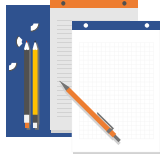




Object counting with Deep Learning

Luca Clissa
INFN Bologna

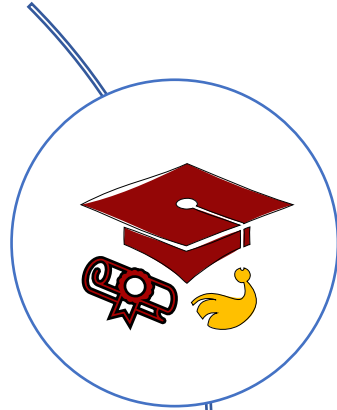
clissa@bo.infn.it



About me



Luca
Clissa



PhD in Data Science & Computation

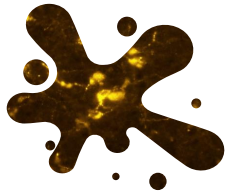


Collaborazioni





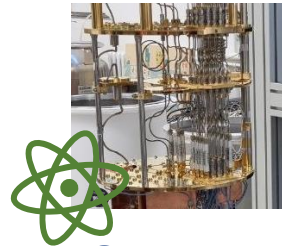
Aree di ricerca



Computer Vision
[[1](#), [2](#), [3](#)]



Text Processing
[[3](#), [4](#), [5](#), [6](#)]



Quantum
Computing
[[7](#), [8](#), [9](#)]



Time Series,
Survival Analysis

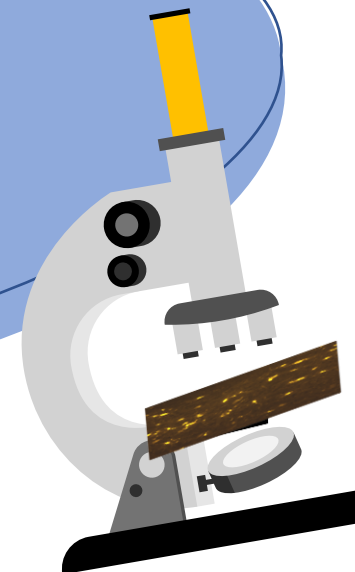


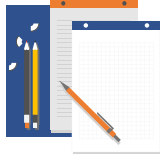
Outline

- Microscopia in fluorescenza
- Fluorescent Neuronal Cells dataset
- Cell ResUnet (c-ResUnet)
- Ispezione risultati

- Possibili sviluppi

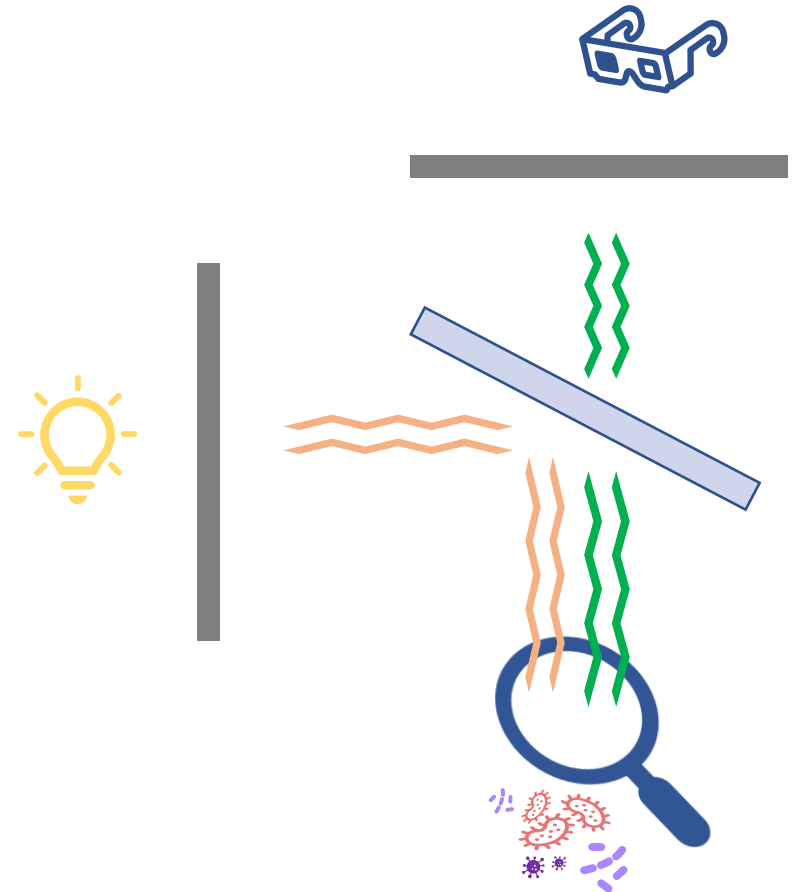
Microscopia in Fluorescenza

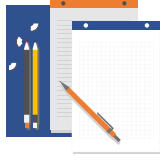




Cos'è?

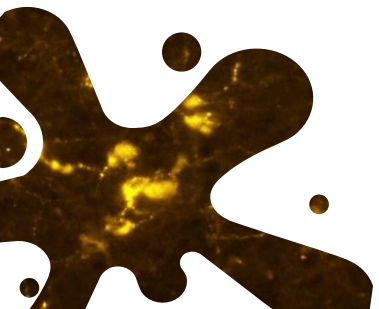
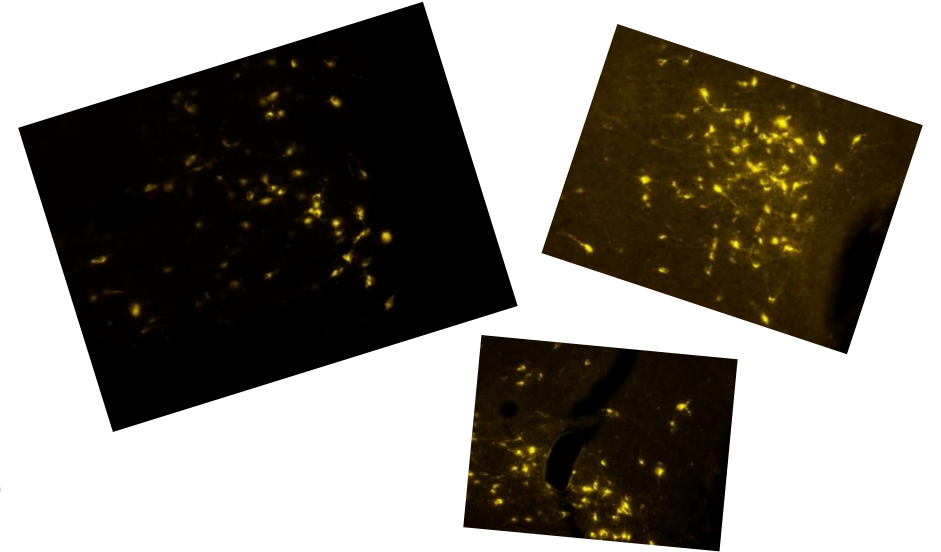
- Tecnica di imaging
- Assorbimento/emissione luce
- Spesso utilizzata in life science

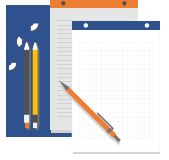




Applicazioni

- Studio meccanismi responsabili del torpore nei roditori [[10](#)]
 - Importanti ricadute umane
- Neuroni marcati con fluoroforo giallo
- Varie tonalità, forme e grandezze
- Obiettivo: conteggio neuroni marcati

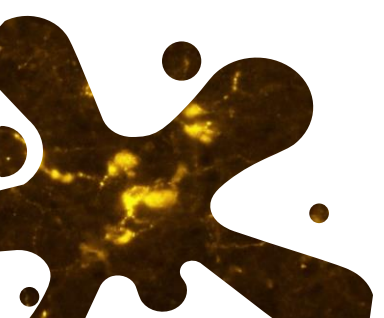


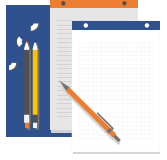


Problema



- Manuale
 - Time-consuming
 - Error-prone
- Interpretazione soggettiva casi limite





Obiettivo

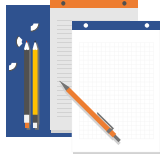


Automazione



Interpretabilità





Contributo

- Approccio Deep Learning per riconoscimento e conteggio
 - cell-ResUnet (c-ResUnet)
 - test against SOTA
- Ablation studies
 - Weight Map (WM)
 - Artifacts Oversampling (AO)
- Fluorescent Neuronal Cells dataset

[[1](#)] Morelli, R. et al. 2021

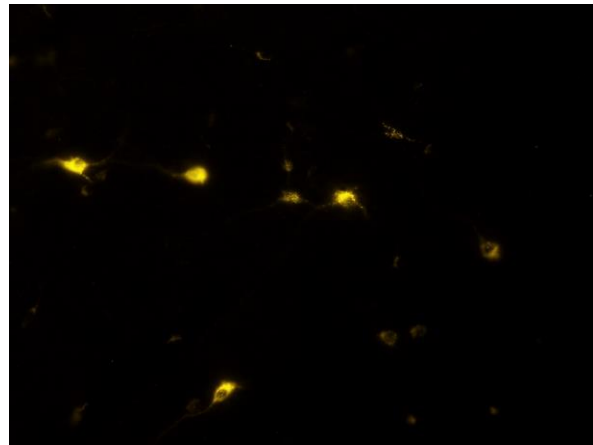
[[2](#)] Clissa, L. et al. 2021

[[3](#)] Clissa, L. et al. 2022



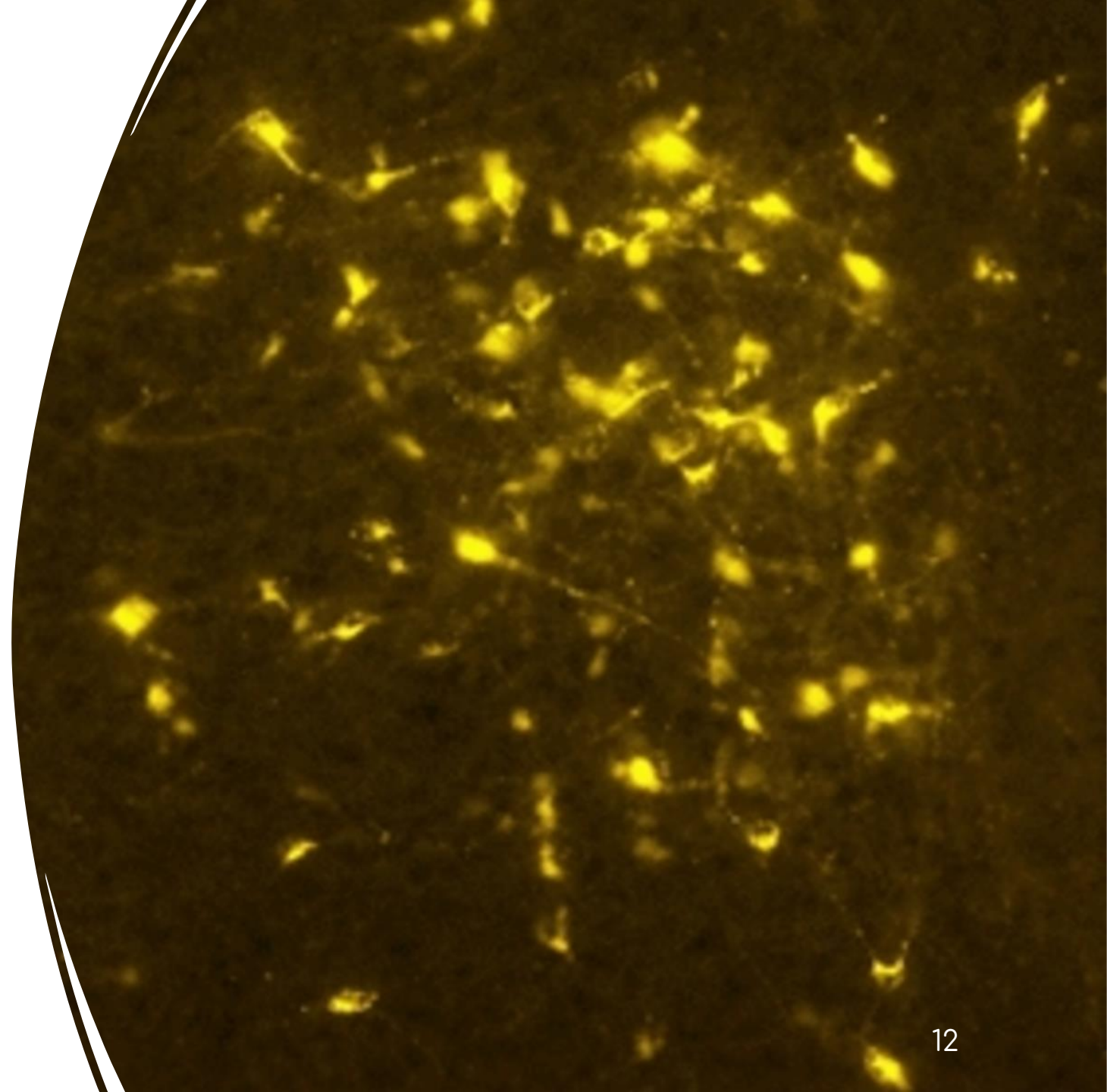
Strategia

- Counting by segmentation
 - Semantic segmentation
 - Convolutional neural network

