A Radically Inexpensive CMOS- and USB-based Inverted Fluorescence Microscope

for exploring medicine and science at a small scale

Brian Rasnow

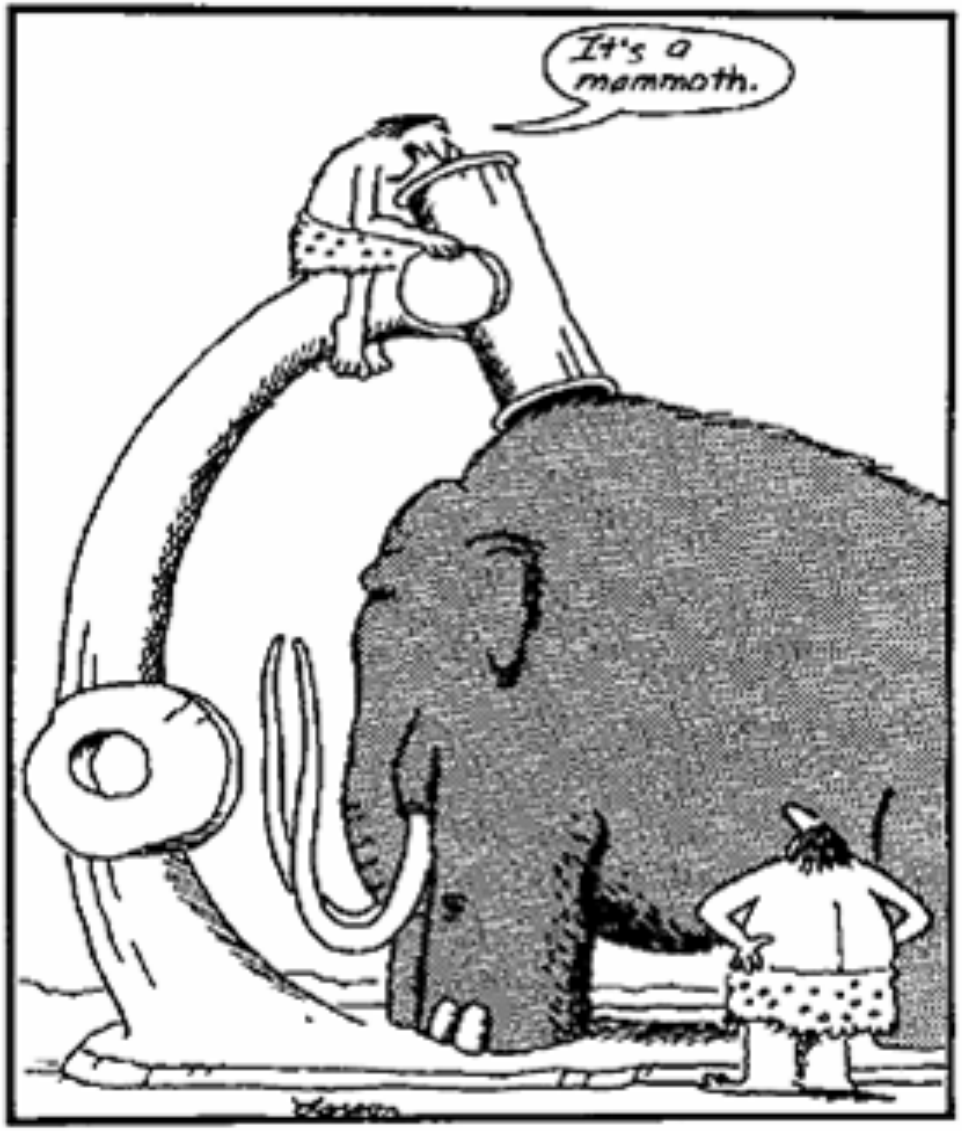
California State University Channel Islands

Etaluma Inc.

brasnow@etaluma.com

The first microscope

- The microscope was invented by many of the same people as the telescope, e.g., Galileo, Hooke, Abbe, Zeiss
- As with the telescope, it revealed new worlds, and profoundly changed our sense of place in our world
  - Pathogens
  - Cells



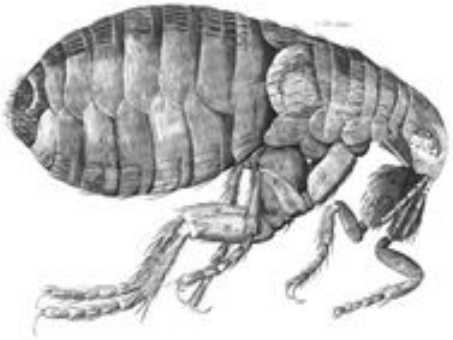
Microscope Evolution

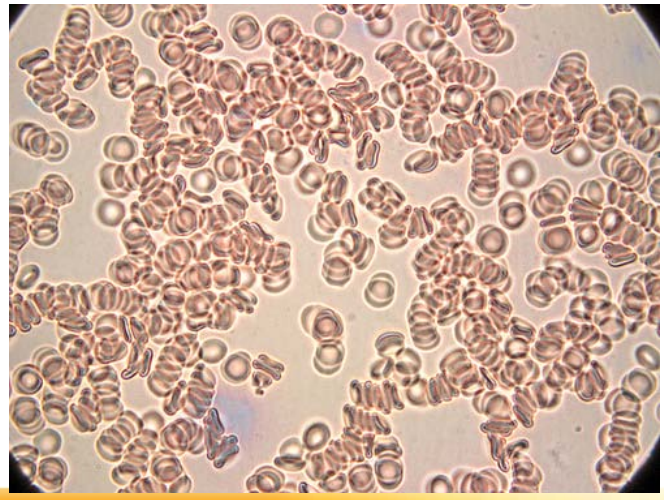
etaluma

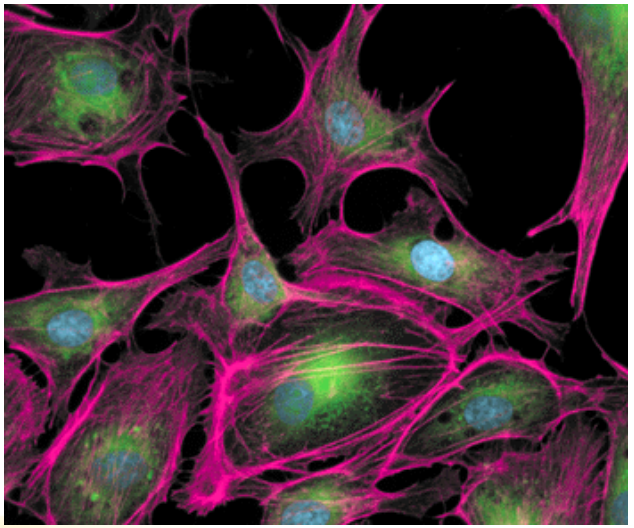
~1660

~1950

~1995







Problem statement

etaluma

- Fluorescence microscopy has become an indispensable tool for medical diagnosis and research
- The prices of modern microscopes are out of reach of many potential users
  - And they are complicated to use and maintain

⇒*How can we make a radically cheap, simple fluorescence microscope suitable for the developing world?*

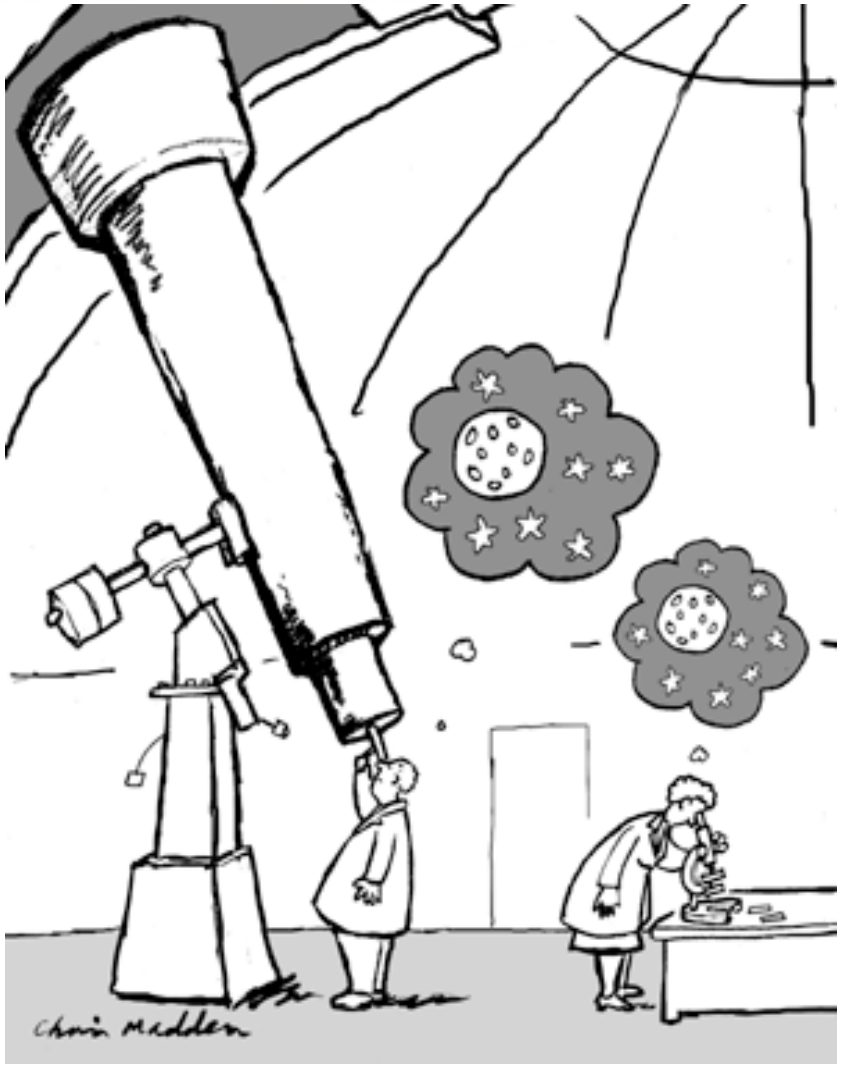
Etaluma's founding

- Etaluma* was formed in Feb. 2009 to solve this problem
- We quickly realized a radically inexpensive digital microscope has broad applications in research, science education, as well as point-of-care medicine

* "Etaluma" means tiny light in Esperanto

Microscopes need to

- Magnify
  - Trade-off between *depth of field* & magnification
  - Trade-off between *field of view* & magnification
  - Resolution fundamentally limited to ~1/3 micron
- Provide contrast
  - Thin samples are nearly transparent
  - Contrast enhancement from subtle changes in refractive index, color, and molecular markers

