

# Optimizing Optical Filter Performance

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## Abstract

Most flow cytometers use multilayer optical thin-film filters to separate the colors from the different fluorescent dyes used in multicolor experiments. These filters usually are built up on a glass substrate by adding many layers of both high and low refractive index hard oxide materials. The wavelength selectivity of the filters comes from the refractive and reflective properties of light at the many interfaces between the layers, causing constructive or destructive interference, according to layer thickness and the wavelength of light. These so-called interference filters are usually named for the 50% transmittance point at their cut-on or cut-off wavelength.

What is not widely known about these filters, but can be easily observed by holding the filter up to a light source and tilting the filter, is that the cut-on or cut-off wavelength varies quickly with changing the angle of incidence of the incoming light. The color of light transmitted by a typical 'dichroic long pass' filter changes from red to blue as the angle of incident light changes from 90 degrees to 45 degrees. This presents the possibility of 'fine-tuning' filter performance, by varying filter angle, with respect to the light colors being separated.

Two aspects of filter performance are profoundly affected by this angle of incidence: the steepness of the cut-on or cut-off transition, and the % transmission of the passed light.

Data will be shown from spectrophotometric scans of filters at different incident light angles, showing the superior performance of filters used at high angles, approaching 90 degrees, compared to the same filters when used at 45 degrees.

## Methods and Materials

A Shimadzu UV-160U UV-Visible Recording Spectrophotometer was used for all filter measurements. The instrument was set to continuously scan the % transmittance of each filter from 1000nm to 300nm. The cuvette holder base plate was removed, and filters were positioned in the light path on a simple bracket which incorporated a protractor, for read-out of the angle of the incident light.

## Results

In both Figure 1, showing % transmittance of a 560 Dichroic Short Pass, and in Figure 2, showing a 620 Dichroic Short Pass, two things are obvious. High angles of incidence produce steeper transitions between reflectance and transmission, and high angles produce higher transmittance. Although reflectance could not be measured in the experimental setup used here, higher reflectance should be expected at higher angles as well. The use of dichroic filters at low incident light angles, such as 45 degrees, allows for ease of manufacturing and a compact optical layout, even for older, larger photomultiplier tubes, but does not provide optimal color separation or sensitivity. Today's smaller PMTs will allow the use of optical filters at higher angles, without increasing instrument size.

