一、光学基础

- 透镜成像、分辨率与衬度
- 显微镜光学系统
- 物镜的构造与技术（数值孔径、像差）
- CCD/CMOS电子影像系统
Magnification in Compound Microscope
(transmission illumination)

\[ M = M_1M_2 = \frac{(v_1 - f_1)(v_2 - f_2)}{f_1f_2} \]

Total mag. is product of mags. for each lens.
人眼所看到的像

目镜

物镜

25 cm
人眼的分辨率

\[ \sim 0.2 \text{ mm @ 25 cm distance} \]
$$R_0 = \frac{0.612\lambda M}{n\sin\alpha}$$
透镜的分辨率极限

\[ \Delta r_0 = \frac{R_0}{M} = \frac{0.612 \lambda}{nsin\alpha} \]

\textit{nsin\alpha}: 数值孔径 (numerical aperture, NA)

<table>
<thead>
<tr>
<th>Visible Light:</th>
<th>( \lambda \sim 6000 \ \text{Å} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-rays:</td>
<td>( \lambda \sim 0.5 - 2.5 \ \text{Å} )</td>
</tr>
<tr>
<td>Electrons:</td>
<td>( \lambda \sim 0.05 \ \text{Å} )</td>
</tr>
</tbody>
</table>
有效放大倍率

Effective magnification = Resolution of naked eye
                           Resolution of microscope

• **Effective magnification** \((M_{eff})\):

\[
= \frac{\text{resolution of eye}}{\text{max. resolution of LOM}} = \frac{0.2 \text{ mm}}{0.00015 \text{ mm}} \approx 1333 \times
\]

• \(M’s > M_{eff}\) make the image bigger, but do not provide any additional details. This is termed “empty magnification.”
显微镜系统

- 物镜
- 光学系统
- 照明系统
- 记录系统
- 载物台
- 控制系统
显微镜的镜头/物镜

Image formation in a reflected light microscope

Similar to transmission optical microscope. Different arrangement of components

- 放大倍数
- 数值孔径
- 分辨率
- Depth of field
- 像差校正
- 物镜类型
像差(aberration)

• 在轴像差
  – 色差、球差
• 离轴像差
  – 慧差、像散
• 畸变
  – 场曲
  – 桶形/枕形畸变
显微镜光学系统
显微镜中的共轭面

25 cm
临界照明

④ Retina
③ Intermediate image
② Object plane
① Field stop diaphragm

科勒照明

④ Iris diaphragm of eye
③ Back focal plane of objective
② Front focal plane of condenser
① Lamp filament

Eye
Eyepiece
Field stop of eyepiece
Objective lens
Stage
Condenser lens
Collector lens
Lamp
建立了两套共轭像平面
光源的像平面
样品的像平面
光阑 (aperture)

• 聚光镜光阑
• 物镜光阑
• 视域光阑

孔径光阑
孔径光阑（aperture diaphragm）

影响分辨率和衬度，但不要轻易试图用调节孔径光阑大小来调节物象的衬度。什么情况下可以调节？
视域光阑(field diaphragm)
景深 (Depth of field)

\[ D_f = \frac{(0.15 \sim 0.30)n}{(N.A.)M} \text{ (μm)} \]

- 0.15~0.30，镜头分辨率，μm
- \(n\)，折射系数
- N.A.，物镜数值孔径
- \(M\)，显微镜放大倍数
像素与镜头分辨率

• Highest useful digital image resolution in optical microscopy is achieved, if the minimal distance between distinguishable 2 points (=optical resolution of the objective) gets detected by 2 or 3 pixels.

