

Nuova strumentazione, upgrade e software di analisi del laboratorio di microscopia confocale



Centro **I**nterdipartimentale **G**randi **S**trumenti
Università degli Studi di Modena e Reggio Emilia



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Ringraziamento

Si ringrazia il Dipartimento di Scienze biomediche, Metaboliche e Neuroscienze, nel quadro del Programma del Dipartimento di eccellenza, per aver messo a disposizione di tutto l'Ateneo strumentazione di avanguardia di microscopia confocale e imaging.



Leica SP8



Microscopio rovesciato da ricerca Leica DMI8 con AFC e XYZ motorizzati

Testa di scansione Leica TCS SP8

Laser bianco (WLL)

Modulo Software Lightning

Modulo FLIM (picoquant)

Sistema di incubazione Top-Stage OKOLAB

Multicolor analysis

FRAP (Fluorescence Recovery After Photobleach)

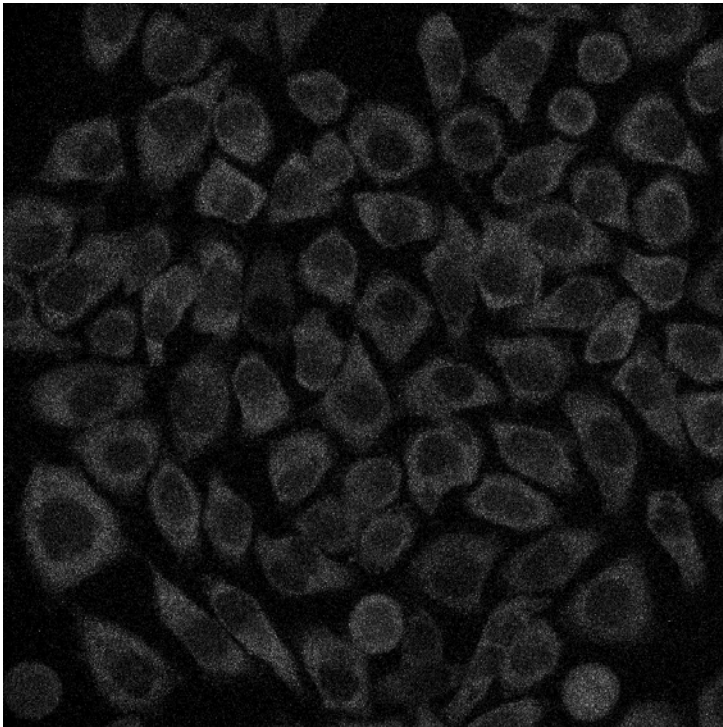
Advanced **time-lapse experiments**

FLIM (Fluorescence Lifetime Imaging Microscopy)

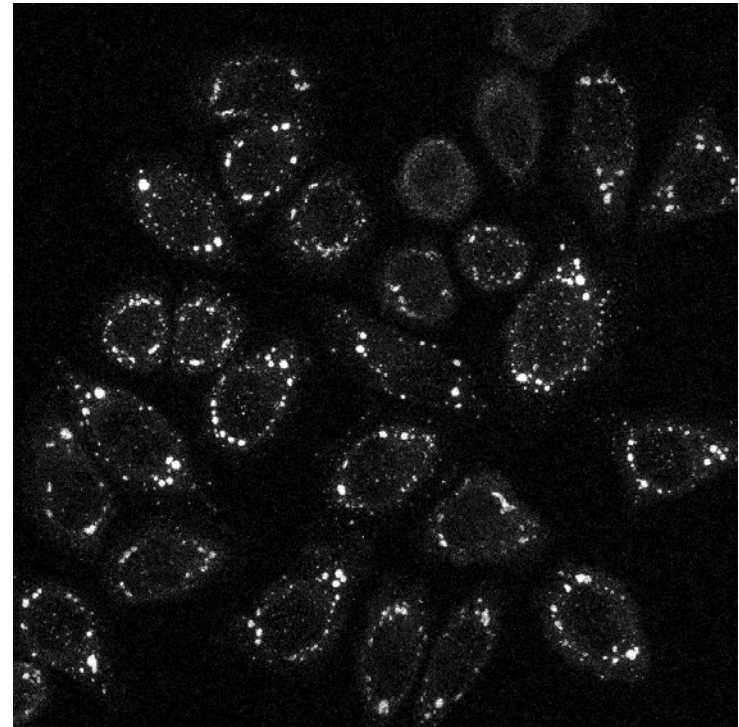
Deconvoluzione (Lightning)

Funzione Mosaic microscopy

Leica SP8 (Time-lapse experiments)



HeLa stably expressing G3BP1-mCherry, treated with sodium arsenite. Z-stack every 5 min for a total of 45min.



Recovery
Z-stack every 10 min for a total of 4 hours

I video sono stati gentilmente fornite dal gruppo Carra.

Accessori e Incubatore



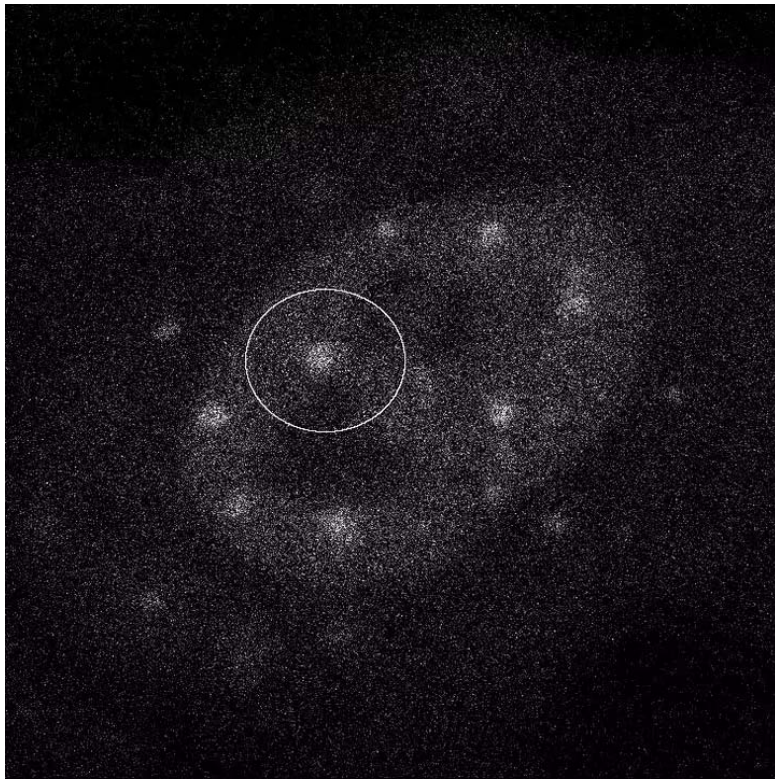
Pompa e mini incubatore per eppendorfs sono presenti per fare lavaggi e cambi di terreno.



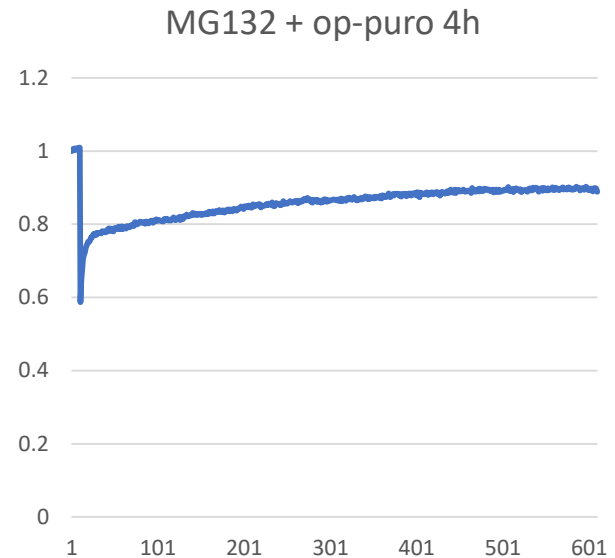
Incubatore già presente nel laboratorio.

Manca la linea di CO₂ che dovrebbe essere in funzione entro fine anno.

Leica SP8 (FRAP)



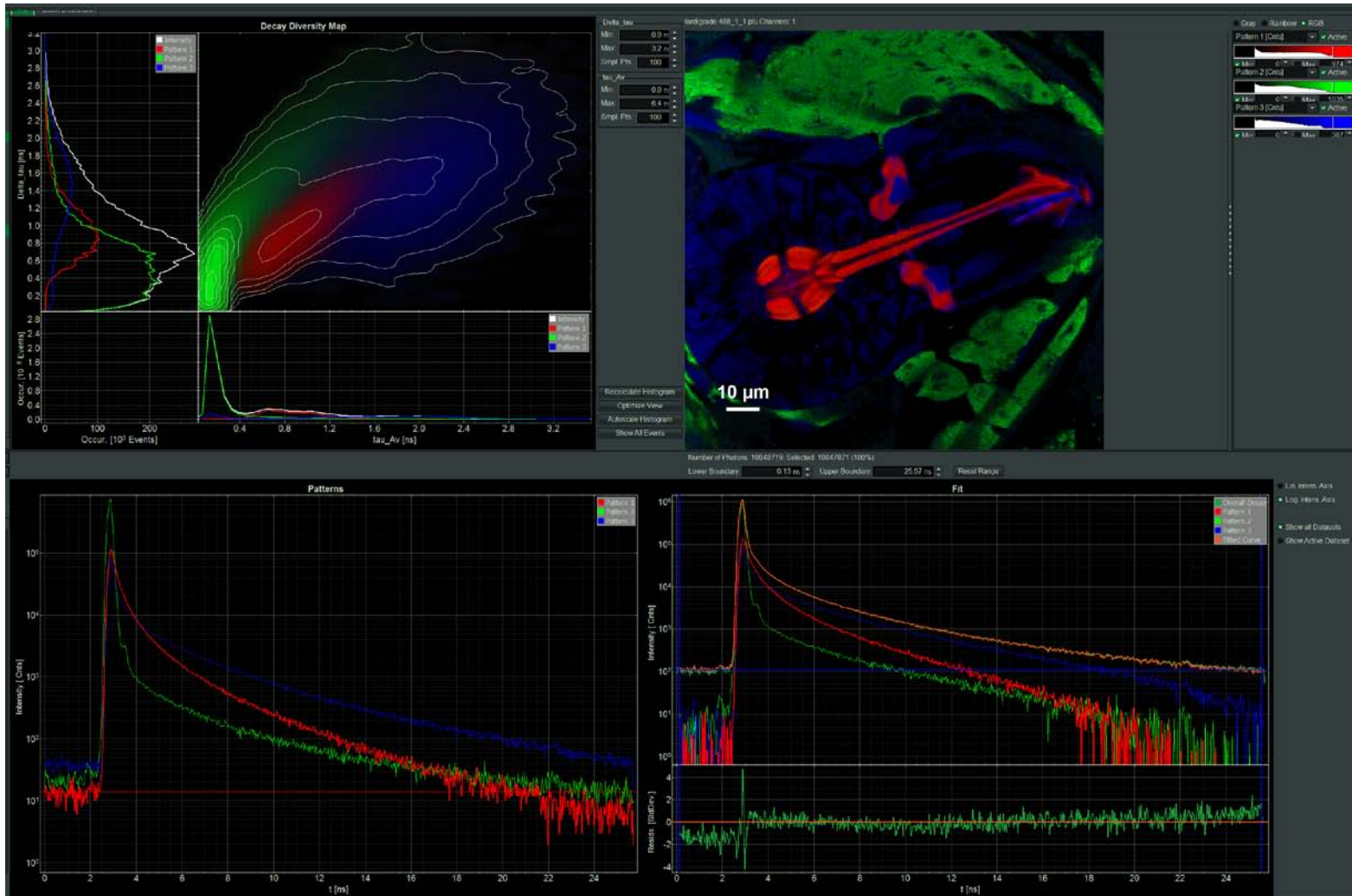
Cellule HeLa che esprimono stabilmente
GFP-PSMA7 (proteasome subunit alpha-7)



Le immagini sono state gentilmente fornite
dal gruppo Carra.

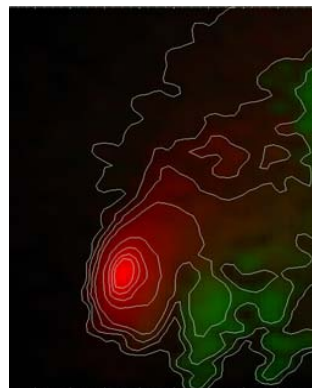
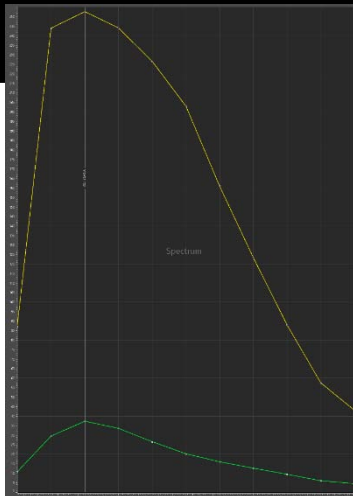
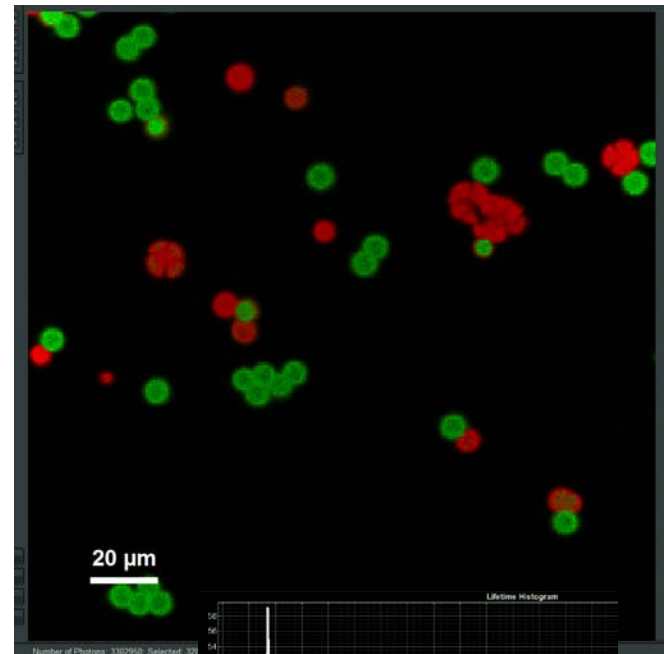
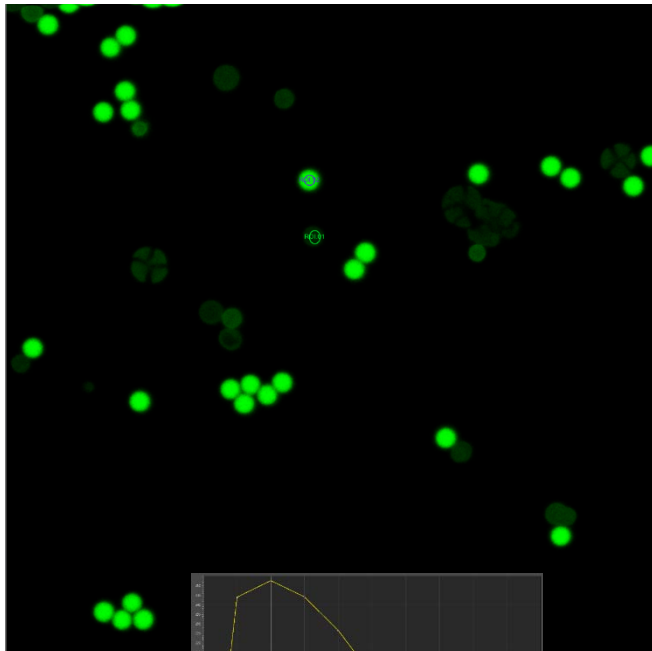
Mediani et al., EMBO J, 2019

Leica SP8 (FLIM)

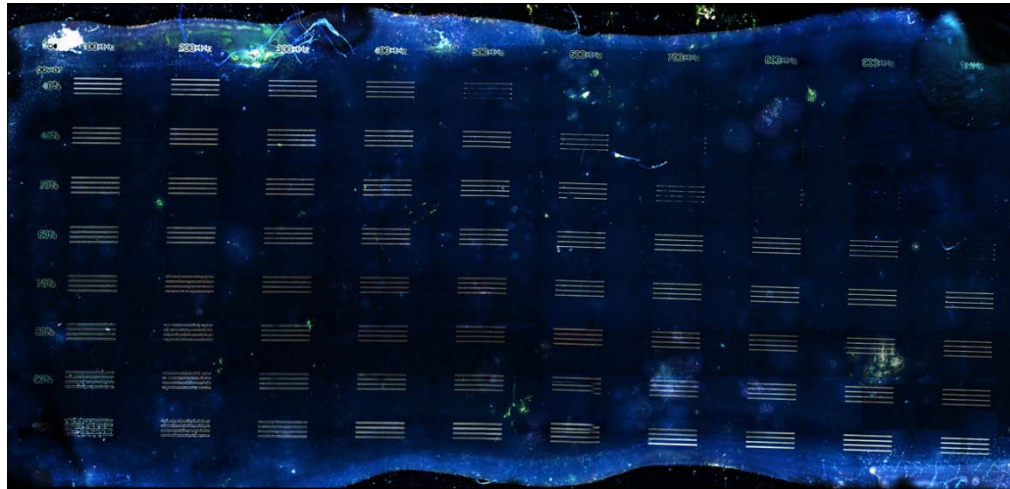


Le immagini sono state gentilmente fornite dal gruppo Guidetti.

