Setting the Standard: Performance Requirements for Fluorescence Imaging

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Optikos

About Optikos

- 40+ Years in business
- Located in Wakefield, MA
- Hardcore optical engineering company
- Products and services
- ISO 9001 and ISO 13485

Product Development

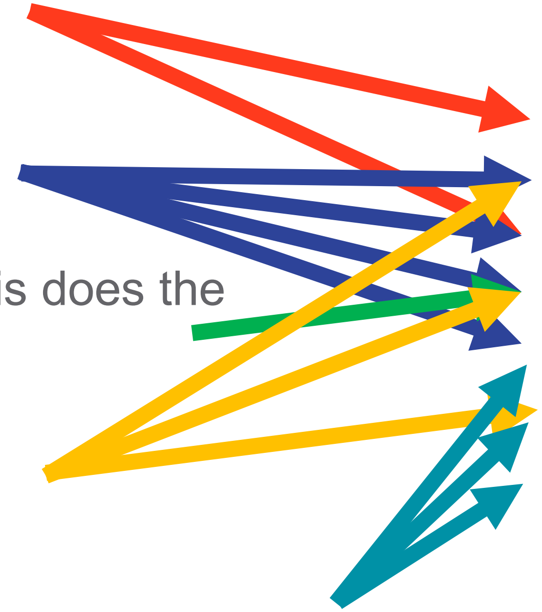
Contract Manufacturing

Metrology Products

Testing Services

First-Order Specs Affect Various System Parameters

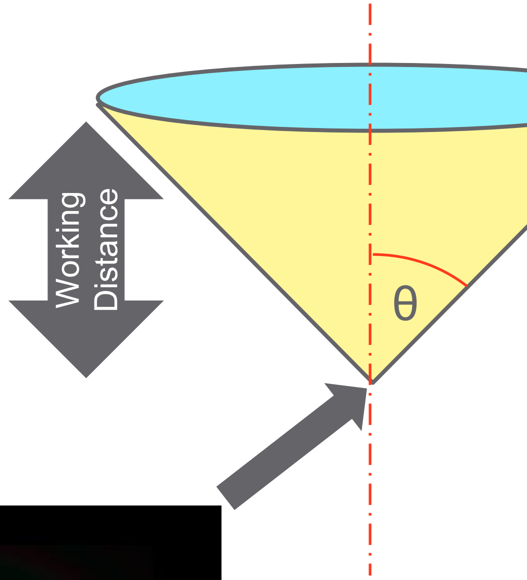
- Field of view — How big is the area that we want to image?
- Resolution — What is the smallest detail we need to resolve?
- Depth of field — How far along the optical axis does the resolution need to hold up?
- Sensitivity — What is the photon budget?
- Spectral considerations — What is the bandwidth and separation of the excitation and emission spectra?



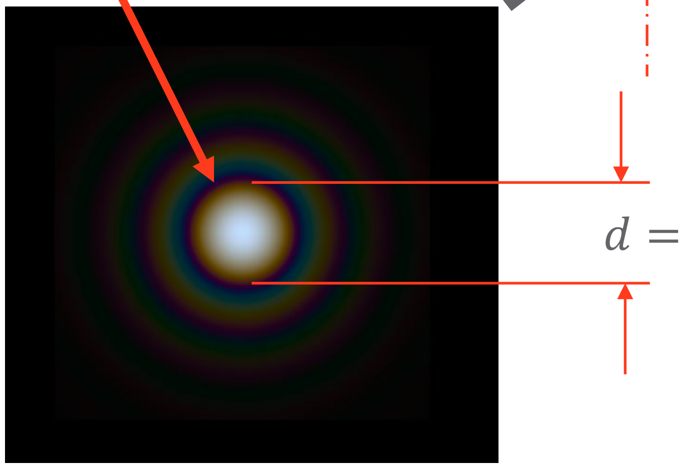
- Sensor size
- Pixel size
- Magnification
- Numerical Aperture (NA)
- Aberration corrections
- Illumination
- Filters

