19th Photonics Workshop: Kopaonik, March 08 – 12, 2026
BioQuantSense Final Workshop

Holographic and Nonlinear Microscopy for Investigating Glial Cell Morphology

Ana Jakovljević, Tanja Pajić, Filip Krajinić, Svetlana Savić, Branka Murić, Dejan Pantelić, Francesca Torrini, Caterina Dallari, Caterina Credi, Pavle Andjus, Branislav Jelenković

Funded by the European Union

HORIZON-WIDERA-2021-ACCESS-03-01 – Twinning
Grant agreement Nº 101079355

Glial cells

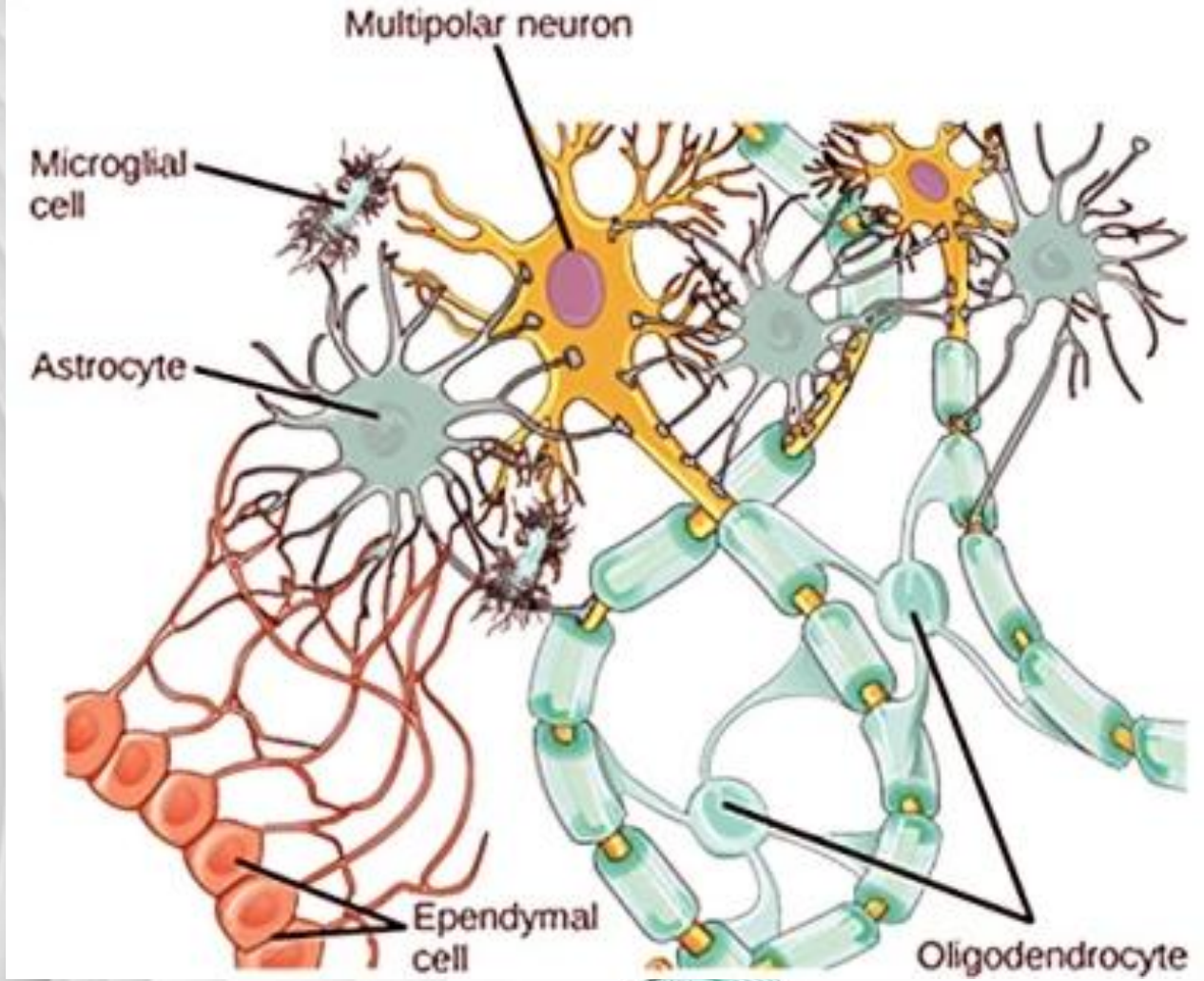
- ~50% of brain cells (von Bartheld et al, 2016)
- neuronal support
- neuroinflammation
- brain homeostasis

Understanding their **morphology** and **dynamics** requires advanced imaging tools.

Limitations of conventional microscopy:

- invasive staining
- limited physical information

https://opentext.csu.edu.au/organisedlifeofanimals/chapter/nervous-tissue/

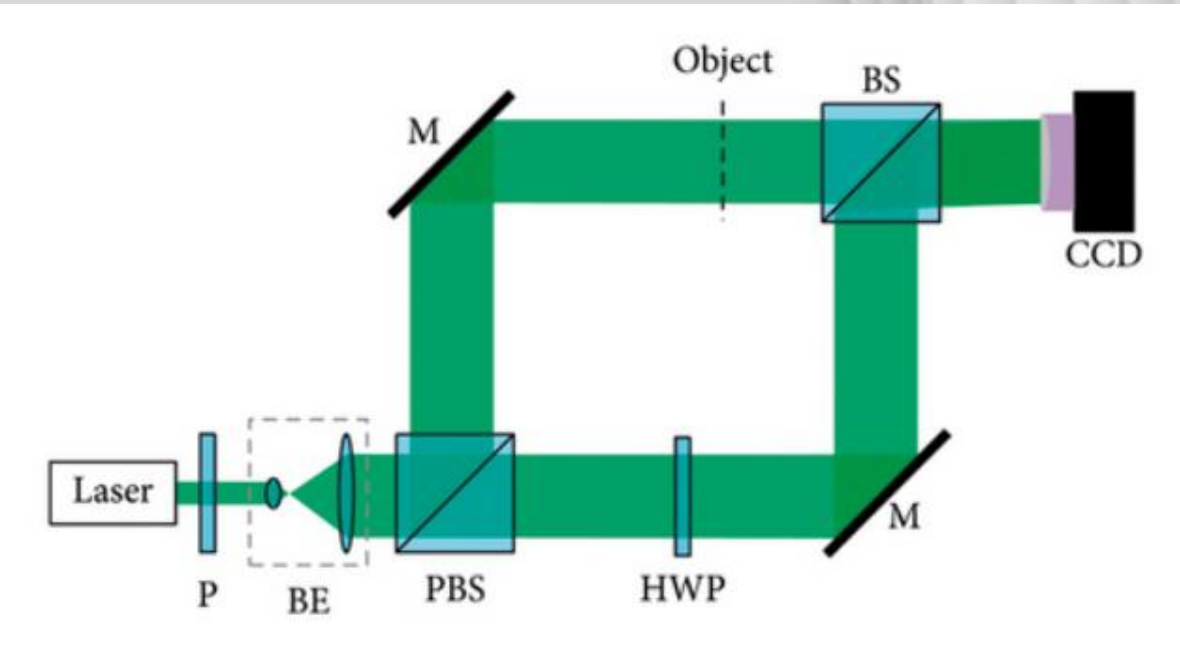


BioQantSense

Complementary Optical Methods

Digital holographic microscopy

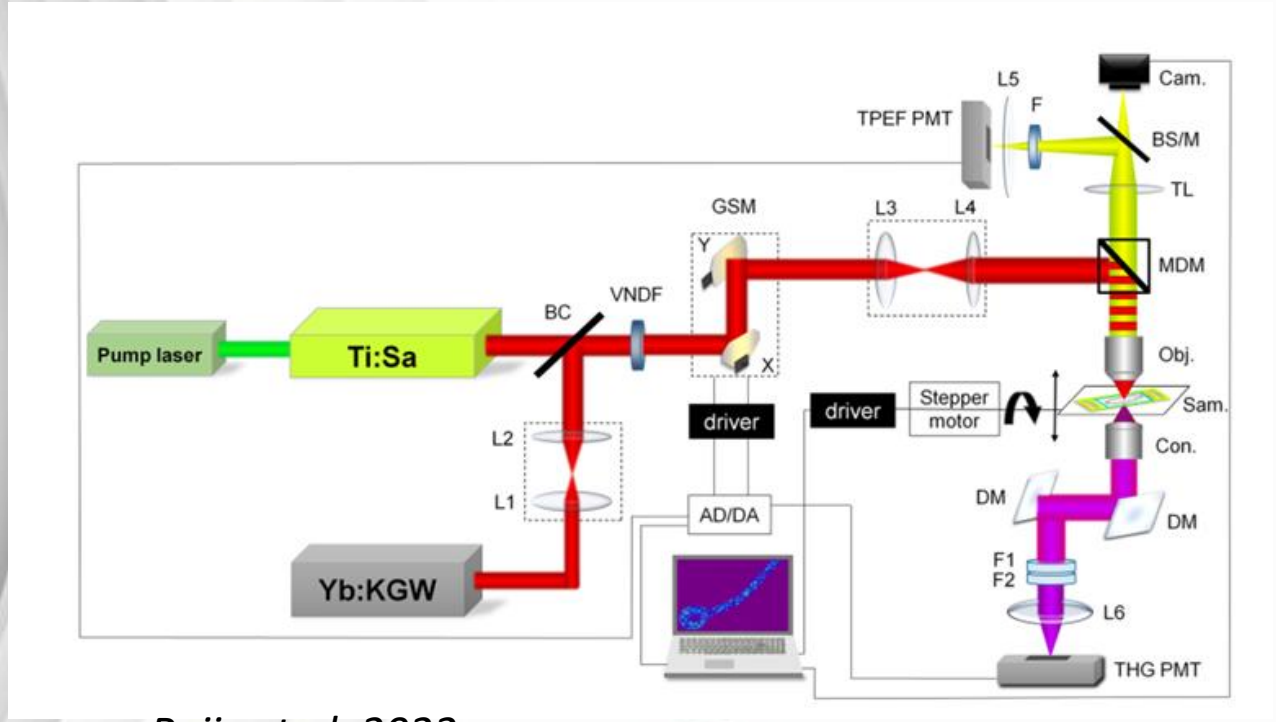
- Label-free morphology information
- Quantitative phase measurement



Wang et al, 2022

Non-linear microscopy

- Two photon excitation
- High spatial resolution



Pajic et al, 2022

Holographic Microscopy

Digital holographic microscopy (DHM):
- records interference pattern of light
- reconstructs **amplitude and phase**

Amplitude images - changes in light intensity caused by absorption or scattering in the sample.
Phase images - how much the light is delayed when passing through the sample, providing information about cell thickness and refractive index.

