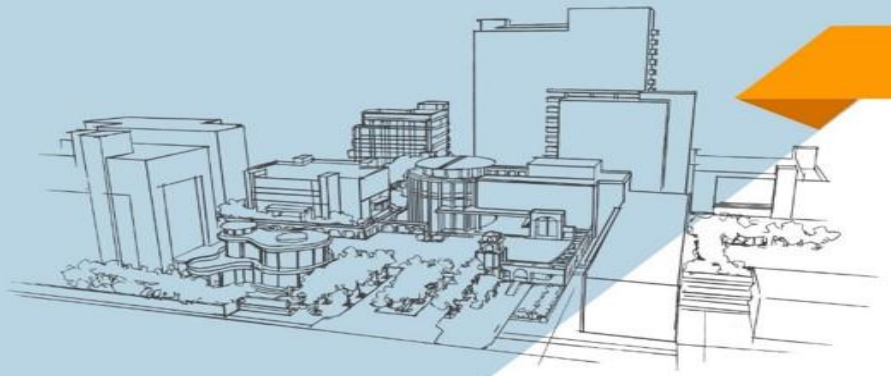
臺北醫學大學
TAIPEI MEDICAL UNIVERSITY

類H&E光學數位化影像應用於人體生物資料庫

劉韻如

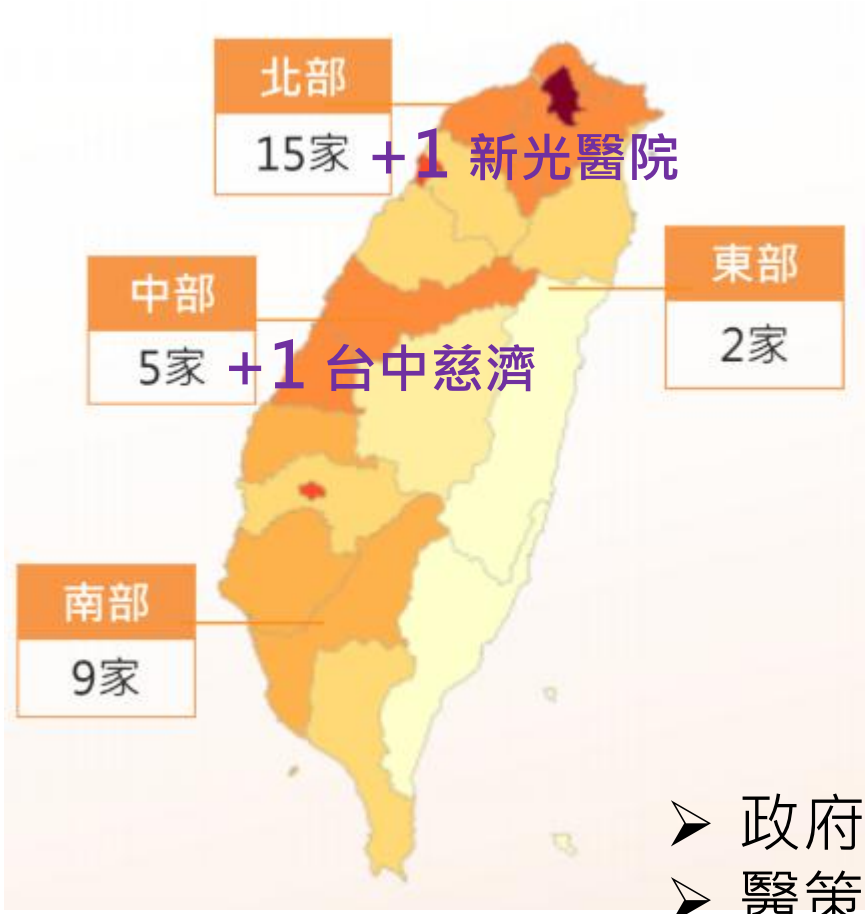


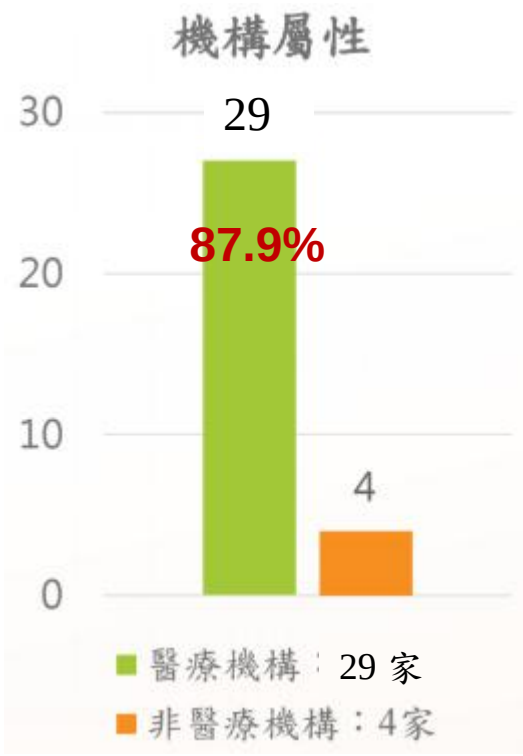
AcuSolutions Inc.

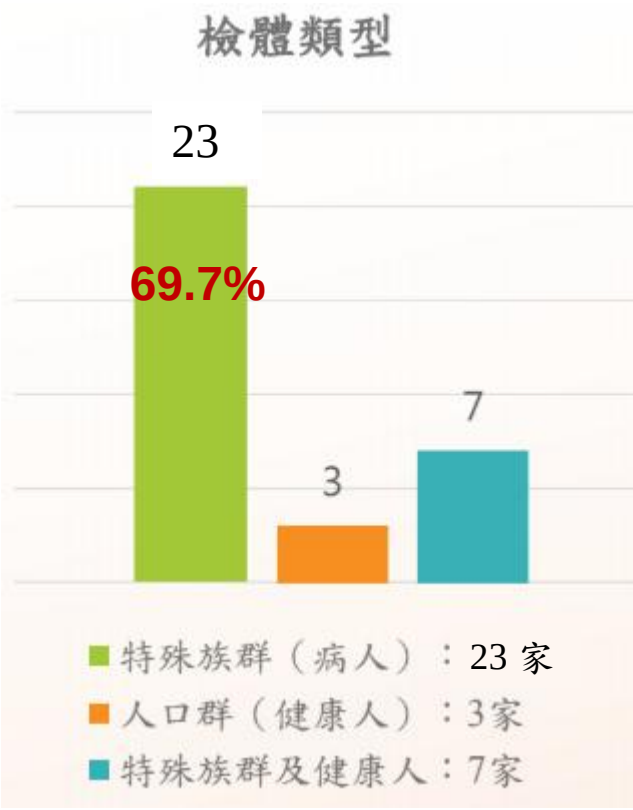
TMU60 1960~2020 北醫六十 邁向榮耀

台灣人體生物資料庫現況

資料來源：醫策會







- 政府經費支持或公立機構的佔 **32%**
- 醫策會辦理生物資料庫查核成功提升收案及出庫機構比例

歐洲/台灣生物資料庫地理分布圖

TMU Biobank – Tissue Quality

表一：聯合人體生物資料庫組織品質品保結果

Tissue	Number	Tissue Quality		
		Good (%)	Fair (%)	Poor (%)
Benign	406	395 (97.3%)	6 (1.5%)	5 (1.2%)
Malignant	2,649	2,425 (91.6%)	165 (6.2%)	59 (2.2%)
Adjacent Normal	2,178	2,067 (94.9%)	85 (3.9%)	26 (1.2%)
Total	5,233	4,887 (93.4%)	256 (4.9%)	90 (1.7%)

TMU Biobank – Tumor Cell %

表二：聯合人體生物資料庫組織中惡性腫瘤細胞百分比之品保結果

Tissue Type	Number	Tumor Cell %			
		Average	No Tumor	1 ~ 49	≥ 50
Brain	85	75.5 %	7 (8.2%)	4 (4.7%)	74 (87.1%)
Breast	460	59.0 %	56 (12.2%)	73 (15.9%)	331 (72.0%)
Esophagus	16	61.9 %	2 (12.5%)	1 (6.3%)	13 (81.3%)
Intestine	879	61.4 %	63 (7.2%)	148 (16.8%)	668 (76.0%)
Kidney	67	70.4 %	4 (6.0%)	10 (14.9%)	53 (79.1%)
Liver	241	73.6 %	19 (7.9%)	30 (12.4%)	192 (79.7%)
Lung	163	61.7 %	14 (8.6%)	35 (21.5%)	114 (69.9%)
Oral Cavity	29	67.8 %	0 (0.0%)	5 (17.2%)	24 (82.8%)
Ovary	71	54.8 %	3 (4.2%)	27 (38.0%)	41 (57.7%)
Pancreas	28	44.1 %	4 (14.3%)	11 (39.3%)	13 (46.4%)
Prostate	44	15.5 %	32 (72.7%)	5 (11.4%)	7 (15.9%)
Soft Tissue	16	76.9 %	0 (0.0%)	3 (18.8%)	13 (81.3%)
Stomach	96	61.7 %	11 (11.5%)	18 (18.8%)	67 (69.8%)
Thyroid	58	71.1 %	2 (3.4%)	10 (17.2%)	46 (79.3%)
Ureter	11	66.8 %	0 (0.0%)	3 (27.3%)	8 (72.7%)
Urinary Bladder	16	45.9 %	1 (6.3%)	7 (43.8%)	8 (50.0%)
Uterus	80	66.4 %	10 (12.5%)	11 (13.8%)	59 (73.8%)
Others	65	75.9 %	1 (1.5%)	8 (12.3%)	56 (86.2%)
Total	2,425	62.6 %	229 (9.4%)	409 (16.9%)	1,787 (73.7%)

REVIEW PAPER: Human Tissues for Discovery Biomarker Pharmaceutical Research: The Experience of the Indiana University Simon Cancer Center–Lilly Research Labs Tissue/Fluid BioBank

G. Sandusky, C. Dumaual, and L. Cheng

Simon Cancer Center and Department of Pathology and Laboratory Medicine, Indiana University, Indianapolis, IN

Vet Pathol **46**:2–9 (2009)

Table 1. Human cancer sample quality control by HE staining.

Tissue type	Total tumor	>65% Tumor	<65% Tumor	Necrosis	No tumor	% of >65% tumor
Breast Ca	171	111	38	1	24	65
Colon Ca	246	161	47	2	37	65
Lung Ca	189	125	36	7	21	73.5
Prostate Ca	277	83	106	0	91	30
Ovary Ca	23	20	3	0	0	87
Liver Ca	42	37	4	0	1	88
Head and Neck Ca	86	59	13	0	13	69
Kidney Ca	34	28	5	0	1	82
Pancreas Ca	70	46	11	4	10	68
Total	1,138	670	263	14	198	59
				1.2 %	17.4%	59%

Histological Assessment of Tumor Tissue Samples via the Mirror Image Method

Haixin Li, Yan Guo, Baocun Sun, and Kexin Chen

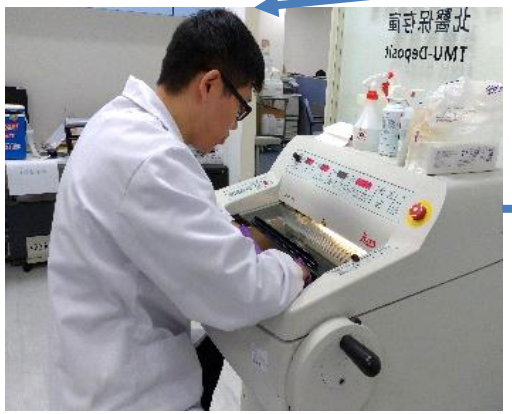
Cancer types	Cases (n=1221)	Percentage of tumor cells (%)			
		>75	51–75	25–50	<25
Solid organs	551	348	83	69	51
Kidney	110	89	10	8	3
Liver	110	74	13	11	12
Lung	331	206	72	28	25
Hollow organs	670	334	152	109	75
Esophagus	95	59	20	14	2
Stomach	273	136	47	49	41
Colorectal	302	139	85	46	32

