cf imager **technologica**

Chlorophyll a fluorescence

Patent No. GB2380790

www.technologica.co.uk

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Fates of excitation energy



Rate constants P + F + D = 1



 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



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.



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*)



Fluorescence terms





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



Fluorescence parameters

Operating efficiency (Genty factor)

Photochemical factor

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

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

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



Fq'/Fv' – PSII efficiency factor

- Fq'/Fv' = (Fm'-F) / (Fm'-Fo')
- Mathematically same as qP
- Changes reflect differences in capacity for photochemistry at PSII



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 nonradiative decay of excitation energy within the pigment matrix associated with PSII.



NPQ – Non photochemical quenching

- **NPQ** = (Fm/Fm')-1
- Non-photochemical quenching of chlorophyll fluorescence is an indicative of the level of nonradiative energy dissipation in the lightharvesting antenna of photosystem II.



Fv/Fm – maximum quantum efficiency of PSII photochemistry

- *Fv/Fm* = (*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



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.



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

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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



STEP 3: Establishing an Experimental Protocol

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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

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7: FvFm (XE) 11:09:18 0.550 Fo link = 5 Fm link = 6	8: F' 50 µmol m ⁻² s ⁻¹ 11:10:26 11:31 11:31 1040 6	9: Fm' 3000 µmol m² s' 11:10:28 1385 1397.5	0.551
10: Fm/Fm'-1 (NPQ) 11:10:28 0.729 Fm link= 6 Fm' link= 9	11: Fq/Fm' (OE) 11:10:28 0.258 F' link = 8 Fm' lin <u>k = 9</u>		Spider Plant Kingston Feb 04.igr
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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

у 343

343

343

60

178

289

X

1

2

3



Colony		х	у		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

Area (mm² Fv/Fm

0.577

0.529

0.551

338.3

487.42

294.82



Colony	x		у	Area (mm ²	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.

Spider Plan	t Kingston Feb 04 - 8 of 12	
Image type:	Fv/Fm (XE) (Copy of 7)	Date: 04-02-2004
Actinic PPFD	0 µmol m ⁻² s ⁻¹	Time: 11:09:18
Camera filter w x h: Active: % Active: Area:	688x500 (81x50) 112055 (4177) 32.6 (103.1) 11.21 (0.42) cm ²	
Mean:	0.550 (0.471)	0.008 to 0.801

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



Colour changes can be copied to all other images

Double click on image change colour palette using palette options



Modifying Images – data range/scale



Copy the data limits from the current image to all images of the same type

Lights: AC: 000.000% MP: 000.00

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.

✓ Deletes pixel(s)
Adds a horizontal zone line
Adds a vertical zone line

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.

