

# Large Histological Serial Sections for Computational Tissue Volume Reconstruction

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# Large Histological Serial Sections for Computational Tissue Volume Reconstruction

## Overview

### 1. Introduction

### 2. Tumour Reconstruction

### 3. Combined Tissue Reconstruction with Alternate Staining H&E/p16<sup>INK4a</sup>/CD3

### 4. Conclusions

Braumann, Kuska, Einenkel et al.:

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

IEEE Transactions on Medical Imaging, vol. 24, no. 10, 2005

Einenkel, Kuska, Braumann et al.:

*Combined Three-Dimensional Microscopic Visualisation of Tumour Invasion-Front of Cervical Carcinoma.*

The Lancet Oncology, vol. 7, no. 8, 2006

# 1 Introduction

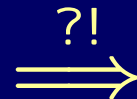
## *General Objective*

Morphometric quantification and classification of multicellular systems

## *Specific Objective (Starting Point)*

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

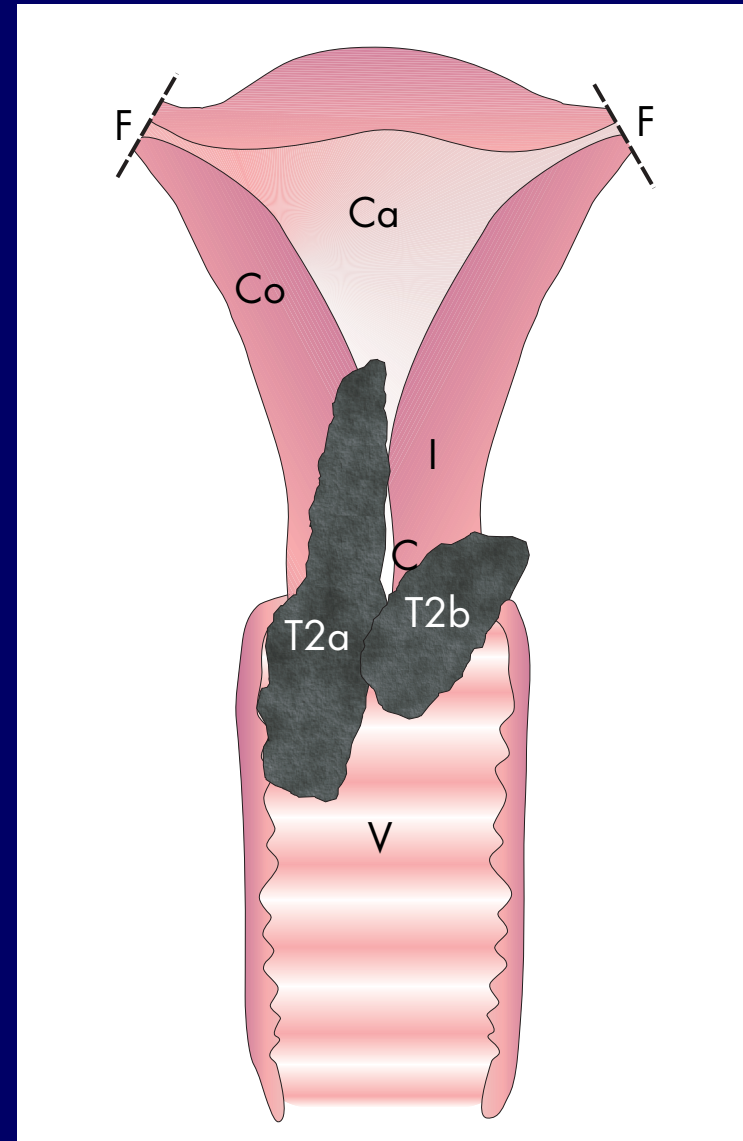
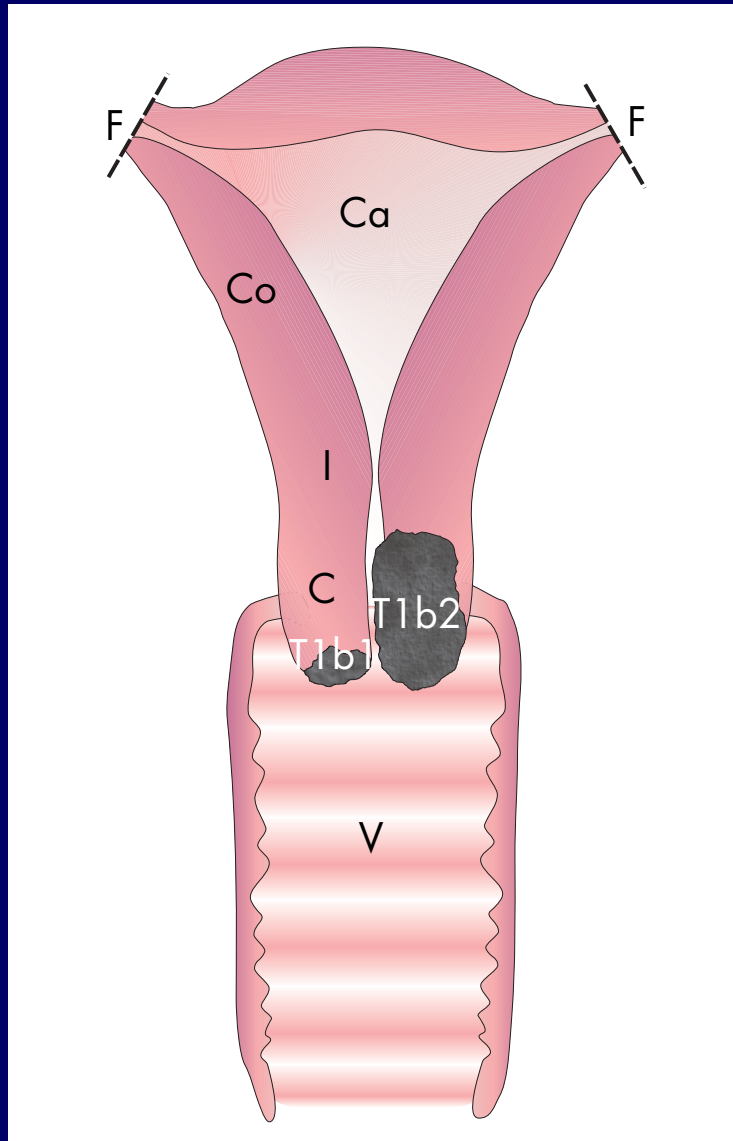
**Tissue specimen**



**Tumour description**

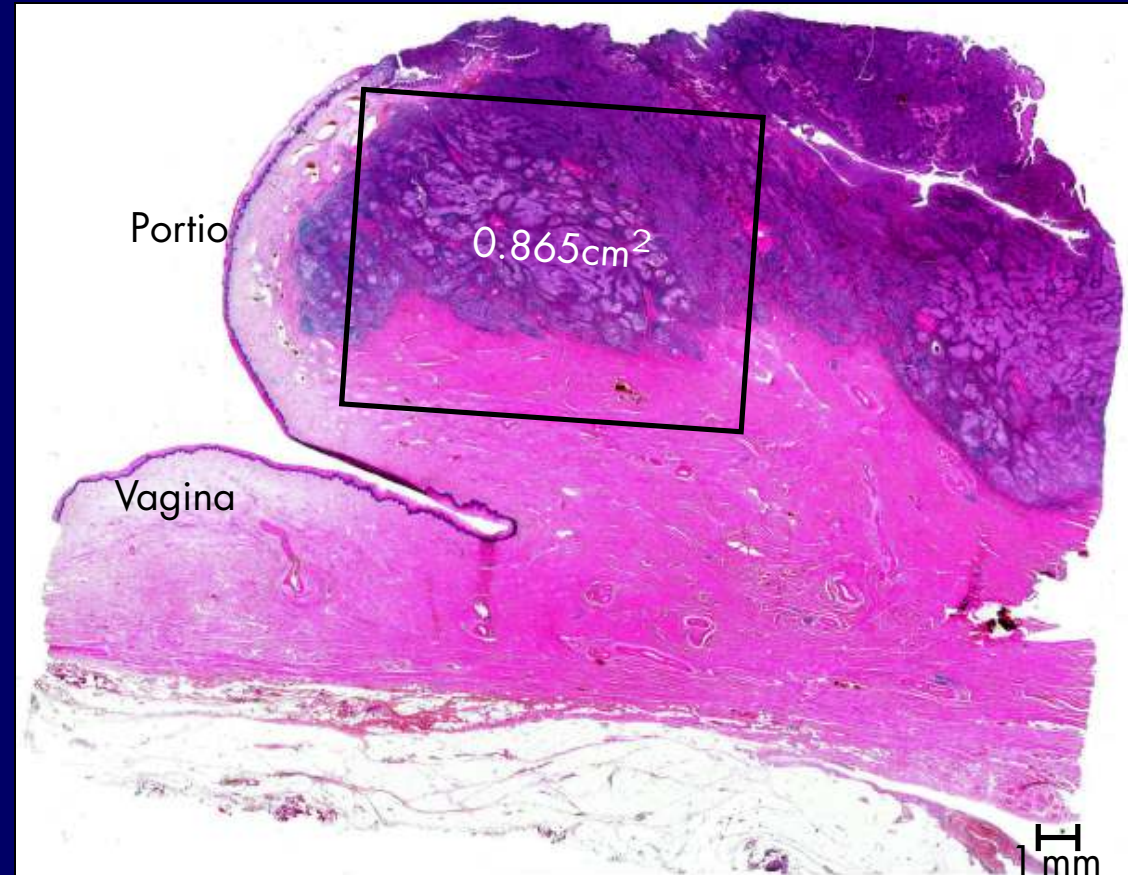
# 1 Introduction (cont'd)

