

Optical Component Design Principles for Cytometry

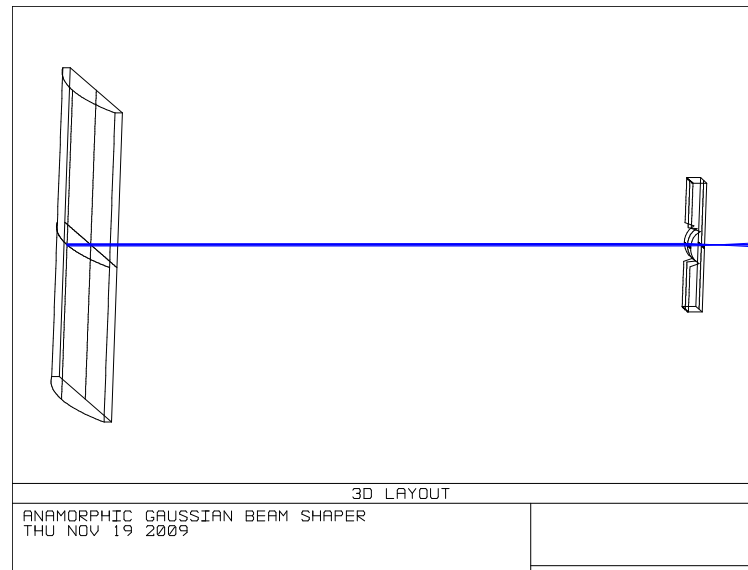
James Byrne

FIU 2009

Anamorphic Laser Beam Shaper

- Cytometry illumination is most advantageously done with a wide thin sheet of light.
- As seen in the last lecture, the beam thickness choice is driven by the particle size, velocity and system bandwidth.
- The width is a compromise between alignment tolerance and stability, and the efficient use of laser light.

Anamorphic Lens



The Mechanical axis of the assembly must coincide with the optical axes of the lenses. Setting accuracy must be about one micron, thermal stability must be in that range over the operating temperature range specified for the instrument.

The focus mechanism acts along the mechanical axis. Positioning axes must act orthogonally.

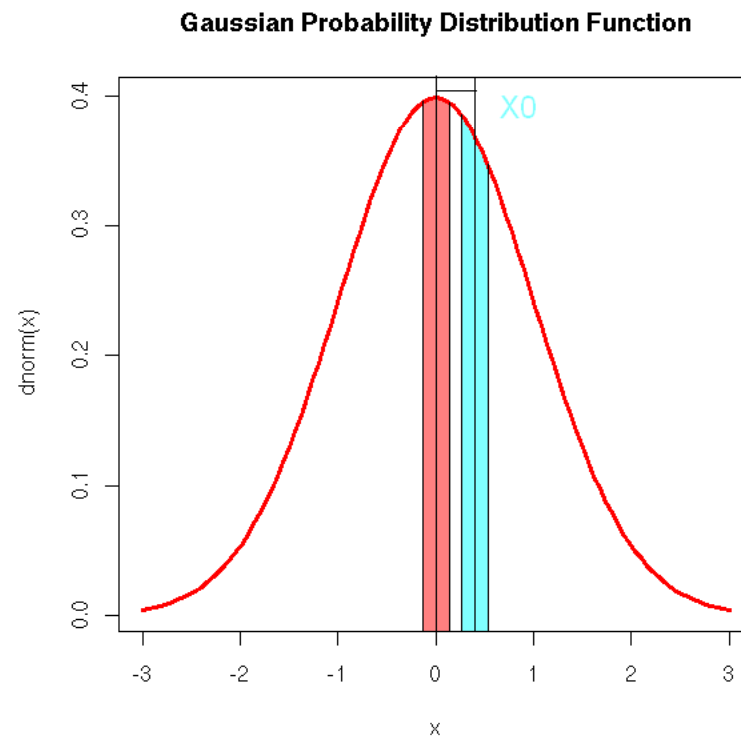
The clear aperture of the bore of the assembly must exceed twice the $1/e^2$ beam width at all points.

Translation of the lens axis parallel to the laser beam axis
Will result in tilting the beam through the target area.

Efficiency of illumination

- The proportion of laser energy delivered to a particle flowing through a laser beam depends upon the particle size the beam width and particle velocity.

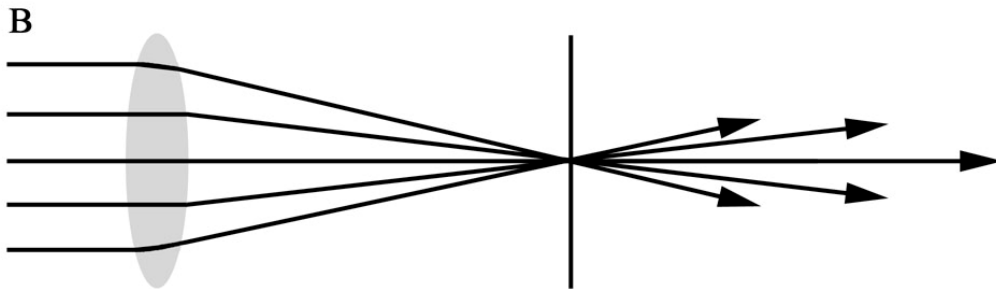
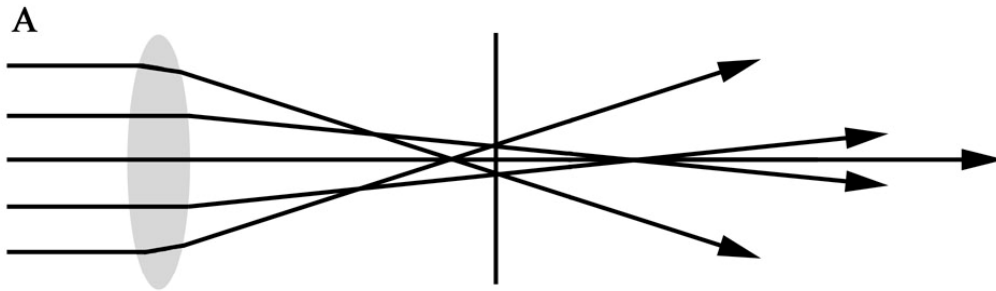
An on axis particle intercepts more energy than an identical particle traversing the beam a distance x_0 off axis.



A 10 micron particle traveling 10 microns off axis in a 150 micron wide Gaussian beam will collect 99% of the energy of an identical particle traveling on axis, adding to measurement uncertainty.

