Système d'Informations Halieutiques

Action Paramètres biologiques

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Protocol for the determination of histological structures found in the ovaries and during the oogenesis of the European plaice, *Pleuronectes platessa* (*Linné*, *1758*)

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The digital version of this document is available on the ARCHIMER Web site (https://archimer.ifremer.fr/doc/00501/61235/)

The digital version of the complementary document « Lexicon of histological structures found in the ovaries and during the oogenesis of the European plaice, *Pleuronectes platessa (Linné, 1758)* » is available on the ARCHIMER Web site (https://archimer.ifremer.fr/doc/00501/61234/)

Version	Date	Comments
1.0	July 2019	Conception of the document

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Context/Generalities/Objectives

This protocol was established during project MATO (MATurité Objective des poissons par l'histologie quantitative) for the evaluation of sexual maturity of exploited stock species, as well as to improve the reading of histological slides needed for this study.

This document follows the work of different workshops that aimed to improve the compilation of data on sexual maturity. The WKMATCH (WorKshop for MATurity staging CHairs, 2012) defined a universal evaluation grid for sexual maturity staging of different species, including the European plaice *Pleuronectes platessa*. During this workshop, two main recommendations were made, underlining the need to improve and complete the knowledge on sexual maturity, as well as to harmonize the practices used to determine these sexual phases.

Another workshop, the WKMATHIS (WorKshop on sexual MATurity staging from HIStological tools) that took place in Caen in 2017, set up different objectives such as : review the current knowledge on gonad histology, determine a sexual maturity scale at a cellular level, and suggested that histological studies should be made in order to improve the determination of sexual maturity on a macroscopic scale.

This protocol, drawn from these workshops and their conclusions, offers a key to the known cellular structures found throughout the gonads of a flatfish, the European plaice *Pleuronectes platessa*. This study will be restricted to the female gonad and the different cellular structures found inside the ovaries of the plaice.

This protocol will present decision trees and reading methods in order to favor a harmonious reading of histological slides, and eventually allow less experienced readers to partake in this exercise.

1. Gathering data

1.1. Terminologies and vocabulary

The specific histological terms, their abbreviations, the definitions for the different types of oocytes, as well as a more accurate description of the identifiable structures, maybe be found in the lexicon : « Lexicon of histological structures found in the ovaries and during the oogenesis of the European plaice, *Pleuronectes platessa (Linné, 1758)* » (Sauger and Kellner, 2019).

During this study, the terminology used was that of Brown-Peterson et al. (2011), since it followed the criteria set by the working groups of the International Council for the Exploration of the Sea (ICES) that defined different maturity scales for European stock species (ICES 2010, 2012a, 2012b, 2016, 2017, 2018a, 2018b).

For the description of the female germinal cells that will become gametes, the terms **oogonia** (before meiosis) or **oocyte** (meiosis has begun) will be used. An oocyte

is characterized by an **ooplasm** encased in an **oolemma** (plasmic membrane) and a **zona pellucida** (Tyler and Sumpter, 1996).

The term **follicle** will be used to designate an oocyte encased in somatic cells: follicular cells and theca cells (Tyler and Sumpter, 1996).

For the description of gamete development, the term **stage** will be used to designate the different gametogenesis stages (Brown-Peterson et al., 2011) :

- Oogonia
- Primary growth oocytes
- Secondary growth oocytes
- Oocyte maturation
- Ovulation stage

The sexual maturity cycle is split into two **states**. The Sexually Immature (**SI**) state and the Sexually Mature (**SM**) state. The terminology **phase** will be used for the gonadal development. The reproduction cycle of fish is divided into different phases (Brown-Peterson et al., 2011, ICES, 2018a).

- Immature
- Developing
- Spawning
- Regressing
- Regenerating
- Omitted spawning
- Abnormal

1.2. Processing of histological slides

For a more detailed explanation on the extraction and processing of the ovaries, please refer to the « Protocole de détermination des critères macroscopiques de gonades de poissons femelles : focus sur les stades I et IIa » (Quinquis et al., 2018).

The specimens were caught in the north sea (ICES division VII d) during the specie's spawning period (20^{th}, 21^{st}, 22^{nd}, 23^{rd} of January 2017, the 7^{th} and 18^{th} of December 2017, the 15^{th} of March, 11^{th} of June, 14^{th} of November and 12^{th} of December 2018, as well as on the 21^{st} of January, 20^{th} of February, and 26^{th} of March 2019). The median section of the ventral ovary was processed in a solution of Davidson, embedded into a paraffin block and cut with a 5µm microtome blade. The sample was then dyed in three-color Prenant-Gabe (1968) before being mounted on a slide.

The pictures found in this protocol are screenshots of the ImageScope interface used during the stereology counting process.

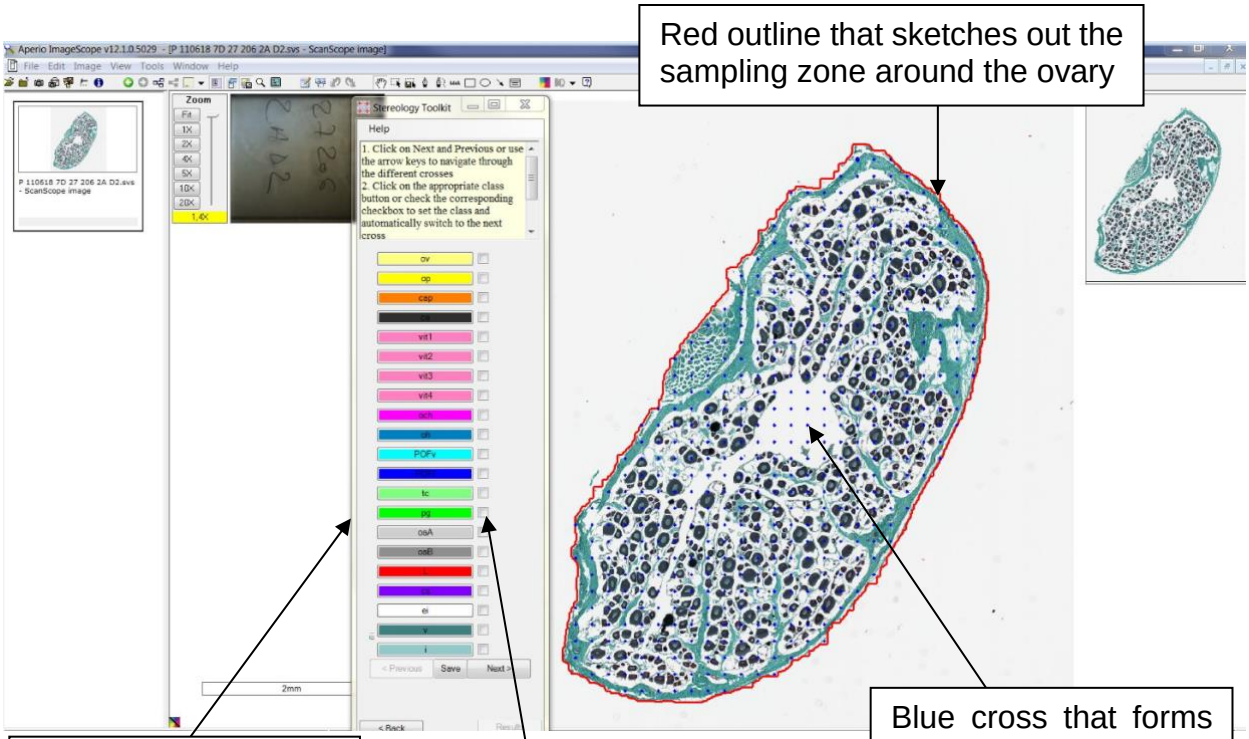
1.3. Stereology Software

For the stereological readings, the software ImageScope (version 12.1.0.5) was used. For each histological slide, the ovaries were outlined (red line) and a grid of crosses (in blue) covered the sampling zone. For each of the 500 to 600 sampling points (blue crosses) a single structure was assigned (box to tick inside Stereology Toolkit window).

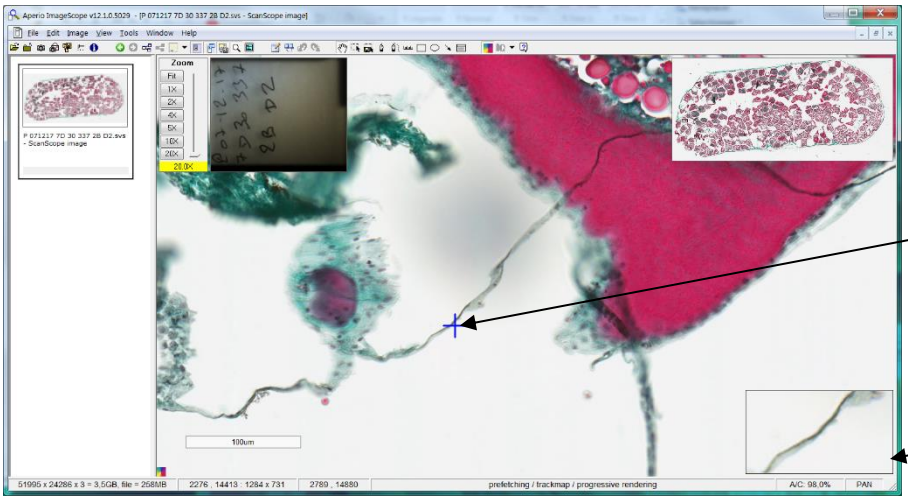
A list of the different cells and structures potentially present inside the ovaries of the plaice was established with the help of the lexicon (Sauger and Kellner, 2019). These 20 identifiable structures inside the plaice ovaries were described in the « Lexicon of histological structures found in the ovaries and during the oogenesis of the European plaice, *Pleuronectes platessa (Linné, 1758)* ». This lexicon allows the identification of the different cell types found throughout the plaice ovaries, and is illustrated with pictures of « perfect looking cells ». Albeit, in a histological slide the presence of artefacts, deformed structures and poorly cut cells (no visible nucleus) are recurring problems.

Other than the lexicon, in order to properly ascertain the different cell types, this software can be used to measure the structure that is to be identified. If the reader hesitates between two different cell types (like between **op1** and **op2**), it is possible to measure the oocyte in order to settle his choice. The measurements of a follicle goes straight across the cell, from the exterior membrane of the theca to the other exterior membrane of the same theca, while passing over the nucleus. If the follicle is distorted (ripped or stretched out), do not measure in the direction of the distortion.

This protocol will facilitate the decision making when the reader encounters these difficult to analyze and categorize elements and structures.



The structures are identified by a blue cross ( ). These crosses are equidistant from one another, and the starting point of the grid is randomize by the software. Each gonad has a sampling grid with 500 to 600 crosses. The center of the cross, on a pixel-precise scale, is used to point out the structure that is to be identified.

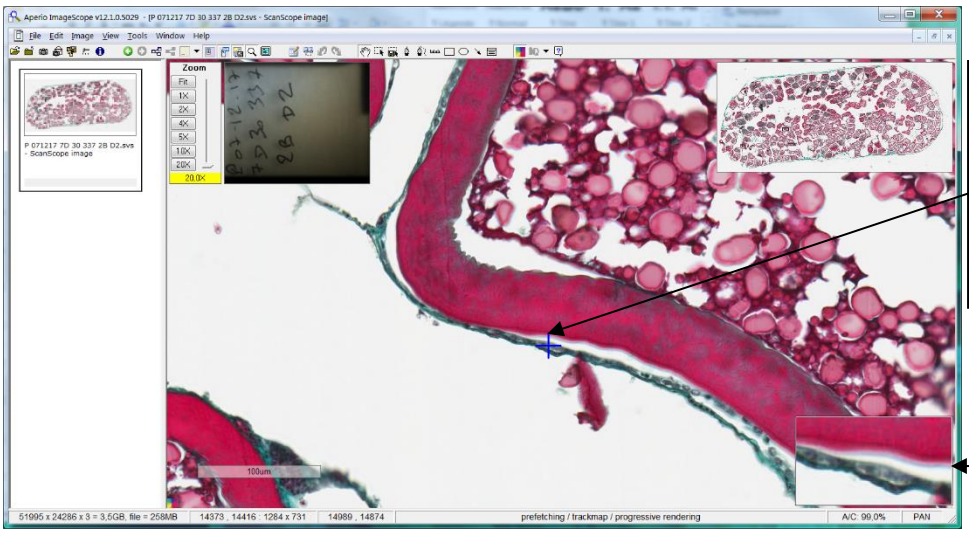


A blue cross next to connective tissue, with the center of the cross in the intercellular space (**ei**). Put this cross in the **ei** category

Widow that zooms on the mouse cursor location

For this study, we will identify the **follicles**. This means that we will take into consideration the **oocyte** and its somatic tissue. If the center of a cross falls on the **theca** of an **oocyte**, we consider that the structure that is to be identified is the **oocyte encased inside this theca**.

Moreover, if the cross falls between the **theca** and the **zona pellucida** (with the **theca** detached from the **zona pellucida**), we still identify the cross as being on the **follicle**.