## Anatomical Overview:



# 1 Introduction (cont'd)

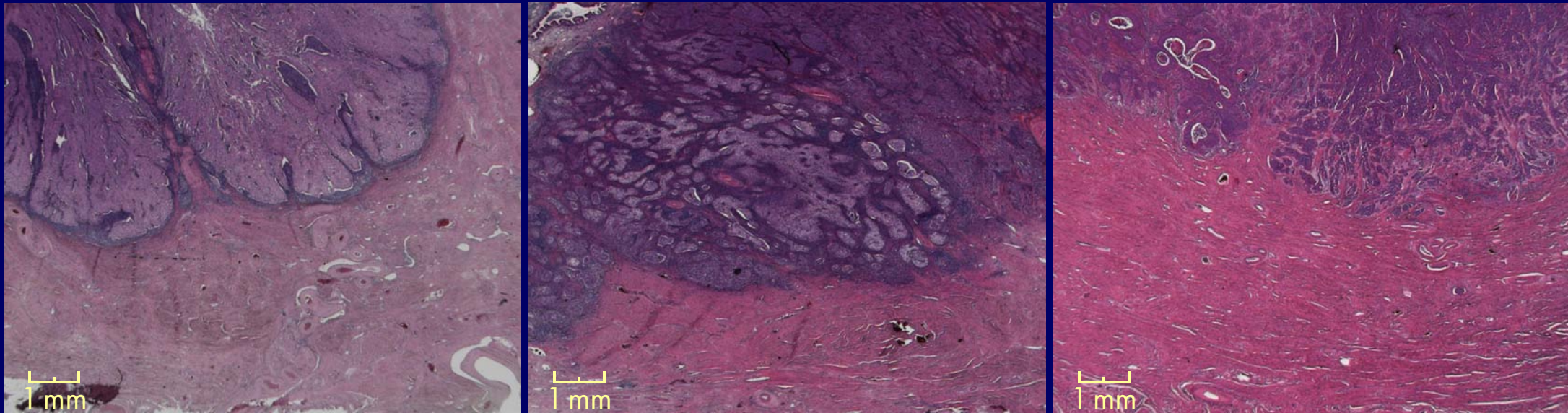
## Material: Paraffin-embedded Sliced Cervix Specimen





# 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

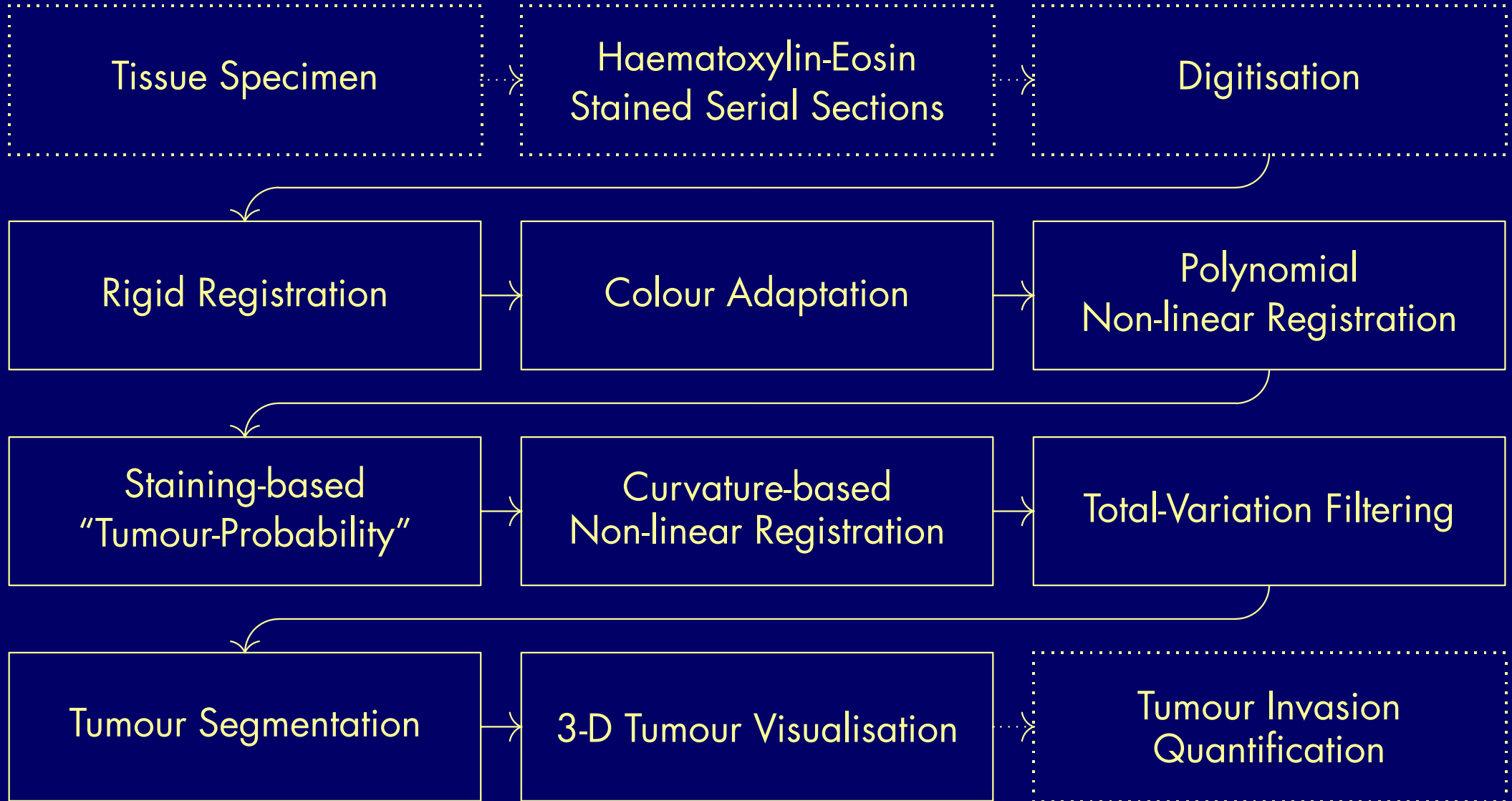
## ***Strategy: procedures for***

- tissue reconstruction
- tumour segmentation
- tumour invasion quantification



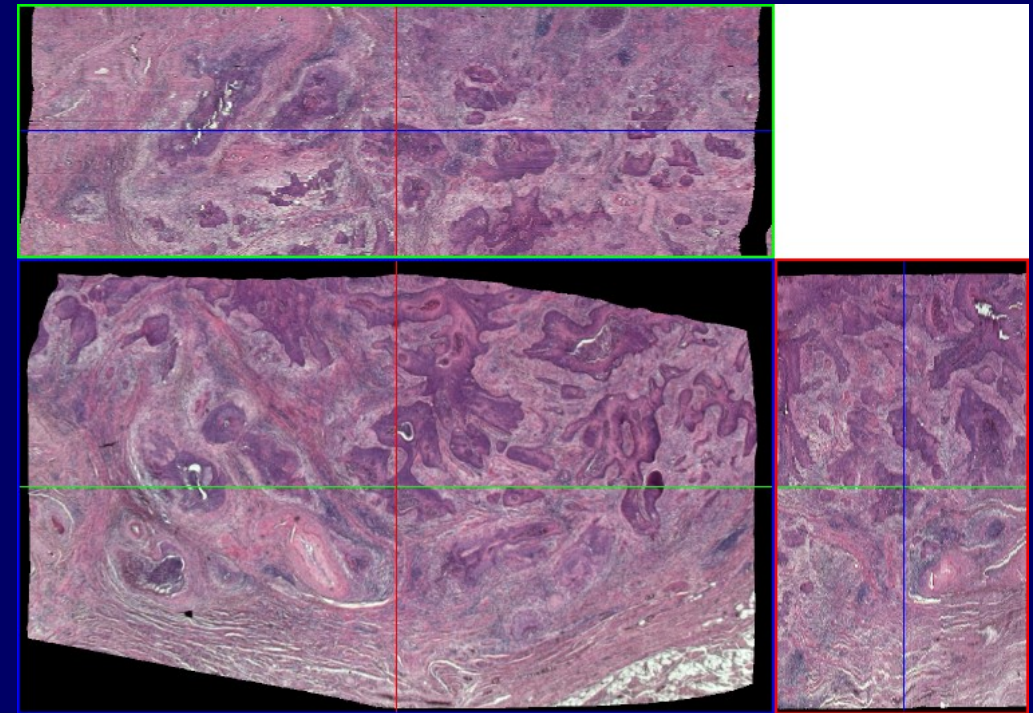
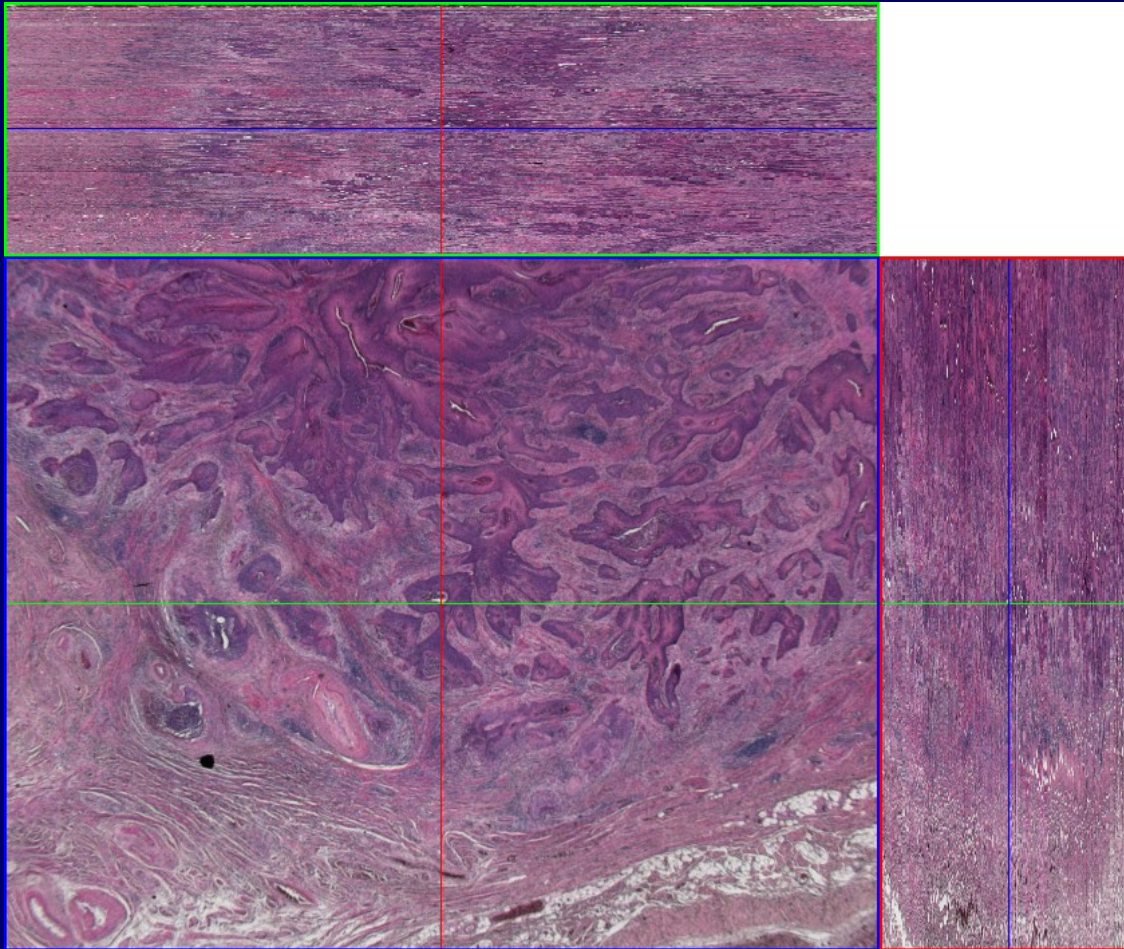
# 2 Tumour Reconstruction

