Automatic measurement of plant features using ImageJ and MRI Cell Image Analyzer

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outline

- Montpellier RIO Imaging
- the image analysis bottleneck
- solution
  - MRI Cell Image Analyzer
  - on demand development
- visual scripting
- measuring plant growth
  - rosettes
    - automatically
    - varying scale, semi-automatically
    - varying scale, automatically
  - cells in epidermis
  - roots
- summery and outlook

Montpellier RIO Imaging

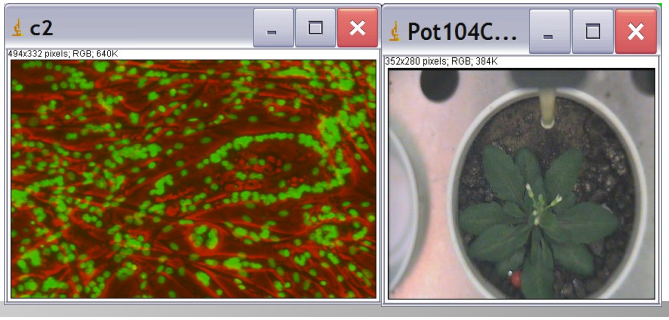
- **Montpellier RIO Imaging** - regional imaging facility

- 7 sites
- 29 microscopes
- 442 users
- 33742 hours/year in 2006/07

- promote the usage of microscopy and imaging
- participate in the development of microscopy and imaging
- provide training

wide range of mostly biological applications

images from sub-cellular to entire organisms



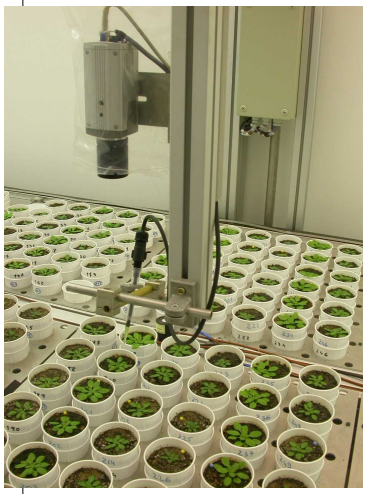
Responsable

PierreTravo, IRCE CNRS

the image analysis bottleneck

robotized acquisition
time series, volume images

- large amounts of data



manual analysis

» time consuming
» biased results?

analysis is the bottleneck

⇨ automatic analysis needed

wide range of
- analysis needs
- image qualities

⇨ analysis must use a-priori knowledge

if automatic analysis not possible
- partial automation can augment efficiency
- let the users only do what the software can't do

⇨ semi-automatic analysis needed

the solution – part I

wanted:

rapid prototyping framework for image analysis applications

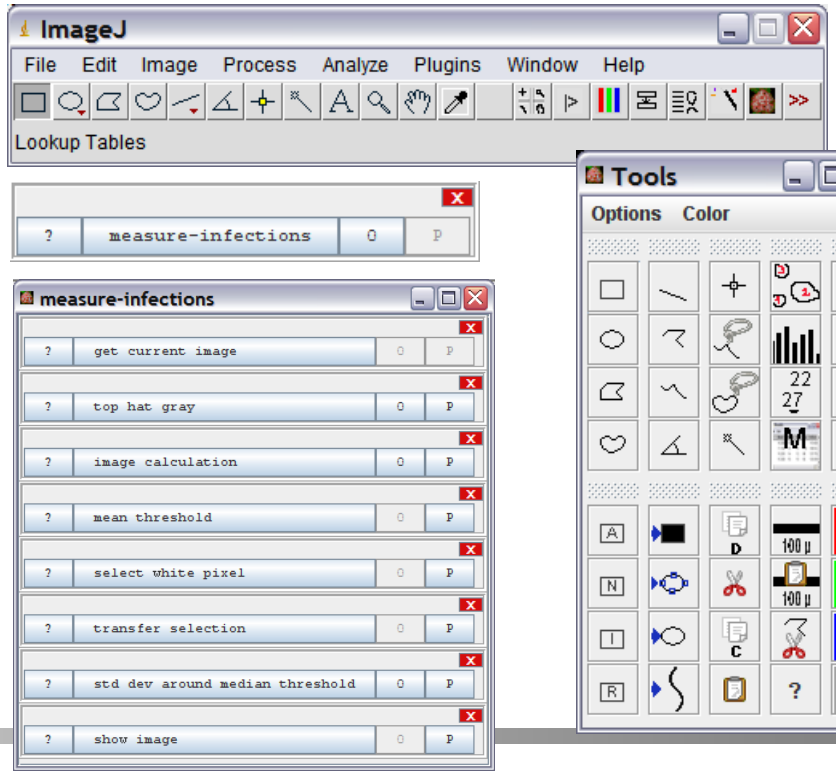
requirements:

- interactive experimentation to find solutions
- interactive and batch applications
- build prototype-applications from existing operations rapidly
- extendable – add new operations
- allow to parametrize and run applications
- easy to use for end user

solution:

MRI Cell Image Analyzer

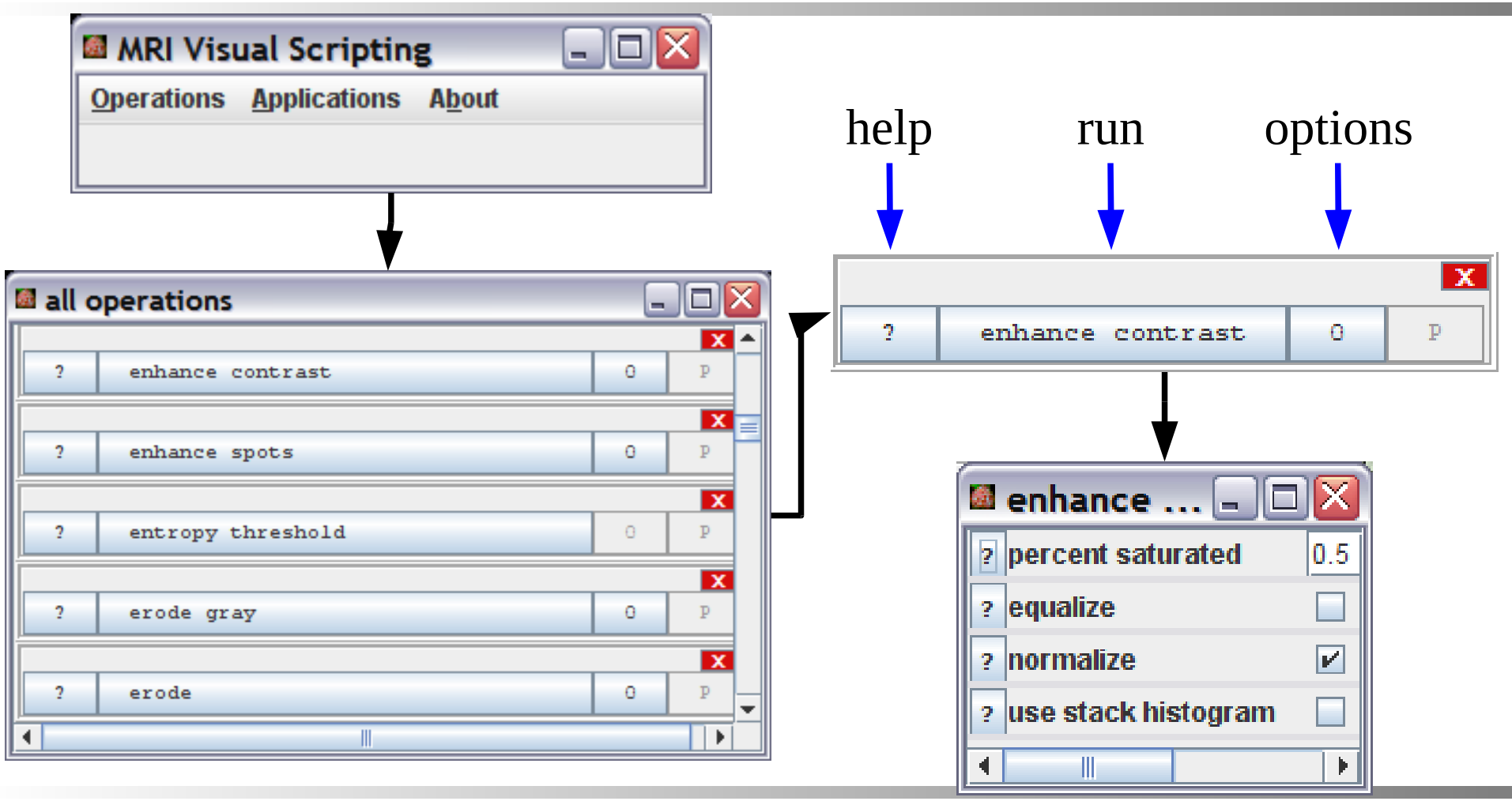
ImageJ + Visual Scripting + Tools



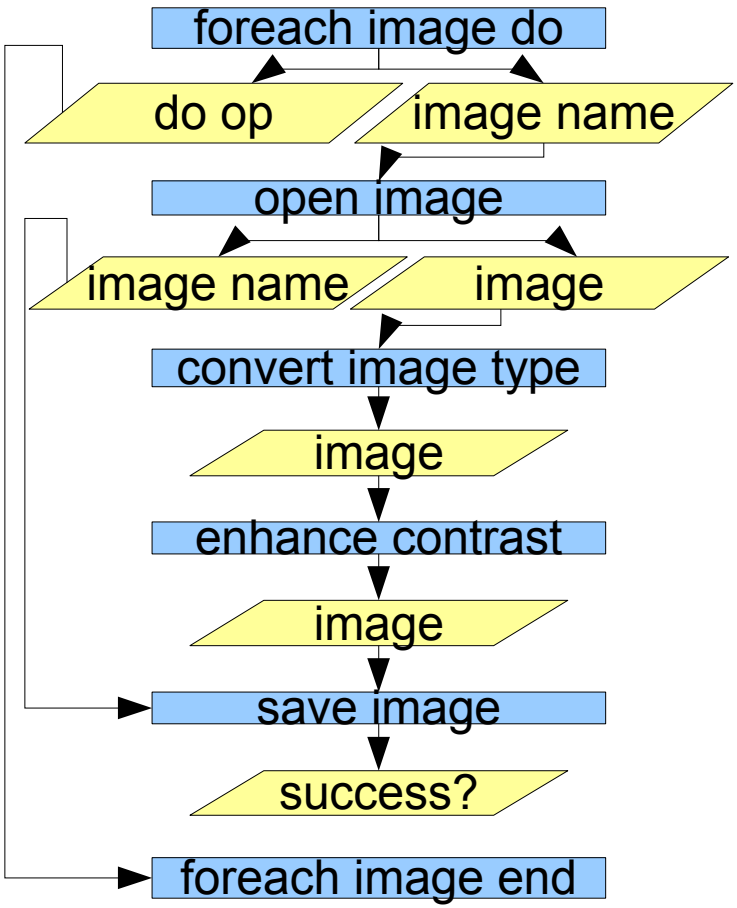
the solution – part II

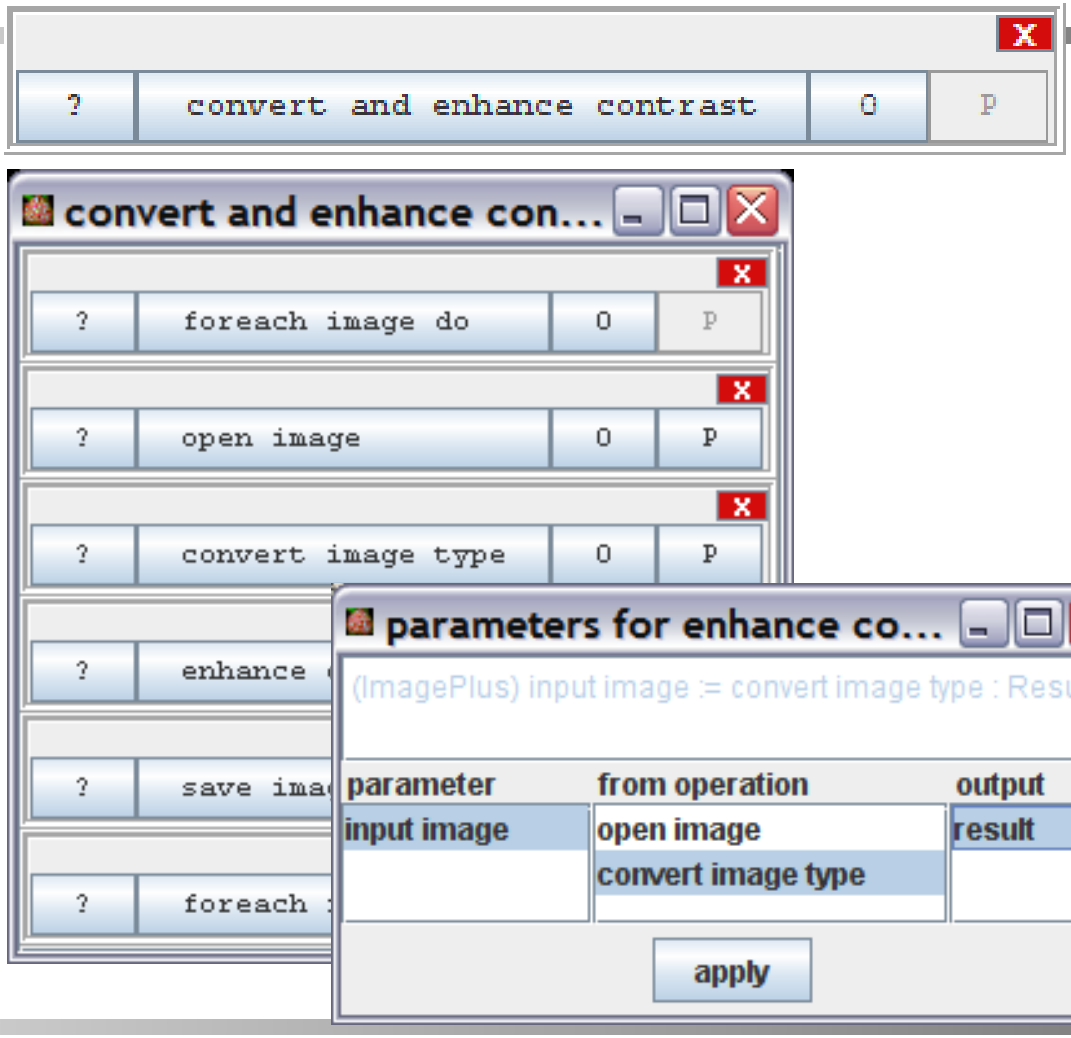
- process
  - use framework
    - to create prototype solutions on demand
    - in close collaboration with biologists
    - extend framework when necessary for a project
      - **only then**
    - eventually create full featured application

visual scripting I



visual scripting II





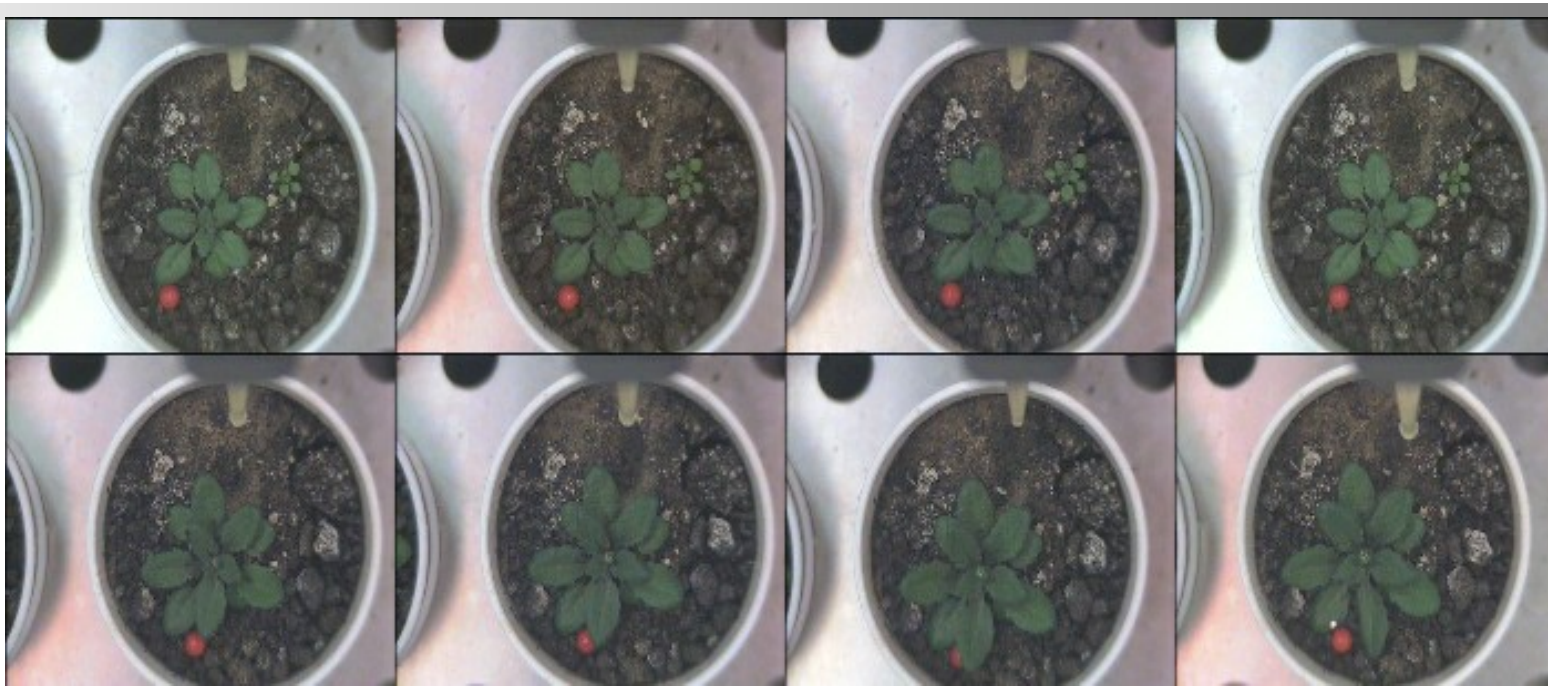
applications

I. measuring rosettes

measuring rosettes automatically

task:

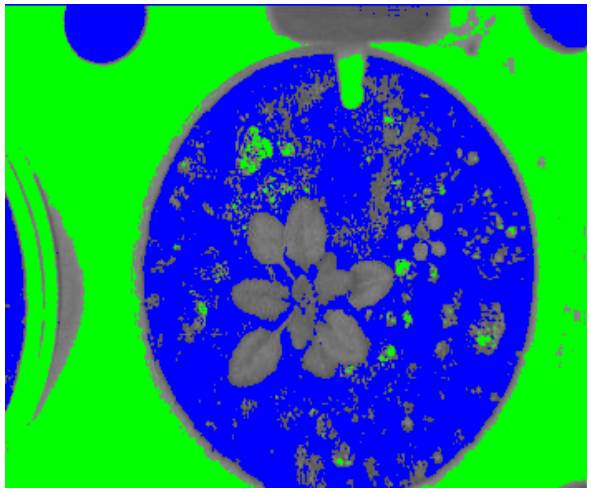
- measure plant surface
- images taken by the PHENOPSIS automaton
- fixed scale

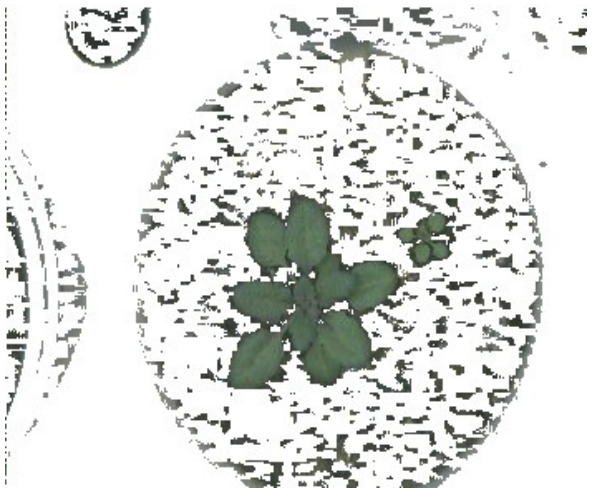


input:

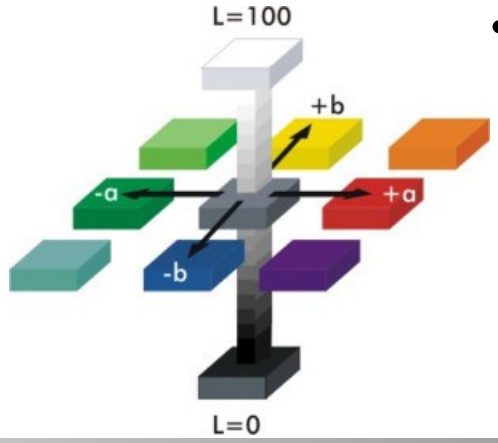
- 352x280 pixel, 8-bit / channel, jpg compressed, RGB color images

