

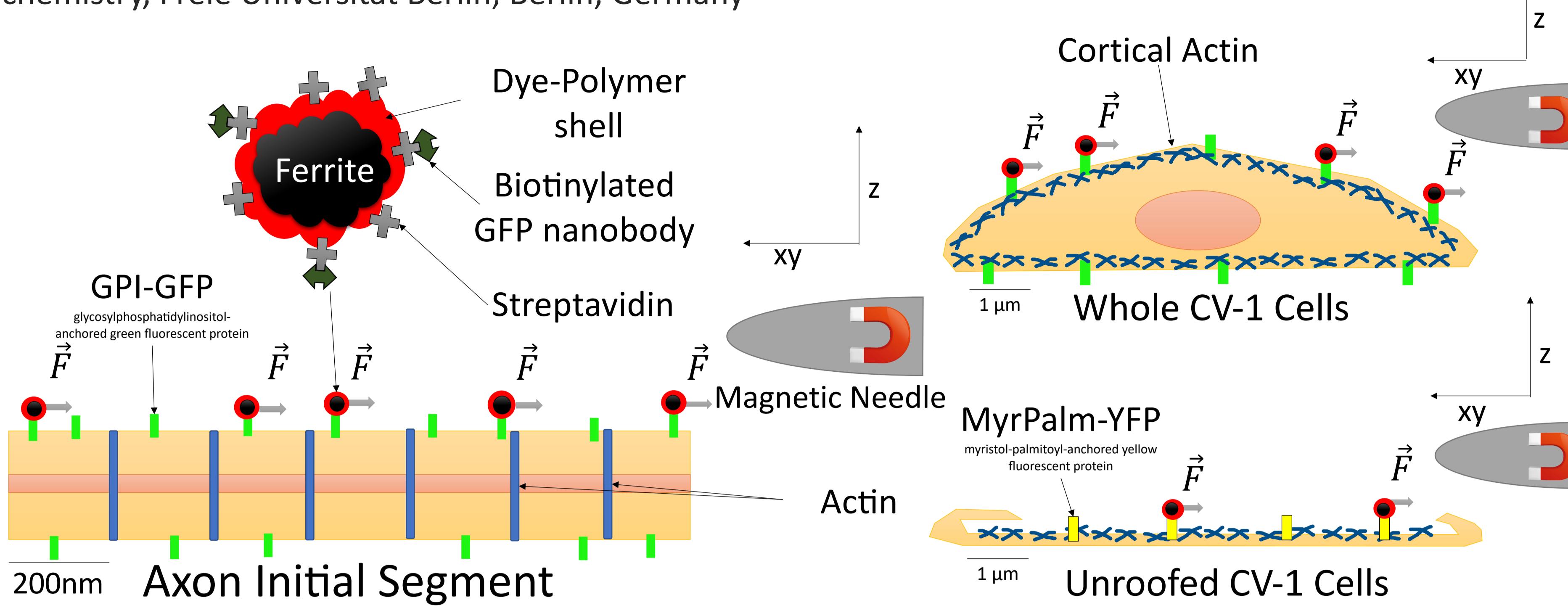


Fluorescent magnetic nanoparticles as a tool to find barriers in membrane diffusion