## Image Processing Chain:



# 2 Tumour Reconstruction (cont'd)

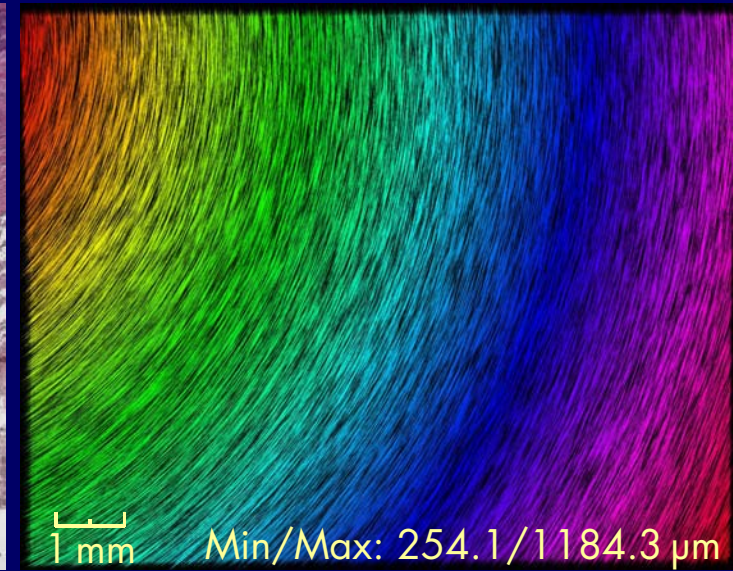
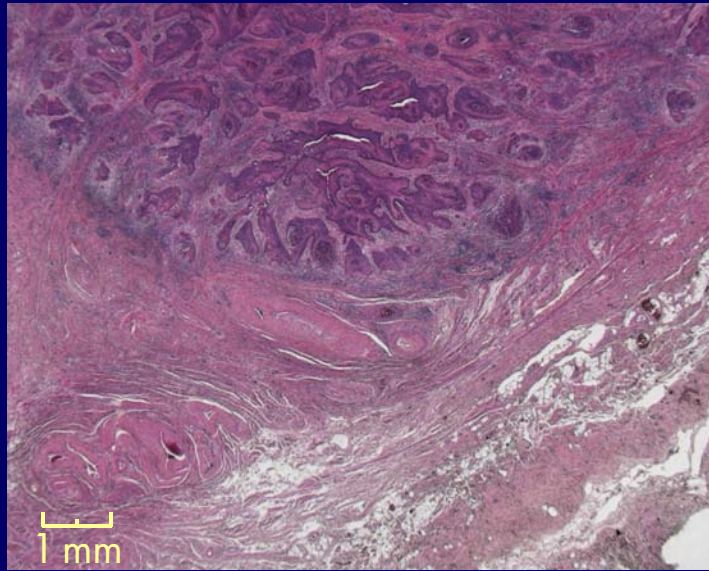
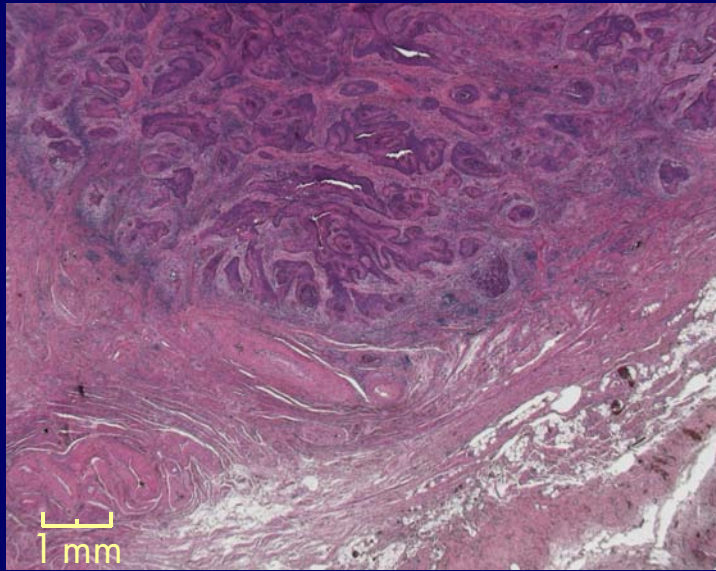
**Before / After:**





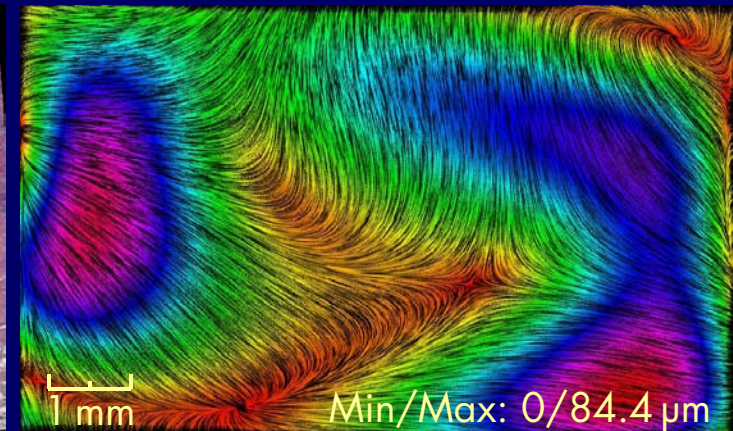
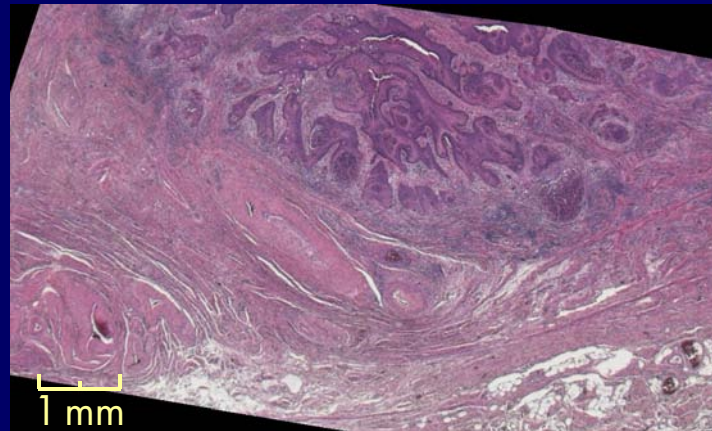
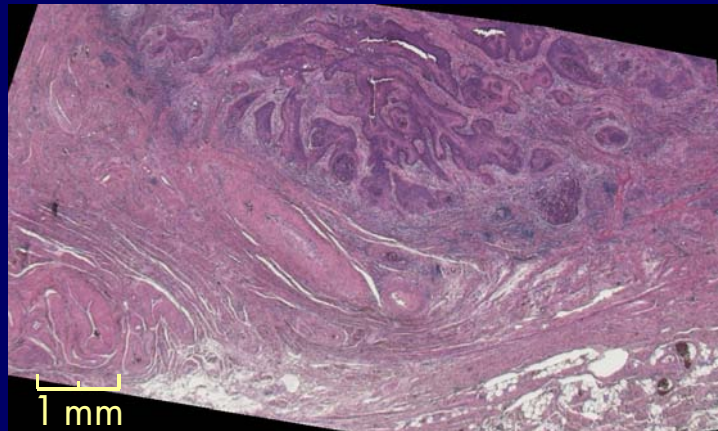
## 2 Tumour Reconstruction (cont'd)

