Light Sources for Microscopy

Focus on Fluorescence Microscopy

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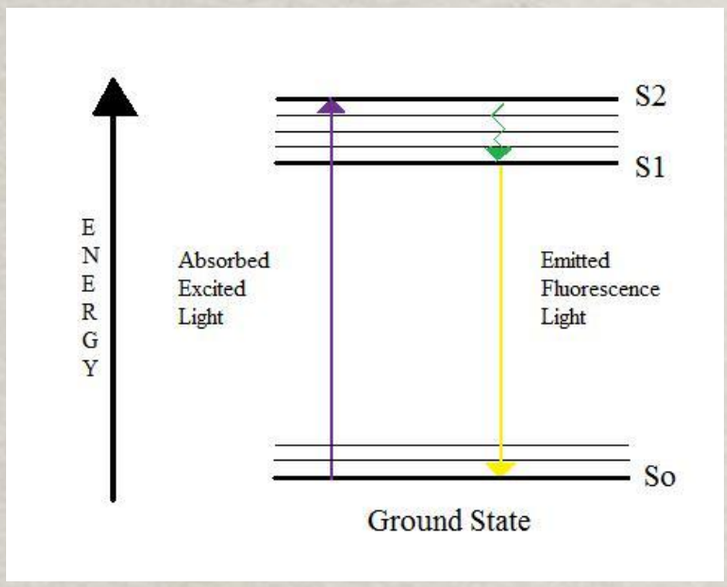
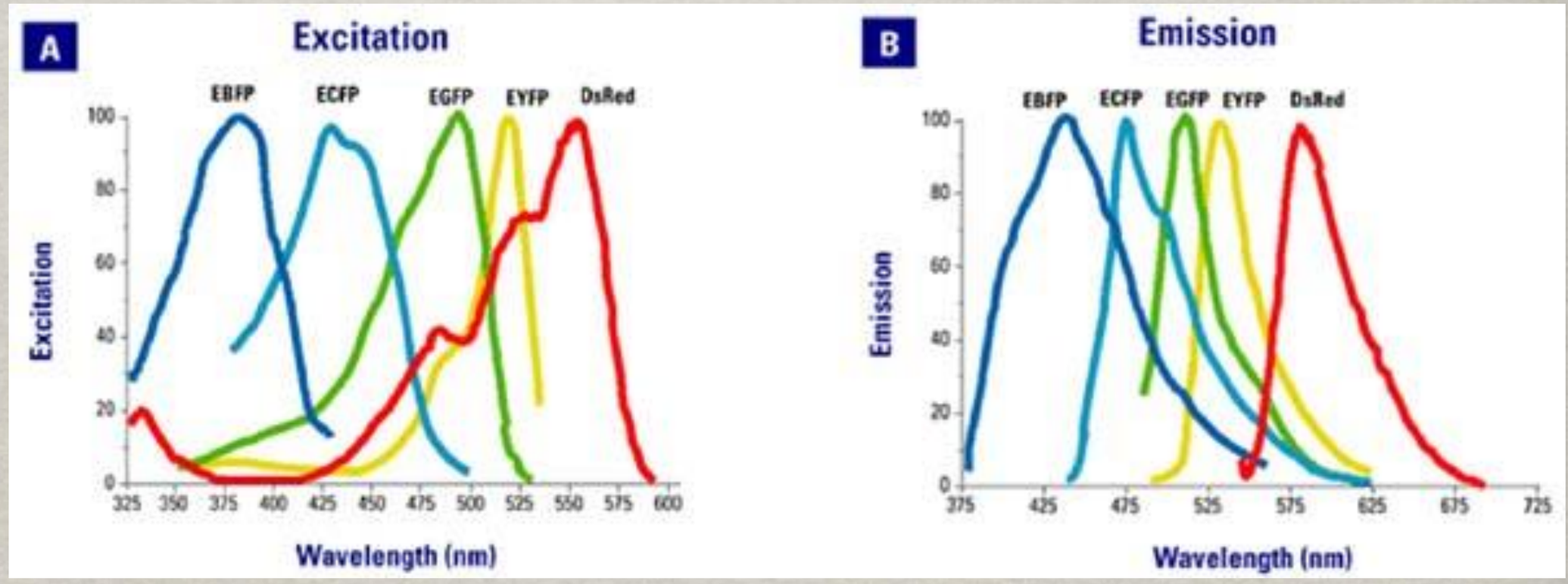
Jablonski Diagram

