DEFINIENS®
the tissue phenomics company

Tissue Phenomics

From Tissue to Biomarkers

Carolina Vanegas
on behalf of

N. Harder, R. Huss and N. Brieu
November 10th, 2016

Tissue Phenomics

Image Analysis and Data Mining for Discovering Novel **Prognostic** and Predictive Markers From Histopathological Data

DEFINIENS®
the tissue phenomics company

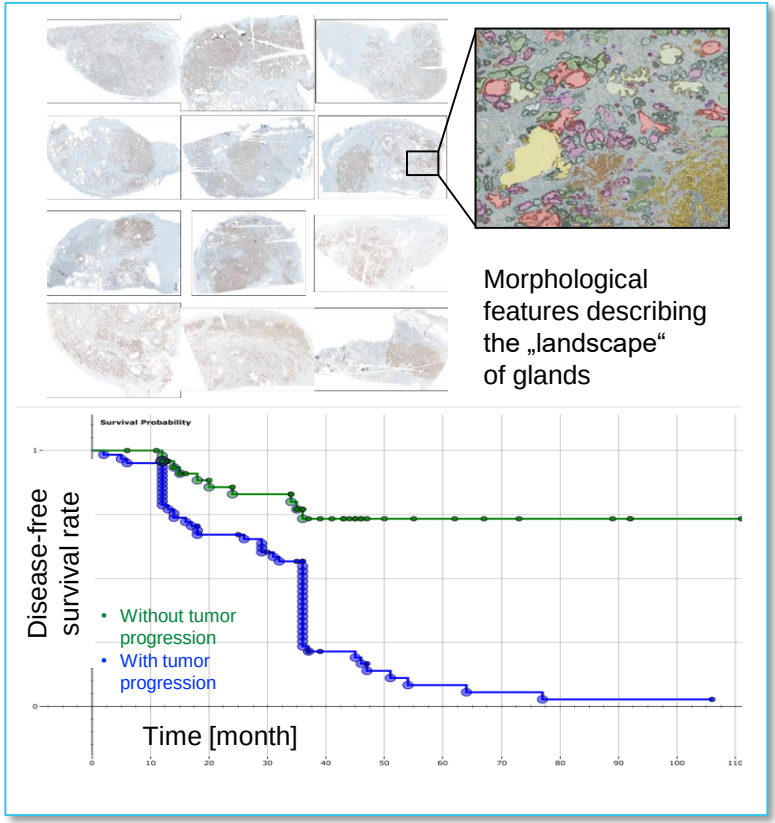
Tissue Phenomics

- Big data approach to quantitative histopathology
- Comprehensive screening of phenes which predict clinical outcome
- Phenes are mathematical descriptions of spatial patterns detected in tissue which have biomedical relevance
- Automated workflow from assay development to phene validation enables automatic multiparametric optimization

DEFINIENS®
the tissue phenomics company

Tissue Phenomics

- Big data approach to quantitative histopathology
- Comprehensive screening of phenes which predict clinical outcome
- Phenes are mathematical descriptions of spatial patterns detected in tissue which have biomedical relevance
- Automated workflow from assay development to phene validation enables automatic multiparametric optimization