Leica SP8 (FLIM)

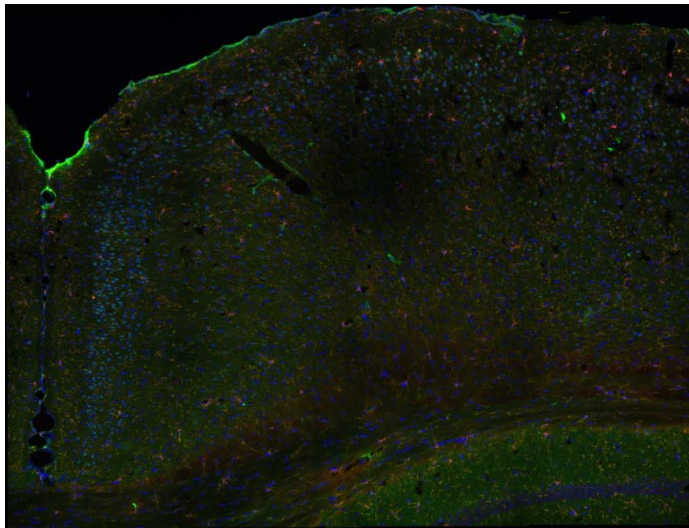


Leica SP8 (Mosaic microscopy)



Polimero bruciato con un laser 405 per depositare dei quantum dots nel tentativo di creare nuovi LED.
Dimensioni del polimero: 3X1 cm
Obiettivo: 10X

Immagine gentilmente fornita dal gruppo Orazi.



Mosaico della corteccia di cervello di topo. Dimensioni del cervello: 1X1 cm
Obiettivo: 20X

Immagine gentilmente fornita dal gruppo Zoli.

Legge di Abbe



Numerical Aperture: $NA = n \sin \alpha$

$$\alpha_{\max} \approx 72^\circ$$

$$NA_{\max \text{ oil}} \approx 1,45$$

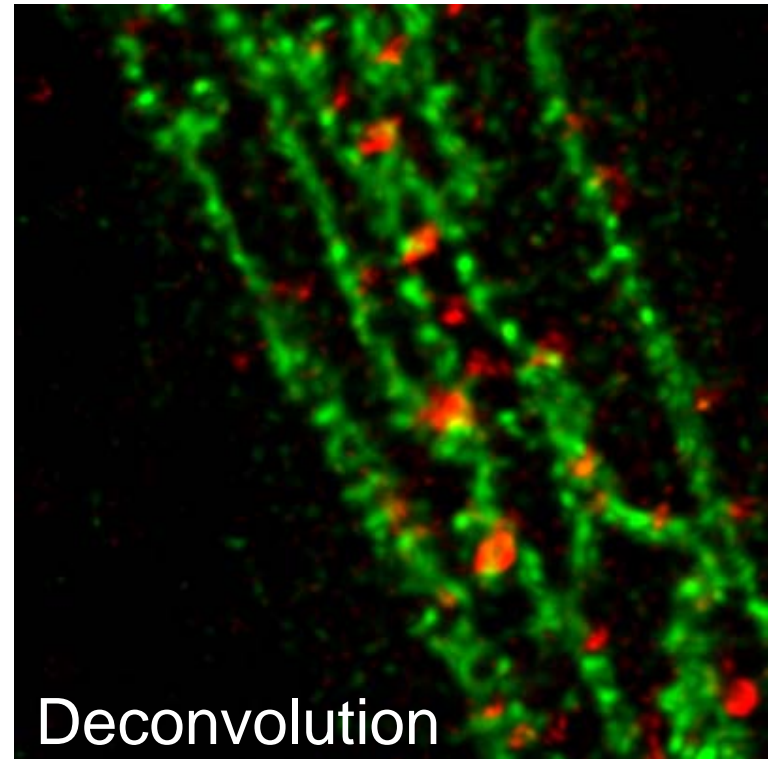
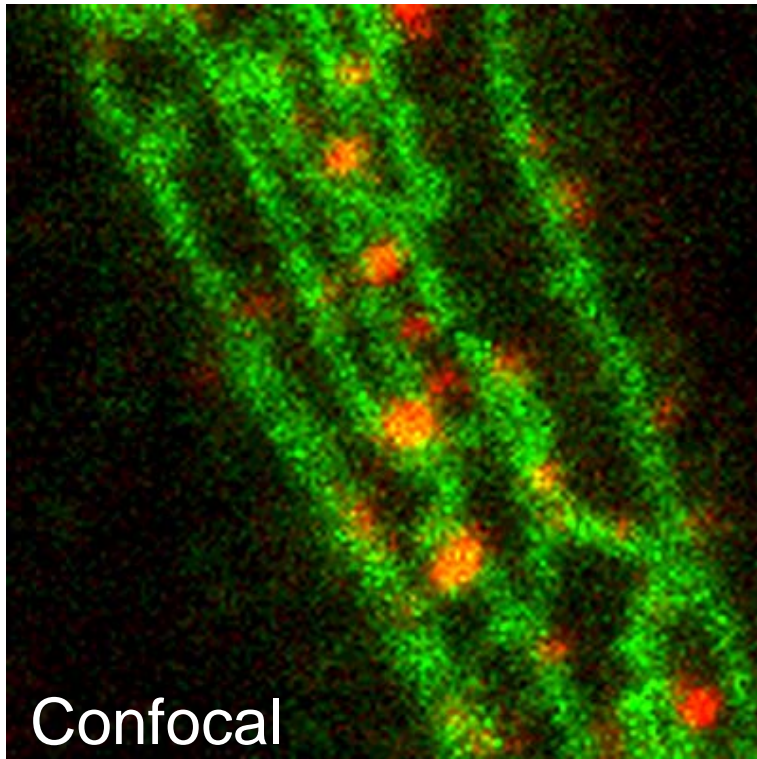
Lateral resolution:

$$\Delta_{xy} = \frac{\lambda}{2n \sin \alpha} \approx 200 \text{ nm}$$

Axial resolution:

$$\Delta_z = \frac{n\lambda}{NA^2} \approx 500 \text{ nm}$$

Leica SP8 (Lightning)

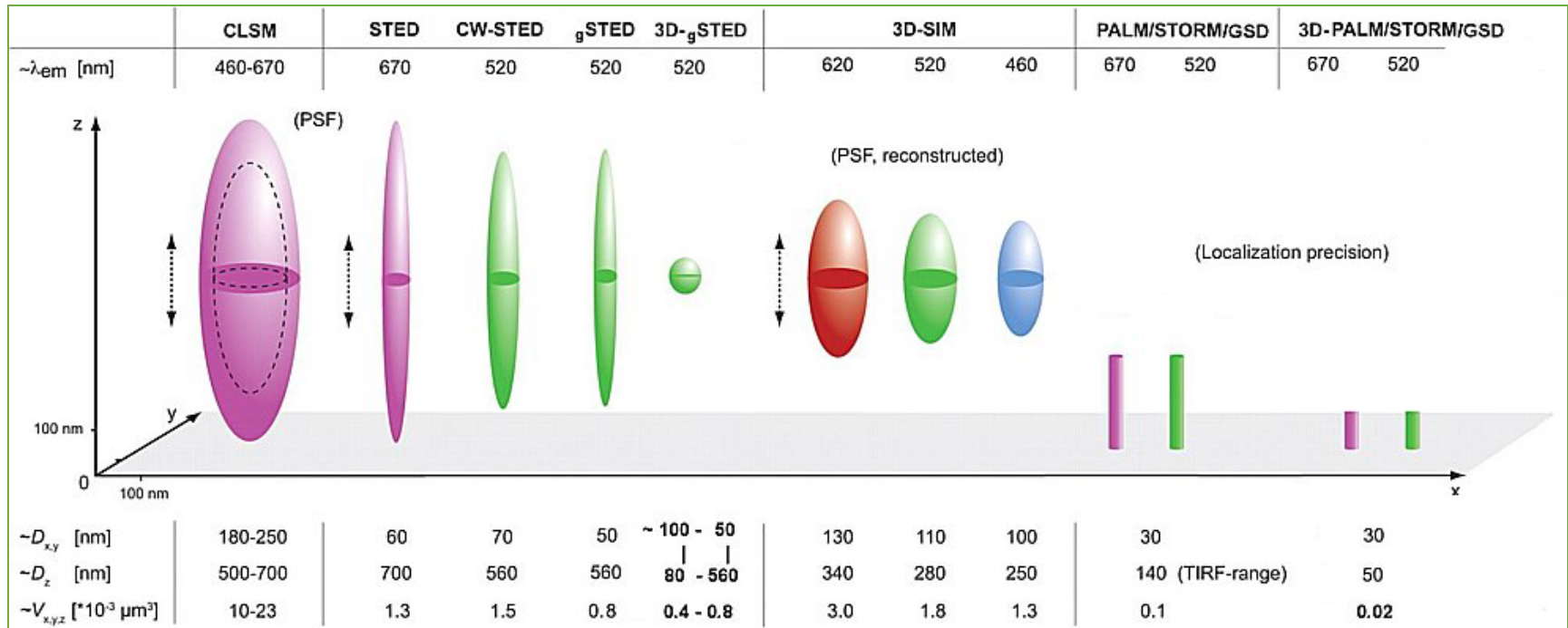


Rosso: dsDNA

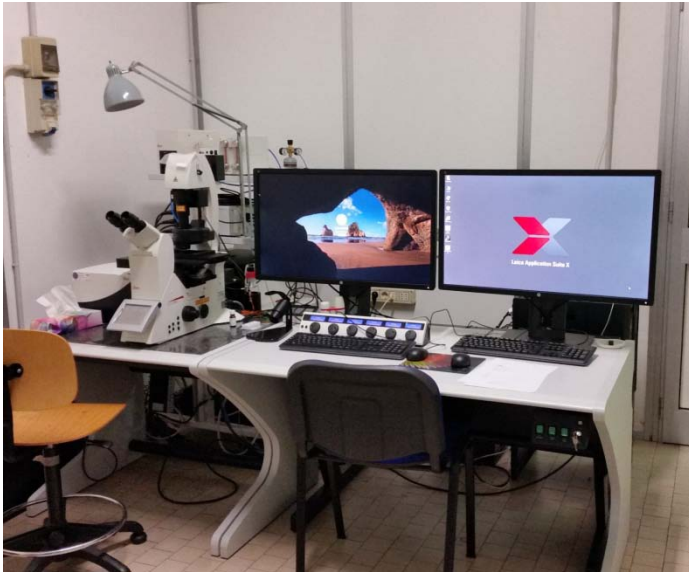
Verde: TOM20

Le immagini sono state gentilmente fornite
dalla dott.ssa Lara Gibellini del gruppo
Cossarizza.

Super-resolution microscopy

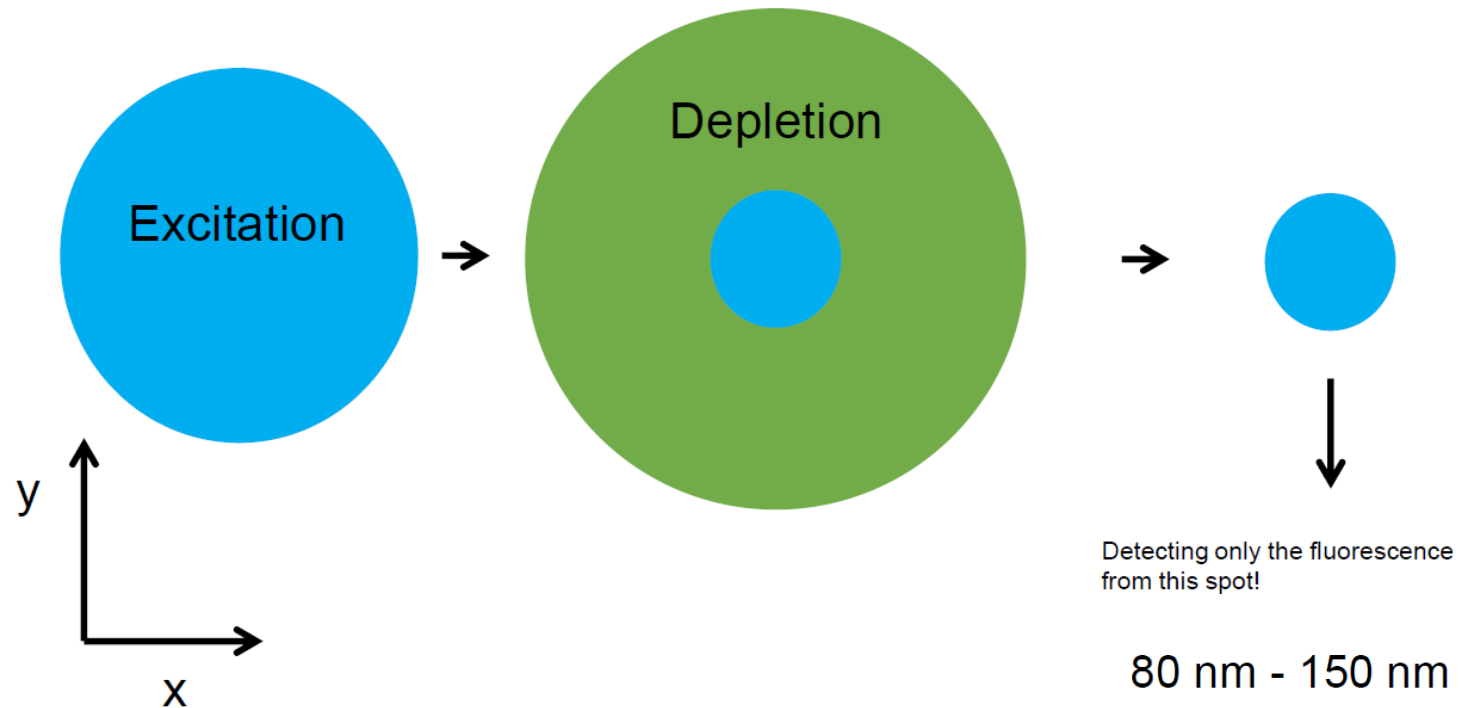


Leica SP8 (STED Upgrade)