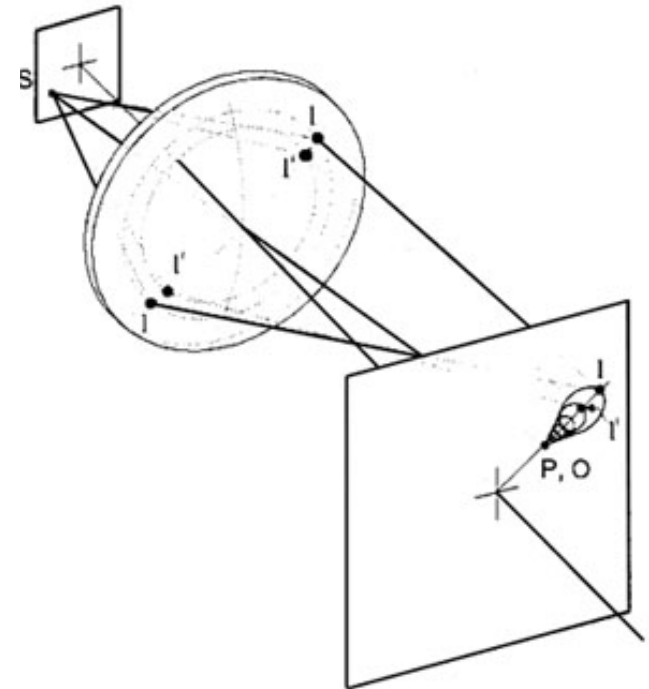
Optical Figures of Merit: Seidel Aberrations

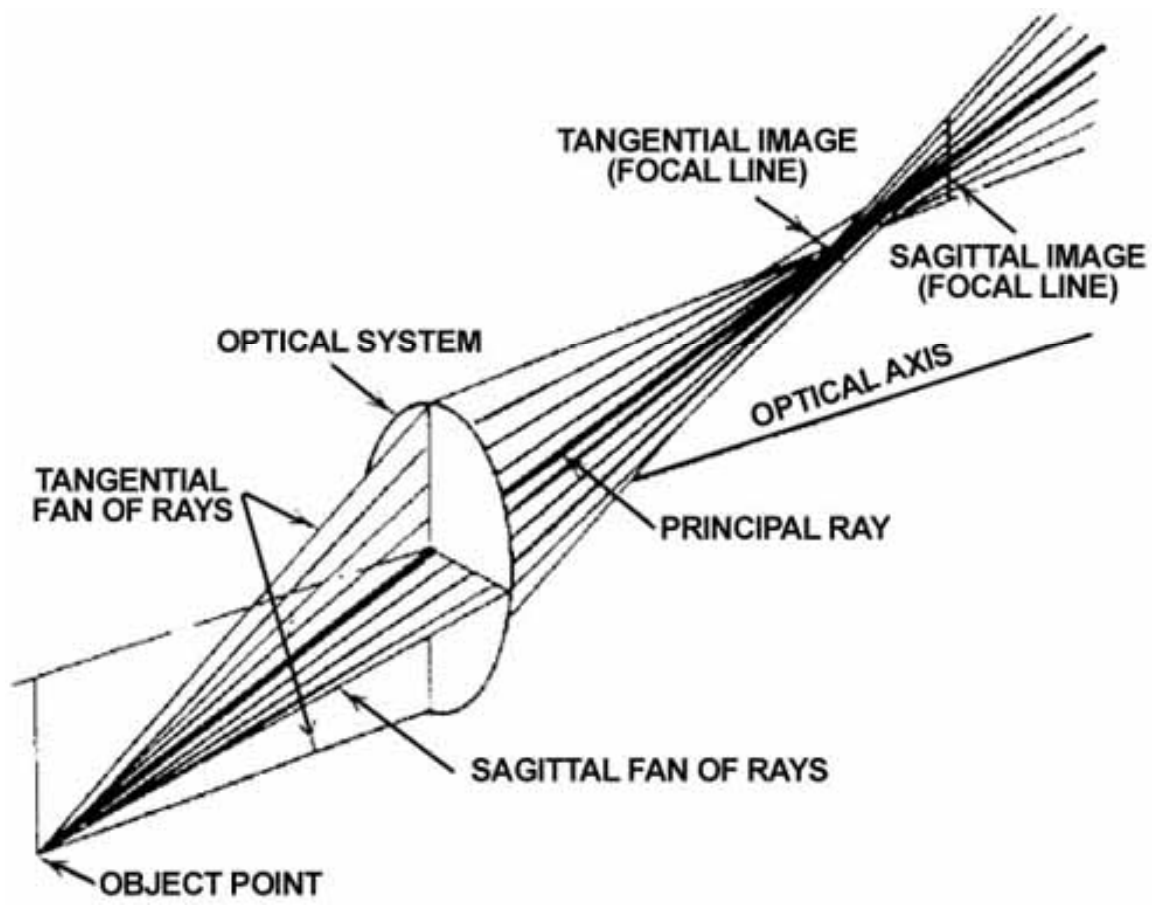
A. Spherical Aberration leads to focal length varying with ray radius.



An ideal lens focuses parallel rays at all radii to a common point.

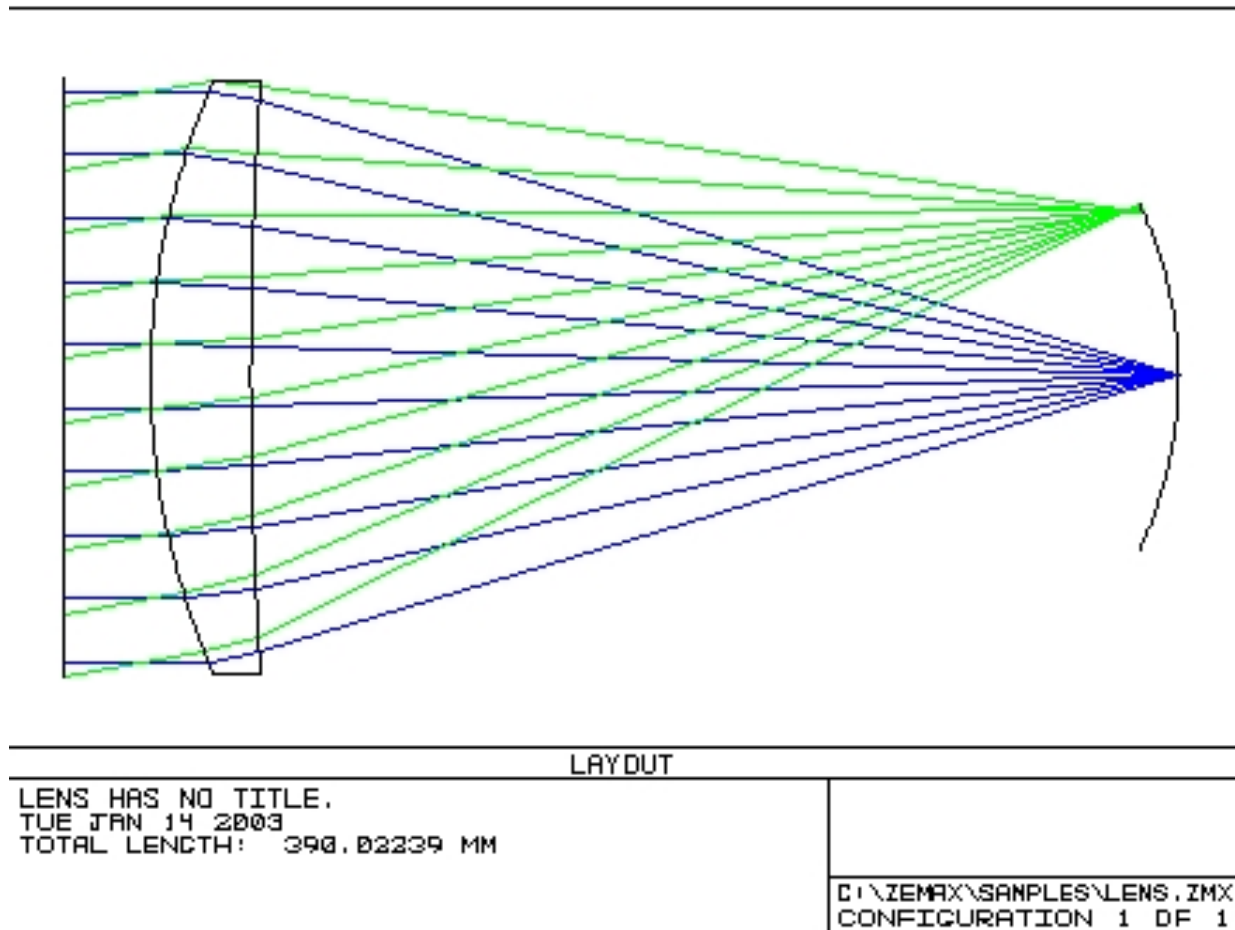
Coma arises from focal length being a function of object field height.