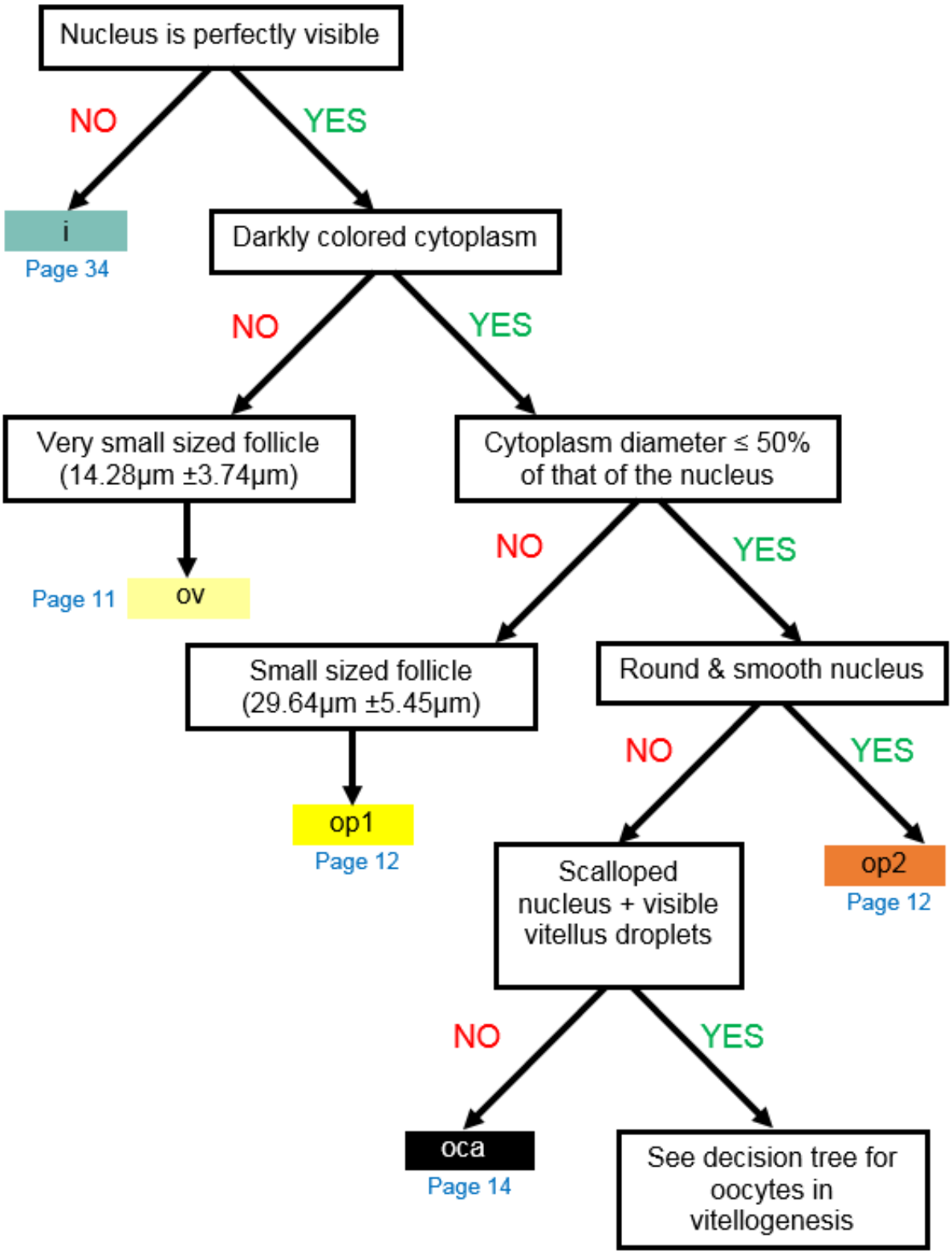


Blue cross on the detached theca of a follicle. Put this cross in the **vit4** category

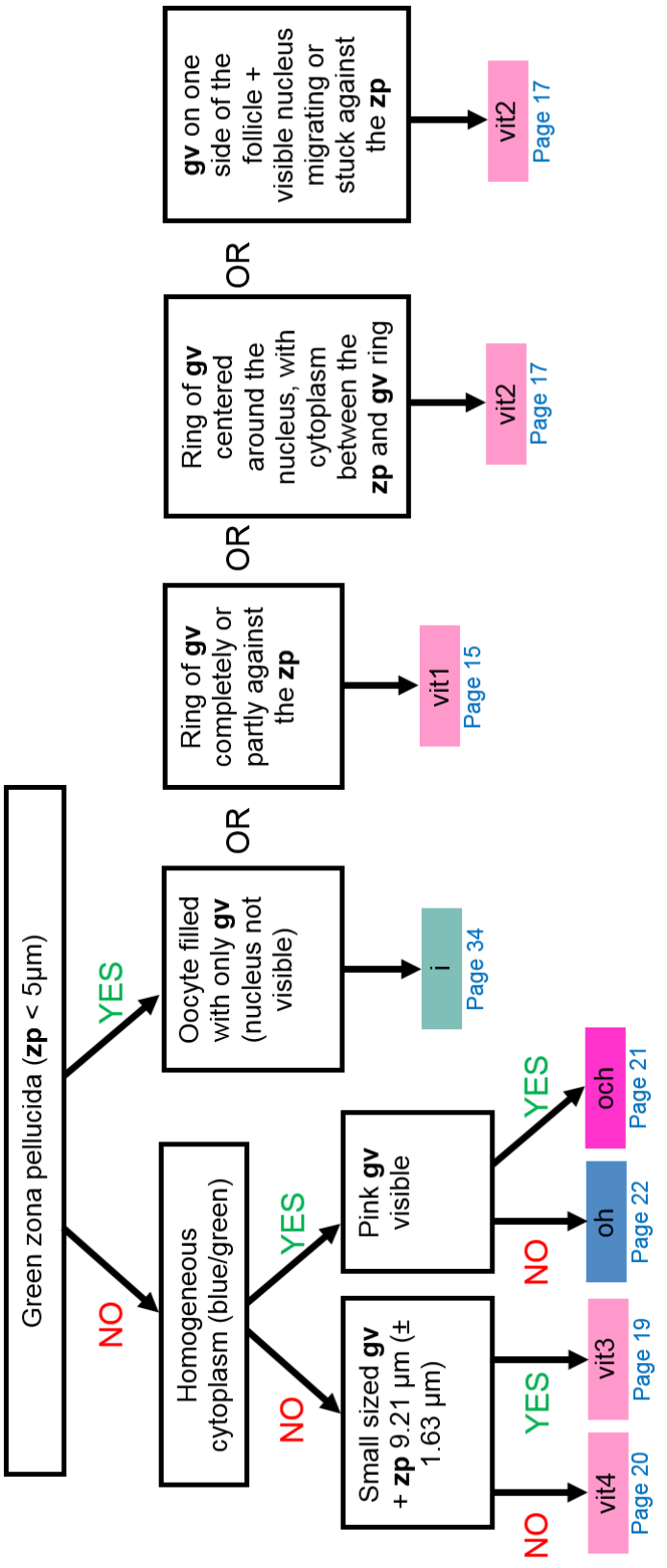
Zoom on the somatic cells of the **vit4** follicle

2. Decision trees

2.1. Previtellogenic oocytes



2.2. Vitellogenic and maturing oocytes

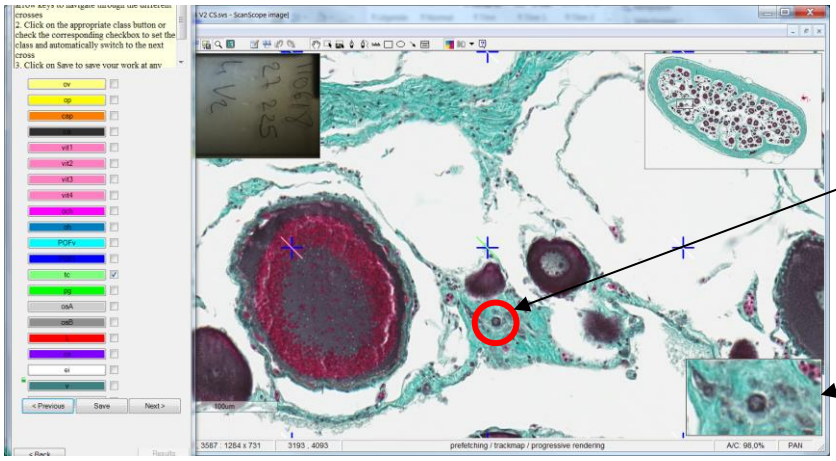


3. Structure identification

3.1. Oogonium (ov)

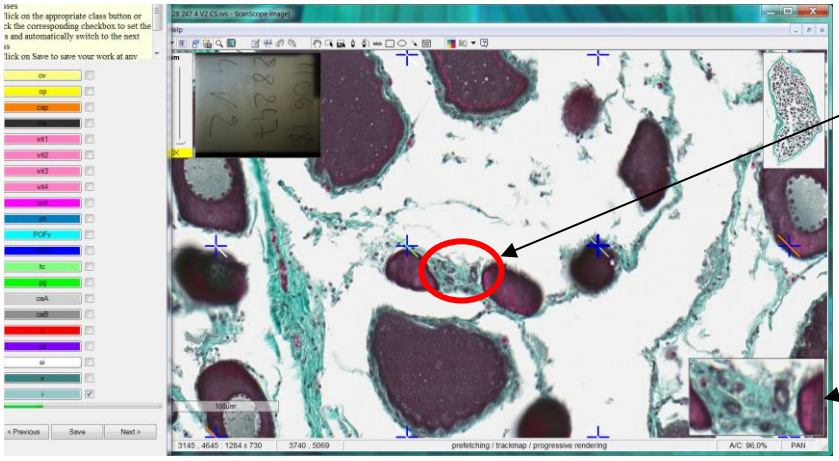
Average size

ov : 14.28µm (±3.74µm)



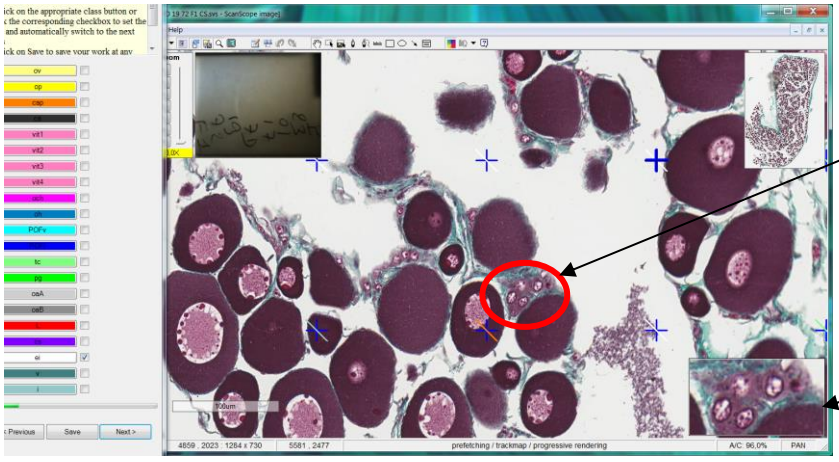
Size of an oogonium (**ov**) in the ImageScope interface

Zoom on the oogonium (**ov**)



Size of oogonia (**ov**) in the ImageScope interface

Zoom on the oogonia (**ov**)



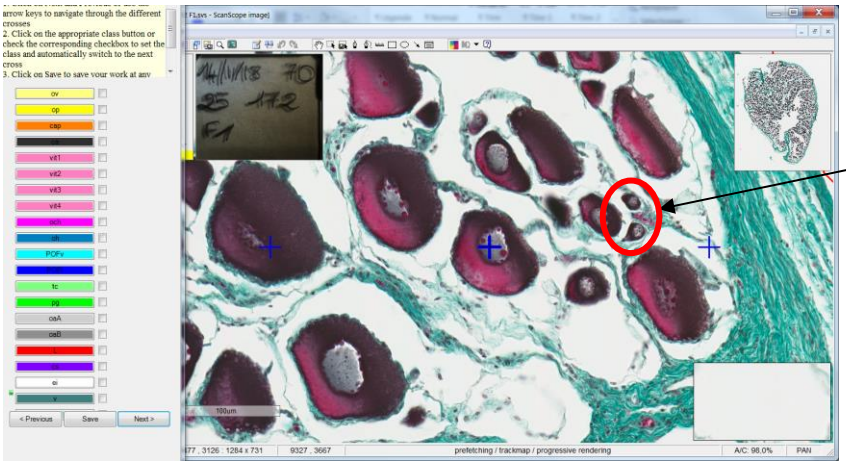
Size of oogonia (**ov**) in the ImageScope interface

Zoom on the oogonia (**ov**)

3.2. Premature oocyte stage 1 (op1)

Average size

op : 29.64µm (±5.45µm)

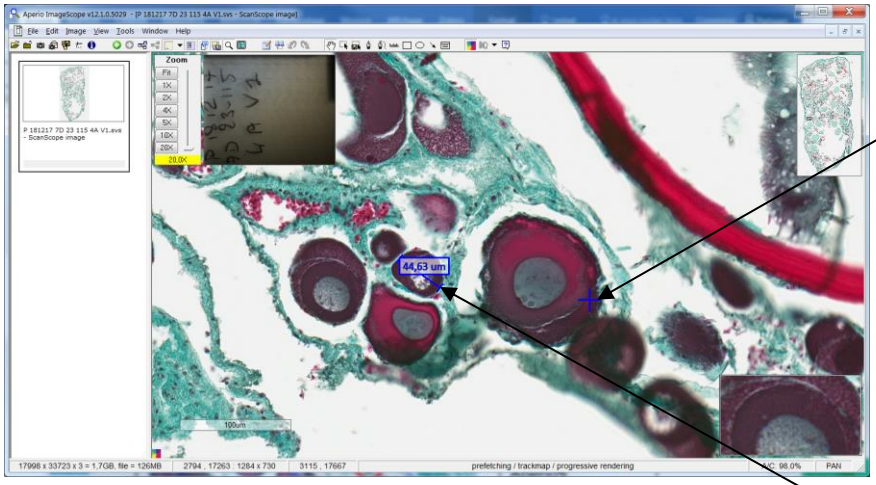


Size of two premature stage 1 oocytes (**op1**) in the ImageScope interface

3.3. Premature oocyte stage 2 (op2)

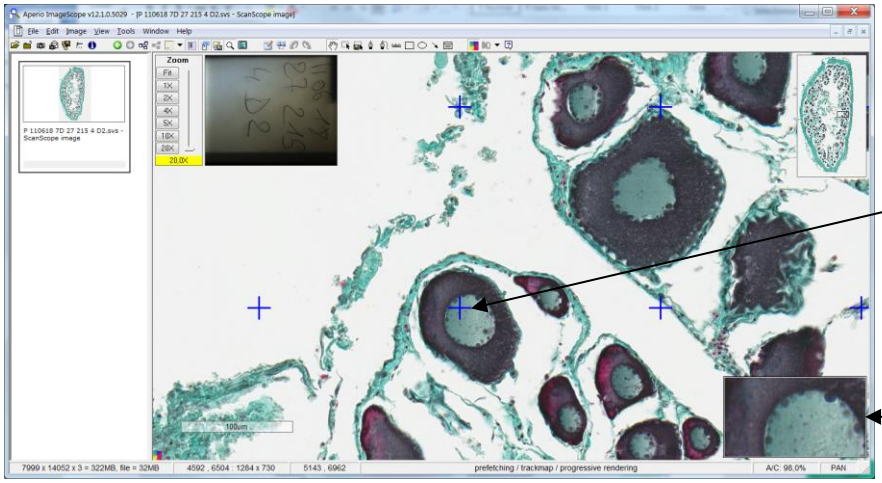
Average size

cap : 82.70µm (±15.89µm)



