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CHEM-E5140 Materials Characterization Laboratory

Basic optical microscope lecture
5.10.2020

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Outline

1. Introduction
2. How do I get the data?
3. Microscope performance
4. What kind of samples can be studied?
5. Quantitative image analysis

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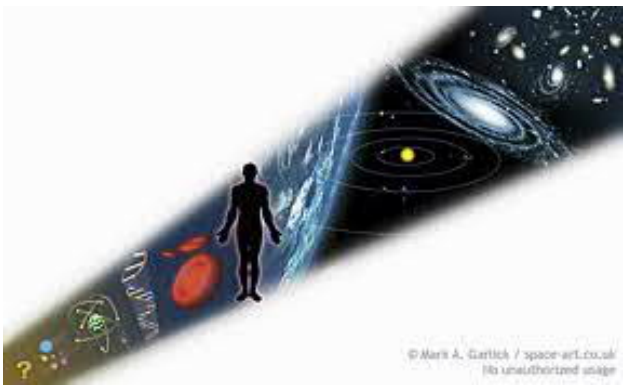
1. Introduction

- A microscope (from the Greek: μικρός, mikrós, "small" and σκοπεῖν, skopeîn, "to look" or "see") is an instrument used to see objects that are too small to be seen by the naked eye.
- The optical microscope uses visible light and a system of lenses to magnify images of small objects.

Why microscopes?



Materials characterization techniques

	Magnification	Resolution	Depth of field	Sample	Other
Basic optical microscope	10-1000x	1-0,2 μm	2-0,2 μm	Flat (polished, etched)	Inexpensive, Reflectivity, FTIR, Raman
Scanning electron microscope	10-200000x	1-100nm	1 mm – 0,1 mm	Usually electrically conductive	**Vacuum,** EDS, WDS, EBSD, CL, EBIC
Transmission electron microscope	>600000x	0,15-0,3 nm	n. 20 nm	Very thin	**Vacuum,** Diffraction, EDS, EELS

Other methods: **XPS (ESCA)**, AES, ...
XRD, XRR,
XRF, **Raman**, AAS, SIMS,PIXE, ...
AFM, STM, μXCT ...

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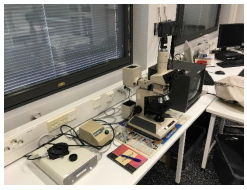
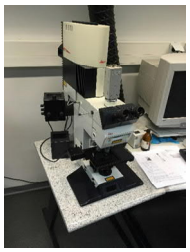
Microscopes



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2. How do I get the data?

- How optical microscope works?
- Equipment technology
- Basic illumination modes

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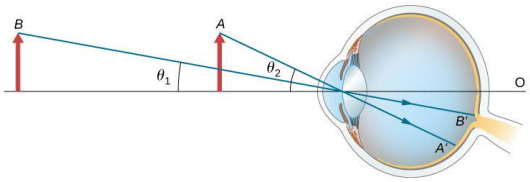
Magnifying images

- Decrease of focal distance
- Increase of the angular size of the object



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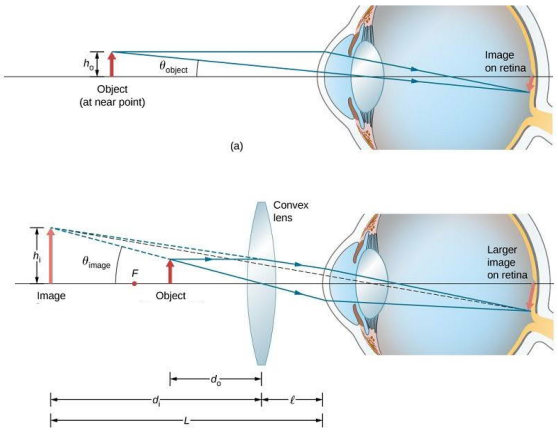
Spectacles and magnifying glasses



