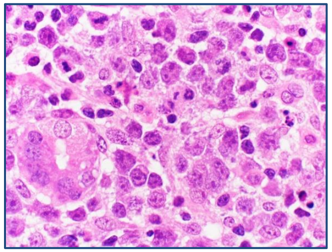
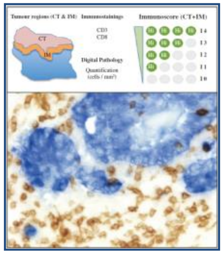
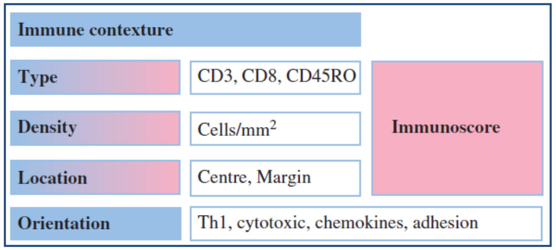
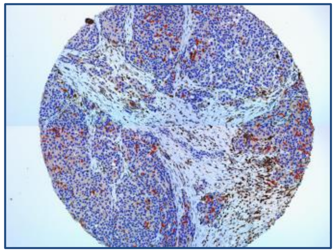
ESMO Preceptorship, 20 November 2014
Session 7: Immunomonitoring

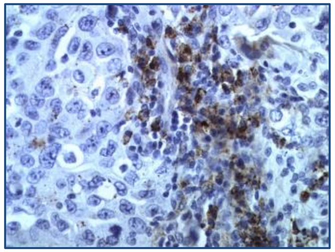
Tumor infiltrating immune cells as biomarkers with prognostic/predictive value







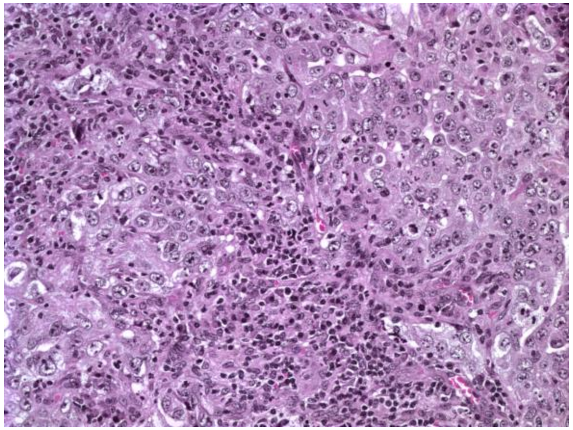
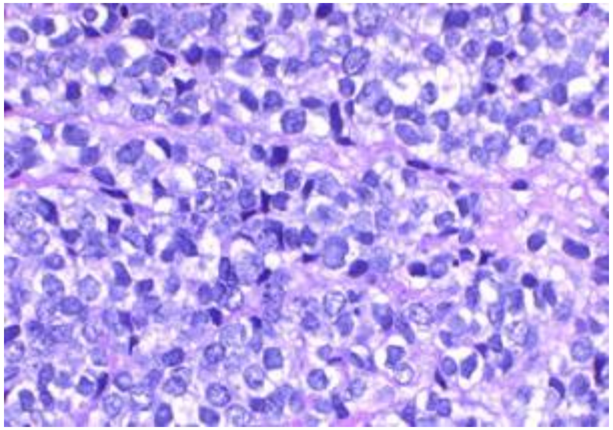
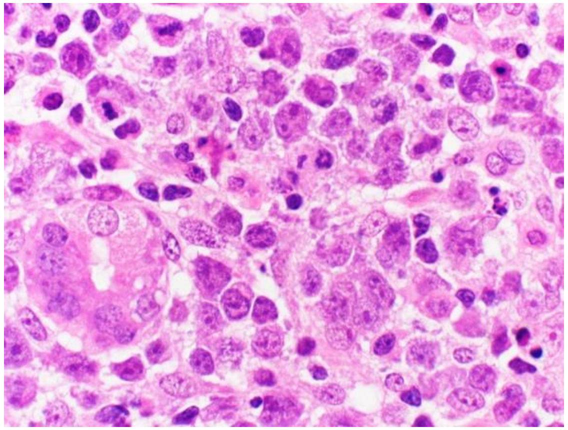




Periklis Foukas, MD, PhD
CTE, DO
CHUV, UNIL

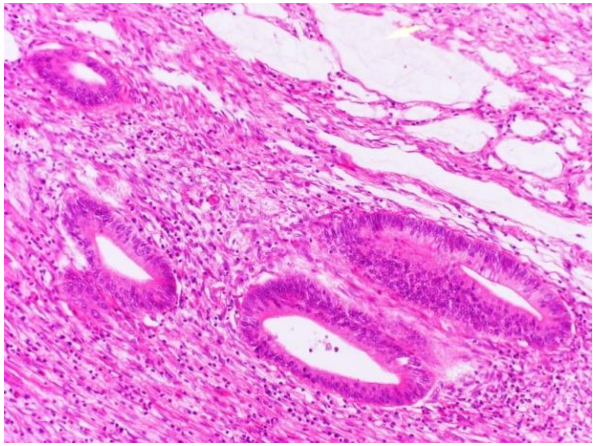
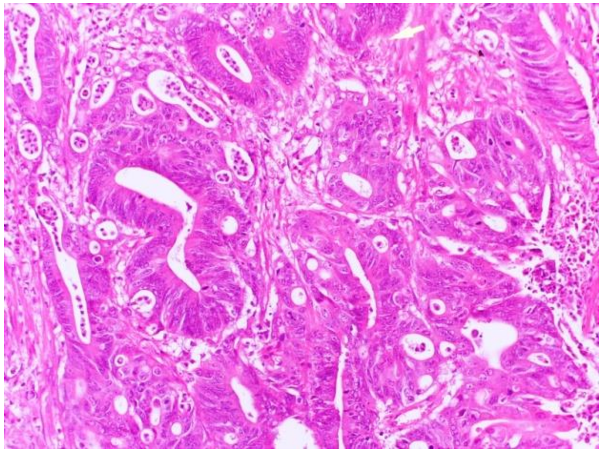
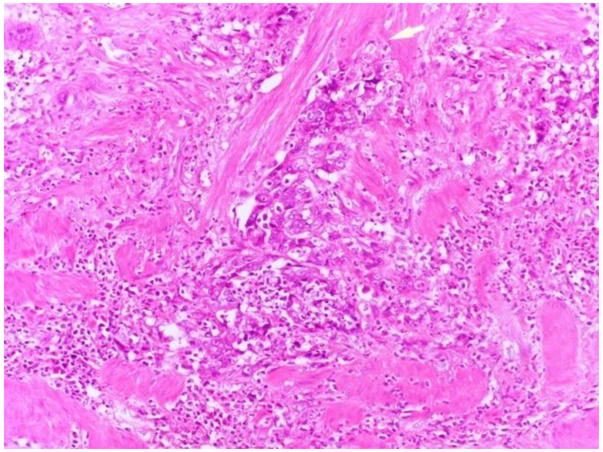
Tumor infiltrating immune cells as biomarkers with prognostic/predictive value

- Tumor type (carcinomas, sarcomas, lymphomas…)

Tumor infiltrating immune cells as biomarkers with prognostic/predictive value

- Tumor type (carcinomas, sarcomas, lymphomas…)
- Differentiation (grading) (well, moderate, poor)

Tumor infiltrating immune cells as biomarkers with prognostic/predictive value

- Tumor type (carcinomas, sarcomas, lymphomas…)
- Differentiation (grading) (low, moderate, high)
- TNM staging information (classification of the extent of spread of cancer)
  - T = Tumor (local extension)
  - N = nodes (LN metastases)
  - M = metastatic spread to distant organs

Tumor infiltrating immune cells as biomarkers with prognostic/predictive value

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} **H&E**

OPINION

Has the new TNM classification for colorectal cancer improved care?

Iris D. Nagtegaal, Phil Quirke and Hans-Joachim Schmoll

Abstract | In 2009, the Union for International Cancer Control issued the seventh edition of the well-used T (tumor), N (node), and M (metastasis) classification guidelines. There has been a continual refinement of the staging for colorectal cancer since this system for assessing tumor stage was initially adopted and it has been used to guide treatment decisions for over 50 years. However, the outcome after therapy for patients with colorectal cancer is very variable, even when patients are assigned to the same TNM category. This article assesses the changes that have been made since the sixth edition and discusses whether they are, in fact, informative improvements for a practicing clinician.

Nagtegaal, I. D. *et al. Nat. Rev. Clin. Oncol.* **9**, 119–123 (2012); published online 18 October 2011;
doi:10.1038/nrclinonc.2011.157

Cell, Vol. 100, 57–70, January 7, 2000, Copyright ©2000 by Cell Press

The Hallmarks of Cancer

Douglas Hanahan* and Robert A. Weinberg†

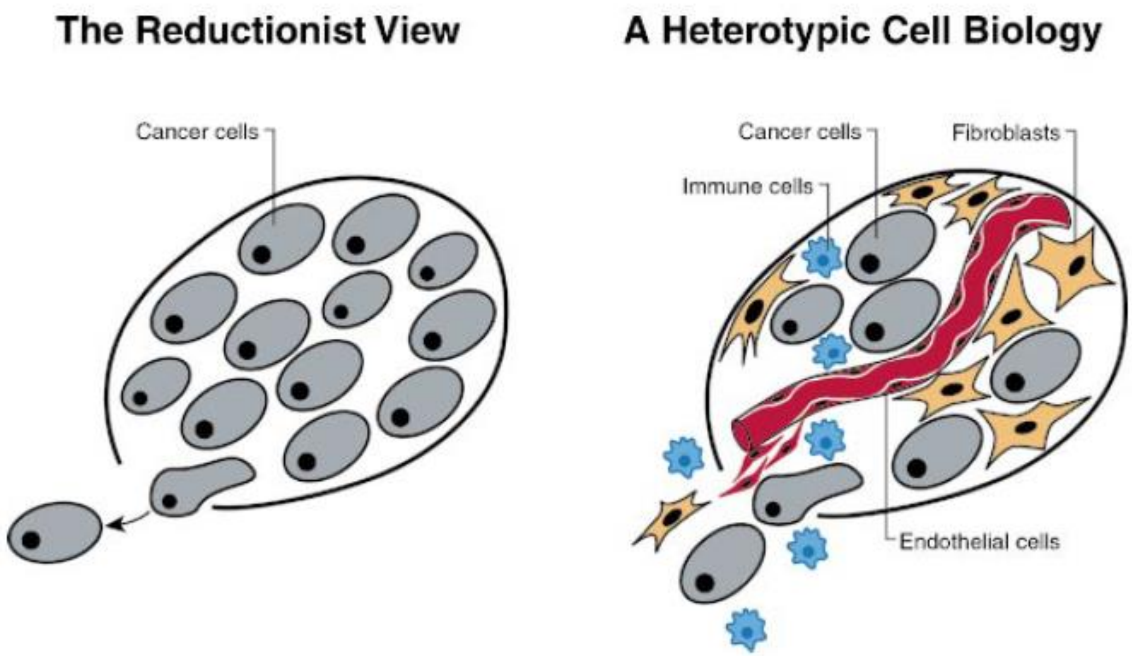

Figure 3. Tumors as Complex Tissues

The field of cancer research has largely been guided by a reductionist focus on cancer cells and the genes within them (left panel)—a focus that has produced an extraordinary body of knowledge. Looking forward in time, we believe that important new inroads will come from regarding tumors as complex tissues in which mutant cancer cells have conscripted and subverted normal cell types to serve as active collaborators in their neoplastic agenda (right panel). The interactions between the genetically altered malignant cells and these supporting coconspirators will prove critical to understanding cancer pathogenesis and to the development of novel, effective therapies.

Cell, Vol. 100, 57–70, January 7, 2000, Copyright ©2000 by Cell Press

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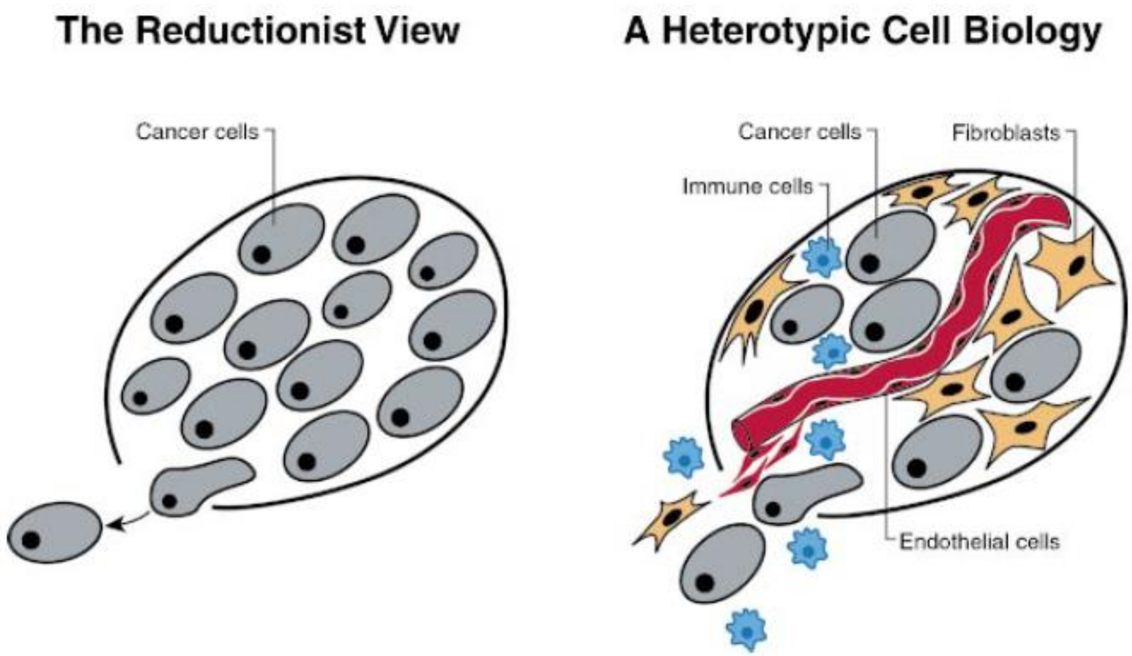

