

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

GUARDANT HEALTH, INC.,
Petitioner,

v.

TEMPUS AI, INC.,
Patent Owner.

Case No. IPR2026-00185
Patent No. 10,991,097

DECLARATION OF DR. NIKOLAOS P. PAPANIKOLOPOULOS

TABLE OF CONTENTS

I.	BACKGROUND QUALIFICATIONS.....	1
II.	SCOPE OF WORK.....	6
III.	LEGAL STANDARDS	9
IV.	BACKGROUND IN THE ART	12
	A. Computational Histopathology	12
	B. Cell Segmentation	16
	C. Lymphocyte and Biomarker Detection	17
V.	OVERVIEW OF THE '097 PATENT	20
VI.	PRIOR ART.....	25
	A. Chukka (EX1005).....	25
	B. Jones (EX1006)	27
	C. Sebastiao (EX1007).....	27
	D. Gallas (EX1010).....	29
VII.	OVERVIEW OF THE LEVEL OF SKILL.....	29
VIII.	CLAIM CONSTRUCTION	31
IX.	GROUND 1: CLAIMS 1, 4, 5, 6, 9-12, 14, 15, 17, AND 18 ARE ANTICIPATED BY CHUKKA	31
	A. Claim 1	36
	1. Preamble.....	36
	2. Element 1[A].....	37
	3. Element 1[B].....	40
	4. Element 1[C].....	45

5.	Element 1[D]	55
6.	Element 1[E]	59
B.	Claim 4	66
C.	Claim 5	68
D.	Claim 6	70
1.	Preamble.....	70
2.	Element 6[A]	71
3.	Element 6[B]	71
4.	Element 6[C]	72
5.	Element 6[D]	72
6.	Element 6[E]	72
7.	Element 6[F]	73
8.	Element 6[G]	85
9.	Element 6[H]	97
E.	Claim 9	105
F.	Claim 10	111
G.	Claim 11	116
H.	Claim 12	117
I.	Claim 14	119
J.	Claim 15	121
K.	Claim 17	125
L.	Claim 18	127
X.	GROUND 2: CLAIMS 2, 3, 7, AND 8 ARE OBVIOUS OVER CHUKKA AND JONES	127

A.	Claim 2	133
B.	Claim 3	146
C.	Claims 7 and 8	148
XI.	GROUND 3: CLAIMS 12, 13, & 16 ARE OBVIOUS OVER CHUKKA AND SEBASTIAO	161
A.	Claim 12	165
B.	Claim 13	170
C.	Claim 16	175
XII.	GROUND 4: CLAIMS 1, 4-6, 9-12, 14, 15, 17, AND 18 ARE OBVIOUS OVER GALLAS AND CHUKKA.....	180
A.	Claim 1	183
1.	Preamble.....	183
2.	Element 1[A].....	186
3.	Element 1[B]	190
4.	Element 1[C]	193
5.	Element 1[D].....	195
6.	Element 1[E]	199
B.	Claim 4	201
C.	Claim 5	203
D.	Claim 6	203
1.	Preamble.....	204
2.	Element 6[A].....	204
3.	Element 6[B]	204
4.	Element 6[C]	204
5.	Element 6[D].....	205

6.	Element 6[E]	205
7.	Element 6[F]	205
8.	Element 6[G].....	206
9.	Element 6[H].....	206
E.	Claim 9	207
F.	Claim 10	208
G.	Claim 11	209
H.	Claim 12	211
I.	Claim 14	211
J.	Claim 15	211
K.	Claim 17	212
L.	Claim 18	212
XIII.	GROUND 5: CLAIMS 2, 3, 7, AND 8 ARE OBVIOUS OVER GALLAS, CHUKKA, AND JONES.....	213
A.	Claim 2	213
B.	Claim 3	213
C.	Claims 7 and 8.....	214
XIV.	GROUND 6: CLAIMS 12, 13, AND 16 ARE OBVIOUS OVER GALLAS, CHUKKA, AND SEBASTIAO.....	214
A.	Claim 12	214
B.	Claim 13	214
C.	Claim 16	215
XV.	CONCLUDING STATEMENTS.....	215
XVI.	APPENDIX - LISTING OF CHALLENGED CLAIMS	217

XVII. EXHIBIT LIST.....	222
-------------------------	-----

I, Nikolaos P. Papanikolopoulos, declare as follows:

I. BACKGROUND QUALIFICATIONS

1. I am the McKnight Presidential Endowed Professor and Distinguished McKnight University Professor in the Department of Computer Science and Engineering of the University of Minnesota in Minneapolis, Minnesota. I am also the Director for the Minnesota Robotics Institute. My curriculum vitae is attached hereto as Exhibit 1003. *See* EX1003.

2. I have been a Professor at the University of Minnesota (originally as an Assistant Professor, and then as an Associate Professor) since the Fall of 1992. Between Fall 2000 and Spring 2004, and between Fall 2010 and Spring 2013, I was the Director of Undergraduate Studies in Computer Science and Engineering at the University of Minnesota.

3. In 1992, I received my Ph.D. in Electrical and Computer Engineering from Carnegie Mellon University. My thesis was entitled “Controlled Active Vision” and focused on using computer vision (including, among other things, methods of acquiring and analyzing digital images to identify and classify objects in the image) in a controlled fashion to detect, track, and manipulate objects in the environment. In 1988, I received my M.S. in Electrical and Computer Engineering from Carnegie Mellon University. My B.S. in Electrical Engineering was received in 1987 from the National Technical University in Athens, Greece.

4. For over thirty years, my research and teaching work has focused on computer vision, machine learning, image processing, and robotics. Since 2010, this research has included the application of machine learning, computer vision and artificial intelligence to the field of histopathology. My research has produced more than 300 journal and conference publications, including in journals such as Journal of Urology, Journal of Endourology, and Medical Image Analysis. Examples of my publications are as follows:

Kaplan, A., Li, X., Sivalingam, R., Somasundaram, G., Banerjee, A., Morellas, V., Papanikolopoulos, N., and Truskinovsky, S., “Computer Vision Methods in Surgical Pathology: Diagnosing Carcinoma of the Breast”, Proceedings of the 2013 United States and Canadian Academy of Pathology Annual Meeting (USCAP), Baltimore, MD, March 2-8, 2013;

Wood, A., Abdallah, N., Benidir, T., Heller, N., Isensee, F., Tejpaul, R., Corrigan, D., Suk-ouichai, C., Struyk, G., Moore, K., Venkatesh, N., Ergun, O., You, A., Campbell, R., Remer, E., Haywood, S., Kirshnamurthi, V., Abouassaly, R., Campbell, S., Papanikolopoulos, N., and Weight, C., “Automated PADUA, C-Index and Tumour CSA Demonstrate Superior Predictive Utility versus Human Scores”, accepted, British Journal of Urology International, 2024;

Abdallah, N., Wood, A., Benidir, T., Heller, N., Isensee, F., Tejpaul, R., Corrigan, D., Suk-ouichai, C., Struyk, G., Moore, K., Venkatesh,

N., Ergun, O., You, A., Campbell, R., Remer, E., Haywood, S., Kirshnamurthi, V., Abouassaly, R., Campbell, S., Papanikolopoulos, N., and Weight, C., “AI-generated R.E.N.A.L.+ Score Surpasses Human-generated Score in Predicting Renal Oncologic Outcomes,” *Journal of Urology*, 18, Jul. 2023;

Benidir, T., Heller, N., Tejpaul, R., Isensee, F., Hofmann, M., Blake, P., Rengel, Z., Moore, K., Sathianathen, N., Kalapara, A., Rosenberg, J., Peterson, S., Walczaka, E., Kutikov, A., Uzzo, R.G., Palacios, D.A., Remer, E.M., Campbell, S., Papanikolopoulos, N., and Weight, C.J., “Computer Generated R.E.N.A.L. Nephrometry Scores Yield Comparable Predictive Results to that of Human-Expert Scores in Predicting Oncologic and Perioperative Outcomes,” *European Journal of Urology*, Dec. 17, 2021;

Rickman, J., Struyk, G., Simpson, B., Byun, B., and Papanikolopoulos, N., “The Growing Role for Semantic Segmentation in Urology”, *European Urology Focus*, Volume 7, No. 4, 2021, pp 692-695;

Heller, N., Isensee, F., Maier-Hein, K., Houd, X., Xied, C., Lid, F., Nand, Y., Mue, G., Ling, Z., Hane, M., Yaoe, G., Gaoe, Y., Zhangh, Y., Wangh, Y., Houh, F., Yang, J., Xiong, G., Tiank, J., Zhongk, C., Mal, J., Rickmana, J., Deana, J., Stai, B., Tejpaul, R., Oestreicha, M., Blake, P., Kaluzniako, H., Razao, S., Rosenberg, J., Moore, K., Walczaka, E., Rengel, Z., Edgerton, Z., Vasdev, R., Peterson, M., McSweeney, S., Peterson, P., Kalapara, A., Sathianathen, N., Papanikolopoulos, N., and Weight, C.J., “The State of the Art in Kidney

and Kidney Tumor Segmentation in Contrast-enhanced CT Imaging: Results of the KiTS19 Challenge”, Medical Image Analysis, January 2021;

Stanitsas, P., Cherian, A., Morellas, V., Tejpaul, R., Papanikolopoulos, N.P., and Truskinovsky, A., “Image Descriptors for Weakly Annotated Histopathological Breast Cancer Data”, Frontiers in Digital Health, Section Connected Health, December 2020;

Stai, B., Heller, N., McSweeney, S., Rickman, J., Blake, P., Vasdev, R., Edgerton, Z., Tejpaul, R., Peterson, M., Rosenburg, J., Kalapara, A., Regmi, S., Papanikolopoulos, N., and Weight, C., “Public Perceptions of Artificial Intelligence and Robotics in Medicine”, Journal of Endourology, Volume 34, No. 10, October 2020;

Li, X., Banerjee, A., Morellas, V., Papanikolopoulos, N., and Truskinovsky, S., “The Use of Computer Vision for the Diagnosis of Benign and Malignant Smooth Muscle Neoplasms of the Uterus”, Proceedings of the 2014 United States and Canadian Academy of Pathology Annual Meeting (USCAP), San Diego, CA, March 1-7, 2014.

5. In my lab’s research, we have applied computer vision and machine learning techniques to histopathological diagnosis, successfully applying computer vision to diagnosing a variety of malignant neoplasms in histopathologic images. Our work focuses on developing computational methods for automated

medical image analysis, particularly in the detection and classification of cancerous tissues in histopathological samples. Our research combines artificial intelligence, pattern recognition, and computer vision technologies to enhance diagnostic accuracy in medical imaging, contributing to the broader field of computer-aided diagnosis in pathology while also working on understanding the decision-making processes of these automated systems.

6. In my lab, we have developed artificial intelligence tools for automated medical image analysis for kidney cancer treatment, particularly through our work on the KiTS19 Challenge (Kidney Tumor Segmentation Challenge). The morphometry of a kidney tumor revealed by contrast-enhanced Computed Tomography (CT) imaging is an important factor in clinical decision-making surrounding the lesion's diagnosis and treatment. Our research addresses the challenge that a quantitative study of the relationship between kidney tumor morphology and clinical outcomes is difficult due to data scarcity and the laborious nature of manually quantifying imaging predictors. Our work focuses on automatic semantic segmentation of kidneys and kidney tumors as a promising tool towards automatically quantifying a wide array of morphometric features. Through the KiTS19 and subsequent challenges, we have contributed to advancing the state-of-the-art in computer-assisted diagnosis and treatment planning for kidney cancer, enabling more accurate and efficient evaluation of

tumor geometry and morphology that directly impacts surgical and treatment outcomes.

7. I am a named inventor of U.S. Patent No. 11,449,985, entitled “Computer Vision for Cancerous Tissue.” The ’985 Patent issued on September 20, 2022 based on Application No. 15/829,562, filed on December 1, 2017. I am an IEEE Fellow and most recently in 2025, I was elected to become a Fellow of the National Academy of Inventors.

8. I also teach two courses related to computer vision: (i) CSci 5561 Computer Vision, and (iii) CSci 8980 Computational Vision and Robotics. I have been teaching these courses for more than 25 years.

II. SCOPE OF WORK

9. I understand that a petition is being filed with the United States Patent and Trademark Office for Inter Partes Review of U.S. Patent No. 10,991,097 to Yip et al. (“the ’097 Patent,” attached as EX1001), entitled “Artificial Intelligence Segmentation of Tissue Images.” I have been retained by Guardant Health, Inc. (“Guardant”) to offer an expert opinion on the validity of claims 1-18 of the ’097 Patent assigned to Tempus AI, Inc. (“Tempus”). I receive \$500 per hour for my services. No part of my compensation is dependent on my opinions or on the outcome of this proceeding.

10. I have been specifically asked to evaluate the patentability of claims 1-18 of the '097 Patent. In connection with this analysis, I have reviewed the '097 Patent. I have also reviewed and considered various other documents in arriving at my opinions and cite to them in this declaration.

11. In my opinion, the claims of the '097 Patent are not novel, and would have been obvious to a person of skill in the art before the critical date of the claims. In particular, it is my opinion that the claims of the '097 Patent are anticipated and/or obvious in view of Chukka (EX1005), alone or in combination with Jones (EX1006) and Sebastiao (EX1007).

12. At a high level, Chukka teaches a “facility” that comprises various components that are able to analyze and score tissue specimen slides, such as slides stained with an immunohistochemical (IHC) assay. Chukka teaches that this facility detects, classifies, and counts cells in such slides, and generates an overlay to label features in those slides. Chukka teaches that in certain embodiments, which it terms an “automated mode,” the facility may first segment the tissue data into tiles and then perform cell classification and counting algorithms with respect to each tile that contains tissue, including by analyzing contextual information in the region surrounding each tile. Chukka teaches that this workflow performs an initial, “coarse resolution” tile analysis followed by a further, finer grained analysis to identify and classify cells. It is my opinion that

this automated workflow taught by Chukka anticipates claims 1, 4, 5, 6, 9-12, 14, 15, 17, and 18.

13. Furthermore, it is my opinion that claims 2, 3, 7, and 8 would have been obvious in view of Chukka and Jones, which together teach using binary foreground masks and detected seed points to define portions of the image that correspond to the boundaries of cells. It is my opinion that claims 12, 13, and 16 would have been obvious in view of Chukka and Sebastiao, which teaches methods of analyzing and scoring images of stained tissue slides for cancer, wherein the images are stained for a “multiplex assay” of lymphocyte markers including CD3, CD8, CD20, FoxP3, and tumor detection markers.

14. Finally, it is my opinion that the claims of the '097 Patent would further have been obvious in view of Gallas (EX1010), in combination with Chukka, Jones, and/or Sebastiao. At a high level, Gallas discloses a platform (eeDAP) which allows for viewing and annotating digital images of tissue slides. Gallas teaches that this platform separates the image into tiles by using a reticle, which superimposes a grid on the image such that each tile may be individually and collectively analyzed. Gallas teaches visual classification of tiles such as identifying mitotic figures, which involves analysis of the evaluation square along with adjacent grid squares. Gallas alone teaches the limitations of claims 1, 4, and 5. Automation of such analysis, such as taught by Chukka, renders claims 1, 4, 5,

6, 9-12, 14, 15, 17, and 18 obvious. Accordingly, Gallas, in combination with Chukka, Jones, and/or Sebastiao would have rendered all challenged claims obvious.

III. LEGAL STANDARDS

15. I understand that a claim is not patentable under 35 U.S.C. §102 as anticipated if the claim as a whole is described, either expressly or inherently, in a single prior art reference. I understand that this also requires that the prior art reference disclose that each claim element is arranged as recited in the claims.

16. I understand that differences between the prior art and a claimed invention, however slight, invoke the question of obviousness, not anticipation. I have been advised that a claimed invention is not patentable under 35 U.S.C. §103 if it is obvious. A patent claim is unpatentable if the claimed invention would have been obvious to a person of ordinary skill in the field at the time the claimed invention was made. This means that, even if all of the requirements of the claim cannot be found in a single prior art reference that would anticipate the claim, a person of ordinary skill in the relevant field who knew about the prior art would have arrived the claimed invention.

17. I have further been advised that the ultimate conclusion of whether a claim is obvious should be based upon several factual determinations. That is, a determination of obviousness requires inquiries into: (1) the level of ordinary skill

in the field; (2) the scope and content of the prior art; (3) what difference, if any, existed between the claimed invention and the prior art; and (4) any secondary evidence bearing on obviousness.

18. I have been advised that, in determining the level of ordinary skill in the field that someone would have had at the time the claimed invention was made, I should consider: (1) the levels of education and experience of persons working in the field; (2) the types of problems encountered in the field; and (3) the sophistication of the technology.

19. I have also been advised that, in determining the scope and content of the prior art, in order to be considered as prior art, a reference must be reasonably related to the claimed invention of the patent. A reference is reasonably related if it is in the same field as the claimed invention or is from another field to which a person of ordinary skill in the field would look to solve a known problem.

20. I have been advised that a patent claim composed of several elements is not proved obvious merely by demonstrating that each of its elements was independently known in the prior art. In evaluating whether such a claim would have been obvious, I may consider whether there is a reason that would have prompted a person of ordinary skill in the field to combine the elements or concepts from the prior art in the same way as in the claimed invention.

21. I have been advised that any obviousness rationale for modifying or combining prior art must include a showing that a person of ordinary skill would have had a reasonable expectation of success.

22. I have been further advised that there is no single way to define the line between true inventiveness on the one hand (which is patentable) and the application of common sense and ordinary skill to solve a problem on the other hand (which is not patentable). For example, market forces or other design incentives may be what produced a change, rather than true inventiveness. I may consider whether the change was merely the predictable result of using prior art elements according to their known functions, or whether it was the result of true inventiveness. I may also consider whether there is some teaching or suggestion in the prior art to make the modification or combination of elements claimed in the patent. I may consider whether the innovation applies a known technique that had been used to improve a similar device or method in a similar way. I may also consider whether the claimed invention would have been obvious to try, meaning that the claimed innovation was one of a relatively small number of possible approaches to the problem with a reasonable expectation of success by those skilled in the art.

23. I understand that hindsight must not be used when opining on the patentability of claims of a patent. Therefore, my opinion will reflect the

understanding of a person of skill in the art as of the relevant patent application filing dates. In this matter, it is my opinion that the relevant critical date for the '097 Patent is December 31, 2018.

IV. BACKGROUND IN THE ART

A. Computational Histopathology

24. The patent identifies several prior art practices reflected in the background knowledge of a person of ordinary skill in the art. As described by the '097 Patent, for example, “[t]o guide a medical professional in diagnosis, prognosis and treatment assessment of a patient’s cancer, it is common to extract and inspect tumor samples from the patient.” EX1001 at 1:29-31.

Conventionally, this was done manually, with pathologists visually inspecting slides of tumor tissue to determine characteristics of the tissue and/or cells of the slide. *Id.* This common practice was similarly reflected in the prior art literature. *See, e.g.*, Giuseppe Musumeci “Past, Present and Future: Overview on Histology and Histopathology.” *J. Histol. Histopathol.* 1.5: 1-3 (2014) (“Musumeci,” EX1008); Peter W. Hamilton et al. “Digital Pathology and Image Analysis in Tissue Biomarker Research.” *Methods* 70:59-73 (2014). (“Hamilton,” EX1009). Hematoxylin and eosin (H&E) and immunohistochemical (IHC) staining are methods of staining tissue and have long been used in the diagnosis of

malignancies, including cancers. EX1008 (Musumeci), 2, 3; EX1009 (Hamilton), 65.

25. It was common in histopathology to use microscopes with reticles representing a grid pattern, to separate the tissue slide into discrete sections or coordinates. See Brandon D. Gallas et al. “eeDAP: an Evaluation Environment for Digital and Analog Pathology.” *Medical Imaging 2014: Image Perception, Observer Performance, and Technology Assessment*. Vol. 9037. SPIE (2014) (“Gallas,” EX1010), Fig. 6;

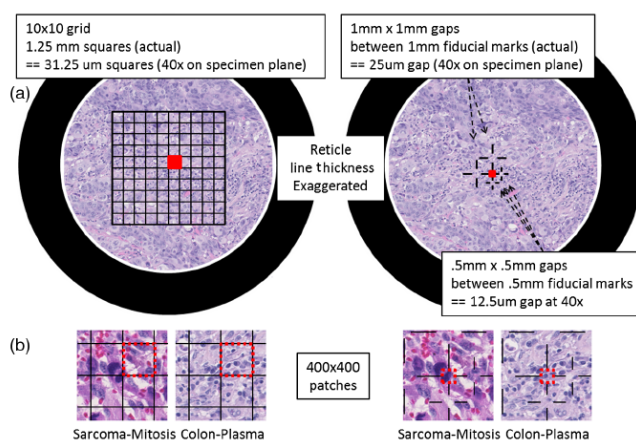


Fig. 6 Reticles (line thicknesses exaggerated) as seen through simulated microscope FOV (a) and 400 × 400 patches as they appear in eeDAP (b). The red squares in the simulated microscope FOVs and the red-dash boundary squares in the 400 × 400 patches indicate the “evaluation” areas. In the 400 × 400 sarcoma patch on the left, a majority of the obvious mitotic figure falls in the grid square to the left of the evaluation square. In the 400 × 400 sarcoma patch on the right, the central cross hairs point to the obvious mitotic figure. In the 400 × 400 colon patch on the left, there are several plasma-cell candidates in the “evaluation” square. In the 400 × 400 colon patch on the right, the cross hairs point to a single plasma-cell candidate to be evaluated.

see also John D. Bancroft, ed. “Theory and Practice of Histological Techniques, Elsevier Health Sciences, 2008 (6th Ed.) (“Bancroft,” EX1029) 649-651 (describing use of a convolutional network to analyze tissue slide images, and showing an overlay map generated with a convolutional network).

26. By the 2010s, technological advancements in digital imaging enabled high resolution scans of stained tissue slides. EX1009 (Hamilton), 60.

Simultaneously, advancements in computer vision and machine learning enabled pathologists to utilize those technologies to analyze high resolution scans of stained pathology slides, including to classify tissues, detect cells, and identify biomarkers. *Id.*, 64. Often, because scans of whole slide images (WSIs) were generally very large in size and pixel count, it was common practice in computational pathology to separate WSIs into discrete image “tiles” or “patches.” See Jun Kong, et al., “Computer-aided Evaluation of Neuroblastoma on Whole-slide Histology Images: Classifying Grade of Neuroblastic Differentiation.” *Pattern Recognition* 42.6: 1080-1092 (2009) (“Kong,” EX1011).

27. Additionally, it was common in the prior art to take a “multi-resolution” or “multi-scale” approach to analysis of pathology slides. *Id.* For example, as early as 2009, Kong et al. described a number of multiscale approaches to feature extraction in digital pathology. EX1011 (Kong), 17. These approaches “mimic[ed] the evaluation of a pathologist such that image analysis starts from the lowest resolution, ... and uses the higher resolution representations for the regions requiring more detailed information for a classification decision.” *Id.*; see also Metin N. Gurcan et al., “Histopathological image analysis: A review.” *IEEE Reviews in Biomedical Engineering* 2: 147-171 (2009) (“Gurcan,”

EX1012) (At lower resolutions, “textural analysis is commonly used to capture tissue architecture, i.e. the overall pattern of glands, stroma and organ organization[;]” while at higher resolutions cellular objects and features such as nuclei may be discerned and used to classify the image.”). This multiscale, “pyramidal” approach to image segmentation was also well-known in the computer vision prior art in a variety of other contexts. See Songfan Yang, et al., “Multi-scale Recognition with DAG-CNNs.” *Proceedings of the IEEE International Conference on Computer Vision* (2015) (“Yang,” EX1013) (“[m]ulti-scale representations are a classic concept in computer vision, dating back to image pyramids, scale-space theory, and multi- resolution models.”); Peter J. Burt, et al., “The Laplacian Pyramid as a Compact Image Code.” *Readings in Computer Vision*. Morgan Kaufmann, 671-679 (1987) (“Burt,” EX1014).

28. In a similar approach, in May of 2016, Barker et al. proposed a method of computerized analysis of digital pathology images that utilized a coarse-to-fine analysis of the localized characteristics in pathology images. See Jocelyn Barker, et al., “Automated Classification of Brain Tumor Type in Whole-Slide Digital Pathology Images Using Local Representative Tiles.” *Medical Image Analysis* 30: 60-71 (2016) (“Barker,” EX1015). Barker taught that analysis of digital pathology slides involved separating WSIs into separate image tiles or patches. See EX1015 (Barker), Abstract (“An initial surveying stage analyzes the

diversity of coarse regions in the whole slide image. This includes extraction of spatially localized features of shape, color and texture from tiled regions covering the slide.”) Barker then taught that representative tiles or patches could be selected for a more detailed, fine feature extraction. *Id.* at 11, Fig. 3:

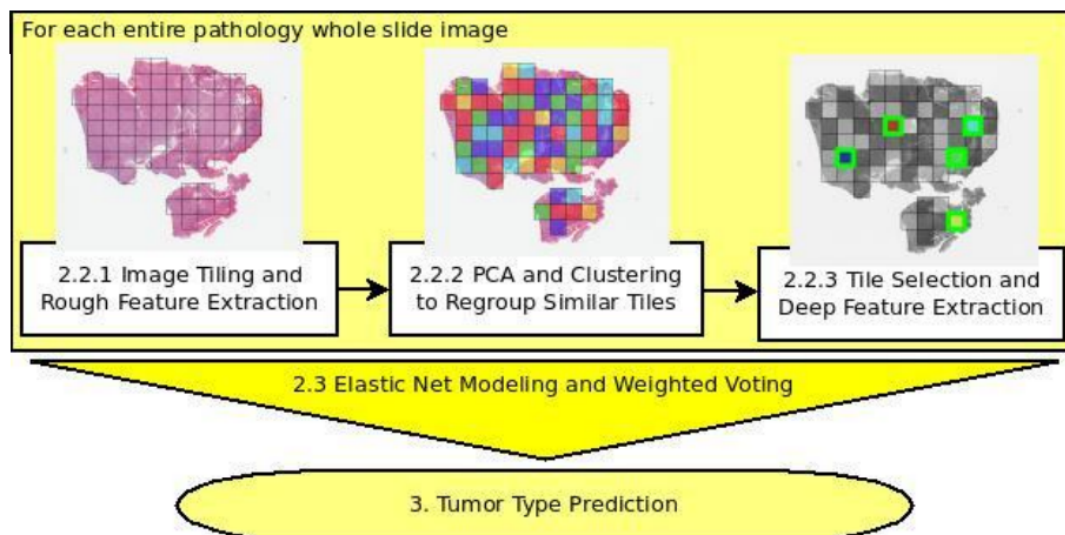


Figure 3. Pipeline for pathology analysis. Images show a scaled down representative whole-slide image with boxes representing tiles. Unique colors represent clusters.

B. Cell Segmentation

29. Prior art also recognized the importance of the detection and identification of cell objects (e.g., cells, nuclei) in computational pathology. *See, e.g.,* Xipeng Pan et al., “Cell Detection in Pathology and Microscopy Images with Multi-scale Fully Convolutional Neural Networks.” *World Wide Web* 21.6:1721-1743 (2018) (“Pan,” EX1016), 2 (“Automated nucleus/cell detection is usually considered as the basis and a critical prerequisite step of computer assisted pathology and microscopy image analysis.”). Therefore, numerous methods of

automated cell segmentation, including by using deep learning, were well-known and routine in the art. See EX1016 (Pan), 2 (describing prior art methods of using convolutional neural networks to segment cells and nuclei); Sahirzeeshan Ali et al., “Segmenting Multiple Overlapping Objects Via a Hybrid Active Contour Model Incorporating Shape Priors: Applications to Digital Pathology.” *Medical Imaging 2011: Image Processing*. Vol. 7962. SPIE (2011) (“Ali,” EX1017), 2-3. In this analysis, it was common and well-known to determine the boundaries of cellular objects such as nuclei, to generate, store, and display outlines or polygons corresponding to those boundaries. See EX1017 (Ali), Fig. 3:

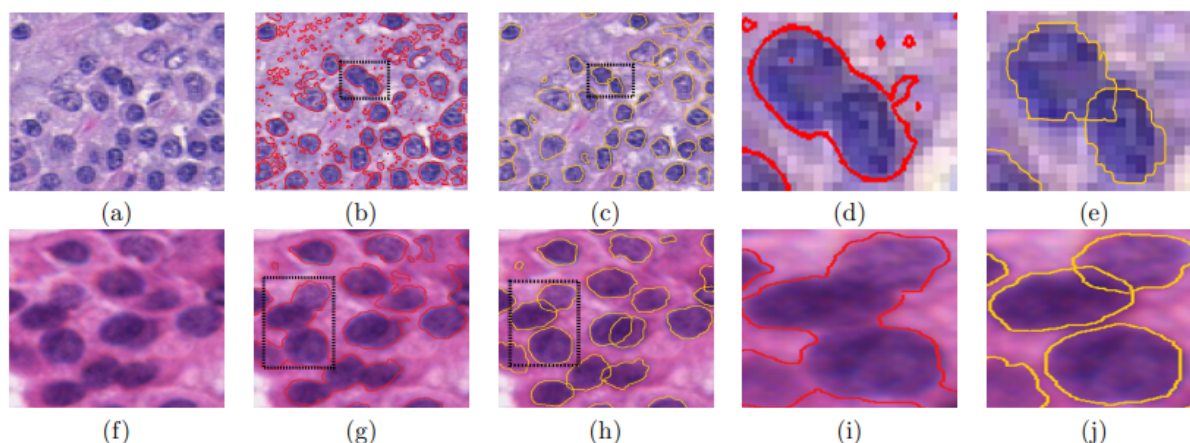


Figure 3. (a), (f) Original histological images corresponding to breast and prostate histology respectively, segmentation results for nuclei and lymphocytes obtained via the (b), (g) Geodesic Active Contour; (c), (h) our integrated AC model; (d), (e) magnified region from (b) and (g); (e), (j) magnified region from (c) and (h).

C. Lymphocyte and Biomarker Detection

30. Numerous studies have recognized the importance of detecting and classifying lymphocytes as relevant biomarkers in immunohistochemical (IHC) and hematoxylin and eosin stained tissue slides. See Michael Barnes, et al.,

“Abstract P5-03-08: Development of a Histology-based Digital Pathology Image Analysis Algorithm for Assessment of Tumor Infiltrating Lymphocytes in HER2+ Breast Cancer.” *Cancer Research* 78.4_Supplement: P5-03 (2018) (“Barnes,” EX1018) (“Histology-based assessment of tumor infiltrating lymphocytes (TILs) as a surrogate of the host immune response has been shown to be prognostic and potentially chemopredictive in triple-negative and HER2-positive breast cancers”); Emilio Garcia, et al., “Automatic Lymphocyte Detection on Gastric Cancer IHC Images using Deep Learning.” *2017 IEEE 30th International Symposium on Computer-Based Medical Systems (CBMS)*. IEEE, 2017 (“Garcia,” EX1019); Keith E. Steele, et al., “Measuring Multiple Parameters of CD8+ Tumor-infiltrating Lymphocytes in Human Cancers by Image Analysis.” *Journal for Immunotherapy of Cancer* 6.1:20 (2018) (“Steele,” EX1020). In particular, studies recognized the importance of detecting and classifying lymphocytes based off surface protein biomarkers such as CD3, CD8, and CD20. See EX1019 (Garcia), 200-01; EX1020 (Steele), Abstract; see also EX1007 (Sebastiao), [0005-0010].

31. Likewise, numerous studies also recognized the importance of staining for and detecting smooth muscle actin and pancytokeratin in diagnosing epithelial tumors and breast cancer. See Thomas Mentzel, et al., “Cutaneous Myoepithelial Neoplasms: Clinicopathologic and Immunohistochemical Study of

20 Cases Suggesting a Continuous Spectrum Ranging from Benign Mixed Tumor of the Skin to Cutaneous Myoepithelioma and Myoepithelial Carcinoma.” *Journal of Cutaneous Pathology* 30.5 (2003): 294-302 (“Mentzel,” EX1021); Michal Zamecnik, “Epithelioid Hemangioendothelioma with Actin+/Cytokeratin+ Myoblast-like Cells: Report of a Case.” *Journal of Interdisciplinary Histopathology*, (2016) (“Zamecnik,” EX1022,); Nemanja Rajković, et al., “The Pan-cytokeratin Staining Intensity and Fractal Computational Analysis of Breast Tumor Malignant Growth Patterns Prognosticate the Occurrence of Distant Metastasis.” *Frontiers in Oncology* 8:348 (2018) (“Rajković,” EX1023); Mami Murakami, et al., “Docetaxel Conjugate Nanoparticles that Target α -smooth Muscle Actin—expressing Stromal Cells Suppress Breast Cancer Metastasis.” *Cancer Research* 73.15: 4862-4871 (2013) (“Murakami,” EX1024); Mahalakshmi Ravi, et al., “Expression of α -smooth Muscle Actin in Benign and Malignant Salivary Gland Tumors: An Immunohistochemical Study.” *Indian Journal of Pathology and Microbiology* 61.4: 479-484 (2018) (“Ravi,” EX1025).

32. Therefore, numerous prior art studies have used computational pathology to analyze stained tissue slides to detect cells and cellular biomarkers such as CD3, CD8, CD20, smooth muscle actin, and pancytokeratin. See Michael J. Gerdes, et al., “Highly Multiplexed Single-Cell Analysis of Formalin-fixed, Paraffin-embedded Cancer Tissue.” *Proceedings of the National Academy of*

Sciences 110.29 (2013): 11982-11987 (“Gerdes,” EX1026); EX1015 (Barker); EX1018 (Barnes); EX1019 (Garcia); EX1020 (Steele); EX1023 (Rajković).

V. OVERVIEW OF THE ’097 PATENT

33. The ’097 Patent is entitled “Artificial Intelligence Segmentation of Tissue Images.” EX1001. The ’097 Patent discloses that it was common practice in pathology for human pathologists to “visually analyze thin slices of tumor tissue mounted on glass microscope slides to classify each region of the tissue as one of many tissue classes that are present in a tumor sample.” *Id.*, 1:29-39. The ’097 Patent further discloses that pathologists “will often assign one or more numerical scores to a slide” and that such scores can, for example, “include tumor purity, which is the percentage of the tissue that is formed by tumor cells.” *Id.*, 1:42-46. The ’097 Patent continues to state that such manual analysis is time consuming and subjective, and that high-resolution digital images of pathology slides “make it possible to use artificial intelligence to analyze the slides and classify the tissue components by tissue class.” *Id.*, 1:60-62. Accordingly, the ’097 Patent presents “[t]echniques for generating an overlay map on a digital medial image of a slide” that include “cell detection and tissue classification processes.” *Id.*, Abstract.

34. The ’097 Patent recites two independent claims (claims 1 and 6). Claim 1’s preamble recites a “method for creating an overlay map on a digital

image of a slide,” whereas claim 6’s preamble recites a “method for tissue classification of a digital image slide.” EX1001, cl. 1, 6. In both claims, the method then recites the following steps, which were all performed conventionally in the art:

[A] receiving the digital image;

[B] separating/segmenting the digital image into a plurality of tiles, each tile of the plurality of tiles containing a respective portion of the digital image of the slide;

[C] for each tile of the plurality of tiles: identifying features of the tile;

[D] identifying structural tissue features of a second portion of the digital image of the slide including at least part of one or more other tiles of the plurality of tiles;

[E] wherein the second portion is larger than the respective portion of the digital image contained in the tile;

[F] identifying the majority class of tissue visible within the tile /

determining a predicted class for each tile based at least in part on the features of the tile and the structural tissue features of the second portion of the digital image of the slide

35. Claim 6 then recites several additional limitations, which were also conventional in the art: [G] identifying a plurality of cell objects in the digital

image; [H] determining a predicted class for each of the plurality of cell objects; and; [I] for each of the plurality of tiles that corresponds to one of the plurality of cell objects, assigning the tile the predicted class of the corresponding cell object in place of the predicted class of the tile. EX1001, cl. 6.

36. The similarities and differences in the language of the independent claims are illustrated in the table below:

Claim 1	Claim 6
[Preamble] A method for creating an overlay map on a digital image of a slide, the method comprising:	[Preamble] A method for tissue classification of a digital image of a slide, the method comprising:
[A] receiving the digital image;	[A] receiving the digital image;
[B] separating the digital image into a plurality of tiles, each tile of the plurality of tiles containing a respective portion of the digital image of the slide; and	[B] segmenting the digital image into a plurality of tiles, each tile of the plurality of tiles containing a respective portion of the digital image of the slide;
[C] for each tile of the plurality of tiles: identifying features of the tile;	[C] for each tile of the plurality of tiles: identifying features of the tile;
[D] identifying structural tissue features of a second portion of the digital image of the slide including at least part of one or more other tiles of the plurality of tiles, wherein the second portion is larger than the respective portion of the digital image contained in the tile; and	[D] identifying structural tissue features of a second portion of the digital image of the slide including at least part of one or more other tiles of the plurality of tiles, wherein the second portion is larger than the respective portion of the digital image contained in the tile; and
[E] identifying the majority class of tissue visible within the tile based at least in part on the features of the tile	[E] determining a predicted class for each tile based at least in part on the features of the tile and the structural

and the structural tissue features of the second portion of the digital image of the slide.	tissue features of the second portion of the digital image of the slide;
	[F] identifying a plurality of cell objects in the digital image;
	[G] determining a predicted class for each of the plurality of cell objects; and
	[H] for each of the plurality of tiles that corresponds to one of the plurality of cell objects, assigning the tile the predicted class of the corresponding cell object in place of the predicted class of the tile.

37. In the paragraphs that follow, I have reproduced the full text of each independent claim and added letters (e.g., [A]) to track the specific steps.

38. Independent claim 1 of the '097 Patent recites:

A method for creating an overlay map on a digital image of a slide, the method comprising:

[A] receiving the digital image;

[B] separating the digital image into a plurality of tiles, each tile of the plurality of tiles containing a respective portion of the digital image of the slide; and

[C] for each tile of the plurality of tiles:

identifying features of the tile;

[D] identifying structural tissue features of a second portion of the digital image of the slide including at least

part of one or more other tiles of the plurality of tiles, wherein the second portion is larger than the respective portion of the digital image contained in the tile; and
[E] identifying the majority class of tissue visible within the tile based at least in part on the features of the tile and the structural tissue features of the second portion of the digital image of the slide.

39. Independent claim 6 of the '097 Patent recites:

A method for tissue classification of a digital image of a slide, the method comprising:

[A] receiving the digital image;

[B] segmenting the digital image into a plurality of tiles, each tile of the plurality of tiles containing a respective portion of the digital image of the slide;

[C] for each tile of the plurality of tiles:

identifying features of the tile;

[D] identifying structural tissue features of a second portion of the digital image of the slide including at least part of one or more other tiles of the plurality of tiles, wherein the second portion is larger than the respective portion of the digital image contained in the tile; and

[E] determining a predicted class for each tile based at least in part on the features of the tile and the structural tissue features of the second portion of the digital image of the slide;

[F] identifying a plurality of cell objects in the digital image;

[G] determining a predicted class for each of the plurality of cell objects; and

[H] for each of the plurality of tiles that corresponds to one of the plurality of cell objects, assigning the tile the predicted class of the corresponding cell object in place of the predicted class of the tile.

40. Dependent claims 2-5 and 7-18 are reproduced further below, where they are discussed in detail. At a high level, dependent claims 2, 3 and 7-15 recite specific additional limitations regarding the overlay image such as displaying outlines or polygons surrounding cells, or including information such as cell counts. Dependent claims 4, 5, and 18 recite specific tissue classes including epithelium, normal epithelium, immune, stroma, necrosis, blood, and fat, or structural tissue features including glands, ducts, vessels, and immune clusters. Dependent claim 16 recites the specific limitation wherein the recited cell objects comprise CD3, CD8, CD20, pancytokeratin, and smooth muscle actin.

41. I understand that the '097 Patent was filed on December 31, 2019, and claims priority to U.S. Prov. Patent Appl. No. 62/787,047, filed December 31, 2018. I therefore understand that at best, the '097 Patent is entitled to a priority date of no earlier than December 31, 2018.

VI. PRIOR ART

A. Chukka (EX1005)

42. U.S. Patent Publication No. 2016/0042511 A1 to Chukka et al. (“Chukka,” EX1005), entitled “Tissue Object-Based Machine Learning System for Automated Scoring of Digital Whole Slides” published on February 11, 2016. Chukka was filed on March 12, 2014, and claims priority to PCT/EP2014/054808. Chukka was filed by Ventana Medical Systems, Inc. I understand that Chukka may be considered in evaluating the patentability of the ’097 Patent claims.

43. Chukka discloses a “facility” including systems and methods “for providing a learning-based image analysis approach for the automated detection, classification, and counting of objects (e.g., cell nuclei) within digital pathology tissue slides.” EX1005 (Chukka), Abstract. Chukka discloses that “[a]t least some embodiments of the disclosed technology are directed to imaging systems for automatically interpreting and scoring tissue specimen slides, for example, specimens stained with an immunohistochemical (IHC) assay.” *Id.*, [0004]. Chukka further discloses creating an overlay drawing to label features of interest on the digital image. *Id.*

44. Chukka teaches that the disclosed system is able to analyze information and characteristics associated with a whole slide image and can identify “cellular structures (e.g., nuclear objects, nuclei seed) and cells ... based at least in part on information pertaining to data associated with tissue containing regions of the slide.” *Id.* Chukka’s disclosed system may also “count cells,

compute various types of local and global features of these cells, identify the cell types, and perform quantitative analysis.” *Id.* This system “automatically count[s] and classif[ies] cells and score the image and/or entire slide.” *Id.* For example, Chukka discloses that embodiments of the system may detect cells and classify them as either positively-stained nuclear objects, negatively-stained nuclear objects, stromata, or lymphocytes. *See id.* at [0006], [0026].

