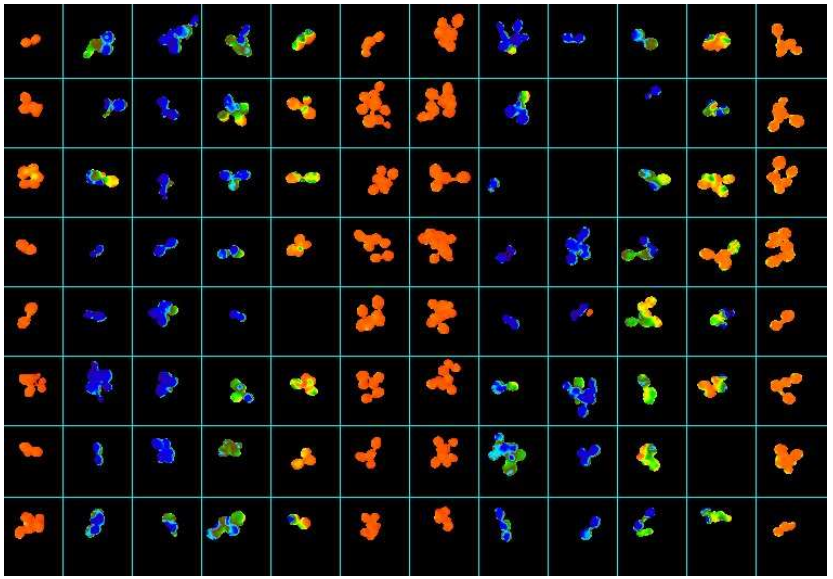
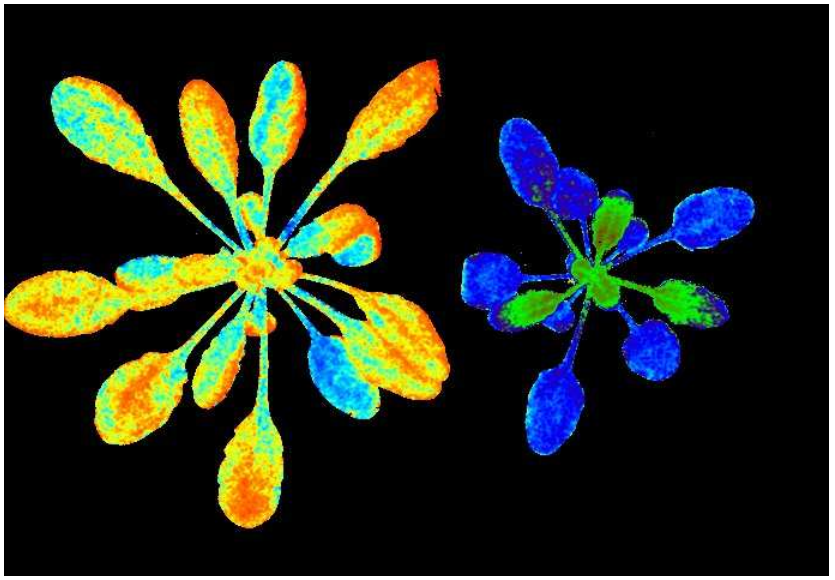
cf imager technologica

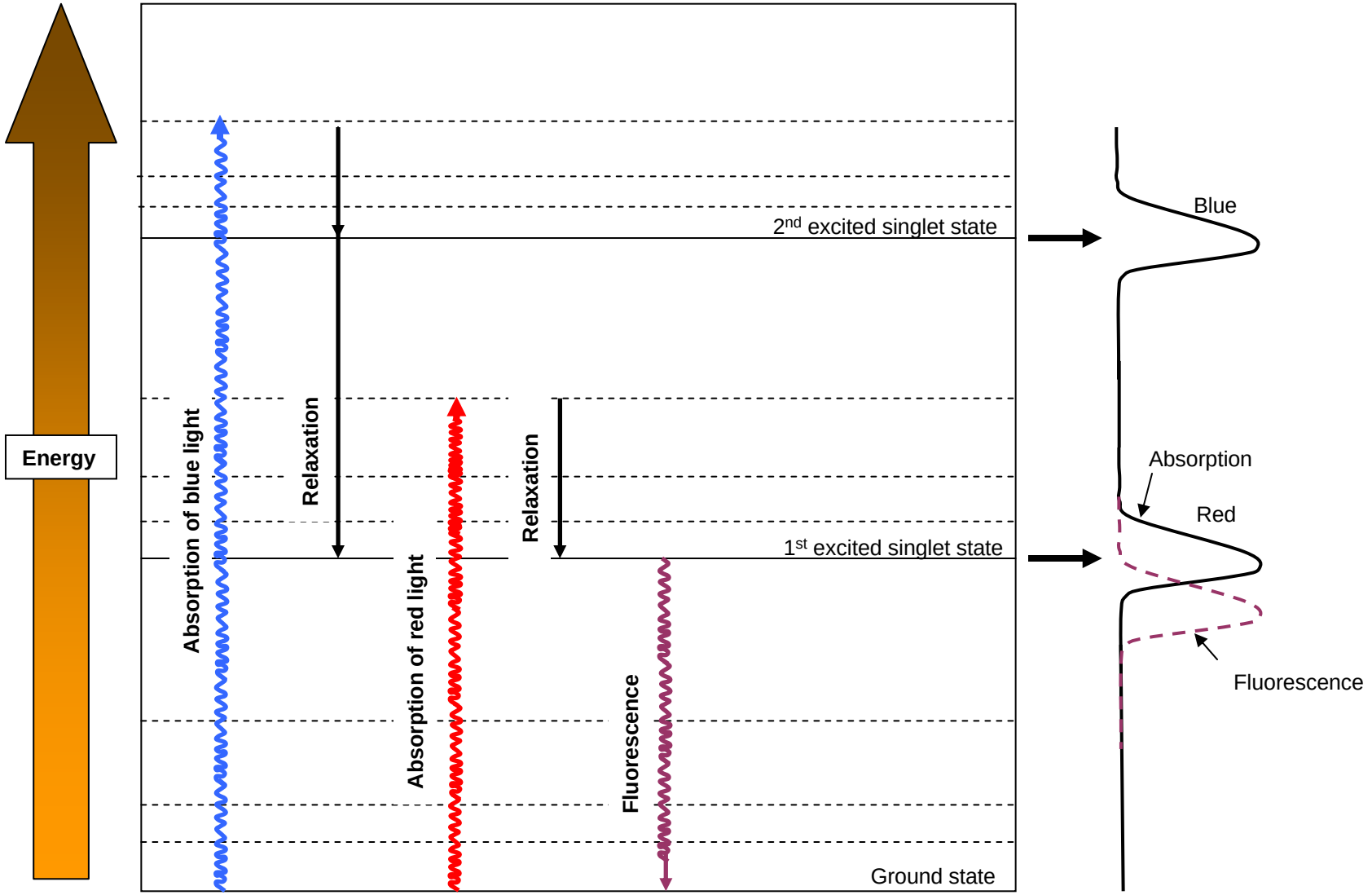


Chlorophyll *a* fluorescence



Patent No. GB2380790

www.technologica.co.uk


Energy
Absorption of blue light
Relaxation
Absorption of red light
Relaxation
Fluorescence
2nd excited singlet state
1st excited singlet state
Ground state
Blue
Absorption
Red
Fluorescence

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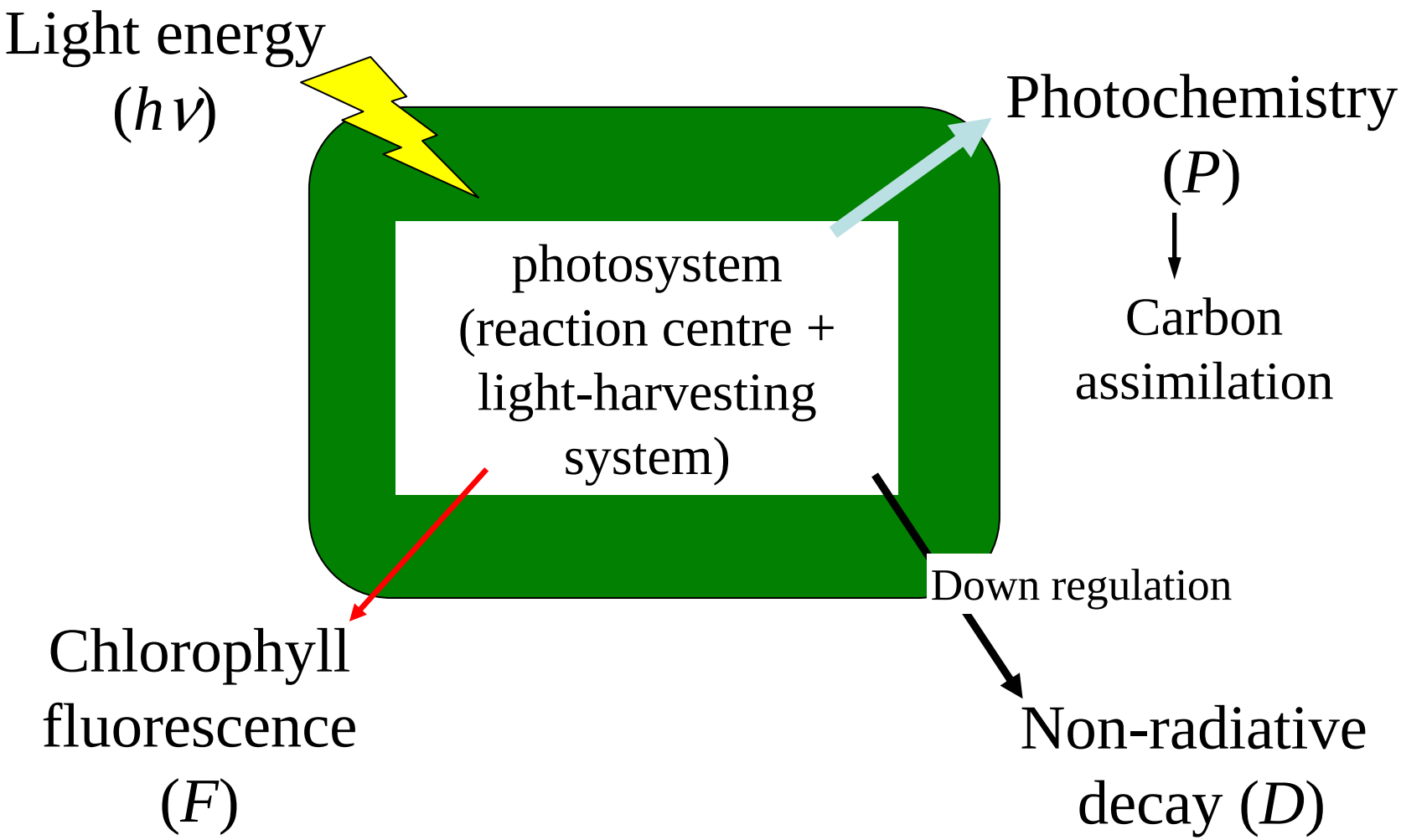
Fates of excitation energy



Rate constants $P + F + D = 1$

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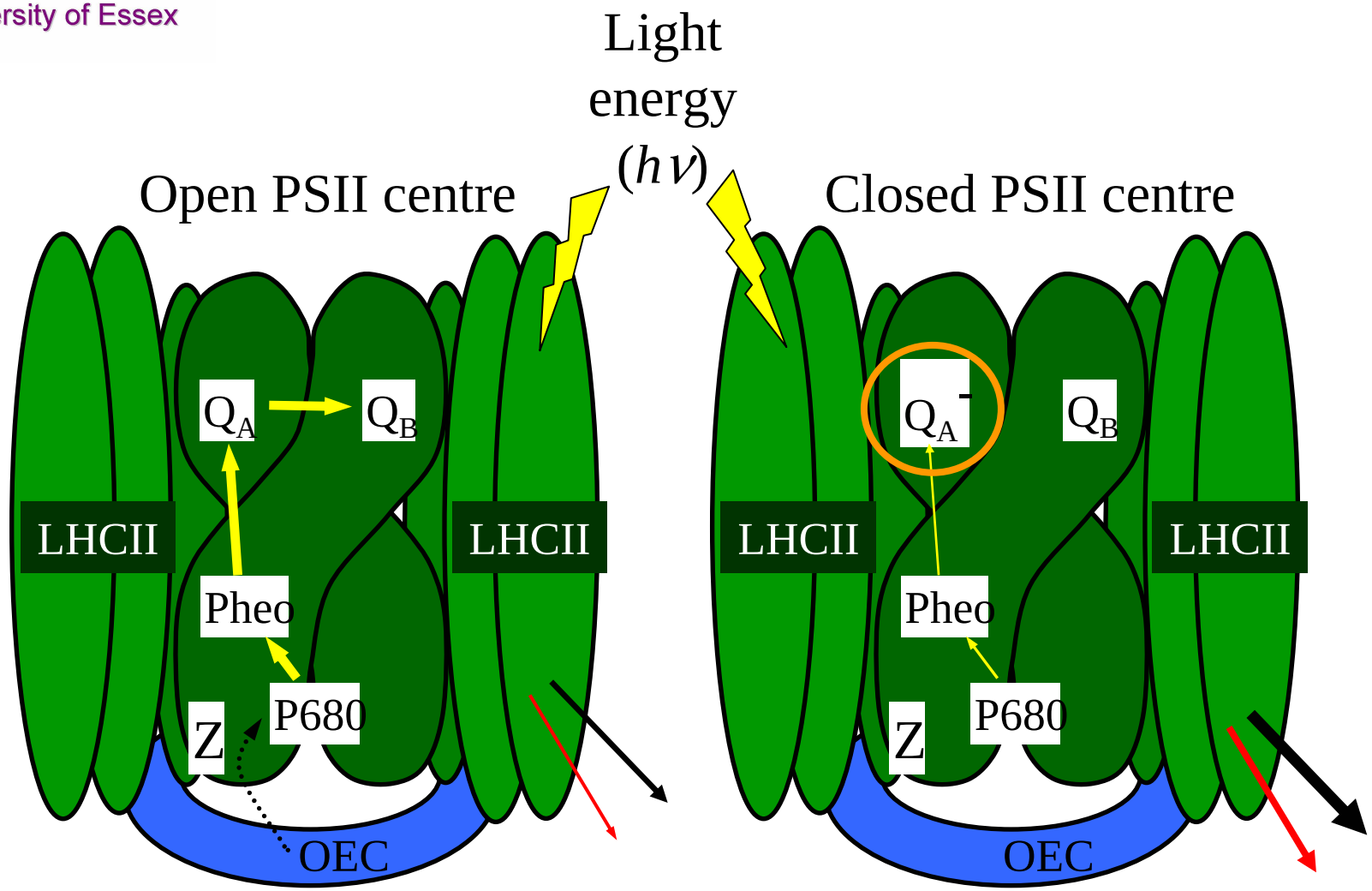

ϕ_P (photochemistry) = very high

ϕ_D (non-radiative decay) = low

ϕ_F (fluorescence) = very low

ϕ_P (photochemistry) = very low

ϕ_D (non-radiative decay) = high

ϕ_F (fluorescence) = high

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Regulation of fluorescence yield

Quenching of fluorescence may result from:

- Photochemical quenching (increase in $[Q_A]$)
- Non-photochemical quenching (increased down-regulation)

Steady-state measurements gives no indication of the extent of each of these processes.

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Solution

Measure system in known states:

- **When $[Q_A] = 1$**
  - all PSII centres are open (***Fo*** or ***Fo'***)
- **When $[Q_A] = 0$**
  - all PSII centres are closed (***Fm*** or ***Fm'***)
- **When down-regulation = 0**
  - dark-adapted state (***Fm*** and ***Fo***)

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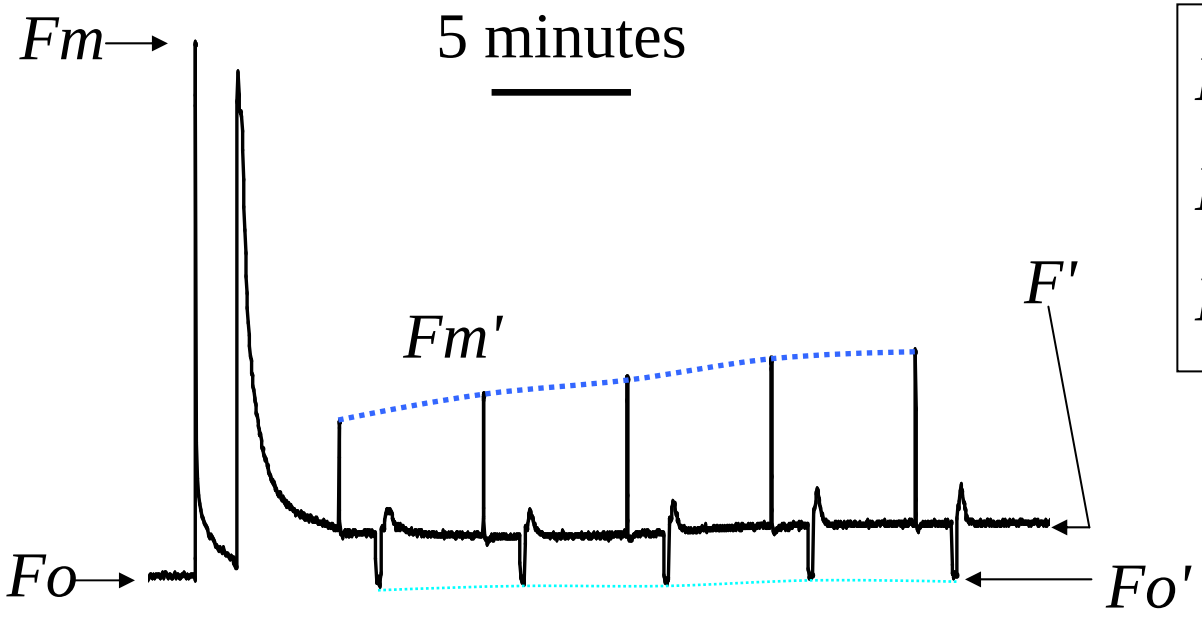
Fluorescence terms



$Fv = Fm - Fo$

$Fv' = Fm' - Fo'$

$Fq' = Fm' - F'$

Baseline

655 µmol m^{-2} s^{-1}

0.1 µmol m^{-2} s^{-1}

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Fq'/Fm' – PSII Quantum efficiency

- ***Fq'/Fm'*** = (*Fm'-F'*) / *Fm'* or $\frac{\Delta F}{Fm'}$

- Also termed: ΦPSII, Genty factor,
- theoretically proportional to the operating quantum efficiency of PSII photochemistry - it is a measure of the proportion of the light absorbed by photosystem II that is used in photochemistry
- Affected by level of electron acceptors, (NADP+) at the acceptor side of PSI.
- Down regulation of PSII – antenna quenching

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Fluorescence parameters

$$\frac{Fq'}{Fm'} = \frac{Fv'}{Fm'} \cdot \frac{Fq'}{Fv'}$$

Operating efficiency
(Genty factor)

Maximum efficiency
(when $[Q_A] = 1$)

Photochemical factor

$$\frac{\Delta F}{Fm'} = \frac{Fv'}{Fm'} \cdot qP$$

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Fq'/Fv' – PSII efficiency factor

- ***Fq'/Fv'*** = (*Fm'*-*F'*) / (*Fm'*- *Fo'*)

- Mathematically same as qP
- Changes reflect differences in capacity for photochemistry at PSII

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Fv'/Fm' – PSII maximum efficiency

- ***Fv'/Fm'*** = (*Fm'*-*Fo'*) / *Fm'*

- Describes energy dissipation – estimate of the PSII quantum efficiency if all PSII centres were in the 'open' state at that point of measurement.
- Value determined by down-regulation – processes which increase rate constant for non-radiative decay of excitation energy within the pigment matrix associated with PSII.

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NPQ – Non photochemical quenching

- **NPQ** = (*Fm*/*Fm*')-1

- Non-photochemical quenching of chlorophyll fluorescence is an indicative of the level of non-radiative energy dissipation in the light-harvesting antenna of photosystem II.

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Fv/Fm – maximum quantum efficiency of PSII photochemistry

- ***FvlFm*** = (*Fm-Fo*) / *Fm*)

- Dark
- Irreversible inhibition of PSII - photoinhibiton
- Dissociation of light harvesting pigment systems of PSII from PSII core
- Stress

The cflmager is built around an array of 1600 LEDs configured in 16 blocks. This arrangement provides even incident irradiance across a 10 x 12 cm standard area.

Individual blocks can be user adjusted for highly non - uniform samples

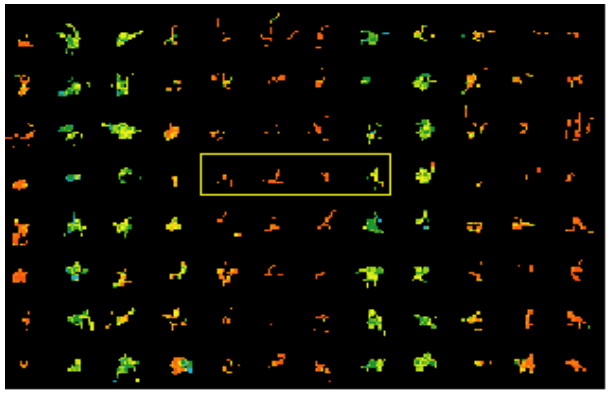
The imager cabinet is sealed against outside light by removable panels on sides and base for measurement of Fo.

Removing the panels can be useful for in-situ measurement and the introduction of attached leaves

Typical Applications

Herbicide Screening

C 8 4 0.8 0.4 C C 8 4 0.8 0.4 C

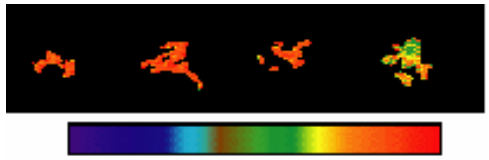


C 8 4 0.8 0.4 C C 8 4 0.8 0.4 C

Seedlings of *Agrostis tenuis* grown in a 96 well plate and treated for 48 h with Imazapyr in 50% Acetone show no *visible* symptoms of inhibition relative to controls.

Imazapyr is an acetolactase inhibitor with no direct effect on the photosynthetic apparatus.

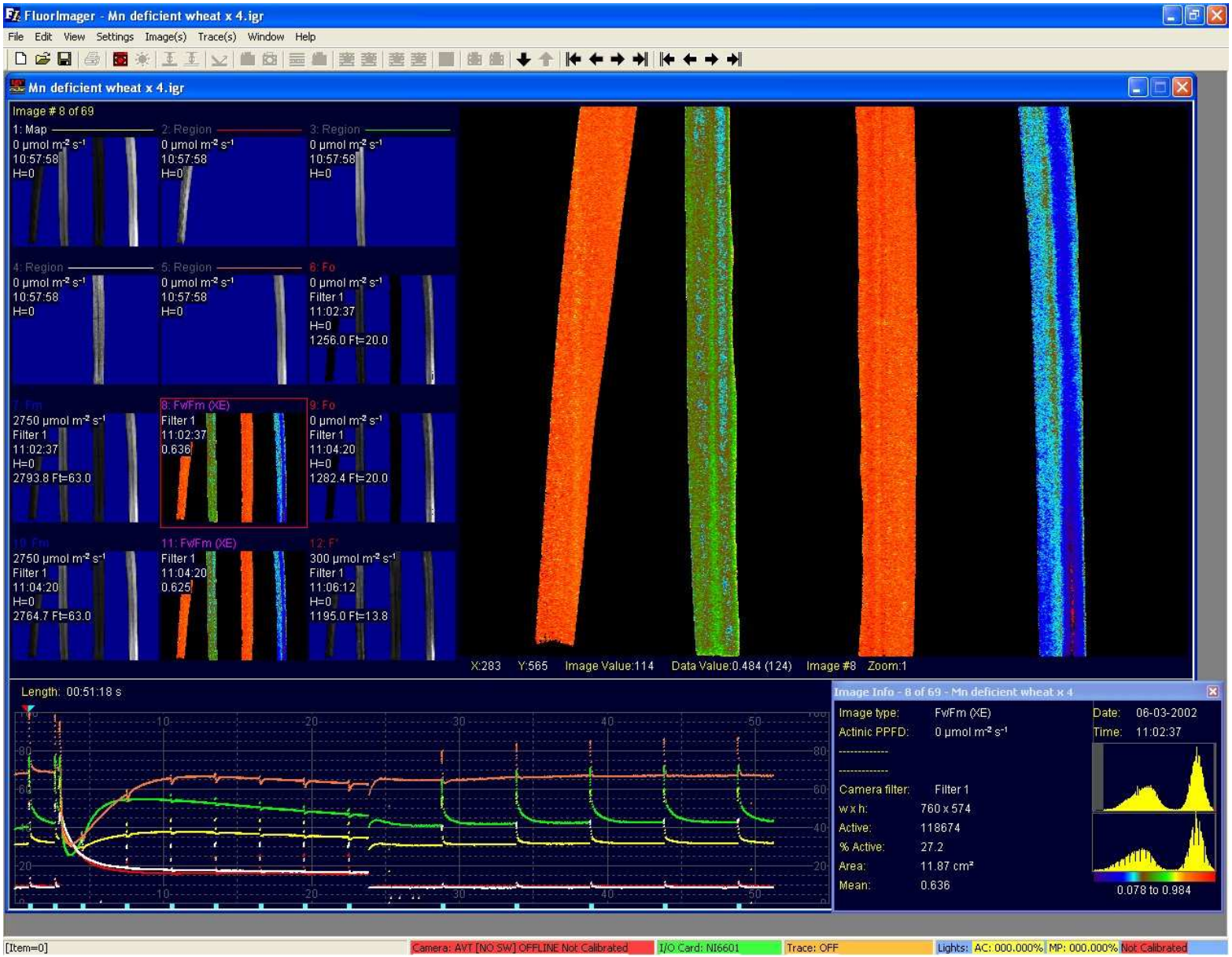
Individual images of *Fv/Fm* for all 96 samples show very clear differences between controls (C) and plants in columns treated with 0.4. 0.8. 4.0 and 8.0 mM Imazapyr. Using conventional observational techniques this screen might take up to 3 weeks. The cf Imager achieves the screen in seconds.



