

# Three-Dimensional Reconstruction and Quantification of Cervical Carcinoma Invasion Fronts from Histological Serial Sections

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# Overview

**1. Introduction**

**2. Tumour Reconstruction**

**3. Tumour Invasion Quantification**

**4. Results**

**5. Conclusions I**

**6. Clinical Applicability?**

**7. Conclusions II**

**8. Advanced Tumour Reconstruction & Analysis**

# 1 Introduction

## ***Carcinoma growth***

- Malignant growth and invasiveness of cancers:
  - intratumoral and stromal factors
- Shape of the tumor invasion front:
  - accessibility to nutrients, oxygen and growth factors
  - stromal composition, interference with the immune system
- Supposed growth pattern-related prognostic differences or surgical relevance

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## **Tissue specimen**

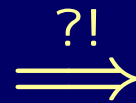
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**Tumour description**

## ***General Objective***

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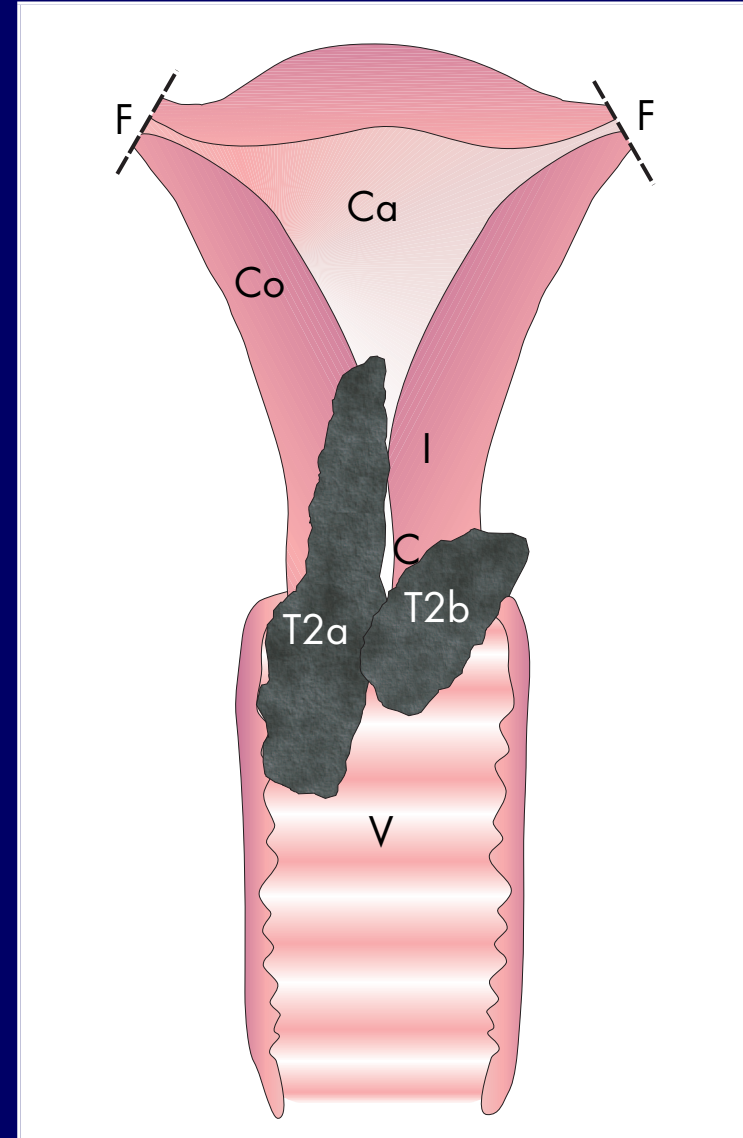
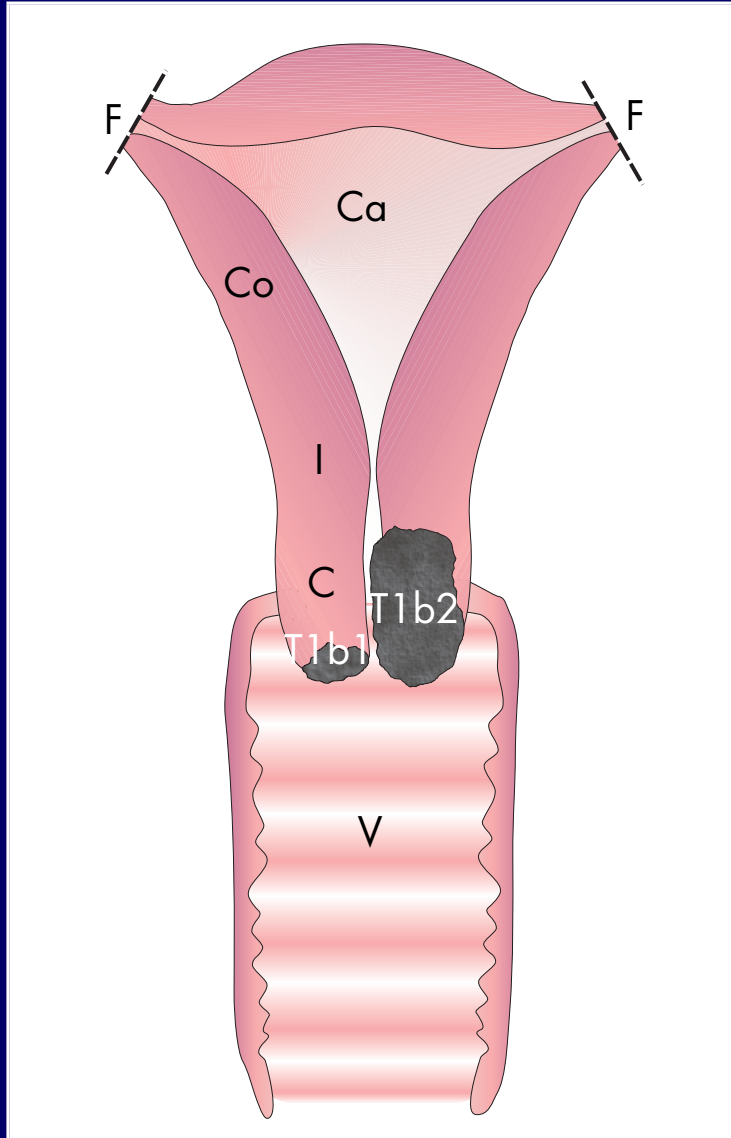
## ***Specific Objective***

3-D characterisation of the invasion pattern of squamous epithelial carcinoma of the uterine cervix (supposed prognostic relevance)

**Tissue specimen**  $\xRightarrow{?!}$  **Tumour description**

# 1 Introduction (cont'd)

## Anatomical Overview:



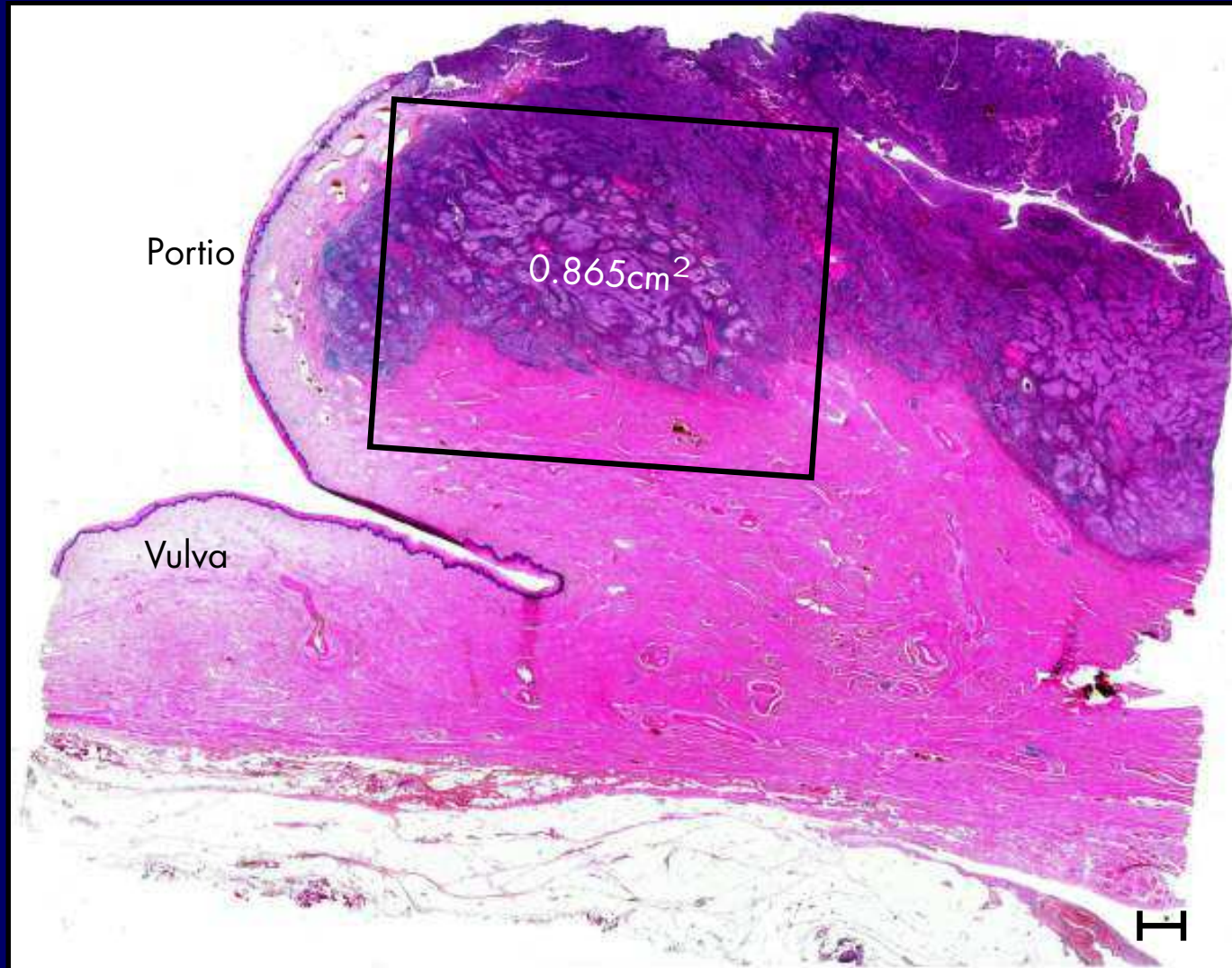
# 1 Introduction (cont'd)

## *Cervix Specimen embedded in Paraffin Wax:*



# 1 Introduction (cont'd)

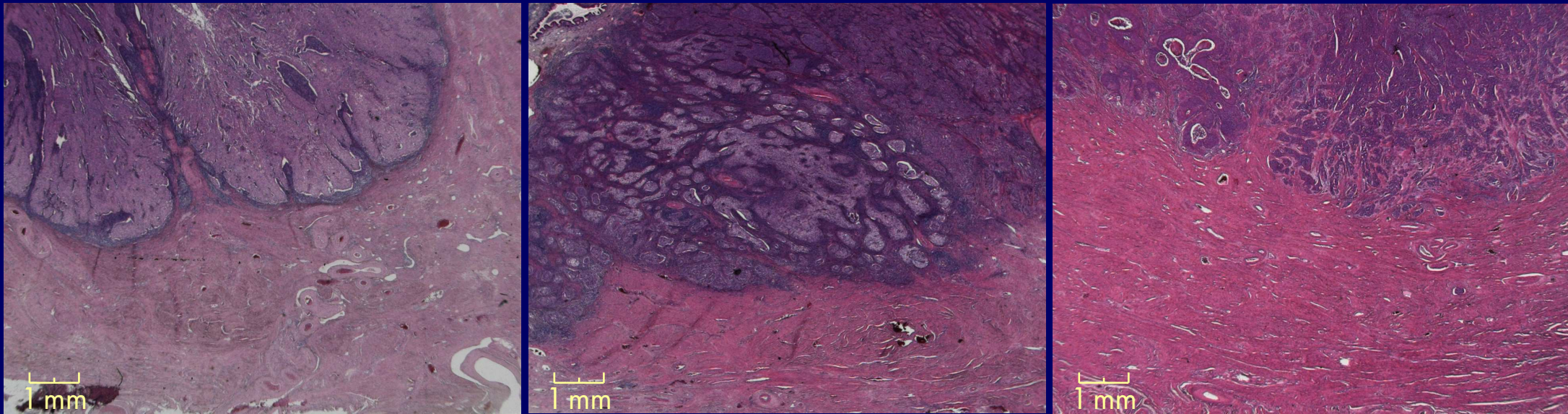
## Material:





# 1 Introduction (cont'd)

## ***Squamous Cell Carcinoma of the Uterine Cervix:***



"closed"

"finger-like"

"diffuse"

1. How to algorithmically quantify tumour invasion?
2. No knowledge about the 3-D invasion front!
3. Do separated tumour islets exist?

# 1 Introduction (cont'd)

## *Imaging Modalities:*

- macroscopic 3-D techniques (CT, MRI, PET, SPECT, US, ...):  
→ too few contrast / spatial resolution
- microscopic 3-D techniques (CLSM, 3-DEM, SFM, ...):  
→ too limited FOV / far sub-cellular resolutions
- transmitted light microscopy:  
→ histological serial sections

# 1 Introduction (cont'd)

## *Problems with Serial Sections: Slicing Artefacts*

- distortions
- slice thickness fluctuations
- damages, fissures, folds

# 1 Introduction (cont'd)

## ***Problems with Serial Sections: Slicing Artefacts***

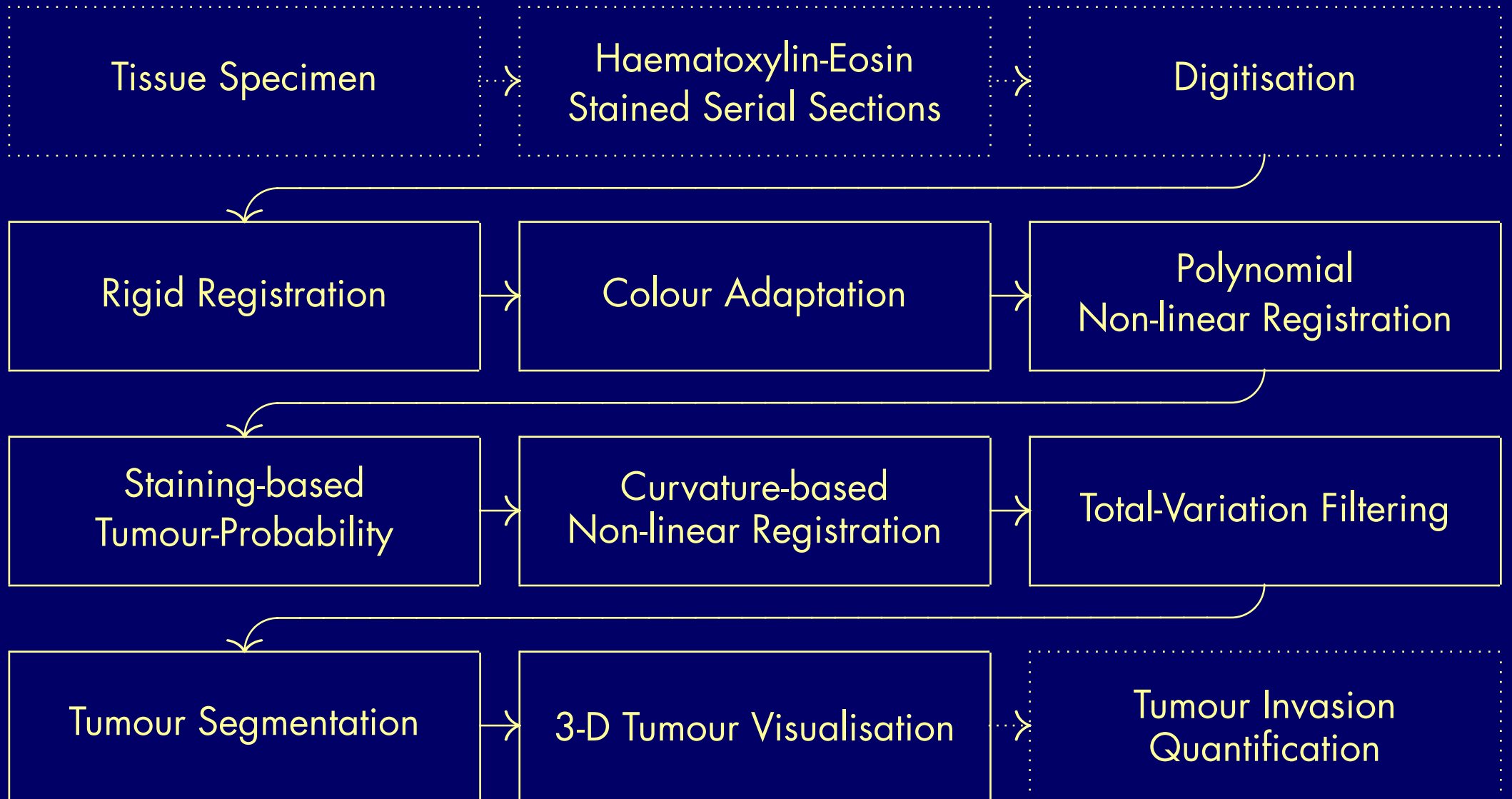
- distortions
- slice thickness fluctuations
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## ***Strategy: procedures for***

- tissue reconstruction
- tumour segmentation
- tumour invasion quantification

# 2 Tumour Reconstruction

## Image Processing Chain:



## 2 Tumour Reconstruction (cont'd)

### ***Rigid Registration:***

- Rough alignment (rotation, translation)
- Fourier-Mellin Invariant & Phase-Only Matched Filtering

## 2 Tumour Reconstruction (cont'd)

### ***Rigid Registration:***

- Rough alignment (rotation, translation)
- Fourier-Mellin Invariant & Phase-Only Matched Filtering

$$r(x, y) = s(x\cos\alpha_0 + y\sin\alpha_0 - x_0, -x\sin\alpha_0 + y\cos\alpha_0 - y_0)$$

*Solution for  $\alpha_0$  and  $x_0, y_0$ :*

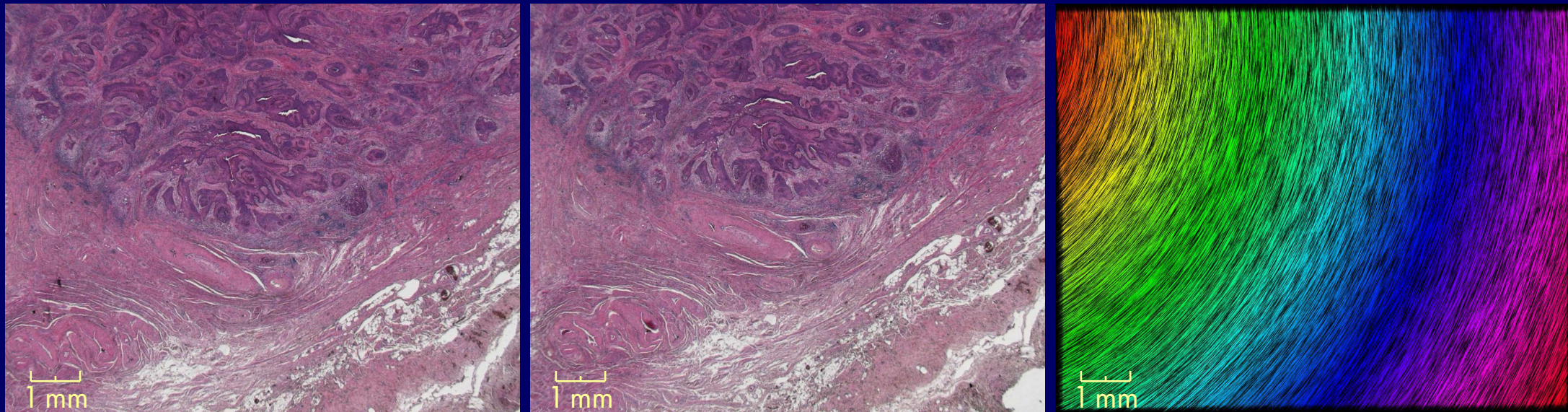
~> Fourier-Mellin-Transformation (Rotation) and

~> Phase-Only Matched Filtering (Rotation & Translation)

