



**Using digital image analysis to
integrate mathematics and
computers into inquiry
laboratories**

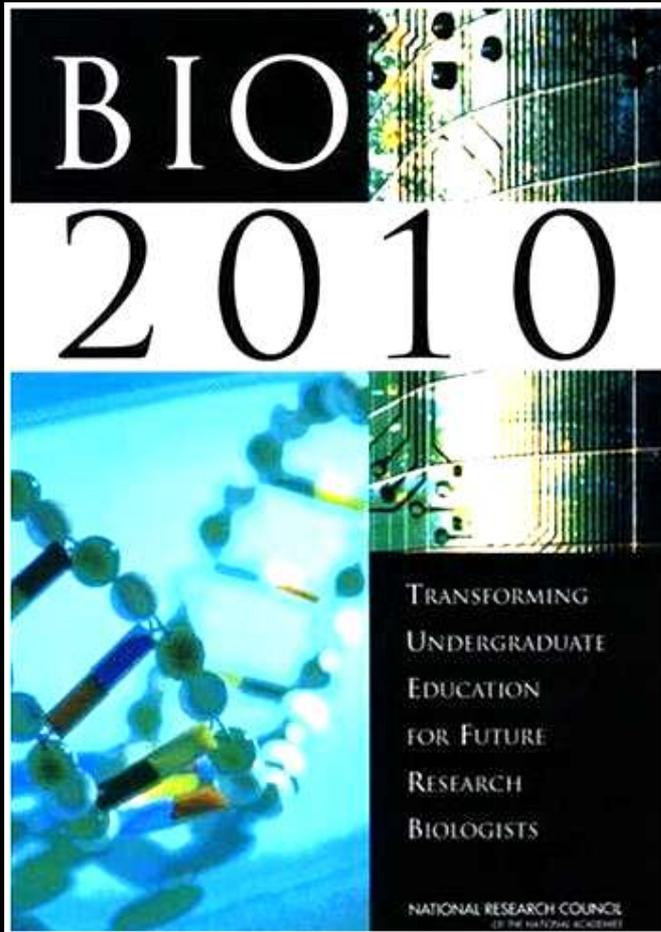
Stokes S. Baker, MS, PhD

Karen Selby, PhD.

Departments of Biology and Education

University of Detroit Mercy

Executive Summary



Supports NRC Bio 2010 recommendations to improve undergraduate curriculum for the next generation of research scientists

1. Increase quantitative skills
2. Increase competence with computers
3. **MCAT and DAT are barriers**

National Research Council. 2003. Bio2010: Transforming Undergraduate Education for Future Research Biologists. National Academy Press, Washington, D.C

Inquiry laboratories can fulfill Bio2010

Inquiry* involves:

- **Students experience the whole cycle of the scientific method**



* National Research Council. 1996. From Analysis to Action: Undergraduate Education in Science, Mathematics, Engineering, and Technology. Washington, D.C.: National Academy Press.

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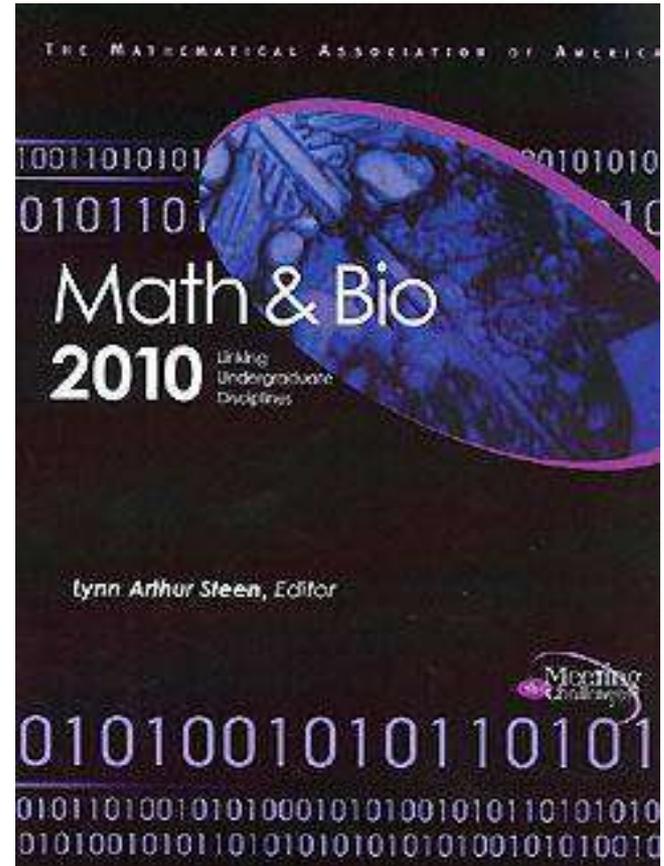
- Students experience the whole cycle of the scientific method**
- Students design & execute their own experiments**
- can mathematics and computer skills in data acquisition and analyze**



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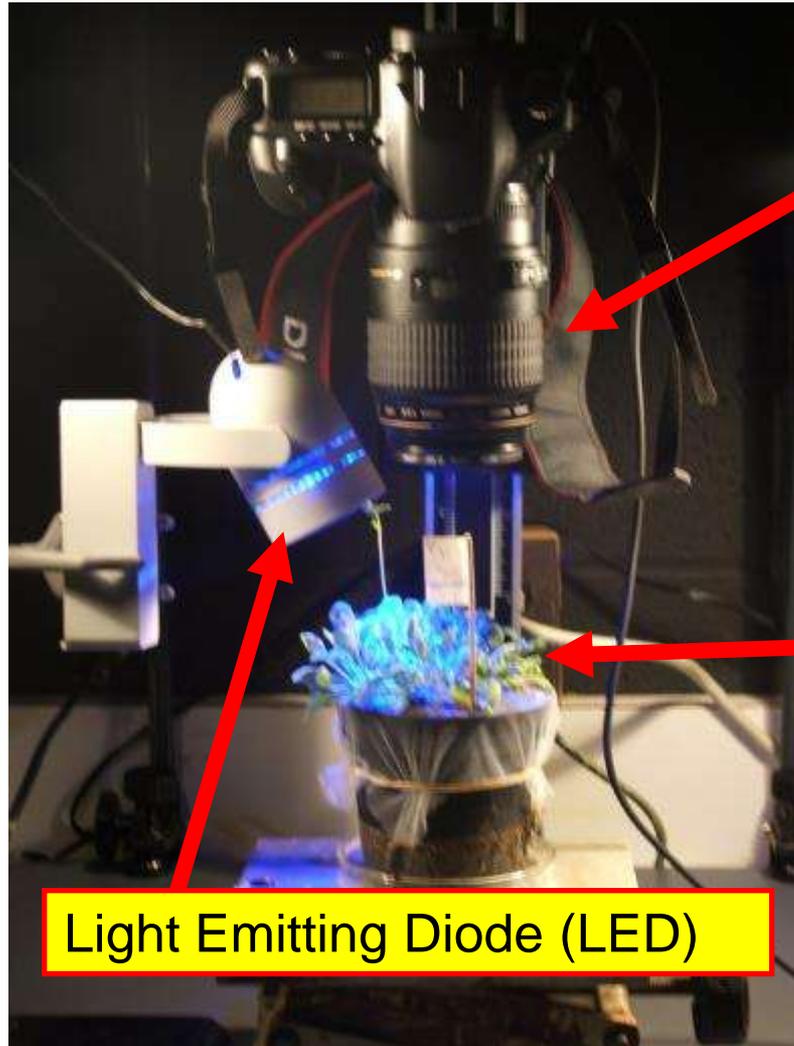
How to overcome the barriers?

