UCSF/QB3 Microscopy Course 2014

Choosing the best fluorescent light source
or *Don't "FRET" about your light source*

Mic Chaudoir, Ph.D. (speaker)
Americas Channel Manager, X-Cite

Kurt Thorn, (organizer)
Associate Professor
Director, QB3 Nikon Imaging Center

EXCELITAS TECHNOLOGIES

Overview

What is X-Cite and who am I ?

Light Sources by Chronology:

- **HBO/Xe Arc Lamps**
- **“Metal Halides”**
  - AC powered
  - DC powered
- **LED light sources**
- **Questions ??**

Who are we?

Lumen Dynamics (an Excelitas company)

- **20,000 + systems worldwide**
- **28+ years experience in light delivery systems**
- **Acquired by Excelitas technologies 2013**
- **X-Cite was THE FIRST Hg arc lamp replacement**

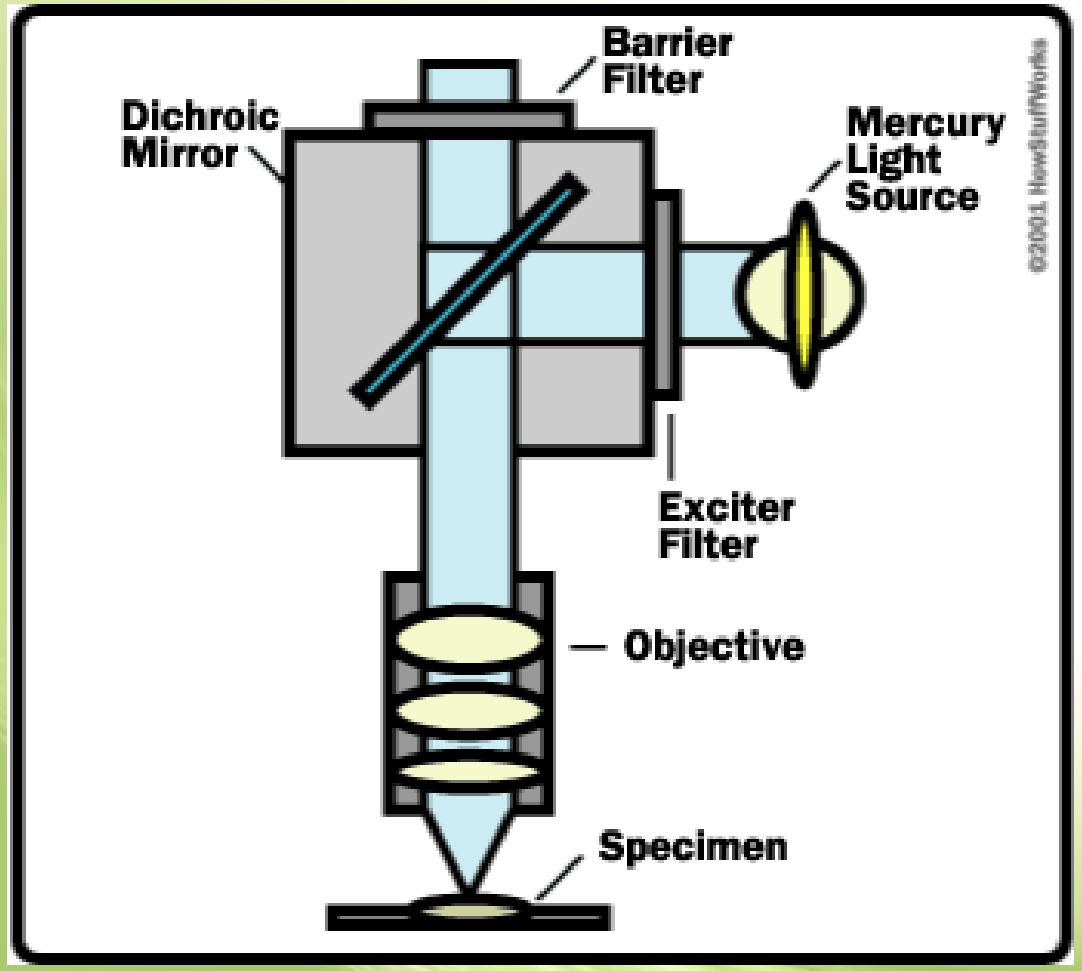
Who am I ?

- Doctorate in Cell and Molecular Biology (Northwestern University, Rex L. Chisholm)

- Extensive experience with imaging Systems design and use (Fryer Company (Nikon), Product Management (Leica), Sales and Product Management (Qimaging/Photometrics)

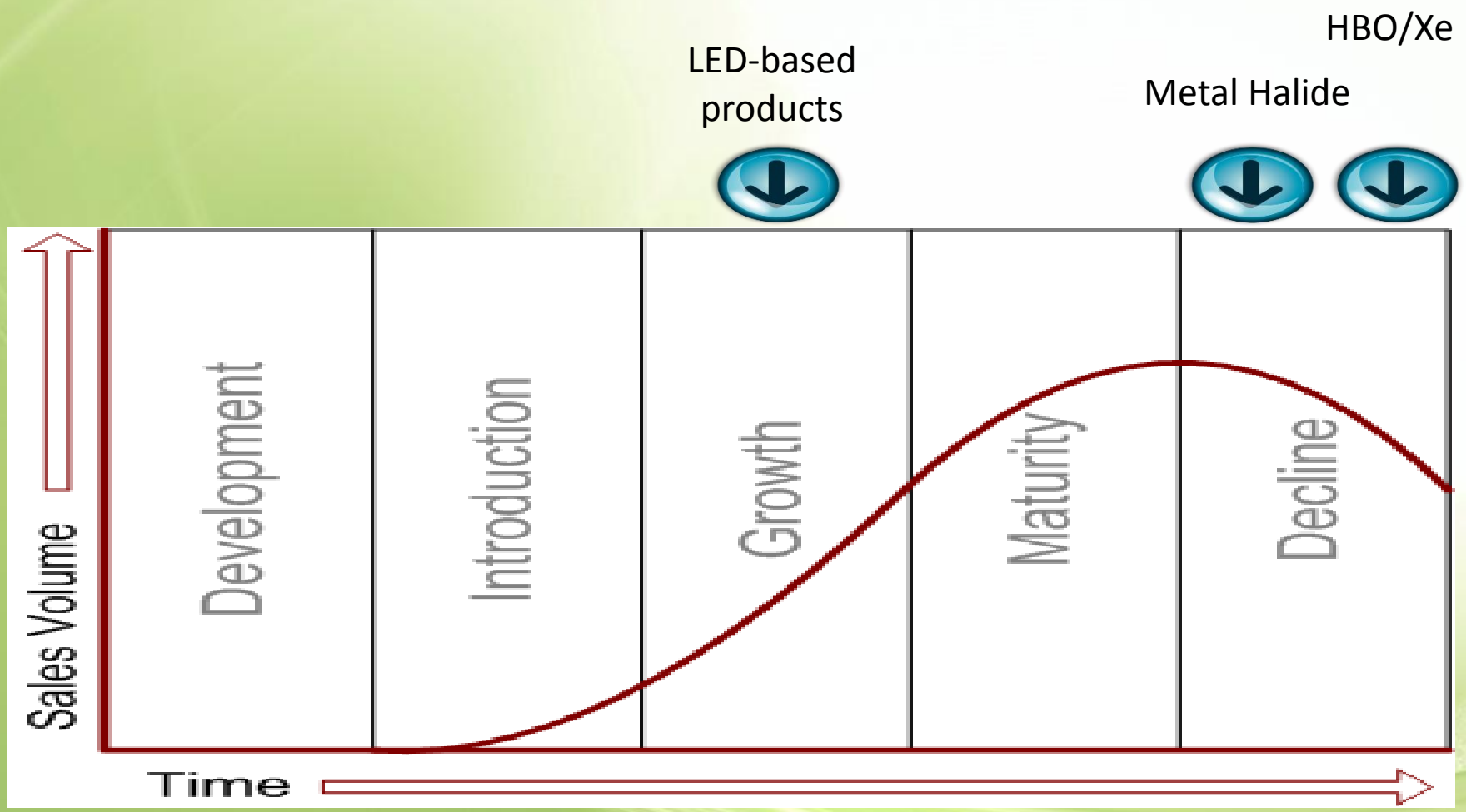
- Currently Americas Sales Manager for X-Cite

