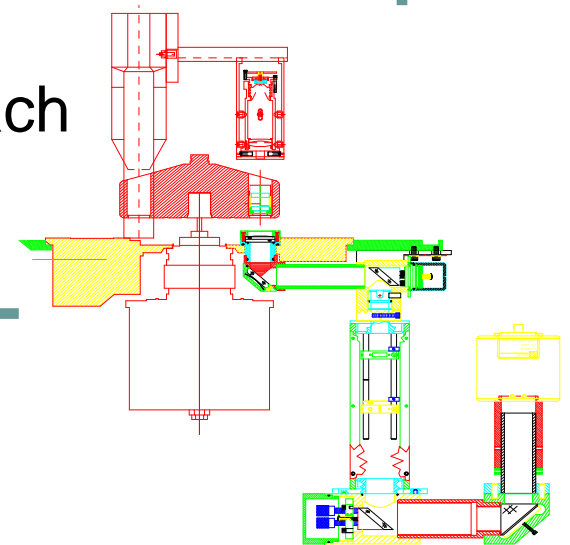
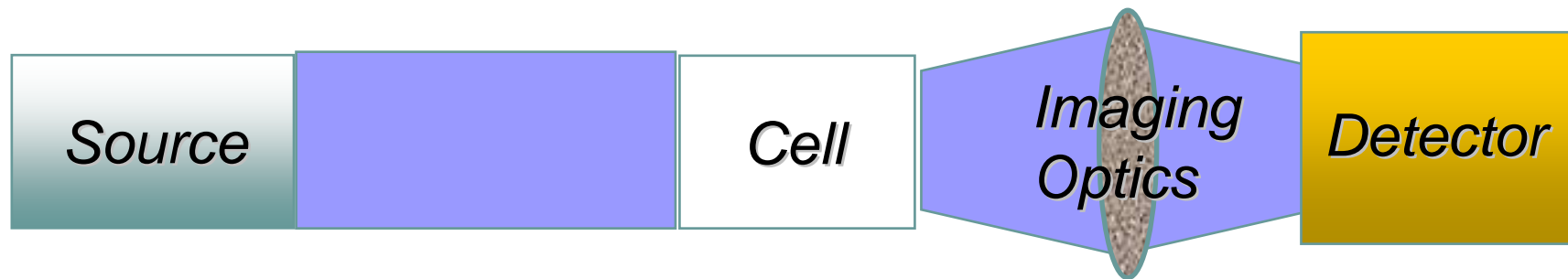


Optical Systems on the XLI

Description of each
How they work
Advantages and disadvantages of each
Choosing which to use



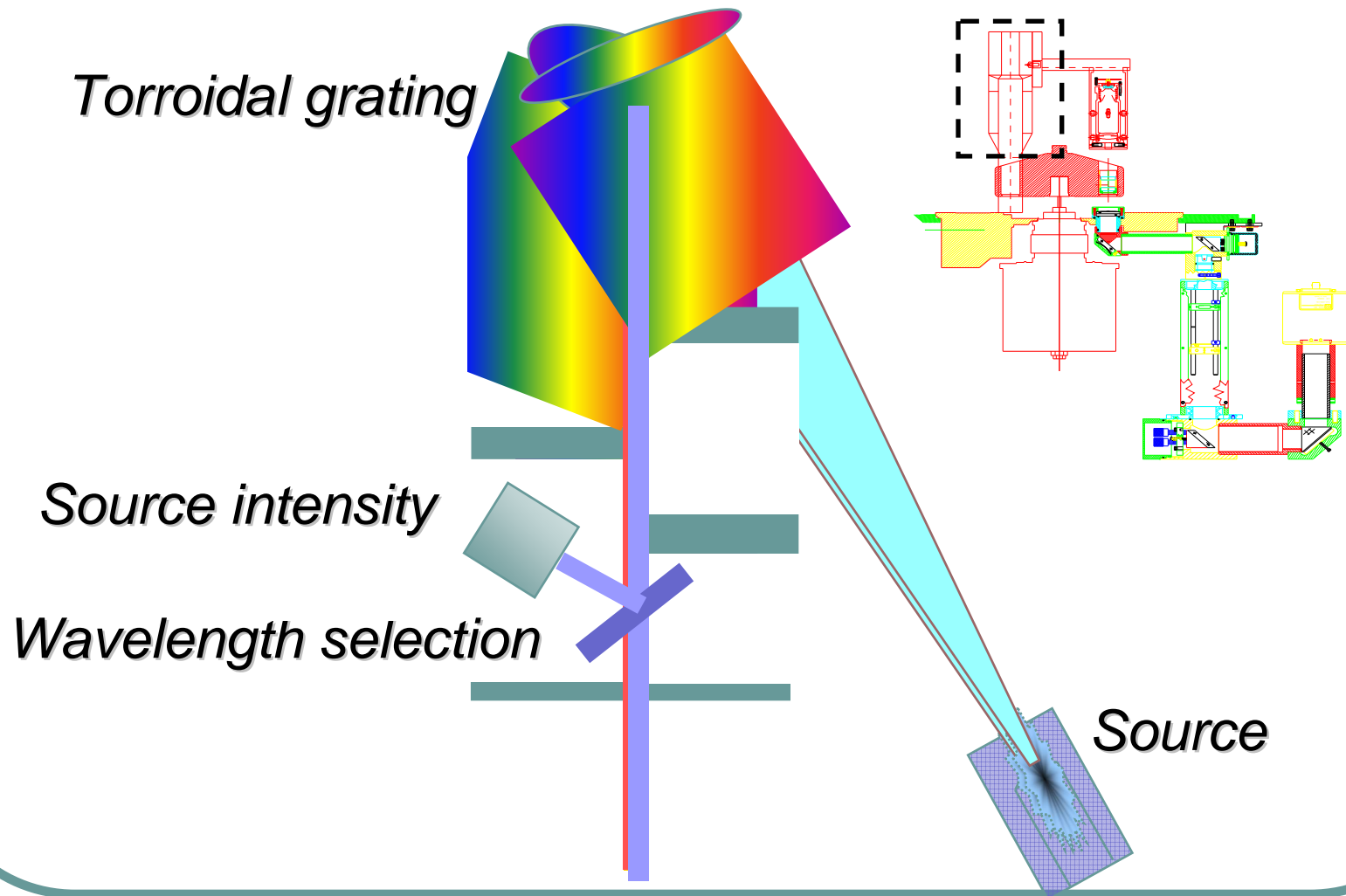
Optical system requirements



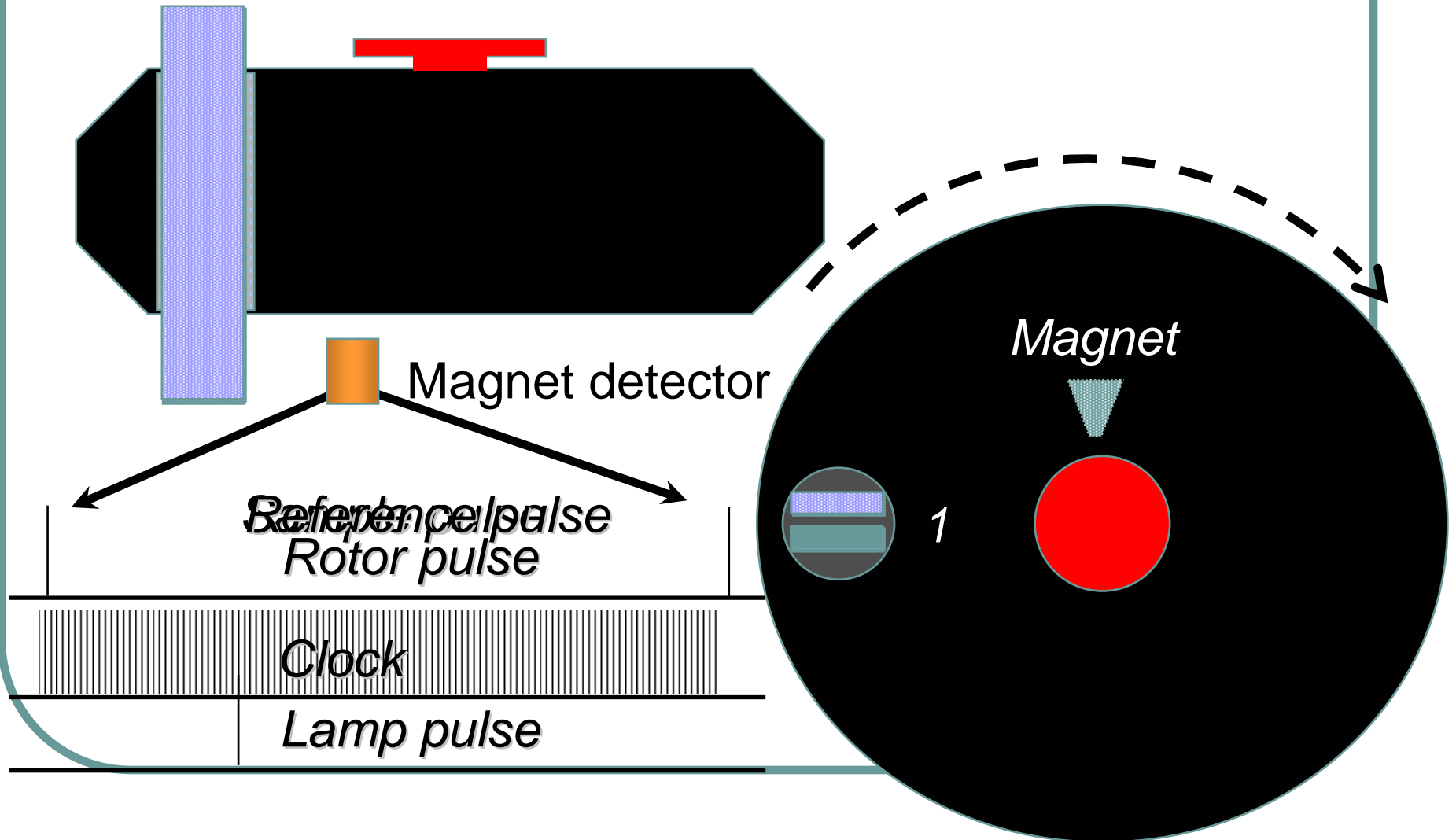
- ***Radial resolution***
- ***Sensitivity***
- ***Range***
- ***Linearity***
- ***Time to scan***