Numerical Aperture (NA) Limits Best-Possible Optical Resolution

This is the sharpness of the pencil with which we're drawing the image



$NA = n \sin\theta$



$$d = \frac{1.22\lambda}{NA}$$

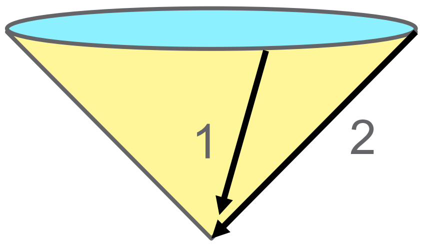
Higher NA → Higher Resolution

NA=0.95 (θ=72°, d=0.70μm @ 546nm)

At the image plane, d= 40 x 0.70μm = 28μm

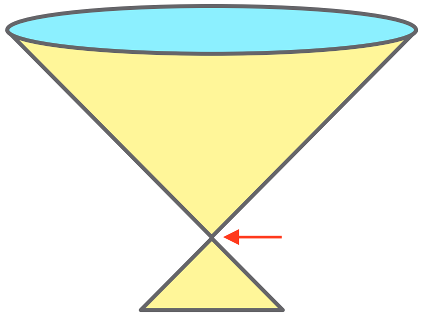
Why Not Just Make All Lenses High NA and High Resolution?

1.The aberrations in Ray 2 are more difficult to correct than those in Ray 1



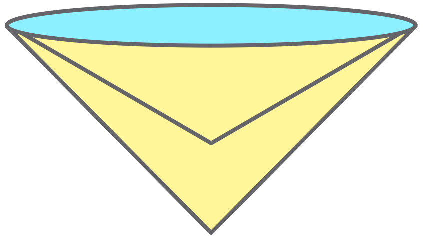
- More Expensive!
- More complicated design (more elements)
- Higher precision lens alignment required to achieve performance

2. The depth of field is shallower in high NA lenses



The spot diameter increases more rapidly with defocus for a larger cone angle/NA

3. Working distances get shorter or optics get larger in diameter with higher NA lenses



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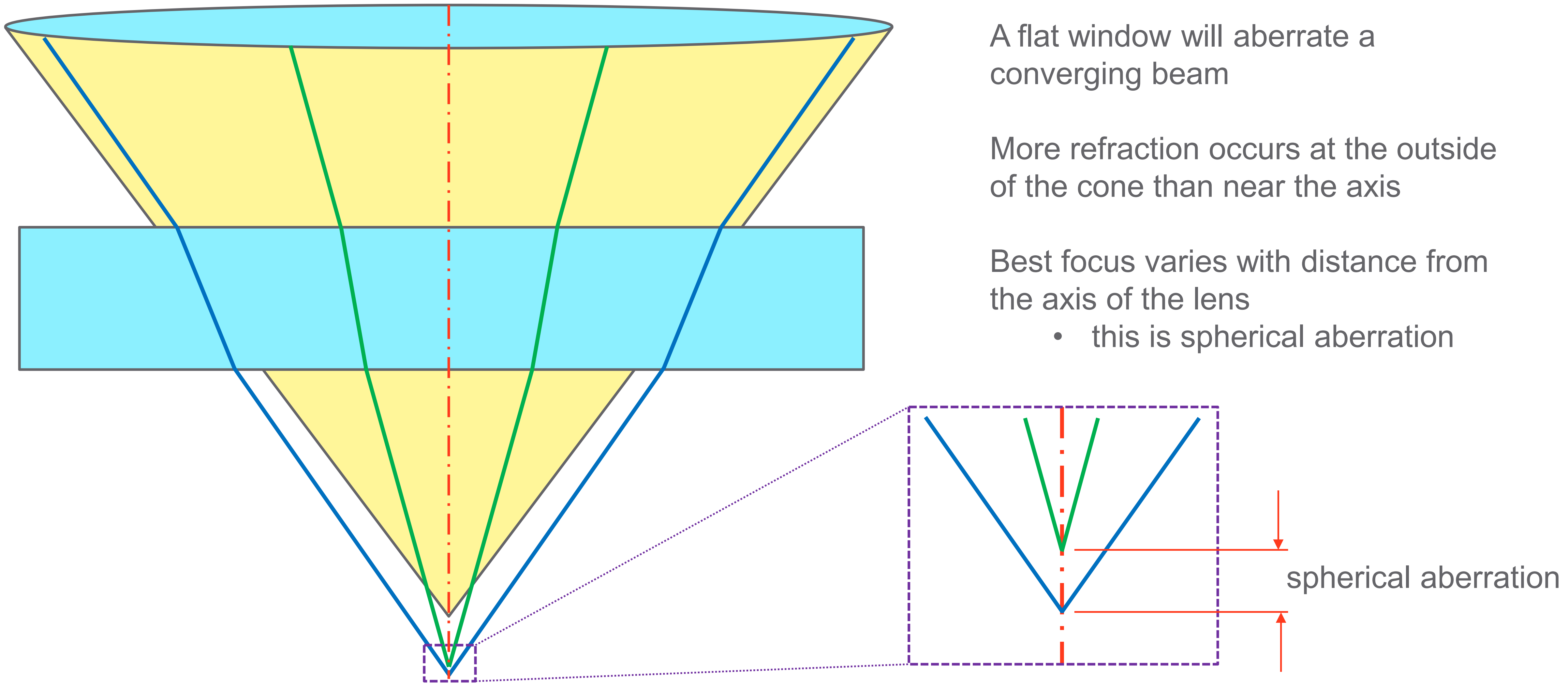
High NA Systems are Designed to Match Cover Glass Thickness