measuring rosettes automatically - problems





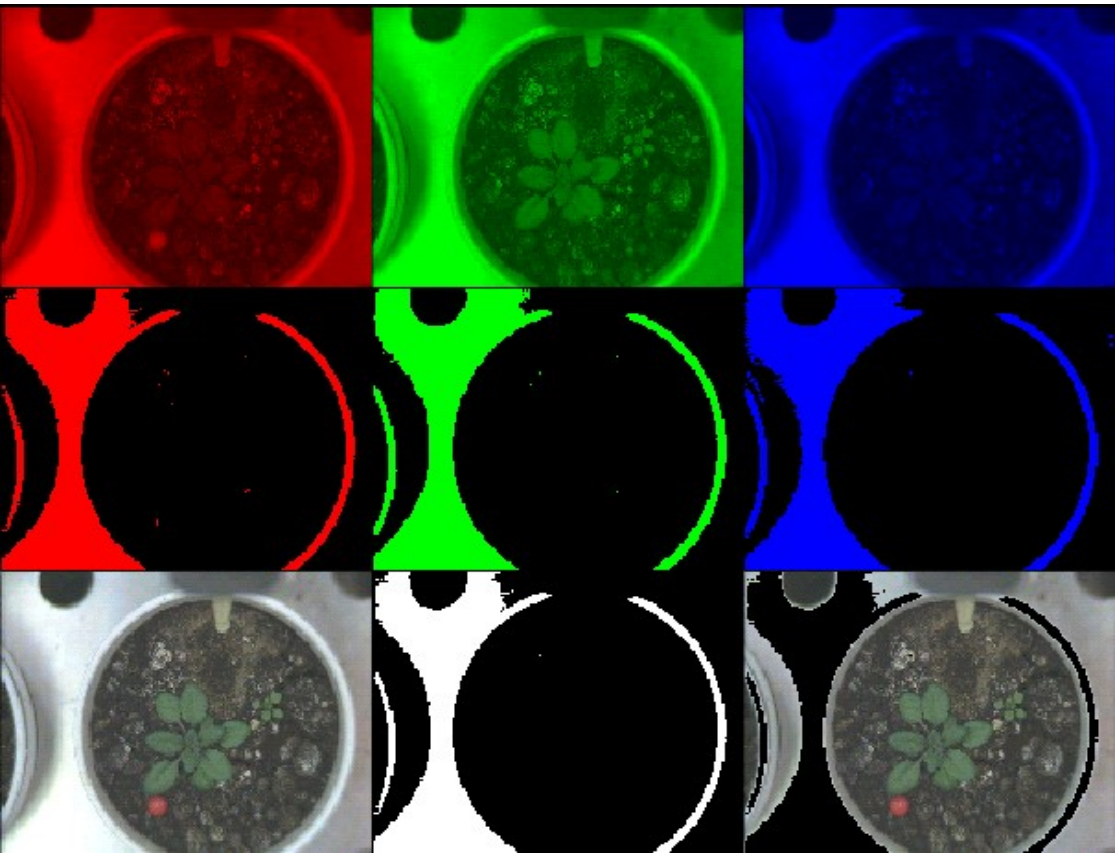
- idea:
  - the plant is green!
- approaches:
  - threshold the green channel
  - use a color threshold in CIELAB color space



- problem
  - same intensities in green channel in plant and:
    - earth
    - tube
    - border of pot

measuring rosettes automatically – the solution

step 1 – exclude « white » regions

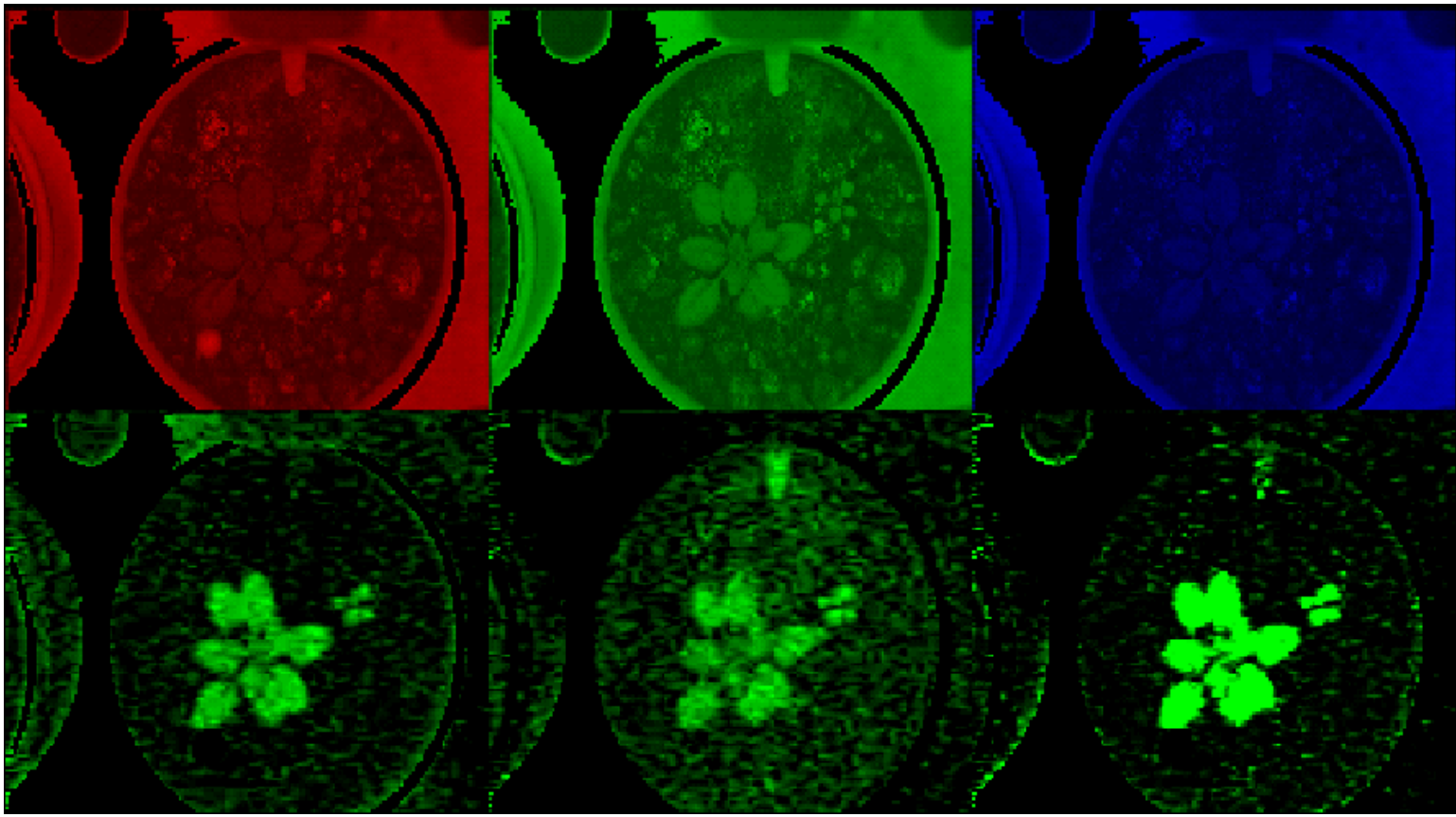


- threshold each channel (t=180)
- combine the masks (AND)
- use resulting mask to exclude bright regions

measuring rosettes automatically – the solution

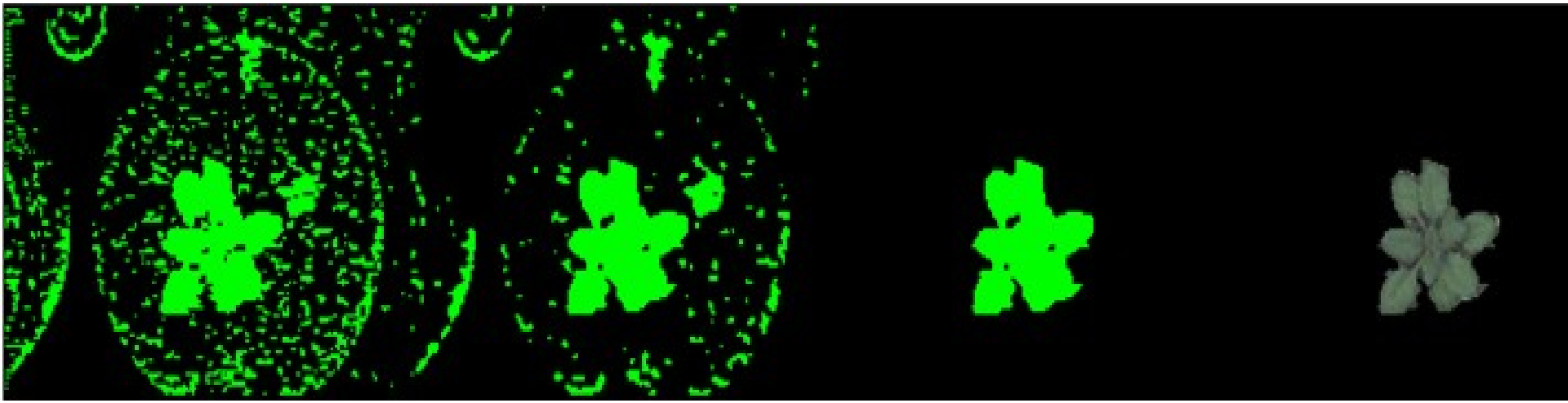
step 2 – increase contrast

- subtract red from green
- subtract blue from green
- multiply results



measuring rosettes automatically – the solution

step 3 – segment plant area



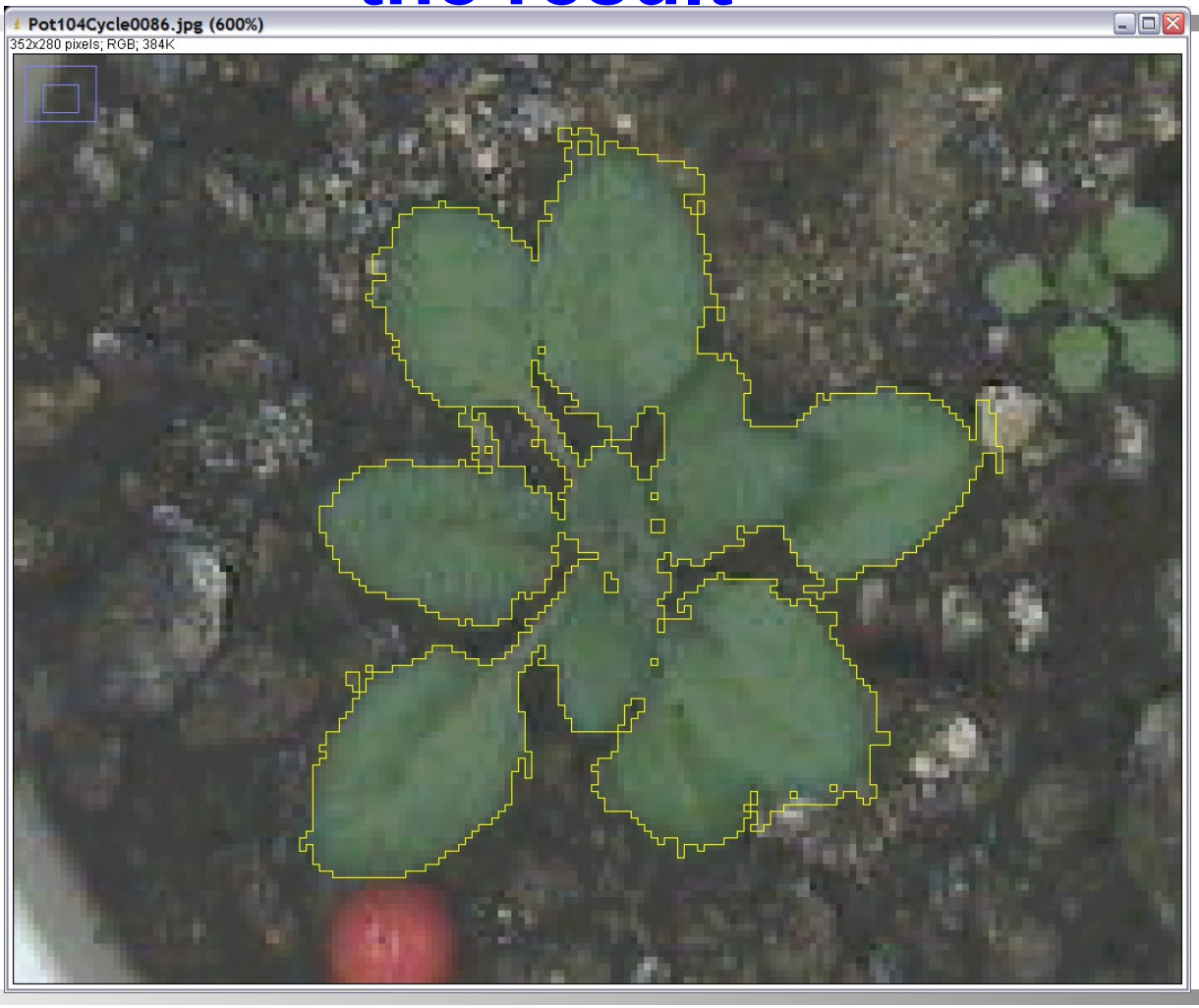
- threshold with mean intensity
- apply median filter (r=3)
- find connected objects and exclude small objects (<1000 pixel²)
- mask original image

