# Multi-Template Matching: a versatile tool for object-localization in microscopy images
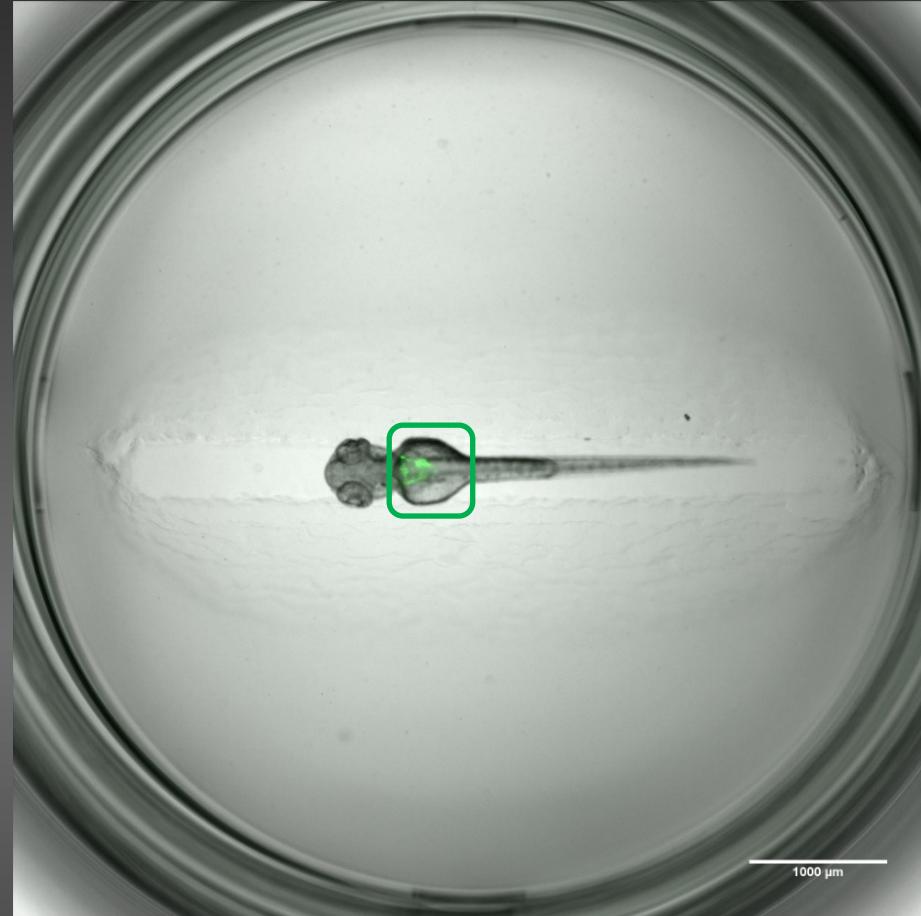

transgenic zebrafish (wt1b:egfp) in a well
2X - Overlay BF + GFP

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# Motivation : High resolution imaging of sample with variable positions

2x
20x
Well 1
Well 2
Well 3
Well 4
Well 5
1 mm

Slight position shift

Random positions
and
orientations
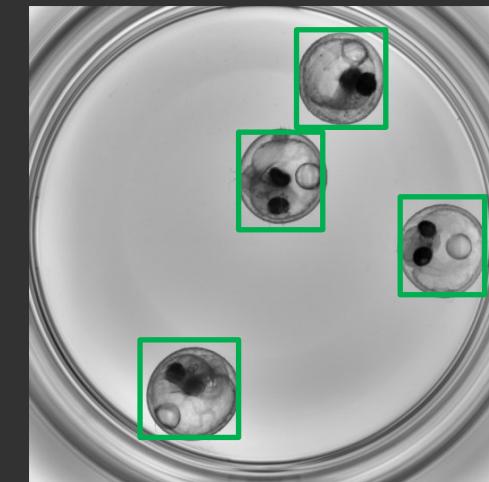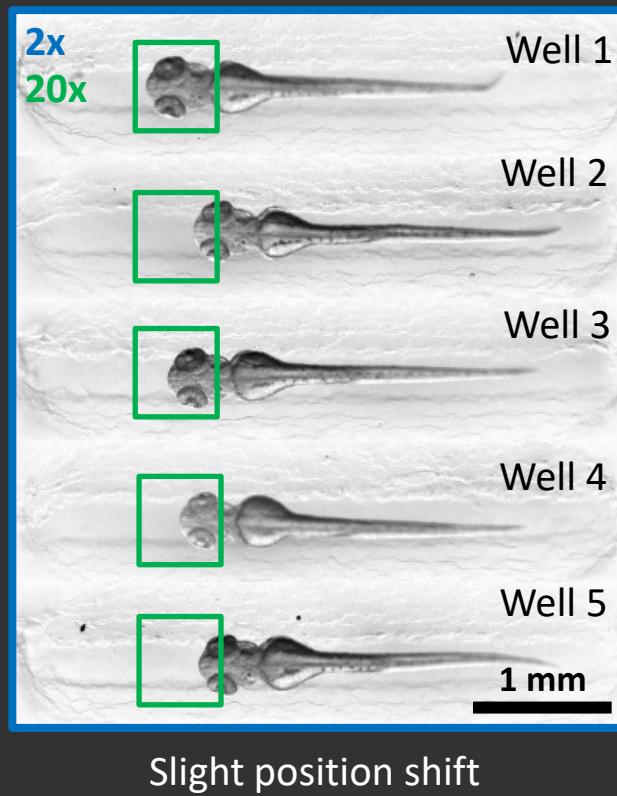
# Using feedback microscopy for guided acquisition