Phase signal provides information on:
- **cell thickness**
- **refractive index**
- **morphology**

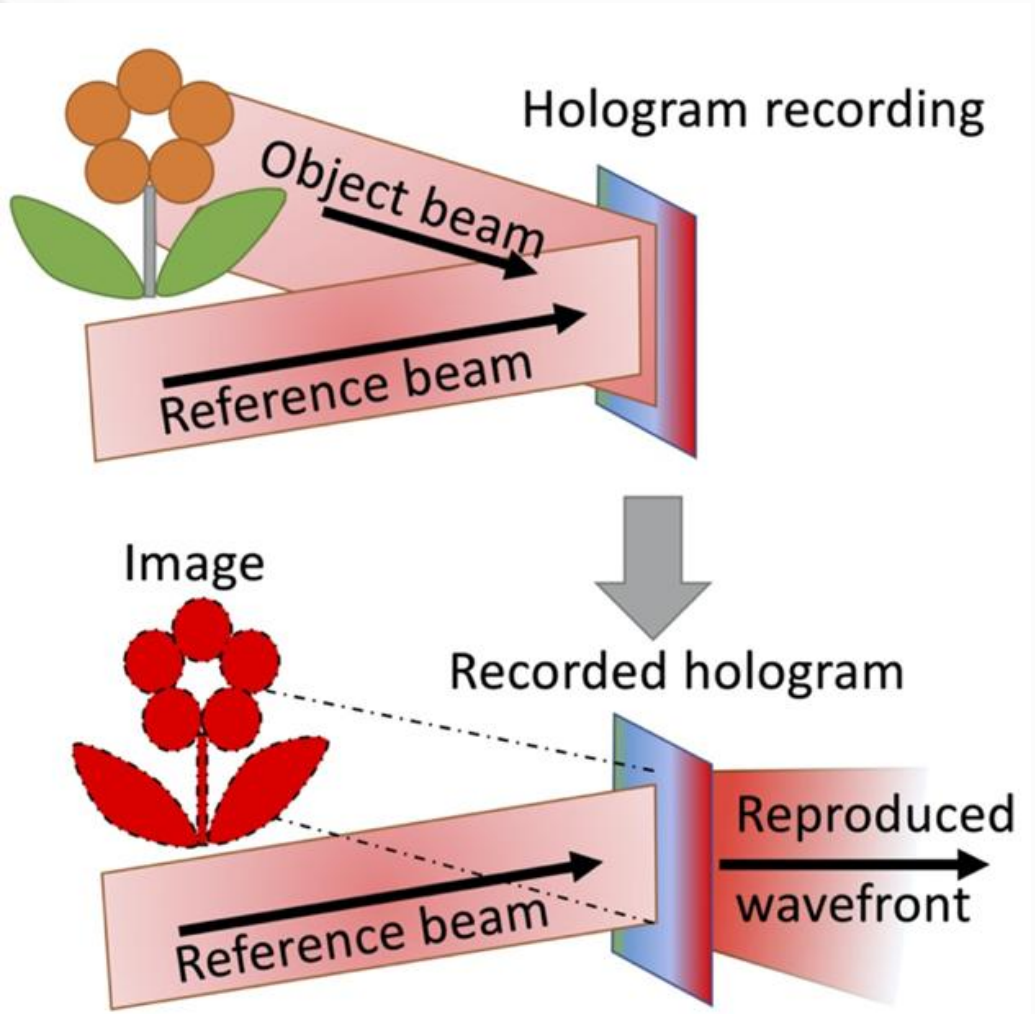


Fig. 1. Principle of holography.

Quan et al, 2022

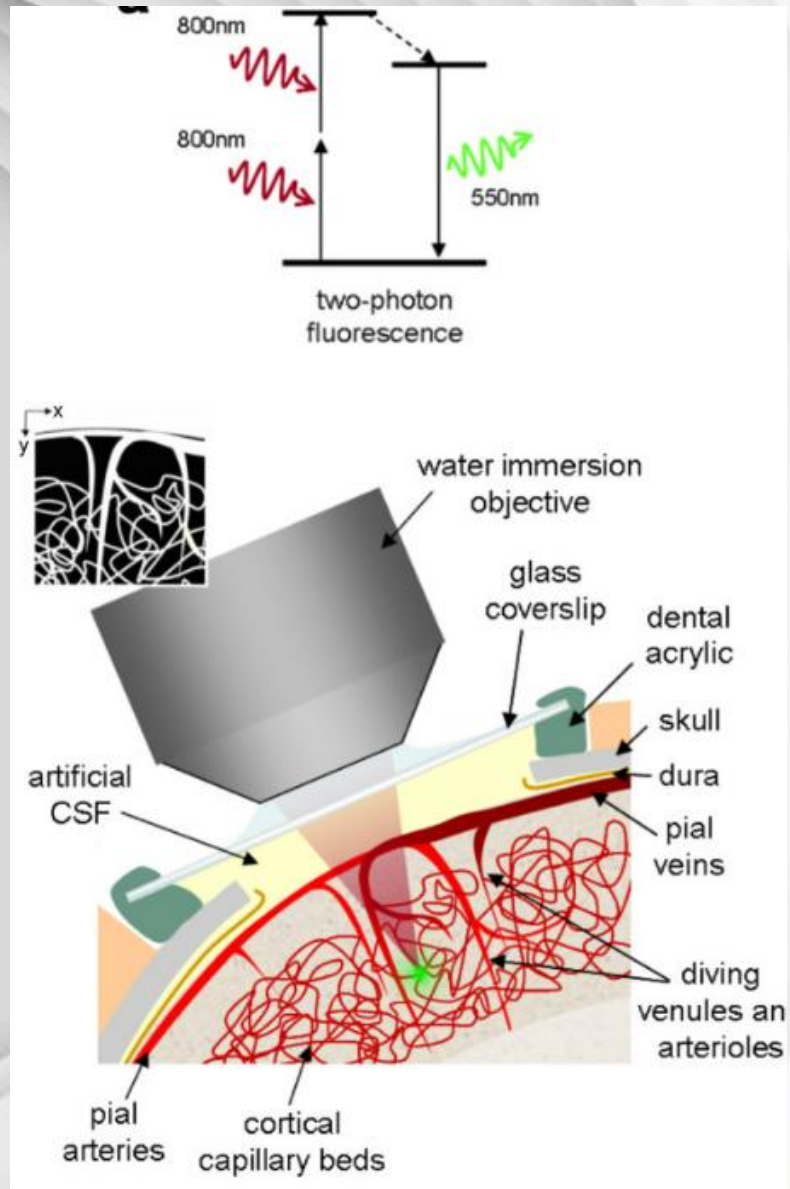
Nonlinear Microscopy

Principle

- non-linear excitation process
- femtosecond pulsed laser

Detected signals

- two-photon fluorescence (TPEF)
- second / third harmonic generation (SHG, THG)
- endogenous autofluorescence



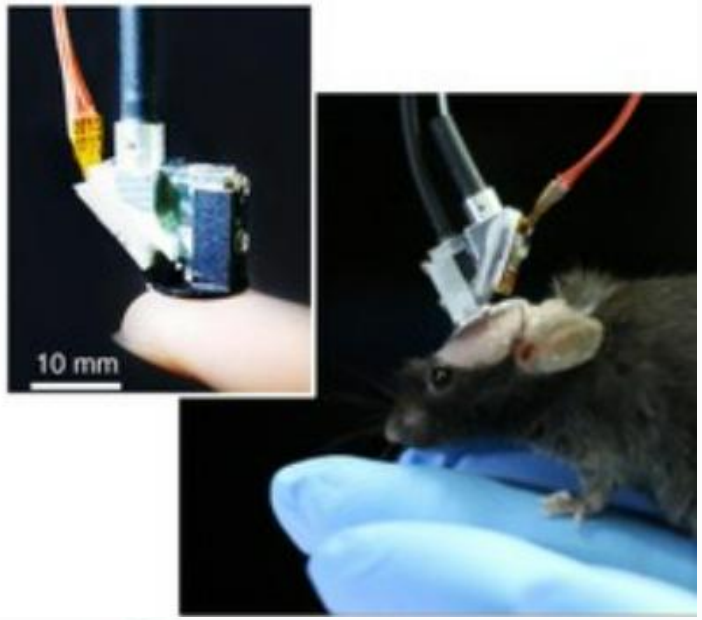
Kherlopian et al, 2008

Advantages

- high spatial resolution
- deep tissue imaging (hundreds of µm)
- localized excitation → reduced scattering

Applications

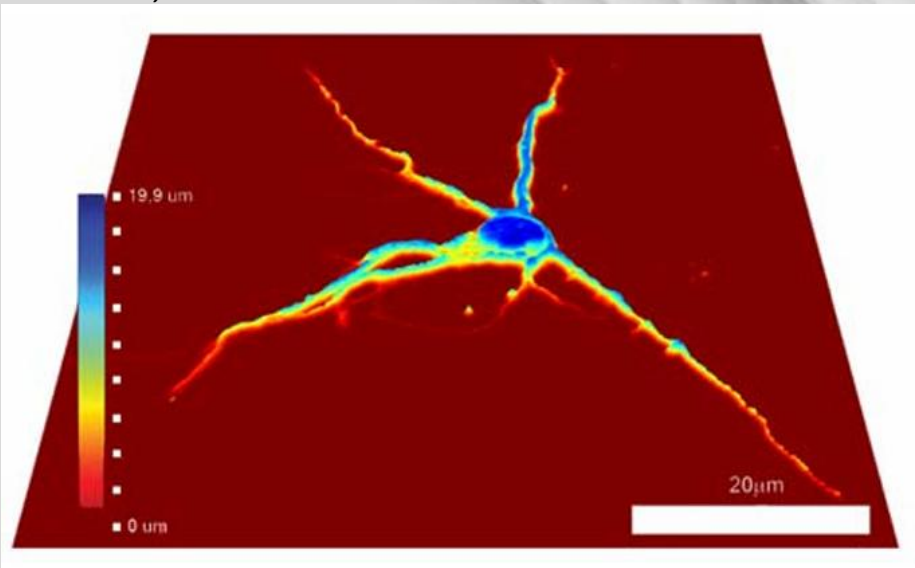
- imaging cellular morphology *in vivo*
- monitoring cell dynamics in living tissue
- studying physiological processes (e.g., calcium signaling)



Zong et al, 2017

Holographic and Non-linear Microscopy in Neuroscience

Alm et al, 2011



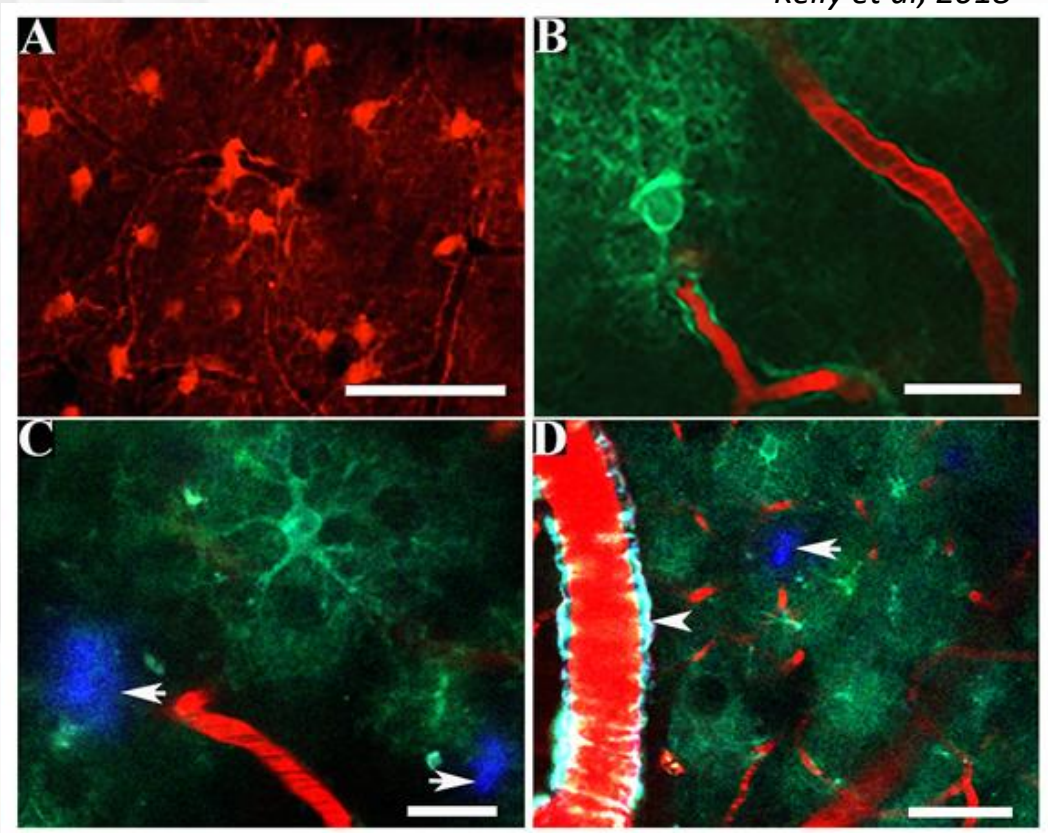
Physiological and pathological changes detected by DHM:

- Cell swelling: neuron after hypotonic shock
- Morphology changes: phase decrease during glutamate-induced excitotoxicity
- Intracelular processes: correlation between calcium increase and cell volume changes (Marquet 2005; Pavillon 2012)

Physiological and pathological changes detected by Non-linear Two Photon Microscopy:

- Astrocyes soma, perivascular endfoot processes
- Blood vessel affected by amyloid formations (cerebral amyloid angiopathy)

Kelly et al, 2018



Aim

To investigate optical signals in different glial cell types using digital holographic microscopy and two-photon microscopy, and evaluate their potential for imaging glial cell morphology

BioQantSense

Biological model

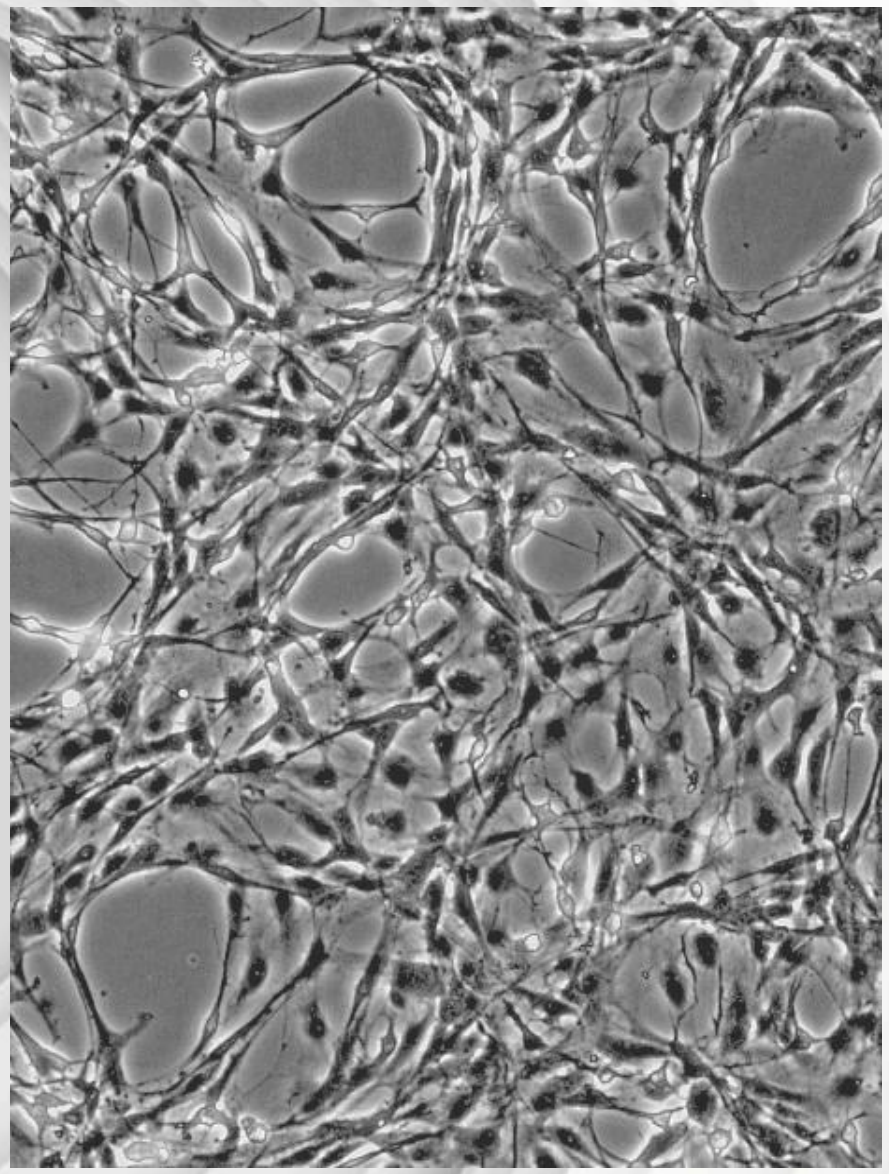
Primary astrocytes:

- Structural, metabolic, and homeostatic support to neurons
- Isolated from rat cortex

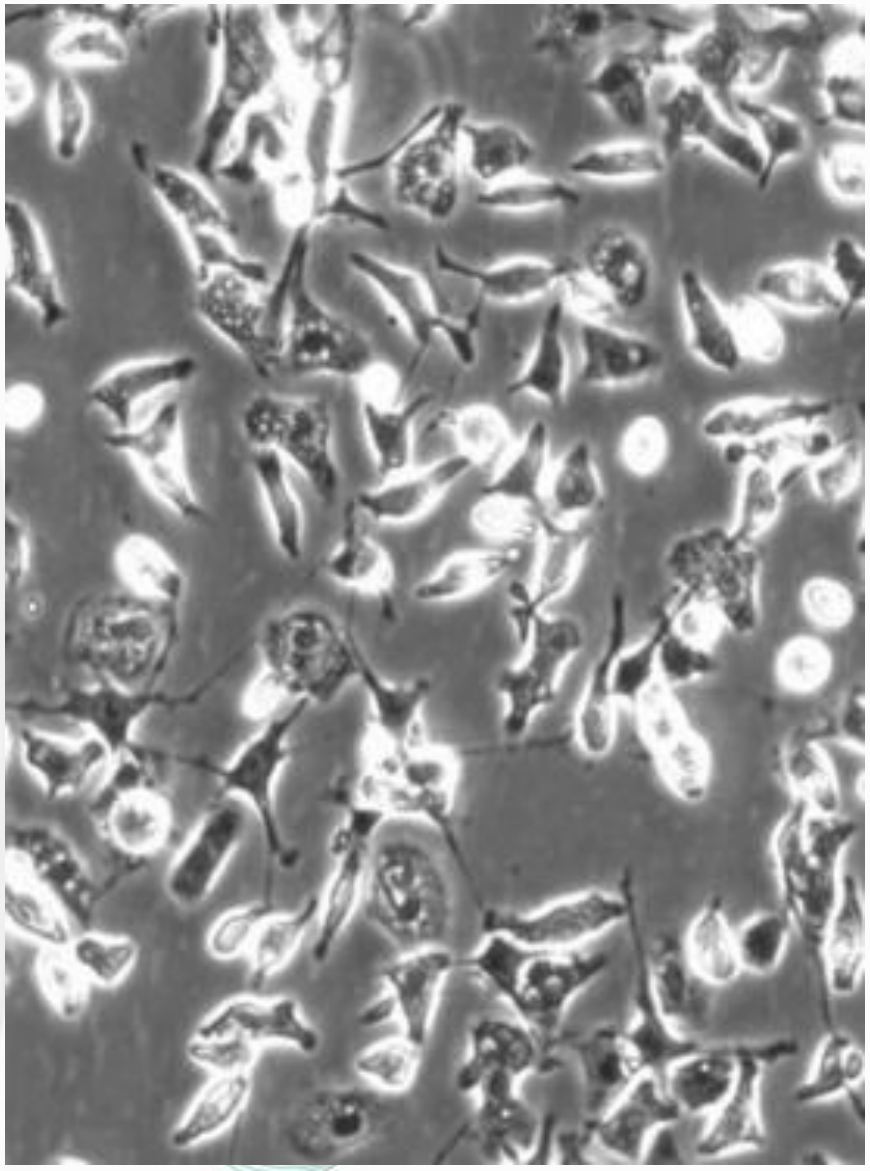
BV2 mice microglial cell line

- Standard *in vitro* model for studying neuroinflammation, neurodegeneration, and phagocytosis

Astrocytes



BV2 cells



The Centre for Laser Microscopy
capacities for the cell and tissue imaging

Cells labeling

Gold nanoparticles (AuNPs) conjugated with **Wheat Germ Agglutinin (WGA)**

AuNP properties

- diameter: 15 nm
- plasmon absorption peak at **~520 nm** (dispersed colloidal state)
- optical properties depend on particle size and aggregation state

WGA targeting

- binds to membrane glycoproteins
- enables membrane labeling of cells

Purpose

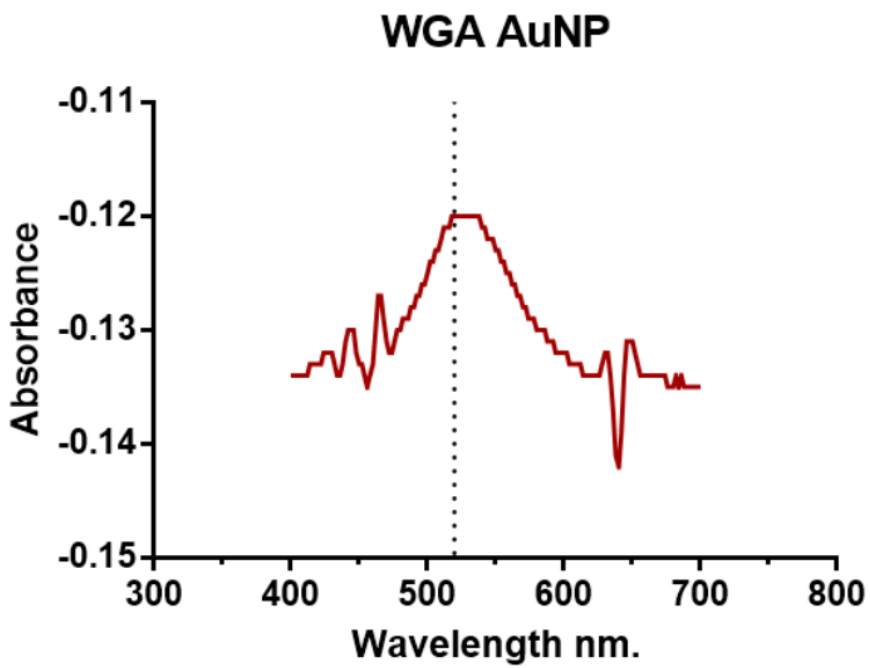
- to test AuNPs as selective optical signal enhancers due to their unique plasmonic properties

