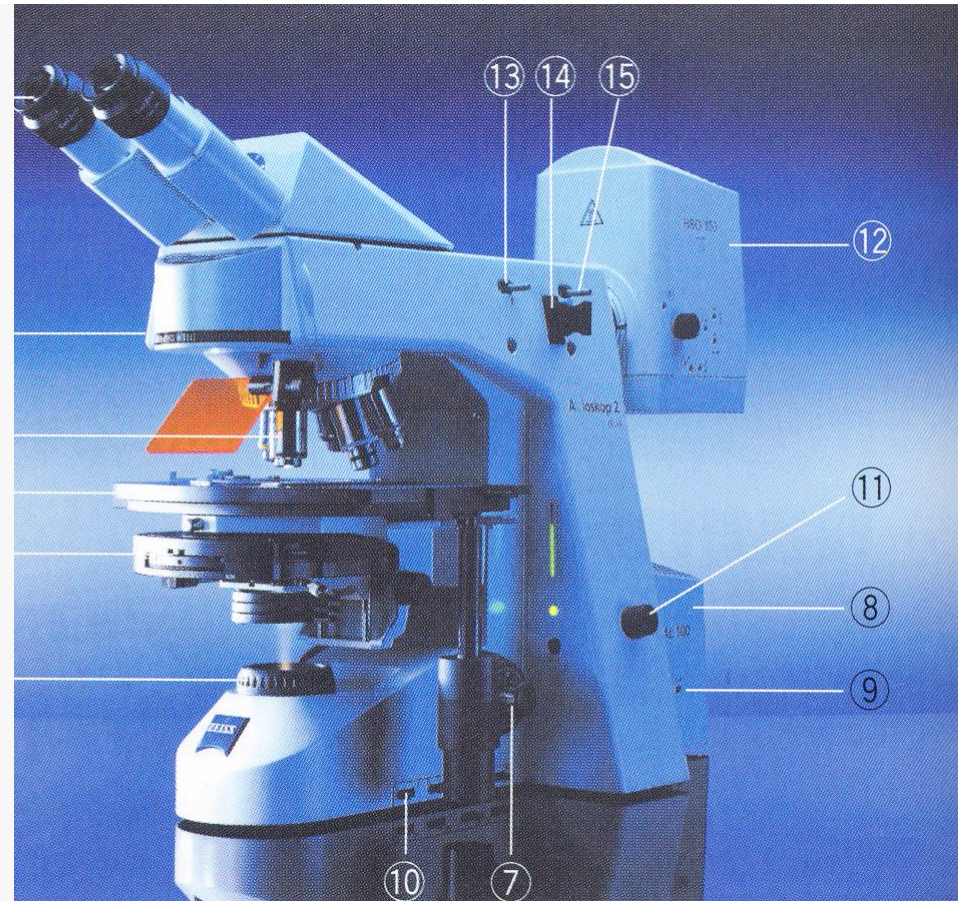
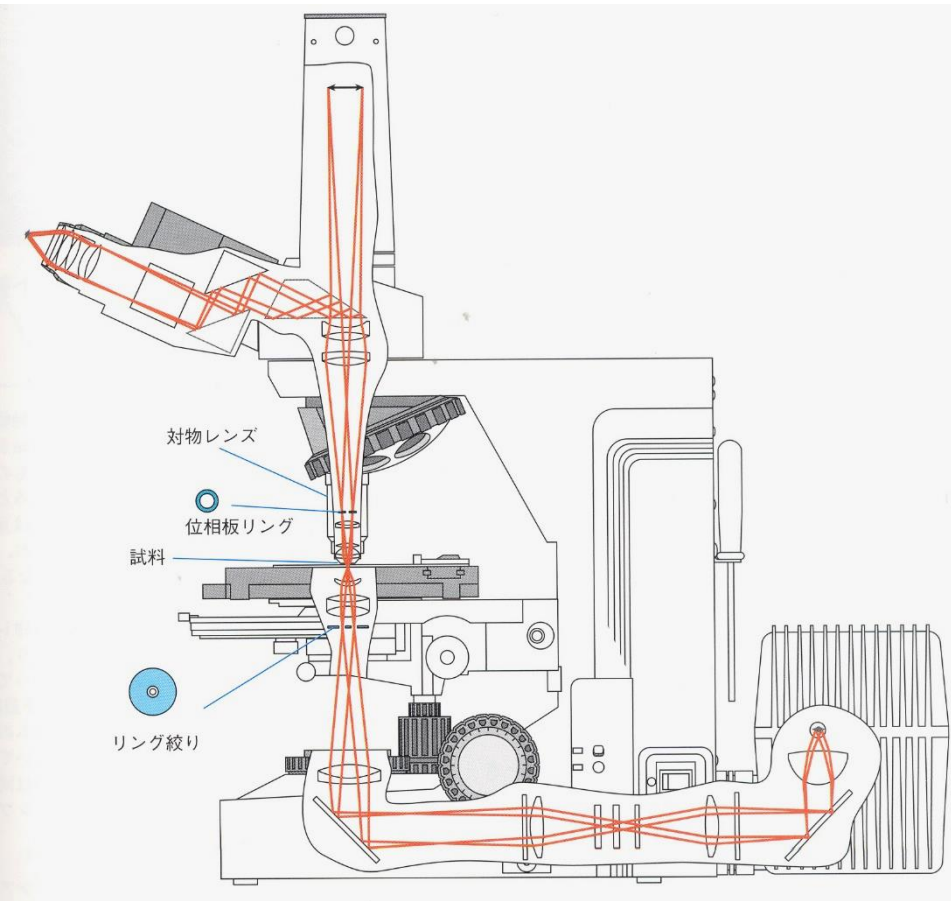


光學顯微鏡 基本原理及運用

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2023

光學顯微鏡



顯微鏡的分類

正立顯微鏡：觀察組織切片

倒立顯微鏡：觀察培養活細胞

實體顯微鏡：解剖及立體定位

第1章 顯微鏡的分類

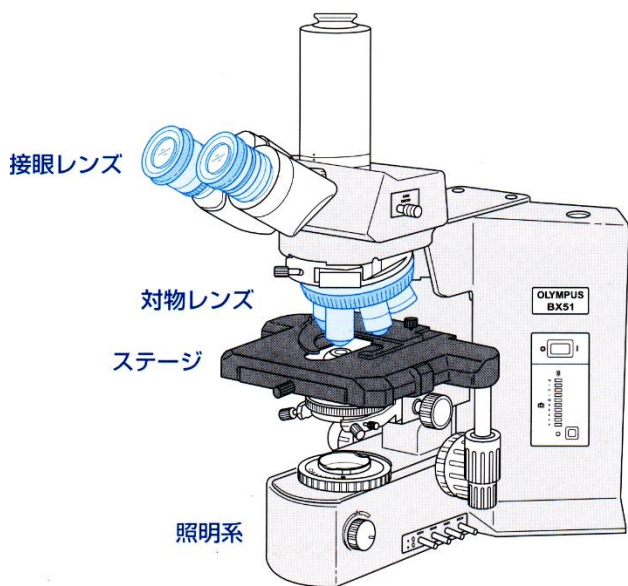


図1 正立型顯微鏡

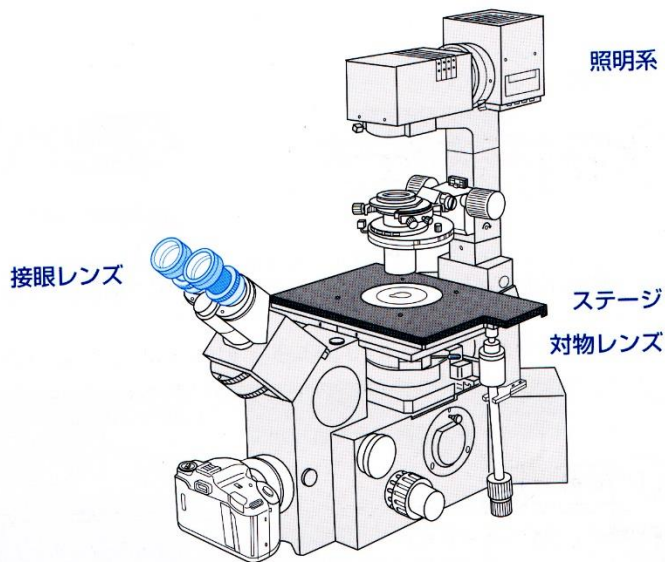
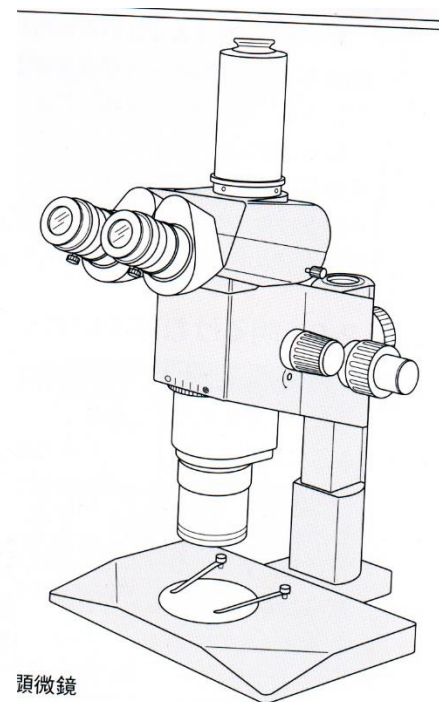


図2 倒立型顯微鏡



頭微鏡

D いろいろな顕微鏡と装置 試料をいろいろな面から観察できるような光学顕微鏡と付属装置が開発されている。

● 微分干涉顕微鏡



細胞分裂・原形質流動・アメーバ運動・繊毛運動の観察に使用。

● 培養倒立顕微鏡



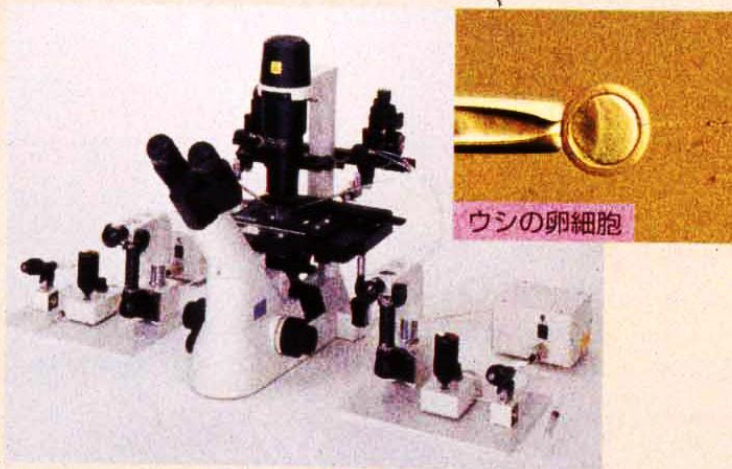
培養容器に入った細胞などの観察に使う。

● 偏光顕微鏡



偏光板を利用した顕微鏡で、筋繊維などの微細構造の観察に使用。

● マイクロマニピュレーター



顕微受精などに利用する付属装置。

● 蛍光顕微鏡



蛍光色素で抗体などを着色し、試料の発する蛍光を観察する。

● 実体顕微鏡

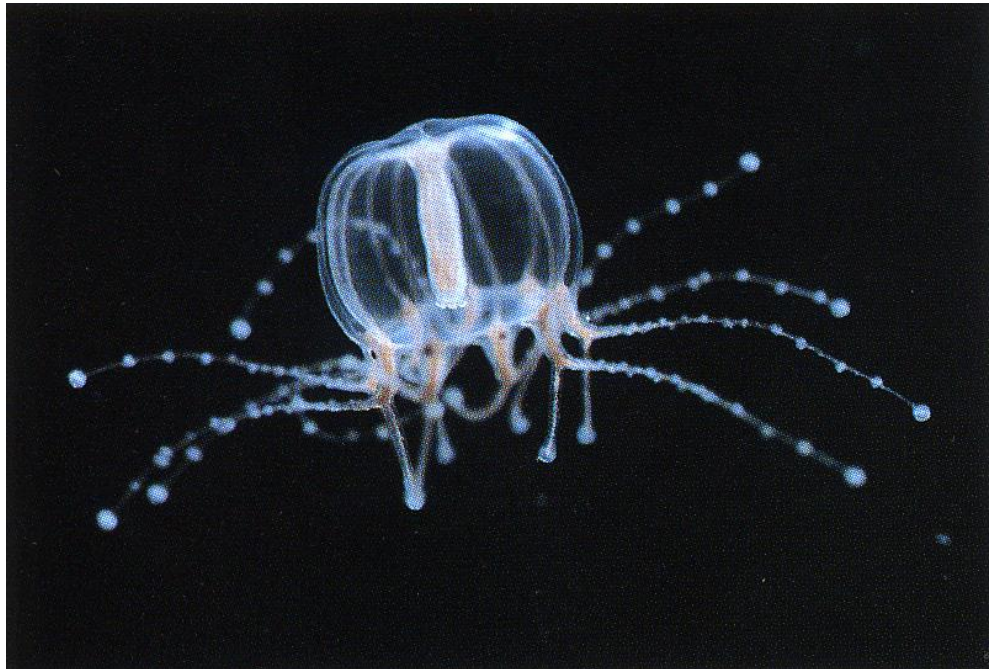
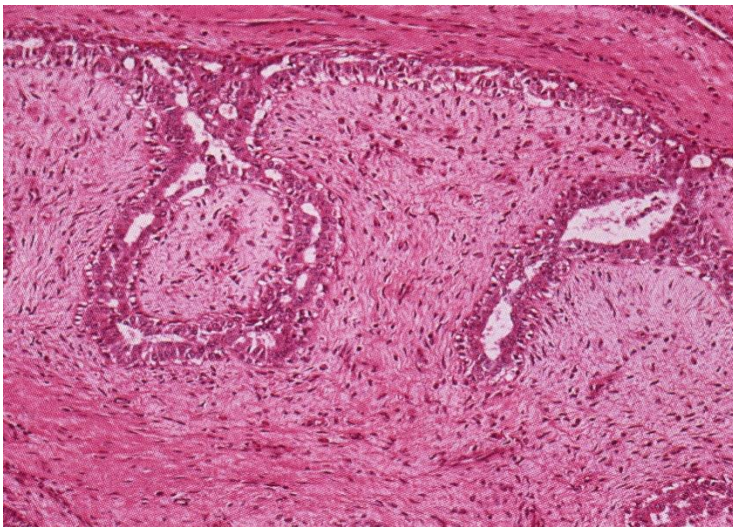
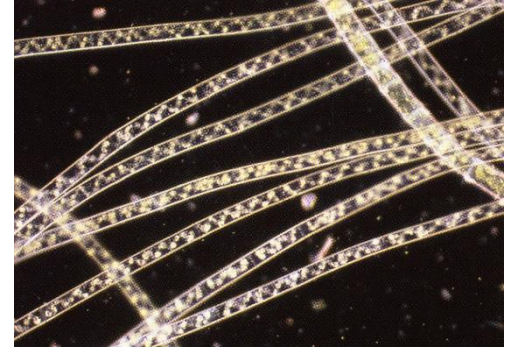
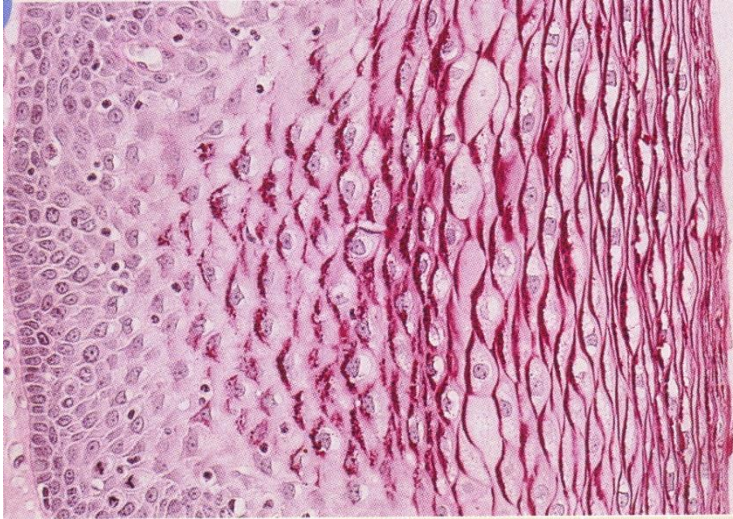