Semi-automated imaging pipeline

2X -Mag

Detection algorithm

Detect ROI
Ex : Kidney

X,Y coordinates

10X mag

Assisted analysis

Classification

Find and …
- Zoom
- Track
- Ablate
- …
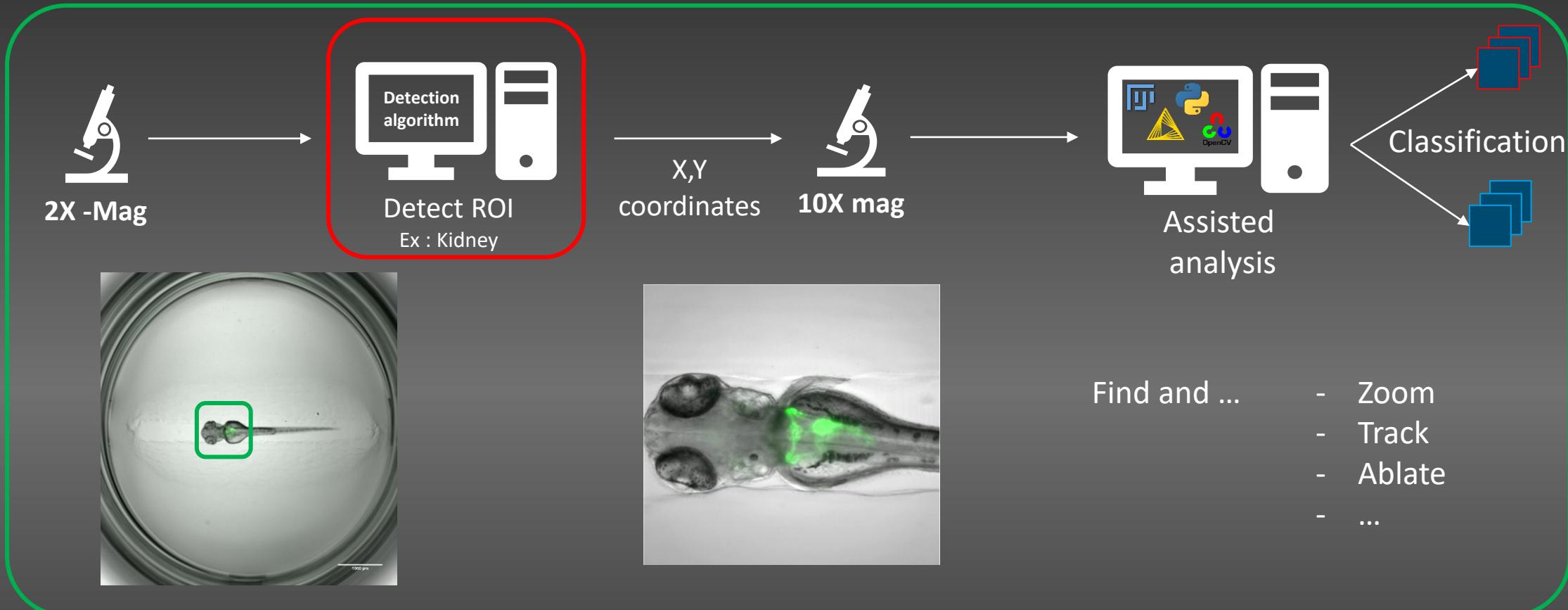
✓ **Time-saving**   ✓ **Reproducible**   ✓ **Easy to use**