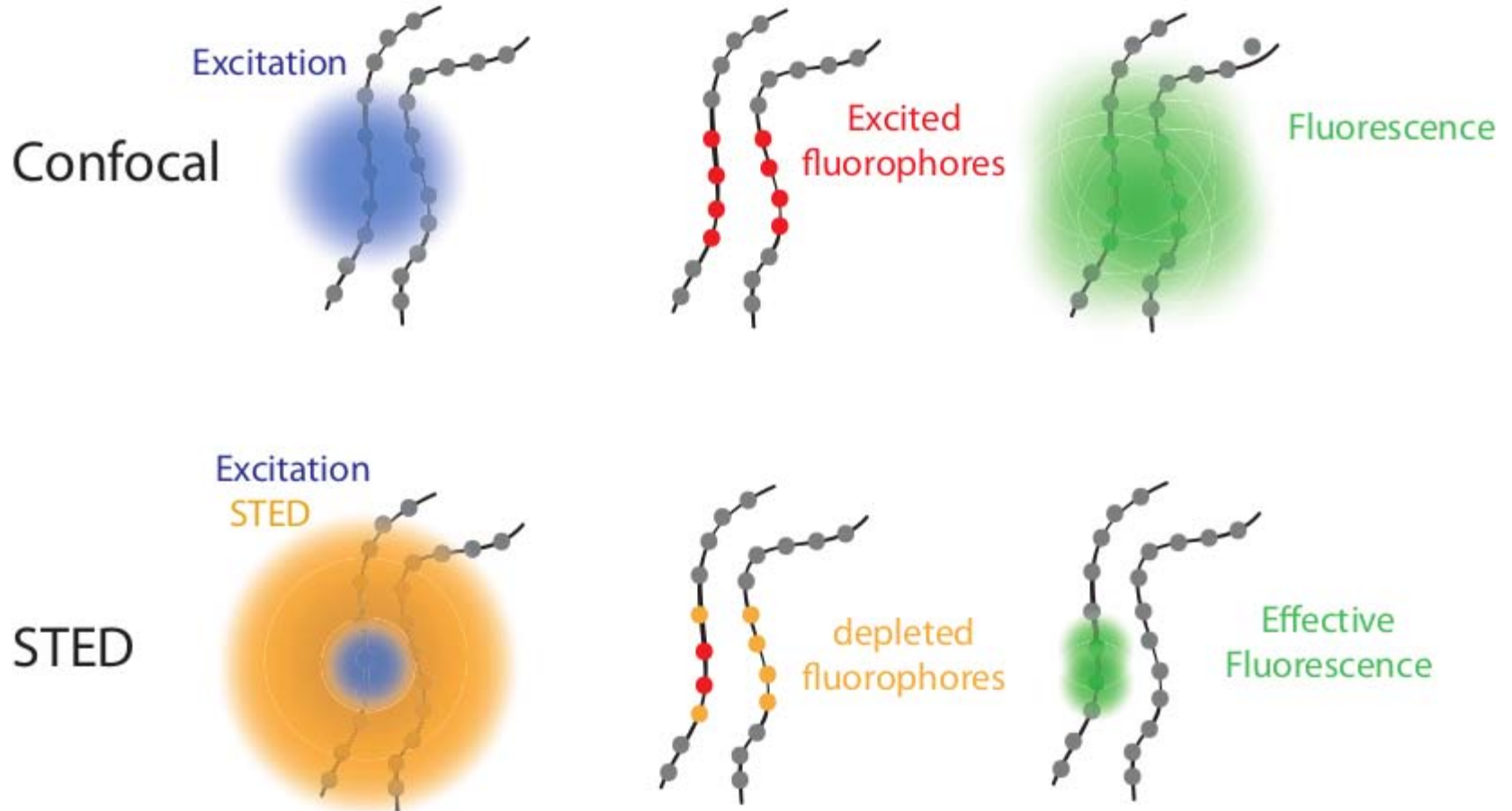


Gated-STED 3D con laser di deplezione 775nm

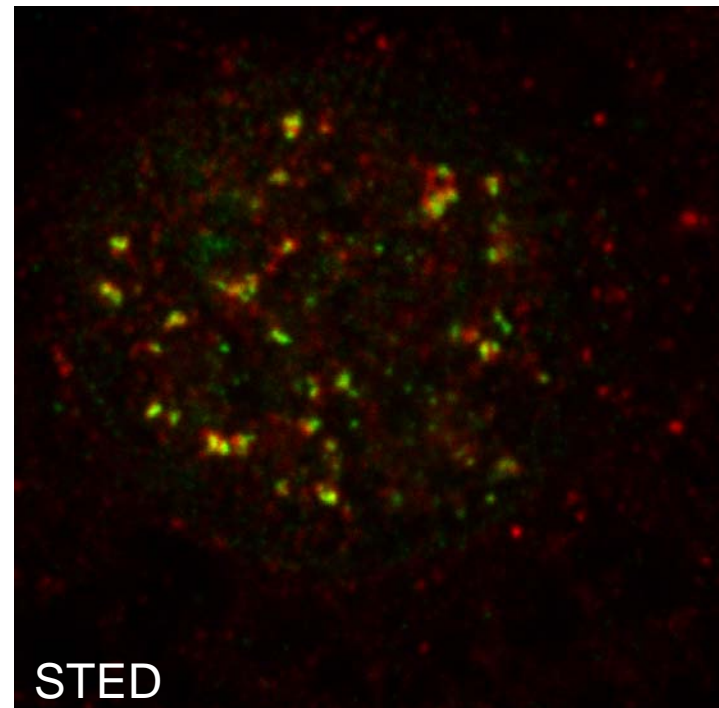
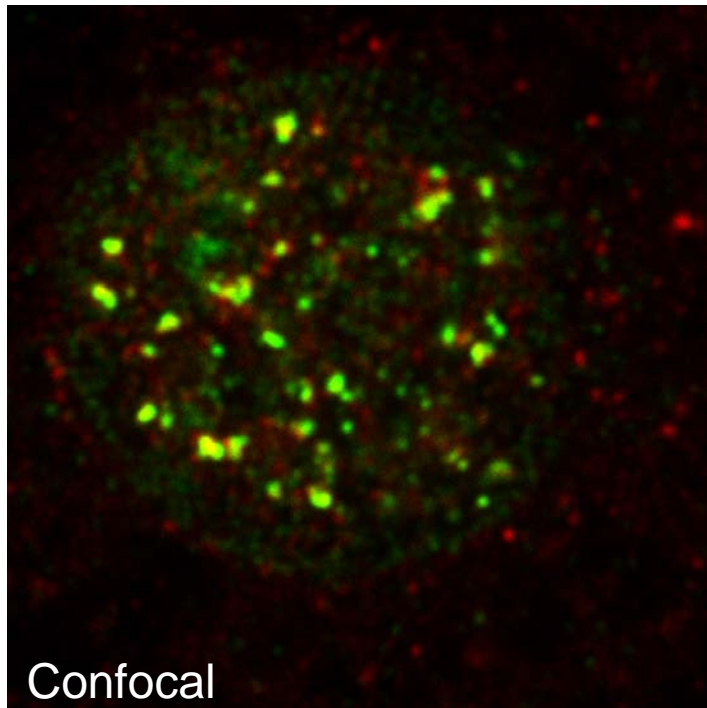
Leica SP8 (STED Upgrade)



Leica SP8 (STED Upgrade)



Leica SP8 (STED)



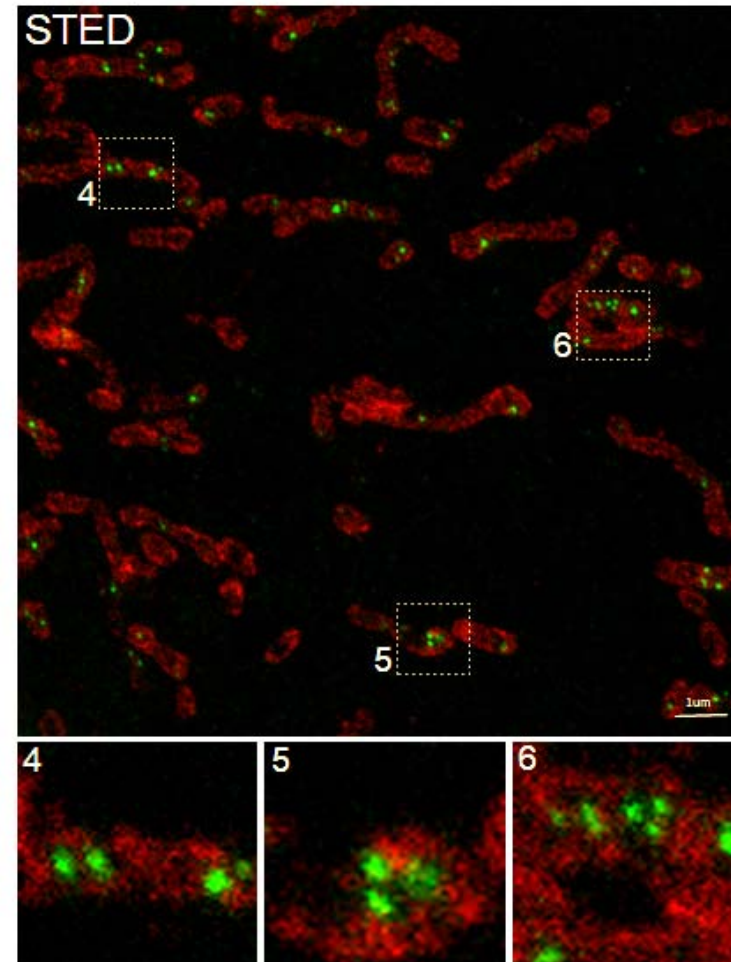
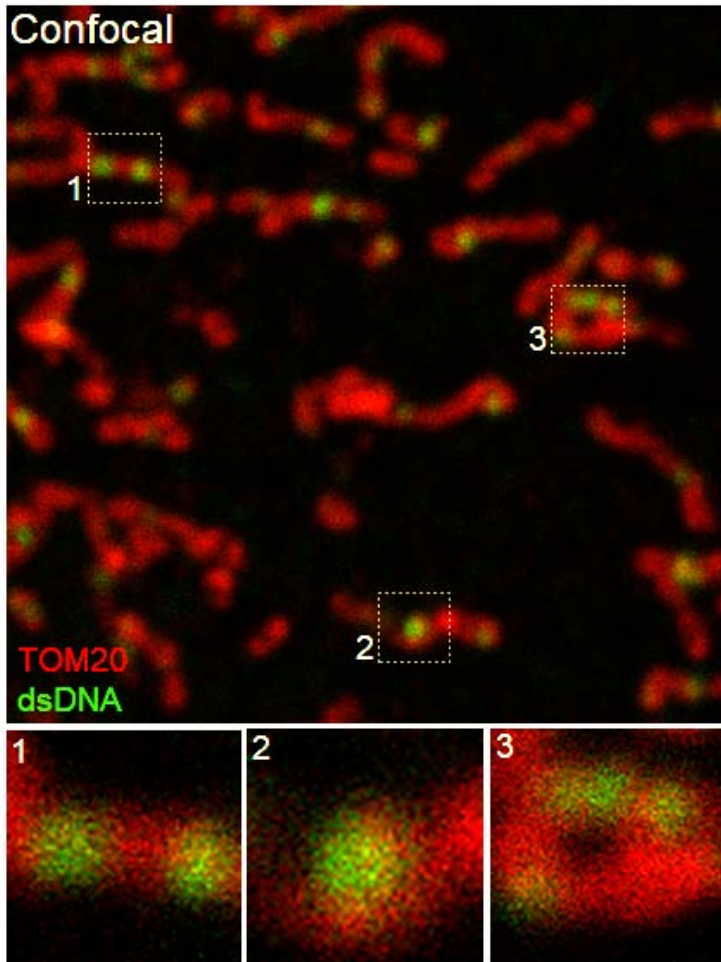
Rosso: DRiPs (azide-594)

Verde: PML (Alexa 647)

Le immagini sono state gentilmente fornite dal gruppo Carra.

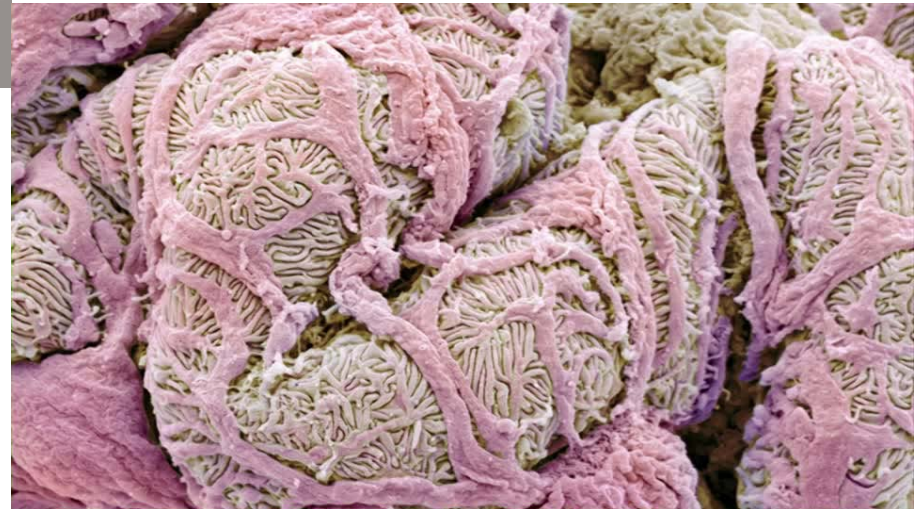
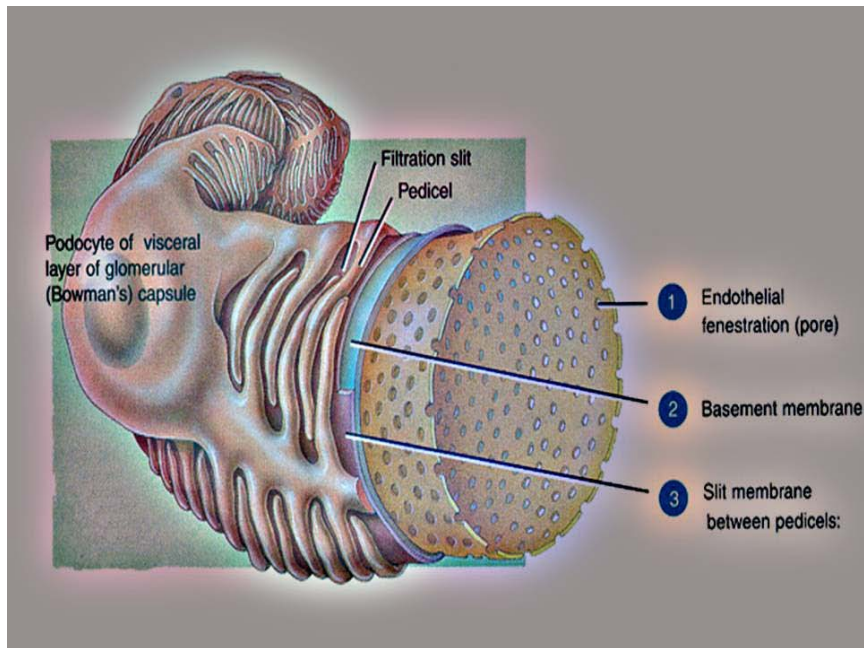
Mediani et al., EMBO J, 2019

Leica SP8 (STED)



Le immagini sono state gentilmente fornite dalla dott.ssa Lara Gibellini del gruppo Cossarizza.

Leica SP8 (STED)



Leica SP8 (STED)

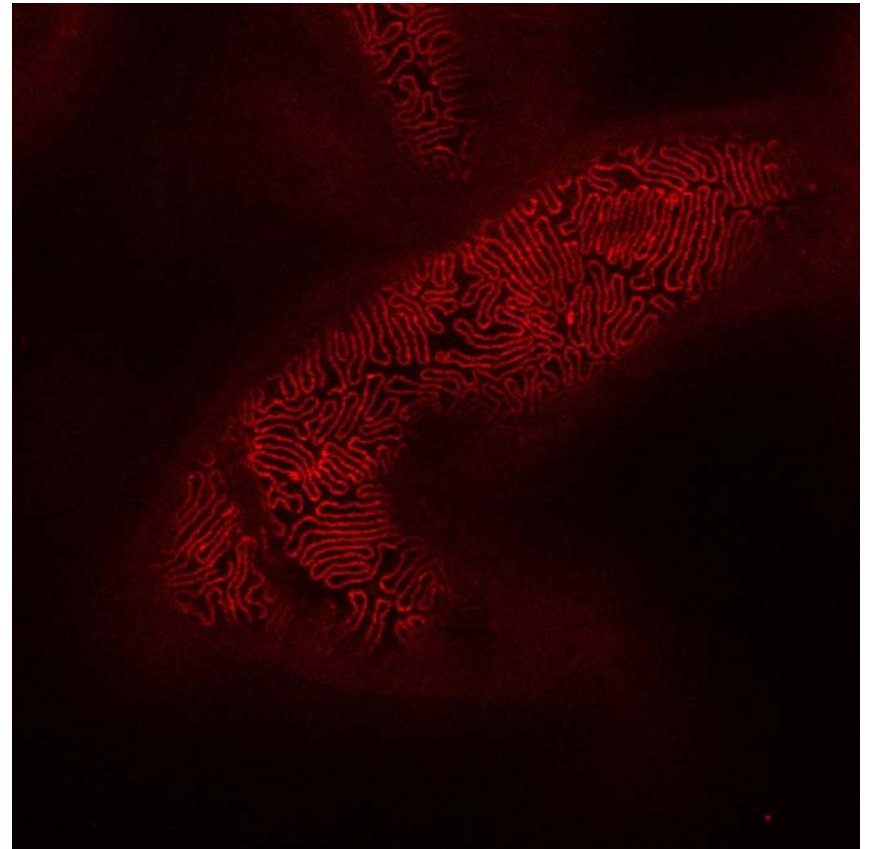
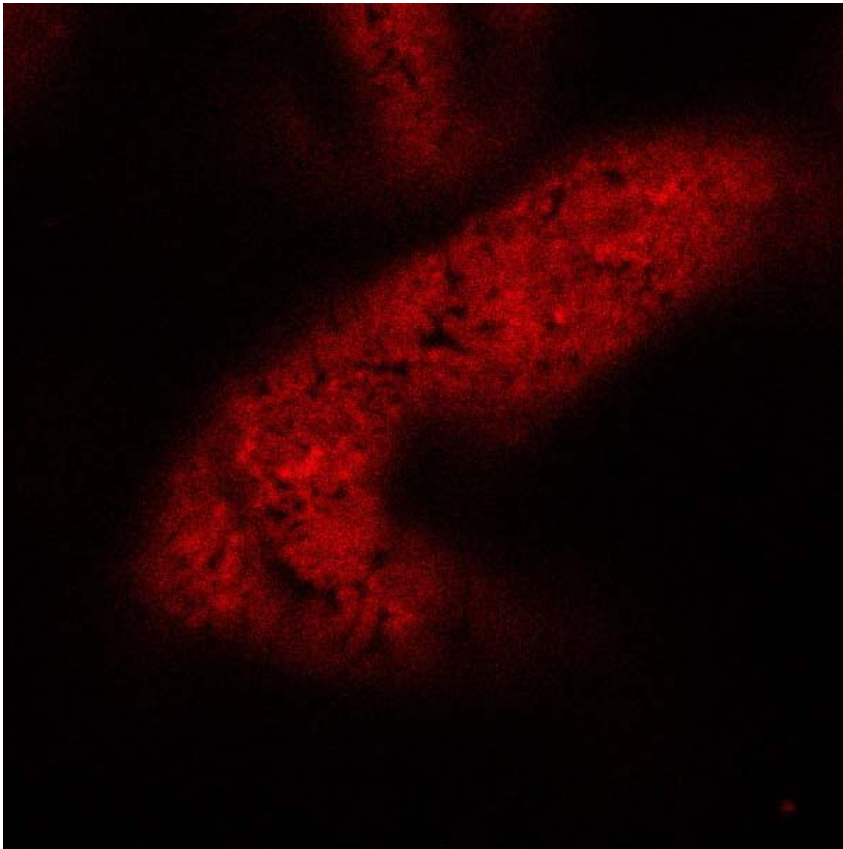