75%

在庫檢體品保/品管作業



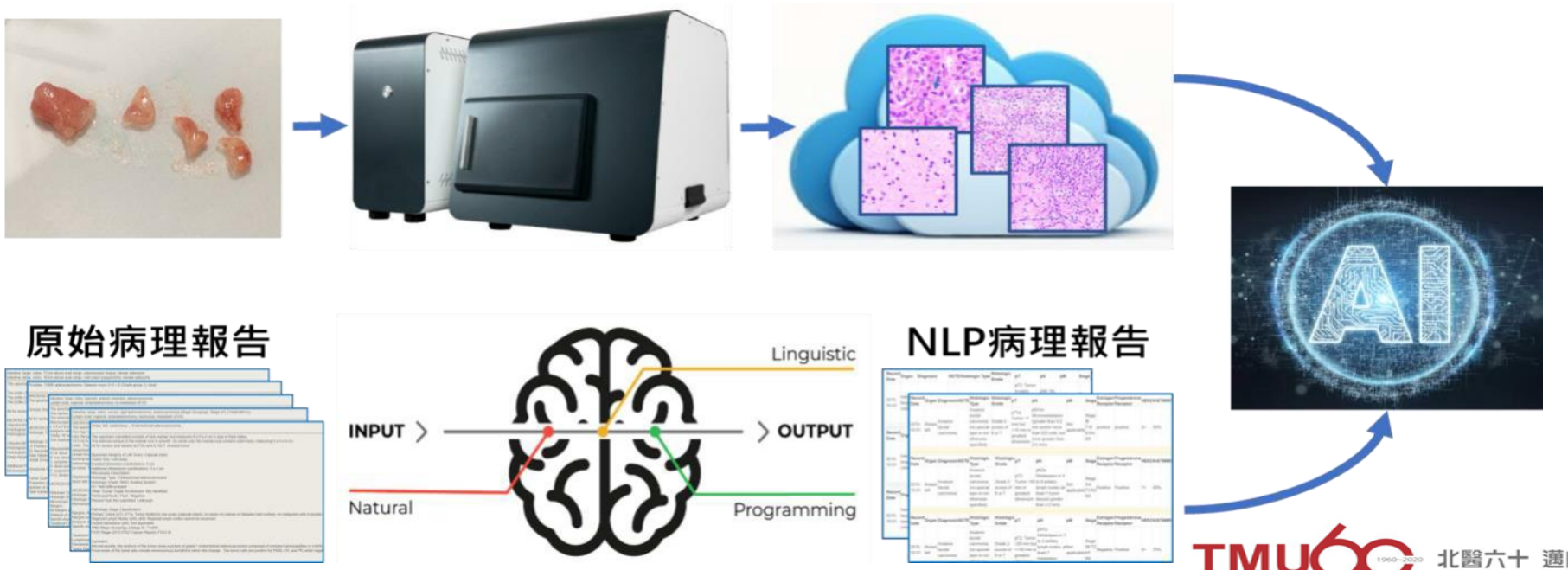





臺北醫學大學 TAIPEI MEDICAL UNIVERSITY

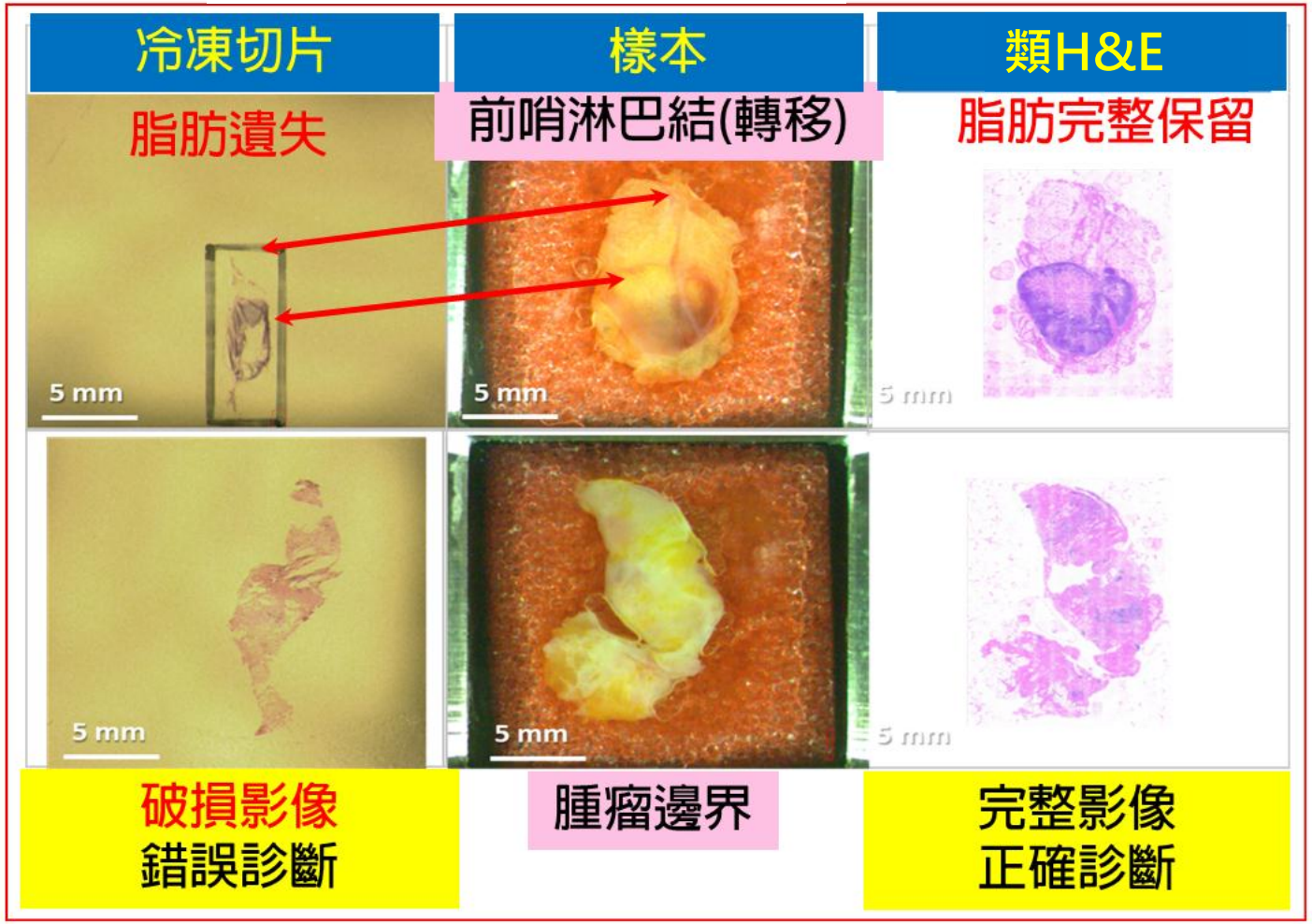
目標

- 以簡單便捷的方式普及人體生物資料庫品質管理及保證
- 以入庫前QA/QC降低人力、耗材及空間成本
- 病理影像數位化可提升服務品質及AI智慧發展

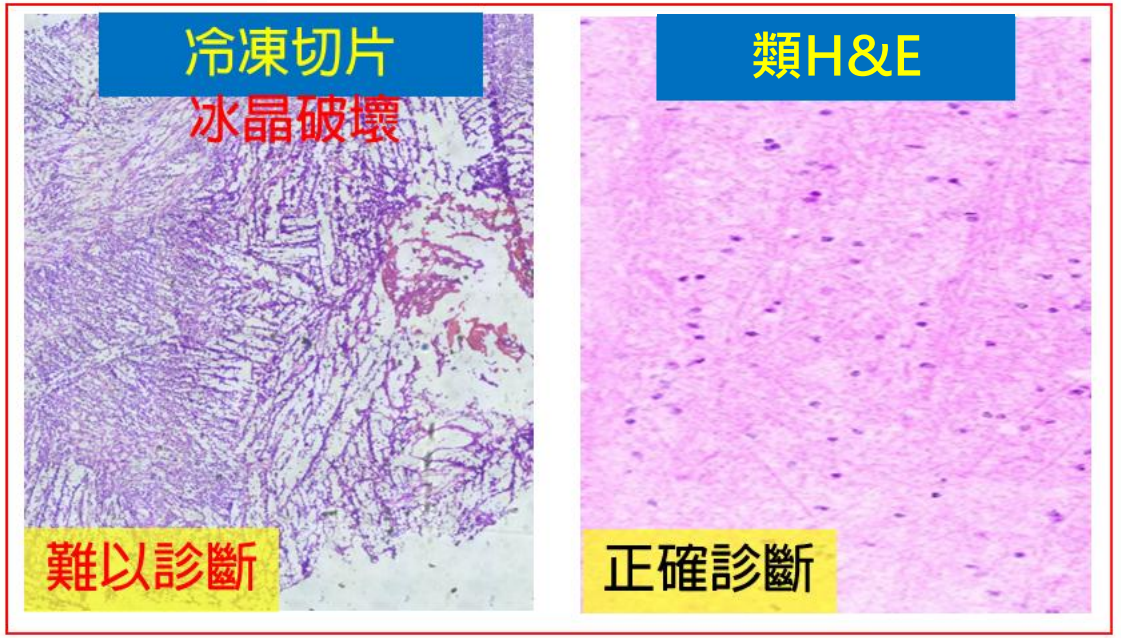


冷凍切片常見問題

- 乳癌 (脂肪遺失)



- 腦腫瘤 (冰晶破壞)



光學切片可解決組織破損問題

樣本處理程序比較

核心技術 – 類H&E光學切片(OCM)

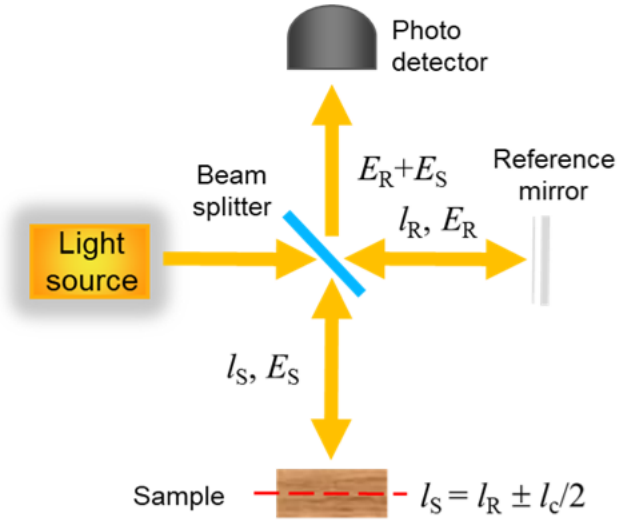
- OCM (Optical Coherence Microscopy) Imaging System 光學同調顯微術影像系統

創新點:整合**兩種光學技術**在同一個系統，完整取得病理醫師診斷所必須的**細胞質**與**細胞核**資訊

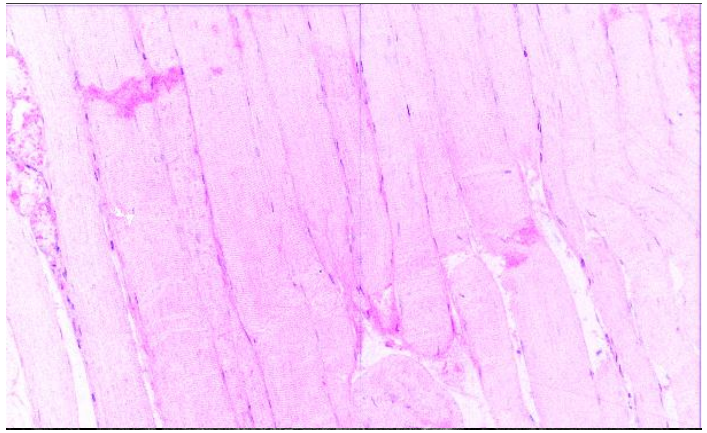
  - Full-field optical coherence tomography (FF-OCT): tissue structure and cytoplasm(細胞質)imaging
  - Another technique for nucleus(細胞核)imaging
  - Software, image processing

