

SEgmentation of Neurons using Standard and supEr-resolution microscopy

Nicola Vanello, Lydia Danglot, Peter Dedecker

# SENSEI

## Rationale & Objectives

Improving the quality of neuronal imaging  
using new imaging techniques and membrane probes

Quantifying neuronal morphology at tissue and molecular levels through the  
development of smart segmentation-based image processing algorithms

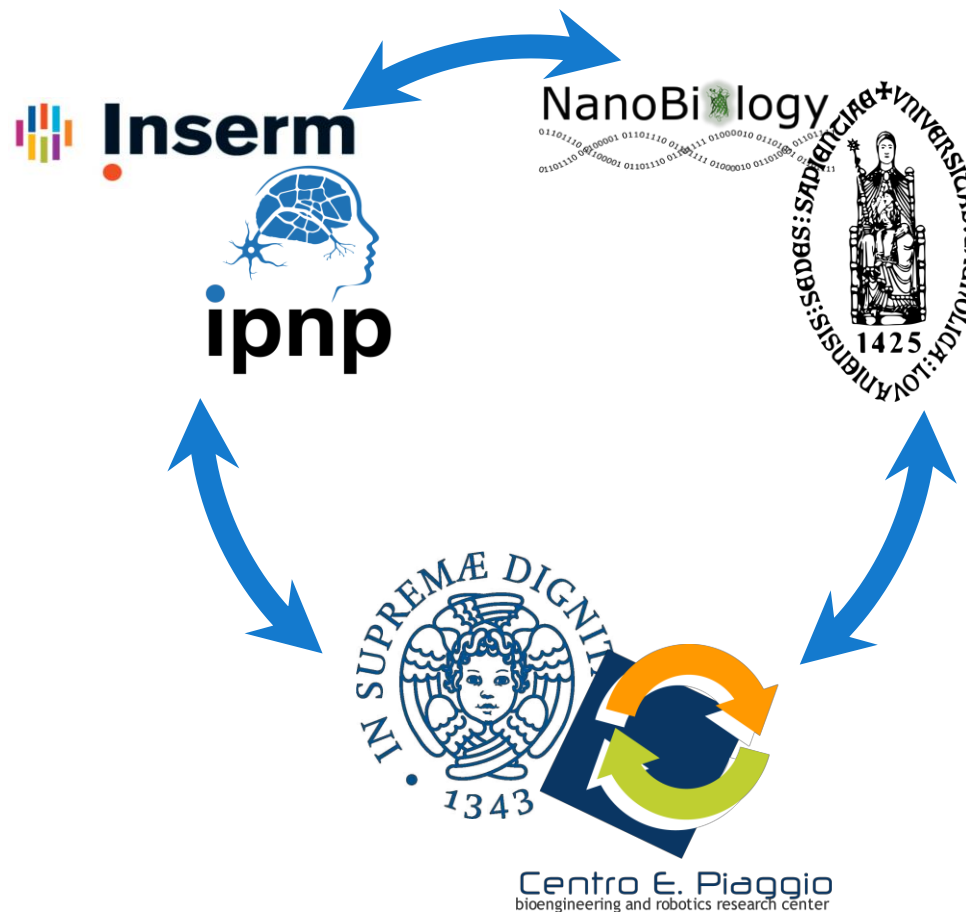
Research Center "E. Piaggio", University of Pisa

Institute of Psychiatry and Neurosciences of Paris (IPNP) – INSERM Unit 1266

KU Leuven, Department of Chemistry, Lab for Nanobiology

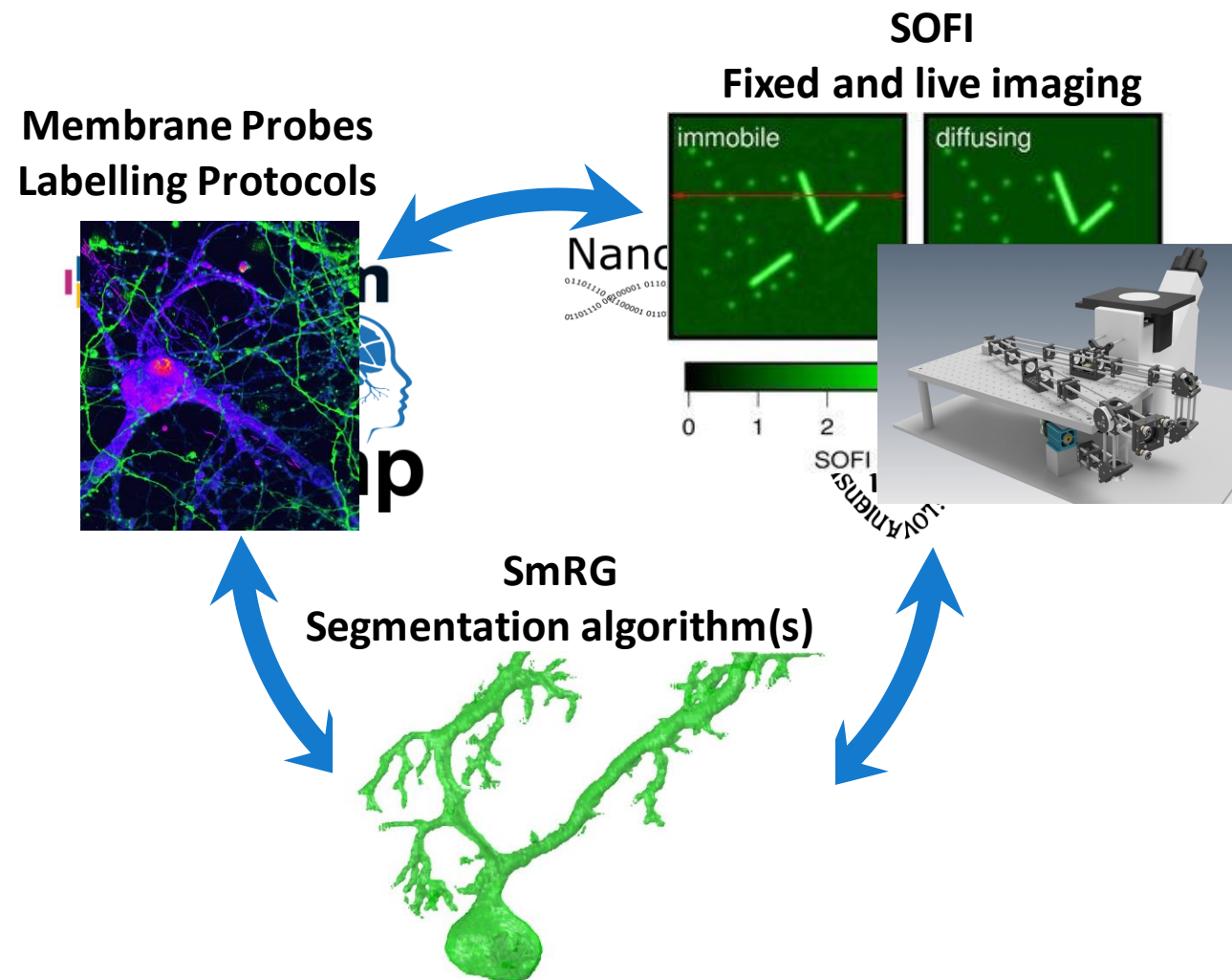
# SENSEI

## The Team



# SENSEI

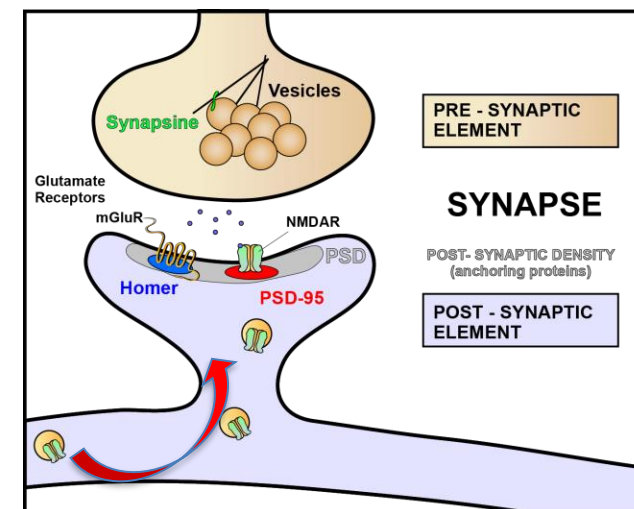
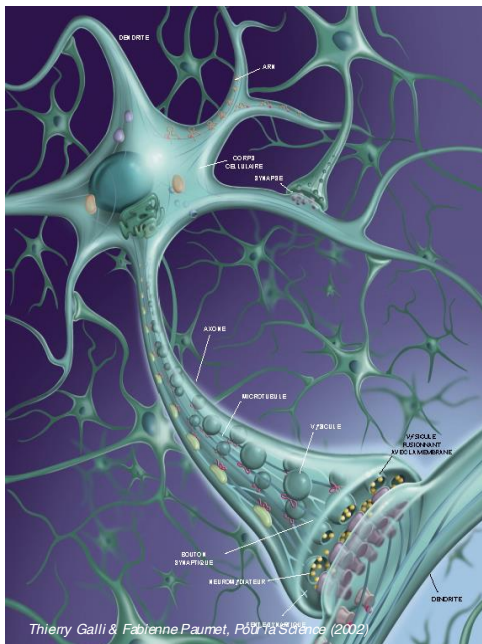
## The Team





