsky M. From space to species: ecological applications for remote sensing. *Trends in Ecology & Evolution*. 2003;18:299–305.
32. Goetz SJ, Steinberg D, Betts MG, et al. Lidar remote sensing variables predict breeding habitat of a Neotropical migrant bird. *Ecology*. 2010;91:1569–1576.
33. Andrew ME, Ustin SL. Habitat suitability modelling of an invasive plant with advanced remote sensing data. *Diversity and Distributions*. 2009;15:627–640.
34. Gustafson EJ. Quantifying landscape spatial pattern: what is the state of the art? *Ecosystems*. 1998;1:143–156.

35. Jobin B, Labrecque S, Grenier M, et al. Object-based classification as an alternative approach to the traditional pixel-based classification to identify potential habitat of the Grasshopper Sparrow. *Environ Manage*. 2008;41:20–31.
36. Naik S, Doyle S, Agner S, et al. Automated gland and nuclei segmentation for grading of prostate and breast cancer histopathology. Biomedical Imaging: From Nano to Macro, 2008. ISBI 2008. 5th IEEE International Symposium on. IEEE.
37. Teverovskiy M, Vengrenyuk Y, Tabesh A, et al. Automated localization and quantification of protein multiplexes via multispectral fluorescence imaging. Biomedical Imaging: From Nano to Macro, 2008. ISBI 2008. 5th IEEE International Symposium on. IEEE.
38. Tansey D, Wells AU, Colby TV, et al. Variations in histological patterns of interstitial pneumonia between connective tissue disorders and their relationship to prognosis. *Histopathology*. 2004;44(; 585–596.
39. Singh S, Janoos F, Pécot T, et al. Identifying nuclear phenotypes using semi-supervised metric learning. *Inf Process Med Imaging*. 2011;22:398–410.
40. Pickett ST, Cadenasso ML. Landscape ecology: spatial heterogeneity in ecological systems. *Science*. 1995;269:331–333.
41. Cushman SA, Gutzweiler K, Evans JS, et al. *"The Gradient Paradigm: A Conceptual and Analytical Framework for Landscape Ecology" Spatial Complexity, Informatics, and Wildlife Conservation*. Japan: Springer; 2010:83–108.
42. Sparks R, Madabhushi A. Novel morphometric based classification via diffeomorphic based shape representation using manifold learning. *Med Image Comput Comput Assist Interv*. 2010;13(pt 3):658–665.
43. Lloyd MC, Allam-Nandyala P, Purohit CN, et al. Using image analysis as a tool for assessment of prognostic and predictive biomarkers for breast cancer: how reliable is it? *J Pathol Inform*. 2010;1:29.
44. Ludwig JA, Wiens JA, Tongway DJ. A scaling rule for landscape patches and how it applies to conserving soil resources in savannas. *Ecosystems*. 2000;3:84–97.
45. Cottam G, Curtis JT. The use of distance measures in phytosociological sampling. *Ecology*. 1956;37:451–460.
46. Fraterrigo J, Turner MG, Pearson SM, et al. Effects of past land use on spatial heterogeneity of soil nutrients in Southern Appalachian forests. *Ecol Monogr*. 2005;75:215–230.
47. Madabhushi A, Agner S, Basavanhally A, et al. Computer-aided prognosis: predicting patient and disease outcome via quantitative fusion of multi-scale, multi-modal data. *Comput Med Imaging Graph*. 2011;35:506–514.
48. Bissell MJ, Rizki A, Mian IS. Tissue architecture: the ultimate regulator of breast epithelial function. *Curr Opin Cell Biol*. 2003;15:753–762.
49. Dorner B, Lertzman K, Fall J. Landscape pattern in topographically complex landscapes: issues and techniques for analysis. *Landsc Ecol*. 2002;17:729–743.
50. Watt AS. Pattern and process in the plant community. *J Ecol*. 1947;35(1/2):1–22.
51. Alfarouk KO, Ibrahim ME, Gatenby RA, et al. Riparian ecosystems in human cancers. *Evol Appl*. 2013;6:46–53.
52. Gatenby RA, Grove O, Gillies RJ. Quantitative imaging in cancer evolution and ecology. *Radiology*. 2013;269:8–14.
53. Lloyd MC, Alfarouk KO, Verduzco D, et al. Vascular measurements correlate with estrogen receptor status. *BMC Cancer*. 2014;14:279.
54. Messina JL, Fenstermacher DA, Eschrich S, et al. 12-chemokine gene signature identifies lymph node-like structures in melanoma: potential for patient selection for immunotherapy? *Sci Rep*. 2012;2:765.