ROI detection algorithm : Expectations

Well 1

Well 2

Well 3

Well 4

Well 5
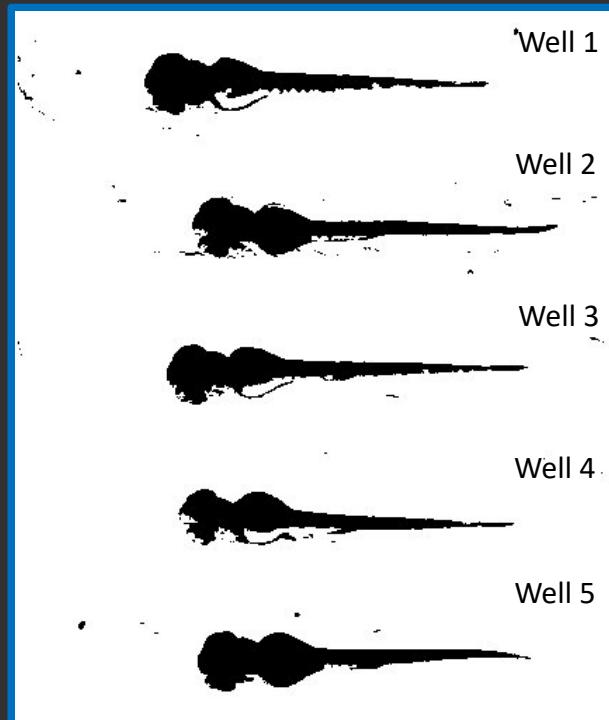
Requirements

- Generic (little tweaking)

- User-friendly (little parameters, simple to understand)

Challenges

- Diffuse fluorescent signal over specimen (not site-specific)

- Variable morphology and/or fluorescence intensity

Template Matching

Template
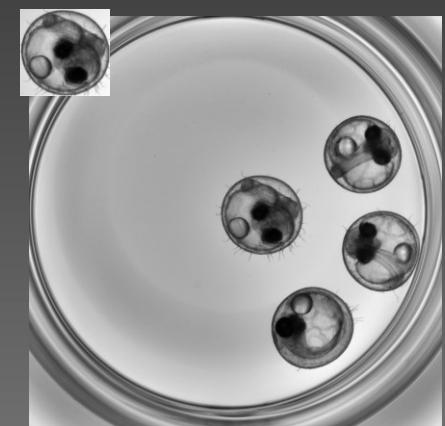
Target image
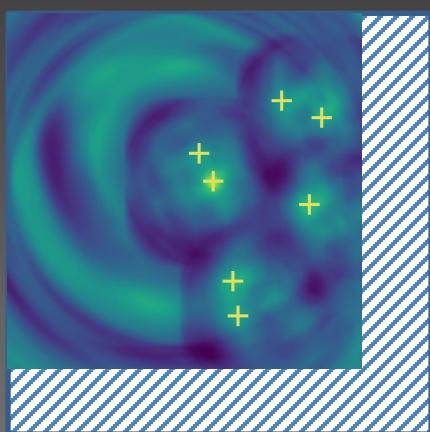
Correlation map

Local maxima detection
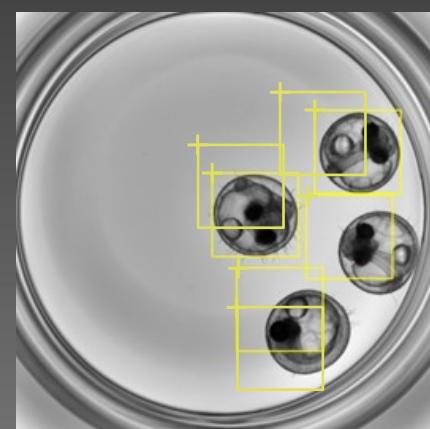
Overlap-based Non-Maxima Suppression
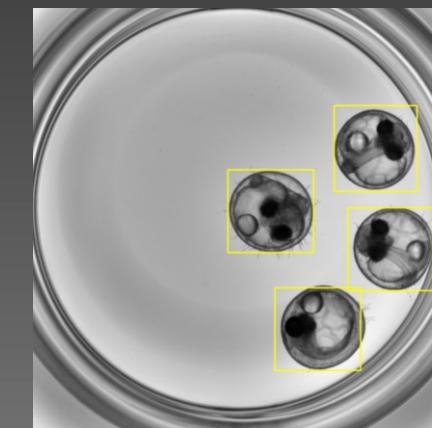
- Simple (pixel-intensity comparison)

BUT

- Limited flexibility (rotation…)

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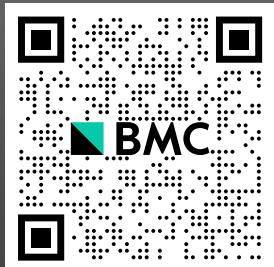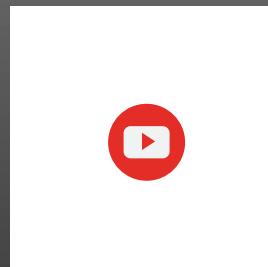
Multi-Template Matching

