

Installing Autocell:

The screenshot shows the Autocell software interface with the following sections:

- Format Selection:** Three buttons for Nikon format: Fluor (.nd2), **Nikon: Cellpose (.nd2)** (highlighted), and Metamorph (.nd). Below them are labels: Fluorescent marked nuclei, Phase contrast widefield image only, and Fluorescent marked nuclei.
- STEP 1: Sort nd2 files into sub-folders, then select the main folder**
 - A button labeled "Select Main Folder" next to an empty text box.
- STEP 2: Cell Tracking**
 - Pixel Size (microns) and Time Step (minutes) input fields.
 - A "START" button.
 - A checkbox for "Crop Timepoints" with start and end input fields (start: 1, end: 50).
 - Cellpose parameters**
 - Model: cyto3 (dropdown menu).
 - Approx cell size: 60 (input field, with text: (default is 60 for 0.650 um pixel, & 120 for 0.325 um pixel)).
 - A "STOP" button.
- STEP 3: Select plotting order and create analysis plots**
 - A list box for file selection.
 - "Move Up" and "Move Down" buttons.
 - A "Select ANALYSIS folder" button.
 - A "Create Plots" button.
- Parameter Adjustment (optional)**
 - Image Processing and Tracking**
 - ☒ **Auto Adjust**
 - Gaussian Filter Sigma Size: 5.0 (input field, with text: (default for dp = 0.65 microns is 6.0))
 - Min Consecutive TrackPoints: 24 (input field, with text: (3 hour minimum length))
 - % Threshold Above Background: 2.0 (input field, with text: (default = 2.0%))
 - Channel Assignment**
 - Widefield: 1 (dropdown menu)
 - Nuclear: 2 (dropdown menu)
 - MSD Criteria**
 - Number of points used in MSD fit **: 10 (input field, with a checked checkbox)
 - ☐ All
- Footer:** **For post-autocell changes, use VIEWCELL to recalculate velocities.

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 relevant 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...