CNR-INO
ISTITUTO NAZIONALE DI OTTICA
CONSIGLIO NAZIONALE DELLE RICERCHE

Francesca Torrini

Caterina Dallari

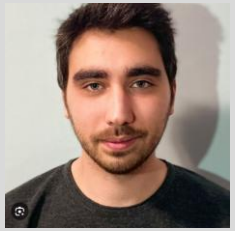
Caterina Credi



Results
Holographic microscopy

BioQantSense

Phase imaging of astrocytes



Filip Krajinić

Svetlana Savić

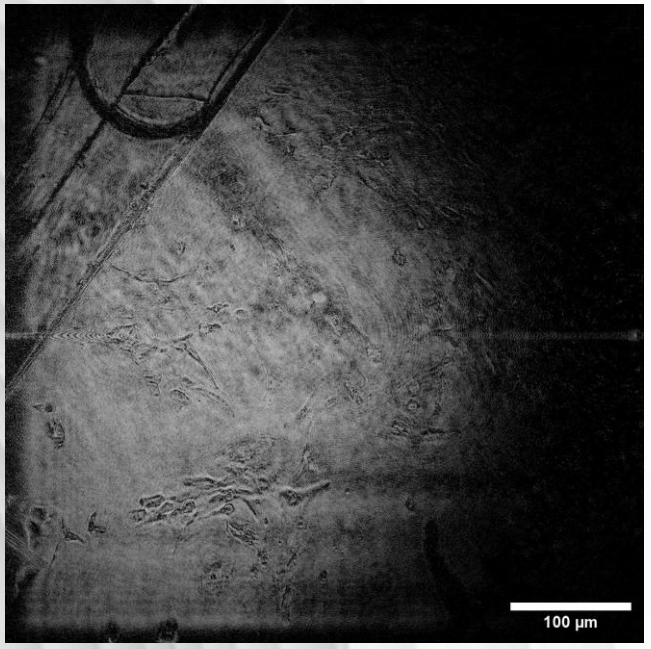
Branka Murić

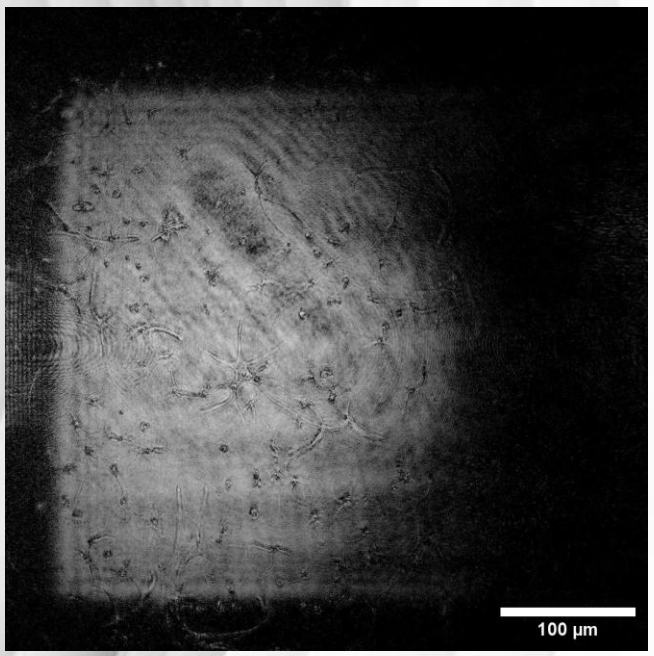
Unlabeled astrocytes

Objective 20X
NA=0.4
532 nm
wavelenght

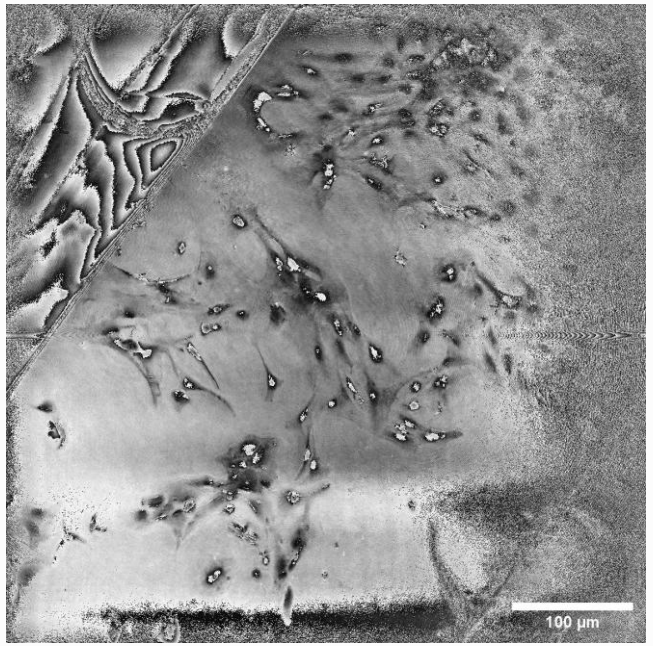
Astrocytes with Au NP

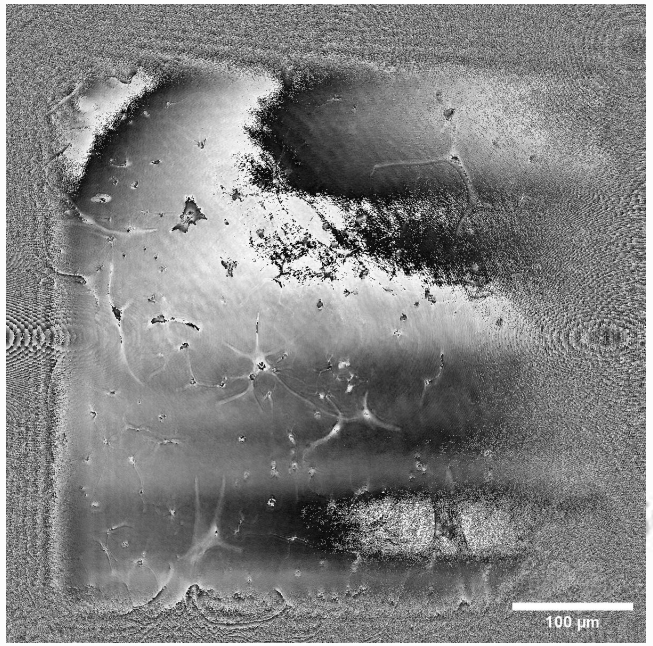
Amplitude image



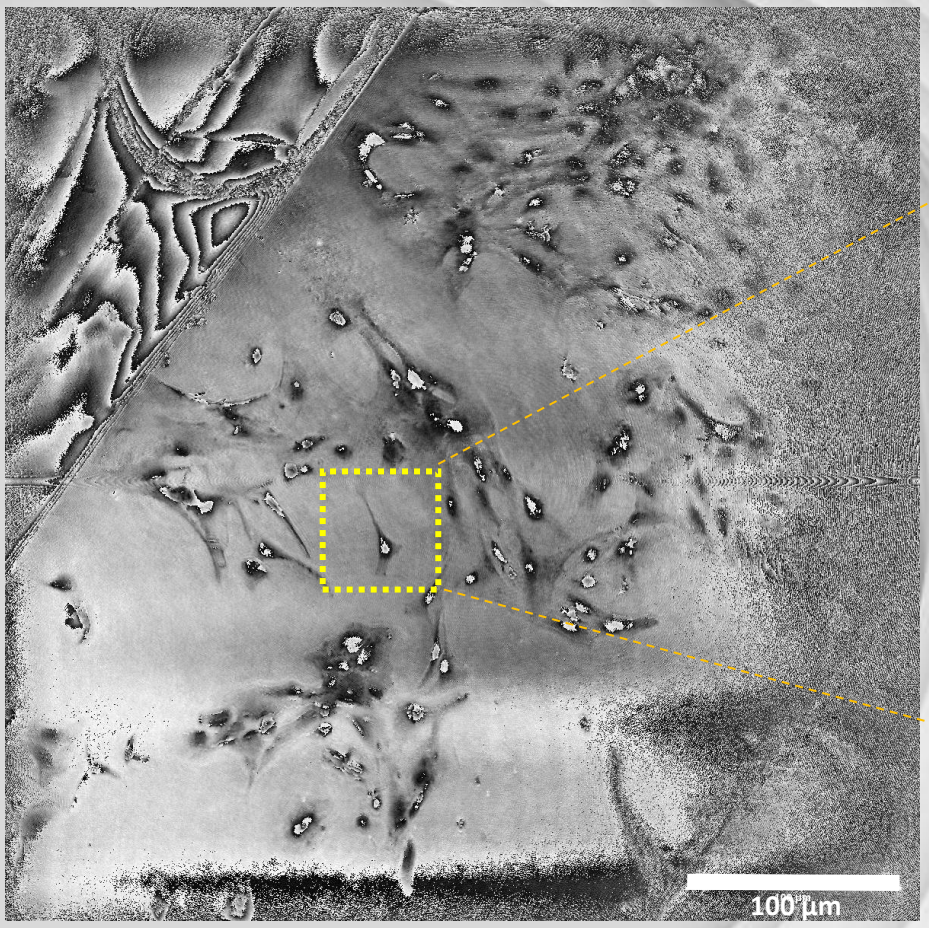


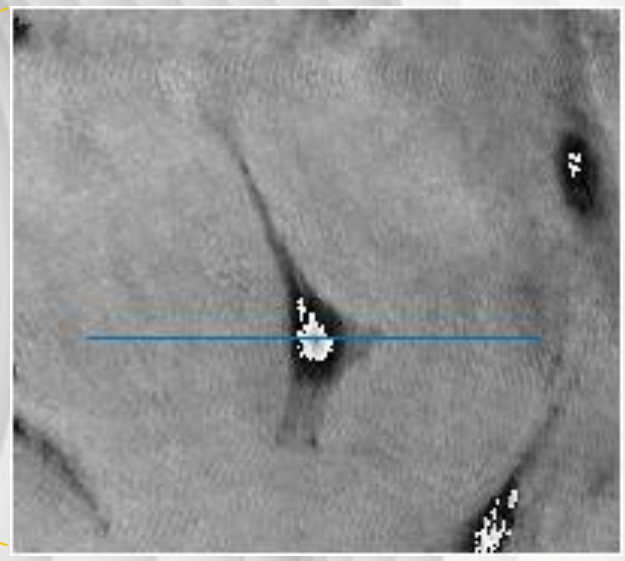
Phase image

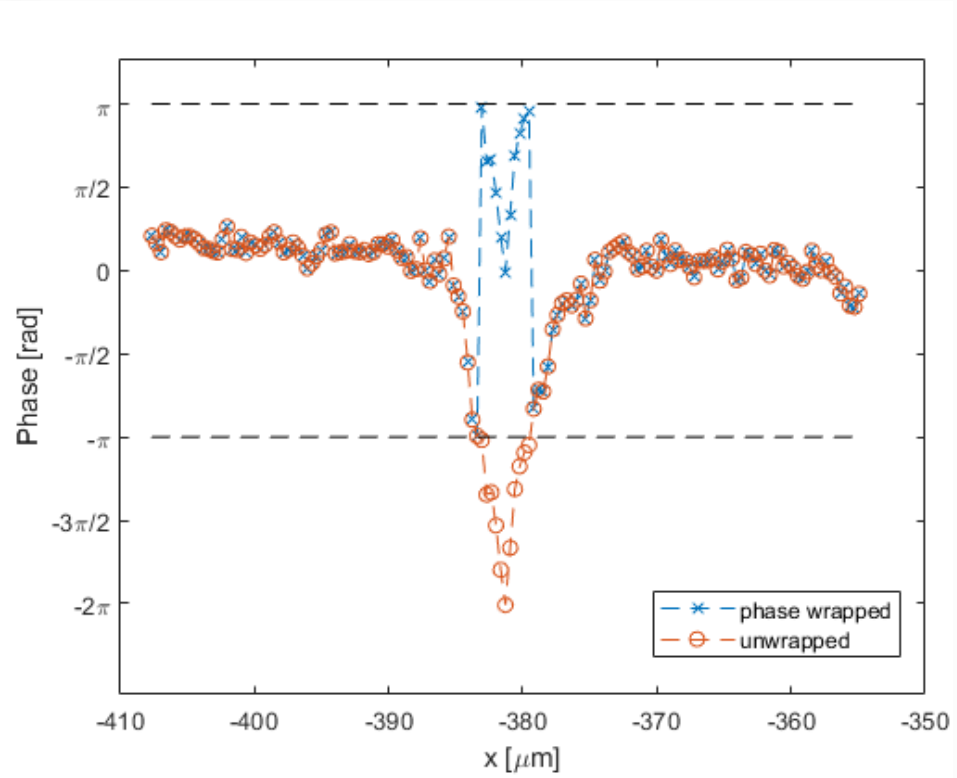




Phase imaging: phase wrapping



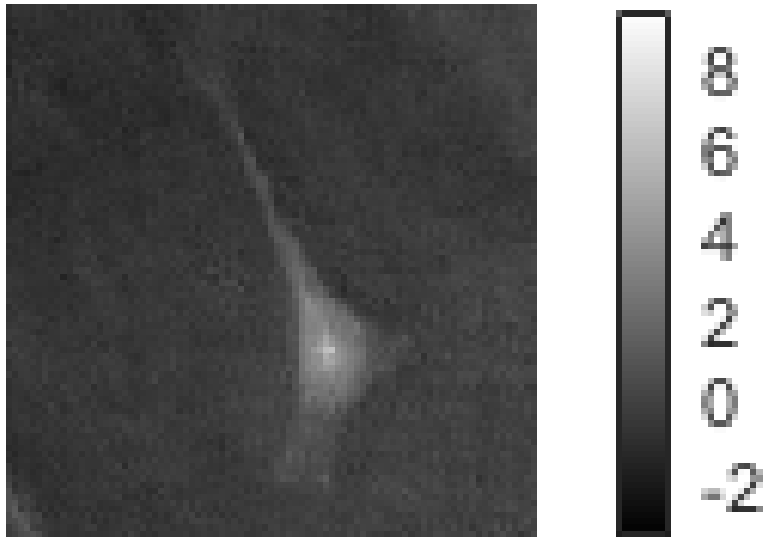




The *astrocyte cross-section (blue line)* displays the phase signal. The sharp change in contrast seen in the nucleus (white) is caused by **phase wrapping**, which occurs when the phase signal exceeds the measurable range. This indicates a stronger phase shift in the nuclear region compared to the surrounding cytoplasm.