Figure 3. Tumors as Complex Tissues

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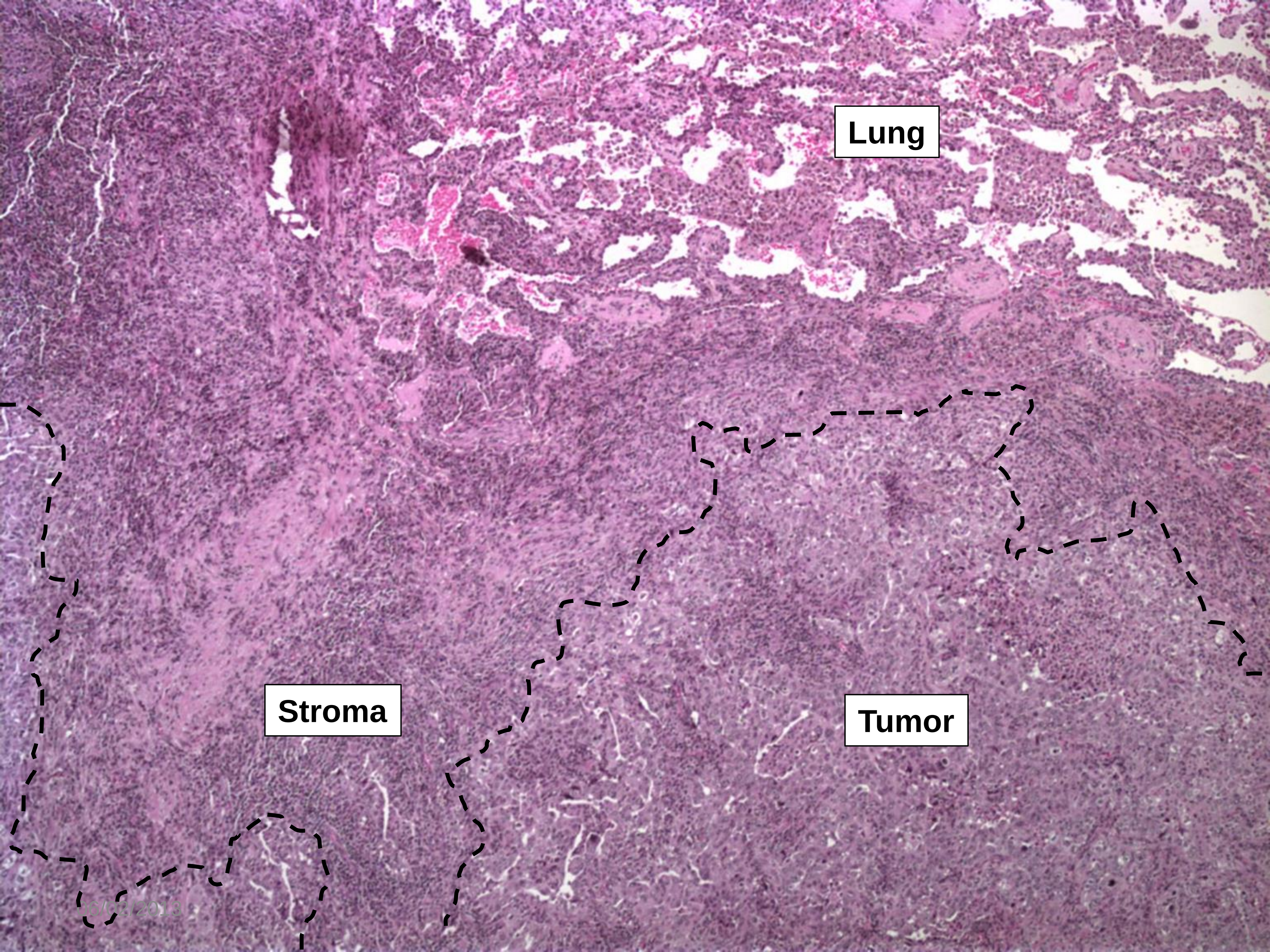
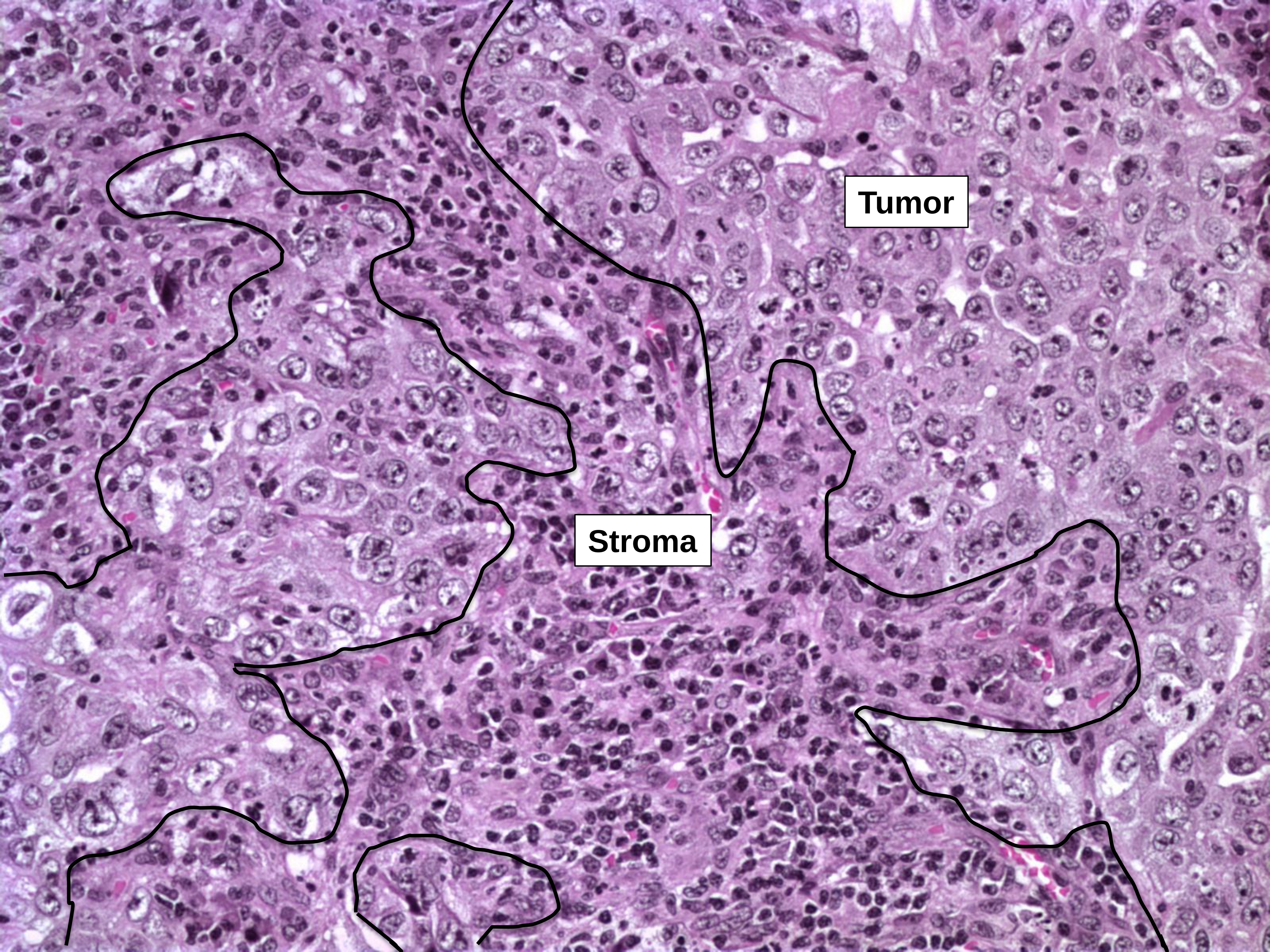
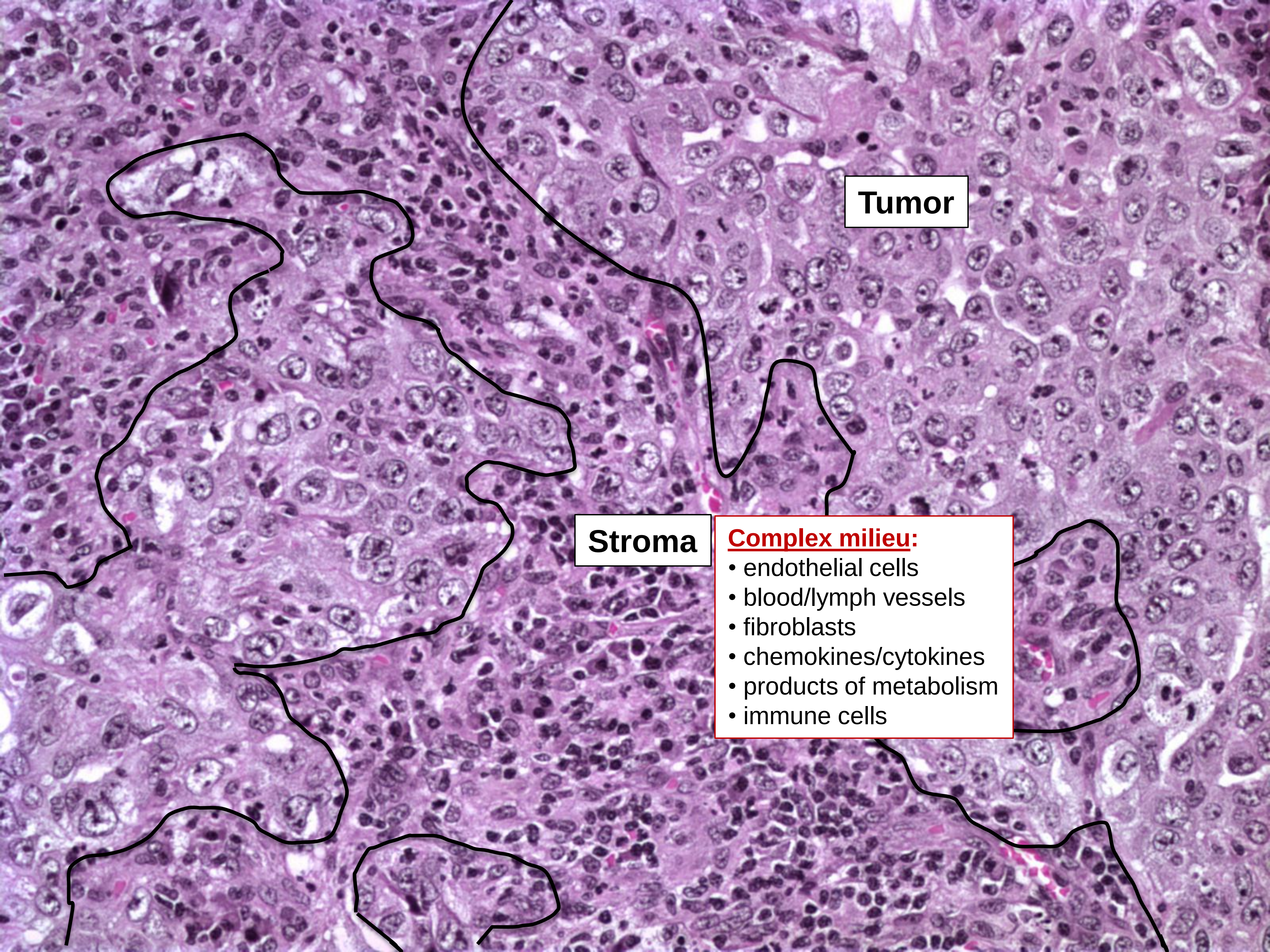
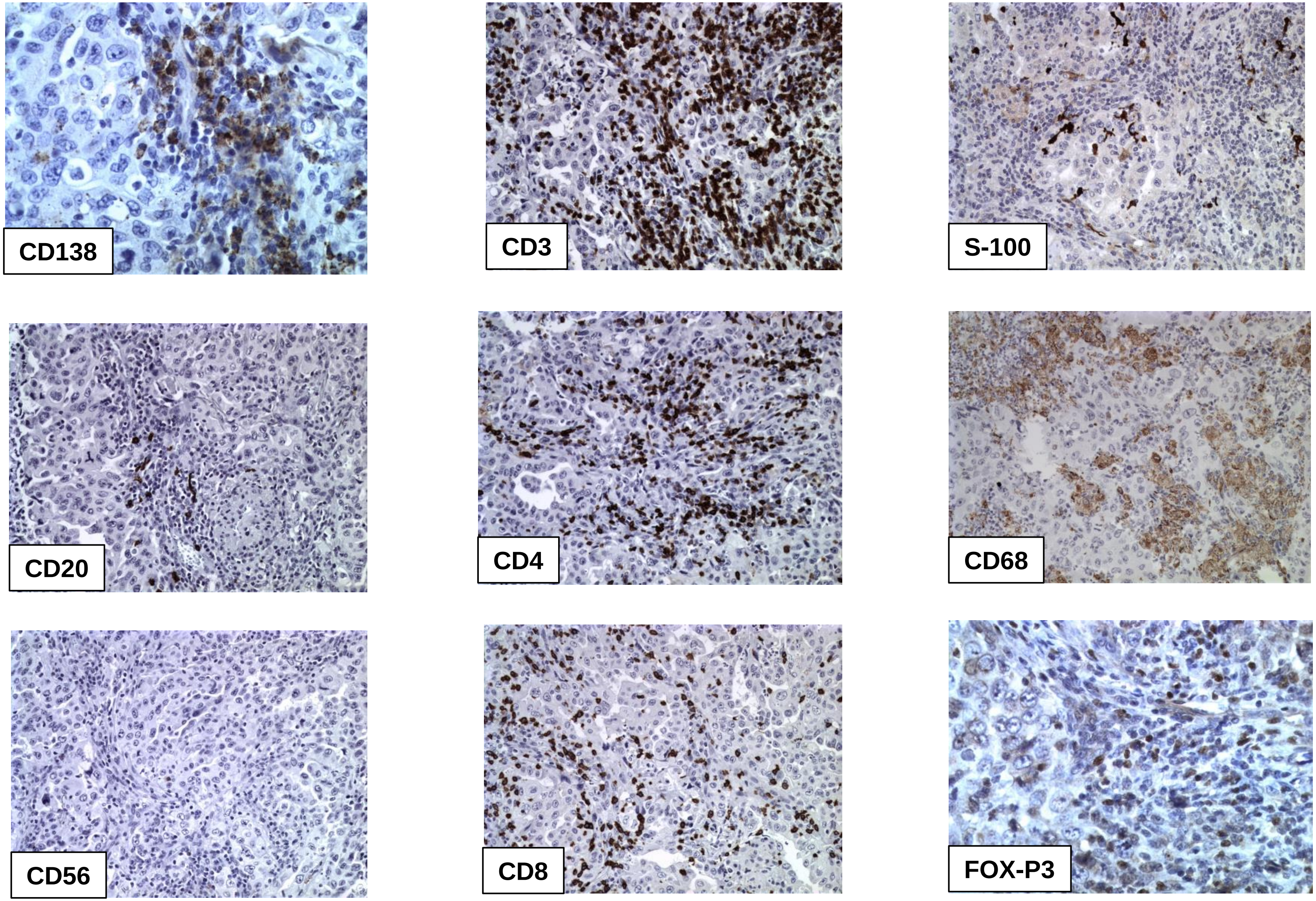
…in other words the TNM classification scheme is based on the assumption that disease progression is a tumor cell-autonomous process


Lung
Stroma
Tumor


Tumor
Stroma


Tumor
Stroma
Complex milieu:
• endothelial cells
• blood/lymph vessels
• fibroblasts
• chemokines/cytokines
• products of metabolism
• immune cells

NSCLC immune infiltrates comprises many different cell types



Understanding the natural (spontaneous) immune response of cancer patients is critical for the design of efficient anticancer immunotherapies

Understanding the natural (spontaneous) immune response of cancer patients is critical for the design of efficient anticancer immunotherapies

…and may be for the prediction of the efficacy of immunotherapies

1. Oncogenesis proceeds in the context of continuous interactions with immunosurveilance
3Es= equilibrium, editing, escape

IFNγ and lymphocytes prevent primary tumour development and shape tumour immunogenicity

Vijay Shankaran*, Hiroaki Ikeda*, Allen T. Bruce*, J. Michael White*, Paul E. Swanson*, Lloyd J. Old† & Robert D. Schreiber*

** Department of Pathology and Immunology, Center for Immunology, Washington University School of Medicine, 660 South Euclid Avenue, St Louis, Missouri 63110, USA*
† Ludwig Institute for Cancer Research, New York Branch at Memorial Sloan-Kettering Cancer Center, New York, New York 10021, USA

Lymphocytes were originally thought to form the basis of a 'cancer immunosurveillance' process that protects immunocompetent hosts against primary tumour development[1,2], but this idea was largely abandoned when no differences in primary tumour development were found between athymic nude mice and syngeneic wild-type mice[3–5]. However, subsequent observations that nude mice do not completely lack functional T cells[6,7] and that two components of the immune system—IFNγ[8,9] and perforin[10–12]—help to prevent tumour formation in mice have led to renewed interest in a tumour-suppressor role for the immune response. Here we show that lymphocytes and IFNγ collaborate to protect against development of carcinogen-induced sarcomas and spontaneous epithelial carcinomas and also to select for tumour cells with reduced immunogenicity. The immune response thus functions as an effective extrinsic tumour-suppressor system. However, this process also leads to the immunoselection of tumour cells that are more capable of surviving in an immunocompetent host, which explains the apparent paradox of tumour formation in immunologically intact individuals.

