

# *Combining Icy and Cytomine for multi-centric digital pathology*

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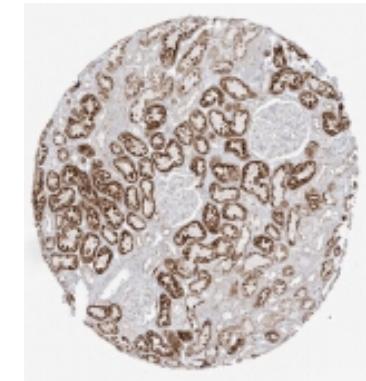
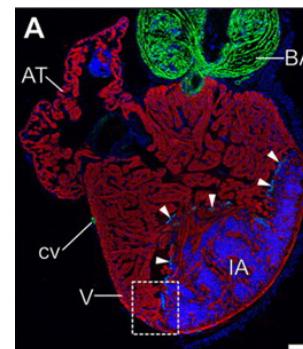
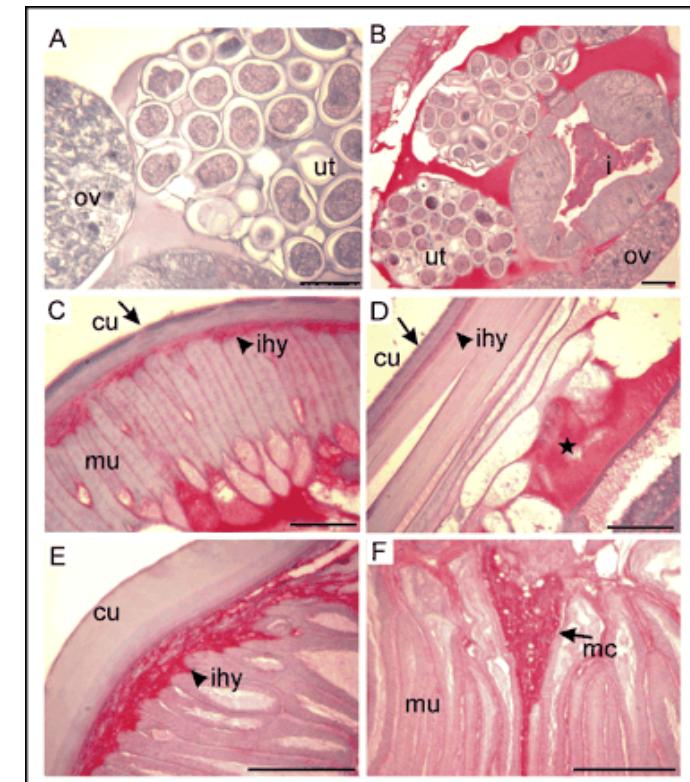
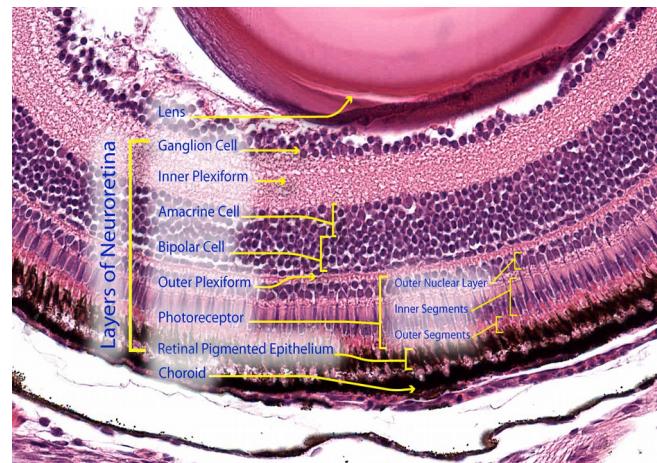
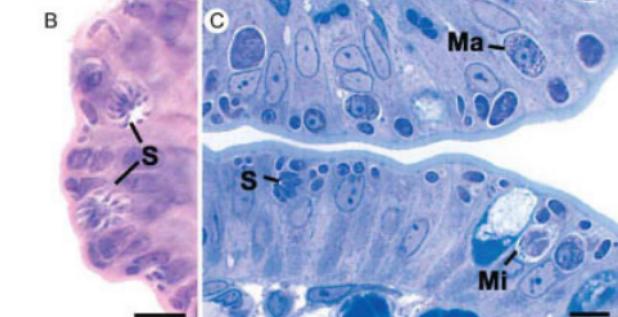
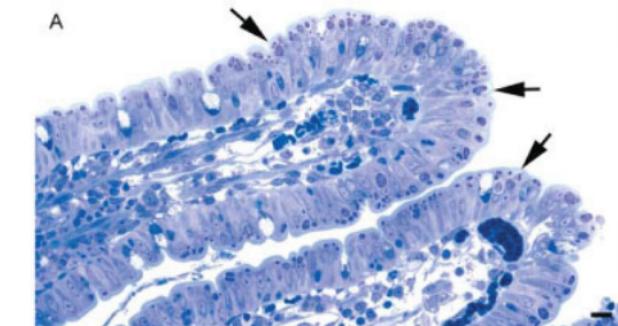
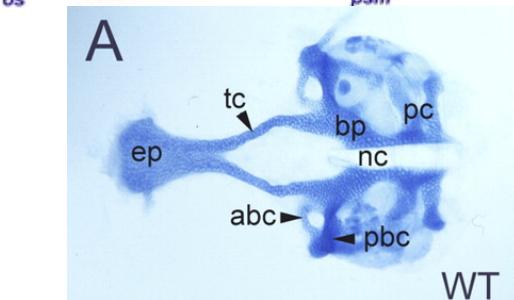
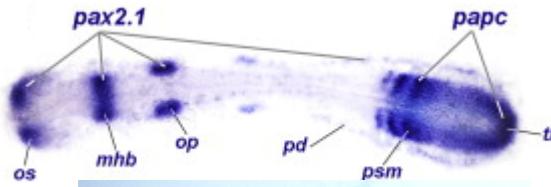
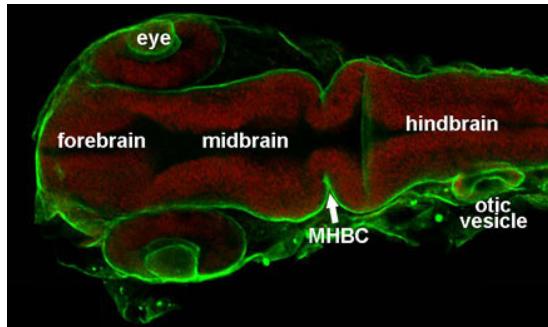
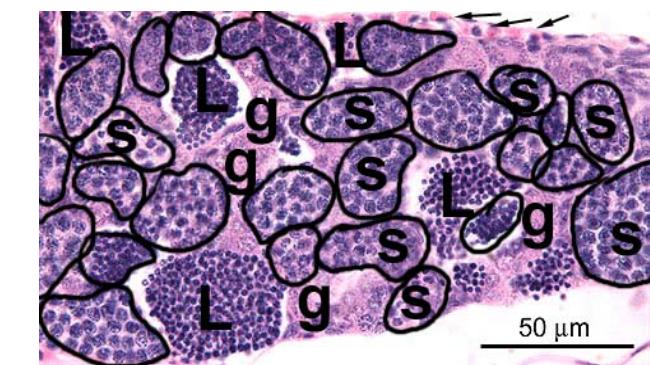
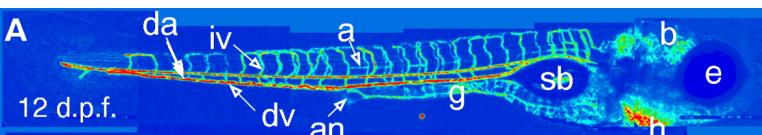
**Raphaël Marée\*, Florian Aubin, Alexandre Gaspard Cilia,  
Jean-Christophe Olivo-Marin, Vannary Meas-Yedid**

**Bioimage Analysis Unit, Institut Pasteur, Paris, France**

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Workshop du GDR ISIS « Analyse de tissu biologique et histopathologie numérique »  
23 juin 2015, Paris

# Biomedical research heavily relies on semantic annotation & quantification of samples



(Source : Google Images)

# Digital pathology studies are multi-centric



# Digital pathology studies are multi-centric



This talk : open-source tools to enable collaborative analysis + an application in nephrology

# Tools



[icy.bioimageanalysis.org](http://icy.bioimageanalysis.org)

Icy combines:

- ✓ A user-friendly software for image acquisition, visualization, quantification
- ✓ An online database to share tools, methods & knowledge

Icy targets:

- ✓ The biology community
- ✓ The imaging community
- ✓ The computer vision community

Icy is:

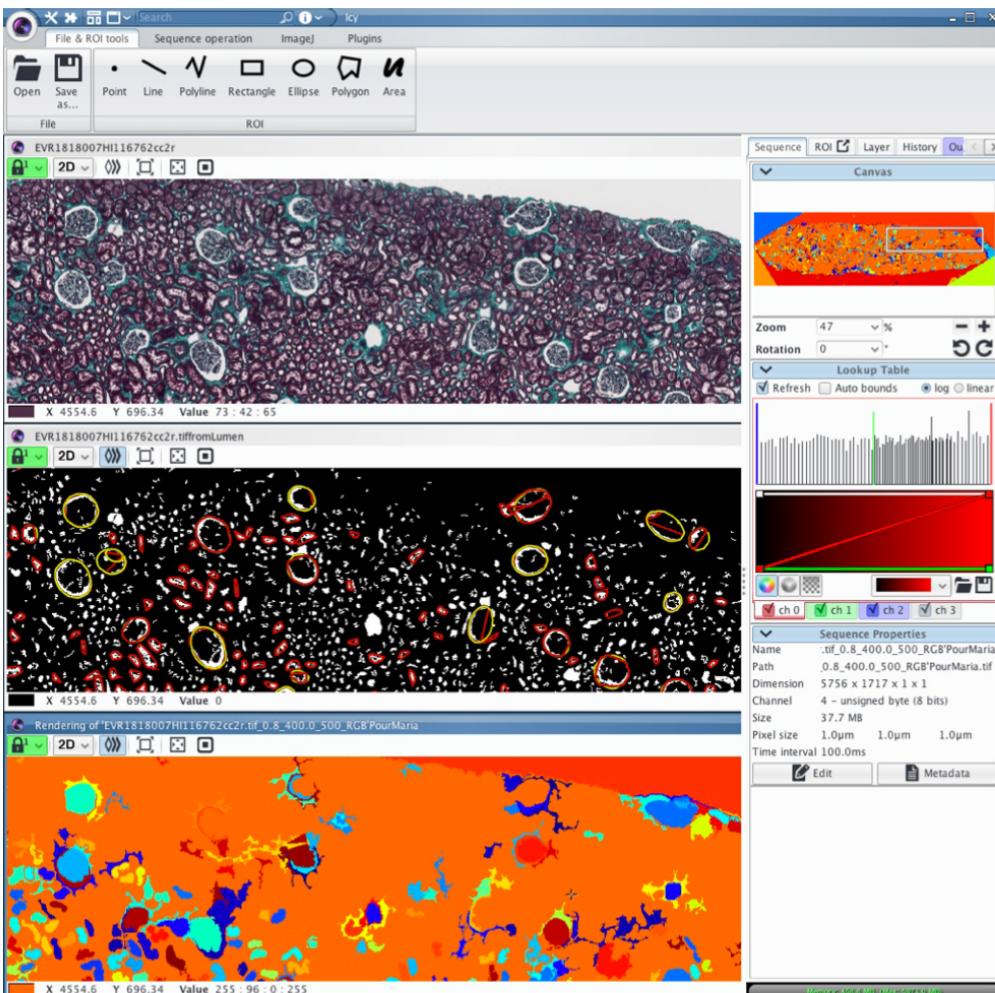
- ✓ Free & Open source
- ✓ ***Stably Funded***



*de Chaumont et al., Nature Methods 2012*



## 1. A powerful & user-friendly software



- User-friendly interface
- Supports many formats
- 250+ plugins
- Automatic updates
- Automatic bug reports

## 2. An online community platform

The website features:

- Mathematical Formula:** A complex equation involving multiple integrals and derivatives of functions  $I_x$  and  $V_x$ .
- STATE-OF-THE-ART IMAGE ANALYSIS METHODS:** A sidebar listing various advanced analysis techniques.
- Most popular plugins:**
  - ImageBrowser**: Nicolas Hervé, TAG: GUI. A visual programming environment for image processing.
  - Protocols**: Alexandre Dufour, TAG: PROTOCOL. A visual programming environment for image processing protocols.
  - EzPlug SDK**: Alexandre Dufour, TAG: LIBRARY. A plugin developer's toolkit.
  - Active Cells**: Biomedical Imaging Group, TAG: SEGMENTATION. A plugin for segmenting active contours.
  - Spot Detector**: Fabrice de Chaumont, TAG: SPOT COUNT. A plugin for detecting and counting spots.
  - Script Editor**: Thomas Provoost, TAG: SCRIPT. A plugin for creating scripts.
  - Protocols SDK**: Alexandre Dufour, TAG: LIBRARY. A software development kit for protocols.
  - Mice Profiler Tracker**: Fabrice de Chaumont, TAG: TRACKING. A plugin for tracking mice.
  - Magnifier**: Alexandre Dufour, TAG: GUI. An extension for the 2D viewer.

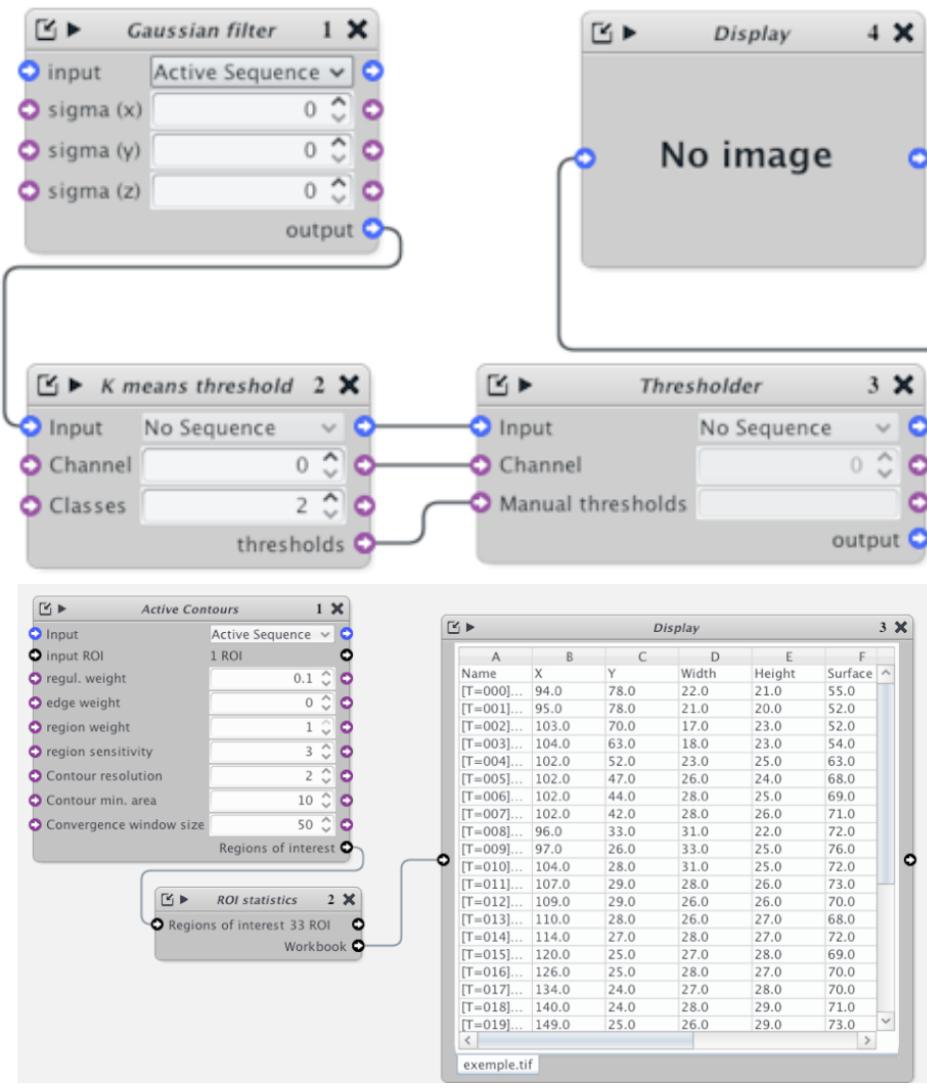
- "All-in-one-place"**
- User & Dev. accounts
  - Plugin ratings
  - Documentation
  - Forum
  - Video tutorials
  - Trainings

[icy.bioimageanalysis.org](http://icy.bioimageanalysis.org)



# Anyone can contribute

## Protocols (Graphical workflows)



## Script editor (Javascript, Python)

The screenshot shows the script editor interface of the icy software. It includes:

- A toolbar with File, Edit, Templates, Tools, Options.
- A list of open files: ROI\_Oval\_Grid.js, ROI\_Oval\_Grid2.js, ROI\_Oval\_Grid3.js, ROI\_Oval\_Grid4.js.
- A dropdown for Lang: JavaScript.
- A code editor window displaying the following JavaScript code:

```
size = 34
space = 56
xa = 191 - 34 / 2
ya = 180 - 34 / 2
xb = 191 + 34 / 2
yb = 180 + 34 / 2

for (i = 0; i < 12; i = i + 1) {
    for (j = 0; j < 8; j = j + 1) {
        xai = xa + space * i
        yai = ya + space * j
        xbi = xb + space * i
        ybi = yb + space * j

        topLeft = new Point2D.Double(xai, yai)
        bottomRight = new Point2D.Double(xbi, ybi)
        roi = new ROI2DEllipse(topLeft, bottomRight)
        ROI2DUtil.add(roi)
    }
}

topLeft = new Point2D.Double(xai, yai)
bottomRight = new Point2D.Double(xbi, ybi)
roi = new ROI2DEllipse(topLeft, bottomRight)
ROI2DUtil.add(roi)

*****
```

Below the code editor is a table of ROI statistics:

4	11	150.86569579288025	
5	11	132.3066343042071	
6	11	75.0331715210356	
7	11	90.0663430420712	
8	11	82.01456310679612	
1	12	90.0663430420712	
2	12	122.39563106796116	
3	12	66.98139158576052	
4	12	110.22168284789645	
5	12	130.3770226537217	

At the bottom right is a Clear button.

# cytominE

Cytomine    Dashboard    Projects    Ontologies    Explore    Storage    Activity    Search    d

About Us    Marée Raphaël (mareae) ▾

ULG-LBTD-NEO04    Images    Annotations    Properties    Jobs    Configuration    NEO4\_CURCU\_INH\_8.20\_01....