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# Unraveling neuronal morphogenesis from whole brain to synaptic molecules



## Neuronal morphogenesis during learning and plasticity



conventional



&



super resolution

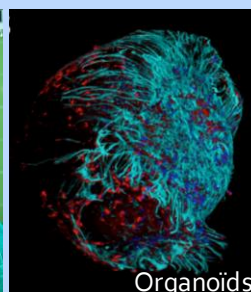
## From Human brain

neuronal networks

to synaptic molecules



## Surgery at the hospital

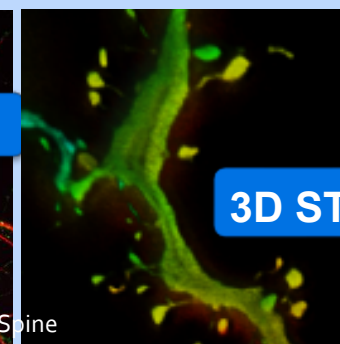


## Organoids



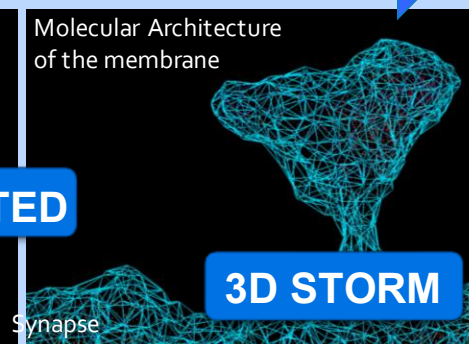
SIM

Neuron Spine



## 3D STED

## Synapse



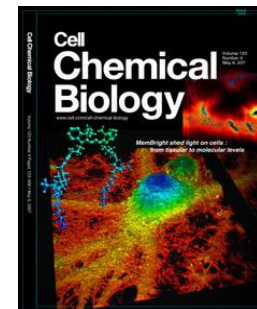
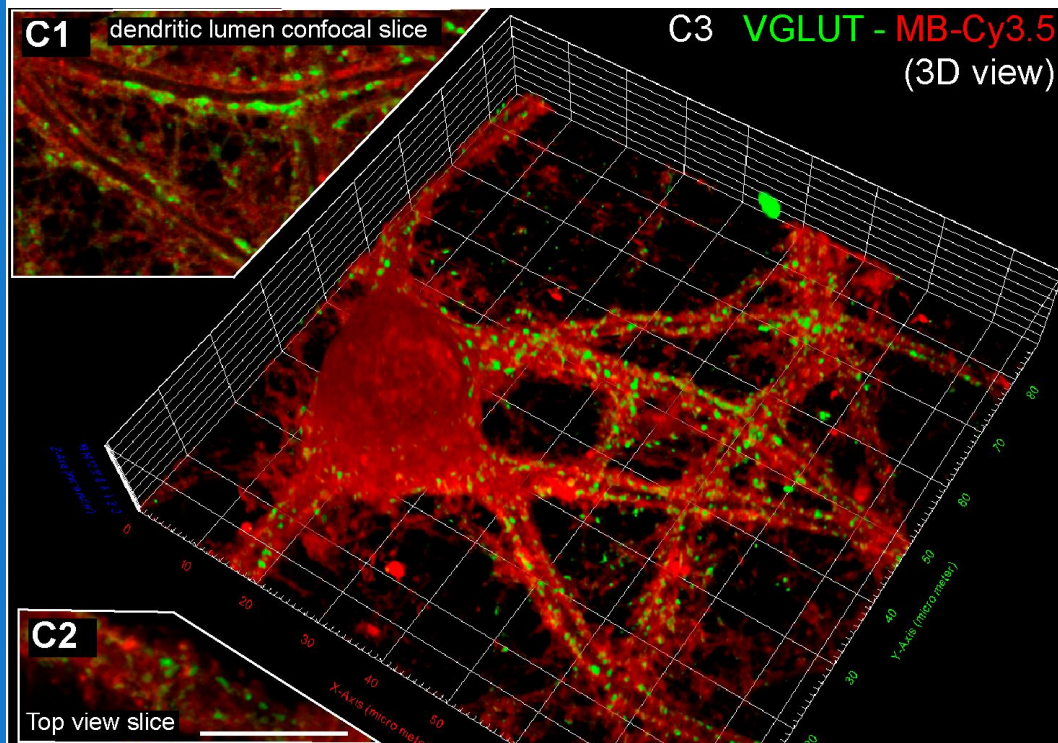
## Molecular Architecture of the membrane

## 3D STORM

## SENSEI

New Probes  
Optimizing  
Labelling  
Procedures  
on Different  
Imaging Modalities

## Membrane labelling with MemBright probes



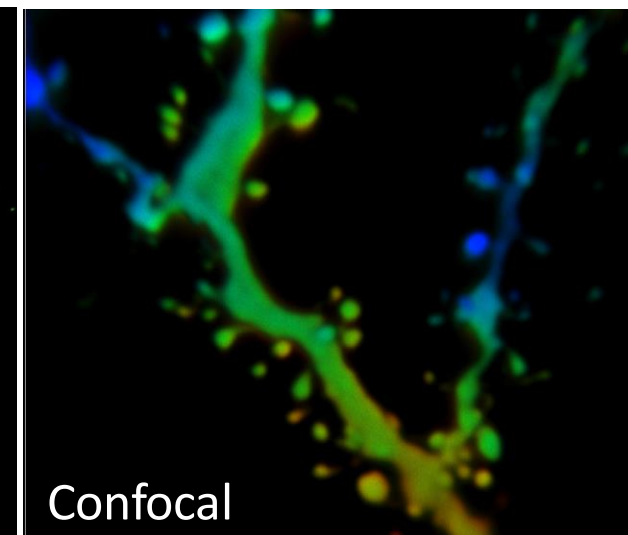
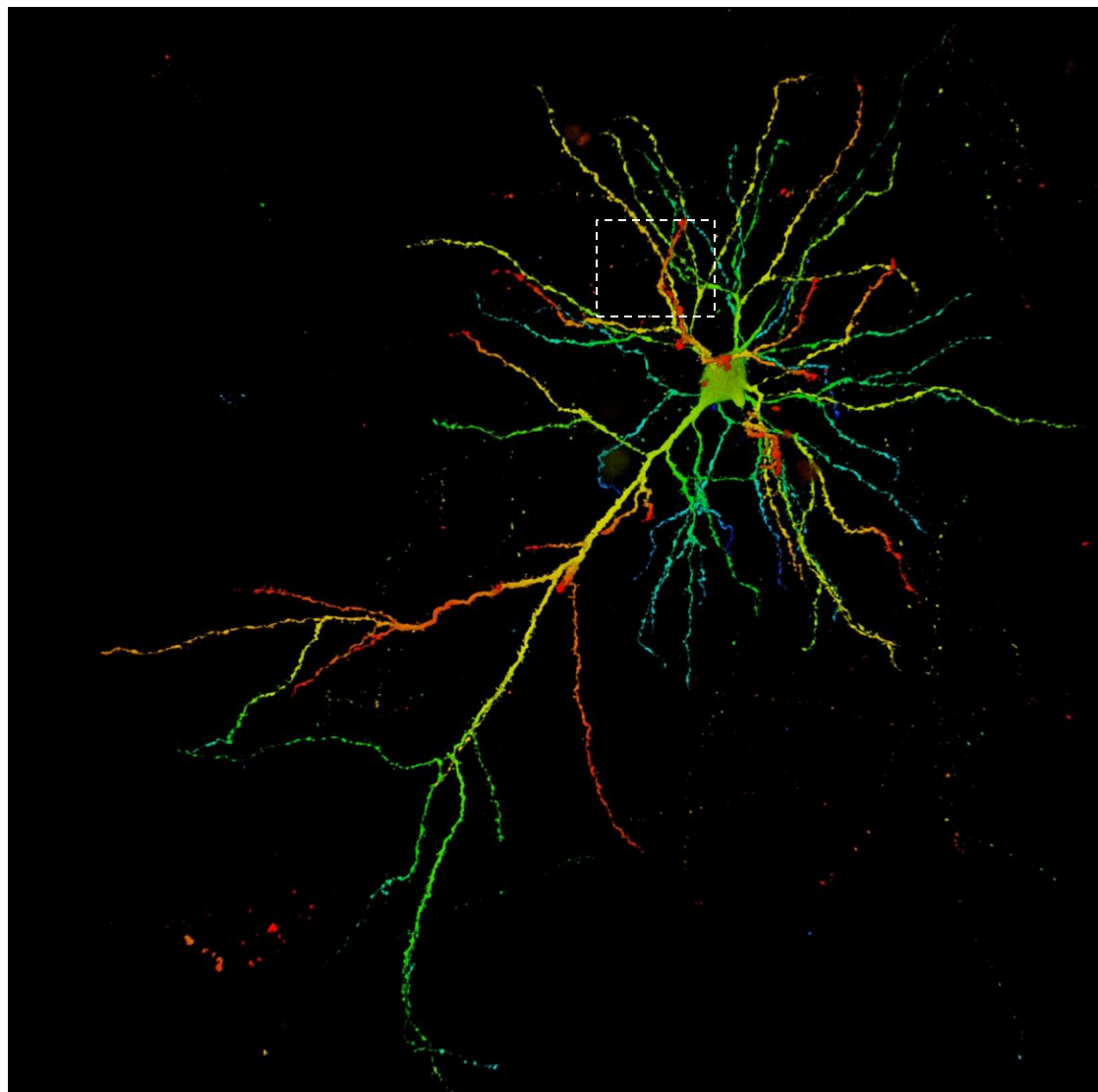
- *in vitro* (live rodent hippocampal neurons)
- *in situ* (fixed rodent brains & human tissue)

