Life Sciences – Light Sources

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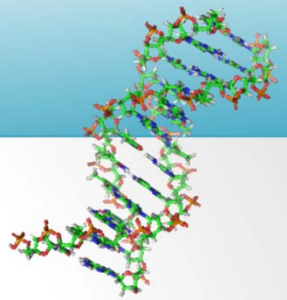
#TheVisionShow

Course Goals

- Almost all imaging systems utilize some type of controlled illumination source.
- Many different technologies and techniques associated with lasers, LEDs, and more traditional light sources are heavily utilized in life sciences and medical applications.
- This course will help you gain a better understanding of Life Science applications and the differences in capabilities between lighting technologies ranging from:
  - Brightness
  - Power outputs
  - Ease of integration
  - Power stability for more reliable results
  - Life time expectations
  - Ease of customization
  - Long term cost controls

“Life Science”



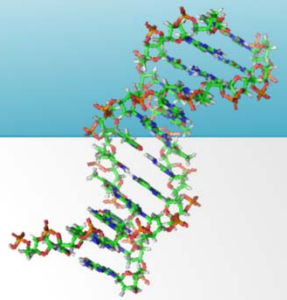
What does this mean?

- Traditionally, the term life sciences has referred to several branches of science, such as biology, medicine, anthropology, or ecology, that describe living organisms and their organization, life processes, and their relationships to each other and their environment.

How does this relate to me?

- “Life Sciences” is defined as companies in the fields of biotechnology, pharmaceuticals, biomedical technologies, life systems technologies, biomedical devices and organizations and institutions that devote the majority of their efforts in the various stages of research, development, technology transfer and commercialization.

"Life Science"



Business potential?

- Life sciences firms are moving beyond traditional industry boundaries to create new health care solutions for patients and providers.

- By combining life sciences' core technologies (diagnostics, devices and drugs) in innovative ways, firms are creating new technology platforms and products that offer improvements in safety, effectiveness, convenience and value.

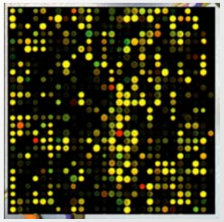
Illumination in Life Science

Lighting subsystems to drive today's bio-analysis and biomedical diagnostics face stringent requirements.

Industry-wide demands for speed, accuracy and portability mean illumination must be intense as well as spectrally pure, switchable, stable, durable and inexpensive.

Ideally, a common lighting solution could service these needs for numerous research and clinical applications.

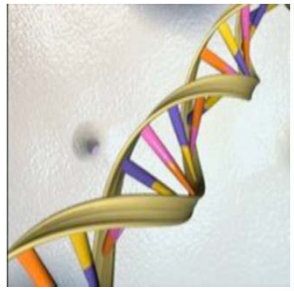
While this is a noble objective, the current technology of arc lamps, lasers, LEDs and most recently, light pipes, have intrinsic spectral and angular traits that make a common solution untenable.



Illumination in Life Science

Any solution begins with a critical understanding of the instrument architecture and specifications for illumination regarding:

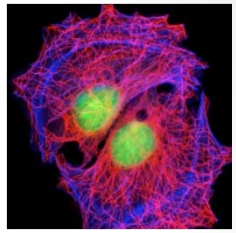
- power
- illumination area
- illumination and emission wavelengths
- numerical aperture



Optimizing signal to noise requires careful optimization of these parameters within the additional constraints of instrument footprint and cost.

Often the illumination design process is confined to maximizing signal to noise without the ability to adjust any of the above parameters.

Illumination in Life Science



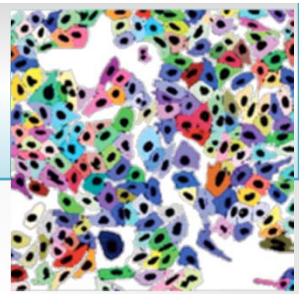
Optimal signal to noise ratios are characterized quite differently for the vast array of life science applications.

For example, fluorescent imaging of live cells generally requires maximum light flux and minimum exposure times timed precisely to the camera's exposure setting. This maximizes the signal while reducing cell toxicity and photo bleaching of the fluorescent tags.

> Only the light flux that reaches the illuminated sample area captured by the CCD camera is of value.

For fluorescing organic molecules, the angular distribution of the illumination is not critical since the absorption process is independent of incident angle. Therefore, in this case, the optimal illumination system will maximize flux over the imaged area or intensity (W/cm2).

Intensity



While optimizing intensity seems straight forward, delivering optimal intensity requires an understanding of a key optical invariant, étendue, in conjunction with the illumination delivery system.

Most simply, étendue determines the maximum light flux through an optical system.

The term étendue comes from the French word for extent or spread. Étendue characterizes the spread of the light in area and angle.

- From the perspective of a chemist, it is the entropy of the ray bundle.
- To a physicist, it is the area of the source times the solid angle.

Étendue

$$\textbf{Etendue} = \pi(Area)NA^2$$

Within any bioanalytical instrument, the étendue is the area of the entrance pupil times the solid angle of the source subtended as seen by the entrance pupil.

Like entropy in a chemical system, étendue can only increase in any optical subsystem.

In a perfect optical system, the image produced possesses the same étendue as the source.

More typically, there are other constraints limiting this ideal circumstance and so optimization requires matching these étendues as best as physically possible.