↪ fast, non-iterative procedure

## 2 Tumour Reconstruction (cont'd)

### Rigid Registration:



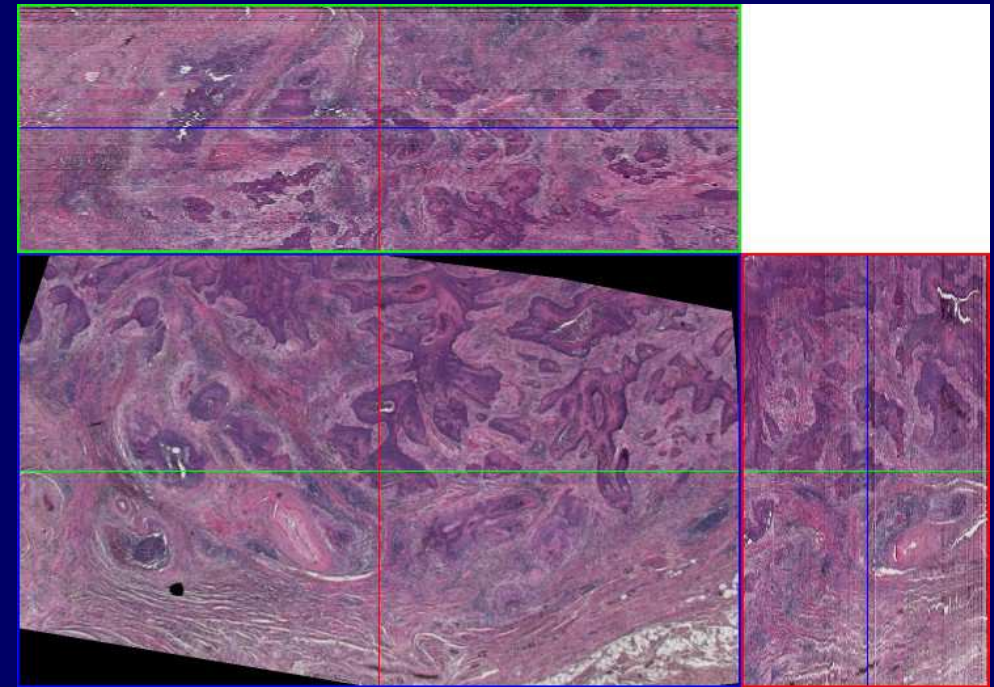
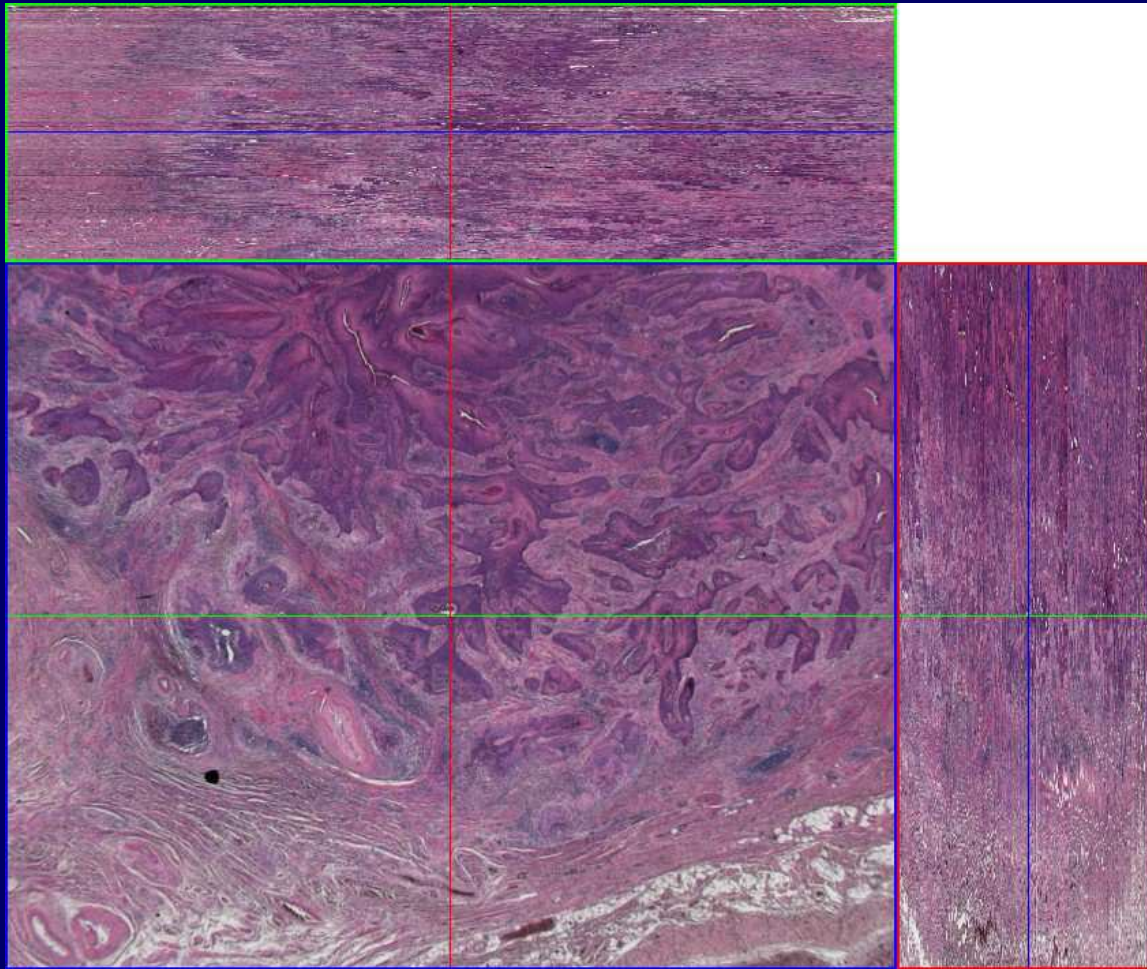
Maximum displacement:  $1184.3\mu\text{m}$  (lower right)

minimum:  $254.1\mu\text{m}$  (upper left, "rotational center", outside image)



# 2 Tumour Reconstruction (cont'd)

## Rigid Registration:



## 2 Tumour Reconstruction (cont'd)

### ***Colour Adaptation:***

- Compensation for saturation/staining fluctuations
- Criterion: reference multivariate distribution in RGB-colour space

~> estimated matrices for offset, scaling, and rotation

## 2 Tumour Reconstruction (cont'd)

### Colour Adaptation:

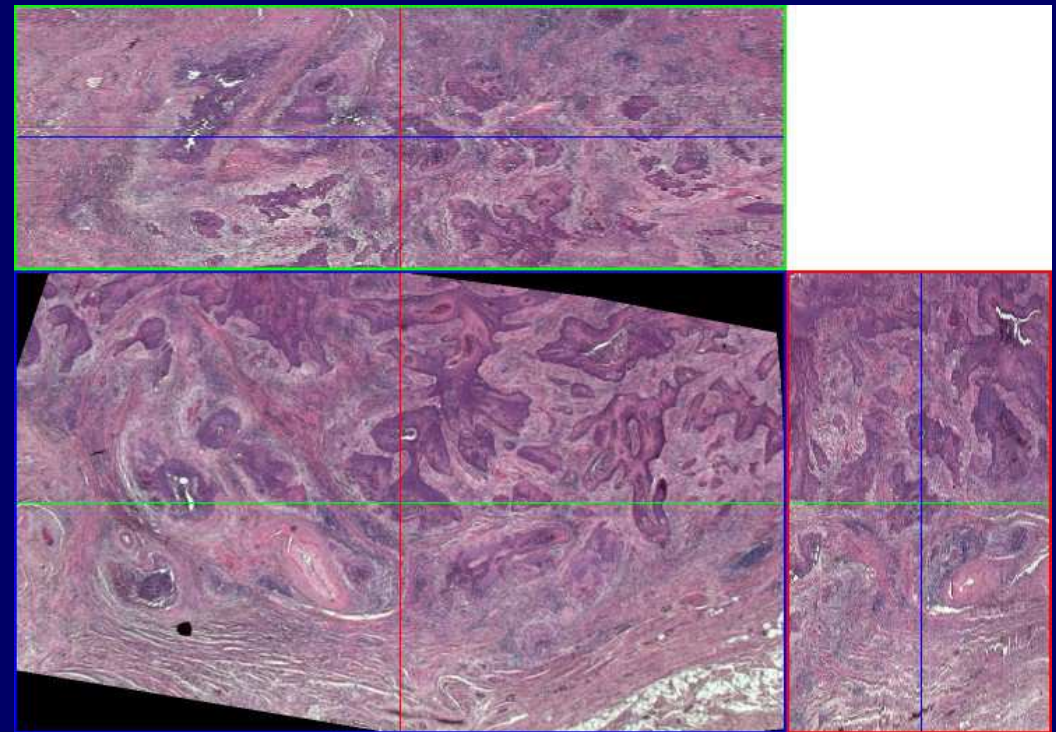
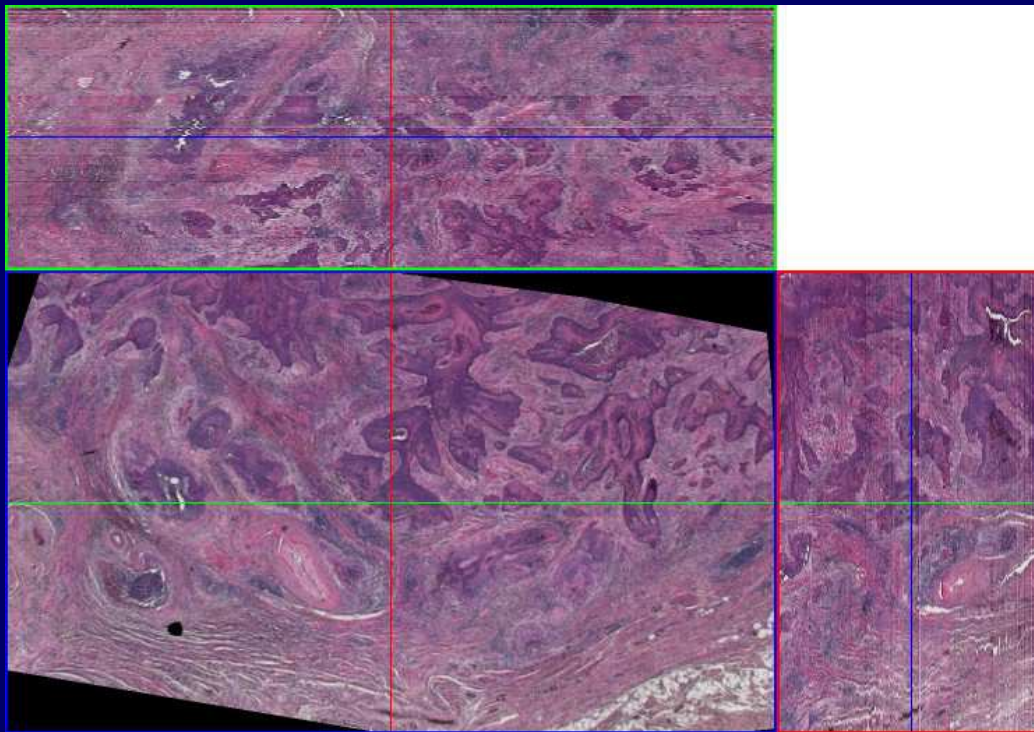
- Compensation for saturation/staining fluctuations
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$$\begin{bmatrix} R \\ G \\ B \\ 1 \end{bmatrix}_{\text{ref}} = O_{\text{ref}}^{-1} \cdot R \cdot S \cdot O_{\text{sam}} \begin{bmatrix} R \\ G \\ B \\ 1 \end{bmatrix}_{\text{sam}}$$

## 2 Tumour Reconstruction (cont'd)

### Colour Adaptation:



## 2 Tumour Reconstruction (cont'd)

### ***Polynomial Non-linear Registration:***

Compensation of slice-global distortions using sparsely-populated displacement vector fields,  $M > (N + 1)^2$  vectors,  $N$ th degree polynomials

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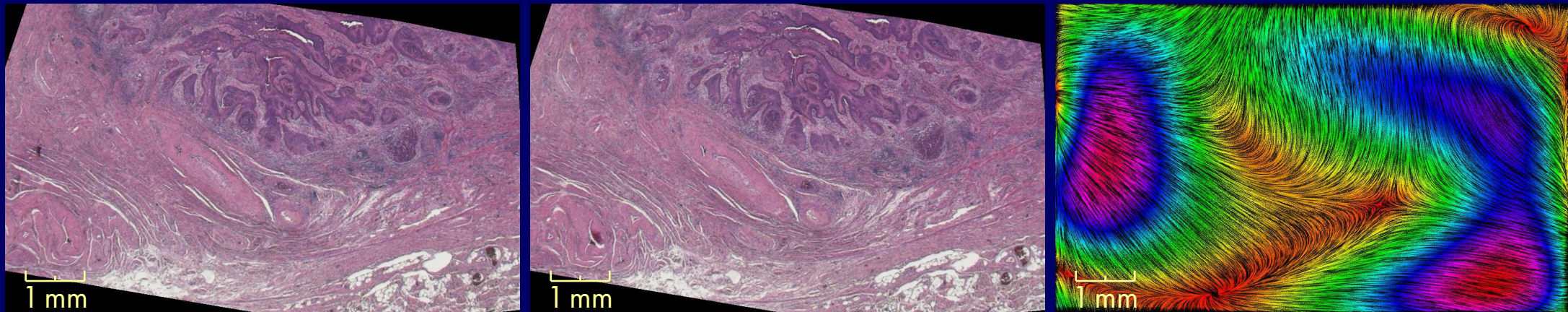
$$r(x, y) = s(a(x, y), b(x, y))$$

$$= s \left( \sum_{i=0}^N \sum_{j=0}^N p_{j(N+1)+i+1} \cdot x^j y^i, \sum_{i=0}^N \sum_{j=0}^N q_{j(N+1)+i+1} \cdot x^j y^i \right)$$

$(N + 1)^2$  coefficients  $p_n$  und  $q_n$  by means of linear regression (least squares estimation),  $N = 5$

## 2 Tumour Reconstruction (cont'd)

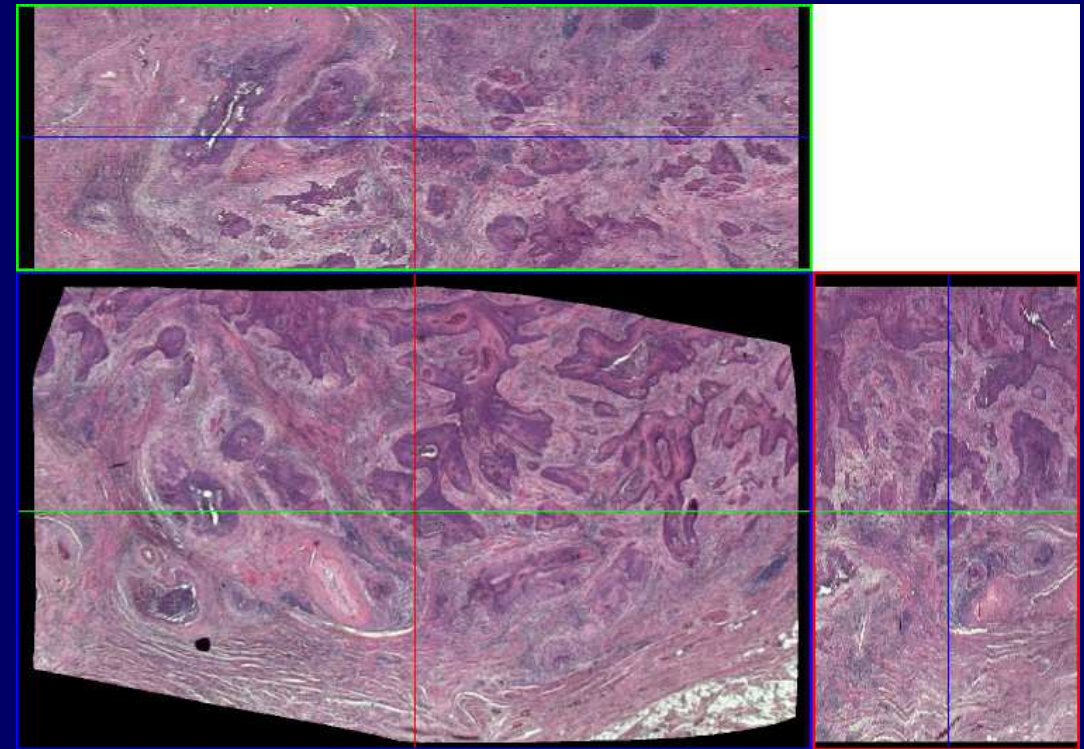
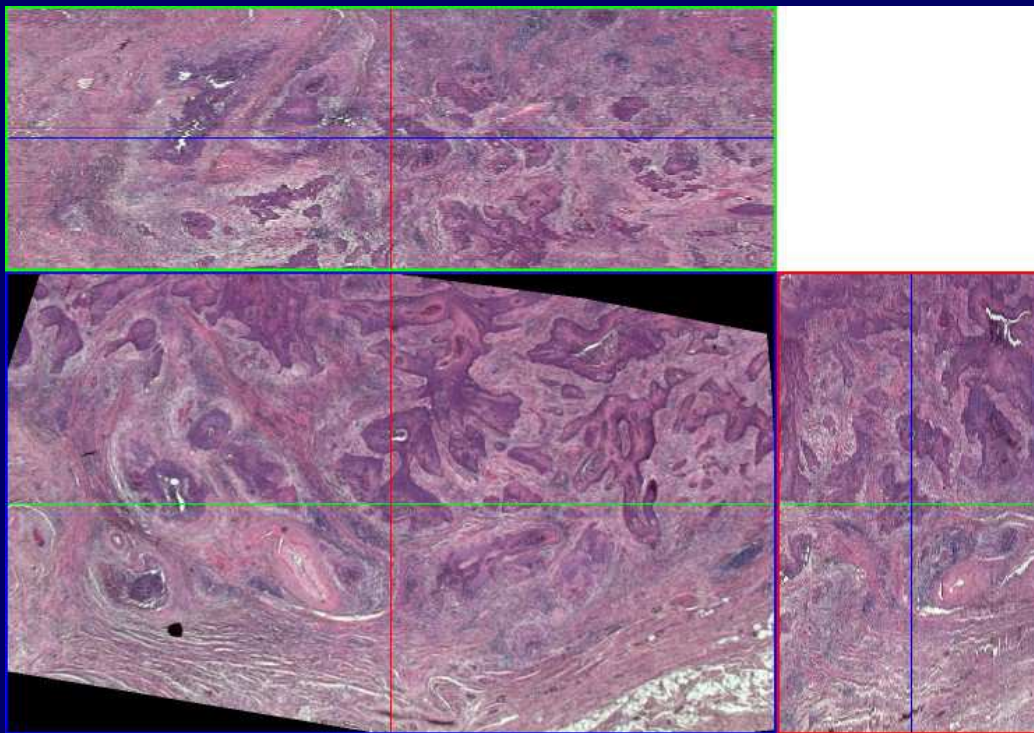
### Polynomial Non-linear Registration:



Maximum displacement:  $84.4\mu\text{m}$ , minimum:  $0\mu\text{m}$ .

