**System requirements:**

- for Matlab Code:
  - Windows 7, 64 bit or later
  - Matlab v. 2017b or later with "Image Processing and Statistics" and "Machine Learning" toolbox
  - No specific hardware required

- for compiled Software:
  - Windows 7, 64 bit or later
  - Matlab Runtime v. 2018a
  - No specific hardware required

**Installation guide:**

- Copy code to Matlab Path, download dependencies (see Wiki on https://github.com/DWALab/Phindr3D) and run from Matlab

**or**

- Install from the provided EXE file and install Matlab runtime if required.

**Demo:**

*To test the functionality of Phindr3D please download an excerpt of the full data sets from Zenodo:* The sample data include:

- neuron data set to test the functionality of all Phindr3D core functions (20 3-channel image stacks from four experimental groups from one experimental replicate; size of the zipped data set: 13.8 GB; https://dx.doi.org/10.5281/zenodo.4064148)
- organoid data set to test the functionality of the organoid selection and cropping feature (4 3-channel image stacks with 81 z-planes; size of the zipped data set: 5.4 GB; https://dx.doi.org/10.5281/zenodo.4384912).

Expected output:

- Neuron data set: visualization of the feature space the can be changed using PCA, t-SNE or Sammon mapping; further analysis of the data using clustering or classification. All output plots can be saved from the Phindr3D GUI.
- Organoid data set: cropped organoid volumes will be saved as individual TIF files into the specified output folder.

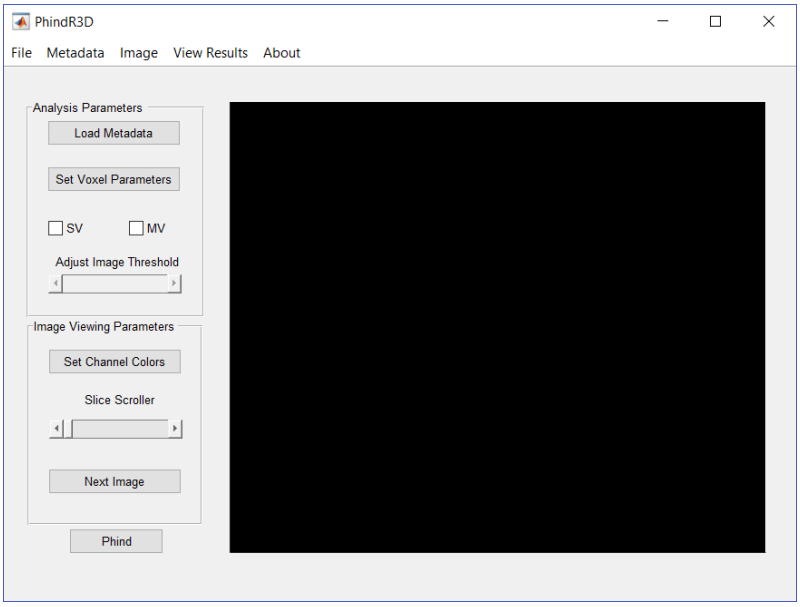
Expected run time on a "normal" desktop computer:

- neuron data set: ~30 minutes to retrieve feature data
- organoid data set: ~20 minutes to crop all organoids