+ Select Point Arrow Rectangle Ellipse Circle Polygon MagicWand + - Fill Ruler Edit Rotate Resize Drag ☰ SHOW TOOLS

a b c

Annotation

1X Magnification  
10X Magnification  
40X Magnification

# cytomine

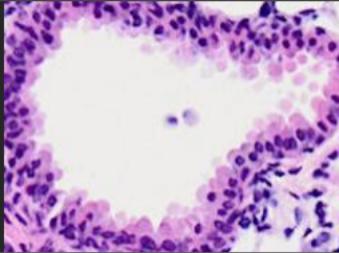
**e**

CURRENT SELECTION +

Area : 12330 micron<sup>2</sup>  
 Perimeter : 0 mm  
 User : Rocks Natacha (nrocks)

You can edit this annotation  
[Comment \(0\)](#)

ANNOTATION PREVIEW +



SIMILARITIES +

Suggested term : Bronche (90%) or Adénocarcinomes (7%)  
[See similar annotations](#)

PROPERTIES +

Add a property

DESCRIPTION +

This is a normal lung bronchus stained with H&E...  
[See full text and edit](#)

**f**

ONTOLOGY -

- ULG-LBTD-TISSUS
  - Artefact (0) Show:
  - Background (0) Show:
  - Bronche (1) Show:
  - Bronche - incidence de coupe (0) Show:
  - Cartilage (0) Show:
  - Cellules graisseuses (0) Show:
  - Cellules nécrosées (0) Show:
  - Coupe (0) Show:
  - Espaces aériens (0) Show:
  - Foyer d'inflammation (0) Show:
  - Globule rouge (0) Show:
  - Mitose (0) Show:
  - Muscle (0) Show:
  - NotAdeno (0) Show:
  - Poumon (0) Show:
  - Poumon non insufflé (0) Show:
  - Unknown (0) Show:
  - Zone floue (0) Show:
- Marquage
  - Tumeurs (0) Show:
- Vaisseaux
  - Adénocarcinomes (0) Show:
  - Cellules tumorales (0) Show:
  - Foyer hyperplasie nodulaire (0) Show:
  - Nécrose tumorale (0) Show:
  - Tumeurs épidermiques (0) Show:
  - Vaisseaux tumoraux (0) Show:

**i**

REVIEW | LAYERS +

Display Review layer :   
 Others layers :  Rocks Natacha (nrocks)

Opacity

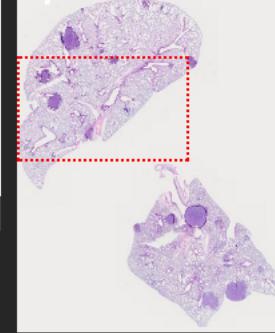
REVIEW | ACTION SELECTION -

Annotation : 426618  
 User : Rocks Natacha (nrocks)  
 Created : 2012-04-03 00h00  
 Term(s) :  Adénocarcinomes

REVIEW | ACTION IMAGE -

**k**

OVERVIEW -



**g**

ANNOTATIONS LAYERS -

Marée Raphaël (rmaree) (10)   
 Rocks Natacha (nrocks) (238)

Opacity

**h**

ANNOTATIONS PROPERTIES -

No Key Selected

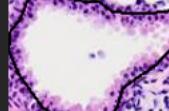
AREA\_OF\_Adénocarcinomes  
 CellCount  
 NUMBER\_OF\_Adénocarcinomes