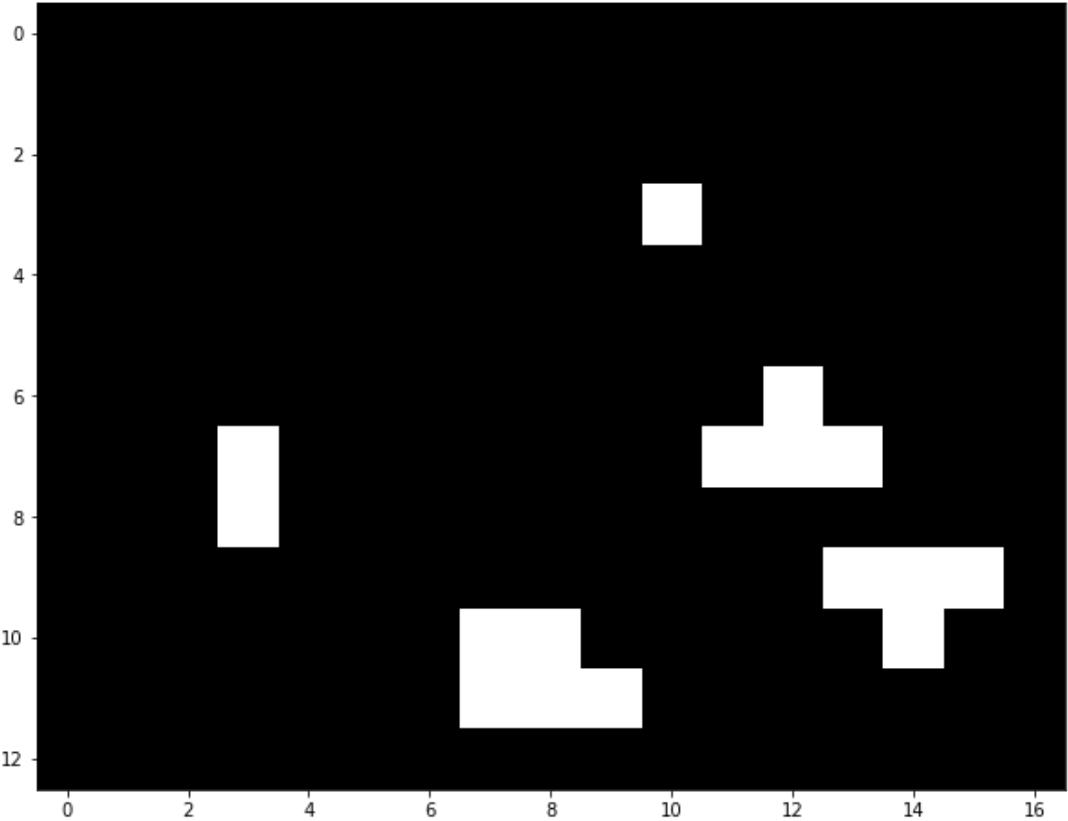
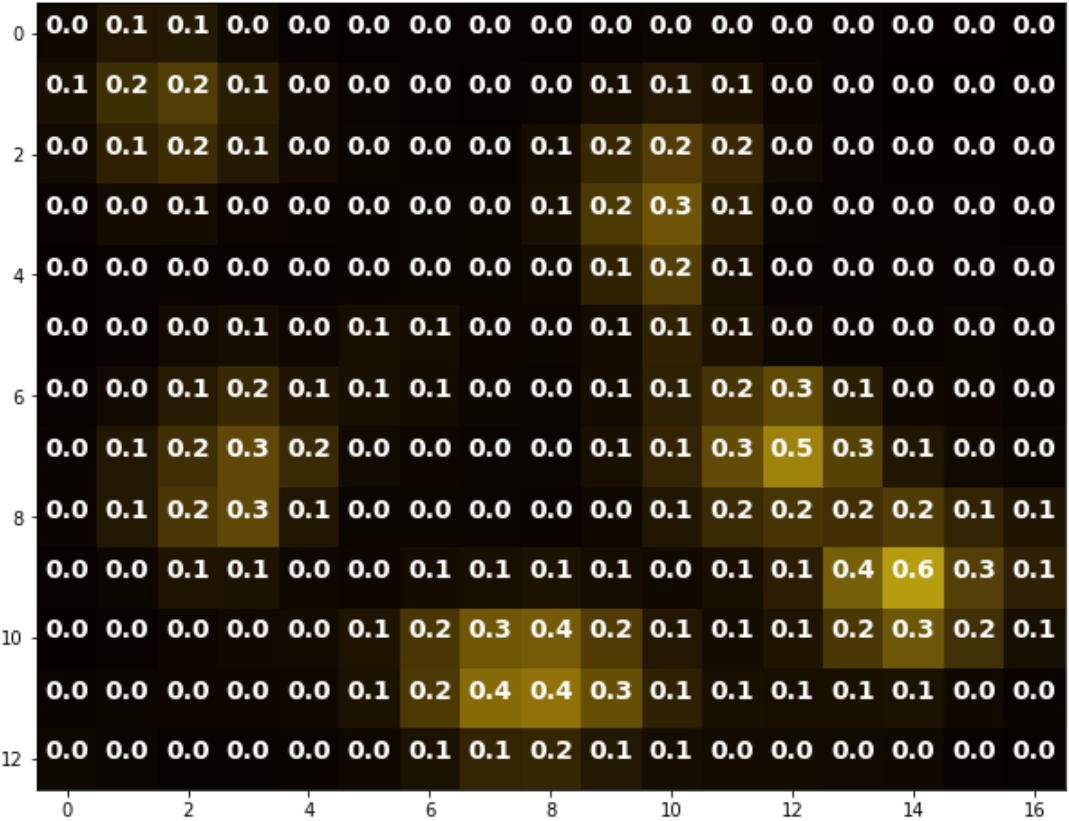


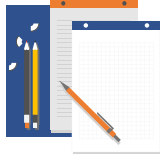
Fluorescent Neuronal Cells dataset

- 283 immagini ad alta risoluzione (1200x1600 px)
- Ottenere annotazioni è molto dispendioso
- Approccio semi-automatico
 - 252 bozze basate su filtro luminosità, rifinite poi da esperti
 - 31 immagini segmentate manualmente



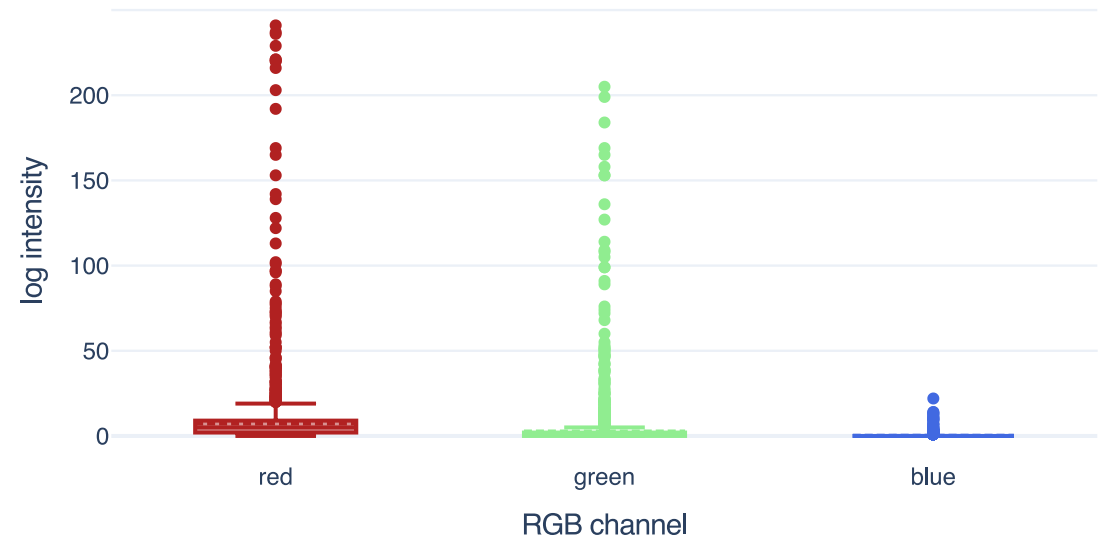
Filtro semi-automatico

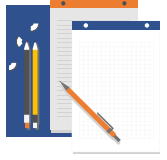




Canali RGB

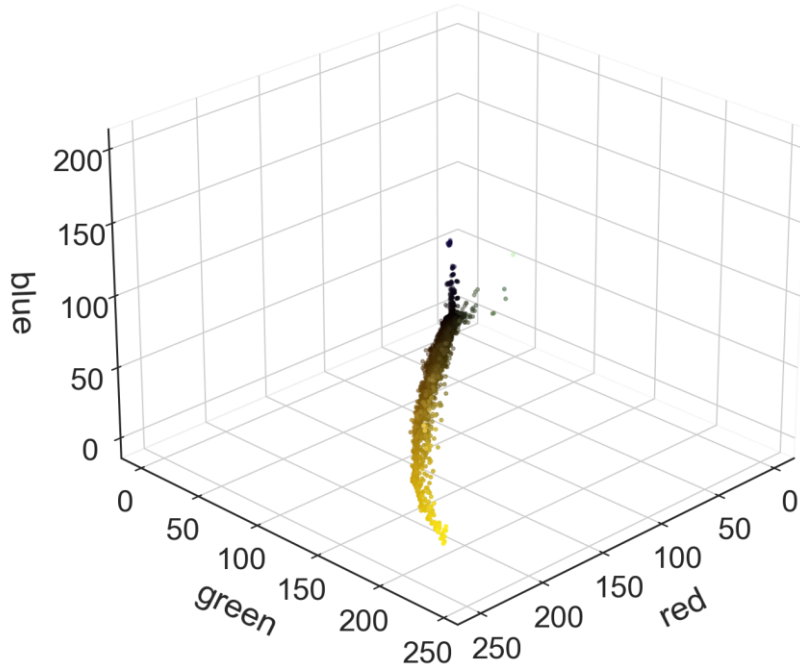
quantile	red	green	blue
mean	7.32	2.83	0.20
s.d.	16.81	13.30	1.43
min	0	0	0
10%	0	0	0
25%	2	0	0
50%	5	2	0
75%	9	3	0
90%	12	4	0
max	252	251	87



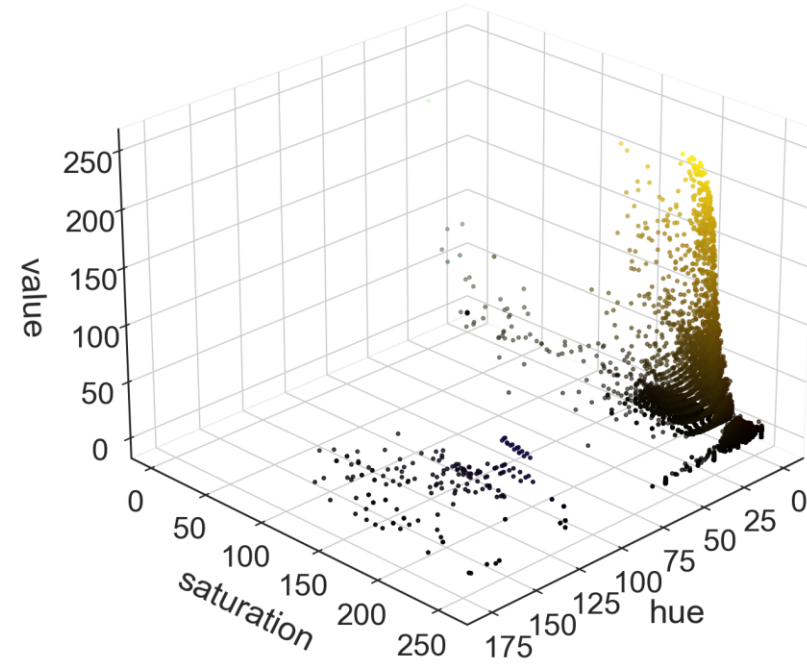


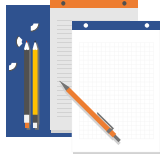
Encoding colore

RGB



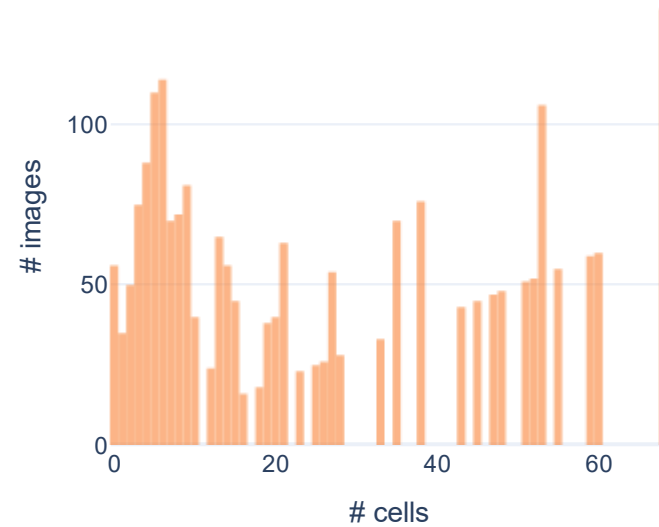
HSV

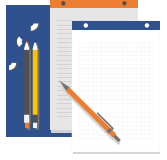




Distribuzione conteggi

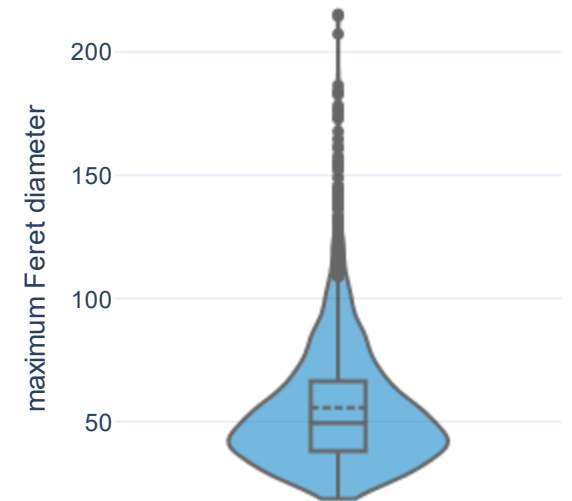
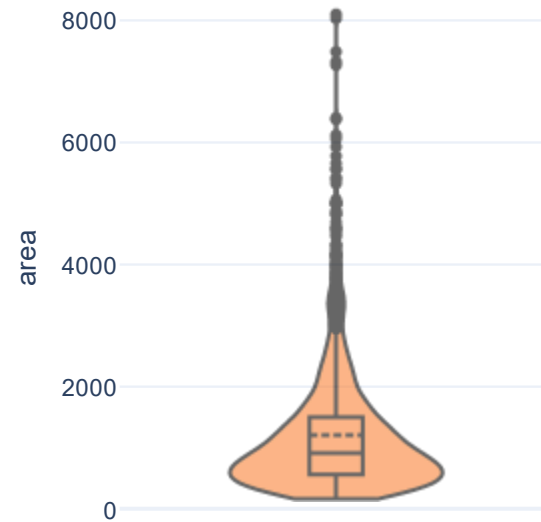
quantile	# cells/image
mean	27.05
s.d.	21.75
min	0
10%	4
25%	7
50%	21
75%	48
90%	59
max	68

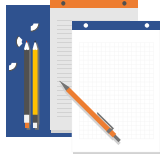




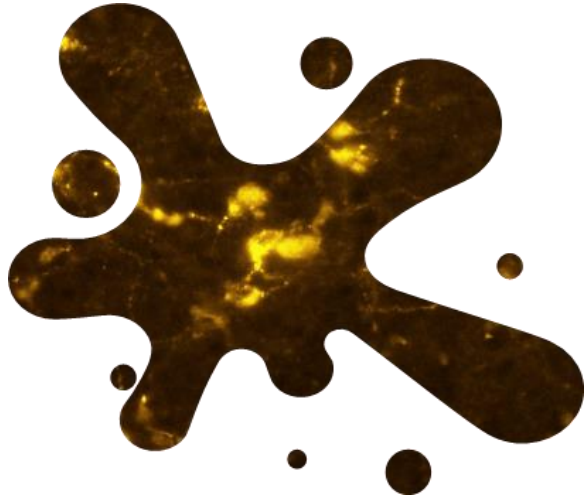
Area e Feret diameter

quantile	area (μm^2)	Feret Diameter (μm)
mean	119.30	17.48
s.d.	97.96	8.20
min	15.94	5.86
10%	35.23	9.42
25%	55.51	11.95
50%	89.86	15.53
75%	148.02	20.86
90%	237.09	27.61
max	796.39	67.48

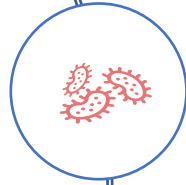




Sfide



Sbilanciamento classi



Overcrowding



Rumore etichette

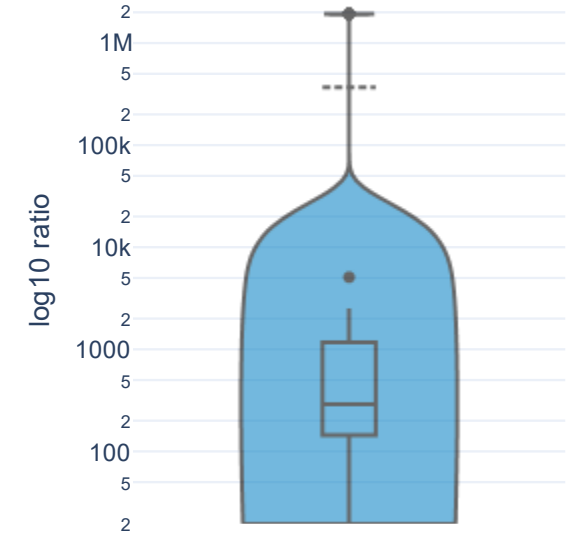
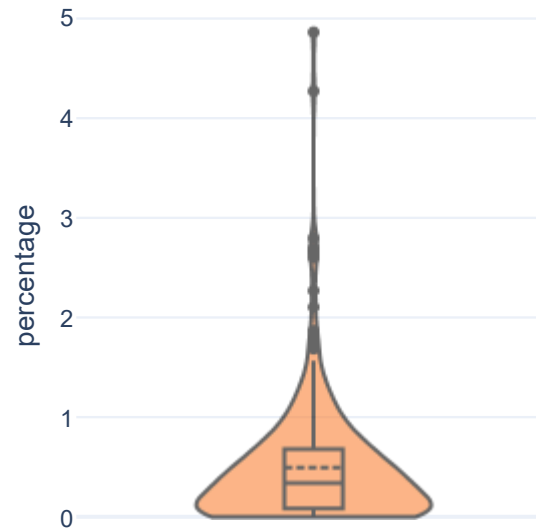


