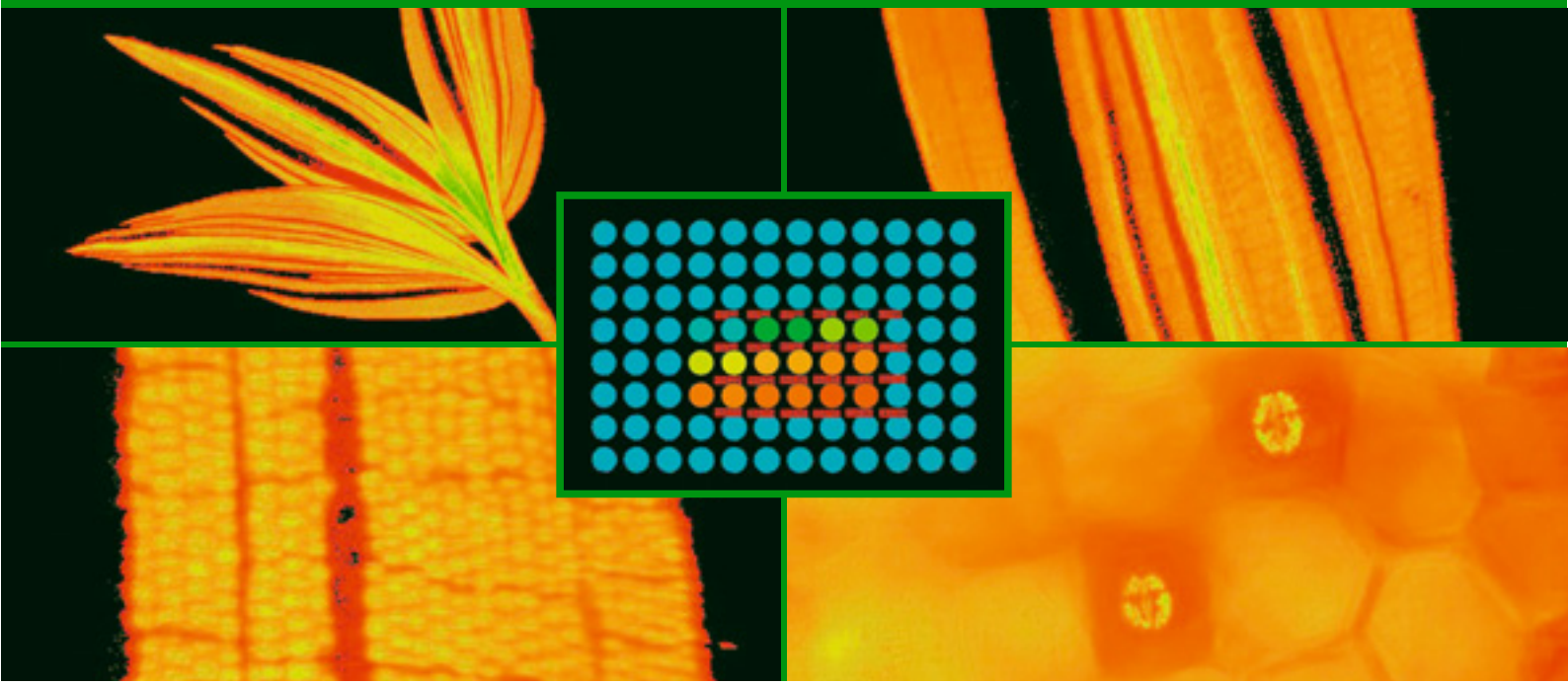
IMAGING-PAM
M-Series

Chlorophyll Fluorescence System



... for a wide range of chlorophyll fluorescence imaging applications

WALZ

Mess- und Regeltechnik

IMAGING-PAM **M**-Series

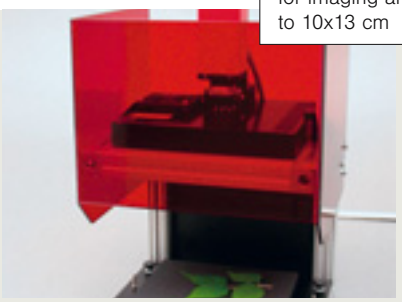
Chlorophyll Fluorescence System

Features

- From intact leaves to single cells using the same Multi Control Unit
- 4 basic versions based on different Measuring Heads
- Largely differing sample areas at a wide range of magnifications
- Fluorescence imaging of multiwell plates
- Major applications in Ecotoxicology, Ecophysiology, Plant Molecular Biology, Phytopathology, Limnology, Photosynthesis Research, Horticulture and Agriculture