Absorbance Optical System

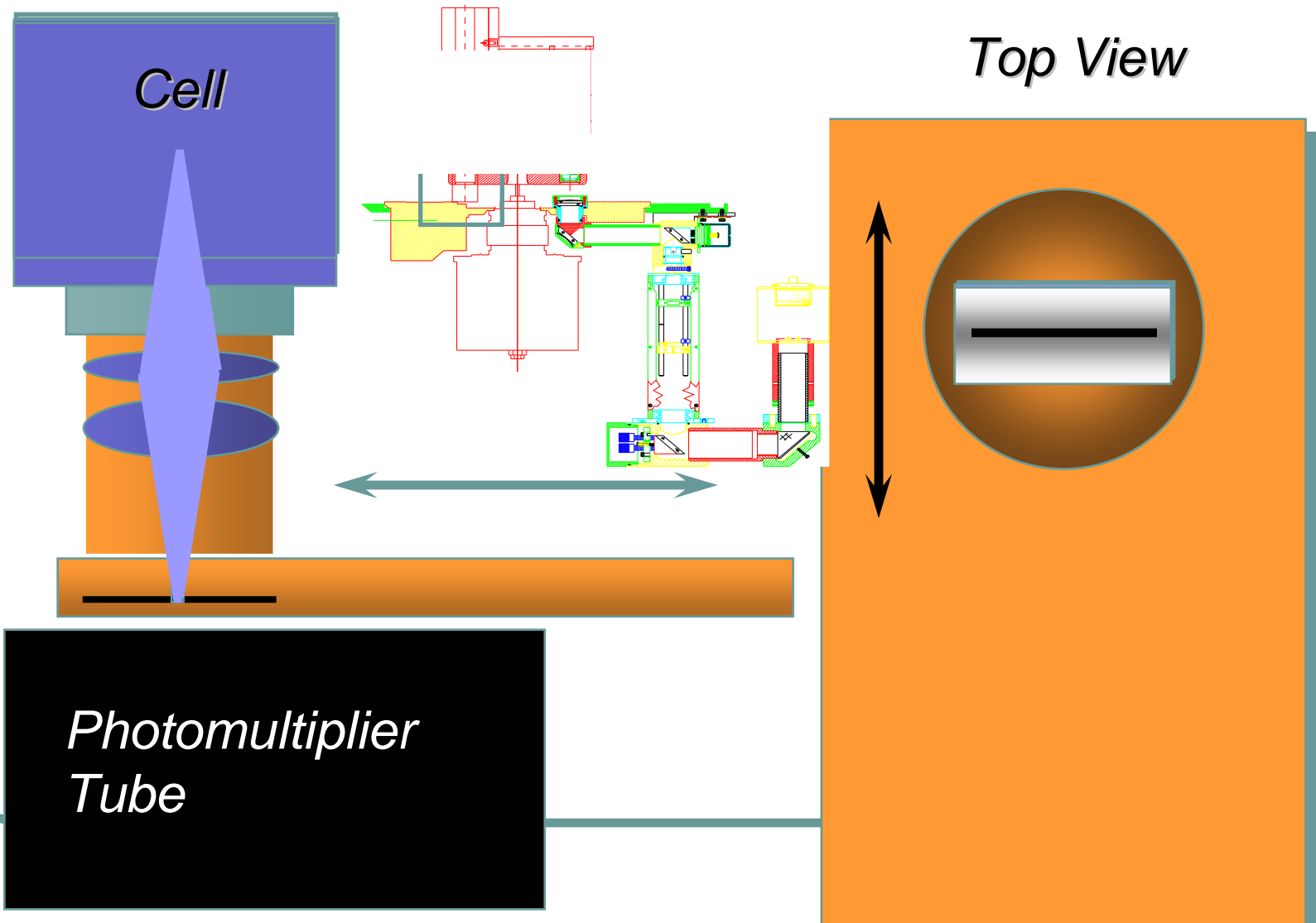
Source overview



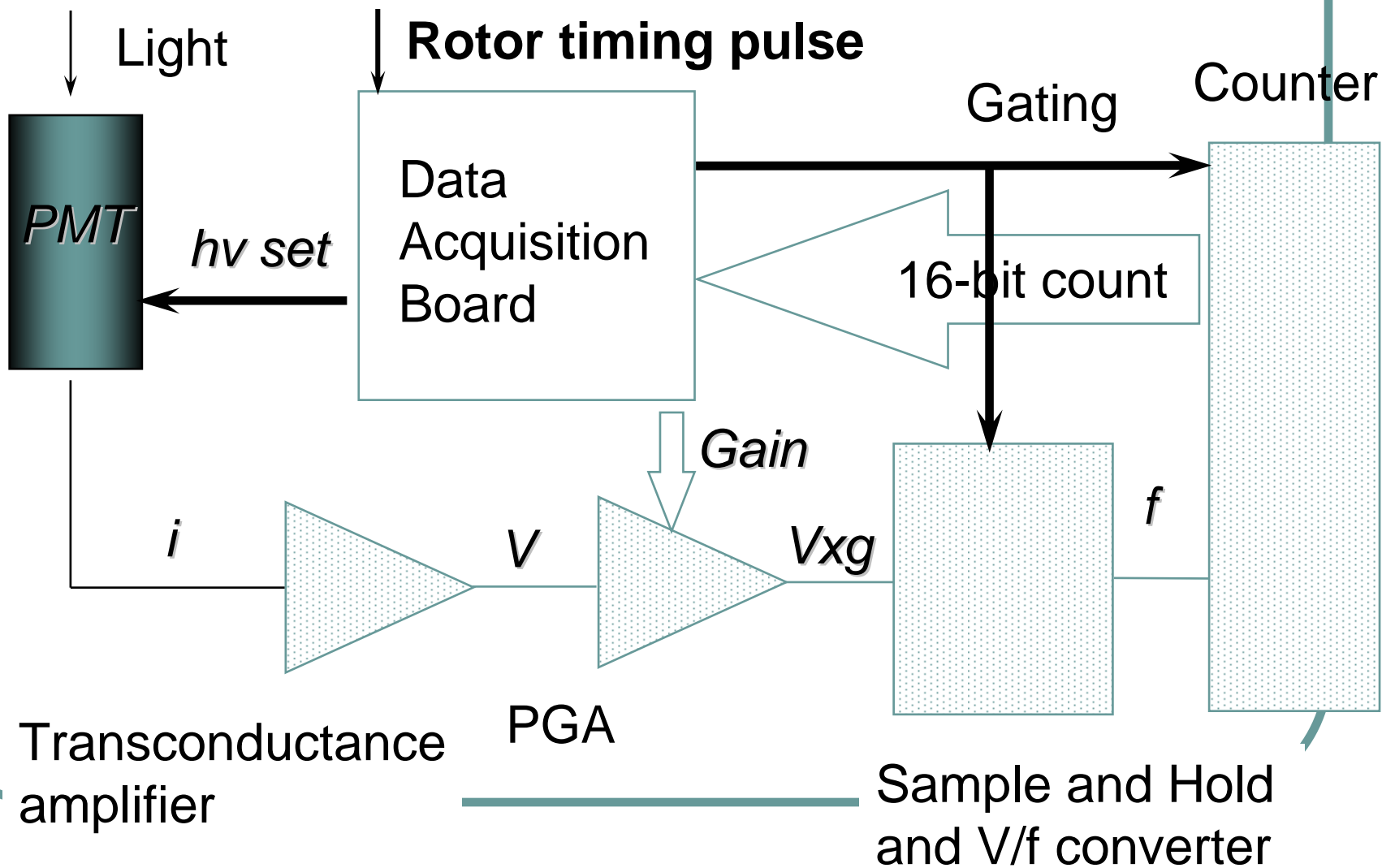
Synchronizing sources



Absorbance detector



Absorbance signal processing



Absorbance signal

- $A = \log (I_0/I)$
 - Low I_0 results in high noise level
 - Save data as intensities
- Noise is high frequency
 - Stochastic- rms $1/N^{1/2}$
 - Diminishing returns beyond $N=9$
 - Dependent on signal levels
 - Best between 0.1-1.0 A