- Pre-health professions entrance exams:
 - Integrate mathematics in pre-existing courses



HYDORN, D., S. S. BAKER, AND J. BOATS. 2005. Quantitative initiatives in college biology: Profiles of projects at undergraduate institutions. *In* L. A. Steen [ed.], *Math & Bio 2010: Linking Undergraduate Disciplines*. Mathematical Association of America, Washington, DC.

The digital age has made photographic data accessible

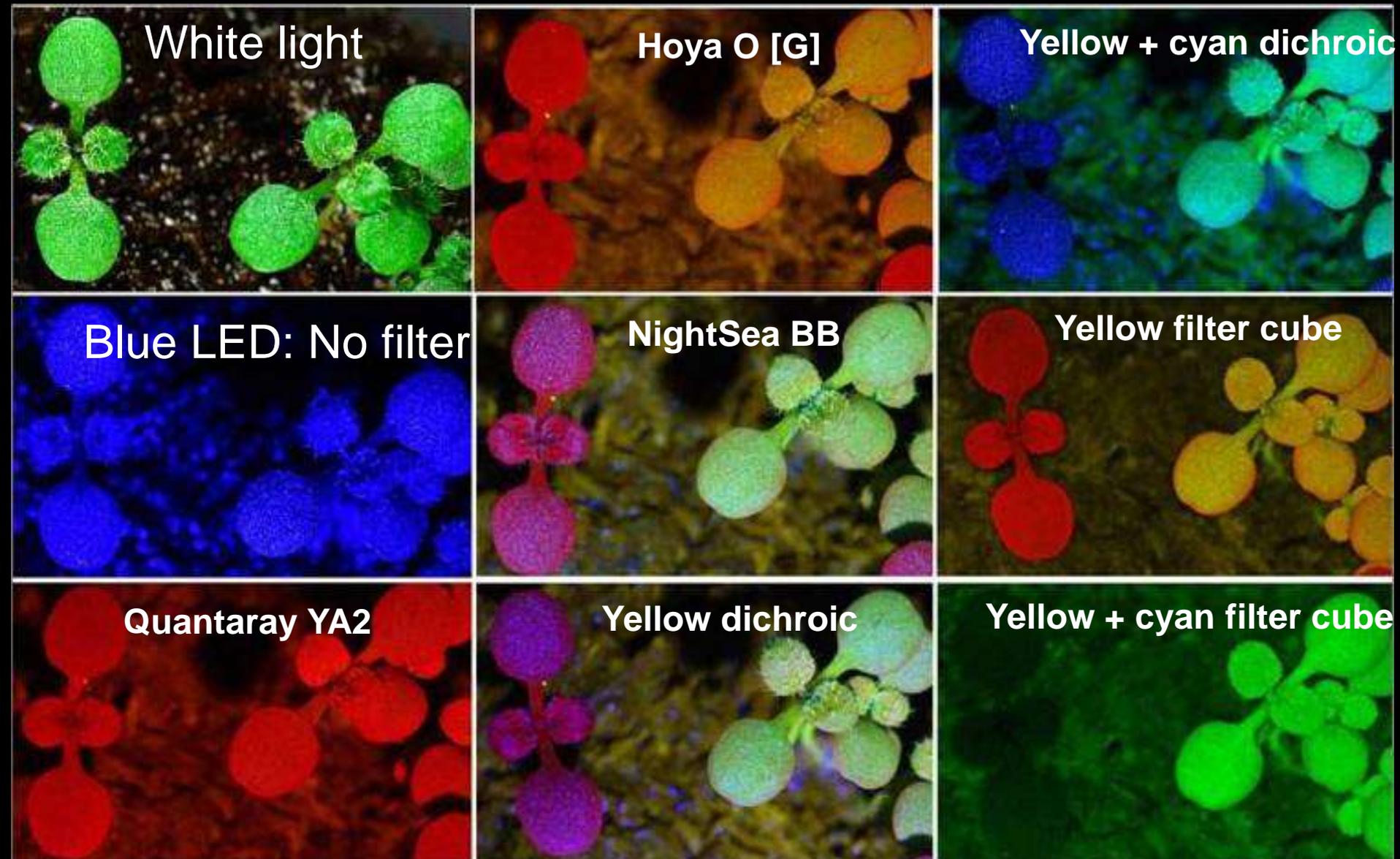


Macro Lens
for close-up
focusing

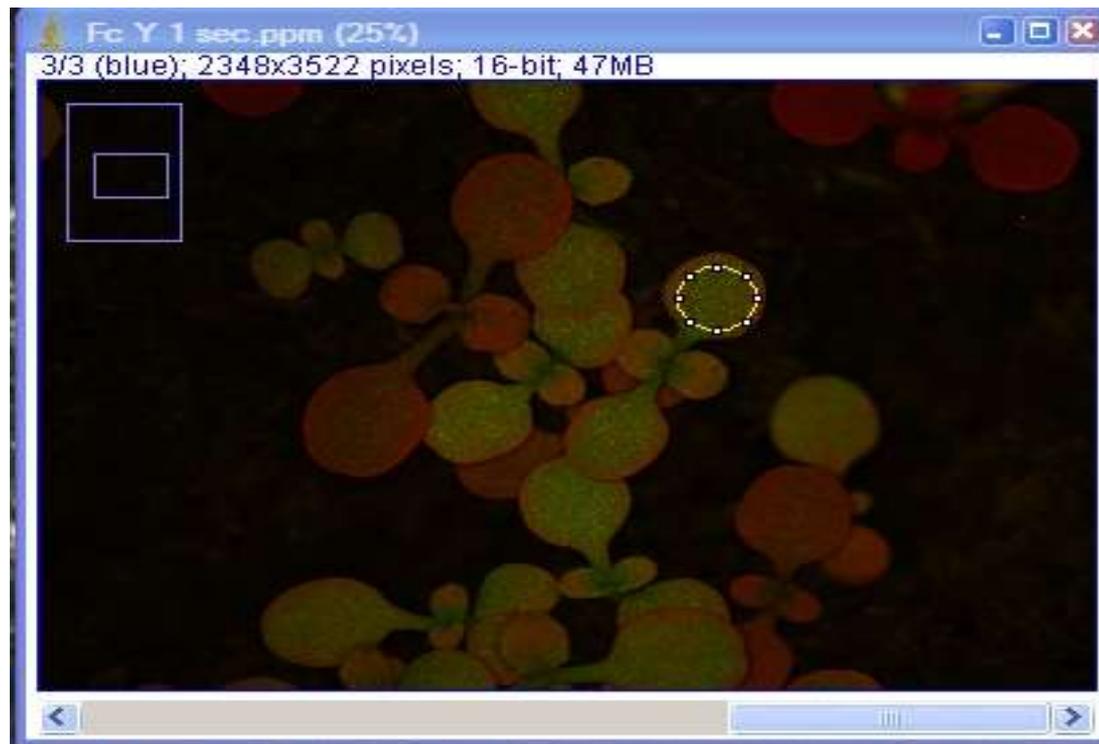
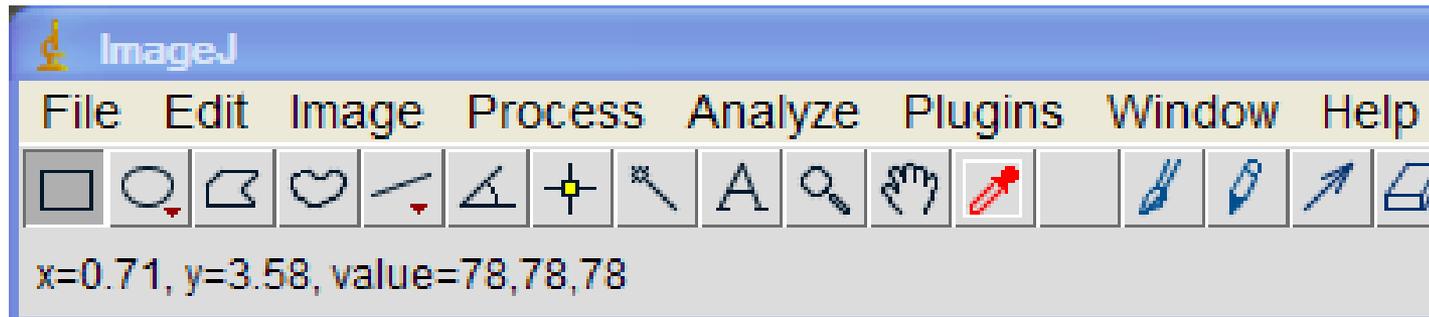
Plant
specimen

Light Emitting Diode (LED)

Images can provide a “hook” to peak the interest of students



Digital photograph allows for quantitative measurements using computer technology



