



電子顯微鏡在細胞學 之應用

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109年2月第一版

摘要

- 電顯技術簡介
- 製作細胞電顯樣本前之準備
- 細胞樣本脫水包埋原則與方式
- 樣本粗切細切比較
- 細胞樣本上機觀察
- 應用於學術研究之潛力

電顯技術簡介

- TEM v.s. SEM
- 超顯微結構 (2K~100K)
- 組織 / 細胞檢體
- 脫水→包埋→切片(染色)→上機觀察

製作細胞電顯樣本前之準備

- 細胞量? Cell line ? Primary culture ?
- 離心速度Max ~2500rpm
- 去除PBS / medium上清液
- 加入電顯固定液(2.5% glutaraldehyde- 3% formaldehyde)固定至少6小時 (pre-fixation)
- 四氧化錳(Osmin) 50~70min (post-fixation)
- 乙酸鈾醯 (Uranyl acetate)



細胞量? Cell line ? Primary culture ?

	Surface Area (mm ²)	Seeding Density	Cells at Confluency ¹	Versene (ml of 0.53 mM EDTA)	Trypsin (ml of 0.05 % trypsin, 0.53 mM EDTA)	Growth Medium (ml)
Dishes						
35 mm	962	0.3×10^6	1.2×10^6	1	1	2
60 mm	2,827	0.8×10^6	3.2×10^6	3	2	3
100 mm	7,854	2.2×10^6	8.8×10^6	5	3	10
150 mm	17,671	5.0×10^6	20.0×10^6	10	8	20
Cluster Plates						
6-well	962	0.3×10^6	1.2×10^6	2	2	3-5
12-well	401	0.1×10^6	0.4×10^6	1	1	1-2
24-well	200	0.05×10^6	0.2×10^6	0.5	0.5	0.5-1.0
Flasks						
T-25	2,500	0.7×10^6	2.8×10^6	3	3	3-5
T-75	7,500	2.1×10^6	8.4×10^6	5	5	8-15
T-160	16,000	4.6×10^6	18.4×10^6	10	10	15-30

¹ The number of cells on a confluent plate, dish, or flask will vary with cell type. For this table, HeLa cells were used.

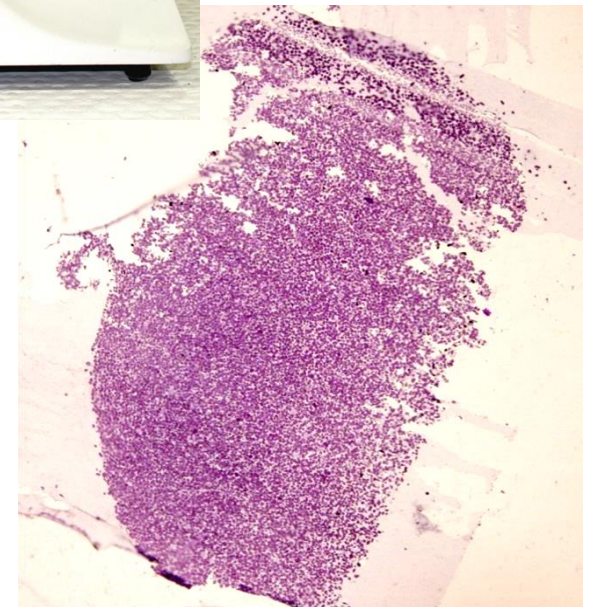
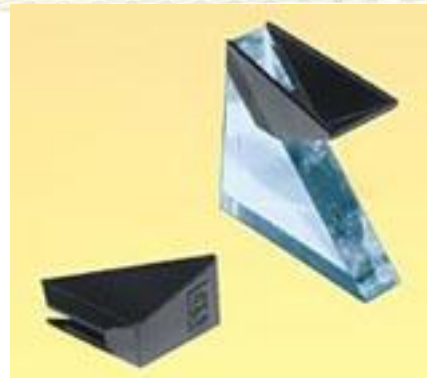
細胞樣本脫水包埋原則與方式

- 梯度酒精50%→75%→85%→95%→100%
- 環氧丙烷 (propylene oxide)作為過渡溶劑
- EPON 812 包埋專用樹脂
- 烤箱(>60°C)靜置48~72小時硬化聚合



樣本粗切細切比較

- 粗切(玻璃刀)
~200nm
- 甲苯胺藍
(Toluidin blue stain)對比染色
- 光學顯微鏡下
觀察切片是否具有目標組織/
細胞

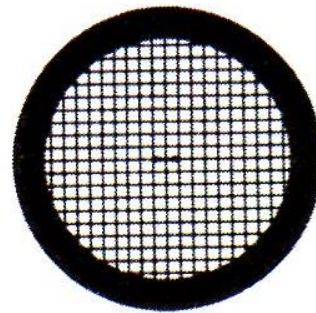


樣本粗切細切比較

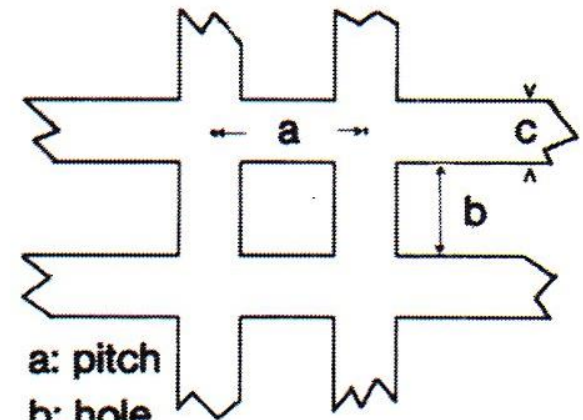
- 細切(鑽石刀) ~80nm
- 200 mesh銅網掛載超薄片 3~4 sections