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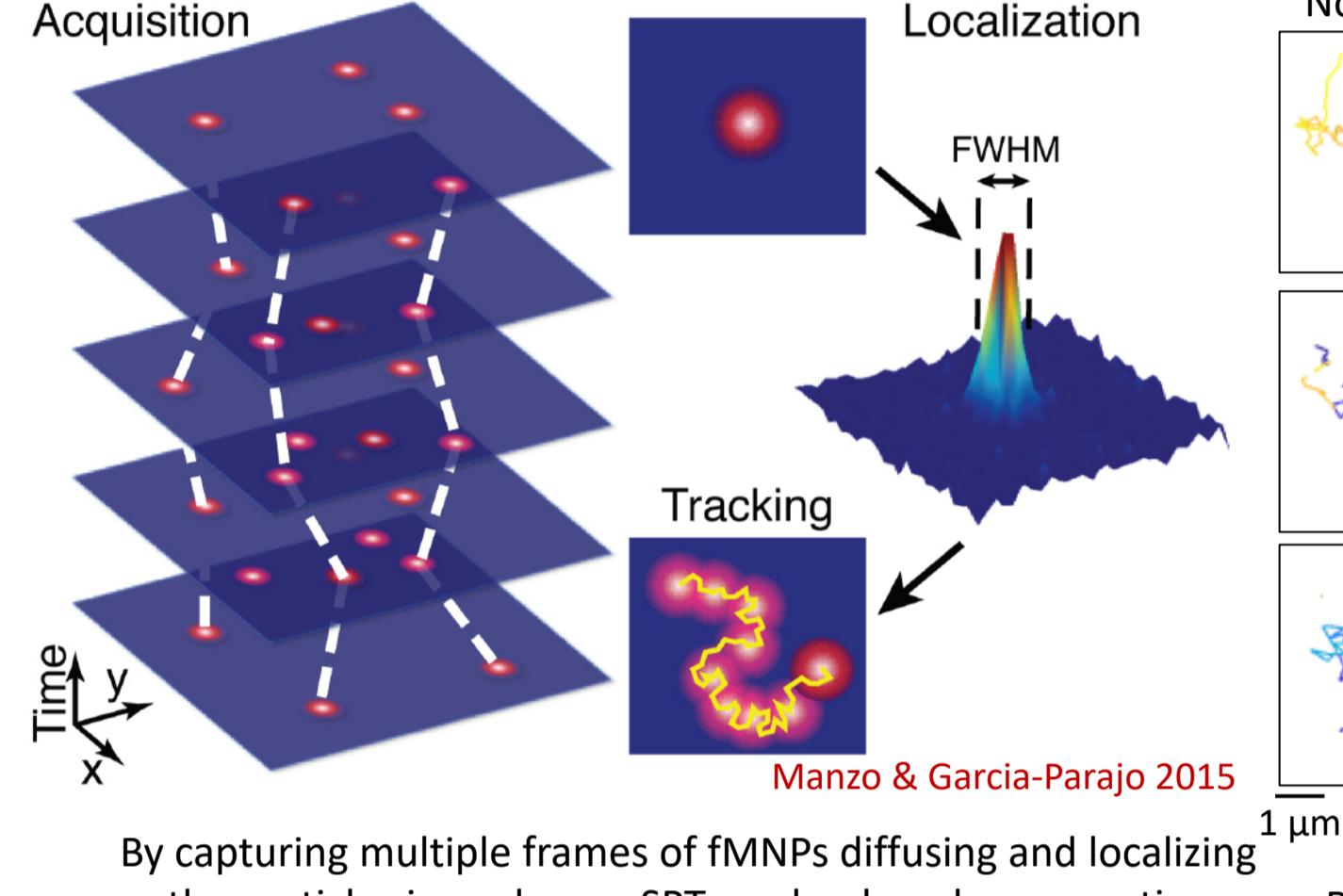
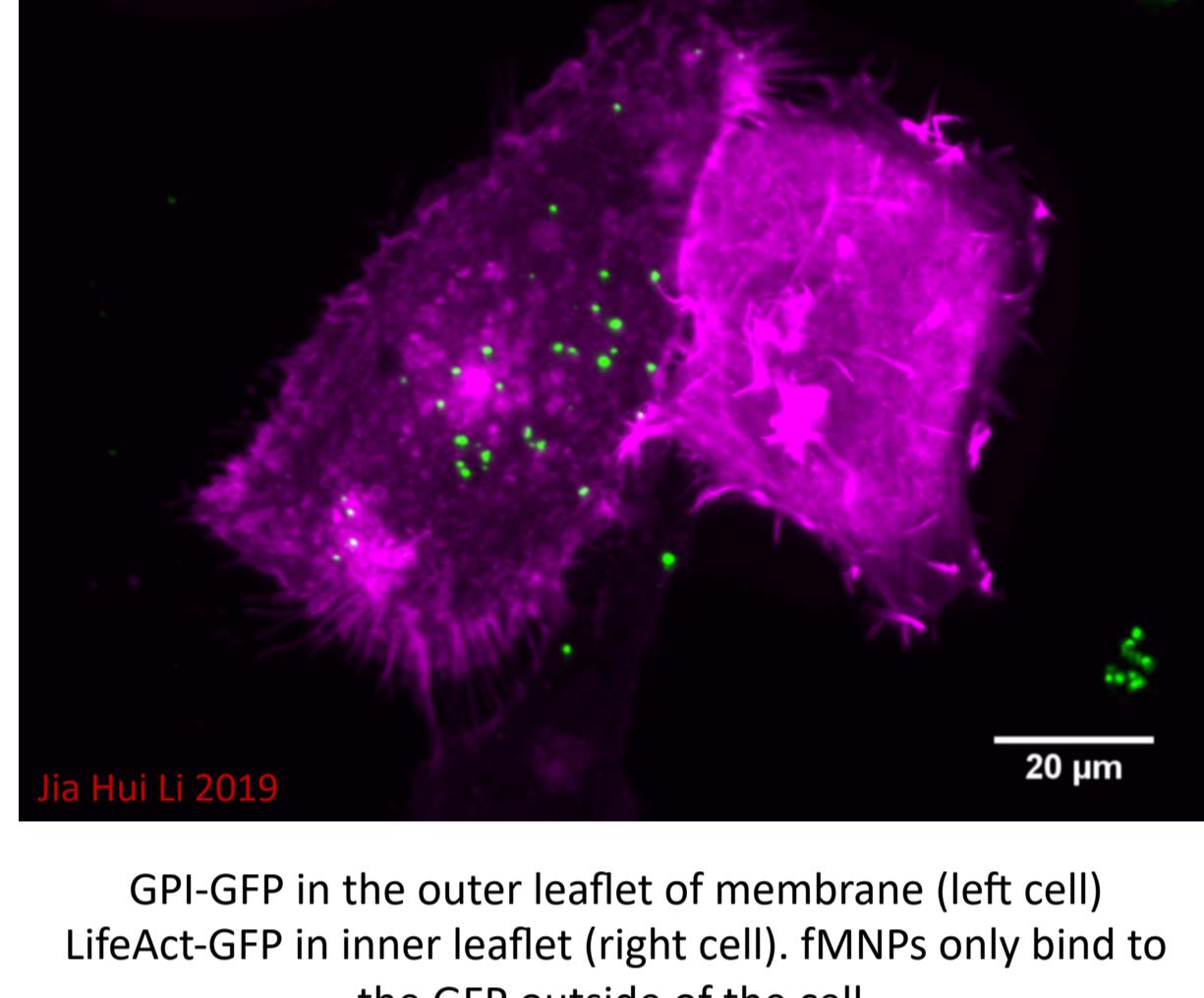
Abstract