Fluorescence Illumination



Reproduced from: http://science.howstuffworks.com/light-microscope4.htm

Product/Technology Lifecycle



AC Powered Metal Halide Sources

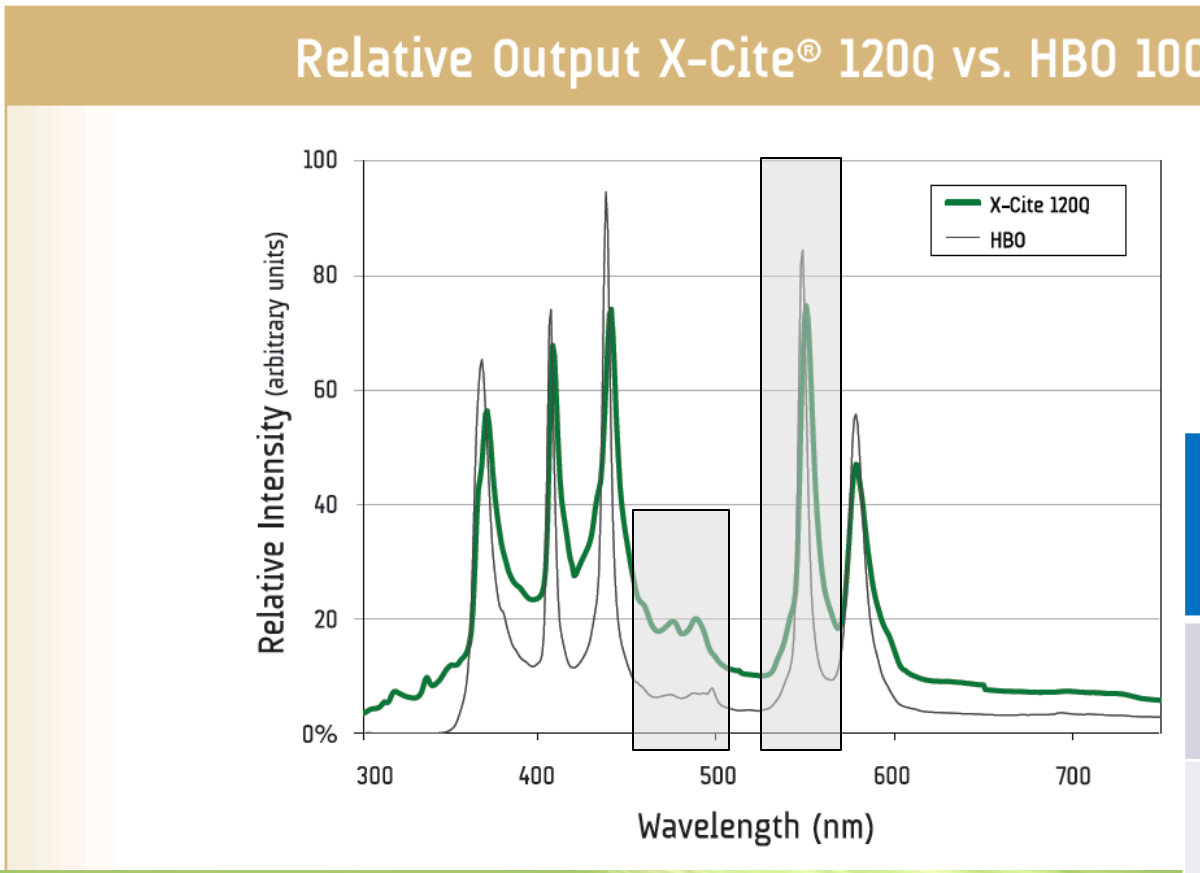
- Original “Metal Halide” Sources introduced circa 2003 under trade-name “XFO”

- AC powered versions now manufactured under a variety of labels and manufacturers (E.g. X-Cite, Prior, Leica, Zeiss, etcetera)

- Advantages: Similar initial cost, lower heat/vibration on scope, lower recurring cost, highest power, automation available, lower maintenance, lower waste

- Disadvantages: Mercury waste, still needs maintenance/recurring cost/waste, mechanical automation, Stability

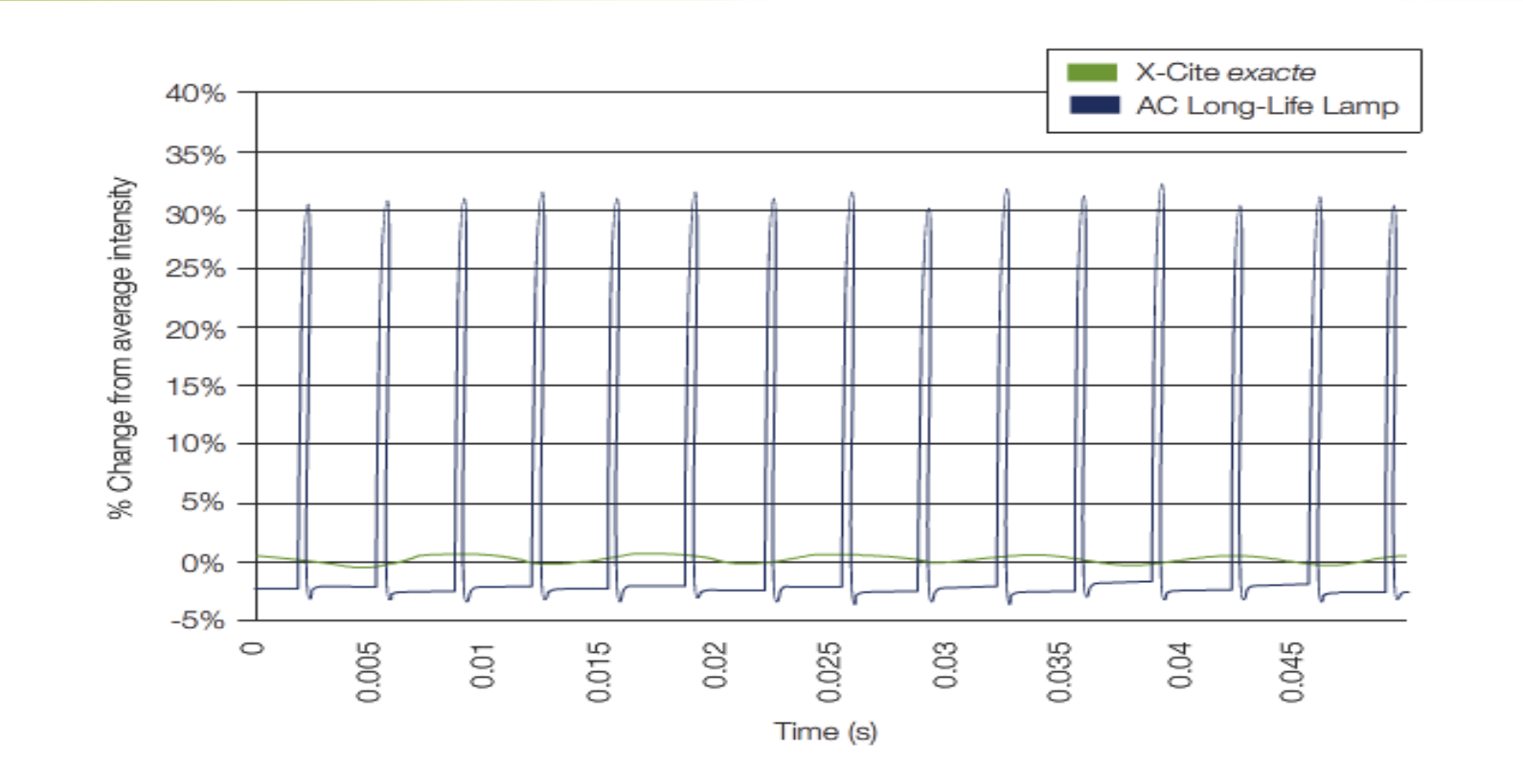
Spectral Comparison of 120 to HBO



	X-Cite	HBO
FITC 480/30	240mW	90mW
TRITC 540/25	360mW	280mW

Stability

- **Short term stability (poor)**
  - AC lamps cycle with a multiple of 120HZ



DC Powered Metal Halide Sources

- “Gen 2” Metal Halide sources, introduced circa 2008

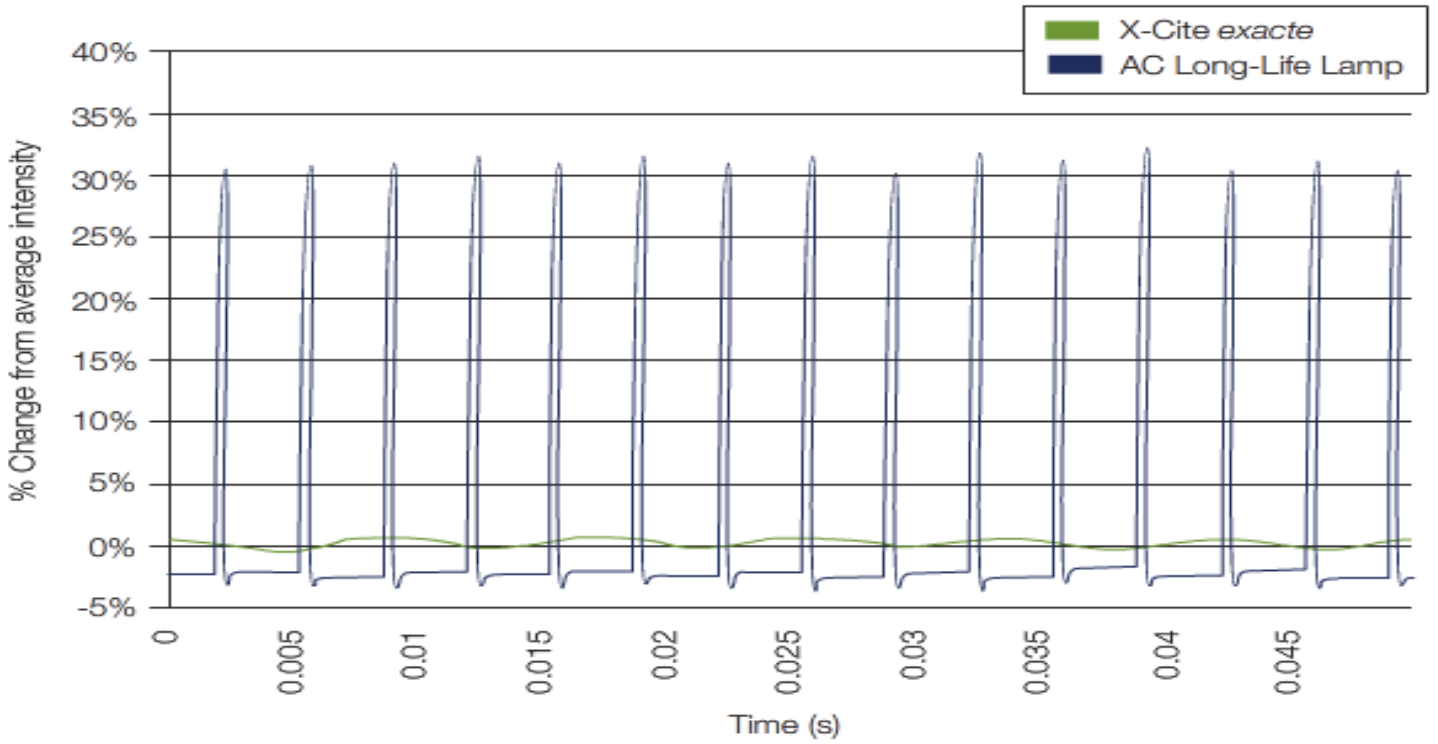
- DC powered versions now manufactured under a variety of labels and manufacturers (E.g. X-Cite, Prior, Leica, Zeiss, etcetera)

- Advantages: Similar initial cost, lower heat/vibration on scope, lower recurring cost, highest power, automation available, lower maintenance, lower waste, better stability