► Standard Square Mesh



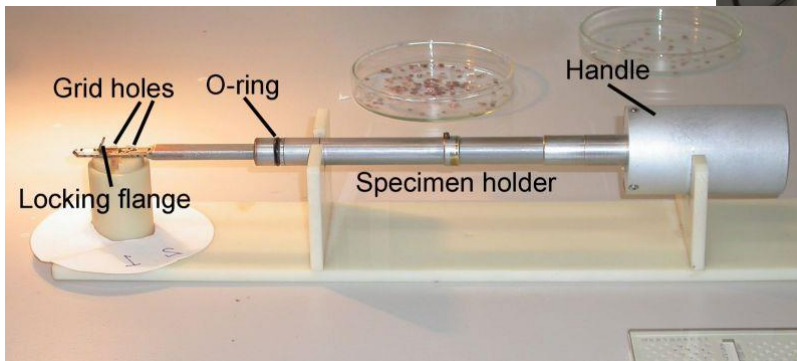
G100 - G400



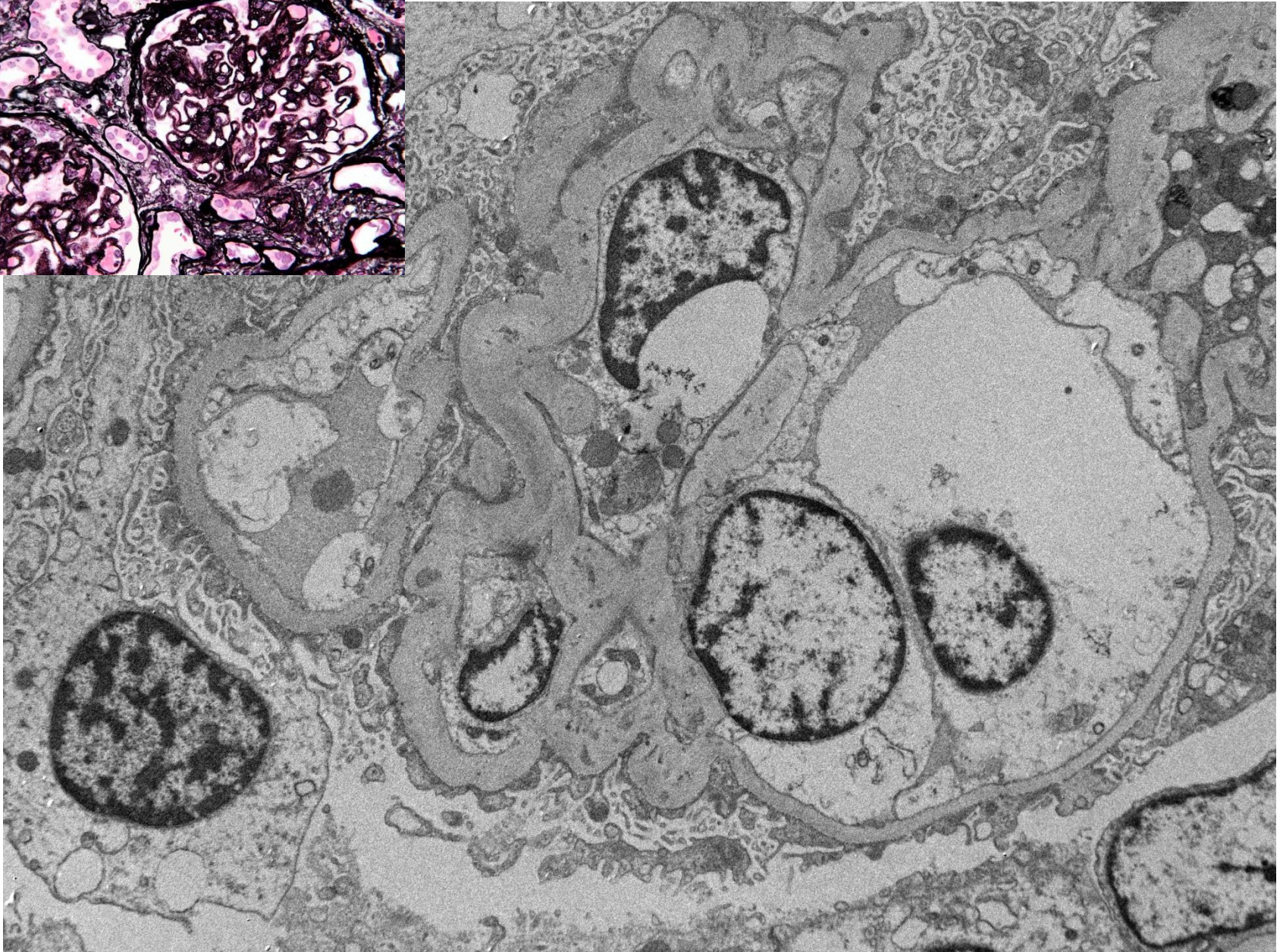
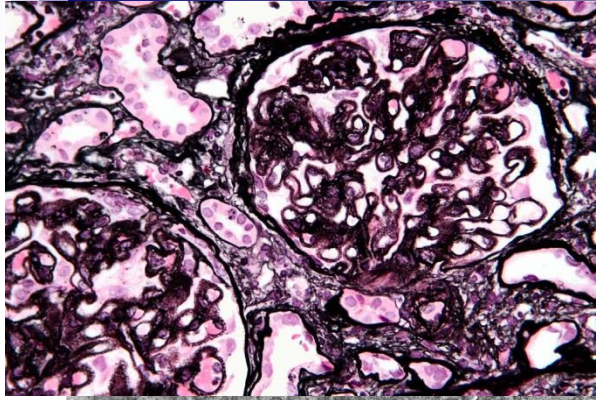
a: pitch
b: hole
c: bar

樣本上機觀察

- HITACHI TEM HT7700
- 75kV (HV)
18~20V (Filament)
6.0 μ A (Beam)

