“One Sees Only What One Knows”
SEM Image Interpretation

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SEM Imaging
- Information required?
  - Signal types
  - Resolution?
- Specimen preparation
  - Preservation?
  - Dehydration?
  - Coating
- Microscope settings
- Interpretation
  - Analysis

Potential SEM Information
- If used incorrectly
  - Very little
  - Waste of time and effort
- If used to its potential
  - Much
  - Dependent upon
    - Specimen preparation
    - Imaging conditions
    - Interpretation and analysis

Quotation

“Man sieht nur was man weiss”
(One sees only what one knows)
Goethe

What is this?

Romanesque Brocolli/Cauliflower (or Romanesco)

Purpose of SEM Studies
- Reveal topographical surface detail
  - SE – low voltage
  - BSE – all voltages
- Detect sub-surface information
  - BSE – optimise voltage
- Detect compositional differences
  - BSE – Atomic number contrast
  - XRA – element specific
Logical Analysis of Micrographs

CONSOLIDATED VIEW

MICROGRAPH

INTERPRETATION

Instrumental effects

Interactions

Specimen preparation

CONSOLIDATED VIEW

Correlative information

Supplementary considerations

INFORMATION RETRIEVAL

Structural

Cytochemical

Numerical

Reasons for Lack of Positive Contrast e.g. BSE imaging

- Microscope settings?
- Fixing/staining agent(s) has not penetrated
- Fixing/staining agent(s) has not reacted
- Coating unsuitable
- … etc.

Calcified/soft Cartilage Interface

Which is the correct image?

Secondary Electron Images

Backscattered Electron Images

Surface of Glass Fibre

Different Signal Comparison

1 kV SE
4nm Pt/Pd

3 kV BSE
4nm Pt/Pd

30 kV BSE
4nm Pt/Pd + 60nm C
Cultured fibroblast Osmium stained BSE images

Images courtesy of R.G.Richards

8kV

15kV

Image Analysis

Quantitative data
Grey level values (256 / pixel)
Measurements
length, breadth, areas etc
Counting
labels

Basic Principles of Image Analysis

Areas of interest defined
expressed as binary (0 & 1) image
Measurements on binary image.

The result of ‘thresholding’

The result of ‘processing’
Some Measurements Possible
1. Count the No of objects
2. Area
3. Length
4. Breadth
5. Perimeter

Others:
6. Equivalent circle
7. Ferets
8. … and more

PC_Image
Select Measurements

3D Rendering by software (Alicona: Mex)

Articular cartilage fracture propagation
Undulation of matrix

Take Home Messages
• Do microscopy
  – Carefully
  – Not a means to ‘decorate’ publications
• Research background
  – Carefully (broad sources)
• Ask a microscopist!
  – Before you start (potential information)
  – To interpret results

Combining Microscopies

Images courtesy of DO Meredith, AO

SEM Workshop
ap Gwynn & Richards 2005