- Pseudo-H&E影像演算法

創新點:影像符合醫學傳統，各國醫師們接受度反應極高

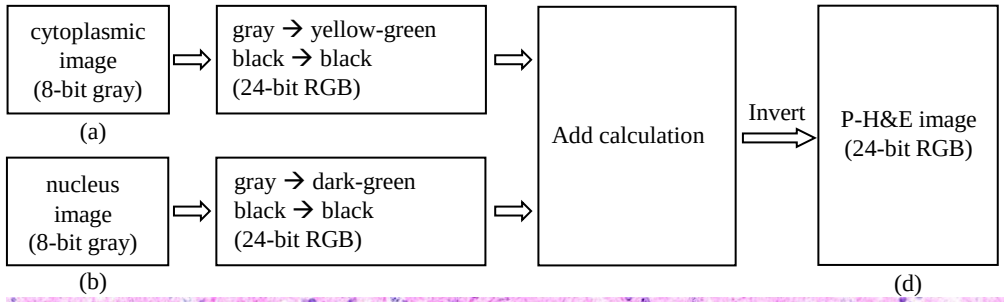


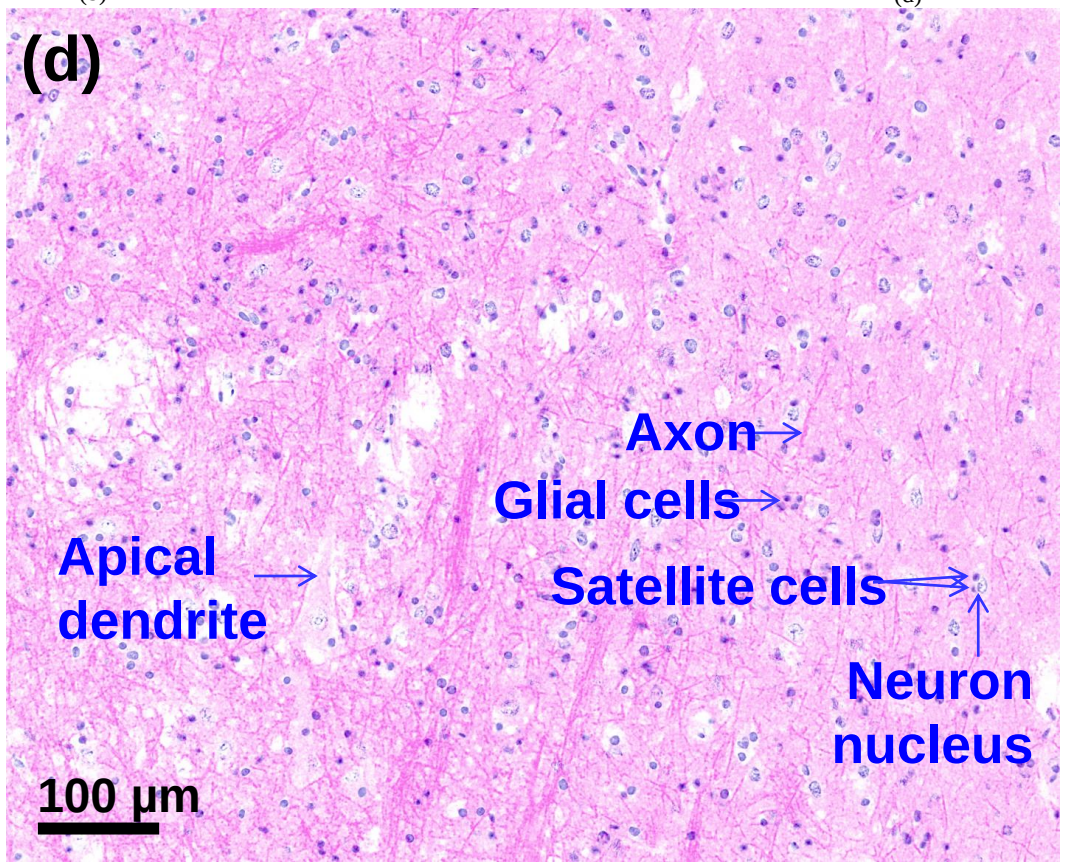
Michelson interferometer

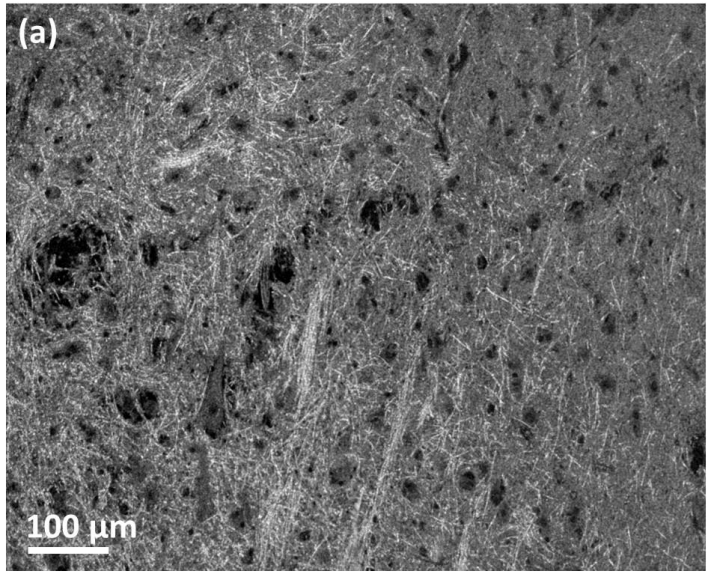


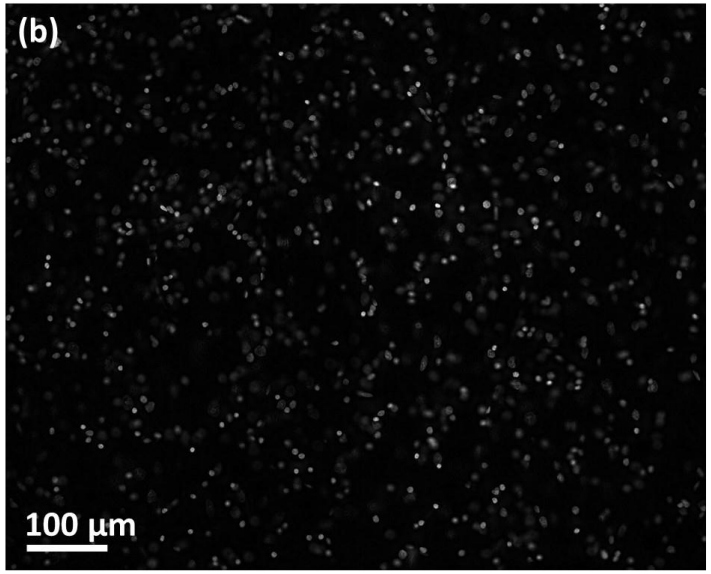
核心技術已受多國近十項專利之保護

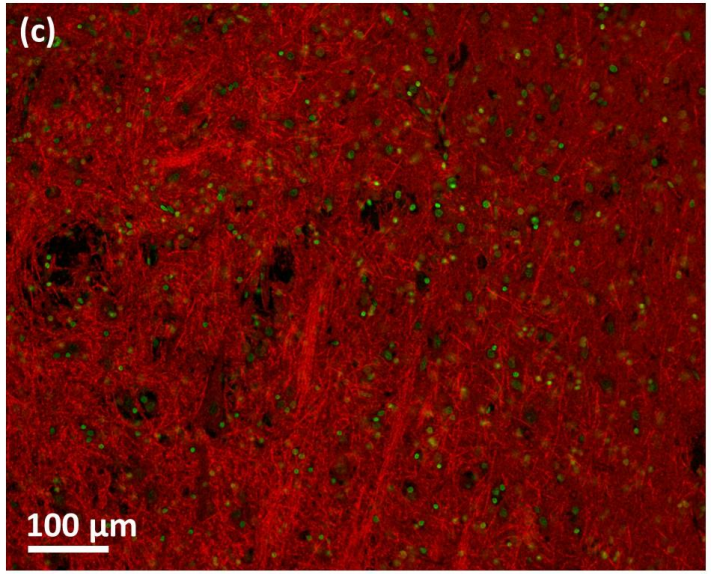
樣本影像產生程序 (例：新鮮豬腦)

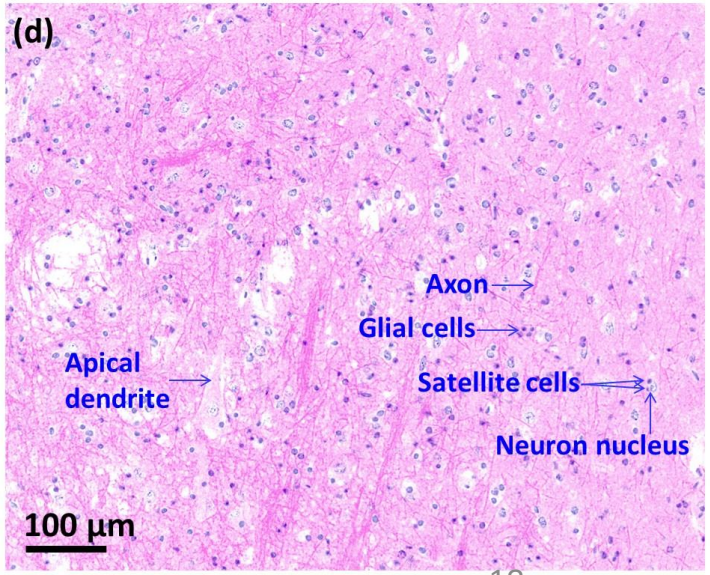












醫學驗證

AcuSolutions OCM 已經過台日美國內外許多醫學驗證

臺北市立萬芳醫院-委託財團法人臺北醫學大學辦理
Taipei Municipal Wanfang Hospital（Managed by Taipei Medical University）

- Clinical Trial at Department of Pathology, Wanfang Hospital (Sept. ’17 ~ Mar. ’18):
  Indication: intra-operative diagnosis, SLN / breast cancer.
- Clinical Trial at Department of General Surgery, ChangGung Memorial Hosp. (Dec. ’18 ~Jan. ’20)
  Indication: breast biopsy, 300 cases
- Research Collaboration at Kyoto Univ. Pathology:
  1st-year: Apr. ’18 ~ Mar. ’19
  2nd-year: Apr. ’19 ~ Mar. ’20
- Department of Pathology, UCSF
  Tryout: Aug. ’18 ~ Nov. ’19
- Department of Ophthalmology, Stanford University (Leasing)
- Biobank, Taipei Medical University (Jan. ’20 ~)
- School of Veterinary Medicine National Taiwan University (May.’20 ~)
- Clinical Trial at Department of Neurosurgery ChangGung Memorial Hosp. (Under Preparation)

醫學驗證 – 京都大學醫學部 USCAP2020 論文公開

京都大学 KYOTO UNIVERSITY

ACUSOLUTIONS

Rapid slide-free pathology diagnostics using full-color optical coherence microscopy (full-color OCM)

Akihiko Yoshizawa[1], Shinji Sumiyoshi[1], Chien-Chung Tsai[2], and Hironori Haga[1]

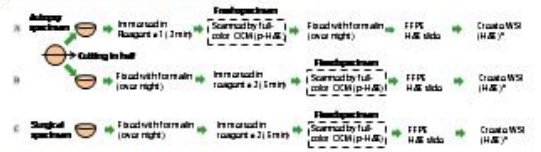
[1] Dept. of Diagnostic Pathology, Kyoto University Hospital, Kyoto, Japan, [2] AcuSolutions Inc., Taipei, Taiwan

Background

Some techniques, which enable visualization of unsectioned blocks or unstained glass slides, have been proposed. Conventional full-field optical coherence microscopy (FF-OCM) [1], which is one of the techniques, is capable of providing cell-level cytoplasmic images without using expensive components; however, it is difficult to visualize nuclei in detail and is not able to create full-color images [2]. We propose a novel slide-free imaging method, **full-color OCM**, combining FF-OCM with fluorescence microscope (FM) to acquire digital images, which can be further synthesized into pseudo-hematoxylin-and-eosin (p-H&E) color histological images. This study demonstrates rapid histological imaging of unsectioned surgical or fresh specimens using full-color OCM and assesses its quality compared to conventional H&E staining of formalin-fixed paraffin-embedded (FFPE) tissues.

Materials and Methods

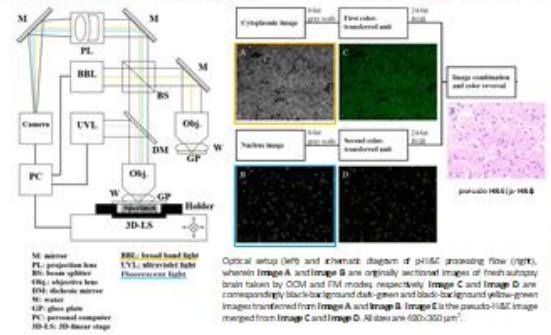
Tissue preparation strategy (Fig. 1)



Scanned by full-color OCM (Fig. 1)

A total of 106 formalin-fixed surgical specimens and 120 fresh specimens from 11 autopsy cases (Figs. 1A-C) were collected. After scan of full-color OCM (Fig. 1D), the FFPE tissues were processed for making H&E slides and then compared to full-color OCM images, correspondingly.

Optical Setup and Image Process (Fig. 2)

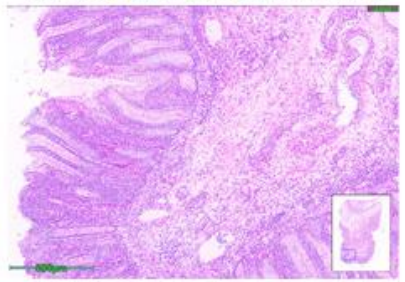


Optical setup (left) and schematic diagram of p-H&E processing flow (right), wherein **Image A** and **Image B** are originally sectioned images of fresh autopsy brain taken by OCM and FM modes respectively. **Image C** and **Image D** are correspondingly black-background dark-green and black-background yellow-green images transferred from **Image A** and **Image B**. **Image E** is the pseudo-H&E image merged from **Image C** and **Image D**. All sizes are 480×360 μm².

Results

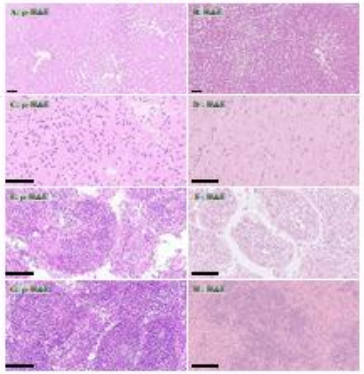
Full-color OCM takes about 4 min for scanning 1 cm² area and totally 15 min from receiving specimen to completion of scan. Pseudo-H&E and H&E images from the same tissues are listed and compared, accordingly.

Fig. 3: Representative image scanned by full-color OCM



This pseudo-H&E image of formalin fixed colon was produced by full-color OCM. All pseudo-H&E images were evaluated by AcuViewer navigation system which was home-developed.

Fig. 4: Fresh autopsy specimens: normal tissue (Strategy A in Fig. 1)



Figs. 4A, 4C, 4E, and 4G were pseudo-H&E images of fresh autopsy specimens of normal liver, brain, testis, and lymph nodes, respectively. Figs. 4B, 4D, 4F, and 4H were H&E images which were made from the formalin-fixed specimens of the same specimens. All the scale bars are 100 um.

Fig. 5: Fresh autopsy specimens: tumor tissue (Strategy A in Fig. 1)

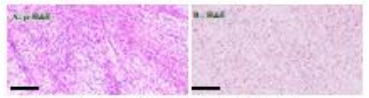
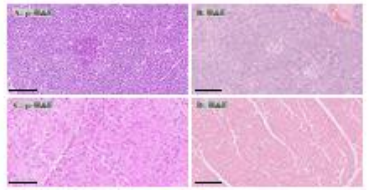


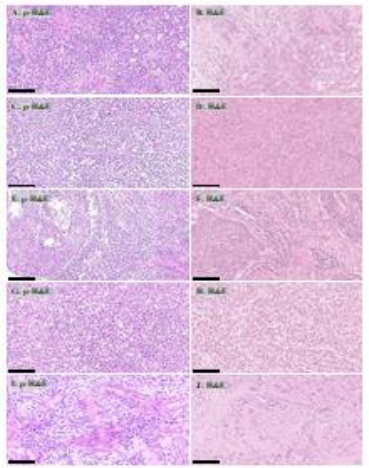
Fig. 5A was pseudo-H&E images of fresh autopsy specimens of tumor tissue (undifferentiated pleomorphic sarcoma of soft tissue). Fig. 5B was H&E images which was made from the formalin-fixed specimens of the same specimens. The scale bars are 100 um.

Fig. 6: Formalin-fixed autopsy specimens: normal tissue (Strategy B in Fig. 1)



Figs. 6A and 6C were pseudo-H&E images of formalin-fixed autopsy specimens of normal pancreas and heart, respectively. Figs. 6B and 6D were H&E images made from the formalin-fixed blocks of the same specimens. All the scale bars are 100 um.

Fig. 7: Fixed surgical specimens: tumor (Strategy C in Fig. 1)



Figs. 7A, 7C, 7E, 7G, and 7I were pseudo-H&E images of formalin-fixed surgical specimens of some kinds of tumor. Figs. 7B, 7D, 7F, 7H, and 7J were H&E images which were made from the formalin-fixed specimens of the same specimens. All the scale bars are 100 um. Figs. 7A and 7B shows pleomorphic adenoma of major salivary gland; Figs. 7C and 7D shows meningioma; Figs. 7E and 7F shows squamous cell carcinoma of lung; Figs. 7G and 7H shows testicular seminoma; Figs. 7I and 7J shows schwannoma of soft tissue. Scale bars are 100 um.

Summary of slide-free pseudo H&E technology

Optical-Sectioning Technology	Full-color OCM	FF-OCT	Light-sheet microscopy	MUSE	Nonlinear microscopy	Raman microscopy	Confocal microscopy
Scanning Speed[1]	Fast (5 min)	Fast	Fair (40s to scan, 15min to extract data)	Very Fast (1.3 min)	Fair (7.9 min)	Very Slow (60 min)	Fair
Image Quality	- cellular crowding	- no nucleus info	- limited resolution* - cellular crowding	- highly depend on surface quality* - too thick DOF (very cellular crowding)	- cellular crowding	- imaged by CH3 & CH2 bonds, not exactly compatible with H&E system	- cellular crowding
Est. Cost[2]	Low	Low	Low	Low	High*	Very High*	Moderate
Remark			*4× objective, NA 0.28	*can only scan specimen surface	*expensive laser	*expensive lasers	
References	—	[3] Mukherjee et al. / Rahul Vallurs, 2015 / [4] Darke et al., Dartmouth Eng. 2014 / [5] Orazi et al., Technol Cancer Res T., 2014	[6] Liu et al., Biomed Opt Express, 2016 / [7] Liu et al., Sci Rep., 2016 / [8] Liu et al., Biomed Opt Express, 2016	[9] Levenson et al., Nat Biotechnol Eng., 2017 / [10] Fereiro et al., Commun Pathology, 2018 / [11] Fujimoto et al., Sci Rep., 2018	[12] Fujimoto et al., PNAS, 2014 / [13] Fujimoto et al., Lab Invest., 2016 / [14] Fujimoto et al., Lab Invest., 2016	[15] Orringer et al., Sci Transl Med., 2015 / [16] Orringer et al., Nat Biomed Eng., 2017 / [17] Orringer et al., Am Soc Clin Res., 2018	[18] L. Orazi et al., J Biomed Opt, 2010 / [19] S. Orazi et al., Dartmouth, 2010

[1] Speed is calculated for 10*10 mm² specimen, only including scanning time.
[2] The cost is only for reference. Definition: Low:50K~150K USD, Moderate: 150K~300K USD, High:300K~500K USD, Very High: over 500K USD.
Abbreviation: **Full-color OCM**, full-color optical coherence microscopy; **FF-OCT**, full-field optical coherence tomography; **MUSE**, microscopy with UV surface excitation; **Raman microscopy**, stimulated Raman scattering microscopy.