DEFINIENS
the tissue phenomics company

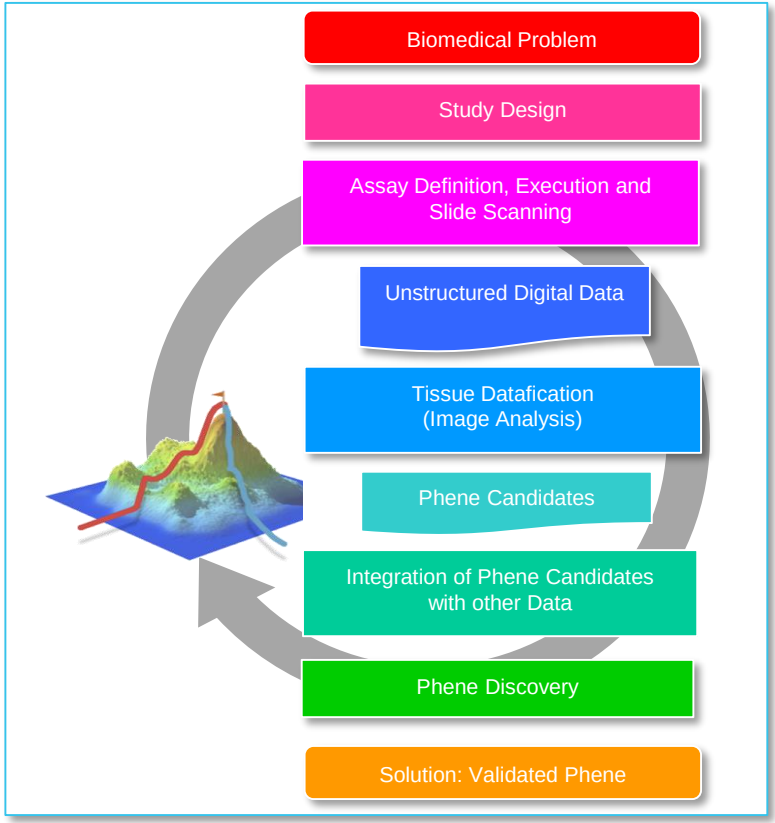
Tissue Phenomics

- Big data approach to quantitative histopathology
- Comprehensive screening of phenes which predict clinical outcome
- Phenes are mathematical descriptions of spatial patterns detected in tissue which have biomedical relevance
- Automated workflow from assay development to phene validation enables automatic multiparametric optimization

DEFINIENS®
the tissue phenomics company

Tissue Phenomics

- Big data approach to quantitative histopathology
- Comprehensive screening of phenes which predict clinical outcome
- Phenes are mathematical descriptions of spatial patterns detected in tissue which have biomedical relevance
- Automated workflow from assay development to phene validation enables automatic multiparametric optimization



DEFINIENS®
the tissue phenomics company

Discovering Prognostic Factors for Prostate Cancer Tumor Progression

DEFINIENS
the tissue phenomics company

Prostate Cancer

Prostate cancer

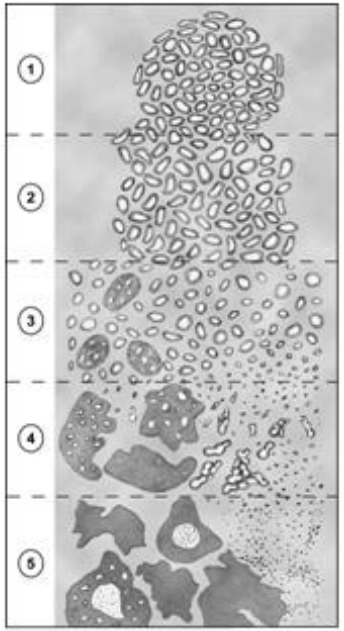
- Second most frequently diagnosed cancer in men in 2012
  (15% of all male cancers) [World Cancer Report 2014, WHO]
- Increasing incidence with increasing age

Gleason score

- Sum of pattern-number of the primary (major) and secondary (minor) grades (e.g., 7a=3+4)

Gleason score	6	7	8-10
	Low risk	Intermediate risk	High risk

[NCCN guidelines, NCCN.org]



[J.I. Epstein,
Am J Surg Pathol, 2005]

- Small, uniform glands
- More space between glands
- Infiltration of cells from glands at margins
- Irregular masses of cells with few glands
- Lack of glands, sheets of cells

DEFINIENS
the tissue phenomics company

Project Goals

Goal 1: Development of a prognostic test to automatically predict tumor progression based on resected prostate tissue with high accuracy and stratify patients into treatment groups

Goal 2: Test should have an improved prognostic power compared to the state-of-the-art method, the Gleason score (assigned by pathologists)

Data: Tissue sections from prostate resections of selected patients (whole slide images)