Multi Control Unit IMAG-C**M**

MAXI-Version for imaging areas up to 10x13 cm



Walz introduces a new family of **IMAGING-PAM** fluorometers, the so-called **M-Series** which covers a wide range of applications. Large scale samples with areas exceeding multiwell plate format can be imaged as well as microscopically small samples at the level of single cells and even chloroplasts. **MAXI-**, **MINI-**, **MICRO-** and **MICROSCOPY-**Versions are available, that are based on the same **Multi Control Unit IMAG-CM** and 4 different Measuring Heads.

For different applications **various sub-versions** are available, differing in optical geometries and excitation wavelengths. While blue excitation normally is used for fluorescence imaging of plants and algae, **red-orange excitation** is required for **cyanobacteria**. Measuring heads can be also equipped with special LEDs and filter sets for imaging fluorescence not originating from chlorophyll, like **GFP-fluorescence**.

The M-Series extends the applications of the IMAGING-PAM, the first version of which was introduced in 2001 and since then has been used with considerable success in such diverse fields of science as leaf electrophysiology, coral research, phytopathology and marine ecophysiology:

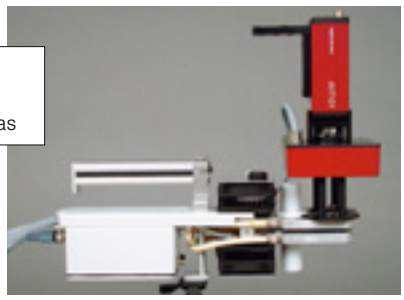
Koziolek C, Grams TEE, Schreiber U, Matyssek R and Fromm J (2003). ***Transient knockout of photosynthesis mediated by electrical signals.*** *New Phytologist 161:715-722*

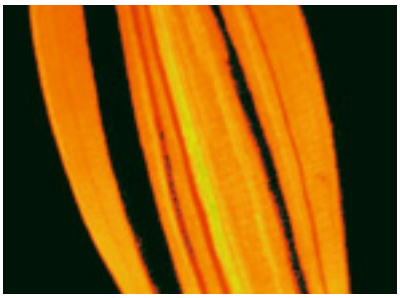
Hill R, Schreiber U, Gademann R, Larkum AWD, Kühl M and Ralph P (2004). ***Spatial heterogeneity of photosynthesis and the effect of temperature-induced bleaching conditions in three species of corals.*** *Marine Biology 144: 633-640*

Berger S, Papadopoulos M, Schreiber U, Kaiser W and Roitsch T (2004). ***Complex regulation of gene expression, photosynthesis and sugar levels by pathogen infection in tomato.*** *Physiologia Plantarum 122 (4), 419-428*

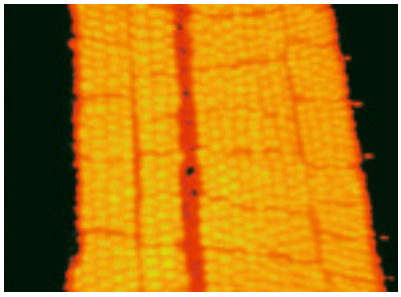
Kühl M, Chen M, Ralph P, Schreiber U and Larkum AWD (2005). ***Niche and photosynthesis of Chlorophyll d-containing cyanobacteria.*** *Nature 433:820*