MEMBRIGHT ALLOWS VISUALIZATION OF  
FAST DYNAMIC PROCESSES

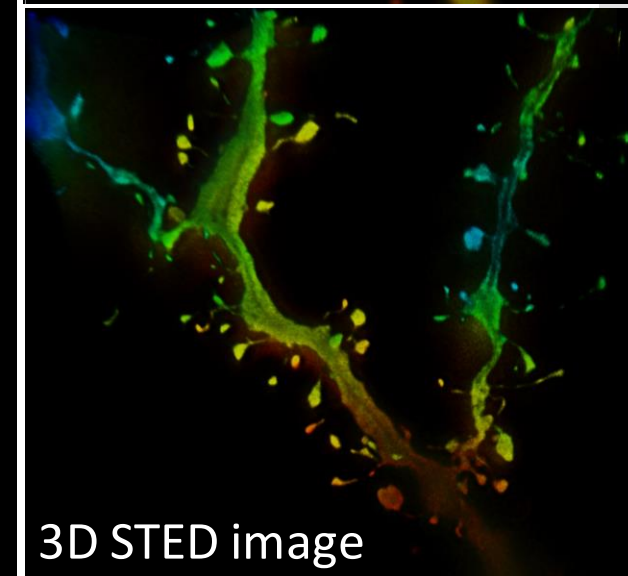
# SENSEI

New Probes  
Optimizing Labelling  
Procedures  
on Different Imaging  
Modalities

- With super resolution microscopy (SIM, STED, STORM, SOFI)



Confocal

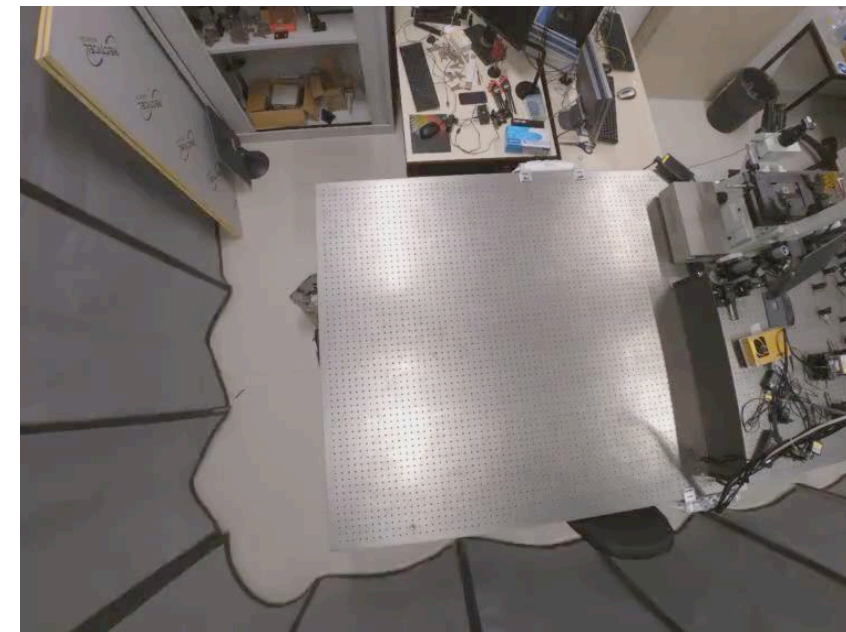
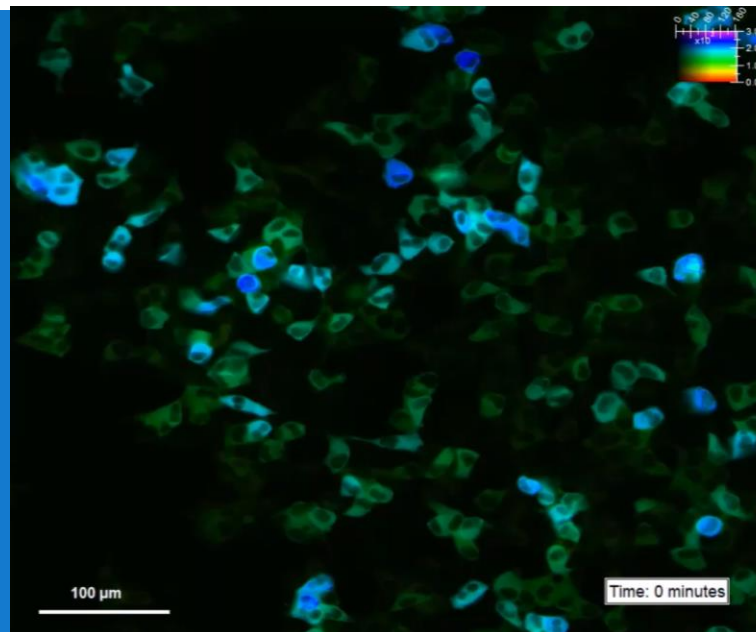


