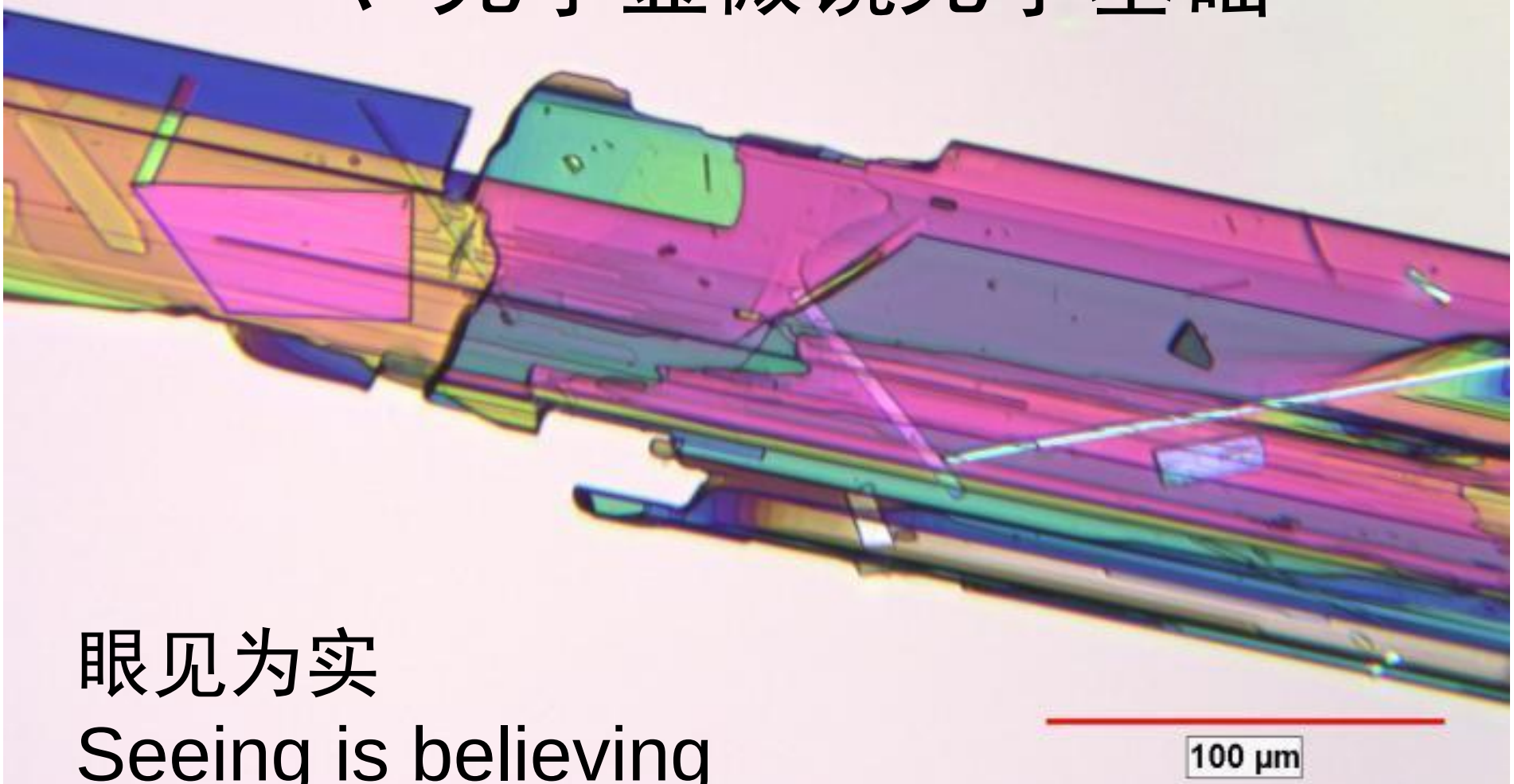
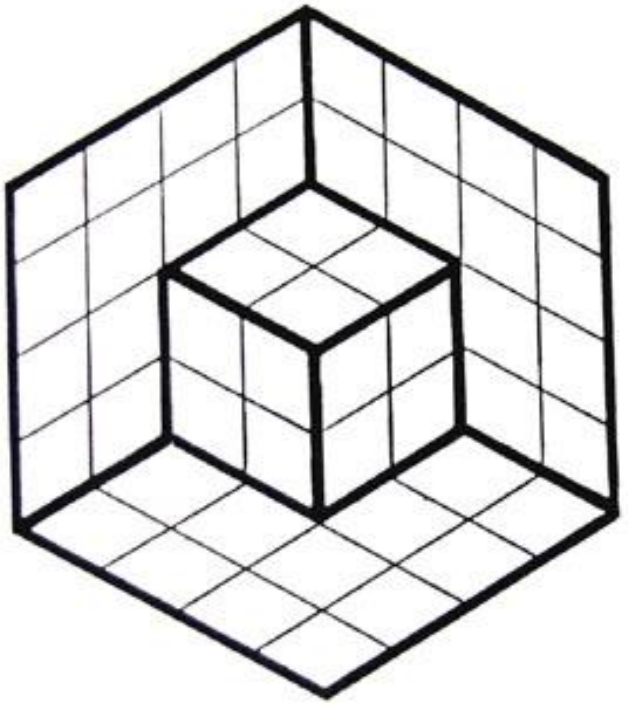
一、光学显微镜光学基础



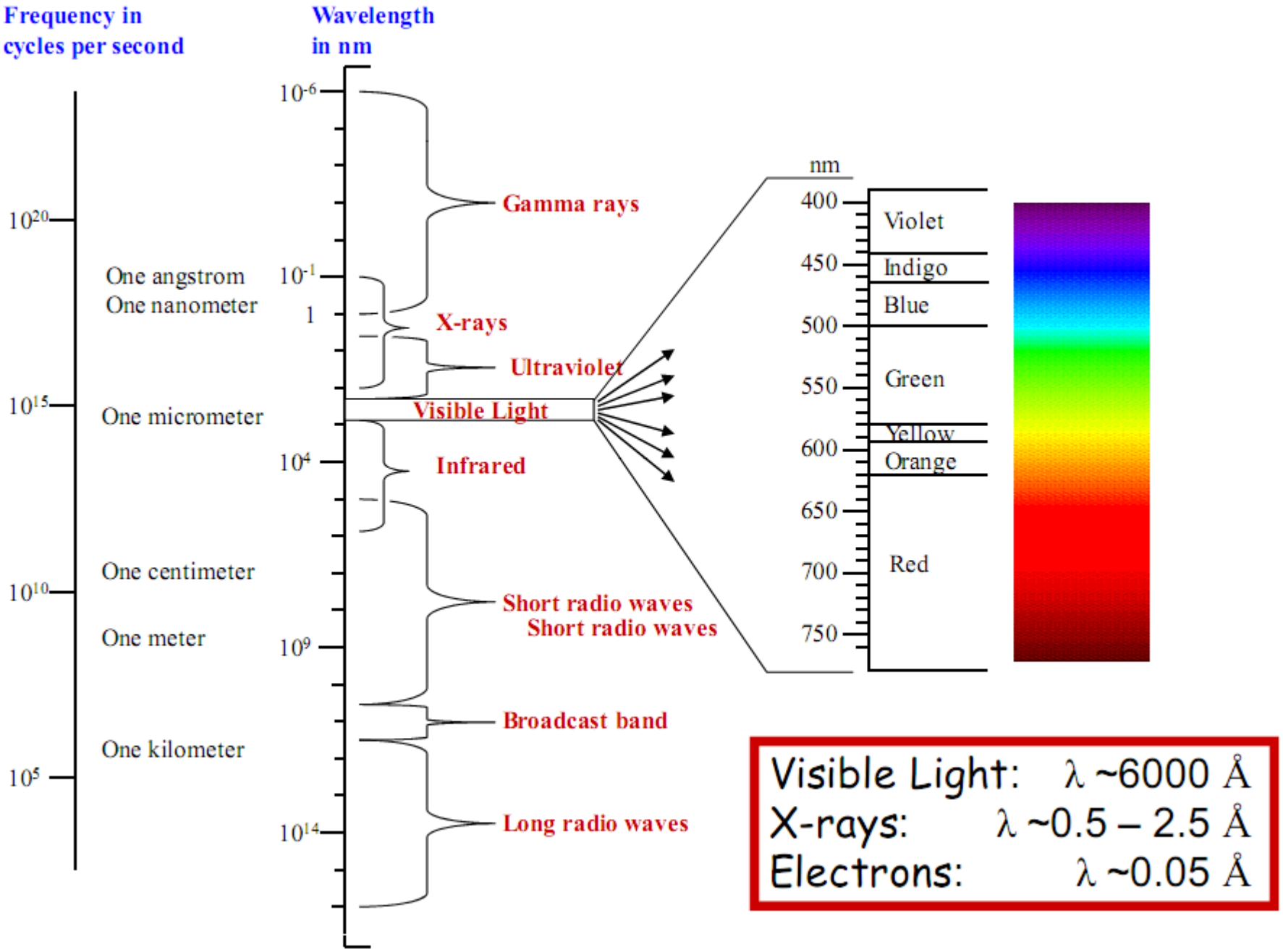
眼见为实

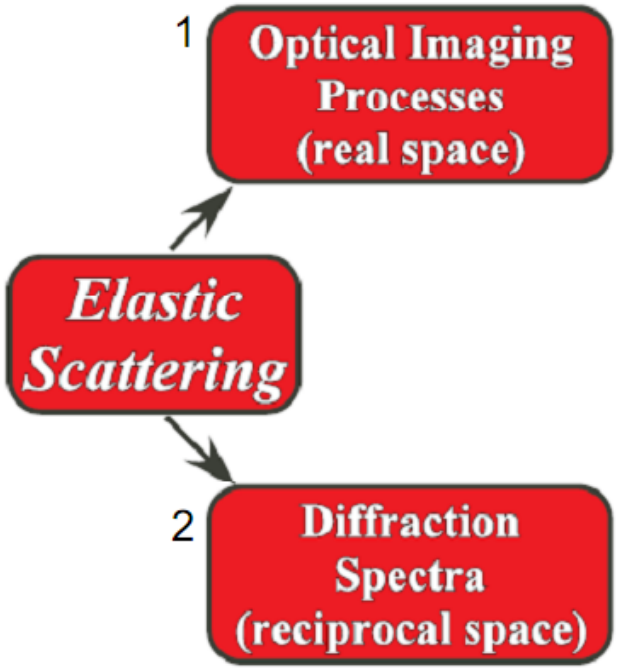
Seeing is believing

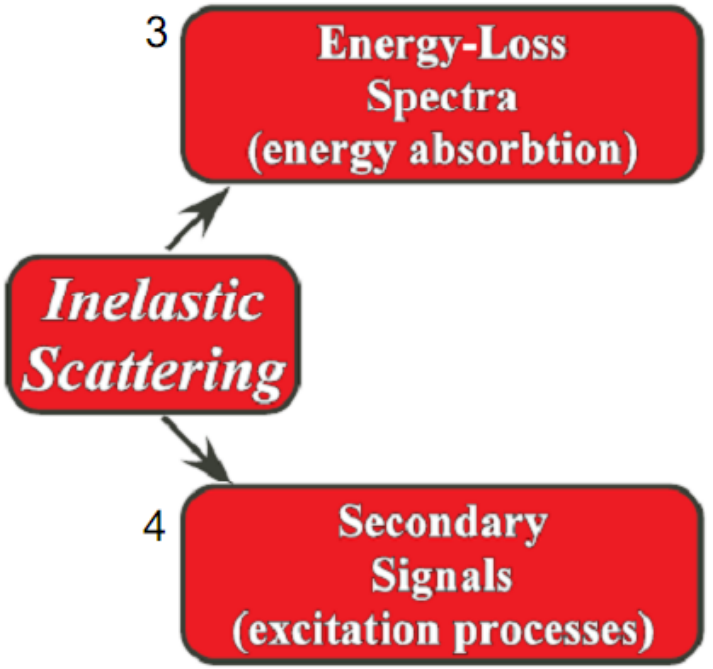


眼见为实

Seeing is believing


Frequency in cycles per second
Wavelength in nm
10^{20}
10^{15}
10^{10}
10^{5}
One angstrom
One nanometer
One micrometer
One centimeter
One meter
One kilometer
10^{-6}
10^{-1}
1
10^{4}
10^{9}
10^{14}
Gamma rays
X-rays
Ultraviolet
Visible Light
Infrared
Short radio waves
Short radio waves
Broadcast band
Long radio waves
nm
400
450
500
550
600
650
700
750
Violet
Indigo
Blue
Green
Yellow
Orange
Red
Visible Light: λ ~6000 Å
X-rays: λ ~0.5 – 2.5 Å
Electrons: λ ~0.05 Å





1. Signal can be focused → real space image (e.g., OM, SEM, TEM)

2. Scattering angles can be collected and analyzed in reciprocal space (e.g., XRD or SAD)

3. Energy loss spectra (*due to absorption of incident radiation*)

4. Secondary signals such as x-rays or secondary electrons (*due to excitation of electrons in material*)

(Figure from D.Brandon and W. Kaplan, *Microstructural Characterization of Materials, 2nd Edition*, Wiley (2008) p. 6)

XRD *vs* OM

XRD

- 结构的信息
  - 晶体结构(相)
  - 晶格常数
  - 应力/应变
  - 倒易空间(reciprocal space)
- 形态的信息
  - 透视
  - 探伤

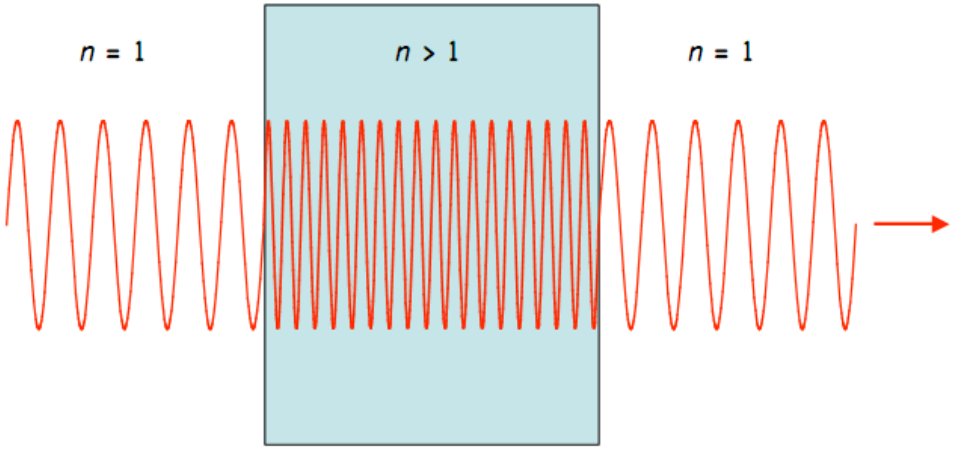
Optical microscope (OM)

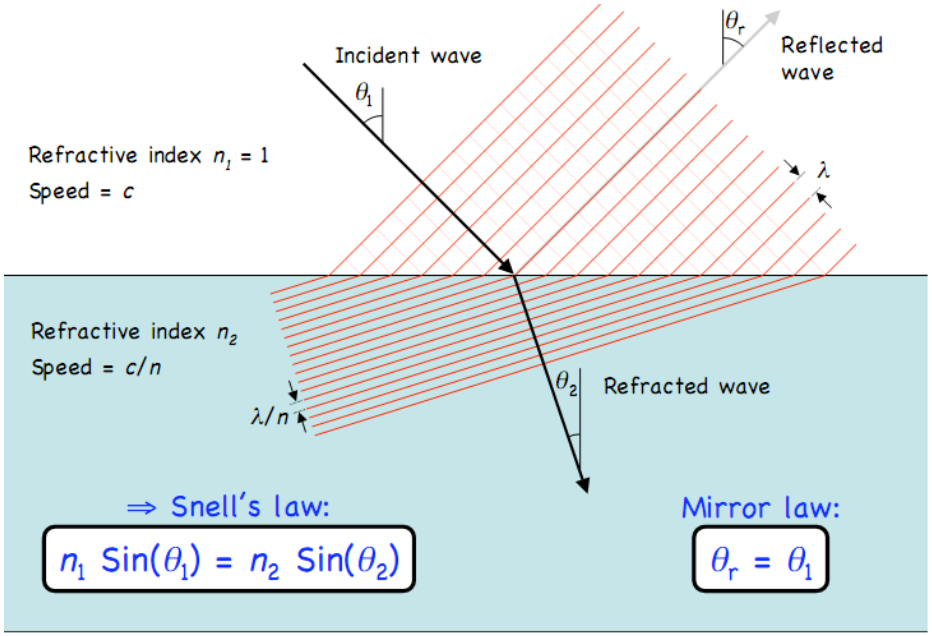
- 形貌的信息
  - 正空间(real space)
- 检查材料微观组织结构的最常用、最基础的手段
- 应用
  - 材料生产制造中的质量控制
  - 材料使用中失效原因分析
  - 建立材料结构-性能关系

一、光学基础

- 透镜成像与分辨率
- 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

折射(refraction)





折射率(refractive index

- Vacuum 1
- Air 1.0003
- Water 1.333
- Cytoplasm 1.35–1.38 ?
- Glycerol 1.475 (anhydrous)
- Immersion oil 1.515
- Fused silica 1.46
- Optical glasses 1.5–1.9
- Diamond 2.417

