System requirements:

- for Matlab Code:
  - Windows 7, 64 bit or later
  - Matlab v. 2017b or later with statistics toolbox and image processing toolbox
  - No specific hardware required

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

Installation guide:

- Extract code from ZIP File and run from Matlab

or

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

Demo:

For reviewing purposes and to test the functionality of Phindr3D please download an excerpt of the data anonymously from

https://owncloud-ext.charite.de/owncloud/index.php/s/PrkuPfOTl0MN1x0 (password: Phindr3D)

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)
- 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).

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.

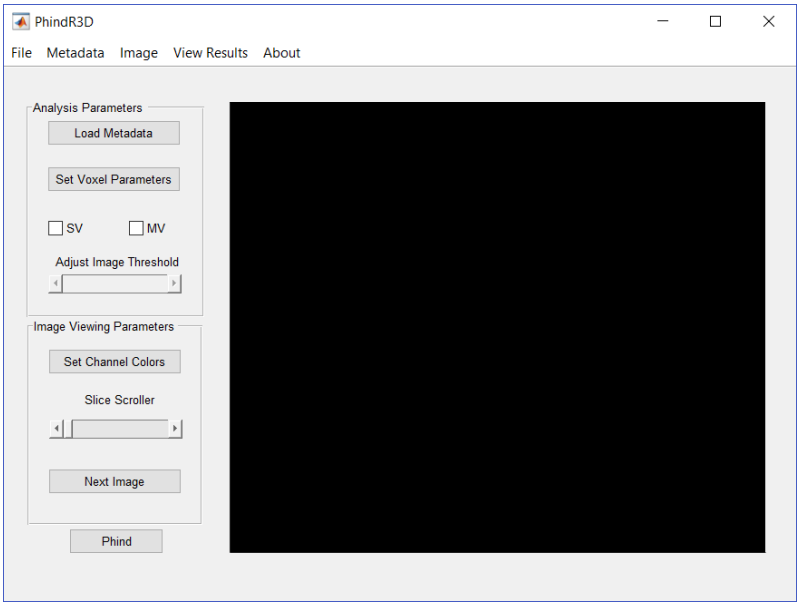
Expected run time on a "normal" desktop computer:

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

Instructions for Use

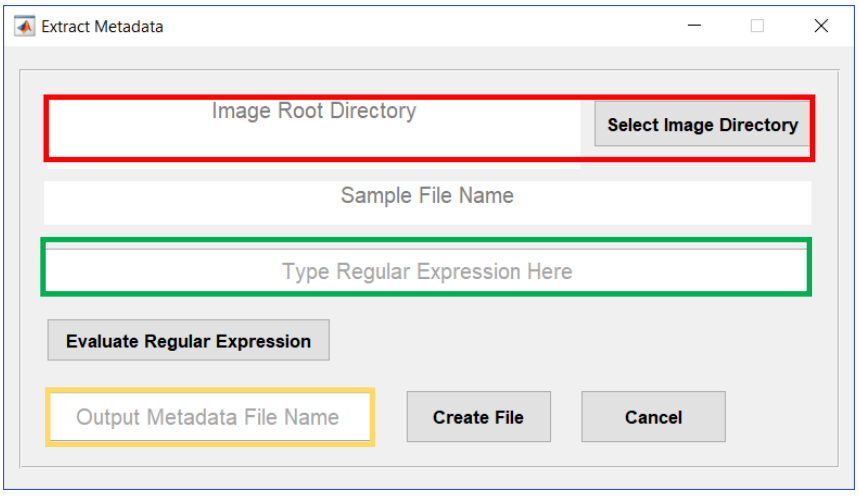
MAIN GUI

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



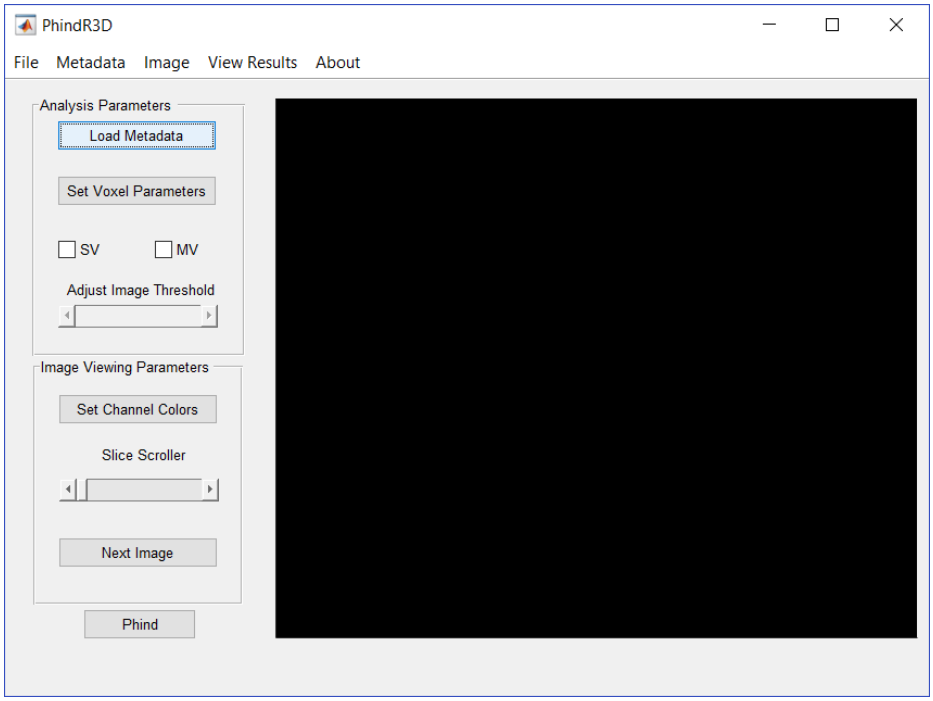
Select root directory where images are located (Red)
The sample file name automatically fills up
Type regular expression to extract metadata from file name (Green).

When using the test data, type

(?<Well>\w+)f(?<Field>\d+)p(?<Stack>\d+)-ch(?<Channel>\d)..tif(f)?*

For tutorial (https://www.mathworks.com/help/matlab/matlab_prog/regular-expressions.html)
You can evaluate the regular expression.
Write a prefix for the file name (Yellow) and hit Create File. A metadata file is created in the same directory as the images

LOAD METADATA

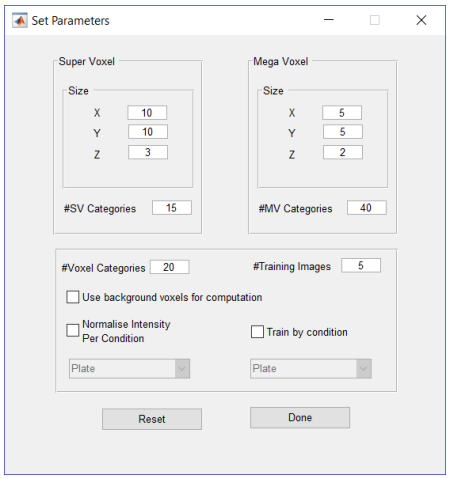


Press Load Metadata and select the metadata file.