B. Jones (EX1006)

45. “Voronoi-based segmentation of cells on image manifolds” by Jones et al. (“Jones,” EX1006) published on October 21, 2005, in *International Workshop on Computer Vision for Biomedical Image Applications*. I understand that Jones may be considered in evaluating the patentability of the ’097 Patent claims.

46. Jones discloses an algorithm for determining cell-to-cell boundaries by finding the Voronoi region of each seed on a manifold with a metric controlled by local image properties. Jones’s method teaches “computing a discretized approximation of the Voronoi regions of each seed on a manifold with a metric controlled by local image features.” EX1006 (Jones), 537. Jones teaches that its method performs well at determining cellular boundaries when compared to manual segmentation. *Id.*, 542.

C. Sebastiao (EX1007)

47. International Publication No. WO 2015/124737 A1 to Sebastiao, et al., (“Sebastiao,” EX1007), entitled “Methods, Kits, and Systems for Scoring the Immune Response to Cancer by Simultaneous Detection of CD3, CD8, CD20, and FoxP3” was published on August 27, 2015. Sebastiao was also filed by Ventana Medical Systems, Inc. Srinivas Chukka, the first named inventor of Chukka, is listed as a named inventor on Sebastiao. I understand that Sebastiao may be considered in evaluating the patentability of the ’097 Patent claims.

48. Sebastiao discloses computer-implemented methods of scoring multiplex stained tissue slides for identifying and classifying multiple lymphocyte markers. EX1007 (Sebastiao), Abstract; *see also id.*, [0005]. Specifically, Sebastiao teaches an image analysis algorithm and system that “automatically computes an immune score from a set images of multiplex [immunohistochemical] slides.” *Id.*, [0014]. Sebastiao teaches that the algorithm receives a tissue image that has been stained with a multiplex assay for lymphocyte markers such as CD3, CD8, CD20, and FoxP3, and unmixes the image into separate image channels for each marker. *Id.* Sebastiao then teaches identifying regions of interest in each image channel, and then applying cell detection and classification algorithms on each image channel, or on combined and/or surrogated images generated from those image channels, to identify and

classify cells as, for example, false positive cells, CD3 only T-cells, CD3 and CD8 T-cells, FP3 T-cells; and CD20 B-cells. *Id.*

D. Gallas (EX1010)

49. “Evaluation environment for digital and analog pathology: a platform for validation studies” by Brandon D. Gallas, et al. (“Gallas,” EX1010) published in November 2014 in the *Journal of Medical Imaging*. I understand that Gallas may be considered in evaluating the patentability of the ’097 Patent claims.

50. Gallas discloses a platform (eeDAP) which allows for viewing and annotating digital histology slides. EX1010 (Gallas), Abstract. Gallas’s platform includes a computer connected to a monitor that displays digital images. EX1010 (Gallas), Fig. 2 (showing eeDAP hardware), Fig. 4 (screenshot showing display of digital images). Gallas describes separating digital images into tiles with reticles comprising “fine lines and grids that appear superimposed on the specimen” that allow the user to “narrow tasks to very small regions and individual cells.” EX1010 (Gallas), 7. Gallas’s reticles separate the digital image into tiles that may be individually and collectively analyzed.

VII. OVERVIEW OF THE LEVEL OF SKILL

51. I have been advised that “a person of ordinary skill in the relevant field” (or “person of ordinary skill in the art,” or “POSA”) is a hypothetical person to whom one could assign a routine task with reasonable confidence that the task

would be successfully carried out. I have been advised further that, in determining the level of ordinary skill in the art that someone would have had at the time the claimed invention was made, I should consider: (1) the levels of education and experience of persons working in the field; (2) the types of problems encountered in the field; and (3) the sophistication of the technology.

52. Based on my specialized education, knowledge, training, and experience, I am familiar with the level of skill of a POSA as of the critical date. It is my opinion that a POSA as of the critical date would have an advanced degree (e.g., Ph.D., M.S., or equivalent) in computer science, electrical engineering, computer engineering, or biomedical engineering focusing on computer vision, image processing, or machine learning. A POSA would have been familiar with prior art literature describing applications of deep learning, including convolutional neural networks, in the field of computer vision. A POSA would also have at least two years of experience working or consulting with others in the field of medical imaging, including digital pathology. A POSA would also have or could have less academic experience but significant professional experience in one or more of these fields. A POSA would have experience applying computer vision and machine learning in the field of “analysis of medical images and, more particularly, to techniques for analysis of histological slides other images of cancerous tissue” (EX1001, 1:15-18), and as

needed, could have consulted a pathologist, oncologist, or literature published by the same regarding histopathology.

VIII. CLAIM CONSTRUCTION

53. I have been advised that the claim terms of the '097 Patent claims are to be given the plain and ordinary meaning that the terms would have to a person of ordinary skill in the art in question at the relevant priority date. I understand that the analysis focuses on how the patentee used the claim term in the claims, specification, and prosecution history. I also understand that dictionaries or other extrinsic sources may assist in determining the plain and ordinary meaning but cannot override a meaning that is unambiguous from the intrinsic evidence. I have followed these principles in my analysis throughout this declaration.

IX. GROUND 1: CLAIMS 1, 4, 5, 6, 9-12, 14, 15, 17, AND 18 ARE ANTICIPATED BY CHUKKA

54. It is my opinion that claims 1, 4, 5, 6, 9-12, 14, 15, 17, and 18 are anticipated by Chukka. Below, I note specific prior art disclosures that satisfy each element of each challenged claim. After each claim element, I provide a chart identifying certain exemplary prior art disclosures that are relevant to that element.

55. At a high level, Chukka discloses every element of independent claim 1. Chukka teaches that “[a]t least some embodiments of the disclosed technology are directed to imaging systems for automatically interpreting and scoring tissue

specimen slides, for example, specimens stained with an immunohistochemical (IHC) assay.” *Id.*, [0004]. Chukka teaches that the system may identify, count, and classify cells in a digitized image of tissue slide. *Id.* For example, Chukka discloses that embodiments of the system may detect cells and classify them as either positively-stained nuclear objects, negatively-stained nuclear objects, stromata, or lymphocytes. *See id.* at [0006], [0026]. Chukka further teaches that in some embodiments, the system may produce an “overlay image” to “label features of interest in the image of a specimen from a subject.” *Id.*, [0004].

56. FIG. 3 of Chukka, reproduced below, is a “flow diagram illustrating the processing of an analyze slide component **112** in some embodiments.” *Id.*, [0039]. According to FIG. 3, component 112 “may tile the whole slide tissue data, or a portion thereof, based at least in part on a fixed or set number of tiles.” *Id.*, [0044]. Chukka teaches that the component may perform a coarse resolution tile-analysis by using “a single tile information from within the tiled region” and also “information from the whole slide context.” *Id.* For example, Chukka teaches that “[a]t a coarse resolution, tile-analysis can find coarse texture-based features to perform a reliable detection of stromal regions.” *Id.* at [0045]. Chukka further teaches that the component may identify structural tissue features such as cell density or lymphocyte clusters, based on contextual information in a second region surrounding each individual tile. *Id.* [0044-45]. Chukka teaches that using

this information to identify the majority tissue class of the tile, as e.g., lymphocyte clusters, positively-stained cells, negatively-stained cells, or stroma. *Id.*, [0044-45].

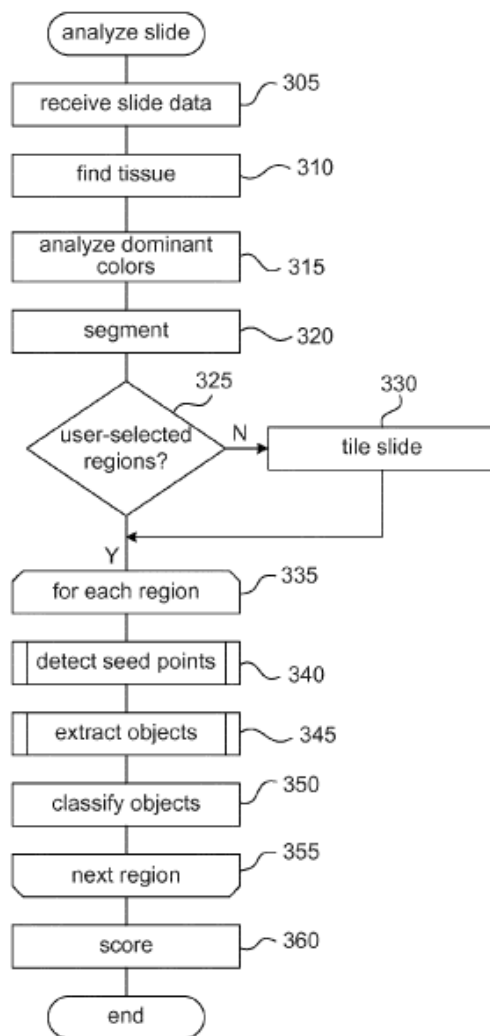


FIG. 3

57. Chukka performs a “quantitative analysis” of tissue features in select regions (e.g., tiles) and larger portions of the tissue slide. EX1005, Abstract, [0004]. Chukka describes analysis of regions (e.g., tiles) in a digital image as

based at least in part on tissue features in regions of the tissue slide other than the selected region. *See, e.g.*, EX1005, [0004] (“The system analyzes a region of an image...based at least in part on information and characteristics associated with the whole slide and selects features for quantitative analysis.”), *id.* (“The feature computation can use information from not only an annotated region of a slide but also information from the whole slide...”), [0020] (“The disclosed techniques can take advantage of the whole slide context...”), [0044] (“...information from the whole slide context...”), [0045] (“...use of context (e.g., surrounding tiles) provides extra information for classifying...”).

58. Chukka also teaches each limitation of independent claim 6. Claim 6 recites substantially the same elements as claim 1, with additional limitations of “[F] identifying a plurality of cell objects in the digital image;” “[G] determining a predicted class for each of the plurality of cell objects; and” “[H] for each of the plurality of tiles that corresponds to one of the plurality of cell objects, assigning the tile the predicted class of the corresponding cell object in place of the predicted class of the tile.” EX1001 (’097 Patent), cl. 6. Chukka teaches that in specific workflows (e.g., an “automated mode”), the tissue data is “segmented into tiles and classification and nuclei counting algorithms are performed with respect to each tile that contains tissue.” EX1005 (Chukka), [0010]. Chukka describes this automated mode in detail in FIG. 3, and in particular, teaches that an “initial

tile-analysis” may perform an “approximate segmentation” based on “coarse, texture-based features” as well as contextual information from surrounding tiles such as structural features (e.g., density, clusters).

59. Chukka teaches that after an initial “coarse-resolution” tile analysis of the tissue slide, the “analyze slide component” in the flow diagram, the component “loops through ... the tiles” and “ (1) “identif[ies] seeds within the [tile]” using a “detect seed points component[,]” (2) identifies objects within the tile using an “extract objects component[,]” and (3) “uses a classifier...to classify each of the extracted objects as a positively stained positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes.” *Id.*, [0046]. Accordingly, Chukka discloses a two-stage approach that teaches every limitation of independent claim 6.

60. Chukka also teaches dependent claims 4-5, 9-11, 14-15, and 17-18. Dependent claims 4, 5, 17 and 18 recite limitations wherein the majority class of tissue is epithelium, normal epithelium, immune, stroma, necrosis, blood, or fat (claim 4), the cell objects are lymphocytes (claim 17) or wherein the structural tissue features comprises at least one of glands, ducts, vessels, and immune clusters (claims 5, 18). Chukka teaches these additional limitations. *See, e.g.*, EX1005 (Chukka), [0006], [0044]. Dependent claims 9-11, and 14-15 recite various aspects of generating a digital overlay drawing for the digital image,

where the overlay drawing displays certain information (e.g., a histology mask, cell counts, percentages of predicted classes, etc.). Chukka expressly teaches that its facility may produce an overlay image to label “features of interest” in the image, and further teaches generating the relevant information such as predicted cell objects, cell classifications, or a histology mask.

A. Claim 1

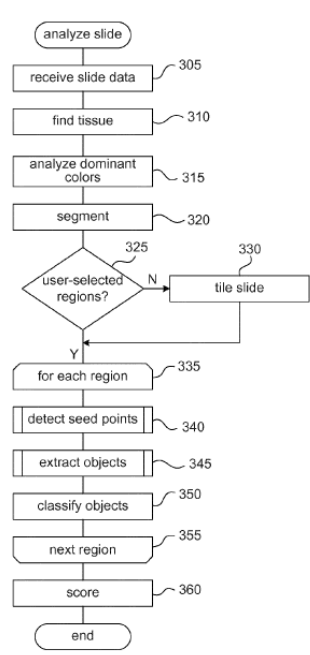
1. Preamble

61. Chukka discloses or teaches the preamble of claim 1, which recites “A method for creating an overlay map on a digital image of a slide, the method comprising:”

62. Chukka teaches that at least some embodiments of its disclosed invention “are directed to imaging systems for automatically interpreting and scoring tissue specimen slides.” EX1005 (Chukka), [0004]. Chukka teaches that this system analyzes the image, or regions of the image, and in some embodiments, produces “an overlay image...to label features of interest” in the image.” *Id.*; *see also id.*, [0010], [0040], [0044], Fig. 3.

63. Chukka therefore teaches a method for creating an overlay map on a digital image of slide; accordingly, Chukka teaches this element.

’097 Patent	Prior Art
A method for creating an overlay map on a digital	EX1005 (Chukka), [0004] (“At least some embodiments of the disclosed technology are

image of a slide, the method comprising:	directed to imaging systems for automatically interpreting and scoring tissue specimen slides, for example, specimens stained with an immunohistochemical (IHC) assay.... In some embodiments, an overlay image is produced to label features of interest in the image of a specimen from a subject.”); <i>see also id.</i>, [0010], [0040], [0044], Fig. 3.  <pre> graph TD 305([analyze slide]) --> 310[receive slide data] 310 --> 315[find tissue] 315 --> 320[analyze dominant colors] 320 --> 325[segment] 325 --> 330{user-selected regions?} 330 -- N --> 330 330 -- Y --> 335[for each region] 335 --> 340[detect seed points] 340 --> 345[extract objects] 345 --> 350[classify objects] 350 --> 355[next region] 355 --> 360[score] 360 --> end([end]) </pre> FIG. 3
---	---

2. Element 1[A]

64. Element 1[A] of claim 1 recites: “receiving the digital image[.]”

Chukka teaches this element.

65. Chukka teaches receiving a digital image of a tissue slide. Chukka teaches a “facility” that includes systems and methods for providing a “learning-based image analysis approach” to analyze digitized tissue slides. EX1005

(Chukka), Abstract. Chukka teaches that this facility “trains an object classifier” and “in response to *receiving a scanned image of a slide containing tissue data*, the facility separates the whole slide into a background region and a tissue region using image segmentation techniques.” *Id.* More specifically, Chukka teaches that the facility comprises, in some embodiments, a “supervised learning system for classifying objects within digitized images of tissue data” which includes, “means for receiving a digitized image of tissue data associated with an input slide” and “means for analyzing the digitized tissue data.” EX1005 (Chukka), [0008].

66. FIG. 3 of Chukka further teaches this element. FIG. 3 “is a flow diagram illustrating the processing of an analyze slide component **112** in some embodiments.” *Id.*, [0039]. Chukka teaches that the component is invoked by the system to analyze slide data. Specifically, Chukka teaches that “[i]n block **305**, the component receives slide data comprising digitized tissue data.” *Id.*

67. Chukka therefore teaches receiving a digital image. Accordingly, Chukka teaches this element.

'097 Patent	Prior Art
receiving the digital image;	EX1005 (Chukka), Abstract (“A facility includes systems and methods for providing a learning-based image analysis approach for the automated detection, classification, and counting of objects (e.g., cell nuclei) within digitized pathology tissue

	slides. The facility trains an object classifier using a plurality of reference sample slides. Subsequently, and in response to <i>receiving a scanned image of a slide containing tissue data</i>, the facility separates the whole slide into a background region and a tissue region using image segmentation techniques.”) EX1005 (Chukka), [0008] (“In further embodiments, a supervised learning system for classifying objects within digitized images of tissue data includes means for training a classifier based at least in part on ground truth slides, <i>means for receiving a digitized image of tissue data associated with an input slide</i>, and means for analyzing the digitized tissue data.”) EX1005 (Chukka), [0010] (“The system, in some embodiments, can receive a digitized image of data associated with an input slide with a sample from a subject.”) EX1005 (Chukka), [0012] (“The facility is further configured to train a tissue-object classifier using the received digitized images of tissue samples. Upon receiving a digitized image of data associated with a first slide, wherein the first slide is not a ground truth slide, the facility identifies 1) tissue within the digitized image of data associated with the first slide, 2) dominant colors within the identified tissue, and 3) regions within the identified tissue based at least in part on the identified dominant colors.”) EX1005 (Chukka), [0024] (“The facility can receive a scanned and digitized image of pathology tissue slide data to be analyzed. The slide data may be magnified (e.g., 1×, 2×, 5×, 20×, 40×, and so on).”)
--	---

EX1005 (Chukka), [0039] (“FIG. 3 is a flow diagram illustrating the processing of an analyze slide component 112 in some embodiments. The system invokes the component to analyze and captured slide data to be analyzed. In block 305, the component receives slide data comprising digitized tissue data.”)

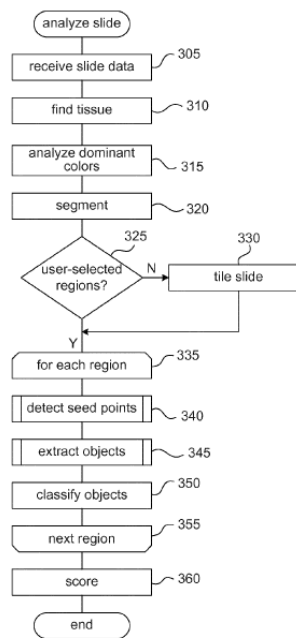


FIG. 3

3. Element 1[B]

68. Element 1[B] of claim 1 recites: “separating the digital image into a plurality of tiles, each tile of the plurality of tiles containing a respective portion of the digital image of the slide; and[.]” Chukka teaches this element.

69. Chukka teaches separating the digital image of a tissue slide into a plurality of tiles. Chukka, in FIG. 3, reproduced below, teaches a flow diagram “illustrating the processing of an analyze slide component 112 in some

embodiments” that analyzes the received slide image data. EX1005 (Chukka), [0039]. Chukka teaches that “In block **330**, the component **112** can use a grid pattern to tile the portion of the slide corresponding to the tissue data.” *Id.*, [0044]. Specifically, Chukka teaches that the component may tile the “whole slide tissue data, or a portion thereof” based on a grid pattern. *Id.* This grid pattern may be based on “a fixed or set number of tiles (e.g., a 5×5 grid, a 10×10 grid, a 3×10 grid, etc.), based at least in part on a number of pixels for each tile (e.g., 60 pixels by 60 pixels, 150 pixels by 150 pixels, 300 pixels by 1000 pixels, etc.), dimensions for each tile (e.g., 1 μm×1 μm, 1 mm×1 mm, etc.)” *Id.*

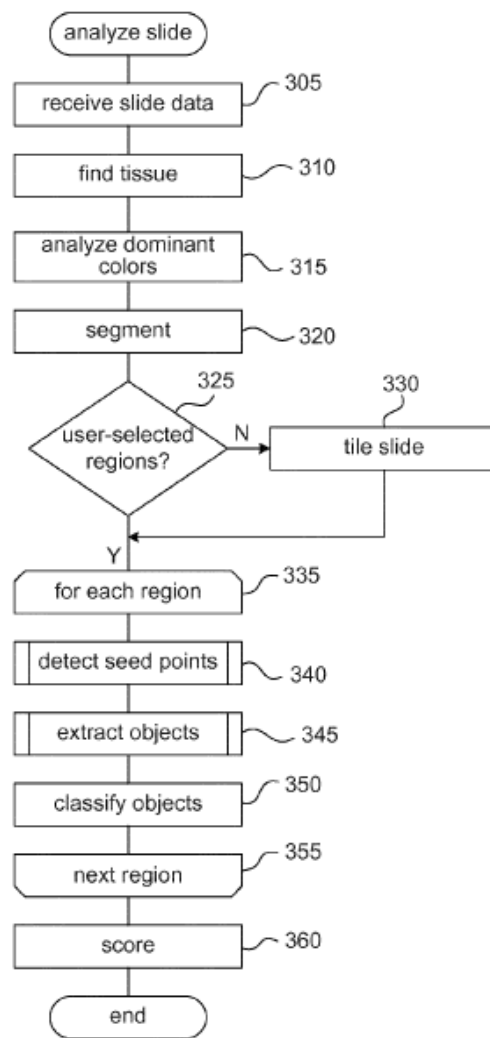
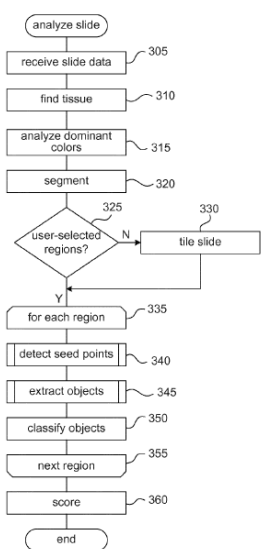


FIG. 3

70. Chukka additionally teaches performing an “image segmentation technique” and teaches the availability of “a wide range of image segmentation techniques (e.g., HSV color-based image segmentation, Lab image segmentation, mean-shift color image segmentation, region growing, level set methods, fast marching methods, etc.).” *Id.*, [0040]

71. Chukka therefore teaches separating the digital image into a plurality of tiles, each tile of the plurality of tiles containing a respective portion of the digital image. Accordingly, Chukka teaches this element.

'097 Patent	Prior Art
separating the digital image into a plurality of tiles, each tile of the plurality of tiles containing a respective portion of the digital image of the slide; and	EX1005 (Chukka), [0039] (“FIG. 3 is a flow diagram illustrating the processing of an analyze slide component 112 in some embodiments. The system invokes the component to analyze and captured slide data to be analyzed. In block 305, the component receives slide data comprising digitized tissue data.”).  <pre> graph TD Start([analyze slide]) --> 305[receive slide data] 305 --> 310[find tissue] 310 --> 315[analyze dominant colors] 315 --> 320[segment] 320 --> 325{user-selected regions?} 325 -- N --> 330[tile slide] 325 -- Y --> 335[for each region] 335 --> 340[detect seed points] 340 --> 345[extract objects] 345 --> 350[classify objects] 350 --> 355[next region] 355 --> 360[score] 360 --> End([end]) </pre> FIG. 3 EX1005 (Chukka) [0040] (“In block 310, the component performs an image segmentation technique to distinguish between the digitized tissue data and the slide in the image, the tissue corresponding to the foreground and the slide corresponding to the background. In some embodiments, the component computes the Area of Interest (AoI) in a whole slide image in order to detect all tissue regions in the AoI while limiting

	the amount of background non-tissue area that is analyzed. A wide range of image segmentation techniques (e.g., HSV color-based image segmentation, Lab image segmentation, mean-shift color image segmentation, region growing, level set methods, fast marching methods, etc.) can be used to determine, for example, boundaries of the tissue data and non-tissue or background data. Based at least in part on the segmentation, the component can also generate a tissue foreground mask that can be use to identify those portions of the digitized slide data that correspond to the tissue data. Alternatively, the component can generate a background mask used to identify those portions of the digitized slide date that do not correspond to the tissue data.”) EX1005 (Chukka), [0043] (“In decision block 325, if the user has identified or selected any FOVs of interest, then the component 112 continues at block 325, else the component continues at block 330. For example, the user may use a selection tool (e.g., mouse, joystick, icon such as a lasso tool or one or more key strokes or other mechanism) to select one or portions of the tissue displayed in the image as an FOV. These portions can be used in lieu of or in addition to automatically generated tiles of the slide.”) EX1005 (Chukka), [0044] (“In block 330, the component 112 can use a grid pattern to tile the portion of the slide corresponding to the tissue data. For example, the component may tile the whole slide tissue data, or a portion thereof, based at least in part on a fixed or set number of tiles (e.g., a 5×5 grid, a 10×10 grid, a 3×10 grid, etc.), based at least in part on a number of pixels for each tile (e.g., 60 pixels by 60 pixels, 150 pixels by 150 pixels, 300 pixels by 1000 pixels, etc.), dimensions for each tile (e.g., 1 μm×1 μm, 1 mm×1 mm, etc.), and so
--	---

	on. In some embodiments, the component may prompt the user to define the size/shape of each tile and/or the overall number of tiles along each dimension. In processing, a single tile image information from within the tiled region and also information from the whole slide context, which is extracted from different magnifications, and image information in the neighbor slides is used. Tile-based processing allows for the use of context-sensitive features that may not be present when using annotated FOV based workflow. For example, tile-based processing may enable the component to distinguish between negative tumor nuclei and lymphocytes, which can be important. Because Lymphocytes occur in clusters, lymphocyte context may or may not be adequately represented in a particular FOV. In contrast, when examining a particular tile, its neighboring tiles can be considered in order to provide additional context and a refined estimate of cell density. This additional context can help to discriminate between lymphocytes (generally densely packed) and negative tumor cells.”).
--	--

4. Element 1[C]

72. Element 1[C] of claim 1 recites: “for each tile of the plurality of tiles: identifying features of the tile[.]” Chukka teaches this element.

73. To begin with, the '097 Patent states that “visually detectable basic features” include “lines, patterns of alternating brightness, and outlineable shapes” and “may be combined to create visually detectable complex features including cell size, cell roundness, cell shape, and staining patterns referred to as texture features.” EX1001 ('097 Patent), 10:4-12.

74. As discussed above, Chukka teaches that the system receives a digitized image of data from a tissue slide, which may comprise tissue regions. Chukka teaches that in certain automated embodiments (e.g., an “automated mode”), the system finds or identifies a tissue region, and that this tissue region “is then segmented into tiles and classification and nuclei counting algorithms are performed with respect to each tile that contains tissue.” EX1005 (Chukka), [0010]. Chukka explains that an “analyze slide component 112” tiles the image into a plurality of tiles. *Id.*, [0044] (discussing “a fixed or set number of tiles...dimensions for each tile...and so on...” and “the size/shape of each tile and/or the overall number of tiles along each dimension.”). Chukka then teaches that “single tile information from within the tiled region” may be “extracted from different magnifications[.]” *Id.*

75. Chukka teaches that the automated workflow may identify regions within tissue “based at least in part on dominant colors.” *Id.*, [0010]. Chukka further teaches that in some embodiments, the automated workflow automatically identifies features “based at least in part on one or more selection criteria” such as “color characteristics, sample morphology ... tissue characteristics ... spatial parameters ... image characteristic parameters” as well as cellular “color characteristics,” “shape, dimensions, composition, ... spatial parameters ... image characteristics, combinations thereof, or the like.” *Id.*, [0011]. Chukka further

teaches that the automated workflow features within tiles such as “coarse texture features” and the color of the stain, e.g., “blue-stained cells.” *Id.*, [0045]. Chukka also explains that a “a pathologist” can replace “the automated score ...with a manual score (e.g., a score based at least in part on visual inspection).” *Id.*, [0005].

76. More specifically, Chukka teaches that “the component performs an image segmentation technique” to distinguish foreground from background pixels using “a wide range of image segmentation techniques (e.g., HSV color-based image segmentation, Lab image segmentation, mean-shift color image segmentation, region growing, level set methods, fast marching methods, etc.).” *Id.* [0040]. Chukka then teaches that the component “performs a dominant color analysis” of the tissue data to “identify those portions of the digitized slide data having an intensity value that exceeds a predetermined threshold in the dominant color space.” *Id.*, [0041]. Chukka then teaches that “[i]n block 320, the component 112 segments the digitized tissue data into glandular and stromal portions based at least in part on, for example, one or more of color, texture, a co-occurrence matrix, multi-scale Haar features, filter banks, and so on.” *Id.*, [0042].

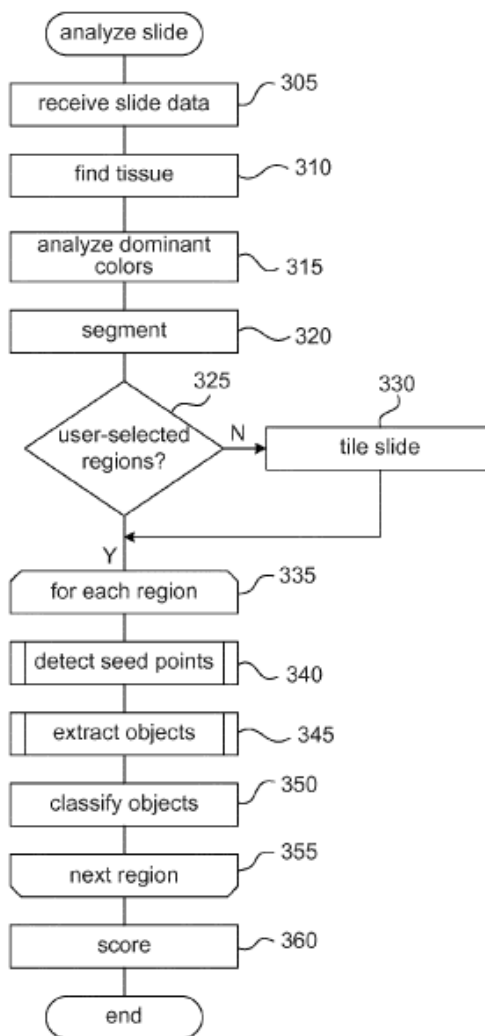


FIG. 3

77. Chukka therefore teaches for each tile of the plurality of tiles: identifying features of the tile. Accordingly, Chukka teaches this element.

'097 Patent	Prior Art
for each tile of the plurality of tiles: identifying features of the tile;	EX1005 (Chukka) [0010] (“In the automated mode, either an Area of Interest (AoI) detector finds or identifies a tissue region in the whole slide image or the tissue annotations are automatically generated by some other image analysis algorithm,

	such as image registration algorithm which maps annotations from the adjacent serial section to the IHC tissue slide. The tissue region is then segmented into tiles and classification and nuclei counting algorithms are performed with respect to each tile that contains tissue. Additionally, a composite score can be obtained based at least in part on the image tiles containing tissue. Though the underlying methodology for detecting, counting, and classifying cells in a given image are similar (the image may be a user annotated region or an automatically obtained tile in the whole slide image after AoI detection), there is at least one difference in the two workflows. The FoV mode relies on manual input in terms of FOV selection while the automated mode does not. The annotated FOV mode is further discussed with respect to FIG. 2 while the automated mode is further discussed with respect to FIG. 3. One or more regions within the identified tissue are identified based at least in part on dominant colors. For identified regions, seed points within the identified region are detected, and objects from the identified regions are extracted. Features of the extracted object(s) are computed such that the trained classifier classifies the extracted object(s) based at least in part on the computed features of the extracted object.”). EX1005 (Chukka) [0011] (“In some embodiments, a computer system can be programmed to automatically identify features in an image of a specimen based at least in part on one or more selection criteria, including criteria based at least in part on color characteristics, sample morphology (e.g., cell component morphology, cell morphology, tissue morphology, anatomical structure morphology, etc.), tissue characteristics (e.g., density, composition, or the like), spatial parameters (e.g., arrangement of tissue structures, relative positions between tissue structures, etc.),
--	---

image characteristic parameters, or the like. If the features are nuclei, the selection criteria can include, without limitation, color characteristics, nuclei morphology (e.g., shape, dimensions, composition, etc.), spatial parameters (e.g., position of nuclei in cellular structure, relative position between nuclei, etc.), image characteristics, combinations thereof, or the like.”).

EX1005 (Chukka), [0039] (“FIG. 3 is a flow diagram illustrating the processing of an analyze slide component **112** in some embodiments. The system invokes the component to analyze and captured slide data to be analyzed. In block **305**, the component receives slide data comprising digitized tissue data.”).

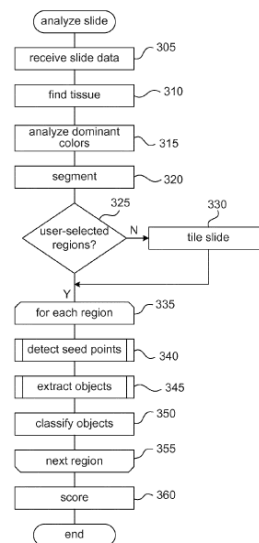


FIG. 3

EX1005 (Chukka) [0040] (“In block **310**, the component performs an image segmentation technique to distinguish between the digitized tissue data and the slide in the image, the tissue corresponding to the foreground and the slide corresponding to the background. In some embodiments, the component computes the Area of Interest (AoI) in a whole slide image in order to

	detect all tissue regions in the AoI while limiting the amount of background non-tissue area that is analyzed. A wide range of image segmentation techniques (e.g., HSV color-based image segmentation, Lab image segmentation, mean-shift color image segmentation, region growing, level set methods, fast marching methods, etc.) can be used to determine, for example, boundaries of the tissue data and non-tissue or background data. Based at least in part on the segmentation, the component can also generate a tissue foreground mask that can be use to identify those portions of the digitized slide data that correspond to the tissue data. Alternatively, the component can generate a background mask used to identify those portions of the digitized slide date that do not correspond to the tissue data.”) EX1005 (Chukka) [0041] (“In block 315, the component performs a dominant color analysis of the foreground data (i.e., the tissue data). The dominant color analysis includes, without limitation, a) determining the dominant colors in the tissue data across the entirety of the digitized slide data, b) projecting the RGB image data onto the dominant color space, c) performing a thresholding technique (such as adaptive color thresholding, Otsu's method, balanced histogram thresholding, or other thresholding techniques), to identify those portions of the digitized slide data having an intensity value that exceeds a predetermined threshold in the dominant color space. For example, if the dominant colors are blue and brown, the component can identify those portions having intensity values in the blue or brown color space that exceed a threshold. The threshold may be established by a user or may be established by the component based at least in part on the range of colors or intensities within each color space. For example, the threshold may be
--	--

	defined as the median color or intensity, the mean color or intensity, the color or intensity that is some predetermined number of standard deviations away from (above or below) the mean color or intensity value, and so on. Other color analysis techniques can be used.”) EX1005 (Chukka), [0042] (“In block 320, the component 112 segments the digitized tissue data into glandular and stromal portions based at least in part on, for example, one or more of color, texture, a co-occurrence matrix, multi-scale Haar features, filter banks, and so on. Moreover, the component may perform the segmentation process at various scales, resolution levels, or magnification levels to reduce the probability of misidentifying different portions.”) EX1005 (Chukka), [0044] (“In block 330, the component 112 can use a grid pattern to tile the portion of the slide corresponding to the tissue data. For example, the component may tile the whole slide tissue data, or a portion thereof, based at least in part on a fixed or set number of tiles (e.g., a 5×5 grid, a 10×10 grid, a 3×10 grid, etc.), based at least in part on a number of pixels for each tile (e.g., 60 pixels by 60 pixels, 150 pixels by 150 pixels, 300 pixels by 1000 pixels, etc.), dimensions for each tile (e.g., 1 μm×1 μm, 1 mm×1 mm, etc.), and so on. In some embodiments, the component may prompt the user to define the size/shape of each tile and/or the overall number of tiles along each dimension. In processing, a single tile image information from within the tiled region and also information from the whole slide context, which is extracted from different magnifications, and image information in the neighbor slides is used. Tile-based processing allows for the use of context-sensitive features that may not be present when using annotated FOV based workflow. For
--	--

	example, tile-based processing may enable the component to distinguish between negative tumor nuclei and lymphocytes, which can be important. Because Lymphocytes occur in clusters, lymphocyte context may or may not be adequately represented in a particular FOV. In contrast, when examining a particular tile, its neighboring tiles can be considered in order to provide additional context and a refined estimate of cell density. This additional context can help to discriminate between lymphocytes (generally densely packed) and negative tumor cells.”) EX1005 (Chukka), [0045] (“Another aspect where context based analysis is useful is that the whole slide image can be initially considered at a coarse resolution (e.g., 2×). Based on the initial tile-analysis, an approximate region segmentation process can be performed based at least in part on, for example, an identification of dense lymphocyte clusters. Accordingly, detected lymphocyte regions can be discarded while searching for, as an example, blue-stained tumor nuclei. Furthermore, reliable stromal region identification can assist in avoiding the counting of negative nuclei in stromal regions. At a coarse resolution, tile-analysis can find coarse texture-based features to perform a reliable detection of stromal regions. Thus, although the annotated FOV based workflow provides a relatively simple workflow, the whole slide based workflow presents many opportunities for intelligent image analysis. For example, use of context (e.g., surrounding tiles) provides extra information for classifying, for example, blue-stained cells (tumor nuclei can be discriminated from lymphocytes) based at least in part on their relative density and other features. As a result, context analysis can be used to perform a wide range of segmentation processes.”)
--	--

	EX1005 (Chukka), [0046] (“In blocks 335 to 355, the component loops through each region (i.e., the user-identified FOVs of interest or the tiles generated in block 330). In block 340, the component invokes a detect seed points component to identify seeds within the region that the component is currently processing. In block 345, the component invokes an extract objects component to identify the objects within the region that the component is currently processing. In block 350, the component uses a classifier, such as a classifier generated according to FIG. 2, to classify each of the extracted objects as a positively-stained nuclear object, a negatively-stained nuclear object, stroma, a lymphocyte, or an extraneous blob. In block 355, the component selects the next region, if any, and then loops back to block 335 for further processing. In block 360, the component generates and stores or displays a score for the slide or one or more regions within the slide by, for example, generating a positivity value (e.g., the ratio of positively-stained nuclear objects to the overall number of positively- and negatively-stained nuclear objects), calculating an H-score, or calculating another metric. Processing of the component is then completed. In some embodiments, the component may loop back to block 305 to continue receiving and analyzing slide data.”) EX1005 (Chukka), [0047] (“In some embodiments, the facility may pre-process a slide by tiling the slide and then detecting and classifying objects within the slide. In the event that a user wishes to perform an analysis of one or more particular regions or FOVs within a slide, the facility may identify all of the pre-processed tiles that intersect those one or more particular regions or FOVs and provide an analysis based at least in part on the
--	--

	intersection, pre-processed tiles rather than separately performing the identification and detection process for the one or more particular regions or FOVs. Moreover, the facility may perform the tiling and analysis at varying levels of granularity to increase accuracy.”)
--	--

5. Element 1[D]

78. Element 1[D] of claim 1 recites:

identifying structural tissue features of a second portion of the digital image of the slide including at least part of one or more other tiles of the plurality of tiles, wherein the second portion is larger than the respective portion of the digital image contained in the tile; and....

Chukka teaches this element.

79. To begin with, the '097 Patent states that “structural tissue features may include glands, ducts, vessels, immune clusters, etc.” EX1001 ('097 Patent), 15:32-34. Chukka teaches analyzing “a region of an image or an entire image.” EX1005 (Chukka), [0004], *see also id.*, [0007], [0010]. Chukka teaches that alongside identifying features within each region or tile, that the processing may also use “information from the whole slide context, which is extracted from different magnifications....” EX1005 (Chukka), [0044]. For instance, Chukka teaches that because lymphocytes occur in clusters, lymphocyte context may not be adequately represented in a particular FOV or tile. Accordingly, Chukka teaches that “when examining a particular tile, its neighboring tiles can be

considered in order to provide additional context and a refined estimate of cell density.” *Id.*, see also *id.*, [0045] (“For example, use of context (e.g., surrounding tiles) provides extra information for classifying, for example, blue-stained cells (tumor nuclei can be discriminated from lymphocytes) based at least in part on their relative density and other features. As a result, context analysis can be used to perform a wide range of segmentation processes.”).

80. Chukka therefore teaches identifying structural tissue features (such as lymphocyte clusters) of a second portion of the digital image including at least part of one or more other tiles of the plurality of tiles, wherein the second portion is larger than the respective portion of the digital image contained in the tile.

Accordingly, Chukka teaches this element.

