

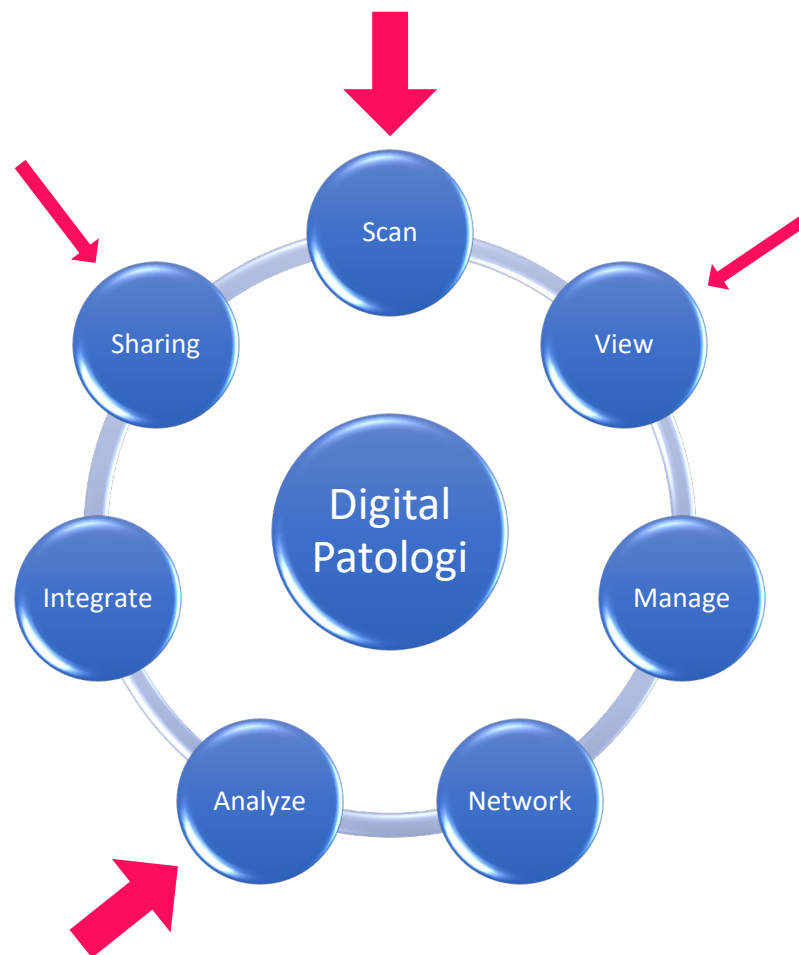
# Introduktion til scannere, billedeanalyse og algoritmer

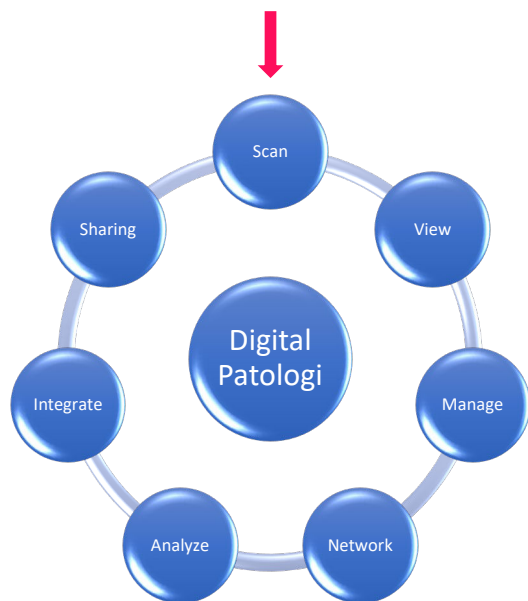
Kristina Lystlund Lauridsen, bioanalytiker  
Patologi, AUH

22-03-2023

Kom godt i gang med digital patologi, Axlabs

## Introduktion

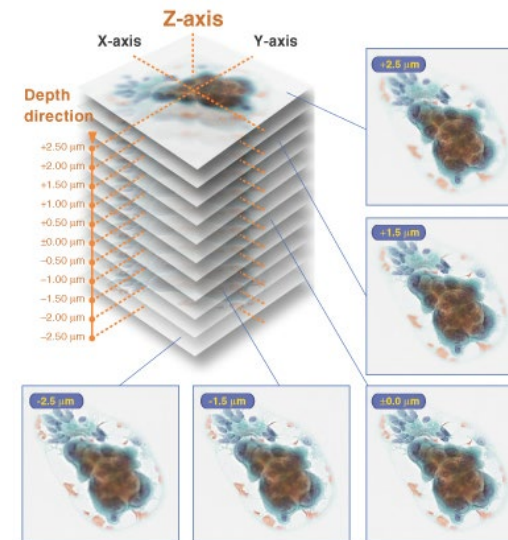
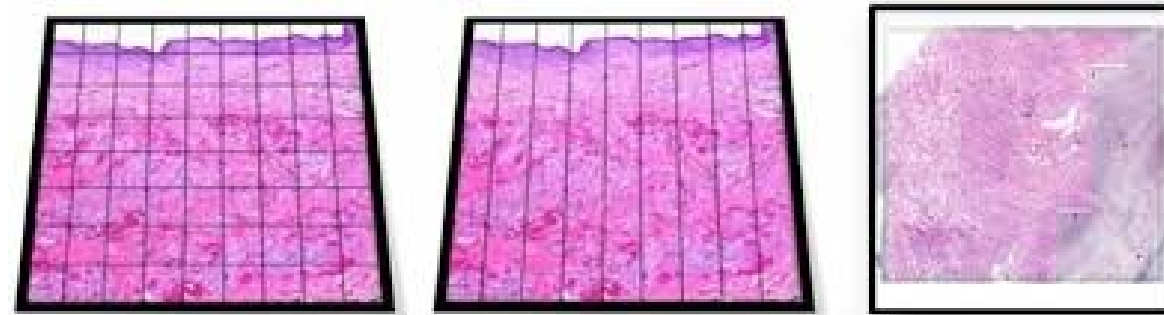




# Skanneren

## Skannertyper

- Line- og tile-skannere
- Z-stacking



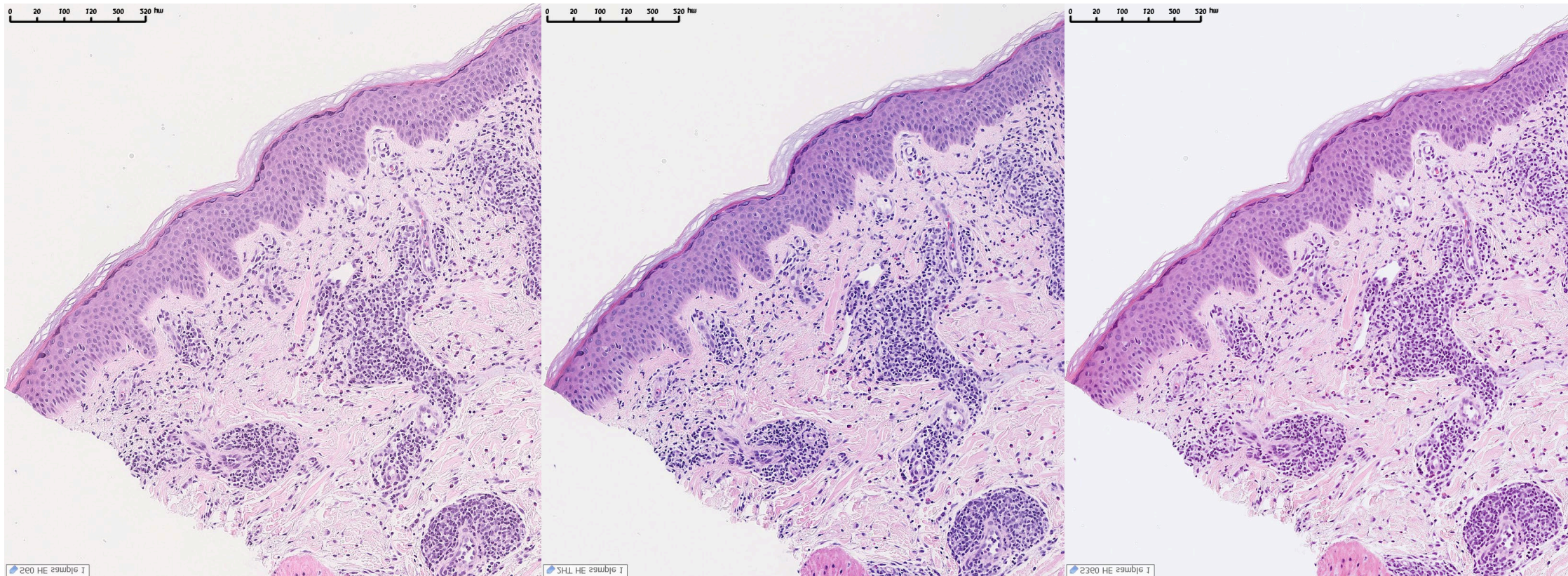
## Rundt om skanneren:

- Hastighed
  - Husk at se på total skantid
  - Hvordan fyldes den
    - Rack eller holder
  - Kontinuert loading
- Kvalitet
  - Fejlrate
  - Farvegengivelse/pixelværdier\*
- Opløsning/forstørrelse
  - Eksempel: Hamamatsu Nanozoomer objektiv 20x/0,75NA
- Fuldautomatisk skanning
- Antal og størrelse glas
  - megaglas
- Oppetid
- Mulighed for service og support
- Fluorescens/cytologi



It is expected that within a **single department, different scanners** will be used for **different purposes**. One vendor may have the best high-throughput scanner for general histology, while another scanner is best for large or mega slides and yet another for cytology or fluorescence staining.

<https://medical.sectra.com/resources/supporting-digital-full-scale-primary-diagnostics-pathology/> Elin Kindberg

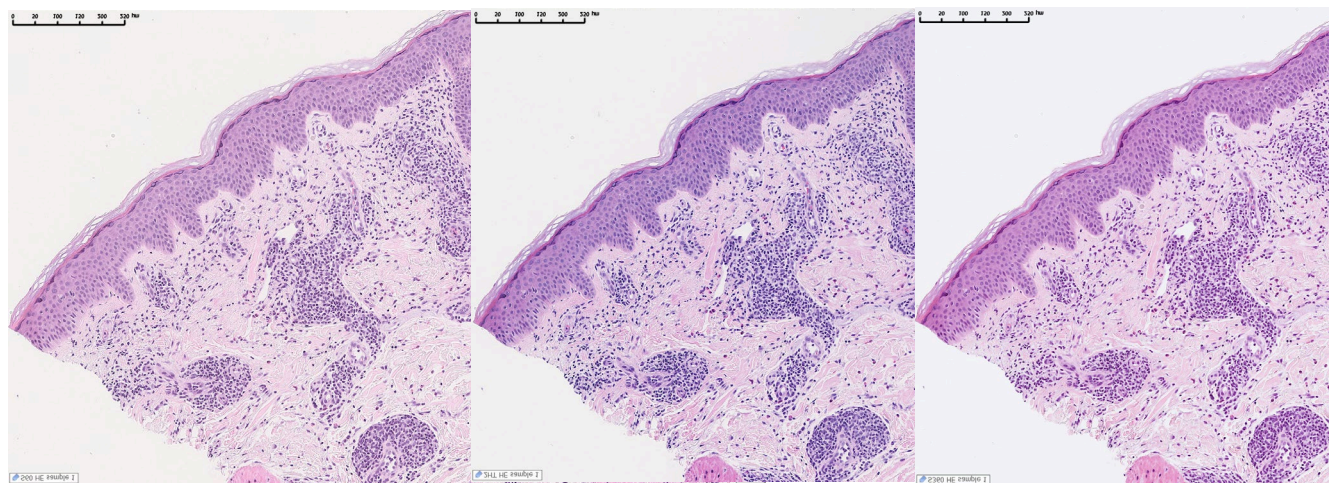


## Share the slide

In a typical scenario, if a pathologist wants to **consult with colleagues** from a different hospital, city, or state, the samples in question have to be **packaged and sent** to the intended specialist. Such shipping is quite **costly**, not to mention that getting a second opinion will take **days to weeks**. Also, there is always a risk of **slide damage** or loss in transit.

But all those hurdles disappear once tissue specimens become available in electronic form allowing them to be exchanged via the Internet. You can **easily share** slides with other specialists for getting a second opinion as well as with patients, research centers, and other stakeholders.

<https://www.altexsoft.com/blog/digital-computational-pathology/>





## Hvad det er værd at vide om skanneren foran dig:

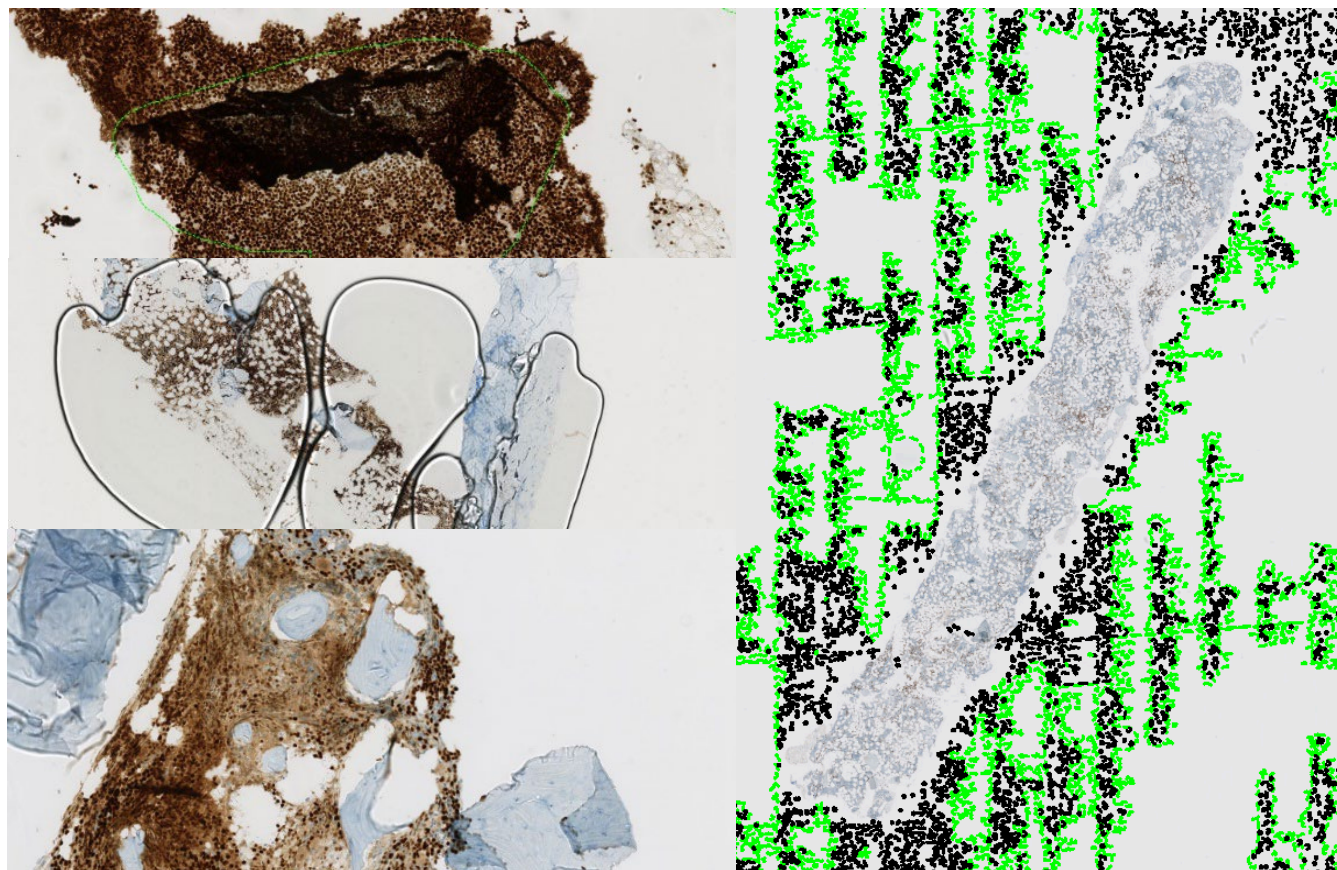
- Hastighed
  - Husk at se på total skantid
  - Hvordan fyldes den
    - Rack eller holder
    - Tab af glas
  - Kontinuert loading
- Kvalitet
  - Fejlrate
  - Farvegengivelse/pixelværdier\*
- Opløsning/forstørrelse
  - Eksempel: Hamamatsu Nanozoomer objektiv 20x/0,75NA
- Fulldautomatisk skanning
- Oppetid
- Antal og størrelse glas
  - megaglas
- Mulighed for service og support
- Fluorescens/cytologi