Immagine di podociti in un glomerulo di rene di topo

Considerazioni tecniche (STED)

Usare anticorpi primari che danno un SNR molto alto

Usare anticorpi/soluzioni non contaminati

Usare coverslips 1.5H

Usare Mowiol come mounting medium

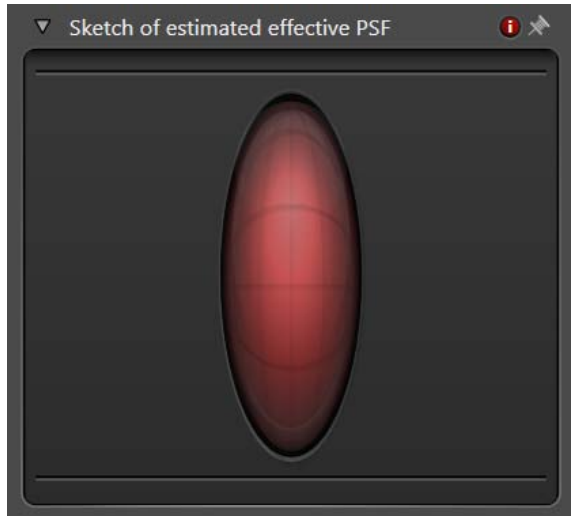
Usare smalto trasparente per sigillare

Non tutti fluorofori sono adatti per la STED:

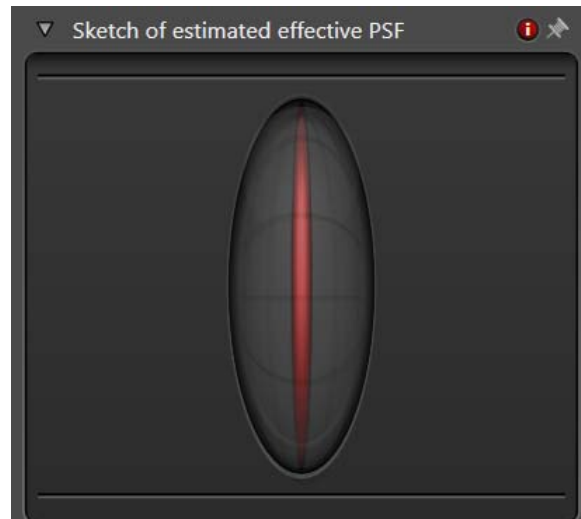
ATTO 647N, STAR 635P, Alexa 594, ATTO 565, Alexa 568

Pensare se si vuole fare STED 2D o STED 3D.

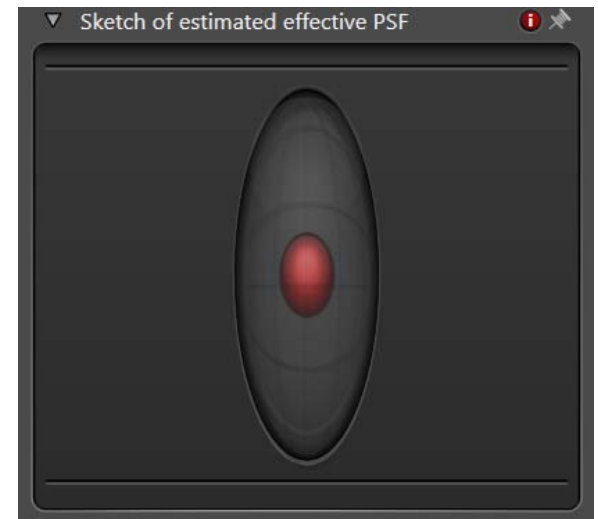
Considerazioni tecniche (STED)



Confocale

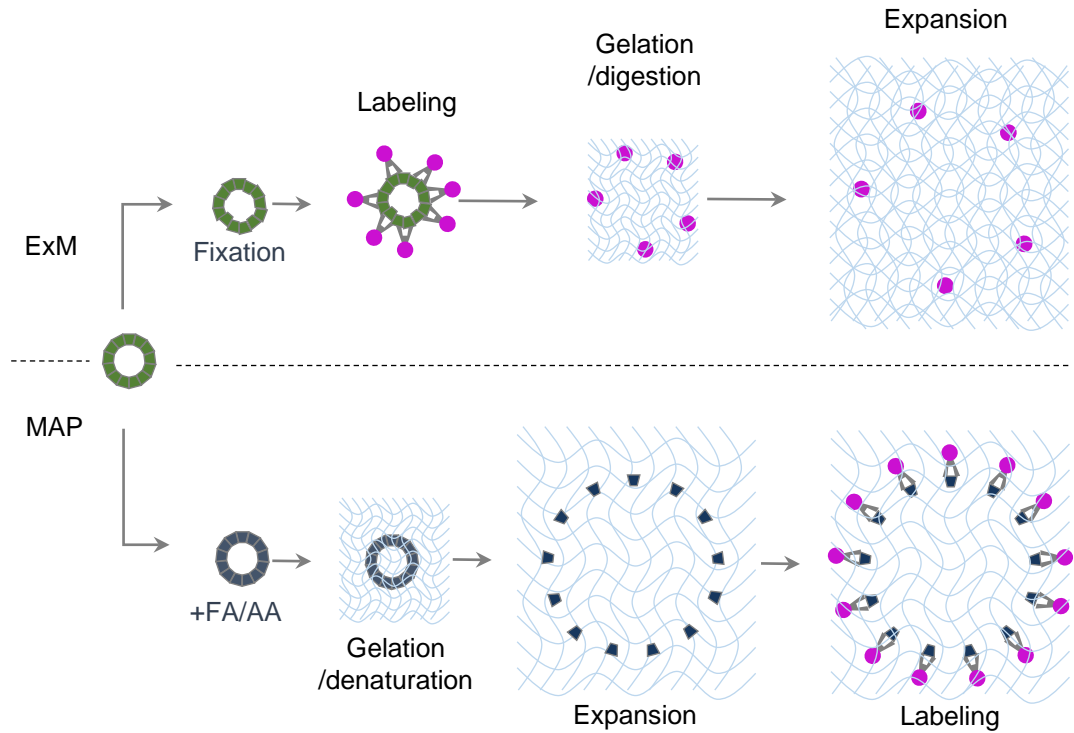
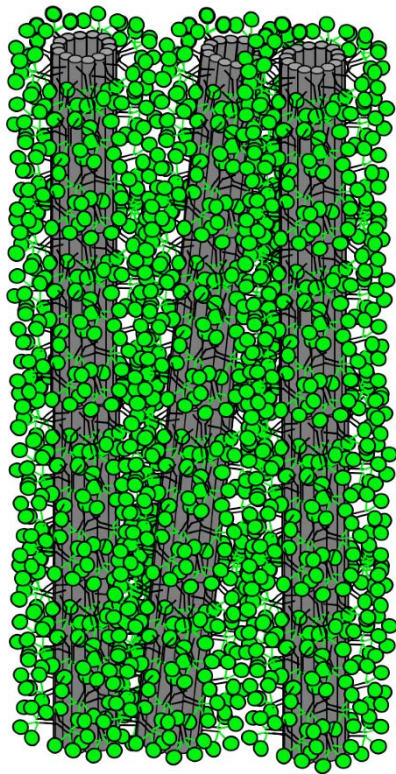


STED 2D



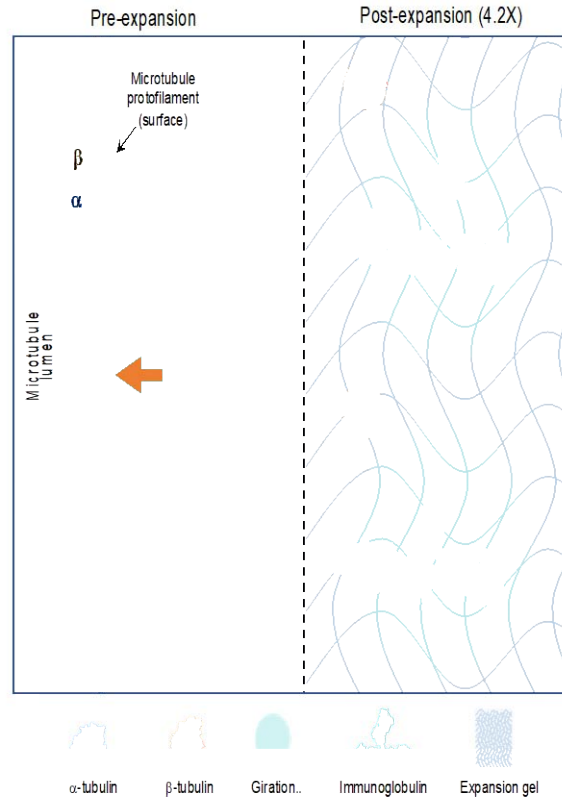
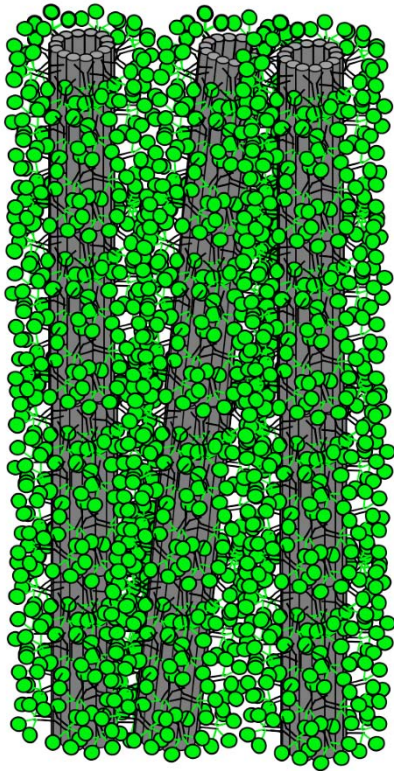
STED 3D

ExM (Expansion Microscopy)

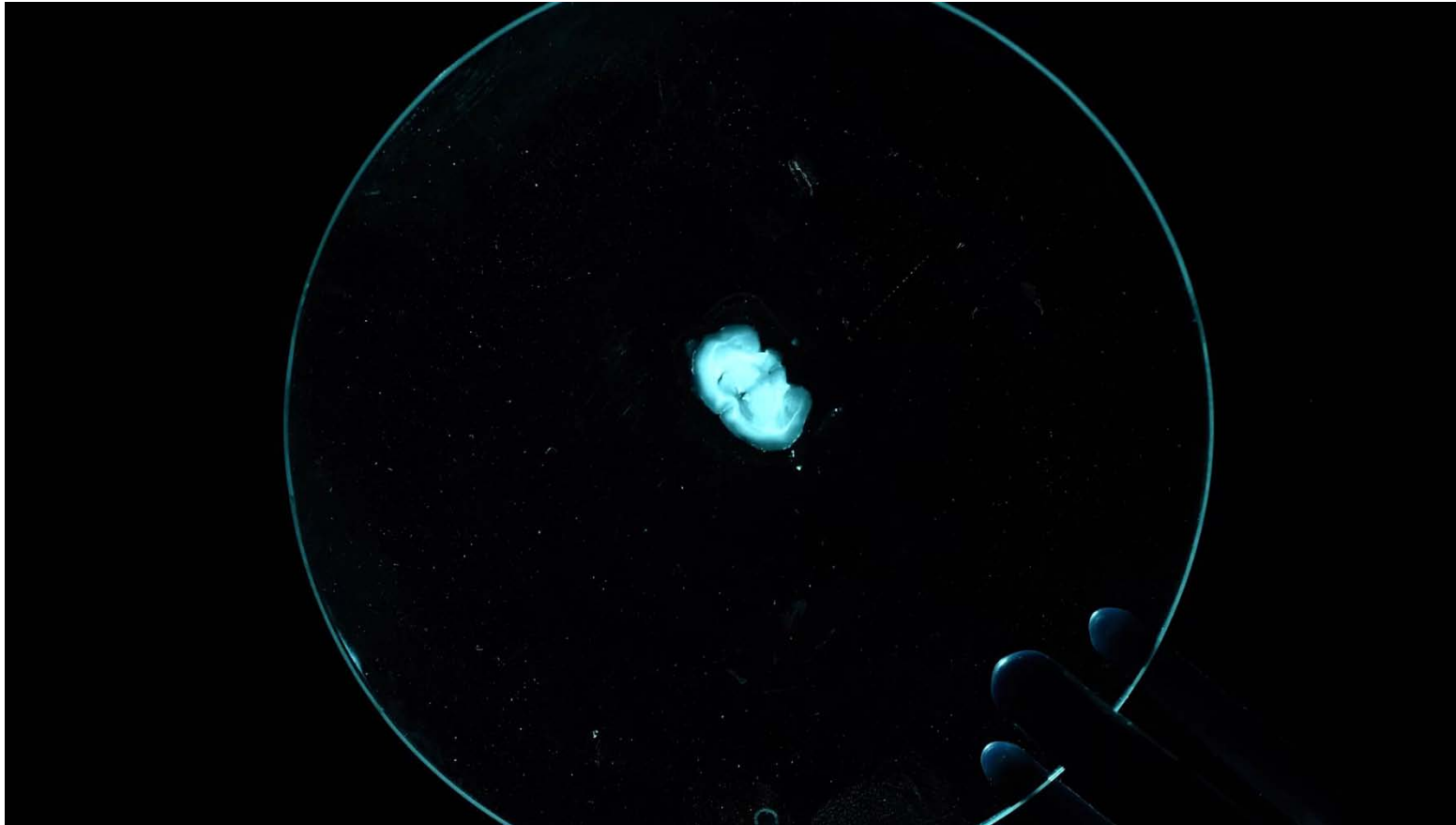


2. NO fluorescence density reduction

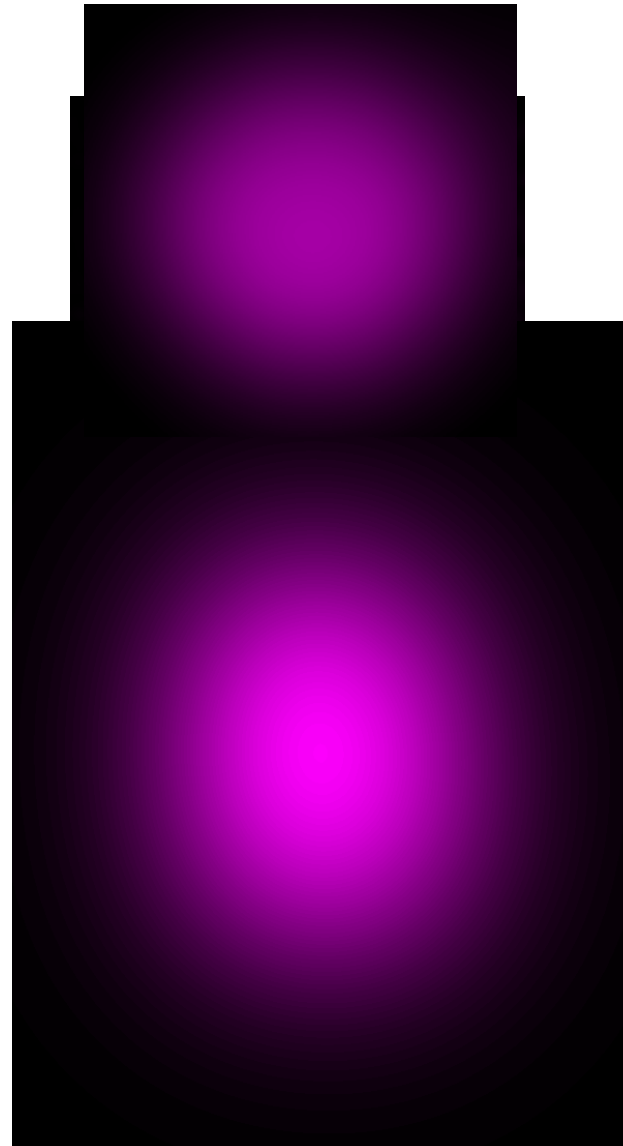
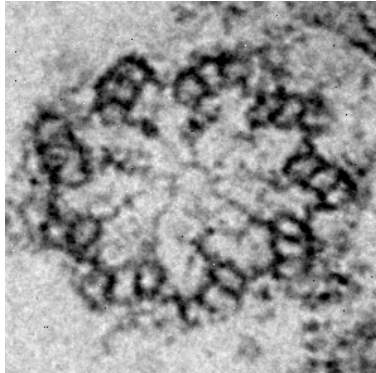
ExM (Expansion Microscopy)



