Image Analysis @ MRI

MRI’s current image analysis service

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Scope

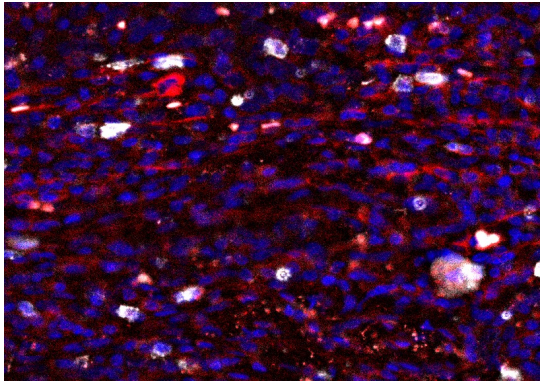
- Support
  - Level 0
    - Planning – What is the best acquisition mode for my project
  - Level 1
    - Point user to the most adequate existing solution / workflow and coach him
  - Level 2
    - Automatize the analysis workflow or parts of it (script, macro)
  - Level 3
    - Develop a specific method / tool for one analysis
  - Level 4
    - Long term cooperation in a project (thesis, R&D, ...)

hours

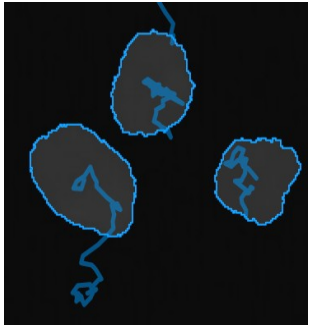
days

weeks

months



Is it possible to count double stained cells (red, gray) in these images (Leica Thunder) ?



I want to track cells in 3D images.

Means

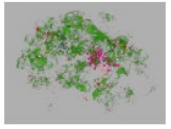
- 11 engineers part time
- 1 engineer full time
- Analysis PCs with proprietary software
  - Imaris
  - Avizo
  - Huygens
  - (Definiens)
  - (Matlab)
- Image db server OMERO

- Open source software
  - FIJI/ImageJ
  - Cellprofiler
  - Ilastik
  - Icy
  - Python
    - Jupyter
    - Cellpose
  - QuPath
- 15 analysis pcs
  - Adapted for 3D and deep learning
  - 2x Intel(R) Xeon(R) E5-2660 v4 CPU @ 2.00 GHz [14 core(s) x64]
  - 128/256 GB RAM, NVIDEA GeForce

Results

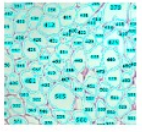
- per year (ex. 2019):
  - 40 demands
  - 25 tools
    - available on github
    - 53 citations
      - Most popular:
        - Scratch assay
        - Intensity ratio nuclei/cytoplasm
        - Adipocytes tool
        - Fibrosis tool
        - Cell invasion in 3D matrix

1. 3D_Nuclei_Clustering_Tool



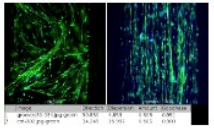
Analyze the clustering behavior of nuclei in 3D images. The centers of the nuclei are detected. The nuclei are filtered by the presence of a signal in a different channel. The clustering is done with the density based algorithm DBSCAN. The nearest neighbor distances between all nuclei and those outside and inside of the clusters are calculated.

2. Adipocytes Tools



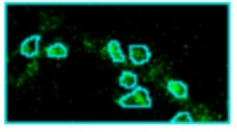
The Adipocytes Tools help to analyze fat cells in images from histological section.

3. MRI_Analyze_Alignment_of_Muscles_Tool



The tool uses the Directionality plugin to measure the main direction of the structures in the image and the dispersion. It is used in this context to analyze to which degree the muscles in the image are vertically aligned. The tool allows to run the Directionality plugin in batch-mode on a series of images. The direction-histograms and the measurements are exported as csv-files.