2. Several immunotherapies taking advantage of the natural (spontaneous) adaptive immune responses achieved remarkable successes

Immunity

Review

Oncology Meets Immunology: The Cancer-Immunity Cycle

Daniel S. Chen[1,3] and Ira Mellman[2,3,*]

Immunity *39*, July 25, 2013

Immunity

Review

Adoptive T Cell Transfer for Cancer Immunotherapy in the Era of Synthetic Biology

Michael Kalos[1,*] and Carl H. June[1,*]

Immunity *39*, July 25, 2013

3. The natural (spontaneous) adaptive immune responses of cancer patients have been shown to influence their survival

Intratumoral T Cells, Recurrence, and Survival in Epithelial Ovarian Cancer

Lin Zhang, M.D., Jose R. Conejo-Garcia, M.D., Ph.D., Dionyssios Katsaros, M.D., Ph.D., Phyllis A. Gimotty, Ph.D., Marco Massobrio, M.D., Giorgia Regnani, M.D., Antonis Makrigiannakis, M.D., Ph.D., Heidi Gray, M.D., Katia Schlienger, M.D., Ph.D., Michael N. Liebman, Ph.D., Stephen C. Rubin, M.D., and George Coukos, M.D., Ph.D.

N Engl J Med 2003;348:203-13.

Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

Jérôme Galon,[1*†] Anne Costes,[1] Fatima Sanchez-Cabo,[2] Amos Kirilovsky,[1] Bernhard Mlecnik,[2] Christine Lagorce-Pagès,[3] Marie Tosolini,[1] Matthieu Camus,[1] Anne Berger,[4] Philippe Wind,[4] Franck Zinzindohoué,[5] Patrick Bruneval,[6] Paul-Henri Cugnenc,[5] Zlatko Trajanoski,[2] Wolf-Herman Fridman,[1,7] Franck Pagès[1,7]†

29 SEPTEMBER 2006 VOL 313 SCIENCE

The immune contexture in human tumours: impact on clinical outcome

Wolf Herman Fridman, Franck Pagès, Catherine Sautès-Fridman and Jérôme Galon

NATURE REVIEWS | CANCER VOLUME 12 | APRIL 2012

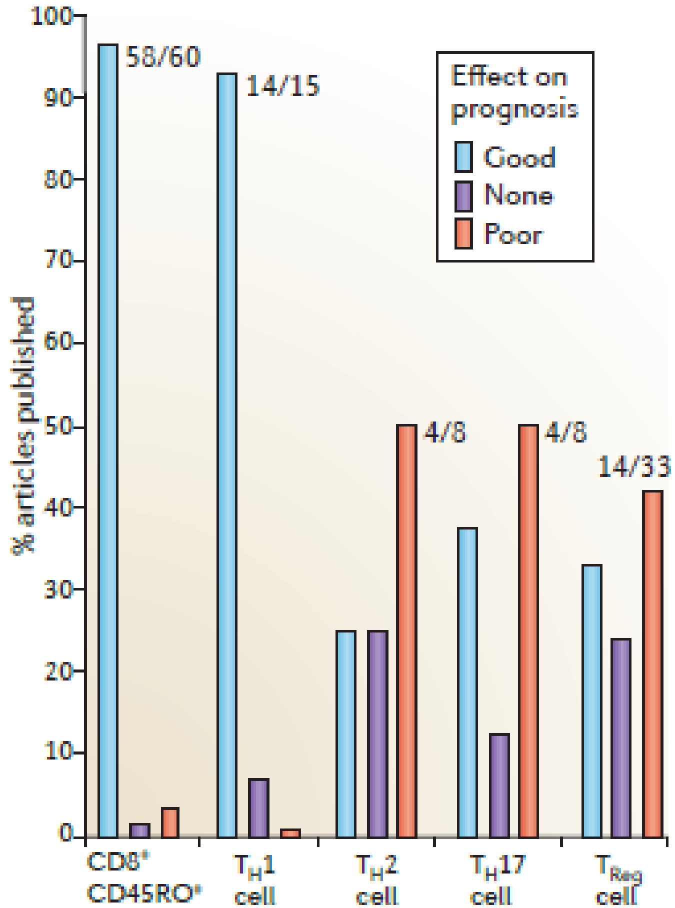


Figure 2 | The association of immune cell infiltrates with prognosis in various types of cancer. The analysis of 124 published articles studying the impact of cytotoxic T cells, memory T cells, regulatory T (T_{Reg}) cells and T helper (T_H) cell subpopulations with regard to prognosis of cancer patients (20 different cancer types were analysed) is represented. 'Good' means that the cell type is associated with a good prognosis, 'none' means that there was no correlation and 'poor' means that the cells are associated with a poor prognosis. Please also refer to TABLE 1 for references.

In different tumors, studies suggesting a protective role of immune infiltrates have been contradicted by others that did not reach this conclusion

⇩

Non-reproducibility

- variable descriptions of the type of lymphocytic infiltrates
- subjective grading of host lymphocytic reaction

In different tumors, studies suggesting a protective role of immune infiltrates have been contradicted by others that did not reach this conclusion

⇓

Non-reproducibility

- variable descriptions of the type of lymphocytic infiltrates
- subjective grading of host lymphocytic reaction

In order to be used globally in a routine manner…..

Characteristics of a good marker: pathology based and feasible in routine settings, simple, inexpensive, rapid, robust, reproducible, quantitative, standardized and powerfull

In different tumors, studies suggesting a protective role of immune infiltrates have been contradicted by others that did not reach this conclusion

⬇

Non-reproducibility
- variable descriptions of the type of lymphocytic infiltrates
- subjective grading of host lymphocytic reaction

⬇

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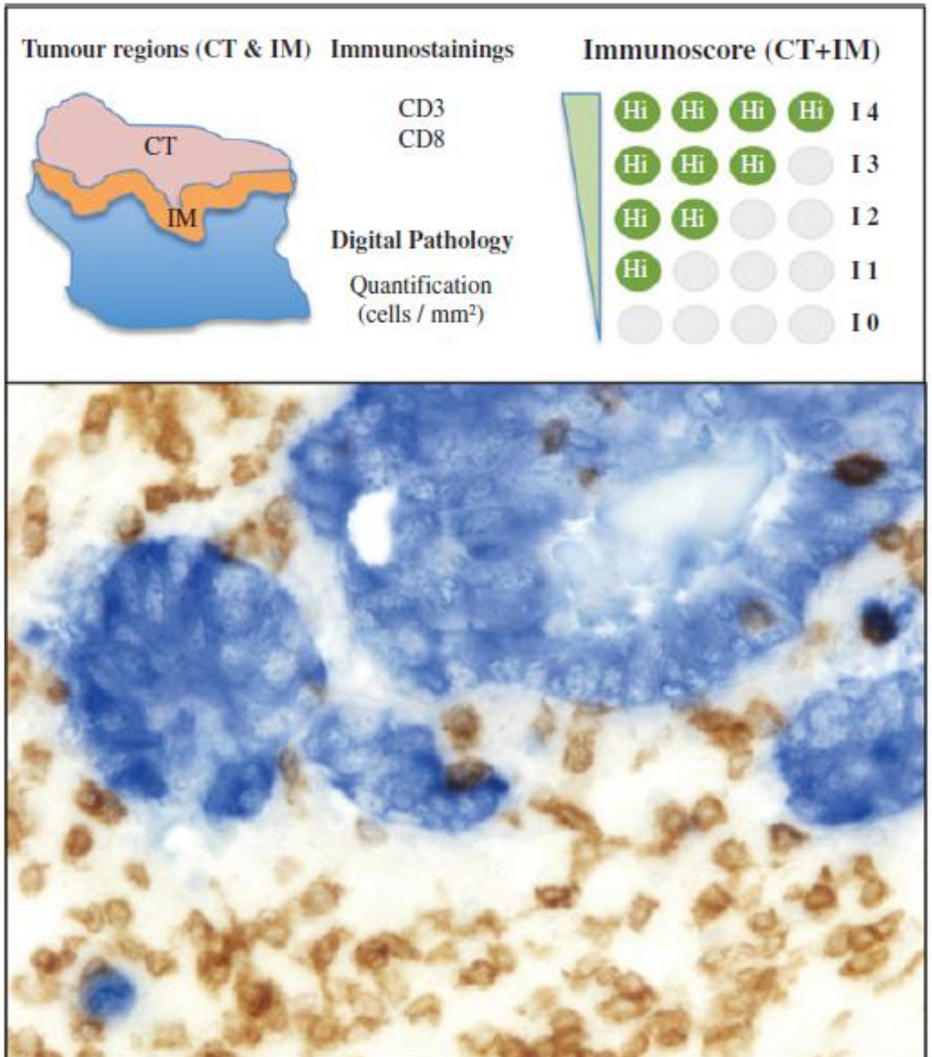
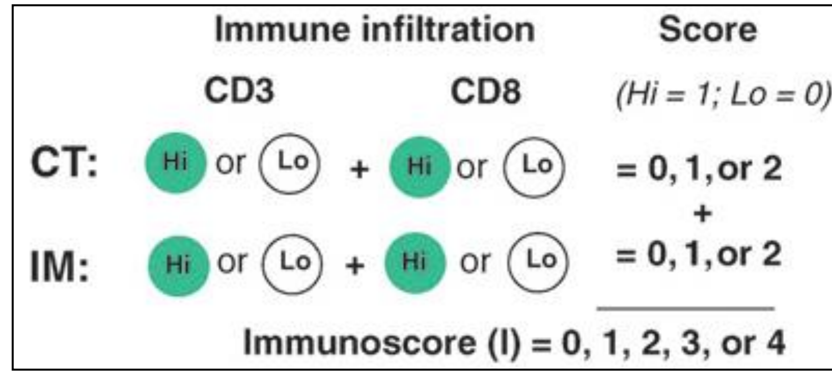
29 SEPTEMBER 2006 VOL 313 **SCIENCE**

Towards the introduction of the 'Immunoscore' in the classification of malignant tumours





Cutoff (Hi vs Lo)= the minimum *p* value

Galon J. et al J Pathol, 2014; 232: 199-209

Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

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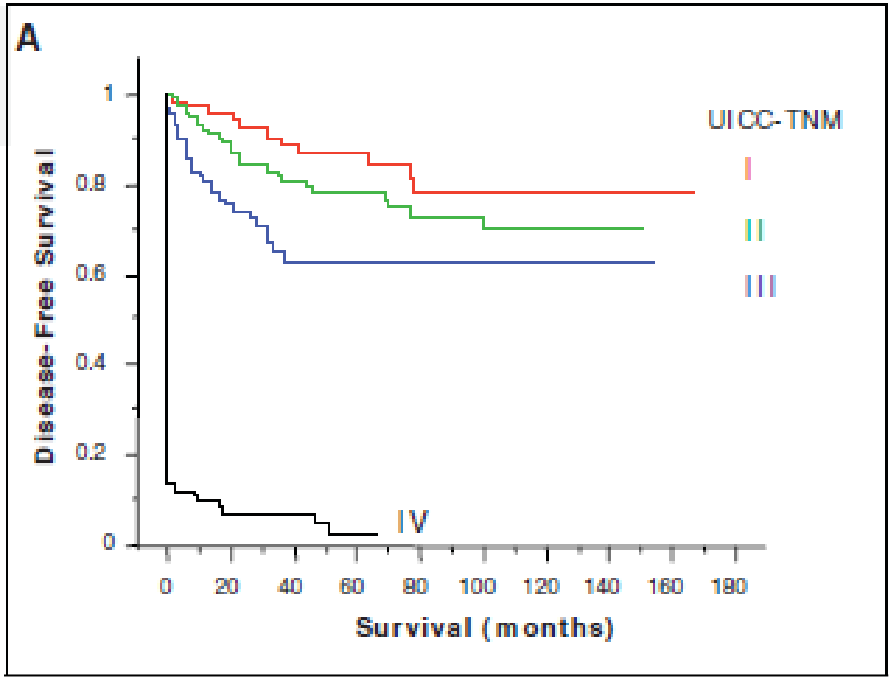
Tumor histopathology