Multi-Template Matching results

Spheroid (single template flipped and rotated)
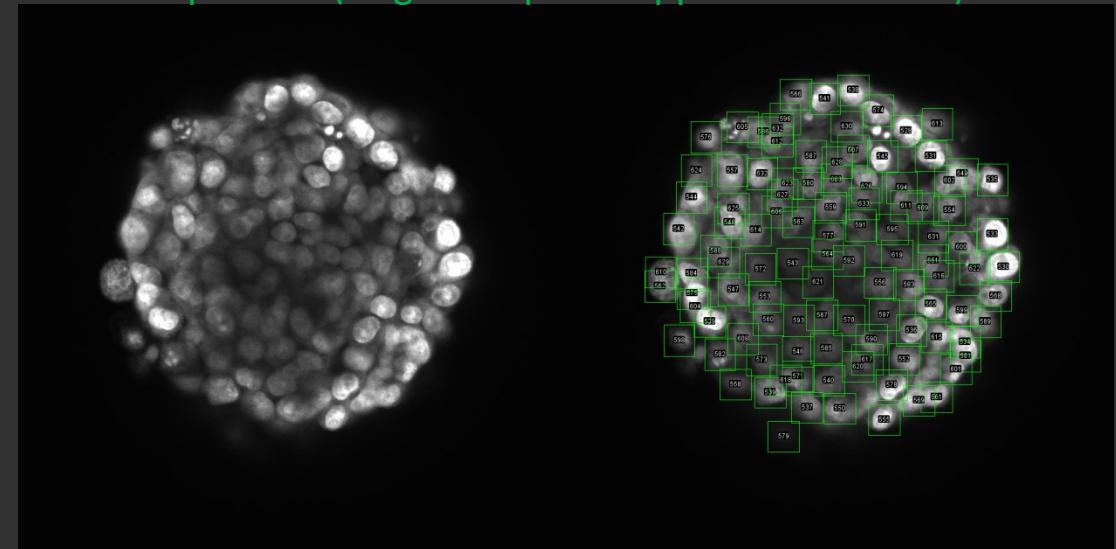
Image courtesy – Riviere C., Tricot V., Rousseau D., Rasti P., – Angers, France

Zebrafish head (single template)

N = 96

Image courtesy Gunjan Pandey

Medaka larvae (single template flipped and rotated)

N = 10 images x 4 eggs

Image courtesy Jakob Gierten

✓ Simple (normalised pixel-pixel comparison)

✓ Robust to change of illumination, to mild morphology changes

✓ Easy to install and (re)-use

✗ Limited tolerance to variability (more templates = longer computation)