ImageJ



- Provided by National Institutes of Health
<http://rsbweb.nih.gov/ij>
- Platform independent: Written in Java
- Can read many formats including TIFF and JPG
- Capabilities extended with a number of plug-ins

ImageJ can measure:



- Lengths
- Areas
- Angles
- Number of particles
- Estimate time in videos
- Light intensities
- More!

Examples where digital photograph has been integrated into inquiry instruction

1. Investigating morphological response to the environment
2. Investigating anatomical adaptation in plants
3. Measuring GFP fluorescence in transgenic organisms

Ecological investigation using ImageJ

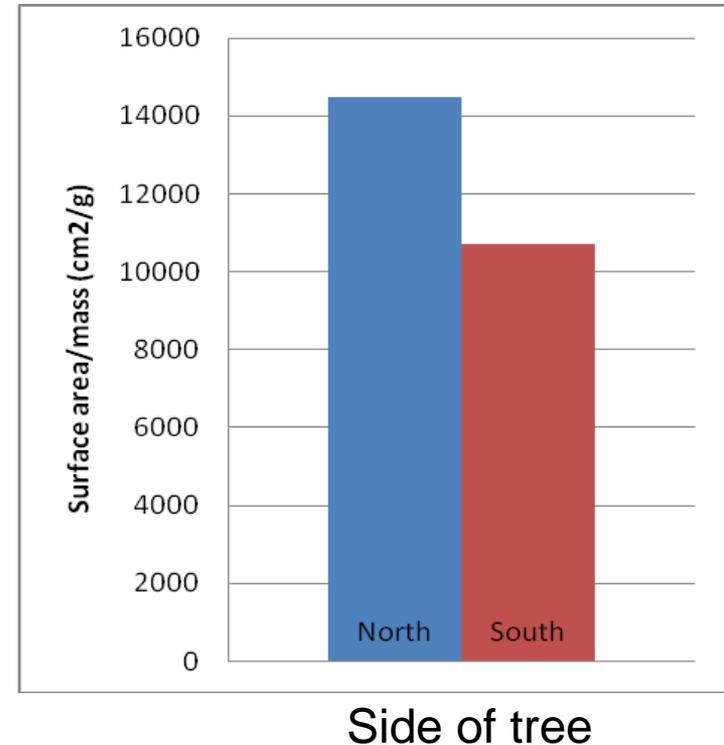
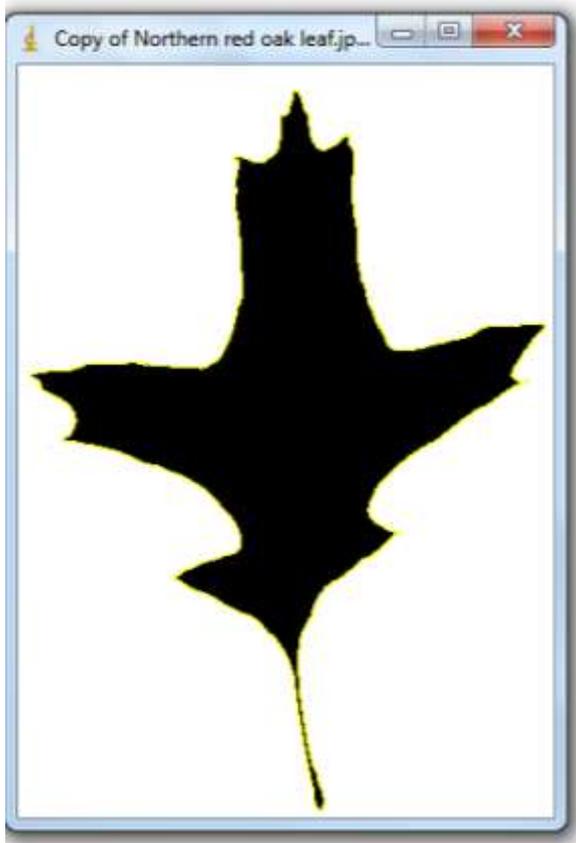
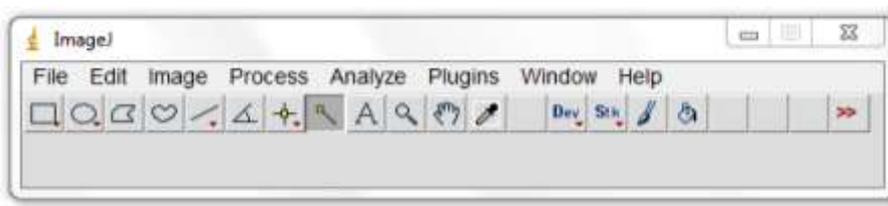


