Platform for single cell optogenetic to study synaptic engrams in vitro

Pisa, 13.09.2022

Clara Zaccaria, University of Trento, Physics Department, NanoScience Laboratory

UNIVERSITY OF TRENTO

Outline:

- Our goal: build up an in-vitro engram
- Top-down excitation: Digital Light Processor (DLP) + optogenetics
- Results

PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

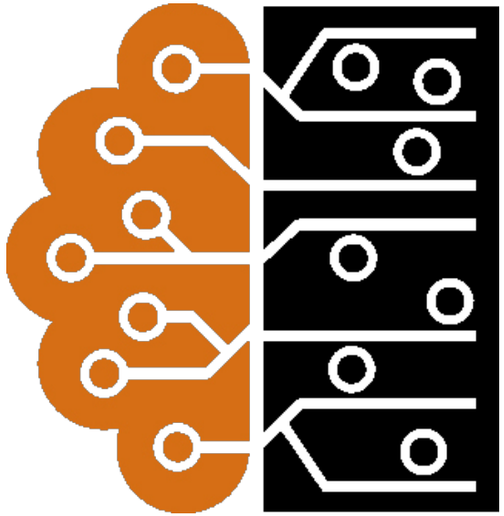
BACKUP neuromorphic photonics and the brain
UNIVERSITY OF TRENTO

BACKUP Project

The aim of the project is to create an **hybrid neuromorphic computing platform**.

Neuronal network + Artificial (photonic) network

Hybrid network, able to **replace malfunctioning brain sectors**



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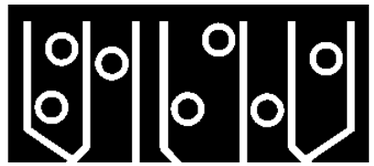
Understand the basic processes of the brain (**memory**, epilepsy..)



Photonic computation

Implement neuromorphic computation in integrated photonics

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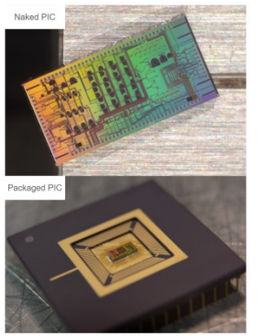

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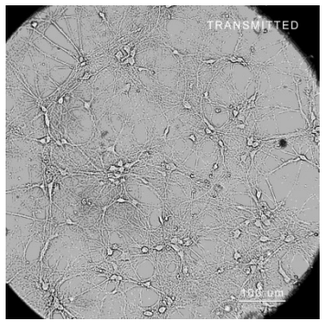

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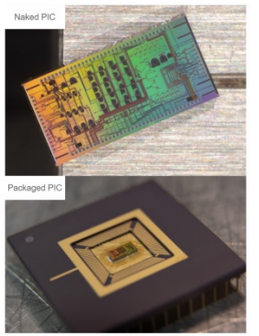

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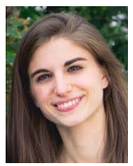

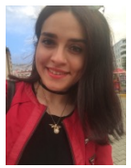


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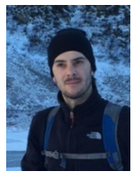
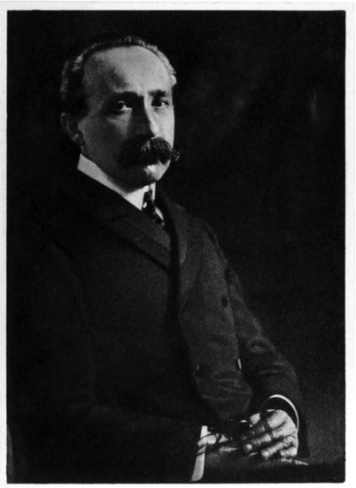



PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

BACKUP neuromorphic photonics and the brain



Richard Semon 1904

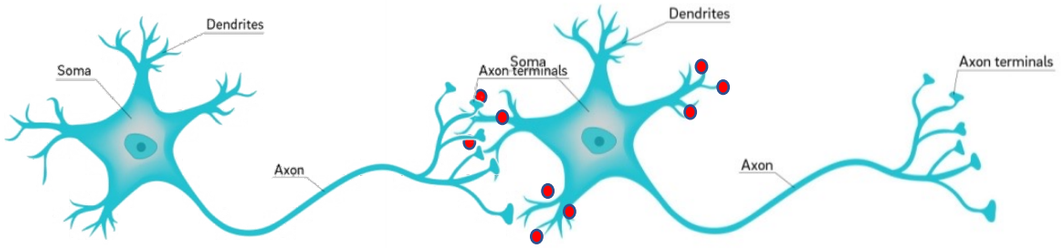
Donald O. Hebb 1949

Memory resides in specific “cell assemblies” (engram) formed by the strengthening of neuronal connections

‘When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased

Neuron A

Neuron B



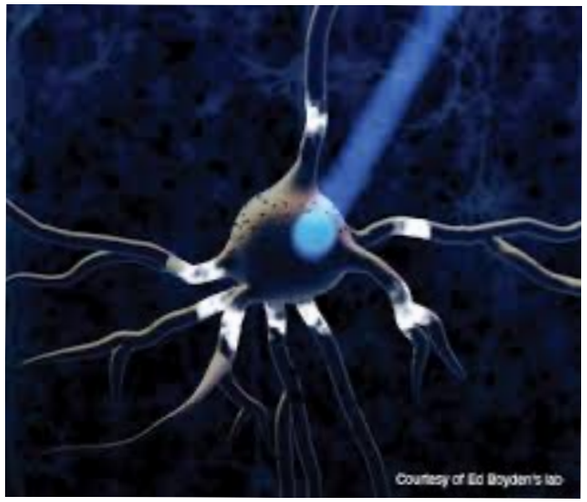
Our goal:
build up an in-vitro engram

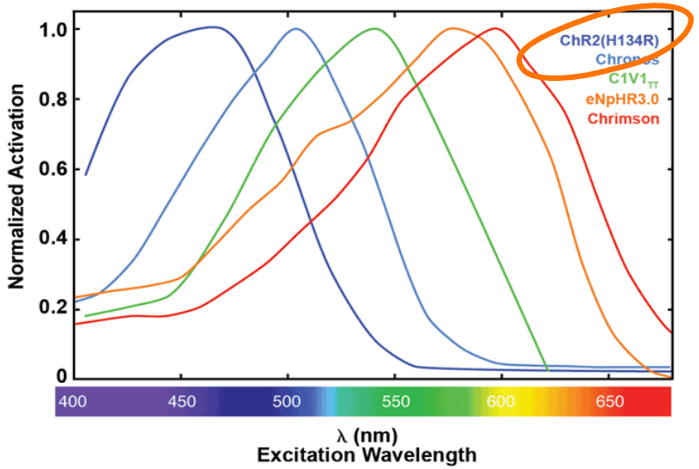
PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

BACKUP neuromorphic photonics and the brain

Inducing memory with light: optogenetics

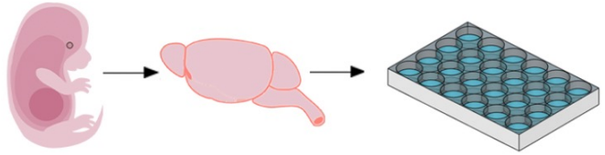
AV (CHR2): pAVV-hSyn-hChR2(H134R)-EYFP





https://www.photonics.com/Articles/Lasers_Optics_Enhance_Optogenetics_Studies/a57283

Wild type mice (C57Bl6)
E 17.5 embryos

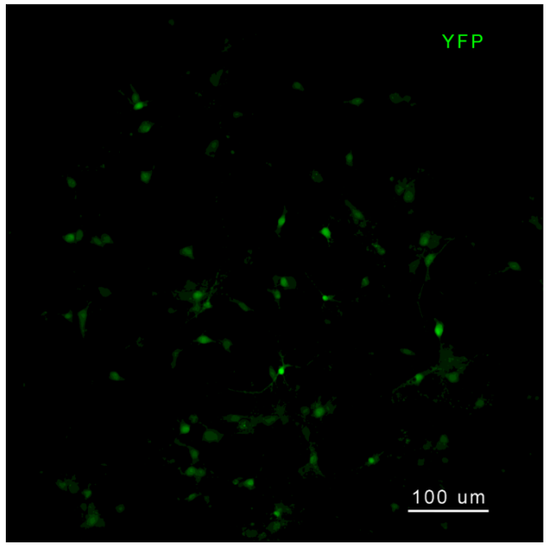


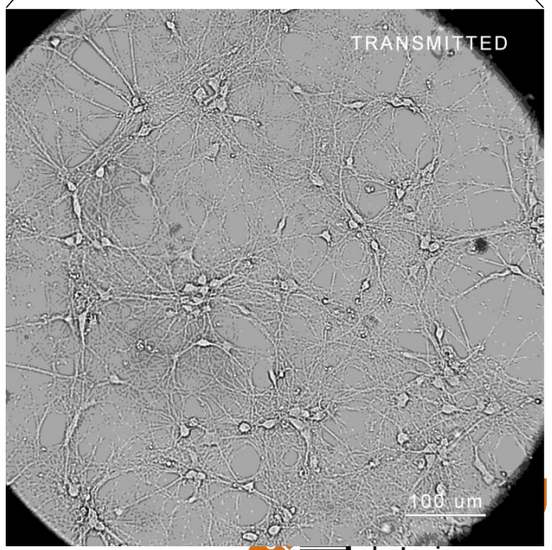
AV
INFECTION

0
DIV

2
DIV

11
DIV

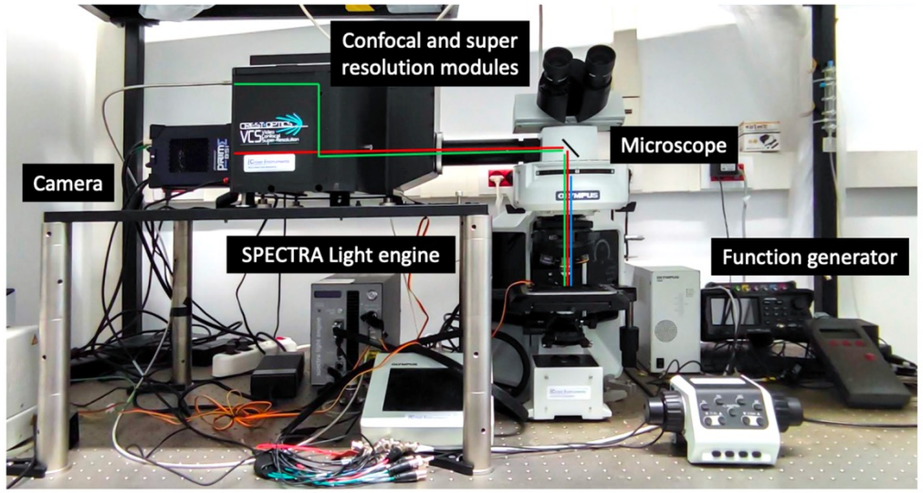


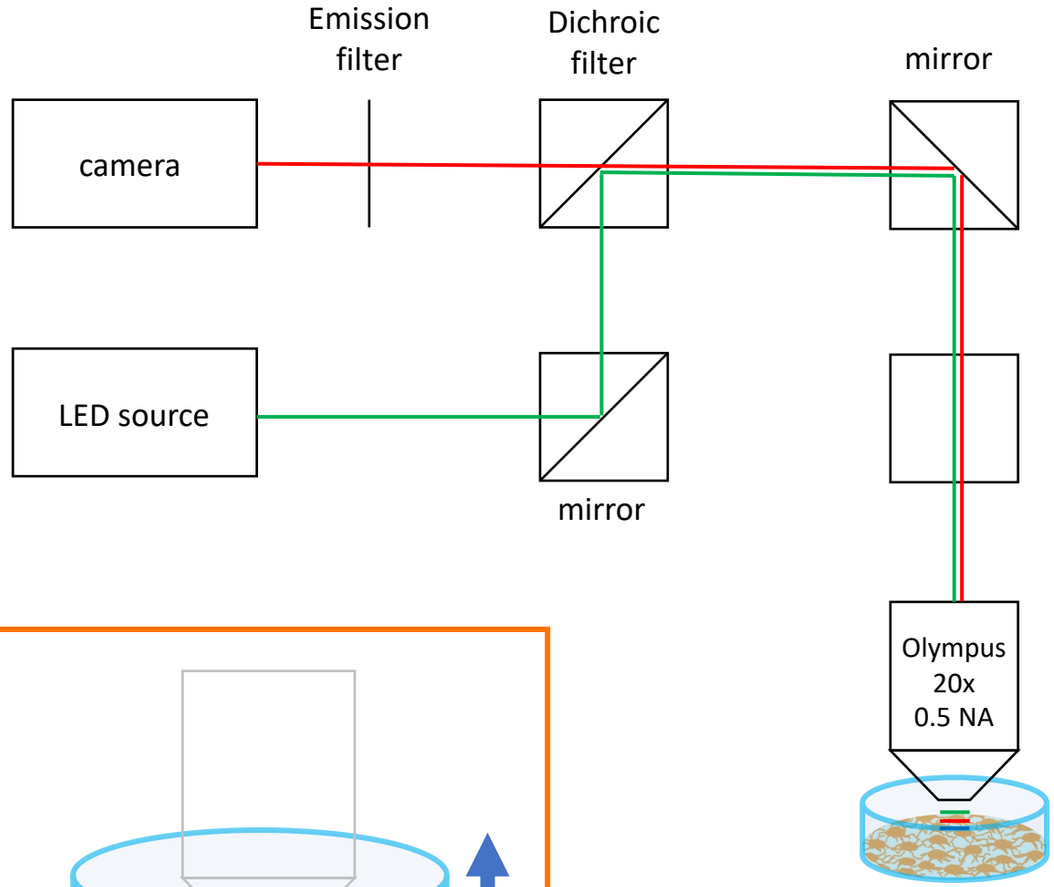


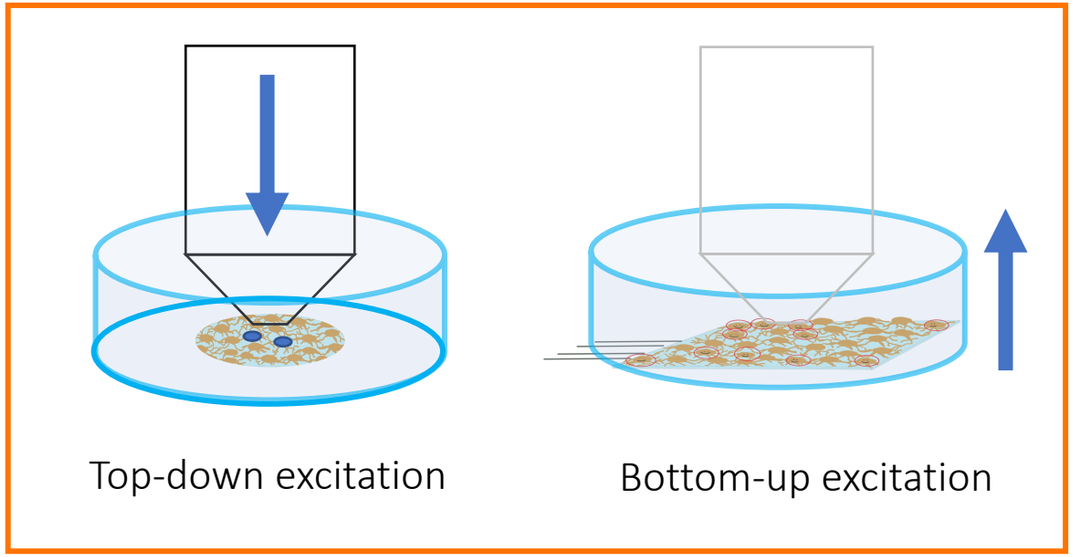
PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

photonics and the brain

Microscope setup: how to create patterned illumination?