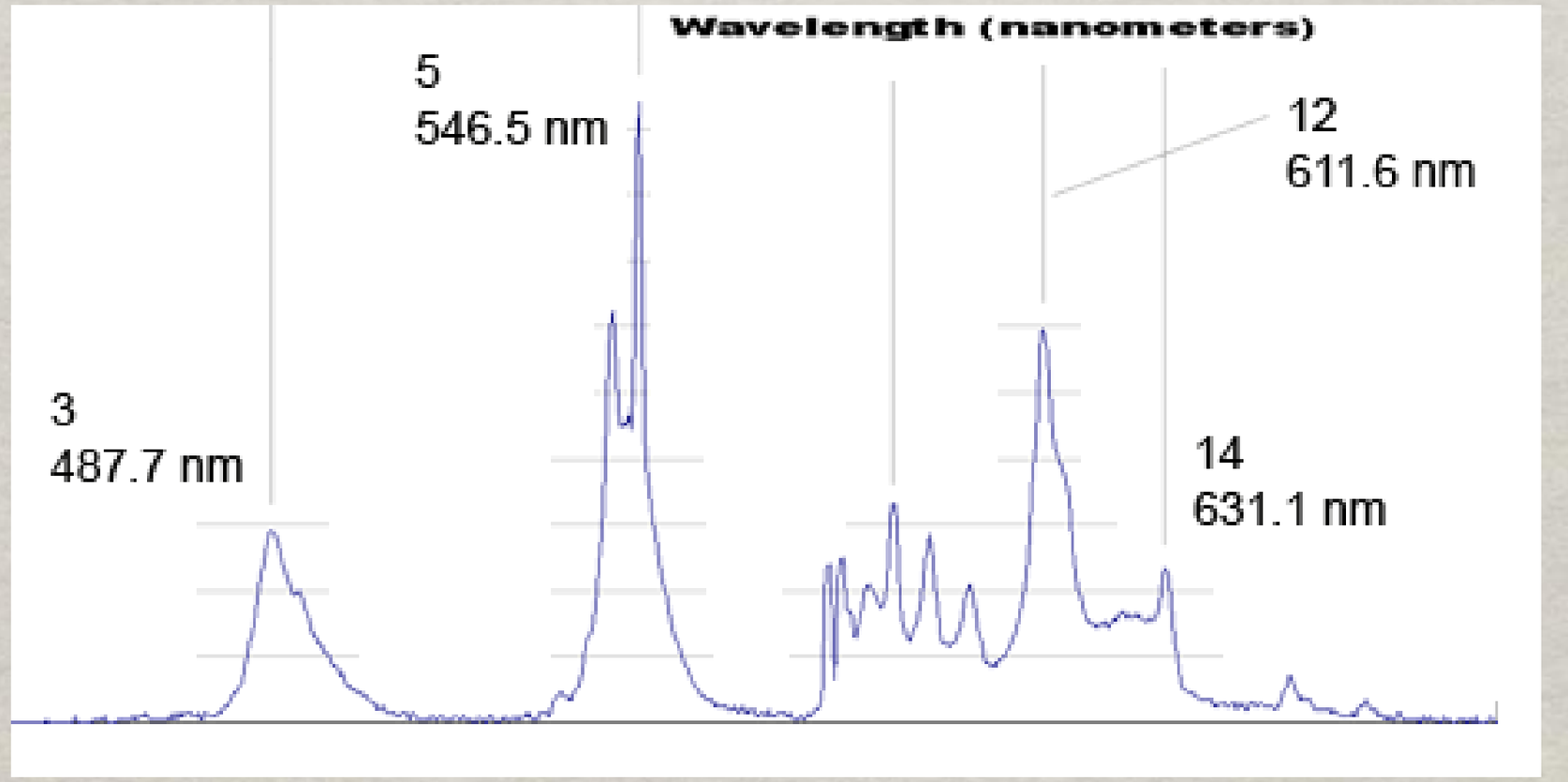


Fluorescence Spectra

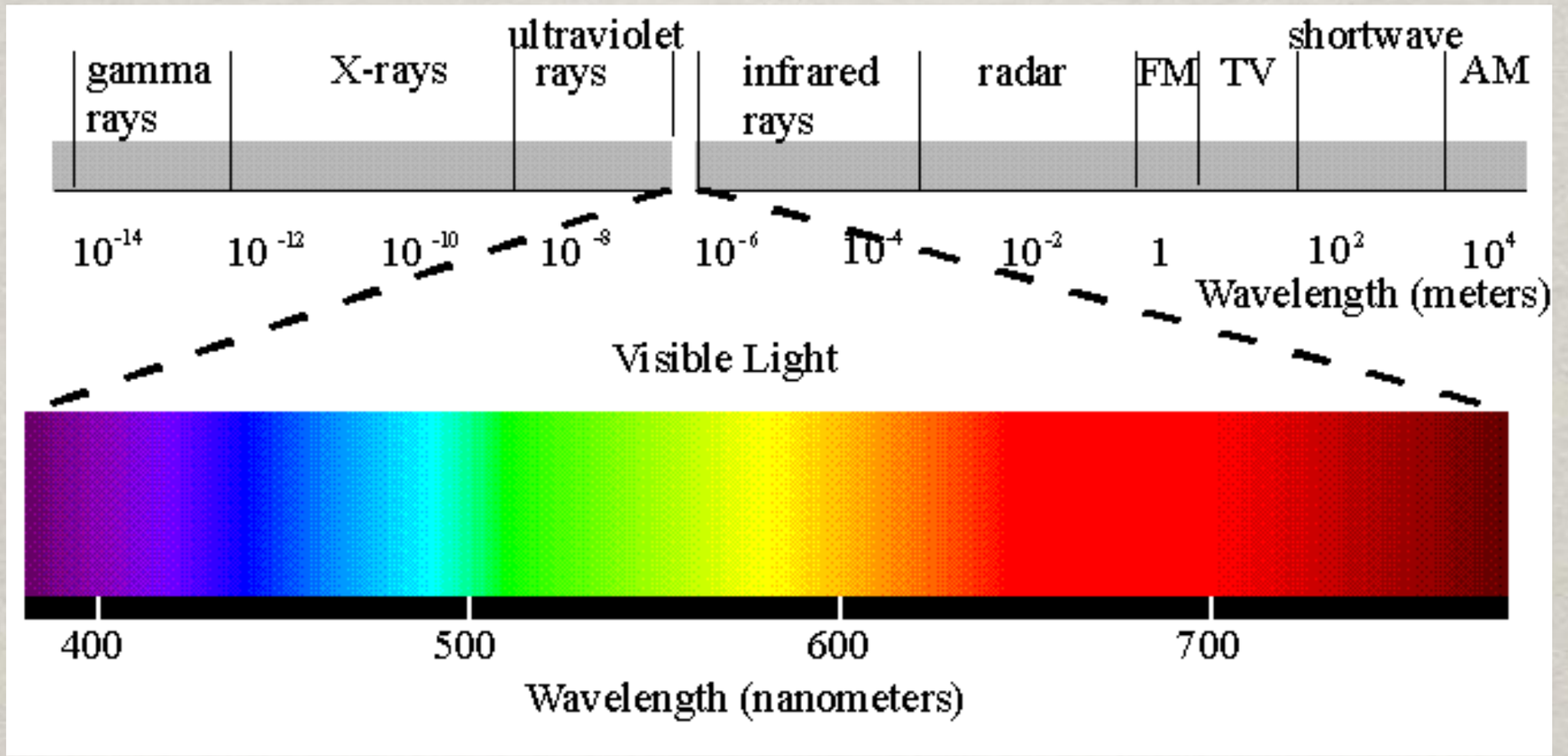


White Light provides All Wavelengths?

Spectra of White Florescent Light



Spectrum of Light



Common Sources In Microscopy

- Natural
- Filament
- Arc
- Solid State

Illumination Needs

- Spectral Requirements
- Directed
- Intense
- Even Illumination

Natural sources of light

Sunlight

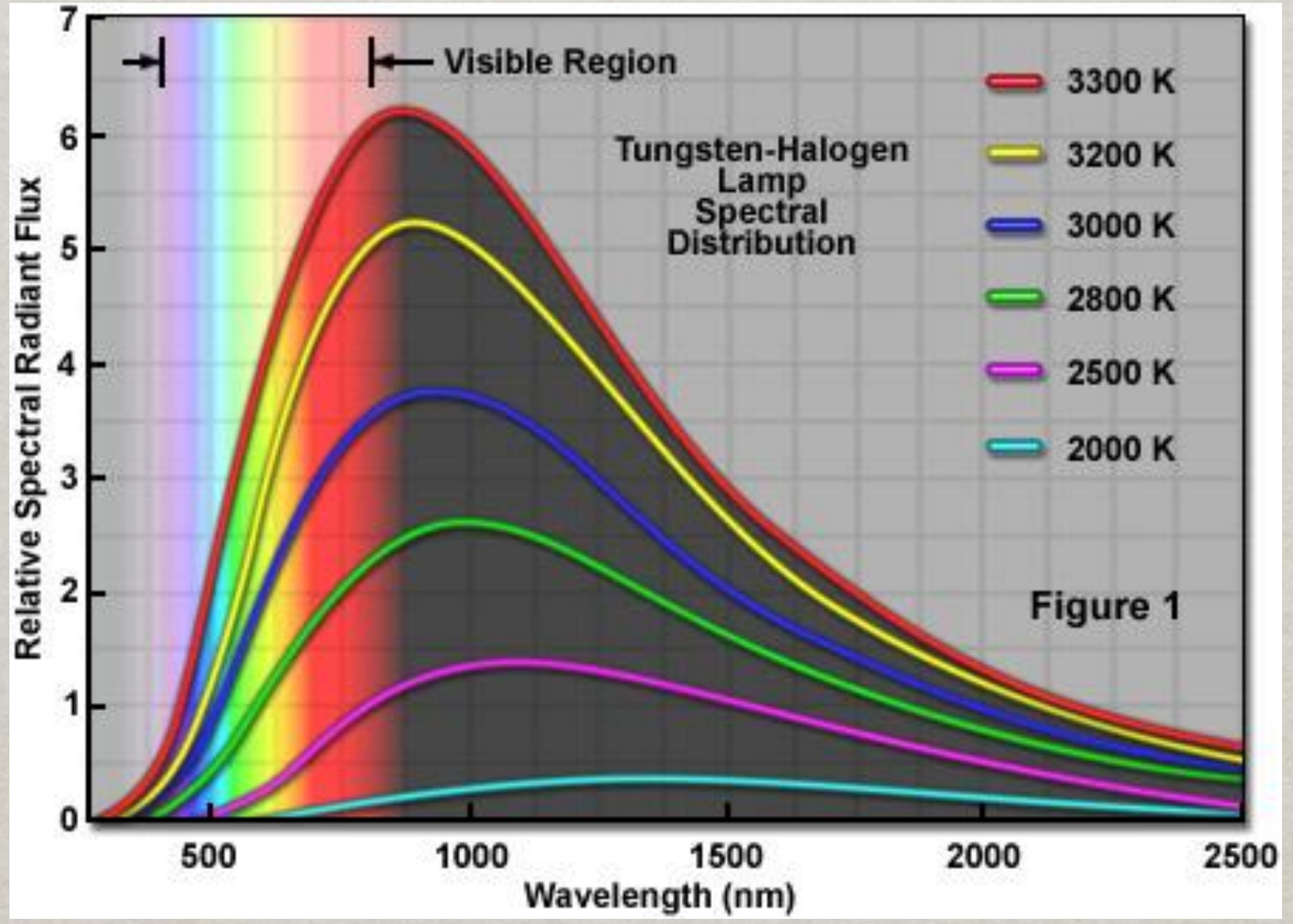
Full Spectrum
Difficult to Harness
No Overnight Experiments