**j**

JOB TEMPLATE -

Choose a ROI

Draw a ROI  Show ROI layer



Annotation 426237

Choose a job shortcut

computeTermStats  
 ComputeAdenocarcinomesStat

Start job

Job status

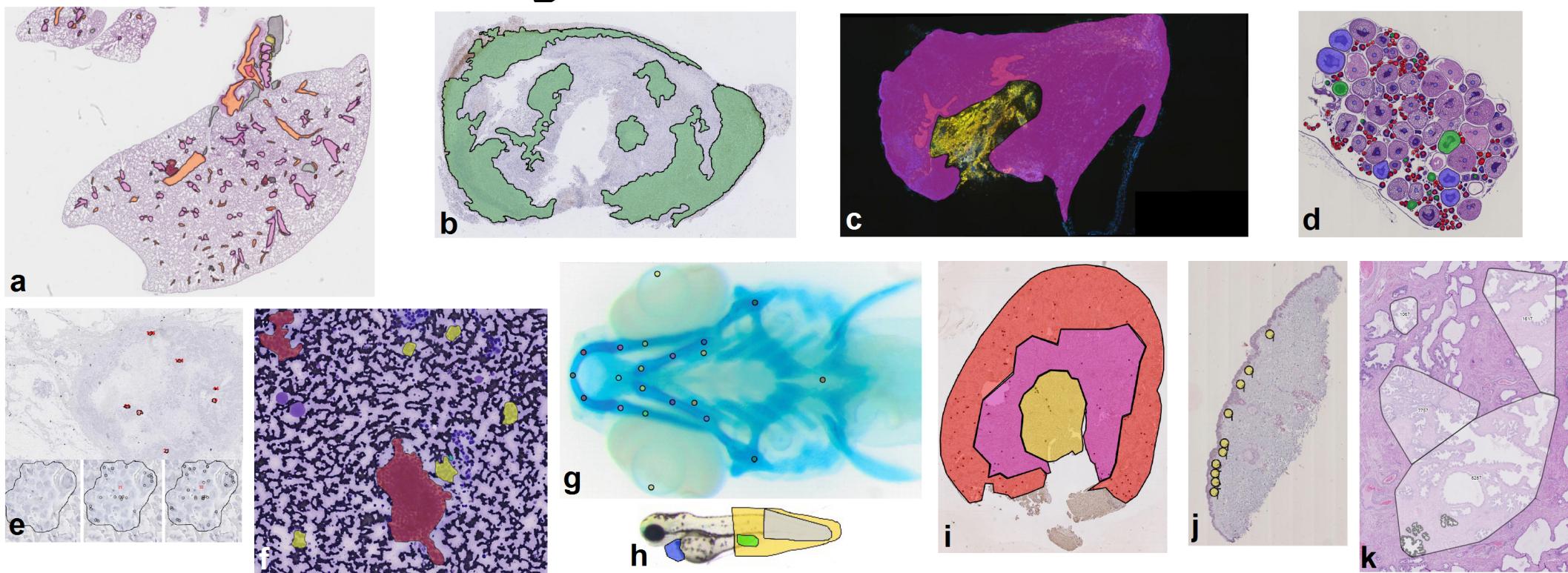
**m**

IMAGE LAYERS -

H&E Haematoxyli  
 H&E Eosin  
 IsoData Thresho  
 Otsu Threshold  
 Original

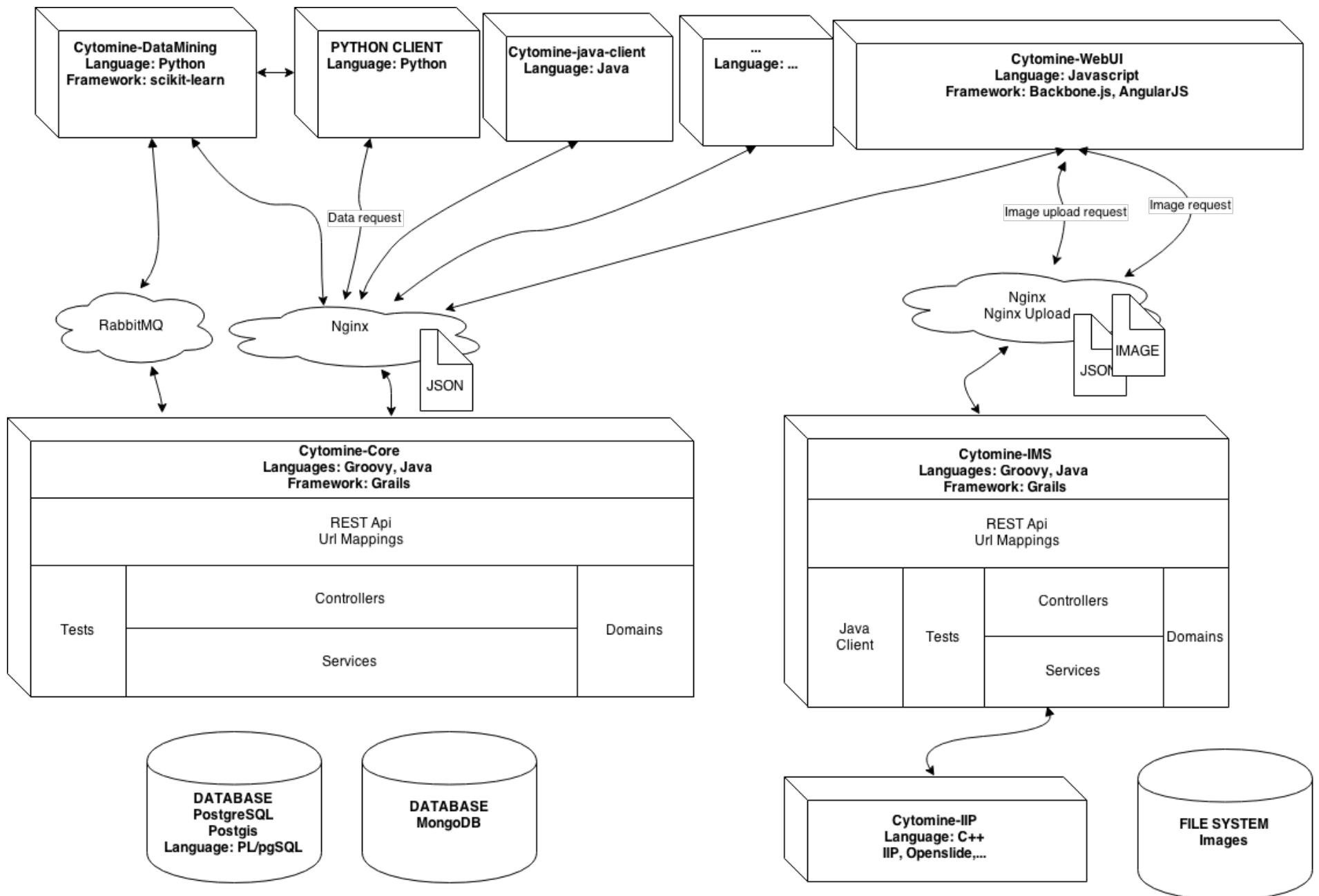
www.cytomine.be

# cytominE

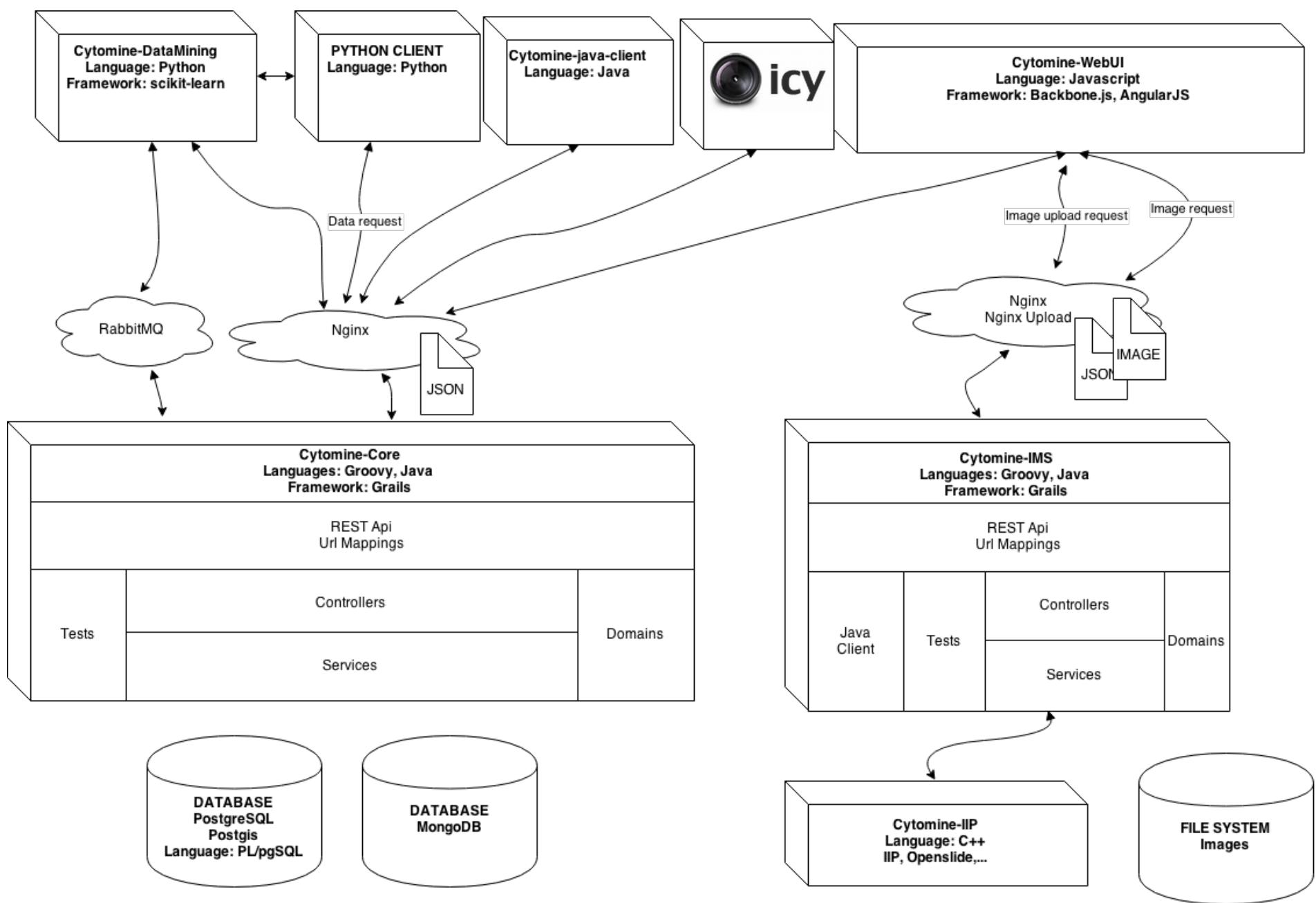


*Figure 2: Examples of annotations created using Cytomine in images from various research fields:*  
(a) H&E mice lung cancer research [9] (D.Cataldo's lab, GIGA-Research). (b) IHC mice lung cancer research (P. Martinive's lab, GIGA-Research). (c) Immunofluorescent mouse ear sponge assays in tumor angiogenesis [14]. (d) H&E Chondrostoma nasus sexual maturation research (Gennotte's lab, CEFRA). (e) in situ hybridization assays in human breast cancer research (C.Josse's lab, GIGA-Research). (f) Human thyroid cytology (I.Salmon's lab, ULB Anatomical Pathology Department). (g) Danio rerio embryo development [10] (M. Muller's lab, GIGA-Research). (h) Danio rerio toxicology research [11] (M.Muller's lab, GIGA-Research). (i) IHC renal ischemia/reperfusion research [12] (F.Jouret's lab, GIGA-Research). (j) IHC in melanoma microenvironment research (P.Quatresooz's lab, GIGA-Research). (k) H&E in human breast cancer research (R. Longuespée, GIGA-Research).

# cytominE

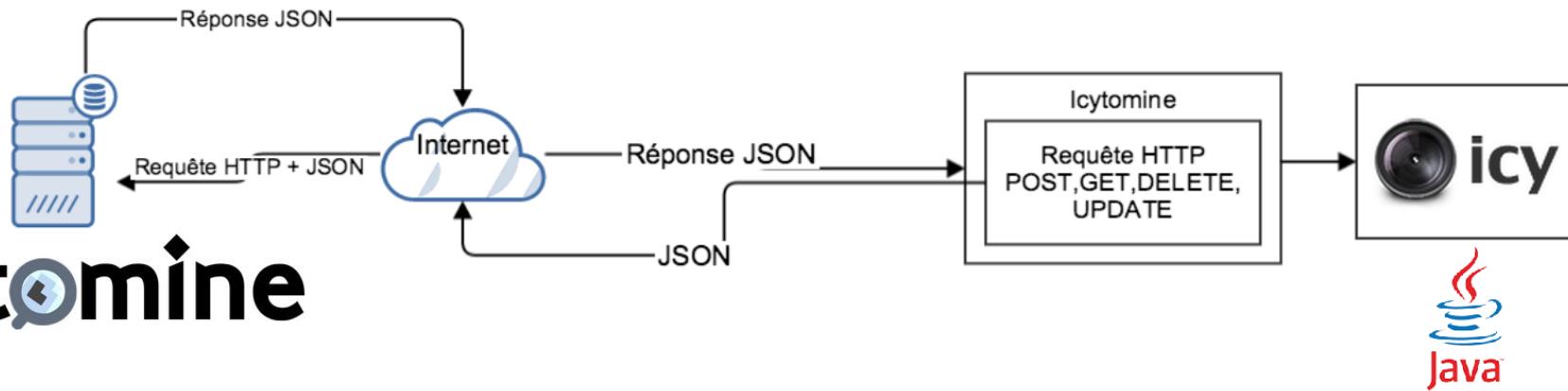


# cytominE



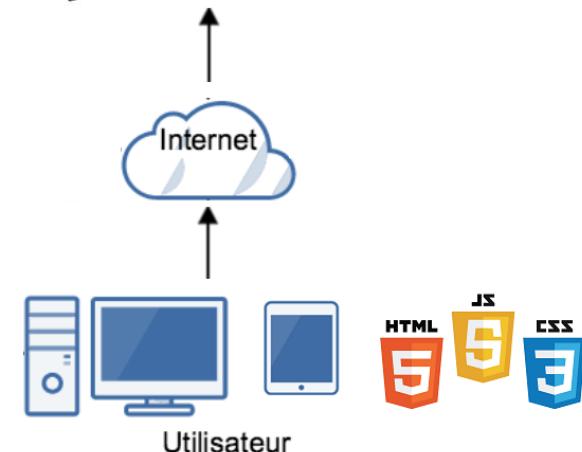


**cytominé**





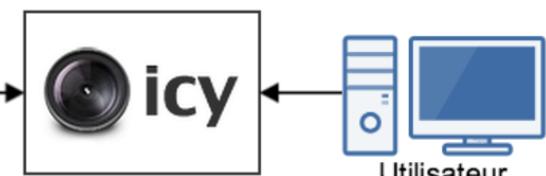
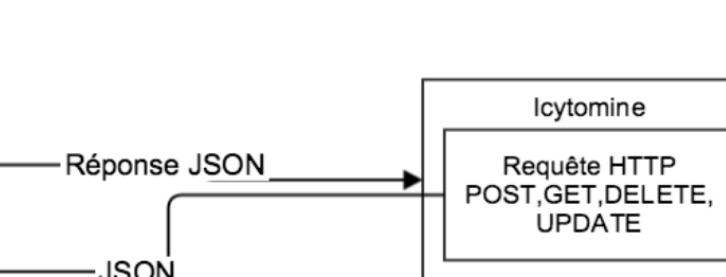
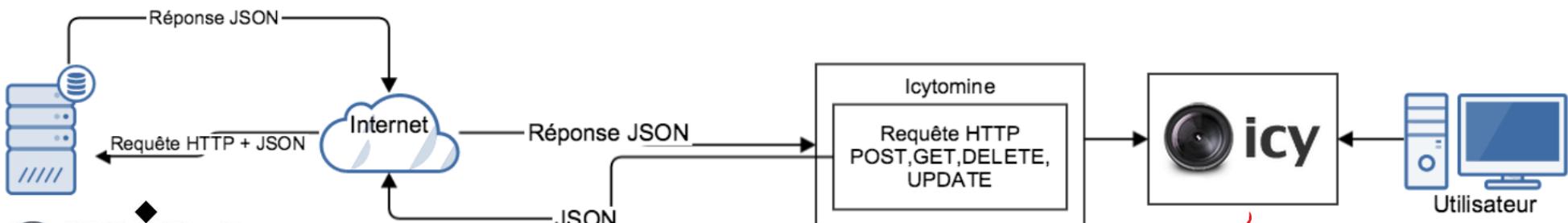
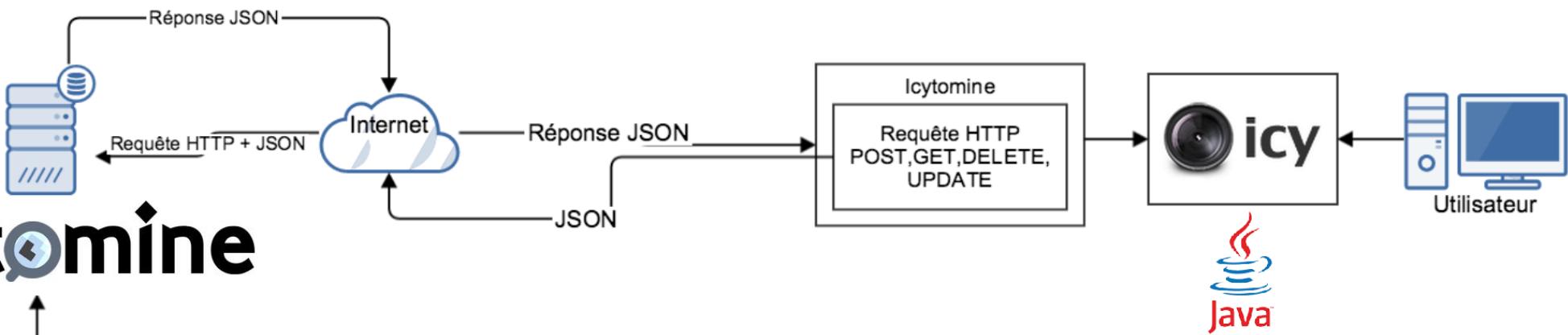
# cytominé