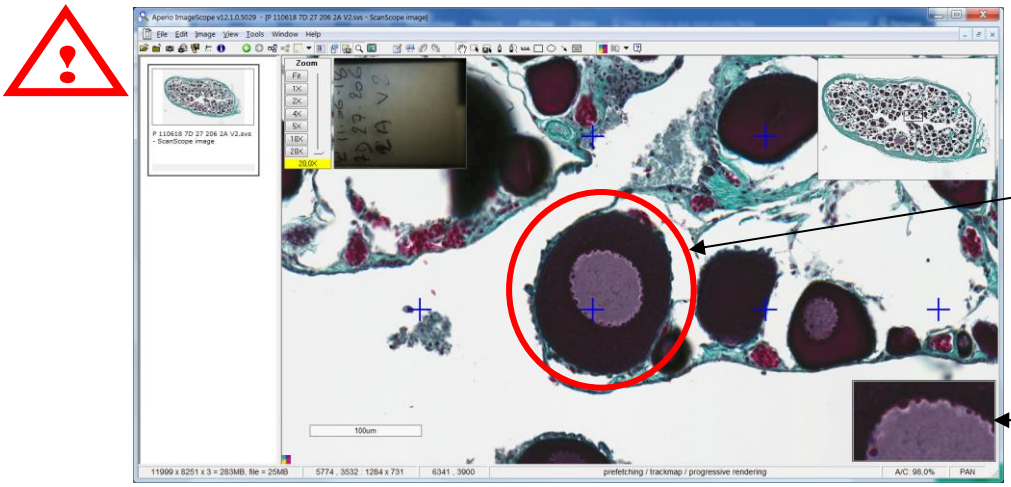
There seems to be either two cytoplasms or an artefact effect on the slide. The nucleus is round and smooth. Put this cross in the **op2** category

Smooth nucleus with a large nucleolus, and a cell to cytoplasm diameter ratio too big to be a **op1**. Put this cross in the **op2** category



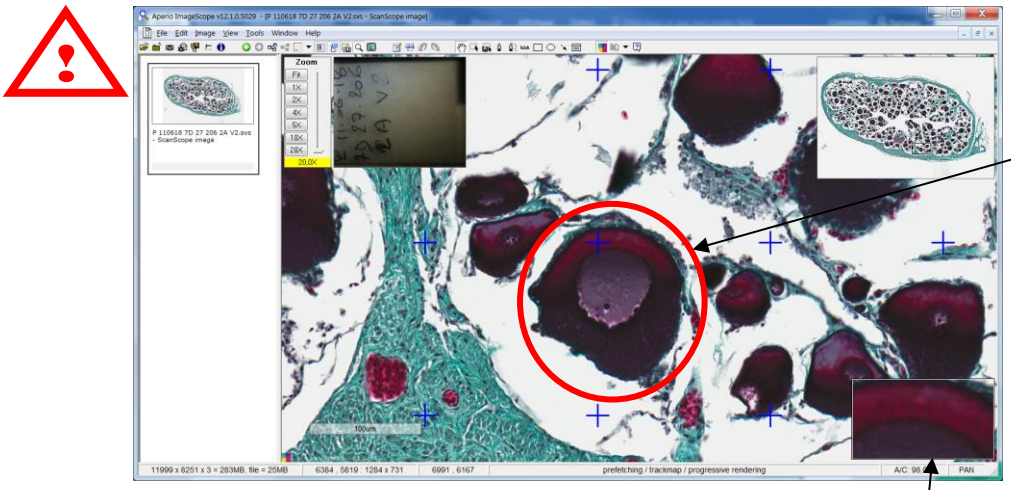
Smooth nucleus. Put this cross in the **op2** category

Zoom on the smooth nucleus



The nucleoli are against the nuclear membrane, but the nucleus is still smooth! Put this cell in the **op2** category

Zoom on the smooth nucleus



The cell is warped (stretched downwards). Look at the unstretched part of the cell, the nucleus is smooth! Put this cell in the **op2** category

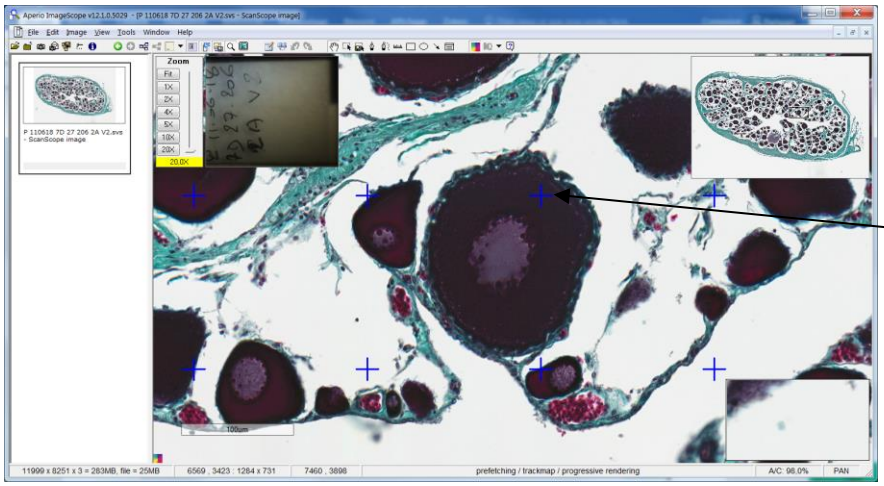
Zoom on the unstretched part of the nucleus

3.4. Cortical alveoli (oca)

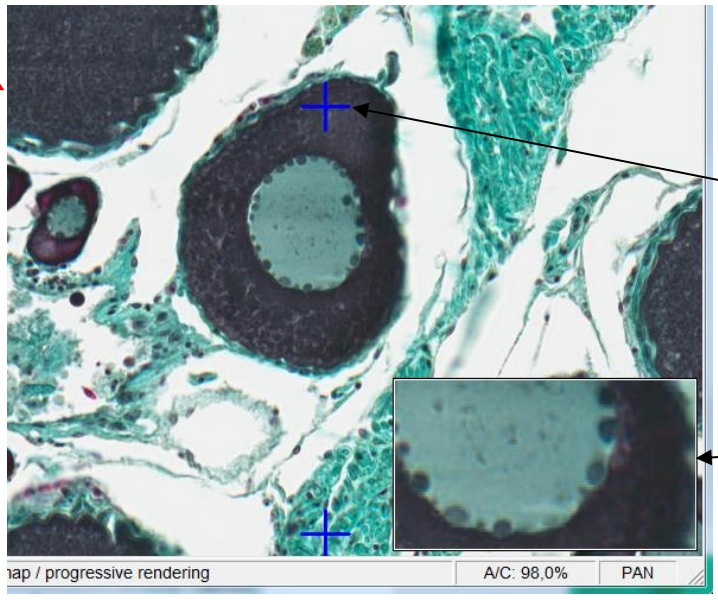
oca = cortical alveoli oocyte

Average size

oca : 153.07µm (±17.83µm)
zp : 0.91µm (±0.23µm)

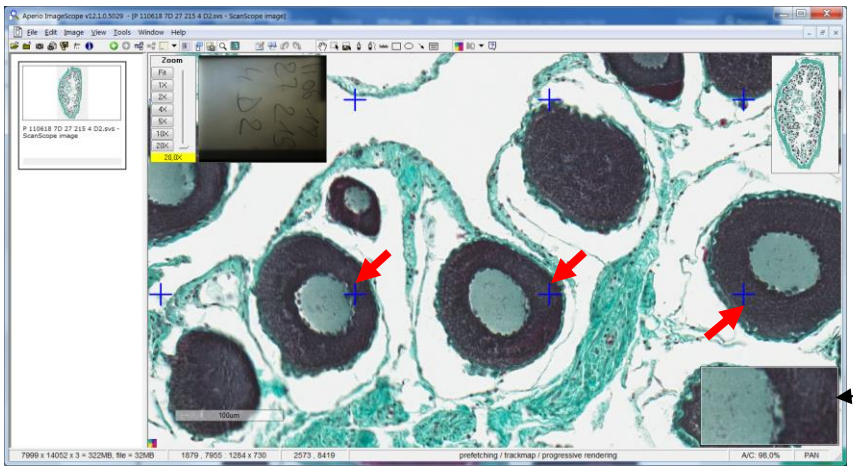


Scalloped nucleus (the nuclear membrane takes a scalloped form). A ring of light colored droplets can be seen near the follicular cells. Put this cross in the **oca** category



The nucleus is beginning to scallop. Put this cross in the **oca** category. Measuring this follicle can help determine the cell type. If there is a doubt, mark this cross as **i**.

Zoom on the nuclear membrane of the nucleus



Scalloped nuclei. Put these three crosses (↙) in **oca**.

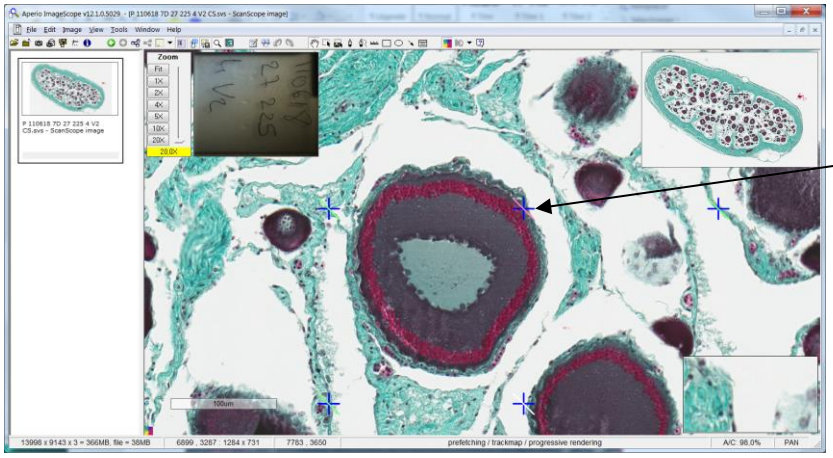
Zoom on a scalloped nucleus

3.5. Vitellogenesis 1 (vit1)

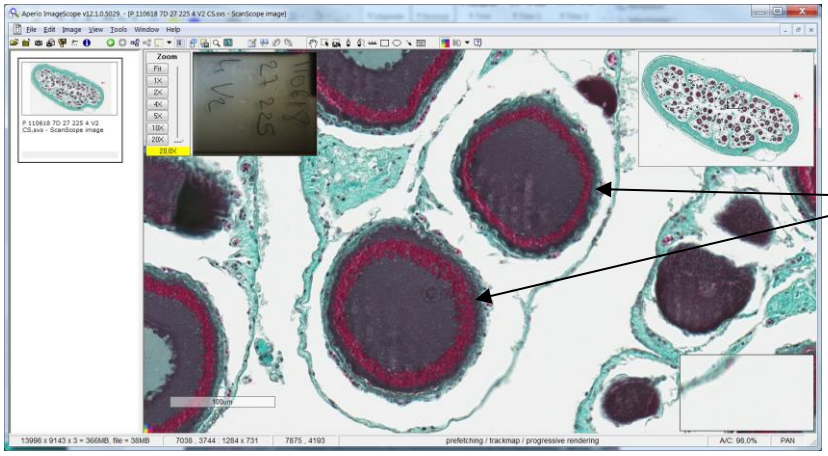
Average size

vit1 : 191.52µm (±29.08µm)
zp : 1.43µm (±0.32µm)

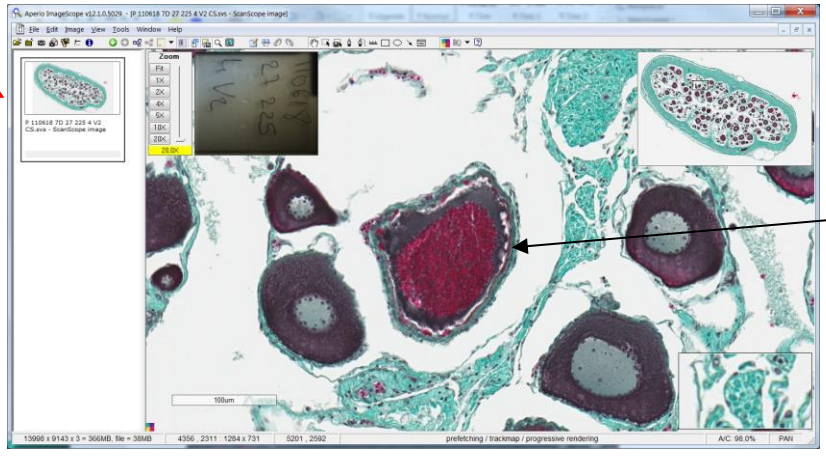
vit1 = oocyte in vitellogenesis with a ring of vitellus droplets against the zona pellucida



