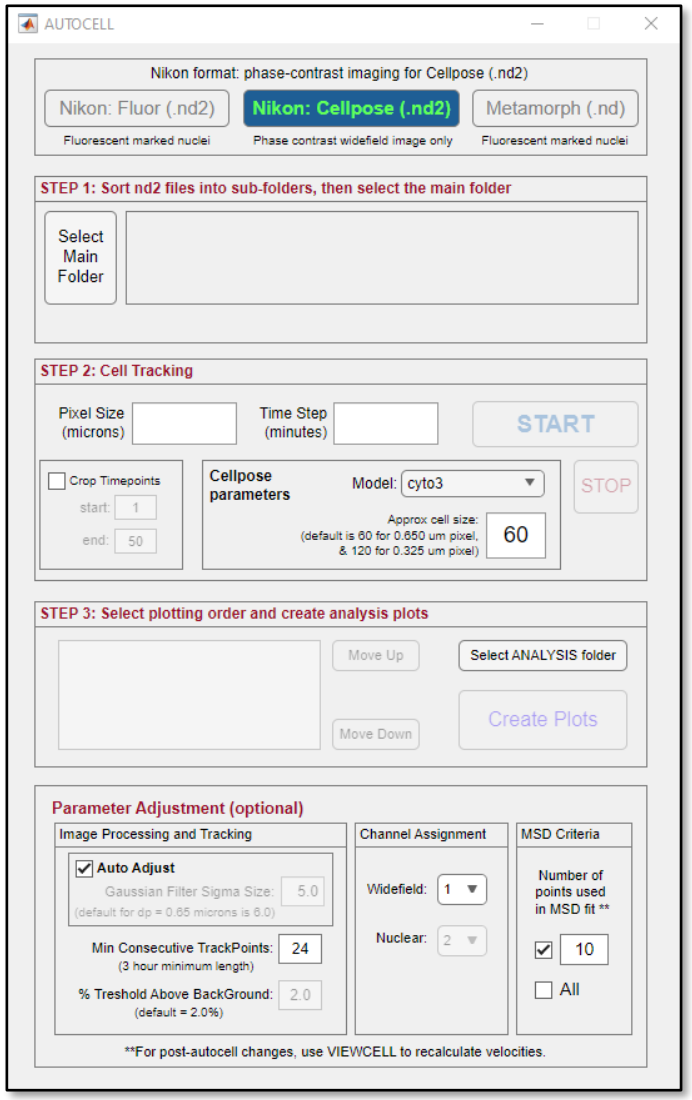
Installing Autocell:



Installing **Autocell** for use in **MATLAB/Windows** environment

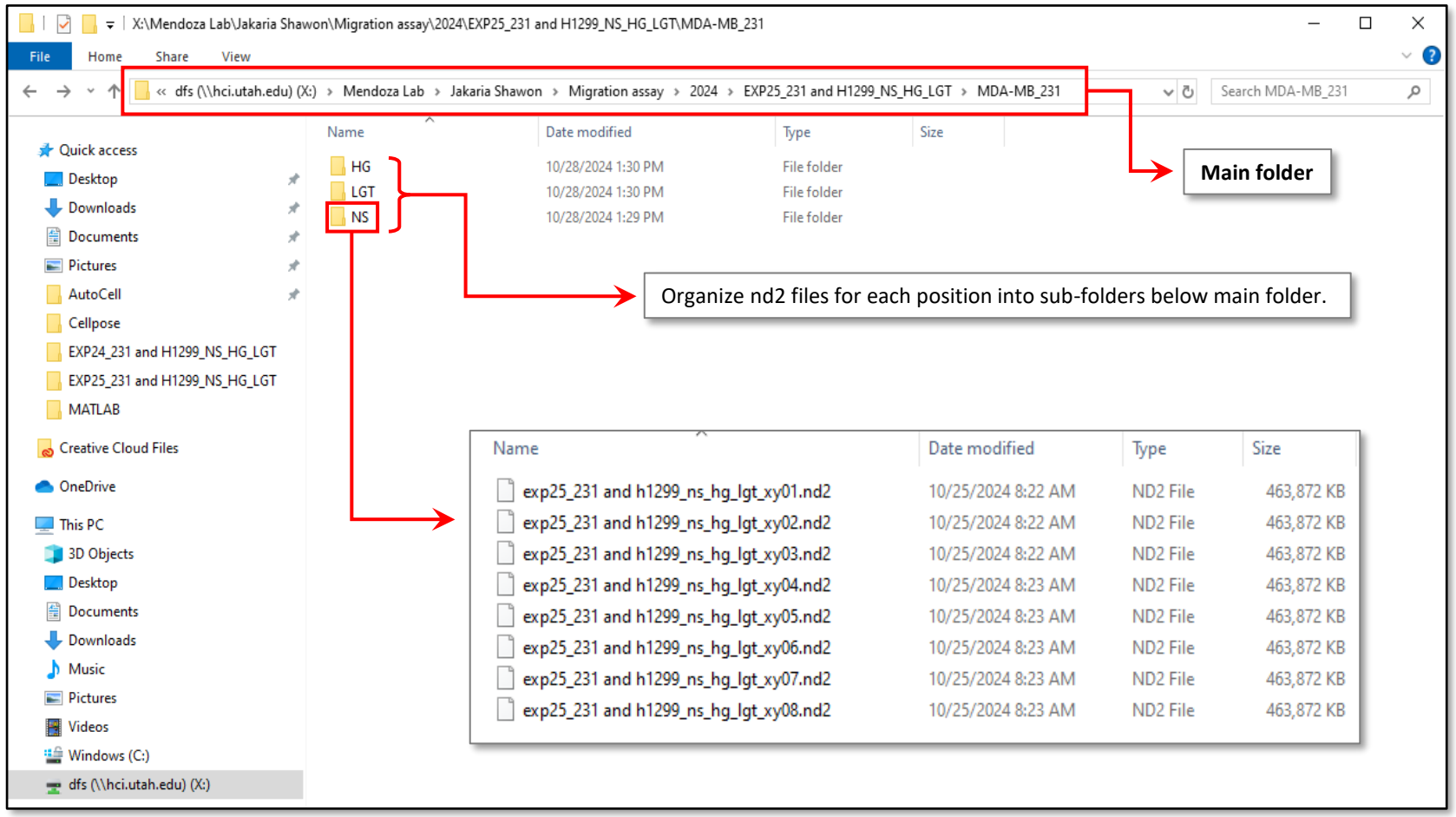
1. **Install MATLAB, and make sure that, at least, the following MATLAB toolboxes are installed as well**
   (type *ver* in MATLAB command window prompt to verify):
   - **1.1. Computer Vision Toolbox**
   - **1.2. Curve Fitting Toolbox**
   - **1.3. Deep Learning Toolbox**
   - **1.4. Image Processing Toolbox**
   - **1.5. Medical Imaging Toolbox**
   - **1.6. Optimization Toolbox**
   - **1.7. Signal Processing Toolbox**
   - **1.8. Statistics and Machine Learning Toolbox**
2. **Download AutoCell functions from GitHub and add download directory to MATLAB paths.**
   - **2.1.** AutoCell GitHub link: *https://github.com/MendozaLabHCI/Autocell*
   - **2.2.** Download AutoCell functions:
     - **2.2.1. autocell.mlapp**
     - **2.2.2. Sort_ndFormat.mlapp**
     - **2.2.3. RosePlotColorSelector.mlapp**
3. **Install MATLAB Add-On: *Medical Imaging Toolbox Interface for Cellpose Library*.**
   - **3.1.** Add-On button is found here: ***MATLAB > Home Tab > Add-Ons***
   - **3.2.** Associated Cellpose paper: *https://www.nature.com/articles/s41592-020-01018-x*
   - **3.3.** If Add-On installation errors, try temporarily turning off any fire-wall/viral protection software.
4. **Add the following directories to the MATLAB paths** (Code/file sources are given but no download necessary):
   (The Set Path button is found here: MATLAB > Home Tab > Set Path > Add With Subfolders)
   - **4.1. X:\Mendoza Lab\MATLAB\Cellpose\cellposeModels**
     - **4.1.1.** Cellpose model source: *https://cellpose.readthedocs.io/en/latest/models.html*
     - **4.1.2.** Include *cyto3* model
   - **4.2. X:\Mendoza Lab\MATLAB\Bio-Formats – OpenMicroscopyDotOrg**
     - **4.2.1.** Bio-formats MATLAB toolbox: *https://www.openmicroscopy.org/bio-formats/downloads/*
   - **4.3. X:\Mendoza Lab\MATLAB\Custom Keith Code\AutoCell**
     - 4.3.1. This is the current location for the Mendoza lab at HCI.
       Change this to relevent folder the code in step 2.2 was downloaded to.
   - ***4.4.* X:\Mendoza Lab\MATLAB\Linear Assignment Cell Tracker**
     - ***4.4.1.*** Associated paper: *https://doi.org/10.1038/nmeth.1237*
     - ***4.4.2.*** MATLAB code: *https://github.com/Biofrontiers-ALMC/cell-tracking-toolbox/wiki*
5. **Test Cellpose install by typing *cellpose()* followed by <enter> in the MATLAB command window.**
   - ***5.1.*** If the following error occurs...