### Rigid Registration:



## 2 Tumour Reconstruction (cont'd)

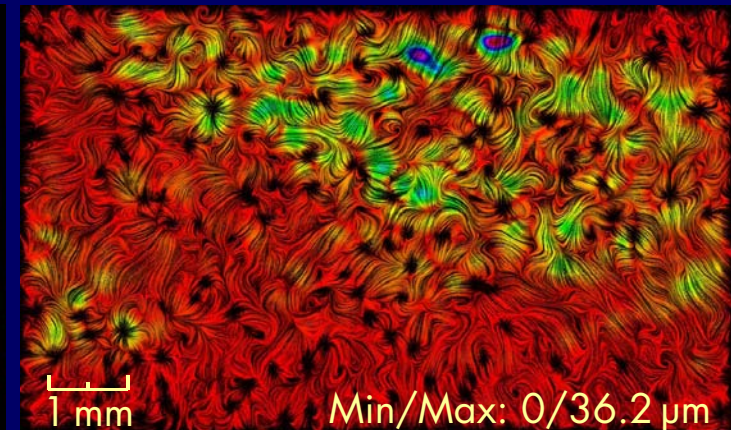
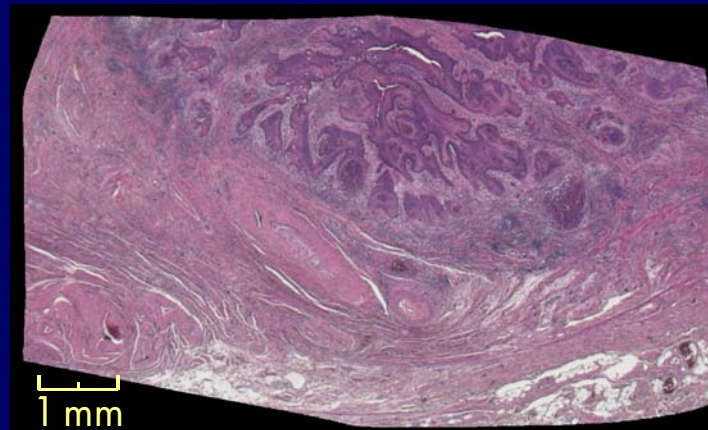
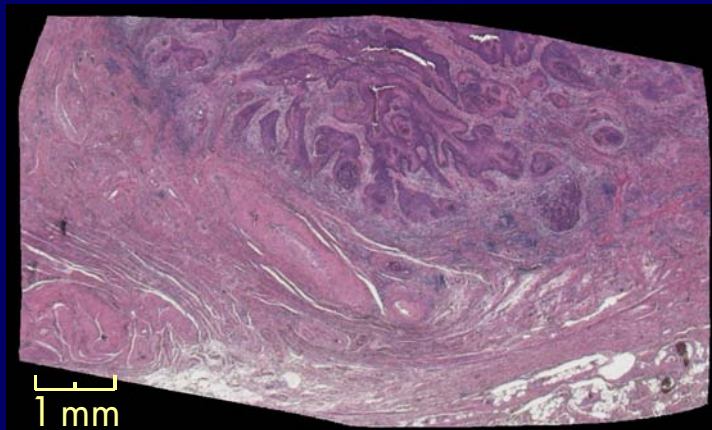
### Polynomial Non-linear Registration:





## 2 Tumour Reconstruction (cont'd)

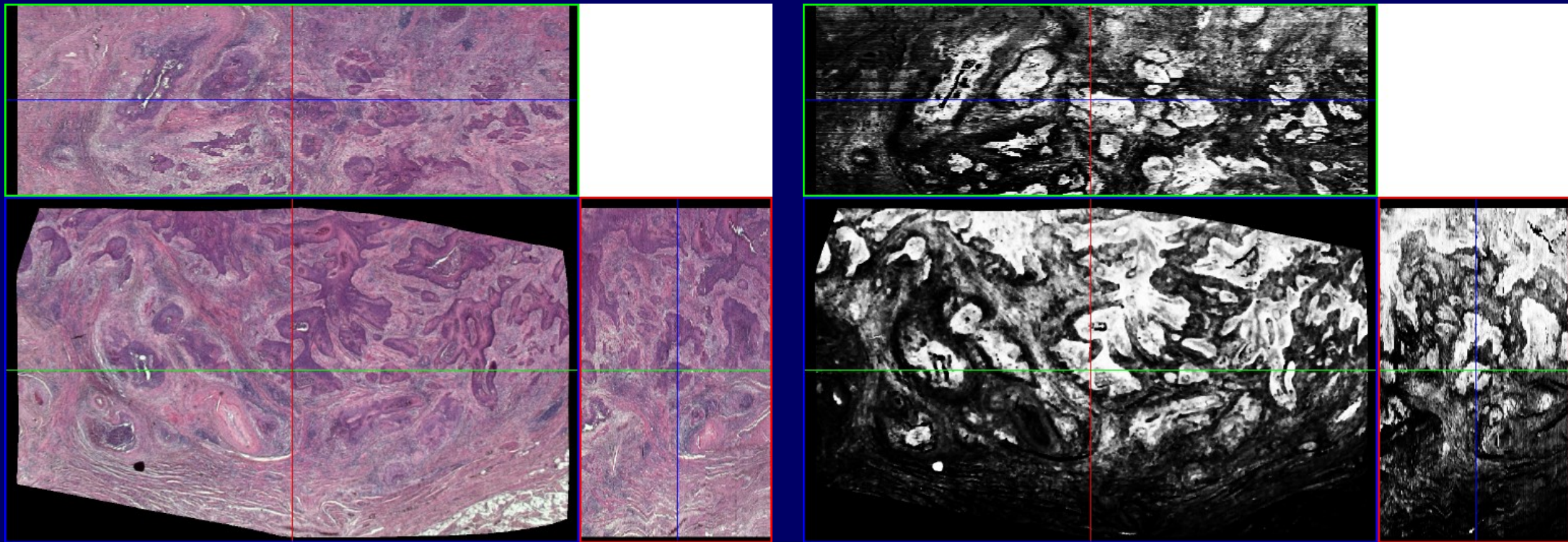
### Non-linear Curvature-based Registration:





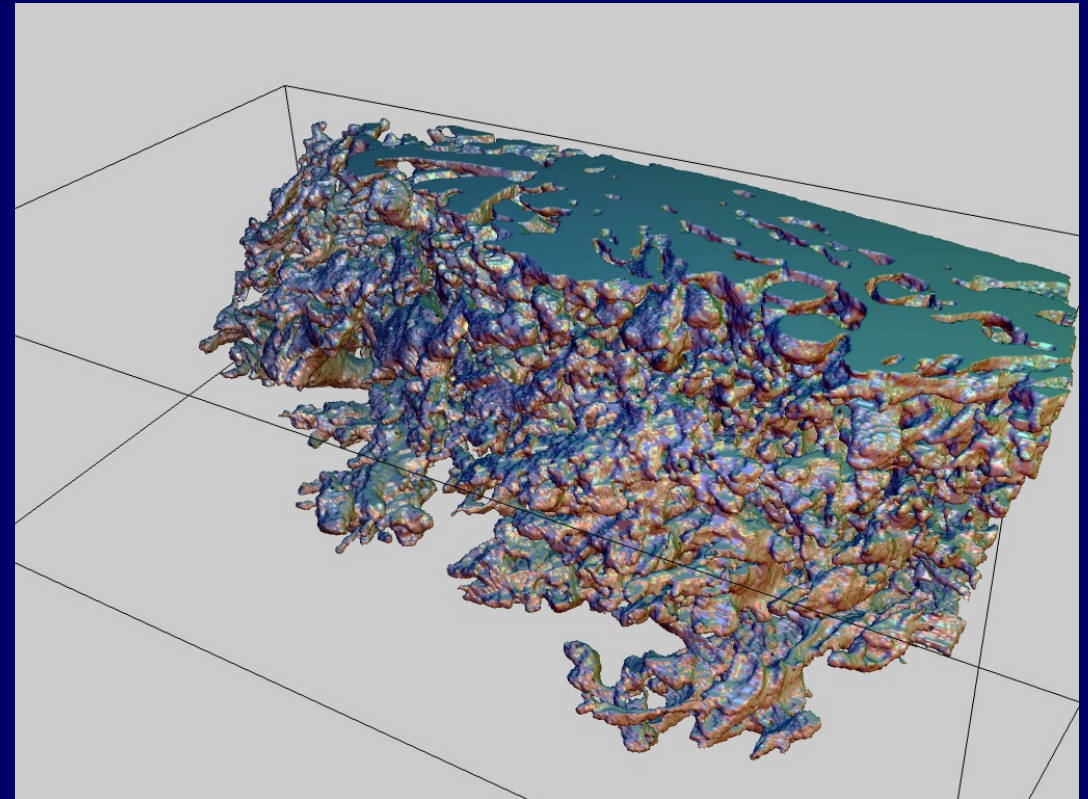
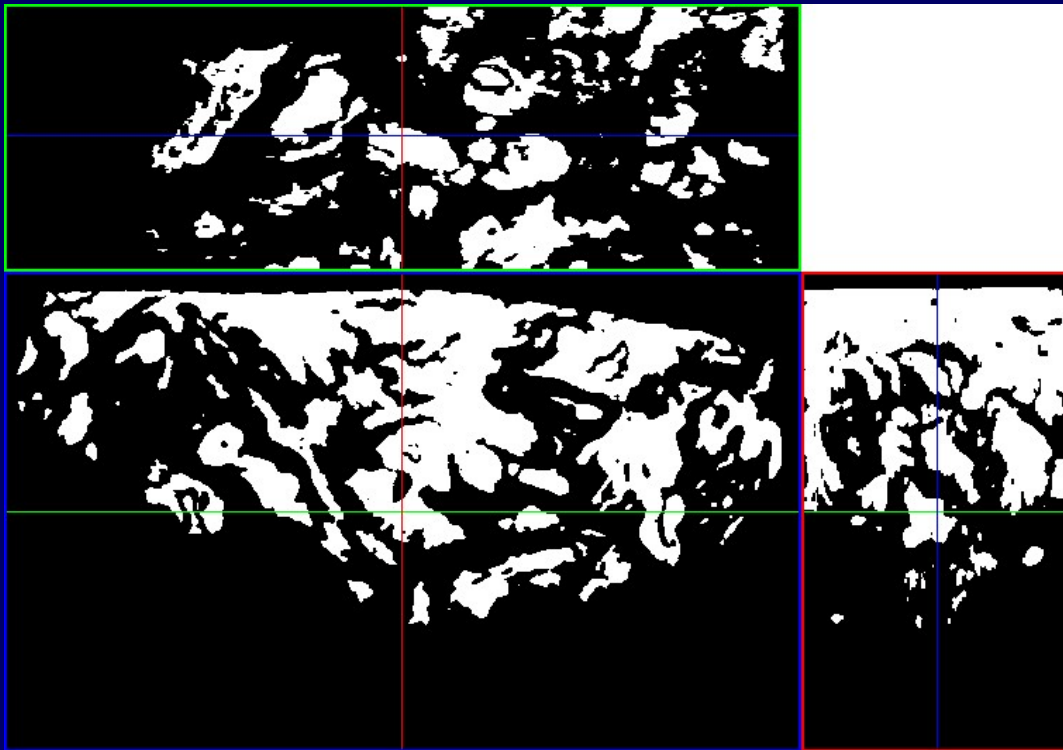
## 2 Tumour Reconstruction (cont'd)