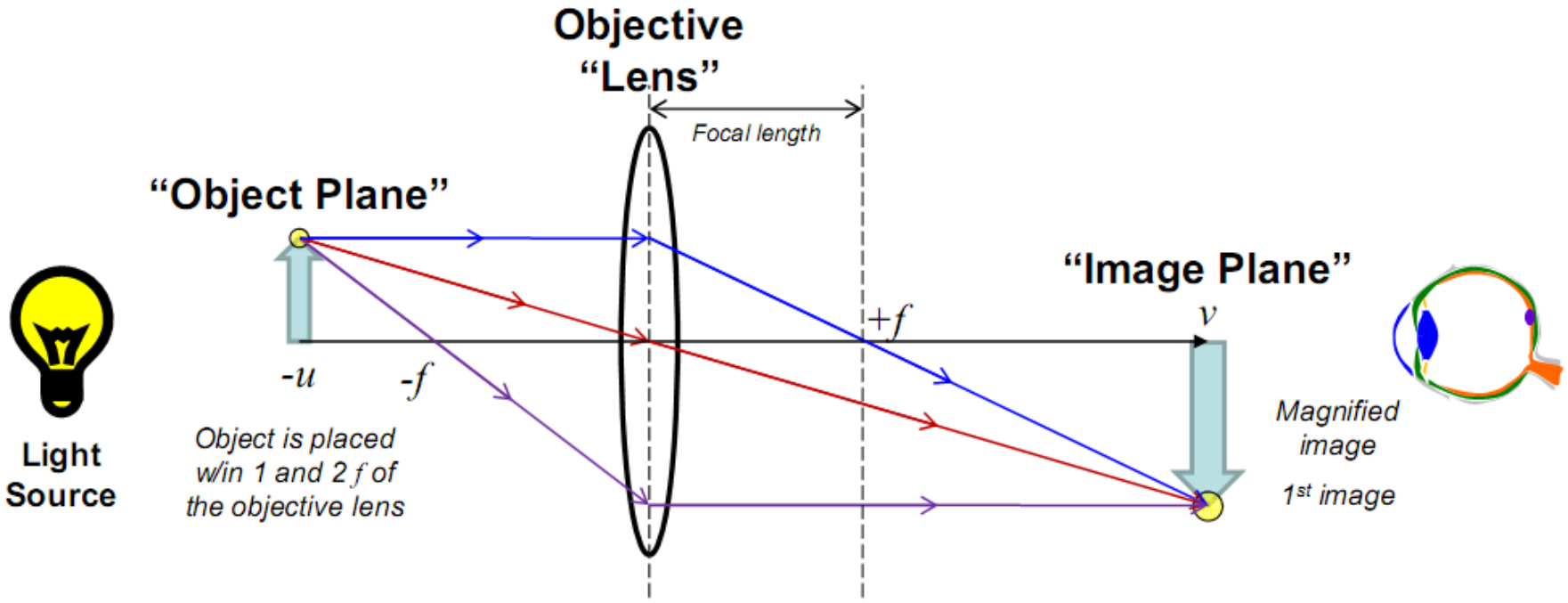
Depends on wavelength and temperature

色散 (dispersion)

- 不同波长光线的折射率*n*不同

http://gallery.hd.org/_c/natural-science/prism-and-refraction-of-light-into-rainbow-AJHD.jpg.html

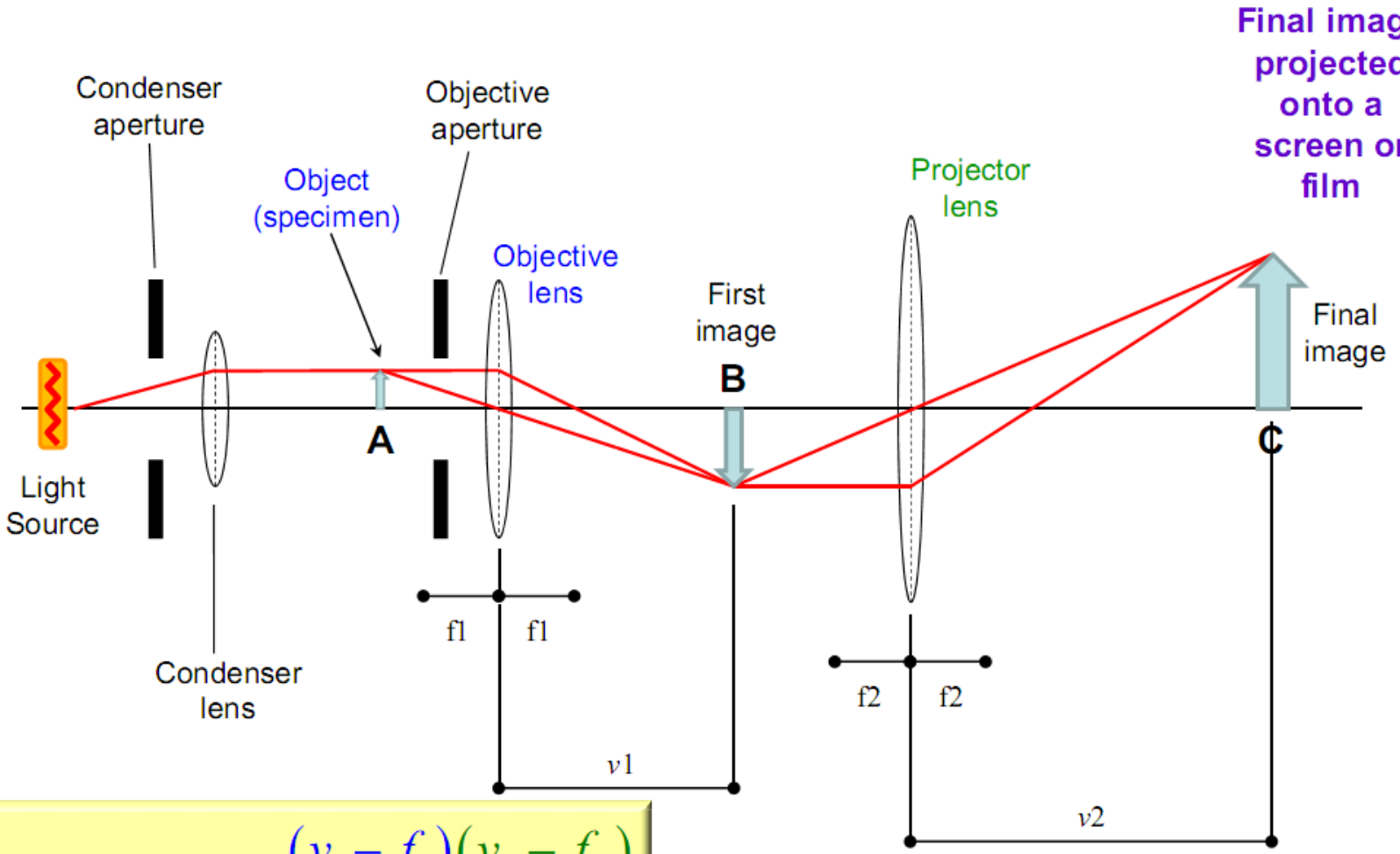
光学成像原理



$$\frac{1}{u}+\frac{1}{v}=\frac{1}{f} \quad \text{and} \quad M=\frac{v-f}{f}=\frac{v}{u}$$

Magnification in Compound Microscope

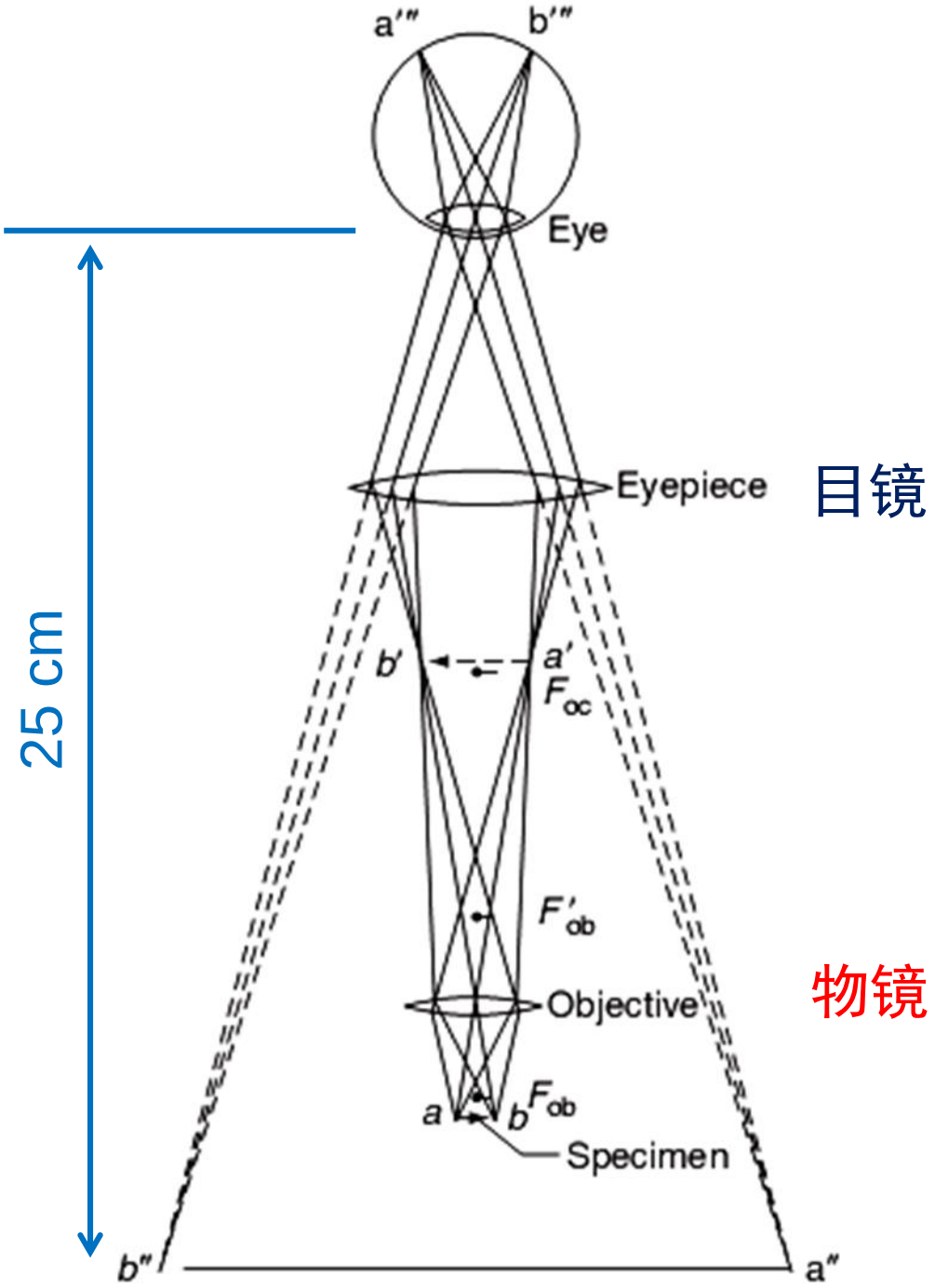
(transmission illumination)



$$M = M_1 M_2 = \frac{(v_1 - f_1)(v_2 - f_2)}{f_1 f_2}$$

Total mag. is product of mags. for each lens.

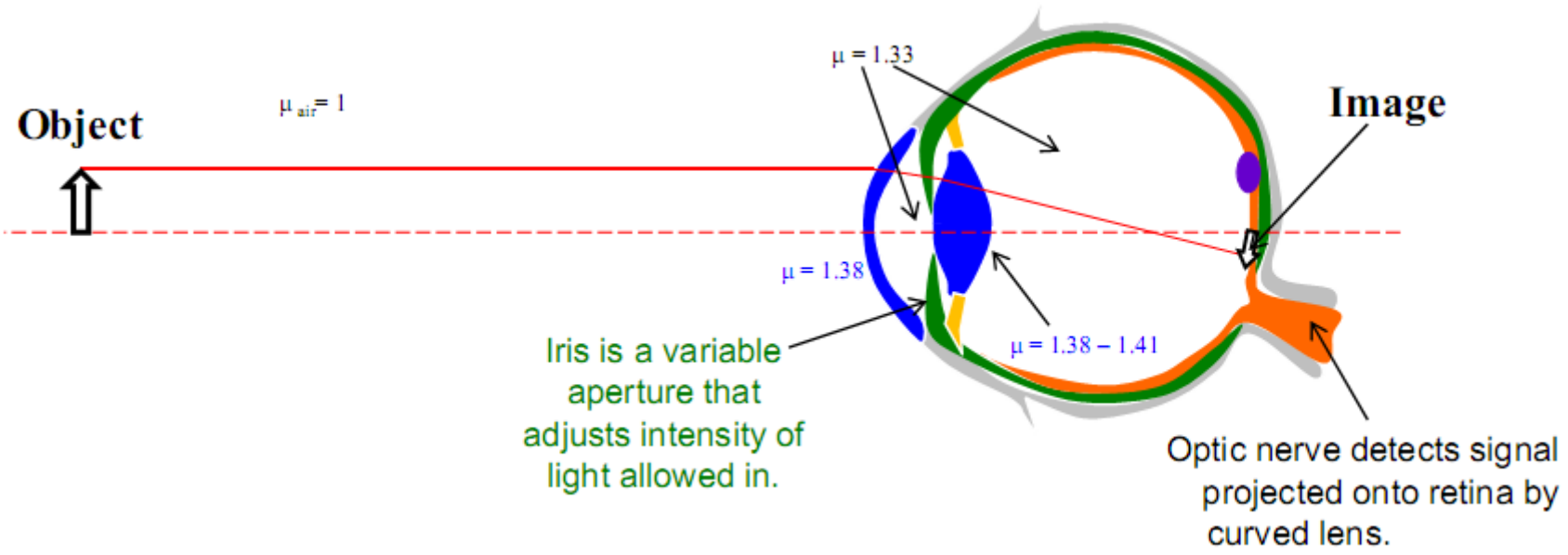
人眼所看到的像


a‴
b‴
Eye
Eyepiece
目镜
25 cm
b′
a′
F oc
F′ ob
Objective
物镜
a
b
F ob
Specimen
b″
a″

放大倍率与分辨率

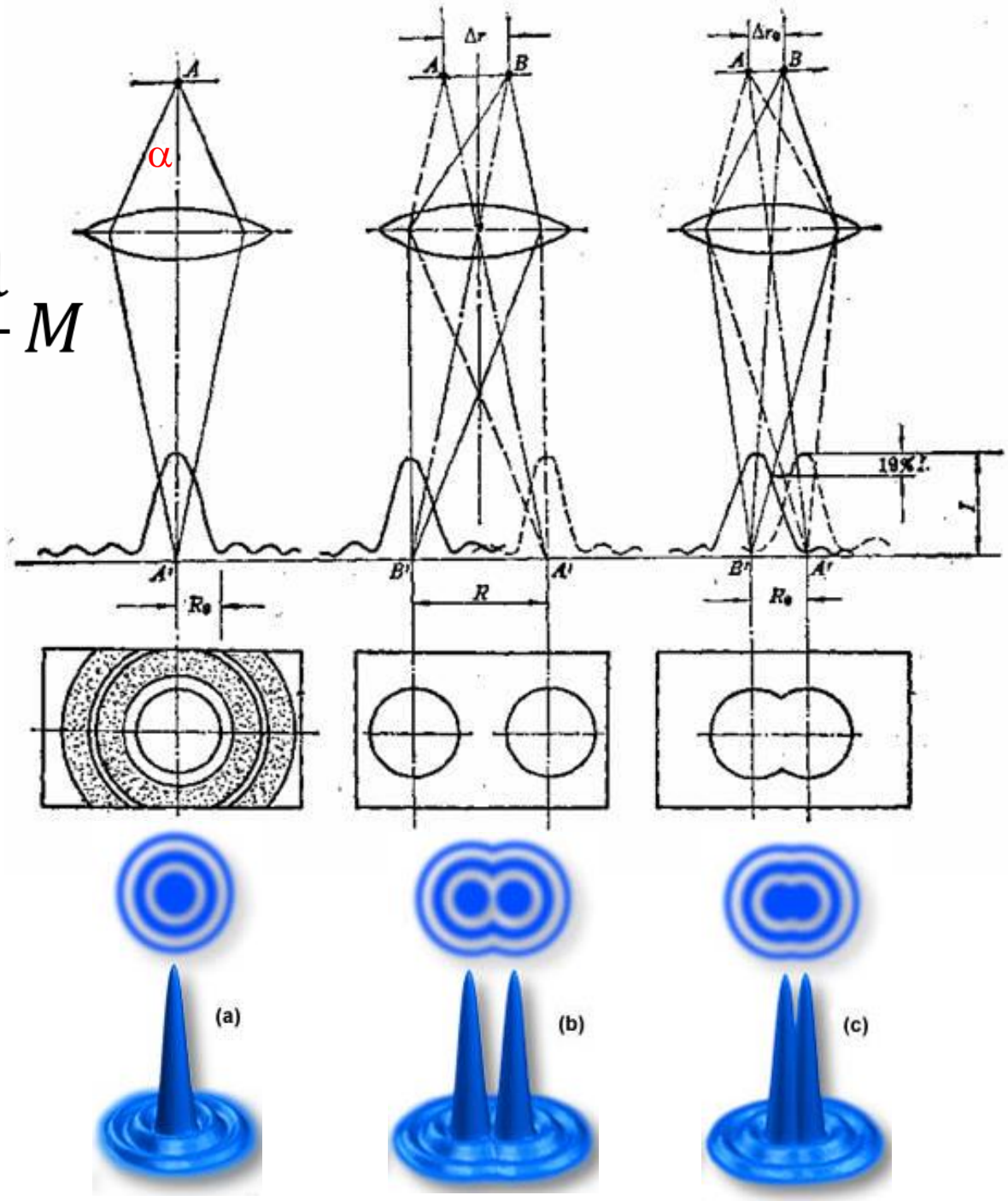
- 放大倍率
  - 人眼从显微镜目镜中所见的被观察物尺寸与原物尺寸的比值。
  - 人眼明视距离除以物镜焦距。
- 分辨率
  - 区分两个相邻点的能力
- 放大倍率上限？

