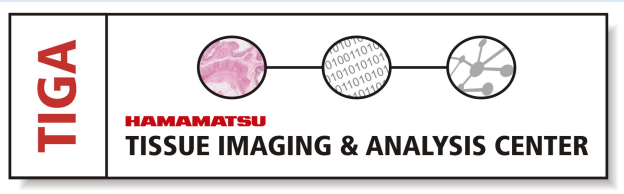
Applications for Virtual Microscopy in the quantitative analysis of the immunological tumor microenvironment

Niels Halama



Part I

„How many birds are in the forest and where are they?“

Immune Cells in Colorectal Cancer

Previous Studies

Intraepithelial CD8$^+$ T-cell-count becomes a prognostic factor after a longer follow-up period in human colorectal carcinoma: possible association with suppression of micrometastasis

T Chiba[1,2], H Ohtani[*,2,3], T Mizoi[4], Y Naito[2], E Sato[2], H Nagura[2], A Ohuchi[5], K Ohuchi[6], K Shiiba[4], Y Kurokawa[1] and S Satomi[1]

[1]Division of Advanced Surgical Science and Technology, Tohoku University Graduate School of Medicine, Sendai, Japan; [2]Department of Pathology, Tohoku University Graduate School of Medicine, Sendai, Japan; [3]Department of Pathology, Mito Medical Center, Ibaraki, Japan; [4]Division of Biological Regulation and Oncology, Department of Surgery, Tohoku University Graduate School of Medicine, Sendai, Japan; [5]Department of Surgery, Tohoku Rosai Hospital, Sendai, Japan; [6]Department of Surgery, Miyagi Cancer Center, Natori, Japan

British Journal of Cancer (2004) 91, 1711–1717

Prognostic Role of CD8+ Tumor-Infiltrating Lymphocytes in Stage III Colorectal Cancer With and Without Microsatellite Instability

FRIEDRICH PRALL, MD, THOMAS DÜHRKOP, VOLKER WEIRICH, MD, CHRISTIANE OSTWALD, PHD, PETER LENZ, MD, HORST NIZZE, MD, AND MALTE BARTEN, MD

HUMAN PATHOLOGY Volume 35, No. 7 (July 2004)

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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†]

The role of the adaptive immune response in controlling the growth and recurrence of human tumors has been controversial. We characterized the tumor-infiltrating immune cells in large cohorts of human colorectal cancers by gene expression profiling and in situ immunohistochemical staining. Collectively, the immunological data (the type, density, and location of immune cells within the tumor samples) were found to be a better predictor of patient survival than the histopathological methods currently used to stage colorectal cancer. The results were validated in two additional patient populations. These data support the hypothesis that the adaptive immune response influences the behavior of human tumors. In situ analysis of tumor-infiltrating immune cells may therefore be a valuable prognostic tool in the treatment of colorectal cancer and possibly other malignancies.

Science 2006

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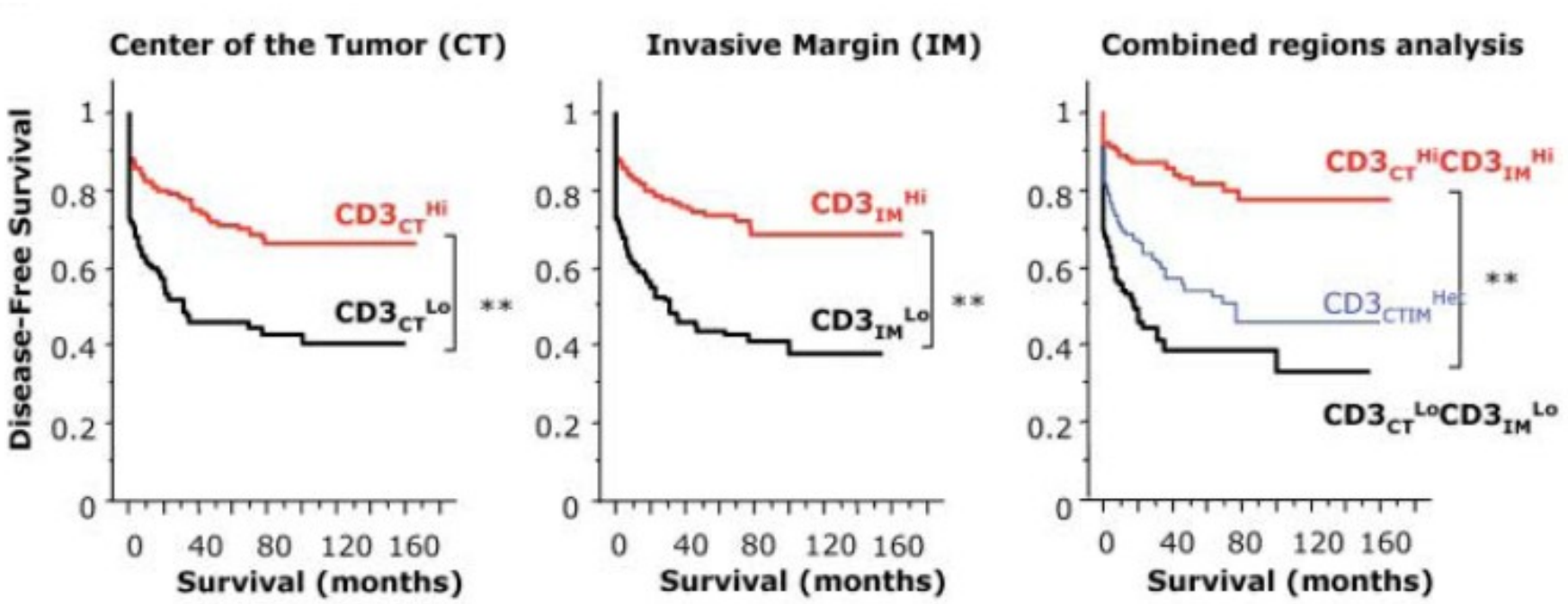
Immunohistochemistry

IHC: CD3, CD8, Granzyme B and CD45RO



Galon et al. in Science 2006

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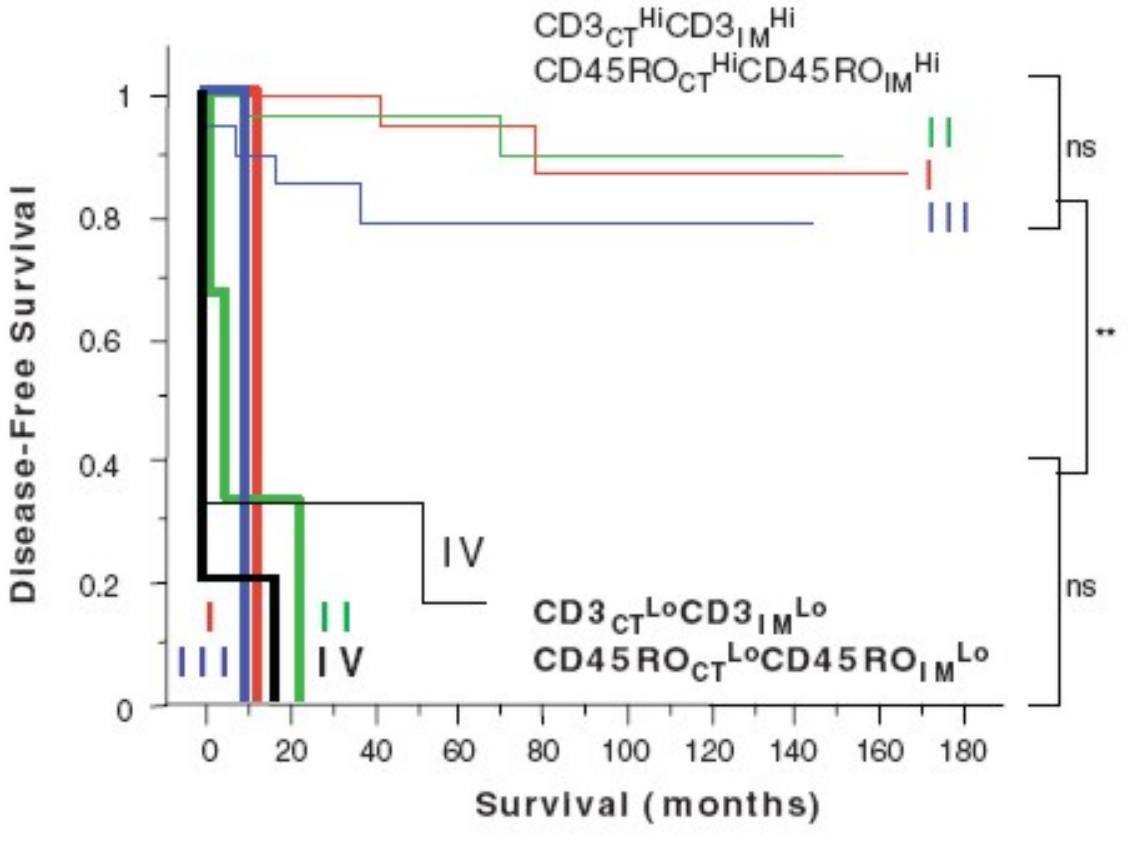
Clinical Impact of Immune Infiltration