Absorbance system facts

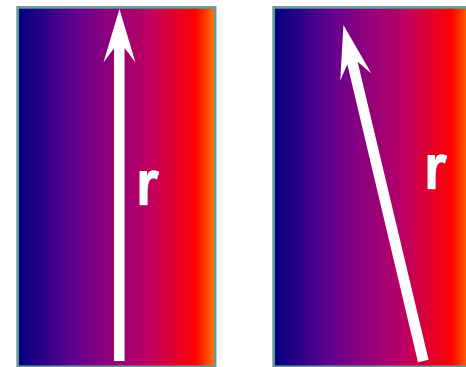
- Xenon lamp intensity 'spiky' and blue
 - Maximum light intensity 230 nm
 - Work at high intensity regime
- Maximum repetition rate 100 Hz
 - Limits scan rate above 6000 rpm
- Scales gain on reference intensity
 - Uses 6.5 cm
 - 'Invert' sample and reference may not work

When to use Absorbance

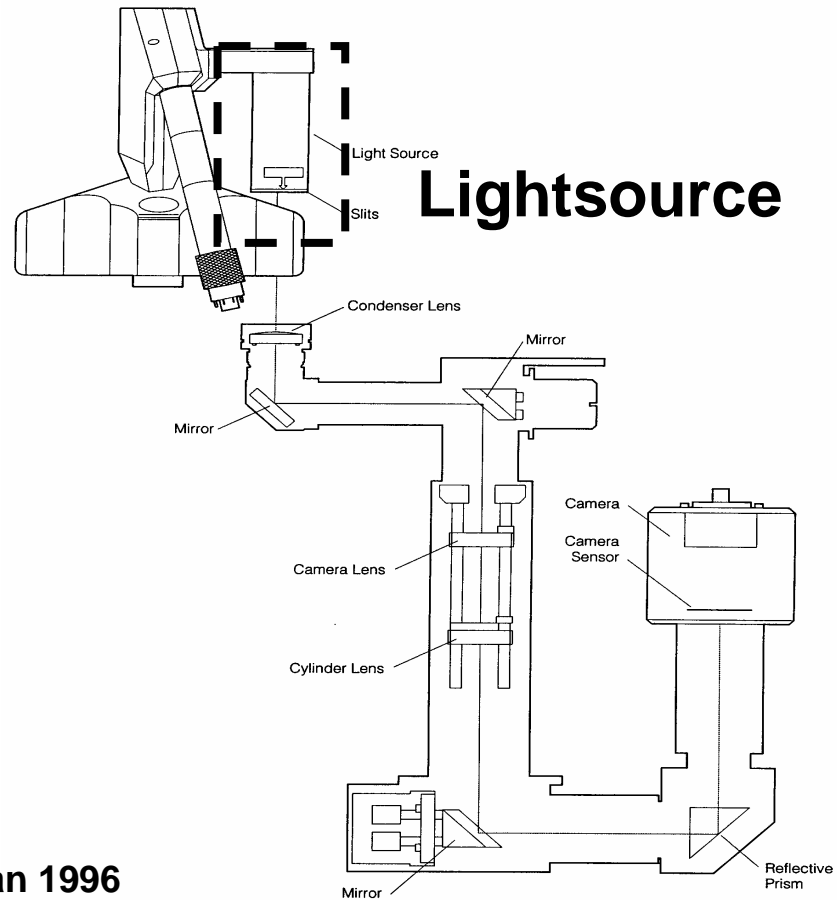
- Need selectivity
- Added sensitivity
- Cannot dialyze sample

Absorbance system 'gotchas'

- Low intensity!
 - Weak lamp output
 - Buffer absorbs
- Schlieren distortion at high gradients
 - Light deviated out of system
 - Typically overestimate $A(r)$
- Slit to lamp image tracking
 - Steep absorbance curve
 - Test holmium oxide cell