# Forudsætninger for god skan-kvalitet

## PROBLEMER

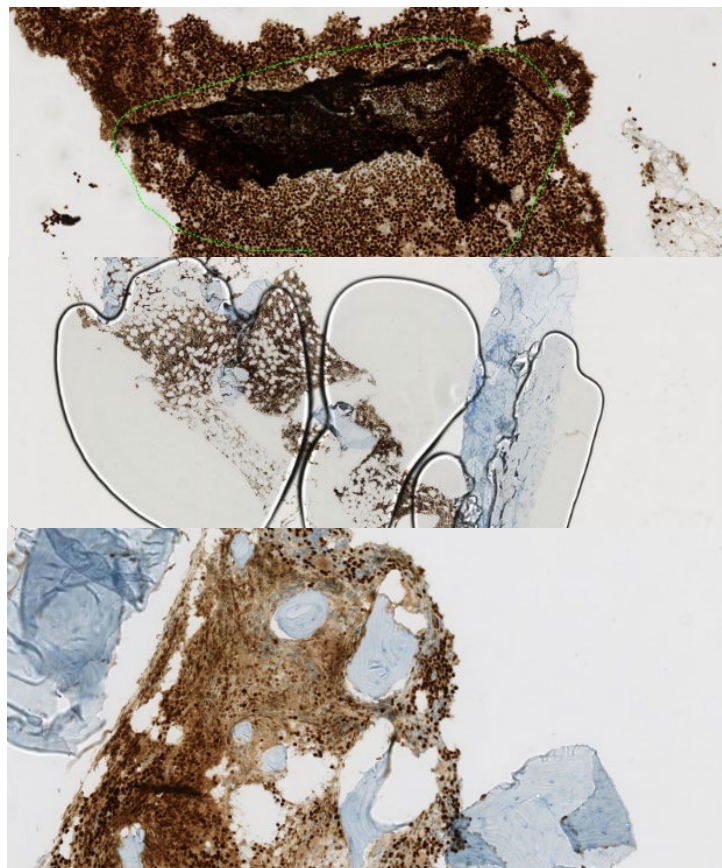
- Artefakter
  - Flydere
  - Ridser
  - Folder
  - Montering
  - Udfældninger
- Tykkelse af vævssnit
- Fedtvæv/meget svagt farvet væv
- Kalibrering/hvidbalance



# Forudsætninger for god skan-kvalitet

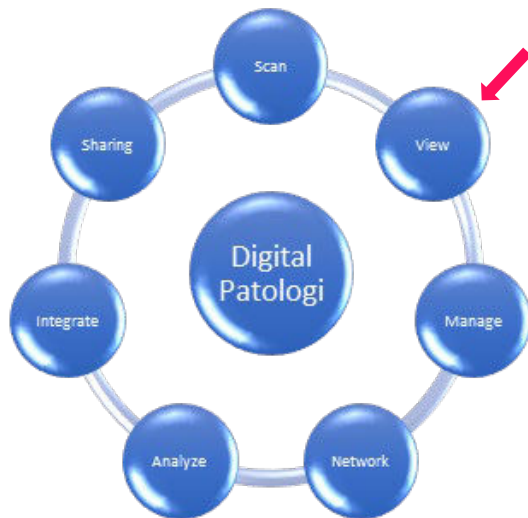
## PROBLEMER

- Artefakter
  - Flydere
  - Ridser
  - Folder
  - Montering
  - Udfældninger
- Tykkelse af vævssnit
- Fedtvæv/meget svagt farvet væv
- Kalibrering/hvidbalance\*



## HVAD KAN VI GØRE?

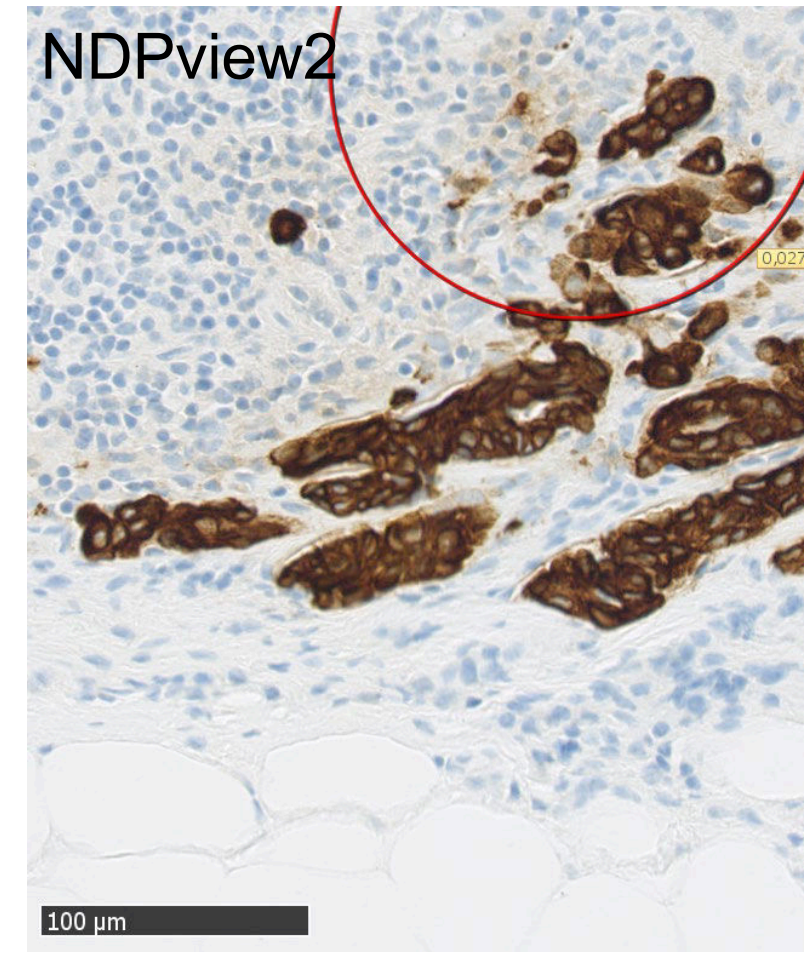
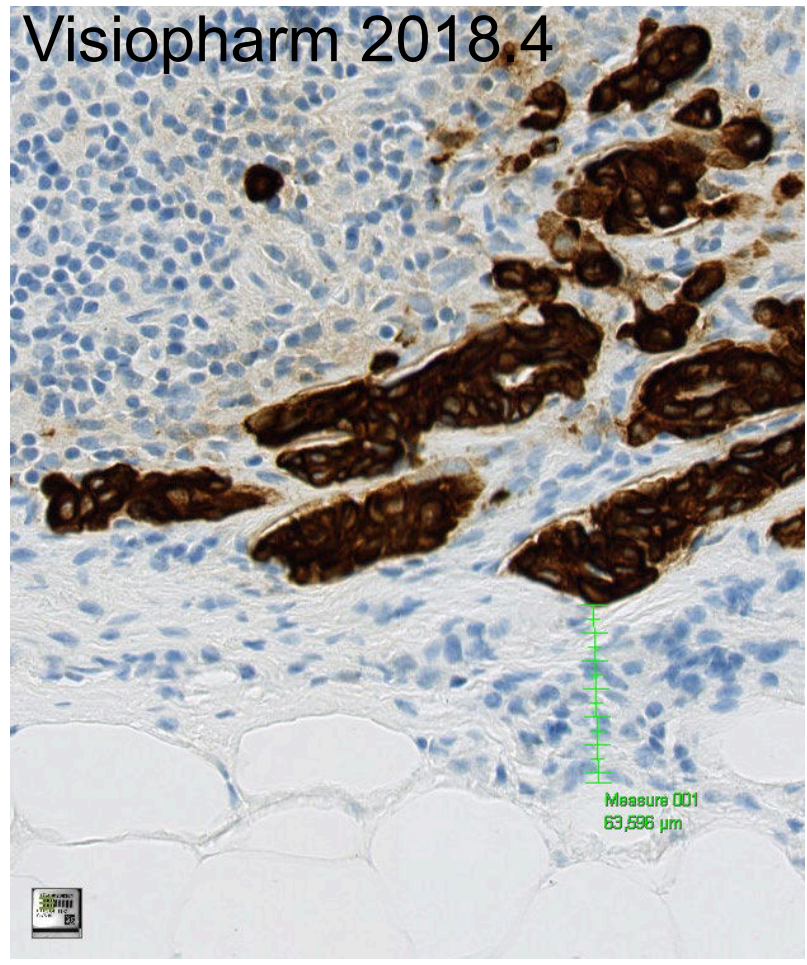
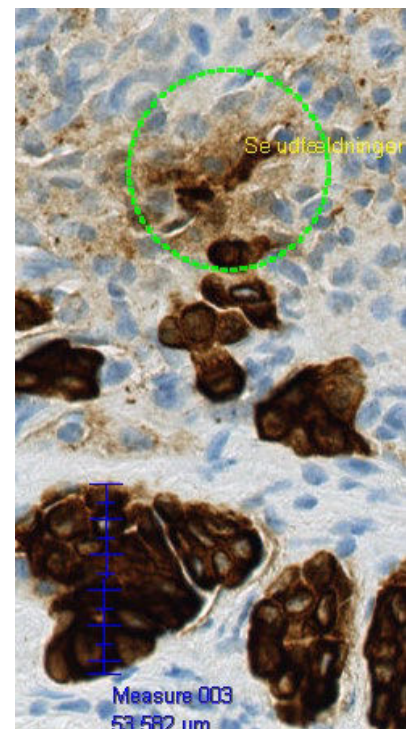
- Skift vandbad
- Ændret fokus på kvalitetssikring
  - mikrotomi
  - montering
- Snittykkelse
  - Mikrotomi-robotter
- Kvalitetskontrol indbygget i skanneren
- Husk at kalibrere/hvidbalance