UICC-TNM Staging system



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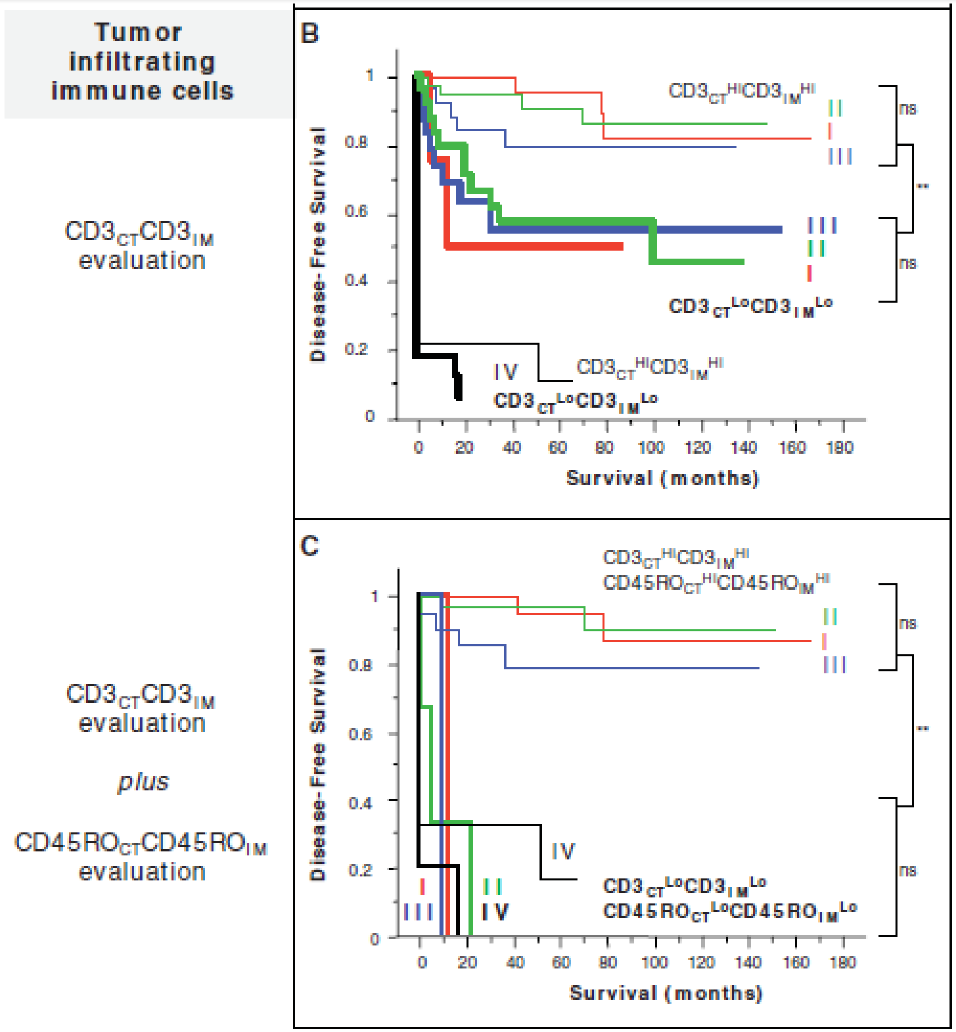

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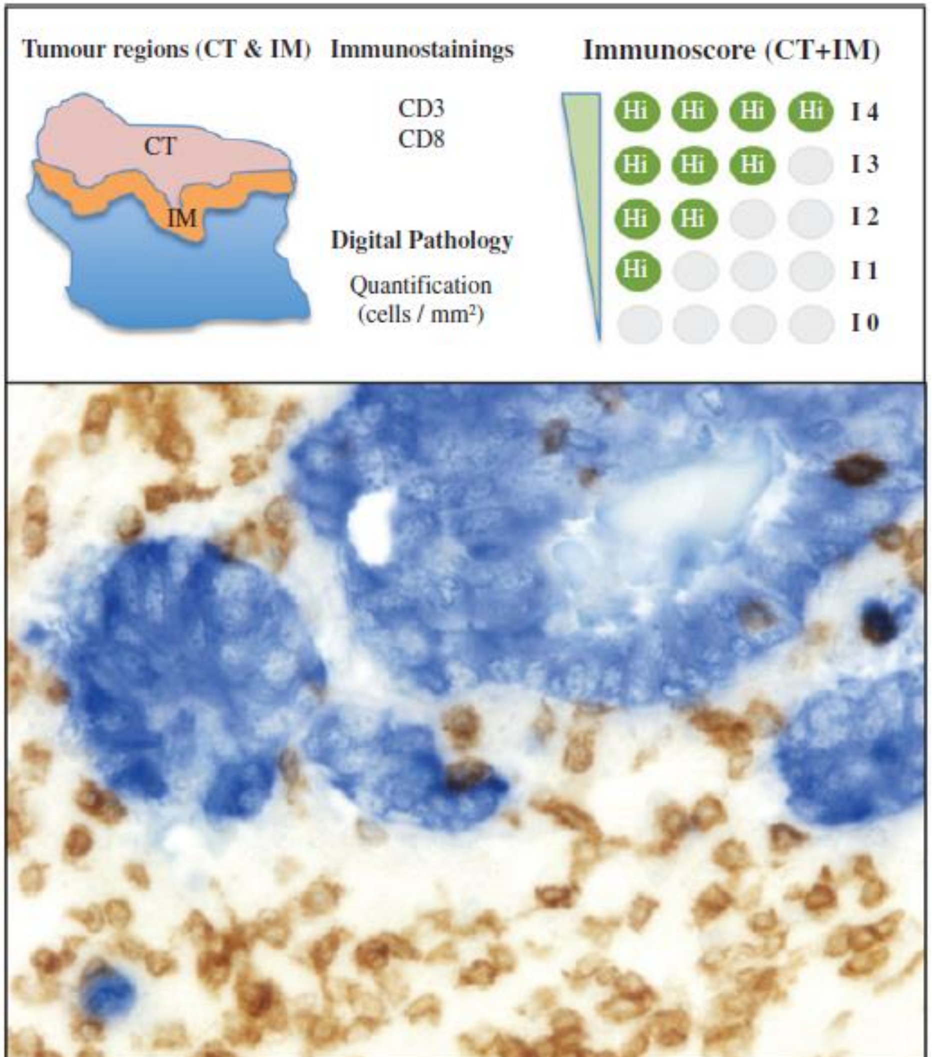


Good biomarker	
Routine	☑
Feasible	☑
Simple	☑
Inexpensive	☑
Rapid	☑
Robust	☑
Reproducible	☑
Quantitative	☑
Standardized	☑
Pathology-based	☑
Powerful	☑

Galon J. et al J Pathol, 2014; 232: 199-209

Towards the introduction of the 'Immunoscore' in the classification of malignant tumours

Immune contexture		
Type	CD3, CD8, CD45RO	**Immunoscore**
Density	Cells/mm^2	
Location	Centre, Margin	
Orientation	Th1, cytotoxic, chemokines, adhesion	

Galon J. et al J Pathol, 2014; 232: 199-209

Immune contexture		
Type	CD3, CD8, CD45RO	**Immunoscore**
Density	Cells/mm^2	
Location	Centre, Margin	
Orientation	Th1, cytotoxic, chemokines, adhesion	

↓ ?

Simple and powerfull immune biomarkers

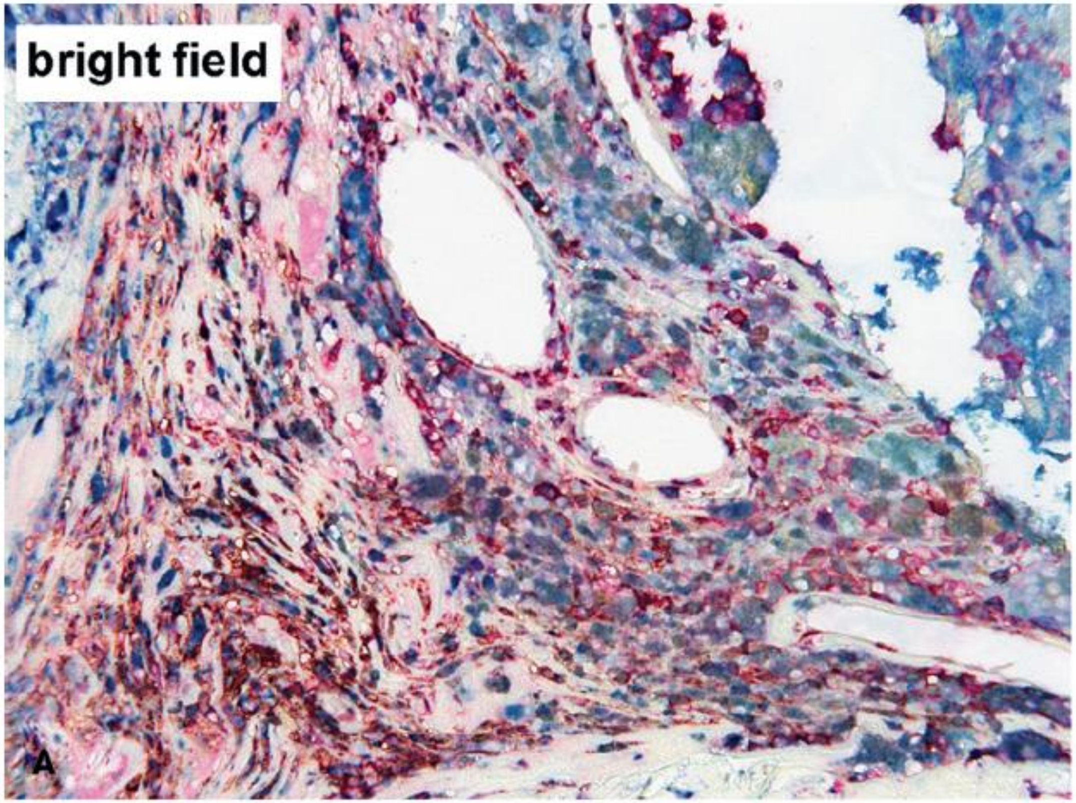
Immune contexture		
Type	CD3, CD8, CD45RO	**Immunoscore**
Density	Cells/mm^2	
Location	Centre, Margin	
Orientation	Th1, cytotoxic, chemokines, adhesion	

?

- Gene expression profile
- TCR (BCR?) sequencing
- In situ multispectral imaging analysis

Simple and powerfull immune biomarkers

IHC is a very important tool but there are limitations inherent to this type of analysis which prevent comprehensive description of leukocytic infiltrates



CD68
CD14
CD163

van der Loos C., The journal of Histotechnology, 2010

Multispectral Imaging

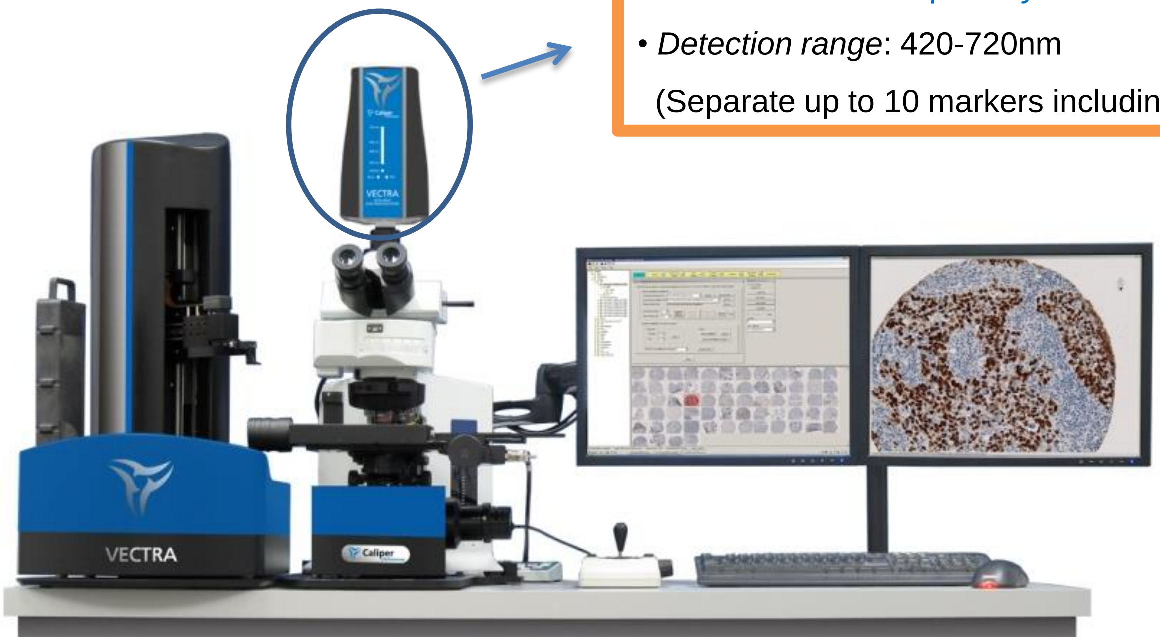
Vectra™ Automated Multi-modal Tissue Analysis System

Multispectral Imaging

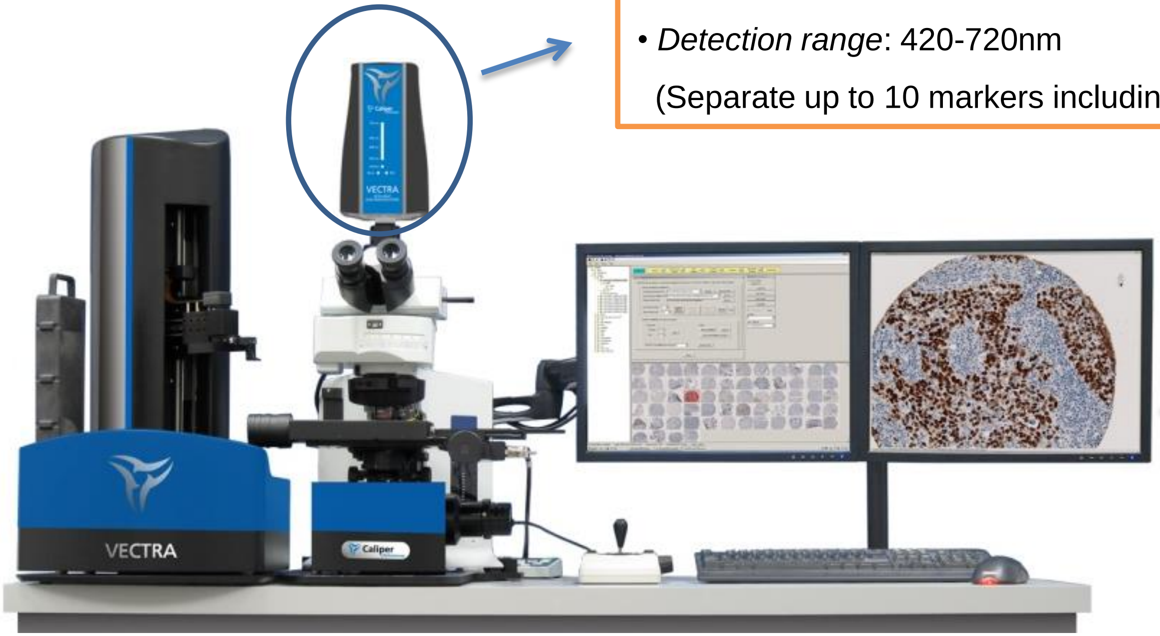
CCD camera with Liquid Crystal Tunable Filter