Fig. 7: Effect of tree aspect on average surface area to mass ratio of *Q. rubra* leaves on north and south sides $p = 0.01$, $t = -2.58$

Investigating involving stem anatomy to evaluate niche

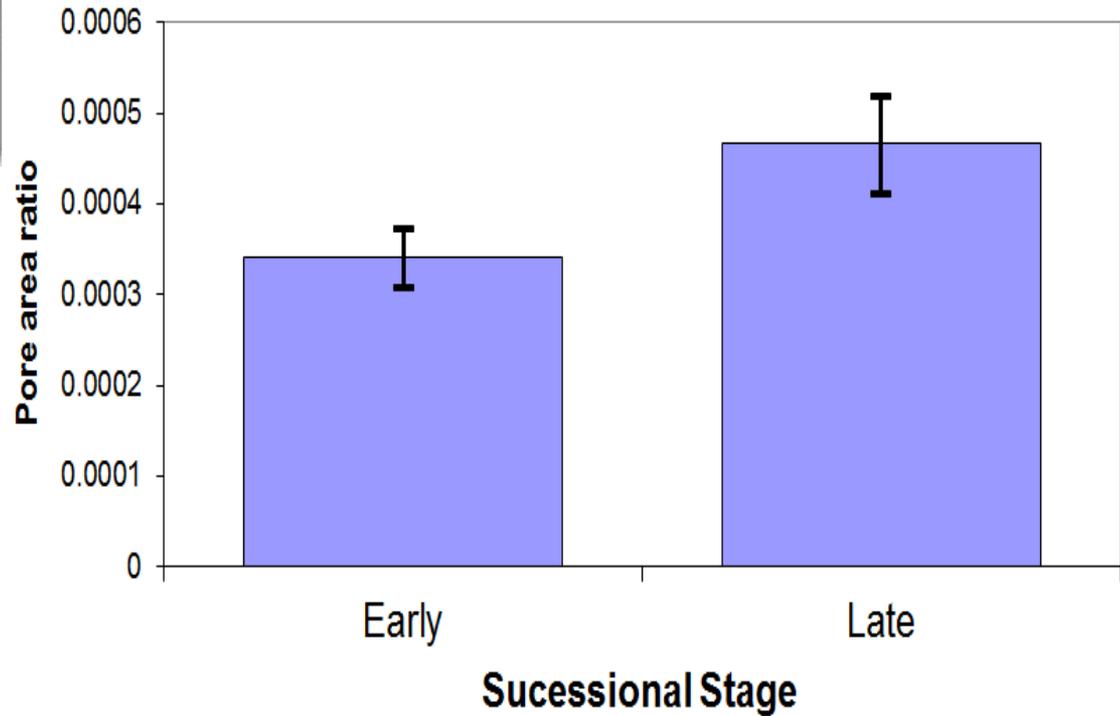
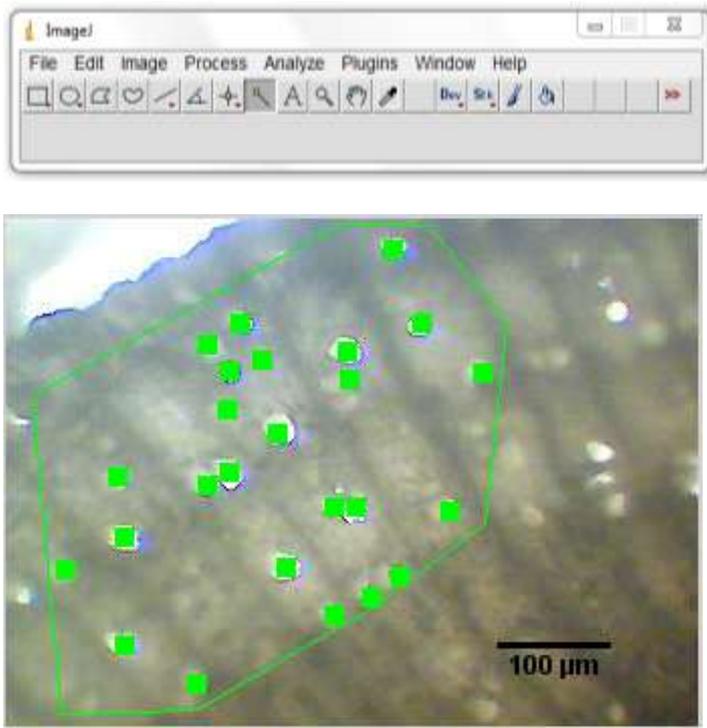


Figure 1: The effects of succession on total pore area to secondary xylem area ratio. Range bars are 95% confidence intervals.

The resulting time-lapse video

- Rapid-cycling *Brassica rapa* germination
- Petri plate contain simple mineral agar
- Time period 48 hours
- Video time is 20 seconds



