THERMOIMAGING – THEORY

THERMOIMAGING - a non-destructive method suitable for monitoring spatial distribution of temperature (Instrument: *Thermal camera – TC)*

THERMAL CAMERA (infrared camera) – detects infrared radiation and uses it for calculation of spatial distribution of temperature (the calculation is made based on physical laws describing *black body radiation*)

BLACK BODY RADIATION (BBR) - every object emits electromagnetic radiation that is directly related to their temperature. Spectrum of black body radiation follows *Planck radiation formula*

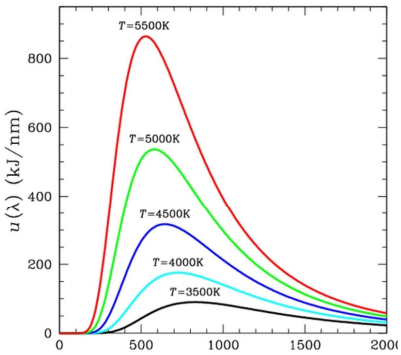
BBR intensity - described by ***Stefan-Boltzman Law***, it is function of emissivity (ε) and temperature (T) and Stefan-Boltzman constant (σ)

$$R = \varepsilon\sigma T^4$$

- True black body ε = 1, Real object ε < 1 (ε = 0.92-0.99 for leaf)
- For correct estimation of temperature by thermal camera knowledge of emissivity is necessary

	Emissivity
Plant leaves	0.95 (0.92–0.99)
Plant canopies	0.98–0.99
Dry leaves	0.96
Dry grass	0.88
Wood	0.90
Bark	0.94–0.97
Dry soil	0.92
Wet soil	0.95
Sand	0.87–0.92
Distilled water	0.96
Water	0.98–0.99

Planck radiation formula



$$I(\lambda, T) = \frac{2hc^2}{\lambda^5} \frac{1}{e^{\frac{hc}{\lambda kT}} - 1}$$

THERMOGRAPHY – PRINCIPLES

Thermocamera detects radiation as a sum of object (R_e), background (R_e) and air path (R_e) radiation

1 – direct radiation of measured object ($\mathbf{R_e}$,T)
- reduced with distance
- Function of object temperature

2 – reflected background radiation (R_A,T_b)
- Function of amb. temp T_A

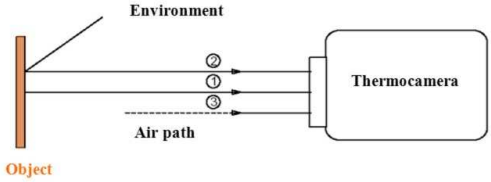
3 – radiation of air path (R_p,T_p)
- Function of path temp T_p

$$R_e \sim \varepsilon_o * \tau_p * P(T_o, \lambda) \text{ with } \tau_p = e^{\frac{-ap*distance}{1000}}$$

$$R_A \sim (1-\varepsilon_o) * \tau_p * P(T_A, \lambda)$$

$$R_p \sim (1-\tau_p) * P(T_p, \lambda)$$

Scheme of radiation pathways



$$R = R_e(T) + R_p + R_A$$

Detection of radiations from object (R_e) with given temperature (T)

THERMAL CAMERAS

Detector type

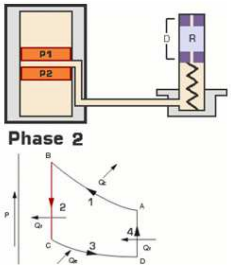
- *Uncooled detectors*
  - pyroelectric materials like Vanadium oxide
  - Lower sensitivity
  - Faster start of camera
- *Cooled detectors*
  - Semiconductor materials like **HgCdTe –** Mercury-cadmium-telluride
  - Higher sensitivity
  - Cooling range 4-110K
  - Slower start of camera because of cooling

Detector arrangement

- *Object scanning*
  - *Varioscan* by Jena Optic
  - Higher sensitivity
  - Lower frame rate
- *Array of detectors*
  - e.g. Thermovision by FTIR systems
  - Lower resolution, higher frame rate