<table>
<thead>
<tr>
<th>Objective (numerical aperture)</th>
<th>Resolution Limit (microns)</th>
<th>Projected Size on CCD (microns)</th>
<th>Required Pixel Size (microns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4x (0.20)</td>
<td>1.5</td>
<td>5.8</td>
<td>2.9</td>
</tr>
<tr>
<td>10x (0.45)</td>
<td>0.64</td>
<td>6.4</td>
<td>3.2</td>
</tr>
<tr>
<td>20x (0.75)</td>
<td>0.39</td>
<td>7.7</td>
<td>3.9</td>
</tr>
<tr>
<td>40x (0.85)</td>
<td>0.34</td>
<td>13.6</td>
<td>6.8</td>
</tr>
<tr>
<td>40x (1.30)</td>
<td>0.22</td>
<td>8.9</td>
<td>4.5</td>
</tr>
<tr>
<td>60x (0.95)</td>
<td>0.31</td>
<td>18.3</td>
<td>9.2</td>
</tr>
<tr>
<td>60x (1.40)</td>
<td>0.21</td>
<td>12.4</td>
<td>6.2</td>
</tr>
<tr>
<td>100x (0.90)</td>
<td>0.32</td>
<td>32.0</td>
<td>16.0</td>
</tr>
<tr>
<td>100x (1.25)</td>
<td>0.23</td>
<td>23.0</td>
<td>11.5</td>
</tr>
<tr>
<td>100x (1.40)</td>
<td>0.21</td>
<td>21.0</td>
<td>10.5</td>
</tr>
</tbody>
</table>
二、金相试样制备

看“真相”不要看“假相”

好样品是成功的一半

什么样的样品是好样品？
取样制样浸蚀观察

金相试样制备“艺术”
取样

图1-1  轧制型材金相试样的切取
1—与轧制表面平行的纵断面   2—与轧制表面垂直的纵断面
3—横断面   4—放射纵断面   5—切向纵断面
损伤层

• 切割是为了取样并使样品留下的残余损伤最小

AISI P20钢电火花切割表面重熔层

退火工业纯钛的切割损伤
制样

• 目的：去除损伤层，得到表面光滑平整的磨面，是显示材料真实组织的前提。

镶嵌（mounting）
- 热镶
- 冷镶
- 夹持

磨光（grinding）
- 粗磨
- 细磨

抛光（polishing）
- 机械抛光
- 电解抛光
浸蚀/显示

- 化学
- 电解
- 特殊方法
Theoretical current density versus voltage curve for electrolytic polishing/etching. From page 159 in Geels.
Example of a welded low-carbon steel etched with Klemm's I showing the clear superiority of color etching in revealing the grain structure
三、各种光学显微术 (显微镜附件)
人眼可以区分振幅变化（单色光）
- eg.: 明场、暗场

人眼不能区分相位、偏振性的变化
- eg.: 偏光、相衬、微分干涉

衬度  $Contrast = \frac{|I(s) - I(b)|}{I(b)} \geq 0.02$
反射式显微镜的衬度模式

- 明场(BF)
- 暗场(DF)
- 相位衬度(Phase contrast)
- 偏振光(Polarized light)
- 微分干涉(DIC)
明场像的衬度原理
暗场像
Incident beam, $\lambda$

