Oxygen Imaging Respiration Tool
oxIR v0.1 – Quick guide

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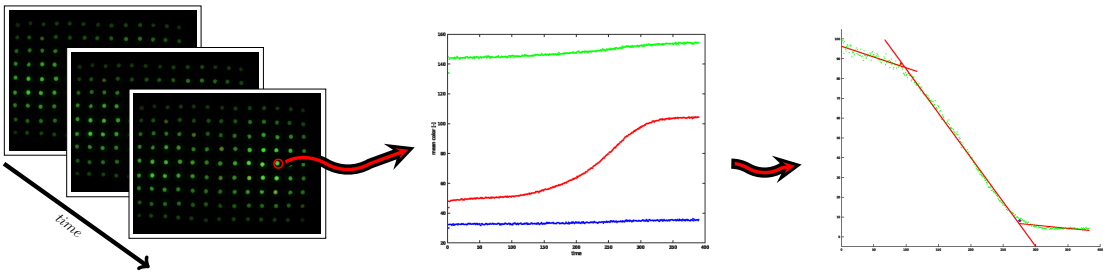
A tool to estimate change of oxygen content for a time series of grid samples



Input images
Time series

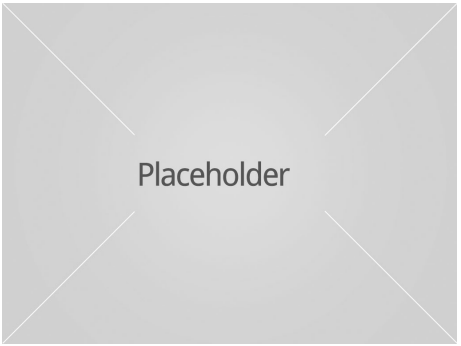
Mean RGB values

Estimated oxygen content

Contents

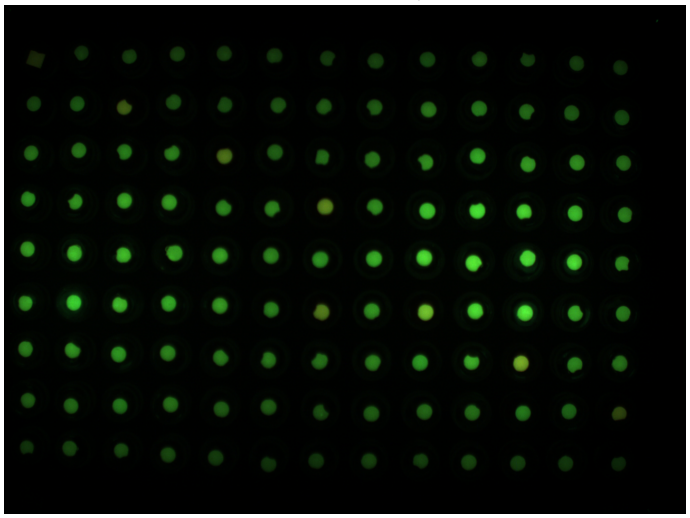
1 Introduction

oxIR tool was developed within the scope of a master thesis [1] aiming to provide an efficient possibility to estimate oxygen content and so respiration rates of seed samples of grid cells over time obtained by abc facility (Fig. 1a). The abc facility generates fluorescence images (Fig. 1c) as time series of a defined grid as exemplary given in Fig. 1b.



(a) abc facility

(b) Standard 96-well microtiter plate on the left and a typical example of an obtained image by the abc facility.