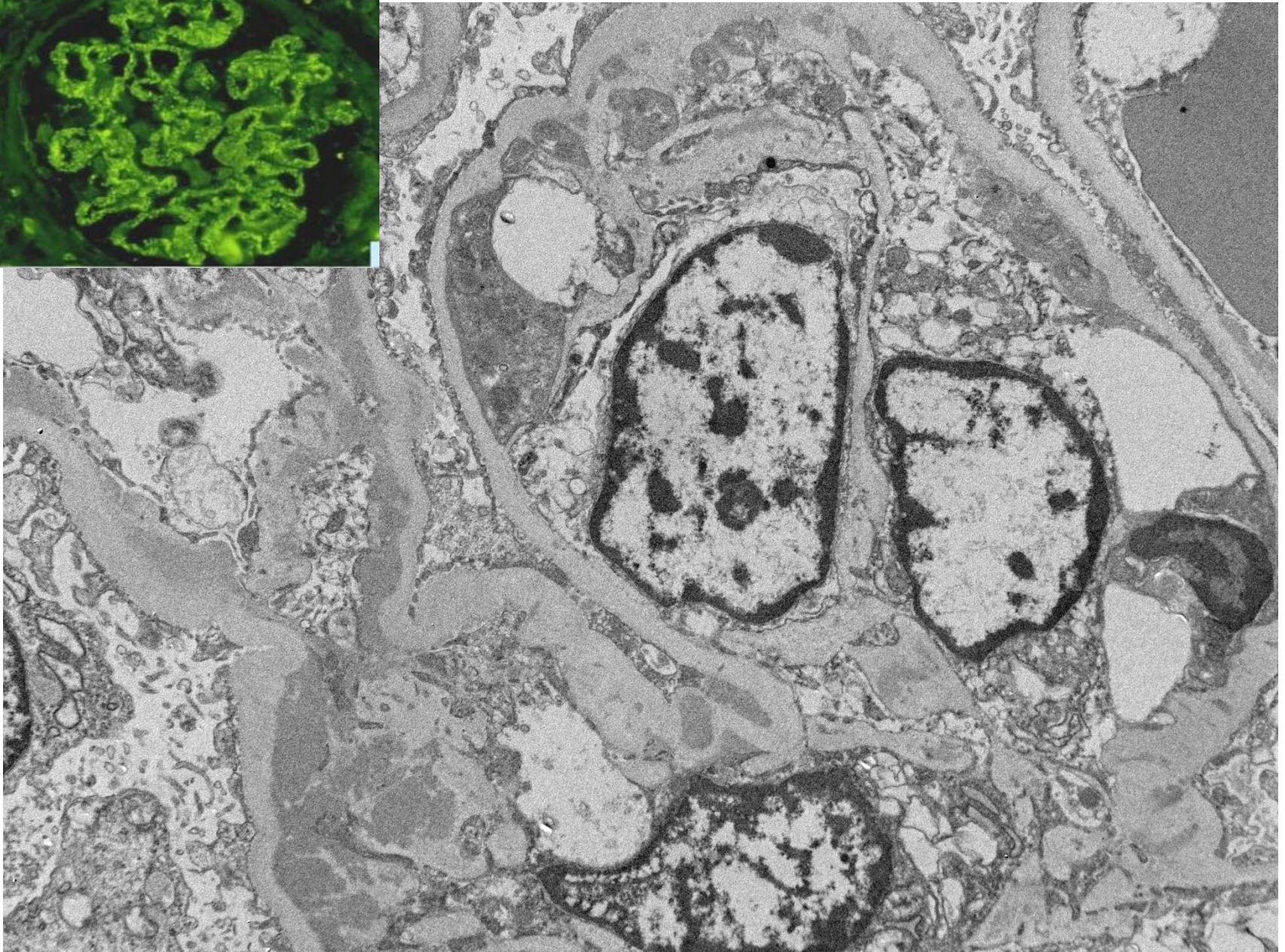
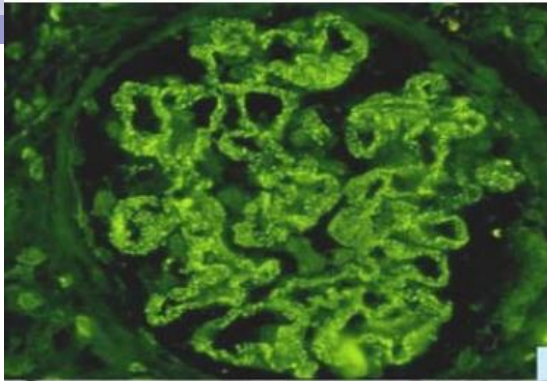


<https://myscope.training/>



x1.2k Zoom-1 HC-1 75.0kV 2019/01/03 11:37:33
Hitachi TEM system.