- *Detection range*: 420-720nm

  (Separate up to 10 markers including autofluorescence)



Multispectral Imaging



CCD camera with Liquid Crystal Tunable Filter

- *Detection range*: 420-720nm

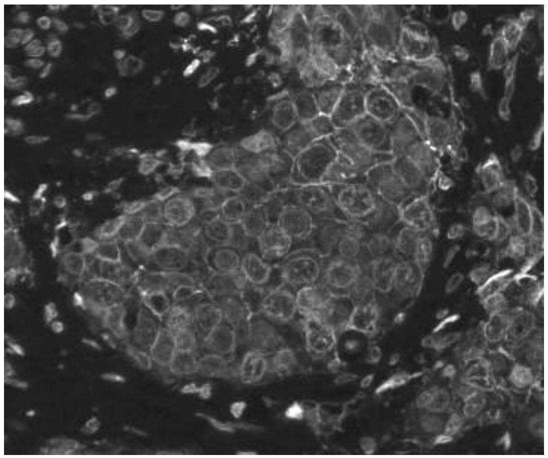
  (Separate up to 10 markers including autofluorescence)

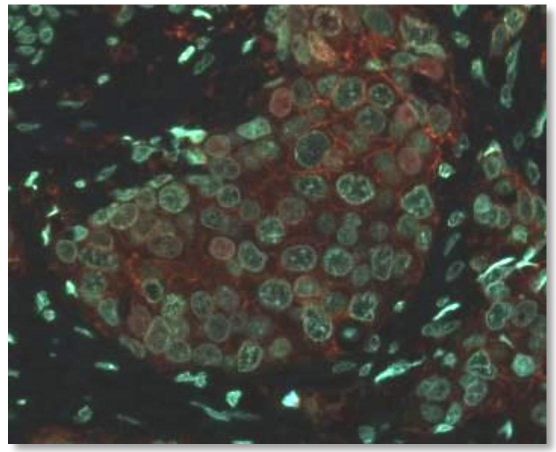
Analysis software

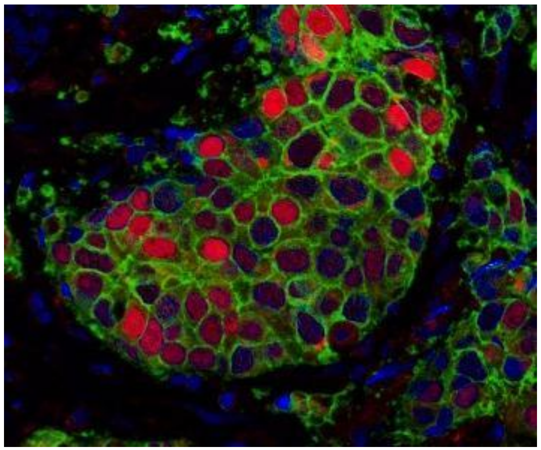
- Spectral unmixing
- Learn by examples algorithm
- Quantitative data output

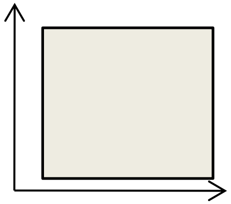
Benefits of using multispectral imaging

Multispectral imaging technology is used to acquire images at many wavelengths (10-30) to better determine fluorophore/stain distribution in cells or tissue

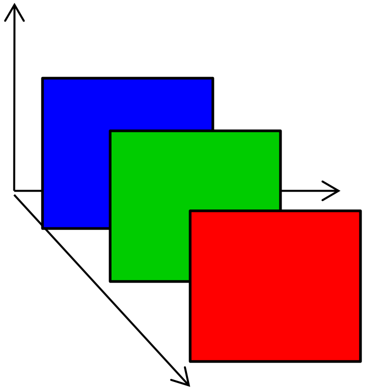




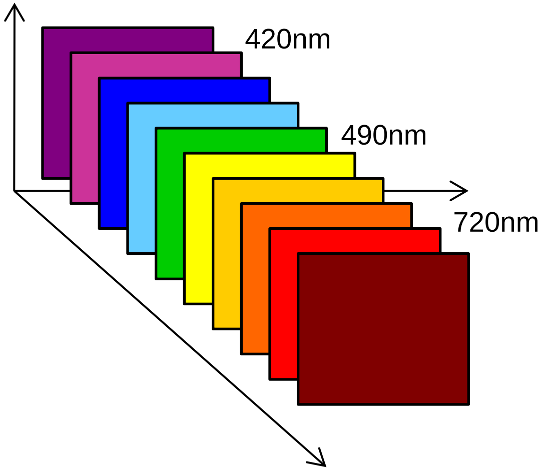




Monochrome

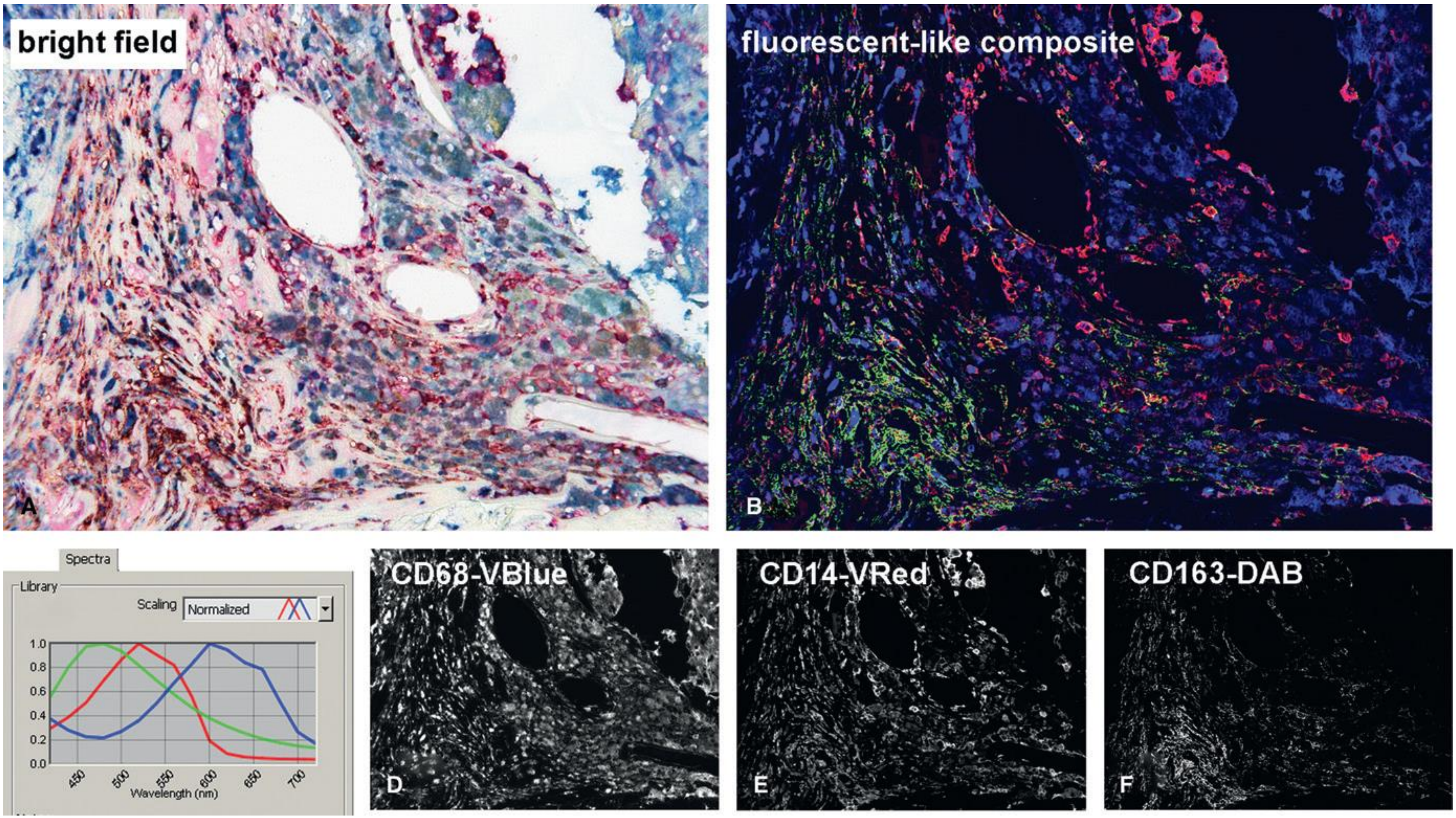


Color (RGB)



Multispectral

Pushing the limits of color separation using spectral unmixing

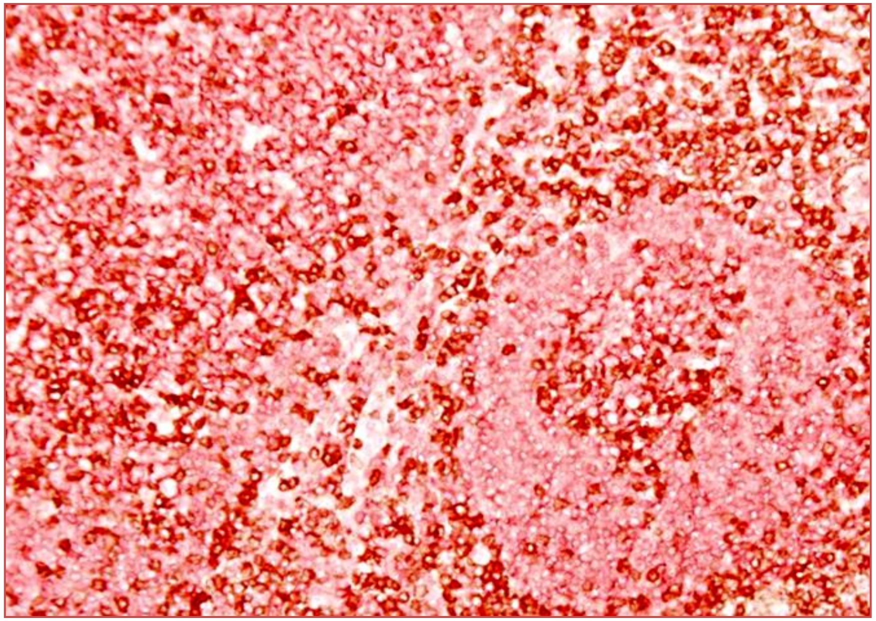


Chris van der Loos, Journal of histotechnology, 2010

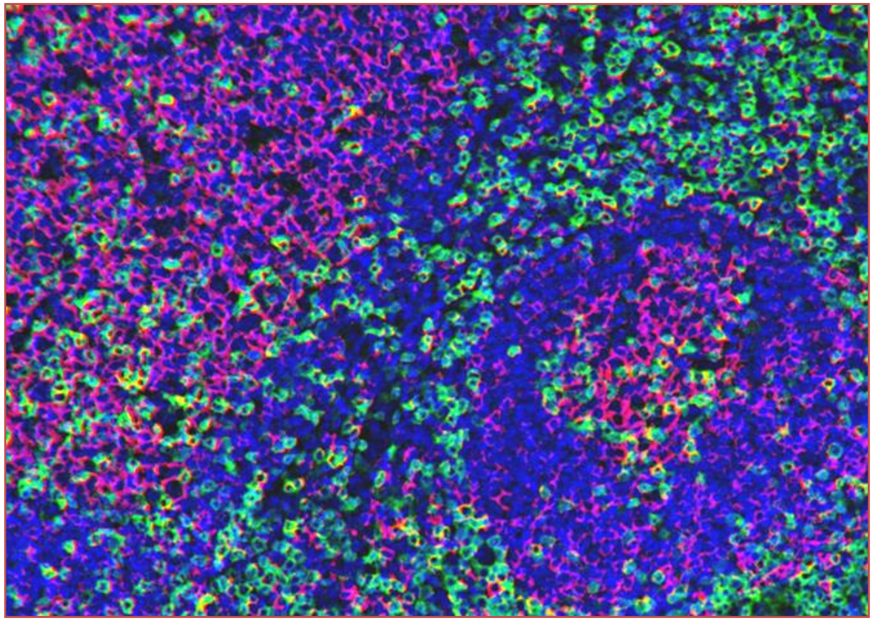
Pushing the limits of color separation using spectral unmixing

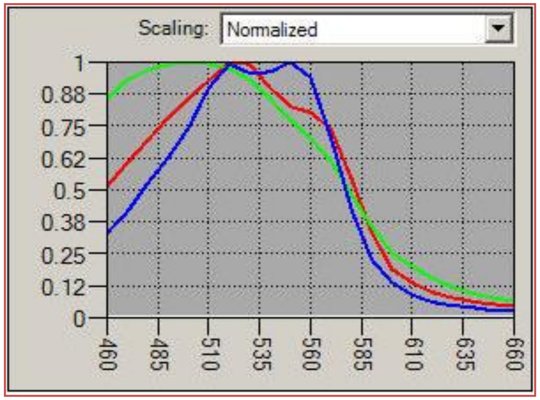
Example: Red vs. Red vs. Red

Bright field



Fluorescent-like composite

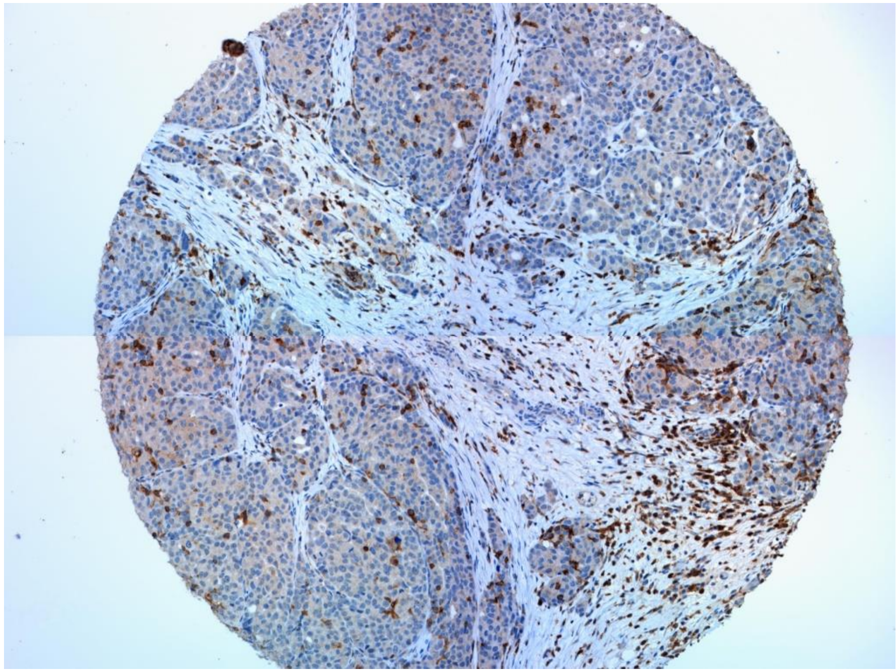




Liquid Permanent Red
Vector Red
AEC

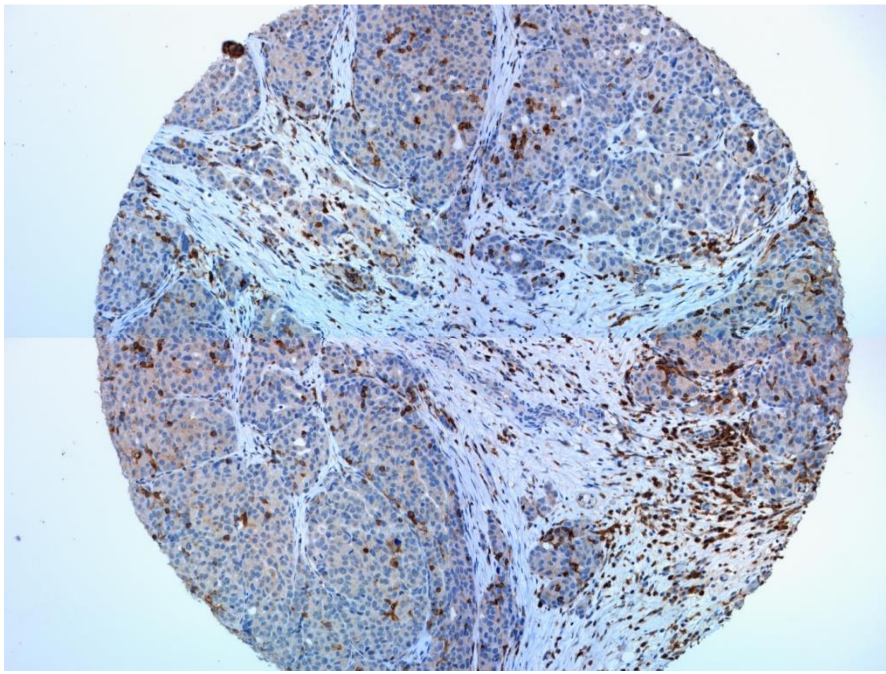
Sample courtesy of Chris van der Loos, University of Amsterdam