2-step template matching for more robust detection

Example : Eye and lens segmentation in Zebrafish larvae

Edges (Canny) → Hough circle
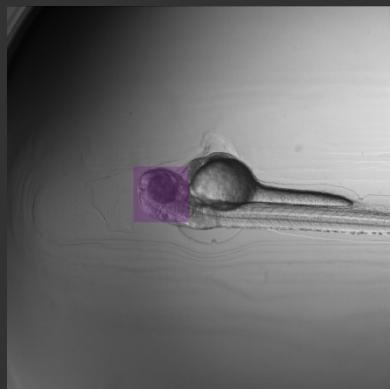
crop

crop

Head detection
Template matching

Eye detection
Template matching

Alexandre
Jeanne

Lens

Eye   Edges (Sobel) → Ellipse fitting
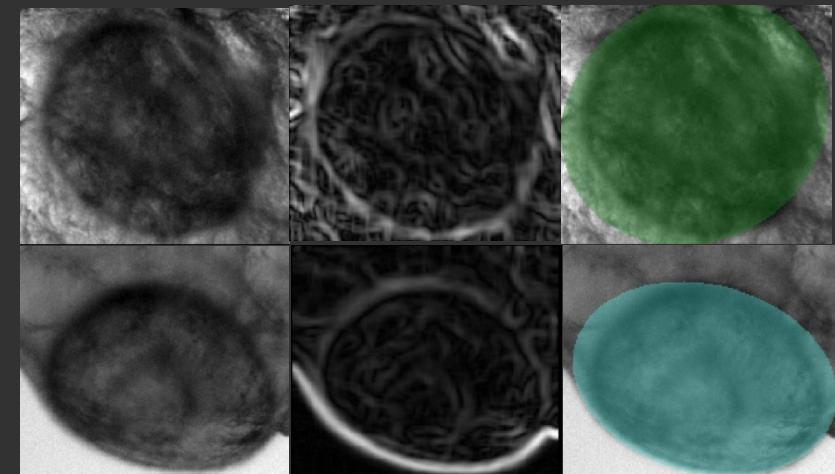
A bit of theory: the correlation map
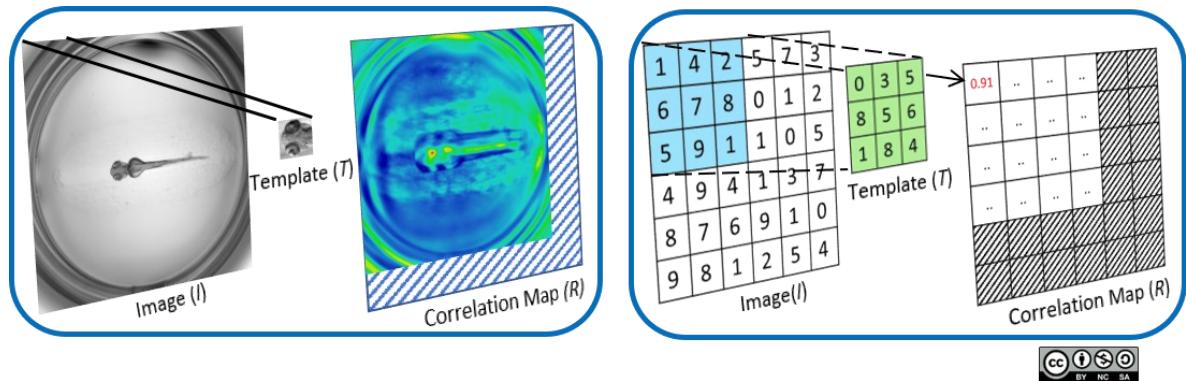
$$CorrMap(x, y) = \frac{Template * ImagePatch\ at\ (x, y)}{\sqrt{\sum_{x',y'} Template^2 * \sum_{x',y'} ImagePatch\ at\ (x,y)^2}}$$

$$CorrMap(x, y) = \frac{\sum_{x'y'} T(x', y') * I(x + x', y + y')}{\sqrt{\sum_{x',y'} T(x', y')^2 * \sum_{x',y'} I(x + x', y + y')^2}}$$

- 1 correlation map/template

$$R(0,0) = \frac{1*0 + 4*3 + 2*5 + 6*8 + 7*5 + 8*6 + 5*1 + 9*8 + 1*4}{\sqrt{(1^2 + 4^2 + 2^2 + 6^2 + 7^2 + 8^2 + 5^2 + 9^2 + 1^2) * (0^2 + 3^2 + 5^2 + 8^2 + 5^2 + 6^2 + 1^2 + 8^2 + 4^2)}} = 0.91$$

A bit of theory: maxima detection

2.a)

2.b)

2.c)
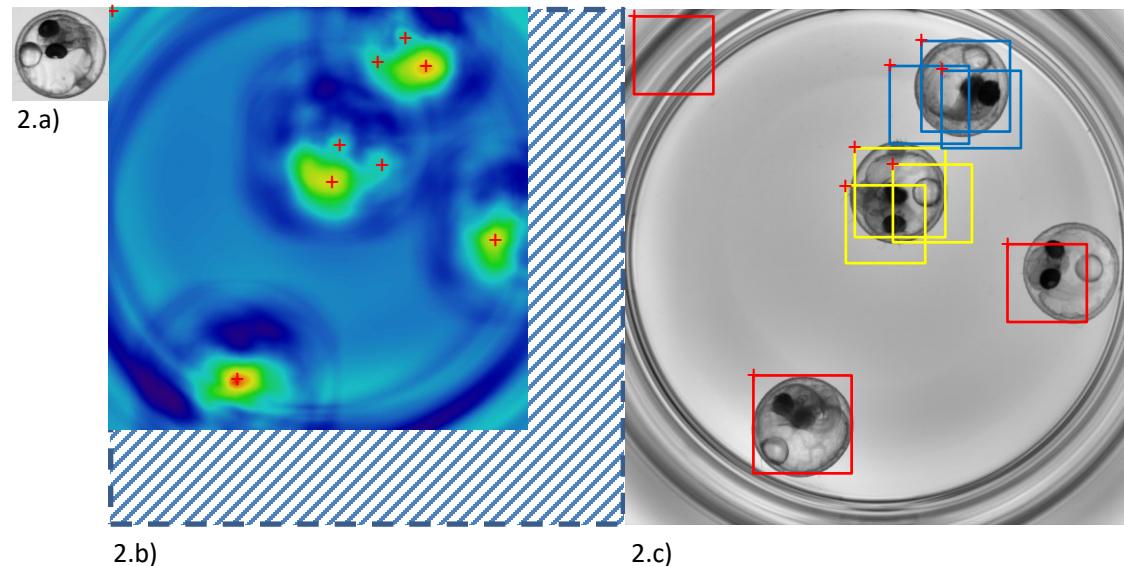
Probable object-location
-> maxima of the correlation map