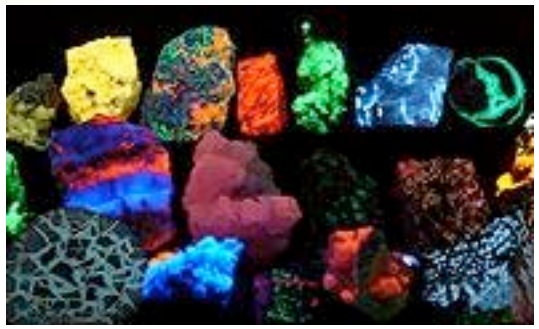


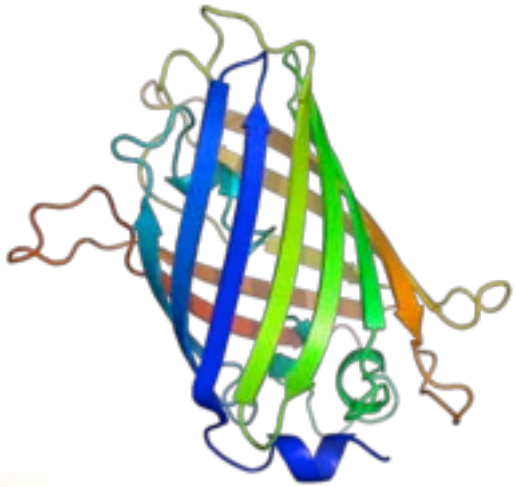
A microscope is a backwards telescope

Fluorescence

- **Fluorescence** is emission of light of a different wavelength than was absorbed.
- Fluorescence is rare in nature. Fluorescent molecules are thus useful labels when endowed with selective affinities
- Fluorescence revolutionized biology with the cloning of green fluorescent protein, which converts blue light to green
- The GFP gene can be coupled to other genes, rendering the “fusion protein” both visible and functional

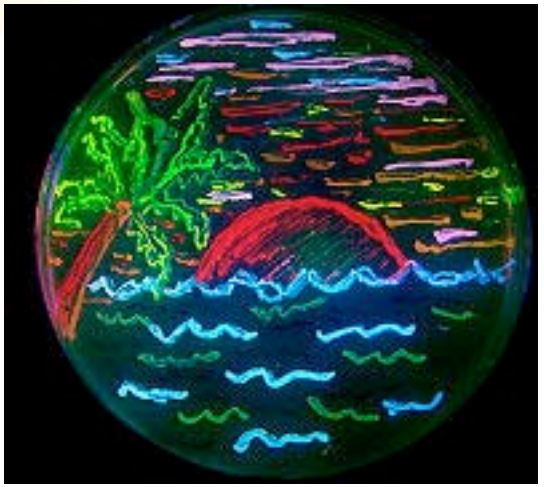
- Note: the jellyfish population that gave us GFP is in decline. There is great value in protecting biodiversity.



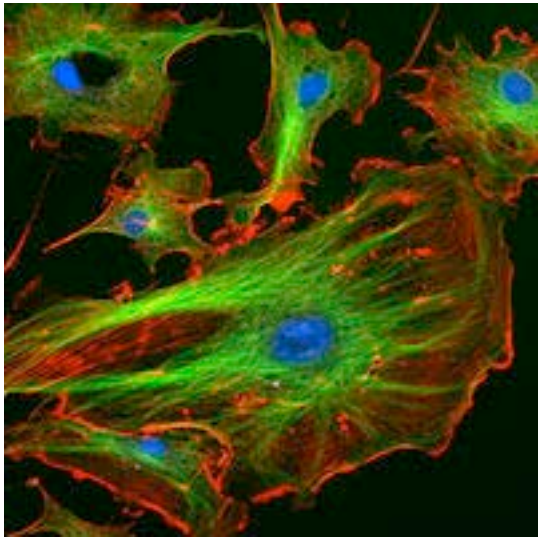


Fluorescence imaging

- Without fluorescence, phase contrast, and other optical tricks, most biology would be flat gray or invisible.
- GFP mutations enable visual colocalization of ~10nm molecules to <1 μm.
- Fluorescence resonance energy transfer (FRET) can reveal differential intermolecular distances <~10nm.
- Such molecular tools enable more specific and sensitive diagnostics



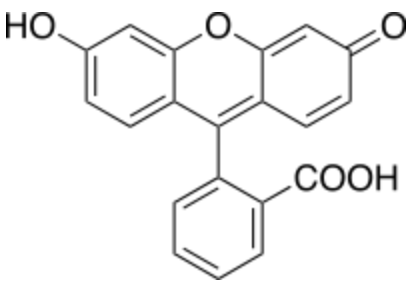
Bacteria expressing FP mutants

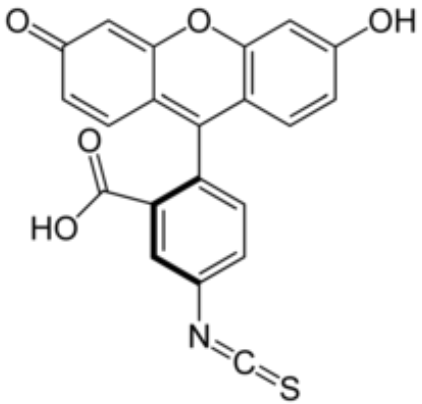


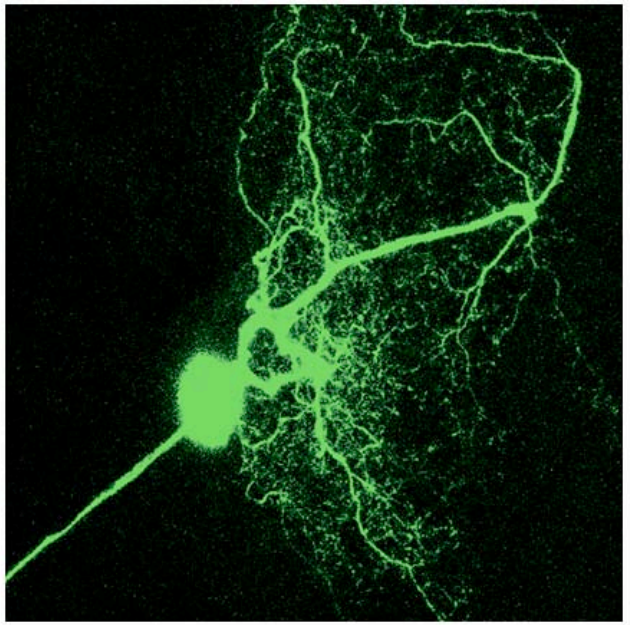
Cells labeled with 3 fluorescent antibodies

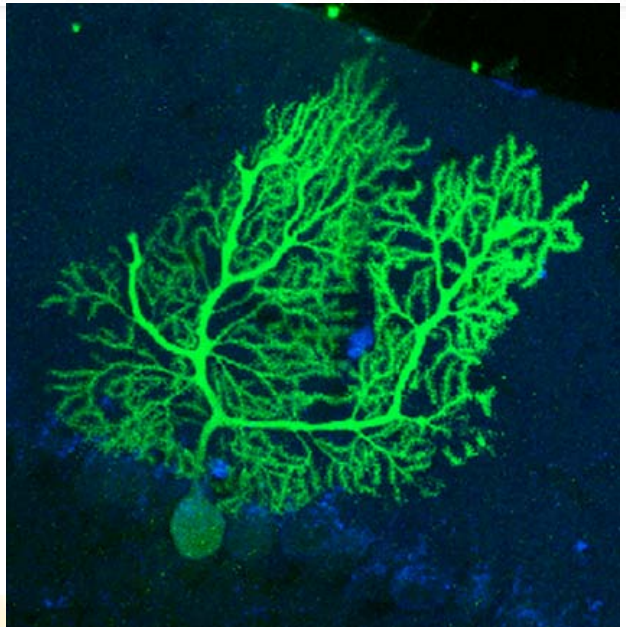
Fluorescein

- Each fluorescein molecule can convert a million blue photons to green photons in one second
- Related compounds like fluorescein isothiocyanate (FITC) easily bind to proteins
- A common infectious bacteria, *Pseudomonas aeruginosa* secretes fluorescein





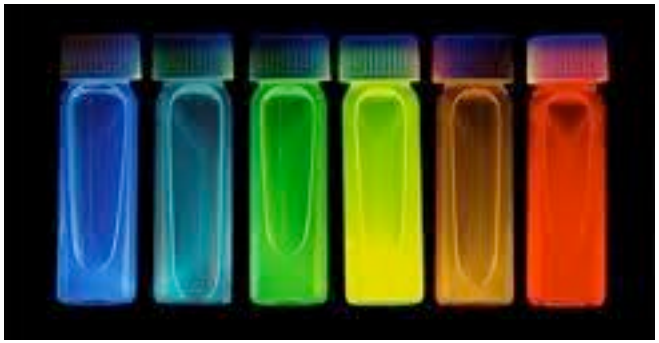


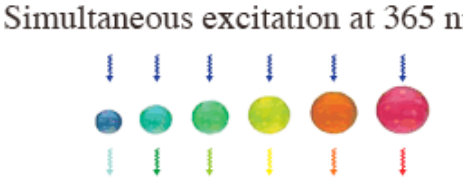


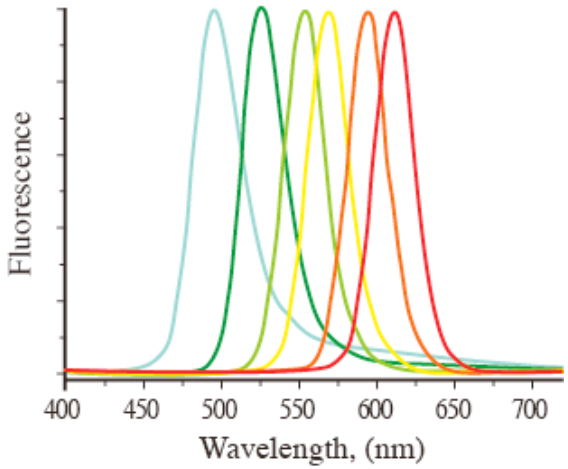
Quantum dots

etaluma

- Semiconductor nanoparticles (~10nm, 10 atoms across) exhibiting size-dependent fluorescent emission
- Can be brighter and more stable than organic fluorophores
  - ➔ May enable more robust and sensitive assays
- Much greater Stokes shift and narrower emission spectra
  - ➔ May enable multiplexing with a color camera and cheaper filters



