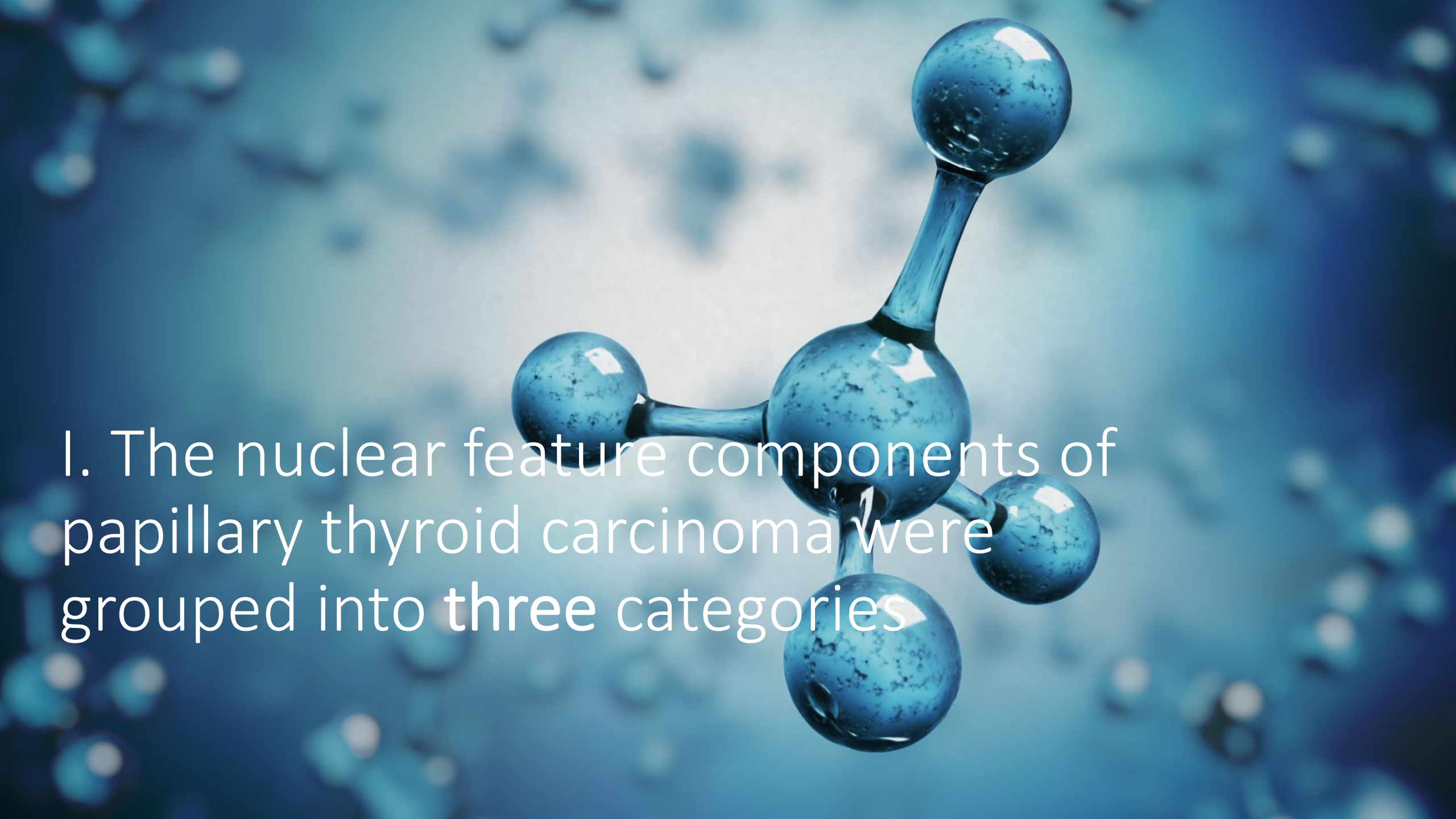
In-depth Tutorial Module: Semiquantitative Assessment PTC-like Nuclear Features on Thyroid Tumor

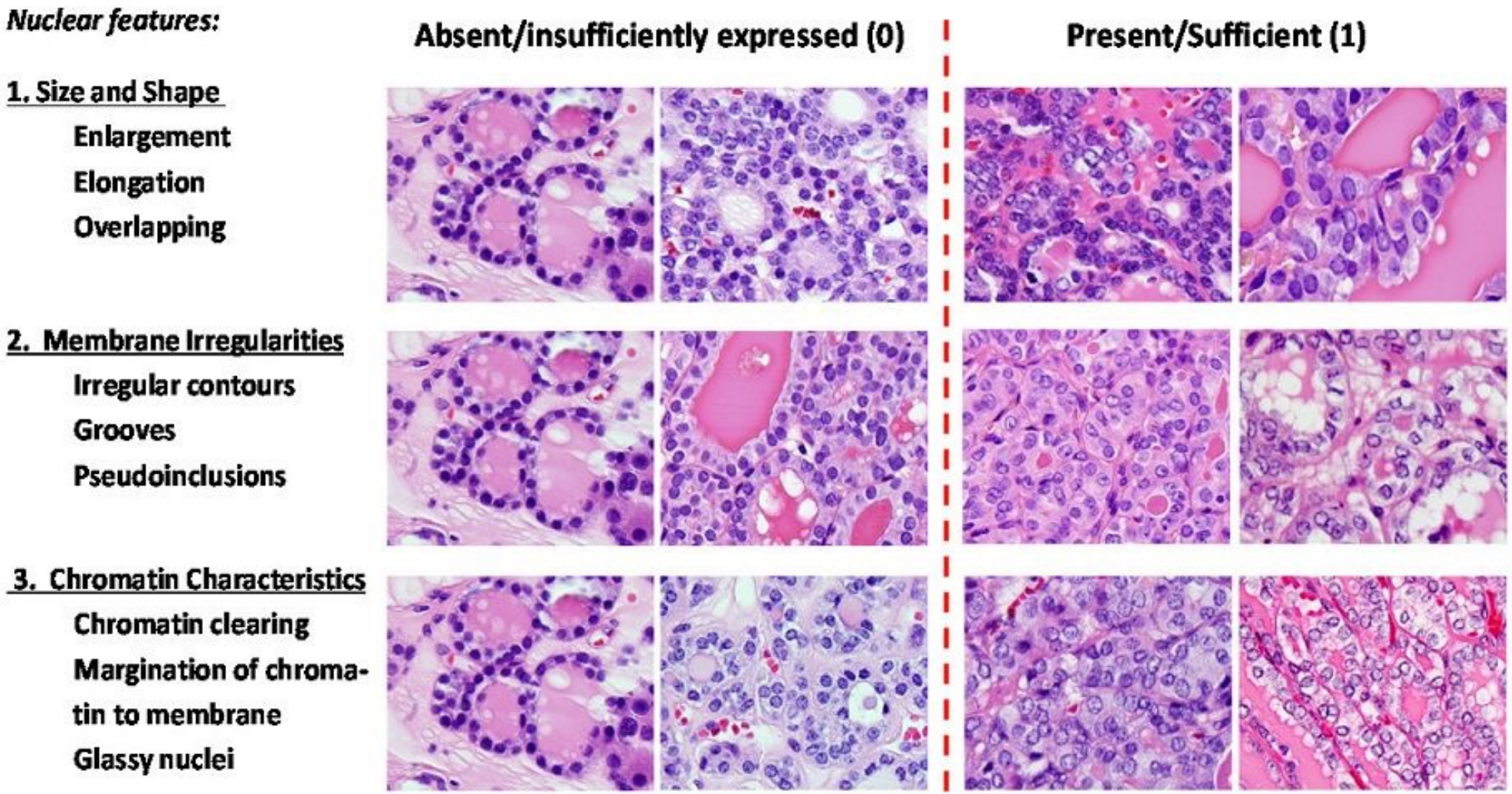
Indonesian Thyroid Multicenter Research



I. The nuclear feature components of papillary thyroid carcinoma were grouped into **three** categories

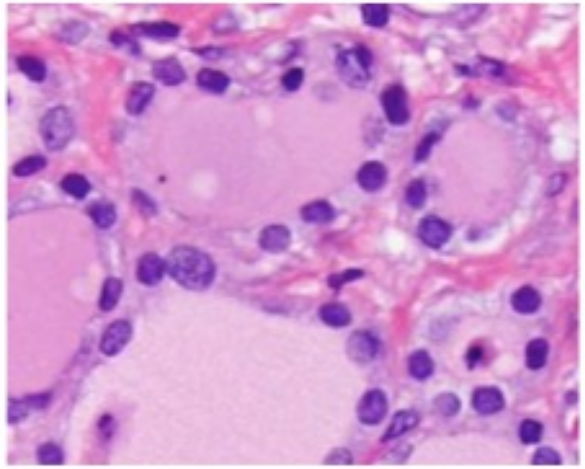
PTC nuclear features

1. Nuclear size and shape: nuclear enlargement, elongation, overlapping, and crowding (Fig. 1).
2. Nuclear membrane irregularities: irregular contours, nuclear grooves, nuclear folds, and intranuclear cytoplasmic inclusions (Fig. 2).
3. Nuclear chromatin characteristics: chromatin clearing, margination to the membranes, and glassy nuclei with fine even delicate chromatin (Fig. 3).



https://doi.org/10.1038/modpathol.2017.130

Normal for reference



Nuclear features:

1. Size and Shape
Enlargement; Elongation; Overlapping — 1=Yes

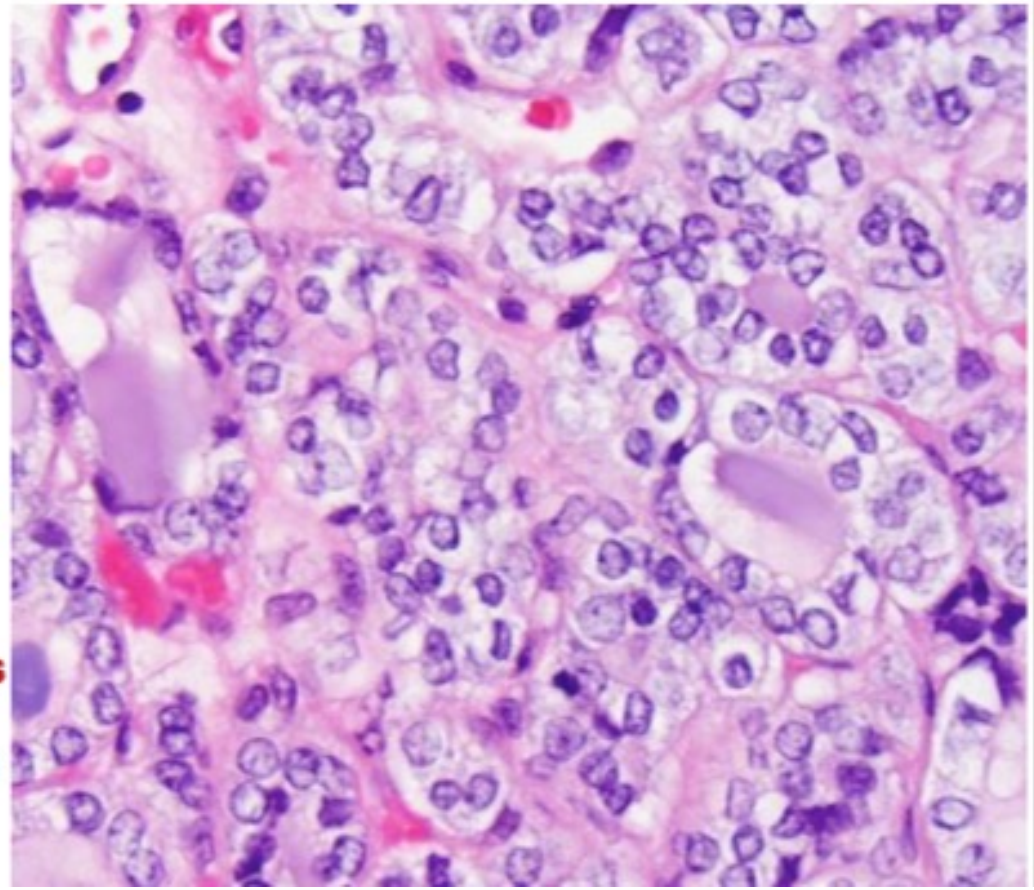
2. Membrane Irregularities
Irregular contours; grooves
Pseudoinclusions — 1=Yes

3. Chromatin Characteristics — 1=Yes
Chromatin clearing; Margination of chromatin to membrane; Glassy nuclei

Total=3

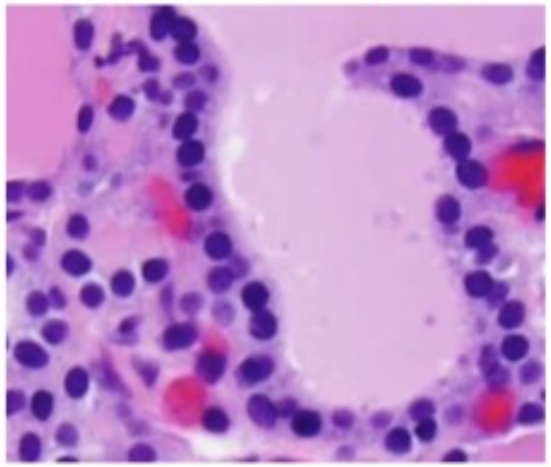
Non-invasive nodule
Evaluation of nuclei

YES "papillary like nuclei"= NIFTP



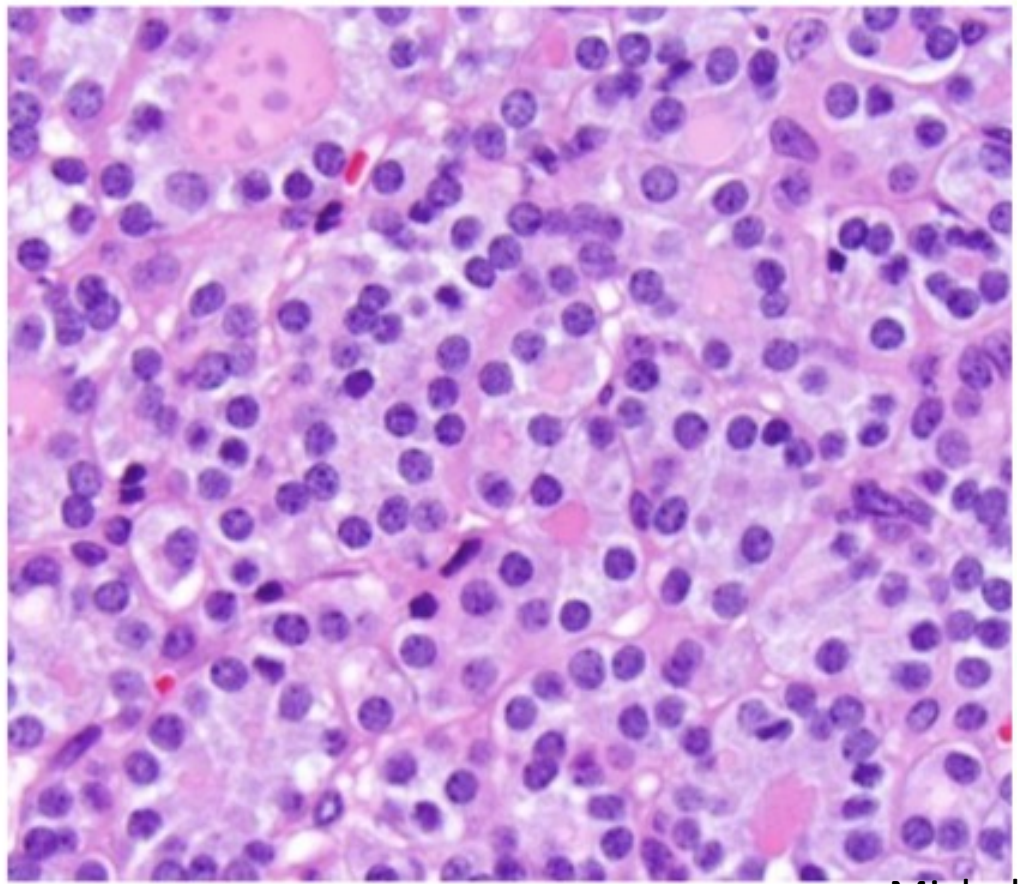
Michelle Williams, APIAP 2017

Normal for reference



Non-invasive nodule
Evaluation of nuclei

No "papillary like nuclei", NOT NIFTP



Nuclear features:

1. Size and Shape
Enlargement; Elongation; Overlapping — **0=Slight (insufficient)**

2. Membrane Irregularities
Irregular contours; grooves; Pseudoinclusions — **0=Slight (insufficient)**

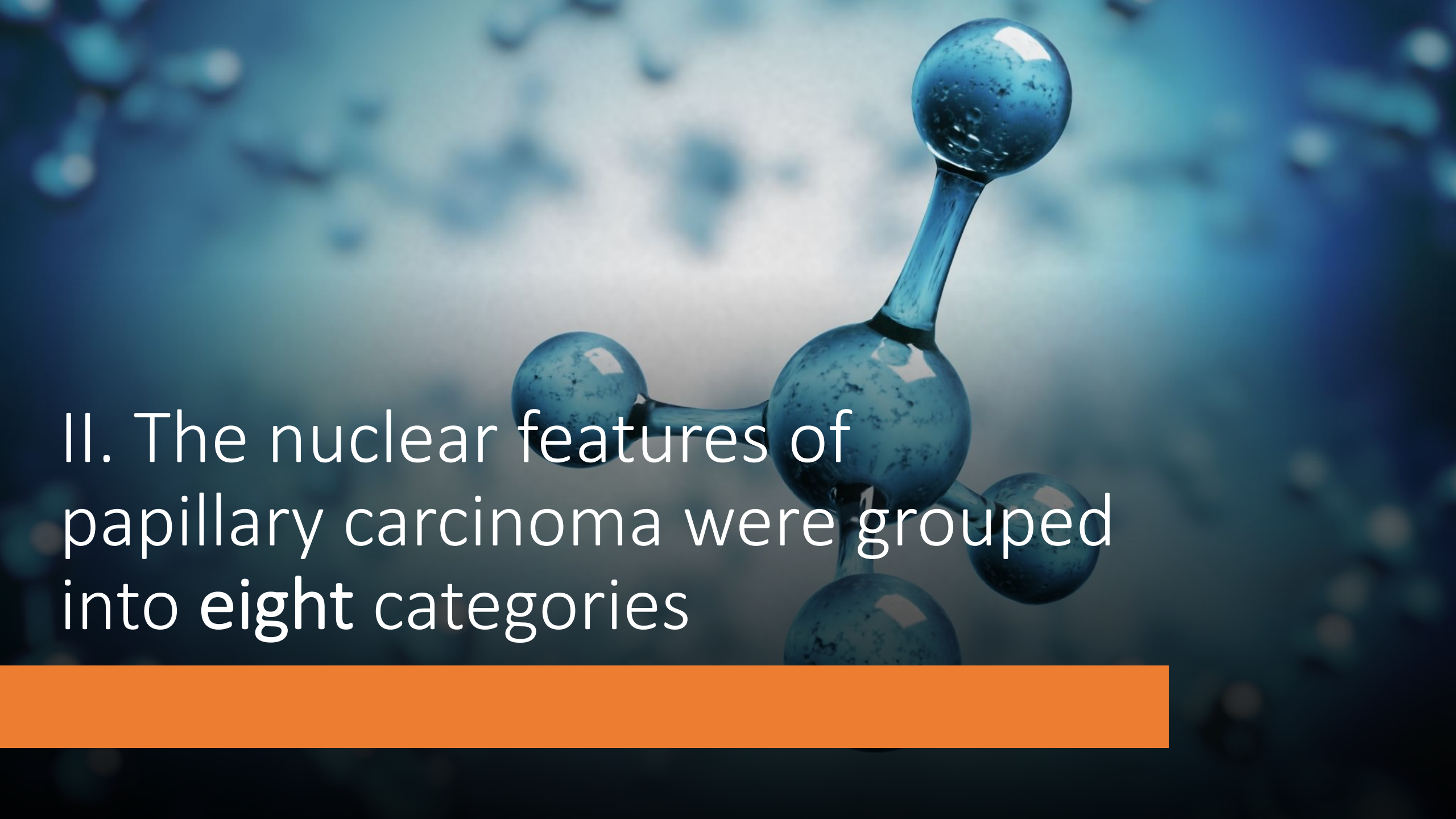
3. Chromatin Characteristics — **0= No**
Chromatin clearing; Margination of chromatin to membrane; Glassy nuclei

Total=0

Michelle Williams, APIAP 2017

Three point scoring system

	A	B	C	D	E	F	G	H	I	J	K	L
		3 POINT			8 POINT							
	CASE NO	Nuclear Size & Shape	Nuclear membran Irregularities	Nuclear Chromatin Characteristic	Nuclear Enlargement	Nuclear Overlapping / Crowding	Nuclear Elongation	Irregular membrane Contour	Nuclear Grooves	Chromatin Clearing	Chromatin Distribution	Nuclear Pseudo-Inclusion
	1											
	2											
	3											
	4											
	5											
	6											
	7											
	8											
	9											
	10											
	11											
	12											
	13											
	14											
		Yes = 0	Yes = 0	Yes = 0	Yes < 10% = 0	Yes < 10% = 0	Yes < 10% = 0	Yes < 10% = 0	Yes < 10% = 0	Yes < 10% = 0	Diffuse = 0	Yes = 0
		No = 1	No = 1	No = 1	Yes > 10% = 1	Yes > 10% = 1	Yes > 10% = 1	Yes > 10% = 1	Yes > 10% = 1	Yes > 10% = 1	Patchy = 1	No = 1
					No = 2	No = 2	No = 2	No = 2	No = 2	No = 2		



II. The nuclear features of papillary carcinoma were grouped into **eight** categories

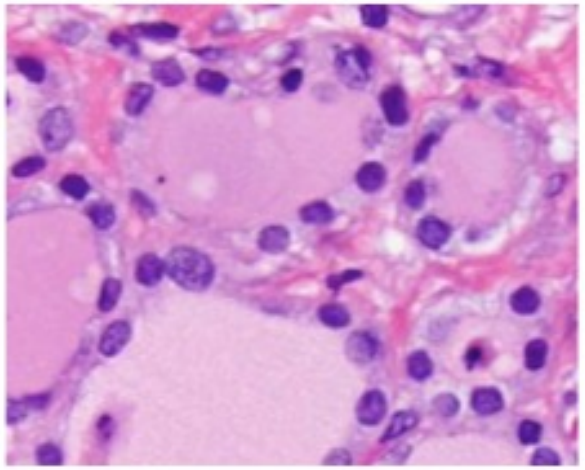
1. Nuclear Enlargement

Definition: Enlarged cell nuclei beyond the size of a normal cell nucleus.

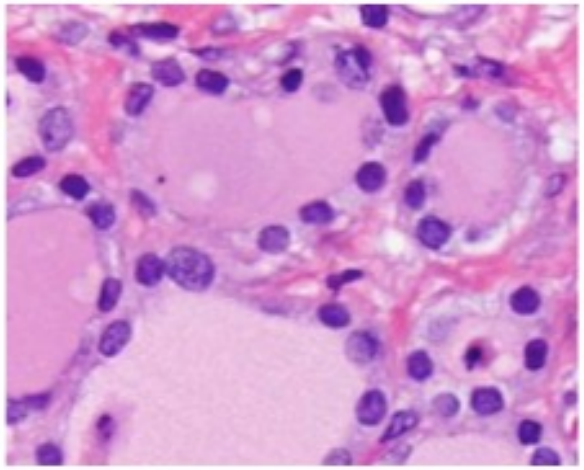
Assessment Method:

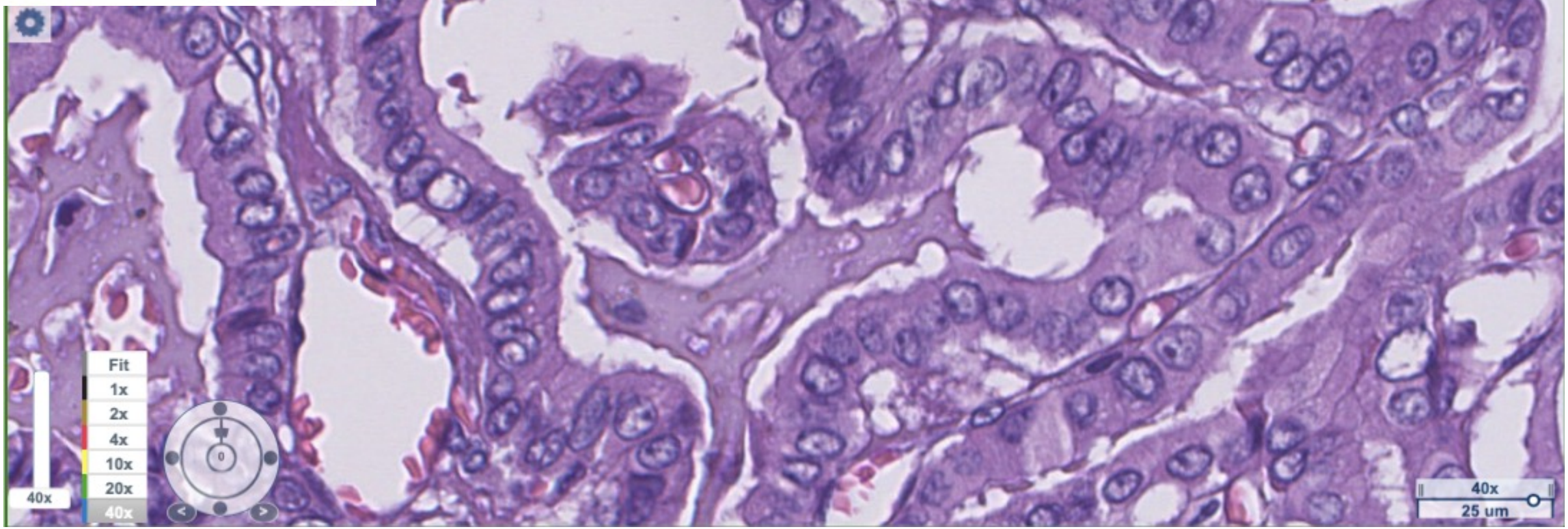
- Compare to lymphocyte cell nuclei as a control.
- Use 40x magnification or more for better detail.

Normal for reference



Normal for reference





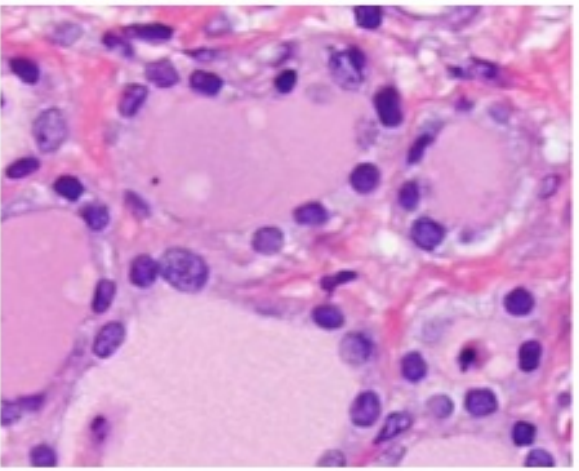
2. Nuclear Crowding/ Overlapping

Definition: Cell nuclei that are closely adjacent or overlapping.

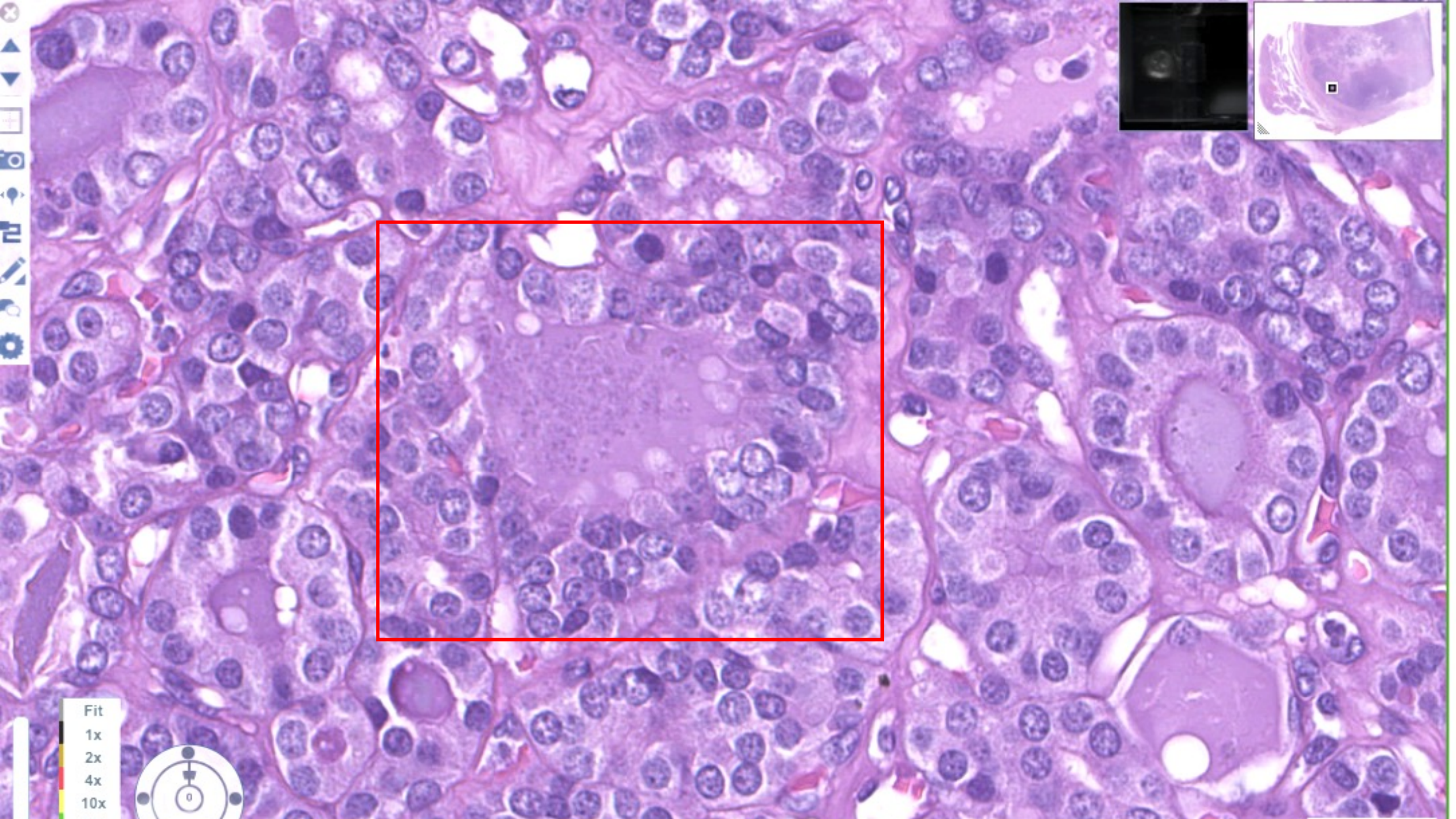
Assessment Method:

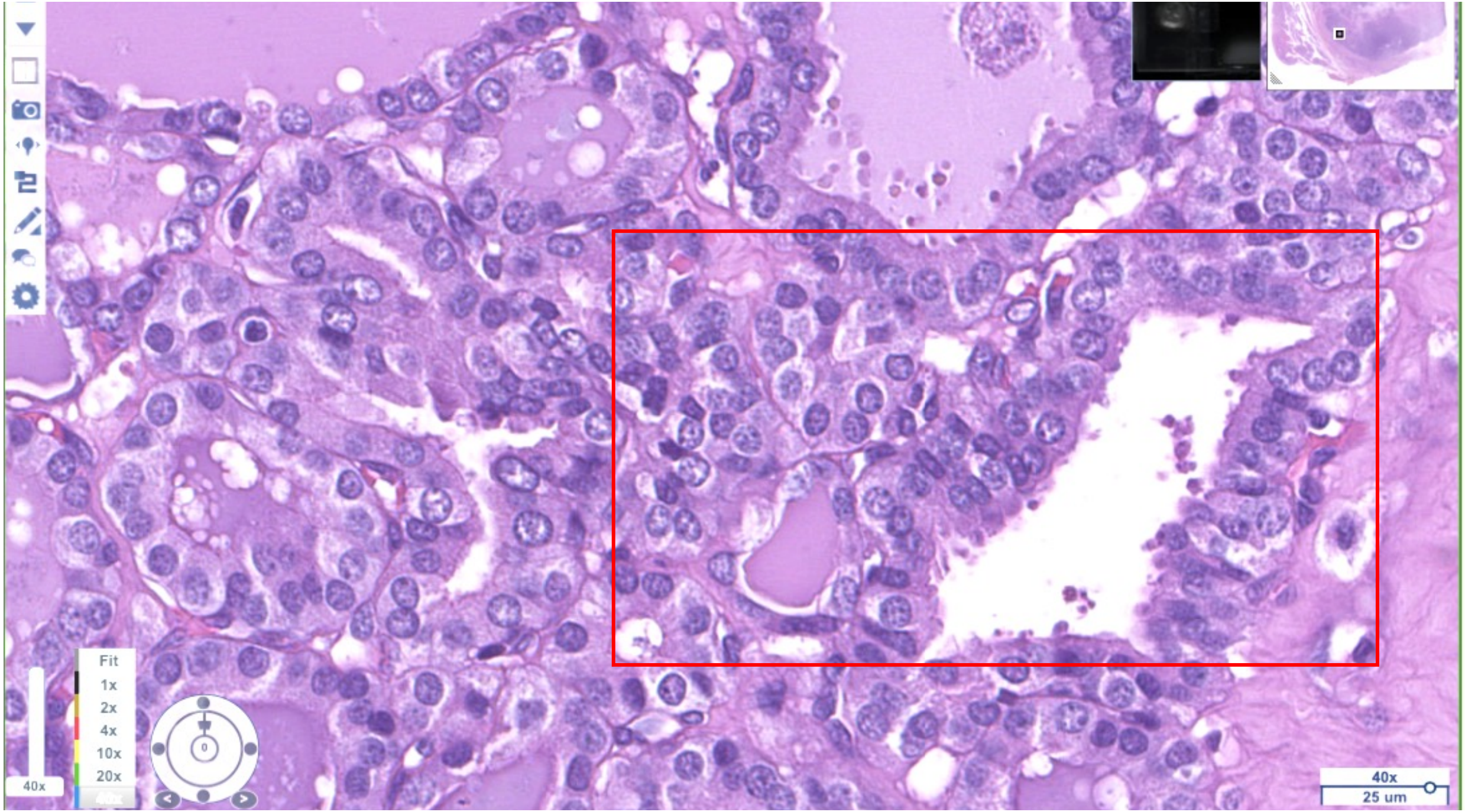
- Focus on areas with dense cells.
- Note the overlap degree and cell distribution.