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

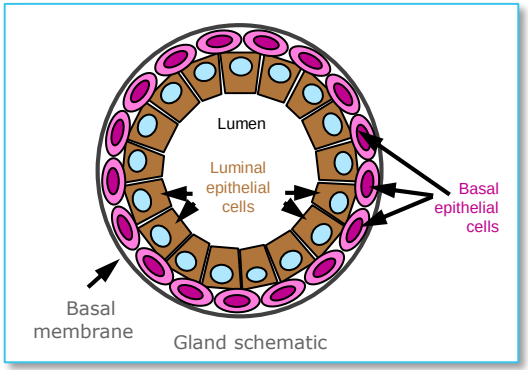
DEFINIENS
the tissue phenomics company

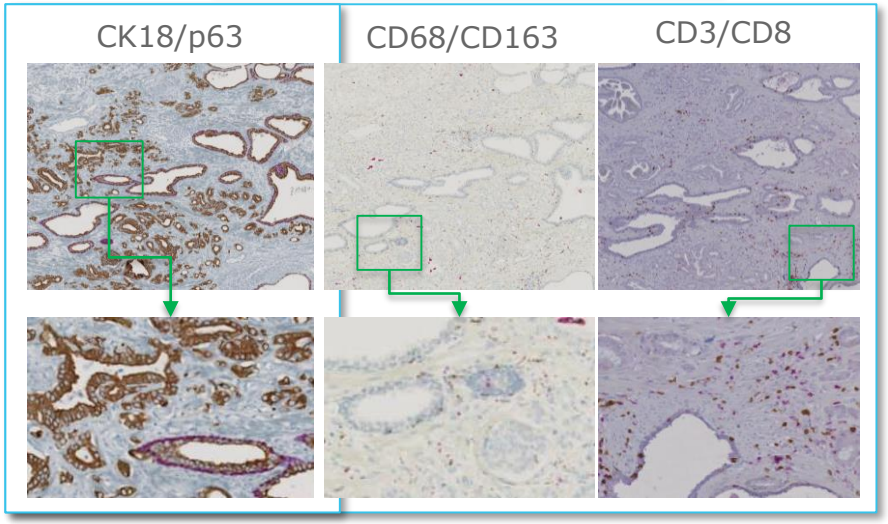
Two Novel Prognostic Markers

For Prostate Cancer Progression

- **Spatial relationship of glands**
  - **CK18/p63**
    Tumor vs. non-tumor glands

- **Tumor-associated Macrophages and T-cells**
  - **CK18/p63**
    Tumor vs. non-tumor glands
  - **CD68/CD163**
    M1/M2 Tumor Associated Macrophages (TMAs)
  - **CD3/CD8**
    Tumor infiltrating T-cells (TILs)





DEFINIENS®
the tissue phenomics company

Prognostic factor I
Spatial Relationship of Glands

ISBI 2016 – Co-occurence features characterizing gland distribution patterns as new prognostic markers in prostate cancer whole slide images