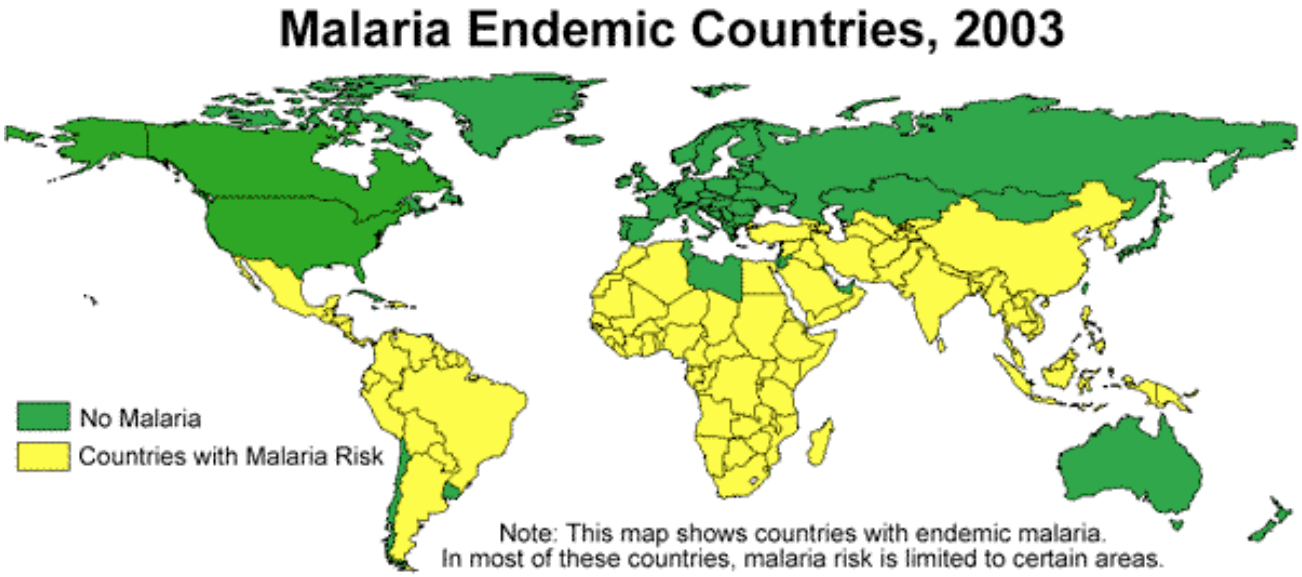


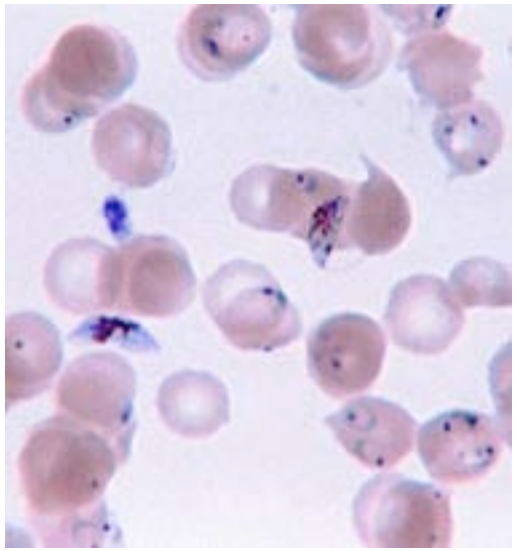


Why am I sick?

etaluma

- Malaria infects 225 million people
- The parasite can be seen in blood with suitable contrast enhancement
  - Red blood cells lack chromosomes so dsDNA stains in RBCs indicate a parasite

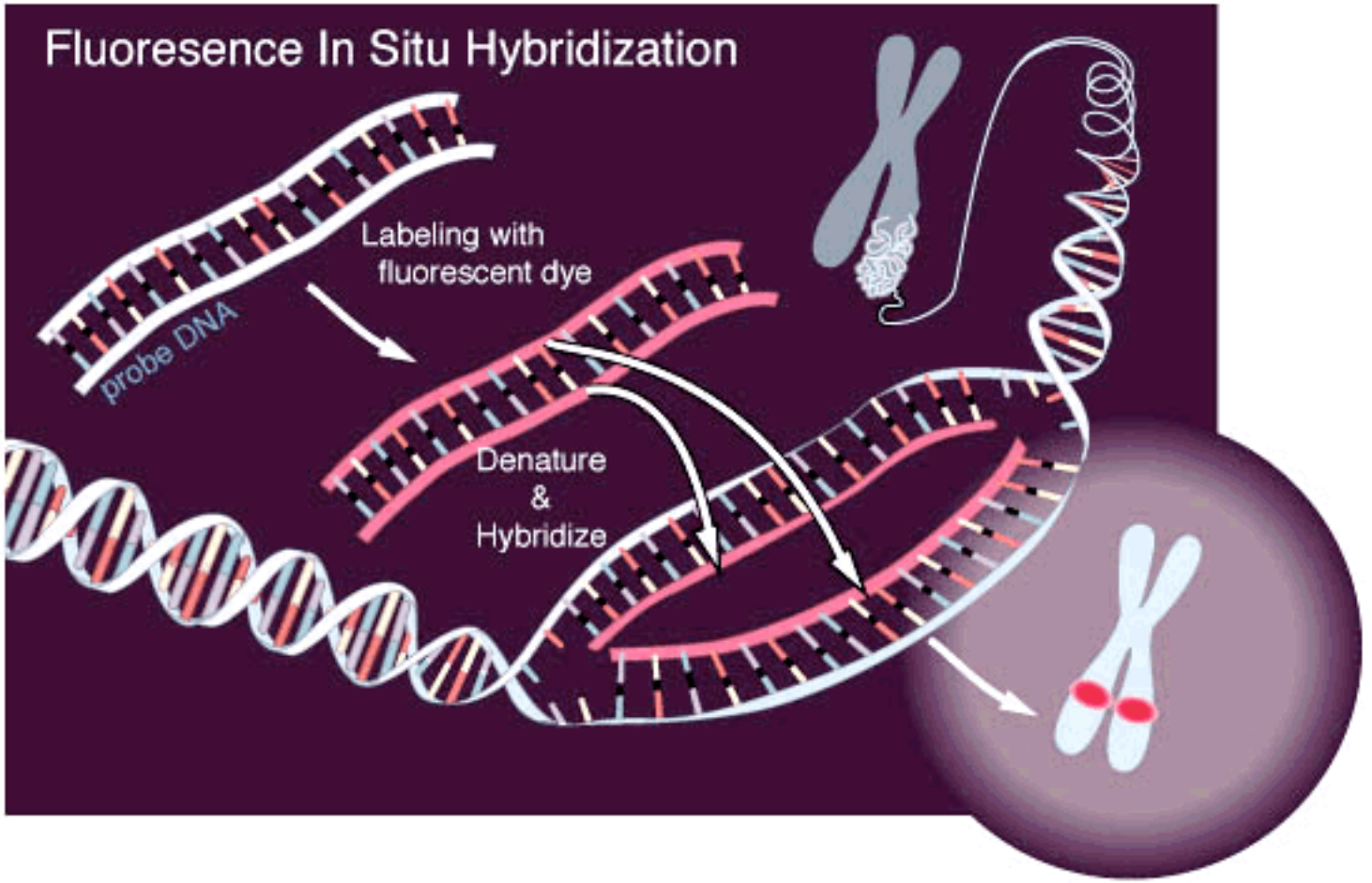




Fluorescence In situ Hybridization

etaluma

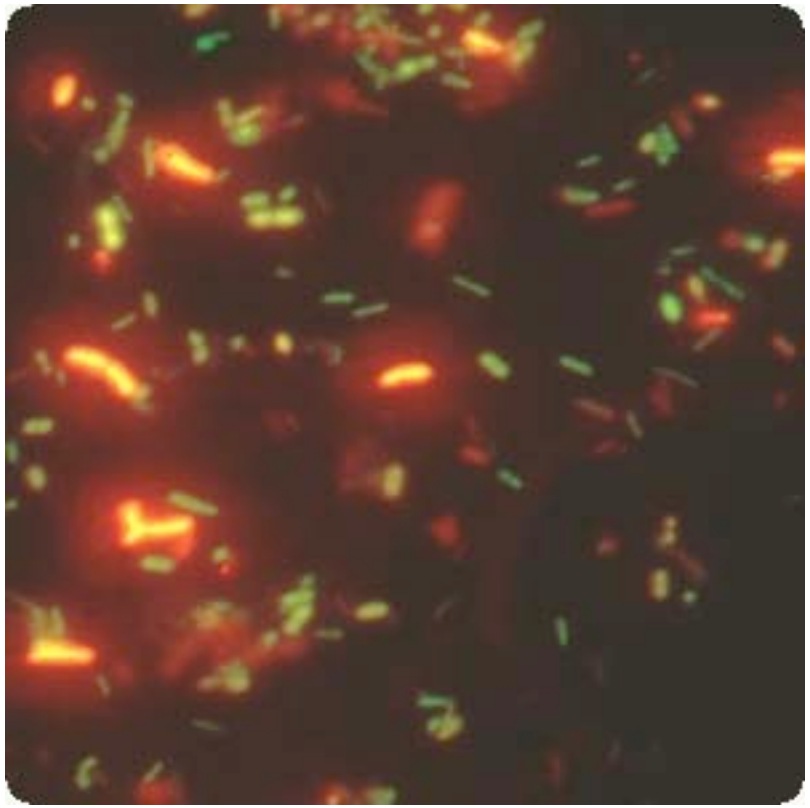
- FISH is a powerful generic method for detecting specific DNA sequences and their location in the cell