MINI-Version
for imaging
24x32 mm areas

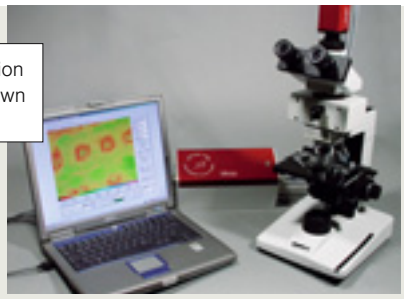


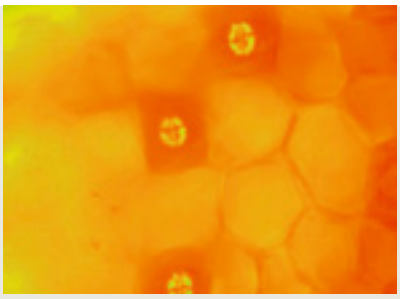


MICRO-Version
for imaging
3.5x4.5 mm areas



MICROSCOPY-Version
for imaging areas down
to 130x150 µm





Chlorophyll fluorescence and PAM fluorometry

Chlorophyll fluorescence is a very sensitive indicator of photosynthesis. Quantitative information on the quantum yield of photosynthetic energy conversion is obtained by PAM fluorometry and the Saturation Pulse method *(Schreiber U (2004)* ***Pulse-Amplitude (PAM) fluorometry and saturation pulse method.*** *In: Papageorgiou G and Govindjee (eds)* ***Chlorophyll fluorescence: A signature of Photosynthesis,*** *pp. 279-319. Kluwer Academic Publishers, Dordrecht, The Netherlands).*

A wide range of photosynthetic parameters can be derived from fluorescence measurements, giving insight into the physiological state of all photosynthetically active organisms, including higher plants, mosses and ferns as well as various types of algae, phytoplankton and biofilms.

17 different parameters

Chlorophyll fluorescence imaging

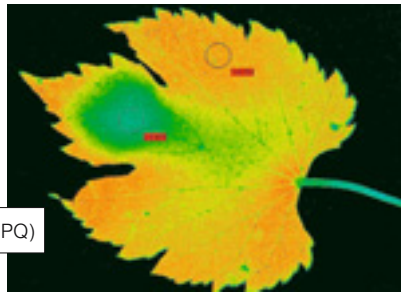
With the advance of highly sensitive CCD cameras and extremely strong light emitting diodes (LED) development of IMAGING-PAM fluorometers has become possible that not only measure images of chlorophyll fluorescence but are also fully competent in providing all relevant chlorophyll fluorescence parameters using the Saturation Pulse method. In this way, **images of photosynthetic activity and its spatiotemporal variations** can be obtained.

All IMAGING-PAM fluorometers provide **images of 17 different parameters**. The fluorescence parameter Ft is continuously monitored. Fo and Fm are assessed after dark adaptation, serving as reference for fluorescence quenching analysis by the Saturation Pulse method. Besides Fv/Fm, the PS II quantum yield after dark adaptation, also the PS II quantum yield during illumination, Y(II), and the quantum yields of regulated and non-regulated energy dissipation, Y(NPQ) and Y(NO), can be imaged.

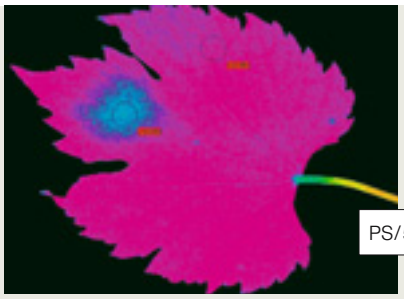
A routine for measurement of a **PAR-absorptivity** image is provided (Abs.-image based on images of NIR and Red light remission). A normalized image of **photosynthetic electron transport rate (PS)** is calculated from Y(II), Abs. and the PAR-value.

MAXI-Version of the IMAGING-PAM

for imaging large areas up to 10x13 cm



Y(NPQ)



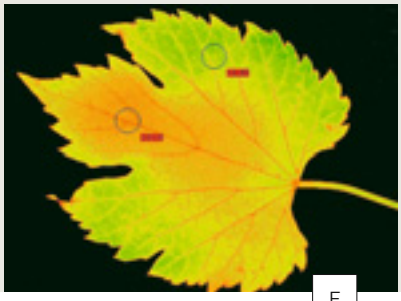
PS/50

The **MAXI-Version** of the IMAGING-PAM employs a very compact and powerful **300 W LED-Array** for homogeneous illumination of up to **10x13 cm** areas with pulse-modulated excitation, actinic and Saturation Pulse light. A special **Mounting Stand with Eye Protection** is provided, which features a red perspex hood, through which the red fluorescence can be viewed.

