Performance PerSPECtives

The Waters® 2475 Multi-λ Fluorescence Detector offers unsurpassed sensitivity for quantitating low levels of target compounds, whether naturally fluorescent or derivatized with a fluorescent tag (e.g., Waters AccQ •Tag™ precolumn derivitization method for protein and peptide hydrolysates). This Performance PerSPECtive describes the technology that provides improved performance of Waters 2475 detector. (See Waters Performance PerSPECtive 720000425EN for data showing the superior results obtained on the 2475 Multi-λ Fluorescence Detector compared to previous generation products).
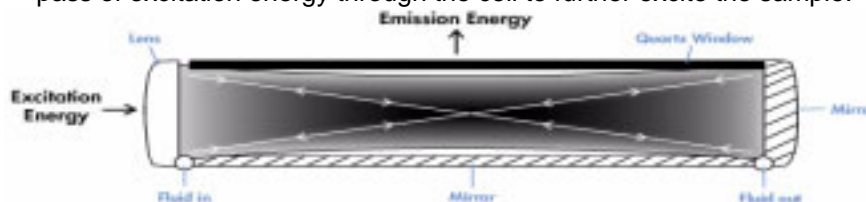
How Flow cell Design Affects Detection Sensitivity:
The superior performance of Waters 2475 detector is due to the unique axially illuminated flow cell design (i.e., excitation occurs along the longest axis of the cell). Detection sensitivity is increased compared to results obtained with traditional fluorescence detectors that use cuvette-shaped flow cells (i.e., excitation across the shortest axis of the cell) because the Xenon lamp excitation energy has an increased pathlength. Additionally, the arrangement of an entrance lens opposite a mirrored rear cell wall provides a second pass of excitation energy to illuminate the sample. More importantly the lens and curved mirrored wall are optically matched, thus the excitation energy is guided across the flow cell, in both directions, minimizing light scattering and reducing detector noise. Finally, the increased efficiency allows the excitation entrance to be smaller, so volume and dispersion are reduced. This effectively increases sensitivity without increasing band broadening of the chromatographically separated peaks. (See Figure 1).

Figure 1: Flowcell technology contained in Waters 2475 detector enhances performance compared to use of conventional flowcell designs.

Waters 2475 Axially Illuminated Flowcell Design

λ_{Em}

Conventional Cuvette-shaped Flowcell Design (Not drawn to scale)