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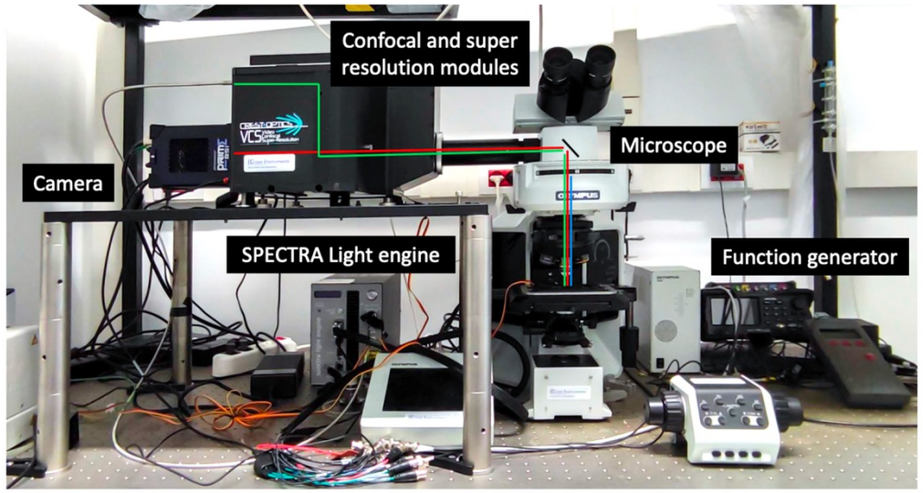
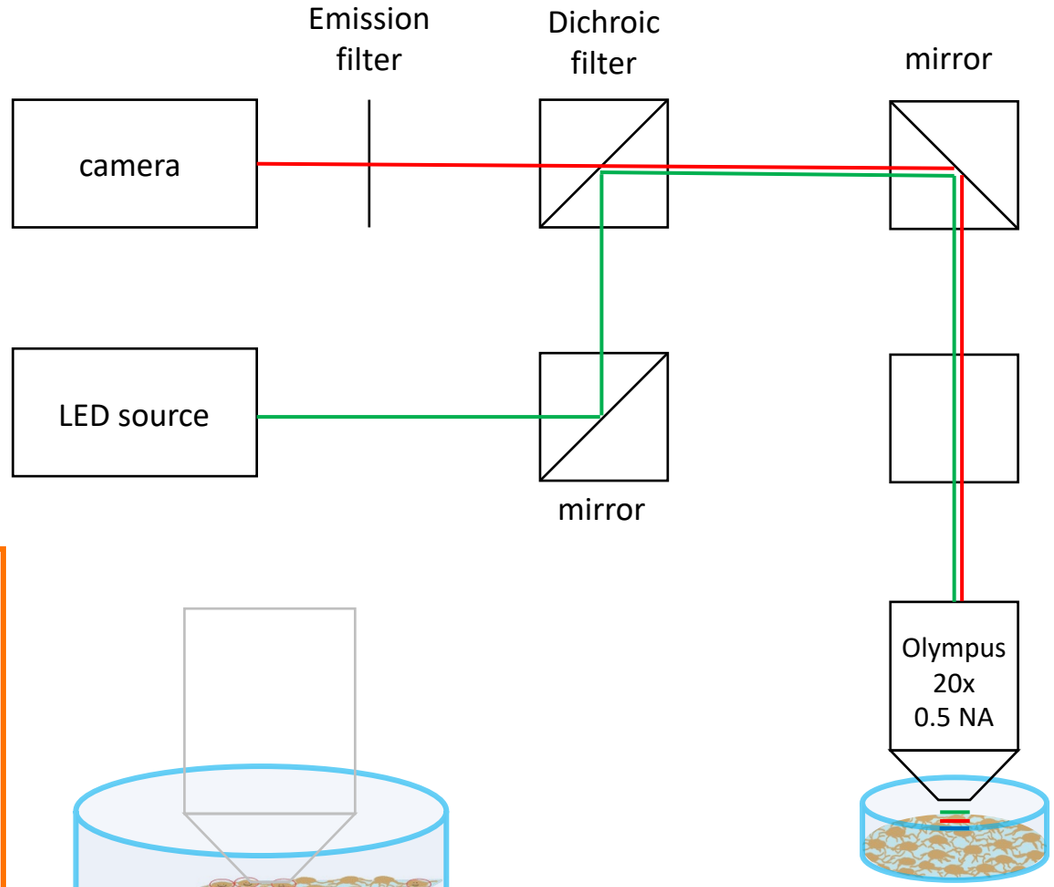
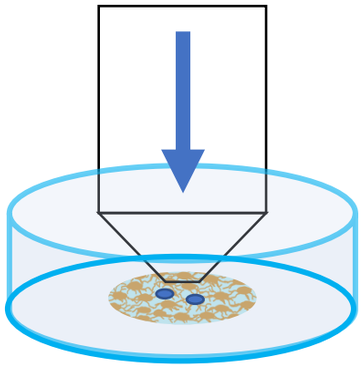


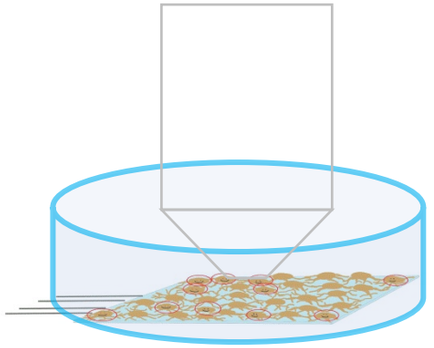




Top-down excitation

Digital Light Projector

(DLP)



Bottom-up excitation

PHOTONIC CHIP

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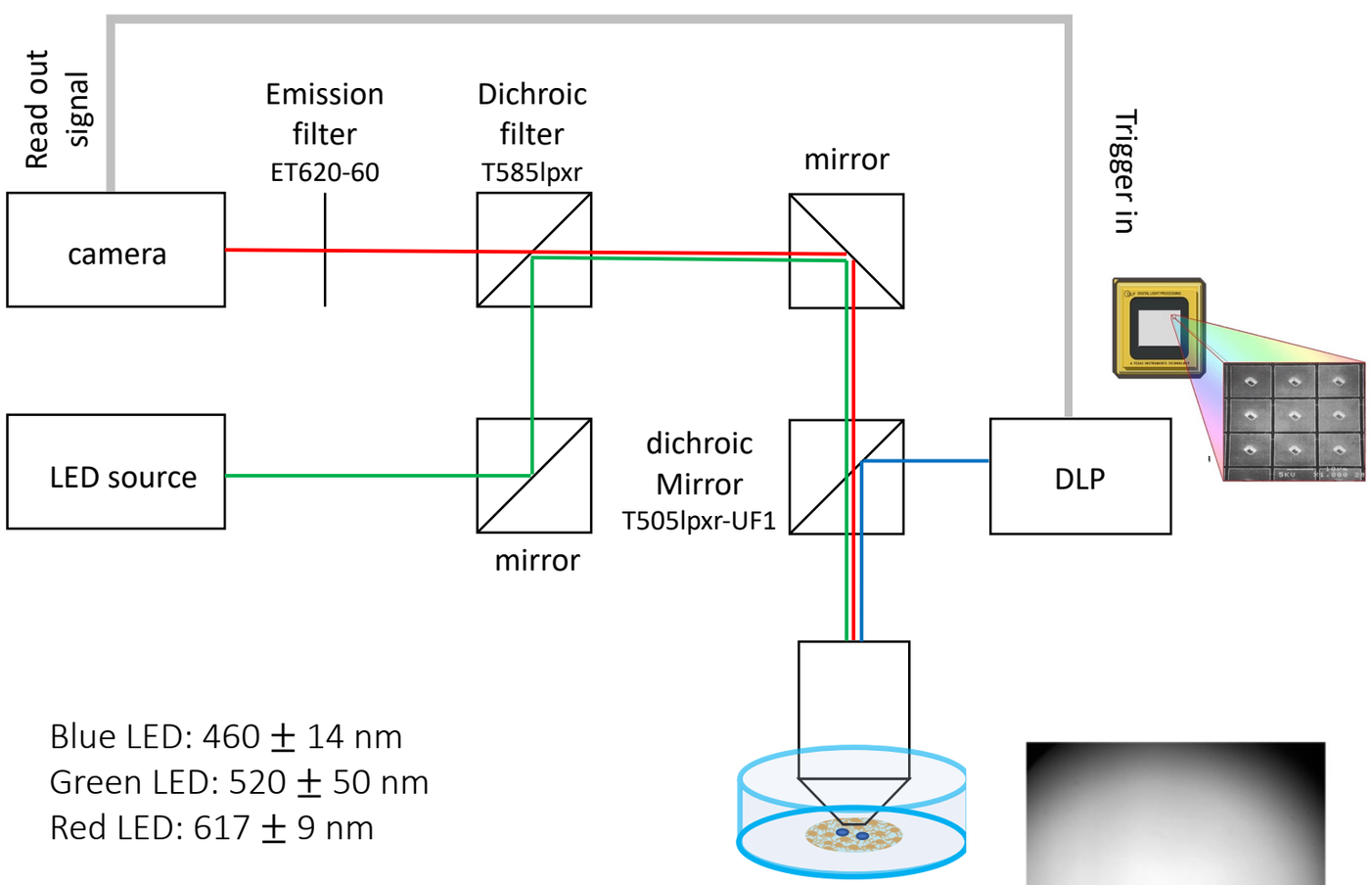
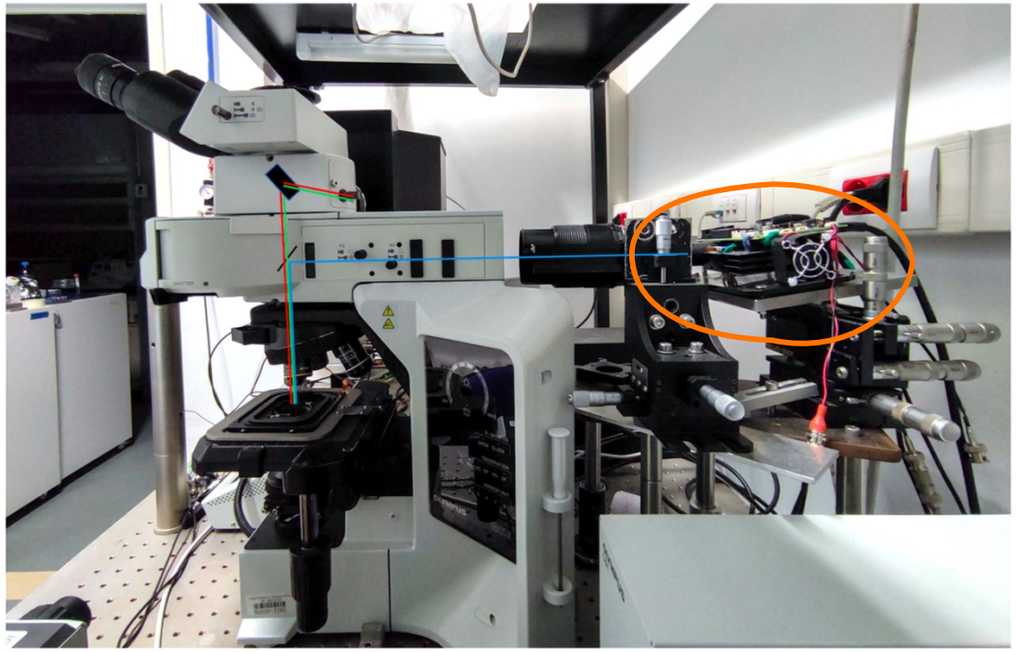
BACKUP neuromorphic photonics and the brain

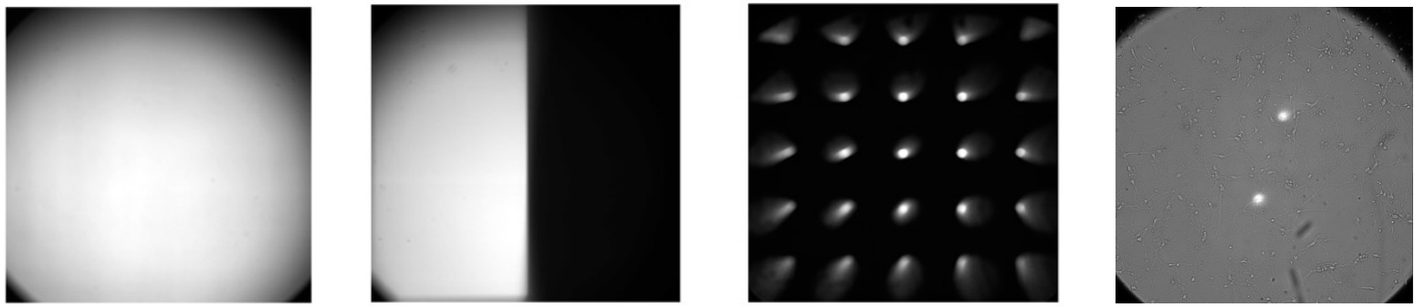
Digital Light Projector (DLP)