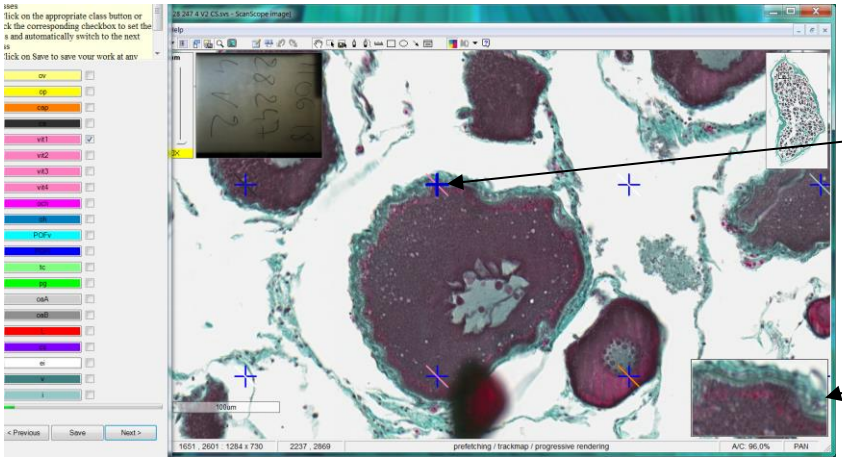
Cross on the theca of a **vit1** follicle. Put this cross in the **vit1** category



Two cells and no visible nuclei. The ring of vitellus is against the **zp**. Put these follicles in the **vit1** category



We cannot see the nucleus in this follicle filled with vitellus droplets (bad cutting angle). Put this follicle in the **i** category



Cross on a follicle that has begun to form a ring of vitellus droplets. Put this follicle in the **vit1** category

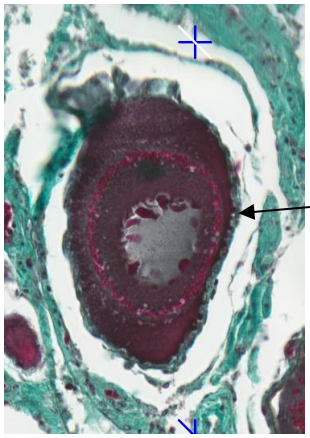
Zoom on the ring of vitellus

3.6. Vitellogenesis 2 (vit2)

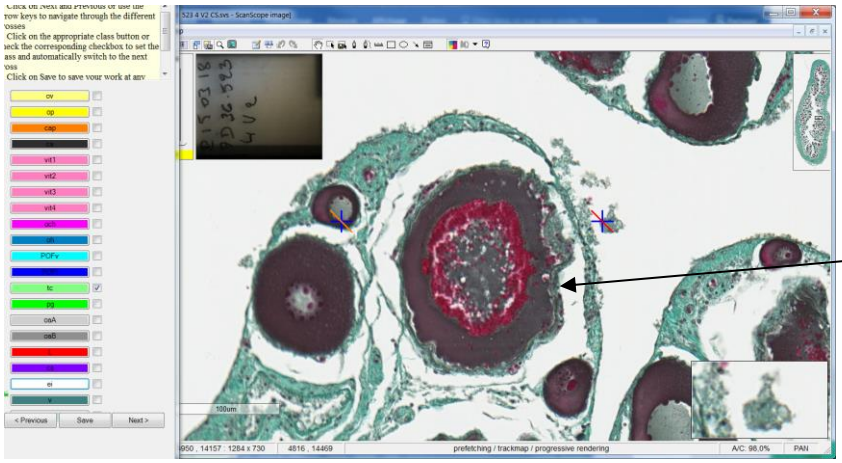
vit2 = oocyte in vitellogenesis with the migration of the nucleus

Average size

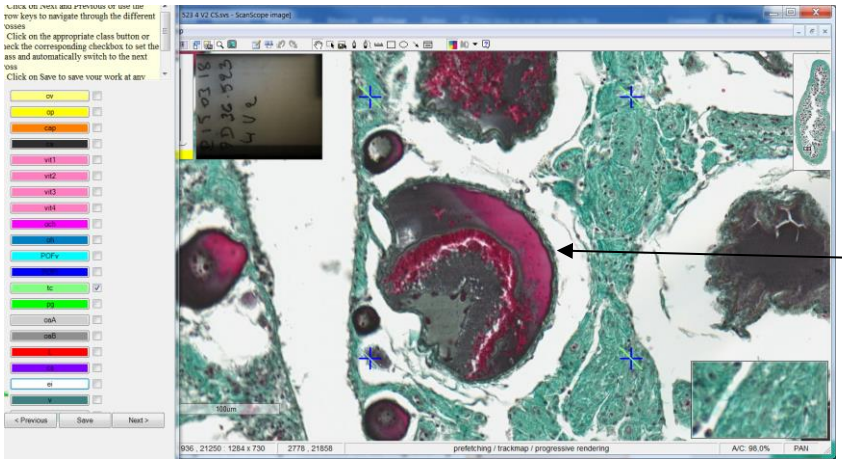
vit2 : 220.19µm (±25.28µm)
zp : 1.45µm (±0.20µm)



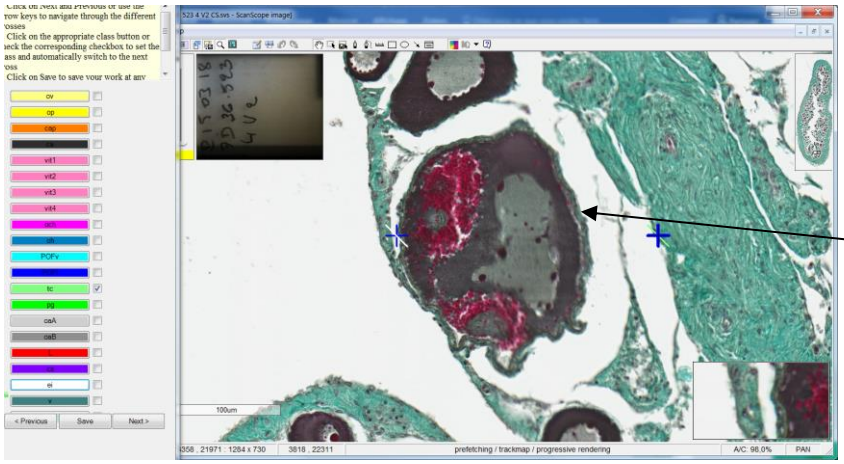
Follicles with a zone of cytroplasm between the **zp** and the ring of vitellus droplets. Put these follicles in the **vit2** category



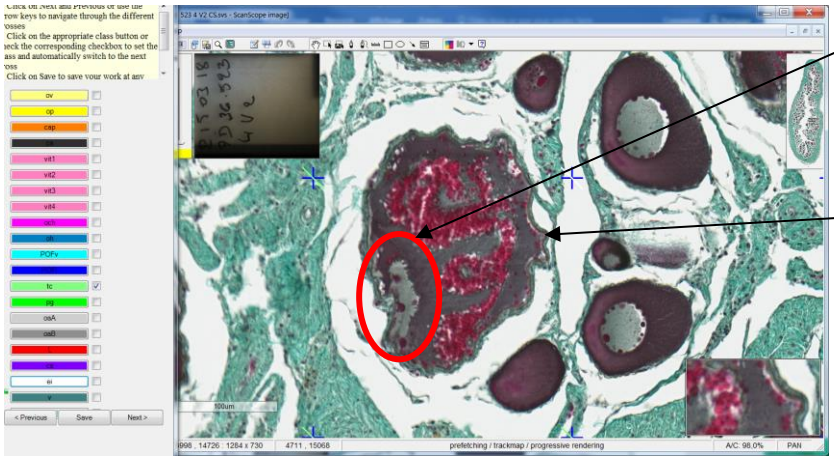
Follicle with a zone of cytroplasm between the **zp** and the ring of vitellus droplets. Even if the nucleus is not visible, put this follicle in the **vit2** category



Follicle with a zone of cytroplasm between the **zp** and the ring of vitellus droplets. The nucleus is migrating through the vitellus ring. Put this follicle in the **vit2** category



Follicle with a zone of cytroplasm between the **zp** and the ring of vitellus droplets. The nucleus is migrating through the vitellus ring. Put this follicle in the **vit2** category



Nucleus against the **zp**

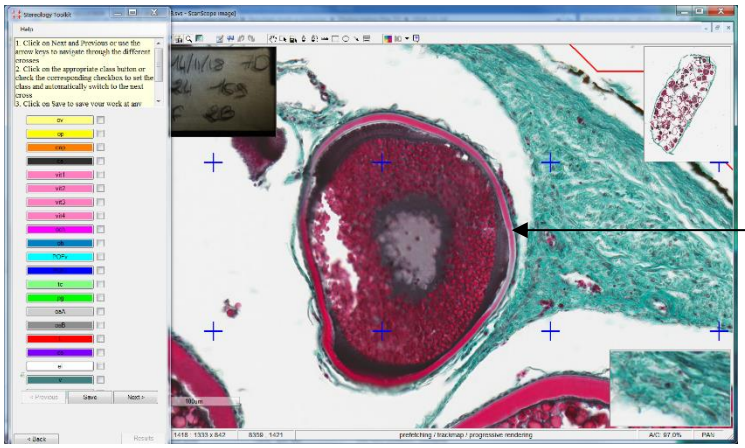
Follicle with the nucleus against the **zp** and the vitellus droplets on the other side. Put this follicle in the **vit2** category

3.7. Vitellogenesis 3 (vit3)

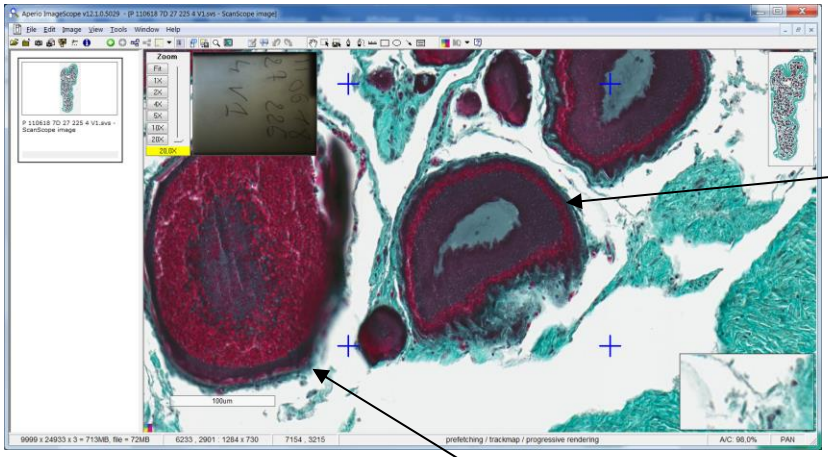
vit3 = oocyte in vitellogenesis with the growth of the zona pellucida

Average size

vit3 : 380.02µm (±57.16µm)
zp : 9.21µm (±1.63µm)



Follicle filled with small **gv** and a thin **zp** that is taking on a pink tinge. Put this follicle in the **vit3** category



Warped follicle and nucleus, but the ring of vitellus droplets is against the **zp**. Put this follicle in the **vit1** category

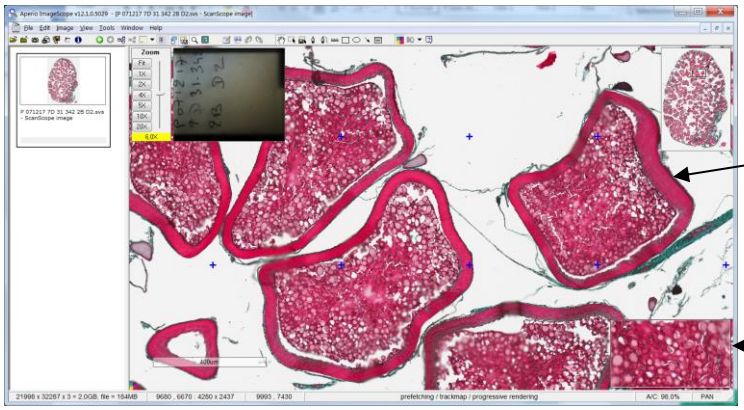
We don't see the nucleus in this follicle. The ring of vitellus is slightly detached from the **zp**, and the **zp** is growing (taking a pink tinge). Put this follicle in the **vit3** category

3.8. Vitellogenesis 4 (vit4)

vit4 = oocytes at the end of their vitellogenesis

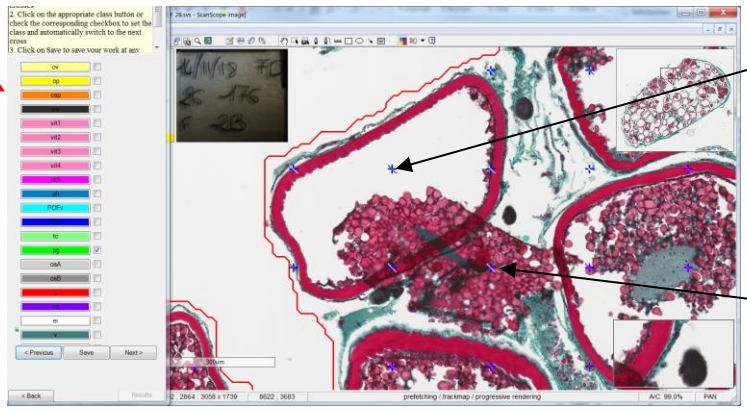
Average size

vit4 : 629.47µm (±88.45µm)
zp : 53.81µm (±5.10µm)



Follicle filled with **gv**, and a thick and pink **zp**. Put this follicle in the **vit4** category

Zoom on the big **gv**



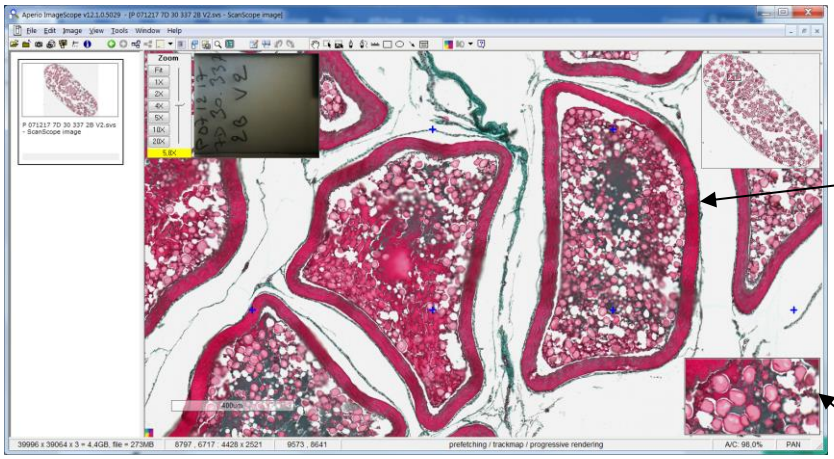
Cross inside a a follicle with the **gv** that have shifted out of the follicular cells. Put this cross in the **v** category