3D STED image

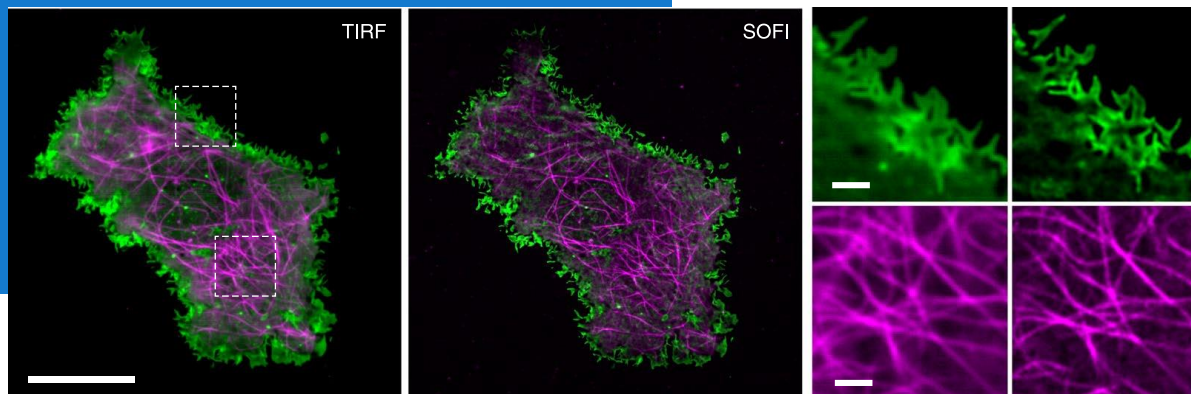


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# Imaging Development

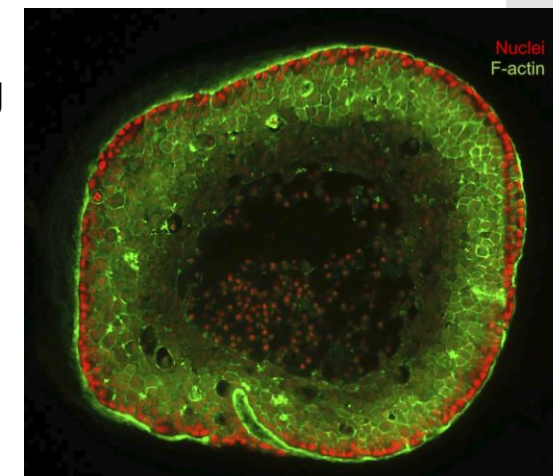


## In-tissue super resolution imaging with SOFI



A. G. Tebo et al, Nature Chem Biol 2020

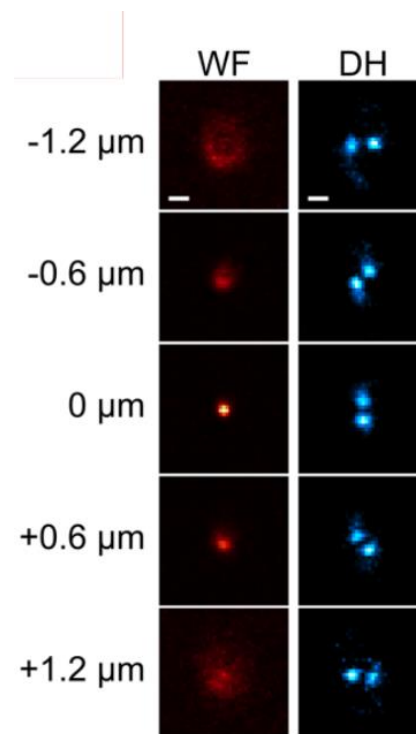
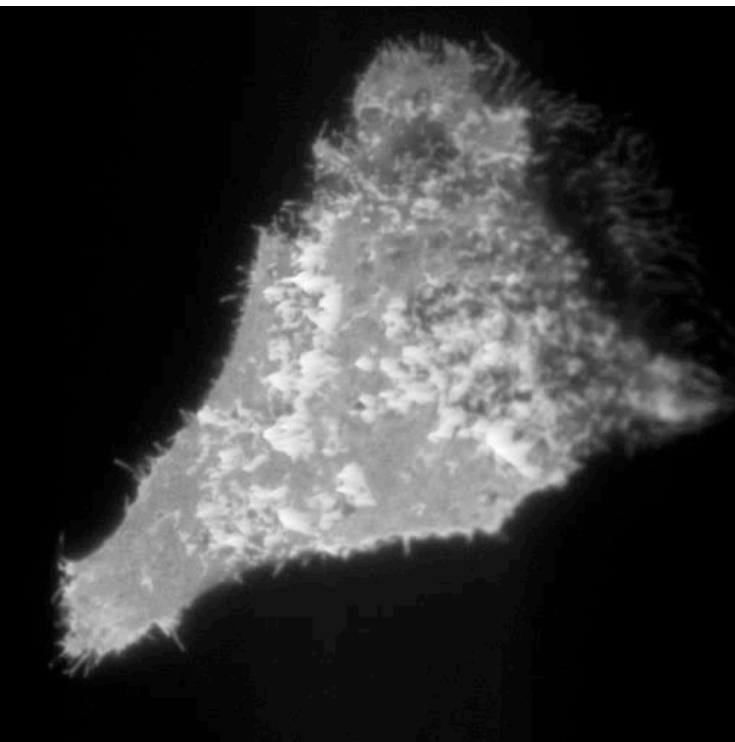
## Fast in-tissue confocal imaging and localization-based super-resolution imaging



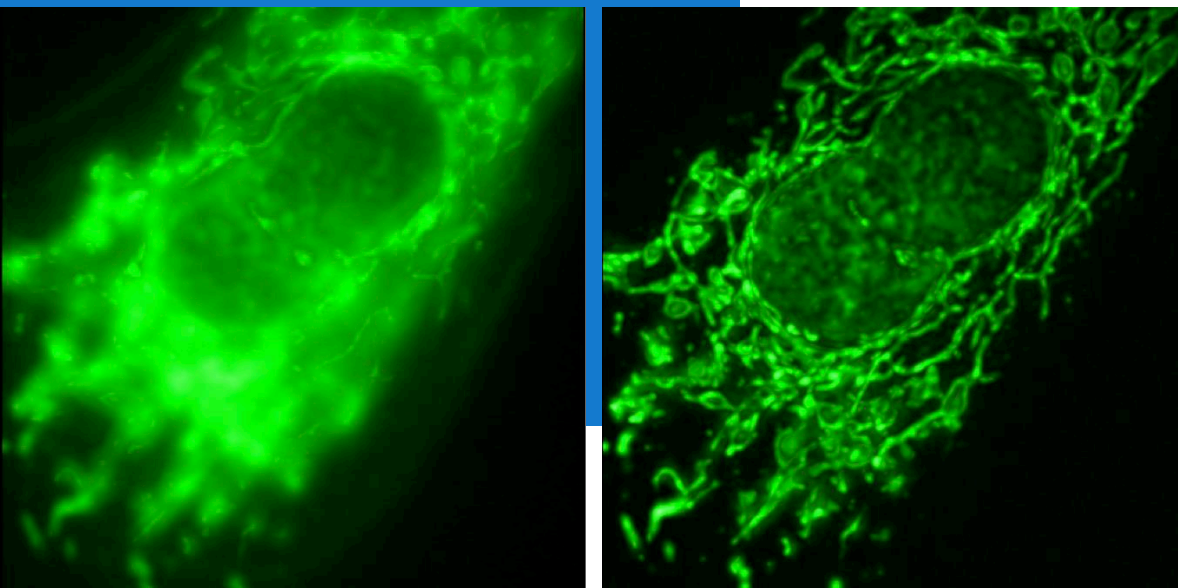
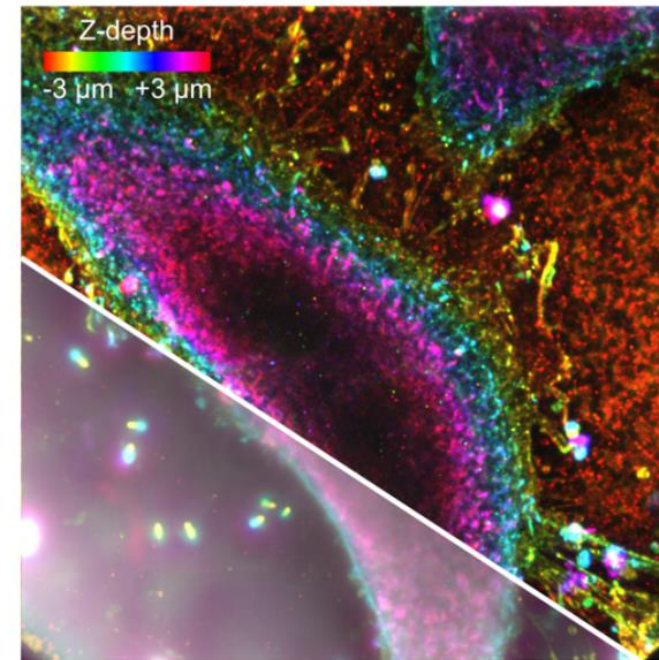
Courtesy F. Pampaloni, Univ. Frankfurt



# 3D sub-diffraction imaging with SOFI



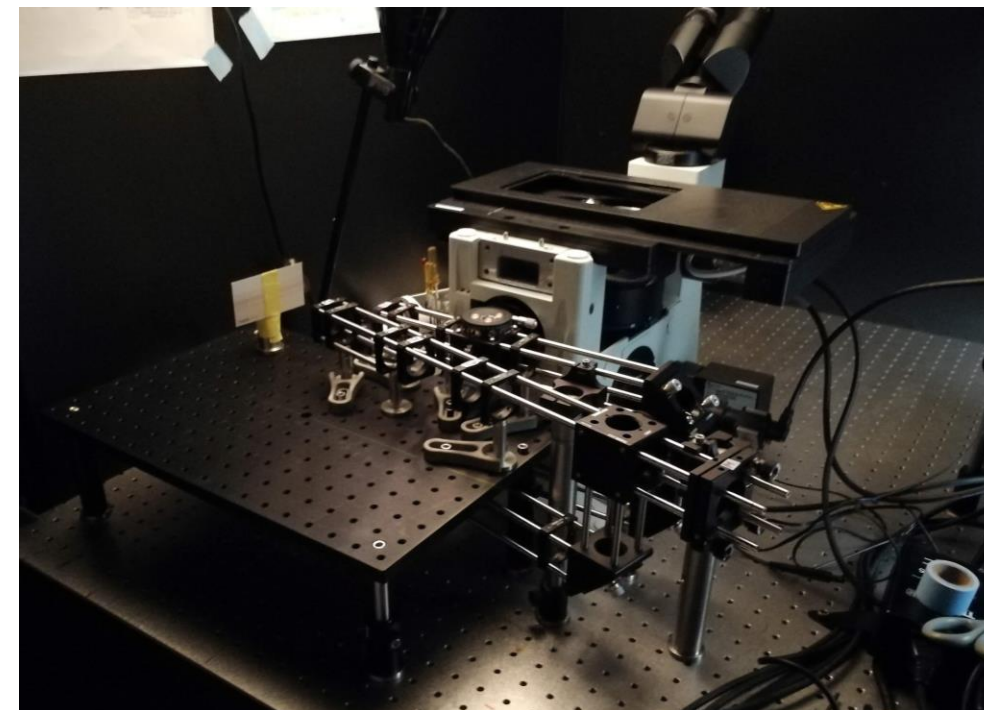
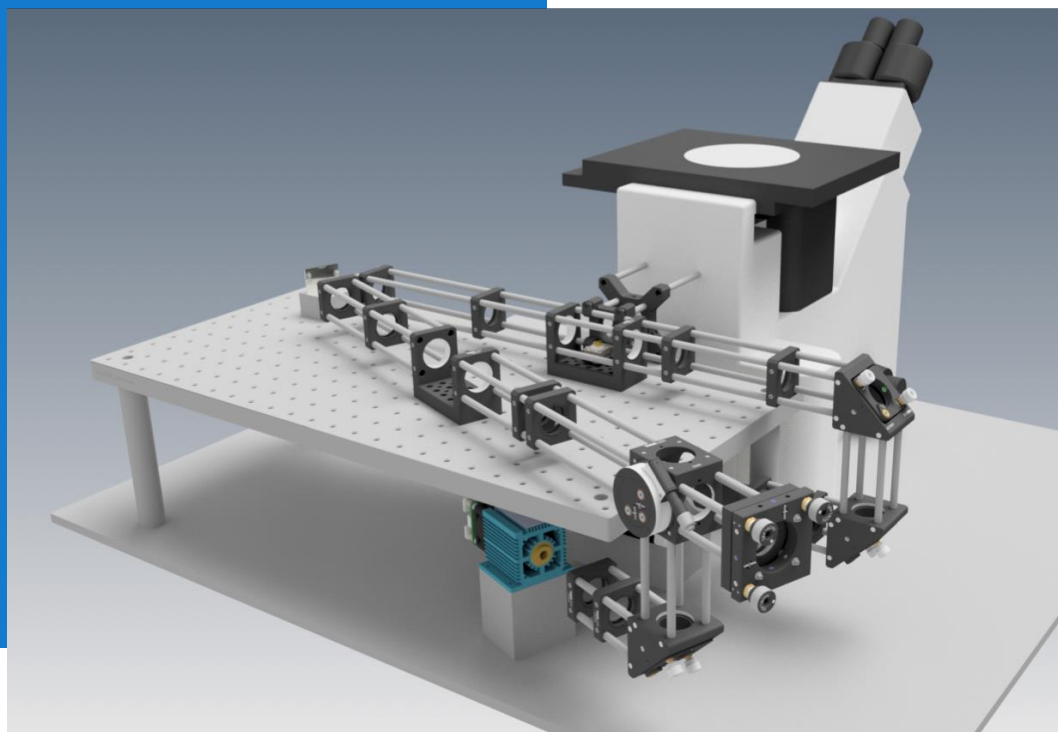
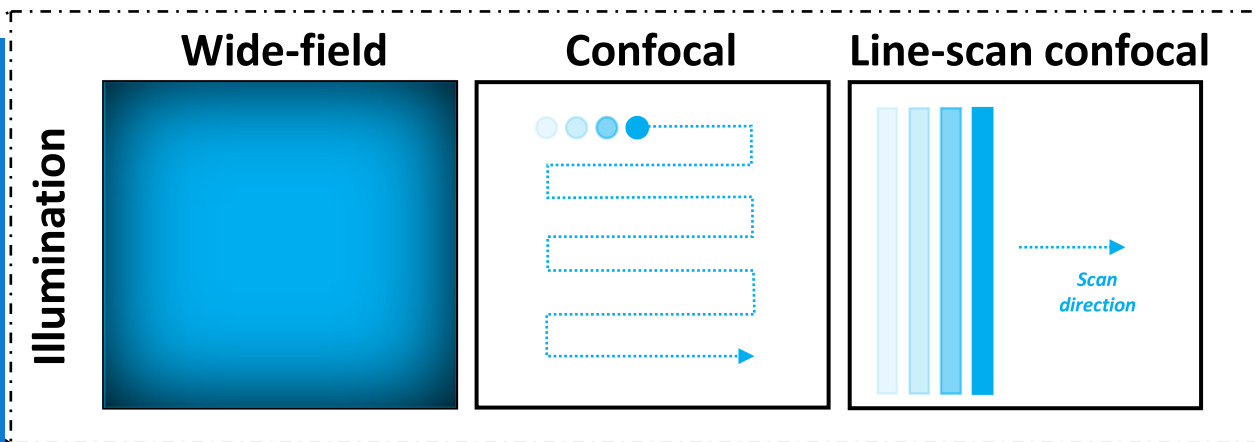
## Simultaneous 16-plane acquisition