Data can become quantitative

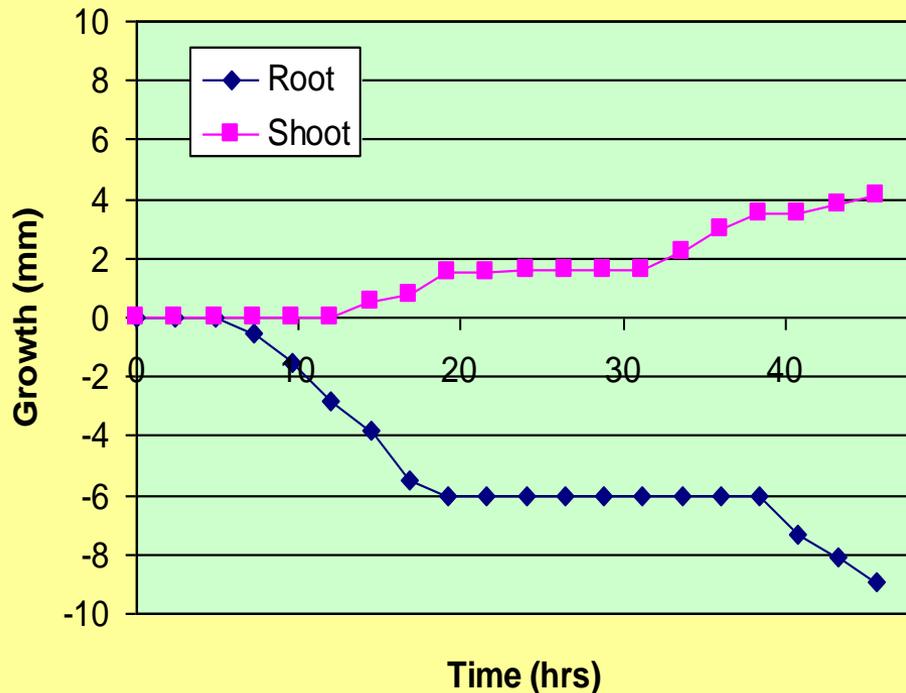
- Print out images at predetermine intervals
- Use ruler to measure growth of roots and shoots
- Convert frame time to plant growth time
- $(48 \text{ hr.} / 20 \text{ sec.}) = 2.4 \text{ hr./sec.}$



Frame time (sec.)	Germination time (hrs)	Root (mm)	Shoot (mm)
0	0	0	0
1	2.4	0	0
2	4.8	0	0
3	7.2	-0.5	0
4	9.6	-1.5	0
5	12	-2.8	0

Data can be quantitative

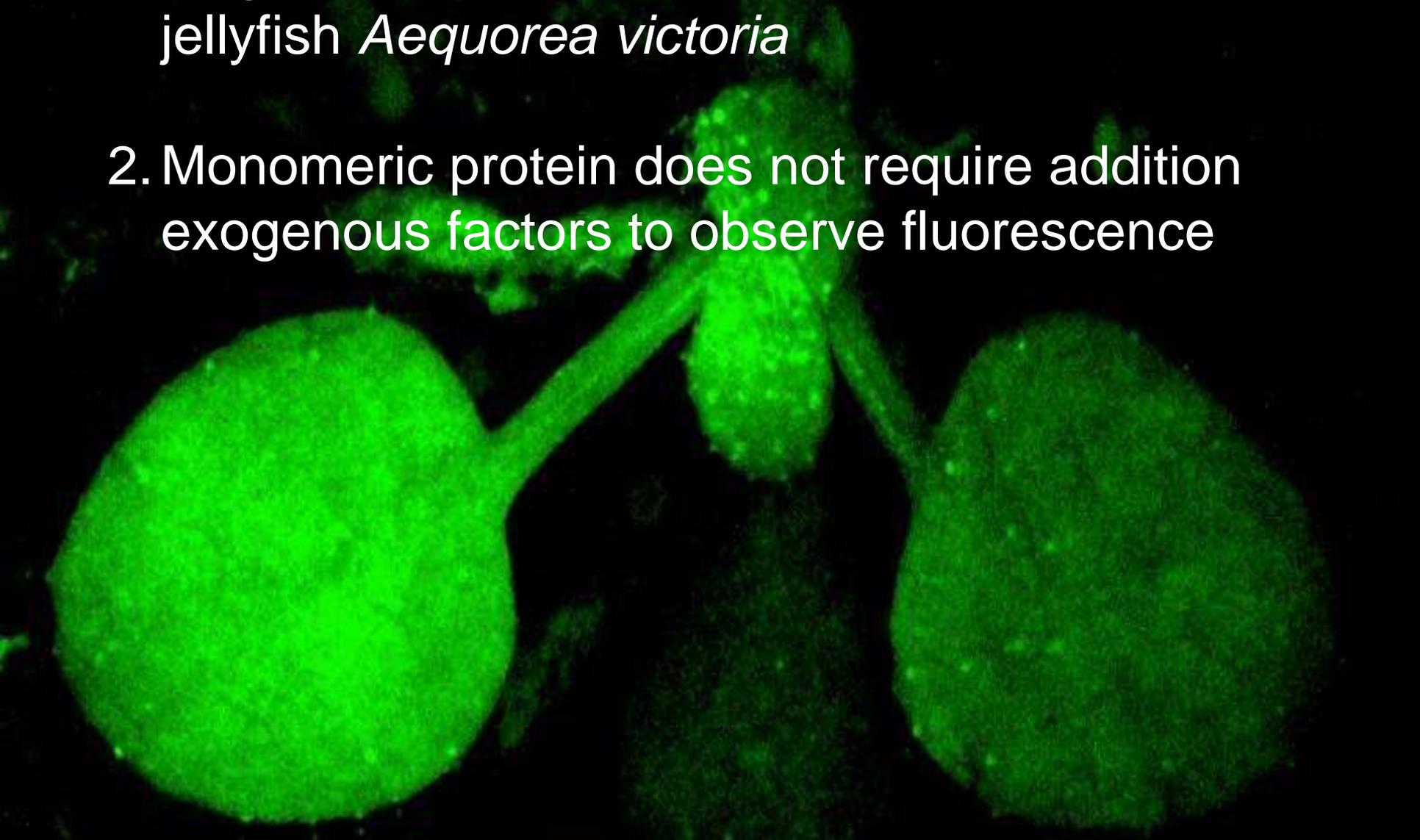
Growth during germination



- Simple experiments can show surprises
- Roots grow faster than shoots: Why? An adaptation?
- Plant growth is in spurts. Why?
- Allows development of future experiments
- Can meet national and state K-12 instruction standard.