5.0µm



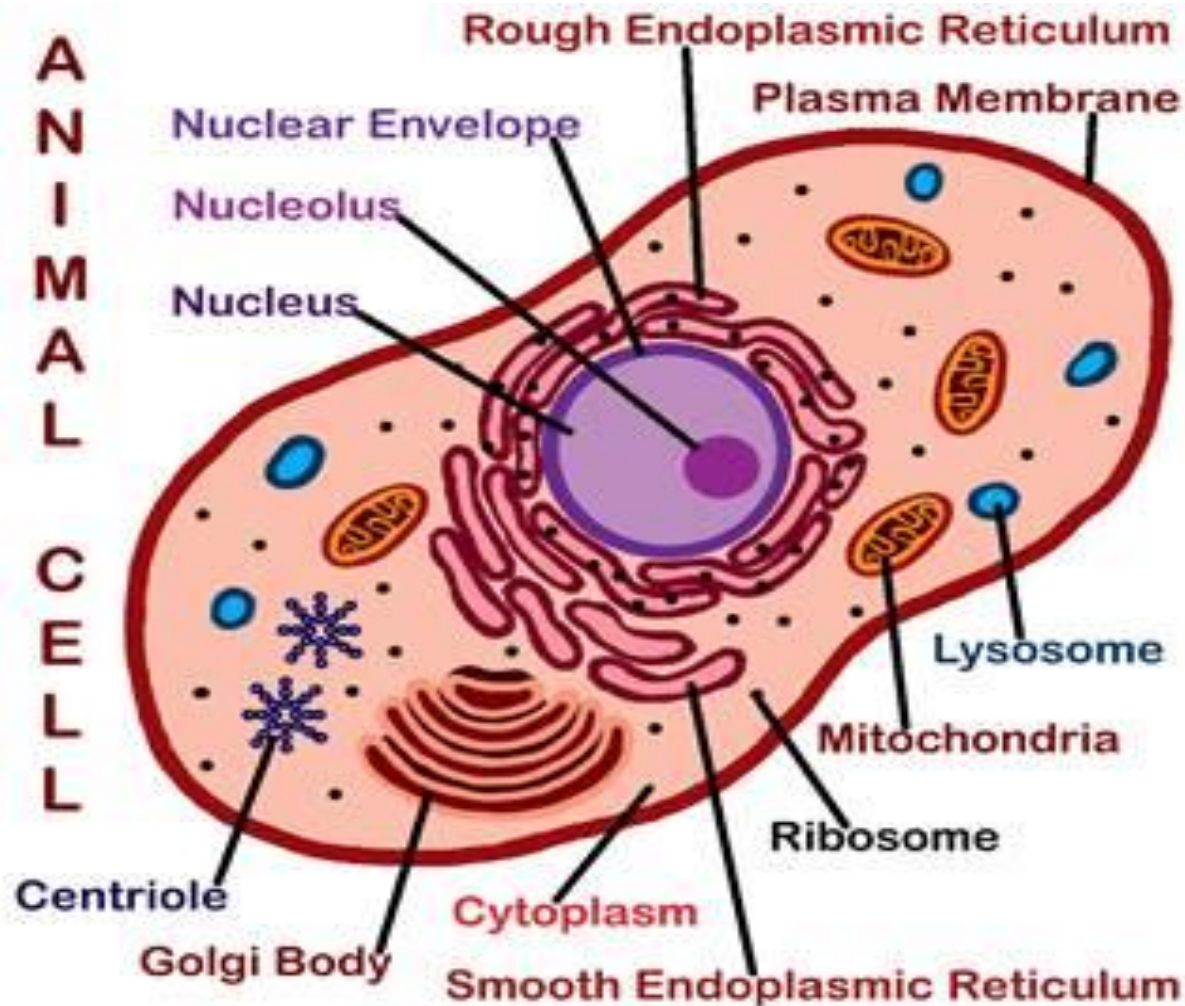
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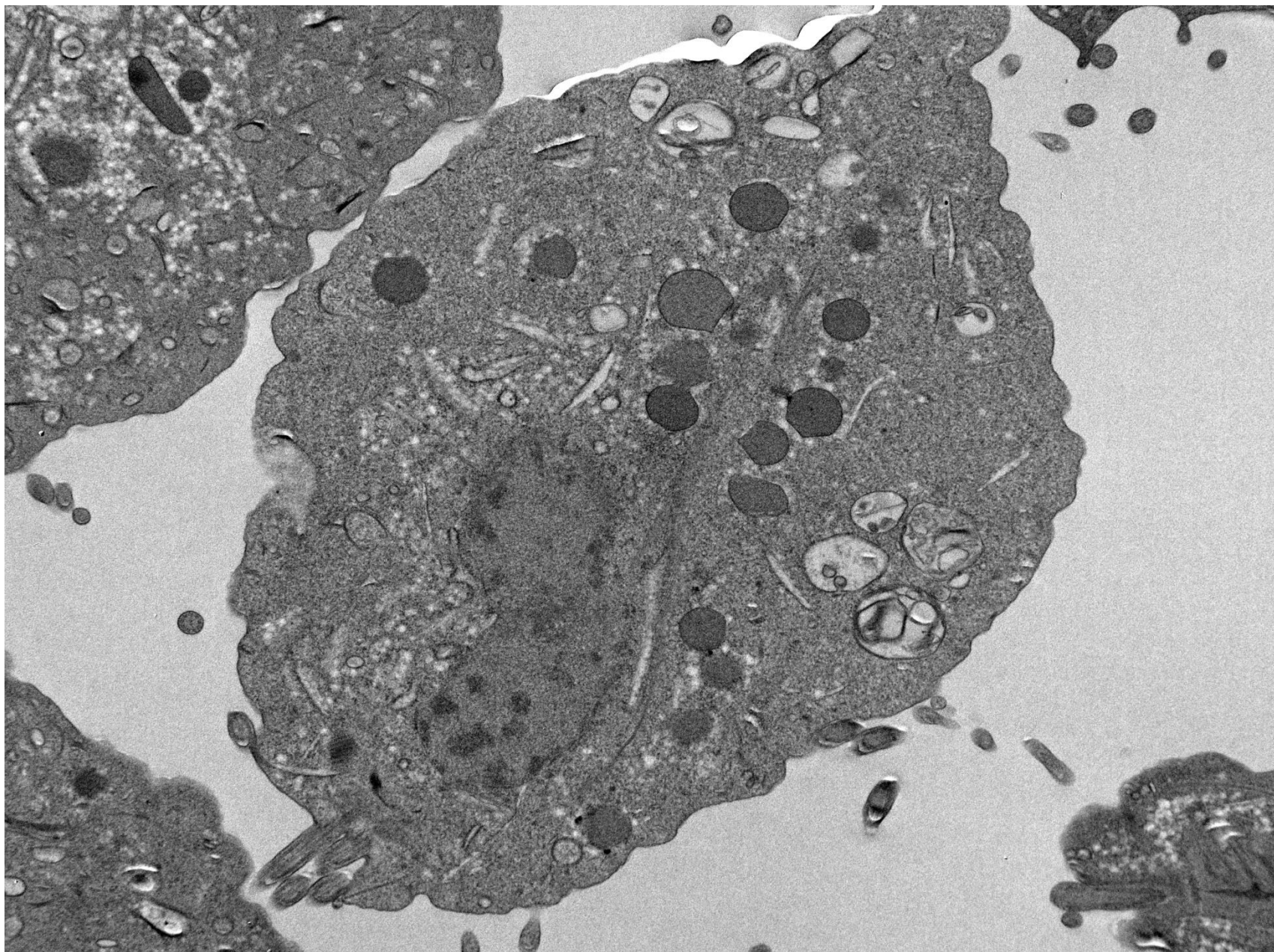
5.0µm