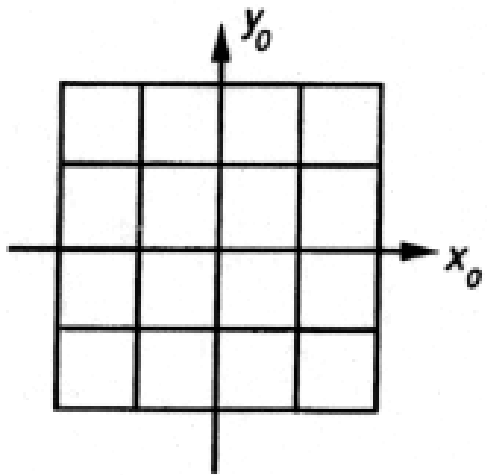


Ref: NASA

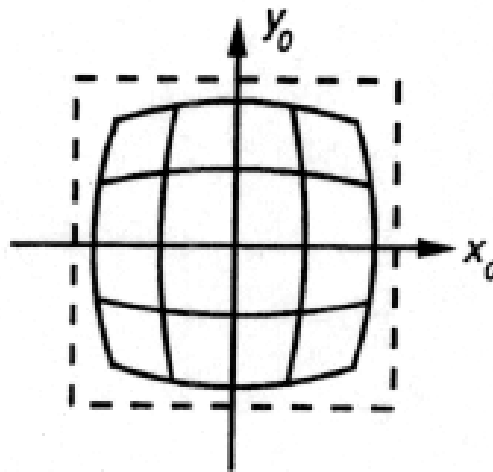
Field Curvature



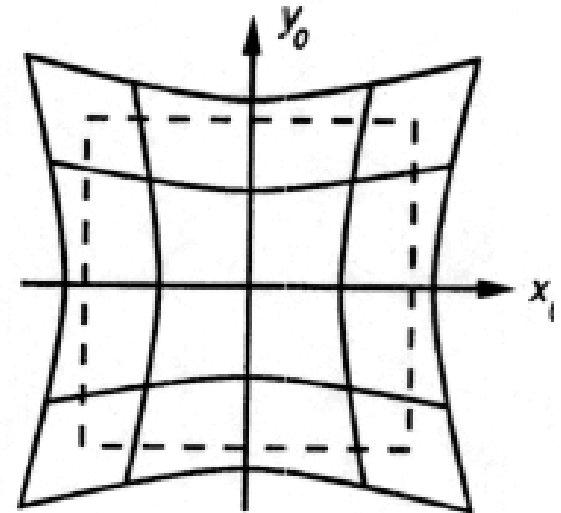
Distortion



No Distortion



Barrel Distortion

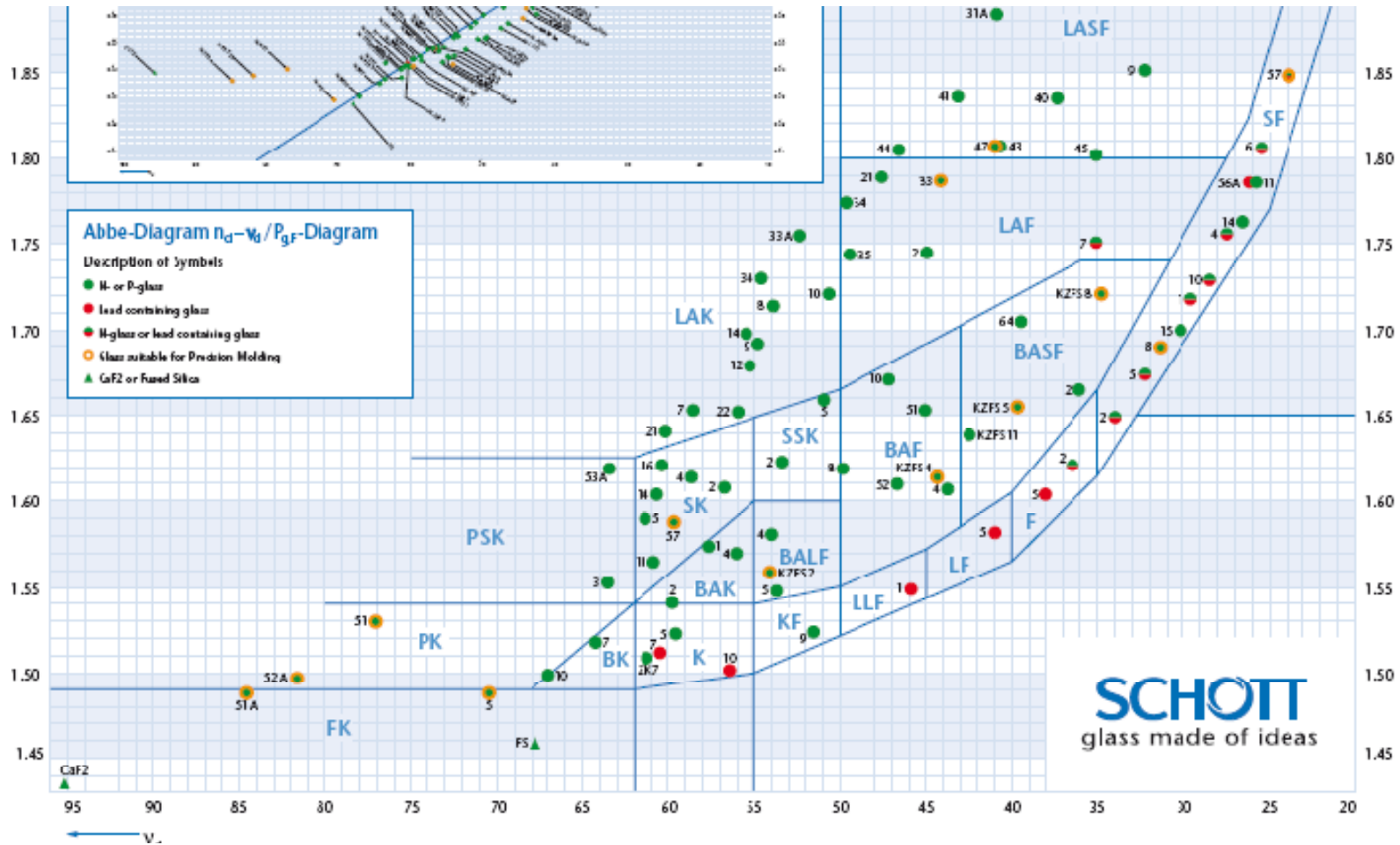


Pincushion Distortion

Objective Lens General Specifications

- Working Distance
 - If a lens is far from the object, say immersed in a flow cell, marginal rays pass farther off axis, giving rise to more spherical aberration.
 - Long WD leads to larger lenses for a given N.A
- Numerical Aperture
 - Light collection increases as NA^2 .
 - Spatial Resolution $\sim 1 / (2 NA)$ is related to time resolution by the particle velocity and size.
- Field of View - This is limited by the magnification and the diameter of the field stop. This limits the amount of background light that may pass through the optical system
- Tube Length- Constrains the effective focal length if the magnification is specified.
- Magnification: High M requires better mechanical stability
- Seidel aberrations must be minimized in the following order
 - Spherical Aberration
 - Coma Astigmatism
 - Curvature of Field
 - Distortion
- Then Chromatic Aberrations may be addressed.

