Human Brain Project

EBRAINS

SMART-BRAIN

Advanced Morphological Reconstruction of Human Brain Tissue by Multimodal Fusion of Multiscale Optical Imaging Technologies

HBP Partnering Projects Meeting: Status quo & outlook

5-7 September 2022 | Nijmegen, The Netherlands

Co-funded by
the European Union

The consortium

UNIVERSITY OF MODENA AND REGGIO EMILIA (Jonathan Mapelli)

LABORATORY OF NON-LINEAR SPECTROSCOPY – LENS (Ludovico Silvestri)

LYON NEUROSCIENCE RESEARCH CENTER & HOSPITAL OF LYON (Sylvain Rheims)

DELFT UNIVERSITY OF TECHNOLOGY (Raf Van De Plas)

Scientific Background (1)

- Data acquisition is a fundamental step to understand brain physiology, pathology and to generate models.
- Optical microscopy applied to Neuroscience is declined in different imaging modalities allowing to generate rich datasets.
- Each imaging modality has its own advantages (high resolution, low invasiveness, in vivo applicability) and disadvantages (sample preparation, fluorophores to be expressed)
- Imaging technology is evolving towards less invasive, larger scale and higher resolution methodologies, however requirements to be fulfilled for the perfect technique cannot be satisfied

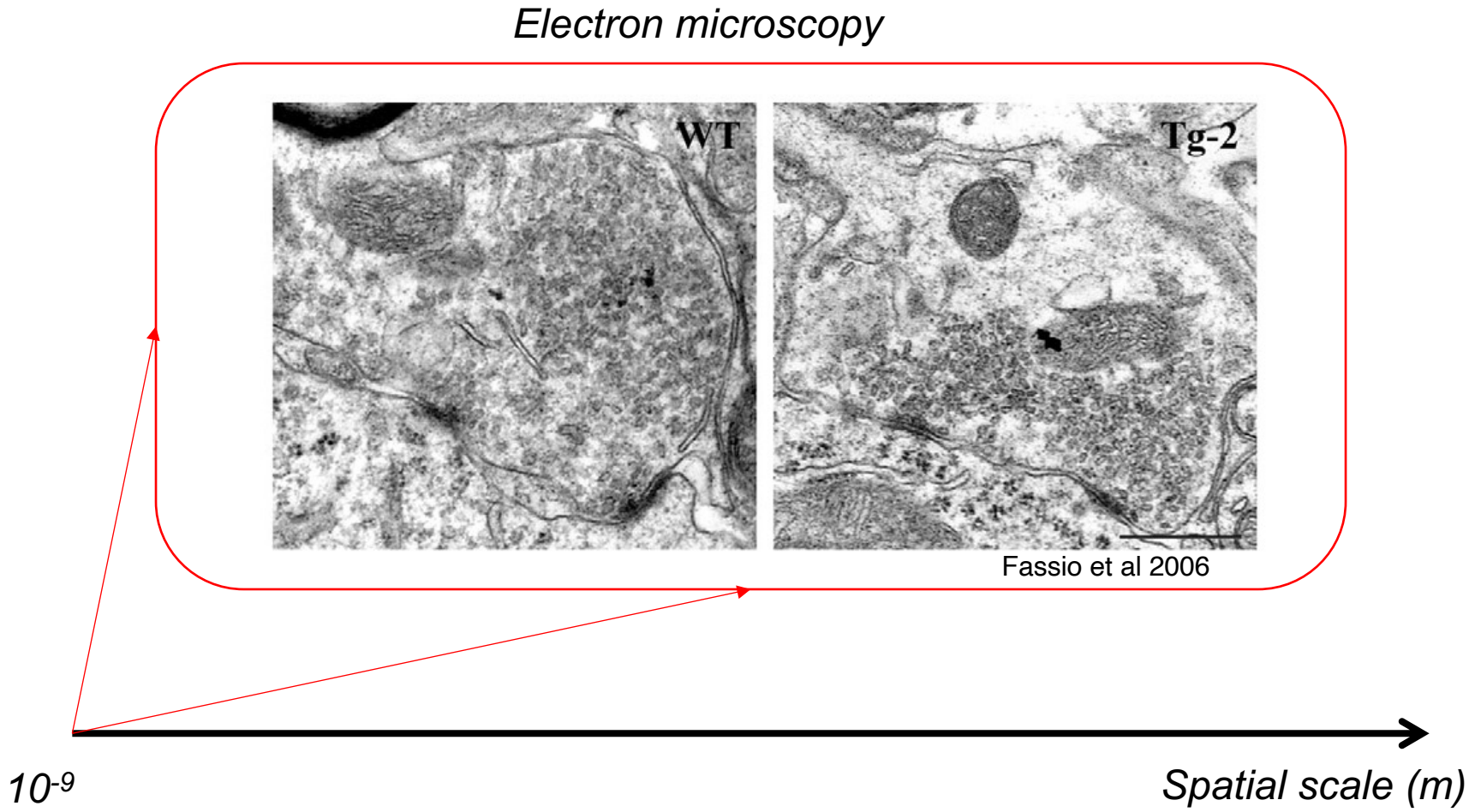
Scientific Background (2)

- The advent of high performance computing and the availability of multi-modal imaging datasets opened the route to *in silico* integration strategies of multiple spatial scale brain circuits data.
- Computational algorithms have been developed to aid brain morphology reconstruction with probabilistic supported information where physical measurements are infeasible
- A crucial step for HBP/EBRAINS would be the development of imaging methods capable of performing detailed multiscale correlations of brain morphology across different resolutions
- Multi-modal integration approaches offer the possibility of side-stepping the viewport *vs* resolution trade-off that hamper imaging technologies

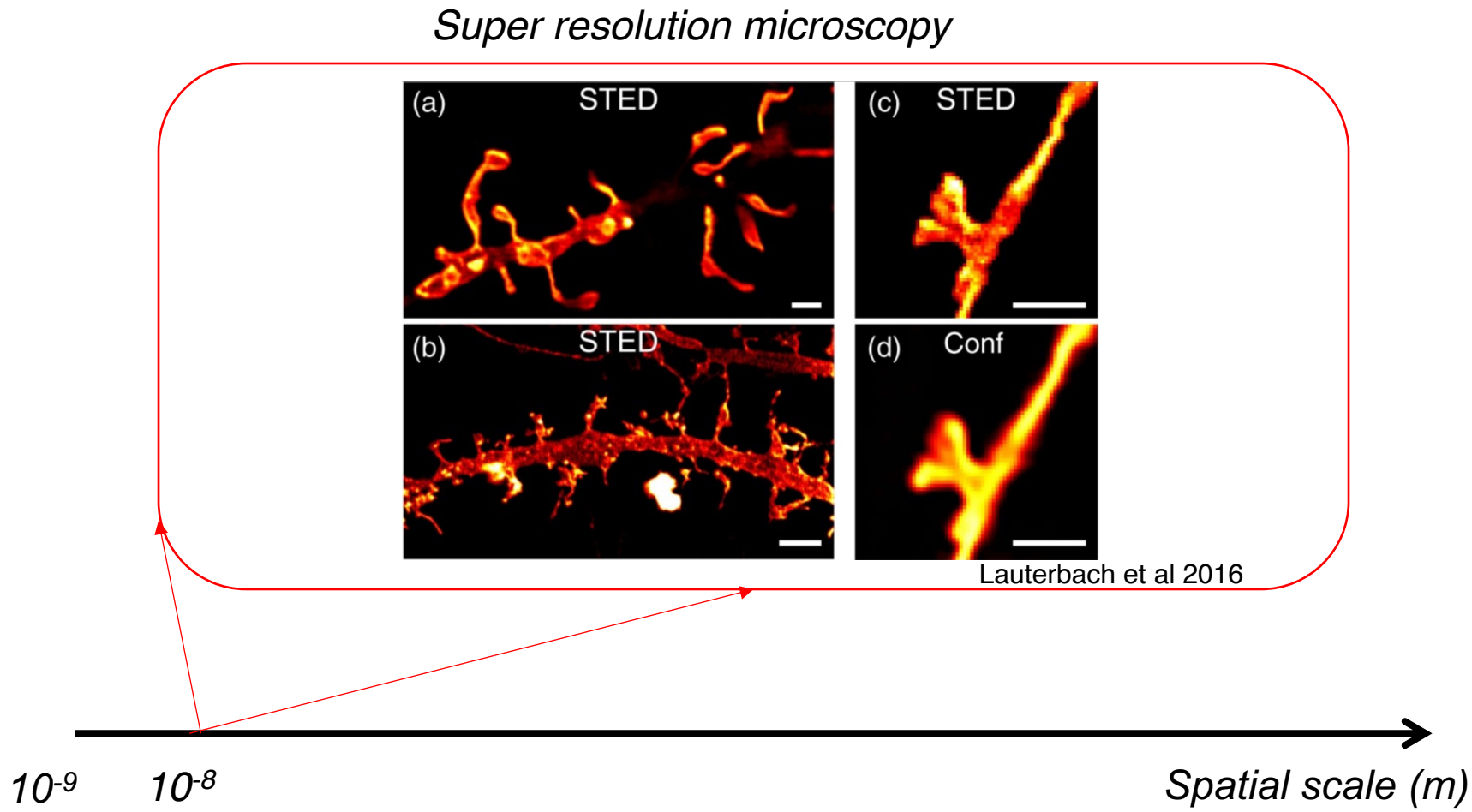
Scientific Background (3)

- Brain morphology is typically obtained through histological procedures that are poorly correlated with clinical imaging data
- Data driven algorithms shows intriguing perspectives for the correlation of clinical low-resolution non-invasive imaging data
- Non invasive diagnostic tools based on multi-modal fusion methodologies offer a path towards imaging that is both non-invasive and highly resolved.
- Observation of neural morphologies in large samples could become feasible and would allow to map whole-brain at high spatial resolution