Utilisateur



Utilisateur

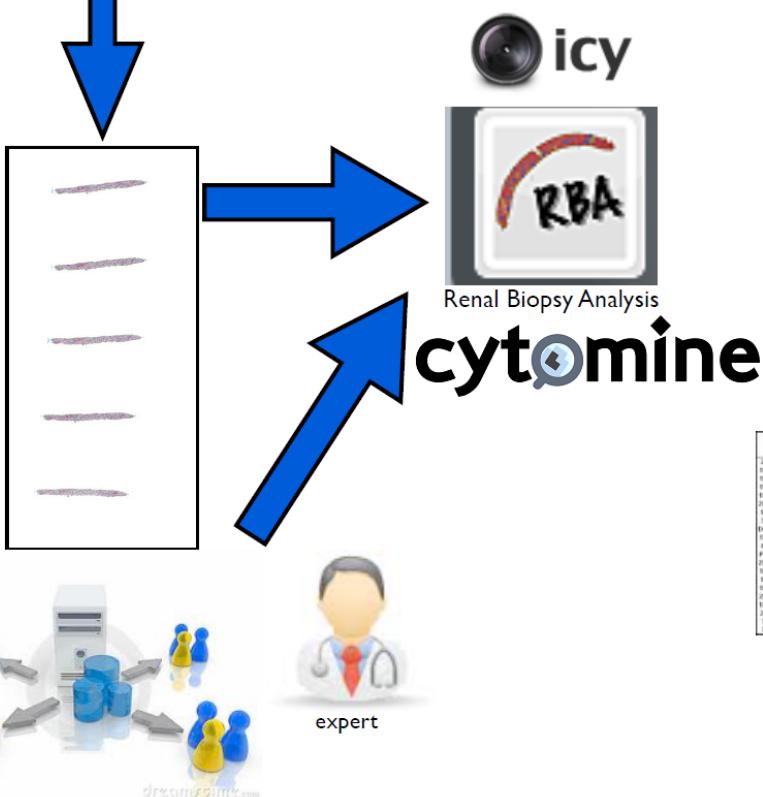


# Application in Nephrology

## Big data



Slide Scanner



# Web

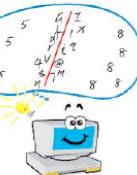
## Clinical data

 **icytominer**

 **icytominer**

## statistical analysis

# Machine learning

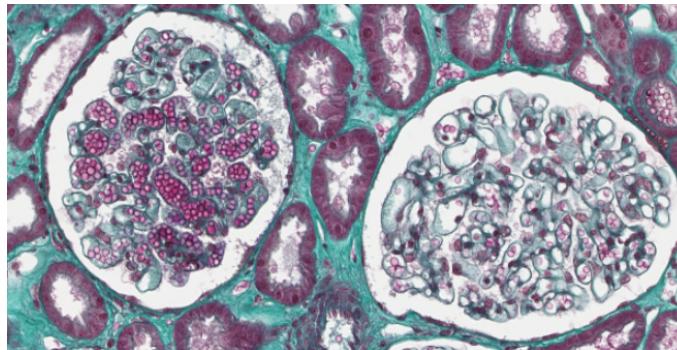
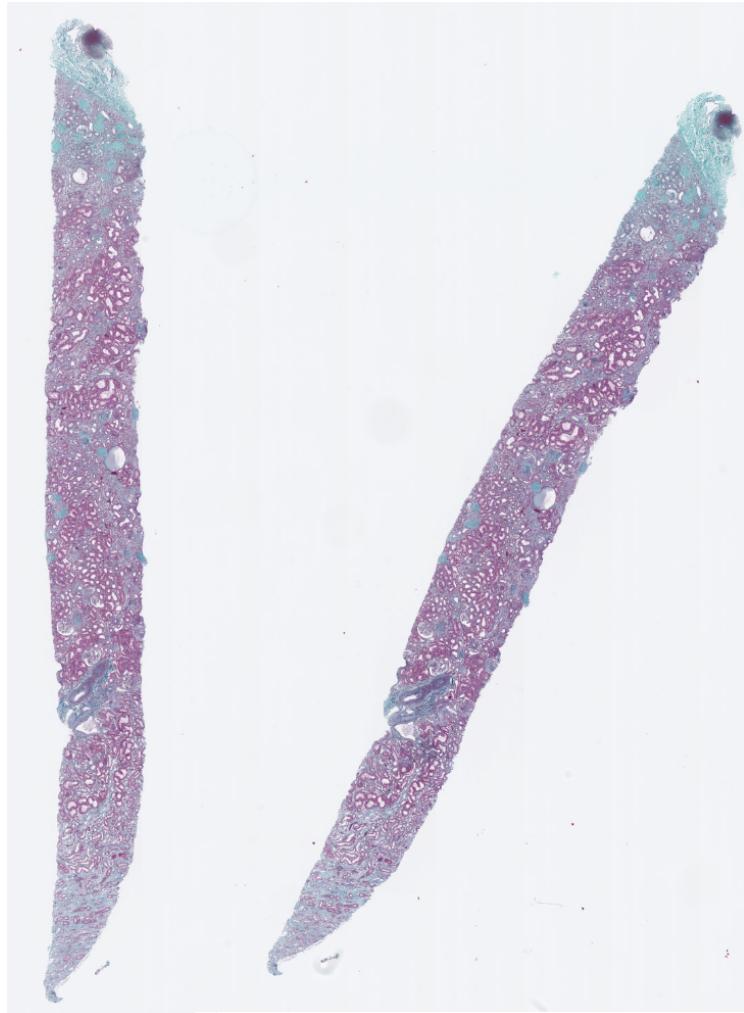


# Biomarker discovery

**Prognosis:**  
predicting the course  
and outcome of a  
medical condition

**Diagnosis:**  
detecting the nature  
or cause of a  
medical condition

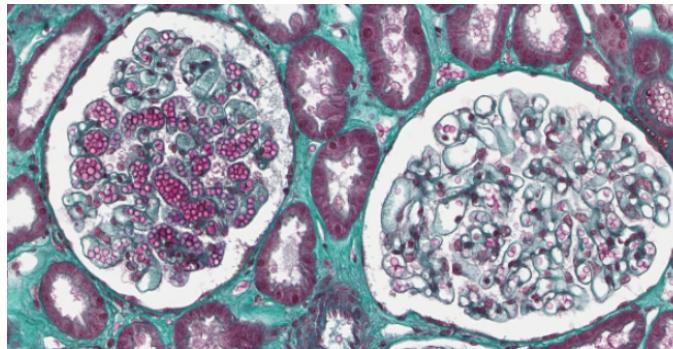
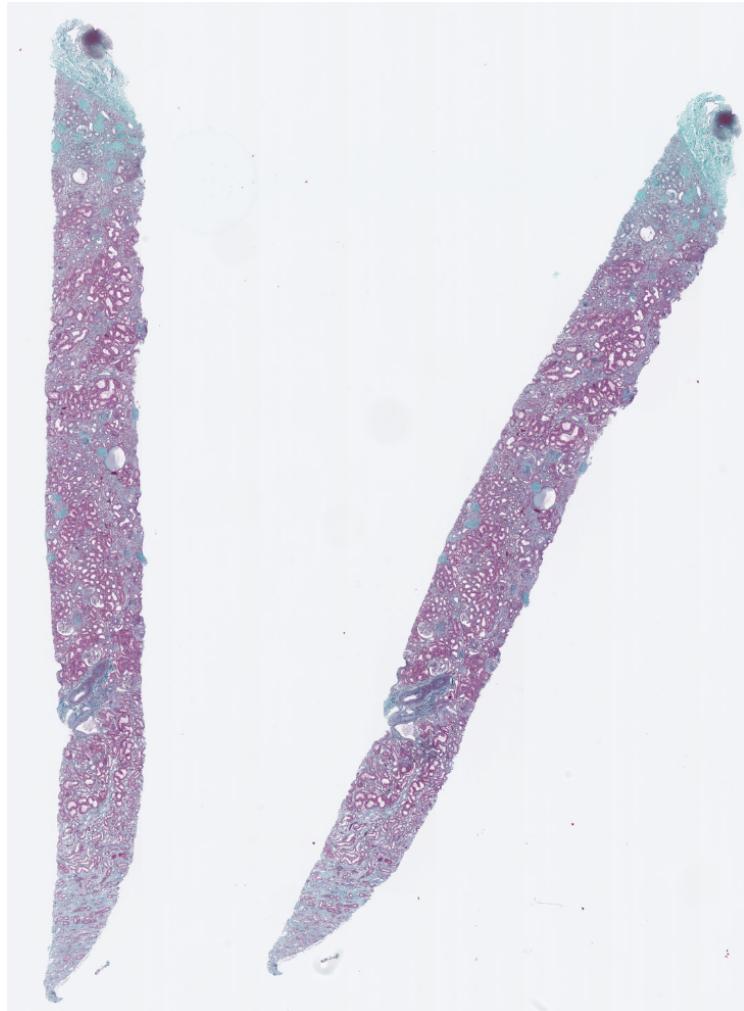
# Glomeruli detection in human nephrology whole tissue