生試料を正立像で立体的に観察できる。

觀察法的顯微鏡分類

明視野 (bright field)：一般光學組織切片

暗視野 (dark field)：小動物如線蟲及纖毛鞭毛結構

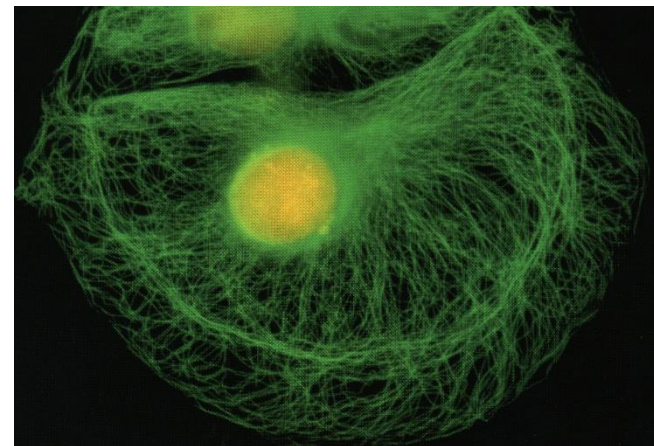
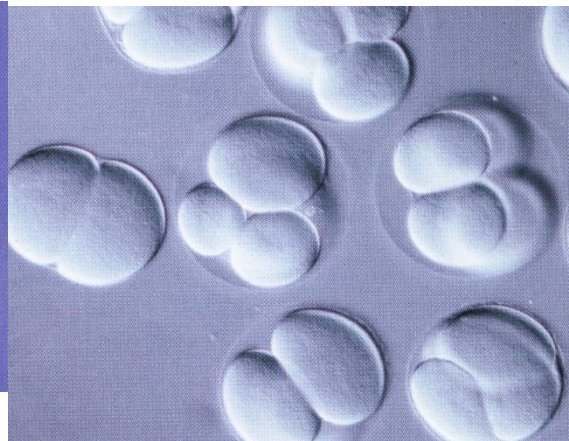
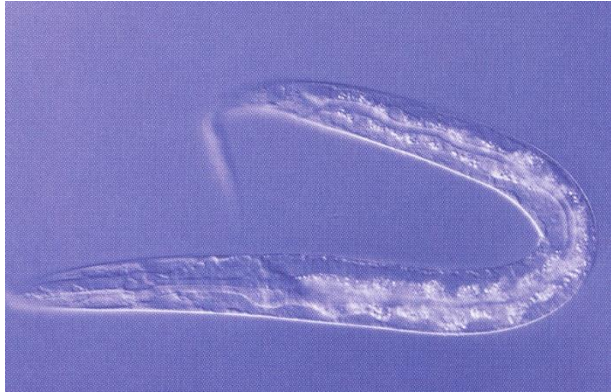
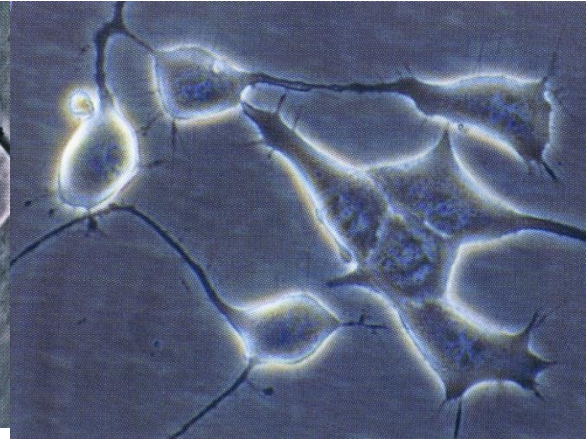


偏光 (polarized light) : 堅硬的結晶結構等

位相差 (phase contrast) : 一般培養活細胞

微分干涉 (differential interference contrast, DIC) : 活體卵細胞及線蟲等

螢光 (fluorescence) : 一般螢光染色標示或活體螢光如GFP等



照明光源路徑:

1. **穿透光照明**：一般光學組織切片，薄具透光性；對比與染色
2. **反射照明**：解剖及實體顯微鏡，螢光顯微鏡；表面光源反射

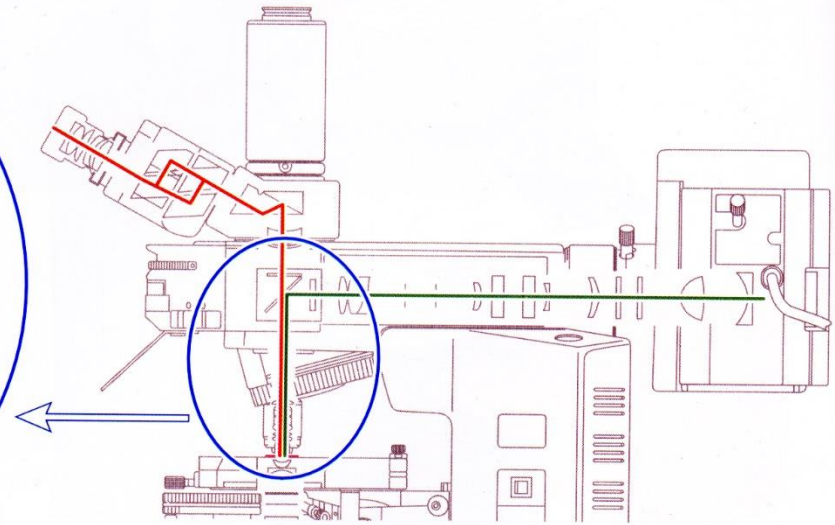
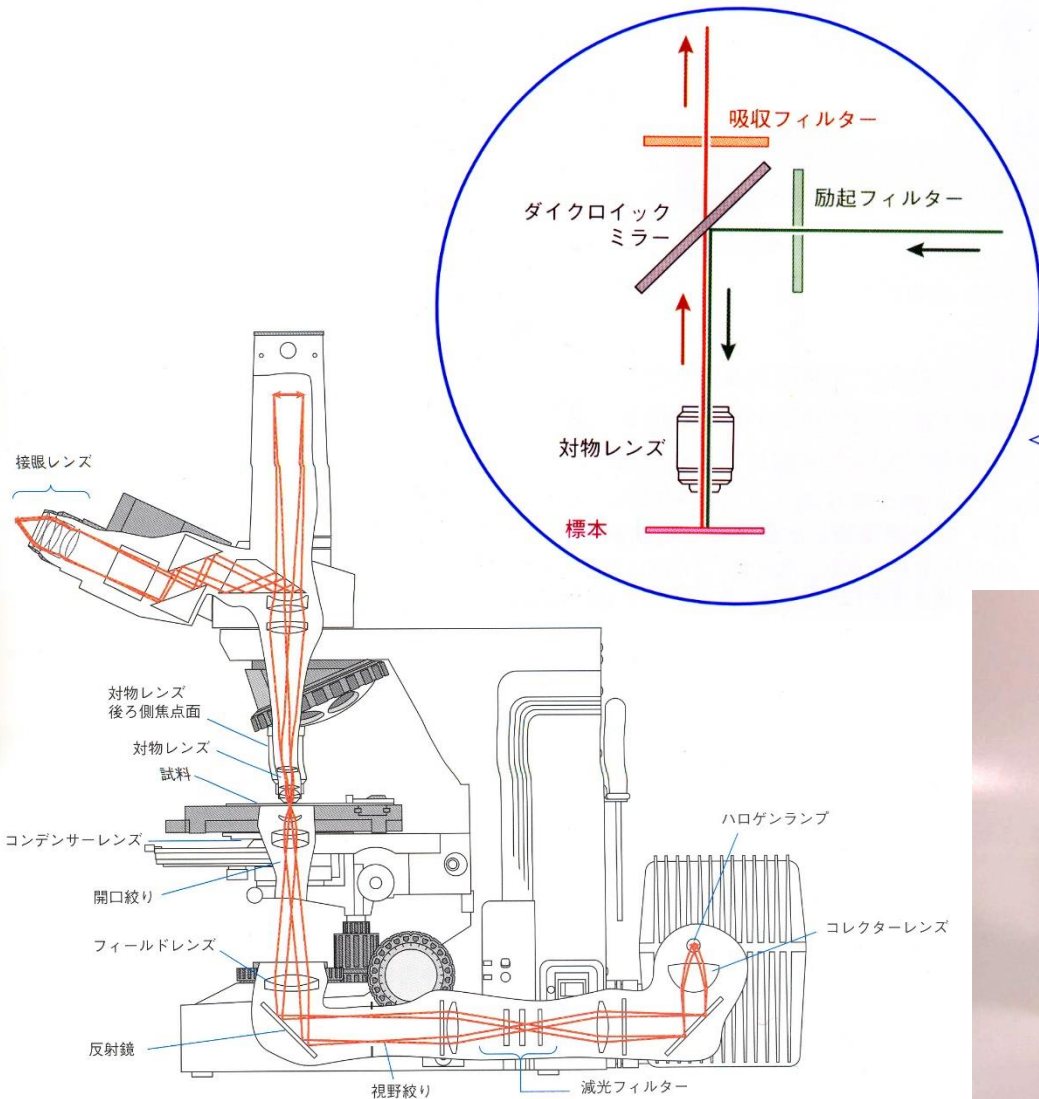
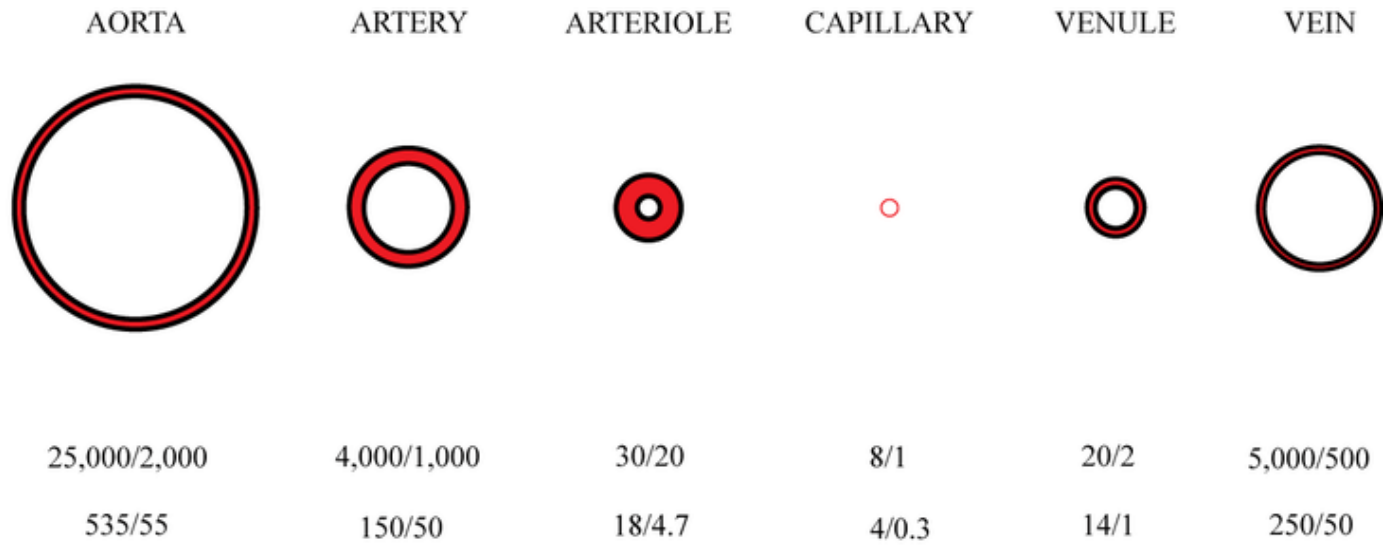
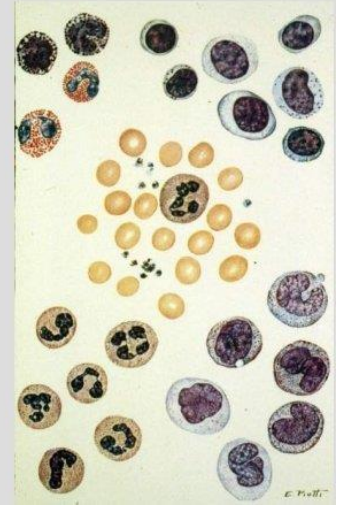


TABLE 1.3. Eye versus Instrument Resolution

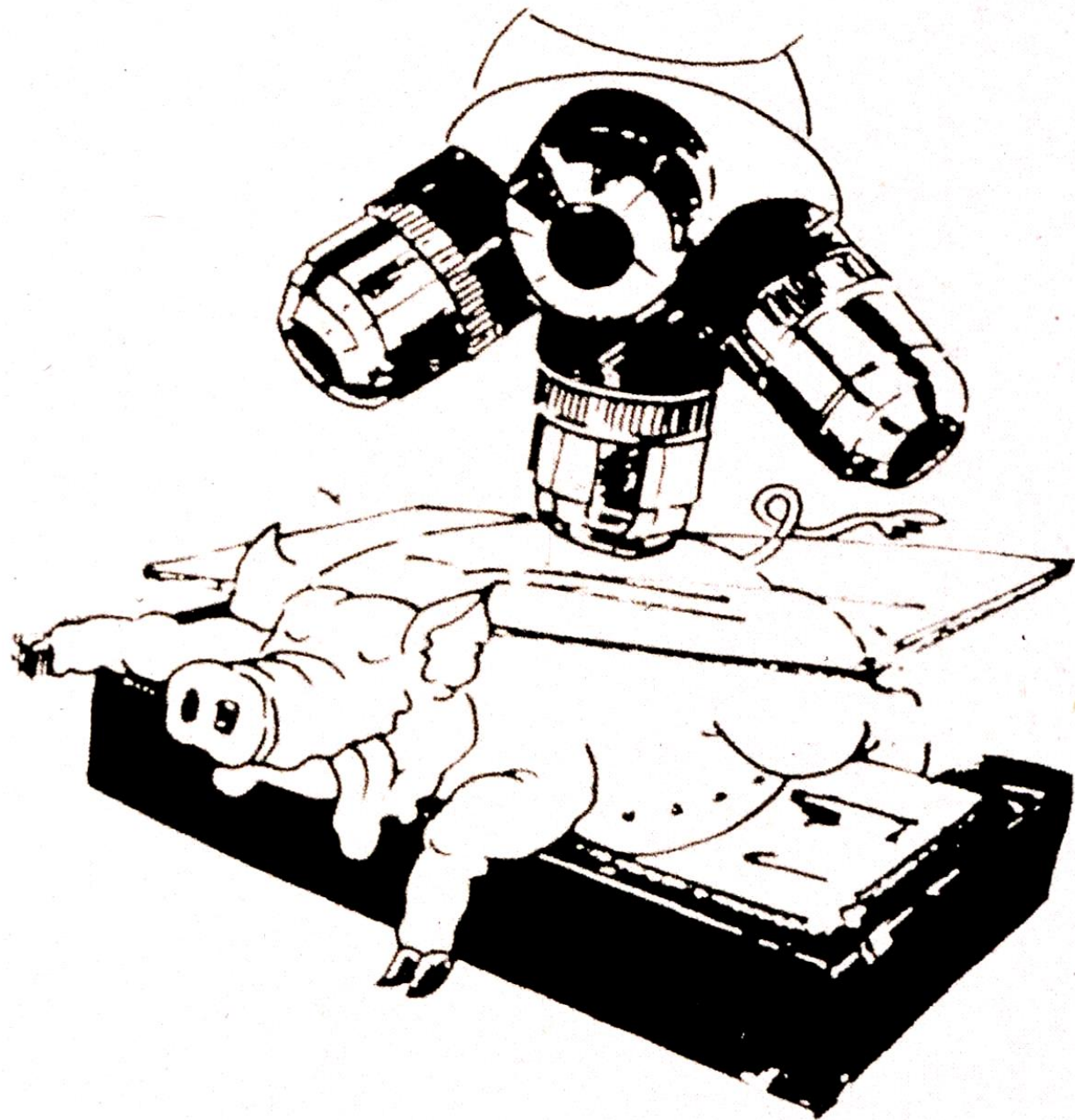
	DISTANCE BETWEEN RESOLVABLE POINTS
Human eye	0.2 mm
Bright-field microscope	0.2 μm
SEM	2.5 nm
TEM	
Theoretical	0.05 nm
Tissue section	1.0 nm

SIZE OF HUMAN BLOOD CELLS

CELL/PLATELET	SIZE
ERYTHROCYTES	6.5-8 μm
LEUKOCYTES (WBC)	
% of WBC	
NEUTROPHIL	12-15 μm
60-70%	
LYMPHOCYTE	6-18 μm
25%	
MONOCYTE	12-20 μm
5%	
EOSINOPHIL	12-15 μm
-4%	
BASOPHIL	12-15 μm
-1%	
PLATELETS	2-4 μm



The typical diameters/wall thicknesses of blood vessel for humans and mice are given in micrometers. The smallest capillaries have diameters of a very few micrometers and a wall thickness of about one micrometer (μm).