Fire

Spectrum Depends On Fuel
Difficult to Control
Avoid Wind!

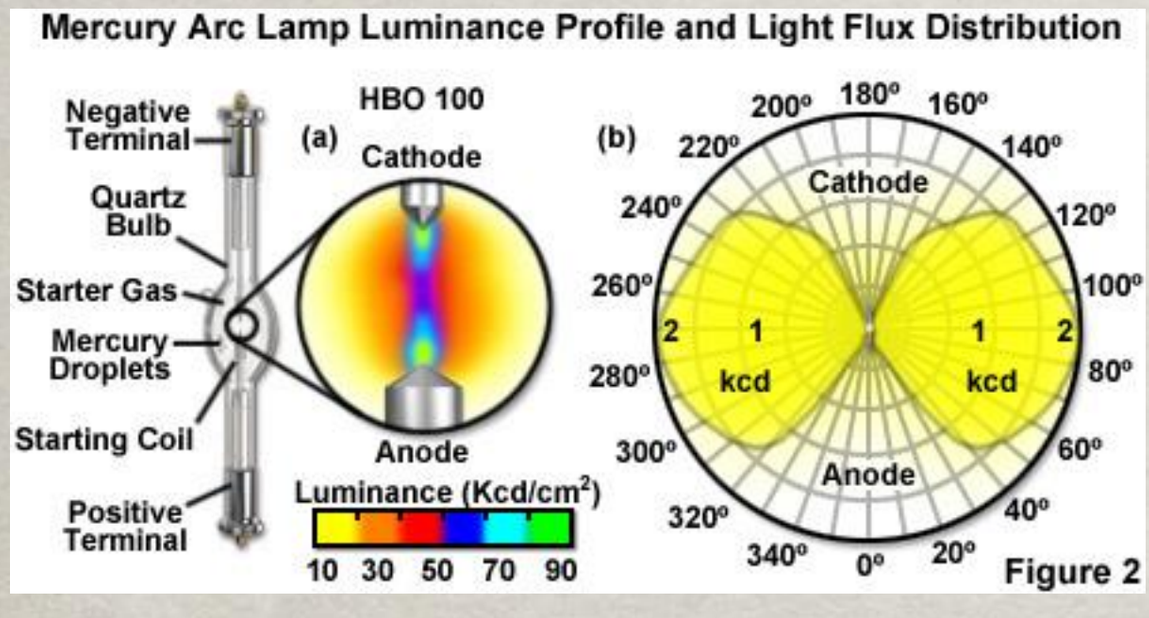
Filament Lamps

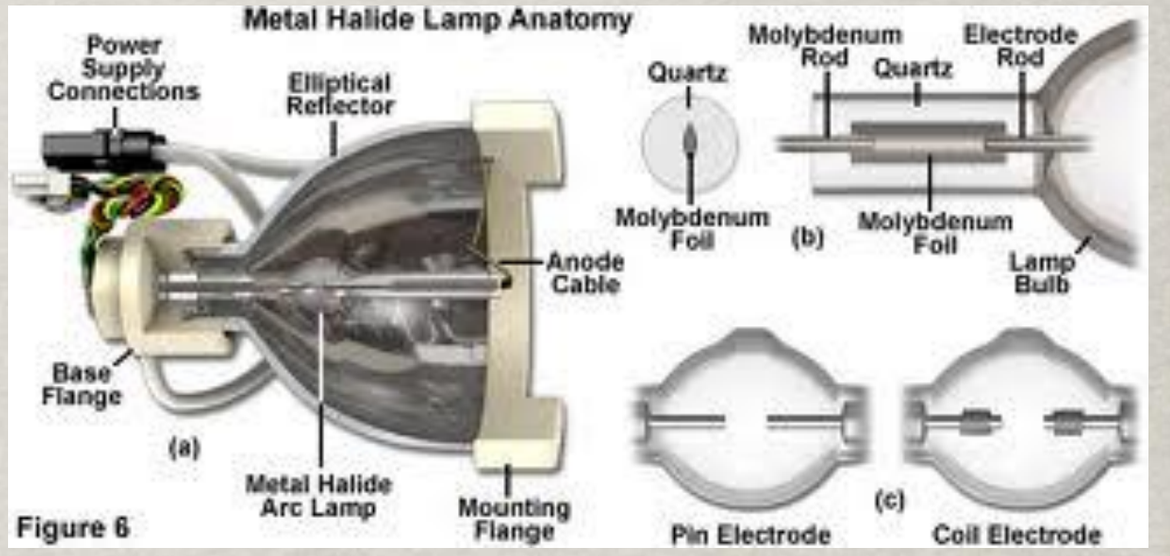
Halogen Lamp Spectra

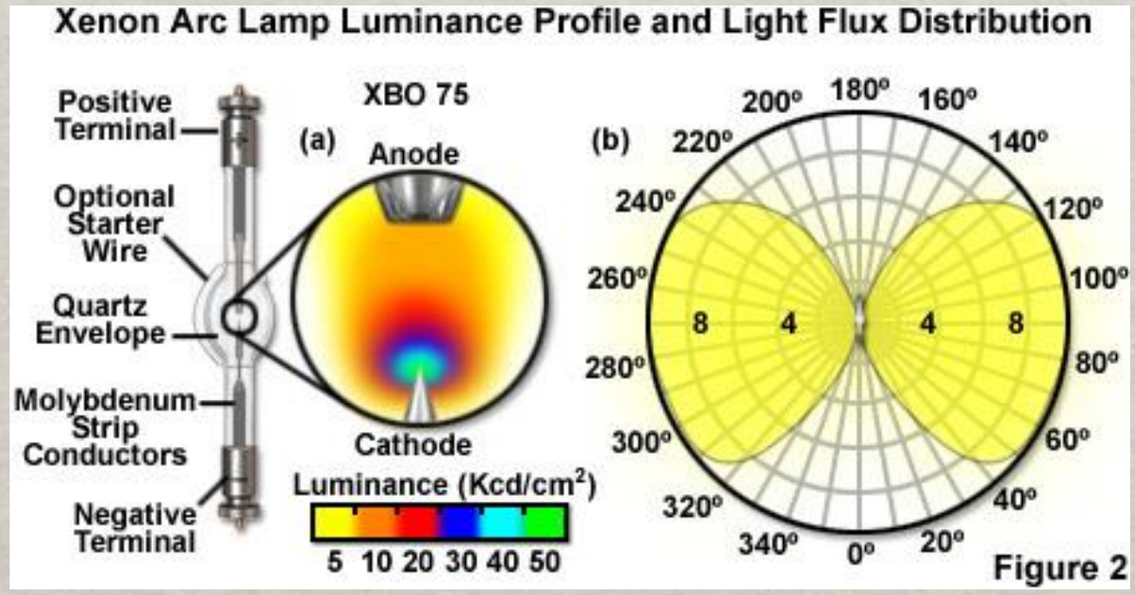