Artefatti



Class imbalance

quantile	signal (%)	signal ratio
mean	0.50	367k
s.d.	0.61	756k
min	0	19.57
10%	0	92.39
25%	0.09	145.35
50%	0.34	291.10
75%	0.68	1k
90%	1.07	1.9M
max	4.86	1.9M



10 μ m

cells agglomerate

cells agglomerate

non-marked cell
type: shaded

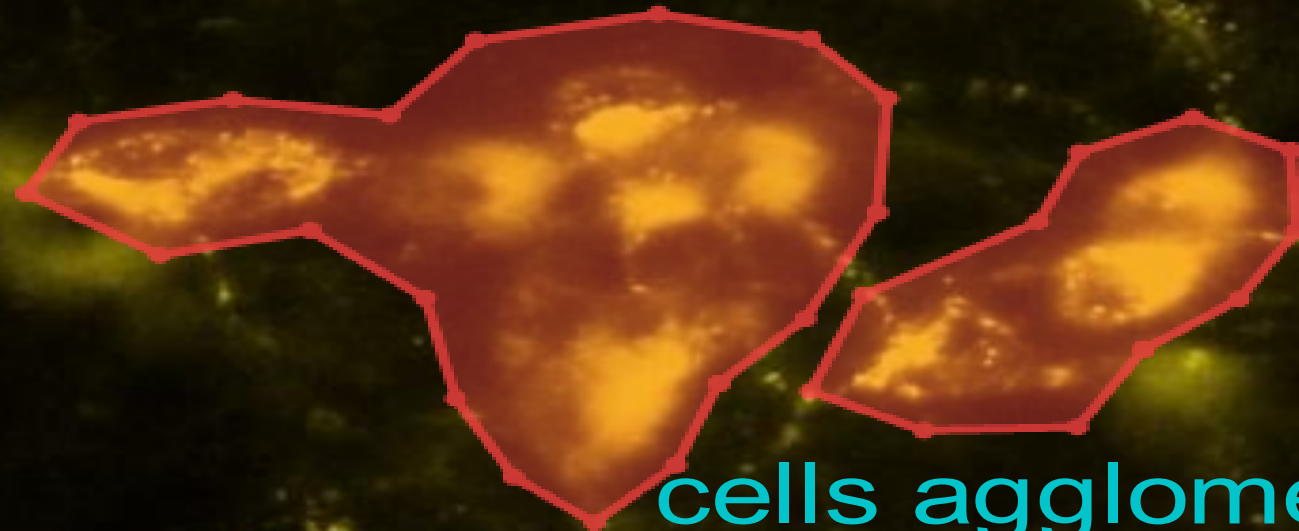
marked cell
type: dotted

cells agglomerate
cells agglomerate

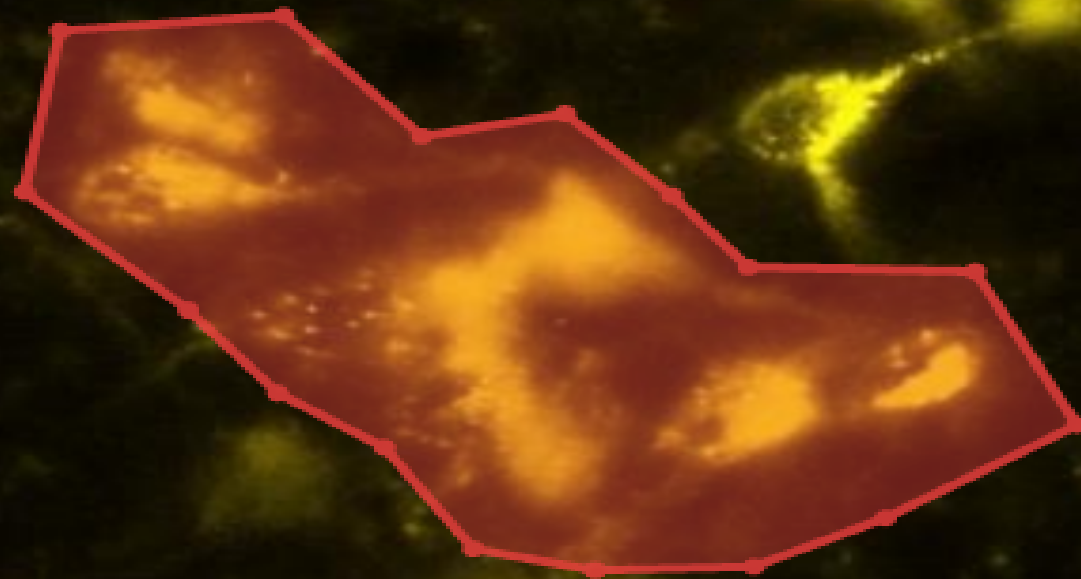
cells agglomerate

non-marked cell
type: dotted

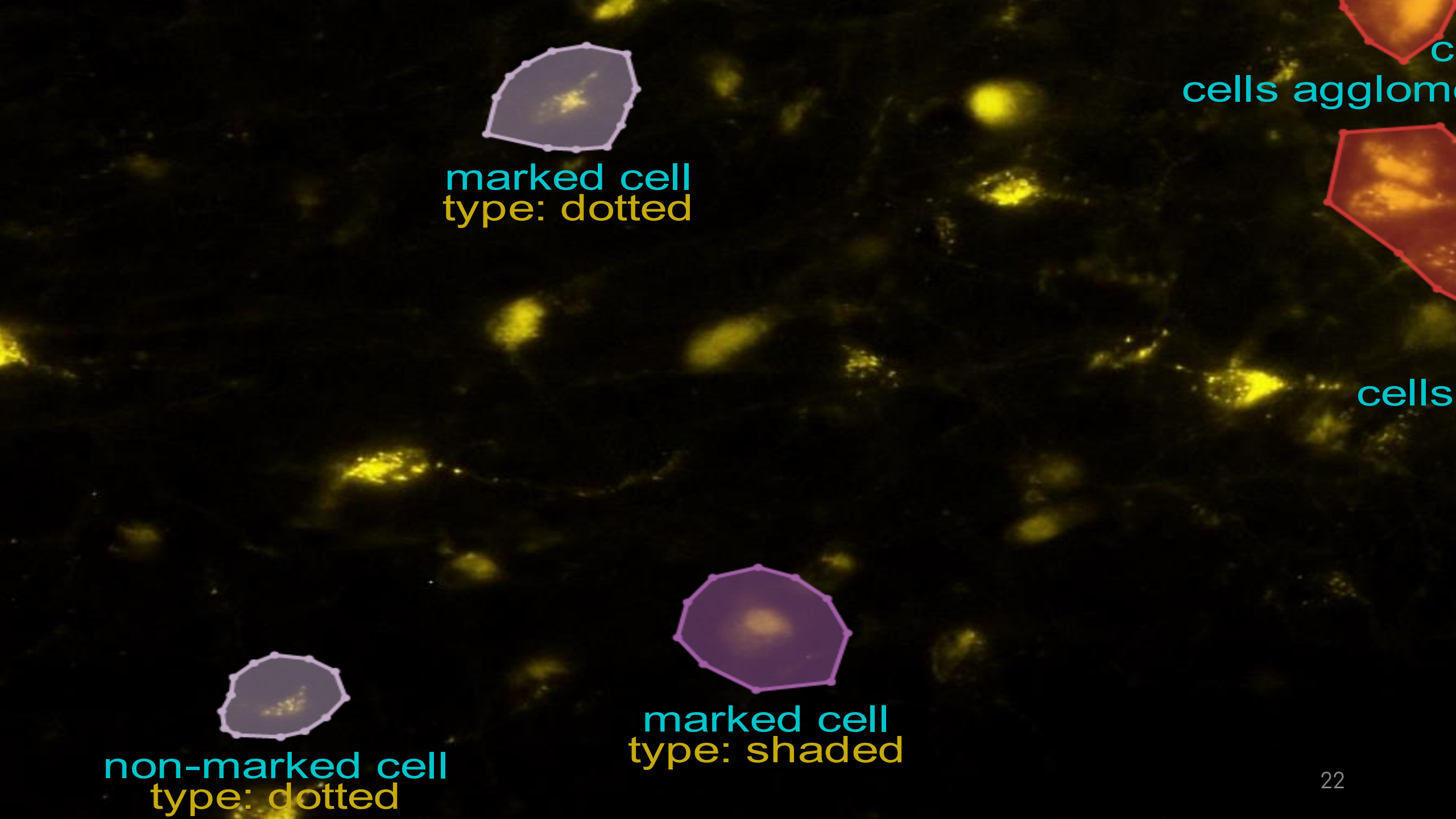
marked cell
type: shaded



cells agglomerate
cells agglomerate



cells agglomerate



marked cell
type: dotted

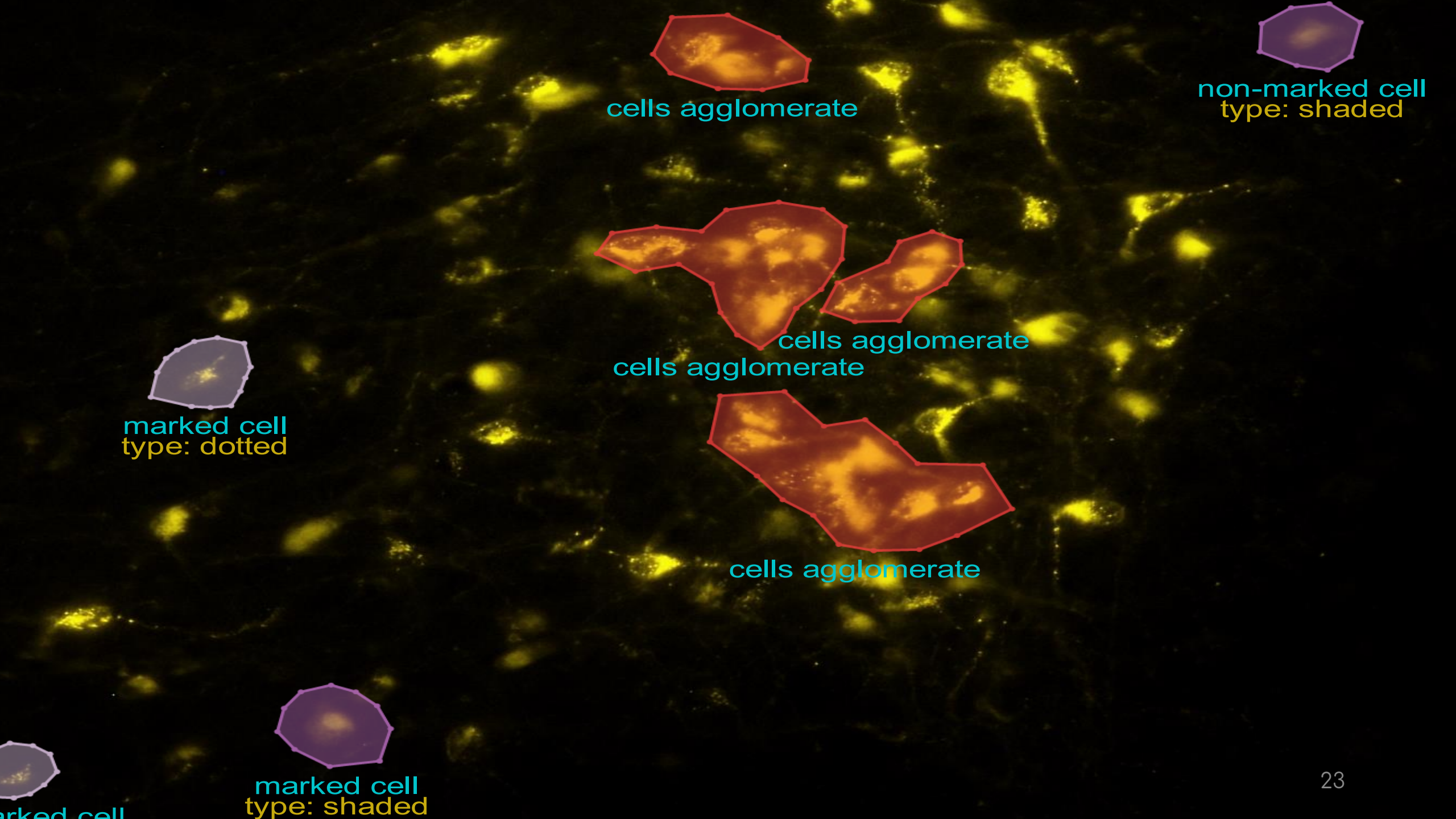
cells agglom



cells

non-marked cell
type: dotted

marked cell
type: shaded



cells agglomerate

non-marked cell
type: shaded

cells agglomerate
cells agglomerate

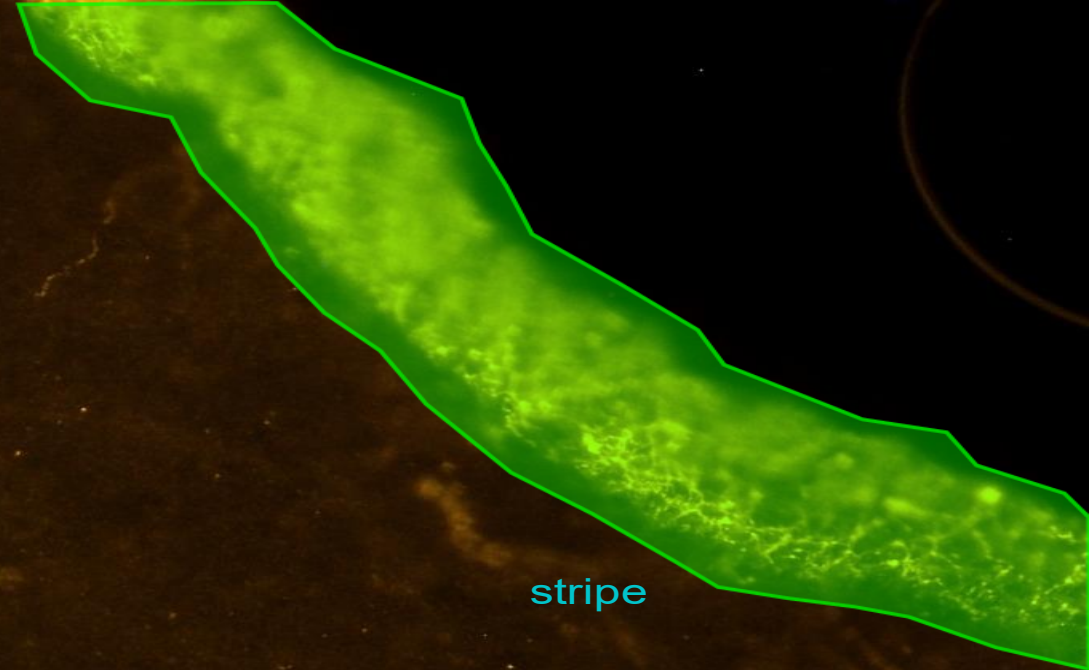
marked cell
type: dotted

cells agglomerate

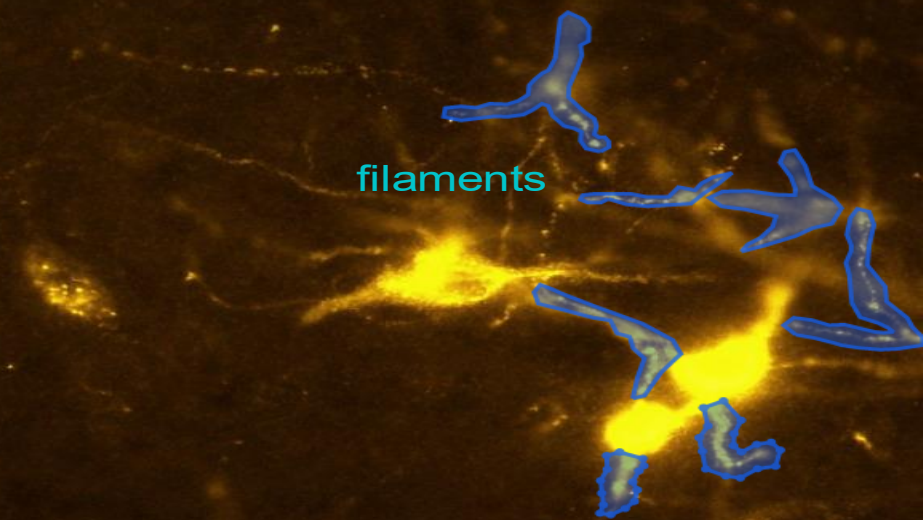