'097 Patent	Prior Art
identifying structural tissue features of a second portion of the digital image of the slide including at least part of one or more other tiles of the plurality of tiles, wherein the second portion is larger than the respective portion of the digital image contained in the tile; and	EX1005 (Chukka), [0044] (“In block 330 , the component 112 can use a grid pattern to tile the portion of the slide corresponding to the tissue data. For example, the component may tile the whole slide tissue data, or a portion thereof, based at least in part on a fixed or set number of tiles (e.g., a 5×5 grid, a 10×10 grid, a 3×10 grid, etc.), based at least in part on a number of pixels for each tile (e.g., 60 pixels by 60 pixels, 150 pixels by 150 pixels, 300 pixels by 1000 pixels, etc.), dimensions for each tile (e.g., 1 μm×1 μm, 1 mm×1 mm, etc.), and so on. In some embodiments, the component may prompt the user to define the size/shape of each tile and/or the overall number of tiles along each dimension. In processing, a single tile image

	information from within the tiled region and also information from the whole slide context, which is extracted from different magnifications, and image information in the neighbor slides is used. Tile-based processing allows for the use of context-sensitive features that may not be present when using annotated FOV based workflow. For example, tile-based processing may enable the component to distinguish between negative tumor nuclei and lymphocytes, which can be important. Because Lymphocytes occur in clusters, lymphocyte context may or may not be adequately represented in a particular FOV. In contrast, when examining a particular tile, its neighboring tiles can be considered in order to provide additional context and a refined estimate of cell density. This additional context can help to discriminate between lymphocytes (generally densely packed) and negative tumor cells.”) EX1005 (Chukka), [0045] (“Another aspect where context based analysis is useful is that the whole slide image can be initially considered at a coarse resolution (e.g., 2×). Based on the initial tile-analysis, an approximate region segmentation process can be performed based at least in part on, for example, an identification of dense lymphocyte clusters. Accordingly, detected lymphocyte regions can be discarded while searching for, as an example, blue-stained tumor nuclei. Furthermore, reliable stromal region identification can assist in avoiding the counting of negative nuclei in stromal regions. At a coarse resolution, tile-analysis can find coarse texture-based features to perform a reliable detection of stromal regions. Thus, although the annotated FOV based workflow provides a relatively simple workflow, the whole slide based workflow presents many opportunities for intelligent image analysis. For example, use of context (e.g., surrounding tiles) provides extra
--	--

	information for classifying, for example, blue-stained cells (tumor nuclei can be discriminated from lymphocytes) based at least in part on their relative density and other features. As a result, context analysis can be used to perform a wide range of segmentation processes.”) EX1005 (Chukka), [0046] (“In blocks 335 to 355, the component loops through each region (i.e., the user-identified FOVs of interest or the tiles generated in block 330). In block 340, the component invokes a detect seed points component to identify seeds within the region that the component is currently processing. In block 345, the component invokes an extract objects component to identify the objects within the region that the component is currently processing. In block 350, the component uses a classifier, such as a classifier generated according to FIG. 2, to classify each of the extracted objects as a positively-stained nuclear object, a negatively-stained nuclear object, stroma, a lymphocyte, or an extraneous blob. In block 355, the component selects the next region, if any, and then loops back to block 335 for further processing. In block 360, the component generates and stores or displays a score for the slide or one or more regions within the slide by, for example, generating a positivity value (e.g., the ratio of positively-stained nuclear objects to the overall number of positively- and negatively-stained nuclear objects), calculating an H-score, or calculating another metric. Processing of the component is then completed. In some embodiments, the component may loop back to block 305 to continue receiving and analyzing slide data.”) EX1005 (Chukka), [0047] (“In some embodiments, the facility may pre-process a slide by tiling the slide and then detecting and classifying objects
--	---

	within the slide. In the event that a user wishes to perform an analysis of one or more particular regions or FOVs within a slide, the facility may identify all of the pre-processed tiles that intersect those one or more particular regions or FOVs and provide an analysis based at least in part on the intersection, pre-processed tiles rather than separately performing the identification and detection process for the one or more particular regions or FOVs. Moreover, the facility may perform the tiling and analysis at varying levels of granularity to increase accuracy.”)
--	---

6. Element 1[E]

81. Element 1[E] of claim recites: “identifying the majority class of tissue visible within the tile based at least in part on the features of the tile and the structural tissue features of the second portion of the digital image of the slide.” Chukka teaches this element.

82. To begin with, the '097 Patent states that “Examples of tissue classes include ... tumor, stroma, normal, immune cluster, necrosis, hyperplasia/dysplasia, red blood cells, and tissue classes or cell types that are positive ... or negative for an IHC stain target molecule....” EX1001 ('097 Patent), 6:32-38. Other examples include “tumor positive, tumor negative, lymphocyte positive, and lymphocyte negative.” *Id.*

83. Chukka discloses quantifying tissue features, including by “automatically count and classify cells and score the image and scor[ing] the

image....” EX1005 (Chukka), [0004]; *see also id.*, 0010] (“...classification and nuclei counting algorithms are performed with respect to each tile that contains tissue.”), [0036] (“each object can be assigned a probability of belonging to a particular one of tissue, stromal, or lymphatic regions based at least in part on the region segmentation.”), [0041] (“dominant color analysis”), [0045] (“use of context (e.g., surrounding tiles) provides extra information for classifying”).

84. As discussed above, Chukka teaches using an “analyze slide component 112” which may classify tissue slides using a tile-based approach. *See* EX1005 (Chukka), [0039-0044]. Chukka teaches that in this processing, “a single tile image information from within the tiled region and also information from the whole slide context, which is extracted from different magnifications, and image information in the neighbor slides is used.” *Id.*, [0044]. Chukka teaches that this tile-based processing may identify the majority tissue class visible within a tile by e.g., “distinguish[ing] between negative tumor nuclei and lymphocytes” using information regarding features of the tile in combination with structural tissue features (e.g., cell density or clustering). Chukka teaches that “[t]his additional context can help to discriminate between lymphocytes (generally densely packed) and negative tumor cells.” *Id.*

85. More specifically, Chukka teaches that in some embodiments, the analyze slide component may perform a “coarse resolution ... tile analysis.” *Id.*,

[0045]. Chukka teaches that “[b]ased on the initial tile-analysis, an approximate region segmentation process can be performed based at least in part on, for example, an identification of dense lymphocyte clusters.” *Id.* Chukka further teaches that “[a]t a coarse resolution, tile-analysis can find coarse texture-based features to perform a reliable detection of stromal regions.” *Id.* Finally, Chukka teaches that this coarse resolution tile analysis may use “context (e.g., surrounding tiles)” which “provides extra information for classifying, for example, blue-stained cells (tumor nuclei can be discriminated from lymphocytes) based at least in part on their relative density and other features.” *Id.*

86. Chukka therefore teaches identifying the majority class of tissue visible within the tile based at least in part on the features of the tile and the structural tissue features of the second portion of the digital image of the slide. Accordingly, Chukka teaches this element.

'097 Patent	Prior Art
identifying the majority class of tissue visible within the tile based at least in part on the features of the tile and the structural tissue features of the second portion of the digital image of the slide.	EX1005 (Chukka), [0039] (“FIG. 3 is a flow diagram illustrating the processing of an analyze slide component 112 in some embodiments. The system invokes the component to analyze and captured slide data to be analyzed. In block 305 , the component receives slide data comprising digitized tissue data.”).

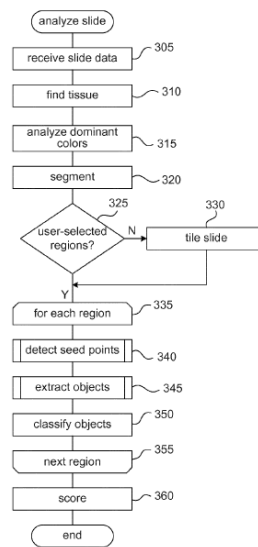


FIG. 3

EX1005 (Chukka) [0040] (“In block **310**, the component performs an image segmentation technique to distinguish between the digitized tissue data and the slide in the image, the tissue corresponding to the foreground and the slide corresponding to the background. In some embodiments, the component computes the Area of Interest (AoI) in a whole slide image in order to detect all tissue regions in the AoI while limiting the amount of background non-tissue area that is analyzed. A wide range of image segmentation techniques (e.g., HSV color-based image segmentation, Lab image segmentation, mean-shift color image segmentation, region growing, level set methods, fast marching methods, etc.) can be used to determine, for example, boundaries of the tissue data and non-tissue or background data. Based at least in part on the segmentation, the component can also generate a tissue foreground mask that can be use to identify those portions of the digitized slide data that correspond to the tissue data. Alternatively, the component can generate a background mask used to identify those portions of

	the digitized slide data that do not correspond to the tissue data.”) EX1005 (Chukka) [0041] (“In block 315, the component performs a dominant color analysis of the foreground data (i.e., the tissue data). The dominant color analysis includes, without limitation, a) determining the dominant colors in the tissue data across the entirety of the digitized slide data, b) projecting the RGB image data onto the dominant color space, c) performing a thresholding technique (such as adaptive color thresholding, Otsu's method, balanced histogram thresholding, or other thresholding techniques), to identify those portions of the digitized slide data having an intensity value that exceeds a predetermined threshold in the dominant color space. For example, if the dominant colors are blue and brown, the component can identify those portions having intensity values in the blue or brown color space that exceed a threshold. The threshold may be established by a user or may be established by the component based at least in part on the range of colors or intensities within each color space. For example, the threshold may be defined as the median color or intensity, the mean color or intensity, the color or intensity that is some predetermined number of standard deviations away from (above or below) the mean color or intensity value, and so on. Other color analysis techniques can be used.”) EX1005 (Chukka), [0042] (“In block 320, the component 112 segments the digitized tissue data into glandular and stromal portions based at least in part on, for example, one or more of color, texture, a co-occurrence matrix, multi-scale Haar features, filter banks, and so on. Moreover, the component may perform the segmentation process at various scales, resolution levels, or magnification
--	--

	levels to reduce the probability of misidentifying different portions.”) EX1005 (Chukka), [0044] (“In block 330, the component 112 can use a grid pattern to tile the portion of the slide corresponding to the tissue data. For example, the component may tile the whole slide tissue data, or a portion thereof, based at least in part on a fixed or set number of tiles (e.g., a 5×5 grid, a 10×10 grid, a 3×10 grid, etc.), based at least in part on a number of pixels for each tile (e.g., 60 pixels by 60 pixels, 150 pixels by 150 pixels, 300 pixels by 1000 pixels, etc.), dimensions for each tile (e.g., 1 μm×1 μm, 1 mm×1 mm, etc.), and so on. In some embodiments, the component may prompt the user to define the size/shape of each tile and/or the overall number of tiles along each dimension. In processing, a single tile image information from within the tiled region and also information from the whole slide context, which is extracted from different magnifications, and image information in the neighbor slides is used. Tile-based processing allows for the use of context-sensitive features that may not be present when using annotated FOV based workflow. For example, tile-based processing may enable the component to distinguish between negative tumor nuclei and lymphocytes, which can be important. Because Lymphocytes occur in clusters, lymphocyte context may or may not be adequately represented in a particular FOV. In contrast, when examining a particular tile, its neighboring tiles can be considered in order to provide additional context and a refined estimate of cell density. This additional context can help to discriminate between lymphocytes (generally densely packed) and negative tumor cells.”) EX1005 (Chukka), [0045] (“Another aspect where context based analysis is useful is that the whole
--	---

	slide image can be initially considered at a coarse resolution (e.g., 2×). Based on the initial tile-analysis, an approximate region segmentation process can be performed based at least in part on, for example, an identification of dense lymphocyte clusters. Accordingly, detected lymphocyte regions can be discarded while searching for, as an example, blue-stained tumor nuclei. Furthermore, reliable stromal region identification can assist in avoiding the counting of negative nuclei in stromal regions. At a coarse resolution, tile-analysis can find coarse texture-based features to perform a reliable detection of stromal regions. Thus, although the annotated FOV based workflow provides a relatively simple workflow, the whole slide based workflow presents many opportunities for intelligent image analysis. For example, use of context (e.g., surrounding tiles) provides extra information for classifying, for example, blue-stained cells (tumor nuclei can be discriminated from lymphocytes) based at least in part on their relative density and other features. As a result, context analysis can be used to perform a wide range of segmentation processes.”) EX1005 (Chukka), [0046] (“In blocks 335 to 355, the component loops through each region (i.e., the user-identified FOVs of interest or the tiles generated in block 330). In block 340, the component invokes a detect seed points component to identify seeds within the region that the component is currently processing. In block 345, the component invokes an extract objects component to identify the objects within the region that the component is currently processing. In block 350, the component uses a classifier, such as a classifier generated according to FIG. 2, to classify each of the extracted objects as a positively-stained nuclear object, a negatively-stained nuclear object, stroma, a lymphocyte, or an
--	--

	extraneous blob. In block 355, the component selects the next region, if any, and then loops back to block 335 for further processing. In block 360, the component generates and stores or displays a score for the slide or one or more regions within the slide by, for example, generating a positivity value (e.g., the ratio of positively-stained nuclear objects to the overall number of positively- and negatively-stained nuclear objects), calculating an H-score, or calculating another metric. Processing of the component is then completed. In some embodiments, the component may loop back to block 305 to continue receiving and analyzing slide data.”) EX1005 (Chukka), [0047] (“In some embodiments, the facility may pre-process a slide by tiling the slide and then detecting and classifying objects within the slide. In the event that a user wishes to perform an analysis of one or more particular regions or FOVs within a slide, the facility may identify all of the pre-processed tiles that intersect those one or more particular regions or FOVs and provide an analysis based at least in part on the intersection, pre-processed tiles rather than separately performing the identification and detection process for the one or more particular regions or FOVs. Moreover, the facility may perform the tiling and analysis at varying levels of granularity to increase accuracy.”)
--	---

B. Claim 4

87. Claim 4 depends on claim 1, and recites as follows: “The method of claim 1, wherein the majority class of tissue is selected from the group consisting

of epithelium, normal epithelium, immune, stroma, necrosis, blood, and fat.”

Chukka teaches this claim.

88. Chukka teaches that the disclosed facility “identif[ies] positive and negative nuclei,” distinguish “positive nuclei ... from the negative nuclei, lymphocytes, and stroma[,]” and distinguish “negative cells and lymphocytes” from stroma. EX1005 (Chukka), [0006]. Chukka also teaches that the facility can, in some embodiments, “classify cell nuclei as positively-stained nuclei or negatively-stained nuclei and can disregard other tissue (e.g., stromata, lymphocytes, etc.)” *Id.*, [0020].

89. Chukka therefore teaches identifying the majority class of tissue, wherein the majority class of tissue is either immune (e.g., lymphocyte clusters) or stroma. Accordingly, Chukka teaches this claim.

'097 Patent	Prior Art
The method of claim 1, wherein the majority class of tissue is selected from the group consisting of epithelium, normal epithelium, immune, stroma, necrosis, blood, and fat.	EX1005 (Chukka), [0006] (“In some embodiments, a multi-stage binary classifier can identify positive and negative nuclei. The positive nuclei can be distinguished from the negative nuclei, lymphocytes, and stroma. Additionally, the negative cells and lymphocytes can be distinguished from stroma.”) EX1005 (Chukka), [0020] (“To analyze a breast tissue sample, for example, the facility can classify cell nuclei as positively-stained nuclei or negatively-stained nuclei and can disregard other tissue (e.g., stromata, lymphocytes, etc.) to

	determine a score based at least in part on, for example, percent positive/negative, H-score, etc. In some embodiments, the facility may further identify extraneous artifacts or ‘junk’ features.”) EX1005 (Chukka), [0023] (“For each object in the ground truth slides, the ground truth data can identify the type of object (e.g., a positively-stained nuclear object, a negatively-stained nuclear object, stroma, or a lymphocyte).”)
--	---

C. Claim 5

90. Claim 5 depends on claim 1, and recites as follows: “The method of claim 1, wherein the structural tissue features comprise at least one of glands, ducts, vessels, and immune clusters.” Chukka teaches this claim.

91. As discussed above, Chukka teaches that its facility may invoke a “analyze cells component” which may use a tile-based approach to distinguish between negative tumor nuclei and lymphocytes, based on features identified in the tiles and contextual information in the region surrounding each tile. *Id.*, [0044]. Chukka teaches that because lymphocytes occur in clusters, lymphocyte context may not be adequately represented in a particular FOV or tile.

Accordingly, Chukka teaches that “when examining a particular tile, its neighboring tiles can be considered in order to provide additional context and a refined estimate of cell density.” *Id.* Chukka further teaches that the “initial tile-

analysis” provides “an approximate region segmentation process” based at least in part on “an identification of dense lymphocyte clusters.” *Id.*, [0045].

92. A POSA would understand that a lymphocyte cluster is an “immune cluster,” as lymphocytes are immune cells. *See* EX1001, 5:47-48 (CD3 associated with “T lymphocyte immune cells”); 6:32-42 (overlay may highlight immune cells such as lymphocytes); 22:24-26 (identifying “clusters of lymphocytes”).

93. Chukka therefore teaches a method wherein the structural tissue features comprise immune (e.g., lymphocyte) clusters. Accordingly, Chukka teaches this claim.

'097 Patent	Prior Art
The method of claim 1, wherein the structural tissue features comprises at least one of glands, ducts, vessels, and immune clusters.	EX1005 (Chukka), [0044] (“For example, tile-based processing may enable the component to distinguish between negative tumor nuclei and lymphocytes, which can be important. Because Lymphocytes occur in clusters, lymphocyte context may or may not be adequately represented in a particular FOV. In contrast, when examining a particular tile, its neighboring tiles can be considered in order to provide additional context and a refined estimate of cell density. This additional context can help to discriminate between lymphocytes (generally densely packed) and negative tumor cells.”) EX1005 (Chukka), [0045] (“Based on the initial tile-analysis, an approximate region segmentation process can be performed based at least in part on, for example, an identification of dense lymphocyte clusters. Accordingly, detected lymphocyte regions

	can be discarded while searching for, as an example, blue-stained tumor nuclei. Furthermore, reliable stromal region identification can assist in avoiding the counting of negative nuclei in stromal regions. At a coarse resolution, tile-analysis can find coarse texture-based features to perform a reliable detection of stromal regions.”)
--	---

D. Claim 6

1. Preamble

94. Chukka teaches the preamble of claim 6, which recites “A method for tissue classification of a digital image of a slide, the method comprising:....” As discussed above with regards to claim 1, Chukka teaches systems and methods of “providing a learning-based image analysis approach for the automated detection, classification, and counting of objects (e.g., cell nuclei) within digitized pathology tissue slides.” EX1005 (Chukka), Abstract; *see also id.*, [0020] (same).

'097 Patent	Prior Art
A method for tissue classification of a digital image of a slide, the method comprising:	EX1005 (Chukka), Abstract (“A facility includes systems and methods for providing a learning-based image analysis approach for the automated detection, classification, and counting of objects (e.g., cell nuclei) within digitized pathology tissue slides.”); <i>see also id.</i>, [0020] EX1005 (Chukka), [0004] (“The system can automatically count and classify cells and score the image and/or entire slide based at least in part on selected fields of view and/or the whole slide based at least in part on information or data associated

	with the whole slide (i.e., all of the tissue containing regions of the slide).”) EX1005 (Chukka), [0006] (“In some embodiments, a multi-stage binary classifier can identify positive and negative nuclei. The positive nuclei can be distinguished from the negative nuclei, lymphocytes, and stroma. Additionally, the negative cells and lymphocytes can be distinguished from stroma. Lymphocytes are then distinguished from the negative nuclei. In further classification, the positive cells can be distinguished from background cells.”)
--	---

2. Element 6[A]

95. Element 6[A] of claim 6 is identical to element 1[A] of claim 1, reciting “receiving the digital image[.]” Accordingly for the same reasons discussed above with regards to claim 1, Chukka teaches element 6[A] of claim 6.

3. Element 6[B]

96. Element 6[B] of claim 6 is substantially identical to element 1[B] of claim 1, reciting “segmenting the digital image into a plurality of tiles, each tile of the plurality of tiles containing a respective portion of the digital image of the slide[.]” It is my opinion that the ’097 Patent does not differentiate between the words “segment” and “separate.” Accordingly for the same reasons discussed above with regards to claim 1, Chukka teaches element 6[B] of claim 6.

4. Element 6[C]

97. Element 6[C] of claim 6 is identical to element 1[C] of claim 1, reciting “for each tile of the plurality of tiles: identifying features of the tile[.]” Accordingly for the same reasons discussed above with regards to claim 1, Chukka teaches element 6[C] of claim 6.

5. Element 6[D]

98. Element 6[D] of claim 6 is identical to element 1[D] of claim 1, reciting “identifying structural tissue features of a second portion of the digital image of the slide including at least part of one or more other tiles of the plurality of tiles, wherein the second portion is larger than the respective portion of the digital image contained in the tile; and...” Accordingly for the same reasons discussed above with regards to claim 1, Chukka teaches element 6[D] of claim 6.

6. Element 6[E]

99. Element 6[E] of claim 6 is substantially identical to element 1[E] of claim 1, reciting “determining a predicted class for each tile based at least in part on the features of the tile and the structural tissue features of the second portion of the digital image of the slide[.]” Accordingly for the same reasons discussed above with regards to claim 1, Chukka teaches element 6[E] of claim 6.

7. Element 6[F]

100. Element 6[F] of claim 6 recites: “identifying a plurality of cell objects in the digital image[.]” Chukka teaches this element.

101. Chukka discloses and teaches this element. Chukka generally teaches that its facility may use trained classifier models to identify “cellular structures (e.g., nuclear objects, nuclei seed) and cells in a region of a slide (e.g., a particular tissue region of the slide) or the whole slide, based at least in part on information pertaining to data associated with tissue containing regions of the slide.” EX1003, [0004]. Chukka’s system can “automatically count and classify cells,” “accurately count nuclear objects to determine information about the tissue,” and in one embodiment, “positively-stained nuclear objects and/or negatively stained nuclear objects to score, for example, a biological specimen (e.g., tumor tissue).” *Id.* Chukka further teaches using a multi-stage binary classifier to “identify positive and negative nuclei” and further distinguish positive nuclei from negative nuclei, lymphocytes, and stroma. *Id.*, [0006].

102. More specifically, and as discussed in reference to claim 1 and elements 6[A]-6[E], Chukka teaches that in the “automated mode” as illustrated in FIG. 3, “the tissue region is ... segmented into tiles and classification and nuclei counting algorithms are performed with respect to each tile that contains tissue.” EX1005 (Chukka), [0010]. Chukka teaches that as part of the flow diagram

illustrated in FIG. 3, the analyze slide component may first separate out the tissue data image into tiles, and perform a “coarse resolution” tile-based analysis of the image. See EX1005 (Chukka), [0044-0045]. Chukka then teaches that, as the next step in the flow diagram, the component “loops through ... the tiles” and invokes a “(1) “identify seeds within the [tile]” using a “detect seed points component[,]” (2) identifies objects within the tile using an “extract objects component[,]” and (3) “uses a classifier...to classify each of the extracted objects as a positively stained positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes.” *Id.*, [0046].

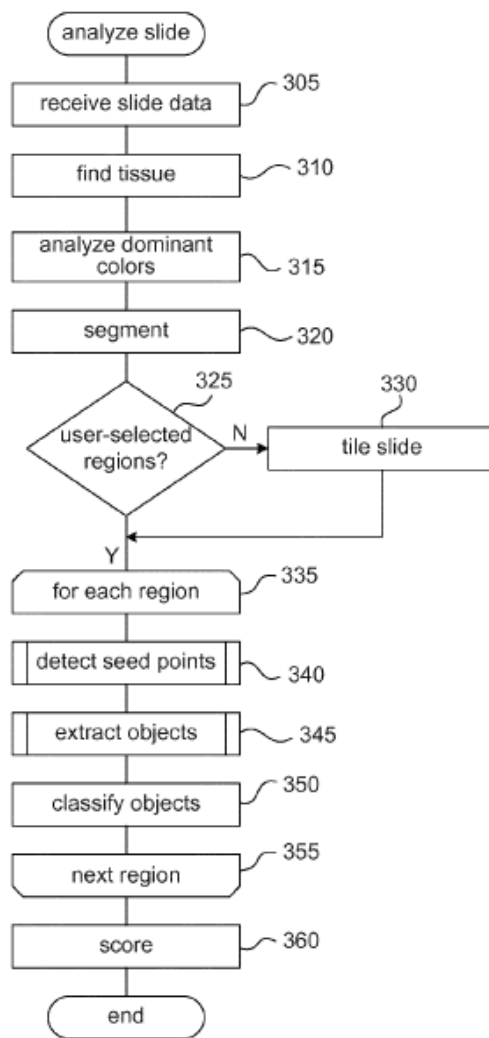


FIG. 3

103. Chukka teaches that identification of cell objects by invoking a “voting kernel process to identify objects or seed points, for example nuclear objects or nuclear seed points” within dominant color regions of the stained tissue slide. *Id.*, [0026]. Chukka teaches that these seed points may be “assumed to lie inside an object and are the starting point for localizing objects” such as nuclear objects or cells. *Id.* Accordingly, Chukka teaches generating a greyscale

representation of the digitized slide data and identifying local maxima as seed locations. *Id.* Chukka teaches that “in a parallel and independent step, for example, an adaptive thresholding technique can be applied to the tissue data to distinguish between darker objects (e.g., cell nuclei) and other objects, (e.g., stroma and slide background) and generate an object foreground mask.” *Id.* The object foreground mask and seed locations are used to create a tessellated version of the foreground image “where each distinct connected area of pixels or cells” “corresponds to a distinct detected object or ‘blob.’” *Id.*

104. Chukka therefore teaches identifying a plurality of cell objects in the digital image. Accordingly, Chukka teaches this element.

'097 Patent	Prior Art
identifying a plurality of cell objects in the digital image;	EX1005 (Chukka), [0004] (“The system can automatically count and classify cells and score the image and/or entire slide based at least in part on selected fields of view and/or the whole slide based at least in part on information or data associated with the whole slide (i.e., all of the tissue containing regions of the slide).”) EX1005 (Chukka), [0006] (“In some embodiments, a multi-stage binary classifier can identify positive and negative nuclei. The positive nuclei can be distinguished from the negative nuclei, lymphocytes, and stroma. Additionally, the negative cells and lymphocytes can be distinguished from stroma. Lymphocytes are then distinguished from the negative nuclei. In further

	classification, the positive cells can be distinguished from background cells.”) EX1005 (Chukka) [0010] (“In the automated mode, either an Area of Interest (AoI) detector finds or identifies a tissue region in the whole slide image or the tissue annotations are automatically generated by some other image analysis algorithm, such as image registration algorithm which maps annotations from the adjacent serial section to the IHC tissue slide. The tissue region is then segmented into tiles and classification and nuclei counting algorithms are performed with respect to each tile that contains tissue. Additionally, a composite score can be obtained based at least in part on the image tiles containing tissue. Though the underlying methodology for detecting, counting, and classifying cells in a given image are similar (the image may be a user annotated region or an automatically obtained tile in the whole slide image after AoI detection), there is at least one difference in the two workflows. The FoV mode relies on manual input in terms of FOV selection while the automated mode does not. The annotated FOV mode is further discussed with respect to FIG. 2 while the automated mode is further discussed with respect to FIG. 3. One or more regions within the identified tissue are identified based at least in part on dominant colors. For identified regions, seed points within the identified region are detected, and objects from the identified regions are extracted. Features of the extracted object(s) are computed such that the trained classifier classifies the extracted object(s) based at least in part on the computed features of the extracted object.”). EX1005 (Chukka), [0026] (“In some embodiments, the facility invokes a voting kernel process to identify objects or seed points, for example, nuclear objects or nuclear seed points, within the two
--	--

	dominant color regions. Seed points can be points that are assumed to lie inside an object and are the starting point for localizing objects (e.g., nuclear objects or other features of interest). In other words, seed identification can identify the approximate center point or other internal point for objects. As discussed in further detail below with reference to FIG. 4A, to identify seed objects, the facility can generate a grayscale representation of the digitized slide data and then computes gradient information from the grayscale representation. The facility then generates a vote response matrix by, for each pixel having a gradient magnitude that exceeds a pre-defined threshold, casting a vote to that pixels local neighborhood. The local neighborhood is determined based at least in part on the local gradient direction and a pre-determined distance or radius range. Subsequently, each local maximum within with the vote response matrix that exceeds a voting threshold can be identified as a seed location. In a parallel and independent step, for example, an adaptive thresholding technique can be applied to the tissue data to distinguish between darker objects (e.g., cell nuclei) and other objects, (e.g., stroma and slide background) and generate an object foreground mask. The object foreground mask and the seed locations are used in combination to generate a tessellated version of the foreground image, where each distinct connected area of pixels or cell in the tessellated version corresponds to a distinct detected object or “blob.” Each “blob” is then classified using a classifier (e.g., a previously-trained classifier) to identify one or more of positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes. Once classified, the detected nuclear objects can be used to score a slide or particular regions within a slide. Thus, the disclosed techniques take advantage of whole slide context and machine learning techniques to improve
--	---

nuclear object detection and automated slide scoring.”)

EX1005 (Chukka), [0039] (“FIG. 3 is a flow diagram illustrating the processing of an analyze slide component **112** in some embodiments. The system invokes the component to analyze and captured slide data to be analyzed. In block **305**, the component receives slide data comprising digitized tissue data.”).

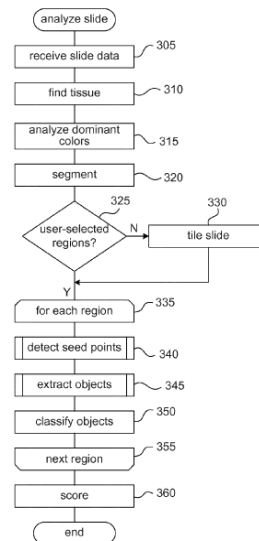


FIG. 3

EX1005 (Chukka) [0040] (“In block **310**, the component performs an image segmentation technique to distinguish between the digitized tissue data and the slide in the image, the tissue corresponding to the foreground and the slide corresponding to the background. In some embodiments, the component computes the Area of Interest (AoI) in a whole slide image in order to detect all tissue regions in the AoI while limiting the amount of background non-tissue area that is analyzed. A wide range of image segmentation techniques (e.g., HSV color-based image segmentation, Lab image segmentation, mean-shift color image segmentation, region growing, level set

	methods, fast marching methods, etc.) can be used to determine, for example, boundaries of the tissue data and non-tissue or background data. Based at least in part on the segmentation, the component can also generate a tissue foreground mask that can be use to identify those portions of the digitized slide data that correspond to the tissue data. Alternatively, the component can generate a background mask used to identify those portions of the digitized slide date that do not correspond to the tissue data.”) EX1005 (Chukka) [0041] (“In block 315, the component performs a dominant color analysis of the foreground data (i.e., the tissue data). The dominant color analysis includes, without limitation, a) determining the dominant colors in the tissue data across the entirety of the digitized slide data, b) projecting the RGB image data onto the dominant color space, c) performing a thresholding technique (such as adaptive color thresholding, Otsu's method, balanced histogram thresholding, or other thresholding techniques), to identify those portions of the digitized slide data having an intensity value that exceeds a predetermined threshold in the dominant color space. For example, if the dominant colors are blue and brown, the component can identify those portions having intensity values in the blue or brown color space that exceed a threshold. The threshold may be established by a user or may be established by the component based at least in part on the range of colors or intensities within each color space. For example, the threshold may be defined as the median color or intensity, the mean color or intensity, the color or intensity that is some predetermined number of standard deviations away from (above or below) the mean color or intensity value, and so on. Other color analysis techniques can be used.”)
--	--

	EX1005 (Chukka), [0042] (“In block 320, the component 112 segments the digitized tissue data into glandular and stromal portions based at least in part on, for example, one or more of color, texture, a co-occurrence matrix, multi-scale Haar features, filter banks, and so on. Moreover, the component may perform the segmentation process at various scales, resolution levels, or magnification levels to reduce the probability of misidentifying different portions.”) EX1005 (Chukka), [0044] (“In block 330, the component 112 can use a grid pattern to tile the portion of the slide corresponding to the tissue data. For example, the component may tile the whole slide tissue data, or a portion thereof, based at least in part on a fixed or set number of tiles (e.g., a 5×5 grid, a 10×10 grid, a 3×10 grid, etc.), based at least in part on a number of pixels for each tile (e.g., 60 pixels by 60 pixels, 150 pixels by 150 pixels, 300 pixels by 1000 pixels, etc.), dimensions for each tile (e.g., 1 μm×1 μm, 1 mm×1 mm, etc.), and so on. In some embodiments, the component may prompt the user to define the size/shape of each tile and/or the overall number of tiles along each dimension. In processing, a single tile image information from within the tiled region and also information from the whole slide context, which is extracted from different magnifications, and image information in the neighbor slides is used. Tile-based processing allows for the use of context-sensitive features that may not be present when using annotated FOV based workflow. For example, tile-based processing may enable the component to distinguish between negative tumor nuclei and lymphocytes, which can be important. Because Lymphocytes occur in clusters, lymphocyte context may or may not be adequately represented in a particular FOV. In contrast, when
--	--

	examining a particular tile, its neighboring tiles can be considered in order to provide additional context and a refined estimate of cell density. This additional context can help to discriminate between lymphocytes (generally densely packed) and negative tumor cells.”) EX1005 (Chukka), [0045] (“Another aspect where context based analysis is useful is that the whole slide image can be initially considered at a coarse resolution (e.g., 2×). Based on the initial tile-analysis, an approximate region segmentation process can be performed based at least in part on, for example, an identification of dense lymphocyte clusters. Accordingly, detected lymphocyte regions can be discarded while searching for, as an example, blue-stained tumor nuclei. Furthermore, reliable stromal region identification can assist in avoiding the counting of negative nuclei in stromal regions. At a coarse resolution, tile-analysis can find coarse texture-based features to perform a reliable detection of stromal regions. Thus, although the annotated FOV based workflow provides a relatively simple workflow, the whole slide based workflow presents many opportunities for intelligent image analysis. For example, use of context (e.g., surrounding tiles) provides extra information for classifying, for example, blue-stained cells (tumor nuclei can be discriminated from lymphocytes) based at least in part on their relative density and other features. As a result, context analysis can be used to perform a wide range of segmentation processes.”) EX1005 (Chukka), [0046] (“In blocks 335 to 355, the component loops through each region (i.e., the user-identified FOVs of interest or the tiles generated in block 330). In block 340, the component invokes a detect seed points component to identify seeds within the region that the
--	---

	component is currently processing. In block 345, the component invokes an extract objects component to identify the objects within the region that the component is currently processing. In block 350, the component uses a classifier, such as a classifier generated according to FIG. 2, to classify each of the extracted objects as a positively-stained nuclear object, a negatively-stained nuclear object, stroma, a lymphocyte, or an extraneous blob. In block 355, the component selects the next region, if any, and then loops back to block 335 for further processing. In block 360, the component generates and stores or displays a score for the slide or one or more regions within the slide by, for example, generating a positivity value (e.g., the ratio of positively-stained nuclear objects to the overall number of positively- and negatively-stained nuclear objects), calculating an H-score, or calculating another metric. Processing of the component is then completed. In some embodiments, the component may loop back to block 305 to continue receiving and analyzing slide data.”) EX1005 (Chukka), [0047] (“In some embodiments, the facility may pre-process a slide by tiling the slide and then detecting and classifying objects within the slide. In the event that a user wishes to perform an analysis of one or more particular regions or FOVs within a slide, the facility may identify all of the pre-processed tiles that intersect those one or more particular regions or FOVs and provide an analysis based at least in part on the intersection, pre-processed tiles rather than separately performing the identification and detection process for the one or more particular regions or FOVs. Moreover, the facility may perform the tiling and analysis at varying levels of granularity to increase accuracy.”) EX1005 (Chukka), [0051] (“FIG. 5 is a flow
--	---

diagram illustrating the processing of an extract objects component **114** in some embodiments. The facility invokes the component to identify boundaries around seed points that correspond to an object. Processing of the component is based at least in part on image information generated in steps **305-320** discussed above (e.g., tissue foreground mask, dominant color analysis, segmentation) and thus is based, in part, on context generated from the whole slide. In block **510**, the component generates an object foreground mask by identifying those pixels that are darker than their local neighborhood intensity distribution and that exceed a global threshold. The object foreground mask may be generated, for example, by assigning a value of 1 for all pixels whose intensity value exceeds a mean intensity value for a local portion of the tissue (e.g., all pixels within the region currently being analyzed) and that also exceed a global threshold (e.g., 0.85). For pixels that do not meet these criteria, the component may assign another value, such as 0. The object foreground mask allows the facility to prune out weak and spurious objects that are unlikely to correspond to any object of interest (e.g., positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes). In block **520**, the component applies the generated mask to the digitized tissue data of the region currently being analyzed. In block **530**, the component retrieves the seed points that correspond to the region currently being analyzed. In block **540**, the component generates a tessellation of the foreground image (e.g., a Voronoi tessellation) data based at least in part on the retrieved seed points to identify those portions of the foreground image data that are associated with each of the seed points. In block **550**, the component identifies, for each seed point, the portion of the foreground image that is associated with the seed point based at least in

	part on the tessellation. This portion or “blob” can be associated with the seed and can define the boundary of a detected object. In block 560, the component characterizes each of the identified objects based at least in part on location and any number of characteristics including, for example, color characteristics, shape and size characteristics, object descriptor characteristics, cytoplasmic characteristics, inter-object and density characteristics, or any of the above-mentioned characteristics. Each of these identified objects is then returned for storage and classification by a trained classifier as discussed above.”)
--	--

8. Element 6[G]

105. Element 6[G] of claim 6 recites: “determining a predicted class for each of the plurality of cell objects; and[.]” Chukka teaches this element.

106. As discussed above, Chukka teaches that in the “automated mode” as illustrated in FIG. 3, “the tissue region is ... segmented into tiles and classification and nuclei counting algorithms are performed with respect to each tile that contains tissue.” EX1005 (Chukka), [0010]. Chukka teaches that in this embodiment, the facility may comprise an “analyze slide component” which tiles the image of the tissue slide. See EX1005 (Chukka), [0044]. Chukka teaches that after tiling the image and performing an initial, coarse resolution tile-based analysis, the component then “loops through” the tiles and invokes a “ (1) “identify seeds within the [tile]” using a “detect seed points component[.]” (2)

identifies objects within the tile using an “extract objects component[,]” and (3) “uses a classifier...to classify each of the extracted objects as a positively stained positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes.” *Id.*, [0046]; *see also id.*, [0026] (teaching to use an object foreground mask and seed locations to identify cellular “blobs” and then classifying each “blob...using a classifier (e.g., a previously-trained classifier) to identify one or more of positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes.”).

107. FIG. 2 of Chukka, reproduced below, is a “block diagram illustrating the processing of a construct classifier component” such as the classifier described in Chukka’s FIG. 3. *See id.*, [0035]. Chukka teaches using this classifier to classify each extracted object in the tiled image of the tissue slide. *Id.*, [0046].

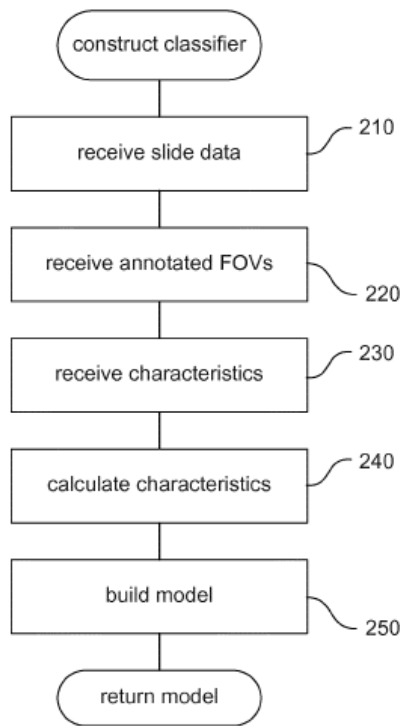


FIG. 2

108. Chukka therefore teaches determining a predicted class for each of the plurality of cell objects. Accordingly, Chukka teaches this element.

'097 Patent	Prior Art
determining a predicted class for each of the plurality of cell objects; and	EX1005 (Chukka) [0010] (“In the automated mode, either an Area of Interest (AoI) detector finds or identifies a tissue region in the whole slide image or the tissue annotations are automatically generated by some other image analysis algorithm, such as image registration algorithm which maps annotations from the adjacent serial section to the IHC tissue slide. The tissue region is then segmented into tiles and classification and nuclei counting algorithms are performed with respect to each tile that contains tissue. Additionally, a composite score can be obtained based at least in part on the image tiles containing tissue. Though

	the underlying methodology for detecting, counting, and classifying cells in a given image are similar (the image may be a user annotated region or an automatically obtained tile in the whole slide image after AoI detection), there is at least one difference in the two workflows. The FoV mode relies on manual input in terms of FOV selection while the automated mode does not. The annotated FOV mode is further discussed with respect to FIG. 2 while the automated mode is further discussed with respect to FIG. 3. One or more regions within the identified tissue are identified based at least in part on dominant colors. For identified regions, seed points within the identified region are detected, and objects from the identified regions are extracted. Features of the extracted object(s) are computed such that the trained classifier classifies the extracted object(s) based at least in part on the computed features of the extracted object.”). EX1005 (Chukka), [0026] (“In some embodiments, the facility invokes a voting kernel process to identify objects or seed points, for example, nuclear objects or nuclear seed points, within the two dominant color regions. Seed points can be points that are assumed to lie inside an object and are the starting point for localizing objects (e.g., nuclear objects or other features of interest). In other words, seed identification can identify the approximate center point or other internal point for objects. As discussed in further detail below with reference to FIG. 4A, to identify seed objects, the facility can generate a grayscale representation of the digitized slide data and then computes gradient information from the grayscale representation. The facility then generates a vote response matrix by, for each pixel having a gradient magnitude that exceeds a pre-defined threshold, casting a vote to that pixels local neighborhood. The local neighborhood is determined based at least in part on the local
--	---

	gradient direction and a pre-determined distance or radius range. Subsequently, each local maximum within with the vote response matrix that exceeds a voting threshold can be identified as a seed location. In a parallel and independent step, for example, an adaptive thresholding technique can be applied to the tissue data to distinguish between darker objects (e.g., cell nuclei) and other objects, (e.g., stroma and slide background) and generate an object foreground mask. The object foreground mask and the seed locations are used in combination to generate a tessellated version of the foreground image, where each distinct connected area of pixels or cell in the tessellated version corresponds to a distinct detected object or “blob.” Each “blob” is then classified using a classifier (e.g., a previously-trained classifier) to identify one or more of positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes. Once classified, the detected nuclear objects can be used to score a slide or particular regions within a slide. Thus, the disclosed techniques take advantage of whole slide context and machine learning techniques to improve nuclear object detection and automated slide scoring.”) EX1005 (Chukka), [0035] (“FIG. 2 is a block diagram illustrating the processing of a construct classifier component 111 in some embodiments. The system invokes the construct classifier component 111 to construct an object classifier model that is used to classify detected objects as, for example, positively-stained nuclear objects, negatively-stained nuclear objects, stromata, or lymphocytes. In block 210, the component receives slide data. The received slide data can correspond to “ground truth” slides that the facility uses to generate the model. In block 220, the component receives information about the slides, such as an
--	--

indication of annotated FOVs for each slide. Each FOV can correspond to a portion of the slide that is interesting to the user, such as portions that contain one or more types of objects. Each object is identified by a particular location within a particular slide and an indication of whether it is a positively-stained nuclear object, a negatively-stained nuclear object, stroma, or a lymphocyte.”).

