

## Default MIINA image file naming conventions

The **calib** folder contains image files for the color shift calibration between the 561-nm and 647-nm fluorescence channels. The two image files are named as **movie\_0001\_561.dax** and **movie\_0001\_647.dax** respectively. These are z-stack image files containing many frames with 5 frames collected at each z height.

The **sequential** folder contains all other image files collected during MIINA imaging. The files are:

**STORM1\_HybNo\_FieldNo.dax**: These are images taken with 750-nm illumination for MERFISH readout. (The MERFISH readout probes are labeled with Alexa Fluor 750.) HybNo is the double-digit serial number for the sequential hybridization. FieldNo is the serial number of the fields of view imaged on the sample. These images are z-stack image files containing many frames with 5 frames collected at each z height.

**STORM2\_HybNo\_FieldNo.dax**: These are multi-channel images taken with 647-nm, 561-nm, and 488-nm illuminations. The 647-nm and 561-nm illuminations allow visualization of the chromatin tracing secondary probes. The 647-nm illumination also allows visualization of the co-immunofluorescence of nucleolar labeling in one round of sequential hybridization (HybNo: 00). The 561-nm illumination also allows visualization of the cell boundary labeling in one round of sequential hybridization (HybNo: 31). The 488-nm illumination allows visualization of the fiducial beads for drift correction. HybNo should have double digits, e.g. from 00 to 40. These images are z-stack image files. At each z height, 647-nm, 561-nm, and 488-nm illuminated images are sequentially taken with 5 frames in each channel, before moving on to the next z height.

**Laser405\_0\_FieldNo.dax** and **STORM2\_0\_FieldNo.dax**: These files correspond to an additional round of sequential imaging (e.g. the 41<sup>st</sup> round) for the visualization of the DAPI staining of whole nuclei. The **Laser405\_0\_FieldNo.dax** file contains a z-stack of the 405-nm illuminated fluorescence images to visualize the DAPI staining, with 5 frames taken at each z height. The **STORM2\_0\_FieldNo.dax** file is a multi-channel z-stack image as introduced in the last paragraph. (Note this file is different from **STORM2\_00\_FieldNo.dax** where the HybNo is double-digit.) Only the 488-nm channel frames in this stack are used to visualize the fiducial beads for the drift correction of this added round of imaging.

All .dax files and the associated auxiliary files with the same names but different extensions (there are five auxiliary files for each .dax file) were collected using open source python codes from <https://github.com/ZhuangLab/storm-control>.

The **sequential** folder of this example dataset contains two fields of view (FieldNo: 00 and 01), a Hyb0 imaging round (HybNo: 00), 40 rounds of secondary hybridization and imaging (HybNo: 01, 02, ..., 40), and an additional imaging round for the DAPI staining.