## 2 Tumour Reconstruction (cont'd)

### Polynomial Non-linear Registration:

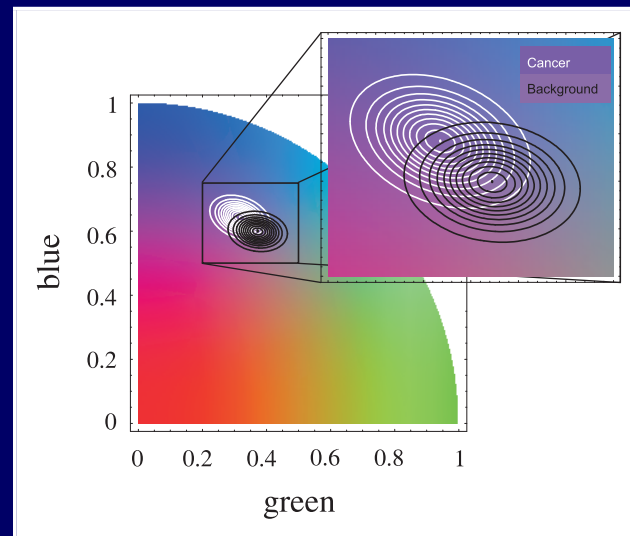




## 2 Tumour Reconstruction (cont'd)

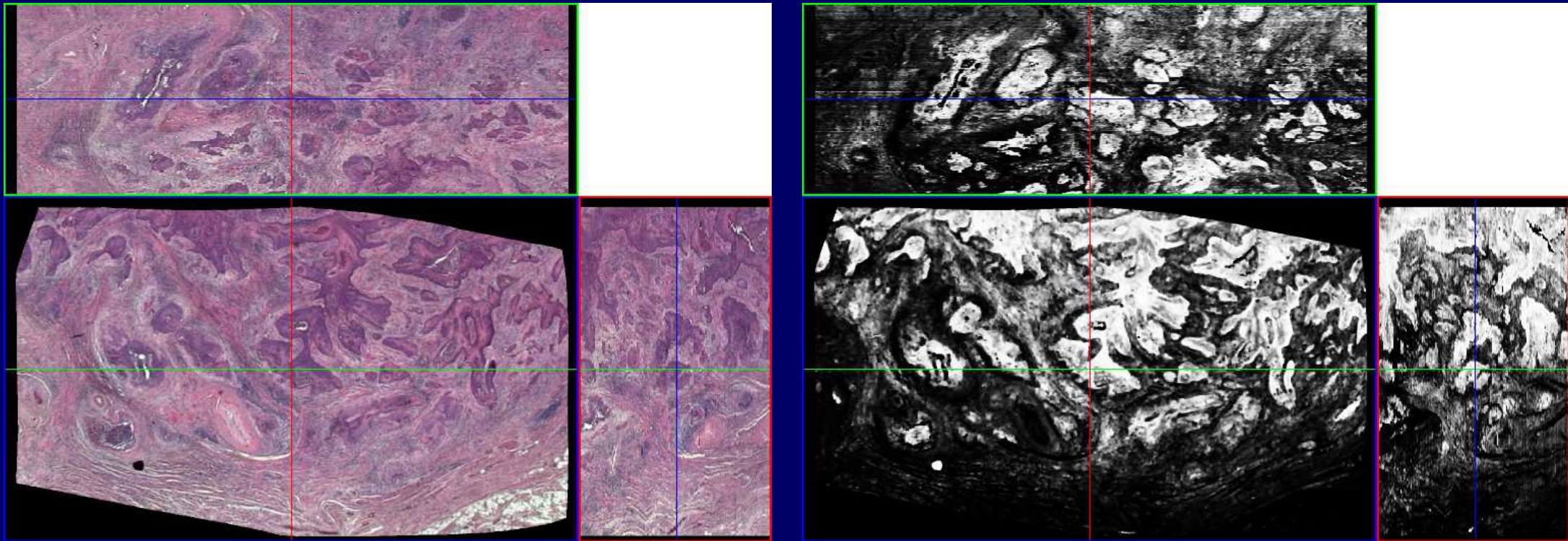
### Staining-based Tumour-Probability:

- Colour samples manually taken from typical slices
- Estimated multivariate densities of tumour  $c$  and background  $m$
- Tumour probability @ pixel  $\xi$ : 
$$\gamma(\xi) = \frac{\rho_c(\xi)}{\rho_c(\xi) + \rho_m(\xi)}$$



## 2 Tumour Reconstruction (cont'd)

### Staining-based Tumour-Probability:



## 2 Tumour Reconstruction (cont'd)

### ***Non-linear Curvature-based Registration:***

- Compensation of remaining local distortions
- Minimisation of curvature of the displacement field components
- 4th order partial differential equation for the displacement field

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### **Non-linear Curvature-based Registration:**

- Compensation of remaining local distortions
- Minimisation of curvature of the displacement field components
- 4th order partial differential equation for the displacement field

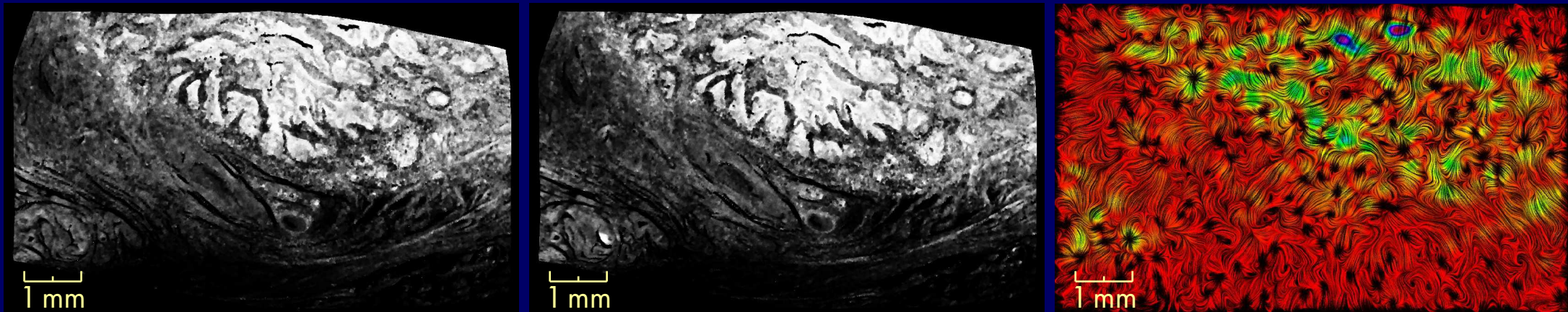
$$\frac{\partial \vec{u}}{\partial t}(x, y, t) = -\alpha \Delta^2 \vec{u}(x, y, t) + \vec{f}(\vec{u}(x, y, t))$$

with

$$\vec{f} = \left( r(x - u_x(x, y), y - u_y(x, y)) - s(x, y) \right) \\ \times \nabla \left( r(x - u_x, y - u_y) - s(x, y) \right)$$

## 2 Tumour Reconstruction (cont'd)

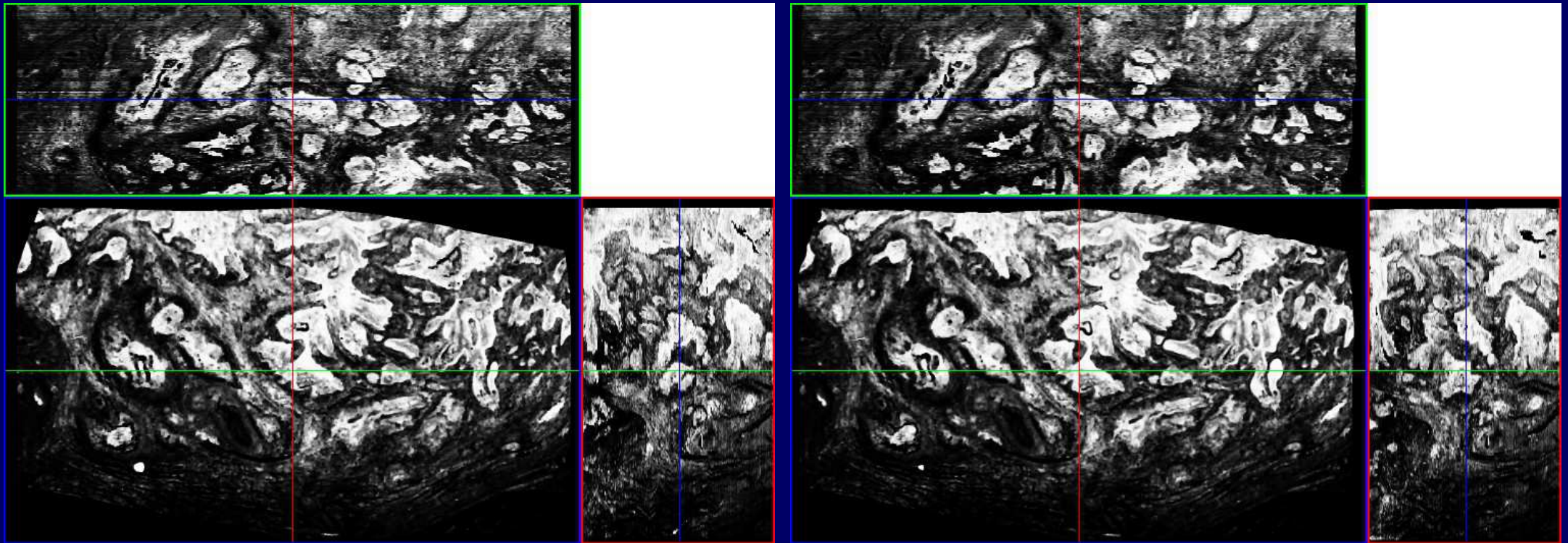
### *Non-linear Curvature-based Registration:*



Maximum displacement:  $36.2\mu\text{m}$ , minimum:  $0\mu\text{m}$ .

## 2 Tumour Reconstruction (cont'd)

### *Non-linear Curvature-based Registration:*



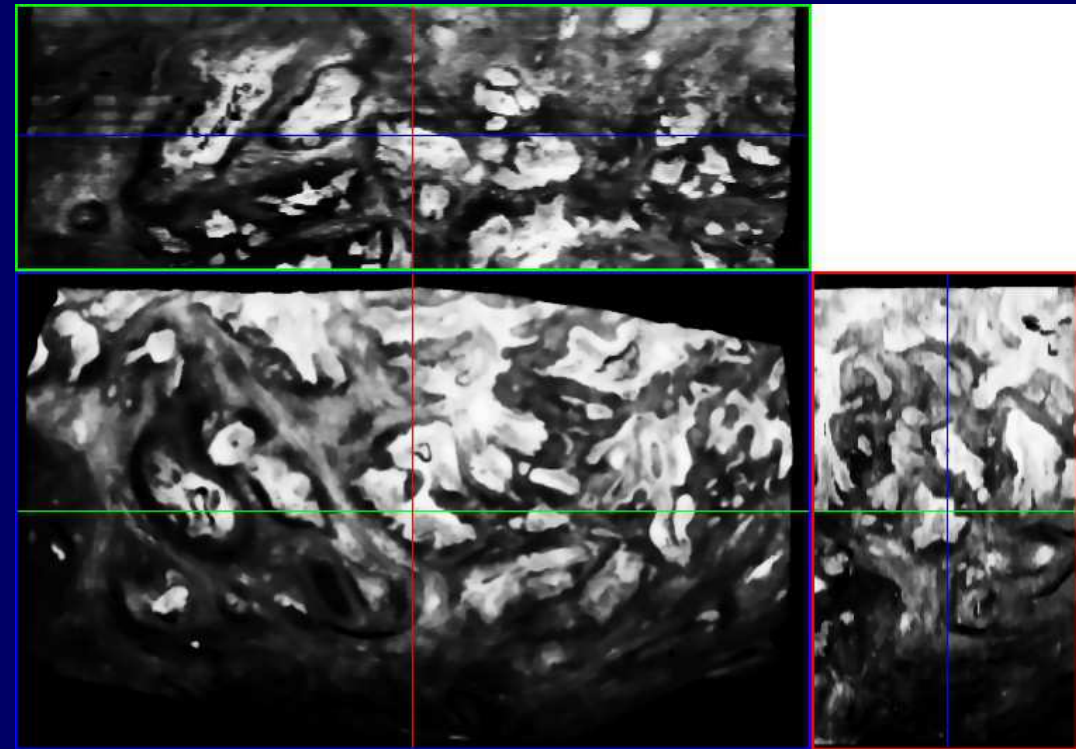
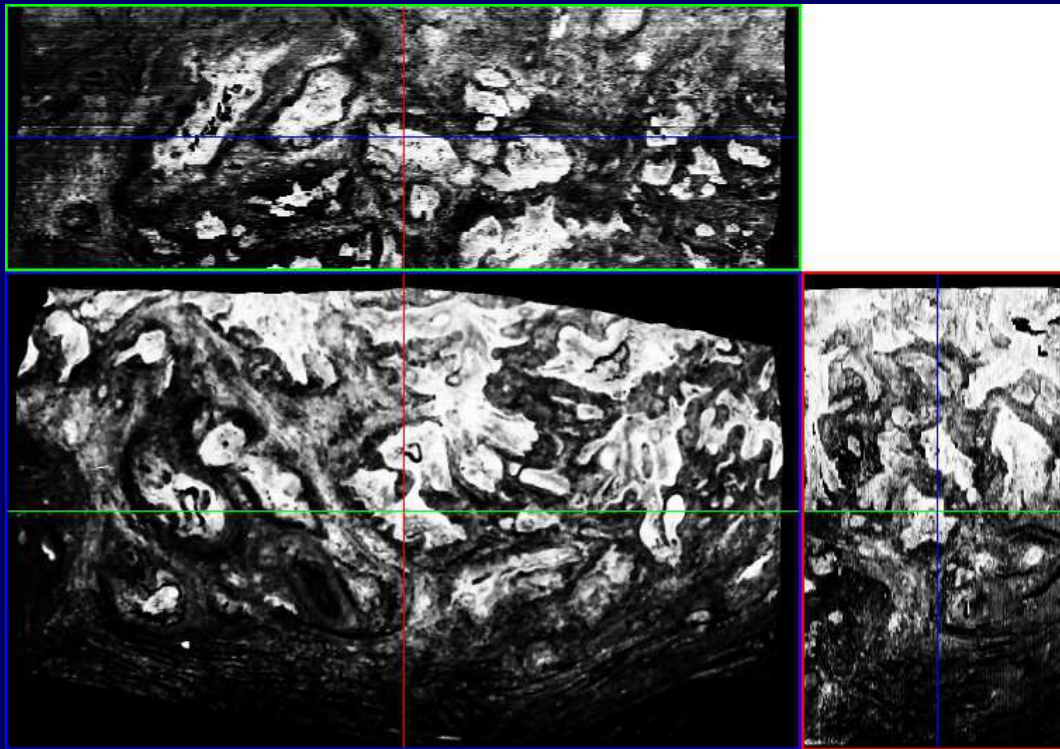
## 2 Tumour Reconstruction (cont'd)

### Total-Variation Filtering:

- non-linear, edge-preserving low-pass filtering
- $J[u] = \int_{\Omega} |\nabla u(r)| dr + \frac{\lambda}{2} \int_{\Omega} (u(r) - u^{(0)}(r))^2 dr \rightarrow \text{Min}$
- solution as time-dependant problem:  
$$\frac{\partial u}{\partial t}(r, t) = \nabla \frac{\nabla u(r, t)}{|\nabla u(r, t)|} + \lambda(u^{(0)}(r) - u(r, t))$$
- discrete solution has just one free parameter: assumed variance of noise

## 2 Tumour Reconstruction (cont'd)

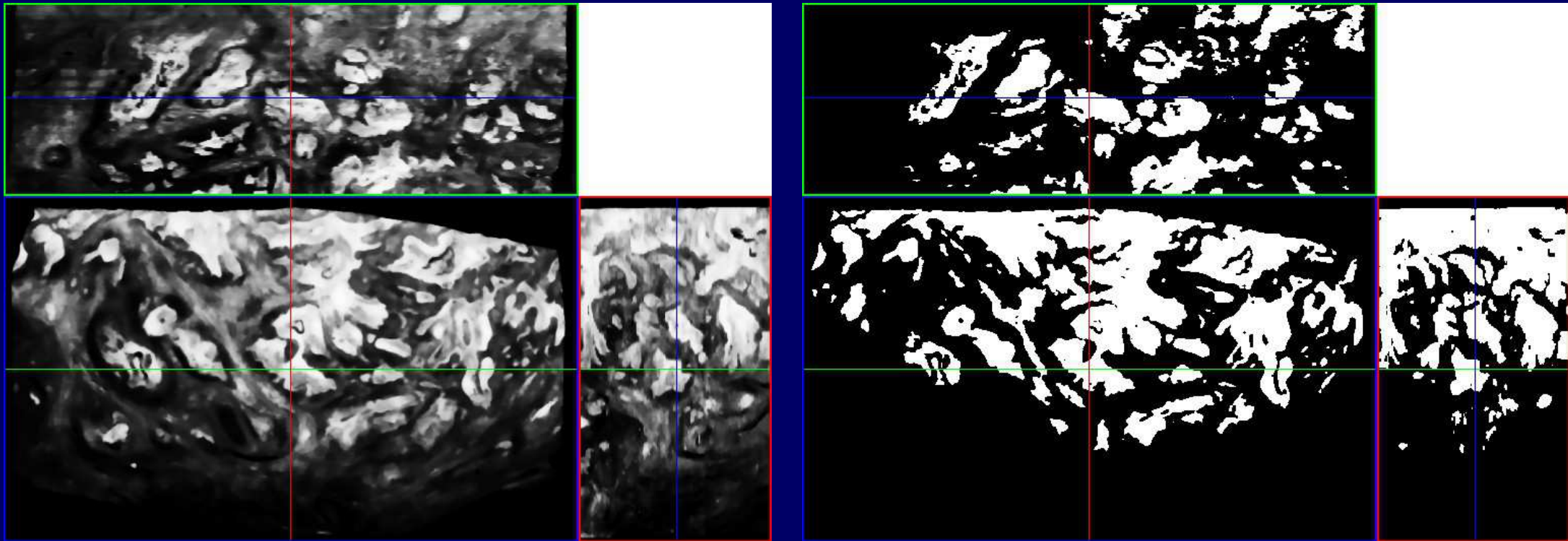
### Total-Variation Filtering:





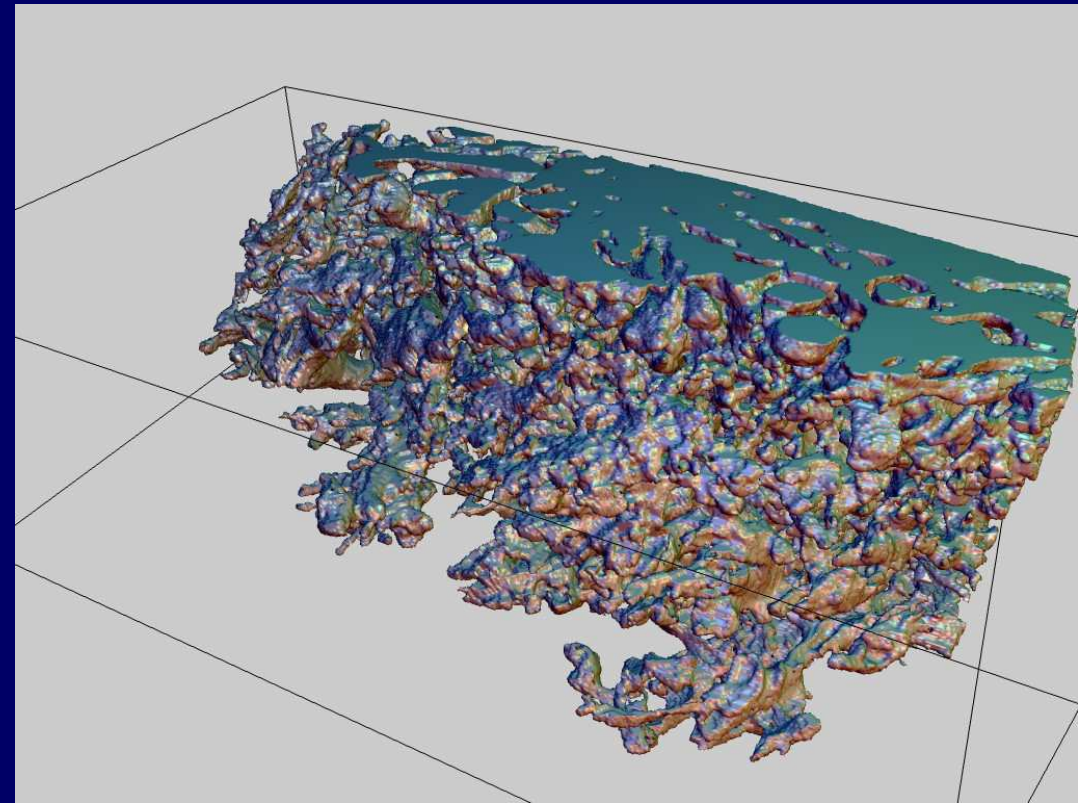
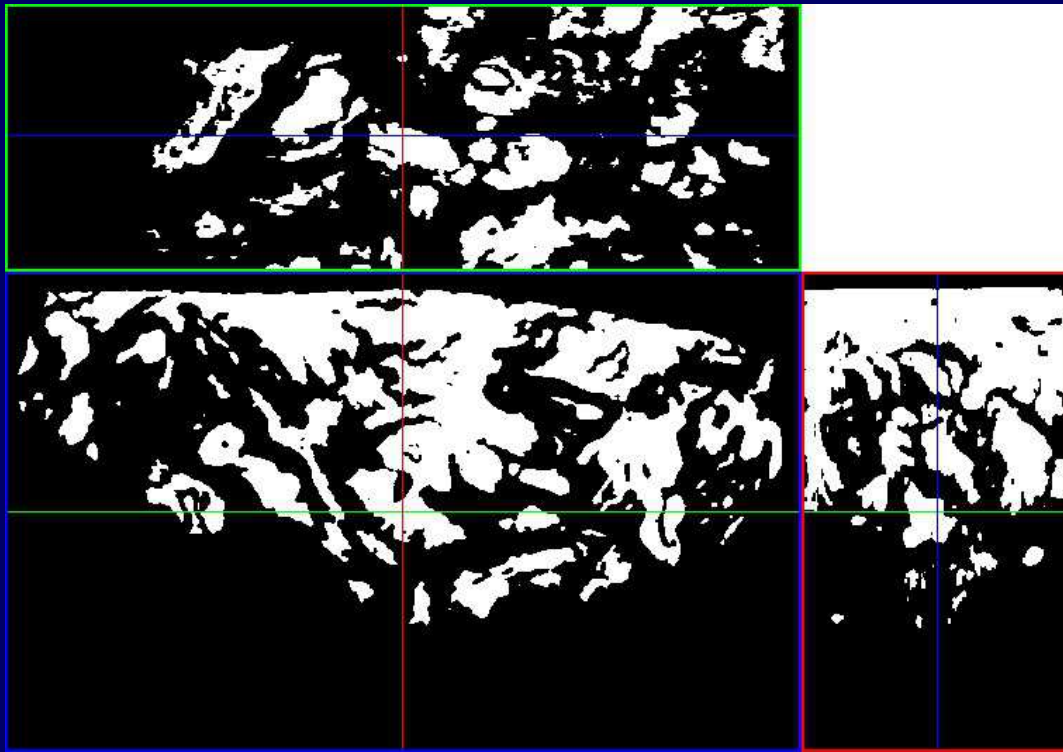
## 2 Tumour Reconstruction (cont'd)