### Staining-based "Tumour-Probability":



## 2 Tumour Reconstruction (cont'd)

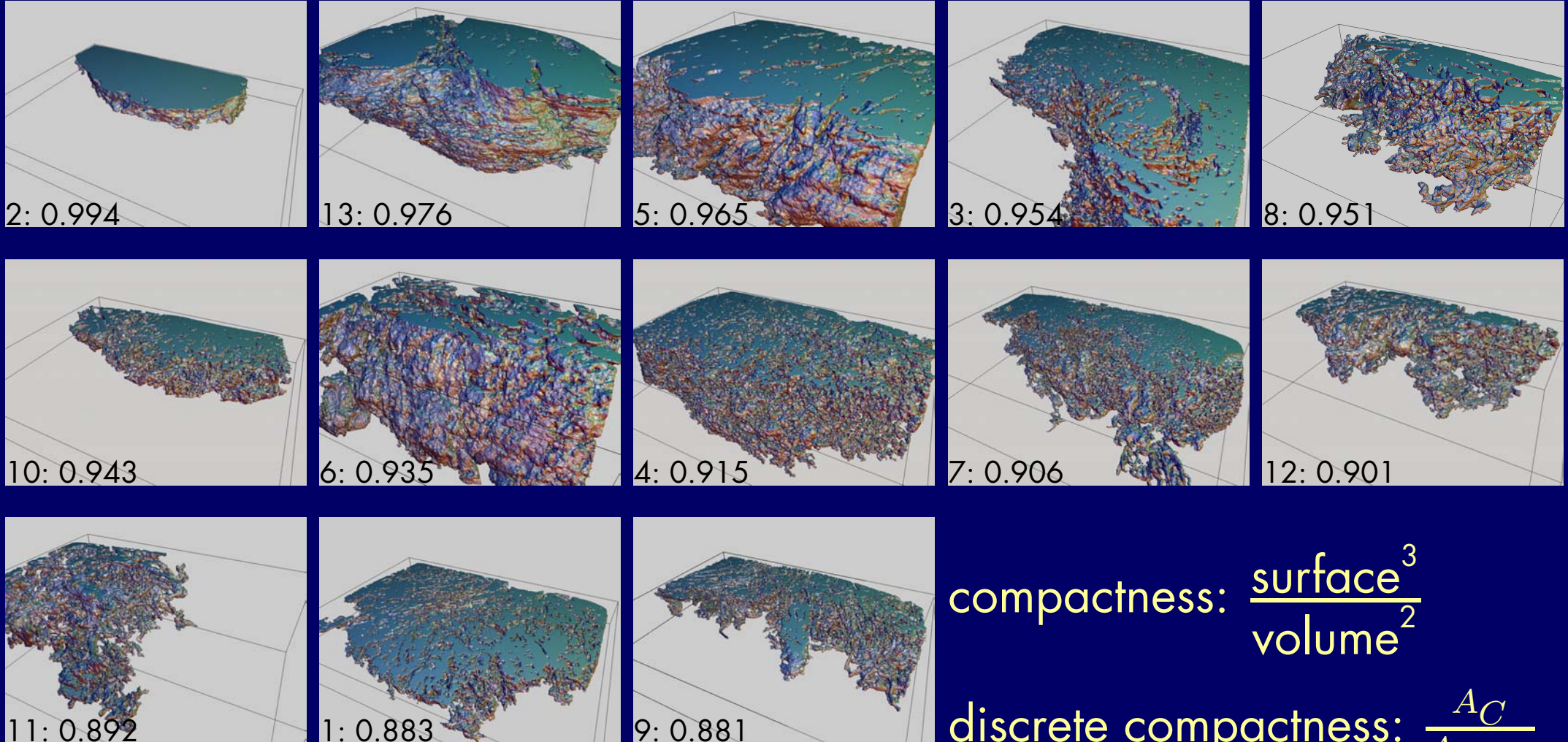
### Segmented Tumour / 3-D Surface Rendering:





# 2 Tumour Reconstruction (cont'd)

## Gallery of 3-D Tumour Invasion Fronts:



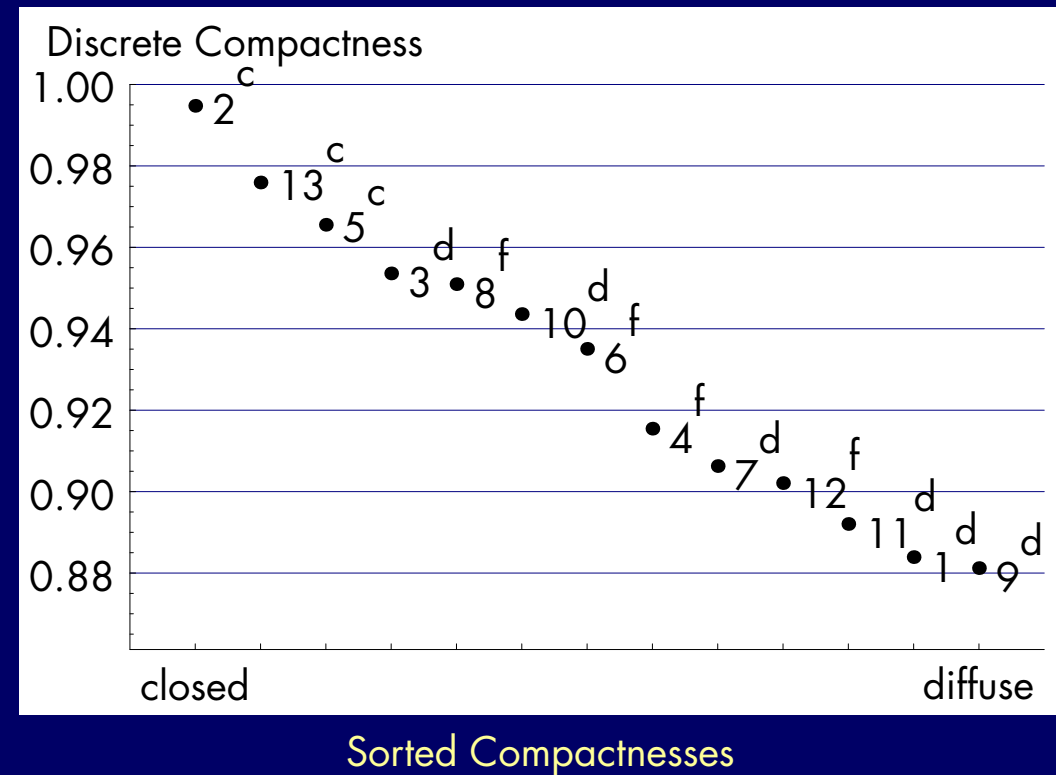
compactness:  $\frac{\text{surface}^3}{\text{volume}^2}$

discrete compactness:  $\frac{A_C}{A_{C_{\max}}}$

## 2 Tumour Reconstruction (cont'd)

### Quantification of Tumour Invasion:

Specimen Number	Number of Slices	Slice Thickness [ $\mu\text{m}$ ]	Reconstructed Volume [ $\text{mm}^3$ ]	Discrete Compactness
1	96	10	60.2	0.884
2	90	6	16.7	0.995
3	230	10	146.1	0.954
4	230	10	133.6	0.915
5	250	10	130.8	0.966
6	300	10	104.7	0.935
7	250	10	148.9	0.906
8	300	10	146.8	0.951
9	150	10	100.5	0.881
10	100	10	62.8	0.944
11	301	10	143.4	0.892
12	260	10	123.8	0.902
13	500	5	89.3	0.976