Typically Used for Brightfield Imaging Techniques

Arc Lamps

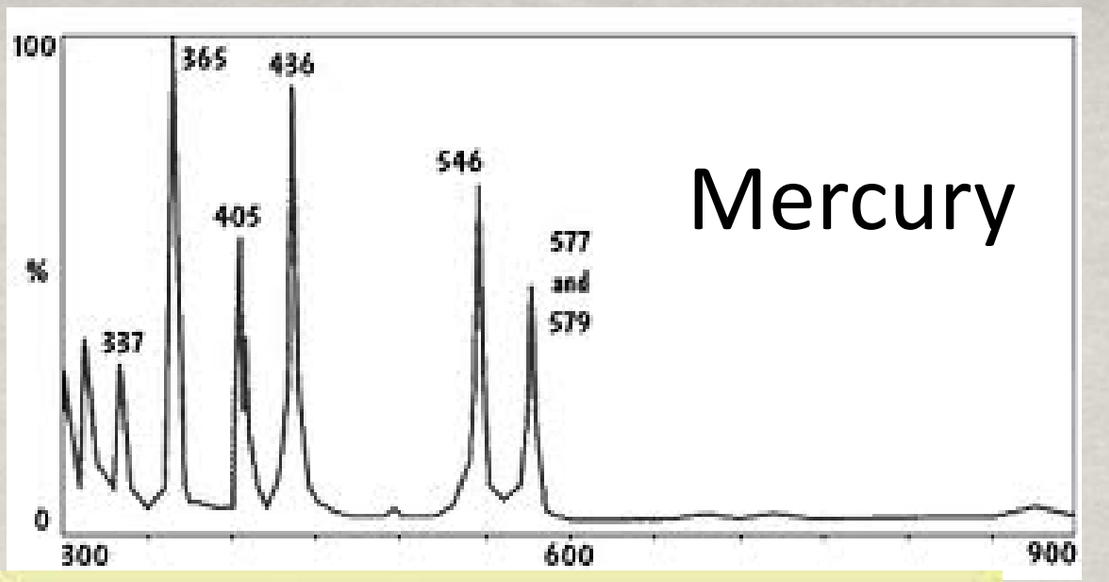




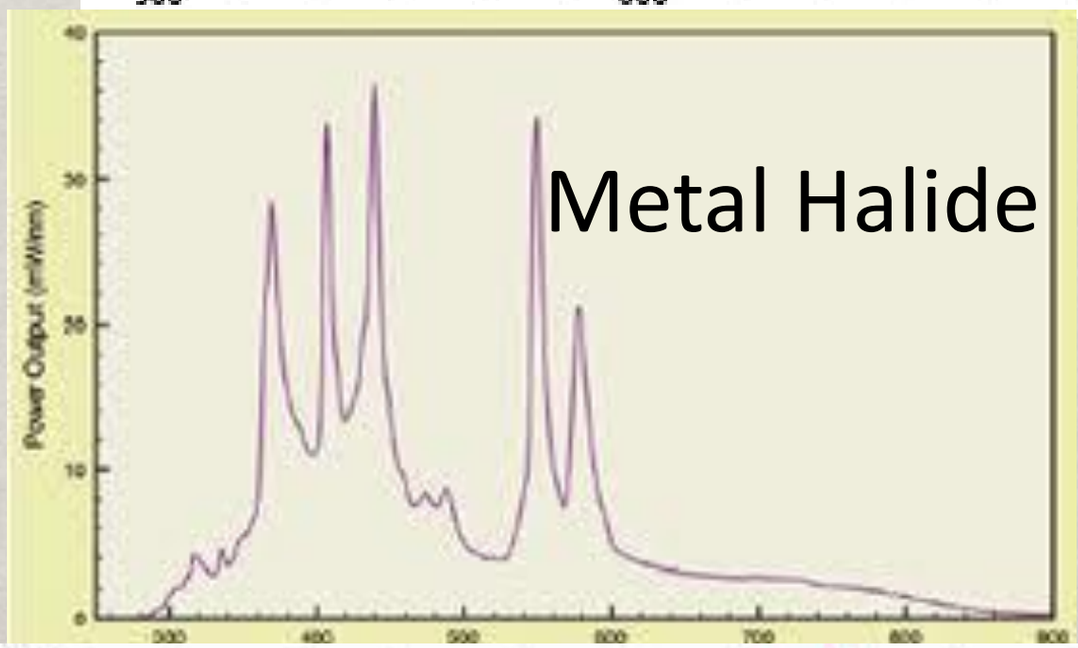


http://zeiss-campus.magnet.fsu.edu/

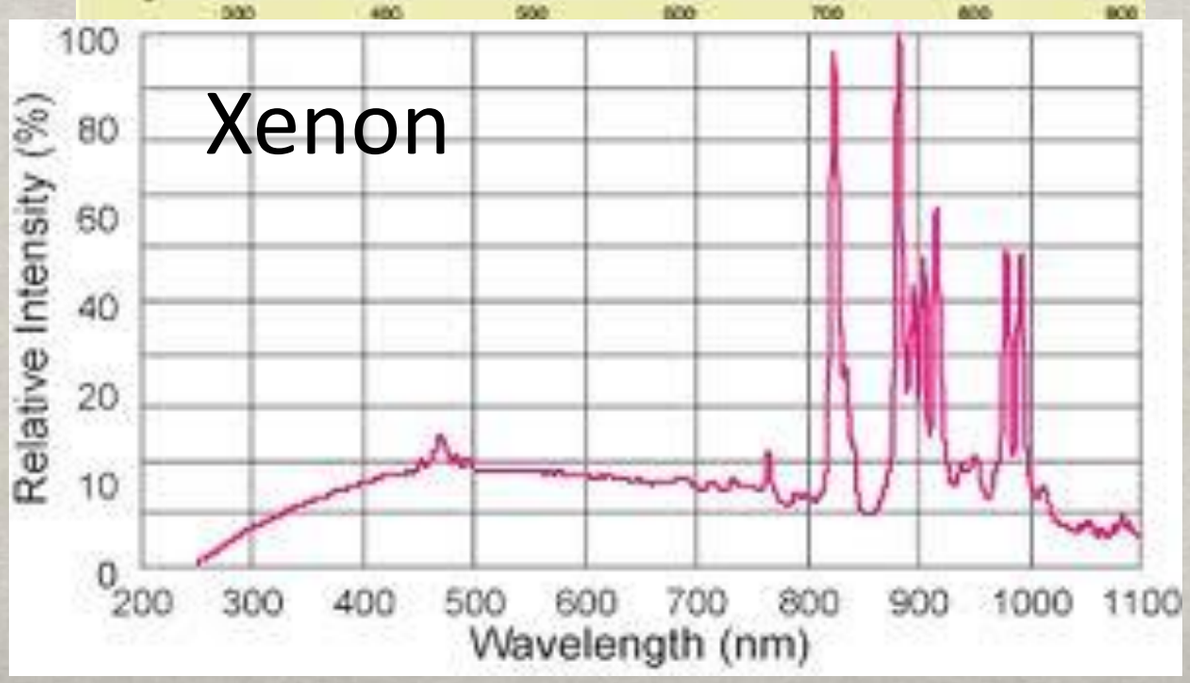
Peak levels are not comparable