- Problem -> Multiple hits per object

- Apply a score-threshold, still redundant detections

Non-Maxima Suppression (NMS)
Removing overlapping detection with NMS
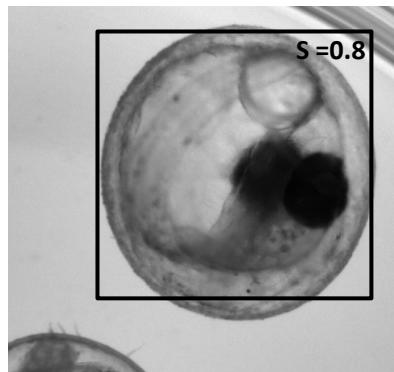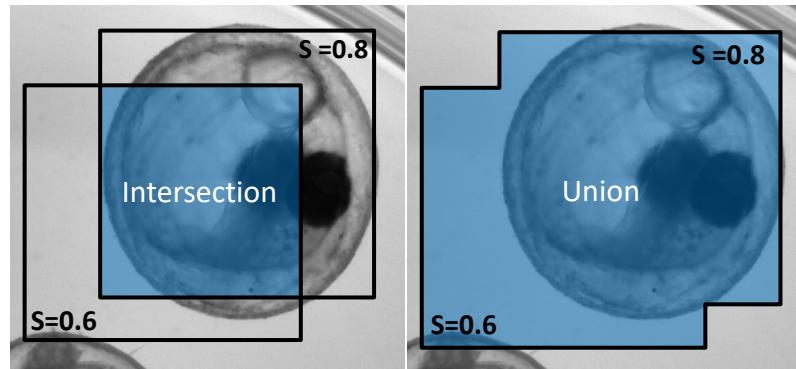
$$Normalized\ overlap = \frac{Intersection}{Union}$$

$Overlap > threshold$

- Redundant detections
- Discard the lower-score detection

$Overlap < threshold$

- Distinct detections
- Keep both detections

Multi-Template matching

As a Fiji plugin
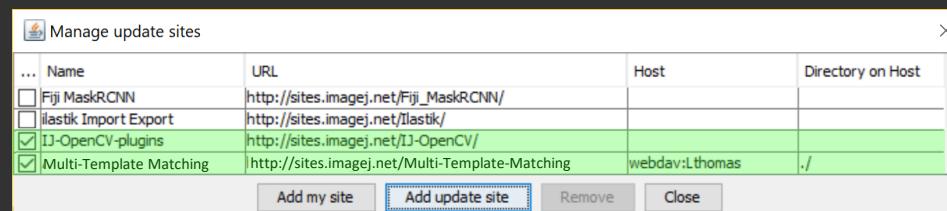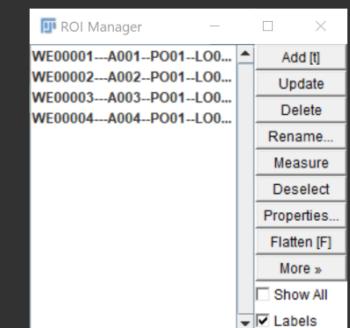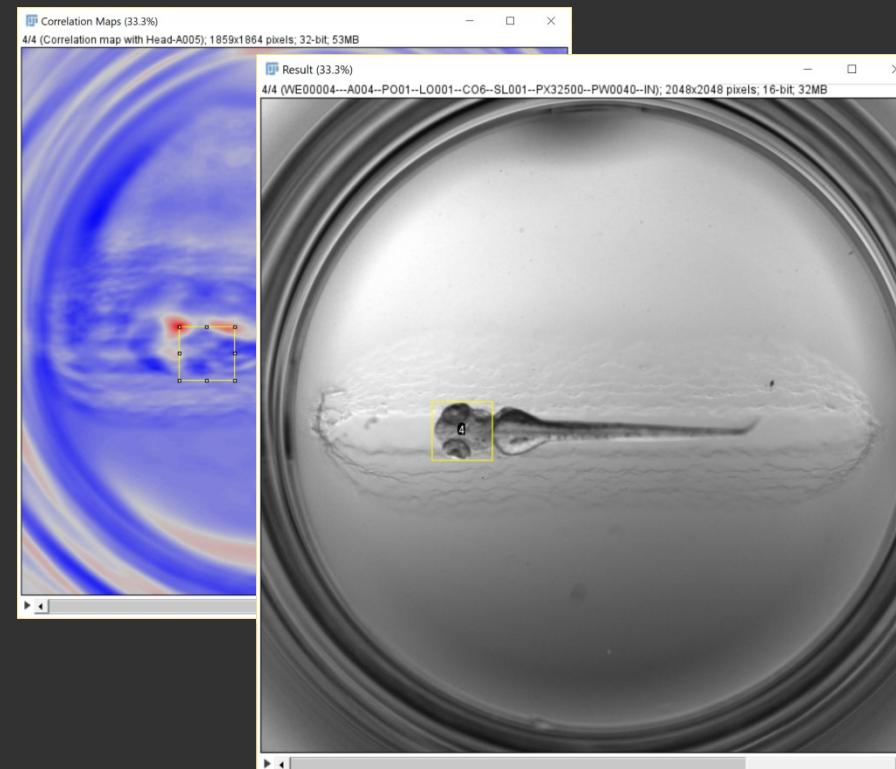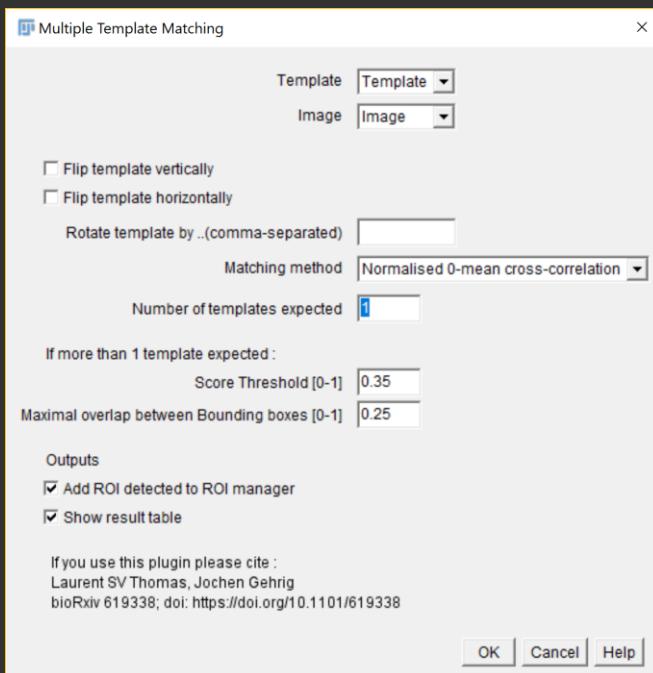
- Macro recordable
- Available via an update site