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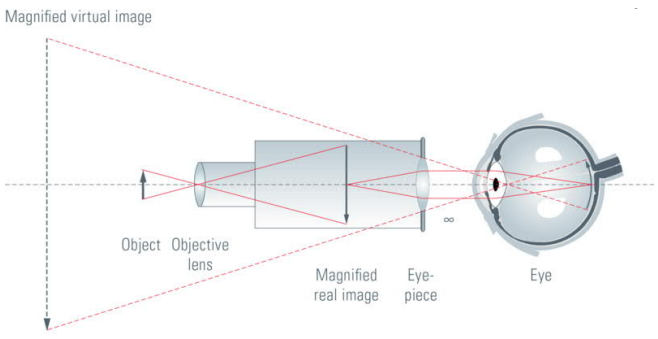
Principle of simple compound microscope



Compound microscope makes two stage magnification

- initial magnification with objective
- further magnification with eyepiece

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Equipment technology

- Main microscope components
- Microscope designs
- Illumination modes

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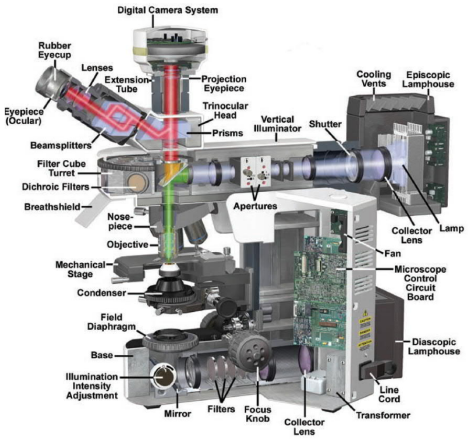
Main microscope components

- **Objective lens**
- Eyepiece
- Filters
- Field diaphragm
- Aperture diaphragm
- Illumination system
- Camera system
- Specimen stage



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Set of objective lenses

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Objective lenses

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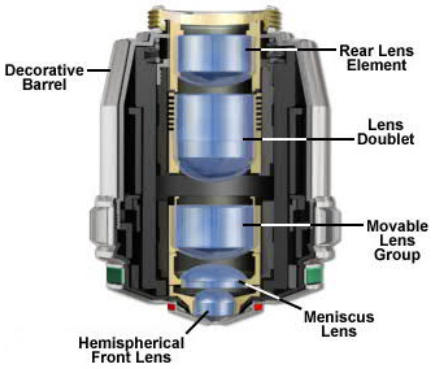
Objective lens



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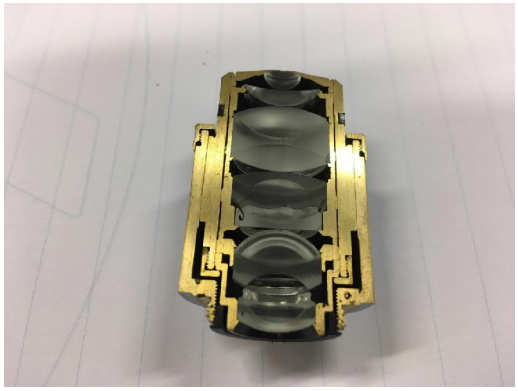
A lens cut in half



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Numerical aperture

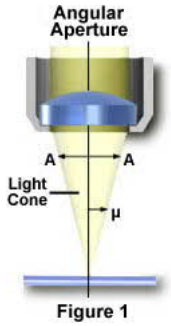

Figure 1

- The numerical aperture of a microscope objective is a measure of its ability to gather light and resolve fine specimen detail at a fixed object distance.
- Numerical Aperture (NA) = n(sin μ)
- The angle μ is one-half the angular aperture A.
- n is the refractive index of the imaging medium between the objective and the sample.
- Working in air, the theoretical maximum value of the numerical aperture is NA = 1 (μ = 90°). The practical limit is NA = 0.95.