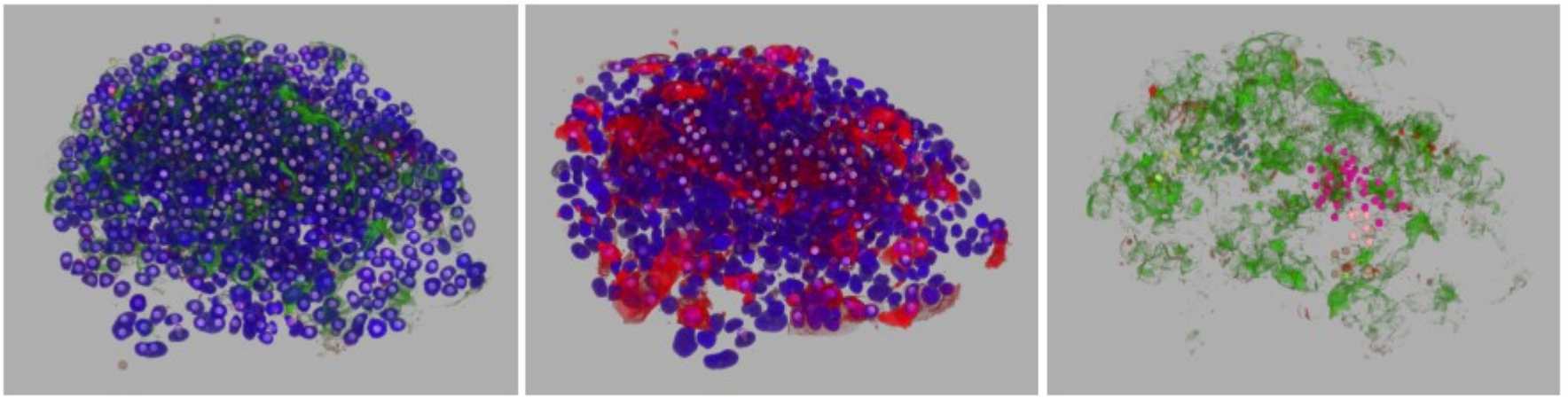
4. Analyze_Calcium_Signals_In_Spines



Analyze calcium signals in dendritic spines. The images consist of time-series of calcium signals. Each image contains a selection that marks the point of stimulation. The tool finds the region to analyze close to the point of stimulation. It measures the intensity of the calcium signal in the whole region of interest and in the segmented spots.

https://github.com/MontpellierRessourcesImagerie/imagej_macros_and_scripts

Results – Examples 1

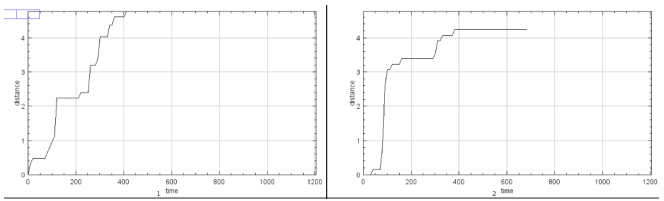


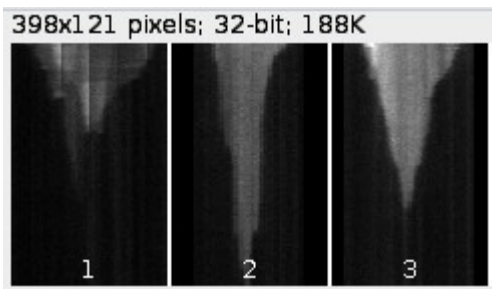
total	nr. of nuclei ab	nr. of clusters	clustered	unclustered	mean nn-distance all	stddev. all	mean nn-distance	stddev. unclustered	mean nn-distance	stddev. clustered
656	249	6	74	175	14.191632201	5.116154692	15.658863498	5.860337659	7.487100518	3.558586574