marked cell
type: shaded

marked cell

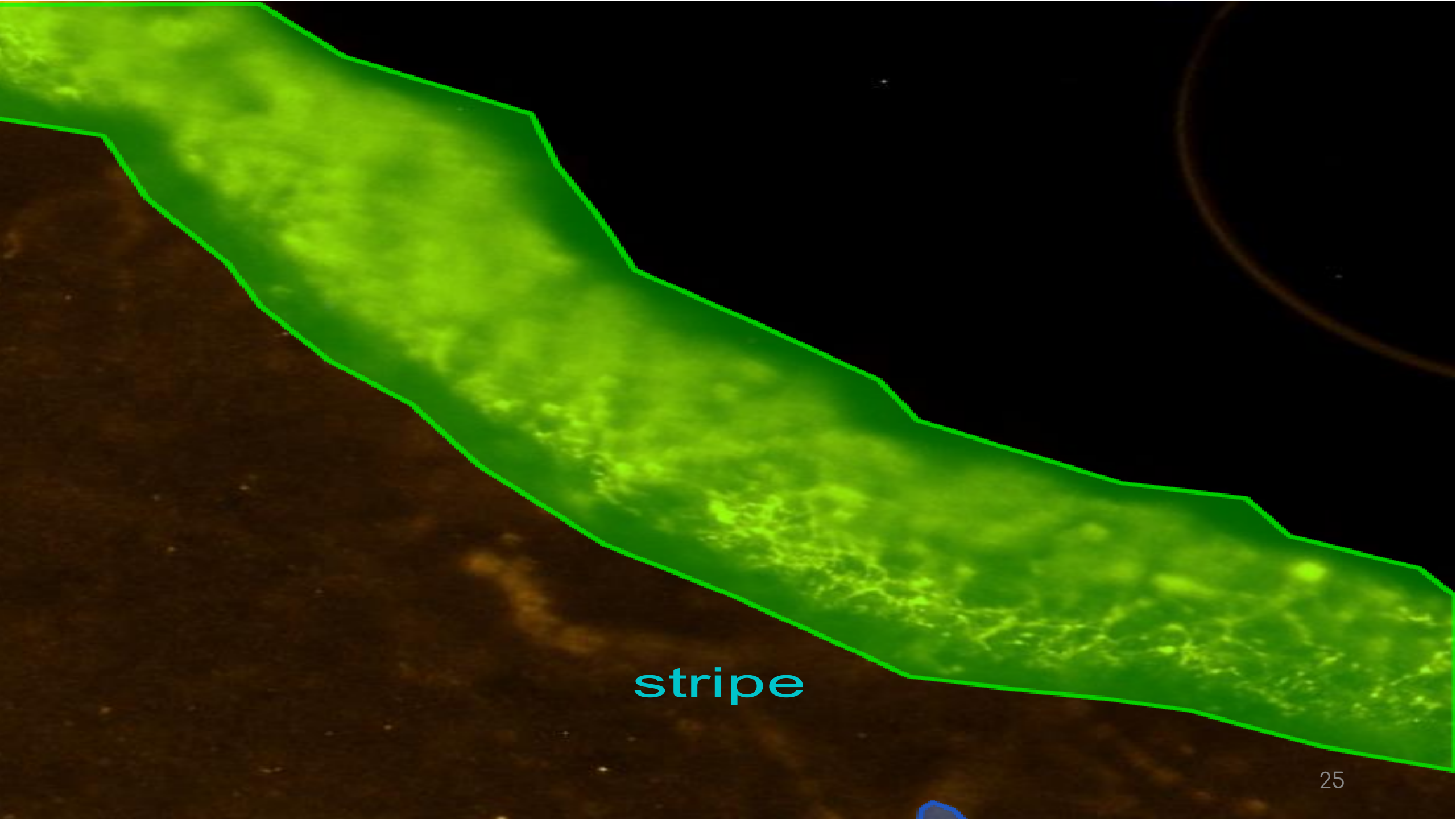
10 μm



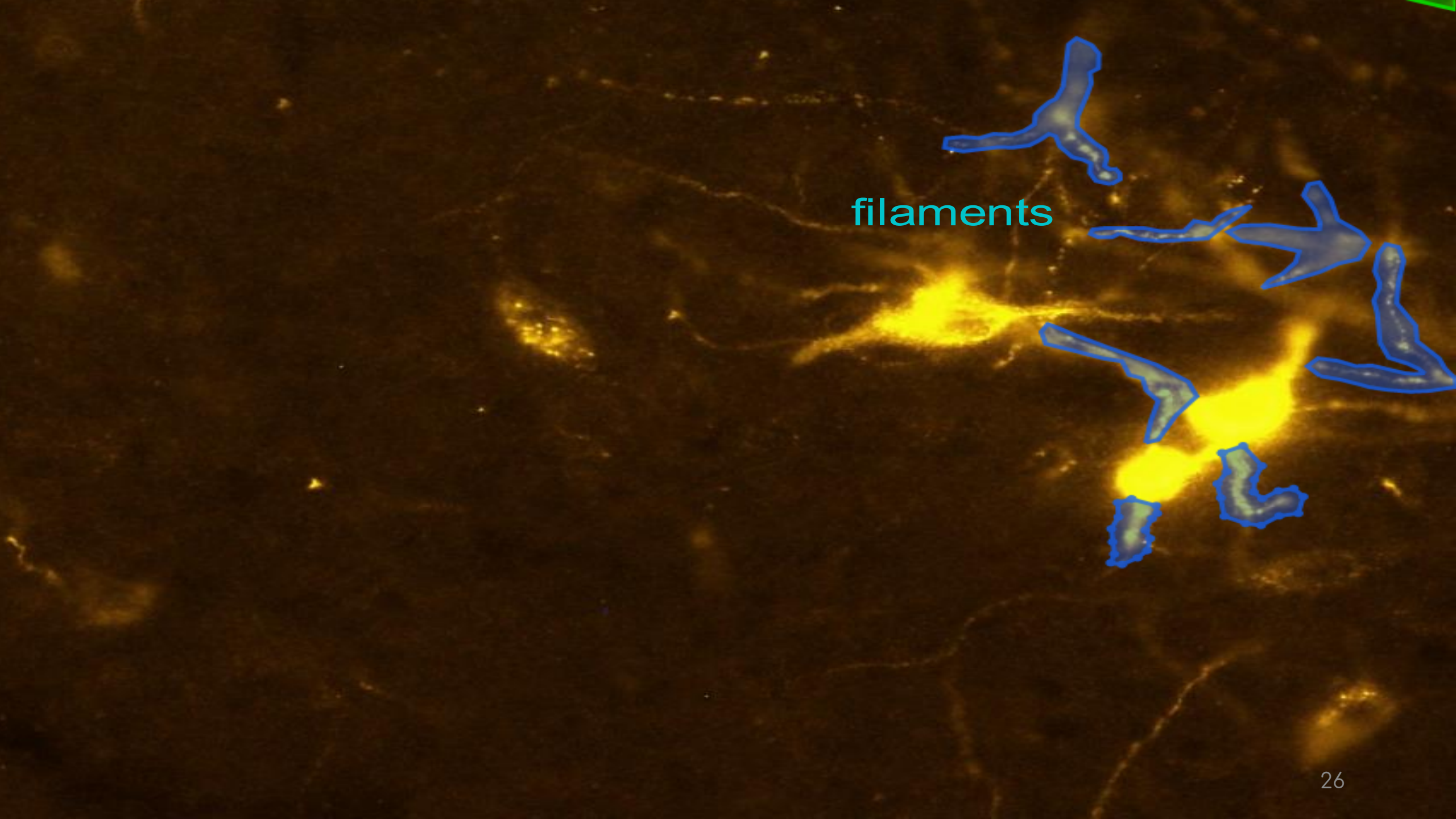
stripe



filaments



stripe



filaments

10 μ m

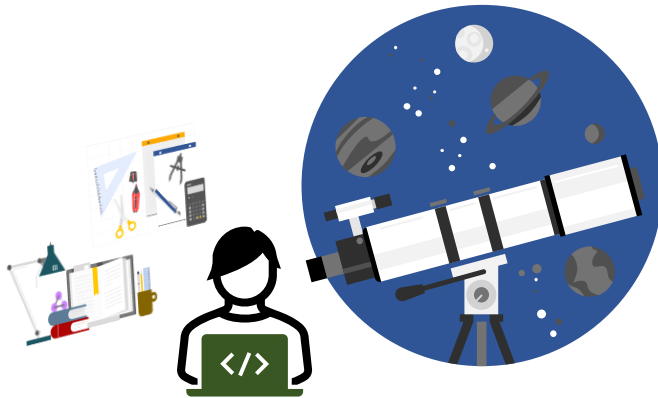
artifact
size: small

artifact
size: small

artifact
size: big



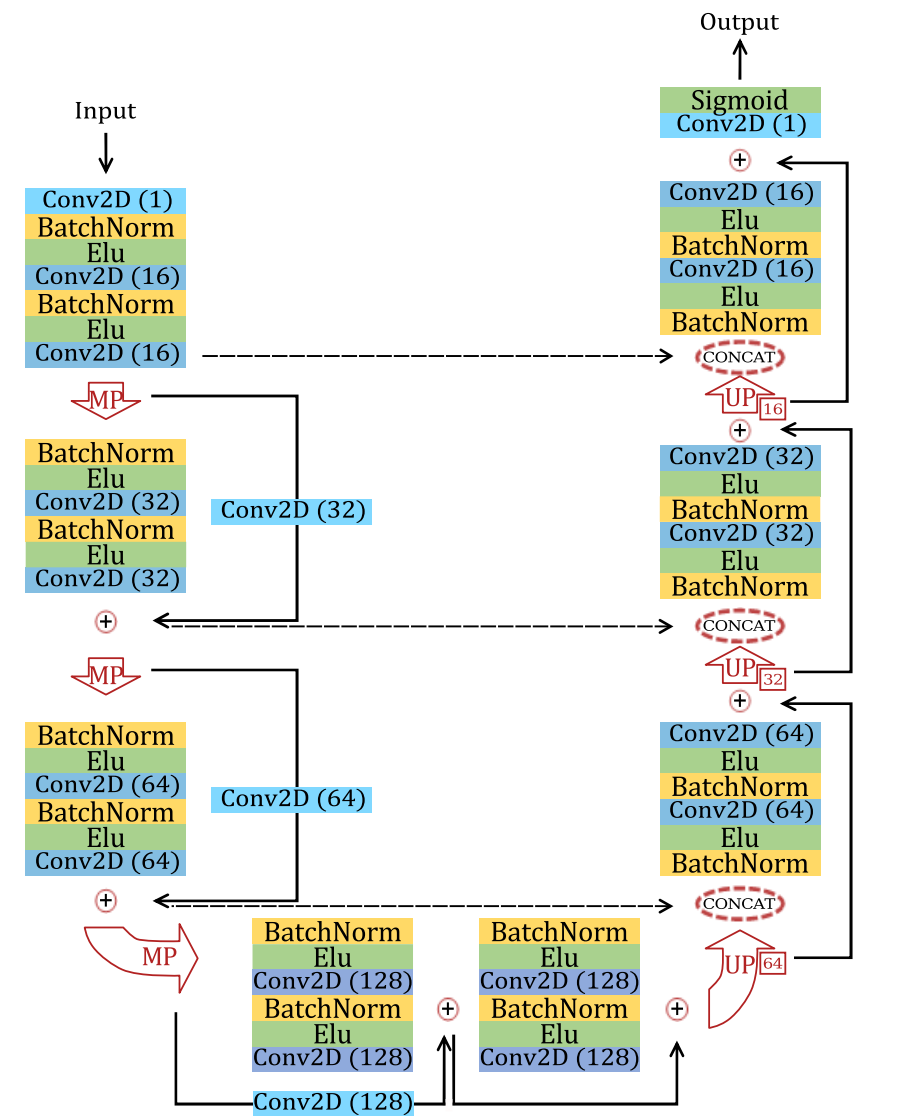
Metodi



- c-ResUnet
- Ablation studies
 - Weight maps (WM)
 - Artifacts oversampling (AO)

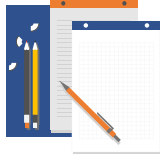
Cell ResUnet

- Convoluzione 1x1 iniziale
 - Conversione grayscale
 - Colourspace appreso
- Bottleneck
 - Blocco residuo aggiuntivo
 - Filtri 5x5 (anzichè 3x3)
 - Maggiore field-of-view



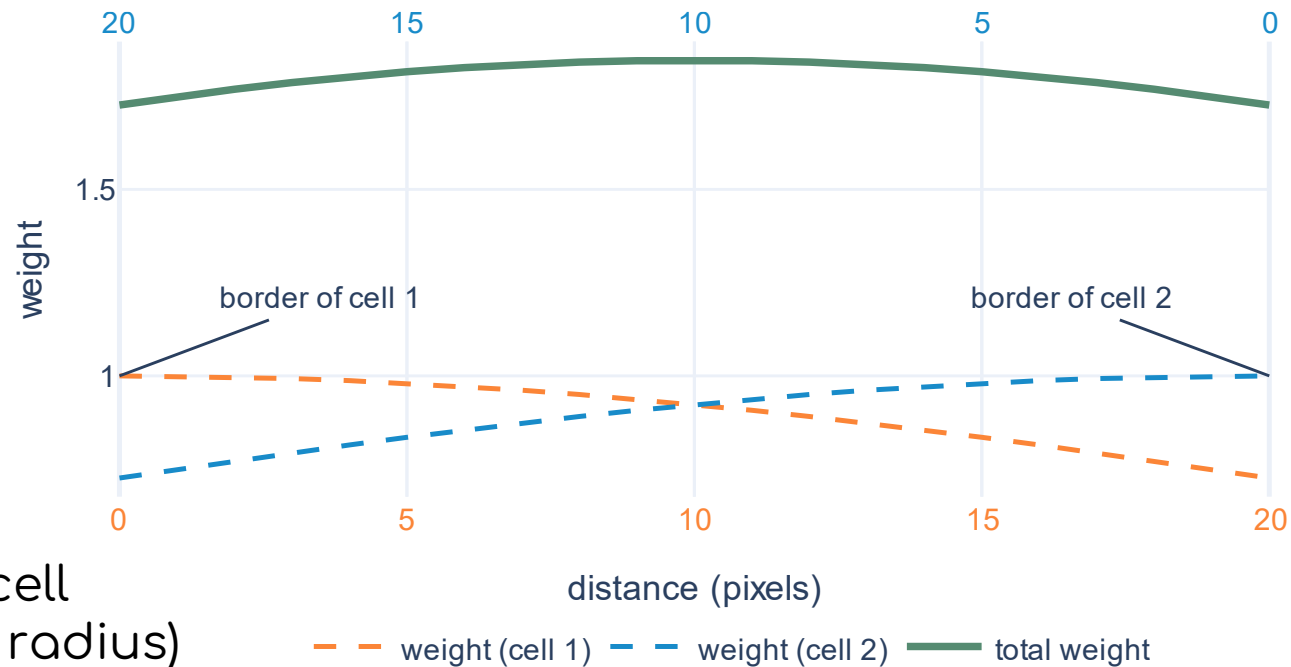
Legend

---→ (CONCAT)	Concatenation	Conv2D (N. filters) 1 x 1	MP	Max Pooling (size = 2, stride=2)
→ (+)	Short-range skip-connection with sum	Conv2D (N. filters) 3 x 3	UP _{N. filters}	Deconvolution (size = 2, stride=2)
		Conv2D (N. filters) 5 x 5		