Select channels in the new window and press ok

SET PARAMETERS

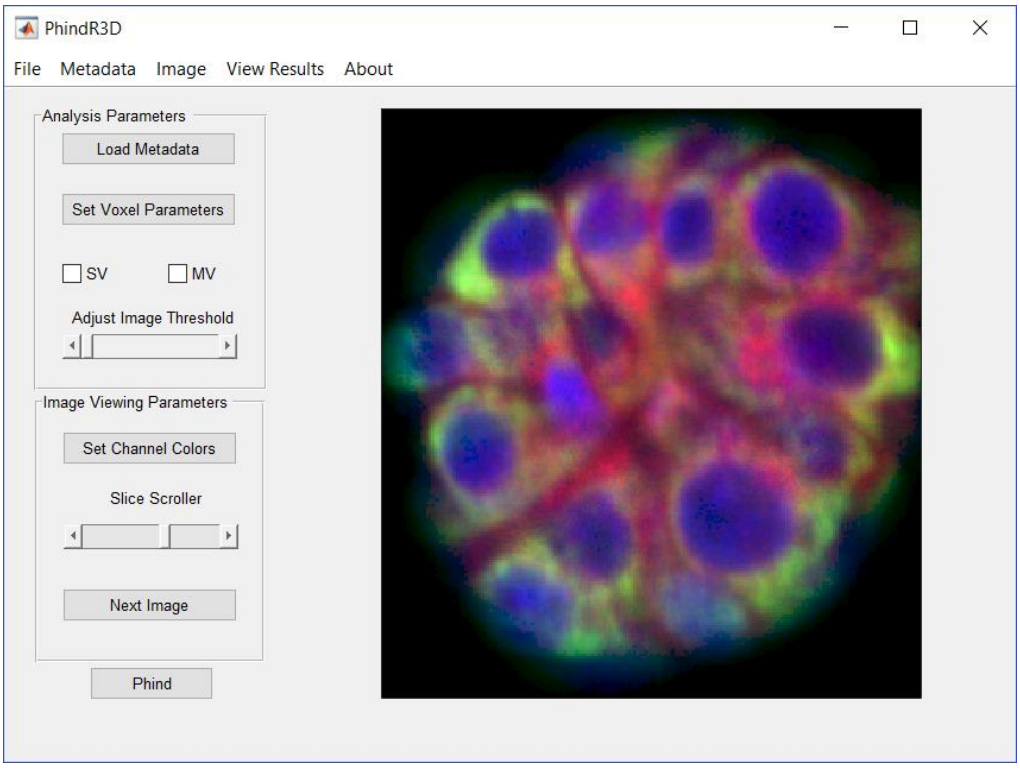
Parameters are automatically set. However, if user needs to change parameters press “Set Voxel Parameters”. A new window pops up where the parameters could be set



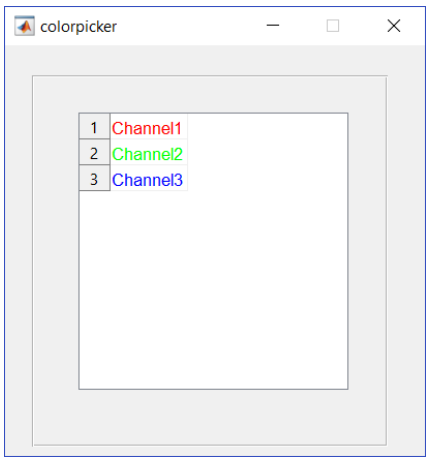
Press DONE

RUNNING PHINDR3D

Multi-channel image is displayed in the main window



To *change channel colors* Press Channel colors



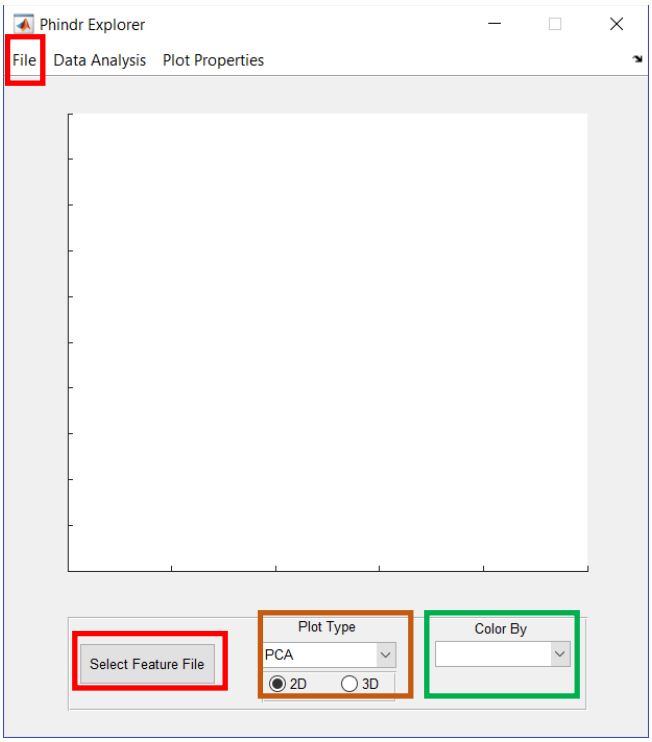
Click on individual channels and select the color for each channel. Close the window once you set colors

To see *SV/MV,* check the SV or MV box

To extract features, Press **Phind** button. Enter file name. Once completed, a feature file is created in the output directory provided by the user