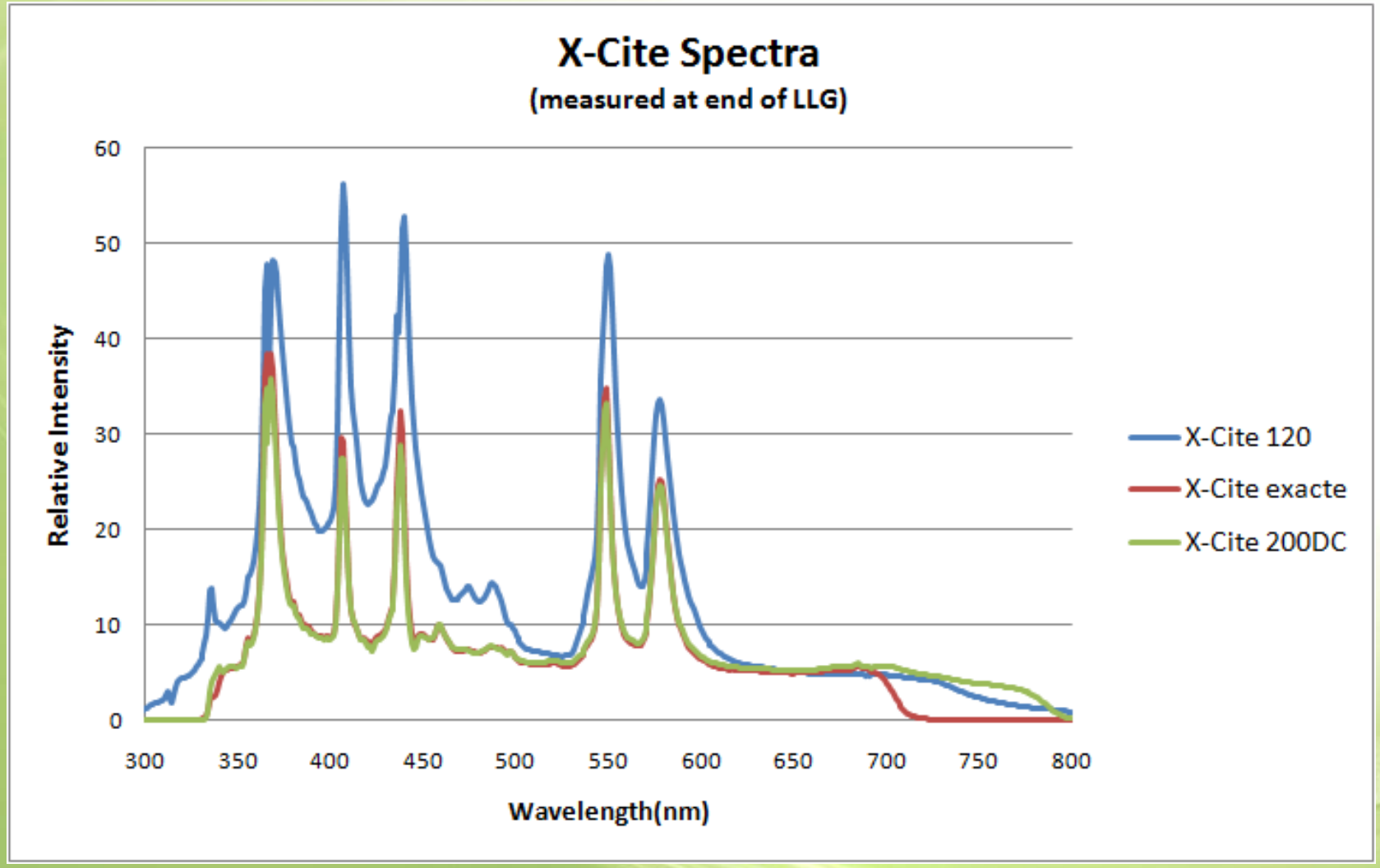
- Disadvantages: Mercury waste, still needs maintenance/recurring cost/waste, mechanical automation, slightly lower power (vs. AC)

Stability

- **Short term stability (good with DC)**
  - AC lamps cycle with a multiple of 120HZ



AC vs. DC Spectra



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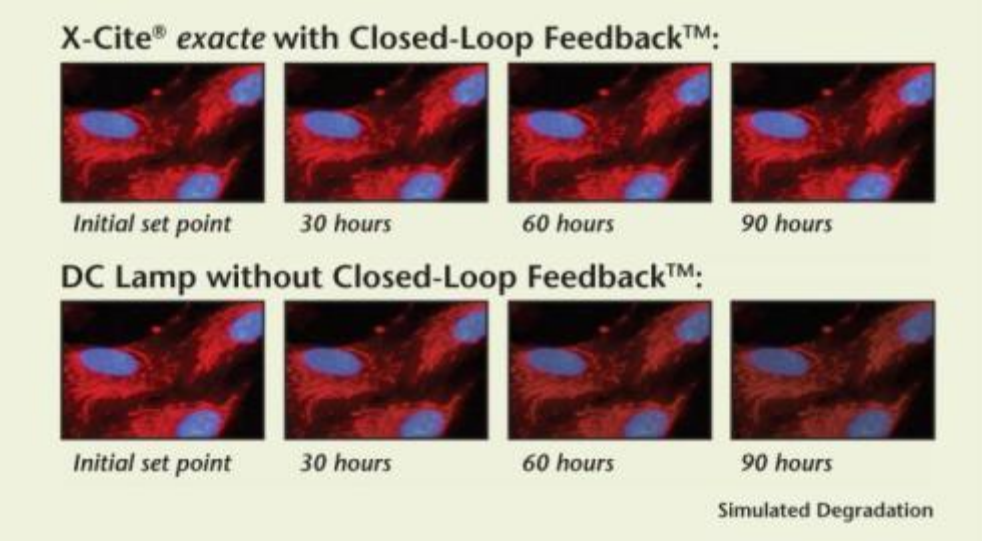
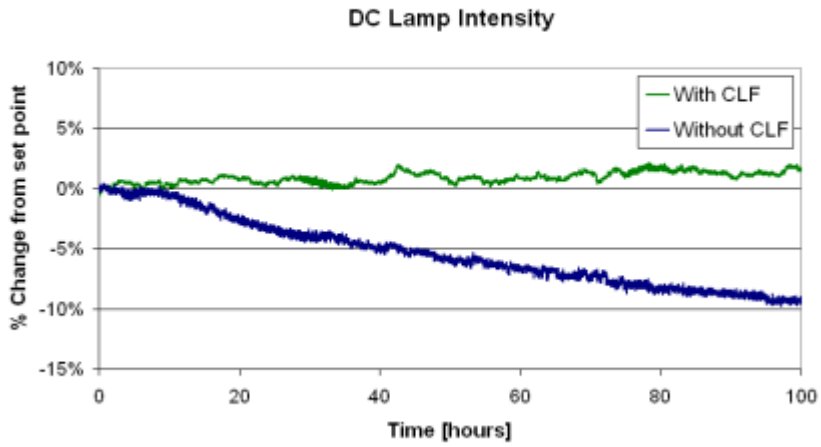
Technology Focus: X-Cite *Exacte*





- Intensity of any mercury (Hg) lamp will decline as the lamp ages
- X-Cite exacte monitors and stabilizes intensity
- As the lamp ages, the iris position is automatically adjusted to maintain intensity at the original set point

RESULT:

Stable intensity for the duration of an experiment, whether it lasts for hours or DAYS!

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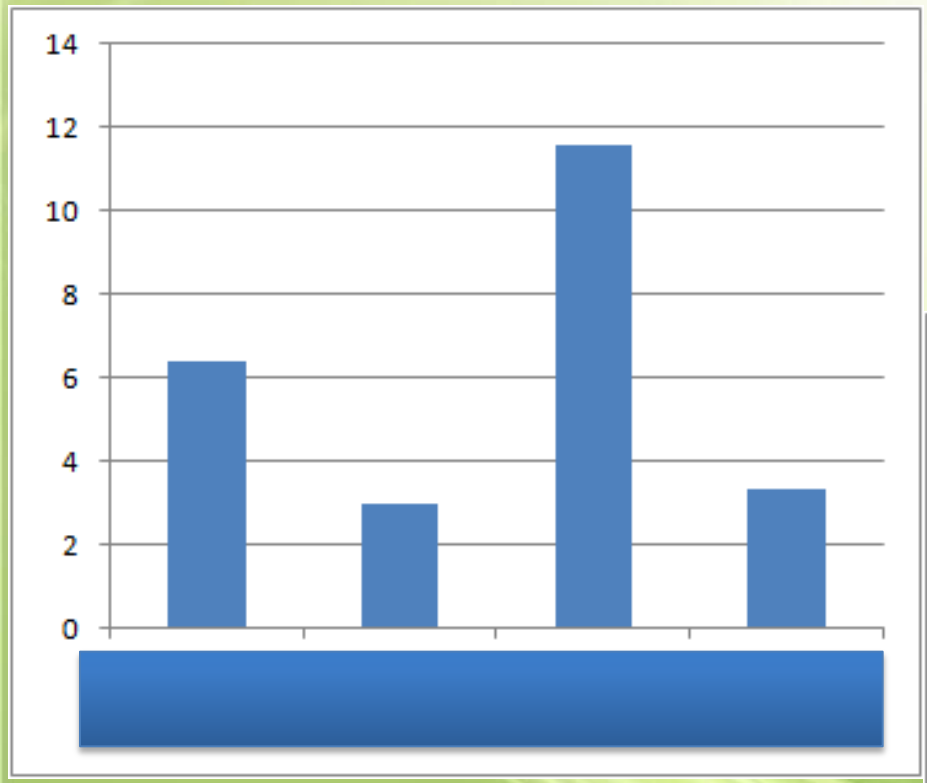
Know your power……