Weight maps

- Penalizzano errore in aree sovrapposte
- Effetto additivo

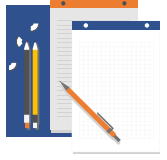


$$w = \exp\left\{-\frac{d^2}{2\sigma^2}\right\},$$

where

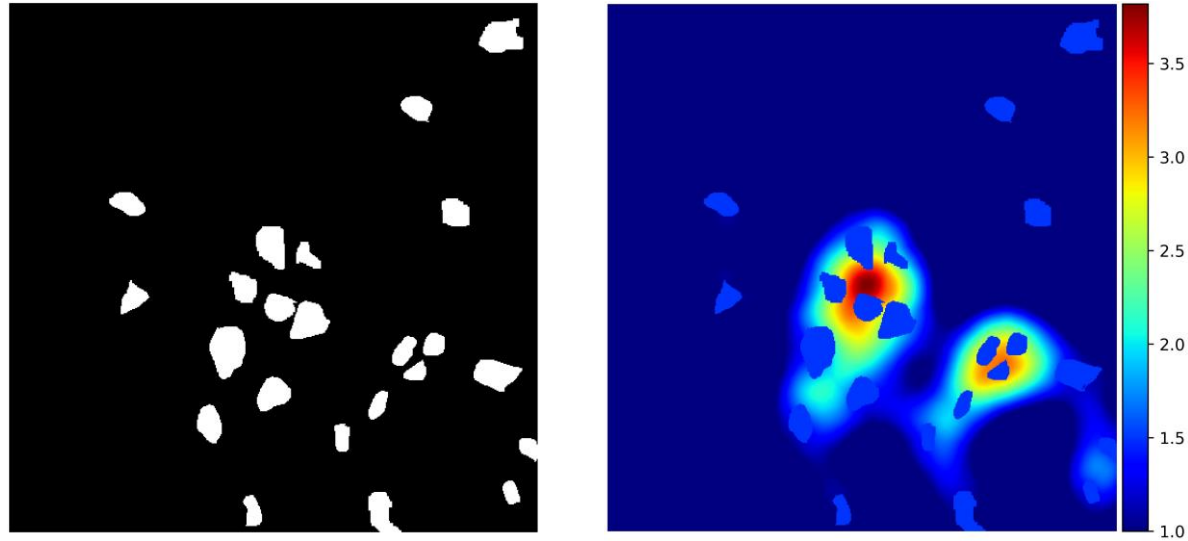
d : distance from a cell

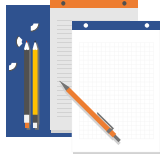
$\sigma = 25\text{px}$ (mean cell radius)



Weight maps

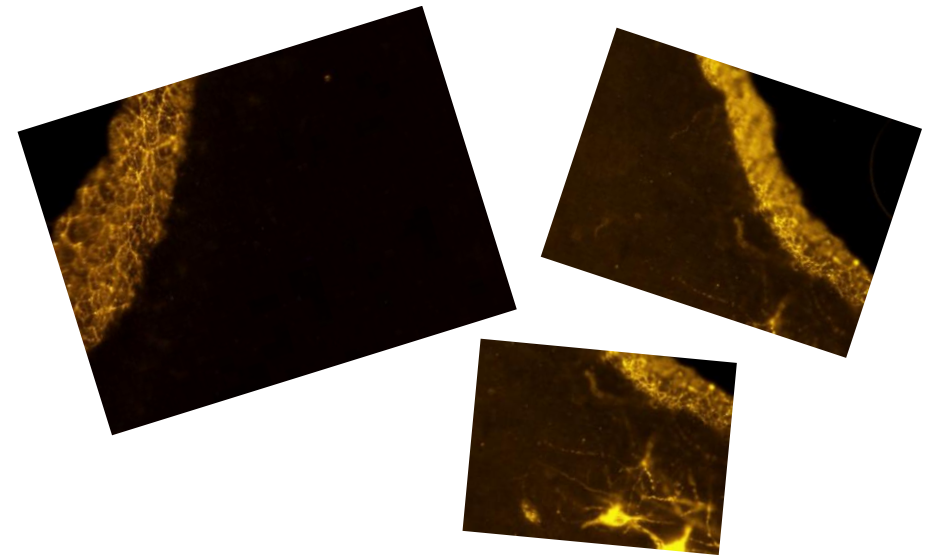
- Penalizzano errore in aree sovrappollate
- Effetto additivo

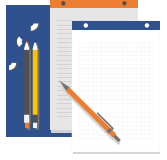




Artifacts oversampling

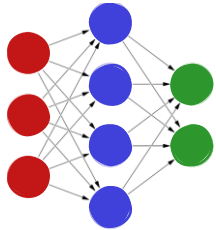
- Difficile da gestire
- Sottorappresentati
- Ricampionati con fattore 6





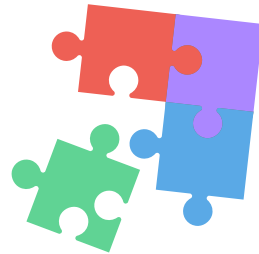
Experiments

Benchmark against SOTA architectures



- c-ResUnet (1.7M params)
- ResUnet (887k params)
- Unet (14M params)
- small Unet (876k params)

Ablation studies

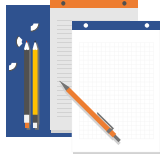


- Full design (WM + AO)
- Weight maps only (no AO)
- Artifacts oversampling only (no WM)

Metrics of performance



- Detection: F_1 score, AUC
- Counting : R^2 , MAE



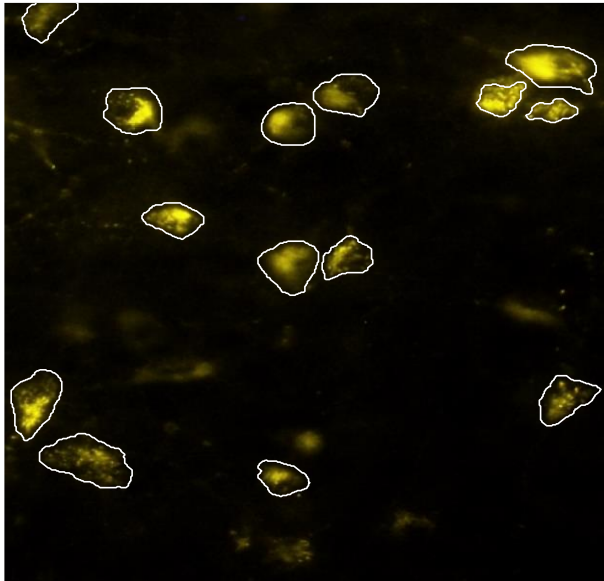
Training pipeline

- 150 training; 63 validation; 70 test
- 512x512 crop
- Augmentation
 - Rotazioni, rumore Gaussiano, variazione luminosità, deformazioni elastiche
 - Fattore re-sampling
 - 4 per immagini segmentate semi-automaticamente
 - 10 per immagini segmentate manualmente
 - 25 per artefatti

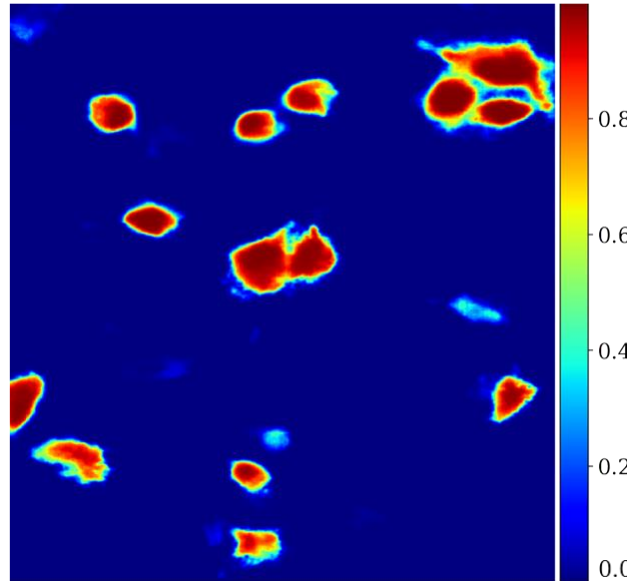


Post-processing

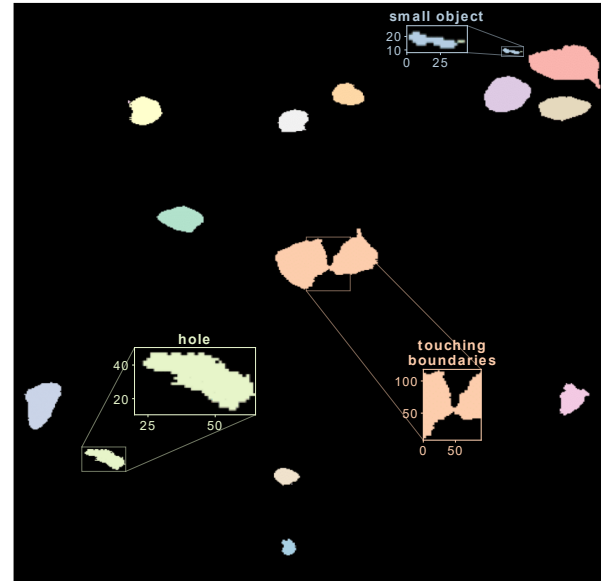
(a) ground-truth



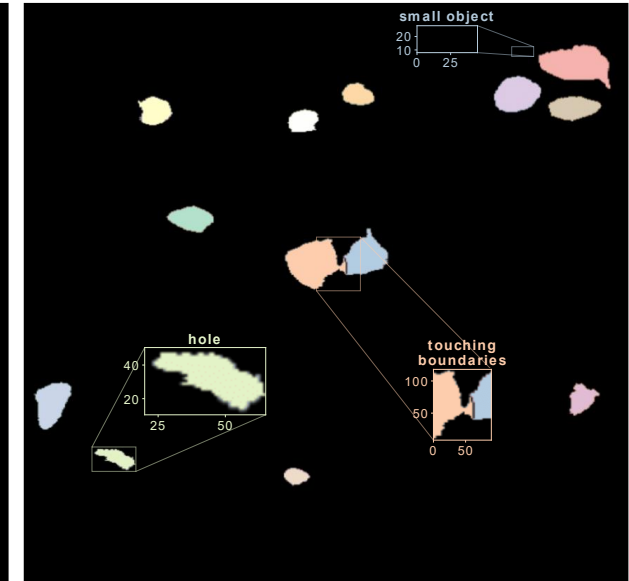
(b) raw heatmap

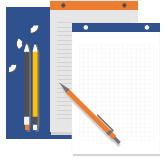


(c) thresholded

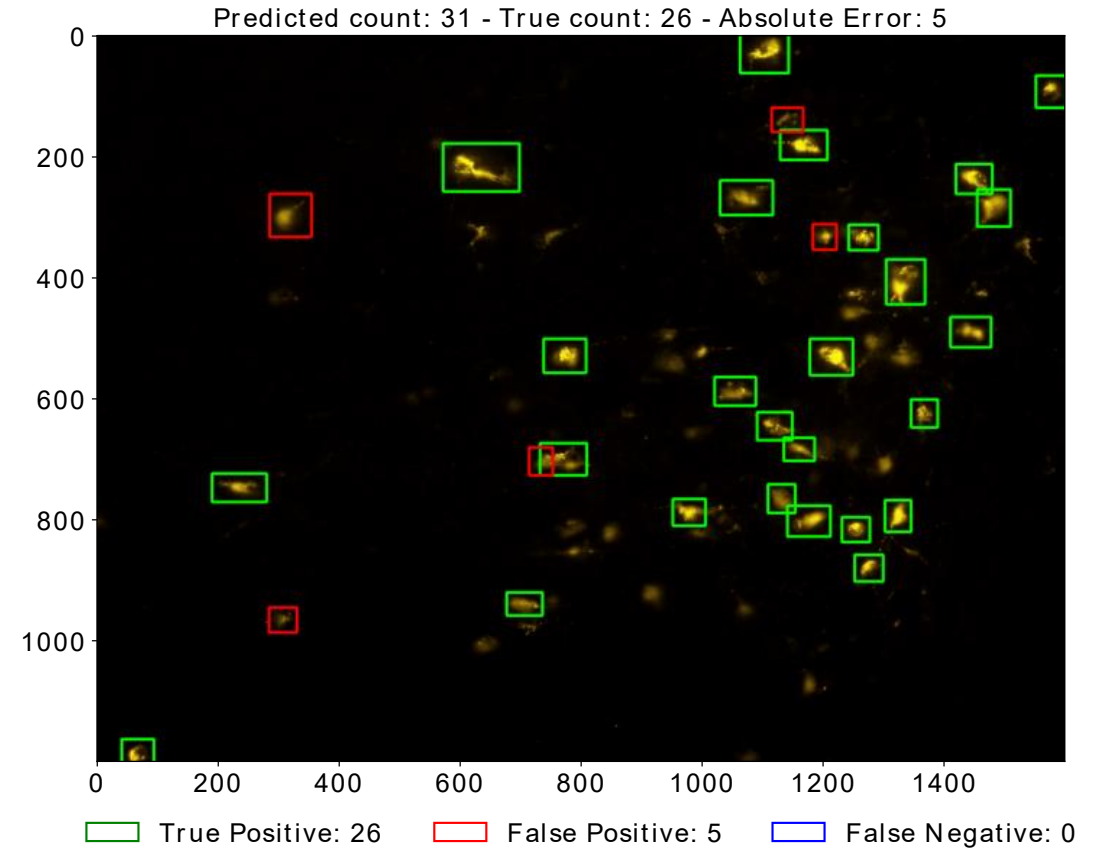
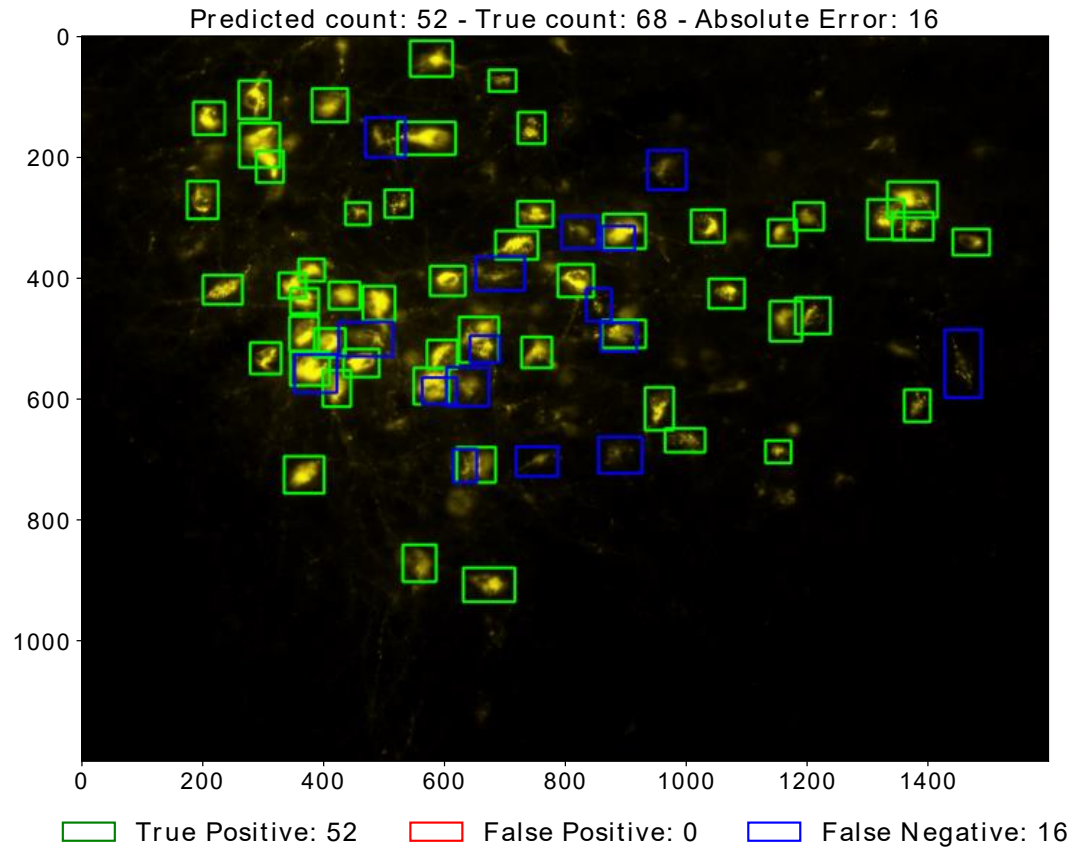