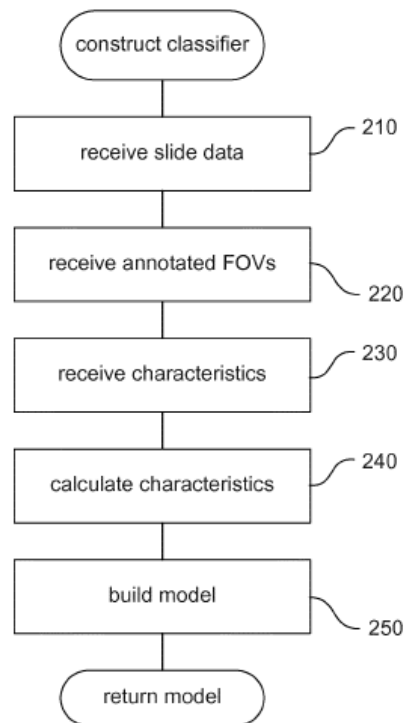


FIG. 2

EX1005 (Chukka), [0039] (“FIG. 3 is a flow diagram illustrating the processing of an analyze slide component **112** in some embodiments. The system invokes the component to analyze and captured slide data to be analyzed. In block **305**, the component receives slide data comprising digitized tissue data.”).

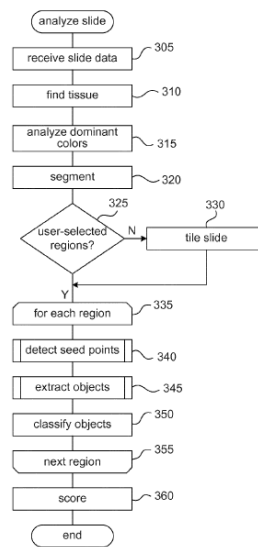


FIG. 3

EX1005 (Chukka) [0040] (“In block **310**, the component performs an image segmentation technique to distinguish between the digitized tissue data and the slide in the image, the tissue corresponding to the foreground and the slide corresponding to the background. In some embodiments, the component computes the Area of Interest (AoI) in a whole slide image in order to detect all tissue regions in the AoI while limiting the amount of background non-tissue area that is analyzed. A wide range of image segmentation techniques (e.g., HSV color-based image segmentation, Lab image segmentation, mean-shift color image segmentation, region growing, level set methods, fast marching methods, etc.) can be used to determine, for example, boundaries of the tissue data and non-tissue or background data. Based at least in part on the segmentation, the component can also generate a tissue foreground mask that can be use to identify those portions of the digitized slide data that correspond to the tissue data. Alternatively, the component can generate a background mask used to identify those portions of

	the digitized slide data that do not correspond to the tissue data.”) EX1005 (Chukka) [0041] (“In block 315, the component performs a dominant color analysis of the foreground data (i.e., the tissue data). The dominant color analysis includes, without limitation, a) determining the dominant colors in the tissue data across the entirety of the digitized slide data, b) projecting the RGB image data onto the dominant color space, c) performing a thresholding technique (such as adaptive color thresholding, Otsu's method, balanced histogram thresholding, or other thresholding techniques), to identify those portions of the digitized slide data having an intensity value that exceeds a predetermined threshold in the dominant color space. For example, if the dominant colors are blue and brown, the component can identify those portions having intensity values in the blue or brown color space that exceed a threshold. The threshold may be established by a user or may be established by the component based at least in part on the range of colors or intensities within each color space. For example, the threshold may be defined as the median color or intensity, the mean color or intensity, the color or intensity that is some predetermined number of standard deviations away from (above or below) the mean color or intensity value, and so on. Other color analysis techniques can be used.”) EX1005 (Chukka), [0042] (“In block 320, the component 112 segments the digitized tissue data into glandular and stromal portions based at least in part on, for example, one or more of color, texture, a co-occurrence matrix, multi-scale Haar features, filter banks, and so on. Moreover, the component may perform the segmentation process at various scales, resolution levels, or magnification
--	--

	levels to reduce the probability of misidentifying different portions.”) EX1005 (Chukka), [0044] (“In block 330, the component 112 can use a grid pattern to tile the portion of the slide corresponding to the tissue data. For example, the component may tile the whole slide tissue data, or a portion thereof, based at least in part on a fixed or set number of tiles (e.g., a 5×5 grid, a 10×10 grid, a 3×10 grid, etc.), based at least in part on a number of pixels for each tile (e.g., 60 pixels by 60 pixels, 150 pixels by 150 pixels, 300 pixels by 1000 pixels, etc.), dimensions for each tile (e.g., 1 μm×1 μm, 1 mm×1 mm, etc.), and so on. In some embodiments, the component may prompt the user to define the size/shape of each tile and/or the overall number of tiles along each dimension. In processing, a single tile image information from within the tiled region and also information from the whole slide context, which is extracted from different magnifications, and image information in the neighbor slides is used. Tile-based processing allows for the use of context-sensitive features that may not be present when using annotated FOV based workflow. For example, tile-based processing may enable the component to distinguish between negative tumor nuclei and lymphocytes, which can be important. Because Lymphocytes occur in clusters, lymphocyte context may or may not be adequately represented in a particular FOV. In contrast, when examining a particular tile, its neighboring tiles can be considered in order to provide additional context and a refined estimate of cell density. This additional context can help to discriminate between lymphocytes (generally densely packed) and negative tumor cells.”) EX1005 (Chukka), [0045] (“Another aspect where context based analysis is useful is that the whole
--	---

	slide image can be initially considered at a coarse resolution (e.g., 2×). Based on the initial tile-analysis, an approximate region segmentation process can be performed based at least in part on, for example, an identification of dense lymphocyte clusters. Accordingly, detected lymphocyte regions can be discarded while searching for, as an example, blue-stained tumor nuclei. Furthermore, reliable stromal region identification can assist in avoiding the counting of negative nuclei in stromal regions. At a coarse resolution, tile-analysis can find coarse texture-based features to perform a reliable detection of stromal regions. Thus, although the annotated FOV based workflow provides a relatively simple workflow, the whole slide based workflow presents many opportunities for intelligent image analysis. For example, use of context (e.g., surrounding tiles) provides extra information for classifying, for example, blue-stained cells (tumor nuclei can be discriminated from lymphocytes) based at least in part on their relative density and other features. As a result, context analysis can be used to perform a wide range of segmentation processes.”) EX1005 (Chukka), [0046] (“In blocks 335 to 355, the component loops through each region (i.e., the user-identified FOVs of interest or the tiles generated in block 330). In block 340, the component invokes a detect seed points component to identify seeds within the region that the component is currently processing. In block 345, the component invokes an extract objects component to identify the objects within the region that the component is currently processing. In block 350, the component uses a classifier, such as a classifier generated according to FIG. 2, to classify each of the extracted objects as a positively-stained nuclear object, a negatively-stained nuclear object, stroma, a lymphocyte, or an
--	--

	extraneous blob. In block 355, the component selects the next region, if any, and then loops back to block 335 for further processing. In block 360, the component generates and stores or displays a score for the slide or one or more regions within the slide by, for example, generating a positivity value (e.g., the ratio of positively-stained nuclear objects to the overall number of positively- and negatively-stained nuclear objects), calculating an H-score, or calculating another metric. Processing of the component is then completed. In some embodiments, the component may loop back to block 305 to continue receiving and analyzing slide data.”) EX1005 (Chukka), [0047] (“In some embodiments, the facility may pre-process a slide by tiling the slide and then detecting and classifying objects within the slide. In the event that a user wishes to perform an analysis of one or more particular regions or FOVs within a slide, the facility may identify all of the pre-processed tiles that intersect those one or more particular regions or FOVs and provide an analysis based at least in part on the intersection, pre-processed tiles rather than separately performing the identification and detection process for the one or more particular regions or FOVs. Moreover, the facility may perform the tiling and analysis at varying levels of granularity to increase accuracy.”) EX1005 (Chukka), [0051] (“FIG. 5 is a flow diagram illustrating the processing of an extract objects component 114 in some embodiments. The facility invokes the component to identify boundaries around seed points that correspond to an object. Processing of the component is based at least in part on image information generated in steps 305-320 discussed above (e.g., tissue foreground mask, dominant color analysis, segmentation) and thus is based, in part, on context
--	--

	generated from the whole slide. In block 510, the component generates an object foreground mask by identifying those pixels that are darker than their local neighborhood intensity distribution and that exceed a global threshold. The object foreground mask may be generated, for example, by assigning a value of 1 for all pixels whose intensity value exceeds a mean intensity value for a local portion of the tissue (e.g., all pixels within the region currently being analyzed) and that also exceed a global threshold (e.g., 0.85). For pixels that do not meet these criteria, the component may assign another value, such as 0. The object foreground mask allows the facility to prune out weak and spurious objects that are unlikely to correspond to any object of interest (e.g., positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes). In block 520, the component applies the generated mask to the digitized tissue data of the region currently being analyzed. In block 530, the component retrieves the seed points that correspond to the region currently being analyzed. In block 540, the component generates a tessellation of the foreground image (e.g., a Voronoi tessellation) data based at least in part on the retrieved seed points to identify those portions of the foreground image data that are associated with each of the seed points. In block 550, the component identifies, for each seed point, the portion of the foreground image that is associated with the seed point based at least in part on the tessellation. This portion or “blob” can be associated with the seed and can define the boundary of a detected object. In block 560, the component characterizes each of the identified objects based at least in part on location and any number of characteristics including, for example, color characteristics, shape and size characteristics, object descriptor characteristics, cytoplasmic characteristics, inter-object and density
--	--

	characteristics, or any of the above-mentioned characteristics. Each of these identified objects is then returned for storage and classification by a trained classifier as discussed above.”)
--	--

9. Element 6[H]

109. Element 6[H] of claim 6 recites: “for each of the plurality of tiles that corresponds to one of the plurality of cell objects, assigning the tile the predicted class of the corresponding cell object in place of the predicted class of the tile.”

Chukka teaches this element.

110. As discussed above, Chukka teaches that in the “automated mode” as illustrated in FIG. 3, “the tissue region is ... segmented into tiles and classification and nuclei counting algorithms are performed with respect to each tile that contains tissue.” EX1005 (Chukka), [0010]. In this “automated mode,” the facility first tiles the image of the tissue data, and performs an initial tile-based processing to classify the tile as, e.g., lymphocyte clusters, based on information within each tile and information surrounding the tile. *See* EX1005 (Chukka), [0044]. Chukka teaches that in some aspects, the tile-based analysis “can be initially considered at a coarse resolution (e.g., 2×).” EX1005 (Chukka), [0045]. Chukka teaches that the system may perform an “initial tile-analysis” wherein “an approximate region segmentation process can be performed” to identify, for example “dense lymphocyte clusters.” *Id.* Chukka teaches that this initial “coarse

resolution, tile-analysis can find coarse texture-based features” such as a “reliable detection of stromal features.” *Id.* Chukka teaches that this coarse resolution tile-analysis is partially based on context information (e.g., surrounding tiles) regarding, for example cell density and other features. *Id.*

111. Chukka then teaches that the component “loops through each ... tile” and performs a subsequent, finer analysis by (1) “identify[ing] seeds within the [tile]” using a “detect seed points component[,]” (2) identifying objects within the tile using an “extract objects component[,]” and (3) “us[ing] a classifier...to classify each of the extracted objects as a positively stained positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes.” *Id.*, [0046]. Accordingly, Chukka teaches that its disclosed system may first conduct a “coarse resolution tile-analysis” that predicts the class of each tile, and then subsequently performs a finer analysis by extracting cellular objects from each tile and classifying those cellular objects individually. *Id.*, [0046]; *see also id.*, [0047] (“In some embodiments, the facility may pre-process a slide by tiling the slide and then detecting and classifying objects within the slide... Moreover, the facility may perform the tiling and analysis at varying levels of granularity to increase accuracy.”).

112. Chukka teaches tile classification as an initial, coarse-resolution analysis that “approximates” region segmentation based on “coarse” texture-based

features. *Id.*, [0045]. Chukka also teaches that the “facility may perform the tiling and analysis at varying levels of granularity to increase accuracy.” *Id.*, [0047]. A POSA would therefore understand that Chukka’s subsequent identification, extraction, and classification of cell objects taught in blocks 335-350 of FIG. 3 to be a more accurate analysis and classification of the tissue data, replacing the initial, coarser texture-based classification with the subsequent, cell classification. This multi-resolution or multistage approach to computational pathology was also well known in the art. *See e.g.*, EX1011 (Kong); EX1012 (Gurcan); EX1015(Barker).

113. Chukka therefore teaches for each of the plurality of tiles that corresponds to one of the plurality of cell objects, assigning the tile the predicted class of the corresponding cell object in place of the predicted class of the tile. Accordingly, Chukka teaches this element.

’097 Patent	Prior Art
for each of the plurality of tiles that corresponds to one of the plurality of cell objects, assigning the tile the predicted class of the corresponding cell object in place of the predicted class of the tile.	EX1005 (Chukka) [0010] (“In the automated mode, either an Area of Interest (AoI) detector finds or identifies a tissue region in the whole slide image or the tissue annotations are automatically generated by some other image analysis algorithm, such as image registration algorithm which maps annotations from the adjacent serial section to the IHC tissue slide. The tissue region is then segmented into tiles and classification and nuclei counting algorithms are performed with respect to each tile that contains tissue. Additionally, a

	composite score can be obtained based at least in part on the image tiles containing tissue. Though the underlying methodology for detecting, counting, and classifying cells in a given image are similar (the image may be a user annotated region or an automatically obtained tile in the whole slide image after AoI detection), there is at least one difference in the two workflows. The FoV mode relies on manual input in terms of FOV selection while the automated mode does not. The annotated FOV mode is further discussed with respect to FIG. 2 while the automated mode is further discussed with respect to FIG. 3. One or more regions within the identified tissue are identified based at least in part on dominant colors. For identified regions, seed points within the identified region are detected, and objects from the identified regions are extracted. Features of the extracted object(s) are computed such that the trained classifier classifies the extracted object(s) based at least in part on the computed features of the extracted object.”). EX1005 (Chukka), [0039] (“FIG. 3 is a flow diagram illustrating the processing of an analyze slide component 112 in some embodiments. The system invokes the component to analyze and captured slide data to be analyzed. In block 305, the component receives slide data comprising digitized tissue data.”).
--	---

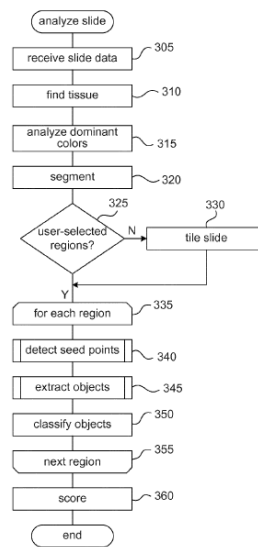


FIG. 3

EX1005 (Chukka) [0040] (“In block **310**, the component performs an image segmentation technique to distinguish between the digitized tissue data and the slide in the image, the tissue corresponding to the foreground and the slide corresponding to the background. In some embodiments, the component computes the Area of Interest (AoI) in a whole slide image in order to detect all tissue regions in the AoI while limiting the amount of background non-tissue area that is analyzed. A wide range of image segmentation techniques (e.g., HSV color-based image segmentation, Lab image segmentation, mean-shift color image segmentation, region growing, level set methods, fast marching methods, etc.) can be used to determine, for example, boundaries of the tissue data and non-tissue or background data. Based at least in part on the segmentation, the component can also generate a tissue foreground mask that can be use to identify those portions of the digitized slide data that correspond to the tissue data. Alternatively, the component can generate a background mask used to identify those portions of

	the digitized slide data that do not correspond to the tissue data.”) EX1005 (Chukka) [0041] (“In block 315, the component performs a dominant color analysis of the foreground data (i.e., the tissue data). The dominant color analysis includes, without limitation, a) determining the dominant colors in the tissue data across the entirety of the digitized slide data, b) projecting the RGB image data onto the dominant color space, c) performing a thresholding technique (such as adaptive color thresholding, Otsu's method, balanced histogram thresholding, or other thresholding techniques), to identify those portions of the digitized slide data having an intensity value that exceeds a predetermined threshold in the dominant color space. For example, if the dominant colors are blue and brown, the component can identify those portions having intensity values in the blue or brown color space that exceed a threshold. The threshold may be established by a user or may be established by the component based at least in part on the range of colors or intensities within each color space. For example, the threshold may be defined as the median color or intensity, the mean color or intensity, the color or intensity that is some predetermined number of standard deviations away from (above or below) the mean color or intensity value, and so on. Other color analysis techniques can be used.”) EX1005 (Chukka), [0042] (“In block 320, the component 112 segments the digitized tissue data into glandular and stromal portions based at least in part on, for example, one or more of color, texture, a co-occurrence matrix, multi-scale Haar features, filter banks, and so on. Moreover, the component may perform the segmentation process at various scales, resolution levels, or magnification
--	--

	levels to reduce the probability of misidentifying different portions.”) EX1005 (Chukka), [0044] (“In block 330, the component 112 can use a grid pattern to tile the portion of the slide corresponding to the tissue data. For example, the component may tile the whole slide tissue data, or a portion thereof, based at least in part on a fixed or set number of tiles (e.g., a 5×5 grid, a 10×10 grid, a 3×10 grid, etc.), based at least in part on a number of pixels for each tile (e.g., 60 pixels by 60 pixels, 150 pixels by 150 pixels, 300 pixels by 1000 pixels, etc.), dimensions for each tile (e.g., 1 μm×1 μm, 1 mm×1 mm, etc.), and so on. In some embodiments, the component may prompt the user to define the size/shape of each tile and/or the overall number of tiles along each dimension. In processing, a single tile image information from within the tiled region and also information from the whole slide context, which is extracted from different magnifications, and image information in the neighbor slides is used. Tile-based processing allows for the use of context-sensitive features that may not be present when using annotated FOV based workflow. For example, tile-based processing may enable the component to distinguish between negative tumor nuclei and lymphocytes, which can be important. Because Lymphocytes occur in clusters, lymphocyte context may or may not be adequately represented in a particular FOV. In contrast, when examining a particular tile, its neighboring tiles can be considered in order to provide additional context and a refined estimate of cell density. This additional context can help to discriminate between lymphocytes (generally densely packed) and negative tumor cells.”) EX1005 (Chukka), [0045] (“Another aspect where context based analysis is useful is that the whole
--	---

	slide image can be initially considered at a coarse resolution (e.g., 2×). Based on the initial tile-analysis, an approximate region segmentation process can be performed based at least in part on, for example, an identification of dense lymphocyte clusters. Accordingly, detected lymphocyte regions can be discarded while searching for, as an example, blue-stained tumor nuclei. Furthermore, reliable stromal region identification can assist in avoiding the counting of negative nuclei in stromal regions. At a coarse resolution, tile-analysis can find coarse texture-based features to perform a reliable detection of stromal regions. Thus, although the annotated FOV based workflow provides a relatively simple workflow, the whole slide based workflow presents many opportunities for intelligent image analysis. For example, use of context (e.g., surrounding tiles) provides extra information for classifying, for example, blue-stained cells (tumor nuclei can be discriminated from lymphocytes) based at least in part on their relative density and other features. As a result, context analysis can be used to perform a wide range of segmentation processes.”) EX1005 (Chukka), [0046] (“In blocks 335 to 355, the component loops through each region (i.e., the user-identified FOVs of interest or the tiles generated in block 330). In block 340, the component invokes a detect seed points component to identify seeds within the region that the component is currently processing. In block 345, the component invokes an extract objects component to identify the objects within the region that the component is currently processing. In block 350, the component uses a classifier, such as a classifier generated according to FIG. 2, to classify each of the extracted objects as a positively-stained nuclear object, a negatively-stained nuclear object, stroma, a lymphocyte, or an
--	--

	extraneous blob. In block 355, the component selects the next region, if any, and then loops back to block 335 for further processing. In block 360, the component generates and stores or displays a score for the slide or one or more regions within the slide by, for example, generating a positivity value (e.g., the ratio of positively-stained nuclear objects to the overall number of positively- and negatively-stained nuclear objects), calculating an H-score, or calculating another metric. Processing of the component is then completed. In some embodiments, the component may loop back to block 305 to continue receiving and analyzing slide data.”) EX1005 (Chukka), [0047] (“In some embodiments, the facility may pre-process a slide by tiling the slide and then detecting and classifying objects within the slide. In the event that a user wishes to perform an analysis of one or more particular regions or FOVs within a slide, the facility may identify all of the pre-processed tiles that intersect those one or more particular regions or FOVs and provide an analysis based at least in part on the intersection, pre-processed tiles rather than separately performing the identification and detection process for the one or more particular regions or FOVs. Moreover, the facility may perform the tiling and analysis at varying levels of granularity to increase accuracy.”)
--	---

E. Claim 9

114. Claim 9 depends on claim 6, reciting as follows:

The method of claim 6, further comprising:

generating a digital overlay drawing for the digital image, where the digital overlay drawing is a histology mask displaying the plurality of tiles and the predict class for each tile.

115. To begin with, the '097 Patent does not define what it means by the word “histology mask,” and in fact only uses the term once in its specification. *See* EX1001, 3:22-25 (“In some examples, the method further includes generating a digital overlay drawing for the digital image, where the digital overlay drawing is a histology mask displaying the plurality of tiles and the predict class for each tile.”). The '097 Patent does state that a mask “may be a copy of an image, where the colors, RGB values, or other values in the pixels are adjusted to show the presence or absence of an object of a certain type to show the location of all objects of that type.” *Id.*, 12:40-44. The '097 Patent also describes an example of a “binary mask” that indicates regions on the image that “may be designated as non-tissue regions.” *Id.* In my opinion, a POSA would understand the term “histology mask” in claim 9 to refer to a copy of the image where the colors, RGB values, or other values in the pixels are adjusted to show the presence or absence of various histological objects or features (e.g., tissues, cell objects).

116. As discussed above, Chukka teaches that the facility may generate an overlay drawing to label features of interest. EX1005 (Chukka), [0004]. Chukka's teaches that this overlay drawing is a “histology mask” as the '097 Patent describes, e.g., to show the presence or absence of an object of a certain

type or to show the location of all objects of that type. For instance, Chukka teaches that its facility may generate an object foreground mask to designate objects of interest. Chukka teaches that “an adaptive thresholding technique can be applied to the tissue data to distinguish between darker objects (e.g., cell nuclei) and other objects, (e.g., stroma and slide background) and generate an object foreground mask.” EX1005 (Chukka), [0026]. Chukka also teaches that the facility “invokes a voting kernel process to identify objects or seed points, for example, nuclear objects or nuclear seed points” and uses these nuclear seed points in combination with the object foreground mask to “generate a tessellated version of the foreground image, where each distinct connected area of pixels or cell in the tessellated version corresponds to a distinct detected object or ‘blob.’” *Id.* Chukka then teaches that each such “blob” is then classified using a trained classifier model as “one or more of positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes.” *Id.*; *see also id.*, [0051] (“extract objects component 114,” may be invoked “to identify boundaries around seed points that correspond to an object[,]” “generate[] an object foreground mask...,” and “characterizes each of the identified objects based at least in part on location and any number of characteristics....”).

117. Chukka therefore teaches generating an object foreground mask (e.g., binary mask) to distinguish between certain cell objects (e.g., cell nuclei vs.

stroma or background). Chukka further teaches using the object foreground mask, in combination with seed points, to generate a further “tessellated copy of the image” that displays detected cellular objects as “blobs” and classifying these objects as certain cellular objects (e.g., positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes). Finally, Chukka teaches displaying information related to features of interest in a digital overlay drawing.

118. Chukka therefore teaches generating a digital overlay drawing for the digital image, where the digital overlay drawing is a histology mask displaying the plurality of tiles and the predict class for each tile. Accordingly, Chukka teaches this claim.

'097 Patent	Prior Art
The method of claim 6, further comprising: generating a digital overlay drawing for the digital image, where the digital overlay drawing is a histology mask displaying the plurality of tiles and the predict class for each tile.	EX1005 (Chukka), [0004] (“At least some embodiments of the disclosed technology are directed to imaging systems for automatically interpreting and scoring tissue specimen slides, for example, specimens stained with an immunohistochemical (IHC) assay.... In some embodiments, an overlay image is produced to label features of interest in the image of a specimen from a subject.”). EX1005 (Chukka), [0026] (“In some embodiments, the facility invokes a voting kernel process to identify objects or seed points, for example, nuclear objects or nuclear seed points, within the two dominant color regions. Seed points can be points

	that are assumed to lie inside an object and are the starting point for localizing objects (e.g., nuclear objects or other features of interest). In other words, seed identification can identify the approximate center point or other internal point for objects. As discussed in further detail below with reference to FIG. 4A, to identify seed objects, the facility can generate a grayscale representation of the digitized slide data and then computes gradient information from the grayscale representation. The facility then generates a vote response matrix by, for each pixel having a gradient magnitude that exceeds a pre-defined threshold, casting a vote to that pixels local neighborhood. The local neighborhood is determined based at least in part on the local gradient direction and a pre-determined distance or radius range. Subsequently, each local maximum within with the vote response matrix that exceeds a voting threshold can be identified as a seed location. In a parallel and independent step, for example, an adaptive thresholding technique can be applied to the tissue data to distinguish between darker objects (e.g., cell nuclei) and other objects, (e.g., stroma and slide background) and generate an object foreground mask. The object foreground mask and the seed locations are used in combination to generate a tessellated version of the foreground image, where each distinct connected area of pixels or cell in the tessellated version corresponds to a distinct detected object or “blob.” Each “blob” is then classified using a classifier (e.g., a previously-trained classifier) to identify one or more of positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes. Once classified, the detected nuclear objects can be used to score a slide or particular regions within a slide. Thus, the disclosed techniques take advantage of whole slide context and machine learning techniques to improve
--	---

	nuclear object detection and automated slide scoring.”) EX1005 (Chukka), [0046] (“In blocks 335 to 355, the component loops through each region (i.e., the user-identified FOVs of interest or the tiles generated in block 330). In block 340, the component invokes a detect seed points component to identify seeds within the region that the component is currently processing. In block 345, the component invokes an extract objects component to identify the objects within the region that the component is currently processing. In block 350, the component uses a classifier, such as a classifier generated according to FIG. 2, to classify each of the extracted objects as a positively-stained nuclear object, a negatively-stained nuclear object, stroma, a lymphocyte, or an extraneous blob.”) EX1005 (Chukka), [0051] (“FIG. 5 is a flow diagram illustrating the processing of an extract objects component 114 in some embodiments. The facility invokes the component to identify boundaries around seed points that correspond to an object. Processing of the component is based at least in part on image information generated in steps 305-320 discussed above (e.g., tissue foreground mask, dominant color analysis, segmentation) and thus is based, in part, on context generated from the whole slide. In block 510, the component generates an object foreground mask by identifying those pixels that are darker than their local neighborhood intensity distribution and that exceed a global threshold. The object foreground mask may be generated, for example, by assigning a value of 1 for all pixels whose intensity value exceeds a mean intensity value for a local portion of the tissue (e.g., all pixels within the region currently being analyzed) and that also exceed a
--	--

	global threshold (e.g., 0.85). For pixels that do not meet these criteria, the component may assign another value, such as 0. The object foreground mask allows the facility to prune out weak and spurious objects that are unlikely to correspond to any object of interest (e.g., positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes). In block 520, the component applies the generated mask to the digitized tissue data of the region currently being analyzed. In block 530, the component retrieves the seed points that correspond to the region currently being analyzed. In block 540, the component generates a tessellation of the foreground image (e.g., a Voronoi tessellation) data based at least in part on the retrieved seed points to identify those portions of the foreground image data that are associated with each of the seed points. In block 550, the component identifies, for each seed point, the portion of the foreground image that is associated with the seed point based at least in part on the tessellation. This portion or “blob” can be associated with the seed and can define the boundary of a detected object. In block 560, the component characterizes each of the identified objects based at least in part on location and any number of characteristics including, for example, color characteristics, shape and size characteristics, object descriptor characteristics, cytoplasmic characteristics, inter-object and density characteristics, or any of the above-mentioned characteristics. Each of these identified objects is then returned for storage and classification by a trained classifier as discussed above.”)
--	--

F. Claim 10

119. Claim 10 depends on claim 6, and recites as follows:

The method of claim 6, further comprising:

generating a digital overlay drawing for the digital image; and displaying, in the digital overlay drawing, the plurality of tiles and the predicted class for each tile that does not correspond to a cell object, and displaying, the plurality of cell objects and the predicted class of each cell object.

120. Chukka teaches this element. As I discussed above regarding claim 9, Chukka teaches that the facility may generate an overlay drawing to label features of interest. EX1005 (Chukka), [0004], [0024], [0026]. Chukka teaches that the “facility can separate the whole slide into a background region (e.g., a glass background region) and a tissue (foreground) region using color image segmentation techniques, such as HSV (hue, saturation, and value)-based image segmentation.” EX1005 (Chukka), [0024]. Chukka then teaches that the system may identify seed points corresponding to cell objects, and use “adaptive thresholding techniques” to distinguish between “darker objects (e.g., cell nuclei) and other objects, (e.g., stroma and slide background)” to generate an “object foreground mask.” *Id.*, [0026]; *see also id.*, [0051] The object foreground mask and seed locations are then used to “generate a tessellated version of the foreground image, where each distinct connected area of pixels or cell in the tessellated version corresponds to a distinct detected object or ‘blob.’” *Id.* Chukka further teaches that these blobs are classified to “identify one or more of

positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes.” *Id.*

121. Chukka thus teaches generating an overlay drawing that displays the plurality of tiles and the predicted class of each tile that does not correspond to a cell object (e.g., stroma or cell background) and displaying the plurality of cell objects and the predicted class of each cell object (e.g., tumor positive, tumor negative, or lymphocytes). Accordingly, Chukka teaches this claim.

'097 Patent	Prior Art
The method of claim 6, further comprising: generating a digital overlay drawing for the digital image; and displaying, in the digital overlay drawing, the plurality of tiles and the predicted class for each tile that does not correspond to a cell object, and displaying, the plurality of cell objects and the predicted class of each cell object.	EX1005 (Chukka), [0004] (“At least some embodiments of the disclosed technology are directed to imaging systems for automatically interpreting and scoring tissue specimen slides, for example, specimens stained with an immunohistochemical (IHC) assay.... In some embodiments, an overlay image is produced to label features of interest in the image of a specimen from a subject.”). EX1005 (Chukka), [0024] (“The facility can separate the whole slide into a background region (e.g., a glass background region) and a tissue (foreground) region using color image segmentation techniques, such as HSV (hue, saturation, and value)-based image segmentation. This process allows the facility to distinguish between the tissue data (the data of interest) and the slide.”) EX1005 (Chukka), [0026] (“In some embodiments, the facility invokes a voting kernel process to identify objects or seed points, for example, nuclear objects or nuclear seed points, within the two

	dominant color regions. Seed points can be points that are assumed to lie inside an object and are the starting point for localizing objects (e.g., nuclear objects or other features of interest). In other words, seed identification can identify the approximate center point or other internal point for objects. As discussed in further detail below with reference to FIG. 4A, to identify seed objects, the facility can generate a grayscale representation of the digitized slide data and then computes gradient information from the grayscale representation. The facility then generates a vote response matrix by, for each pixel having a gradient magnitude that exceeds a pre-defined threshold, casting a vote to that pixels local neighborhood. The local neighborhood is determined based at least in part on the local gradient direction and a pre-determined distance or radius range. Subsequently, each local maximum within with the vote response matrix that exceeds a voting threshold can be identified as a seed location. In a parallel and independent step, for example, an adaptive thresholding technique can be applied to the tissue data to distinguish between darker objects (e.g., cell nuclei) and other objects, (e.g., stroma and slide background) and generate an object foreground mask. The object foreground mask and the seed locations are used in combination to generate a tessellated version of the foreground image, where each distinct connected area of pixels or cell in the tessellated version corresponds to a distinct detected object or “blob.” Each “blob” is then classified using a classifier (e.g., a previously-trained classifier) to identify one or more of positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes. Once classified, the detected nuclear objects can be used to score a slide or particular regions within a slide. Thus, the disclosed techniques take advantage of whole slide context and machine learning techniques to improve
--	---

	nuclear object detection and automated slide scoring.”) EX1005 (Chukka), [0051] (“FIG. 5 is a flow diagram illustrating the processing of an extract objects component 114 in some embodiments. The facility invokes the component to identify boundaries around seed points that correspond to an object. Processing of the component is based at least in part on image information generated in steps 305-320 discussed above (e.g., tissue foreground mask, dominant color analysis, segmentation) and thus is based, in part, on context generated from the whole slide. In block 510, the component generates an object foreground mask by identifying those pixels that are darker than their local neighborhood intensity distribution and that exceed a global threshold. The object foreground mask may be generated, for example, by assigning a value of 1 for all pixels whose intensity value exceeds a mean intensity value for a local portion of the tissue (e.g., all pixels within the region currently being analyzed) and that also exceed a global threshold (e.g., 0.85). For pixels that do not meet these criteria, the component may assign another value, such as 0. The object foreground mask allows the facility to prune out weak and spurious objects that are unlikely to correspond to any object of interest (e.g., positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes). In block 520, the component applies the generated mask to the digitized tissue data of the region currently being analyzed. In block 530, the component retrieves the seed points that correspond to the region currently being analyzed. In block 540, the component generates a tessellation of the foreground image (e.g., a Voronoi tessellation) data based at least in part on the retrieved seed points to identify those portions of the foreground image data that are
--	--

	associated with each of the seed points. In block 550, the component identifies, for each seed point, the portion of the foreground image that is associated with the seed point based at least in part on the tessellation. This portion or “blob” can be associated with the seed and can define the boundary of a detected object. In block 560, the component characterizes each of the identified objects based at least in part on location and any number of characteristics including, for example, color characteristics, shape and size characteristics, object descriptor characteristics, cytoplasmic characteristics, inter-object and density characteristics, or any of the above-mentioned characteristics. Each of these identified objects is then returned for storage and classification by a trained classifier as discussed above.”)
--	--

G. Claim 11

122. Claim 11 depends on claim 6, and recites as follows:

The method of claim 6, further comprising:

generating a digital overlay drawing for the digital image, wherein the digital overlay drawing includes the digital image; and
displaying the digital overlay drawing.

123. Chukka teaches this claim. As I discussed above regarding claim 9, Chukka teaches that the facility may generate an overlay drawing to label features of interest. EX1005 (Chukka), [0004], [0024], [0026]. An “overlay image” that “label[s] features of interest” in a digital image includes the digital image (the image being labelled). Chukka teaches that the system “displaying the images to the user” and “can assist a user ... by detecting and classifying objects and scoring

the whole slide and/or regions...of the slide.” *Id.*, [0027]. Chukka therefore teaches generating a digital overlay drawing for the digital image, wherein the digital overlay drawing includes the digital image, and displaying the digital overlay drawing. Accordingly, Chukka teaches this claim.

'097 Patent	Prior Art
The method of claim 6, further comprising: generating a digital overlay drawing for the digital image, wherein the digital overlay drawing includes the digital image; and displaying the digital overlay drawing.	EX1005 (Chukka), [0004] (“For example, the system can accurately count nuclear objects to determine information about the tissue to assist with reliable and reproducible slide interpretation. In one embodiment, the system counts positively-stained nuclear objects and/or negatively-stained nuclear objects to score, for example, a biological specimen (e.g., tumor tissue). In some embodiments, an overlay image is produced to label features of interest in the image of a specimen from a subject. Scoring of the tissue may be performed to predict and/or generate a prognosis for the tissue sample.”); <i>see also id.</i>, [0024], [0026]. EX1005 (Chukka), [0027] (“FIG. 1 illustrates a computer-based system and environment for analyzing tissue specimens in accordance with an embodiment of the disclosed technology. An analyzing system 100 includes an imaging apparatus 120 and a computer system 110. Specimen-bearing microscope slides can be loaded into the imaging apparatus 120. The imaging apparatus 120 can produce the images of the specimens. The images are sent to a computer system 110 either through a direct connection or via a network 130. The computer system 110 displays the images to a user.”)

H. Claim 12

124. Claim 12 depends from claim 6, reciting as follows:

The method of claim 6, further comprising:

generating a digital overlay drawing for the digital image, wherein the digital overlay drawing includes a generated version of the digital image; and

displaying the digital overlay drawing.

125. Chukka teaches this claim. As I discussed above regarding claim 9,

Chukka teaches that the facility may generate an overlay drawing to label features of interest. EX1005 (Chukka), [0004], [0024], [0026]. Chukka further teaches that the facility “can generate a grayscale representation of the digitized slide data[.]” *Id.*, [0026]. Chukka teaches that the system “displaying the images to the user” and “can assist a user ... by detecting and classifying objects and scoring the whole slide and/or regions...of the slide.” *Id.*, [0027]. Accordingly, Chukka teaches generating a digital overlay drawing for the digital image, wherein the digital overlay drawing includes a generated version of the digital image; and displaying the digital overlay drawing.

'097 Patent	Prior Art
The method of claim 6, further comprising: generating a digital overlay drawing for the digital image, wherein the digital overlay drawing includes a generated	EX1005 (Chukka), [0004] (“For example, the system can accurately count nuclear objects to determine information about the tissue to assist with reliable and reproducible slide interpretation. In one embodiment, the system counts positively-stained nuclear objects and/or negatively-stained nuclear objects to score, for example, a biological specimen (e.g., tumor tissue). In some

version of the digital image; and displaying the digital overlay drawing.	embodiments, an overlay image is produced to label features of interest in the image of a specimen from a subject. Scoring of the tissue may be performed to predict and/or generate a prognosis for the tissue sample.”); <i>see also id.</i> , [0024], [0026]. EX1005 (Chukka), [0026] (“As discussed in further detail below with reference to FIG. 4A, to identify seed objects, the facility can generate a grayscale representation of the digitized slide data and then computes gradient information from the grayscale representation. The facility then generates a vote response matrix by, for each pixel having a gradient magnitude that exceeds a pre-defined threshold, casting a vote to that pixels local neighborhood.”)
--	--

I. Claim 14

126. Claim 14 depends on claim 6, and recites as follows:

The method of claim 6, further comprising:

generating a digital overlay drawing for the digital image, where the digital overlay drawing comprises percentages of predict classes corresponding to the digital image.

127. Chukka teaches this element. As I discussed above regarding claim 9, Chukka teaches that the facility may generate an overlay drawing to label features of interest. EX1005 (Chukka), [0004], [0024], [0026]. Chukka teaches that, as background, “pathologists often report a score for the slide by visually reviewing and estimating the percentage of positively-stained (e.g., brown-colored) nuclear objects to the total number of positively-stained and negatively-

stained (e.g., blue-colored) nuclear objects.” EX1005 (Chukka), [0003] As discussed above, Chukka teaches that in some embodiments, the disclosed facility may generate “an overlay image” which “label[s] features of interest” in the tissue slide image. *Id.*, [0004]. Chukka further teaches that the facility can classify cells in the tissue image as positive or negative for cancer and “to determine a score based on ... percent positive/negative[.]” *Id.*, [0020].

128. Chukka’s therefore teaches generating an overlay drawing for the digital image, where the digital overlay drawing comprises percentages of predict classes corresponding to the digital image. Accordingly, Chukka teaches this claim.