Multispectral Imaging



1. Load image

Multispectral Imaging



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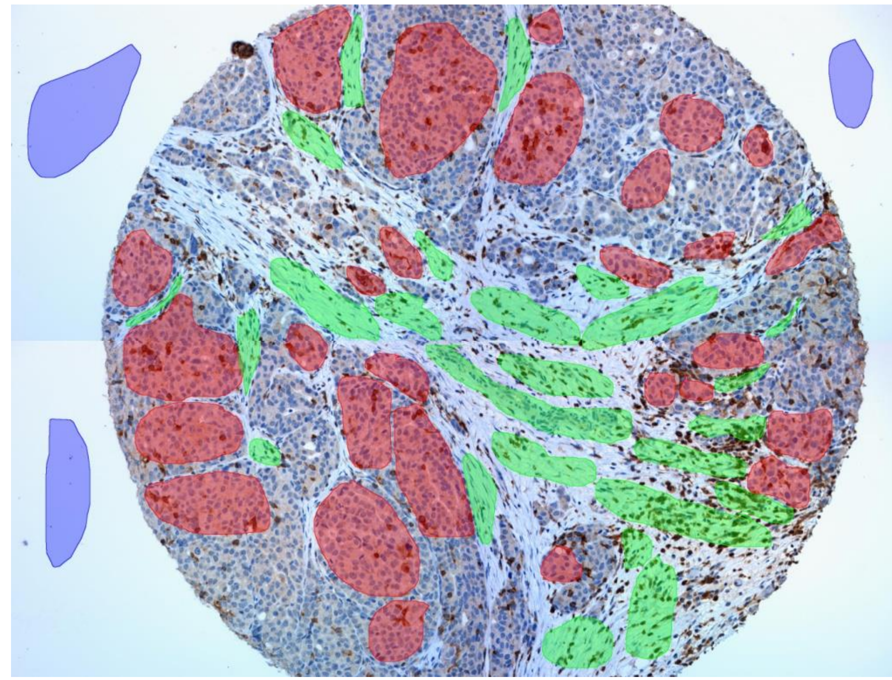
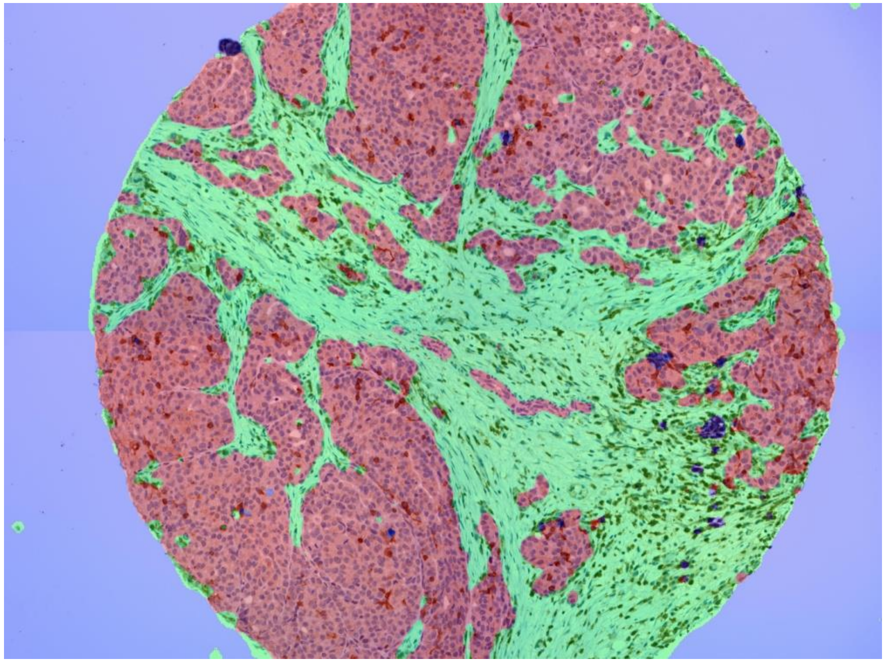


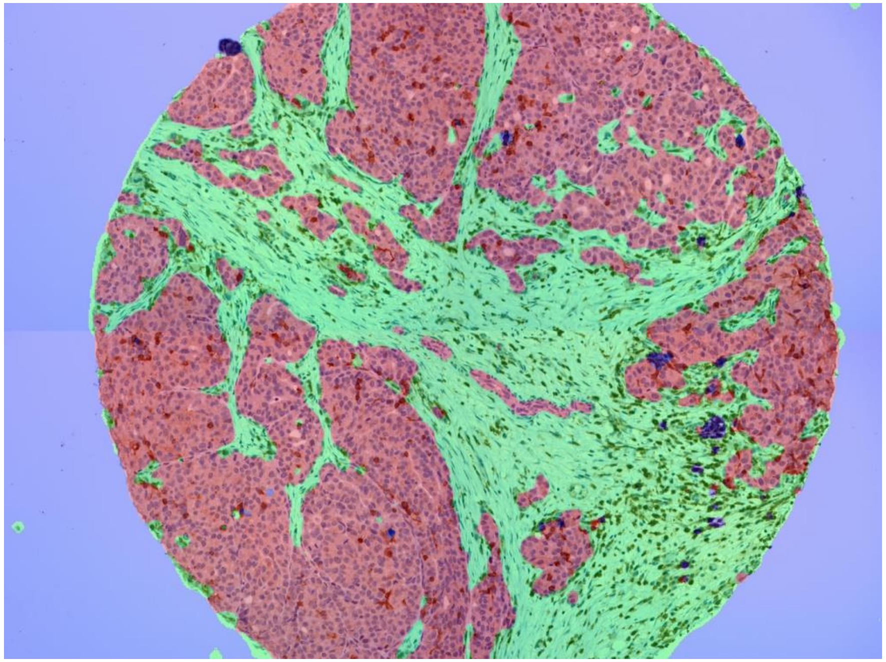
2. Draw training regions and “train” algorithm