UICC-TNM stadium as reference

$CD3_{CT}CD3_{IM}$ evaluation

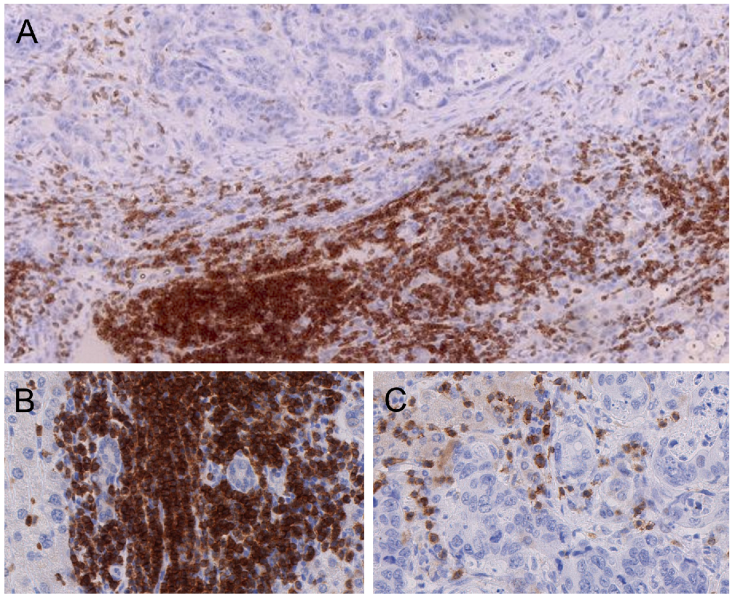
plus

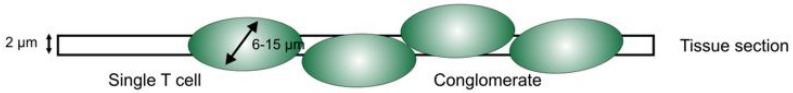
$CD45RO_{CT}CD45RO_{IM}$ evaluation

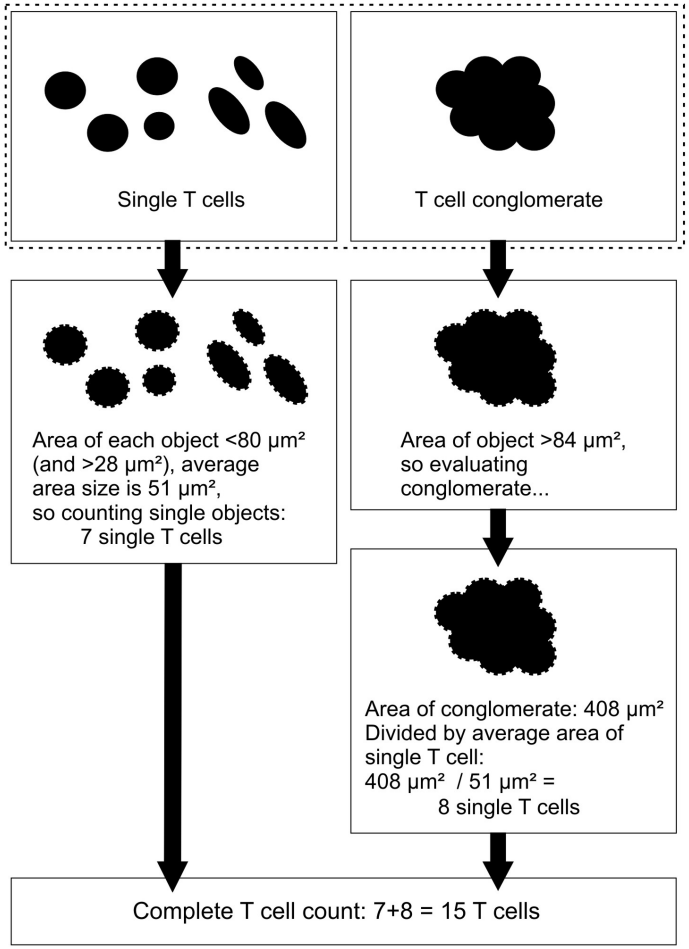


Galon et al. in Science 2006

Quantification of Immune Cells (I)

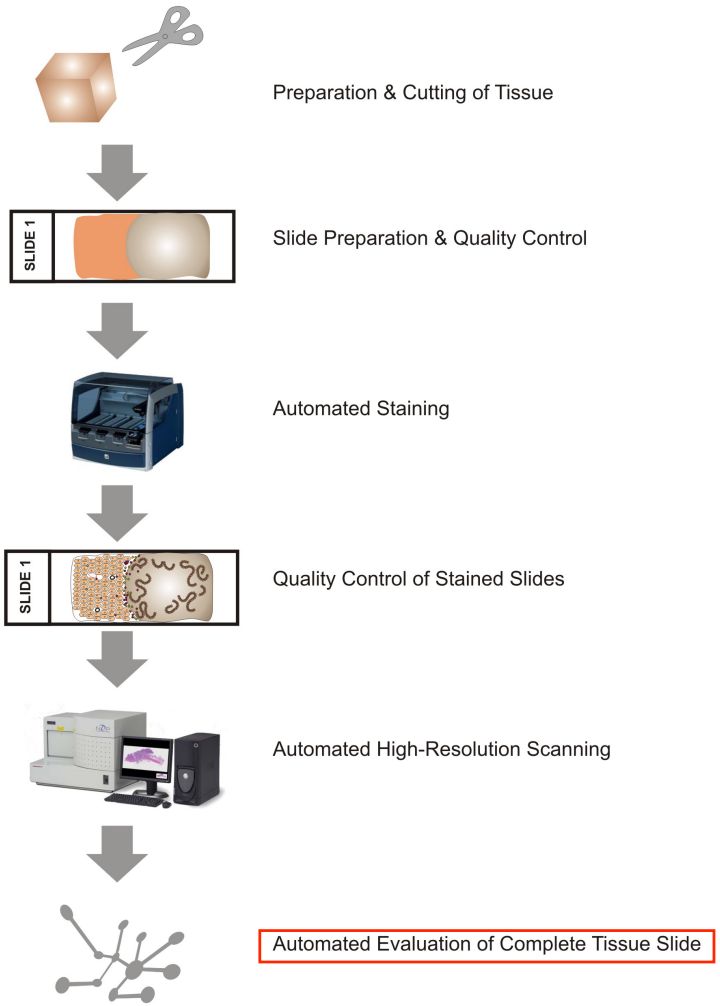




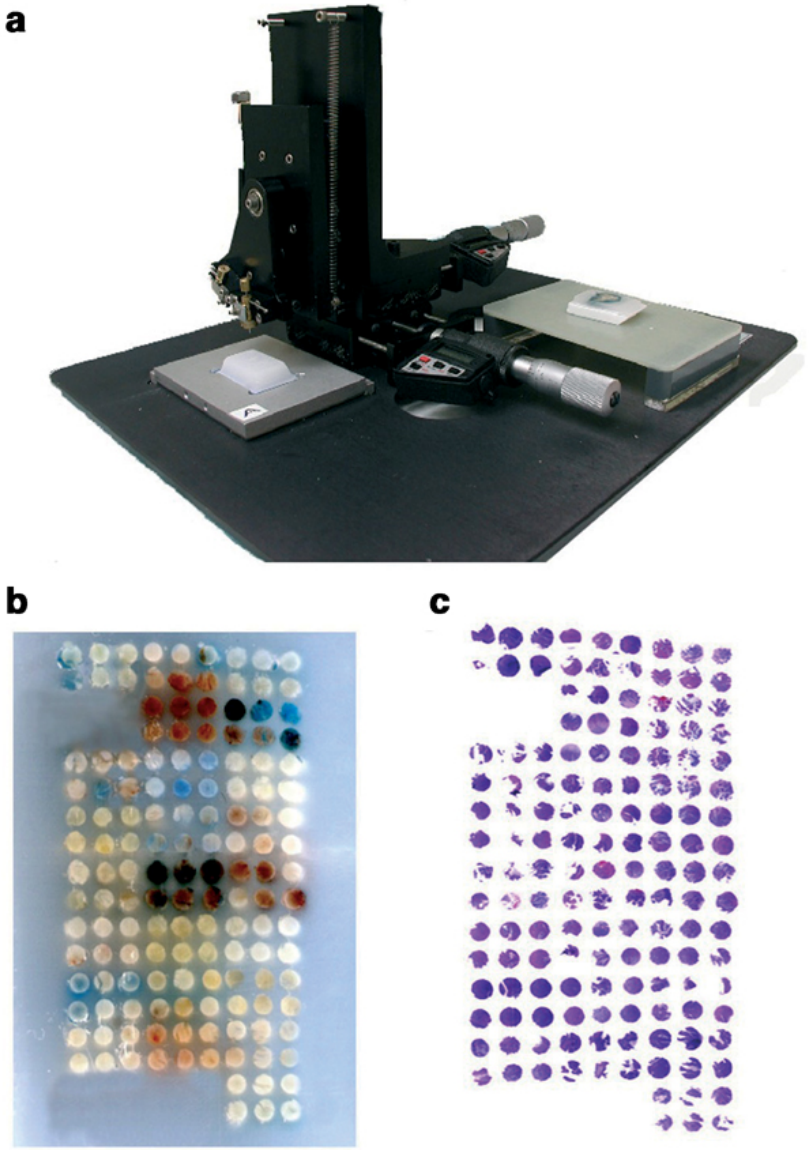


Quantification of Immune Cells (II)