measuring rosettes automatically – the solution

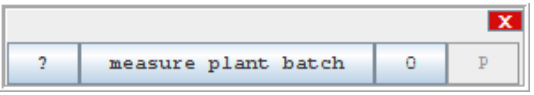
step 4 – segment plant

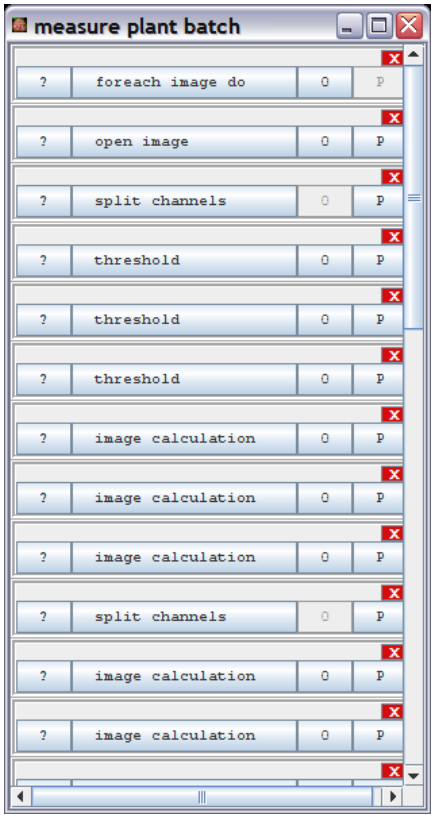
- sqrt green channel
- auto-threshold
- find connected objects and exclude small objects (<250 pixel²)
- measure surface

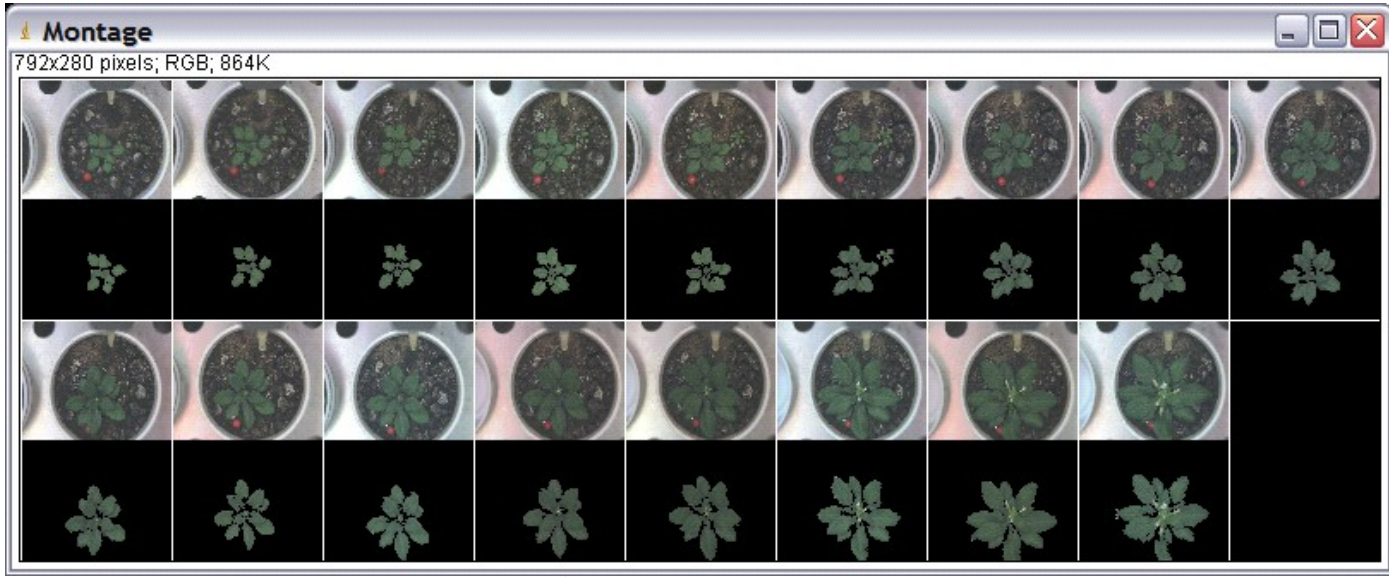
measuring rosettes automatically – the result



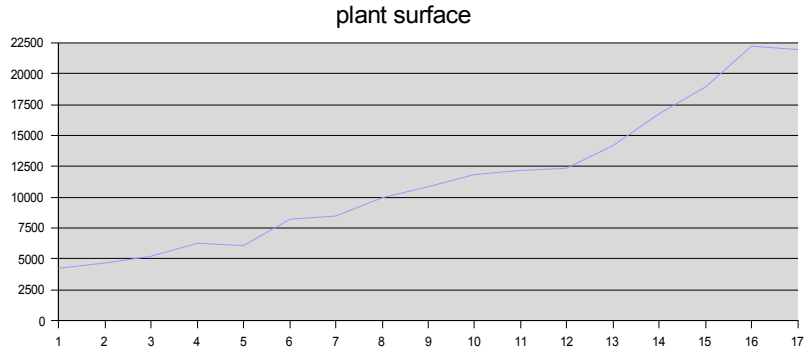
measuring rosettes automatically – the application







- run batch on all images
- results:
  - control images
  - measurements (spreadsheet file)



rosettes, varying scale 1

task:

- measure plant surface
- images taken manually
- varying scale

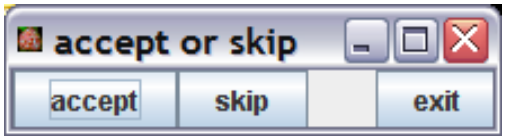
- 1600x1200 pixel, 8-bit / channel, jpg compressed, RGB color images

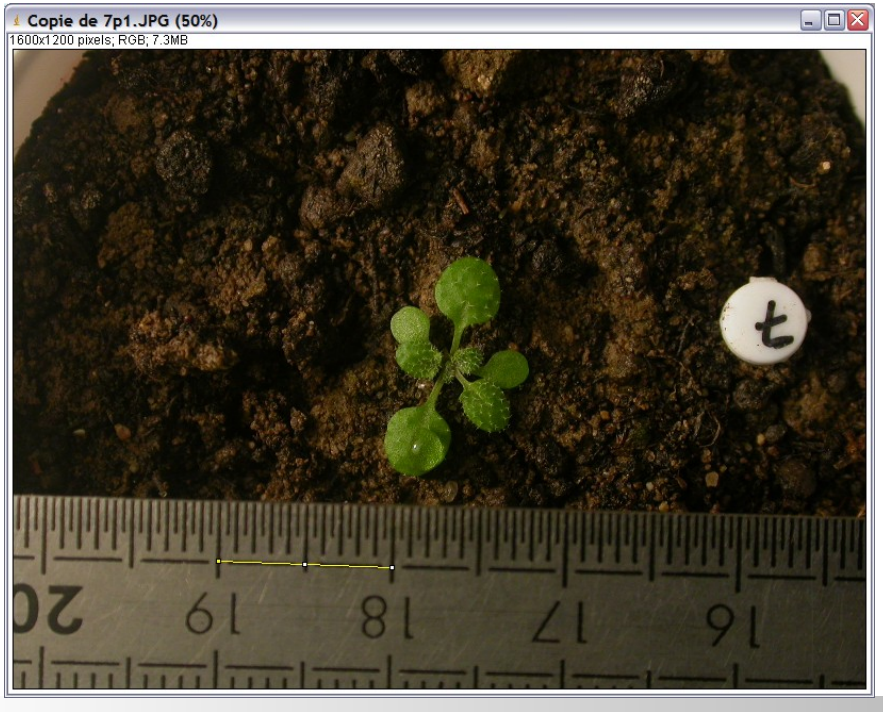
rosettes, varying scale 1 - segmentation

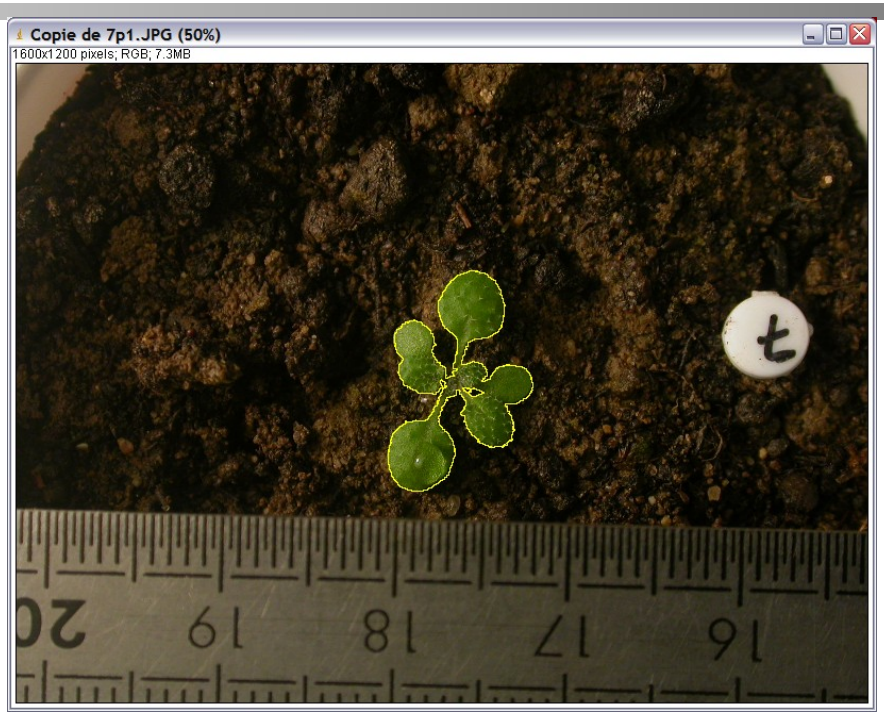
- `green*(green-red)`
- `apply threshold (t=128)`
- `median filter (r=3)`
- `find connected objects and exclude small objects (<3000 pixel²)`

rosettes, varying scale 1 - interactive

- select 1 cm to calculate scale

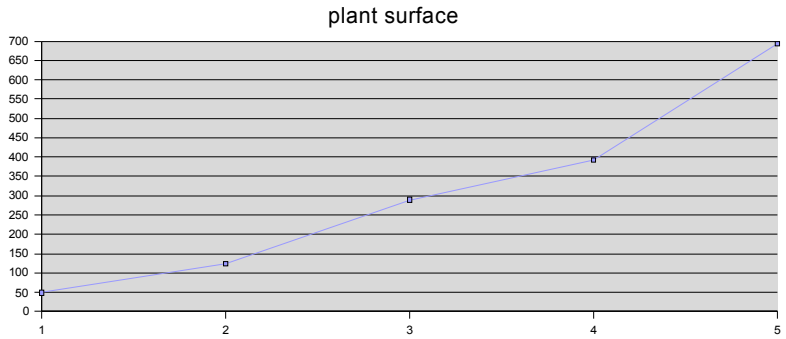






- correct selection if necessary

rosettes, varying scale 1 - results



- results
  - control images
  - measurements in cm² in spreadsheet file

rosettes, varying scale 2 - automatic

UMR - BGPI
Biologie et Génétique
des Interactions Plante-Parasite

task:

- measure plant surface
- images taken manually
- varying scale

input:

- 1600x1200 pixel, 8-bit / channel, jpg compressed, RGB color images

work automatically !

rosettes, varying scale 2 - approach

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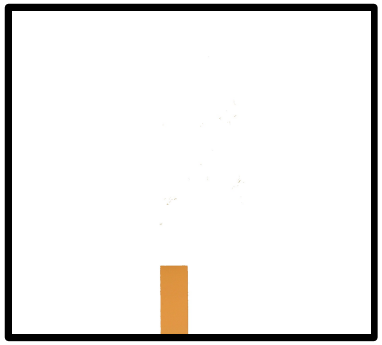
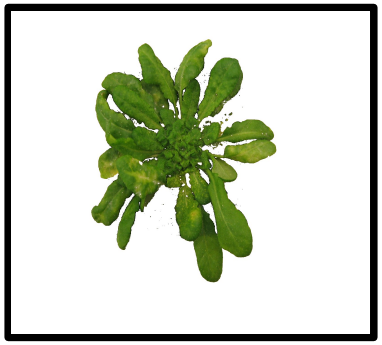
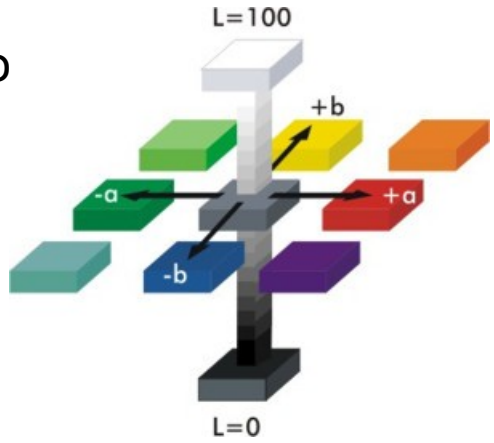
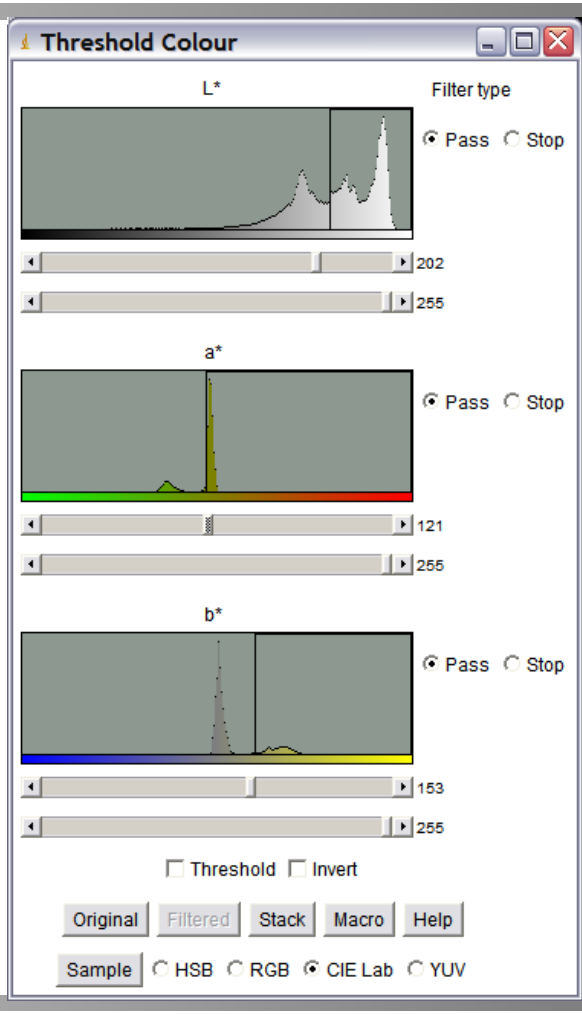
des Interactions Plante-Parasite

- idea:
  - use object of known size to calculate scale
- approaches:
  - color threshold reference object
  - color threshold plant





rosettes, varying scale 2 - results

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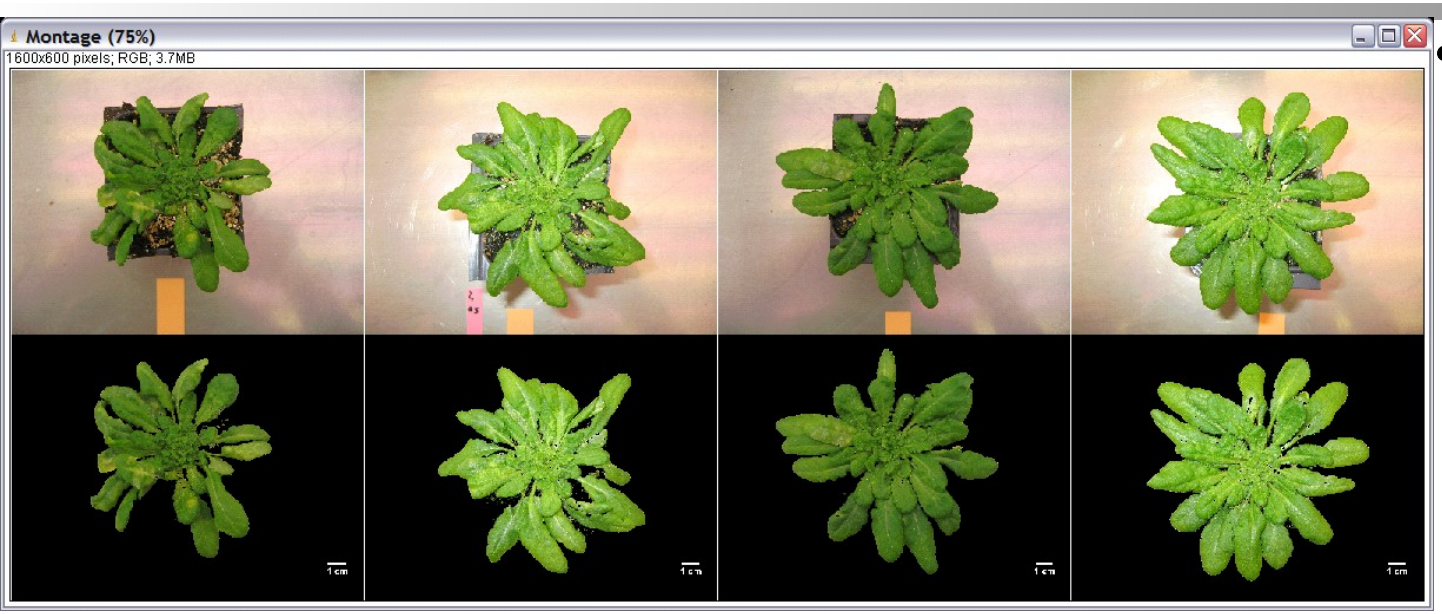
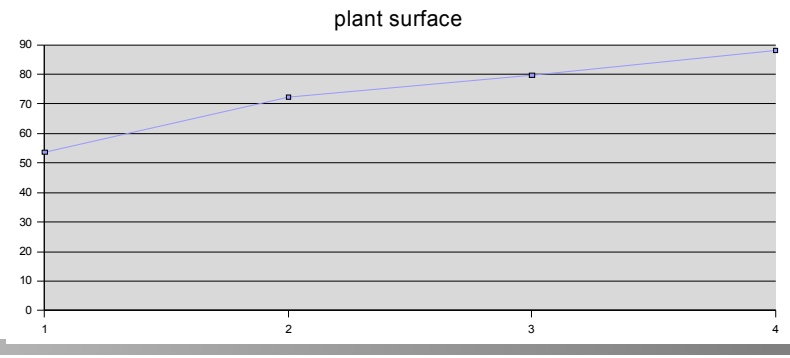


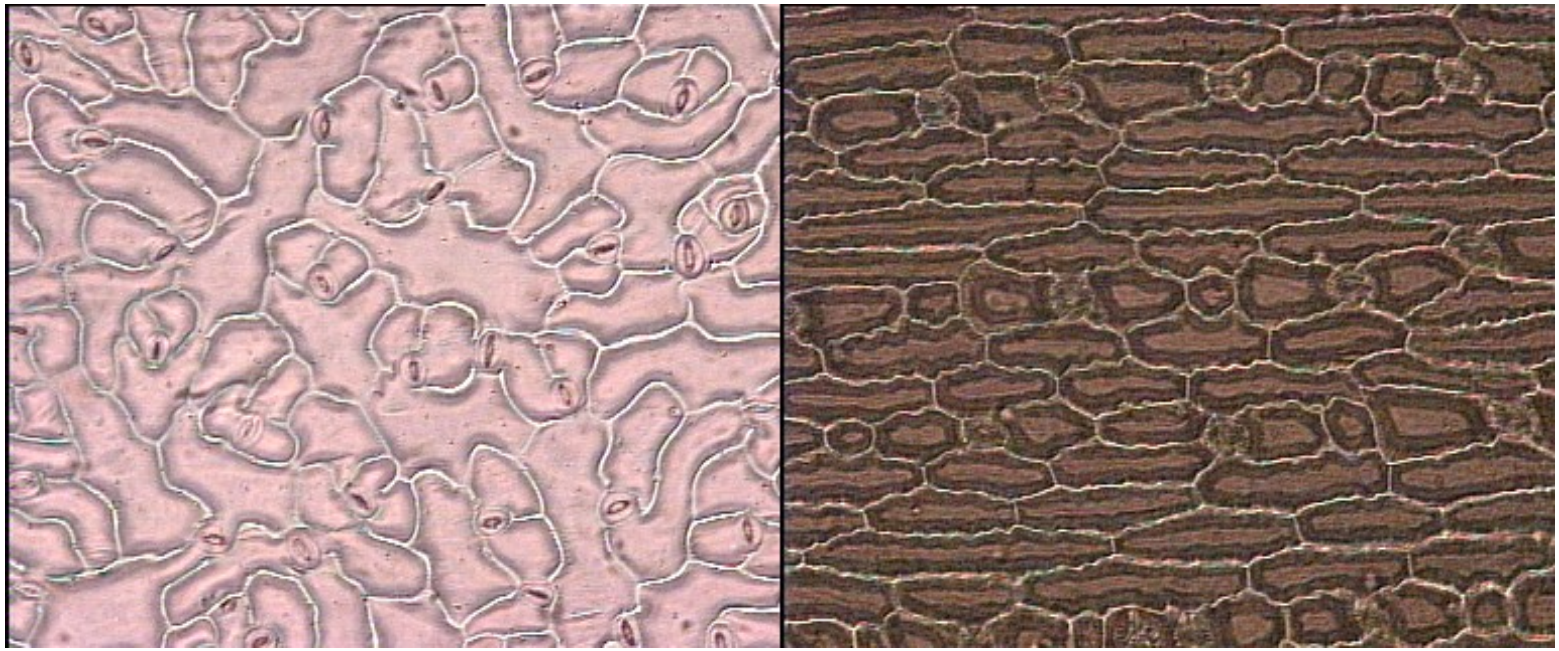
- problems:
  - ref. object must be parallel to image axis
  - ref. object should be longer