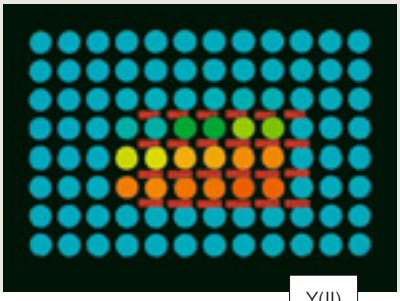
On the bottom of this stand an x-y stage for variable sample positioning or a **multi well plate** can be placed at defined working distance of 18.5 cm. The bottom can be removed and the whole stand jacked up for imaging of plants growing on trays or in pots.

Two different CCD-cameras are avail-able. For **high sensitivity** applications the IMAG-MAX/K (2/3" chip, 1392x1040 pixel with **4-pixel-binning**) is recommended. For standard applications the IMAG-MAX/K2 (1/2" chip, 640x480 pixel) is available, which can be used in conjunction with the powerful IMAG-MAX/K2Z **zoom objective** (F1.0/f=8-48mm).

Images of the various fluorescence parameters are depicted in false colors coding from 0.0 (black) to 1.0 (purple)

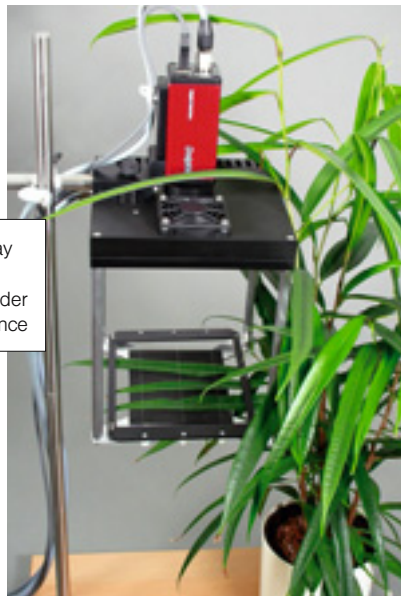


F



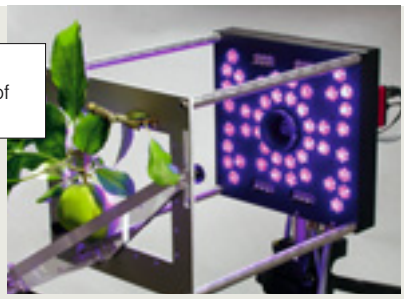
Y(II)

Different Configurations of the **MAXI**-Version



Measuring Head (LED-Array plus camera) mounted on separate stand with leaf holder providing fixed working distance

With Mounting Stand being jacked up for studying potted plants

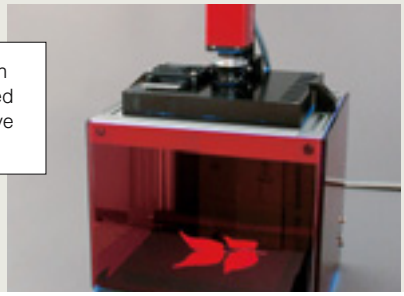


Special head mounted on tripod for assessment of fruit in the field

Standard configuration with 96-well microtiter plate

The **MAXI-Version** of the IMAGING-PAM can be used in a variety of different configurations for a wide field of applications in the laboratory and under field conditions. Due to the very powerful **LED-Array Illumination Unit**, in all applications steps must be taken to avoid looking directly into the LED-Array.

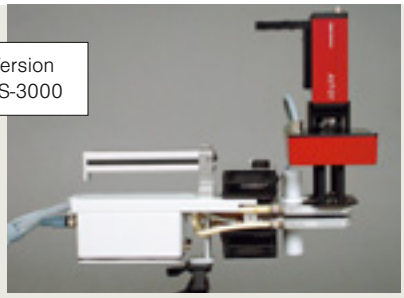
For laboratory applications, the **Mounting Stand with Eye Protection** is ideally suited, as it not only protects the eyes, but also allows to view directly the red chlorophyll fluorescence via the red perspex hood.



