How much is enough?

ESTIMATING TUMOR FRACTION
Hey Rocky! Watch me pull a rabbit out of this hat!
Significance of tumor cell fraction on molecular analysis

- Sanger sequencing sensitivity ~20%

- Consider allele fraction:
  - 2 WT alleles / non malignant cell
  - 1 wt and 1 mutant allele / malignant cell*
  - 20% tumor cell fraction yields 10% allele fraction!

*a significant assumption (MASI, amplification, etc.)
Professional responsibility

- **CPT 88381** Microdissection (ie sample preparation of microscopically identified target); manual
  - Professional component  PFS
  - Technical component  CLFS

- **CPT 88363** Examination and selection of retrieved archival (ie, previously diagnosed) tissue(s) for molecular analysis (eg, KRAS mutational analysis)
  - Professional component  PFS
<table>
<thead>
<tr>
<th>Percentage Range (%)</th>
<th>No. Labs (88)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 - 13.0</td>
<td>5</td>
</tr>
<tr>
<td>11.0 - 20.0</td>
<td>5</td>
</tr>
<tr>
<td>21.0 - 30.0</td>
<td>16</td>
</tr>
<tr>
<td>31.0 - 40.0</td>
<td>11</td>
</tr>
<tr>
<td>41.0 - 50.0</td>
<td>12</td>
</tr>
<tr>
<td>51.0 - 60.0</td>
<td>13</td>
</tr>
<tr>
<td>61.0 - 70.0</td>
<td>8</td>
</tr>
<tr>
<td>71.0 - 80.0</td>
<td>7</td>
</tr>
<tr>
<td>81.0 - 90.0</td>
<td>6</td>
</tr>
<tr>
<td>91.0 - 99.9</td>
<td>5</td>
</tr>
</tbody>
</table>

6. Estimation of the percentage of neoplastic cells for the entire section.
Methods for estimating tumor cell fraction

- Count them
- Subjective assessment
- Imaging assisted morphometrics
- Manual morphometric assessment
  - i.e. *point counting method*
Point Counting Method for Estimating Tumor Cell Fraction

Calculate fraction of malignant nuclei = \( p \)

Standard error = \( (p (1-p) / N)^{1/2} \)

e.g. 40% tumor fraction

\[
p = 0.4 \quad 1-p = 0.6 \quad N = 200
\]

\[
SE = 0.03
\]

Tumor fraction +/- 2 SE = 40% +/- 6

i.e. 34 – 46%
A semi-quantitative approach

- Estimate the ratio of malignant to non-malignant cells in the focus of interest

- The error of your estimate is + or – 10% (at best), and more typically, 15 – 20%

- Aim for >50% tumor fraction