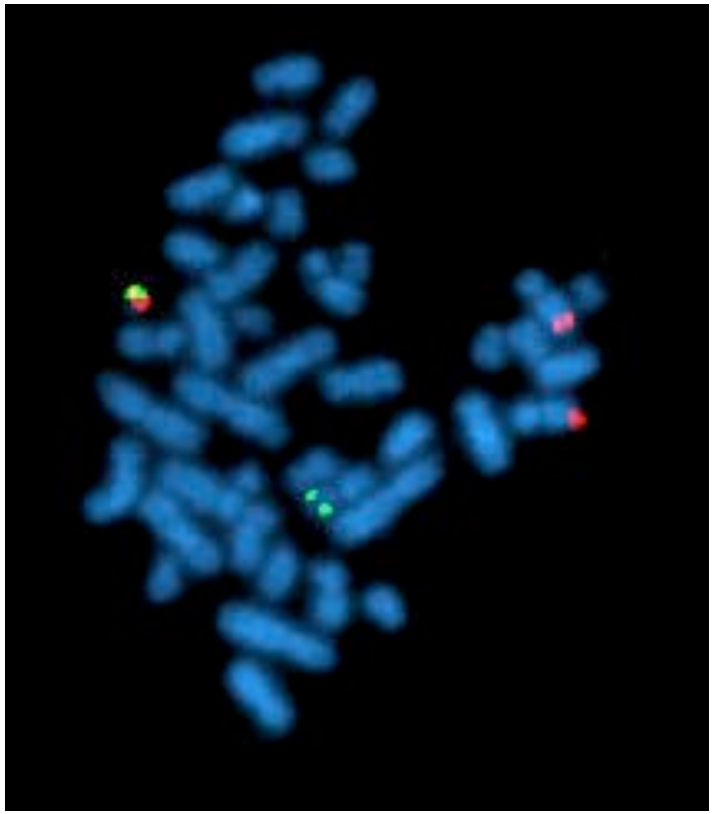
FISH images

etaluma

- Taken with conventional fluorescence microscopes



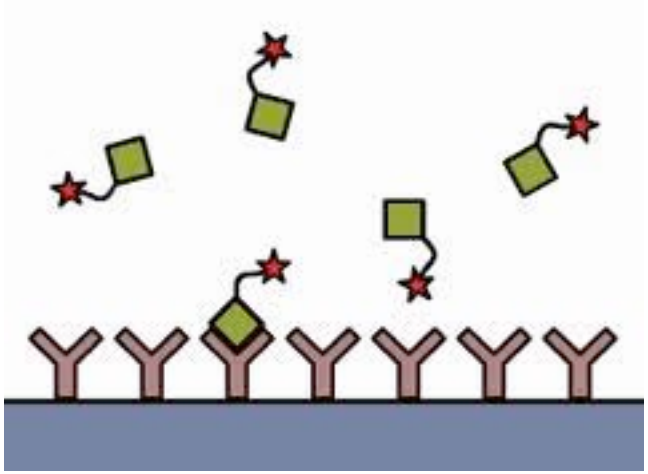
bacteria

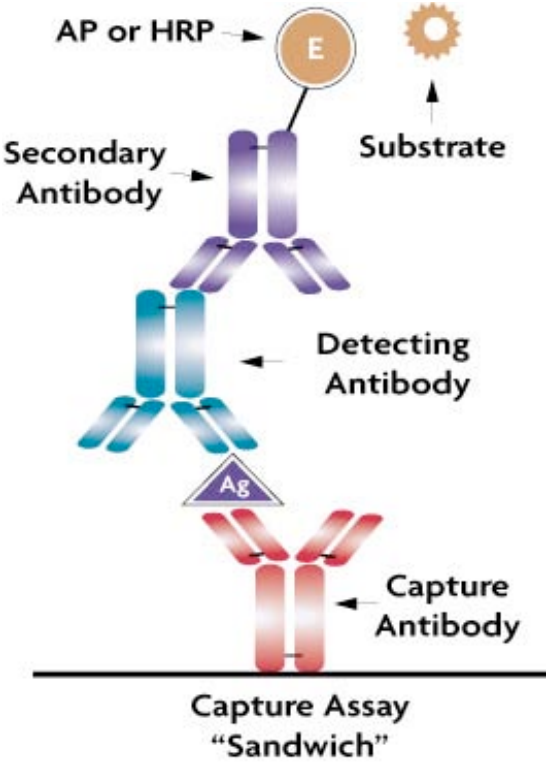


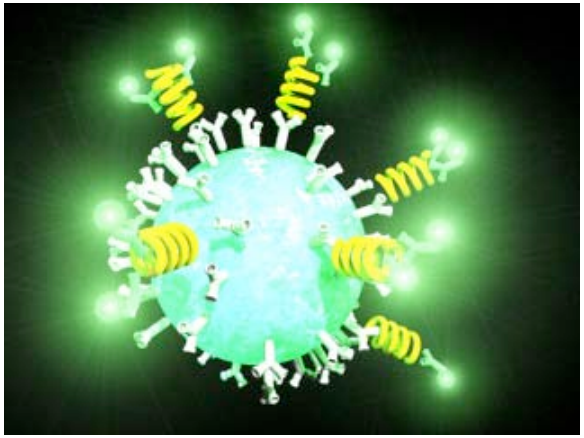
bca/abl in leukemia

Antibody assays

- Antibodies are large, diverse molecules with high affinities to specific antigens
- Antibodies can be directly labeled with fluorophores
- ELISA & Sandwich assays can amplify the fluorescence







Other fluorescence assays

- MAb's for flu, cold, measles, pathogenic E. coli, Salmonella…
  - It doesn't matter that the target is smaller than the resolving power of the microscope

Microscopes in the classroom

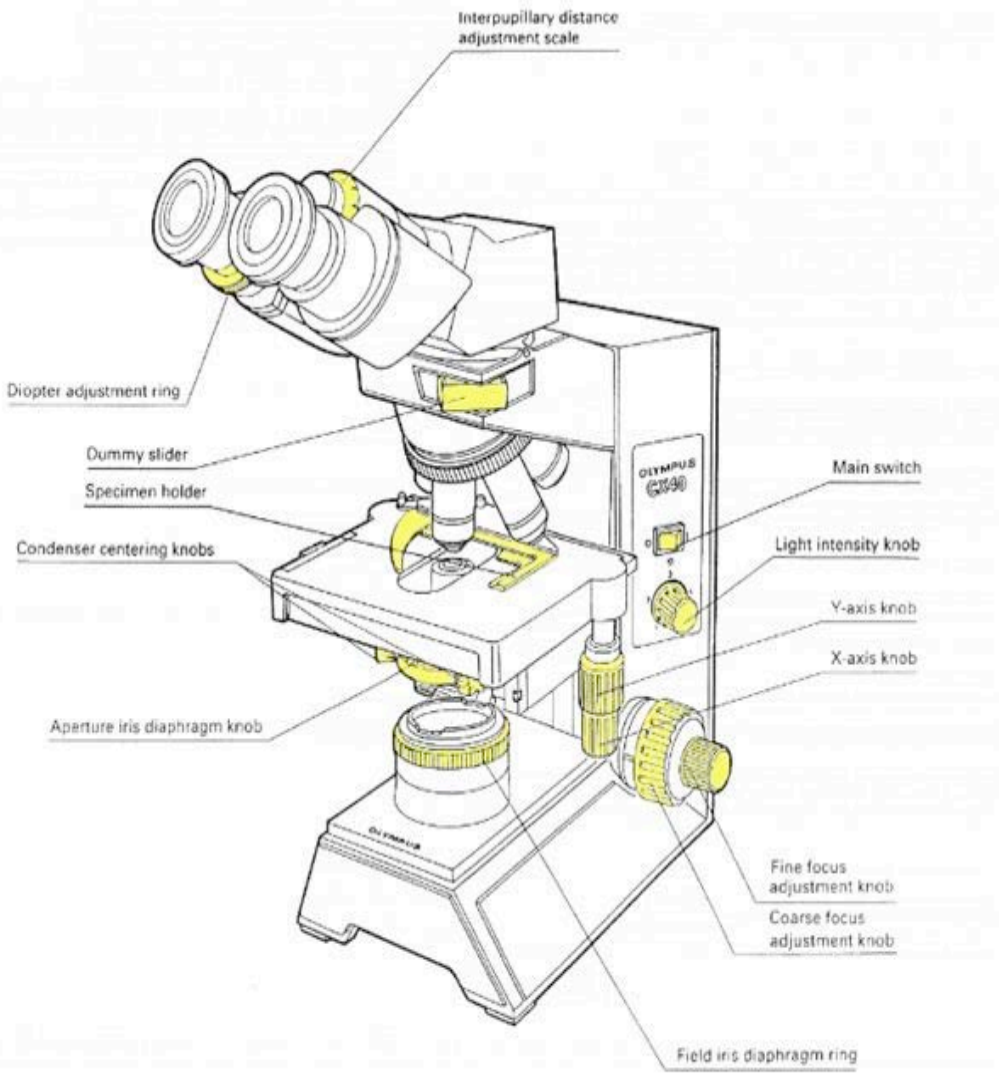
etaluma

- Schools struggle with conventional microscopes
  - They are foreign and intimidating to modern students
  - They are complicated; frequently misaligned interoccular distance, condenser focus, microscope focus; damaged or dirty (the objective isn't easily visible)
  - Students' drawings don't reflect what they see

Microscopes in the classroom

etaluma

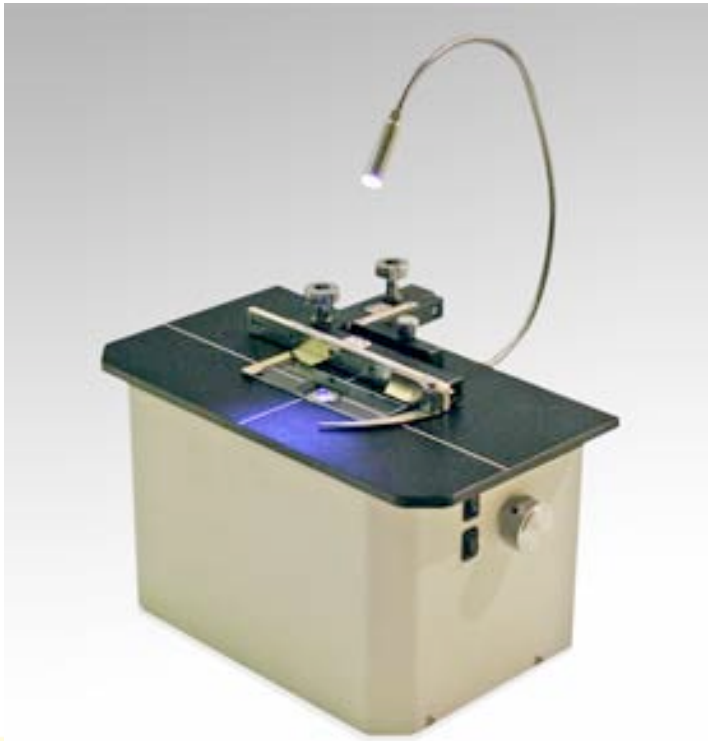
- There aren't enough teachers to assist 30+ students and verify what they are (not) seeing
- http://virtualurchin.stanford.edu/microtutorial.htm and others have developed sophisticated microscope simulations, because despite the challenges, using a microscope is so inspirational



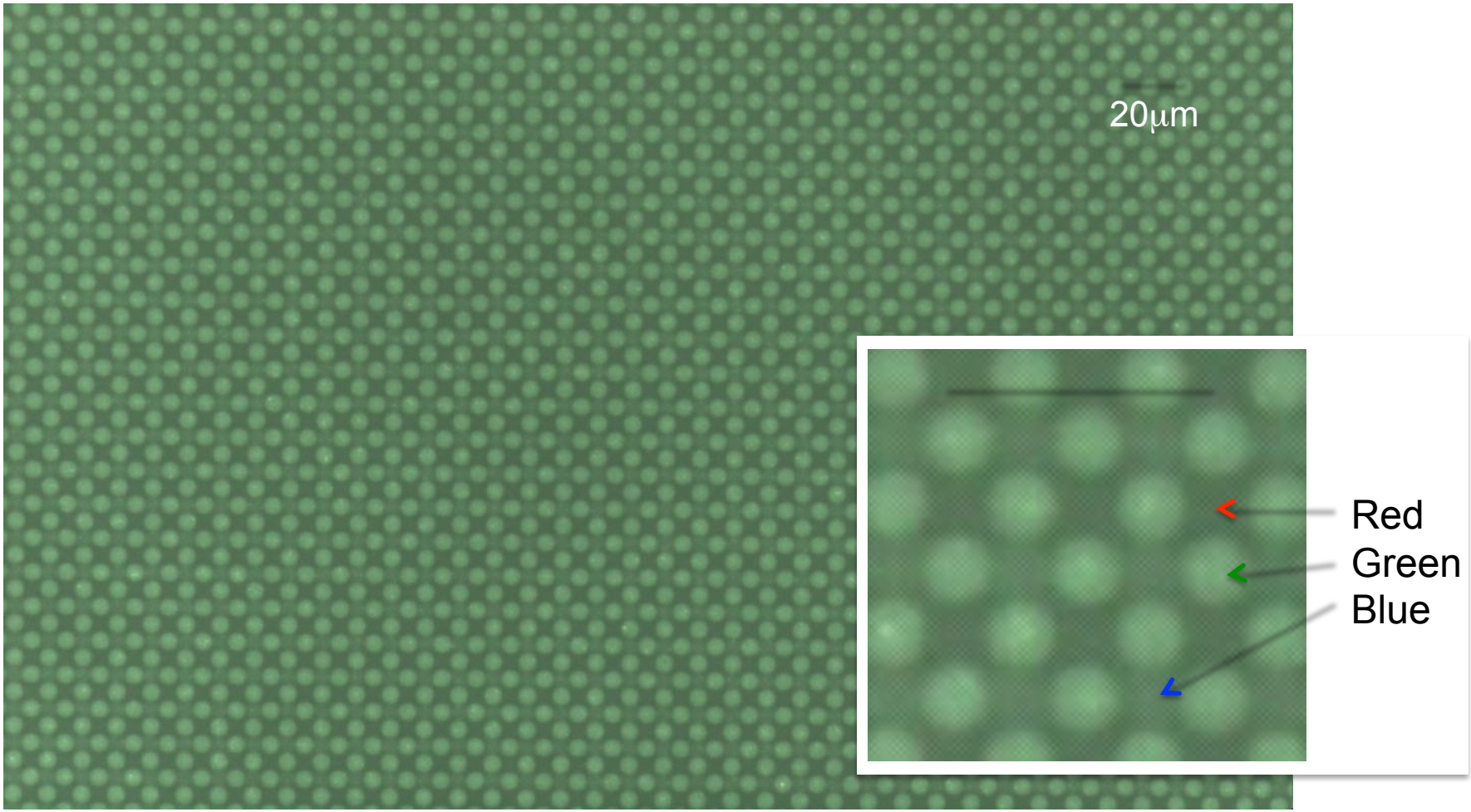
LumaScope in the classroom

etaluma

- LumaScopes are simple and intuitive to use
  - Kids intuitively know what to do with a USB port & 1 knob
  - The focus knob and switches controlling 2 light sources each provide immediate visual feedback of their functions
  - The software is simple
  - Cut & paste images into lab reports
- LumaScope enables teachers to focus on applications and content of the lab, rather than debugging the apparatus on each student's desk
- LumaScopes are compact to store, and stackable, reducing their likelihood of getting damaged