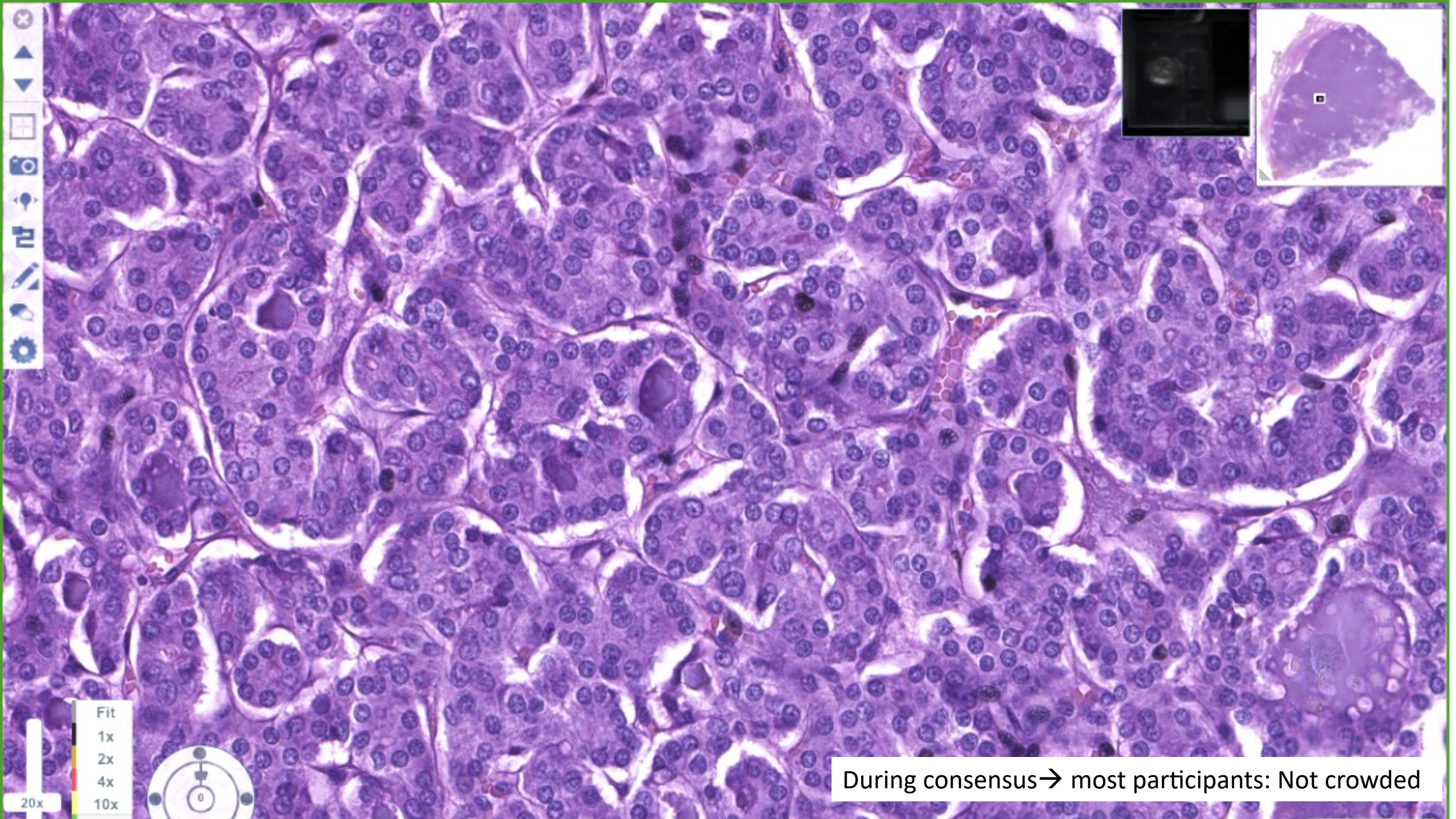
Normal for reference



40x
25 um


Fit
1x
2x
4x
10x


Fit
1x
2x
4x
10x
20x
40x
40x
25 um

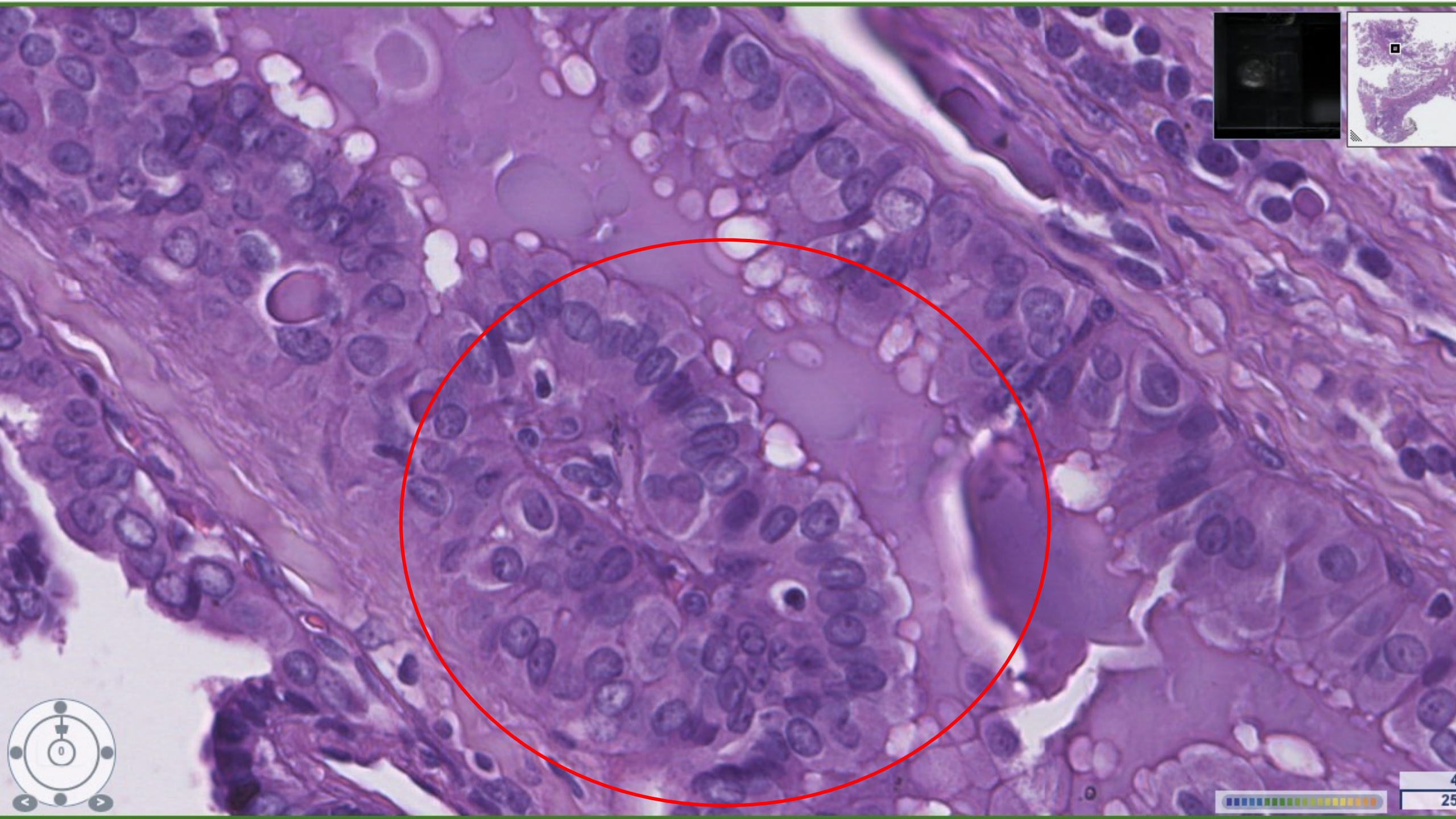

Fit
1x
2x
4x
10x
20x
During consensus→ most participants: Not crowded

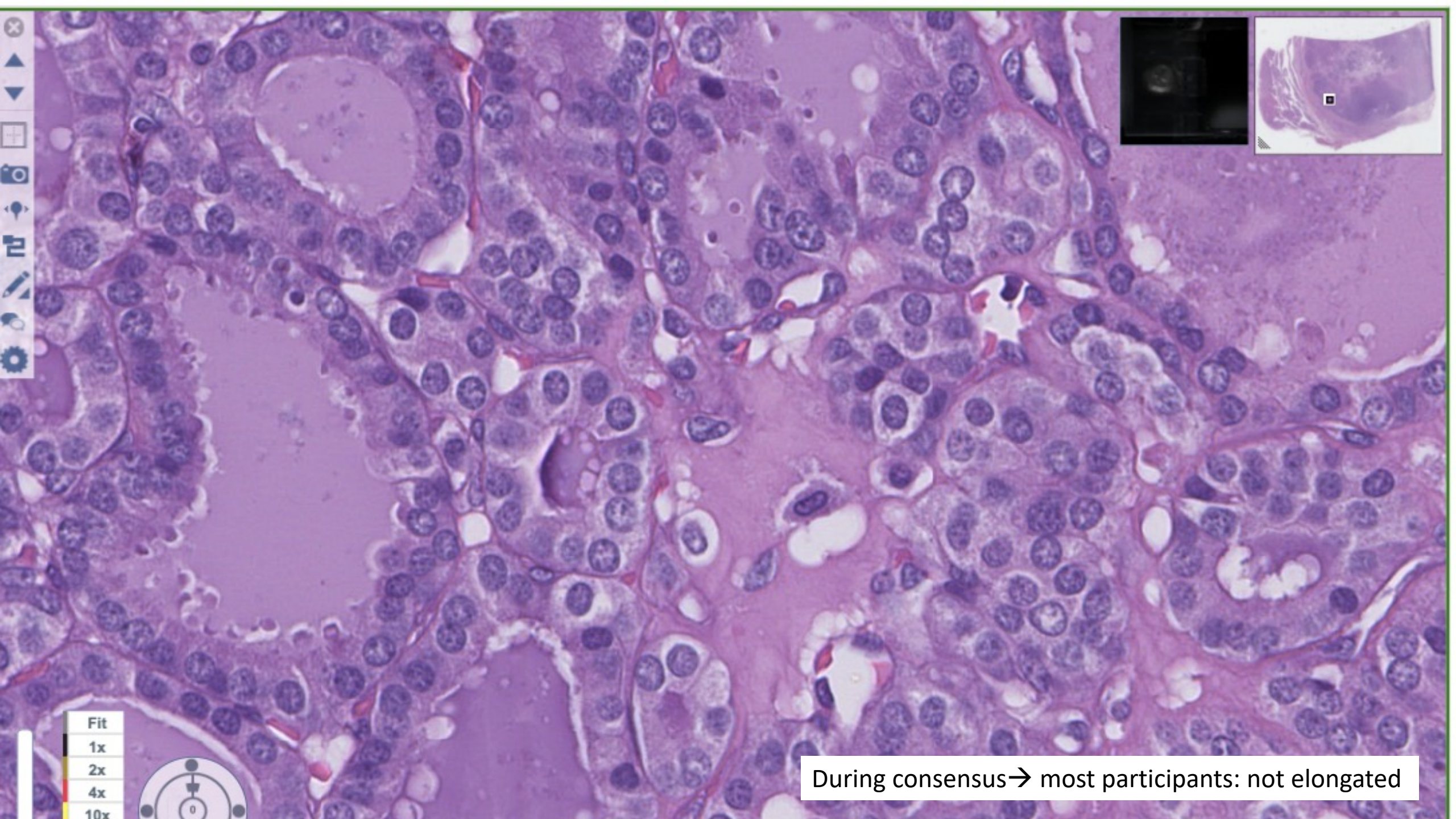
3. Nuclear Elongation

Definition: Cell nuclei that are elongated beyond its normal shape.

Assessment Method:

- Find nuclei that have a length more than twice its width.
- Not to be confused with truncation artifacts.