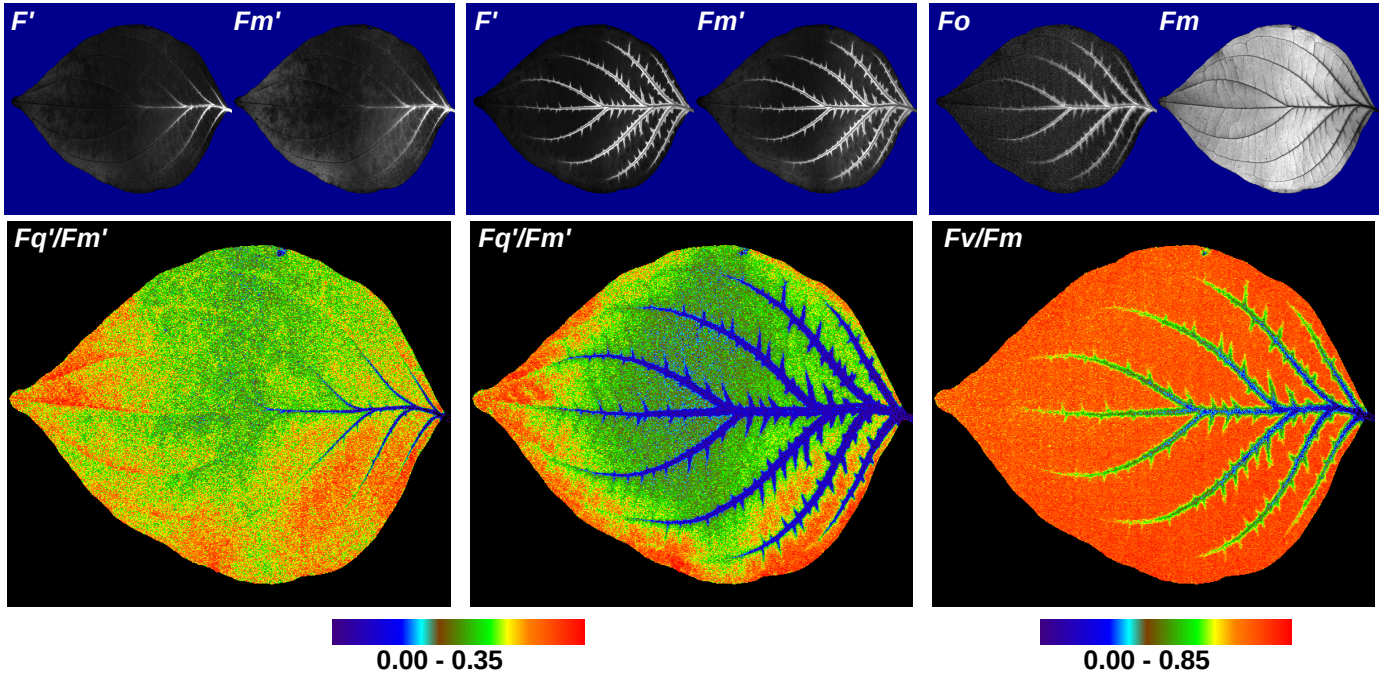
***Fv/Fm* palette**

0.020 to 0.977

Nutrient Effects

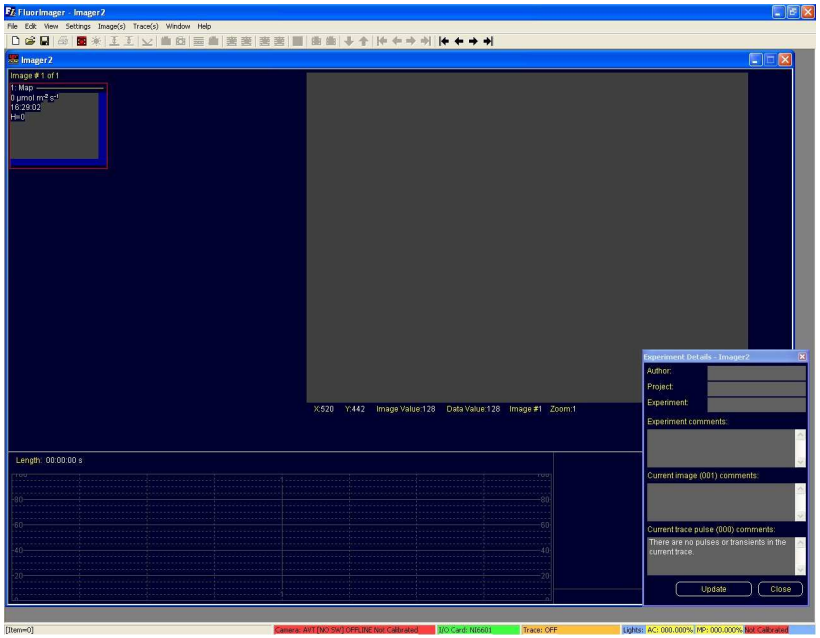


Development of Photosynthetic Heterogeneity within a Sample



The images above illustrate the ability of the cf imager system to resolve heterogeneous patterns of photosynthetic performance within a sample. The petiole of a detached leaf of *Cornus* sp. was placed in a 10mM solution of DCMU (a PS II herbicide). During the 100 minute illumination period, DCMU was taken up through the transpiration stream. When the leaf was illuminated (left and middle), the penetration of DCMU is evident in the *F'*, *Fm'* and *Fq'/Fm'* images, reflecting and inhibition of both photochemical and non-photochemical PSII processes. After 1 h dark-recovery (right), DCMU has impacted on *Fo* and *Fv/Fm*, but not on *Fm*. This indicates that non-photochemical quenching has reversed during the dark period, but that the impact of DCMU on the photochemical capacity of PSII has not.

Basic setup procedures and button menu bar



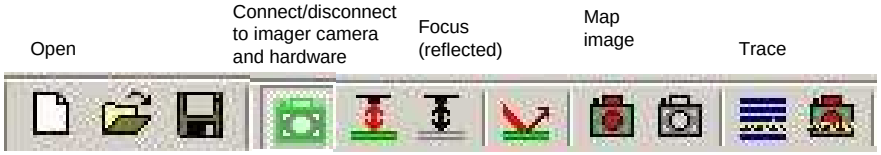
Connect to camera

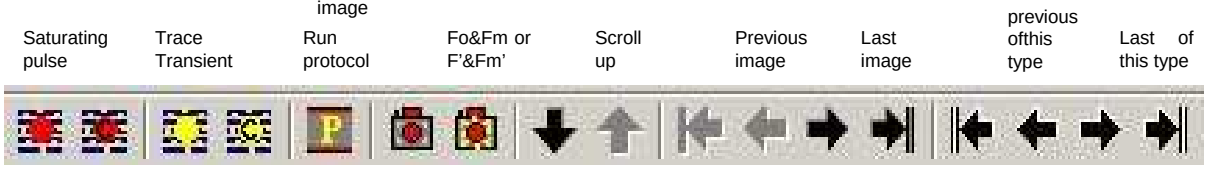
Focus

When focussed, take map image

Order of commands to set up

Menu Bar – see below for details

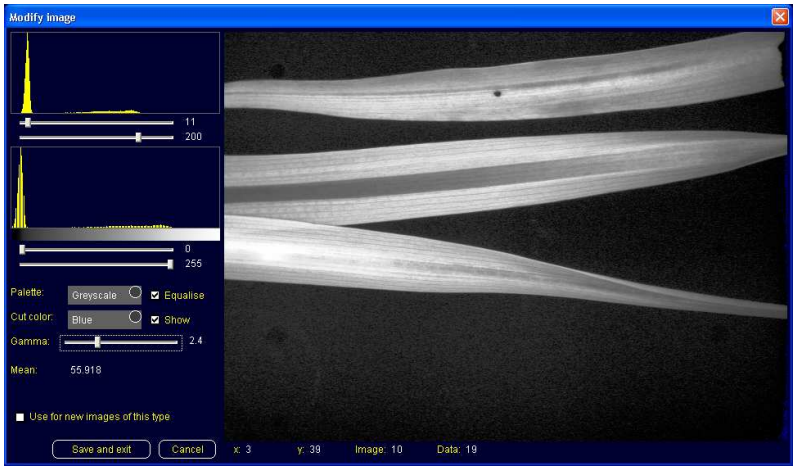




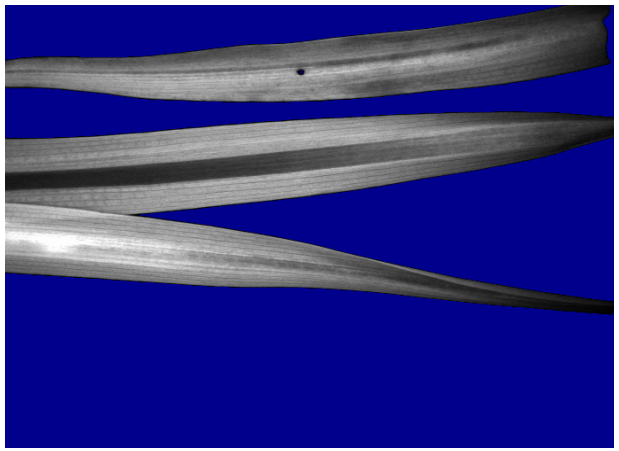
Experimental Procedures

Analyzing Fluorescence of Single or Multiple Leaves

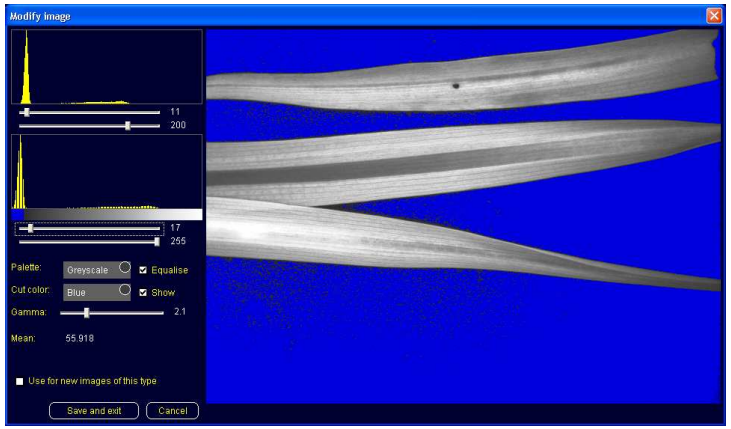
STEP 1: Attached or detached leaves are placed within the camera field of view, and a MAP IMAGE is taken by clicking on a program icon. This image is used to determine which camera pixels within the field of view are to remain active while measuring fluorescence parameters.



The initial map image (left upper) may be modified by several image manipulation tools within the imager program. The simplest way to isolate pixels that make up the images of the leaves is to click on Apply Isolation within the Image(s) menu. This deactivates pixels with low fluorescence values, clearly defining the leaves (left lower).

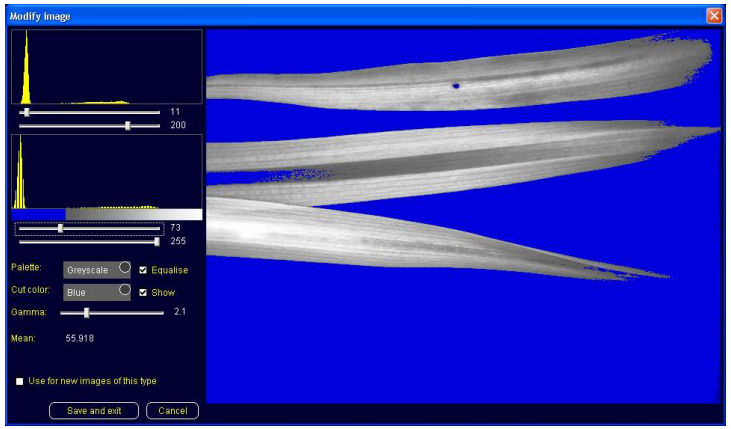


For more complex images where there is a risk that the Apply Isolation command may deactivate potentially important pixels, the Modify Image dialog box allows the map image to be adjusted with a fine degree of control by the user, as shown on the next slide.

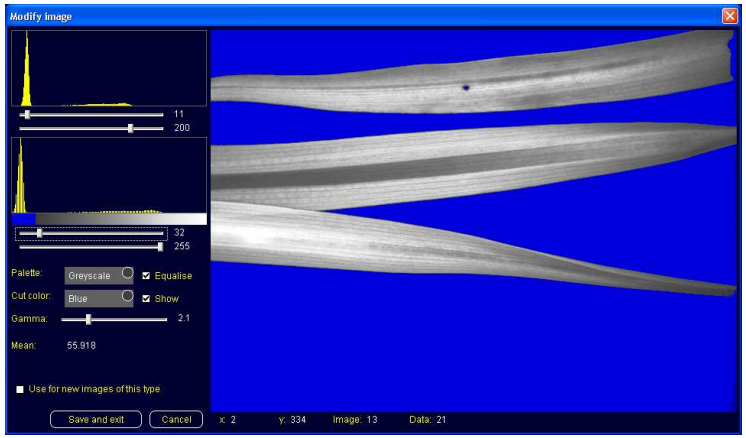


Active pixels are shown in grey scale. Inactive areas are shaded blue. A slider is used to activate and deactivate pixels.

Two few pixels have been deactivated in the upper image. Signal scattering is seen around the leaves.

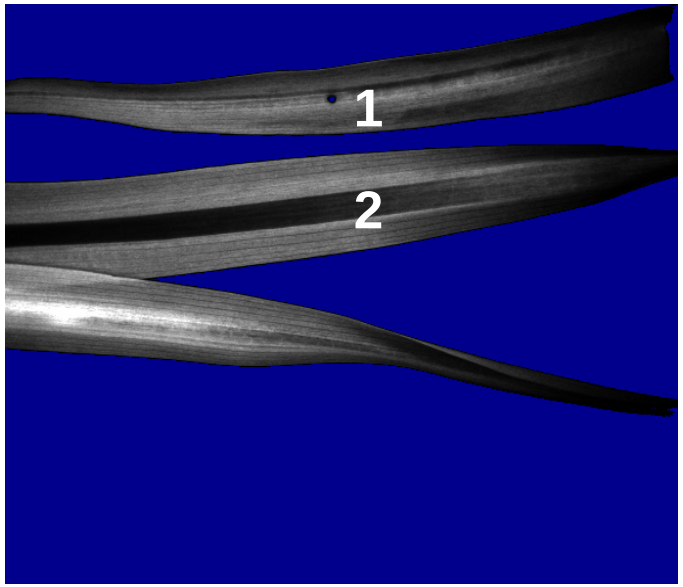


Too many pixels have been deactivated in the middle image. Areas of the leaf have been cut, especially those where variegation has resulted in low signals.

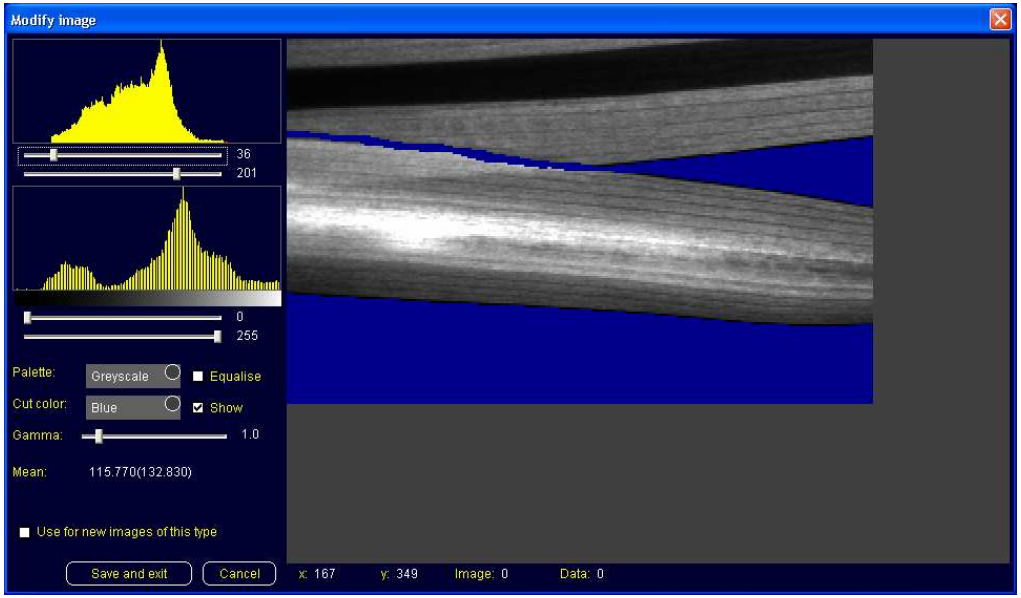


The correct degree of isolation has been attained. Leaf margins are distinct, no part of the leaf body has been deleted and there is no scatter about the leaves.

STEP 2: Isolate Region Images

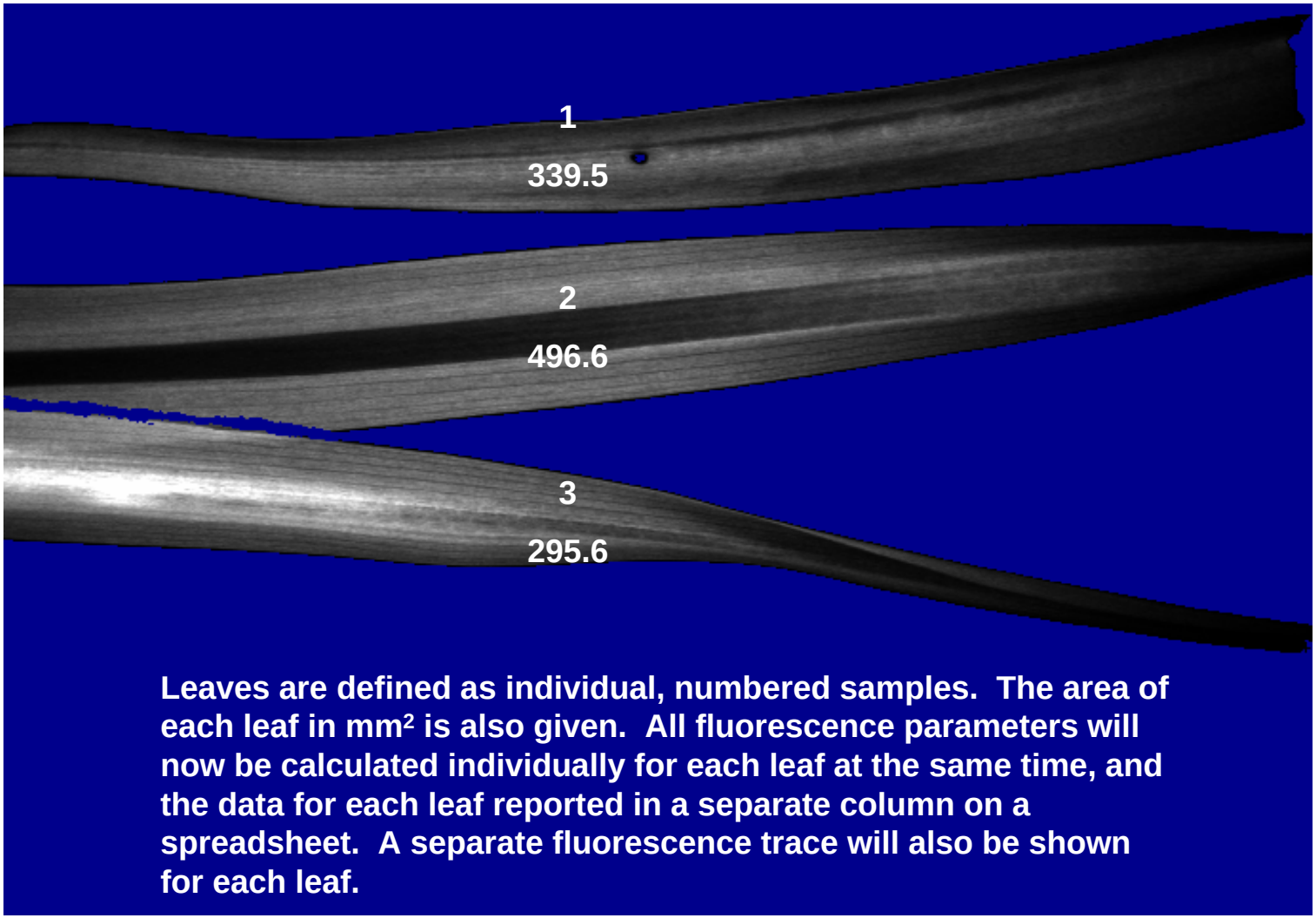


If separate fluorescence data is required for each sample in the field of view, the Auto Region Images command may be used to identify each individual sample. However, the command functions by isolating areas within a continuous circumference of pixels. Therefore, if leaves are over-lapping, as in the example shown at left, the overlapping leaves will be identified as a single unit. To overcome this, the Modify Image dialog box is activated that allows the user to cut pixels from the map Image and separate the leaves.



Here, the zoom function has been used to zoom in on the area where the two lower leaves overlap, a line of pixels has been cut to separate the leaves. The Auto Region Images command may now be used to identify the three leaves as individuals as shown on the next slide.

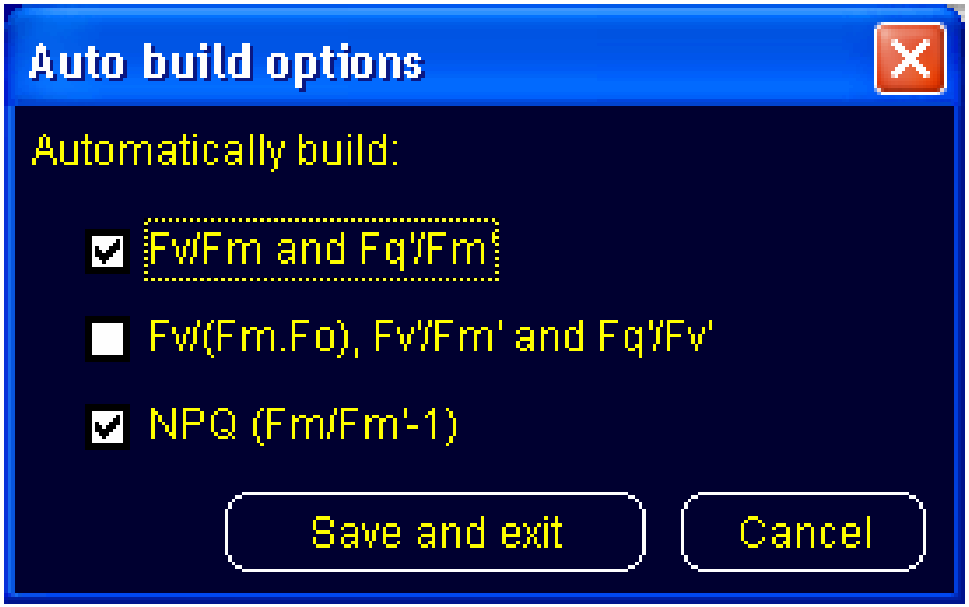
The Auto Region Images Command



Leaves are defined as individual, numbered samples. The area of each leaf in mm^2 is also given. All fluorescence parameters will now be calculated individually for each leaf at the same time, and the data for each leaf reported in a separate column on a spreadsheet. A separate fluorescence trace will also be shown for each leaf.

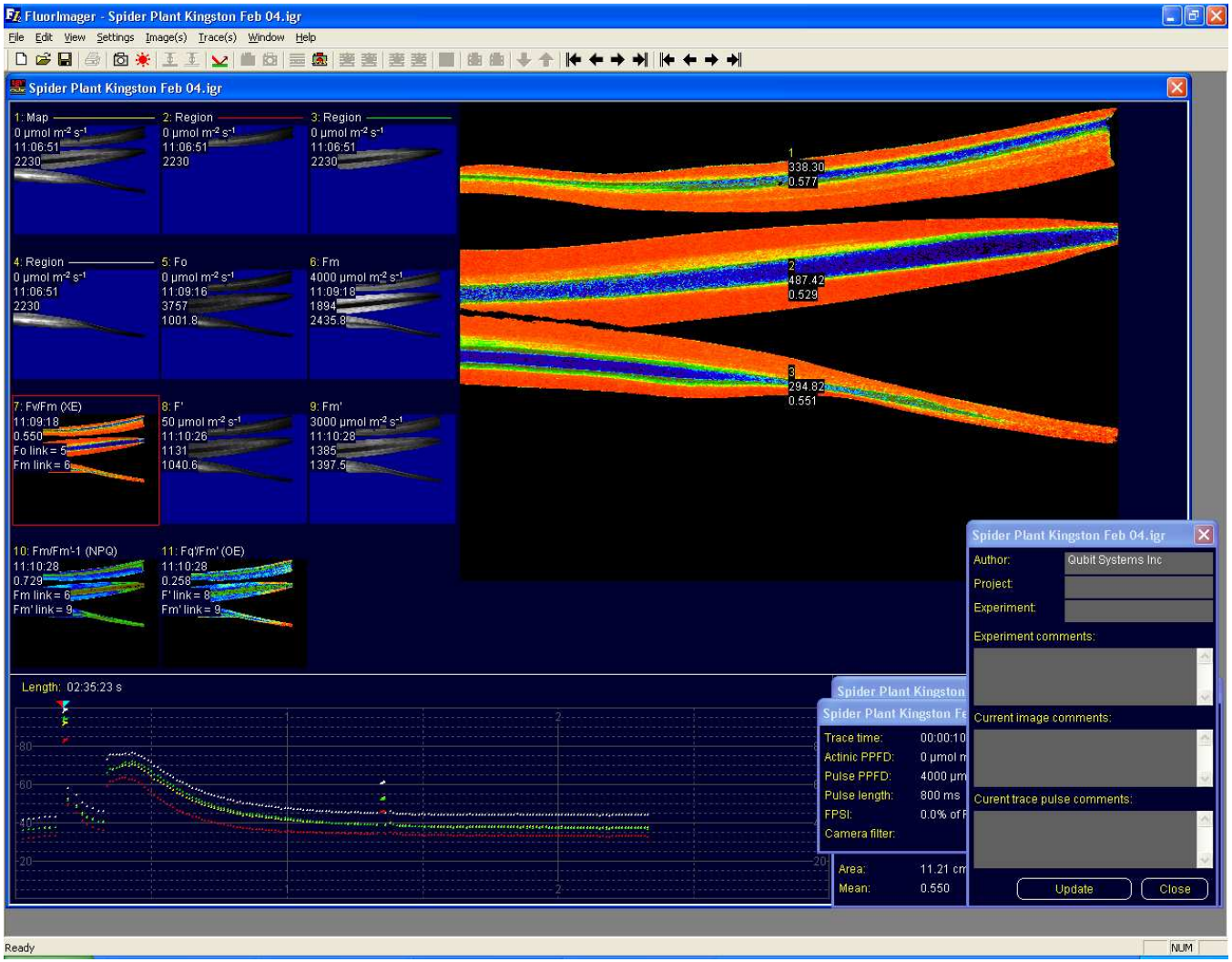
STEP 3: Establishing an Experimental Protocol

STEP 4: Selecting Automatic Construction of Images



During the experiment, data are used to construct images within 1 second of the data being collected. Thumb nail images (Fo, Fm, F' and Fm') appear on screen as the experiment progresses. The user has the option of displaying up to 6 different parameter images based on these values. The selection is made under Auto Build Options in the Image(s) menu.

STEP 5: Running the Protocol

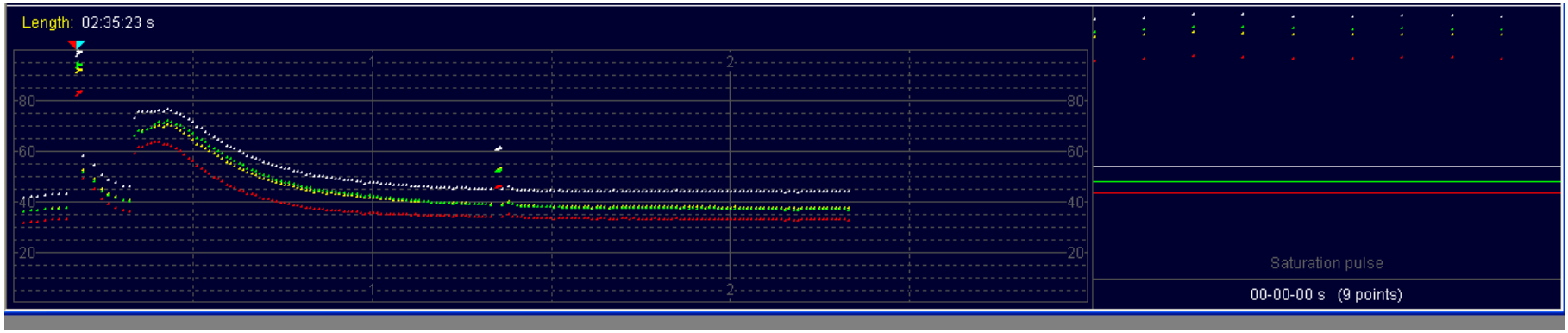


The protocol starts immediately after the user clicks on the Start Protocol icon. Thumbnail images appear to the left of the screen, complete fluorescence traces for all regions appear at the bottom of the screen, and a main image appears as the central feature of the screen. The main image can be selected from the thumb nail images by clicking on any one of them.

The main image in the example at left shows Fv/Fm values for the three leaves.