### *Tumour Segmentation (Thresholding):*



# 3 Tumour Invasion Quantification

## Segmented Tumour / 3-D Surface Rendering:



### 3 Tumour Invasion Quantification (cont'd)

#### Ways:

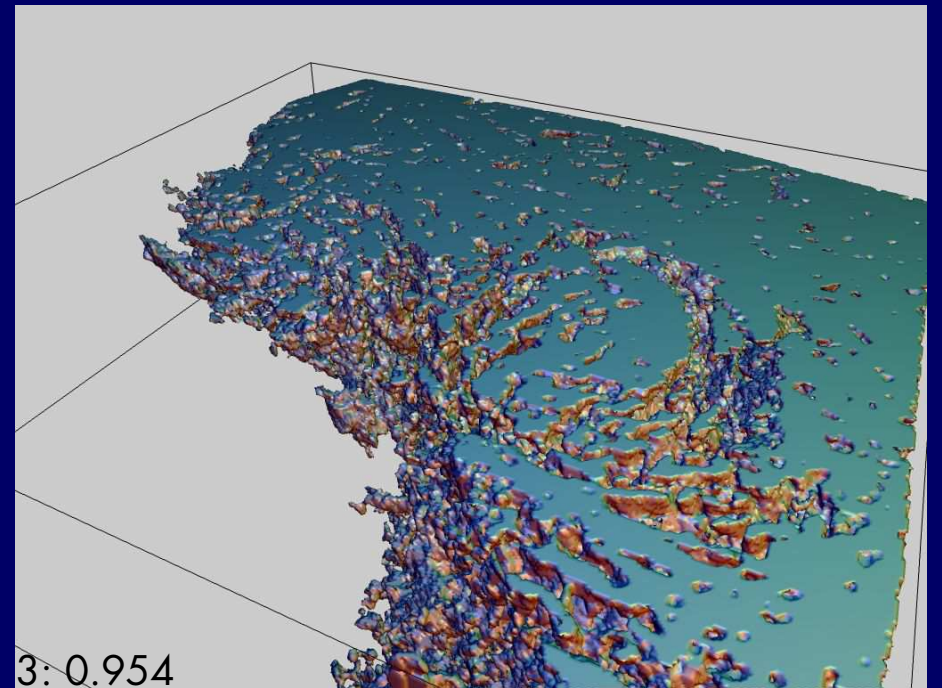
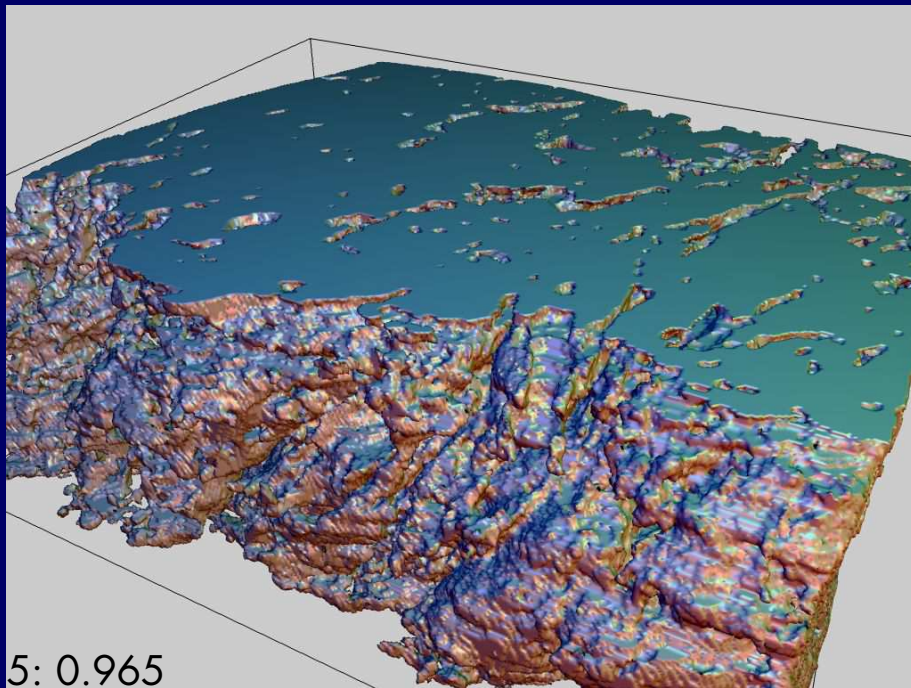
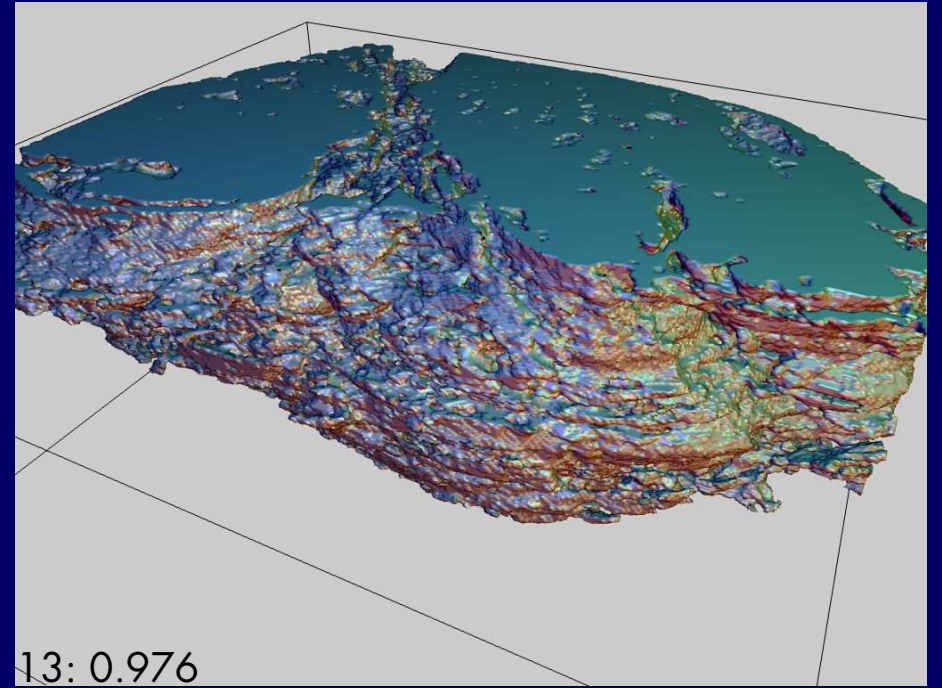
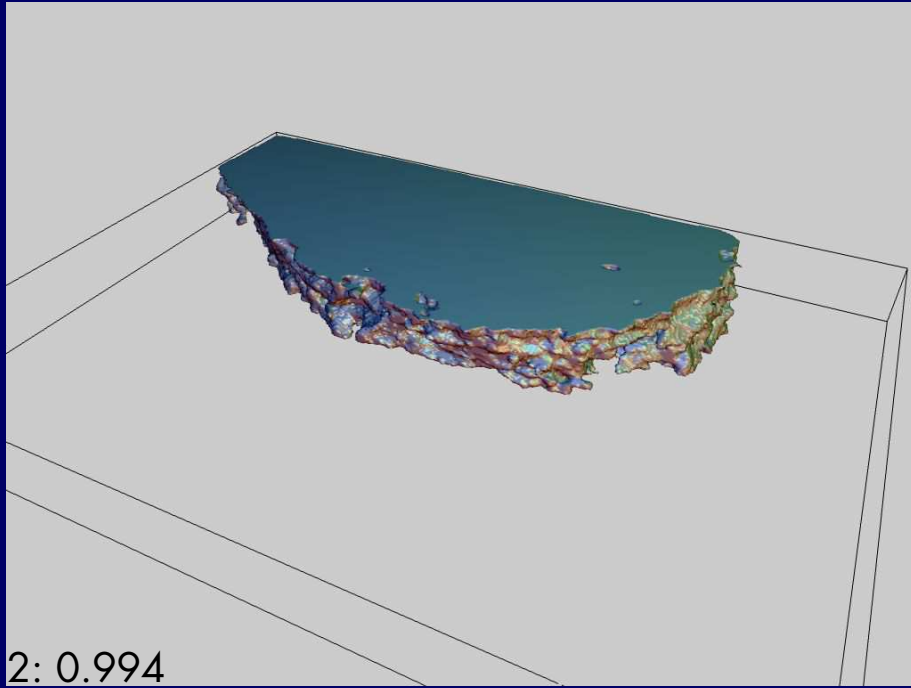
- differential-geometric surface properties
- fractal surface properties
- ...
- surface-volume ratios
- compactness:  $\frac{\text{surface}^3}{\text{volume}^2}$
- discrete compactness:  $C_D = \frac{A_C - A_{C_{\min}}}{A_{C_{\max}} - A_{C_{\min}}}$

# 4 Results

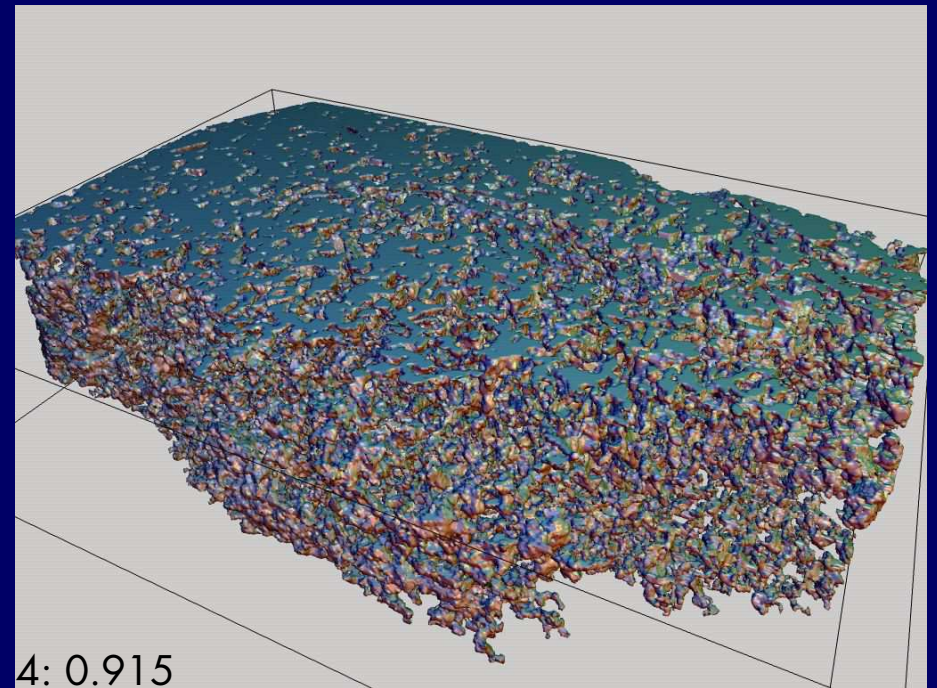
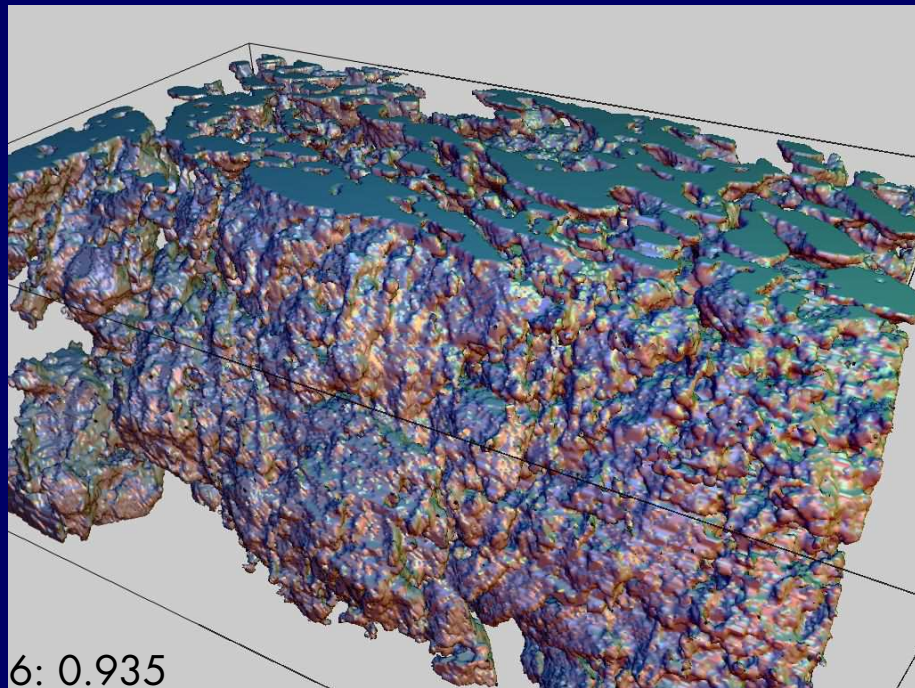
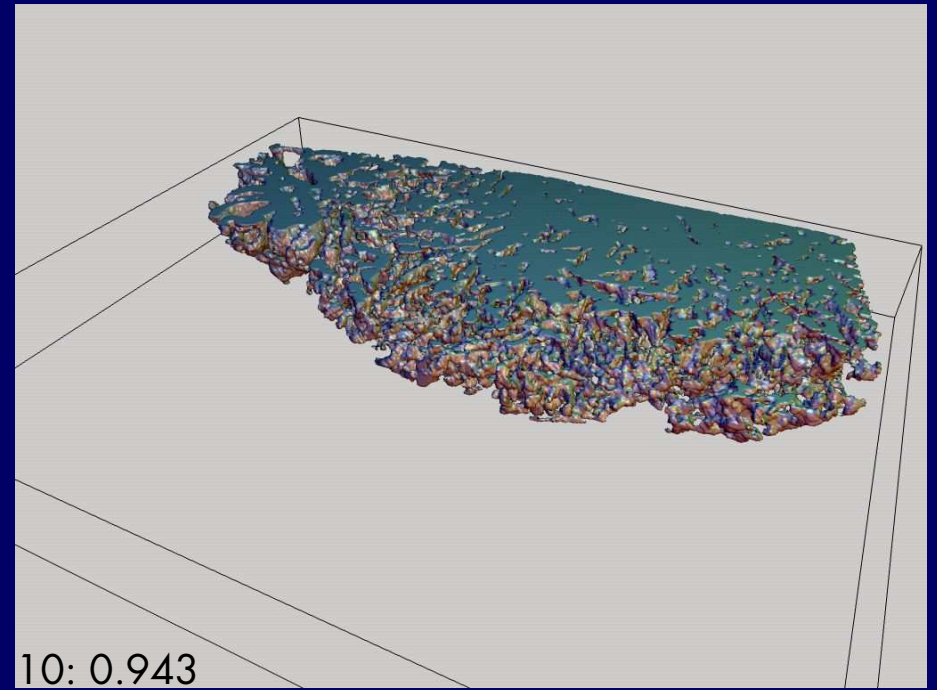
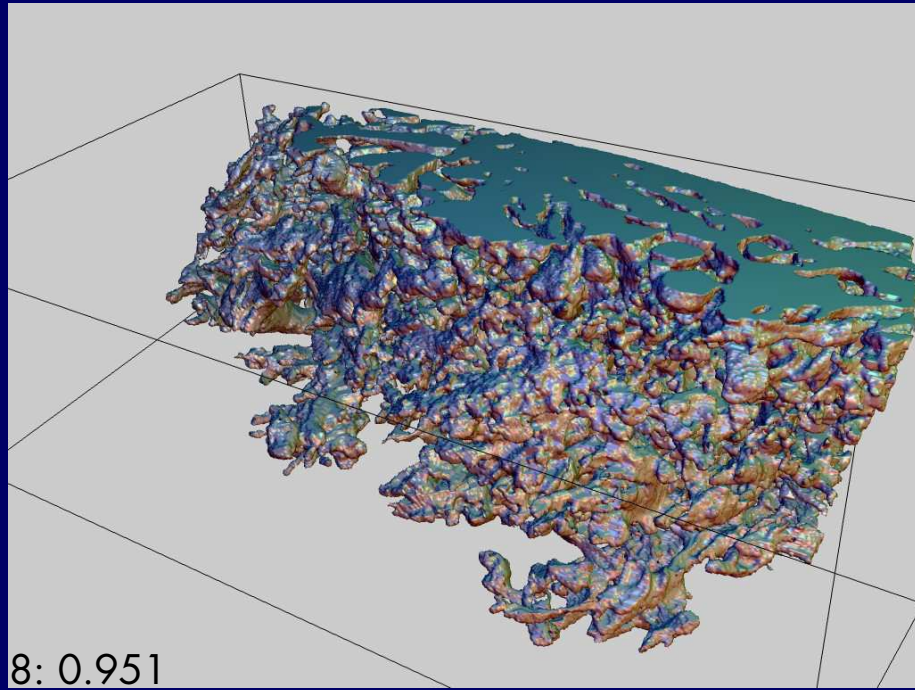
## Overview:

Specimen Number	Number of Slices	Slice Thickness [ $\mu\text{m}$ ]	Reconstructed Volume [ $\text{mm}^3$ ]	Mean Residual Error [ $\mu\text{m}$ ]			Discrete Compactness
				Rigid R.	Polyn. R.	Curv. R.	
1	96	5	30.1	20.1	12.9	5.9 (9.6)	0.884
2	90	6	16.7	13.2	7.5	7.1 (12.9)	0.995
3	230	10	146.1	15.7	7.8	5.6 (11.5)	0.954
4	230	10	133.6	12.9	5.9	3.5 (6.6)	0.915
5	250	10	130.8	10.7	9.8	6.2 (6.8)	0.966
6	300	10	104.7	10.9	7.0	3.9 (5.4)	0.935
7	250	10	148.9	14.7	7.7	4.8 (9.0)	0.906
8	300	10	146.8	10.6	5.9	3.5 (6.3)	0.951
9	150	10	100.5	15.9	8.6	5.0 (8.7)	0.881
10	100	10	62.8	14.1	7.5	5.2 (10.4)	0.944
11	301	10	143.4	11.5	8.0	5.9 (9.2)	0.892
12	260	10	123.8	15.2	10.3	7.5 (11.7)	0.902
13	500	5	89.3	13.0	7.5	5.1 (9.1)	0.976