(図：カールツァイス社)

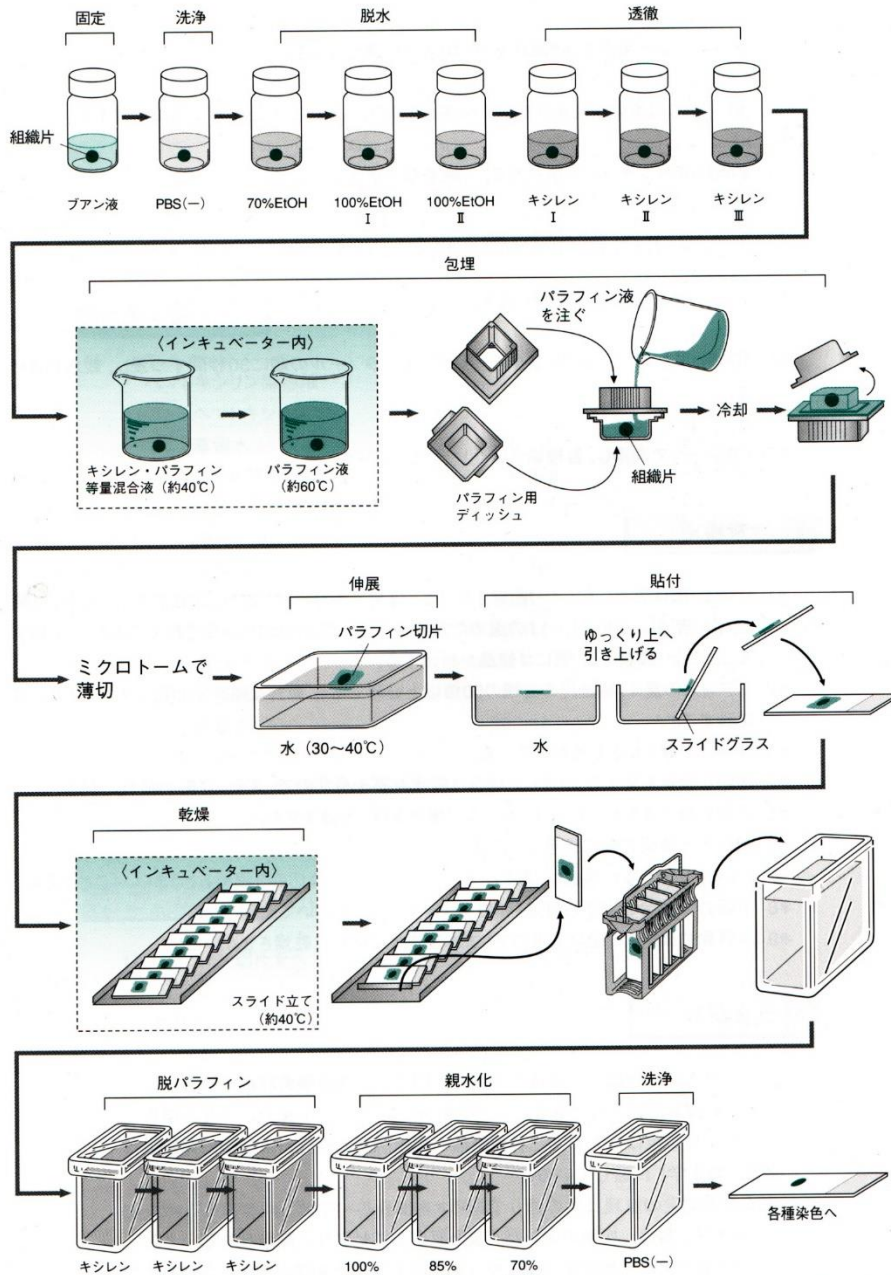


図2-8 パラフィン切片の作製

Cryostat sectioning



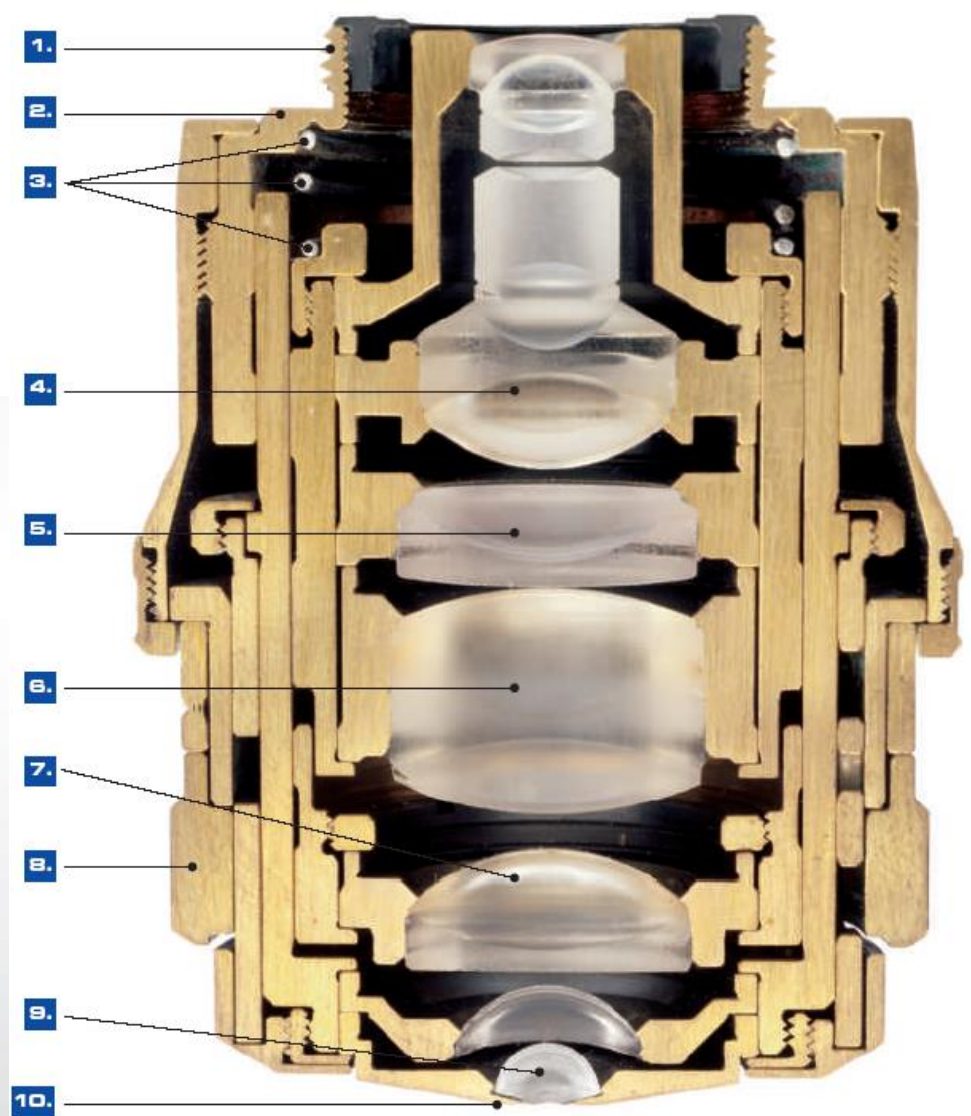
物鏡

- 開口值 /NA
- 消色差
- 工作距離
- 色帶
- 蓋玻片厚度



Lens:

Most important part of Microscope

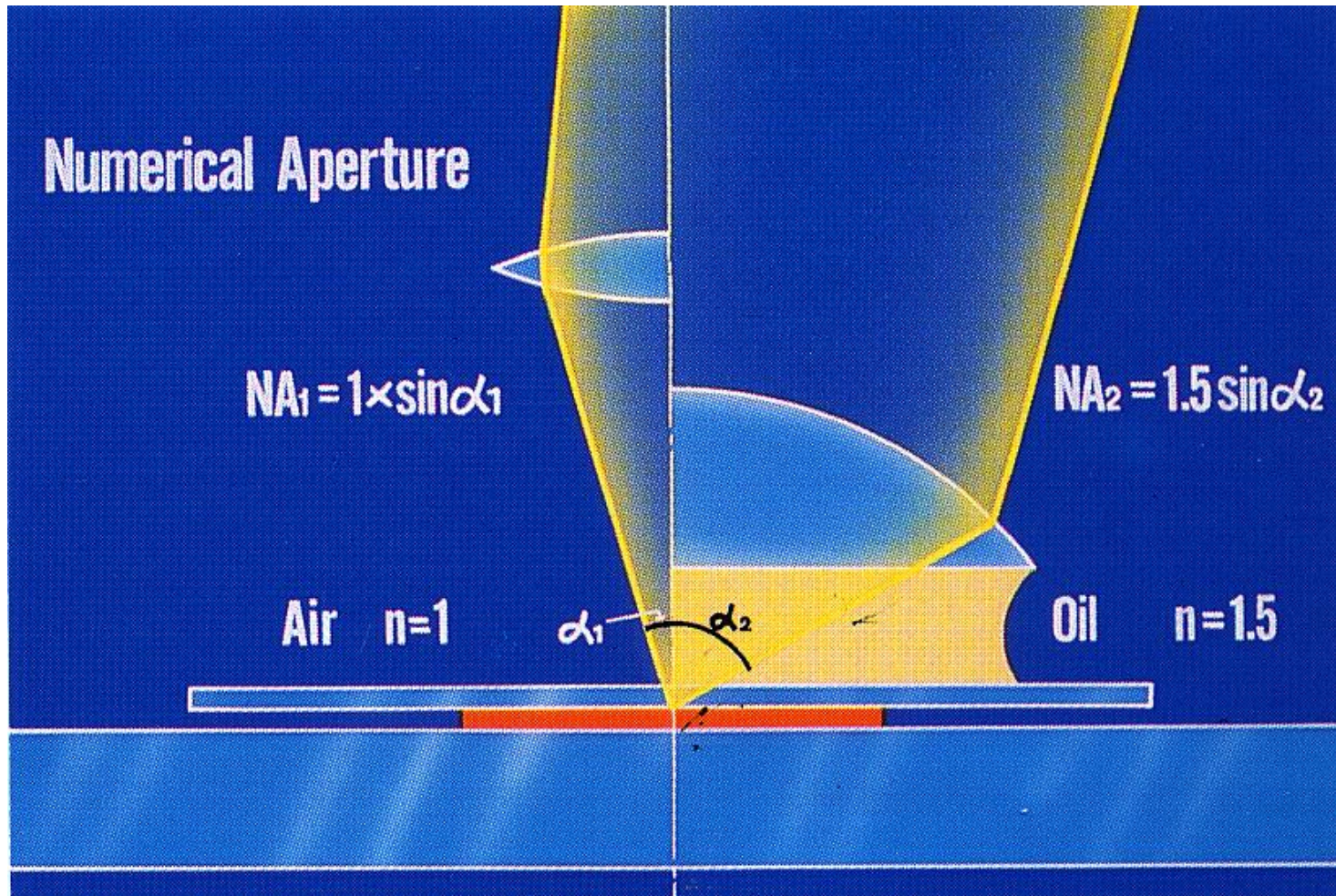


Cross section of an objective

1. Objective thread
2. Stop face of the objective
3. Spring system for the specimen-protection mechanism
- 4-7. Lens groups for the correction of image errors
8. Correction collar for adapting to deviating cover glass thicknesses or temperatures
9. Front lens system
10. Front lens holder

開口值 (NA)

- 開口值越大，鏡頭聚光能力越強，解析能力越強。



A

開口数

倍率



種類

対応するリングスリット
位相差観察用対物レンズにのみ表示がある

浸液

- Oil イマージョンオイル
- W 水
- Gly グリセリン

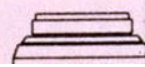
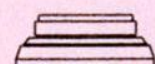
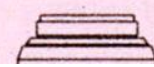
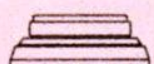
浸液表示色帯

イマージョン
オイル

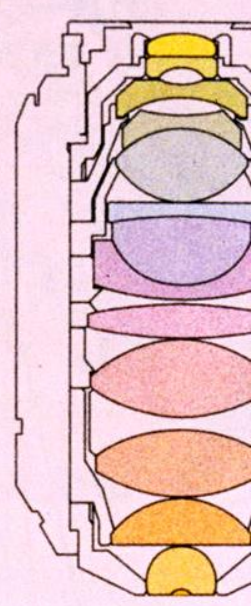
水

グリセリン

複数



B



- Correction efficiency (UV – VIS – IR)

- N. A.

- Type (What's CS Objectives ?) - Quality

PL-APO (Delta, HCS)

PL-Fluotar

N Plan

C Plan

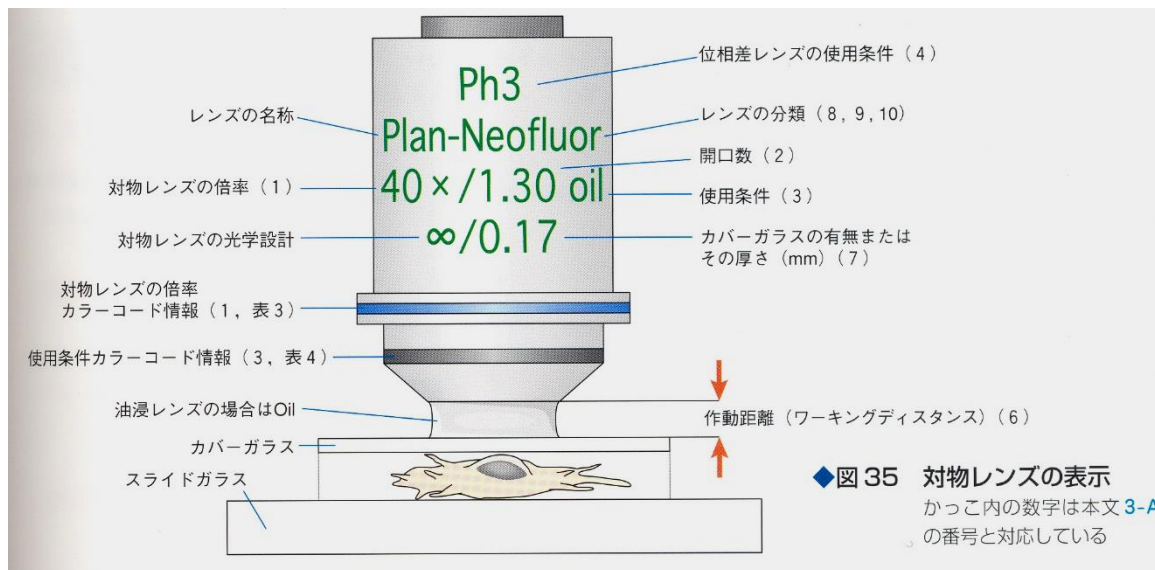
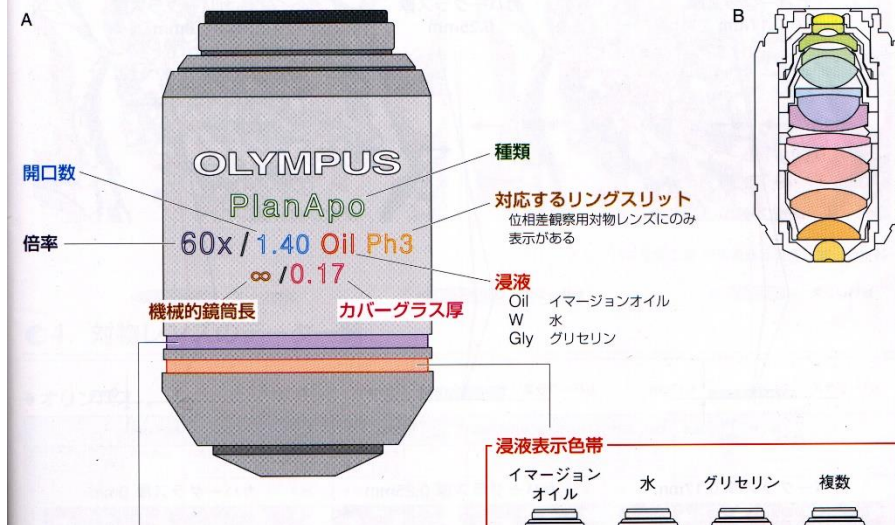
Air, Water, Oil (Imm)

Cover slip thickness correction ?

Chamber Type ?

2. 対物レンズの外と内

対物レンズの外には、種類、倍率、開口数、カバーガラス厚などが記されている。また、対物レンズの先端側には倍率と浸液を示す色帯(カラーリング)が付いており、外面の文字が見えなくてもカラーリングの色から倍率や浸液がわかるようになっている(図1A)。対物レンズの内部は、10枚前後のガラスが図1Bのように複雑に組み合わされている。



The Best Lens: Apochromat



Plan-APOCHROMAT: protects sensitive samples
Plan-APOCHROMAT objectives demonstrate top-class optical performance. They make it possible to see structures at the boundary of what is visible. Their outstanding performance features include: excellent correction, extremely high apertures and maximum resolution, color purity, contrast and flatness of field. All this combines to produce brilliant, needle-sharp images for observation, digital documentation and, in particular, fluorescence applications. The i Plan-APOCHROMAT of the 63x objective has been developed specifically for Live Cell Imaging – for optimal focus stability for time-lapse experiments.

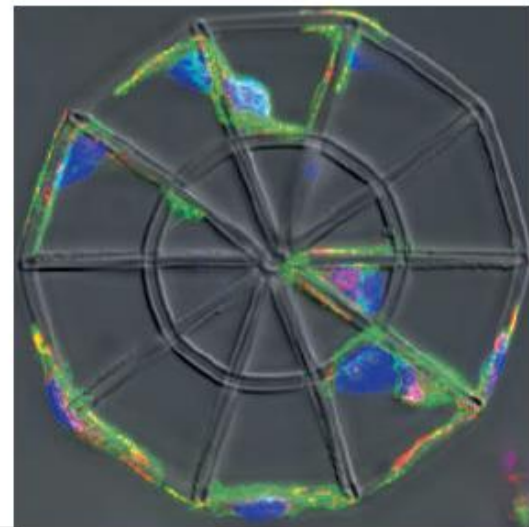


Image courtesy of Martin Bastmeyer und Franziska Klein, University of Karlsruhe, Germany

Resolution table using green light with
 $\lambda = 0.550 \mu\text{m}$:

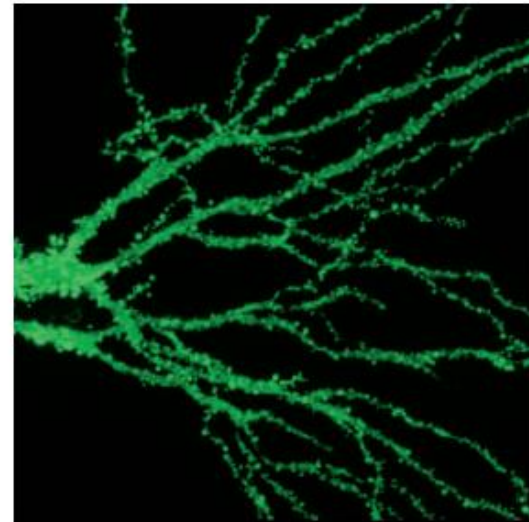
Magnification	/	NA	Resolution (μm)
10x	/	0.30	1.10
40x	/	0.75	0.45
63x	/	1.40 Oil	0.24
100x	/	1.30 Oil	0.26

- Field of view: 25 mm
- Flatness: ★★★★★
- Color correction: ★★★★★

Objectives with optimum correction of flatness of field and color; suitable for Digital Imaging



W Plan-APOCHROMAT: apochromatically correct
The immersion variant of the Plan-APOCHROMAT series – an addition to the water objectives of the ACHROPLAN class – has been specifically designed for electrophysiology. W Plan-APOCHROMAT objectives have apochromatic correction from visible light to the near infrared (VIS - IR) and are intended for use without a cover glass. Typical transmission values are greater than 80% from 450 nm to 1,000 nm and greater than 50% at 365 nm. These are also ideal prerequisites for use in 2-photon microscopy. The front of this slender objective is made of a special inert plastic that was originally developed for food technology.