BRAIN IMAGING

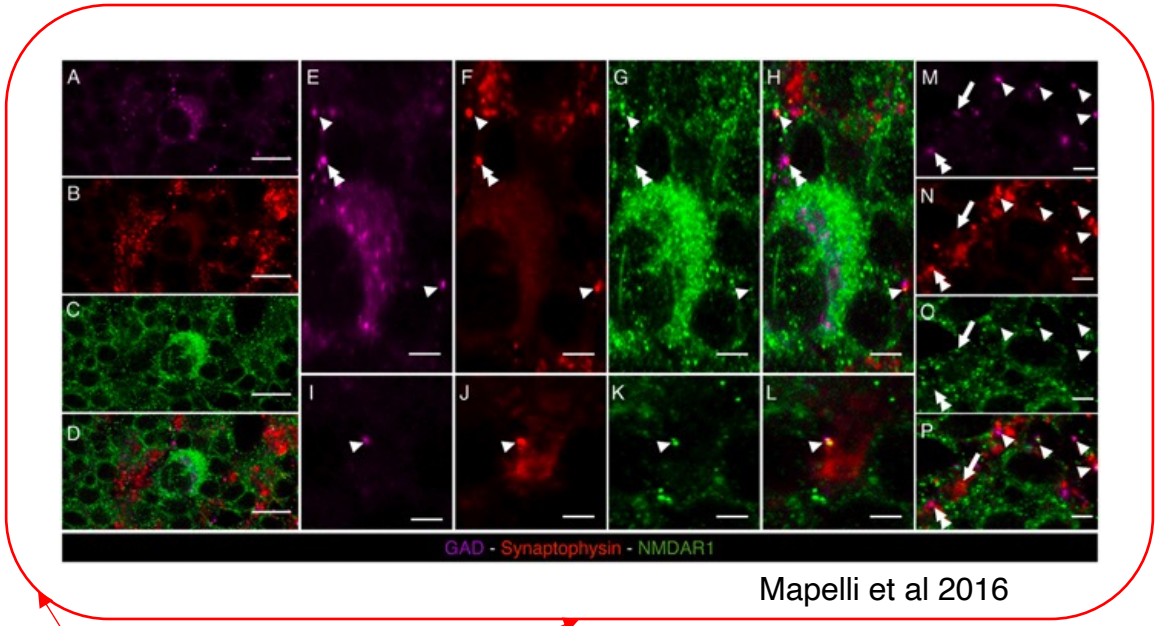


BRAIN IMAGING



BRAIN IMAGING

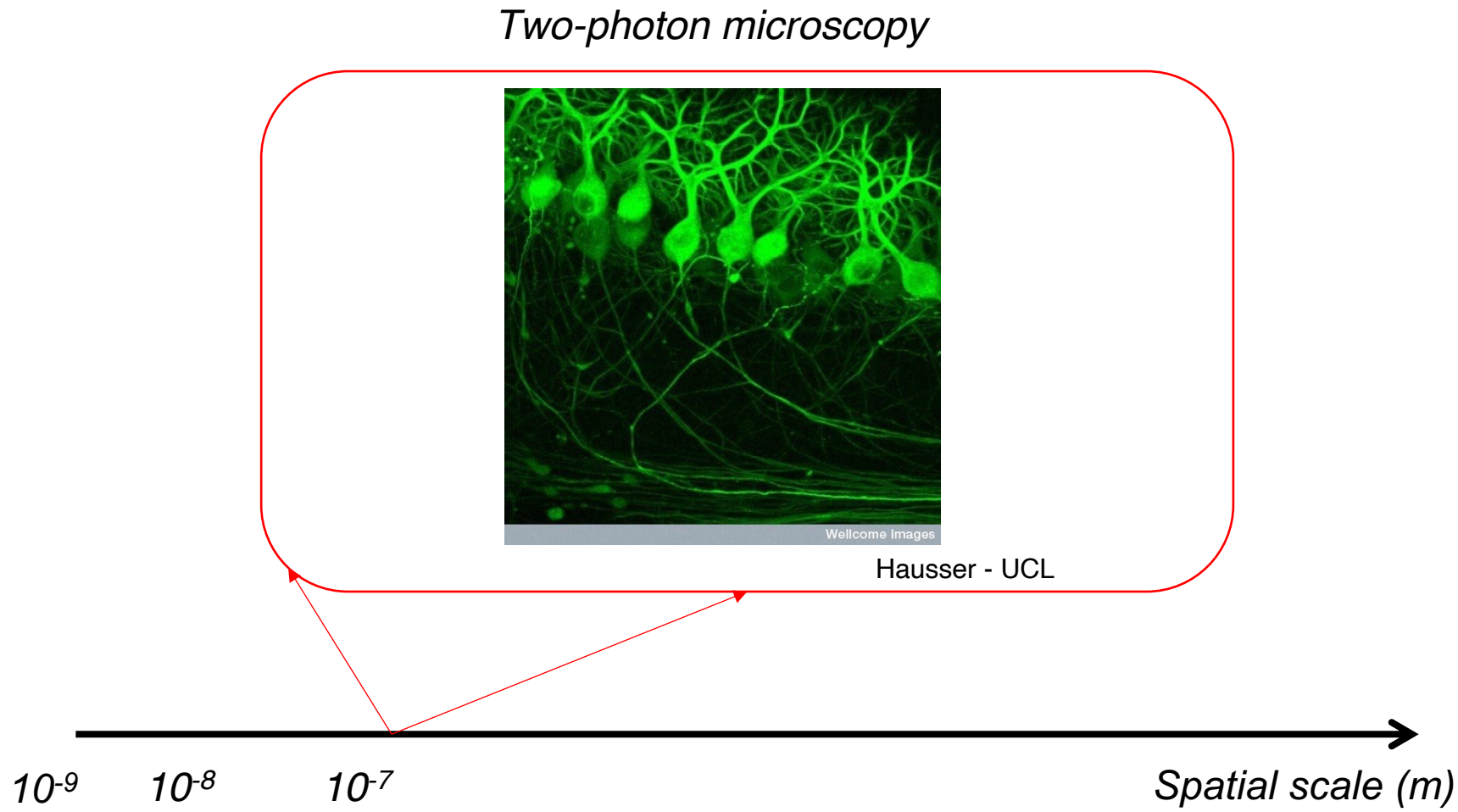
Confocal microscopy



Mapelli et al 2016

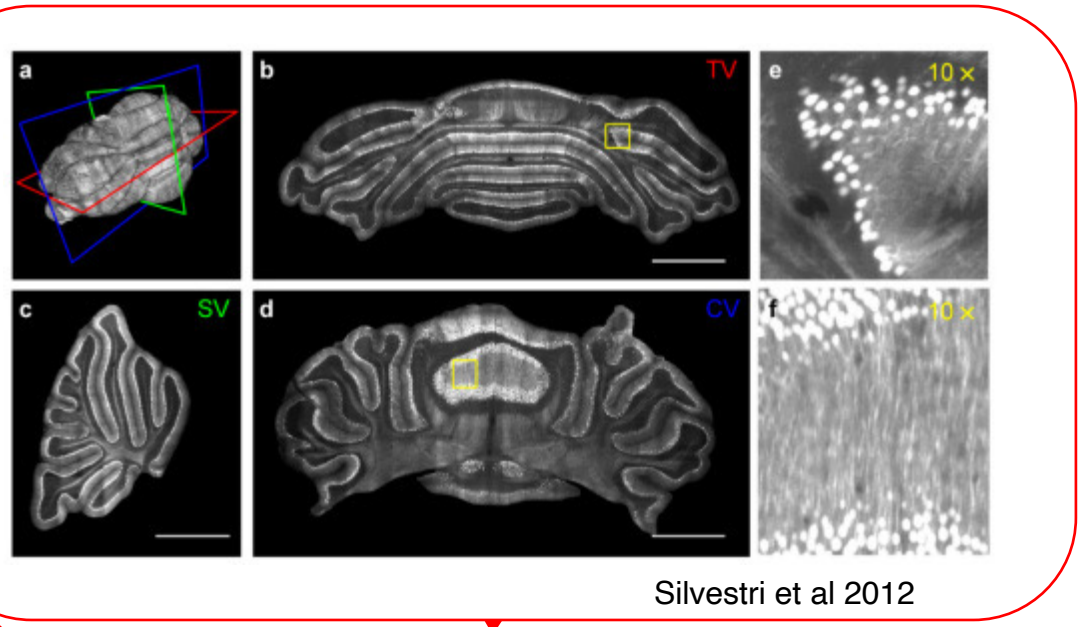
10^{-9} 10^{-8} 10^{-7} *Spatial scale (m)*

BRAIN IMAGING



BRAIN IMAGING

Light-sheet microscopy



Silvestri et al 2012

10^{-9} 10^{-8} 10^{-7} 10^{-6} *Spatial scale (m)*

BRAIN IMAGING

- The complementary measurements of neuronal morphologies and circuit architectures are performed separately.

- Each imaging modality is acquired and data integration is generally obtained through manual procedure.

- Our idea was to apply a particular machine learnign algorithm, '*image fusion*', which proved useful to integrate optical microscopy and mass spectroscopy

ADDRESSING THE SCIENTIFIC QUESTIONS

- Identify brain imaging modalities
  - Super resolution microscopy (STED)
  - Multiphoton microscopy (MPM)
  - Light-sheet microscopy (LSM)

BRAIN IMAGING

Some issues (national administration, the pandemic, fundings) required to change the project implementation strategy and WPs have been rearranged and parallelized

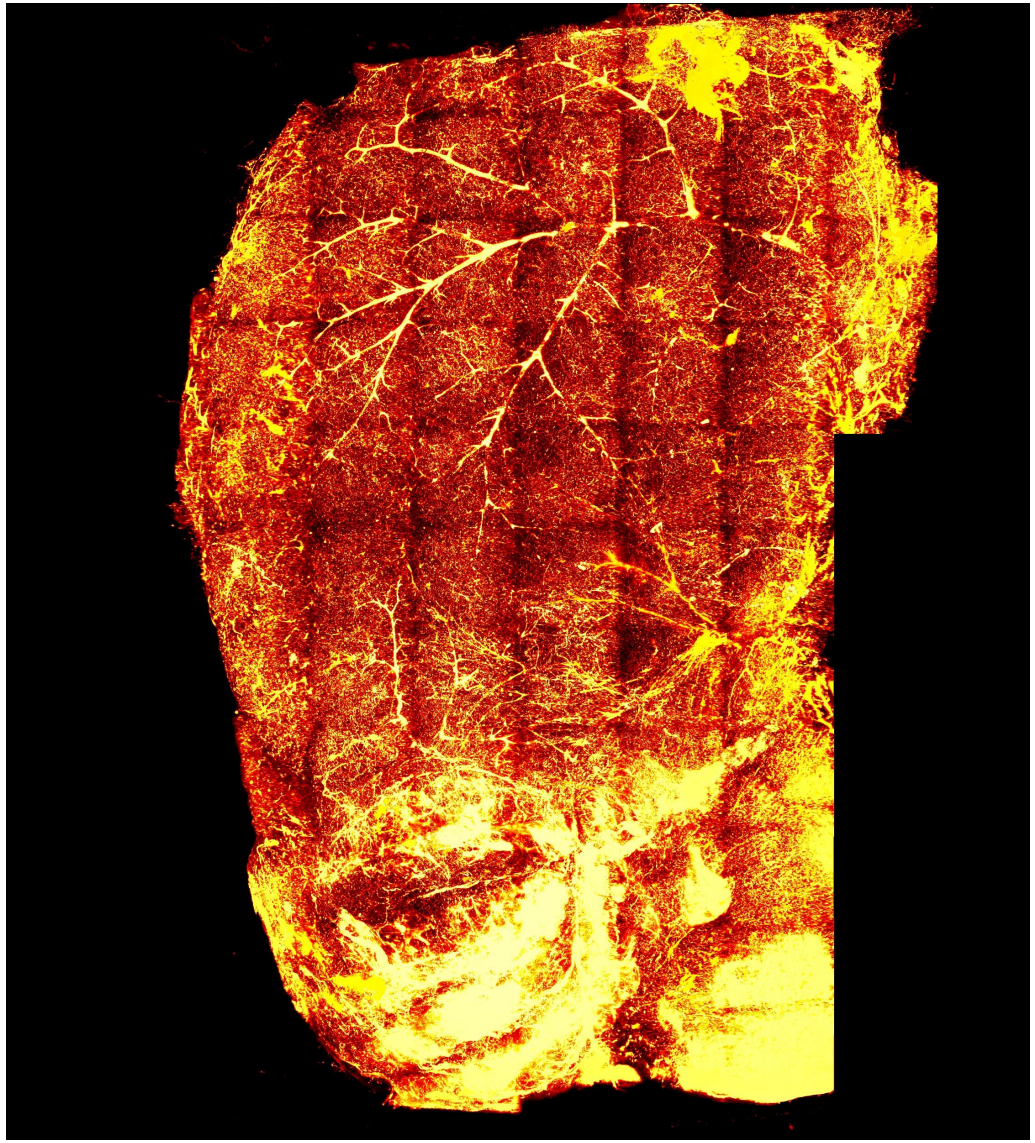
- **WP1 Validation of image fusion algorithms on murine samples**
  - Integration of super resolution, multiphoton and light sheet microscopy.
- **WP2 Human tissue sample collection**
  - Fixed tissues selected from biobanks and fresh samples from neurosurgery department
- **WP3 Image fusion on human samples**
  - Translation of WP1 protocols to human samples.
- **WP4 Extension of data driven image fusion methods**
  - Application of fusion methods to human tissues and database creation.