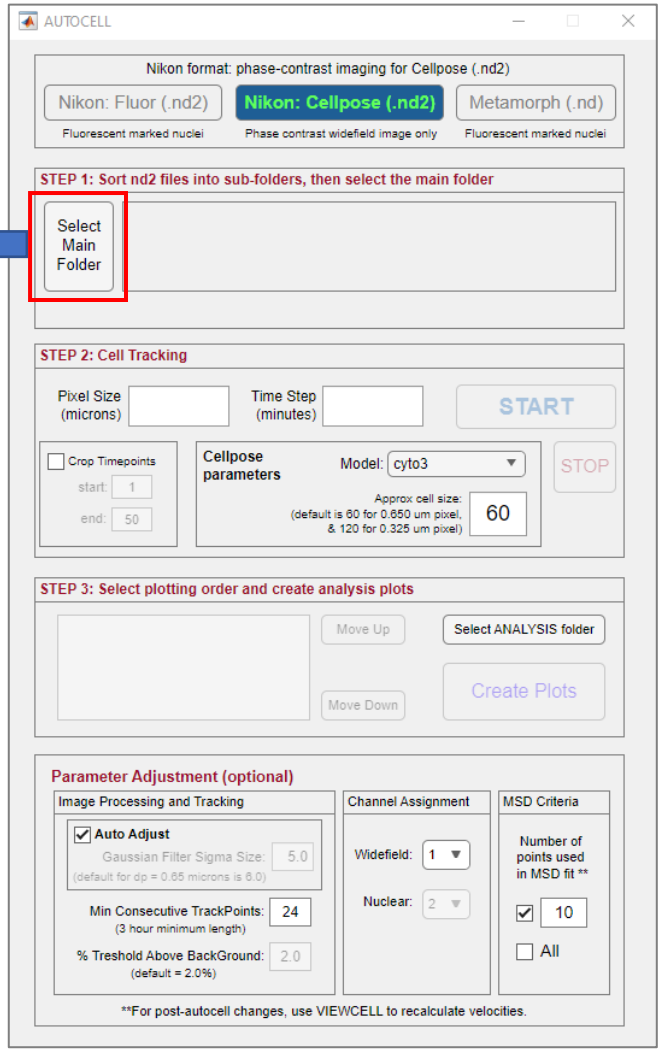
```
>> CP = cellpose()
Unable to resolve the name 'py.torch.set_num_threads'.

Error in cellpose.setupCellposePythonEnvironment

Error in cellpose
```

   try the solution found here: *X:\Mendoza Lab\MATLAB\Cellpose**CellposePermissionsErrorFix.pdf***
   (Also found on page 21 of lab notebook)
6. **To open autocell software, type *autocell* and press <enter> at the MATLAB command window prompt.**

