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The images acquired from the llsm needs to be preprocessed. Currently, we use the code “preprocessing_perAcquisition_ver3.m“, which will do the preprocessing when all images have completed the acquisition (in a single Ex folder). To carry this out successfully, we will need to correctly set up the folder structure.

Folder structure for the LLSM:

- D:
    - {[Year][Month][Day]_[InnerNA]_[OuterNA]_[Camera]_[Biologist]}
        - LLSCalibrations
            - illum
                - sB_noDither
                    - {[sBnD_...].tif}
                - nB_noDither
                    - {[nBnD_...].tif}
                - nB_Dither
                    - {[nBD_...].tif}
            - chroma
                - {Ex[%02d]chroma{_{wavelength}nm_{power}mW}_[s-plane_spacing]}
                    - {[chroma_...].tif}
            - bk
                - [bk_..._CamA_…].tif
                - [bk_.._.CamB_…].tif
            - PSF
                - {Ex[%02d]psf{_{wavelength}nm_{power}mW}_[s-plane_spacing]}
                    - {psf_..._].tif}
            - XZPSF
                - {[wavelength]XZPSF}
        - {CS[%1d]_[sampleName]_{sampleInfo}}
            - {Ex[%02d][keyword]{_{wavelength}nm_{power}mW}_[s-plane_spacing]}
                - {[ex[%02d]_..._].tif}

Example

- D:
  - 20210210_p5_p55_sCMOS_Gustavo
    - LLSCalibrations
      - illum
        - sB_noDither
          - sBnD_…_CamA_…_488nm_… .tif
          - sBnD_…_CamB_…_560nm_… .tif
          - sBnD_…_CamA_…_642nm_… .tif
        - nB_noDither
          - sBnD_…_CamA_…_488nm_… .tif
          - sBnD_…_CamB_…_560nm_… .tif
          - sBnD_…_CamA_…_642nm_… .tif
        - nB_Dither
          - sBnD_…_CamA_…_488nm_… .tif
          - sBnD_…_CamB_…_560nm_… .tif
          - sBnD_…_CamA_…_642nm_… .tif
      - chroma
        - Ex00chroma_488nm_300mW_560nm_500mW_642nm_500mW_z0p2
          - chroma_…._488nm_... .tif
          - chroma_…._560nm_... .tif
          - chroma_…._642nm_... .tif
      - bk
        - bk_..._CamA_… .tif
        - bk_..._CamB_… .tif
      - PSF
        - Ex488psf1_488nm_300mW_z0p2
          - psf_...488nm_… .tif
        - Ex560psf1_488nm_300mW_z0p2
          - psf_...560nm_… .tif
        - Ex642psf1_488nm_300mW_z0p2
          - psf_...642nm_… .tif
      - XZPSF
        - 488XZPSF
        - 560XZPSF
        - 642XZPSF
    - CS1_SVGA_eGFP_NUP133_JF649_NUP107
      - Ex01_488nm_300mW_560nm_500mW_z0p2
      - Ex02_488nm_300mW_560nm_500mW_z0p2
      - Ex03_488nm_300mW_560nm_500mW_z0p2

- CS2_SVGA_eGFP_NUP133_JF649_NUP205
    - Ex04_488nm_300mW_560nm_500mW_z0p2
    - Ex05_488nm_300mW_560nm_500mW_z0p2
    - Ex06_488nm_300mW_560nm_500mW_z0p2

How to read:

- {values}
    - Means that there are multiple DEFINED value within values.
        - For example, an experiment with the channels: 488, 560 and 642; powers: 300, 500, 500
            - {wavelength} = 488560642
            - {_{wavelength}nm} = _488nm_560nm_642nm
            - {_{wavelength}nm_{power}mW} = _488nm_300mW_560nm_500mW_642nm_500mW
- [variable]
    - Means it is a NON-DEFINED variable. We also use regular expressions
        - Source folder name
            - For example, if todays date is 2021, Feb 10th
                - [Year] = 2021
                - [Month] = 02
                - [Day] = 10
            - For example, if the inner NA is 0.5 and the outer is 0.55
                - [innerNA] = p5
                - [outerNA]= p55
            - Camera will be either sCMOS or EMCCD. Lets say we are using sCMOS
                - [Camera] = sCMOS
            - Biologist will be the name of the biologist, lets say its Gustavo, then
                - [Biologist] = Gustavo
        - Experiments
            - [%02d] means any two digit number, so can be 00, 10, 32, 99, … etc
                - Ex[%02d] means it can any of, but not limited to: Ex00, Ex10, Ex32, Ex99,... etc
                - Of course, if you have more than 100 unique experiments, you can use Ex[%03d]_ format
                    - Ex100_

            - Ex999_
        - [keyword]
            - Your personal keyword. You don't have to use it. You can use it to distinguish experiments. For example
                - good
                - nup205
                - lowSignal
                - incubated30min
                - '' (empty string)
        - Ex[%02d][keyword]_. THIS MUST BE UNIQUE
            - Ex01good_
            - Ex10lowSignal_
            - Ex15SNR10_
            - Ex43incubated30min_
            - Ex90_
- Combination of Both
    - Source folder
        - Examples of {[Year][Month][Day]_[InnerNA]_[OuterNA]_[Camera]_[Biologist]}:
            - 20200210_p5_p55_sCMOS_Gustavo
            - 20201222_p6_p5_EMCCD_Alex
            - 20210123_p5_p55_sCMOS_Anwesha

Notes

- If any of [Year][Month][Day]_[InnerNA]_[OuterNA]_[Camera]_[Biologist] are different, create a new folder
    - Do not combine same day experiments into a single folder if you have different biologist
    - You can copy and paste the calibrations folder from one to another if you are using exactly the same condition (same filter, imaging media, etc..)
- Each Ex[%02d][keyword] should have a unique cell/sample to be imaged
    - For example, if you image the same sample 3 times in a different folder, then you can name your folder something like (this is an example):
        - Ex01_…._
        - Ex01second_ ...
        - Ex01third_ …
    - For example, when we ran NUP experiments, we first took a volume scan, then bleached the cell, then ran a time-series acquisition. The folder names can be:
        - Ex01initialVol_

        - Ex01bleach_
        - Ex01timeSeries_
- Each Ex[%02d][keyword]_ MUST BE UNIQUE
- If you acquire a volume scan (sample scan or objective scan) you must create a folder with “Ex … “ and save the tif images there