ADDRESSING THE SCIENTIFIC QUESTIONS

LIGHT-SHEET MICROSCOPY

LS imaging of murine brain vasculature obtained with dextrane conjugated rodamine perfusion

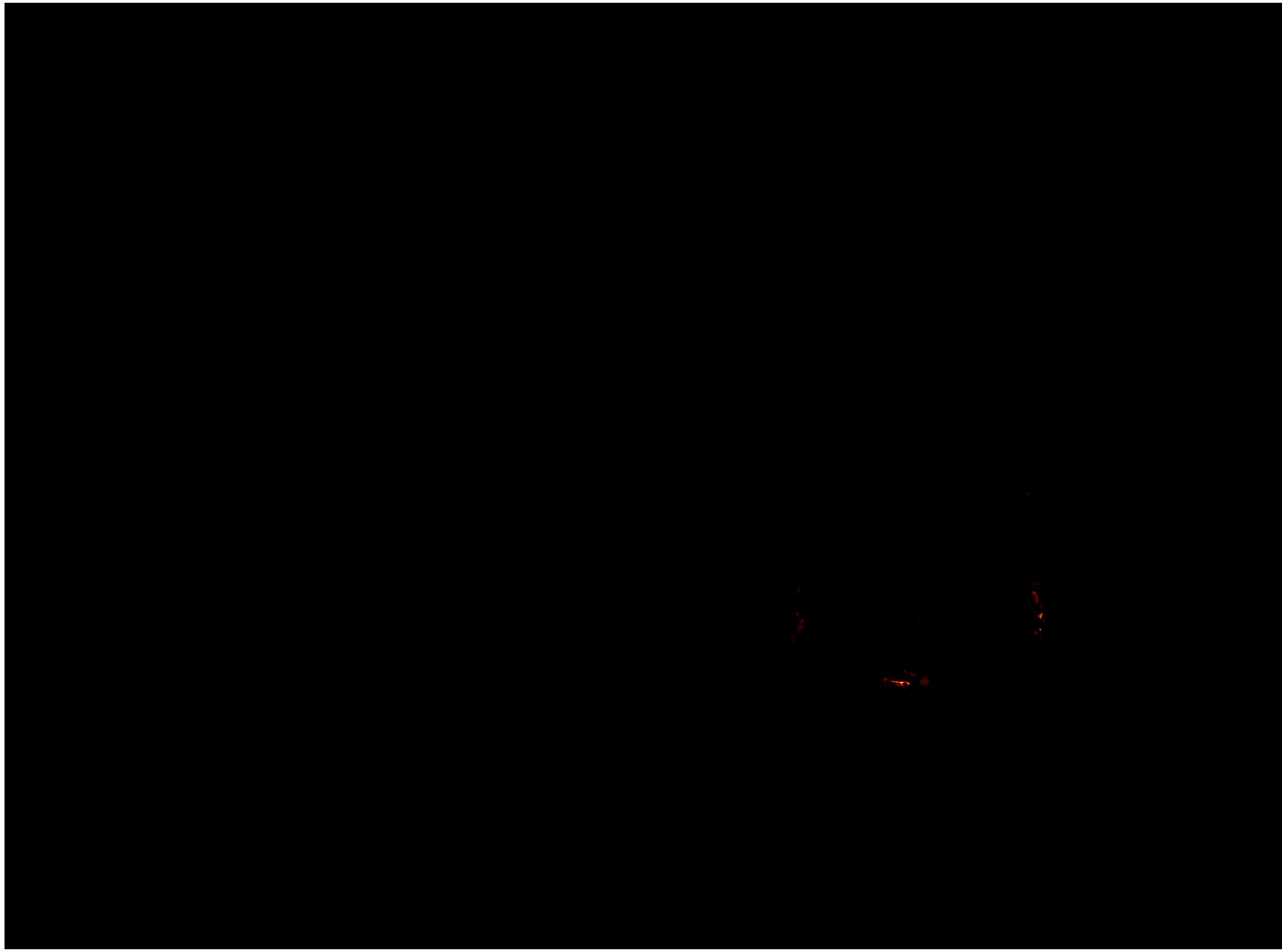
field of view (x,y,z):
12953 x 9562 x 6040 pixels
Resolution 1.5x1.5x3 micrometers



ADDRESSING THE SCIENTIFIC QUESTIONS

LIGHT-SHEET MICROSCOPY

LS imaging of murine brain vasculature obtained with dextrane conjugated rodamine perfusion

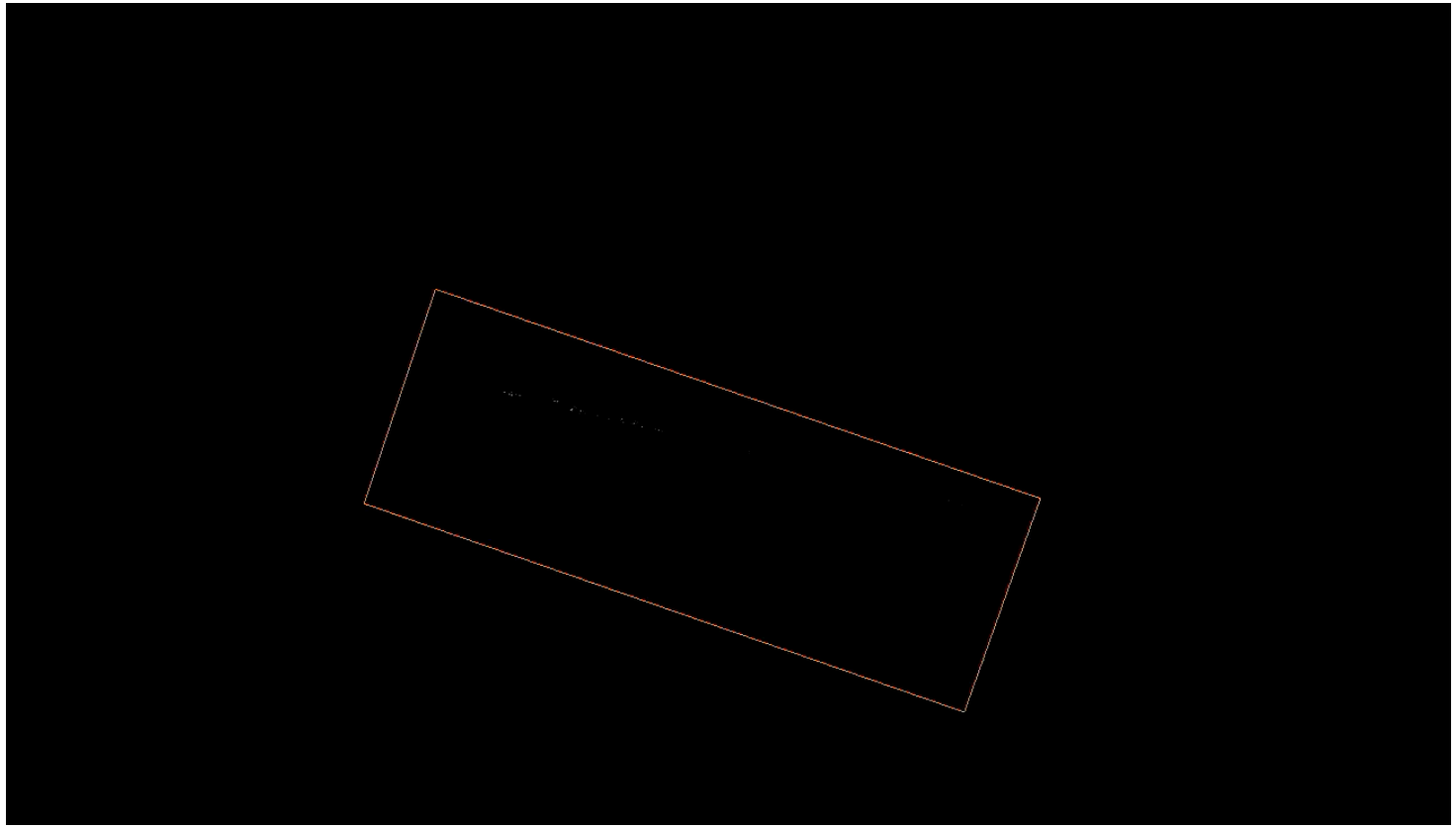


field of view (x,y,z):
12953 x 9562 x 6040 pixels
Resolution 1.5x1.5x3 micrometers

ADDRESSING THE SCIENTIFIC QUESTIONS

LIGHT-SHEET MICROSCOPY

LS imaging of murine brain cleared and stained with neuronal markers (anti-synapsin)

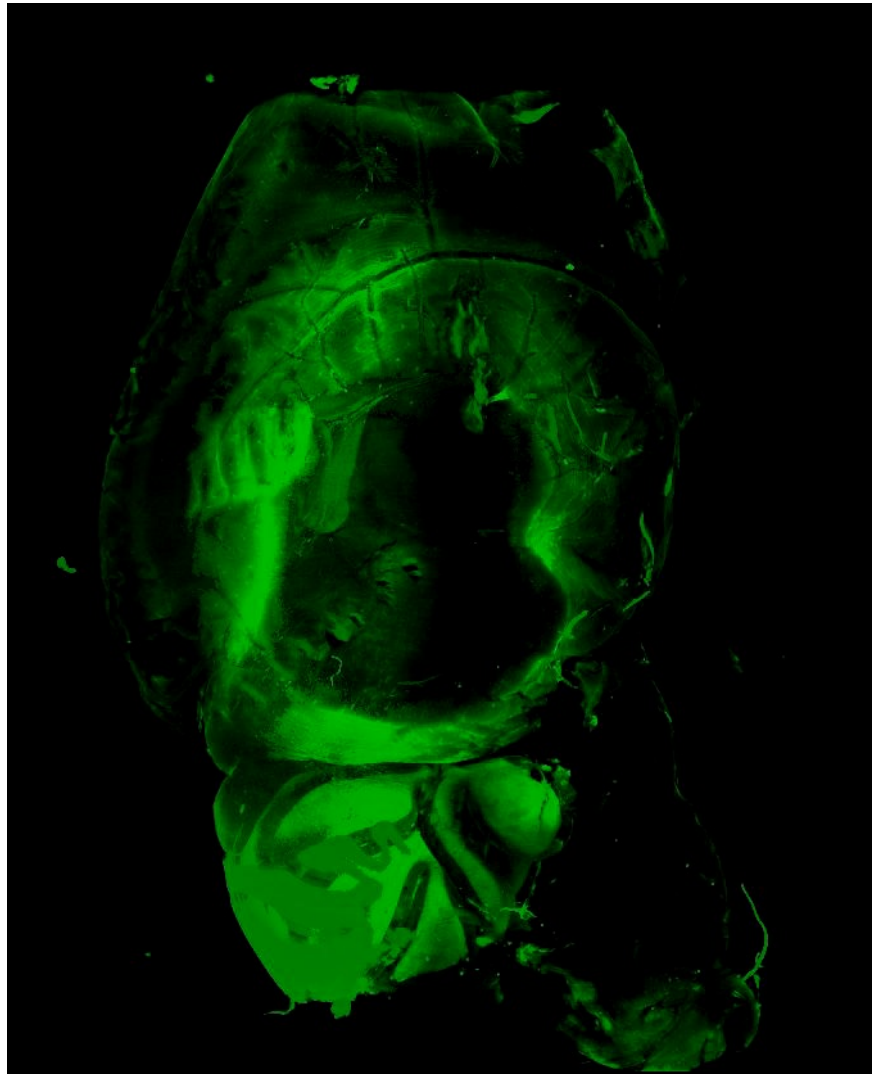


field of view (x,y,z):
12953 x 9562 x 6040 pixels
Resolution 1.5x1.5x3 micrometers