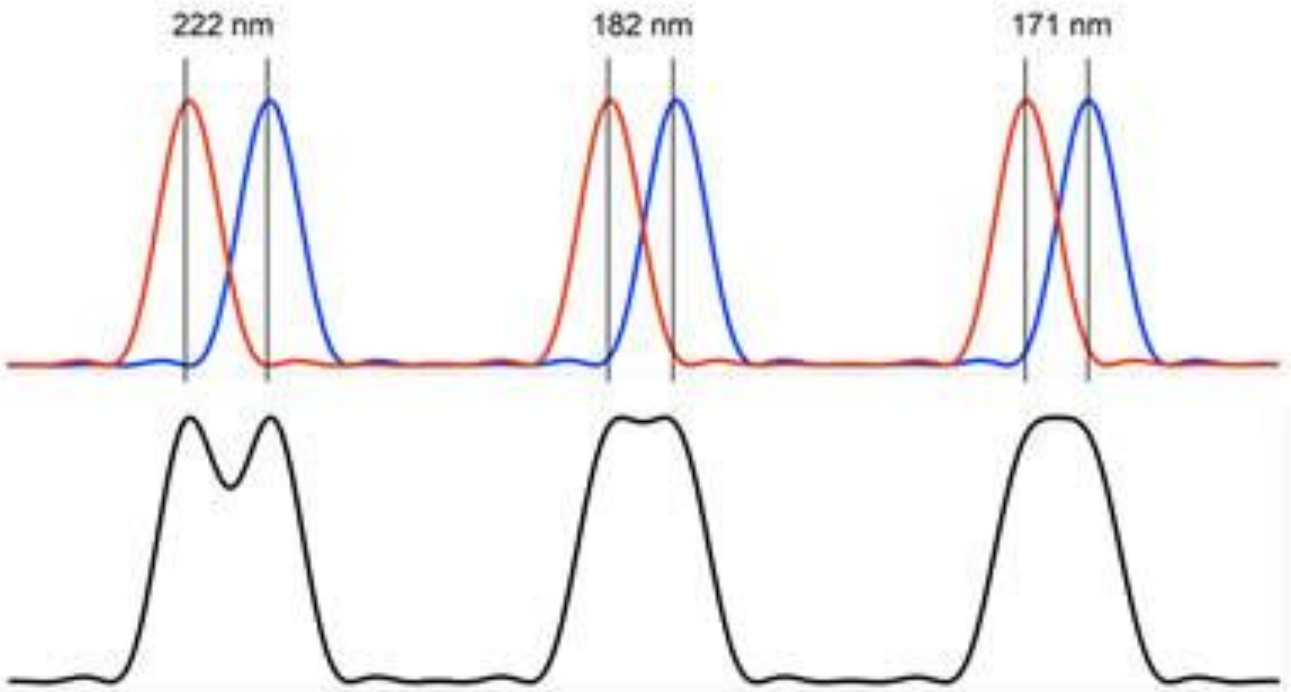
人眼的分辨率



~0.2 mm @ 25 cm distance

$$R_0 = \frac{0.612\lambda}{n sin\alpha} M$$




222 nm
182 nm
171 nm
Rayleigh limit
d = 0.61•λ / NA
Abbe limit
d = 0.50• λ / NA
Sparrow limit
d = 0.47•λ / NA
λ = 510 nm
NA = 1.4

透镜的分辨率极限

$$\Delta r_0 = \frac{R_0}{M} = \frac{0.612\lambda}{n\sin\alpha}$$

nsinα: 数值孔径(numerical aperture, NA)

Visible Light:	λ ~6000 Å
X-rays:	λ ~0.5 – 2.5 Å
Electrons:	λ ~0.05 Å

数值孔径与分辨率

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

数值孔径(N.A.)

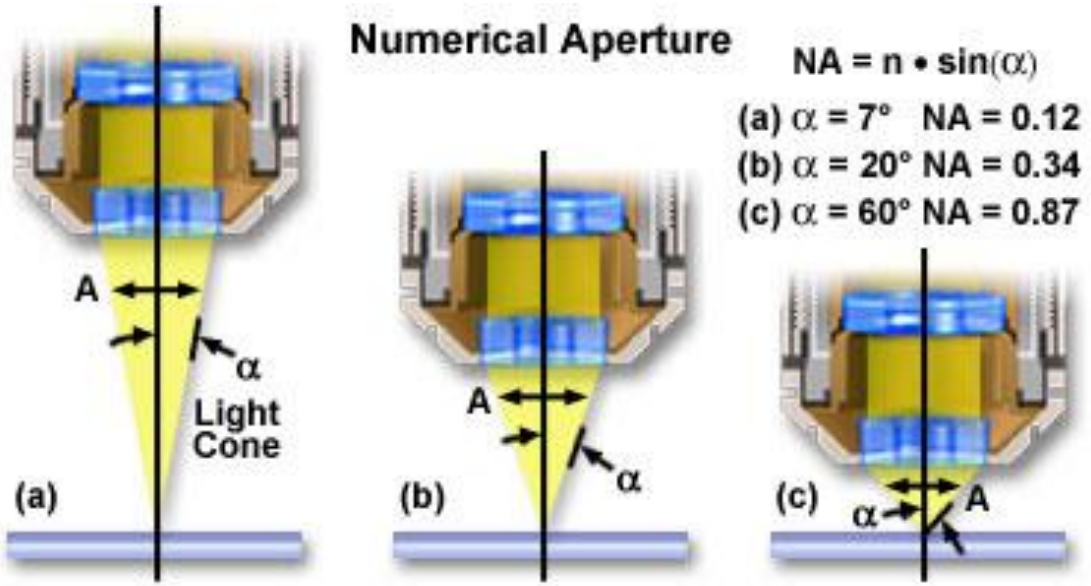
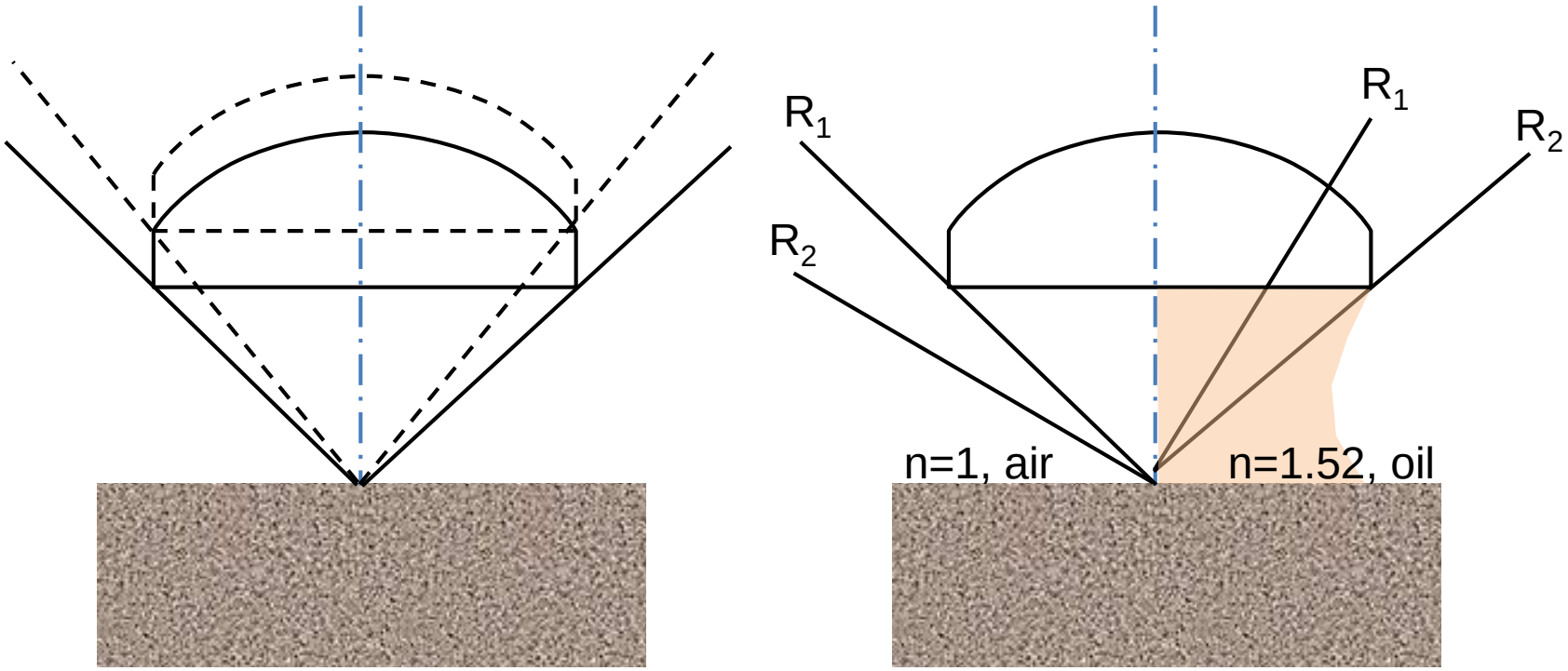
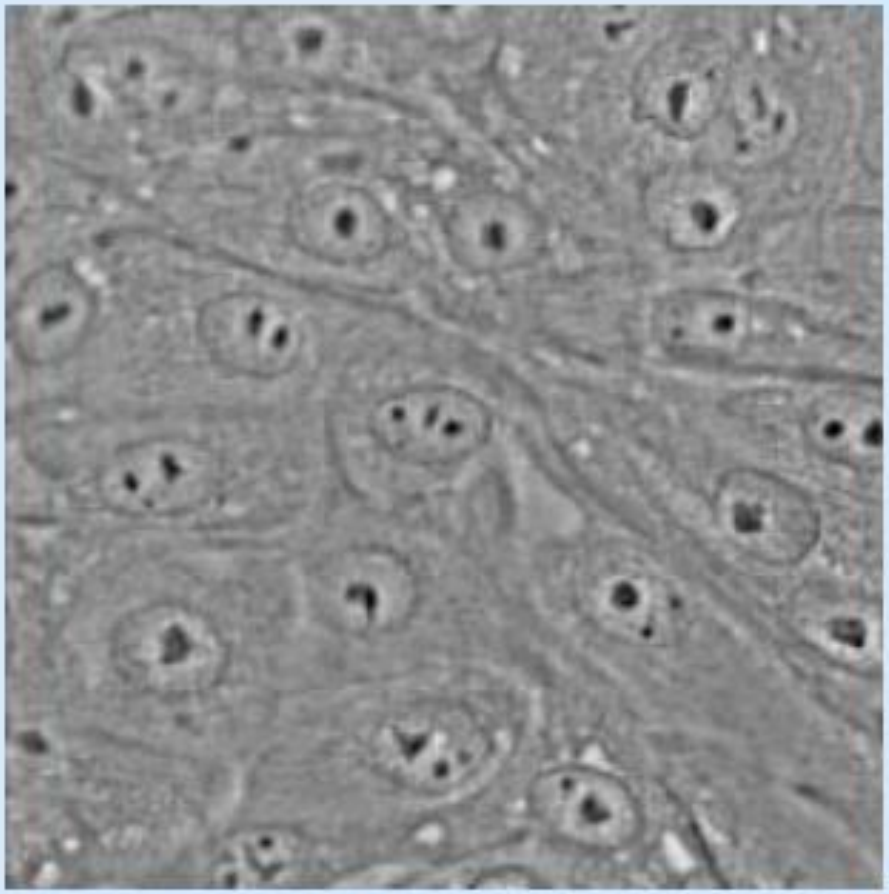


Figure 1

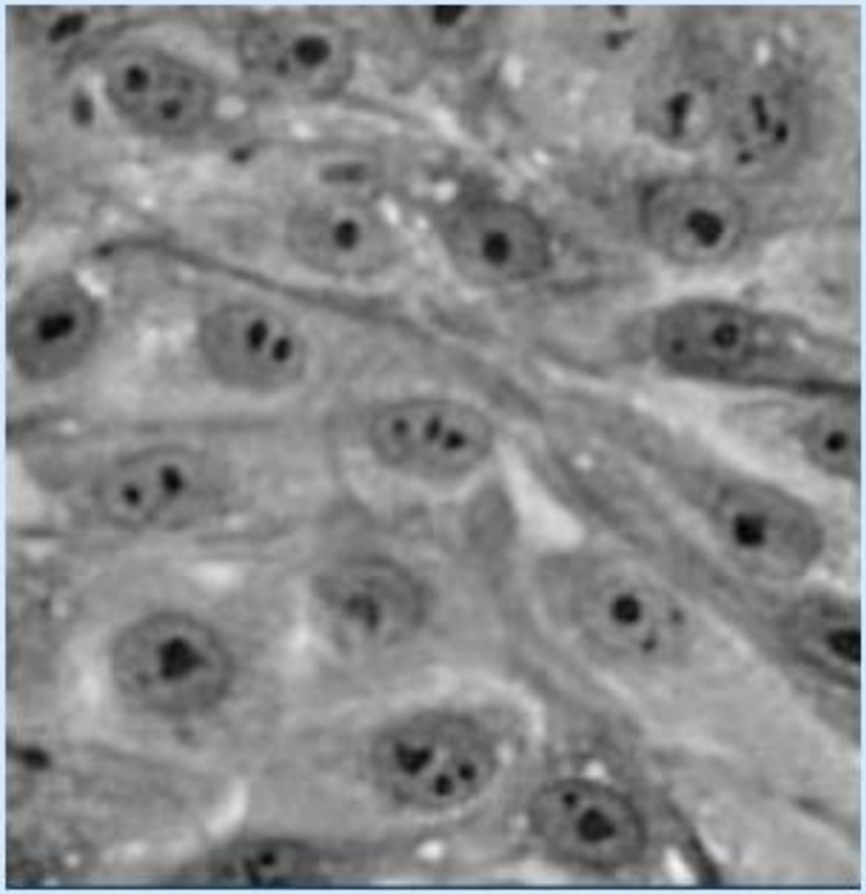
数值孔径的大小代表了镜头的集光能力

$$N.A. = n\sin\alpha$$





NA 1.25



NA 0.3

有效放大倍率

$$\text{Effective magnification} = \frac{\text{Resolution of naked eye}}{\text{Resolution of microscope}}$$

- *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."

有效放大倍率与数值孔径

$$M_{eff} = \frac{\Delta r_{eye}}{\Delta r_0}$$

$$M_{eff} \approx (550\sim1100)\text{N.A.}$$

显微镜放大倍率=物镜放大倍率x目镜放大倍率

从透镜到显微镜

Some Types of Optical Microscopes

1. Simple optical microscope → One lens; 25x; 10 μm resolution
2. Stereoscopic microscope → Two lens trains; 6-8x
3. Compound optical microscope → Objective + eyepiece + condenser lenses; 1300x; 1 μm resolution.