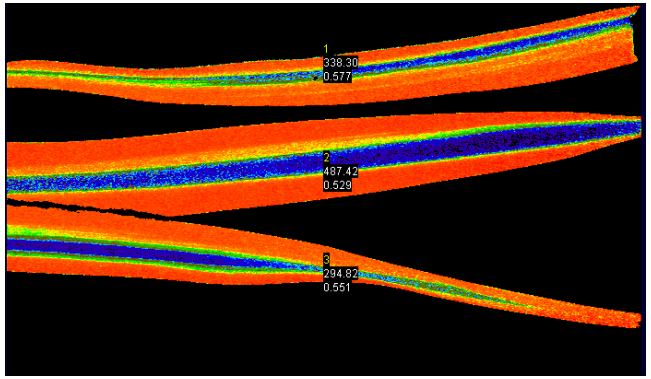
STEP 6A: Extracting Data

Numerical data may be extracted from the imager program in several ways. Right clicking on the main fluorescence trace opens a dialog box in which the user can select Copy Trace Data to the Clipboard. The data can then be pasted into a spreadsheet program such as Excel. All experimental conditions, raw fluorescence data, and parameter data for all regions are recorded as shown below.

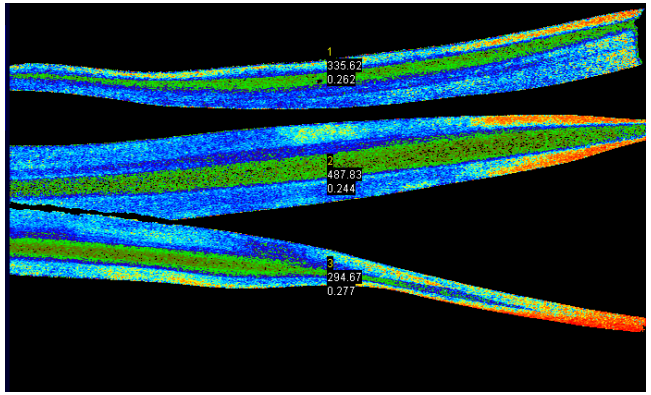


Pulse data															
Based		Trace	Trace	Camera	Actinic	Pulse	Pulse	Fo &	Fm &	Fv/	Fv/Fm or		(qP)	(NPQ)
on		time	time (s)	filter	PPFD	PPFD	length	F'	Fm'	(Fm.Fo)	Fq'/Fm'	Fv'/Fm'	Fq'/Fv'	Fm/Fm'-1
Map (1)														
	1	0:00:10	10		0	4000	800	1007	2495	1	0.596	0.596	1	0
	2	0:01:21	81		50	3000	800	1045	1421	0.43	0.265	0.457	0.579	0.76
Region (2)														
	1	0:00:10	10		0	4000	800	889	2252	1	0.605	0.605	1	0
	2	0:01:21	81		50	3000	800	912	1240	0.43	0.265	0.458	0.578	0.82
Region (3)														
	1	0:00:10	10		0	4000	800	999	2553	1	0.609	0.609	1	0
	2	0:01:21	81		50	3000	800	1039	1409	0.41	0.263	0.462	0.568	0.81
Region (4)														
	1	0:00:10	10		0	4000	800	1158	2676	1	0.567	0.567	1	0
	2	0:01:21	81		50	3000	800	1209	1648	0.45	0.266	0.447	0.596	0.62

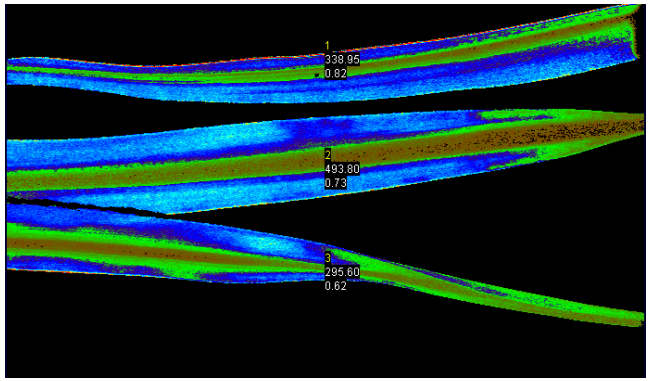
STEP 6B: Extracting Data



Colony	x	y	Area (mm²	Fv/Fm
1	343	60	338.3	0.577
2	343	178	487.42	0.529
3	343	289	294.82	0.551



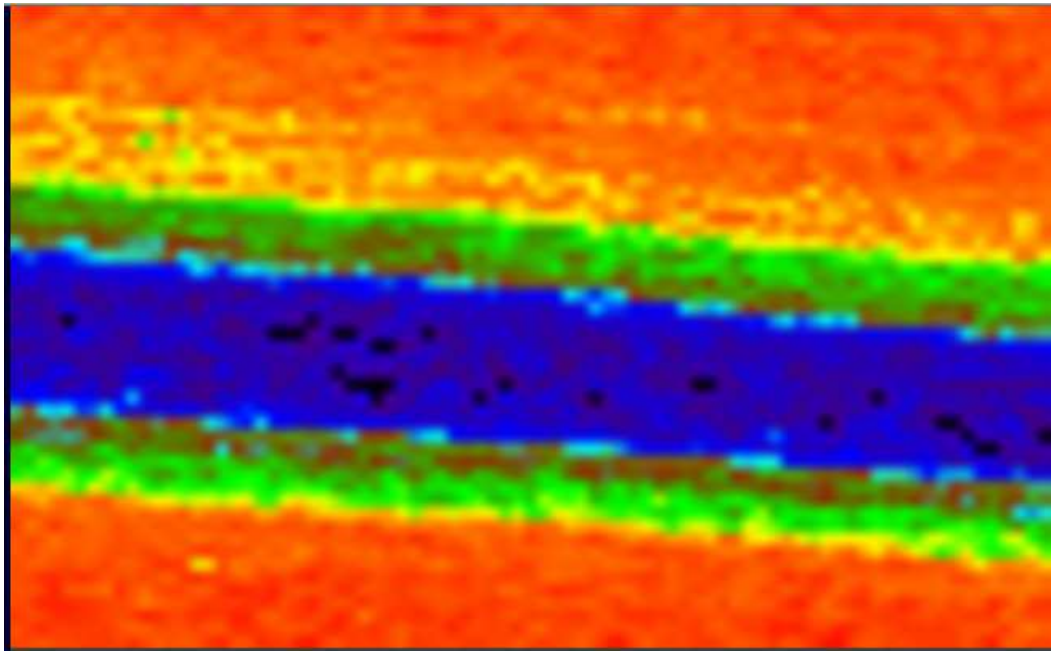
Colony	x	y	Area (mm²	Fq'/Fm'
1	343	60	335.62	0.262
2	343	178	487.83	0.244
3	343	289	294.67	0.277



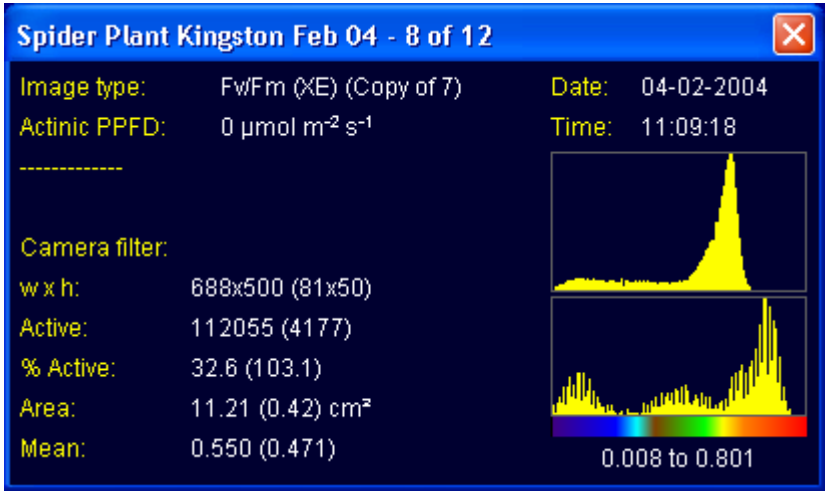
Colony	x	y	Area (mm²	NPQ
1	343	60	338.95	0.82
2	343	178	493.8	0.73
3	343	289	295.6	0.62

Data from individual images can be downloaded by selecting the desired image as the main image and then right clicking on the image. Selecting Copy Colony Data to the Clipboard allows the user to copy the data into Excel or other spreadsheet program in the format shown at left.

Zooming In

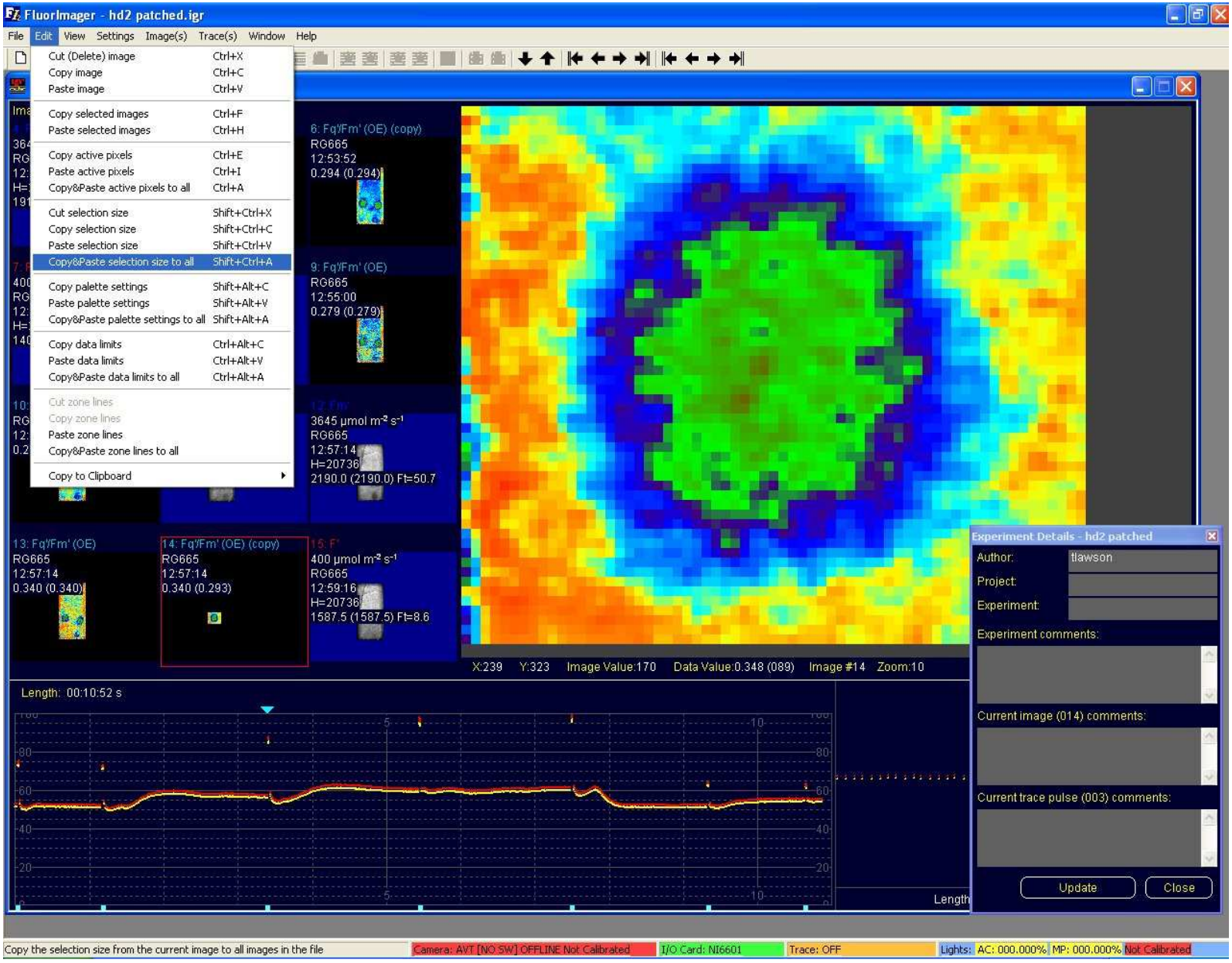


Dragging the cursor across the image while holding down the Shift and Control keys zooms in on the area selected. This is shown left for one of the leaves on the previous slide.

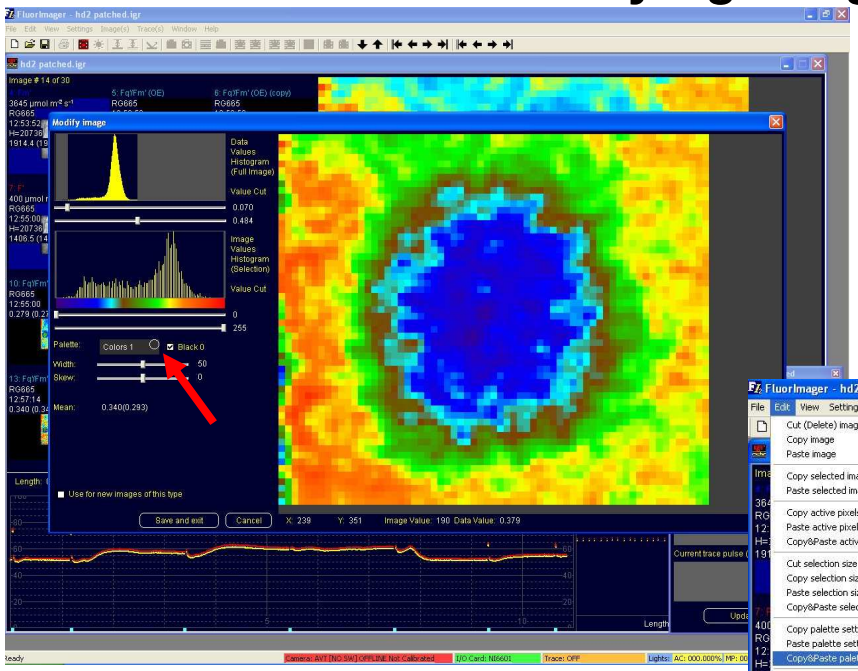


The imager software calculates a new average value for the selected area and displays this along with the value for the original entire image in the Image Information display box. The physical areas of the original and selected portion of the image are also displayed.

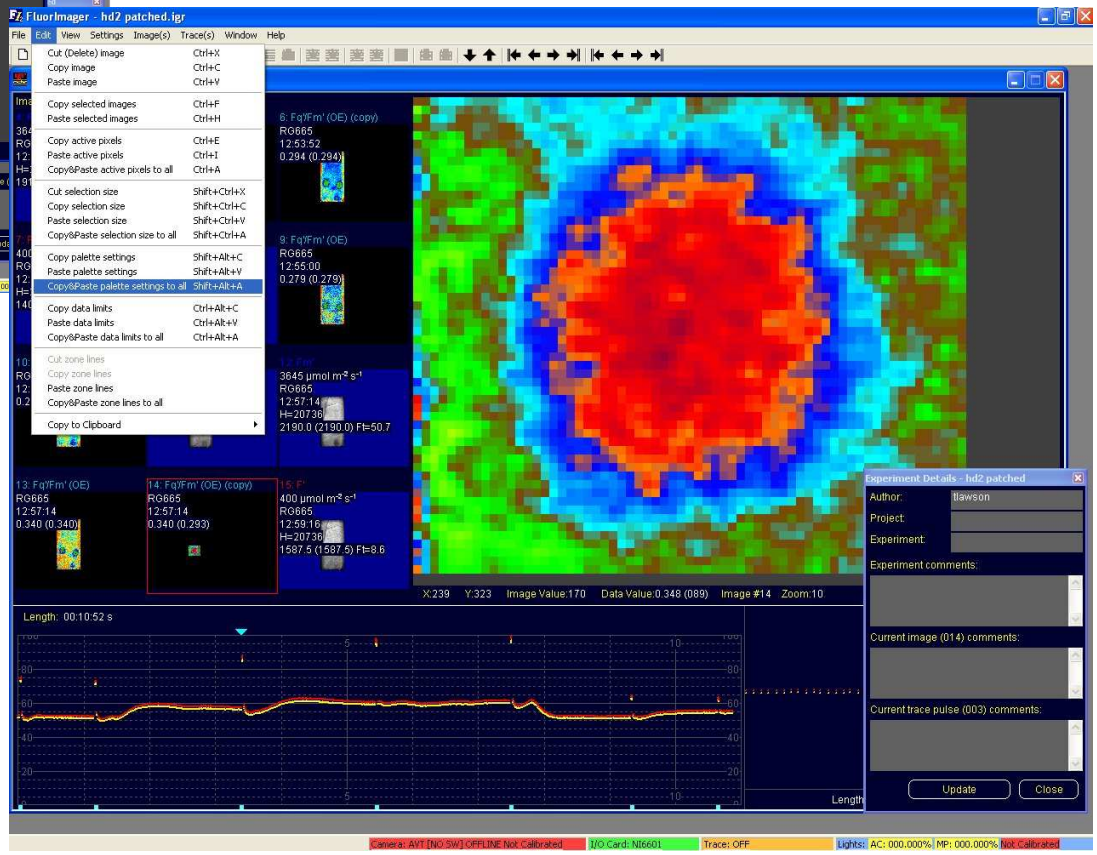
Modifying Images- selection



Modifying Images – colour palette

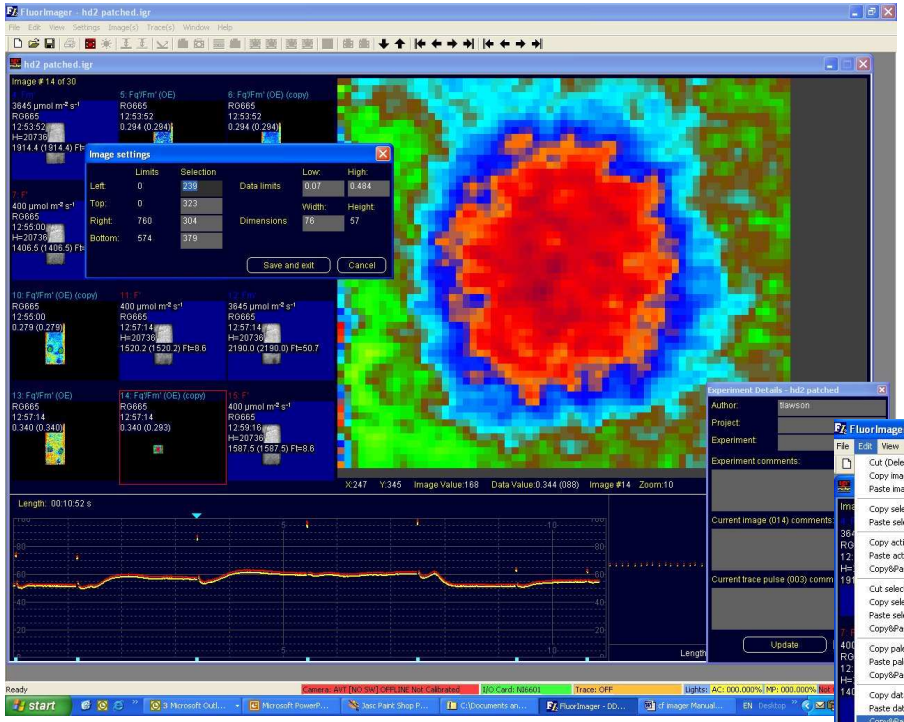


Double click on image - change colour palette using palette options

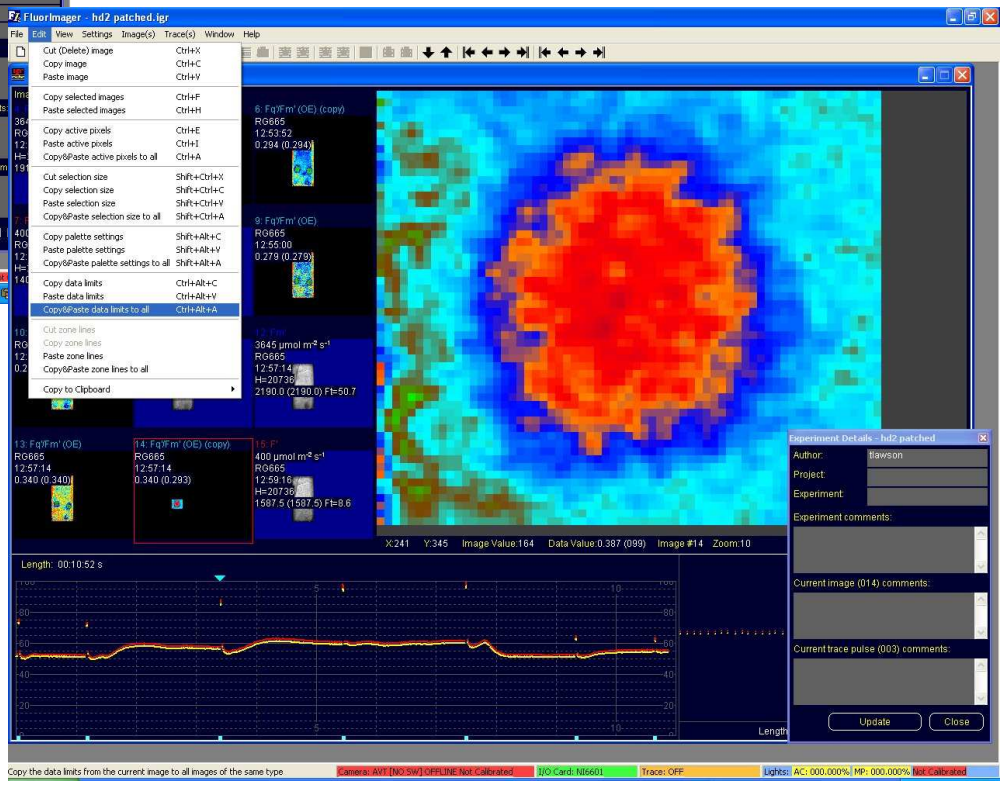


Colour changes can be copied to all other images

Modifying Images – data range/scale



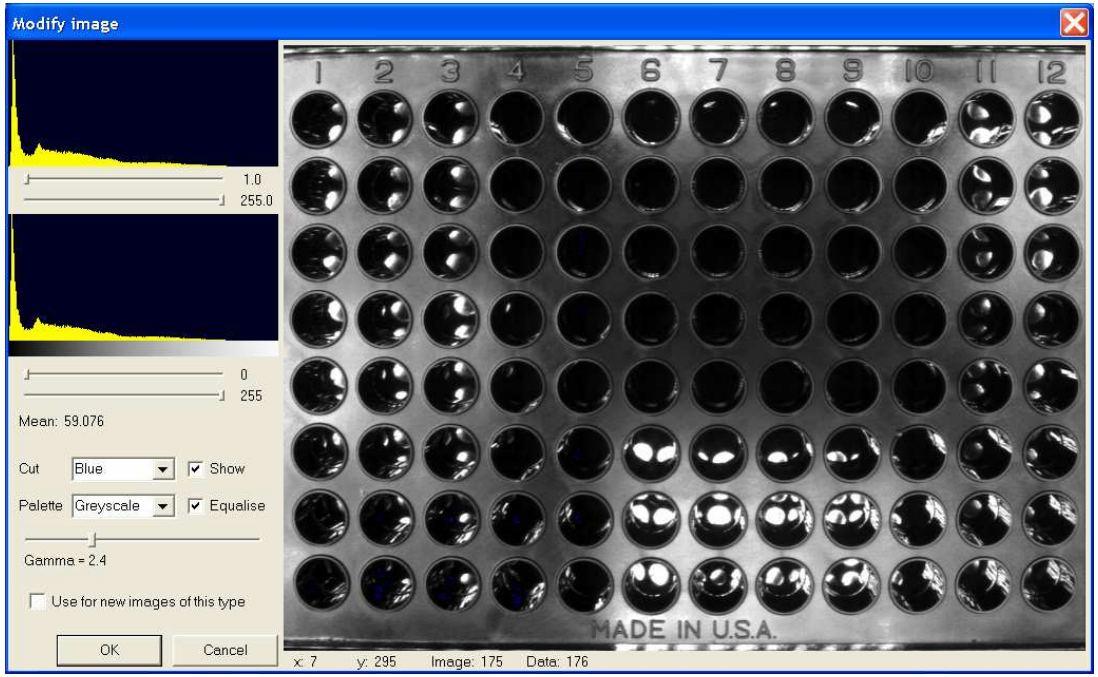
Data limits can be altered using Image setting dialogue, accessed through Image main menu.



After changing the data limits, these can be copied to all other images.

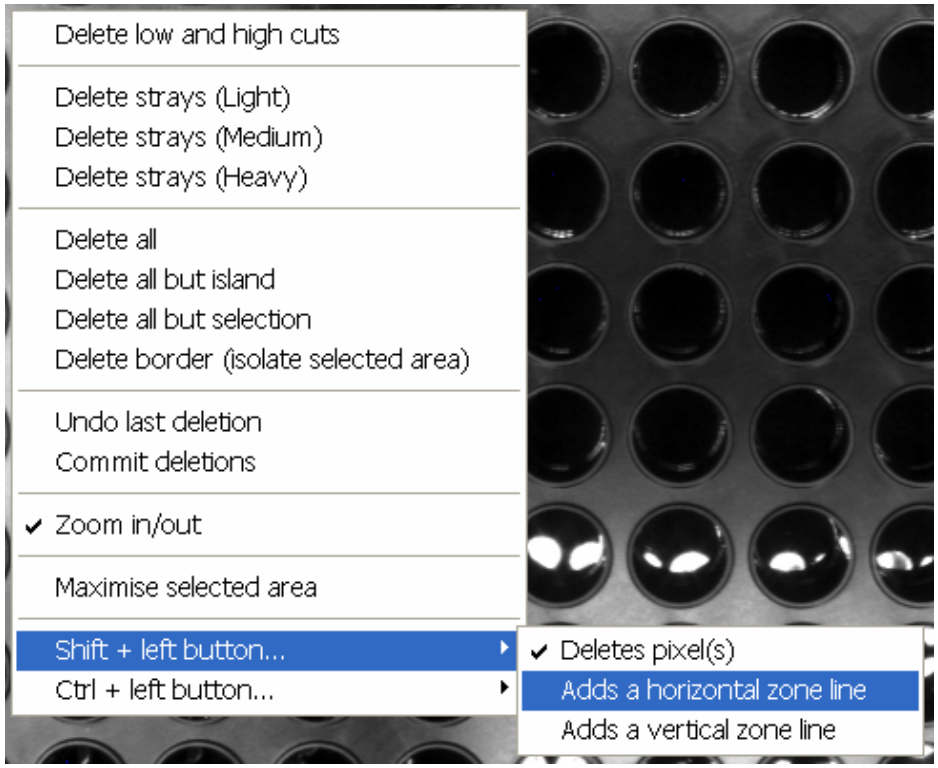
Setting up and Running a Protocol to Screen a 96 Well Plate

Use of Zone Lines

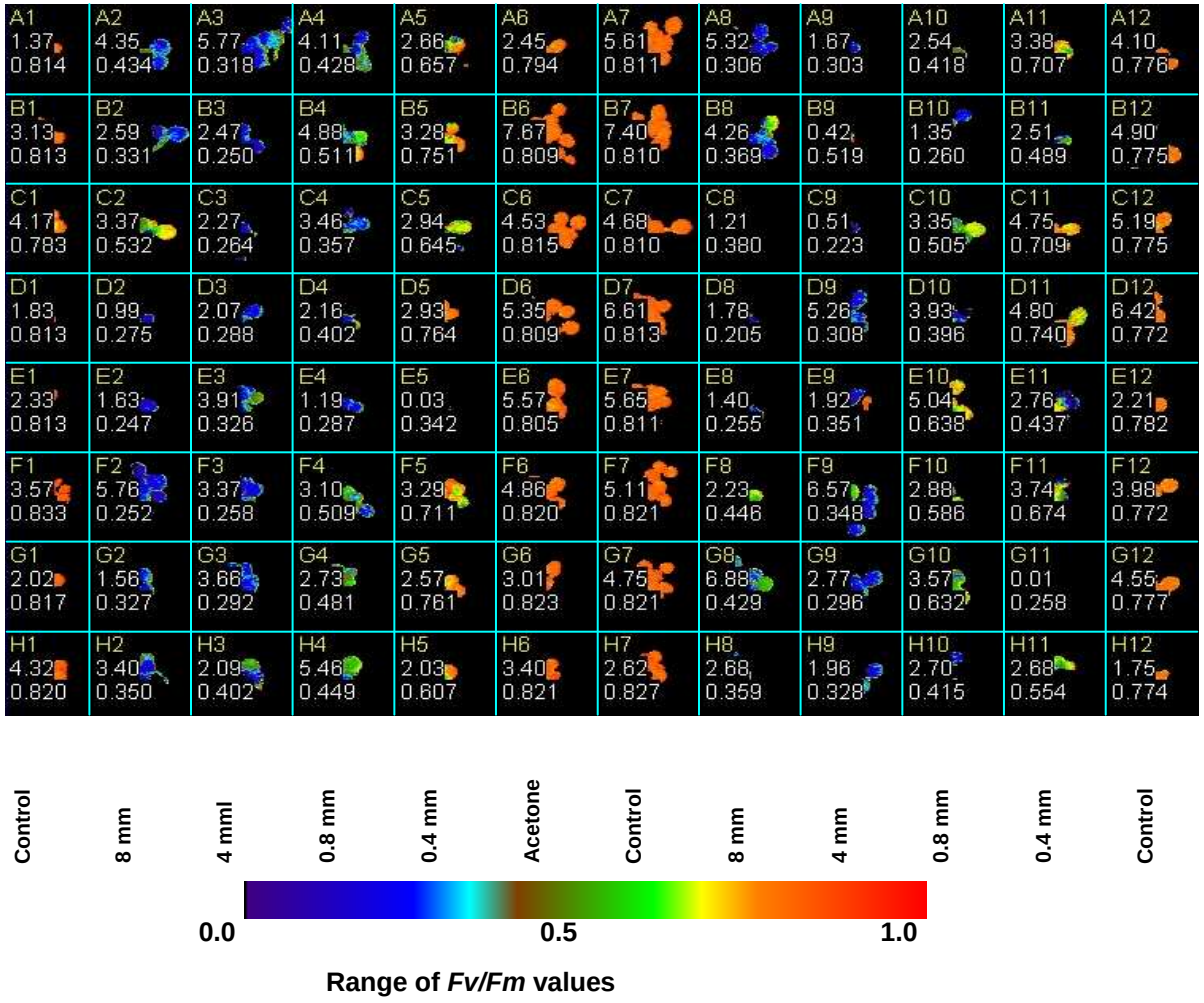


A 96 well plate is useful for growing small plants such as Arabidopsis for screening. The Imager software can calculate fluorescence values for all 96 plants simultaneously.