Challenges :

- Large whole tissue images (e.g. 50K x 30K, 20X objective, 0.45 $\mu$ m/pixel)
- Glomeruli appearance variability (color, texture, size, shape) due to preparation protocols, tissue (pathological or not), image acquisition equipment

# Glomeruli detection in human nephrology whole tissue



Challenges :

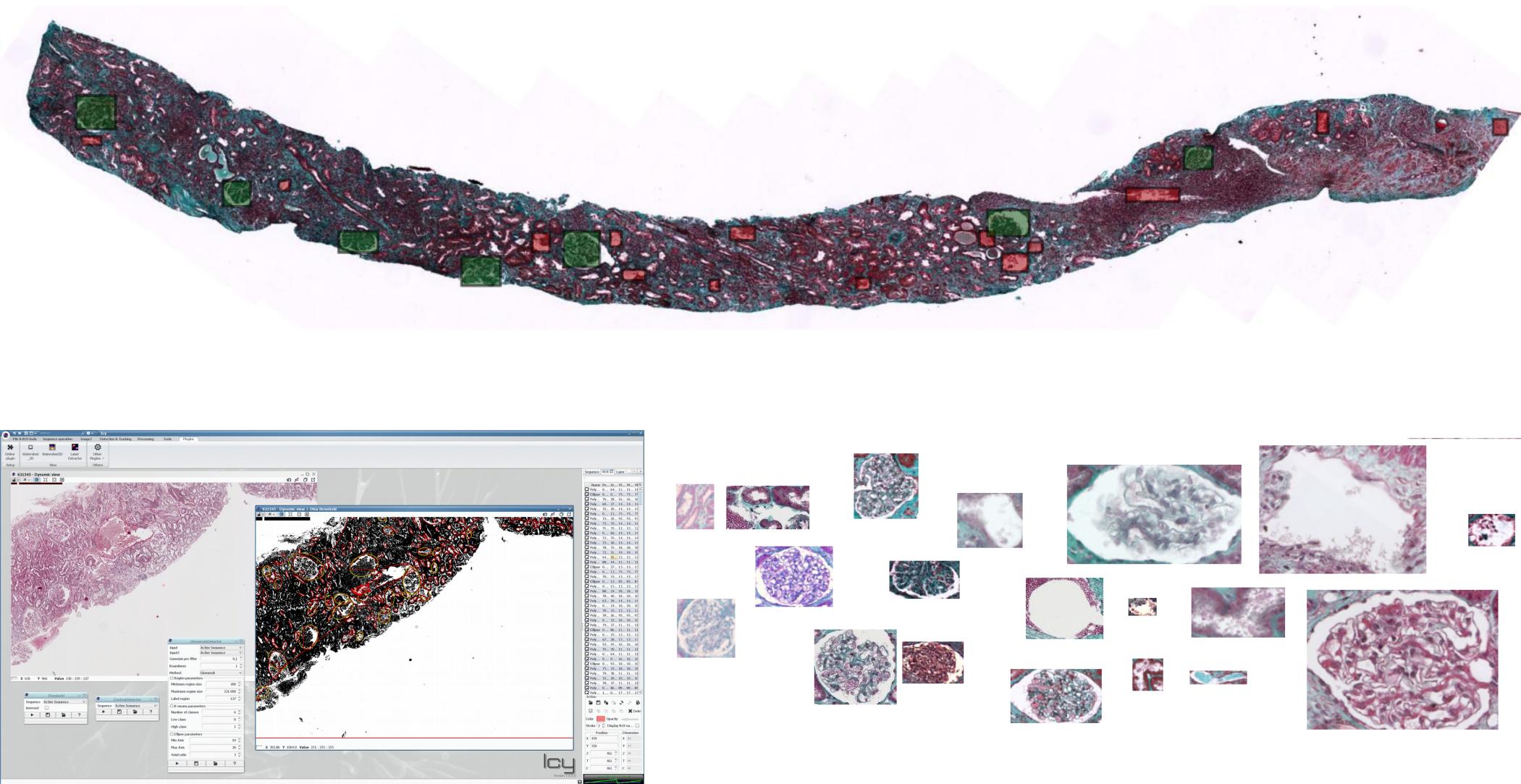
- Large whole tissue images (e.g. 50K x 30K, 20X objective, 0.45 $\mu$ m/pixel)
- Glomeruli appearance variability (color, texture, size, shape) due to preparation protocols, tissue (pathological or not), image acquisition equipment

**Proposed approach : Two-tier (segmentation + classification)**

# Proposed approach :

## 1. Segmentation of candidates within whole slides using Icy algorithms

Otsu thresholding + Convex Hull on lumen region + Ellipse detection (Fitzgibbon, 1999)  
Superpixel segmentation (Felzenszwalb, 2004)



# Proposed approach :

## 2. Supervised classification of candidates using Cytomine algorithms

**Training set:** candidates generated by Step 1 classified manually using Cytomine on the subset of training images:

Cytomine    Dashboard    Projects    Explore    Storage    Activity

PASTEUR-LAIQ-EVEROLD    Images    Annotations    Properties    Jobs    Configuration    Review

About Us    Marée Raphaël (rmaree) ▾

Review for project PASTEUR-LAIQ-EVEROLD

You are reviewing image EVR01013V0 - 2015-01-05 19.44.58.ndpi.

User: Icy\_Glomeruli\_Finder 2015-06-19 16h52   Term: All

Icy\_Glomeruli\_Finder 2015-06-19  
16h52: 99 / 240 reviewed  
Icy\_Section\_Finder 2015-06-19  
16h52: 0 / 6 reviewed

Explore

Glomerule

Annotation details

Created by Icy\_Glomeruli\_Finder  
Date 2015-06-19 16h53  
Term associated Icy\_Glomeruli\_Finder has associated Glomerule