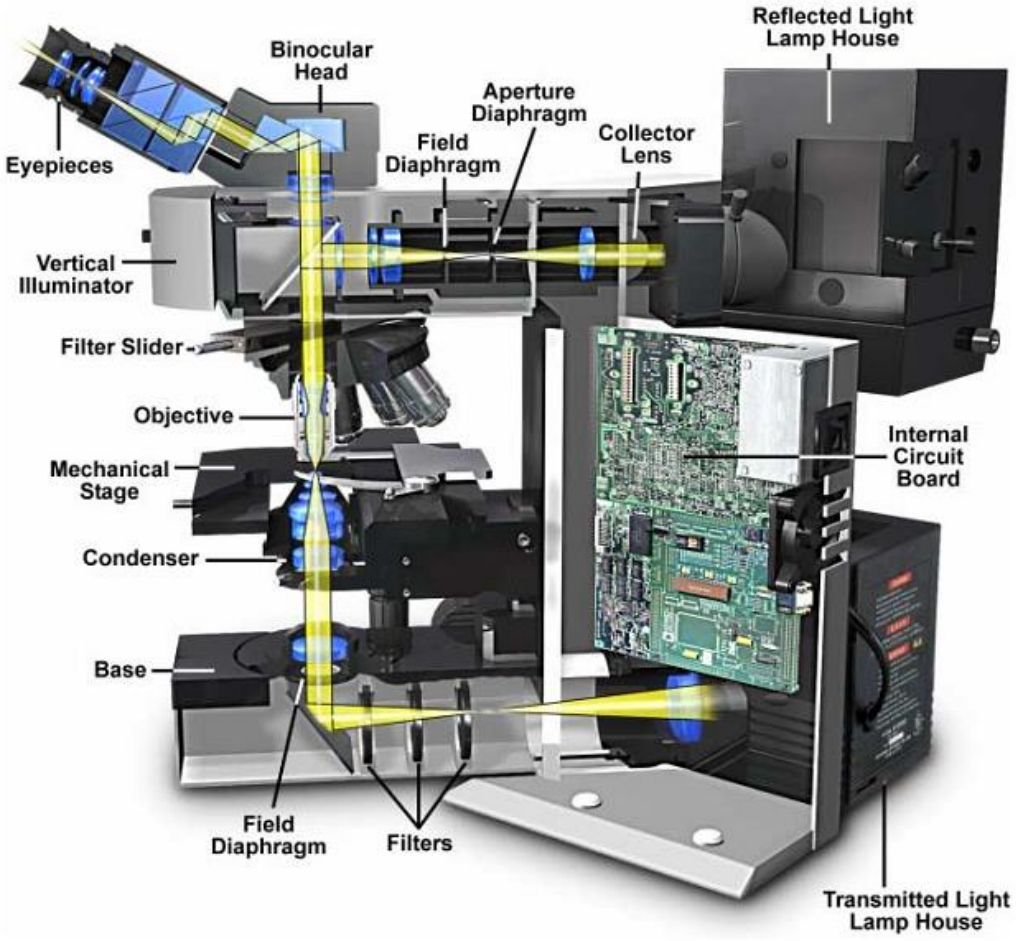
1

2

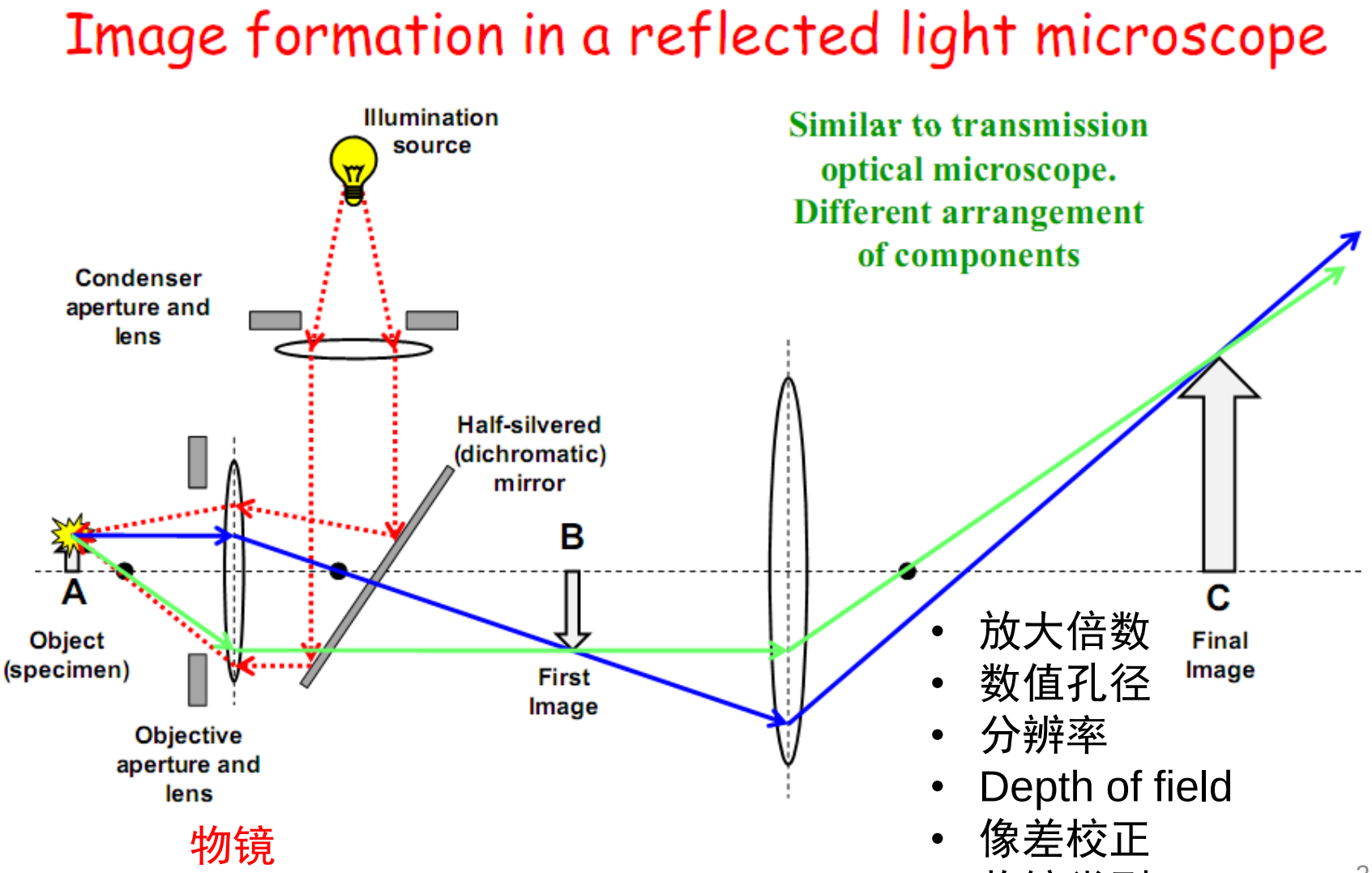
3

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



- 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

色差(chromatic aberr.)

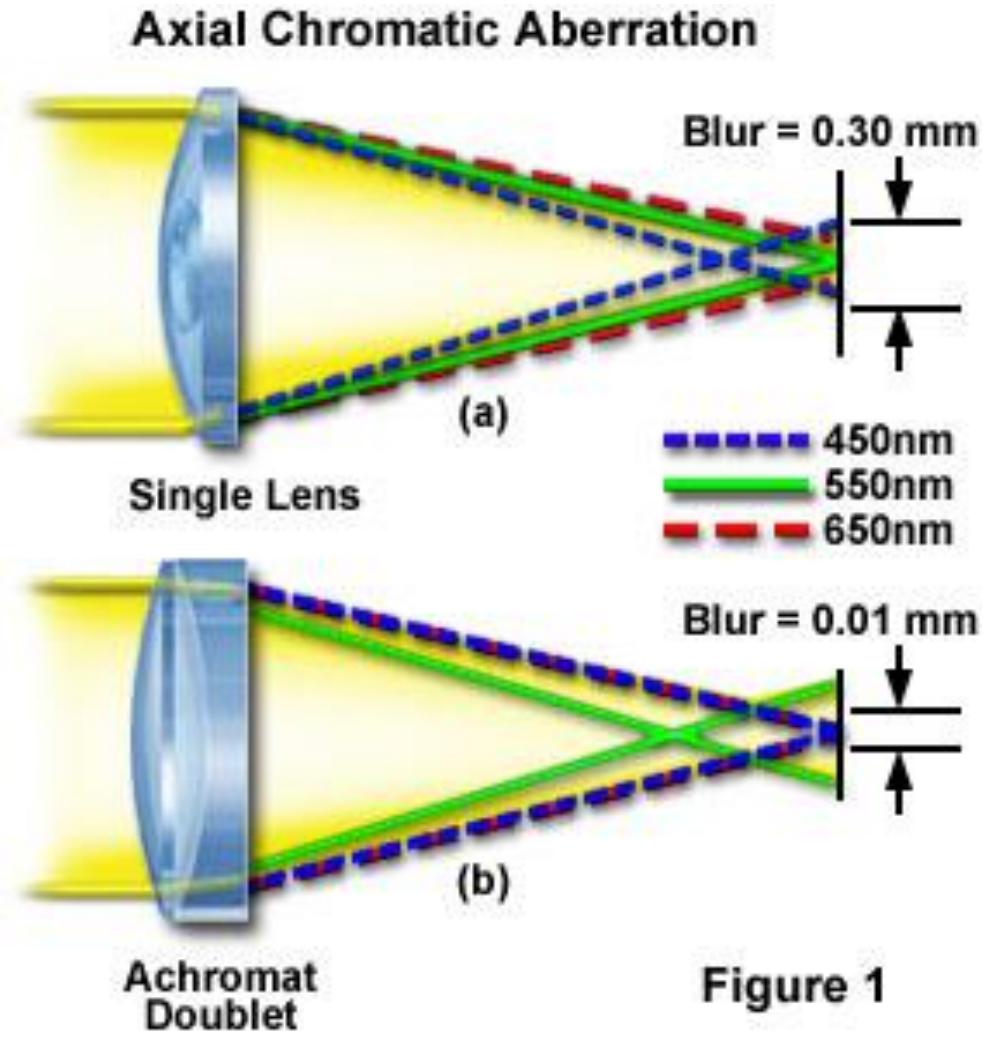
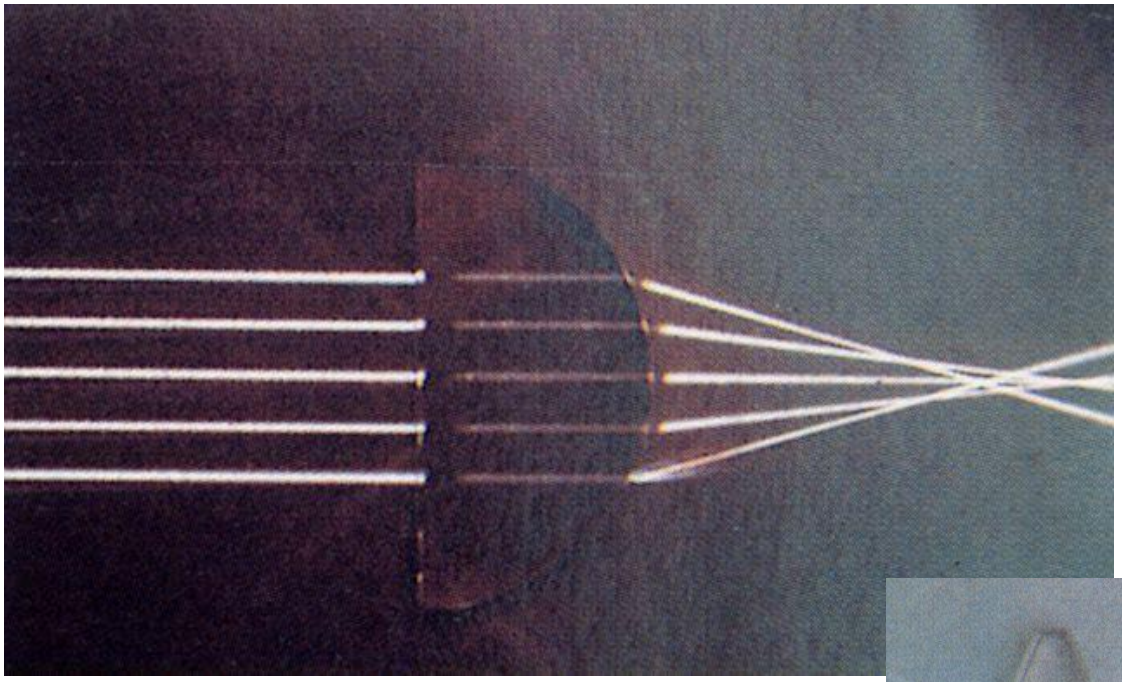
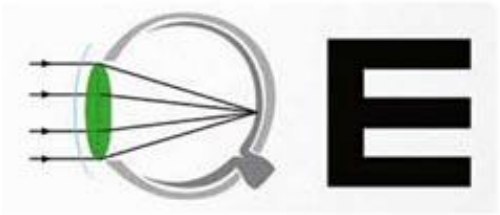


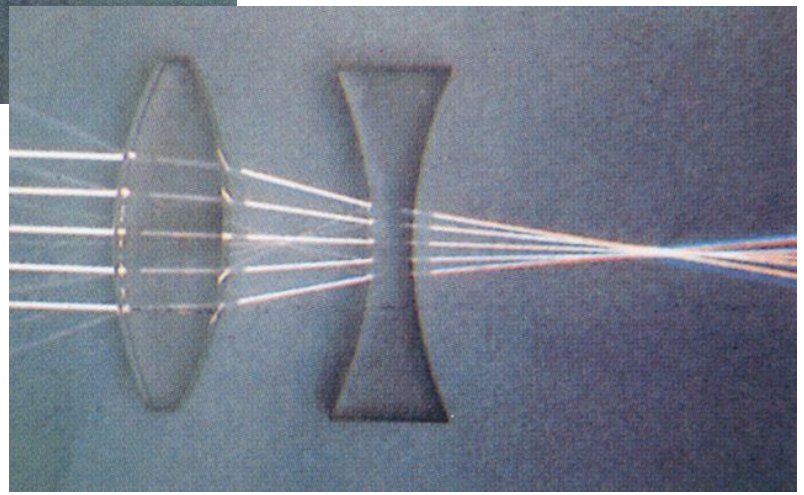
Figure 1

- 解决方案
  - 单色光
  - 采用折射率不同的玻璃制作镜片

球差(spherical aberr.)

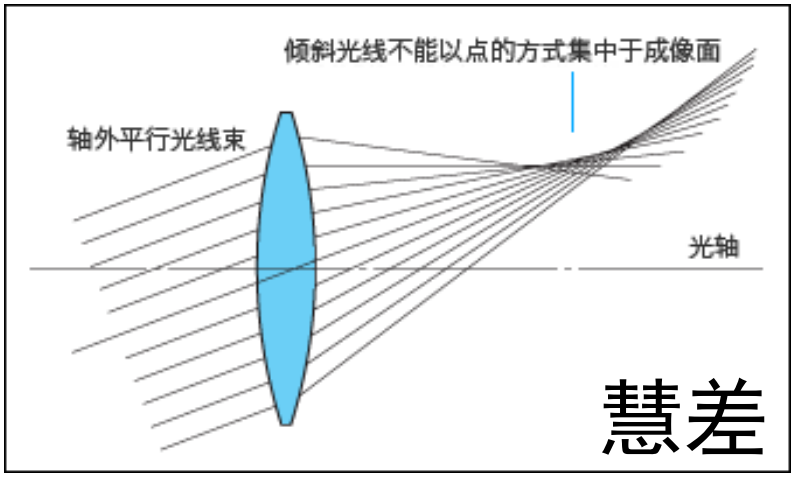


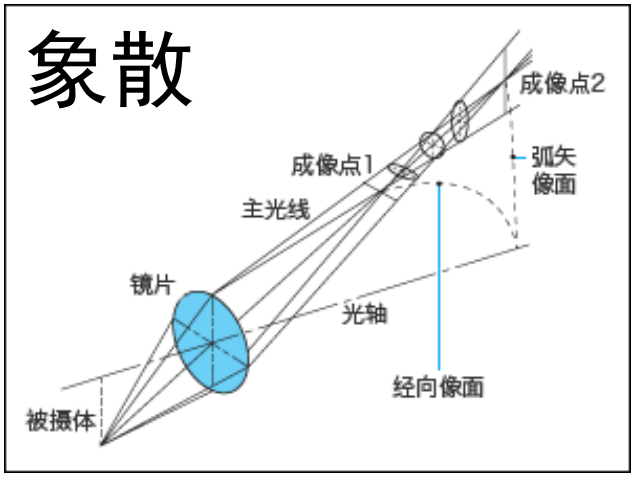


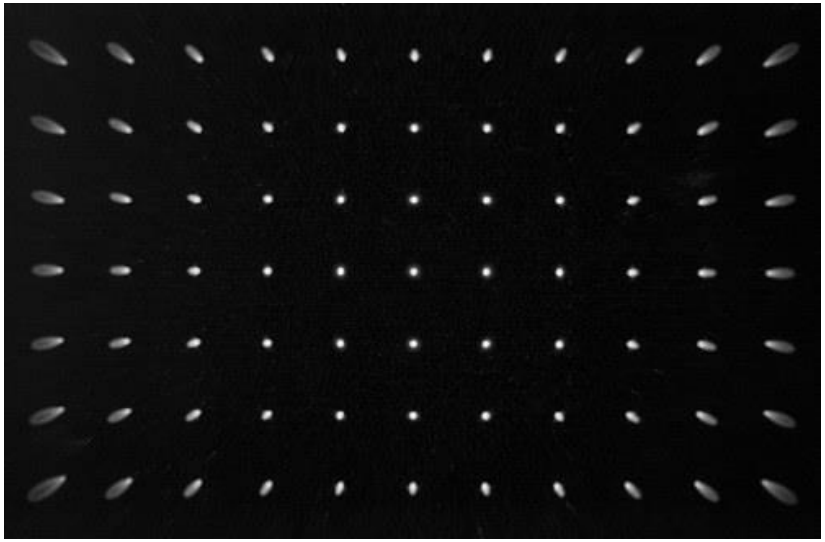


- 解决方案
  - 减小孔径
  - 采用不同曲率的透镜组合

离轴像差

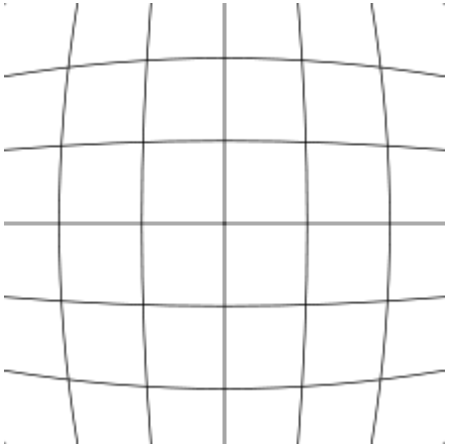




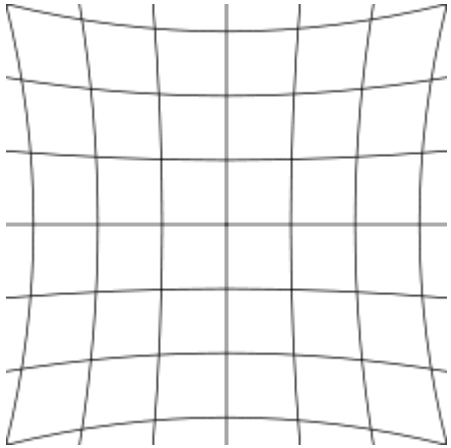


象散(astigmatism)

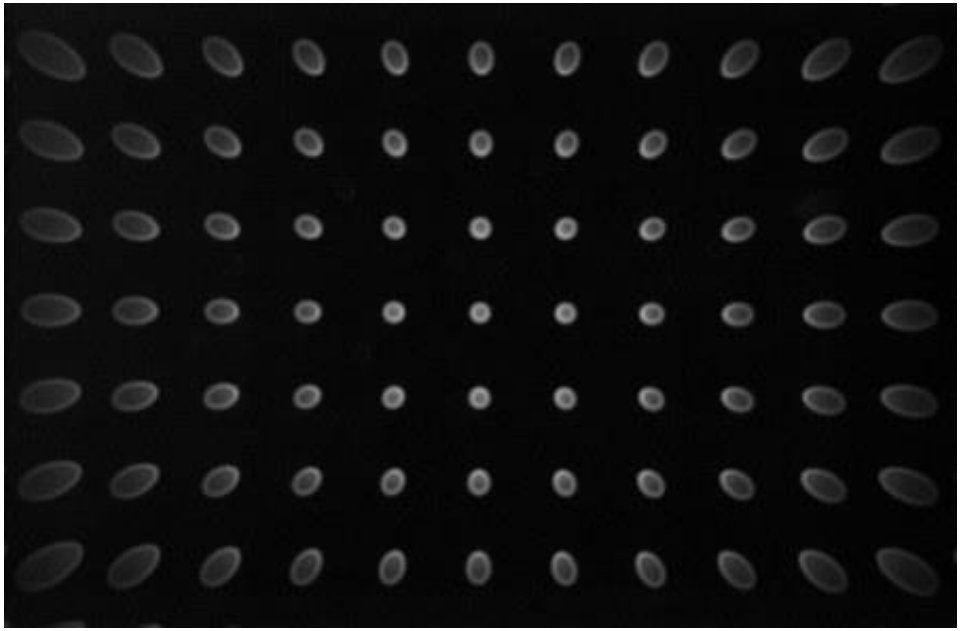
场曲(Distortion)