Conclusion:

Full-color OCM images closely resembled FFPE-H&E images and enabled rapid assessment of normal and pathological histology in both fixed and fresh specimens. This technology provides a way of fast imaging, leading to increasing opportunities of intra-operative consultation and saving cost by skipping FFPE sectioning procedure. Furthermore, it has the potential to provide a digital pathologic platform for telepathology and rapid artificial intelligence assisted diagnosis.

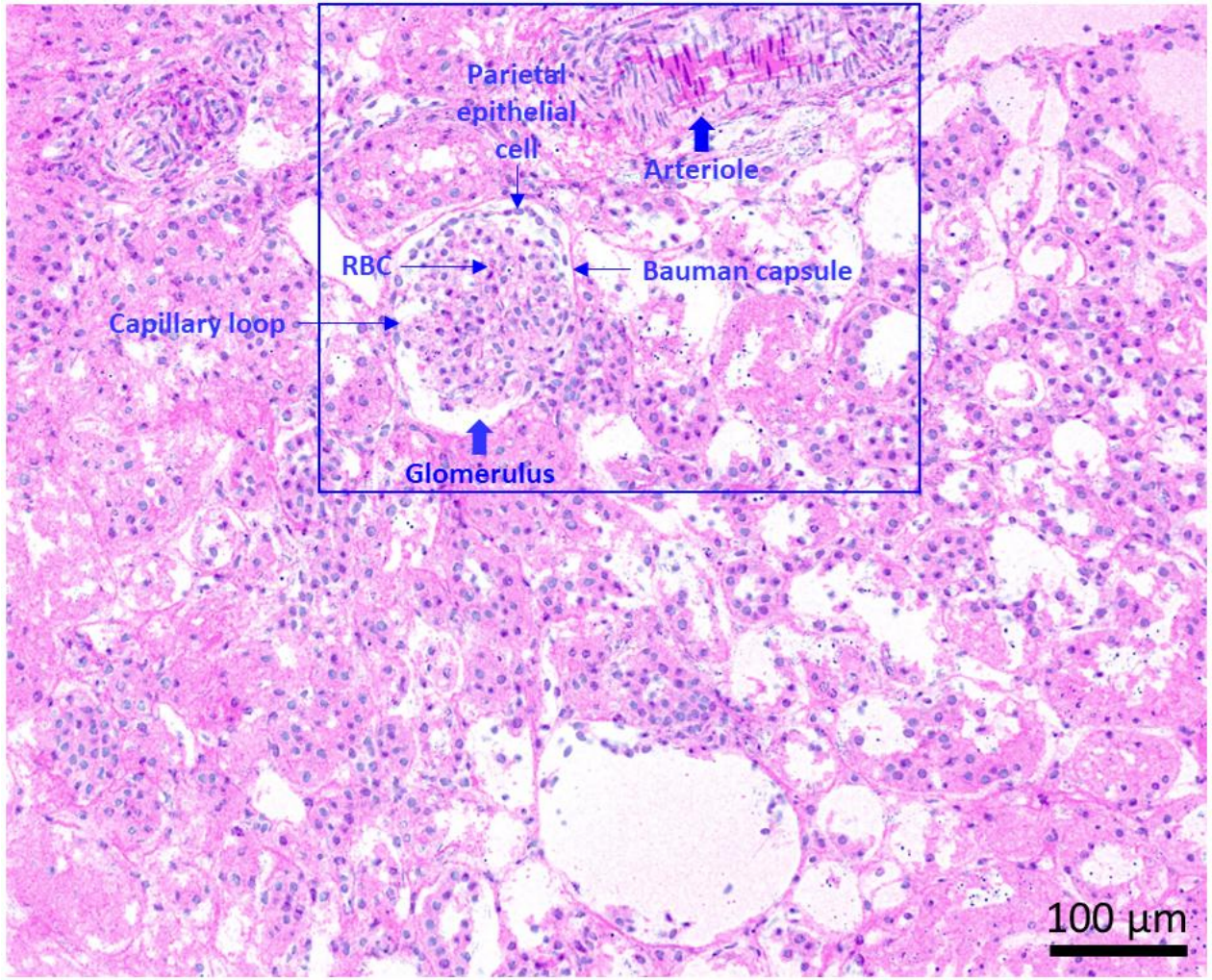
3/1 ~ 3/5, 2020 Meeting of United States & Canadian Academy of Pathology (USCAP 2020) 美國洛杉磯

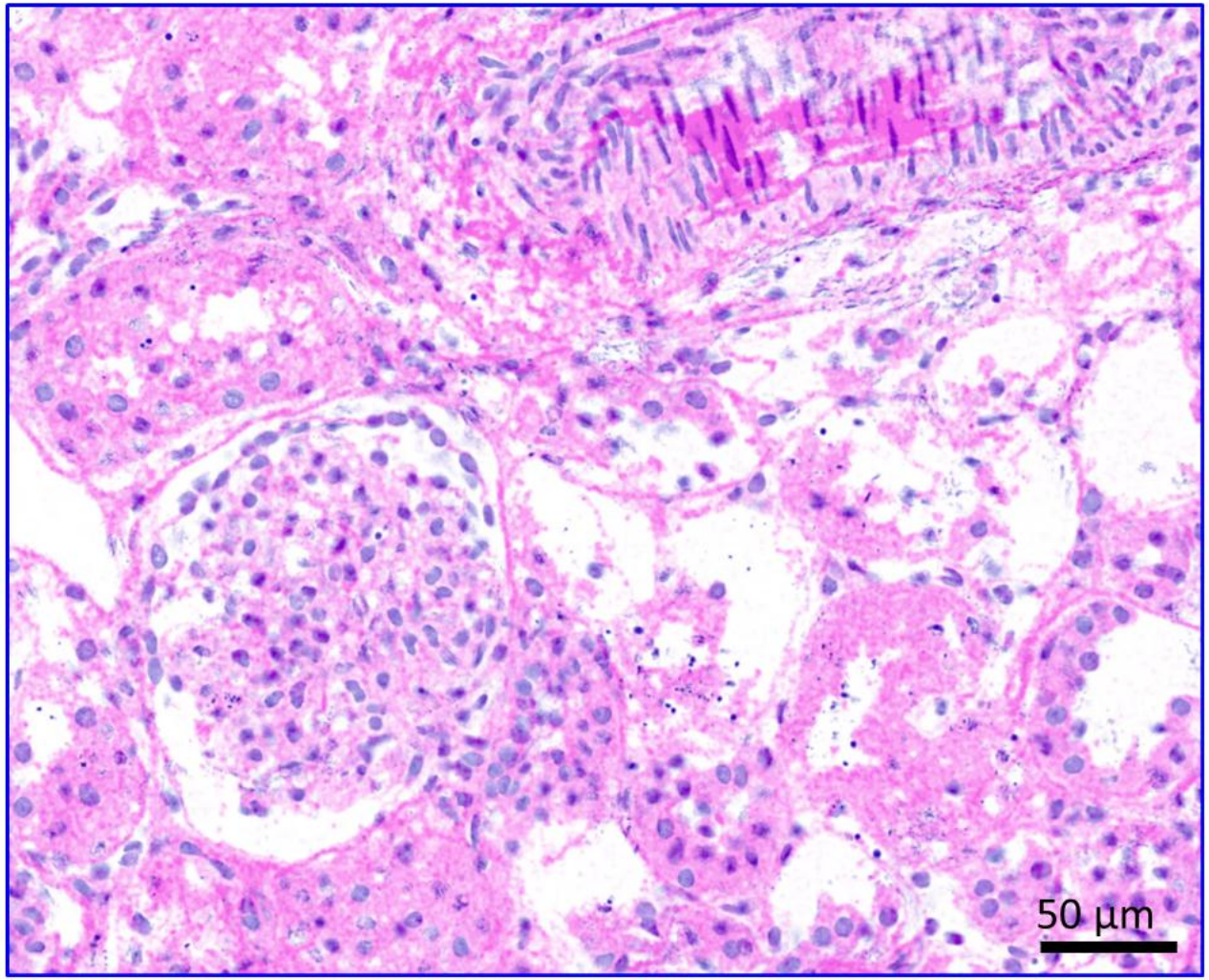
Digitimes: https://www.digitimes.com.tw/tech/dt/n/shwnws.asp?cnlid=13&id=0000580988_AWD8JYWN9WCF5H7SWNKUW

與京都大學共同研究之主題為廣範圍檢體之新鮮（由解剖組織取得）與福馬林固定(由外科腫瘤組織取得)組織，各取得超過100例之影像。於各部位器官之中取得不同種類之一般組織與腫瘤，與同樣本之FFPE H&E 相似度高且可診斷。

ACUSOLUTIONS

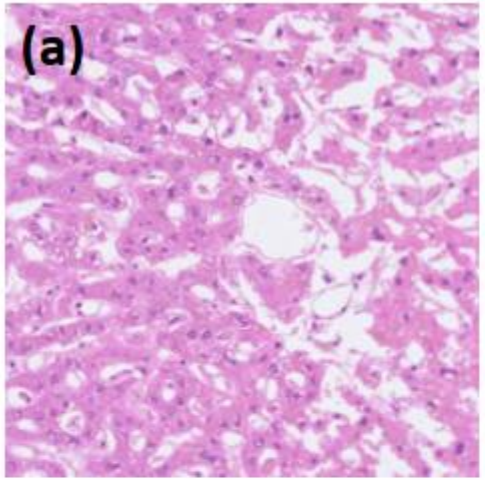
新鮮豬腎 (動物醫院驗證)