'097 Patent	Prior Art
The method of claim 6, further comprising: generating a digital overlay drawing for the digital image, where the digital overlay drawing comprises percentages of predict classes corresponding to the digital image.	EX1005 (Chukka), [0003] (“In a clinical reading, pathologists often report a score for the slide by visually reviewing and estimating the percentage of positively-stained (e.g., brown-colored) nuclear objects to the total number of positively-stained and negatively-stained (e.g., blue-colored) nuclear objects.”) EX1005 (Chukka), [0004] (“For example, the system can accurately count nuclear objects to determine information about the tissue to assist with reliable and reproducible slide interpretation. In one embodiment, the system counts positively-stained nuclear objects and/or negatively-stained nuclear objects to score, for example, a biological specimen (e.g., tumor tissue). In some embodiments, an overlay image is produced to

	label features of interest in the image of a specimen from a subject. Scoring of the tissue may be performed to predict and/or generate a prognosis for the tissue sample.”); <i>see also id.</i>, [0024], [0026]. EX1005 (Chukka), [0020] (“The facility can classify positively-stained nuclear objects, negatively-stained nuclear objects, tissue (including non-cell tissue), or other features in order to, for example, assign a score to an area of interest of an image, a given field of view (FOV), and/or an entire slide or group of slides. The facility is configured to detect different types of cell nuclei in a FOV and classify each cell nuclei. To analyze a breast tissue sample, for example, the facility can classify cell nuclei as positively-stained nuclei or negatively-stained nuclei and can disregard other tissue (e.g., stromata, lymphocytes, etc.) to determine a score based at least in part on, for example, percent positive/negative, H-score, etc. In some embodiments, the facility may further identify extraneous artifacts or “junk” features.”) EX1005 (Chukka), [0027] (“FIG. 1 illustrates a computer-based system and environment for analyzing tissue specimens in accordance with an embodiment of the disclosed technology. An analyzing system 100 includes an imaging apparatus 120 and a computer system 110. Specimen-bearing microscope slides can be loaded into the imaging apparatus 120. The imaging apparatus 120 can produce the images of the specimens. The images are sent to a computer system 110 either through a direct connection or via a network 130. The computer system 110 displays the images to a user.”)
--	---

J. Claim 15

129. Claim 15 depends on claim 6, and recites as follows:

The method of claim 6, further comprising:

generating a digital overlay drawing for the digital image, where the digital overlay drawing comprises total counts of predict classes corresponding to the digital image.

130. Chukka teaches this element. As I discussed above regarding claim 9, Chukka teaches that the facility may generate an overlay drawing to label features of interest. EX1005 (Chukka), [0004], [0024], [0026]. Chukka further teaches that the facility “can automatically count and classify cells” such as counting “positively-stained nuclear objects and/or negatively-stained nuclear objects to score, for example, a biological specimen (e.g., tumor tissue).” *Id.* Chukka then teaches that the system can score the slide “[b]ased at least in part on the number of positive/negative nuclei.” *Id.*, [0006]; *see also id.*, [0010] (“The tissue region is then segmented into tiles and classification and nuclei counting algorithms are performed with respect to each tile that contains tissue.”).

131. Chukka therefore teaches determining the total counts of predicted classes corresponding to the digital image and displaying the total counts of predicted classes as part of its overlay drawing. According, Chukka teaches this claim.

'097 Patent	Prior Art
-------------	-----------

The method of claim 6, further comprising: generating a digital overlay drawing for the digital image, where the digital overlay drawing comprises total counts of predict classes corresponding to the digital image.	EX1005 (Chukka), [0004] (“Furthermore, the disclosed system may count cells, compute various types of local and global features of these cells, identify the cell types, and perform quantitative analysis. The feature computation can use information from not only an annotated region of a slide but also information from the whole slide (e.g., tissue-containing regions of the slide analyzed at multiple magnifications). The system can automatically count and classify cells and score the image and/or entire slide based at least in part on selected fields of view and/or the whole slide based at least in part on information or data associated with the whole slide (i.e., all of the tissue containing regions of the slide). The score can be used for slide interpretation. For example, the system can accurately count nuclear objects to determine information about the tissue to assist with reliable and reproducible slide interpretation. In one embodiment, the system counts positively-stained nuclear objects and/or negatively-stained nuclear objects to score, for example, a biological specimen (e.g., tumor tissue). In some embodiments, an overlay image is produced to label features of interest in the image of a specimen from a subject. Scoring of the tissue may be performed to predict and/or generate a prognosis for the tissue sample.”); <i>see also id.</i>, [0024], [0026]. EX1005 (Chukka), [0006] (“In some embodiments, a multi-stage binary classifier can identify positive and negative nuclei. The positive nuclei can be distinguished from the negative nuclei, lymphocytes, and stroma. Additionally, the negative cells and lymphocytes can be distinguished from stroma. Lymphocytes are then distinguished from the negative nuclei. In further classification, the positive cells can be
--	--

	distinguished from background cells. For example, if the positive cells have brown stained nuclei, the background cells may be cytoplasmic blush that can be filtered out. Based at least in part on the number of positive/negative nuclei, a score (e.g., a whole-slide score) can be determined.”) EX1005 (Chukka), [0010] (“The tissue region is then segmented into tiles and classification and nuclei counting algorithms are performed with respect to each tile that contains tissue. Additionally, a composite score can be obtained based at least in part on the image tiles containing tissue. Though the underlying methodology for detecting, counting, and classifying cells in a given image are similar (the image may be a user annotated region or an automatically obtained tile in the whole slide image after AoI detection), there is at least one difference in the two workflows.”) EX1005 (Chukka), [0021] (“The disclosed detection and classification process can be extended to digitized whole-slide images to generate a score for the whole slide (e.g., by counting nuclei without selecting regions of interest, based on information from the whole slide).”) EX1005 (Chukka), [0027] (“FIG. 1 illustrates a computer-based system and environment for analyzing tissue specimens in accordance with an embodiment of the disclosed technology. An analyzing system 100 includes an imaging apparatus 120 and a computer system 110. Specimen-bearing microscope slides can be loaded into the imaging apparatus 120. The imaging apparatus 120 can produce the images of the specimens. The images are sent to a computer system 110 either through a direct connection or via a network 130. The computer system 110 displays the images to a user.”)
--	---

K. Claim 17

132. Claim 17 depends on claim 6, reciting as follows:

The method of claim 6, wherein the cell objects include lymphocyte cells and not lymphocyte cells.

133. Chukka teaches this element. Chukka teaches that in some embodiments, the disclosed system identifies “positive and negative nuclei” and distinguish positive nuclei “from the negative nuclei, lymphocytes, and stroma.” EX1005 (Chukka), [0006]. Chukka further teaches that lymphocytes may also be distinguished from negative nuclei. *Id.* Chukka further describes that the disclosed facility “can classify cell nuclei as positively-stained nuclei or negatively-stained nuclei and can disregard other tissue (e.g., stromata, lymphocytes, etc.).”

134. More specifically, Chukka teaches that the analyze cell component 112 can use a tile-based approach to distinguish between negative tumor nuclei and lymphocytes, based on features identified in the tiles and contextual information in the region surrounding each tile. *Id.*, [0044]. Chukka teaches that “[b]ecause Lymphocytes occur in clusters ... neighboring tiles can be considered in order to provide additional context and a refined estimate of cell density” which “can help to discriminate between lymphocytes (generally densely packed) and negative tumor cells.” *Id.*

135. Chukka therefore teaches a method wherein the cell objects include lymphocyte cells and not lymphocyte cells. Accordingly, Chukka teaches this claim.

'097 Patent	Prior Art
The method of claim 6, wherein the cell objects include lymphocyte cells and not lymphocyte cells.	EX1005 (Chukka), [0006] (“In some embodiments, a multi-stage binary classifier can identify positive and negative nuclei. The positive nuclei can be distinguished from the negative nuclei, lymphocytes, and stroma. Additionally, the negative cells and lymphocytes can be distinguished from stroma. Lymphocytes are then distinguished from the negative nuclei. In further classification, the positive cells can be distinguished from background cells. For example, if the positive cells have brown stained nuclei, the background cells may be cytoplasmic blush that can be filtered out. Based at least in part on the number of positive/negative nuclei, a score (e.g., a whole-slide score) can be determined.”) EX1005 (Chukka), [0044] (“For example, tile-based processing may enable the component to distinguish between negative tumor nuclei and lymphocytes, which can be important. Because Lymphocytes occur in clusters, lymphocyte context may or may not be adequately represented in a particular FOV. In contrast, when examining a particular tile, its neighboring tiles can be considered in order to provide additional context and a refined estimate of cell density. This additional context can help to discriminate between lymphocytes (generally densely packed) and negative tumor cells.”) EX1005 (Chukka), [0045] (“Another aspect where context based analysis is useful is that the whole

	slide image can be initially considered at a coarse resolution (e.g., 2×). Based on the initial tile-analysis, an approximate region segmentation process can be performed based at least in part on, for example, an identification of dense lymphocyte clusters. Accordingly, detected lymphocyte regions can be discarded while searching for, as an example, blue-stained tumor nuclei... or example, use of context (e.g., surrounding tiles) provides extra information for classifying, for example, blue-stained cells (tumor nuclei can be discriminated from lymphocytes) based at least in part on their relative density and other features.”)
--	--

L. Claim 18

136. Claim 18 depends on claim 6, reciting as follows:

The method of claim 6, wherein the structural tissue features comprises at least one of glands, ducts, vessels, and immune clusters.

137. As discussed above with regards to claim 5, Chukka teaches that the structural tissue features may comprise at least one of glands, ducts, vessels, and immune clusters. Accordingly for the same reasons discussed above with regards to claim 5, Chukka teaches this claim.

X. GROUND 2: CLAIMS 2, 3, 7, AND 8 ARE OBVIOUS OVER CHUKKA AND JONES

138. It is my opinion that claims 2, 3, 7, and 8 would further have been obvious over Chukka in view of Jones. Below, I note specific prior art disclosures that satisfy each element of each challenged claim. After each claim element, I

provide a chart identifying certain exemplary prior art disclosures that are relevant to that element.

139. Claims 2, 3, 7, and 8 recite limitations relating to generating, displaying, and storing outlines (e.g., polygons) of the detected cells or cell objects. The '097 Patent does not purport to have invented a new or novel method of outlining cell objects, and it was well-known and conventional practice in computational histopathology to outline cell objects. *See* EX1017 (Ali) Fig. 3:

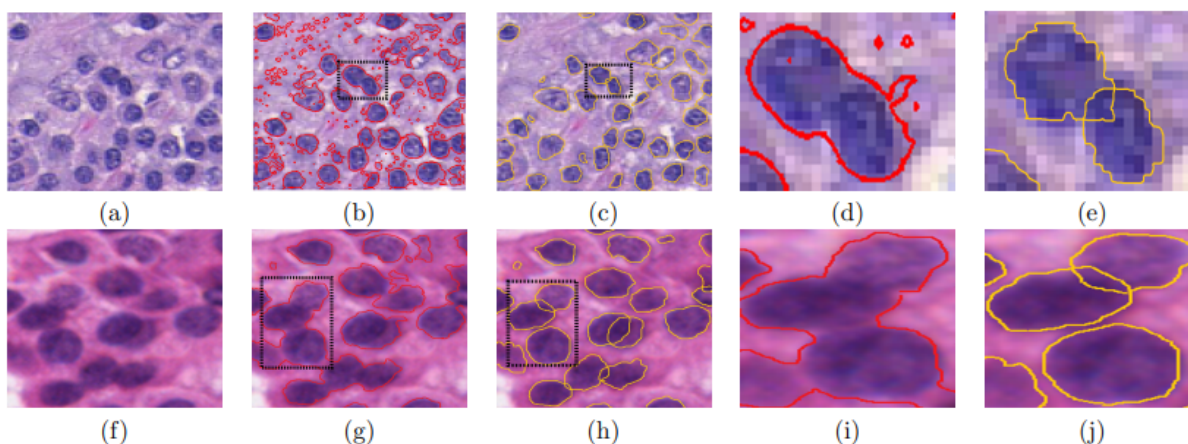


Figure 3. (a), (f) Original histological images corresponding to breast and prostate histology respectively, segmentation results for nuclei and lymphocytes obtained via the (b), (g) Geodesic Active Contour; (c), (h) our integrated AC model; (d), (e) magnified region from (b) and (g); (e), (j) magnified region from (c) and (h).

140. Chukka teaches that its facility may invoke an “extract objects component” to “identify boundaries around seed points that correspond to an object.” EX1005 (Chukka), [0051]. Chukka teaches that the component “generates an object foreground mask by identifying those pixels that are darker than their local neighborhood intensity distribution and that exceed a global threshold.” *Id.* Chukka then teaches that the component retrieves “seed points”

corresponding to the region and “generates a tessellation of the foreground image (e.g., a Voronoi tessellation)” based on the object foreground mask and seed points. *Id.* Finally, Chukka teaches that “the component identifies, for each seed point, the portion [or ‘blob’] of the foreground image that is associated with the seed point based at least in part on the tessellation.” *Id.* Chukka teaches that this “portion” or “blob” is “associated with the seed and can define the boundary of the detected object.” *Id.*

141. Jones discloses and teaches a specific method of defining such boundaries. Jones teaches that a “fundamental operation in cell-image analysis is identifying individual cell boundaries” and that this “is often difficult because there are many different staining protocols” and the difference between cell interior and cell border may not be very pronounced.” EX1006 (Jones), 535. Jones further teaches that in most cases, “the nuclei of cells are more easily identifiable,” and that the difficult problem is identifying boundaries between cells. *Id.* Accordingly, Jones teaches “a method for finding the boundaries between adjacent regions in an image, where ‘seed’ areas have already been identified in the individual regions to be segmented.” *Id.*, Abstract.

142. Jones specifically teaches that this method “operates by computing a discretized approximation of the Voronoi regions of each seed on a manifold with a metric controlled by local image features.” *Id.*, 537. Jones teaches using a

specific “Riemannian metric” defined below, “in terms of the image I and a regularization parameter λ ,” and “where I is the 2×2 identity matrix.” *Id.*

$$\mathbf{G} = \frac{\nabla \mathbf{g}(I) \nabla \mathbf{g}^T(I) + \lambda \mathbf{I}}{1 + \lambda}, \quad (1)$$

143. Jones then teaches defining a manifold by the following formula:

$$\|d\mathbf{x}\|_{\mathbf{G}}^2 \equiv d\mathbf{x}^T \mathbf{G} d\mathbf{x} = \frac{(d\mathbf{x}^T \nabla \mathbf{g}(I))^2 + \lambda (d\mathbf{x}^T d\mathbf{x})^2}{\lambda + 1}. \quad (2)$$

wherein G becomes more Euclidian as λ increases. *Id.* Jones teaches that the “computation of Voronoi regions places the boundary between two adjacent regions at pixels that are equidistant from each seed, as measured on the manifold from (2).” *Id.*, 538. In computing these Voronoi regions, “the inter-pixel distance is larger where the image is changing more according to $\nabla \mathbf{g}(I)$, so boundaries tend to align with image differences.” *Id.*

144. As demonstrated in Fig. 1 of Jones, reproduced below, Jones teaches that its method is able to generate an outline of the cell from pre-determined seed regions (e.g., cell nuclei). Jones teaches that its method performs well at determining cell-to-cell boundaries. *Id.*, 536, 539-541.

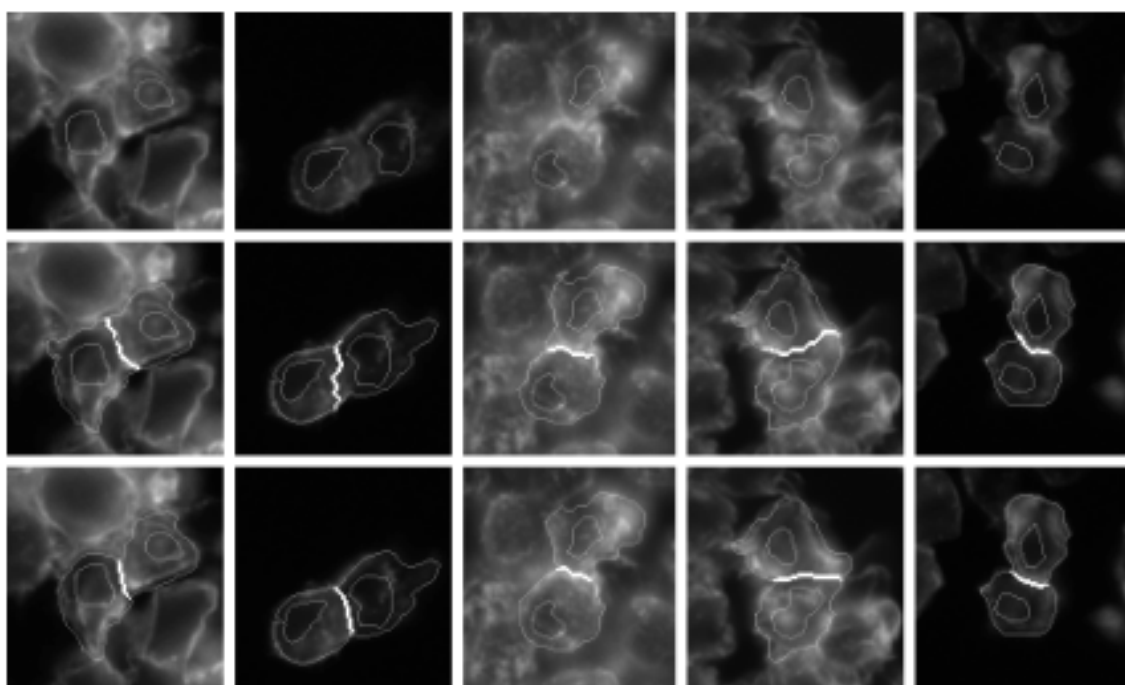


Fig. 1. Typical results from our algorithm. Internal shapes are seed regions, taken from manual segmentation of nuclei, and are the same in both rows. Bold lines show cell/cell boundaries that are compared. Top row: Cell images, with nuclei outlined. Middle row: Automatic segmentation with our method. Bottom row: Manual segmentation.

145. Moreover, as demonstrated in FIG. 3 of Jones, reproduced below, Jones teaches that its method is capable of effectively identifying boundaries between multiple different seed points or seed regions. Accordingly, a POSA would understand that Jones's method could be used to identify cell-to-cell boundaries in an identified region, e.g., a foreground of a tissue slide image.

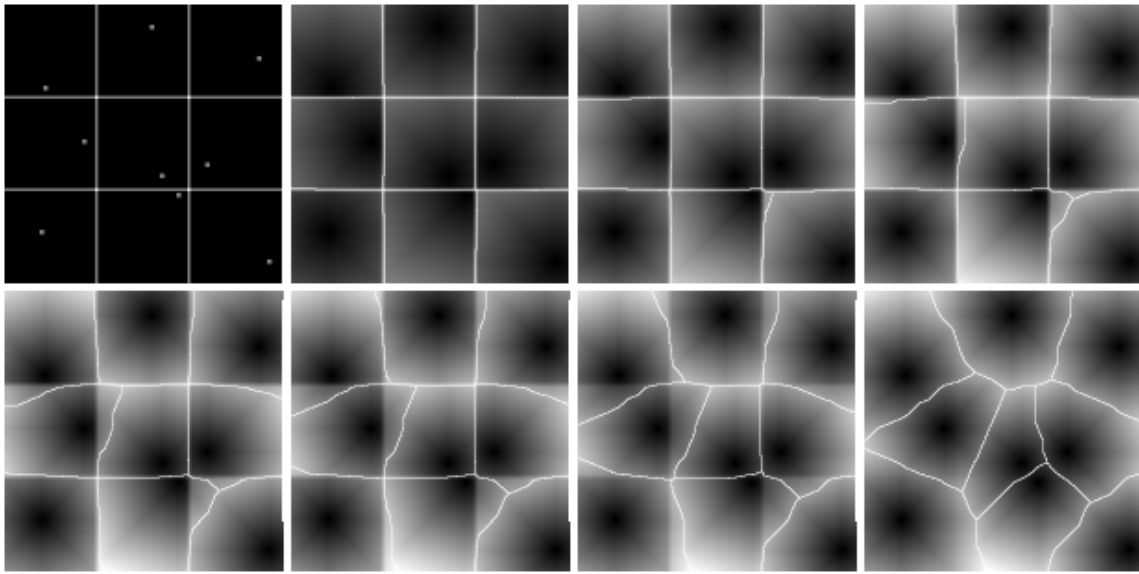


Fig. 3. Synthetic example. The input image is in the upper left, with seed sites marked with dots. From left to right across the two rows, the resulting distances calculated with our metric are shown, with the resulting segmentation overlaid white lines, for λ equal to 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, and 3.0. The segmentation lines follow the ridges in the distances function. As can be seen, as λ increases, the segmentation approaches the Voronoi diagram of the seed regions.

146. A POSA would have had a good reason to combine the teachings of Chukka and Jones. Chukka teaches that its facility can identify portions of the image (e.g., a “blob”) that corresponds to the boundaries of a specific object (e.g., a cell) based on retrieved seed points and an object foreground mask. Chukka teaches that the component does this by generating, for example, a Voronoi tessellation of the foreground image. Jones teaches a known method of doing so. Jones teaches effectively approximating cell-to-cell boundaries by computing a Voronoi tessellation of pre-determined seeds on a manifold based on a its defined regularization parameters. A POSA looking to implement Chukka’s facility, and

specifically its “extract objects component” to identify portions of the image corresponding to boundaries around detected cellular objects would have had a good reason to look at teachings of Jones to do so.

147. A POSA would also have a reasonable expectation of success in combining the teachings of Chukka and Jones. Chukka teaches that the facility includes a “detect seed points component” that is able to “identify the location of seed points within a specified region of tissue data, each seed point corresponding to a potential object.” EX1005 (Chukka), [0048]. Chukka further teaches that the “extract objects component” can generate an “object foreground mask by identifying those pixels that are darker than their local neighborhood intensity distribution and that exceed a global threshold.” *Id.*, [0051]. Jones teaches that its method solves the “problem of segmenting cells as one of identifying boundaries between regions given ‘seeds’ in individual regions from which to start the segmentation.” EX1006 (Jones), 1. Jones further teaches that its method “does not compute a foreground/background separation, but instead relies on a such a label for each pixel to be given as input.” *Id.*, 4. Chukka therefore provides precisely the information Jones’ method requires, i.e., a seed point and an object foreground mask, to effectively outline the boundaries of cells.

A. Claim 2

148. Claim 2 depends on claim 1, and recites as follows: “The method of claim 1, further comprising: generating a digital overlay drawing of an outer edge of each cell in the image.” This claim would have been obvious over Chukka in view of Jones.

149. As discussed above, Chukka teaches that in some embodiments, the disclosed facility may generate “an overlay image” which “label[s] features of interest” in the tissue slide image. *Id.*, [0004]. Chukka further teaches that the facility includes an “extract objects component 114,” the processing of which is illustrated in FIG. 5, reproduced below. *Id.* [0051]. Chukka teaches invoking this component “to identify boundaries around seed points that correspond to an object.” *Id.* Chukka teaches that the component “generates an object foreground mask by identifying those pixels that are darker than their local neighborhood intensity distribution and that exceed a global threshold.” *Id.* This object foreground mask outlines cellular “objects of interest” such as positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes.” *Id.*

150. Chukka then teaches that this component retrieves detected seed points corresponding to each object, and generates a tessellation of the foreground image “based at least in part on the retrieved seed points to identify those portions of the foreground image data that are associated with each of the seed points.” *Id.*

The component then identifies a portion or “blob” of the foreground image that “can be associated with the seed and can define the boundary of a detected object.” *Id.* Finally, “the component characterizes each of the identified objects based at least in part on location and any number of characteristics.” *Id.*

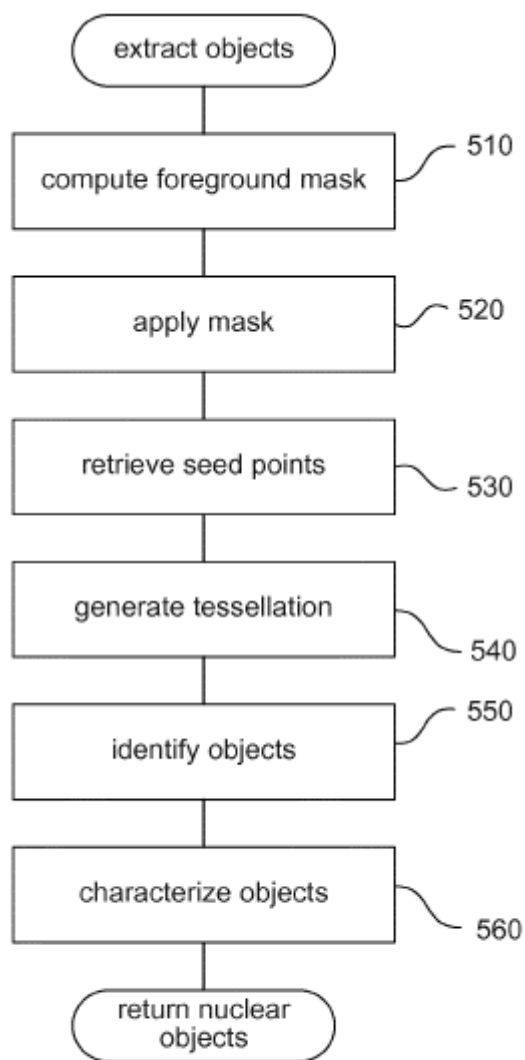


FIG. 5

151. Jones teaches an algorithm to calculate cell-to-cell boundaries by “computing a discretized approximation of the Voronoi regions of each seed on a manifold with a metric controlled by local image features.” EX1006 (Jones), 537. Jones teaches that this approach “define[s] a metric in the image plane” and “calculate[s] distances from seed regions according to this metric.” *Id.* “Pixels are then assigned to cells according to their distance from the corresponding nucleus under that metric. The metric uses information about image edges, both their strength and their orientation, as well as a regularization term corresponding to inter-pixel distance within the image.” *Id.*

152. Jones teaches that its algorithm performs well at identifying cell boundaries. *Id.*, 8. For example, Jones teaches that its algorithm has been successfully implemented in several experiments “with a variety of cell types and morphologies.” *Id.* As illustrated in Jones’s Fig. 1, Jones teaches that its algorithm can generate a digital overlay drawing of an outer edge of each cell in

the image. *Id.*, 536, Fig. 1.

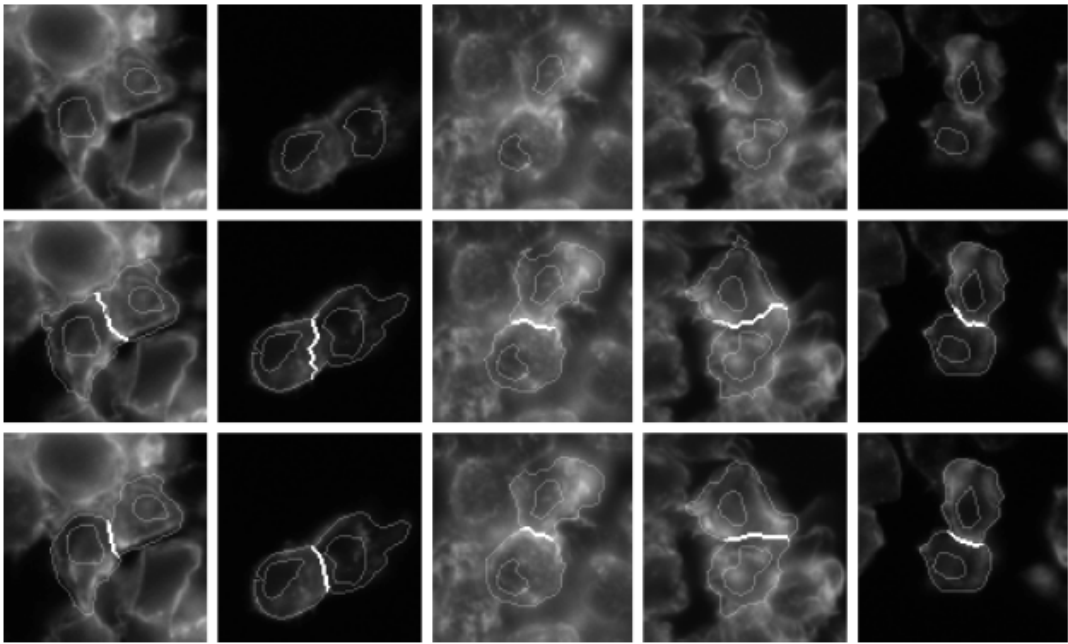


Fig. 1. Typical results from our algorithm. Internal shapes are seed regions, taken from manual segmentation of nuclei, and are the same in both rows. Bold lines show cell/cell boundaries that are compared. Top row: Cell images, with nuclei outlined. Middle row: Automatic segmentation with our method. Bottom row: Manual segmentation.

153. Jones therefore teaches generating a digital overlay drawing of an outer edge of each cell in the image. As discussed above, a POSA would have good reason to combine the teachings of Chukka and Jones with a reasonable expectation of success. Accordingly, to the extent claim 2 is not anticipated by or obvious in view of Chukka alone, claim 2 would have been obvious in view of Chukka and Jones.

'097 Patent	Prior Art
-------------	-----------

The method of claim 1, further comprising: generating a digital overlay drawing of an outer edge of each cell in the image.	EX1005 (Chukka), [0004] (“For example, the system can accurately count nuclear objects to determine information about the tissue to assist with reliable and reproducible slide interpretation. In one embodiment, the system counts positively-stained nuclear objects and/or negatively-stained nuclear objects to score, for example, a biological specimen (e.g., tumor tissue). In some embodiments, an overlay image is produced to label features of interest in the image of a specimen from a subject. Scoring of the tissue may be performed to predict and/or generate a prognosis for the tissue sample.”) EX1005 (Chukka), [0026] (“In some embodiments, the facility invokes a voting kernel process to identify objects or seed points, for example, nuclear objects or nuclear seed points, within the two dominant color regions. Seed points can be points that are assumed to lie inside an object and are the starting point for localizing objects (e.g., nuclear objects or other features of interest). In other words, seed identification can identify the approximate center point or other internal point for objects. As discussed in further detail below with reference to FIG. 4A, to identify seed objects, the facility can generate a grayscale representation of the digitized slide data and then computes gradient information from the grayscale representation. The facility then generates a vote response matrix by, for each pixel having a gradient magnitude that exceeds a pre-defined threshold, casting a vote to that pixels local neighborhood. The local neighborhood is determined based at least in part on the local gradient direction and a pre-determined distance or radius range. Subsequently, each local maximum within with the vote response matrix that exceeds a voting threshold can be identified as a seed location. In a parallel and independent step, for example, an adaptive thresholding technique can be applied to the tissue
--	--

	data to distinguish between darker objects (e.g., cell nuclei) and other objects, (e.g., stroma and slide background) and generate an object foreground mask. The object foreground mask and the seed locations are used in combination to generate a tessellated version of the foreground image, where each distinct connected area of pixels or cell in the tessellated version corresponds to a distinct detected object or “blob.” Each “blob” is then classified using a classifier (e.g., a previously-trained classifier) to identify one or more of positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes. Once classified, the detected nuclear objects can be used to score a slide or particular regions within a slide. Thus, the disclosed techniques take advantage of whole slide context and machine learning techniques to improve nuclear object detection and automated slide scoring.”) EX1005 (Chukka), [0044] (“In some embodiments, an overlay image is produced to label features of interest in the image of a specimen from a subject. Scoring of the tissue may be performed to predict and/or generate a prognosis for the tissue sample.”) EX1005 (Chukka), [0051] (“FIG. 5 is a flow diagram illustrating the processing of an extract objects component 114 in some embodiments. The facility invokes the component to identify boundaries around seed points that correspond to an object. Processing of the component is based at least in part on image information generated in steps 305-320 discussed above (e.g., tissue foreground mask, dominant color analysis, segmentation) and thus is based, in part, on context generated from the whole slide. In block 510, the component generates an object foreground mask by identifying those pixels that are darker than their local neighborhood intensity distribution and that exceed a global threshold. The object foreground mask may be generated, for
--	--

	example, by assigning a value of 1 for all pixels whose intensity value exceeds a mean intensity value for a local portion of the tissue (e.g., all pixels within the region currently being analyzed) and that also exceed a global threshold (e.g., 0.85). For pixels that do not meet these criteria, the component may assign another value, such as 0. The object foreground mask allows the facility to prune out weak and spurious objects that are unlikely to correspond to any object of interest (e.g., positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes). In block 520, the component applies the generated mask to the digitized tissue data of the region currently being analyzed. In block 530, the component retrieves the seed points that correspond to the region currently being analyzed. In block 540, the component generates a tessellation of the foreground image (e.g., a Voronoi tessellation) data based at least in part on the retrieved seed points to identify those portions of the foreground image data that are associated with each of the seed points. In block 550, the component identifies, for each seed point, the portion of the foreground image that is associated with the seed point based at least in part on the tessellation. This portion or “blob” can be associated with the seed and can define the boundary of a detected object. In block 560, the component characterizes each of the identified objects based at least in part on location and any number of characteristics including, for example, color characteristics, shape and size characteristics, object descriptor characteristics, cytoplasmic characteristics, inter-object and density characteristics, or any of the above-mentioned characteristics. Each of these identified objects is then returned for storage and classification by a trained classifier as discussed above.”).
--	--

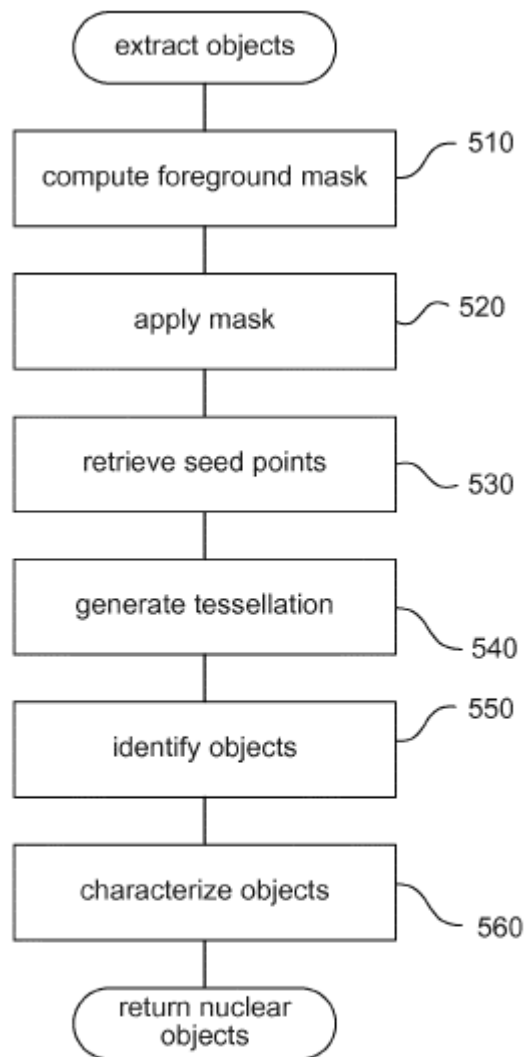


FIG. 5

EX1006 (Jones), Abstract (“We present a method for finding the boundaries between adjacent regions in an image, where “seed” areas have already been identified in the individual regions to be segmented. This method was motivated by the problem of finding the borders of cells in microscopy images, given a labelling of the nuclei in the images. The method finds the Voronoi region of each seed on a manifold with a metric controlled by local image properties. We discuss similarities to other methods

based on image-controlled metrics, such as Geodesic Active Contours, and give a fast algorithm for computing the Voronoi regions. We validate our method against hand-traced boundaries for cell images.”).

EX1006 (Jones), 537 (“Our method operates by computing a discretized approximation of the Voronoi regions of each seed on a manifold with a metric controlled by local image features. The metric defines the incremental distance in a particular direction in the image/manifold. Its behavior is such that adjacent pixels with similar surrounds are close to one another, while pixels whose surrounds differ are treated as farther apart.

We introduce a Riemannian metric defined in terms of the image I and a regularization parameter λ , as

$$\mathbf{G} = \frac{\nabla \mathbf{g}(I) \nabla \mathbf{g}^T(I) + \lambda \mathbf{I}}{1 + \lambda}, \quad (1)$$

where $\mathbf{I}$ is the 2×2 identity matrix. The function $\mathbf{g}$ maps images to images, and in our application is generally a small-radius blur. The effect of this blur is to combine a weighted neighborhood in the gradient computation, to avoid relying too much on single (noisy) pixel values. Infinitesimal distances under $\mathbf{G}$ are measured by

$$\|d\mathbf{x}\|_{\mathbf{G}}^2 \equiv d\mathbf{x}^T \mathbf{G} d\mathbf{x} = \frac{(d\mathbf{x}^T \nabla \mathbf{g}(I))^2 + \lambda (d\mathbf{x}^T d\mathbf{x})^2}{\lambda + 1}. \quad (2)$$

The first term in the numerator of (2), $\|d\mathbf{x}^T \nabla \mathbf{g}(I)\|^2$, increases distances measured parallel to large gradients in $\mathbf{g}(I)$. The regularization effect of λ can be seen by

$$\lim_{\lambda \rightarrow \infty} \|d\mathbf{x}\|_{\mathbf{G}}^2 = d\mathbf{x}^T d\mathbf{x} = \|d\mathbf{x}\|_2^2, \quad (3)$$

	i.e., $\mathbf{G}$ becomes more Euclidean as λ increases.” EX1006 (Jones), 3 (“Pixels overlapping seed regions are initialized to be distance 0 from their seed, and the distances of the remaining pixels are computed by Dijkstra’s algorithm [6]. Each pixel is labelled with the seed it is closest to, i.e., the Voronoi region of that seed in the manifold defined by (2). Computing the seed-to-pixel distances in this manner also makes it trivial to limit the segmentation to a predetermined foreground region (for example, the result from a global thresholding step). The computation of Voronoi regions places the boundary between two adjacent regions at pixels that are equidistant from each seed, as measured on the manifold from (2). The inter-pixel distance is larger where the image is changing more according to $\nabla \mathbf{g}(I)$, so boundaries tend to align with image differences. The regularizing parameter λ allows the user to make the manifold more ‘flat’ according to prior knowledge about region shapes, and the choice of $\mathbf{g}$ (e.g., the radius of smoothing) controls how sensitive distances are to small features. In this work, we use a narrow (3- or 5-pixel radius) Gaussian blur for $\mathbf{g}$.”) EX1006 (Jones), 539 (“Our algorithm is currently part of an automatic image cytometry program [4], and has been used in a variety of experiments with several cell types of varying morphologies. To evaluate our algorithm on real world data, we compare it to the manual segmentation of cell images. Sixteen images taken from <i>Drosophila</i> cells stained for DNA (to label nuclei) and Actin (a cytoskeletal protein, to show the cell body) were outlined. First, nuclei were outlined by hand. The nuclear outlines were overlaid on the cell images, and one cell per nucleus was outlined. Our algorithm was then applied to the same cell images and nuclear-
--	---

outlines data, and the results compared by computing the signed distance between boundary pixels. It effectively computes the distance for each pixel in the automatic segmentation boundary to the corresponding manual segmentation boundary. Hand-outlined nuclei were used, rather than automatically segmented nuclei, since our algorithm does not address nuclei segmentation, and we want the comparison to be as meaningful as possible. In general, automatic segmentation of nuclei is fairly simple (See section 1.)

Our algorithm does not compute a foreground/background separation, but instead relies on a such a label for each pixel to be given as input. For comparing our algorithm to the manual segmentation, we compute the foreground pixels as the union of cells identified in the manual segmentation. Methods exist for automatically choosing a foreground/background labelling, but distinguishing foreground from background is not part of our algorithm.”).

EX1006 (Jones), Fig.1:

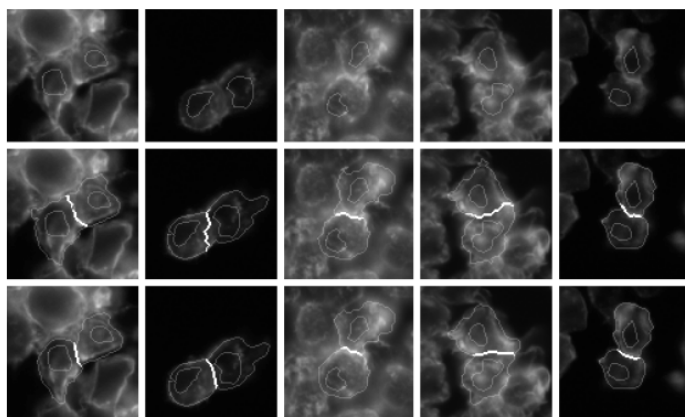


Fig. 1. Typical results from our algorithm. Internal shapes are seed regions, taken from manual segmentation of nuclei, and are the same in both rows. Bold lines show cell/cell boundaries that are compared. Top row: Cell images, with nuclei outlined. Middle row: Automatic segmentation with our method. Bottom row: Manual segmentation.

	EX1006 (Jones), 542 (“We have demonstrated an algorithm for segmentation of image regions based on seed areas that respects image boundaries as defined by a difference operator. The algorithm computes an approximation of the Voronoi region of a seed on a manifold, implicitly defined by a difference operator that operates on image neighborhoods. The only assumption that the algorithm relies on is that images change more near the borders of regions. This is similar to the behavior of Geodesic Active Contours, with an inversion in the behavior of the metrics near edges. We approximate distances using a chamfer-like difference operator, and use Dijkstra’s algorithm for computing individual regions quickly. Our algorithm is currently implemented in an automatic image cytometry package [4], and has been used successfully in several experiments with a variety of cell types and morphologies. We have compared our algorithm to manual segmentation by an expert of microscopy cell images. Our algorithm performs well. There are a few failure cases, perhaps due to the fairly simple prior on cell shape and size that our metric implies. A more complex prior could incorporate measures of, e.g., cell roundness or cell area. Although our method is more accurate for segmenting cells than others, such as using fixed offsets from nuclei or the watershed transform, the true test of its usefulness is in whether it produces more accurate measurements of cellular phenotypes. In order to understand how segmentation accuracy affects measurement accuracy, we plan to validate against data from flow cytometry or another method not based on image processing.”).
--	--

B. Claim 3

154. Claim 3 depends on claim 2, and recites as follows: “The method of claim 2, wherein the digital overlay drawing is prepared at the resolution level of an individual pixel.” Both Chukka and Jones teach this additional limitation.

