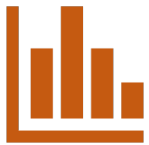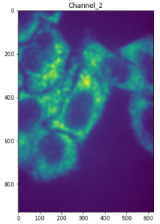# Python module
to process FISH (Fluorescence In Situ Hybridization) images

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Motivation

- Extracting quantitative data from FISH images.

   – Multi-step process. (Segmentation, spot detection, quantification).
       – Requires a skilled user.
       – Labor intensive.
       – Multiple steps require user input (to select thresholds).
       – User-to-user variability.

   – Large and complex datasets that are:
       – Difficult to transfer data between computers (local/remote).
       – Difficult to interpret.
       – Difficult to apply (new/retrospective) changes.

Code architecture
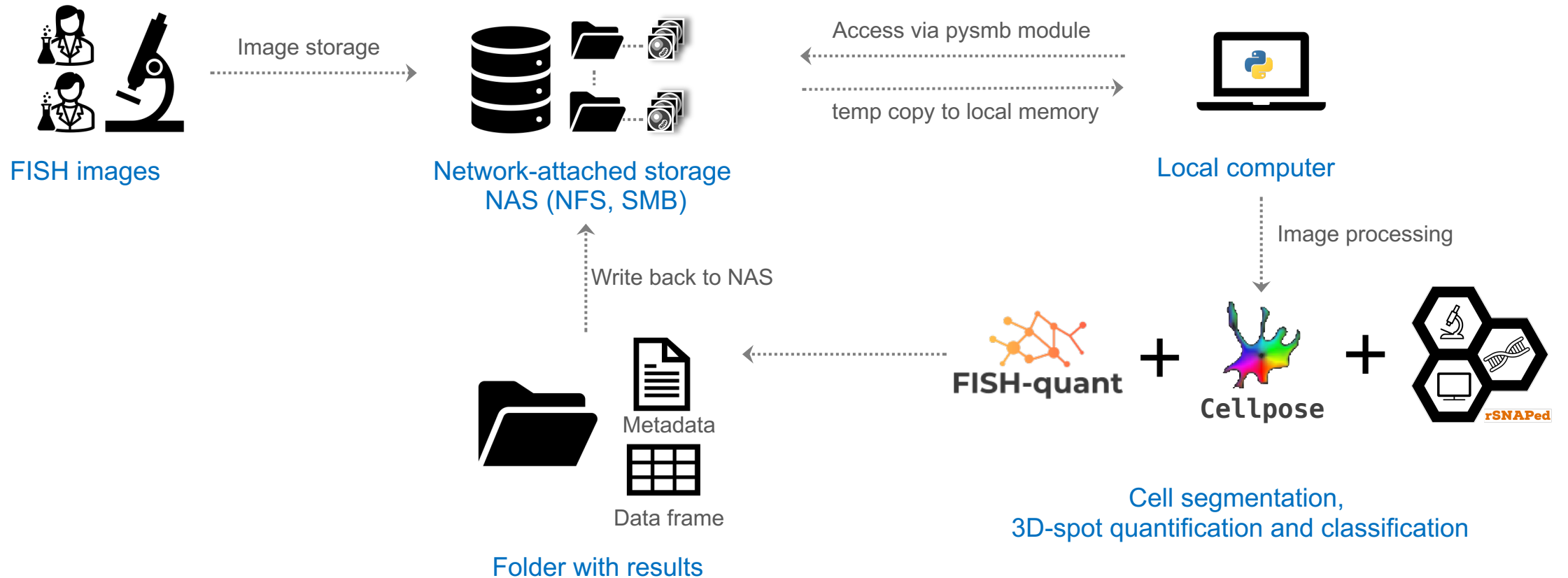
Easy to use

- Experimental parameters

```
# Path to folder with images
  data_dir                        # path to a folder with images.
# Cell Segmentation
  channels_with_cytosol = [1,2]   # list or int indicating the channels where the cytosol is detectable
  channels_with_nucleus = 0       # list or int indicating the channels where the nucleus is detectable
  channels_with_FISH = [1,2]      # list or int with the channels with FISH spots.
  diamter_nucleus = 200           # approximate nucleus size in pixels
  diameter_cytosol = 250          # approximate cytosol size in pixels
# Parameters for spot detection
  voxel_size_z = 500              # Microscope conversion px to nanometers in the z axis.
  voxel_size_yx = 103             # Microscope conversion px to nanometers in the xy axis.
  psf_z = 600                     # PSF emitted by a [rna] spot in the z plan, in nanometers.
  psf_yx = 150                    # PSF emitted by a [rna] spot in the yx plan, in nanometers.
# Cluster Detection
  minimum_spots_cluster = 7       # The number of intensities for a point to be considered as a cluster.
```