DEFINIENS®
the tissue phenomics company

Spatial relationship of glands

- **The patient cohort**

  98 selected patients

  Age <= 75 years

  Staging: pT2

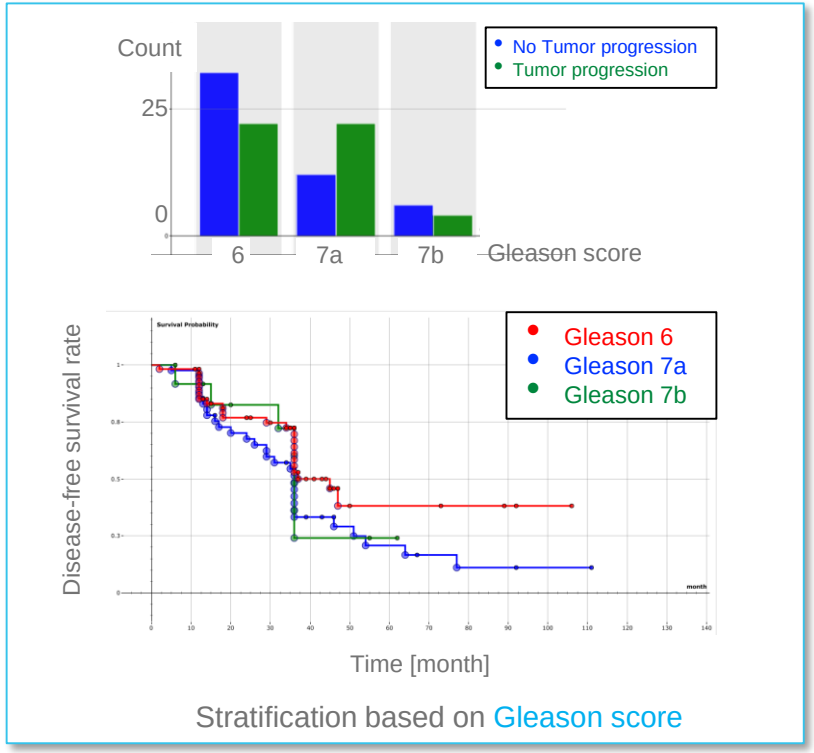
  Resection border: R0

  Gleason-Score: 3+3 (6), 3+4 (7a), 4+3 (7b)

  Low- to intermediate-risk patients after Radical Prostatectomy

  Clinical outcome data available

  Observation times of 2 to 118 month

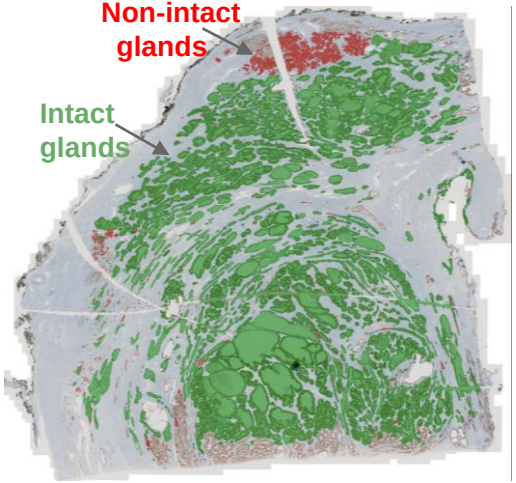


Stratification based on Gleason score

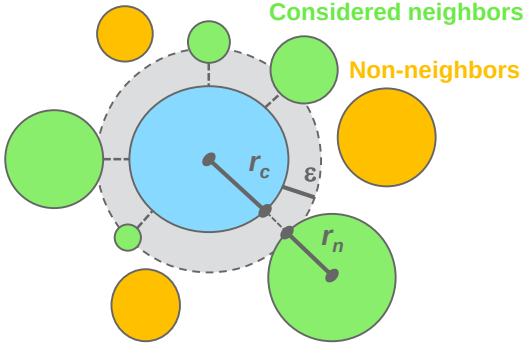
DEFINIENS
the tissue phenomics company

Spatial relationship of glands

- **Image Analyis**
  - **Automatic Segmentation**
    - P63 marked regions
    - Glands (epithelium and lumen)
  - **Classification of glands**
    Size: Small, Medium, and Large
    AND
    Functional: Intact (with p63)/ Non Intact (no p63)
  - **Co-occurence features**
    - Analogy to classical Haralick features [1]
    - Co-occurrence matrices of differently classified neighboring glands are computed
    - Different distances and directions are considered
    - 2140 features are generated in total



Segmentation Result



Gland Neighborhood Graph

[1] Harder N. *et al.*, "Co-occurrence features characterizing gland distribution patterns as new prognostic markers in prostate cancer whole-slide images," *Proc. IEEE ISBI 2016*, pp. 807-810, 2016.