55. Haase P. Spatial pattern analysis in ecology based on Ripley's K-function: introduction and methods of edge correction. *J Veg Sci*. 1995;6(: 575–582.
56. Wiegand T, Moloney. KA. Rings, circles, and null–models for point pattern analysis in ecology. *Oikos*. 2004;104:209–229.
57. de Oliveira Martins L, da Silva EC, Silva AC, et al. *"Classification of Breast Masses in Mammogram Images Using Ripley's K Function and Support Vector Machine" Machine Learning and Data Mining in Pattern Recognition*. Berlin Heidelberg: Springer; 2007:784–794.
58. Kim HL, Vander Griend DJ, Yang X, et al. Mitogen-activated protein kinase kinase 4 metastasis suppressor gene expression is inversely related to histological pattern in advancing human prostatic cancers. *Cancer Res*. 2001;61(: 2833–2837.
59. Thomlinson RH, Gray LH. The histological structure of some human lung cancers and the possible implications for radiotherapy. *Br J Cancer*. 1955;9(: 539–549.
60. Moran PA. Notes on continuous stochastic phenomena. *Biometrika*. 1950;37:17–23.
61. da Rocha SV, Braz Junior G, Silva AC, et al. Texture analysis of masses in digitized mammograms using Gleason and Menhinick diversity indexes. *Rev Bras Eng Biomed*. 2014;30:27–34.
62. Estrella V, Chen T, Lloyd M, et al. Acidity generated by the tumor microenvironment drives local invasion. *Cancer Res*. 2013;73:1524–1535.
63. Delahunt B, Bethwaite PB, Thornton A. Prognostic significance of microscopic vascularity for clear cell renal cell carcinoma. *Br J Urol*. 1997;80(: 401–404.
64. Jellinger K, Slowik F. Histological subtypes and prognostic problems in meningiomas. *J Neurol*. 1975;208(: 279–298.
65. González-García I, Solé RV, Costa J. Metapopulation dynamics and spatial heterogeneity in cancer. *Proc Natl Acad Sci U S A*. 2002;99:13085–13089.
66. Epstein JI, Pizov G, Walsh PC. Correlation of pathologic findings with progression after radical retropubic prostatectomy. *Cancer*. 1993;71(), 3582–3593.
67. Johnson JB, Omland KS. Model selection in ecology and evolution. *Trends Ecol Evol*. 2004;19:101–108.
68. Haslberger A, Varga F, Karlic H. Recursive causality in evolution: a model for epigenetic mechanisms in cancer development. *Med Hypotheses*. 2006;67:1448–1454.
69. Jansson B, Révész L. Cell ecology: deductive and dynamic models for proliferation, differentiation and competition of tumor cell populations. *J Theor Biol*. 1977;68:43–51.
70. Kim M, Gillies RJ, Rejniak KA. Current advances in mathematical modeling of anti-cancer drug penetration into tumor tissues. *Front Oncol*. 2013;3:278.
71. Rejniak KA, Anderson AR. Hybrid models of tumor growth. *Wiley Interdiscip Rev Syst Biol Med*. 2011;3:115–125.
72. Rejniak KA, McCawley LJ. Current trends in mathematical modeling of tumor–microenvironment interactions: a survey of tools and applications. *Exp Med Biol*. 2010;235:411–423.
73. With KA, Pavuk DM, Worchuck JL, et al. Threshold effects of landscape structure on biological control in agroecosystems. *Ecological Applications*. 2002;12(), 52–65.
74. Araújo MB, Peterson AT. Uses and misuses of bioclimatic envelope modeling. *Ecology*. 2012;93:1527–1539.
75. Dormann CF, Schymanski SJ, Cabral J, et al. Correlation and process in species distribution models: bridging a dichotomy. *J Biogeogr*. 2012;39:2119–2131.
76. Peterson AT. Predicting the geography of species' invasions via ecological niche modeling. *Q Rev Biol*. 2003;78:419–433.
77. Axelrod R, Axelrod DE, Pienta KJ. Evolution of cooperation among tumor cells. *Proc Natl Acad Sci U S A*. 2006;103: 13474–13479.
78. Anderson RP, Gomez-Laverde M, Peterson AT. Geographical distributions of spiny pocket mice in South America: insights from predictive models. *Global Ecol Biogeogr*. 2002;11:131–141.
79. MacArthur RH, Wilson EO. *The Theory of Island Biogeography, Volume 1*. Princeton, NJ: Princeton University Press; 2001.
80. Rouget M, Richardson DM. Inferring process from pattern in plant invasions: a semimechanistic model incorporating propagule pressure and environmental factors. *Am Nat*. 2003;162: 713–724.