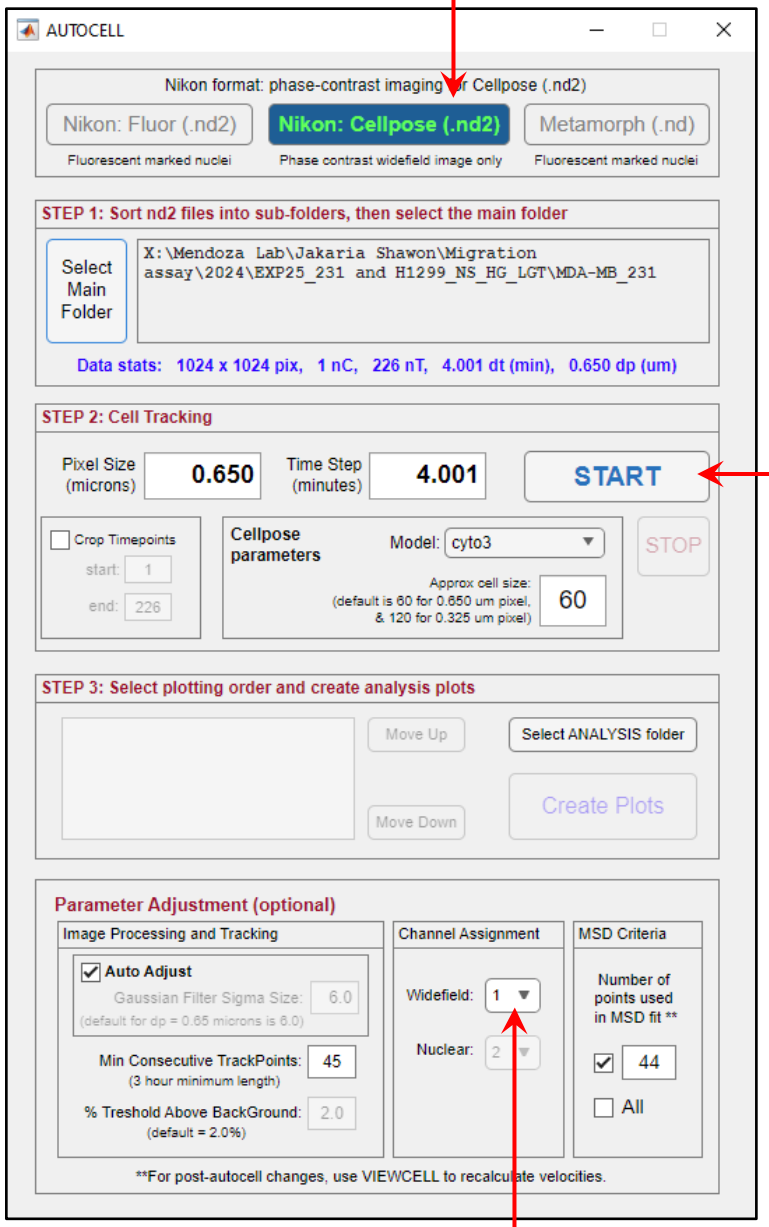
Using Autocell:

1. Acquire timelapse cell migration image data. (suggested time step < 5 min)
2. Sort nd2 files into sub-folders.
3. **Press "Select Main Folder" button** in Autocell and select Main Folder location. After selecting Main Folder, Autocell should populate image stats.