DEFINIENS®
the tissue phenomics company

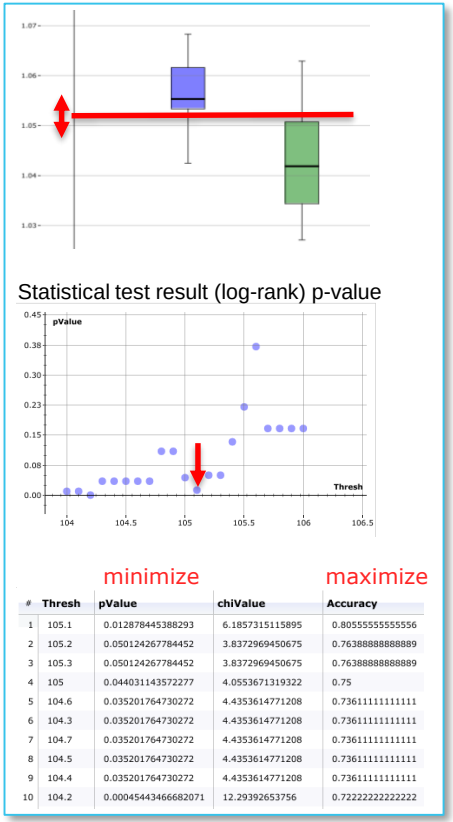
Spatial relationship of glands

Data Mining

The stratification performance is systematically tested for each feature with log-rank test

Cross validation

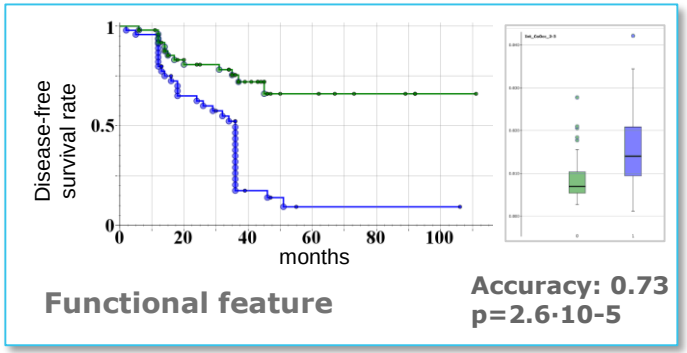
1. **Optimize the threshold** for classifying patients per feature w.r.t. the mean accuracy and the log-rank test p-value
2. **Rank features** according to their best classification accuracy



#	Thresh	pValue	chiValue	Accuracy
1	105.1	0.012878445388293	6.1857315115895	0.80555555555556
2	105.2	0.050124267784452	3.8372969450675	0.76388888888889
3	105.3	0.050124267784452	3.8372969450675	0.76388888888889
4	105	0.044031143572277	4.0553671319322	0.75
5	104.6	0.035201764730272	4.4353614771208	0.73611111111111
6	104.3	0.035201764730272	4.4353614771208	0.73611111111111
7	104.7	0.035201764730272	4.4353614771208	0.73611111111111
8	104.5	0.035201764730272	4.4353614771208	0.73611111111111
9	104.4	0.035201764730272	4.4353614771208	0.73611111111111
10	104.2	0.00045443466682071	12.29392653756	0.72222222222222

Prognostic Biomarkers

Co-occurrence probability of small-non-intact to medium-intact



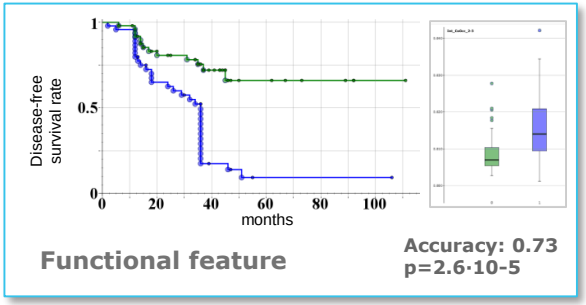
- No tumor progression
- Tumor progression