Optical Glass



Schott Glass, A.G.

Color Correction

- Principal Dispersion, $n_f - n_c$
- Abbe Number $V_d = (n_d - 1) / (n_f - n_c)$

- Relative Partial Dispersion: $P_{x,y} = (n_x - n_y) / (n_f - n_c)$
- X and y are usually for wavelengths below
- f = 486.133 nm
- g = 435.834 nm
- c = 656.273 nm
- d = 587.562 nm

Objective Layout

Common Objective Optical Correction Factors

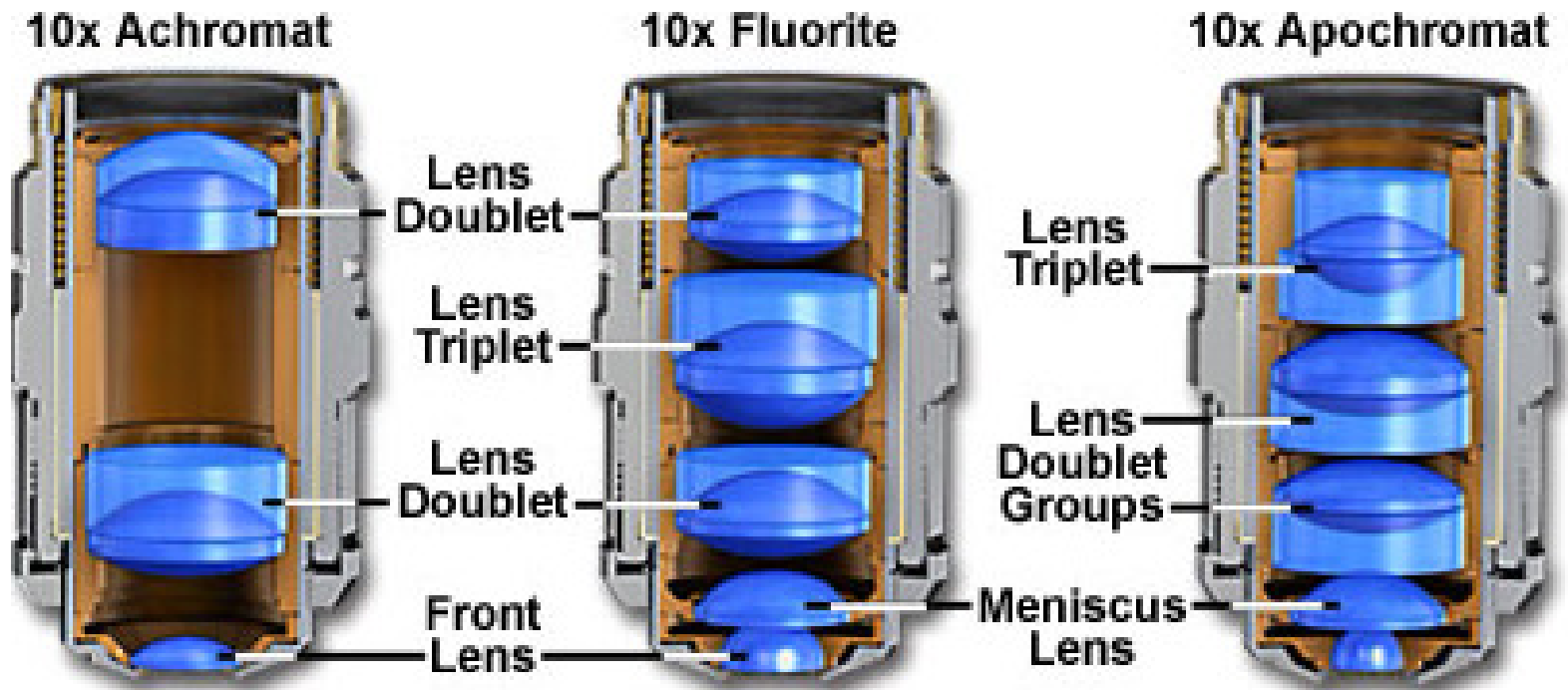
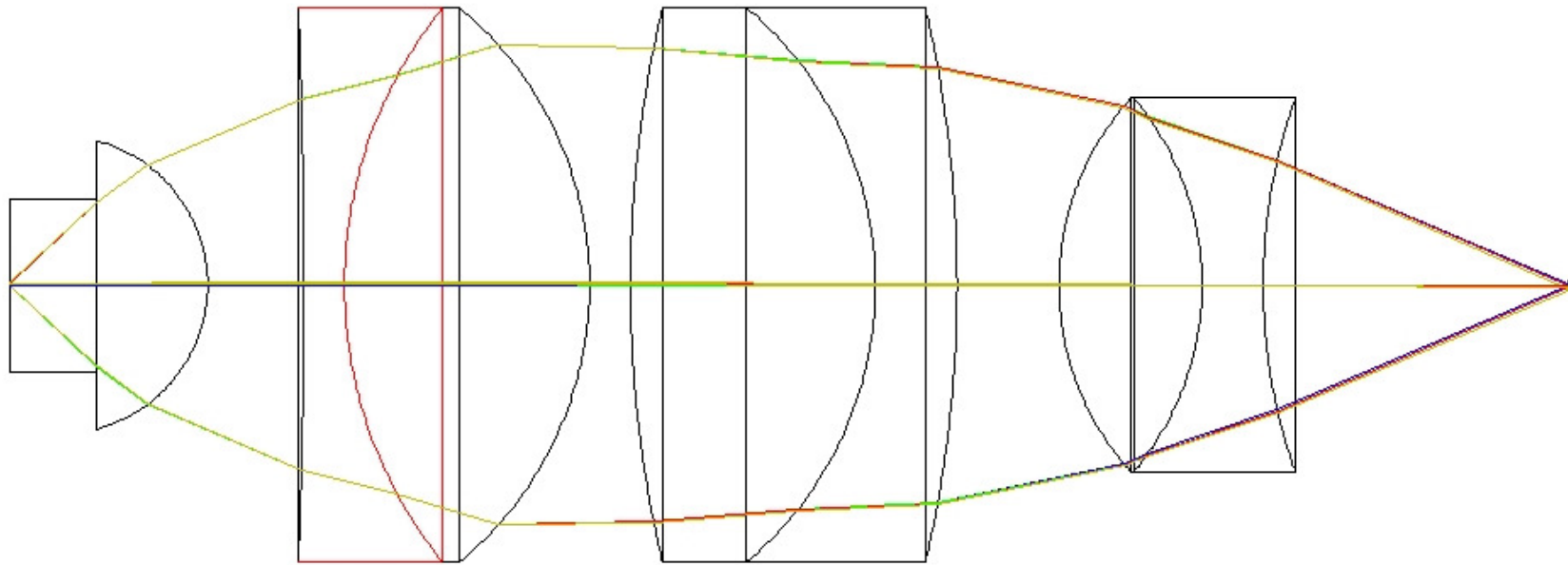