Cross outside of the cell membranes, but inside the shifted **gv**. Put this follicle in the **vit4** category

3.9. Oocyte hydration (och)

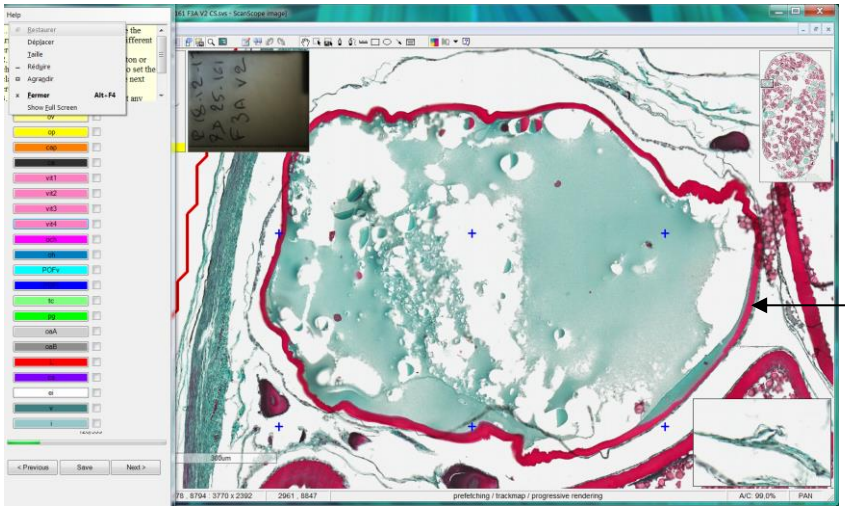
Average size

och : 847.21µm (±104.01µm)
zp : 33.11µm (±3.50µm)



Follicle with a thick and pink **zp**. Homogeneous vitellus (blue/green coloration) between large **gv**. Put this follicle in the **och** category

Zoom on large **gv** in a zone of homogeneous vitellus

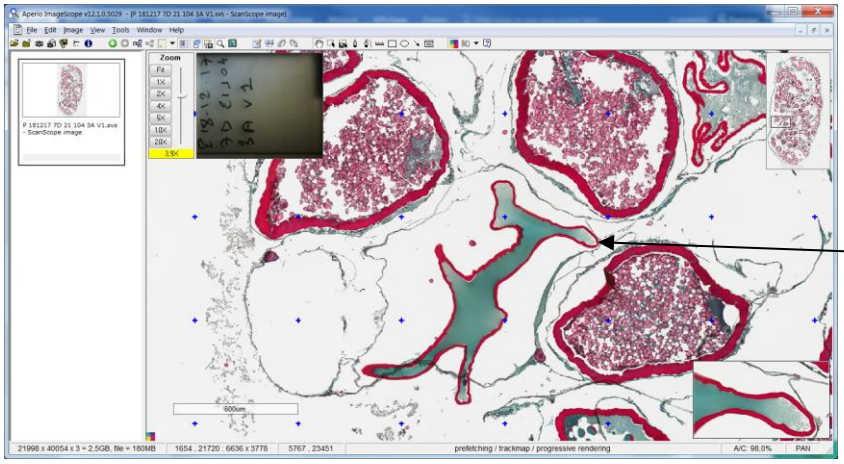


Follicle with a lot of homogeneous vitellus and still a few pink **gv**. Put this follicle in the **och** category

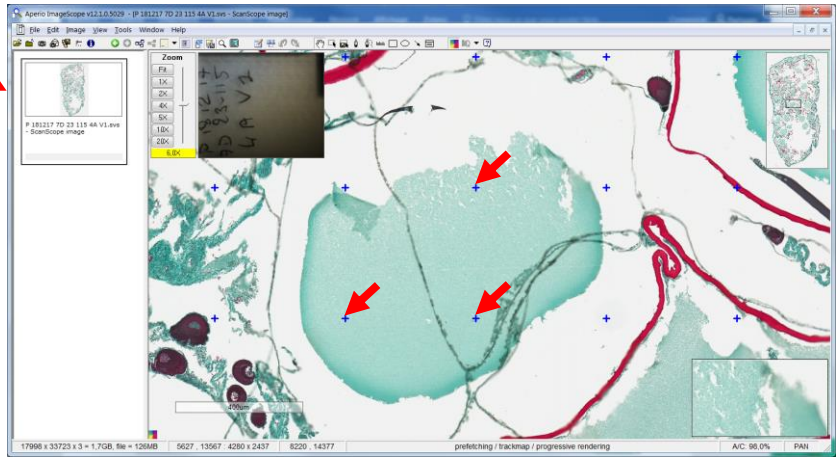
3.10. Hydrated oocyte (oh)

Average size

oh : 958.66µm (±60.66µm)
zp : 40.41µm (±5.00µm)

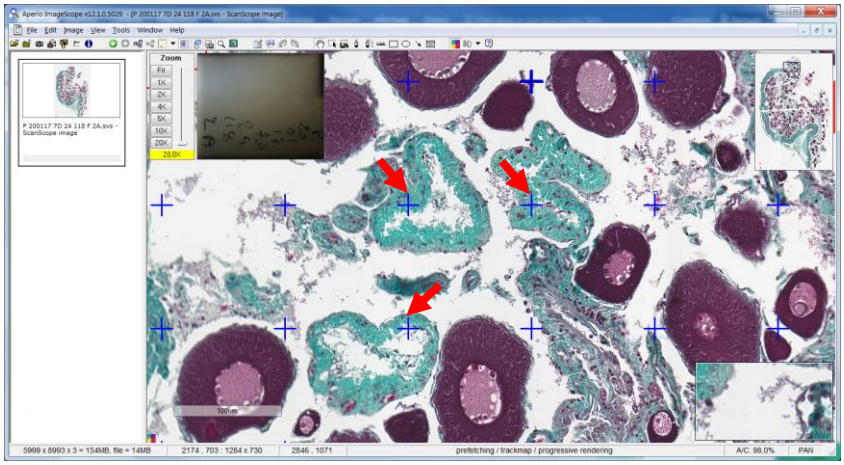


Follicle with a thick and pink **zp**. Only homogeneous vitellus (blue/green) is found inside the cell. The theca can still be seen around the warped cell. Put this follicle in the **oh** category

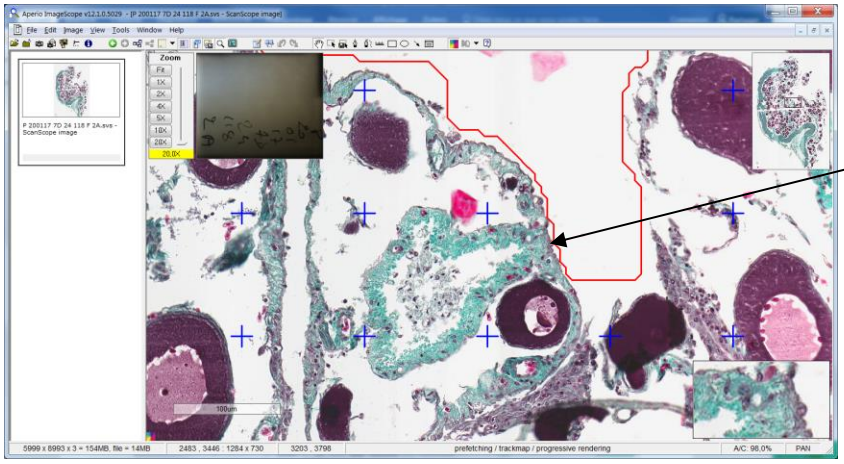


Crosses (↙) in a zone of homogeneous vitellus that was torn from its **zp** and/or theca. Put this follicle in the **oh** category

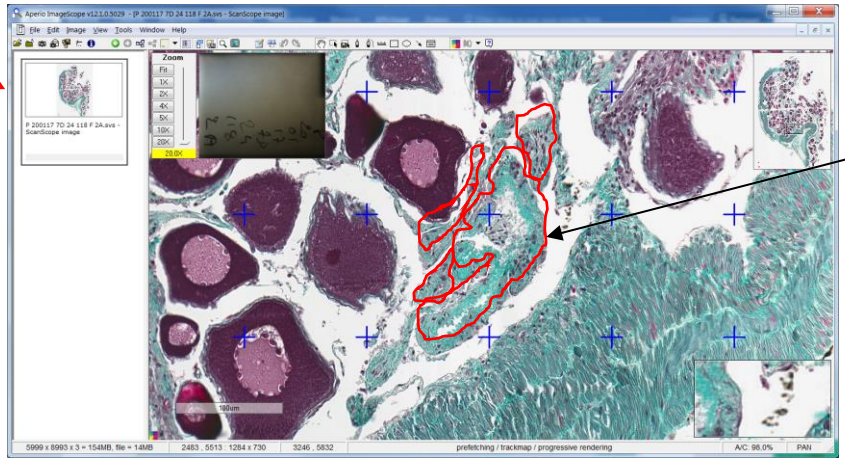
3.11. Post-Ovulatory Follicle (POF)



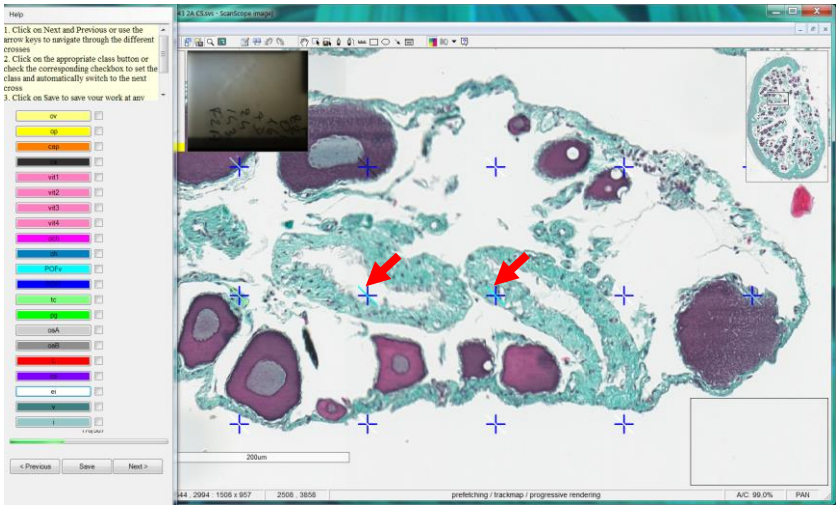
POF containing macrophages for these 3 crosses (↙)



POF containing macrophages. Here no cross falls on this structure

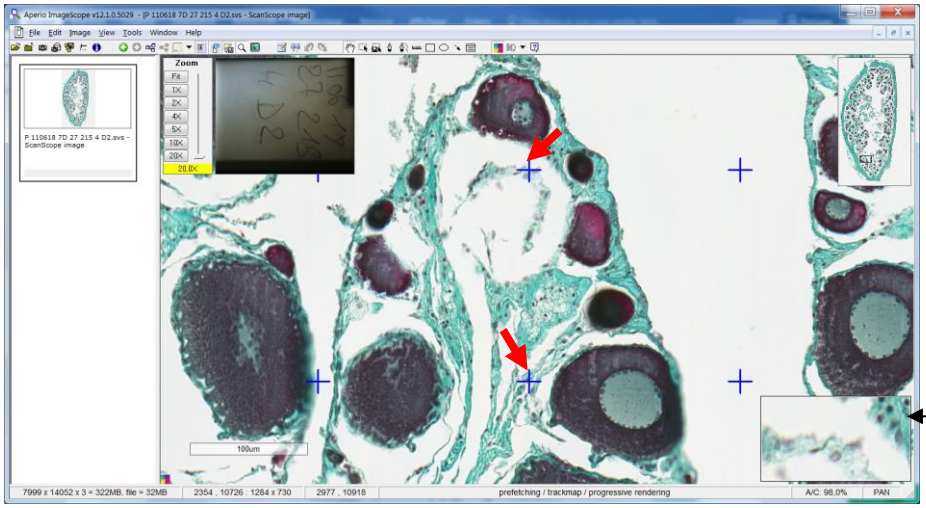


POF (outlined in red) encased in **tc**. Be careful not to mix up the two ! In certain cases, it is difficult to see where one ends and the other begins



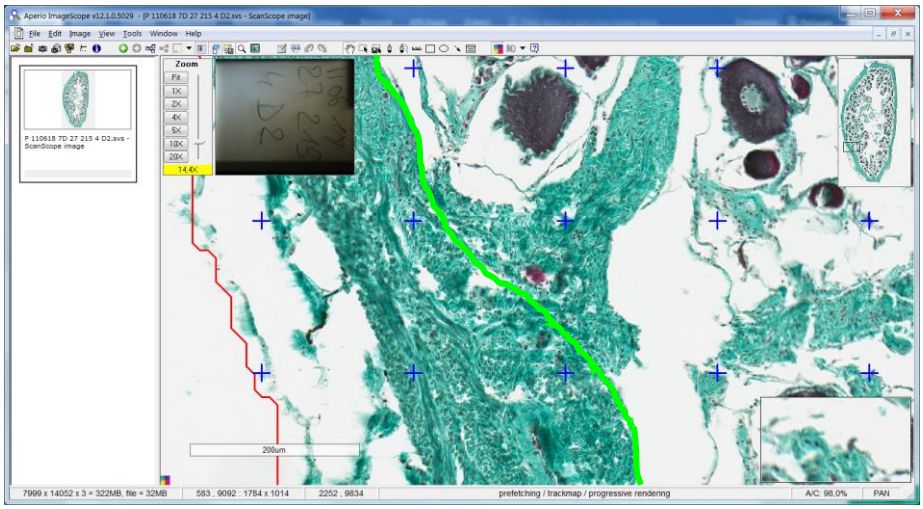
POF for these 2 crosses (↙)