ADDRESSING THE SCIENTIFIC QUESTIONS

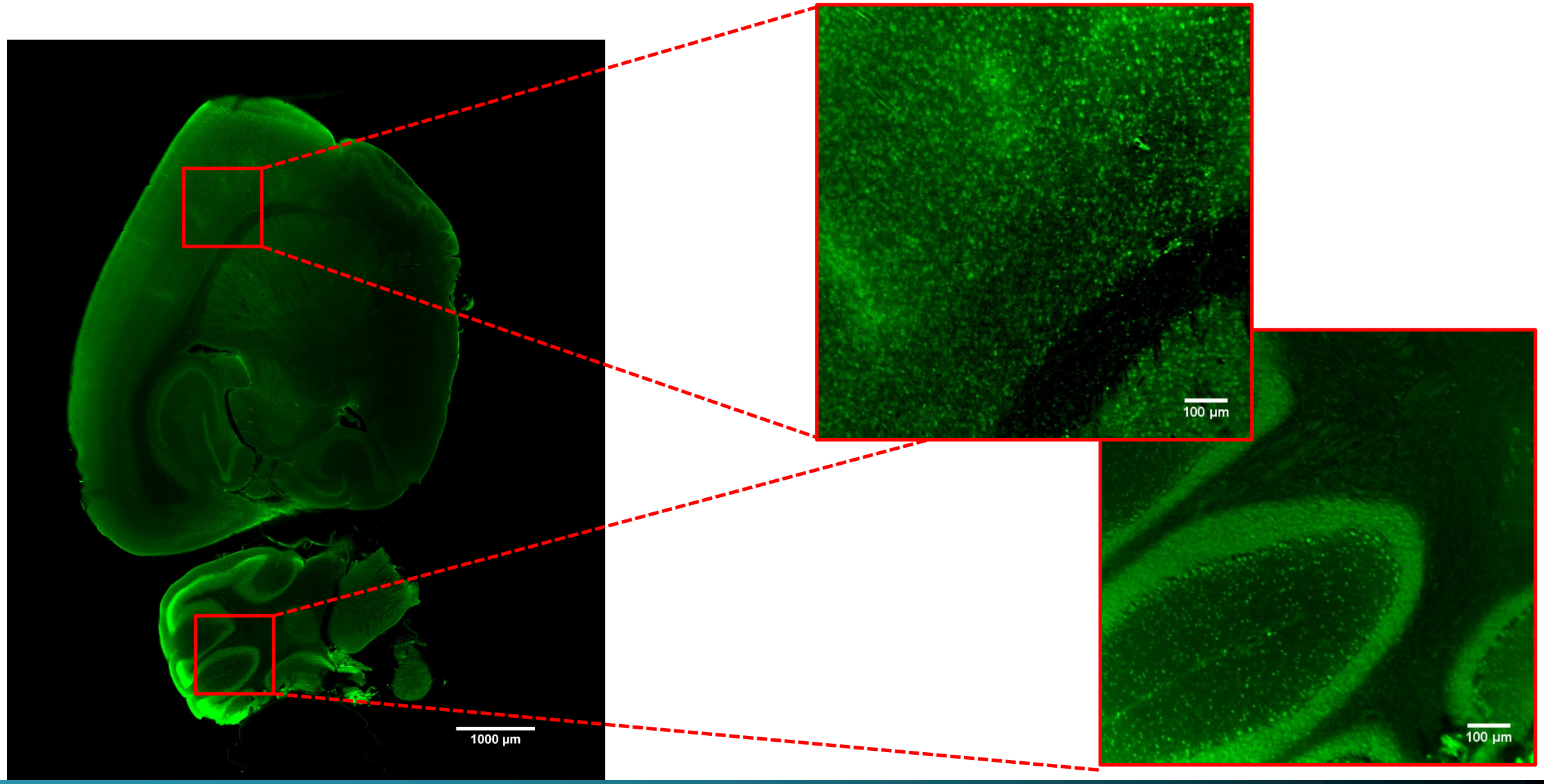
LIGHT-SHEET MICROSCOPY

LS imaging of murine brain cleared and stained with neuronal markers (anti-synapsin)



field of view (x,y,z):
12953 x 9562 x 6040 pixels
Resolution 1.5x1.5x3 micrometers

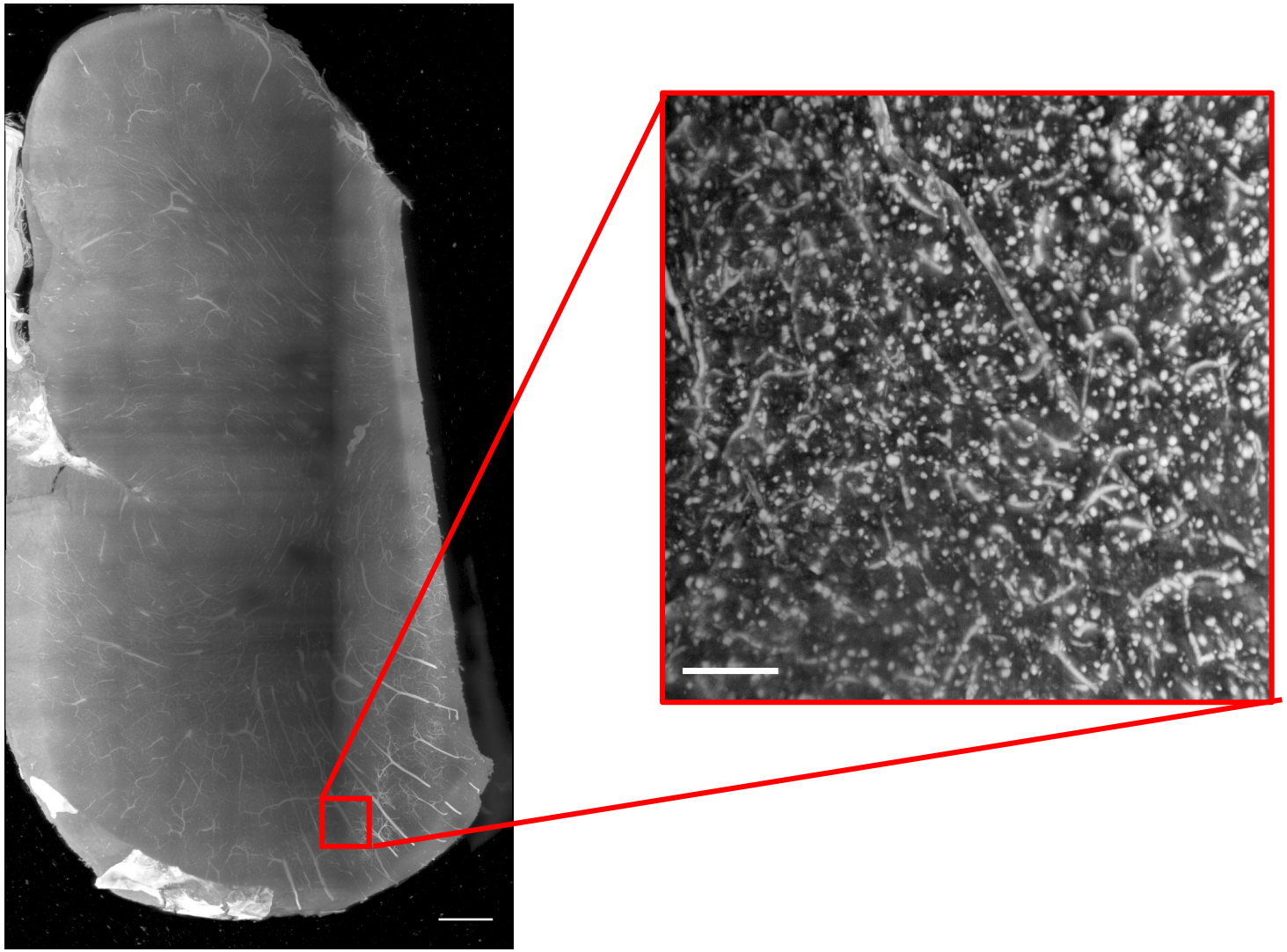
ADDRESSING THE SCIENTIFIC QUESTIONS



ADDRESSING THE SCIENTIFIC QUESTIONS

LIGHT-SHEET MICROSCOPY

LS imaging of human brains cleared and stained with eosin and sytox blue)



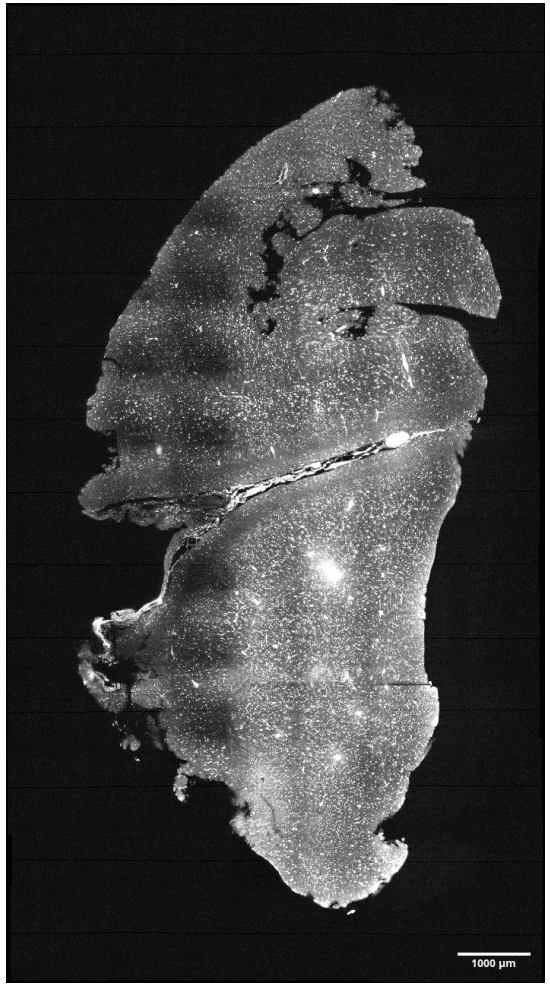
field of view (x,y,z):
12953 x 9562 x 6040 pixels
Resolution 1.5x1.5x3 micrometers

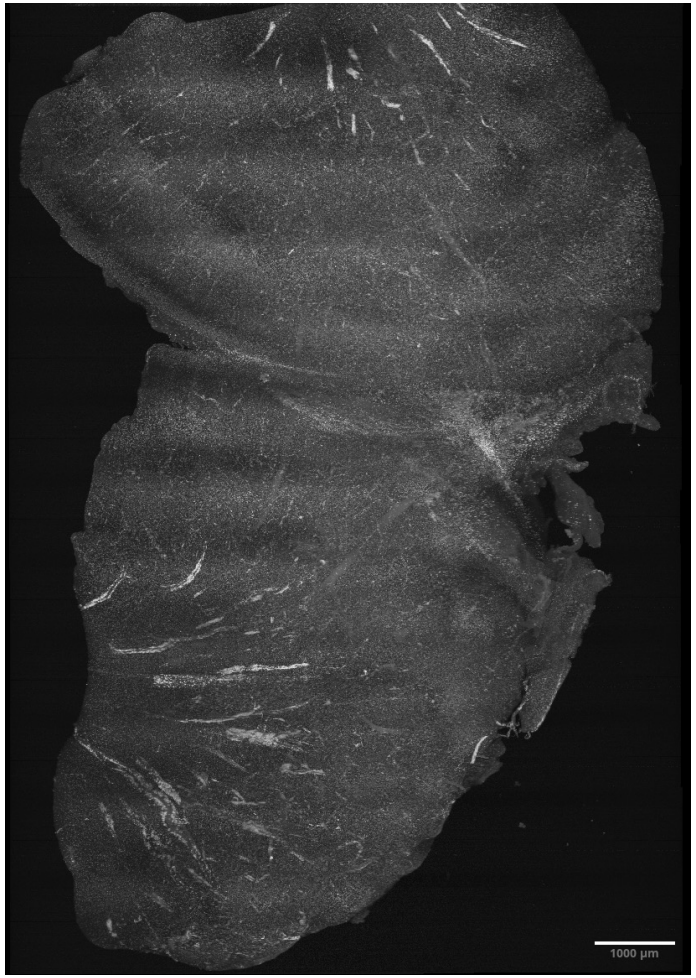
ADDRESSING THE SCIENTIFIC QUESTIONS

LIGHT-SHEET MICROSCOPY

LS imaging of human brains cleared and stained with eosin and sytox blue)