Mercury

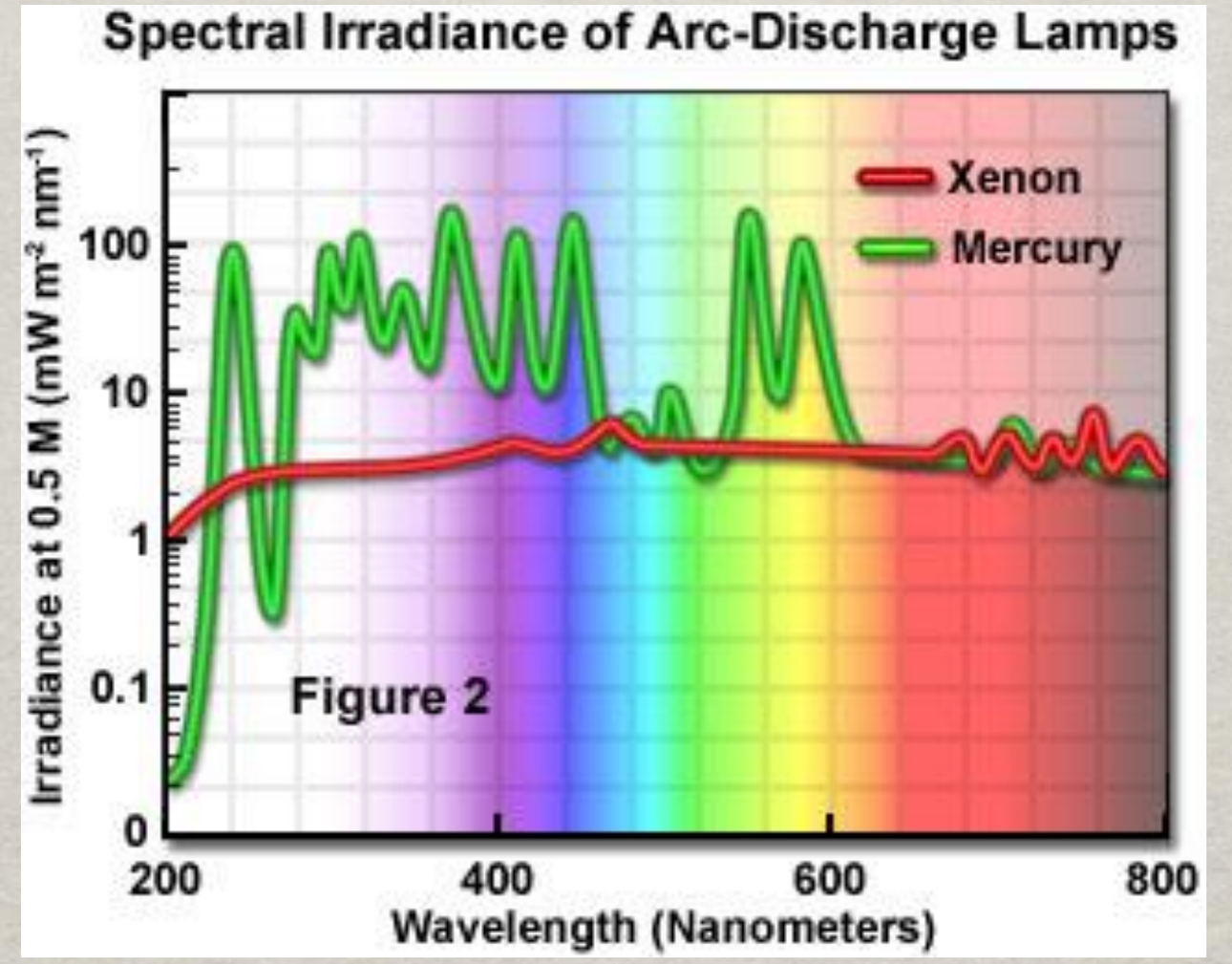


Metal Halide



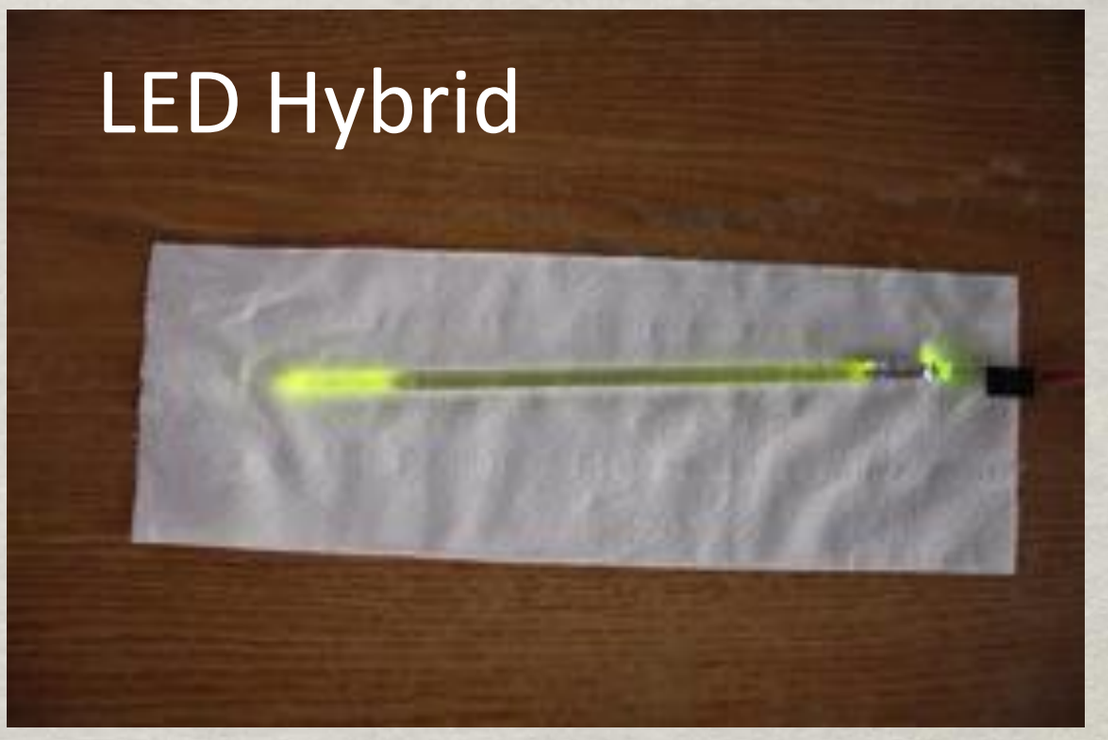
Xenon

Spectral Comparison



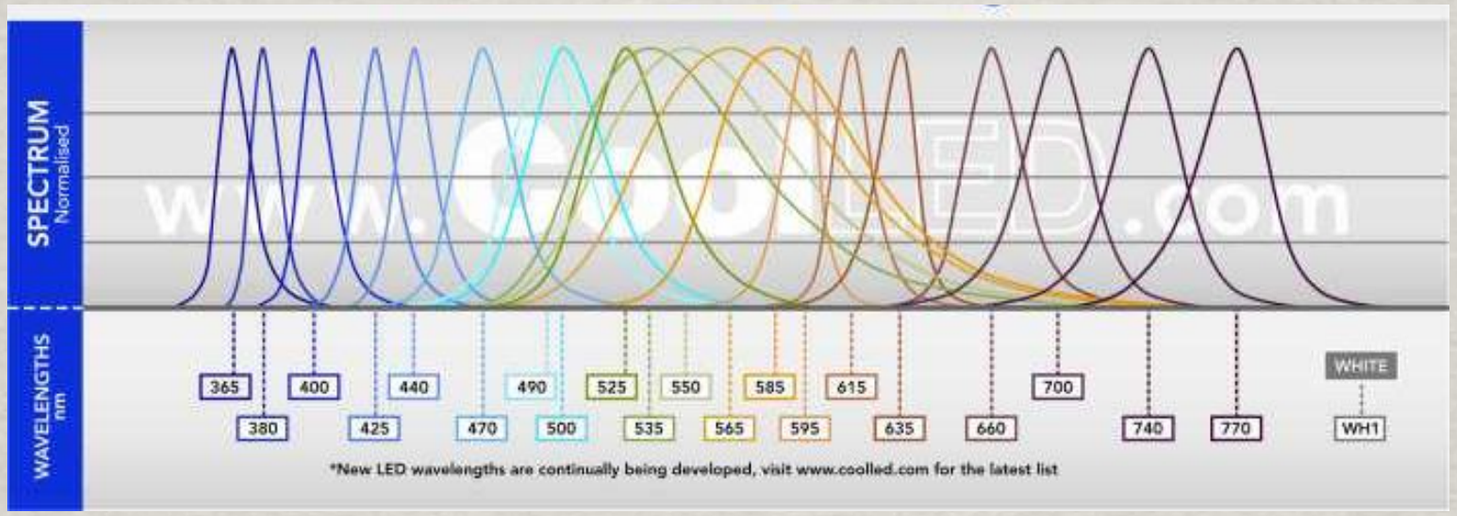
340nm and 380nm make Xenon ideal for Fura-2 imaging (Ca++)