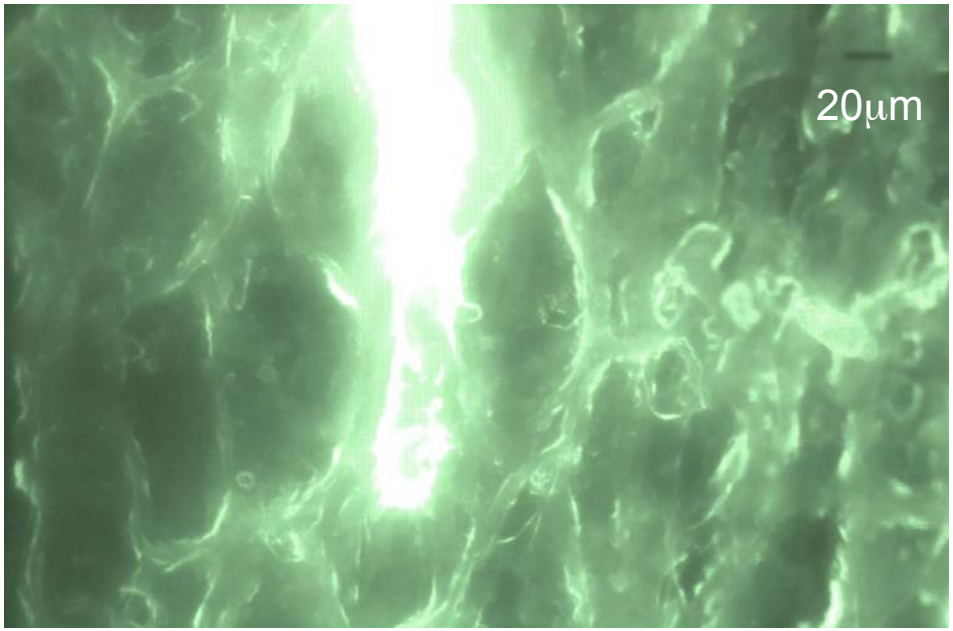


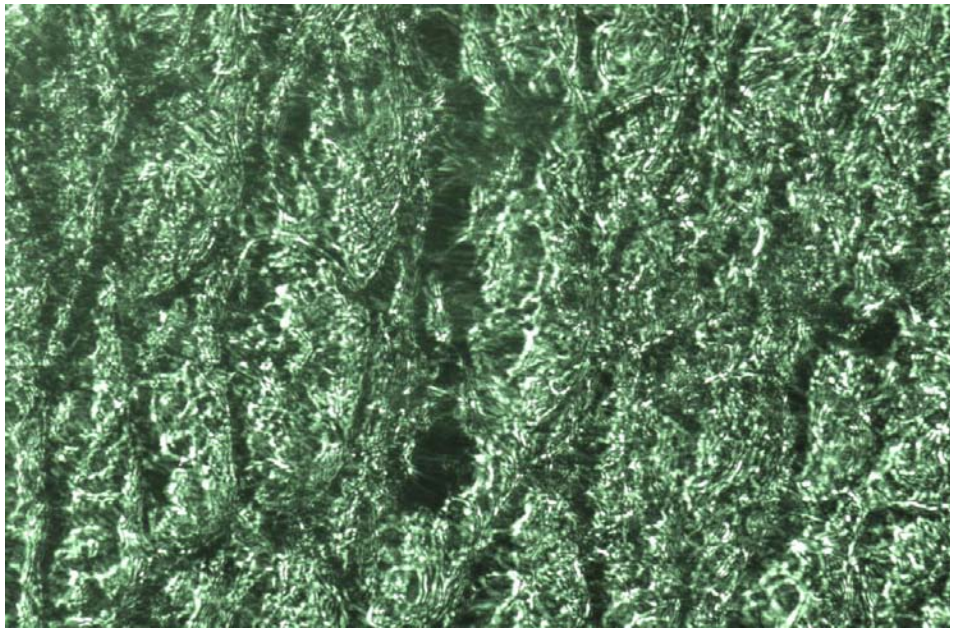
Bayer pixel pattern



- LumaScope only sees green light, but that is adequate to reveal the Bayer pixel pattern on a color CMOS sensor.

Microfluidics in a flower

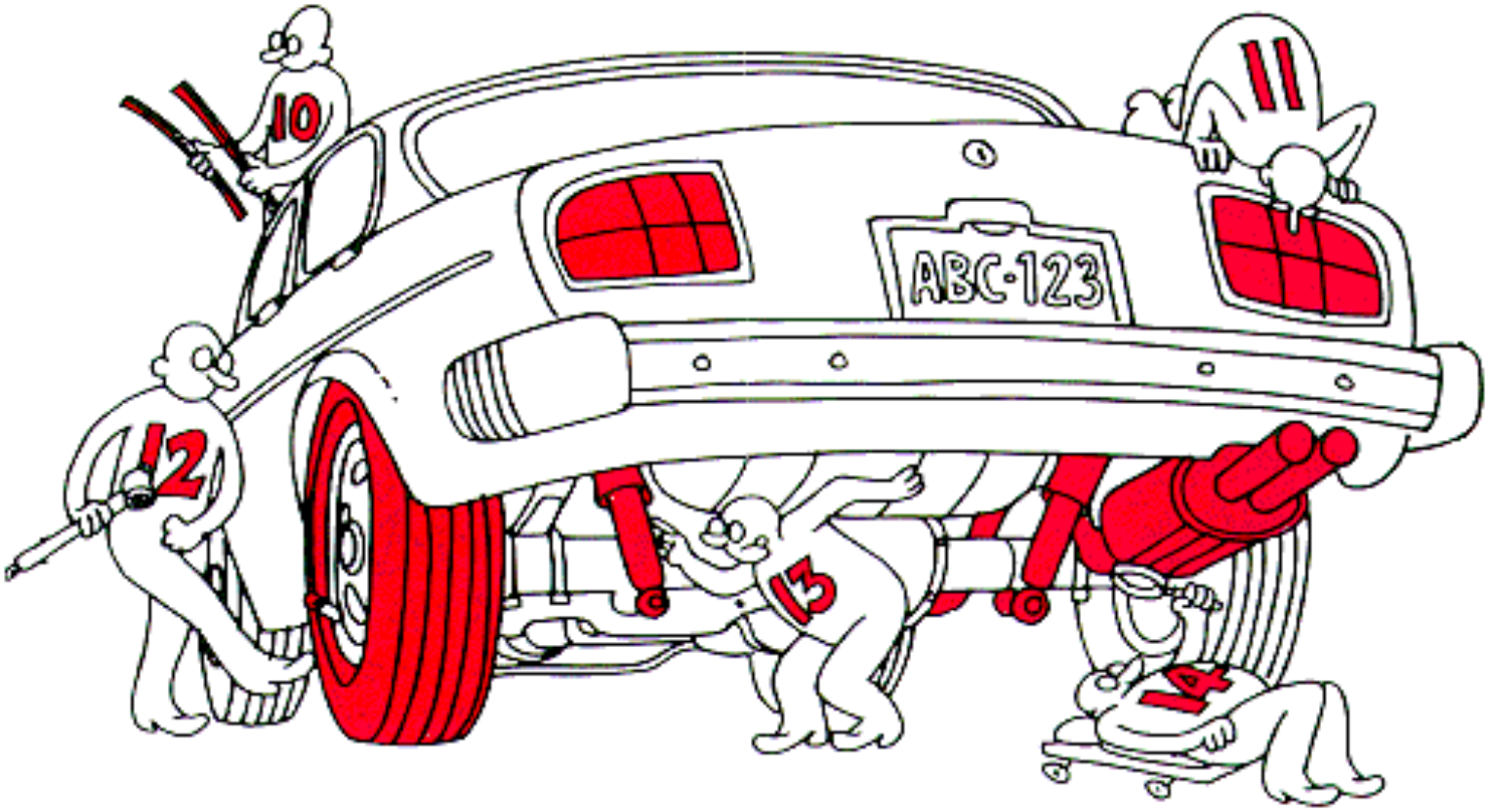




- Fluorescein transport in a flower petal was driven by capillary action. The epifluorescence initially increased rapidly and then was constant (left image).
- The identical field of view illuminated with the gooseneck white LED reveals the capillary canyon (right image).
- These profound differences in contrast are from the angle and spectra of the light sources.