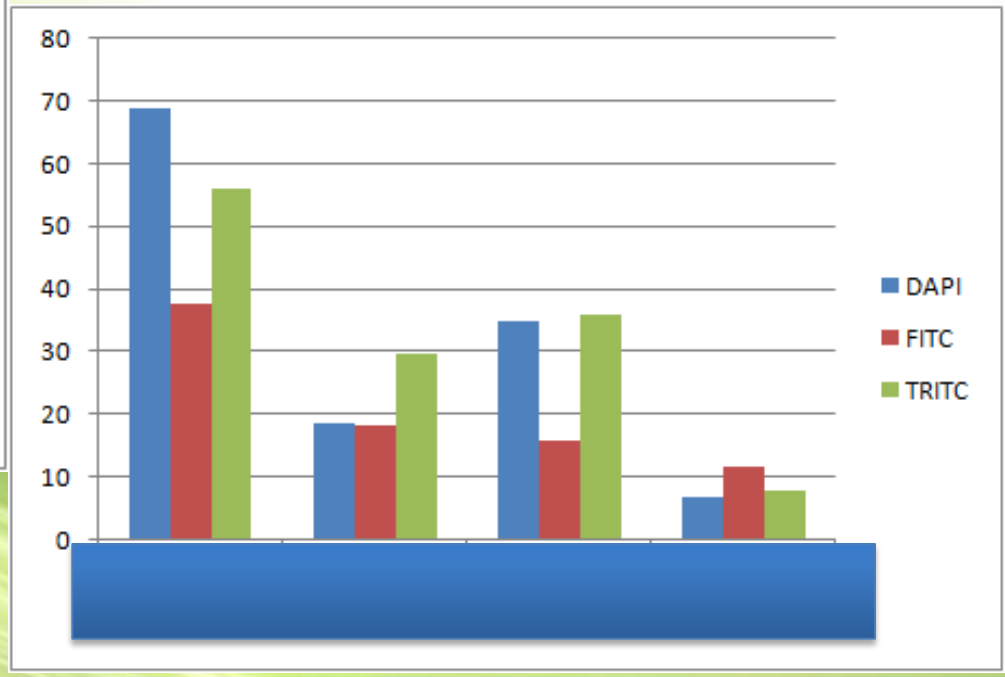
- Slide format power meter
- 320-750nm
- Usable for all light sources
- Ability to calibrate with the exacte

Technology Focus: Collimation!

Total LLG Power (W)



Sample Plane (mW)



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LED light Sources – revolution underway!

- Improved LED power technology driving a new wave of light sources…. Currently underway!

- Two varieties: Switchable, and White light. For technical reasons (power), switchable sources pre-dated white light sources

- Advantages: No consumables, electronic control, electronic switching, long lifetime, lower power consumption, cell viability

- Disadvantages: Lower power and spectral options, lower maturity level, higher initial cost

Switchable LED Sources

- Long lifetime
- Stability
- Low vibration, shutterless design
- Reduced photo-toxicity and bleaching
- Less heat
- Energy efficiency
- Licensed
  - Carl Zeiss owns the patent for switchable

  LED use in microscopy

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X-Cite® XLED1

Microscopy TODAY 2012 Innovation Award

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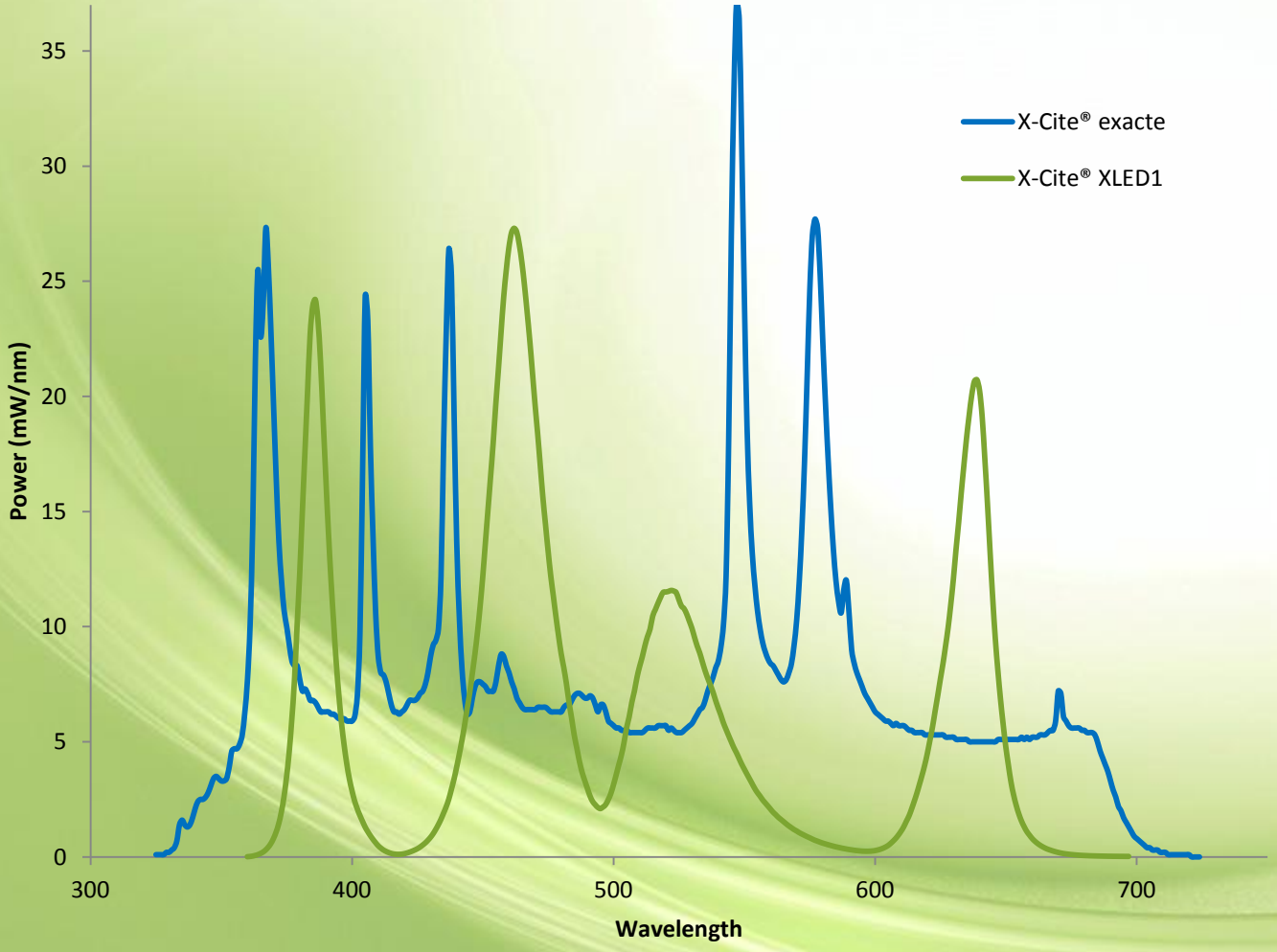
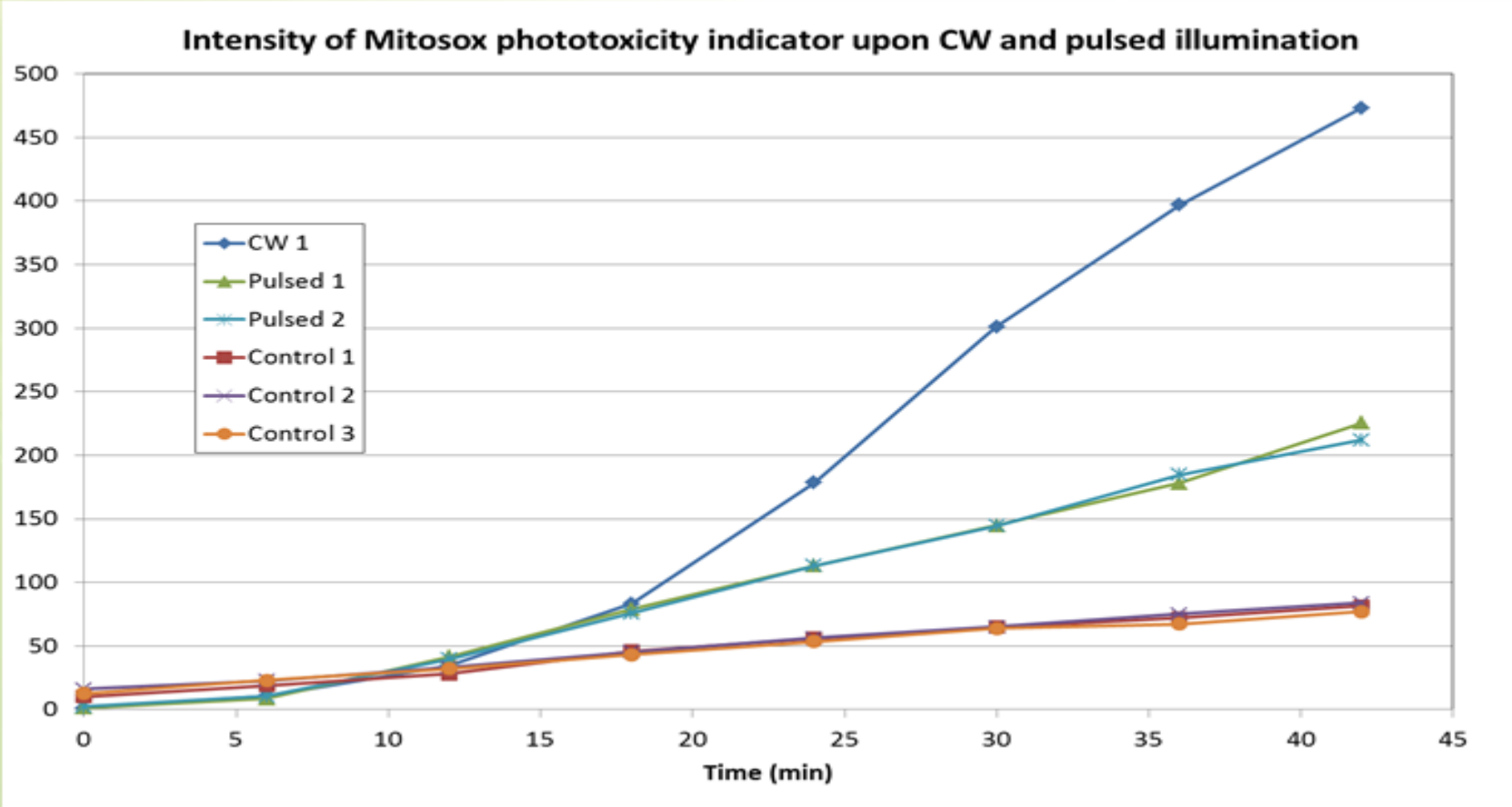
X-Cite® XLED1 power output



Exit power measured from a 3mm liquid light guide

Effect of LED pulsing on Phototoxicity



Data provided by James Jonkman, AOMF in collaboration with Kavita Aswani, LDGI - unpublished

AOMF ADVANCED OPTICAL MICROSCOPY FACILITY

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Switchable LED Sources – disadvantages

So why not use for everything ?