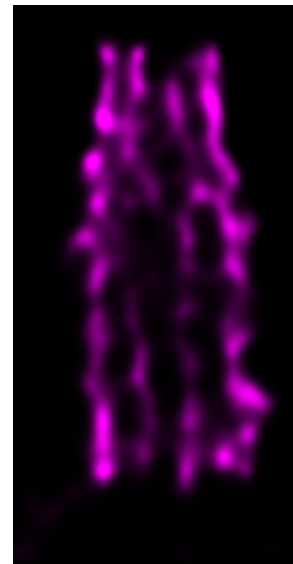
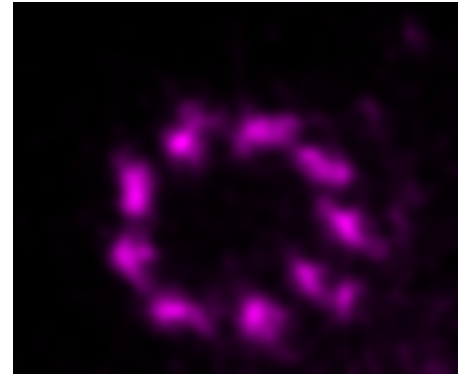
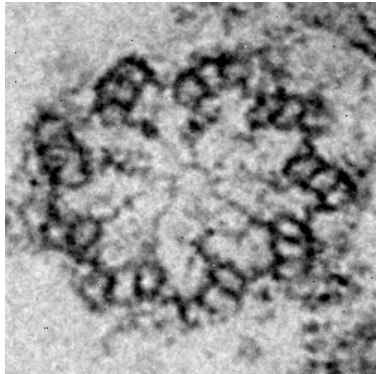
ExM (Expansion Microscopy)



ExM (Expansion Microscopy-Centrosome)



ExM (Expansion Microscopy-Centrosome)



Nikon A1



Microscopio rovesciato da ricerca Nikon Ti2-e con AFC e XYZ motorizzati

Testa di scansione A1R Resonant 512x512 (32fps) o 1024x1024 (16fps)

Sistema con 4 laser e filtri (blu, verde, rosso, farRed)

Sistema di incubazione Top-Stage OKOLAB

Advanced **time-lapse experiments**

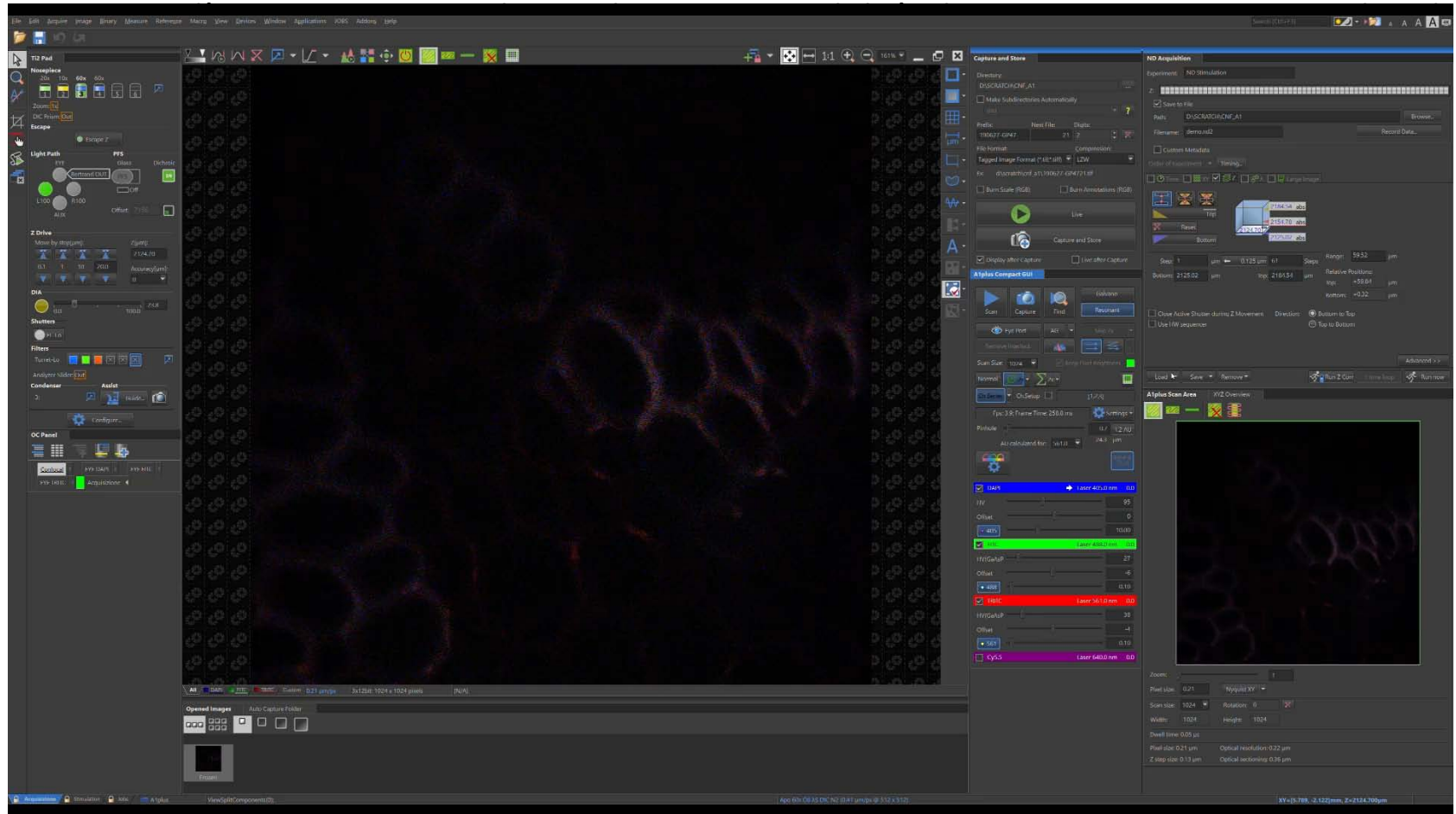
Funzione Mosaic microscopy

FRAP (Fluorescence Recovery After Photobleach)

Multicolor analysis

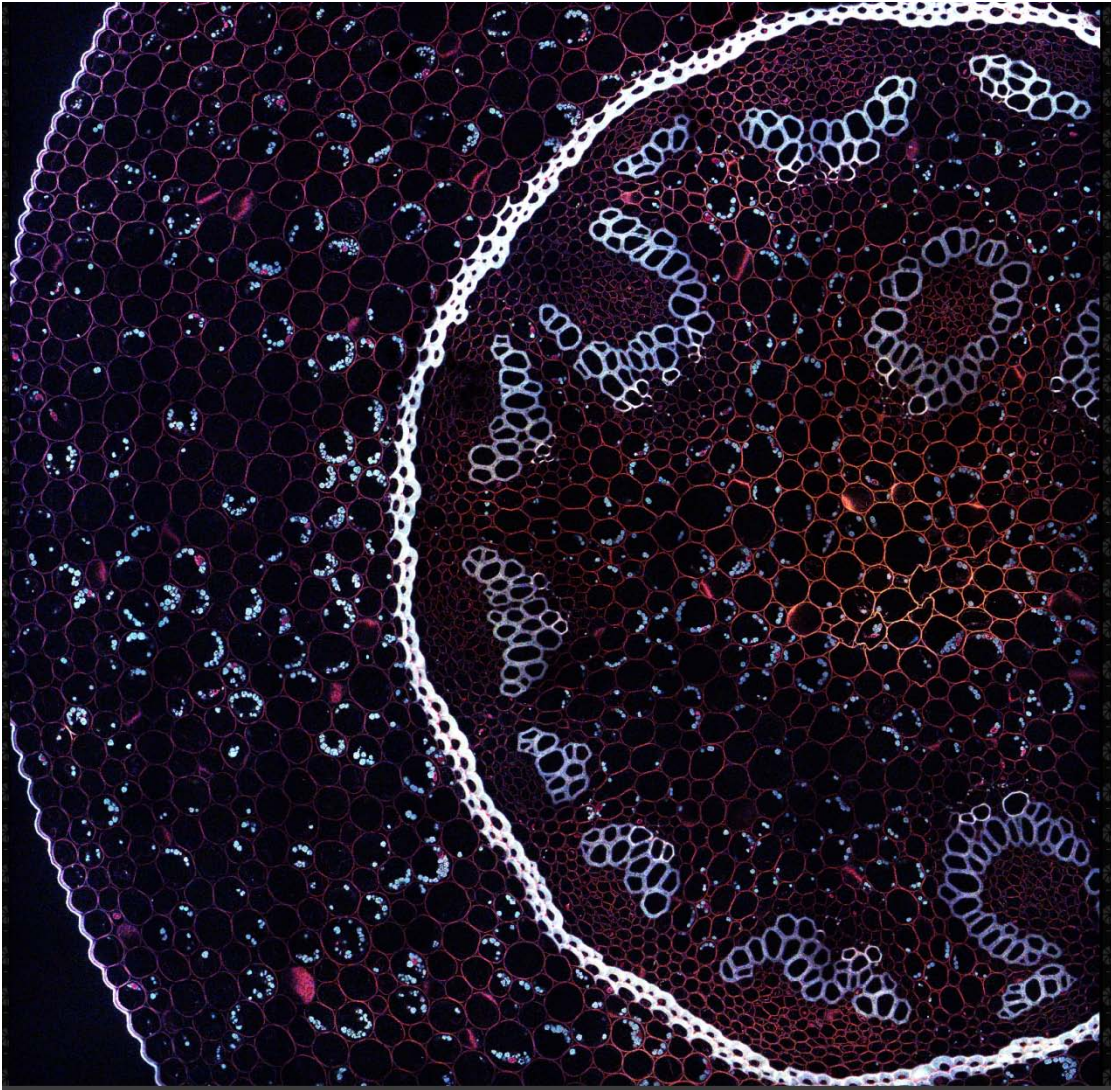
Photo Activation/Conversion Experiments

Nikon A1 (Resonant Scanner)



Z-stack di 60 immagine (3 canali, avg 2) acquisite in ca 30 sec

Nikon A1 (Resonant Scanner)



Mosaico 8X8 acquisito con obiettivo
60X

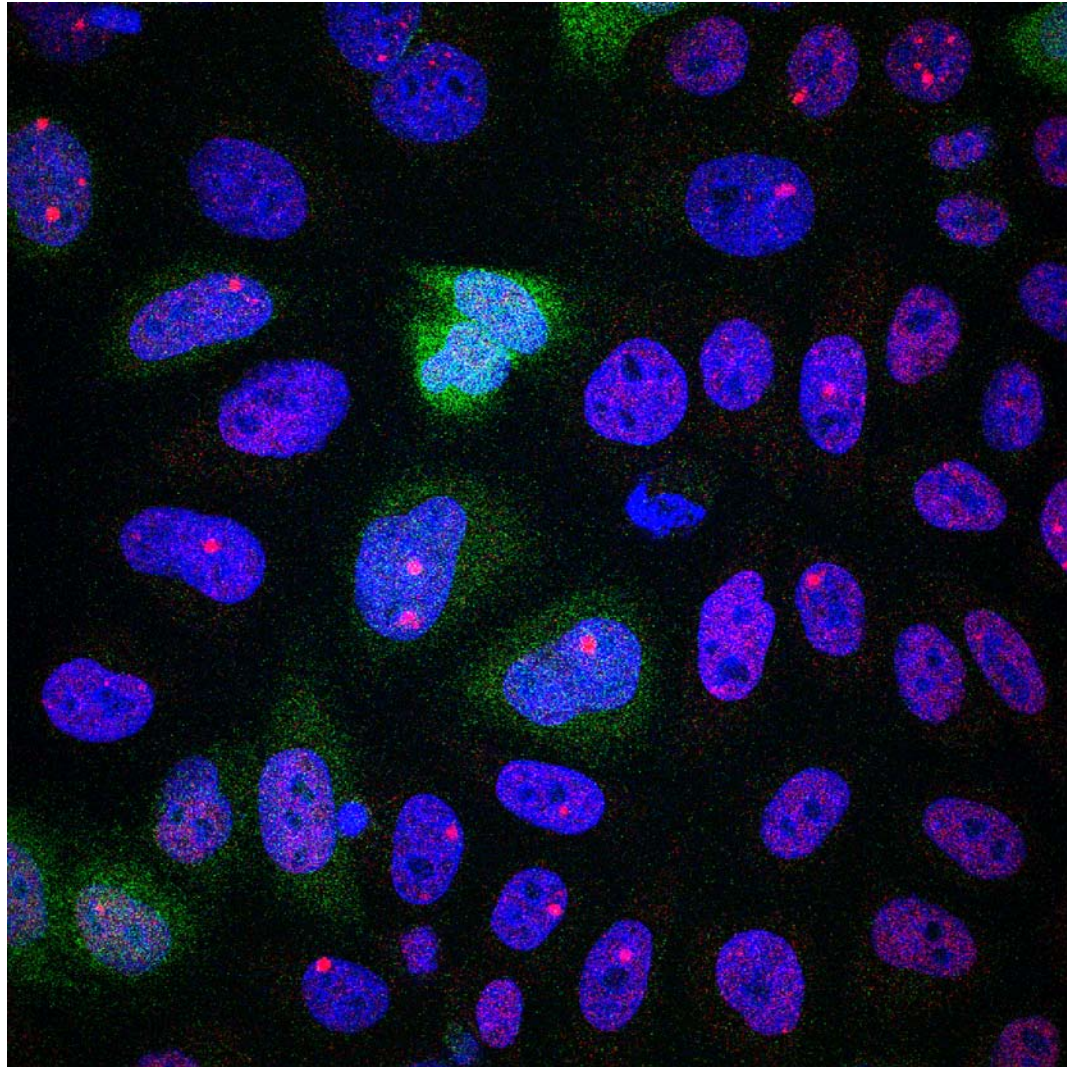
Durata del processo: 30sec

Nikon A1 (JOBS)

The screenshot displays the Nikon A1 software interface, organized into several functional panels:

- Well Plates:** Includes options like Define Plate, Select Wells, Loop over Wells, Label Wells, Align Plate, Manual Plate List, Loop over Plates, Move to Well, Move to Well Center, and Move to Previous/Next Well.
- Sample Holder:** Includes Define Slide, Define Stage Area, Define Stage Area - Legacy, Align Slide, Manual Slide List, Loop over Slides, Time Lapse, Redefine Time Lapse, Time Sequence, Repeat N Times, Generate Points, Predefined Points, New Point Set, Loop over Points, Move to Point, Move to Previous/Next, Offset Point Set, Add Point to Point Set, Remove Point from Point Set, Append Point Set to Point Set, Clear Point Set, Add/Edit Points Manually, Append Point Set to Point Set, Clear Point Set, Export Point Set to ND, and Import Point Set from ND.
- Large Images:** Includes Scan Large Image, Scan Large Image in Slide/Well/Area, Scan Large Image in Region, Draw Regions, Shift Region, Edit Regions, Region List, Loop over Regions, Move to Region Center, Append Region List to Region List, and Clear Region List.
- Z-Stack:** Includes Define Z-Stack, Z-Stack, Z-Stack Loop, Assign Z-Stack to Point/Well/ND Acq, and Move to Z-Stack home.
- ROIs:** Includes Define ROI, Use ROI from Analysis, and Assign ROI to Point/Well.
- Autofocus + Focus Surface:** Includes Autofocus Settings, Autofocus, Create Focus Surface, Move to Focus Surface, Offset Focus Surface, Assign Current Z to Point/Well/Point, Escape Z, Refocus Z, Get Current Z Position, and Move to Z Position.
- PFS (Point Focus Surface):** Includes PFS On and Focus, PFS Off, PFS Offset from the current z position, Auto PFS Focus Setup, Auto PFS Focus, Assign PFS to Point/Well, Create PFS Surface, Move to PFS Surface, and Offset PFS Surface.
- Acquisition:** Includes Capture Definition, Capture, Capture Current OC, Capture HDR, Capture Current OC HDR, ND Acquisition, Fast Timelapse, Triggered Experiment, Live Window, Merge and Store Captured Images, Detect Background, and Save Captured Image.
- Stimulation:** Includes Sequential Stimulation and Simultaneous Stimulation.
- Optical Configurations:** Includes Select Optical Configuration, Auto Brightness, Set Exposure to Optical Configuration, Adjust Camera Settings Manually in C, Assign Camera Settings to Point/Well, and Interpolate Exposure between Wells.
- Conditions:** Includes Close Shutter and Open Shutter.
- Conditions Panel (Right):** Includes IF Condition (If), IF ELSE Condition (If-Else), Every nth, Break, System (Question, Macro, Expression, Variables, Custom Metadata), Wait, Comment, Send E-mail Notification, Send SMS Notification, Create Labels, Assign Label, Debug, Phase, Finish, Abort, Execute Macro After Run, Alternative Storage Location, Storage, Analysis (Define Analysis, General Analysis, Cell Count Analysis, Intensity Analysis, General Analysis RGB, Out Proc, Wound Healing, Live/Dead Analysis), and Device Control (Wait for I/O Event).

Nikon A1 (JOBS)



Nikon A1 (Piano Sviluppo)

Acquistare una telecamera per poter fare foto sia in fluorescenza (non confocale) che in luce trasmessa. Questa telecamera sarà compatibile/controllabile con il software Nikon.

Acquistare un sistema DIC (per tutti gli obiettivi) per poter fare contrasto di fase.

IL SISTEMA È A USO GRATUITO FINO A GIUGNO 2020



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Olympus ScanR (analisi d'immagini)

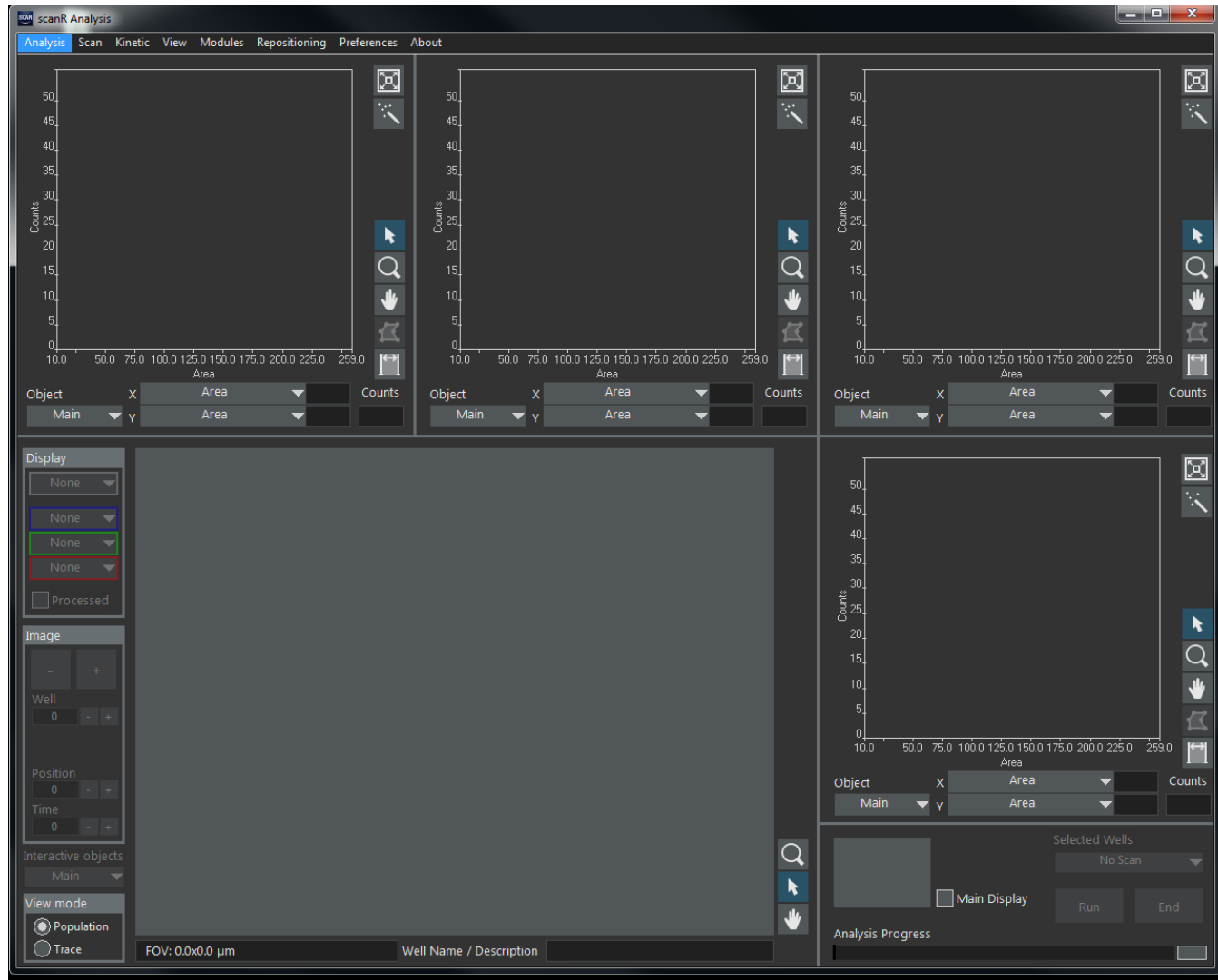


Sistema di analisi collegato a microscopio a fluorescenza x analisi di screening.

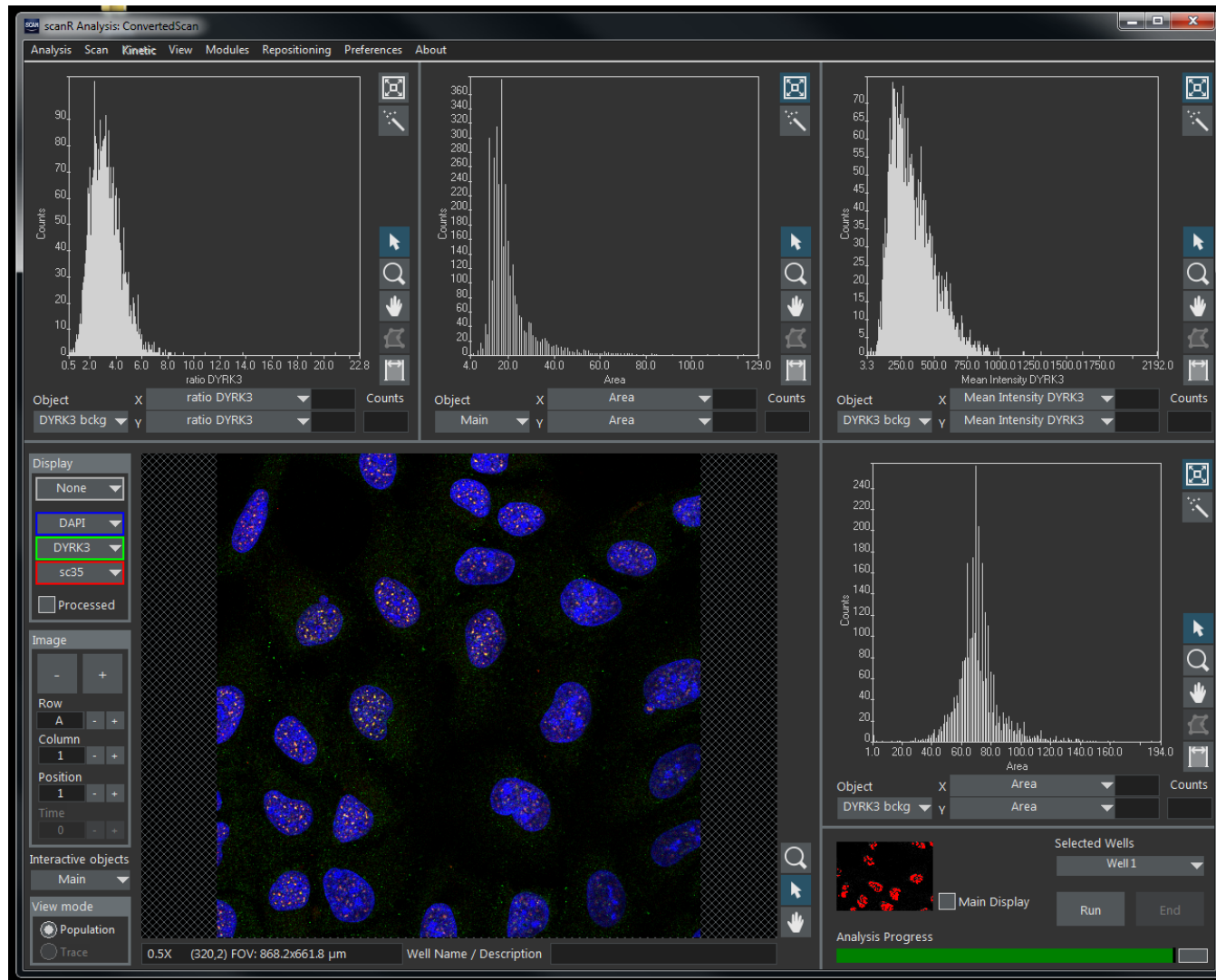
Software di analisi molto potente che gli utenti hanno chiesto di poter usarlo offline.

Olympus ha prodotto un sistema di conversione d'immagine per poter analizzare le immagini acquisite da qualsiasi sistema.

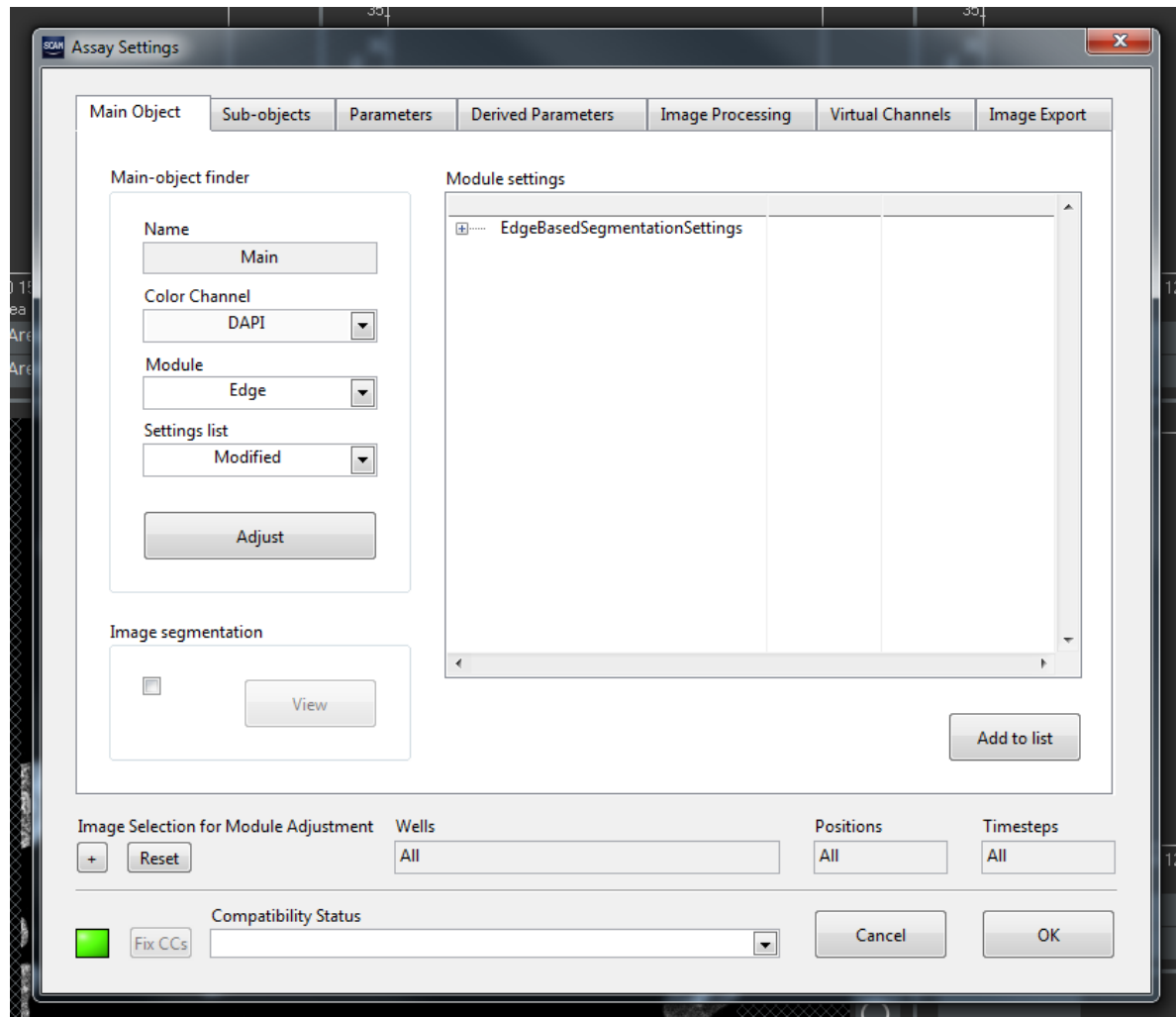
Olympus ScanR (analisi d'immagini)



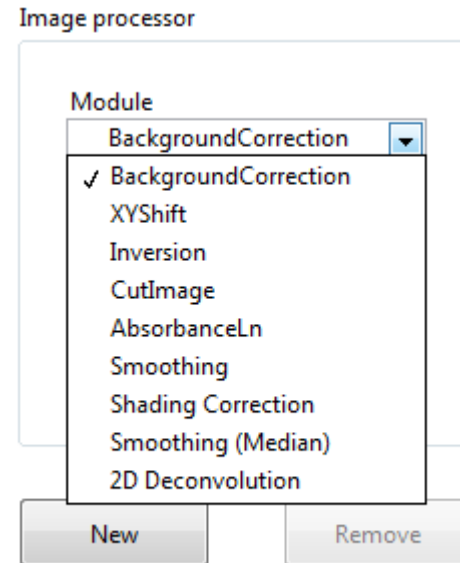
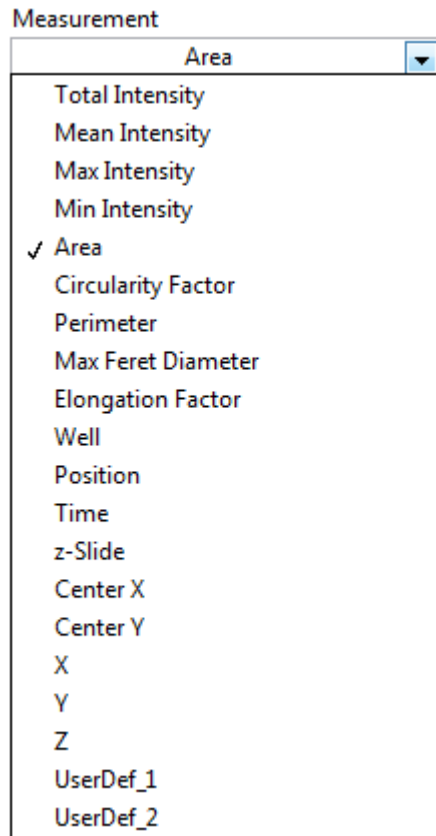
Olympus ScanR (analisi d'immagini)