3.12. Connective tissue (tc)



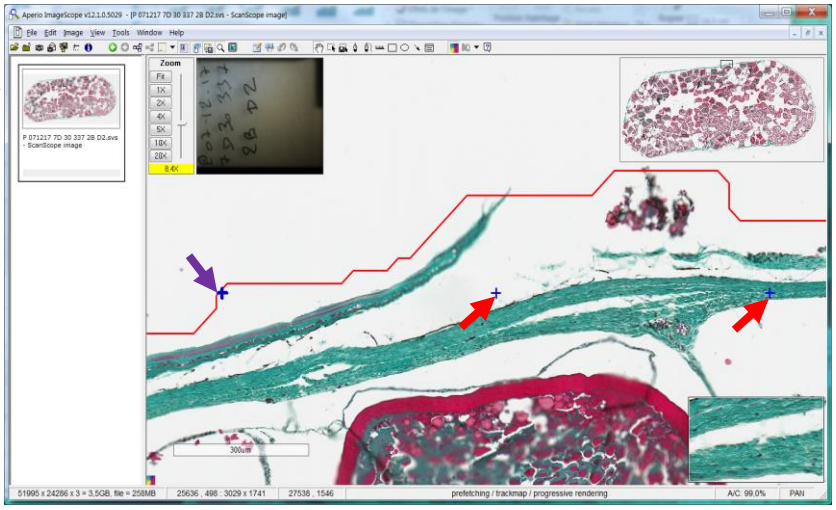
Cosses on **tc** ( ). Put these structures in the **tc** category

Zoom on the top cross

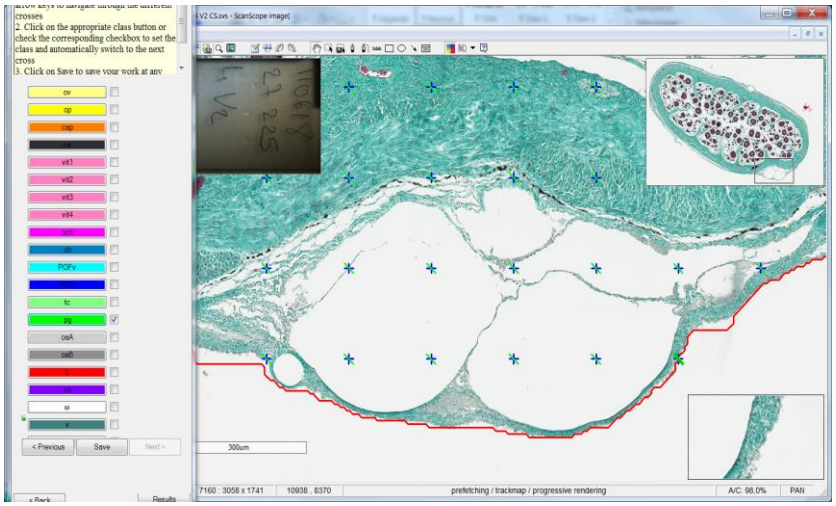


Green line = boundary between the **pg** (left) and the **tc** (right) that will lead into the ovarian lamella

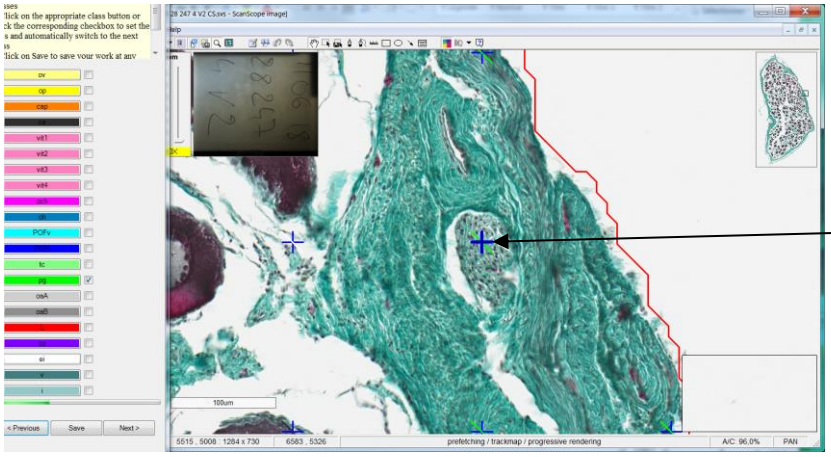
3.13. Gonadal wall (pg)



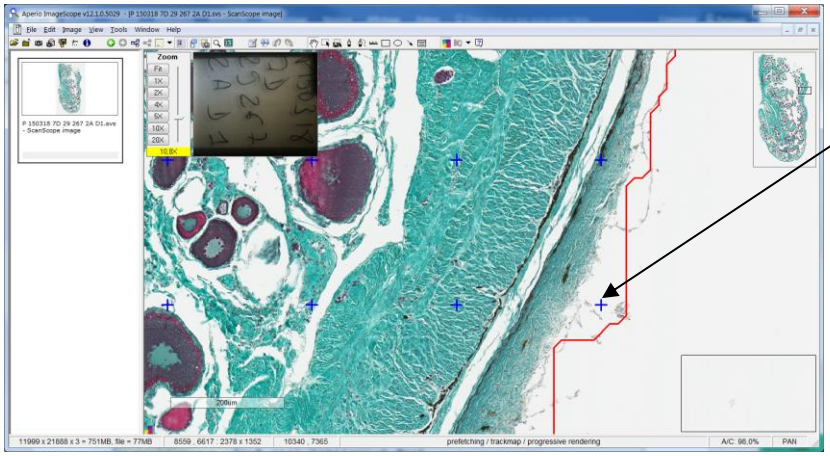
Cross on the **pg** ( ). The middle cross is under a layer of cell from the **pg**, so even if it's in a « white zone » it will be categorized as **pg**. On the other hand, the cross on the left ( ) is above the external cell layer of the **pg**, and under the sampling grid boundary (red line). Put this cross in the **v** category



All the crosses here are between the external and internal cell layer of the **pg**. Put all of these crosses into the **pg** category

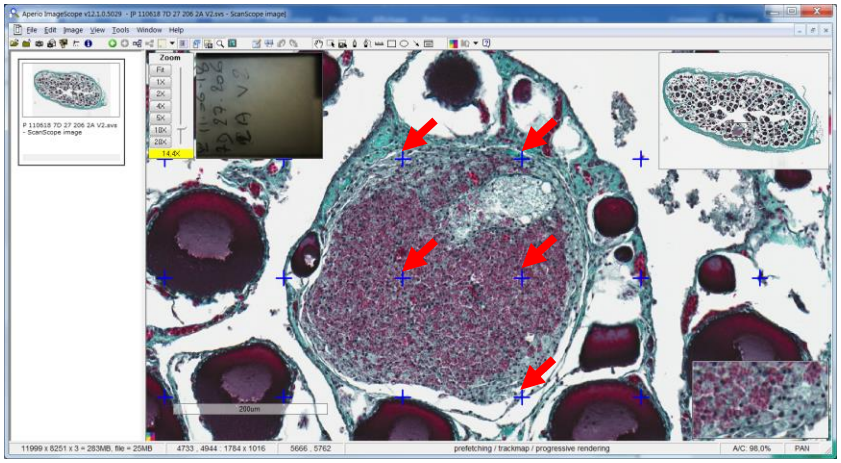


Cross on muscle tissue inside the **pg**. Put this cross in the **pg** category

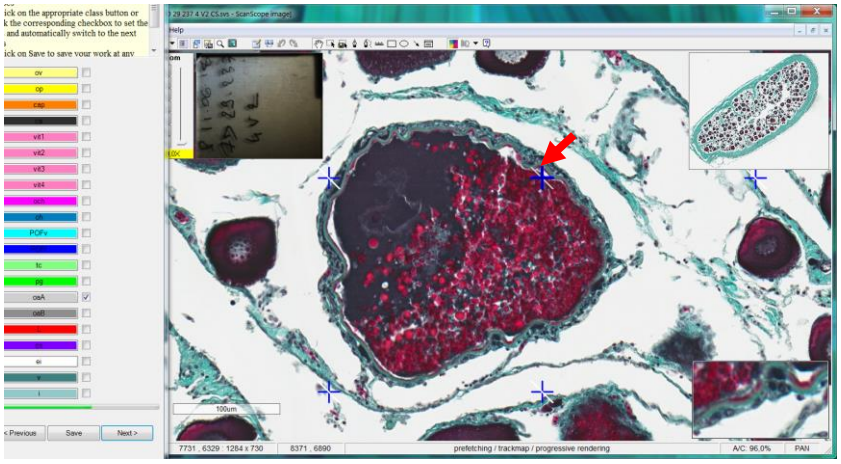


Cross under cells from the external layer of **pg**. Put this cross in the **pg** category

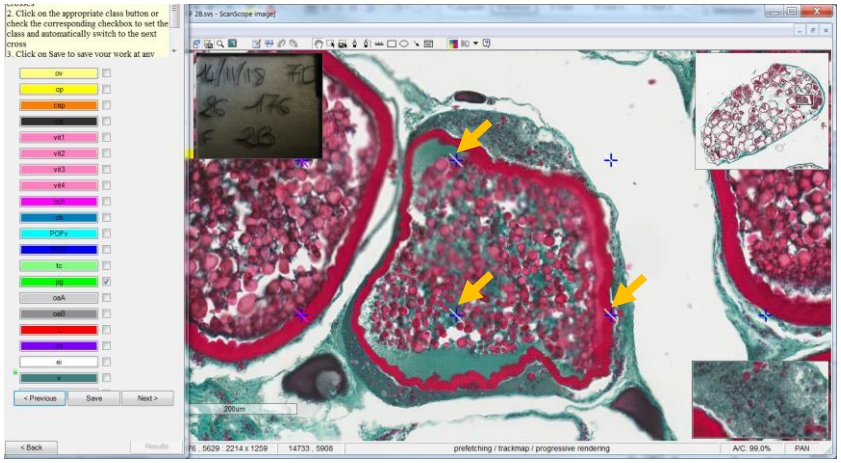
3.14. Atretic oocyte alpha (oaA)



Cross on a **oaA** ( ), oocyte encased in its theca, inside the ovarian lamella

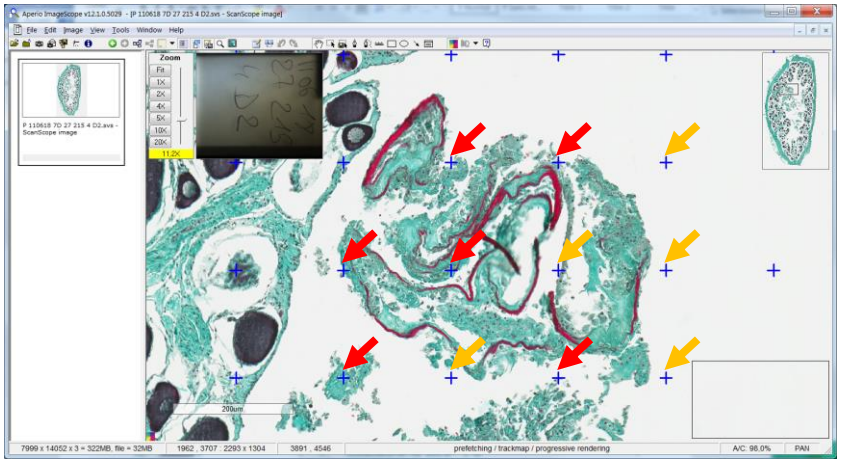


Cross on a **oaA** ( ), oocyte encased in its theca, inside the ovarian lamella



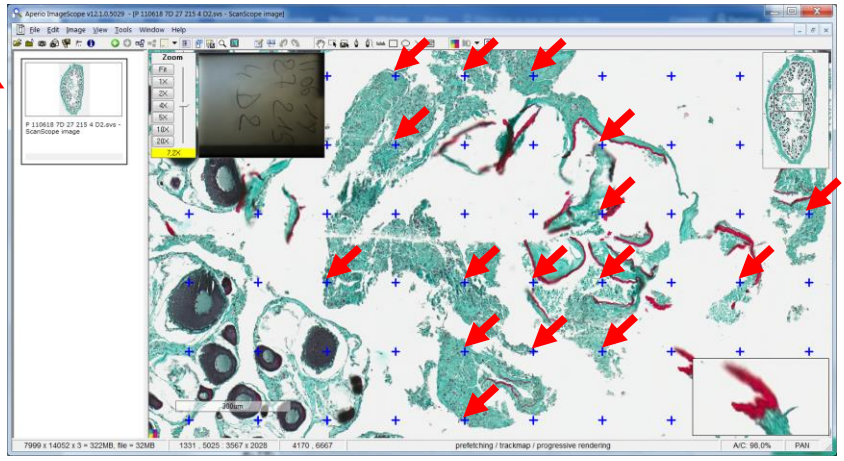
Cross on a **oaA** ( ), oocyte encased in its theca, inside the ovarian lamella. Macrophages can be seen between the **zp** and the theca