Figure 2

Ref Nikon

Cytometer Objective Layout



3D LAYOUT

SNOUT_S
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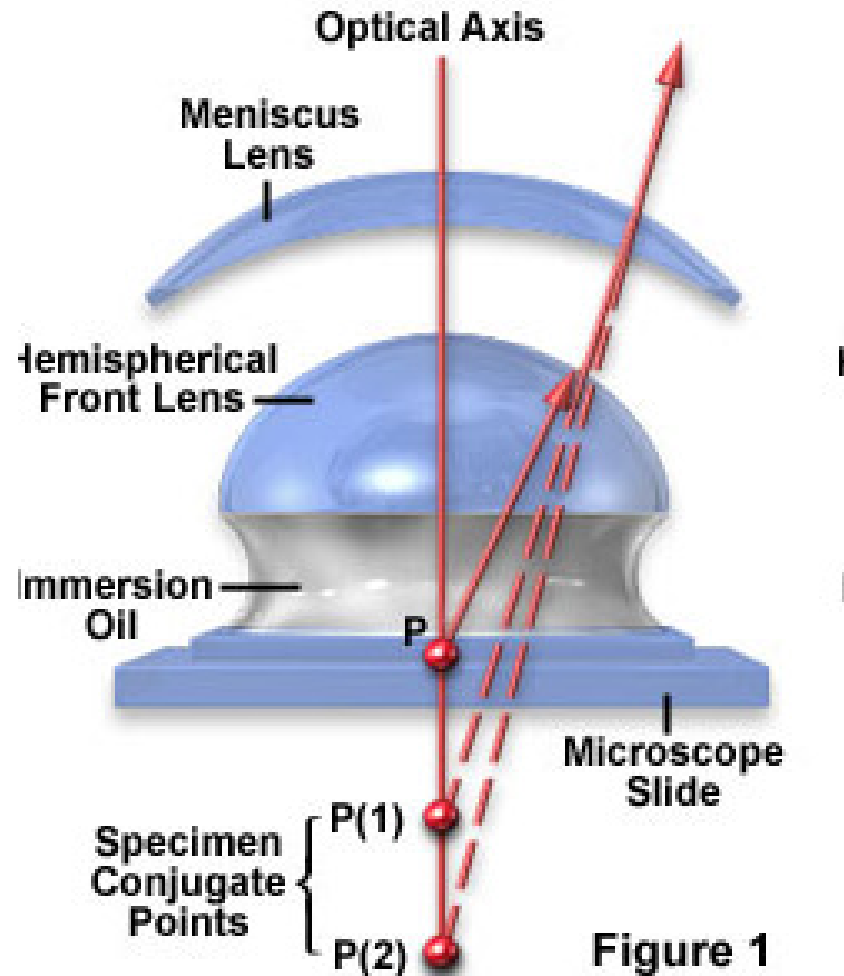
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CONFIGURATION 1 OF 1

Objective lens components

- Fluid medium and Flow cell material
- Air or Immersion? If air lies between the object and the lens, the max NA is 1
- Optical Sine Condition
 - $n y \sin(a) = n_1 y_1 \sin(a_1)$,
 - For $y/y_1 = \text{const} = \text{transverse Mag}$ then $n/n_1 = \sin(a)/\sin(a_1)$
- Rays Satisfying the sine condition will not exhibit spherical or coma type aberrations.
- Hemispherical Element.
 - When the lens is immersed in an index matching medium the plane surface does not refract.
 - The first refractive surface is spherical, angles of incidence are low. To satisfy the sine condition, the object is placed at point t so that $t + r = -r(n_1/n)$ or $t = -r(1 + n_1/n)$, with an origin at the vertex of the refracting sphere.
 - For a hemisphere, the Max NA is given by the n of the embedding medium times the sine of the half angle subtended by the objective aperture as observed from the object.
 - If the medium is aqueous, $n \approx 1.33$ and NA required is 1.2 with $t = 2$ mm what is r ?
 - $\text{Arcsine}(1.2/1.33) = 64.5$ degrees, $\tan \theta = 2.09$, so $r = -4.18$ mm.

Aplanatic Points

Oil-Immersion Microscope Objective



Meniscus Element

- For an object placed at the aplanatic point of the hemisphere light appears to radiate from a point farther from the hemispheric surface. This point will be the center of curvature of the first surface of the meniscus, so no refraction takes place there. Refraction takes place at the second surface according to the sine condition, resulting in a second aplanatic point conjugate to the first two but farther along the optical axis.

