Press ANALYZE EXISTING IMAGES on http://www.arctoa.ru/areoana/ You can select projects for only one species from dropping menu of species or look throughout the table for the list of existing images/species. Green mark means that project has an open status.

log list o spec	out <u>user'</u> f project ciesall	<u>s form</u>	Y				
sho	ort_form						
N	ID_PID	master_login	species	sample	shoot	leaf	frame
1	<u>31</u>	ivanov_ivanov	Cyrtomnium_hymenophyllum	Fedosov_5_103	1	4	m 1 2 3 4 5 6 7 8 9 31
2	<u>51</u>	ivanov_ivanov	Bryum_cryophilum	Afonina_28_07_76	1	<u>6</u>	<u>m 1 2 51</u>
3	<u>58</u>	ivanov_ivanov	Bryum_cyclophyllum	Fedosov_08_14	1	2	<u>m 1 2</u> 58
4	<u>61</u>	ivanov_ivanov	Bryum_cyclophyllum	Fedosov_08_14	1	3	<u>m 1 2</u> 61
5	67	ivanov_ivanov	Bryum_cyclophyllum	Fedosov_08_14	1	5	<u>m 1 1</u> 67
б	<u>70</u>	ivanov_ivanov	Bryum_cyclophyllum	Fedosov_08_14	1	2	m 1 2 70
7	<u>78</u>	ivanov_ivanov	Cyrtomnium_hymenophylloides	Pisarenko_12_09_09	1	4	m12345 78
8	84	ivanov_ivanov	Cyrtomnium_hymenophylloides	Pisarenko_12_09_09	1	5	m12345 84
9	<u>119</u>	ivanov_ivanov	Cyrtomnium_hymenophyllum	Malashkina_08_08_11	1	6	<u>m 1 2 3 4 119</u>
10	13	ivanov_ivanov	Rhizomnium_andrewsianum	Ignatov_11_4131	1	5	m 1 2 3 143
11	147	ivanov_ivanov	Rhizomnium_andrewsianum	Ignatov_11_4131	1	2	m1 2 3 147
10	155	4	mt :	1	2	2	La treel

To have a look at the digitized image of a leaf, press a number in the column `leaf''. This is a demo mode: here you are able to see digitized image with previous analysis of the specimen (if it has been done), but cannot mark areas of your own interest.

To put and keep your marks and parameters of the analysis, you need to go to the column ID_PID, which shows technical details of the specimen, <u>and duplicate</u> the project for personal analysis (press DUPLICATE PROJECT). After doing this, the file with your login will appear at the end of the project list table. This file has two numbers: the latter number is that of original project, and the former being new unique number of your project.

Dulpication with this option allows you to save all marks and parameters which you have put for image analysis (they are saving by pressing button CONNECT SERVER TO UPDATE). All these parameters of your analysis will be unseen for other users, unless you like to share them; for the latter put 1 in the box "shared", where as default is 0 "not shared".

logout	
name	T_mnioides_bakalin_18_20_07_01_02
species-sample	Tetraplodon_mnioides_Bakalin_18_20_07
shoot	
leaf	2
shared	

You will see a digitized image of leaf and a table below it.

To start the analysis, please select a number in a column "leaf".

log list c	out <u>user'</u> f project	<u>s form</u>					
spe	ciesall						
sh	ort_form		a Commission	Energy and the second sec	Transactor	The second	
И	ID_PID	master_login	species	sample	shoot	leaf	frame
1	<u>31</u>	ivanov_ivanov	Cyrtomnium_hymenophyllum	Fedosov_5_103	1	4	m 1 2 3 4 5 6 7 8 9 31
2	51	ivanov_ivanov	Bryum_cryophilum	Afonina_28_07_76	1	<u>6</u>	m 1 2 51
3	58	ivanov ivanov	Bryum cyclophyllum	Fedosov 08 14	1	2	m 1 2 58

Then decide how many areas and parameters you would like to analyze, put a corresponding number of lines in a box "NUMBER OF CELL STATISTICS" and press the button "CLICK TO CONNECT SERVER FOR UPDATE".

If during the analysis you find that you need more lines, just change this number. Then in

set number 1-6 1 and click to mark selected area mark to unselect all marked areas number of cell statistics 7											
i	marked area	parameter	from	to	N_cells	Mean	Sigma	plot number	line color	line width	line smoothnes
0	0	area 💌	1	1000	873	524.875	235.29	0	/home/	2	20
1	0	length 🗾	1	100	960	38.2634	12.7547	p i	1	2	0.3
2		width	1	100	957	20.2738	5.7901	0	1	2	0.3
3	0	orientation	0	90	967	45.6809	29.1032	0	1	2	0.1
4	0	area/boxarea 💌	0.5	1	861	0.750019	0.0737899	0	1	2	0.1
5	D	n_apex	3	10	923	5.52871	1.09663	0	1	2	0.1
6	0	length/width	0	4	928	1.9101	0.567988	0	1	2	0.1

For each parameter the area for measurements and range of values must be indicated.