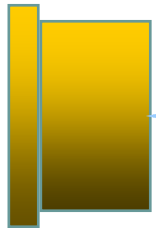
Interference optical system



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Interference source

Laser Diode



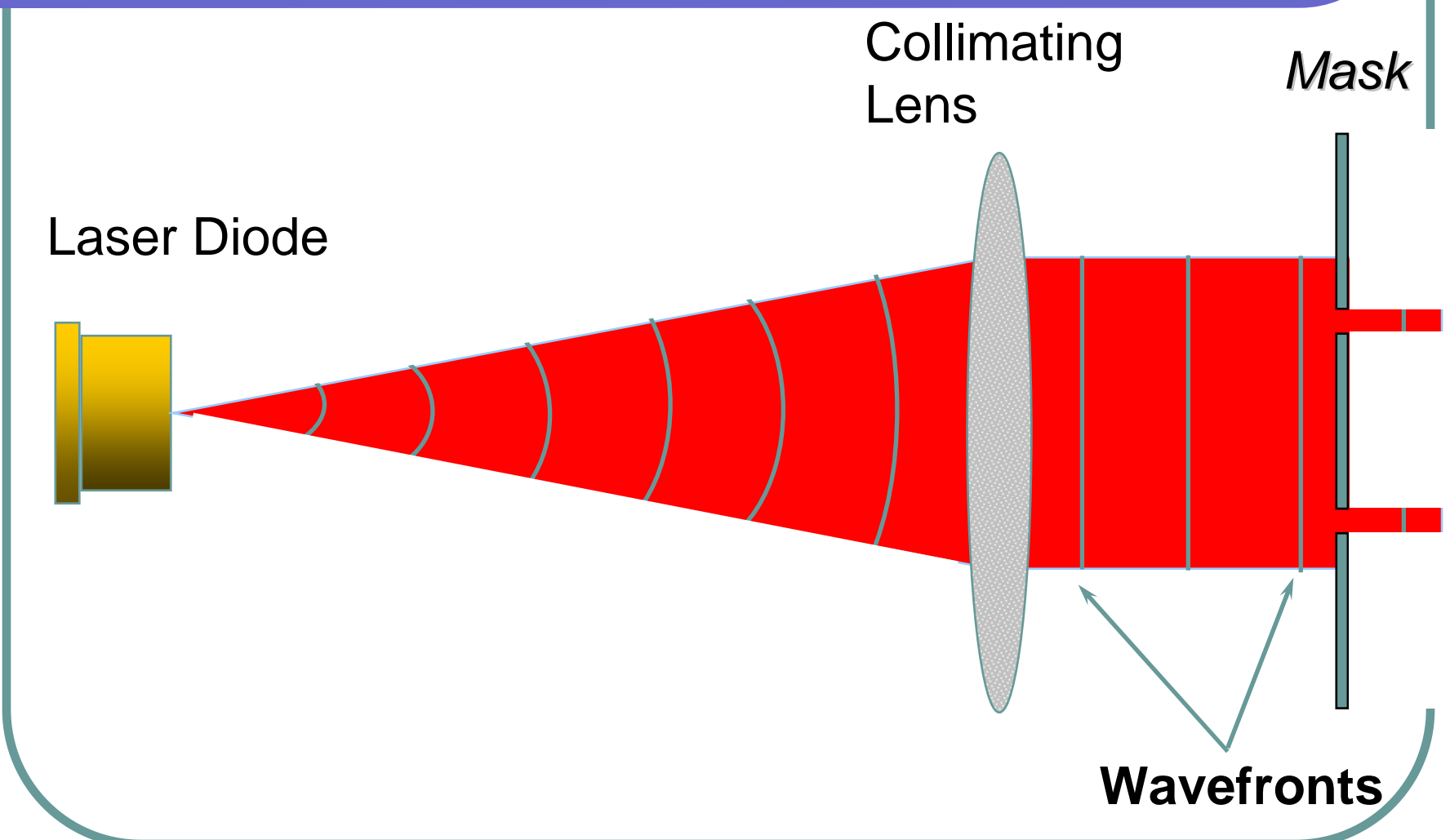
Collimating
Lens



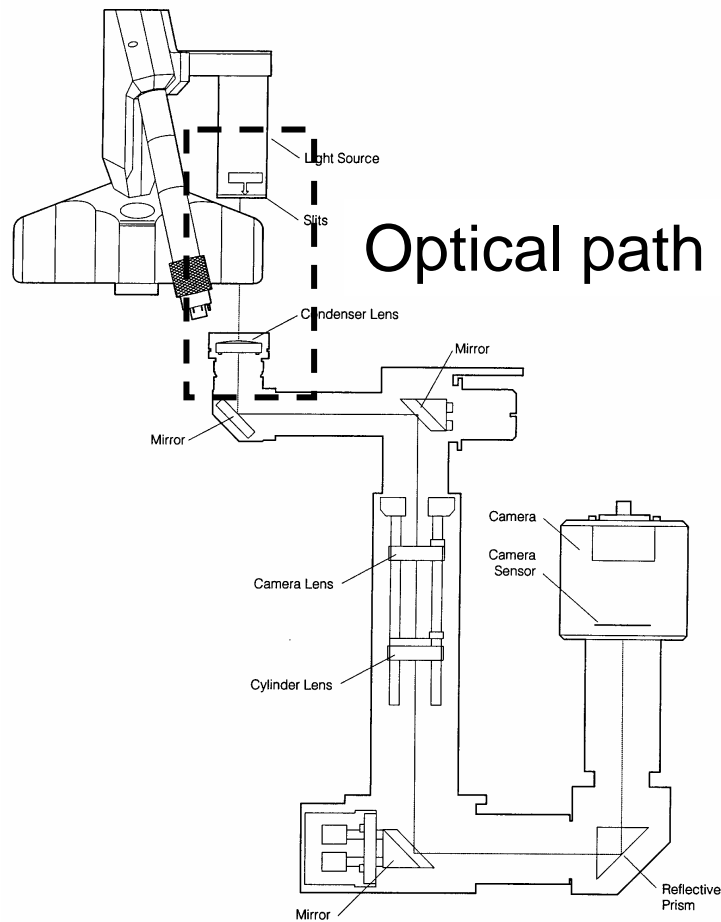
Mask



Wavefronts

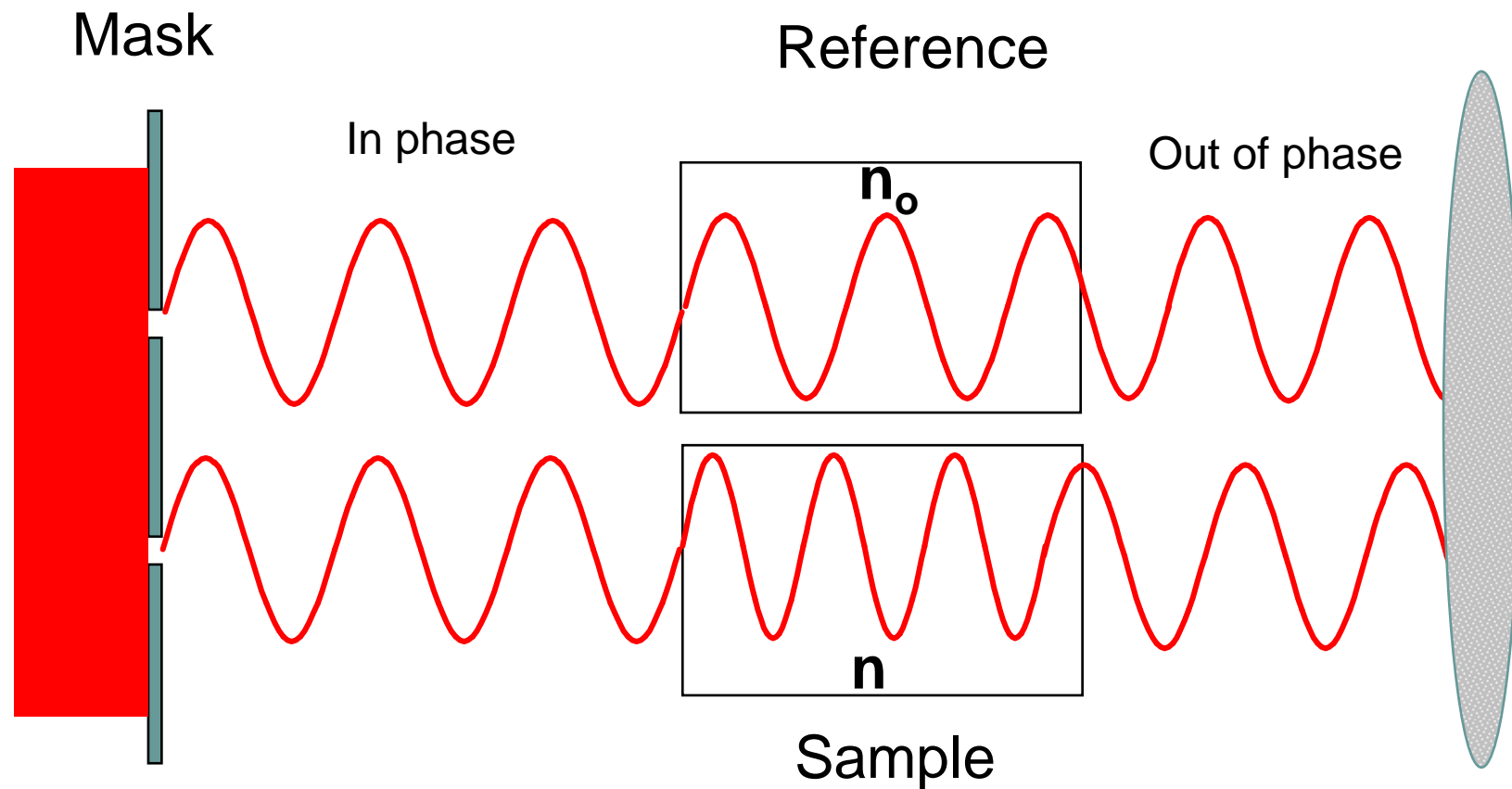


Optical path difference