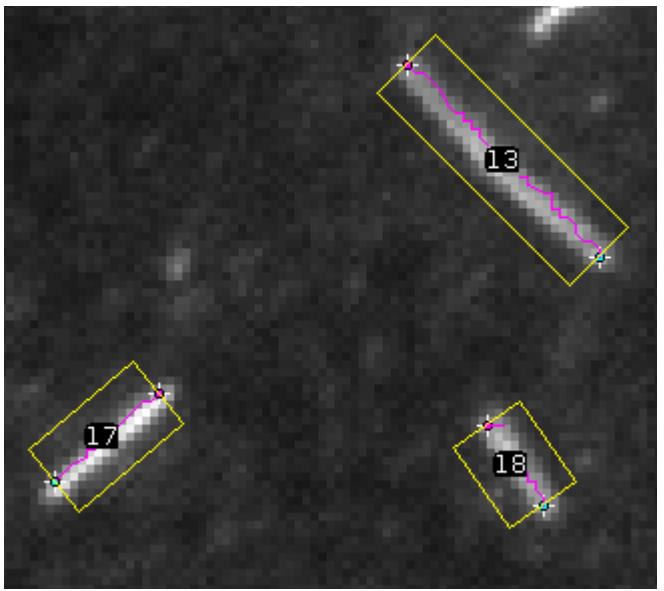
Analyze the clustering behavior of nuclei in 3D images. The centers of the nuclei are detected. The nuclei are filtered by the presence of a signal in a different channel. The clustering is done with the density based algorithm DBSCAN. The nearest neighbor distances between all nuclei and those outside and inside of the clusters are calculated.

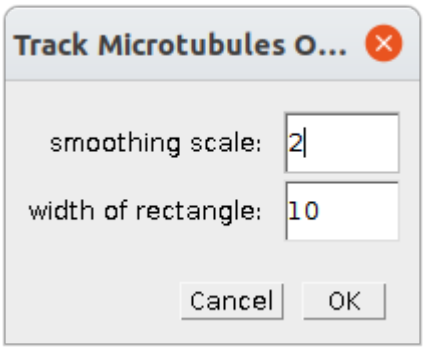
group Francois Fagotto

Results – Examples 2





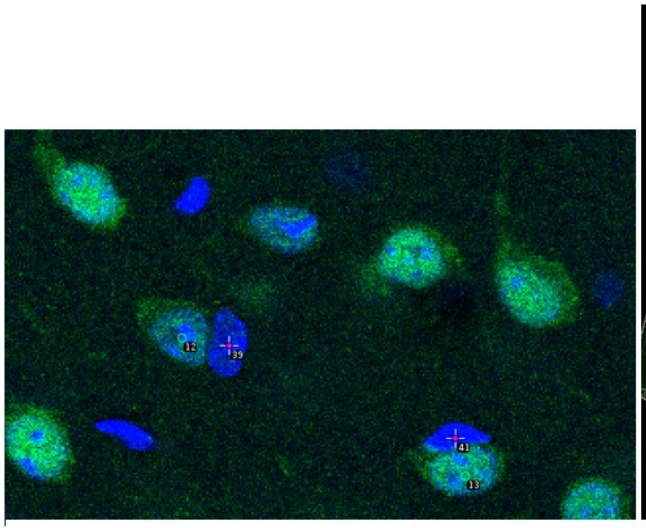


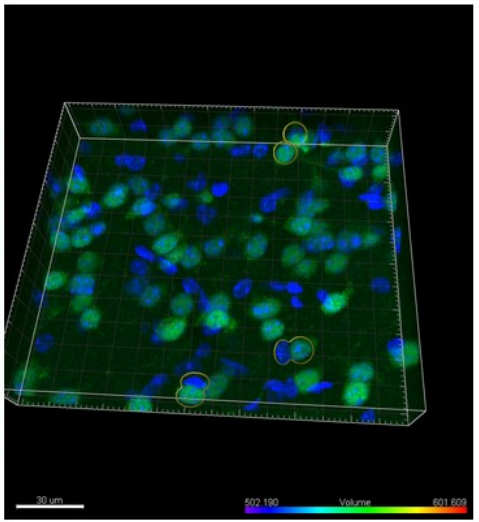


The tool allows to track the ends of fluorescently labelled microtubules, which are becoming shorter and to measure the speed of the movement of each end. It also creates kymograms and plots distance-per-time.