We can excite any desired position of the field of view

Blue LED: 460 ± 14 nm
Green LED: 520 ± 50 nm
Red LED: 617 ± 9 nm

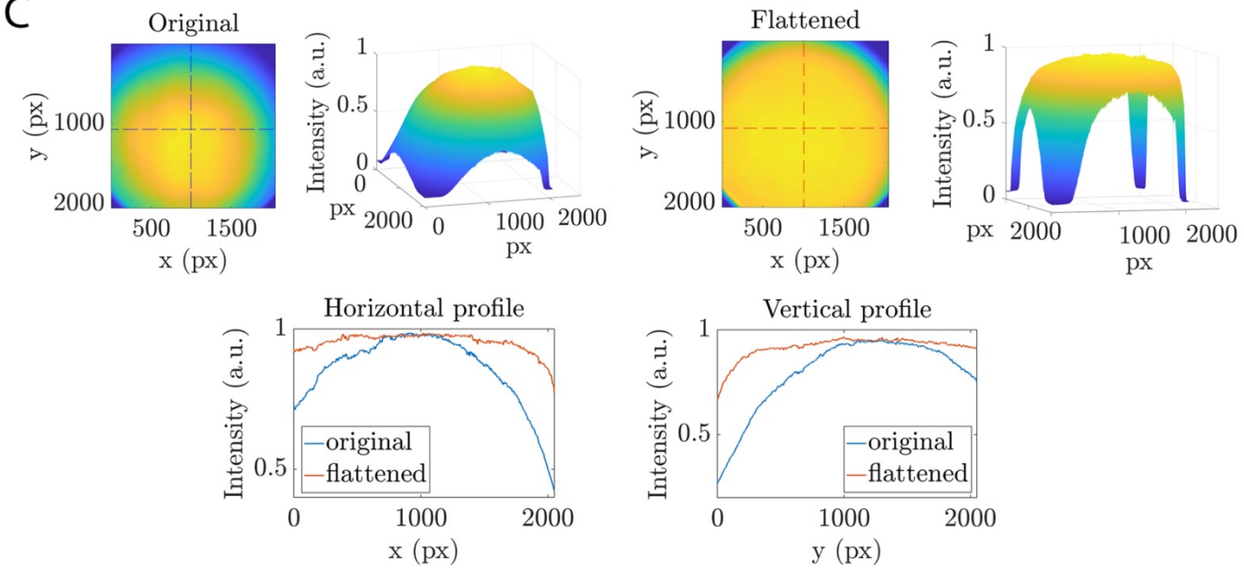


Temporalresolution~µs

PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

BACKUP neuromorphic photonics and the brain

C



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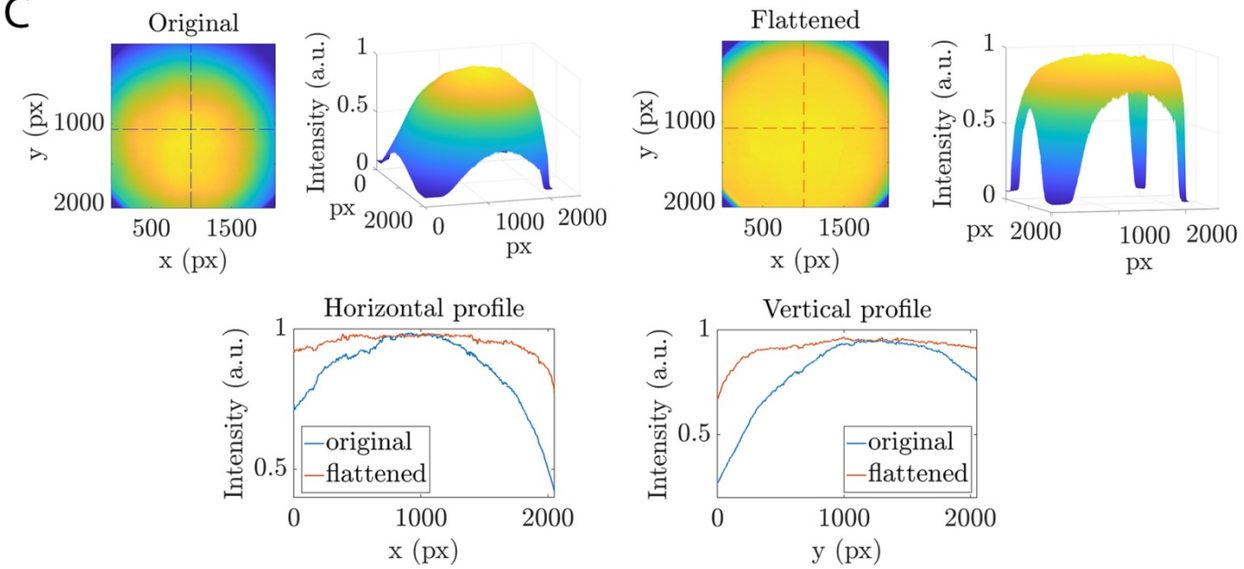
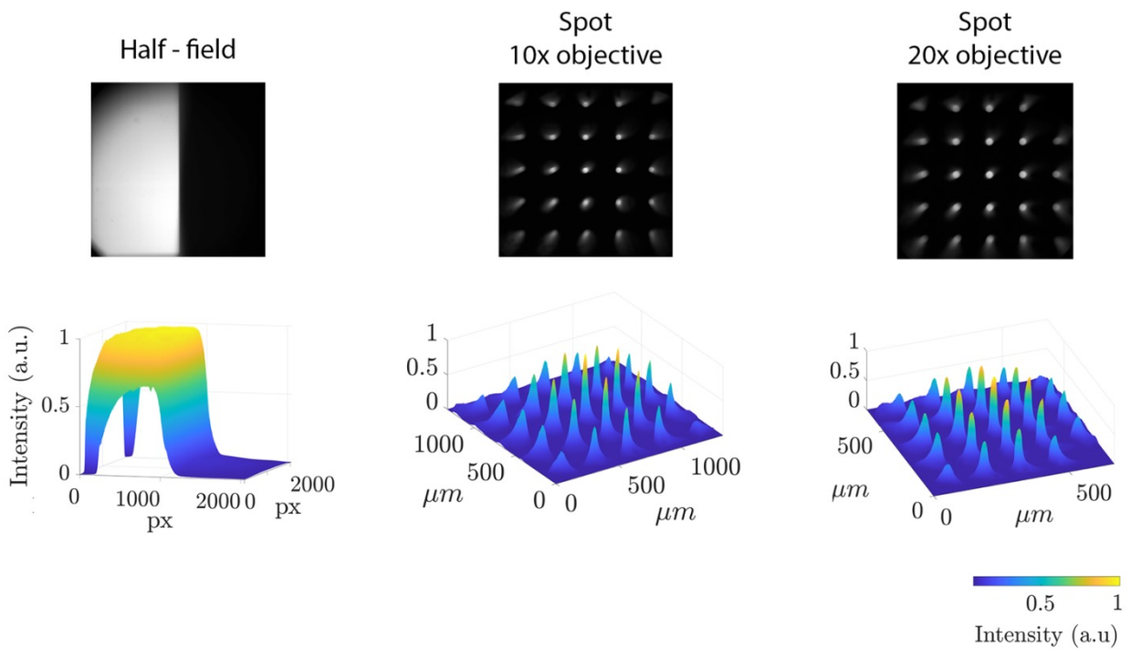


D



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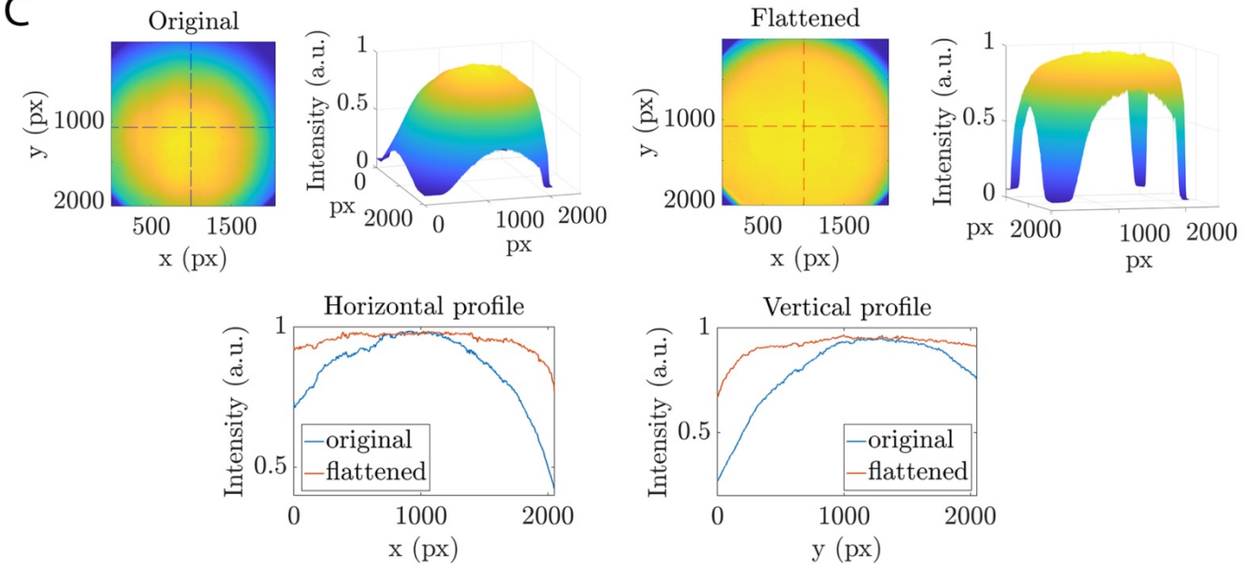
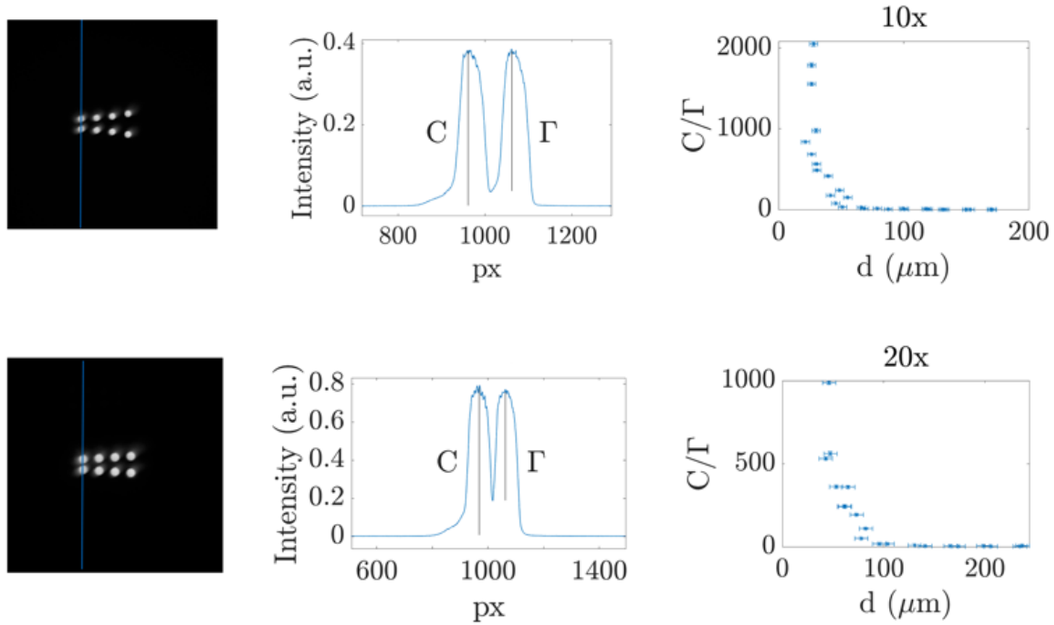
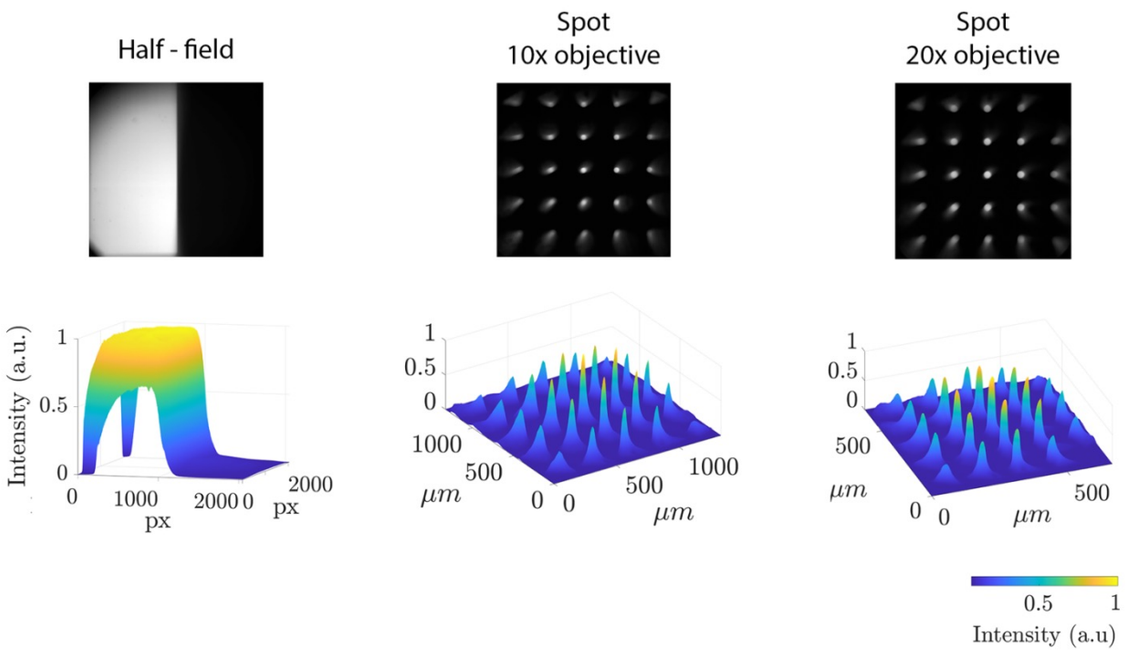


E



D



$$C = \frac{\mathbf{Max} - \mathbf{Min}}{\mathbf{Min}} \times 100$$

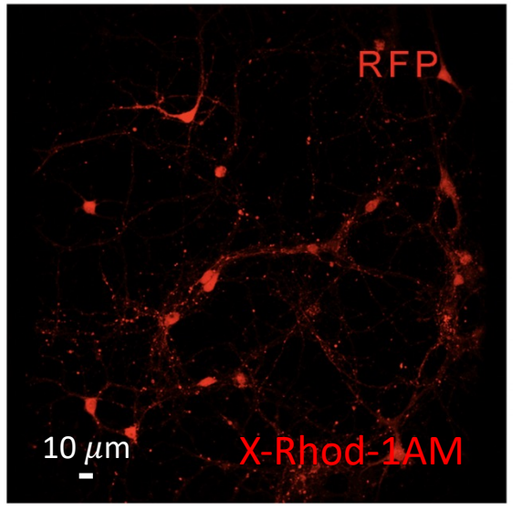
OBJ	Minimum resolution (FWHM)	Spot FWHM	Spot contrast (C)	Minimum distance
10x dry	6 ± 2 μm	47 ± 8 μm	$(14 \pm 6)\times 10^3$	90 ± 7 μm
20x W	3 ± 1 μm	27 ± 7 μm	$(17 \pm 8)\times 10^3$	60 ± 3 μm

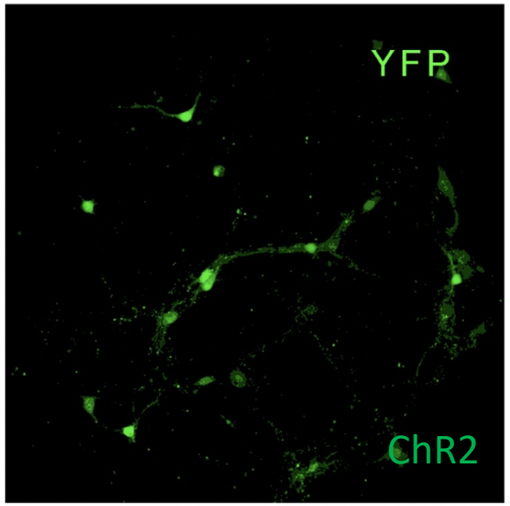
We have enough resolution and contrast to get localized illumination on single cells.

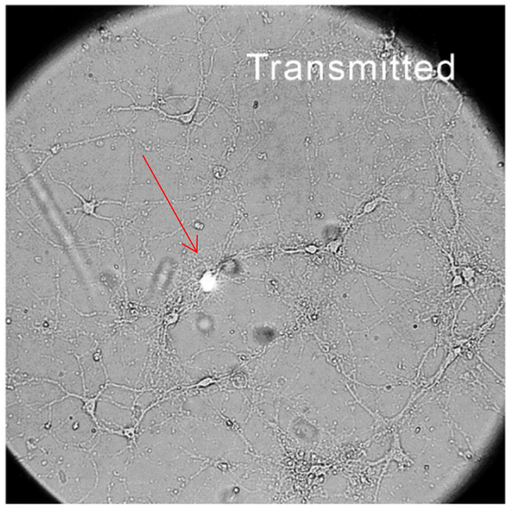
PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

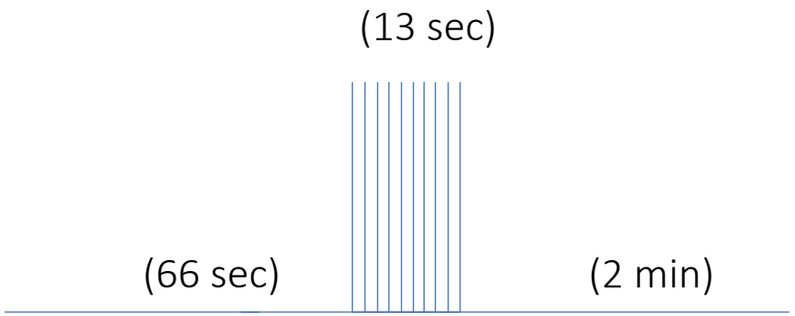
BACKUP neuromorphic photonics and the brain

Are we able to excite neurons?