Standard configuration with LED-Array and camera fixed on Mounting Stand with Eye Protection

MINI-Version of the IMAGING-PAM

for imaging 24x32 mm areas (6x magnification)



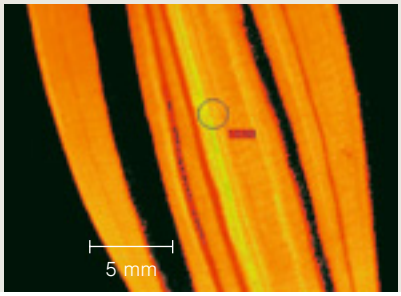
MINI-Version on GFS-3000

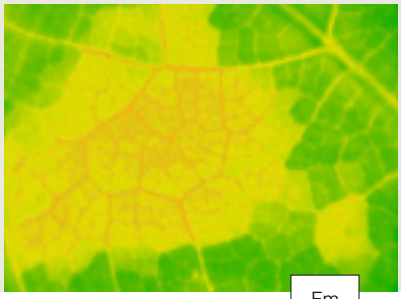
The 24x32 mm area imaged by the **MINI-Version** is illuminated by a very powerful Luxeon LED array consisting of 4 groups of 3 LEDs equipped with 4 individual short-pass filters. Red (650 nm) and NIR (780 nm) LEDs (8 each) serve for assessment of **PAR-Absorptivity images**.

Three different versions are available:
IMAG-MIN/B (blue, 450 nm, standard leaf applications;
IMAG-MIN/R (red, 620 nm, for cyanobacteria);
IMAG-MIN/GFP (blue, 480 nm, GFP imaging).

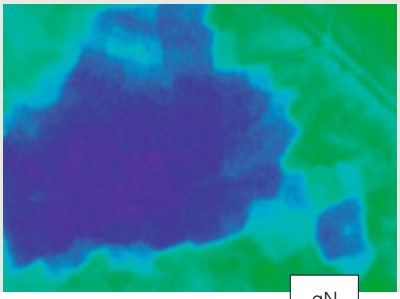
Due to the compact design, the MINI-Version is well suited for field applications. As the imaged area is much smaller than that of the MAXI-Version (factor of 16), maximal intensities are higher, whereas power consumption is lower. It can be mounted on the Standard measuring Head 3010-S of the Portable Gas Exchange Fluorescence System **GFS-3000**.

The MINI-Version employs a **1/3" CCD camera (640x480 pixel)** with a F1.2/f=12mm objective lense. It is designed for measurements at fixed working distance.





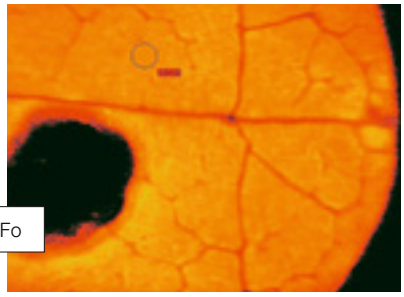
Fm



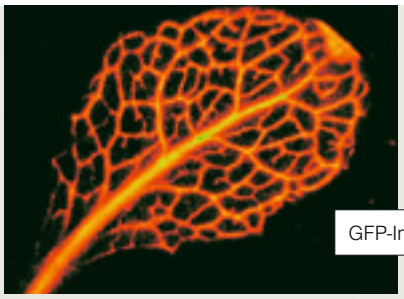
qN

MICRO-Version of the IMAGING-PAM

for imaging 3.5x4.5 mm areas (45x magnification)



Fo



GFP-Imaging

The **MICRO-Version** of the IMAGING-PAM features an extremely compact Measuring Head with integrated Cosmicar-Pentax CCTV objective lens (F1.4/f=16mm). It is directly mounted on the CCD camera (1/3" chip with 640x480 pixel).

A single high power Luxeon LED (blue, 450 nm) in conjunction with a special **dichroic beam splitter** is employed, similarly as in an epifluorescence microscope.