**Instructions for Use**

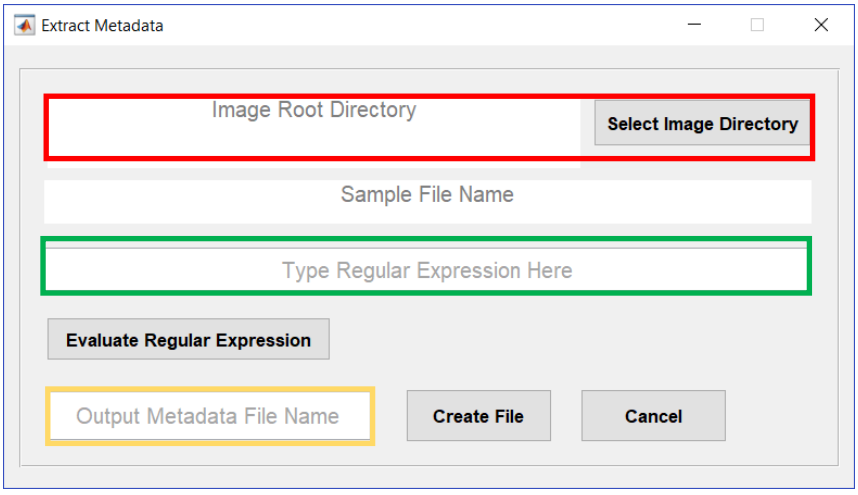
**MAIN GUI**

- Go to folder where code resides. Type <Phindr3D> from Matlab command prompt
- The following window opens up:




**CREATE METADATA**

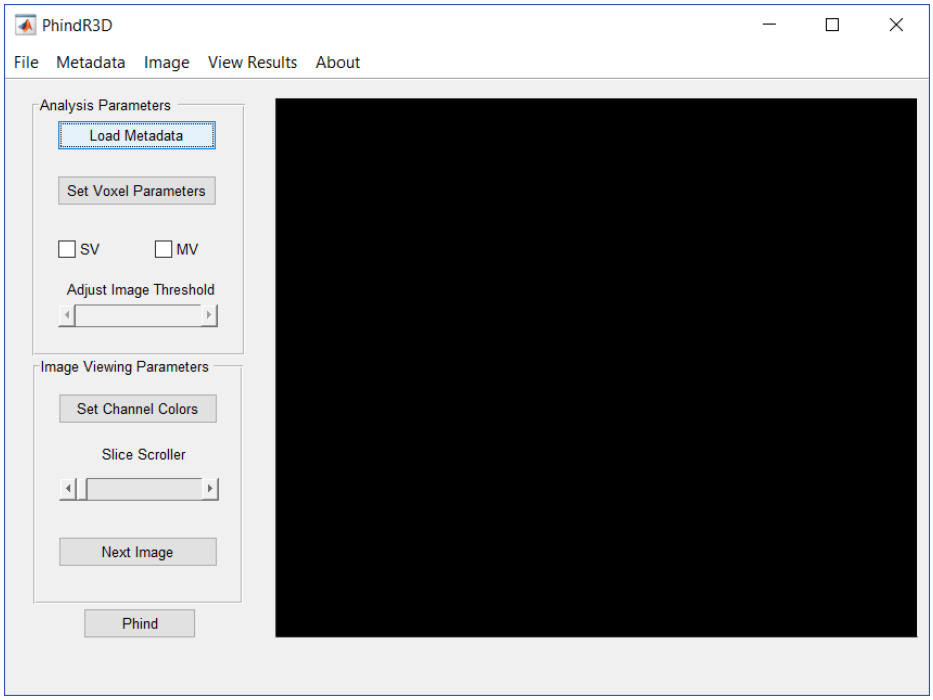
- Click on Metadata from Menu > Metadata > Create Metadata




- Select root directory where images are located (Red box)
- The sample file name automatically fills up
- Type regular expression to extract metadata from file name (Green box).
- *When using the test data or other data generated using PerkinElmer Harmony, type:*
  *(?<Well>\w+)f(?<Field>\d+)p(?<Stack>\d+)-ch(?<Channel>\d).*.tif(f)?*

- For a tutorial see https://www.mathworks.com/help/matlab/matlab_prog/regular-expressions.html
- You can now evaluate the regular expression
- Write a prefix for the file name (Yellow box) and press “Create File”. A metadata file is created in the same directory as the images
- Close this window manually