應用於細胞學術研究之潛力

- 觀察細胞超顯微結構：粒腺體、高基氏體、脂肪小體。
- 觀察細胞生理機能：胞噬作用、自噬作用、細胞凋亡現象。

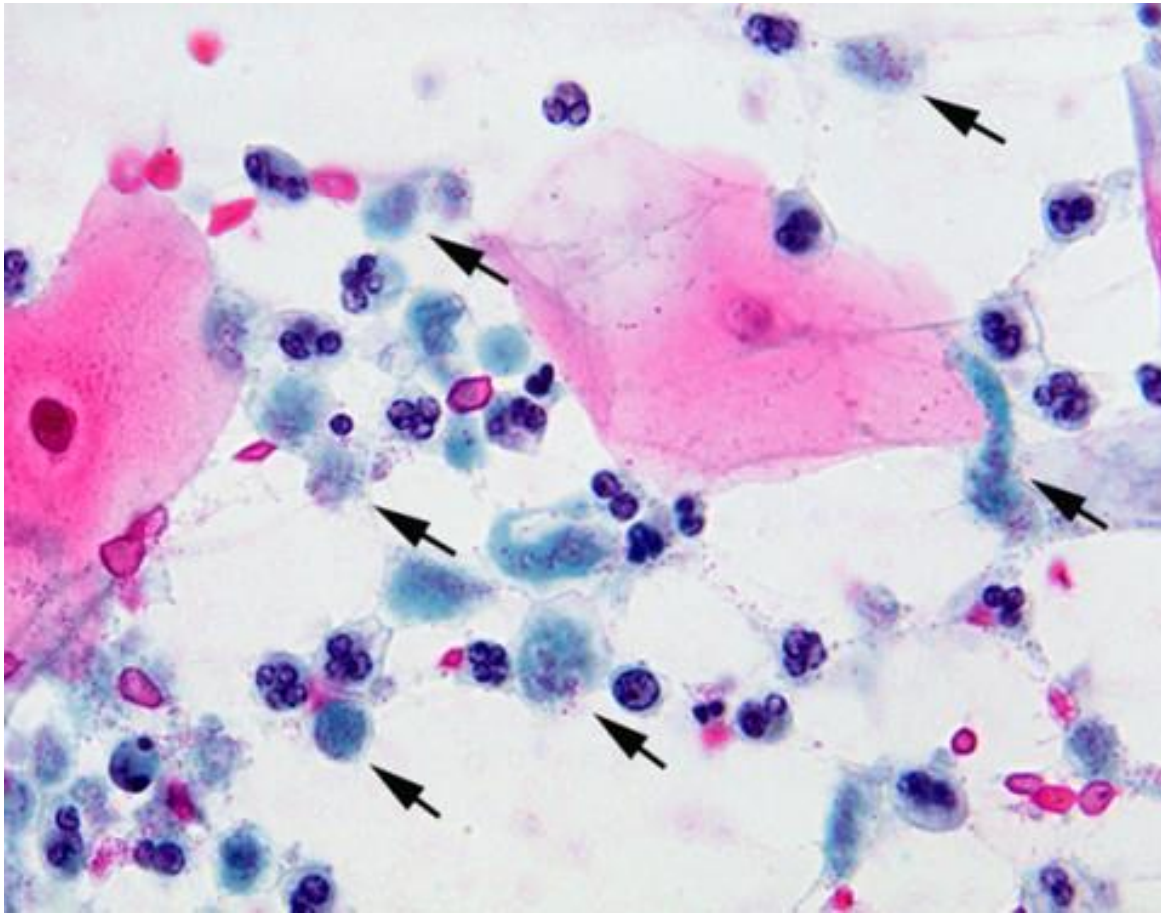
細胞顯微結構





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Hitachi TEM system.