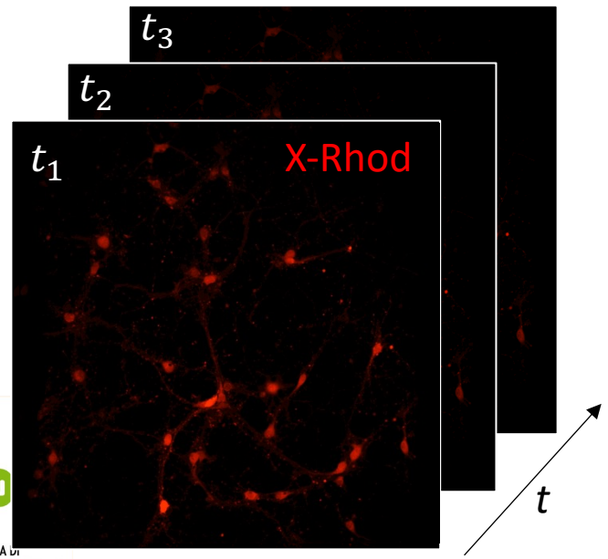




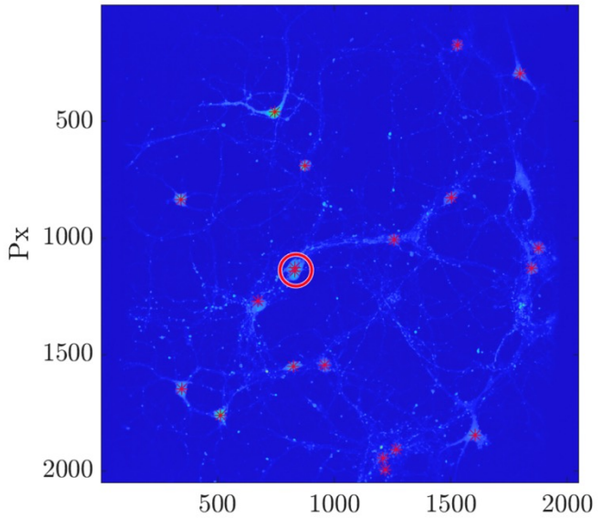


20 ms long pulses, 3Hz

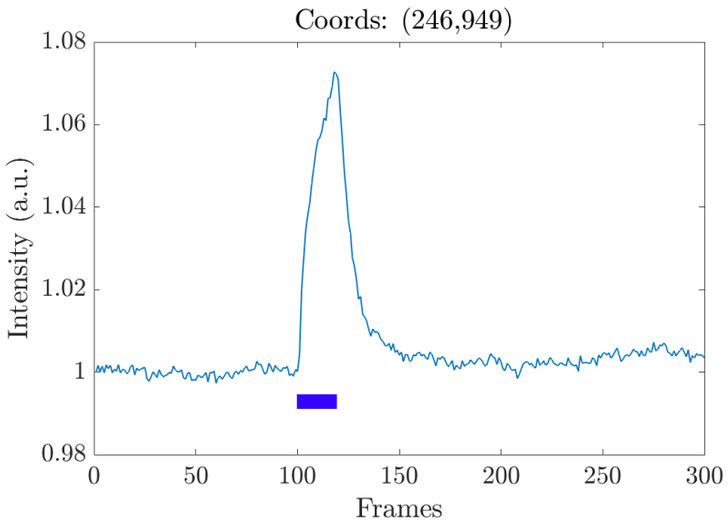
Collect time-lapse data



Define multiple ROIs



Extract temporal data



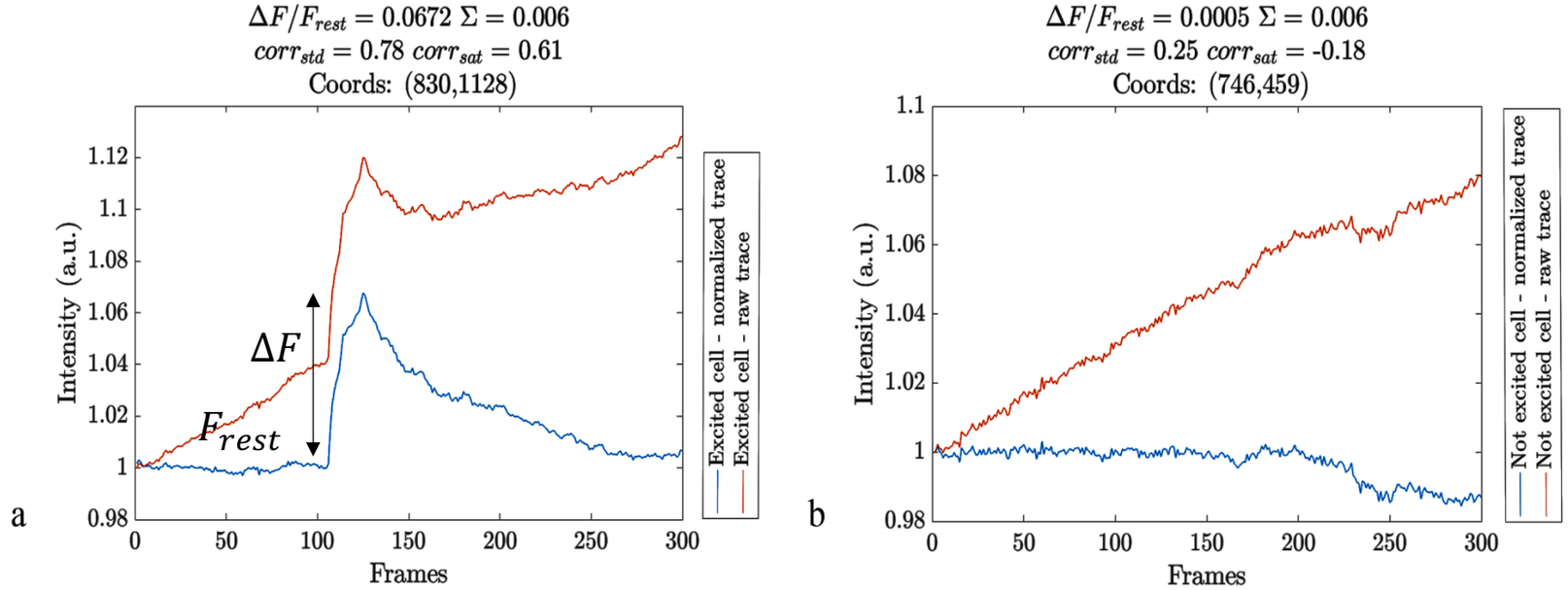
PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

photonics and the brain

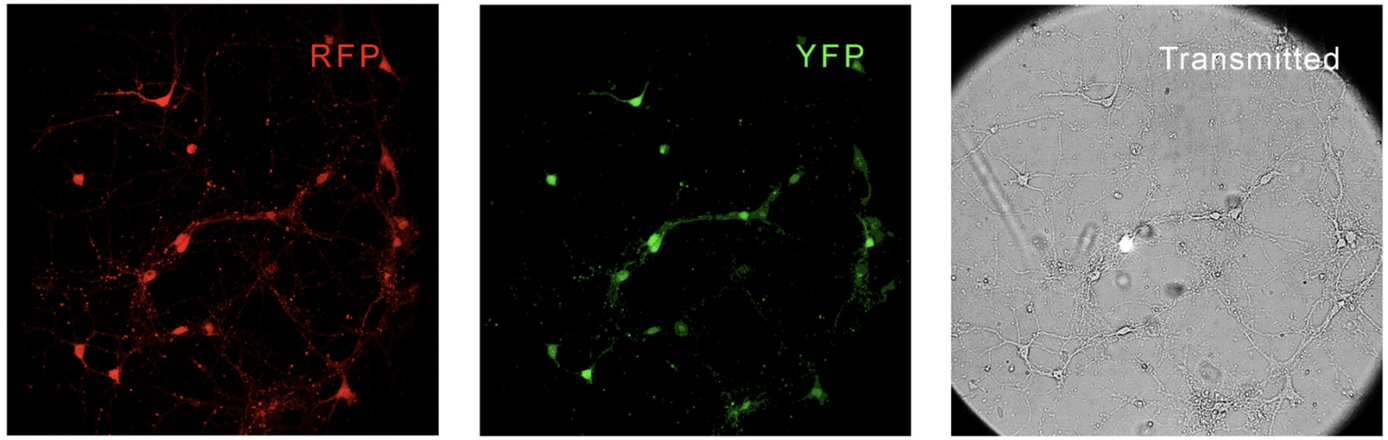
ROIs analysis

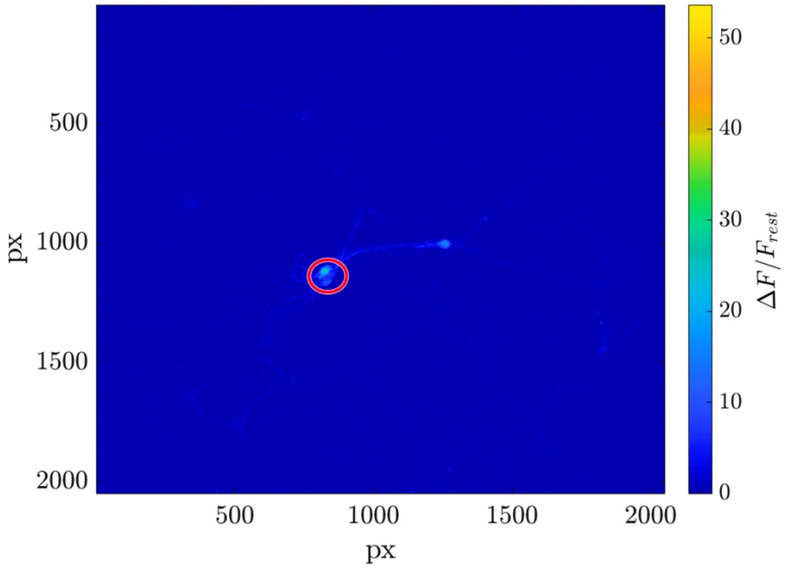
Raw trace

Normalized trace



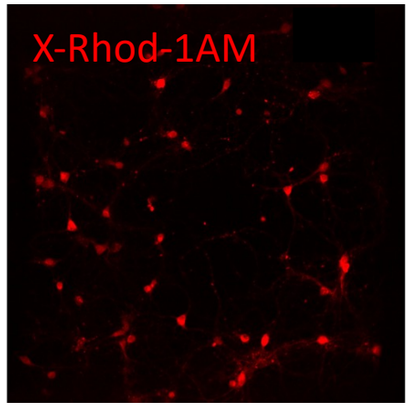
Pixel by pixel analysis

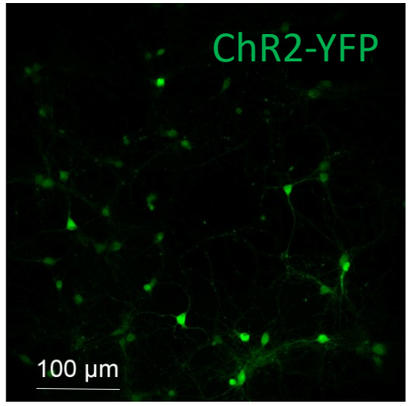


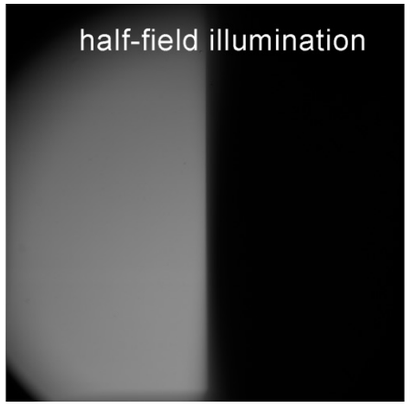


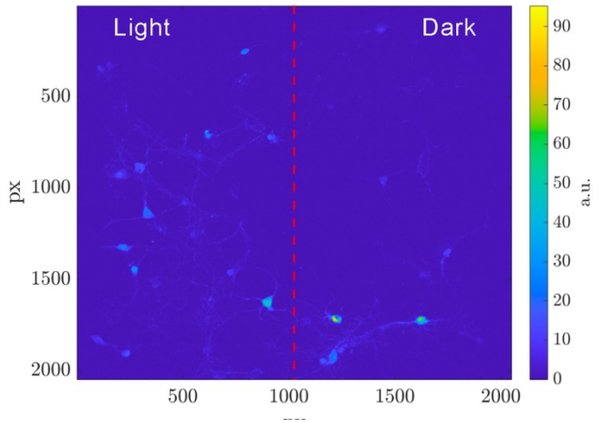
PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

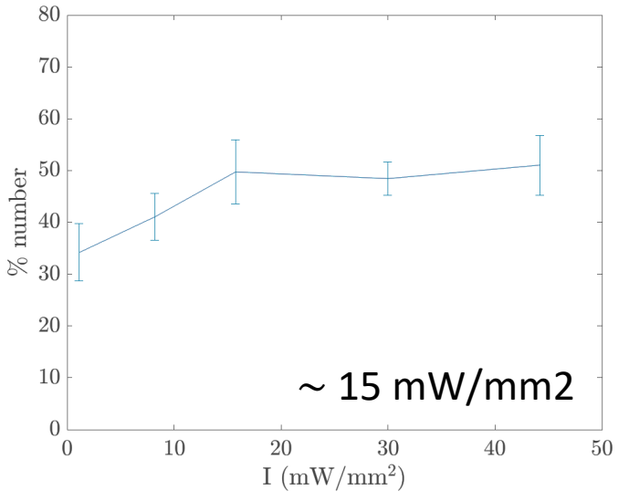
BACKUP neuromorphic photonics and the brain

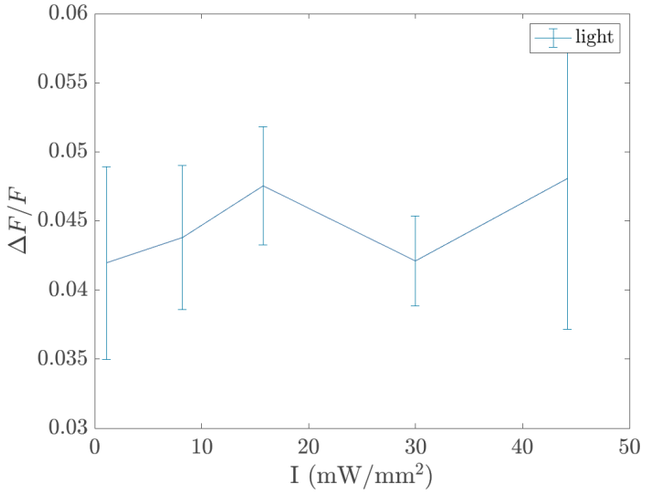


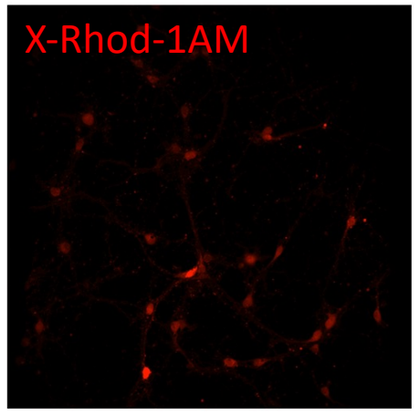


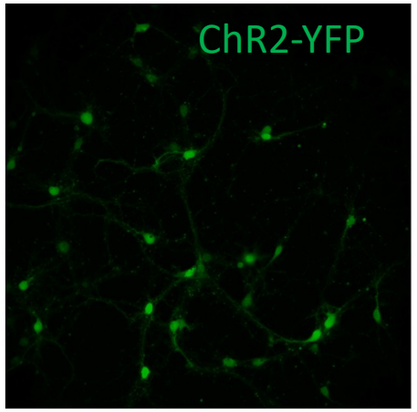


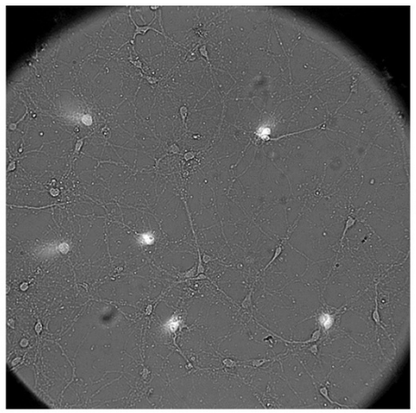


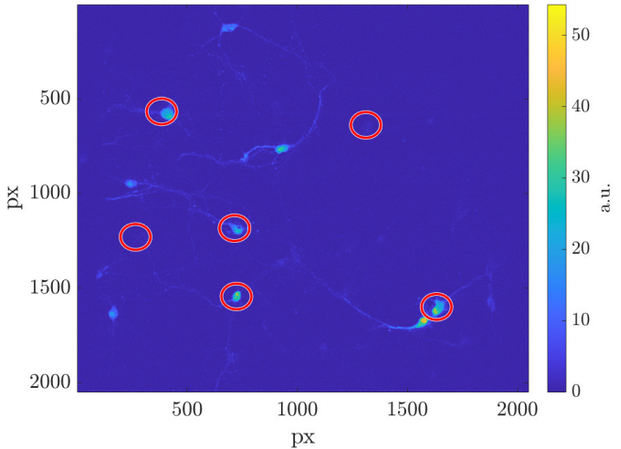






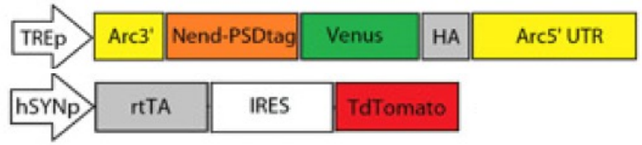




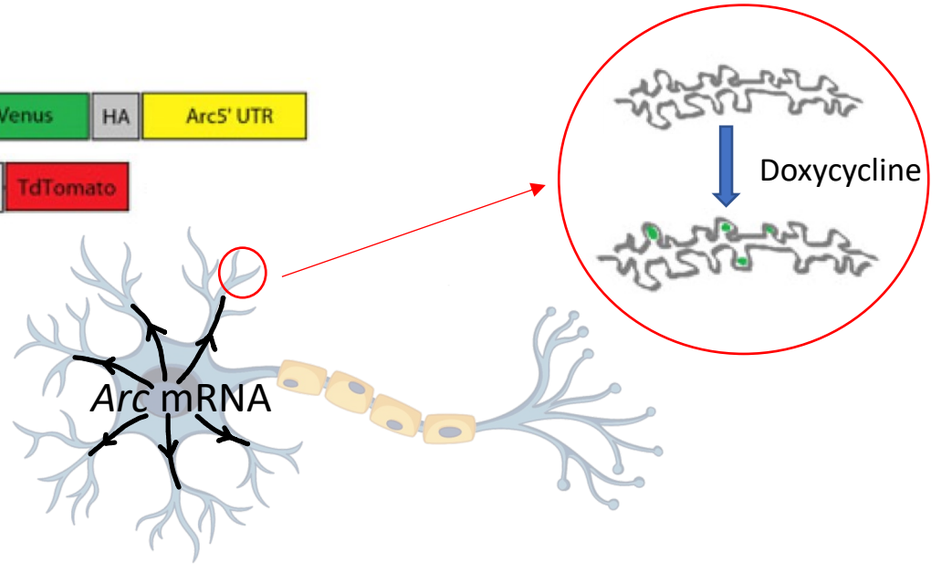


PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

SynActive (SA)

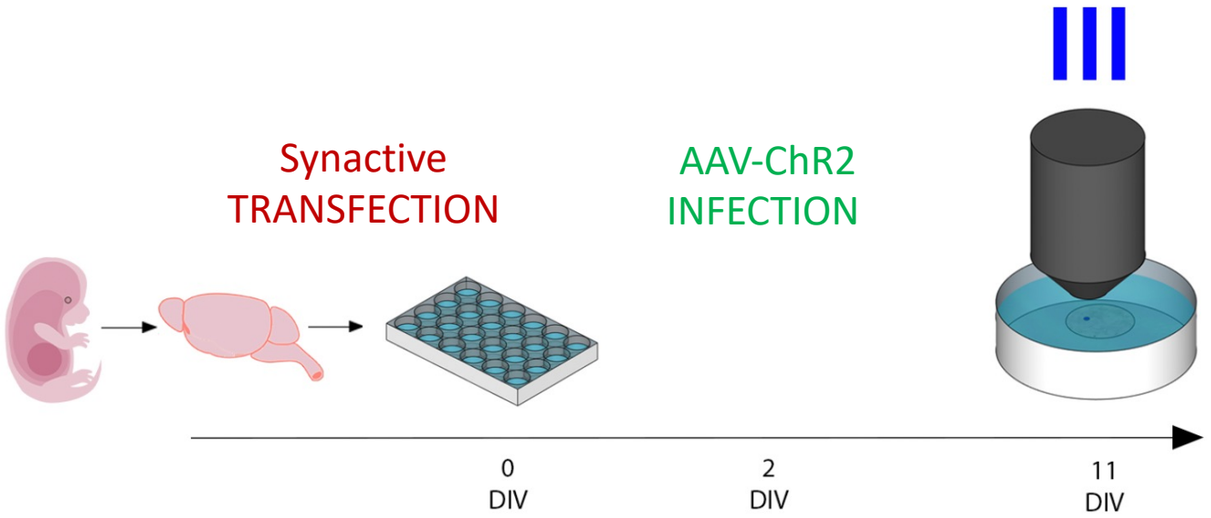


SA-Venus



Are we able to potentiate neurons?