155. As discussed above, Chukka teaches that in some embodiments, the facility may generate an overlay drawing to label features of interest in the image. Chuka further teaches that the facility may generate an object foreground mask by “identifying those pixels that are darker than their local neighborhood intensity distribution and that exceed a global threshold” and assigning “a value of 1 for all pixels whose intensity value exceeds a mean intensity value for a local portion of the tissue (e.g., all pixels within the region currently being analyzed) and that also exceed a global threshold (e.g., 0.85).” EX1005 (Chukka), [0051]. Chukka therefore teaches that the facility may analyze the digital image at the resolution level of the individual pixel.

156. Jones also teaches that the digital overlay drawing may be at the resolution level of an individual pixel. For example, Jones teaches that in its method, “pixels are assigned to the nearest seed region measured in the image plane” and “pixels are then assigned to cells according to their distance from the corresponding nucleus under that metric.” EX1006 (Jones), 537. Jones teaches that its method “effectively computes the distance for *each pixel* in the automatic

segmentation boundary to the corresponding manual segmentation boundary.”

Id., 539; *see also id.*, 537 (calculating “distance between boundary pixels.”).

157. Accordingly, Chukka, in combination with Jones, teaches generating a digital overlay drawing wherein the digital overlay drawing is prepared at the resolution level of an individual pixel. This claim is therefore obvious over Chukka, in view of Jones.

'097 Patent	Prior Art
The method of claim 2, wherein the digital overlay drawing is prepared at the resolution level of an individual pixel.	EX1005 (Chukka), [0004] (“At least some embodiments of the disclosed technology are directed to imaging systems for automatically interpreting and scoring tissue specimen slides, for example, specimens stained with an immunohistochemical (IHC) assay.... In some embodiments, an overlay image is produced to label features of interest in the image of a specimen from a subject.”). EX1005 (Chukka), [0044] (“In some embodiments, an overlay image is produced to label features of interest in the image of a specimen from a subject. Scoring of the tissue may be performed to predict and/or generate a prognosis for the tissue sample.”) EX1005 (Chukka), [0051] (“FIG. 5 is a flow diagram illustrating the processing of an extract objects component 114 in some embodiments. The facility invokes the component to identify boundaries around seed points that correspond to an object. Processing of the component is based at least in part on image information generated in steps 305-320 discussed above (e.g., tissue foreground mask, dominant color analysis, segmentation) and thus is based, in part, on context

	generated from the whole slide. In block 510, the component generates an object foreground mask by identifying those pixels that are darker than their local neighborhood intensity distribution and that exceed a global threshold. The object foreground mask may be generated, for example, by assigning a value of 1 for all pixels whose intensity value exceeds a mean intensity value for a local portion of the tissue (e.g., all pixels within the region currently being analyzed) and that also exceed a global threshold (e.g., 0.85). For pixels that do not meet these criteria, the component may assign another value, such as 0. The object foreground mask allows the facility to prune out weak and spurious objects that are unlikely to correspond to any object of interest (e.g., positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes).”). EX1006 (Jones).
--	--

C. Claims 7 and 8

158. Claim 7 depends from claim 6, and recites as follows:

The method of claim 6, further comprising:

storing, in a first file, for each tile, a tile position and the predicted class of the tile; and

storing, in a second file, for each cell object, a polygon outlining the cell object and the predicted class of the cell object.

159. Claim 8 depends from claim 6, and recites as follows:

The method of claim 6, further comprising:

generating a digital overlay drawing for the digital image, where the digital overlay drawing is a cell mask displaying a polygon around each cell object.

160. Claim 7 is directed to the storage of tile position and class in one file, and cell outline (e.g., polygon) and class in a second file. Claim 8 is directed to displaying a “cell mask displaying a polygon around each cell object.” The ’097 Patent does not purport to have invented a new or inventive technology to generate the polygon outline, only stating that in some embodiments, the method “outlines a shape with a closed polygon.” EX1001, 14:12-13. As discussed above, outlining cells and cell objects using a polygon was well-known and conventional in histopathology. See EX1017 (Ali), Fig. 3:

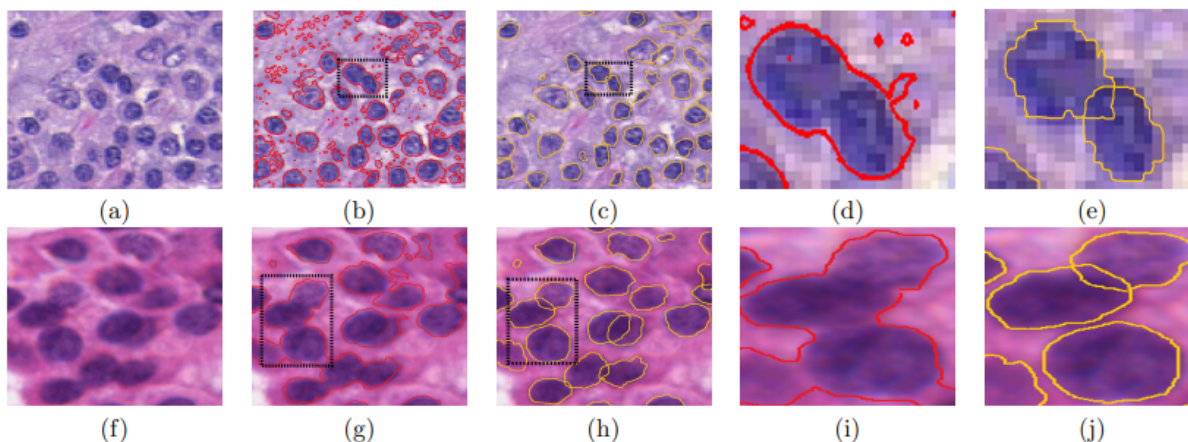


Figure 3. (a), (f) Original histological images corresponding to breast and prostate histology respectively, segmentation results for nuclei and lymphocytes obtained via the (b), (g) Geodesic Active Contour; (c), (h) our integrated AC model; (d), (e) magnified region from (b) and (g); (e), (j) magnified region from (c) and (h).

161. As discussed above, Chukka teaches generating a digital overlay drawing for the digital image. *Id.*, [0004]. Chukka further teaches storing in a first file, for each tile, a tile position and predicted class of the tile. Chukka

teaches that the facility may comprise a “computer-readable storage medium that can store data[.]” EX1005 (Chukka), [0007]; *see also id.*, [0029], [0030], [0031]. Chukka also teaches that the facility may store information about the tiles including the predicted class of the tile. Chukka teaches that in the tile-analysis embodiments, the “analyze slide component” “loops through each region (i.e., ...the tiles generated in block 330)” to identify seeds, extract objects, and classify each extracted object. *Id.*, [0046]. Chukka then teaches that the component “generates and stores...a score for the slide or one or more regions within the slide by....” *Id.* Chukka then teaches that the processing may loop back to continue with other regions or tiles of the slide. *Id.* Chukka therefore teaches storing, in a first file, a tile position and the predicted class of the tile.

162. Chukka also teaches storing, in a second file, for each cell object, a polygon outlining the cell object and the predicted class of the cell object. Chukka teaches that in some embodiments, the facility invokes an “extract objects component” “to identify boundaries around seed points that correspond to an object.” *Id.*, [0051]. Chukka then teaches that “the component generates a tessellation of the foreground image (e.g., a Voronoi tessellation) data based at least in part on the retrieved seed points to identify those portions of the foreground image data that are associated with each of the seed points.” *Id.* A Voronoi tessellation is a polygon around a cell object. *See* EX1027 (Du), 637

(“Depending on the application, there exist many different names for Voronoi regions, including Dirichlet regions, area of influence ***polygons***, Meijering cells, Thiessen ***polygons***, and S-mosaics.” (emphasis added)).

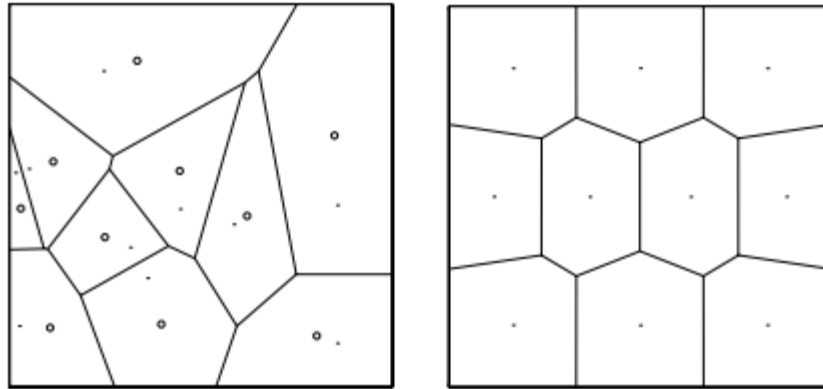


Fig. 1.1 *On the left, the Voronoi regions corresponding to 10 randomly selected points in a square; the density function is a constant. The dots are the Voronoi generators and the circles are the centroids of the corresponding Voronoi regions. Note that the generators and the centroids do not coincide. On the right, a 10-point centroidal Voronoi tessellation. The dots are simultaneously the generators for the Voronoi tessellation and the centroids of the Voronoi regions.*

163. Chukka then teaches that the component identifies “the portion of the foreground image data that is associated with the seed point based at least in part on the tessellation,” and that this “portion or ‘blob’ can...define the boundary of the detected object.” EX1005 (Chukka), [0051]. Chukka then teaches that the component “characterizes each of the identified objects based at least in part on location and any number of characteristics....” *Id.* Finally, Chukka teaches that “each of these identified objects then returned for ***storage*** and classification by a trained classifier as discussed above.” *Id.* Chukka therefore teaches storing, in a

second file, for each cell object, a polygon outlining the cell object and the predicted class of the cell object.

164. Jones further explains generating a polygon around each cell object and/or outlining each cell object. As discussed above, Jones teaches using Voronoi tessellations of seed regions to determine cellular boundaries. EX1006 (Jones), 535-39. Jones teaches that its algorithm has been successfully implemented in several experiments “with a variety of cell types and morphologies.” *Id.*, 542. As illustrated in Jones’s Fig. 1, Jones teaches that its algorithm can generate a digital overlay drawing of an outer edge of each cell in the image. *Id.*, 5, Fig. 1.

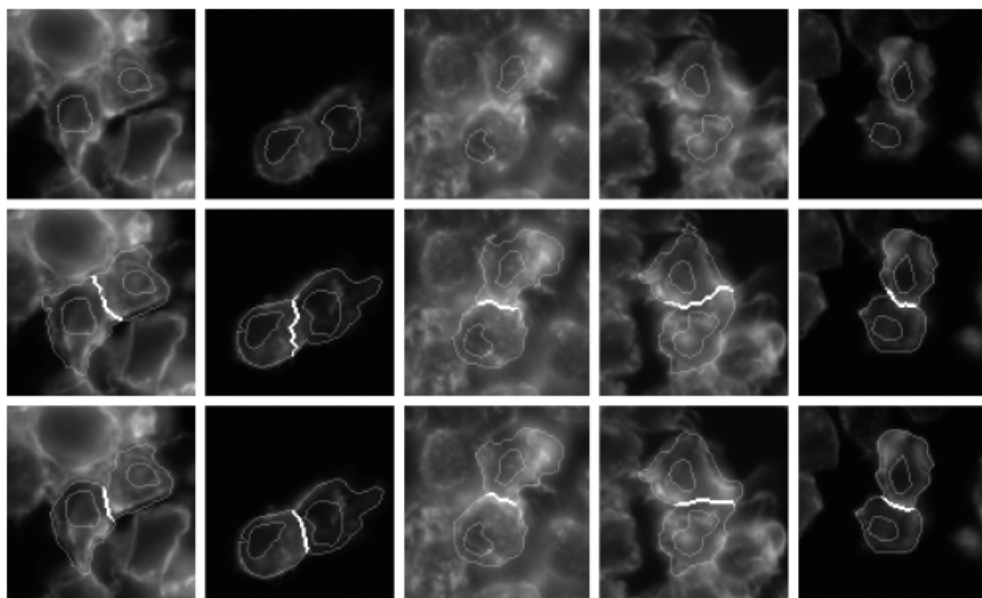


Fig. 1. Typical results from our algorithm. Internal shapes are seed regions, taken from manual segmentation of nuclei, and are the same in both rows. Bold lines show cell/cell boundaries that are compared. Top row: Cell images, with nuclei outlined. Middle row: Automatic segmentation with our method. Bottom row: Manual segmentation.

165. Jones therefore teaches generating a digital overlay drawing of an outer edge of each cell in the image. As discussed above, a POSA would have good reason to combine the teachings of Chukka and Jones with a reasonable expectation of success. Accordingly, claims 7 and 8 would have been obvious over Chukka, in view of Jones.

'097 Patent	Prior Art
The method of claim 6, further comprising: storing, in a first file, for each tile, a tile position and the predicted class of the tile; and storing, in a second file, for each cell object, a polygon outlining the cell object and the predicted class of the cell object.	EX1005 (Chukka), [0004] (“For example, the system can accurately count nuclear objects to determine information about the tissue to assist with reliable and reproducible slide interpretation. In one embodiment, the system counts positively-stained nuclear objects and/or negatively-stained nuclear objects to score, for example, a biological specimen (e.g., tumor tissue). In some embodiments, an overlay image is produced to label features of interest in the image of a specimen from a subject. Scoring of the tissue may be performed to predict and/or generate a prognosis for the tissue sample.”)
The method of claim 6, further comprising: generating a digital overlay drawing for the digital image, where the digital overlay drawing is a cell mask displaying a polygon around each cell object.	EX1005 (Chukka), [0007] (“A computer-readable storage medium can store data (e.g., classifiers, algorithms, etc.) and instructions that, if executed by a computing system having a processor, cause the computing system to perform such methods.”); <i>see also id.</i>, [0029], [0030], [0031]. EX1005 (Chukka), [0026] (“In some embodiments, the facility invokes a voting kernel process to identify objects or seed points, for example, nuclear objects or nuclear seed points, within the two dominant color regions. Seed points can be points that are assumed to lie inside an object and are the starting point for localizing objects (e.g., nuclear objects or other features of interest). In other words, seed

	identification can identify the approximate center point or other internal point for objects. As discussed in further detail below with reference to FIG. 4A, to identify seed objects, the facility can generate a grayscale representation of the digitized slide data and then computes gradient information from the grayscale representation. The facility then generates a vote response matrix by, for each pixel having a gradient magnitude that exceeds a pre-defined threshold, casting a vote to that pixels local neighborhood. The local neighborhood is determined based at least in part on the local gradient direction and a pre-determined distance or radius range. Subsequently, each local maximum within with the vote response matrix that exceeds a voting threshold can be identified as a seed location. In a parallel and independent step, for example, an adaptive thresholding technique can be applied to the tissue data to distinguish between darker objects (e.g., cell nuclei) and other objects, (e.g., stroma and slide background) and generate an object foreground mask. The object foreground mask and the seed locations are used in combination to generate a tessellated version of the foreground image, where each distinct connected area of pixels or cell in the tessellated version corresponds to a distinct detected object or “blob.” Each “blob” is then classified using a classifier (e.g., a previously-trained classifier) to identify one or more of positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes. Once classified, the detected nuclear objects can be used to score a slide or particular regions within a slide. Thus, the disclosed techniques take advantage of whole slide context and machine learning techniques to improve nuclear object detection and automated slide scoring.”) EX1005 (Chukka), [0044] (“In some embodiments, an overlay image is produced to label features of interest in the image of a specimen from a subject.
--	--

	Scoring of the tissue may be performed to predict and/or generate a prognosis for the tissue sample.”) EX1005 (Chukka), [0051] (“FIG. 5 is a flow diagram illustrating the processing of an extract objects component 114 in some embodiments. The facility invokes the component to identify boundaries around seed points that correspond to an object. Processing of the component is based at least in part on image information generated in steps 305-320 discussed above (e.g., tissue foreground mask, dominant color analysis, segmentation) and thus is based, in part, on context generated from the whole slide. In block 510, the component generates an object foreground mask by identifying those pixels that are darker than their local neighborhood intensity distribution and that exceed a global threshold. The object foreground mask may be generated, for example, by assigning a value of 1 for all pixels whose intensity value exceeds a mean intensity value for a local portion of the tissue (e.g., all pixels within the region currently being analyzed) and that also exceed a global threshold (e.g., 0.85). For pixels that do not meet these criteria, the component may assign another value, such as 0. The object foreground mask allows the facility to prune out weak and spurious objects that are unlikely to correspond to any object of interest (e.g., positively-stained nuclear objects, negatively-stained nuclear objects, stromata, and lymphocytes). In block 520, the component applies the generated mask to the digitized tissue data of the region currently being analyzed. In block 530, the component retrieves the seed points that correspond to the region currently being analyzed. In block 540, the component generates a tessellation of the foreground image (e.g., a Voronoi tessellation) data based at least in part on the retrieved seed points to identify those portions of the foreground image data that are associated with each of the seed points. In block 550, the component identifies, for each seed
--	---

point, the portion of the foreground image that is associated with the seed point based at least in part on the tessellation. This portion or “blob” can be associated with the seed and can define the boundary of a detected object. In block **560**, the component characterizes each of the identified objects based at least in part on location and any number of characteristics including, for example, color characteristics, shape and size characteristics, object descriptor characteristics, cytoplasmic characteristics, inter-object and density characteristics, or any of the above-mentioned characteristics. Each of these identified objects is then returned for storage and classification by a trained classifier as discussed above.”).

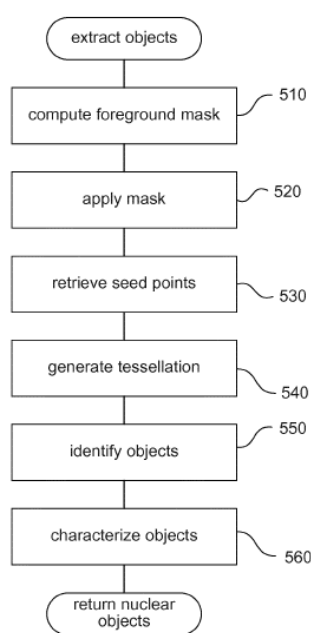


FIG. 5

EX1006 (Jones), Abstract (“We present a method for finding the boundaries between adjacent regions in an image, where “seed” areas have already been identified in the individual regions to be segmented. This method was motivated by the problem of finding the borders of cells in microscopy images,

given a labelling of the nuclei in the images. The method finds the Voronoi region of each seed on a manifold with a metric controlled by local image properties. We discuss similarities to other methods based on image-controlled metrics, such as Geodesic Active Contours, and give a fast algorithm for computing the Voronoi regions. We validate our method against hand-traced boundaries for cell images.”).

EX1006 (Jones), 537 (“Our method operates by computing a discretized approximation of the Voronoi regions of each seed on a manifold with a metric controlled by local image features. The metric defines the incremental distance in a particular direction in the image/manifold. Its behavior is such that adjacent pixels with similar surrounds are close to one another, while pixels whose surrounds differ are treated as farther apart.

We introduce a Riemannian metric defined in terms of the image I and a regularization parameter λ , as

$$\mathbf{G} = \frac{\nabla \mathbf{g}(I) \nabla \mathbf{g}^T(I) + \lambda \mathbf{I}}{1 + \lambda}, \quad (1)$$

where $\mathbf{I}$ is the 2×2 identity matrix. The function $\mathbf{g}$ maps images to images, and in our application is generally a small-radius blur. The effect of this blur is to combine a weighted neighborhood in the gradient computation, to avoid relying too much on single (noisy) pixel values. Infinitesimal distances under $\mathbf{G}$ are measured by

$$\|d\mathbf{x}\|_{\mathbf{G}}^2 \equiv d\mathbf{x}^T \mathbf{G} d\mathbf{x} = \frac{(d\mathbf{x}^T \nabla \mathbf{g}(I))^2 + \lambda (d\mathbf{x}^T d\mathbf{x})^2}{\lambda + 1}. \quad (2)$$

The first term in the numerator of (2), $\|d\mathbf{x}^T \nabla \mathbf{g}(I)\|^2$, increases distances measured parallel to large gradients in $\mathbf{g}(I)$. The regularization effect of λ can

	be seen by $\lim_{\lambda \rightarrow \infty} d\mathbf{x} _{\mathbf{G}}^2 = d\mathbf{x}^T d\mathbf{x} = d\mathbf{x} _2^2, \quad (3)$ i.e., $\mathbf{G}$ becomes more Euclidean as λ increases.” EX1006 (Jones), 3 (“Pixels overlapping seed regions are initialized to be distance 0 from their seed, and the distances of the remaining pixels are computed by Dijkstra’s algorithm [6]. Each pixel is labelled with the seed it is closest to, i.e., the Voronoi region of that seed in the manifold defined by (2). Computing the seed-to-pixel distances in this manner also makes it trivial to limit the segmentation to a predetermined foreground region (for example, the result from a global thresholding step). The computation of Voronoi regions places the boundary between two adjacent regions at pixels that are equidistant from each seed, as measured on the manifold from (2). The inter-pixel distance is larger where the image is changing more according to $\nabla \mathbf{g}(I)$, so boundaries tend to align with image differences. The regularizing parameter λ allows the user to make the manifold more ‘flat’ according to prior knowledge about region shapes, and the choice of $\mathbf{g}$ (e.g., the radius of smoothing) controls how sensitive distances are to small features. In this work, we use a narrow (3- or 5-pixel radius) Gaussian blur for $\mathbf{g}$.”) EX1006 (Jones), 539 (“Our algorithm is currently part of an automatic image cytometry program [4], and has been used in a variety of experiments with several cell types of varying morphologies. To evaluate our algorithm on real world data, we compare it to the manual segmentation of cell images. Sixteen images taken from <i>Drosophila</i> cells stained for DNA (to label nuclei) and Actin (a cytoskeletal protein, to show the cell body) were outlined. First, nuclei were outlined by hand. The
--	---

	nuclear outlines were overlaid on the cell images, and one cell per nucleus was outlined. Our algorithm was then applied to the same cell images and nuclear-outlines data, and the results compared by computing the signed distance between boundary pixels. It effectively computes the distance for each pixel in the automatic segmentation boundary to the corresponding manual segmentation boundary. Hand-outlined nuclei were used, rather than automatically segmented nuclei, since our algorithm does not address nuclei segmentation, and we want the comparison to be as meaningful as possible. In general, automatic segmentation of nuclei is fairly simple (See section 1.) Our algorithm does not compute a foreground/background separation, but instead relies on a such a label for each pixel to be given as input. For comparing our algorithm to the manual segmentation, we compute the foreground pixels as the union of cells identified in the manual segmentation. Methods exist for automatically choosing a foreground/background labelling, but distinguishing foreground from background is not part of our algorithm.”). EX1006 (Jones), Fig.1:
--	---

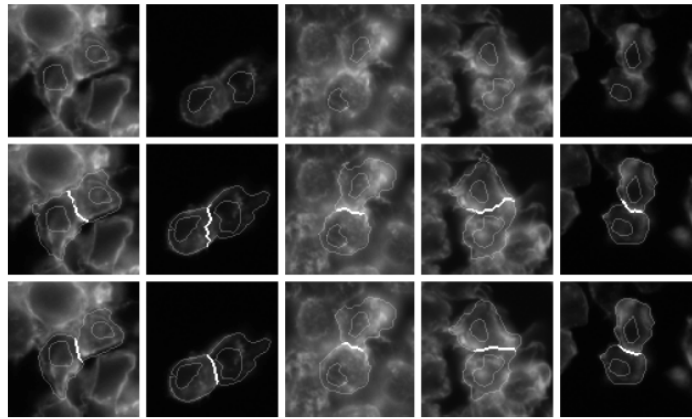


Fig. 1. Typical results from our algorithm. Internal shapes are seed regions, taken from manual segmentation of nuclei, and are the same in both rows. Bold lines show cell/cell boundaries that are compared. Top row: Cell images, with nuclei outlined. Middle row: Automatic segmentation with our method. Bottom row: Manual segmentation.

EX1006 (Jones), 542 (“We have demonstrated an algorithm for segmentation of image regions based on seed areas that respects image boundaries as defined by a difference operator. The algorithm computes an approximation of the Voronoi region of a seed on a manifold, implicitly defined by a difference operator that operates on image neighborhoods. The only assumption that the algorithm relies on is that images change more near the borders of regions. This is similar to the behavior of Geodesic Active Contours, with an inversion in the behavior of the metrics near edges. We approximate distances using a chamfer-like difference operator, and use Dijkstra’s algorithm for computing individual regions quickly.

Our algorithm is currently implemented in an automatic image cytometry package [4], and has been used successfully in several experiments with a variety of cell types and morphologies. We have compared our algorithm to manual segmentation by an expert of microscopy cell images. Our algorithm performs well. There are a few failure cases, perhaps due to the fairly simple prior on cell shape and size

	that our metric implies. A more complex prior could incorporate measures of, e.g., cell roundness or cell area. Although our method is more accurate for segmenting cells than others, such as using fixed offsets from nuclei or the watershed transform, the true test of its usefulness is in whether it produces more accurate measurements of cellular phenotypes. In order to understand how segmentation accuracy affects measurement accuracy, we plan to validate against data from flow cytometry or another method not based on image processing.”).
--	---

XI. GROUND 3: CLAIMS 12, 13, & 16 ARE OBVIOUS OVER CHUKKA AND SEBASTIAO

166. As discussed in detail below, Chukka in view of Sebastiao would have rendered claims 12, 13 and 16 obvious. Below, I note specific prior art disclosures that satisfy each element of each challenged claim. After each claim element, I provide a chart identifying certain exemplary prior art disclosures that are relevant to that element.

167. Claims 12, 13, and 16 all depend on claim 6. Claim 12 recites the additional limitations of “generating a digital overlay drawing for the digital image, wherein the digital overlay drawing includes a generated version of the digital image; and displaying the digital overlay drawing.” Claim 13 similarly recites “generating a plurality of digital overlay drawings for the digital image, where each digital overlay drawing corresponds to a different predicted class” and then “selectively displaying one of the plurality of digital overlay drawings.”

EX1001 ('097 Patent) cl. 12, 13. Claim 16, for its part recites, a method of claim 6 wherein the “cell objects comprise CD3, CD8, CD20, pancytokeratin, and smooth muscle actin.” EX1001 ('097 Patent), cl. 16.

168. As discussed above, Chukka teaches every limitation of independent claim 6. Chukka also teaches generating and displaying an overlay drawing of the digital image. Accordingly, a POSA would have understood that Chukka teaches every element of claims 12 and 13 except possibly for “wherein the digital overlay drawing includes a generated version of the digital image,” (claim 12) or “generating a **plurality** of digital overlay drawings ... where each digital overlay drawing corresponds to a different predicted class” and “**selectively** displaying one” of those overlay drawings (claim 13). Moreover, a POSA would have understood that Chukka also teaches every element of claim 16 except possibly identifying cell objects comprising CD3, CD8, CD20, pancytokeratin, and smooth muscle actin.

169. Sebastiao teaches those elements. At a high level, Sebastiao teaches methods of analyzing and scoring images of stained tissue slides for cancer, wherein the images are stained for a “multiplex assay” of lymphocyte markers including CD3, CD8, CD20, FoxP3, and tumor detection markers, including cytokeratin EX1005 (Sebastiao), [0014], [0037], [0048]. Sebastiao teaches “unmixing” the tissue image stained with this multiplex assay into “separate

image channels for each marker for the multiplex assay,” and identifying regions of interest in each image channel. *Id.* Sebastiao then teaches generating a single “surrogated image” that is a combination of the image channel information for all of the lymphocyte markers. *Id.* Sebastiao then teaches applying a cell detection algorithm to detect lymphocytes in the surrogated image or image channels, and to classify the detected cells “as at least one of false positive cells, CD3 only T-cells, CD3 and CD8 T-cells, FP3 T-cells; and CD20 B-cells[.]” *Id.*

170. A POSA would have had a good reason to combine the teachings of Chukka and Sebastiao. First, both Chukka and Sebastiao were filed by the same applicant—Ventana Medical Systems, Inc.— and share a named inventor—Srinivas Chukka. A POSA would therefore have had a good reason to look to combine Chukka with other prior art by the same inventor and applicant, and vice versa for Sebastiao. Both Chukka and Sebastiao teach that histopathological examination of immunohistochemical (IHC) slides is a known method of identifying biomarkers to characterize and diagnose cancers such as breast cancer. *See* EX1005 (Chukka), [0002]; EX1005 (Sebastiao), [0005]. In particular, both Chukka and Sebastiao teach identifying and classifying lymphocytes. *See* EX1005 (Chukka), [0044]; EX1005 (Sebastiao), [0030].

171. Sebastiao teaches that a multiplexed assay tissue slide is stained for multiple lymphocyte markers such as CD3, CD8, CD20, FoxP3, and others.

Sebastiao then teaches using an image analysis algorithm to unmix the image into separate channels for each marker, and to then apply a “cell detection algorithm” to identify features of lymphocytes and combinations of lymphocytes in each image channel. EX1005 (Sebastiao), [0014]. Chukka teaches one method of detecting lymphocytes, by analyzing stained tissue slides through a tile-based approach, where the system iteratively analyzes each tile and features in neighboring tiles to identify lymphocytes. EX1005 (Chukka), [0044]. Chukka teaches that this method more effectively identifies lymphocytes, as it enables the system to analyze the whole slide in a context-based manner while also at a coarse resolution. *Id.*, [0045]. A POSA would therefore understand that applying Chukka’s tile-based approach to identifying and classifying lymphocytes would be the application of a known technique to Sebastiao’s multiplex assay of lymphocyte markers.

172. A POSA would also have a reasonable expectation of success in combining Chukka and Sebastiao. Again, both Chukka and Sebastiao were filed by Ventana Medical Systems, and therefore reference the same example systems for, e.g., staining and scanning pathology slides. *See* EX1005 (Chukka), [0039] (“The digitized tissue data may be generated, for example, by an iSCAN COREO™ by VENTANA MEDICAL SYSTEMS of Tucson, Ariz. or other suitable imaging equipment.”); EX1005 (Sebastiao), [0081] (“Exemplary

automated systems available through Ventana Medical Systems, Inc., Tucson, AZ include SYMPHONY® Staining System, catalog #: 900-SYM3, VENTANA® BenchMark Automated Slide Preparation Systems, catalog #s: N750-BMKXT-FS, N750-BMKU-FS, VENTANA, and VENTANA® BenchMark Special Stains automated slide stainer.”).

A. Claim 12

173. Claim 12 depends from claim 6, reciting as follows:

The method of claim 6, further comprising:

generating a digital overlay drawing for the digital image, wherein the digital overlay drawing includes a generated version of the digital image; and

displaying the digital overlay drawing.

174. Chukka, alone or in combination with Sebastiao, teaches this element. As discussed above, Chukka teaches that in some embodiments, the disclosed facility may generate “an overlay image” which “label[s] features of interest” in the tissue slide image. EX1005 (Chukka), [0004]. Chukka also teaches that in some embodiments, the “facility can generate a grayscale representation of the digitized slide data and then computes gradient information from the grayscale representation.” *Id.*, [0026]. Chukka further teaches that the facility can comprise an “imaging apparatus” to capture images of the tissue specimen, and that an image of the specimen “can be produced by combining” a plurality of “snapshots.” *Id.*, [0028].

175. Sebastiao teaches creating a generated version of the digital image. Sebastiao teaches that the image analysis algorithm may receive a set of images of multiplex IHC slides and unmix the images into “separate image channels for each marker of the multiplex assay.” EX1005 (Sebastiao), [0014]. Sebastiao also teaches combining these separate channels into a surrogated image, wherein “the surrogated image is a combination of the image channel information of all the lymphocyte markers.” *Id.* Sebastiao further teaches that the image may be transformed into a “grayscale or absorbance image.” *Id.*

176. More specifically, Sebastiao teaches that generating a surrogated image of the slide for each region of interest. Sebastiao teaches that “[t]o generate the surrogated image, a log transform is utilized to convert the RGB image into absorbance space, and then the Oscar channel may be utilized to remove the noise [tumor cells].” *Id.*, [0056]. Sebastiao explains that the surrogated image may be advantageous by reducing “color variation” and “removing noise.” *Id.* Sebastiao also teaches that the “cell detection algorithm ... is then applied to the surrogated image to detect the lymphocytes.” *Id.*, [0057]. Sebastiao then teaches that in exemplary embodiments, the score for each marker may be reported, along with the tissue context information, as output.” *Id.*, [0051].

177. As discussed above, a POSA would have had a good reason to combine the teachings of Sebastiao and Chukka, in order to detect and count

lymphocytes in a multiplex stained tissue slide image, with a reasonable expectation of success. A POSA would therefore have had a good reason to generate an overlay image as taught in Chukka, wherein the overlay image comprises a generated (e.g., surrogated) version of the digital image as taught in Sebastiao. Accordingly, this claim would have been obvious over Chukka in view of Sebastiao.

'097 Patent	Prior Art
The method of claim 6, further comprising: generating a digital overlay drawing for the digital image, wherein the digital overlay drawing includes a generated version of the digital image; and displaying the digital overlay drawing.	EX1005 (Chukka), [0004] (“For example, the system can accurately count nuclear objects to determine information about the tissue to assist with reliable and reproducible slide interpretation. In one embodiment, the system counts positively-stained nuclear objects and/or negatively-stained nuclear objects to score, for example, a biological specimen (e.g., tumor tissue). In some embodiments, an overlay image is produced to label features of interest in the image of a specimen from a subject. Scoring of the tissue may be performed to predict and/or generate a prognosis for the tissue sample.”) EX1005 (Chukka), [0026] (“As discussed in further detail below with reference to FIG. 4A, to identify seed objects, the facility can generate a grayscale representation of the digitized slide data and then computes gradient information from the grayscale representation.”) EX1005 (Chukka), [0028] (“One method of producing a digital image includes determining a scan area comprising a region of the microscope slide that includes at least a portion of the specimen. The scan area may be divided into a

	plurality of snapshots. An image can be produced by combining the snapshots. In some embodiments, the imaging apparatus 120 produces a high-resolution image of the entire specimen and/or an image of the entire mounting area of a slide.”) EX1007 (Sebastiao), [0014] (“An image analysis algorithm and/or system has been developed that automatically computes an immune score from a set images of multiplex IHC slides and/or fluorescent stained slides. The image analysis algorithm involves a computer-implemented method for counting a number of types of cells in a single tissue specimen that has been stained with a multiplex assay, comprising: imaging the single tissue specimen that has been stained with the multiplex assay that includes lymphocyte markers CD3, CD8, CD20, FoxP3, and tumor detection markers; unmixing the image of single tissue specimen that has been stained with a multiplex assay into separate image channels for each marker of the multiplex assay; identifying regions of interest in each image channel based on intensity information in each channel, wherein regions of low intensity in each channel are removed, and regions of high intensity represent cell signals; generating a single surrogated image, wherein the surrogated image is a combination of the image channel information of all the lymphocyte markers; applying a cell detection algorithm, wherein the cell detection algorithm is a membrane finding algorithm or a nucleus finding algorithm; identifying features of the lymphocytes and combinations of lymphocytes in each image channel or image of combined channels, or a transformed image such as grayscale or absorbance image, or a surrogated image; training a classification algorithm based on features of known lymphocytes and lymphocyte combinations; applying the trained algorithm to features of the
--	---

	lymphocytes and combinations of lymphocytes in each image channel or in each image of combined channels, or in a transformed image such as grayscale or absorbance image, or in a surrogated image, that were identified to classify the detected cells as at least one of false positive cells, CD3 only T-cells, CD3 and CD8 T-cells, FP3 T-cells; and CD20 B-cells; counting a number of each different type of cell classified; generating a score of the tissue specimen, wherein the score is based on the number of each type of cell counted.”). EX1007 (Sebastiao), [0051] (“ To analyze the generated or obtained images, the image analysis algorithm and/or system of the present invention includes a color unmixing algorithm, which may be included in an unmixing module, that identifies the individual constituent dyes for the biomarkers and the proportions in which they appear in the color images....In an exemplary embodiment, image analysis is performed, after unmixing, to identify, quantify and/or generate a score for a biomarker, for example, Oscar, CD3, CD8 and FoxP3 markers. In an exemplary embodiment of the present invention, the score for each marker may be reported, along with the tissue context information, as output.”). EX1007 (Sebastiao), [0056] (“A surrogated image of lymphocytes [i.e., an image that contains all the T-cells] may be obtained first. By doing so, we can first detect all the T-cells and then use the classification algorithm to identify the cell types. To generate the surrogated image, a log transform is utilized to convert the RGB image into absorbance space, and then the Oscar channel may be utilized to remove the noise [tumor cells] as shown in Fig. 9, since we are not detecting the tumor cells. In an exemplary embodiment of the present invention, the Oscar channel information,
--	---

	including the tumor cell information is saved in a memory in order to count the tumor cells. A surrogated image is utilized to do detection, instead of each unmixed channel, because unmixing multiplex assays, for example assays having five or more markers, may not be reliable due to the huge color variation across the image. In exemplary embodiments of the present invention, a combined image of some other sort may be utilized, for example, a transformed image [e.g., an absorbance image or grey scale image] or an image that combines various channels. Then the input image [e.g., the RGB image] may be unmixed into different channels of biomarkers, for example, the Oscar, FoxP3, CD3, CD8, HTX and CD20 channels.”) EX1007 (Sebastiao), [0057] (“The cell detection algorithm [e.g., corresponding to the ring detector in Fig. 10], may be included in a cell detection module, and is applied to the surrogated image to detect the lymphocytes. In an exemplary embodiment of the present invention, the ring shape of the cell detection algorithm provides for capturing ring shape membrane markers [e.g. all the possible T-cell candidates and may include false positive detections]. We use the absorbance image with Oscar correction as the surrogated image in the implementation [Fig. 9] to detect the ring shape membrane markers. In the implementation stage, a cell detection algorithm that is very sensitive, is desirable, e.g., an algorithm that captures all possible cells and may include the false positive detections. In an exemplary embodiment of the present invention, a ring detector may be applied to the surrogate image to detect the membrane markers, which are utilized to identify cells.”)
--	---

B. Claim 13

178. Claim 13 depends from claim 6, reciting as follows:

The method of claim 6, further comprising:

generating a plurality of digital overlay drawings for the digital image, where each digital overlay drawing corresponds to a different predicted class; and

selectively displaying one of the plurality of digital overlay drawings.

179. The combination of Chukka and Sebastiao teaches this element. As discussed above, Sebastiao teaches receiving and analyzing a digital image of a multiplex stained tissue slide. EX1007 (Sebastiao), [0014]. Sebastiao teaches unmixing this multiplexed assay image into “separate image channels for each marker of the multiplex assay” and that the system can then identify “regions of interest in each image channel based on intensity information in each channel.” *Id.*, [0014]. Sebastiao further teaches applying a “cell detection algorithm” to each image channel or a combination of the image channels to identify and classify the detected cells, count the number of each type of cell, and generate a score of the tissue. *Id.* Sebastiao further teaches that “[i]n an exemplary embodiment of the present invention, the score for each marker may be reported, along with the tissue context information, as output.” *Id.*, [0051]. Chukka, for its part, teaches that its disclosed facility may generate “an overlay image” which “label[s] features of interest” in the tissue slide image. EX1005 (Chukka), [0004].

180. Therefore, Chukka teaches generating digital overlay drawings for the digital image, and Sebastiao teaches that these digital overlay drawings may correspond to a different predicted class (e.g., separate image channels that correspond to different lymphocyte markers). It would have been obvious to a POSA to combine Chukka's teachings of generating overlay drawings with Sebastiao's separate image channels to generate a plurality of digital overlay drawings, a plurality of digital overlay drawings for the digital image, where each digital overlay drawing corresponds to a different predicted class; and selectively displaying one of the plurality of digital overlay drawings. Accordingly, this claim would have been obvious over Chukka in view of Sebastiao.

