出國報告（出國類別：見習）

無國界文資教師見習方案

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摘要

本出國計畫主要到美國芝加哥 McCrone Research Institute 學習光學顯微鏡的原理與其應用技法。McCrone Research Institute 可說是光學顯微鏡學重要的研究機構，定期開授顯微鏡相關課程。光學顯微鏡常應用於文物鑑定，可說是材料分析最基礎的儀器。課程包括光學原理、顯微鏡結構、晶體結構、異向性、折射率、雙折射率、光學成像原理、採樣與基礎鑑定等內容。此課程為採密集上課，為期五天，通過測試者則頒發結業證書。

目次

一、目的

McCrone Research Institute 是美國芝加哥著名的非營利教育與科學研究組織，創始人 Walter C.McCrone 博士是知名的顯微鏡學家，曾參與有名的文蘭地圖 (Vinland map)與都靈裹屍布(Shroud of Turin)等鑑定案件。因博士致力推廣有效地應用光學顯微鏡，以解決實際的問題。因此 McCrone Research Institute 在顯微鏡學領域佔有非常重要的地位。此次選擇學習偏光顯微鏡的基礎課程主因是國內多數專家偏向於使用高科技分析儀器如 FTIR、拉曼光譜儀等。但這類分析儀器設備除了需要專人管理，且價格非一般機構所能負擔。相較於此，光學顯微鏡結構簡單，若能掌握光學顯微鏡各項應用技術，亦能精準地鑑定文物材料。


圖 1. McCrone Research Institute 大門入口

二、過程

此次上課主題為偏光顯微鏡/鑑識學的應用可說是 McCrone Research Institute 的基礎課程，期程五天，第一天到第四天早上八點到下午五點，一天八個鐘點，總計三十二個小時，第五天八點到十二點共四小時，課程總時數為三十六小時，等於兩個學分。

第一天上課內容包括鑑識科學及歷史介紹、光學原理、顯微鏡種類等內容；下午課程介紹晶體結構、型態、結晶等；實際操作並認識顯微鏡的功能。

第二天上課內容包括折射率、光波、溫度等對折射率的影響、偏光介紹、分散染色。實驗課程內容包括等向性與非等向性晶體的折射率測量與分散染色操作。

第三天上課內容以再次認識晶體結構、雙折射率、消色(extinction)、光向方位

與補償濾鏡。操作課程包括各向異性材料(anisotropic materials)、雙折射率(birefringence)、消色、光向方位等練習。

第四天早上實為測試，老師不動聲色地將玻片發給每位學員，要求學員在顯微鏡觀察下鑑定玻片的內含物。考試完後繼續上錐光偏振顯微鏡觀察法；其後在講述微化學測試的應用。

第五天上課內容主要集中於採樣與各式樣品的融合測試 (Fusion method)。
上述課程內容相當紮實且可實際應用於了解古物與修護顏料的認識與應用，藉由採樣與檢視分析與測試，可謂是鑑定材料的基礎。


圖 2. 折射率觀察使用橙色濾片

圖 3. 測試合格後領到的結業證書

三、心得與建議

就分析儀器而言，光學顯微鏡對文物材質鑑定貢獻是不容小覷的，與貴重儀器比較，成本低廉，可說是文保修護工作室必備之工具。此次赴美學習偏振光原理與其鑑定技法，不但可以應用於文物材料的檢視，同時材料學課程也安排偏光顯微理論之內容，充實學生顯微專業的知識。

四、附件

課堂筆記

Microscopy
Lecturer: Sebastian Sparenga
ssparenga@mri.org

How to set up microscope
Thing align properly, esp. for photographing

Physical optics review
Light is electromagnetic radiation

electromagnetic spectrum
CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=2521356

visible light spectrum
By Gringer - Own work, Public Domain,
https://commons.wikimedia.org/w/index.php?curid=4639774

Properties:

1. Propagation quality: linear direction →

2. Vibrating component (wave)
   Wave length λ (nm)

Light wave
By No machine-readable author provided. Gpvos assumed (based on copyright claims). - No machine-readable source provided. Own work assumed (based on copyright claims)., CC BY-SA 3.0,
https://commons.wikimedia.org/w/index.php?curid=1864272

Wave length λ (nanometer, nm)
Amplitude

Interaction of light with matter: absorption, reflection, refraction

Absorption

a light wave of a given frequency strikes a material with electrons having the same vibrational frequencies, then those electrons will absorb the energy of the light wave and transform it into vibrational motion.

During its vibration, the electrons interact with neighboring atoms in such a manner as to convert its vibrational energy into thermal energy. Subsequently, the light wave with that given frequency is absorbed by the object, never again to be released in the form of light.