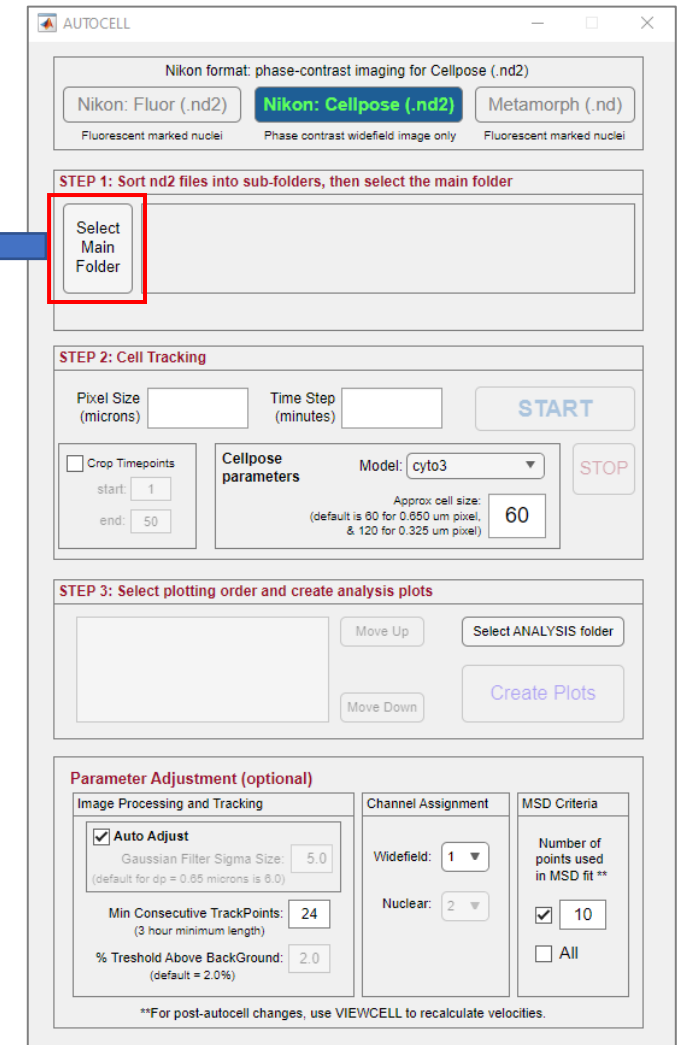
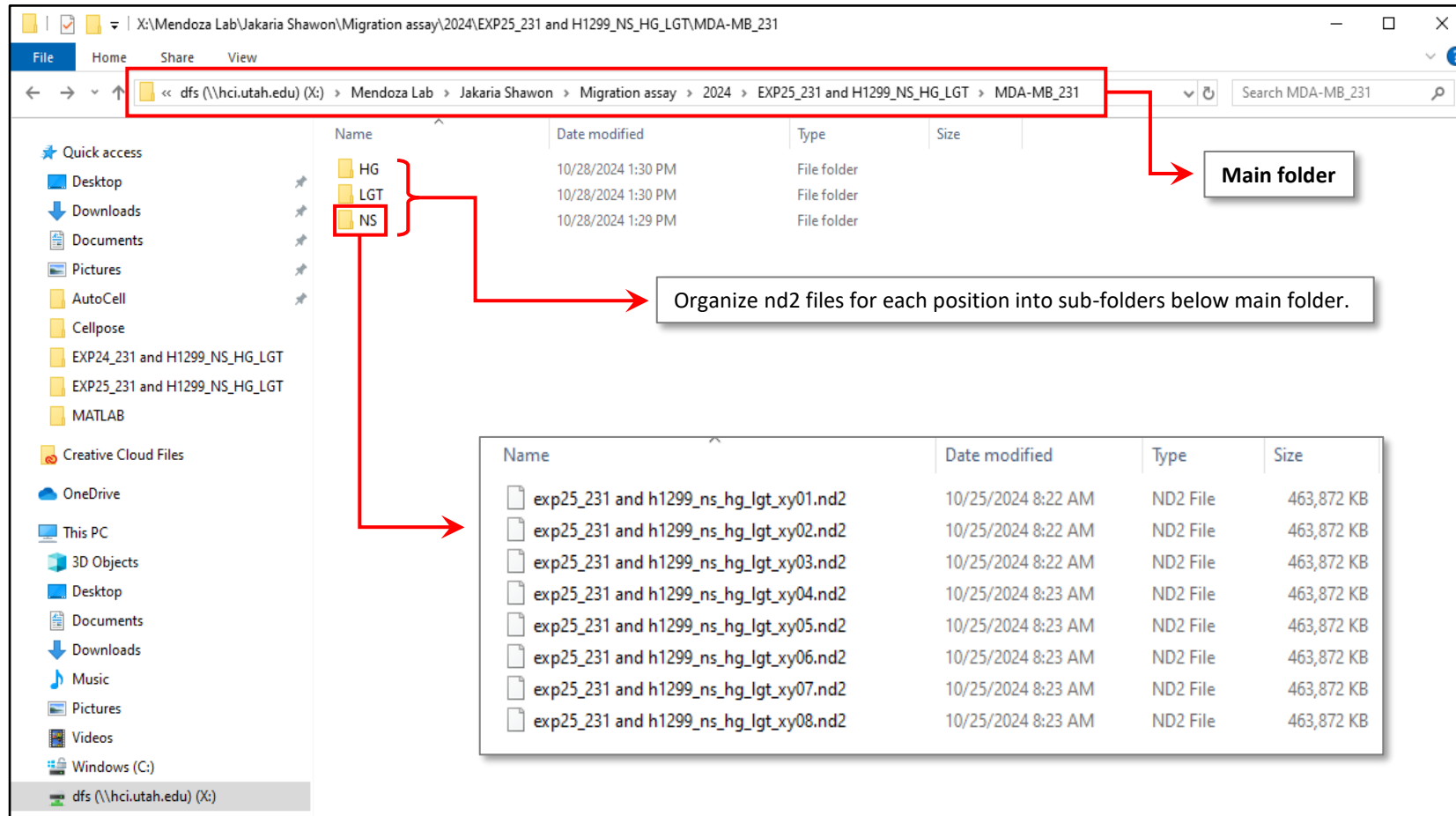
```
>> CP = cellpose()
Unable to resolve the name 'py.torch.set_num_threads'.

Error in cellpose/setupCellposePythonEnvironment
Error in cellpose
```