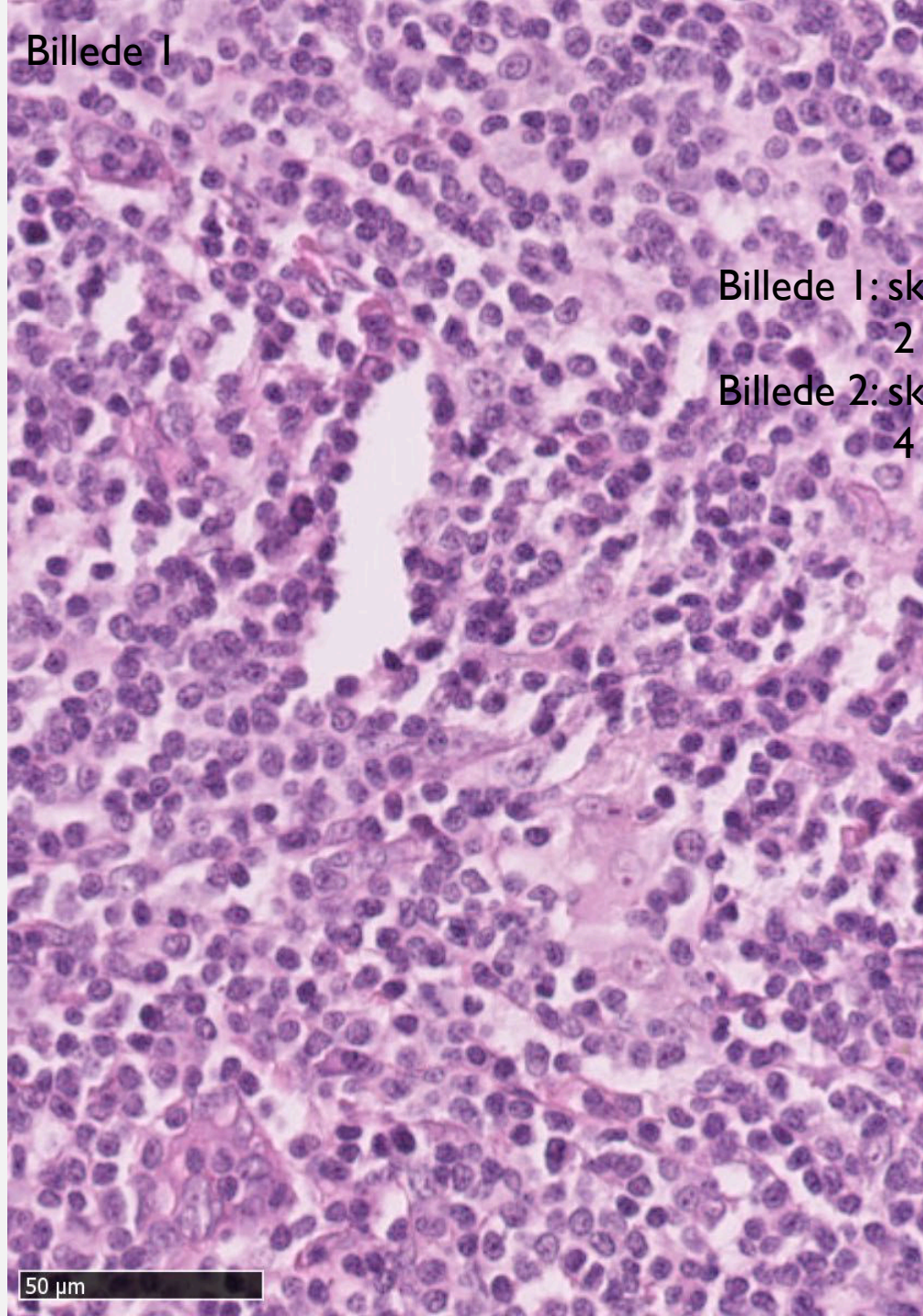
# Viewer

# Viewer

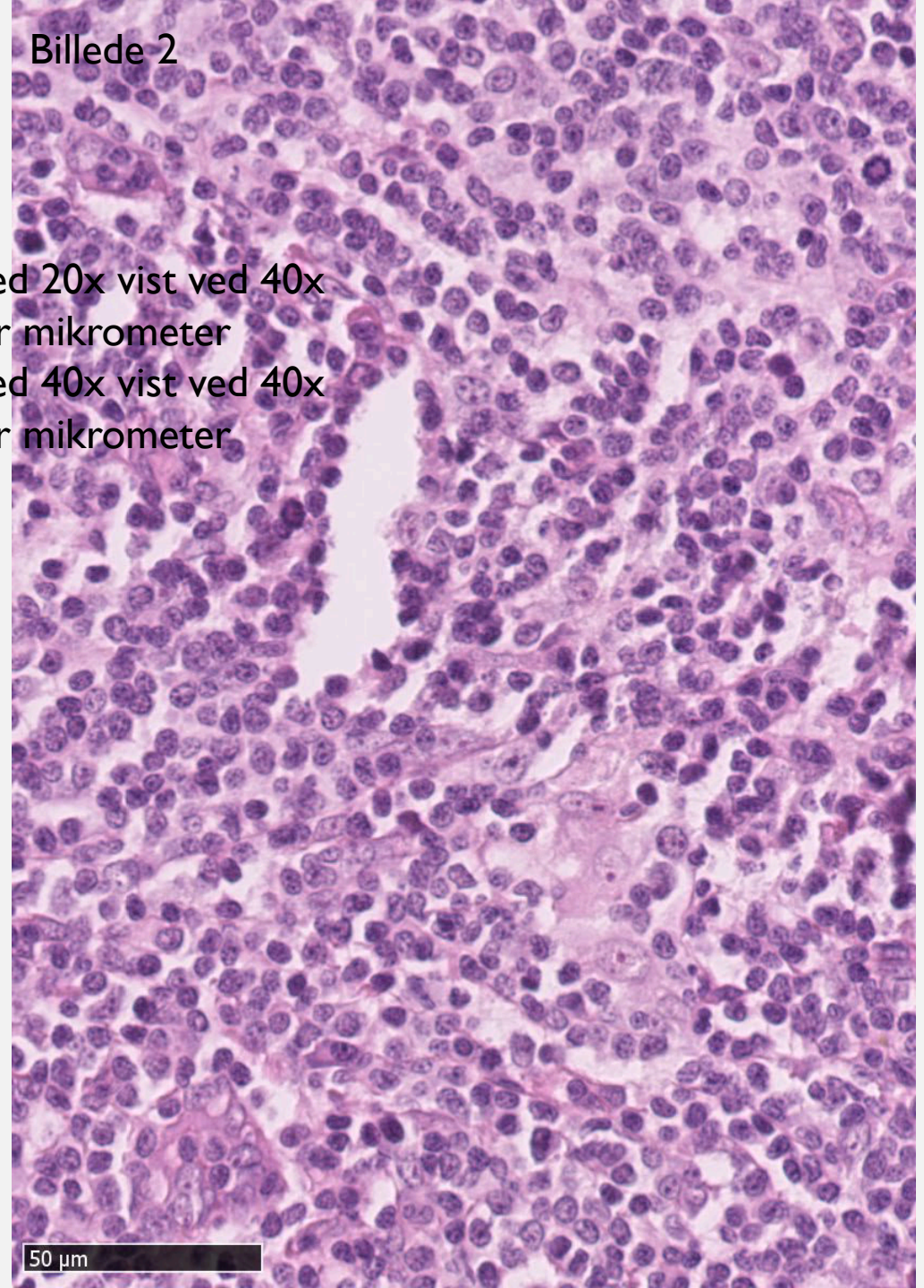
## Annotationer



Billede 1



Billede 2

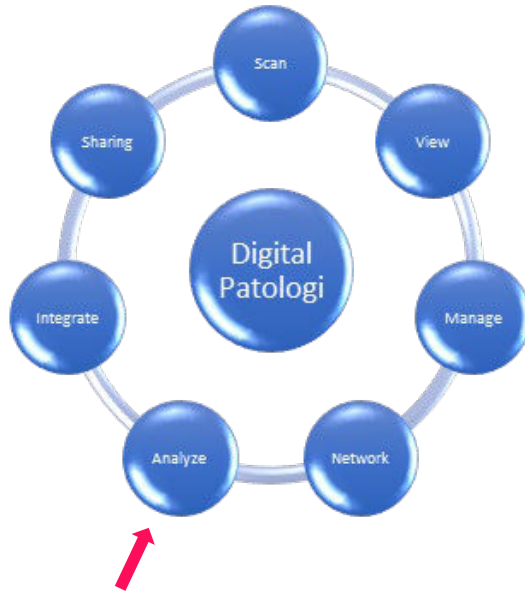


Billede 1: skannet ved 20x vist ved 40x  
2 pixels pr mikrometer

Billede 2: skannet ved 40x vist ved 40x  
4 pixels pr mikrometer

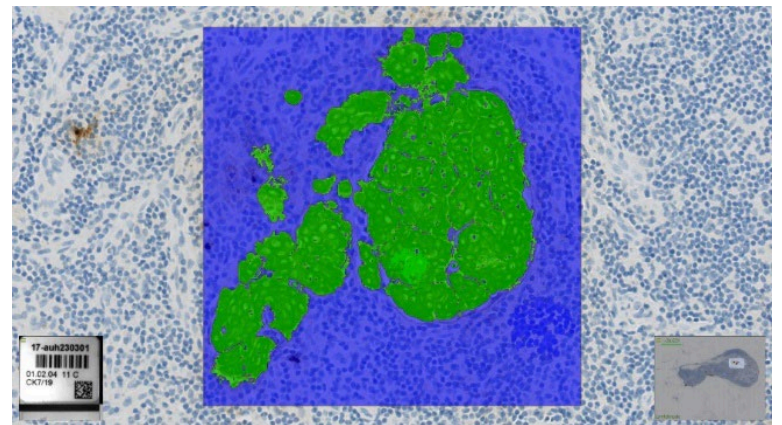
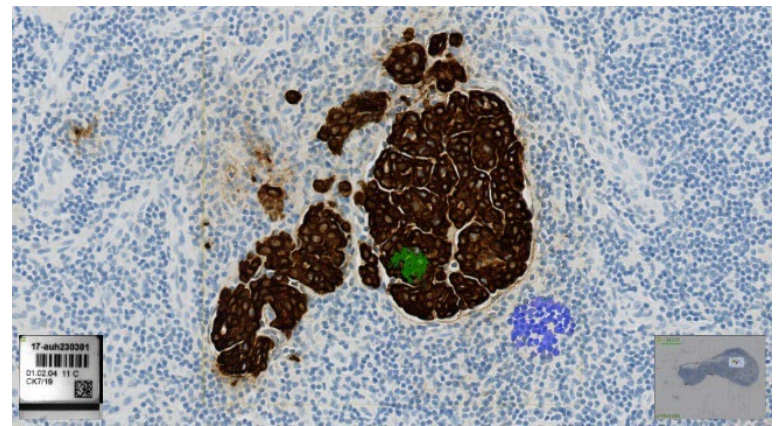
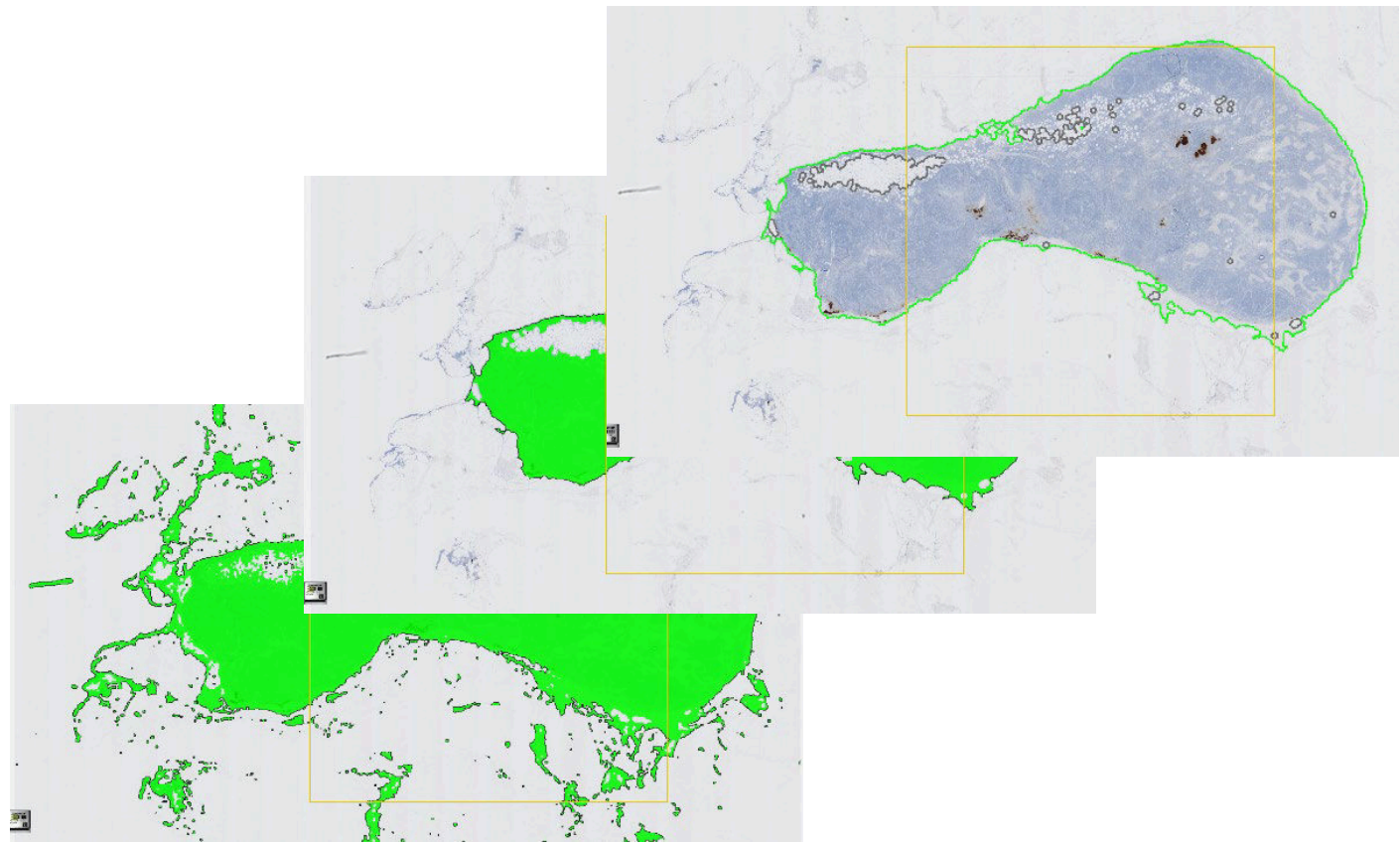
50 μm

50 μm



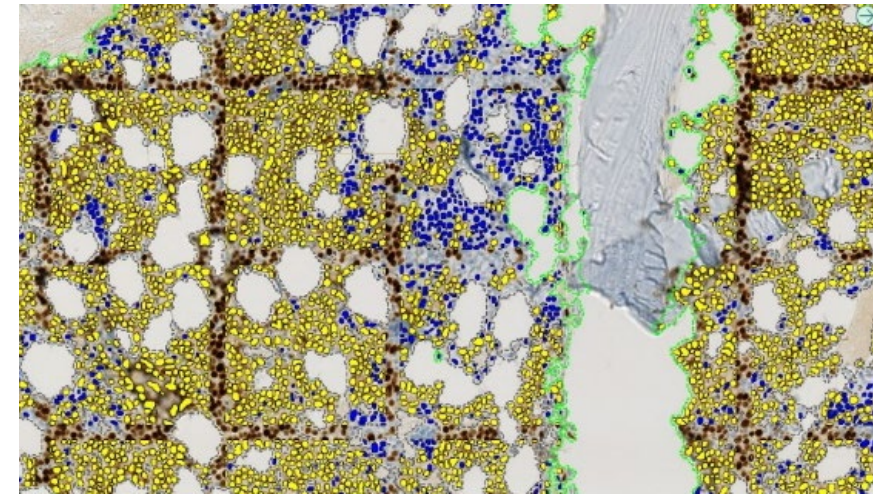
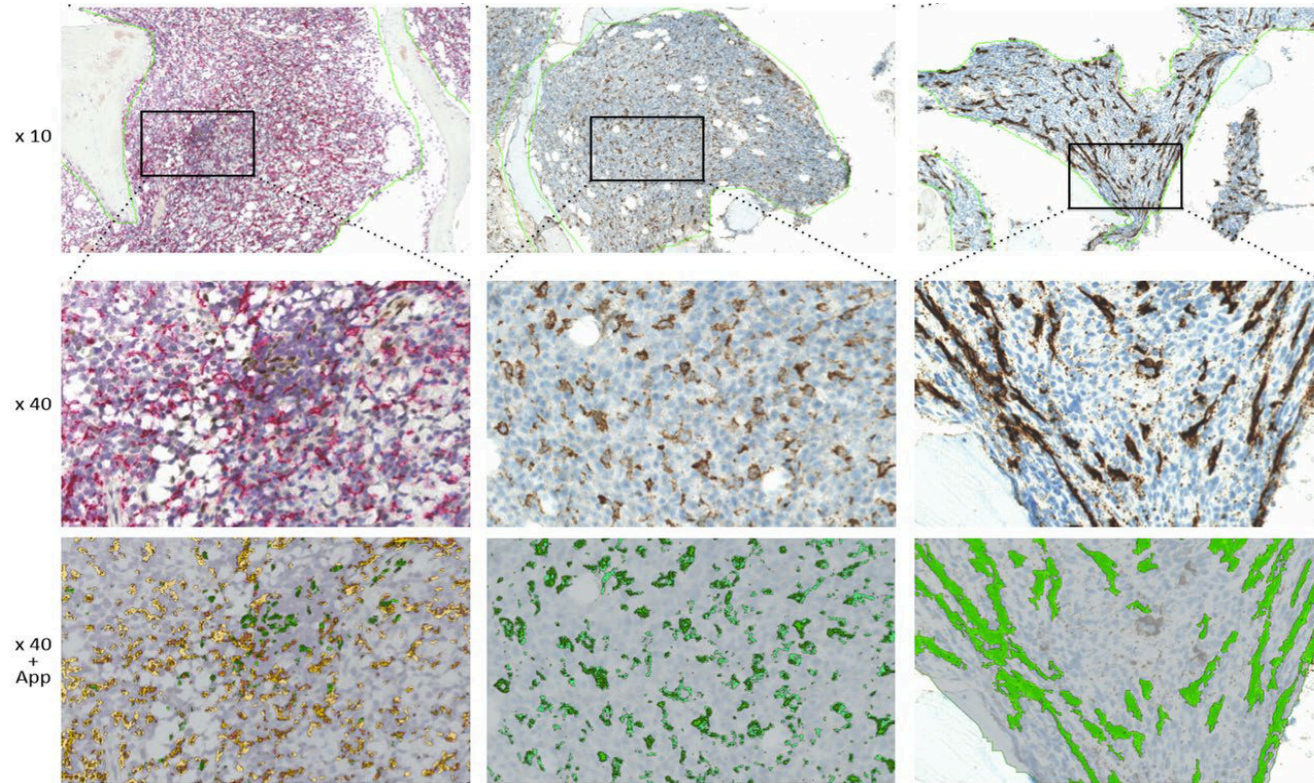
# Digital billedanalyse

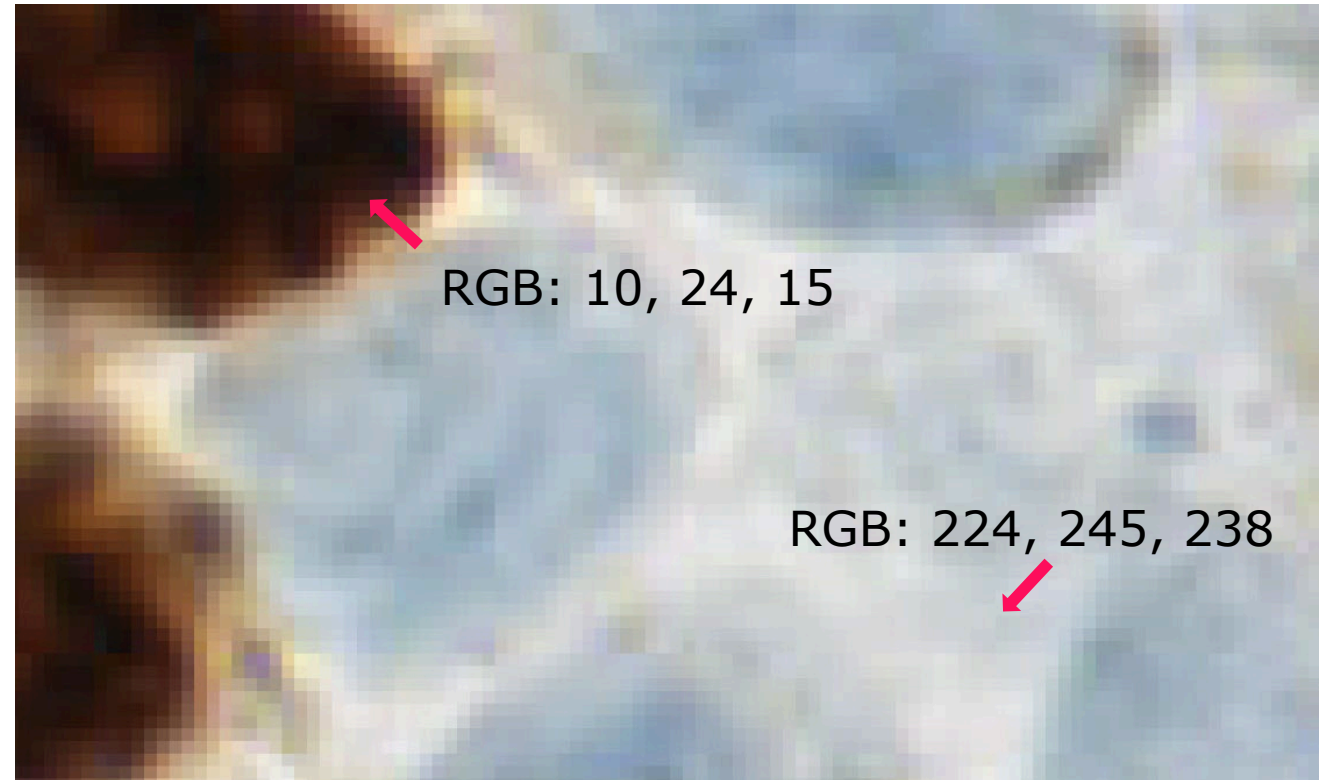
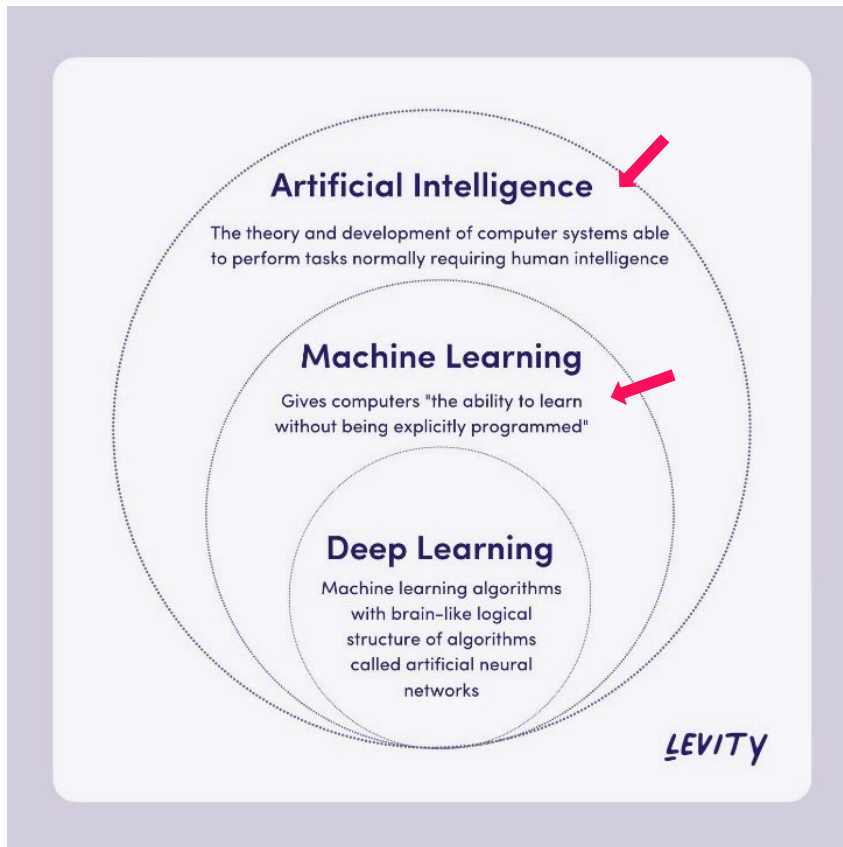
# Digital billedanalyse