Table of Typical Étendue

System	Size (mm)	Area (mm^2)	NA	Coll. Angle	Etendue ($mm^2 \bullet sr$)
Delivery Light Guide					
Single Fiber	0.1	0.008	0.22	12.7	0.0012
Single Fiber	1	0.79	0.39	23	0.38
Liquid Light Guide	3	7.07	0.3	17.5	2
Fiber Bundle	5	19.6	0.6	36.9	22.21
Solid State Source					
Fiber Laser	0.2	0.031	0.22	12.7	0.0048
Single LED	1	1	0.9	64.2	2.54
LED 2x2 Array	2.1	4.4	0.9	64.2	11.22
Light Pipe	1	1	0.6	36.9	1.13
Microscope Objective					
Objective (10x/0.45)	2.5	4.91	0.45	26.7	3.12
With square diaphragm	1	1	0.45	26.7	0.64
Objective (20x/0.80)	1.25	1.23	0.8	53.1	2.47
With square diaphragm	0.75	0.56	0.8	53.1	1.13
Objective (40x/0.95)	0.625	0.31	0.95	71.8	0.87

Étendue

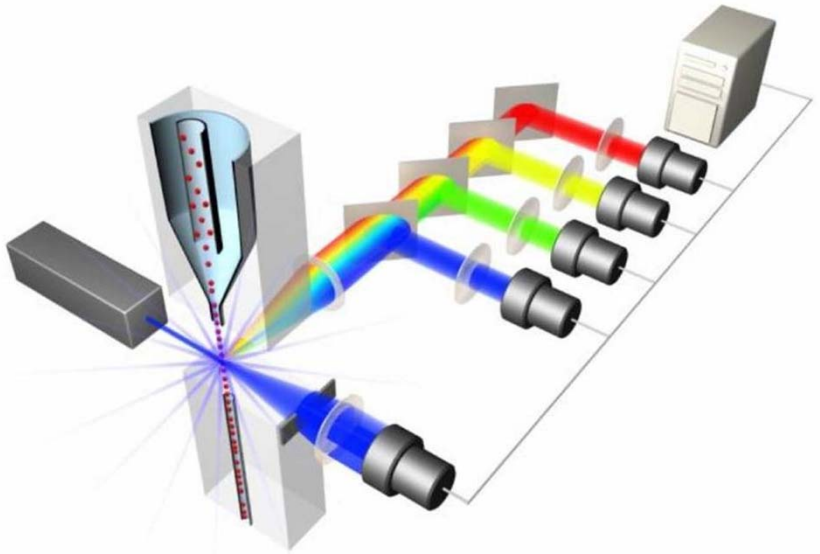
The table shows a wide range of étendue for fairly typical systems employed across the life sciences. It is the match between optical source and sink that is critical to providing the maximum intensity for the range of life science tools.

Maximizing intensity of the sample region necessitates taking into account the étendue of the illumination source **and** sink.

Key Applications – Flow Cytometry

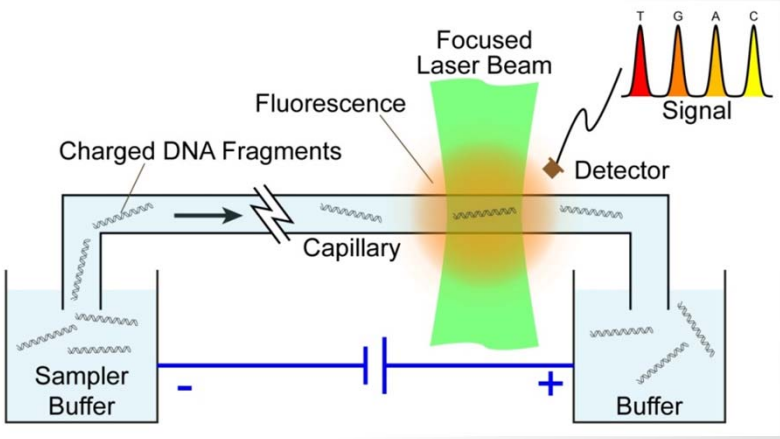
Flow cytometry is a technique for analyzing biological samples. The laser beam is directed to a hydro dynamically focused stream of samples stained with fluorescent components. An analyzer tool captures the information while a sorter features sample subset selection.

- Sources used are continuous wave visible and UV lasers, LEDs
- Powers from 20mW up to 5W dependent on throughput needs
- Target application areas : clinical diagnostics, sperm sorting, stem cell applications, research



Key Applications – DNA Sequencing

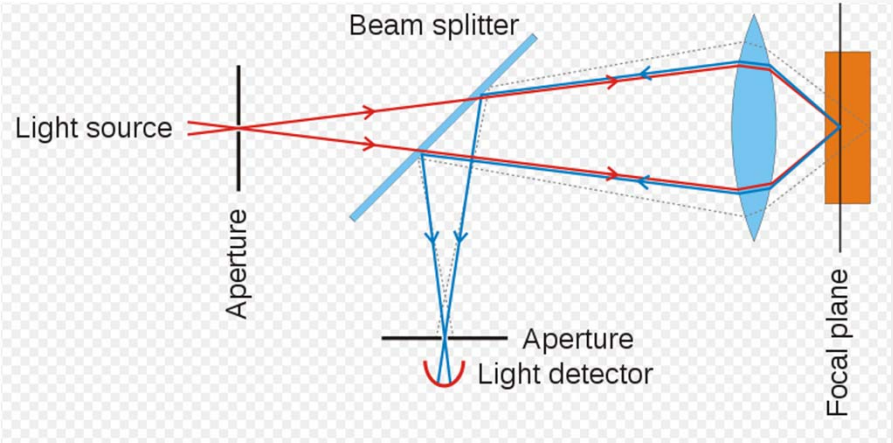
- The common goal is to use DNA polymerase and fluorescently labeled nucleotides to produce DNA fragments of every possible length. These are labeled at their primary or terminal end with a fluorescently labeled nucleotide.
- Since each of the four nucleotides (AGCT) is labeled with a different fluorophore, laser excitation can be used to indentify the terminal (or primary) base for each different length strand.
- Initially, DNA sequencers used a single 488 nm ion laser to produce 10's or 100's of mW. To enable lower cost sequencing, today's machines can use multiple lasers/LEDs (e.g., 488/532/red).
- Power density is key to enhance read lengths and speed and accordingly reduce cost and time needed for sequencing.



Key Applications – Microscopy

Confocal Laser Scanning Microscopy uses a point illumination and a pinhole in an optically conjugate plane to capture images from selected depths. Fluorescent components attached to the sample offer characterization and tracking of samples.

- Lasers used are continuous wave visible and UV lasers.
- Powers from 20mW (standard confocal), 100mW (live imaging) to 2W (Super Resolution Microscopy)
- Target application areas: Cell Biology and Research. Clinical diagnostics



Illumination Sources

- The light source can be a laser, an arc lamp or even an LED. Today, the majority of instruments use a laser.
- Lasers are chosen because they produce a high intensity beam of monochromatic light. They also have a small ‘spot’ size, which is important since the light needs to be focused into a small volume to obtain maximum excitation of a single cell and to minimize the probability of there being more than one cell in the laser beam.
- Arc lamps need optical filters to select the appropriate wavelength. They do not give the sensitivity needed to observe weak fluorescence but offer a cheaper alternative for observing strong fluorescences, for example, in DNA analysis.