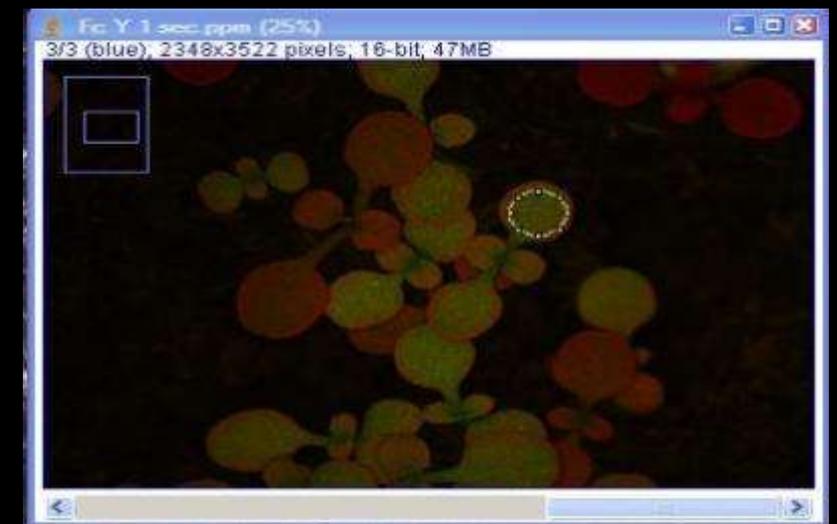
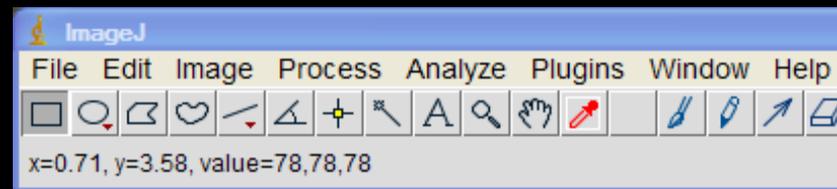
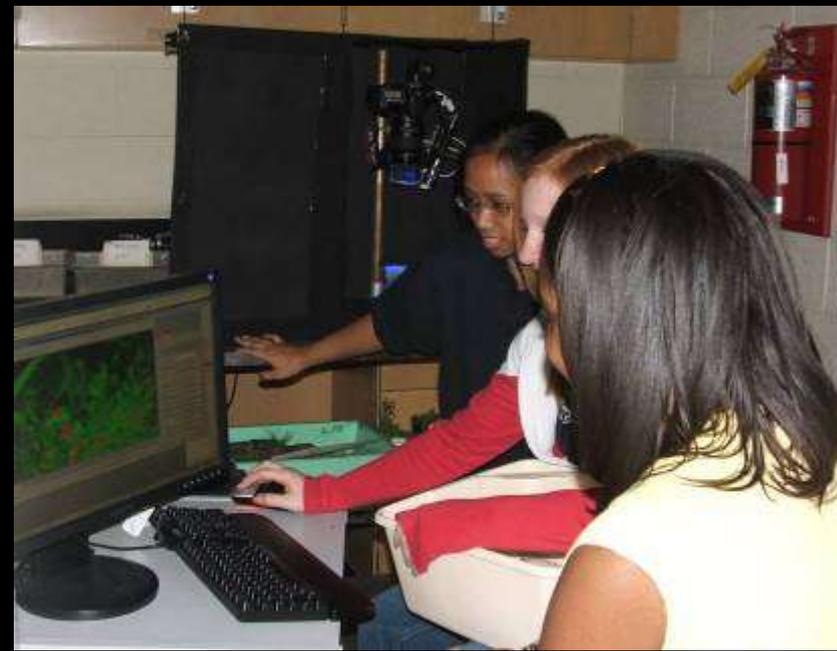
Green Fluorescent Protein

1. Originally cloned from the bioluminescent jellyfish *Aequorea victoria*
2. Monomeric protein does not require addition exogenous factors to observe fluorescence

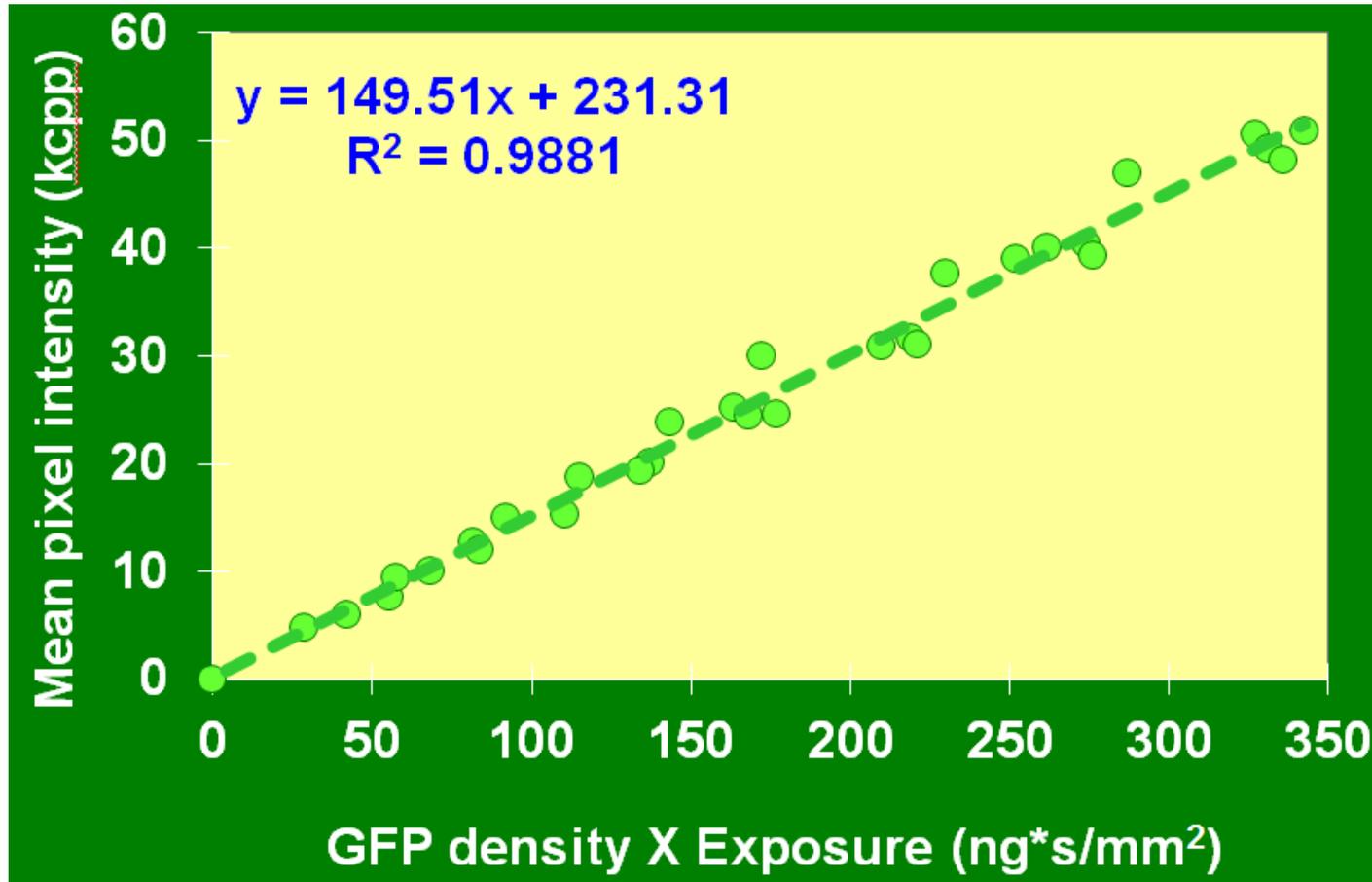


GFP's Advantages

- Quantitative assay.
- Real-time data collection with digital camera's:
No histochemistry!
- Allows for *in-vivo* detection:
 - * **Greater array of experiments possible.**
 - * **Supporting inquiry**
- Allow for the integration of computers:
Fulfills one of the goals of Bio2010.
- **Downside: Expensive instrumentation**