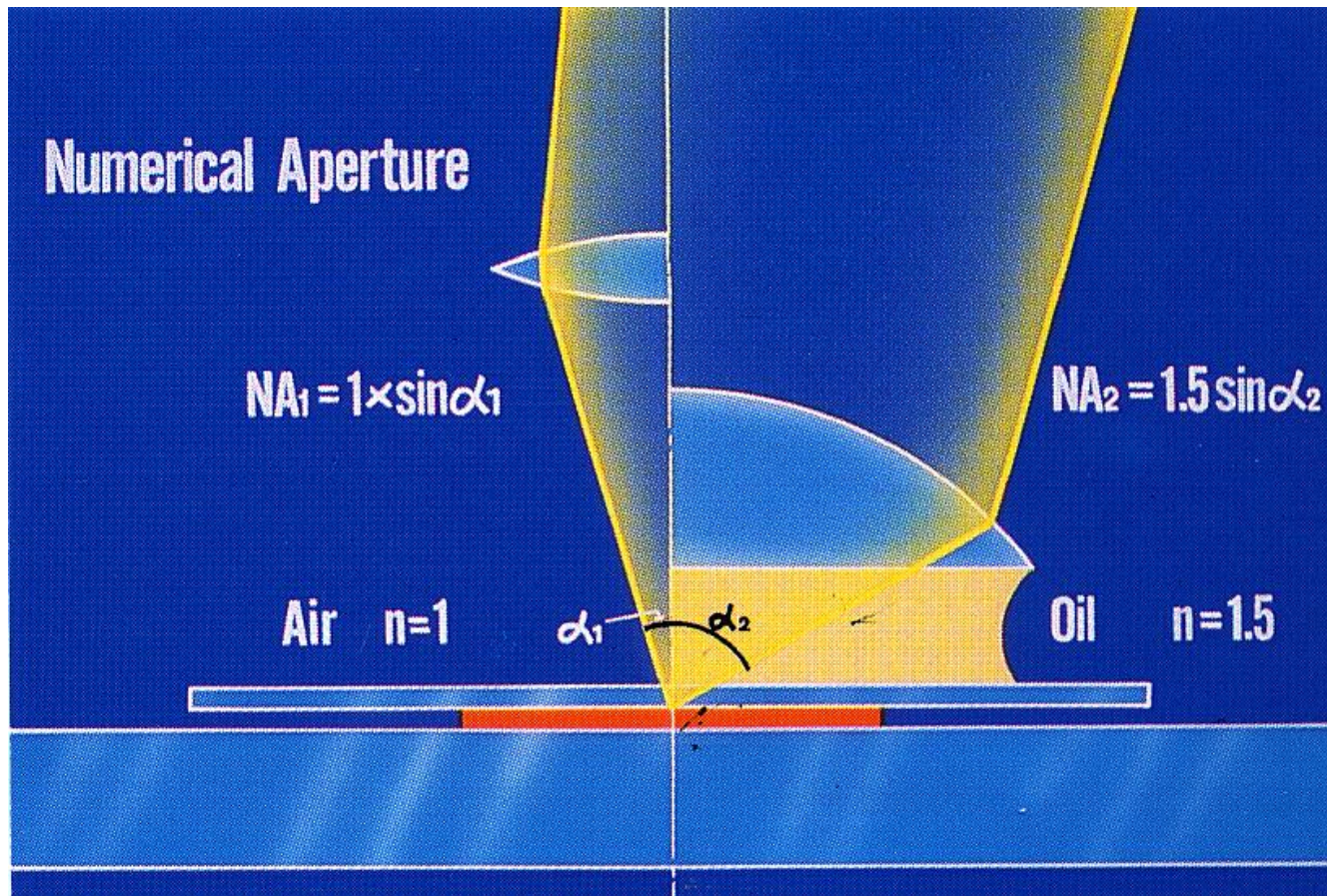


- Field of view: 20 mm
- Flatness: ★★★★★
- Color correction: ★★★★★

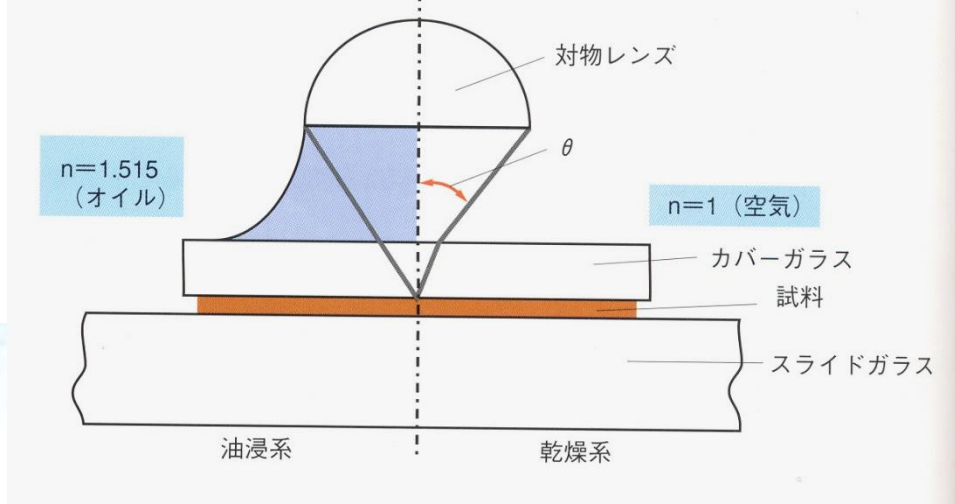
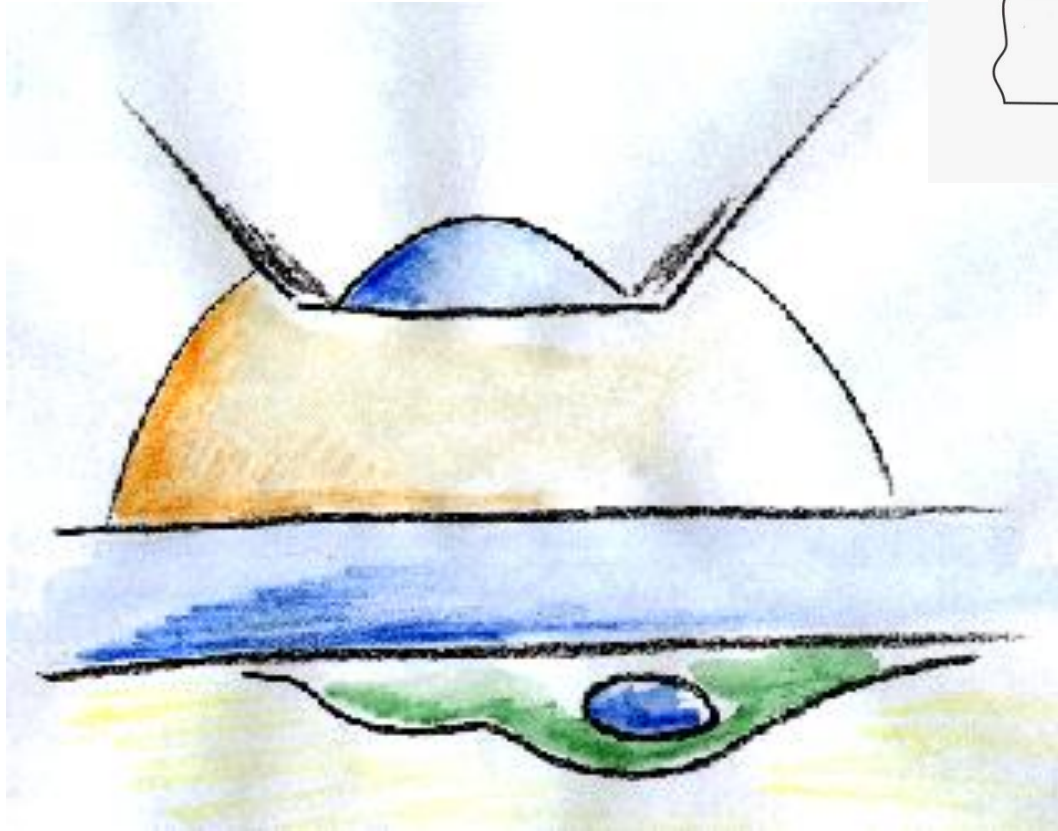
Apochromatically corrected immersion objectives for applications in physiology

介質的種類

- 乾式物鏡
- 油鏡
- 水鏡



- **Observation and optical setup**



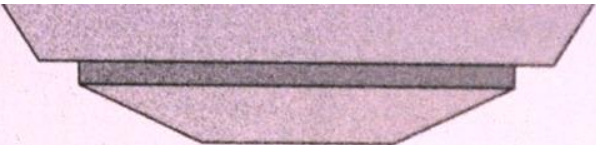
Micro objective

Immersion

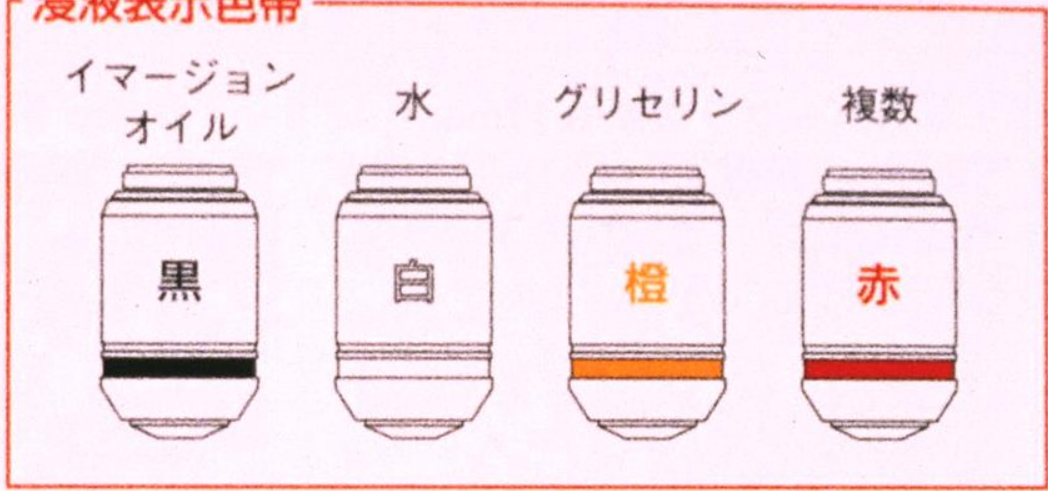
Cover slip

Sample

The last 3 optical elements are usually added by the user !



浸液表示色帯



示色帯

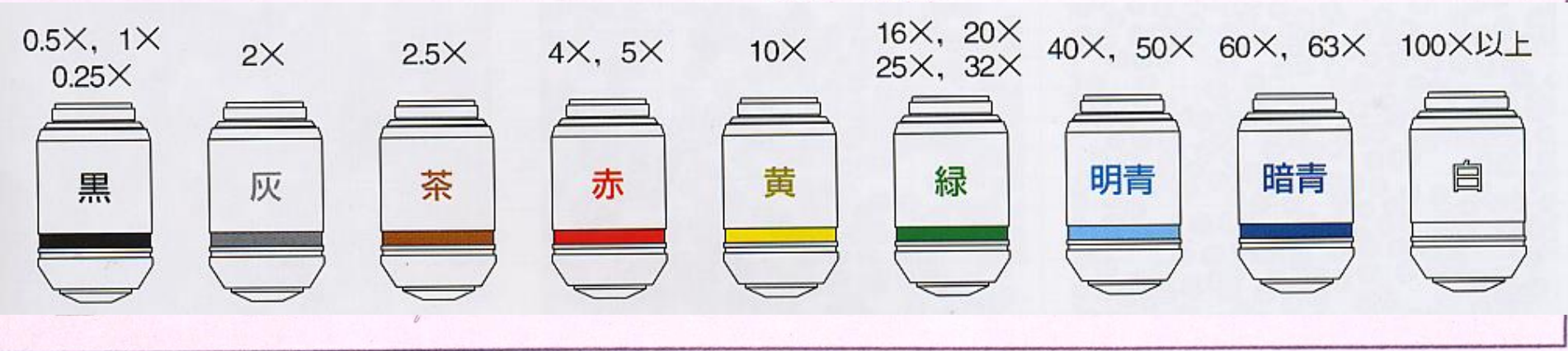
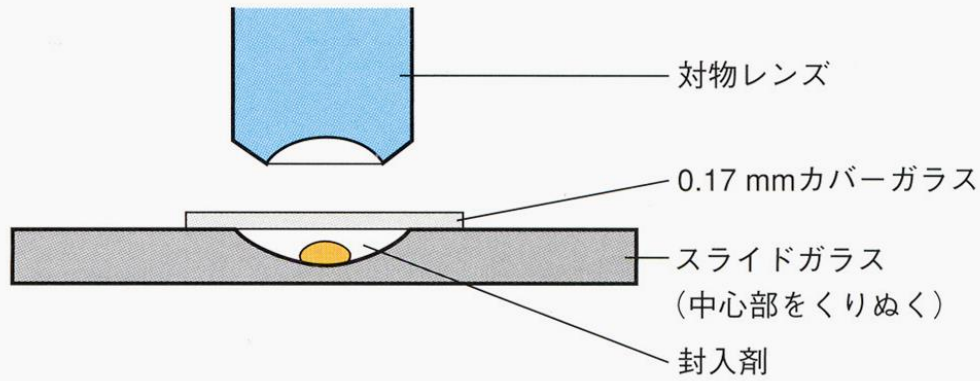


図3 対物レンズの外と内

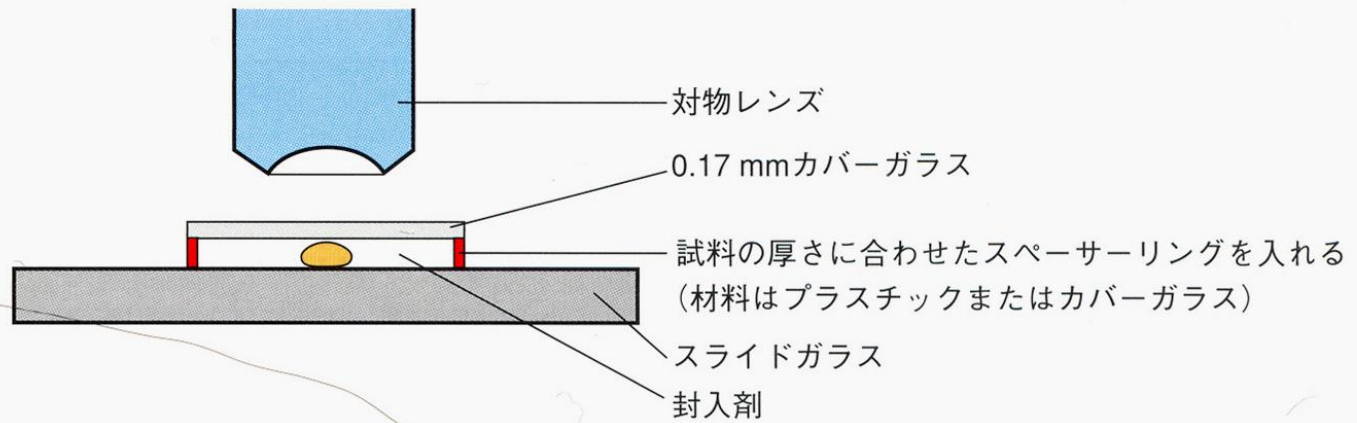
A: 60×のプランアポクロマート油浸対物レンズの外表面。外表面に記されている文字は、明視野観察用では黒、位相差観察用では緑、偏光観察用では赤に色分けされている。また、機械的鏡筒長は対物レンズの