Figure 1

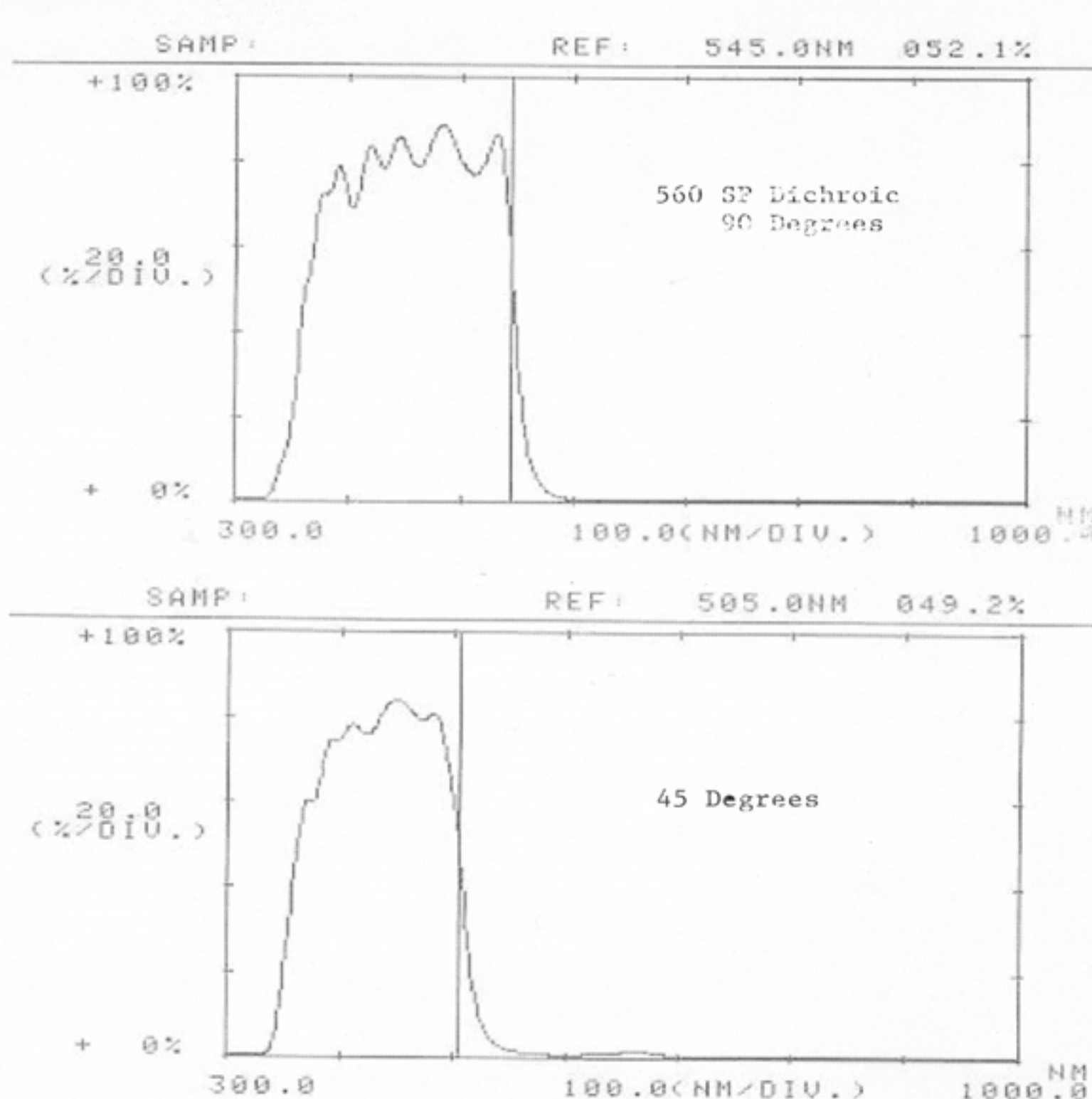
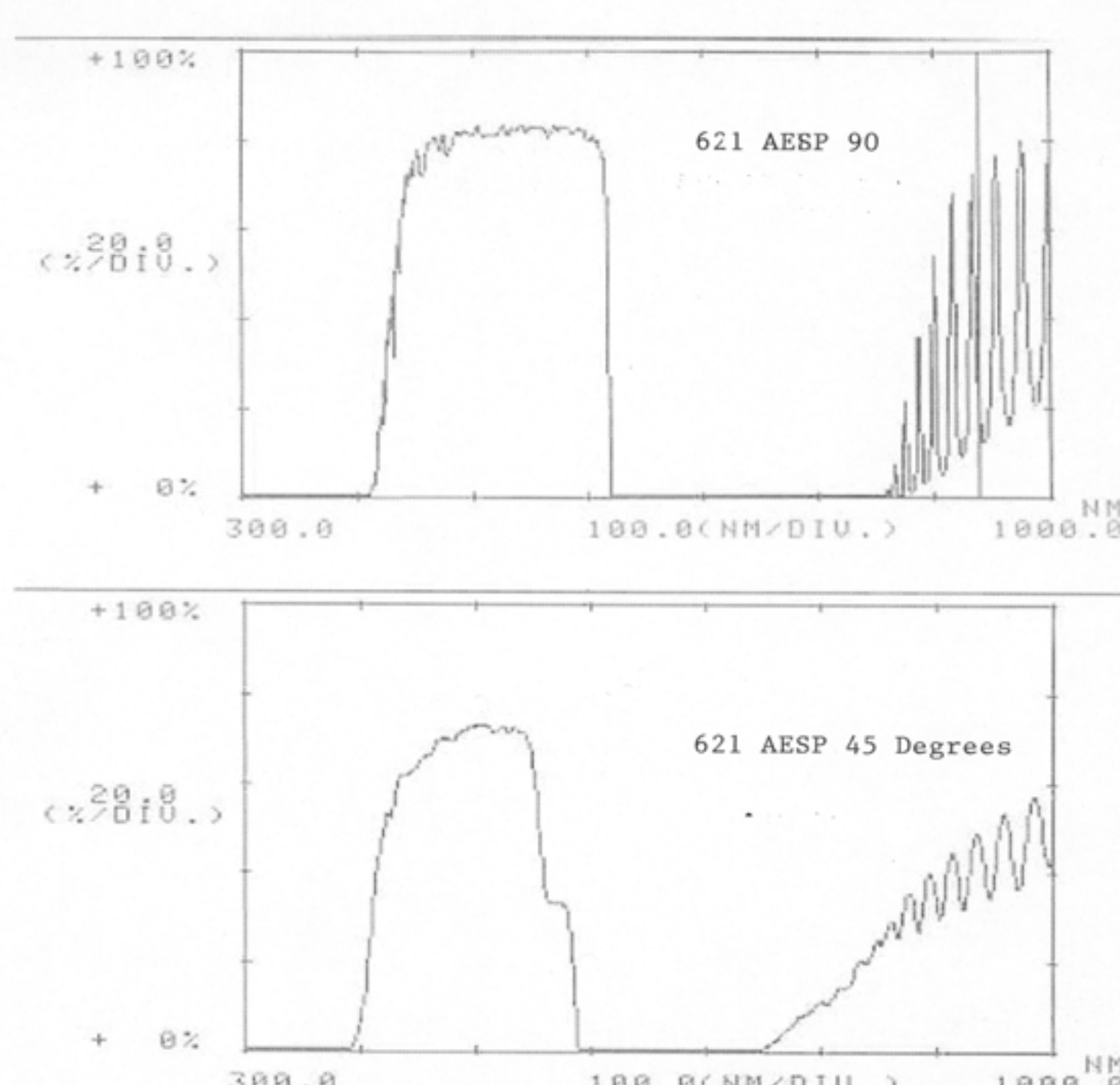


Figure 2



## Discussion

Limiting detector spectral bandwidth has become increasingly important as the number of fluorochromes used simultaneously has increased (1.) Attempts to achieve the needed color separation using interferometers and prisms have been costly and no more efficient than dichroic filters (2, 3.) Using dichroic filters at high incident light angles results in better color separation and better sensitivity, but requires smaller detectors to avoid increasing instrument size. Because % reflection is almost always higher than % transmission, using filters in an arrangement where most of the light is reflected at each dichroic filter has been the standard in the telecom industry for nearly three decades, because this results in additional sensitivity improvements (4.)

## Conclusions

Newer, smaller detectors now allow optical filters to be used at higher angles of incident light, without increasing instrument size.

Using dichroic mirrors at high incident light angles improves color separation and sensitivity.

Filter arrangements that reflect the majority of colors while minimizing the number of transmittances will improve sensitivity.

## References

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