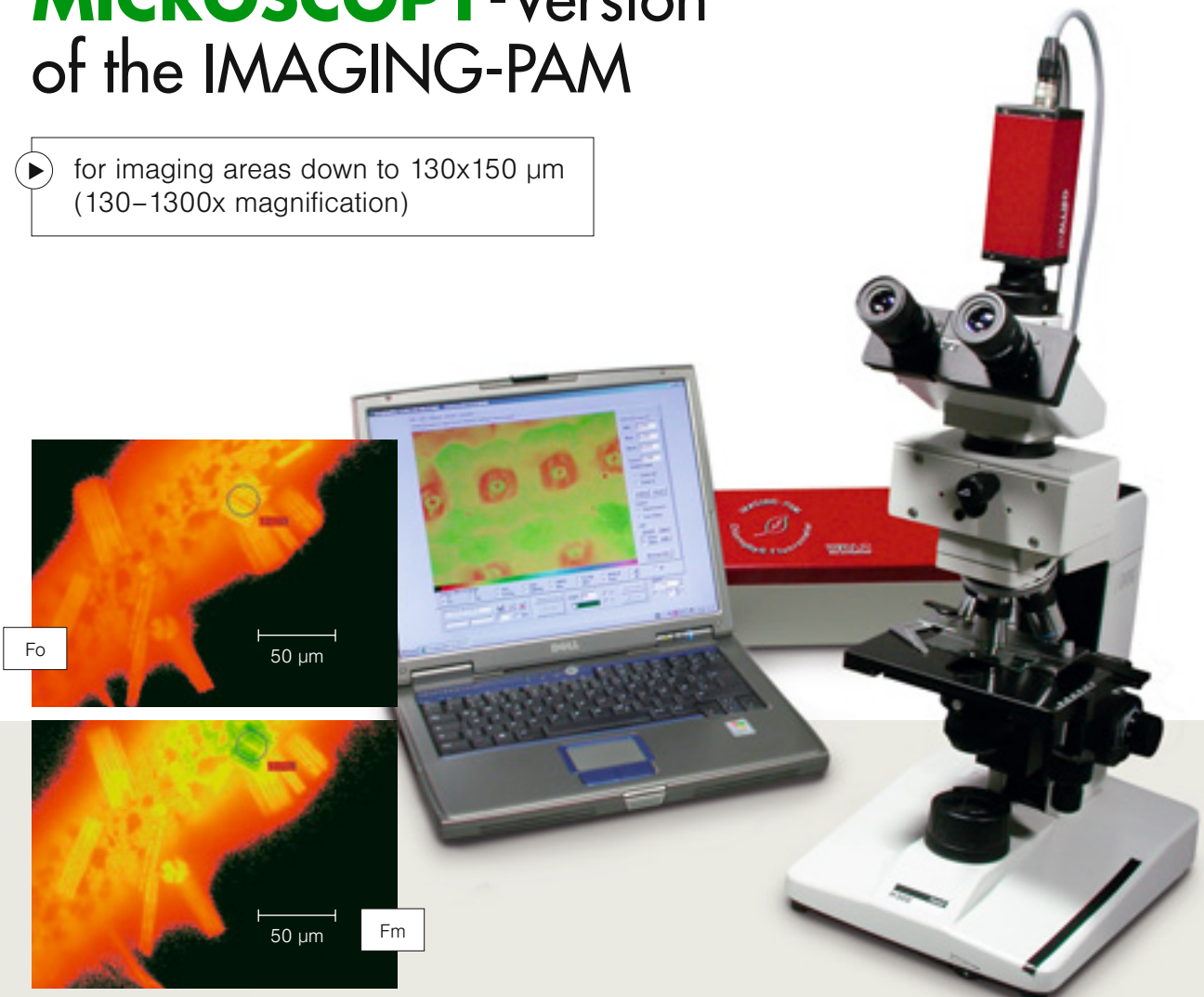
With an imaged area of 3.5x4.5 mm **(45x magnification)** the resulting high spatial resolution allows imaging heterogeneities at the level of the **minor veins** of leaves. A special version for **GFP imaging** is available.

The MICRO Measuring Head can be also connected to the original Standard IMAGING-PAM with the IMAG-C Control Unit. It features a miniature x-y stage and is designed for fixed working distance.