1) 正立型顕微鏡の場合

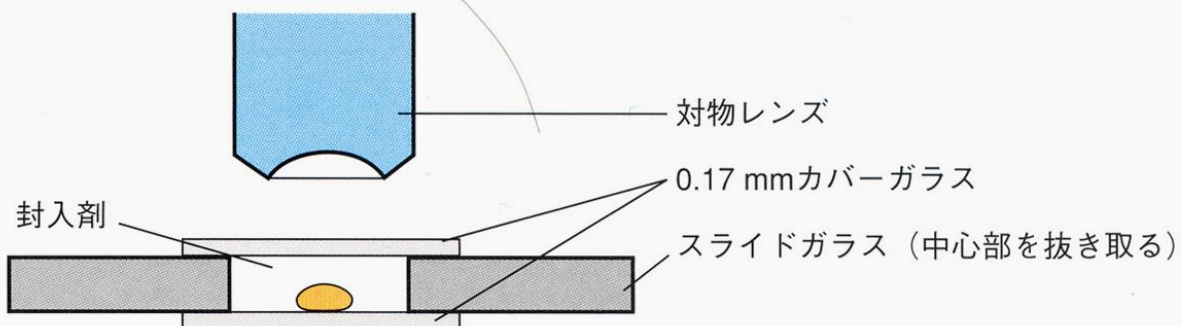
a)



b)

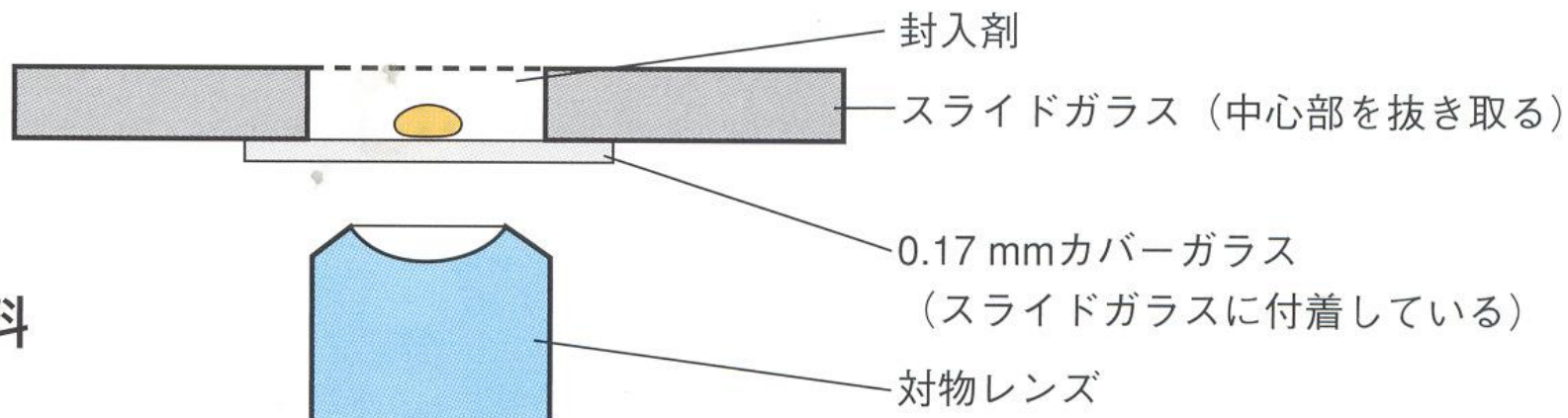


c)