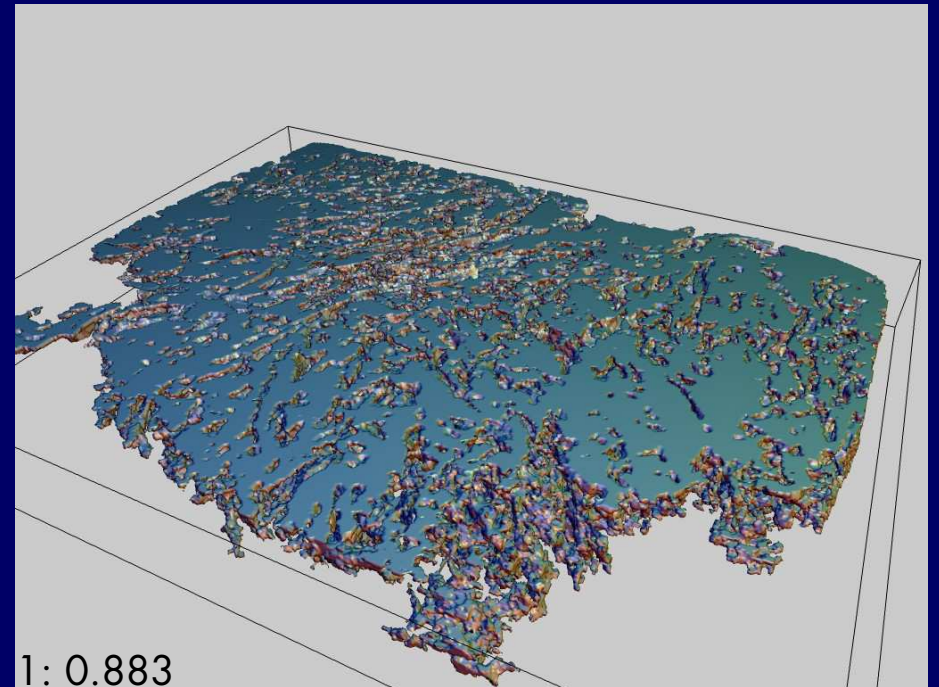
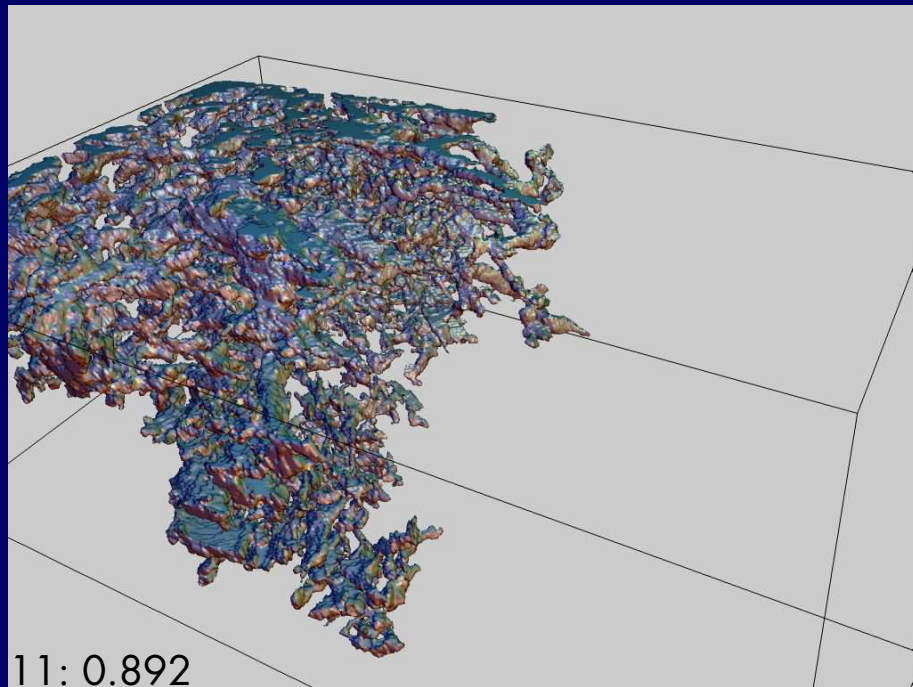
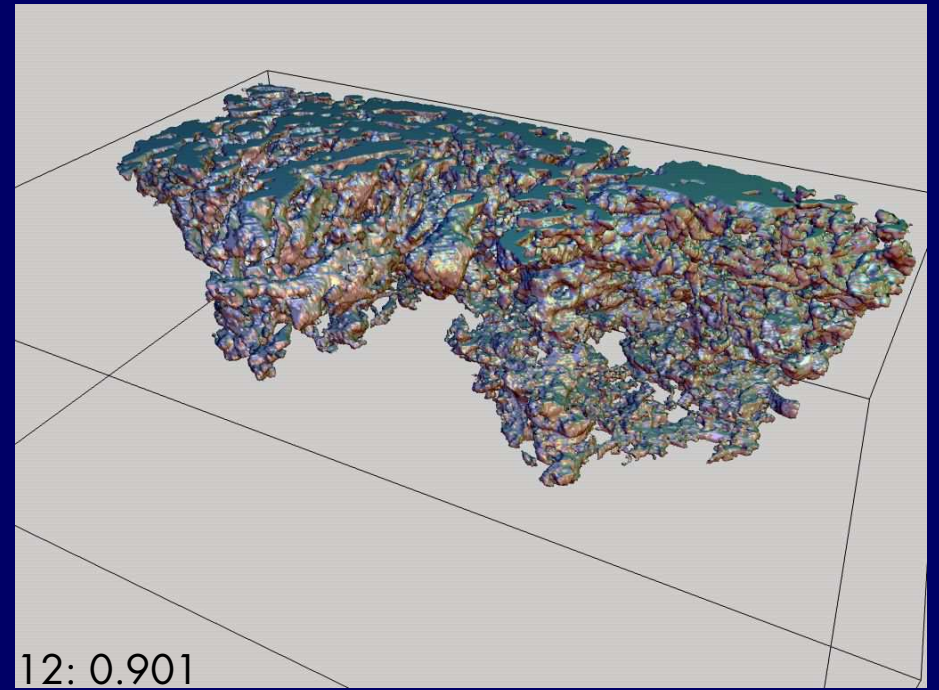
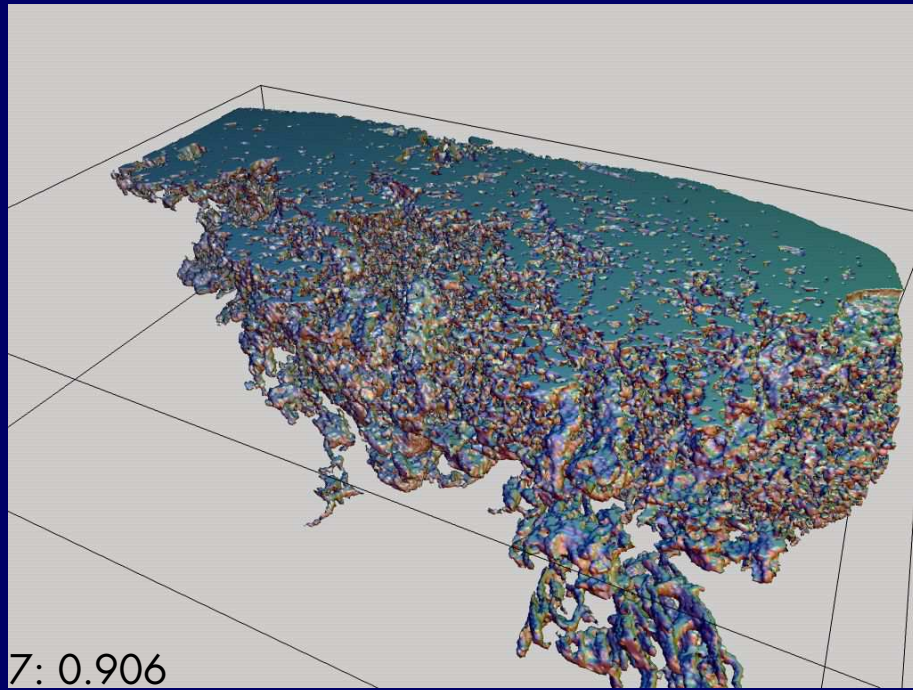
# 5 Results (cont'd)



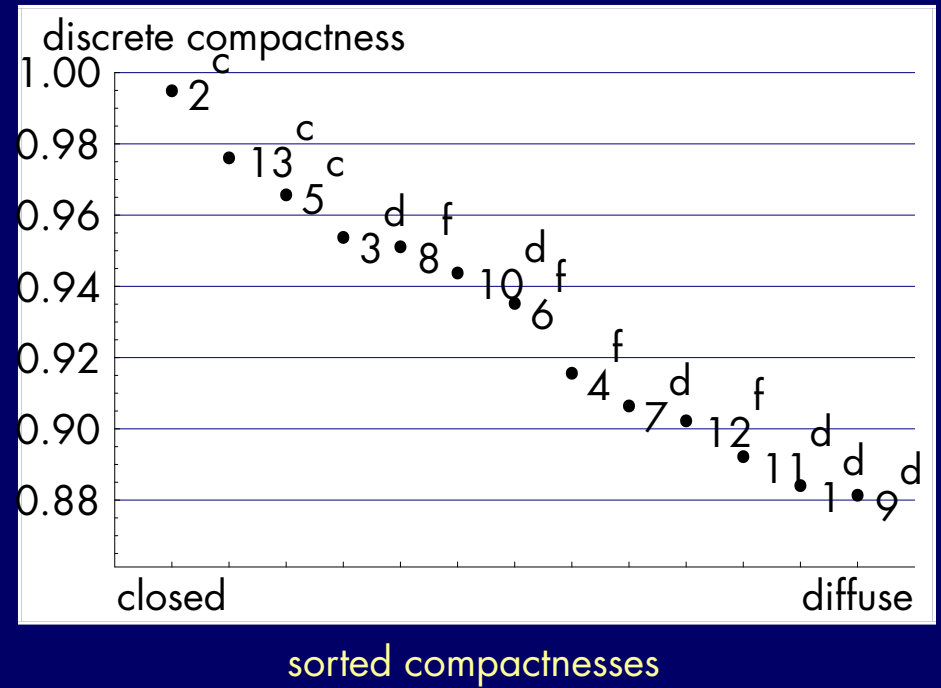
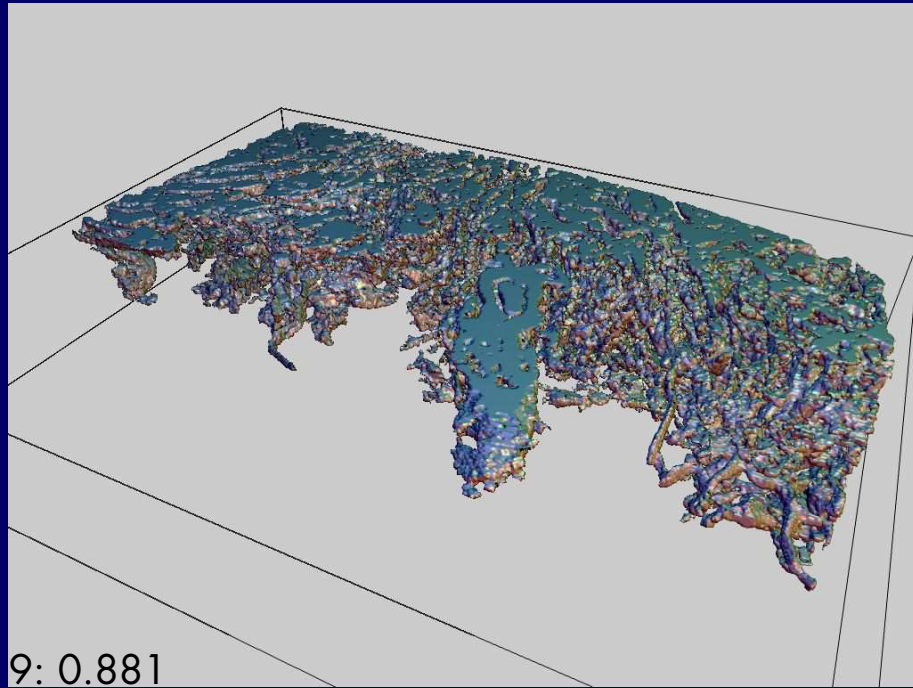
# 5 Results (cont'd)



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# 5 Results (cont'd)





## 6 Conclusions I

- 3-D reconstruction feasible at  $10\mu\text{m}$  resolution
- invasion 'per continuitatem', no separated islets
- invasion patterns form a 'continuum' of compactnesses
- compactness basically corresponds to pathologist's assessment

# Movie

# 7 Clinical Applicability?

***Main Problem: 3-D Reconstruction Complexity***

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# 7 Clinical Applicability?

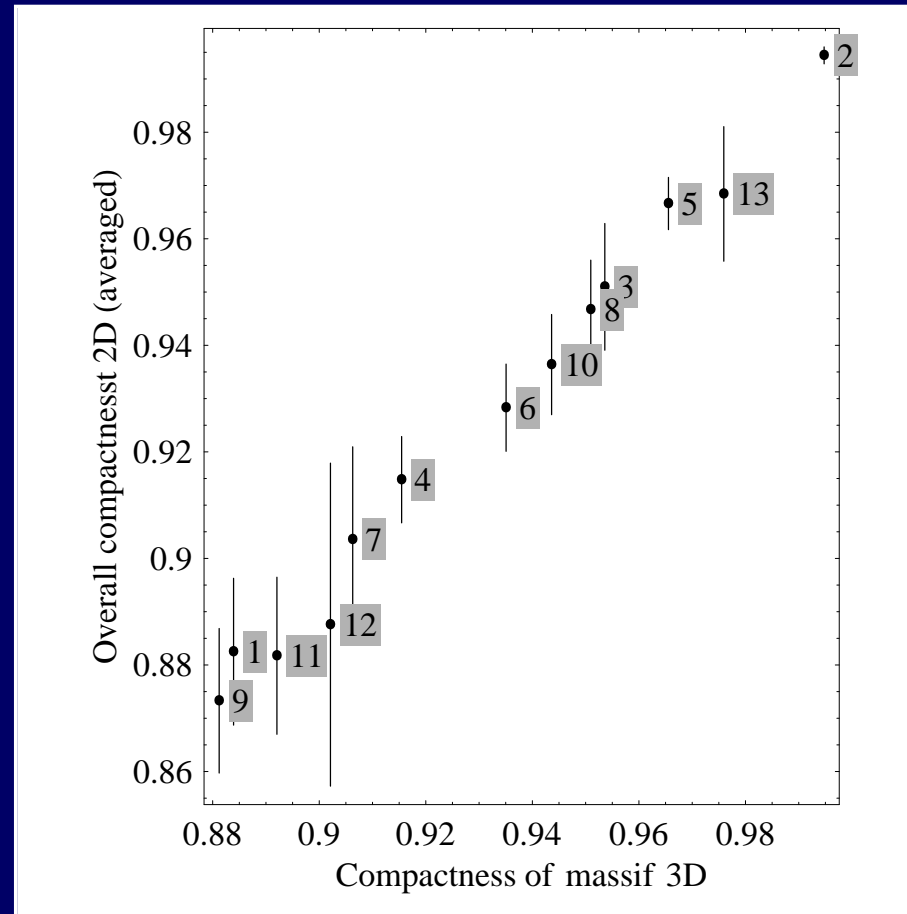
**Main Problem:** 3-D Reconstruction Complexity

**Trade-Off:** Options for 2-D?

**Comparison:** Compactness 3-D vs. 2-D

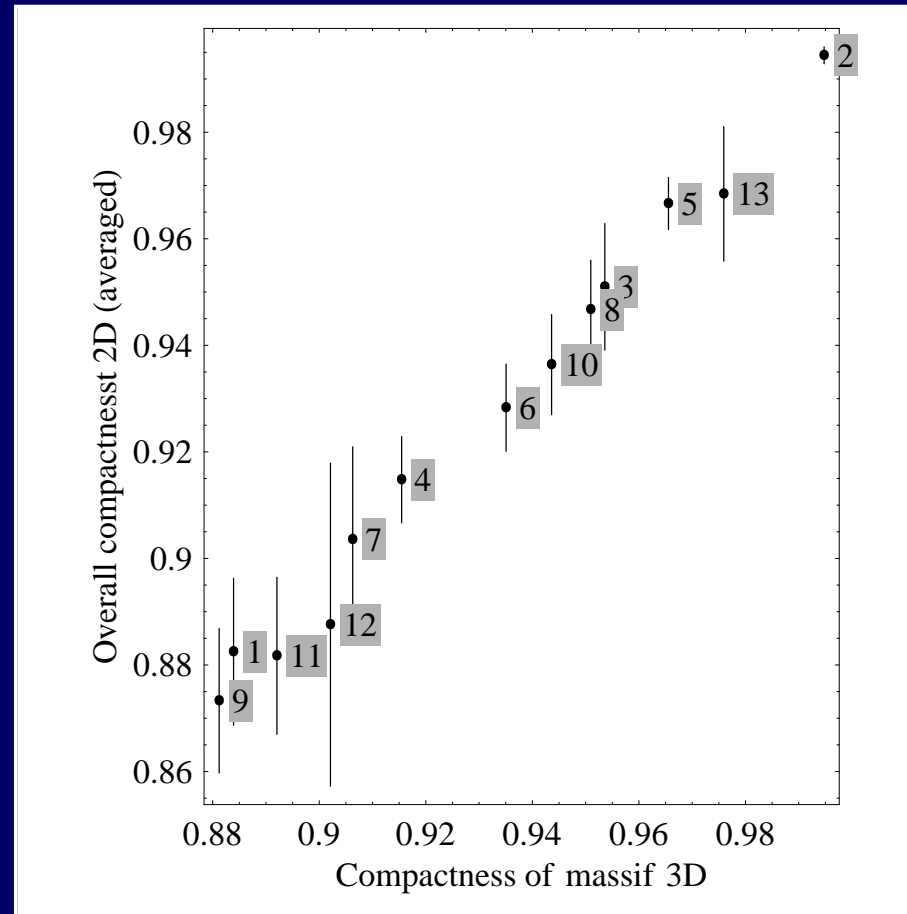
# 7 Clinical Applicability? (cont'd)

## Comparison:



# 7 Clinical Applicability? (cont'd)

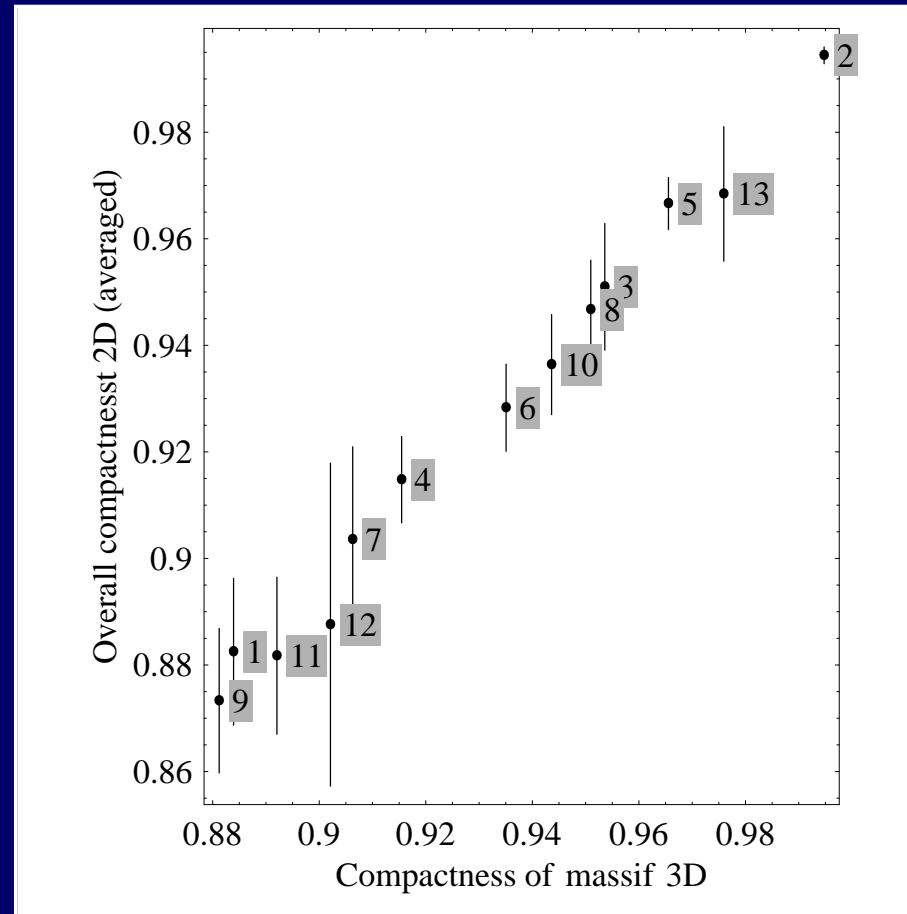
## Comparison:



Correlation Coefficient: 0.994

# 7 Clinical Applicability? (cont'd)

## Comparison:



Correlation Coefficient: 0.994  $\rightsquigarrow$  practically equivalent



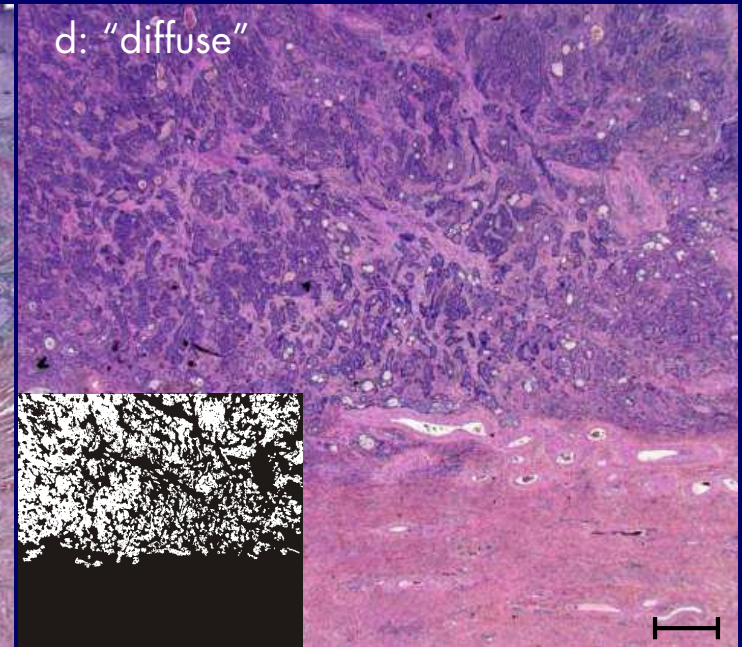
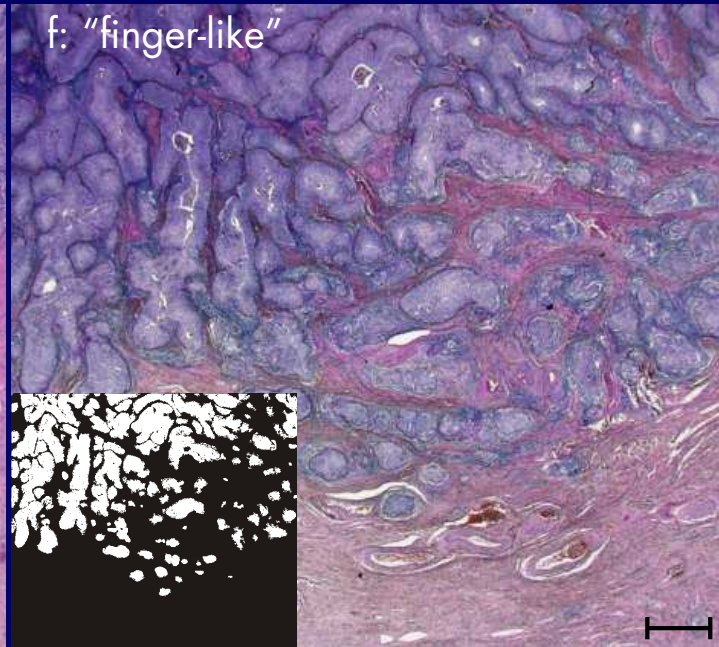
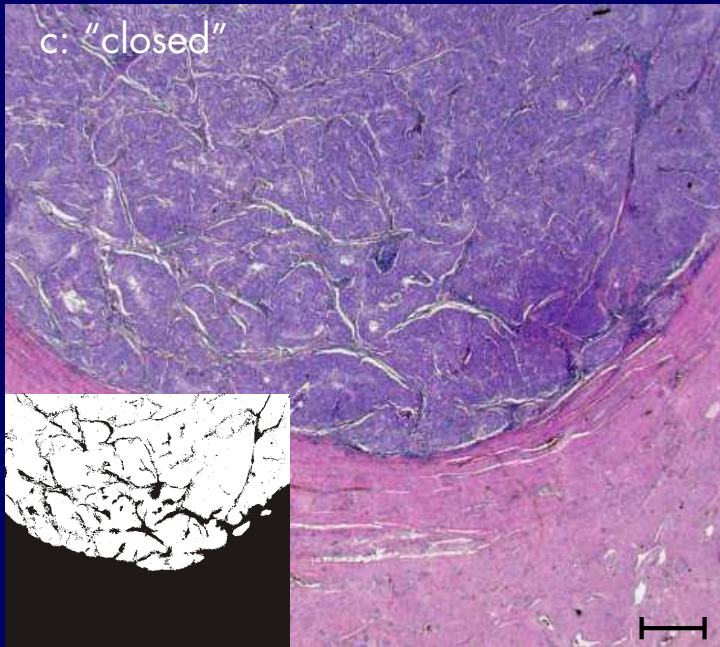
# 7 Clinical Applicability? (cont'd)

## *Analysis of 76 Cases:*

# 7 Clinical Applicability? (cont'd)

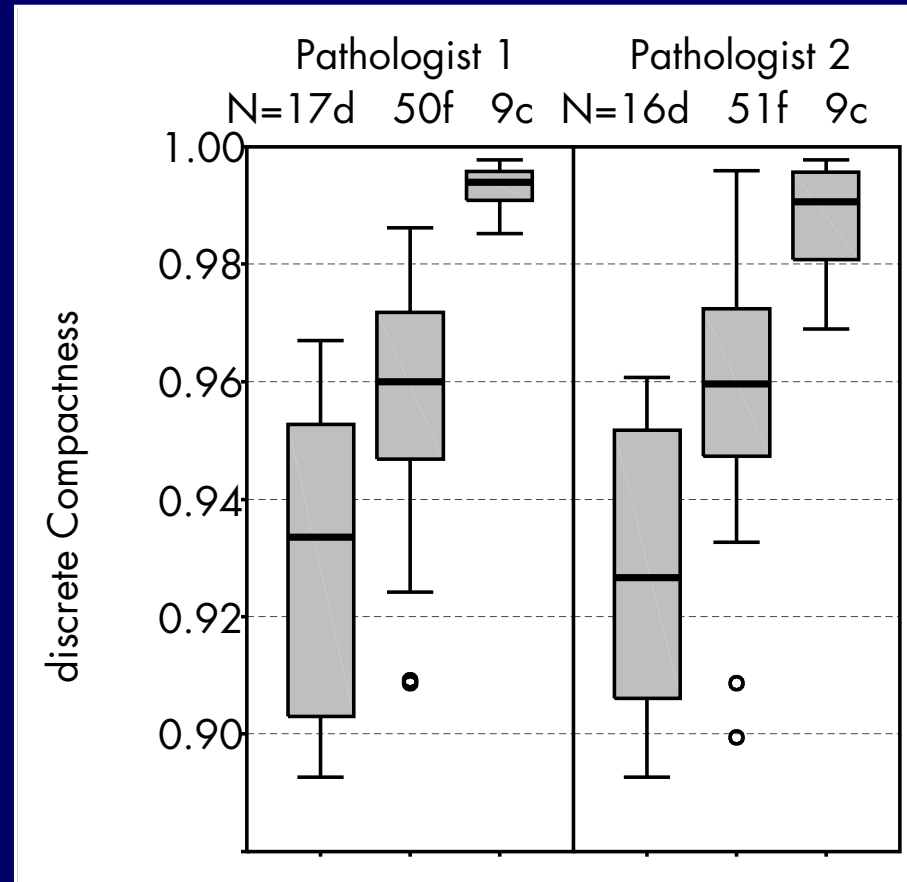
## Analysis of 76 Cases:

### Examples



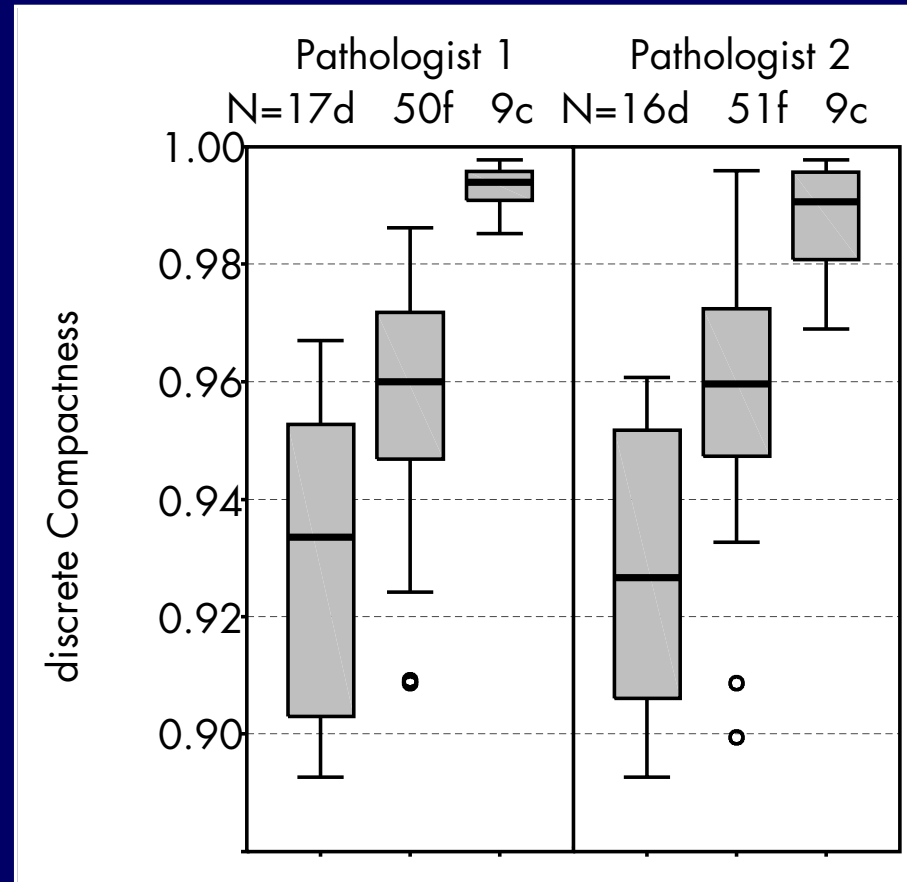
# 7 Clinical Applicability? (cont'd)

## Analysis of 76 Cases:



# 7 Clinical Applicability? (cont'd)

## Analysis of 76 Cases:



Compactness intervals significantly different ( $p \leq 0.0001$ )

# 7 Clinical Applicability? (cont'd)

## Analysis of 76 Cases: Results

- Parametrial involvement vs. Compactness:  
present/not present: 23/53, medians: 0.9478/0.9637,  
 $p \leq 0.028$
- Lymphatic vessel invasion vs. Compactness:  
present/not present: 59/17, medians: 0.9559/0.9661,  
 $p \leq 0.033$
- $\forall$  other characteristics no non-random compactness differences:  
age (35a), pT, pN, rel. tumour invasion depth, G, V, inflamm.  
reaction, recurrence (5a)

# 7 Clinical Applicability? (cont'd)

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# 7 Clinical Applicability? (cont'd)

## Analysis of 76 Cases: Results

- Parametrial involvement vs. Compactness:  
present/not present: 23/53, medians: 0.9478/0.9637,  
 $p \leq 0.028$
- Lymphatic vessel invasion vs. Compactness:  
present/not present: 59/17, medians: 0.9559/0.9661,  
 $p \leq 0.033$
- $\forall$  other characteristics no non-random compactness differences:  
age (35a), pT, pN, rel. tumour invasion depth, G, V, inflamm.  
reaction, recurrence (5a)

## 7 Clinical Applicability? (cont'd)

### ***Analysis of 76 Cases: Interpretation***

Lower compactness for present parametrial involvement and lymphatic vessel invasion: *diffuse invasion forms*



## 7 Clinical Applicability? (cont'd)

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- faster penetration of cervical stroma
  - more frequent affection of lymphatic vessels
- ~> discrete compactness might represent some *motile phenotype* (in analogy to micro vessel density as angiogenic phenotype)

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## 8 Conclusions II

- discrete compactness realisable & meaningful for tumour invasion quantification
- illustrative morphometric measure
- simple procedure, fully automatable

# 9 Advanced Tumour Reconstruction & Analysis

## 9 Advanced Tumour Reconstruction & Analysis (cont'd)

- Specific question:
  - the spatial organization of a cervical cancer
  - ⇒ the relation of the tumor invasion front vs. the infiltration with  $CD3^+$  T-cells.

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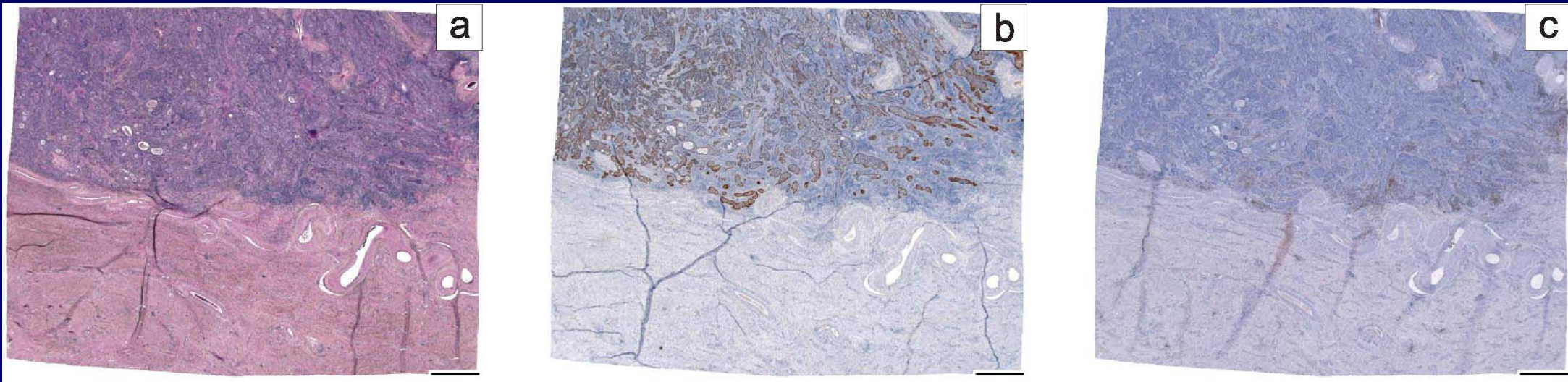
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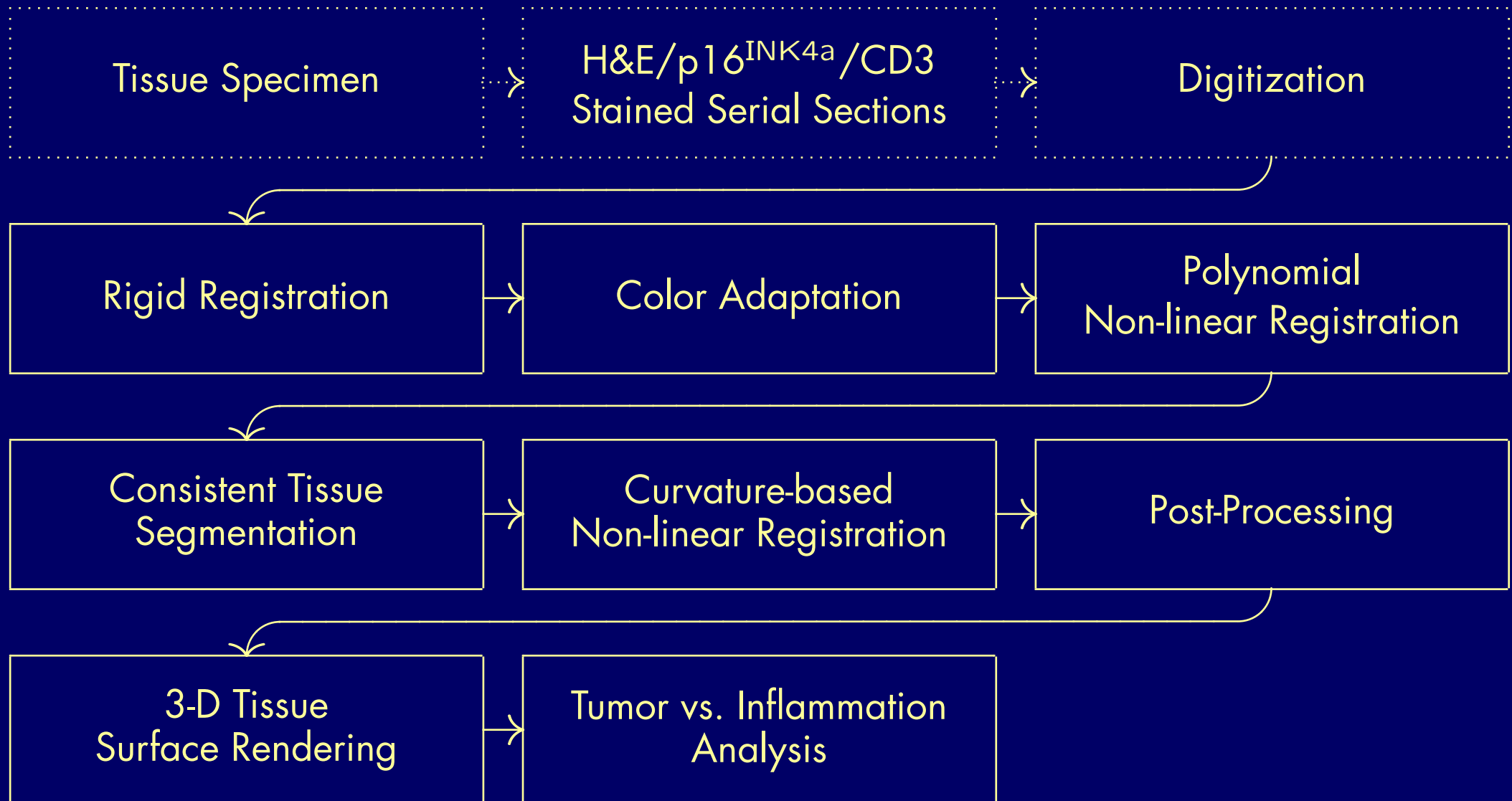
## 9 Advanced Tumour Reconstruction & Analysis (cont'd)

- Cervical squamous cell carcinoma specimen
  - serial section with 84 slices, three interleaving subsets stained with
    - a H&E (routine reference stain)
    - b the cervical carcinoma biomarker p16<sup>INK4a</sup>
    - c the T-cell marker CD3