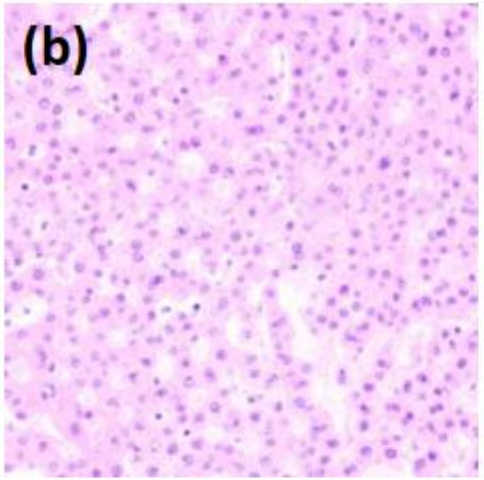




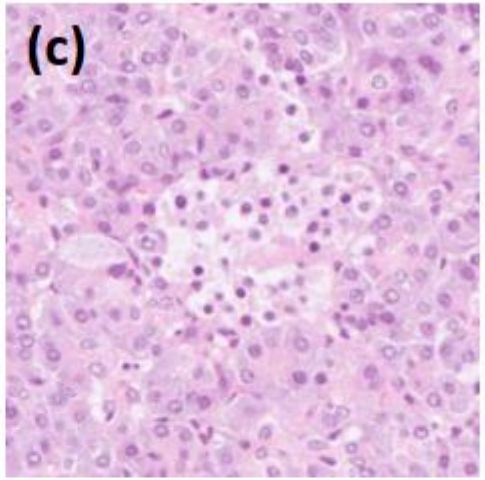
人類正常組織

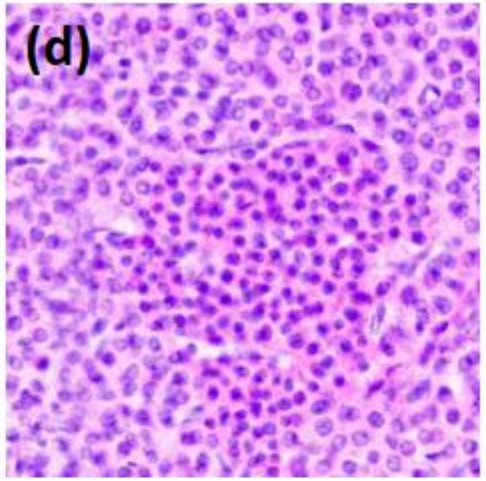
肝臟組織



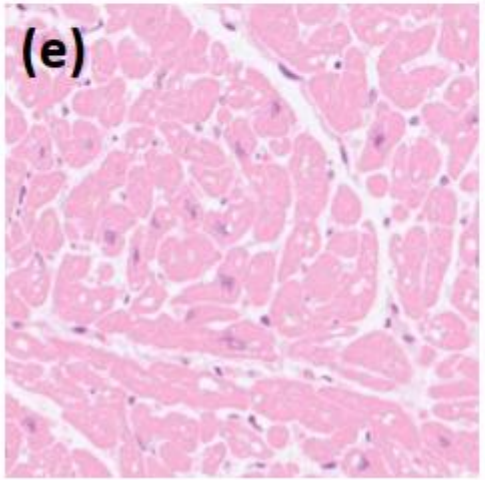


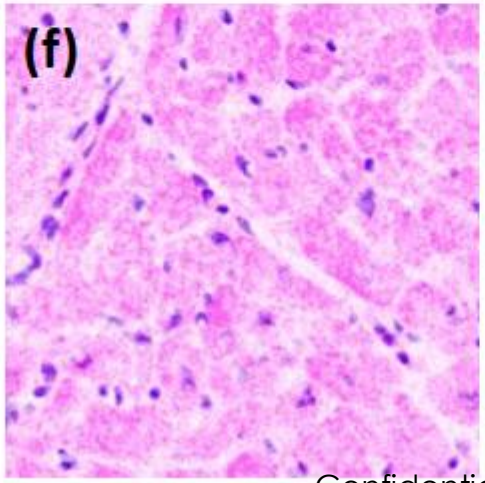
胰臟組織（圖中央為蘭氏小島）



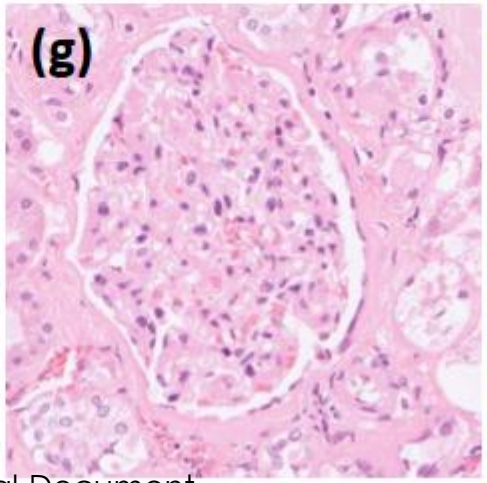


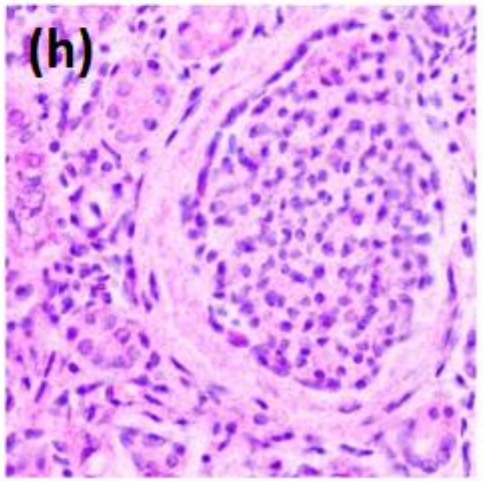
肌肉組織





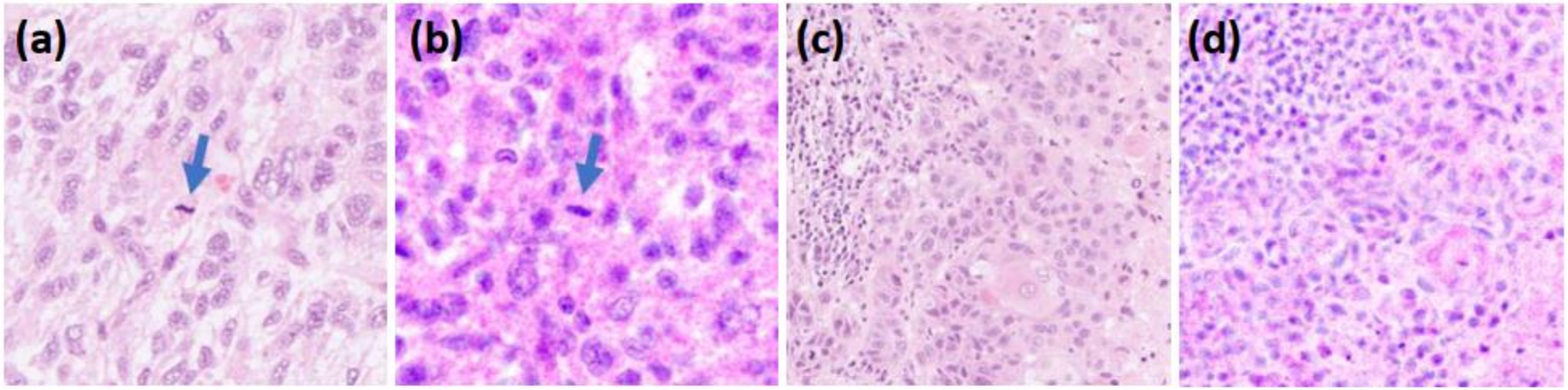
腎臟組織（圖中央為腎絲球）



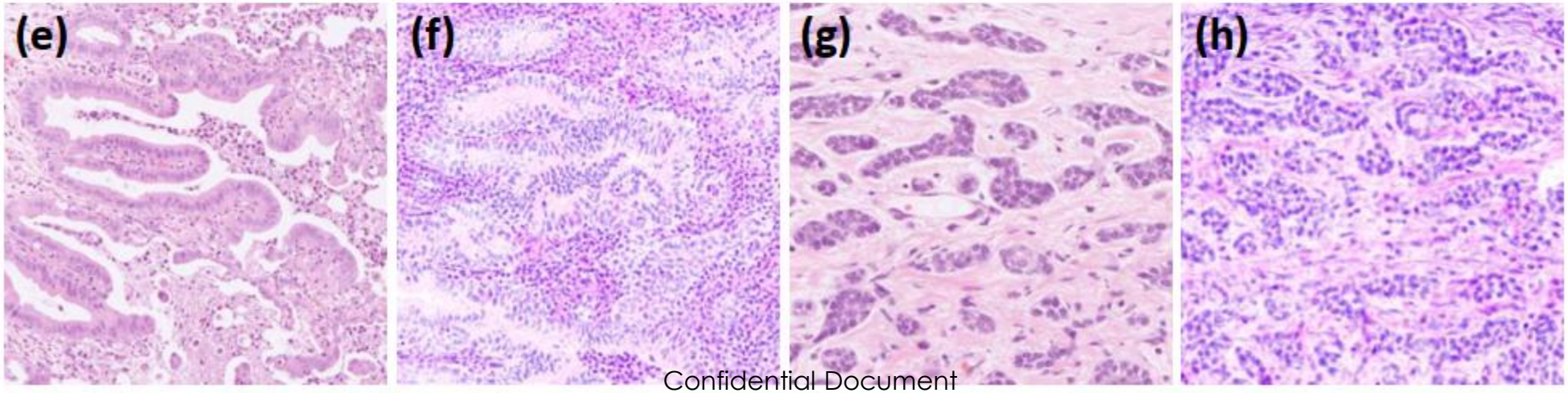


人類腫瘤組織

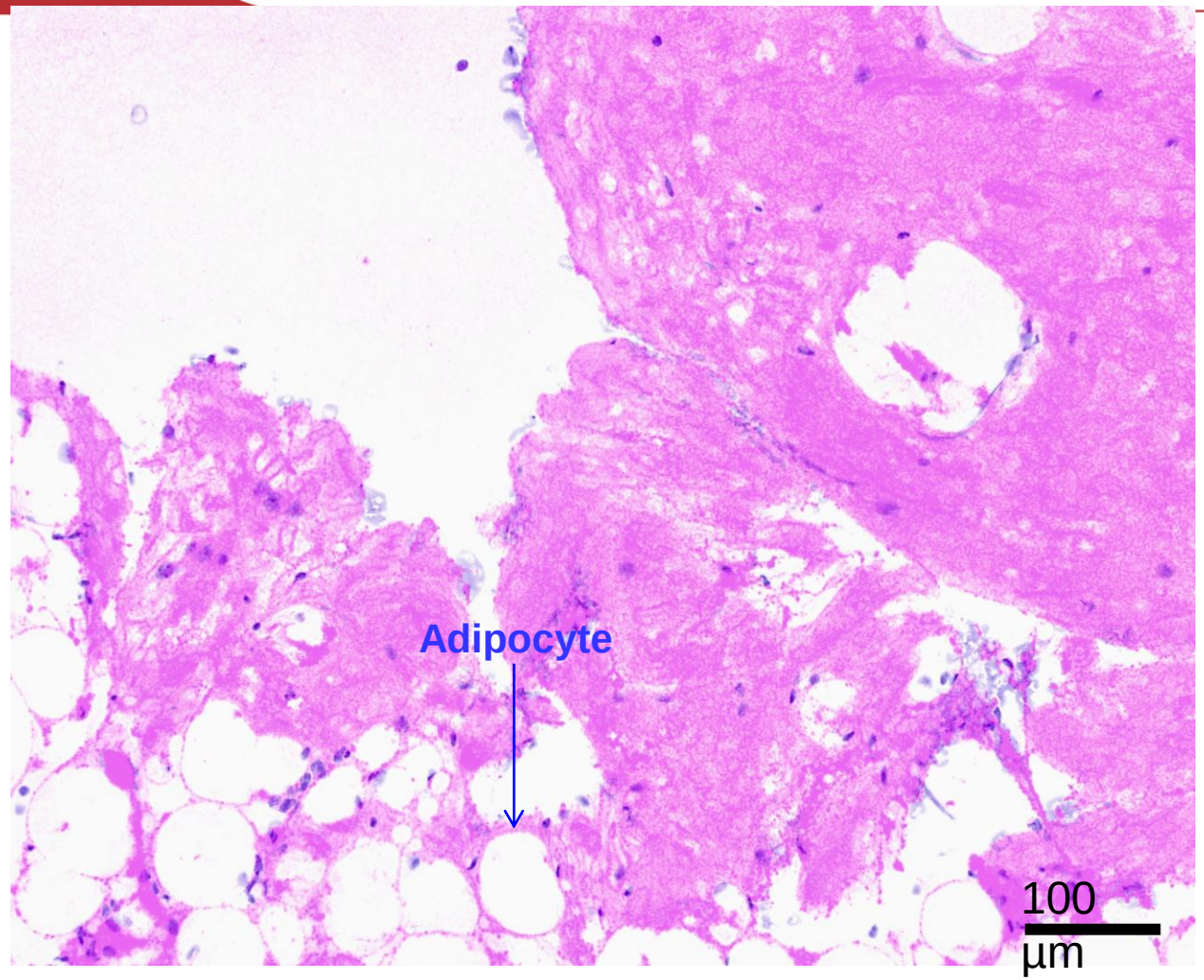
肉瘤 (sarcoma，箭頭處為 mitosis 特徵)　　鱗狀上皮癌 (squamous cell carcinoma)



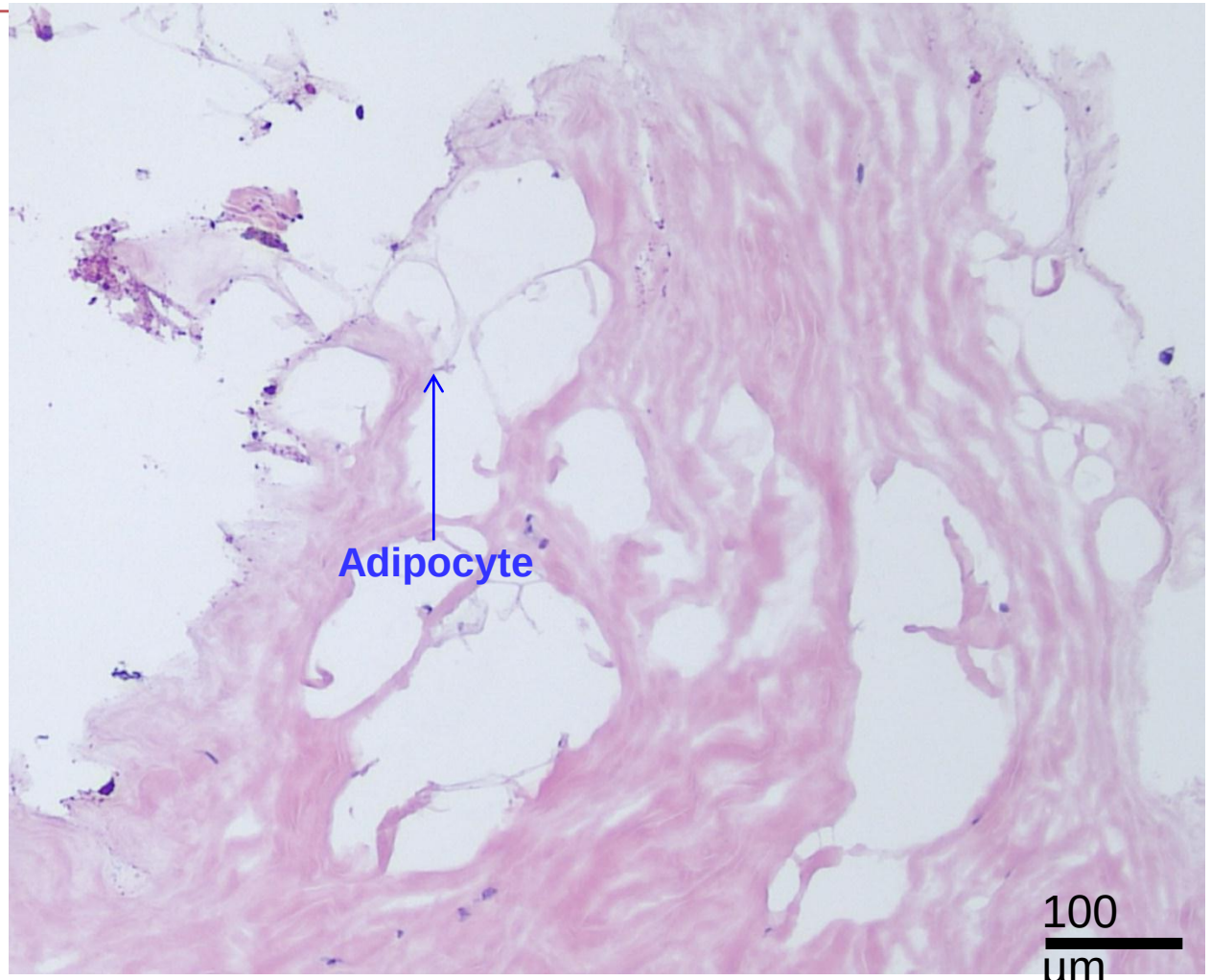
腺癌 (adenocarcinoma)　　腺樣囊狀癌 (adenoid cystic carcinoma)



入庫前乳腺結締/脂肪組織 (北醫Biobank)