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Lens aberrations

- Chromatic aberration
- Spherical aberration
- Curvature of field

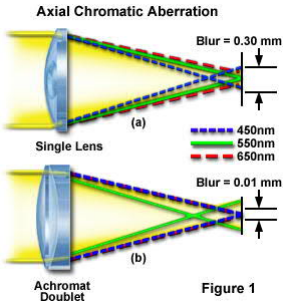

Figure 1

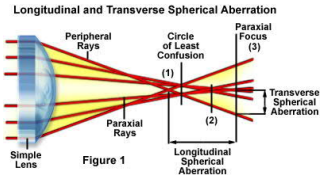

Figure 1

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Microscope designs

	Transmitted light	Reflected light
Upright		
Inverted		

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Digital camera

- A digital camera captures photographs in digital memory
- Cameras are based on CCD or CMOS sensors
- Today most high-resolution microscope cameras will provide full microscope resolution with good dynamic range and signal-to-noise characteristics
- Most of the recent development in basic optical microscopy has taken place due to digitalization

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Software for image acquisition and analysis

- Image aquisition
- Image processing
- Feature extraction
- Representation of microstructural geometry
  - Features: volume, surface area, size, shape, orientation etc.
  - How much?
  - Distribution
  - Clustering correlations

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Basic illumination modes in research microscopes

- **Brightfield**
- Darkfield
- Polarized light
- Differential interference contrast
- Fluorescence microscopy

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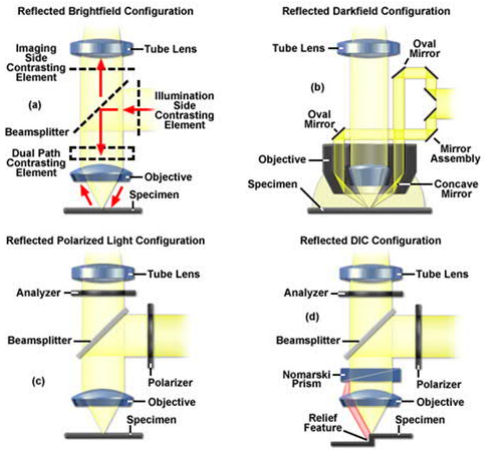
Contrast mechanisms in reflected light microscopy



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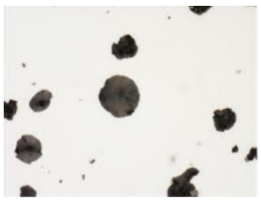
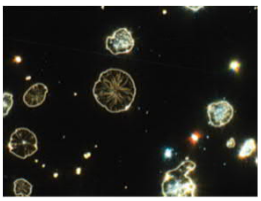
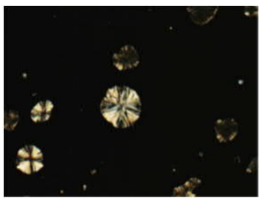
Images from nodular cast iron



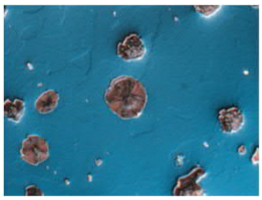
Brightfield



Darkfield



Polarized light



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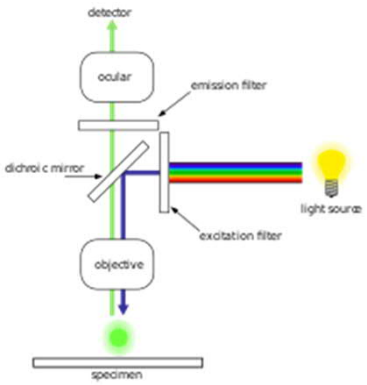
Fluorescence microscope



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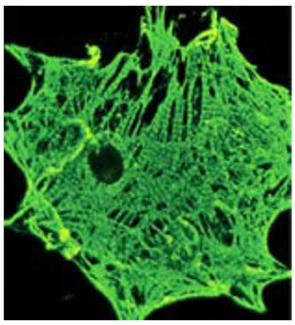
Cell with fluorescent dye staining



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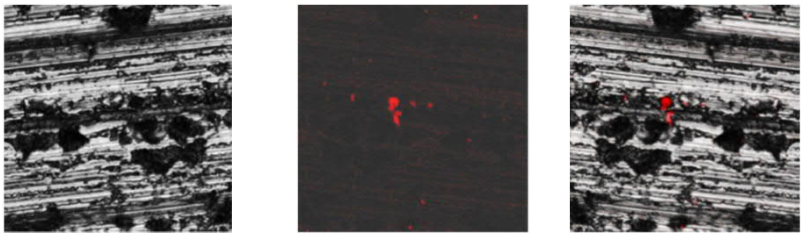
Investigation of cracks



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3. Microscope performance

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Microscope performance

- Magnification
- Resolution
- Depth of field
- Contrast

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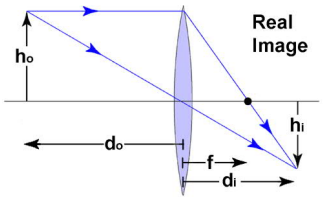
Magnification

Magnification is the ability of a microscope to produce an image of an object at a scale larger than its actual size.

