

The Relationship of Noise, Linear Dynamic Range, Optical Resolution, and Number Of Diodes on Resolution in Photodiode Array Detectors

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INTRODUCTION

The application of photodiode array technology to absorbance detection combines the benefits of sensitivity and highly linear detection of chromatographic peaks. The collection of the absorbance spectra of eluted peaks provides an opportunity to detect co-elution as well as confirm the identity of eluted peaks based on library matching. The design of an absorbance detector for liquid chromatography requires a balancing of several factors:

- Low noise
- Wide linear dynamic range
- Data rate
- Spectral resolution for photodiode array based detectors

This white paper will discuss the relationship between noise, linear dynamic range and optical resolution. It will also consider the implications of the number of diodes used in the diode array to capture the spectral data.

OPTICAL RESOLUTION - NOISE

Because absorbance detectors use broad band sources, such as a deuterium lamp or a tungsten lamp, a monochromator is required to select the wavelength(s) of interest from the full source spectrum. Modern instruments use diffraction gratings to disperse the various wavelengths contained in the source's spectrum. In the case of a diode array spectrophotometer, the image of the source is projected onto the photodiode array device. The resolution of the spectrometer is determined by several factors, such as the slit width used to illuminate the flow cell and grating as well as the groove density of the grating. The optical resolution of the spectrometer is described by the full width at half height of an emission line (typically from a mercury discharge lamp) as observed by the detector. The optical resolution is the ultimate limit of a spectrophotometer's ability to detect differences in spectral details. Optical resolution has influence on both detector linearity and detector noise. To a first approximation, absorbance noise has three principal sources. They include **read noise**, **source fluctuation noise**, and **shot noise**.

1. **Read noise:** the electronic noise associated with measuring the photocurrent. In a well-designed instrument, that value is small and fixed, but it becomes significant when the light levels are low.
2. **Source fluctuation noise:** the outcome of variation in lamp intensity or light transmission through the flow cell.
3. **So-called shot noise:** the quantum mechanical noise associated with the behavior of the photodiode (a semiconductor) at ambient temperature. It represents the ultimate noise limit of an absorbance detector.

OPTICAL RESOLUTION - LINEARITY AND SLIT WIDTH

The slit width has a direct influence on the amount of light reaching the photodiodes and a strong influence of linear dynamic range.

A wider slit admits more light while a narrow slit admits less. The optimal slit width should be seen as balancing of low noise against optical resolution. A description of how in practice the slit width impacts linearity is depicted in Table 1.

TIP:

You can minimize your source fluctuation noise by:

- Controlling the lamp's power supply
- Reducing flow fluctuations
- Reducing mobile phase compositional variations (to minimize refractive index changes)

Table 1. The impact of slit width on the linear range

< 1 nm	1 – 5 nm	> 5 nm
<ul style="list-style-type: none"> ■ Compromised ability of detector to provide acceptable noise at moderate sampling rates (10–40 Hz) ■ Not optimal for general chromatographic use 	<ul style="list-style-type: none"> ■ Linearity is well behaved ■ Balance between noise and linearity* 	<ul style="list-style-type: none"> ■ Decreases the linear dynamic range ■ Limits the accuracy of quantitation

*The trade-off between noise and linearity becomes significant as the sampling rate is increased.

OPTICAL RESOLUTION - SPECTRAL QUALITY

Because the smallest chromatographically appropriate slit widths is 1–2 nm (full width at half height), there is a choice of diode pixel density that is required. For photodiode array detectors which are expected to cover the UV and visible light spectrum (190–800 nm), the two most common choices which can cover that wavelength range are 512 and 1024 element arrays. The 512 pixel photodiode array has a diode resolution of 1.2 nm per pixel while the 1024 pixel array has a diode resolution of 0.6 nm per pixel. With a 512 element photodiode array, the image of a 1.2 nm slit completely fills a single diode while with a 1024 element pixel array the slit image covers two pixels.

It should be noted that the resolution of spectral features is entirely determined by the optical resolution and that increasing the number of pixels associated with the recording of the spectra does not change the actual resolution and/or appearance of the spectra.

To illustrate this we collected benzene spectra with two different Waters® photodiode array detectors, an ACQUITY® PDA and an ACQUITY e λ PDA which have the same optical resolution, a 1.2 nm slit width, but differ in their wavelength range. The ACQUITY PDA has a wavelength range of 190–500 nm while the ACQUITY e λ PDA has a wavelength range of 190–800 nm. The detectors have diode resolution values of 0.6 nm/pixel (ACQUITY PDA) and 1.2 nm/pixel (ACQUITY e λ PDA). This difference in diode resolution is identical to the difference between a 512 and a 1024 pixel photodiode array.