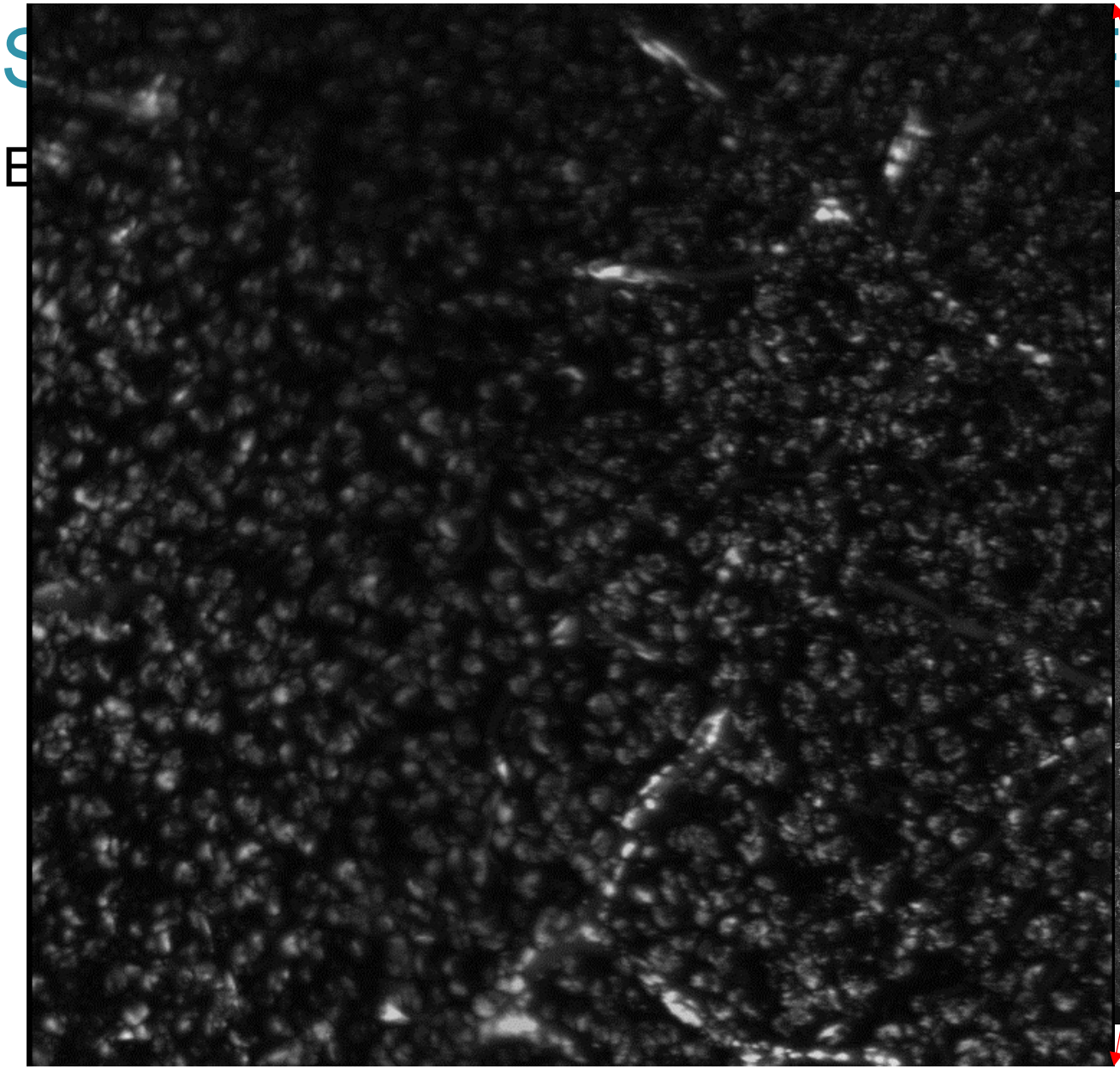
field of view (x,y,z):
12953 x 9562 x 6040 pixels
Resolution 0.6x0.6x2 micrometers

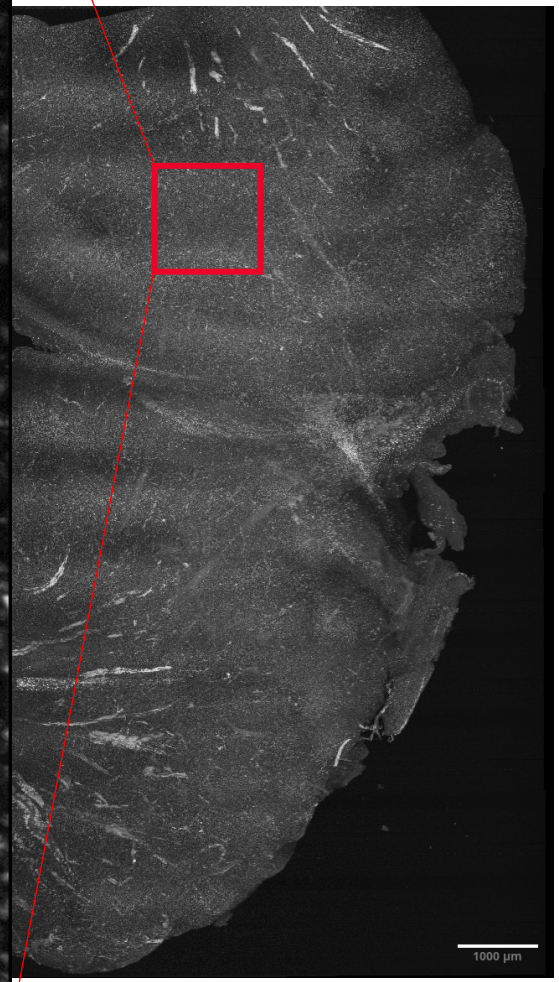




ADDRES ESTIONS

LIGHT-SHEE

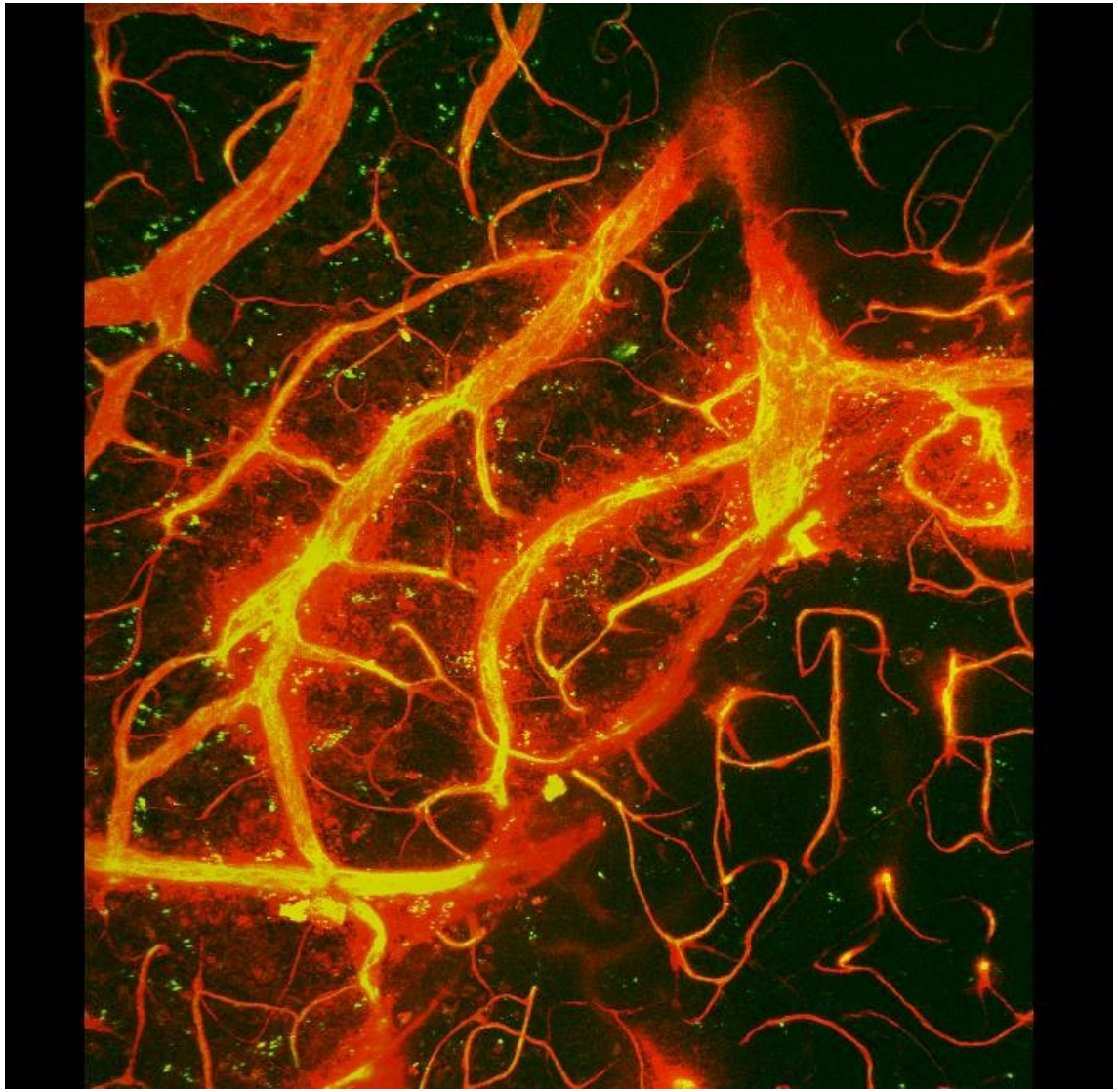




ADDRESSING THE SCIENTIFIC QUESTIONS

MULTI-PHOTON MICROSCOPY

MP imaging of murine brain vasculature obtained with dextrane conjugated rodamine perfusion

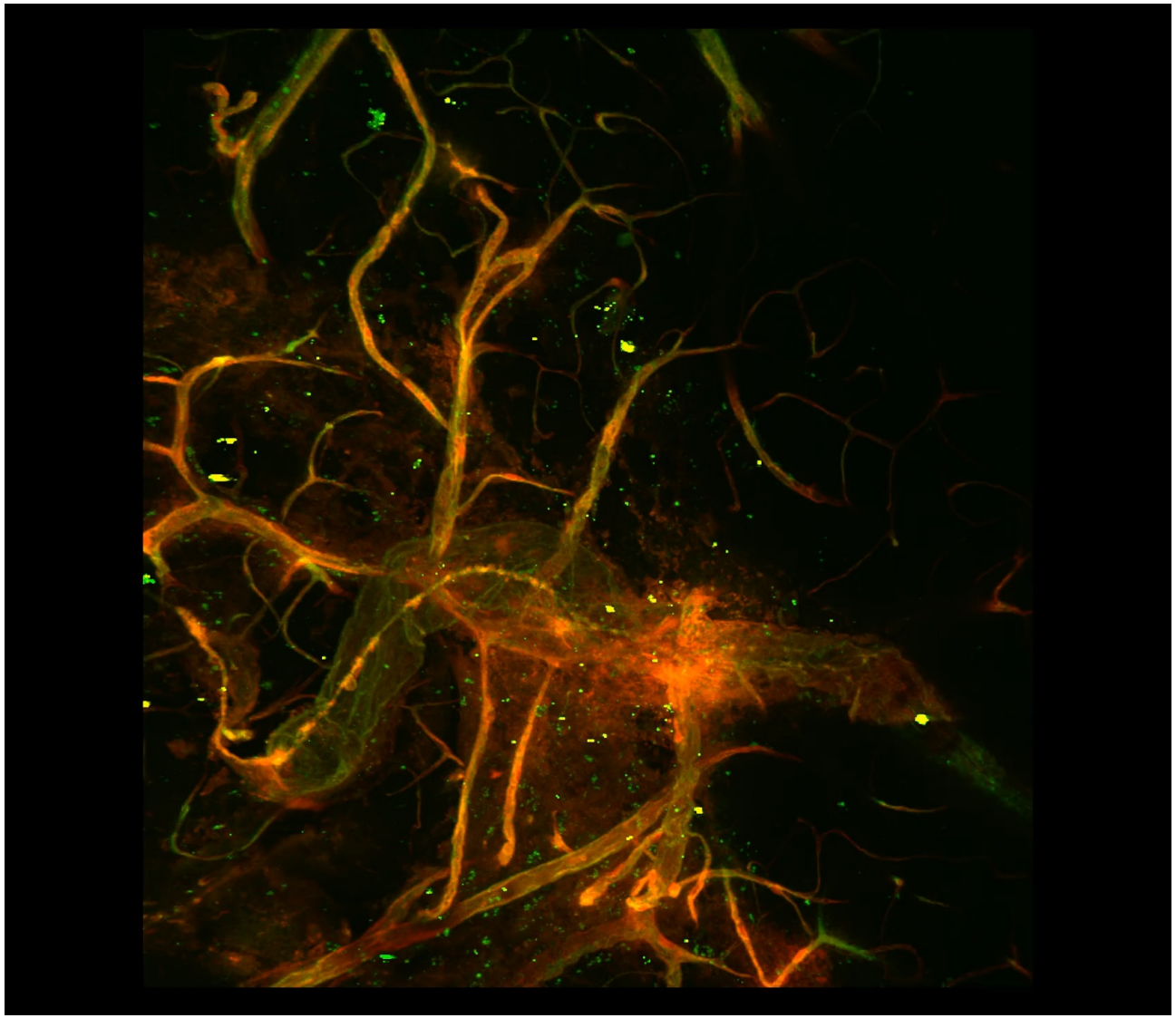


field of view (x,y) :
556 x 692 micrometers.
380 images
Resolution 0.5x0.5x1 micrometers