桶形

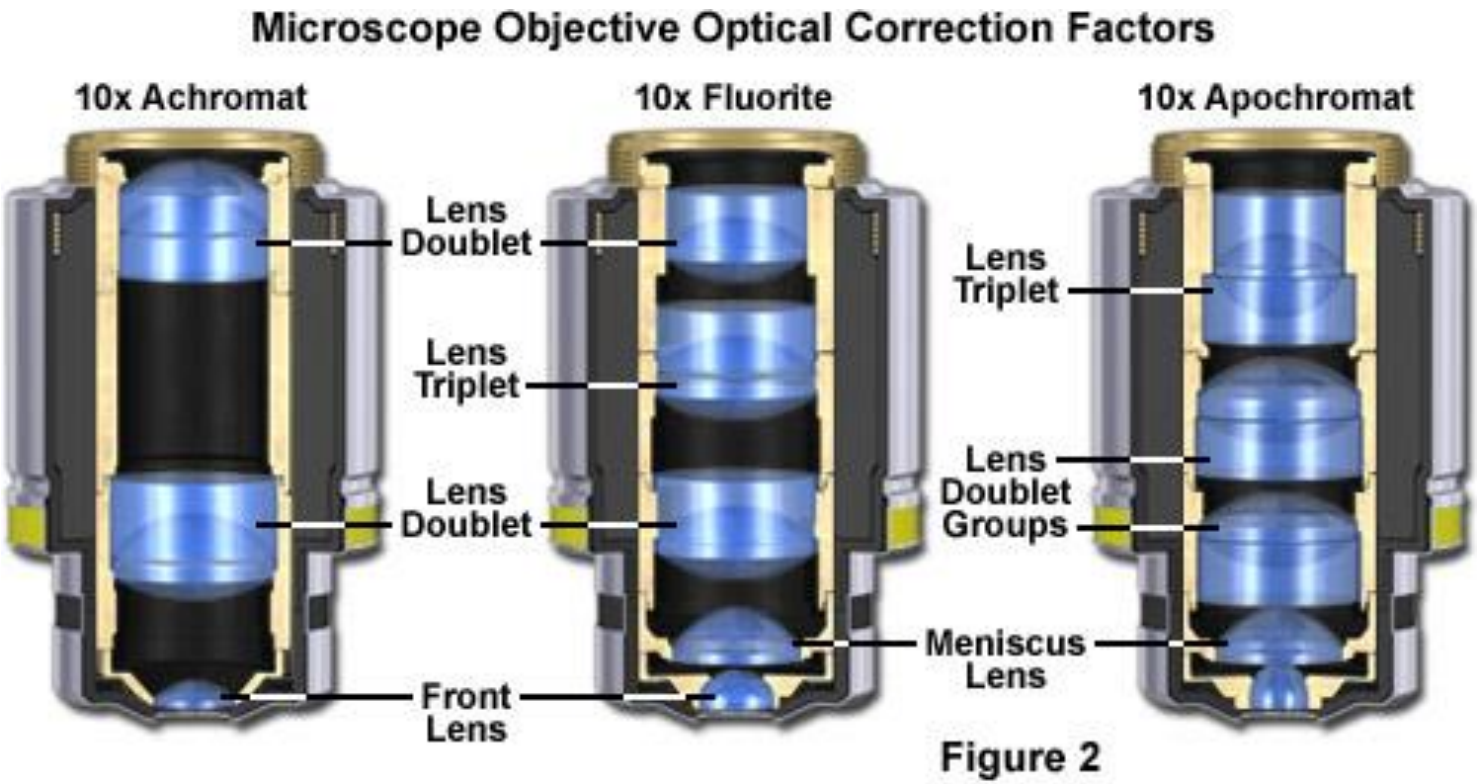


枕形



场曲+象散

显微镜物镜


Microscope Objective Optical Correction Factors
10x Achromat
10x Fluorite
10x Apochromat
Lens Doublet
Lens Triplet
Lens Doublet
Front Lens
Lens Triplet
Lens Doublet Groups
Meniscus Lens
Figure 2

像差校正程度 / 像差 / 物镜类型及标记	球面像差	纵向色差	像域弯曲	备注
消色差物镜（无标记，Achromat）	黄、绿光	红、绿光	-	加黄绿光滤色片，观察用，不适于彩色金相
复消色差物镜（Apochromat 或 Apo）	绿、紫光	红、绿、紫光	-	可加任意滤色片，观察用，适用于彩色金相
平场消色差物镜（PC，Planachromat 或 Pl）	黄、绿光	红、绿光	校正	加黄绿光滤色片，照相用，不适于彩色金相
平场复消色差物镜（PF，Planapochromat）	绿、紫光	红、绿、紫光	校正	可加任意滤色片，照相用，适用于彩色金相

60x Plan Apochromat Objective

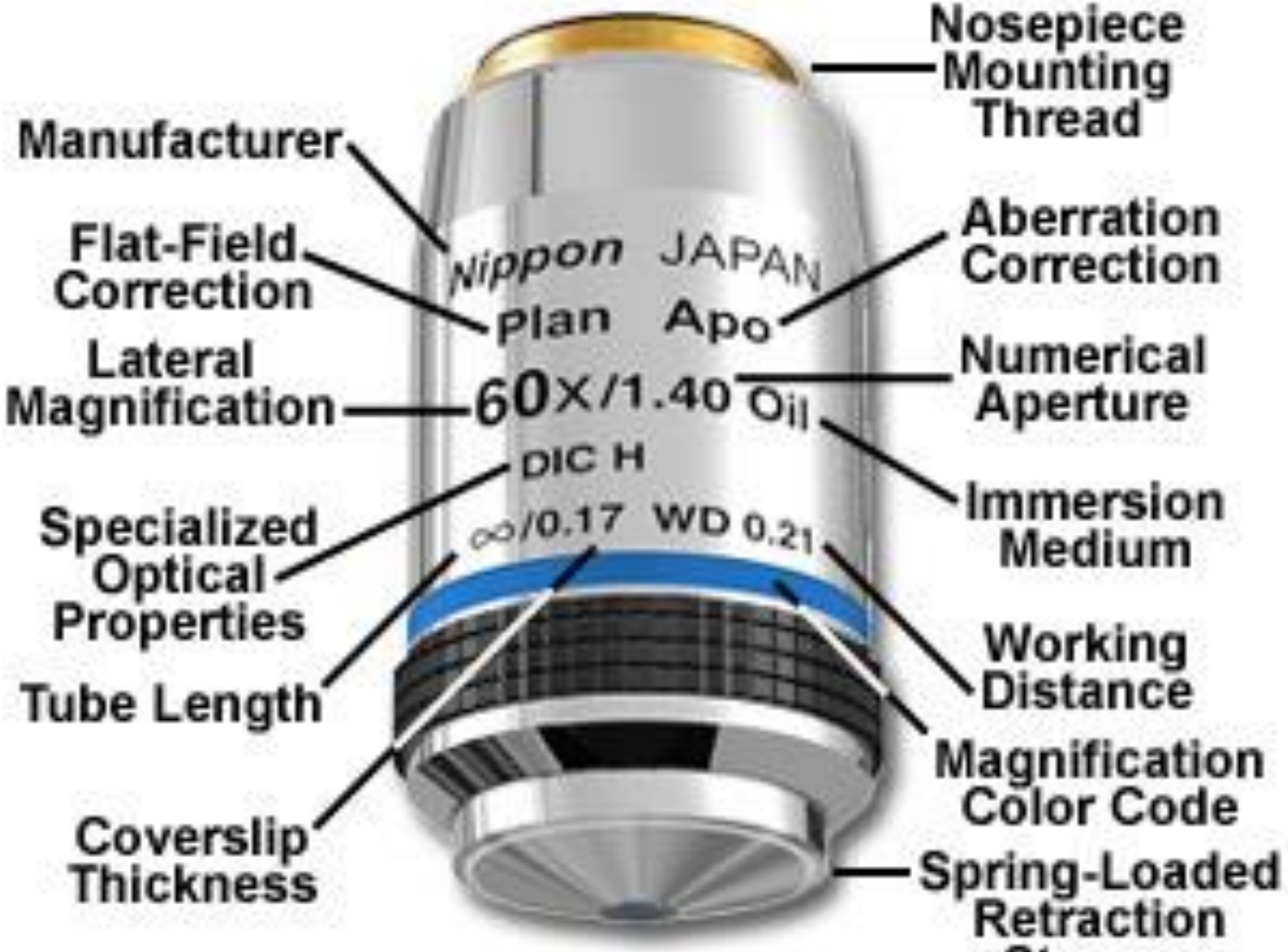
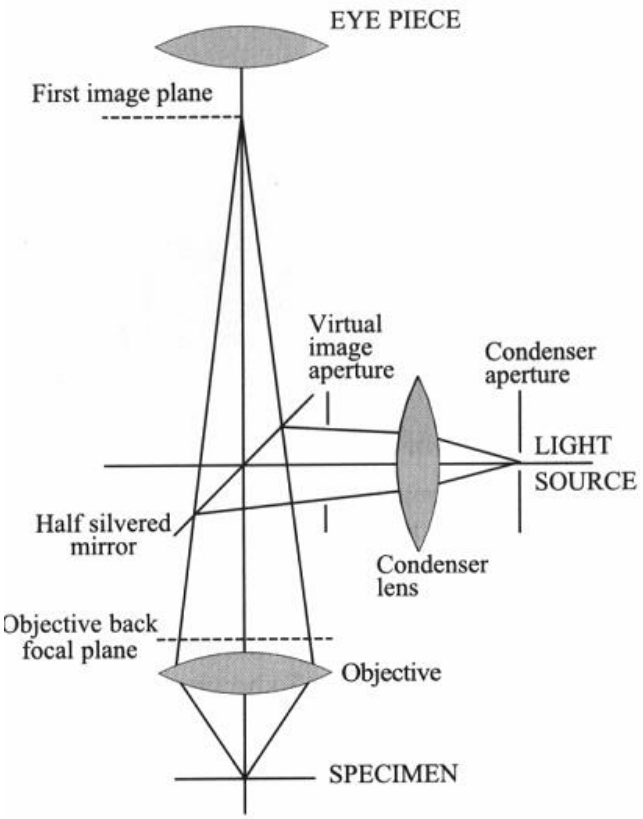
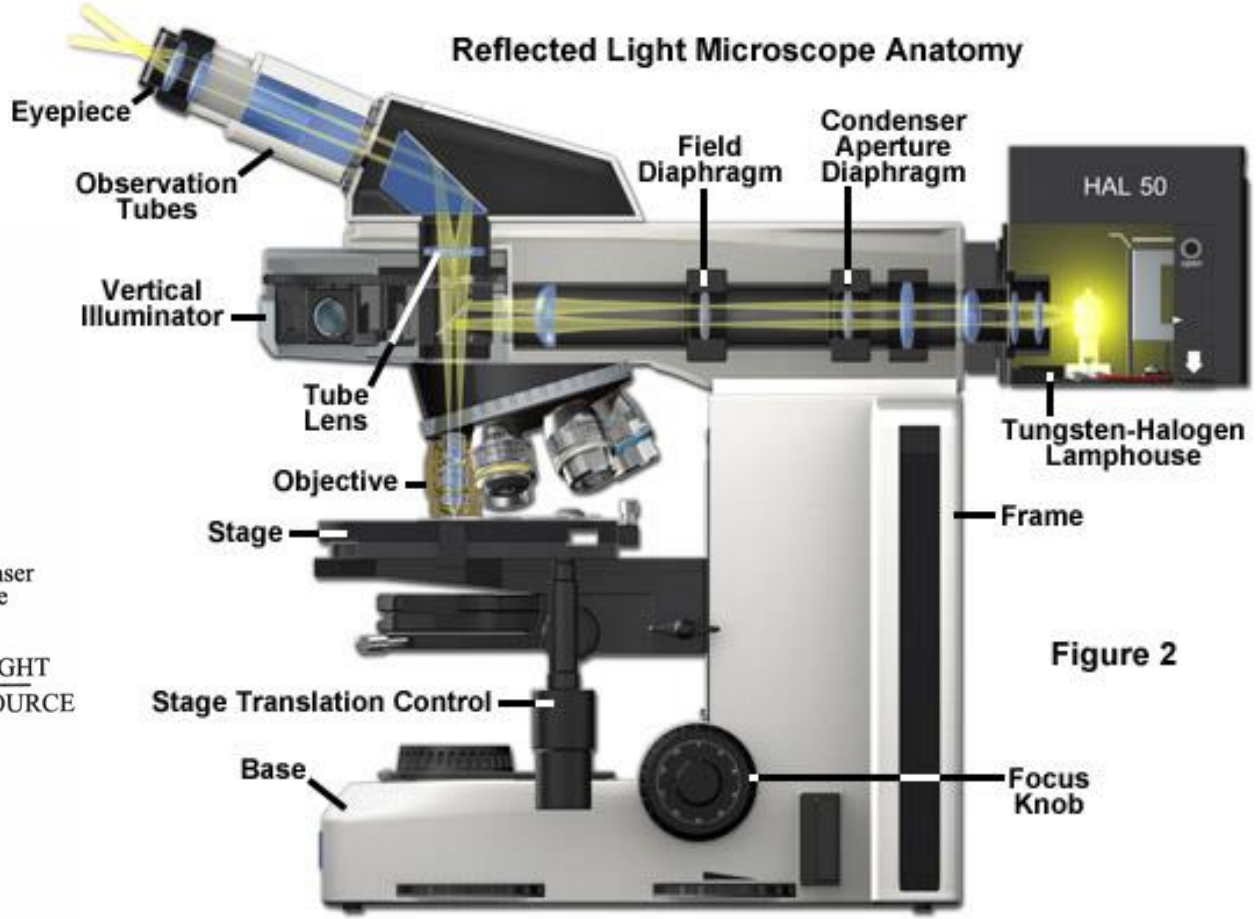


Figure 1


ZEISS
Epiplan-APOCHROMAT
100x/0.95 HD DIC
∞/0
ZEISS
EC Epiplan-APOCHROMAT
50x/0.95 HD DIC
∞/0
ZEISS
EC Epiplan-APOCHROMAT
20x/0.6 HD DIC
∞/0

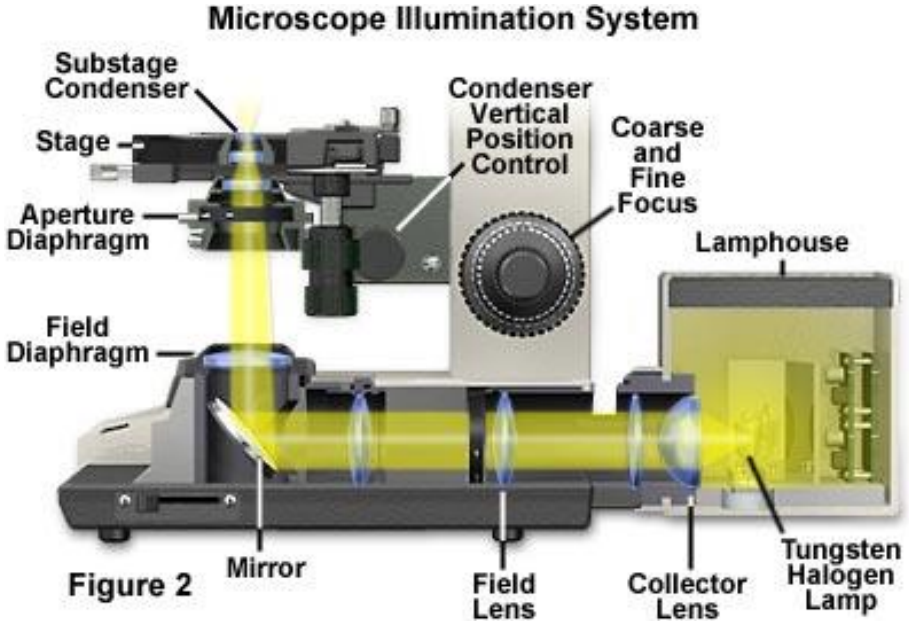
显微镜光学系统


EYE PIECE
First image plane
Virtual image aperture
Condenser aperture
LIGHT SOURCE
Half silvered mirror
Condenser lens
Objective back focal plane
Objective
SPECIMEN

