**Vjestica Lab Software Module**

**Image Registration, ver01**

The image registration software module calculates and performs image registration on a timeseries of images.

The module is started by running the python script imageRegistration\_master.py

The script dependencies are *itertools, matplotlib, matplotlib.backends.backend\_qt5agg, matplotlib.figure, matplotlib.pyplot, matplotlib.use("Qt5Agg"), napari, nd2, numpy, os, pandas, PIL, PyQt5, PyQt5.QtCore, PyQt5.QtGui, PyQt5.QtWidgets, pystackreg, sewar.full\_ref, skimage.metrics, sklearn.preprocessing, sys, tifffile, time*

Table

Description automatically generated

The scripts are reliant on a file-naming convention where:

1. All files taken from the same field of view have the same base name. The base name must be unique and mustnot contain underscores, dots, or spaces.
2. The underscore symbol (\_) is used to separate the basename (left) from other pieces of information about the file.
3. The channel or other information about the file comes after the underscore.
4. The file extension indicates the format of the file. The current version of the scripts can process .nd2 and .tif files. All functions in the module can handle 3-dimensional (tyx-axes order) and some can process multidimensional files as long as the axes are specified within the file.

Graphical user interface, text, application

Description automatically generated

**Module functions:**

**Calculate & Perform registration** functions uses only 3-dimensional reference images to:

1. Calculate the registration matrices (see below) used for alignment, and
2. Evaluate and report the quality of alignment.

The function includes an options to:

1. Specify the reference file identifier (e.g. channel such as \_BF)
2. To align and save all images that share the basename with reference file.

Specific functions allow different kinds of input, including:

1. Individual image files
2. Folders of image files
3. Collection of folders of image files

**Align with registration matrices** functions uses registration matrices located in the same folder as the image files to align 3- and multi-dimensional files.

Specific functions allow users to

1. View aligned file without saving it. Works only with 3-dimensional TYX images.
2. Align and save a single image or a set of images associated with a single registration matrix.
3. Align and save a folder of image files containing corresponding registration matrices.
4. Align and save a collection of folders of image files containing corresponding registration matrices.

**Edit registration matrices** functions allows modification of the information inside registration matrices which is used then used to perform the registration.

Specific functions allow users to:

1. Edit the range of an individual registration matrix.
2. Edit the range of an individual registration matrix.

Folders order : registration, alignment, registrationEditing

**Text

Description automatically generated with low confidenceRegistration matrices**

The module relies on registration matrices that carry the information on how much images should be moved in x and y directions to compensate for the sample drift. The names of registration matrix files follow the format:

1. **Basename**, which establishes it’s link with the set of images that they are related to.
2. **Identifier** *‘-RegistrationMatrix.txt’*, which includes the file extension

Each registration matrix consists of:

1. **Header information**, which includes all lines starting with the hashtag character.
2. **Alignment matrix**, which includes columns:
   1. **timePoint**: timepoint of the original image, starting with index 1
   2. **align\_t\_x** : x-axis displacement, which can be modified by users and used when performing alignment. Integer values.
   3. **align\_t\_y** :y-axis displacement, which can be modified by users and used when performing alignment. Integer values.
   4. **align\_0\_1** : inclusion value used in alignment scripts to either include the timepoint into the alignment (value 1) or not to include it (value 0). Value can be modified by users
   5. **align\_t\_x** : x-axis displacement calculated during registration, the values should NOT be modified by the user
   6. **align\_t\_y** : -axis displacement calculated during registration, the values should NOT be modified by the user

Importantly, users should not modify values of columns

inclusion