A software pipeline for image processing and cell segmentation in biomedical microscopy

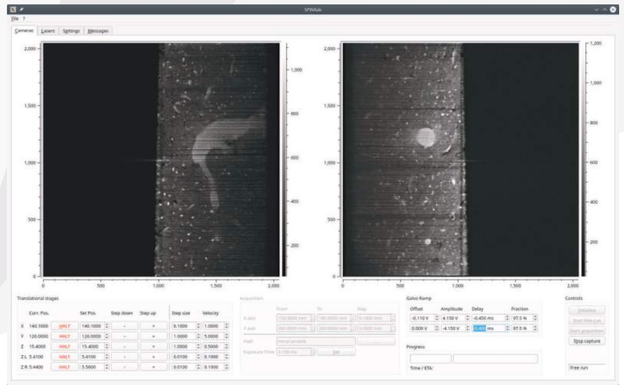
Giacomo Mazzamuto[1,2], Irene Costantini[1,2], La Ode H. Z. Toresano[3], Vladislav Gavryusev[1], Matteo Roffilli[4], Paolo Frasconi[3], Ludovico Silvestri[1] and Francesco S. Pavone[1,2]

[1] European Laboratory for Non-Linear Spectroscopy (LENS), University of Florence, Sesto Fiorentino (FI), Italy
[2] National Institute of optics (INO-CNR), Sesto Fiorentino (FI), Italy
[3] Department of Information Engineering (DINFO), University of Florence, Italy
[4] Bioretics srl, Corte Zavattini, 21 - 47522 Cesena (FC), Italy

In the field of high-resolution biomedical imaging, several challenges arise for what concerns data handling and image processing. In particular, imaging whole organs (e.g. a whole mouse brain) with Light Sheet Fluorescence Microscopy (LSFM) at sub-micron resolution easily results in three-dimensional datasets of several TB in terms of storage. Here we present the software tools that we have developed and deployed in order to tackle the aforementioned challenges, from high speed data acquisitioin in the laboratory, to volumetric stitching of large datasets and feature extraction using neural networks.

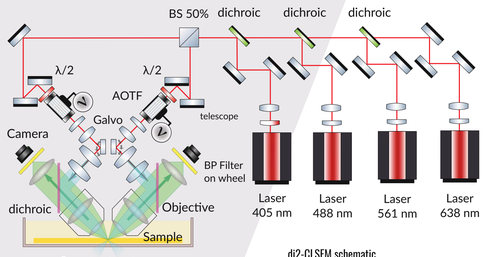
SPIMlab: data acquisition and control software

- Developed in C++ using Qt, powered by openSuse
- 7000 Single Lines of Code (SLOC)
- Multi-threaded
- Flexible and modular architecture



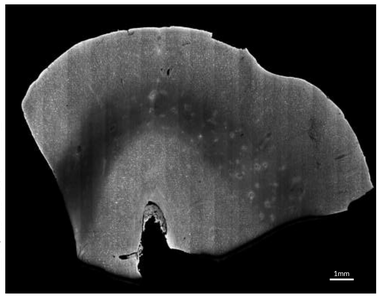
di2-CLSFM

- Dual-view, inverted Confocal Light Sheet Microscope
- Data rate: 800 MiB/s with two cameras
- Volumetric rate: 0.1 mm^3/s
- Isotropic 1 µm resolution
- Designed to image large samples, up to 1 mm in thickness



Sample chamber

di2-CLSFM schematic
Objective specs: WD=9mm; MAG=12x; NA=0.53;
BFP det=17.8mm; BFP exc=1.4mm; f=167mm; length=105mm



1-mm-thick slice of human brain cortex stained for NeuN with Alexa Fluor™ 488, imaged with di2-CLSFM. Gray and white matter can be distinguished. Stripes have been stitched using ZetaStitcher and resampled to compensate tilt.

Automatic neuron segmentation in the human brain cortex

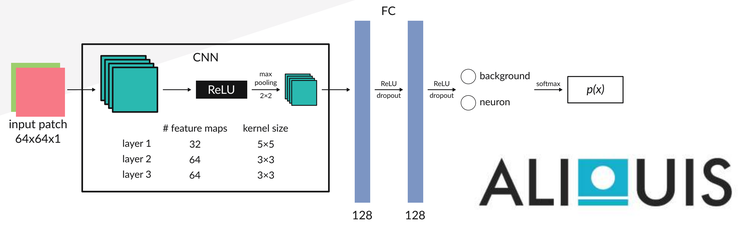
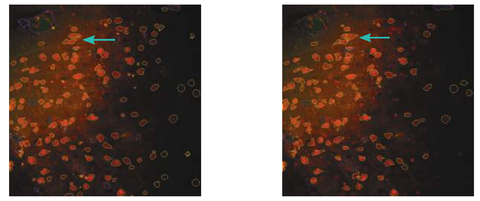
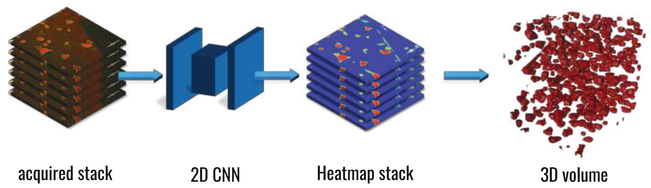


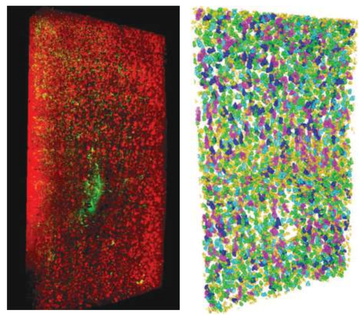
Diagram of the CNN used for pixel classification. A standard 3-layered Convolutional Neural Network classifier is adopted in order to independently classify each single pixel of each input image. The ALIQUIS™ framework was used to implement the CNN.