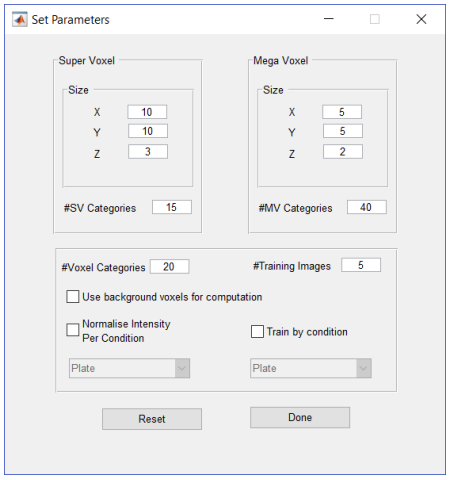
**LOAD METADATA**




- Press Load Metadata and select the metadata file.
- Select channels in the new window and press ok

**SET PARAMETERS**

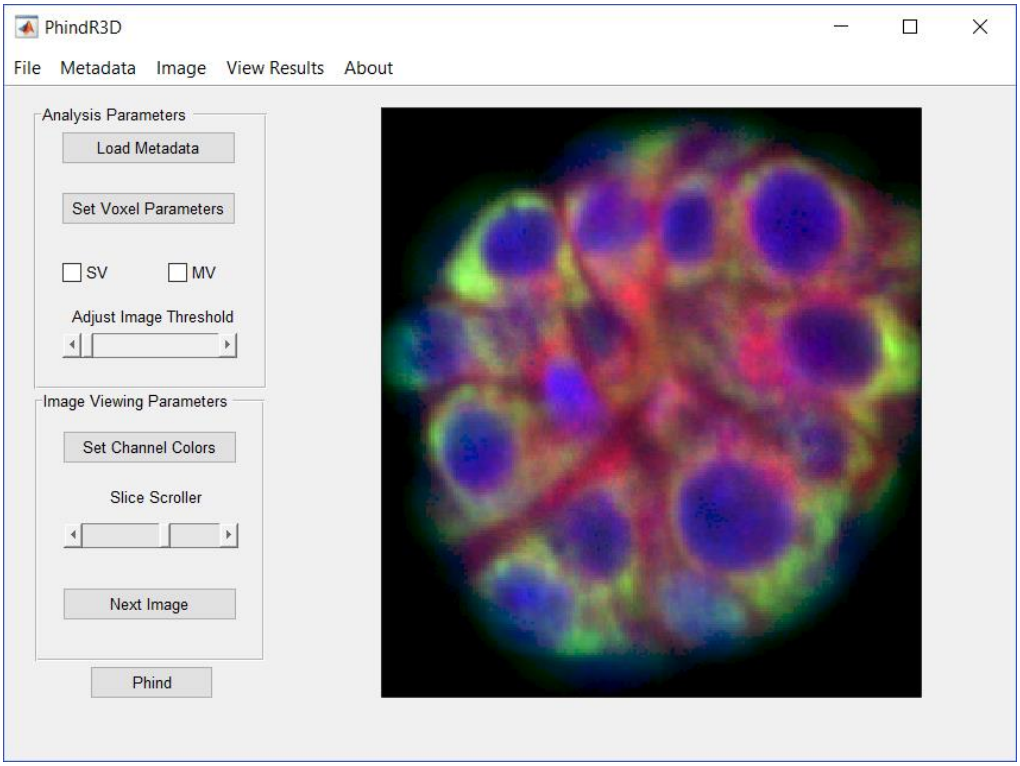
- Standard parameters are set automatically and work fine for many situations.
- However, if the user needs to change parameters press “Set Voxel Parameters”. A new window pops up where the parameters can be changed.