Under the hood …

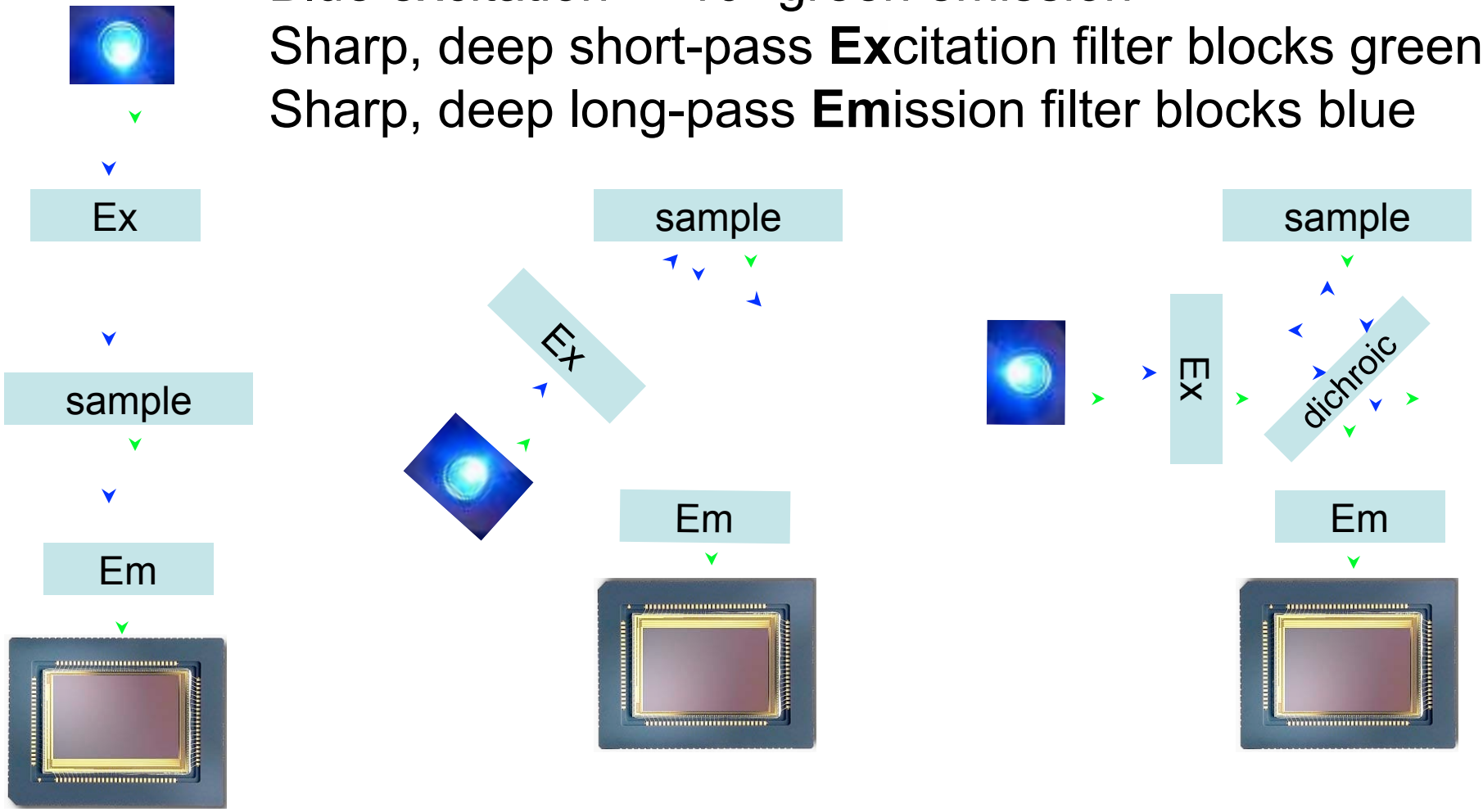
etaluma



Fluorescence μscope designs

etaluma

Blue excitation ~>10^6 green emission
Sharp, deep short-pass **Ex**citation filter blocks green
Sharp, deep long-pass **Em**ission filter blocks blue

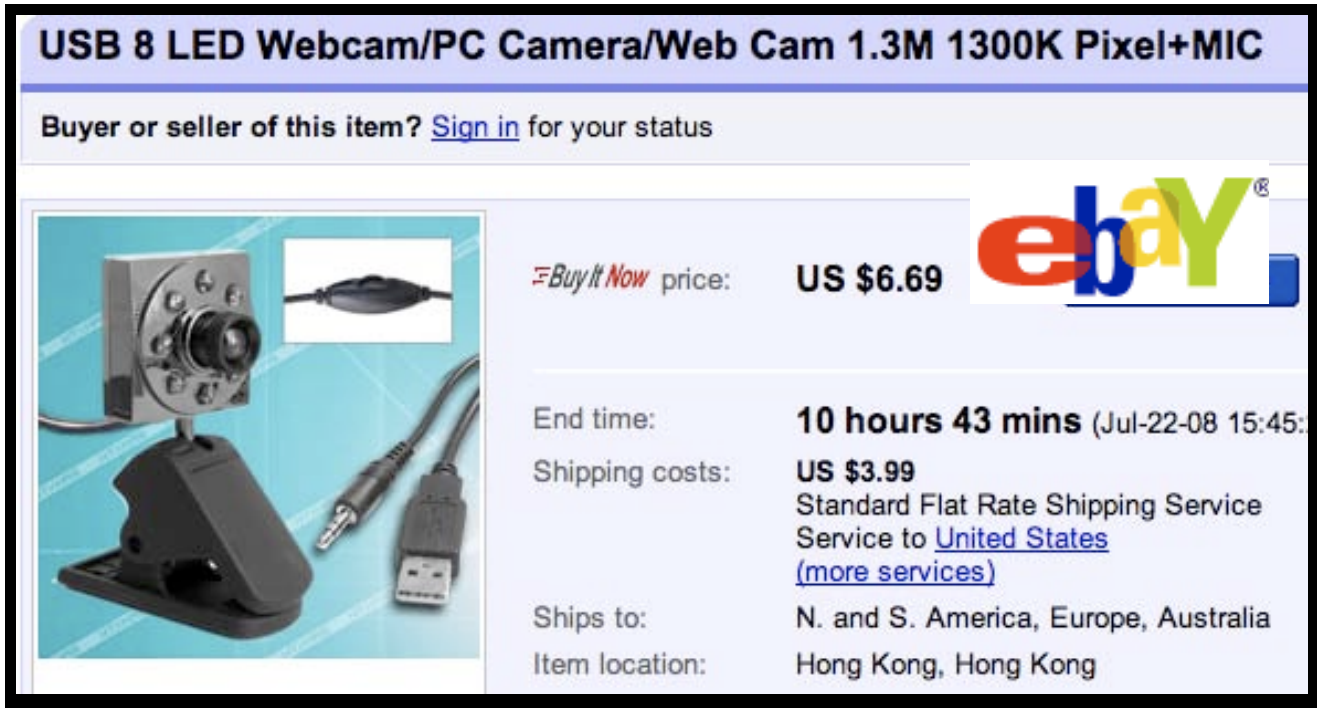


Trans Oblique Epi

Making it radically cheap

etaluma

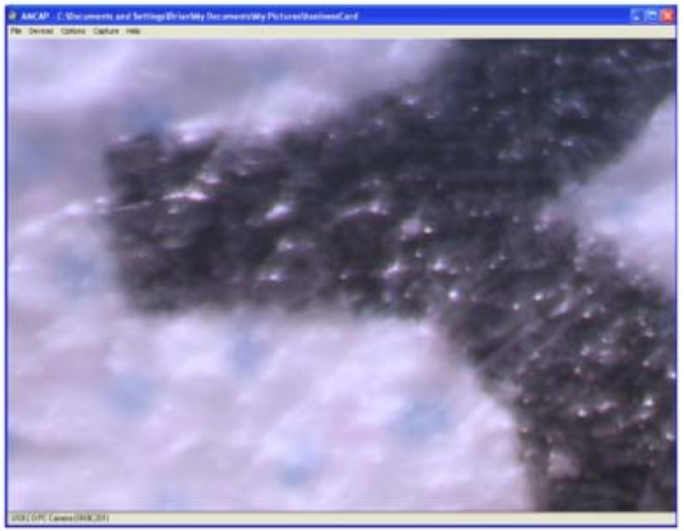
- Webcams are simple, cheap, and contain most essential components of a microscope
  - Lens
  - Light
  - Detector
  - Computer interface
- The ~5μm pixels are diffraction limited with ~10x magnification



Proof of concept, version 1

Webcam into light microscope

etaluma



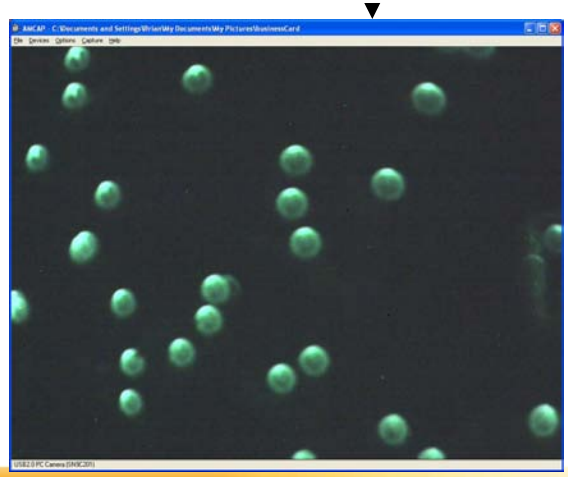
"3" at
1.3 μm
per pixel

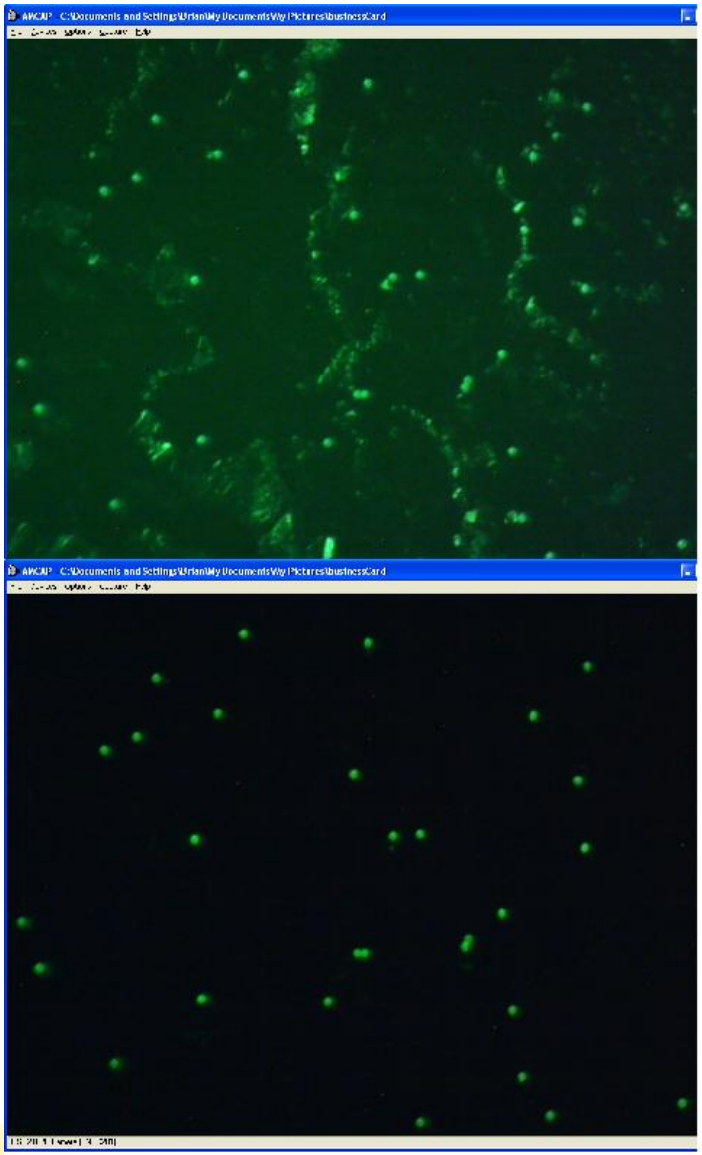
Proof of concept, version 2
Epifluorescence

10 μm FITC beads in saline

Same FOV with and w/o broadband illumination

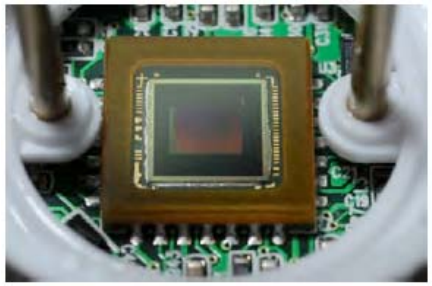
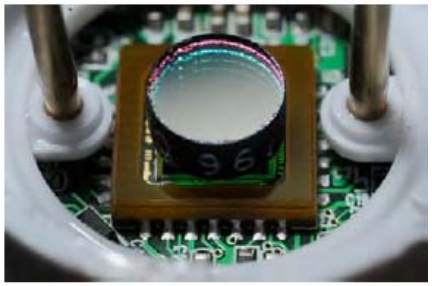
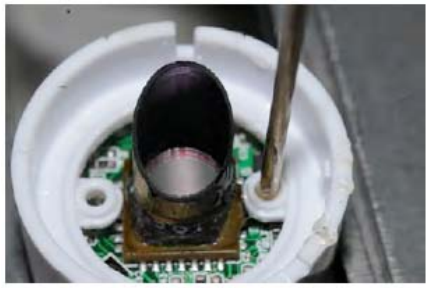
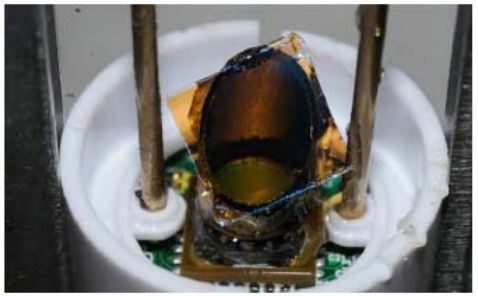
Zoom shows light is from bead's spherical surface