Compact Measuring Head with integrated Cosmicar-Pentax CCTV objective lens, directly mounted on CCD camera

MICROSCOPY-Version of the IMAGING-PAM

for imaging areas down to 130x150 µm (130–1300x magnification)



The **MICROSCOPY-Version** of the IMAGING-PAM operates in conjunction with special **Epifluorescence Microscopes** that are adapted for optimal excitation intensity and fluorescence collection.

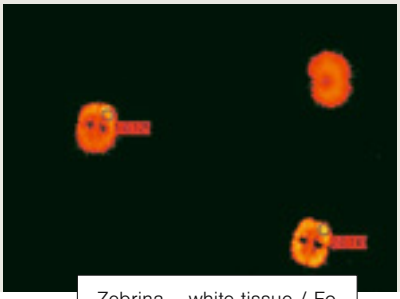
For this purpose, relatively simple microscopes with short optical pathlengths, as **Axiostar** (Zeiss, Göttingen) and **H600AFL** (Hund, Wetzlar), are best suited, which are available with appropriately adapted components.

The IMAG-MAX/K CCD Camera (1392x1040 pixel with 4-pixel-binning) provides high sensitivity.

For standard applications a single high power **Luxeon LED** (450-480 nm) is provided for excitation, actinic illumination and Saturation Pulses. Alternatively a sophisticated **Red-Green-Blue-White LED Lamp** with separate drivers soon will become available. This lamp is controlled via the RGB-output of the IMAG-CM. RGB fluorescence excitation allows to differentiate between various types of algae and cyanobacteria in biofilms, in analogy to the PHYTO-PAM.

An extended ImagingWin software takes account of the particular needs of microscopy applications. A **Life Video image** of the object can be obtained using the standard through-light condenser-illuminator of the microscope. A **special saturation pulse routine** is provided for optimal assessment of Fo, Fm and Fv/Fm at low levels of excitation intensity.

Zebrina – green tissue / Fo



Zebrina – white tissue / Fo

Optical Specifications of the IMAGING-PAM **M**-Series

MAXI-Version

IMAG-MAX/L

LED-Array Illumination Unit

44 blue Luxeon LEDs (450 nm) with individual collimator optics; 16 red (650 nm) and 16 NIR (780 nm) LEDs for measuring PAR-absorptivity; max. actinic intensity, 1200 µE/m²s; max. Saturation Pulse intensity, 2800 µE/m²s; optional filter plate with 44 individual blue filters for high sensitivity applications; 2/3" (1392x1040 pixel with 4-pixel-binning) or 1/2" (640x480 pixel with zoom option) CCD camera; optimal working distance 18.5 cm using special Mounting Stand with Eye Protection; sample areas up to 10x13 cm; 1.5x magnification

MINI-Version

IMAG-MIN/B (or /R, or /GFP)

MINI-Head blue (or red, or GFP)

12 Luxeon LEDs (450 or 620 or 480 nm) with individual short pass filters and collimator optics; 16 red (650 nm) and 16 NIR (780 nm) LEDs for measuring PAR-absorptivity; max. actinic intensity, 2000 µE/m²s; max. Saturation Pulse intensity, 6000 µE/m²s; 1/3" CCD camera (640x480 pixel); fixed working distance; 24x32 mm sample area; 6x magnification

MICRO-Version

IMAG-MIC (or /GFP)

MICRO-Head blue (or GFP)

Single Luxeon LED (450 or 480 nm) with short-pass filter, dichroic beam splitter and collimator optics; max. actinic intensity, 2000 µE/m²s; max. Saturation Pulse intensity, 6000 µE/m²s; integrated F1.4/f=16mm objective lens; 1/3" CCD camera (640x480 pixel); fixed working distance; 3.5x4.5 mm sample area; 45x magnification

MICROSCOPY-Version

IMAG-L450

Microscopy LED Lamp (blue)

Single Luxeon LED (450-480 nm) with blue filter

IMAG-RGB

Microscopy LED Lamp (red-green-blue)

LED Array with 2x620 nm, 3x525 nm and 2x470 nm LEDs that can be driven separately (red, green, blue) or together (white)