# Flere eksempler



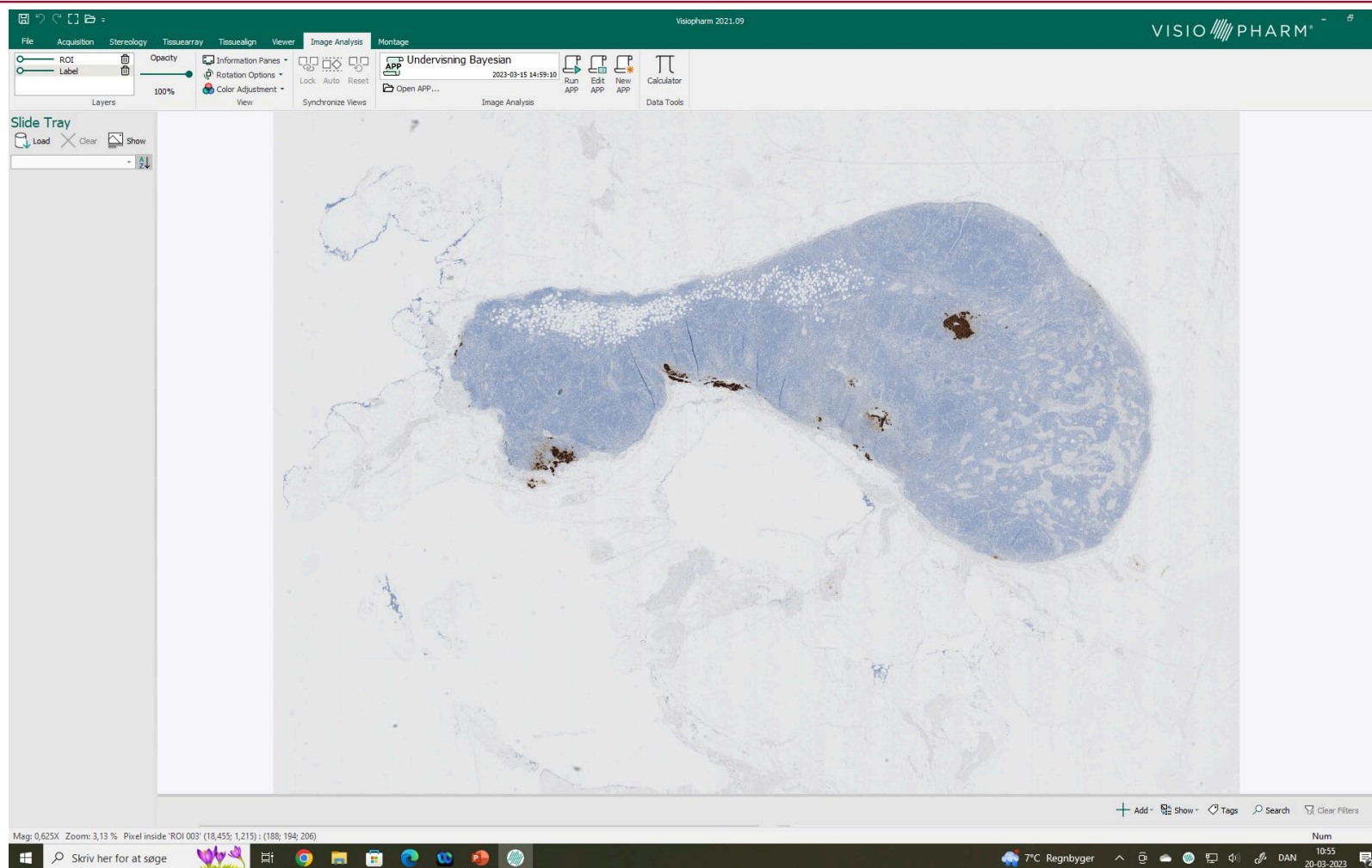


Hvad vi ser: En sentinel node lymfeknude fra en patient med mamma-cancer. Vævssnittet er farvet med CK7/19

Vi har brug for 2 algoritmer:

Formål 1: Identificer Region of Interest (ROI)

Formål 2: Identificer evt. metastaser

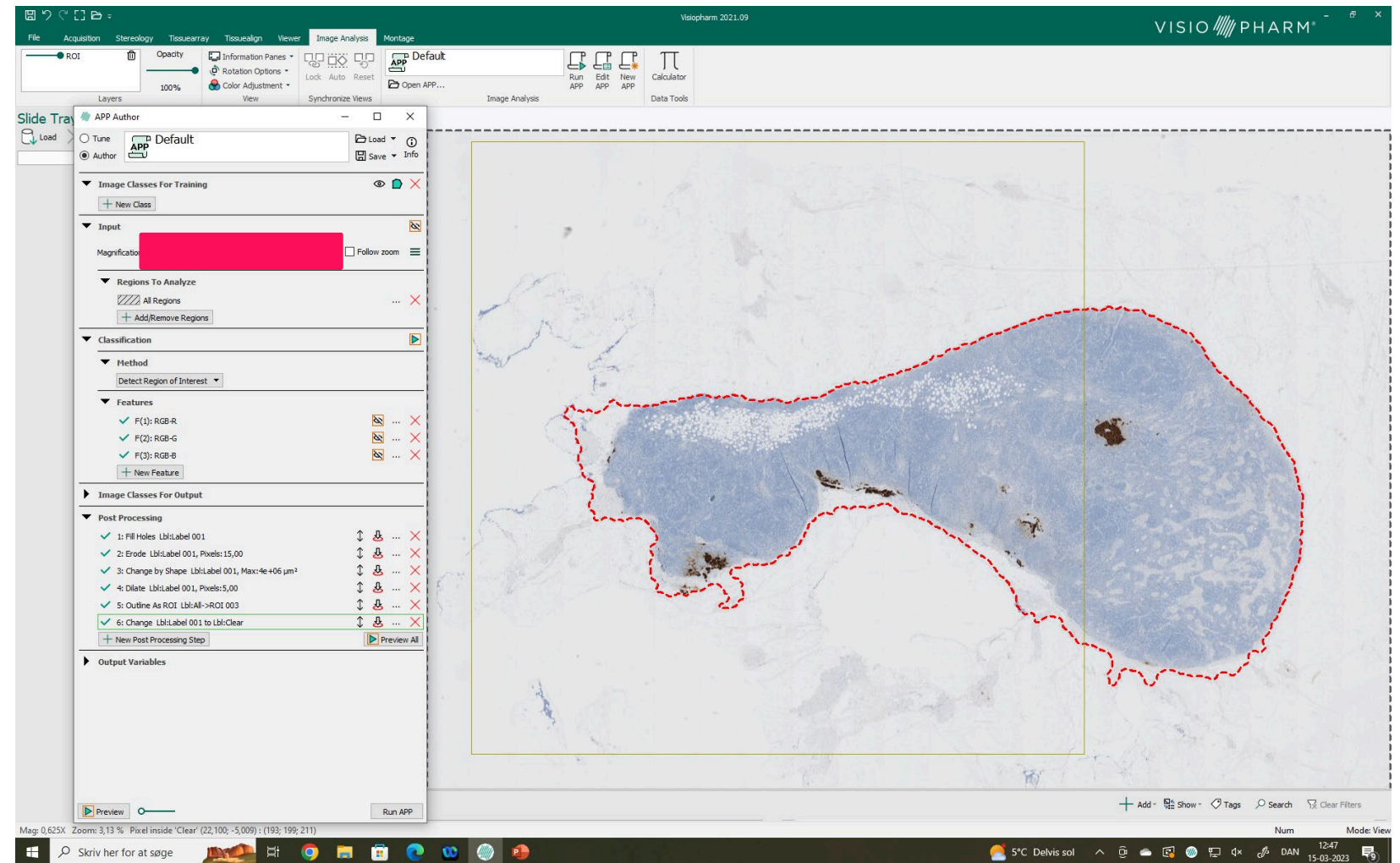


## Formål 1: Identificer Region of Interest (ROI)

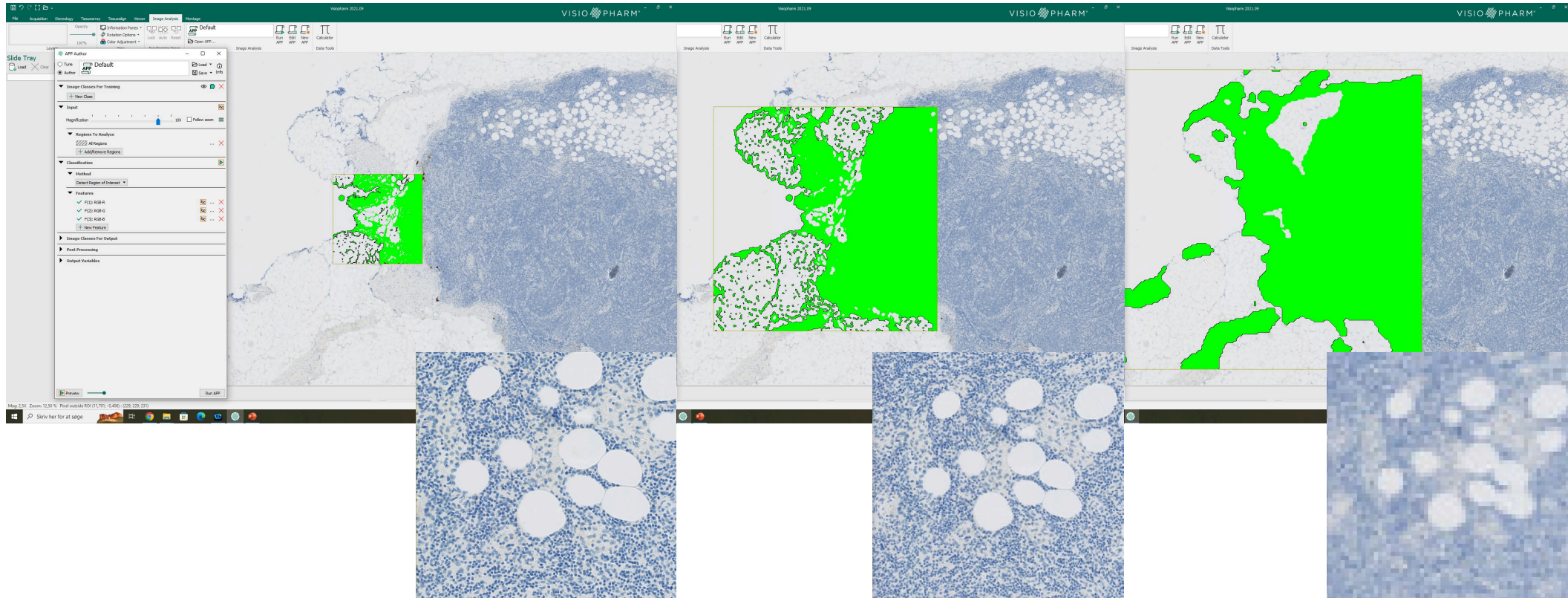
I dette tilfælde er ROI selve lymfeknuden

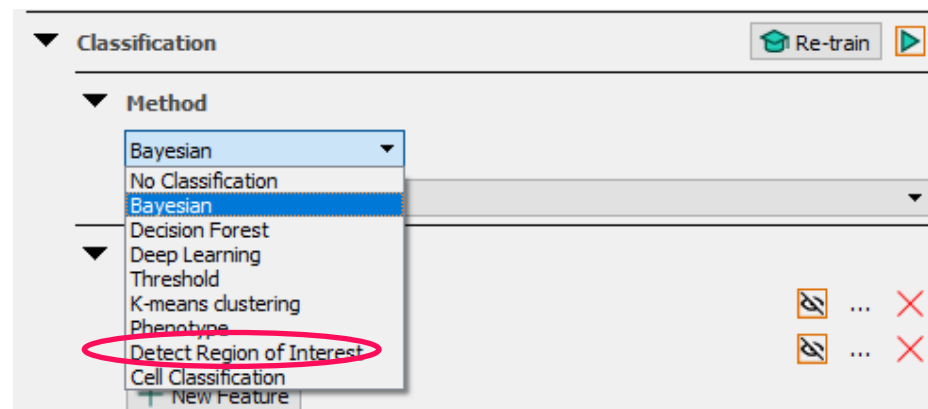
Det kunne være:

- Alt væv
- Tumorstof
- Hotspot
- En vævskomponent



## Forstørrelse: Vælg forstørrelse





File Acquisition Stereology Tissuearray Tissuealign Viewer **Image Analysis** Montage

Label Opacity 100% Information Panes Rotation Options Color Adjustment View Lock Auto Reset APP Default Open APP... Run APP Edit APP New APP Calculator Data Tools