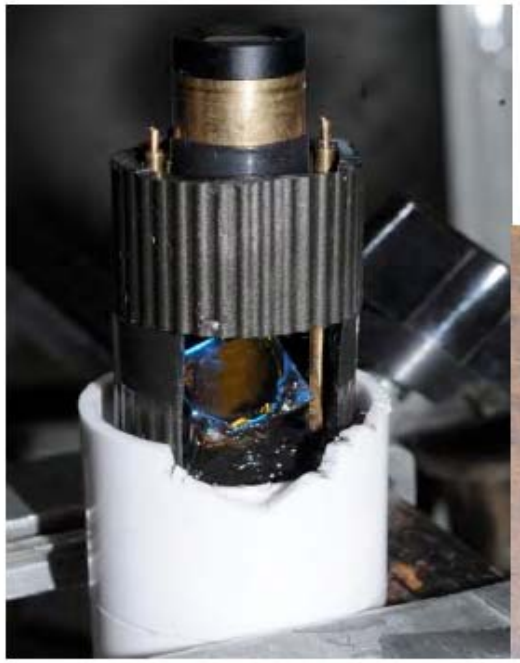


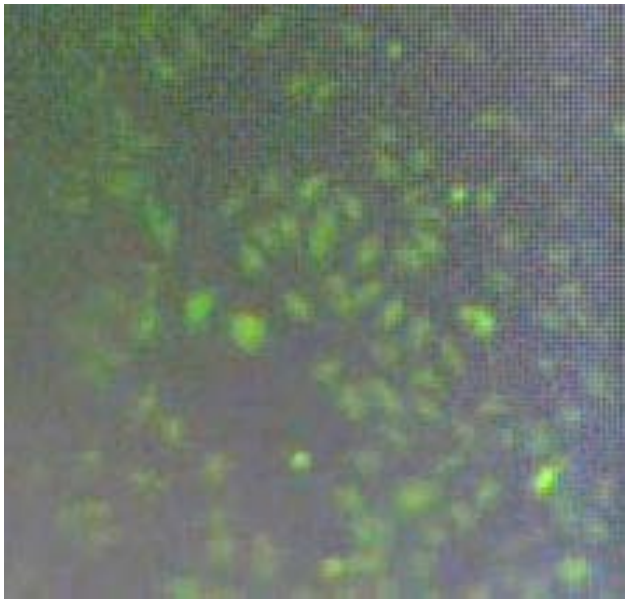


Proof of concept, version 3
Biological validation

etaluma

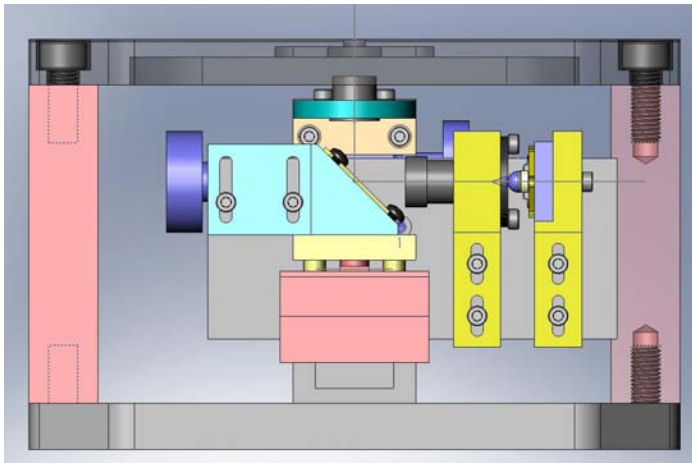


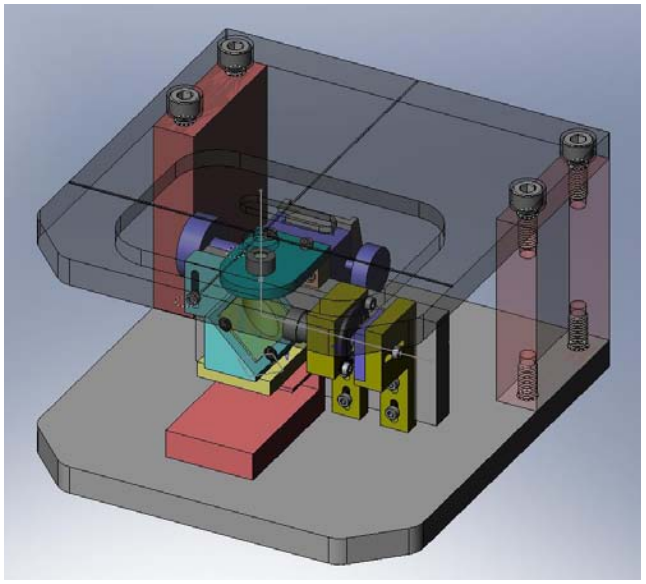



Calcein stained HeLa cells

Pre-Production

etaluma

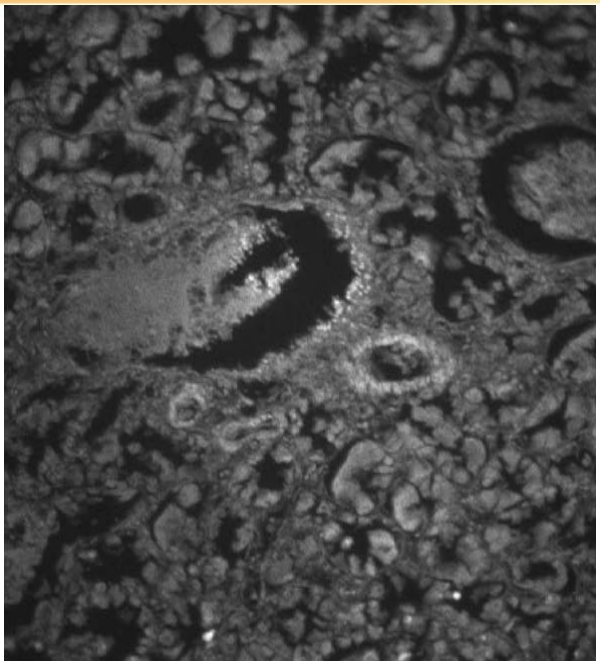


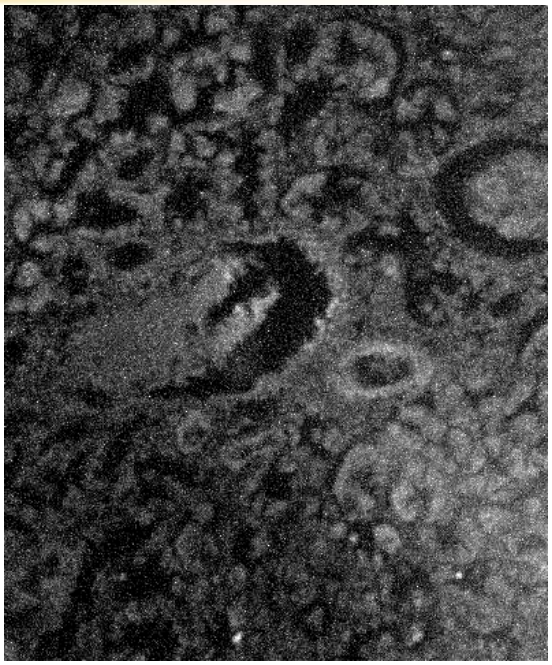


Fluorescent tissue slice

etaluma

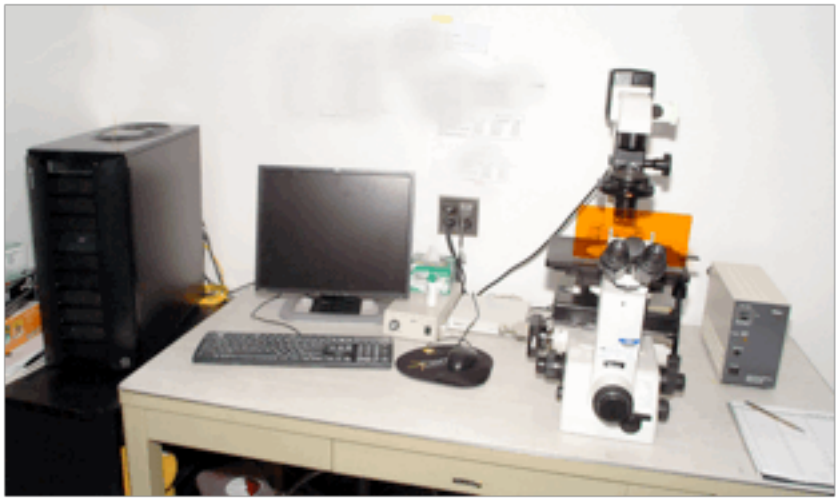
Zeiss Fluorescence test slide
(P/N 1213-943)