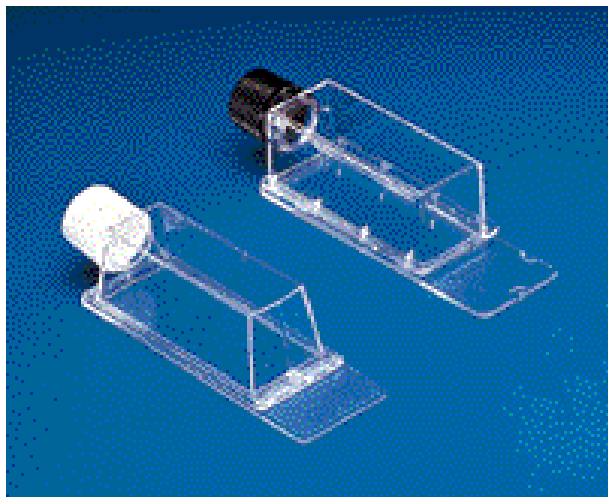


2) 倒立型顕微鏡の場合

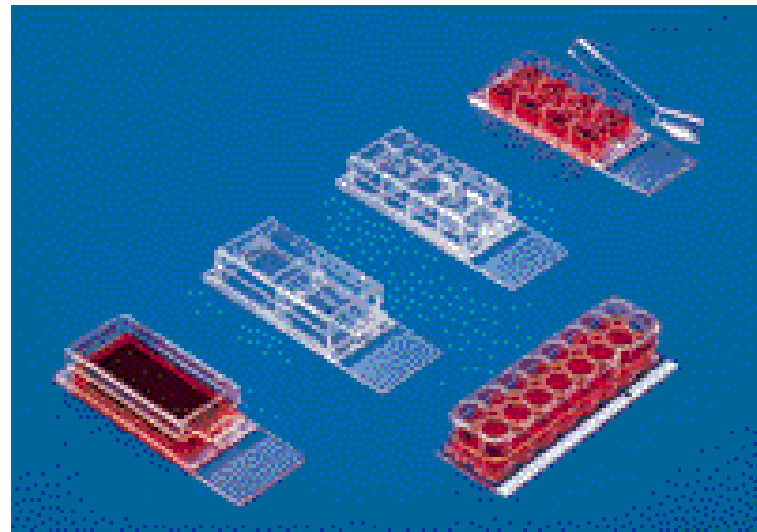
ための試料



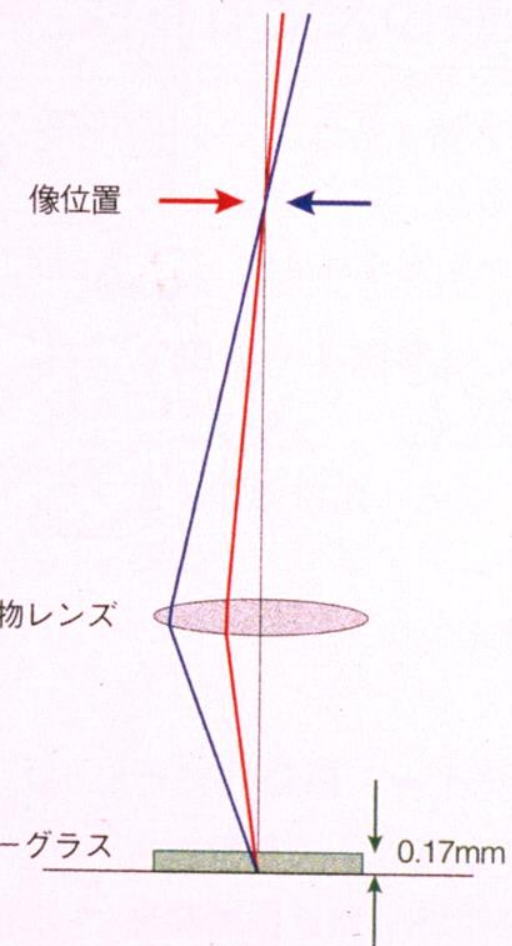
NUNC FLASKETTE® CHAMBER SLIDE/FLASKS



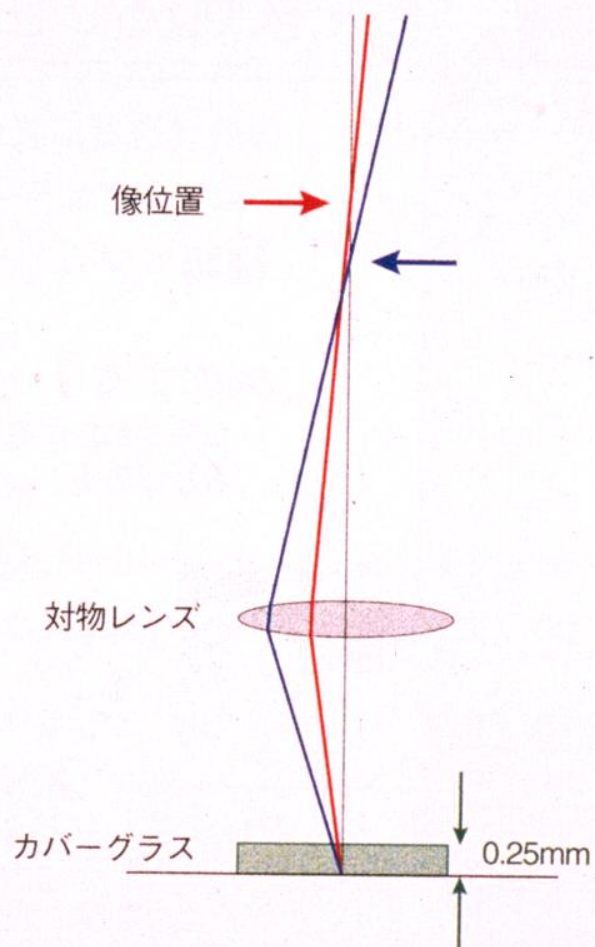
LAB-TEK® II CHAMBERED COVERGLASS



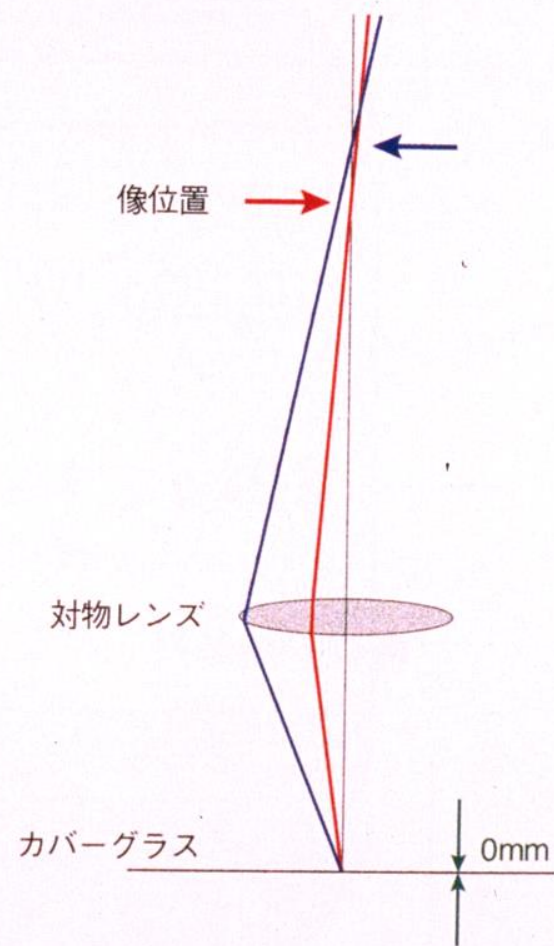
カバーガラス厚
0.17mm



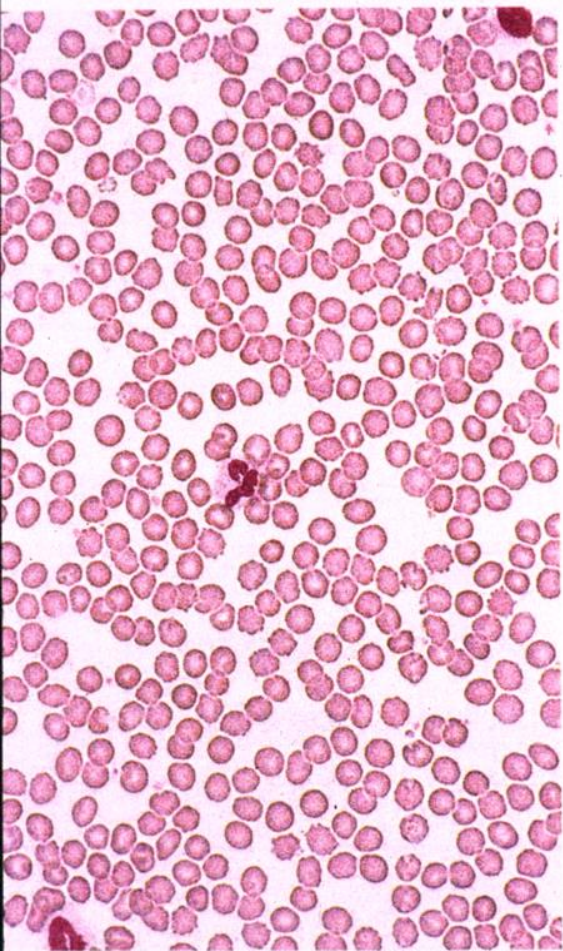
カバーガラス厚
0.25mm



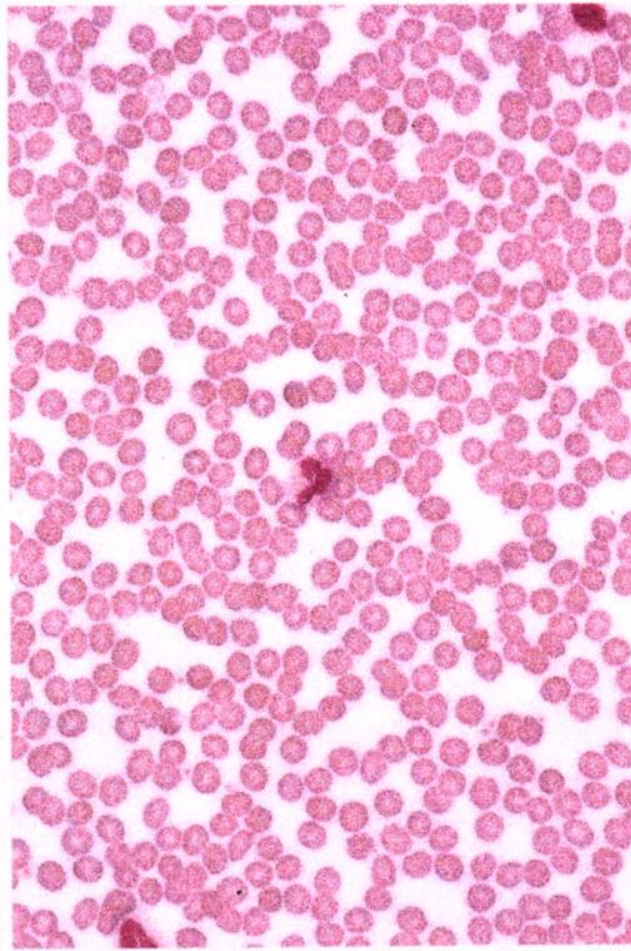
カバーガラス厚
0mm



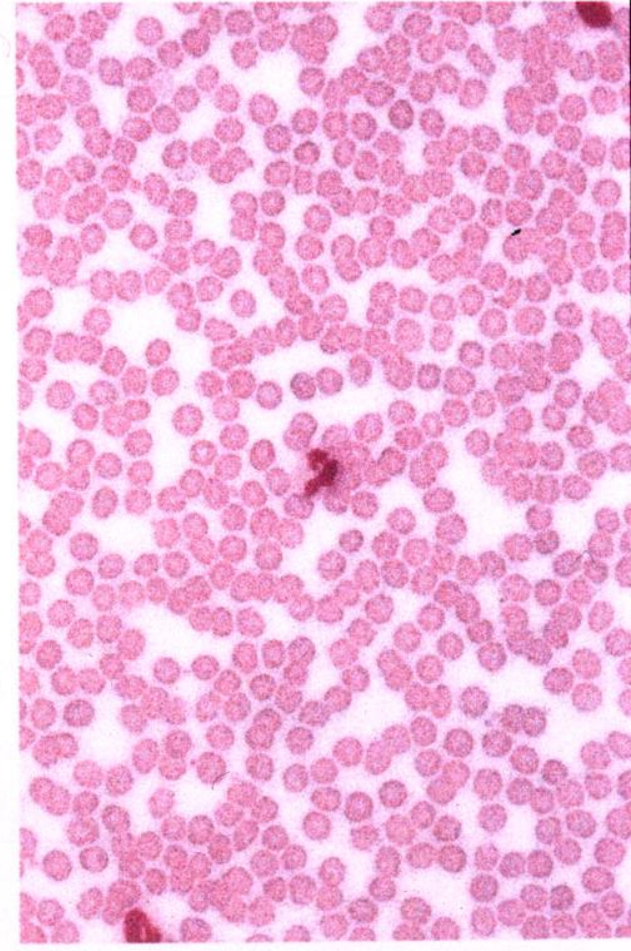
カバーガラス厚 0.17mm



カバーガラス厚 0.25mm



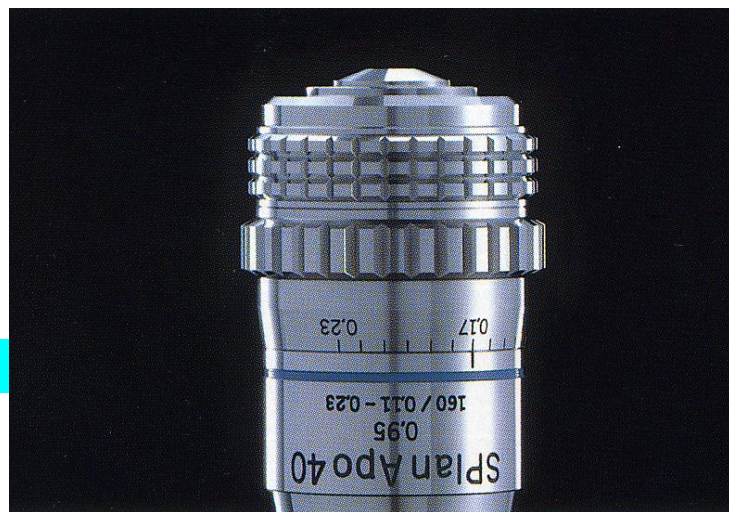
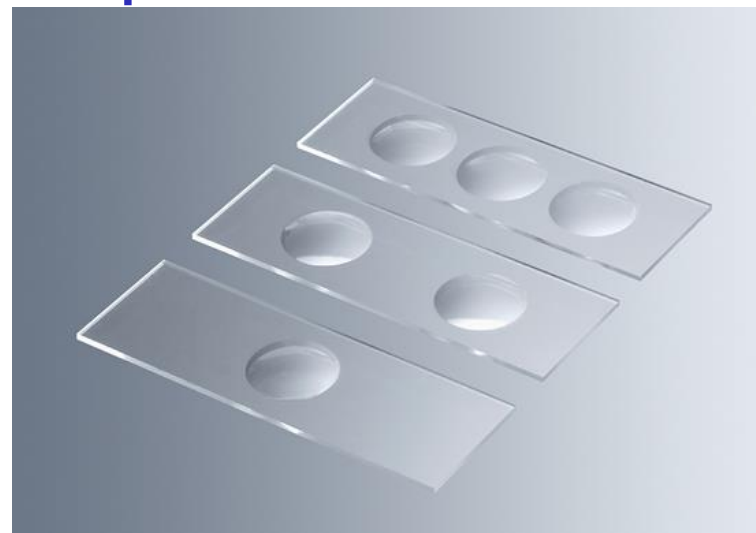
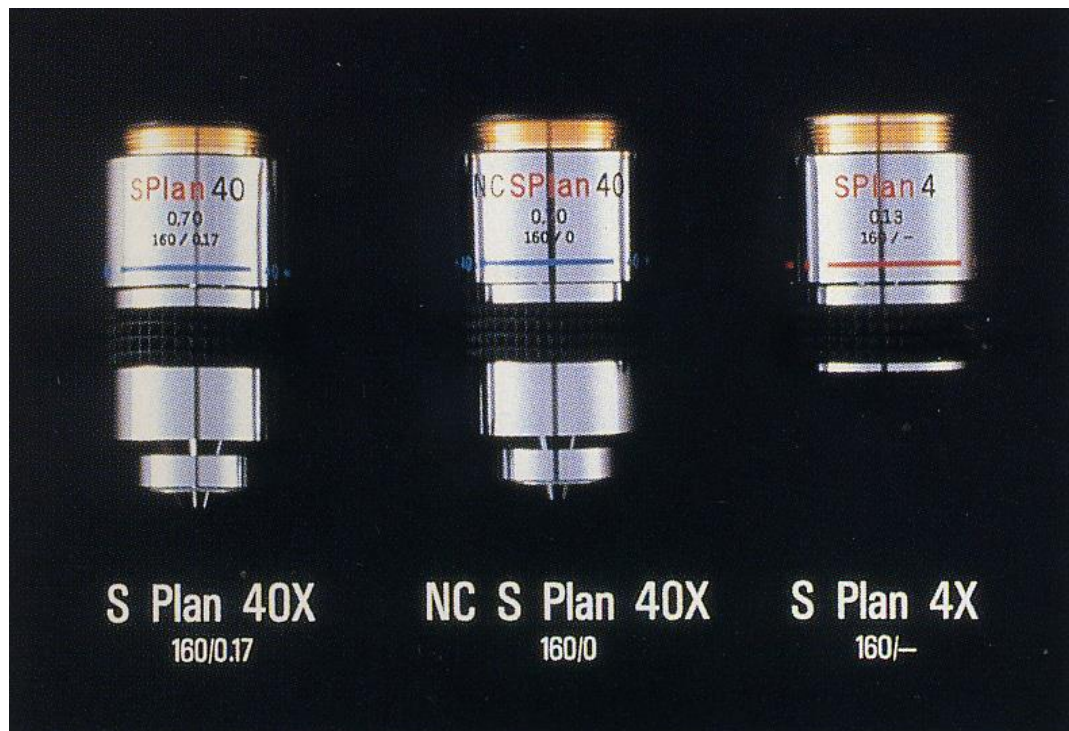
カバーガラス厚 0mm



可修正蓋玻片的物鏡

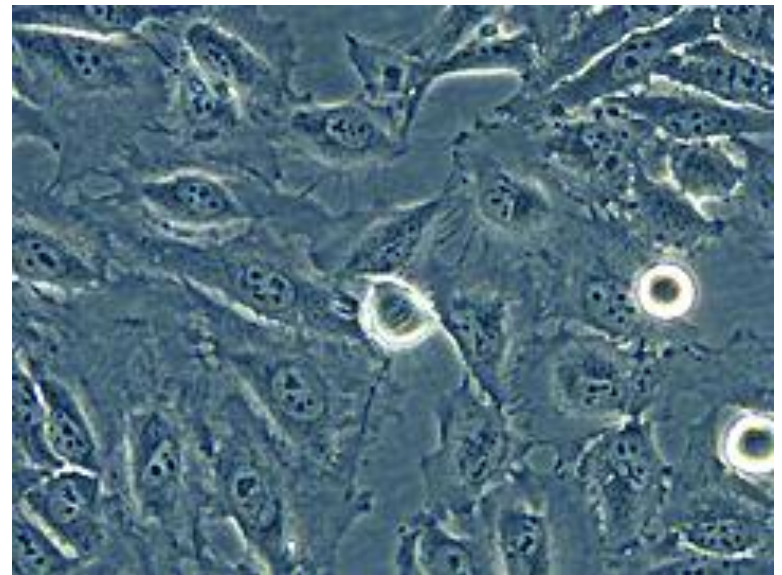
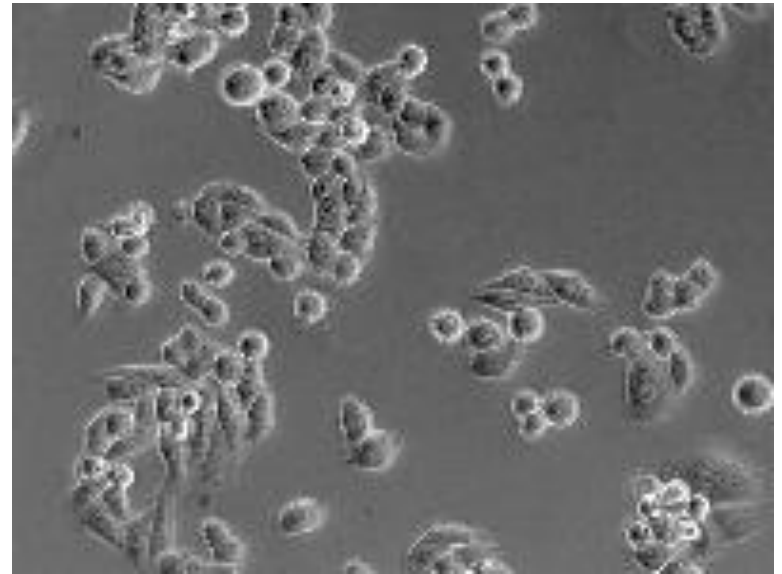
- 高開口值物鏡需要可調整蓋玻片厚度，以取得最佳影像 · (0.11-0.23mm)

Depression slide



使用0.17mm 蓋玻片 不使用蓋玻片 蓋玻片厚度無關

位相差顯微鏡 (Phase Contrast)



位相差的配件

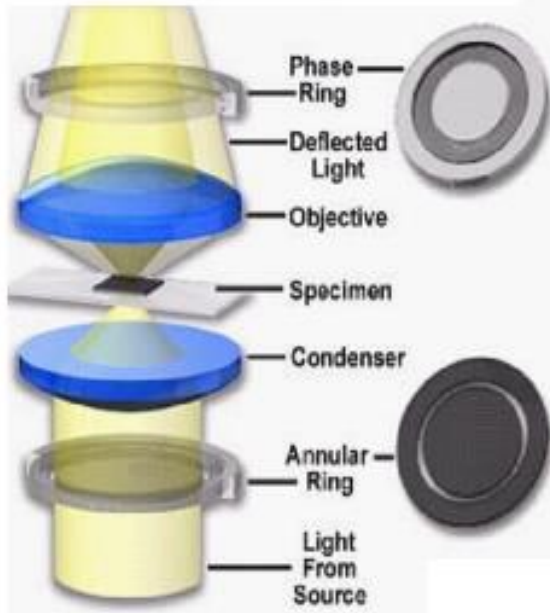
- 位相差專用物鏡
- 位相差環
- 萬用聚光鏡
- 調整用目鏡



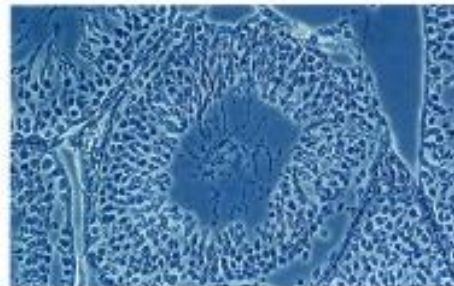
Light rings PH0, PH1, PH2



Phase contrast 光路 / 光學組件



相位差光學組件 (Phase contrast)



Phase contrast

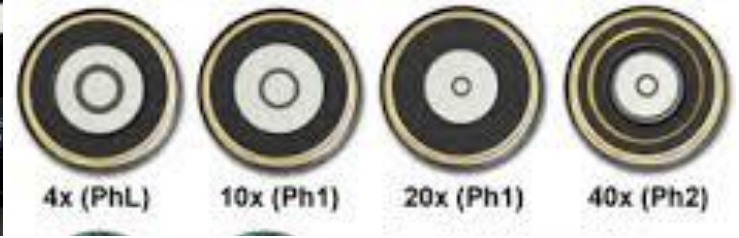


DIC

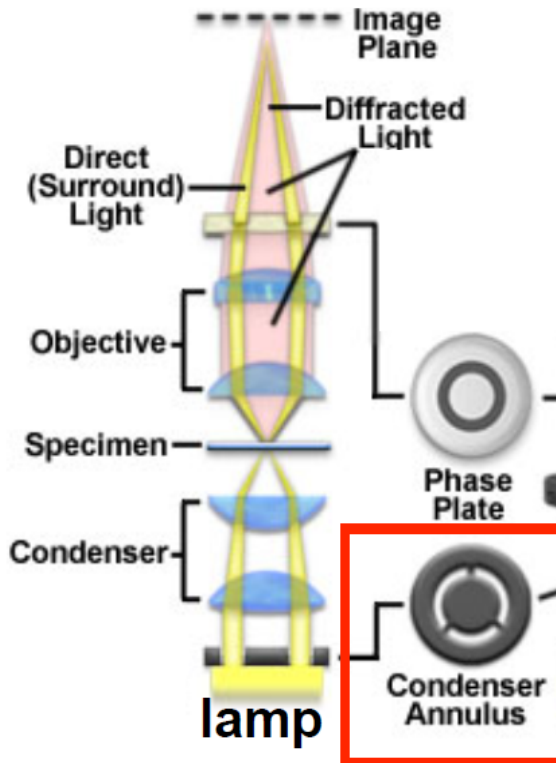
位相差的原理



Objective Apertures and Phase Contrast Optics



Phase Contrast Microscope Configuration

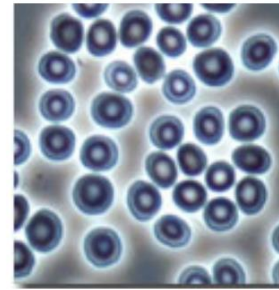
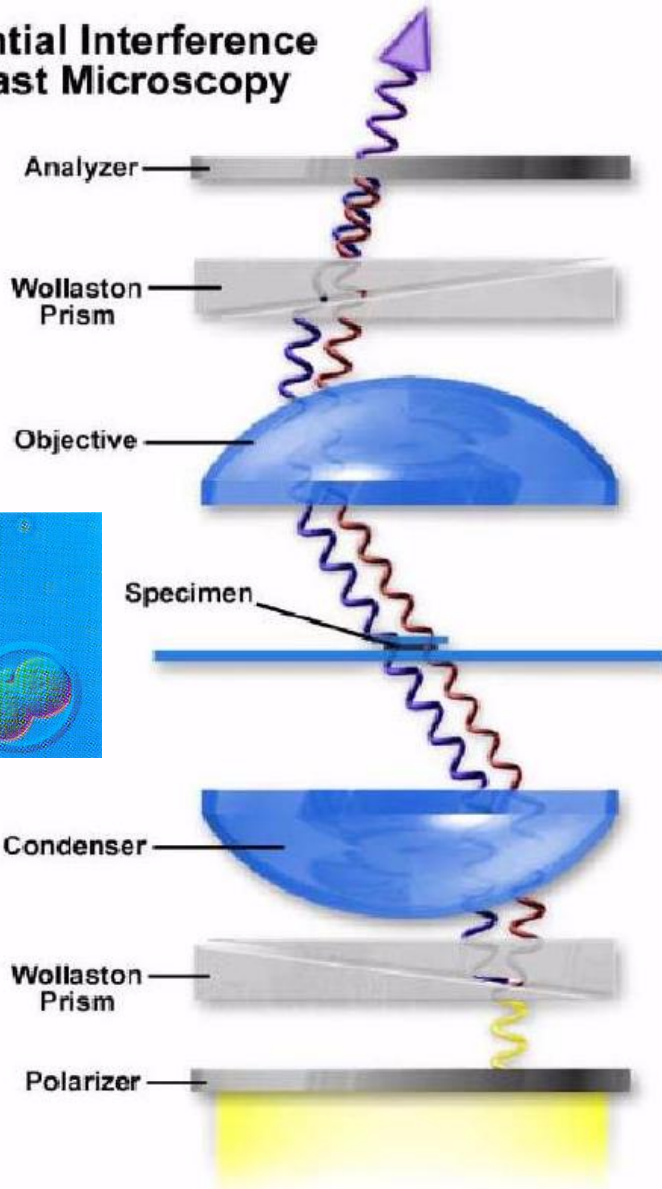


Restricts angles of illumination so diffracted and undiffracted light can be selectively modulated at phase plate

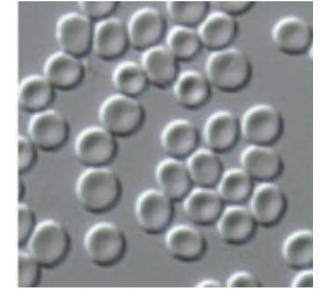
微分干涉顯微鏡

DIC: an alternative technique for enhancing contrast

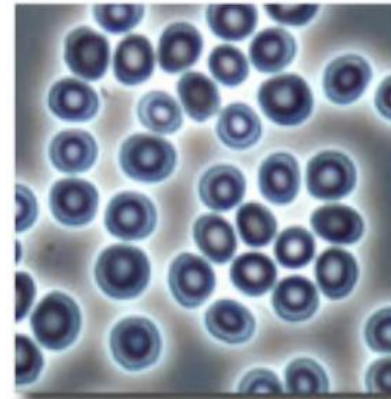
Differential Interference Contrast Microscopy



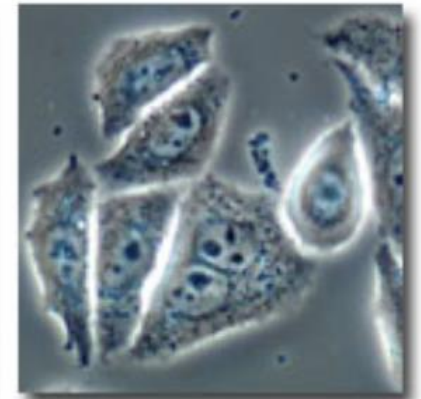
Phase



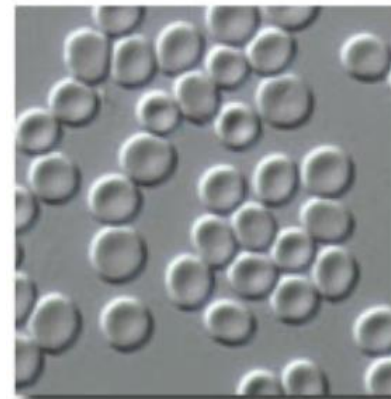
DIC (Differential Interference Contrast)



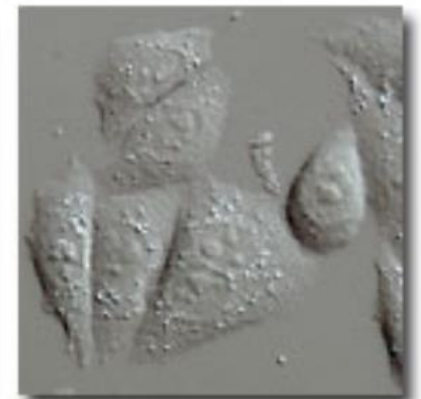
(a)



(c)



(b)



(d)