10 trains of 13 pulses at
100 Hz repeated at 0.5 Hz

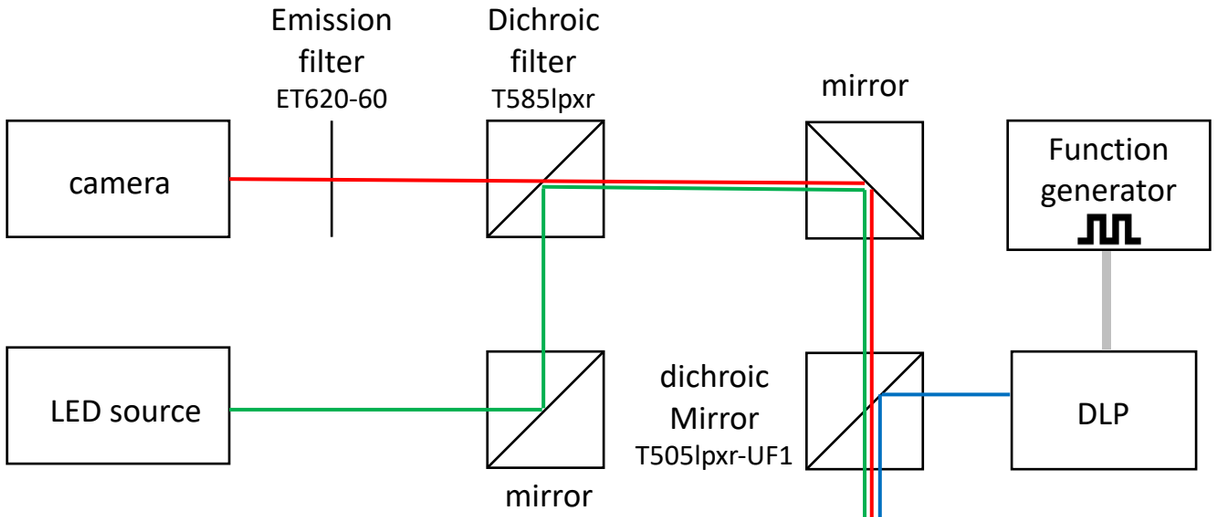


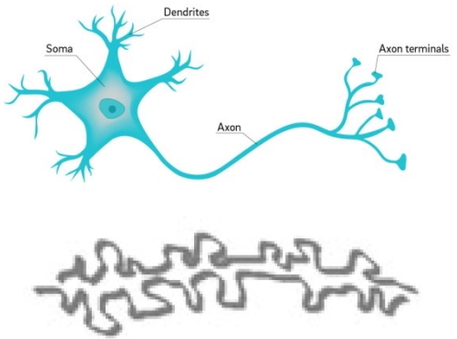
AAV-CHR2: pAVV-hSyn-hChR2(H134R)-EYFP

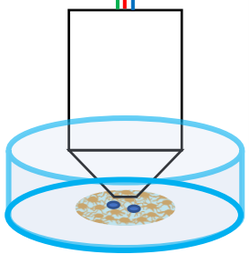
Synactive: TREp-Arc3'-NendPSDTag-
Venus-HA-Arc5'UTR
CAG::rtTA- IRES-tdTomato

PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

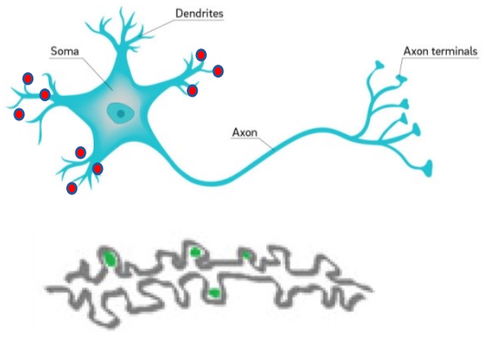
BACKUP neuromorphic photonics and the brain



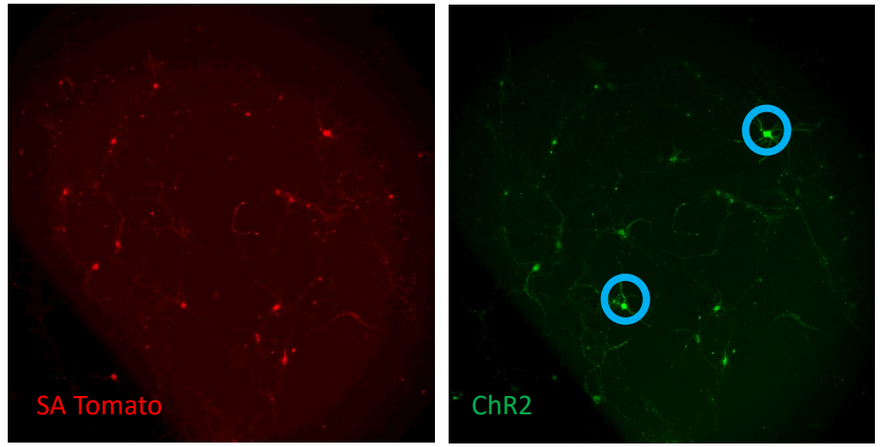




24h Doxycycline



2 spots stimulation



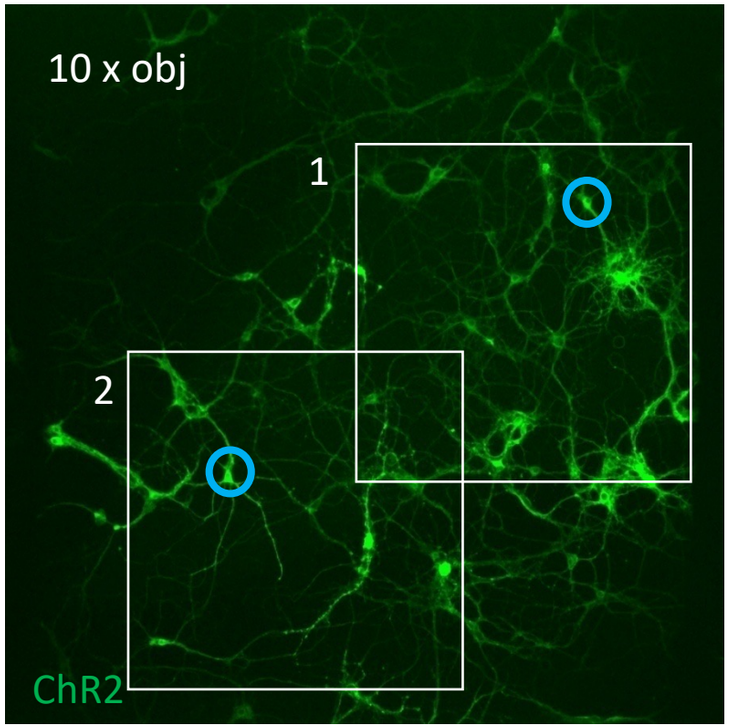
stimulus → PFA fixing → Immunocytochemistry

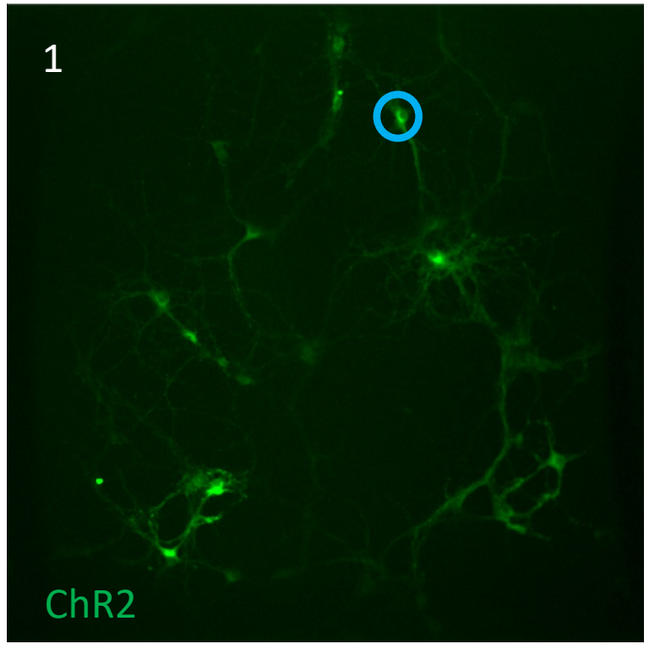
t=0 — 90 min

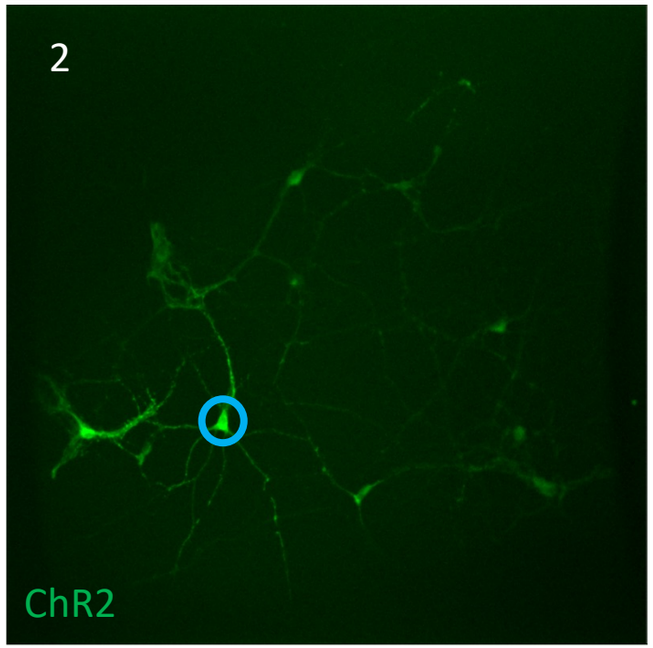
PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

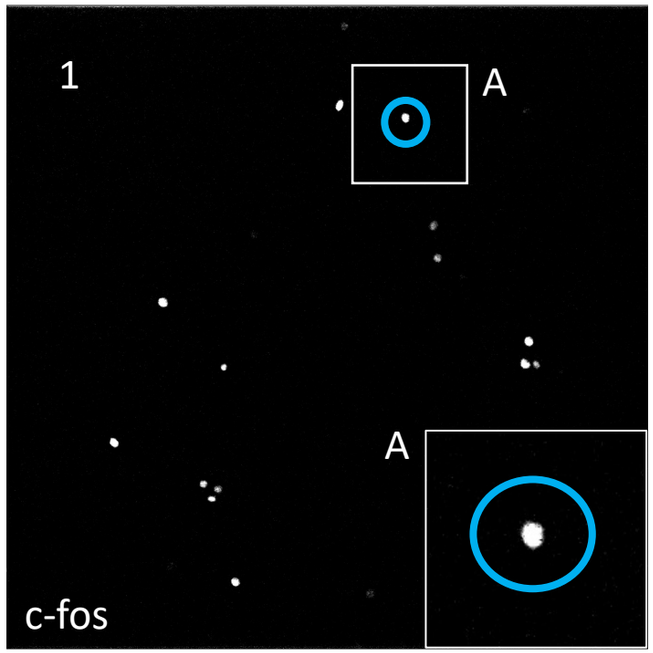
BACKUP neuromorphic photonics and the brain

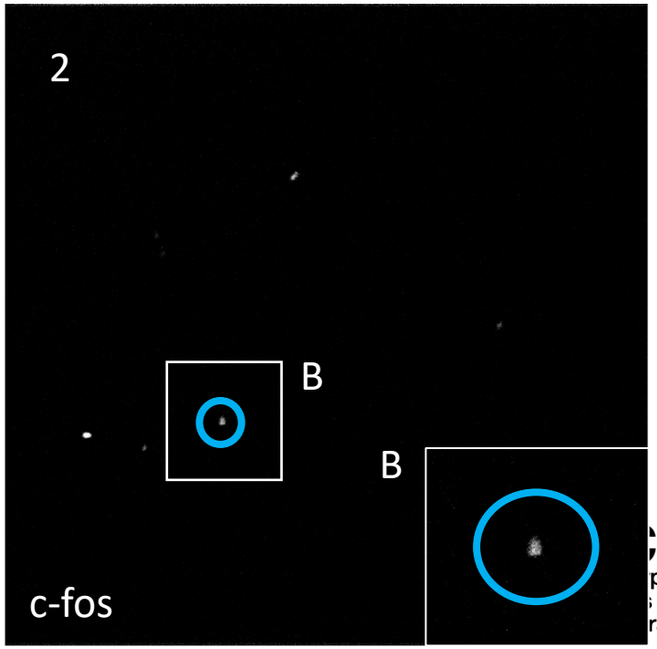
Neuronal engram







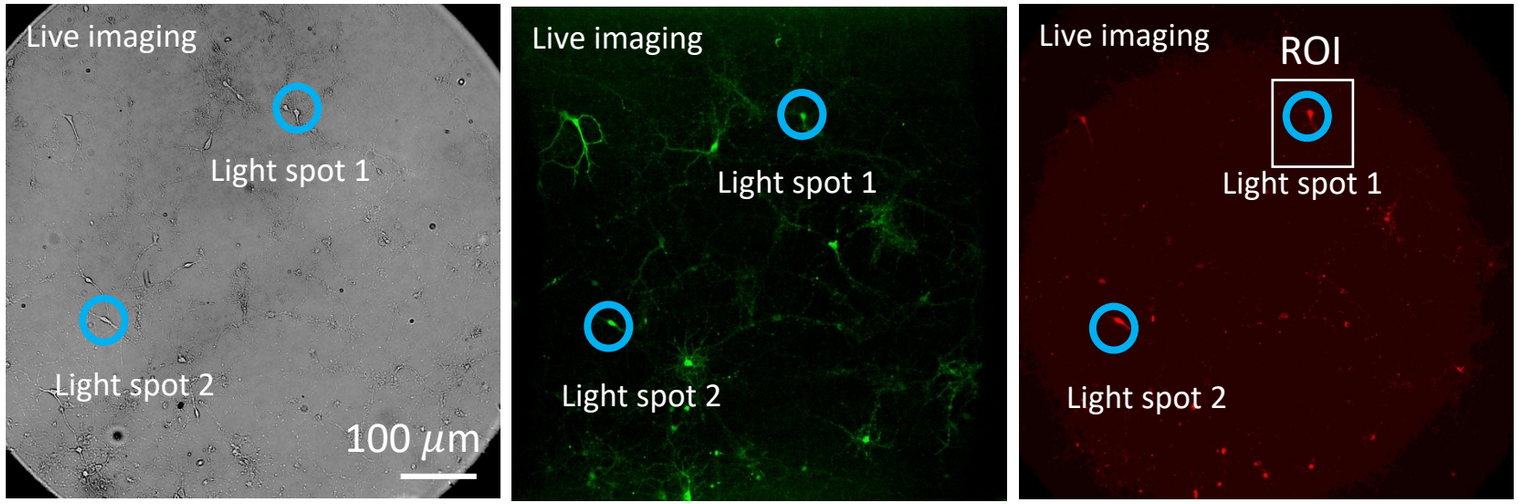




PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

SynActive localization in dendritic spines

A

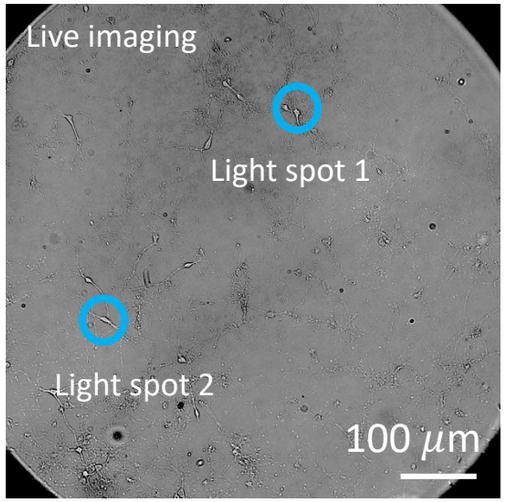


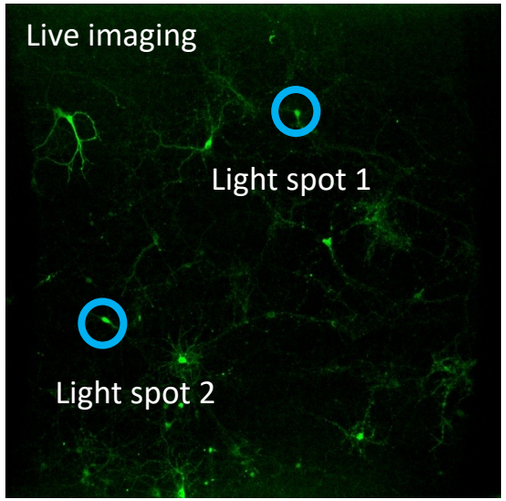
PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