## 2 Tumour Reconstruction (cont'd)

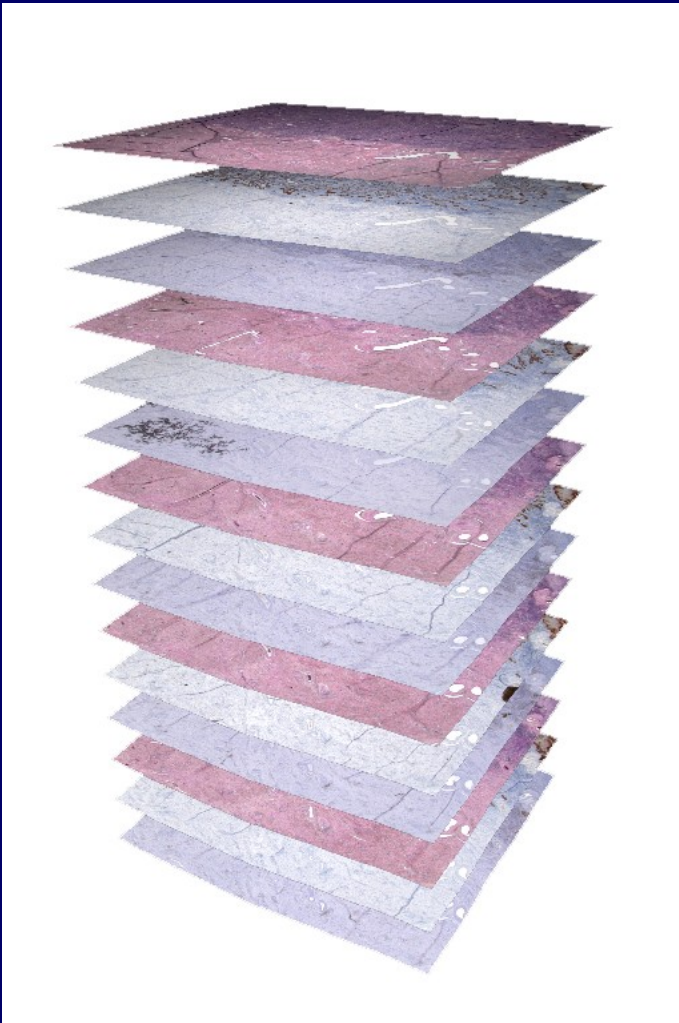
### **Achievements:**

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



### 3 Combined Tissue Reconstruction with Alternate Staining H&E/p16<sup>INK4a</sup>/CD3

#### *Idea/Challenge:*

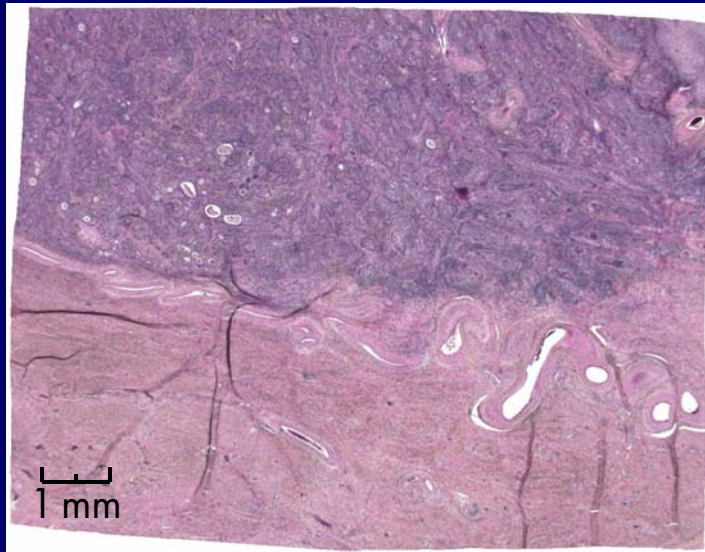


#### **We pursue**

- 3-D cervical tumour invasion analysis
- **co-localisation of different tissue structures in 3-D**
- feasibility study for 3-D reconstructions based on triple alternating stainings (84 slices)

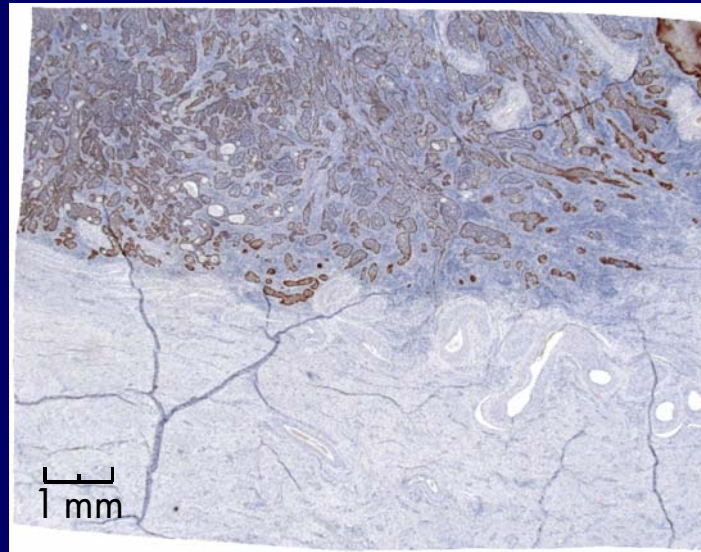
# 3 Combined Tissue Reconstruction with Alternate Staining H&E/p16<sup>INK4a</sup>/CD3 (cont'd)

## Applied Stainings:



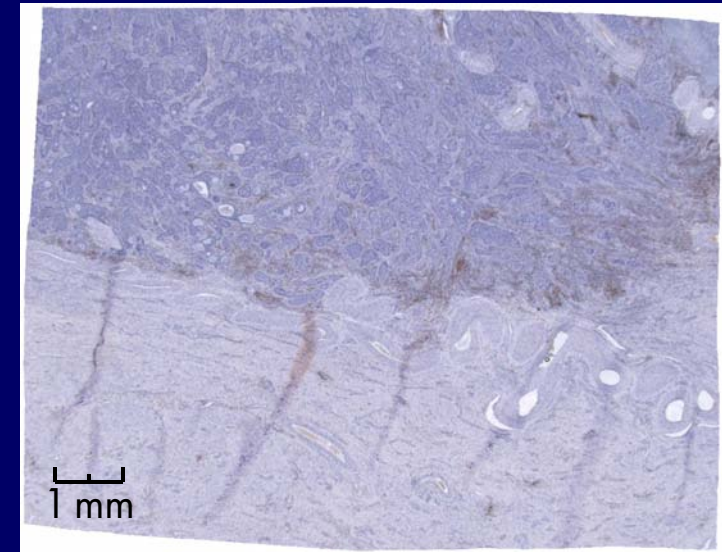
H&E

Routine reference



p16<sup>INK4a</sup>

Cervical tumour marker



CD3

T-Lymphocyte marker

# 3 Combined Tissue Reconstruction with Alternate Staining H&E/p16<sup>INK4a</sup>/CD3 (cont'd)

## 3-D Reconstruction results: Tilted virtual sections



# 3 Combined Tissue Reconstruction with Alternate Staining H&E/p16<sup>INK4a</sup>/CD3 (cont'd)

## Segmentation: Feature vector



### 3 Combined Tissue Reconstruction with Alternate Staining H&E/p16<sup>INK4a</sup>/CD3 (cont'd)

#### **Segmentation: Distributions**

- identification of different tissue types by estimating their respective  $d$ -dimensional normal distributions
- overall distribution of all realisations given by

$$P(\vec{y}) = \sum_{k=1}^K \alpha_k p(\vec{y} | \vec{\mu}_k, \Sigma_k)$$

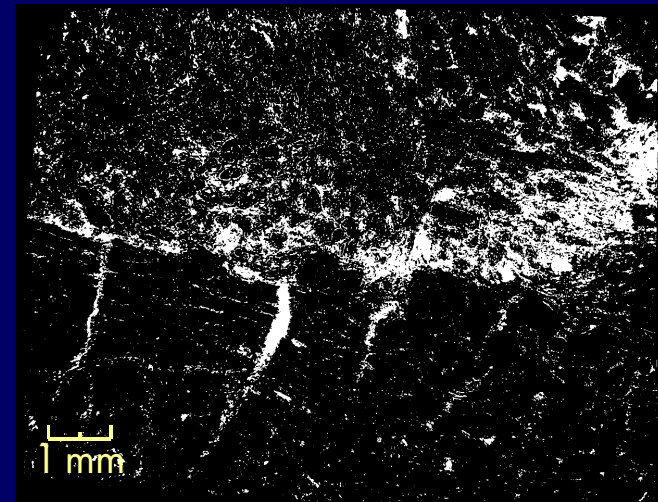
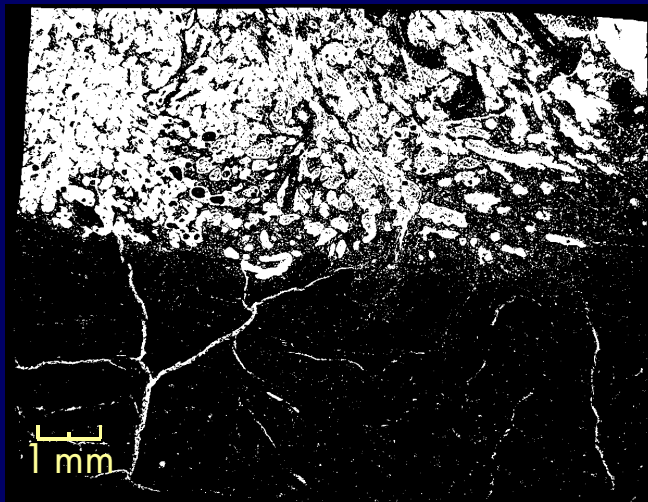
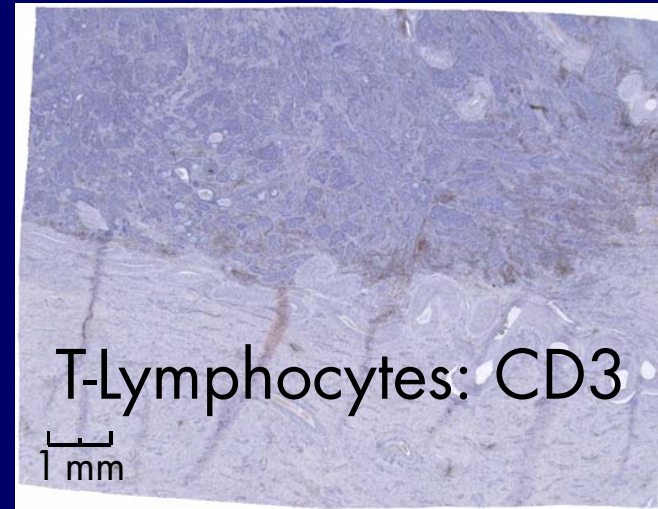
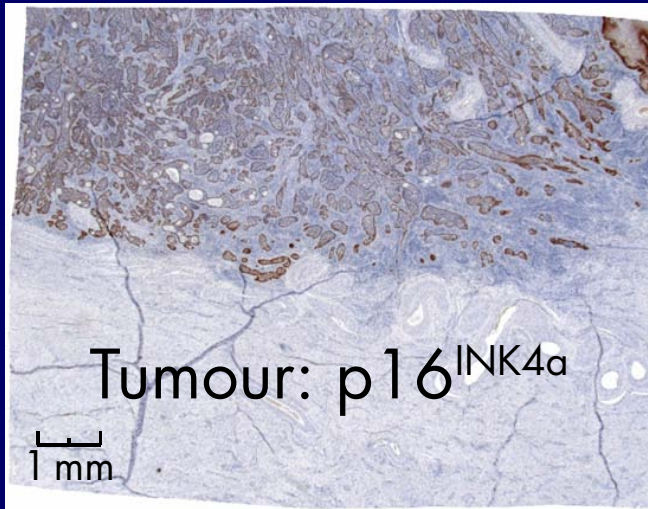
- class membership  $k$  for some feature vector  $x$  is determined as

$$\arg \max_k \frac{\alpha_k p(\vec{x} | \vec{\mu}_k, \Sigma_k)}{\sum_{k=1}^K \alpha_k p(\vec{x} | \vec{\mu}_i, \Sigma_i)}$$