Illumination Sources

- LED can be used as a medium ground between laser and ARC lamp, as they have more controlled waveband that ARC lamps though not as narrow as laser. LED cannot be focused as small as lasers, as a result the efficiency of the illumination delivered to the sample is lower than laser sources.

- While a higher power output improves sensitivity, there is a penalty to pay in terms of increased cost, maintenance and size. A few specialized applications need higher laser powers, such as chromosome analysis and sorting, and lasers producing 200 mW or more are used.

Basic Lighting Scheme

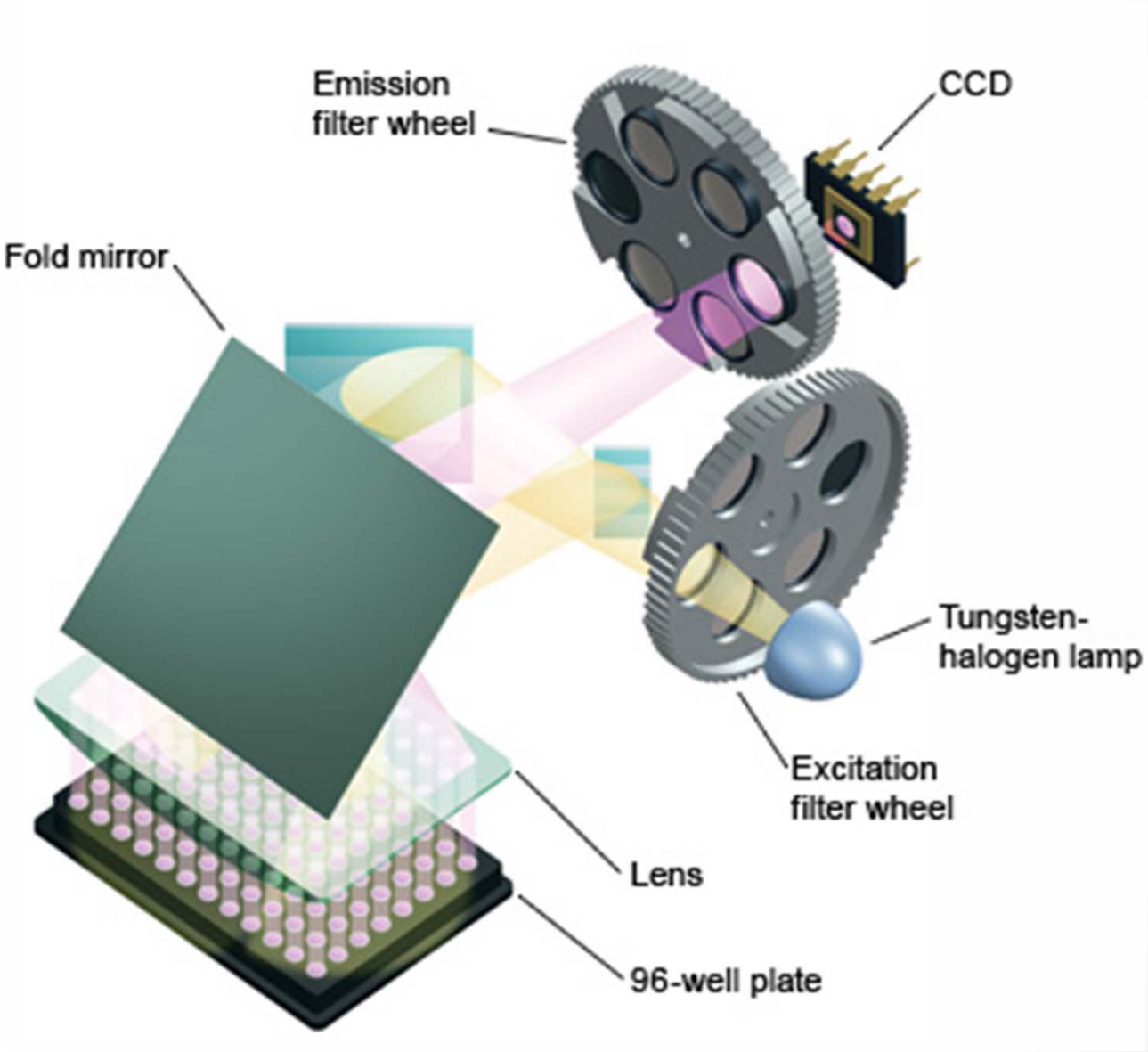
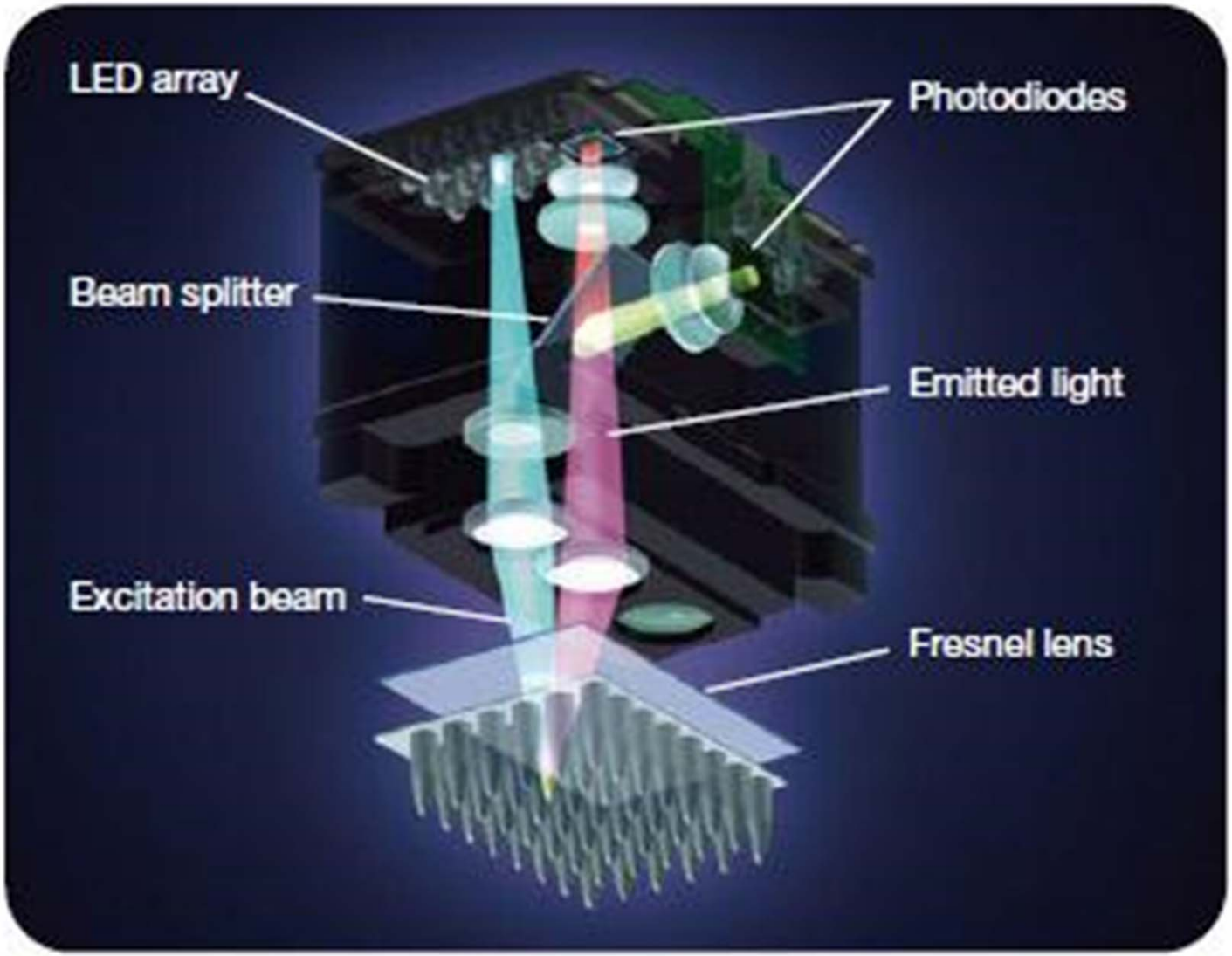