ADDRESSING THE SCIENTIFIC QUESTIONS

MULTI-PHOTON MICROSCOPY

MP imaging of murine brain vasculature obtained with dextrane conjugated rodamine perfusion



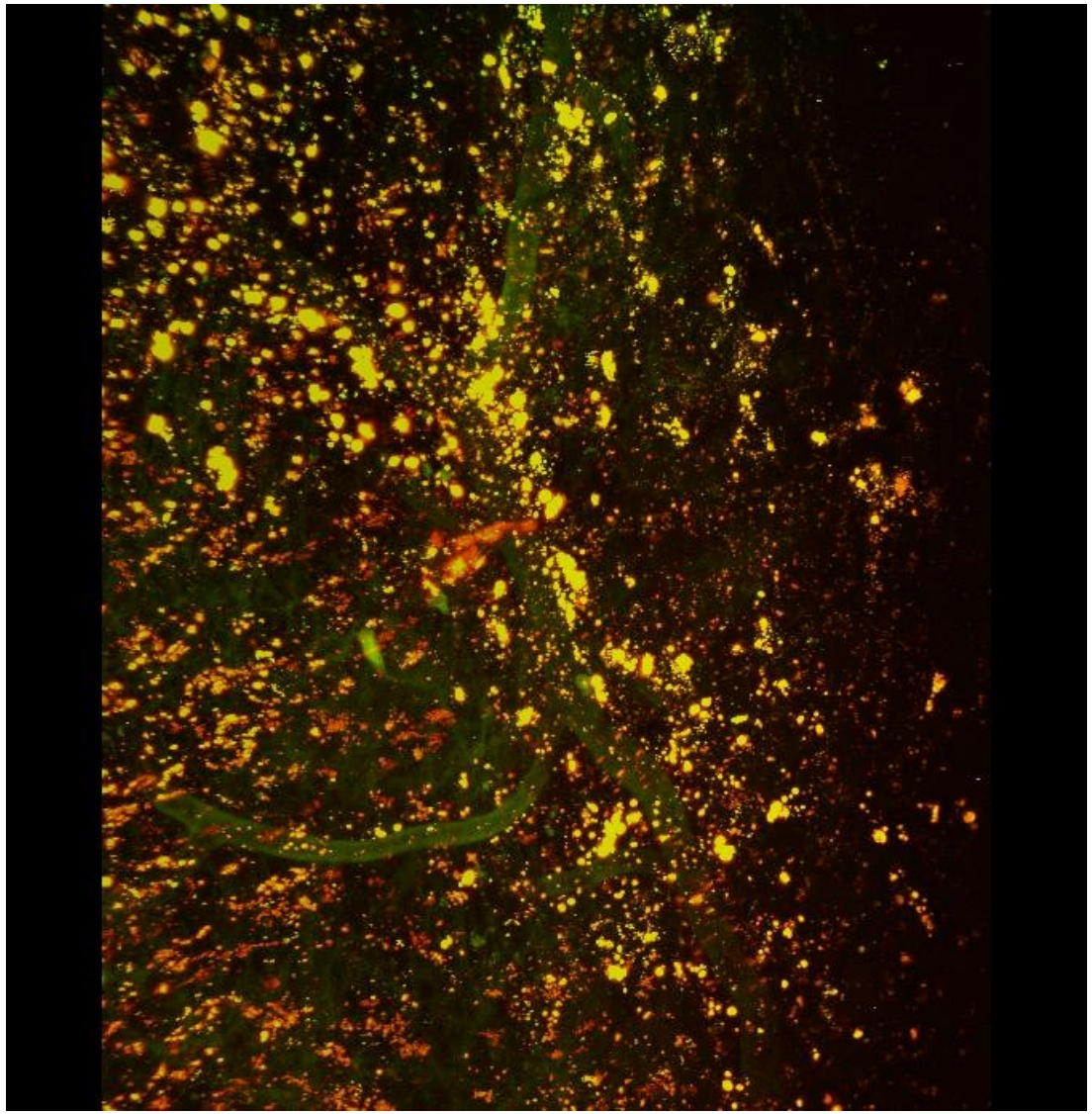
field of view (x,y) :
556 x 692 micrometers.
380 images
Resolution 0.5x0.5x1 micrometers

ADDRESSING THE SCIENTIFIC QUESTIONS

MULTI-PHOTON MICROSCOPY

MP imaging of human brain. Cortical region in the temporal lobe obtained through autofluorescent emission

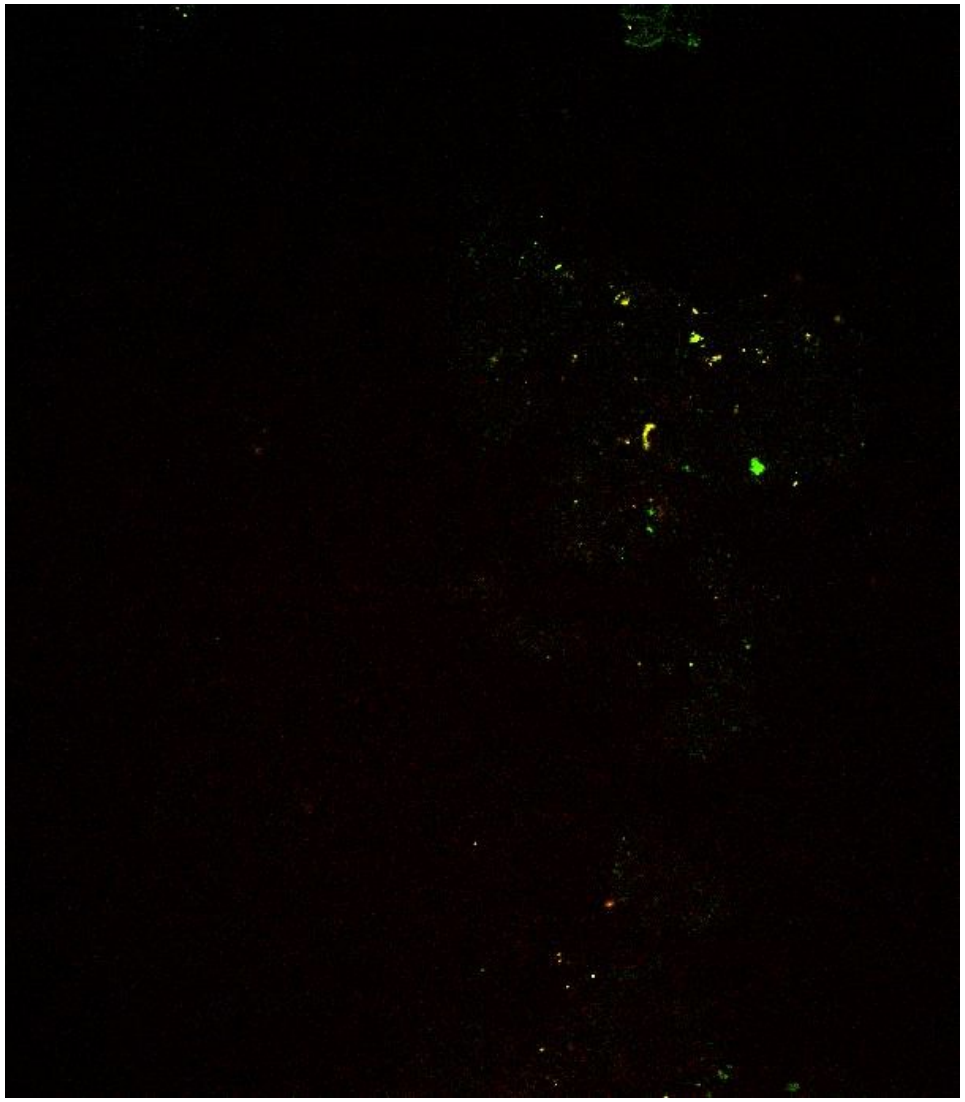
field of view (x,y,z):
556 x 692 x 380 pixels
Resolution 0.5x0.5x1 micrometers



ADDRESSING THE SCIENTIFIC QUESTIONS

MULTI-PHOTON MICROSCOPY

MP imaging of human brain. Cortical region in the temporal lobe obtained through autofluorescent emission

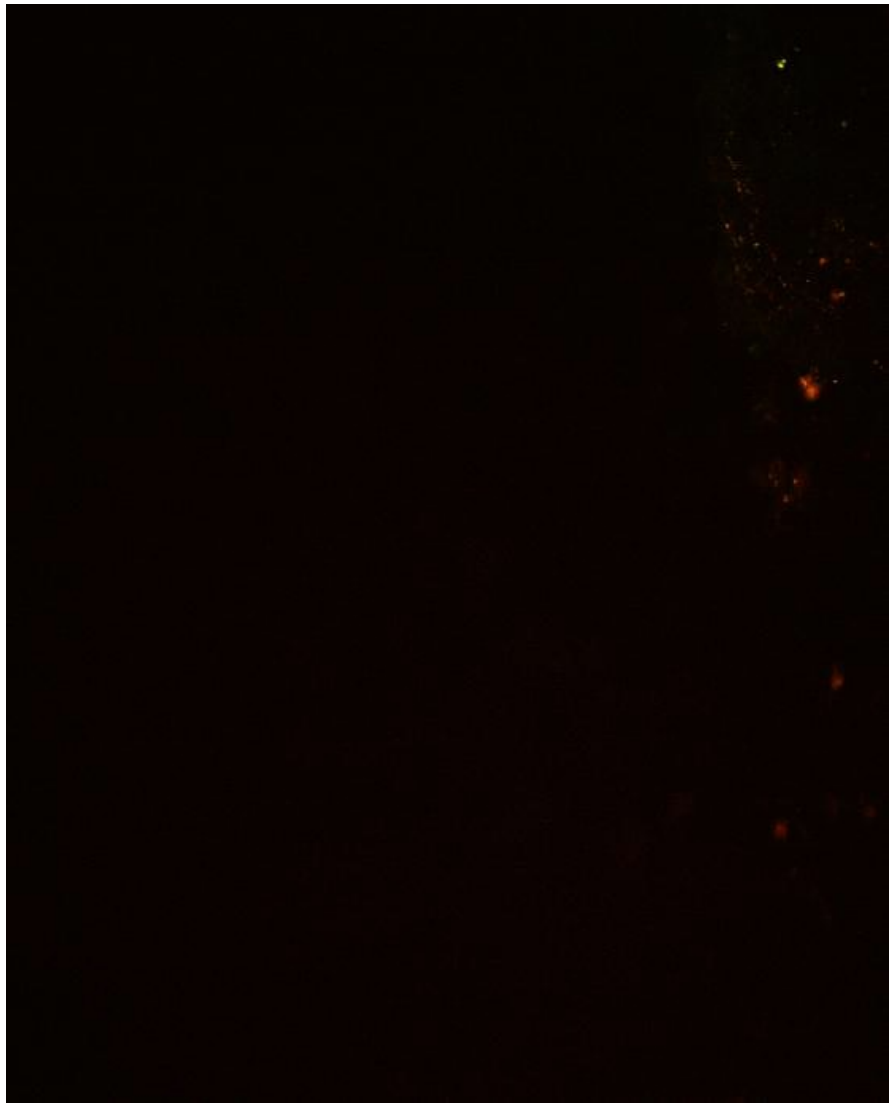


field of view (x,y,z):
556 x 692 x 380 pixels
Resolution 0.5x0.5x1 micrometers

ADDRESSING THE SCIENTIFIC QUESTIONS

MULTI-PHOTON MICROSCOPY

MP imaging of human brain. Cortical region in the temporal lobe obtained through autofluorescent emission



field of view (x,y,z):
556 x 692 x 380 pixels
Resolution 0.5x0.5x1 micrometers

EBRAINS SERVICES

- The project is running and due to the reorganization of WPs data are being acquired