Reflection

Reflection of light, depending on the nature of the interface,

Law of reflection

i (incident angle) = r (reflected angle)

specular reflection: mirror-like reflection (think about mirror, you will see the image) on glare and shiny surface

diffuse reflection: image not formed on rough surface

Fig. reflection of light

http://www.collectionscanada.gc.ca/eppp-archive/100/205/301/ic/cdc/science/english/phys/projects/inline/rainbo2.jpg

By GianniG46 - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=10635585

Where does color come from?

The color of the objects that we see is largely due to the way those objects interact with light which is **reflected** by the object or **transmitted** by a solution to our eyes.

Credit: Dorling Kindersley Ltd.

Exercise

http://www.physicsclassroom.com/class/light/Lesson-2/Light-Absorption,-Reflection,-and-Transmission

Refraction (Transmission)

Refraction is an effect that occurs between transparent materials of different densities.

Transmission of light to the sample.

The change in direction of propagation of a wave due to a change in its transmission medium

That is light bends

Example, light slows down in glass.

The example above, light slows down while entering glass due to molecule density
Refractive index (n): measure how light passes through the substance

$n = V_{vac}/V_{subs}$
V= velocity

$n_{glass} = 3.0x\ 10^{10}\ cm/s\ /2.0x\ 10^{10} cm/s = 1.5$
$n_{air} \approx 1.0$
$n_{water} \approx 1.3$
$n_{glass} \approx 1.5$
$n_{diamond} \approx 1.4$
The bigger n# , the slower light passes the substances

Light into a substance with higher density, it bends toward to the perpendicular. When light pass through, it will bend the other way (away from perpendicular because lower density of media)

http://www.physicsclassroom.com/class/refrn/Lesson-2/Snell-s-Law

Snell' s law:

$$n_i * \text{sine}(\Theta_i) = n_r * \text{sine}(\Theta_r)$$

where Θ_i ("theta i") = angle of incidence

Θ_r ("theta r") = angle of refraction

n_i = index of refraction of the incident medium

n_r = index of refraction of the refractive medium

Excises

How about light refracts out of the water into air?

Things to remember

1. Light travels from lower n to higher n, light bends toward normal (perpendicular line)
2. Light travel from higher n to lower n, light bends away from normal

What affects n?

Density

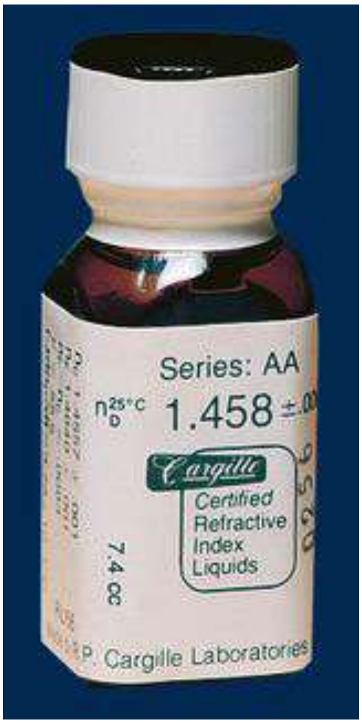
Density ↑, velocity↓, n↑

Chemical composition

Molecular weight (big influence)

Chemical bonding

How to read the refractive index label



$n^{25℃}$ keep in 25 ℃

n will change by T dramatically

As T ↑, D↓, Velocity↑, n↓

n = 1.605 (0.0004 T coefficient)

while in 30℃, 30-25=5 x 0.0004 =0.002

n 30℃ = 1.605-0.002 = 1.603

when light bends into different wave length (700nm to 400nm)

http://jm-derochette.be/index_measurement/Theory.htm

subscript "D" refers to λ
specific wave length = 581.3 nm in orange spectrum, which is a major source wavelength for dispersion staining.
In addition
HC 656nm in red
D stands for Sodium D 589.3nm in orange
Hydrogen F 486nm in blue
Refers to Fraunhoffer lines

https://upload.wikimedia.org/wikipedia/commons/2/2f/Fraunhofer_lines.svg

No condenser with condenser

http://edafologia.ugr.es/optmine/xplconos/consecuw.htm

Optical aberration:

Spherical aberration v.s. ideal lens

Ideal lens: all incoming rays are focused in the focal point .