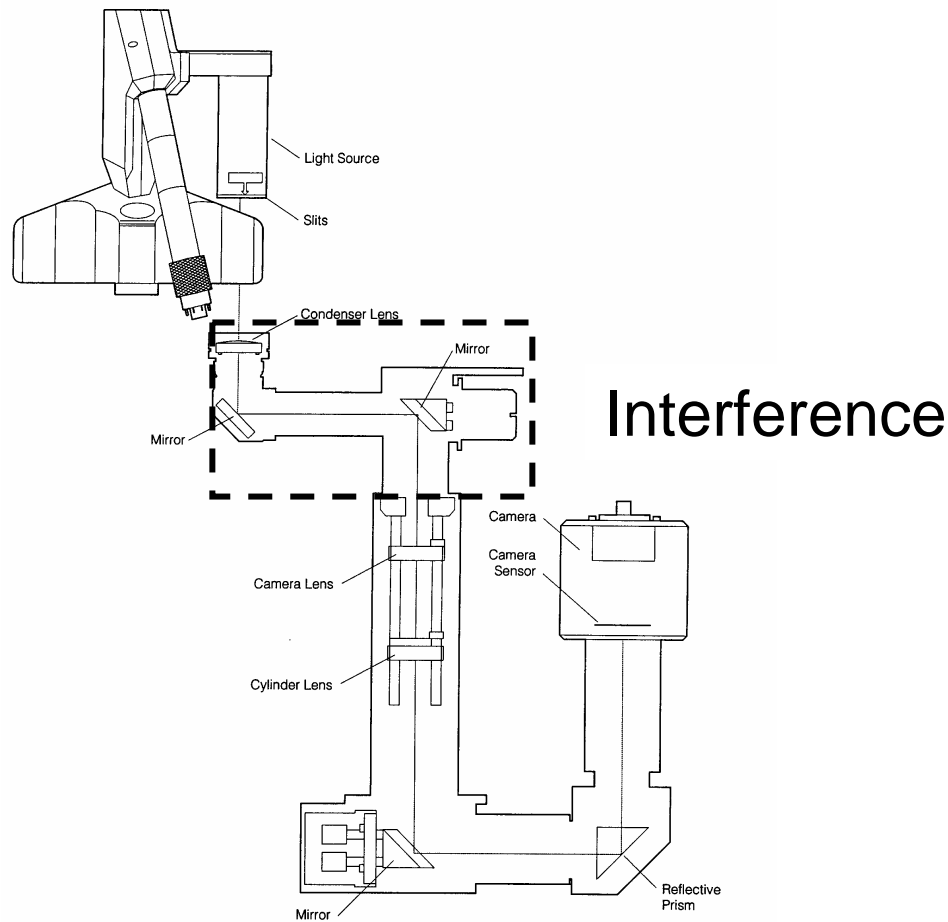


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Pathlength difference



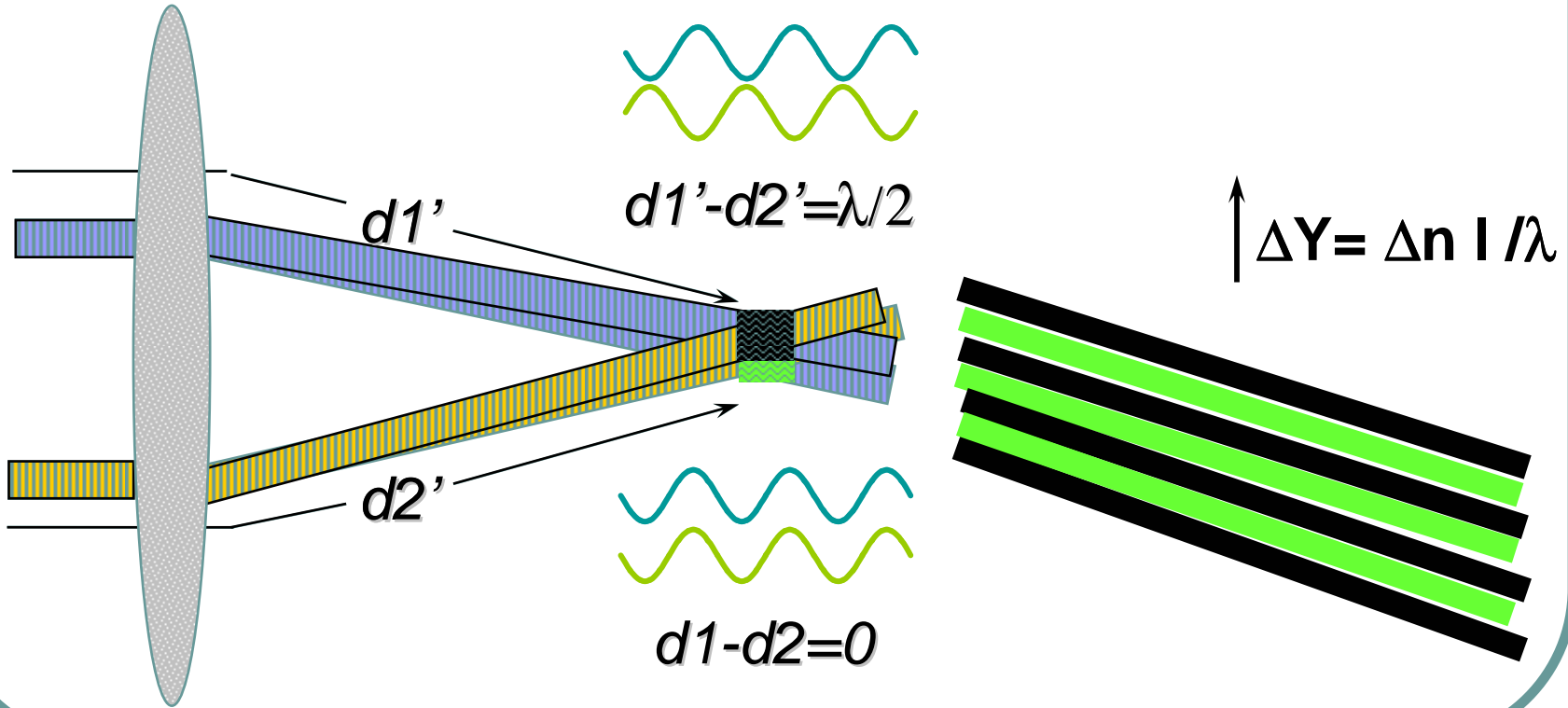
Interference optical system



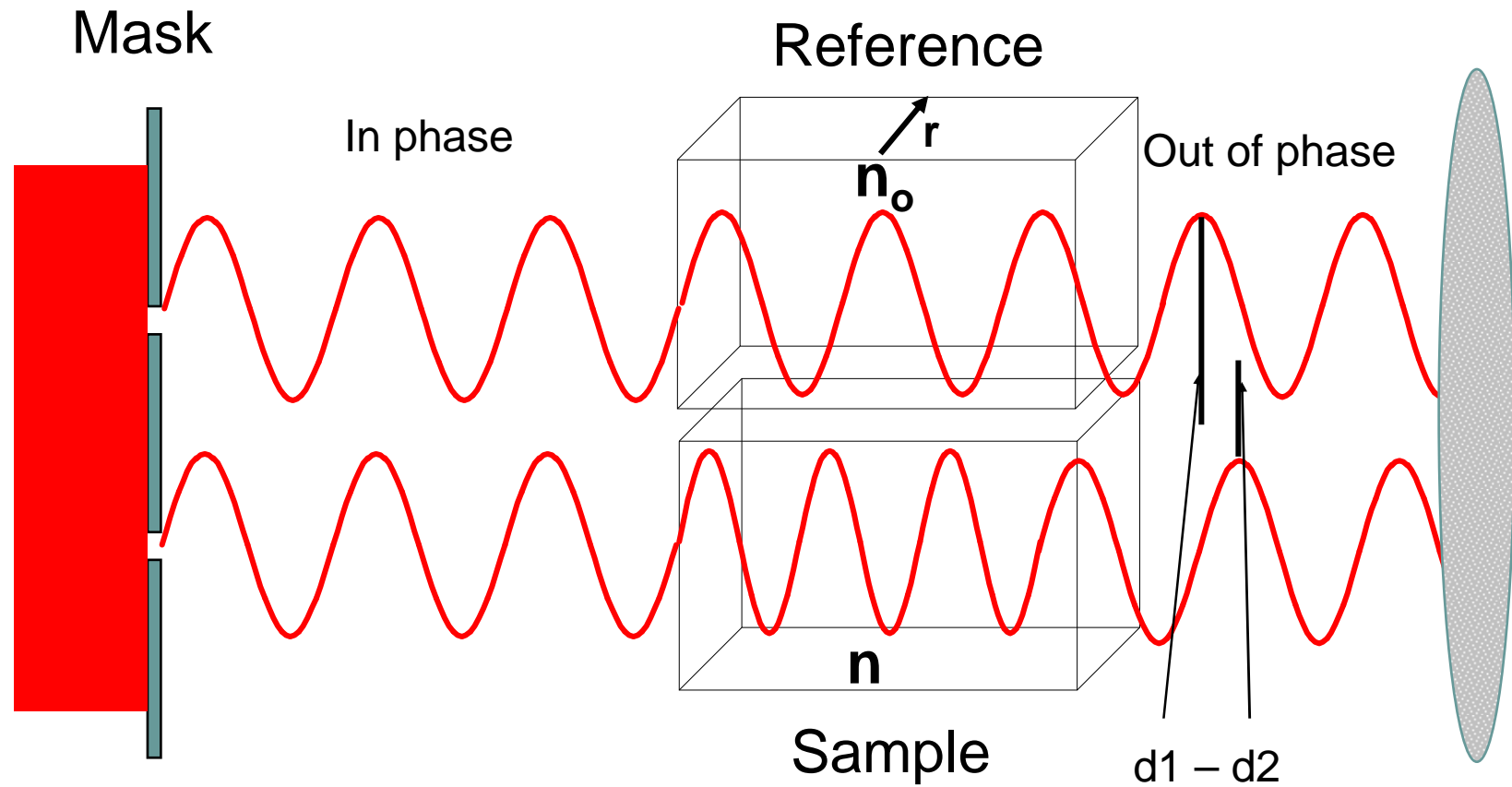
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Pathlength difference leads to fringe displacement