- results
  - control images
  - measurements in cm² in spreadsheet file
  - scale bar in control image



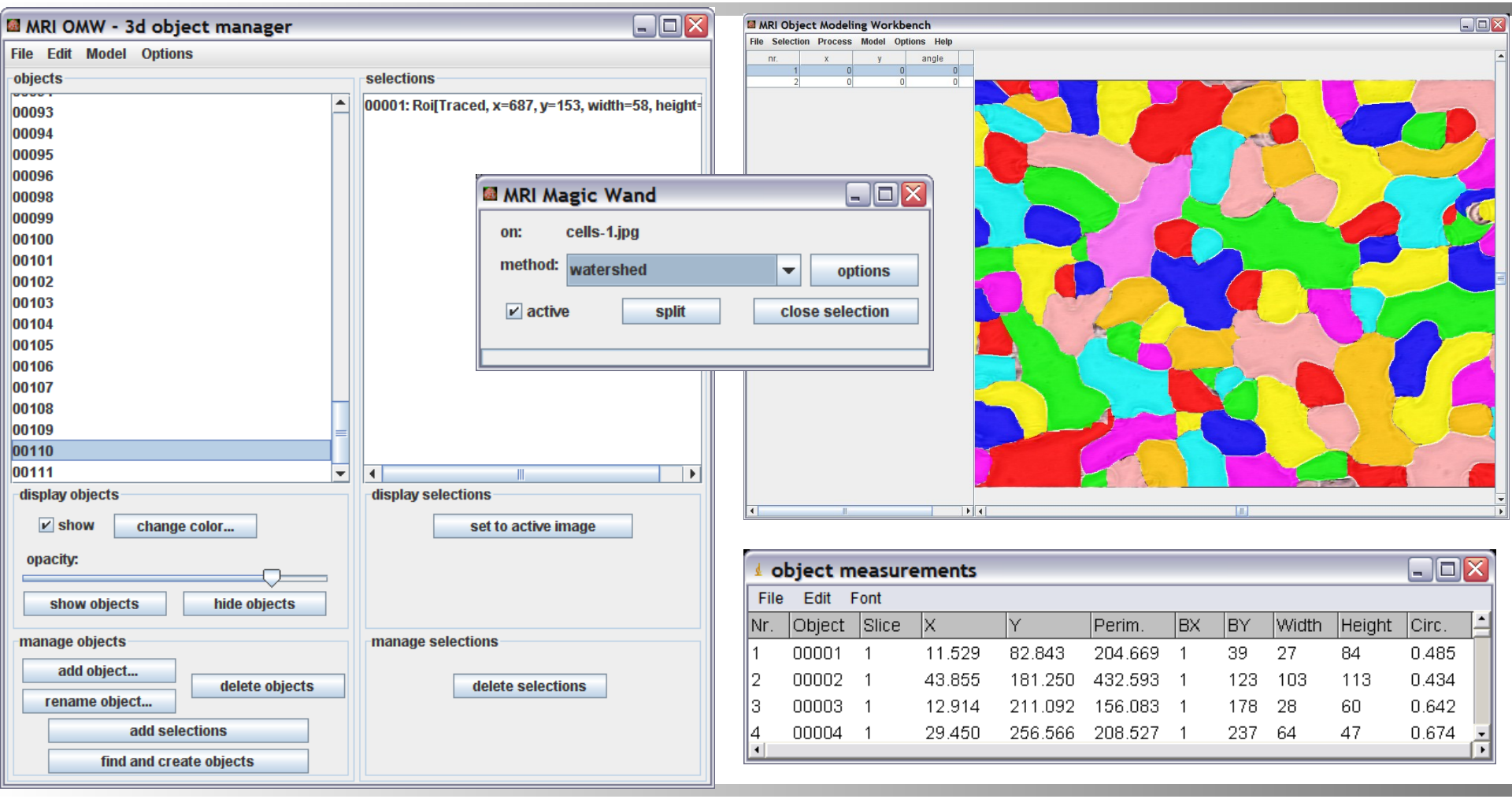
applications
II. measuring cells in the epidermis

measuring plant cells in the epidermis

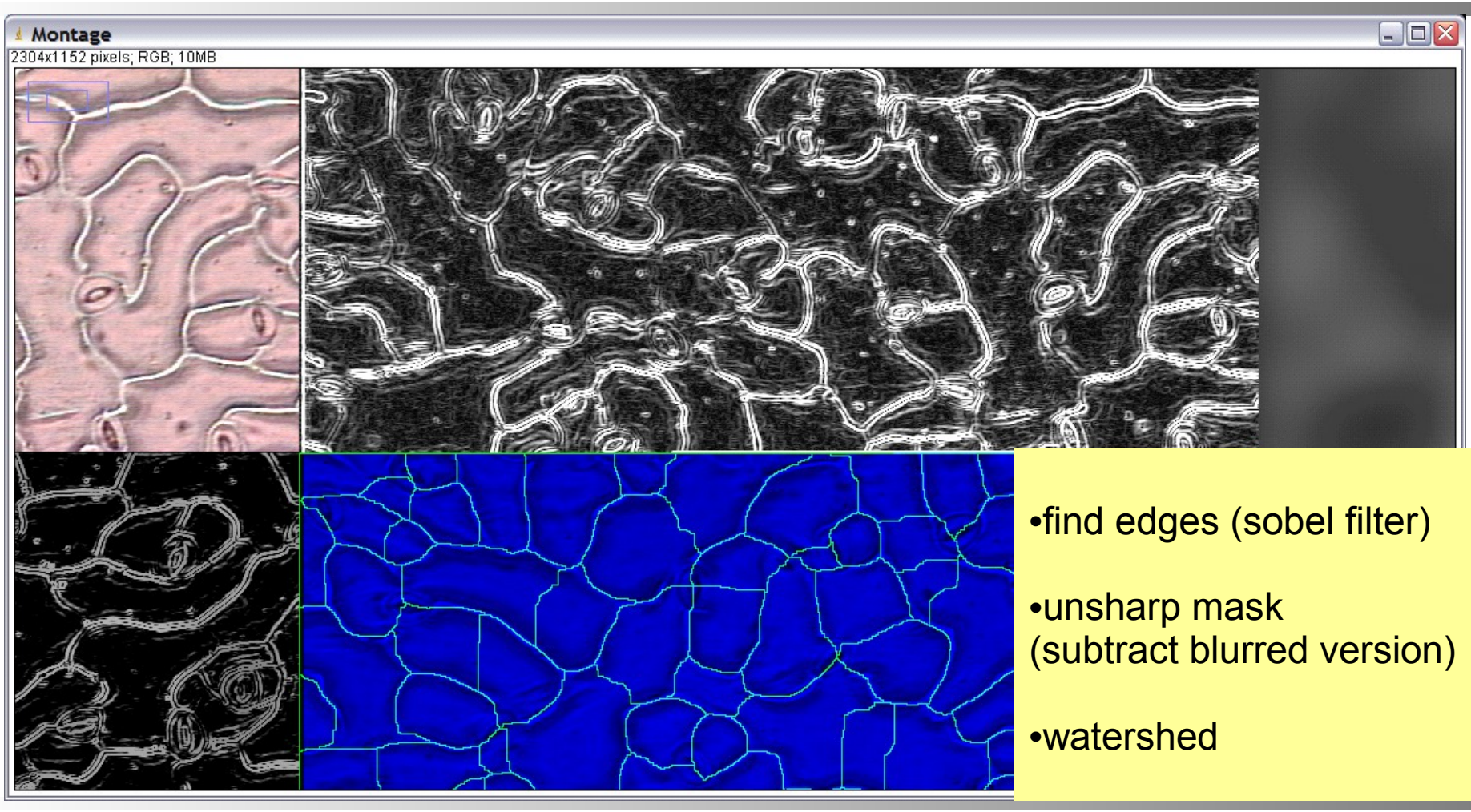


- measure properties of the individual epidermis cells
- problem:
  - segmentation
  - stomata

measuring cells– object modeling workbench

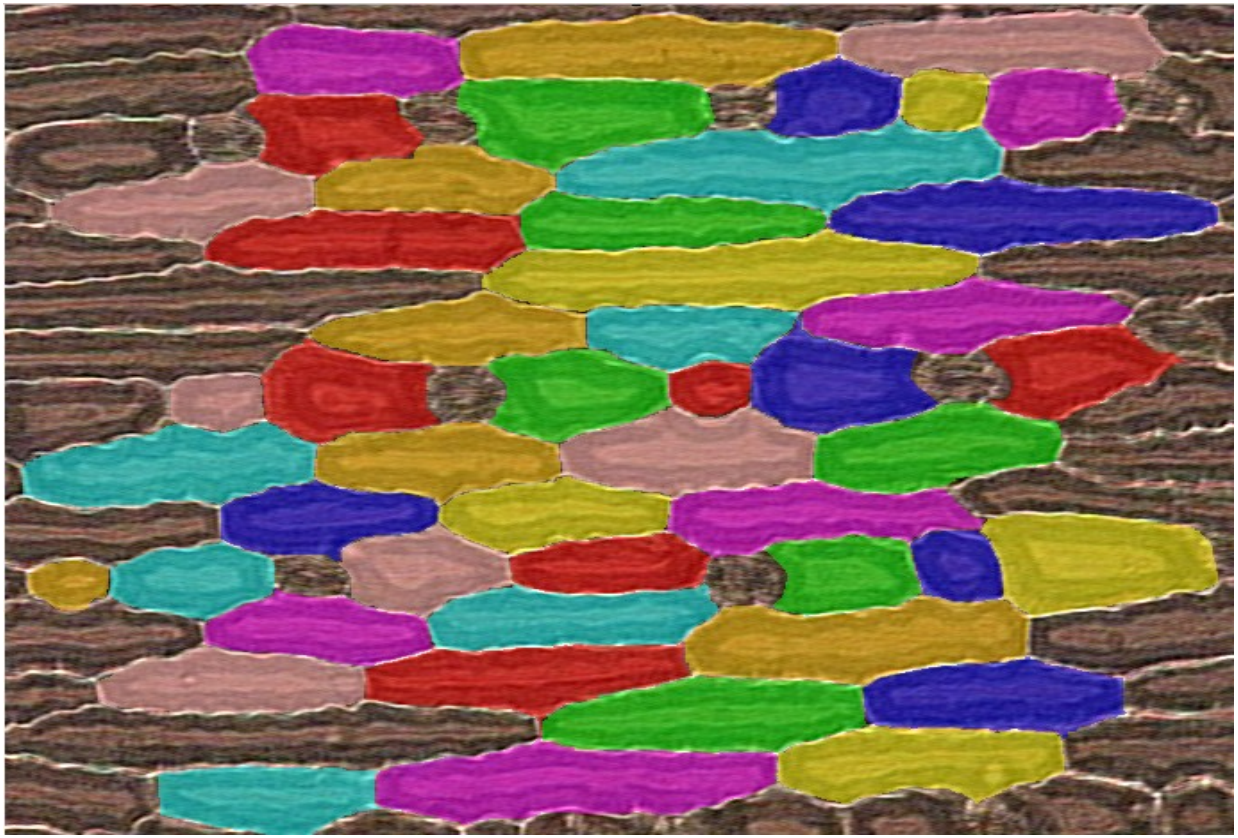


measuring cells– the algorithm



- find edges (sobel filter)
- unsharp mask (subtract blurred version)
- watershed