Image from BioRad

Basic Lighting Scheme



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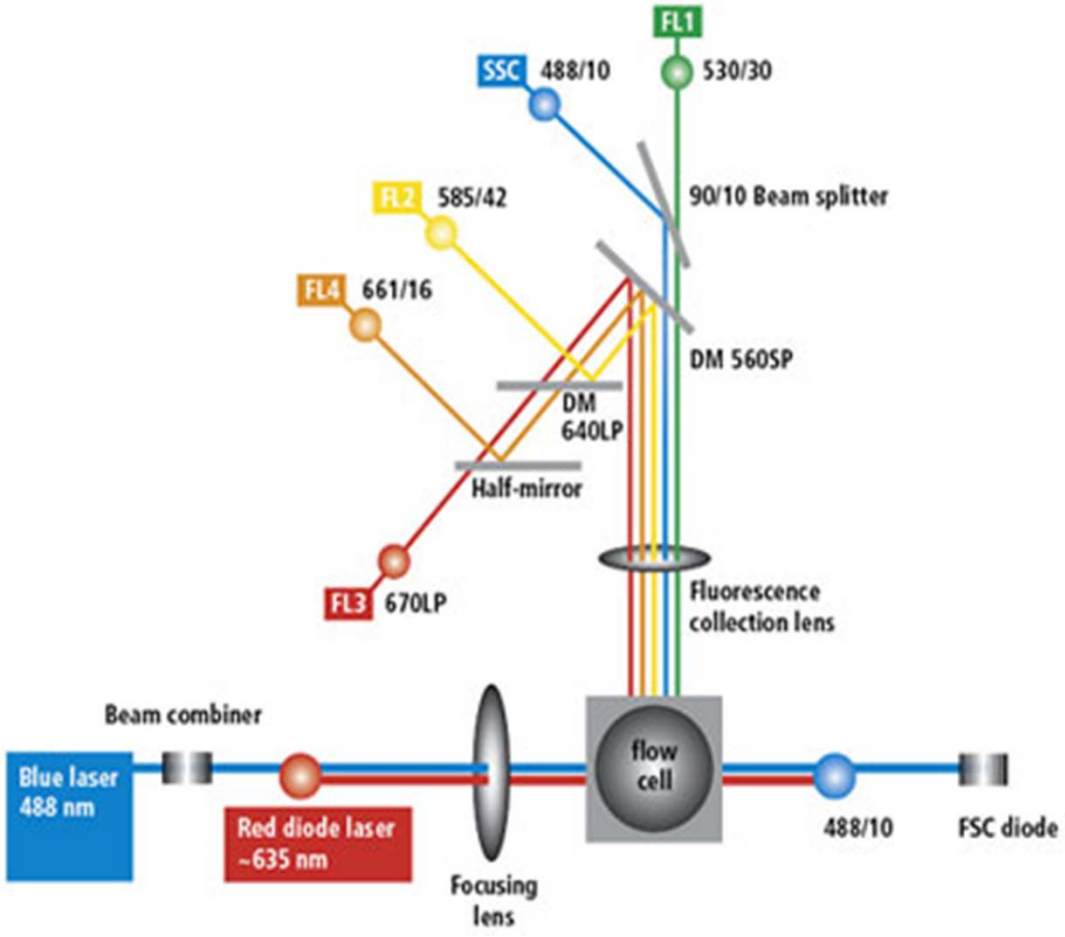
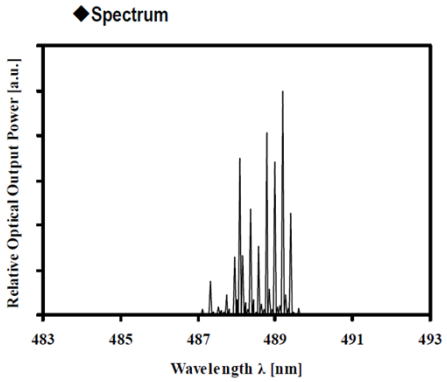
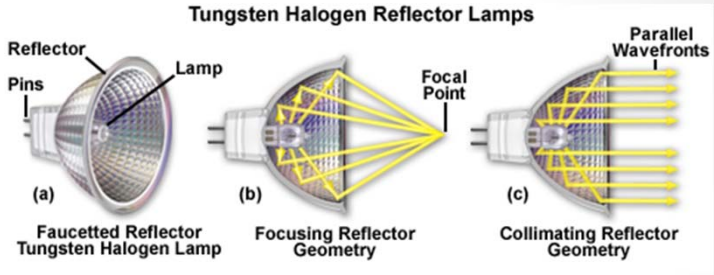