- **28**

- Limited Spectra
  - No 340nm, poor power above 700nm

- High cost >10k (typically 18k) vs. 8-10k

(filter wheel and other source)

X-Cite® XLED1

Microscopy TODAY 2012 Innovation Award

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White light LED sources

- Later availability vs. switchable sources; primarily driven by power of LEDs. First high power units Lumencor SOLA (2011) and X-Cite 120LED (2013).

- White light sources are less expensive, easier to integrate and more accessible than switchable sources, and represent a "2nd Generation" of LEDs for fluorescence illumination

- Advantages: No consumables, electronic control, long lifetime, lower power consumption, cell viability and easy integration with existing systems

- Disadvantages: Lower power and spectral options, lower maturity level, higher initial cost (vs. Metal Halide/HBO)

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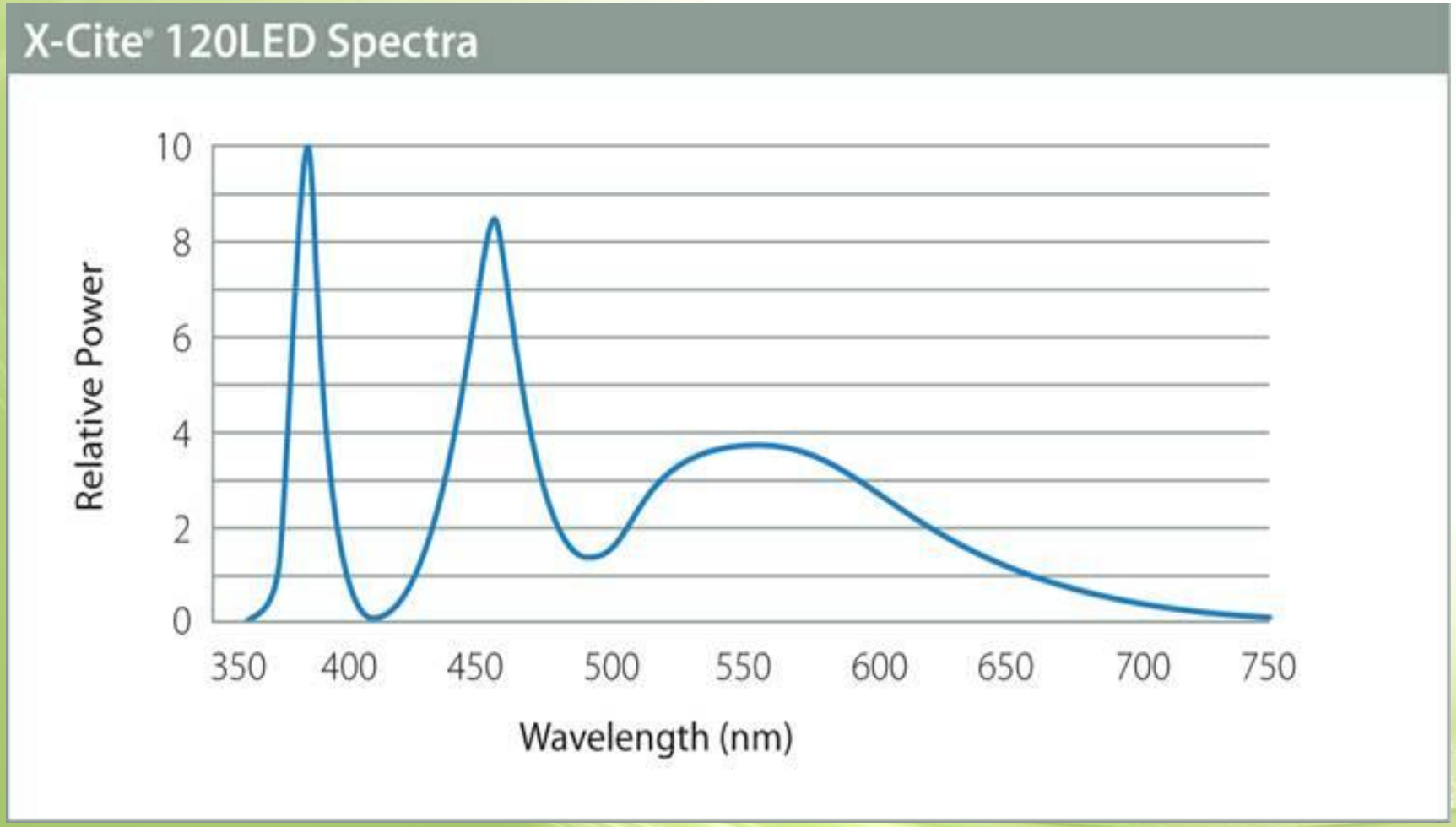
White Light LED sources - Spectra



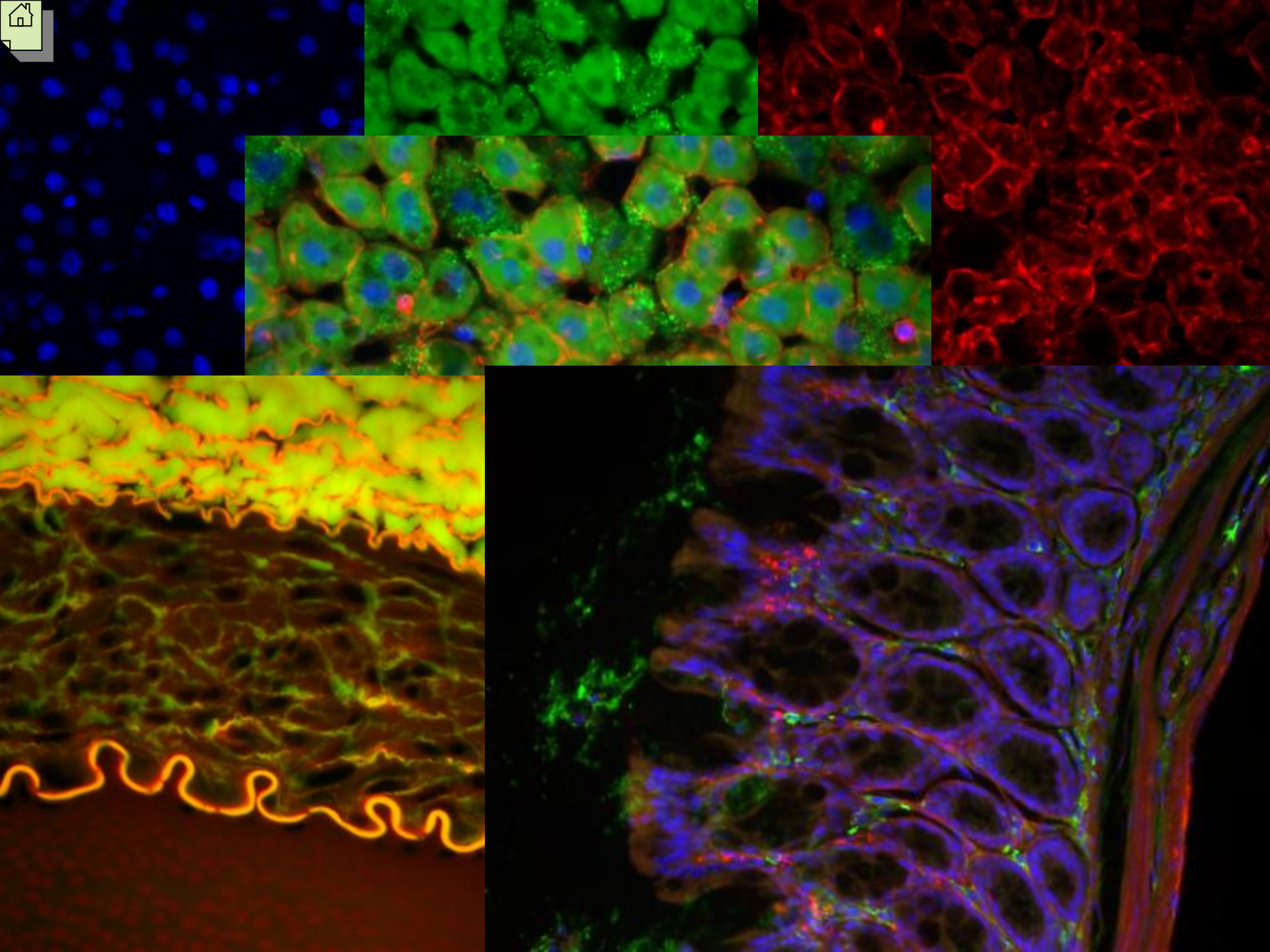
Summary

Traditional arc lamp technology (Hg, Xe) is a proven technology suitable for a wide range of experiments

Metal Halide sources have largely supplanted traditional arc lamps due to lower maintenance, lower cost and better stability

LED sources are emerging to as a solid-state alternative with no maintenance, high power, superior stability and fully electronic control

Thank you



Addendums:

- LED powers
- User endorsements
- Cost of ownership

Happy Users

"The X-Cite® 120LED provides accurate FRET measurements and works well with all of our fluorophores. We find it bright, uniform and easy to use."

