**11. Applying Flat-Field Corrections to Data Images**

The equation for a flat-field correction is as follows:

$$Image\_{ij}^{\*}= \frac{(Image \_{ij}- DarkNoise\_{ij})}{(FFImage \_{ij}- DarkNoise\_{ij})} × <FFImage - DarkNoise> $$

where Imageis the sample to be flat-field corrected, DarkNoise is a background image detailing noise in the camera, and FFImage is the correction image. To summarize the equation above, the corrected image is the sample image multiplied element-wise to a normalized correction image. In reality, the DarkNoise is so low in our setups, that we omit that image completely. For information on how to prepare a FFImage, refer to Section 10.

 **Apply corrections with FFCorrection.m**

FFCorrection.m is a Matlab script used to scan the metadata of the images to be corrected, select the appropriate correction image, apply it, save the corrected images and optionally stitch them using MIJI.

There are two modes that can be called in Matlab:

Automatic

FFCorrection(NumFrames);

where NumFrames is an integer representing the number of frames that are to be corrected.

Manual Override

FFCorrection(NumFrames,{'Channel1','Channel2', ... , 'ChannelN'});

where 'Channel#' is the string identifier for the fluorescence channel to correct with - i.e. the GFP image would be 'eGFP' and the Cy5 image would be 'Cy5'. The manual mode is useful if the settings for the setup have changed (thereby changing the metadata), but it is important to load the directories in the appropriate order - i.e. you don't want to correct a GFP channel with a 'Cy5' correction image.