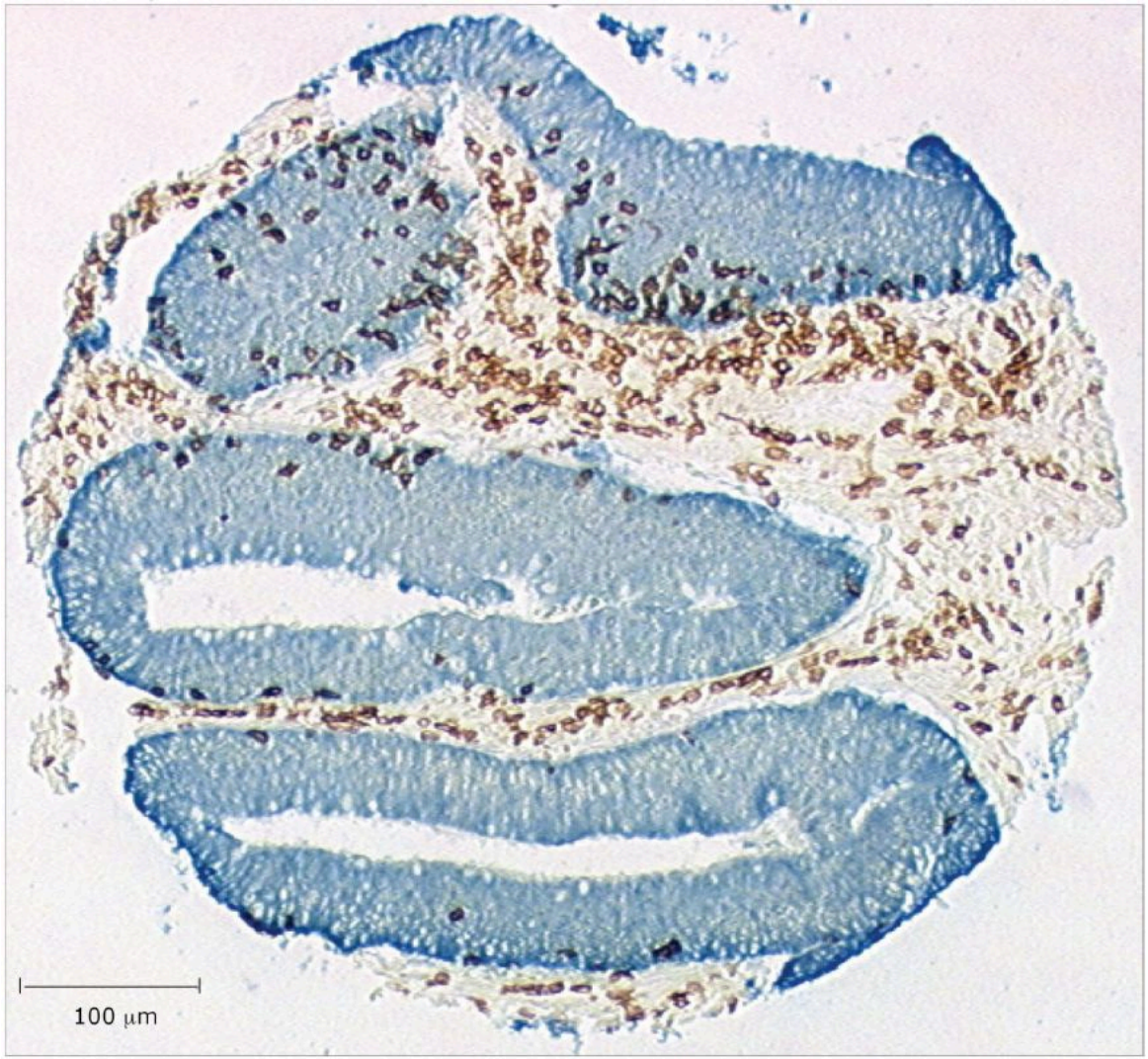
Workflow of Standardized Tissue Evaluation