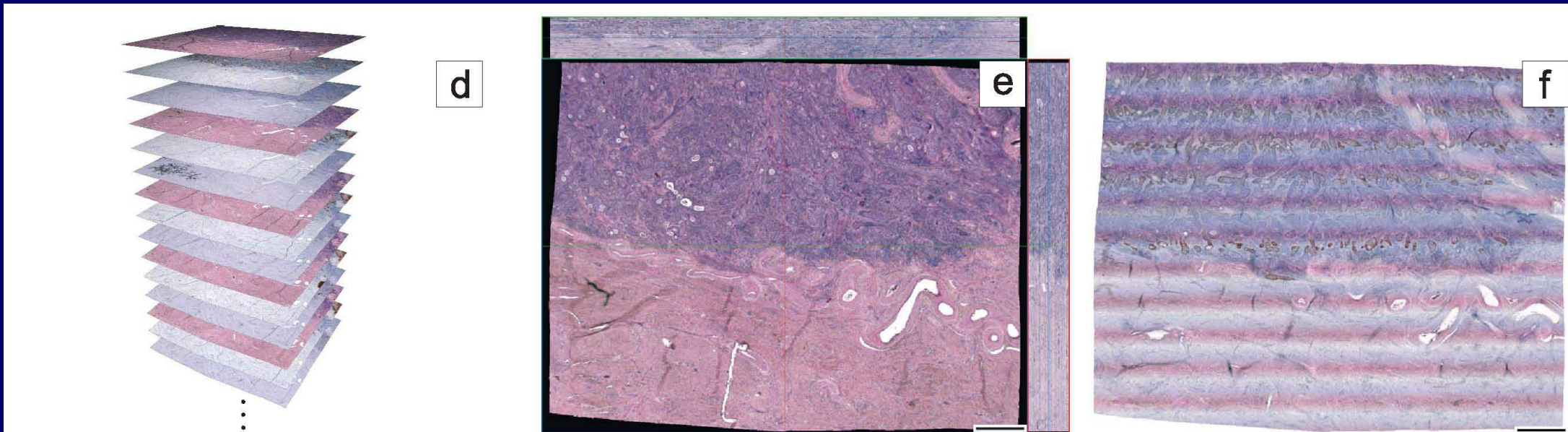
# 9 Advanced Tumour Reconstruction & Analysis (cont'd)

- Adapted Image Processing Chain



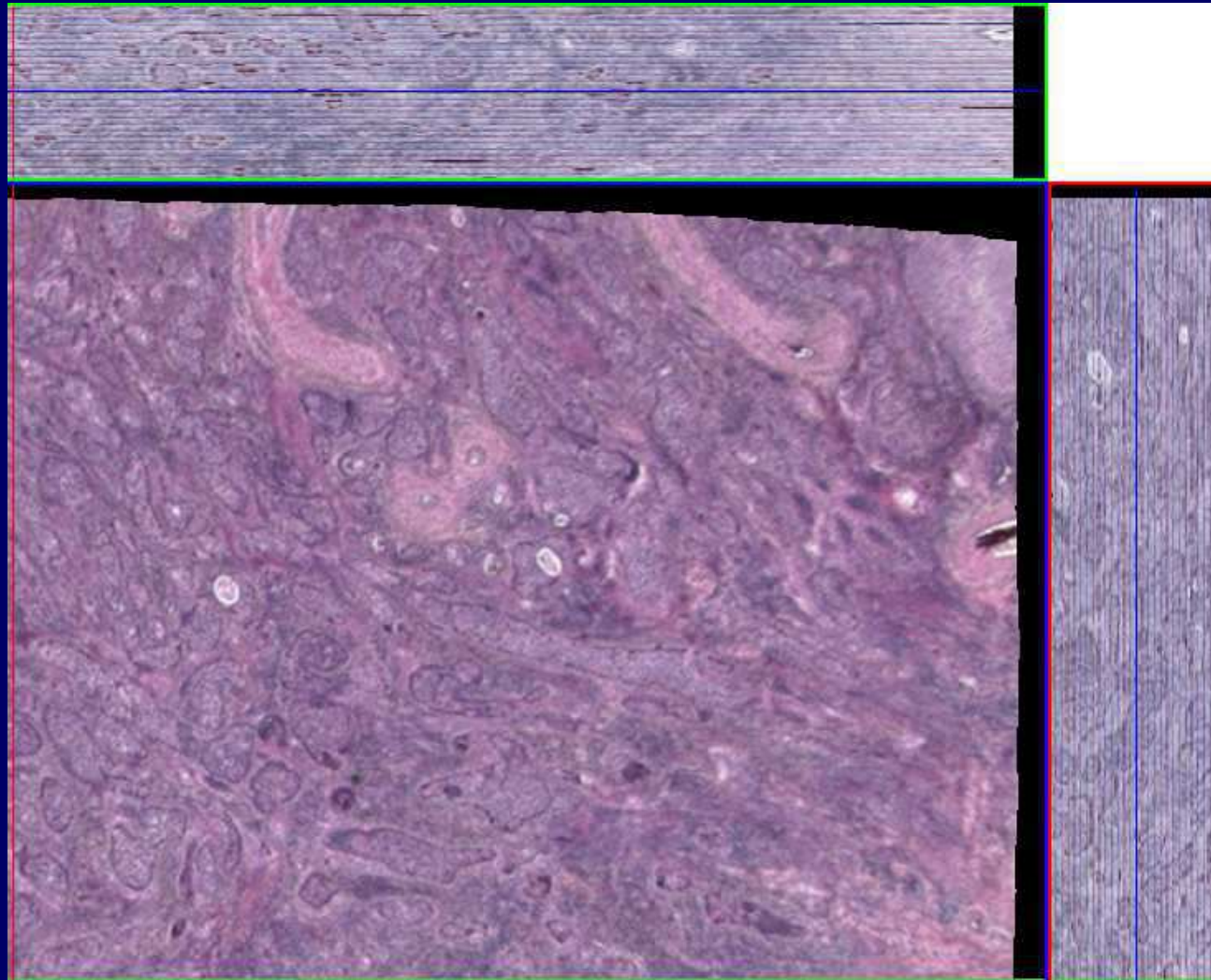
# 9 Advanced Tumour Reconstruction & Analysis (cont'd)

- 3-D Tissue Reconstruction



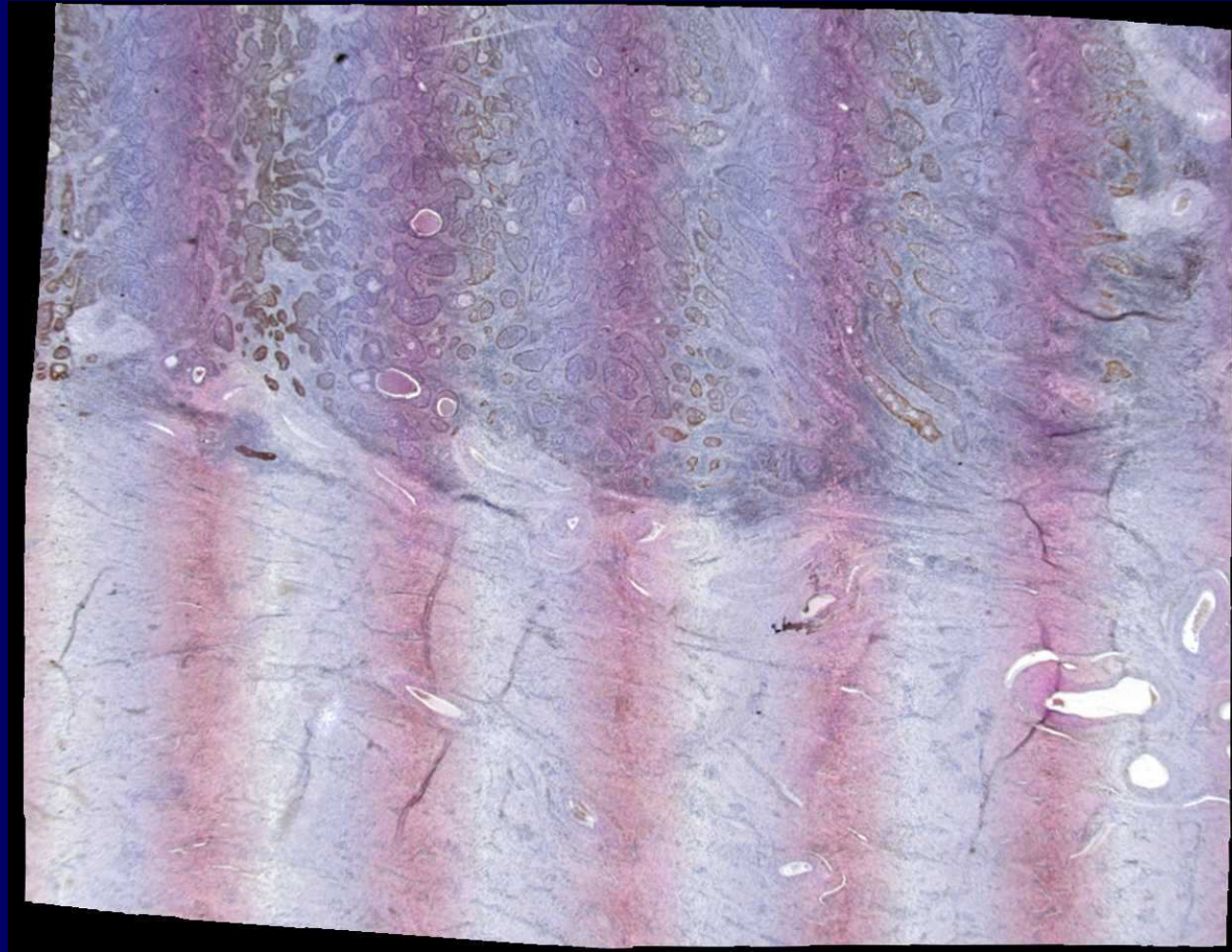
# 9 Advanced Tumour Reconstruction & Analysis (cont'd)

- 3-D Tissue Reconstruction



# 9 Advanced Tumour Reconstruction & Analysis (cont'd)

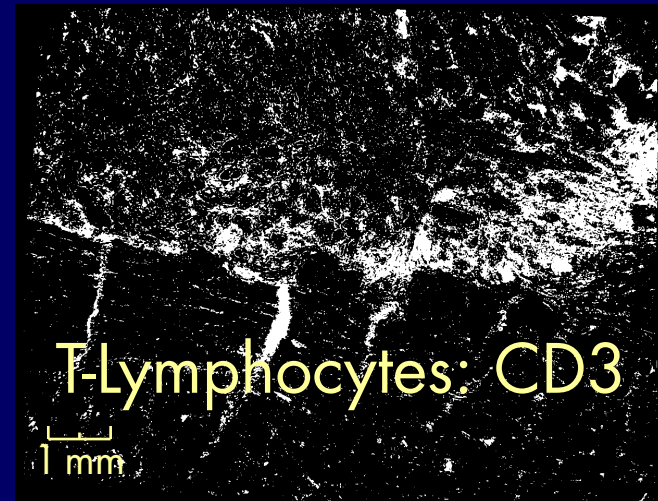
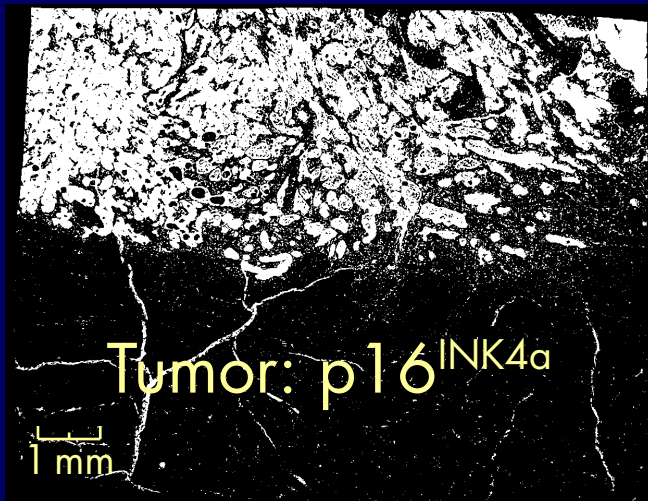
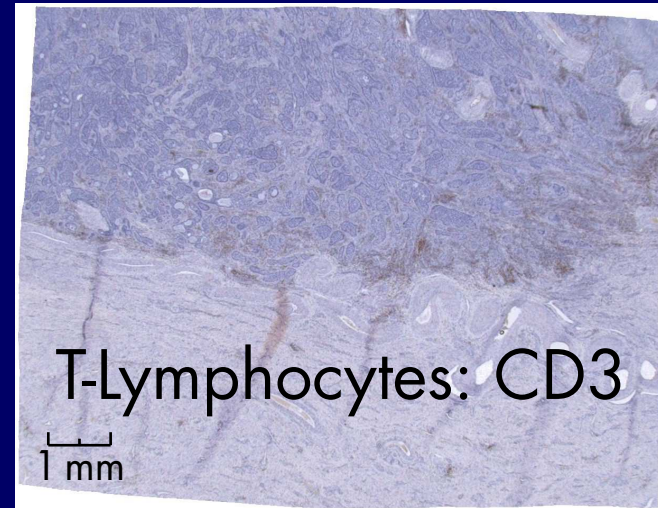
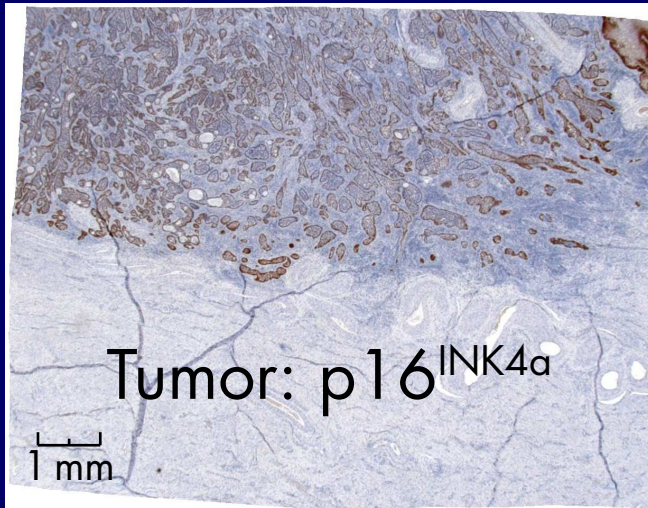
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# Movie

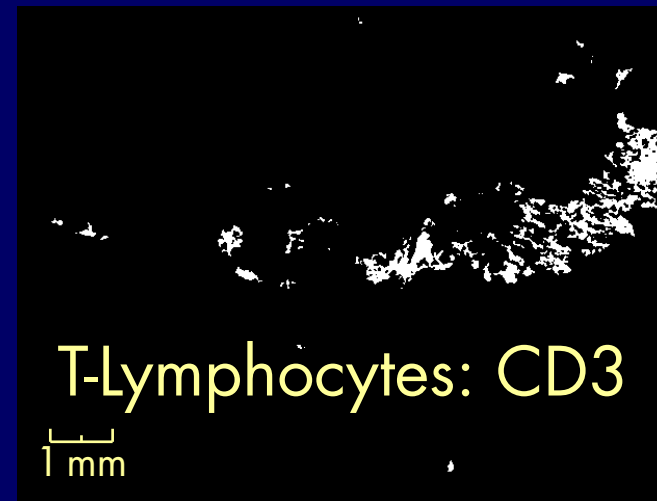
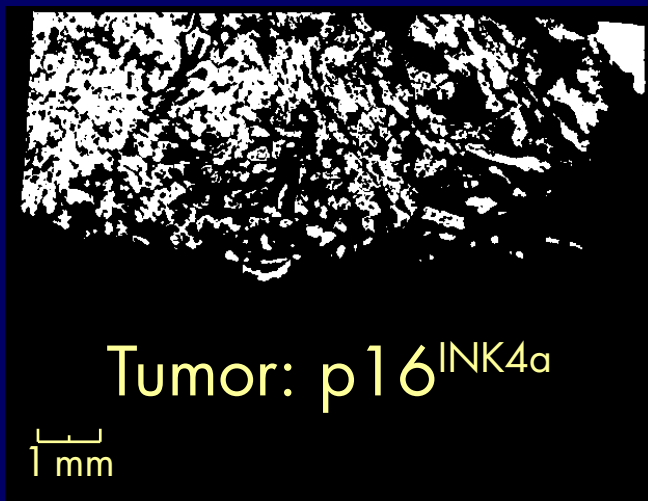
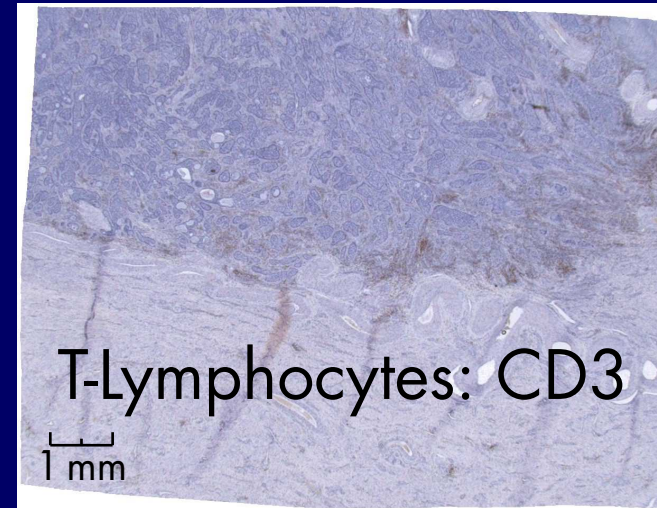
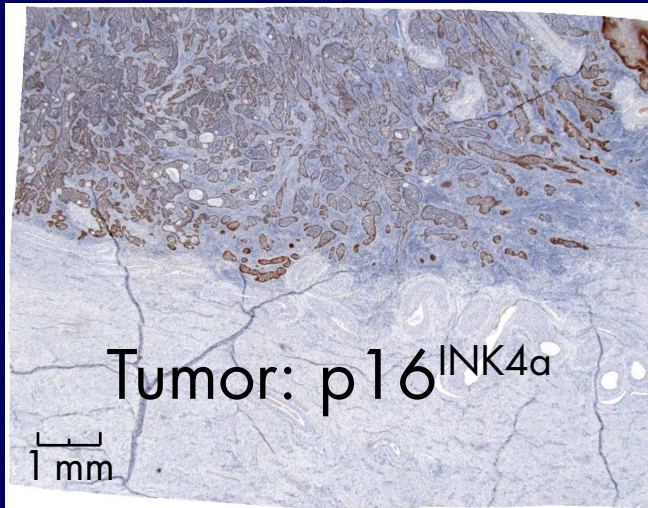
# 9 Advanced Tumour Reconstruction & Analysis (cont'd)

- Automatic Segmentation Examples



# 9 Advanced Tumour Reconstruction & Analysis (cont'd)

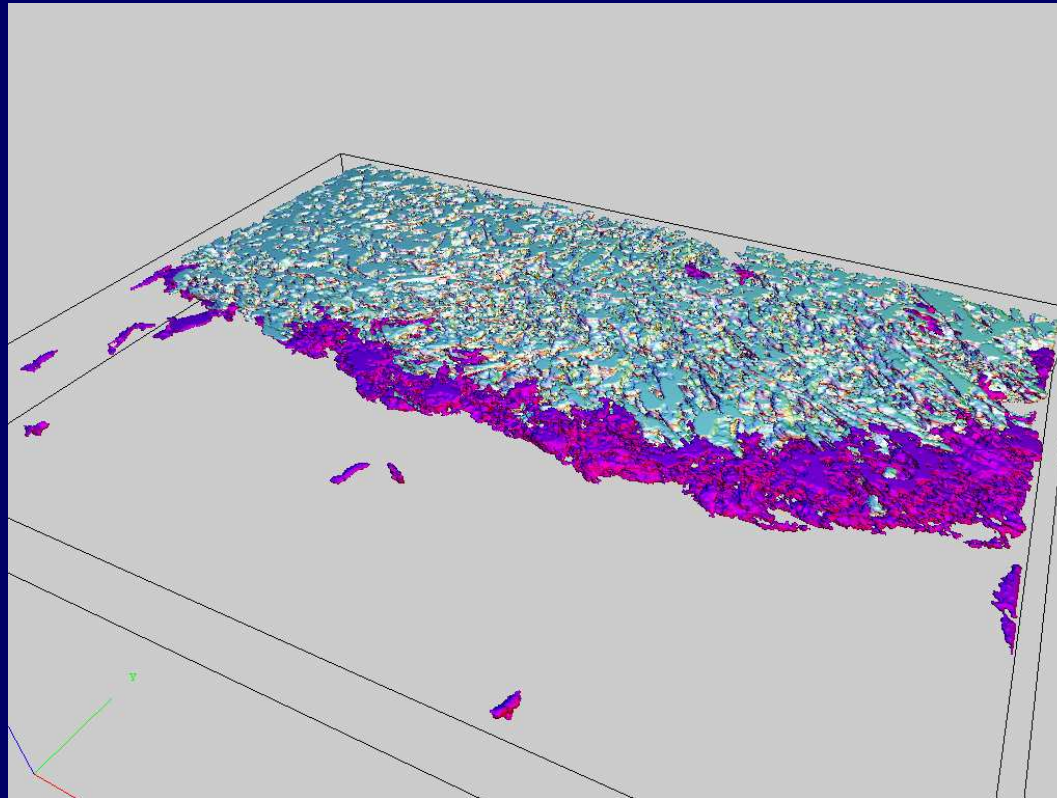
- Automatic Segmentation Examples: Post-Processing