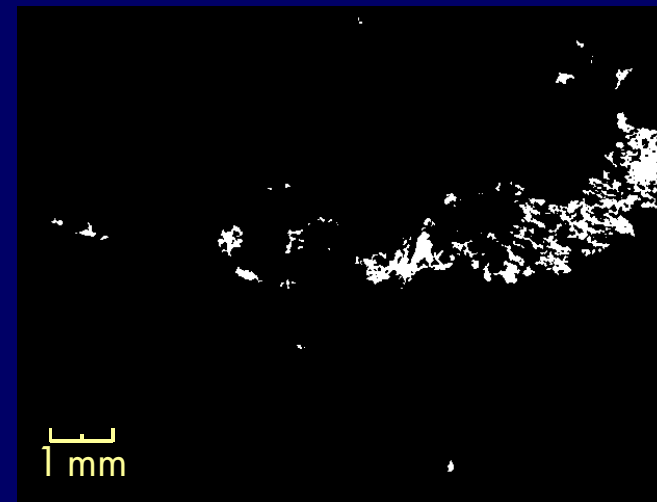
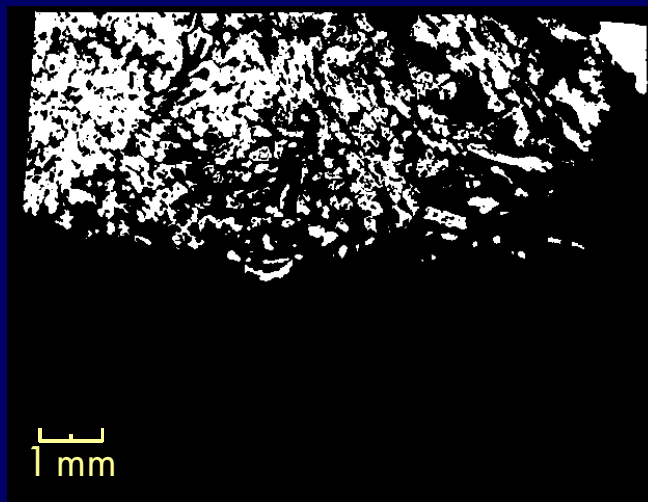
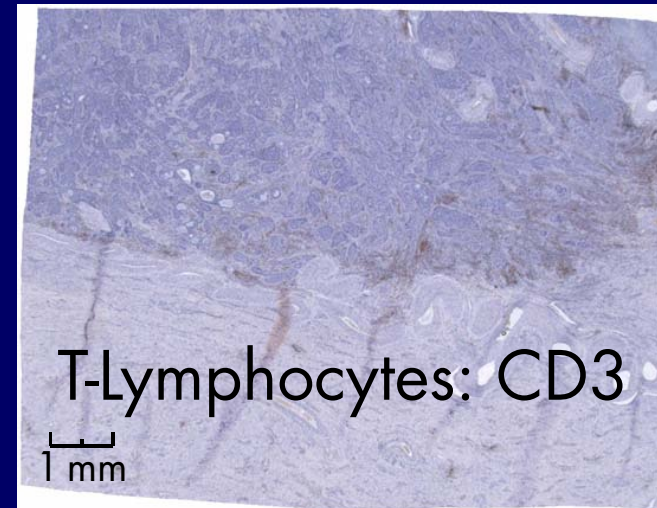
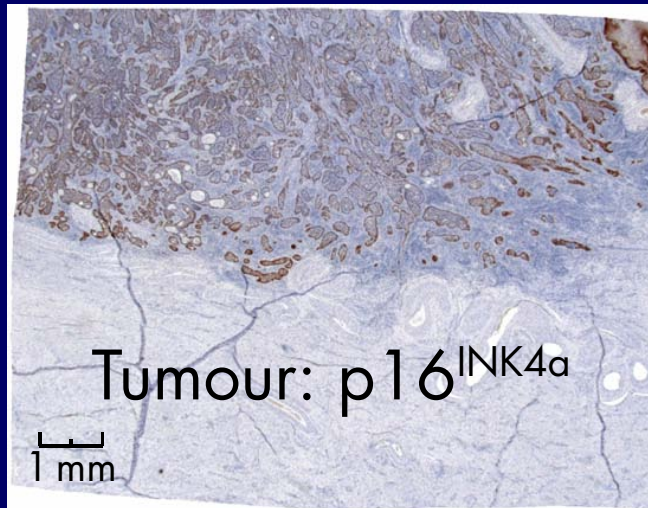
### 3 Combined Tissue Reconstruction with Alternate Staining H&E/p16<sup>INK4a</sup>/CD3 (cont'd)

#### Automatic Segmentation Examples:



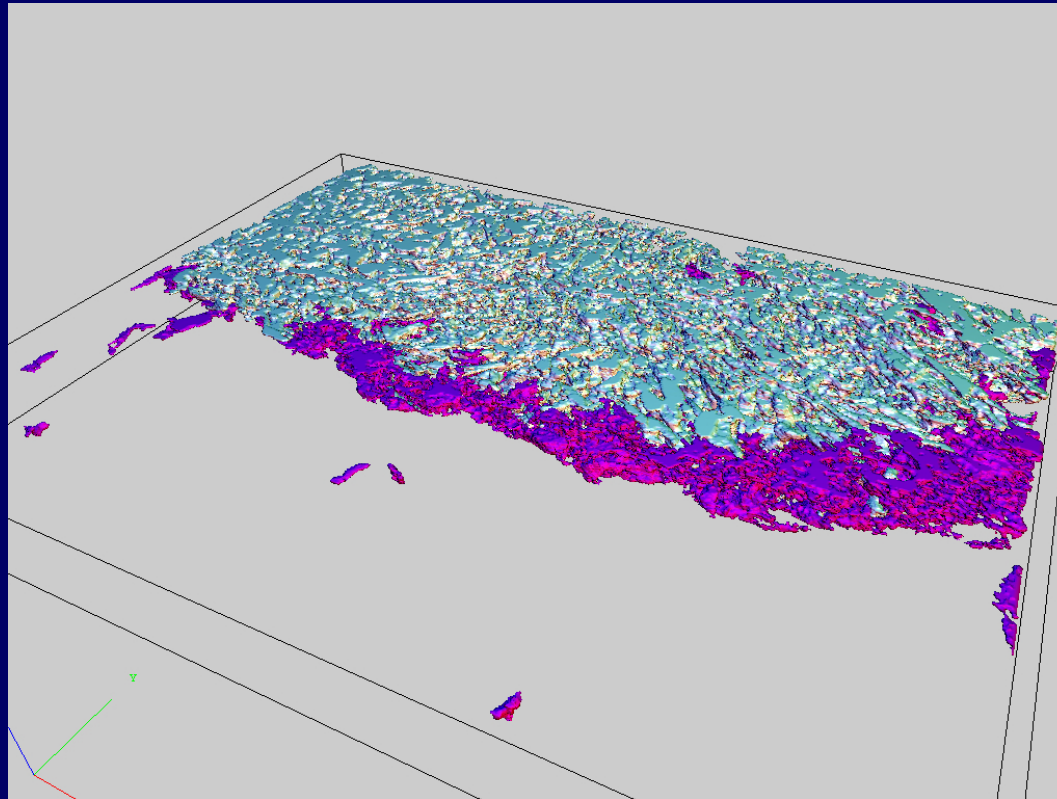
### 3 Combined Tissue Reconstruction with Alternate Staining H&E/p16<sup>INK4a</sup>/CD3 (cont'd)

#### Automatic Segmentation Examples: Post-Processing



### 3 Combined Tissue Reconstruction with Alternate Staining H&E/p16<sup>INK4a</sup>/CD3 (cont'd)

#### 3-D Reconstruction results: Surface rendering

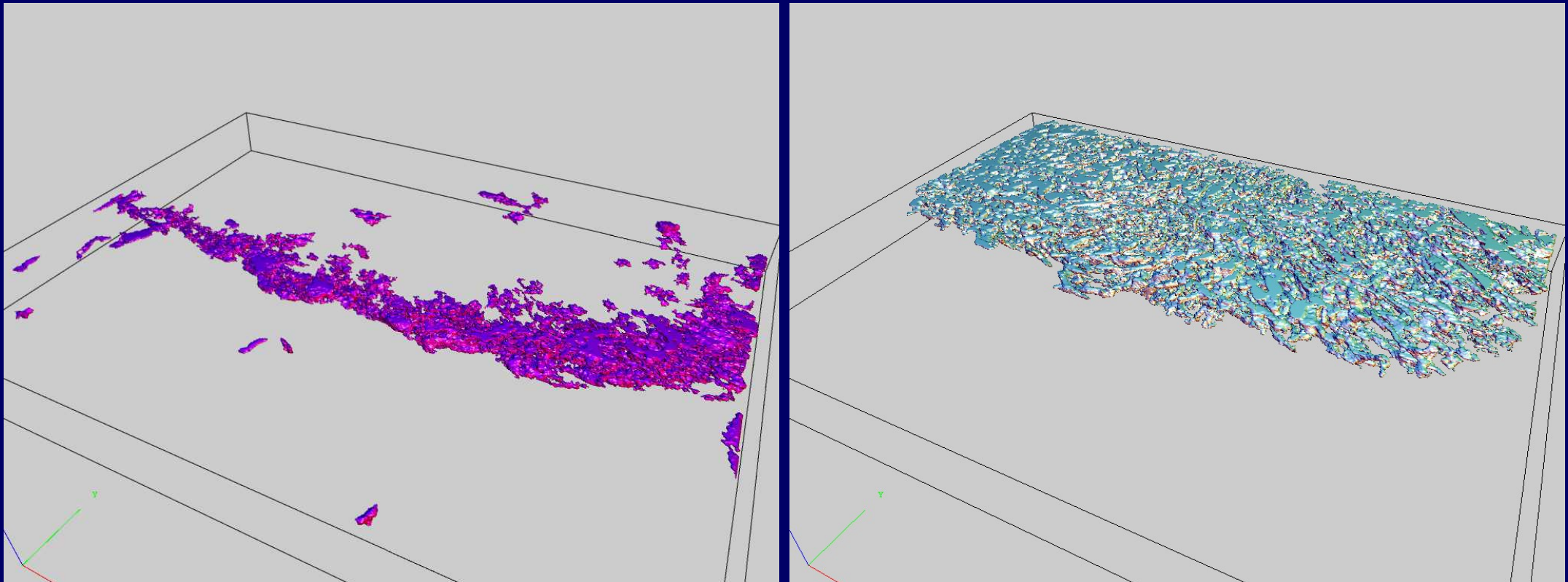


Overall reconstructed tissue volume:  $60.9\text{mm}^3$ , Tumour Compactness: 0.89, Tumour volume:  $11.6\text{mm}^3$ , T-Lymphocyte volume:  $1.1\text{mm}^3$