Tissue Microarray (TMA) Technology

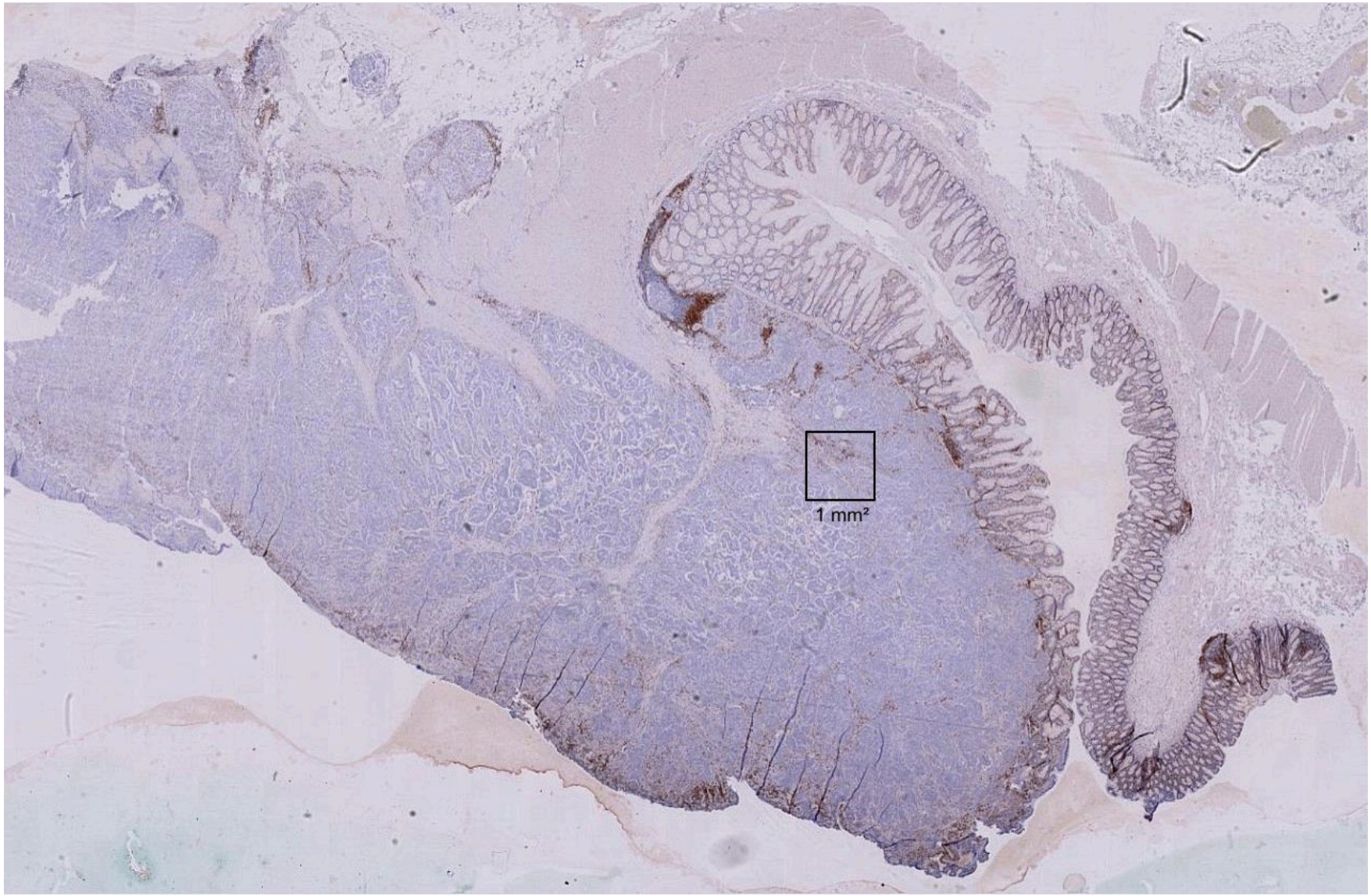


Tissue Microarray on large sample numbers

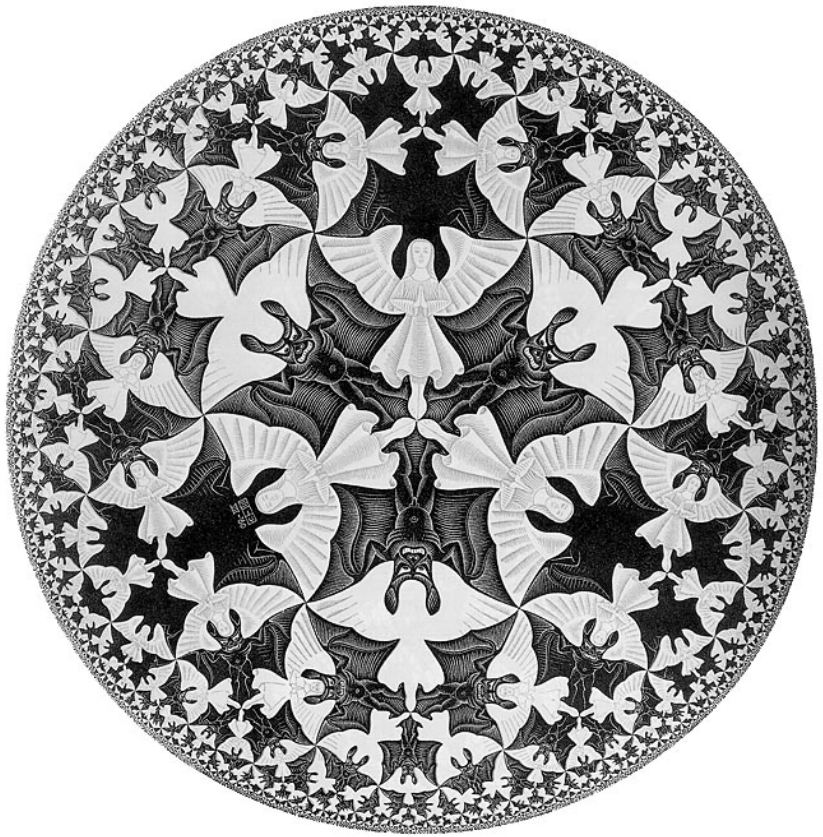


CD3

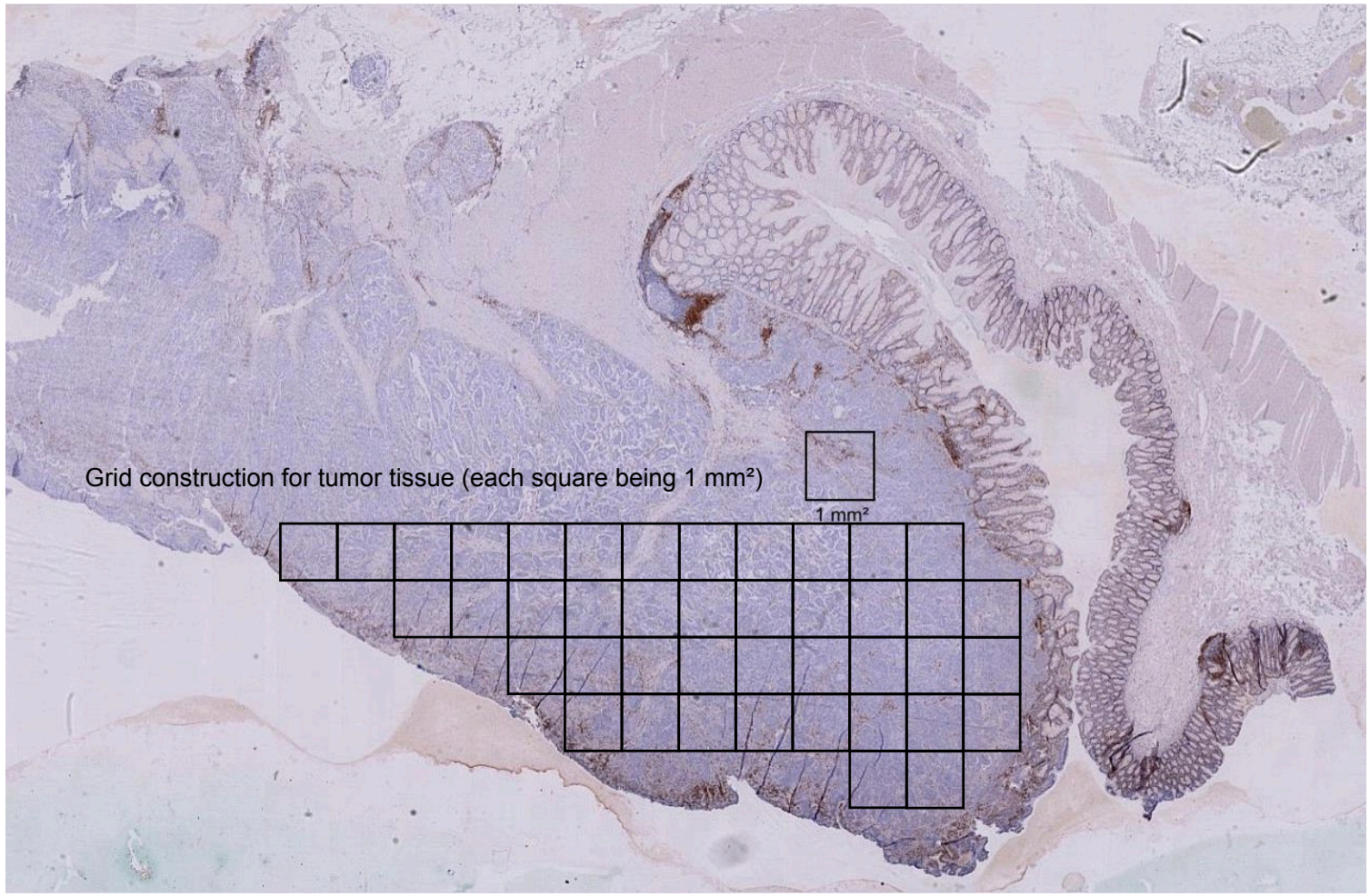
Tissue Microarray Platform



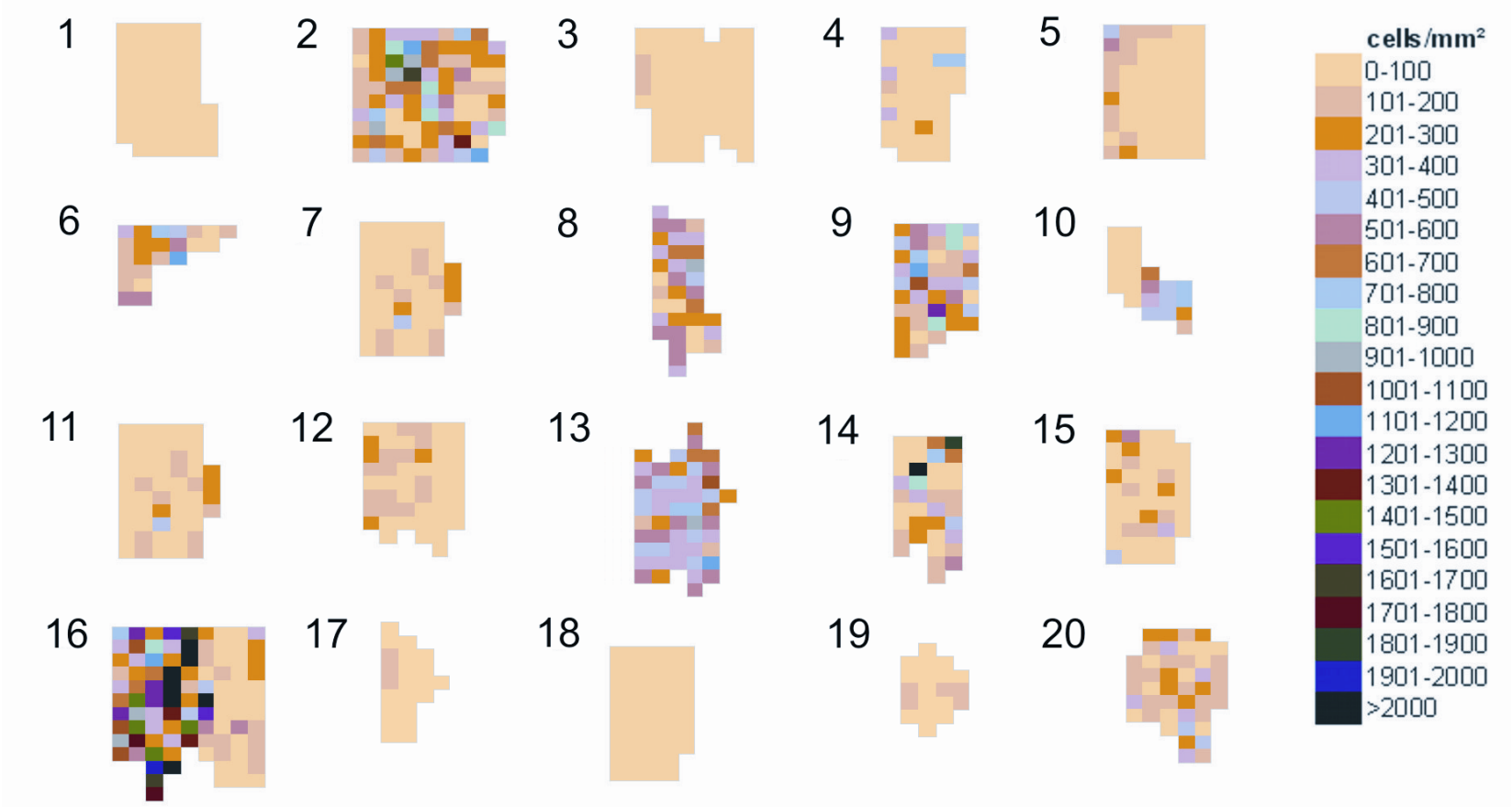
Selecting a representative region…?