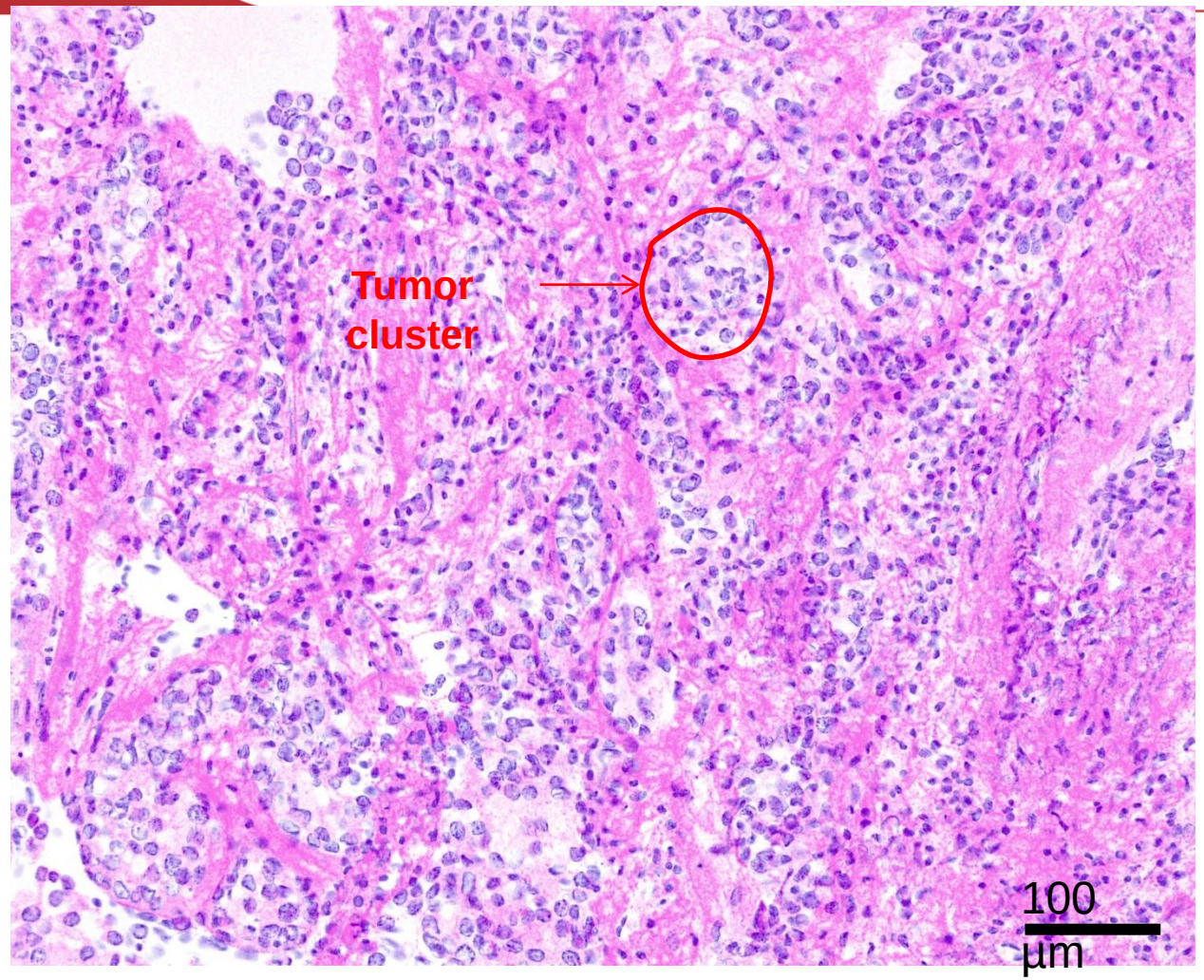


光學切片



冷凍切片

入庫前乳腺腫瘤組織 (北醫Biobank)



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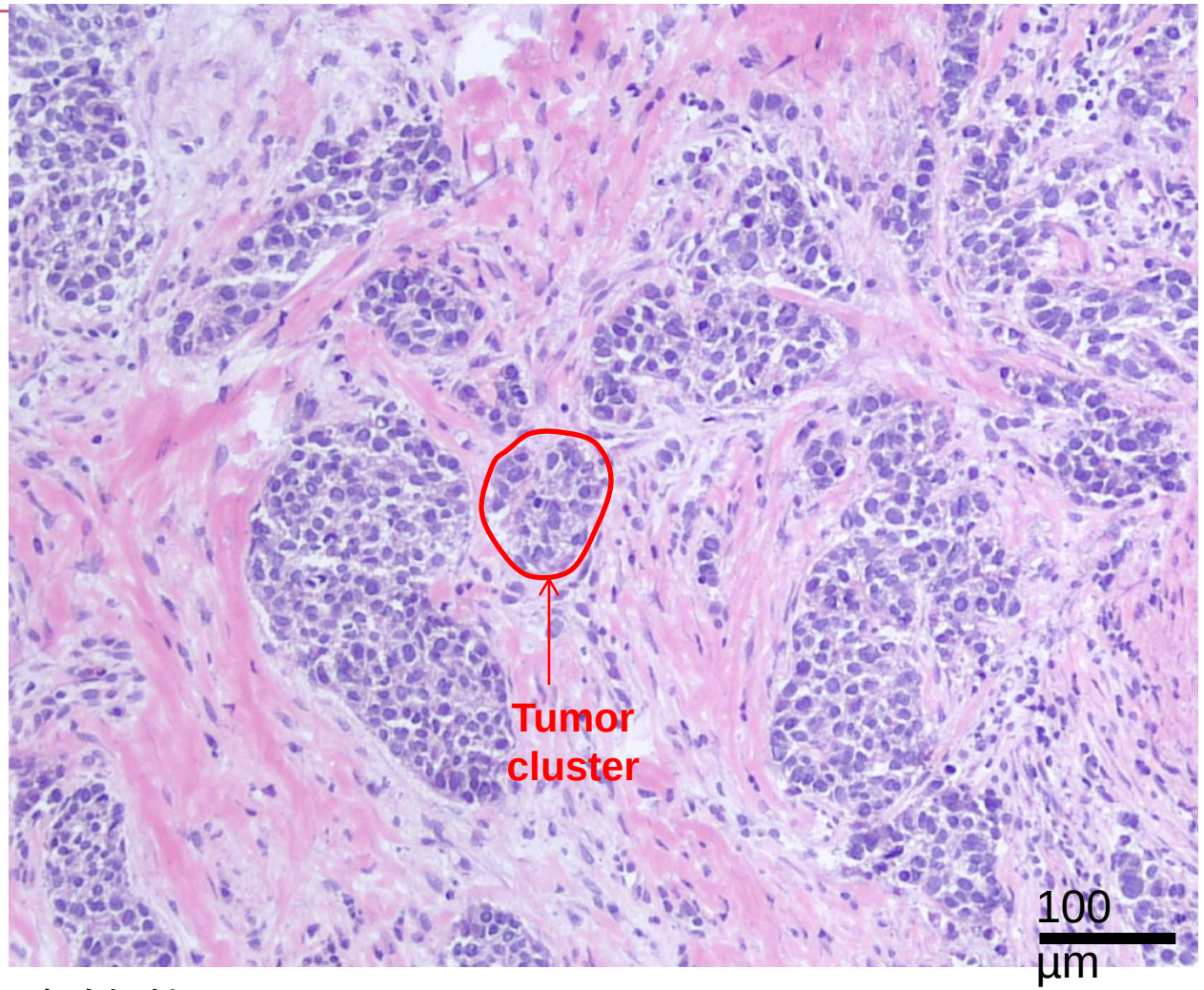
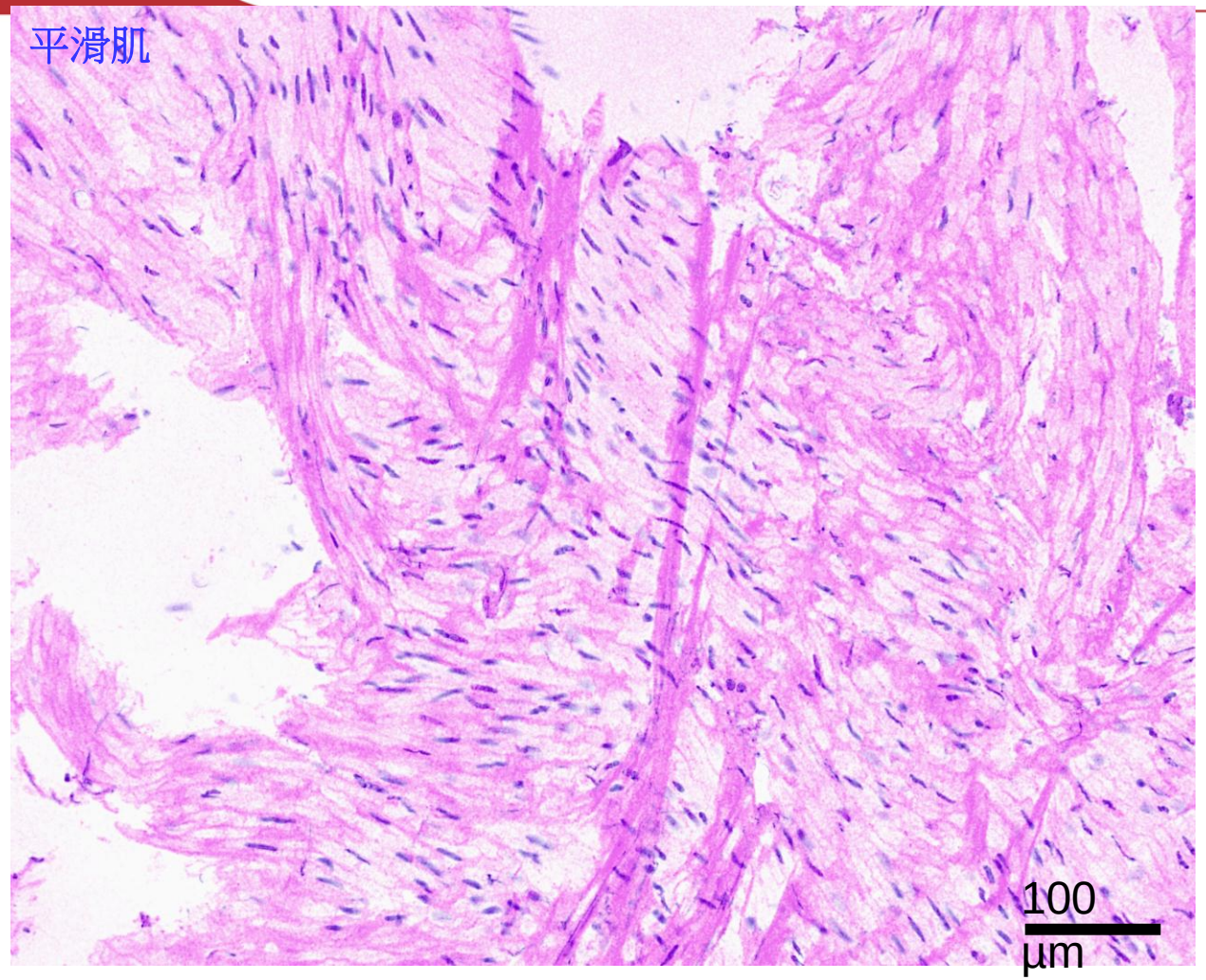
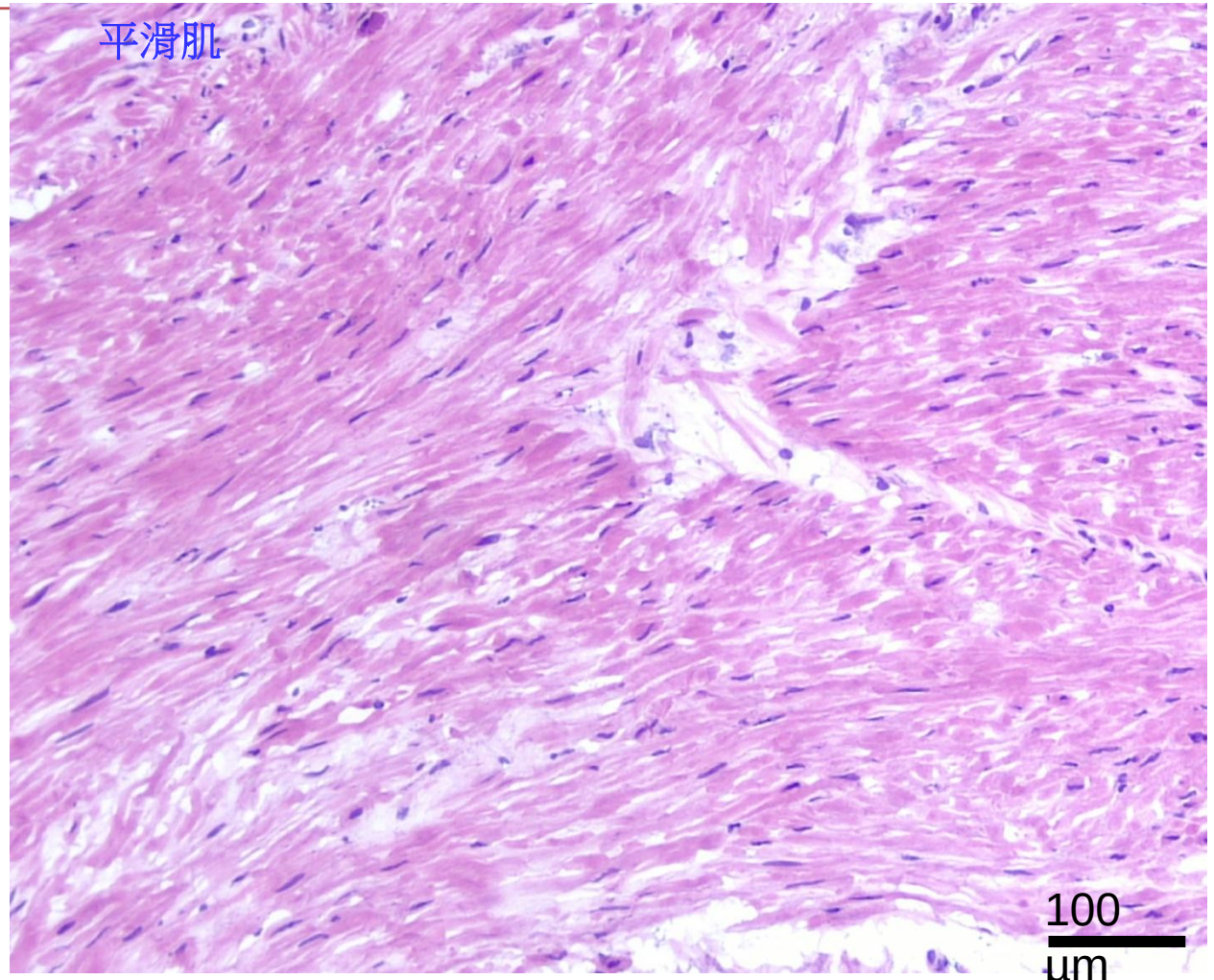


冷凍切片

入庫前大腸結締組織 (北醫Biobank)