'097 Patent	Prior Art
The method of claim 6, further comprising: generating a plurality of digital overlay drawings for the digital image, where each digital overlay drawing corresponds to a different predicted class; and selectively displaying one of the plurality of digital overlay drawings.	EX1005 (Chukka), [0004] ("For example, the system can accurately count nuclear objects to determine information about the tissue to assist with reliable and reproducible slide interpretation. In one embodiment, the system counts positively-stained nuclear objects and/or negatively-stained nuclear objects to score, for example, a biological specimen (e.g., tumor tissue). In some embodiments, an overlay image is produced to label features of interest in the image of a specimen from a subject. Scoring of the tissue may be performed to predict and/or generate a prognosis for the tissue sample.") EX1007 (Sebastiao), [0014] ("An image analysis algorithm and/or system has been developed that automatically computes an immune score from a

	set images of multiplex IHC slides and/or fluorescent stained slides. The image analysis algorithm involves a computer-implemented method for counting a number of types of cells in a single tissue specimen that has been stained with a multiplex assay, comprising: imaging the single tissue specimen that has been stained with the multiplex assay that includes lymphocyte markers CD3, CD8, CD20, FoxP3, and tumor detection markers; unmixing the image of single tissue specimen that has been stained with a multiplex assay into separate image channels for each marker of the multiplex assay; identifying regions of interest in each image channel based on intensity information in each channel, wherein regions of low intensity in each channel are removed, and regions of high intensity represent cell signals; generating a single surrogated image, wherein the surrogated image is a combination of the image channel information of all the lymphocyte markers; applying a cell detection algorithm, wherein the cell detection algorithm is a membrane finding algorithm or a nucleus finding algorithm; identifying features of the lymphocytes and combinations of lymphocytes in each image channel or image of combined channels, or a transformed image such as grayscale or absorbance image, or a surrogated image; training a classification algorithm based on features of known lymphocytes and lymphocyte combinations; applying the trained algorithm to features of the lymphocytes and combinations of lymphocytes in each image channel or in each image of combined channels, or in a transformed image such as grayscale or absorbance image, or in a surrogated image, that were identified to classify the detected cells as at least one of false positive cells, CD3 only T-cells, CD3 and CD8 T-cells, FP3 T-cells; and CD20 B-cells; counting a number of each different type of cell classified; generating a score of the
--	--

	tissue specimen, wherein the score is based on the number of each type of cell counted.”). EX1007 (Sebastiao), [0051] (“ To analyze the generated or obtained images, the image analysis algorithm and/or system of the present invention includes a color unmixing algorithm, which may be included in an unmixing module, that identifies the individual constituent dyes for the biomarkers and the proportions in which they appear in the color images....In an exemplary embodiment, image analysis is performed, after unmixing, to identify, quantify and/or generate a score for a biomarker, for example, Oscar, CD3, CD8 and FoxP3 markers. In an exemplary embodiment of the present invention, the score for each marker may be reported, along with the tissue context information, as output.”). EX1007 (Sebastiao), [0056] (“A surrogated image of lymphocytes [i.e., an image that contains all the T-cells] may be obtained first. By doing so, we can first detect all the T-cells and then use the classification algorithm to identify the cell types. To generate the surrogated image, a log transform is utilized to convert the RGB image into absorbance space, and then the Oscar channel may be utilized to remove the noise [tumor cells] as shown in Fig. 9, since we are not detecting the tumor cells. In an exemplary embodiment of the present invention, the Oscar channel information, including the tumor cell information is saved in a memory in order to count the tumor cells. A surrogated image is utilized to do detection, instead of each unmixed channel, because unmixing multiplex assays, for example assays having five or more markers, may not be reliable due to the huge color variation across the image. In exemplary embodiments of the present invention, a combined image of some other sort may be utilized, for
--	--

	example, a transformed image [e.g., an absorbance image or grey scale image] or an image that combines various channels. Then the input image [e.g., the RGB image] may be unmixed into different channels of biomarkers, for example, the Oscar, FoxP3, CD3, CD8, HTX and CD20 channels.”) EX1007 (Sebastiao), [0051] (“Based on the separated image channels for each marker, detection algorithms are developed to automatically identify different combination of T and B lymphocytes with respect to each of the differentiating IHC markers. For example, different T-cell markers might co-localize and create different combination of the stain amounts. Additionally, a tumor marker, for example a cytokeratin marker [e.g., Oscar], may also be included in the assay to identify the tumor, intratumoral and/or peritumoral regions to get the tissue context [for example, to identify the nuclei and the tumor regions]. In an exemplary embodiment, image analysis is performed, after unmixing, to identify, quantify and/or generate a score for a biomarker, for example, Oscar, CD3, CD8 and FoxP3 markers. In an exemplary embodiment of the present invention, the score for each marker may be reported, along with the tissue context information, as output.”) EX1007 (Sebastiao), [0097] (“Based on the separated image channels for each marker, detection algorithms were developed to automatically identify different types of T-cells. The image analysis was done for Oscar, CD3, CD8 and FP3 markers. As the final output, the score for each marker was reported.”)
--	--

C. Claim 16

181. Claim 16 depends from claim 6, reciting as follows:

The method of claim 6, wherein the cell objects comprise CD3, CD8, CD20, pancytokeratin, and smooth muscle actin.

182. Sebastiao teaches this element. Sebastiao teaches an image analysis algorithm or system that “automatically computes an immune score from a set images of multiplex IHC slides.” EX1007 (Sebastiao), [0014]. Sebastiao teaches that the system comprises a computer-implemented method of counting cell objects in a “single tissue specimen that has been stained with the multiplex assay that includes lymphocyte markers CD3, CD8, CD20, FoxP3, and tumor detection markers.” *Id.* Sebastiao also teaches detecting cytokeratin to identify epithelial tumor cells. *Id.*, [0051] (“Additionally, a tumor marker, for example a cytokeratin marker (e.g., Oscar), may also be included in the assay to identify the tumor....”); [0037] (identifying epithelial carcinomas using cytokeratin staining). As reflected in the prior art, a POSA would have understood that the cytokeratin marker disclosed by Sebastiao (Oscar) is a pancytokeratin marker. EX1030 (Zeta), 1 (“Cytokeratin, Pan (OSCAR) Mouse Monoclonal Primary Antibody is intended for laboratory professional use in the detection of the Broad spectrum of cytokeratin proteins.... Anti-cytokeratin clone OSCAR (CK OSCAR) demonstrates a broad spectrum of cytokeratin reactivity.”).

183. Sebastiao teaches unmixing the image data into separate image channels or each marker of the multiplex assay, and analyzing these image channels or a combined, “surrogated” image using cell detection and classification

algorithms to “classify the detected cells as at least one of false positive cells, CD3 only T-cells, CD3 and CD8 T-cells, FP3 T-cells; and CD20 B-cells.” *Id.*, [0014].

184. Chukka and Sebastiao both further suggest detecting smooth muscle actin. Chukka teaches using its facility to classify cells in breast cancer, including differentiating stroma. The prior art reflects that stromal cells in breast cancers are “characterized by high α -smooth muscle actin (α -SMA) expression.” EX1024 (Murakami), 4862. Similarly, Sebastiao teaches including in its multiplex assay various tumor markers for epithelial cells, and prior art literature teaches that that “[o]ne of the most common immunohistochemical markers for [myoepithelial cells] is smooth muscle actin.” EX1025 (Ravi), 479-80. Thus, a POSA, referencing the prior art literature, would understand that “tumor detection markers” varied by tumor type and that α -SMA is a marker of tumors. EX1007 (Sebastiao), [0037] (describing markers useful for “epithelial” and “neuroectodermal” tumors); EX1025(Ravi), 484 (“We found α -SMA to be an efficient immunohistochemical marker for ME [“myoepithelial cells”] and endorse that its differential expression in the epithelial and stromal compartments of the selected salivary gland lesions influences their morphological patterning, especially in ACC [“adenoid cystic carcinoma.].”).

185. Chukka and Sebastiao therefore teach or suggest a method of identifying cell objects, wherein the cell objects comprise CD3, CD8, CD20, pancytokeratin, and smooth muscle actin. As discussed above, a POSA would have had a good reason to combine the teachings of Sebastiao and Chukka, in order to detect and count lymphocytes in a multiplex stained tissue slide image, with a reasonable expectation of success. Accordingly, this claim would have been obvious over Chukka in view of Sebastiao.

'097 Patent	Prior Art
The method of claim 6, wherein the cell objects comprise CD3, CD8, CD20, pancytokeratin, and smooth muscle actin.	EX1007 (Sebastiao), [0014] (“An image analysis algorithm and/or system has been developed that automatically computes an immune score from a set images of multiplex IHC slides and/or fluorescent stained slides. The image analysis algorithm involves a computer-implemented method for counting a number of types of cells in a single tissue specimen that has been stained with a multiplex assay, comprising: imaging the single tissue specimen that has been stained with the multiplex assay that includes lymphocyte markers CD3, CD8, CD20, FoxP3, and tumor detection markers; unmixing the image of single tissue specimen that has been stained with a multiplex assay into separate image channels for each marker of the multiplex assay; identifying regions of interest in each image channel based on intensity information in each channel, wherein regions of low intensity in each channel are removed, and regions of high intensity represent cell signals; generating a single surrogated image, wherein the surrogated image is a combination of the image channel information of all the lymphocyte markers;

	applying a cell detection algorithm, wherein the cell detection algorithm is a membrane finding algorithm or a nucleus finding algorithm; identifying features of the lymphocytes and combinations of lymphocytes in each image channel or image of combined channels, or a transformed image such as grayscale or absorbance image, or a surrogated image; training a classification algorithm based on features of known lymphocytes and lymphocyte combinations; applying the trained algorithm to features of the lymphocytes and combinations of lymphocytes in each image channel or in each image of combined channels, or in a transformed image such as grayscale or absorbance image, or in a surrogated image, that were identified to classify the detected cells as at least one of false positive cells, CD3 only T-cells, CD3 and CD8 T-cells, FP3 T-cells; and CD20 B-cells; counting a number of each different type of cell classified; generating a score of the tissue specimen, wherein the score is based on the number of each type of cell counted.”). EX1007 (Sebastiao), [0037] (“[0037] In epithelial tumors [carcinomas], cytokeratin staining identifies tumor cells as well as the normal epithelium. This information, together with the fact that tumor cells abnormally overexpress the cytokeratins compared to normal epithelial cells, allows one to identify tumor vs. normal tissue. For melanoma tissue of neuroectodermal origin, the SI 00 biomarker serves a similar purpose. Primary Staining/Blueing/Counterstaining”). EX1007 (Sebastiao), [0051] (“Based on the separated image channels for each marker, detection algorithms are developed to automatically identify different combination of T and B
--	--

	lymphocytes with respect to each of the differentiating IHC markers. For example, different T-cell markers might co-localize and create different combination of the stain amounts. Additionally, a tumor marker, for example a cytokeratin marker [e.g., Oscar], may also be included in the assay to identify the tumor, intratumoral and/or peritumoral regions to get the tissue context [for example, to identify the nuclei and the tumor regions]. In an exemplary embodiment, image analysis is performed, after unmixing, to identify, quantify and/or generate a score for a biomarker, for example, Oscar, CD3, CD8 and FoxP3 markers. In an exemplary embodiment of the present invention, the score for each marker may be reported, along with the tissue context information, as output.”)
--	--

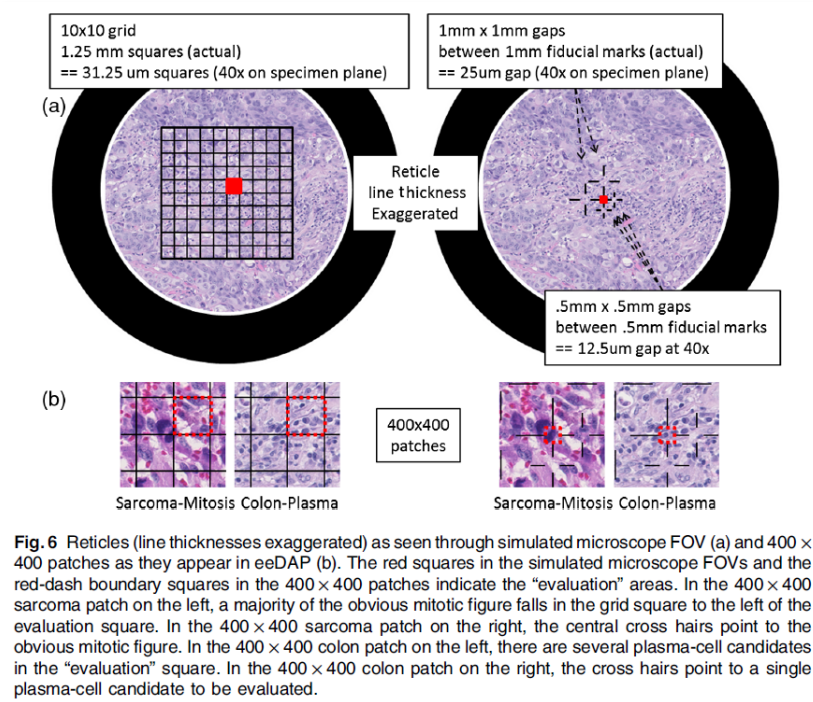
XII. GROUND 4: CLAIMS 1, 4-6, 9-12, 14, 15, 17, AND 18 ARE OBVIOUS OVER GALLAS AND CHUKKA.

186. At a high level, claims 1-4, 6, 9-12, 14, 15, 17, and 18 of the '097 Patent are further obvious over Gallas, in view of Chukka. As discussed in Section II, above, the prior art reflects that analysis of tissue slides was conventionally performed using overlays, or “reticles” that were routinely included within the eyepiece of a microscope or applied as a digital overlay when viewing whole slide images (“WSI”) on a computer screen. *See §II, supra*. These reticles allowed visual inspection of an individual evaluation square (e.g., tile)

along with tissue surrounding that evaluation square, as illustrated in Gallas.

EX1010 (Gallas), Fig. 6.

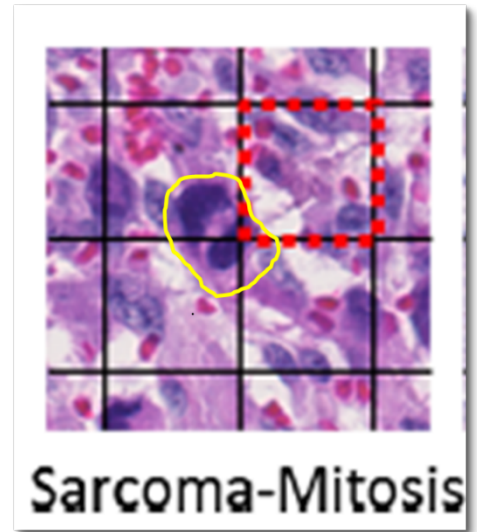
187. As illustrated in Fig. 6(a), Gallas describes separating digital images of tissue slides into tiles with a digital overlay of a reticle. Fig. 6(a) shows a reticle overlaying an image of a tissue slide obtained from a digital camera mounted on a microscope, and Fig. 6(b) shows a “patch” obtained from a WSI derived from the same slide as shown in (a) which also includes a reticle overlaying the image. The reticle overlays shown in Fig. 6, separate the tissue image into grid squares which facilitate evaluation of the tissue slide.



188. Gallas teaches using the platform to evaluate sarcoma tissue for mitotic figures or colon tissue for the presence of plasma-cells. With respect to the sarcoma tissue slide, Gallas describes evaluation of features such as a mitotic

figure (e.g., tissue feature) that spans multiple squares. Gallas explains that the evaluation square, as represented by the red-dashed outline, includes a portion of the mitotic figure but the majority “falls in the grid square to the left.”

EX1010 (Gallas), Fig. 6 (reproduced in part herein with mitotic figure outlined in yellow);



see also id., Fig. 1 (showing screenshots of GUI used for identifying features).

Accordingly, Gallas teaches evaluating of tissue features such as mitotic figures involves analysis of both the evaluation square (i.e., tile) along with adjacent grid squares.

189. While Gallas describes visual identification of slide features by a user, it would have been obvious to conduct a known approach to tissue analysis using a computer-implemented approach, such as described in Chukka. As discussed extensively above, Chukka describes methods for automated tissue slide analysis. *See*, §VII.A (Ground 1), *supra*; EX1005 (Chukka), [0005], [0010], [0022], [0035]-[0046], Figs 2 & 3. Chukka describes that a classifier that may be trained with user annotated digital slides. EX1005 (Chukka), [0035]-[0038].

190. A POSA would have been motivated to look to the teachings of Gallas and Chukka. Both Gallas and Chukka teach methods of automating

evaluation of digital pathology slides. *See e.g.*, EX1010 (Gallas), 8; EX1005 (Chukka) [0003], [0047]. Chukka teaches that automated evaluation of tissue slides may “augment the user’s own assessment” and therefore reduce bias in the analysis. EX1005 (Chukka), [0003], [0047]. Likewise, Gallas teaches that “future work may include the incorporation of algorithms for the automated identification of candidate cells to be classified or histopathological features to be evaluated.” EX1010 (Gallas), 8. Gallas further explains that such automated algorithms “may be less biased and more objective in creating the study sets.” *Id.*

191. A POSA would have recognized that the shift from the digital tools of Gallas to the automation in digital pathology of Chukka represents an evolution necessary to address the challenges faced in healthcare. While digital slide scanners and viewing platforms improved accessibility and collaboration, they still relied on manual pathologist review. This is a bottleneck that could not scale with the growth in diagnostic demand, aging populations, and global pathologist shortages.

A. Claim 1

1. Preamble

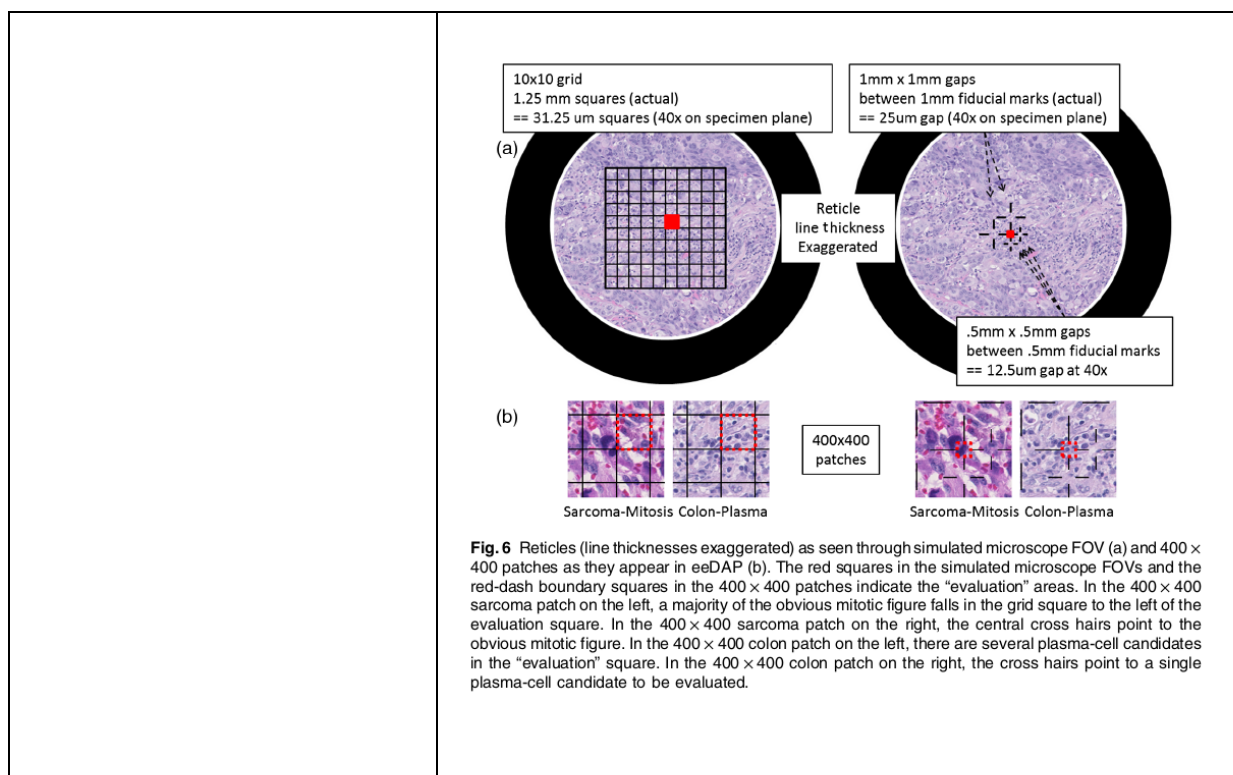
192. As discussed above in Ground 1, Chukka discloses or teaches the preamble of claim 1. *See* §IX.A.1, *supra*.

193. Gallas further describes overlaying reticles on digital images of tissue slides to facilitate analysis. Gallas explains that use of reticles is important for “narrowing the evaluation area to a small ROI or an individual cell.” EX1010 (Gallas), 2. Gallas further illustrates in Fig. 6(a) a reticle mask overlayed on a digital camera image obtained from a slide. According to Gallas, Fig. 6(a) demonstrates “[r]eticles...as seen through simulated microscope FOV” and Fig. 6(b) shows “400 × 400 patches as they appear in eeDAP[.]” EX1010 (Gallas), 7. Gallas teaches that the reticle mask allows a portion of the digital image (e.g., red-filled square of Fig. 6(a) to be mapped to the corresponding portion of the digital representation of the WSI (e.g., red-dash boundary of Fig. 6(b)). *Id.* Gallas teaches that the red squares in the simulated microscope FOVs and the red-dash boundary squares in the 400 × 400 patches indicate the “evaluation” square. *Id.*

194. Accordingly, this element is obvious in view of Gallas and Chukka.

'097 Patent	Prior Art
A method for creating an overlay map on a digital image of a slide, the method comprising:	See §IX.A.1 disclosure of EX1005 (Chukka). EX1010 (Gallas), 2 (“In this paper, we outline the key software and hardware elements of eeDAP. First, we discuss the eeDAP software as a publicly available resource and describe software specifications and requirements. We next talk about the tone reproduction curves that characterize eeDAP and the native viewers: the curves showing the lightness in the output image given the transmittance of the input slide. In Sec. 2.3, we

	summarize the local and global registration methods that are key to pairing ROIs across the digital and microscope domains. In Sec. 2.4, we provide the key hardware specifications that eeDAP requires and then demonstrate the differences in FOVs and image sizes between the two domains: the digital image and the glass slide. These calculations and corresponding representative images help to provide a sense of scale across the digital and analog domains. Finally, we talk about reticles and their important role in narrowing the evaluation area to a small ROI or an individual cell.”). EX1010 (Gallas), 7 (“The instructions for this study were to score the reticle square that was immediately above and to the right of the center cross (red squares in Fig. 6). Identifying the center cross in the 10×10 grid in MicroRT mode is challenging; it is accomplished by rotating the eyepiece as the center cross remains fixed. Additionally, the instructions to score a square were to score the cell that was most likely the target (mitotic figure or plasma cell as shown in Fig. 1), considering cells with at least half their nuclei in the square.”).
--	---



2. Element 1[A]

195. As discussed above in Ground 1, Chukka teaches element 1[A]:
 “receiving the digital image[.]” See §IX.A.2, *supra*.

196. The prior art literature describe the widespread use of commercial platforms for digitizing tissue slides as far back as at the late 1990s. See EX1031 (Pantanowitz), Abstract. Gallas describes an “evaluation environment for digital and analog pathology” referred to as “eeDAP” that receives digital images from whole slide images (“WSI”) and real-time images from a microscope-mounted digital camera. EX1010 (Gallas), 1 (“The quantitative assessment of a pathologist’s ability to evaluate histopathology features in WSI compared to the microscope”), 1-2 (“where the digital image is registered to the real-time view on

the microscope”), 2 (“allowing for a tight comparison between WSI and optical microscopy”), 3 (“eeDAP hardware consists of an upright brightfield microscope, a digital camera”). Fig. 2 of Gallas illustrates the eeDAP hardware as including a microscope, camera, computer controlled stage with joystick, and a computer with a monitor (not shown). Figs. 1, 4, 5, and 6 of Gallas illustrate how received digital images of slides appear on the computer screen. EX1010 (Gallas), Figs. 1, 2, 4, 5, 6.

197. Accordingly, this element is obvious in view of Gallas and Chukka.

'097 Patent	Prior Art
A method for creating an overlay map on a digital image of a slide, the method comprising:	See §IX.A.1 disclosure of EX1005 (Chukka). EX1010 (Gallas), 2 (“In this paper, we outline the key software and hardware elements of eeDAP. First, we discuss the eeDAP software as a publicly available resource and describe software specifications and requirements. We next talk about the tone reproduction curves that characterize eeDAP and the native viewers: the curves showing the lightness in the output image given the transmittance of the input slide. In Sec. 2.3, we summarize the local and global registration methods that are key to pairing ROIs across the digital and microscope domains. In Sec. 2.4, we provide the key hardware specifications that eeDAP requires and then demonstrate the differences in FOVs and image sizes between the two domains: the digital image and the glass slide. These calculations and corresponding representative images help to provide a sense of scale across the digital and analog domains. Finally, we talk about reticles and their important role in

narrowing the evaluation area to a small ROI or an individual cell.”).

EX1010 (Gallas), 7 (“The instructions for this study were to score the reticle square that was immediately above and to the right of the center cross (red squares in Fig. 6). Identifying the center cross in the 10×10 grid in MicroRT mode is challenging; it is accomplished by rotating the eyepiece as the center cross remains fixed. Additionally, the instructions to score a square were to score the cell that was most likely the target (mitotic figure or plasma cell as shown in Fig. 1), considering cells with at least half their nuclei in the square.”).

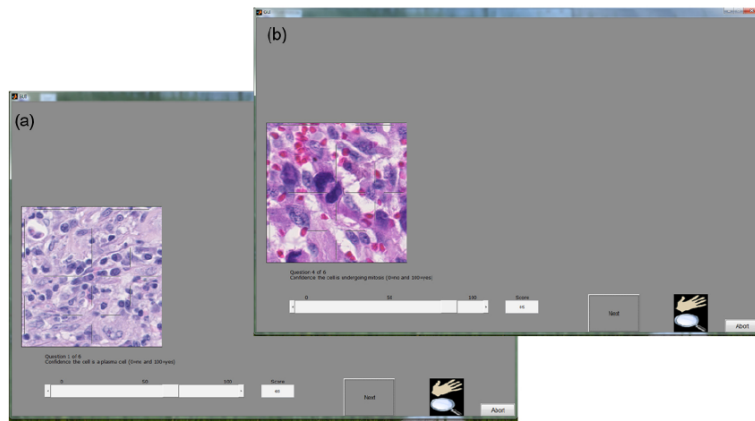


Fig. 1 Here are the two windows, each showing the eeDAP presentation of a slider task: the image on the left is of colon tissue, the image on the right is of sarcoma.

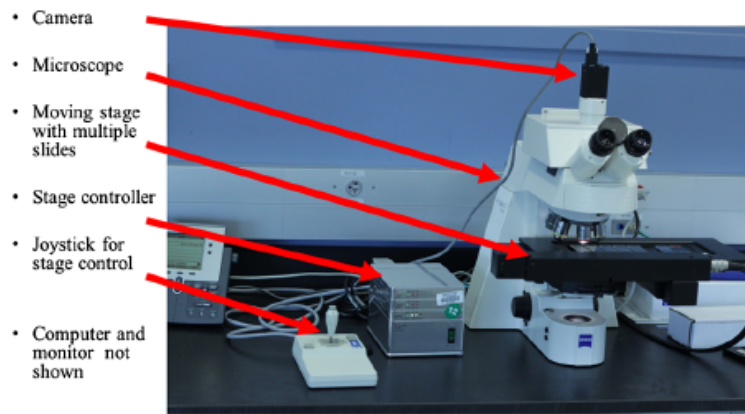


Fig. 2 The evaluation environment for digital and analog pathology (eeDAP) hardware: microscope, camera, computer-controlled stage with joystick, and a computer with monitor (not shown).

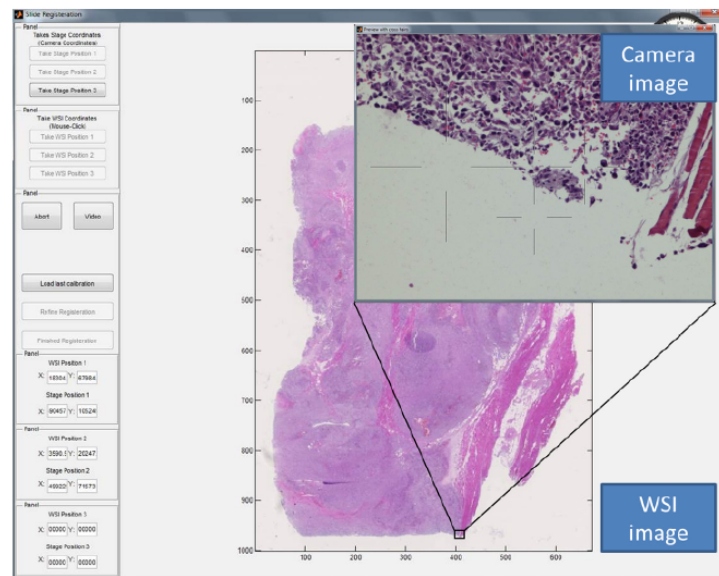
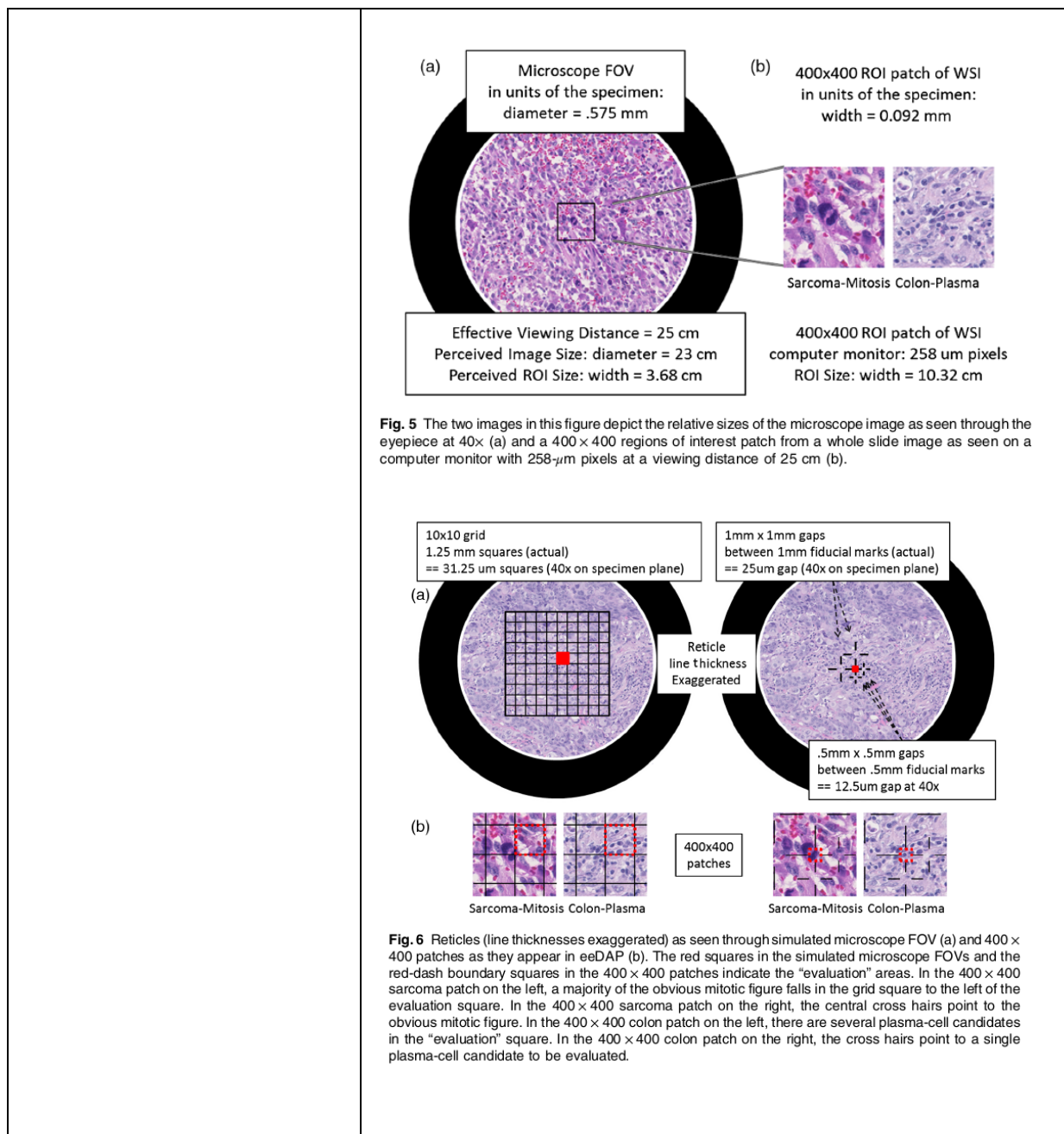


Fig. 4 Screen shot of the registration interface including the real-time microscope field of view (FOV) as seen with the mounted camera ("Camera image").



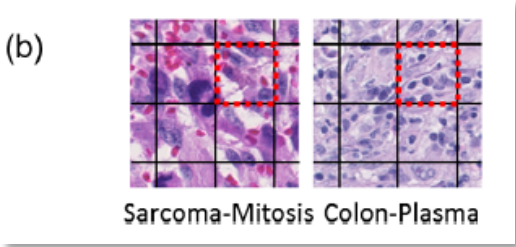
3. Element 1[B]

198. As discussed above in Ground 1, Chukka teaches element 1[B]:

“separating the digital image into a plurality of tiles, each tile of the plurality of tiles contains a respective portion of the digital image of the slide[.]” See

§IX.A.3, *supra*.

199. Gallas teaches separating slide images into a plurality of tiles. For example, Gallas describes the use of a reticle overlay to separate digital images into a plurality of grid squares. *E.g.*, EX1010 (Gallas), 7 (“2.5 Reticles”).



According to Gallas, a portion of the digital image (e.g., a 400 × 400 patch) is divided into a plurality of “grid square[s].” EX1010 (Gallas), Fig. 6b (reproduced in part herein). Each of Gallas’s grid squares corresponds to a portion of the digital image of the slide. EX1010 (Gallas), Fig. 6 (“a majority of the obvious mitotic figure falls in the grid square to the left of the evaluation square”).

200. Accordingly, this element is obvious in view of Gallas and Chukka.

'097 Patent	Prior Art
separating the digital image into a plurality of tiles, each tile of the plurality of tiles containing a respective portion of the digital image of the slide; and	See §IX.A.2 disclosure of EX1005 (Chukka). EX1010 (Gallas), 2 (“In this paper, we outline the key software and hardware elements of eeDAP. First, we discuss the eeDAP software as a publicly available resource and describe software specifications and requirements. We next talk about the tone reproduction curves that characterize eeDAP and the native viewers: the curves showing the lightness in the output image given the transmittance of the input slide. In Sec. 2.3, we summarize the local and global registration methods that are key to pairing ROIs across the digital and microscope domains. In Sec. 2.4, we provide the key hardware specifications that eeDAP

requires and then demonstrate the differences in FOVs and image sizes between the two domains: the digital image and the glass slide. These calculations and corresponding representative images help to provide a sense of scale across the digital and analog domains. Finally, we talk about reticles and their important role in narrowing the evaluation area to a small ROI or an individual cell.”).

EX1010 (Gallas), 7 (“The instructions for this study were to score the reticle square that was immediately above and to the right of the center cross (red squares in Fig. 6). Identifying the center cross in the 10×10 grid in MicroRT mode is challenging; it is accomplished by rotating the eyepiece as the center cross remains fixed. Additionally, the instructions to score a square were to score the cell that was most likely the target (mitotic figure or plasma cell as shown in Fig. 1), considering cells with at least half their nuclei in the square.”).

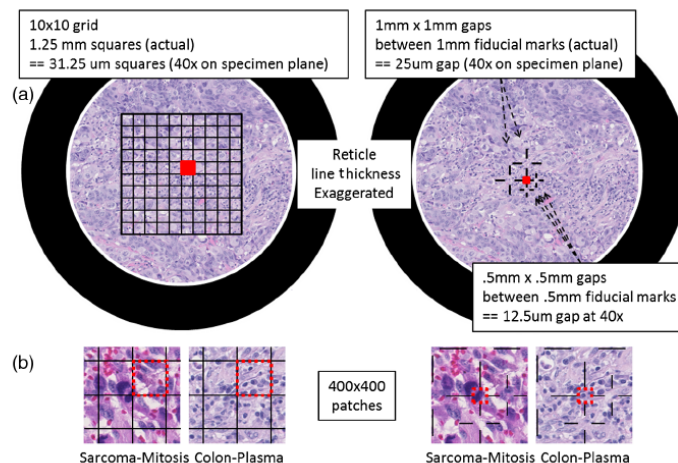


Fig. 6 Reticles (line thicknesses exaggerated) as seen through simulated microscope FOV (a) and 400 × 400 patches as they appear in eeDAP (b). The red squares in the simulated microscope FOVs and the red-dash boundary squares in the 400 × 400 patches indicate the “evaluation” areas. In the 400 × 400 sarcoma patch on the left, a majority of the obvious mitotic figure falls in the grid square to the left of the evaluation square. In the 400 × 400 sarcoma patch on the right, the central cross hairs point to the obvious mitotic figure. In the 400 × 400 colon patch on the left, there are several plasma-cell candidates in the “evaluation” square. In the 400 × 400 colon patch on the right, the cross hairs point to a single plasma-cell candidate to be evaluated.

4. Element 1[C]

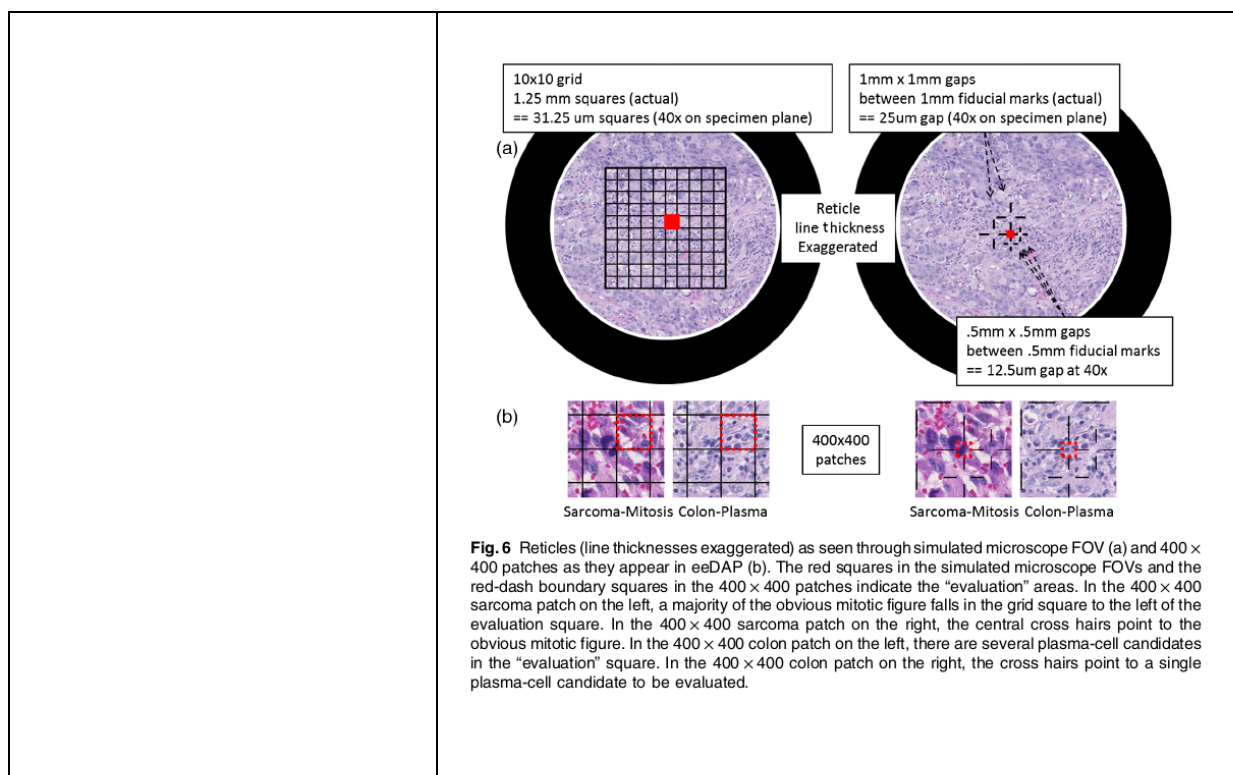
201. As discussed above in Ground 1, Chukka teaches element 1[C]: for each tile of the plurality of tiles: identifying features of the tile[.]” See §IX.A.4, *supra*.

202. As illustrated in Fig. 6(a), Gallas demonstrates a grid overlay with identified features of the separated tiles. In one example, Gallas describes “a reticle containing a 10×10 grid with squares that are 1.25 mm on a sides....” EX1010 (Gallas), 7. In Fig. 6, Gallas describes identifying cells and nuclei in tiles, as well as identifying “obvious mitotic figure[s]” and “several plasma-cell candidates.” *Id.*, Fig. 6. Gallas further describes scoring the square by “scor[ing] the cell that was mostly likely the target (mitotic figure or plasma cell as shown in Fig. 1)” and annotating identified features as part of the eeDAP platform. EX1010 (Gallas), 7.

203. Accordingly, this element is obvious in view of Gallas and Chukka.

'097 Patent	Prior Art
for each tile of the plurality of tiles: identifying features of the tile;	See §IX.A.3 disclosure of EX1005 (Chukka). EX1010 (Gallas), 2 (“In this paper, we outline the key software and hardware elements of eeDAP. First, we discuss the eeDAP software as a publicly available resource and describe software specifications and requirements. We next talk about the tone reproduction curves that characterize eeDAP and the native viewers: the curves

	showing the lightness in the output image given the transmittance of the input slide. In Sec. 2.3, we summarize the local and global registration methods that are key to pairing ROIs across the digital and microscope domains. In Sec. 2.4, we provide the key hardware specifications that eeDAP requires and then demonstrate the differences in FOVs and image sizes between the two domains: the digital image and the glass slide. These calculations and corresponding representative images help to provide a sense of scale across the digital and analog domains. Finally, we talk about reticles and their important role in narrowing the evaluation area to a small ROI or an individual cell.”). EX1010 (Gallas), 7 (“The instructions for this study were to score the reticle square that was immediately above and to the right of the center cross (red squares in Fig. 6). Identifying the center cross in the 10×10 grid in MicroRT mode is challenging; it is accomplished by rotating the eyepiece as the center cross remains fixed. Additionally, the instructions to score a square were to score the cell that was most likely the target (mitotic figure or plasma cell as shown in Fig. 1), considering cells with at least half their nuclei in the square.”).
--	---

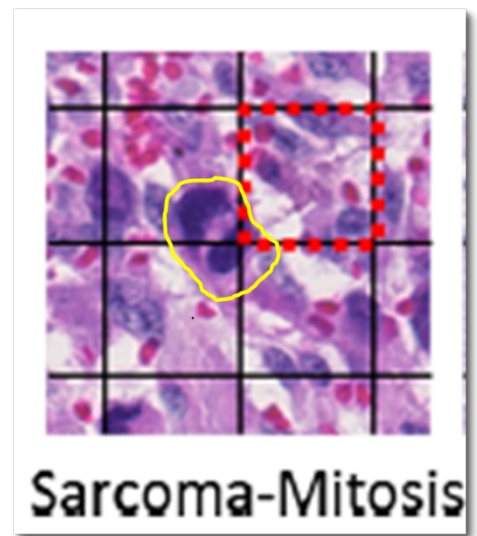


5. Element 1[D]

204. As discussed above in Ground 1, Chukka teaches element 1[D]:

“identifying structural tissue features of a second portion of the digital image of the slide including at least a part of one or more tiles of the plurality of tile, wherein the second portion is larger than the respective portion of the digital image contained in the tile[.]” See §IX.A.5, *supra*.