A basic definition of optical magnification is the ratio between the size of an object in an image and its true size. However, it can be expressed in other terms as well.

Magnification of single lens:

$$M = h_i/h_0 = d_i/d_0 = f/(d_0 - f) = (d_i - f)/f$$



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Magnification in basic optical microscope

When observing the image through the eyepieces of a microscope for visual observation, the total (lateral) magnification is defined as:

$$M_{TOT\,VIS} = M_O \times q \times M_E$$

where
- $M_{TOT\,VIS}$ is the total lateral magnification observed through the eyepiece,
- M_O is the objective lens magnification,
- q is the total tube factor (zoom and other tube lenses), and
- M_E = eyepiece lens magnification.

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Magnification in compound optical microscope with digital camera

For digital microscopes, an image is projected onto an electronic sensor of a digital camera, and then displayed onto an electronic monitor for observation. Thus, the final total magnification for digital microscopy will depend also on the actual pixel size of the monitor. The total magnification can be defined as:

$$M_{DIS} = M_{TOT\,PROJ} \times \text{Pixel ratio}$$

The pixel size ratio is determined by the ratio of the pixel size of the monitor to that of the camera sensor:

$$\text{Pixel ratio} = \frac{\text{Pixel size monitor}}{\text{Pixel size sensor}}$$

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Resolution

Resolution R is smallest distance between two points on a specimen that can still be seen as separate entities.

Resolution in basic optical microscopy is subject to not technical but fundamental physical limits. It is diffraction limited.

R is determined essentially by following parameters:
- the wavelength λ of the illuminating light,
- and the numerical aperture (NA) of the system

In reflected light microscopy the equation reads:

$$R = 0.61 * \lambda / NA_{obj}$$

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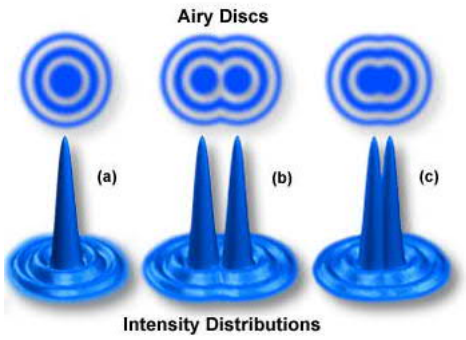
Resolution



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Depth of field

Depth of field d describes the range along the optical axis in which the specimen can move or have topography without the image losing its sharpness.

Depth of field is determined essentially by same parameters than resolution but in different ways:

Mathematically depth of field is directly proportional to:

$$d \sim \lambda/2*NA^2$$

Consequently, depth of field and resolution are dependent.

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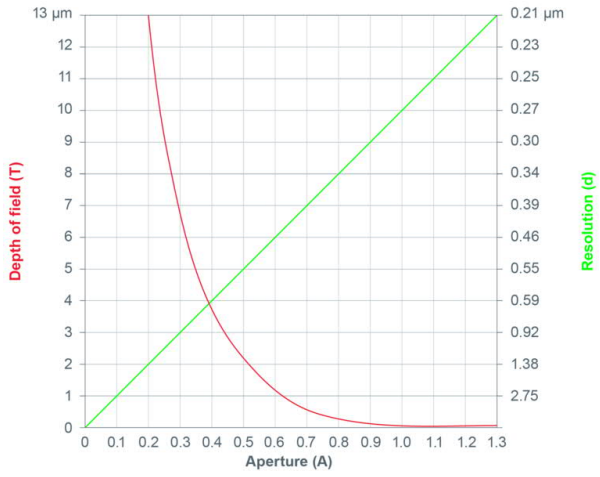
Resolution vs. depth of field