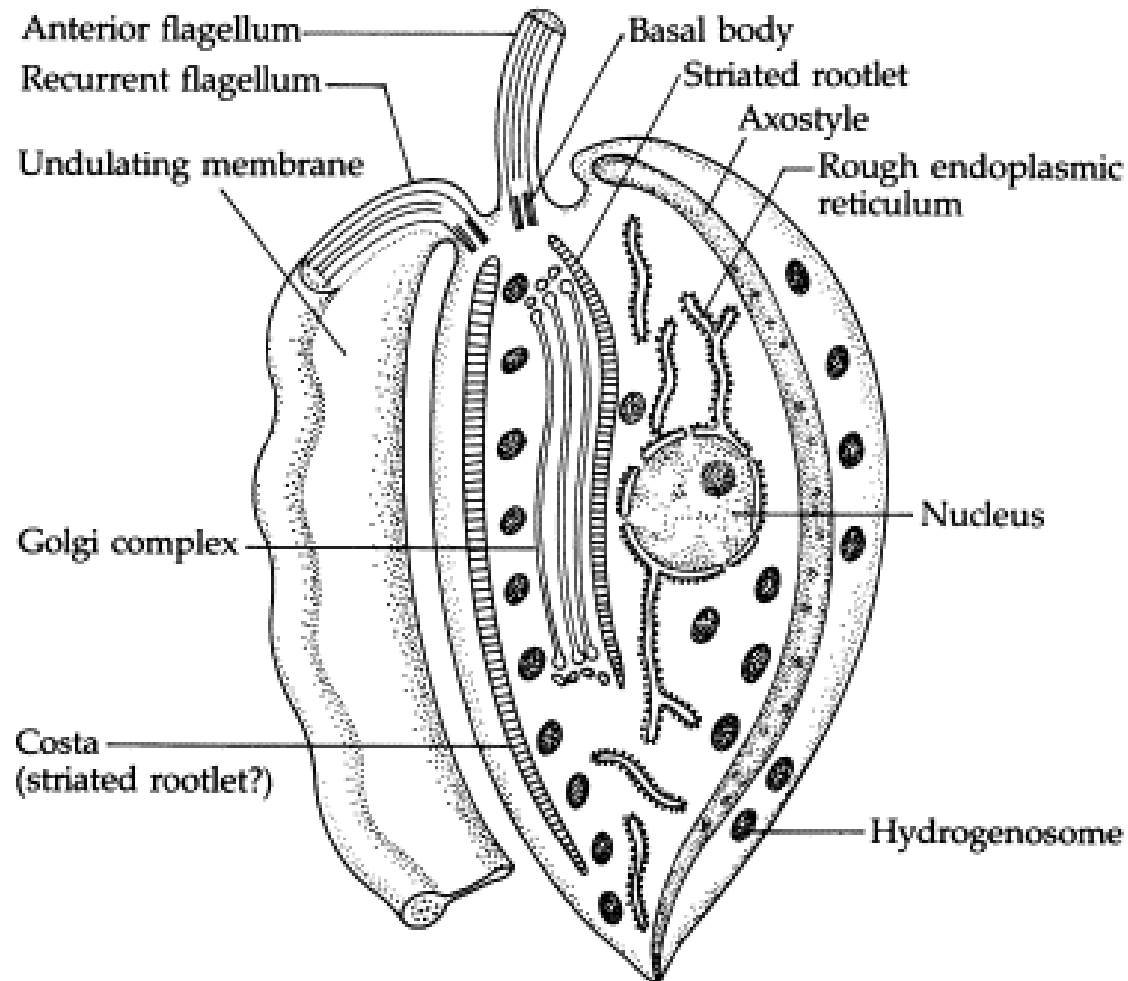


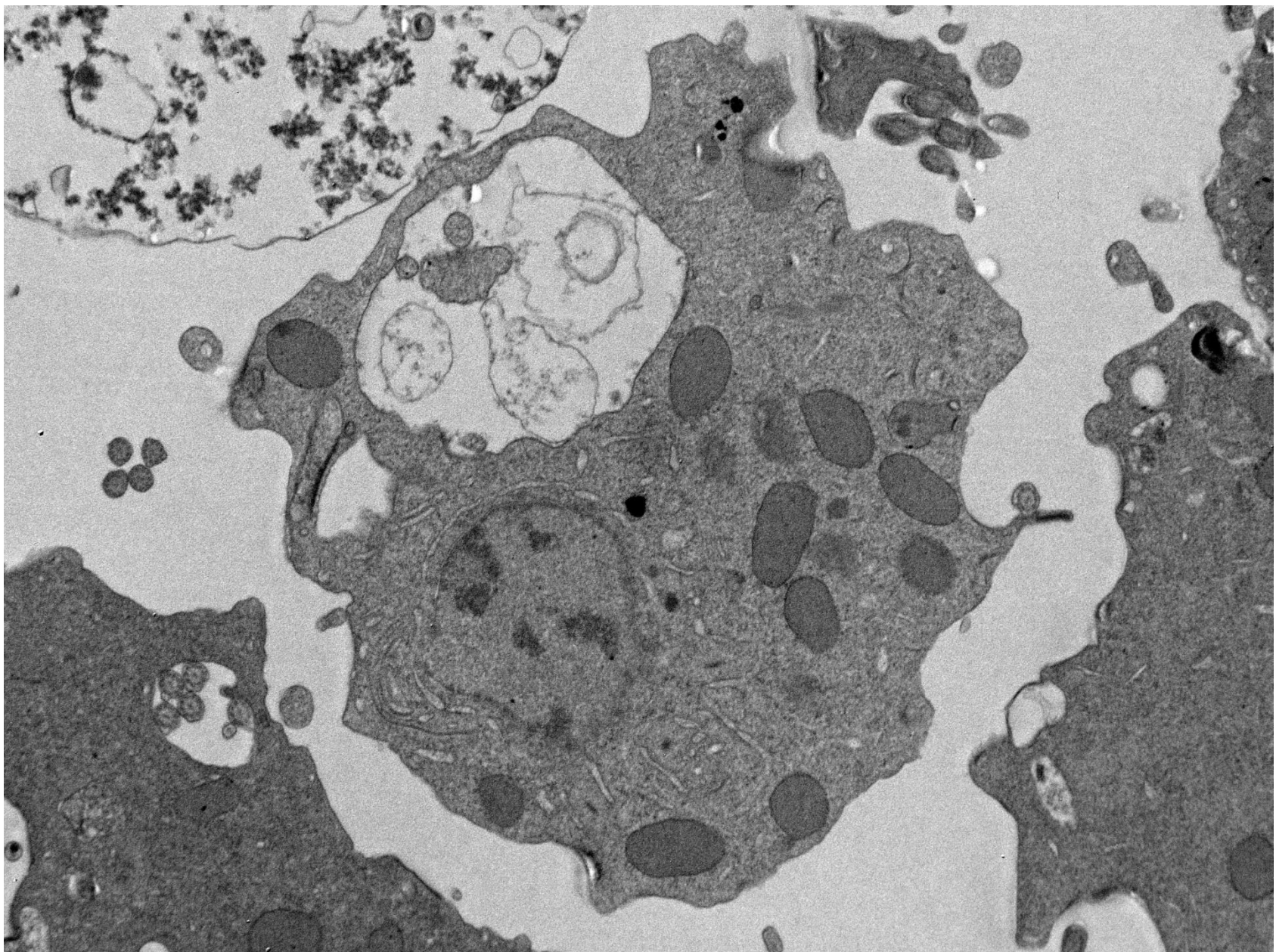
<https://obgynupdated.blogspot.com/>



<https://www.researchgate.net/>