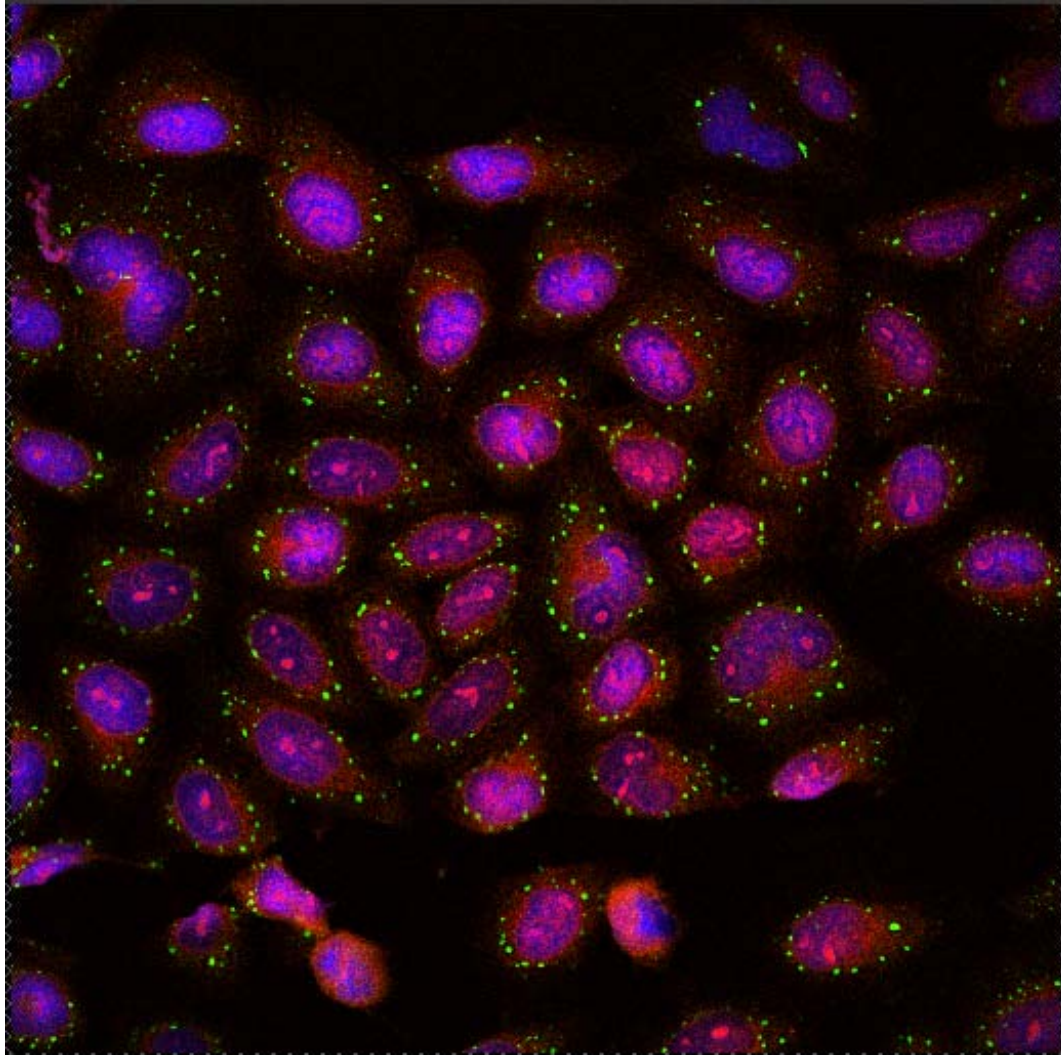
Olympus ScanR (analisi d'immagini)



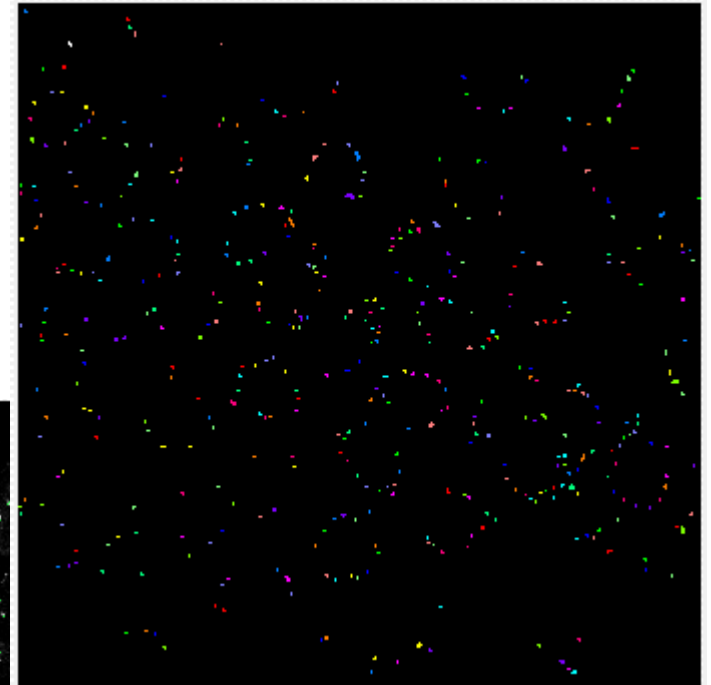
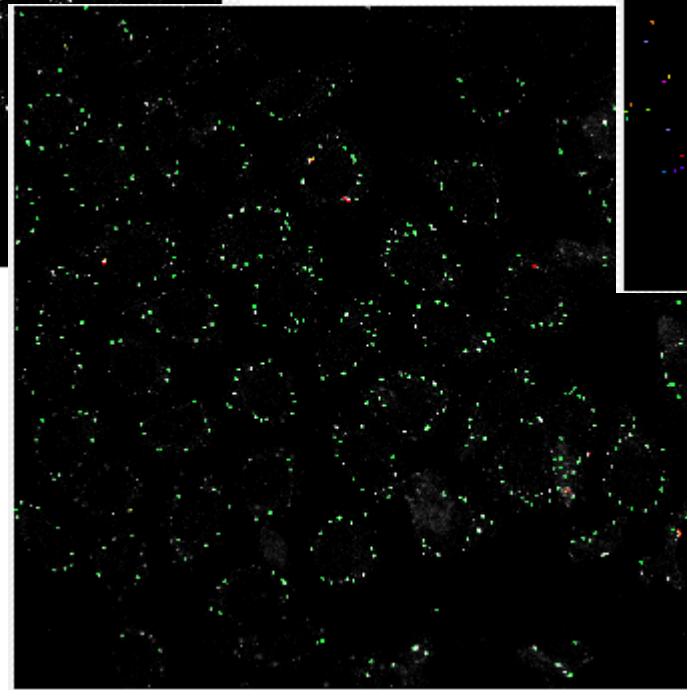
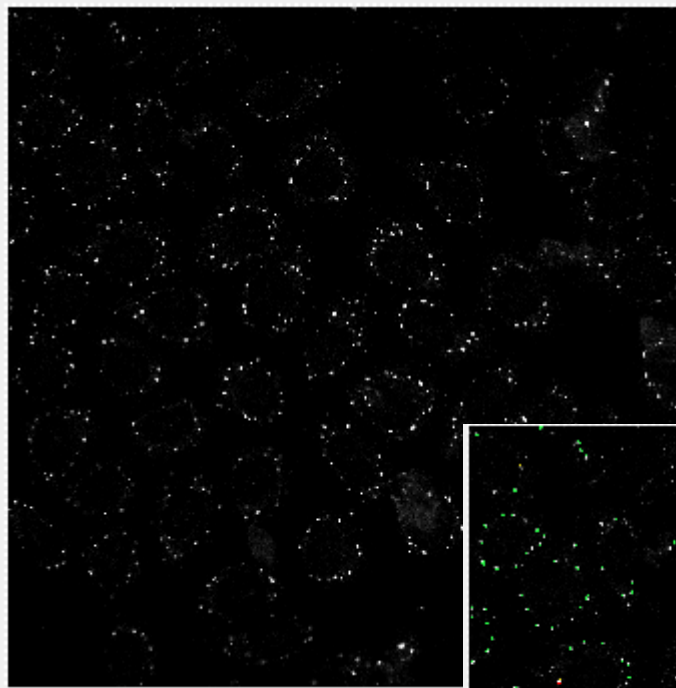
Olympus ScanR (analisi d'immagini)



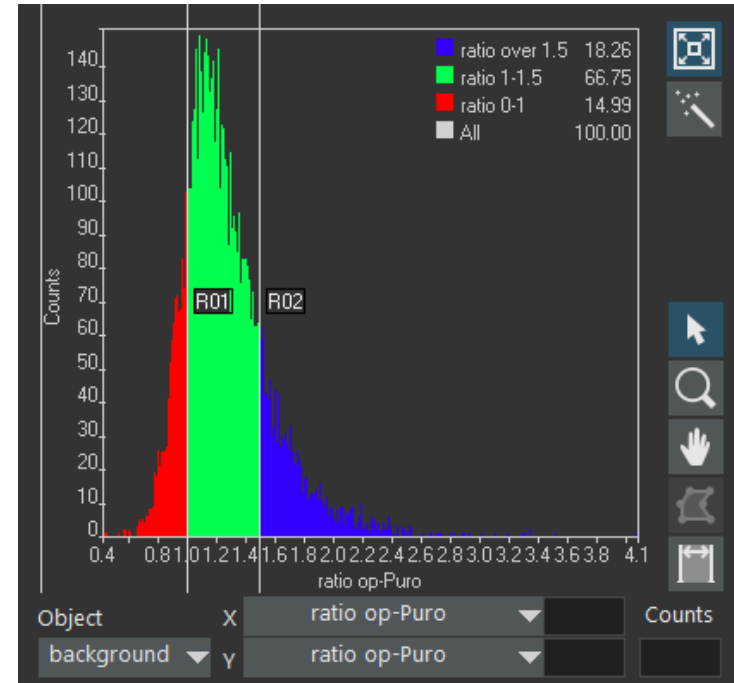
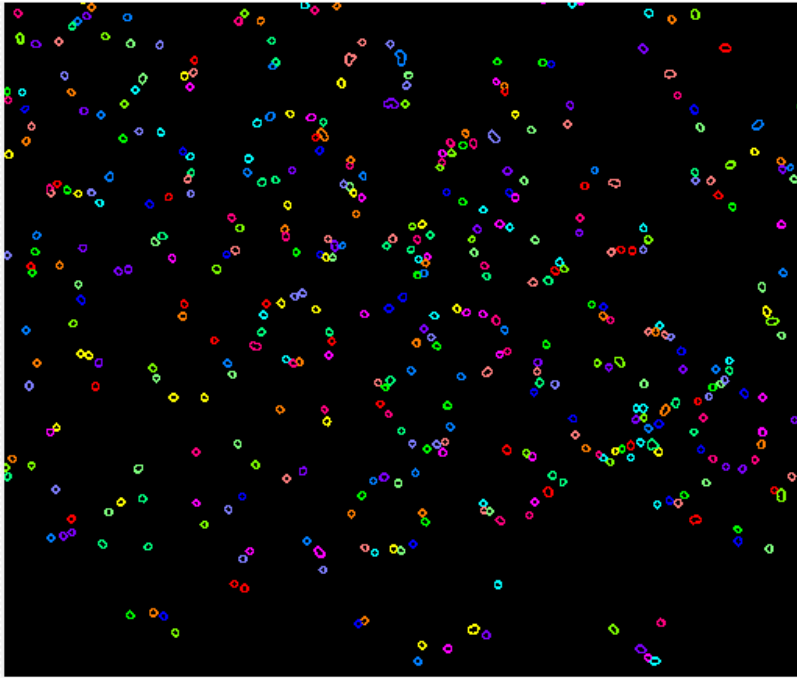
Olympus ScanR (analisi d'immagini)



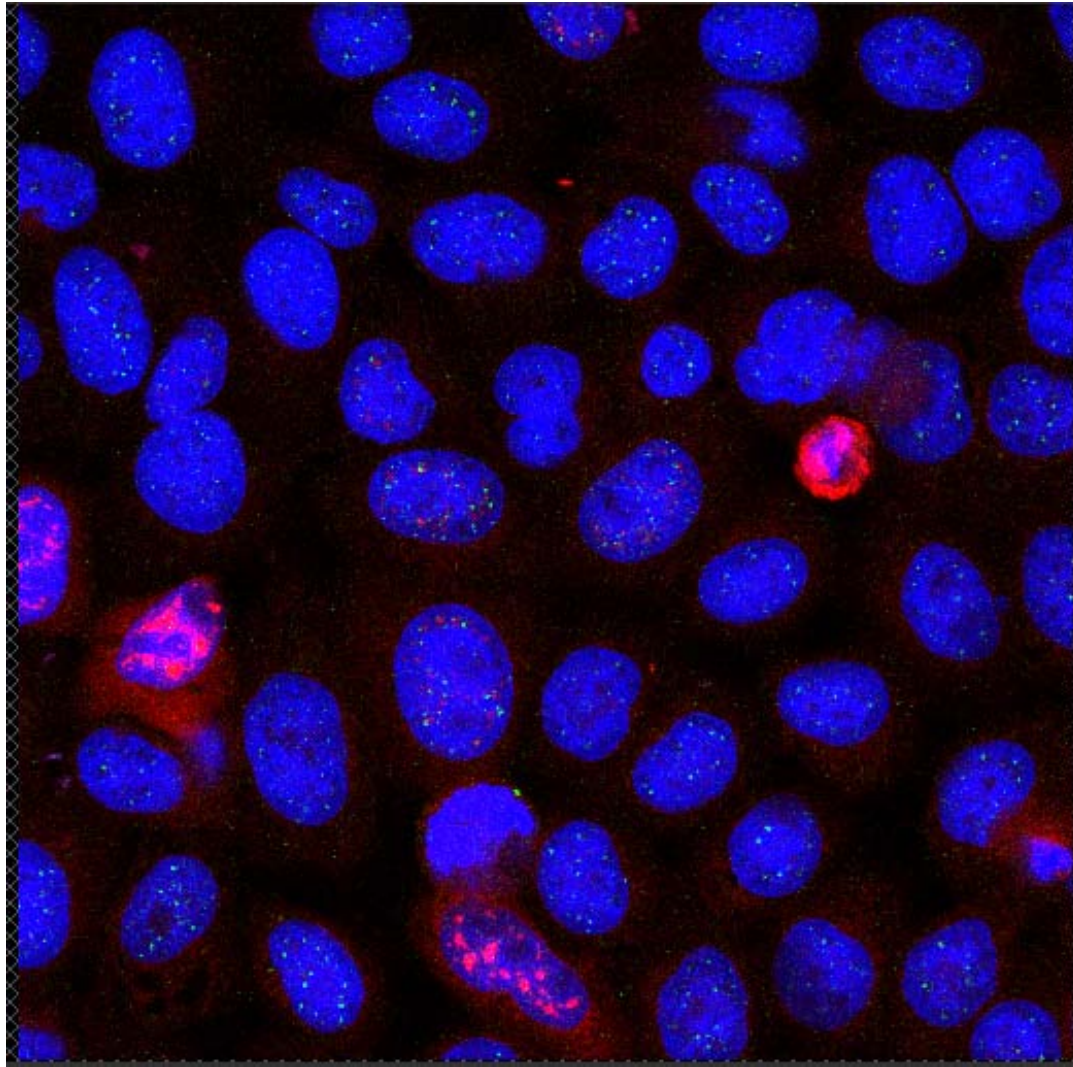
Olympus ScanR (analisi d'immagini)