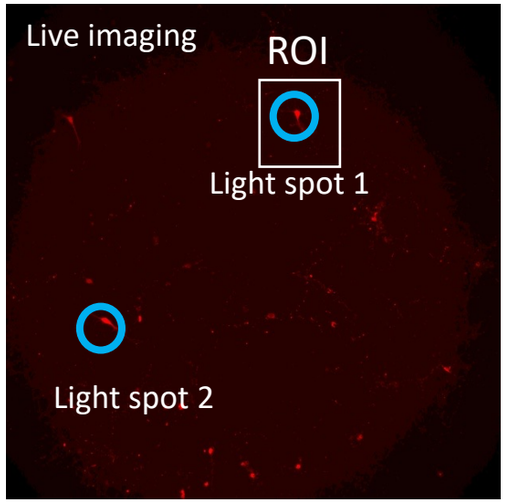
BACKUP neuromorphic photonics and the brain

SynActive localization in dendritic spines

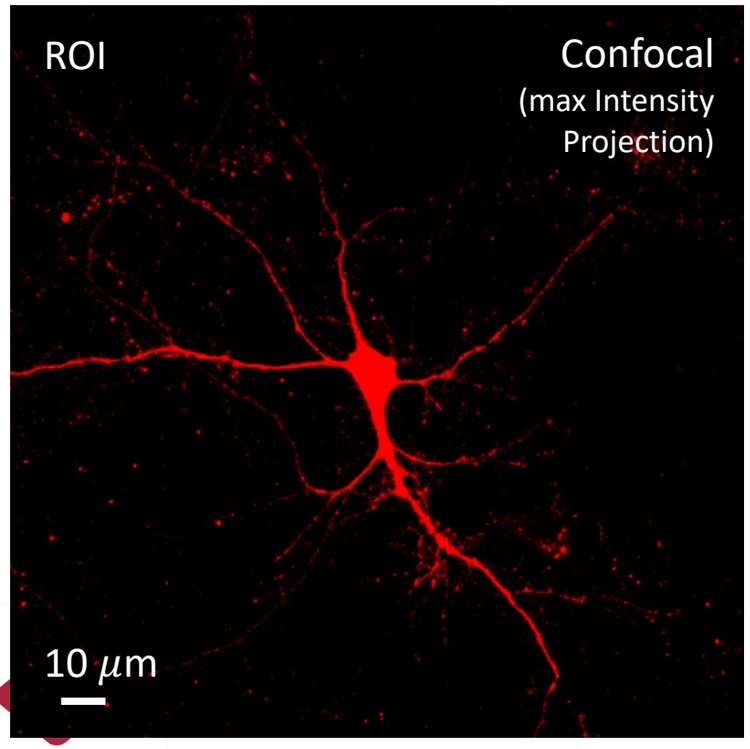
A

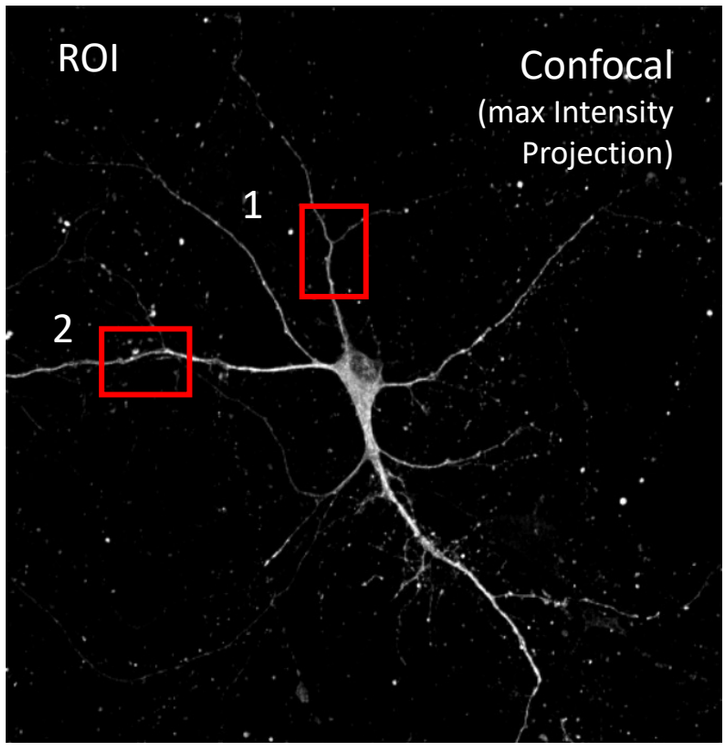






B





PROGETTI DI RICERCA DI
RILEVANTE INTERESSE NAZIONALE

BACKUP
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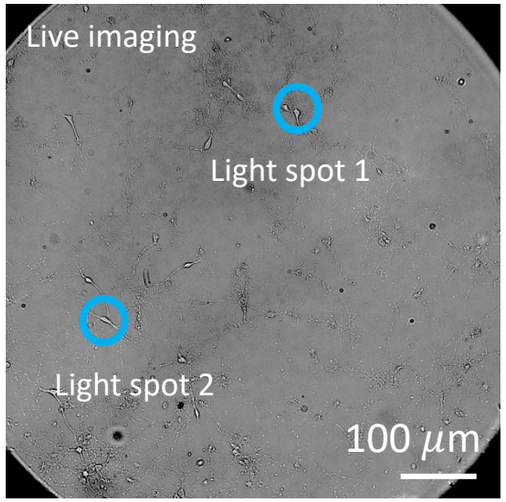
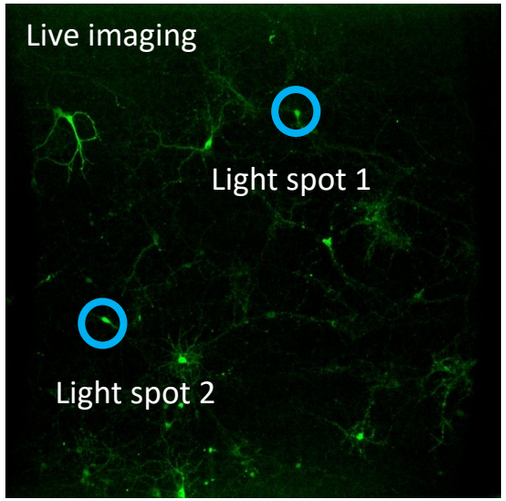
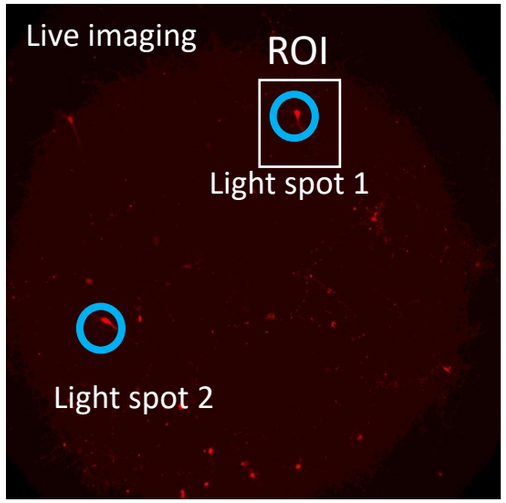
and the brain

SynActive localization in dendritic spines

A







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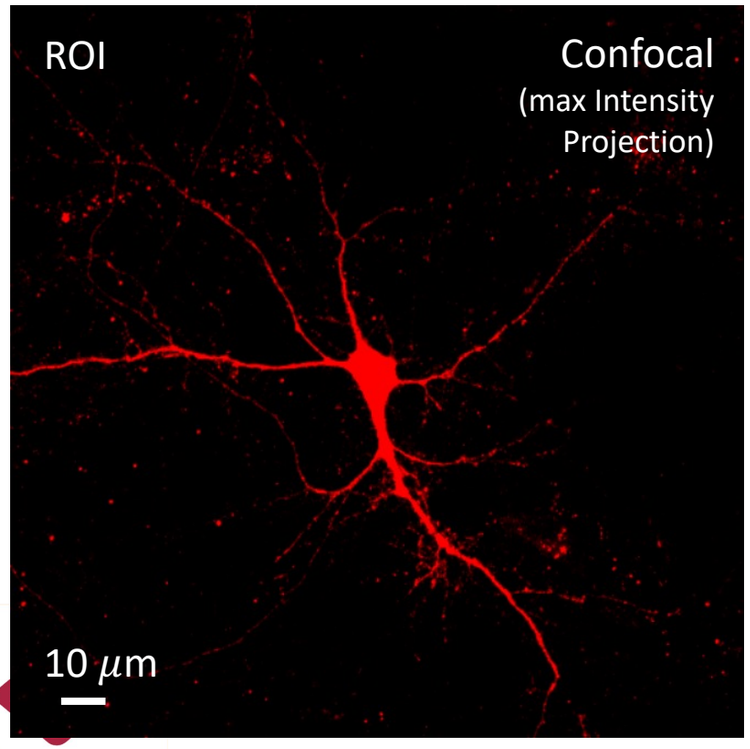
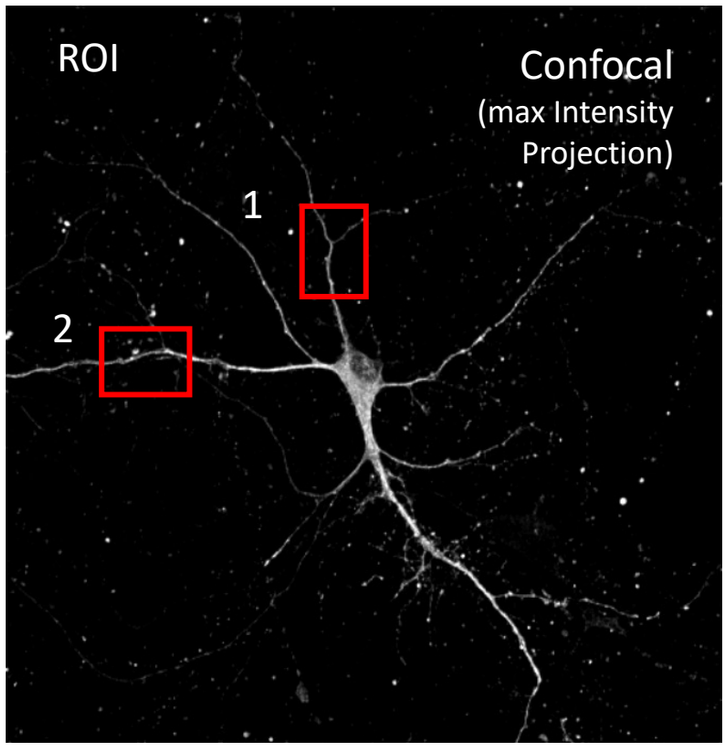
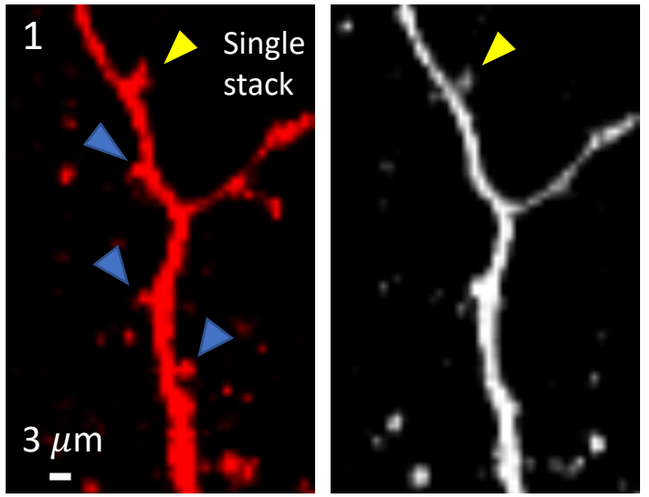


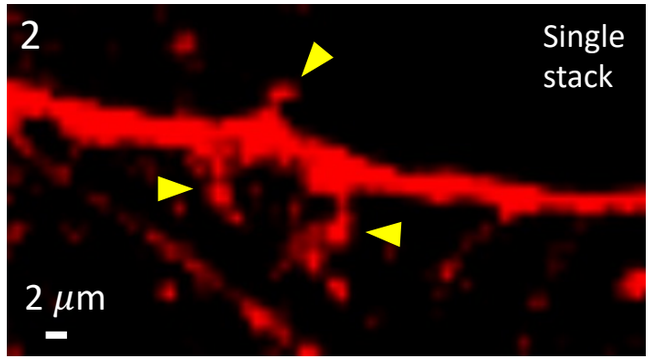


C

HA +

HA -





PROGETTI DI RICERCA DI
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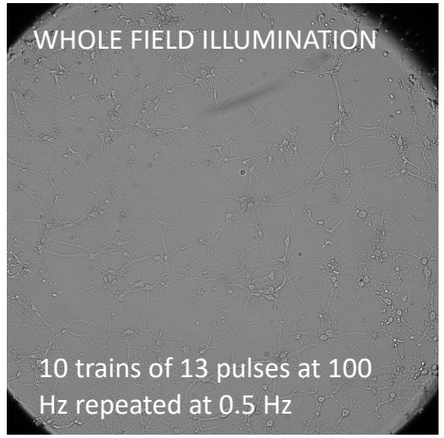
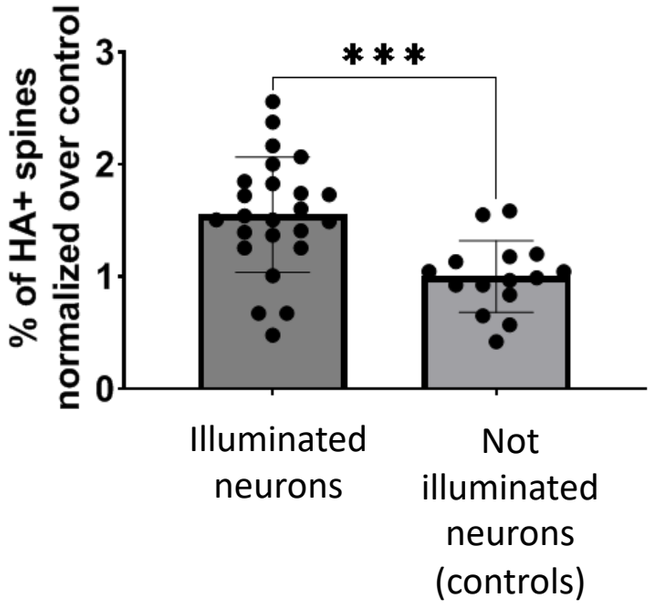
and the brain

