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

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Overview

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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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General Objective

Morphometric quantification and classification of multicellular systems
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Specific Objective

3-D characterisation of the invasion pattern of squamous epithelial carcinoma of the uterine cervix (supposed prognostic relevance)
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Tissue specimen
**General Objective**

Morphometric quantification and classification of multicellular systems

**Specific Objective**

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

Tissue specimen \(\Rightarrow\) Tumour description
General Objective
Morphometric quantification and classification of multicellular systems

Specific Objective
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Tissue specimen → Tumour description
1 Introduction (cont’d)

Anatomical Overview:

Diagram showing anatomical structures with labels such as Ca, Co, I, C, T1b1, T1b2, T2a, and T2b.
Cervix Specimen embedded in Paraffin Wax:
Material:
Squamous Cell Carcinoma of the Uterine Cervix:

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

• damages, fissures, folds

Strategy: procedures for

• tissue reconstruction

• tumour segmentation

• tumour invasion quantification
2 Tumour Reconstruction

Image Processing Chain:

- Tissue Specimen
- Haematoxylin-Eosin Stained Serial Sections
- Digitisation
- Rigid Registration
- Colour Adaptation
- Polynomial Non-linear Registration
- Staining-based Tumour-Probability
- Curvature-based Non-linear Registration
- Total-Variation Filtering
- Tumour Segmentation
- 3-D Tumour Visualisation
- Tumour Invasion Quantification
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 \( x_0, y_0 \):

\( \rightarrow \) Fourier-Mellin-Transformation (Rotation) and

\( \rightarrow \) Phase-Only Matched Filtering (Rotation & Translation)

\( \rightarrow \) fast, non-iterative procedure
Rigid Registration:

Maximum displacement: 1184.3\,\mu m (lower right)
minimum: 254.1\,\mu 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

\[ \sim \text{estimated matrices for offset, scaling, and rotation} \]
Colour Adaptation:

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

\[
\begin{bmatrix}
R \\
G \\
B \\
1
\end{bmatrix}_\text{ref} = O_{\text{ref}}^{-1} \cdot R \cdot S \cdot O_{\text{sam}}
\]

\[
\begin{bmatrix}
R \\
G \\
B \\
1
\end{bmatrix}_\text{sam}
\]
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
Polynomial Non-linear Registration:

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

\[
r(x, y) = s(a(x, y), b(x, y))
\]

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

\((N + 1)^2\) coefficients \( p_n \) und \( q_n \) by means of linear regression (least squares estimation), \( N = 5 \)
Polynomial Non-linear Registration:

Maximum displacement: 84.4\mu m, minimum: 0\mu m.
Polynomial Non-linear Registration:
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:
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
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

\[
\vec{f} = \left( r(x - u_x(x, y), y - u_y(x, y)) - s(x, y) \right) \\
\times \nabla \left( r(x - u_x, y - u_y) - s(x, y) \right)
\]
Non-linear Curvature-based Registration:

Maximum displacement: 36.2µm, minimum: 0µm.
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
Total-Variation Filtering:
2 Tumour Reconstruction (cont’d)

Tumour Segmentation (Thresholding):
3 Tumour Invasion Quantification

Segmented Tumour / 3-D Surface Rendering:
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:

<table>
<thead>
<tr>
<th>Specimen Number</th>
<th>Number of Slices</th>
<th>Slice Thickness [µm]</th>
<th>Reconstructed Volume [mm³]</th>
<th>Mean Residual Error [µm]</th>
<th>Discrete Compactness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rigid R.</td>
<td>Polyn. R.</td>
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<td>1</td>
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<td>30.1</td>
<td>20.1</td>
<td>12.9</td>
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<td>90</td>
<td>6</td>
<td>16.7</td>
<td>13.2</td>
<td>7.5</td>
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<tr>
<td>3</td>
<td>230</td>
<td>10</td>
<td>146.1</td>
<td>15.7</td>
<td>7.8</td>
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<tr>
<td>4</td>
<td>230</td>
<td>10</td>
<td>133.6</td>
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<tr>
<td>5</td>
<td>250</td>
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<td>130.8</td>
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<tr>
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</tr>
<tr>
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<td>260</td>
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<td>123.8</td>
<td>15.2</td>
<td>10.3</td>
</tr>
<tr>
<td>13</td>
<td>500</td>
<td>5</td>
<td>89.3</td>
<td>13.0</td>
<td>7.5</td>
</tr>
</tbody>
</table>
5 Results (cont’d)
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8: 0.951
6: 0.935
4: 0.915
10: 0.943
5 Results (cont’d)
5 Results (cont’d)

9: 0.881
6 Conclusions I

- 3-D reconstruction feasible at 10µm resolution
- invasion ‘per continuitatem’, no separated islets
- invasion patterns form a ‘continuum’ of compactnesses
- compactness basically corresponds to pathologist’s assessment
7 Clinical Applicability?