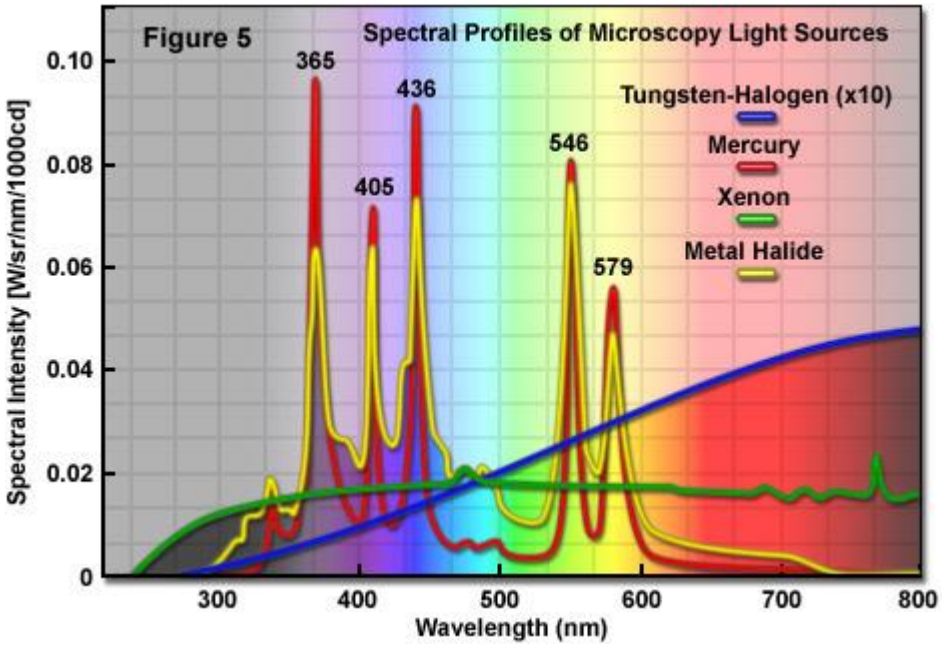

Reflected Light Microscope Anatomy
Eyepiece
Observation Tubes
Field Diaphragm
Condenser Aperture Diaphragm
HAL 50
Vertical Illuminator
Tube Lens
Tungsten-Halogen Lamphouse
Objective
Stage
Frame
Stage Translation Control
Base
Focus Knob
Figure 2

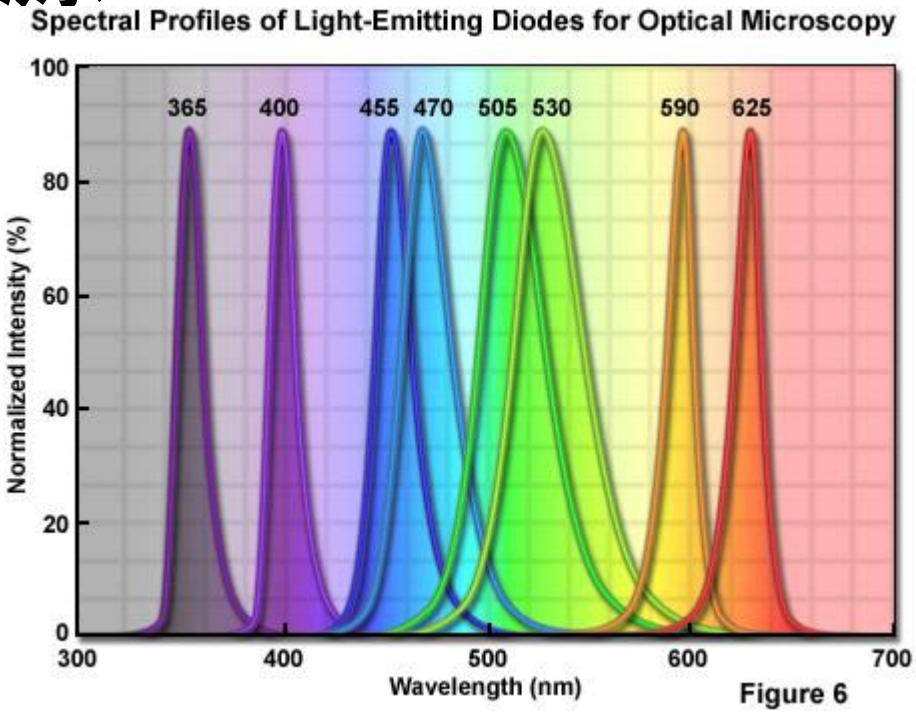
照明系统

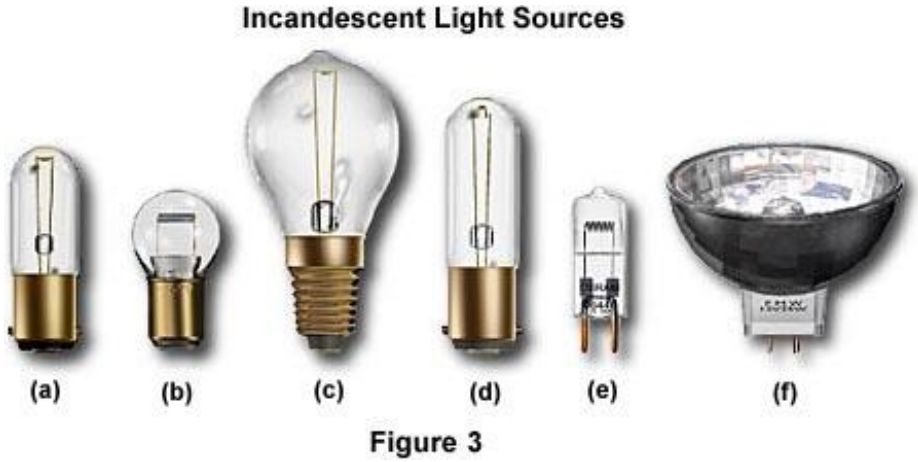
- 对样品提供均匀、明亮的照明，高性能显微镜的前提
- 科勒照明(Koehler illumination)
- 共轭面(Conjugate planes)



光源

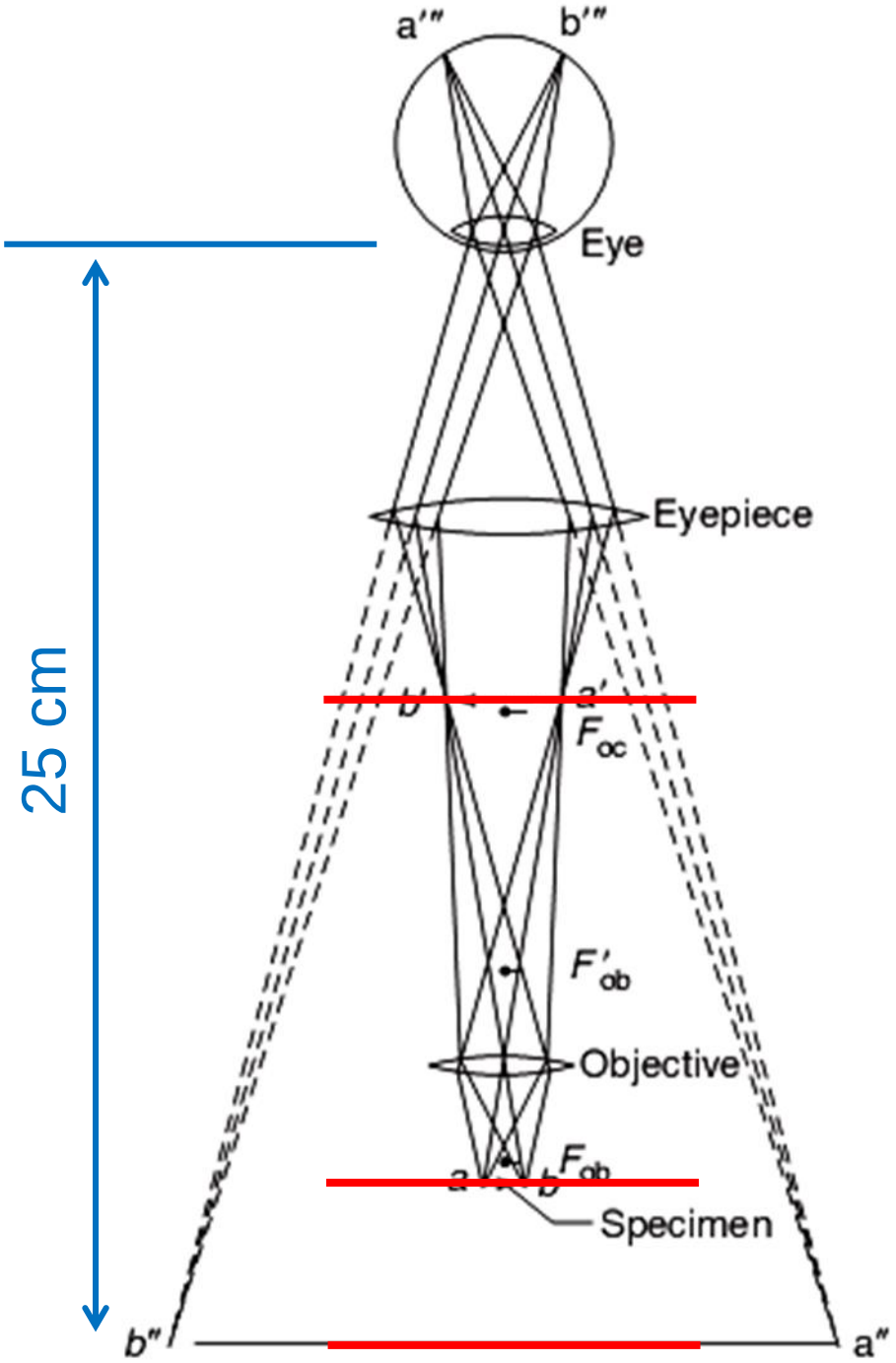




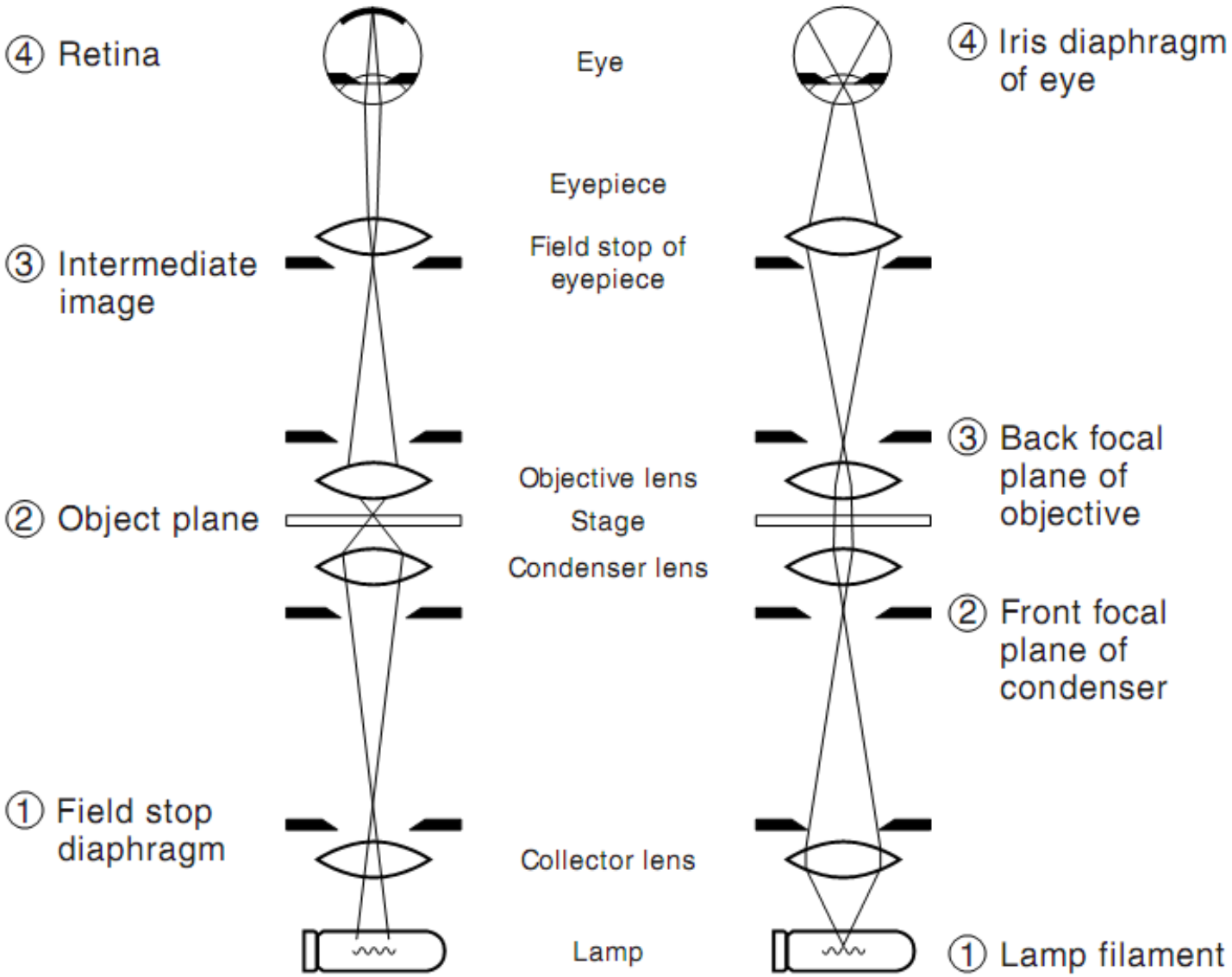


- 钨-卤灯
- LED灯
- 激光(单色性)

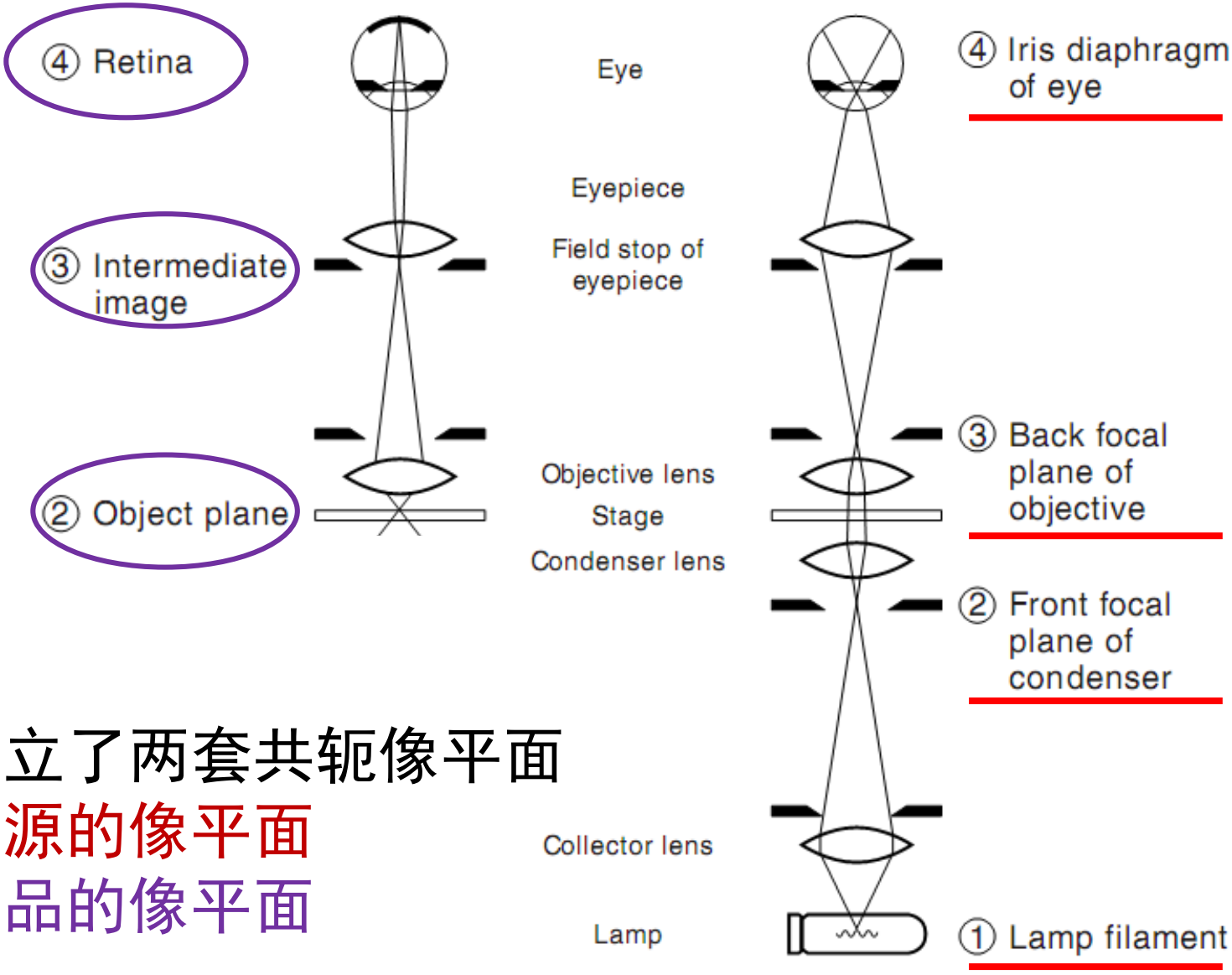
显微镜中的共轭面



临界照明　　科勒照明


④ Retina
③ Intermediate image
② Object plane
① Field stop diaphragm
Eye
Eyepiece
Field stop of eyepiece
Objective lens
Stage
Condenser lens
Collector lens
Lamp
④ Iris diaphragm of eye
③ Back focal plane of objective
② Front focal plane of condenser
① Lamp filament