Dr. Ammasi Periasamy,
W.M. Keck Center for Cellular Imaging, University of Virginia, USA

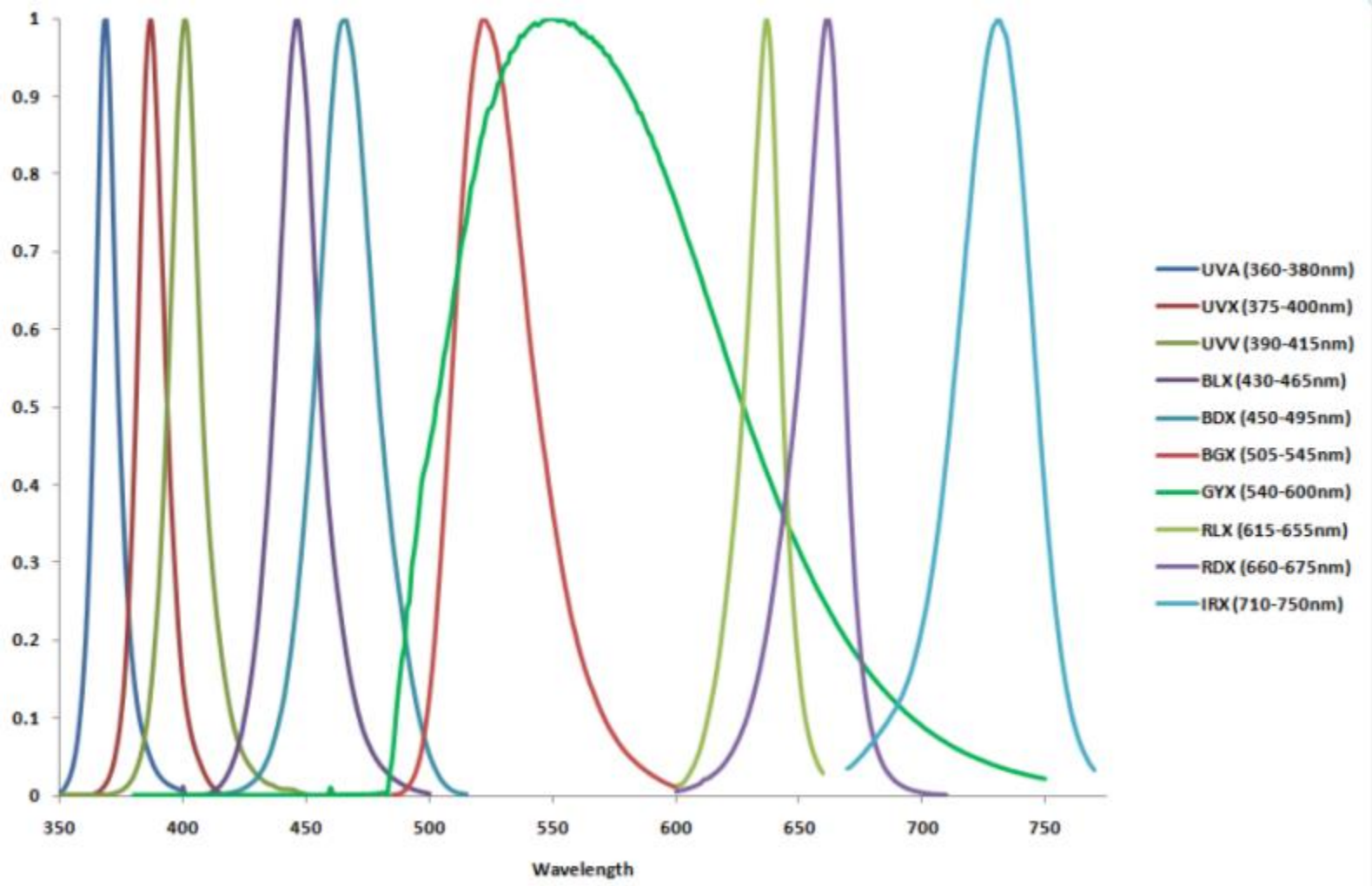
"We achieved excellent results when performing time-lapse imaging on live cells over 24 hours with the X-Cite® 120LED. These results combined with a much longer lamp life time and less maintenance compared to conventional lamps make the 120LED particularly attractive in the context of running a microscope facility."

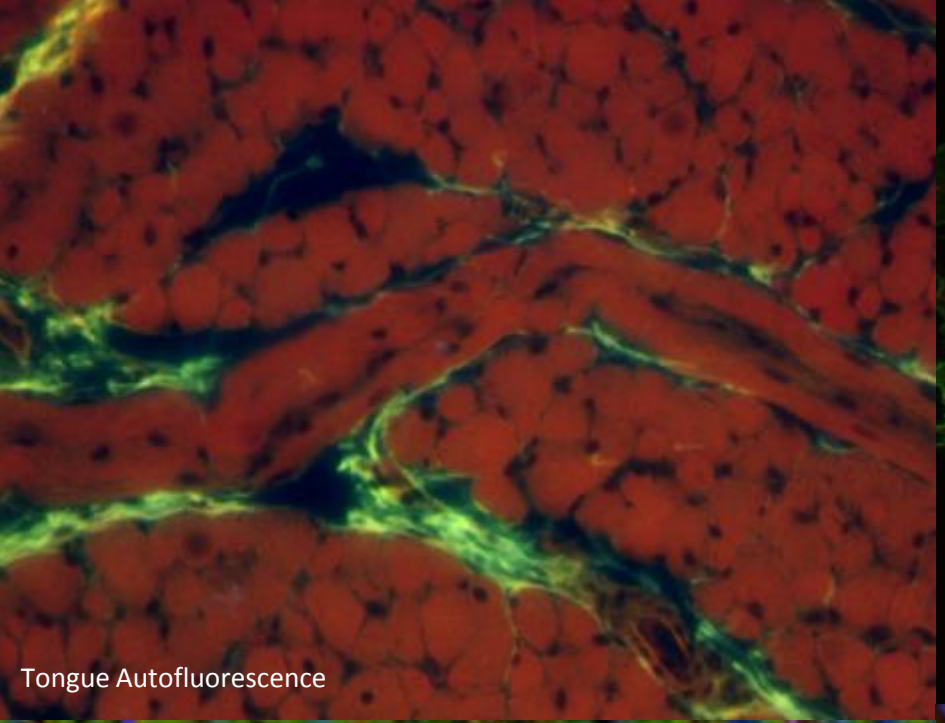
Dr. Beate Neumann,
Advanced Light Microscopy Facility, EMBL Heidelberg.

Cost of ownership vs. HBO+shutter

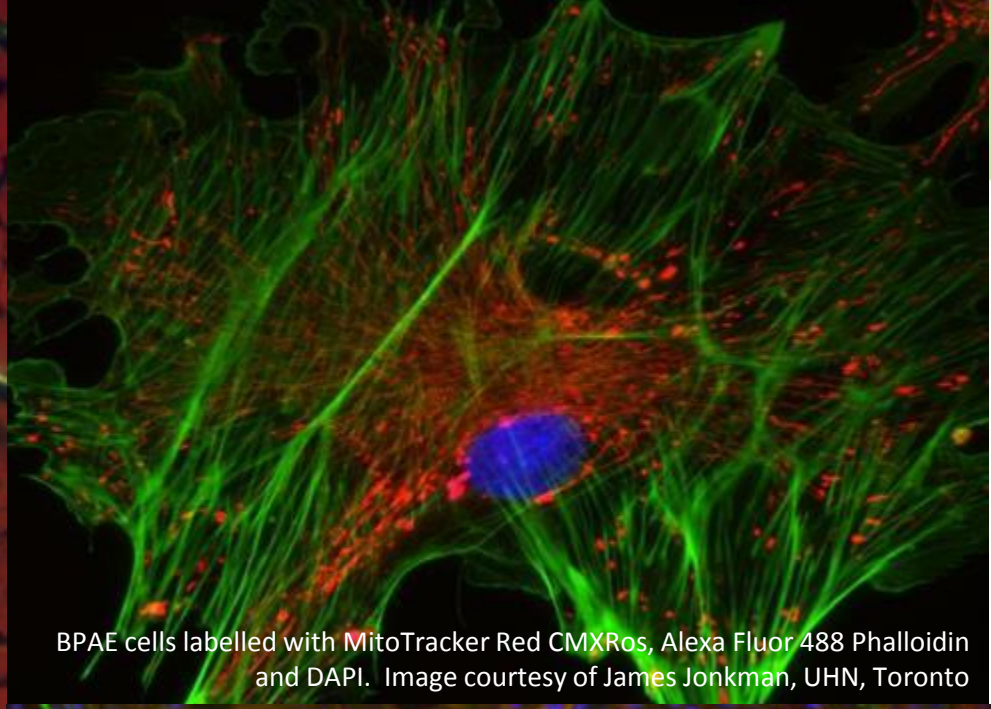
	Initial cost	1 year	3 year	5 year	10 year
HBO system	$4000	$5300	$7900	$10500	$17000
HBO with shutter	$6000	$7612	$10836	$14060	$22120
X-Cite® 120Q	$4995	$5847	$7641	$9435	$13920
X-Cite ® 120LED	$6495	-	-	-	-