Slide Tray APP Author

○ Tune **APP Default** Load Save Info

● Author

Image Classes For Training  
+ New Class

Input  
Magnification 0,5X Follow zoom

Regions To Analyze  
All Regions Add/Remove Regions

Classification  
Method: Detect Region of Interest

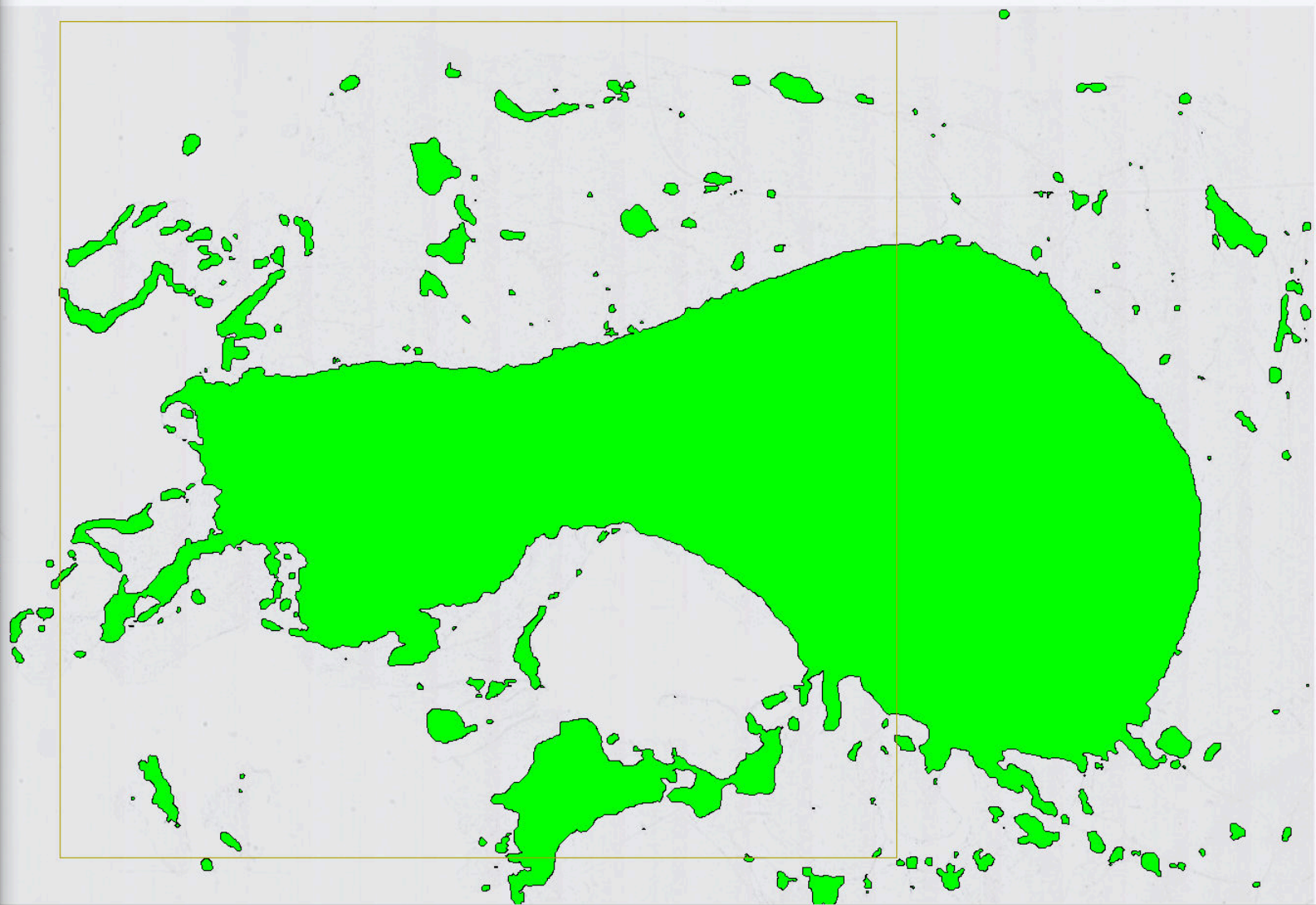
Features  
 F(1): RGB-R  
 F(2): RGB-G  
 F(3): RGB-B  
 + New Feature

Image Classes For Output

Post Processing  
 1: Fill Holes Lbl:Label 001  
 + New Post Processing Step Preview All

Output Variables

Preview Run APP



+ Add Show Tags Search Clear Filters

Mag: 0,625X Zoom: 3,13 %

File Acquisition Stereology Tissuearray Tissuealign Viewer **Image Analysis** Montage

Label  Opacity 100%

Information Panes  Rotation Options  Color Adjustment

Lock Auto Reset Synchronize Views

APP Default Open APP...

Run APP Edit APP New APP Calculator

Image Analysis Data Tools

Slide Tray APP Author

Tune  Author

Default Load Save Info

**Image Classes For Training**

+ New Class

**Input**

Magnification 0,5X Follow zoom

**Regions To Analyze**

All Regions Add/Remove Regions

**Classification**

**Method**

Detect Region of Interest

**Features**

- F(1): RGB-R
- F(2): RGB-G
- F(3): RGB-B

+ New Feature

**Image Classes For Output**

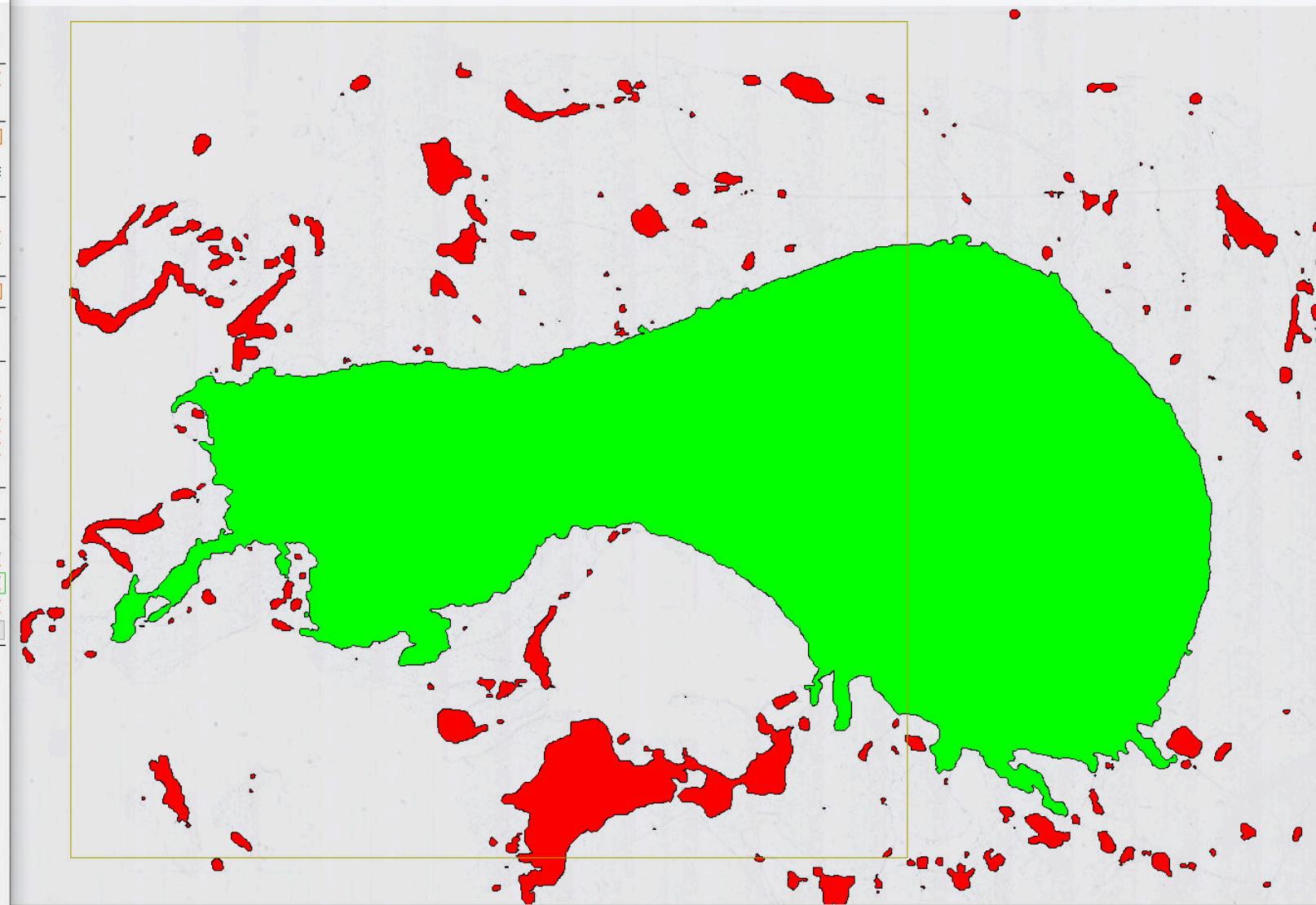
**Post Processing**

- 1: Fill Holes Lbl:Label 001
- 2: Change by Shape Lbl:Label 001, Max:4e+06  $\mu\text{m}^2$
- 3: Erode Lbl:Label 001, Pixels:10,00

+ New Post Processing Step Preview All

**Output Variables**

Preview Run APP



Mag: 0,625X Zoom: 3,13 % Pixel outside ROI (10,525; -4,878) : (229; 231; 232)

+ Add Show Tags Search Clear Filters



File Acquisition Stereology Tissuearray Tissuealign Viewer Image Analysis Montage

Layers: ROI, Label, Opacity: 100%

Information Panes, Rotation Options, Color Adjustment

Lock Auto Reset

APP Default, Open APP...

Run APP, Edit APP, New APP

Calculator

Image Analysis, Data Tools

Slide Tray

APP Author

Tune, Author

Image Classes For Training

+ New Class

Input

Magnification: 0,5X Follow zoom

Regions To Analyze

All Regions

+ Add/Remove Regions

Classification

Method: Detect Region of Interest

Features

- F(1): RGB-R
- F(2): RGB-G
- F(3): RGB-B

+ New Feature

Image Classes For Output

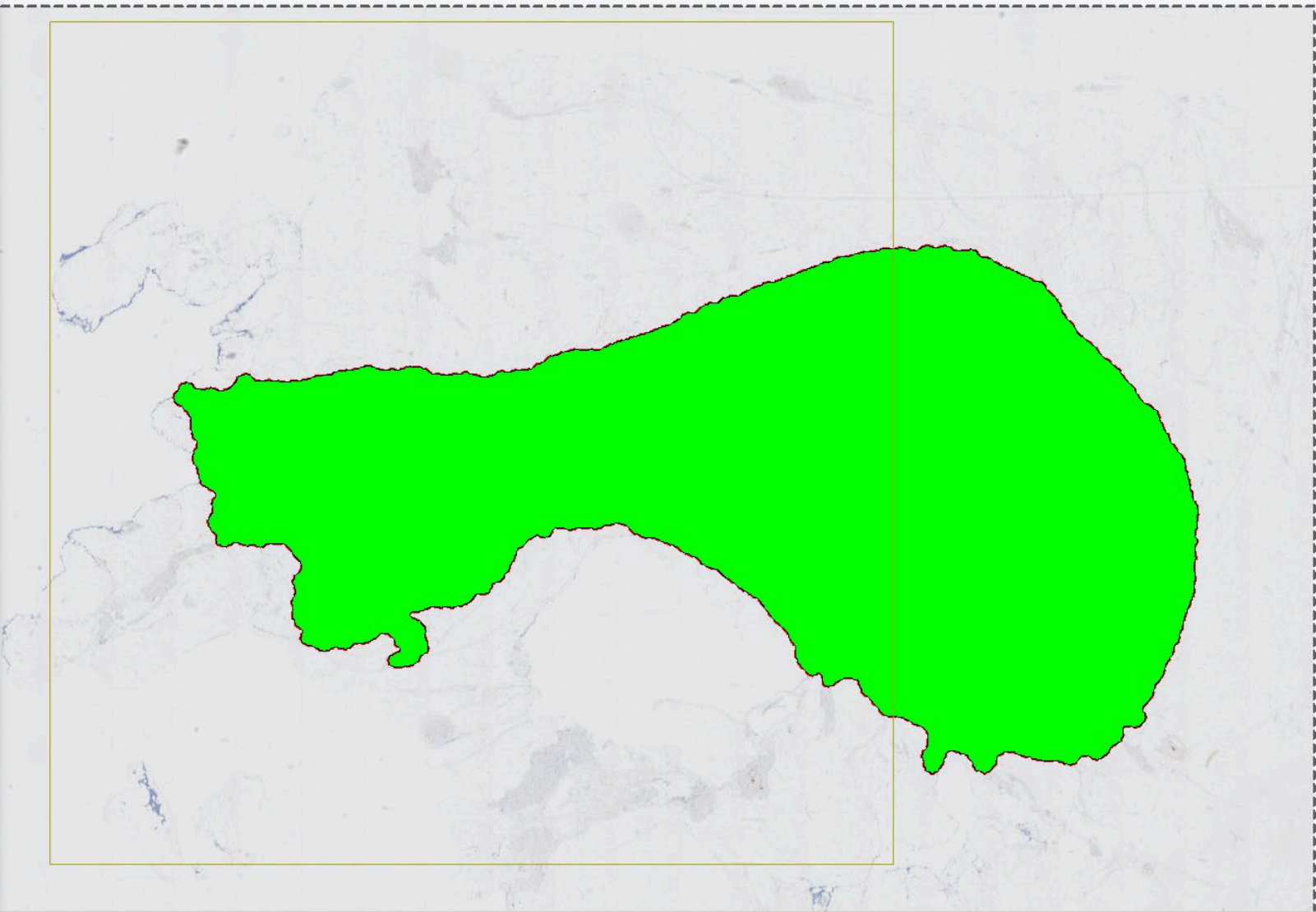
Post Processing

- 1: Fill Holes Lbl:Label 001
- 2: Erode Lbl:Label 001, Pixels: 15,00
- 3: Change by Shape Lbl:Label 001, Max: 4e+06  $\mu\text{m}^2$
- 4: Dilate Lbl:Label 001, Pixels: 10,00
- 5: Outline As ROI Lbl:All->ROI 003

+ New Post Processing Step

Output Variables

Preview, Run APP

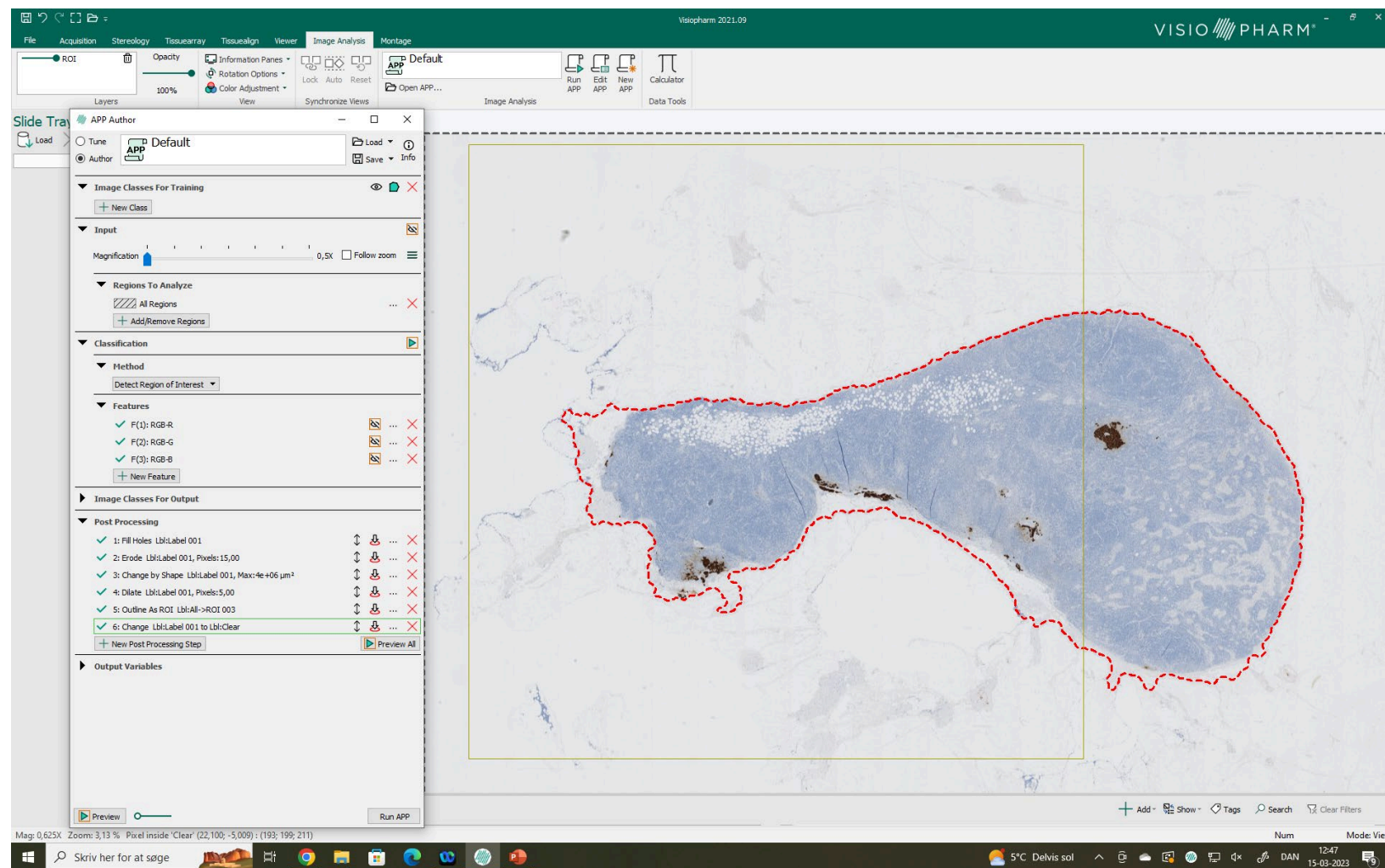


+ Add Show Tags Search Clear Filters

Mag: 0,625X Zoom: 3,13 % Pixel inside 'Clear (27,458; -2,422) : (230; 230; 232)