BioQantSense

Phase unwrapping



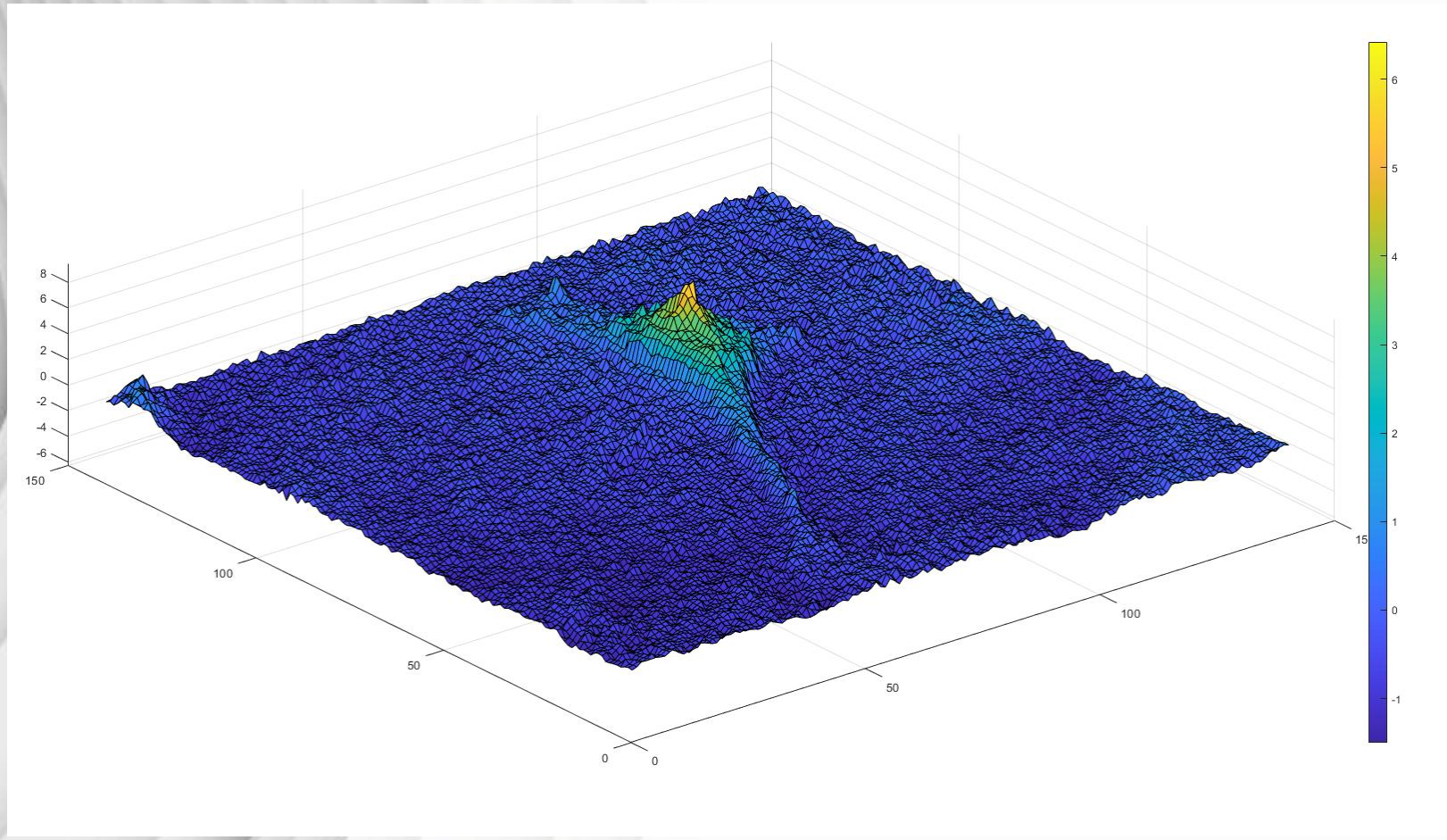
Phase = (n_cell − n_bg) · (2π / λ) · h

n_cell is the refractive index of the cell
n_bg the refractive index of the surrounding medium
λ (532 nm) the illumination wavelength
h the cell thickness.

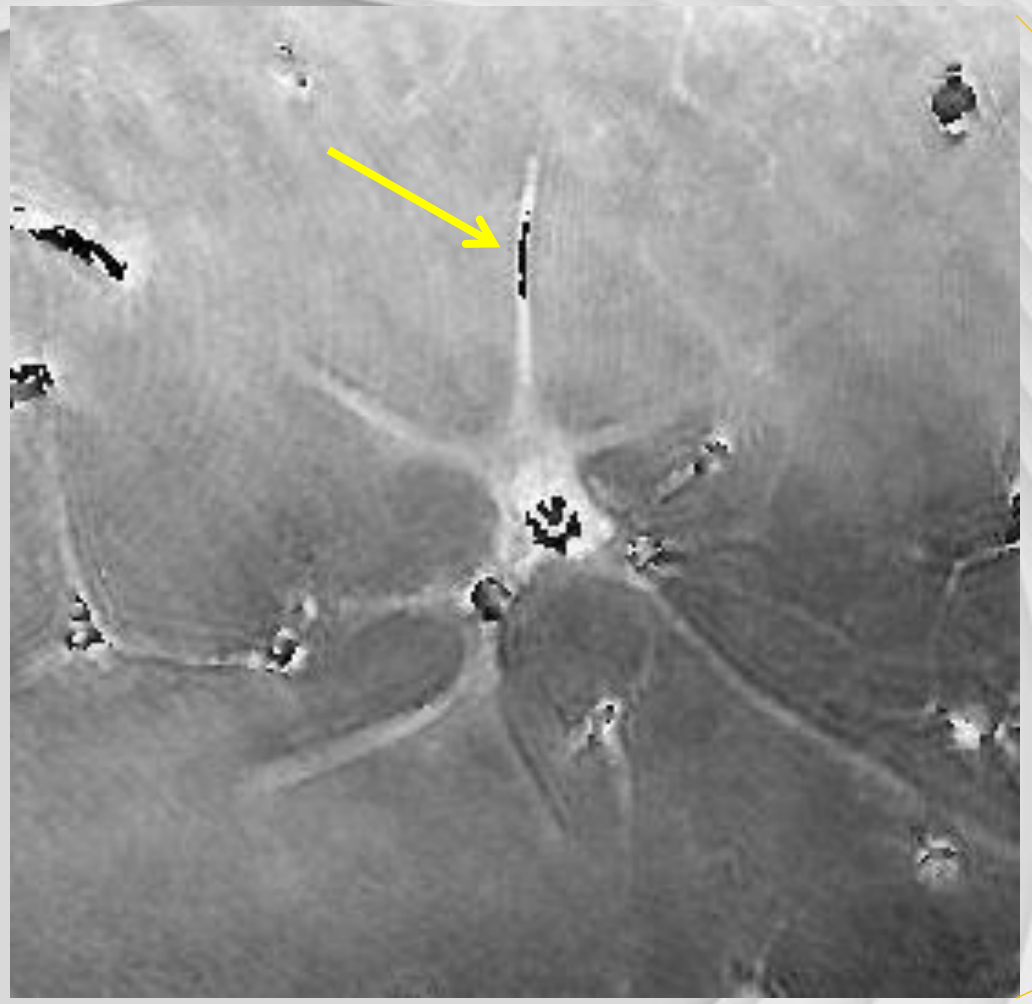
When the phase exceeds the measurable range (−π to +π), **phase wrapping** occurs, causing an apparent jump in the phase signal.

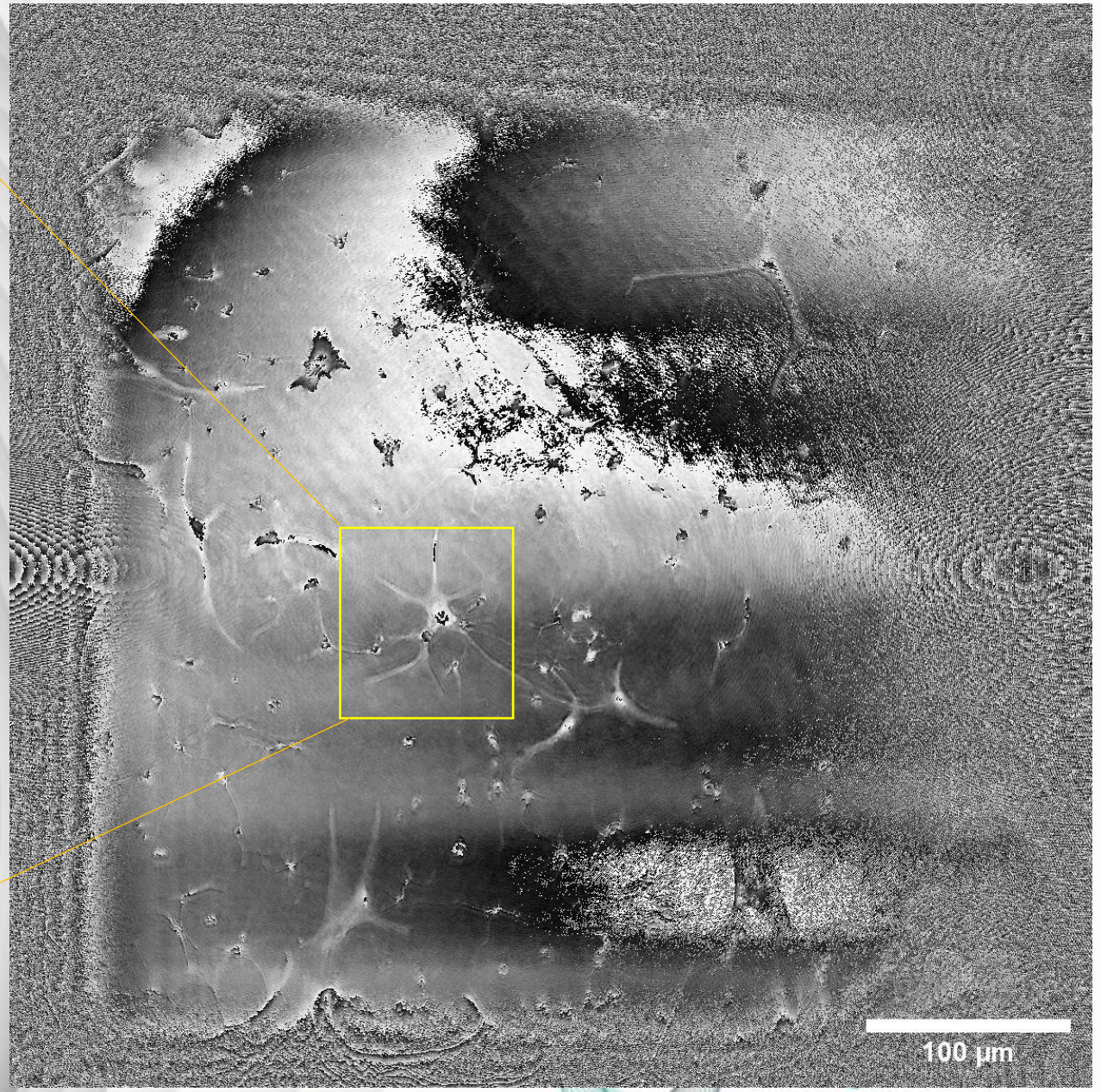
3D phase image of the astrocyte

- Does not indicate the real shape and depth of the cells since the phase change can also be due to lateral dependence of the index of refraction.