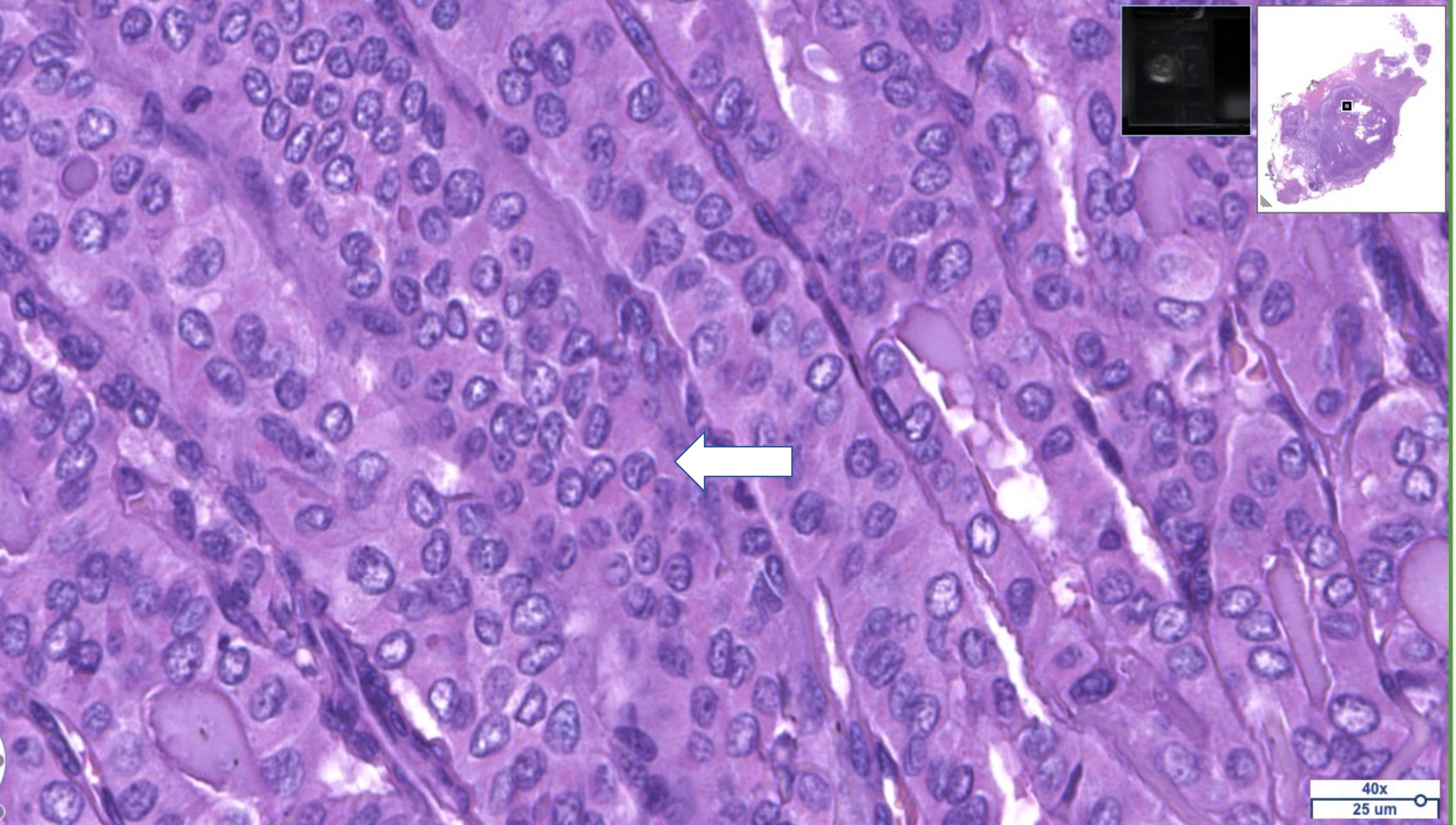

Fit
1x
2x
4x
10x
During consensus→ most participants: not elongated

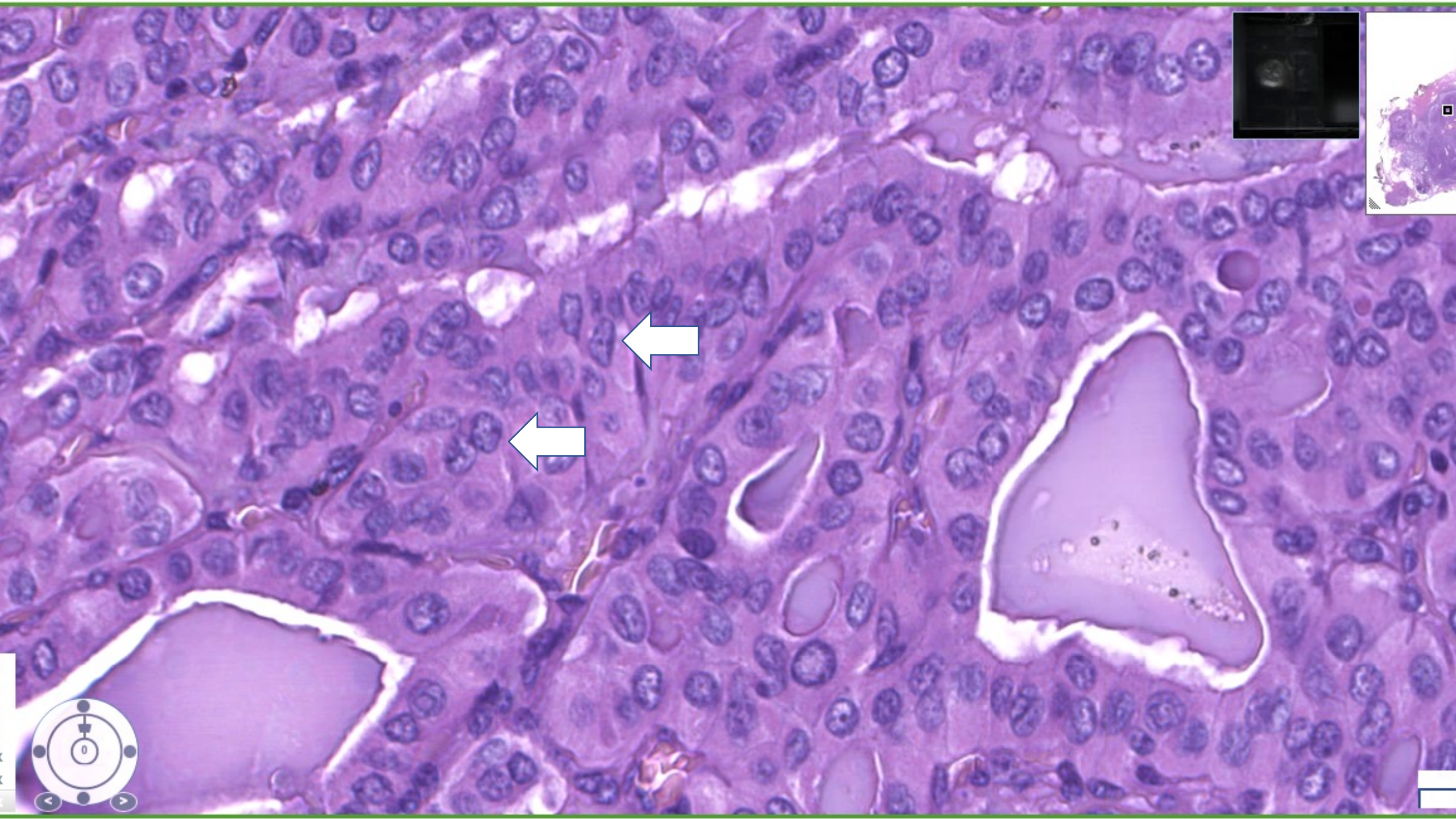
4. Irregular Membrane Contour

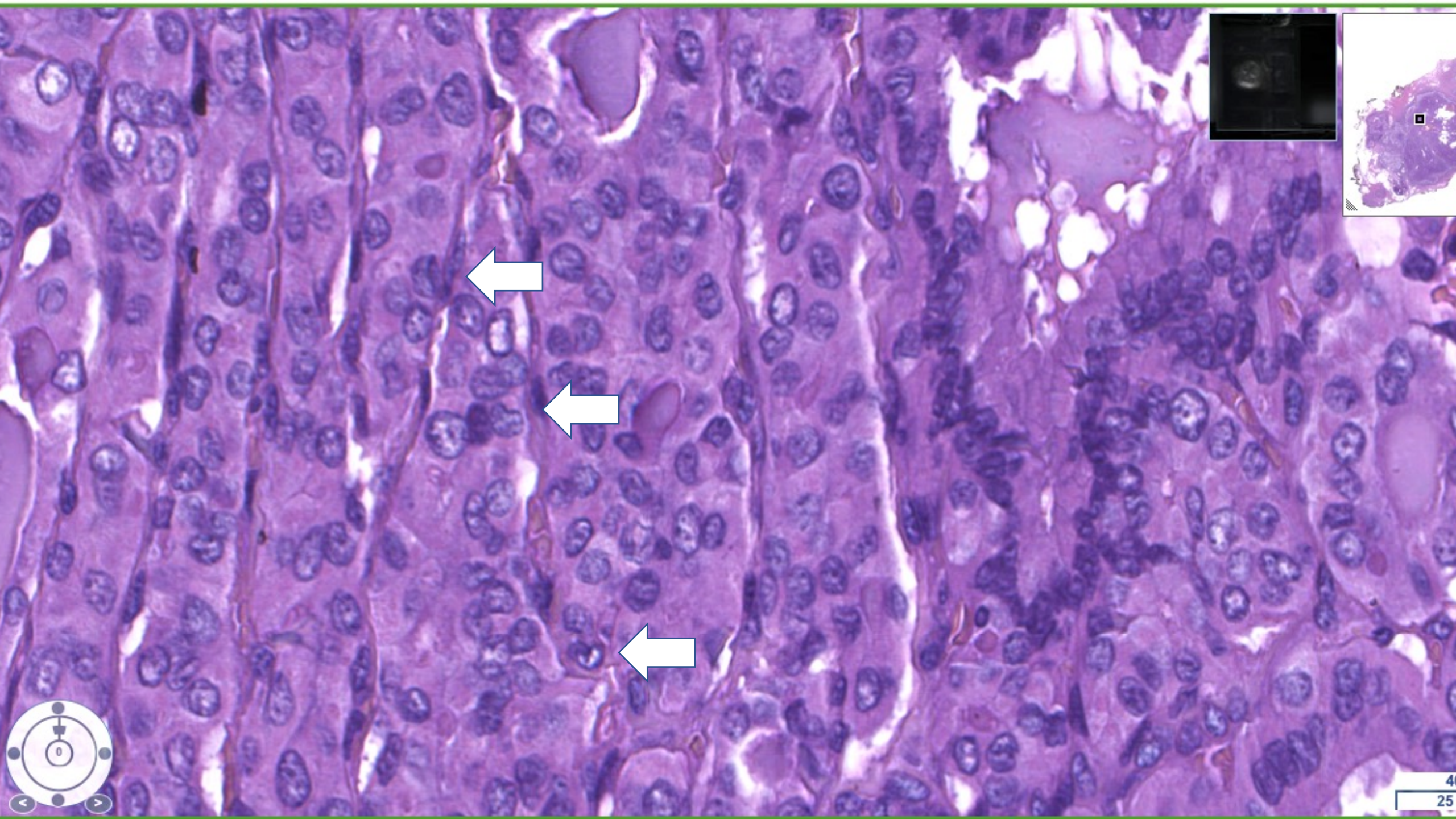
Definition: Uneven or irregular cell nuclei contour.

Assessment Method:

- Look for protruding or inconsistencies on the nuclei surface.
- Compare with surrounding cells to ensure this is not an artifact.