Num Mode: View

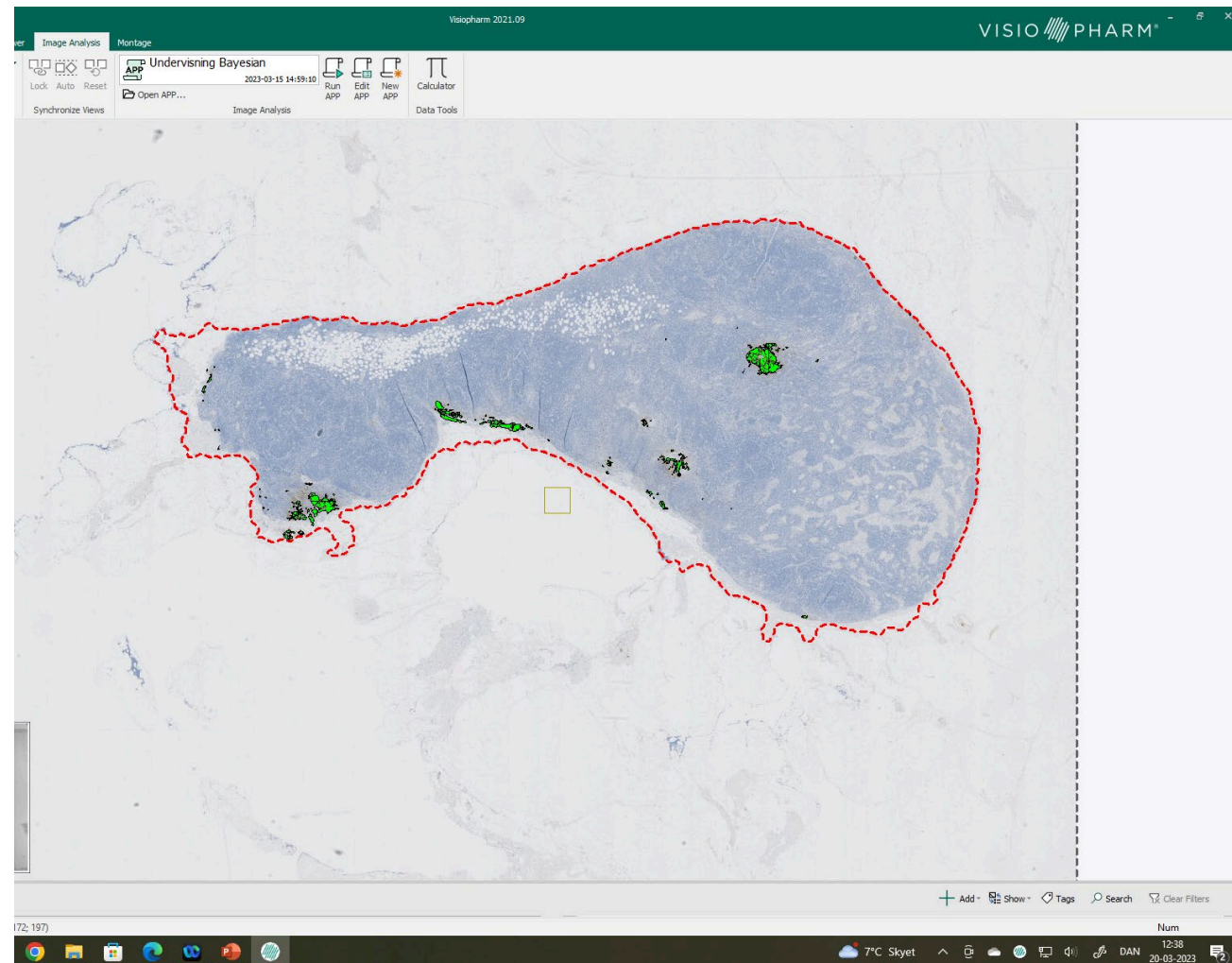
# Formål 1: Identifier Region of Interest (ROI)

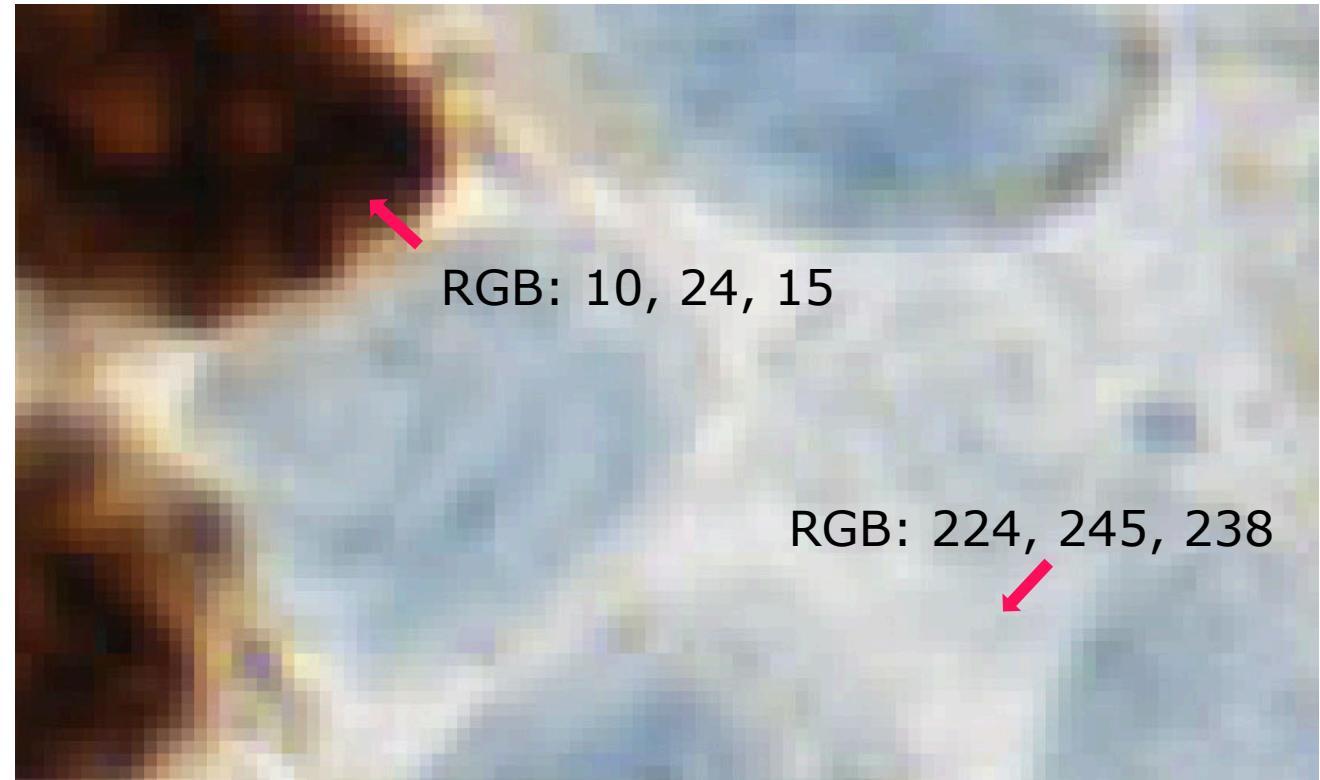
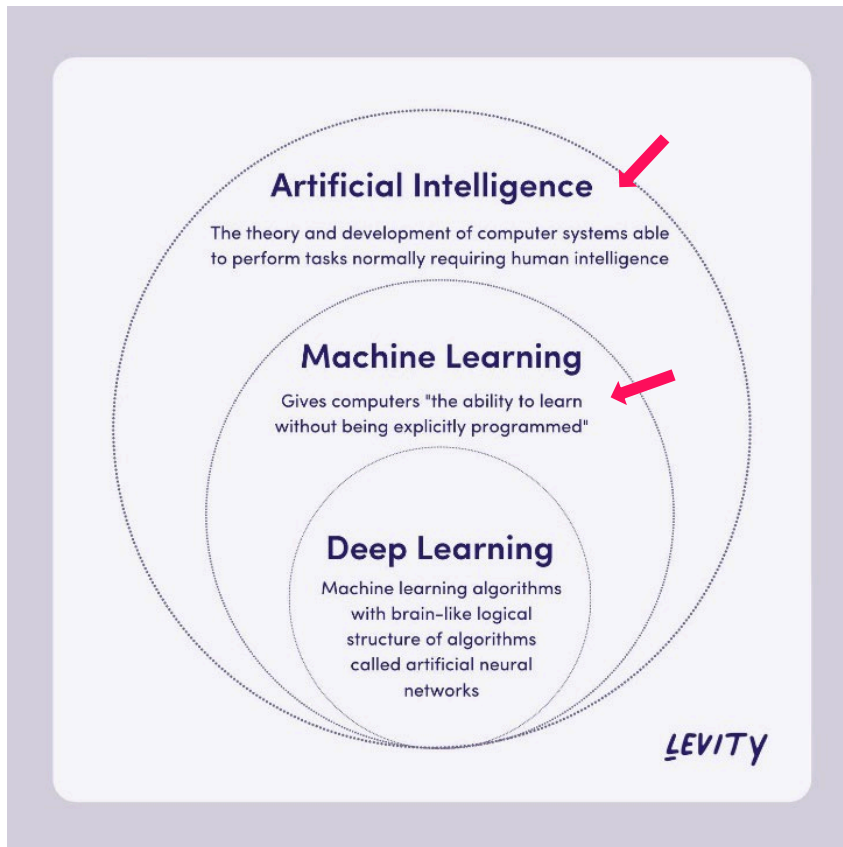


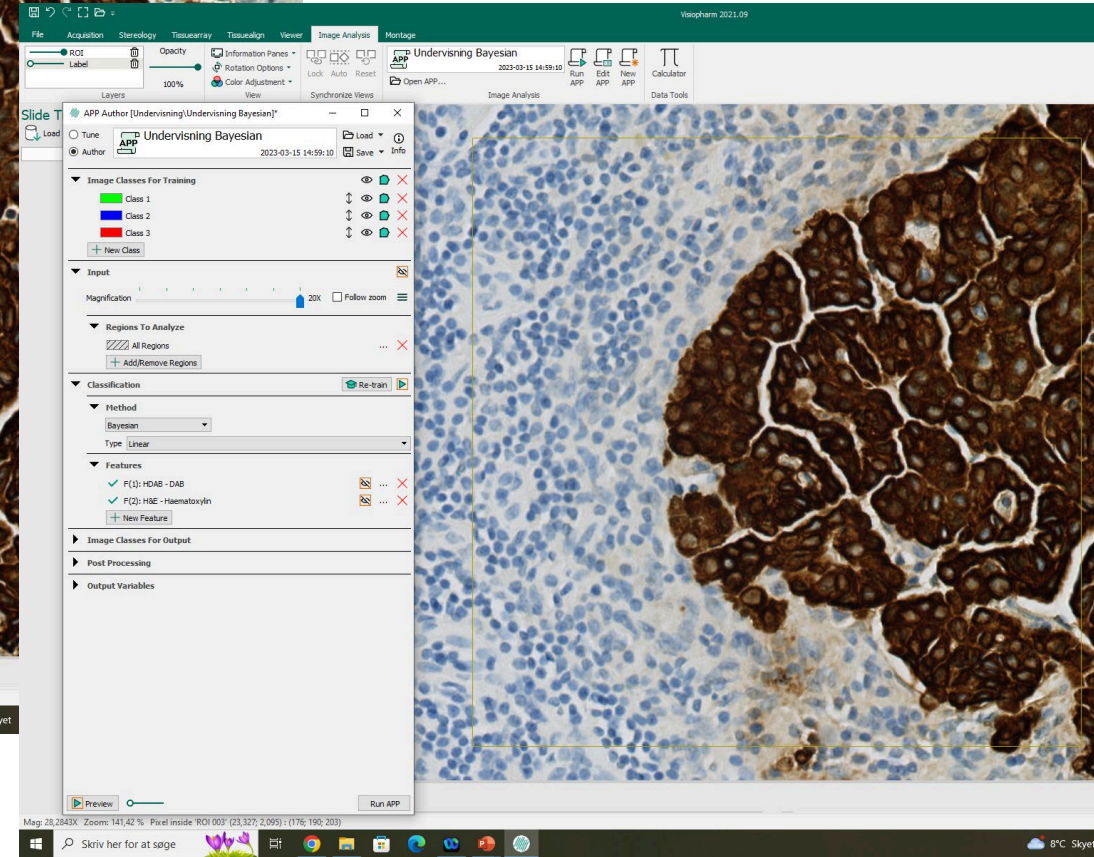
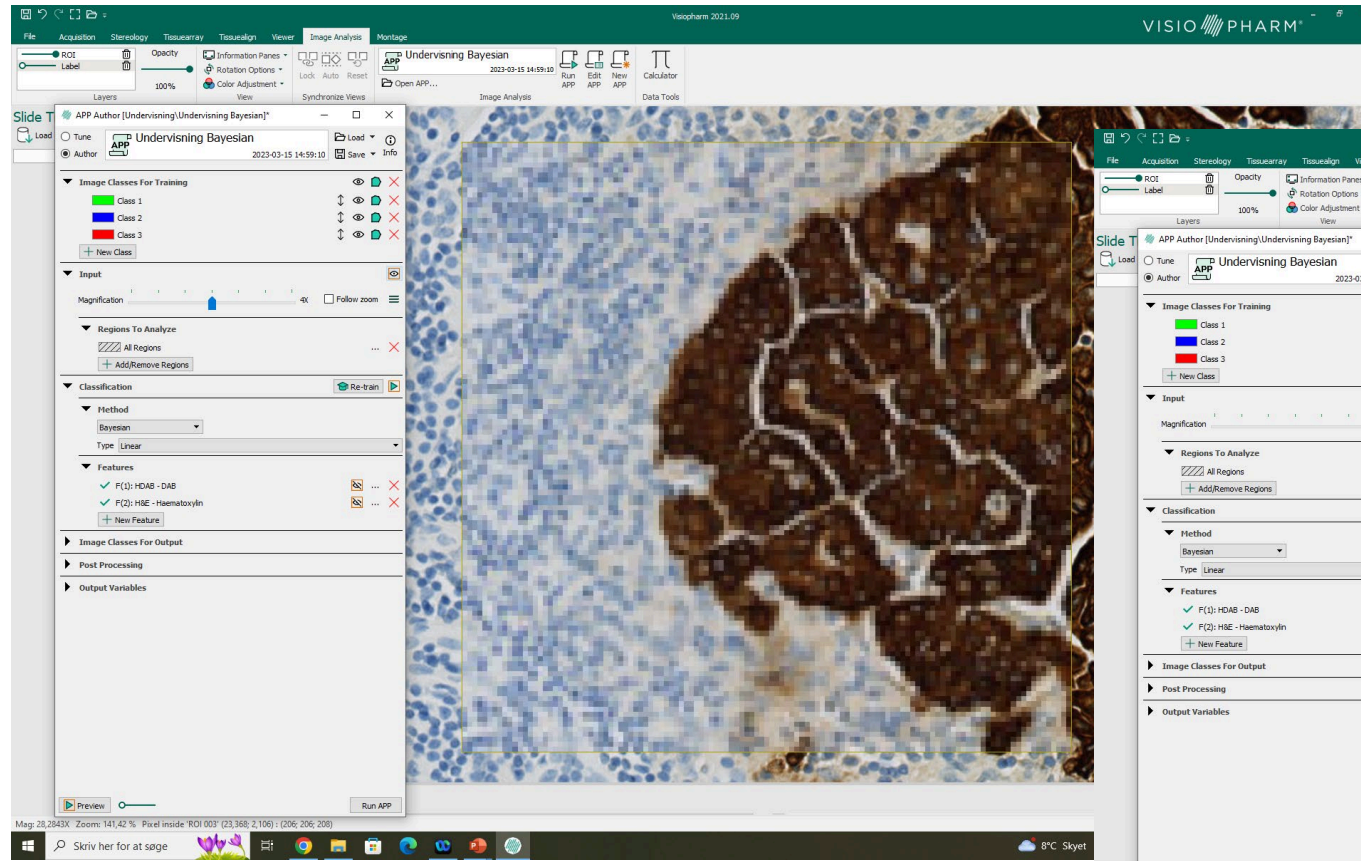
## Formål 2: Identificer evt. metastaser