Phase imaging of astrocytes

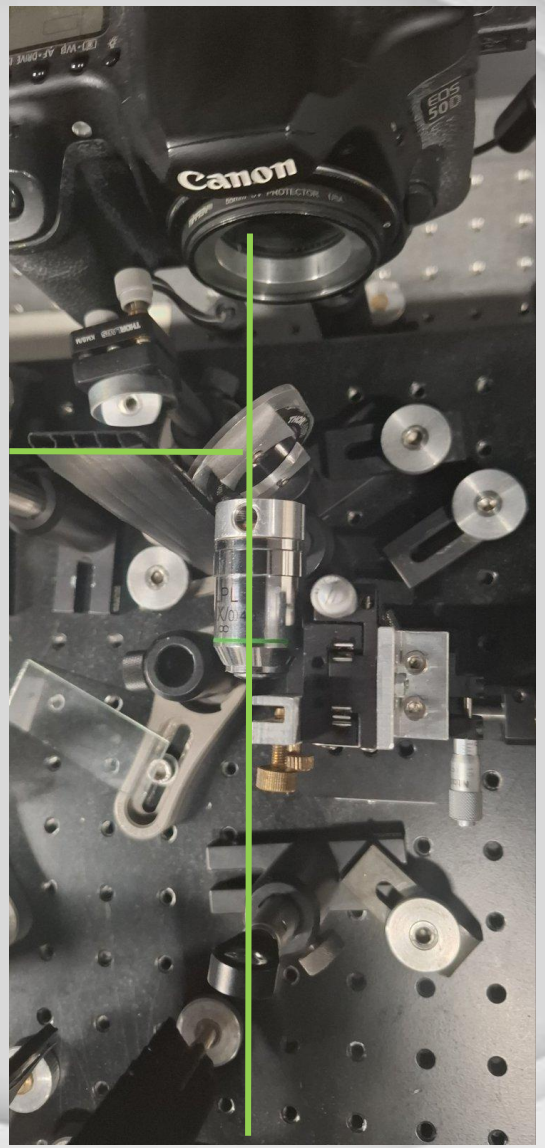




- Thicker astrocytic endfoot are clearly distinguished in phase images

Objective 20x, NA=0,4

Holographic imaging of astrocytes



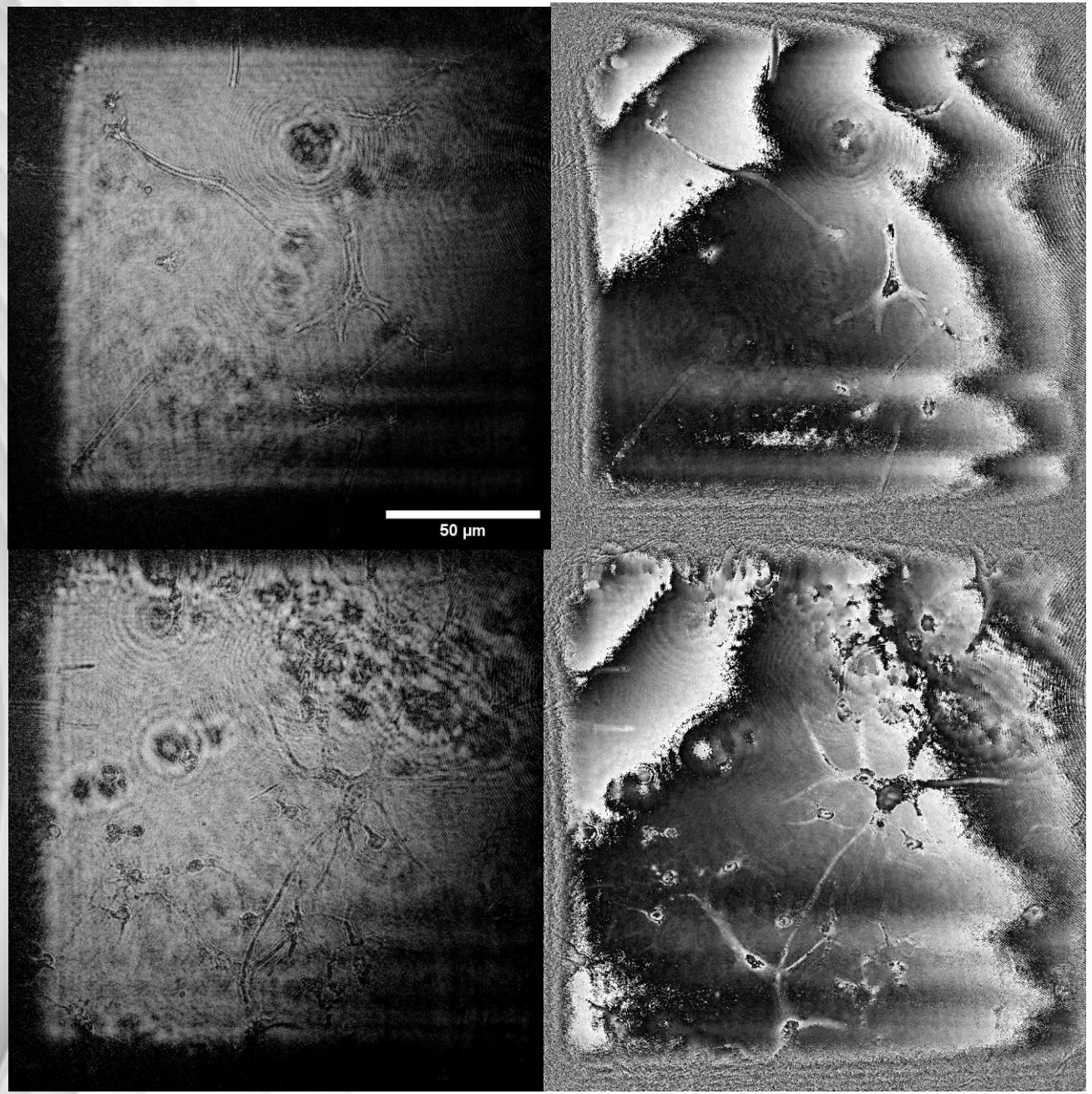
Au NP
1nM

Objective 40X ,
NA=0.65
532 nm
wavelenght

Au NP
2 nM

Amplitude image

Phase image



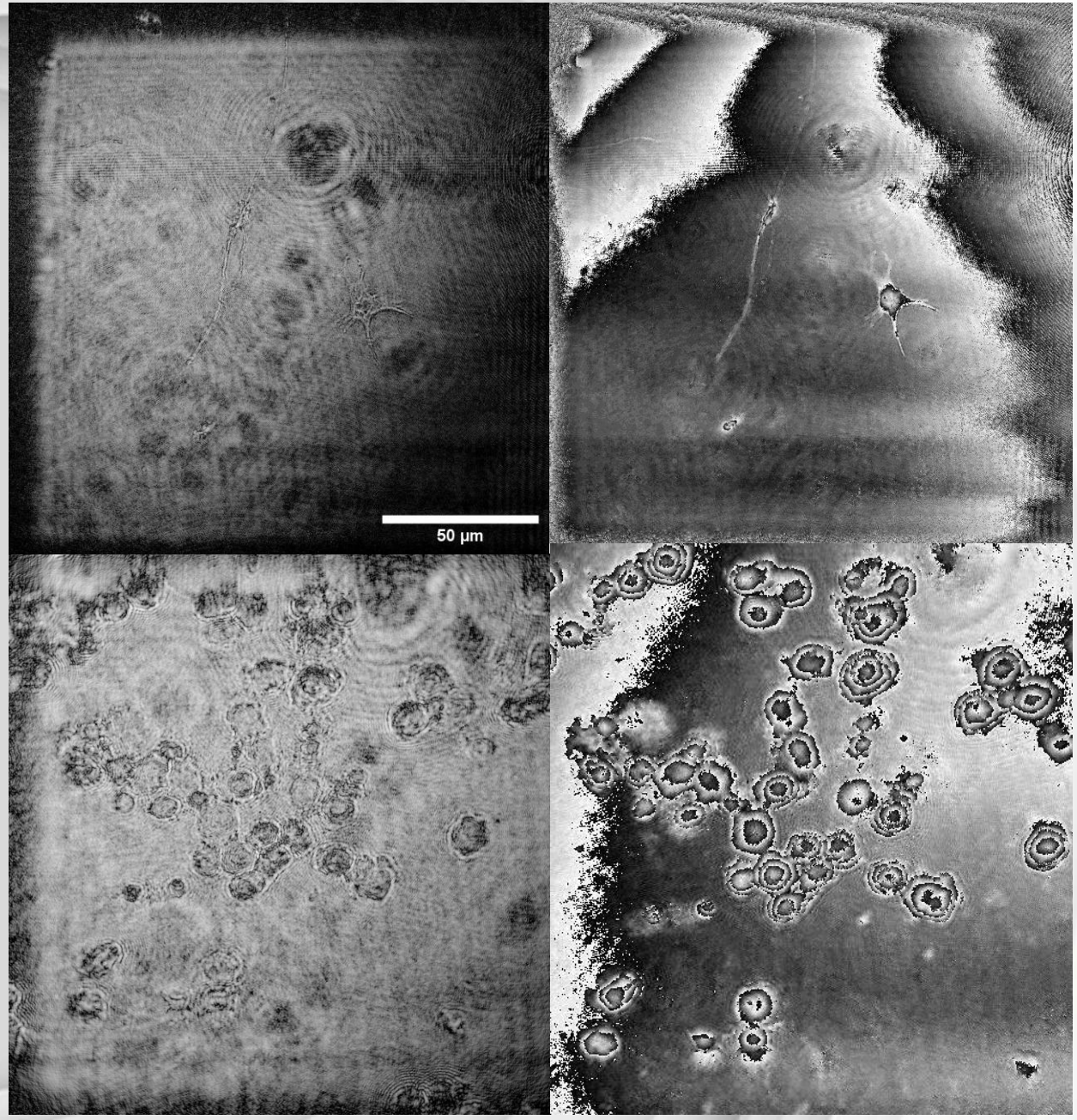
Holographic imaging of glial cells morphology