Zone lines are used to divide the plate into 96 separate sample areas. Zone lines may also be used to divide the plate into 12 vertical zones or 8 horizontal zones. In these cases, the average value for all plants in each zone will be calculated. This is useful if treatments are arranged in vertical or horizontal arrays.



Zone lines are added and deleted using mouse clicks. Line positions selected using a reflected light image of an empty plate can be copied and pasted onto the image of a plate containing samples. In this way the sample plate does not need to be exposed to light before the experiment, and dark adaptation is not disturbed.

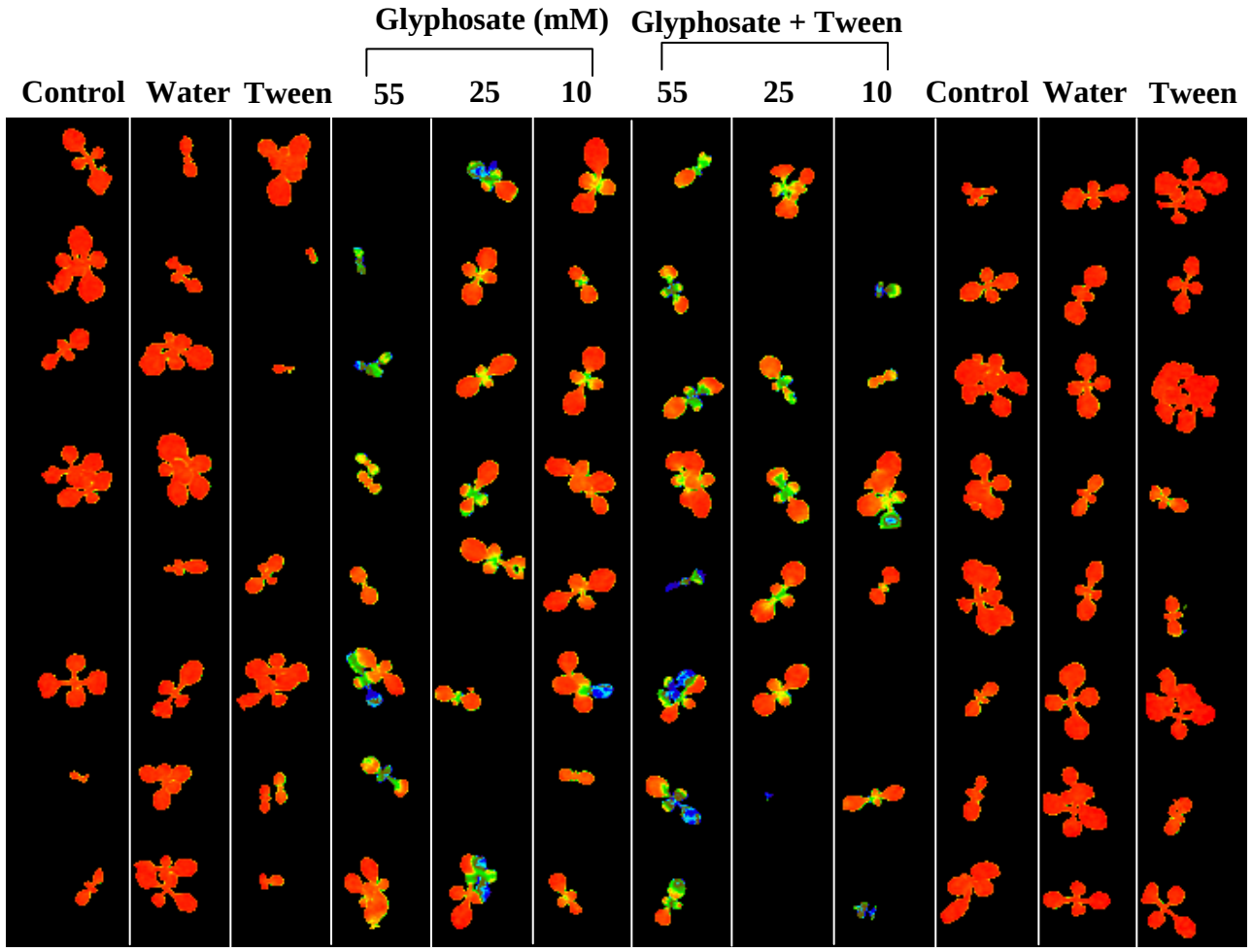


When zone lines are in place, the user has the option of displaying zone data with the Image. The Image opposite shows Fv/Fm data for Arabidopsis plants grown under different concentrations of the herbicide Imazapyr.

Within one second of the *Fo* and *Fm* images being taken, the program had automatically:

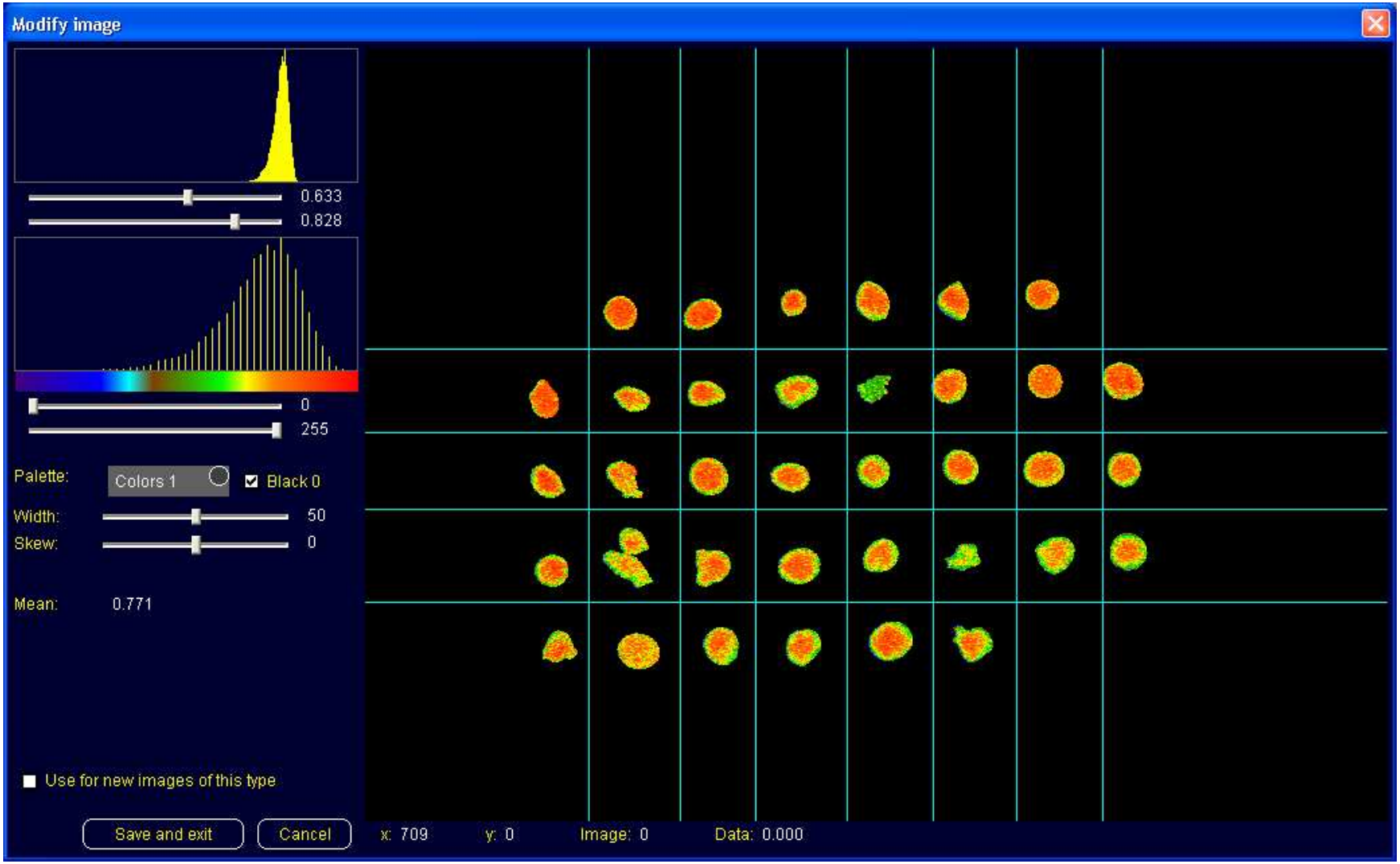
1. **Constructed the *Fv/Fm* image**
2. **Mapped the data values to the palette**
3. **Isolated the plant material within each zone**
4. **Calculated the plant area and mean *Fv/Fm* value for each zone**

With treatments arranged in columns, vertical zone lines allow calculation of the average data for each vertical zone, such as in the glyphosate sensitivity study shown below. Data were collected 24h after exposure to glyphosate at the levels indicated.

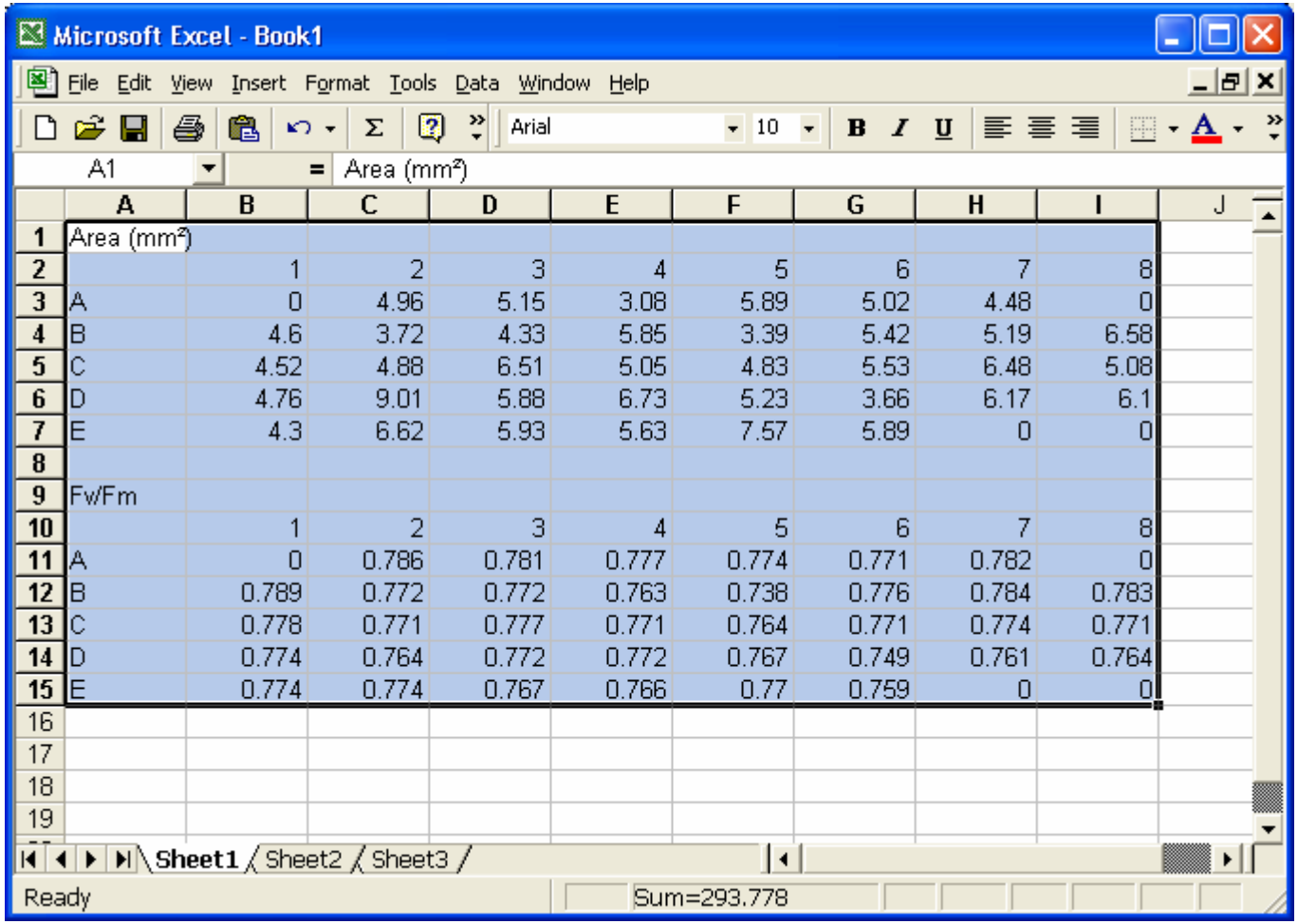


Fv/Fm	0.78	0.78	0.77	0.63	0.67	0.73	0.61	0.66	0.69	0.78	0.78	0.79

Zone lines can be added to any image to separate samples for individual analysis of fluorescence parameters. The image below shows Chalmydamonas colonies grown in a regular array on a Petri plate.



Values for sample area and for the imaged data (from the previous slide) have been copied to the Clipboard and pasted into Excel. The Excel data has an array corresponding to that of the zone data. Zero values indicate empty zones in the array.



Microsoft Excel - Book1

File Edit View Insert Format Tools Data Window Help

Arial 10 B I U

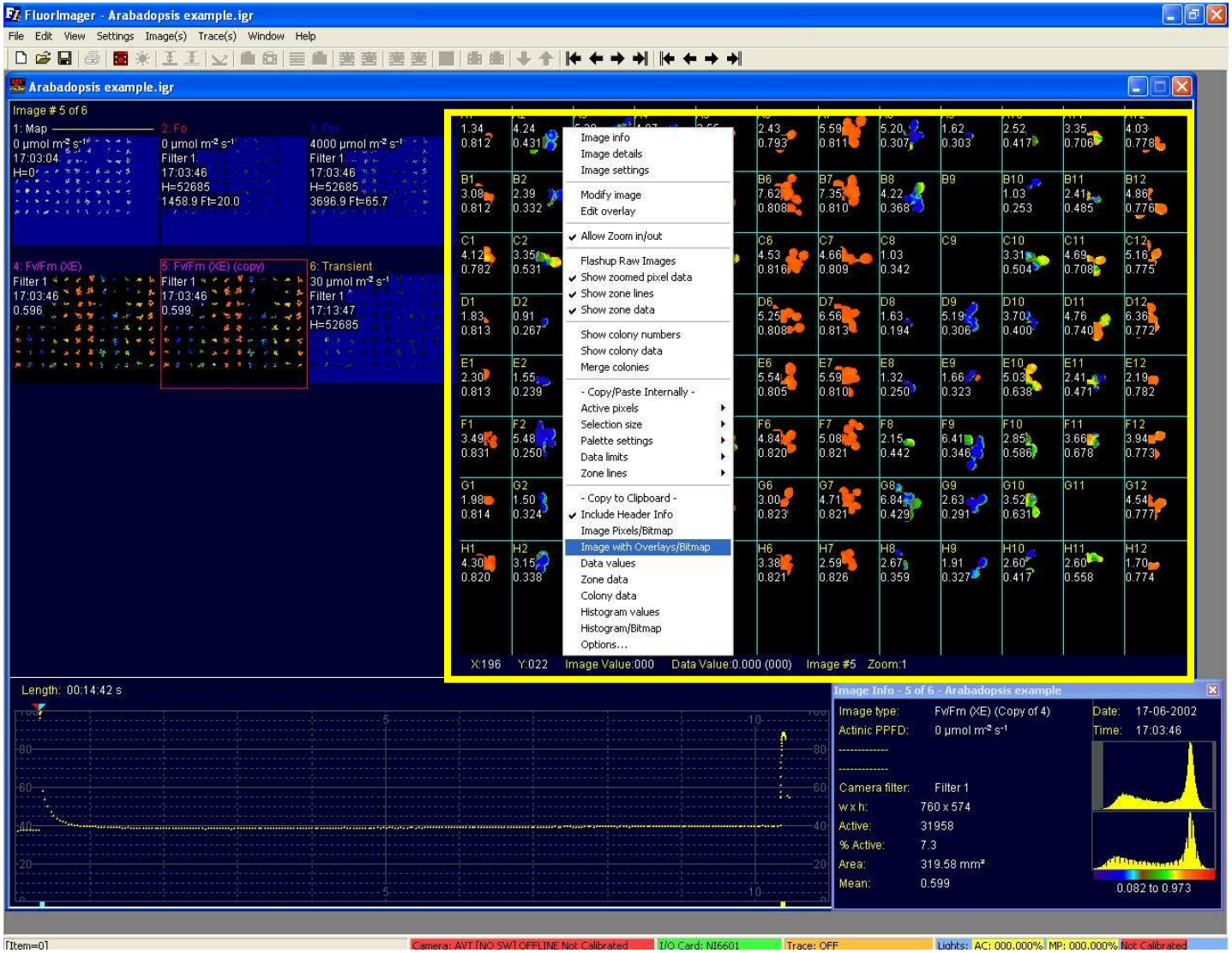
A1 = Area (mm²)

	A	B	C	D	E	F	G	H	I	J
1	Area (mm²)									
2		1	2	3	4	5	6	7	8	
3	A	0	4.96	5.15	3.08	5.89	5.02	4.48	0	
4	B	4.6	3.72	4.33	5.85	3.39	5.42	5.19	6.58	
5	C	4.52	4.88	6.51	5.05	4.83	5.53	6.48	5.08	
6	D	4.76	9.01	5.88	6.73	5.23	3.66	6.17	6.1	
7	E	4.3	6.62	5.93	5.63	7.57	5.89	0	0	
8										
9	Fv/Fm									
10		1	2	3	4	5	6	7	8	
11	A	0	0.786	0.781	0.777	0.774	0.771	0.782	0	
12	B	0.789	0.772	0.772	0.763	0.738	0.776	0.784	0.783	
13	C	0.778	0.771	0.777	0.771	0.764	0.771	0.774	0.771	
14	D	0.774	0.764	0.772	0.772	0.767	0.749	0.761	0.764	
15	E	0.774	0.774	0.767	0.766	0.77	0.759	0	0	
16										
17										
18										
19										

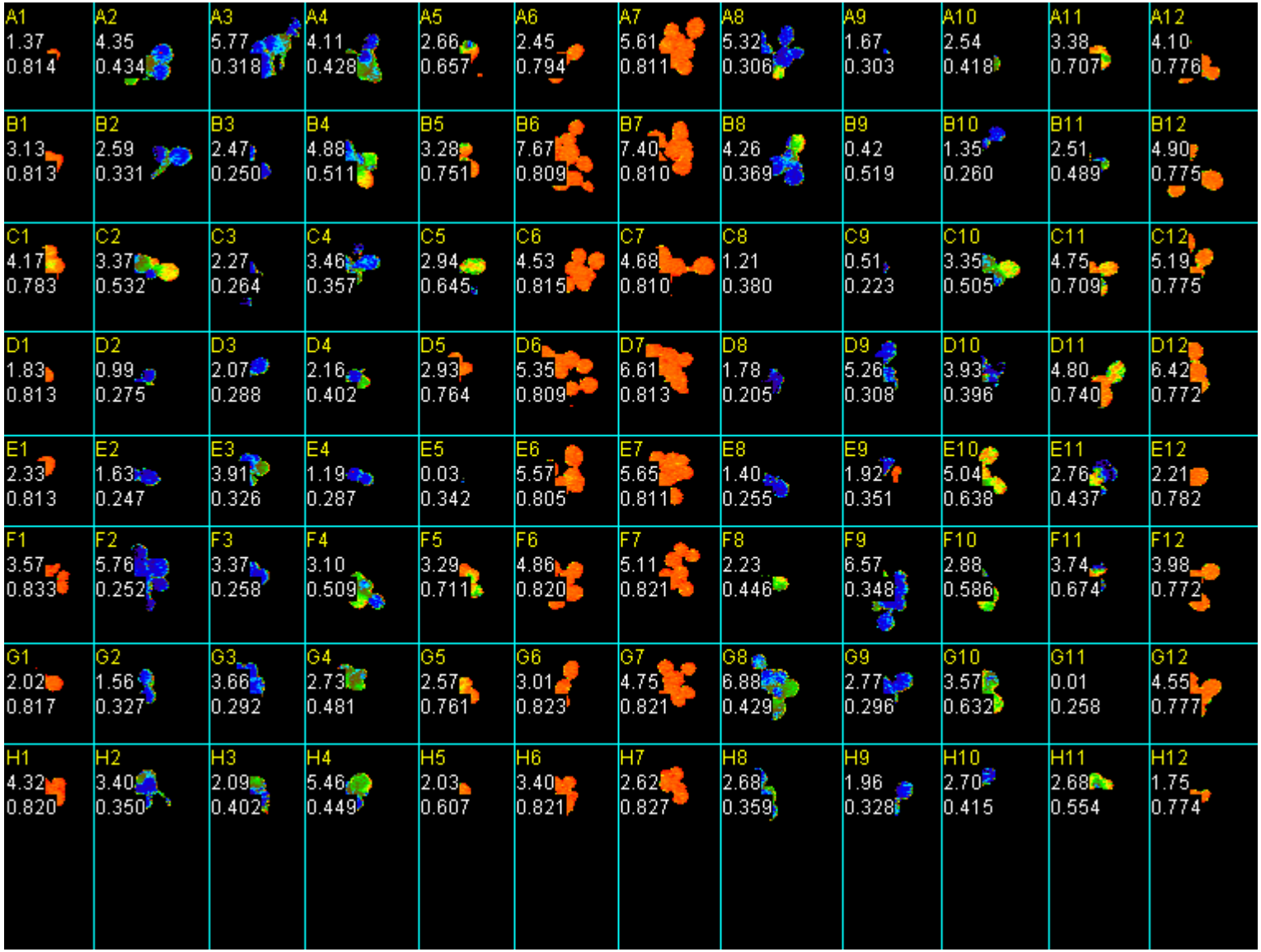
Sheet1 Sheet2 Sheet3

Ready Sum=293.778

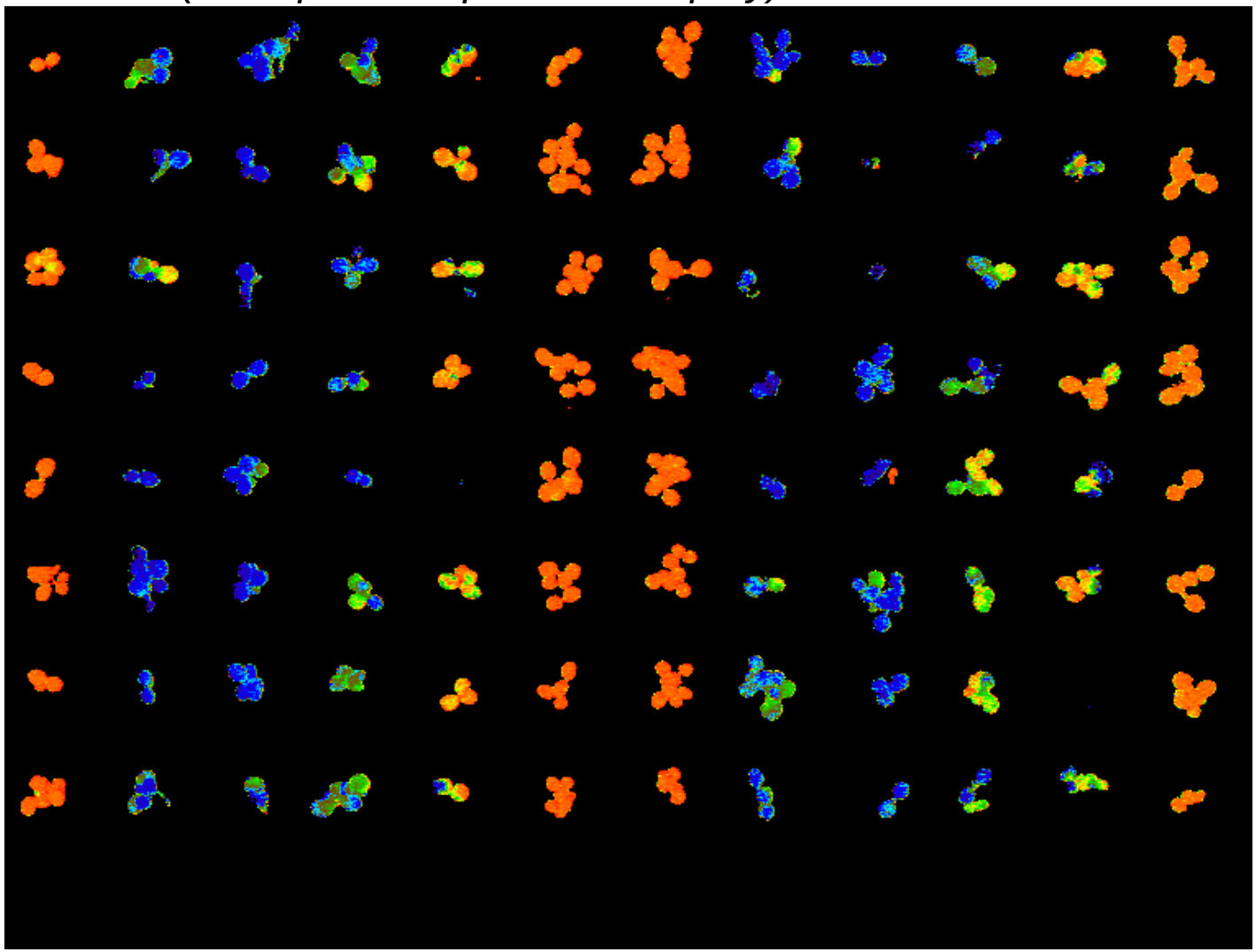
Zone lines and zone data displayed on the image can be copied as one image. Right hand click on the image and select copy image with overlays/bitmap under the Copy to Clipboard options. *This command copies all the information highlighted in the yellow box below. See next slide for example.*



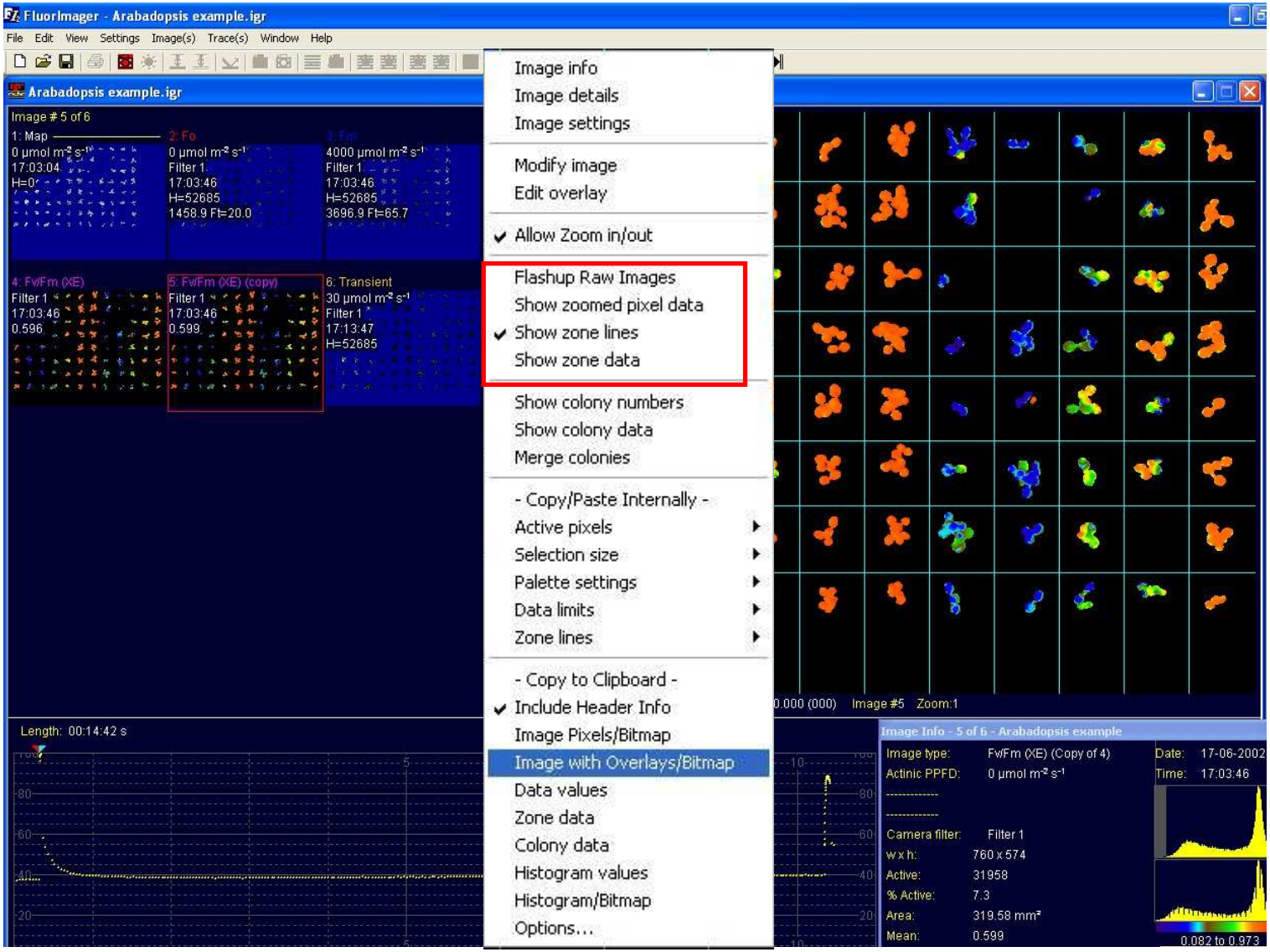
Example from previous slide of copied image including zone lines and zone data.