The main EBRAINS Service that will be used are

- Data and knowledge service. Data collected with different imaging modalities from both mouse and human will be uploaded in the EBRAINS KG and shared with the Neuroscience community
- Live paper. A paper recently published by one of the group involved in the SMART-BRAIN project has been uploaded in the EBRAINS live paper service (Gandolfi et al 2022. Sci Rep)

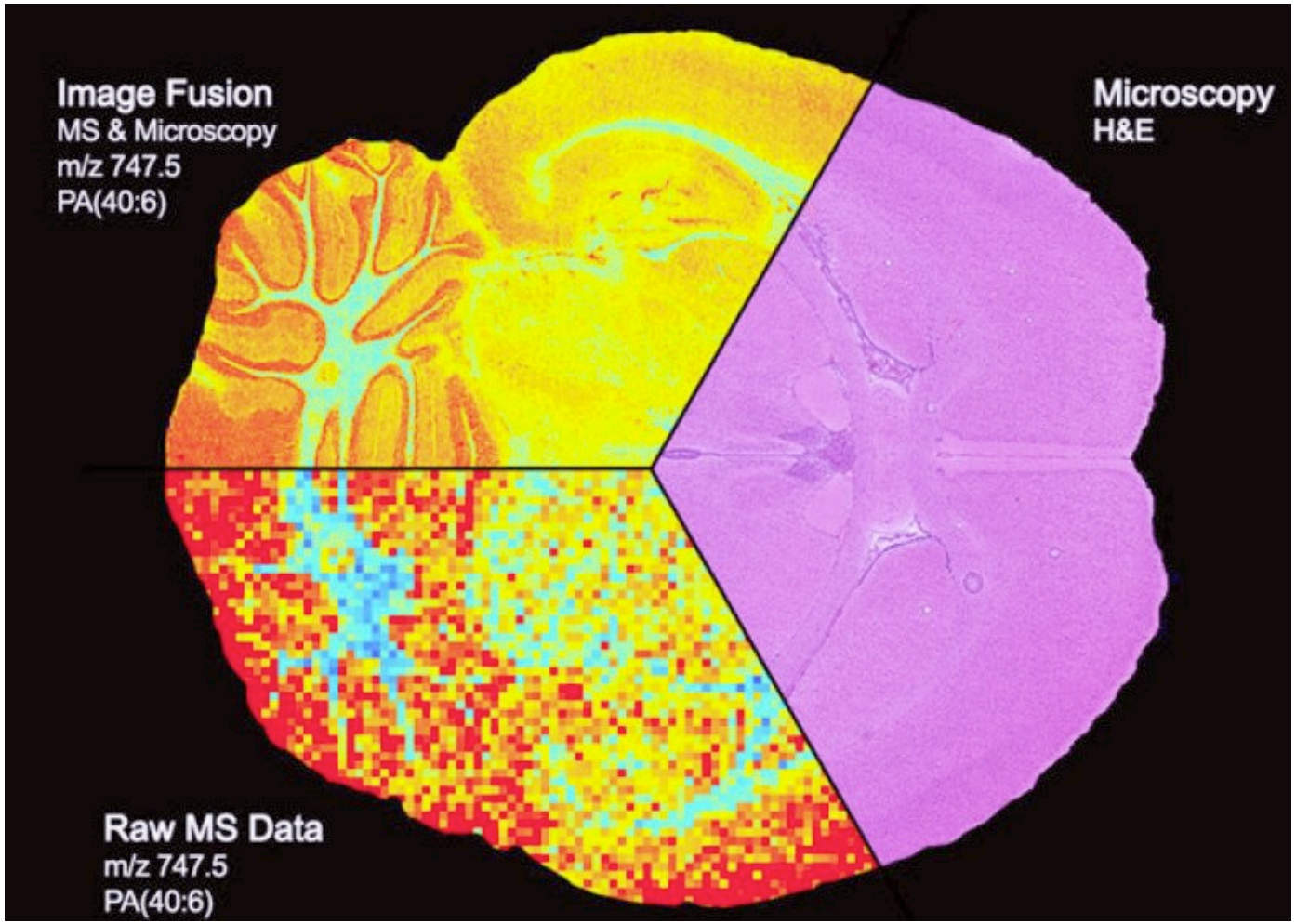
PROJECT OUTCOMES

- The SMART BRAIN outcomes that are now in process of being delivered are:
  - Multimodal database of microscopy images reporting morphology in human brain samples (datasets will be uploaded in the EBRAINS KG in the next weeks).
  - Fusion predicted datasets combining information from different techniques
  - Cross-modal fusion models binding different imaging modalities to be exploited by the EBRAINS and neuroscience community

CROSS MODAL FUSION MODEL

- Proof of Concept of image fusion algorithm.
- Out-of-sample prediction using data-driven image fusion.
- In Out-of-sample prediction a mathematical fusion model si used in an area where both modalities are measured, and then we take that model to another part of the tissue where only one of the two modalities has been measured, and use the model to predict the other modality there.

CROSS MODAL FUSION MODEL



Van de Plas et al. Nature Methods 2015

Image fusion of IMS and stained microscopy.
Fused image of ion image for m/z 747.5 (identified as lipid PA(40:6)) at 100 µm spatial resolution with a measured H&E microscopy image at 10 µm resolution (right), predicting the ion distribution of m/z 747.5 at 10 µm resolution.
Image fusion can be applied for:

1. **Spatial sharpening (prediction at higher resolution)**
2. **Noise removal**
3. **Out of sample prediction (pure prediction of a tissue for a non performed modality)**

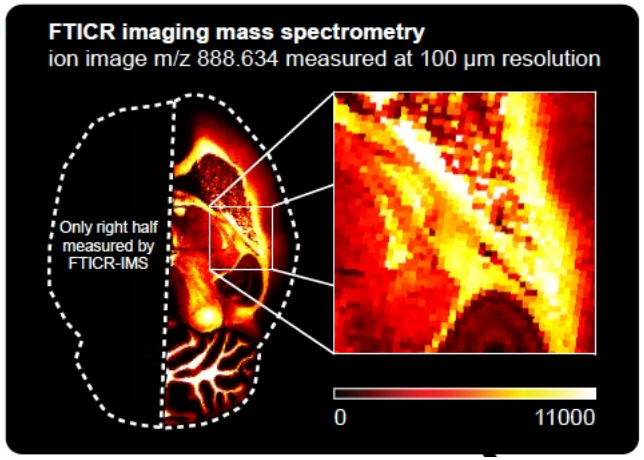
CROSS MODAL FUSION MODEL

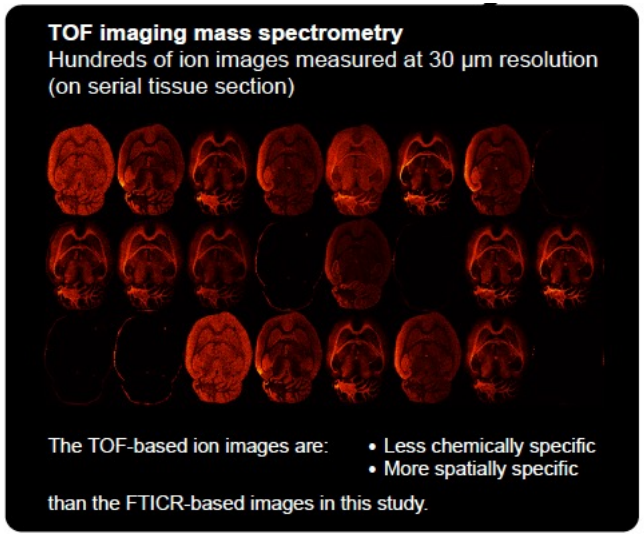
- Fusion example of FTICR and TOF MS imaging.

Both modalities are based on Matrix Assisted Laser desorption /ionization (MALDI).

- FTICR (Fourier Transform Ion Cyclotron Resonance) mass spectrometry imaging allows to detect mass/charge ratio of ions with a maximum spatial resolution of about 100 μm
- TOF (Time of Flight) mass spectrometry imaging is faster, has a larger field of view and a higher spatial (30 μm) and mass resolution (14000 *vs* 200000 of FTICR).
- In principle, the fusion algorithm developed for these fusion can be translated to optical fluorescence microscopy.

CROSS MODAL FUSION MODEL

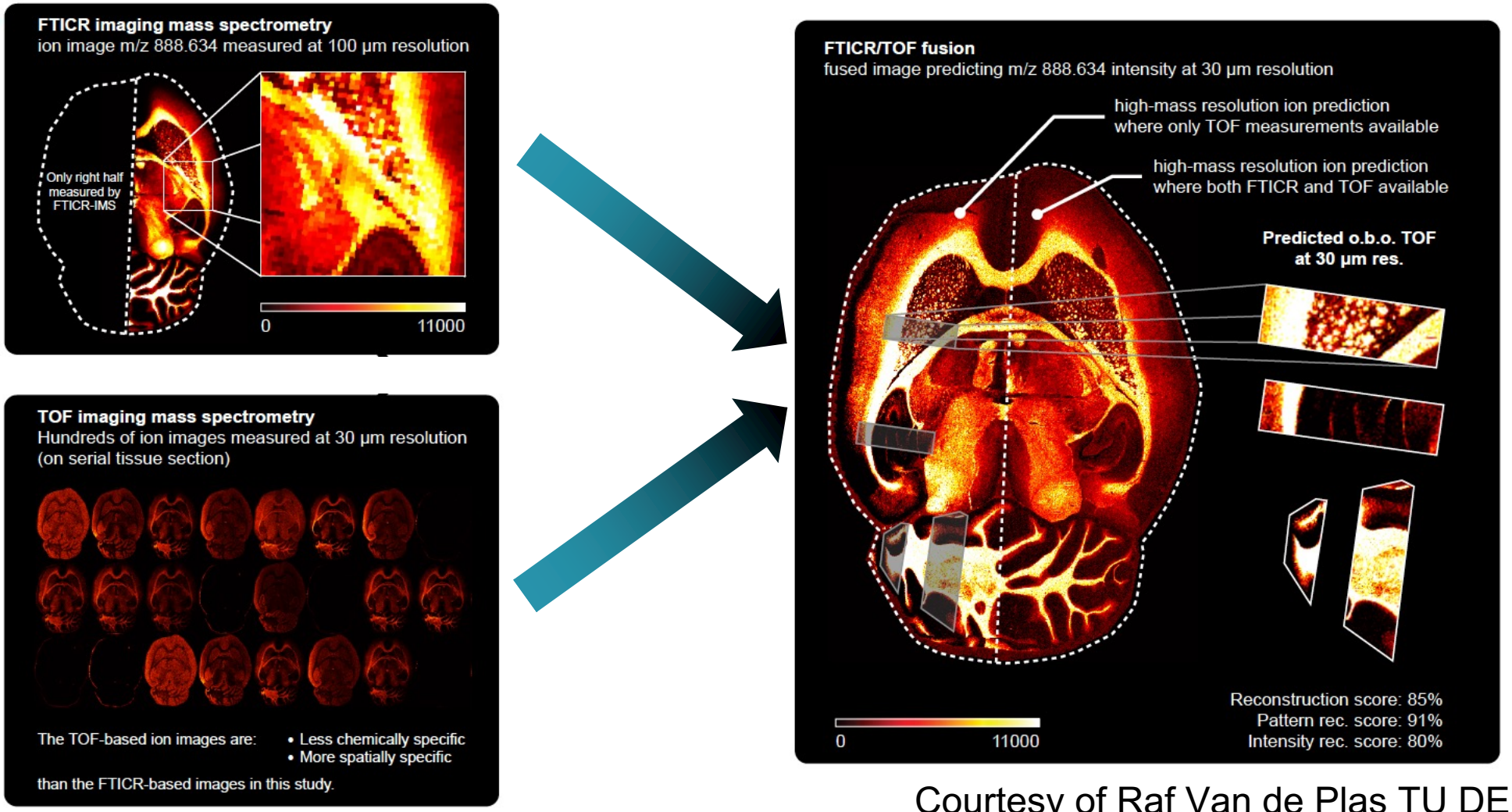




Top. MALDI FTICR imaging mass spectrometry measurements (large pixel sizes, but better chemical specificity). The shown image depicts the spatial distribution of a lipid with mass 888.634 Dalton
Bottom. MALDI TOF imaging mass spectrometry measurements (small pixel size, but lesser chemical specificity). Entire brain tissue section (on a neighboring section of the FTICR section used above)