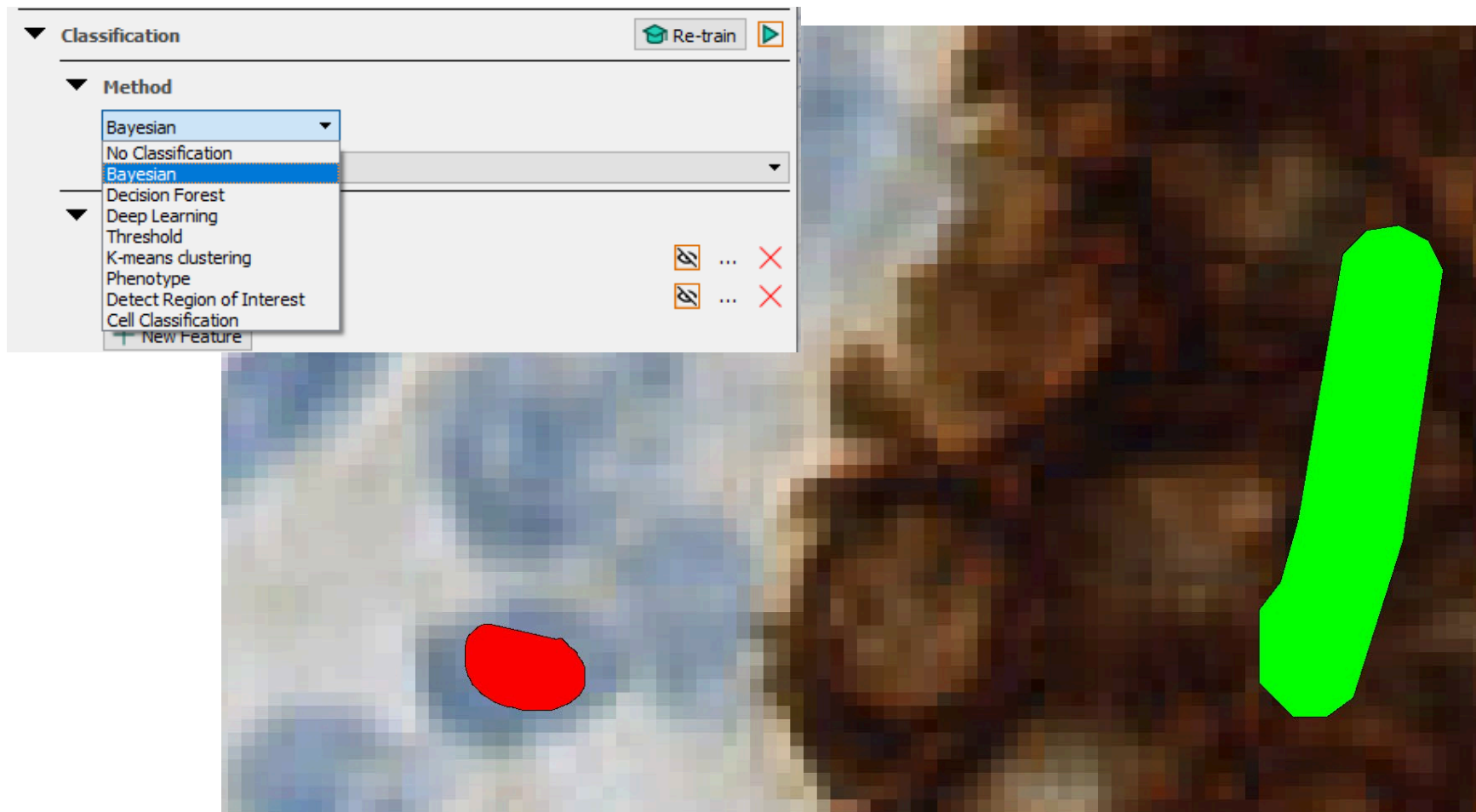
Det kunne være:

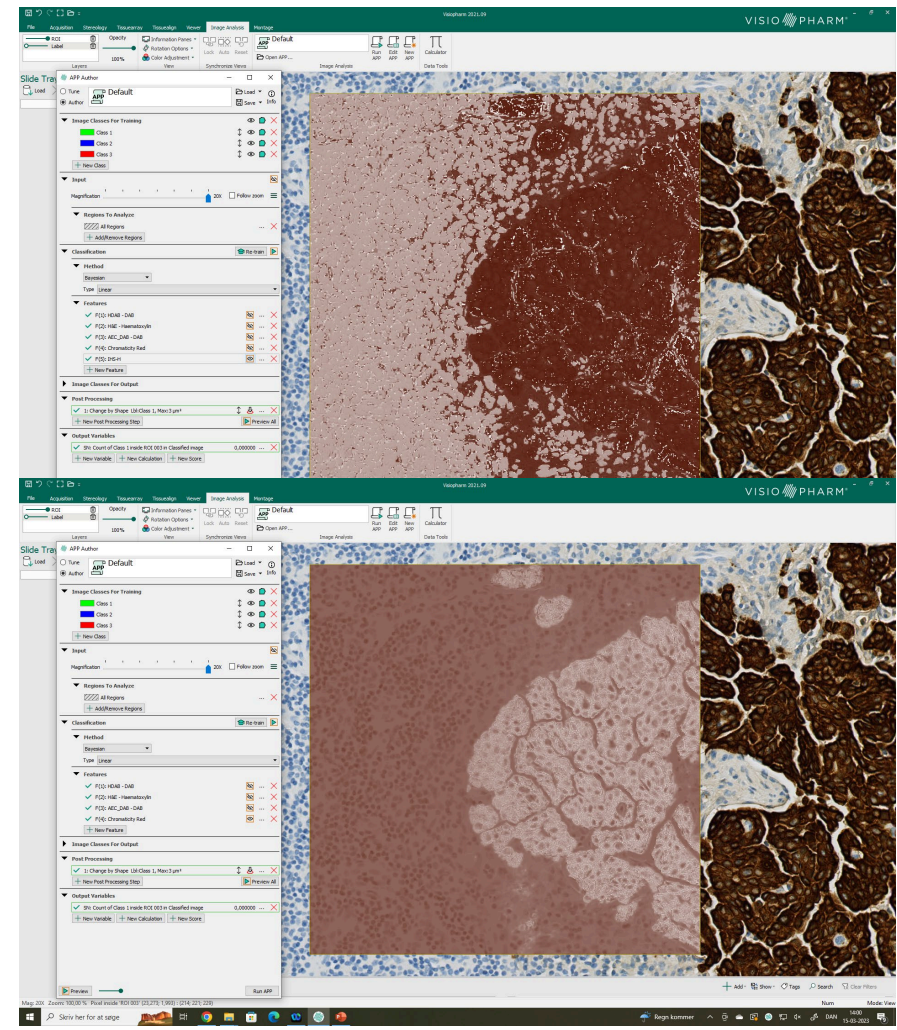
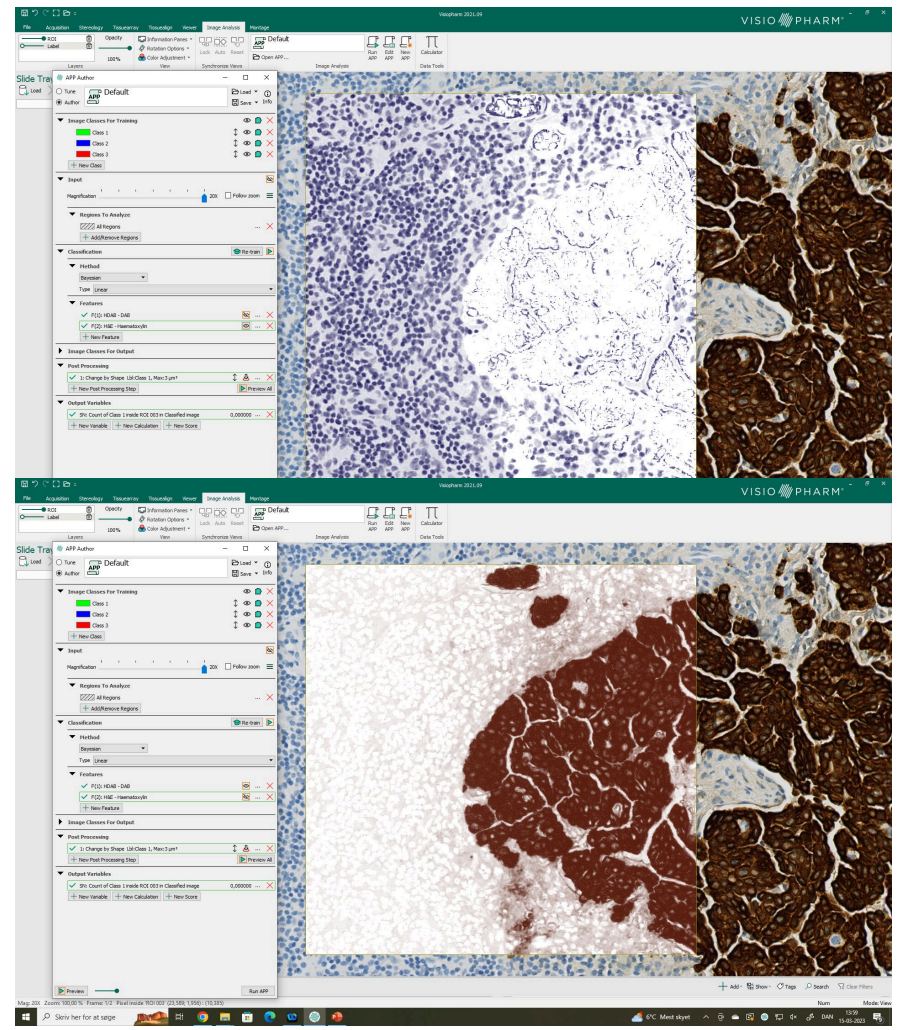
- Arealfraktioner
- Index











File Acquisition Stereology Tissuearray Tissuealign Viewer **Image Analysis** Montage

ROI Label Opacity 100%

Information Panes Rotation Options Color Adjustment View

Lock Auto Reset Synchronize Views

APP Default Open APP...

Run APP Edit APP New APP Calculator Data Tools

Slide Tray APP Author

Load Tune Author

Image Classes For Training

- Class 1
- Class 2
- Class 3
- + New Class

Input

Magnification 20X Follow zoom

Regions To Analyze

- All Regions
- + Add/Remove Regions

Classification Re-train

Method

Bayesian

Type Linear

Features

- F(1): HDAB - DAB
- F(2): H&E - Haematoxylin
- + New Feature

Image Classes For Output

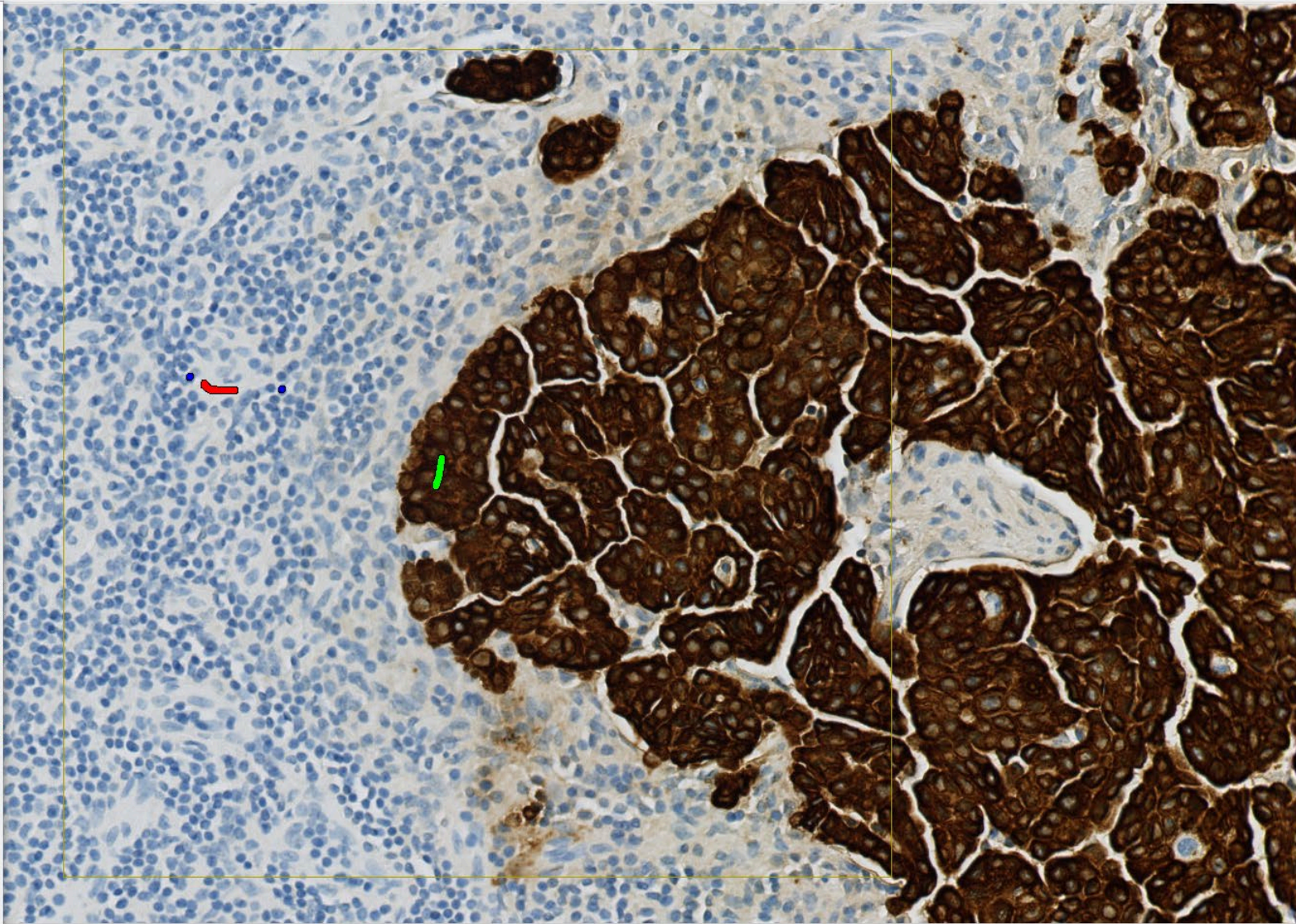
Post Processing

- 1: Change by Shape Lbl:Class 1, Max:3  $\mu\text{m}^2$
- + New Post Processing Step
- Preview All

Output Variables

- SN: Count of Class 1 inside ROI 003 in Classified image 0,000000
- + New Variable + New Calculation + New Score

Preview Run APP



Mag: 20X Zoom: 100,00 % Pixel inside 'ROI 003' (23,317; 2,053) : (157; 177; 201)

+ Add Show Tags Search Clear Filters

Num Mode: View



File Acquisition Stereology Tissuearray Tissuealign Viewer Image Analysis Montage

ROI Label Opacity 100%

Information Panes Rotation Options Color Adjustment View

Lock Auto Reset Synchronize Views

APP Default Open APP...