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

Trade-Off: Options for 2-D?
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:

![Graph showing comparison of compactness of massif 3D and overall compactness 2D (averaged).]
7 Clinical Applicability? (cont’d)

**Comparison:**

![Graph showing correlation between compactness of 3D massif and overall compactness of 2D averaged. The graph includes a scatter plot with data points and a trend line indicating a high correlation coefficient of 0.994.]

Correlation Coefficient: 0.994
7 Clinical Applicability? (cont’d)

Comparison:

![Graph showing correlation between compactness of massif 3D and overall compactness 2D (averaged).]

Correlation Coefficient: 0.994 → practically equivalent
Clinical Applicability? (cont’d)

Analysis of 76 Cases:
7 Clinical Applicability? (cont’d)

Analysis of 76 Cases:

Examples

c: “closed”
f: “finger-like”
d: “diffuse”
7 Clinical Applicability? (cont’d)

Analysis of 76 Cases:

[Box plot image showing the analysis of 76 cases by two pathologists.]

- Pathologist 1: N=17d, 50f, 9c
- Pathologist 2: N=16d, 51f, 9c

Discrete Compactness

Values range from 0.90 to 1.00.
7 Clinical Applicability? (cont’d)

Analysis of 76 Cases:

![Box plot image showing compactness intervals for Pathologist 1 and Pathologist 2.]

Compactness intervals significantly different (p ≤ 0.0001)
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, \( p \leq 0.033 \)

- ∀ other characteristics no non-random compactness differences: age (35a), pT, pN, rel. tumour invasion depth, G, V, inflamm. reaction, recurrence (5a)
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Analysis of 76 Cases: Interpretation

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

- 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
• 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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9 Advanced Tumour Reconstruction & Analysis (cont’d)

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  → the spatial organization of a cervical cancer

  ⇒ the relation of the tumor invasion front vs. the infiltration with CD3\(^+\) T-cells.
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\textsuperscript{INK4a}
    - c the T-cell marker CD3
9 Advanced Tumour Reconstruction & Analysis (cont’d)

- Adapted Image Processing Chain

```
Tissue Specimen → H&E/p16^{INK4a} /CD3 Stained Serial Sections → Digitization

Rigid Registration → Color Adaptation → Polynomial Non-linear Registration

Consistent Tissue Segmentation → Curvature-based Non-linear Registration → Post-Processing

3-D Tissue Surface Rendering → Tumor vs. Inflammation Analysis
```
• 3-D Tissue Reconstruction
• 3-D Tissue Reconstruction
• 3-D Tissue Reconstruction
• Automatic Segmentation Examples

![Tumor: p16\textsuperscript{INK4a}](image1)

![T-Lymphocytes: CD3](image2)

![Tumor: p16\textsuperscript{INK4a}](image3)

![T-Lymphocytes: CD3](image4)
9 Advanced Tumour Reconstruction & Analysis (cont’d)

- Automatic Segmentation Examples: Post-Processing

![Tumor: p16^INK4a](image1)

![T-Lymphocytes: CD3](image2)

![Tumor: p16^INK4a](image3)

![T-Lymphocytes: CD3](image4)
9 Advanced Tumour Reconstruction & Analysis (cont’d)

- 3-D Reconstruction results: Surface rendering

Overall reconstructed tissue volume: 60.9mm$^3$, Tumor Compactness: 0.89, Tumor vol.: 11.6mm$^3$, T-Lymphocyte vol.: 1.1mm$^3$
• 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
• Mean curvature of tumor surface
3-D Reconstruction results: T-Cell ↔ Tumor Distances
3-D Reconstruction results: T-Cell → Tumor Diffusion
• Conditional probability density $p_d(\kappa|d)$ for the mean curvature $\kappa$ at a certain distance $d$ from the T-cells

\begin{align*}
\kappa &> 0.032 \\
&< 0.032 \\
&< 0.030 \\
&< 0.028 \\
&< 0.026 \\
&< 0.024 \\
&< 0.022 \\
&< 0.019 \\
&< 0.017 \\
&< 0.015 \\
&< 0.013 \\
&< 0.011 \\
&< 0.009 \\
&< 0.007 \\
&< 0.005 \\
&< 0.003 \\
&< 0.001
\end{align*}

• the longer $d$, the more surface regions with a high magnitude of $\kappa$ occur (neg. $\kappa$: convex curv.)
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 $d$)
• Probability density $p_s(\kappa, c_s)$ for curvature $\kappa$ and substance concentration $c_s$ (subst. const. emitted by T-cells)

\begin{center}
\includegraphics[width=0.6\textwidth]{probability_density.png}
\end{center}

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