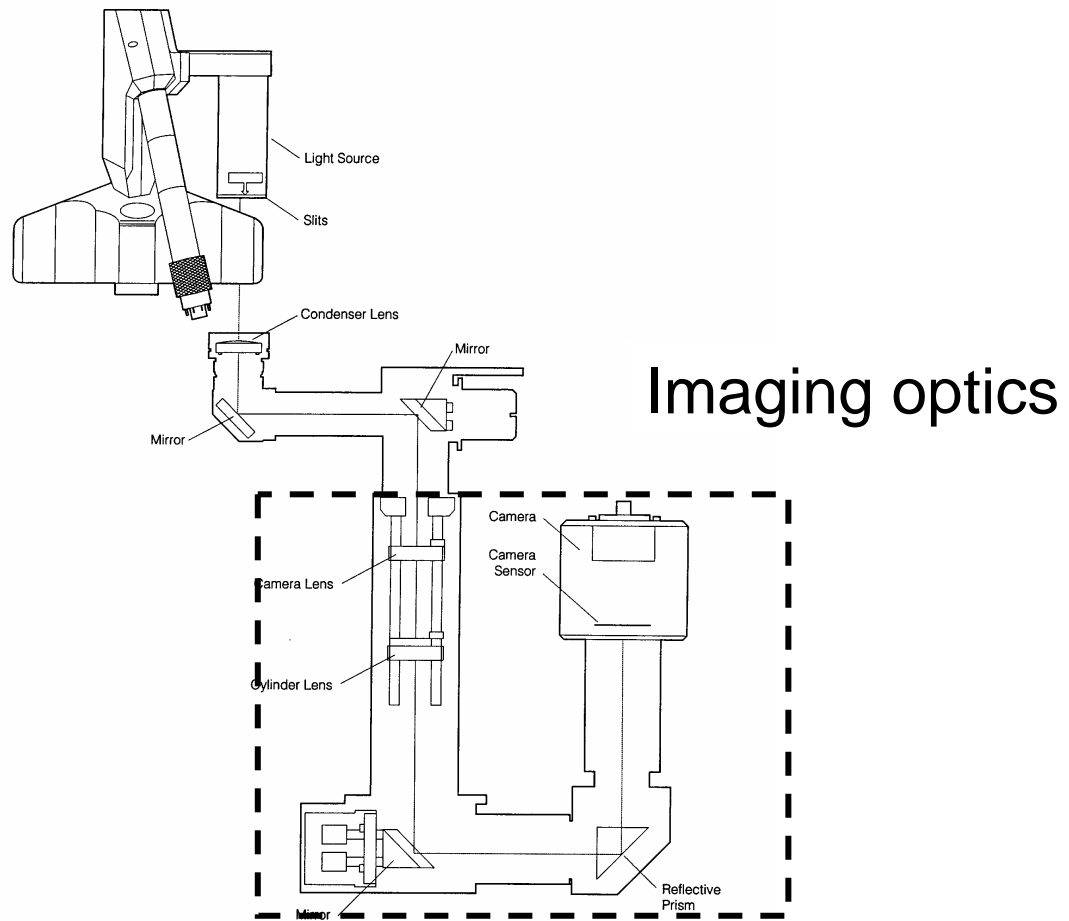
Condensing lens



Pathlength difference

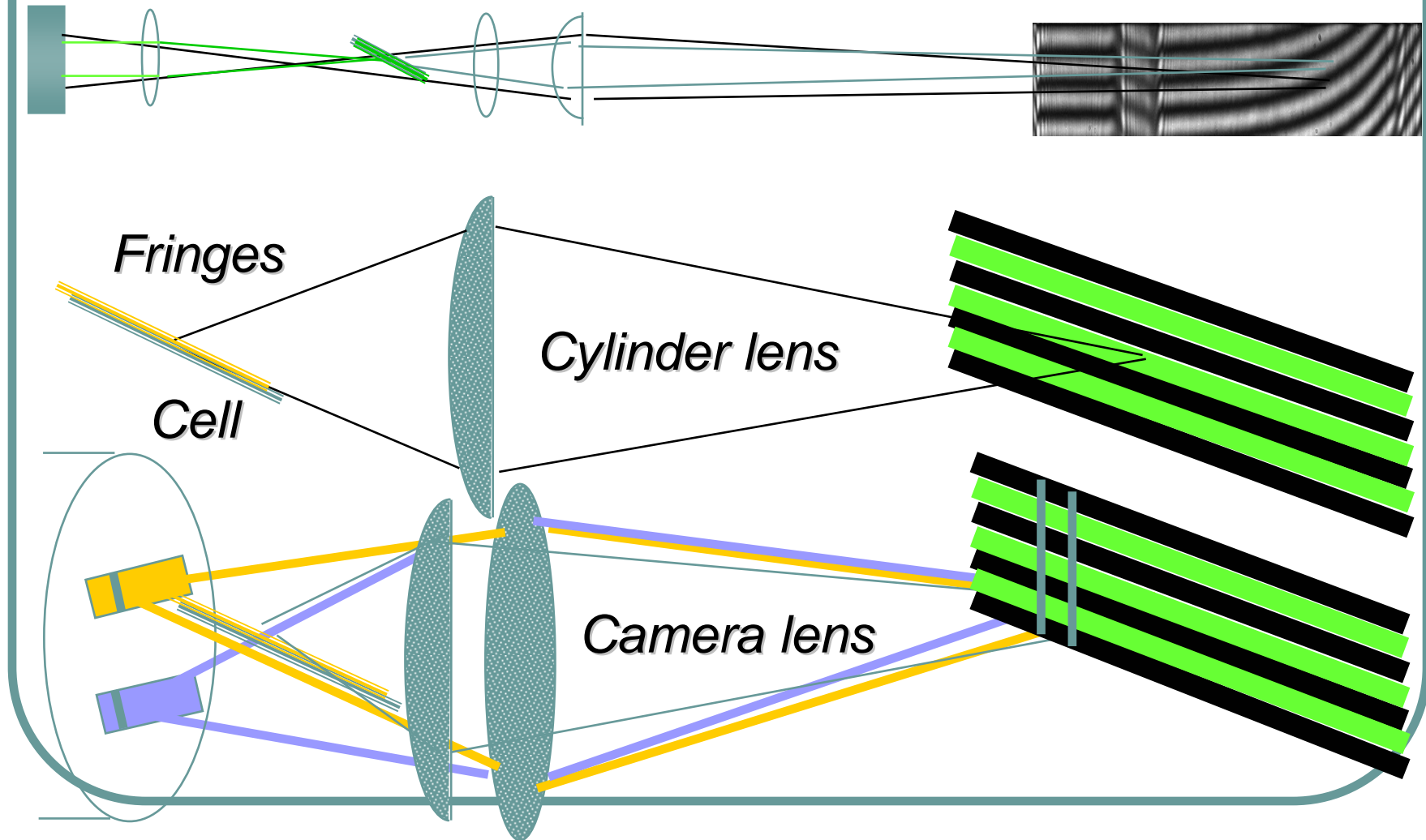


Optical path difference



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Superposition of images



Interference optical system facts

- Everything that contributes to d1-d2 will show up in fringe displacement
 - Only need to alter d1-d2 by $\lambda * 0.01$
- Must use sapphire windows
- Noise tends to be systematic, low frequency
- $\Delta Y = \Delta n l / \lambda$
 - Shorter wavelength laser will increase sensitivity
- Radial resolution 10 μm

Interference system 'gotchas'

- Incomplete dialysis of sample
 - Buffer components contribute equally and are in much higher concentrations
 - Watch out for detergents!
- Improper optical focus and alignment
 - Image looks fine... just inaccurate!
- Inappropriate blank correction
 - Water blanks should be used
 - Not needed for $\Delta c/\Delta t$, Sedanal

When to use interference optics

- Buffer absorbs
- Sample does not
- Precision required
- Extinction coefficient varies
- Short columns

Comparison of optical signals

- Absorbance signal: $A = \epsilon c l$
 - Note: $l = 1.2 \text{ cm}$
 - Can 'tune' to get acceptable ϵ
 - ϵ varies for different biological materials
- Interference signal: $Y = \Delta c l \text{ dn/dc}$
 - Note: Only difference in c is observed
 - dn/dc fairly constant for biological materials
- Roughly comparable sensitivity for protein at 280 nm and interference

Linearity issues

- Absorbance limits at low and high A
 - At 280- seems OK to 1.5 A
 - At 230- seems OK to 1.2 A
 - Quick check using absorbance ratios
- Interference
 - Linearity good to very high concentration
 - Depends on focus

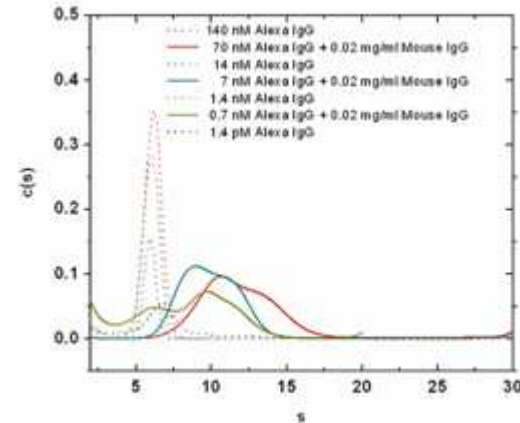
Choosing optical system

- Use absorbance if:
 - Need selectivity
 - Added sensitivity
 - Cannot dialyze sample
- Use both:
 - Determine extinction coefficient
 - Test for sample purity
 - Extend concentration range
- Use interference if:
 - Buffer absorbs
 - Sample does not
 - Precision required
 - $g(s)$
 - Extinction coefficient varies
 - Short columns