3.15. Atretic oocyte beta (oaB)



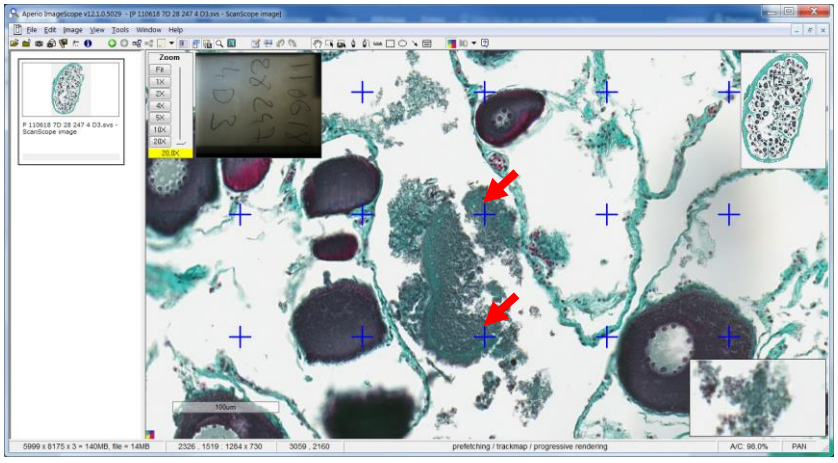
Crosses on a **oaB** (➘)

Crosses in **ei** (➘)



Crosses on a **oaB** (➘)
The other crosses in this zone are in **ei**

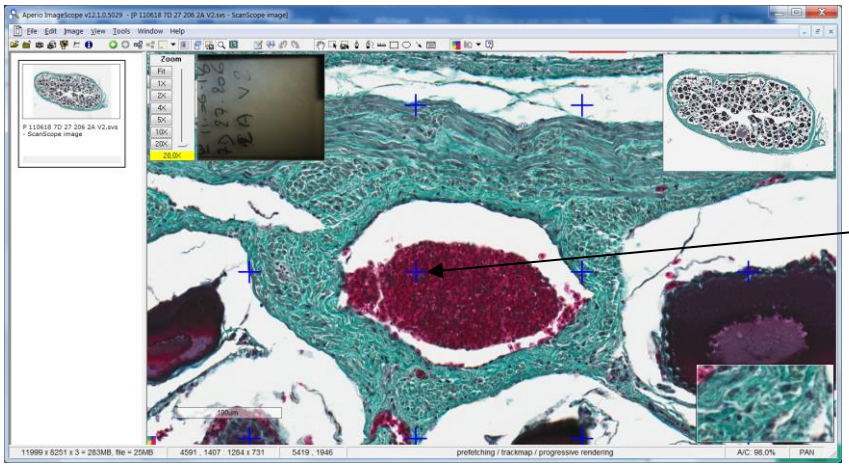
3.16. Lysis (L)



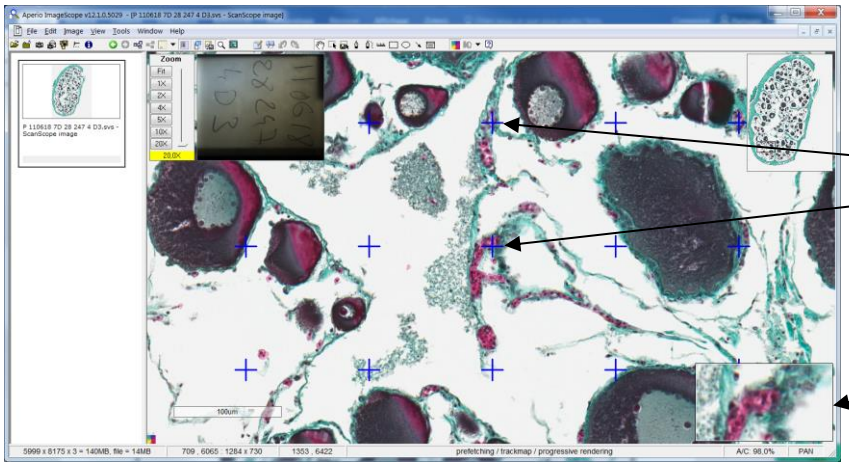
Crosses in a zone of **L** (➘)

3.17. Blood vessels (cs)

cs = blood capillaries and/or blood vessels



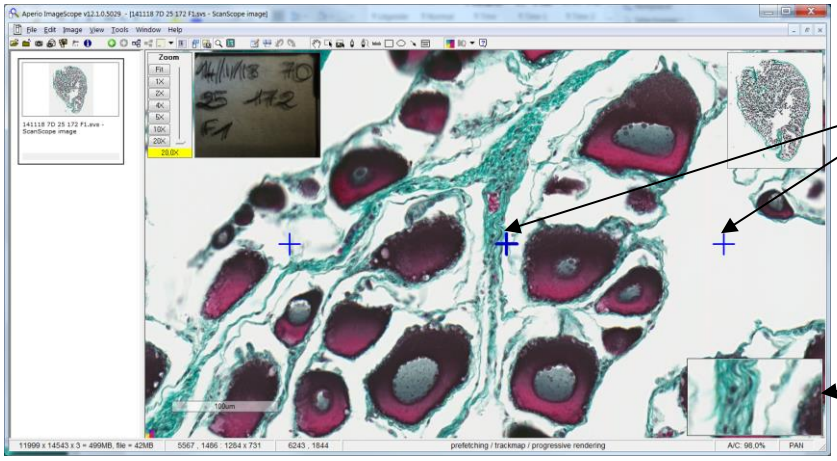
Cross in a blood vessel. Put this cross in the **cs** category



Crosses in blood capillaries. Put these crosses in the **cs** category

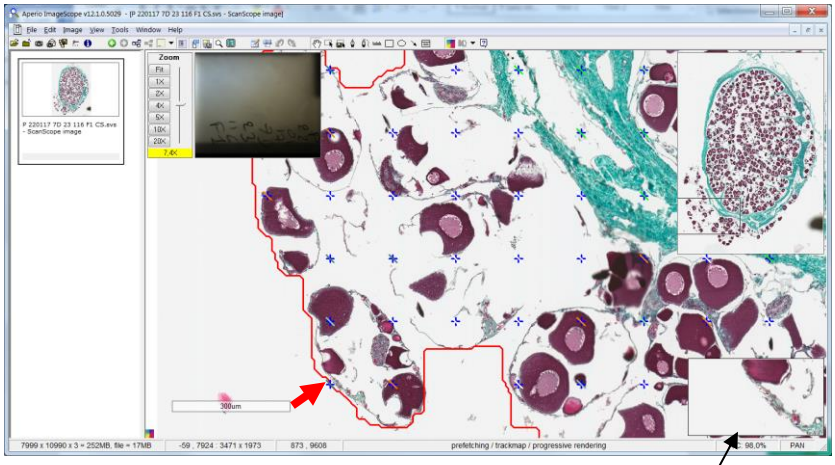
Zoom on the blood cells

3.18. Intercellular space (ei)



Crosses on **ei**. Put these crosses in the **ei** category

Zoom on the middle cross. The center of the cross lands between two **tc** structures

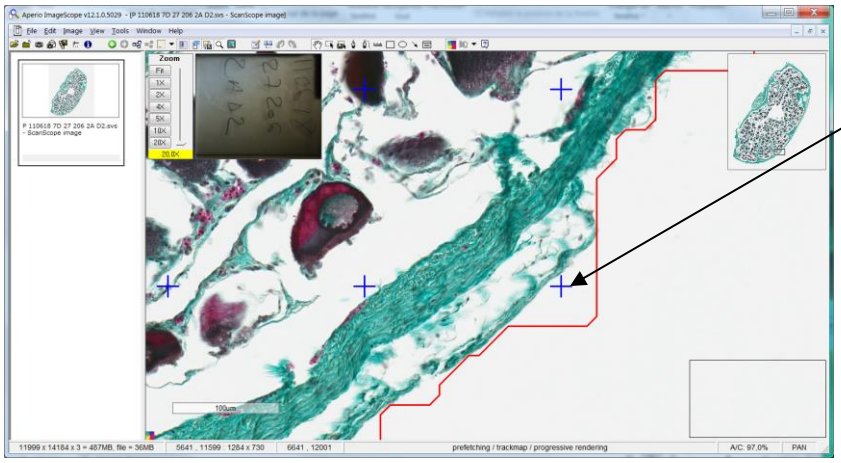


Follicles are outside of the gonadal wall. The crosses that fall between the follicles are categorized under **ei**. Only the bottom left cross (↙) will be categorized under **v** because it falls between the **tc** and the boundary of the sampling grid (red line)

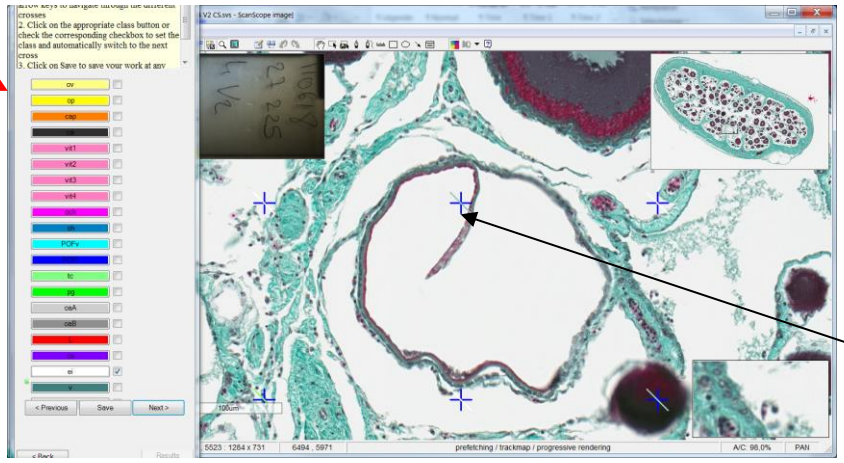
Zoom on the bottom left cross

3.19. Unnatural emptiness (v)

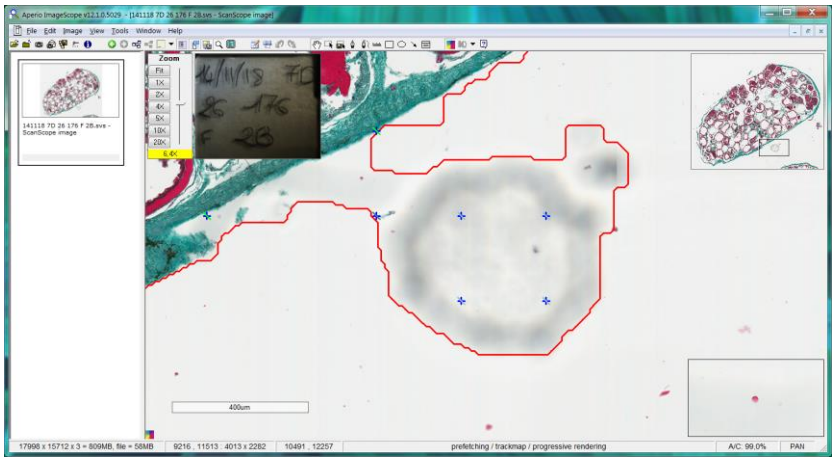
v = empty zone on the grid due to the setup of the slide



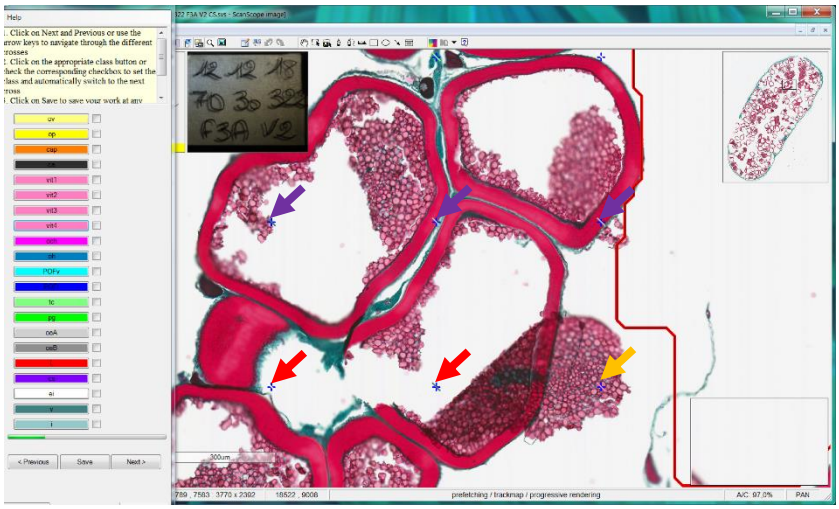
Cross outside of the outer layer of **pg** cells. Put this cross in the **v** category



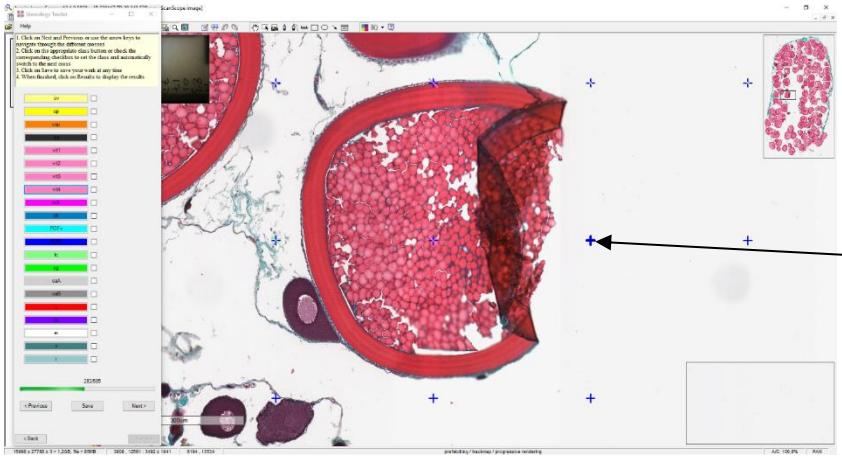
Cross inside a zone encased by a theca and **zp**. The rest of the cell was ripped away. Put this cross in the **v**



These 5 crosses are in a zone outside of the **pg**. Put these crosses in the **v** category