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Microscope calibration

- Stage micrometer

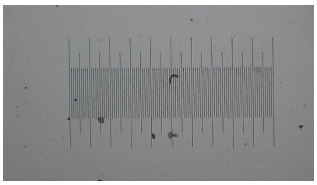

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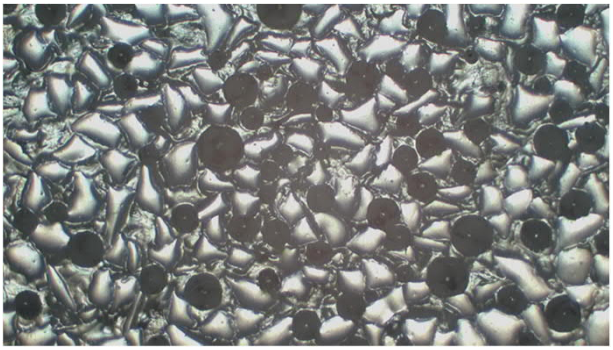
Image without scale



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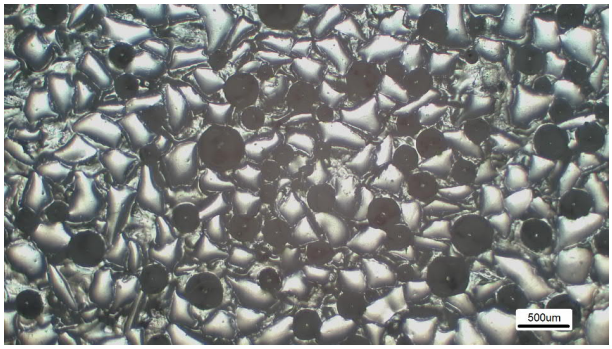
Scale marker



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Empty magnification

The useful range of magnification depends on the maximum resolving power of the microscope system.

In optical microscope, magnification should not be higher than 1000x the NA of the objective

When the magnification passes beyond the useful range, the image will be only enlarged but no additional details can be seen. This situation is referred to as empty magnification

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Useful lens combinations

Objective (NA)	Eyepieces				
	10x	12.5x	15x	20x	25x
2.5X (0.08)	---	---	---	x	x
4X (0.12)	---	---	x	x	x
10X (0.35)	x	x	x	x	x
25X (0,55)	x	x	x	x	---
50X (0,80)	x	x	x	---	---
100X (0,95)	x	---	---	---	---

(x= good combiation, total magnification 500-1000 x NA of Objective)

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Contrast enhancement

- Critical factor when determining whether useful information can be extracted from an image is whether there is sufficient contrast between the features of interest and the background.
- In the bright field illumination only structural details that differ in reflectivity from one another can be distinguished from each other
- With other illumination modes image contrast can be enhanced
- To obtain necessary contrast, sample surface can also be treated.
- Most common materialographic treatments are preferential etchings.
- In fluorescence microscopy, specific areas of the structure can be marked with a fluorescence dye. These areas will absorb light at a specific wavelength and re-emit light at longer wavelength. Especially in examination of biological and medical specimen, fluorescence is often used, as specific dyes are suited for specific constituents in the sample. In this way an exact microscopic identification can be performed.

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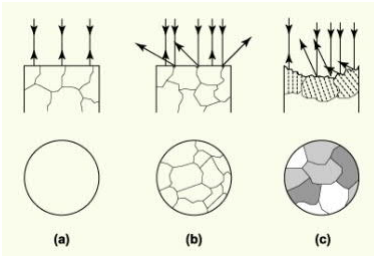
Preferential etching



a) Polished surface gives an image without details about the structure
b) Mildly etched surface: only grain boundaries are visible
c) Etched surface: each grain reacts differently producing varying contrast

Etching recipes are material dependent.

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Advanced optical microscopy

- Computer assisted microscopy
- Confocal microscopy
- Optical profilometry
- Spectral reflectometric microscopy
- Raman microscopy
- FTIR-microscopy
- Scanning near-field optical microscopy
- Super-resolved fluorescence microscopy

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4. What kind of samples can be studied?