Membrane diffusion barriers play a vital role in maintaining the identities of different compartments within a cell, and defective barriers are associated with ailments such as bipolar disease. Yet, the nanoscopic location and mechanisms of these barriers are mostly unknown. Fluorescent magnetic nanoparticles (fMNPs) are a promising tool for research in molecular and cell biology. When attached to proteins of interest in the plasma membrane, we can use single-particle tracking on a fluorescent microscope to follow its motion with nanometer and millisecond resolution. Furthermore, by applying an external magnetic field we can manipulate the protein's motion such that when moved against a barrier, this motion will be affected. A possible cause of diffusion barriers is the actin cytoskeleton of cells. However, actin can be found in arrangements so dense that the novel techniques of super-resolution microscopy must be used for nanometer resolution imaging. Here, we established a workflow that combines single-particle tracking of directed fMNPs and super-resolution microscopy with stochastic optical reconstruction microscopy (STORM) methods to search for a link between protein diffusion and actin structures.



Methods

Single Particle Tracking (SPT)

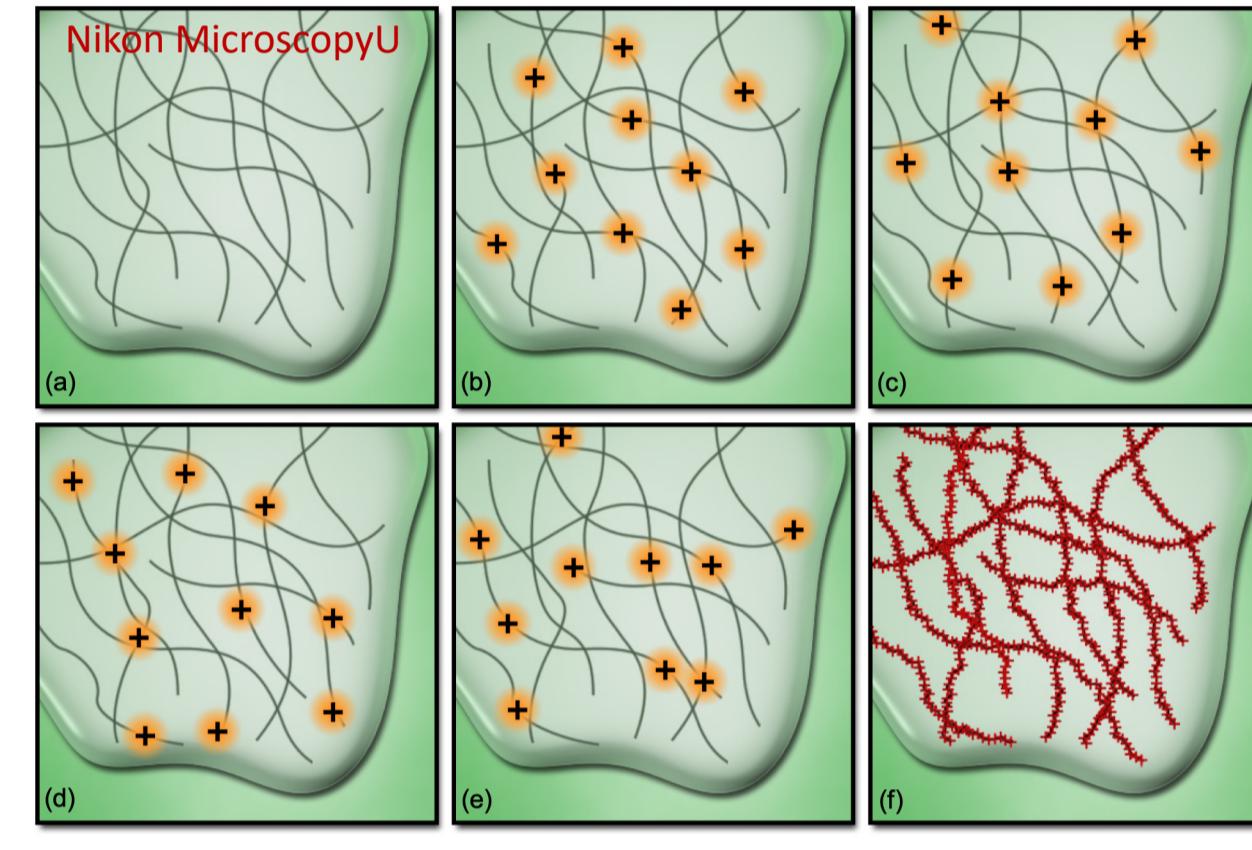


By capturing multiple frames of fMNPs diffusing and localizing the particles in each one, SPT can be done by connecting moving localizations in time to form tracks.

Manzo & Garcia-Parajo 2015
1 μm fMNP tracks on a supported lipid bilayer display Brownian motion before an external magnetic field is applied and directed motion after application.

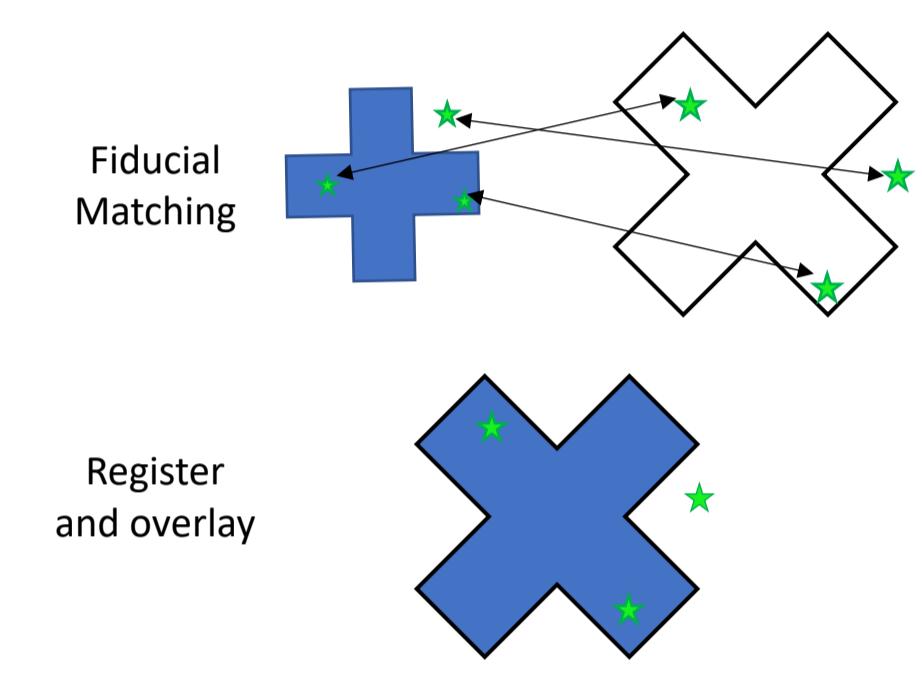
STORM

Stochastic Optical Reconstruction Microscopy



STORM uses single-molecule localization microscopy techniques by capturing multiple frames in which only a handful of fluorophores are activated at a time. In each frame, the fluorophores are localized individually and then all compiled into a super-resolution image.