measuring plant cells in the epidermis – outlook



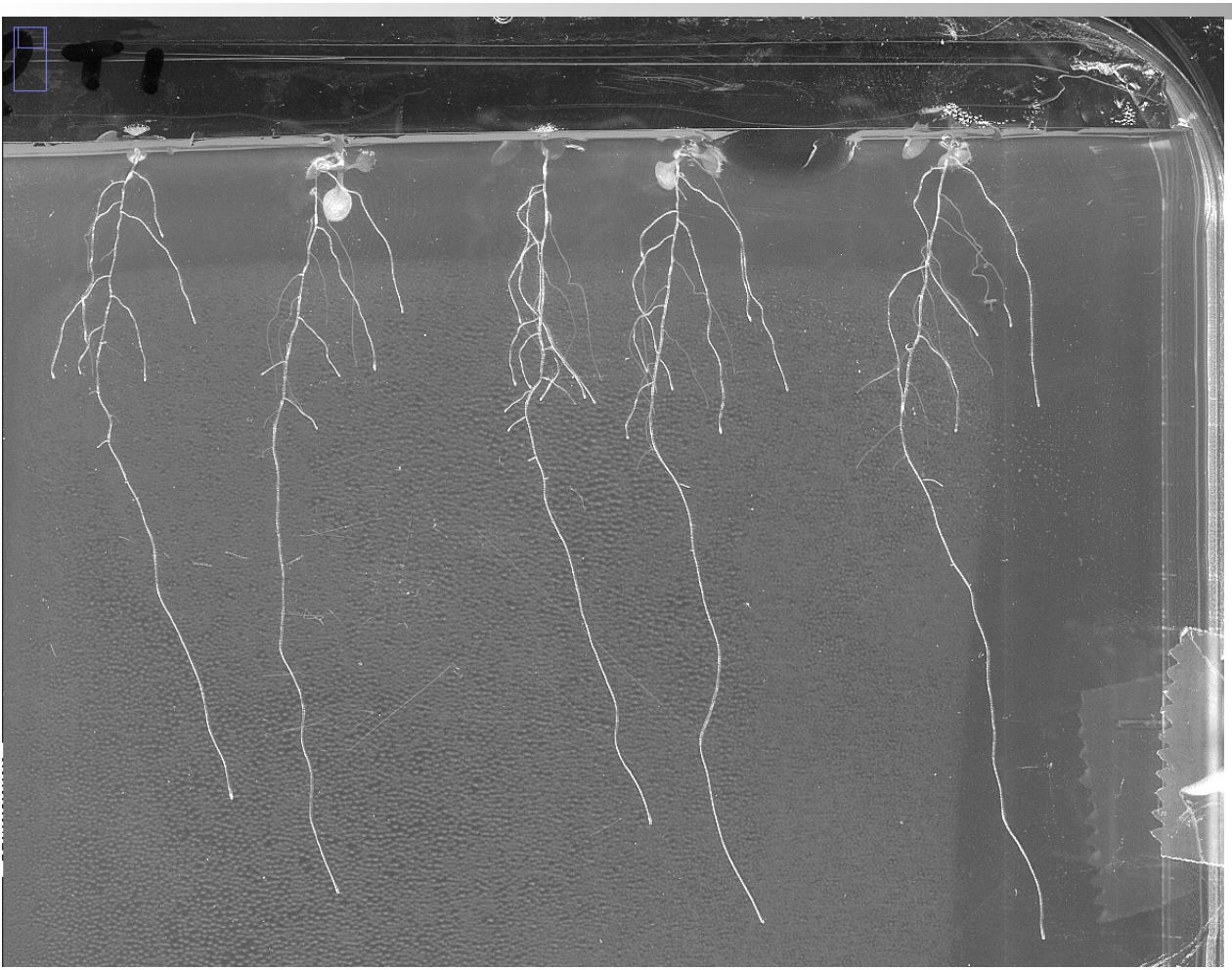
futur versions:

- automatically detect stomata
  - texture threshold
- automatically merge after watershed

applications

III. measuring roots

measuring roots

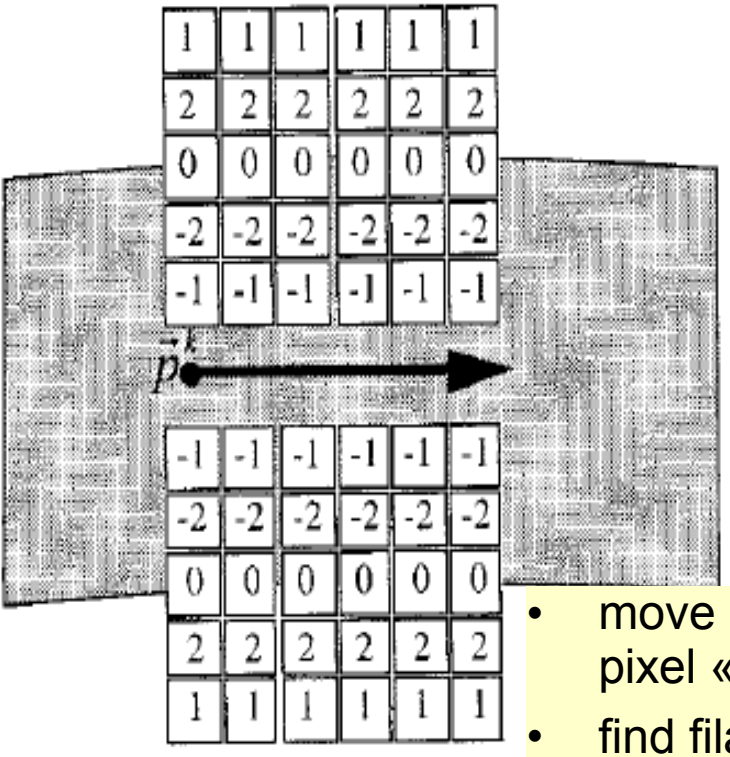


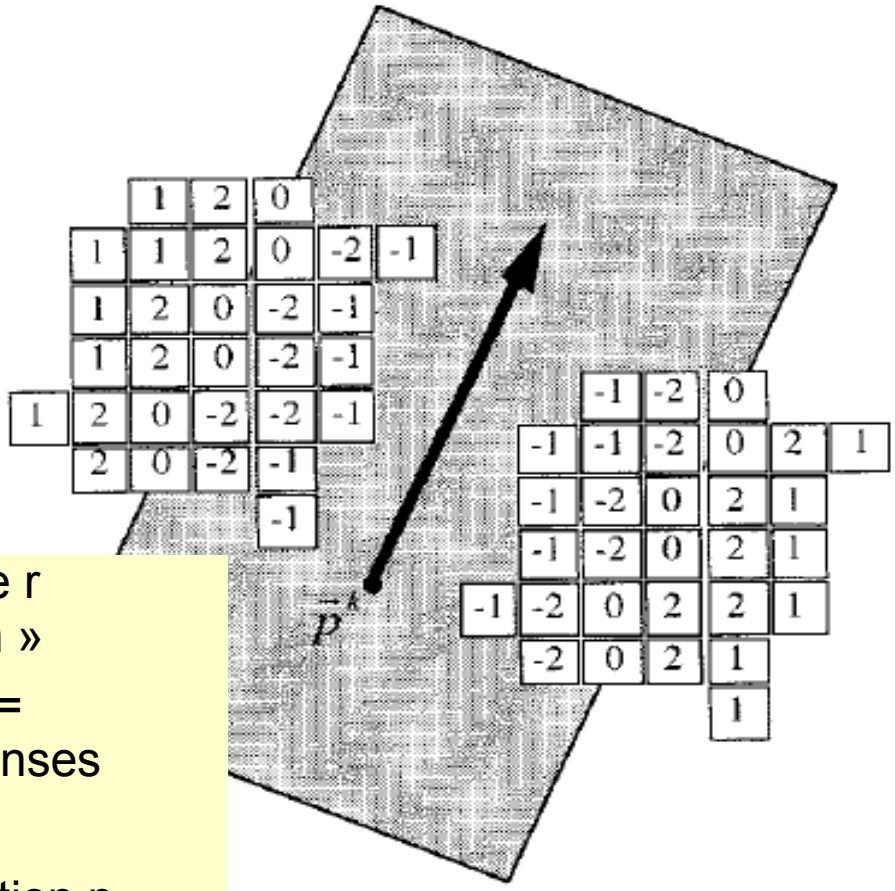
- similar problem as
  - neurite tracing
  - tracing of retinal vasculature

➔ use known algorithm from these fields

➔ direct exploratory tracing algorithm
  - ➔ Can et al. 1999
  - ➔ Y. Zhang et al. 2007