group Nathalie Morin

Results – Example 3





nr.	image	region	neurons	satellites
1	TNE P60 Medial A Cortex Frontal 50x0.67 20x NeuN Dapi-1.czi	x=1232, y=5872, w=512, h=448	34	3

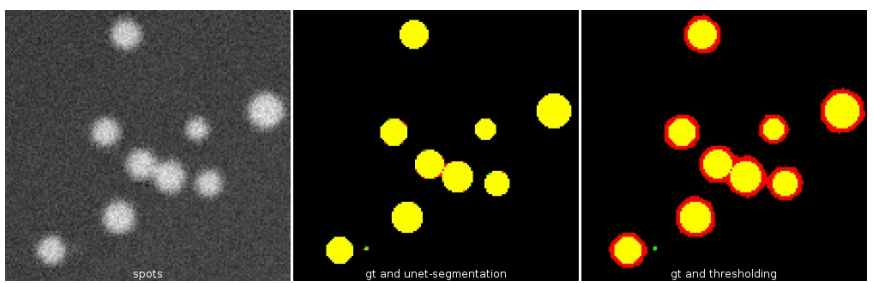
nr.	neuron	nx	ny	nz	satellite	sx	sy	sz	color
1	12	38.224	98.148	10.750	39	44.809	99.237	10.461	1
2	13	78.045	117.635	10.801	41	76.717	112.425	10.977	2
3	14	45.906	7.450	12.872	54	42.257	7.891	26.081	3

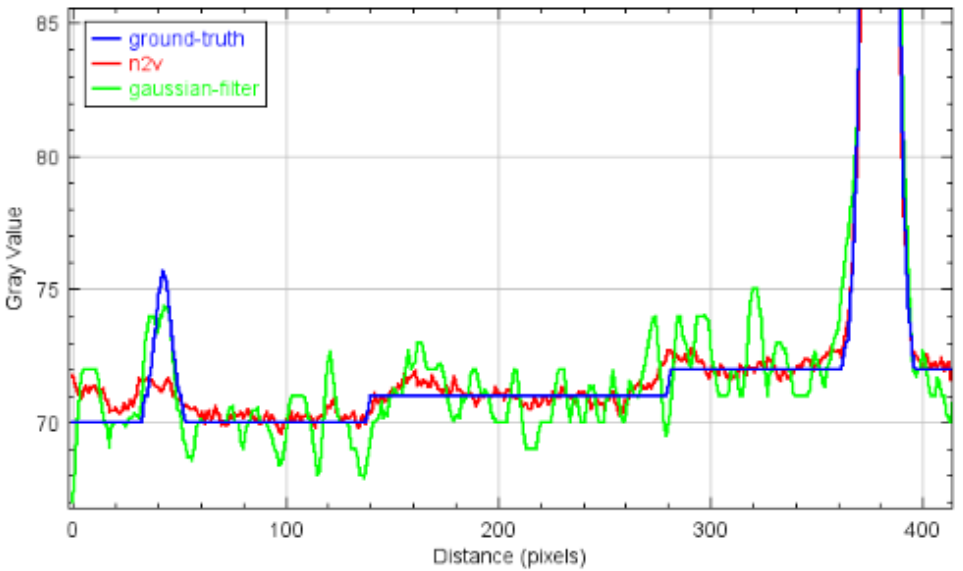
The tool detects and counts the neurons and the neurons with satellite cells. The first channel is supposed to contain a staining of the neurons and the second channel a staining of all nuclei (neurons and satellite cells).