Image Registration

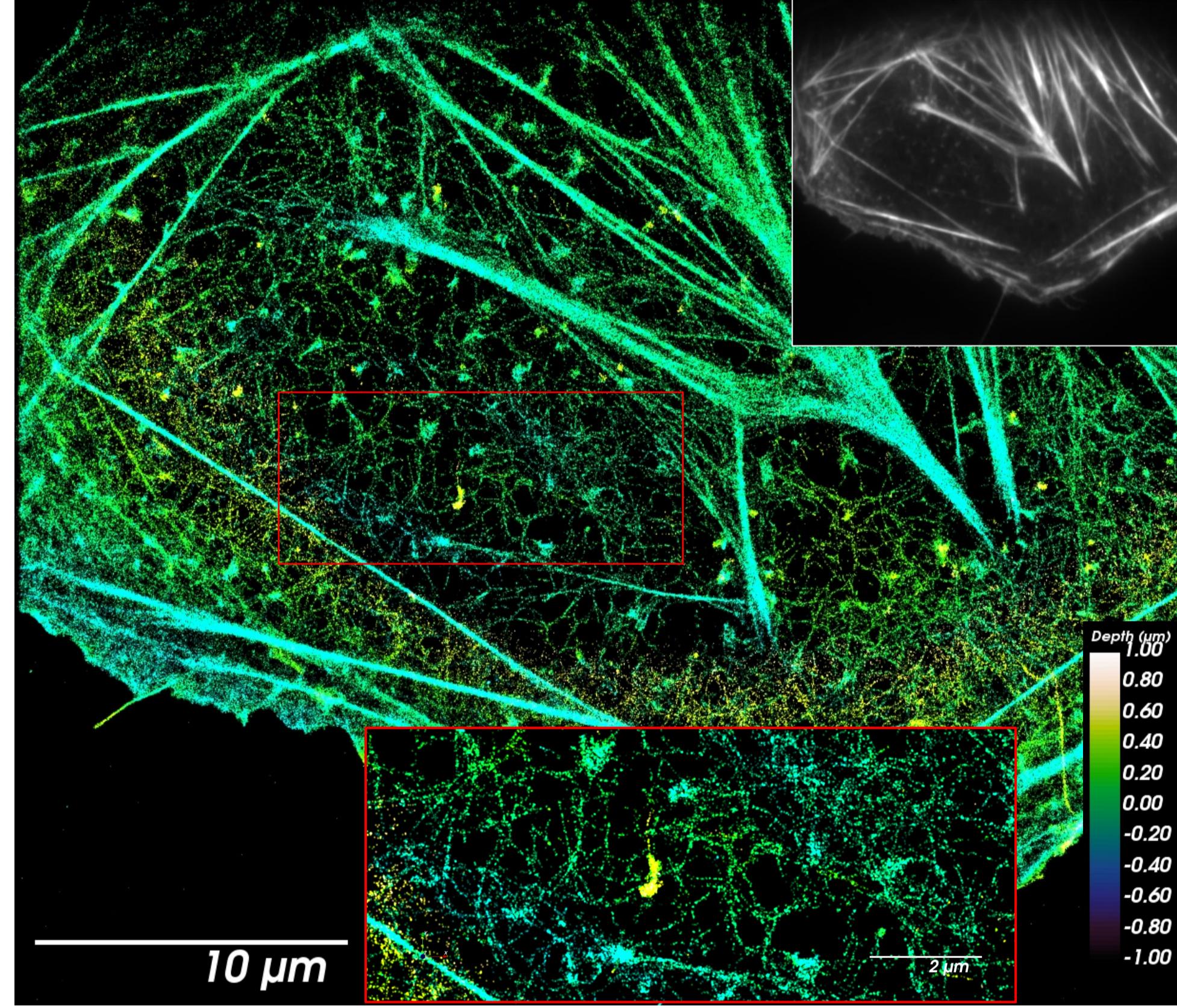


With the tracks and super-resolution image constructed, the two are registered based on fiducials to spatially match our data. As seen here, fiducials (marked by stars) are matched together. From there, a transformation matrix is calculated which transforms the first shape to match the second.