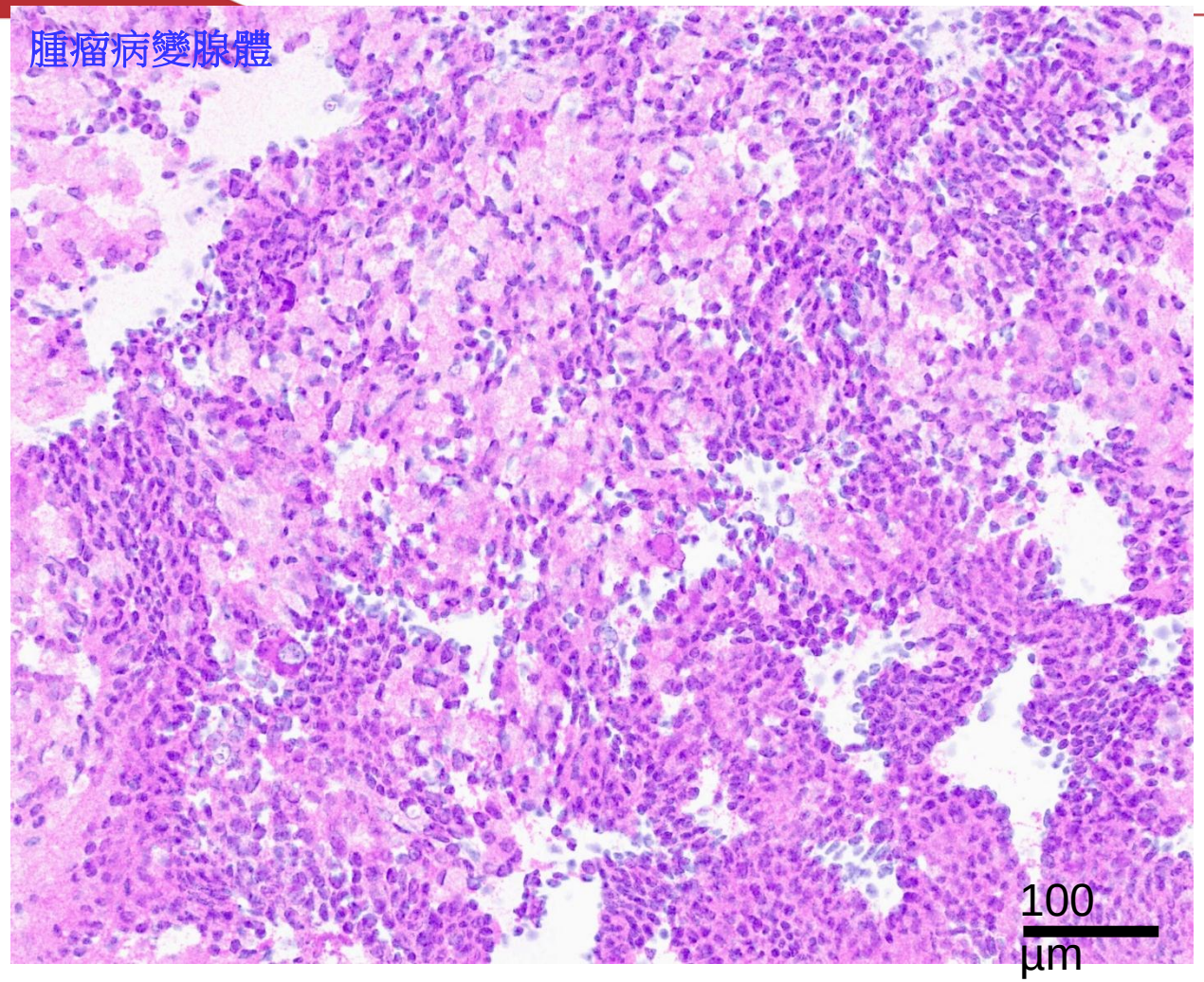


光學切片



冷凍切片

入庫前大腸腫瘤組織 (北醫Biobank)



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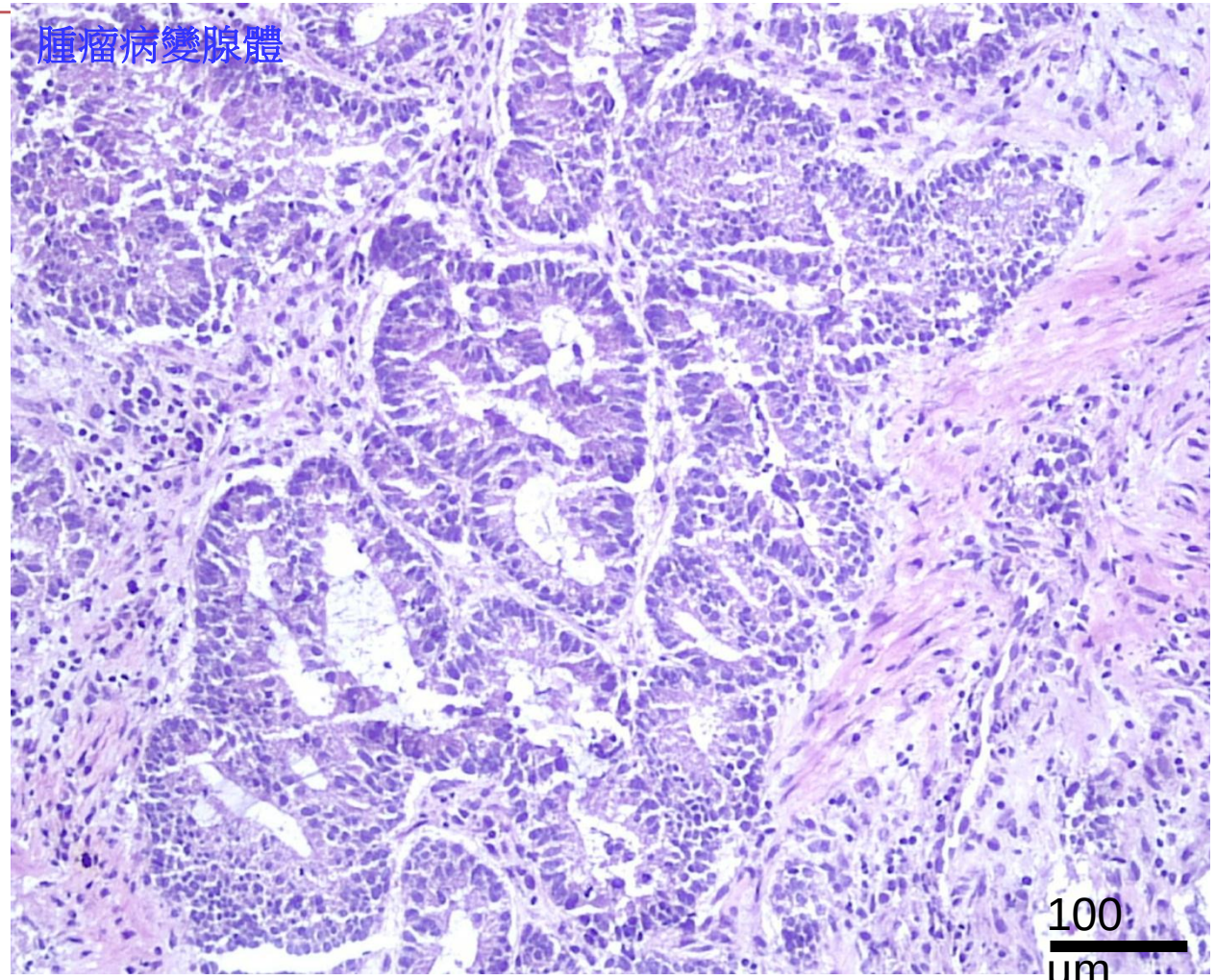
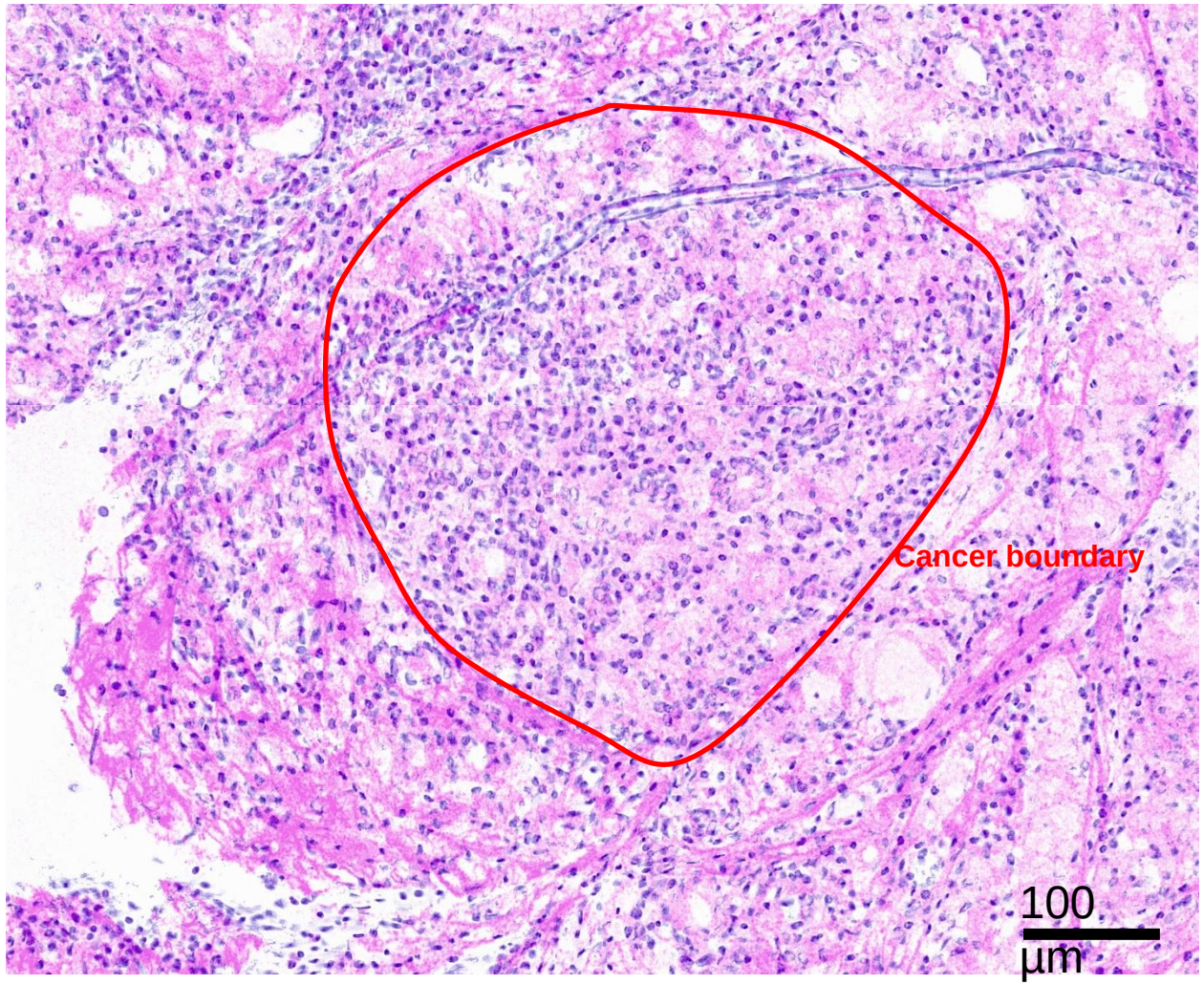
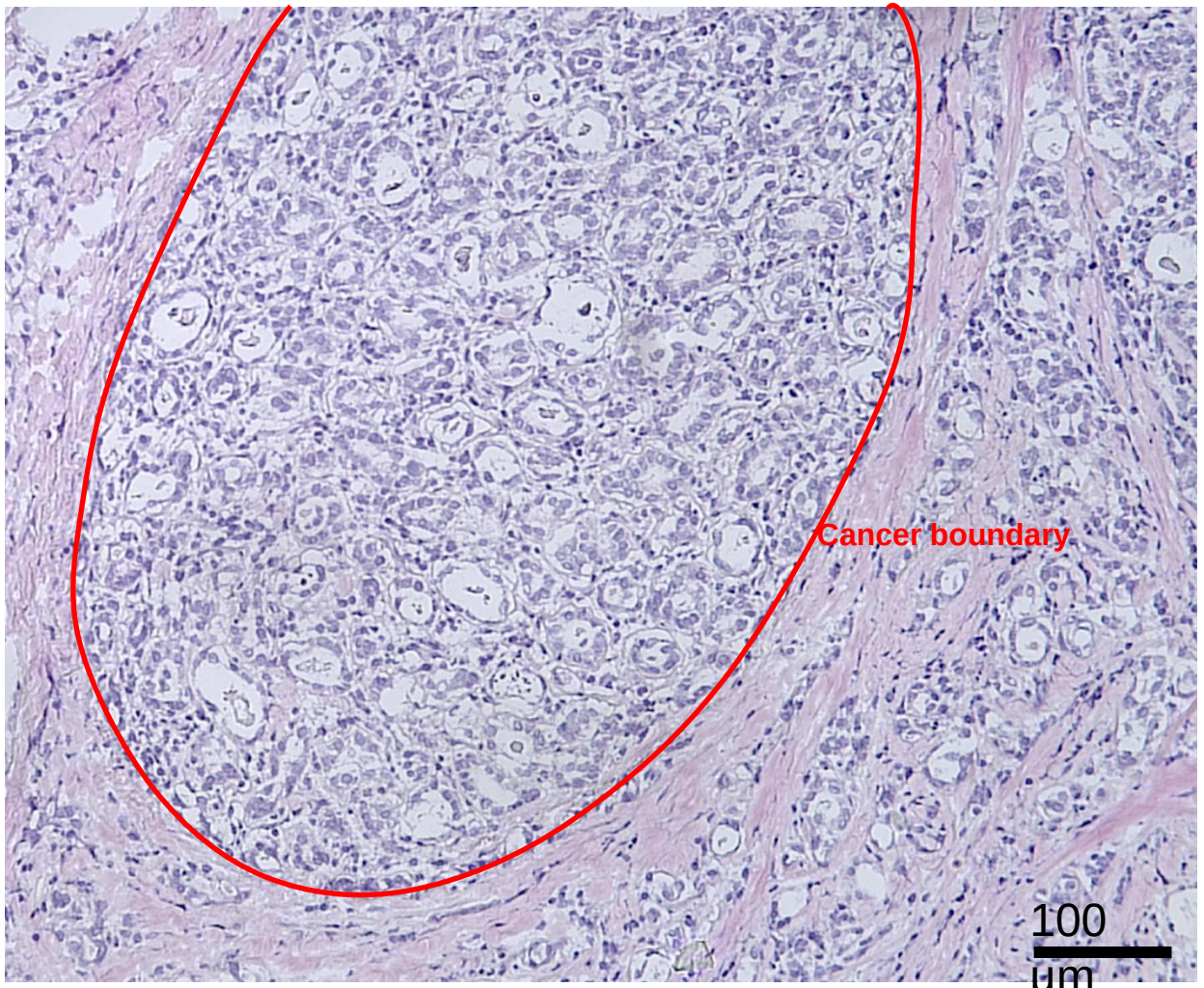