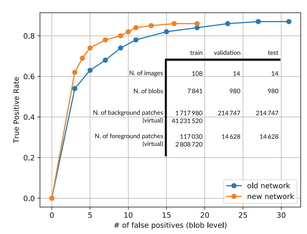
Left: result of neuron segmentation using the old neural network (only red channel).
Right: result of neuron segmentation using the new improved version of the neural network (red and green channels). The arrow highlights a region of clustered neurons that are correctly segmented with the new version of the CNN.

A 2.5D approach to volume reconstruction. Individual frames are fed to the 2D CNN to create a stack of class membership probability images. Using isosurface search algorithms (Marching Cubes), a 3D representation can be reconstructed.





Left: slice of human brain cortex at the TPFM.
Right: morphological classification of neurons.



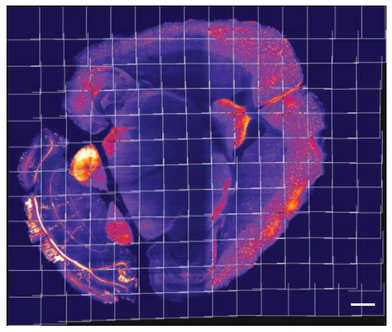
Comparison of FROC curves between old and new version of the neural network.

Stitching TeraVoxel-sized microscopy images

- **ZetaStitcher** is a software tool designed to stitch large volumetric images such as those produced by Light-Sheet Fluorescence Microscopes
- 100% Python
- Able to handle datasets as big as 10^{12} voxels and multichannel images
- Pairwise alignment is determined by evaluating the cross correlation (by means of FFT) between adjacent stacks
- **High throughput**: alignment is computed at selected stack depths only, then applied to the entire stack. Example: total time to stitch the dataset shown on the right, sampling at 15 different depths: 60 minutes.
- Global optimization of the final stack coordinates
- **Powerful** and simple Python **API** to query arbitrary regions within the fused volume.

```
>>> from zetastitcher import VirtualFusedVolume
>>> vfv = VirtualFusedVolume('stitch.yml')
>>> vfv.shape
(2985, 18924, 23486)

a = vfv[2000:2500, 12000:13000, 15500:16500]
```



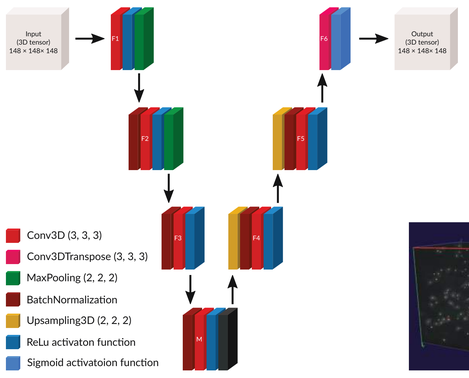
Tomography of a whole mouse brain acquired with a Light-Sheet Microscope. Stacks boundaries are highlighted. Scale bar: 1mm.
Mosaic size: 15 × 12 stacks (2950 × 2048 × 2048 each) ≈ 4 TiB
Fused volume: 2985 × 18924 × 23486 px (Z × Y × X) ≈ 1.3 10^{12} voxels.

python™ ZetaStitcher

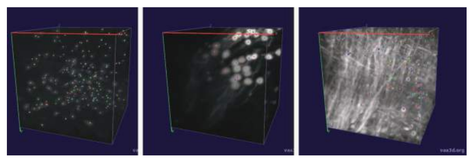
https://github.com/lens-biophotonics/ZetaStitcher

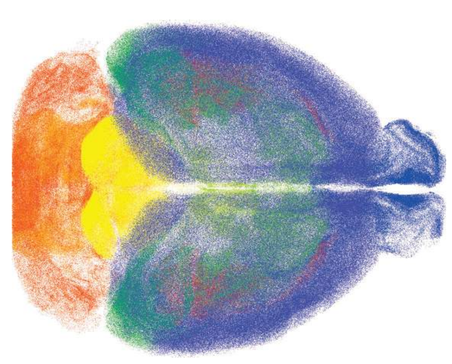
Fork me on GitHub

Semantic deconvolution for neuron localization in the whole mouse brain



Left: Diagram of the 3D Fully Convolutional Neural Network used for semantic deconvolution. After image enhancement, centroids are determined using a mean-shift algorithm.
Bottom: Challenges of localizing neurons in different background scenarios.
Right: Point cloud of 1.5 10^6 neurons automatically localized in the whole mouse brain.





Acknowledgements
EU H2020 research and innovation programme: grant agreements n. 785907 (HBP-SGA2) and 654148 (Laserlab-Europe); EU H2020 EXCELLENT SCIENCE programme - European Research Council (ERC), grant agreement n. 692943 (BrainBIT); General Hospital Corporation Center of the National Institutes of Health award number 1U01MH117023-01; Italian Ministry of Health (RF-2013- 02355240) and Eurobioimaging Italian Nodes (ESFRI research infrastructure); Ente Cassa di Risparmio di Firenze (private foundation).

Contact Information:
Giacomo Mazzamuto
INO - CNR & LENS
National Institute of Optics,
European Laboratory
for Non-Linear Spectroscopy

mazzamuto@lens.unifi.it
www.lens.unifi.it/bio

bioretics BEYOND EXPERIENCE

Human Brain Project

Co-funded by the European Union