group Karine Loulier, INM

Results – DL4Mic

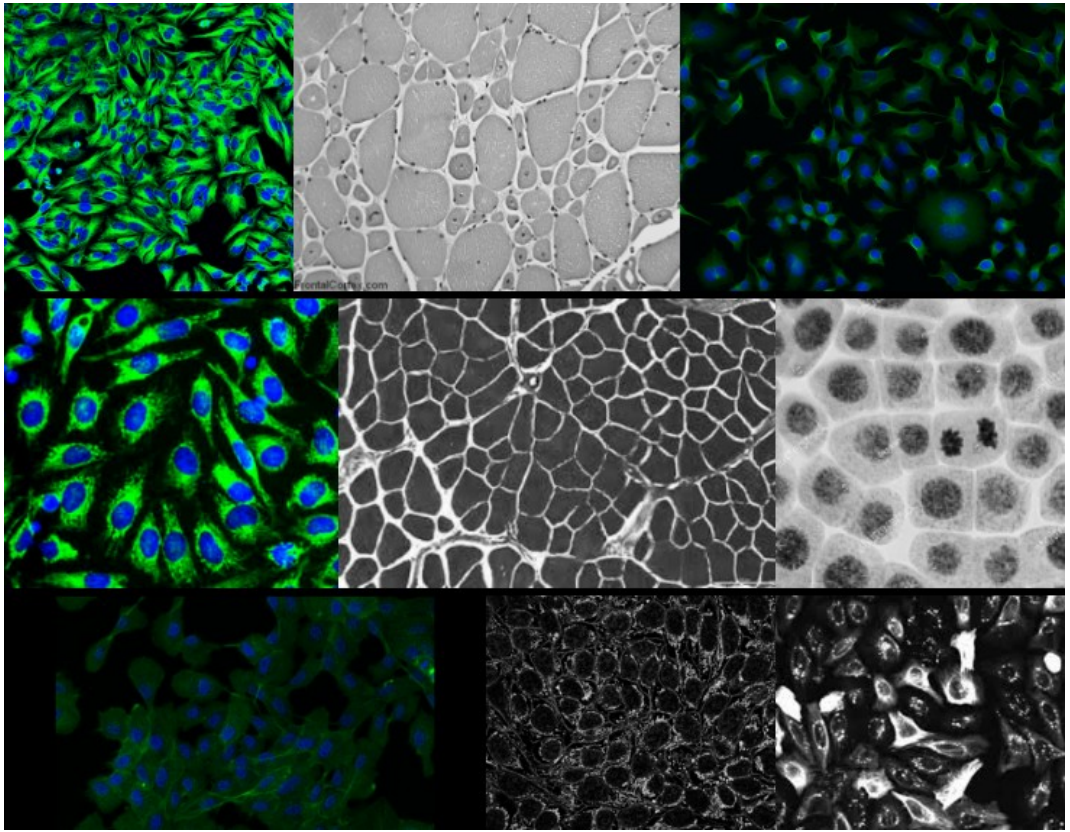
- A framework to make DL-methods available
  - including training on the users image
    - Two networks availabl
      - Noise2Void
      - U-Net segmentation
      - more to come...





Results - Cellpose

- DL network (U-Net), trained to segment nuclei and cells
- Trained with different imaging modalities and cell types
- Installed on analysis pc(s)
- Added batch-mode to cellpose (a new button in the gui) + batch conversion to ij-rois of the results