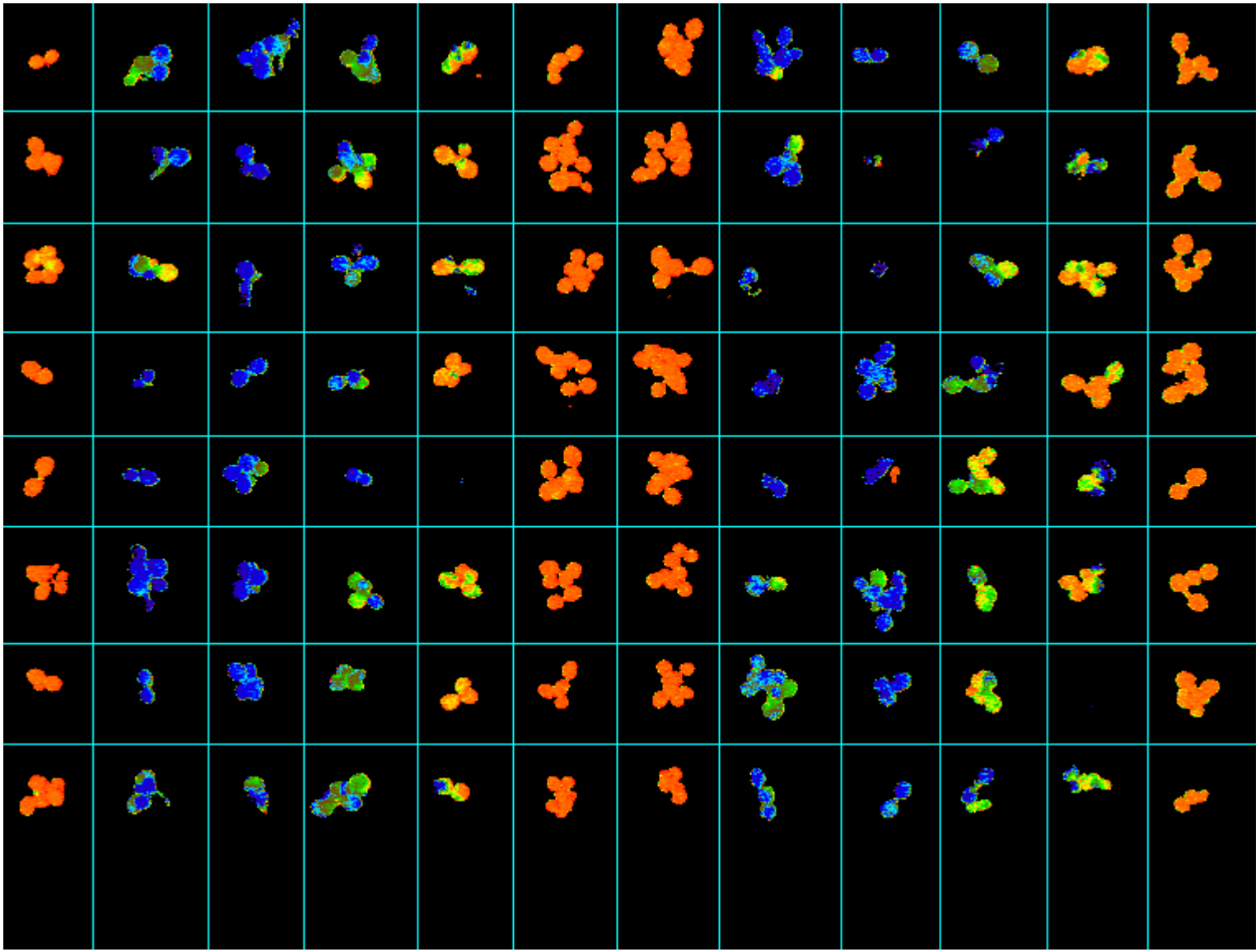
Copying the image only. Right hand click on the image and select copy image pixels/bitmap to the clipboard. *This command copies only the image to the clip board excluding the zone lines and zone data regardless of what is displayed on the screen (example from previous display).*



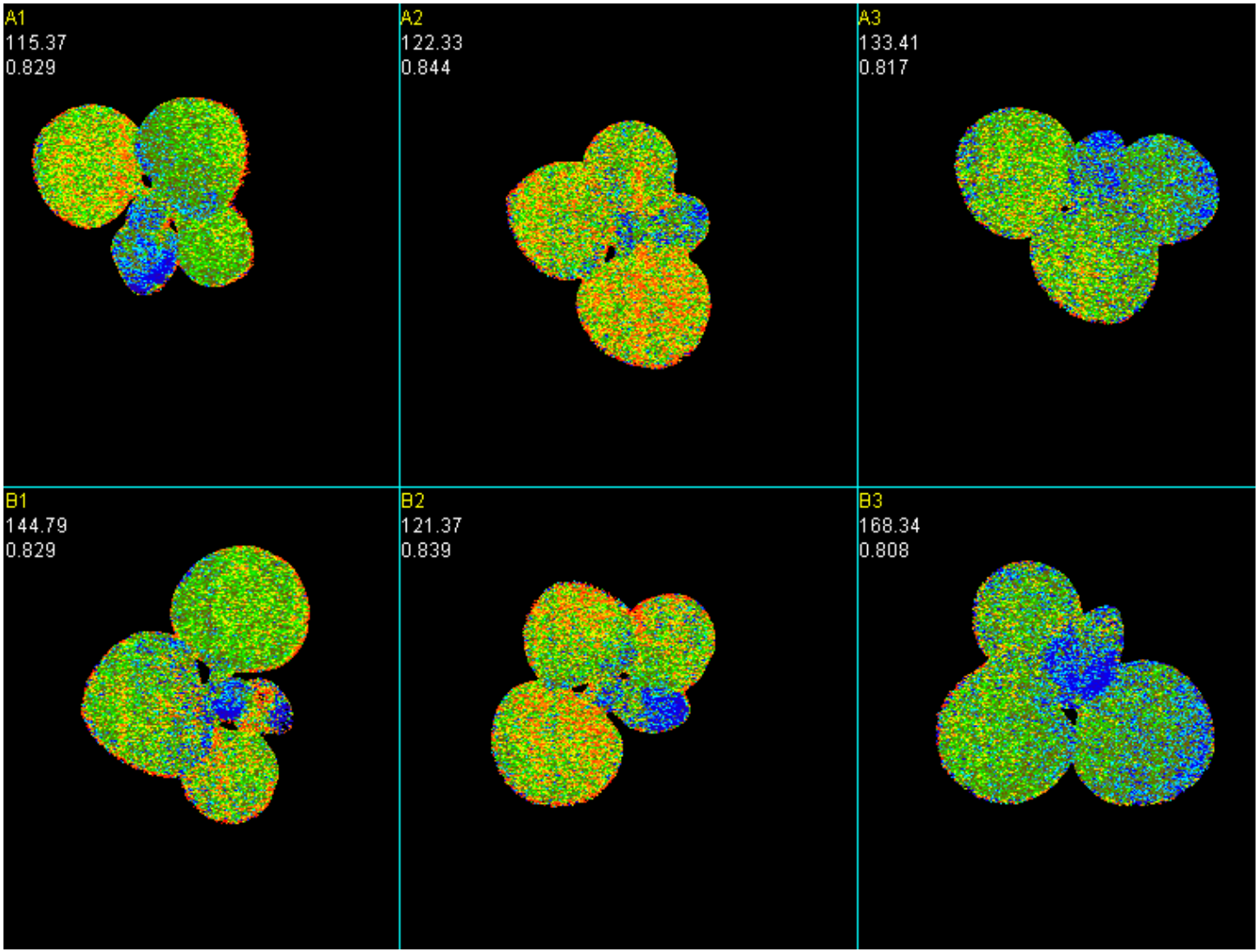
Copy image and zone lines only. Right hand click on the image and select view zone lines, ensure that view zone data is not ticked. Then select Image with Overlays/Bitmap from the Copy to Clipboard options.



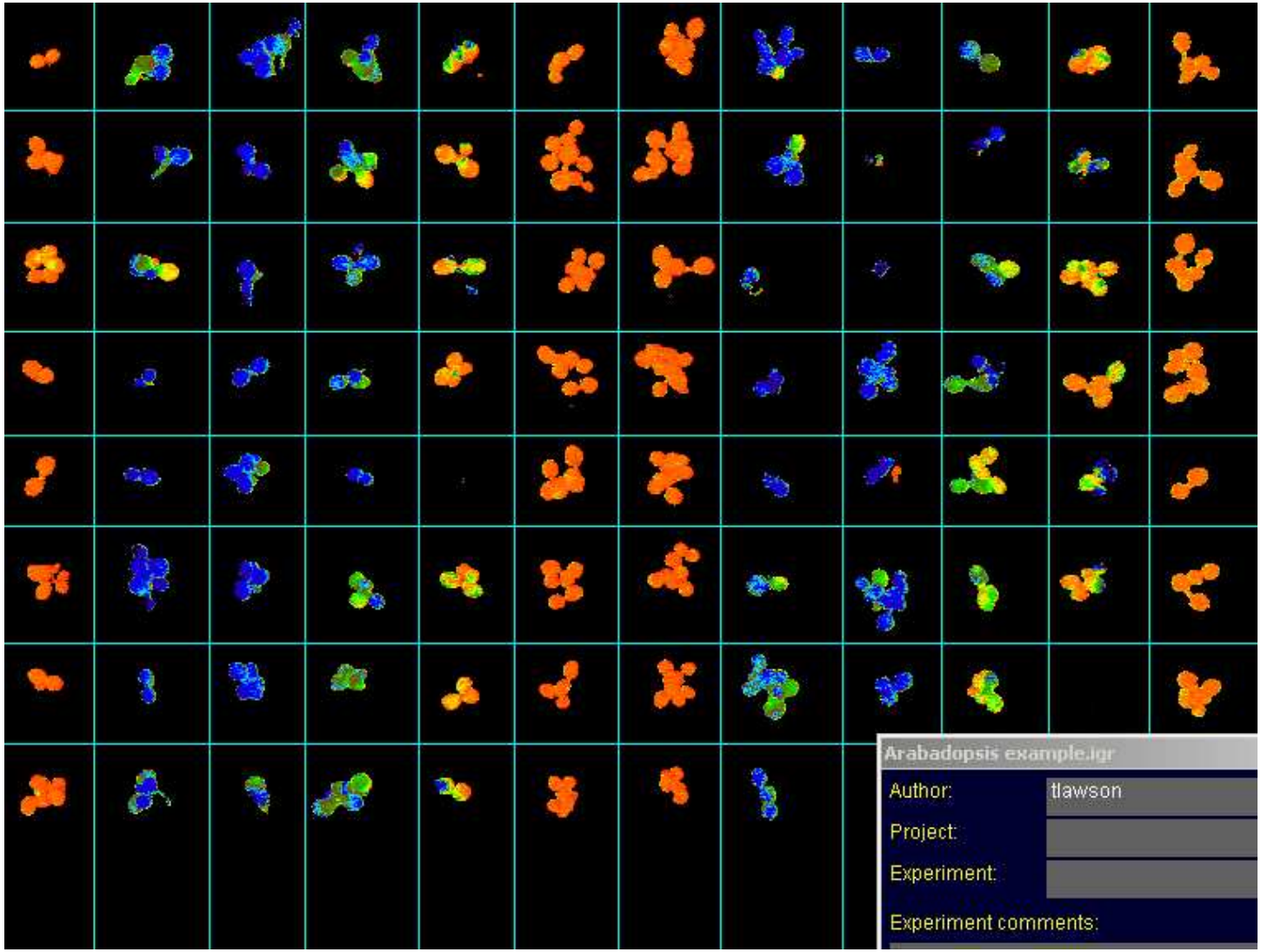
Example of copied image and zone lines only (previous slide).



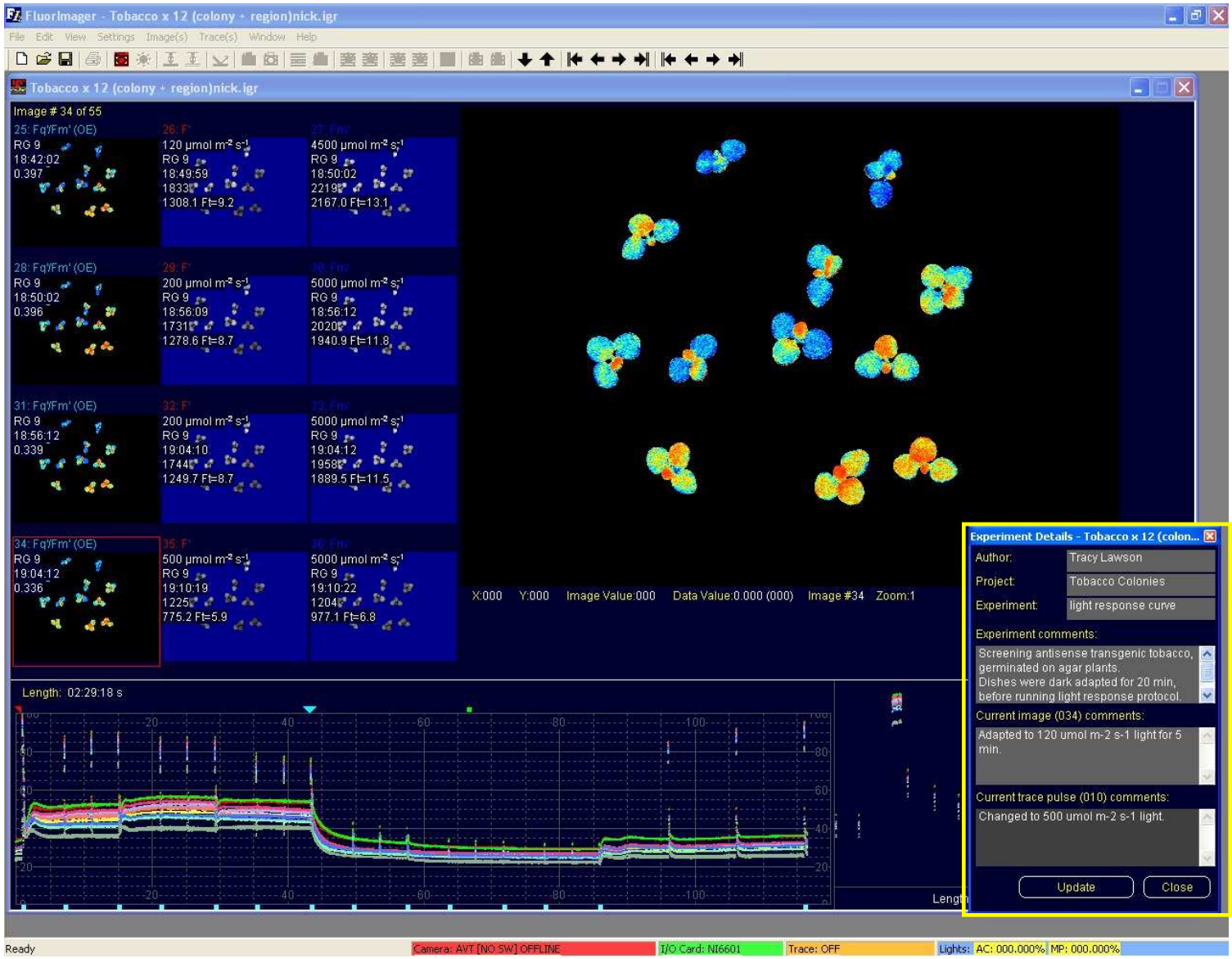
Copying zone lines and zone data along with the image is not restricted to 96 well plates. This example shows images of well established seedling grown and measured in pots.



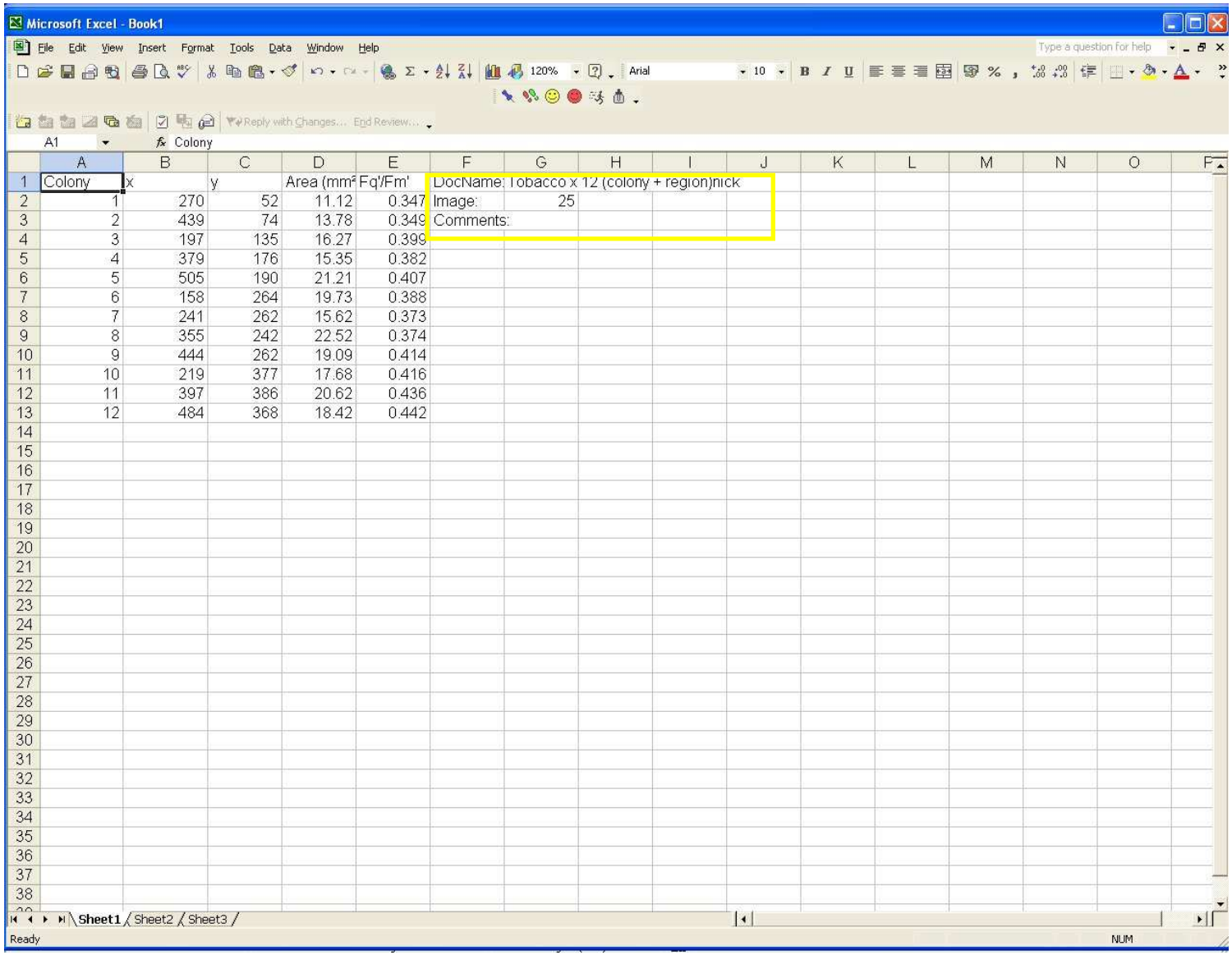
When copying images with overlays take care to ensure that no popup menus are visible within the image as these will be copied along with all the information (see below for example).



Inserting information and comments. Experimental details including Author, Project and Experimental comments can be inserted into a popup menu *(view, experiment details).* Comments can be included with any of the images using the “current image comments” area. All comments can be copied with the data into a spreadsheet.



Document name, image number is copied with image data into the spreadsheet. (*right click image, select copy colony data, paste into Excel).* Data taken from previous slide image.



Microsoft Excel - Book1

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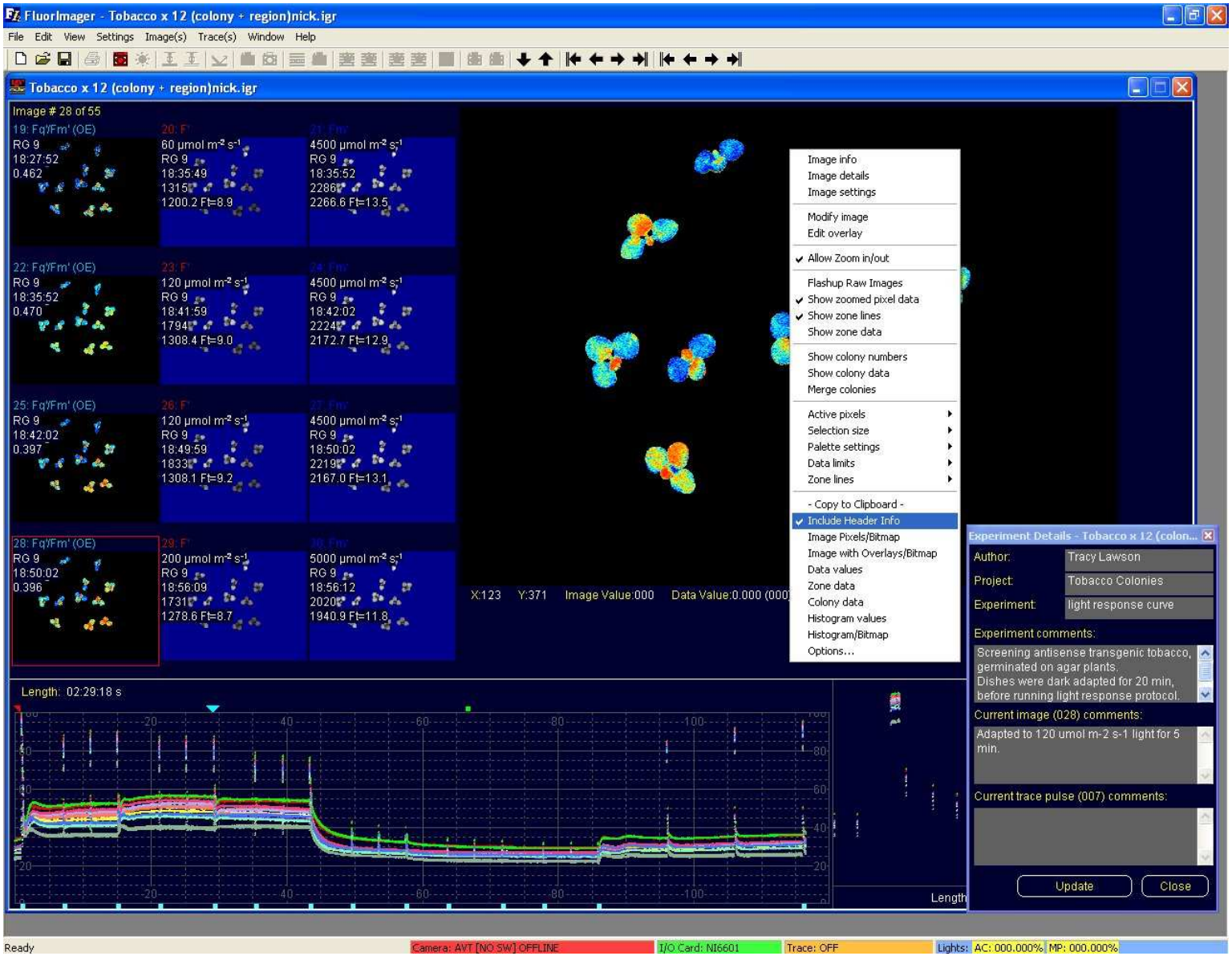
A1 Colony

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Colony	x	y	Area (mm²	Fq'/Fm'	DocName:	Tobacco x 12 (colony + region)nick								
2	1	270	52	11.12	0.347	Image:	25								
3	2	439	74	13.78	0.349	Comments:									
4	3	197	135	16.27	0.399										
5	4	379	176	15.35	0.382										
6	5	505	190	21.21	0.407										
7	6	158	264	19.73	0.388										
8	7	241	262	15.62	0.373										
9	8	355	242	22.52	0.374										
10	9	444	262	19.09	0.414										
11	10	219	377	17.68	0.416										
12	11	397	386	20.62	0.436										
13	12	484	368	18.42	0.442										