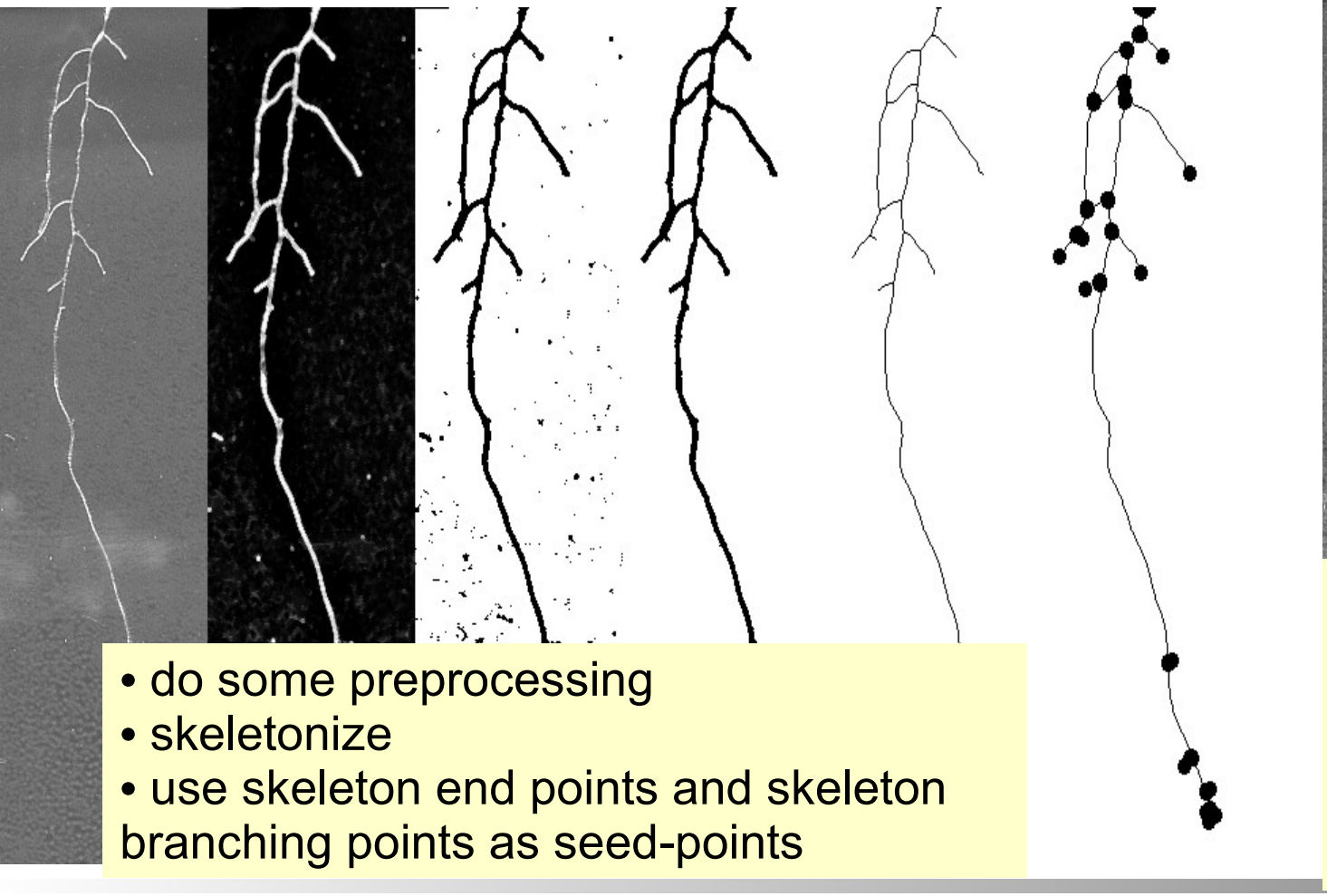
measuring roots - tracing

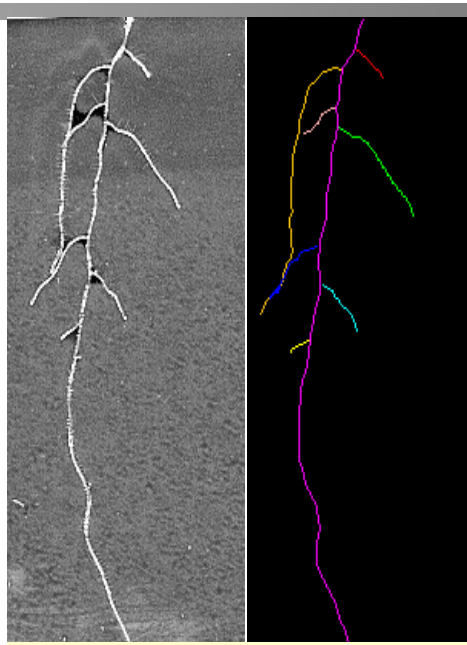




- move each template r pixel « up and down »
- find filament edges = best template responses
- adjust center point
- go one step in direction p

measuring roots – preprocessing and seed points



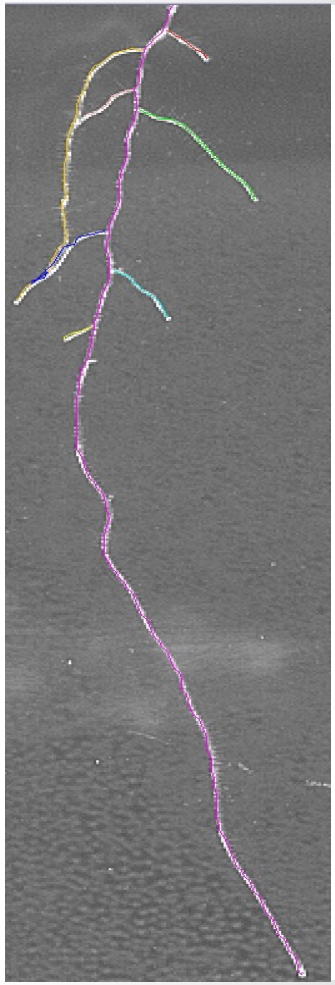


- do some preprocessing
- skeletonize
- use skeleton end points and skeleton branching points as seed-points

before tracing:

do morphological contrast enhance

measuring roots – results



possible applications

- measure total length
- measure main root

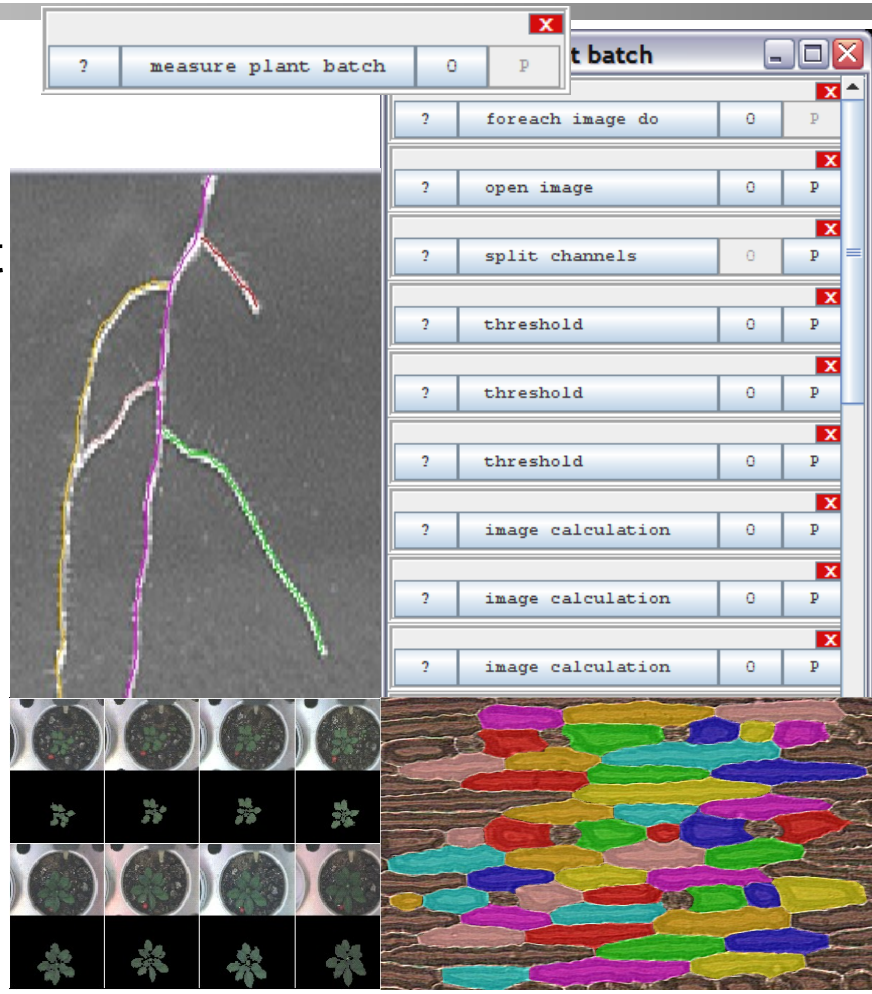
remaining problems

- stop conditions
- overlapping filaments
  - reconstruct tree structure
  - measure second order root lengths

summary and outlook

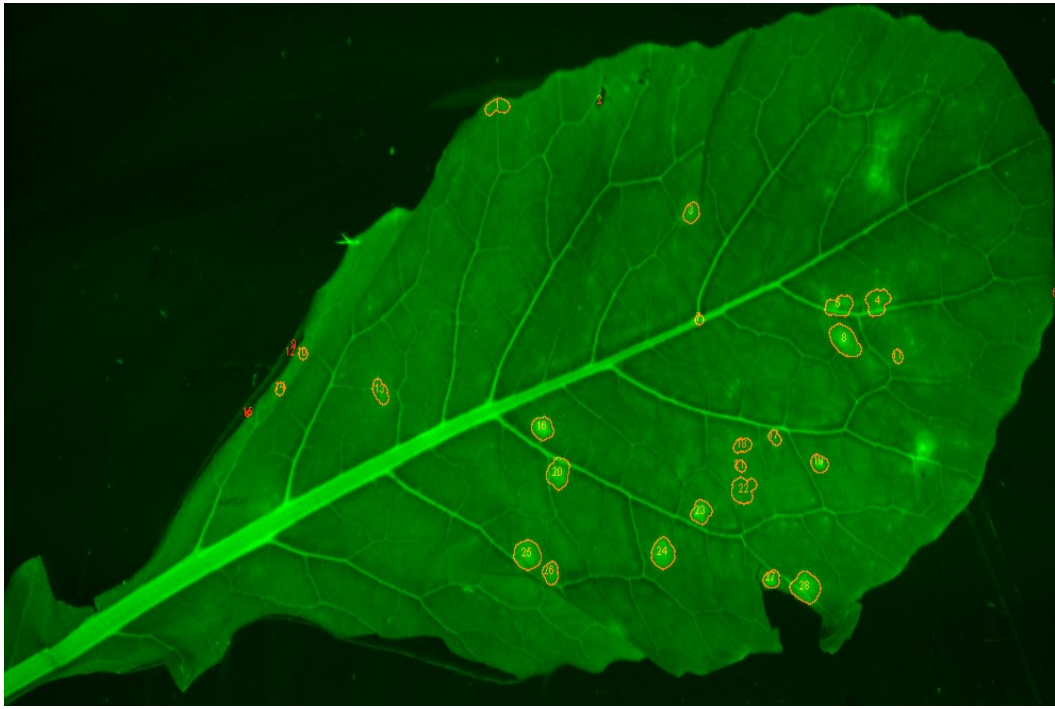
summary

- image analysis is the bottleneck
- MRI Cell Image Analyzer
  - a rapid prototyping environment for image analysis applications
  - visual scripting
- on demand development of image analysis applications
- plant growth applications:
  - measure rosettes of Arabidopsis plants
  - measure cells in epidermis
  - measure roots



outlook

- refine the existing applications
  - cells in epidermis
  - measure roots
- new applications
  - measure leaves
  - measure infections
  - ...
- applications for other techniques
  - infrared images
  - ...



the last slide

Thank you!

? Questions ?

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Literature

- Cell Image Analyzer - A visual scripting interface for ImageJ and its usage at the microscopy facility Montpellier RIO Imaging, Volker Baecker and Pierre Travo, in Proceedings of the ImageJ User and Developer Conference, 2006, Edition 1, p. 105-110, Centre de Recherche Public Henri Tudor, Andreas Jahnen, Christian Moll, 29, Avenue John F. Kennedy, L-1855 Luxembourg, ISBN: 2-919941-01-1, EAN : 9782919941018
- Cross JM, von Korff M, Altmann T, Bartzetko L, Sulpice R, Gibon Y, Palacios N, Stitt M, Variation of enzyme activities and metabolite levels in 24 Arabidopsis accessions growing in carbon-limited conditions, Plant Physiol. 2006 Dec;142(4):1574-88.
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Literature

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- Threshold Color plugin for ImageJ: http://www.dentistry.bham.ac.uk/landinig/software/software.html
- Watershed plugin for ImageJ: http://bigwww.epfl.ch/sage/soft/watershed/
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