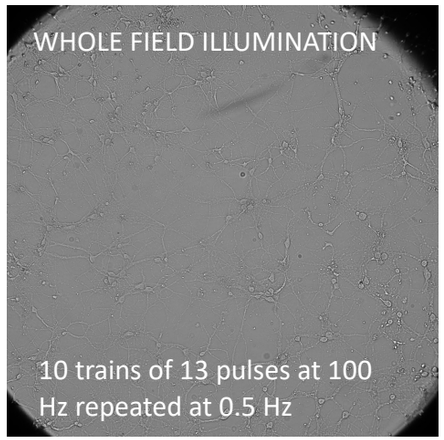


10 trains - doxy

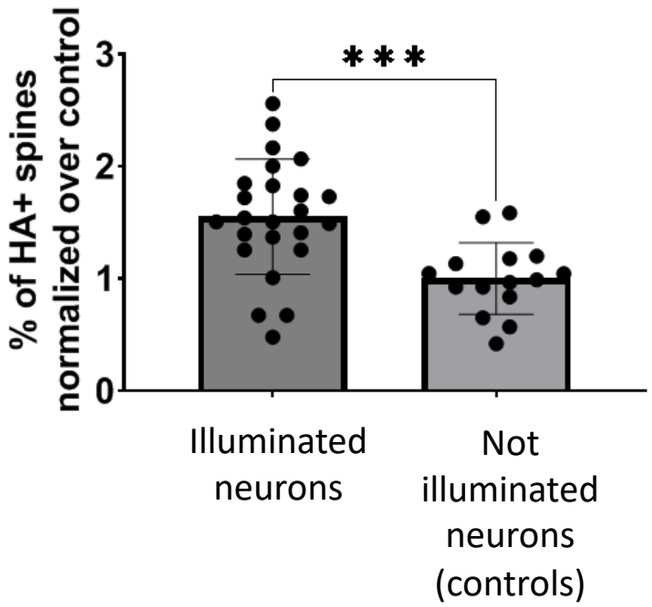


PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

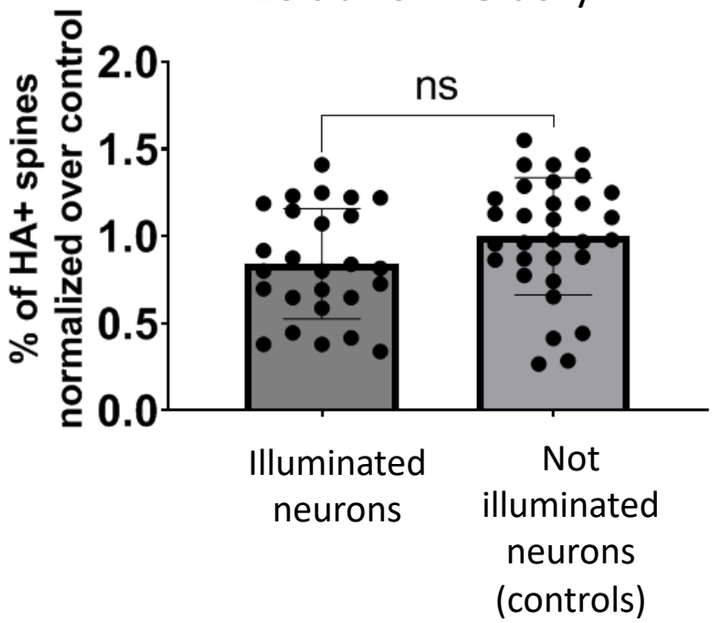
BACKUP neuromorphic photonics and the brain



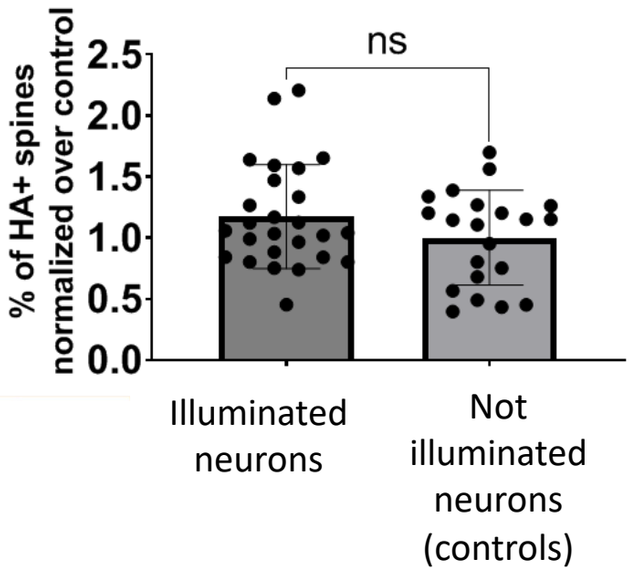
10 trains - doxy



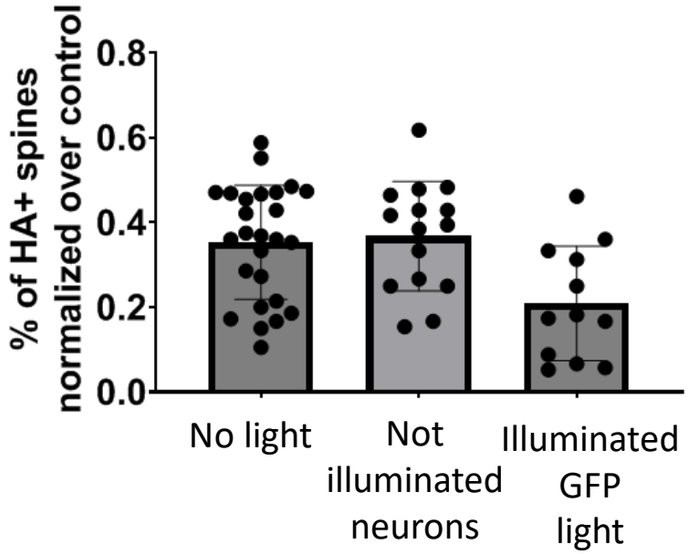
10 trains - NO doxy

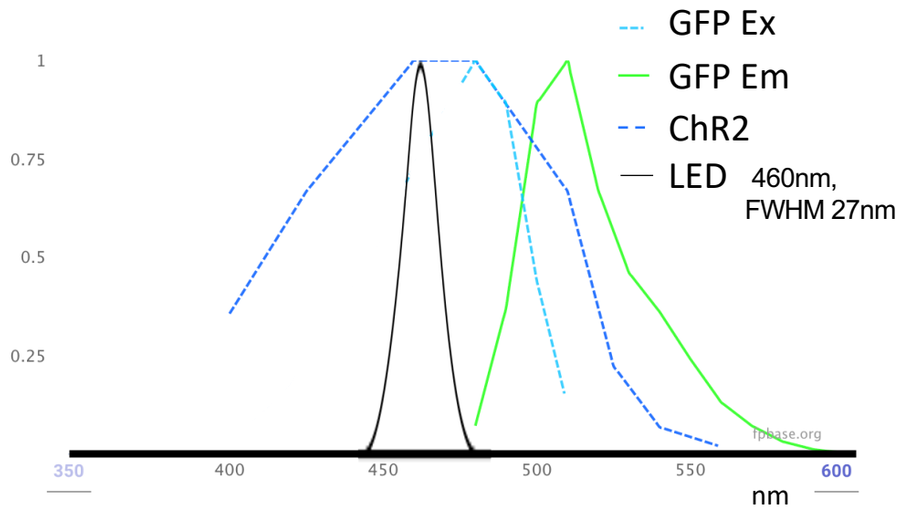


1 train - doxy

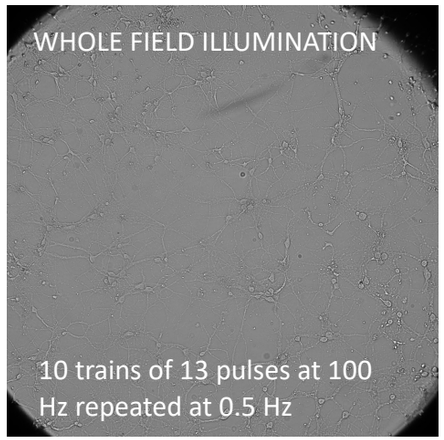


Selective activation check

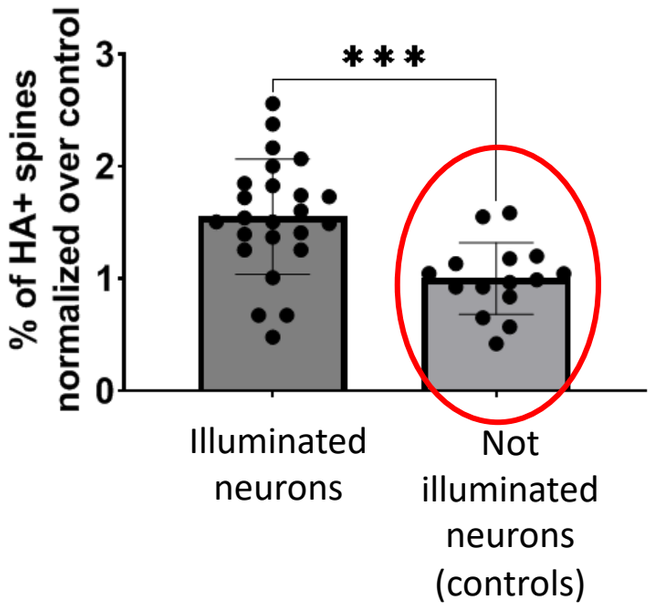




BACKUP neuromorphic photonics and the brain



10 trains - doxy



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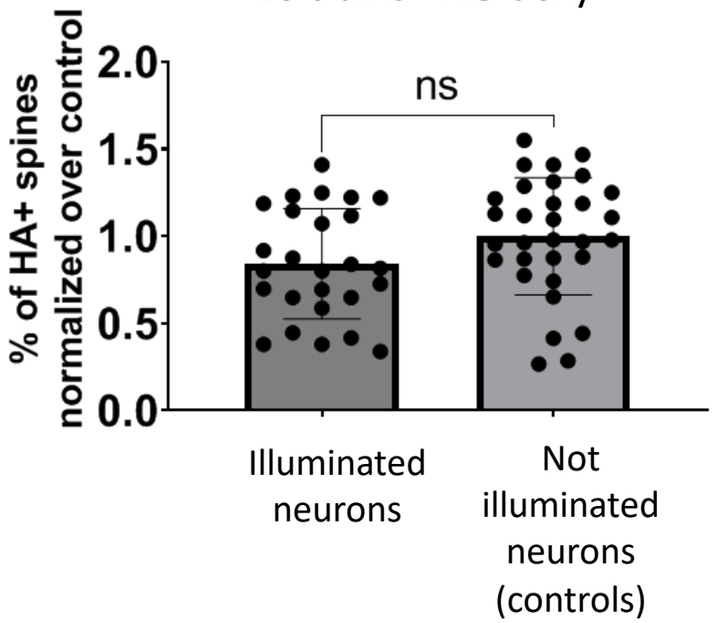
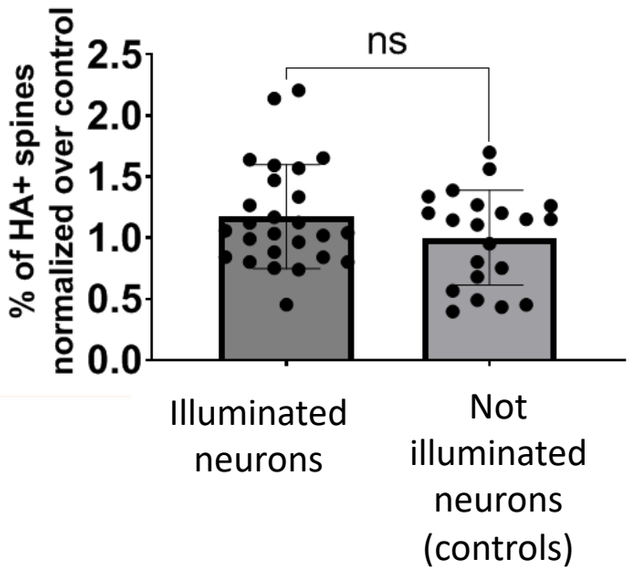


1 train - doxy



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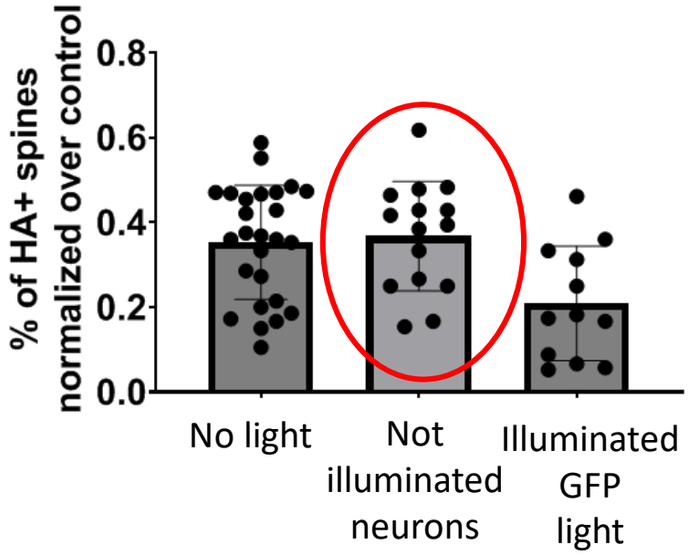
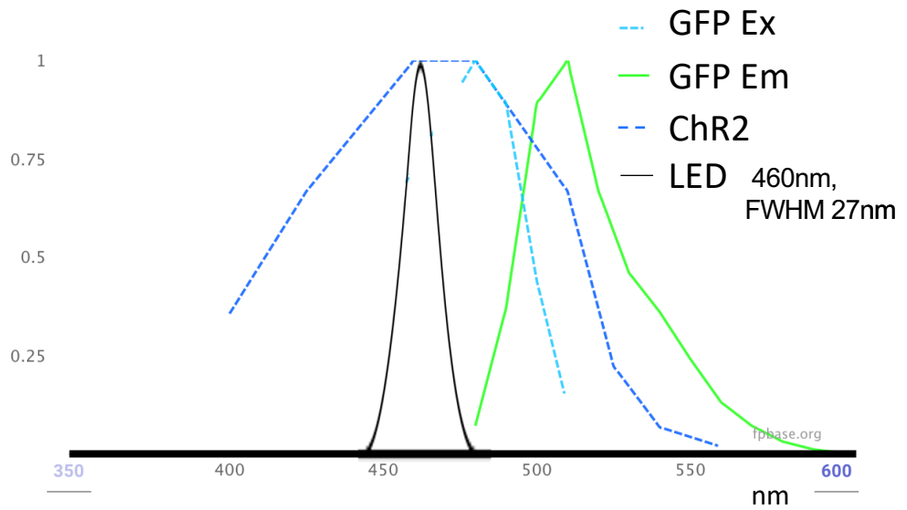
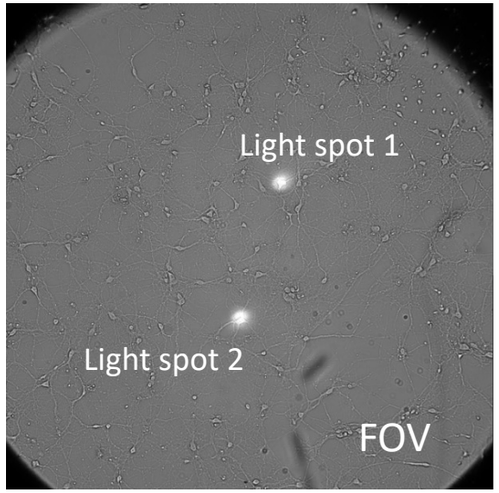




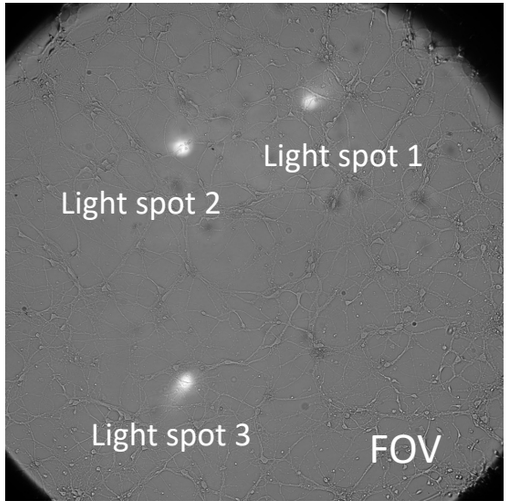
BACKUP neuromorphic photonics and the brain

FOV: not illuminated neurons in the field of view

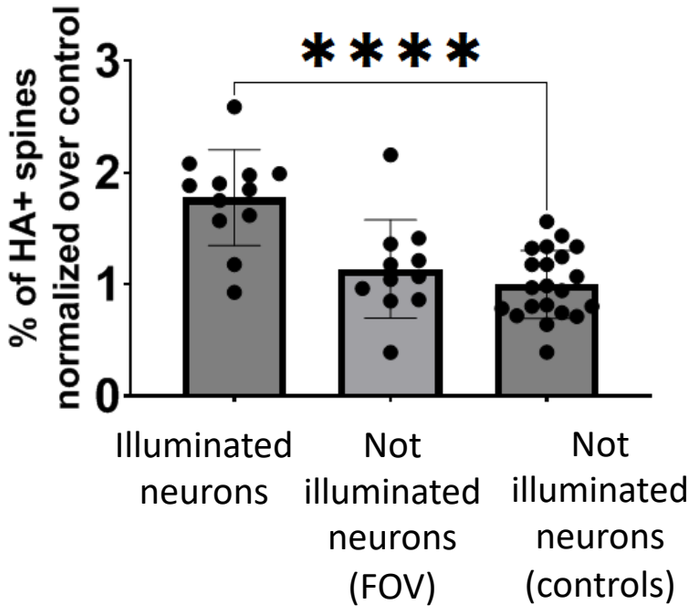
2 LIGHT SPOTS



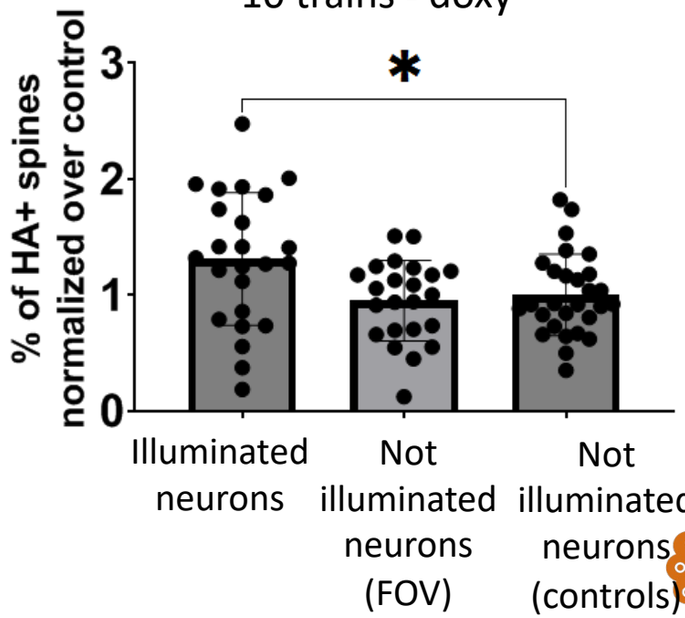
3 LIGHT SPOTS



10 trains - doxy



10 trains - doxy



PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

BACKUP neuromorphic photonics and the brain

Conclusions

We integrated a DLP platform in a spinning disk confocal microscope to perform selective single-cell excitation in in-vitro neuronal cultures.