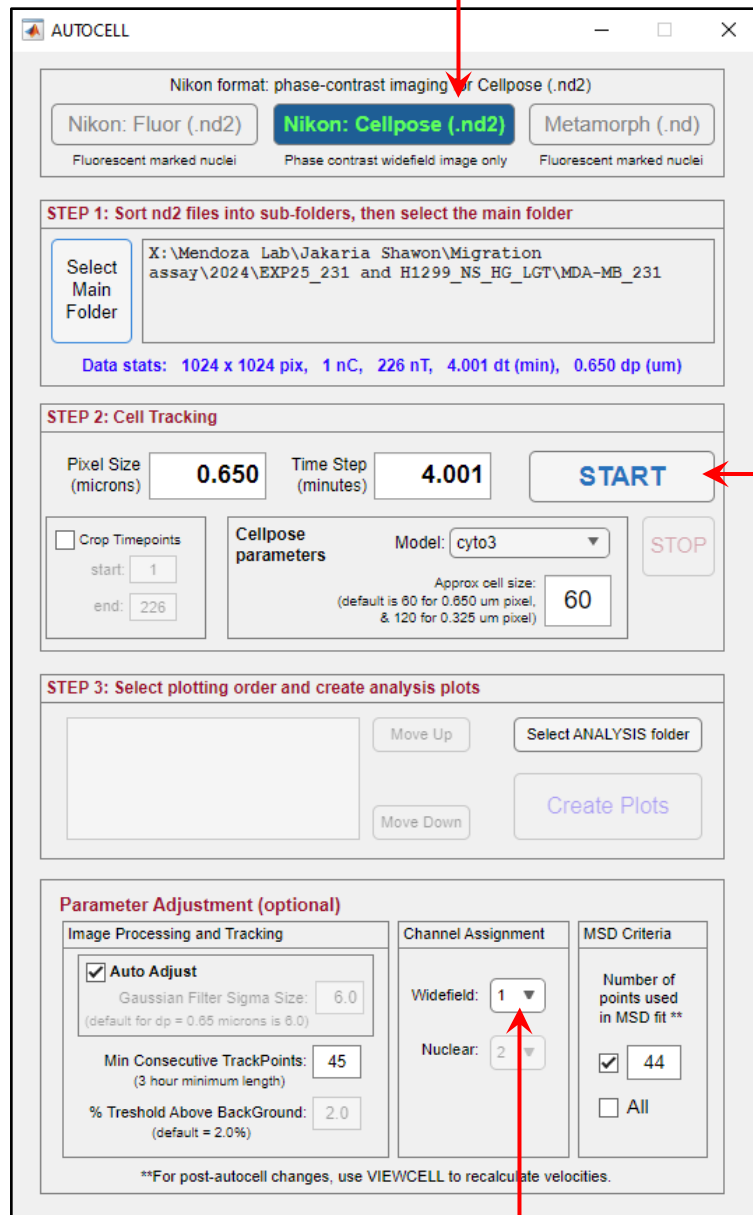
try the solution found here: X:\Mendoza Lab\MATLAB\Cellpose\CellposePermissionsErrorFix.pdf
(Also found on page 21 of lab notebook)
6. **To open autocell software, type `autocell` and press <enter> at the MATLAB command window prompt.**

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.