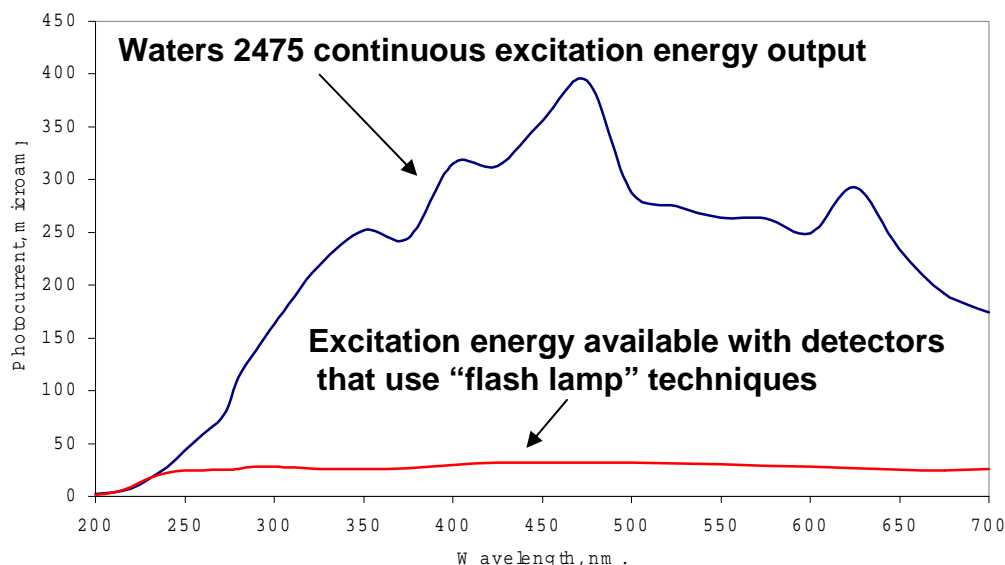λ_{Em}

λ_{Ex}

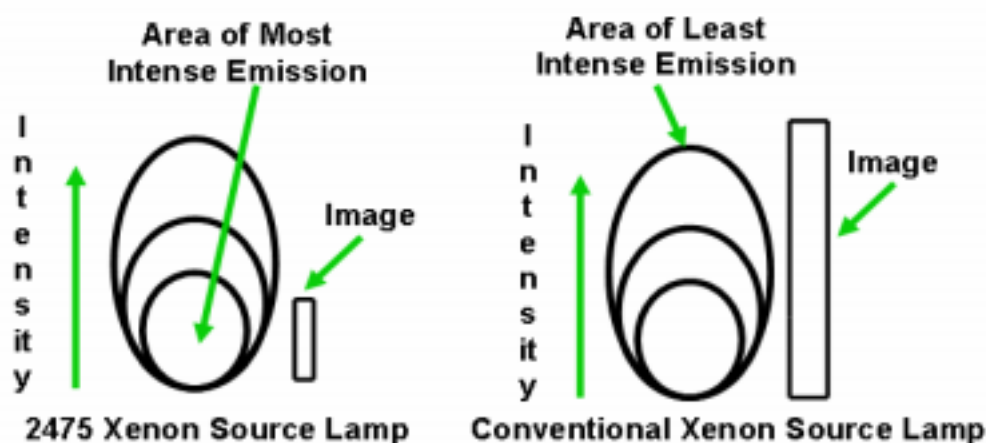λ_{Ex}
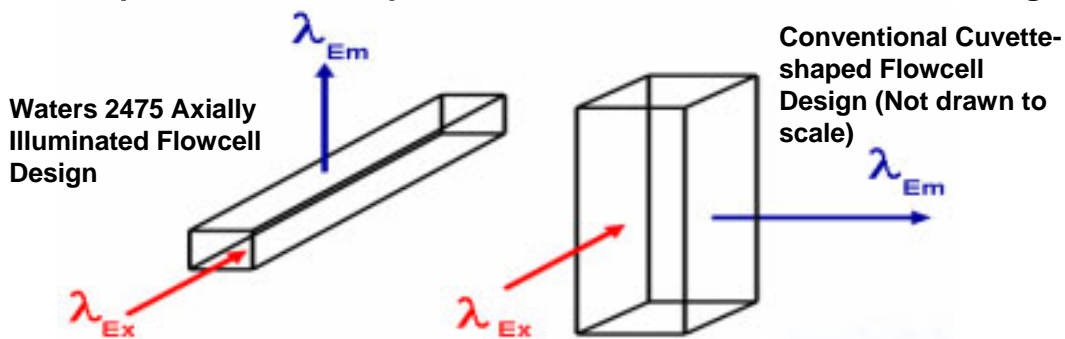
Reflected light from the rear mirror of Waters 2475 flowcell allows a second pass of excitation energy through the cell to further excite the sample.

Lens   Emission Energy   Quartz Window

Excitation Energy →   Mirror

Fluid in   Mirror   Fluid out

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Novel Use of Xenon Lamp Source:

The sensitivity of a detector is dependent on the amount of energy available to excite a sample contained in the flowcell. As shown in Figure 2, the intensity of excitation energy from a Xenon lamp decreases with increased distance from the lamp source. Waters 2475 excitation optics uses a curved mirror to collect and focus the most intense part of the Xenon lamp emission into the flowcell entrance. Traditional detector designs use lenses to collect and focus a larger, yet less intense image into the flowcell. In addition, the continuous firing of the Xenon lamp in Waters 2475 detector results in more available energy at excitation wavelengths above 250 nm compared to detectors that use "flash lamp" techniques. (See Figure 2).

Figure 2: The quality of Xenon lamp energy available for sample excitation is critical in maximizing detection sensitivity.

Area of Most Intense Emission

Area of Least Intense Emission

Intensity — Image

2475 Xenon Source Lamp

Intensity — Image

Conventional Xenon Source Lamp

Waters 2475 continuous excitation energy output

Excitation energy available with detectors that use "flash lamp" techniques

Photocurrent, microamp

Wavelength, nm.

Summary:

• Waters 2475 Multi-λ Fluorescence Detector uses a combination of unique flowcell design and Xenon lamp energy capturing techniques to maximize the ability to detect and quantitate low levels of target analytes. (See Waters Performance PerSPECtive 720000425EN for application examples).