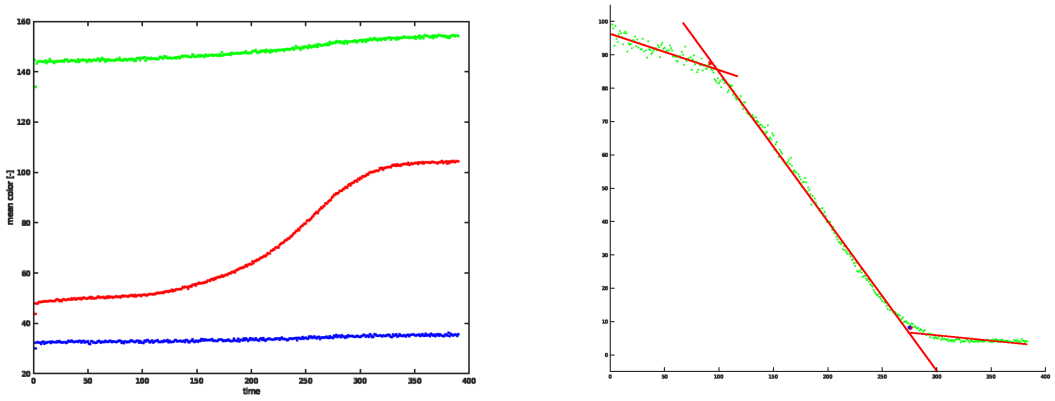
(c) Example fluorescence image.

Figure 1: The abc facility as it was used to produce our sample images.

The change in mean colour channels (i.e. red, green, blue, Fig. 2a) of each sample sport over time can be used to calculate the oxygen content at each time point and so the respiration rate for each sample Fig. 2b). Please refer to [?, ?] for a detailed explanation for the underlying formulas.

1.1 Key Features

The oxIR tool is implemented to semi-automatically process a time series of abc images (a set of images) consisting of an arbitrary number of samples in order to estimate the oxygen content for each time point and further to calculate the rate of change during time, i.e. the respiration rate of seed samples.



(a) Example of the changes in mean red/green/blue values of one particular sample spot.

(b) Oxygen content over time (green dots) together with three linear regessions one for the beginning, one for the middle and one for the last part of the data set.

Figure 2: todo

- user defined grid layout (as optional input)
- automated detection of sample points
- calibration to K0 and K100 reference spots (defined within the grid layout)
- calculation and storage of (done for each spot and for the for the whole time series):
  - mean red and green values
  - green/red ration
  - oxygen content
  - rate of change in oxygen content, i.g. slope of fitted linear functions

The user can adjust diverse algorithmic parameters to influence the behaviour of the calculation.

2 Quick Start

2.1 How to install?

After unpacking the zip archive following two folders will be generated:

```
root
├── oxIR
└── quickGuide
```

The oxIR tool folder contains the pre-compiled executable of the computer program, example grid layout files, a readme- and a license file. Please, read both text files carefully before starting the program. The *quickGuide* folder contains a copy of this file. Example data can be download from the project page as described in Sec. 3.

2.2 How to run?

The oxIR tool comes compiled in two versions, one for Linux- and one for Windows-based operation systems, respectively. To run the program the user has to install the MATLAB Runtime Environment. Since the oxIR tool was developed, tested and compiled under MATLAB 2018b, we recommend to install exactly the same version, i.e. MCR 2018b, which can be downloaded from the official MATLAB side Install and Configure the MATLAB Runtime.

2.2.1 Linux

Under Linux-based operation systems one has to open a terminal and switch to the folder which contains the oxIR tool . Then type

```
./run_oxIR.sh /path/to/your/MATLAB/Runtime/v95
```

where */path/to/your/MATLAB/Runtime/v95* specifies the path to the locally installed MATLAB Runtime Environment (version 2018b - v95).

2.2.2 Windows

To run the program under Windows double-click on the icon of the provided executable in the Windows file explorer or start it with its name from the command line.

2.3 The Interface Layout

The major elements of the software interface include an **Input-area** at the upper part and an **Output-area** at the lower part of the GUI as shown in Fig. 3.
At the input area (Fig. 3 - I), the user can select the folder containing the input images, define the underlying grid layout and set the algorithmic parameters. By selecting the *Start* button all images of the input folder are processed according to the defined settings. A live feedback at the lower part of the GUI (Fig. 3 - II) is given during the whole process of calculation.
Fig. 3 - III provides an example image of the time series with the applied coordinate system (A-H and 1-13 labels) as well as the internal naming defined within the grid layout file (see Section 2.4 below for details about "How to define a grid layout" file.

