Automated microscopy analysis of this touch prep allows for rapid evaluation of surgical margins for invasive breast cancer
Introduction

• Touch preparation have proven nearly ideal for detecting invasive carcinoma but ductal carcinoma in-situ (DCIS) remains challenging.

• Tissue sections provide us definitive pathology and allow us to gain insight in identifying DCIS from other types of cancer cells.

• 30 tissue sections were examined by pathologist to identify normal and cancer cells.

• These calibrated tissue sections were then used to identify characteristics of cancer cells.

Representative Tissue Sections

(Upper left): Normal Breast Tissue
(Upper middle): Low Grade Invasive Carcinoma
(Upper right) High Grade Invasive Carcinoma
(Lower left) Low Grade Ductal carcinoma in-situ
(Lower Right) High Grade Ductal carcinoma in-situ
Automated Feature Extraction

Single Cell Characteristics

- Shape Parameters:
  - Area, Perimeter, Circularity, Feret
  \[ \text{Circ.} = 4\pi \left( \frac{\text{area}}{\text{perimeter}} \right)^2 \]
- Pixel Intensity Measurements:
  - Mean, Min, Max, StdDev.
- Positions

Novel Group Characteristics

- Local Nuclear Density
- Distance to Nearest Neighbor
- Summed Nuclear Area/Neighborhood Area
- Number of Nuclei
- Distributions of Isolated Characteristics
- Architecture
- Of the Shape Parameters, only area and circularity are truly independent.
- Of the Intensity Parameters, only mean and standard deviation are truly independent.
- 4 group parameters are independent.
- Group parameters more than double the number of independent observables.

### Feature Correlations

#### Cell Shapes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>0.90</td>
</tr>
<tr>
<td>Perim.</td>
<td>0.30</td>
</tr>
<tr>
<td>Circ.</td>
<td>0.12</td>
</tr>
<tr>
<td>Feret</td>
<td></td>
</tr>
</tbody>
</table>

#### Cell Intensity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.88</td>
</tr>
<tr>
<td>Min</td>
<td>0.53</td>
</tr>
<tr>
<td>Max</td>
<td>0.66</td>
</tr>
<tr>
<td>StdDev</td>
<td></td>
</tr>
</tbody>
</table>

### Group Properties

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local SD of circularity</td>
<td>0.15</td>
</tr>
<tr>
<td>Local SD of Feret Diameter</td>
<td>0.22</td>
</tr>
<tr>
<td>Local Area Faction</td>
<td>0.076</td>
</tr>
<tr>
<td>Minimum distance to nearest neighbor</td>
<td>0.17</td>
</tr>
<tr>
<td>Number of nearest neighbors</td>
<td></td>
</tr>
</tbody>
</table>

### Correlation Analysis

- Of the Shape Parameters, only area and circularity are truly independent.
- Of the Intensity Parameters, only mean and standard deviation are truly independent.
- 4 group parameters are independent.
- Group parameters more than double the number of independent observables.
Local Group Distributions

- Distributions demonstrate separation of cancer subtypes
- Lines denote simple cutoffs from normal
- Certain distributions are better suited for separation of different cancer types

Max Area separates Normal from HGDCIS

- Mean Standard Deviation of Intensity separates Normal from Cancer
- Circularity separates LGDCIS

Standard Deviation of Nuclear Intensity separates Normal from Cancer
Linear Discrimination Analysis

- Linear Discrimination Analysis (LDA) optimizes for separating predefined classes. Maximizes between class and minimizes in-class variance.
- Even with just single cells properties, using only 10-20 cells, cancer tissue can be distinguished from normal with high certainty.

LDA with single cell properties

- Normal is best separated from HGInv as expected.

LDA single + 100um group

- Group properties increase separation to DCIS since these cells are at very high density.

LDA single + 250um group

- Group properties provide almost complete separation of all cancer types with respect to normal.
Conclusions

• Single cell parameters alone allow separation of normal vs cancer thus allowing us to separate DCIS in our touch preparations

• Identification of the different types of cancer is possible from H&E based image analysis

• Including local neighborhood parameters provides a better separation of the different cell populations

• As a pathology assist tool, we can search large sections for distant micro-metastasis that can be further queried by pathologist

• Enables remote pathology analysis
• Isolate a high affinity nanoparticle (NP) specific to a cell population of interest
• Approached by a NP Library based selection scheme

1. Start with NP Library
2. Exposure to Cells
3. Isolation
4. Amplification

Future: Nanoparticle Libraries for Cancer Identification

Preliminary Results

Advantages
• Discovery of novel markers for detection of breast cancer ex-vivo.
• Can correlate novel markers to future recurrence.
• Conjugation with cancer drugs can provide avenues for novel therapeutics.

Next Steps
• Application of selection scheme to breast cancer cells.
• Verify specificity of selected NP for target cells.

Flow cytometry signal shift can be seen for nanoparticle binding to dendritic cells.
Thank you for your time!

Financial Support

• NanoTumor Center: 5 U54 CA119335-02
  Center of Nano-technology for Treatment, Understanding, and Monitoring of Cancer"
• CURE Research Supplements to Promote Diversity in Health Related Research