Results

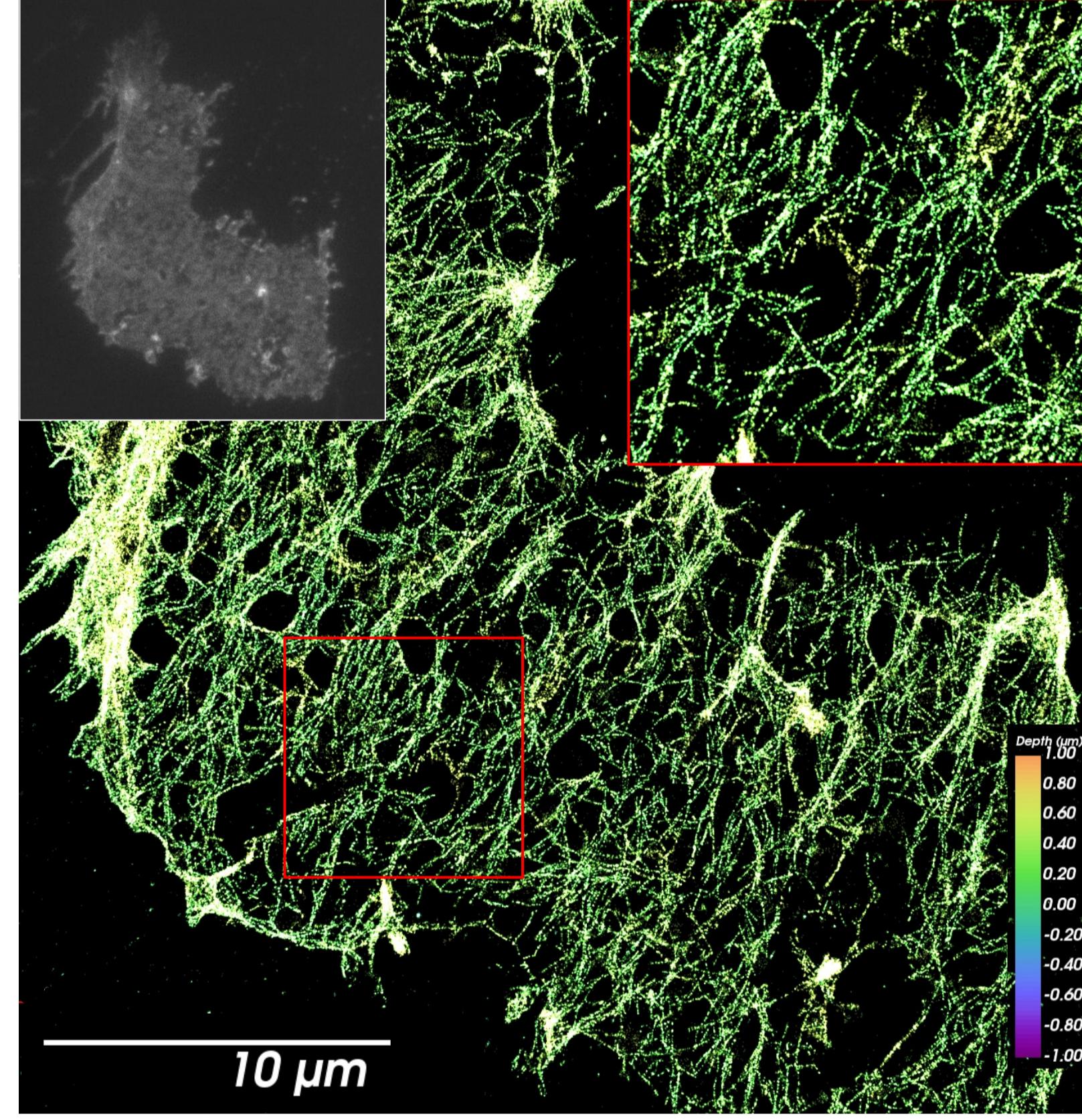
Super-Resolution Imaging Optimization

Whole CV-1 Cells



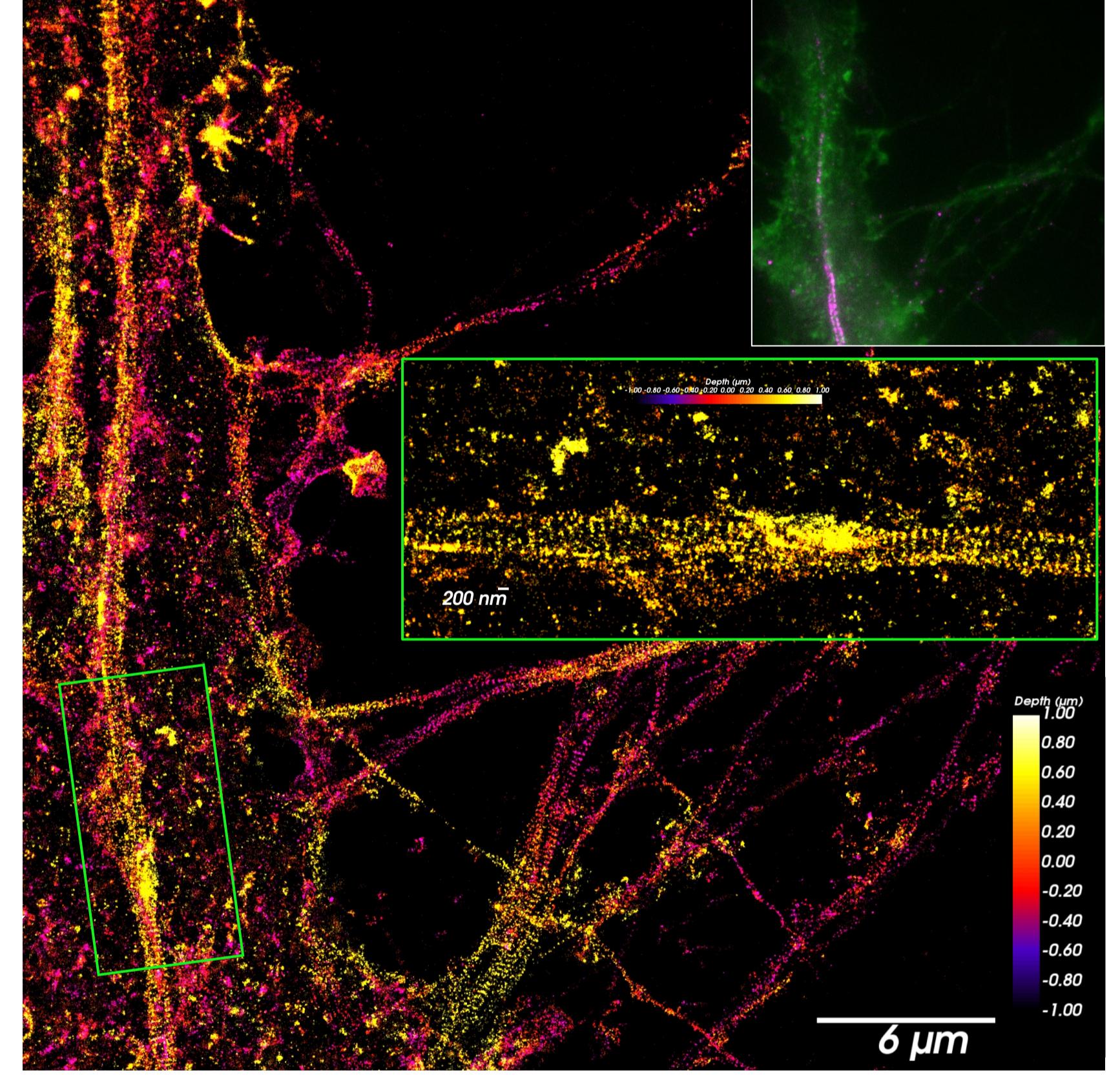
Fixed with glutaraldehyde in cytoskeletal buffer (CB)
Quenched with NaBH₄ in phosphate-buffered saline (PBS)
Actin labelled with phalloidin conjugated Alexa Fluor 647 (reference image)
Imaged for 20 000 frames at 20ms exposure