2.4 First Run

The typical steps to analyse an experiment (a time series of images) are:

1. select an input folder (containing a time series of images)
2. (optional) change the grid layout
3. (optional) adapt the parameter settings
4. run the analysis
5. define the cut-off values according to the given preview data (to get rid of leading and ending problematic measurements)
6. the linear regressions are calculated and presented

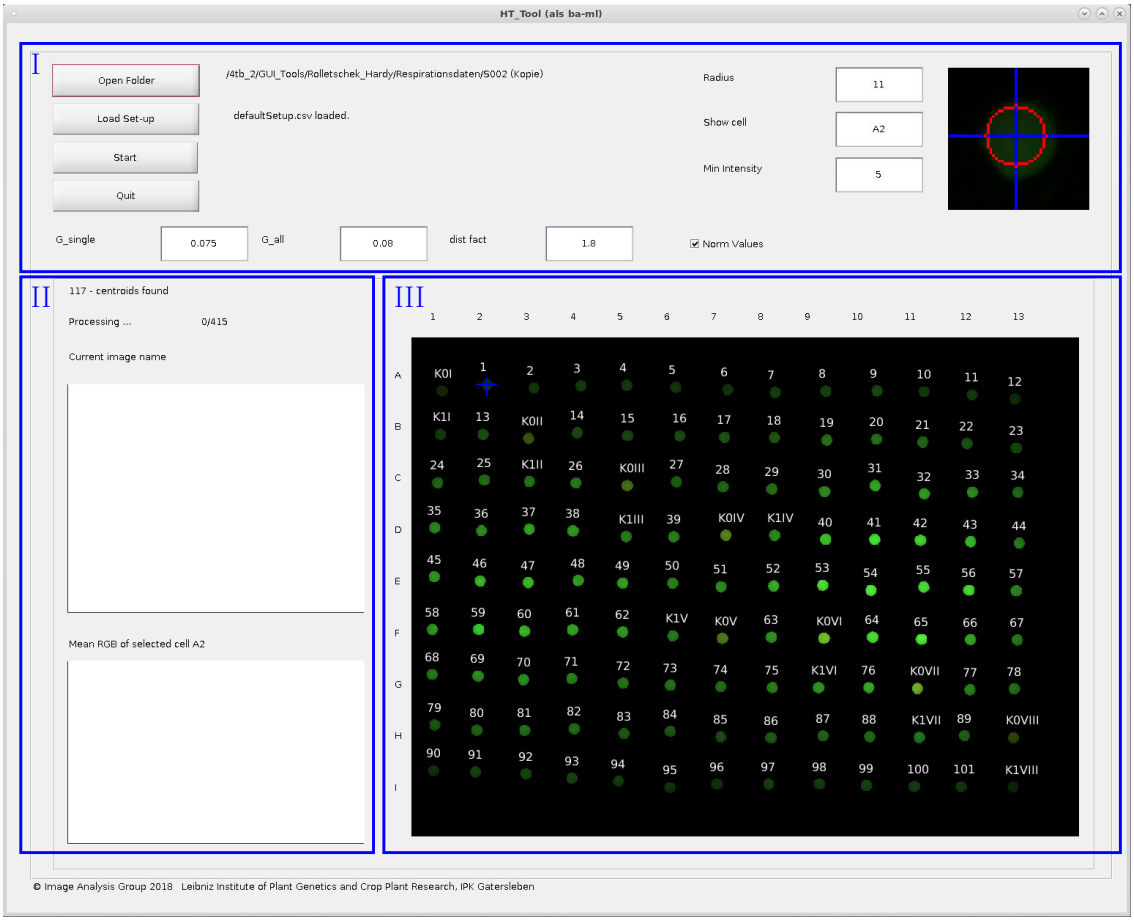


Figure 3: The graphical user interface (GUI) of the oxIR tool .

7. accept or withdraw these calculations; when accepted the results are stored within the input folder, if not the user has possibility to go back to step 5 to define new cut-off values

To run the program, the user has to select the input folder of the time series of images. Once the folder was found and successfully imported, the images of the selected folder are automatically analysed to determine the position (and number) of sample spots with the used configuration. The user now has the chance to adapt some algorithmic parameters. These parameters are:

radius radius $[pixel]$ of the sample spot; all pixel within a circle of the defined radius are considered for calculation. The preview of the selected sample spot and the main overview image are automatically updated after every change.

min intensity minimal intensity applied to each colour channel separately; defines a threshold value

Show cell defines the sample spot which is chosen/selected to be visualised as direct feedback. The images of the sample spot is updated during the whole calculation.