40x
25 um



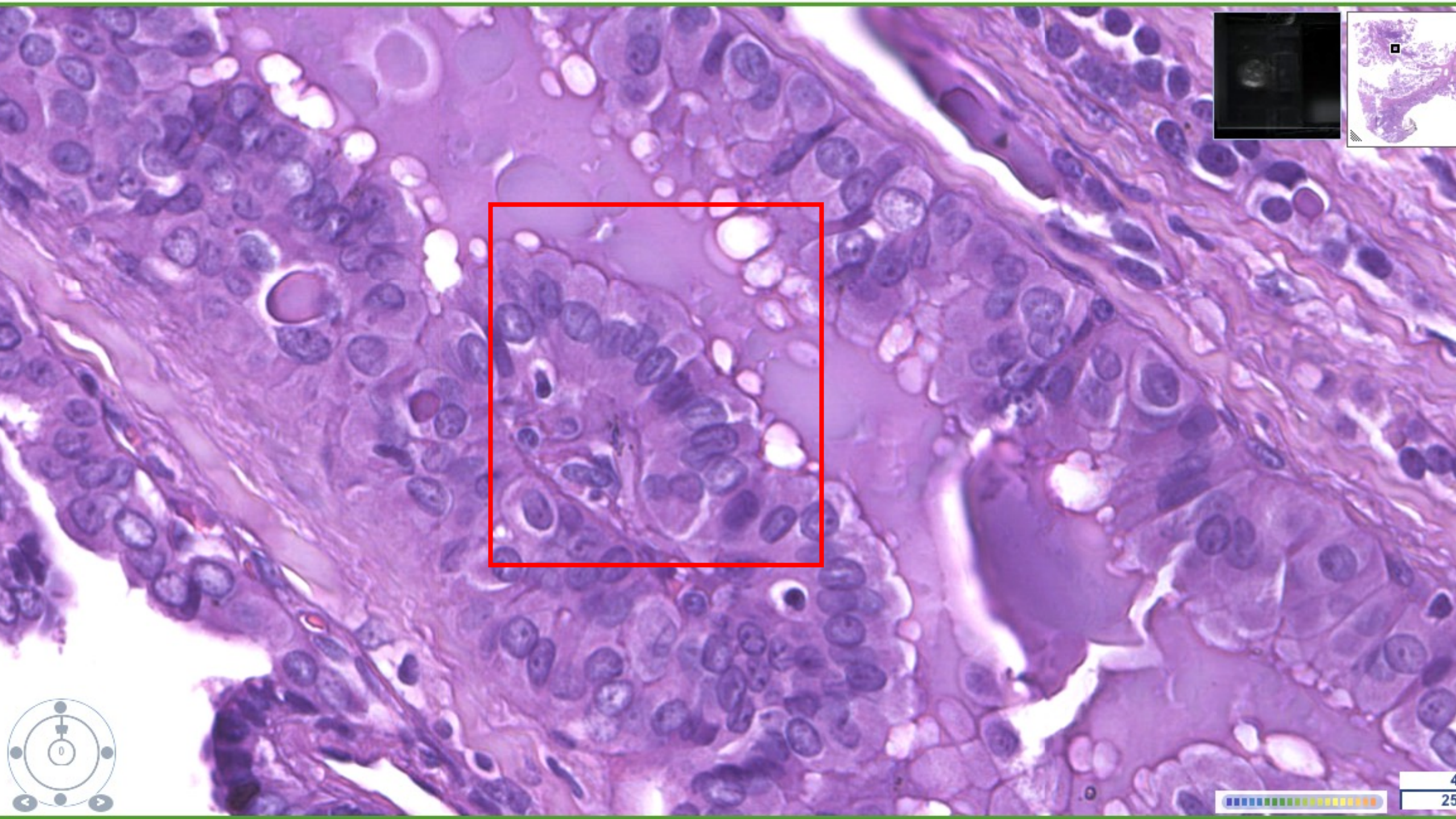


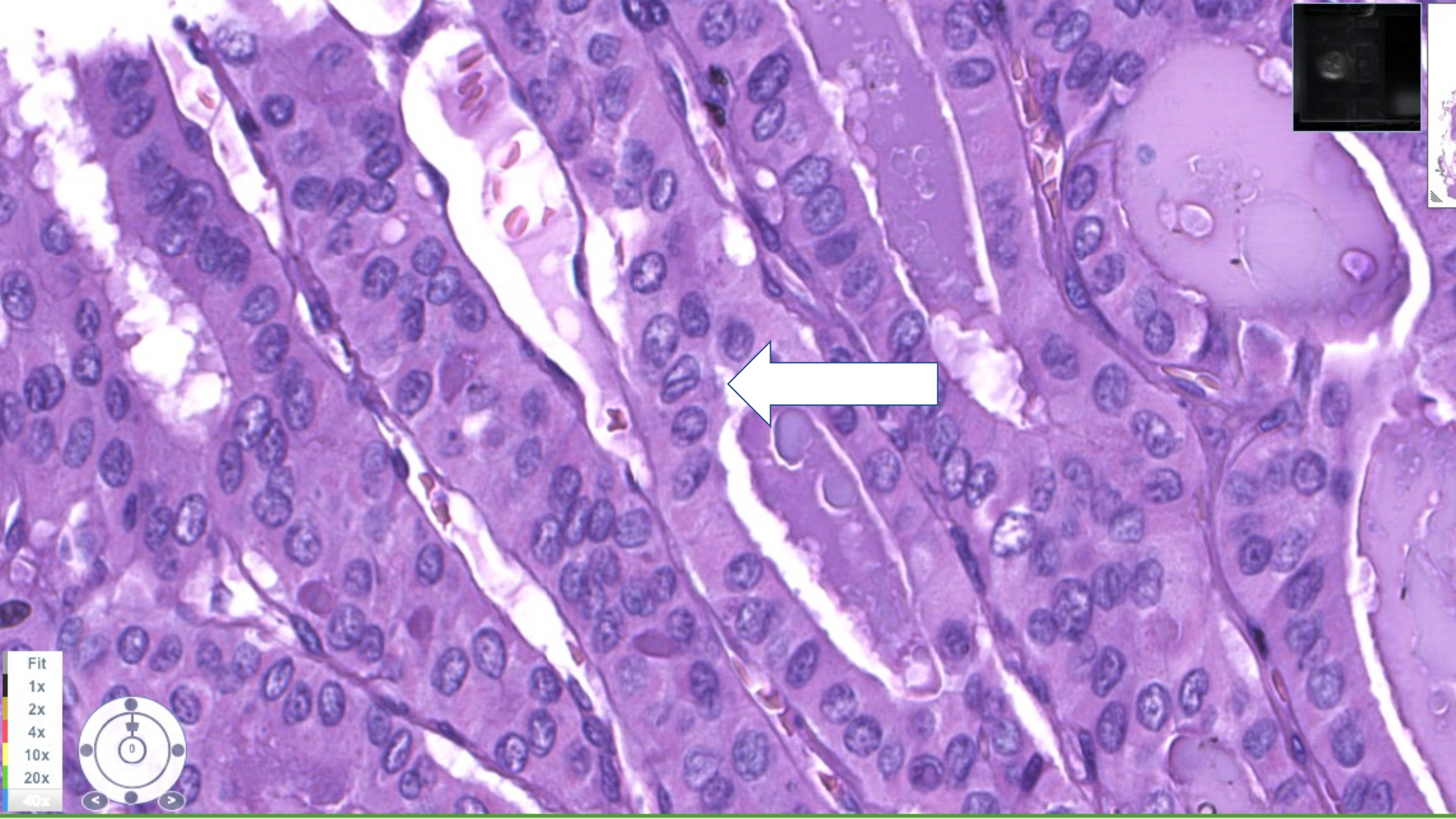
5. Nuclear Grooves

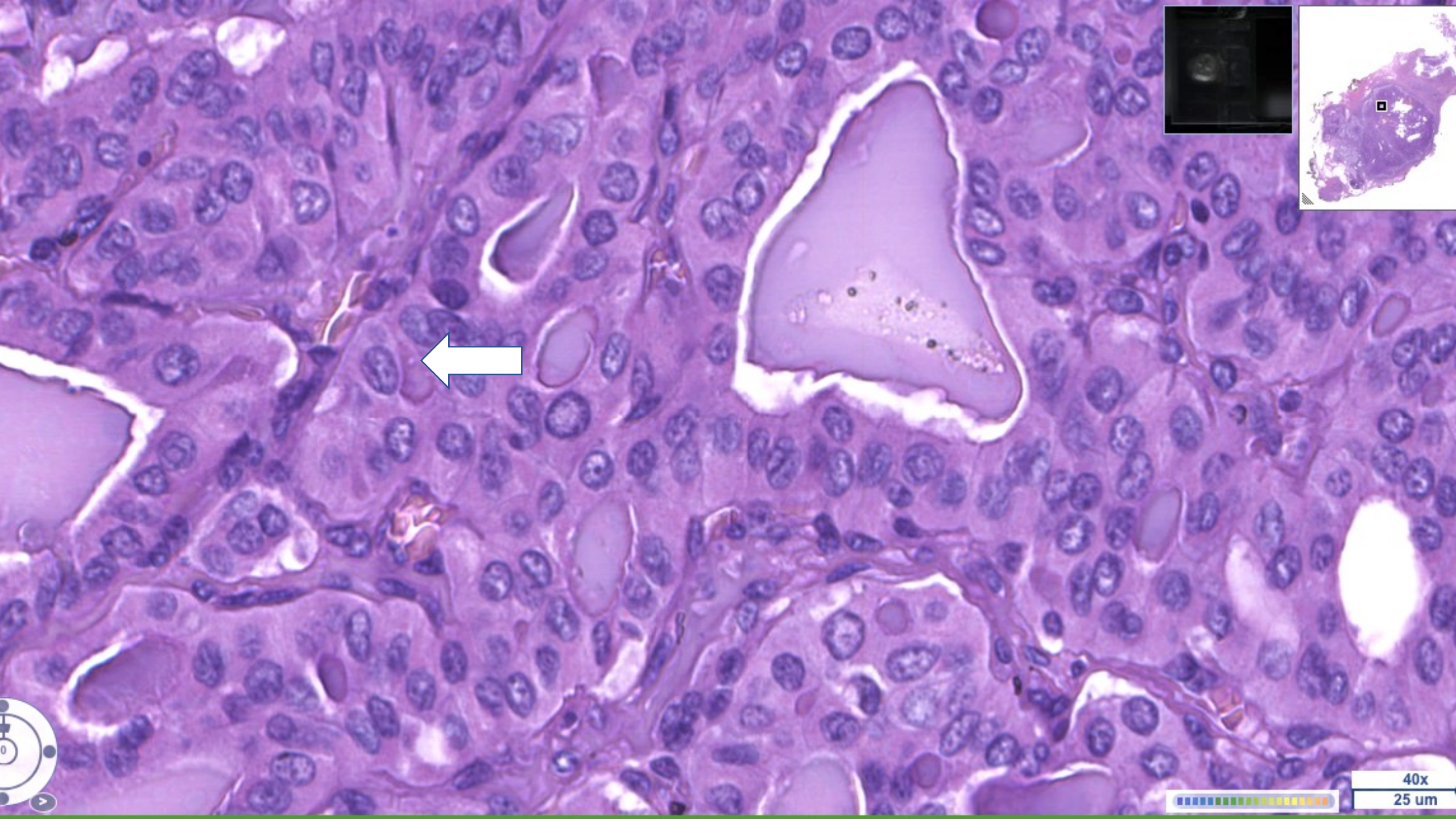
Definition: Linear grooves or folds in the cell nuclei.

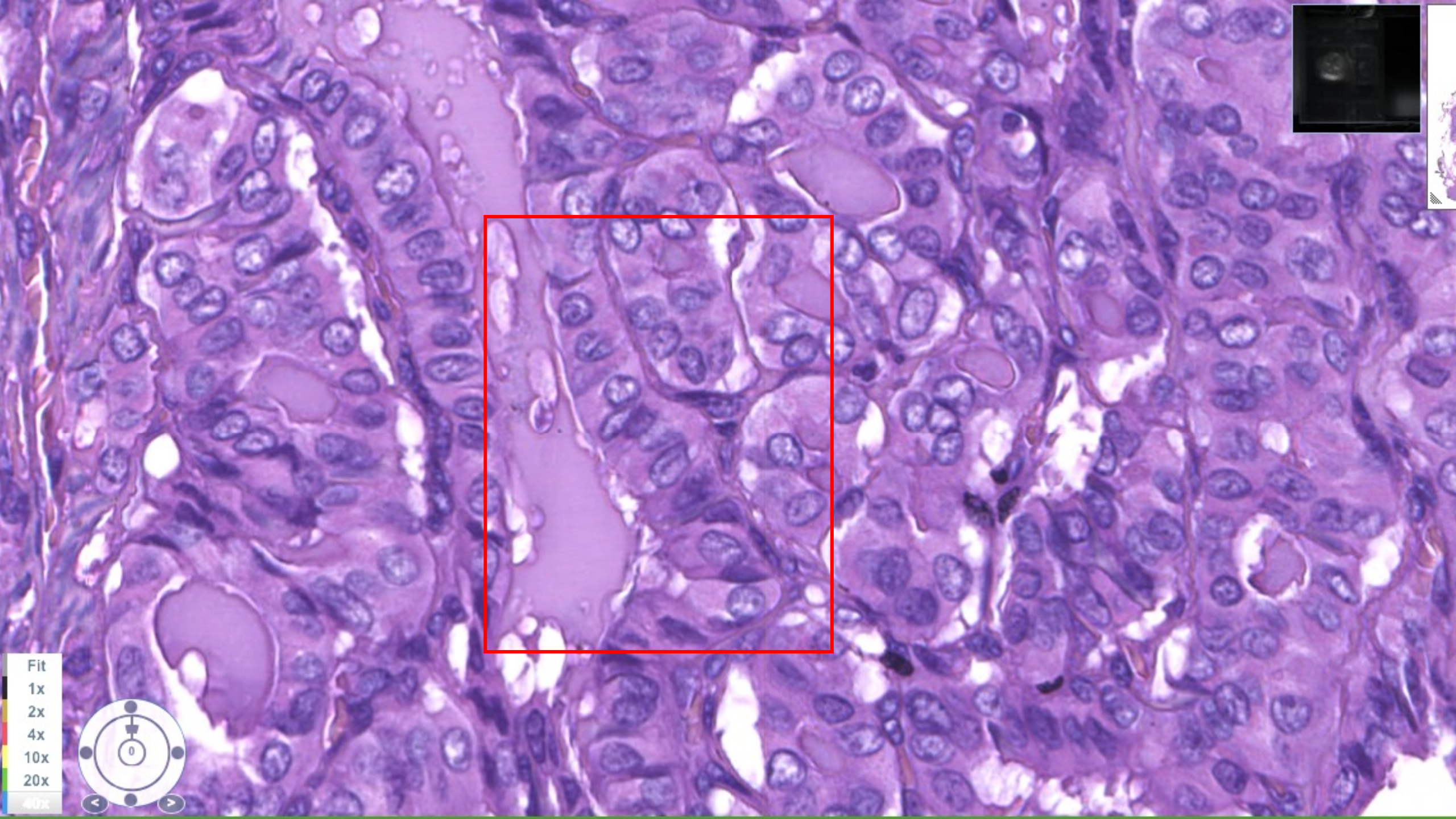
Assessment Method:

- Grooves usually appear as fine longitudinal lines.
- Note to the depth and length of the groove.




Fit
1x
2x
4x
10x
20x
40x


40x
25 um


Fit
1x
2x
4x
10x
20x
40x

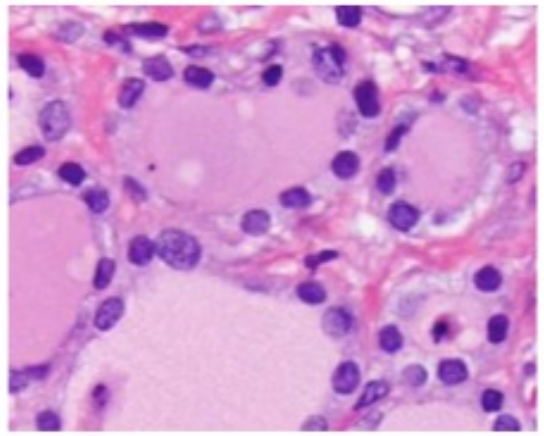
6. Chromatin Clearing

Definition: Chromatin fades and gives the appearance of a lighter nucleus.

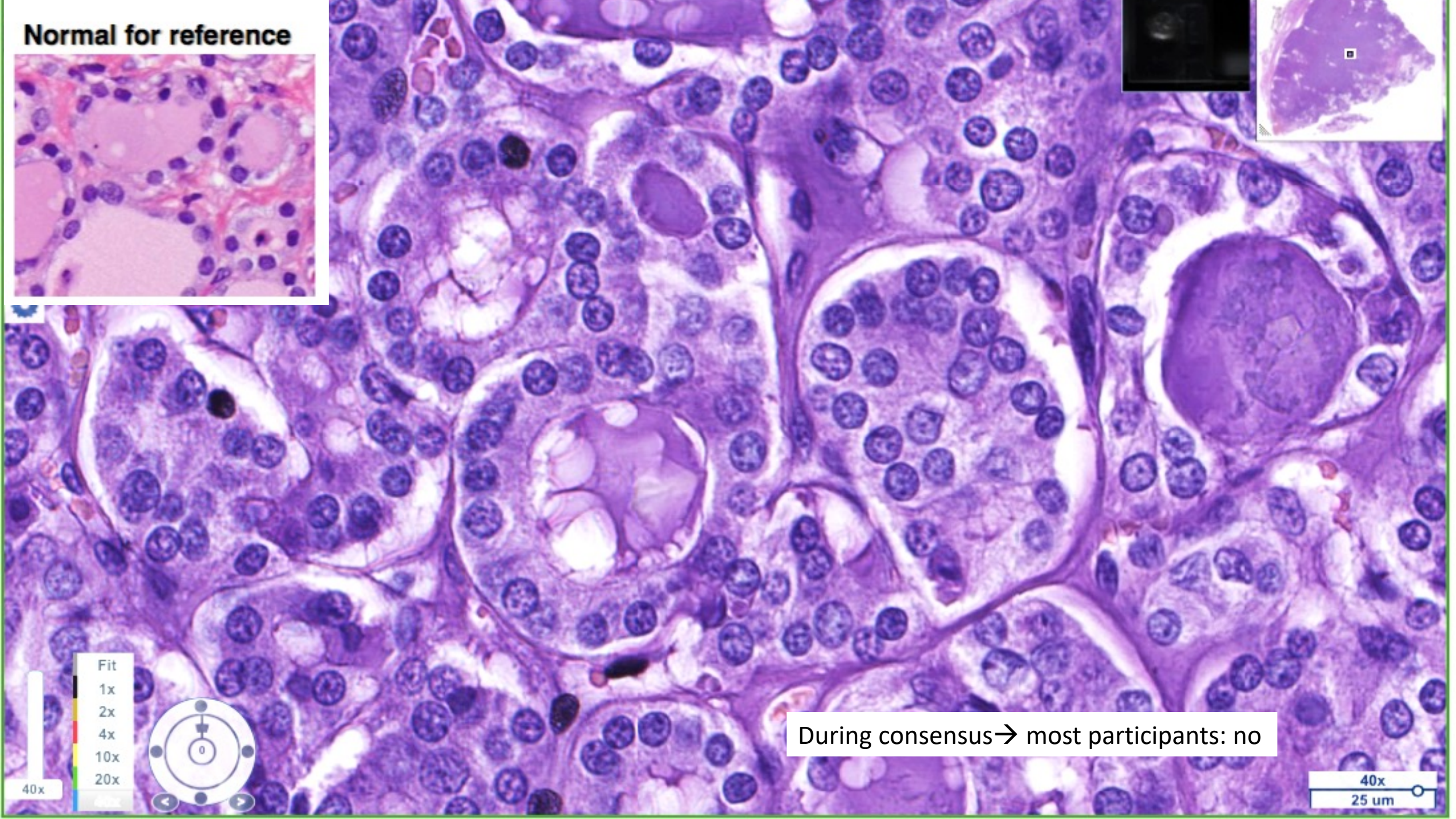
Assessment Method:

- Compare chromatin density with surrounding cells.
- Note the area distribution with chromatin clearing.

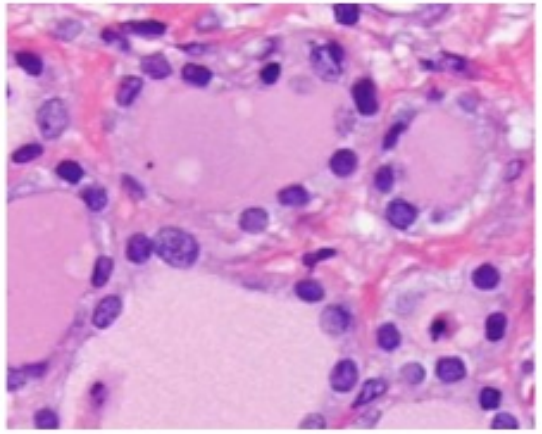
Normal for reference

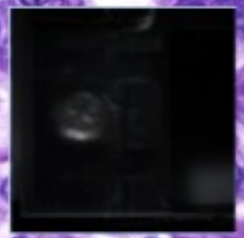


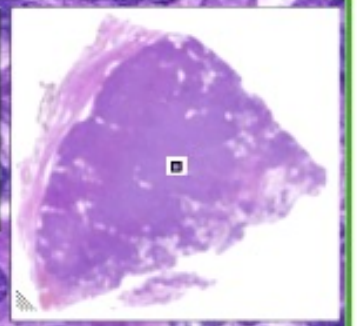
During consensus→ most participants: no


Normal for reference
Fit
1x
2x
4x
10x
20x
40x
40x
25 um
During consensus→ most participants: no