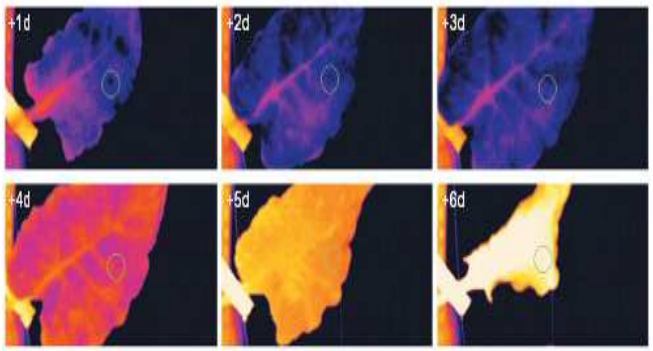
Cooling system

- *Liquid nitrogen, Helium*
- *Stirling Cooling*

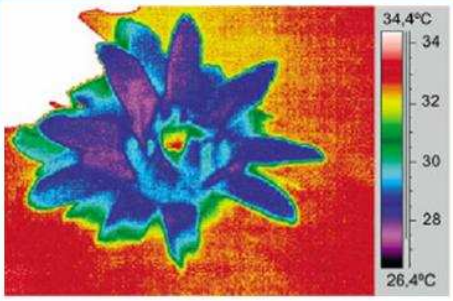


APPLICATION OF THERMOGRAPHY IN PLANT RESEARCH

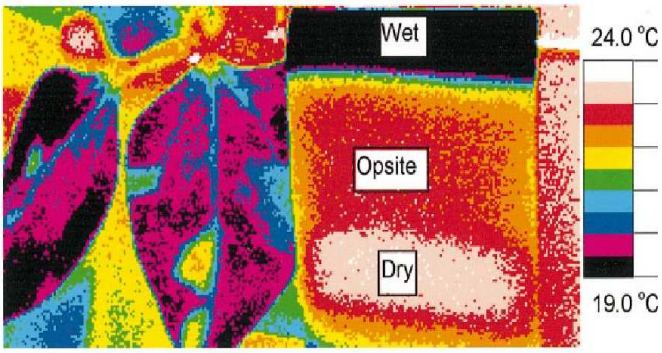
1. **Water stress detection**
2. **Calculation of stomatal conductance**
3. **Plant pathogenesisx**
4. **Mutant selection**
5. **Plant thermogenesis**
6. **Temperature distribution in canopy**
7. **Photosynthesis research**



Water status of a leaf



Thermogenesis in flowers of *V. Cruciana*



Stomatal conductance

THERMOIMAGING OF LEAVES - PRACTICAL

I. Camera setup *(see below)*

- Camera initialisation
- Start IrbisControl software
- Setup of camera parameters
    - Emissivity
    - Temperature of ambient radiation (detected by thermocamera + mirror)
    - Temperature of air path between leaf and camera

II. Sample adjustment

- Fixing of leaf
- Camera focusing on chosen object
- Setup of Camera Zoom (pixel size)

Zoom Step	FOV [°H x °V]
1	30 x 20
2	21 x 14
3	15 10
4	8 x 5
5	5 x 4
6	freely selectable

$$w = 2z \tan(\alpha/2)$$

$$p = \frac{w}{n}$$

a – angle of camera view
w - width of the imaged area
z - object distance
n – number of pixels

III. Measurement of leaf temperature

- Control tobacco leaf
- Elimination of evaporation by Vaseline jelly
- Role of irradiation on leaf temperature
- Water dropping on the leaf surface

IV. Data processing

- Export *.irb data files into text image (data matrix 360x240) *.txt (by ***Irb2ASCI*** software)
- Convert text image (matrix of temperature) into tiff-image (***ImageJ*** software)
- Import pictures into a ***ImageJ*** software (see below)
- Making stack of pictures

IV. Image processing

- Made by ***ImageJ*** software
- <u>Operations with picture</u>
    - Transformation from Kelvin's to Celsius temperature
    - Choosing of the "look-up tables"
    - Image adjustment
        - Brightness
        - Contrast
        - Range of values
    - Calibration bar
    - Scale bar
    - Image export (to *.jpg)
    - Surface plot of image
    - Measuring of temperature values
    - Measurement of distances and areas
- <u>Operation with stack</u>
    - Convert images to stacks
    - Transformation from Kelvin's to Celsius temperature
      Choosing a tables for false colours
    - Stack adjustment
        - Brightness