Image from BCI

Lighting Technologies

Coherent OBIS Laser





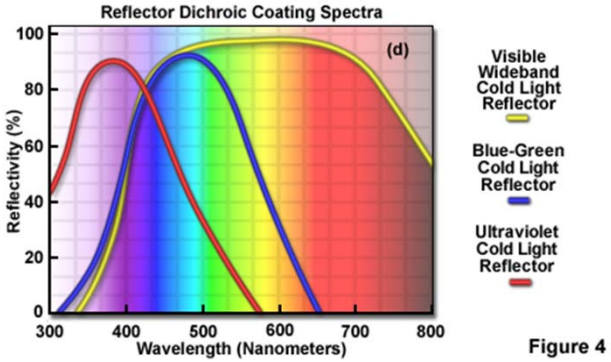
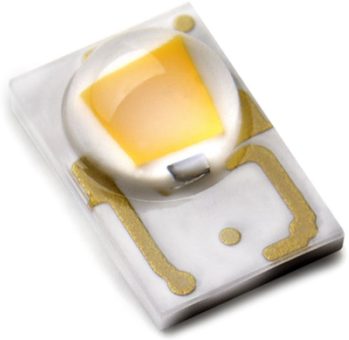


Figure 4



Phillips Luxeon LED

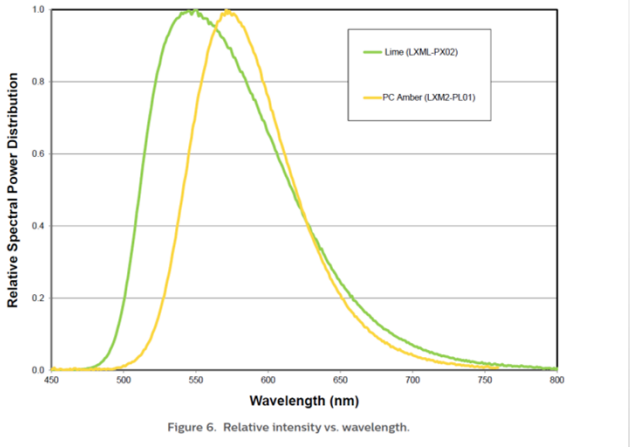


Figure 6. Relative intensity vs. wavelength.