Open   Accept   Open   Accept   Open   Accept

1 / 141   Check & Accept All

Section

Glomerule

Reviewed Other 2015-06-20 14h27

Reviewed Other 2015-06-20 14h27

Reviewed Other 2015-06-20 14h27

N O N      G L O M

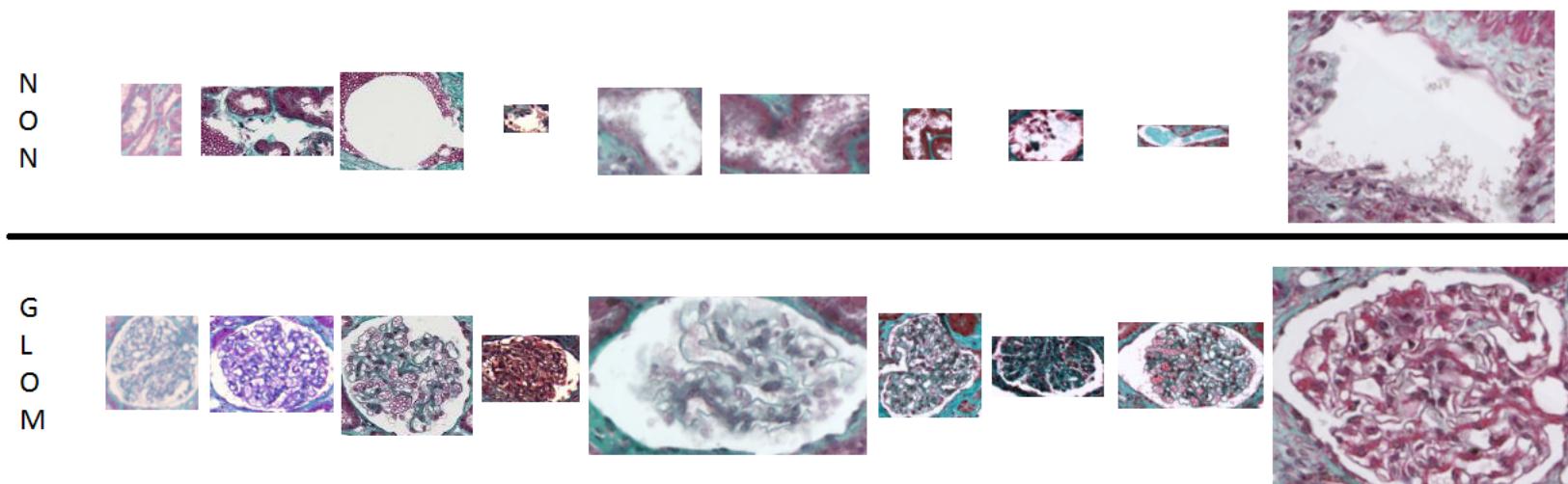
→

# Proposed approach :

## 2. Supervised classification of candidates using Cytomine algorithms

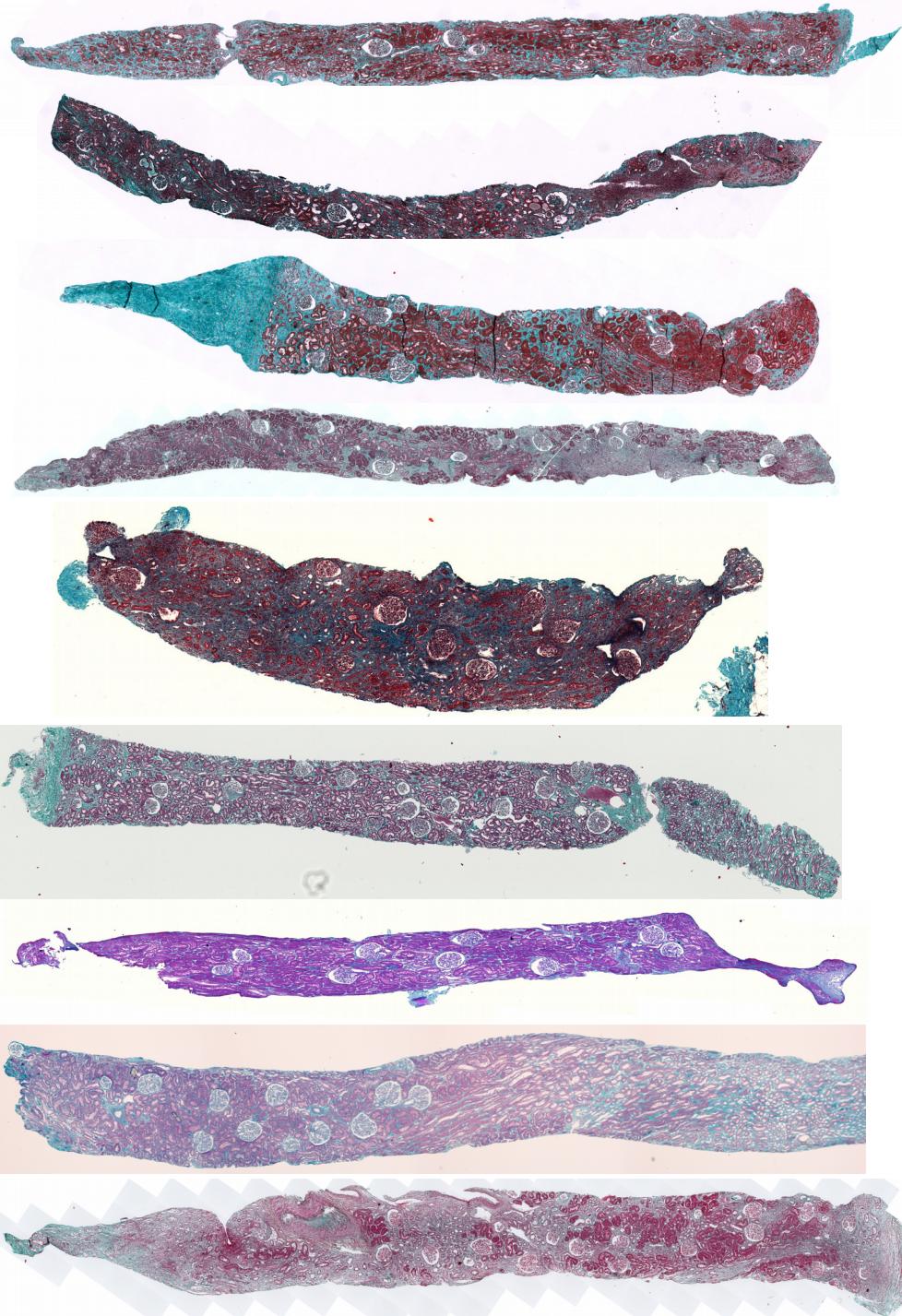
**Learning algorithm** (Marée et al., 2005-2014):

1000 random subwindows (5-50%) extracted in candidates described by normalized pixel values, Extra-Trees learning for feature construction, final linear SVM classifier



**Prediction :** Two-tier procedure applied on new images  
Classifier applied on candidates generated by Step 1

# Results on a preliminary dataset



9 sections (Trichrome Masson)

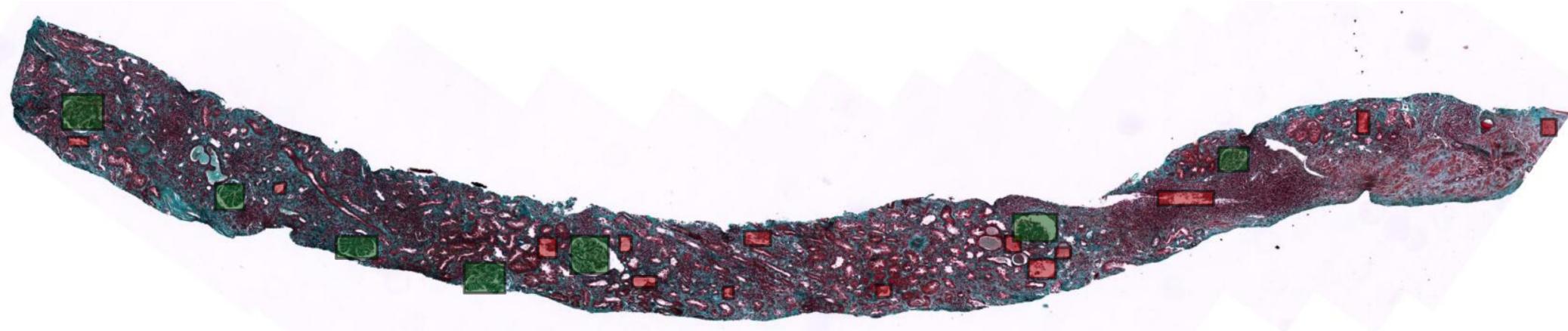
3 slide scanners :

Nanozoomer Hamamatsu (20X, NA=0.75, 0.46 $\mu$ m/pixel)  
MiraxScan 3Dhistech (20X, NA=0.8, 0.33 $\mu$ m par pixel)  
AxioScan.Z1 Zeiss (20X, NA=0.80, 0.22 $\mu$ m)

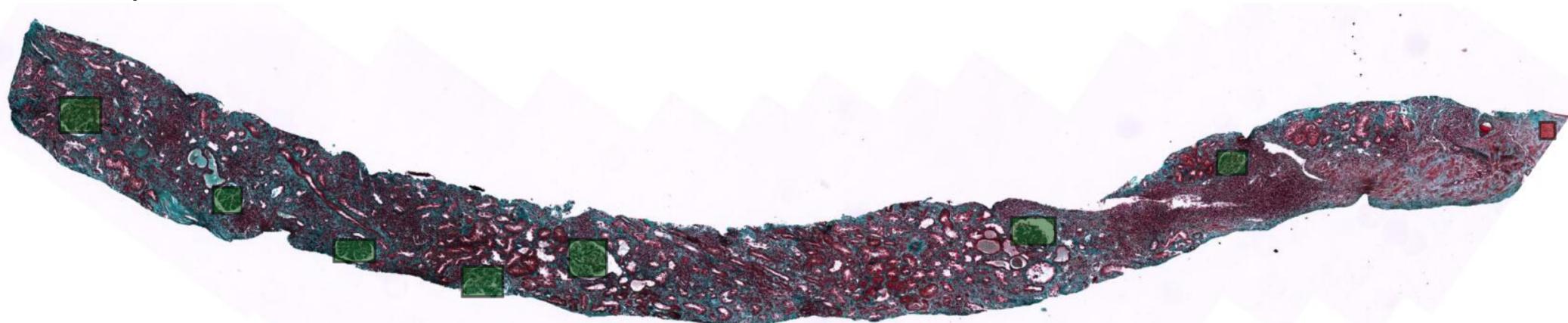
Protocol :  
Leave-one-section-out

# Qualitative results

Step 1 :

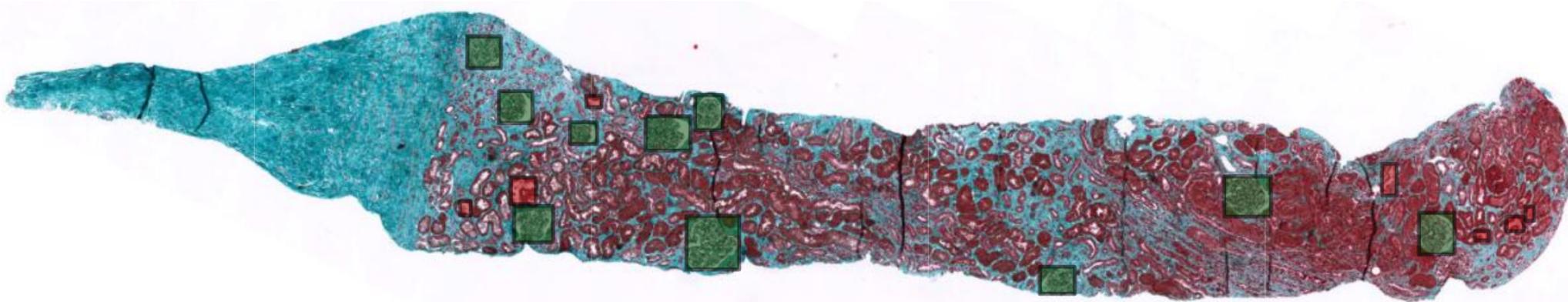


Step 2 :

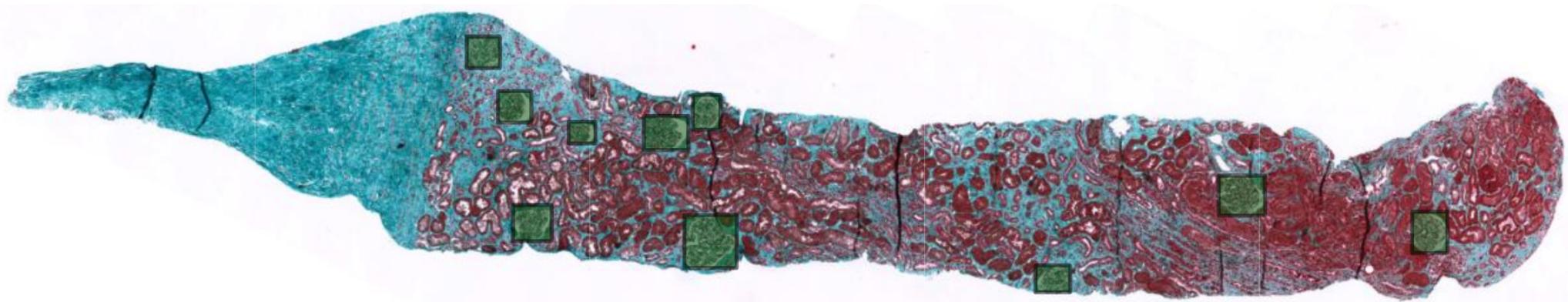


# Qualitative results

Step 1 :