Status: proof-of-concept complete, working on general analysis framework

Expected performance:

- Two- to three-fold resolution increase at up to 16 focal planes at once
- Second to tens of seconds measurement time per full set of 16 planes



Current status: optical design complete, prototype construction underway

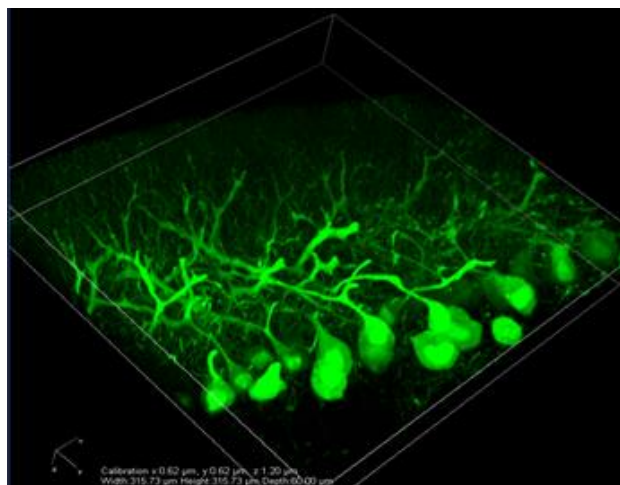
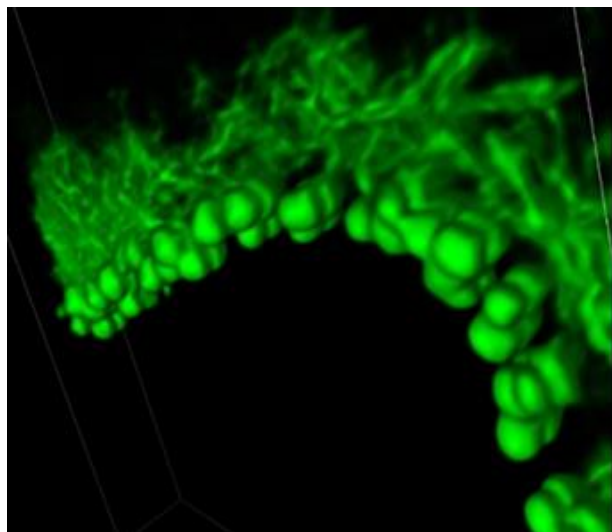
Expected performance:

- Over order of magnitude faster than classical confocal
- Similar resolution in xy
- Slightly reduced sectioning in z
- Single-molecule sensitive

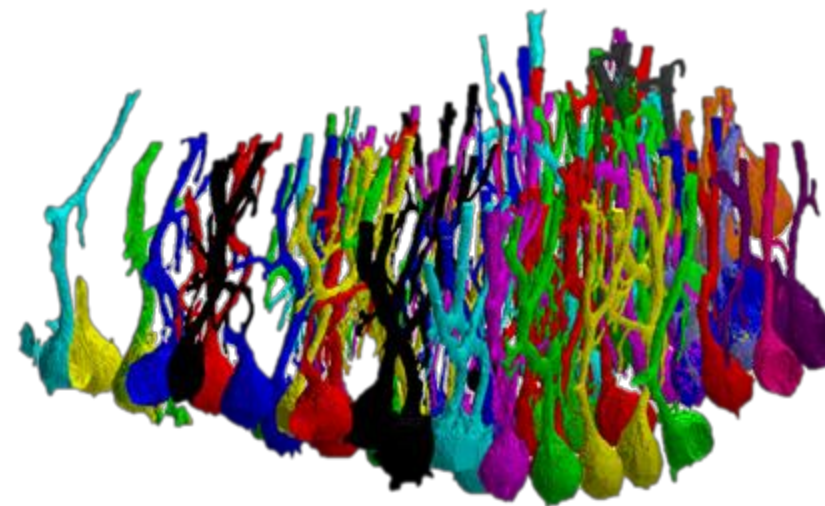


Research  
Center  
"E. Piaggio"

Optimization of Tissue  
Clarification protocols...



Open Access segmentation  
algorithms and 3D neuron  
reconstructions

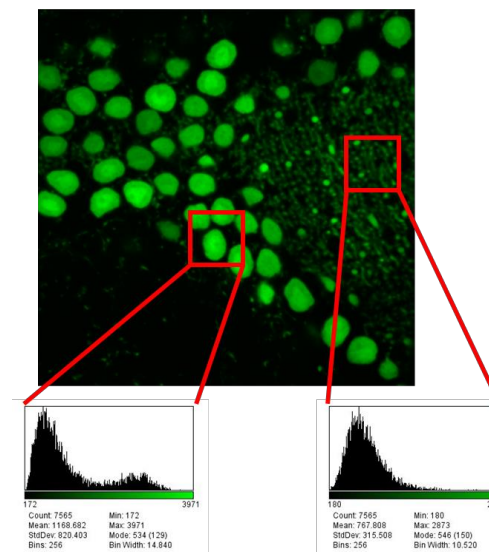
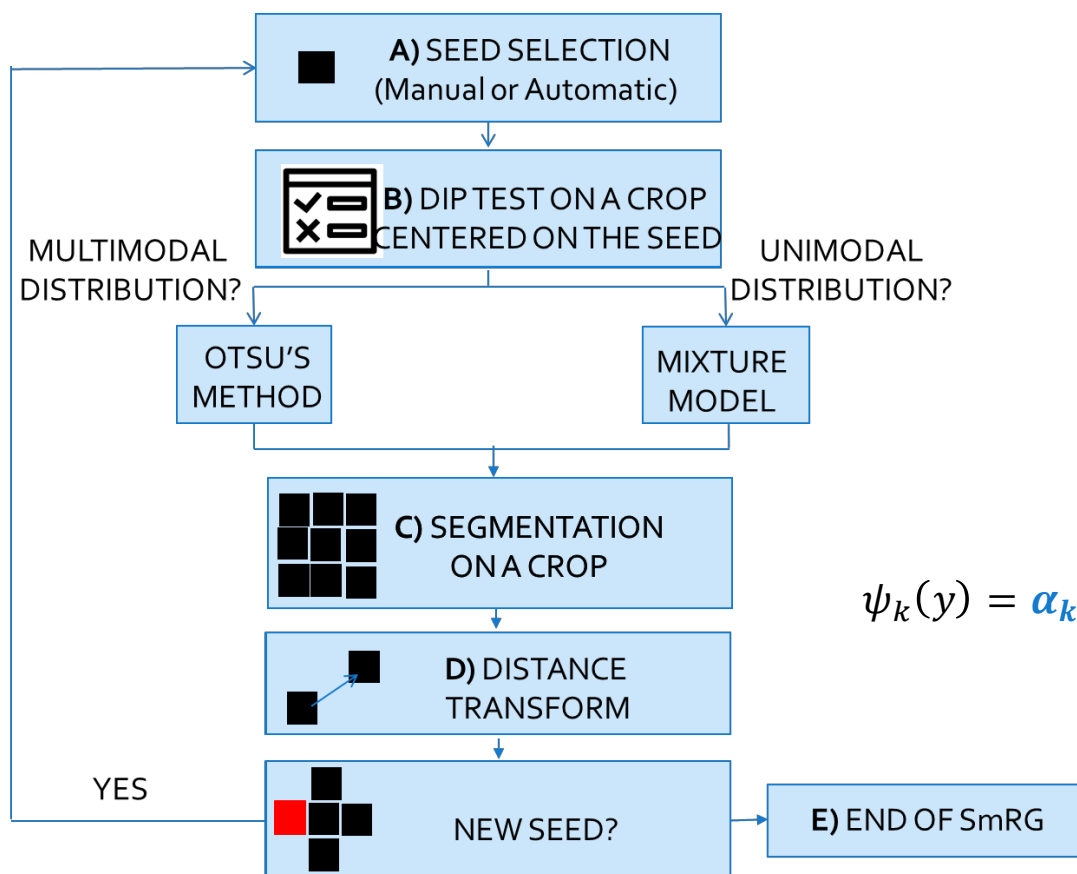