- Basic requirements for suitable samples
- Sample preparation

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Typical application examples of optical microscopy in materials science and engineering

- Structural examination of microstructural features of metallographically prepared samples
- Structural examination of cross-sectional samples of coatings
- Morphological analysis of particles, fibres and porous structures
- Hardness testing (Vickes, Knoop)

- Optical microscopy is minimally invasive

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Materialographic sample preparation

- Sectioning
- (Mounting if needed)
- Grinding
- Polishing
- Etching

- Several cleaning steps in between

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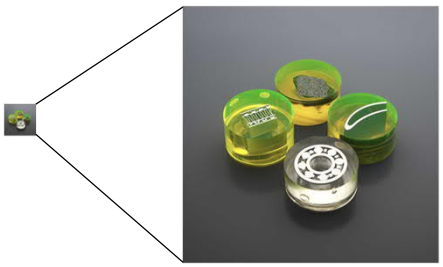
Materialographic sample preparation equipment







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5. Quantitative image analysis

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Quantitative image analysis

- Quantitateve image analysis in this context is extraction of numerical data from microscope images
- It is essentially a data reduction task

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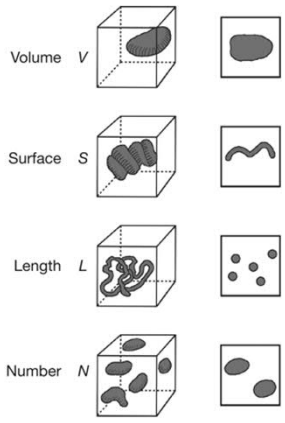
Stereological methods

- Stereology can be considered as science of geometric sampling
- Stereology provides techniques for extracting quantitative information about a three-dimensional structures from measurements performed on two-dimensional planar sections.
- Stereology is based on fundamental principles of geometry and statistics.



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Quantitative image analysis stages

- Image aquisition
- Image processing
- Feature extraction
- Representation of microstructural geometry
  - Features: volume, surface area, size, shape, orientation etc.
  - How much?
  - Distribution
  - Clustering correlations

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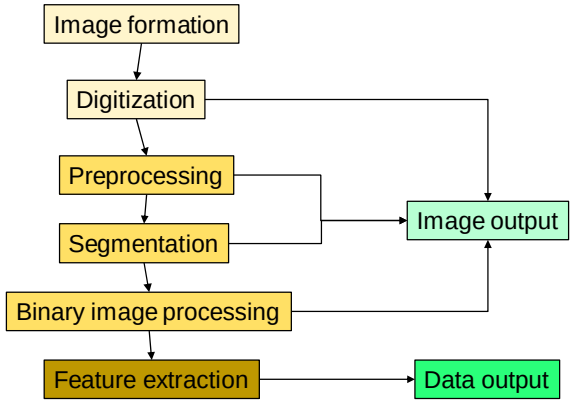
The sequence of digital image acquisition, processing and analysis



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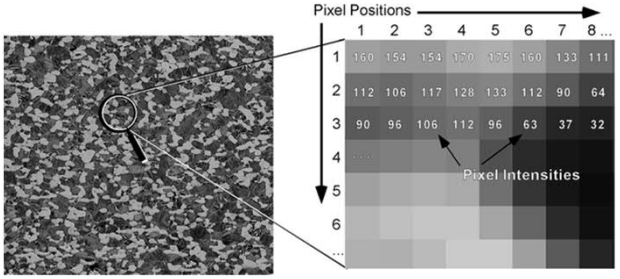
Grayscale digital image



- A digital image is a matrix of pixels with intensities
- Another way of showing the data is numerical table
- Sampling frequency in spatial axis is called resolution
- Sampling frequency in the intensity axis is called quantization

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Gray-level histogram

- By counting number of pixels at each intensity value (gray-level) a distribution histogram can be produced that is yet another basic way of presenting the data.
- Gray-level histogram can be used to optimize image capture.
- It is also important in segmention step.
- The shape and position of the gray-level histogram provides information about brightness, contrast and measurability of the image.