Normal for reference



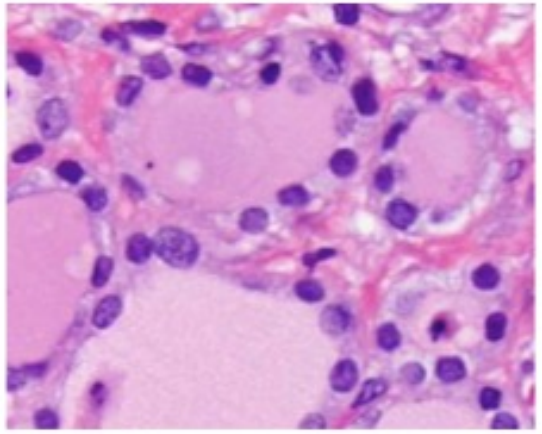




Fit
1x
2x
4x
10x
23x

During consensus→ most participants: no

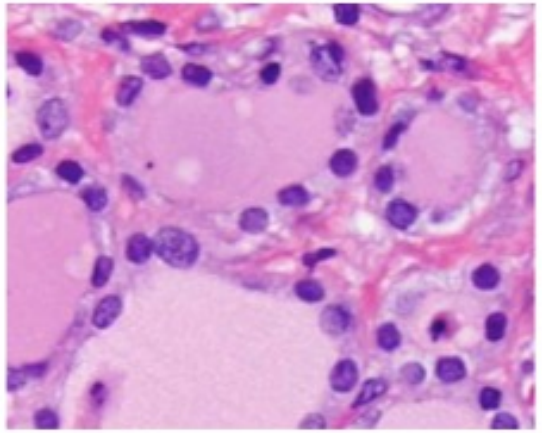
Normal for reference



Fit
1x
2x
4x
10x

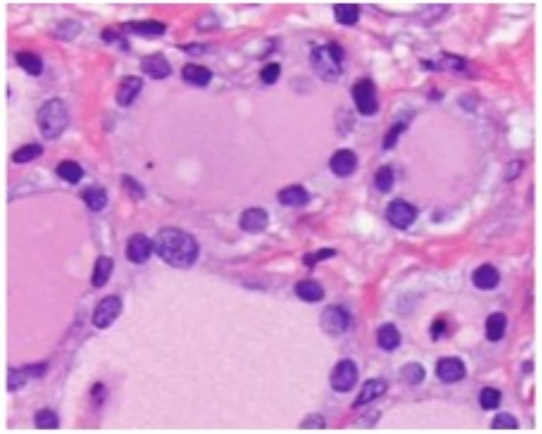
During consensus→ most participants: no

Normal for reference



During consensus→ most participants: yes, <10%

Normal for reference



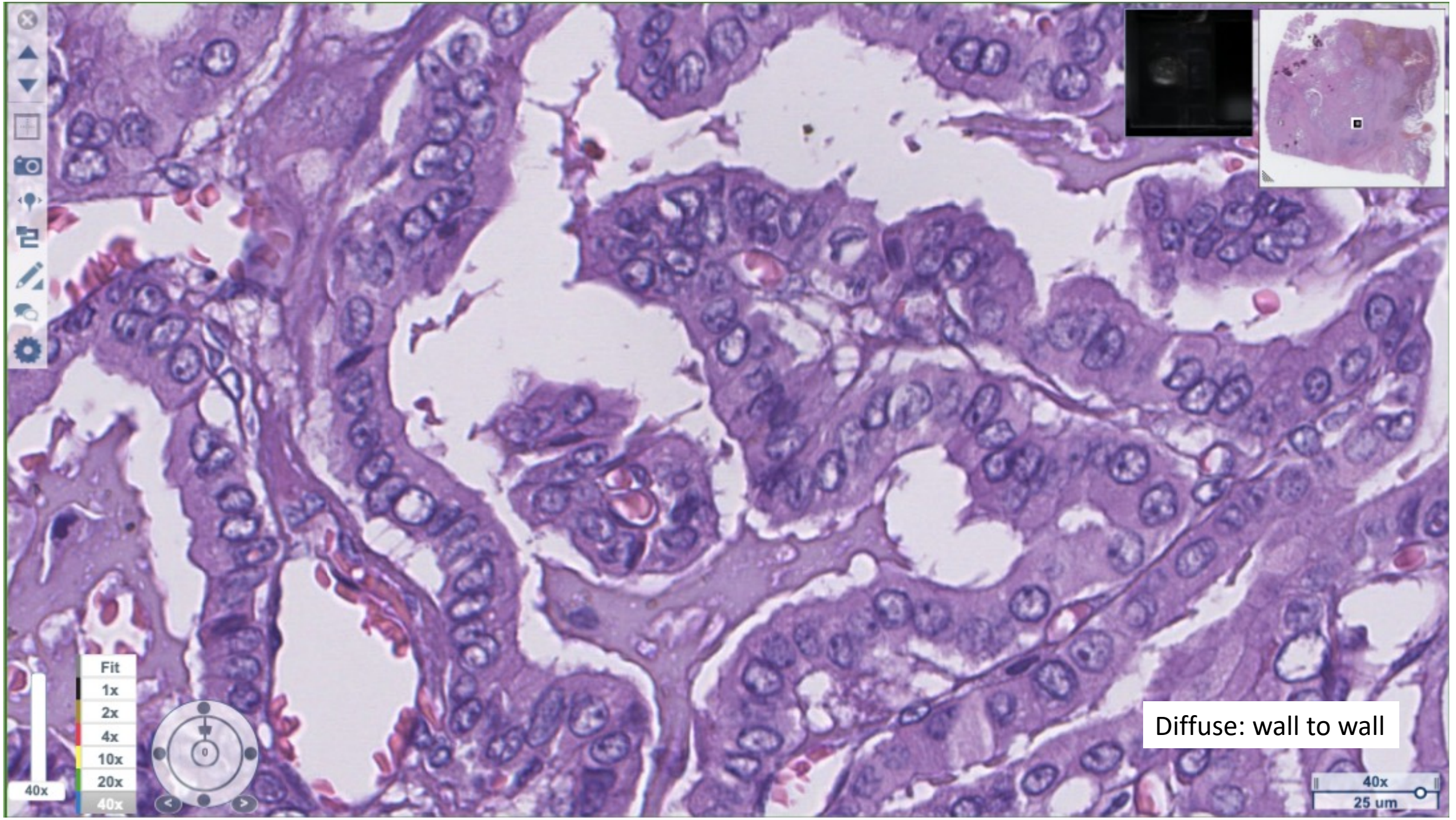
During consensus→ most participants: mostly yes

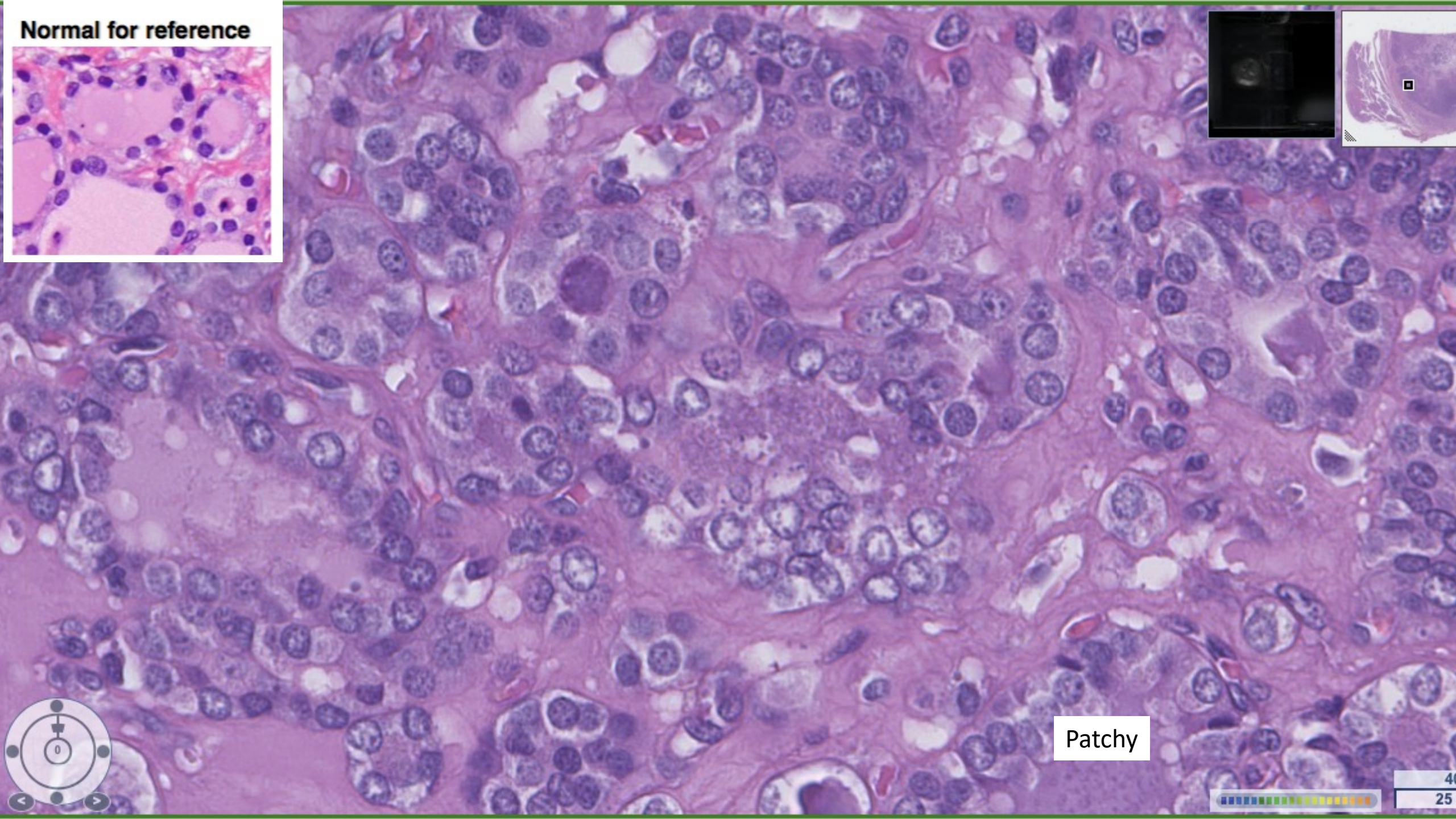
7. Distribution

Definition: Distribution of PTC nuclear features in thyroid tumors (whether it is diffusely distributed or not).

Assessment Method:

- Look for PTC nuclear features.
- Assess whether it is diffuse or patchy.


Fit
1x
2x
4x
10x
20x
40x
40x
Diffuse: wall to wall
40x
25 um

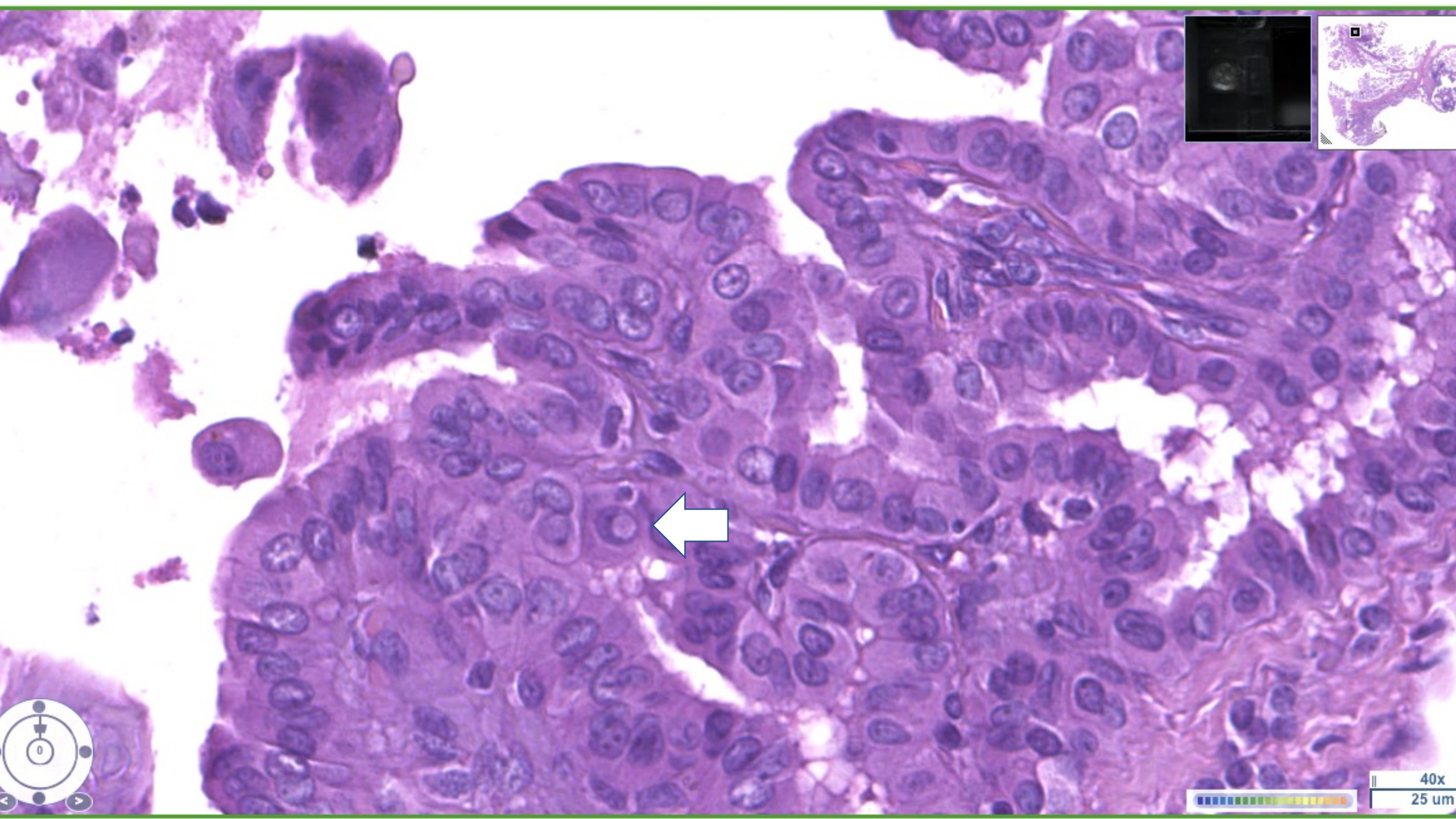

Normal for reference
Patchy

8. Nuclear Pseudo-inclusions

Definition: Structures that appear as cell nuclei inclusions, but actually are not.

Assessment Method:

- Pseudo-inclusions appear as vacuoles or vesicles in the nucleus.
- Distinguish from true nuclear inclusions by looking at the contour and contents of the inclusion.