DEFINIENS
the tissue phenomics company

Spatial relationship of glands

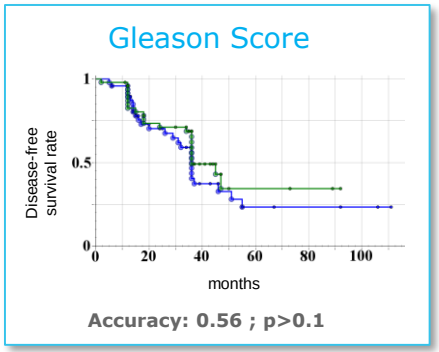
- **Comparison to clinical features**

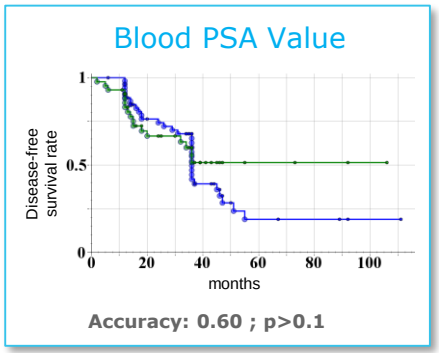
Image Analysis & Data Mining

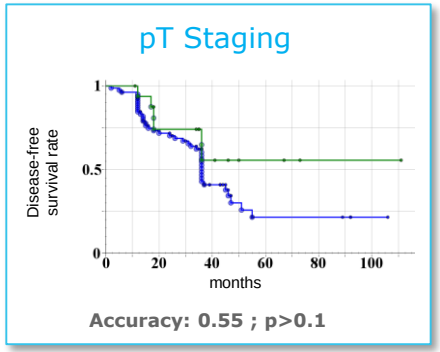


- No tumor progression
- Tumor progression

Clinical Features







DEFINIENS
the tissue phenomics company

Prognostic factor II
Tumor-Associated Macrophages and T-cells

SITC 2016 - Tumor-associated Macrophages (TAMs) as a prognostic marker for prostate cancer progression

DEFINIENS
the tissue phenomics company

Tumor-associated macrophages and T-cells

- **The patient cohort**

  89 selected patients

  Age <= 75 years

  Staging: pT2

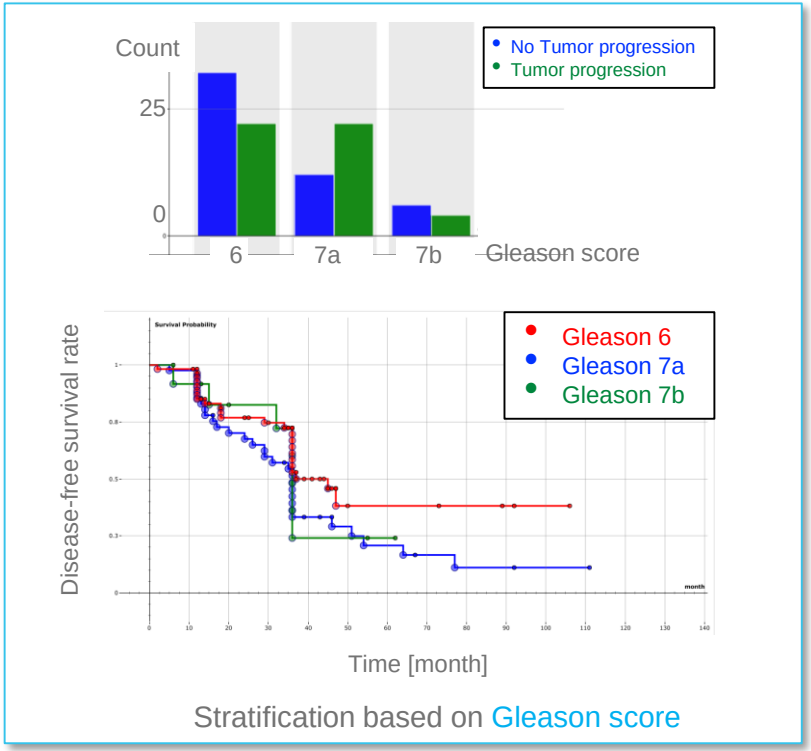
  Resection border: R0

  Gleason-Score: 3+3 (6), 3+4 (7a), 4+3 (7b)