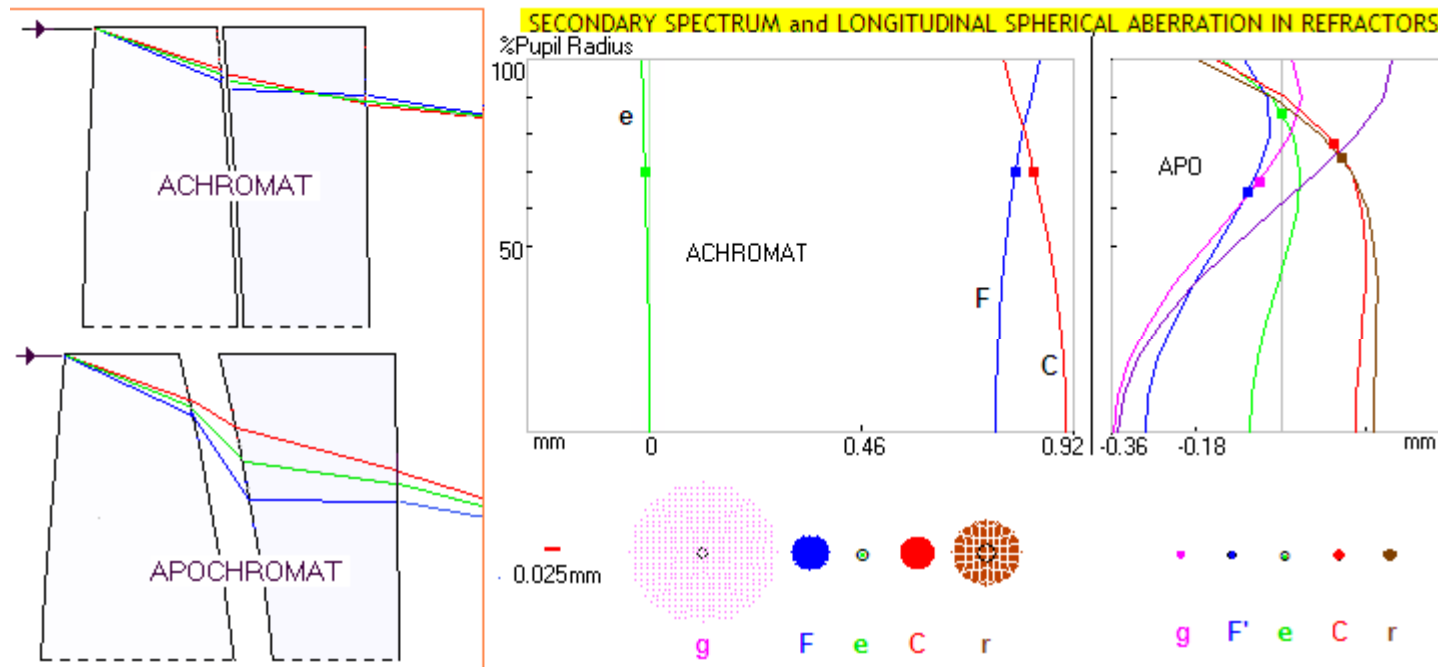
Achromatic Elements

- Normal dispersion is true of most glasses, ie $n_{351} > n_{586} > n_{633}$
- For a given surface curvature and glass thickness, this results in a different focal length for each color, that is called axial chromatic aberration.
- Glasses are usually specified as xxxyyy where xxx is the refractive index and yyy is $V \times 10$
- Catalog tolerances for n are .001 and for V are .002

Cemented Achromat Design Principles

- For a given focal length, SA and Coma are corrected by relative power of the two elements and the bending of the lens as a whole, LCA is controlled by choosing the indices and dispersion of the glasses.
- Combining a low dispersion strong positive crown with a high dispersion Flint element result in a mild positive lens, color corrected at two wavelengths.
- Reducing curvature of glass air interfaces and thickness will reduce path differences at different colors, but may leave spherical aberrations under corrected.
- First and last surface power leads to under corrected spherical aberration, buried surface curvature leads to overcorrected spherical aberration.
- $-f_{y2}/f_{y1} = V_1/V_2 = r$ where f is the focal length of the cemented elements leads to an achromatic design.
- If d is element thickness along the axis and D ray path length through the element then
- $r = -((nd-1)_a(d-D)_a)/((nd-1)_b(d-D)_b)$
- Chose a trial starting point and iterate.

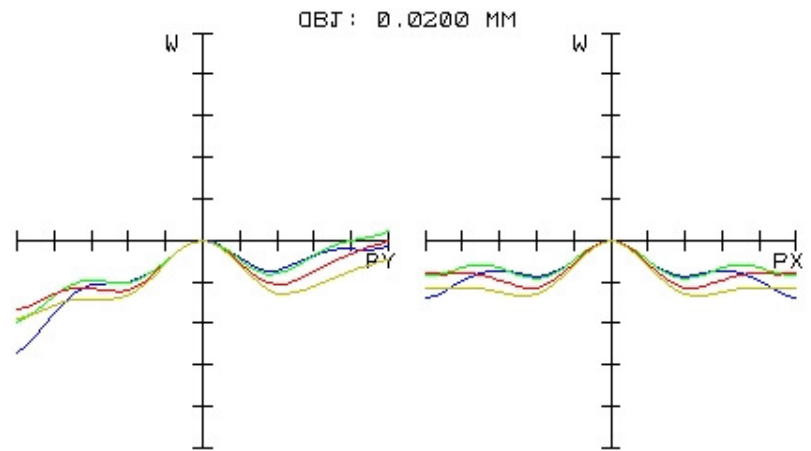
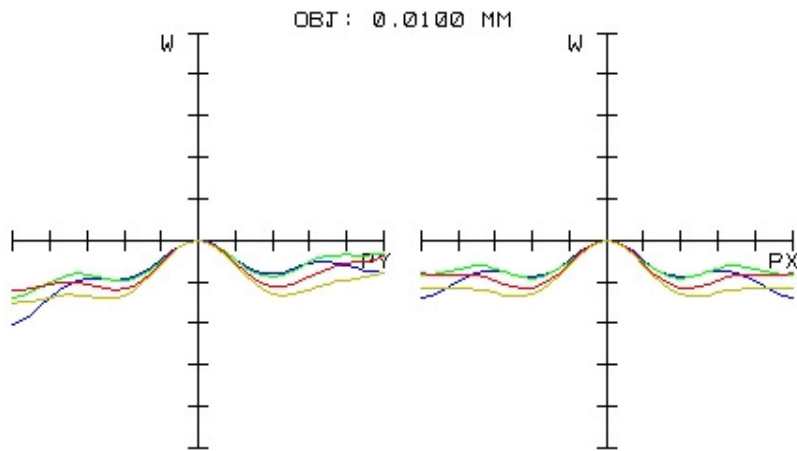
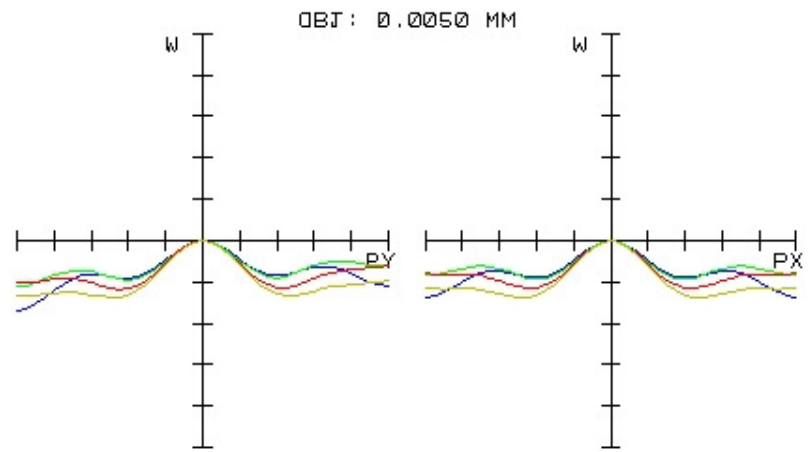
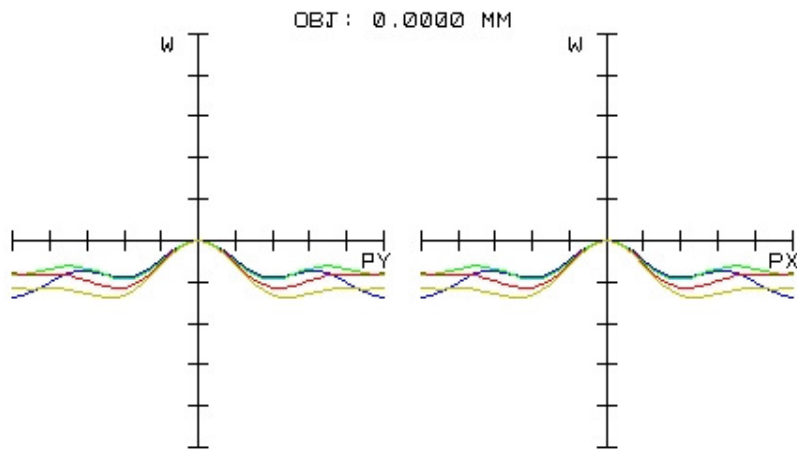
Achromat vs. Apochromat



PREFERRED Glass Selection

Optimax Systems maintains a sizable inventory of standard grade optical materials, as identified in the highlighted column below. These glasses are recommended when quick delivery is critical. As required, optical crystals and ceramics are also processed at Optimax. At the time of order placement, some consideration should be given to material delivery. Price shifts with market conditions.