微分干涉顯微鏡的配件

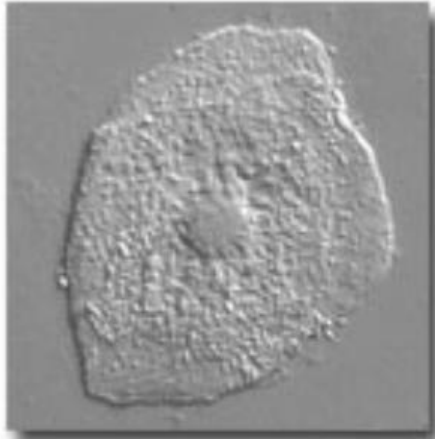
- 萬物聚光鏡
- 微分干涉鍍鏡
- 起偏鏡
- 檢偏鏡



微分干涉 位相差 的比較

	位相差	微分干涉
對比方式	以標本厚度轉換成對比	以厚度變化轉換成對比
對比的調整	無法調整	以鍍鏡調整
影像的特性	以明暗度表現	以立體感及明暗表現
解析度	比微分干涉差	高
合適的標本	細微構造 標本厚度 $10\mu\text{m}$	細微構造，外觀。 標本厚度數百 μm
容器	可使用塑膠類	不可使用塑膠類

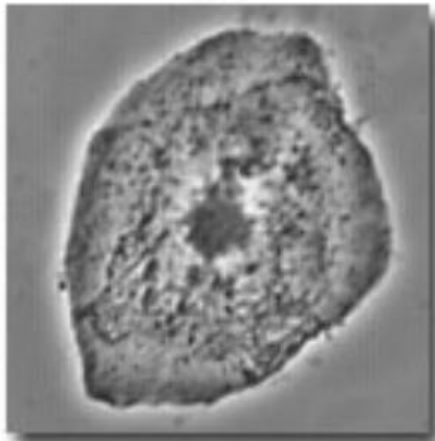
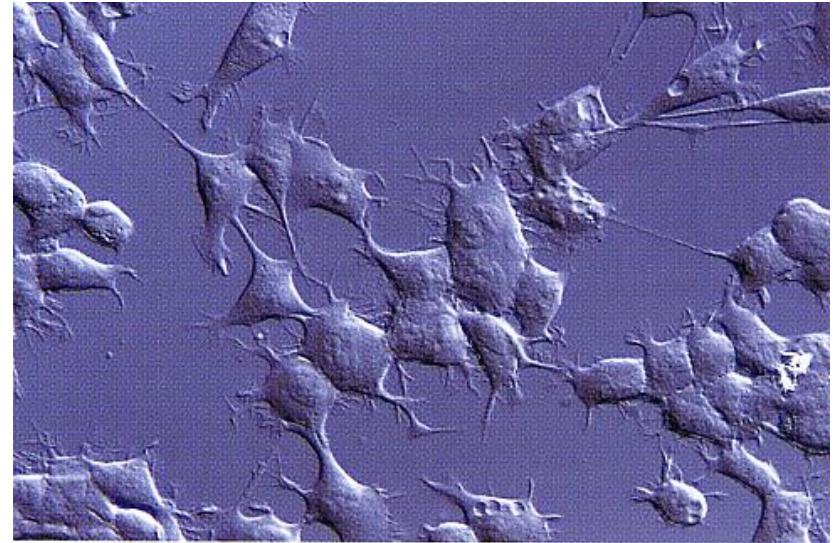
微分干涉及位相差的實例



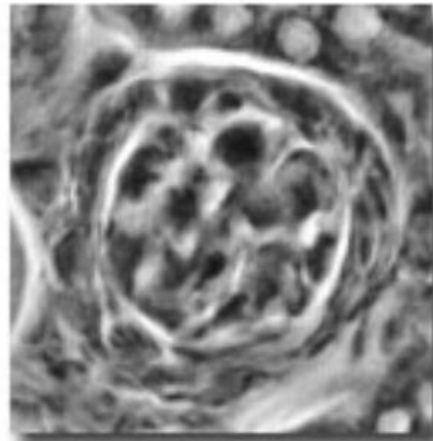
(a)



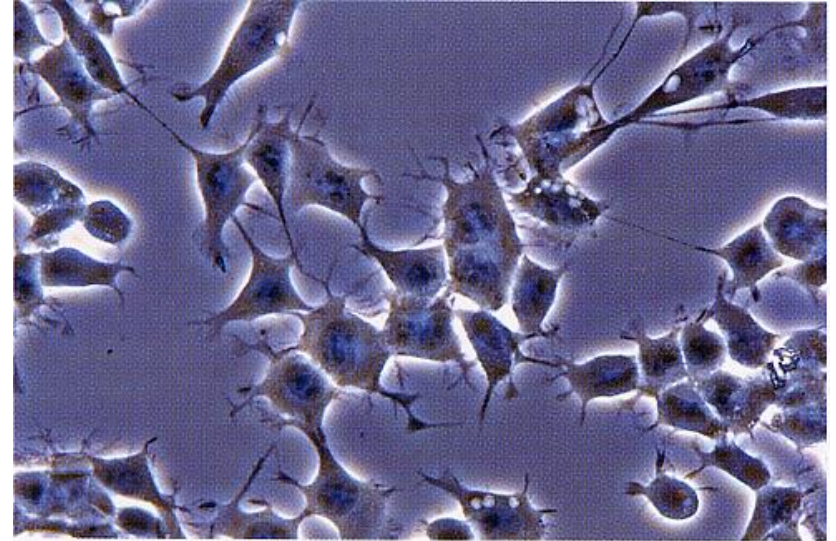
(c)



(b)

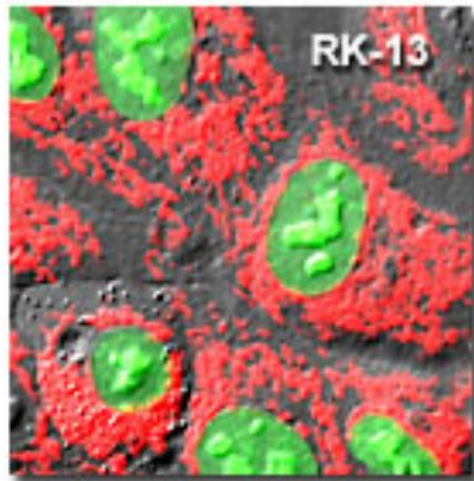


(d)

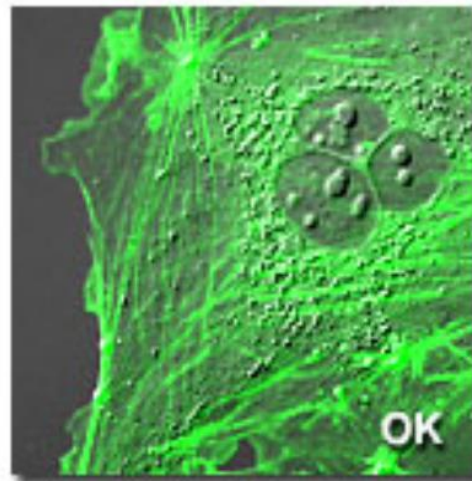


微分干涉及位相差的實例

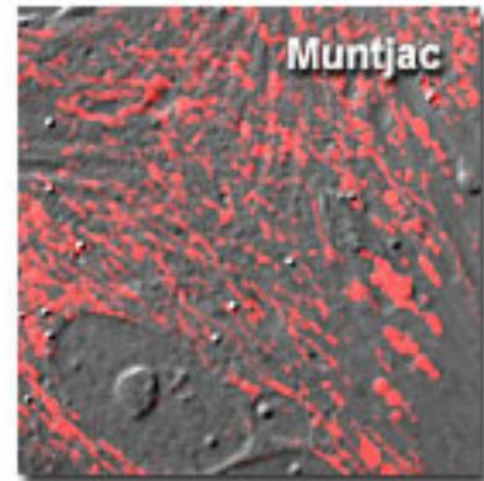
Live-Cell Imaging with Fluorescent Proteins and DIC



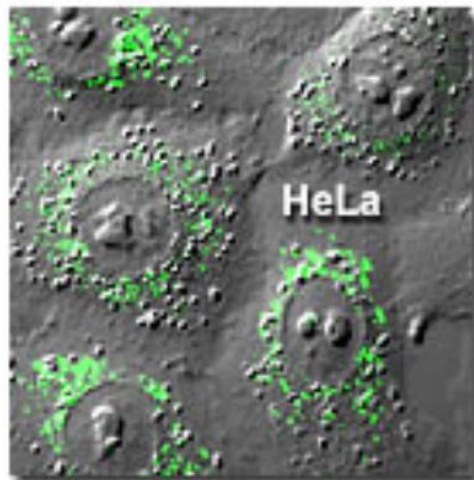
(a)



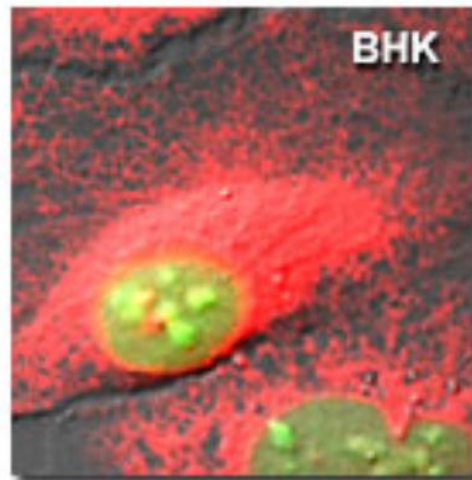
(b)



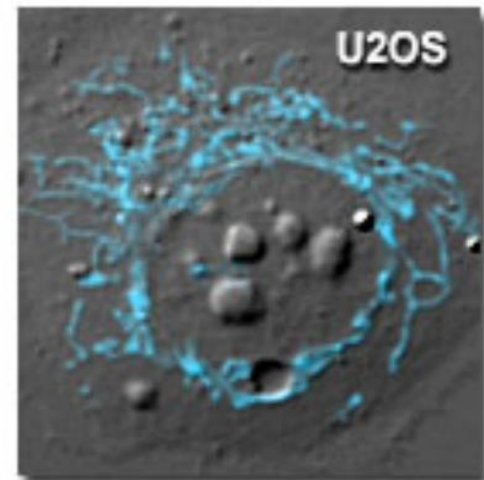
(c)



(d)

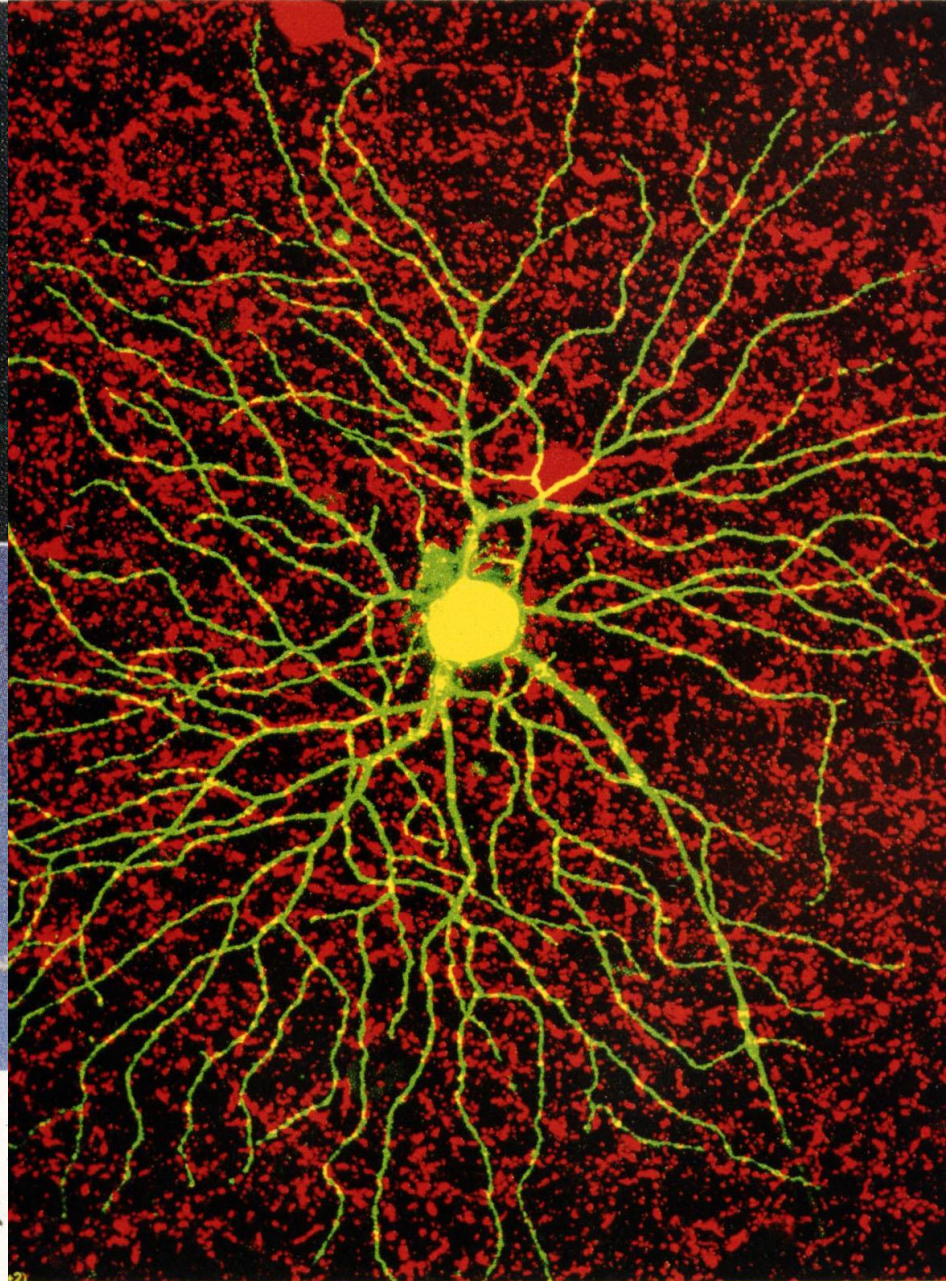
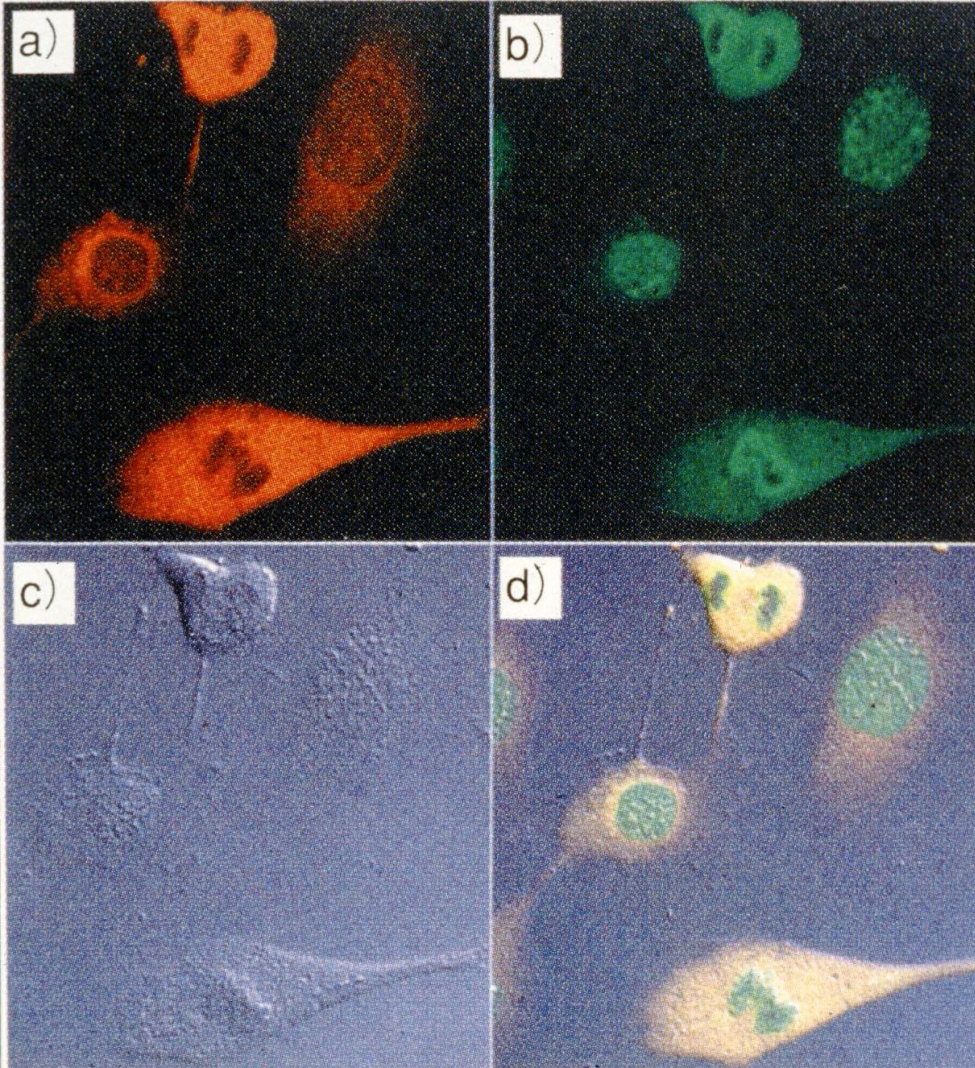


(e)



(f)

Figure 1



12 FITC, Rhodamine, 微分干渉および合成像
 a) F-アクチン/Rhodamine 染色像, b) クリスタリン/FITC 染色像, c) 微分干渉像, d) 合成像.
 ラット由来の筋芽細胞 (L-6) の観察像.
 東京大学大学院総合文化研究科広域科学専攻生
 環境科学系跡見順子先生より提供

細胞とドーパミン作動性アマクリン細胞のレーザー顕微鏡エクスタ
 像. Lucifer Yellowで網膜神経節細胞, Texas Redでドーパミン
 を染色.