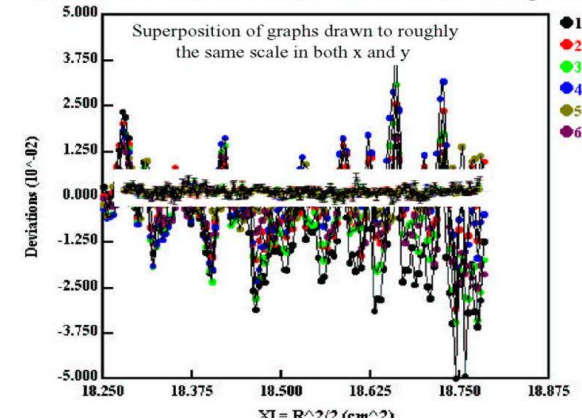
Aviv Biomedical

Improved optics XLI Retrofits

- **Fluorescence optics**
 - Available now
 - pM to μ M
 - Complex systems
- **Absorbance optics**
 - Under development
 - Signal acquisition same as fluorescence optics
- **Interference optics**
 - Under development
 - Larger camera & new laser mount
 - Noise \downarrow 100-fold



Comparison of MATCH Deviations for Data Obtained with usual XLI camera and 1024x2048 Philips camera

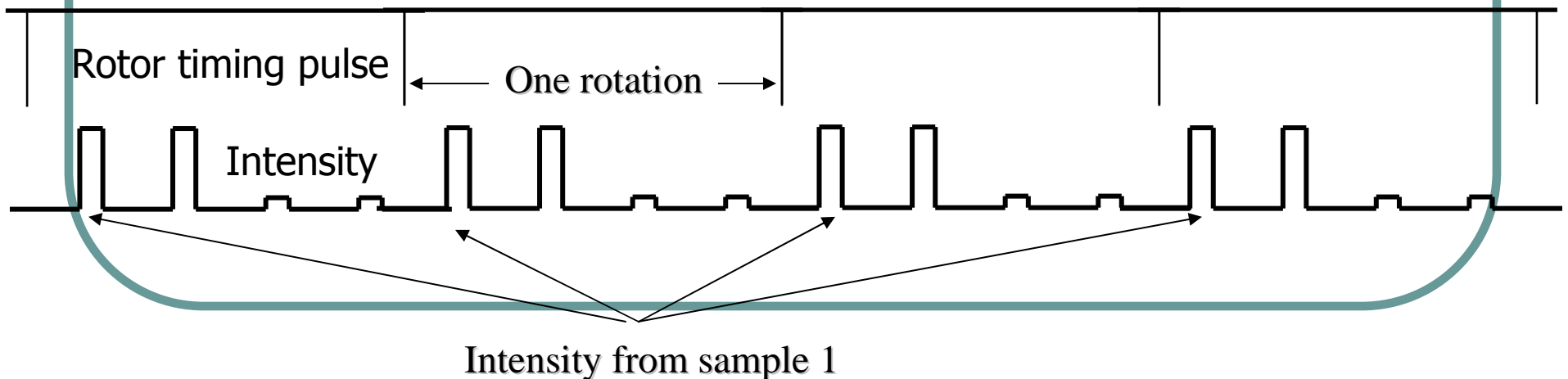


Courtesy of J. Lary & D. Yphantis

AU-FDS & AU-RSA

Synchronizing

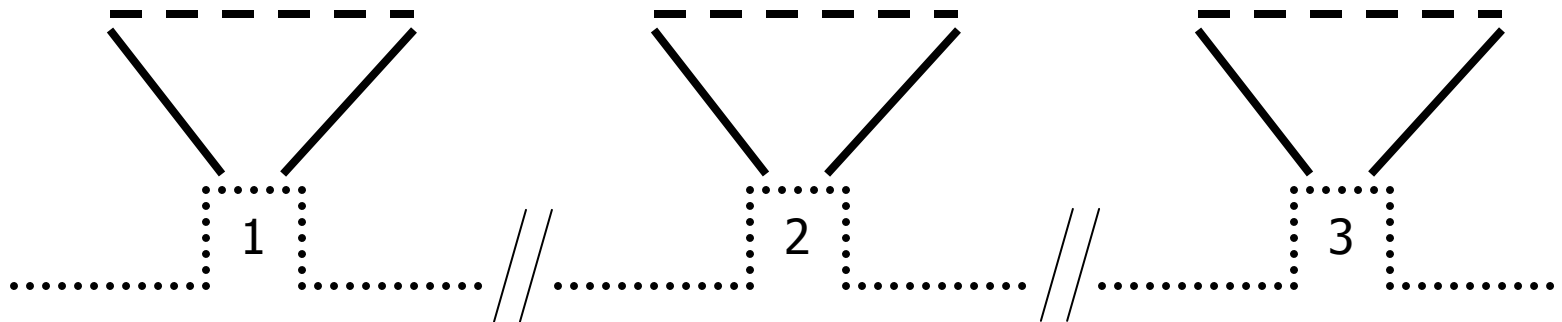
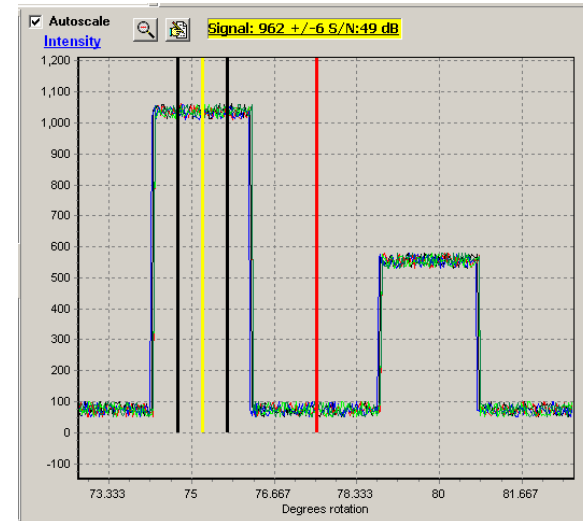
- Use a continuous light source
- Acquire light intensities and rotor timing pulse
 - Simultaneously from FDS and RSA detectors
 - Asynchronously with the rotor spinning
 - For several turns of the rotor for averaging
- Sort out the data in computer memory



Data averaging

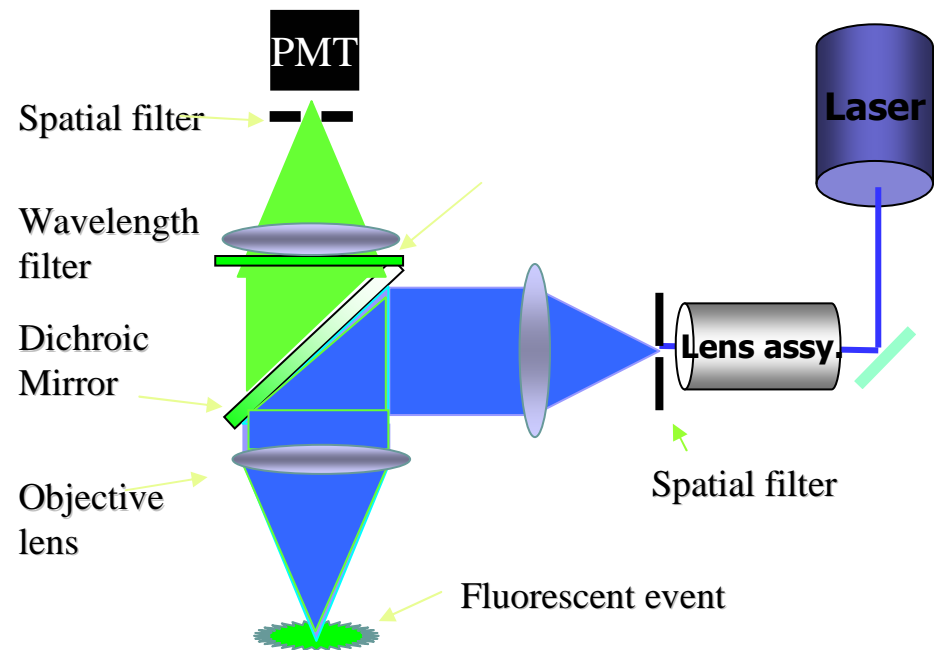
Multiple rotations and multiple intensities

- FDS and RSA data
- Data for all samples
 - Speeds up scanning
- Signal averaging