40x
25 um

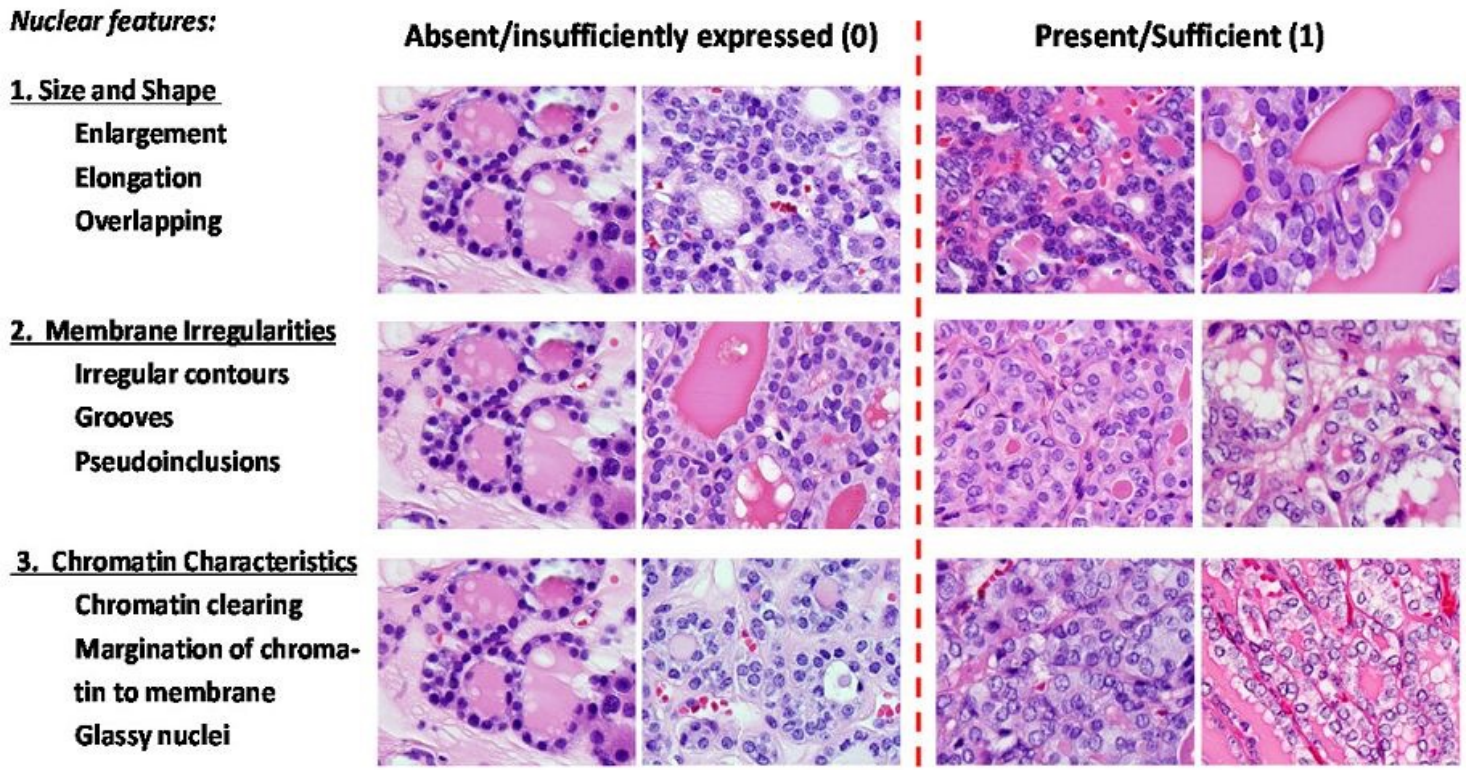
Methodology

Three Points Assessment

Nuclear size and shape : yes or no

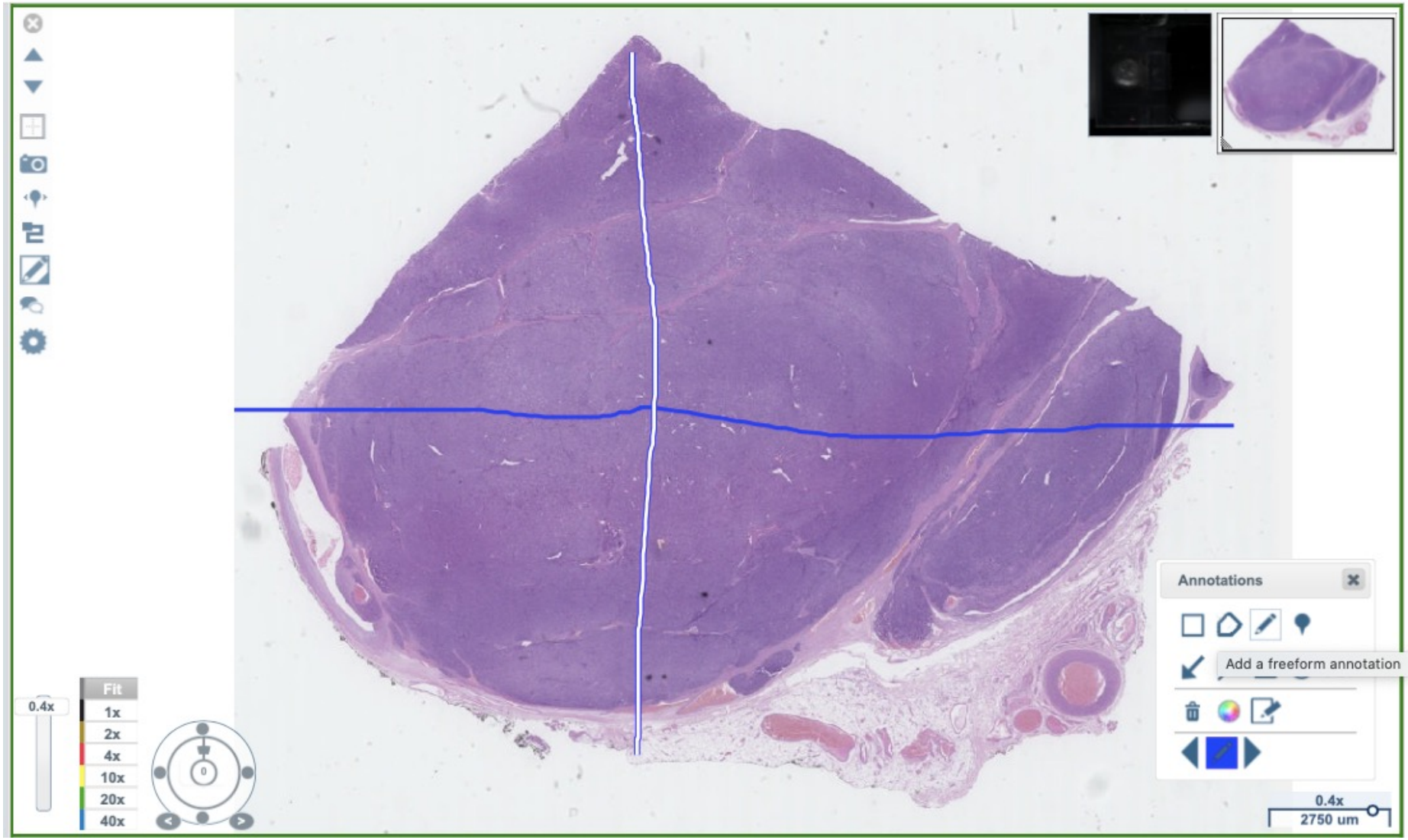
Nuclear membrane irregularities: yes or no

Nuclear chromatin characteristics: yes or no



Eight Points Assessment

1. Nuclear Enlargement : ideal 40x **Yes or No (if yes : <10% or >10%?)**
2. Nuclear Crowding/ Overlapping: ideal 20x **Yes or No (if yes : <10% or >10%?)**
3. Nuclear Elongation: ideal 20x **Yes or No (if yes : <10% or >10%?)**
4. Irregular Membrane Contour: ideal 40x **Yes or No (if yes : <10% or >10%?)**
5. Nuclear Grooves: ideal 40 x **Yes or No (if yes : <10% or >10%?)**
6. Chromatin Clearing: ideal 20-40x **Yes or No (if yes : <10% or >10%?)**
7. Chromatin distribution: ideal 20x **diffuse or patchy**
8. Nuclear Pseudo-inclusions: ideal 40x **yes or no**



Divide the field of view into 4 as a guide. Write down the percentage of each area then calculate the mean value. Assess each case in 30 minutes.

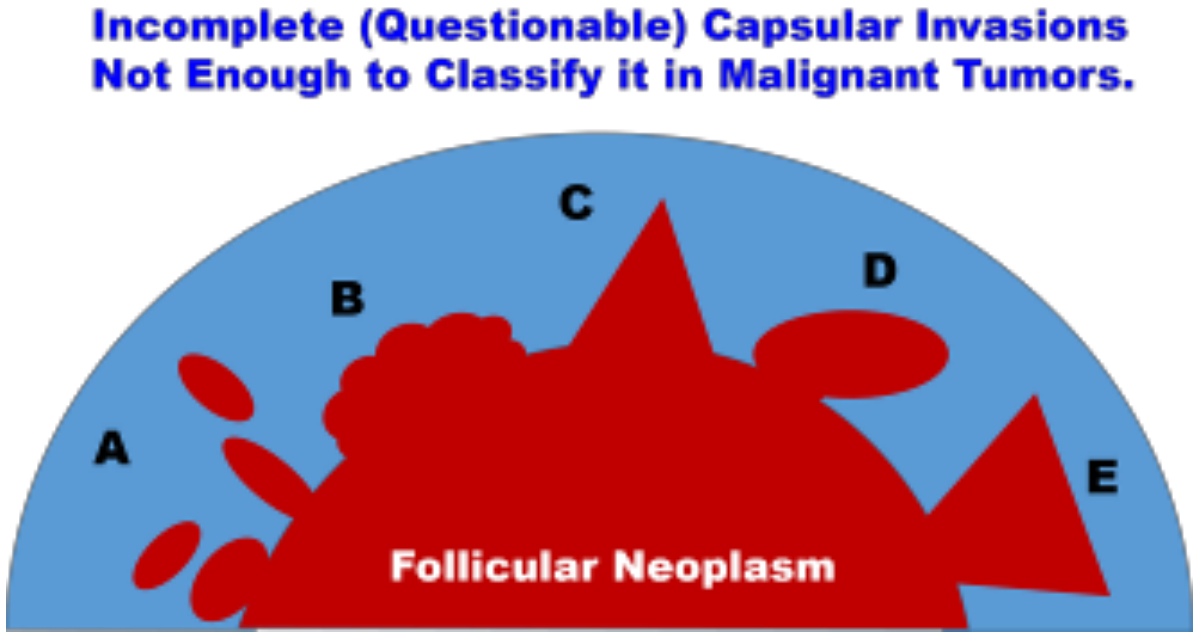
Data Table

	A	B	C	D	E	F	G	H	I	J	K	L
		3 POINT			8 POINT							
	CASE NO	Nuclear Size & Shape	Nuclear membran Irregularities	Nuclear Chromatin Characteristic	Nuclear Enlargement	Nuclear Overlapping / Crowding	Nuclear Elongation	Irregular membrane Contour	Nuclear Grooves	Chromatin Clearing	Chromatin Distribution	Nuclear Pseudo-Inclusion
	1											
	2											
	3											
	4											
	5											
	6											
	7											
	8											
	9											
	10											
	11											
	12											
	13											
	14											
		Yes = 0	Yes = 0	Yes = 0	Yes < 10% = 0	Yes < 10% = 0	Yes < 10% = 0	Yes < 10% = 0	Yes < 10% = 0	Yes < 10% = 0	Diffuse = 0	Yes = 0
		No = 1	No = 1	No = 1	Yes > 10% = 1	Yes > 10% = 1	Yes > 10% = 1	Yes > 10% = 1	Yes > 10% = 1	Yes > 10% = 1	Patchy = 1	No = 1
					No = 2	No = 2	No = 2	No = 2	No = 2	No = 2		

CAPSULAR INVASION

- The following common scenarios were considered as **questionable invasive**:
  - cells connected with the main tumor nodule invade into but not completely through the capsule,
  - thick and irregular capsule pushed by the tumor cells,
  - small nests of tumor cells aligned parallel to the capsule without a connection with the main tumor mass.

Capsular Invasion (CI)



Incomplete (questionable) capsular invasions in uncertain malignant potential. They are not enough to classify in malignant tumors (minimally invasive follicular thyroid carcinoma or invasive encapsulated follicular variant papillary thyroid carcinoma).

Kakudo et al, Journal of Basic & Clinical Medicine 2018; 7(1):10-19

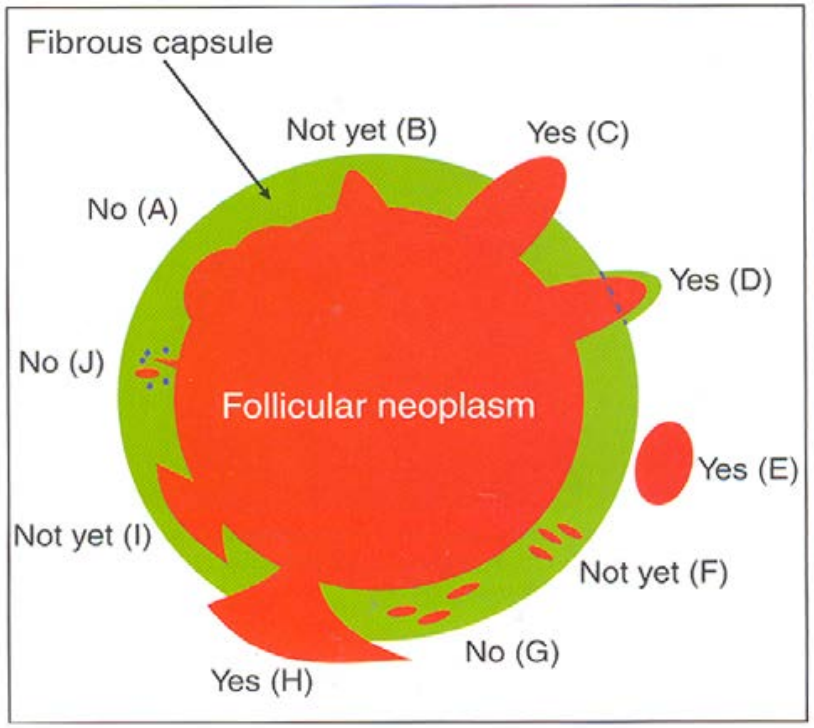
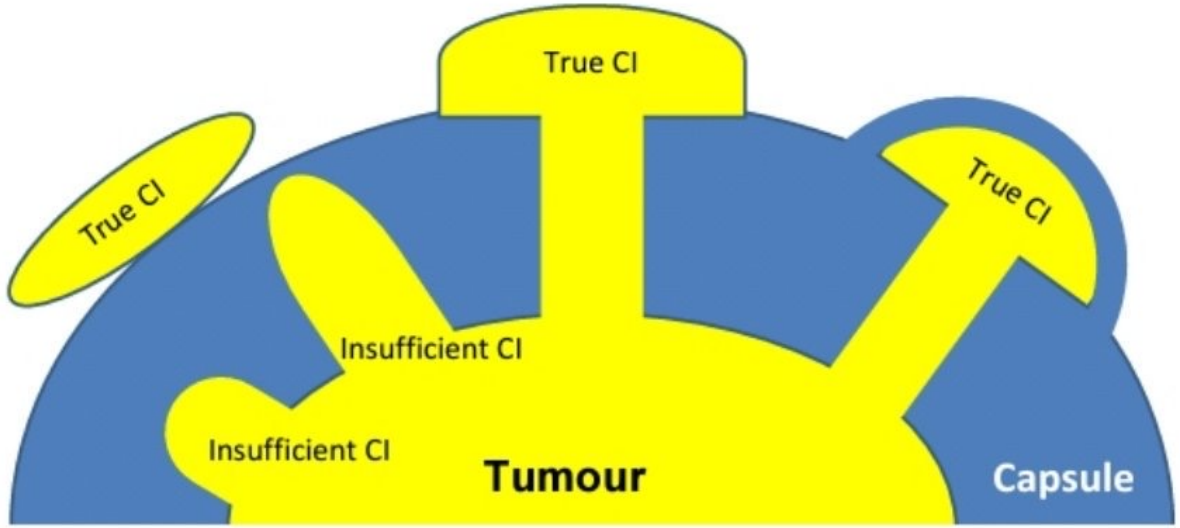


Figure 2. Capsular invasion (CI). Schematic drawing for the interpretation of the presence or absence of CI. The diagram depicts a follicular neoplasm (orange) surrounded by a fibrous capsule (green). A. Bosselation on the inner aspect of the capsule does not represent CI. B. Sharp tumor bud invades into but not through the capsule suggesting invasion requiring deeper sections to exclude. C. Tumor totally transgresses the capsule invading beyond the outer contour of the capsule qualifying as CI. D. Tumor clothed by thin (probably new) fibrous capsule but already extending beyond an imaginary (dotted) line drawn through the outer contour of the capsule qualifying as CI. E. Satellite tumor nodule with similar features (architecture, cytomorphology) to the main tumor lying outside the capsule qualifying as CI. F. Follicles aligned perpendicular to the capsule suggesting invasion requiring deeper sections to exclude. G. Follicles aligned parallel to the capsule do not represent CI. H. Mushroom-shaped tumor with total transgression of the capsule qualifies as CI. I. Mushroom-shaped tumor within but not through the capsule suggests invasion requiring deeper sections to exclude invasion. J. Neoplastic follicles in the fibrous capsule with a degenerated appearance accompanied by lymphocytes and siderophages does not represent CI but rather capsular rupture related to prior fine-needle aspiration.

From Fletcher CDM, ed. *Diagnostic Histopathology of Tumours*. 3rd ed. Edinburgh: Churchill Livingstone Elsevier; 2007. Modified with permission. © Elsevier.

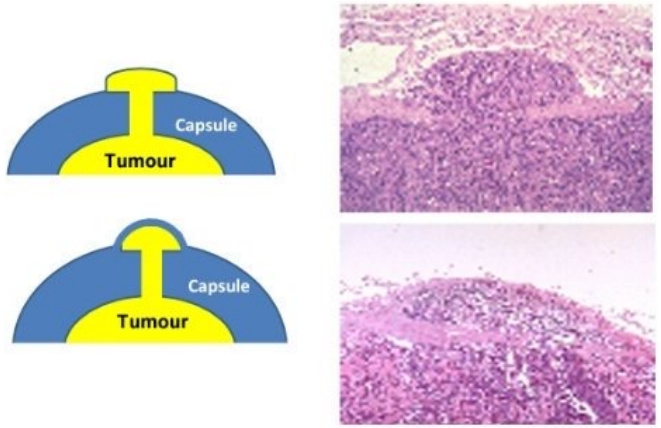
Patterns of Capsular Invasion (CI)



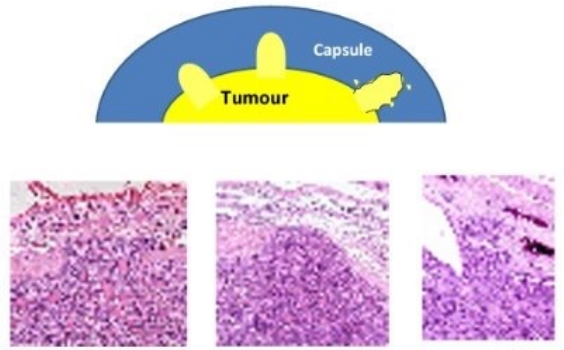
Capsular invasion is defined by **tumour penetration** through the tumour capsule unassociaqted with the site of a previous fine needle aspiration biopsy. (WHO 2004)

Patterns of Capsular Invasion (CI)

Tumour penetration through the tumour capsule



Patterns of Capsular Pseudoinvasion (CPI)



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- Capsular invasion, according to the degree, was graded into three categories based on the 2017 WHO classification, including:
  - non-invasive
  - questionable invasive
  - clear-cut invasive.