Immunological Tumor Maps



Immunological tumor maps: Colorectal Cancer



CD3+ T Cell densities, primary CRC, no MSI

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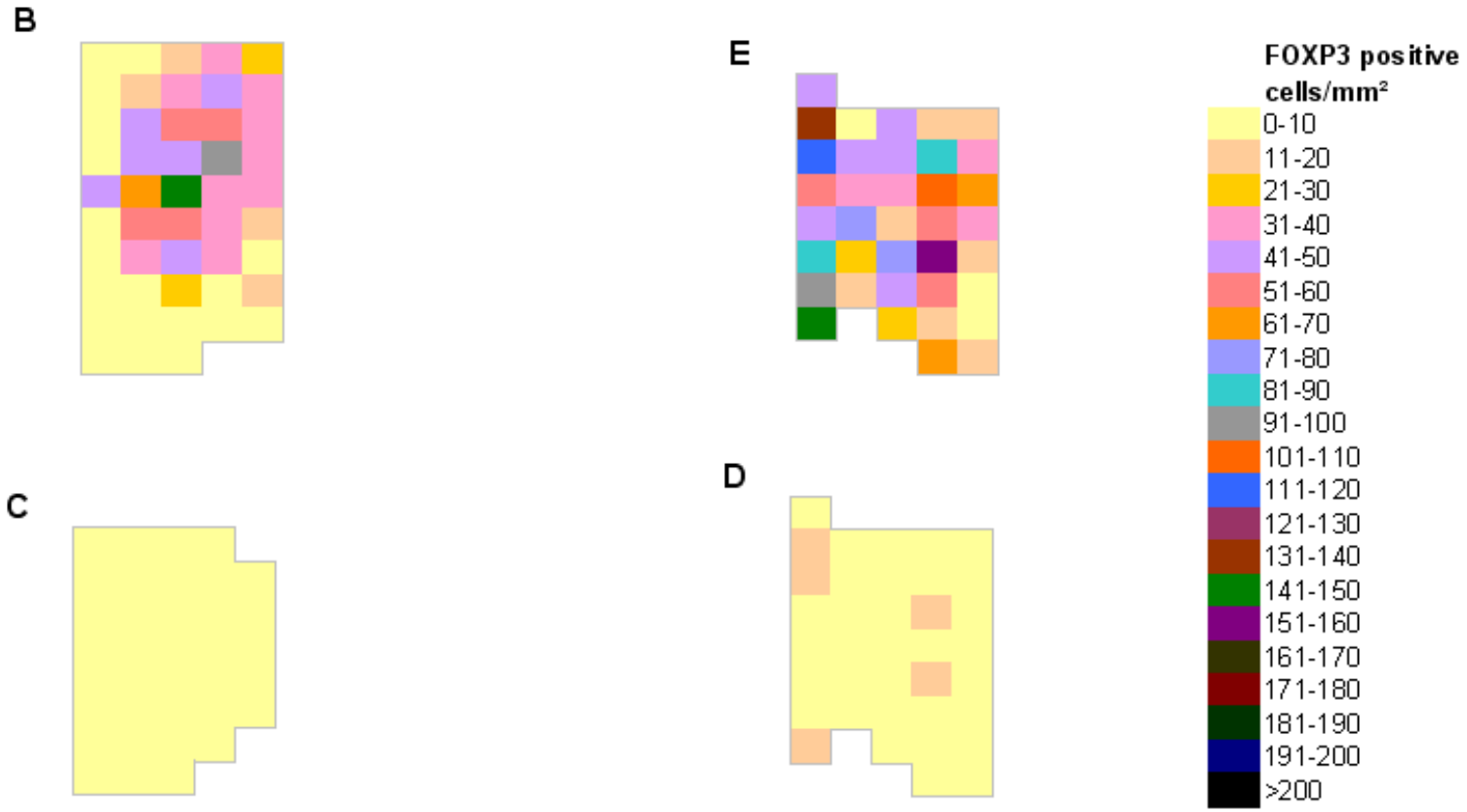
Immunological tumor maps: Breast Cancer



FOXP3+ Treg Cell densities, primary BrCa, all subtypes (ER+,PR+, HER2/neu+, etc.)

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Where is the Tissue Microarray…?

Journal of Pathology
J Pathol 2006; **208**: 607–614
Published online 24 January 2006 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/path.1934

Original Paper

A new approach to the validation of tissue microarrays

L Goethals,[1]* C Perneel,[2] A Debucquoy,[1] H De Schutter,[1] D Borghys,[2] N Ectors,[3] K Geboes,[3] WH McBride[4] and KM Haustermans[1]

[1]*Department of Radiation Oncology, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium*
[2]*Department of Applied Mathematics, Royal Military Academy, Av de la Renaissance 30, B-1000 Brussels, Belgium*
[3]*Department of Pathology, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium*
[4]*Department of Radiation Oncology, David Geffen school of Medicine, UCLA, Los Angeles, USA*

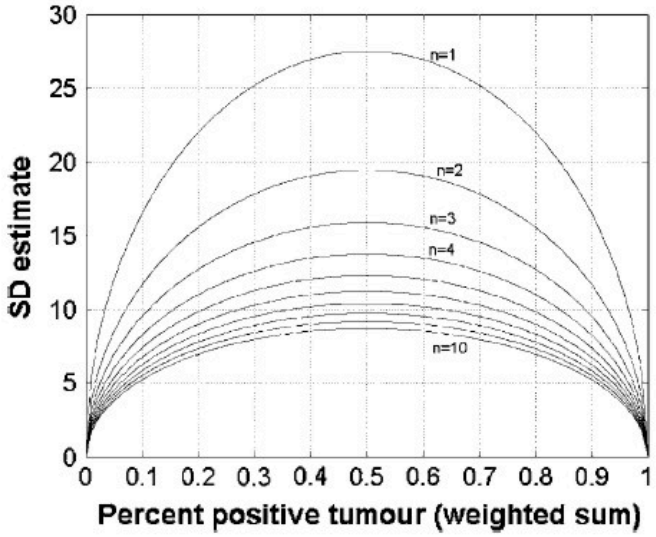

Figure 5. Standard error in function of the mean percentage positivity for a number of punches varying between 1 and 10

The fitted model for the number of cores varying between 1 and 10 is represented in Figure 5. All curves are defined by the same function with constant value 55 ($\sigma = 55\sqrt{\{[\text{mean}\ (1 - \text{mean})]/n\}}$; this constant is derived from the data represented in Table 2). Each curve represents a different number of hypothetical core biopsies that has been taken. So, when the number of core biopsies is high, the SD decreases. From this graph (as from Table 2) it is possible to conclude that the maximal spread (highest SD) is seen around 50% positivity and this decreases for values closer to 0% and 100%. The theoretical variation coefficient for the worst-case situation (50% positivity) with only one punch is 55%, decreasing to 17% using ten punches. Based on the law of diminishing returns, at some point increasing the number of cores will reduce the variability by such a small amount that the cost of the additional core is not worthwhile. Based on these results, we advise taking four or five punches where possible, keeping in mind that even with this number the SD remains substantial.

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Tissue Microarrays are efficient…

…when the investigated object has a homogeneous distribution.

…when large sample numbers are available to make general investigations.

Virtual Microscopy on Whole Slide Images is efficient…

..when distributions or patterns in tissues have to be investigated.

…when individual analyses have to be performed („individualized medicine“).

