



Diffraction Optics Technology—dot[®]

Introduction

Diffraction optics technology, or dot, brings together two mature, well-understood technologies: grating-based light diffraction and immobilized capture surfaces. This combination produces a sensitive and very simple technique for the detection of molecular binding events without the use of fluorescent labels.

Capture molecules are immobilized on a flat surface in an optimized grating that produces a strong diffraction pattern when illuminated. Binding of biomolecules to the patterned capture molecules causes a change in the diffraction efficiency of the pattern, which increases the diffracted signal intensity. Release of the interacting species conversely leads to a measurable change in signal.

Principles of Diffraction

Diffraction occurs because of the wave nature of light: when coherent light strikes a non-random pattern of obstacles, the resulting constructive and destructive interference produces a diffraction image.

Capture molecules, such as antibodies, are immobilized in a specific pattern of lines on the surface of the prism-shaped dotLab™ Sensor. The sensor surface forms the base of a low volume flow cell. A series of discrete diffraction beams is generated when the patterned molecules are illuminated with a laser. Since the illumination occurs through an optical prism, the laser beam does not pass through the bulk solution in the flow channel (see Figure 1).

When a flowing stream of biological sample is introduced into the sensor's flow channel, target molecules bind to the patterned capture molecules, or assay spots. This process is shown in Figure 2.

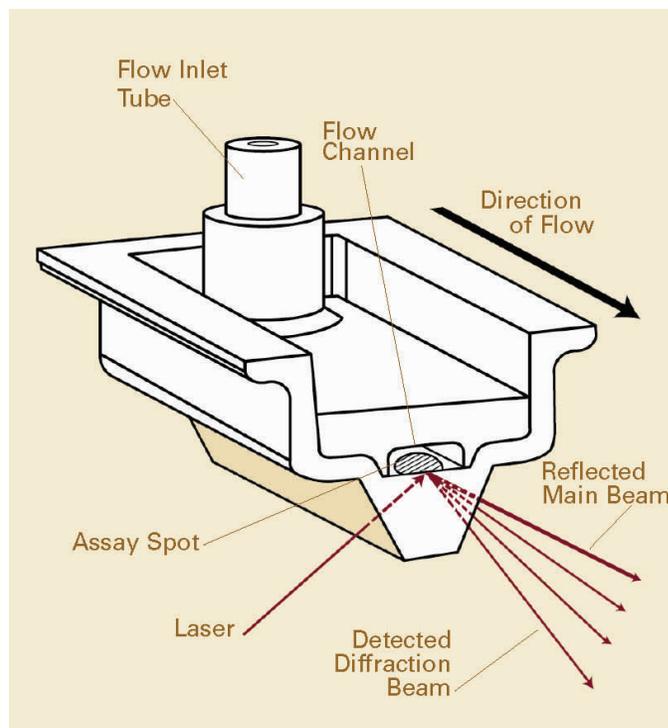


Figure 1. Schematic of dotLab Sensor.

An antigen binding to a patterned antibody is a typical example. The interaction of the antigen to the antibody increases the height of the surface pattern, producing an increased phase shift in the reflected beams, which in turn causes an increase in the diffraction signal intensity.

It is also important to note that diffraction is inherently self-referencing. Since the transduction of binding events is dependent on the initial pattern, an increase in diffractive order intensity will only occur if molecules bind exclusively to the patterned capture reagents. Therefore, non-specific binding to both the patterned and non-patterned regions will not significantly affect the signal. This is a significant advantage over other technologies such as SPR, where any surface binding event will cause an increase in signal.

dotLab Sensors

Each polystyrene dotLab Sensor contains eight assay spots located along its linear flow channel, as shown in Figure 3a and b. The dotLab System introduces samples and assay reagents into the sensor using an automated sampling system and high-precision fluidic controller. Binding of target molecules to the assay spots is detected by illuminating the underside of each spot with a focused laser (see Figure 3c and d). Binding events cause an increase in the diffraction signal, which is detected using photodiodes.

Conclusion

Since the detection beam never passes through the flow channel, the dotLab System is ideal for complex biological samples such as serum, plasma or crude cell lysates, and minimal sample preparation is required. The dotLab Sensors are disposable, and need only a few microliters of sample for analysis. Analyte detection can be performed across a broad dynamic range, which permits truly useful multiplex assays and the measurement of picomolar and micromolar target levels in the same sample. The real-time observation of binding interactions provides scientists with an immediate understanding of complex assays.

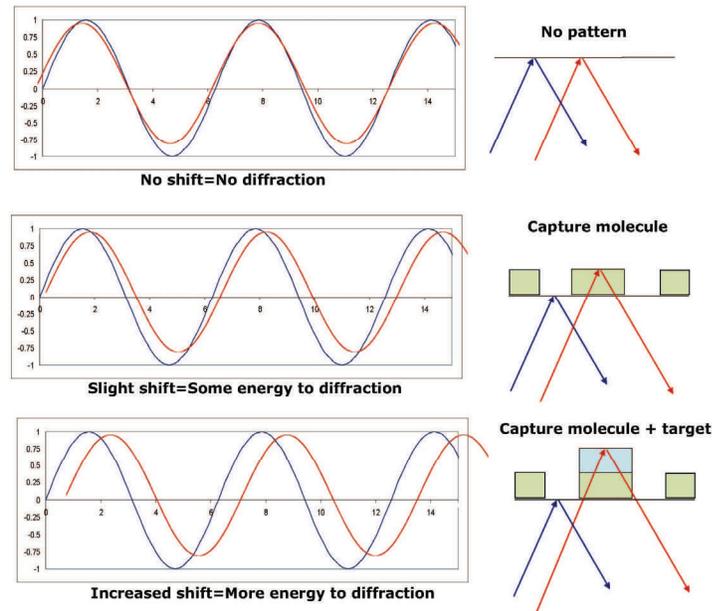
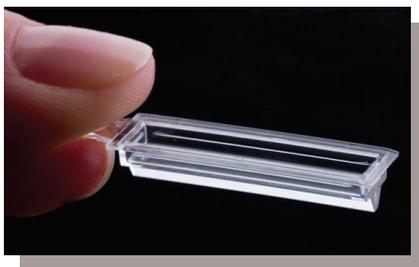


Figure 2. Surface binding produces phase shift that increase the diffraction signal intensity.

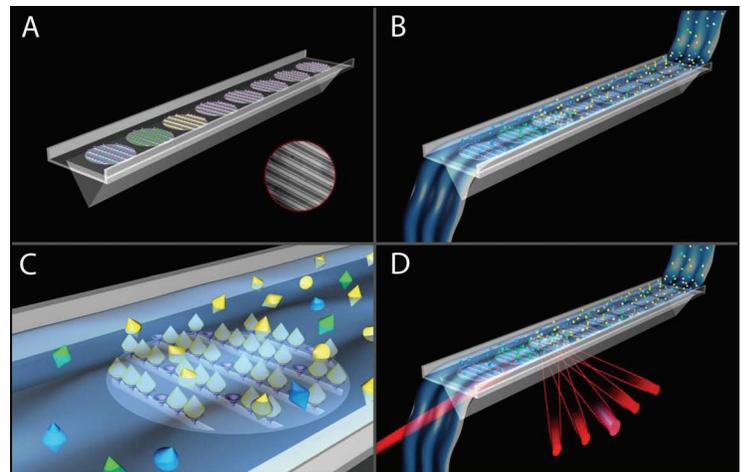


Figure 3. (a) dotLab Sensor and photo of actual assay spot, (b) introduction of flowing sample stream, (c) binding of targets to assay spots, and (d) detection of binding events using laser illumination.