# 3 Combined Tissue Reconstruction with Alternate Staining H&E/p16<sup>INK4a</sup>/CD3 (cont'd)

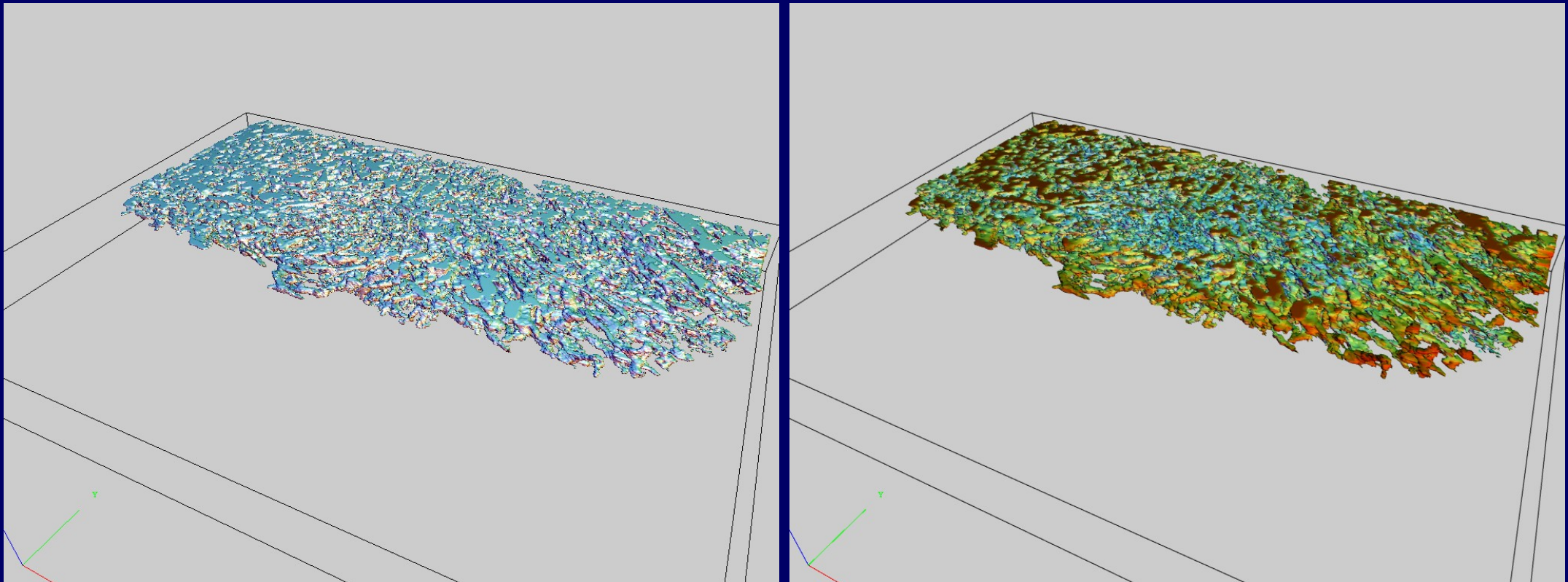
## 3-D Reconstruction results: Volumes





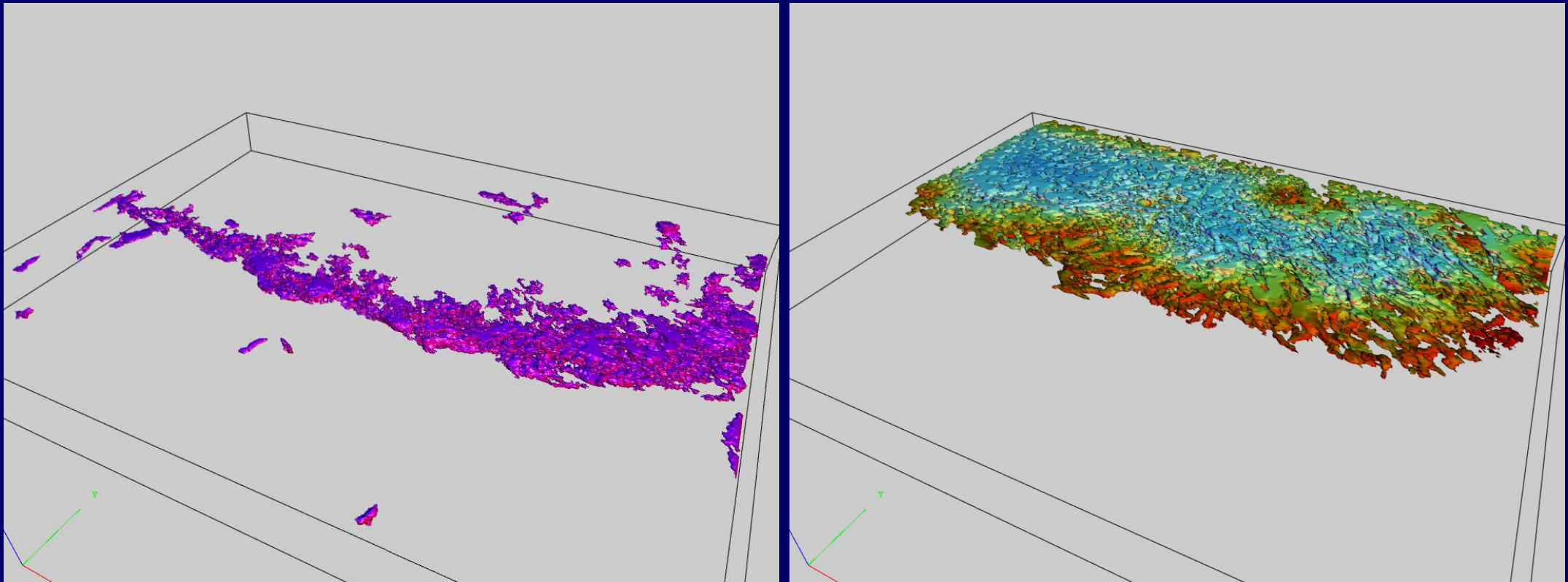
# 3 Combined Tissue Reconstruction with Alternate Staining H&E/p16<sup>INK4a</sup>/CD3 (cont'd)

## 3-D Reconstruction results: Local Compactness



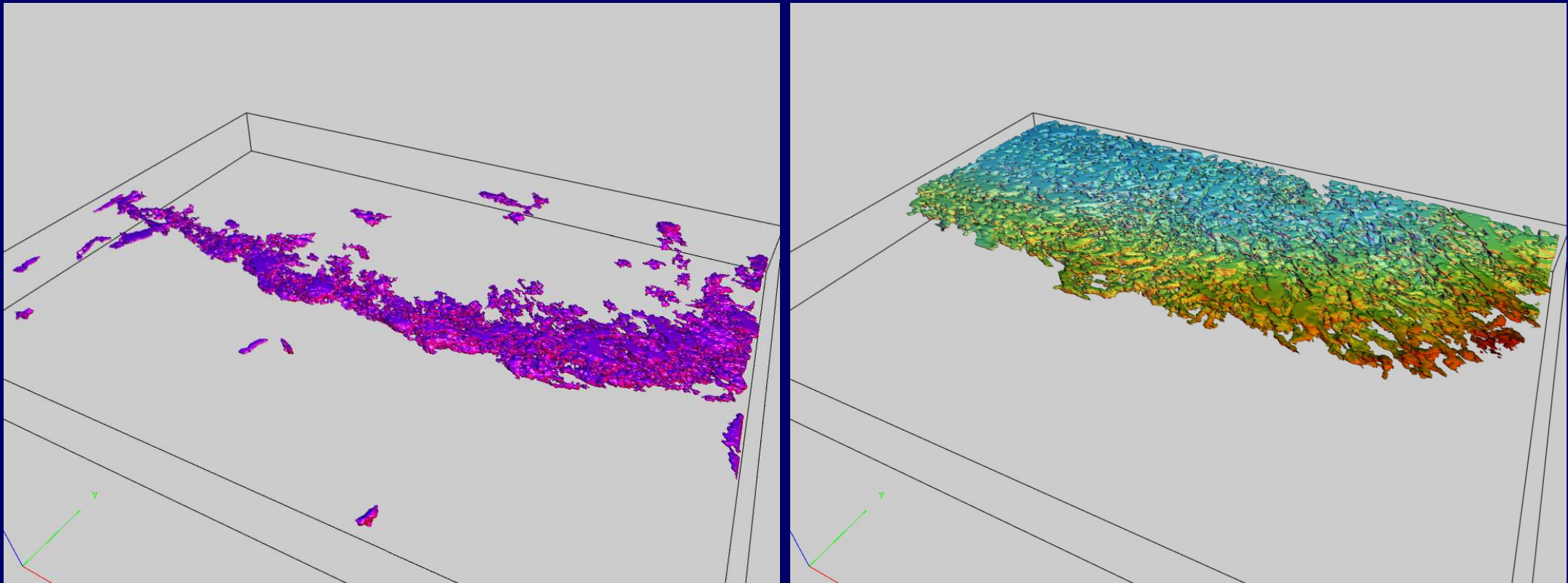
### 3 Combined Tissue Reconstruction with Alternate Staining H&E/p16<sup>INK4a</sup>/CD3 (cont'd)

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



### 3 Combined Tissue Reconstruction with Alternate Staining H&E/p16<sup>INK4a</sup>/CD3 (cont'd)

**3-D Reconstruction results: T-Cell → Tumour Diffusion**





# 4 Conclusions

## *The present work*

- exemplifies the large potential for serial-section based 3-D tissue analyses
- will continue with the inclusion of further stainings, e.g. concerning vascularisation

# Merci!