			Rang	e of <i>Fv/</i>	F <i>m</i> value	es					
		0.0			0.5			1.	.0		
Control	8 mm	4 mml	0.8 mm	0.4 mm	Acetone	Control	8 8	4 mm	0.8 mm	0.4 mm	Control
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12
4.32 <mark>1</mark>	3.40	2.09	5.46)	2.03 <mark>)</mark>	3.40 <mark>8</mark>	2.62	2.68,	1.96	2.70	2.68 **	1.75 -
.0.820	0.350	0.402	0.449	0.607	0.821	0.827	0.359	0.328	0.415	0.554	0.774
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
2.02	1.56	3.66	2.73	2.57	3.01 7	4.75	6.88	2.77	3.57	0.01	4.55
0.817	0.327	0.292	0.481	0.761	0.823	0.821	0.429	0.296	0.632	0.258	0.777
F1	F2	F3	F4	F5	F6_	F7	F8	F9	F10	F11	F12
3.57 6	5.76	3.37	3.10	3.29	4.86 (5.11	2.23 5	6.57)	2.88 <u></u>	3.74 2	3.98/
0.833	0.252	0.258	0.509	0.711	0.820	0.821	0.446	0.348	0.586	0.674	0.772
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
2.33 ¹	1.63	3.91	1.19	0.03	5.57	5.65	1.40	1.92 %	5.04	2.76 6	2.21
0.813	0.247	0.326	0.287	0.342	0.805	0.811	0.255	0.351	0.638	0.437	0.782
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
1.83,	0.99	2.07 <i>2</i>)	2.16	2.93	5.35	6.61	1.78	5.26	3.93	4.80	6.42
0.813	0.275	0.288	0.402	0.764	0.809	0.813	0.205	0.308	0.396	0.740	0.772
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
4.17	3.37	2.27	3.46	2.94,	4.53	4.68	1.21	0.51	3.35	4.75	5.19
0.783	0.532	0.264	0.357	0.645	0.815	0.810	0.380	0.223	0.505	0.709	0.775
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
3.13	2.59	2.47	4.88	3.28	7.67	7.40	4.26	0.42⊾	1.35	2.51	4.90
0.813	0.331	0.250	0.511	0.751	0.809	0.810	0.369	0.519	0.260	0.489	0.775
A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
1.37	4.35	5.77	4.11	2.66	2.45	5.61 11	5.32	1.67 ₅	2.54	3.38	4.10
0.814	0.434	0.318	0.428	0.657 -	0.794	0.811	0.306	0.303	0.418	0.707	0.776

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.

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	<u>File E</u> dit <u>V</u> i	iew <u>I</u> nsert f	F <u>o</u> rmat <u>T</u> ools	; <u>D</u> ata <u>W</u> in	dow <u>H</u> elp					_ 8	×
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	A1	-	= Area (m	m²)							
	Α	B	С	D	E	F	G	Н	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	В	4.6	3.72	4.33	5.85	3.39	5.42	5.19	6.58		-
5	С	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	В	0.789	0.772	0.772	0.763	0.738	0.776	0.784	0.783		-
13	С	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	ļ	-
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Rea	dy				Sun	n=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.*

<mark>Z</mark> Fluorimager - Arabado	psis example.igr											
le Edit View Settings Im	age(s) Trace(s) Window H	elp : Alla Indornator Indornator II	ini i anto anto									
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mage # 5 01 6 : Map —) μmol m ² s ⁻¹⁰ - 2 - 1 - 1 7:03:04	2: Fo 0 µmol m² s ⁻¹ Filter 1.	4000 µmol m ⁻² s ⁻¹	1.34 0.812	4.24 0.431 <mark>/</mark> 8	Image info Image details	2.43 0.793	5.59 % 0.811	5.20 2 0.307,	1.62 0.303	2.52 0.417	3.35 0.706	4.03 0.778
=0, , , , , , , , , , , , , , , , , , ,	17:03:46 H=52685 1458.9 Ft=20.0	17:03:46 H=52685 3696.9 Ft=65.7	B1 3.08 0.812	82 2.39 0.332	Image settings Modify image Edit overlay	86 7.62 0.808	B7 7.35 0.810	B8 4.22 0.368	89	B10 1.03 0.253	B11 2.41 0.485	B12 4.86 0.776
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ilter 1 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	Filter 1 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	30 µmol m ⁻² s ⁻¹ Filter 1 17:13:47 H=52685	D1 1.83 0.813	D2 0.91 0.267	Show zoone pixe data Show zone lines Show zone data Show colony numbers	D6 5.25	D7 6.56 0.813	D8 1.63 0.194	D9 5.19 0.306	D10 3.701 0.400	D11 4.76 0.740	D12 6.36 0.772
1 1 1 1 2 2 2 2 4 9 9 4 9 9 1 2 3 4 1 2 3 5 5 5 2 * *			E1 2.30 0.813	E2 1.55 0.239	Show colony data Merge colonies - Copy/Paste Internally -	E6 5.54 0.805	E7 5.59 0.810	E8 1.32 0.250	E9 1.66 🥠 0.323	E10 5.03 0.638	E11 2.41 0.471	E12 2.19 0.782
			F1 3.49 0.831	F2 5.48 0.250	Selection size	F6 4.84 0.820	F7 5.08 0.821	F8 2.15 0.442	F9 6.41 0.346	F10 2.85 0.586	F11 3.66 <mark>%</mark> 0.678	F12 3.94 # 0.773)
			G1 1.98 0 0.814	G2 1.50 } 0.324	Zone lines	G6 3.00 0.823	G7 4.71 0.821	G8 6.84 0.429	69 2.63 0.291	G10 3.52 0.631	G11	G12 4.54 0.777
			H1 4.30 0.820	H2 3.15 0.338	Image vikelybundge Image vikelybundge Data values Zone data Colony data Histogram values Histogram values Histogram values Histogram Values Data Value	H6 3.38 0.821	H7 2.59 0.826	H8 2.67 0.359	H9 1.91 0.327	H10 2.60 0.417	H11 2.60 0.558	H12 1.70 0.774
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Example from previous slide of copied image including zone lines and zone data.