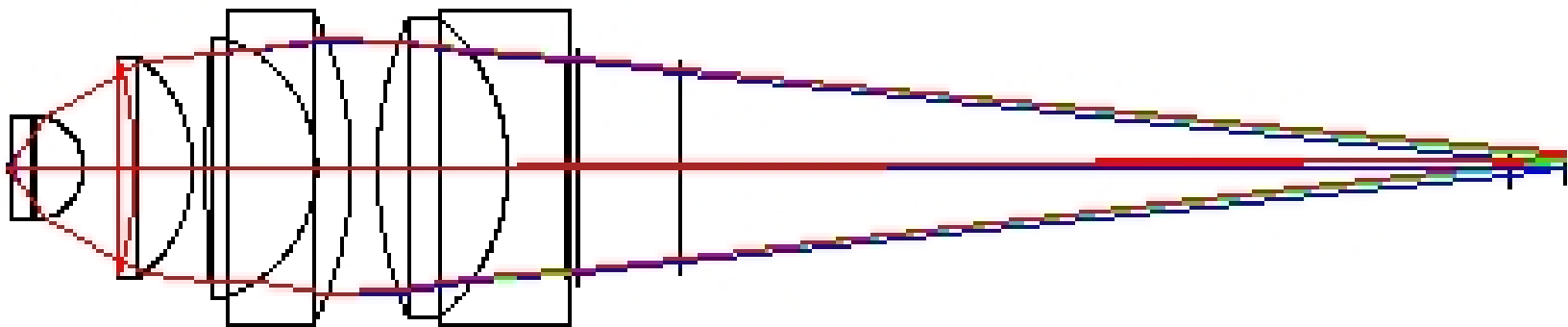
±001002	Optimax Preferred	Hikari	Hoya	Ohara	Schott	CDGM
439950	S-FPL53			S-FPL53		
*458678	7980 OF	Fused Silica			ML-Q#	JGS2
*474660	PYREX					
487704	S-FSL5	E-FK5	E-FC5	S-FSL5	N-FK5	QK3
517642	S-BSL7	E-BK7	E-BSC7	S-BSL7	N-BK7	K9
522597	S-NSL5	E-K5		S-NSL5	N-K5	K50
532488	S-TIL6	E-LLF6	E-FEL6	S-TIL6		QF6
540596	S-BAL12	E-BAK-2		S-BAL12	N-BAK2	BAK2
547536	N-BALF5				N-BALF5	BAF1
548458	S-TIL1	E-LLF1	E-FEL1	S-TIL1	LLF1	QF1
552635	N-SPK3				N-PSK3	BaK4
569562	S-BAL14	E-BAK4	E-BaC4	S-BAL14	LF5	QF50/QF12



OPTICAL PATH DIFFERENCE

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 MAXIMUM SCALE: +/- 20.000 WAVES.
0.532 0.575 0.675 0.750

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 CONFIGURATION 1 OF 1



3D LAYOUT

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CONFIGURATION 1 OF 1

Design Constraints and degrees of Freedom

- One degree of freedom exists for each variable in the lens system, less one.
- A target value of these is necessary to begin optimization. Some may be fixed tightly in order reduce the complexity of the solution.
- Scores of optimization targets must be iteratively specified.
- Tolerances will critically effect cost and performance.
- Achromat degrees of freedom are the radii of 3 surfaces, the center thickness of the elements, the index and Abbe number of the glasses. Glasses are limited to commercial glass grades.
- Glass n and V are guaranteed WITHIN LIMITS! Often lens spacing or curvature must be re-optimized for each lot of glass. This may mean new tooling, so lens spacing is a less expensive variable to leave with residual tolerance. This is not always enough.

Beam Shaper Mounting and Positioning

- The optical axes of the illumination and collection systems must cross at the center line of the fluidic system that carries the sample.
- The designer has to control six degrees of freedom of motion of each of these.
- It is simpler to fix as many of these as possible, but manufacturing tolerances intrude. Some adjustment is inevitable.
- Collimated laser light should enter the lens on the most curved surface to maximize the refractive power of the element.
- Cylinder lenses may be mounted by pressing the cylinder surface against the edges of a rectangular aperture, or by pressing the flat surface against a mounting plane.
- Wedge error in lens manufacture leads to tilt of the beam axis in the plane of the surface normal of the wedge.
- The accuracy of setting is on the order of tenths of a micrometer in some axes. Thus the precision and stability of positioning is of critical concern and require fine thread anti-backlash mounts.

Mechanical Mounting

- The fluorescence collection system is large and heavy. It is best to provide a means of defining a coordinate system for laying out the components.
- Filters can be made with a slight excess diameter to trade cost of components with cost of mounting precision tolerance.
- The excess diameter is useful as a mounting surface.
- Adjustment of filter mounting angle may be provided, if detectors are to be fixed.
- If filter position is fixed, then detector position adjustments will be needed.
- The fluidic system can be mounted to and adjusted upon the front surface of the objective lens.
- For the objective lens, the focus axis requires micron or submicron precision, temperature compensation is mandatory. Tight constraint of all five other axis is required as these form the center line of all other subsystem layouts.
- Expensive fixtures, QC instruments, and trained technicians are required to assemble and mount objective lenses elements in barrels in order to assure the instrument stability and sensitivity.

Light Scatter Optics

- Light diffracted from particles and refracted by flow system components is intercepted by a lens which directs it at near normal incidence upon a detector array.
- A simple lens is usually used for low angle scattering.
- The fluorescence objective is used for higher angle scatter collection.
- The detector array is set up to collect light scattered into different angular zones by different classes of cells. These zones are determined by experiment, frequently a cell will scatter into a number of these zones and principal component analysis in a multi dimensional space is required to classify the results.
- Compromises in angular zone discrimination are made for reasons of economy. A significant one is allowing the light to suffer internal reflection before reaching the lens, thus increasing light losses from smaller particles.

Fluorescence Objective Lens

Choices:

Working distance:

- A long WD makes flow cell design and assembly less difficult.

- A long WD increases spherical aberration

- A long WD increases the diameter and costs of all other optical components.

Numerical aperture

- Fluorescence light collection increases as the square of NA, as does cost.

- Focus tolerance is reduced as NA increases.

Field of View: This is constrained by the spatial filtering aperture.

Wavelength Band:

- Color correction for more than three wavelengths is very expensive.

- Full correction may not be needed if tolerance in focal length with wavelength can be accommodated by shifting sensor positions.

- Hybrid diffractive-refractive lenses can be made to cover ~20nm bandwidth.

Magnification for spatial filtering: this controls tube length, or vis a vis.