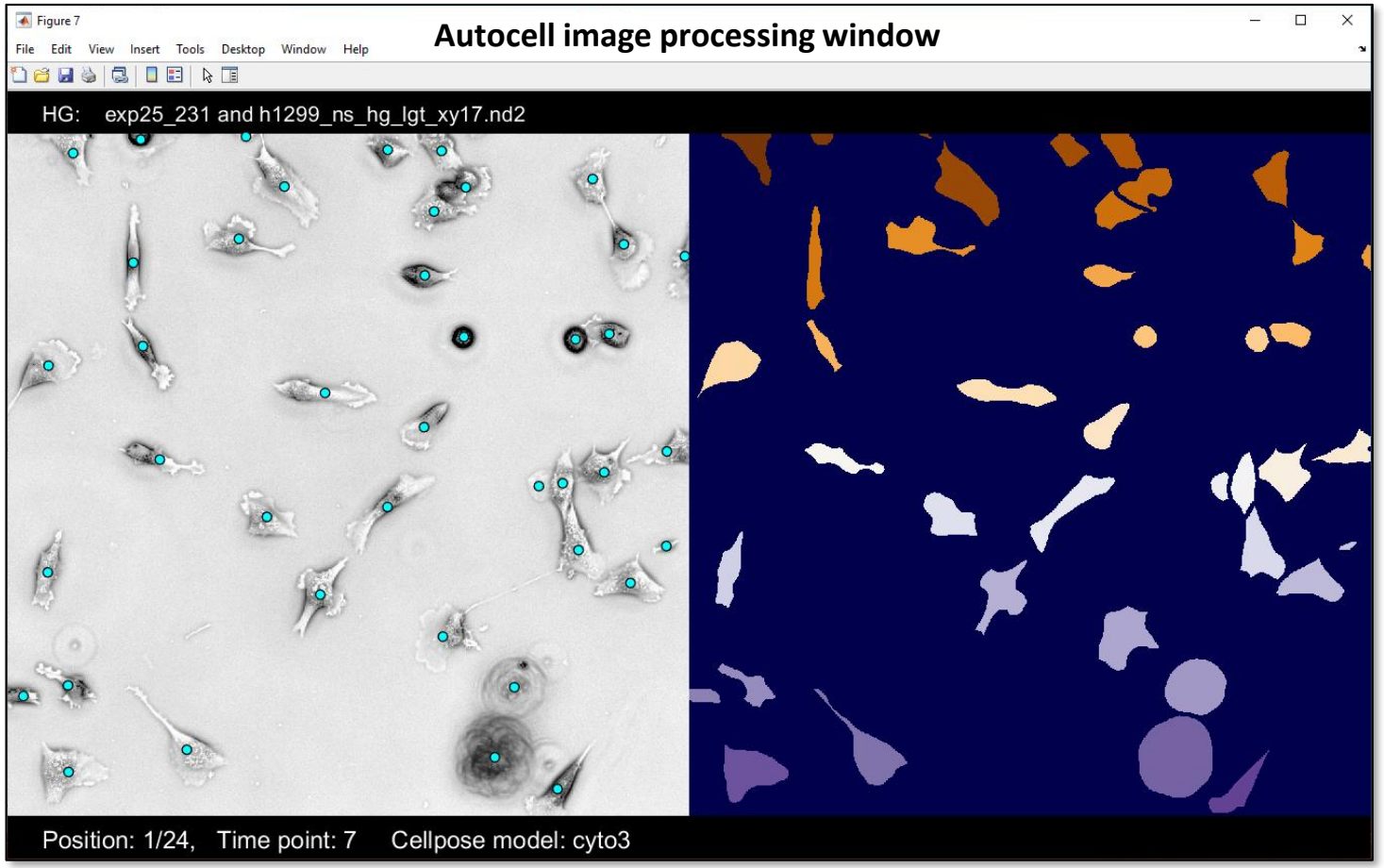


Using Autocell:



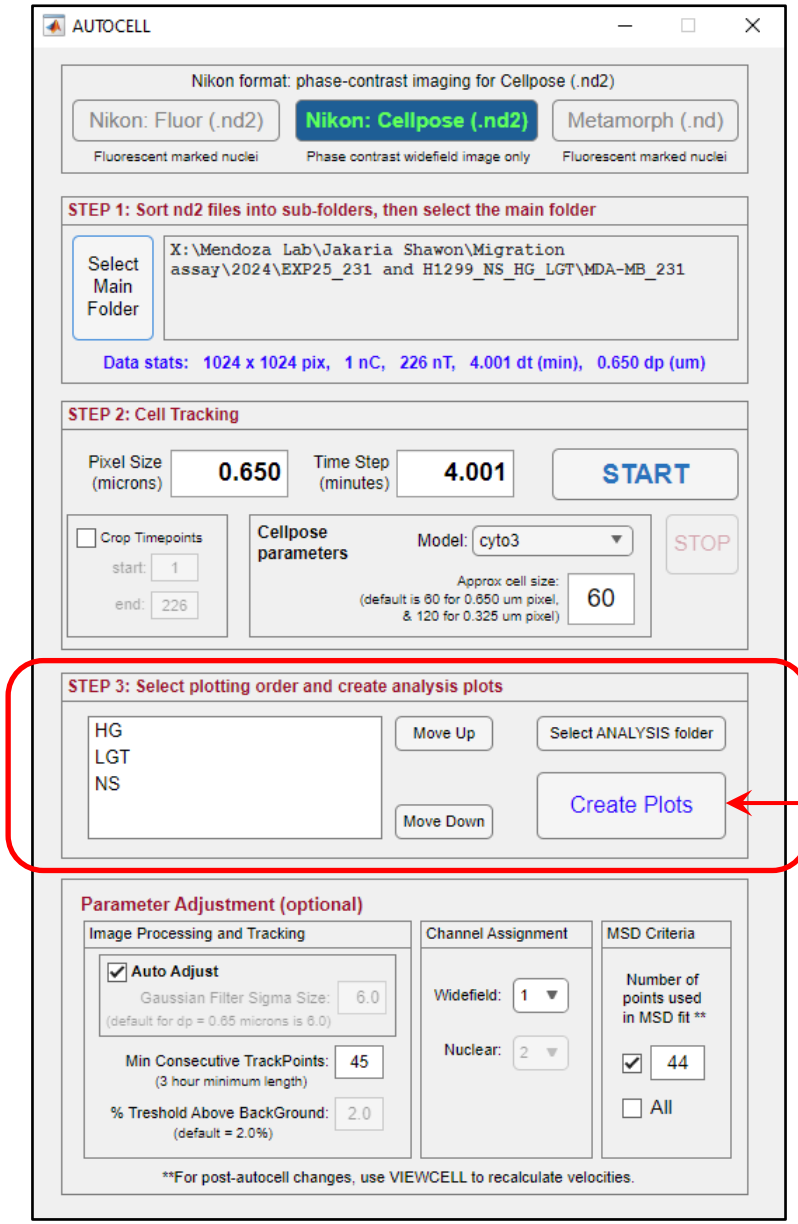
4. **Select <u>Nikon: Cellpose (.nd2)</u>** (default). It assumes single channel wide field image data, and uses Cellpose AI software to segment cells.
   - Select <u>Nikon: Fluor (.nd2)</u> for cell migration data having a nuclear fluorescent marker channel.
5. **Press "START" button** in Autocell to begin cell migration analysis.