Amplitude image

Phase image

Astrocytes

BV2 cells



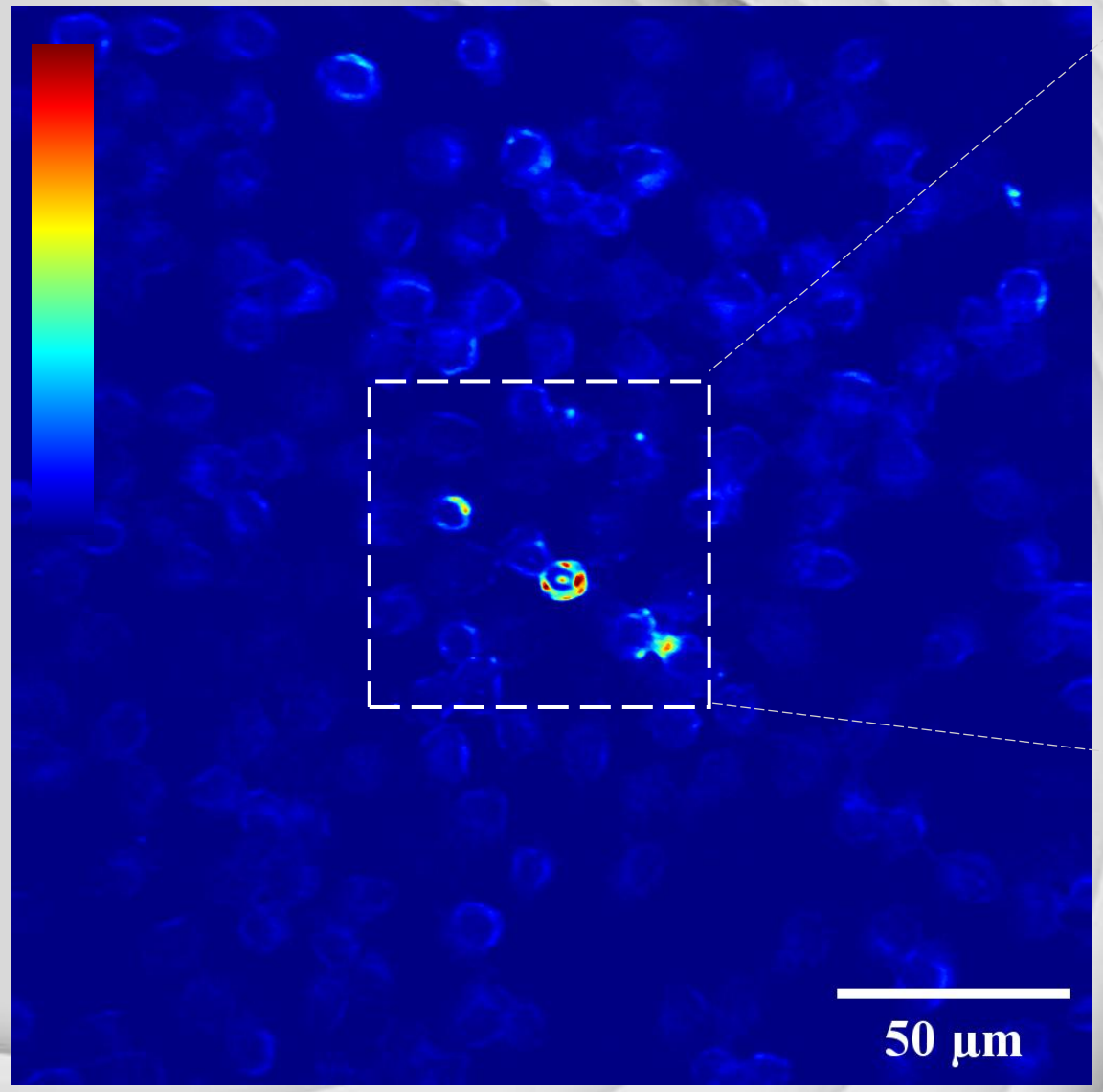
Objective 40X ,
NA=0.65
532 nm
wavelenght

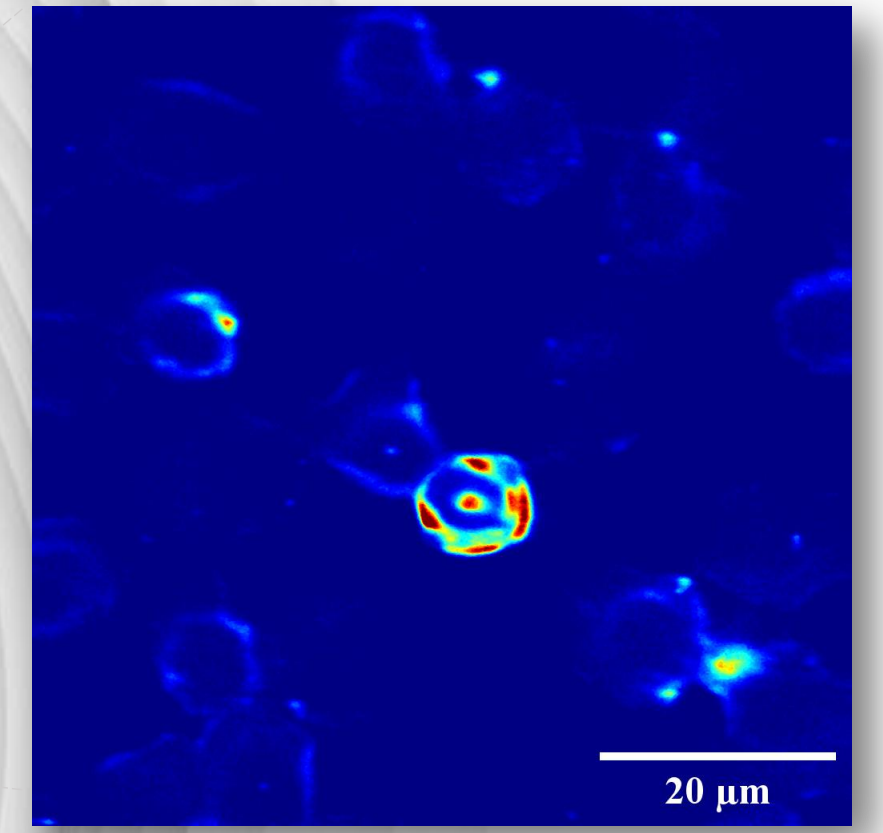
BioQantSense

Results
Nonlinear two-photon microscopy

BioQantSense

Two-photon microscopy, BV2 cells labeled with Au NP





Microscope objective 20x 0.8
Wavelength 800 nm
Laser power 37 mW
filter 700 sp

University in Beograd
FACULTY OF BIOLOGY

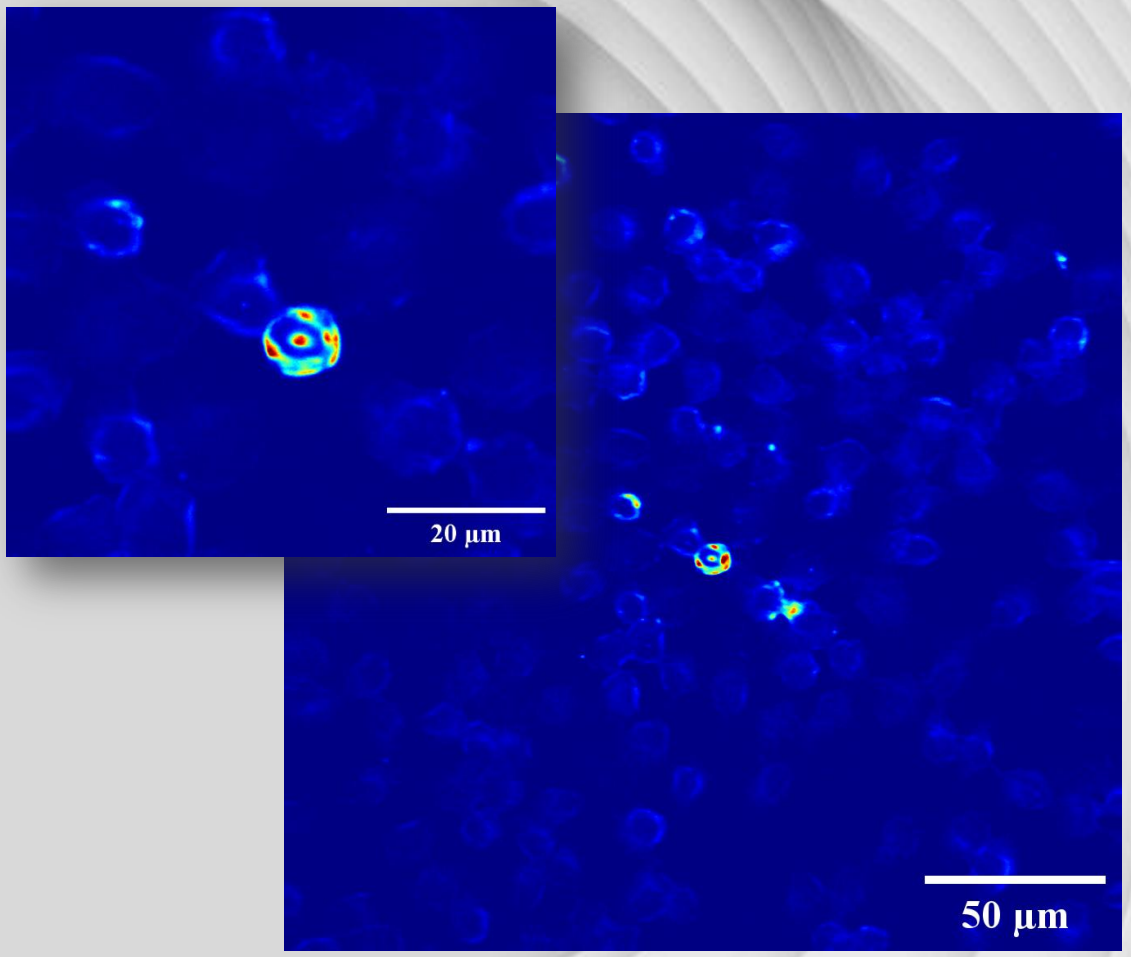
UNIVERSITY OF BELGRADE
INSTITUTE OF PHYSICS | BELGRADE
NATIONAL INSTITUTE OF THE REPUBLIC OF SERBIA