A flat window will aberrate a converging beam

More refraction occurs at the outside of the cone than near the axis

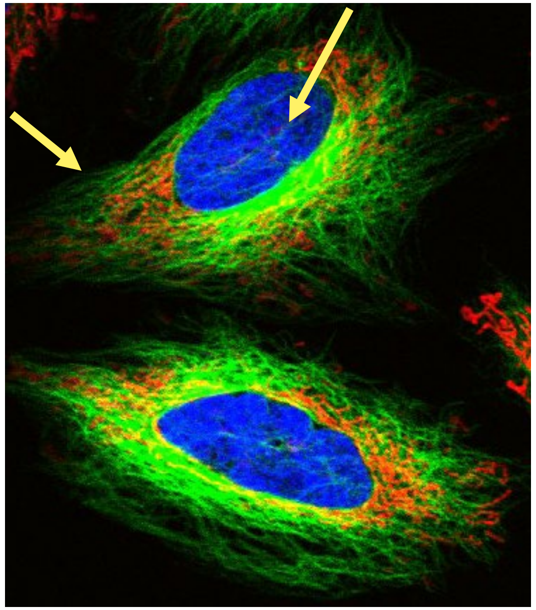
Best focus varies with distance from the axis of the lens

- this is spherical aberration

How Much Resolution is Needed?

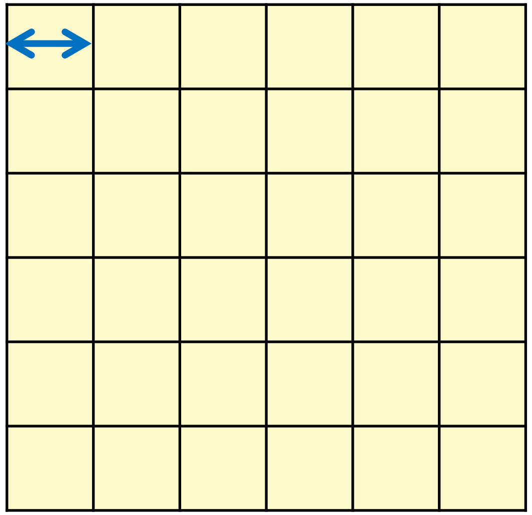
From the sample side:

- What is the smallest feature of the sample that needs to be resolved?
- How far apart are these features?
- Does structure need to be well defined, or is finding a center of mass enough for the application?
- Take inverse of size/separation to define a spatial frequency of interest



From the sensor side:

- What is the pixel size of the sensor?
- Maximum spatial frequency is the Nyquist frequency: $\frac{1}{2*(pixel\ size)}$
- The sensor will not reliably record structures higher than this frequency
- Need at least 2 pixels per structure of interest, preferably more

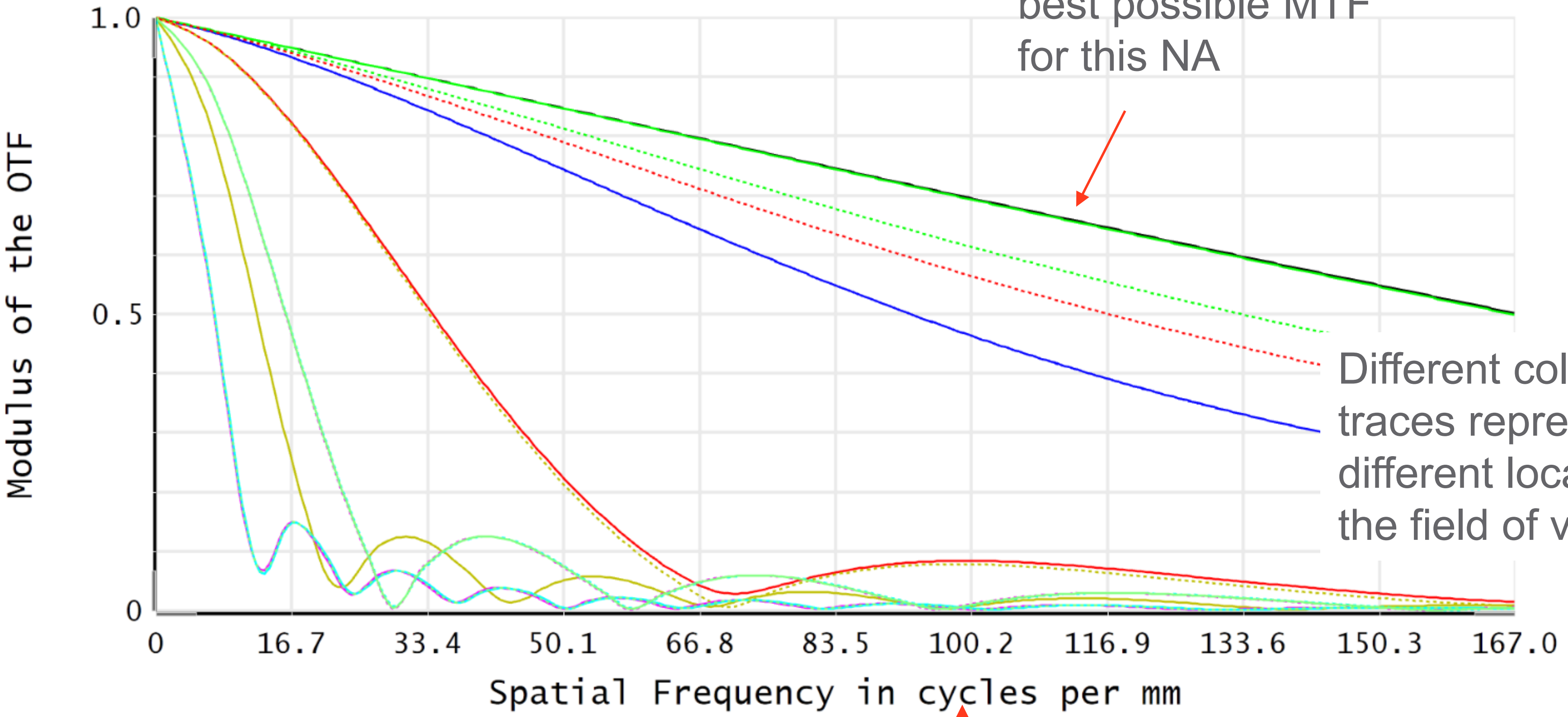


Crop of cell image published on Wikipedia. Original available at: https://commons.wikimedia.org/wiki/File:Multicolor_fluorescence_image_of_living_HeLa_cells.jpg#file