Autocell image processing window

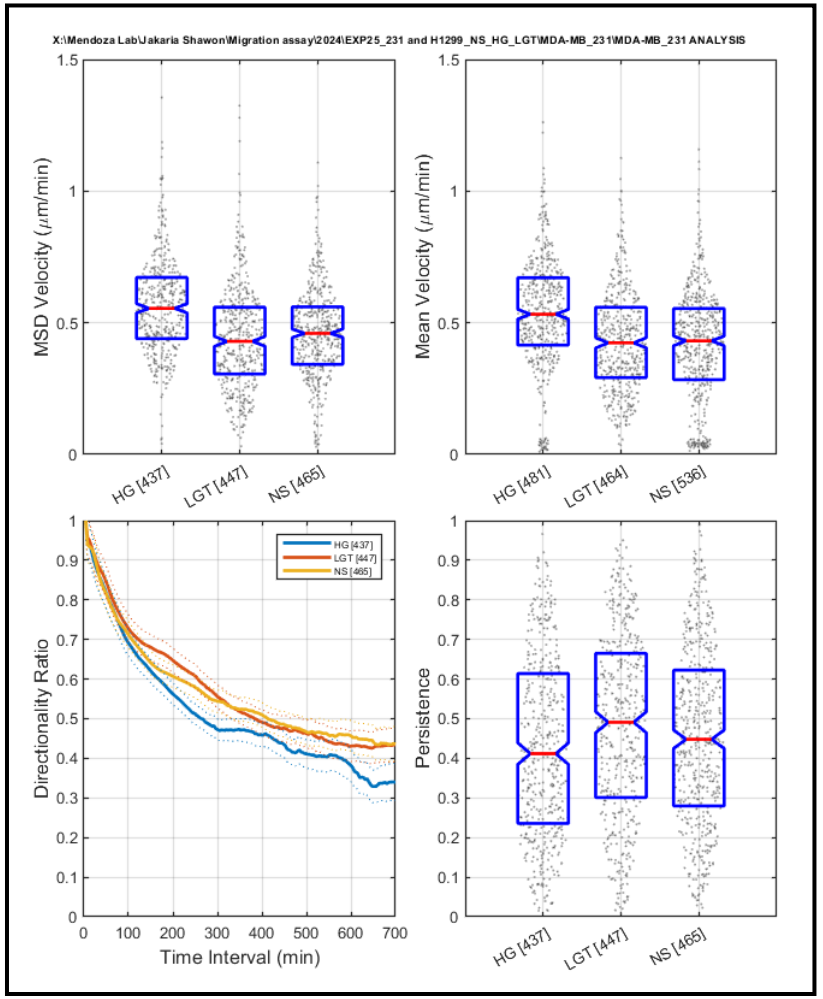


6. If nd2 file contains more than one channel, select the channel that contains the wide field image data.

Using Autocell:



7. After Autocell's cell migration analysis is complete the analysis window will be populated with categories containing cell track data. **Press "Create Plots"** to create velocity and persistence box plots of cell track data as well as median speeds and p-values.



Median of Calculated Cell Speeds (microns/min)			
Category	MSD Speed	Mean Speed	
HG	0.5549	0.5325	
LGT	0.4295	0.4237	
NS	0.4600	0.4313	
Two-Sample Kolmogorov-Smirnov test (for MSD speed values)			
Category 1	Category 2	Pass?	P-value
HG	LGT	TRUE	8.949E-17
HG	NS	TRUE	8.644E-13
LGT	NS	TRUE	1.667E-02
Two-Sample Kolmogorov-Smirnov test (for Mean speed values)			
Category 1	Category 2	Pass?	P-value
HG	LGT	TRUE	1.082E-14
HG	NS	TRUE	1.348E-14
LGT	NS	TRUE	3.413E-04