冷凍切片

入庫前甲狀腺腫瘤 (北醫Biobank)

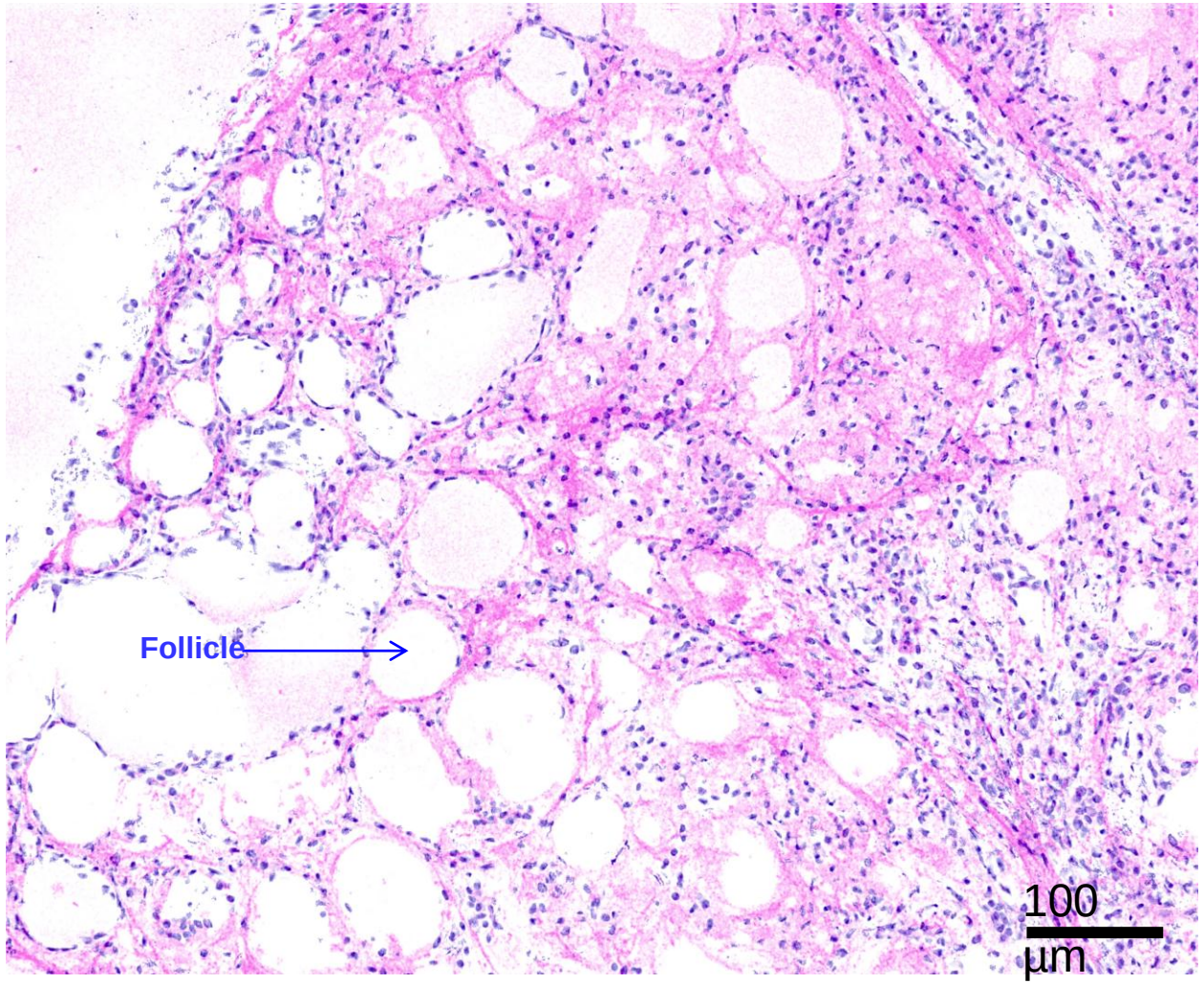


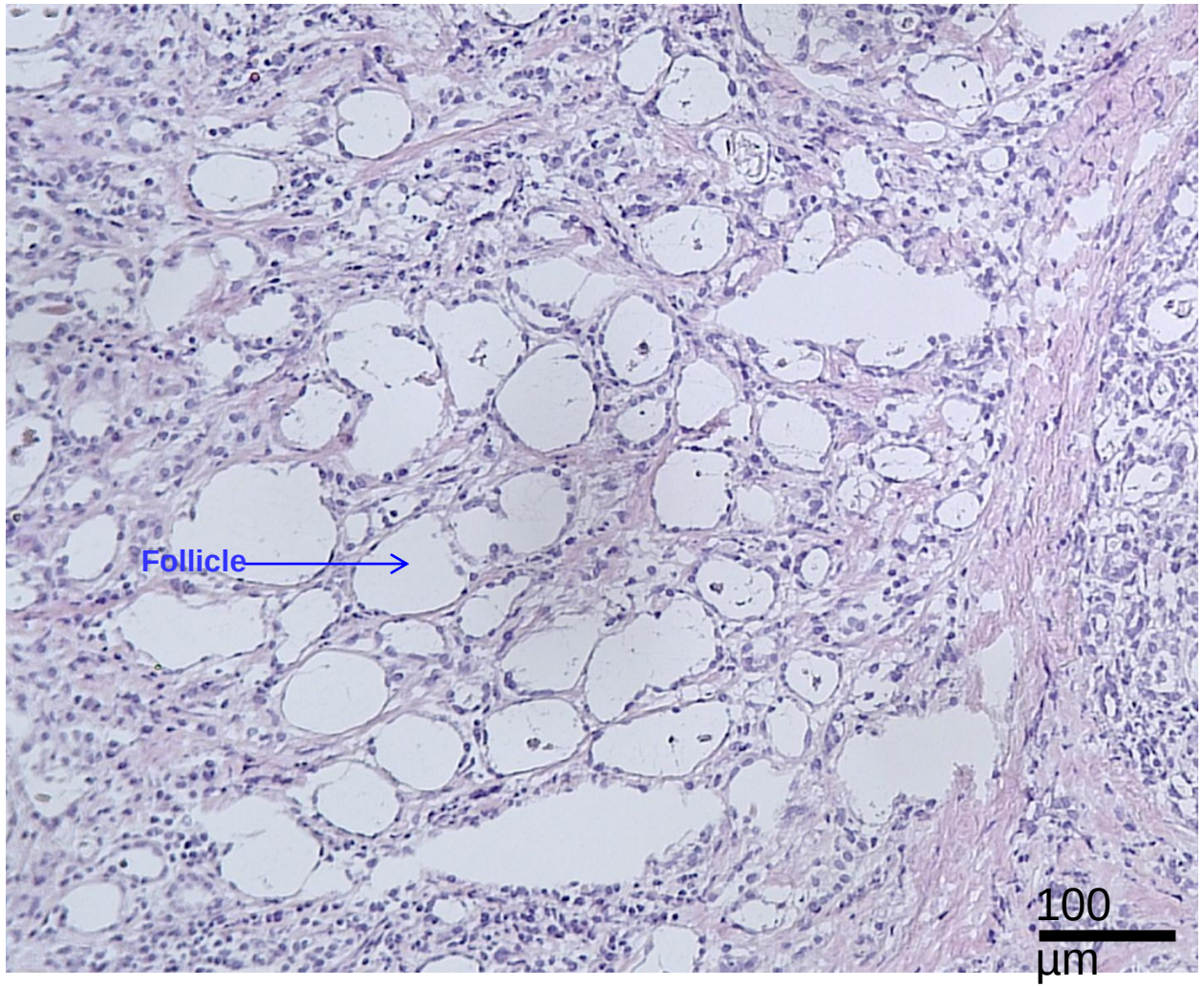
光學切片



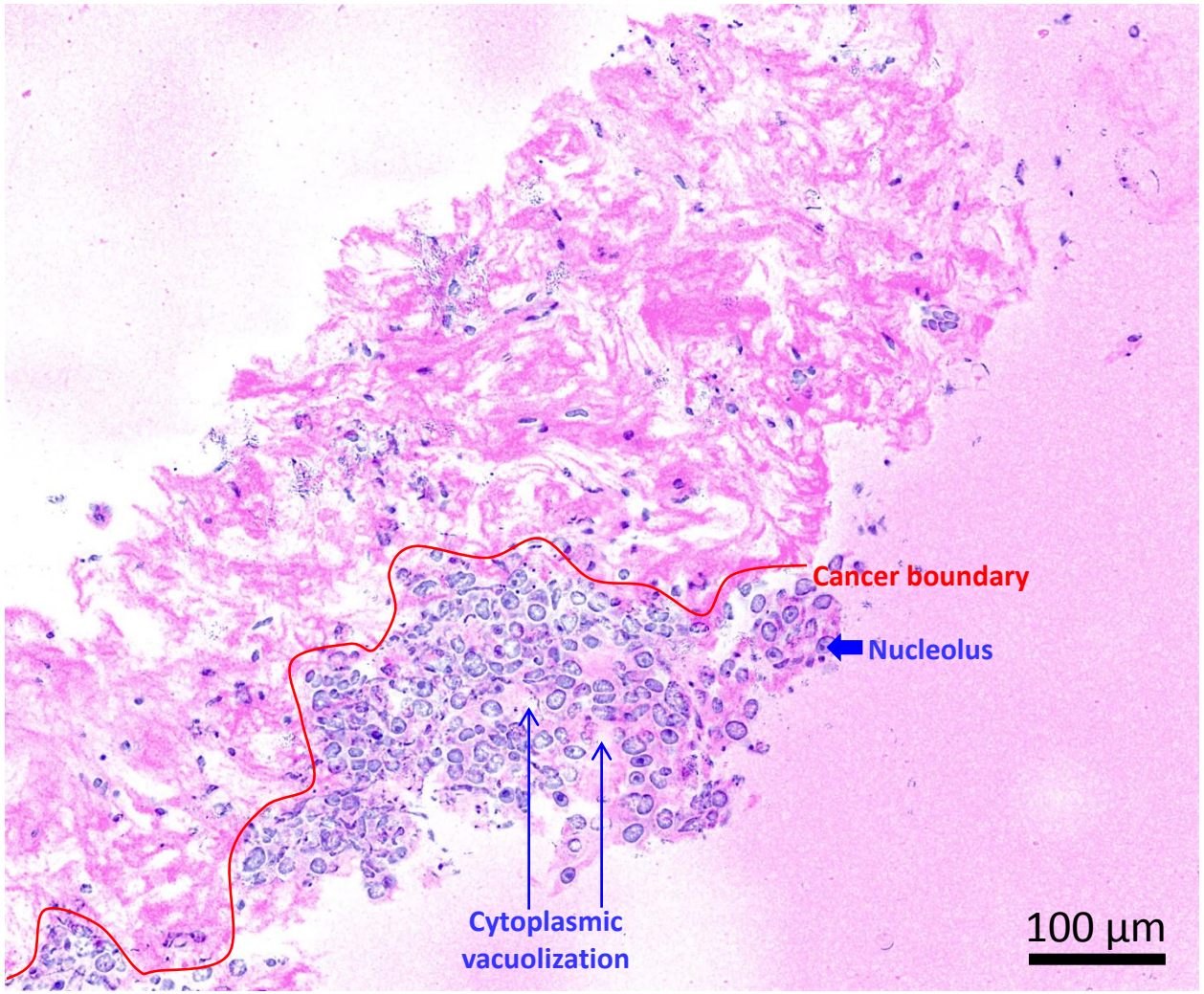
石蠟切片

入庫前甲狀腺正常組織 (北醫Biobank)





醫學驗證 – 乳房門診新鮮組織穿刺試驗 (乳房外科)



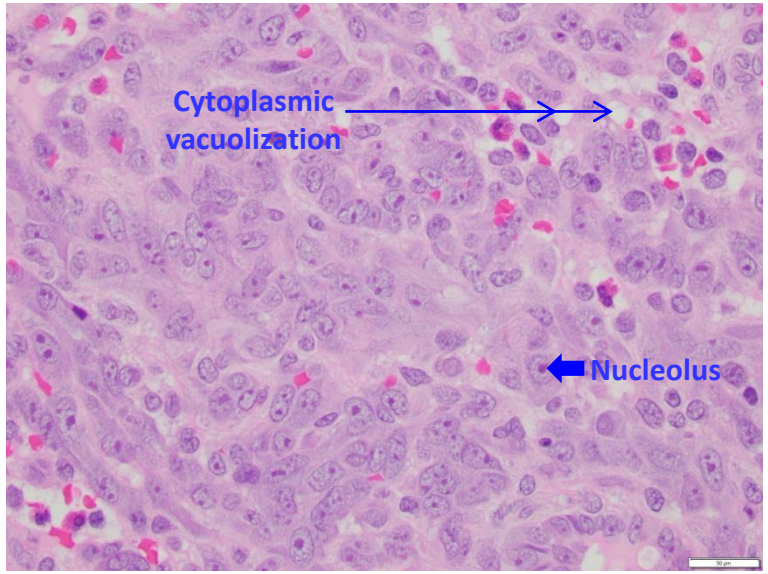
Invasive ductal carcinoma

		Pass
No. of cases		127/134
%	準確度	94.8

敏感度：95.8%；特異性：87.3%

目前已分析300個案例之中的134個

Invasive ductal carcinoma (石蠟切片 H&E)



https://www.urmc.rochester.edu/pathology-labs/education/pathology-now/case-33-painless-breast-nodule.aspx

總結

- **類H&E光學切片應用於生物資料庫之組織檢體品質保證：**
  - 操作簡便可增加生物資料庫QA/QC意願
  - 節省至少三成儲存耗材及設備空間
  - 操作技術門檻較冷凍切片低，克服脂肪及冰晶問題
  - 可減少組織之浪費以及增加品質確認之準確度
  - 數位化病理影像未來可隨同檢體出庫及具有AI潛力
  - 未來有機會發展於臨床手術中及切片採檢之病理初步判讀