

# Guide to folders and files

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mm3 creates many folders and files in your experimental directory during the course of processing. This page enumerates and explains those items. (Explicitly, this explains the way your experimental and analyzed data is organized by mm3, not how the folders and files are organized in the mm3 repository).

The paths of the folders and subfolders should be defined in your parameters.yaml file, though most of the scripts will make the folders for you if you have not already. Be very sure that you put in the right names, as the easiest bugs to get when processing images are path errors!

## Experimental Directory

`/full/path/to/experimental/directory/`

Defined in the parameters.yaml file as `experimental_directory`. Type the whole path in quotes. This is main folder where all subfolders should go that contain raw images, analysis, and movies. Good practice is to begin this folder name with `yyyymmdd`, and make it very similar to the name of the experiment in your Evernote.

Some things besides subfolders go directly into your experimental directory. These include:

- `parameters.yaml` : This holds a adjustable parameters and file names. Definitely fill this out to the best of your ability before you run any scripts, and better yet determine what parameters are used by each script.

## Image Directory

`/experimental_directory/TIFF/`

Defined in the parameters.yaml file as `image_directory`. Type the path from the experimental directory with a trailing forward slash but not a starting one, again in quotes. This folder should contain all your TIFF images, but you should keep `.nd2` files in the experimental directory.

TIFF files have a specific naming scheme:

`yyyymmdd_experiment_name_t0000xy000.tif` OR `yyyymmdd_experiment_name_t0000xy000c0.tif`

The file postfix, with information about the timepoint `t`, the FOV number `xy` and the color channel `c` are particularly important. mm3 scripts expect this format when searching and retrieving metadata from image files.

## Analysis Directory

/experimental\_directory/analysis/

This is where most metadata and processed images go that are accumulated during processing. This includes:

- TIFF\_metadata.pkl and .txt : Python dictionary of metadata associated with each TIFF file. Created by mm3\_Compile.py.
- channel\_masks.pkl and .txt : Python dictionary that records the location of the channels in each FOV. Is a nested dictionaries of FOVs and then channel peaks. The final values are 4 pixel coordinates, ((y1, y2), (x1, x2)). Created by mm3\_Compile.py.
- crosscorrs.pkl and .txt : Python dictionary that contains image correlation value for channels over time. Used to guess if a channel is full or empty. Same structure as channel\_masks. Created by mm3\_ChannelPicker.py.
- specs.pkl and .txt : Python dictionary which is the specifications of channels as full (1), empty (0), or ignore (-1). Same structure as channel\_masks. Created by mm3\_ChannelPicker.py.

The analysis directory also contains subfolders which contain image data, namely the stacked channel images.

### **Channel stacks**

/experimental\_directory/analysis/channels/

Contains the sliced and stacked channel information as created by mm3\_Compile.py. Each stack is for a single channel for a single color plane for all time points. They are named with the following scheme:

experiment\_name\_xy000\_p0000\_c0.tif

Where experiment\_name is from the parameters file. xy is the 3 digit FOV number, p is the four digit peak ID (channel ID) and c is the single digit color plane. FOV number is 1 indexed, color plane is 0 indexed, and the peak ID comes from the X pixel location of the channel midline in the original TIFF images.

### **Averaged empty channels**

/experimental\_directory/analysis/empties/

Contains the averaged empty channel templates, as created by mm3\_ChannelPicker.py, to be used during subtraction. There should be one empty channel stack per FOV. Uses the naming convention:

experimental\_name\_xy000\_empty.tif

### **Subtracted**

/experimental\_directory/analysis/

Contains the subtracted phase contrast images as created by mm3\_Subtract.py. Uses the naming convention:

experimental\_name\_xy000\_p0000\_sub.tif

## **Movie Directory**

*/experimental\_directory/movies/*

Holds movie files made by mm3\_MovieMaker.py.