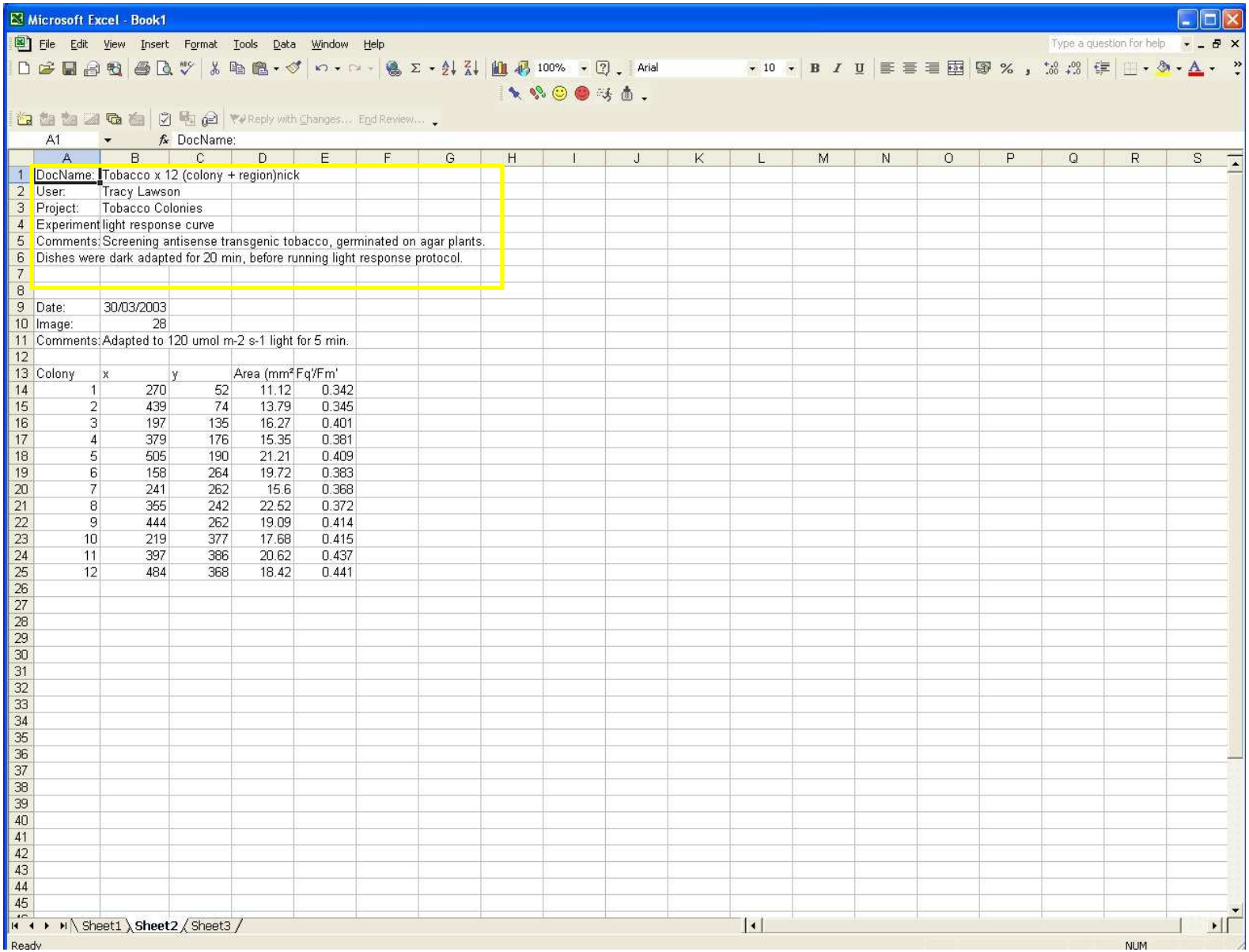
Sheet1 Sheet2 Sheet3

Ready NUM

To copy experimental comments with the data "include header info" must be selected *(right click image, select include header info, a tick will indicate its selection).*



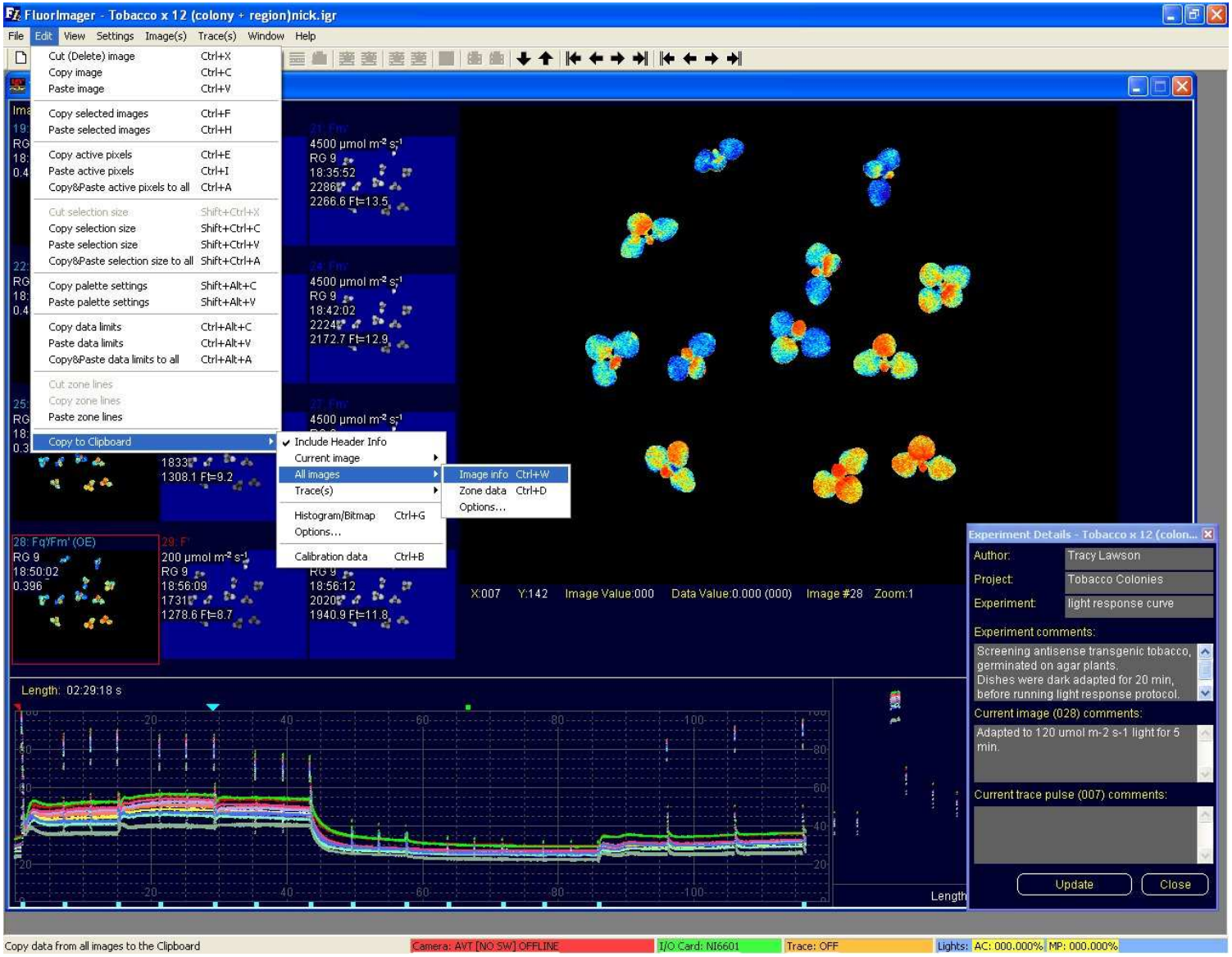
With “header info” selected all experimental details and current image info will be copied with the data and pasted at the top of the spreadsheet.



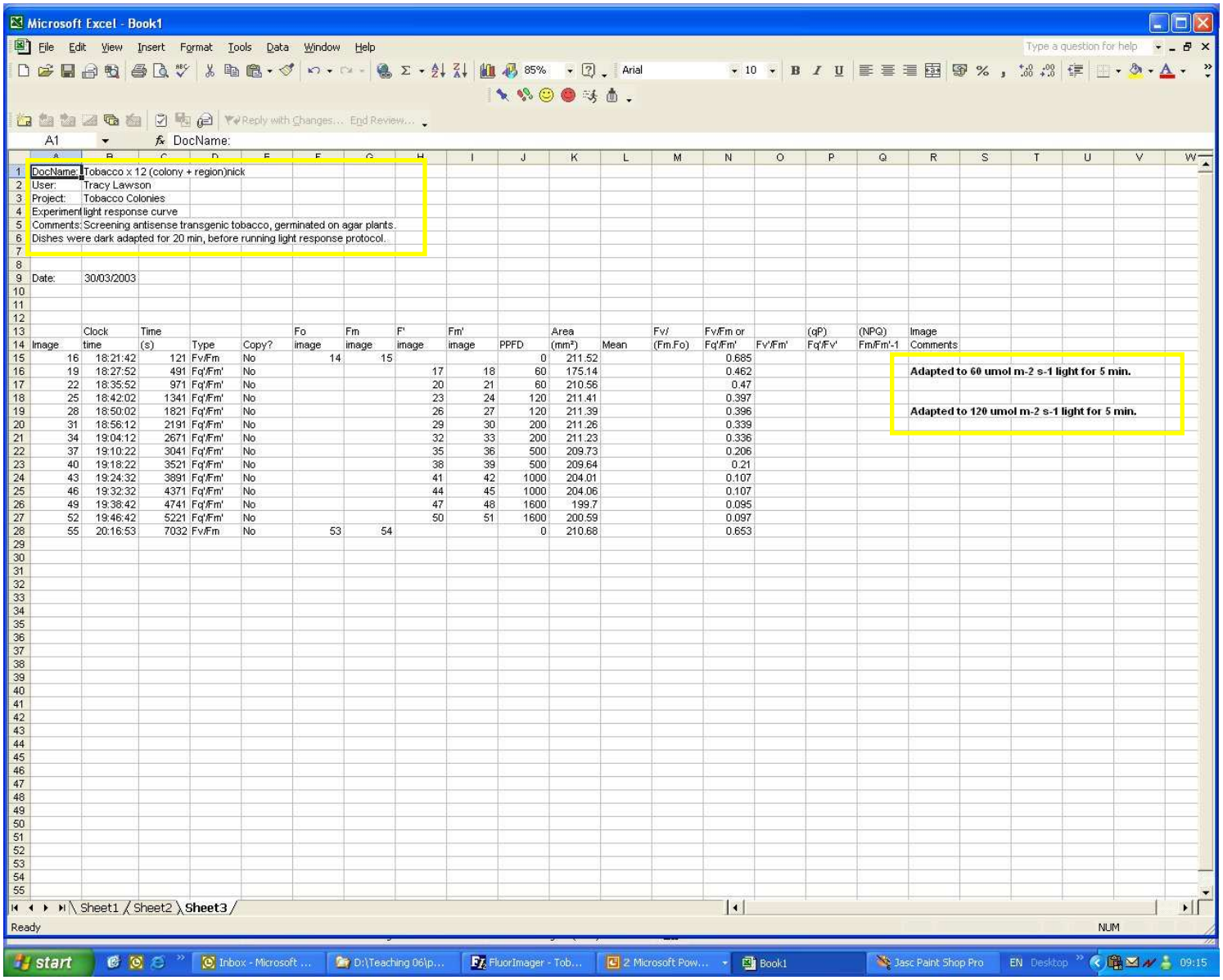
Microsoft Excel - Book1

	A	B	C	D	E
1	DocName:	Tobacco x 12 (colony + region)nick			
2	User:	Tracy Lawson			
3	Project:	Tobacco Colonies			
4	Experiment	light response curve			
5	Comments:	Screening antisense transgenic tobacco, germinated on agar plants.			
6	Dishes were dark adapted for 20 min, before running light response protocol.				
7					
8					
9	Date:	30/03/2003			
10	Image:	28			
11	Comments:	Adapted to 120 umol m-2 s-1 light for 5 min.			
12					
13	Colony	x	y	Area (mm²	Fq'/Fm'
14	1	270	52	11.12	0.342
15	2	439	74	13.79	0.345
16	3	197	135	16.27	0.401
17	4	379	176	15.35	0.381
18	5	505	190	21.21	0.409
19	6	158	264	19.72	0.383
20	7	241	262	15.6	0.368
21	8	355	242	22.52	0.372
22	9	444	262	19.09	0.414
23	10	219	377	17.68	0.415
24	11	397	386	20.62	0.437
25	12	484	368	18.42	0.441

With “header info” selected, all image data copy *(edit, clipboard copy, all images, image info)* **allows comments for all images to be copied and place along side data in the spreadsheet (see next slide)**.



The experimental details and user information is placed at the start of the spreadsheet, whilst current image information is placed in the last column in the corresponding data row.



Microsoft Excel - Book1

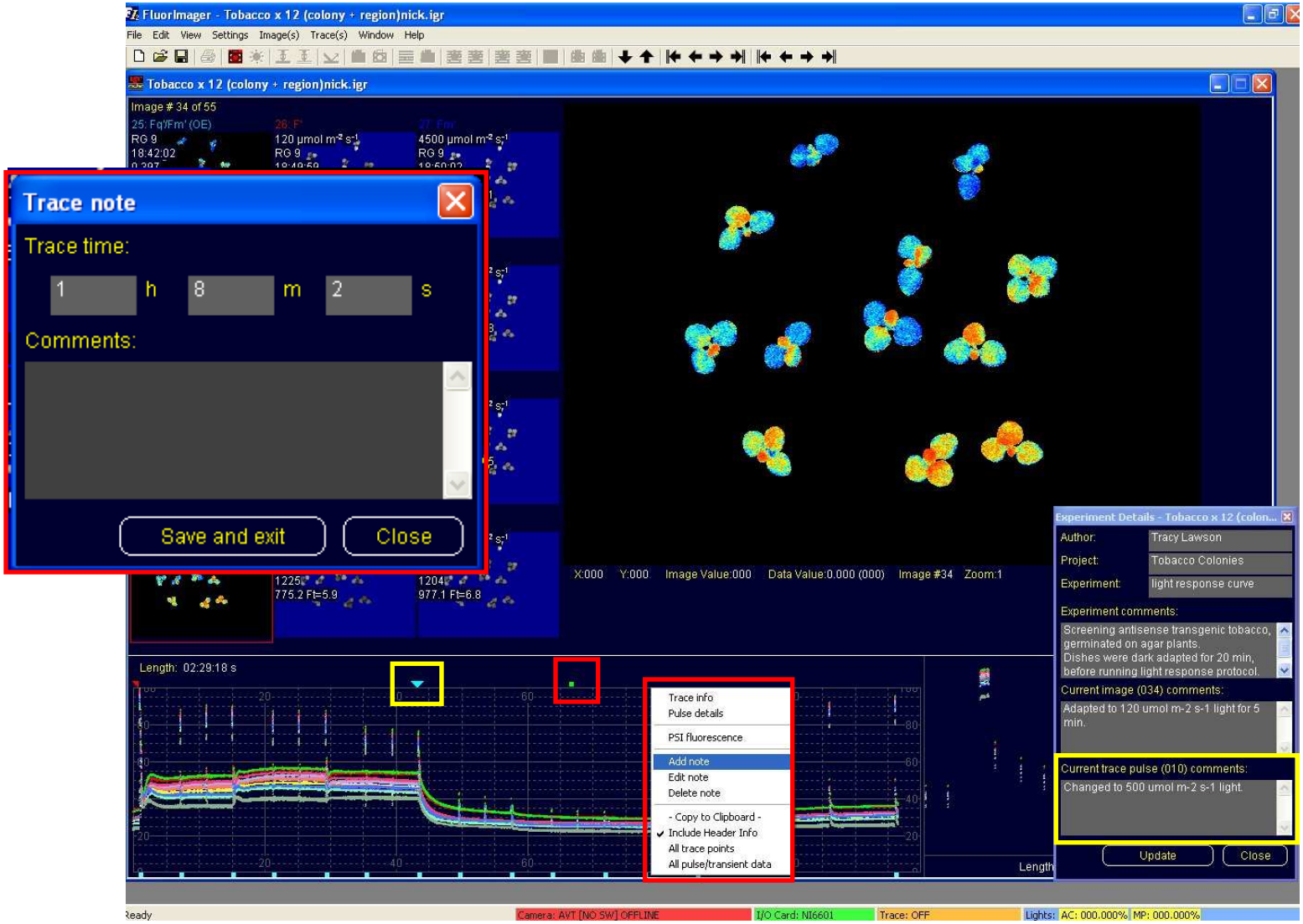
DocName:	Tobacco x 12 (colony + region)nick	
User:	Tracy Lawson	
Project:	Tobacco Colonies	
Experiment	light response curve	
Comments:	Screening antisense transgenic tobacco, germinated on agar plants.	
Dishes were dark adapted for 20 min, before running light response protocol.		
Date:	30/03/2003	

Image	Clock time	Time (s)	Type	Copy?	Fo image	Fm image	F' image	Fm' image	PPFD	Area (mm²)	Mean	Fv/ (Fm.Fo)	Fv/Fm or Fq'/Fm'	Fv'/Fm'	(qP) Fq'/Fv'	(NPQ) Fm/Fm'-1	Image Comments
16	18:21:42	121	Fv/Fm	No	14	15			0	211.52			0.685				
19	18:27:52	491	Fq'/Fm'	No			17	18	60	175.14			0.462				Adapted to 60 umol m-2 s-1 light for 5 min.
22	18:35:52	971	Fq'/Fm'	No			20	21	60	210.56			0.47				
25	18:42:02	1341	Fq'/Fm'	No			23	24	120	211.41			0.397				
28	18:50:02	1821	Fq'/Fm'	No			26	27	120	211.39			0.396				Adapted to 120 umol m-2 s-1 light for 5 min.
31	18:56:12	2191	Fq'/Fm'	No			29	30	200	211.26			0.339				
34	19:04:12	2671	Fq'/Fm'	No			32	33	200	211.23			0.336				
37	19:10:22	3041	Fq'/Fm'	No			35	36	500	209.73			0.206				
40	19:18:22	3521	Fq'/Fm'	No			38	39	500	209.64			0.21				
43	19:24:32	3891	Fq'/Fm'	No			41	42	1000	204.01			0.107				
46	19:32:32	4371	Fq'/Fm'	No			44	45	1000	204.06			0.107				
49	19:38:42	4741	Fq'/Fm'	No			47	48	1600	199.7			0.095				
52	19:46:42	5221	Fq'/Fm'	No			50	51	1600	200.59			0.097				
55	20:16:53	7032	Fv/Fm	No	53	54			0	210.68			0.653				

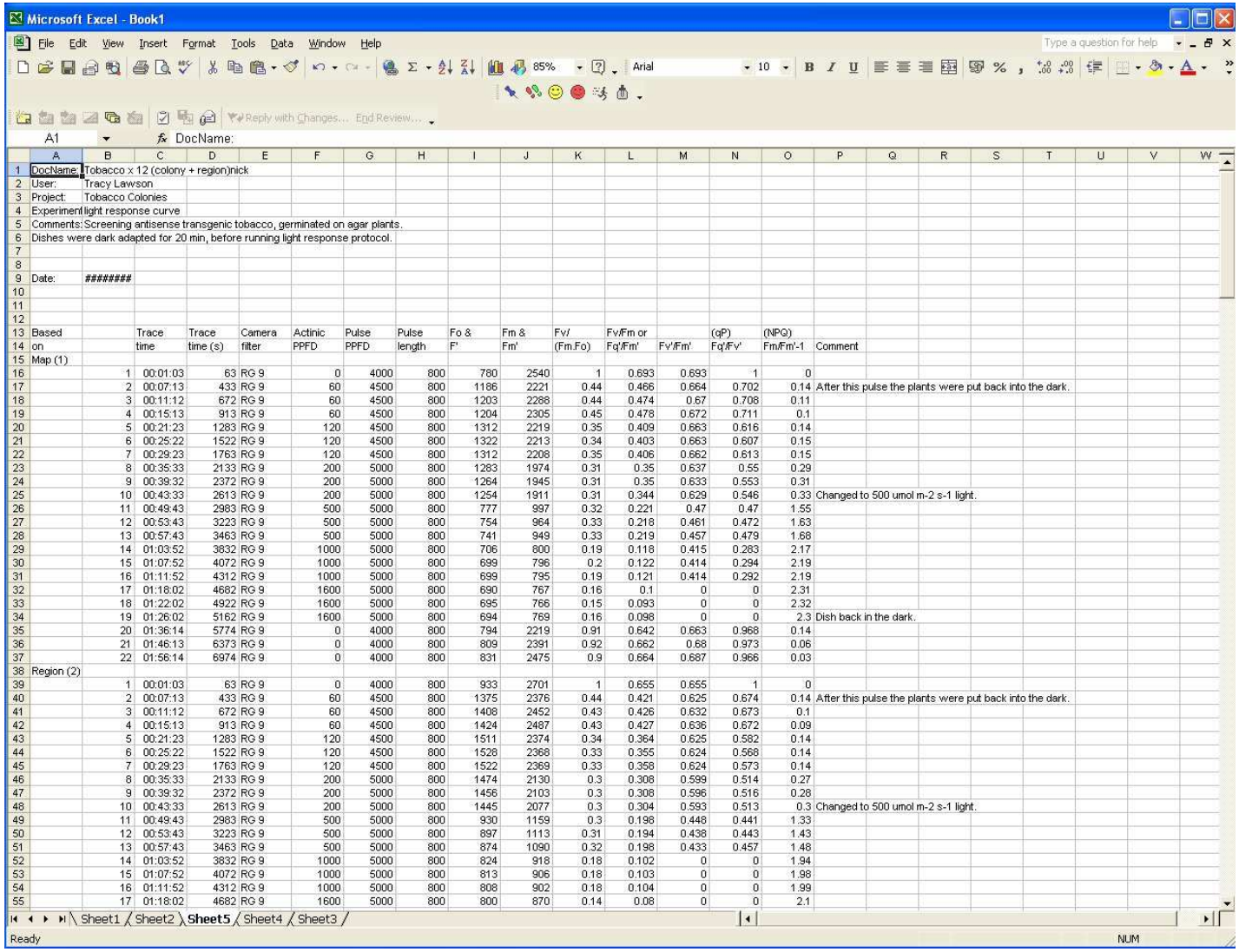
Sheet1 Sheet2 Sheet3

Ready NUM

Trace comments can be added to any saturating pulse in the “current trace comments” of the Experimental details popup dialogue (the position on the trace is marked by a blue triangle). Additionally, comment notes can be added to any position on the trace (*right click, select add note, a trace note box will appear).*** The position of trace comments is marked on the trace by a green square, which can be edited at any time *(right hand click green square and select “edit note”).*



Copying trace data. Copying “all pulse/transient data” (*right click on trace, select “all pulse/transient data” under clipboard copy menu) w*ith “header info” selected (*right click on trace, select “include header info” under copy to clipboard)* all experimental details, current trace comments are copied along with data and can be pasted with the data into excel.



Microsoft Excel - Book1

File Edit View Insert Format Tools Data Window Help

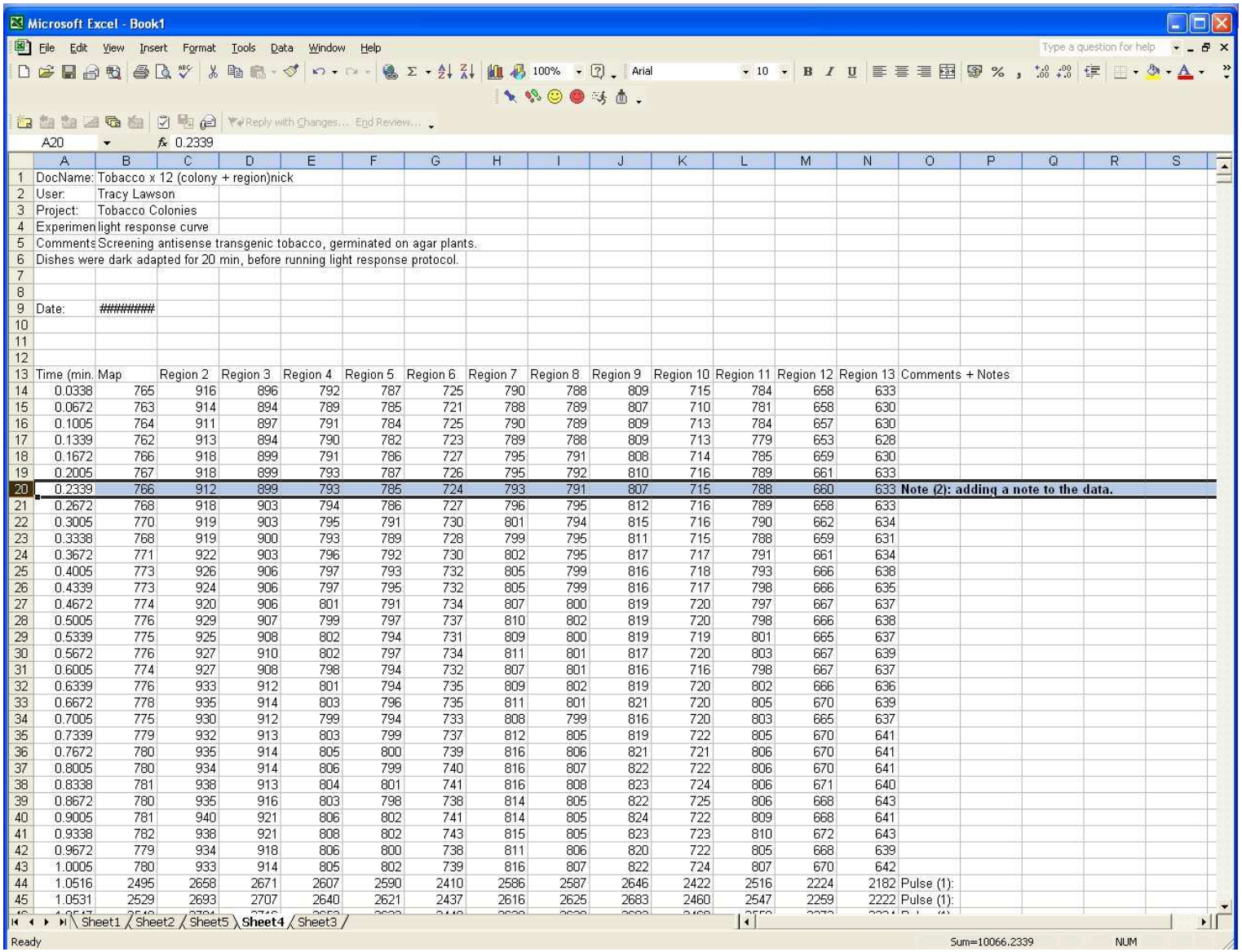
A1 DocName:

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	DocName:	Tobacco x 12 (colony + region)nick																					
2	User:	Tracy Lawson																					
3	Project:	Tobacco Colonies																					
4	Experiment	light response curve																					
5	Comments:	Screening antisense transgenic tobacco, germinated on agar plants.																					
6		Dishes were dark adapted for 20 min, before running light response protocol.																					
7																							
8																							
9	Date:	########																					
10																							
11																							
12																							
13	Based		Trace	Trace	Camera	Actinic	Pulse	Pulse	Fo &	Fm &	Fv/	Fv/Fm or		(qP)	(NPQ)								
14	on		time	time (s)	filter	PPFD	PPFD	length	F'	Fm'	(Fm.Fo)	Fq'/Fm'	Fv'/Fm'	Fq'/Fv'	Fm/Fm'-1	Comment							
15	Map (1)																						
16		1	00:01:03	63	RG 9	0	4000	800	780	2540	1	0.693	0.693	1	0								
17		2	00:07:13	433	RG 9	60	4500	800	1186	2221	0.44	0.466	0.664	0.702	0.14	After this pulse the plants were put back into the dark.							
18		3	00:11:12	672	RG 9	60	4500	800	1203	2288	0.44	0.474	0.67	0.708	0.11								
19		4	00:15:13	913	RG 9	60	4500	800	1204	2305	0.45	0.478	0.672	0.711	0.1								
20		5	00:21:23	1283	RG 9	120	4500	800	1312	2219	0.35	0.409	0.663	0.616	0.14								
21		6	00:25:22	1522	RG 9	120	4500	800	1322	2213	0.34	0.403	0.663	0.607	0.15								
22		7	00:29:23	1763	RG 9	120	4500	800	1312	2208	0.35	0.406	0.662	0.613	0.15								
23		8	00:35:33	2133	RG 9	200	5000	800	1283	1974	0.31	0.35	0.637	0.55	0.29								
24		9	00:39:32	2372	RG 9	200	5000	800	1264	1945	0.31	0.35	0.633	0.553	0.31								
25		10	00:43:33	2613	RG 9	200	5000	800	1254	1911	0.31	0.344	0.629	0.546	0.33	Changed to 500 umol m-2 s-1 light.							
26		11	00:49:43	2983	RG 9	500	5000	800	777	997	0.32	0.221	0.47	0.47	1.55								
27		12	00:53:43	3223	RG 9	500	5000	800	754	964	0.33	0.218	0.461	0.472	1.63								
28		13	00:57:43	3463	RG 9	500	5000	800	741	949	0.33	0.219	0.457	0.479	1.68								
29		14	01:03:52	3832	RG 9	1000	5000	800	706	800	0.19	0.118	0.415	0.283	2.17								
30		15	01:07:52	4072	RG 9	1000	5000	800	699	796	0.2	0.122	0.414	0.294	2.19								
31		16	01:11:52	4312	RG 9	1000	5000	800	699	795	0.19	0.121	0.414	0.292	2.19								
32		17	01:18:02	4682	RG 9	1600	5000	800	690	767	0.16	0.1	0	0	2.31								
33		18	01:22:02	4922	RG 9	1600	5000	800	695	766	0.15	0.093	0	0	2.32								
34		19	01:26:02	5162	RG 9	1600	5000	800	694	769	0.16	0.098	0	0	2.3	Dish back in the dark.							
35		20	01:36:14	5774	RG 9	0	4000	800	794	2219	0.91	0.642	0.863	0.968	0.14								
36		21	01:46:13	6373	RG 9	0	4000	800	809	2391	0.92	0.662	0.68	0.973	0.06								
37		22	01:56:14	6974	RG 9	0	4000	800	831	2475	0.9	0.664	0.687	0.966	0.03								
38	Region (2)																						
39		1	00:01:03	63	RG 9	0	4000	800	933	2701	1	0.655	0.655	1	0								
40		2	00:07:13	433	RG 9	60	4500	800	1375	2376	0.44	0.421	0.625	0.674	0.14	After this pulse the plants were put back into the dark.							
41		3	00:11:12	672	RG 9	60	4500	800	1408	2452	0.43	0.426	0.632	0.673	0.1								
42		4	00:15:13	913	RG 9	60	4500	800	1424	2487	0.43	0.427	0.636	0.672	0.09								
43		5	00:21:23	1283	RG 9	120	4500	800	1511	2374	0.34	0.364	0.625	0.582	0.14								
44		6	00:25:22	1522	RG 9	120	4500	800	1528	2368	0.33	0.355	0.624	0.568	0.14								
45		7	00:29:23	1763	RG 9	120	4500	800	1522	2369	0.33	0.358	0.624	0.573	0.14								
46		8	00:35:33	2133	RG 9	200	5000	800	1474	2130	0.3	0.308	0.599	0.514	0.27								
47		9	00:39:32	2372	RG 9	200	5000	800	1456	2103	0.3	0.308	0.596	0.516	0.28								
48		10	00:43:33	2613	RG 9	200	5000	800	1445	2077	0.3	0.304	0.593	0.513	0.3	Changed to 500 umol m-2 s-1 light.							
49		11	00:49:43	2983	RG 9	500	5000	800	930	1159	0.3	0.198	0.448	0.441	1.33								
50		12	00:53:43	3223	RG 9	500	5000	800	897	1113	0.31	0.194	0.438	0.443	1.43								
51		13	00:57:43	3463	RG 9	500	5000	800	874	1090	0.32	0.198	0.433	0.457	1.48								
52		14	01:03:52	3832	RG 9	1000	5000	800	824	918	0.18	0.102	0	0	1.94								
53		15	01:07:52	4072	RG 9	1000	5000	800	813	906	0.18	0.103	0	0	1.98								
54		16	01:11:52	4312	RG 9	1000	5000	800	808	902	0.18	0.104	0	0	1.99								
55		17	01:18:02	4682	RG 9	1600	5000	800	800	870	0.14	0.08	0	0	2.1								

Sheet1 Sheet2 Sheet5 Sheet4 Sheet3

Ready NUM

Copying All trace point. Copying “all trace points” (*right click on trace, select “all trace points” under clipboard copy menu)* will copy all trace points, added comments are pasted alongside corresponding trace data position.



Microsoft Excel - Book1

File Edit View Insert Format Tools Data Window Help

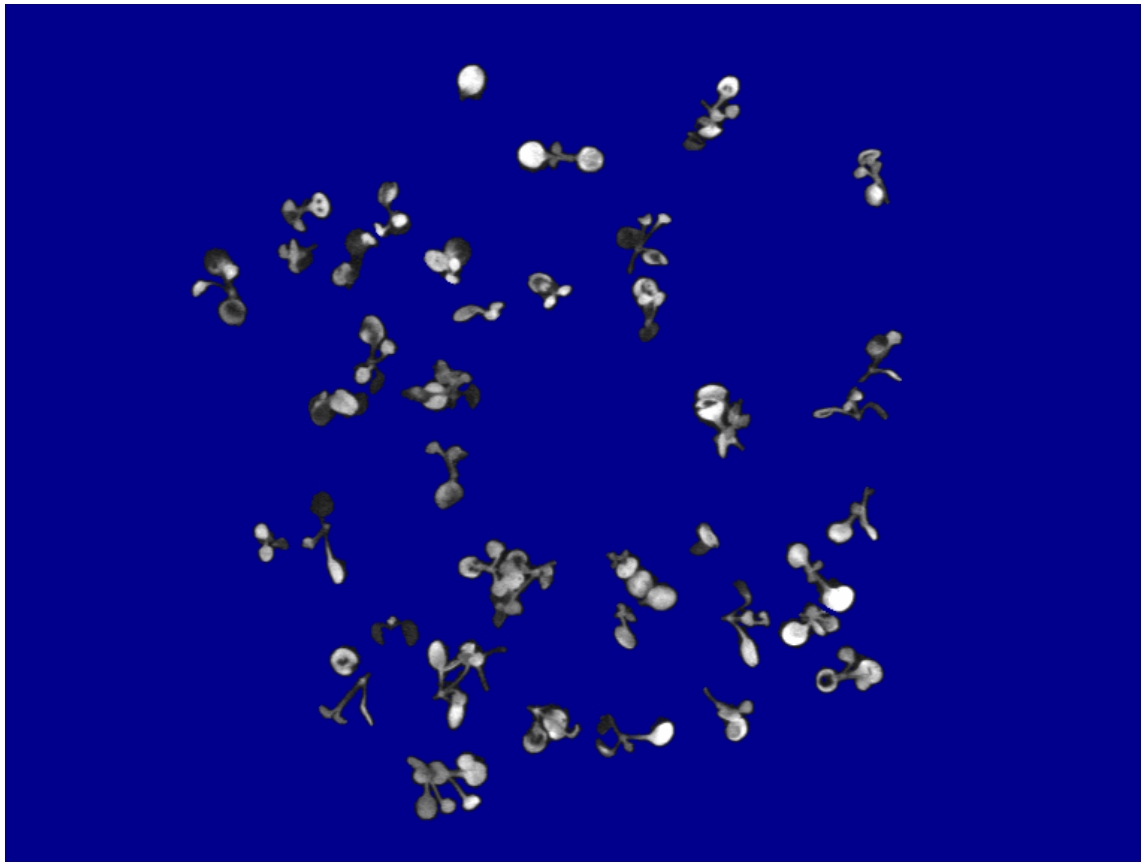
A20 0.2339

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	DocName:	Tobacco x 12 (colony + region)nick																	
2	User:	Tracy Lawson																	
3	Project:	Tobacco Colonies																	
4	Experimen	light response curve																	
5	Comments	Screening antisense transgenic tobacco, germinated on agar plants.																	
6	Dishes were dark adapted for 20 min, before running light response protocol.																		
7																			
8																			
9	Date:	#########																	
10																			
11																			
12																			
13	Time (min.	Map	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7	Region 8	Region 9	Region 10	Region 11	Region 12	Region 13	Comments + Notes				
14	0.0338	765	916	896	792	787	725	790	788	809	715	784	658	633					
15	0.0672	763	914	894	789	785	721	788	789	807	710	781	658	630					
16	0.1005	764	911	897	791	784	725	790	789	809	713	784	657	630					
17	0.1339	762	913	894	790	782	723	789	788	809	713	779	653	628					
18	0.1672	766	918	899	791	786	727	795	791	808	714	785	659	630					
19	0.2005	767	918	899	793	787	726	795	792	810	716	789	661	633					
20	0.2339	766	912	899	793	785	724	793	791	807	715	788	660	633	Note (2): adding a note to the data.				
21	0.2672	768	918	903	794	786	727	796	795	812	716	789	658	633					
22	0.3005	770	919	903	795	791	730	801	794	815	716	790	662	634					
23	0.3338	768	919	900	793	789	728	799	795	811	715	788	659	631					
24	0.3672	771	922	903	796	792	730	802	795	817	717	791	661	634					
25	0.4005	773	926	906	797	793	732	805	799	816	718	793	666	638					
26	0.4339	773	924	906	797	795	732	805	799	816	717	798	666	635					
27	0.4672	774	920	906	801	791	734	807	800	819	720	797	667	637					
28	0.5005	776	929	907	799	797	737	810	802	819	720	798	666	638					
29	0.5339	775	925	908	802	794	731	809	800	819	719	801	665	637					
30	0.5672	776	927	910	802	797	734	811	801	817	720	803	667	639					
31	0.6005	774	927	908	798	794	732	807	801	816	716	798	667	637					
32	0.6339	776	933	912	801	794	735	809	802	819	720	802	666	636					
33	0.6672	778	935	914	803	796	735	811	801	821	720	805	670	639					
34	0.7005	775	930	912	799	794	733	808	799	816	720	803	665	637					
35	0.7339	779	932	913	803	799	737	812	805	819	722	805	670	641					
36	0.7672	780	935	914	805	800	739	816	806	821	721	806	670	641					
37	0.8005	780	934	914	806	799	740	816	807	822	722	806	670	641					
38	0.8338	781	938	913	804	801	741	816	808	823	724	806	671	640					
39	0.8672	780	935	916	803	798	738	814	805	822	725	806	668	643					
40	0.9005	781	940	921	806	802	741	814	805	824	722	809	668	641					
41	0.9338	782	938	921	808	802	743	815	805	823	723	810	672	643					
42	0.9672	779	934	918	806	800	738	811	806	820	722	805	668	639					
43	1.0005	780	933	914	805	802	739	816	807	822	724	807	670	642					
44	1.0516	2495	2658	2671	2607	2590	2410	2586	2587	2646	2422	2516	2224	2182	Pulse (1):				
45	1.0531	2529	2693	2707	2640	2621	2437	2616	2625	2683	2460	2547	2259	2222	Pulse (1):				

Sheet1 Sheet2 Sheet5 Sheet4 Sheet3

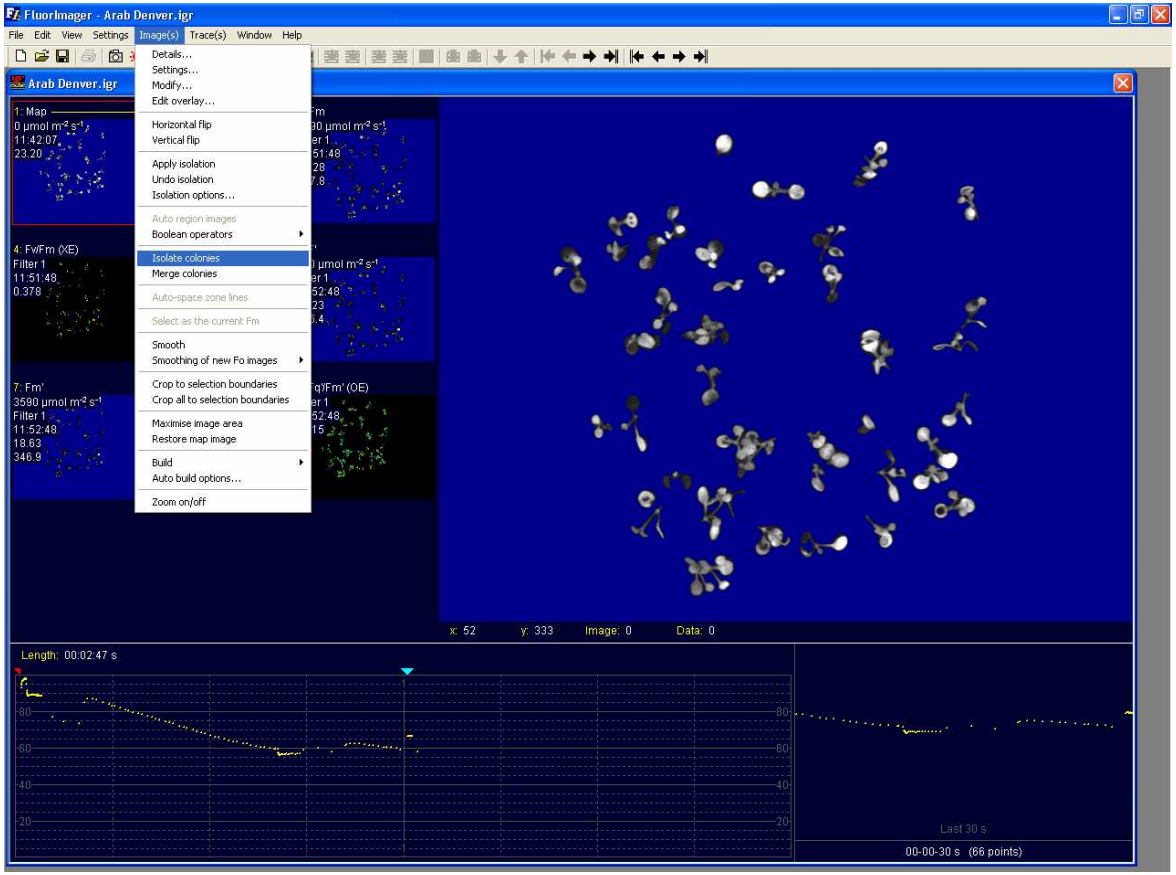
Ready Sum=10066.2339 NUM

Working with Samples Grown Randomly



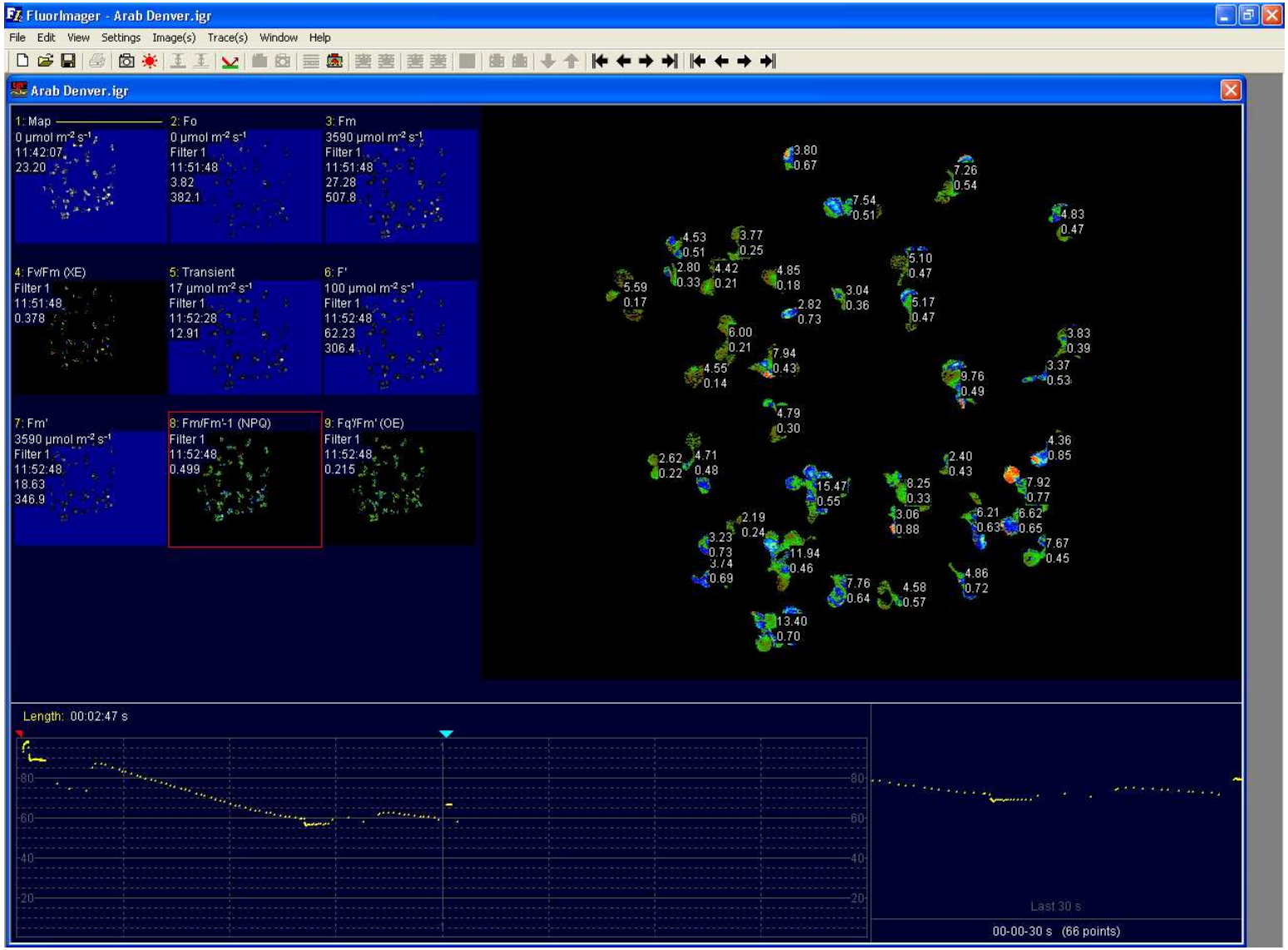
Often, it is not possible, or desirable, to grow samples in an organized array, such as on a 96 well plate. However, the cf imager software allows easy analysis of individual samples even when they are grown randomly as with the Arabidopsis seedlings shown above.

It has been captured as described previously, and Image Isolation has been employed to isolate the samples. The ISOLATE COLONIES command can now be used to identify and number each plant. Up to 250 separate colonies can be identified using the isolate colonies command.



If necessary, Image Modification tools can be used to separate plants that overlap. Also a MERGE COLONIES command allows several colonies to be grouped as one sample if this is required.

Each plant is now numbered as a separate colony, and colony data are shown on the Image (if required)



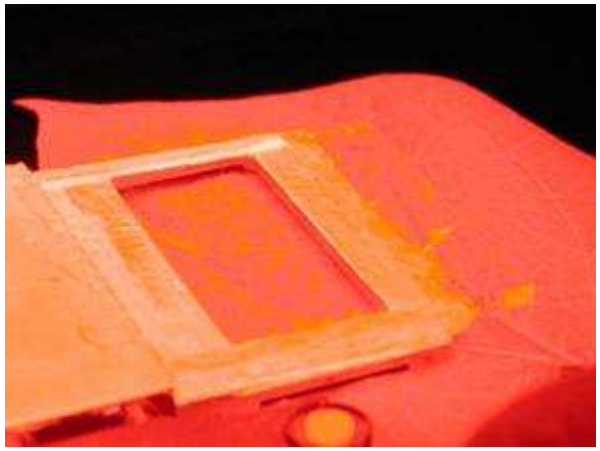
Colony	x	y	Area (mm²	NPQ
1	312	52	3.8	0.67
2	472	72	7.26	0.54
3	371	102	7.54	0.51
4	579	116	4.83	0.47
5	201	139	4.53	0.51
6	258	137	3.77	0.25
7	195	169	2.8	0.33
8	233	170	4.42	0.21
9	295	172	4.85	0.18
10	427	160	5.1	0.47
11	142	189	5.59	0.17
12	316	206	2.82	0.73
13	364	192	3.04	0.36
14	430	204	5.17	0.47
15	247	234	6	0.21
16	291	255	7.94	0.43
17	585	235	3.83	0.39
18	222	270	4.55	0.14
19	479	278	9.76	0.49
20	565	267	3.37	0.53
21	295	315	4.79	0.3
22	177	360	2.62	0.22
23	213	357	4.71	0.48
24	467	358	2.4	0.43
25	566	342	4.36	0.85
26	335	388	15.47	0.55
27	425	385	8.25	0.33
28	414	416	3.06	0.88
29	495	414	6.21	0.63
30	537	415	6.62	0.65
31	545	384	7.92	0.77
32	260	419	2.19	0.24
33	228	465	3.74	0.69
34	227	439	3.23	0.73
35	308	455	11.94	0.46
36	365	485	7.76	0.64
37	483	475	4.86	0.72
38	564	445	7.67	0.45
39	421	489	4.58	0.57
40	295	523	13.4	0.7

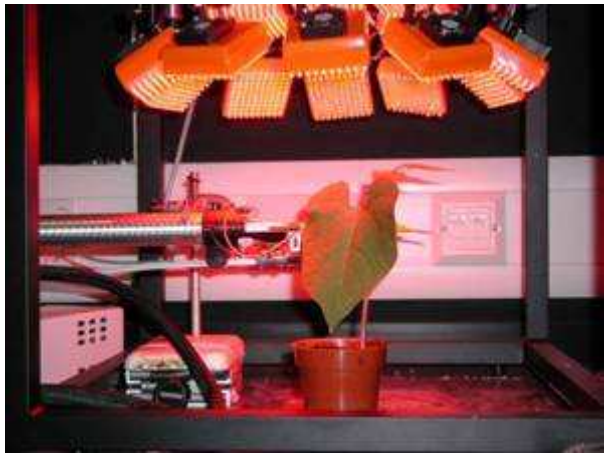
Colony data is copied to the Clipboard and pasted into Excel. The x/y co-ordinates show the location of each colony on the image. Colony area and the selected fluorescence parameter data are shown

University of Essex

Combined with Infra-red gas analysis

Mapping C_i using chlorophyll fluorescence





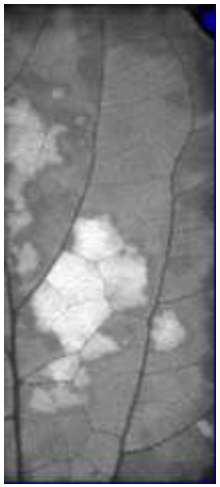
- Photosystem II quantum yield : F_q'/F_m'
- CO_2 assimilation rate : A
  (µmol $CO_2.m^{-2}.s^{-1}$)
- Stomatal conductance : g_s
  (µmol $CO_2.m^{-2}.s^{-1}$)
- Internal CO_2 partial pressure : C_i (ppm)