AXIOSTAR/M

Epifluorescence Microscope I

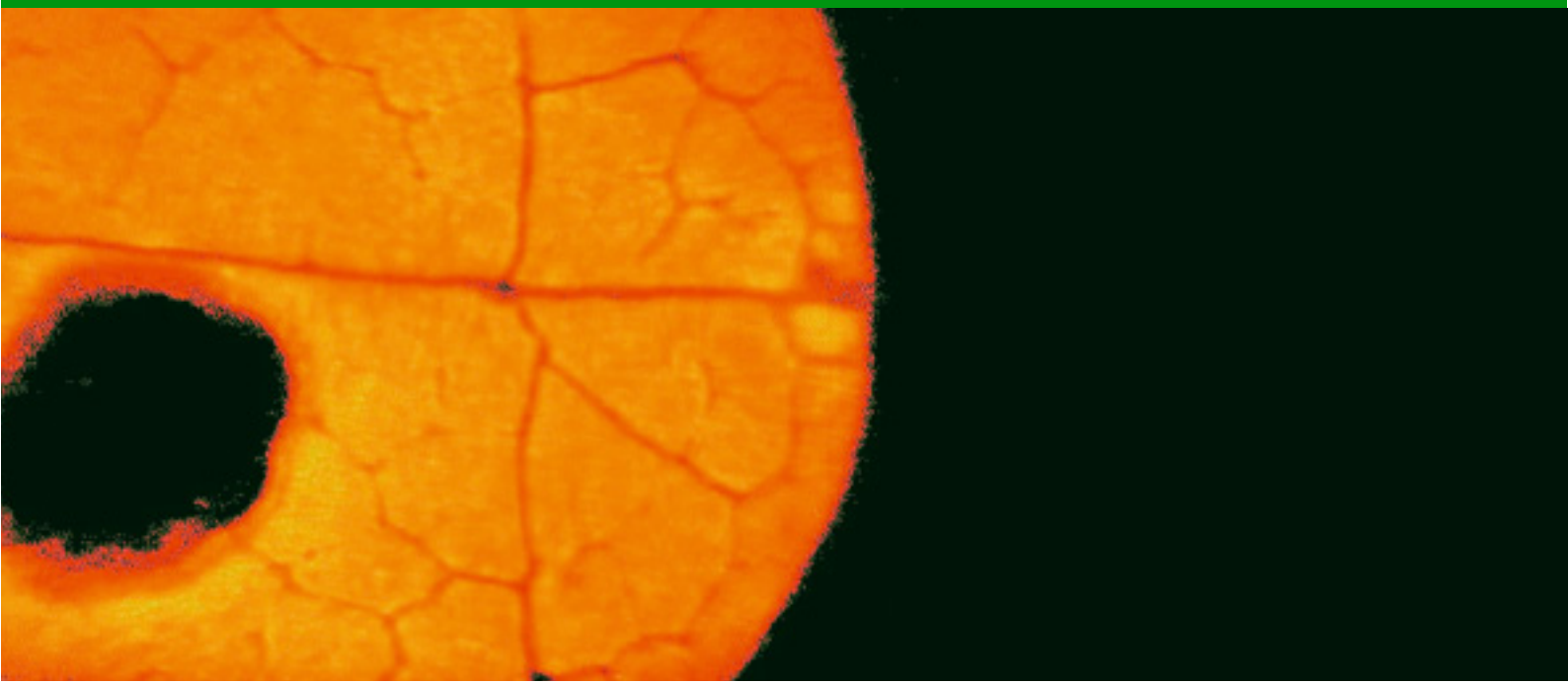
based on Axiostar (Zeiss) with LED Lamp collimator optics, dichroic beamsplitter and 2/3" CCD camera (4-pixel binning)

MC-FMH/M

Epifluorescence Microscope II

based on H600AFL (Hund) with LED Lamp collimator optics, dichroic beamsplitter and 2/3" CCD camera (4-pixel binning)

Max. actinic and Sat. Pulse intensity (depending on particular microscope, objective lens and LED Lamp) in the order of 2000 and 5000 µE/m²s, respectively



High Quality Instrumentation for Plant Sciences

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