Tanja Pajić

BioQantSense

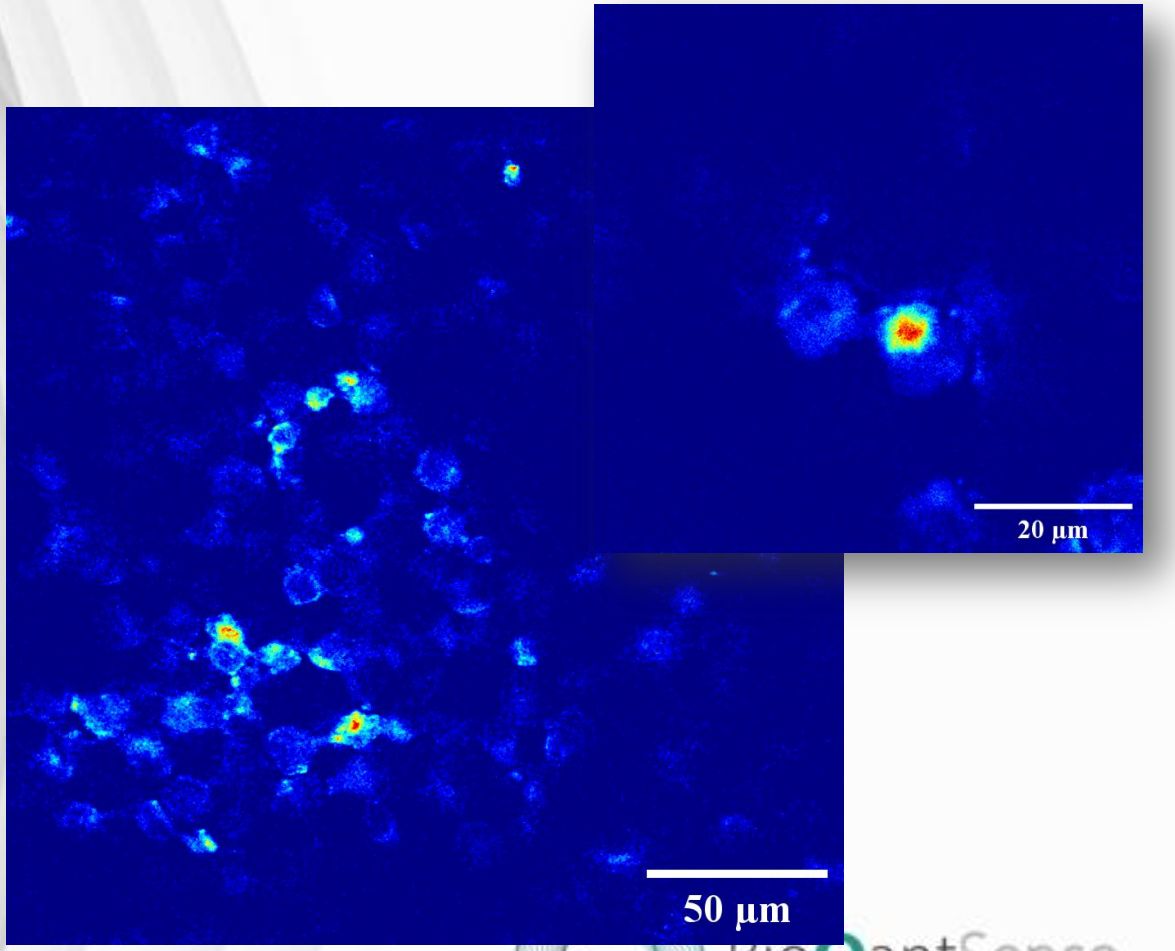
BV2 cells, TPEF, 800 nm, comparison

WGA Au NP



Laser power 37 mW

Negative control

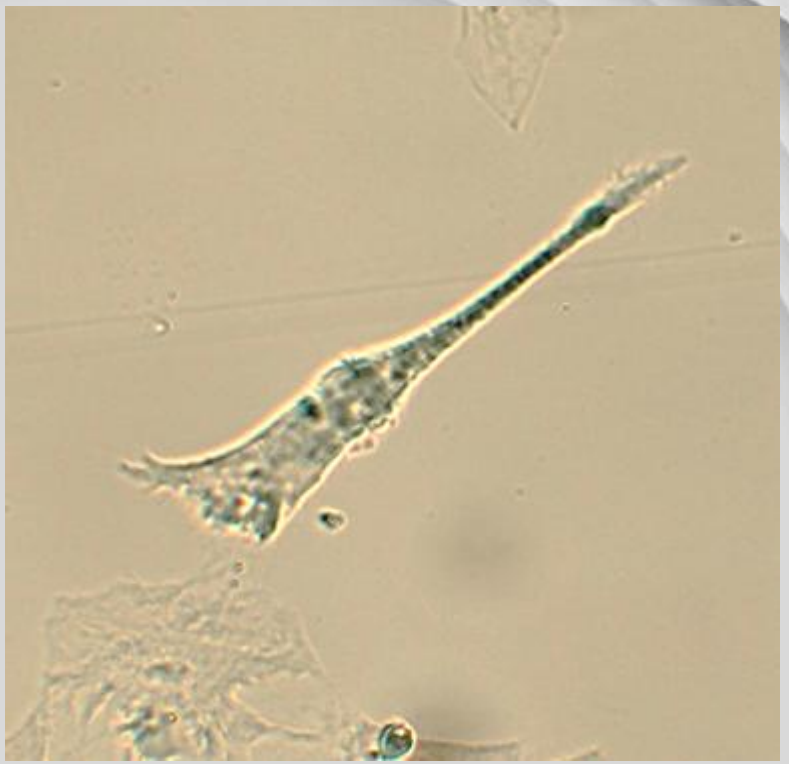


Laser power 51 mW

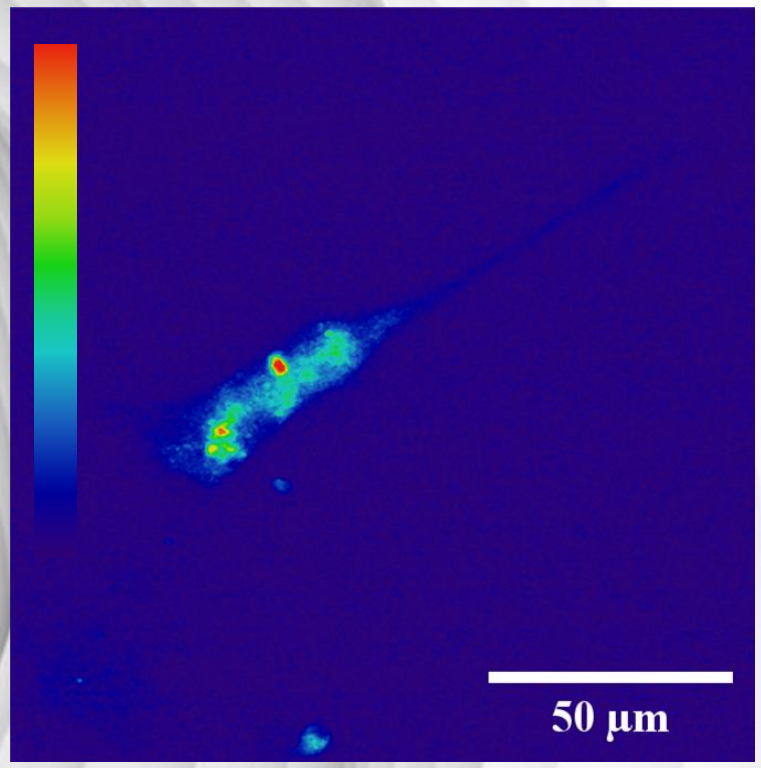
Objective 20X NA=0.8

BioQantSense

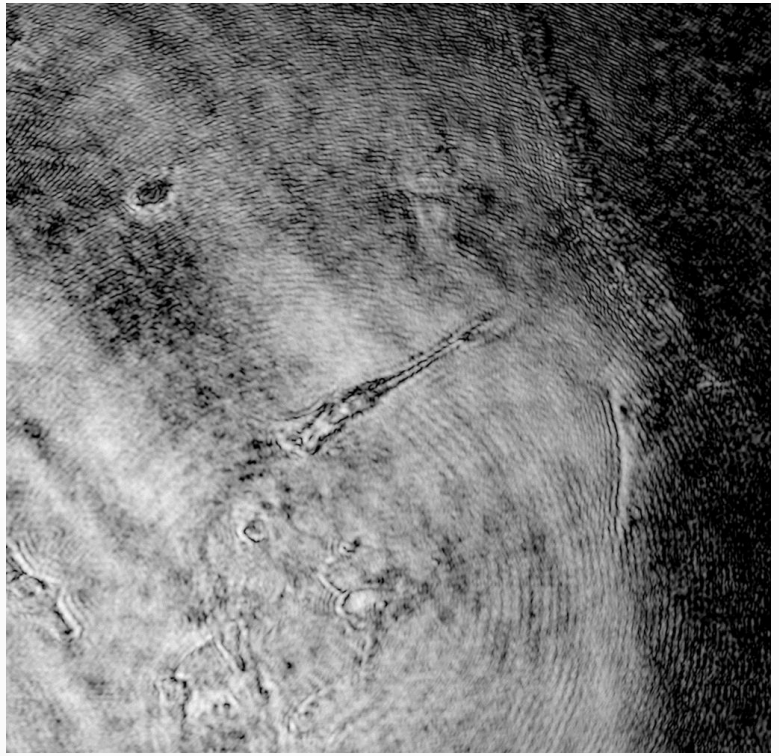
Two-photon and holographic imaging of astrocytes



Brightfield image of astrocytes seeded on a glass slide at a density of 10,000 cells/slide



Two-photon excitation: *laser* **730 nm**, *objective* **20x** 0.8 NA, *laser power on the sample* 24.5 mW, ***Result:*** *Autofluorescence signal at 730 nm (possibly from NADH)*



Holographic image (amplitude image) imaged with 532 nm laser, 20x objective, ~2 µm resolution.

BioQantSense

Conclusions and Outlook

Holographic microscopy

- Phase images provide more **structural information** than amplitude images
- Structural features such as **thicker astrocytic endfeet** were distinguishable
- Gold nanoparticle labeling did not produce a detectable signal at **532 nm**

Two-photon microscopy

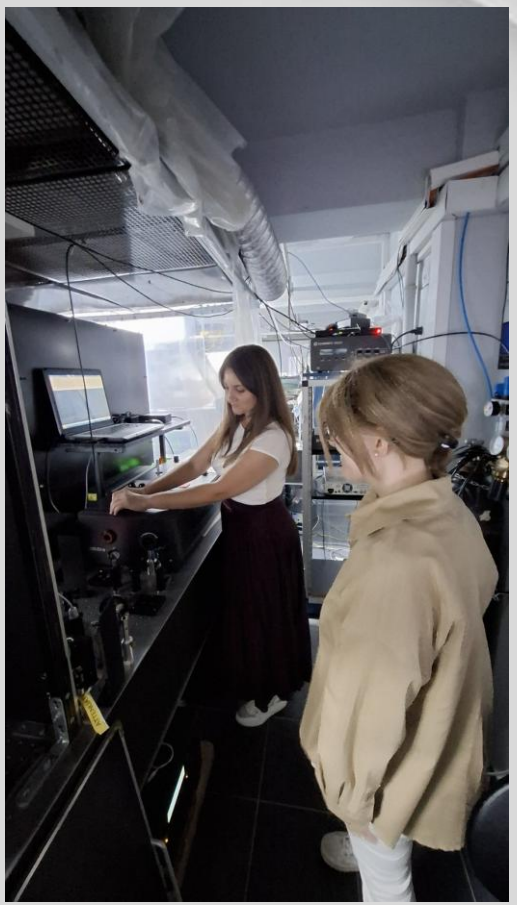
- BV2 cells labeled with AuNP showed possible membrane-associated signal detected at **800 nm excitation**
- Unlabeld astrocytes and BV2 cells showed **autofluorescence signal**

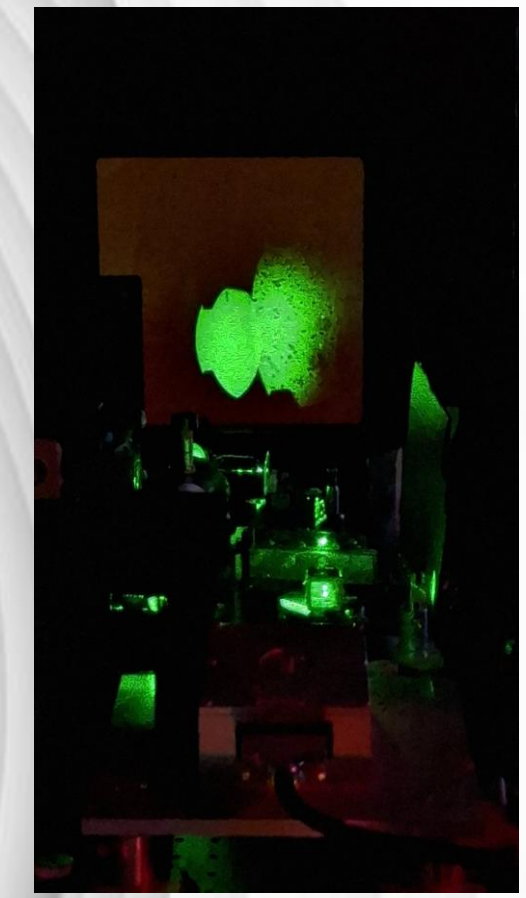
Future steps

- Optimization of the holographic setup for **live-cell imaging**
- Stimulation of cells with neuroinflamation markers for specific signature response

BioQantSense

Acknowledgments





Funded by the European Union

HORIZON-WIDERA-2021-ACCESS-03-01 – Twinning
Grant agreement Nº 101079355

PRIZMA NIMOCHIP Grant agreement4242 Science Fund RS

Thank you for your attention

BioQantSense