Domínguez, César, Jónathan Heras, and Vico Pascual. "Ij-opencv: Combining imagej and opencv for processing images in biomedicine." *Computers in biology and medicine* 84 (2017): 189-194.

Faster detection

1) Down resolution

Downscale Template
and Image
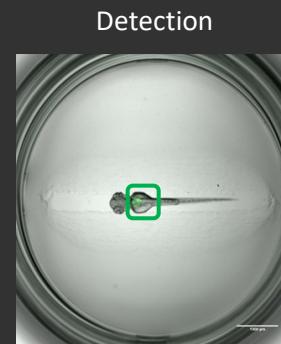
Detection

Rescale bounding boxes
to original image size
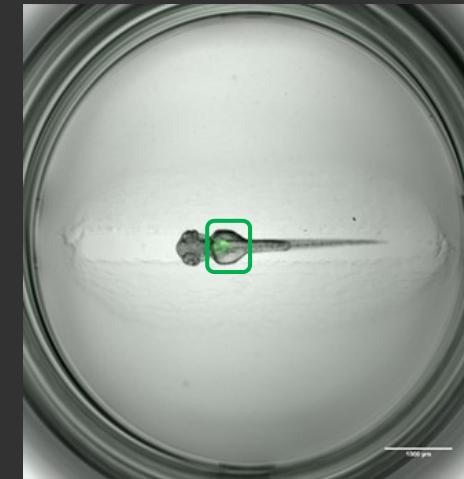
Image-classification with template matching
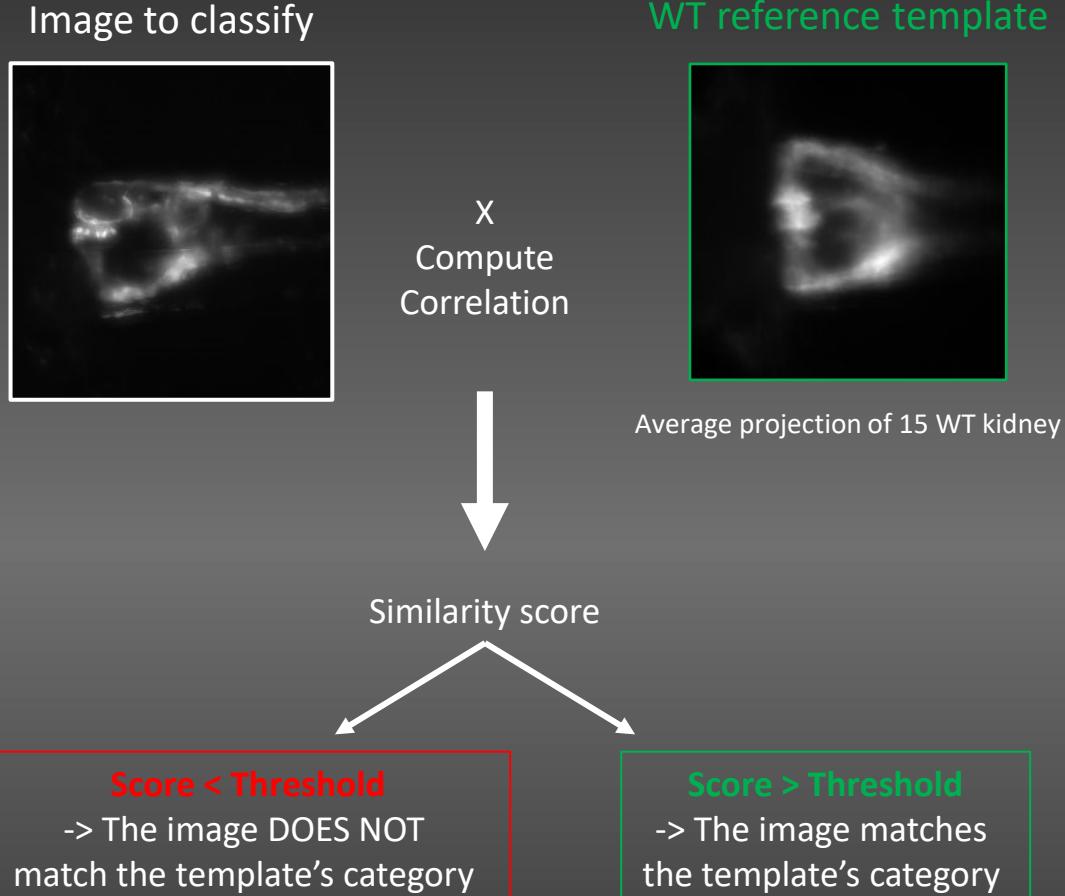
Score distribution for a fraction of the annotated dataset ("Training set": 310 images)

Image to classify

WT reference template

X
Compute
Correlation