$\Delta d \ll \lambda$

$\phi = \frac{2\pi\delta}{\lambda} = \frac{4\pi\Delta d}{\lambda}$

如何将相位差转转变为亮度差？
$S + D = P$
关键：
1. S波与D波分离
2. 使S波移相（超前或滞后π/2）
3. 降低S波振幅

拉大S波与P波的振幅相对差距
两相的表面高度差在10-150 nm情况下“相衬法”辨别两相组织较适宜，如采用特殊相位板可以分辨到5nm左右。

18CrNiW钢高温淬火及冰冷处理后的显微组织

两相的表面高度差在10-150 nm情况下“相衬法”辨别两相组织较适宜，如采用特殊相位板可以分辨到5nm左右。
我们能用偏振光干些什么？

只有立方晶体是各向光学同性的，其余均光学各向异性。

<table>
<thead>
<tr>
<th>Crystal system</th>
<th>Axial constants</th>
<th>Angular constants</th>
<th>Optical symmetry</th>
<th>Refractive indices and optic sign</th>
<th>Birefringence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isometric</td>
<td>a = b = c</td>
<td>α=β=γ =90°</td>
<td>Isotropic</td>
<td>n; (n_ε &gt; n_ω)</td>
<td>n_ε - n_ω</td>
</tr>
<tr>
<td>Hexagonal</td>
<td>a = b ≠ c</td>
<td>γ=β=90°, α = 120°</td>
<td>Uniaxial</td>
<td>+; (n_ε &gt; n_ω)</td>
<td>n_ε - n_ω</td>
</tr>
<tr>
<td>Tetragonal</td>
<td>a = b ≠ c</td>
<td>α=β=γ = 90°</td>
<td>Uniaxial</td>
<td>-; (n_ε &lt; n_ω)</td>
<td>n_ε - n_ω</td>
</tr>
<tr>
<td>Orthorhombic</td>
<td>a ≠ b≠ c</td>
<td>α=β=γ = 90°</td>
<td>Biaxial</td>
<td>n_α &lt; n_β &lt; n_γ</td>
<td>n_γ - n_α</td>
</tr>
<tr>
<td>Monoclinic</td>
<td>a ≠ b≠ c</td>
<td>α=γ=90°, β ≠ 90°</td>
<td>Biaxial</td>
<td>(+); (n_γ-n_β) &gt; (n_β&gt;n_α)</td>
<td>n_γ-n_α</td>
</tr>
<tr>
<td>Triclinic</td>
<td>a ≠ b≠ c</td>
<td>α ≠ β ≠ γ ≠ 90°</td>
<td>Biaxial</td>
<td>(-); (n_γ-n_β) &lt; (n_β&gt;n_α)</td>
<td>n_γ-n_α</td>
</tr>
</tbody>
</table>
我们能用偏振光干些什么？

- 正交偏光镜设置

![偏振光镜图示](image)

- A: direction of progression of analyzer
- P: direction of progression of polarizer
- C: direction of optical axis of anisotropy
我们能用偏振光干些什么？

• 偏振光在各向异性金属表面上的反射

双反射：指一束线偏振光经反射后形成两束振动平面相互垂直的o光和e光。各向异性表面对o光和e光的反射率不同。

仪器：正交偏光镜设置
我们能用偏振光干些什么？

- 偏振光在各向同性金属表面上的反射

高低差形成衬度
微分干涉衬度显微术

偏光 + 干涉

相位梯度

衬度差

Figure 1

Light from Semi-Coherent Source
• 起偏
• 分裂成两束光线：O光线和E光线
• O光线和E光线经样品反射后再合成
• 检偏器与起偏器正交
DIC中衬度形成原理

仪器：起偏器与检偏器正交设置。

O光与E光干涉，无相位差，形成线偏振光。

O光与E光干涉，有相位差，形成椭圆偏振光。
DIC中衬度形成原理
四、共聚焦扫描显微镜

成像原理
构造
应用
共聚焦光学

- 1957年由马文·闵斯基（Marvin Lee Minsky）提出。
- The first commercial instruments appeared in 1987.
传统明场成像
In a conventional widefield optical epi-fluorescence microscope, secondary fluorescence emitted by the specimen often occurs through the excited volume and obscures resolution of features that lie in the objective focal plane.
共聚焦成像

非焦平面
杂散光
荧光显微镜

- The entire specimen is subjected to intense illumination from an incoherent mercury or xenon arc-discharge lamp, and the resulting image of secondary fluorescence emission can be viewed directly in the eyepieces or projected onto the surface of an electronic array detector or traditional film plane.
The scan head is at the heart of the confocal system and is responsible for rasterizing the excitation scans, as well as collecting the photon signals from the specimen that are required to assemble the final image.
深度方向上的成像原理

光切片
optical slicing
The relationship between contrast and resolution with regard to the ability to distinguish two closely spaced specimen features implies that resolution cannot be defined without reference to contrast, and it is this interdependence that has led to considerable ambiguity involving the term resolution and the factors that influence it in microscopy.
The Nobel Prize in Chemistry 2014
Eric Betzig, Stefan W. Hell, William E. Moerner

The Nobel Prize in Chemistry 2014 was awarded jointly to Eric Betzig, Stefan W. Hell and William E. Moerner "for the development of super-resolved fluorescence microscopy".