# SENSEI

## SmRG Smart Region Growing

### Model Based Segmentation Approach Based on statistical model of signal and noise intensity

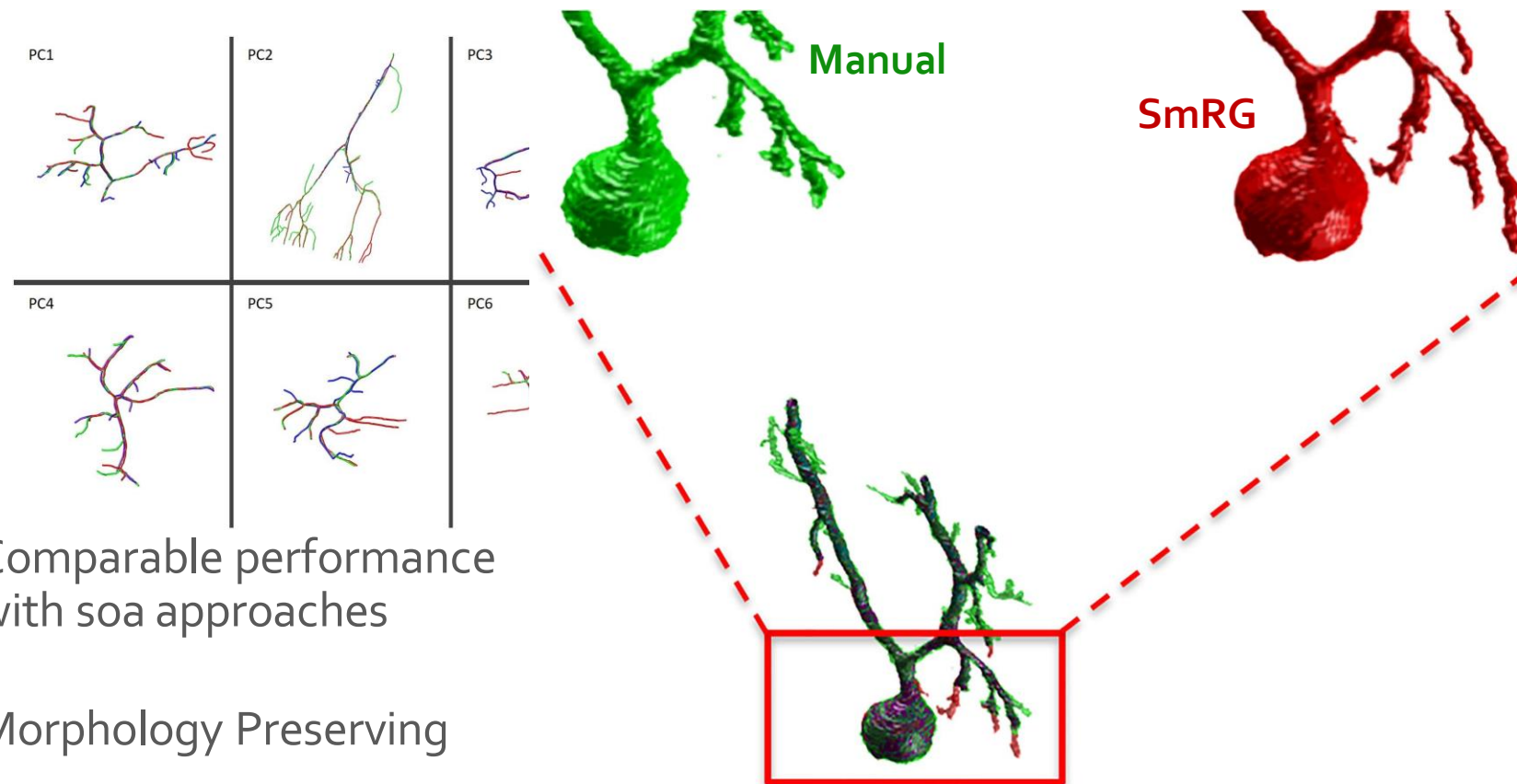


$$\psi_k(y) = \alpha_k \psi_B(y - K_0) + (1 - \alpha_k) \psi_{Sk}(y - K_0)$$

SENSEI

SmRG  
Smart Region  
Growing  
Testing

Tested against ManSegTool (Manual Segmentation)  
and Neuron GPS



Comparable performance  
with soa approaches

Morphology Preserving

Very good performances  
with Animal-derived samples

Callara et al.,(2020) A Smart Region-Growing Algorithm for Single-Neuron Segmentation From Confocal and 2-Photon Datasets. *F. in Neuroinformatics*

# SENSEI

## SmRG Work in Progress

### Next Improvements

Improving the performance of dendritic tree segmentation

Increasing the adaptability to image quality/characteristics changes across volume

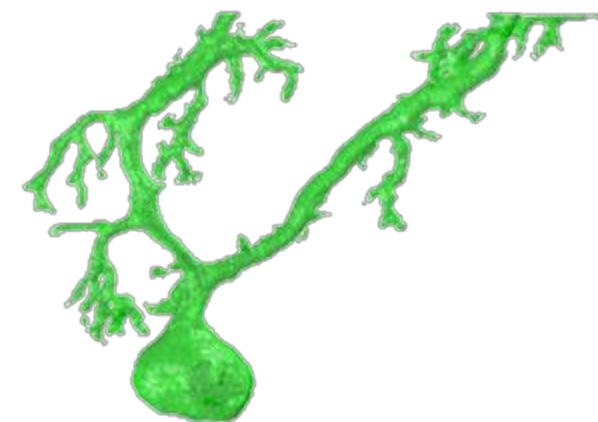
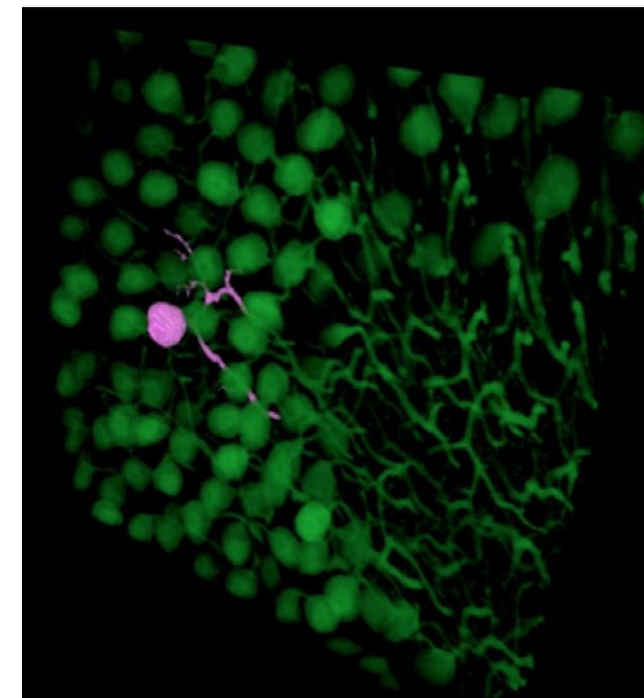
Integrating SmRG with other tools/strategies for separating individual neurons

Applying/adapting to other imaging modalities/  
Different neuronal cells/rodent and human

**This will lead to a robust tool for**

Densely-packed neurons  
(e.g. Purkinje cells)