Average projection of 15 WT kidney

Similarity score

Score < Threshold
-> The image DOES NOT match the template's category

Score > Threshold
-> The image matches the template's category

Correlation with WT template

- 0.93
- 0.87
- 0.84
- 0.8
- 0.72
- 0.7
- 0.63
- 0.56
- 0.43
- 0.25

Impaired   Normal   Class
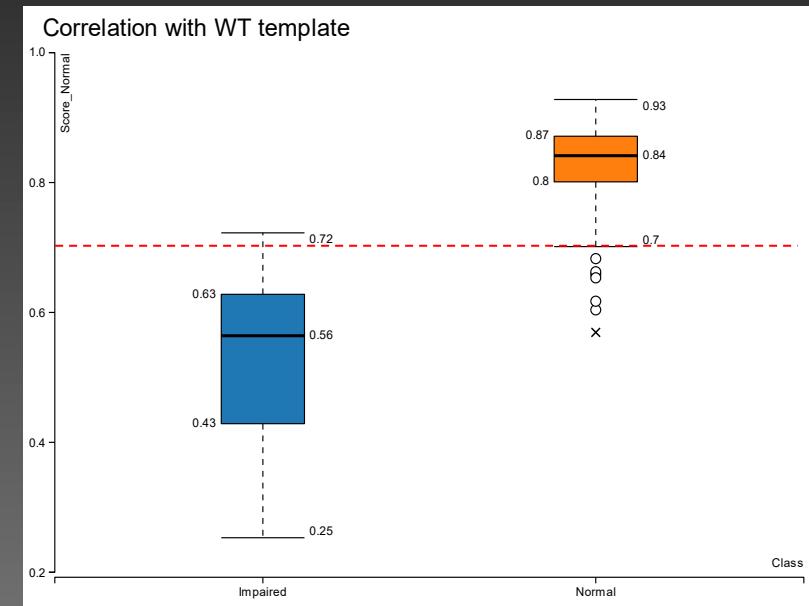
Using 0.7 as a threshold between the normal and impaired category ("Test set": 930 other images)

Class \ Predicted_Class	Normal	Impaired
Normal	603	23
Impaired	16	288

Correct classified: 891   Wrong classified: 39

Accuracy: 95.806 %   Error: 4.194 %

Cohen's kappa (κ) 0.905