VIEW PHINDR RESULTS

Click <View Results> from the main window



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

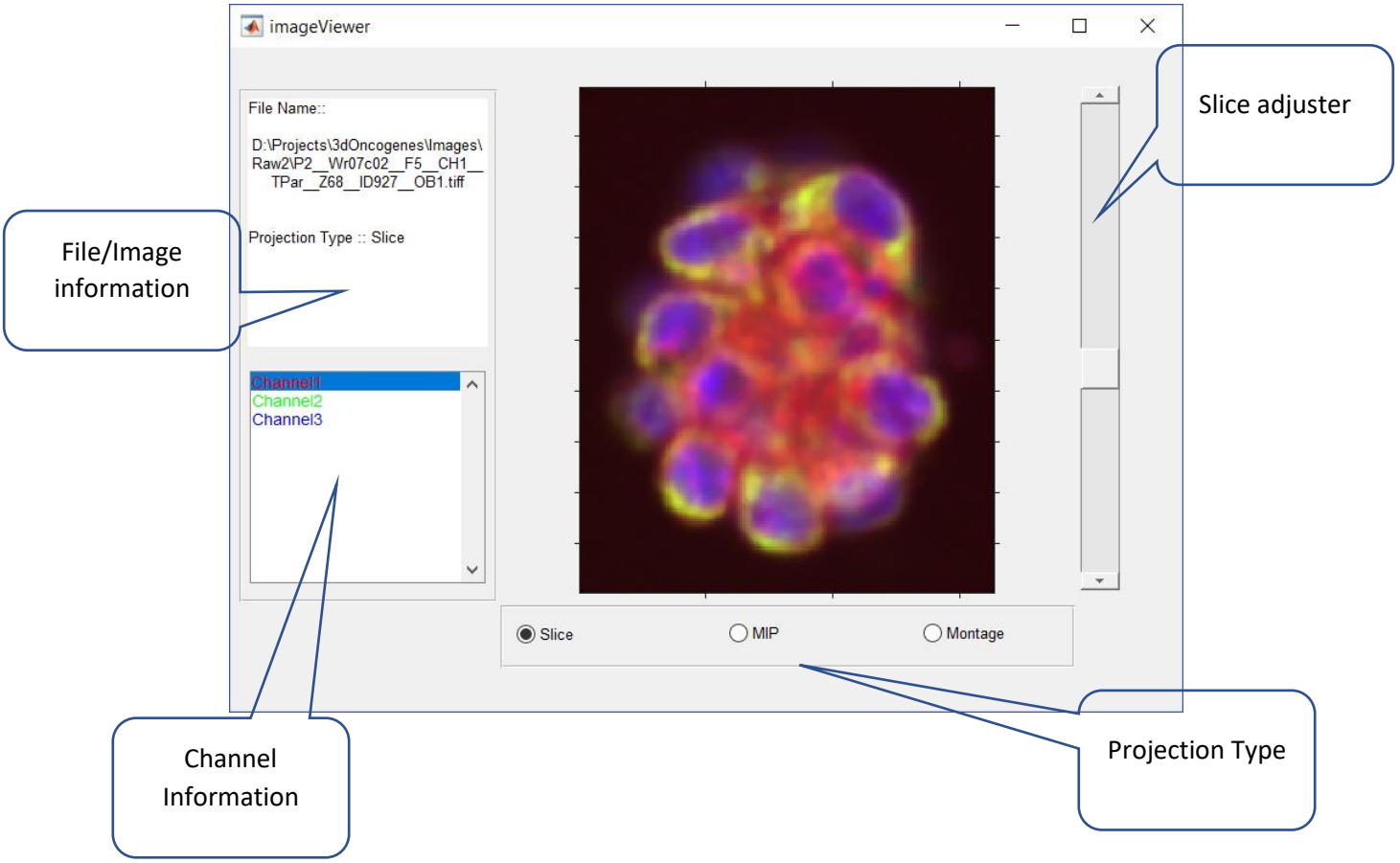
Once the feature file is selected, the software will ask user to define Metadata (i.e. all coloumns except the features [MV1, MV2, etc]) from the feature file. After this, the software will ask to select the Channel columns

PhindR performs as 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)

User can color the points using one of the metadata Columns (Green box)

SHOW IMAGE

To see image for any point. 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