Lens Resolution and MTF (Modulation Transfer Function)

Diffraction limit. The best possible MTF for this NA

MTF (often expressed as a percentage)



Different colored traces represent different locations in the field of view

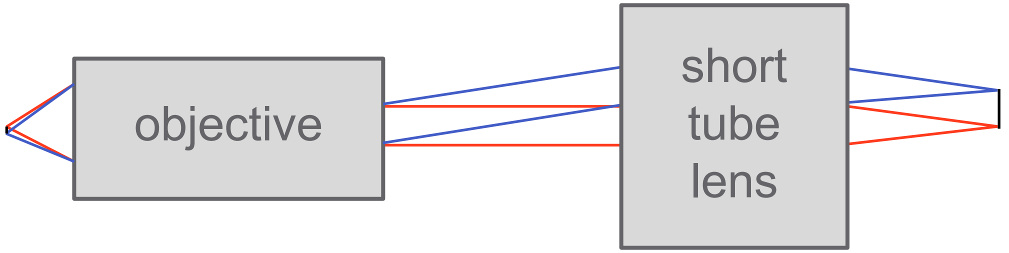
spatial frequencies at the object and image are related by the system magnification

Nyquist frequency for 5 µm pixels

Magnification Affects Both Image Size and Numerical Aperture

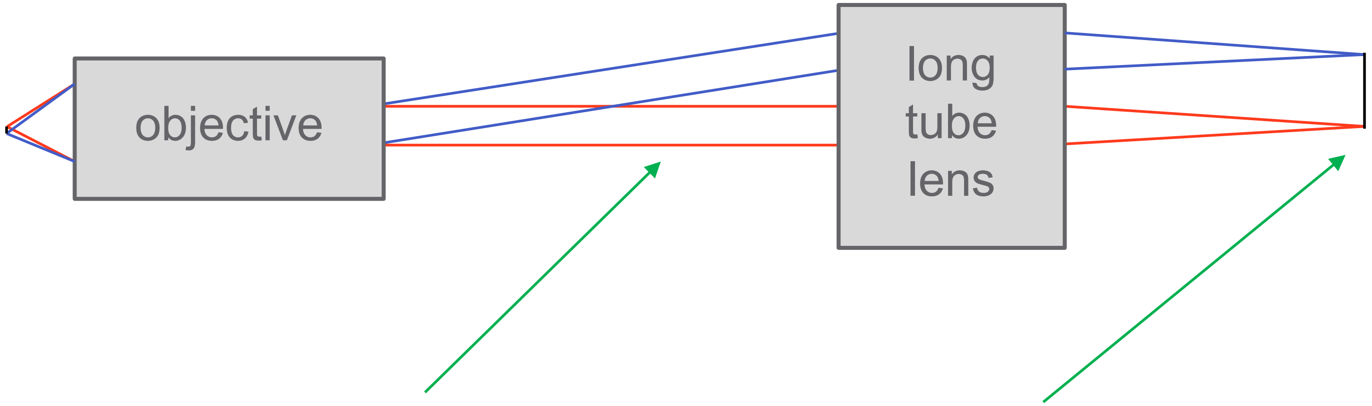
magnification labels on commercial objectives are based on an assumed tube lens

$$m = \frac{image\ size}{object\ size} = \frac{EFL_{tube\ lens}}{EFL_{objective}}$$



short tube lens effective focal length (EFL)