科勒照明

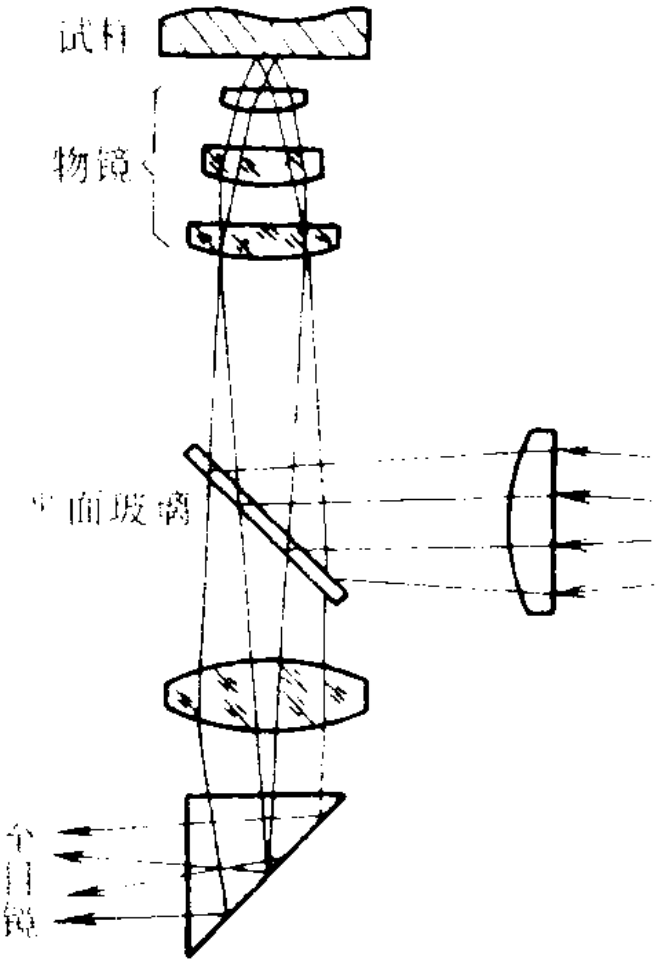


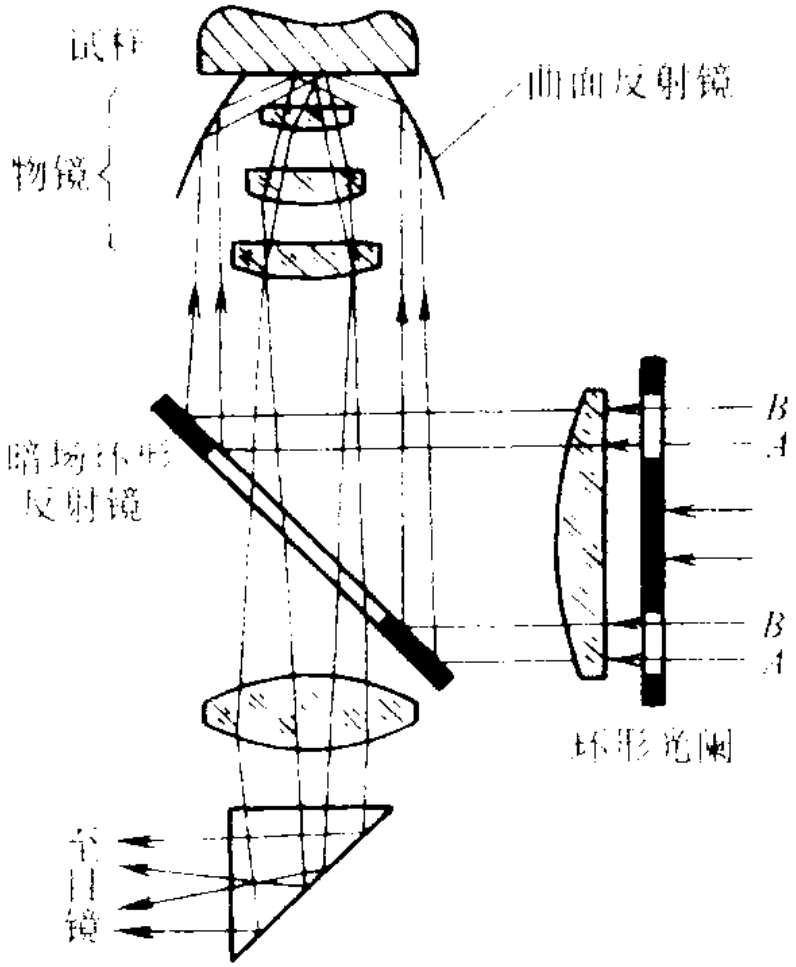
建立了两套共轭像平面

光源的像平面

样品的像平面

明场 *vs* 暗场照明





光阑(aperture)

- 聚光镜光阑
- 物镜光阑
- 视域光阑

孔径光阑

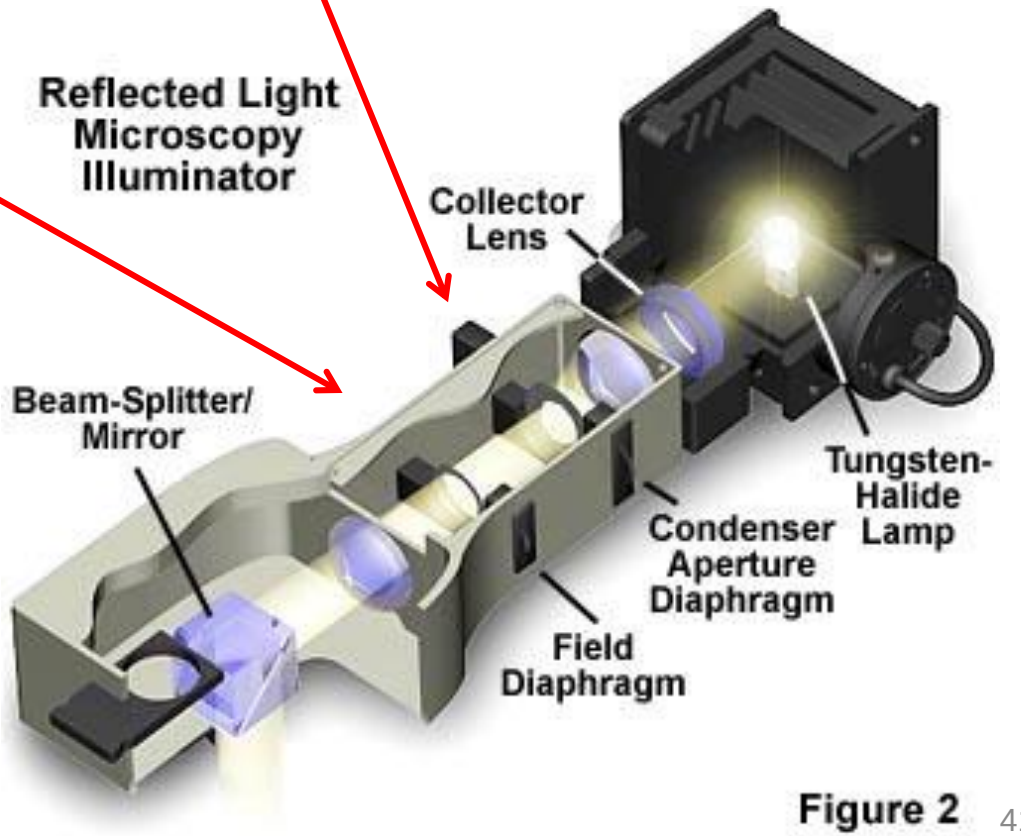
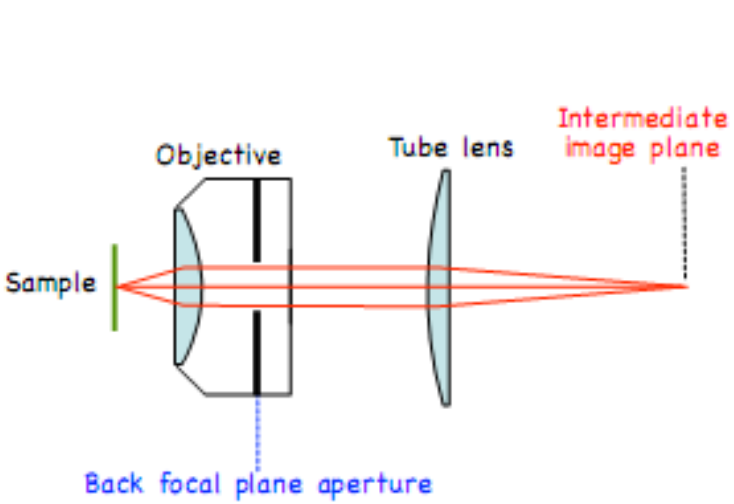
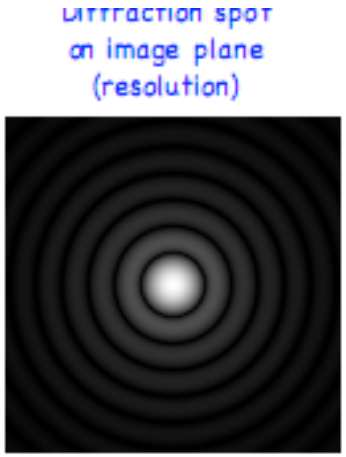
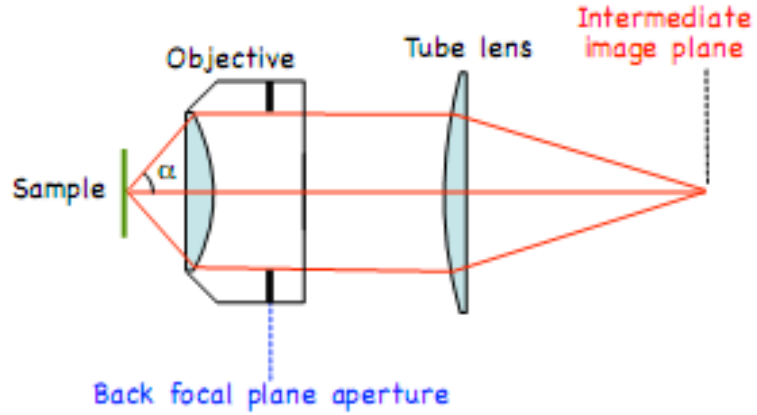


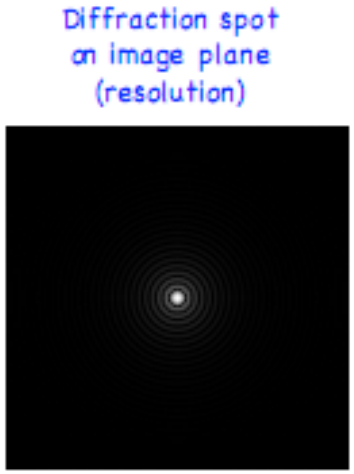
Figure 2

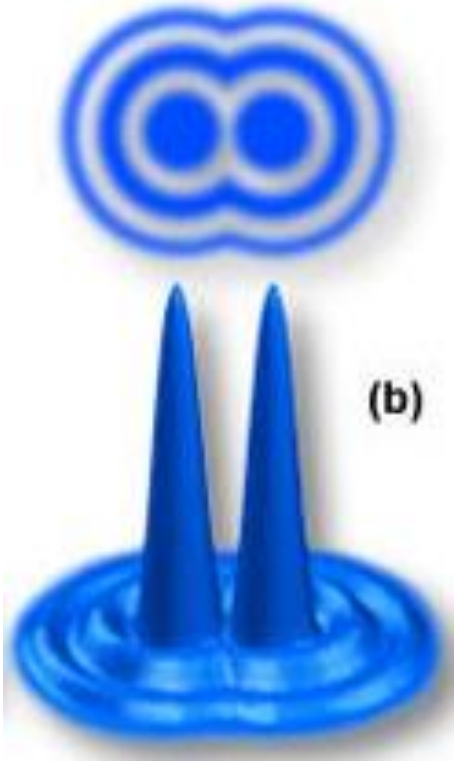
孔径光阑(aperture diaphragm)





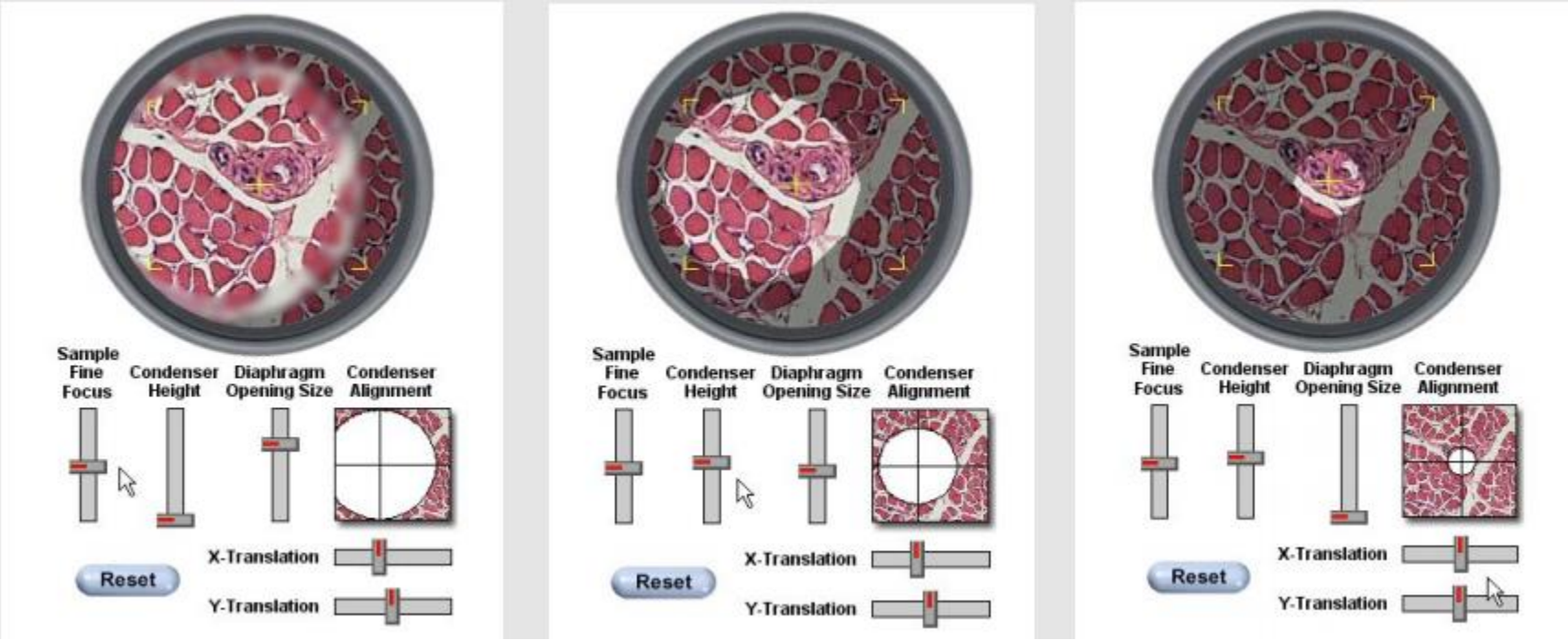






影响分辨率和亮度，但不要试图用调节孔径光阑大小来调节物象的亮度

视域光阑(field diaphragm)



景深(Depth of field)

- 表征物镜对位于样品不同高度平面上细节分辨的能力。The distance along the optic axis over which image details can be observed with acceptable clarity.

$$D_f = \frac{(0.15\sim0.30)n}{(N.A.)M}\ (\mu m)$$

- 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

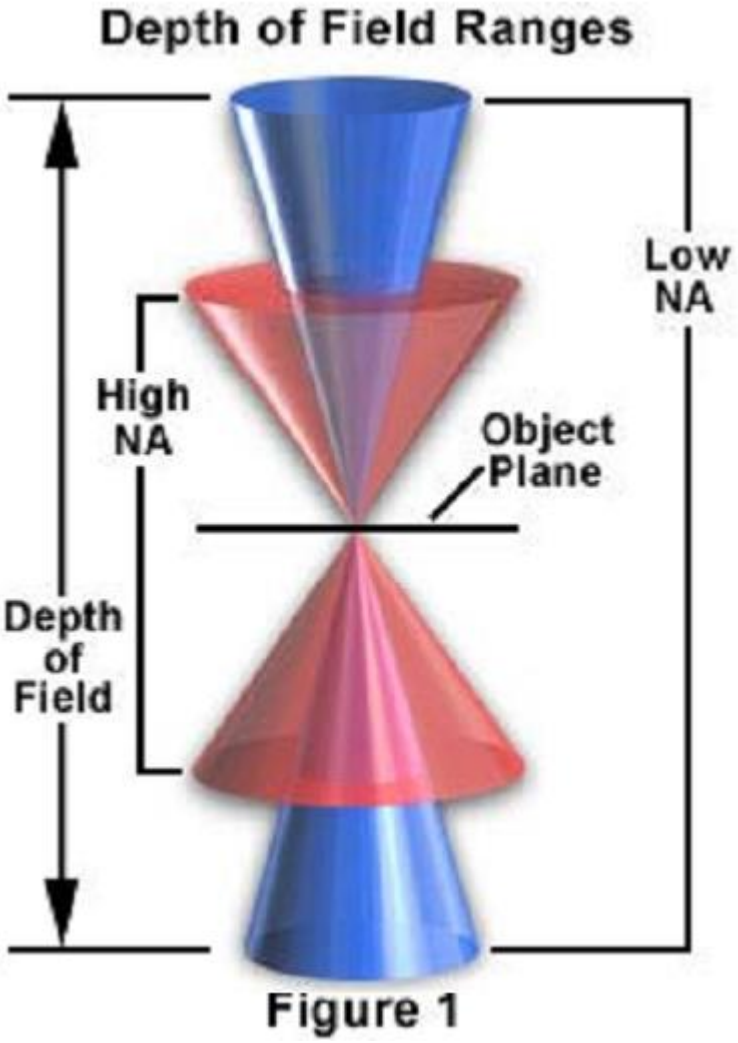
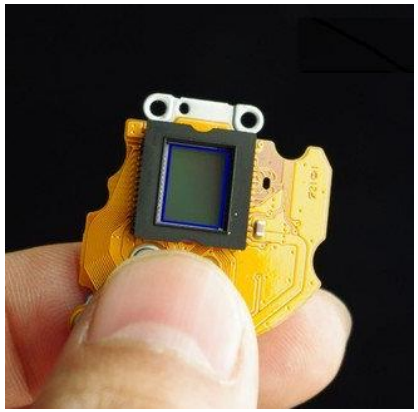
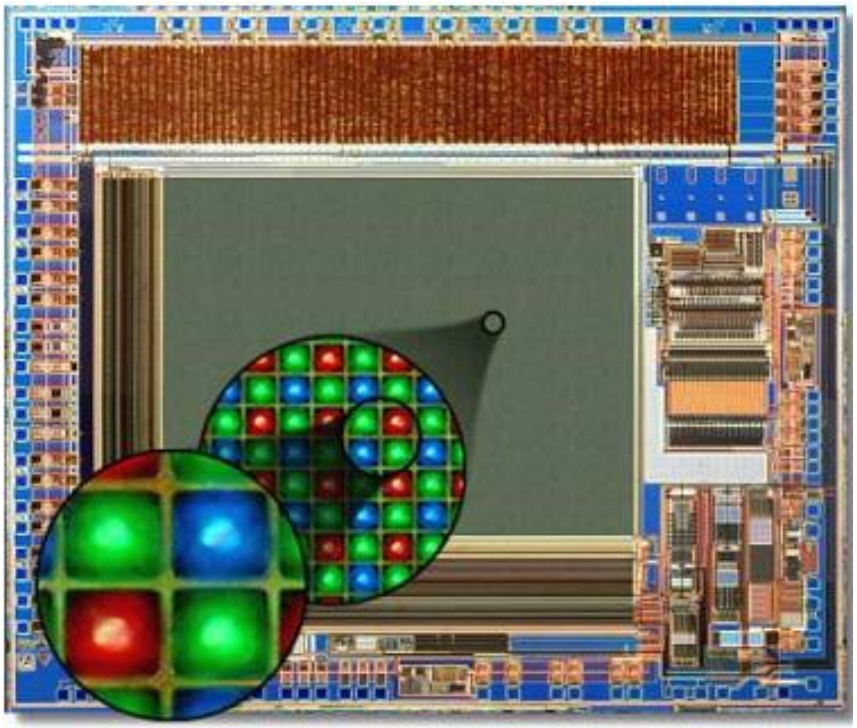