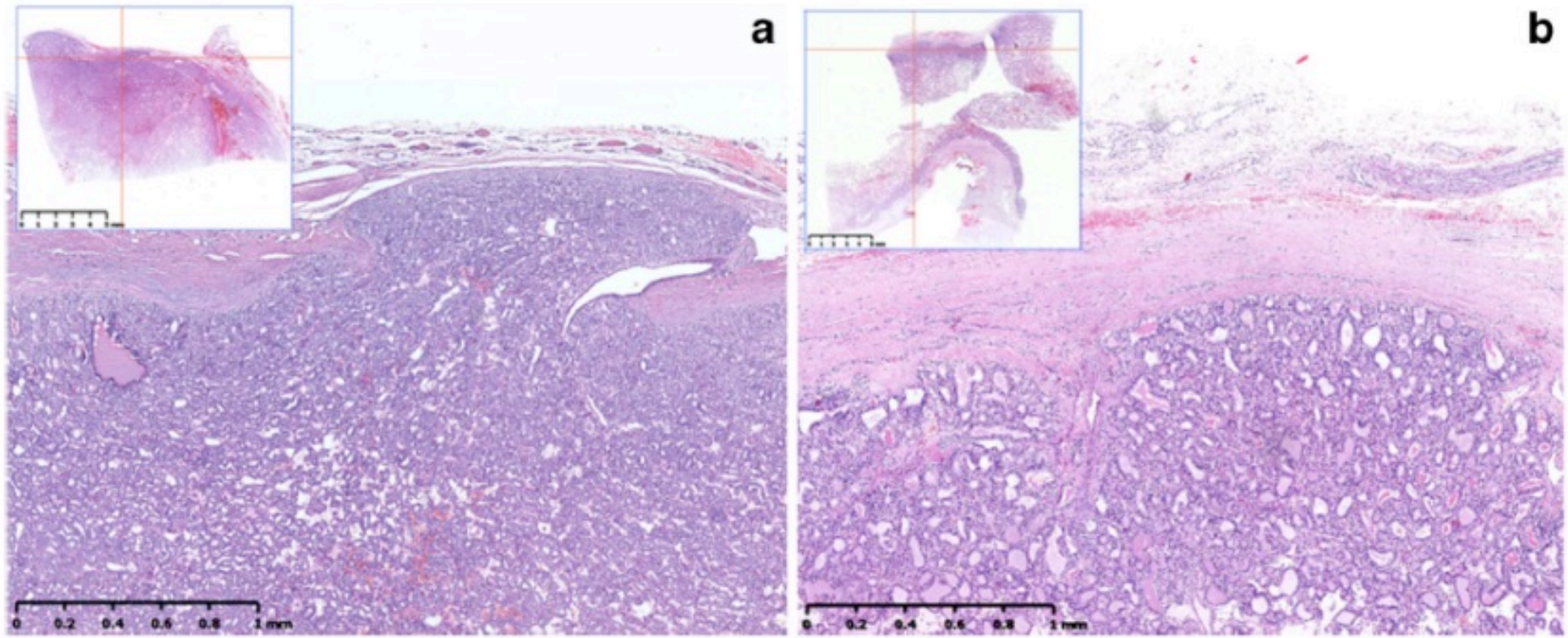

Fig. 1 Evaluation of capsular invasion in follicular thyroid neoplasms. **a** Neoplastic tumor nests penetrate the entire thickness of the tumor capsule. All pathologists diagnosed this area as clear-cut invasive (case 12). **b** The encapsulated lesion shows an irregularly follicular architecture surrounded by a well-formed fibrous capsule (case 17). All participants regarded this lesion as non-invasive

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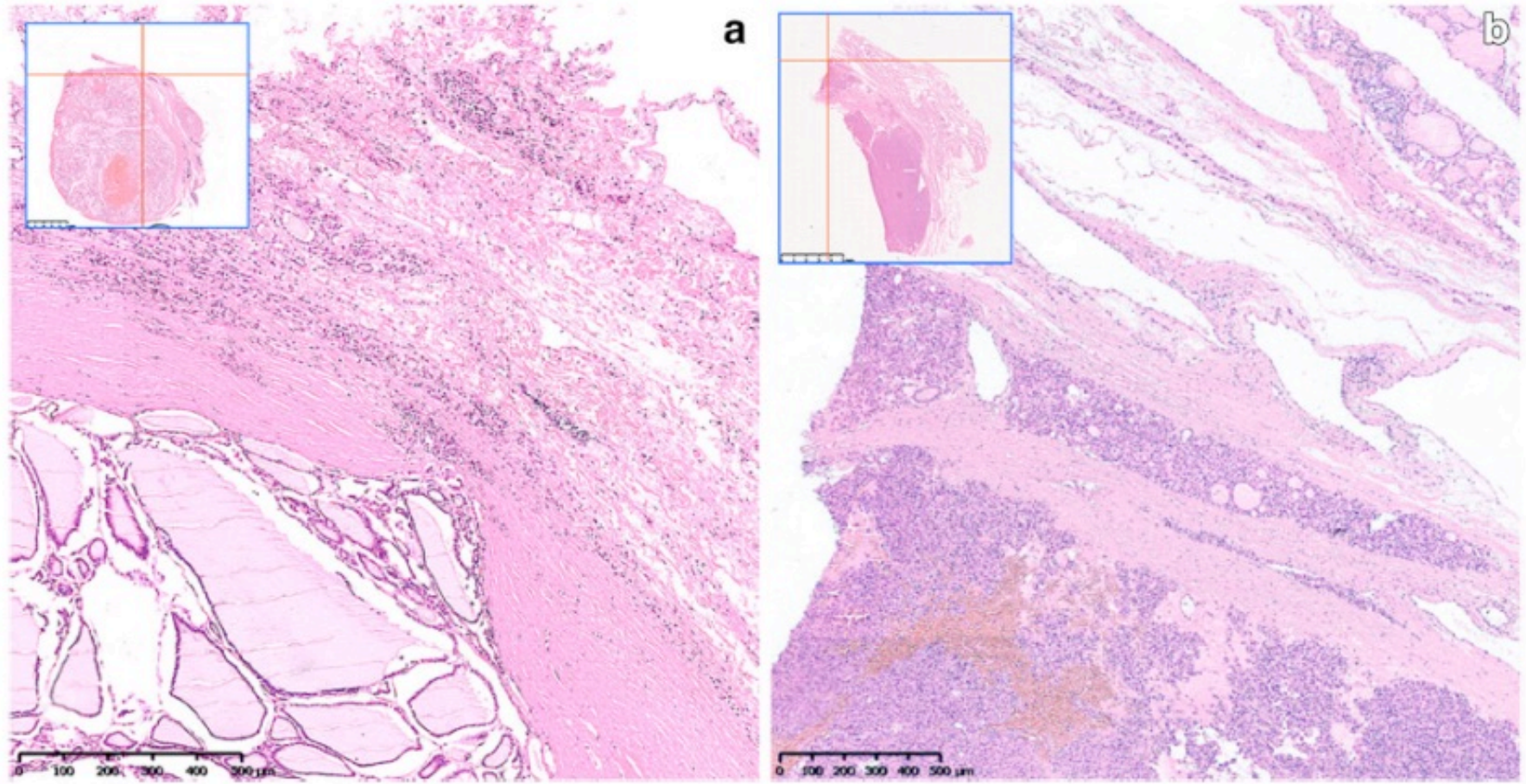

Fig. 3 Evaluation of questionable capsular invasion in follicular thyroid neoplasms. **a** Four pathologists regarded this area as questionable, incomplete invasive because the sharp bud has partially penetrated the fibrous capsule but seems not to reach beyond an imaginary line drawn along the external border of the capsule (case 1). **b** Tumor cell nests are parallel aligned and are tightly clung to the outer edge of the capsule. Note that the growth pattern of the tumor bud is similar to that of the primary tumor mass (case 5). Five pathologists considered this area as questionable, favor non-invasive.

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Angioinvasion

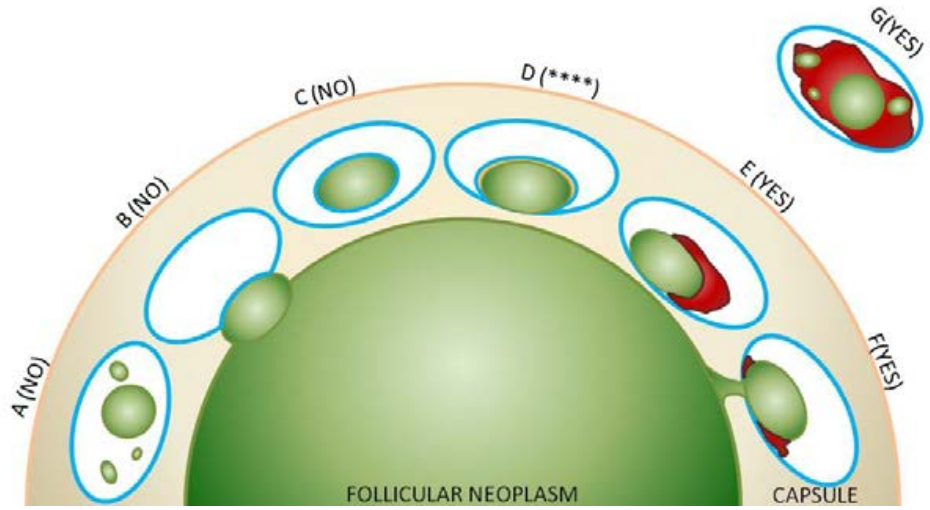


Figure 3. Vascular invasion (VI): Schematic drawing for the interpretation of the presence or absence of significant VI. The diagram depicts a follicular neoplasm (green) surrounded by a fibrous capsule (tan). The driving concepts behind significant VI are penetration through the vessel wall and a reaction to the vascular deposit, namely thrombus formation, which may range from subtle and fibrinoid in nature to large and heavily organized.[27]

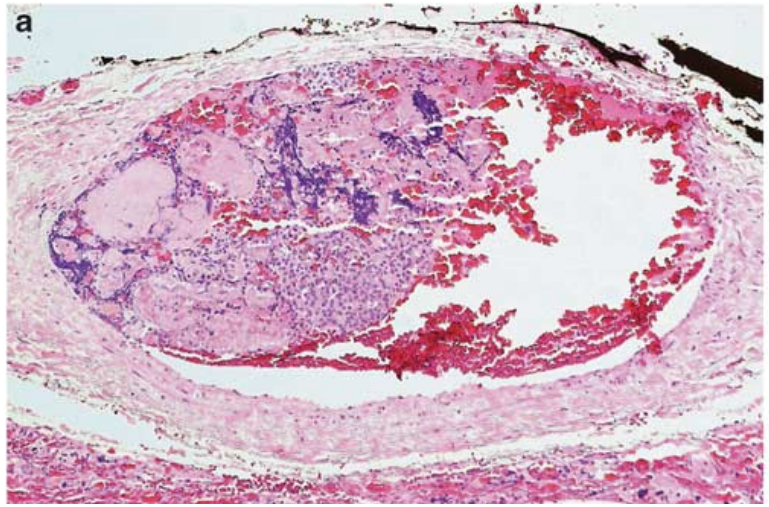
A through C represent scenarios where tumor in vessels are not counted as VI. A. Free-floating irregular tumor fragments often result from artifactual displacement. B. Tumor bulging and indenting the vessel wall does not count as VI. C. Endothelialized tumor floating in an intracapsular vessel may result from tangential sectioning of tumor bulging into a vessel, often at a branch or bifurcation. These findings can however prompt deeper levels (at least by 3) to exclude definitive VI (see E through G).

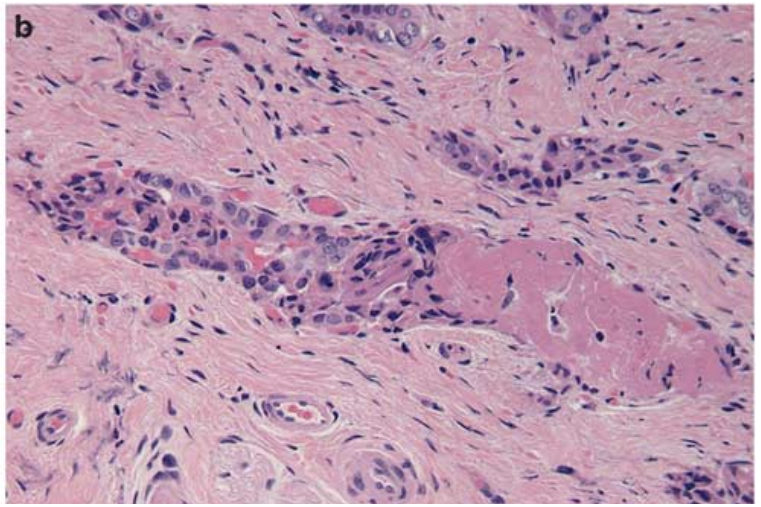
**** D represents a common but contentious scenario among experts, in light of these new proposed criteria for significant VI. This endothelialized tumor deposit is juxtaposed to the vessel wall. As this is somewhat similar to C, and there is no obvious thrombus, technically this would not count as significant VI. One counterargument is that the endothelialized appearance represents “organization” of a tumor thrombus and is thus still significant. While deeper levels may help, this scenario may still be considered a “judgment call” based on current level of evidence.

E through G represent unequivocal VI. E. Tumor is juxtaposed to vessel wall and is associated with a thrombus. F. Tumor penetrating vessel wall also demonstrating thrombus formation at its neck. G. Tumor fragments in intermingled with an organized thrombus and adherent to vessel wall.

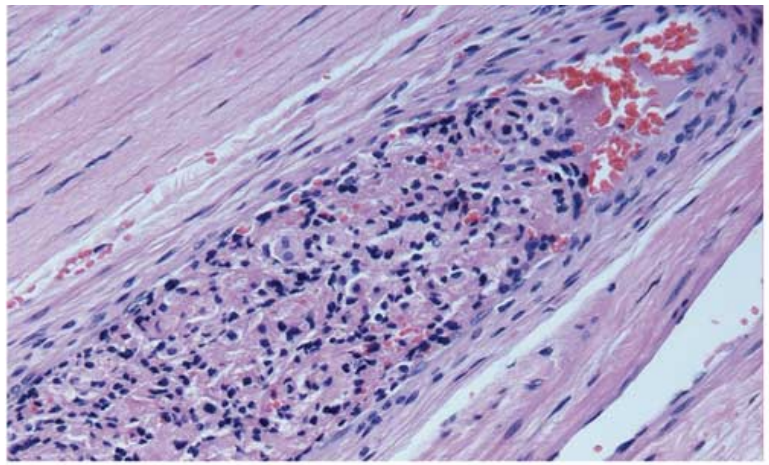
Note: While there is no standard definition of “deeper levels,” generally, each level is at a certain interval (ie, 3 serial sections deeper or 15-micron intervals) below the original H&E rather than an immediate serial section.

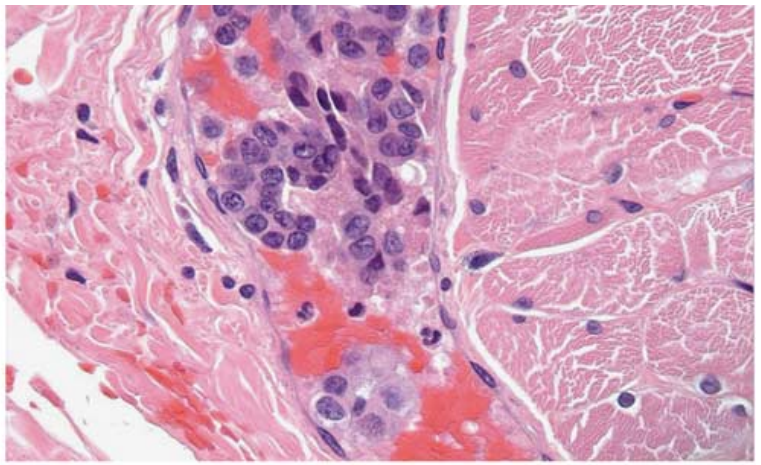
Original concept for schematic from Fletcher CDM, ed. *Diagnostic Histopathology of Tumours.* 3rd ed. Edinburgh; Churchill Livingstone Elsevier; 2007. Modified with permission. © Elsevier.





True angioinvasion is characterized by thrombus adherent to intravascular tumor





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Thank you