(d) post-processed





Qualitative evaluation: prediction

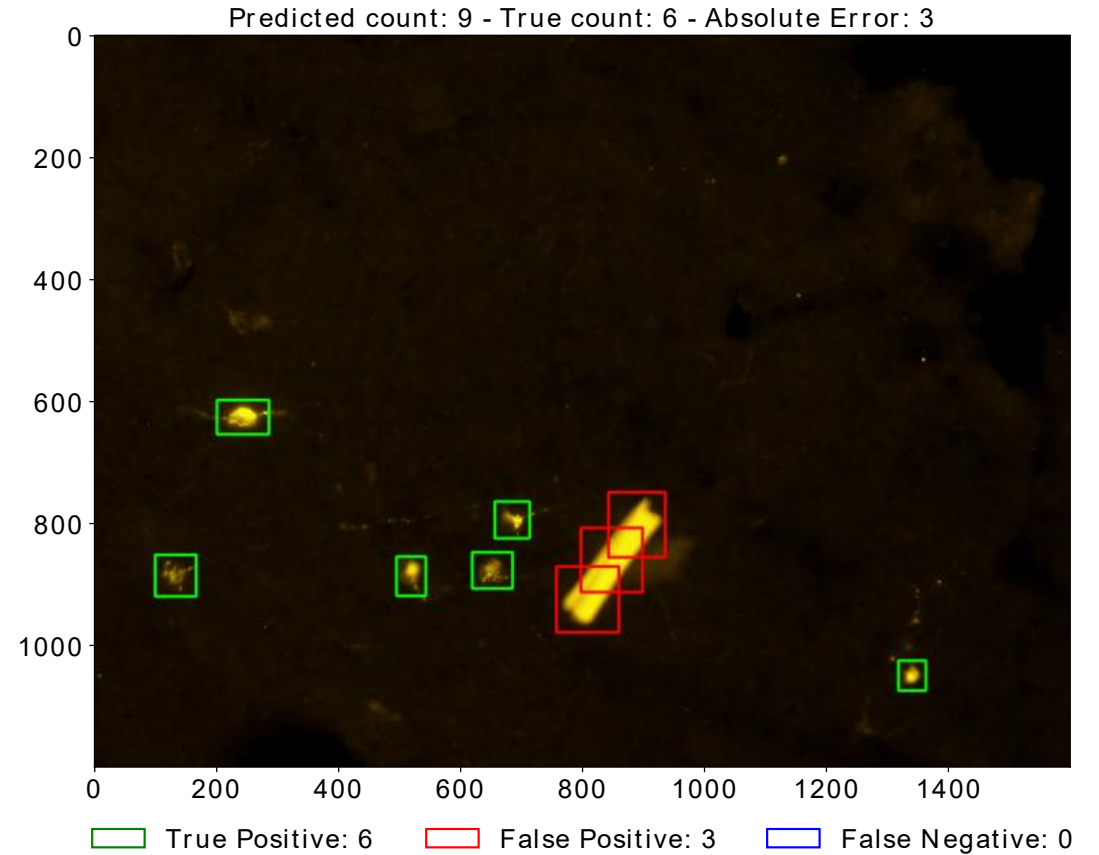
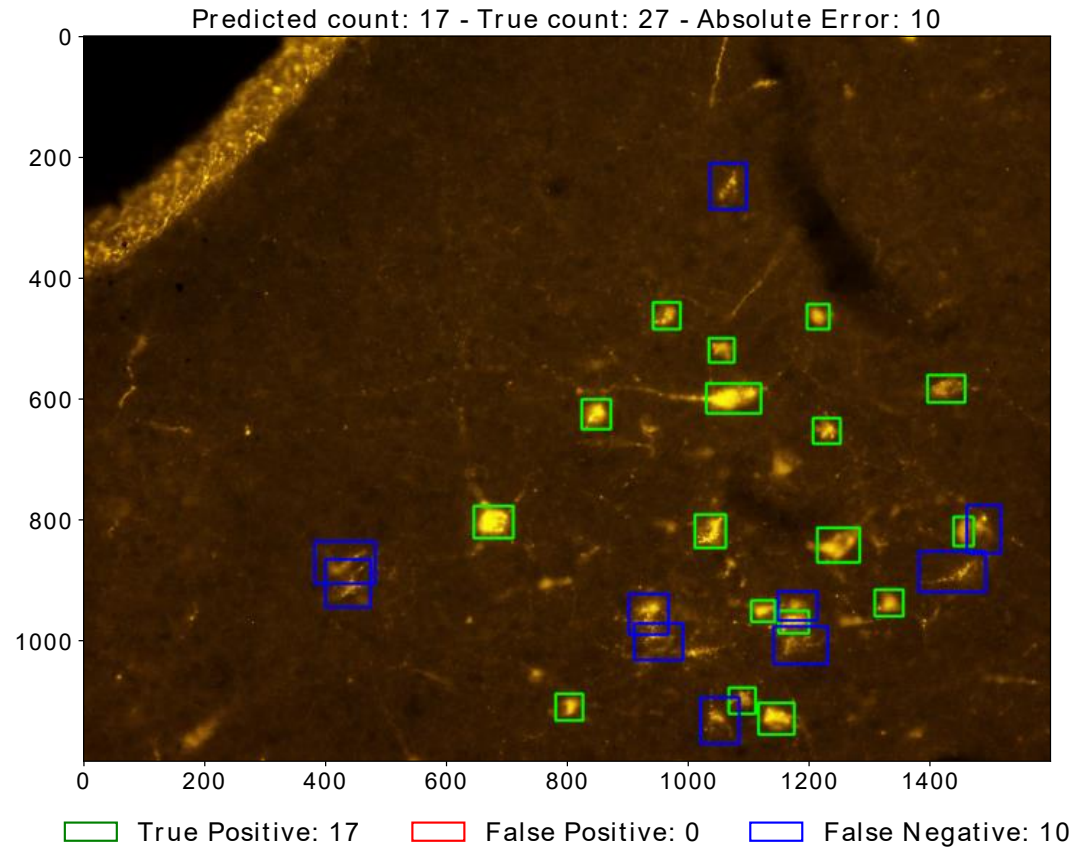


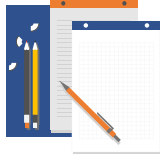


Qualitative evaluation: artifacts

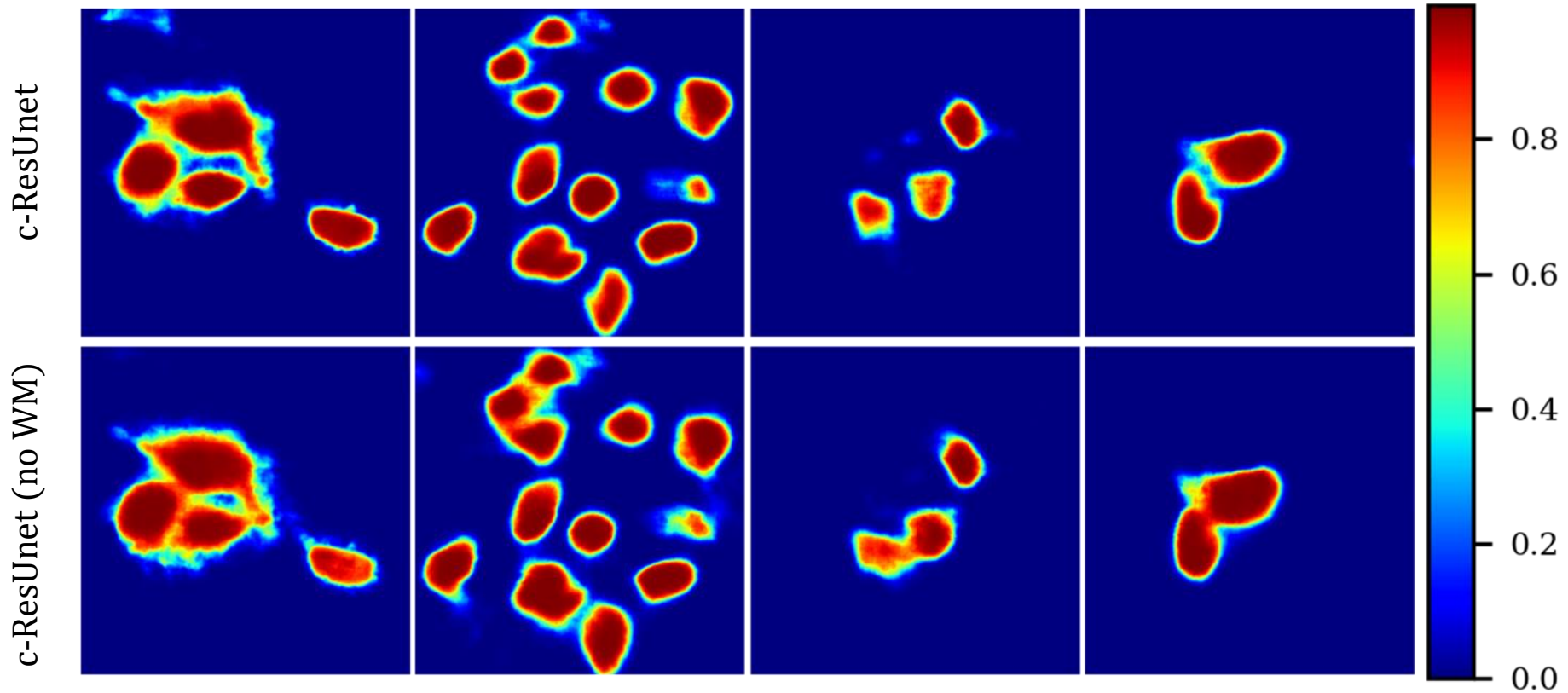
c-ResUnet (no A0)

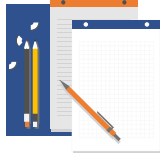
c-ResUnet





Qualitative evaluation: weight maps

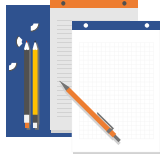




Quantitative evaluation

Model	Threshold	F_1	AUC	Accuracy	Precision	Recall	R^2	MAE	MedAE	MPE (%)
c-ResUnet	0.875	0.8149	0.8705	0.6877	0.9081	0.7391	0.8215	3.0857	1.0	-5.13
c-ResUnet (no AO)	0.875	0.8047	0.8741	0.6732	0.9019	0.7264	0.8077	3.0857	1.5	-6.24
c-ResUnet (no WM)	0.875	0.7613	0.8594	0.6147	0.9418	0.6389	0.7048	3.6857	1.0	-19.14
ResUnet	0.850	0.7855	0.8579	0.6468	0.8865	0.7052	0.7831	3.3286	1.0	-4.84
ResUnet (no WM)	0.850	0.7513	0.8643	0.6016	0.9387	0.6262	0.6955	4.0571	2.0	-24.12
Unet	0.875	0.7724	0.8609	0.6291	0.9117	0.6700	0.7560	3.5143	1.5	-14.36
Unet (no WM)	0.850	0.7886	0.8461	0.6510	0.8989	0.7024	0.8069	3.1571	2.0	-9.23
small Unet	0.875	0.7563	0.8691	0.6081	0.9264	0.6389	0.7682	3.5714	2.0	-21.37
small Unet (no WM)	0.825	0.6697	0.8326	0.5034	0.9483	0.5176	0.5723	4.7714	2.0	-32.01
Adaptive Threshold	0.994	0.6106	0.0865	0.4394	0.5680	0.6601	0.3565	8.0143	6.0	78.26

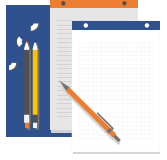
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Quantitative evaluation

Model	Threshold	F_1	AUC	Accuracy	Precision	Recall	R^2	MAE	MedAE	MPE (%)
c-ResUet	0.875	0.8149	0.8705	0.6877	0.9081	0.7391	0.8215	3.0857	1.0	-5.13
c-ResUet (no AD)	0.875	0.8047	0.8741	0.6732	0.9019	0.7264	0.8077	3.0857	1.5	-6.24
c-ResUet (no WM)	0.875	0.7613	0.8594	0.6147	0.9418	0.6389	0.7048	3.0857	1.0	-19.14
ResUet	0.850	0.7855	0.8579	0.6468	0.8865	0.7052	0.7831	3.3296	1.0	-4.84
ResUet (no WM)	0.850	0.7513	0.8643	0.6016	0.9387	0.6262	0.6955	4.0571	2.0	-24.12
Uet	0.875	0.7724	0.8609	0.6291	0.9117	0.6700	0.7560	3.5143	1.5	-14.26
Uet (no WM)	0.850	0.7886	0.8461	0.6510	0.8989	0.7024	0.8069	3.1571	2.0	-9.23
small Uet	0.875	0.7563	0.8691	0.6081	0.9264	0.6389	0.7082	3.5714	2.0	-21.37
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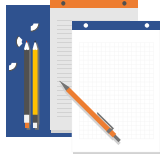
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Uet	0.875	0.7724	0.8689	0.6291	0.9117	0.6790	0.7560	3.5143	1.5	-14.36
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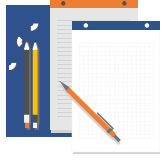
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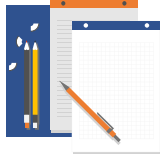
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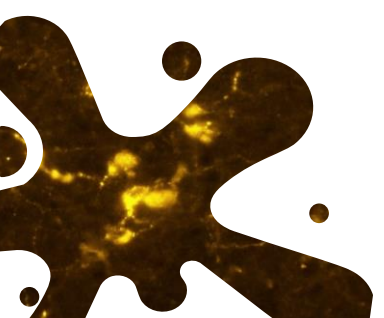
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Recap

- 👍 c-ResUnet performa meglio dei competitor
- 👍 Prestazioni simili ad operatore umano
- 👍 “Effetto operatore” sistematico
- 👍 Weight map utili
- 👍 Pubblicazione dataset e modello allenato
- 👎 Artefatti





Lifecycle completo:

- Messa in produzione
- Labeling



Punti aperti: produzione

Poco fruibile senza un punto d'accesso per utilizzatori finali (e.g. servizio web)