- Contrast
- Range of values

- Calibration bar
- Scale bar
- Time stamp
- Visualisation of temperature kinetics of different areas

IV. Distribution of temperature

THERMOCAMERA SETUP

Camera+Control function in IRBIScontrol software

(A) *Scanner* – adjustment of type of scanning (line scan vs. image scan)

- Image – 1x1
- Linescan OFF
- Compensation – 1 sec (time for compensation wit shutter temperature)
- Interval – Minimal

(B) *Storage* – destination for image saving

- Modus – by user or automatic in different interval (minimal 1 sec)
- Directory
- Name – Mask of file name (e.g. List ***)
- Format – IRB (raw data)
- Background memory – number of image stored in Camera (in the case of no automatic saving), maximal 2000

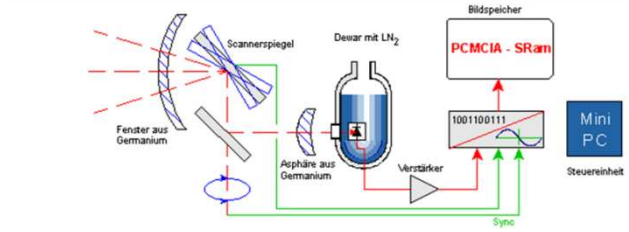
(C) *Automatic*

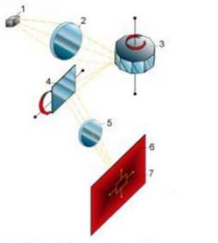
- Aperture adjustment (inactive)

(D) *Parameters of camera* - ***Varioscan 3200***

- Emissivity – number dependent on the type of the surface
- Path temperature (independent detection)
- Environmental temperature (measured by means of mirror)
- Transmisivity (for shorter distances 1)

- Producer infratech (optic by Jenoptik)
- Principle of object scanning - image size 360x240 pixels
- Chopper, vertical and horizontal scanner (mirrors)
- Germanium lenses, silicon-germanium (anti-refraction coated)
- Detection at 8-12µm (one MCT detector)
- Sterling-cooled detector (or nitrogen)
- Geometrical resolution of 1.5 mrad with 30°x20° maximal field of view
- Temperature resolution – 0.03K (high resolution camera)
- Absolute accuracy < ±2K
- Frame frequency 1Hz (for 360x240 image)
- Electro-optical zoom (reduction in field view), maximal 6x





Principle ray path

1 detector, 2+5 lenses, 3 horizontal deflection mirror, 4 vertical deflection mirror, 6 object, 7 measuring spot

DATA PROCESSING - IMAGEJ SOFTWARE

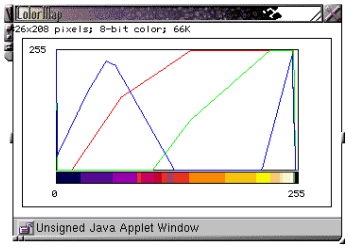
- Open source software for image processing
- Based on Java, macros, plugins
- http://rsb.info.nih.gov/ij/index.html

Transformation from Kelvin's to Celsius temperature scale

- By function "Math operations" (process + math + add) or by means of macro

Look-up tables (LUT)

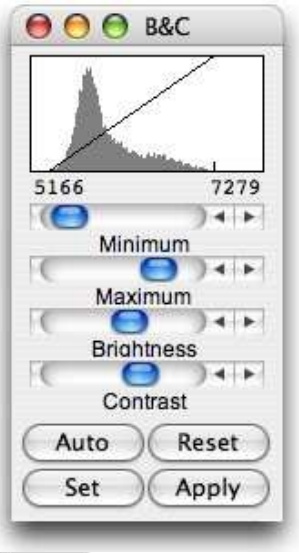
- ***LUT*** is set-up by *Image + lookup Tables*
- Function *Image+Colour+Show LUT* displays a plot of



the active image's lookup table

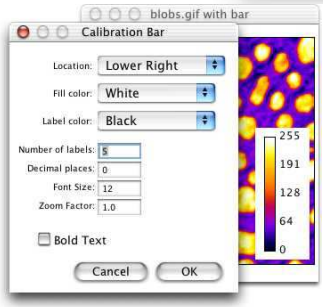
Image adjustment (Brightness, contrast)

