

Usage Instructions for Micro-CT Plant Images

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1 Releases

This software works as a iteration-based release, adding new features and optimising and as such please refer to releases for the latest stable version.

2 Usage

Usage of this software is straightforward. Inputting a directory, a voxel size and a minimum size of expected grain objects will output and write grain statistics and image to file.

2.1 Setup variables

A brief setup of environment variables are required, this is an example:

```
1 voxelSize = 68.8; % or whatever micro-meter to voxel ratio was used in scanning
2 minimumGrainSize = 10000; % a minimum grain size of interest
3 structEleSize = 5; % a size of structuring element to use for morphological operations
4
5 % Every folder in CT-Scans folder and every ISQ file in them
6 directory = '/home/files/CT-Scans/*.ISQ';
7
8 % More optional parameters which change type of scanning
9 startFrom = 1;
10 endAt = 0;
11 watershed = true;
```

2.2 Optional Parameters

- **startFrom** indicates file to start at from the directory indicated, almost always use '1'. It's useful for if processing fails partway through
- **endAt** is where the process will stop, useful for if you'd only like to use a few scans for testing. Default is 0 and this will process everything
- **watershed** will be a boolean value which will toggle if the more robust and strict watershed is triggered - this will make processing take a lot longer and at times may over-segment. Generally only use if scans are failing to separate properly, or are particularly small (i.e. wild types / barley)

2.2.1 N.B.

Scanco has been known to alter the filenames for a reason known only Minerva would know. So be on the lookout for:

```
1 C0000123.ISQ;1
```

To get around this I recommend running a script something like this (or a sed equivalent):

```
1 from glob import glob
2 from os import rename
3
4 [rename(f, f.replace(';', '')) for f in glob('*.ISQ;1')]
```

2.3 Running

Running the program is as simple as calling the `processDirectory` function.

```

1 % Will process all files found by rdir function
2 processDirectory(directory, structEleSize, voxelSize, minimumGrainSize);

```

3 Files and Functions

3.1 cleanWheat

`cleanWheat` is a function which takes as input a filename location on disk of an ISQ raw image, it processes it and outputs a binary 3D image and a greyscale 3D image which has been cleaned and segmented.

3.2 countGrain

`countGrain` takes cleaned image data, separates each identified grain and computes statistics on a grain-per-grain basis. It returns two statistics objects, one with raw pixel data counted and another with computed metric values.

3.3 filterSmallObjects

`filterSmallObjects` attempts to remove all objects which are smaller than the specified parameter during setup. This uses pixel size **not** metric sizes for this.

3.4 imSurface

`imSurface` is a library originally by David Legland. It measures the surface area in pixels of a 3D object.

3.5 processDirectory

`processDirectory` is the main controlling function of this software, it moves image data around from function to function, gathers image results/measurements and saves it to disk from here.

3.6 subdir

`subdir` is a function which recursively finds files, it is used to find files in sub-directories by using the '*' wildcard in the directory name parameter.

- This function was redone as the previous 'rdir' has operating issues with certain versions of windows.

3.7 readISQ

`readISQ` originally developed by Johan Karlsson, we have modified it to make speed increases and added specific slice loading, this helps for increased speed when processing larger images

3.8 segmentRachis

`segmentRachis` finds locations of nodes along the rachis of spikes of wheat, oats etc. Use of this data is primarily for locating joining points of split scans.

3.9 watershed3D

`watershed3D` incorporates traditional watershedding techniques and has adapted them to work in 3D. It also makes use of modernised distance-based watershed methods, by way of chessboard distance technique.

3.10 writeTif








`writeTif` writes image stacks to disk as TIF formatted files.

4 Output

From successful running of this software output will be:

- A statistics of grains CSV with metric values
- A statistics of grains CSV with raw values
- A TIF file of the segmented image
- A statistics file of the rachis top and bottom points.
- A folder of 2D cross sectional images, for each grain
- A folder of 3D TIF files, each a individual grain

The output folder should look similar to this:

Name	Size
 C0001375.ISQ.csv	7.3 kB
 C0001375.ISQ	533.7 MB
 C0001375.ISQcleaned.tif	267.3 MB
▶  C0001375.ISQ-grains	37 items
▶  C0001375.ISQ-grain-stacks	37 items
 C0001375.ISQ-raw_stats.csv	3.4 kB
 C0001375.ISQ-rstats.csv	32 bytes