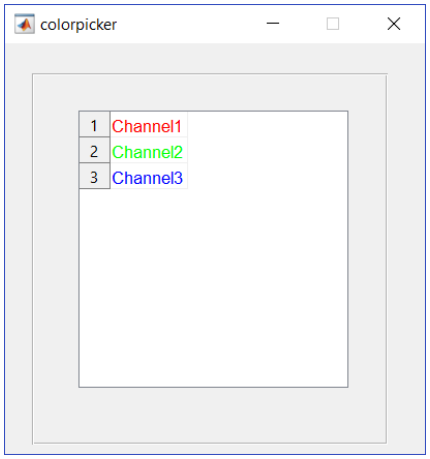

- Press “DONE”

**RUNNING PHINDR3D**

- Multi-channel image is displayed in the main window
- Press “Next Image” to view another image in the folder



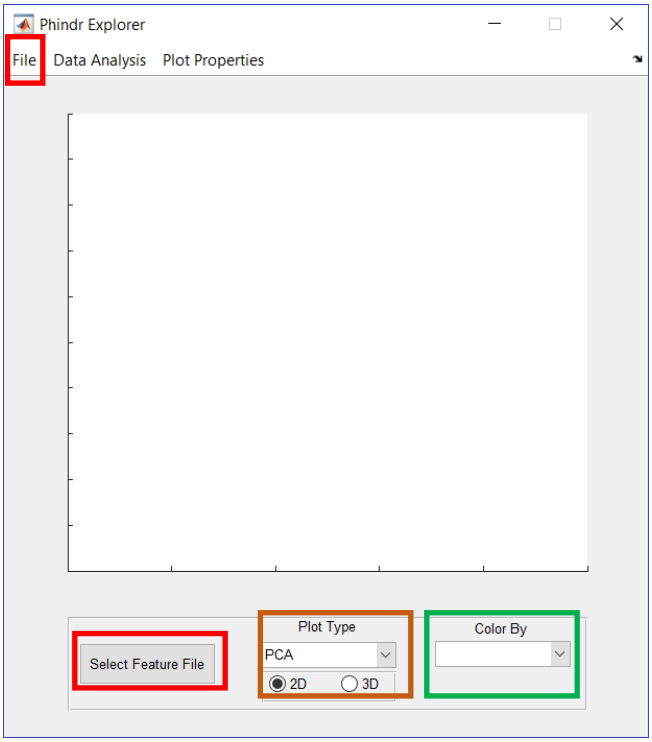
- To *change channel colors* Press Channel colors




- Click on individual channels and select the color for each channel. Close the window once you set colors
- To see *SV/MV,* check the SV or MV box
- To extract features, Press **Phind** button. Enter file name for the output of the features
- Once completed, a feature file is created in the output directory provided by the user