Unroofed CV-1 Cells



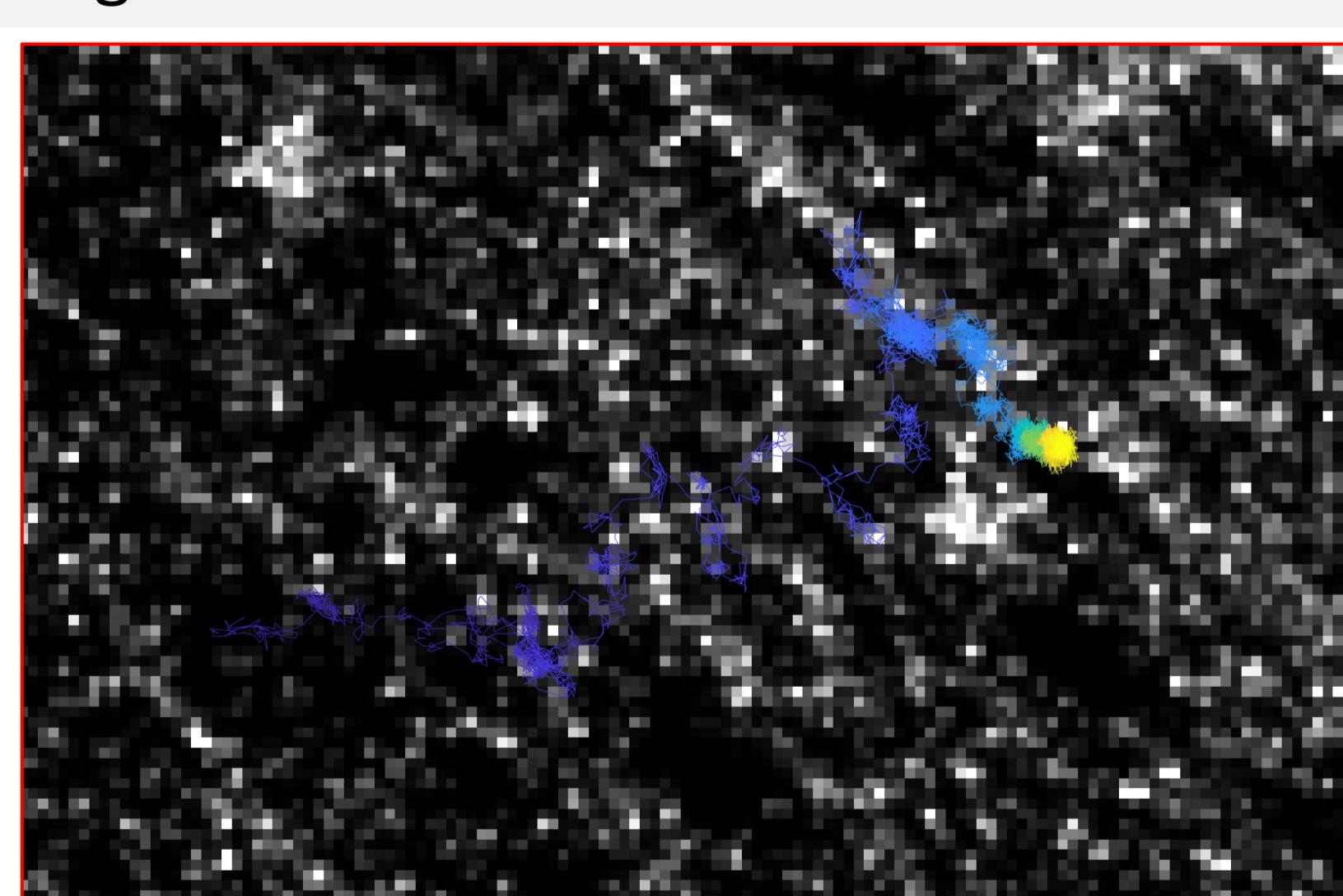
GPI-GFP CV-1 cell on poly-L-lysine coated coverslips
Unroofing was done by ultrasonication in sonication buffer (reference image)
Fixed with glutaraldehyde in CB
Quenched with NaBH₄ in PBS
Actin labelled with phalloidin conjugated Alexa Fluor 647
Imaged for 20 000 frames at 20ms exposure

Neuron Cells



Rat Hippocampal Neurons 28 DIV
Fixed with paraformaldehyde in CB
Quenched with NH₄Cl in CB
Blocked with horse serum and bovine serum albumin in CB
Actin labelled with phalloidin-AF647 (green) and neurofascin by ATTO488 immunofluorescence (pink)
Imaged for 20 000 frames at 20ms exposure

Registered Whole CV-1 Image



Left: Registered image of single-particle tracks on their respective super-resolution image of the actin cytoskeleton in whole GPI-GFP CV-1 cell. Registration is done based on fiducial beads, which have been circled for identification. The magnetic tip has been placed on the right respective to the image shown here. Three fMNP tracks can be distinguished on the cell, two of which display surprising results by diffusing away from the magnetic source. One track diffuses towards the magnet.
Above: Magnified view of the fMNP track that diffuses towards the magnet. Besides the general direction, the track takes a very indirect route. The track then slows down upon reaching an actin filament and remains in a pocket where no actin is detected based on phalloidin-AF647 signal.
Data from Jia Hui Li 2019

Conclusions

- By combining single-particle tracking of fMNPs and super-resolution microscopy, we have successfully established a workflow to find membrane diffusion barriers.
- With super-resolution microscopy protocols established for neurons and CV-1 cells both whole and unroofed, all three experiments can run in parallel.
- The shown result on whole CV-1 cells confirms a degree of correlation between fMNP tracks and actin cytoskeleton presence, but we must conduct more trials before making a conclusion about the role actin plays in membrane diffusion.
- This workflow can be used to test other candidates for membrane diffusion barriers.

References

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