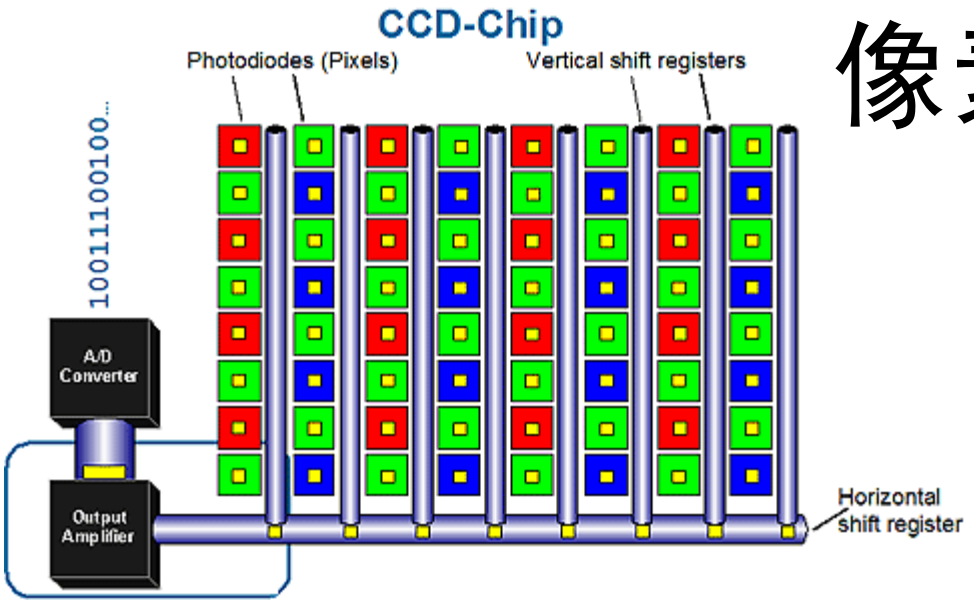
Figure 1

CCD/CMOS电子影像系统

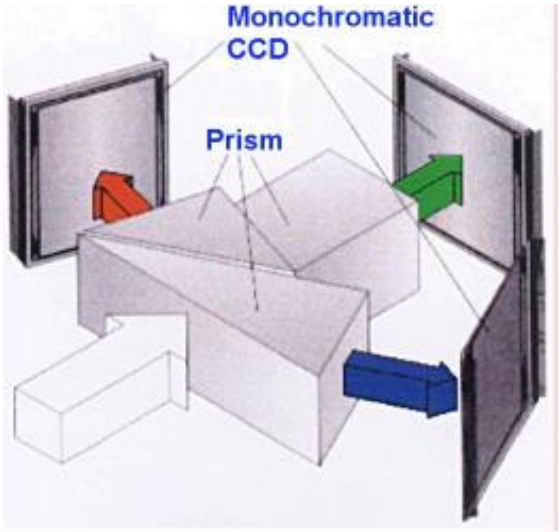
- 虽然数码影像系统已成为显微镜拍摄照片的标准装置，正确认识和使用数码电子影像装置对获得高质量照片依然重要。
- CCD
- CMOS

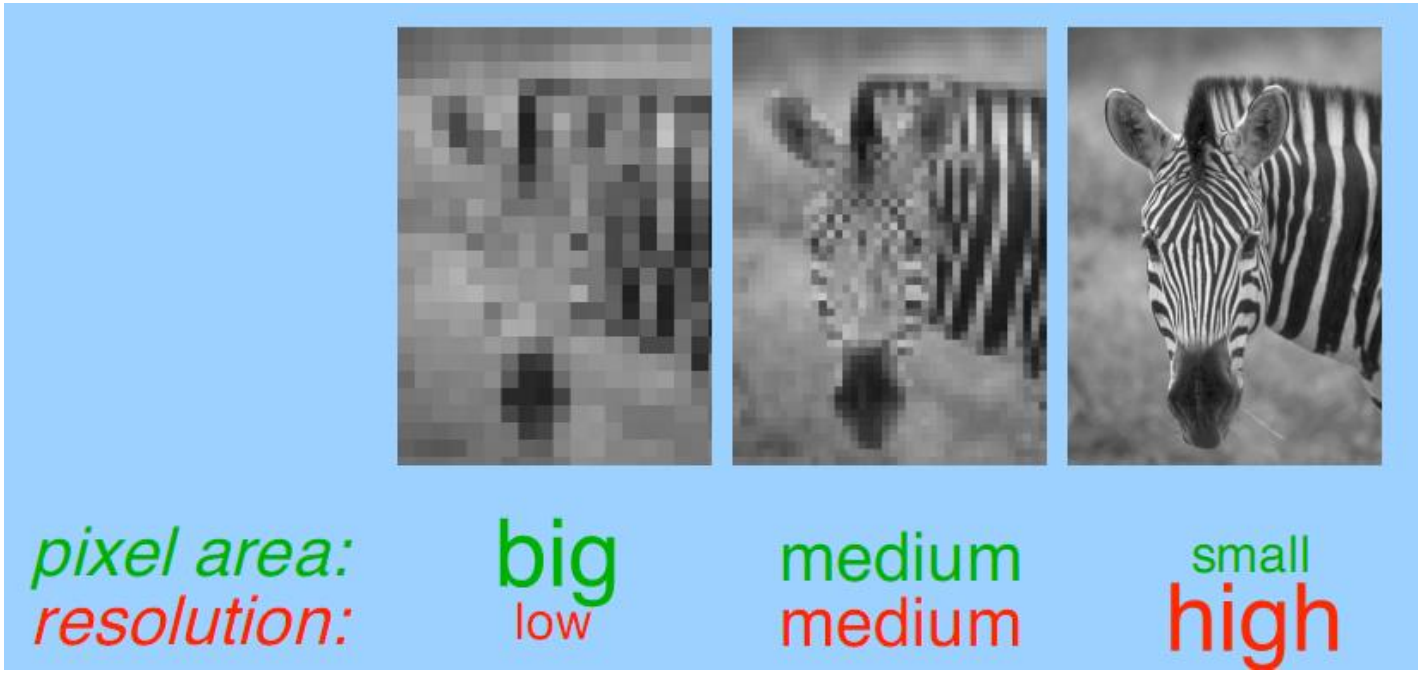




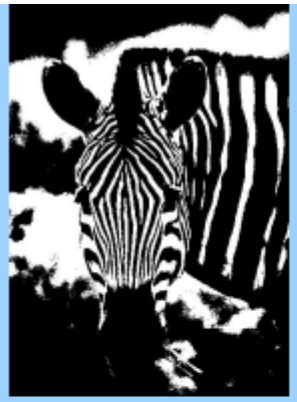

CCD-Chip
Photodiodes (Pixels)
Vertical shift registers
10011100100...
A/D Converter
Output Amplifier
Horizontal shift register

像素


Monochromatic CCD
Prism

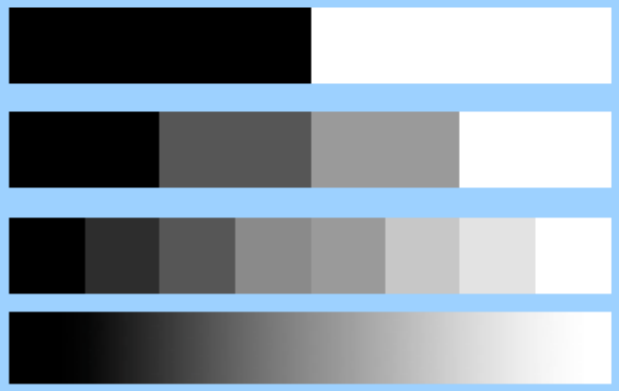

pixel area:
resolution:
big
low
medium
medium
small
high

比特数/位数



1 bit

8 bit



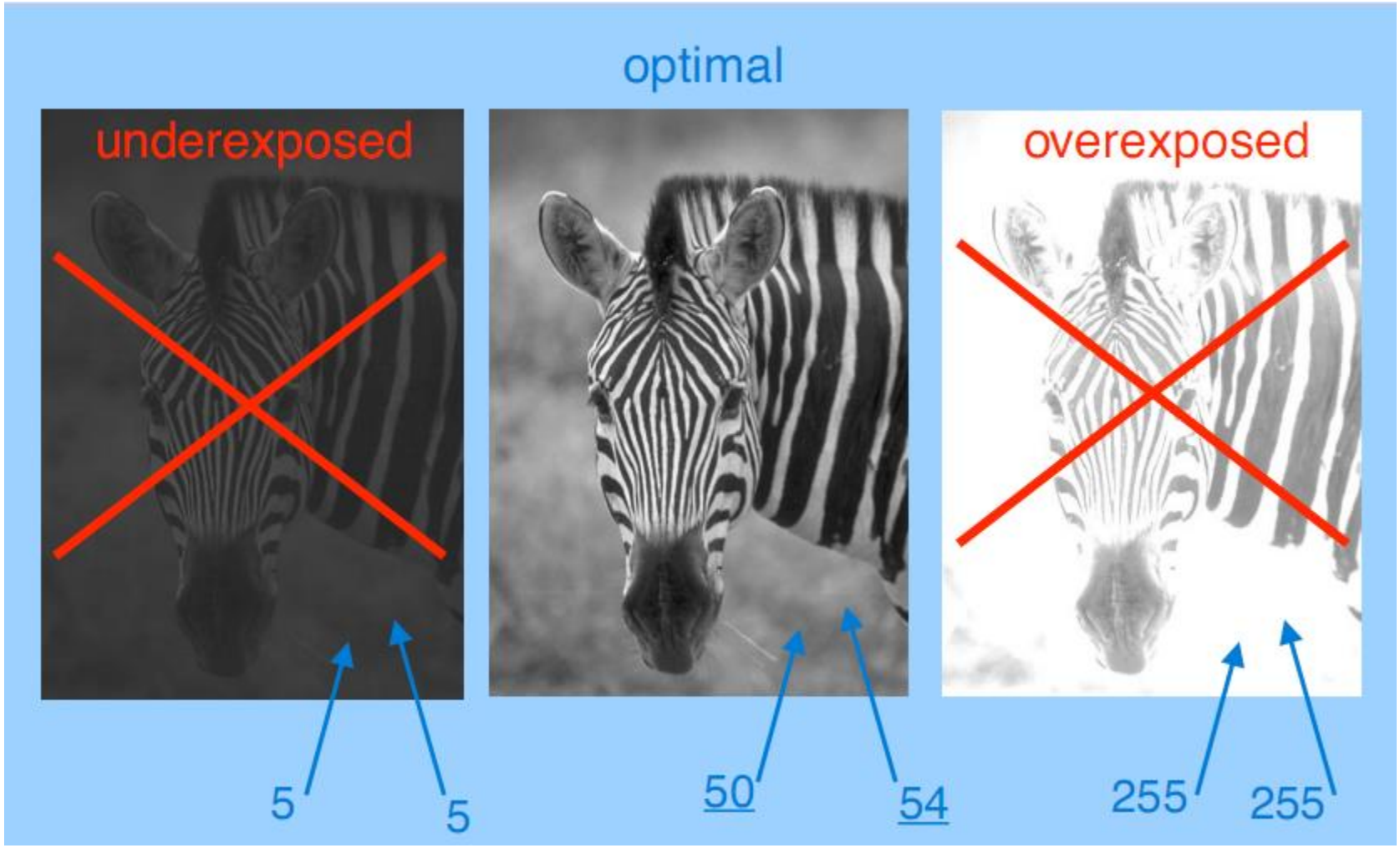
1 bit (2^1 = 2 intensity levels)

2 bits (2^2 = 4 intensity levels)

3 bits (2^3 = 8 intensity levels)

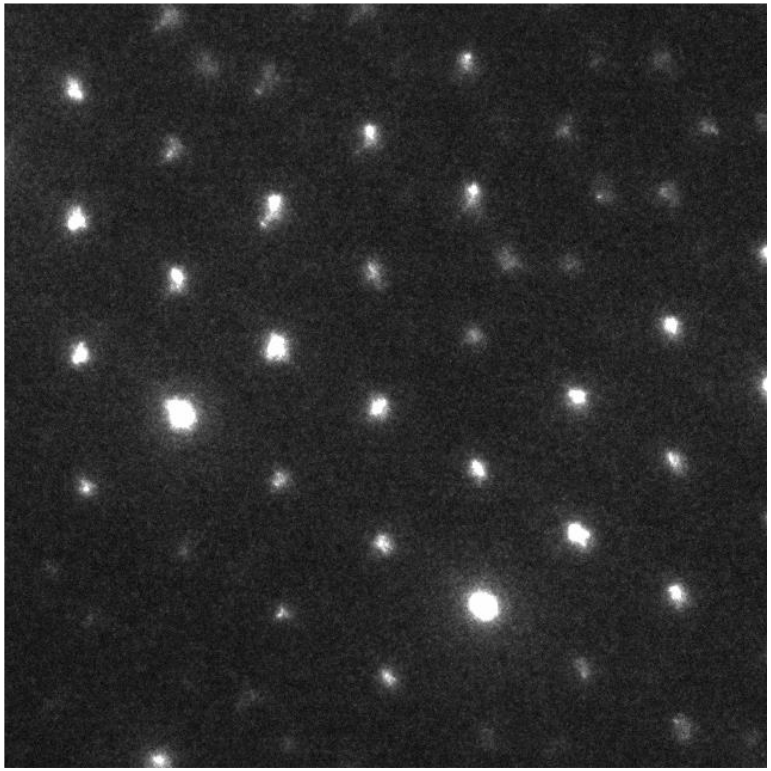
8 bits (2^8 = 256 intensity levels)

动态范围

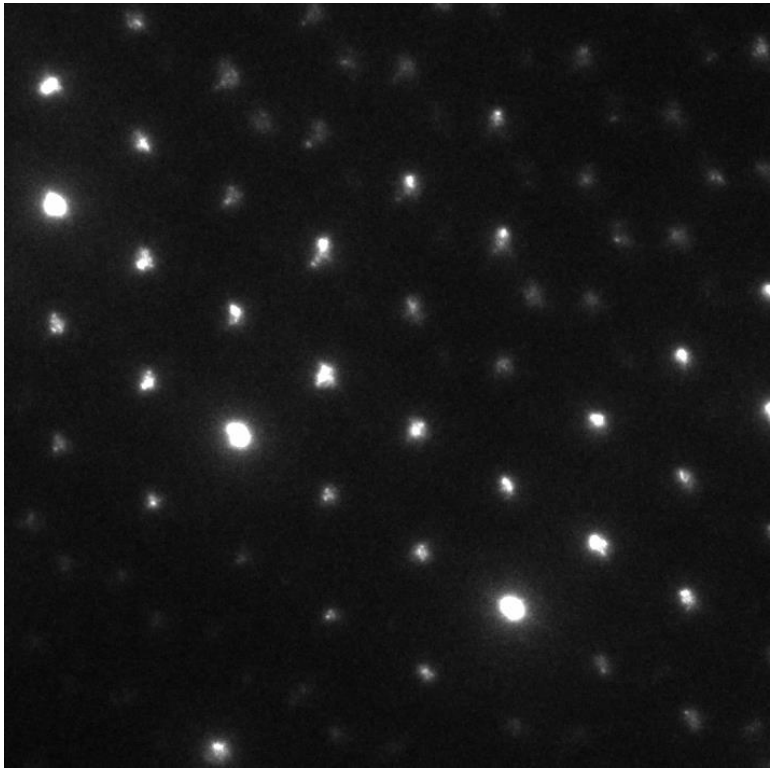


信噪比

- 曝光时间对信噪比的影响



短



长

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.

Pixel Size Requirements for Maximum Resolution in Optical Microscopy

Objective (numerical aperture)	Resolution Limit (microns)	Projected Size on CCD (microns)	Required Pixel Size (microns)
4x (0.20)	1.5	5.8	2.9
10x (0.45)	0.64	6.4	3.2
20x (0.75)	0.39	7.7	3.9
40x (0.85)	0.34	13.6	6.8
40x (1.30)	0.22	8.9	4.5
60x (0.95)	0.31	18.3	9.2
60x (1.40)	0.21	12.4	6.2
100x (0.90)	0.32	32.0	16.0
100x (1.25)	0.23	23.0	11.5
100x (1.40)	0.21	21.0	10.5