  Low- to intermediate-risk Patients after Radical Prostatectomy

  Clinical outcome data available

  Observation times of 2 to 118 month

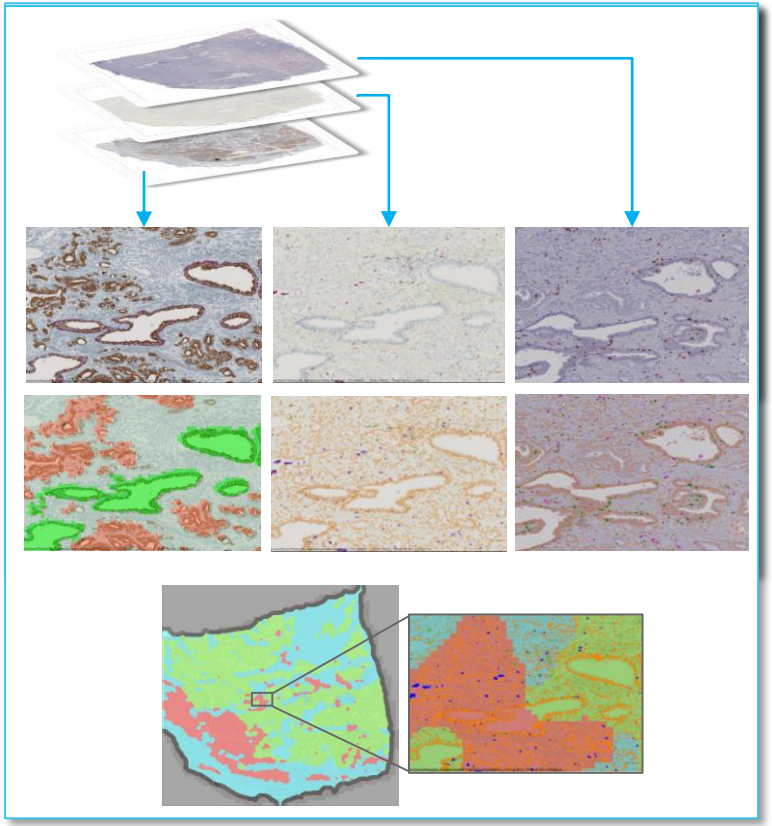


Stratification based on Gleason score

DEFINIENS
the tissue phenomics company

Tumor-associated macrophages and T-cells

- **Image Analyis**
  - **Three double stain images** CK18/p63, CD68/CD163, and CD3/CD8
  - **Object detection** in each image
    - CK18/p63
      - Segmentation of tumor and non-tumor regions
    - CD68/CD163
      - Detection of M1 (cytotoxic) and M2 (tumor growth promoting) macrophages
    - CD3/CD8
      - Detection of cytotoxic and other T cells
  - **Co-Registration**
  - **Joint region-based quantification** of TAMs and Tumor Infiltrating Lymphocytes

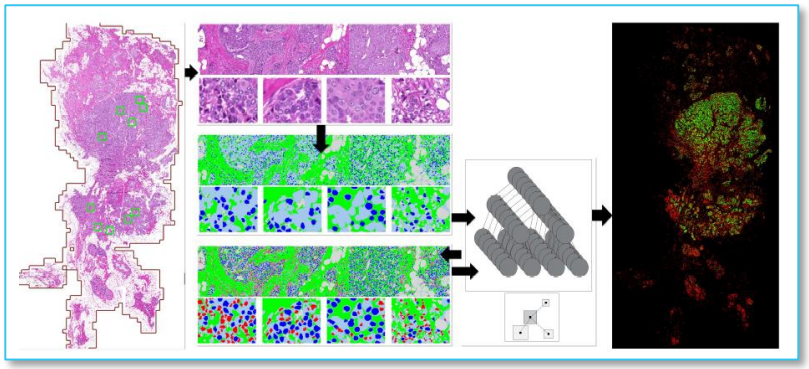


DEFINIENS
the tissue phenomics company

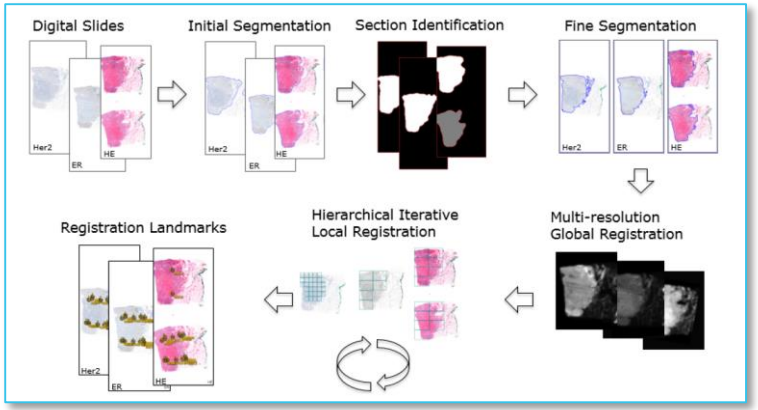
Tumor-associated macrophages and T-cells