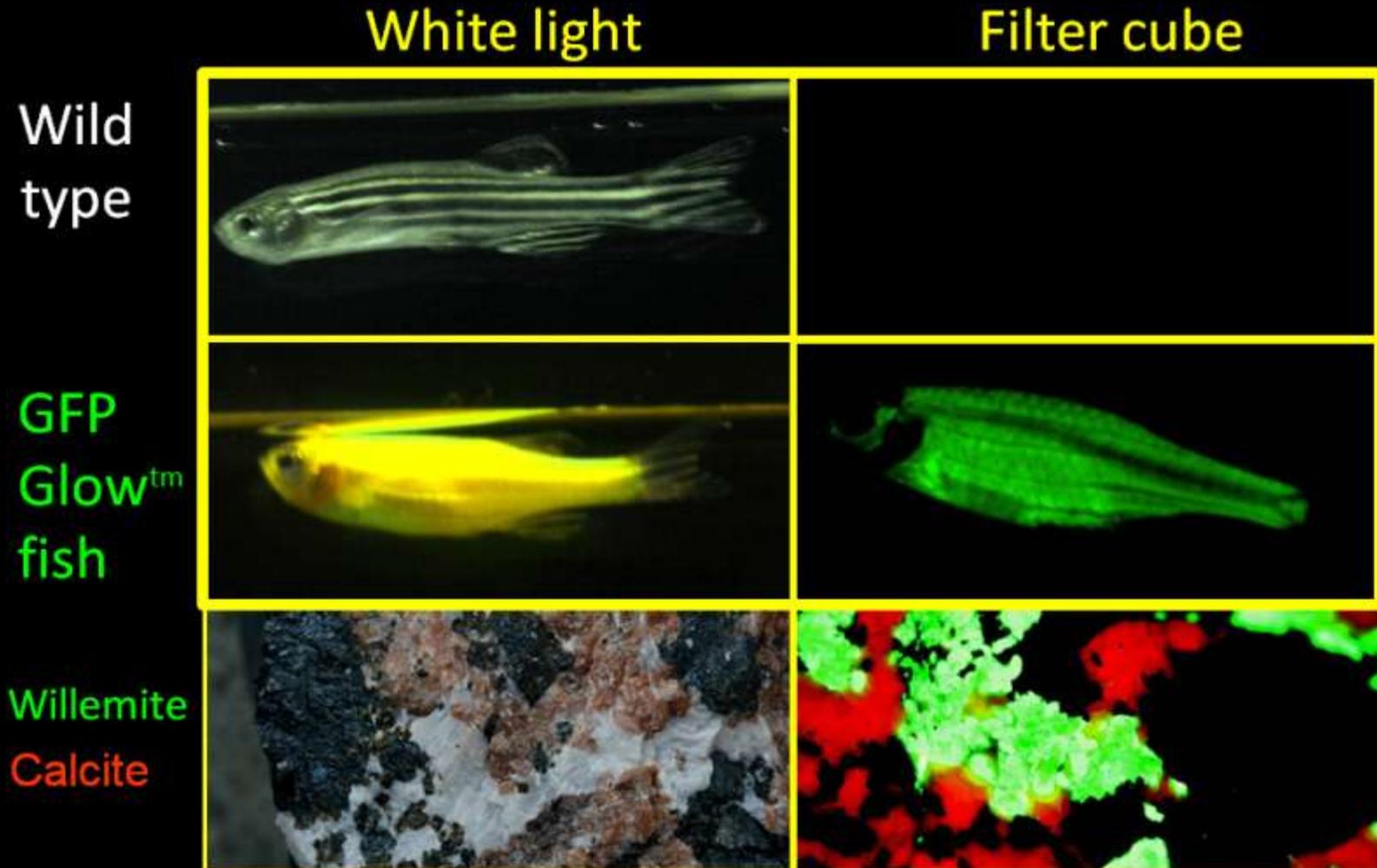


Consumer SRL cameras can be used as a quantitative instrument



Baker *et al.* 2012 doi:10.1093/aobpla/pls003

Epifluorescent camera attachment is a flexible experimental system



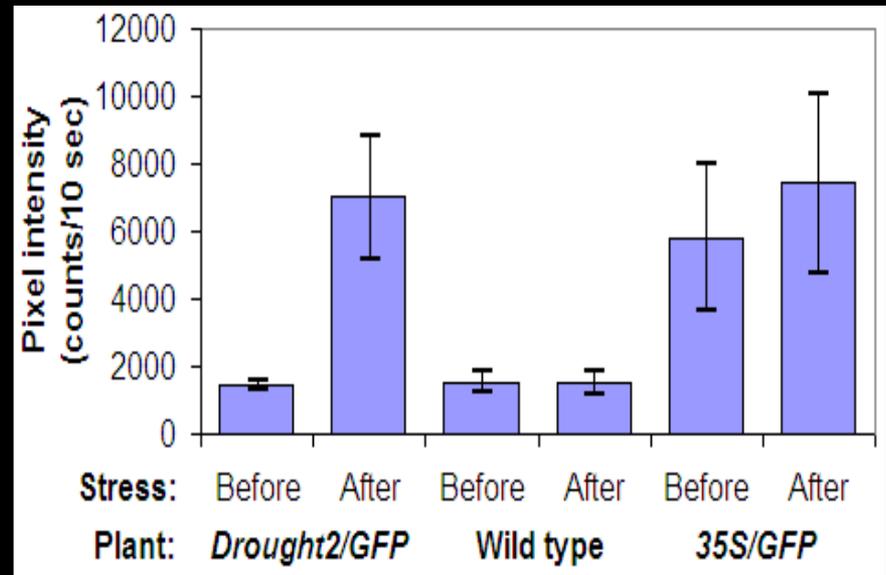
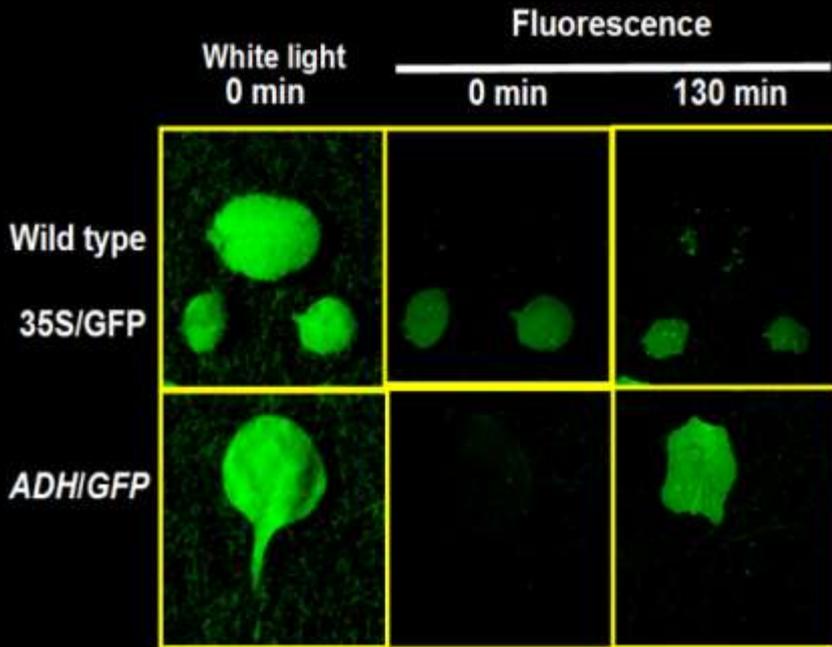
**Is it an effective
teaching tool?**