- Messa in produzione modello

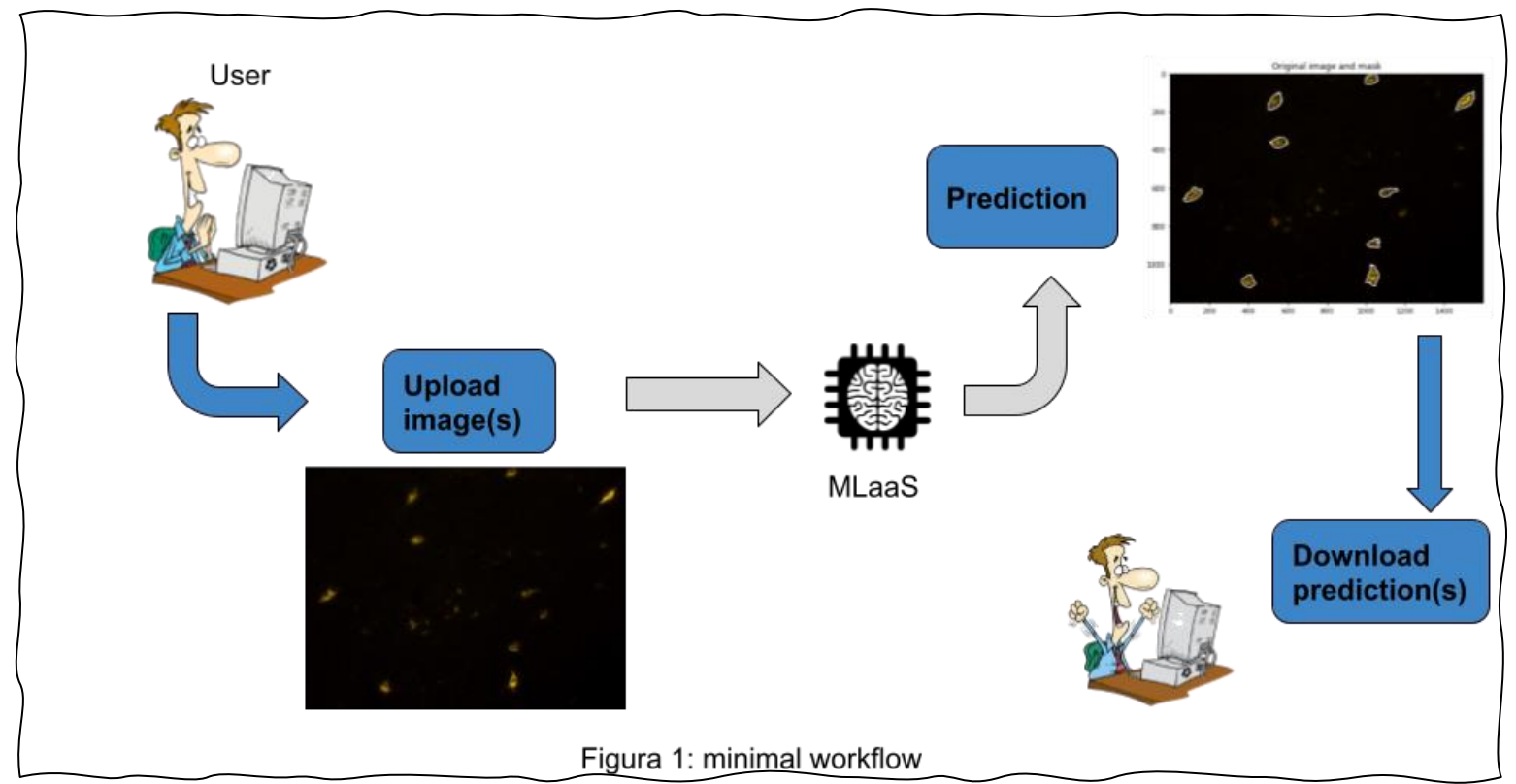


Figura 1: minimal workflow



Punti aperti: produzione

Poco fruibile senza un punto d'accesso per utilizzatori finali (e.g. servizio web)

- Messa in produzione modello
- Data access

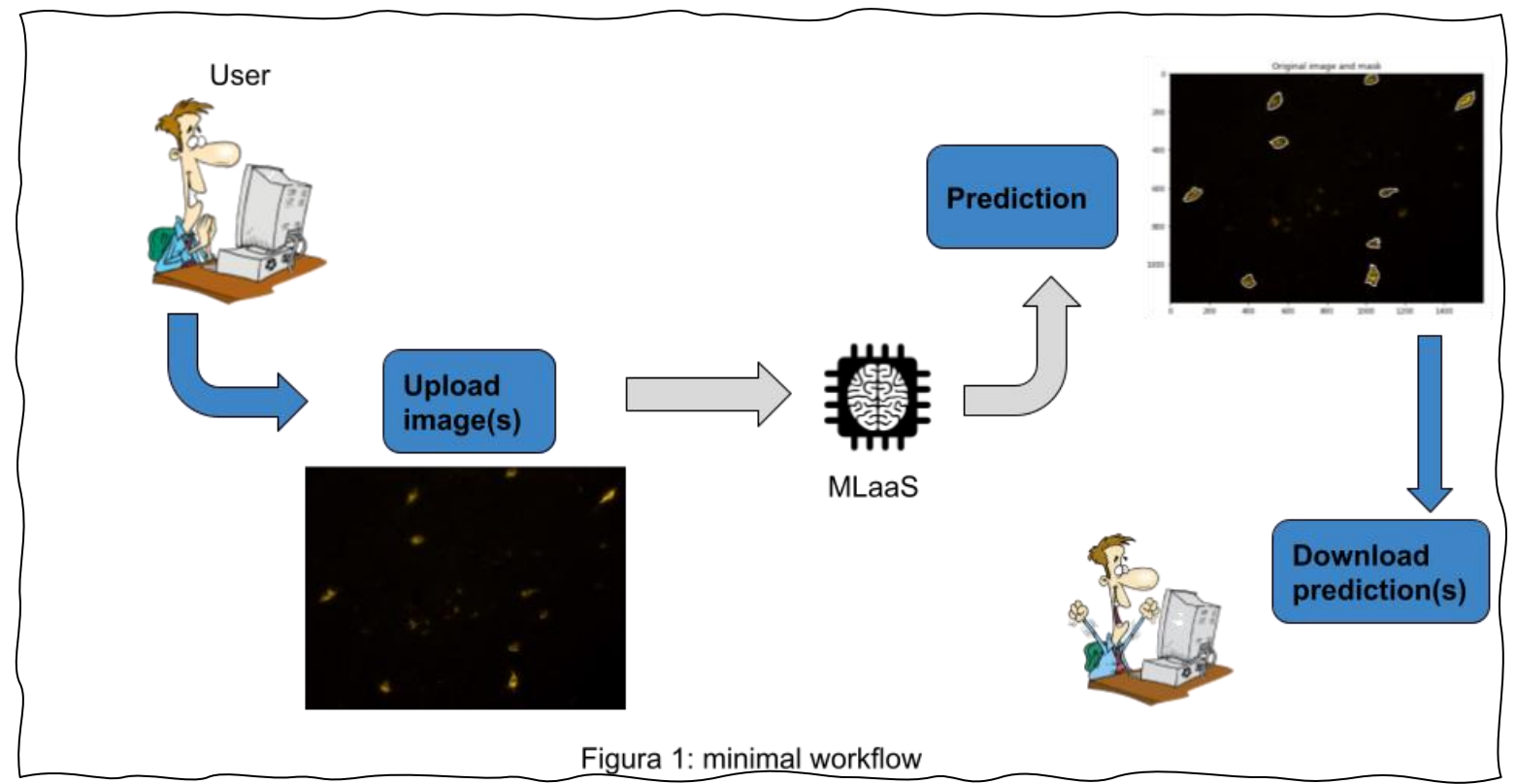
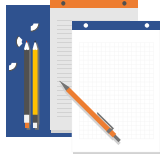


Figura 1: minimal workflow



Punti aperti: labeling

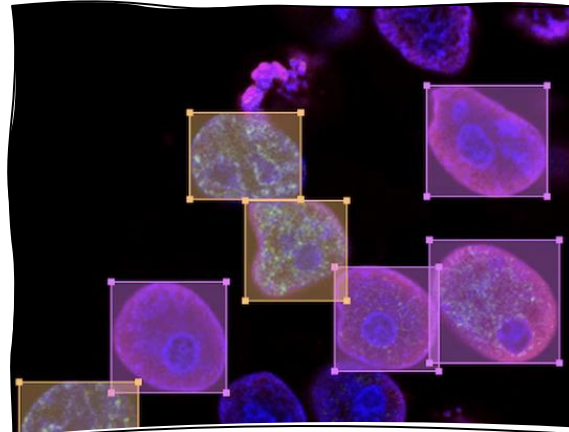
- Annotazioni fondamentali per supervised learning
- Labeling richiede più iterazioni
 - Rifinitura maschere
 - Annotazioni multiple
- In generale, molto dispendioso

→ Tool per labeling



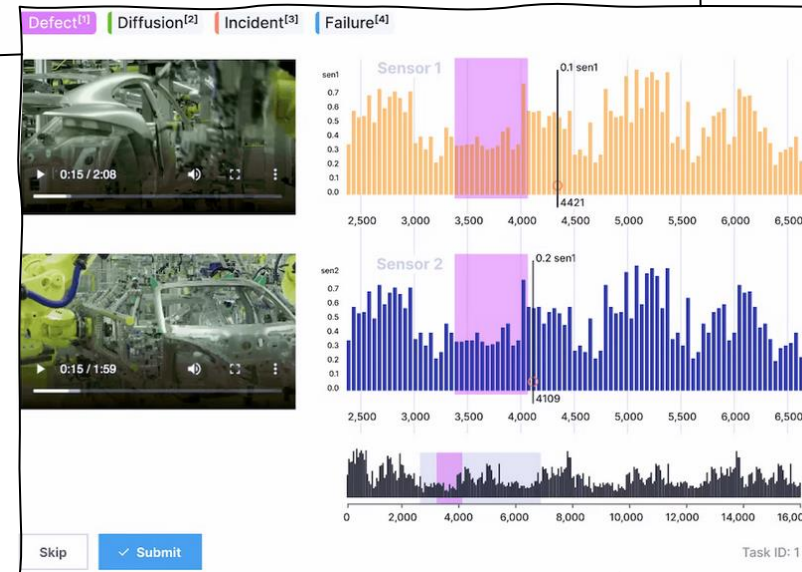
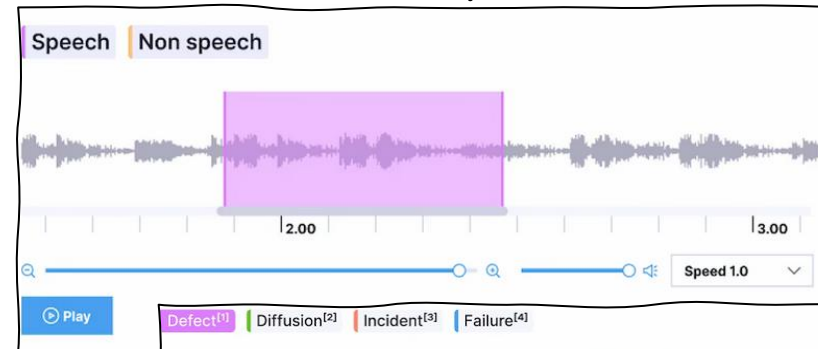
Punti aperti: labeling

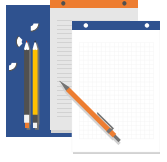
- Esistono molti strumenti commerciali (e.g. [Label Studio](#))



Person^[1] Fact^[2] Date^[3] Time^[4] Ordinal^[5] Product^[6] Language^[7] Location^[8]

Opossums^[Person] are usually solitary^[Fact] and nomadic, staying in one area as long as food and water are easily available. Some families will group together in ready-made burrows or even under houses. Though they will temporarily occupy abandoned burrows, they do not dig or put much^[Ordinal] effort into building their own. As nocturnal animals, they favor dark, secure areas. These^[Date] areas may be below ground or above. When threatened or harmed, they will “play possum”, mimicking the appearance and smell of a sick or dead animal.^[Product] This physiological response is involuntary (like fainting), rather than a conscious act. In the case of baby opossums, however, the brain does not always react this way at the appropriate moment. and therefore they often fail to “play dead” when threatened.^[Language] When





Punti aperti: labeling

- Esistono molti strumenti commerciali (e.g. [Label Studio](#))
- Principali necessità
 - Collaborativo e accessibile
 - Authentication & Authorization
 - Configurabile
- Nice-to-have
 - CI/CD (e.g. ML-assisted labeling, support active/online learning)



End-to-end lifecycle

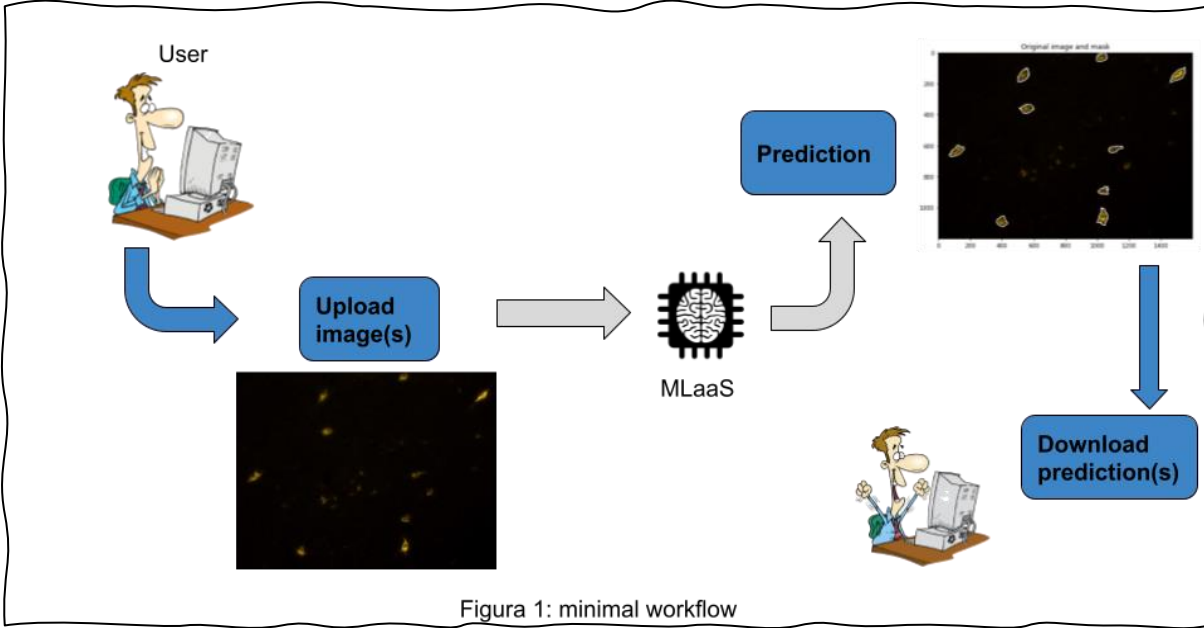


Figura 1: minimal workflow

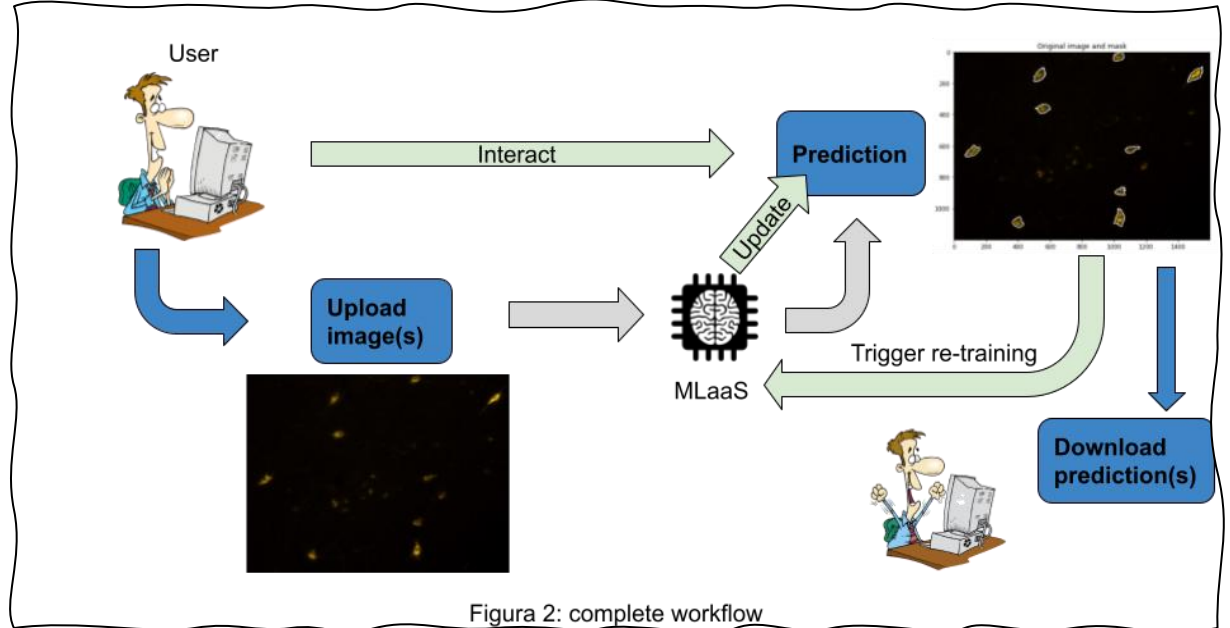
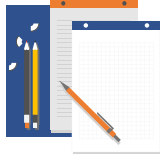
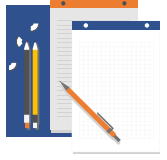