Trichomonas vaginalis

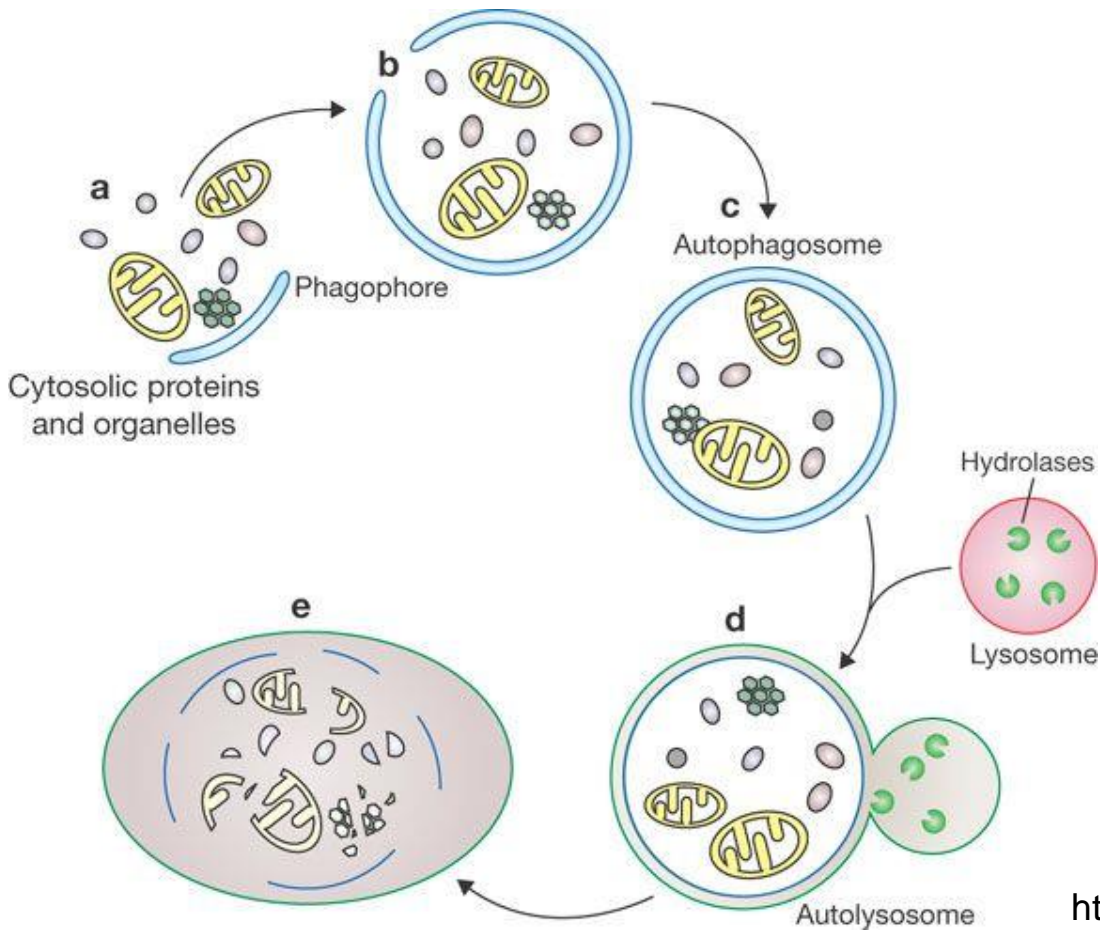


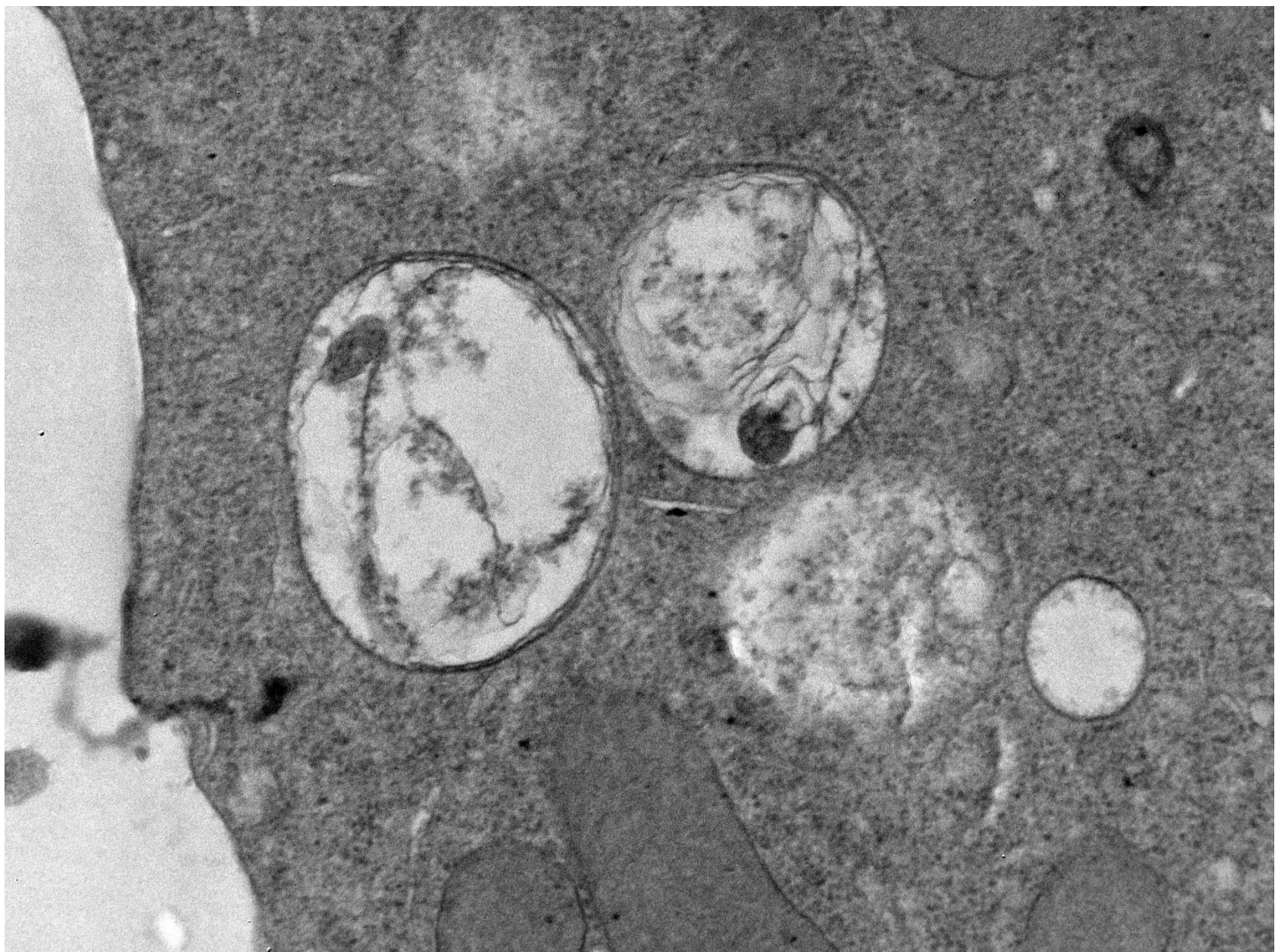