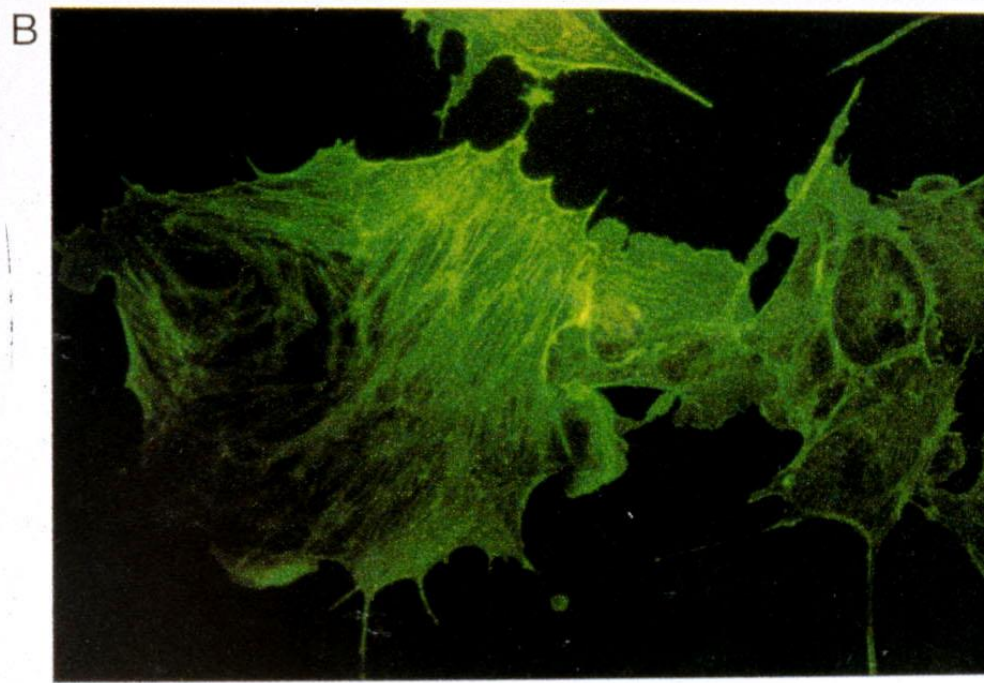
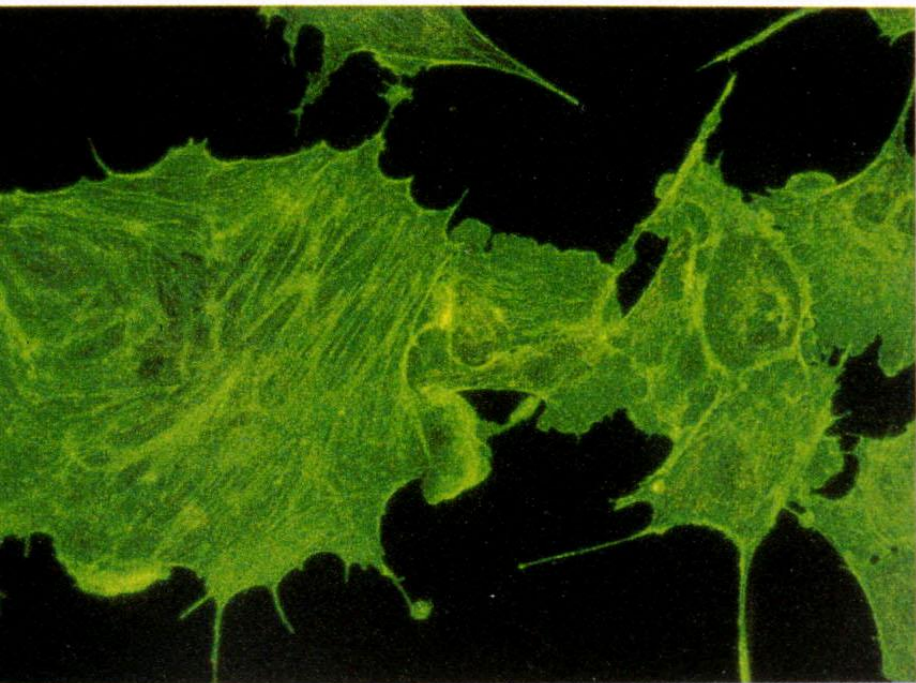


図7 水銀ランプの心出しの効果

水銀ランプの心出しを行った蛍光像 (A) と行っていない蛍光像 (B) . A : きちんと心出しが行われている場合は、視野全体が均一に照明されるため、蛍光像も均一な明るさになる。B : 心出しが不十分な場合は、視野内が均一に照明されないため、蛍光像の中に明るい部分と暗い部分が生じてしまう。

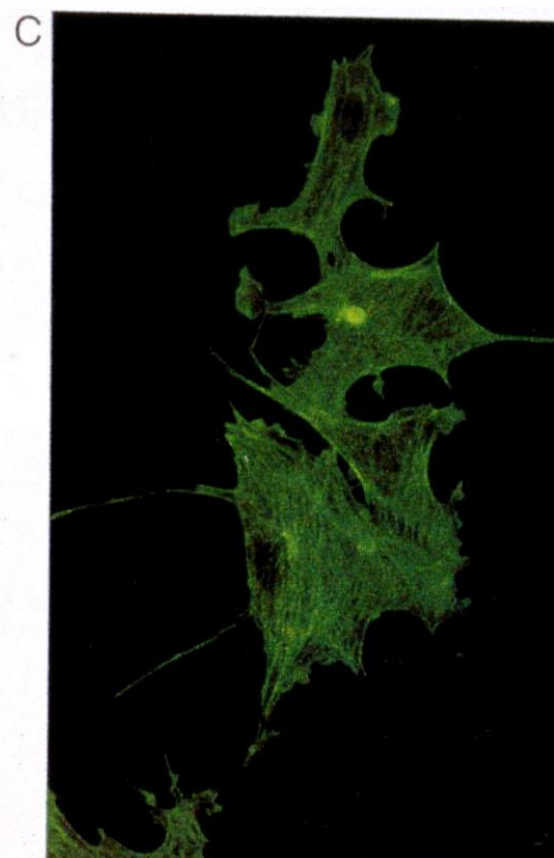
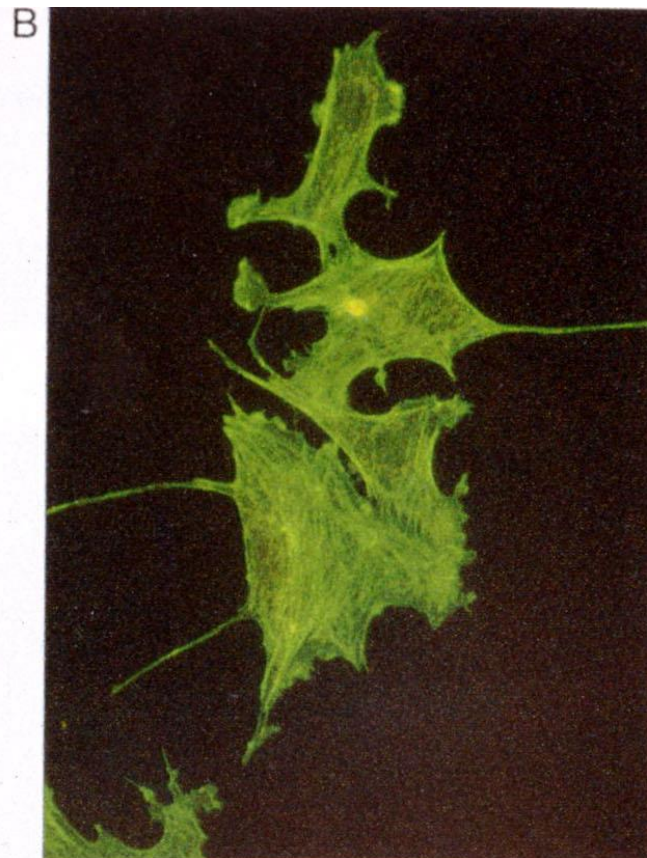
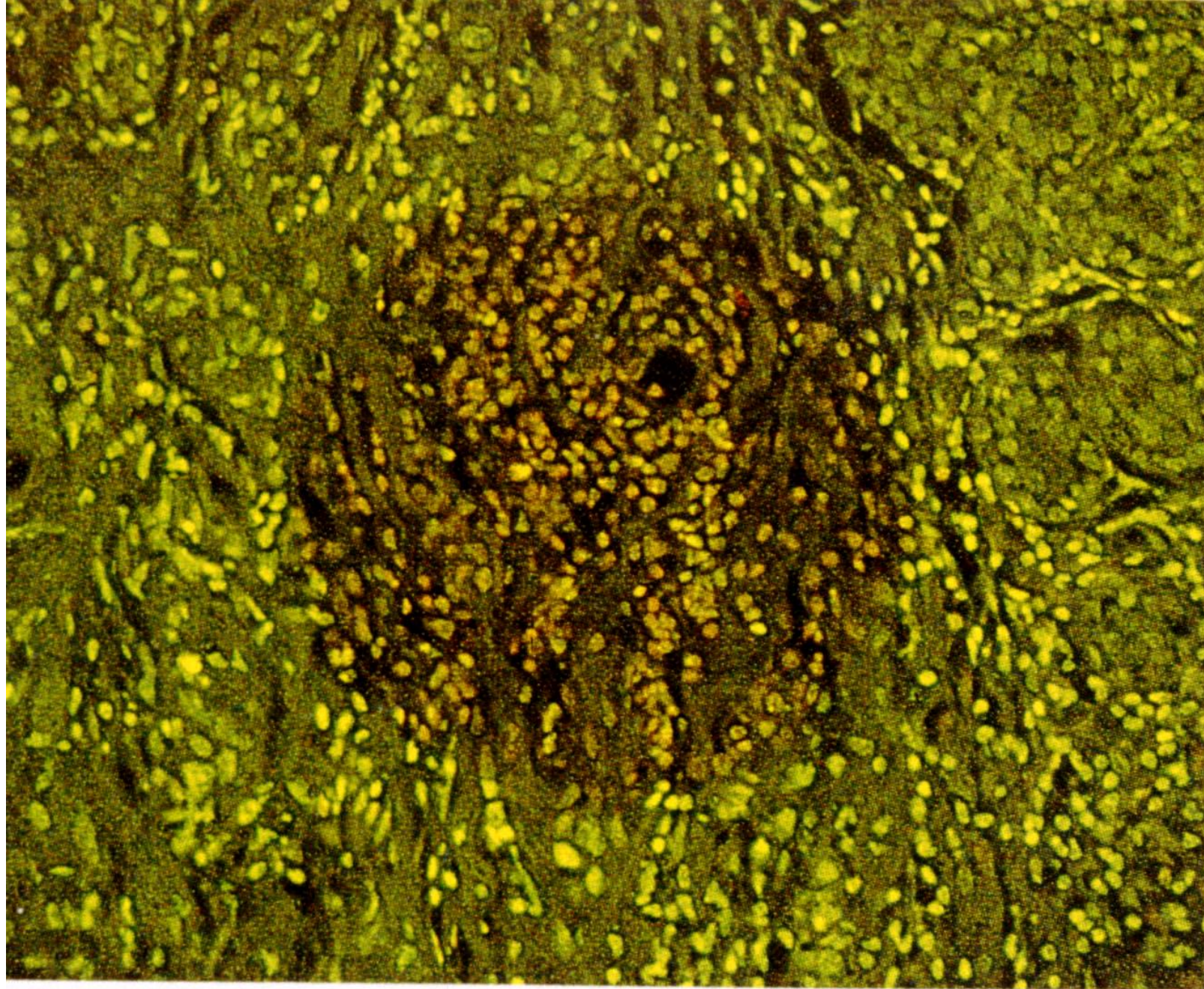


図9 励起フィルターの波長域の効果

A：蛍光キューブにU-MSWB2を使用したときのFITC蛍光像，励起フィルターの波長域が420～480nmと広いため，蛍光像は最も明るい，同時にバックグラウンドも高くなっている．B：蛍光キューブにU-MWIB2を使用したときのFITC蛍光像，励起フィルターの波長域が460～490nmと中程度なので，蛍光像はほどほどに明るく，バックグラウンドもほどほどに出ている．C：蛍光キューブにU-MNIB2を使用したときのFITC蛍光像，励起フィルターの波長域が470～490nmと狭いため，蛍光像は最も暗いが，同時にバックグラウンドも低くなっている．これらのバックグラウンドは，主に対物レンズと培地の自家蛍光によるものである．



褪色してしまった例

常見的抗退劑

- P-phenylenediamine
- DABCO
- N-propylgallate
- 2-mercaptoethylamine

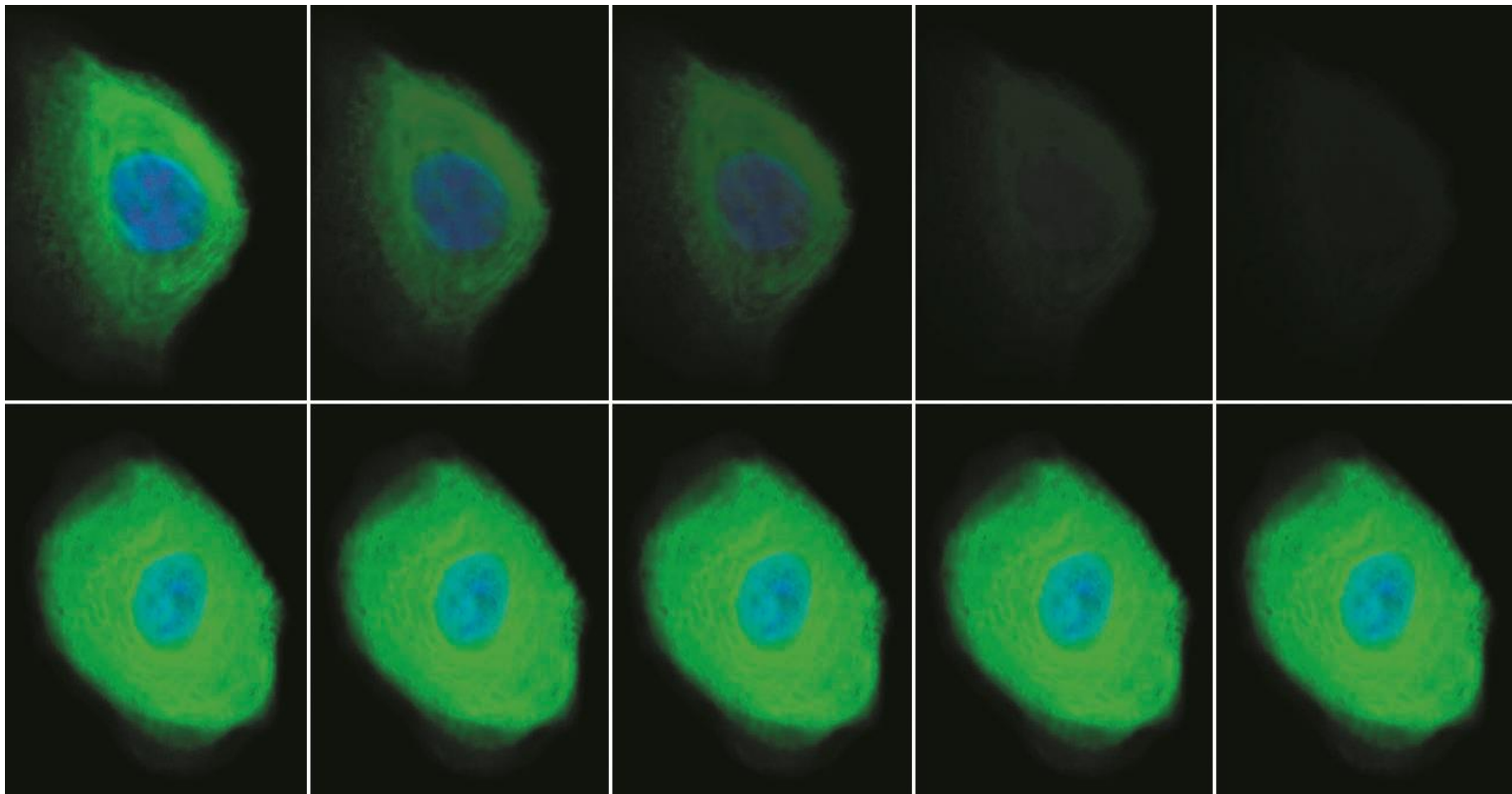
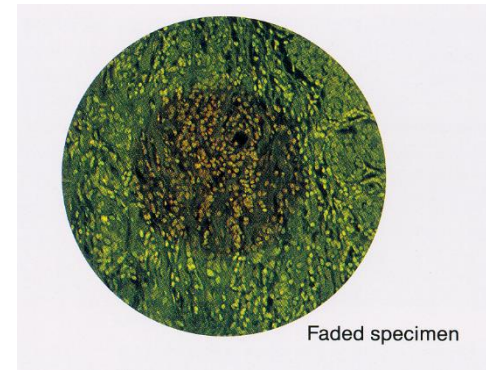


Figure 1. Immunofluorescence imaging of a human keratinocyte stained with FITC-labeled antibodies without (top) and with (bottom) PromoFluor Antifade Reagent at different excitations intervals (5, 10, 15, 20, 25 seconds). **PromoFluor Antifade Reagent**

Thank you for your attention