LED Spectrum vs Emission Spectrum

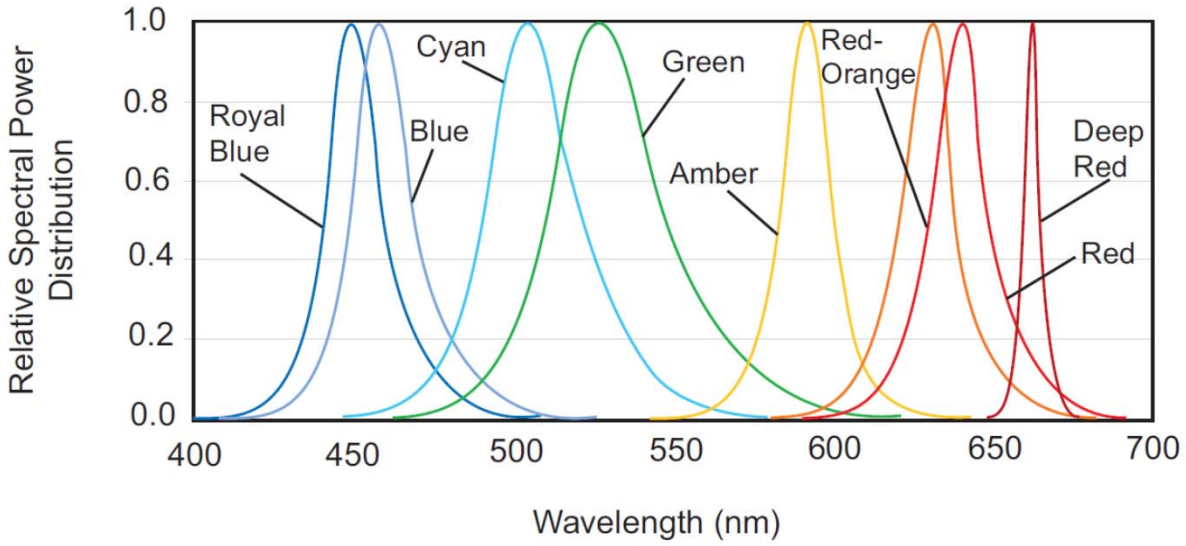
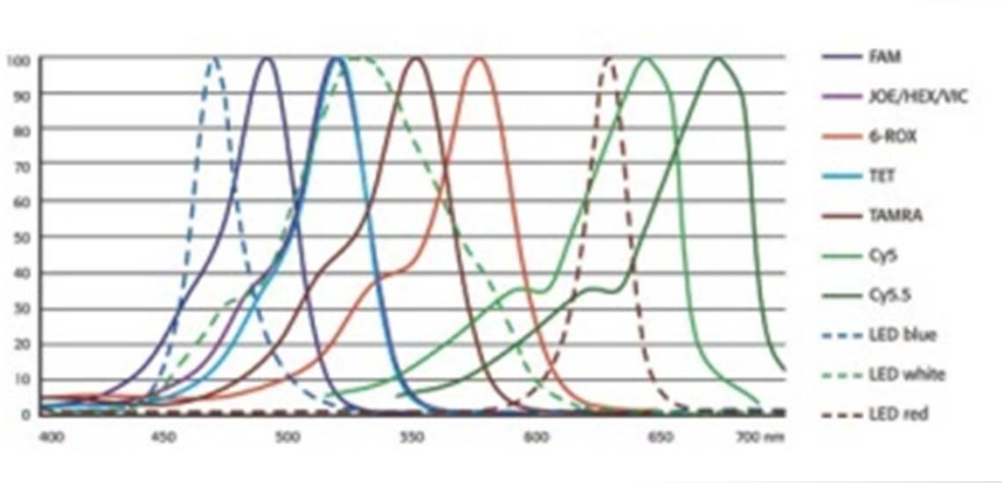
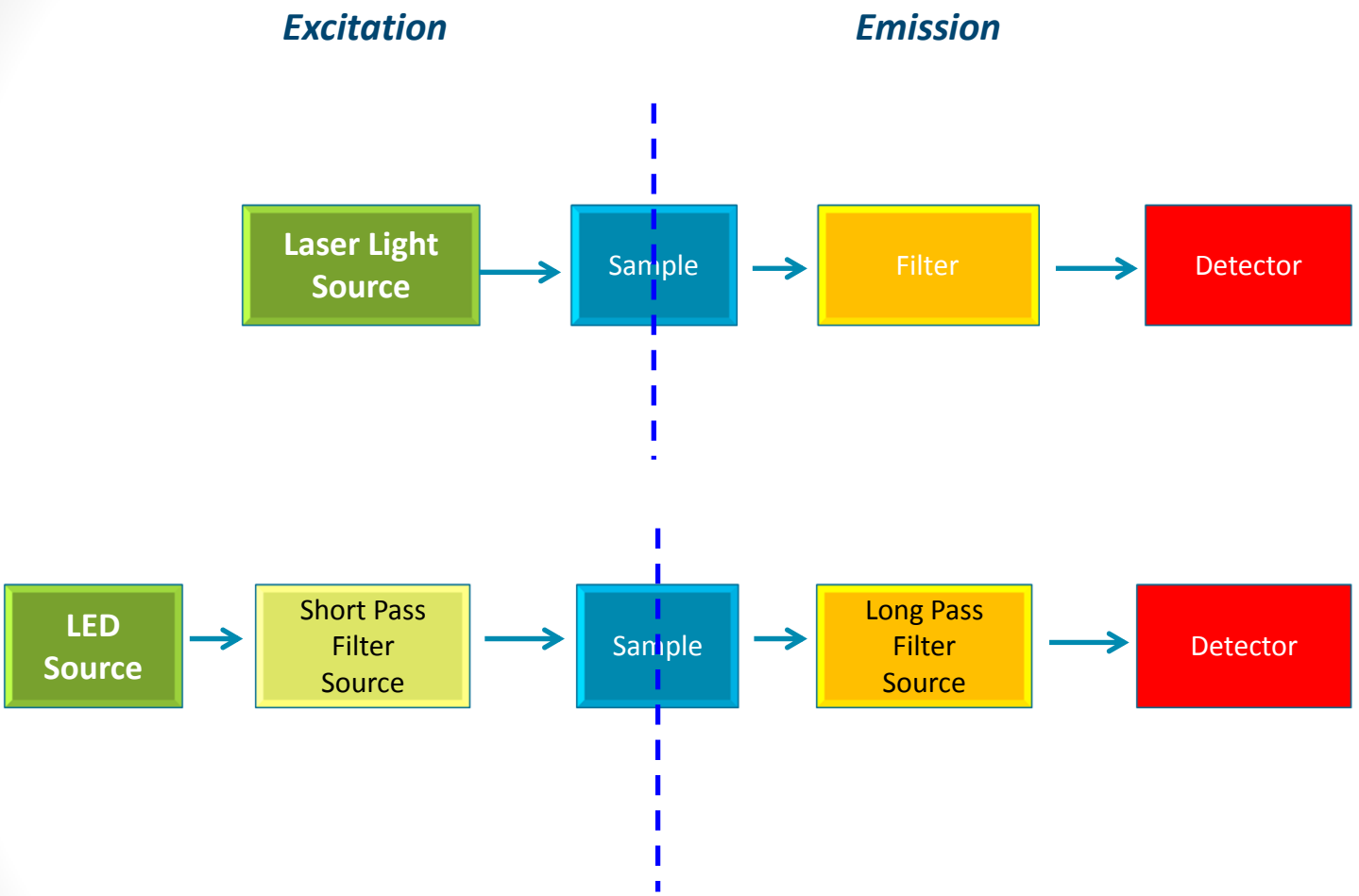


Figure 5. Relative intensity vs. wavelength.



Need for Filtering



One filter after the sample to keep laser light that bounces around the instrument from hitting the detector. The filter may be a long pass or laser line blocking filter.

TTwo sets of filters are required, one on the excitation side and one on the emission side.