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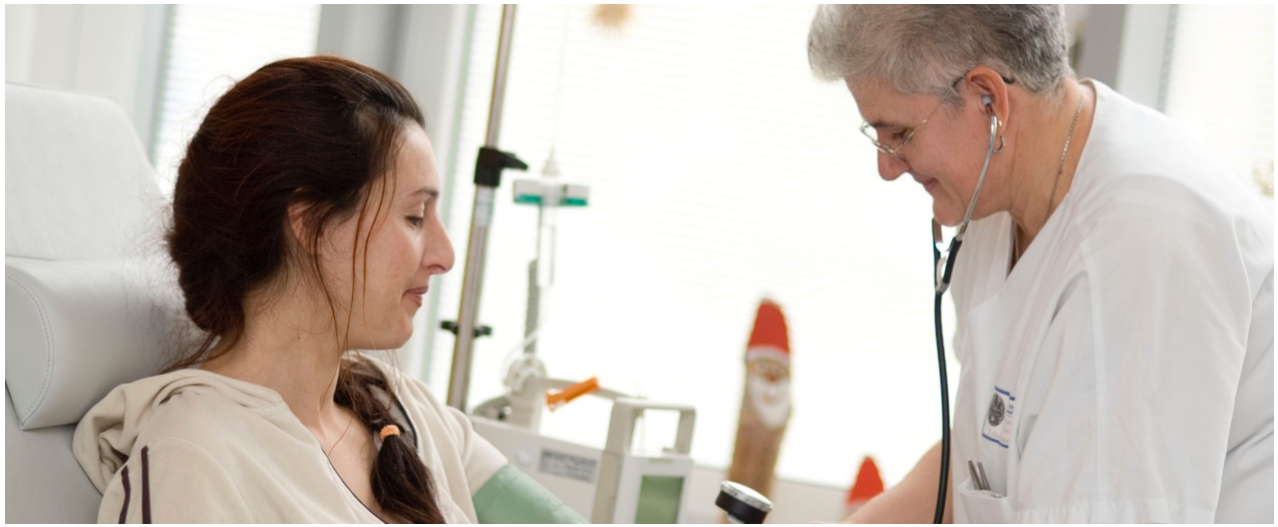
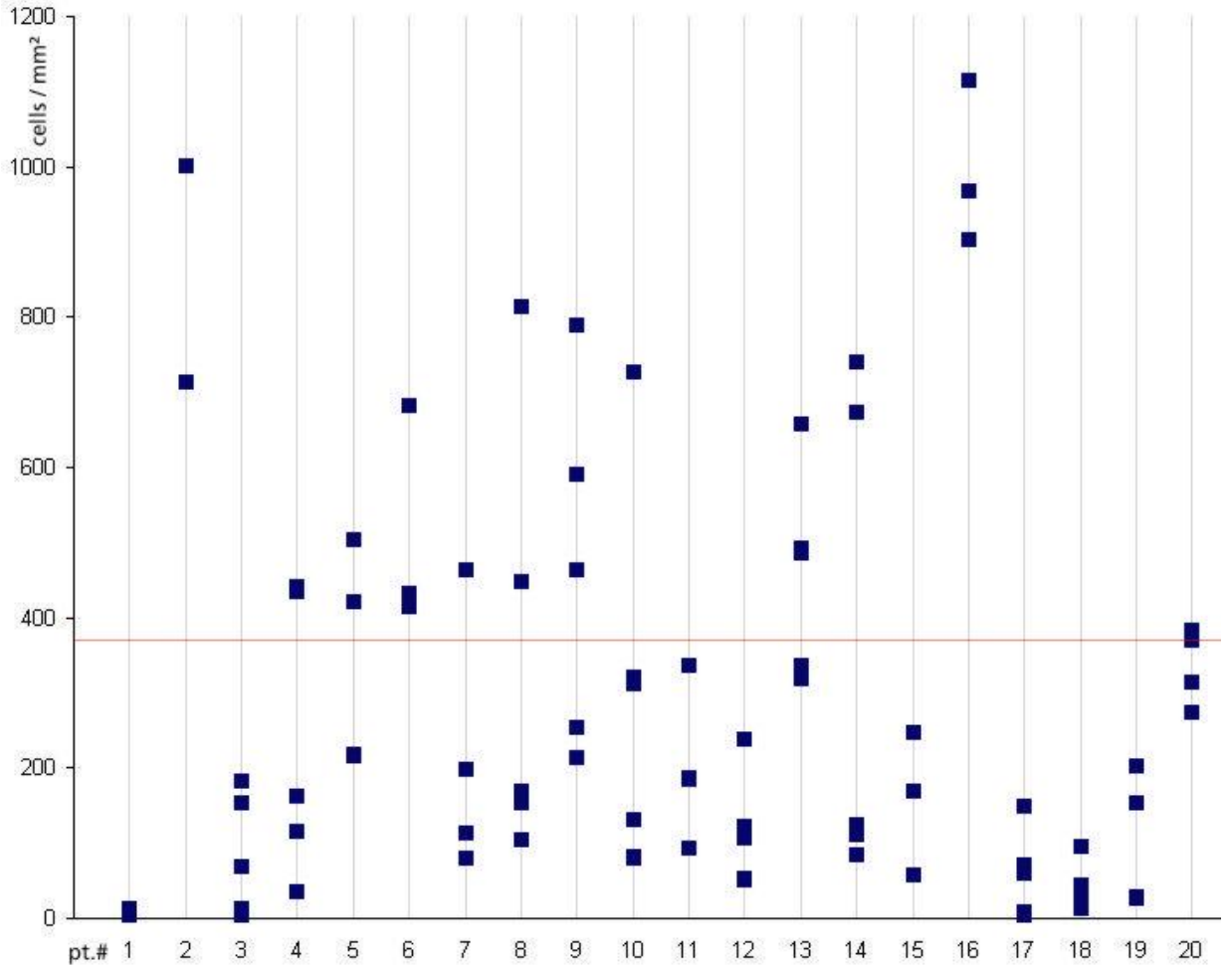


What about the individual patient?

Distribution of immune cells within the tumor: primary CRC

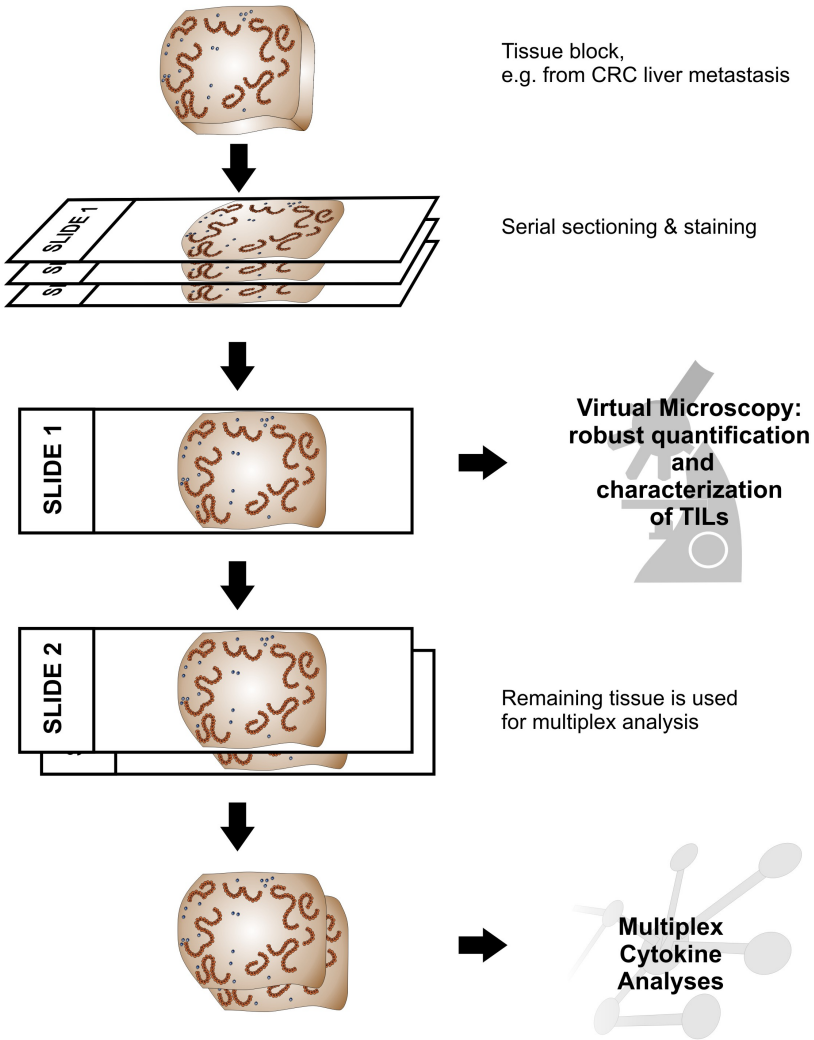


CD3 staining (5 fields of 1mm²)