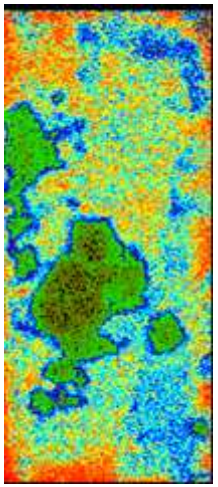
Morison et al., 2005

University of Essex

Visualising natural and artificial patches

Natural patchiness

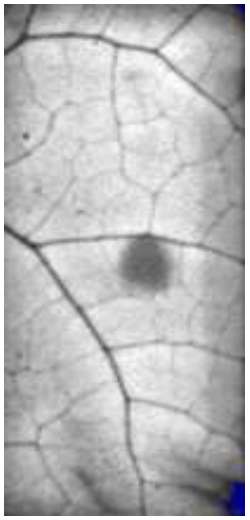


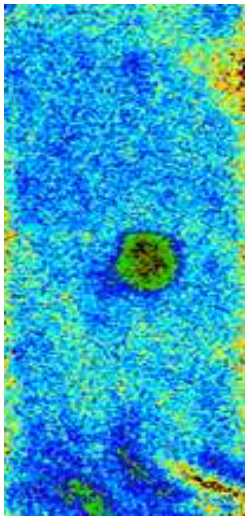


PSII efficiency

Helianthus annuus
Natural patchiness induced by rapid decrease in humidity

Artificial patchiness

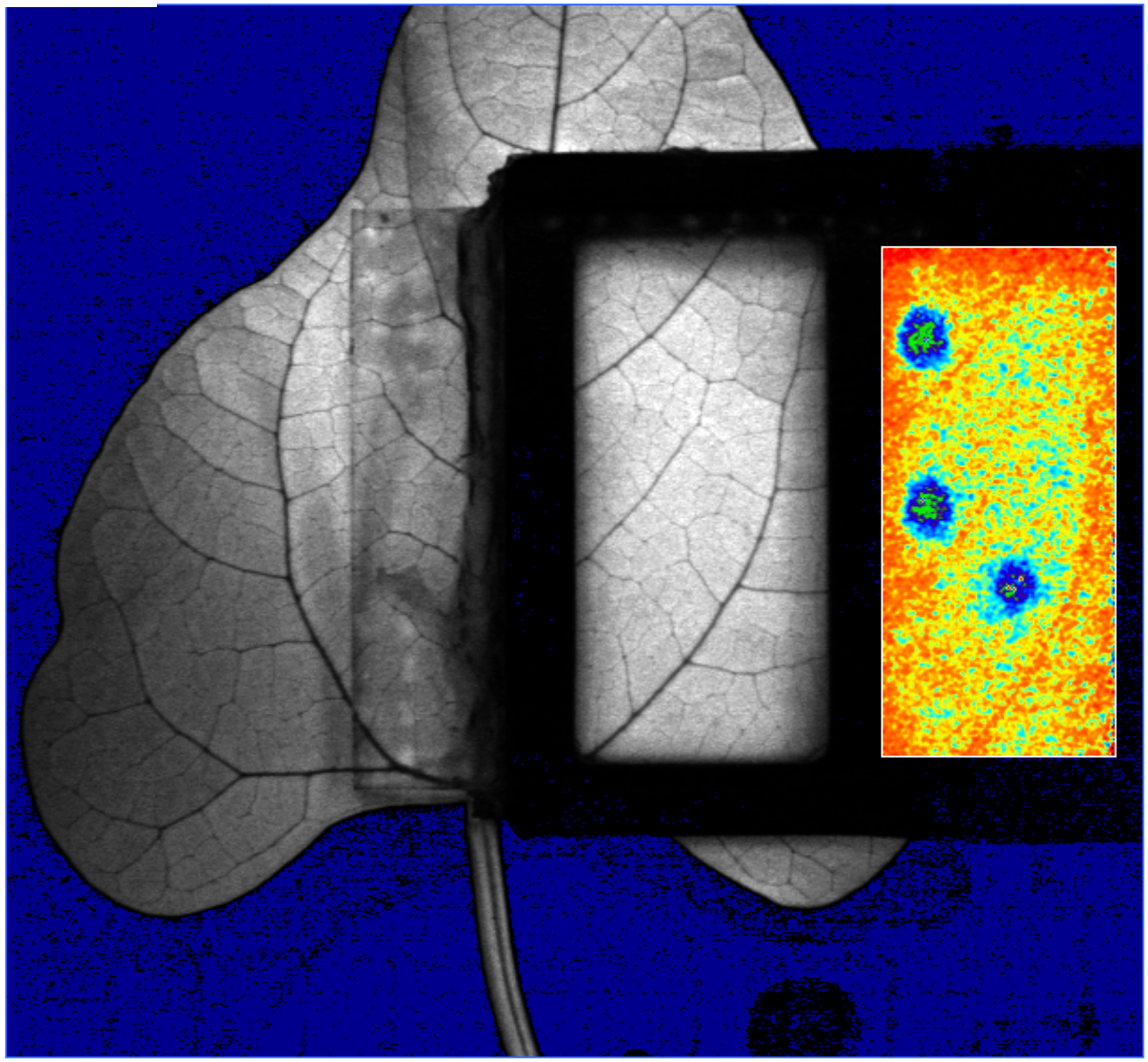




PSII efficiency

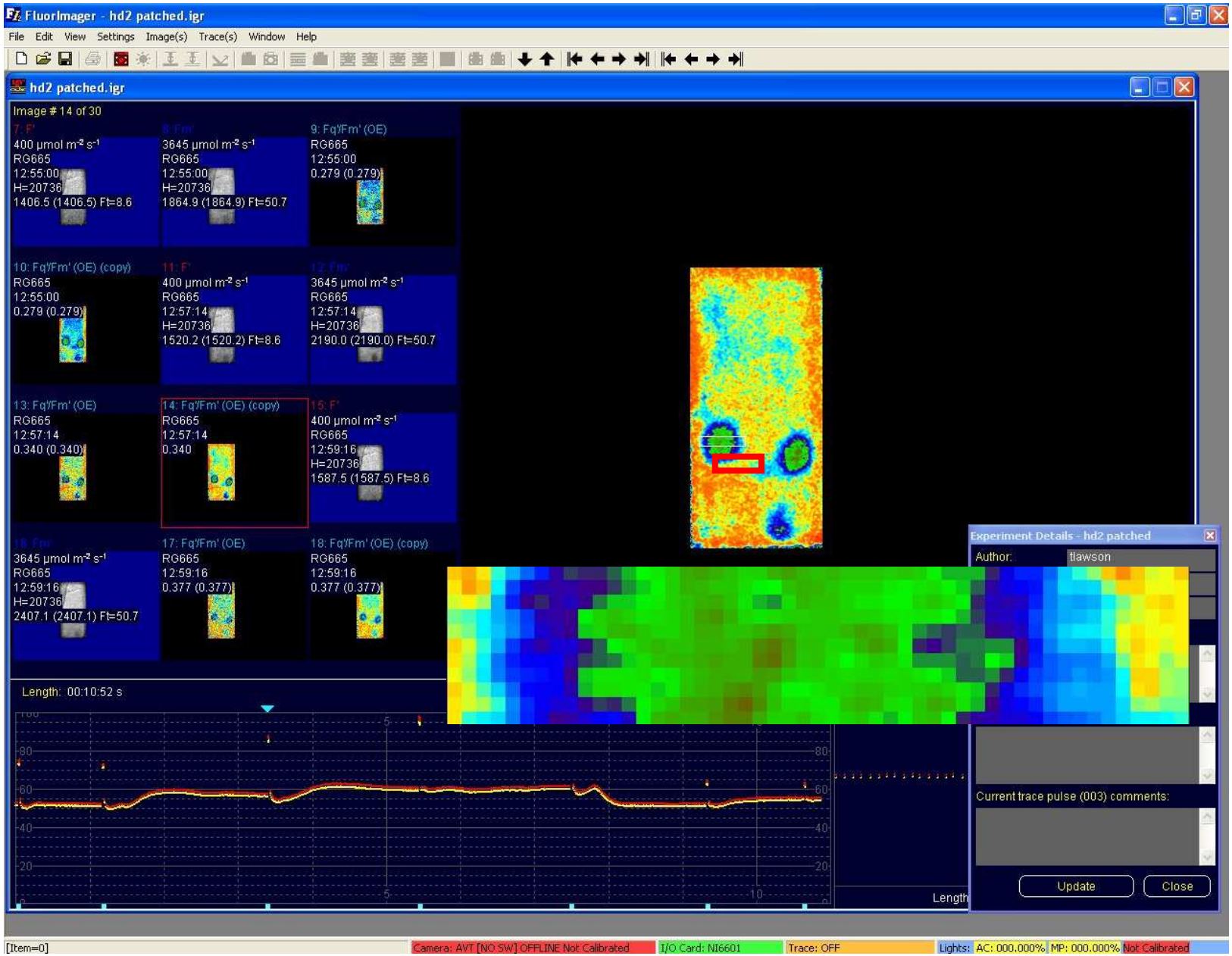
Phaseolus vulgaris
Artificial patch (4mm diameter silicon grease applied on both sides)

University of Essex

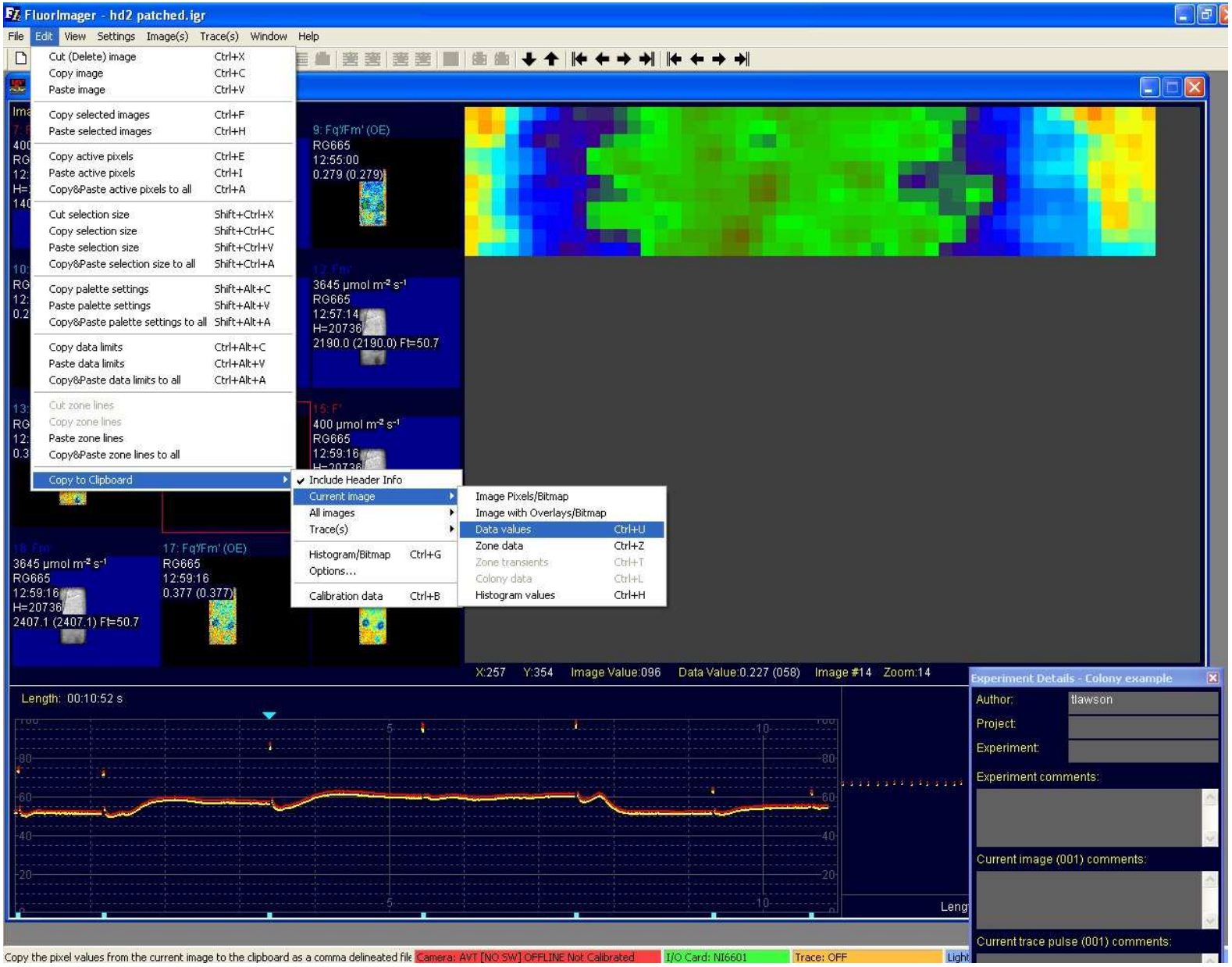


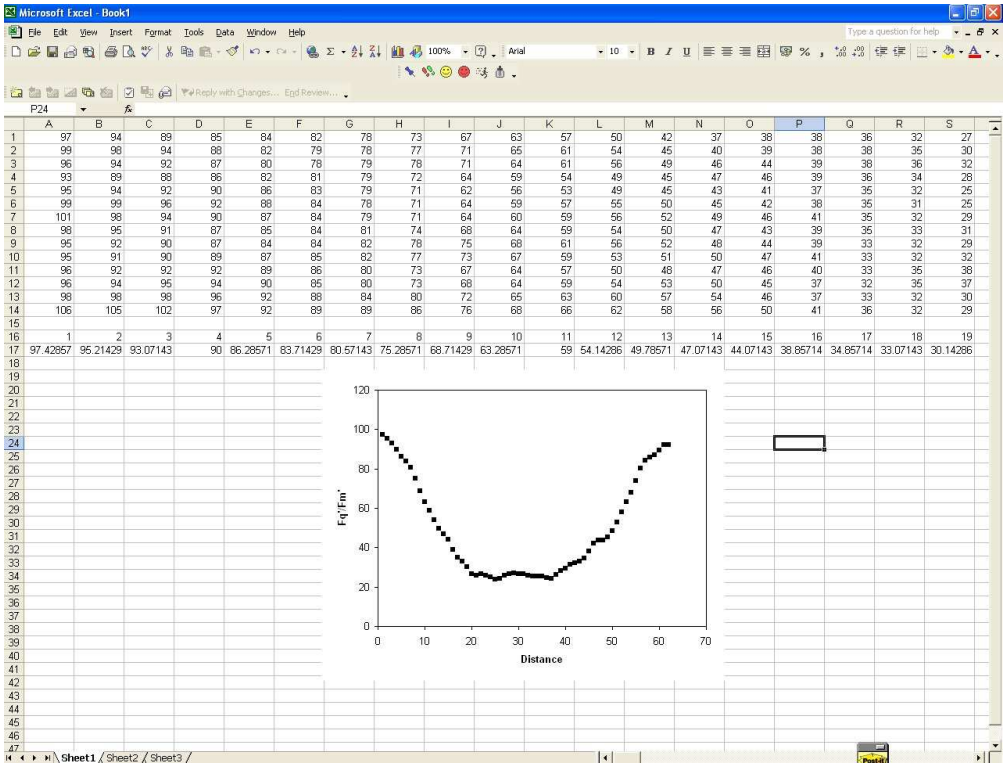
Morison et al., 2005

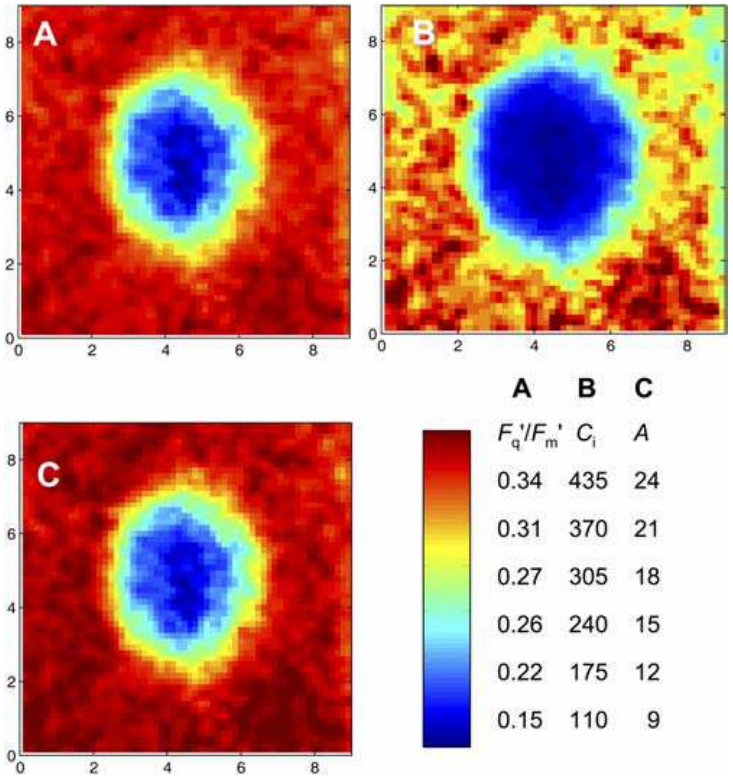
Selected area(s) (red box) can be zoomed in on any image.



The pixel data from such areas can be copied into data processing applications.

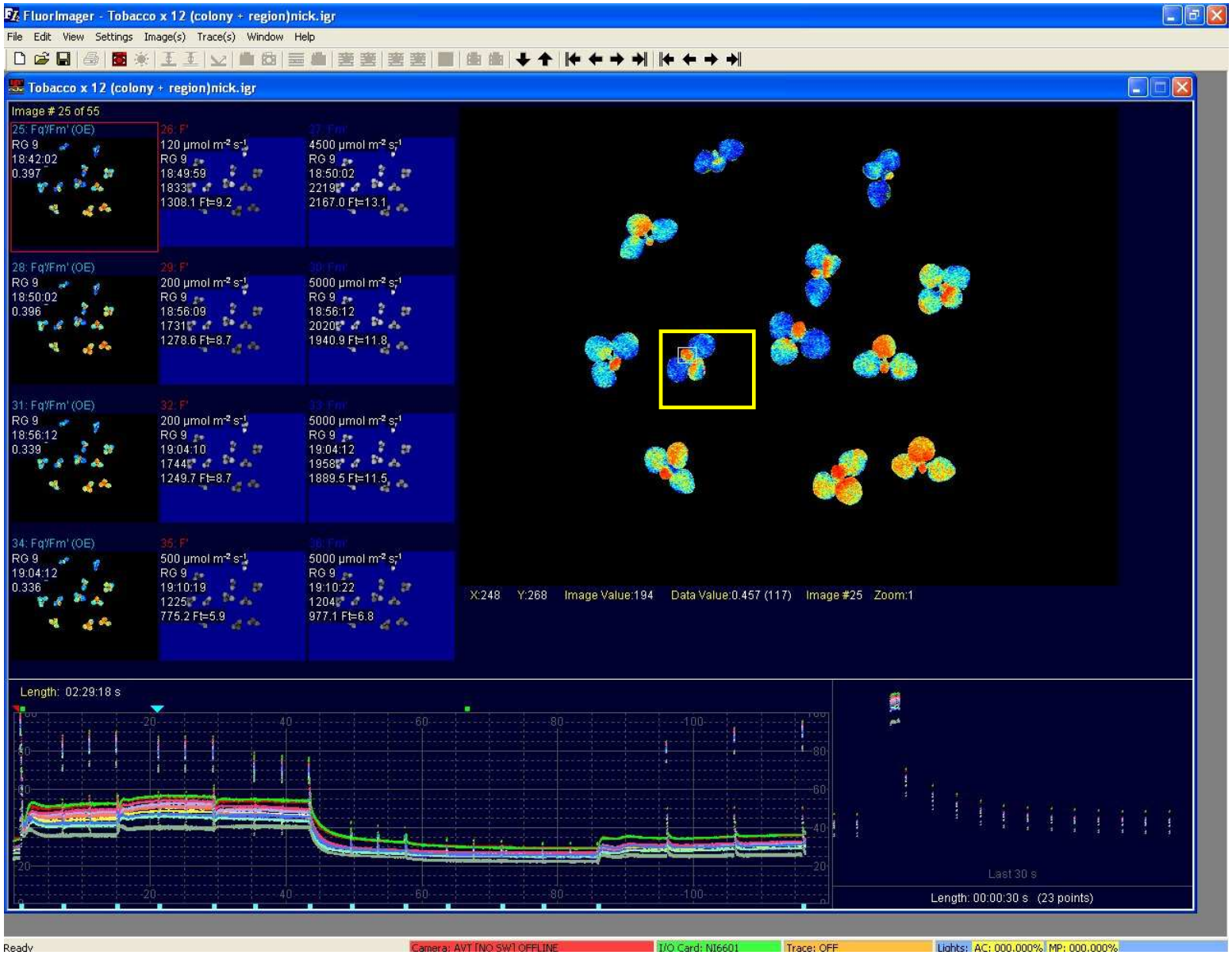




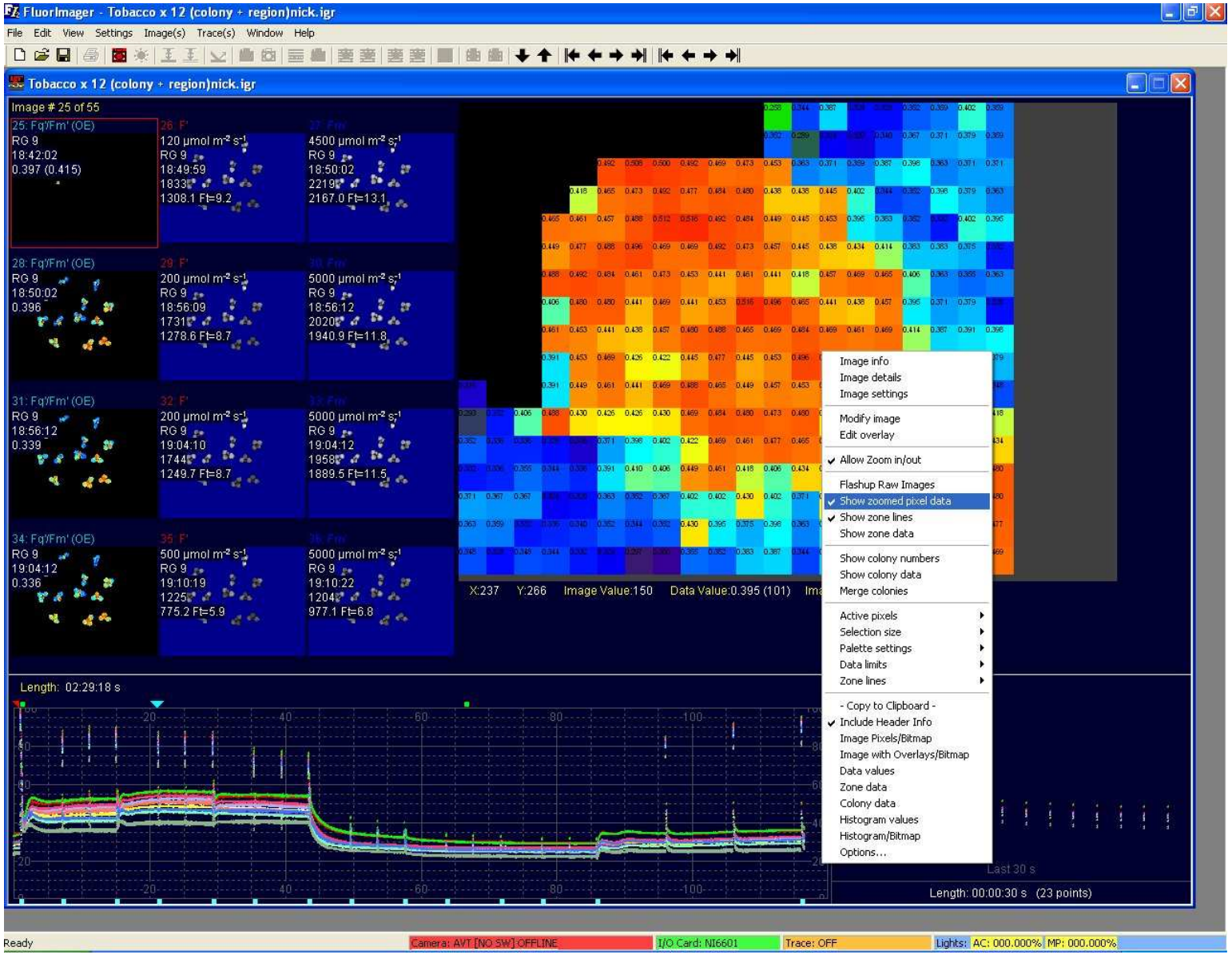


Morison et al., 2005

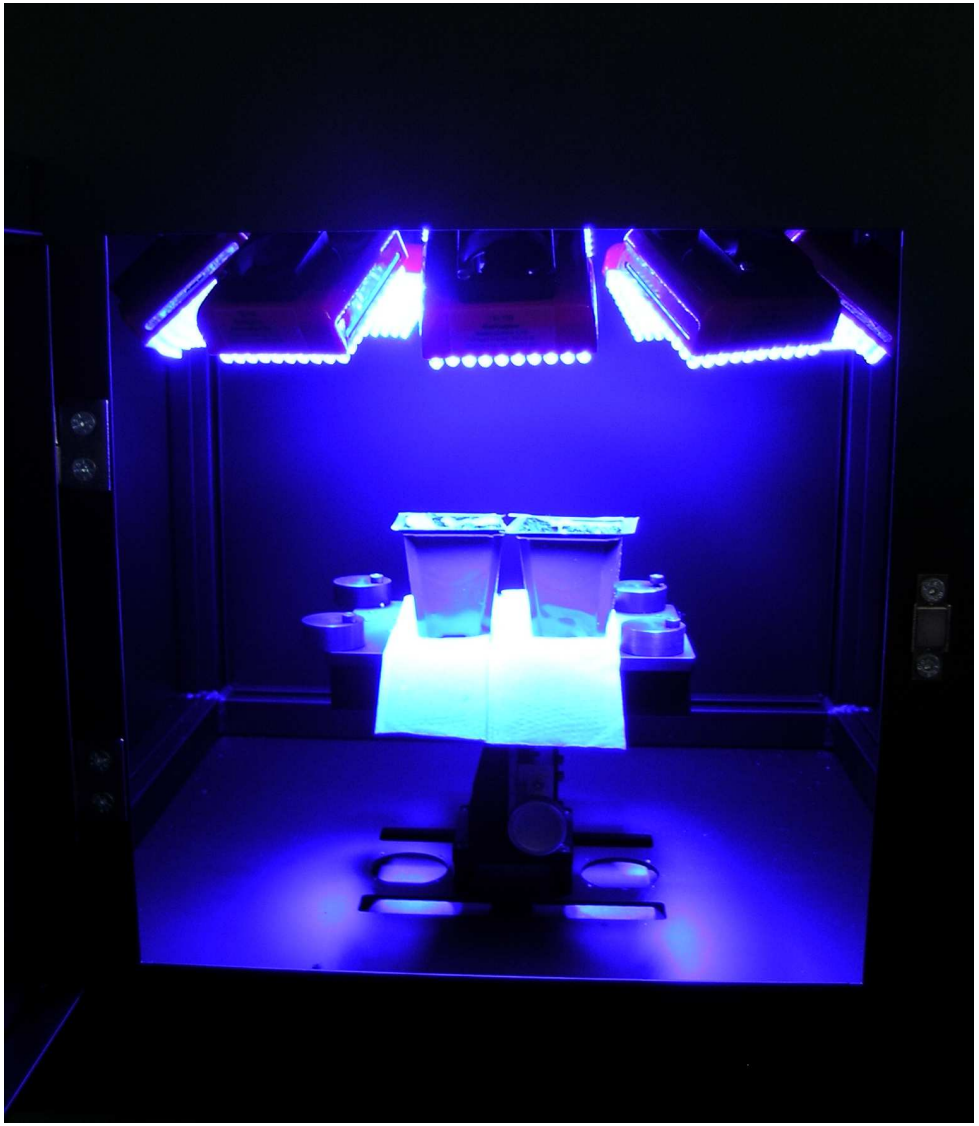
Show zone pixel values. An area of an image can be selected and zoom (inside yellow box). See next image.



Show zone pixel values. With “zoomed pixel data” selected values for each pixel within the zoomed area are displayed on screen.



New system



Blue LED lighting – 6000+ saturating pulse

Less filtering – greater signal:noise at the same price as an orange system

New high resolution camera

-features 2/3” CCD - twice the image area

New high resolution mega pixel lens – greater image quality

Software – gridline export, free upgrades for future developments – simplified installation

Some Published Research Featuring the cfImager

Barbagallo RP et al. (2003) Rapid, Noninvasive Screening for Perturbations of Metabolism and Plant Growth Using Chlorophyll Fluorescence Imaging. Plant Physiol., 132, 485-493

Fryer MJ et al. (2003) Control of ascorbate peroxidase 2 expression by hydrogen peroxide and leaf water status during excess light stress reveals a functional organisation of Arabidopsis leaves. The Plant Journal, 33, 691-705

Lawson et al. (2002) Response of photosynthetic electron transport in stomatal guard cells and mesophyll cells in intact leaves to light, CO2 and humidity. Plant Physiol 128: 1-11.

Baker NR et al. (2001) High resolution imaging of photosynthetic activities of tissues, cells and chloroplasts in leaves. J Exp Bot, 52, 615-621.

Leipner J et al. (2001) Primary sites of ozone-induced perturbations of photosynthesis in leaves: Identification and characterisation in Phaseolus vulgaris using high resolution chlorophyll fluorescence imaging. J Exp Bot, 52, 1-8

Oxborough K et al. (2000) In situ measurement of photosynthetic performance of individual microphytobenthic cells using high-resolution imaging of chlorophyll a fluorescence. Limnology and Oceanography, 45, 1420-1425.

Some key features.

- PWM blue lighting,- PPFD over *6000 µmol/m-2/s-1*
- Minimum 150cm2 imaged area. Precision, calibrated, PWM lighting -16 self-contained light panels for optimum uniform illumination over a standard 96 well-plate or individually adjusted for non-uniform samples.
- Very stable irradiance +/- 2% throughout the entire lighting regime.
- Advanced software - identification and analysis of individual samples in a well plate. User- programmable analysis routines for automated analysis.
- High performance AVT Dolphin SXGA 2/3” progressive scan Fire Wire camera with Tamron High Resolution (Megapixel) 100l/mm Lens.
- Unique virtually light-tight design with re-entrant Dutch-Folded panels - easily removed for in-situ applications.
- Precision Rack and Pinion camera and sample stage adjustment.
- Simple competitive pricing with no hidden extras.

Technologica cfImager

Specifications.

Electrical.	
Mains input	IEC connector
Voltage	85-264 VAC
Frequency	47-440 Hz
Inrush Current	40A peak maximum
Power Factor	0.99 typical-meets EN61000-3-2
Average Current	2-3A typical
Radiated EMI	See Technologica Manual - CE conformity certification
Fuse	15A internal
Safety and Disposal	See Technologica Customer Safety Notice TD002/003
Lighting.	
Source	Blue LED 470 nm. 16 TSL100© panels
Control	PWM- software control
Calibration	Pre-calibrated and pre-aligned
Irradiance	6000 µmol/ m^{-2}/s^{-1} minimum saturating pulse
Stability	+/- 2% typical for entire lighting regime
Uniformity	Uniform for 120mm (4.7”) x 170mm (6.7”) sample stage
Camera.	Pre-calibrated AVT Dolphin SXGA+ 2/3” CCD progressive scan camera mounted on precision +/-20mm adjustable rack and pinion
Imaged area	Minimum 150 cm2 (23in2)
Software.	Fluorimager Two©
Mechanical	
Construction	Lightweight Dutch-Folded light- excluding panels - tubular frame
Finish	Satin black stove enamelled
Ventilation	Axial fan
Standard sample stage	120mm (4.7”) x 170mm (6.7”) aluminium with lockable sample location cams for 96 Well Plates or user configured. Mounted on precision 20mm adjustable rack and pinion.
User access	Dutch-Folded 250mm (9.9”) x 250mm (9.9”) x detachable hinged door
External dimensions	Overall height-580mm (22.8”) Width 450mm (17.7”) Depth 450mm (17.7”)
Internal volume	0.085 m3 (3ft3)
Height above Sample Stage	120mm (4.7”) typical
Net weight	24Kg (53lb)
Environmenta	
Operating temperature	0- 40°C ambient 32° – 100oF
Storage temperature	-40-70°C 100- 160°F
Operating humidity	5%-95% non-condensing

Design and specification of the described product are subject to change without notice.