**VIEW PHINDR3D RESULTS**

- Click <View Results> from the main window

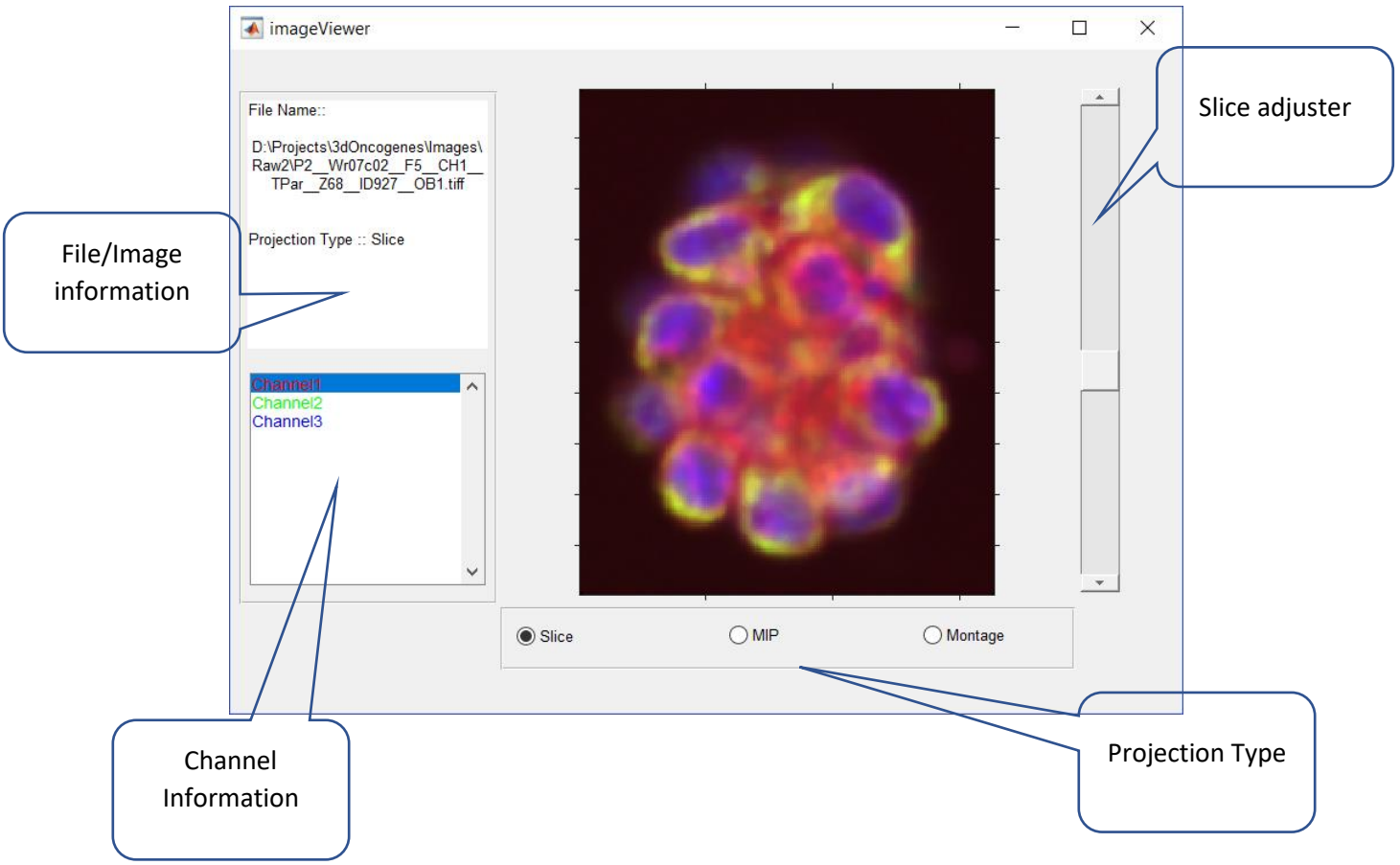



- Select output feature file by clicking either <*Select Feature File*> or *File>> Input Feature File* from the main menu (Red).

- Once the feature file is selected, the software will ask the user to define Metadata (i.e. all coloumns except the features [MV1, MV2, etc]) from the feature file. After this, the software will ask to select the Channel columns.
- Phindr3D performs a PCA and loads the plot on the plot window. The user can change the type of plot using the drop down menu as well as choose between 2D/3D plots (Brown box)
- The user can color the points using one of the metadata Columns (Green box)
- The plots can be exported as vector graphics (SVG file)

**SHOW IMAGE**

- To see the corresponding image for any point on the plot, hover over the point, right click and click show image. A new image window is shown:



**CLUSTER IMAGE DATA**

- *Automated Cluster Estimation*: Click Data Analysis >> Clustering >> Estimate Clusters
- *Manual Clustering*: Click Data Analysis >> Clustering >> Set Number of Clusters
- *Pie Charts*: Click Data Analysis >> Clustering >> Pie Maps (Only available after clustering)
- *Export*: Click Data Analysis >> Clustering >> Export Clustering Results (Only available after clustering)

**CLASSIFY IMAGE DATA**

- Click Data Analysis >> Classification >> Select Classes
- Select the classes and click OK. Once the classifier is built, the output is saved at a user-defined location