Part II

„What else is there in the forest?“

Combining Virtual Microscopy with Multiplex Bead Protein Measurements



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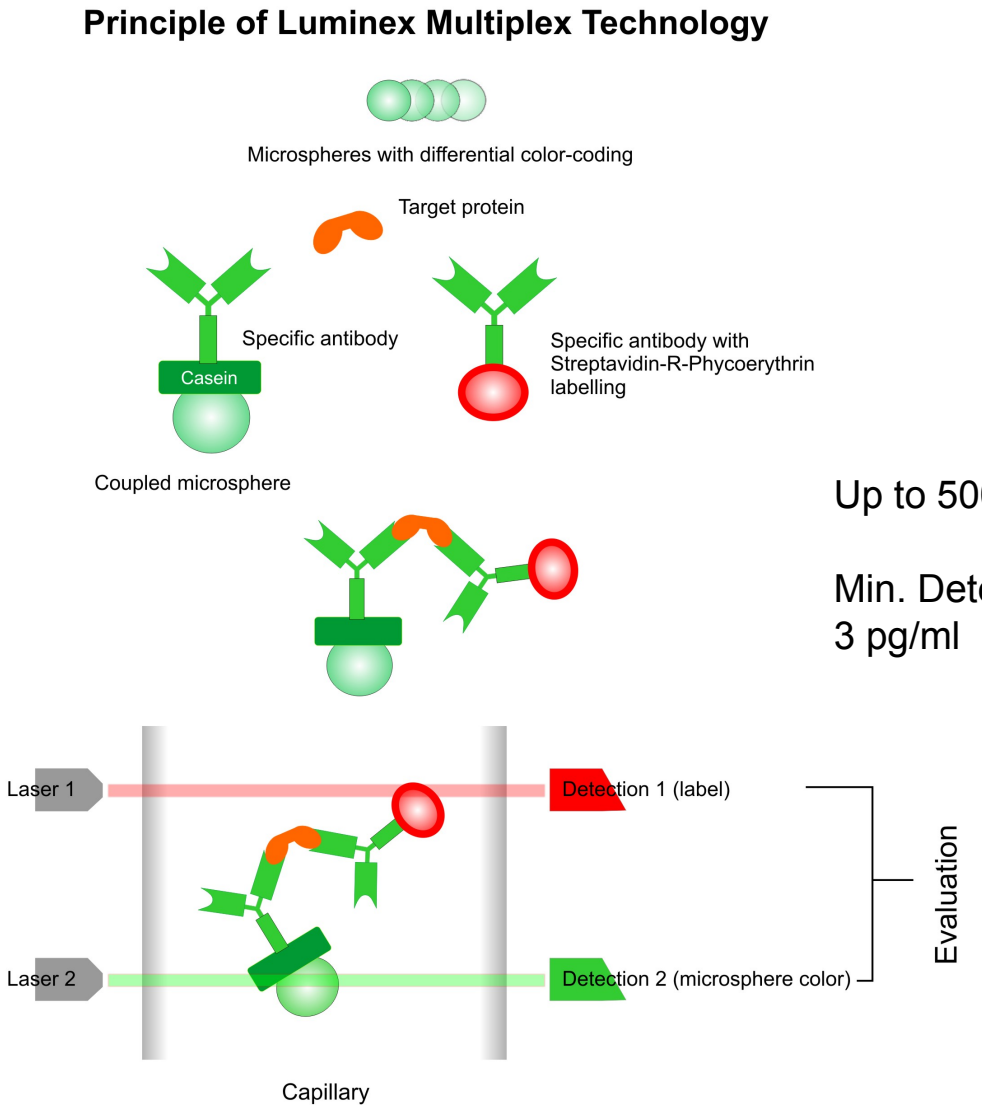
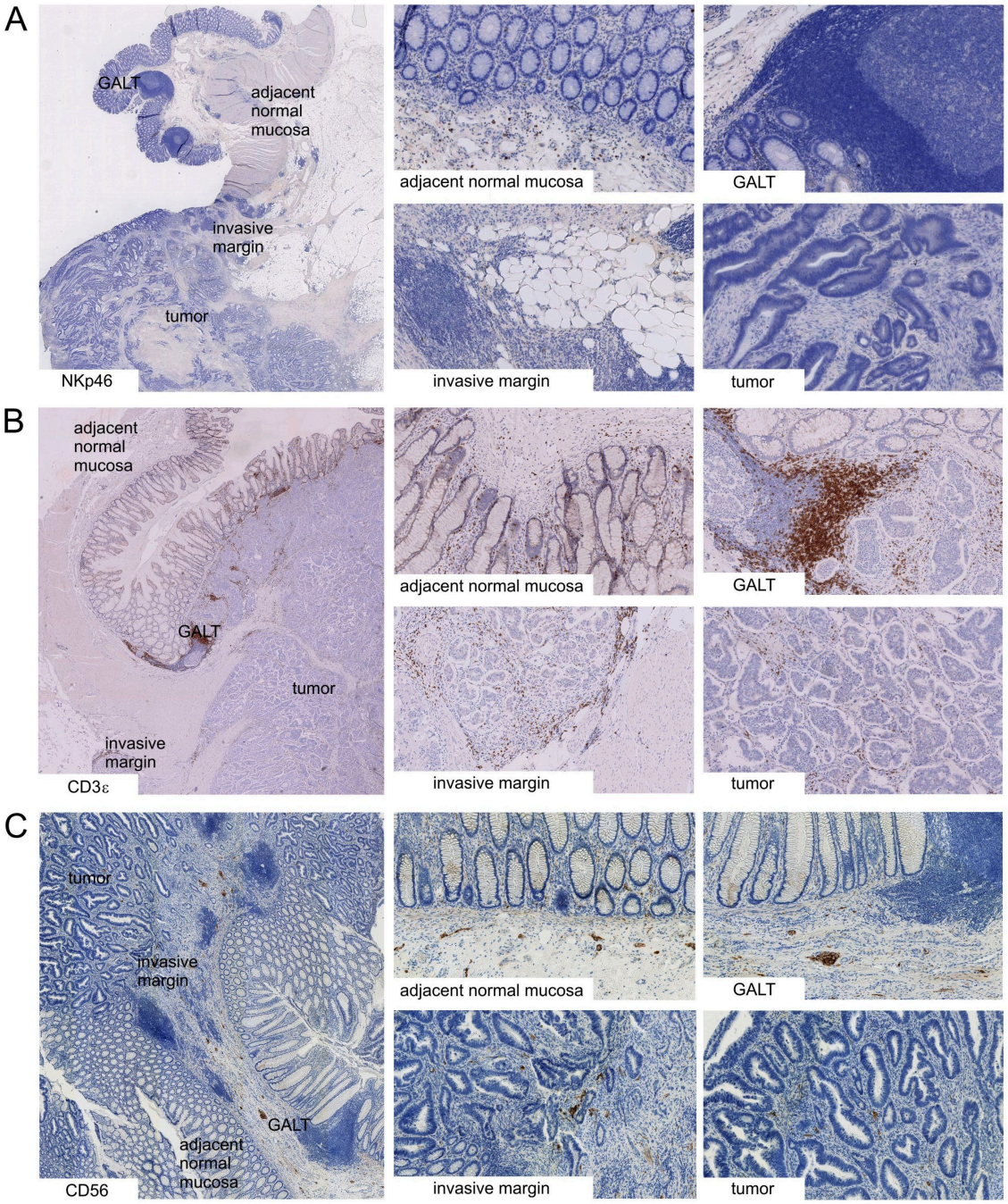
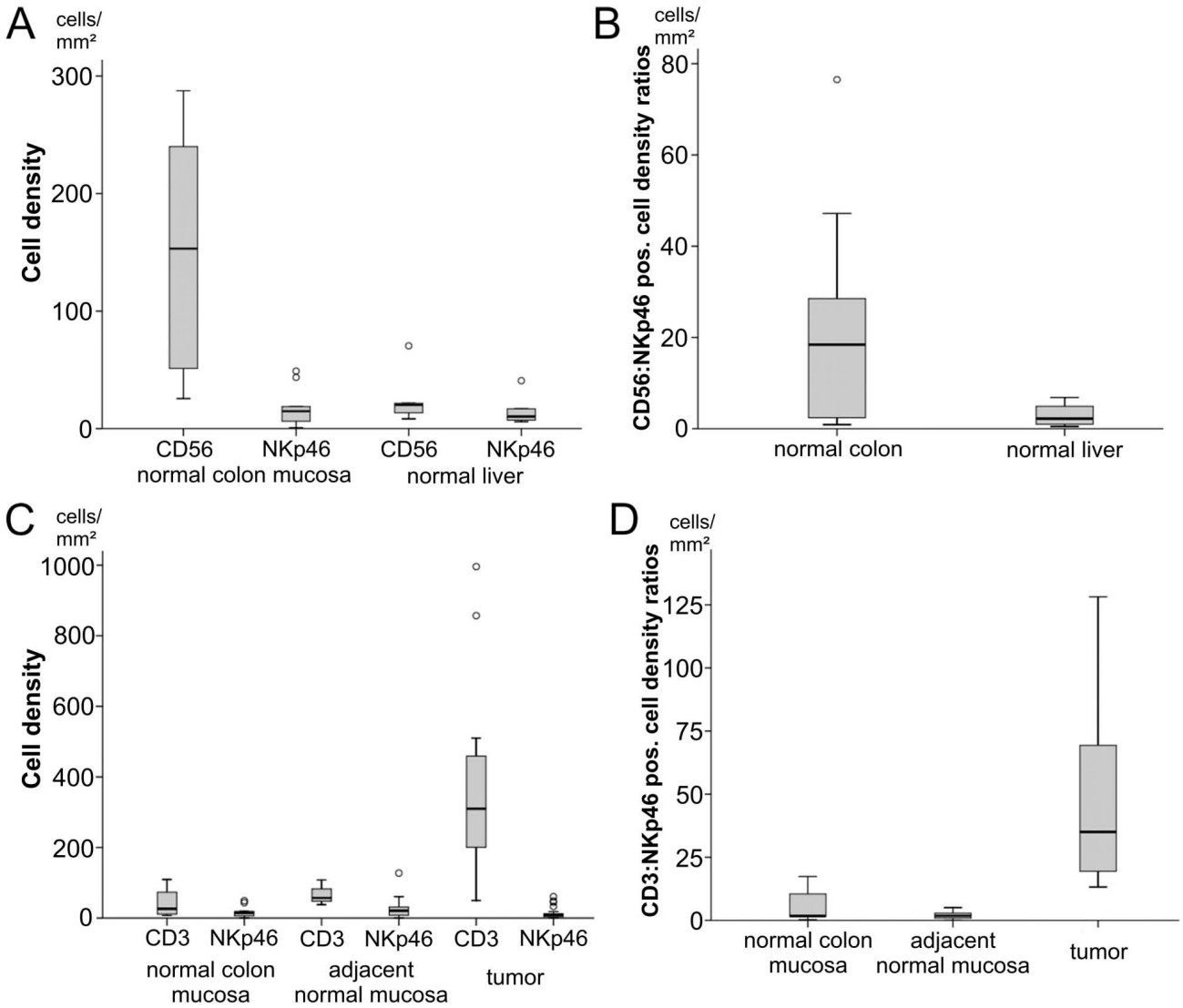
Multiplex Bead Based Analyses