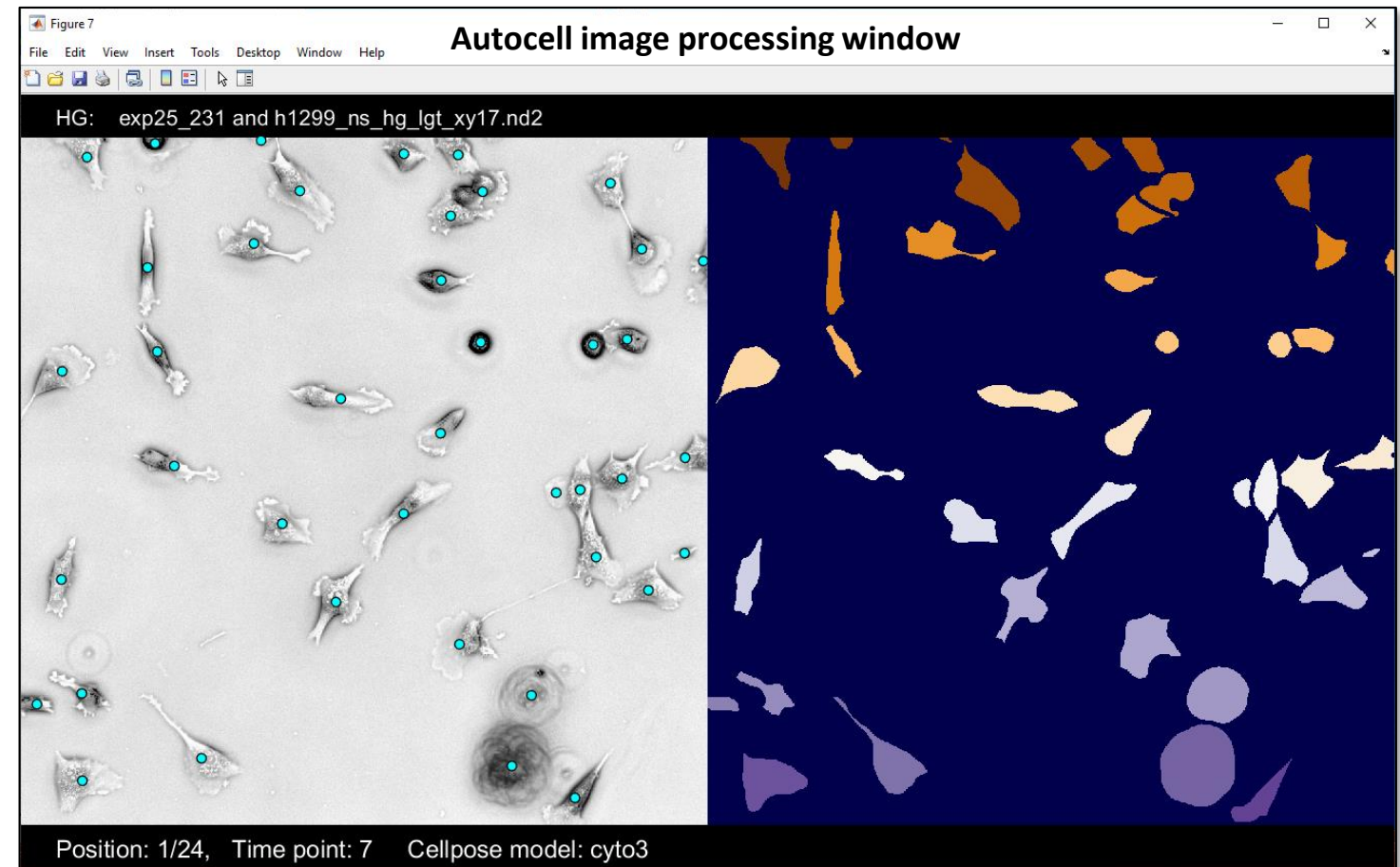
Using Autocell:



The screenshot shows the Autocell software interface with several sections:

- Top Section:** "Nikon format: phase-contrast imaging" with a dropdown menu set to "Cellpose (.nd2)". Below this are three buttons: "Nikon: Fluor (.nd2)", "Nikon: Cellpose (.nd2)" (highlighted with a red arrow), and "Metamorph (.nd2)".
- STEP 1: Sort nd2 files into sub-folders, then select the main folder**
 - A "Select Main Folder" button is next to a text box containing the path: "X:\Mendoza Lab\Jakaria Shawon\Migration assay\2024\EXP25_231 and H1299_NS_HG_LGT\MDA-MB_231".
 - Below the path is a "Data stats" line: "1024 x 1024 pix, 1 nC, 226 nT, 4.001 dt (min), 0.650 dp (um)".
- STEP 2: Cell Tracking**
 - Parameters: "Pixel Size (microns)" is 0.650, "Time Step (minutes)" is 4.001. A "START" button is highlighted with a red arrow.
 - Below these are checkboxes for "Crop Timepoints" (start: 1, end: 226) and "Cellpose parameters" (Model: cyto3, Approx cell size: 60).
 - A "STOP" button is also present.
- STEP 3: Select plotting order and create analysis plots**
 - Buttons for "Move Up", "Move Down", "Select ANALYSIS folder", and "Create Plots".
- Parameter Adjustment (optional)**
 - Image Processing and Tracking:** "Auto Adjust" is checked. "Gaussian Filter Sigma Size" is 6.0. "Min Consecutive TrackPoints" is 45. "% Threshold Above Background" is 2.0.
 - Channel Assignment:** "Widefield" is set to 1 (highlighted with a red arrow) and "Nuclear" is set to 2.
 - MSD Criteria:** "Number of points used in MSD fit" is 44 (checked).
- Footer:** "**For post-autocell changes, use VIEWCELL to recalculate velocities."

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



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.

AUTOCELL

Nikon format: phase-contrast imaging for Cellpose (.nd2)

Nikon: Fluor (.nd2)

Nikon: Cellpose (.nd2)

Metamorph (.nd)

Fluorescent marked nuclei

Phase contrast widefield image only

Fluorescent marked nuclei

STEP 1: Sort nd2 files into sub-folders, then select the main folder

Select Main Folder

X:\Mendoza Lab\Jakaria Shawon\Migration assay\2024\EXP25_231 and H1299_NS_HG_LGT\MDA-MB_231

Data stats: 1024 x 1024 pix, 1 nC, 226 nT, 4.001 dt (min), 0.650 dp (um)

STEP 2: Cell Tracking

Pixel Size (microns)0.650Time Step (minutes)4.001START

☐ Crop Timepoints

start: 1end: 226

Cellpose parametersModel: cyto3Approx cell size: (default is 60 for 0.650 um pixel, & 120 for 0.325 um pixel)60STOP

STEP 3: Select plotting order and create analysis plots

HG
LGT
NS

Move Up
Move Down

Select ANALYSIS folder

Create Plots

Parameter Adjustment (optional)

Image Processing and Tracking

☒ Auto AdjustGaussian Filter Sigma Size: 6.0 (default for dp = 0.65 microns is 6.0)Min Consecutive TrackPoints: 45 (3 hour minimum length)% Threshold Above Background: 2.0 (default = 2.0%)

Channel Assignment

Widefield: 1Nuclear: 2

MSD Criteria

Number of points used in MSD fit **☒ 44☐ All

**For post-autocell changes, use VIEWCELL to recalculate velocities.

X:\Mendoza Lab\Jakaria Shawon\Migration assay\2024\EXP25_231 and H1299_NS_HG_LGT\MDA-MB_231 ANALYSIS

The figure displays four plots related to cell migration analysis. The top-left plot shows MSD Velocity (μm/min) for three categories: HG [437], LGT [447], and NS [465]. The top-right plot shows Mean Velocity (μm/min) for three categories: HG [481], LGT [464], and NS [536]. The bottom-left plot shows Directionality Ratio over Time Interval (min) for three categories: HG [437], LGT [447], and NS [465]. The bottom-right plot shows Persistence for three categories: HG [437], LGT [447], and NS [465].

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