F# for illuminating filters:

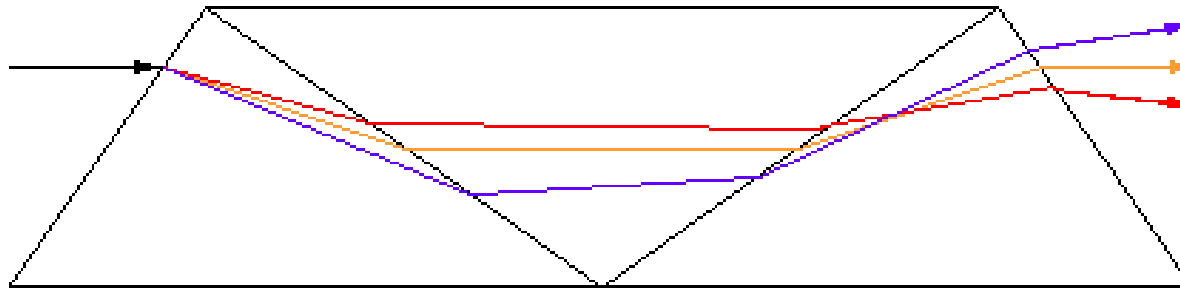
- Filter pass bands shift ~1 nm to the blue

- for each degree of tilt away from the design point.

Dispersive System

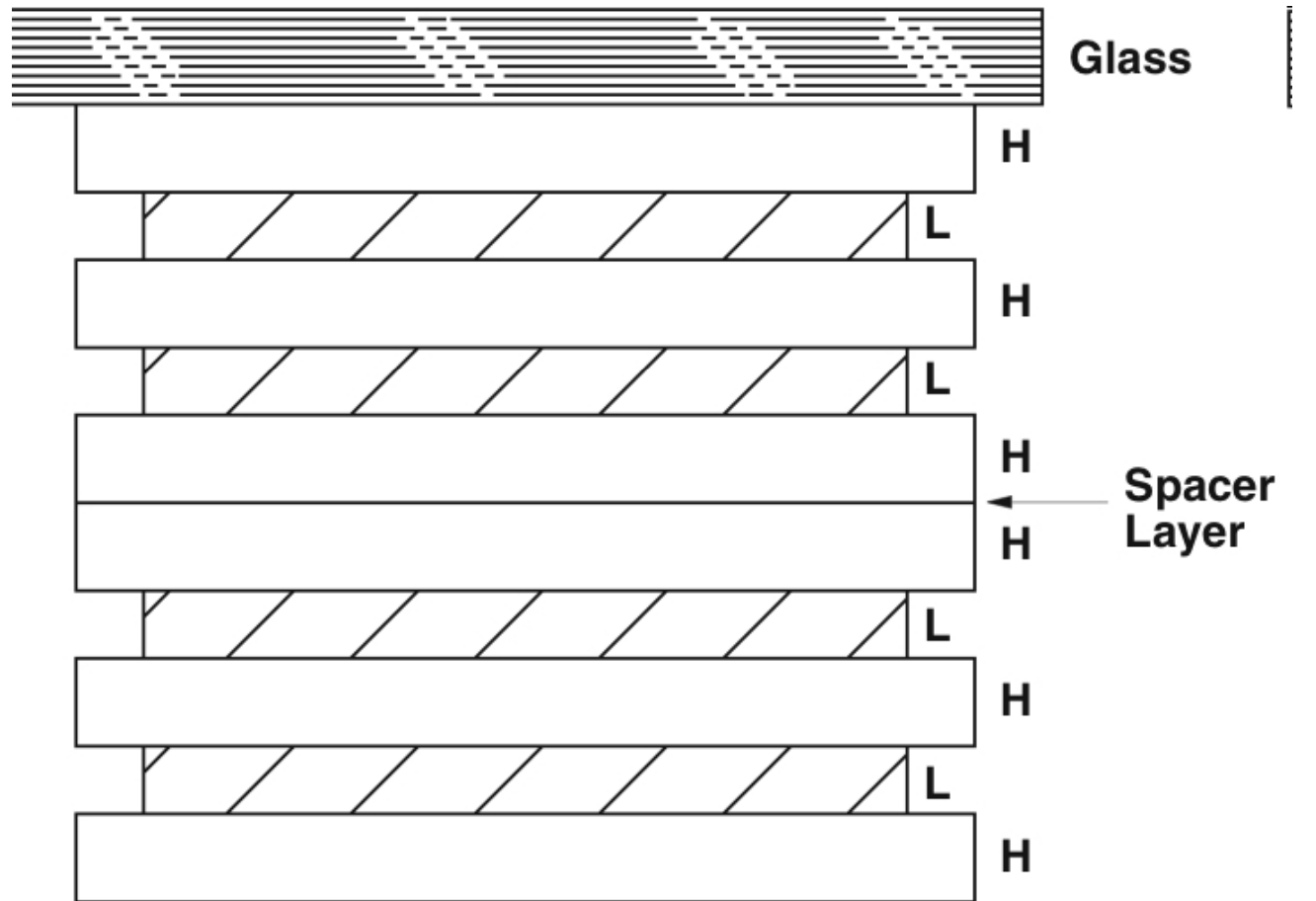
- Prism
 - For an efficient layout, a double Amici Prism arrangement has been used. Ref US Patent *4609286*. Angular deviation of the red and blue beams may be a few degrees, requiring a long focal length relay lens to allow separation between detector apertures.
- Filter Stack
 - Multilayer filter materials absorb strongly at wavelengths shorter than ~400 nm. Short wavelengths may cause some filter materials to fluoresce. Reflecting those wavebands out of the beam early in the path reduces losses in those bands and contamination of other signal channels. Cost ~ a few hundreds of dollars each. Requires a high F# in the filter section to maintain sharp band edge cutoff.
- Grating efficiency varies with λ by a factor of ~5 over the UV to near IR, with a maximum at the blaze wavelength. Additional relay lens needed to image the aperture on a detector aperture.

Double Amici Prism Spectroscope



I.N. Galidakis

Interference Filter



Air/Spacer

JML Optics

Filter Design and Mfg

- Multilayer dielectric coatings are used to create interference filters.
- Ideal coating material $n_2 = (n_1 n_3)^{1/2}$ for layer order 1,2,3.
- The substrates must transmit the desired wavelengths without attenuation or unwanted autofluorescence.
- There is a phase change of pi radians for light entering a higher index medium, no phase change when entering low index media.
- Alternating high and low index materials have thicknesses that form $\frac{1}{4}$ wave stacks. The reflected wave will be $\frac{1}{2}$ wave out of phase and interfere destructively to reduce reflection, and by compliment enhance transmission at a given wavelength.
- Placing a $\frac{1}{2}$ wave spacer computed for the center of the pass band creates the transmission spectral window.
- Transmission of multi cavity band pass stacks ~90%.
- Increasing spacer thickness and the number of quarter wave layers.

Filter Manufacturing Process

- Layers are created by sequential evaporation of materials, usually cryolite (Na_3AlF_6) $n=2.35$ and ZnS $n=1.35$, TiO_2 $n=2.903$, SiO_2 $n=1.46$,
- Al_2O_3 , ZrN , Si_3N_4 etc and others are also used.
- Absorption vs. λ is considered. Deposition temperature and chamber pressure and gas mix is critical.
- Microstructure of the layers impacts hardness, humidity sensitivity, scattering.
- Low pressure systems ($p \leq 10^{-5}$ torr) with filament, e-beam Ion assisted deposition or RF magnetron excited heat sources may be used. High Energy sputtered material gives harder coatings at lower process temperatures.
- Work Pieces are mounted in clips on rotating plates driven with planetary gears to achieve uniform coating on all pieces.
- Stationary witness pieces are monitored automatically by spectral lamp transmission or reflection by counting monochromatic interference fringes as thickness changes.
- Band edge position tolerances of 0.1 nm can be routinely achieved.