Marked area can be: ALL cells (marked area = 0), ALL UNMARKED CELLS (marked area = -1) MARKED AREAS (marked area = 1,2,3,4,5,6) within the leaf

To mark an area for analyzing, you should select it by highlighting no less than three cell angles in the corners of this area. For this, click on red dots of cell angles in clockwise order (the order is important!), after successful click the red dot will enlarge. Note that clicks may highlight not only cell corners but also cell walls, which is not what you need for marking area (but needed for other options). If cell wall get highlighted, unselect it by additional click. If image is too small for relevant clicks, enlarge image by Ctrl+. After area outlining, choose the number from 1 to 6 which will be attributed to this area



Marked cells (after updating image by pressing MARK SELECTED AREA) will have corresponding color marks. If you like to exclude some marked cells or include any unmarked cells to the analyzed area, just click to corresponding cell numbers: in example below #263 is unselected (number becomes not boldfaced), while #388 and #422 are additionally selected (their numbers become boldfaced).



Press again "MARK SELECTED AREA": then red dot disappears for #263 and dots appear for #388 and #422. This way you will have marked area more specifically selected. This option allows to exclude cells suspected as wrongly recognized, e.g. too big, etc.



- COLORS OF MARKS:
- 1 red
- 2 -yellow
- 3 green
- 4 cyan
- 5 blue
- 6 purple

You need also to put the range of parameters.

set ma nu	click to connect server for update set number 1-6 1 and click to mark selected area mark to unselect all marked areas number of cell statistics 7											
i	marked area	parameter	from	to	N_cells	Mean	Sigma	plot number	line color	line width	line smoothness	
0	0	area 💌	1	1000	873	524.875	235.29	0	/home/	2	20	
1	0	length 💽	1	100	960	38.2634	12.7547	0	1	2	0.3	
2	0	width	1	100	957	20.2738	5.7901	0	1	2	0.3	
3	0	orientation	0	90	967	45.6809	29.1032	D.	1	2	0.1	
4	0	area/boxarea 💌	0.5	1	861	0.750019	0.0737899	0	1	2	0.1	
5	þ	n_apex 💌	3	10	923	5.52871	1.09663	0	1	2	0.1	

Parameters are shown in μ m (cell length and width) or square μ m (area), if size of pixel has been indicated when file was uploaded; otherwise it is shown in pixels (allowing subsequent recalculation). Indicating FROM and TO, you may select cells within certain intervals (for example, checking difference in orientation or length to width ratio between large and small cells). If interval is unknown, you may put the broad interval, and then adjust to proper values. Usually all values are of interest, so the range can be set *a priori* broader; however, too broad range reduces plot clearness, because the axis X in plots is restricted by values indicated in FROM and TO.

Length and width are accepted here as longer and shorter sides of minimal rectangle enclosing a cell. Orientation is the angle (in degree) between costa and side of minimal rectangle around cell (ranging from 0 – the direction of costa, to 90 – perpendicular to costa). Area/boxarea means ratio of cell area to area of minimal rectangle enclosing this cell; it ranges from 0.5 in triangle to 1 in rectangle, showing, among others, how much cell shape deviates from rectangle. "N_angles" means number of cell angles (ranging from (3-)4 to 9(?more).

STATISTICS show number of cells used for parameter calculation, their mean and sigma for the chosen parameters.

Plots below the table show one to six distributions. More than two distributions within one plot can be obtained by indicating the same plot number for the same parameter of different areas. Plot design options include line color, line width, and line smoothness. The latter by default is 20 for areas, and 0.1 for other parameters. However in case of too few cells, the distribution may look too saw-like; it can be smoothed by increasing the line smoothness value. Axis X shows cell parameters values in the interval set in

FROM to TO), axis Y displays number of cells in arbitrary units, smoothed by Gaussian function. Please adjust to you own data, considering the following example:

Area smoothness value 1 provides the following plot (number of cells 2838):



Area smoothness value 7 provides the following plot for the same example:



Area smoothness value 30 provides the following plot for the same example:



To obtained the better performed distributions for areas with contrastingly different number of cell, the smoothness value should be the higher the more cells are in the selection.