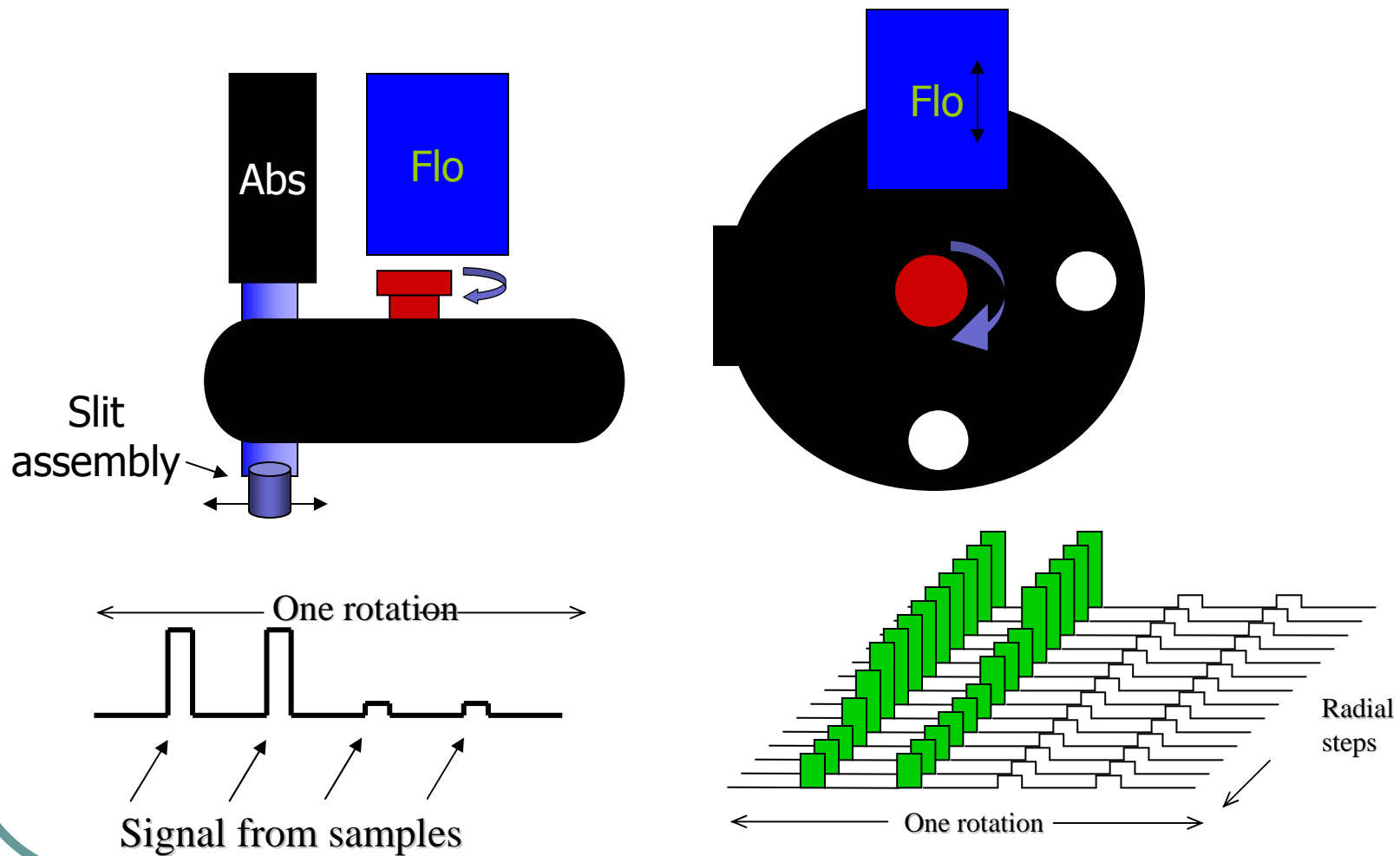


AU-FDS

- Excitation: 10 mW 488 nm
 - Target: fluorescein-like dyes and GFP
 - Protein intrinsic fluorescence not accessible
- Emission: >505 nm
 - Bandpass filters
- Optics move as a unit
 - Radial positioning by stepping motor
- Objective lens focused by stepping motor



Collect data at one radial position at a time

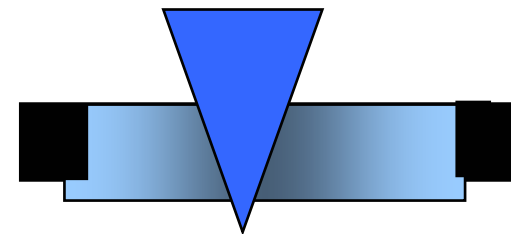
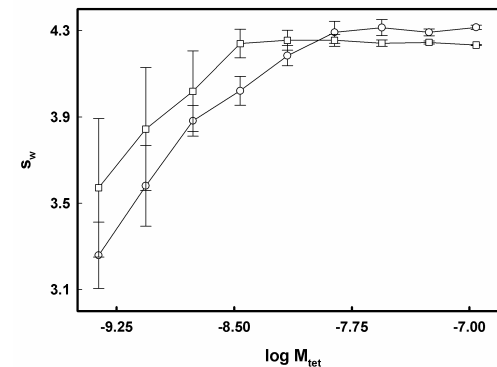


Applications for FDS

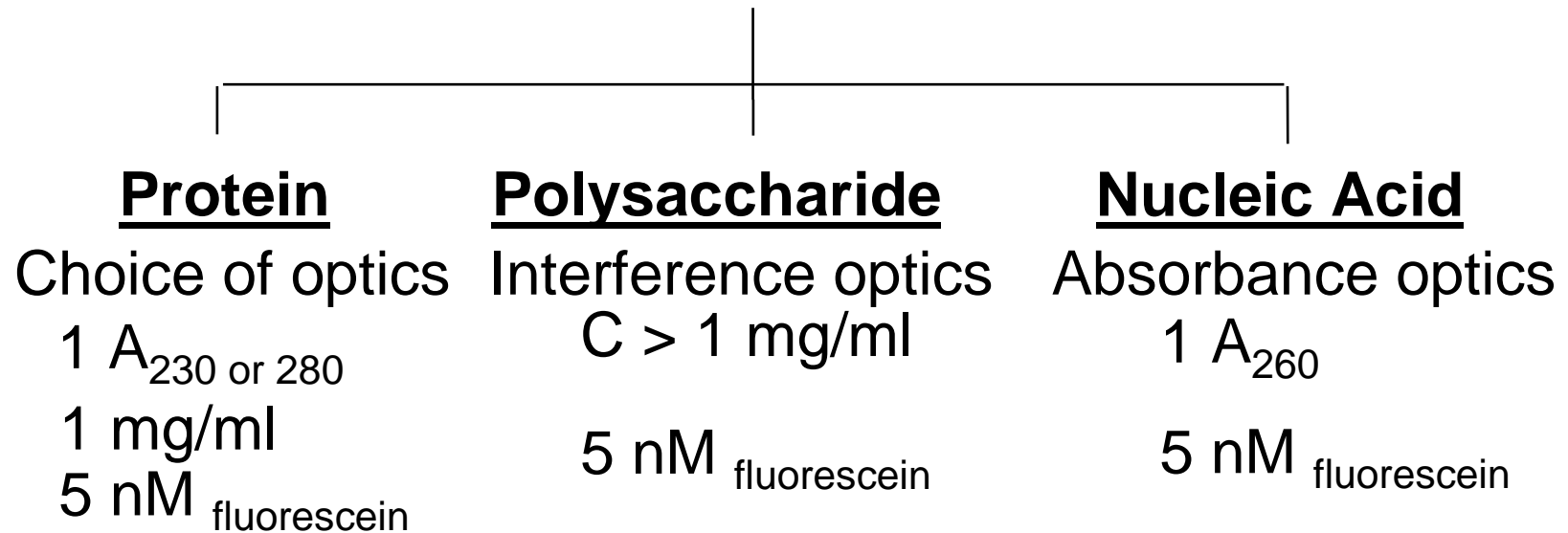
- Extrinsicly labeled compounds
 - 488 nm excitation (fluorescein, Alexa, etc.)
 - Green fluorescent protein
- High selectivity and sensitivity
 - Trace materials
 - Complex fluids (serum, sputum, urine)
- Tight associations ($K_d < 10^{-9}$ M)
- Broad concentration range
 - 6 - 8 orders of magnitude

Fluorescence system gotchas

- $F = I_0 Q \epsilon c$
 - Q is quantum yield
 - Quenching- remove O_2
 - Photobleaching
- Have to label a molecule
 - Changes to function?
- Sample adsorption at low concentrations
 - Meniscus, windows, centerpiece
 - Makes $F \rightarrow c$ difficult to obtain
- Inner filter effect
- Signal roll-off at base
 - Can use FC 43



Optical system choices by sample type and desired initial concentrations



	Absorbance	Interference	Fluorescence
Sensitivity	0.1 OD	0.05 mg/ml	100 pM fluorescein
Range	2-3 logs	3-4 logs	6-8 logs
Precision	Good	Excellent	Good

Summary comparison

	<u>Absorbance</u>	<u>Fluorescence</u>	<u>Interference</u>
Sensitivity	0.1 OD	100 pM fluorescein	$10^{-6} \Delta n$
Radial Resolution	20-50 μm	20-50 μm	10 μm
Scan time	1-5 minutes	60 seconds (all cells)	Seconds
When to Use	<ul style="list-style-type: none"> • Selectivity • Sensitivity • Non-dialyzable 	<ul style="list-style-type: none"> • Selectivity • Sensitivity • Non-dialyzable • Small quantities 	<ul style="list-style-type: none"> • Buffer absorbs • Sample doesn't • Variable ϵ • Accurate C • Short column equilibrium