Min intensity The minimal intensity which is taken into account for mean colour calculation for each colour channel. Each pixel within the defined radius with a lower intensity the "min intensity" is removed from the calculation for each colour channel individually.

G-single Grenzwert singel ... todo

G-all grenzwert all ... todo

dist fact distance factor ... todo

Norm Values normalise RGB values ... todo

By default the default grid layout is loaded and applied. To change the gird layout one can select a new layout file by clicking on the "Load Set-up" button. A layout file is a simple CSV-file containing the label for each spot, separated by colons or tabs:
defaultSetup.csv

```
K0I,1,2,3,4,5,6,7,8,9,10,11,12
K1I,13,K0II,14,15,16,17,18,19,20,21,22,23
24,25,K1II,26,K0III,27,28,29,30,31,32,33,34
35,36,37,38,K1III,39,K0IV,K1IV,40,41,42,43,44
45,46,47,48,49,50,51,52,53,54,55,56,57
58,59,60,61,62,K1V,K0V,63,K0VI,64,65,66,67
68,69,70,71,72,73,74,75,K1VI,76,K0VII,77,78
79,80,81,82,83,84,85,86,87,88,K1VII,89,K0VIII
90,91,92,93,94,95,96,97,98,99,100,101,K1VIII
```

3 Provided example data

The oxIR tool comes with two set of example images consisting of 100 or 200 images of the same experiment.
The images can be download from the project page at https://ag-ba.ipk-gatersleben.de/oxir.html

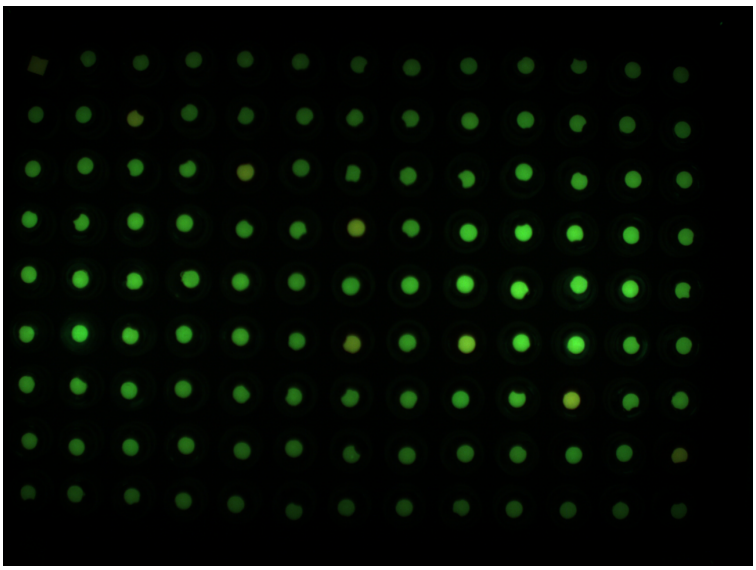


Figure 4: Example of a shoot image of a mid-age wheat plant.

A Funding

todo

B Acknowledgments

We would like to thank ... gott und der welt für alles und nichts ... todo.

C Links

For details of the abc facility we refer to the description on companies web side (https://www.abc.com/products/image.html).

D References

[1] Zornow, Master Thesis, todo

[2] Henke, M., Zornow, R., Rolletschek, H., Gladilin, E., paper: todo, *in preparation*

[3] Rolletschek, H. and Liebsch, G. (2017) A Method for Imaging Oxygen Distribution and Respiration at a Microscopic Level of Resolution, Kapuganti Jagadis Gupta (ed.), Plant Respiration and Internal Oxygen: Methods and Protocols, Methods in Molecular Biology, vol. 1670, DOI 10.1007/978-1-4939-7292-0_3, Springer Science+Business Media LLC 2017

E Terms of use

1. The oxIR tool and the example image data are distributed for non-commercial usage WITHOUT ANY WARRANTY under the terms described in the EULA license. See the included

EULA.txt file for details.

2. The user manual is intellectual property of the Image Analysis Group of the IPK Gatersleben. The user may download and use the tool and information available on our web site.

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