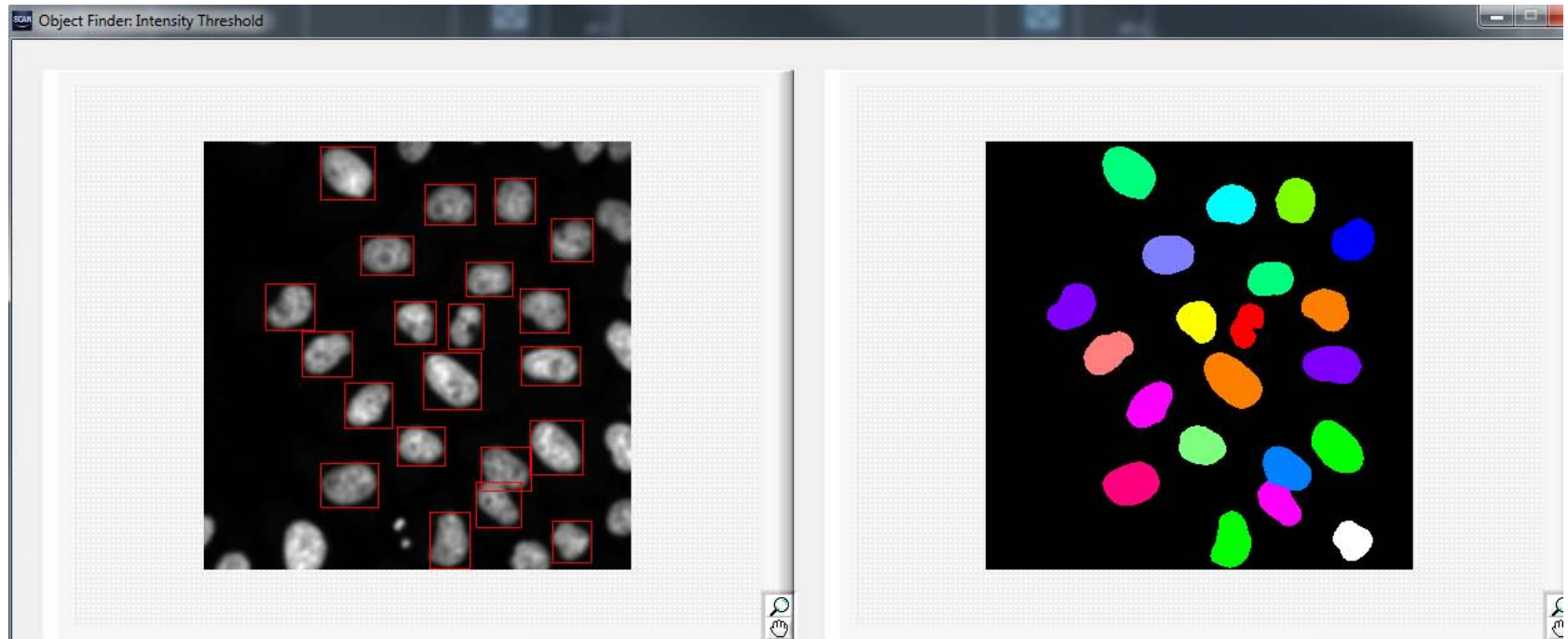
Olympus ScanR (analisi d'immagini)



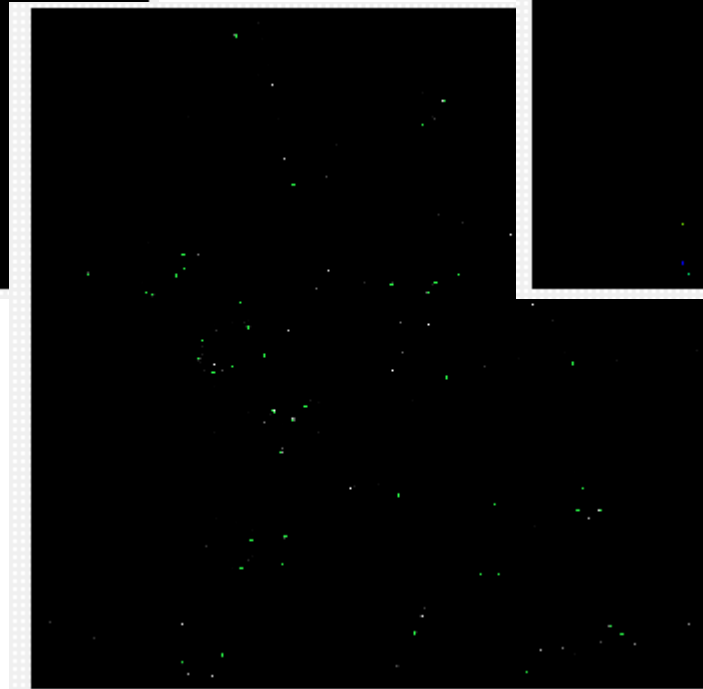
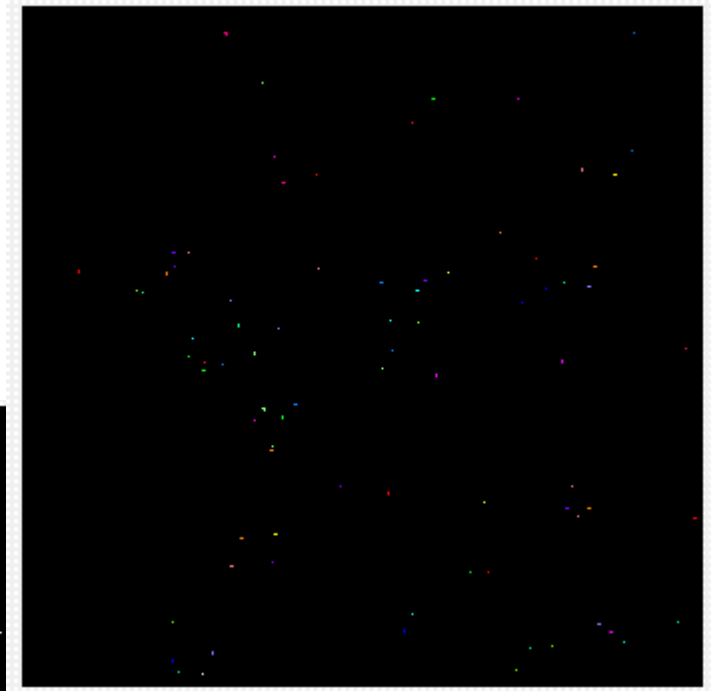
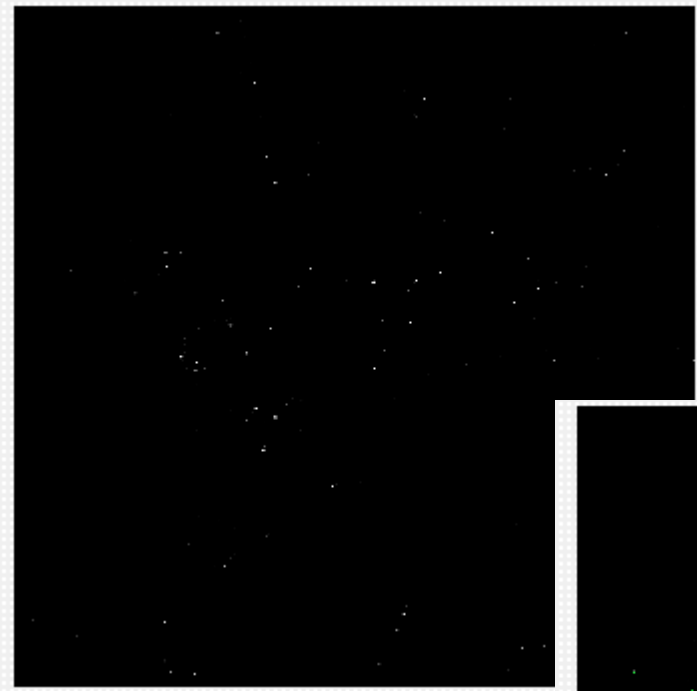
Olympus ScanR (analisi d'immagini)



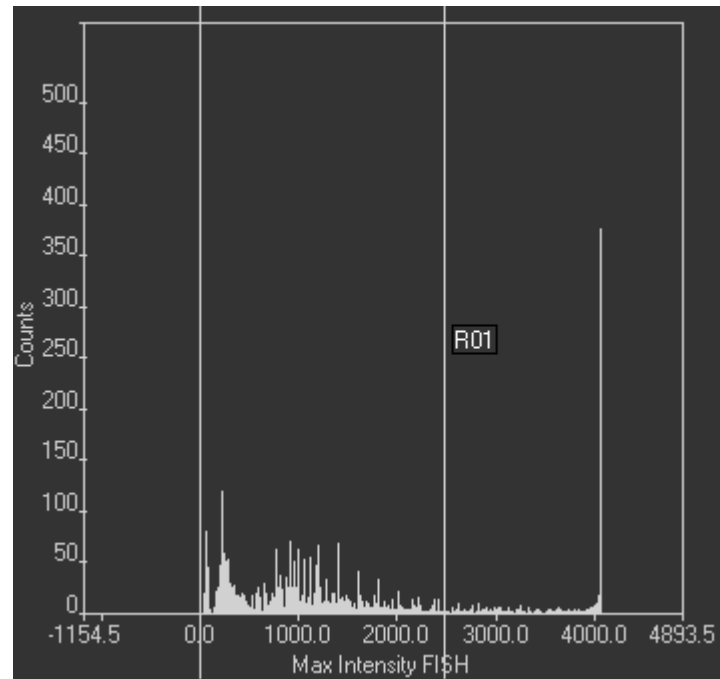
Olympus ScanR (analisi d'immagini)



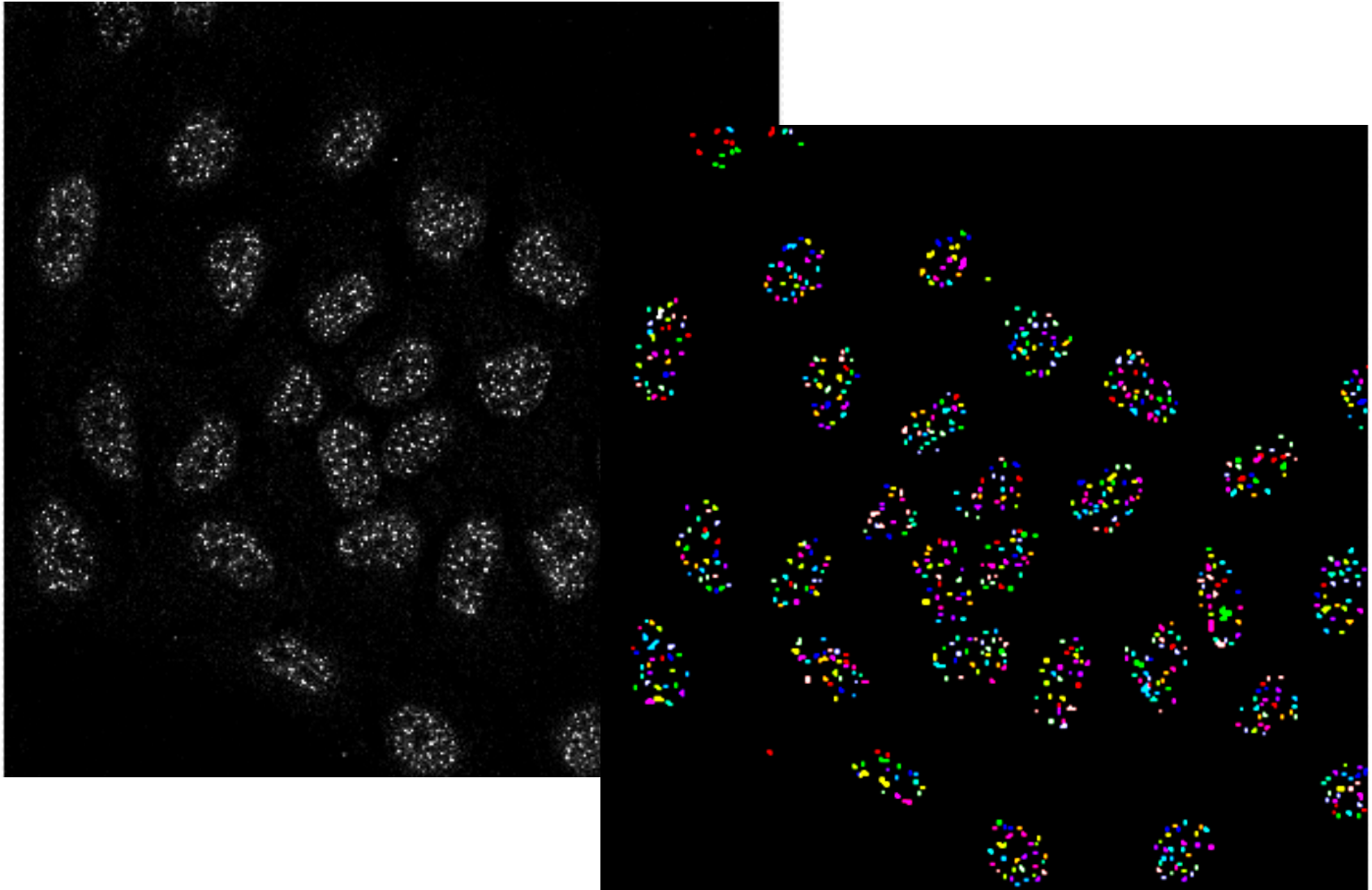
Olympus ScanR (analisi d'immagini)



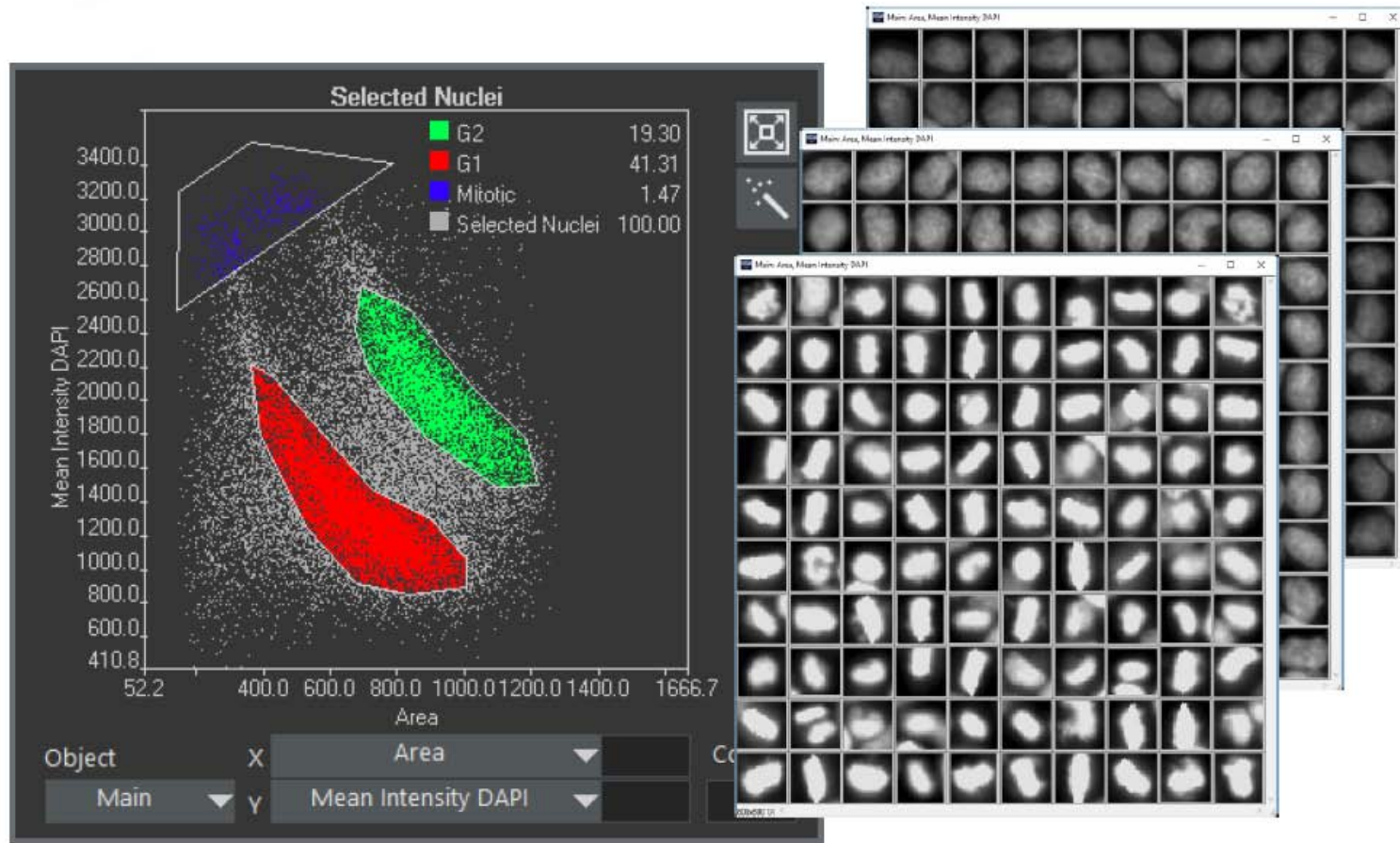
Olympus ScanR (analisi d'immagini)



Olympus ScanR (analisi d'immagini)



Olympus ScanR (analisi d'immagini)



GRAZIE



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