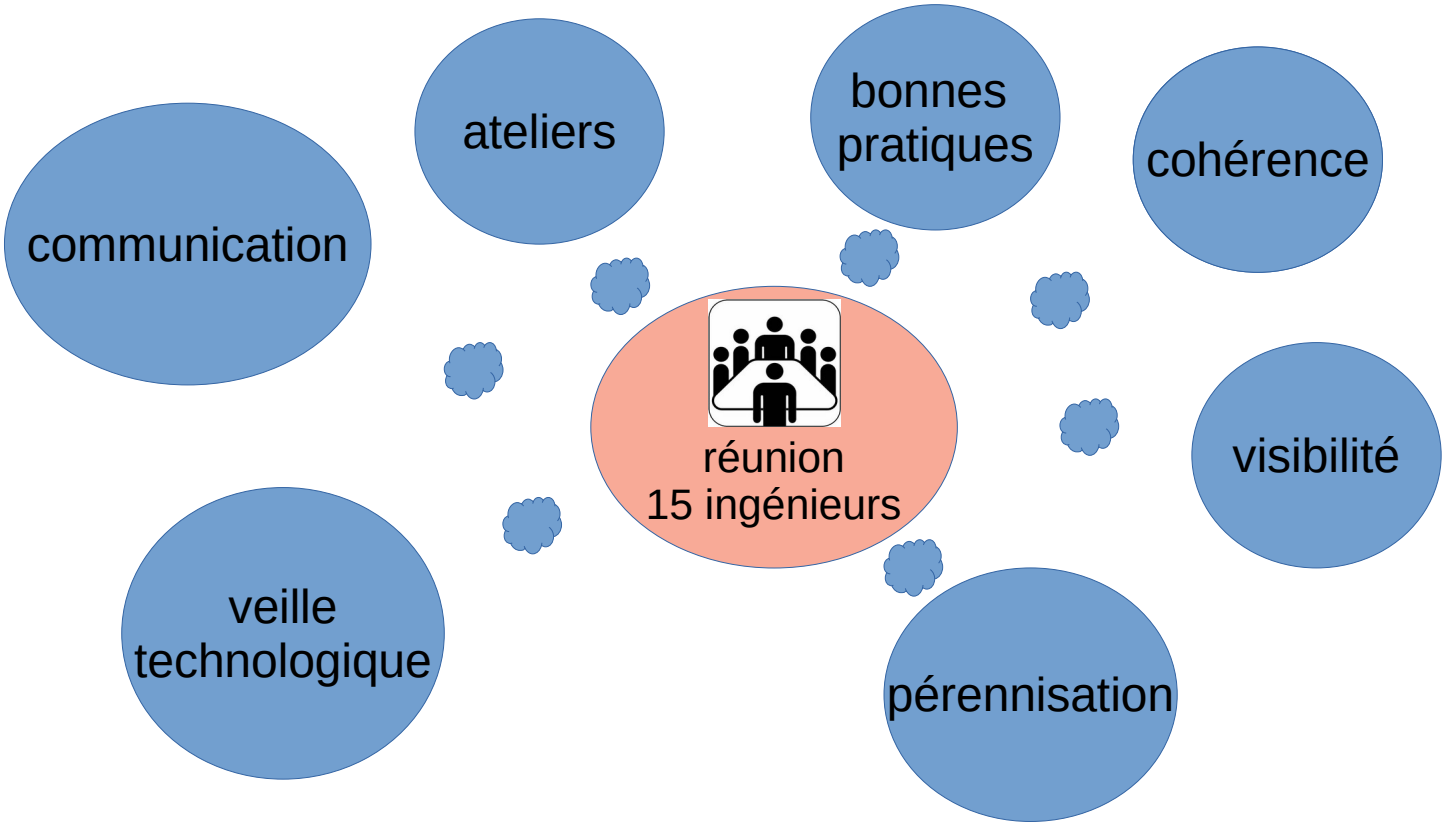


Stringer, C., Wang, T., Michaelos, M. et al. Cellpose: a generalist algorithm for cellular segmentation. Nat Methods 18, 100–106 (2021).

Communication - Workshops

- Atelier Biocampus
  - Image Analysis with ImageJ
  - Macro Programming
  - Machine Learning for Bio-Image Analysis
  - To come in autumn 2021: 3D Image Analysis

Communication - Image Analysis Workgroup



Summary and Conclusions

- image analysis well organized inside MRI
- can handle a number of small/medium/long term demands
- mainly service, on demand development
- little time for R&D

- how to bring image analysis at MRI to the next level ?
  - do we miss needs because users do not now the possibilities ?
  - connect with R&D teams and image analysts outside MRI ?!
  - create a structure to define the IA strategy with main users and other image analysts in Montpellier ?
  - work more on project/grant basis ?
  - are more (human) resources needed ?

Francois Fagotto

- We do lot of image analysis with
  - ImageJ, CellProfiler and Imaris
- Imaris for 3D segmentation / measurements
  - Limited - not open
  - More versatile 3D software needed

- large variety of types of analysis.
- not one test/model to be used over and over
- rather assays with different approaches for analysis
- => time to invest in the optimization / development of automated image analysis is limited
- classical ways to do automated measurements often don't work
- too much variability
- => manual quantification
- Started with WEKA segmentation (machine learning)