- Running the pipeline

```
dataframe_FISH = PipelineFISH(data_dir, channels_with_cytosol, channels_with_nucleus,
channels_with_FISH, diamter_nucleus, diameter_cytosol, voxel_size_z, voxel_size_yx, psf_z,
psf_yx, minimum_spots_cluster).run()
```

Easy to interpret

- Data-frame with all information (Pandas)

	image_id	cell_id	spot_id	nucleus_y	nucleus_x	nuc_area_px	cyto_area_px	cell_area_px	z	y	x	is_nuc	is_cluster	cluster_size	spot_type	is_cell_fragmented
0	0	0	0	260	587	10427	13213	22623	5	262	615	1	0	0	0	1
1	0	0	1	260	587	10427	13213	22623	5	296	607	1	0	0	0	1
2	0	0	2	260	587	10427	13213	22623	6	210	582	1	0	0	0	1
3	0	0	3	260	587	10427	13213	22623	7	273	577	1	0	0	0	1
4	0	0	4	260	587	10427	13213	22623	7	286	602	1	0	0	0	1

- Metadata for reproducibility (text file)

```
#############################################################################
AUTHOR INFORMATION
    Author: luisaguilera
    Created at: 10 Nov 2021
    Time: 17:4
    Operative System: darwin
    Hostname: Luis.local
#############################################################################
PARAMETERS USED
    channels_with_cytosol: [1, 2]
    channels_with_nucleus: 0
    channels_with_FISH: [1, 2]
    diamter_nucleus: 200
    diameter_cytosol: 250
    voxel_size_z: 500
    voxel_size_yx: 103
    psf_z: 600
    psf_yx: 150
    minimum_spots_cluster: 7
#############################################################################
FILES AND DIRECTORIES USED
    Directory path: /Users/luisaguilera/Desktop/FISH_Processing/dataBases/GAPDH_Exon_Cy5_short
    Folder name: GAPDH_Exon_Cy5_short
    Images in directory :
        ROI003_XY1620755767_Z00_T0_merged.tif
#############################################################################
REPRODUCIBILITY
    Platform:
        Python: 3.6.13
    Dependancies:
        tqdm==4.62.3
```

Connection to NAS

NAS (NFS, SMB)   Local computer
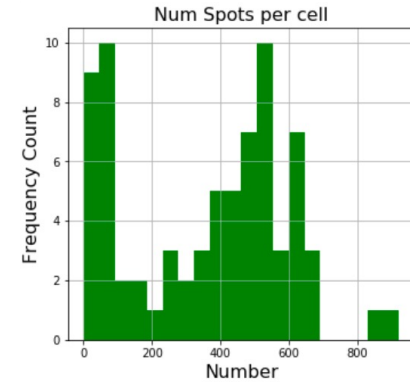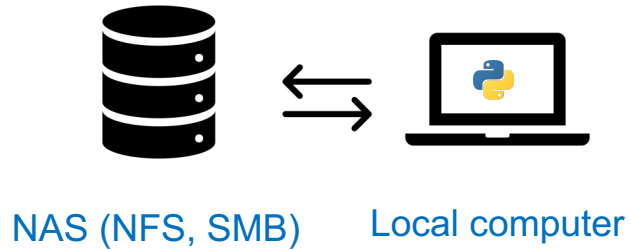
- Configuration file (.yaml)


```
user:
    username: user_name
    password: user_password
    remote_address : munsky-nas.engr.colostate.edu
    domain: engr_dom
```

```
NASConnection(config_file, share).copy_files(remote_folder, local_folder, timeout=60)
```

- Options to interact with NAS.
  - Download images to local computer.
  - Write .tex and .csv files to NAS.

Try it in Google Colab!

https://colab.research.google.com/drive/1CQx4e5MQ0ZsZSQgqtLzVVh53dAg4uaQj?usp=sharing