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
1.37	4.35	5.77	4.11	2.66	2.45	5.61	5.32)	1.67	2.54	3.38	4.10
0.814	0.434	0.318	0.428	0.657	0.794	0.811	0.306	0.303	0.418▶	0.707	0.7 <u>7</u> 6
B1	82	⊟3	84	85	86	87	88	89	810	B11	B12
3.13	2.59	2.47≱	4.88	3.28	7.67 5	7.40	4.26	0.42	1.35 ⁹	2.51,	4.90
0.813	0.331 🏸	0.250⋗	0.511	0.751	0.809	0.810	0.369	0.519	0.260	0.489	0.775
C1 4.17 <mark>5</mark> 0.783	C2 3.37 0.532	C3 2.27 0.264	C4 3.46	C5 2.94 <i>c</i> 0.645₅	C6 4.53 0.815	C7 4.68 0.810	C8 1.21 0.380	C9 0.51, 0.223	C10 3.35 0.505	C11 4.75 0.709)	C12 5.19 0.775
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
1.83 <mark>)</mark>	0.99	2.07 <i>2</i>	2.16	2.93	5.35	6.61	1.78	5.26	3.93	4.80,	6.42
0.813	0.275	0.288	0.402	0.764	0.809	0.813	0.205	0.308	0.396	0.740	0.772
E1	E2	E3	E4	<mark>E5</mark>	E6	E7	E8	E9	E10	E11	E12
2.33	1.63x>	3.91	1.19 	0.03,	5.57 <mark>12</mark>	5.65	1.40	1.927	5.04	2.76 <mark>\$</mark>	2.21
0.813	0.247	0.326	0.287	0.342	0.805	0.811	0.255	0.351	0.638	0.437	0.782
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
3.57	5.76	3.37	3.10	3.29	4.86	5.11	2.23	6.57	2.88	3.74 🚁	3.98
0.833	0.252	0.258	0.509	0.711	0.820	0.821	0.446	0.348	0.586	0.674)	0.772
G1_	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
2.02 —	1.56	3.66	2.73	2.57 <mark>2</mark>	3.01	4.75)	6.88	2.77	3.57	0.01	4.55
0.817	0.327	0.292	0.481	0.761	0.823	0.821	0.429	0.296	0.632	0.258	0.777
H1 4.32 0.820	H2 3.40 0.350	H3 2.09 0.402	H4 5.46 () 0.449	H5 2.03⊾ 0.607	H6 3.40 0.821	H7 2.62 4 0.827	H8 2.68 0.359	H9 1.96 0.328	H10 2.70₹ 0.415	H11 2.68	H12 1.75 0.774

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.

🗗 Fluorimager - Arabado	opsis example.igr									
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Example of copied image and zone lines only (previous slide).

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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).

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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.

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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.

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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.

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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) with "header info" selected (<i>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.