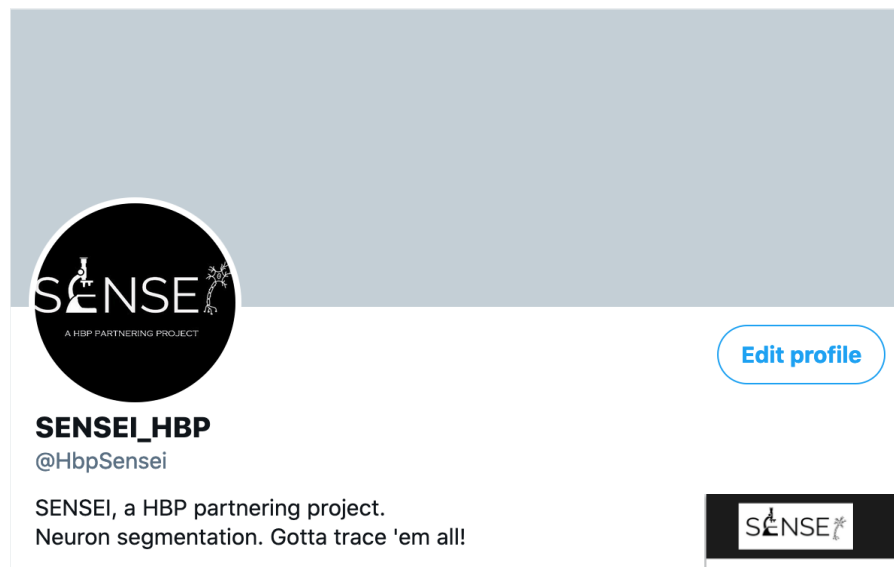
Clarified brain samples



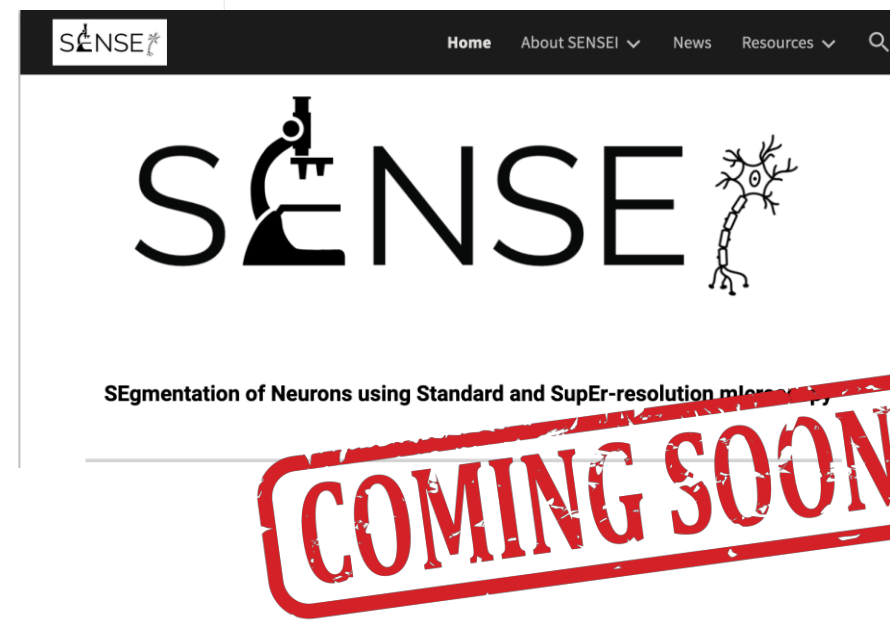


# Social SENSEI

## Twitter account



## Website



# Social SENSEI



← **Danglot**  
357 Tweets [Suivre](#)



**Danglot** @LydiaDanglot · 6 févr. 2020

Thanks @Flag-Era for granting and @nicvanel @DedeckerLab for being such great collaborators. We will be happy to collaborate together with @HumanBrainProj core partners.



💬 ↺ ❤️ 3 ↗

[Afficher cette discussion](#)



**Danglot** @LydiaDanglot · 6 févr. 2020

On the way back to Paris 🇫🇷  
Pretty happy for having presented our Sensei project at @HumanBrainProj summit concerning new segmentation methods for conventional and super resolution imaging of neurons. It's time now to enjoy chromatic aberrations 😊



← **Danglot**  
357 Tweets [Suivre](#)



**Bruker Fluorescence Microscopy** @BrukerFM · 10 nov. 2020

Our first speaker to start our 3-day Neuroscience Virtual Symposium is @LydiaDanglot! There is still time to register and watch her live presentation that kicks off at 8AM PST | 11AM EST! Register bit.ly/3nfbRtb



💬 ↺ ❤️ 4 ↗



**Danglot** @LydiaDanglot · 9 nov. 2020

Seems cool !

**Christophe Leterrier** @christlet · 9 nov. 2020

📢 We have a call for starting a new team or joining an existing team at our Institute @inp\_marseille! DM me if you're interested and want more info. RT appreciated 🙌 [twitter.com/SocNeuro\\_Tweet...](https://twitter.com/SocNeuro_Tweet...)

💬 ↺ ❤️ 1 ↗



**Danglot** @LydiaDanglot · 9 nov. 2020

# RRI

# Responsible Research and Innovation (RRI)

# Open Science

SENSEI is Open :  
Data and algorithm is or will be shared  
e.g. SmRG <https://github.com/AlejandroCallara/SmRG>

## Publication of picture and algorithm on Zenodo.org

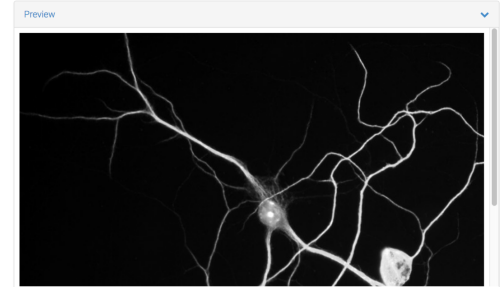
December 15, 2020

19 views 1 download

Neuronal cell labelled with anti MAP2 antibody - sample image for software testing

by DANGLOT Lydia

**Hippocampal neurons** in culture seeded on glass coverslip. Dendritic extensions are labeled with antibody against **MAP2**, a protein associated with microtubules and nucleus is labeled with **DAPI**. The resulting signal give access to the neuronal cell body and dendrite. Here the axon is not labeled. This picture has been taken on a Leica DMIRE microscope with a 40x objective. This is a sample demo to be used to test our automatic segmentation of neuronal cells thanks to the **Easy cell shape protocol**, available freely on icy software. You can download the software (PC, Mac or linux) and have access to the full documentation of this solution on <http://icy.biomeanalysis.org/protocol/easy-cell-shape-with-hk-means/> (Publication ID: ICY-77638). It segments in a user friendly mode the cell shape using the hk-mean plugin that applies a N-class thresholding based on a K-Means classification of the image histogram.



Files (921.8 kb)

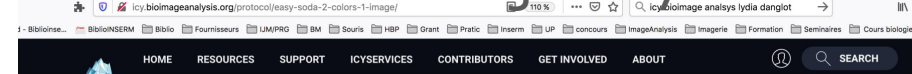
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indexed in  
**OpenAIRE**

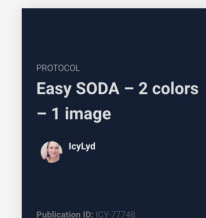
**Publication date:**  
December 15, 2020  
**DOI:**  
[10.5281/zenodo.4322944](https://doi.org/10.5281/zenodo.4322944)  
**Keyword(s):**  
neuron hippocampal confocal cell shape  
**Related identifiers:**  
Supplementary material  
[10.5281/zenodo.4317783](https://doi.org/10.5281/zenodo.4317783) (Software)  
**Communities:**  
[icy.biomeanalysis.org](#)  
**License (for files):**  
[CC Creative Commons Attribution 4.0 International](#)

**Versions**  
Version 1  
[10.5281/zenodo.4322944](https://doi.org/10.5281/zenodo.4322944) Dec 15, 2020

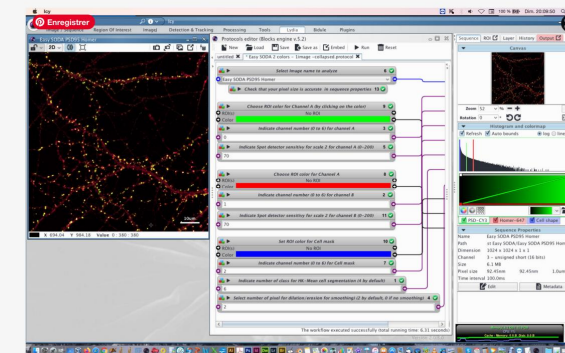
## Publication of free image analysis solution



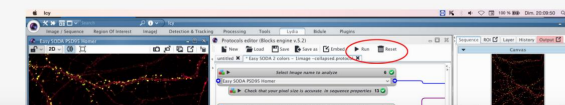
This particular protocol will allow you to analyse 1 image (2D or 3D) using spot detector, HKmean and icy SODA plugins. It will adapt automatically to 3D if your data is in 3D.