Count: 823536
Mean: 0.881
StdDev: 3.601

Min: 0
Max: 44
Mode: 0 (763610)

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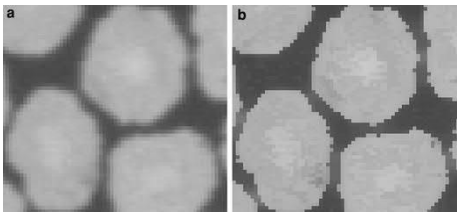
Preprocessing

- Noise reduction
- Background correction
- Delineation
- (Contrast stretching)



a) Magnified original gray-level image of particles showing gradual transition of gray levels along the feature edges.
b) The same image after using a delineation filter

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Segmentation

- Segmentation is the term used for recognition of objects in an image
- It is made through classification of each pixel of the image as pertaining of not to an object
- The simplest and most commonly used method is intensity thresholding
- Segmentation results binary image (black and white)

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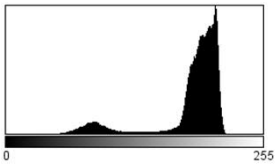
Intensity thresholding

- The process in which grayscale is reduced black and white, which represent features and backround, is called thresholding
- Bimodal gray-level histogram is a proper starting point for thresholding



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Max: 225
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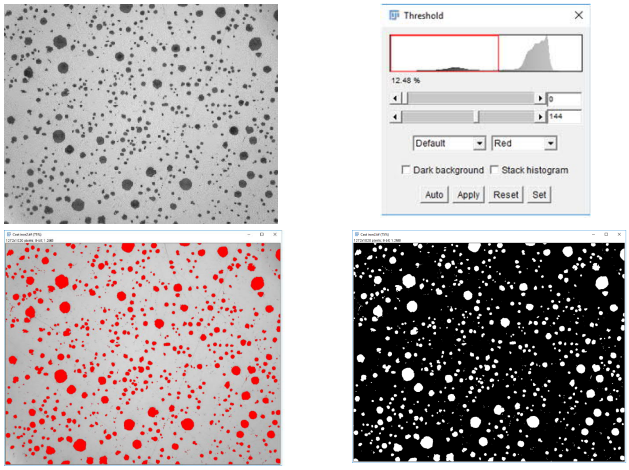
Intensity thresholding



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Binary image processing

- Even with the best conditions, segmentation is seldom a single-step procedure

- Hole filling
- Erosion and dilation

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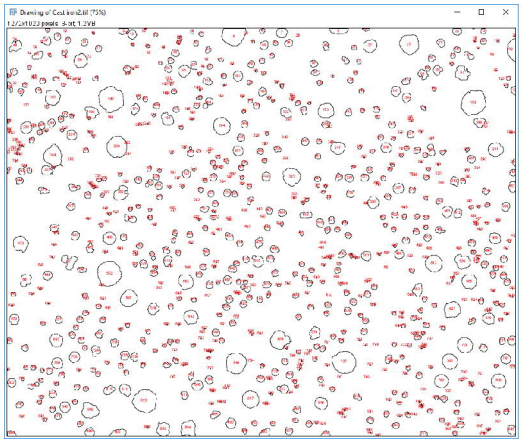
Feature extraction



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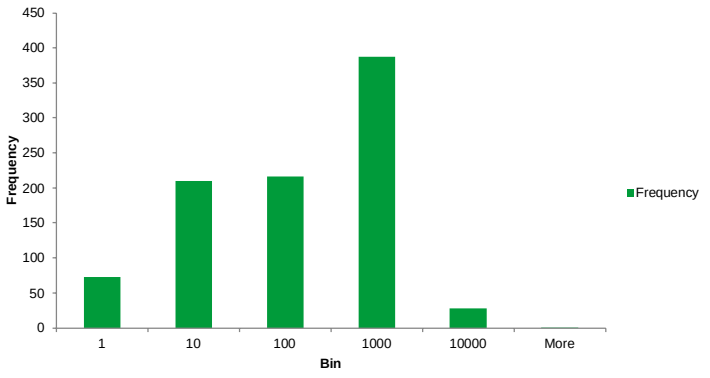
Data output



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Pretask:
How to prepare

terrapinsa005.weebly.com

- Prepare 4-6 slides
  - What information the method provides and how does it work?
  - What kind of samples can be analysed?
  - Is the method destructive for the sample?
  - Your picture of the operating mechanism of the device (drawn with hand or by yourself with computer)

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Thank you for your attention

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