Generic Image Analysis Solutions

- **Object Detection [1]**
  - Auto-adaptive machine learning
  - Selection of representative tiles
  - Fishing of well-defined objects using shape and size constraints
  - Training slide-specific Random Forest models



- **Registration [2]**
  - Global initial alignment of the tissue sections
  - Local refinement of interesting regions
  - Multiresolution approach



DEFINIENS
the tissue phenomics company

[1] Brieu, N. et al. "Slide specific models for segmentation of differently stained digital histopathology whole slide images", SPIE Medical Imaging, 2016
[2] Yigitsoy, M. et al. "Hierarchical patch-based co-registration of differently stained histopathology slides", To Appear in SPIE Medical Imaging, 2017

Tumor-associated macrophages and T-cells

Features

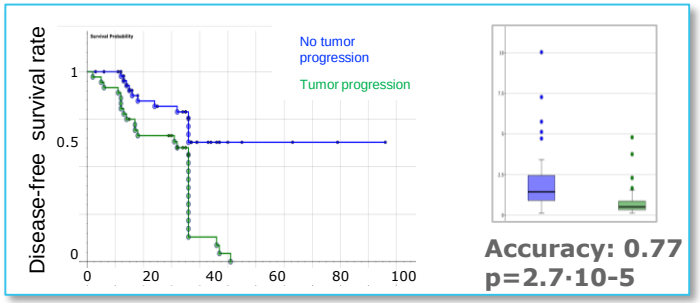
- Features are generated by **combining** the image based measurements
- For instance,
  Densities (number positive cells per area)
  Ratios of positive cells in different tissue sections

Data Mining

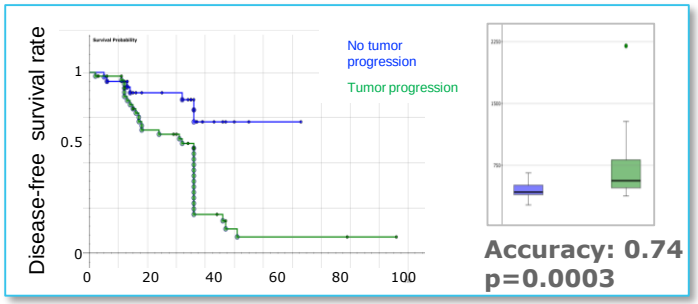
- The stratification performance is systematically tested for each feature with **log-rank** test
- **Optimize the threshold**
- **Rank features w.r.t accuracy and p-value**

Prognostic Biomarkers

Prostate cancer patients without tumor progression show a significantly higher ratio of CD8+ (cytotoxic t-cells) to CD163+ (M2) cell densities in the tumor microenvironment



Prostate cancer patients without tumor progression show a significantly lower average distance of CD3+ (non-cytotoxic t-cells) to CD8+ (cytotoxic t-cells) cells in the tumor microenvironment



DEFINIENS
the tissue phenomics company

Conclusion

DEFINIENS
the tissue phenomics company

Conclusion

- **Tissue Phenomics:**
  - Generic Image analysis and Data mining enable the discovery of better biomarkers
  - Extract features from Whole Slide Images → image analysis
  - Correlate with clinical outcome data → data mining

- As an example, the discovery of **two biomarkers** showing **prognostic potential** to predict tumor progression and survival time
  - Based on spatial organization of glands
  - Based on co-localized TILs and TAMS

- Potential high impact for prostate cancer patient treatment decisions

DEFINIENS®
the tissue phenomics company

Thank you

Contacts:

Nathalie Harder - nharder@definiens.com

Ralf Huss- rhuss@definiens.com

DEFINIENS
the tissue phenomics company