UDM General Biology Laboratory

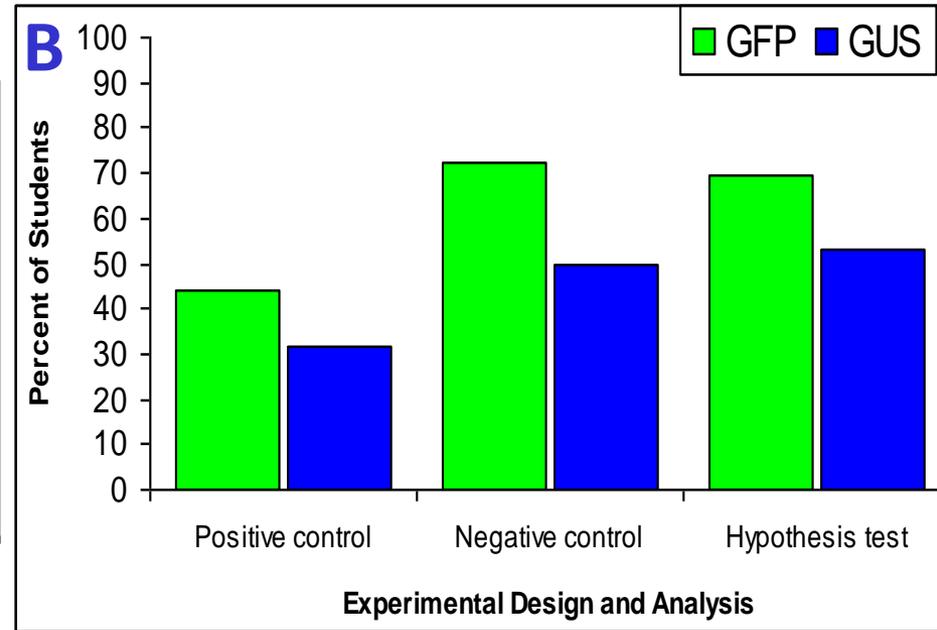
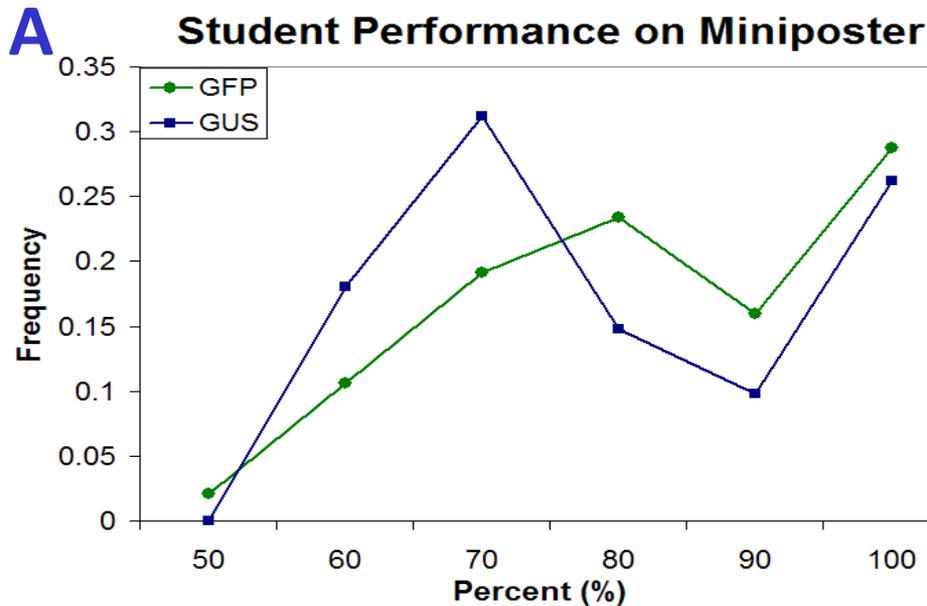
1. Students learned how to detect reporter gene (either **chemically** or **digitally**).
2. Students observed induced expression of *cor15a/gusA* (**qualitative assay**) or *ADH/sGFP* (**quantitative assay**).
3. Students performed growth study involving abiotic stresses: **Used 95% confidence intervals.**
4. Assigned into cooperative learning groups:
Designed controlled experiment **to determine what environmental factor controlled the expression of the reporter gene.**
5. Groups executed experiments.
6. Individuals wrote “Miniposters”.

ADH/GFP chimeric

- ADH induced by several abiotic factor's like dehydration
- Demonstration experiment can be completed during single class period
- Procedure highly quantitative



Analysis of grading rubric



Panel A: Proportion of students with desirable scores (>70%) was statistically significant ($\chi^2 = 4.65$, $p = 0.031$).

Panel B: Proportion of students that used:

- positive controls ($\chi^2 = 2.29$, $p = 0.130$)
- negative controls ($\chi^2 = 7.92$, $p = 0.005$) to evaluate reporter gene expression
- a hypothesis test to evaluate data ($\chi^2 = 3.92$, $p = 0.048$)

Acknowledgements

Robert J. Ferl

Assistant Director of the Biotechnology Program
Professor Department of Horticultural Sciences
University of Florida



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Course Curriculum and Laboratory
Improvement
Award numbers 0442771 and
9952366

Question for the discussion

What sort of inquiry investigations can you perform:

- With digital images?
- With fluorescence?
- With ImageJ?

How can you:

- Develop students' computer skills?
- Improve mathematical thinking?