- Brightness and contract can be changed by *Image+adjust* (SHIFT + C) function - updating the image's look-up table (LUT), so pixel values are unchanged
- ImageJ displays images by linearly mapping pixel values in the display range to display values in the range 0-255
- Pixels with a value less than the minimum are displayed as black and those with a value greater than the maximum are displayed as white
- ***Brightness*** increases or decreases image brightness by moving the display range
- ***Contrast*** increases or decreases contrast by varying the width of the display range



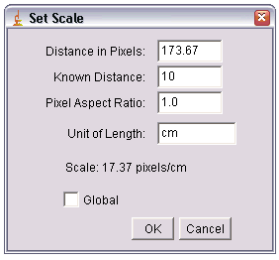
Calibration bar

- For indication of temperatures
- *Analyse + Tool + Calibration Bar*
- Visualisation of temperature range



Scale bar

- For indication of distances
  - Putting of scale line into the picture (stack) – *Analyze+tool+scale bars*



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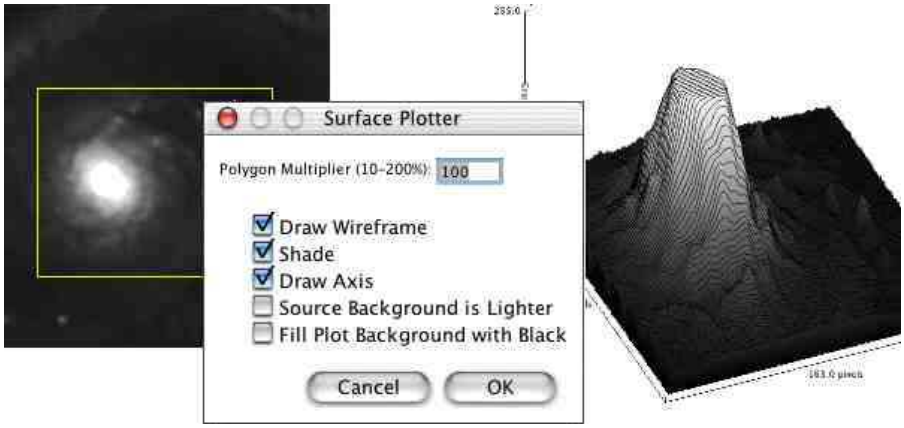
Zoom	Pixel size from 1 m [mm]	Pixel size from 0.75 m [mm]	Pixel size from 0.5 m [mm]	Pixel size from 0.25 m [mm]
0	1.488606625	1.116454968	0.744303312	0.372151656
1	1.043537968	0.782653476	0.521768984	0.260884492
2	0.731402764	0.548552073	0.365701382	0.182850691
3	0.517246077	0.387934557	0.258623038	0.129311519
4	0.364130349	0.273097762	0.182065174	0.091032587
5	0.257151797	0.192863848	0.128575899	0.064287949

Time stamper

- characterization of time for video exported from stack (*plugins + stack-time stamper*)

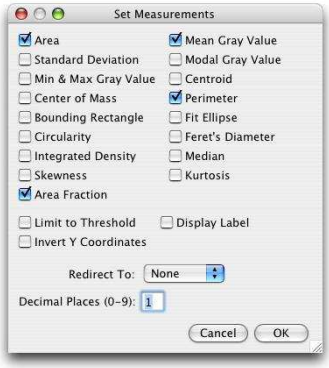
Making of the surface plot

- making of 3D plot from 2D thermopicture (*analyze + surface plot*)
- maximal 300 picture in stacks and reduction in the resolution is necessary



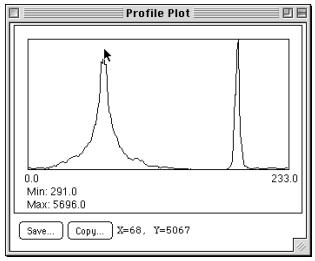
Measuring of temperature values

- possibility of measurement of mean values of temperature in given area of
- picture (CTRL+M) or for the whole stack (*Image+Stacks+PlotZ axis profile*)
- set-up of measured parameters by *Analyze + set measurement*



Measuring of distances

- Several methods, calibration of scale is necessary
- *Analyze + plot profiles* - Displays a two-dimensional graph of the intensities of pixels along a line within the image



Temperature histograms

- Characterisation of temperature distribution
- *Analyse +histogram*