Wavelength choices

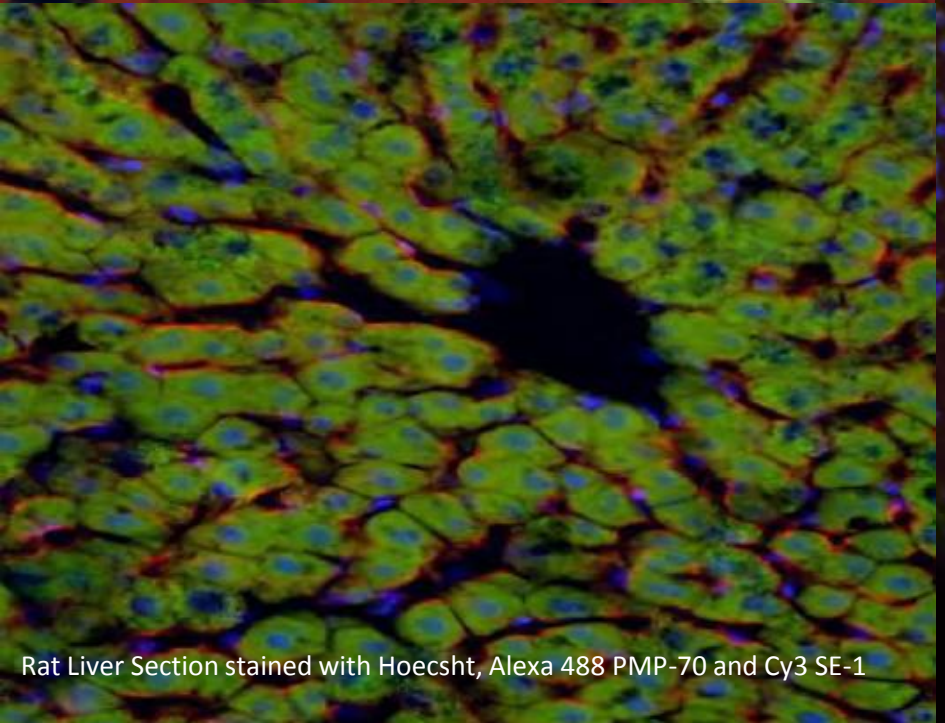




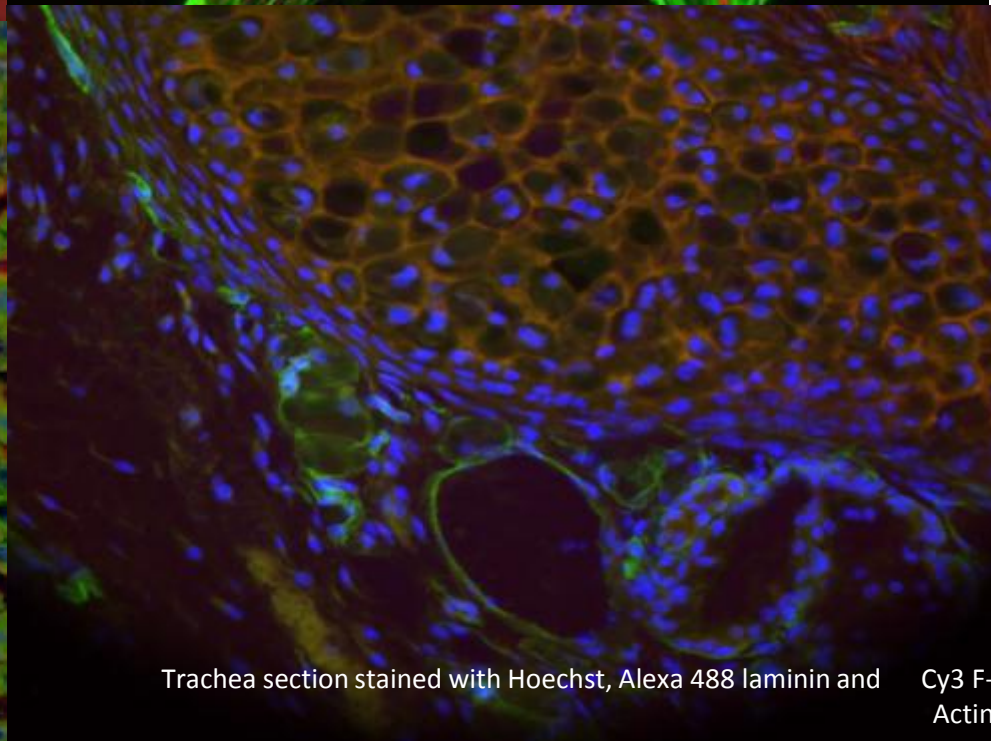
Tongue Autofluorescence



BPAE cells labelled with MitoTracker Red CMXRos, Alexa Fluor 488 Phalloidin and DAPI. Image courtesy of James Jonkman, UHN, Toronto

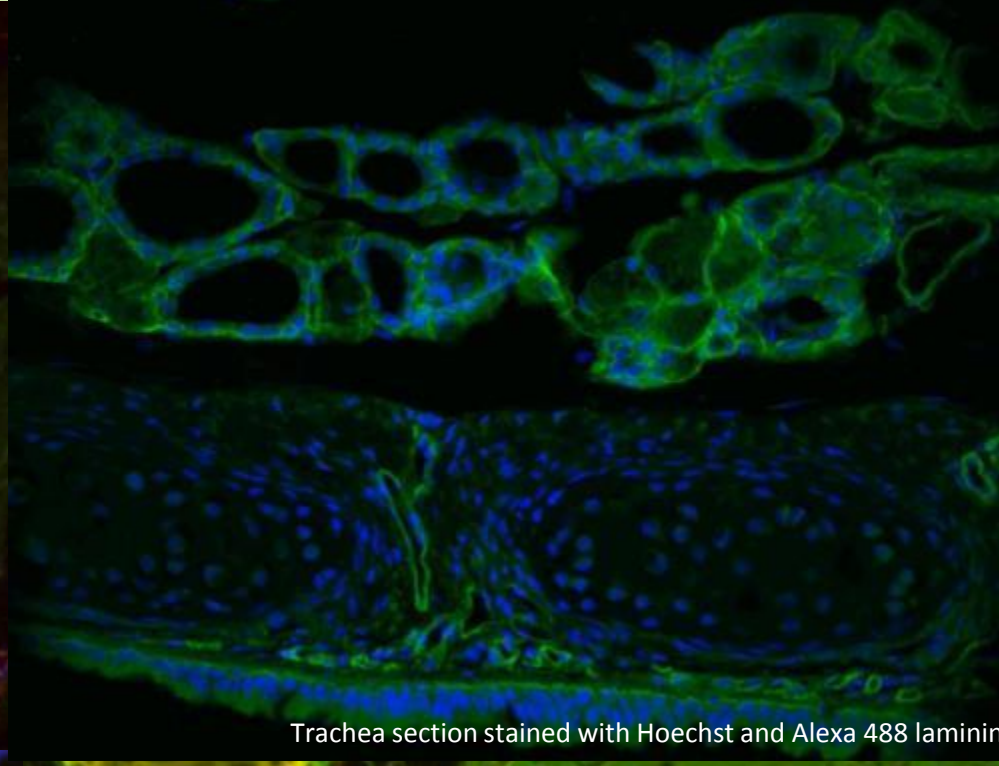


Rat Liver Section stained with Hoecsht, Alexa 488 PMP-70 and Cy3 SE-1

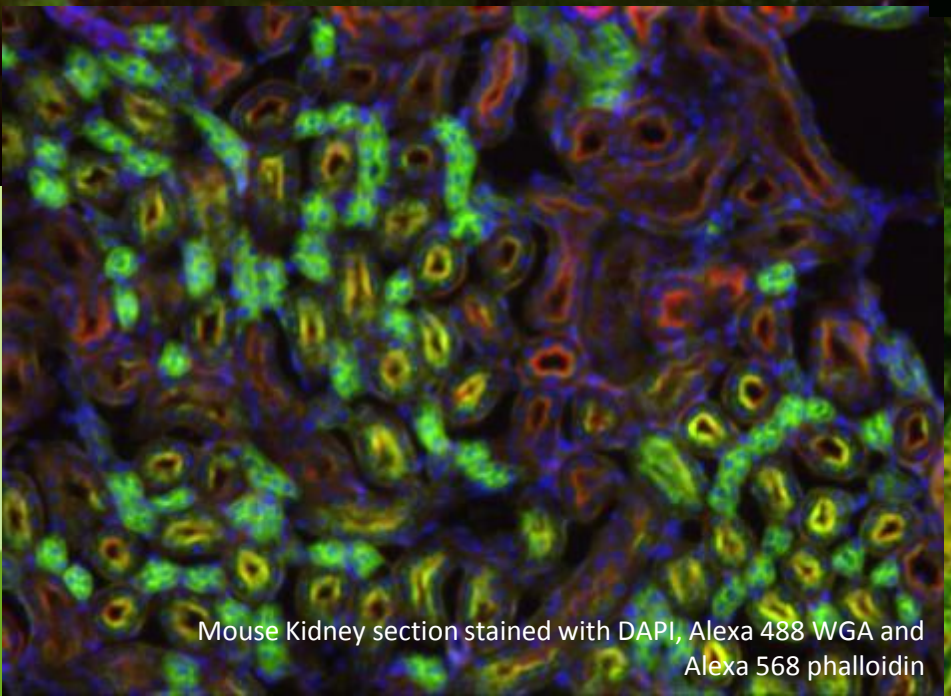


Trachea section stained with Hoechst, Alexa 488 laminin and Cy3 F-Actin

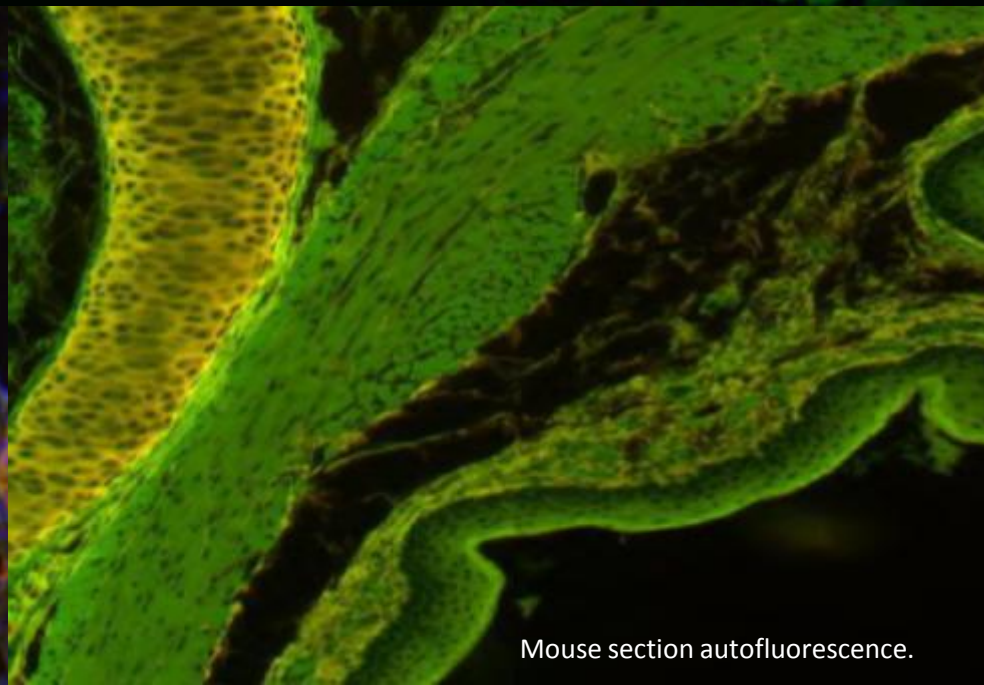
Skin section stained with Hoechst, Alexa 488 F-Actin, Cy3 Collagen IV