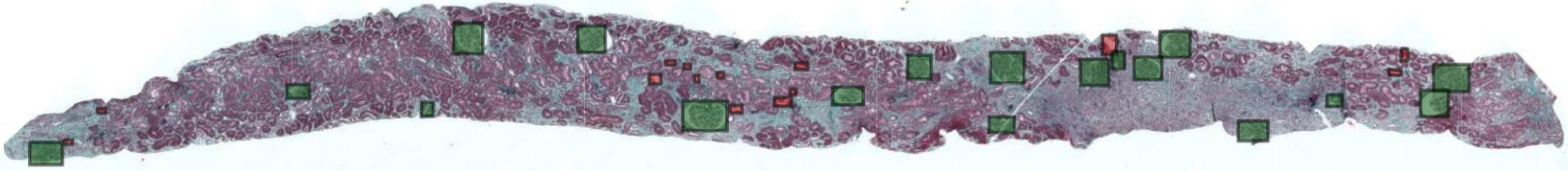


Step 2 :

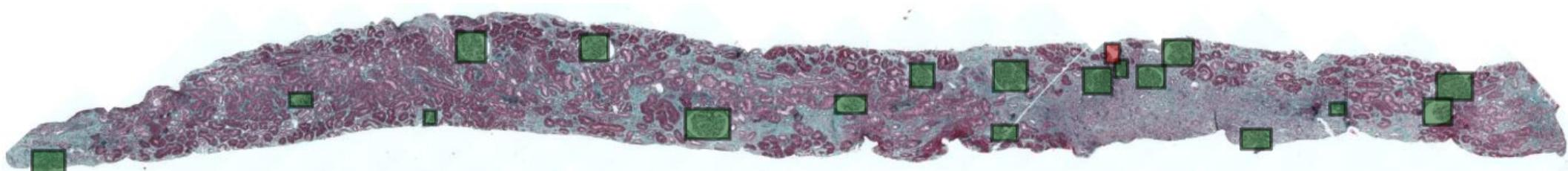


# Qualitative results

Step 1 :



Step 2 :



# Quantitative results

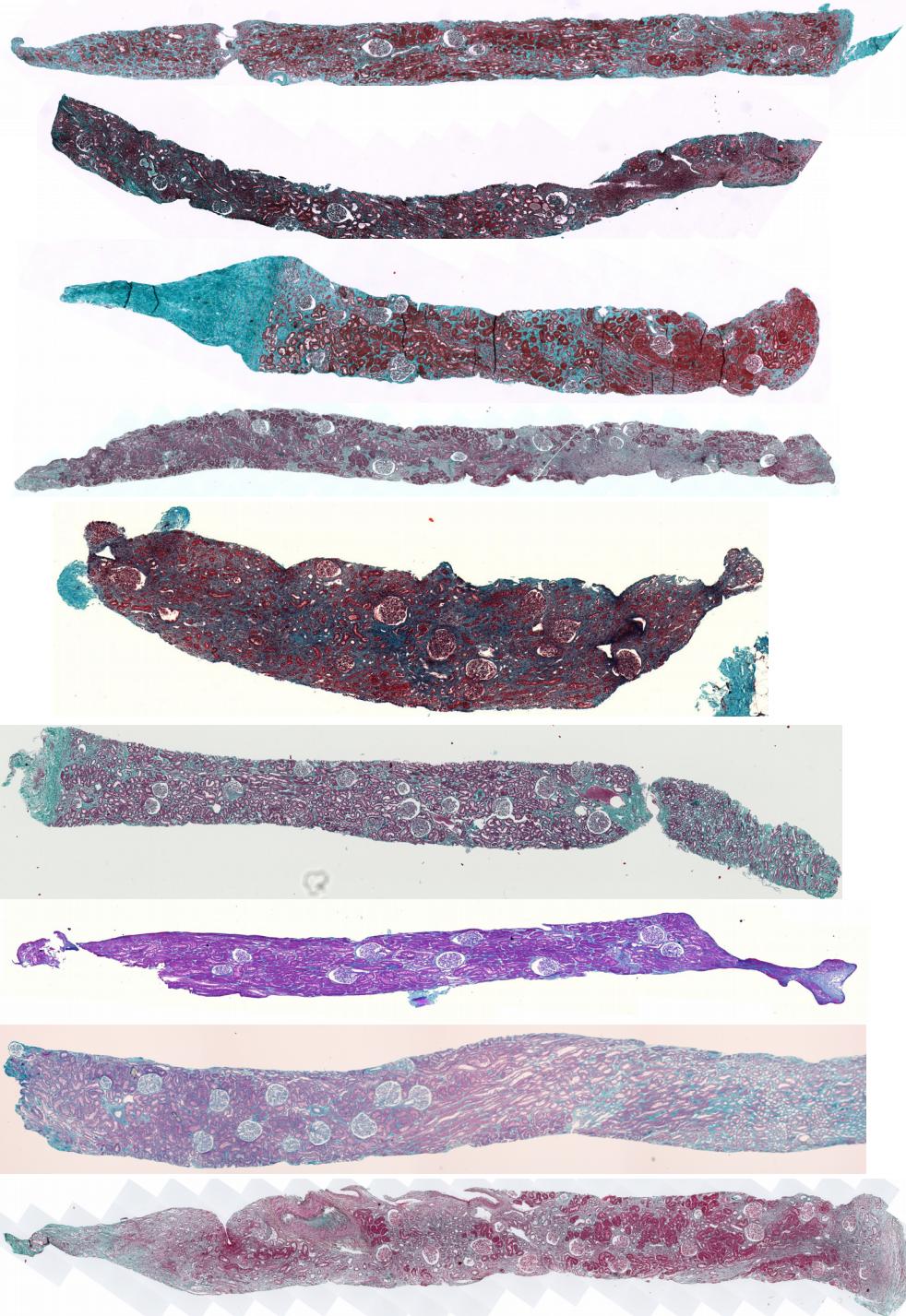


TABLE 1 – Matrice de confusion cumulée pour l'ensemble des imagettes des 9 sections selon le protocole “leave-one-section-out”.

	NONGLOM	GLOM
NONGLOM	<b>55</b>	14
GLOM	5	<b>109</b>

79.7 %  
95.6 %

TABLE 2 – Nombre (NB) de glomérules détectées pour chaque section par l'expert, l'étapes 1 seule, les étapes 1 et 2 combinées, vrais positifs (TP), faux positifs (FP), vrais négatifs (TN), et faux négatifs (FN).

Section	# Expert	# Etape 1	# Etape 1+2
	NB	NB (TP/FP/TN/FN)	NB (TP/FP/TN/FN)
1	6	8 (6/2/0/0)	6 (6/0/2/0)
2	7	21 (7/14/0/0)	8 (7/1/13/0)
3	10	17 (10/7/0/0)	10 (10/0/7/0)
4	18	32 (18/14/0/0)	19 (18/1/13/0)
5	12	16 (12/4/0/0)	12 (11/1/3/1)
6	17	26 (17/9/0/0)	22 (16/6/3/1)
7	9	9 (9/0/0/0)	9 (9/0/0/0)
8	13	16 (13/3/0/0)	14 (13/1/2/0)
9	22	38 (22/16/0/0)	23 (19/4/12/3)

# Ongoing and future work

Evaluation on a larger set (currently : > 600 whole slides) reveals lack of robustness:

- Section detection
- Glomeruli detection
- Glomeruli classification



Expert proofreading of classifications to refine training sets and models

The screenshot shows the Cytomine software interface for a project titled "PASTEUR-LAIQ-EVEROLD". The main window displays a review session for the image "EVRO1013V0 - 2015-01-05 19:44:58.ndpi". The interface includes a header bar with navigation links like "Dashboard", "Projects", "Explore", "Storage", "Activity", "About Us", and a user profile. Below the header, there's a toolbar with buttons for "Images", "Annotations", "Properties", "Jobs", "Configuration", and "Review". A central panel shows a grid of histological images. One image is selected and has a green border around a specific area. A tooltip for this image provides annotation details: "Created by Icy\_Glomeruli\_Finder Date 2015-06-19 16h53 Term associated Icy\_Glomeruli\_Finder has associated Glomerule". To the right of the images, there are buttons for "Open" and "Accept" for each row. A progress bar at the bottom indicates "1 / 141" and a "Check & Accept All" button. On the far right, a sidebar titled "Your last review for this project:" lists three previous reviews, each with a thumbnail, a "Reviewed" status, and an "Other" category. The dates for these reviews are 2015-06-20 14h27, 2015-06-20 14h27, and 2015-06-20 14h27.

Other image analysis tasks and data mining for biomarker discovery

# Acknowledgments

- Systems and Modeling (GIGA-Research / Montefiore Institute, ULg):
  - Cytomine core development : Loïc Rollus, Renaud Hoyoux, Benjamin Stévens
  - Cytomine algorithms : Gilles Louppe, Jean-Michel Begon, Rémy Vandaele  
Pierre Geurts, Louis Wehenkel
- Nephrologists and Pathologists  
@ HEGP, Necker, Hospices Civils de Lyon
- Unité d'Analyse d'Images Biologiques (Pasteur) : Fabrice de Chaumont, Stéphane Dallongeville, Alexandre Dufour, Thibault Lagache

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Institut Pasteur Carnot Maladies Infectieuses  
Research grants of the Wallonia (DGO6) :  
CYTOMINE (2010-2015) n° 1017072  
HISTOWEB (2014-2017) n° 1017072



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