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Dased	-	time	time (s)	filter	PPED	PUISE	length	F0 &	Fm & Fm'	(Em Eo)	FO/Fm or	Ev'/Em'	(qP) Ed/EV	Em/Em'-1	Comment	-		-				-
Map (1)	\$						1.196			2	1.10		180									
	1	00:01:03	63	8 RG 9	0	4000	800	780	2540	1	0.693	0.693	1	0							-	
-	2	00:07:13	433	RG9	60	4500	800	1186	2221	0.44	0.466	0.654	0.702	0.14	After this	puise the pi	ants were	put back in	to the dark.			-
	4	00:15:13	913	RG9	60	4500	800	1204	2305	0.45	0.478	0.672	0.711	0.1								+
	5	00:21:23	1283	8 RG 9	120	4500	800	1312	2219	0.35	0.409	0.663	0.616	0.14				-			1	
-	6	00:25:22	1522	2 RG 9	120	4500	800	1322	2213	0.34	0.403	0.663	0.607	0.15				-		-		-
	8	00:35:33	2133	8 RG 9	200	5000	800	1283	1974	0.33	0.35	0.637	0.55	0.29	1				1			-
	9	00:39:32	2372	2 RG 9	200	5000	800	1264	1945	0.31	0.35	0.633	0.553	0.31							-	
	10	00:43:33	2613	RG 9	200	5000	800	1254	1911	0.31	0.344	0.629	0.546	0.33	Changed t	o 500 umol	m-2 s-1 ligi	ht.				_
	12	00:53:43	3223	RG 9	500	5000	800	754	964	0.32	0.221	0.47	0.47	1.55	1				-			-
	13	00:57:43	3463	8 RG 9	500	5000	800	741	949	0.33	0.219	0.457	0.479	1.68	1						1	
	14	01:03:52	3832	2 RG 9	1000	5000	800	706	800	0.19	0.118	0.415	0.283	2.17								_
	15	01:07:52	4072	RG9 RG9	1000	5000	800	699	796	0.2	0.122	0.414	0.294	2.19					1		-	-
	17	01:18:02	4682	2 RG 9	1600	5000	800	690	767	0.16	0.1	0.111	0101	2.31								+
	18	01:22:02	4922	2 RG 9	1600	5000	800	695	766	0.15	0.093	0	0	2.32				_				
	19	01:26:02	5162	RG9	1600	5000	800	694 704	2210	0.16	0.098	0 663	0	2.3	Dish back	in the dark.	4					-
	20	01:46:13	6373	RG9	0	4000	800	809	2391	0.92	0.662	0.68	0.973	0.06							-	-
	22	01:56:14	6974	RG 9	0	4000	800	831	2475	0.9	0.664	0.687	0.966	0.03	1							
Region (2)	00:01:02		RGO		4000	800	000	2704	4	0.655	0.644	1	0	1		-	-				-
	2	00:07:13	433	8 RG 9	60	4500	800	1375	2376	0.44	0.035	0.625	0.674	0.14	After this i	pulse the pl	ants were	put back in	to the dark.			+
	3	00:11:12	672	2 RG 9	60	4500	800	1408	2452	0.43	0.426	0.632	0.673	0.1								
	4	00:15:13	913	RG 9	60	4500	800	1424	2487	0.43	0.427	0.636	0.672	0.09								-
	5	00:21:23	1283	RG9 RG9	120	4500	800	1511	2374	0.34	0.364	0.625	0.582	0.14								-
	7	00:29:23	1763	RG 9	120	4500	800	1522	2369	0.33	0.358	0.624	0.573	0.14		1		-	-			t
	8	00:35:33	2133	8 RG 9	200	5000	800	1474	2130	0.3	0.308	0.599	0.514	0.27								
	9	00:39:32	2372	2 RG 9	200	5000	800	1456	2103	0.3	0.308	0.596	0.516	0.28		500 1	0.45			-	-	-
	10	00:43:33	2613	RG 9 RG 9	200	5000	800	1445	2077	0.3	0.304	0.593	0.513	0.3	Changed t	o 500 umol	m-2 s-1 ligi	ητ.				+
	12	00:53:43	3223	RG 9	500	5000	800	897	1113	0.31	0.194	0.438	0.443	1.43								t
	13	00:57:43	3463	8 RG 9	500	5000	800	874	1090	0.32	0.198	0.433	0.457	1.48	1							T
	14	01:03:52	3832	2 RG 9	1000	5000	800	824	918	0.18	0.102	0	0	1.94			-	-	-		-	+
	15	01:07:52	4072	RG 9	1000	5000	800	813	906	0.18	0.103	0	0	1.98			-	-				+
	17	01:18:02	4682	2 RG 9	1600	5000	800	800	870	0.10	0.04	0	0	2.1								+
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19 20 21 22 23 24	0.2005 0.2339 0.2672 0.3005 0.3338 0.3672	767 768 770 768 770 768 771	918 912 918 919 919 919 922	899 899 903 903 900 900	793 793 794 795 793 793	785 786 791 789 792	728 724 727 730 728 730	795 793 796 801 799 802	792 791 795 794 795 795	807 812 815 811 817	716 715 716 716 715 715 717	789 788 789 790 788 791	660 658 662 659 661	633 633 634 631 634	Note (2): a	idding a n	ote to the	data.		
19 20 21 22 23 24 25	0.2005 0.2339 0.2672 0.3005 0.3338 0.3672 0.4005	767 766 768 770 768 771 773	918 912 918 919 919 919 922 926	899 899 903 903 900 903 900 903	793 793 794 795 795 793 796 797	787 785 786 791 789 792 793	726 724 727 730 728 730 730 732	795 793 796 801 799 802 802 805	792 791 795 794 795 795 795 799	810 807 812 815 815 811 817 816	716 715 716 716 715 715 717 718	789 788 789 790 788 791 793	660 658 662 659 661 666	633 633 634 634 634 634 634	<u>Note (2): a</u>	idding a n	ote to the	data.		
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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	у	Area (mm ²	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