Multispectral Imaging

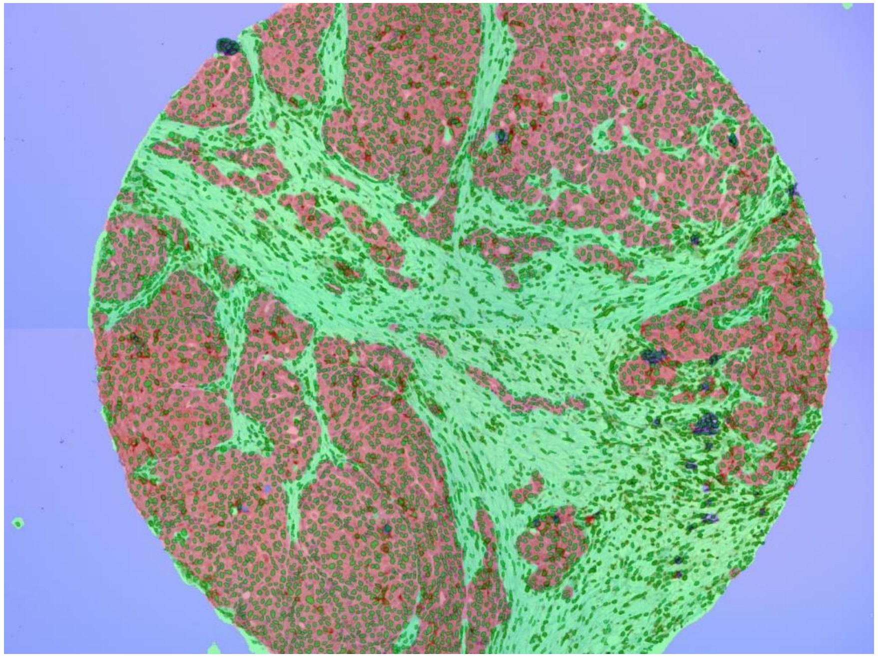


3. Segment image

Multispectral Imaging

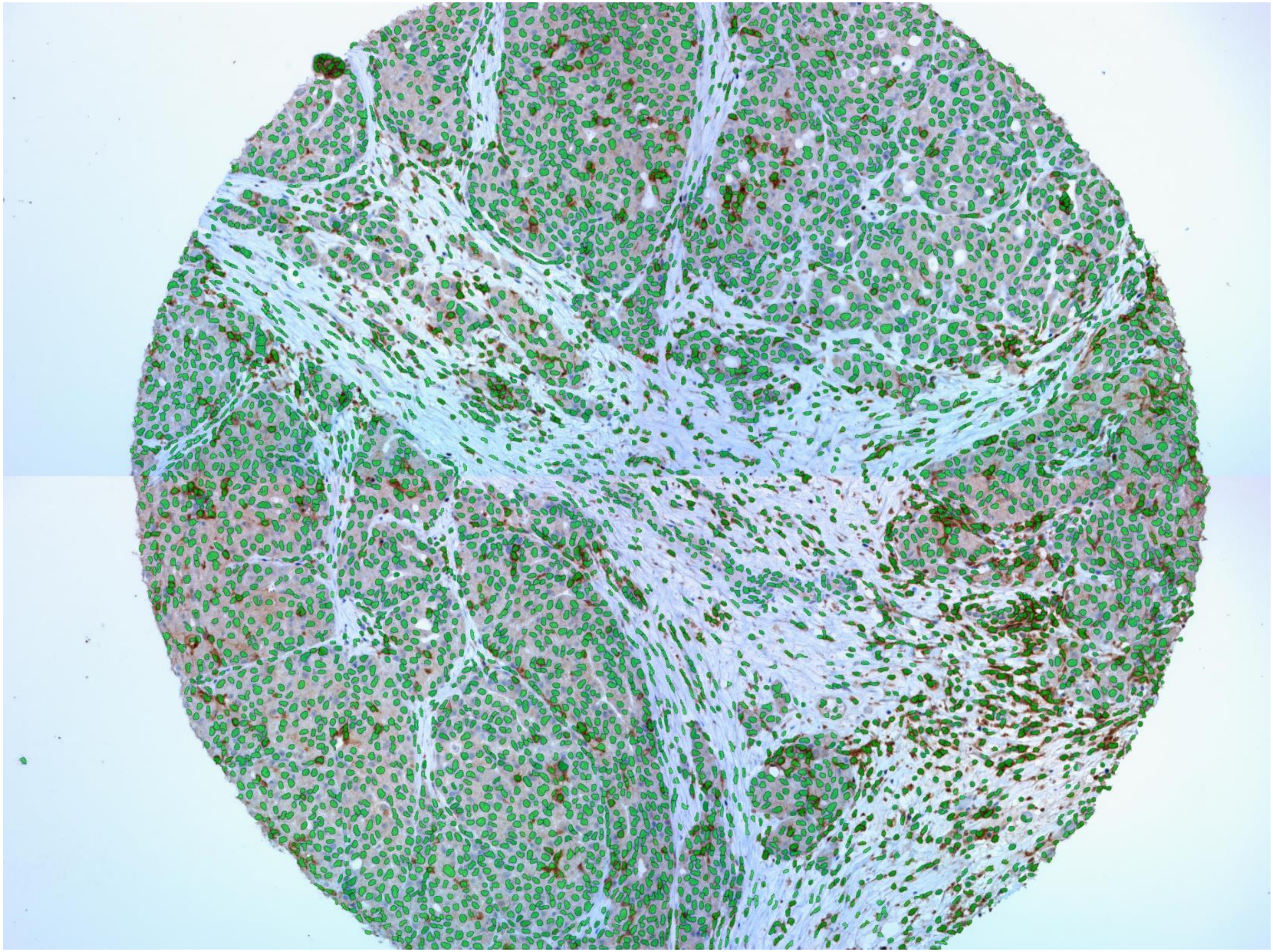


3. Segment image

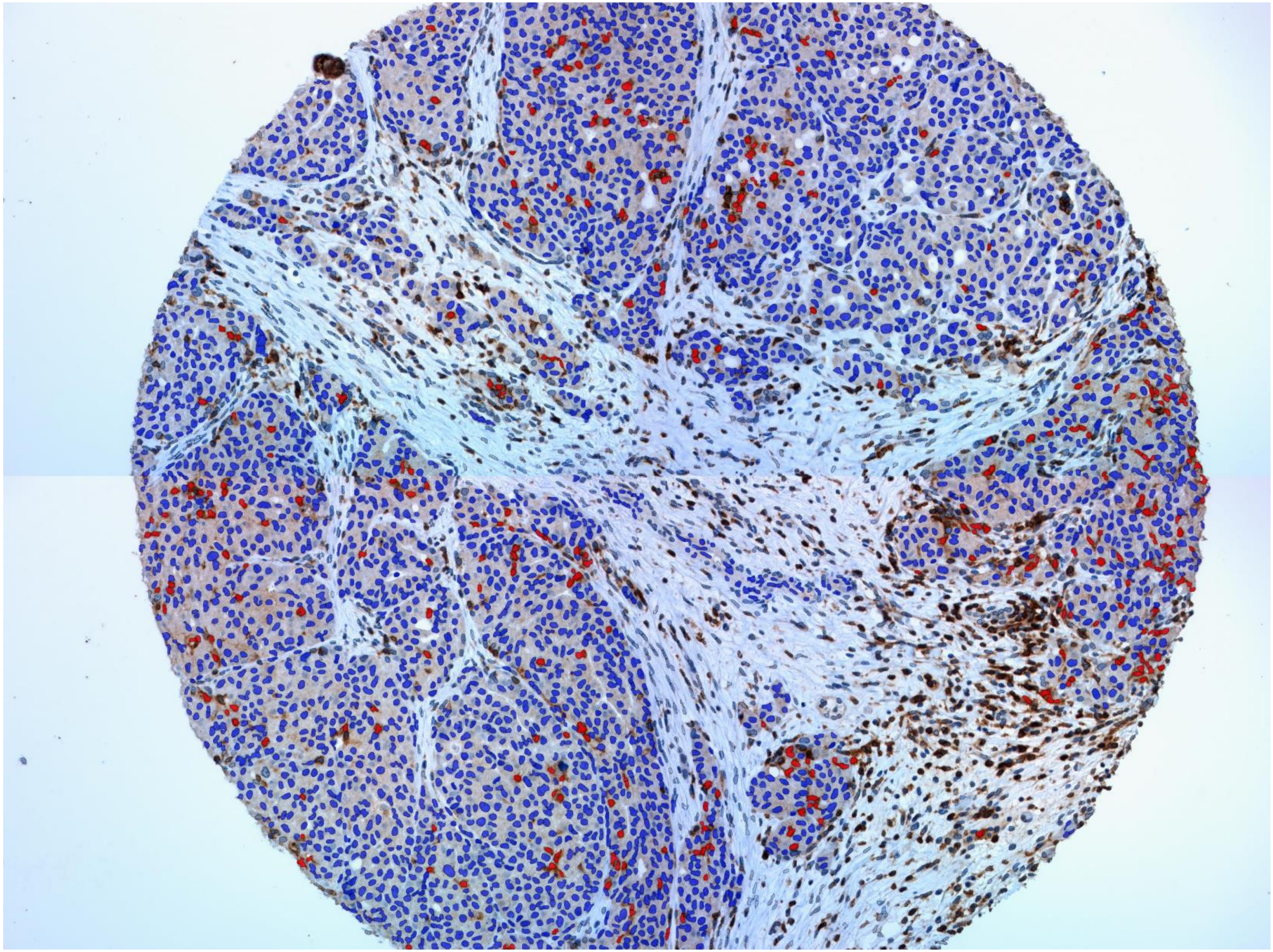


4. Segment cells (nuclei, cytoplasm, membrane)

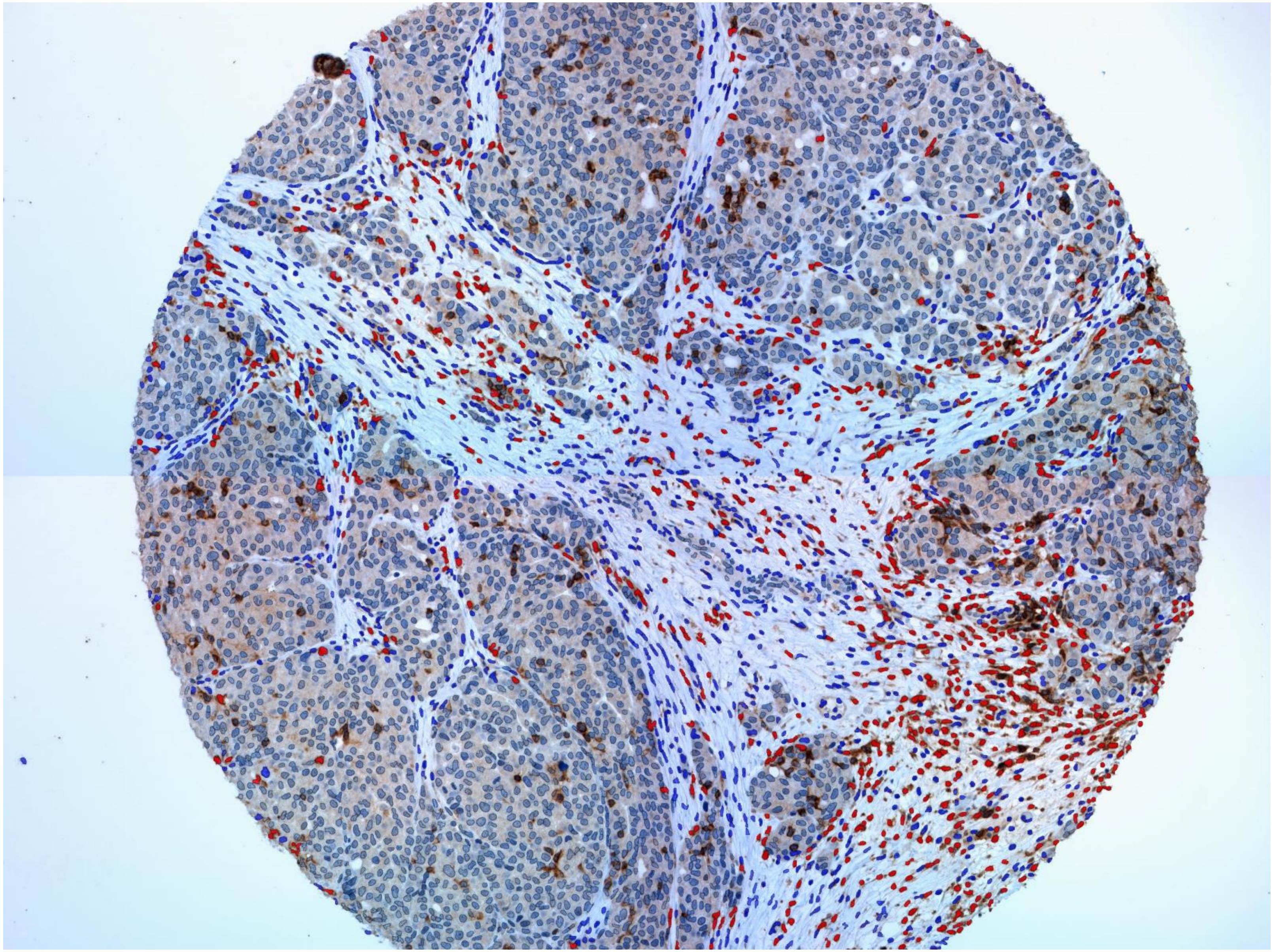
Multispectral Imaging



Multispectral Imaging



Multispectral Imaging



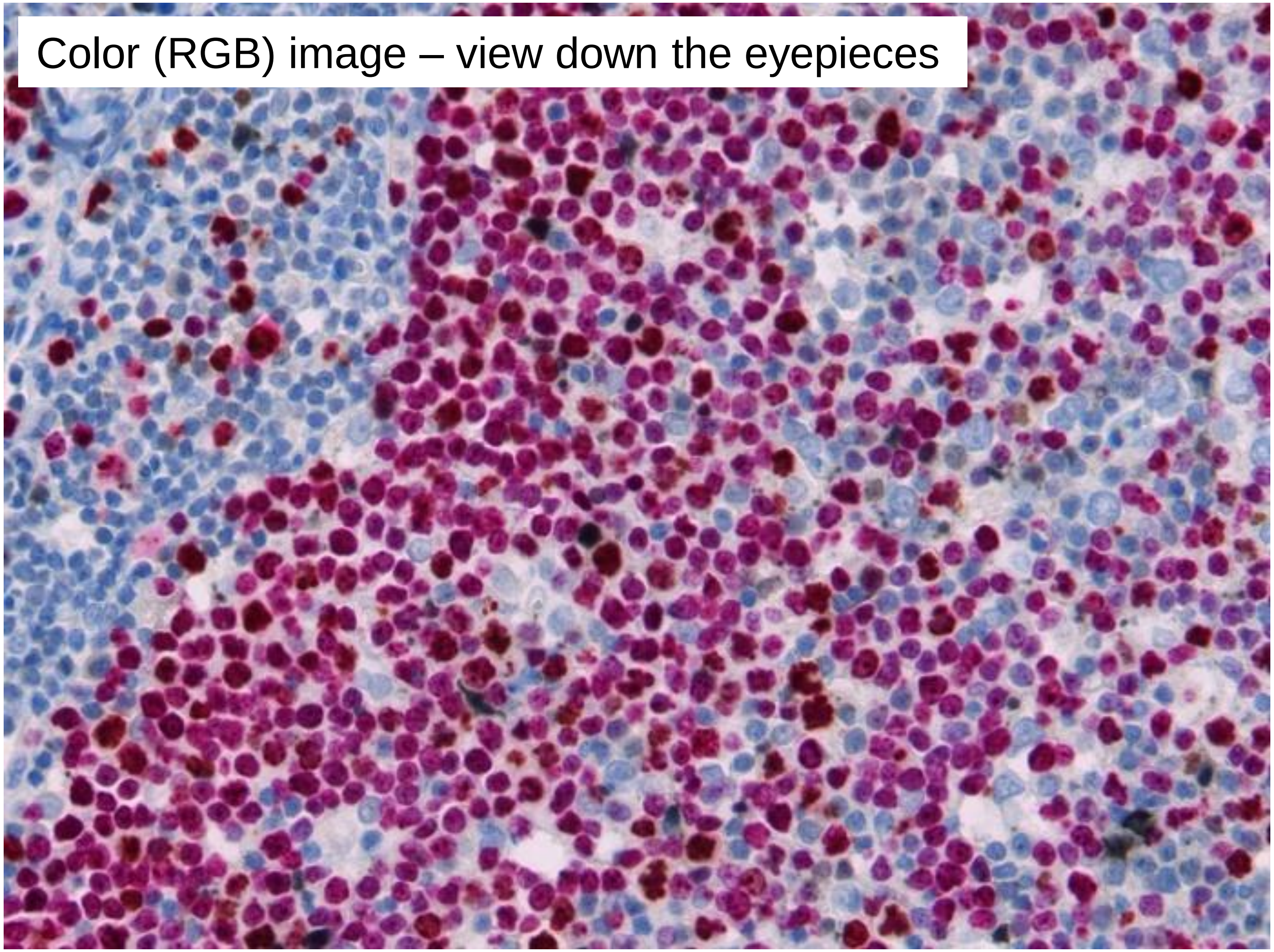
Separation and quantitation of 4 chromogens in brightfield

Hematoxylin: blue

Ki67: red
- Proliferation

pHH3: brown
- Phospho-histone 3
- Cell cycle marker

CC3: gray/black
- Cleaved caspase 3
- Apoptosis marker



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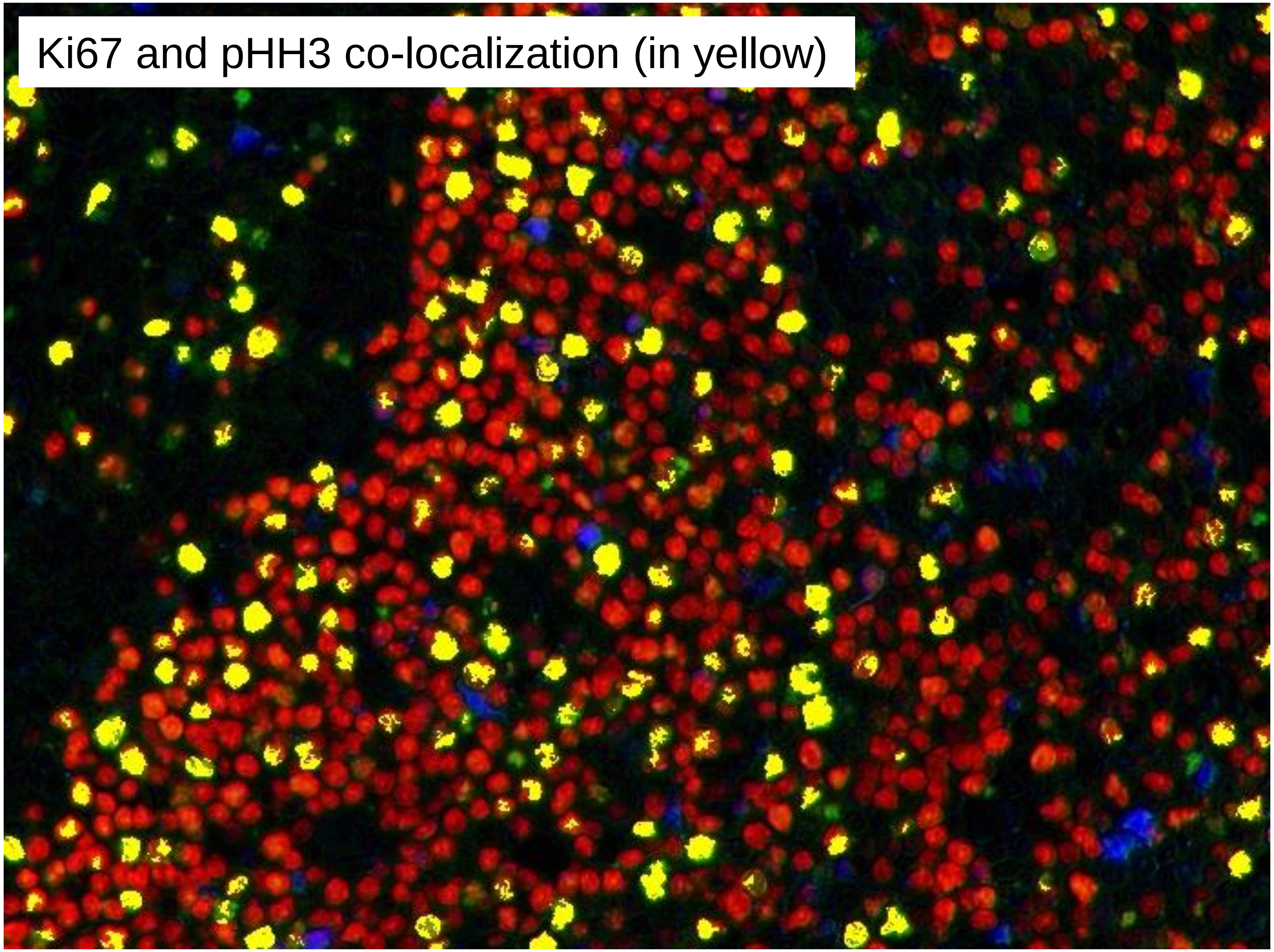