Spherical aberration: the different rays do not meet after the lens in one focal point.

https://en.wikipedia.org/wiki/Spherical_aberration#/media/File:Spherical_aberration_2.svg

Chromatic aberration

a failure of a lens to focus all colors to the same convergence point because lenses have different refractive indices for different wavelengths of light.

http://photographyelement.com/what-is-chromatic-aberration/

https://en.wikipedia.org/wiki/Chromatic_aberration#/media/File:Chromatic_aberration_lens_diagram.svg

https://en.wikipedia.org/wiki/Chromatic_aberration#/media/File:Chromatic_aberration_(comparison).jpg

Field curvature

Also "Petzval field curvature", is a common optical problem that causes a flat object to appear sharp only in a certain part(s) of the frame, instead of being uniformly sharp across the frame.

https://en.wikipedia.org/wiki/Petzval_field_curvature#/media/File:Field_curvature.svg

http://www.handprint.com/ASTRO/ae4.html

Corrected lenses (PLAN) to correct false
Achromat
Fluorites
Apochromats
Plan- (plan-achromats or plan-apochromats)

Types of corrected objective lenses: depending to the degree of correction

objective	Spherical correction	Chromatic correction
Achromat (like Toyota)	1 wave length	2
Fluorite	2	2
Apochromat highly corrected objective lenses you can purchase	2	3

Apochromat lense: long-crown glass (fluor crown glass and calcium fluoride) and special short flint glass

Any of these can be corrected for filed curvature
Just add a plane-prefix?

Strain free objectives should be used for PLM

Limits of accommodation of human eyes
Healthy eye 250mm
M=250/f
Image formed by a positive lens- Ray diagrams
The line of sight principle suggests that in order to view an image of an object in a mirror, a person must sight along a line at the image of the object. When sighting along such a line, light from the object reflects off the mirror according to the law of reflection and travels to the person's eye. This process was discussed and explained **earlier in this lesson**. One useful tool that is frequently used to depict this idea is known as a ray diagram. A **ray diagram** is a diagram that traces the path that light takes in order for a person to view a point on the image of an object. On the diagram, rays (lines with arrows) are drawn for the incident ray and the reflected ray. Complex objects such as people are often represented by stick figures or arrows. In such cases it is

customary to draw rays for the extreme positions of such objects.

Bibliography
NIKON
https://www.microscopyu.com/techniques/polarized-light/polarized-light-microscopy

http://www.physicsclassroom.com/

Compound microscope p. 15?
Lamp housing
Lamp condensing lens
Color balancing filter (blue) to compensate tungsten light bulb' s color

Diffuser
Par focal
Started with lower magnification to higher magnification
Field dual lens
Close to the field
Condenser
Condenser aperture
Nose pieces
Dispersion staining 10x with screw holes blocking light for a special purposes
Neck area: intermediate tubes
Binocular head
Trinocular head
Eye pieces 10x

Image forming rays fig.1

Illumination rays fig.2 Bertrand lens inserted to bring up the focus

Kohler Illumination
Purpose
Masimize control over
Contrast
Resolution

Depth of field
Even illumination over entire field of view
Set up manual appendix 2-17

Contrast

https://www.microscopyu.com/techniques/phase-contrast/specimen-contrast-in-optical-microscopy

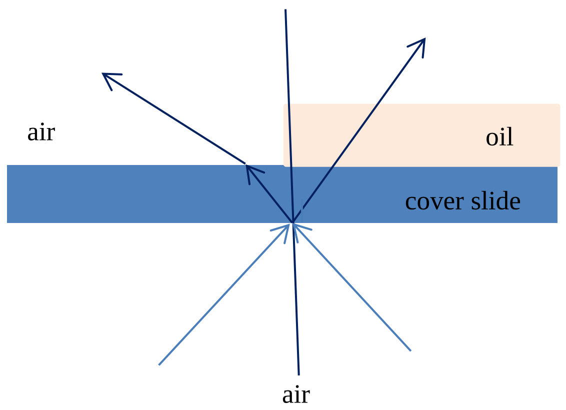
Resolution and numerical (numerical aperture, N.A.)
the ability to distinguish between two closely spaced Airy disks in the diffraction pattern

High objective resolution aperture
Low objective resolution aperture

Resolution X = (0.61*λ)/N.A. the shorter wave length, the better resolution
X = the smallest distance between two objects
See https://www.microscopyu.com/microscopy-basics/resolution

light gathering ability of lens N.A. = n*sin(AA/2)
n refractive index cover slip and objective

n_{oil} = 1.52
n_{glass} = 1.5



Angular Aaperture (A.A.)

Higher magnification, higher A.A.

A.A. 140 the highest so far you can get
Larger # refers to magnification ex. MAG 40
N.A. 0.65

160/0.17
0.17 thickness of cover slip
160 length of mechanical tube, make sure the same
∞ stands for infinity corrected optics
Keep system together
Ie. ∞ to ∞

Condenser #0.9 to 1.25 refer to NA

Open (left)	Close (right)
High resolution	Low resolution
Low contrast	High contrast
Low depth of field	High depth of field

The morning section introduce basic theory and light property of microscope field, explaining how, whay the effect of looking at the microscope
1. Knowing structure of microscope

2. Set up microscope, align all lenses
3. Stage centered/ nosepiece centered

Micrometry
Know scale
1mm = 1000µm
10µm is good size to work on microscope, too big won't be suitable
Eyepiece #100 stage micrometer
 100 x 0.01 = 1 mm
 Each division = 10 µm