The majority of the samples to be analyzed by LC with photodiode array detection have spectra which do not have fine structure and a typical full width at half height for most small organic molecules is 20 nm. Such molecules show no functional differences in their spectra and the differences in diode resolution between a 1024 and 512 pixel array are not visible. This is shown in Figure 1 which displays caffeine spectra collected on an ACQUITY PDA (0.6 nm/pixel) and an ACQUITY e λ PDA (1.2 nm/pixel). The spectra are virtually identical. This will be the case for the vast majority of the samples analyzed by liquid chromatography.

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The spectrum of benzene contains significant fine structure in the 220–300 nm region and has been used as a test for spectral resolution. Figure 2 shows a benzene spectrum as collected with the ACQUITY PDA and the ACQUITY e λ PDA for equivalent concentrations. The spectra show the same number of peaks at the same λ_{max} values with less than ± 1 nm which is the wavelength accuracy specification for both instruments. Figure 3 shows the same spectra with smoothing. In both figures the peak widths are equivalent and the spectra are clearly equivalent.

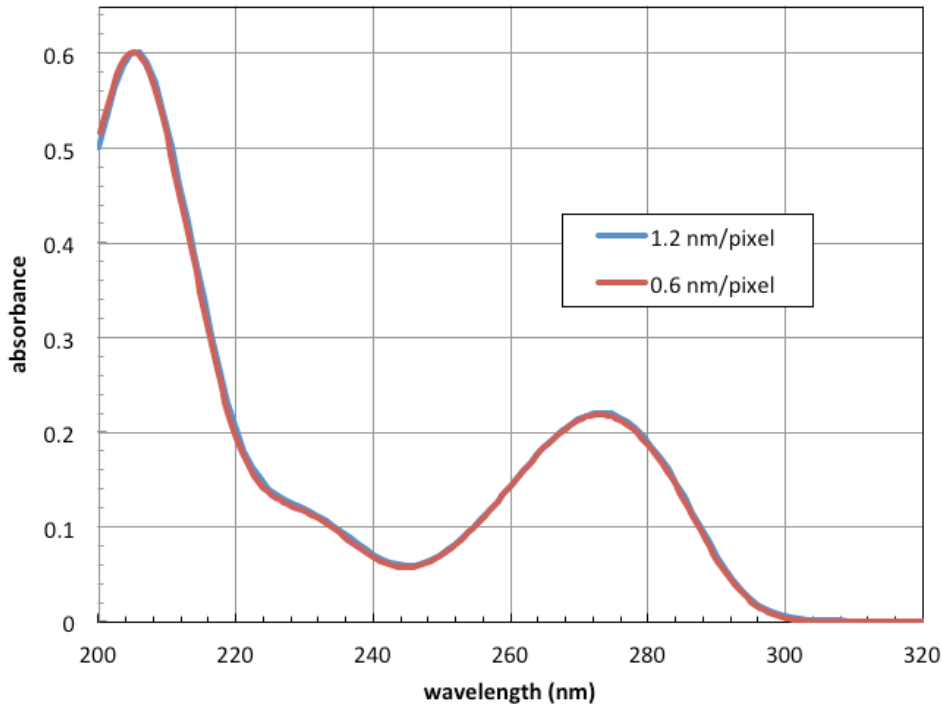


Figure 1. Caffeine Spectra with 1.2 nm/pixel and 0.6 nm/pixel ACQUITY PDAs.