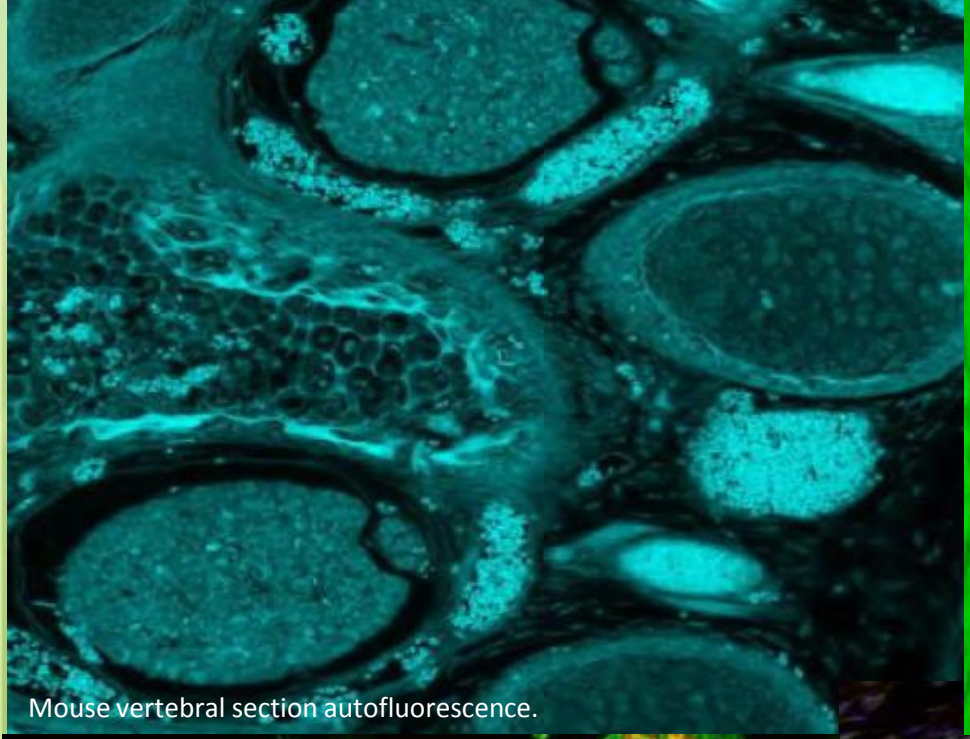
Trachea section stained with Hoechst and Alexa 488 laminin



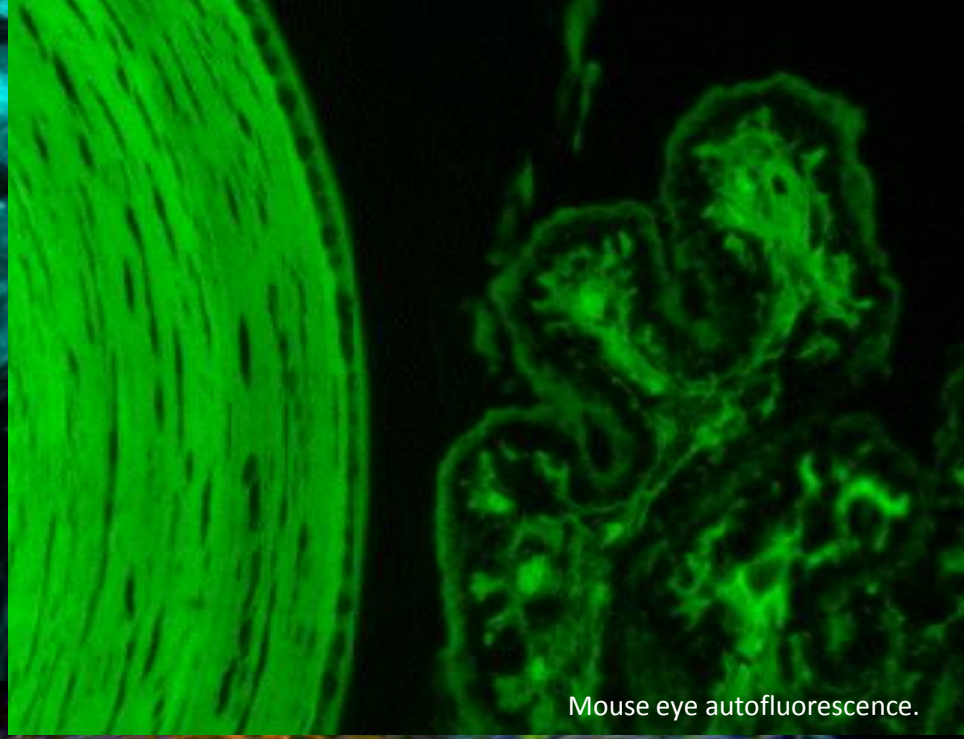
Mouse Kidney section stained with DAPI, Alexa 488 WGA and Alexa 568 phalloidin



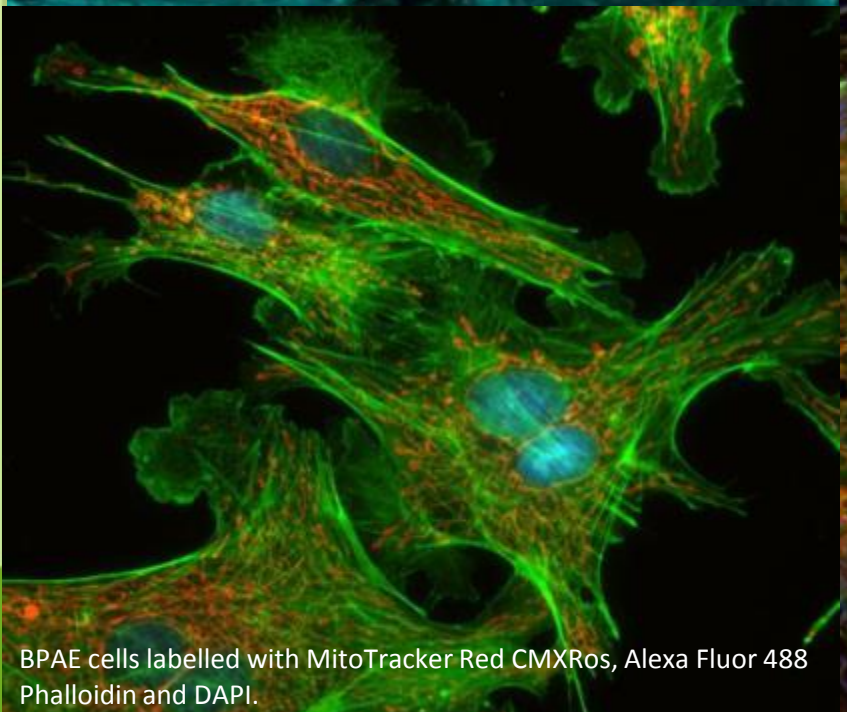
Mouse section autofluorescence.



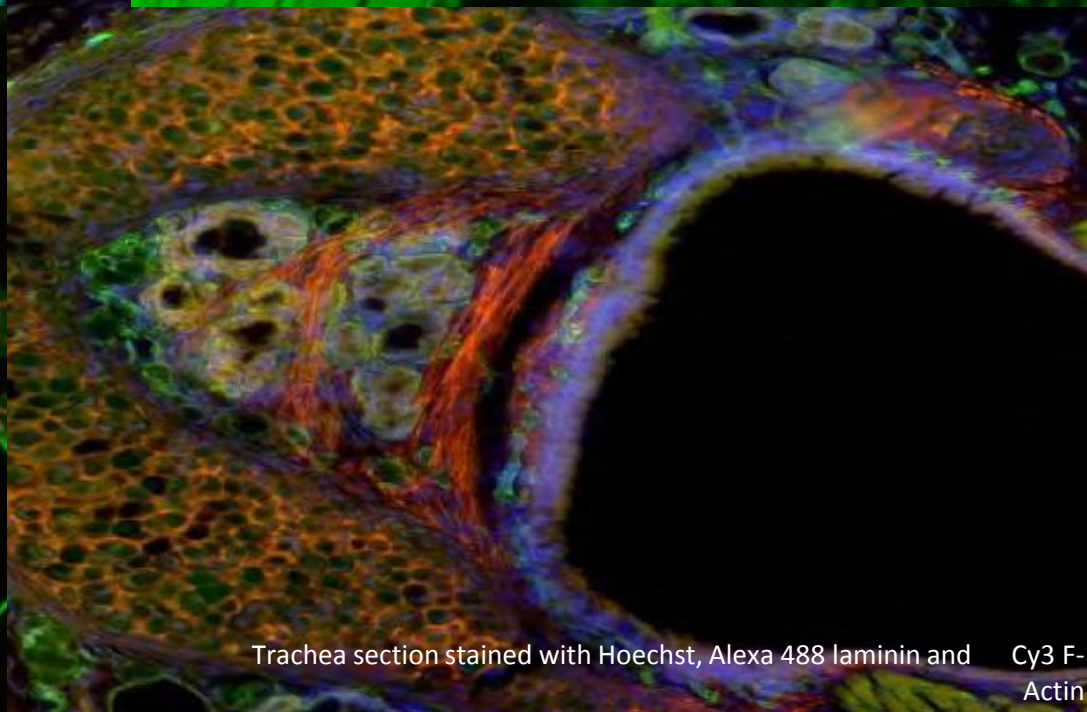
Mouse vertebral section autofluorescence.



Mouse eye autofluorescence.



BPAE cells labelled with MitoTracker Red CMXRos, Alexa Fluor 488 Phalloidin and DAPI.



Trachea section stained with Hoechst, Alexa 488 laminin and Cy3 F-Actin