Left (stage scale one to rotate)	right
SS (Stage Scale) = 0	SS = 80
OS (Ocular Scale) = 10	OS = 89

SS = 80-0 = 80 x 10 µm = 800
OS = 89-10 = 79
800/79 = 10µm/division

http://microbiologyconcepts.blogspot.tw/2016/04/micrometry.html

How to measure
Various statistical diameter
Feret's diameter
    Maximum horizontal intercept
    Projected area diameter
Martin diameter
    Measure the center of particle no rotation, take right in the mid point

http://www.pharmacopeia.cn/v29240/usp29nf24s0_c776.html

Statistical purpose: at least 100 to few hundreds
Check on image J free online

Calibration of micrometry is basically same that is
10x 10µm

20x 5µm
40x 2.5µm
Except Leica OS 0 to 60

Crystal morphology
Shape and size in mind
Crystalline
One that has 3D order to the spacing of orientation of its chemical binding blocks
Constant through entire substance with highly order arrangement

Amorphous structure: randomly positioned chemical building block with no pattern

Degree pf crystallinity
Fiber is in between crystalline and amorphous

Polymorphism
Examples:
Bricks can be arranged different shape
C could be diamond could be graphite

https://en.wikipedia.org/wiki/Diamond_cubic#/media/File:Diamond_cubic_animation.gif

https://en.wikipedia.org/wiki/Graphite#/media/File:Graphite_stereo_animation.gif
Crystal system

http://www.luckysci.com/2014/01/best-trick-for-learning-the-seven-crystal-systems/

Rotation symmetry
Cubic can rotate 360° at 4 times to see same shape, called 4-fold
Rotate 180° at 2 times to see same shape, called 2-fold

4-fold: 90° / 360°
2-fold: 180° / 360°
3-fold: 120° / 360°
6-fold: 60° / 360°

Mirror planes: cut in the center representing same shape

Symmetry element
1. Cubic system: all axis are perpendicular, can be 4-fold or 3-fold with a tilted point, 1 spacing
2. Tetragonal system: existing 4-fold and 2-fold, 2 spacing
3. Hexagonal system (including trigonal and hexagonal): 6-fold and 2-fold, 2 spacing
4. Orthorhombic system: 3 existing 2-fold, 3 spacing
5. Monoclinic: 1 existing 2-fold, 1 mirror plane
6. Triclinic: no perpendicular angle, no symmetry, no rotation semmetry, most crystal have it

Habit: refer to a crystal's general shape, such as equant, massive, needle, rods, plate, tablet etc. It can occur in any of the six crystal systems.

equant, Peridot. taken by Azuncha
https://en.wikipedia.org/wiki/Crystal_habit#/media/File:Peridot2.jpg

fibrous
https://en.wikipedia.org/wiki/Crystal_habit#/media/File:Byssolite_France.jpg

botryoidal or globular
https://en.wikipedia.org/wiki/Crystal_habit#/media/File:Hematite09.jpg

Massive
https://en.wikipedia.org/wiki/Crystal_habit#/media/File:Turquoise_with_quartz.jpg

Type of cleavage
Important feature of many crystalline substances

mica: basal cleavage, can be peel into thin sheet
https://en.wikipedia.org/wiki/Mica#/media/File:MicaSheetUSGOV.jpg

Fluorite
https://en.wikipedia.org/wiki/Cleavage_(crystal)#/media/File:Fluorita_green.jpeg

Ways to recrystallize compound on a microscope slide

1. Sublimation

Take some crystal on slide, heat the slide and ethanol? might be added, crystal will be reform and condense in coverslip

2. Evaporation

Saturated drop with material, more materials should be added than sublimationee

Check on the edge of water – crystal reform

Materials should be soluble in water

3. Hoffing technique

Exercise

Recrystallization observation using Evaporation techniques (p.14-3)

NaCl

$Pb(NO_3)2$

$NH_4H_2PO_4$

Na_2SO_3

$NaNO_3$

NH_4ClO_4

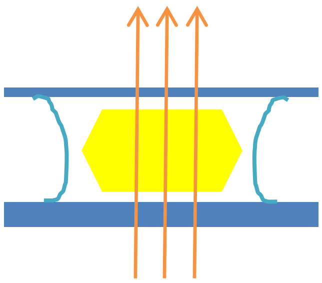
Borax

$CuSO_4.5H_2O$

Refractive Index Measurement

Low contract - n of mounting is closer to particles

$n_{solid} \approx n_{liquid}$



High contract – n of mounting media is different from particles

Mounting media

AROCCOR n= 1.66

Meltmount different n

Sample (mounting media n=1.66)

Mounting media n =1.66

Mineral	contrast	n
Fluorite	High	1.44
Quartz	Medium	1.55g
Apatite	Low	1.64
Corondum	Medium	1.77