Combined with Infra-red gas analysis Mapping C_i using chlorophyll fluorescence





- Photosystem II quantum yield : F_a'/F_m'
- CO₂ assimilation rate : A (µmol CO₂.m⁻².s⁻¹)
- Stomatal conductance : g_s (µmol CO₂.m⁻².s⁻¹)
- Internal CO₂ partial pressure : C_i (ppm)

Morison et al., 2005



Visualising natural and artificial patches

Natural patchiness





PSII efficiency

Helianthus annuus Natural patchiness induced by rapid decrease in humidity Artif

Artificial patchiness





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





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.





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11	96	92	92	92	89	86	80	73	67	64	57	50	48	47	46	40	33	35	38	
12	96	94	95	94	90	85	80	73	68	64	59	54	53	50	45	37	32	35	37	
13	98	98	98	96	92	88	84	80	72	65	63	60	57	54	46	37	33	32	30	
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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 cflmager

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 cflmager

Specifications.

Electrical. Mains input IEC connector 85-264 VAC Voltage 47-440 Hz Frequency 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 Pre-calibrated AVT Dolphin SXGA+ 2/3" CCD progressive scan camera mounted on precision +/-20mm Camera. adjustable rack and pinion Minimum 150 cm2 (23in2) Imaged area Software. Fluorimager Two© Mechanical Construction Lightweight Dutch-Folded light- excluding panels - tubular frame Finish Satin black stove enamelled Axial fan Ventilation 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. Dutch-Folded 250mm (9.9") x 250mm (9.9") x detachable hinged door User access Overall height-580mm (22.8") Width 450mm (17.7") Depth 450mm (17.7") External dimensions 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 -40-70°C 100- 160°F Storage temperature Operating humidity 5%-95% non-condensing

Design and specification of the described product are subject to change without notice. ©~05/2006