Pre-production LumaScope

Nikon Eclipse
20x Objective
SPOT camera

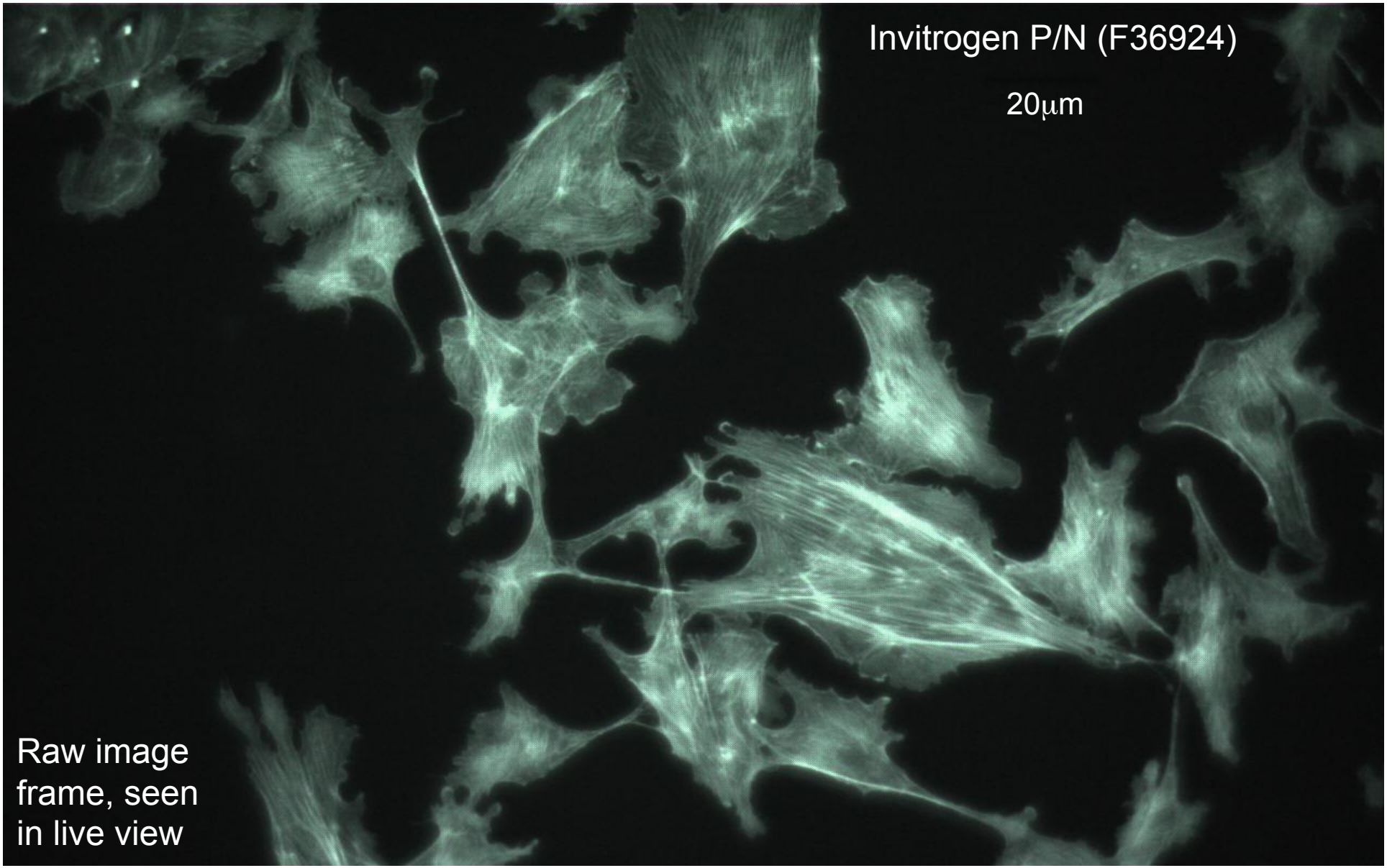


Optimizing performance

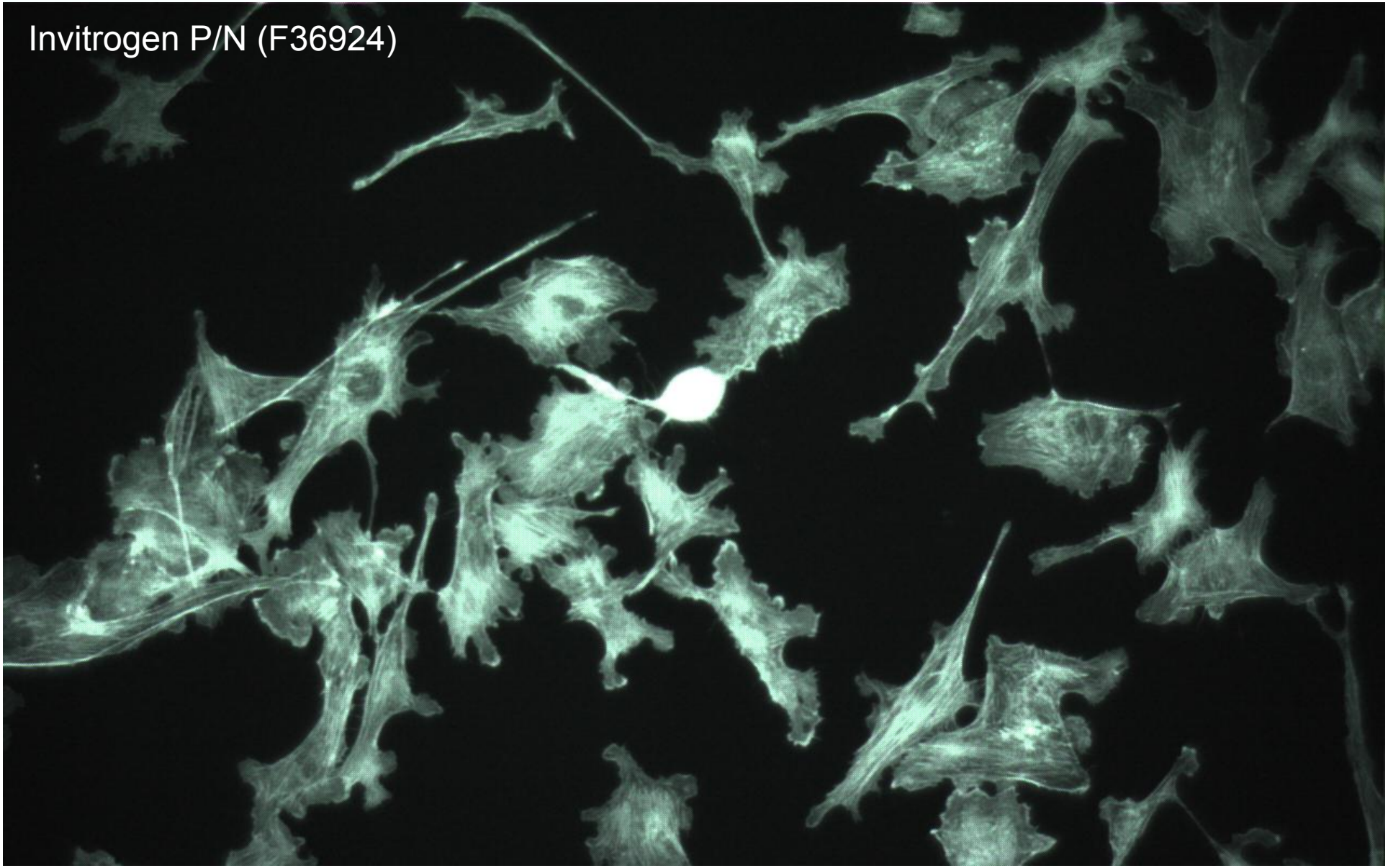
- For a biopharmaceutical research tool, we've traded off some cost for maximum fluorescence performance
  - Higher N.A. and magnification microscope objective vs. cheap lens
  - "Industrial" CMOS sensor and USB electronics vs. surplus webcams
  - Register-level software control of sensor to manually override exposure, frame rate, binning, etc.
  - Machined aluminum stage, baseplate, and internals vs. injection molding
- The resulting image quality is incredible (especially at ~ $4K)

Endothelial cells with Actin Ab

etaluma



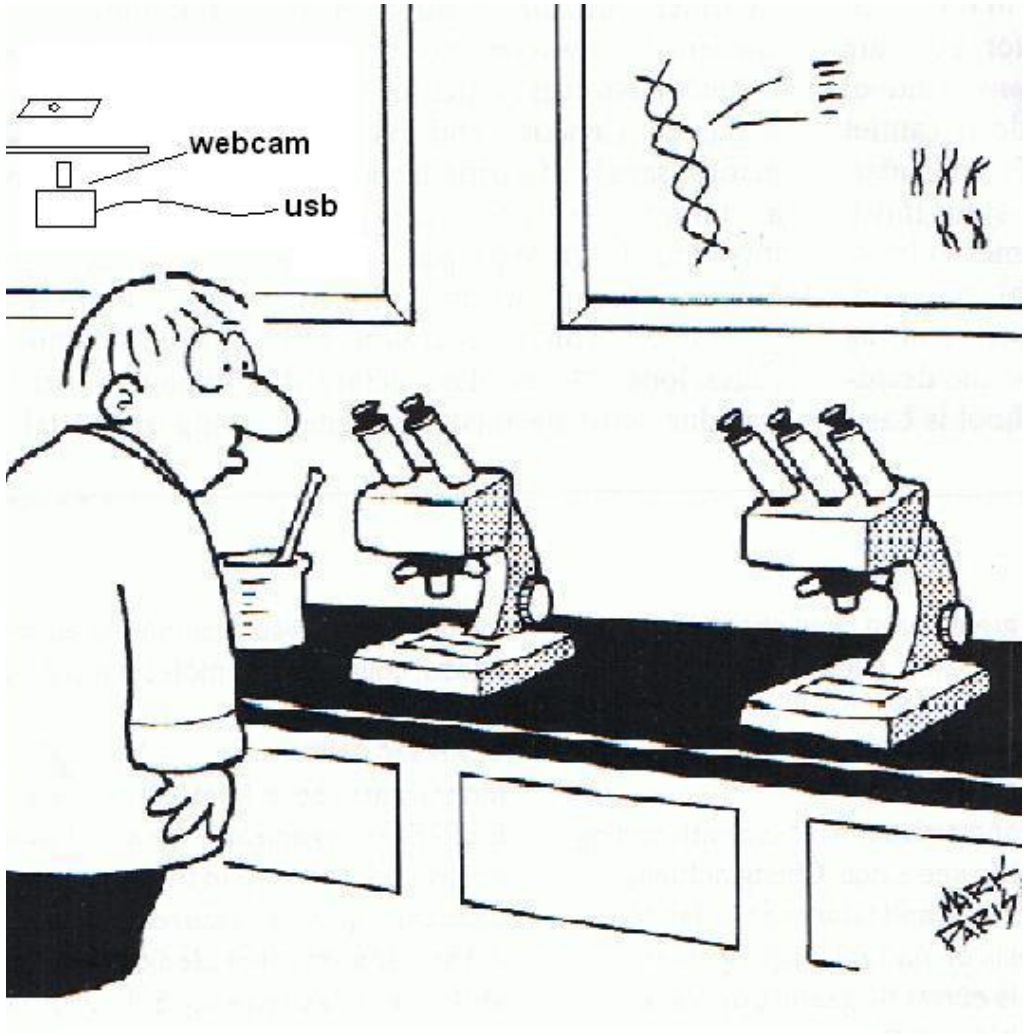
Endothelial cells with Actin Ab



Why is LumaScope so good?

- The LumaScope is around an order of magnitude *more* sensitive than a conventional fluorescence microscope
  - Our 1W LED and CMOS sensor are much closer to the sample than the 150W lamp and chilled CCD on the Nikon
  - LumaScope doesn't require as dark a room and dark-adapted eyes
  - The faster frame rate allows real time focusing and positioning
  - The image quality is exquisite
- Giving up a direct interface to the human eye
  - dramatically simplified the optics
  - enabled a more compact and efficient design
  - made a more intuitive instrument for teachers and students to use

etaluma



Sometimes more is not better...

Conclusions

- LumaScope is a radically different “personal microscope”
- Its advantages for schools and colleges include
  - Inexpensive and robust
  - Easy and intuitive to set up, understand, and use
  - Inverted design images from below the subject
  - Great sensitivity and resolution
  - Versatile oblique illumination and fluorescence for contrast enhancement
  - Integrates well with other software, facilitating data analysis and lab reports
  - Small, stackable
- By making the mechanics of microscopy orders of magnitude easier, LumaScope creates new possibilities for exploring multidisciplinary sciences in the classroom.

Acknowledgements

- Etaluma
  - Boris Gites
  - Jennifer Kahle
  - Kenji Levin
  - Lane Niles
  - Frank Ryan
  - Mel Schehlein
  - Chris Shumate

- Questions? www.etaluma.com