Figura 2: complete workflow



Punti aperti: labeling

- Esistono molti strumenti commerciali (e.g. [Label Studio](#))
- Principali necessità
 - Collaborativo e accessibile
 - Authentication & Authorization
 - Configurabile
- Nice-to-have
 - CI/CD (e.g. ML-assisted labeling, support active/online learning)
- Principali difficoltà
 - Self-hosting (in particolare storage/calcolo)
 - Authentication & Authorization



References

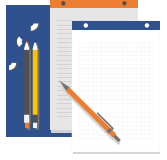


- [1] Morelli R., et al., (2021), Automating cell counting in fluorescent microscopy through deep learning with c-ResUnet, *Scientific Reports*
- [2] Clissa L., et al., (2021), Fluorescent Neuronal Cells dataset, *AMS Acta*
- [3] Clissa L., (2022), Supporting Scientific Research Through Machine and Deep Learning: Fluorescence Microscopy and Operational Intelligence Use Cases, *AMS Dottorato*
- [4] Clissa L., et al., (2022), Analyzing WLCG File Transfer Errors Through Machine Learning, *Computing and Software for Big Science*
- [5] Di Girolamo A., et al., (2022), Preparing Distributed Computing Operations for the HL-LHC Era With Operational Intelligence, *Frontiers in Big Data*
- [6] Di Girolamo A., et al., (2020), for Distributed Computing Systems for Exascale Science, CHEP
- [7] Macaluso A., et al., (2020), A Variational Algorithm for Quantum Neural Networks, *International Conference on Computational Science*
- [8] Macaluso A., et al., (2020), Quantum Ensemble for Classification, *arXiv*
- [9] Macaluso A., et al., (2020), Quantum splines for non-linear approximations, *International Conference on Computing Frontiers*
- [10] Hitrec T., et al., (2019), Neural control of fasting-induced torpor in mice, *Scientific Reports*
- [11] Clissa L., (2023), Survey of Big Data sizes in 2021, *arXiv*

GitHub: https://github.com/robomorelli/cell_counting_yellow



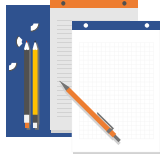
Backup



Training pipeline

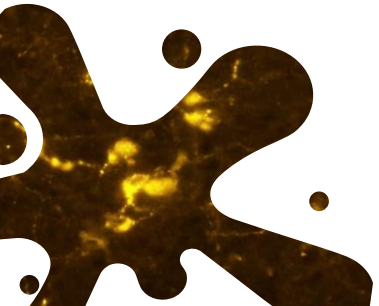
- Crop images in 512x512 partially overlapping crops
- Augmentation
 - rotations, Gaussian noise, brightness variation, elastic transformation
 - Augmentation factors set to:
 - 4 for semi-automatically segmented images
 - 10 for manually segmented images
 - 25 for crops in AO
- Train from scratch





Training settings

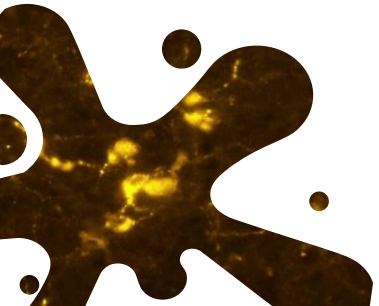
- Adam optimizer
- Learning rate (lr) starts at 0.06
- Decrease lr by 30% if no improvement in 4 epochs
- Weighted Binary Cross Entropy loss (1.5 weight for cells, 1 for background)
- Train until no improvement in 20 epochs
- TensorFlow implementation through Keras
- 4 NVIDIA V100 GPUs hosted at CNAF

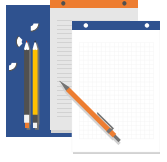




Post-processing

- Output grezzo: heatmap
- Thresholding
 - Introduce iper-parametro
 - Ottimizzazione threshold
- Pulizia output



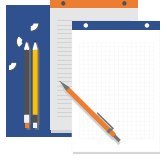


Metriche performance

- True positives (TP), False Positives (FP) e False Negatives (FN)

```
for center in predicted_centers:  
    compute distance (px) from all true_centers  
    if minimum_distance < 50:  
        increase TP count  
        remove corresponding true center  
FN = n_true - TP  
FP = n_pred - TP
```

- Detection: F_1 score, AUC
- Conteggio: R^2 , MAE



Metriche detection: F_1 score e AUC

- F_1 score come metrica principale:

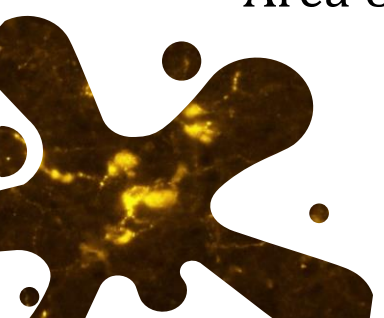
$$accuracy = \frac{TP}{TP + FP + FN},$$

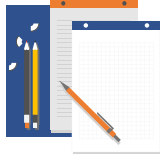
$$precision = \frac{TP}{TP + FP},$$

$$recall = \frac{TP}{TP + FN},$$

$$F_1 = \frac{2 \cdot precision \cdot recall}{precision + recall},$$

- Area Under precision/recall Curve (AUC)





Counting metrics: MAE and R²

Errore assoluto (AE) e percentuale (PE):

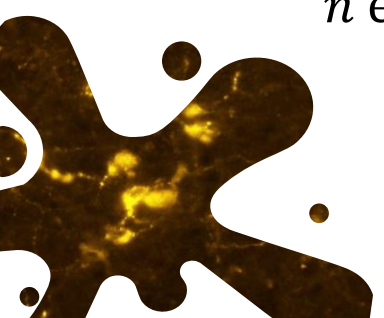
$$AE = |n_{true} - n_{pred}|,$$

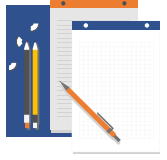
$$PE = \frac{n_{true} - n_{pred}}{n_{true}}$$

Coefficiente di determinazione, R²:

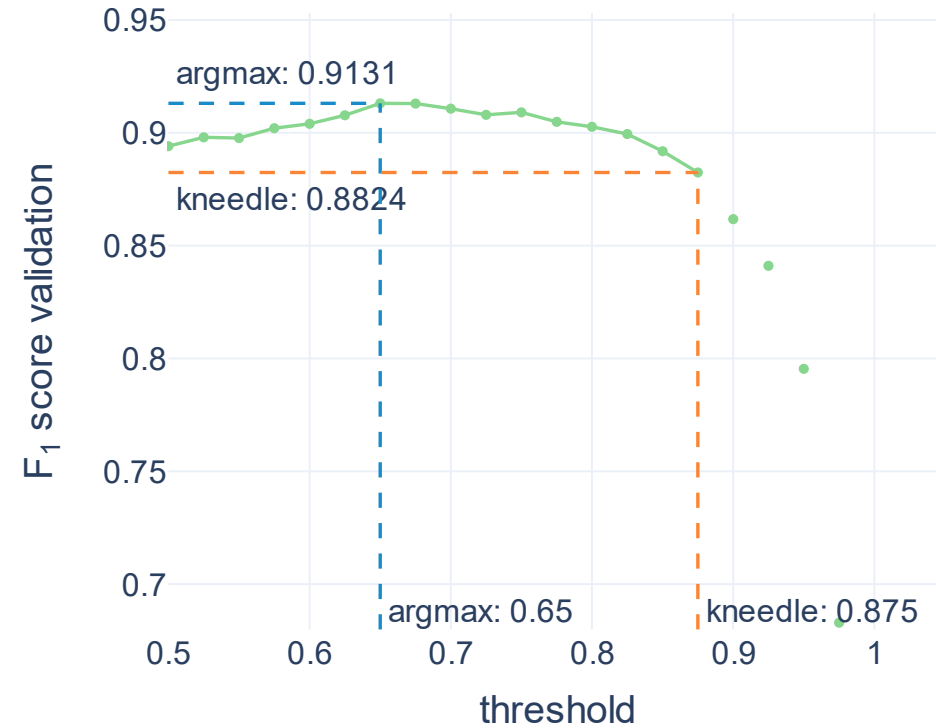
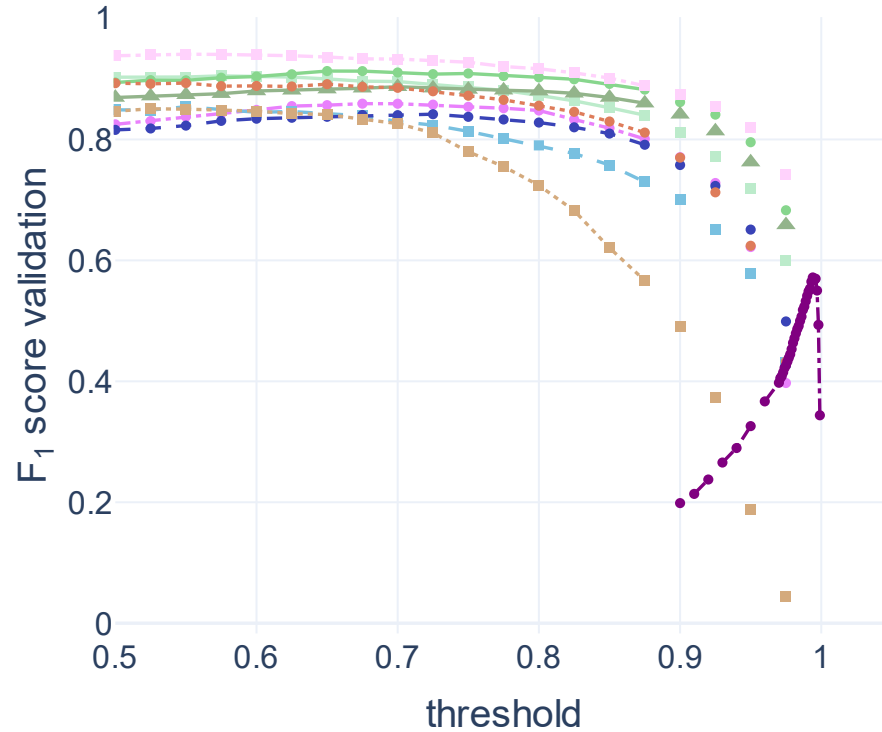
$$R^2 = 1 - \frac{\sum_i (n_{true}^i - n_{pred}^i)^2}{\sum_i (n_{true}^i - \bar{n})^2},$$

Dove n_{true}^i , n_{pred}^i sono il numero di cellule vere e predette per l'immagine i ,
 \bar{n} è il numero medio di cellule per immagine

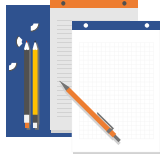




Threshold optimization



- c-ResUnet (no WM)
- Unet (no WM)
- ResUnet (no WM)
- small Unet (no WM)
- Adaptive Threshold
- c-ResUnet
- Unet
- ResUnet
- small Unet
- c-ResUnet (no AO)



ROC curve

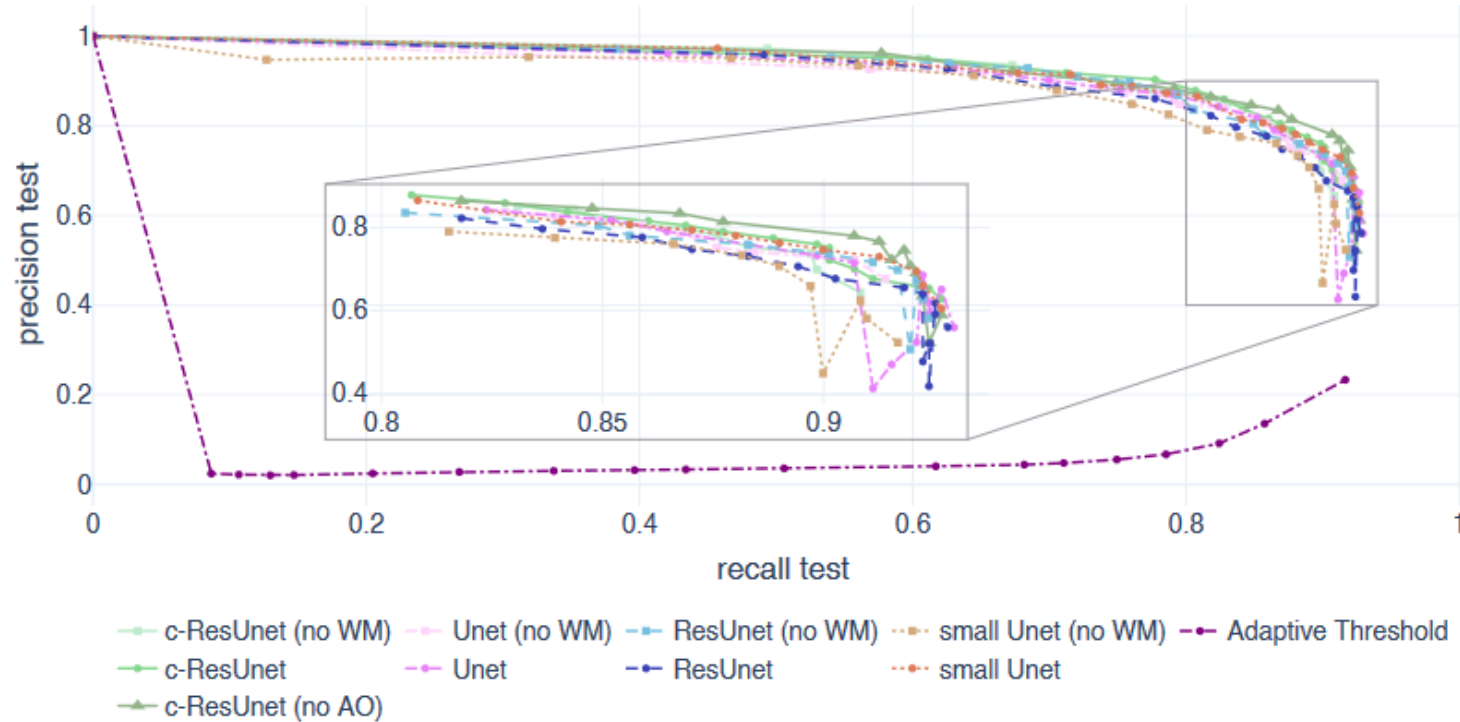
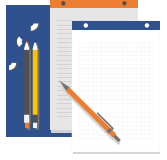


Figure 5.1: **Precision/Recall plot.** Test set precision/recall curves varying the threshold for predicted heatmap binarization. The inset plot reports a zoom of the top right corner to highlight differences of the various curves.



Quantitative evaluation

Model	Threshold	F_1	AUC	Accuracy	Precision	Recall	R^2	MAE	MedAE	MPE (%)
c-ResUnet	0.875	0.8149	0.8705	0.6877	0.9061	0.7201	0.8215	3.0857	1.0	-5.13
c-ResUnet (no AO)	0.875	0.8047	0.8741	0.6732	0.9019	0.7264	0.8077	3.0857	1.5	-6.24
c-ResUnet (no WM)	0.875	0.7613	0.8594	0.6147	0.9418	0.6389	0.7048	3.6857	1.0	-19.14
ResUnet	0.850	0.7855	0.8579	0.6488	0.8965	0.7052	0.7831	3.3286	1.0	-4.84
ResUnet (no WM)	0.850	0.7513	0.8643	0.6016	0.9367	0.6302	0.6955	4.0571	2.0	-24.12
Unet	0.875	0.7724	0.8609	0.6291	0.9117	0.6790	0.7560	3.5143	1.5	-14.26
Unet (no WM)	0.850	0.7886	0.8461	0.6510	0.8989	0.7024	0.8069	3.1571	2.0	-9.23
small Unet	0.875	0.7563	0.8691	0.6081	0.9264	0.6389	0.7682	3.5714	2.0	-21.37
small Unet (no WM)	0.825	0.6697	0.8326	0.5034	0.9483	0.5176	0.5723	4.7714	2.0	-32.01
Adaptive Threshold	0.994	0.6196	0.8965	0.4394	0.5690	0.6001	0.2365	8.0143	6.0	78.26

Table 5.1: Performance metrics. Test set performance using the optimal *kneel* threshold. The first five columns report the detection metrics, while the latter ones evaluate counting performance.