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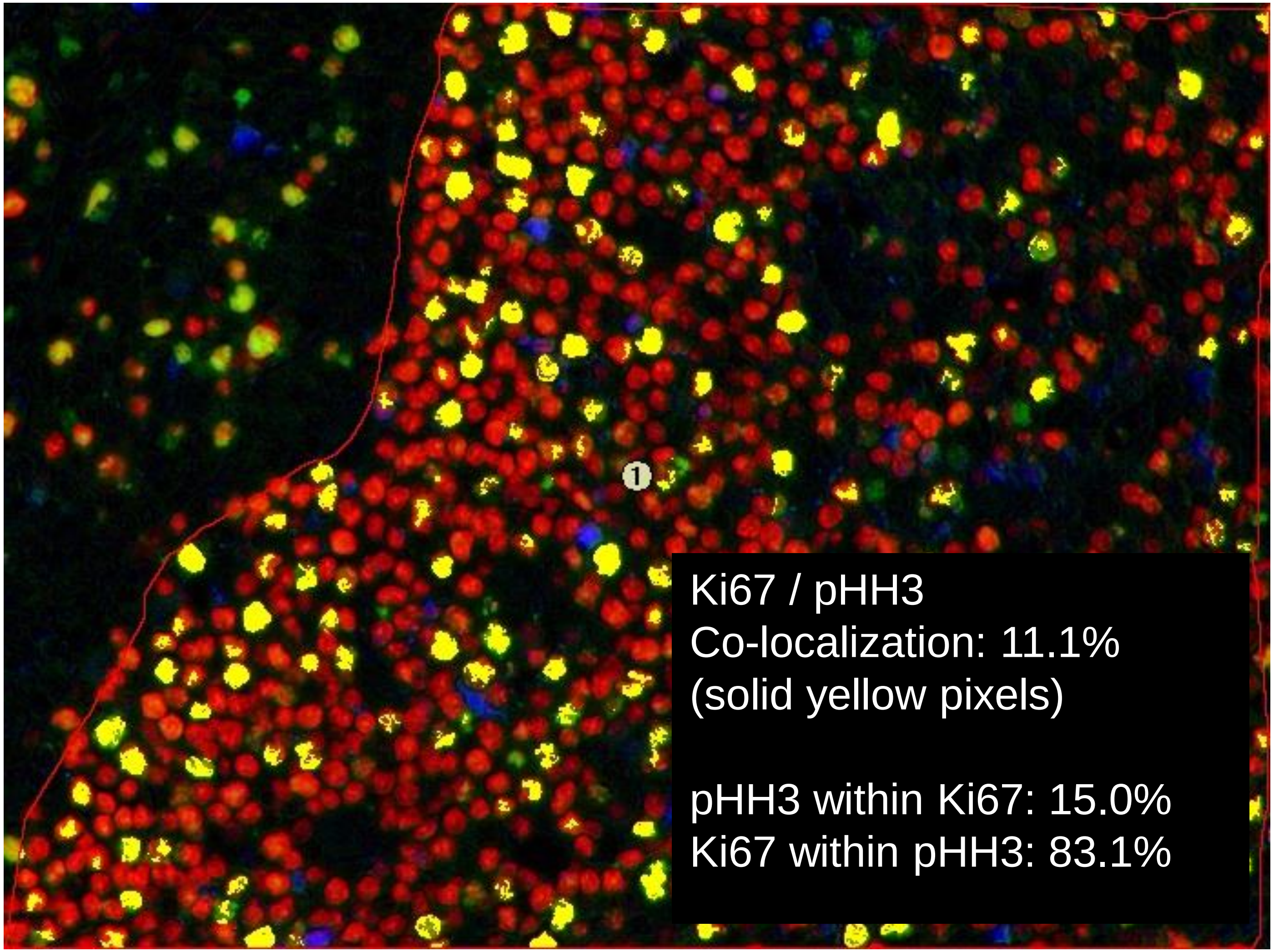
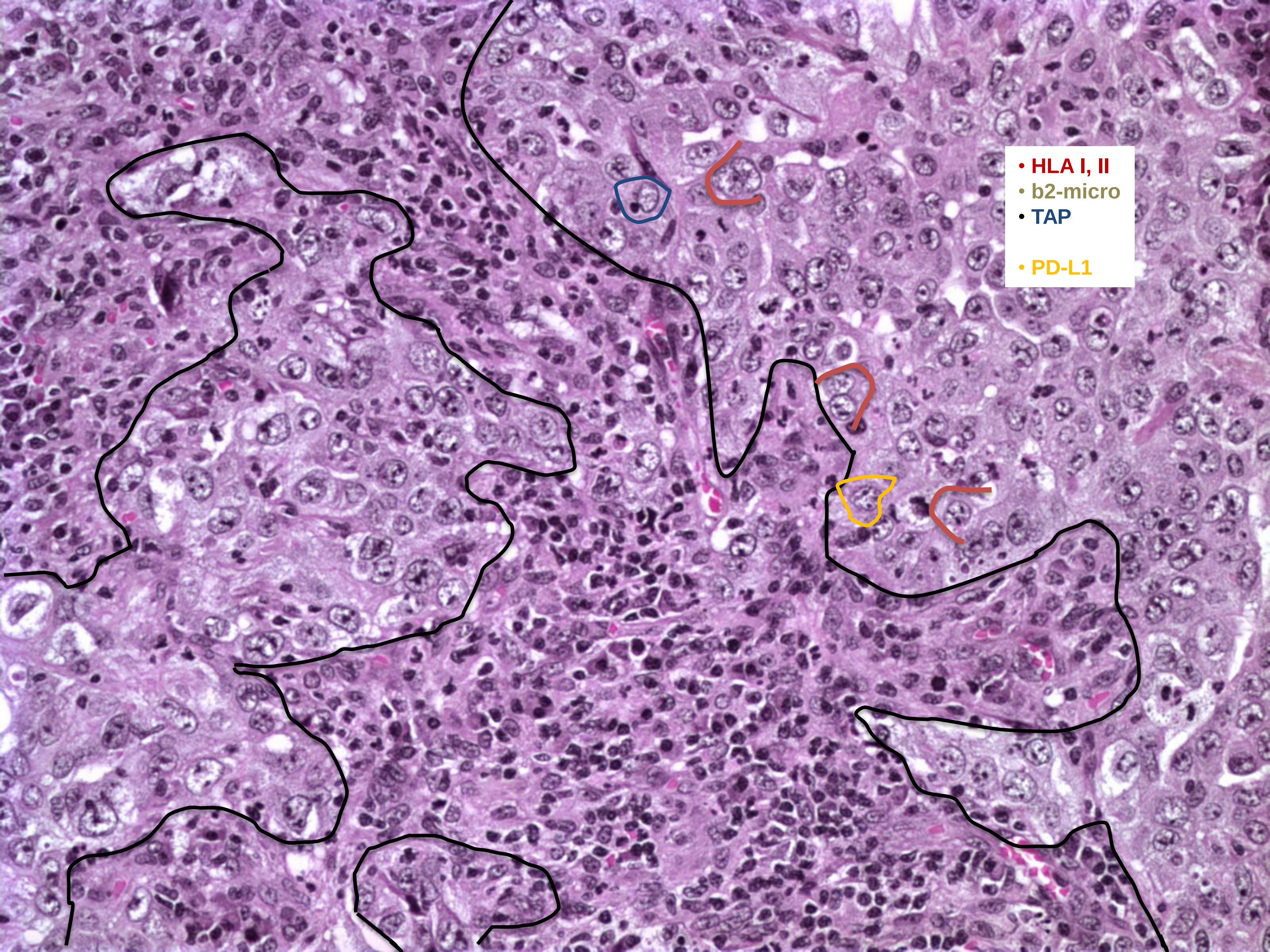
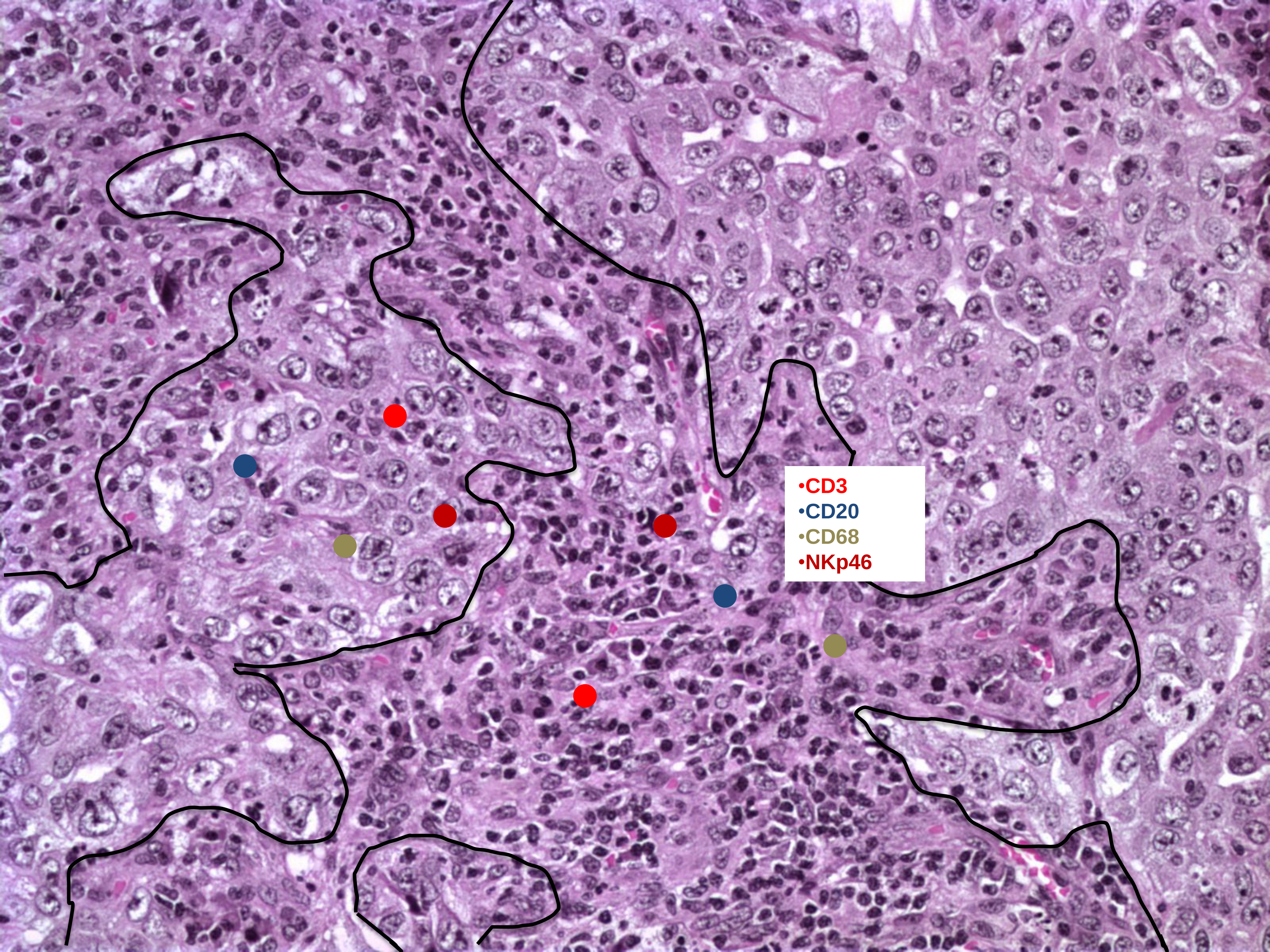


- antibody panels

- CD3, CD4, CD8, CD45RO (for T cell subpopulations and memory).
- Tbet, GATA3, IL17 and FoxP3 (for T cell polarization i.e. Th1, Th2, Th17 and Tregs).
- CD103 (intraepithelial resident memory T cells), E-cadherin (ligand for CD103), TCRγ (γδT cells), TCRβ (αβT cells).
- CD3, Ki67, CD137, Granzyme B, CTLA-4, PD-1, ICOS, CD38, HLADR (functional status of TILs).
- Tryptase, granulocytes (BM-2), CD68, CD163 (for innate immunity cells and M1-M2 macrophage polarization).
- CD20, CD138, CD33 (for B cells, plasma cells and MDSC).
- CD141/DNGR1 (CD141+ DCs), CD123 (plasmacytoid DCs), CD1a (Langerhans cells), CD83, CD86 (mature DCs)
- NKp46, CD137, NKG2D, KLRG1 (presence and activity of NK cells).
- Cytokeratin, CD45, TAP1, TAP2, tapasin, β2-microglobulin, HLA-I, HLA-II (antigen presentation by tumor cells)
- Cytokeratin, PD-L1, PD-L2, IDO-1, CD39, CD73, COX1, COX2, TGF-b, IL-10 (immunosuppressive factors by tumor cells)
- PD-1, LAG-3, TIM-3, ICOS, CD160 (inhibitory receptors on T cells)
- Calreticulin and HMGB1, elF2a (immunogenic cell death, ER stress), Beclin-1 (autophagy),
- Ki67, cleaved caspase 3 (cell kinetics)
- CD31, CD34, D2-40, CD105, VCAM-1, Ang2 (Angiogenesis, lymphangiogenesis)
- γH2AX, Chk2, pATM, p53, BRCA1, Rad51 (DDR, HR)
- a-SMA, FAP (cancer associated fibroblasts)

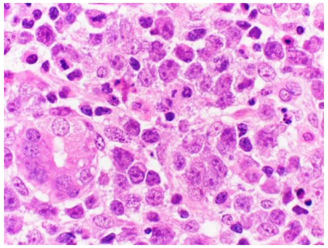

• HLA I, II
• b2-micro
• TAP
• PD-L1

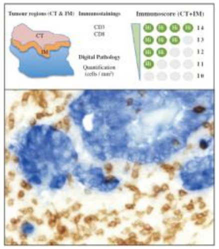


•CD3
•CD20
•CD68
•NKp46

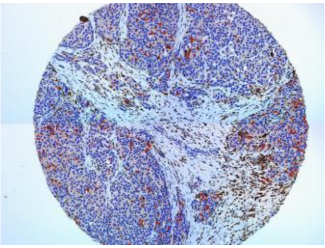
ESMO Preceptorship, 20 November 2014
Session 7: Immunomonitoring

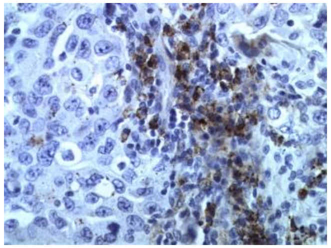
Tumor infiltrating immune cells as biomarkers with prognostic/predictive value





Immune contexture		
Type	CD3, CD8, CD45RO	Immunoscore
Density	Cells/mm^2	
Location	Centre, Margin	
Orientation	Th1, cytotoxic, chemokines, adhesion	





Thank you

Periklis Foukas, MD, PhD
CTE, DO
CHUV, UNIL