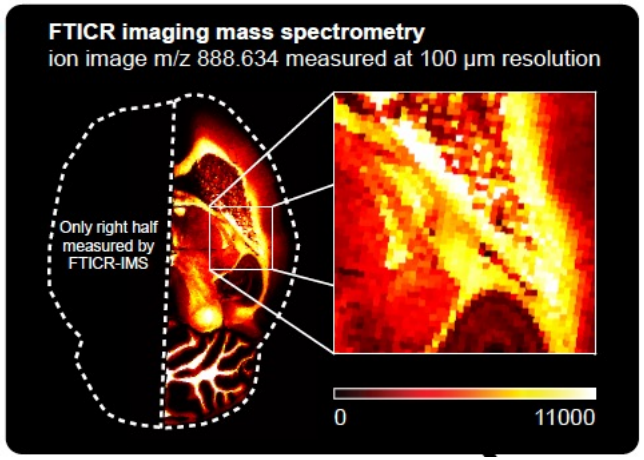
Courtesy of Raf Van de Plas TU DElft

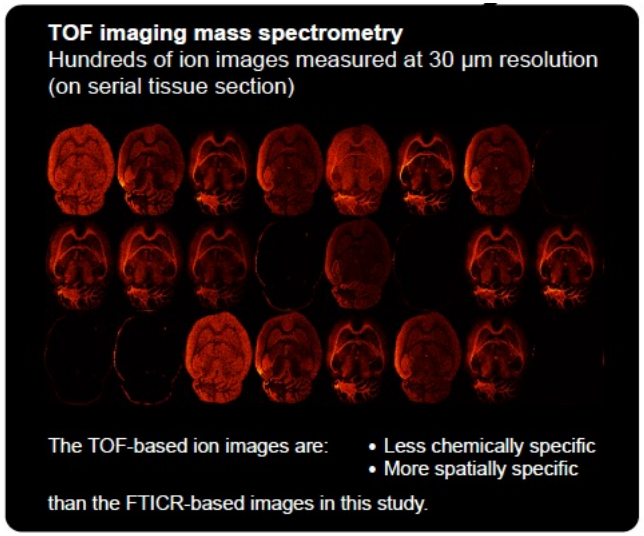
CROSS MODAL FUSION MODEL

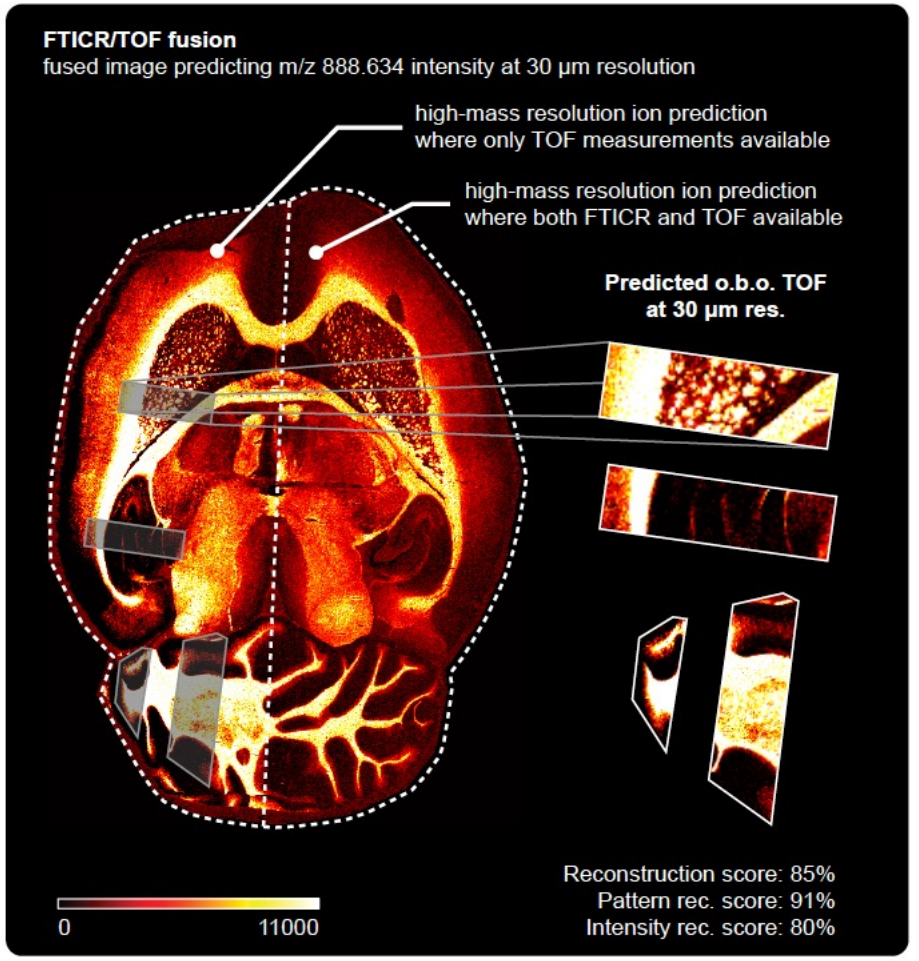


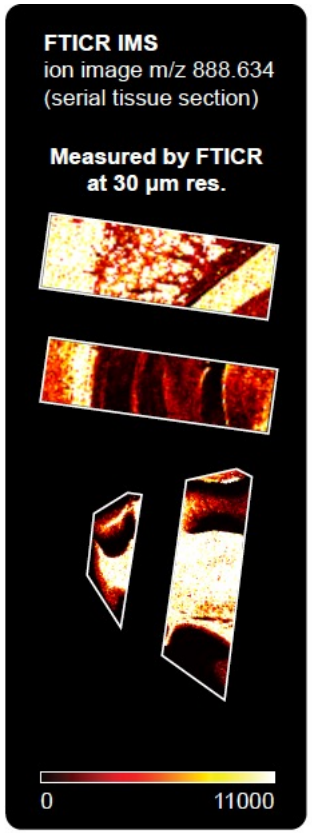
Courtesy of Raf Van de Plas TU DElft

CROSS MODAL FUSION MODEL



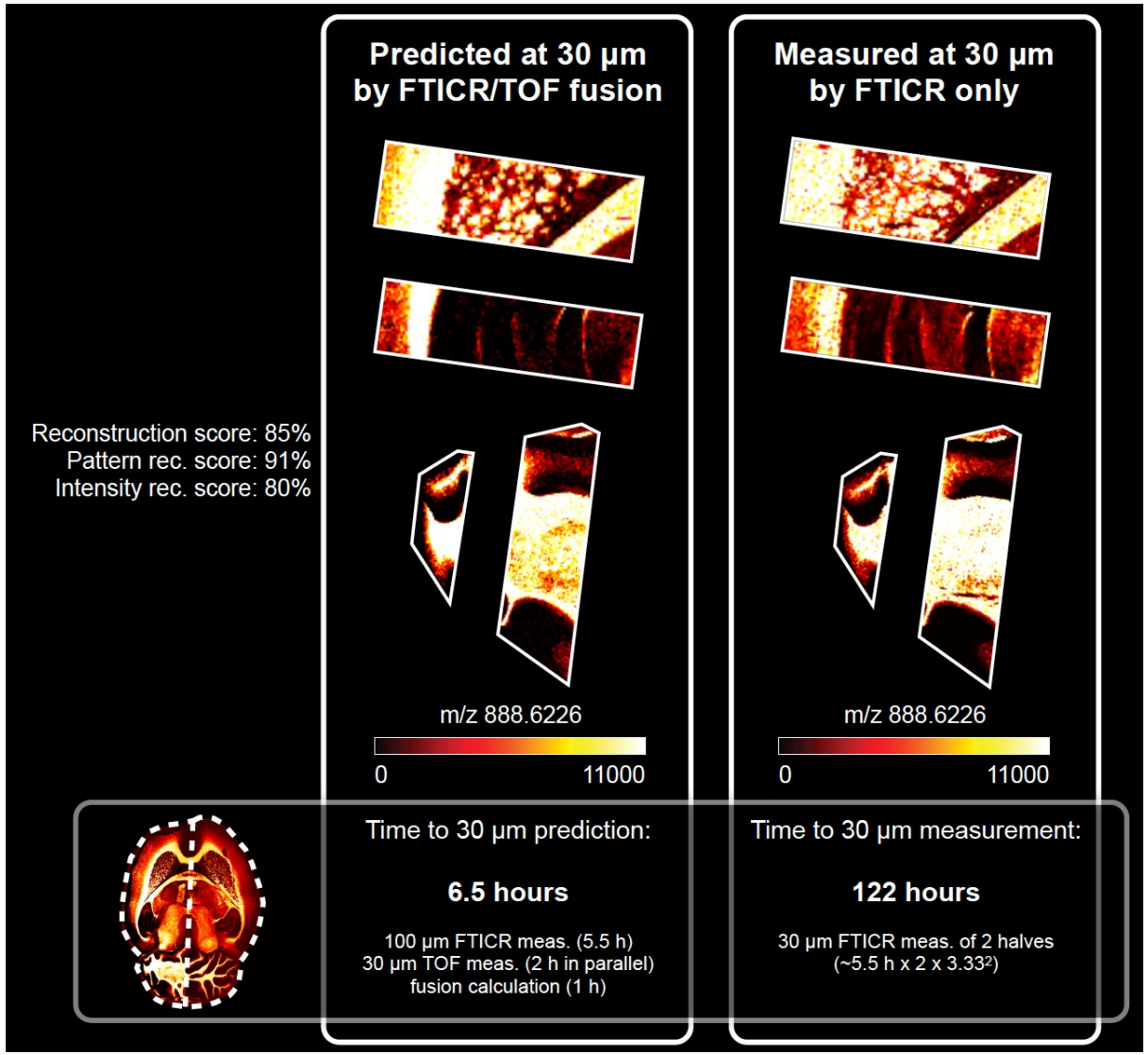






Courtesy of Raf Van de Plas TU DElft

PREDICTION IS BETTER THAN MEASURE?



- When measures are too expensive, not feasible with certain requirementes (pixel to large) simply take too much time.
- **The tissue area that was not measured by FTICR, if you would need to measure it at 30um pixel size, it would take 122 hours of instrument time. Prediction with uncertainty for that same tissue area, also at 30um, would take only 6.5 hours.**

The model

- R. Van de Plas, J. Yang, J. Spraggins, and R. M. Caprioli, Nature Methods, vol. 12, nr. 4, pp. 366-72, Apr 2015.
- L. Migas, L. Tideman, and R. Van de Plas (Delft University of Technology, Delft, Netherlands) J. Spraggins and R. M. Caprioli (Vanderbilt University, Nashville TN, U.S.A.)

CONCLUSION and PERSPECTIVES

- The project outcomes will be integrated into EBRAINS services as data sharing, live papers and share models

- We expect to exploit EBRAINS services to further accelerate data sharing

- Most importantly we aim at providing the integrated image fusion algorithm as an integrated EBRAINS service to be shared with EBRAINS community

People involved

Alessandra Franceschini

Miriam Cavagnini

Paolo Pozzi

Daniela Gandolfi

Giulia Boiani

Lukasz Migas

Leonore Tideman

Marc Guenot

Hayet Kouchi

Nathalie Streichenberger

Human Brain Project

EBRAINS

Thank you

www.humanbrainproject.eu

www.ebrains.eu

Co-funded by
the European Union

CONCLUSIONS and PERSPECTIVES

Template

- 1) What was the scientific question?
- 2) How did you/project partners address it?
  - Did you use EBRAINS tools/services ? If so, which ones? Any particular challenges they experienced?
- 3) What are the main outcomes of the project?
  - How did you share scientific data amongst partners ?
- 4) What are the next steps of the project?
  - E.g How EBRAINS could help you in your scientific endeavours?

Reminder : 25 min presentations; 5 min Q&A