Run APP Edit APP New APP Calculator Data Tools

Slide Tray APP Author

Tune Author

Image Classes For Training

- Class 1
- Class 2
- Class 3

Input

Magnification 20X Follow zoom

Regions To Analyze

- All Regions

Classification

Method Bayesian

Type Linear

Features

- F(1): HDAB - DAB
- F(2): H&E - Haematoxylin

Image Classes For Output

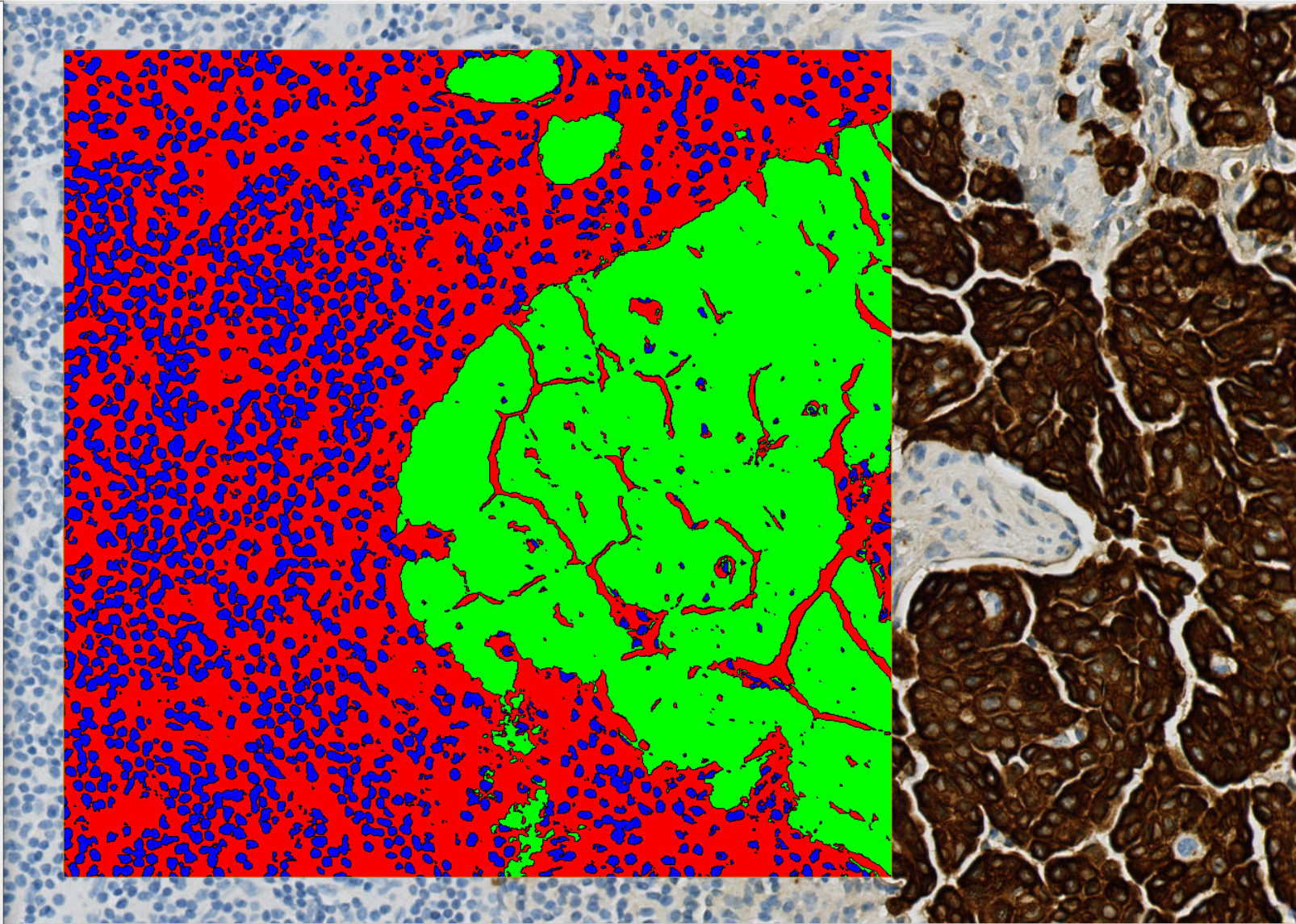
Post Processing

- 1: Change by Shape Lbl:Class 1, Max:3  $\mu\text{m}^2$

Output Variables

- SN: Count of Class 1 inside ROI 003 in Classified image 0,000000

Preview Run APP



File Acquisition Stereology Tissuearray Tissuealign Viewer Image Analysis Montage

ROI Label Opacity 100%

Information Panes Rotation Options Color Adjustment

Lock Auto Reset Synchronize Views

APP Undersiving Bayesian 2023-03-15 14:19:47

Open APP... Run APP Edit APP New APP Calculator

Image Analysis Data Tools

Slide Tray APP Author [Undersiving\Undersiving Bayesian]\*

Load Undersiving Bayesian 2023-03-15 14:19:47 Save Info

**Image Classes For Training**

- Class 1
- Class 2
- Class 3
- + New Class

**Input**

Magnification 20X Follow zoom

**Regions To Analyze**

- All Regions
- + Add/Remove Regions

**Classification** Re-train

**Method**

Bayesian

Type Linear

**Features**

- F(1): HDAB - DAB
- F(2): H&E - Haematoxylin
- + New Feature

**Image Classes For Output**

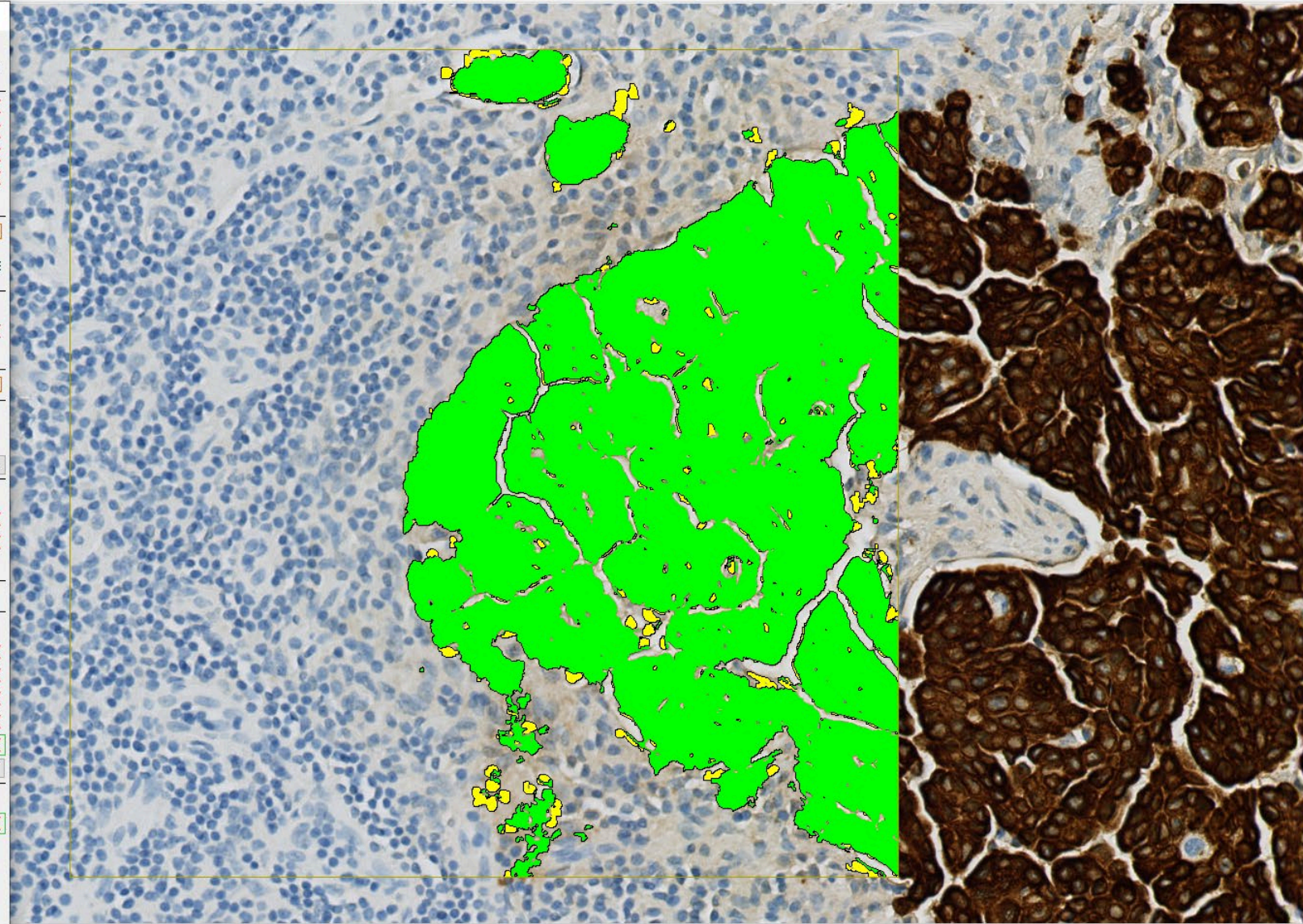
**Post Processing**

- 1: Change by Shape Lbl:Class 1, Max:3  $\mu\text{m}^2$
- 2: Change Lbl:Class 3 to Lbl:Clear
- 3: Dilate Lbl:Class 2, Pixels:3,00
- 4: Change Surrounded Lbl:Class 2, Min:0,1
- 5: Change Lbl:Class 2 to Lbl:Clear
- + New Post Processing Step
- Preview All

**Output Variables**

- SN: Count of Class 1 inside ROI 003 in Classified image 32,000000
- + New Variable + New Calculation + New Score

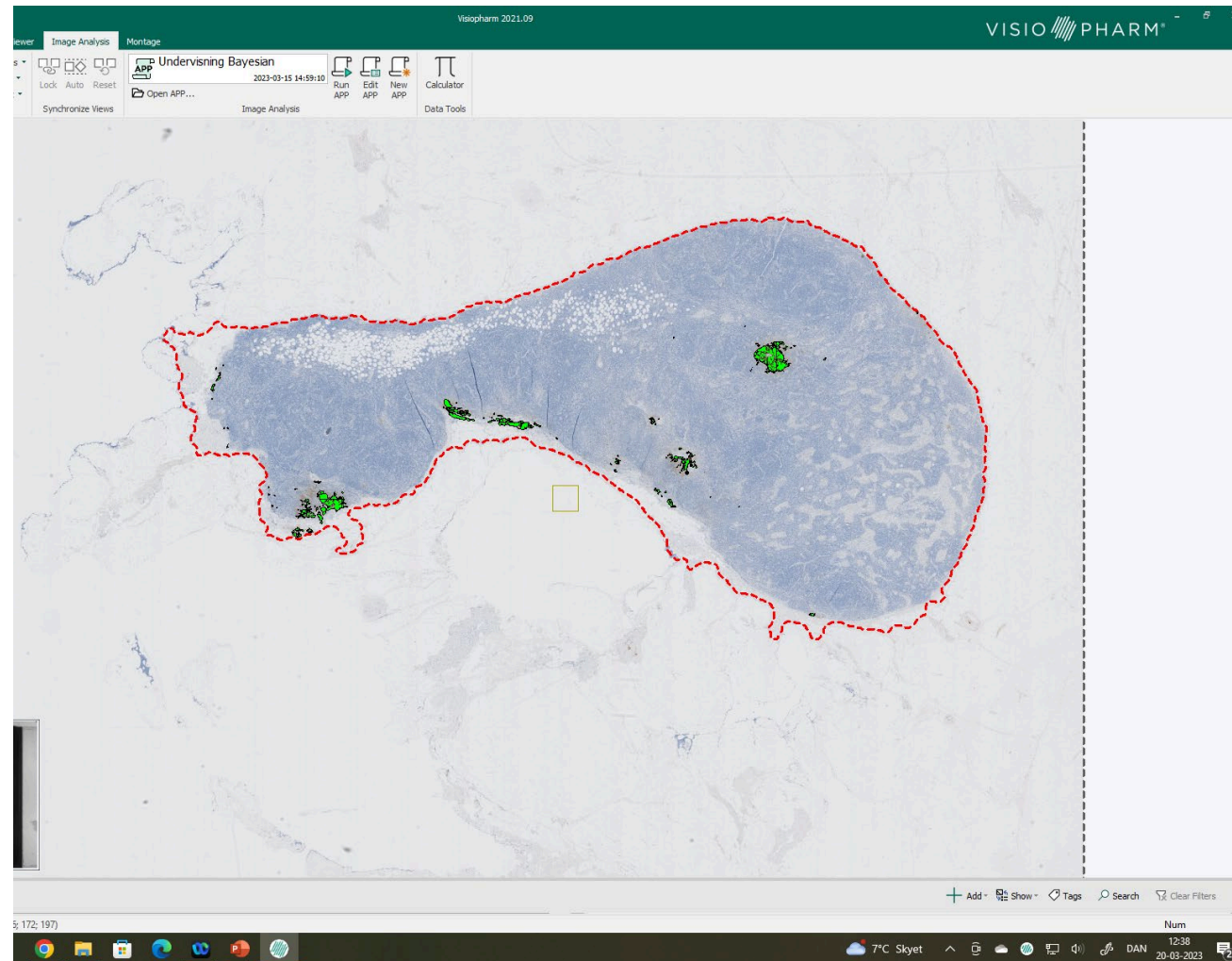
Preview Run APP

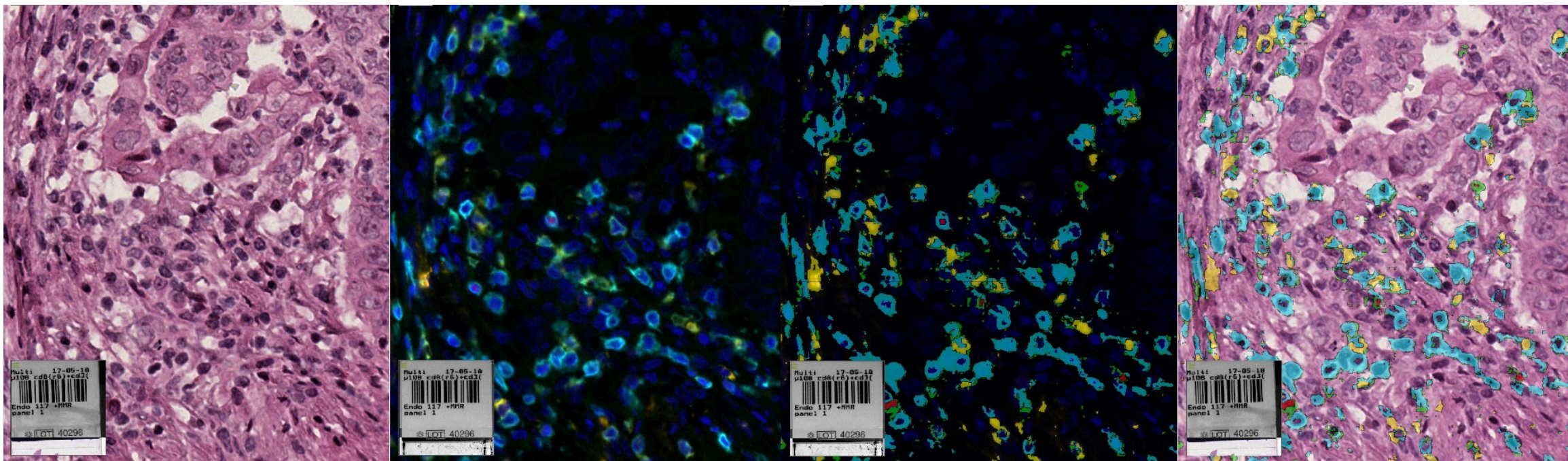


Mag: 20X Zoom: 100,00 % Pixel inside 'ROI 003' (23,261; 1,944) : (132; 160; 197)

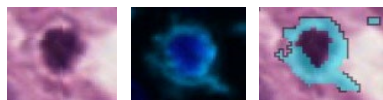
+ Add Show Tags Search Clear Filters

## Formål 2: Identificer evt. metastaser

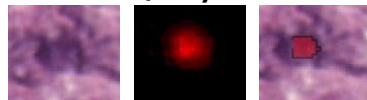




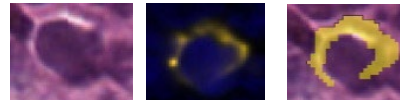
CD3/DCC



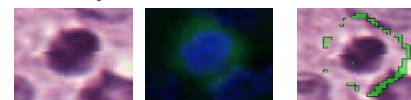
FOXP3/Cy5



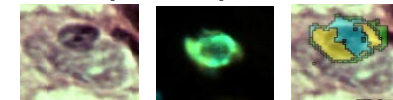
CD8/Rhodamin



PD1/FITC



CD3/CD8/PD1



# TAK