LED spectrum enforces a minimum of two sets of filters

Need for Filtering

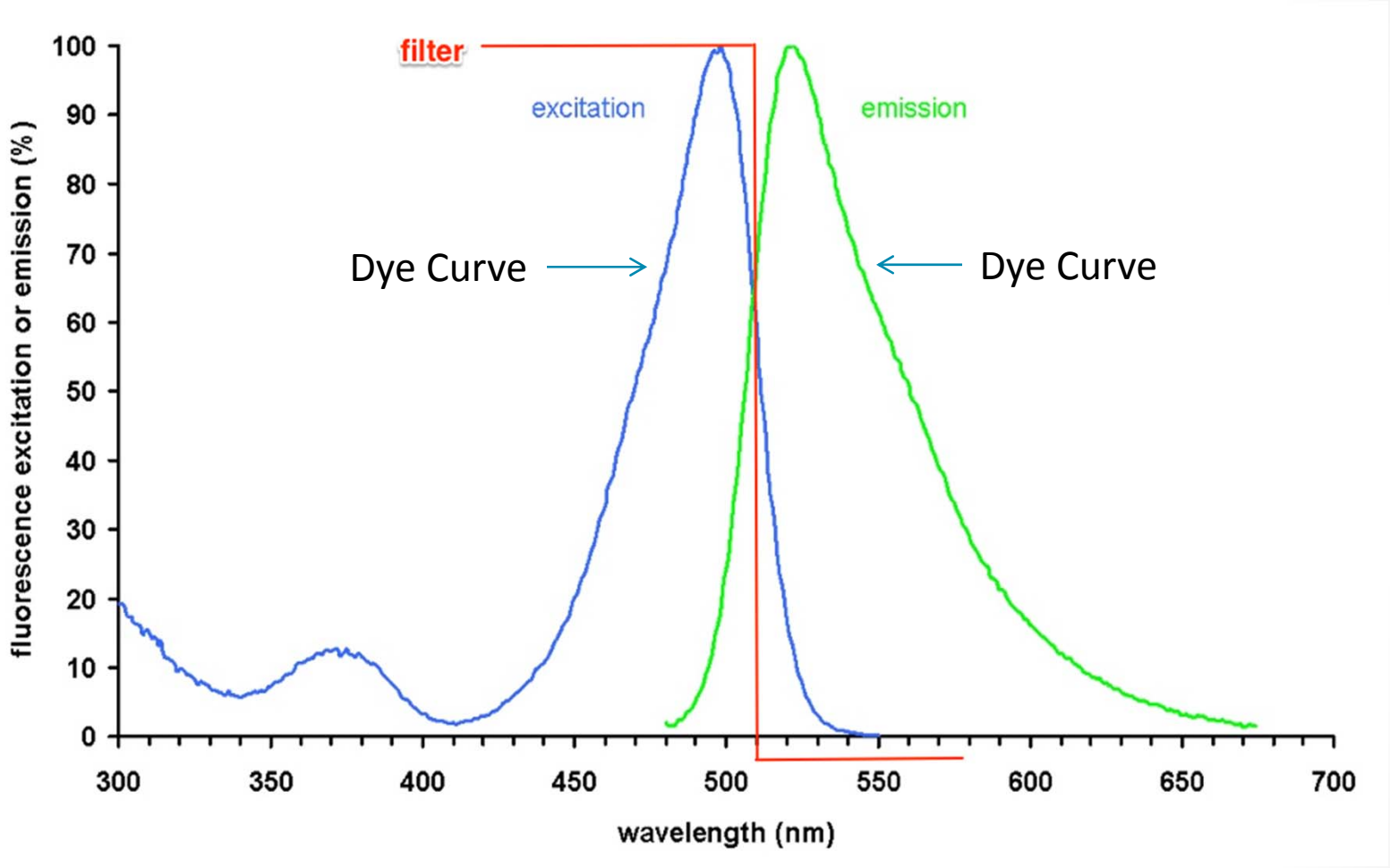
LED:

- When using multiple dyes, the filtering with LEDs is an issue. More photons are “thrown away” using filters on the input and output to the sample.
- Sourcing the right filters for longterm production is a longterm risk that might require redesigns in the future.

Laser:

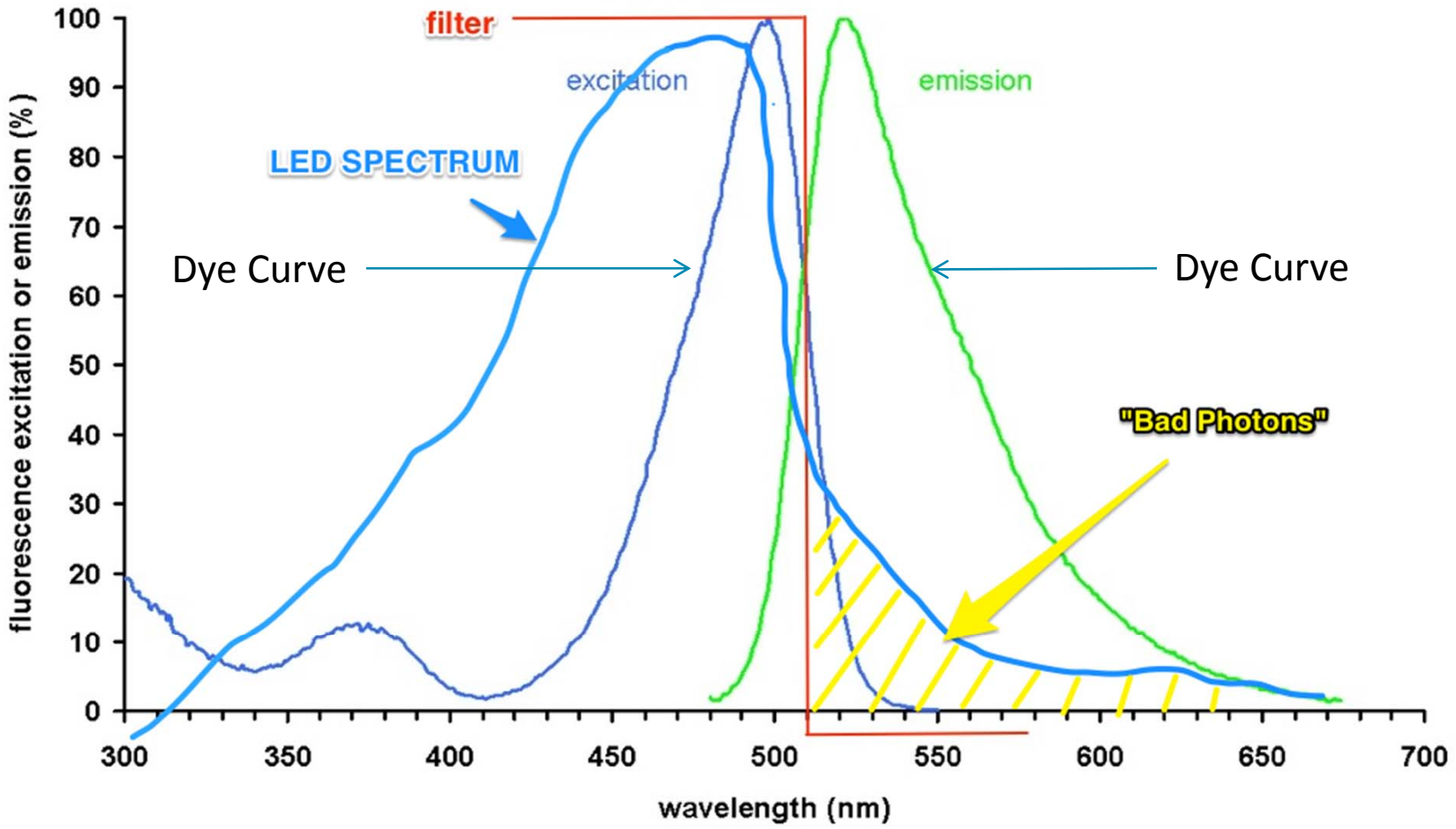
- Because lasers provide so much power and all the photons are useful in exciting exactly where the designer needs and nowhere else, there is a lot of emission signal.
- So the designer can afford to throw away photons on filtering to minimize cross-talk.