- lower magnification
- smaller image at sensor
- higher NA at sensor

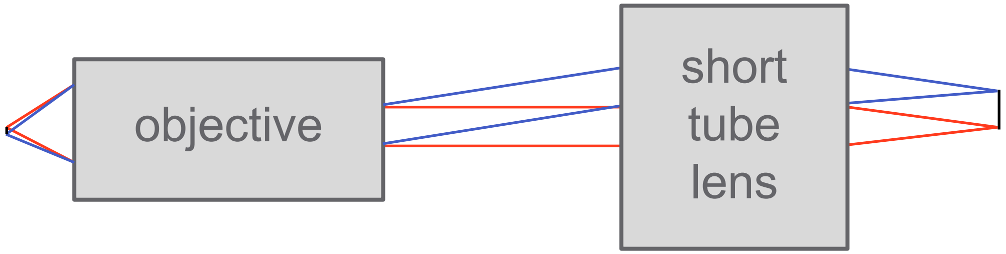


longer tube lens EFL

- higher magnification
- larger image at sensor
- lower NA at sensor

beam size unchanged, distance to image increased => smaller cone angle

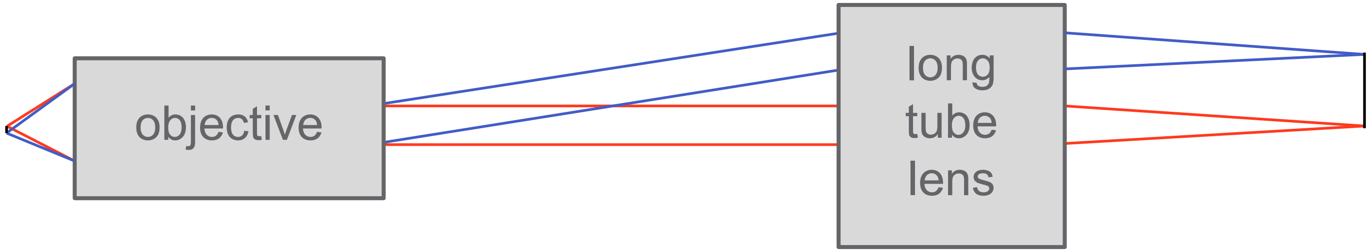
Irradiance at the Sensor Goes as the Square of Image NA



Irradiance (E)

- power per area on a surface
- can be used to calculate photons per pixel

$$E \propto NA^2$$



higher magnification creates a larger image with lower power per unit area

total energy of the image doesn't change, it's just distributed differently

Excitation Geometry Can Impact Lens Requirements

Are imaging optics used in the excitation path?

- requires excitation bandwidth to be included in performance analysis
- may restrict material selection as some glasses and epoxies will fluoresce, increasing background noise in the image

Glass Selection Affects Performance and Cost

- n_d : refractive index at 587.56 nm
- V_d : Abbe number ($\frac{n_d - 1}{n_F - n_C}$)
  - measure of wavelength dispersion
- Combining glasses from different areas of the map can improve spectral performance
- Glasses with very high index or Abbe number tend to be more expensive