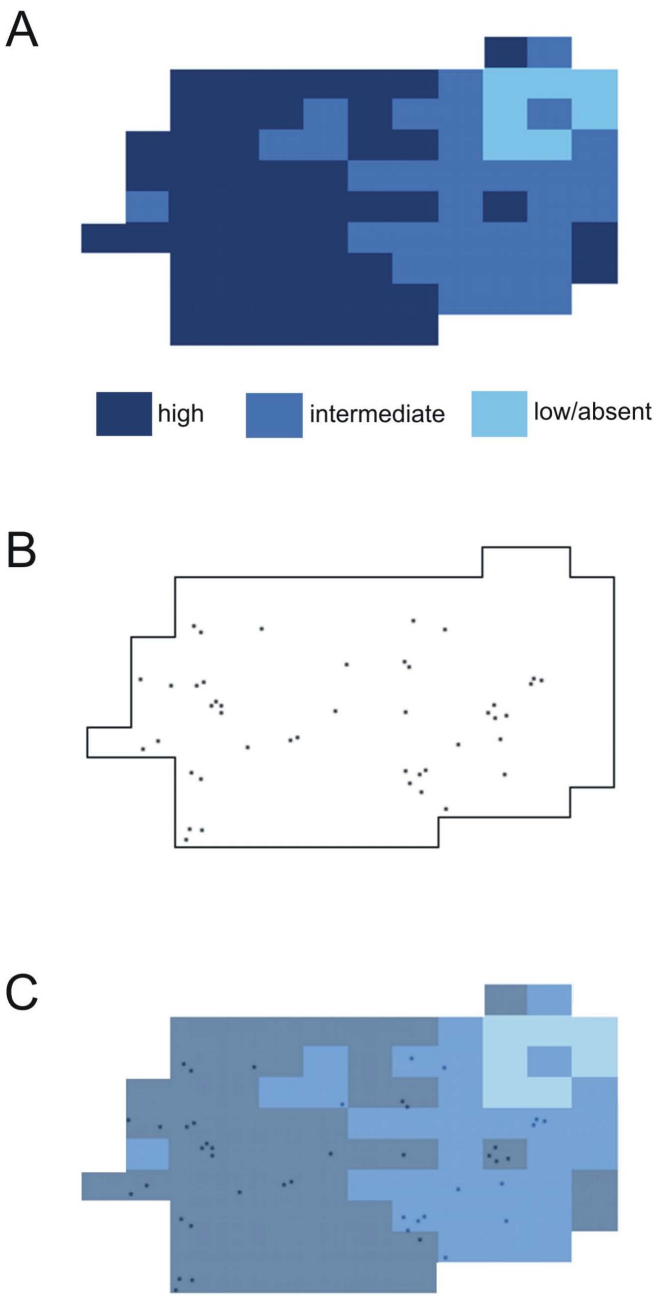


Up to 500 target proteins

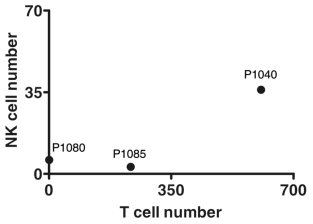
Min. Detection Level:
3 pg/ml

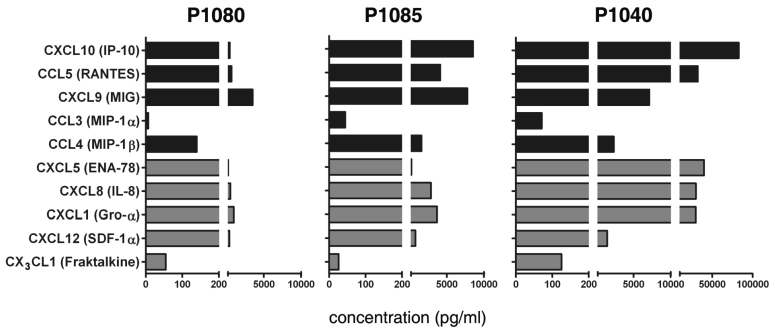

A
GALT
adjacent normal mucosa
invasive margin
tumor
NKp46
adjacent normal mucosa
GALT
invasive margin
tumor
B
adjacent normal mucosa
GALT
tumor
invasive margin
CD3ε
adjacent normal mucosa
GALT
invasive margin
tumor
C
tumor
invasive margin
GALT
adjacent normal mucosa
CD56
adjacent normal mucosa
GALT
invasive margin
tumor


A
cells/
mm²
Cell density
300
200
100
0
CD56
NKp46
CD56
NKp46
normal colon mucosa
normal liver
B
cells/
mm²
CD56:NKp46 pos. cell density ratios
80
60
40
20
0
normal colon
normal liver
C
cells/
mm²
Cell density
1000
800
600
400
200
0
CD3
NKp46
CD3
NKp46
CD3
NKp46
normal colon mucosa
adjacent normal mucosa
tumor
D
cells/
mm²
CD3:NKp46 pos. cell density ratios
125
100
75
50
25
0
normal colon mucosa
adjacent normal mucosa
tumor

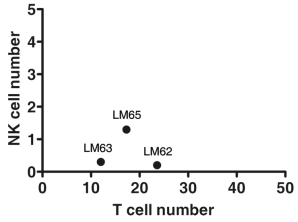

A
high
intermediate
low/absent
B
C

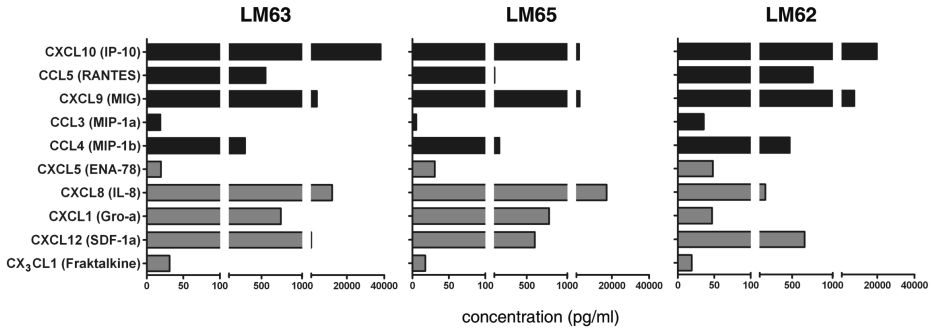
A


NK cell number
70
35
0
P1040
P1080
P1085
0
350
700
T cell number

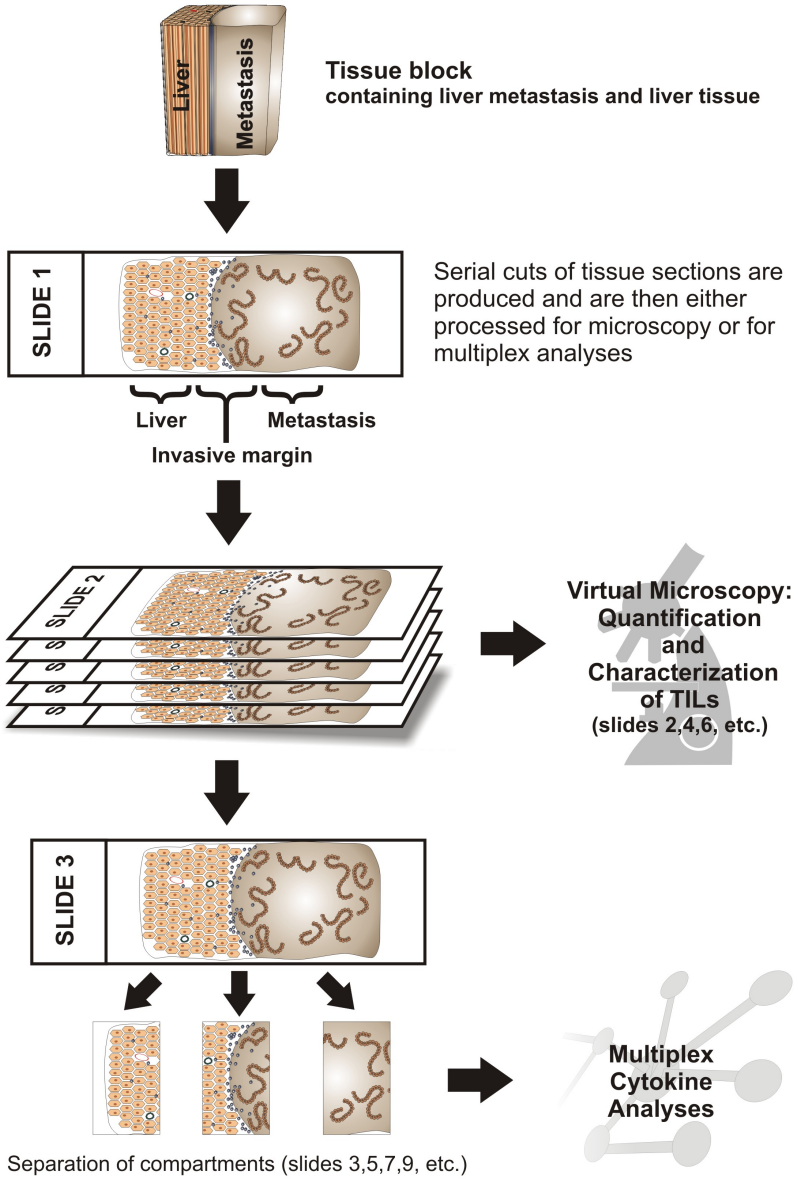

P1080
P1085
P1040
CXCL10 (IP-10)
CCL5 (RANTES)
CXCL9 (MIG)
CCL3 (MIP-1α)
CCL4 (MIP-1β)
CXCL5 (ENA-78)
CXCL8 (IL-8)
CXCL1 (Gro-α)
CXCL12 (SDF-1α)
CX3CL1 (Fraktalkine)
0 100 200 5000 10000
0 100 200 5000 10000
0 100 200 5000 10000 50000 100000
concentration (pg/ml)

B


NK cell number
5
4
3
2
1
0
LM65
LM63
LM62
0 10 20 30 40 50
T cell number


LM63
LM65
LM62
CXCL10 (IP-10)
CCL5 (RANTES)
CXCL9 (MIG)
CCL3 (MIP-1a)
CCL4 (MIP-1b)
CXCL5 (ENA-78)
CXCL8 (IL-8)
CXCL1 (Gro-a)
CXCL12 (SDF-1a)
CX3CL1 (Fraktalkine)
0 50 100 500 1000 20000 40000
0 50 100 500 1000 20000 40000
0 50 100 500 1000 20000 40000
concentration (pg/ml)

Differential Profiling & Cell Densities



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Isabella Gosch
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Jutta Funk

Jürgen Weitz
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Karsten Brand

Christine Falk
Monika Braun
Christian Quack

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