Excitation vs. Emission



The sharper the cutoff the more expensive the filter

Bad Photons



- Short-pass filter well-matched to the specific LED used, as well as the absorption/emission spectra of the dyes needed to block the long tail of the LED spectrum.
- Filter cuts out a significant portion of the light from the LED.

Source Properties

- Power
  - ARC (100s Watts)
  - LED (10s Watts)
  - Laser (100s mW)
- Usable Power
  - ARC (10s mW)
  - LED (1s mW)
  - Laser (100s mW)

How to go from 5W to 1mW?

“False Hope” 5W to 1mW!

Component

Source

Instrument

LED 5W

LED 3.5W

- Electrical, CW ~70%

LED 175 to 350mW

- Optical conversion ~5-10%

LED 100 to 200mW

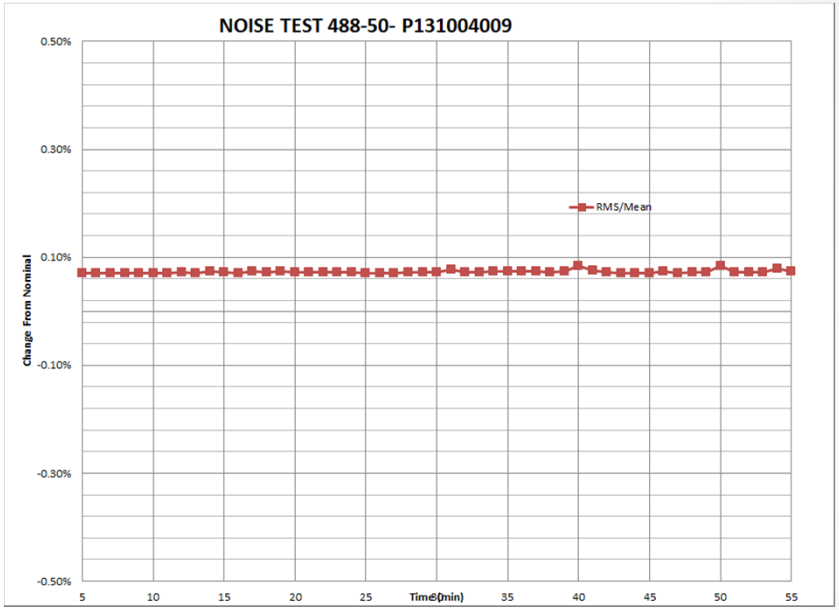
- Filters ~75% for excitation and emission

LED 1-2mW

- Étendue

Performance

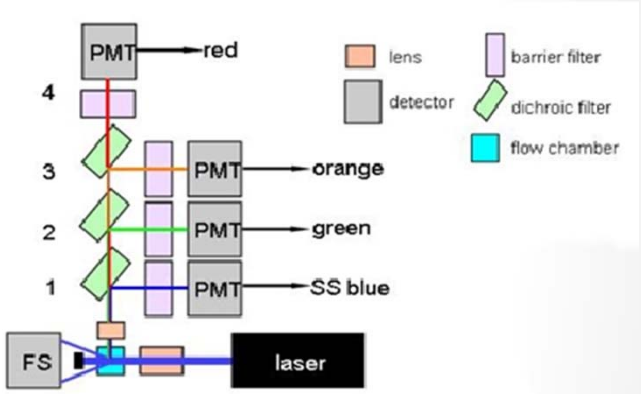
- Power Stability
  - Halogen
    - Drive Electronics
    - Temperature
  - LED
    - Drive Electronics
  - Laser
    - Feedback loop is internal
    - Temperature



- Lifetime
  - Halogen
    - 100W – 2k hrs
  - LED
    - 5W – 50k hrs
  - Laser
    - 100mW – 15k hrs

Performance

- Ease of integration
  - Halogen
    - Overhead in filter management with both excitation and detection
    - Voltage stability relates directly to emission spectra
    - Heat load is very high
  - LED
    - Less filter management than Halogen
    - Fixed filters for excitation and detection
    - Availability of correct BINs
    - More and more expensive optical components
    - Good thermal properties
  - Laser
    - Minimum filter management
    - Narrow and stable spectrum
    - Package is larger that either above
    - Heat load needs management
    - Less optical components and less expensive requirements



Flow Cytometry – a basic introduction Dr. Michael Ormerod

- Ease of customization
  - Halogen
    - Fixed bulbs and reflectors
  - LED
    - Fixed BINs (power/wavelength)
  - Laser
    - Fixed packages with some flexibility on delivery package

Performance

- Cost comparisons
  - Source
    - Halogen
      - $
    - LED
      - $$
    - Laser
      - $$$$
  - Optics
    - Halogen
      - $$$
    - LED
      - $$
    - Laser
      - $
- Comparable BOM
  - Halogen
    - $$$$
  - LED
    - $$$$
  - Laser
    - $$$$$
- $/Usable Photon
  - Halogen
    - $$
  - LED
    - $$$
  - Laser
    - $$

Conclusion

The design of lighting subsystems for bioinstruments is being pushed to more and more demanding performance specifications including **spectral purity**, **spectral breadth**, **high power**, fast switching, durability, ease of use, **compact size**, and **reduced cost**.

Technology	Spectral Purity	Spectral Breadth	Brightness	Size	Cost
Lamp	-	+	-	+	+/-
LED	-	-	-	+	+/-
Laser	+	+	+	-	-
Hybrid	+	+	+/-	-	+

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