**Signal Generation & Optimal Imaging of Biomaterial Surfaces**

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Comparison of electron sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Beam Diameter (nm)</th>
<th>Operation Temp. (°C)</th>
<th>Emission Current Density (A/cm²)</th>
<th>Energy Spread ΔE (eV)</th>
<th>Total Emission Current (µA)</th>
<th>Max Probe Current (nA)</th>
<th>Emission Current Drift (%/hr)</th>
<th>Tip Life (hrs)</th>
<th>Vacuum (Torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tungsten</td>
<td>20 000-50 000</td>
<td>10 000-20 000</td>
<td>10-20</td>
<td>3-10</td>
<td>200</td>
<td>1000</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>10-5</td>
</tr>
<tr>
<td>LaB₆</td>
<td>10000</td>
<td>8000</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schottky (ZrO/W)</td>
<td>1000</td>
<td>1000</td>
<td>10</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFE W</td>
<td>5000</td>
<td>10000</td>
<td>10-20</td>
<td>0.1</td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

**Control of interaction vol., signal & final image**

Input Variables
- Electron source & emission current
- Position, type & wd of detector
- Condenser lens current
- Condenser lens aperture size
- Accelerating Voltage (kV)
- Type & thickness of coating
- Z variation of the sample

Output: Signals
- AE
- SE
- BSE
- Characteristic X-Ray
- Continuum X-Ray
- Fluorescent X-Ray
- Light

**Monte Carlo**

Electron beam

- High density
- Low density

Probe becomes diffuse in sample from elastic scattering. Shape changes with spec z. Vol dec with increasing spec z. Vol size proportional to beam energy.

**Specimen Interaction : kV**

<table>
<thead>
<tr>
<th>High kV</th>
<th>Low kV</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSE₁</td>
<td>BSE₁</td>
</tr>
<tr>
<td>BSE</td>
<td>BSE₂</td>
</tr>
<tr>
<td>SE</td>
<td>SE</td>
</tr>
<tr>
<td>SE</td>
<td>SE</td>
</tr>
</tbody>
</table>

**Signal emergence volumes from a biological sample**

- Primary electron beam
- Auger e
- Continuum X-rays
- Characteristic X-rays
- Fluorescent X-rays
- Typical 5-10µm
**Electron - Specimen Interactions**

- **Elastic Scattering**
  - Close to atomic nucleus
  - Retarded by electro-magnetic field of nucleus
  - Little loss of energy by electron
  - Large deflection
    - Proportional to atomic number
    - Related to energy on electron (acc. voltage)
  - = Backscattered Electron (BSE)

- **Inelastic Scattering**

- **Secondary Electrons** 20-50eV

**BSE (basic) Imaging (composition)**

- **BSE images**
  - Embedded fibroblast cell undersurface

- **Immunolabelling**
  - Gold enhanced 5nm gold (~30nm particles)
    - 0eV (upper detector) mainly SE
    - -10eV (upper detector) removed low energy SE
5nm Colloidal gold – practical resolution

SE / BSE

Contrast Formation (lateral detector)

Topography & Zone of Primary excitation

Optimal Fixation & Imaging
Stereo pairs

-3 tilt

+3 tilt

5
Use many electron-optic conditions to obtain the most information from your sample.

Lecture series: Richards & ap Gwynn