**SHORT DESCRIPTION**  
**DOCUMENTATION**  
**ARTICLES ON THIS**  
**REVIEW THIS**  
**DEPENDENCIES**  
**CHANGELOG**



- Open protocol editor.
- Load Easy icy SODA protocol

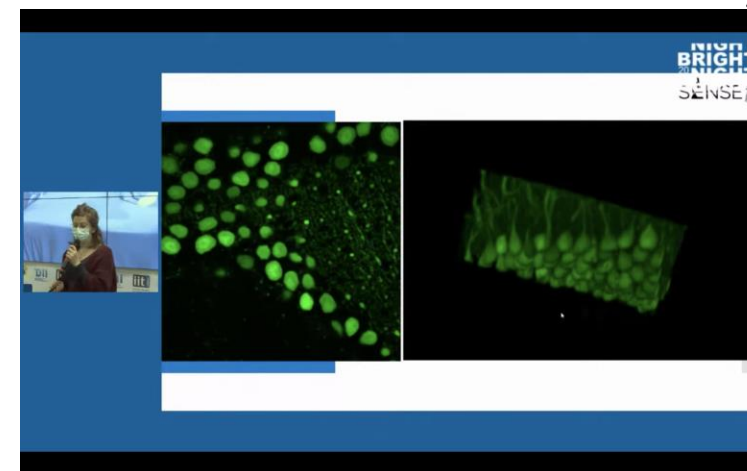




RRI

# Responsible Research and Innovation (RRI) Communication

SENSE is communicating with  
Students and general public  
(interactive live presentation  
at Bright)



- Brain Awareness week with IPNP animations, this Friday 2pm

**Inserm Workshop 249**  
Multidimensional imaging and quantitative Analysis of cell dynamic's: focus on traffic and cell migration

**REGISTRATION DEADLINE: September 14, 2018**

**ORGANIZERS:** Lydia DANGLOT (Institut de Psychiatrie et Neurosciences de Paris), Alexandre DUFOUR (Institut Pasteur, Paris), Franck DEBARBIEUX (Institut de Neurosciences de la Timone, Marseille)

**AIMS:** To expose all methods available to image and analyze quantitatively cellular dynamics (organelles and traffic, cell migration and deformation). It will cover both imaging and data analysis of cultured cells but also tissular level with 2 photons in vivo imaging.

**PHASE I - CRITICAL ASSESSMENT**  
November 26-28, 2018 in Bordeaux

**DYNAMICS OF THE CYTOSKELETON AND CELLULAR MORPHOGENESIS**  
Isabelle TARDIEUX (Institute for Advanced Bioscience, FRA), Robert MURPHY (Carnegie Mellon University, USA), Ferreol SOULEZ (Université Lyon 1, FRA), Xavier GIDROL (CEA, FRA)

**3D IMAGING OF CELLS AND DENDRITIC SPINE**  
Jean-Yves TINEVEZ (Institut Pasteur, FRA), Nicolas HECK (Paris Sorbonne University, FRA), Harold MAC GILLAVRY (Utrecht University, NLD), Flavie LAVOIE-CARDINAL (Laval University, CAN)

**INTRACELLULAR TRAFFICKING: DYNAMICS OF ORGANELLES AND MOLECULES AT THE CELL SURFACE**  
Franck PEREZ (Institut Curie, FRA), Mayeul COLLOT (Université de Strasbourg, FRA), Thibault LAGACHE (Columbia University, USA), Lydia DANGLOT (Institut de Psychiatrie et Neurosciences de Paris, FRA), Yves DE KONINCK (Laval University, CAN)

**IMAGING AND CELLULAR MONITORING IN VIVO: THE CHALLENGE OF COMPLEX ENVIRONMENTS WITH FOCUS ON 2 PHOTONS IMAGING**  
Craig JENNE (Calgary University, CAN), Franck DEBARBIEUX (Institut de Neurosciences de la Timone, FRA), Chris XU (Cornell University, USA), Laurent BOURDIEU (Ecole Normale Supérieure, FRA)

**SEMAINE DU CERVEAU 2021**

du 15 au 21 mars

**LA SEMAINE DU CERVEAU**  
Société des Neurosciences

Sous le haut patronage de  
Mme Frédérique Vidal  
Ministre de l'Enseignement Supérieur  
de la Recherche et de l'Innovation

#SDC2021  
www.semaineducerveau.fr

## RRI

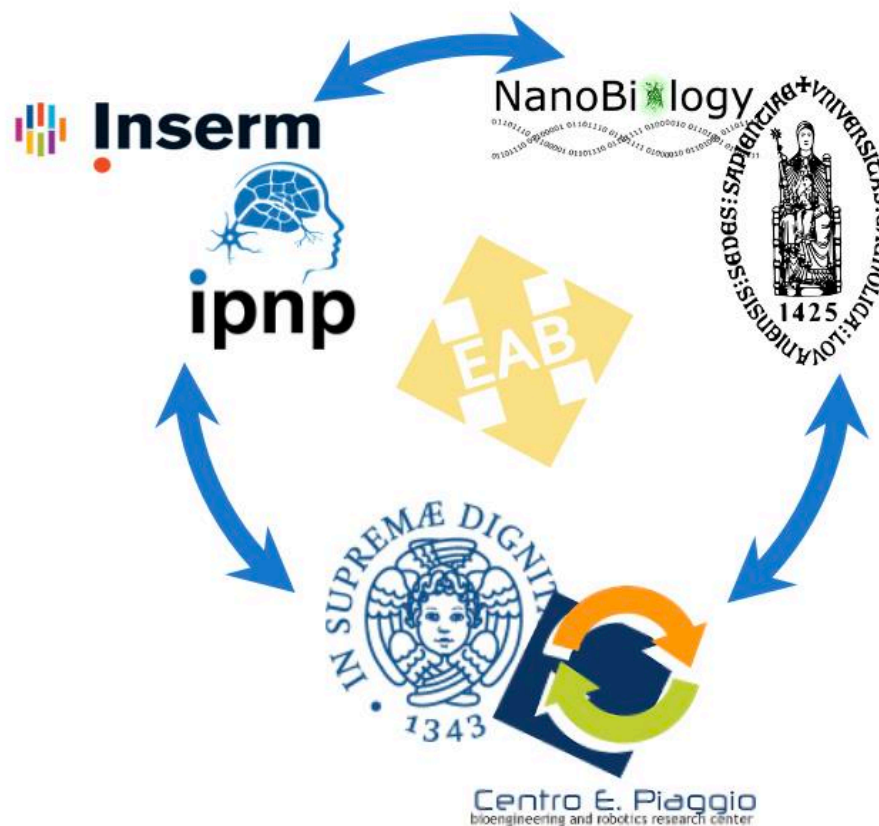
Responsible  
Research and  
Innovation  
(RRI)

## Ethics – 3R

SENSEI adheres to 3Rs principles.  
UNIPi staff: membership of Centro 3R  
Reduction-Refinement-Replacement



SENSEI collaborates with an Independent Ethics Advisory Board



## Ethics Advisory Board



Univ. Genova



Azienda Osp.  
Univ. Pisa



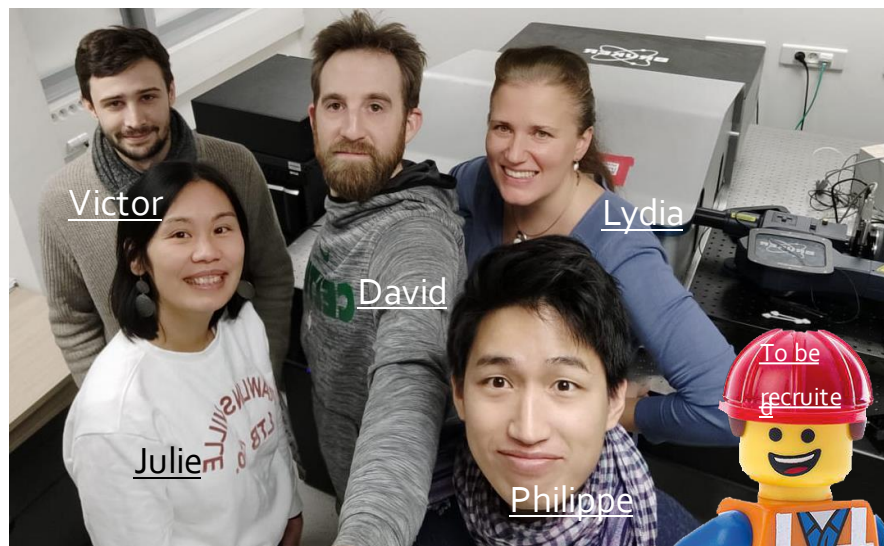
## RRI

# Responsible Research and Innovation (RRI)

# Research and Innovation

# Innovation

(RRI)



Victor

Lydia

# David

Julie

Philippe

To be  
recruite



NanoBiology



Daniela

Chiara

Alejandro

Arti

Carla

Nicola

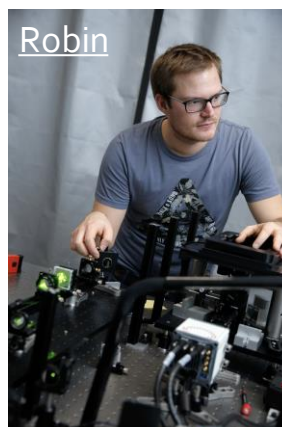
## Francesca



**CENITTO E. PLAGGIO**  
bioengineering and robotics research center



## Franziska

Robin

Wim



Peter



Thank You!!!