205. Gallas teaches that when evaluating a tile to identify tissue features, users should evaluate surrounding tiles to identify structural tissue features. Specifically, Gallas instructs users to only “consider[] cells with at least half their nuclei in the [evaluation] square.” EX1010 (Gallas), 7. Gallas therefore teaches that each time a tile is evaluated, the user must consider “at least half” of the nucleus is in the evaluation tile or a neighboring tile. *Id.* Gallas further describes identifying structural tissue features including mitotic figures and immune clusters (e.g., groupings of plasma cells) among the tiles. Gallas describes identifying structural tissue features that span multiple tiles. For example, in Fig. 6(b) (which I reproduced in part herein with mitotic figure annotated in yellow), Gallas explains that “the red-dash boundary squares in the 400×400 patches indicate the ‘evaluation’ areas.” Gallas further explains that “a majority of the obvious mitotic figure falls in the grid square to the left of the evaluation square.” *Id.* The mitotic figure described by Gallas spans four grid squares and is outlined in yellow.

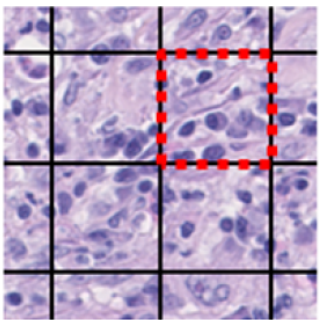


Thus, identifying the mitotic figure requires evaluating an area that includes the evaluation tile (red-dash boundary) and the additional surrounding tiles occupied by the mitotic figure. The portion of the digital image occupied by the mitotic

figure (i.e., 4 tiles) is larger than the respective portion (i.e., the evaluation tile) of the image. Gallas further discloses identifying “several plasma-cell candidates” in the patch displayed in Fig. 6(b).

206. Gallas likewise teaches evaluating colon tissue features such as such as a cluster of plasma cells in evaluation square. EX1010 (Gallas), Fig. 6.

As shown in the excerpt of Fig. 6, Gallas illustrates a plasma cell cluster with a larger portion including



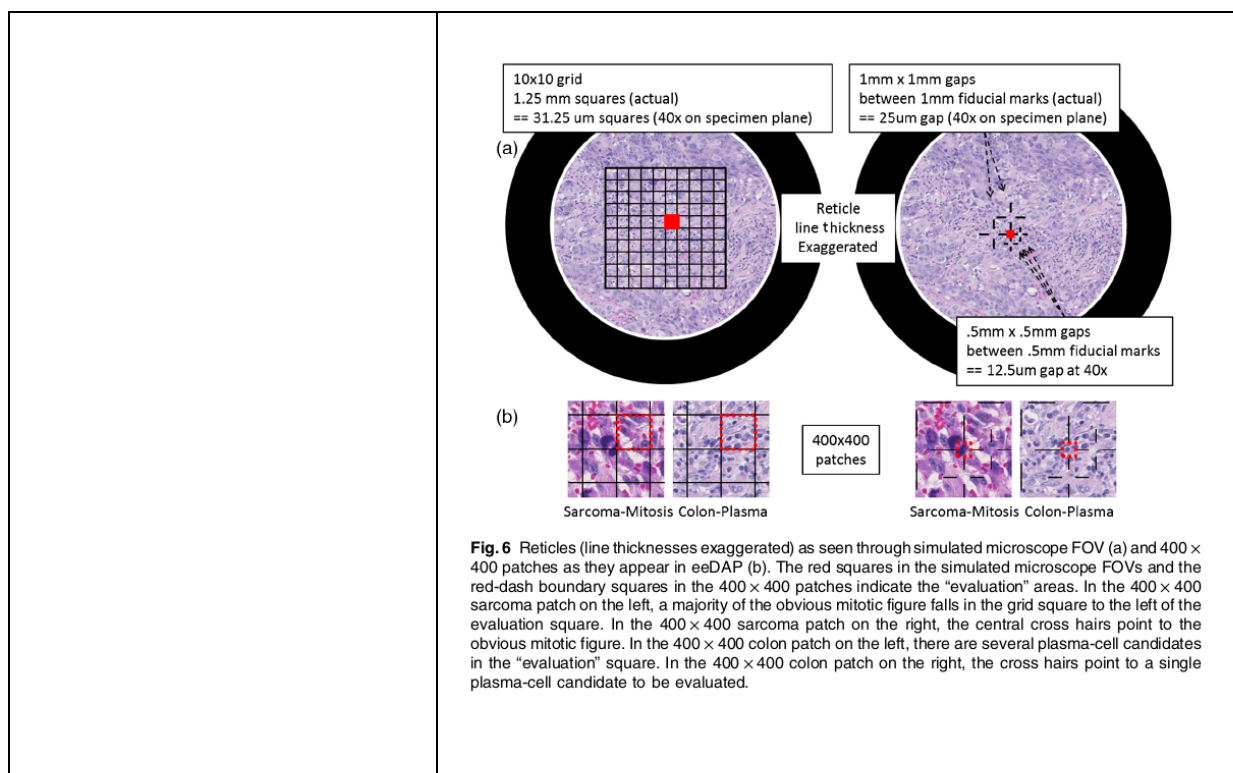
; Colon-Plasma

tiles surrounding the evaluation tile (denoted by the red dashed line). A POSA would have understand that evaluation of the plasma cluster in the evaluation tile would involve evaluating tissue features of the larger overall cluster, in surrounding tiles.

207. Accordingly, this element is obvious in view of Gallas and Chukka.

'097 Patent	Prior Art
identifying structural tissue features of a second portion of the digital image of the slide including at least part of one or more other tiles of the plurality of tiles, wherein the second portion is larger than the respective portion of the	See §IX.A.4 disclosure of EX1005 (Chukka). EX1010 (Gallas), 2 (“In this paper, we outline the key software and hardware elements of eeDAP. First, we discuss the eeDAP software as a publicly available resource and describe software specifications and requirements. We next talk about the tone reproduction curves that characterize eeDAP and the native viewers: the curves showing the lightness in the output image given the transmittance of the input slide. In Sec. 2.3, we

digital image contained in the tile; and	summarize the local and global registration methods that are key to pairing ROIs across the digital and microscope domains. In Sec. 2.4, we provide the key hardware specifications that eeDAP requires and then demonstrate the differences in FOVs and image sizes between the two domains: the digital image and the glass slide. These calculations and corresponding representative images help to provide a sense of scale across the digital and analog domains. Finally, we talk about reticles and their important role in narrowing the evaluation area to a small ROI or an individual cell.”). EX1010 (Gallas), 7 (“The instructions for this study were to score the reticle square that was immediately above and to the right of the center cross (red squares in Fig. 6). Identifying the center cross in the 10×10 grid in MicroRT mode is challenging; it is accomplished by rotating the eyepiece as the center cross remains fixed. Additionally, the instructions to score a square were to score the cell that was most likely the target (mitotic figure or plasma cell as shown in Fig. 1), considering cells with at least half their nuclei in the square.”).
---	---



6. Element 1[E]

208. As discussed above in Ground 1, Chukka teaches element 1[E]:

“identifying the majority class of tissue visible within the tile based at least in part on the features of the tile and the structural tissue features of the second portion of the digital image of the slide[.]” See §IX.A.6, *supra*.

209. As I discuss above, Gallas teaches separating a digital image of a tissue slide using a tiled overlay reticle and evaluating the tissue features in an evaluation tile in the context of surrounding tiles. Gallas teaches to identify the majority class of tissue (e.g., sarcoma and plasma cells) in a tile based on the features of the tile and structural tissue features identified in the second portion. For example, in Fig. 6(b), Gallas illustrates sarcoma tissue that is not undergoing

mitosis. Gallas identifies this by stating that “a majority of the obvious mitotic figure falls in the grid square to the left of the evaluation square,” confirming that this identification is based on analysis of both the evaluation tile and tiles in a surrounding second portion (e.g., including surrounding tiles). EX1010 (Gallas), 7, Fig. 6. Gallas further describes identifying plasma-cell candidates as the majority class of tissue in the evaluation tile. EX1010 (Gallas), Fig. 6 (“there are several plasma-cell candidates in the ‘evaluation’ square”).

210. Accordingly, this element is obvious in view of Gallas and Chukka.

'097 Patent	Prior Art
identifying the majority class of tissue visible within the tile based at least in part on the features of the tile and the structural tissue features of the second portion of the digital image of the slide.	See §IX.A.5 disclosure of EX1005 (Chukka). EX1010 (Gallas), 2 (“In this paper, we outline the key software and hardware elements of eeDAP. First, we discuss the eeDAP software as a publicly available resource and describe software specifications and requirements. We next talk about the tone reproduction curves that characterize eeDAP and the native viewers: the curves showing the lightness in the output image given the transmittance of the input slide. In Sec. 2.3, we summarize the local and global registration methods that are key to pairing ROIs across the digital and microscope domains. In Sec. 2.4, we provide the key hardware specifications that eeDAP requires and then demonstrate the differences in FOVs and image sizes between the two domains: the digital image and the glass slide. These calculations and corresponding representative images help to provide a sense of scale across the digital and analog domains. Finally, we talk about

reticles and their important role in narrowing the evaluation area to a small ROI or an individual cell.”).

EX1010 (Gallas), 7 (“The instructions for this study were to score the reticle square that was immediately above and to the right of the center cross (red squares in Fig. 6). Identifying the center cross in the 10×10 grid in MicroRT mode is challenging; it is accomplished by rotating the eyepiece as the center cross remains fixed. Additionally, the instructions to score a square were to score the cell that was most likely the target (mitotic figure or plasma cell as shown in Fig. 1), considering cells with at least half their nuclei in the square.”).

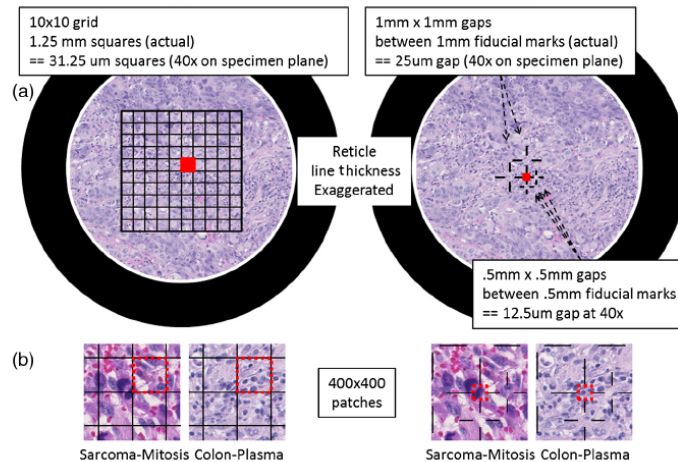


Fig. 6 Reticles (line thicknesses exaggerated) as seen through simulated microscope FOV (a) and 400 × 400 patches as they appear in eeDAP (b). The red squares in the simulated microscope FOVs and the red-dash boundary squares in the 400 × 400 patches indicate the “evaluation” areas. In the 400 × 400 sarcoma patch on the left, a majority of the obvious mitotic figure falls in the grid square to the left of the evaluation square. In the 400 × 400 sarcoma patch on the right, the central cross hairs point to the obvious mitotic figure. In the 400 × 400 colon patch on the left, there are several plasma-cell candidates in the “evaluation” square. In the 400 × 400 colon patch on the right, the cross hairs point to a single plasma-cell candidate to be evaluated.

B. Claim 4

211. As discussed above in Ground 1, Chukka teaches claim 4. *See* § IX.B, *supra*. Gallas further teaches identifying plasma cells as the majority class of tissue. EX1010 (Gallas), Fig. 6. A POSA would understand, referring to prior art, that plasma cells such as those described in Gallas are immune cells. *See* EX1032 (Kindt), 13, 15. Accordingly, this claim is obvious in view of Gallas and Chukka.

'097 Patent	Prior Art
The method of claim 1, wherein the majority class of tissue is selected from the group consisting of epithelium, normal epithelium, immune, stroma, necrosis, blood, and fat.	<i>See</i> §IX.B disclosure of EX1005 (Chukka). EX1010 (Gallas), 2 (“In this paper, we outline the key software and hardware elements of eeDAP. First, we discuss the eeDAP software as a publicly available resource and describe software specifications and requirements. We next talk about the tone reproduction curves that characterize eeDAP and the native viewers: the curves showing the lightness in the output image given the transmittance of the input slide. In Sec. 2.3, we summarize the local and global registration methods that are key to pairing ROIs across the digital and microscope domains. In Sec. 2.4, we provide the key hardware specifications that eeDAP requires and then demonstrate the differences in FOVs and image sizes between the two domains: the digital image and the glass slide. These calculations and corresponding representative images help to provide a sense of scale across the digital and analog domains. Finally, we talk about reticles and their important role in narrowing the evaluation area to a small ROI or an individual cell.”).

EX1010 (Gallas), 7 (“The instructions for this study were to score the reticle square that was immediately above and to the right of the center cross (red squares in Fig. 6). Identifying the center cross in the 10×10 grid in MicroRT mode is challenging; it is accomplished by rotating the eyepiece as the center cross remains fixed. Additionally, the instructions to score a square were to score the cell that was most likely the target (mitotic figure or plasma cell as shown in Fig. 1), considering cells with at least half their nuclei in the square.”).

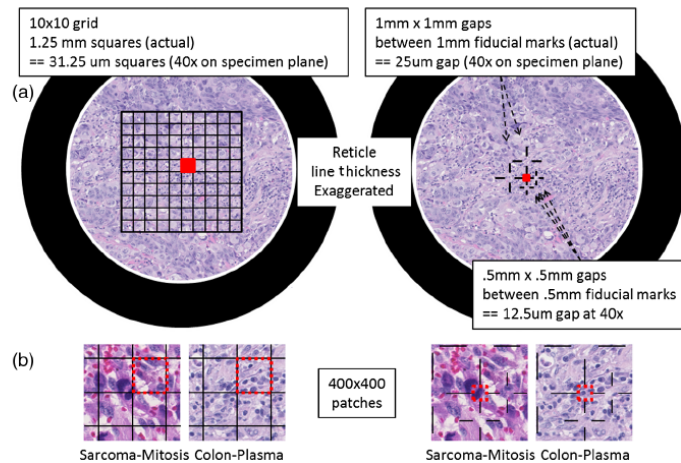


Fig. 6 Reticles (line thicknesses exaggerated) as seen through simulated microscope FOV (a) and 400 × 400 patches as they appear in eeDAP (b). The red squares in the simulated microscope FOVs and the red-dash boundary squares in the 400 × 400 patches indicate the “evaluation” areas. In the 400 × 400 sarcoma patch on the left, a majority of the obvious mitotic figure falls in the grid square to the left of the evaluation square. In the 400 × 400 sarcoma patch on the right, the central cross hairs point to the obvious mitotic figure. In the 400 × 400 colon patch on the left, there are several plasma-cell candidates in the “evaluation” square. In the 400 × 400 colon patch on the right, the cross hairs point to a single plasma-cell candidate to be evaluated.

C. Claim 5

212. As discussed above in Ground 1, Chukka teaches claim 5. See § IX.C, *supra*. Accordingly, this claim is obvious in view of Gallas and Chukka.

D. Claim 6

1. Preamble

213. As discussed above in Ground 1, Chukka teaches the preamble of claim 6. *See* § IX.D.1, *supra*. Accordingly, this element is obvious in view of Gallas and Chukka.

2. Element 6[A]

214. As discussed above in Ground 1, Chukka teaches element 6[A]. *See* § IX.D.1, *supra*. Accordingly, this element is obvious in view of Gallas and Chukka.

3. Element 6[B]

215. As discussed above in Ground 1, Chukka teaches element 6[B]. *See* § IX.D.1, *supra*. Accordingly, this element is obvious in view of Gallas and Chukka.

4. Element 6[C]

216. As discussed above in Ground 1, Chukka teaches element 6[C]. *See* § IX.D.1, *supra*. Accordingly, this element is obvious in view of Gallas and Chukka.

5. Element 6[D]

217. As discussed above in Ground 1, Chukka teaches element 6[D]. *See* § IX.D.1, *supra*. Accordingly, this element is obvious in view of Gallas and Chukka.

6. Element 6[E]

218. As discussed above in Ground 1, Chukka teaches element 6[E]. *See* § IX.D.1, *supra*. Accordingly, this element is obvious in view of Gallas and Chukka.

7. Element 6[F]

219. As discussed above in Ground 1, Chukka teaches element 6[F]. *See* § IX.D.1, *supra*. Gallas also teaches identifying cell objects such as cells and nuclei. For example, Gallas explains that users may “consider[] cells with at least half their nuclei in the square.” EX1010 (Gallas), 7. Gallas further describes identifying mitotic figures and plasma cells. EX1010 (Gallas), Fig. 6. Accordingly, this element is obvious in view of Gallas and Chukka.

'097 Patent	Prior Art
identifying a plurality of cell objects in the digital image;	<i>See</i> §IX.D.1 disclosure of EX1005 (Chukka). EX1010 (Gallas), 7 (“The instructions for this study were to score the reticle square that was immediately above and to the right of the center cross (red squares in Fig. 6). Identifying the center cross in the 10 × 10 grid in MicroRT mode is

challenging; it is accomplished by rotating the eyepiece as the center cross remains fixed. Additionally, the instructions to score a square were to score the cell that was most likely the target (mitotic figure or plasma cell as shown in Fig. 1), considering cells with at least half their nuclei in the square.”).

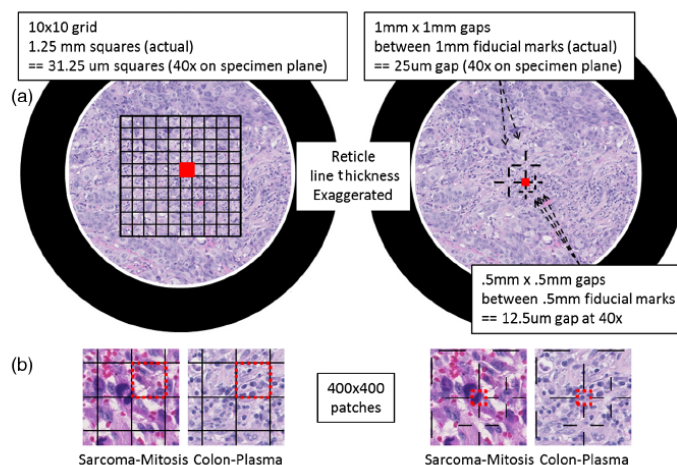


Fig. 6 Reticles (line thicknesses exaggerated) as seen through simulated microscope FOV (a) and 400 × 400 patches as they appear in eeDAP (b). The red squares in the simulated microscope FOVs and the red-dash boundary squares in the 400 × 400 patches indicate the “evaluation” areas. In the 400 × 400 sarcoma patch on the left, a majority of the obvious mitotic figure falls in the grid square to the left of the evaluation square. In the 400 × 400 sarcoma patch on the right, the central cross hairs point to the obvious mitotic figure. In the 400 × 400 colon patch on the left, there are several plasma-cell candidates in the “evaluation” square. In the 400 × 400 colon patch on the right, the cross hairs point to a single plasma-cell candidate to be evaluated.

8. Element 6[G]

220. As discussed above in Ground 1, Chukka teaches element 6[G]. See § IX.D.1, *supra*. Accordingly, this element is obvious in view of Gallas and Chukka.

9. Element 6[H]

221. As discussed above in Ground 1, Chukka teaches element 6[H]. See § IX.D.1, *supra*. Moreover, to the extent Patent Owner argues that Chukka does

not expressly disclose 6[H] “assigning each tile to the predicted class of a cell...***in place*** of the predicted class of the tile,” it is my opinion that it would have been obvious for a POSA to do so. As discussed above, Chukka discusses how “a pathologist” can replace “the automated score ...with a manual score (e.g., a score based at least in part on visual inspection).” EX1005, [0005]. Chukka also teaches multi-stage tile analysis where the system first performs a “coarse” texture-based classification of the tiled image, and then subsequently performing a second, finer classification by looping through each tile and identifying and classifying the cells within each tile. EX1005 (Chukka), [0045]-[0046]. A POSA would have understood Chukka’s description of the first stage as an initial “coarse-resolution” analysis followed by further “analysis at varying levels of granularity to increase accuracy” to involve replacing the initial, coarser texture-based classification with the subsequent, cell classification. EX1005 (Chukka), [0044], [0047]. As I discuss above, such multi-stage approaches to analysis were known in the prior art. See EX1011 (Kong), 1081-82; EX1012 (Gurcan), 159-60; EX1015 (Barker), 11.

222. Accordingly, this element is obvious in view of Gallas and Chukka.

E. Claim 9

223. As discussed above in Ground 1, Chukka teaches claim 9. See § IX.E, *supra*. To the extent that Patent Owner argues Chukka does not expressly

teach the “a histology mask **displaying** the plurality of tiles and the predict class for each tile,” it is my opinion that it would have been obvious for a POSA to do so. As I discuss in Ground 1, Chukka teaches generating digital overlay drawings to label features of interest, and Chukka teaches identifying features of interest in tiles, such as identifying portions of the image that correspond to predicted classes (e.g., lymphocyte, stromata). *See, e.g.*, EX1005 (Chukka), [0004], [0051].

Therefore, it would have been readily apparent to a POSA that that Chukka’s digital overlay drawing would be used to display the plurality of tiles, and label features of interest such as the predicted class of each tile.

224. Accordingly, this claim is further obvious in view of Gallas and Chukka.

F. Claim 10

225. As discussed above in Ground 1, Chukka teaches claim 10. *See* § IX.F, *supra*. To the extent that Patent Owner argues Chukka does not expressly teach “**displaying**, in the digital overlay drawing, the plurality of tiles and the predicted class for each tile that does not correspond to a cell object, and **displaying** the plurality of cell objects and the predicted class of each cell object,” it is my opinion that it would have been obvious for a POSA to do so. As I discuss in Ground 1, Chukka discloses generating digital overlay drawings to label features of interest, and Chukka discloses identifying features of interest in

tiles, such as identifying portions of the image that correspond to predicted classes (e.g., lymphocyte, stromata). *See, e.g.*, EX1005 (Chukka), [0004], [0051].

Therefore, it would have been readily apparent to a POSA that that Chukka's digital overlay drawing would be used to display the plurality of tiles, and label features of interest including by displaying both the predicted class for each tile that does not correspond to a cell object, and the predicted class of each cell object.

226. Accordingly, this claim is further obvious in view of Gallas and Chukka.

G. Claim 11

227. As discussed above in Ground 1, Chukka teaches claim 11. *See* § IX.G, *supra*. Gallas further teaches generating a digital overlay drawing for a digital image. For example, Gallas describes overlaying a reticle on a digital image. EX1010 (Gallas), Fig. 6. Gallas further describes displaying the digital overlay drawings on a computer monitor. EX1010 (Gallas), 3 (“The eeDAP hardware consists of an upright brightfield microscope, a digital camera, a computer-controlled stage with a joystick, a computer monitor, and a computer (see Fig. 2).”), 6 (“The size of a patch seen by a pathologist depends on the computer monitor.”), Fig. 5 (“as seen on a computer monitor”).

228. Accordingly, this claim is further obvious in view of Gallas and Chukka.

'097 Patent	Prior Art
The method of claim 6, further comprising: generating a digital overlay drawing for the digital image, wherein the digital overlay drawing includes the digital image; and displaying the digital overlay drawing.	See §IX.G disclosure of EX1005 (Chukka). EX1010 (Gallas), 3 (“The eeDAP hardware consists of an upright brightfield microscope, a digital camera, a computer-controlled stage with a joystick, a computer monitor, and a computer (see Fig. 2). The microscope requires a port for mounting the camera that allows for simultaneous viewing with the eyepiece. eeDAP currently supports a Ludl controller and compatible xy stage, and an IEEE 1394 FireWire camera communicating according to a DCAM interface (RGB, 8 bits-per-channel, minimum width 640, minimum height 480). Setup instructions and example specifications can be found in the user manual.”). 10x10 grid 1.25 mm squares (actual) == 31.25 μm squares (40x on specimen plane) 1mm x 1mm gaps between 1mm fiducial marks (actual) == 25μm gap (40x on specimen plane) (a) Reticle line thickness Exaggerated .5mm x .5mm gaps between .5mm fiducial marks == 12.5μm gap at 40x (b) 400x400 patches Sarcoma-Mitosis Colon-Plasma Sarcoma-Mitosis Colon-Plasma Fig. 6 Reticles (line thicknesses exaggerated) as seen through simulated microscope FOV (a) and 400 × 400 patches as they appear in eeDAP (b). The red squares in the simulated microscope FOVs and the red-dash boundary squares in the 400 × 400 patches indicate the “evaluation” areas. In the 400 × 400 sarcoma patch on the left, a majority of the obvious mitotic figure falls in the grid square to the left of the evaluation square. In the 400 × 400 sarcoma patch on the right, the central cross hairs point to the obvious mitotic figure. In the 400 × 400 colon patch on the left, there are several plasma-cell candidates in the “evaluation” square. In the 400 × 400 colon patch on the right, the cross hairs point to a single plasma-cell candidate to be evaluated.

H. Claim 12

229. As discussed above in Ground 1, Chukka teaches claim 12. See § IX.H, *supra*. Accordingly, this claim is further obvious in view of Gallas and Chukka.

I. Claim 14

230. As discussed above in Ground 1, Chukka teaches claim 14. See § IX.I, *supra*. To the extent that Patent Owner argues Chukka does not expressly teach that generating a digital overlay drawing that “***comprises percentages of predict classes corresponding to the digital image,***” it is my opinion that it would have been obvious for a POSA to do so. As I discussed in Ground 1, Chukka discloses generating digital overlay drawings to label features of interest. EX1005 (Chukka), [0004]. Chukka also teaches reporting a score that comprises a percentages of predicted classes in the slide (e.g., percentages of positively-stained cells). EX1005 (Chukka), [0003]. Therefore, it would have been readily apparent to a POSA that Chukka’s digital overlay drawing would comprise percentages of the predicted classes corresponding to the digital image.

231. Accordingly, this claim is further obvious in view of Gallas and Chukka.

J. Claim 15

232. As discussed above in Ground 1, Chukka teaches claim 15. See § IX.J, *supra*. To the extent that Patent Owner argues Chukka does not expressly

teach that generating a digital overlay drawing that “***comprises total counts of predict classes corresponding to the digital image***,” it is my opinion that it would have been obvious for a POSA to do so. As I discussed in Ground 1, Chukka discloses generating digital overlay drawings to label features of interest. EX1005 (Chukka), [0004]. Chukka also teaches counting and classifying cells as, for example, positively-stained tumor cells. *Id.* Therefore, it would have been readily apparent to a POSA that Chukka’s digital overlay drawing would comprise total counts of predicted classes corresponding to the digital image.

233. Accordingly, this claim is further obvious in view of Gallas and Chukka.

K. Claim 17

234. As discussed above in Ground 1, Chukka teaches claim 17. *See* § IX.K, *supra*. Gallas describes a tissue slide comprising colon cells and sarcoma cells, in other words, “not lymphocyte cells”. *See* EX1010, Fig. 6 (“In the 400 × 400 colon patch on the left, there are several plasma-cell candidates in the ‘evaluation’ square.”). Gallas also describes identifying plasma cells, e.g., lymphocytes. *Id.*; *see also* EX1032 (Kindt), 13, 15. Accordingly, this claim is further obvious in view of Gallas and Chukka.

L. Claim 18

235. As discussed above in Ground 1, Chukka teaches claim 18. *See* § IX.L, *supra*. Gallas further describes a tissue slide comprising colon cells and a cluster of plasma cells (e.g., an immune cluster). EX1010, Fig. 6 (“In the 400 × 400 colon patch on the left, there are several plasma-cell candidates in the ‘evaluation’ square.”); EX1032 (Kindt), 13, 15. Accordingly, this claim is further obvious in view of Gallas and Chukka.

XIII. GROUND 5: CLAIMS 2, 3, 7, AND 8 ARE OBVIOUS OVER GALLAS, CHUKKA, AND JONES.

236. As I discuss above, independent claims 1 and 6 are obvious over Gallas and Chukka. *See* § XII, *supra*. Further, as I explained above, Jones teaches or renders obvious the additional limitations of claims 2, 3, 7, and 8, and a POSA would have been motivated to combine the teachings of Jones in implementing Chukka’s automated object extraction and classification methods. *See* § X, *supra*. Accordingly, claims 2, 3, 7, and 8 would further have been obvious in view of Gallas, Chukka, and Jones.

A. Claim 2

237. As discussed above in Ground 2, Chukka, in view of Jones, teaches claim 2. *See* § X.A, *supra*. Accordingly, this claim is further obvious in view of Gallas, Chukka, and Jones.

B. Claim 3

238. As discussed above in Ground 2, Chukka, in view of Jones, teaches claim 3. *See* § X.B, *supra*. Accordingly, this claim is further obvious in view of Gallas, Chukka, and Jones.

C. Claims 7 and 8

239. As discussed above in Ground 2, Chukka, in view of Jones, teach claims 7 and 8. *See* § X.C, *supra*. Accordingly, these claims are further obvious in view of Gallas, Chukka, and Jones

XIV. GROUND 6: CLAIMS 12, 13, AND 16 ARE OBVIOUS OVER GALLAS, CHUKKA, AND SEBASTIAO.

240. As I discuss above, independent claim 6 is obvious over Gallas and Chukka. *See* § XII, *supra*. Further, as I explained above, Sebastiao teaches or renders obvious the additional limitations of claims 12, 13, and 16, and a POSA would have been motivated to combine the teachings of Sebastiao and Chukka. *See* § XI, *supra*. Accordingly, claims 12, 13, and 16 would further have been obvious in view of Gallas, Chukka, and Sebastiao.

A. Claim 12

241. As discussed above in Ground 3, Chukka, in view of Sebastiao, teaches claim 12. *See* § XI.A, *supra*. Accordingly, this claim is further obvious in view of Gallas, Chukka, and Sebastiao.

B. Claim 13

242. As discussed above in Ground 3, Chukka, in view of Sebastiao, teaches claim 13. *See* § XI.A, *supra*. Accordingly, this claim is further obvious in view of Gallas, Chukka, and Sebastiao.

C. Claim 16

243. As discussed above in Ground 3, Chukka, in view of Sebastiao, teaches claim 16. *See* § XI.A, *supra*. Accordingly, this claim is further obvious in view of Gallas, Chukka, and Sebastiao.

XV. CONCLUDING STATEMENTS

244. In signing this declaration, I understand that the declaration will be filed as evidence in a contested case before the Patent Trial and Appeal Board of the United States Patent and Trademark Office. I acknowledge that I may be subject to cross-examination in this case and that cross-examination will take place within the United States. If cross-examination is required of me, I will appear for cross-examination within the United States during the time allotted for cross-examination.

245. I declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: December 30, 2025

By: _____

Nikolaos P. Papanikolopoulos

XVI. APPENDIX - LISTING OF CHALLENGED CLAIMS

1. A method for creating an overlay map on a digital image of a slide, the method comprising:

[A] receiving the digital image;

[B] separating the digital image into a plurality of tiles, each tile of the plurality of tiles containing a respective portion of the digital image of the slide; and

[C] for each tile of the plurality of tiles: identifying features of the tile;

[D] identifying structural tissue features of a second portion of the digital image of the slide including at least part of one or more other tiles of the plurality of tiles, wherein the second portion is larger than the respective portion of the digital image contained in the tile; and

[E] identifying the majority class of tissue visible within the tile based at least in part on the features of the tile and the structural tissue features of the second portion of the digital image of the slide.

2. The method of claim 1, further comprising:

generating a digital overlay drawing of an outer edge of each cell in the image.

3. The method of claim 2, wherein the digital overlay drawing is prepared at the resolution level of an individual pixel.

4. The method of claim 1, wherein the majority class of tissue is selected from the group consisting of epithelium, normal epithelium, immune, stroma, necrosis, blood, and fat.

5. The method of claim 1, wherein the structural tissue features comprises at least one of glands, ducts, vessels, and immune clusters.

6. A method for tissue classification of a digital image of a slide, the method comprising:

[A] receiving the digital image;

[B] segmenting the digital image into a plurality of tiles, each tile of the plurality of tiles containing a respective portion of the digital image of the slide;

[C] for each tile of the plurality of tiles: identifying features of the tile;

[D] identifying structural tissue features of a second portion of the digital image of the slide including at least part of one or more other tiles of the plurality of tiles, wherein the second portion is larger than the respective portion of the digital image contained in the tile; and

[E] determining a predicted class for each tile based at least in part on the features of the tile and the structural tissue features of the second portion of the digital image of the slide;

[F] identifying a plurality of cell objects in the digital image;

[G] determining a predicted class for each of the plurality of cell objects;
and

[H] for each of the plurality of tiles that corresponds to one of the plurality of cell objects, assigning the tile the predicted class of the corresponding cell object in place of the predicted class of the tile.

7. The method of claim 6, further comprising:

storing, in a first file, for each tile, a tile position and the predicted class of the tile; and

storing, in a second file, for each cell object, a polygon outlining the cell object and the predicted class of the cell object.

8. The method of claim 6, further comprising:

generating a digital overlay drawing for the digital image, where the digital overlay drawing is a cell mask displaying a polygon around each cell object.

9. The method of claim 6, further comprising:

generating a digital overlay drawing for the digital image, where the digital overlay drawing is a histology mask displaying the plurality of tiles and the predict class for each tile.

10. The method of claim 6, further comprising:

generating a digital overlay drawing for the digital image; and

displaying, in the digital overlay drawing, the plurality of tiles and the predicted class for each tile that does not correspond to a cell object, and displaying, the plurality of cell objects and the predicted class of each cell object.

11. The method of claim 6, further comprising:

generating a digital overlay drawing for the digital image, wherein the digital overlay drawing includes the digital image; and displaying the digital overlay drawing.

12. The method of claim 6, further comprising:

generating a digital overlay drawing for the digital image, wherein the digital overlay drawing includes a generated version of the digital image; and displaying the digital overlay drawing.

13. The method of claim 6, further comprising:

generating a plurality of digital overlay drawings for the digital image, where each digital overlay drawing corresponds to a different predicted class; and selectively displaying one of the plurality of digital overlay drawings.

14. The method of claim 6, further comprising:

generating a digital overlay drawing for the digital image, where the digital overlay drawing comprises percentages of predict classes corresponding to the digital image.

15. The method of claim 6, further comprising:

generating a digital overlay drawing for the digital image, where the digital overlay drawing comprises total counts of predict classes corresponding to the digital image.

16. The method of claim 6, wherein the cell objects comprise CD3, CD8, CD20, pancytokeratin, and smooth muscle actin.

17. The method of claim 6, wherein the cell objects include lymphocyte cells and not lymphocyte cells.

18. The method of claim 6, wherein the structural tissue features comprises at least one of glands, ducts, vessels, and immune clusters.

XVII. EXHIBIT LIST

Exhibit No.	Description
1001	U.S. Patent No. 10,991,097
1002	Papanikolopoulos Declaration
1003	Papanikolopoulos CV
1004	'097 Patent Prosecution History
1005	U.S. Patent Publication No. 2016/0042511 A1 to Chukka et al.
1006	Thouis R. Jones, et al., "Voronoi-based segmentation of cells on image manifolds." <i>International Workshop on Computer Vision for Biomedical Image Applications</i> . Berlin, Heidelberg: Springer Berlin Heidelberg, 2005
1007	International Publication No. WO 2015/124737 A1 to Sebastiao, et al.
1008	Giuseppe Musumeci "Past, Present and Future: Overview on Histology and Histopathology." <i>J. Histol. Histopathol.</i> 1.5: 1-3 (2014)
1009	Peter W. Hamilton et al. "Digital Pathology and Image Analysis in Tissue Biomarker Research." <i>Methods</i> 70:59-73 (2014).
1010	Brandon D. Gallas et al. "eeDAP: an Evaluation Environment for Digital and Analog Pathology." <i>Medical Imaging 2014: Image Perception, Observer Performance, and Technology Assessment</i> . Vol. 9037. SPIE (2014)
1011	Jun Kong, et al., "Computer-aided Evaluation of Neuroblastoma on Whole-slide Histology Images: Classifying Grade of Neuroblastic Differentiation." <i>Pattern Recognition</i> 42.6: 1080-1092 (2009)
1012	Metin N. Gurcan et al., "Histopathological image analysis: A review." <i>IEEE Reviews in Biomedical Engineering</i> 2: 147-171 (2009)

1013	Songfan Yang, et al., “Multi-scale Recognition with DAG-CNNs.” <i>Proceedings of the IEEE International Conference on Computer Vision</i> (2015)
1014	Peter J. Burt, et al., “The Laplacian Pyramid as a Compact Image Code.” <i>Readings in Computer Vision</i> . Morgan Kaufmann, 671-679 (1987)
1015	Jocelyn Barker, et al., “Automated Classification of Brain Tumor Type in Whole-Slide Digital Pathology Images Using Local Representative Tiles.” <i>Medical Image Analysis</i> 30: 60-71 (2016)
1016	Xipeng Pan et al., “Cell Detection in Pathology and Microscopy Images with Multi-scale Fully Convolutional Neural Networks.” <i>World Wide Web</i> 21.6:1721-1743 (2018)
1017	Sahirzeeshan Ali et al., “Segmenting Multiple Overlapping Objects Via a Hybrid Active Contour Model Incorporating Shape Priors: Applications to Digital Pathology.” <i>Medical Imaging 2011: Image Processing</i> . Vol. 7962. SPIE (2011)
1018	Michael Barnes, et al., “Abstract P5-03-08: Development of a Histology-based Digital Pathology Image Analysis Algorithm for Assessment of Tumor Infiltrating Lymphocytes in HER2+ Breast Cancer.” <i>Cancer Research</i> 78.4_Supplement: P5-03 (2018)
1019	Emilio Garcia, et al., “Automatic Lymphocyte Detection on Gastric Cancer IHC Images using Deep Learning.” <i>2017 IEEE 30th International Symposium on Computer-Based Medical Systems (CBMS)</i> . IEEE, 2017
1020	Keith E. Steele, et al., “Measuring Multiple Parameters of CD8+ Tumor-infiltrating Lymphocytes in Human Cancers by Image Analysis.” <i>Journal for Immunotherapy of Cancer</i> 6.1:20 (2018)
1021	Thomas Mentzel, et al., “Cutaneous Myoepithelial Neoplasms: Clinicopathologic and Immunohistochemical Study of 20 Cases Suggesting a Continuous Spectrum Ranging from Benign Mixed Tumor of the Skin to Cutaneous Myoepithelioma and Myoepithelial Carcinoma.” <i>Journal of Cutaneous Pathology</i> 30.5 (2003): 294-302

1022	Michal Zamecnik, “Epithelioid Hemangioendothelioma with Actin+/Cytokeratin+ Myoblast-like Cells: Report of a Case.” <i>Journal of Interdisciplinary Histopathology</i> , (2016)
1023	Nemanja Rajković, et al., “The Pan-cytokeratin Staining Intensity and Fractal Computational Analysis of Breast Tumor Malignant Growth Patterns Prognosticate the Occurrence of Distant Metastasis.” <i>Frontiers in Oncology</i> 8:348 (2018)
1024	Mami Murakami, et al., “Docetaxel Conjugate Nanoparticles that Target α -smooth Muscle Actin-expressing Stromal Cells Suppress Breast Cancer Metastasis.” <i>Cancer Research</i> 73.15: 4862-4871 (2013)
1025	Mahalakshmi Ravi, et al., “Expression of α -smooth Muscle Actin in Benign and Malignant Salivary Gland Tumors: An Immunohistochemical Study.” <i>Indian Journal of Pathology and Microbiology</i> 61.4: 479-484 (2018)
1026	Michael J. Gerdes, et al. “Highly Multiplexed Single-Cell Analysis of Formalin-fixed, Paraffin-embedded Cancer Tissue.” <i>Proceedings of the National Academy of Sciences</i> 110.29 (2013): 11982-11987
1027	Qiang Du, et al., “Centroidal Voronoi tessellations: Applications and algorithms.” <i>SIAM Review</i> 41.4: 637-676 (1999)
1029	John D. Bancroft, ed. “Theory and Practice of Histological Techniques, Elsevier Health Sciences, 2008 (6th Ed.).
1030	Zeta Corp. Pancytokeratin brochure
1031	Liron Pantanowitz, et al., “Twenty Years of Digital Pathology: An Overview of the Road Travelled, What is on the Horizon, and the Emergence of Vendor-Neutral Archives.” <i>Journal of Pathology Informatics</i> 9.1:40 (2018)
1032	Thomas J. Kindt, et al., Kuby Immunology, 2007 (6th Ed.)