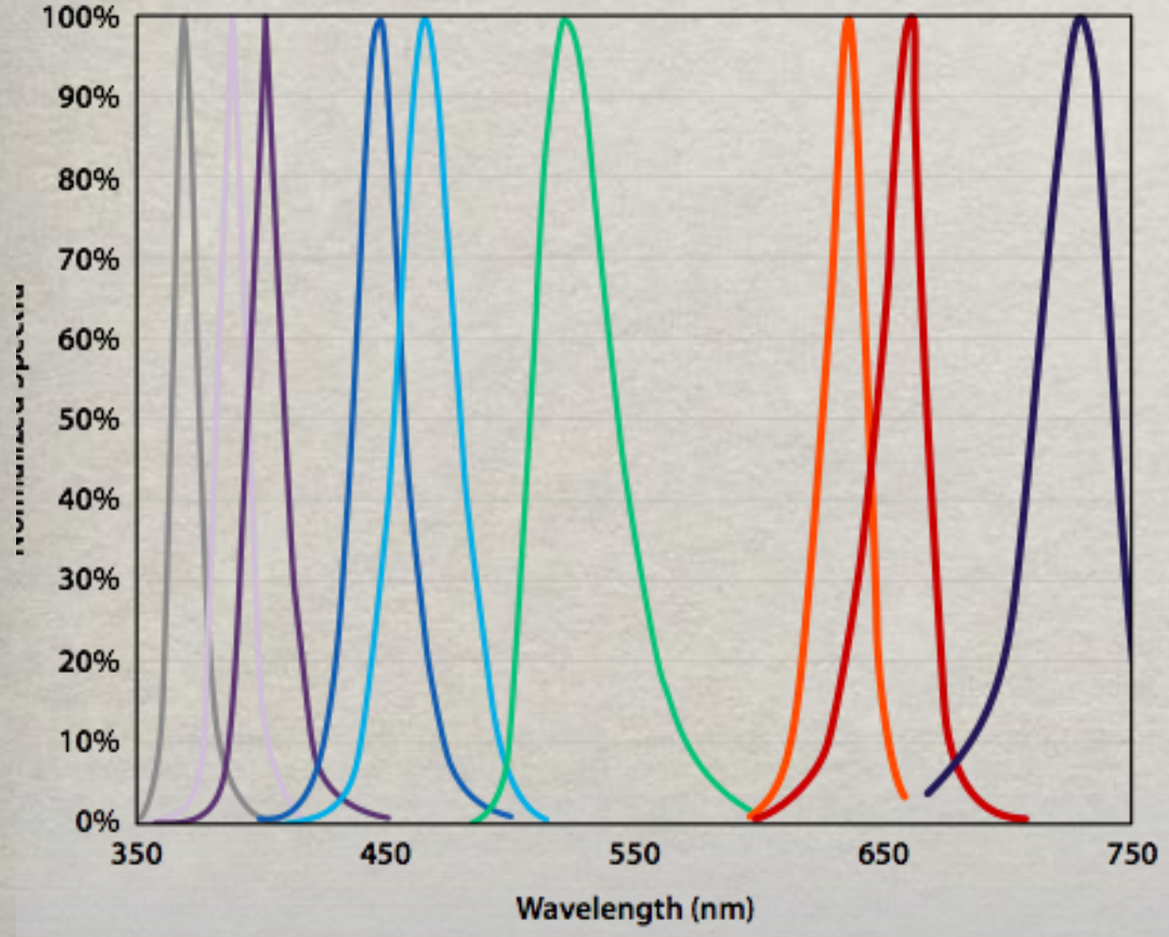
Solid State Sources

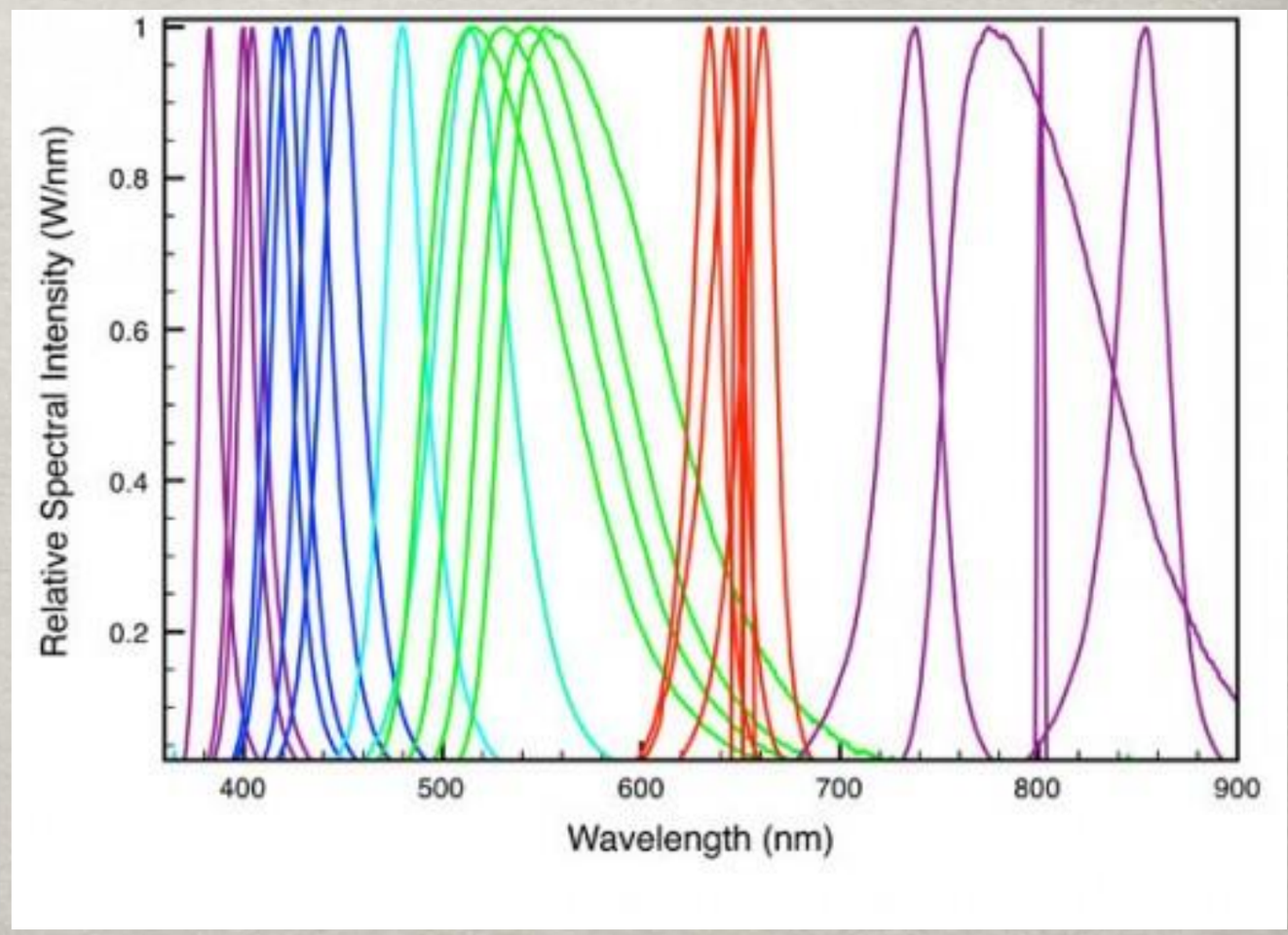


Light Emitting Diode

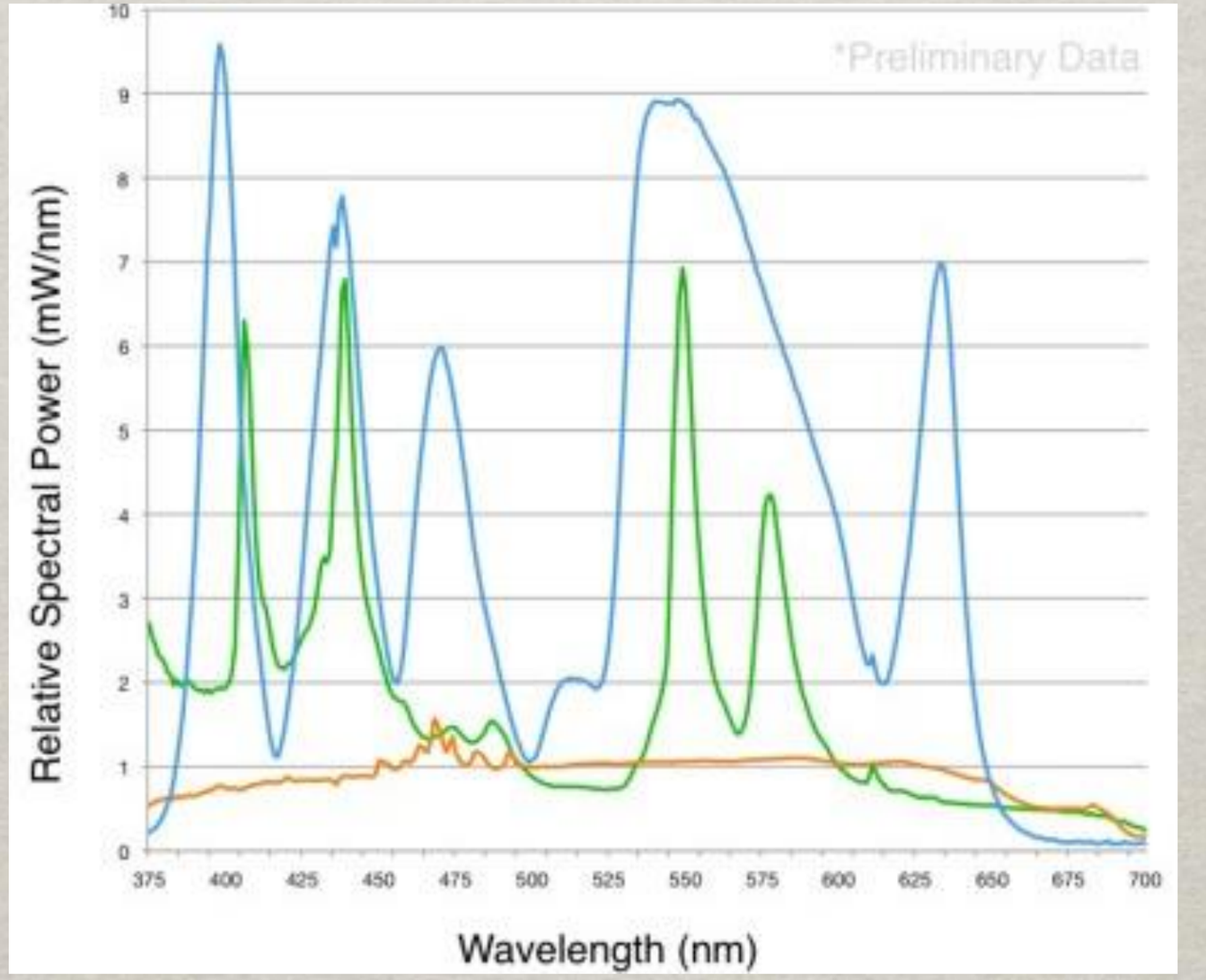
Don’t be fooled by the peak heights



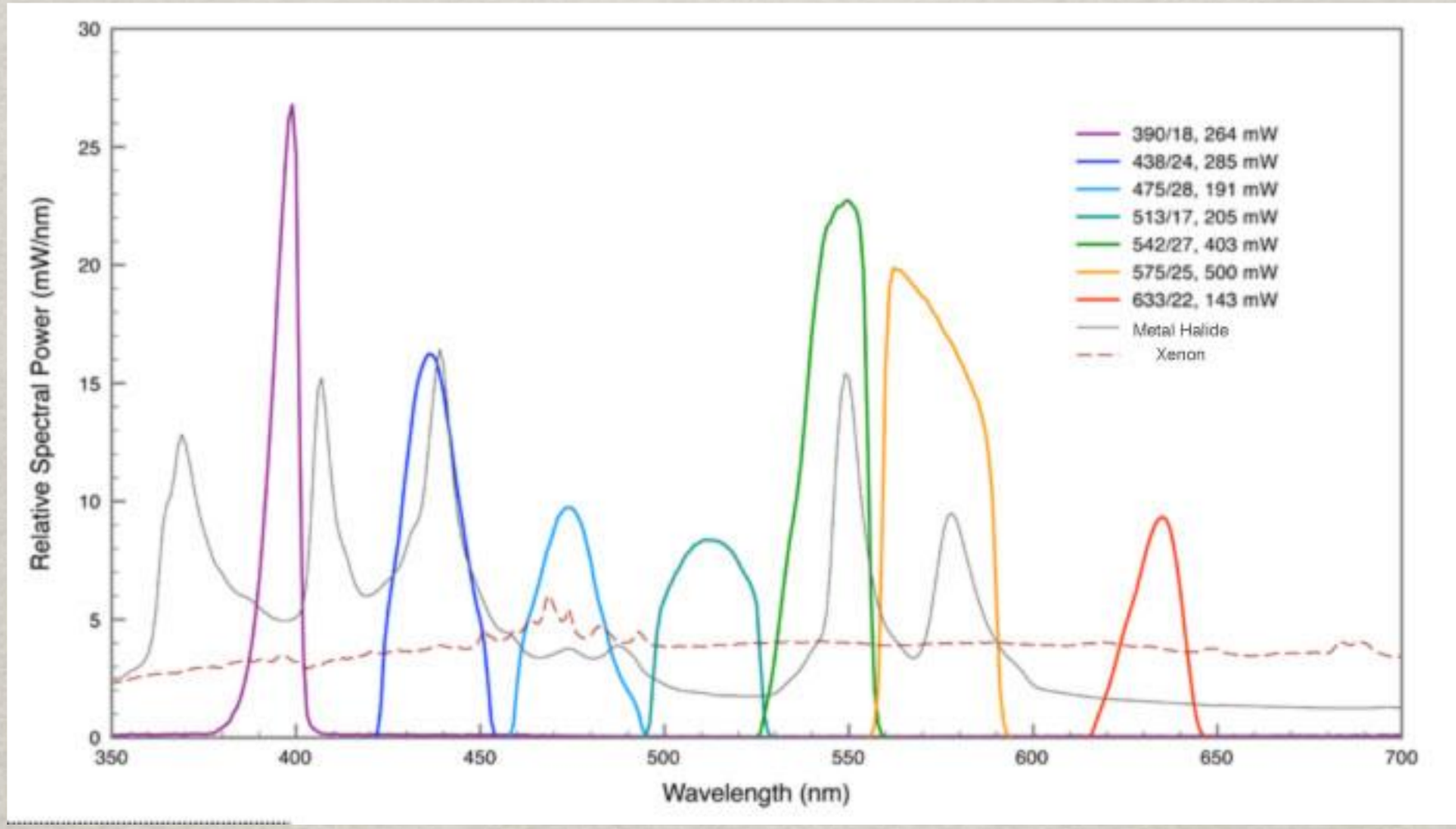




Light source Comparison



Filtered LED Source



Light Source Comparison

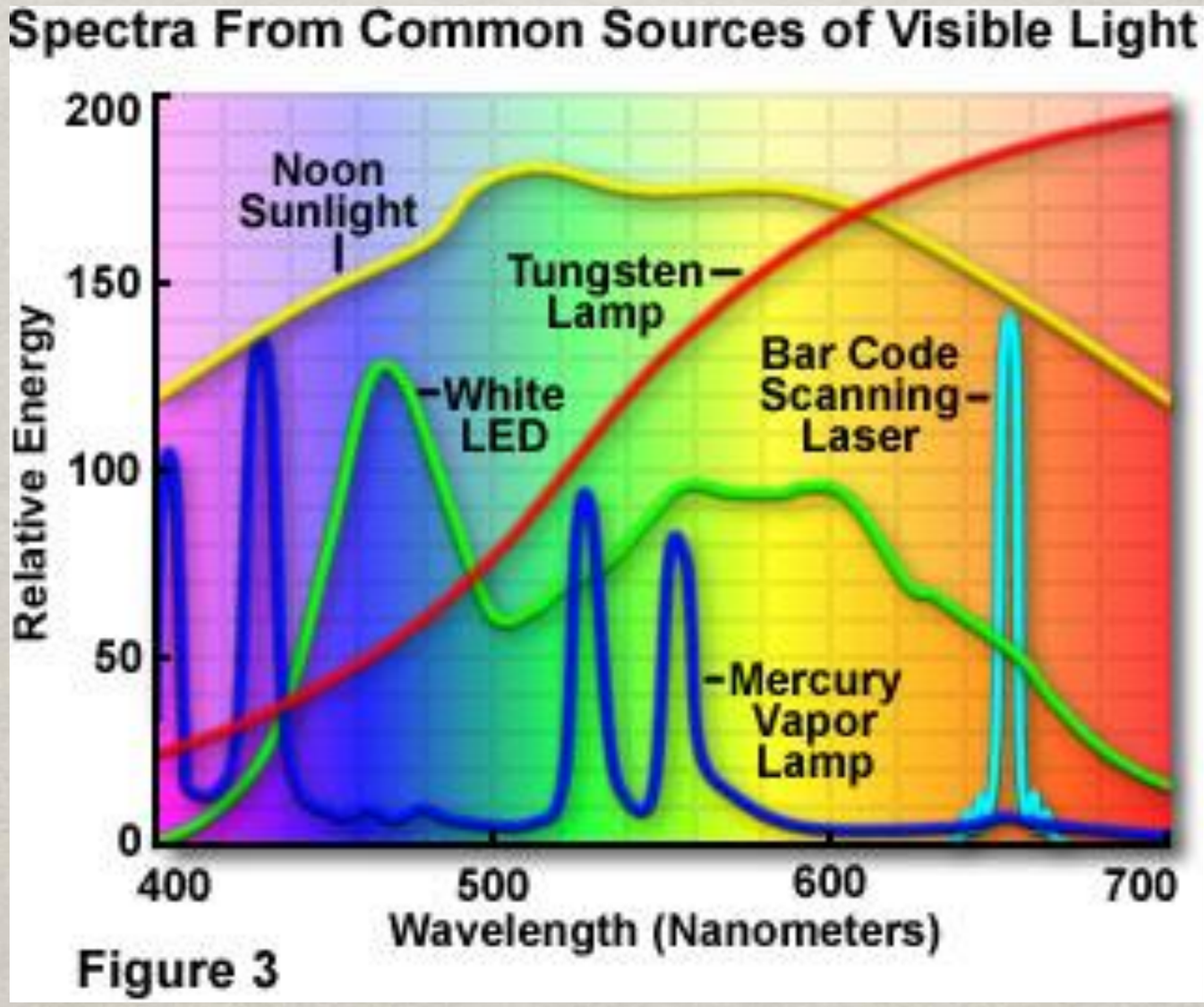


Figure 3

Common Fluorescence SOURCES

Source	Lifetime	Stability	Consumable Cost	Wavelength Range	Warm-up	Shutter/Switch Speed	Alignment	Time to 50% degradation (hr)	Flicker	Hazard	Heat	Green
Mercury	100	-	$200	UV - 600	30 min	External	+++	Varies	++++	++++	++++	NO
Metal Halide	2000	+	$600	350-600	5 min	External	-	200	++	+++	++++	NO
Xenon	1000	++	$600	340-1000	5 min	External	-	500	+	++++	++++	NO
LED	20000	++++	0	360-800	>1 min	5kHZ	-	20000	--	---	-	YES
Lasers	10000	++++	0	UV-IR	> 1 min	1MHz	-	10000	-	---	-	Yes

Thoughts on Light Source Comparisons

- Use Compatible Filters!!!
- Where does the measurement matter most?
- What is the unit of measure?
- What is the area of illumination?
- What is the hour number on the source?
- What is the cost of ownership?