## 9 Advanced Tumour Reconstruction & Analysis (cont'd)

- 3-D Reconstruction results: Surface rendering



Overall reconstructed tissue volume:  $60.9\text{mm}^3$ , Tumor Compactness: 0.89, Tumor vol.:  $11.6\text{mm}^3$ , T-Lymphocyte vol.:  $1.1\text{mm}^3$

# Movie

## 9 Advanced Tumour Reconstruction & Analysis (cont'd)

- How to do a **local** tumor invasion front analysis:

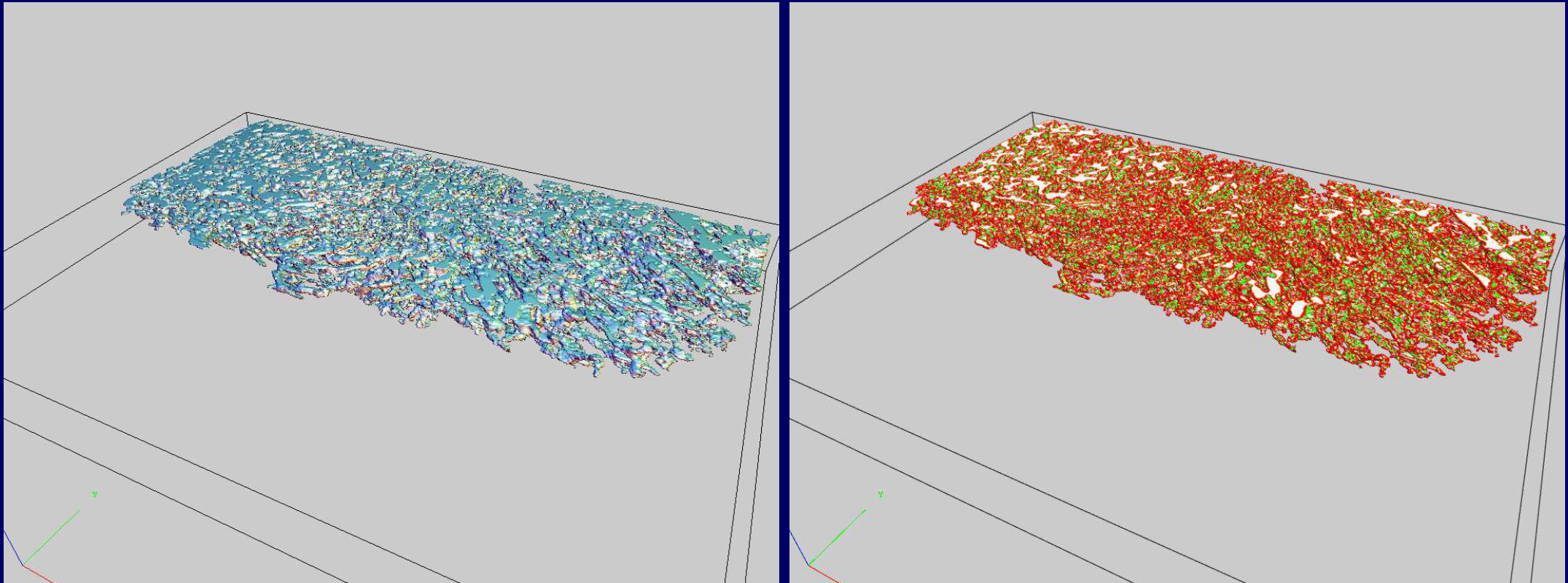
**Mean surface curvature**, related to

→ the respective local minimum tumor to T-cell distance

→ a T-cell originated diffusing substance's concentration at the tumor surface

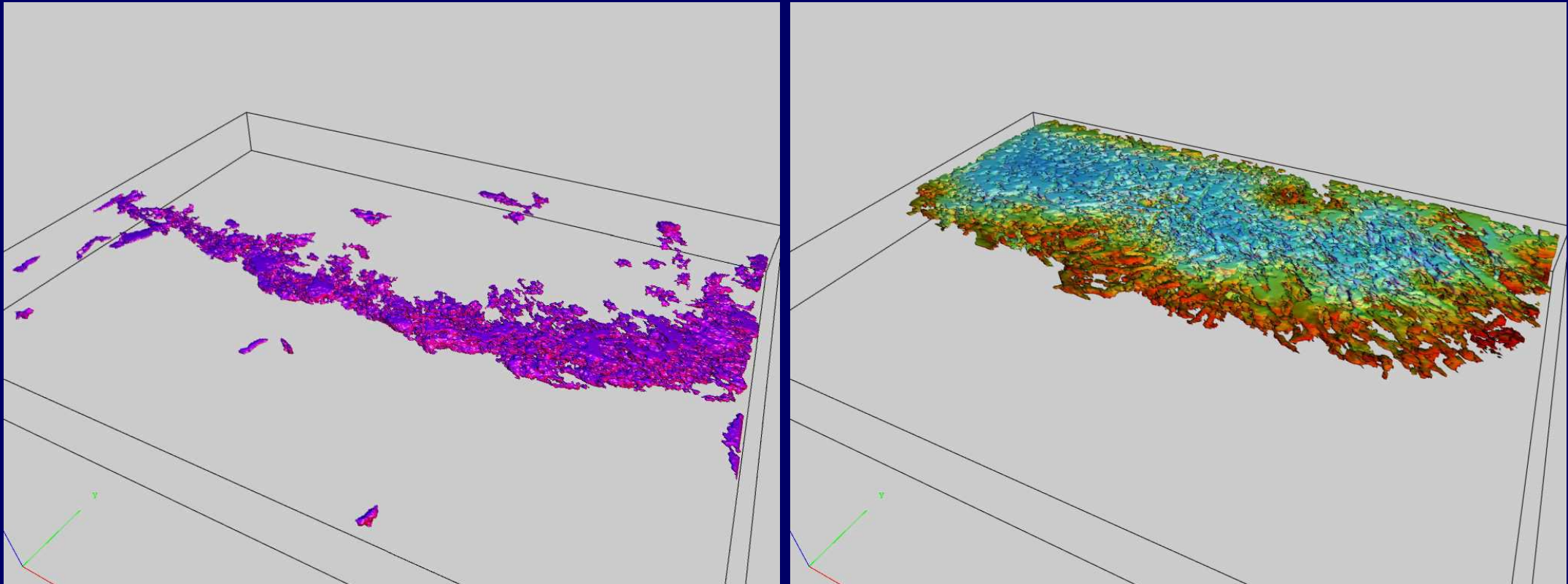
# 9 Advanced Tumour Reconstruction & Analysis (cont'd)

- Mean curvature of tumor surface



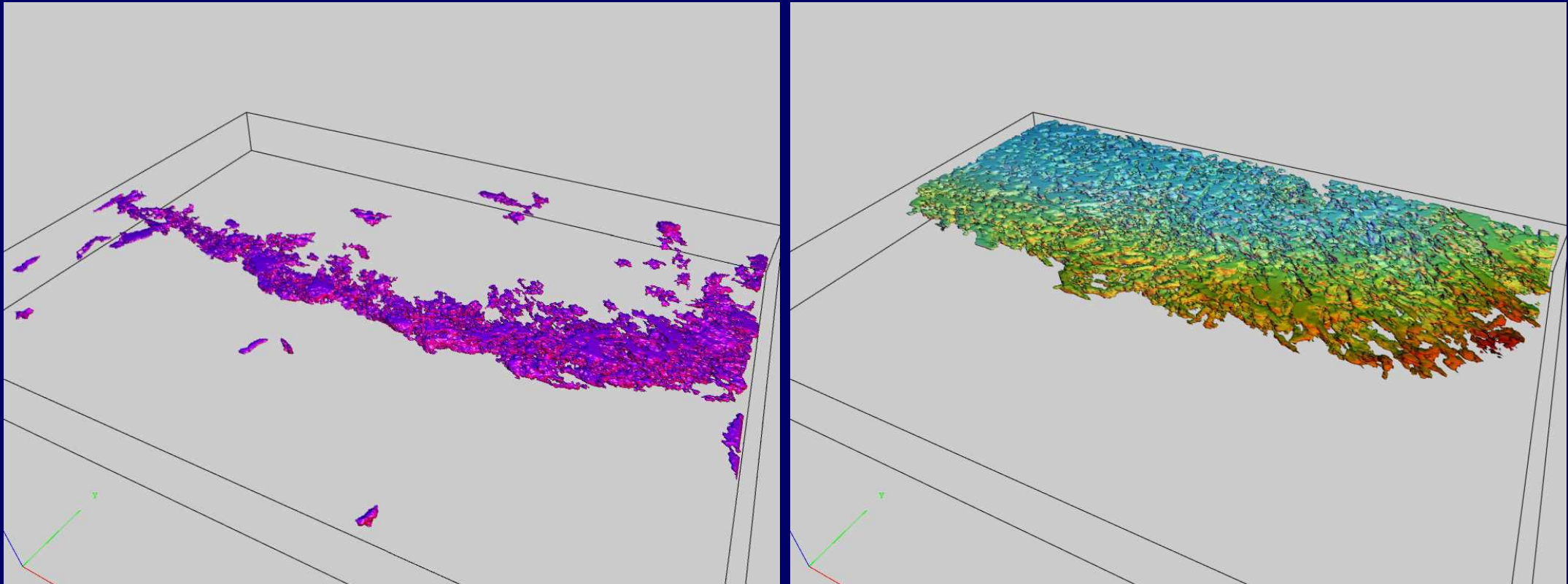
# 9 Advanced Tumour Reconstruction & Analysis (cont'd)

- 3-D Reconstruction results: T-Cell  $\leftrightarrow$  Tumor Distances



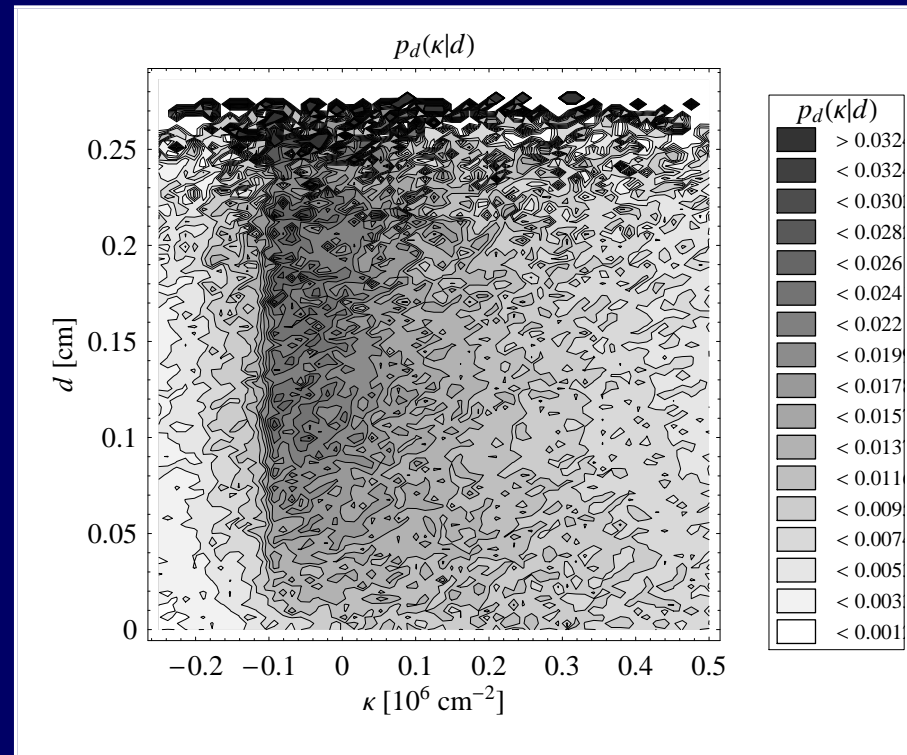
# 9 Advanced Tumour Reconstruction & Analysis (cont'd)

- 3-D Reconstruction results: T-Cell  $\rightarrow$  Tumor Diffusion



## 9 Advanced Tumour Reconstruction & Analysis (cont'd)

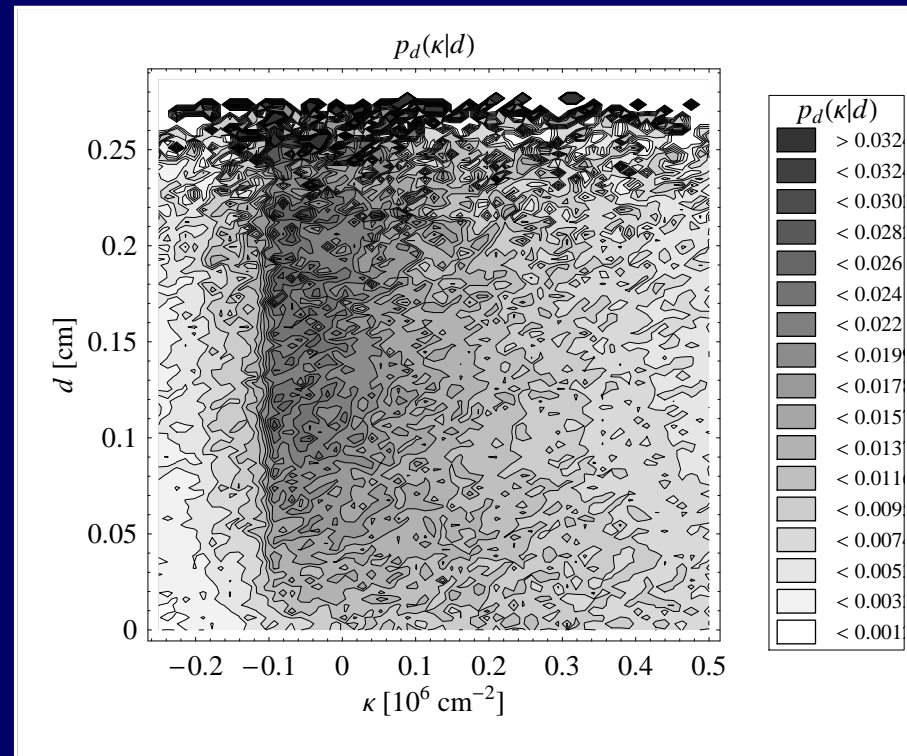
- Conditional probability density  $p_d(\kappa|d)$  for the mean curvature  $\kappa$  at a certain distance  $d$  from the T-cells



- the longer  $d$ , the more surface regions with a high magnitude of  $\kappa$  occur (neg.  $\kappa$ : convex curv.)

## 9 Advanced Tumour Reconstruction & Analysis (cont'd)

- Conditional probability density  $p_d(\kappa|d)$  for the mean curvature  $\kappa$  at a certain distance  $d$  from the T-cells

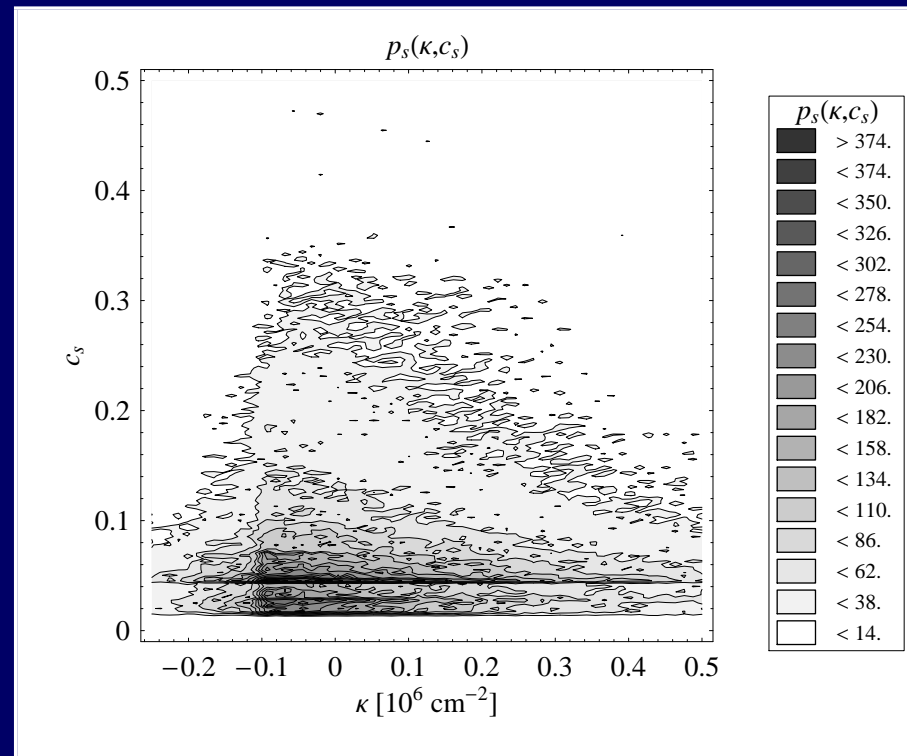


~> T-cells seem to cause a smoothing of the tumor surface (the smaller the  $d$ )



## 9 Advanced Tumour Reconstruction & Analysis (cont'd)

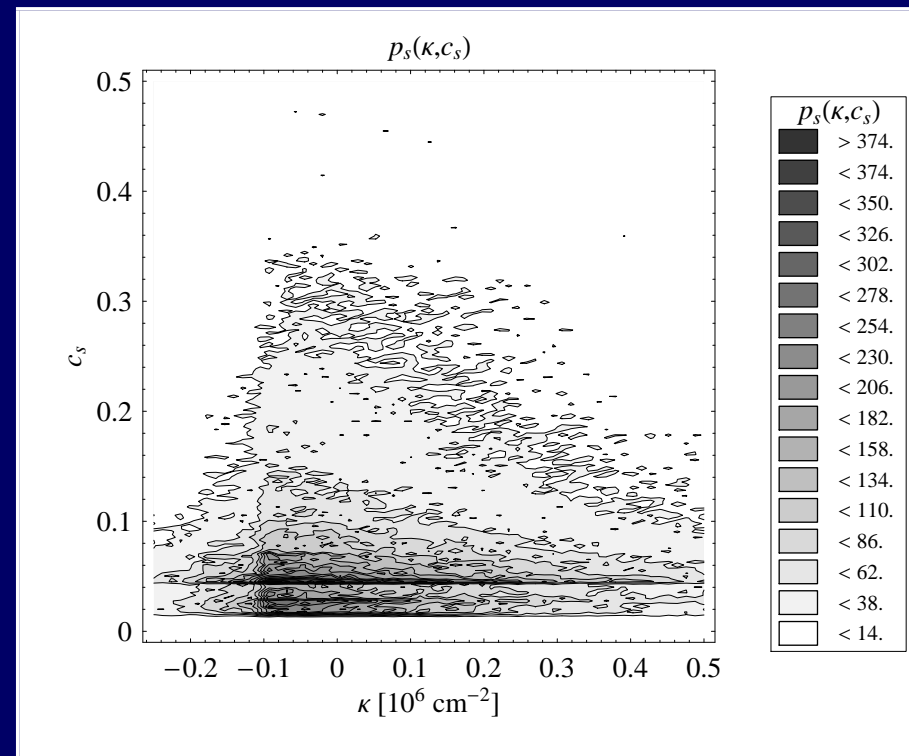
- Probability density  $p_s(\kappa, c_s)$  for curvature  $\kappa$  and substance concentration  $c_s$  (subst. const. emitted by T-cells)



- at low  $c_s$ , a broad range of curvatures  $\kappa$  occurs (expressing an irregular tumor surface shape)

## 9 Advanced Tumour Reconstruction & Analysis (cont'd)

- Probability density  $p_s(\kappa, c_s)$  for curvature  $\kappa$  and substance concentration  $c_s$  (subst. const. emitted by T-cells)



- with rising  $c_s$ , this range shrinks to low  $|\kappa|$  (increasing tumor smoothness)

# Movie

Merci!