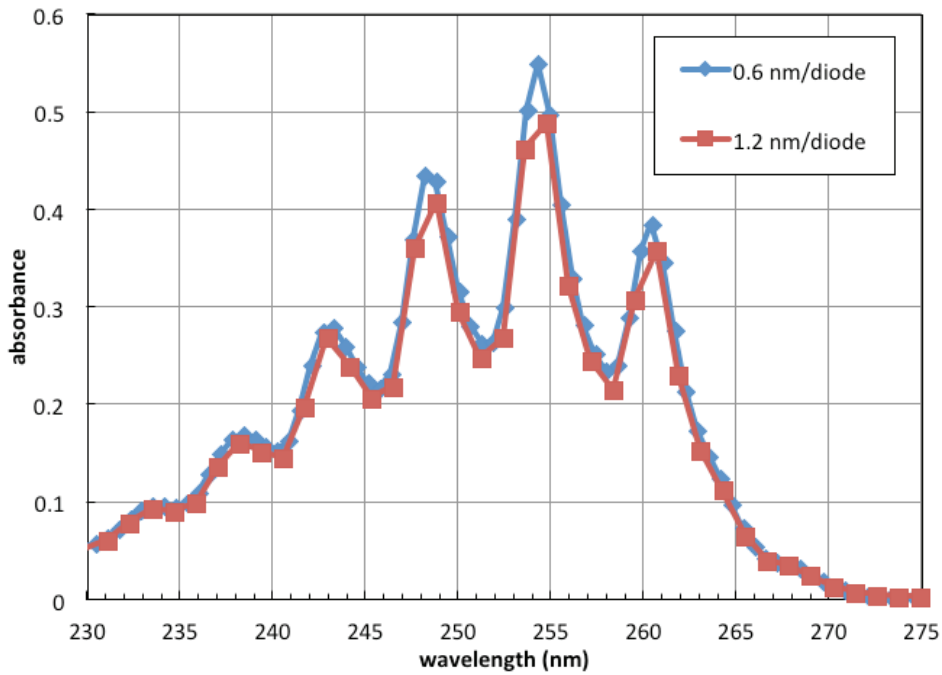


Figure 2. Comparison of Benzene Spectra vs. Diode Resolution.

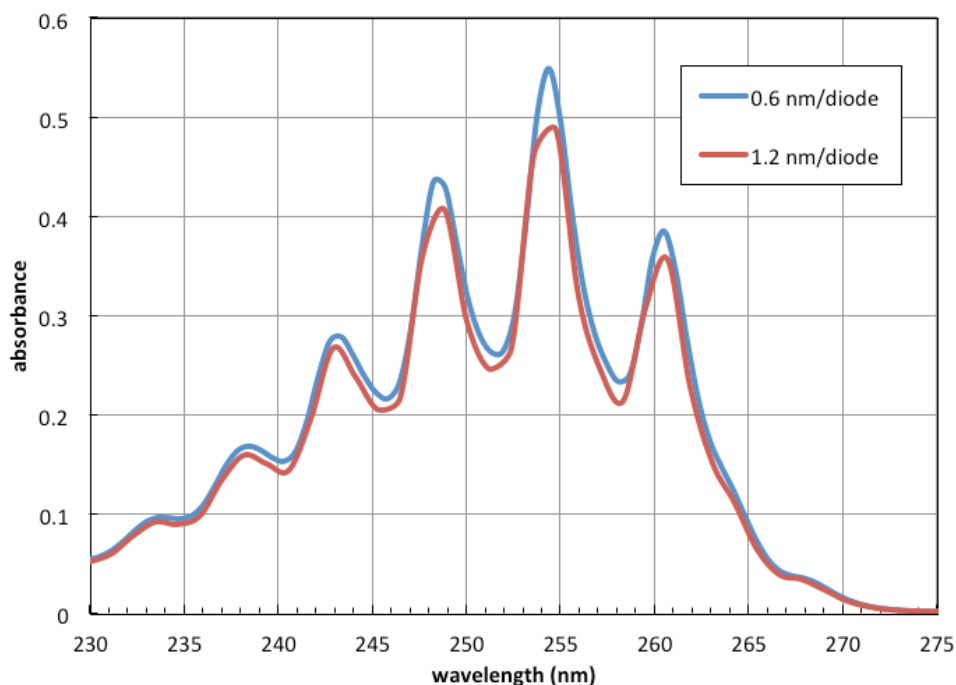


Figure 3. Comparison of Smoothed Benzene Spectra.

The minor differences in the benzene spectra do not impact library matching, nor do they impact linearity or sensitivity. It should be noted, that these differences would NOT be observed if the slit width were increased to 5 nm or greater and the number of “peaks” in the spectrum would decrease with a degradation of optical resolution.

In summary, increasing the diode resolution does not impact the spectral quality when the optical resolution is fixed at 1.2 nm. For typical LC analytes, the spectra are not impacted by diode resolution at constant optical slit width.

CONCLUSIONS

- Diode resolution has little impact on the quality of spectra generated by photodiode array detectors.
- Increasing the slit width (in nm) tends to improve noise at the expense of linear dynamic range and spectral quality. Decreasing the slit width (in nm) tends to improve spectral resolution and linearity at the expense of detector noise.
- Matching the size of the diodes (in nm) to the slit width balances noise performance, linear dynamic range and spectral resolution.
- Increasing the number of pixels, which is equivalent to enlarging the slit width, does not improve the spectral resolution and may degrade noise performance.

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