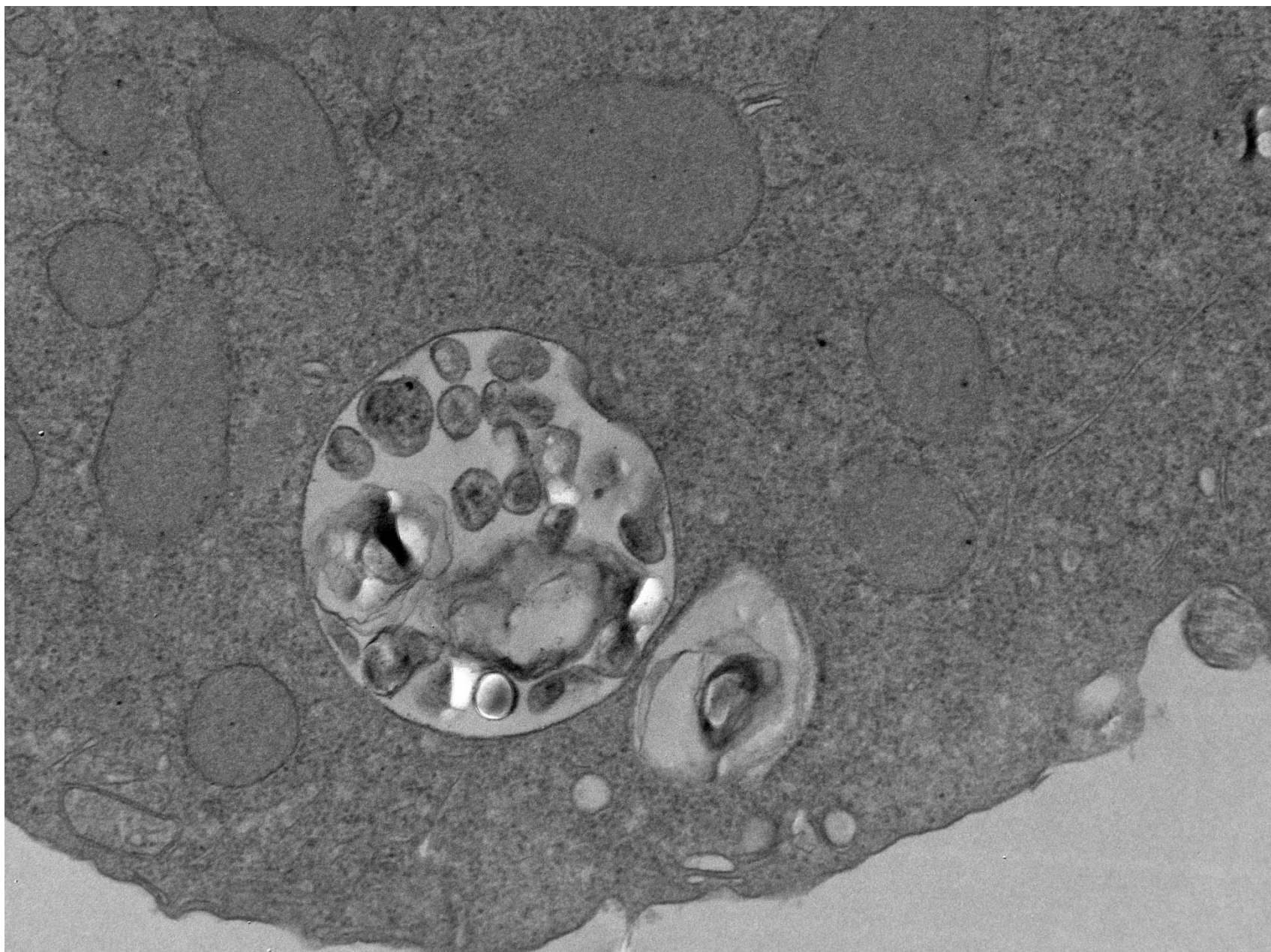
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Hitachi TEM system.

2.0µm

Autophagy (自噬作用)









謝謝聆聽
恭請指教