Example Glass Map from Ohara

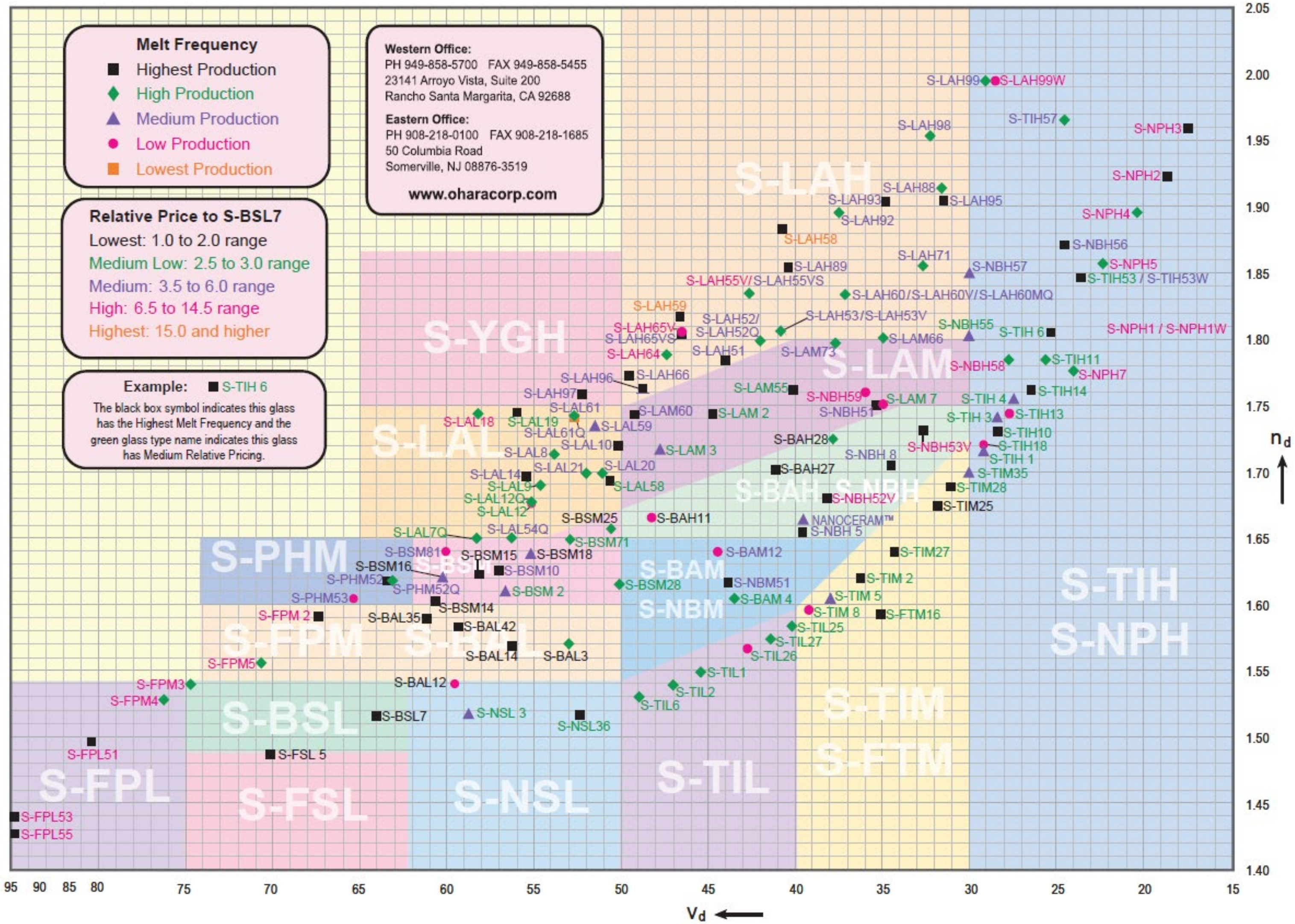


Figure available from Ohara:
https://oharacorp.com/optical-glass/

Once Specs are Set, How Will the Optical System be Built?

Optics Sourcing	Pros	Cons
Catalog lens assemblies (Photographic lenses, microscope objectives etc.)	▪ Reasonable cost ▪ Rapid delivery	• Fixed form factor • Fixed optical parameters • Mixed performance
Catalog components (singlets, doublets, mirrors etc.)	▪ Modest cost ▪ Rapid delivery ▪ Flexible assembly options	• Limited design performance • Modest fabricated performance • Limited offerings
Custom lens assemblies	▪ Designed for purpose ▪ Assembled performance is controlled ▪ Potentially molded optics	• Expensive in low volumes • Long lead times

Key Take Aways

- Many important optical system parameters are interrelated
  - magnification affects both image size and NA
  - NA affects resolution, depth of field/focus, working distance and sensitivity

- Lens resolution requirements can be based on the application requirements or the limit of a given sensor
  - application-based requirements are less likely to result in an overspecified lens system

- Excitation and emission geometry can impact glass selection and design constraints
  - sharing optics between excitation and emission paths tends to increase cost

- Custom optics can provide better performance and design flexibility than commercial options
  - development costs and lead times are significantly higher

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