LTP-like pattern → A subset of spines of the illuminated neurons (c-fos positive) show potentiation (synactive positive)

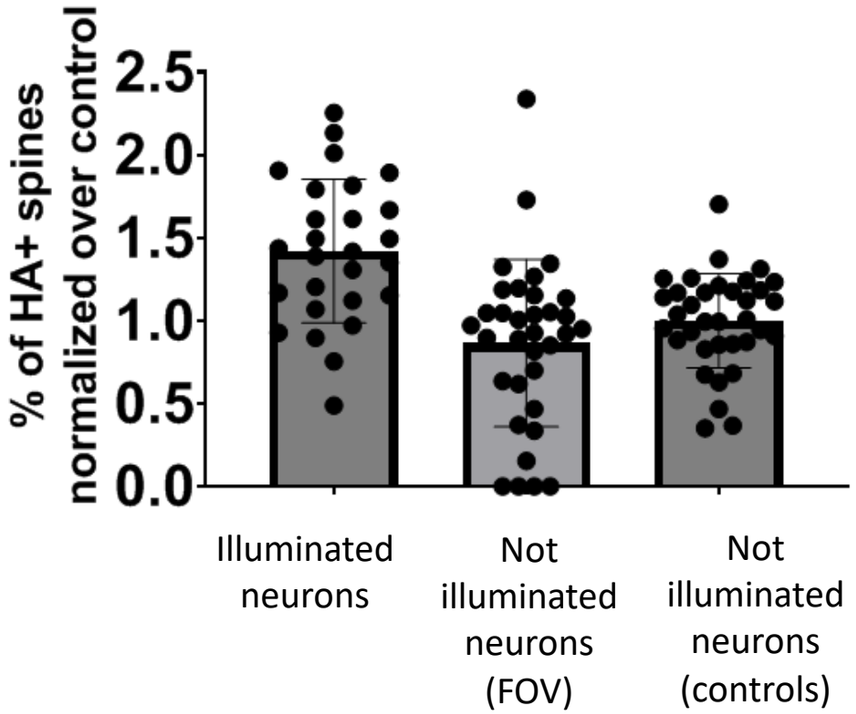
PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

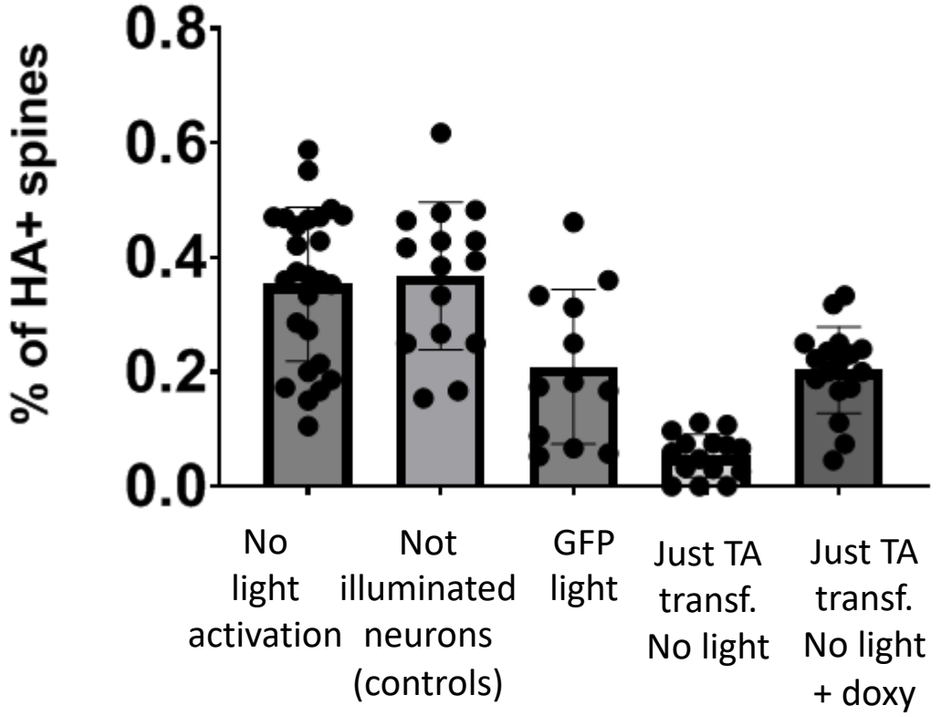
BACKUP neuromorphic photonics and the brain

Acknowledgments

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 788793-BACKUP)

TTX 20 min

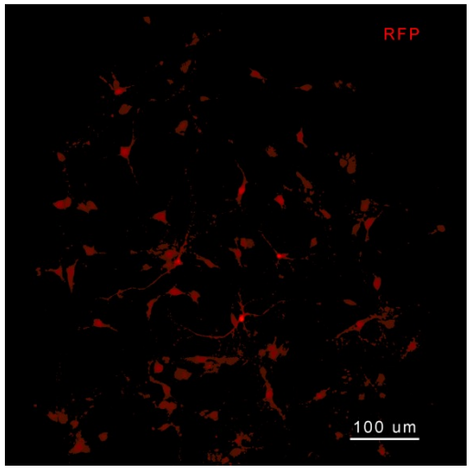




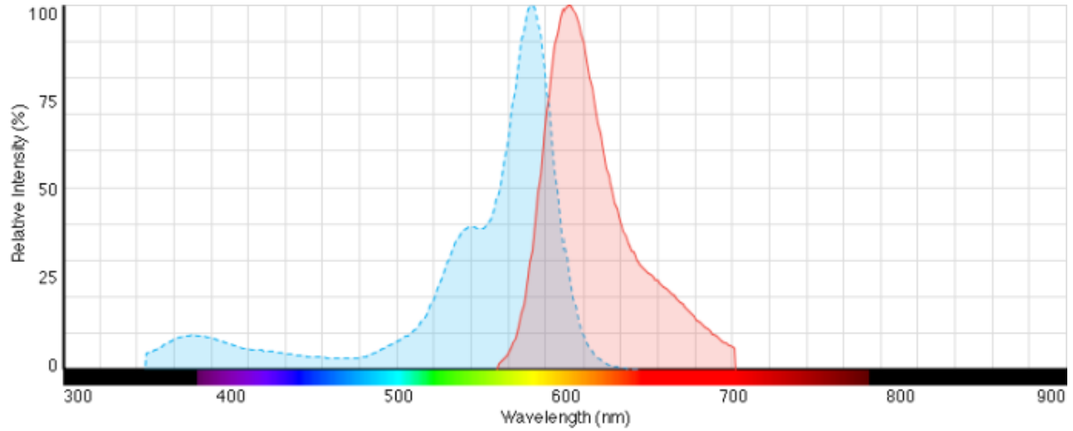
PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE

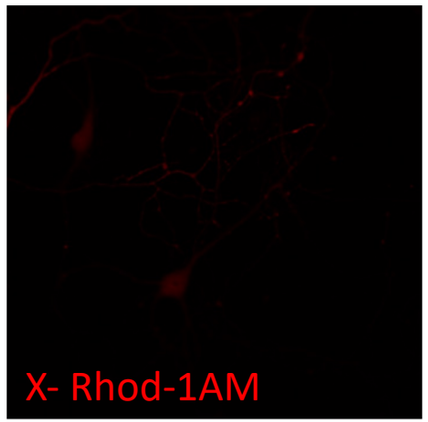
BACKUP neuromorphic photonics and the brain

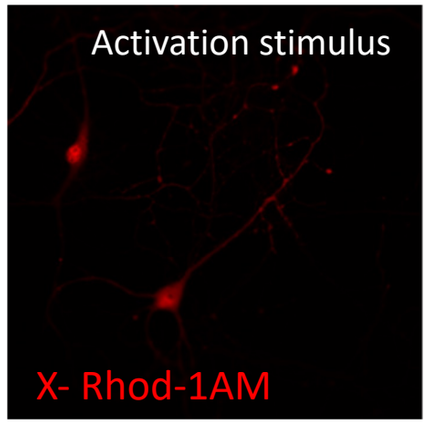
Optical reading of neuronal signals: calcium indicator

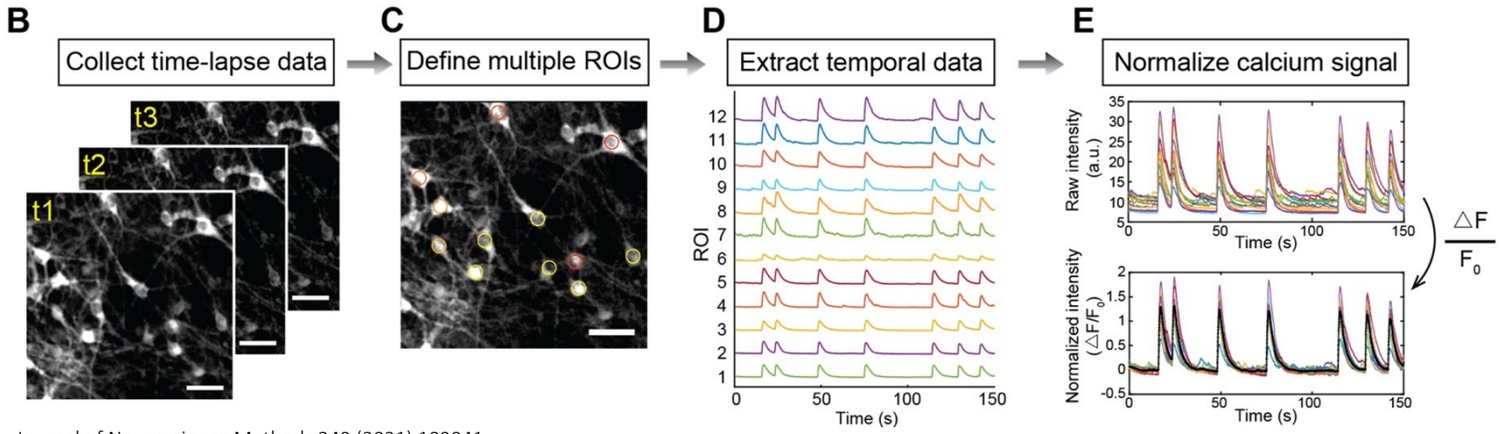


X-Rhod-1AM









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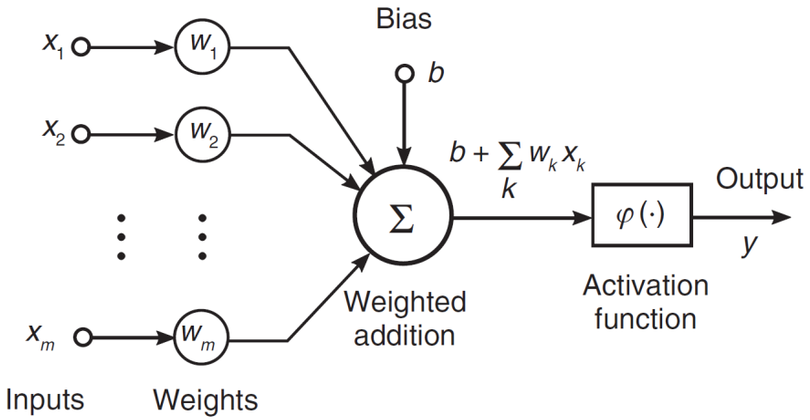
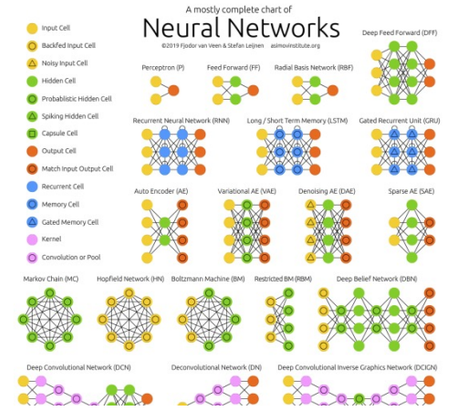
BACKUP neuromorphic photonics and the brain

UP

Artificial Intelligence → neuromorphic computing

investigates novel **architectures** to improve efficiency, by imitating the mechanisms of **biological neural networks.**

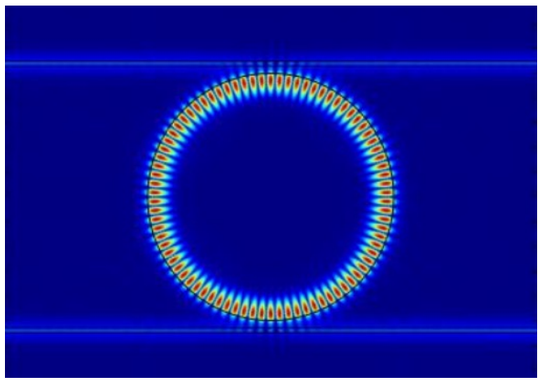


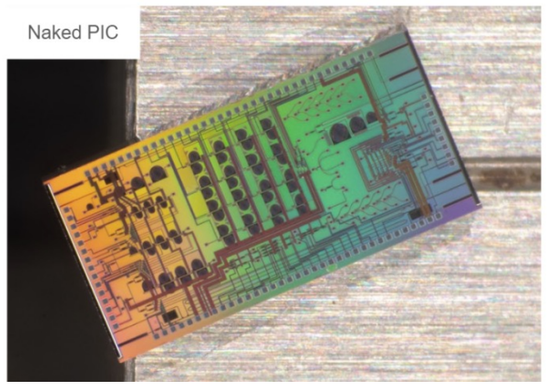


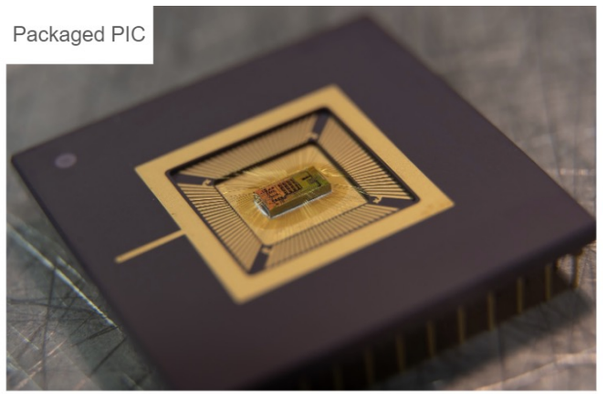
https://www.asimovinstitute.org/neural-network-zoo/

Artificial photonic neuronal networks

Energy efficiency (low losses) and high bandwidth







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