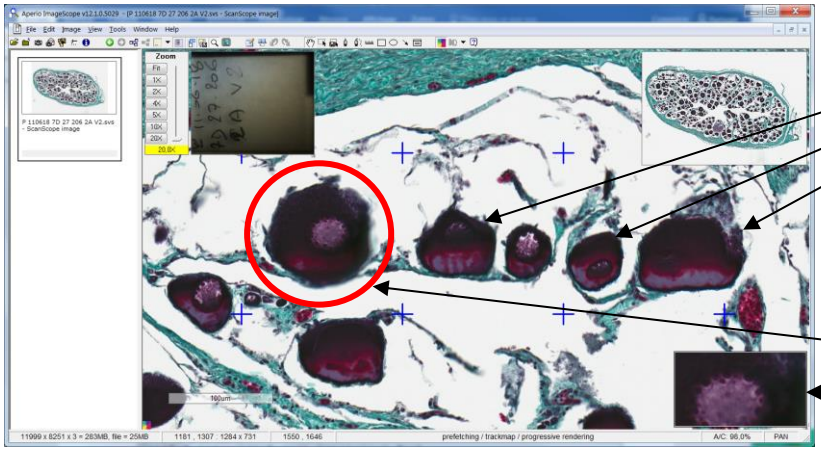


Put these crosses in the **v** category ( ) since they fall in a zone where the inner structures of the cell have been ripped away.
Cross on **gv** that have shifted out of the follicle ( ). Put this cross in the **vit4** category
Cross on the **zp** of follicles that are missing too much vitellus to be able to determine the stage ( ). Put these crosses in the **i** category



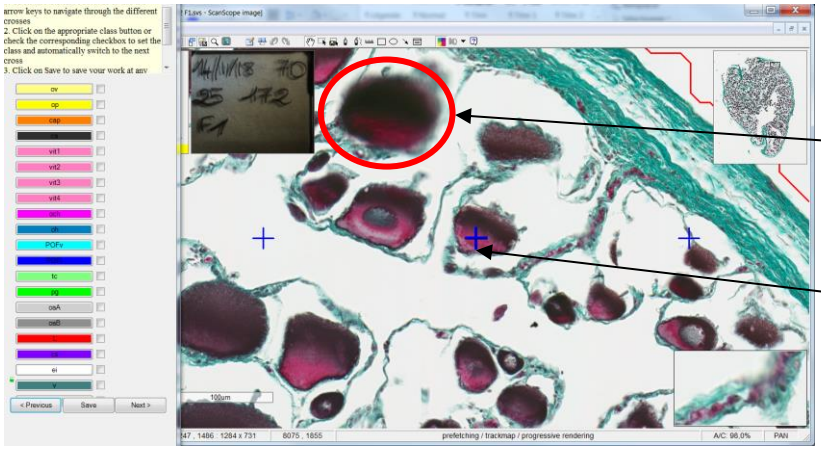
Put this cross in the **v** category since the follicle has been folded back onto itself

3.20. Undetermined (i)



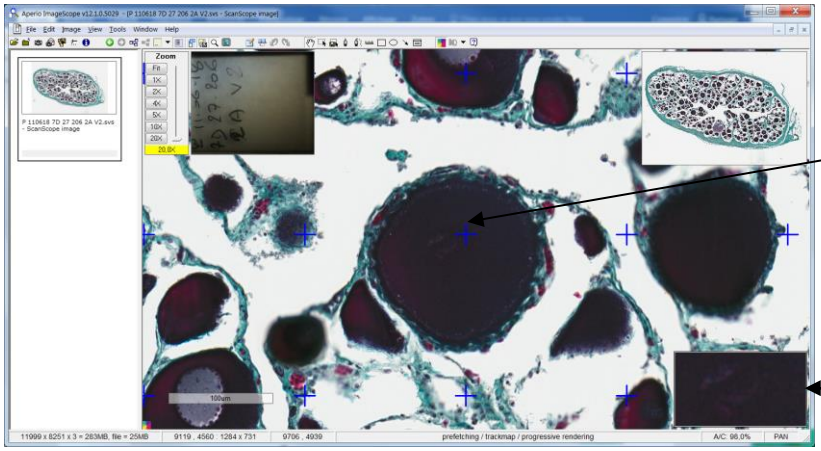
Follicles for which we cannot clearly see the nucleus. Put these cells in the **i** category

Zoom on the nucleus. We cannot see the edges of the nuclear membrane



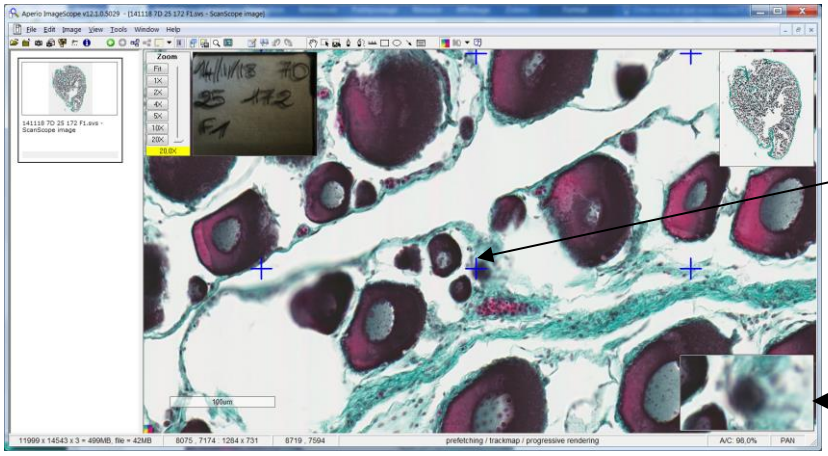
Blurry structure, put it in the **i** category

Cross on a follicle with no visible nucleus. Put this cross in the **i** category



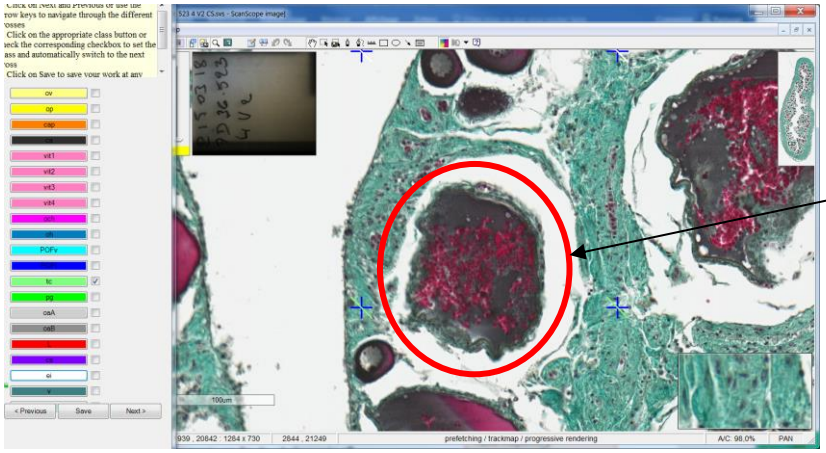
Cross on a follicle with no visible nucleus. Put this cross in the **i** category

Zoom on the cross

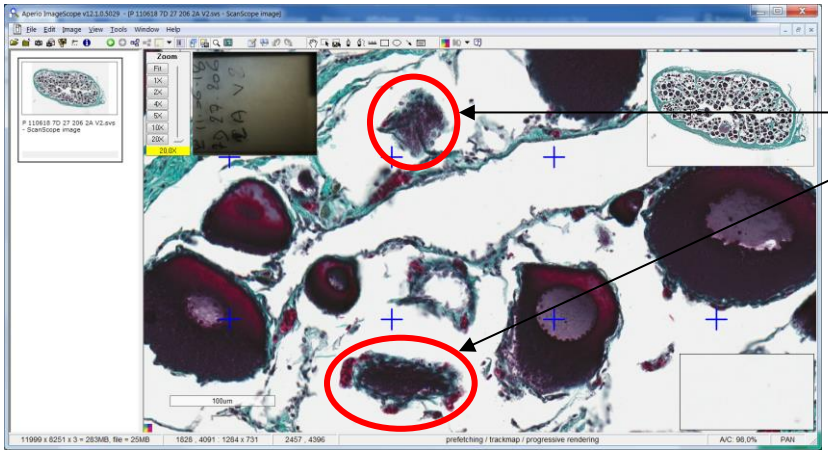


Cross on a blurry structure. Put this cross in the **i** category

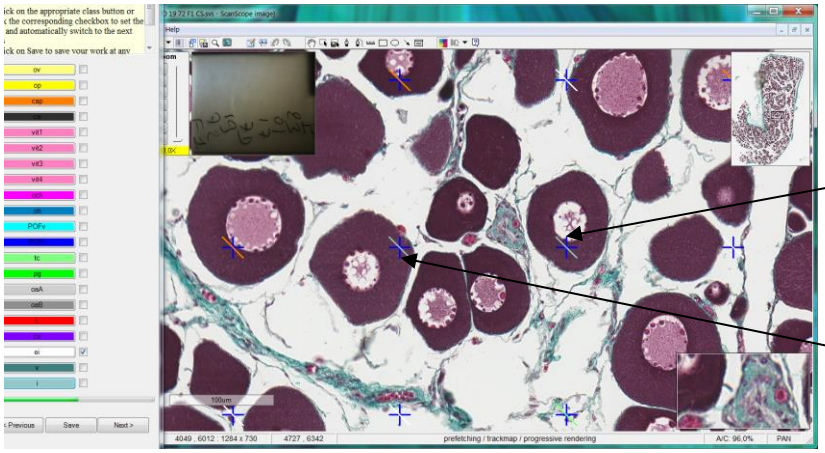
Zoom on the cross



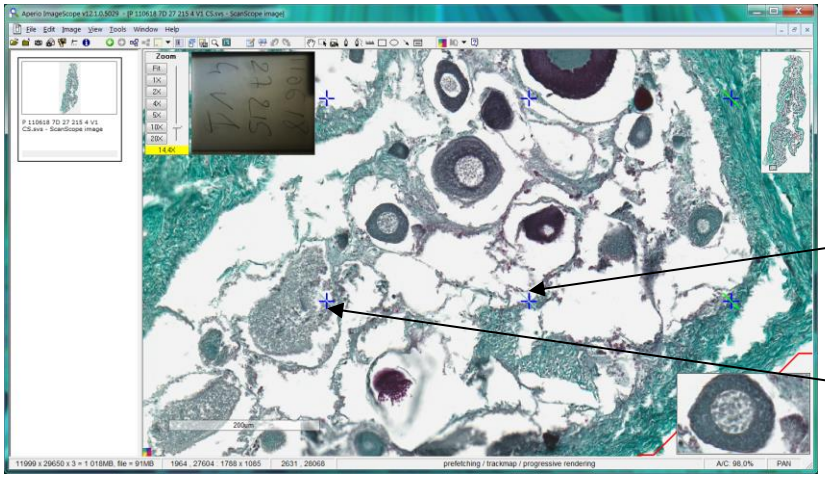
Follicle in vitellogenesis, but no visible nucleus and no ring of vitellus. Put this follicle in the **i** category



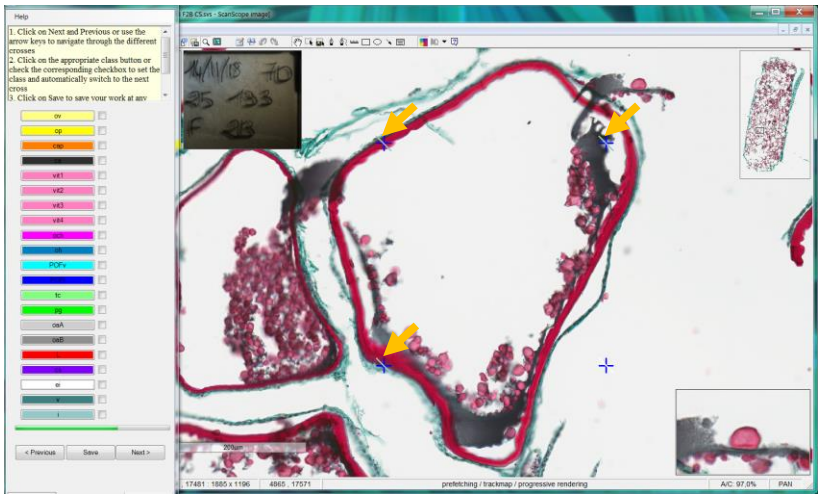
Structures that have not been defined in the protocol or in the lexicon. Put these structures in the **i** category



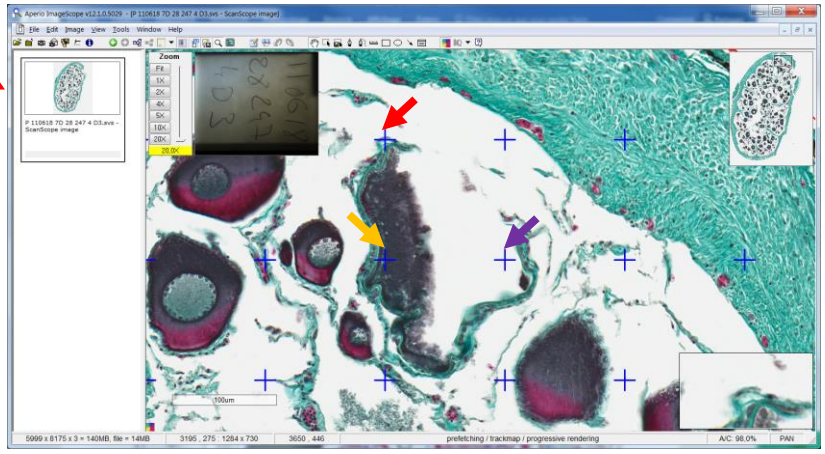
Crosses on follicles that had their nucleus ripped or damaged. We cannot see the edges of the nuclear membrane. Put these crosses in the **i** category



Discolored zone. Some structures might be mislabeled without the help of coloration. Put these crosses in the **i** category



Crosses (↙) on the theca of a follicle that cannot be identified. Put these crosses in the **i** category



Cross ( ) on the theca of a follicle that cannot be identified. Put this cross in the **i** category

Cross ( ) on a follicle that cannot be identified. Put this cross in the **i** category

Cross ( ) in a zone where the oocyte has been ripped away, with a part of the theca and **zp** still attached. Put this cross in the **i** category

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