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Applications of Filters to Biochemical & Pathological Detection

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Introduction

Bandpass filters are essential optical components in multiple applications, including remote climate monitoring, planetary exploration, commercial imaging, biomedical and product quality inspection, autonomous car navigation, and reconnaissance and surveillance. Other applications utilizing filters include archeology, geology and planetary, and asteroid surface topography.

In this issue, we will concentrate on filters for medical research and diagnostics. We will briefly review some of the roles that precision bandpass filters play to parse, measure and monitor essential properties to aid in the detection, diagnosis, tracking, and conquering of the coronavirus.

Optical Techniques Applied to Biochemistry and Pathology

Biochemical techniques based on the analysis of blood serum are frequently used for detection and progression monitoring of the coronavirus 2 (SARS-CoV-2), which is the etiological agent of the COVID-19 pandemic. Optical techniques that rely on spectral signals isolated by precision filters find immediate application to the COVID-19 pandemic. Figure 1, based on SEM images, is a model of the 50 – 100 nm body and shows the 20 nm tall spikes (of the "crown") that attach to and destroy respiratory cells.

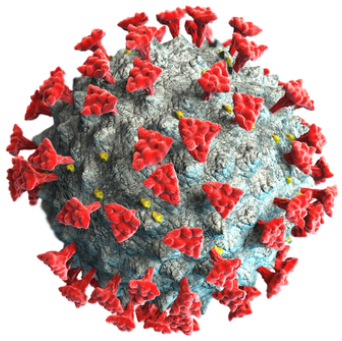

Figure 1. Model of the COVID-19 virus.

Optical techniques vary in their sensitivities and detection rates and are complementary in the clinical setting. Fluorescence microscopy (FM), chemiluminescent antiviral immune response (CLIA), flow cytometry (FC), and surface plasma resonance (SPR) are several techniques used for antibody immunoassay and detection of DNA and RNA strands.

The detection basis is the presence of fluorochromes added or generated in the test specimen.

Fluorochromes are stains designed to attach themselves to cellular material. When illuminated, the DNA/RNA and receptors/structures emit a quantifiable quantum yield. FM and its sister technology, FC, both require separation and high isolation of excitation and emission wavelengths to illuminate, detect, and reconstruct the cells or material(s) on the slide with precision and accuracy. FM images the spatial distribution of reaction sites, whereas the multi-parameter FC technique can assay reacted cell population and characteristic fluorescent emissions.

Let's briefly explore each technique below.

Fluorescence Microscopy (FM)

Applications of FM include cancer and virology research, diagnostics and detection. Using the gross analog of lasing a target for an advanced munition launched from air, land or sea, FM uses a primary wavelength that interacts with the specimen. The detector reads the response and zeroes in on very specific embedded signals, ignoring all else. Digging deeper however, this technique is confined to an enhanced light microscope (Figure 2 shows the basic components of a fluorescence microscope). The specimen is treated with a fluorescent dye (fluorochrome) that binds to specific receptors or components of intracellular material specific to the virus, nucleotide fragment, antigen or tissue site of interest (tumor). When stimulated by shorter wavelength (excitation) light, the bound dye interacting with the object fluoresces at a longer characteristic emission wavelength. Light from a short wavelength source is isolated by a narrow bandpass filter to remove energy that can appear at longer wavelengths where the emission occurs. A dichroic beam-divider reflects this excitation light to the specimen and the fluorescent light emitted is transmitted by the dichroic beam divider to the detector. The stimulating light has a greater intensity than the fluorescent signal, so a final long wavepass (LWP) filter is used to remove any residual stimulation light.

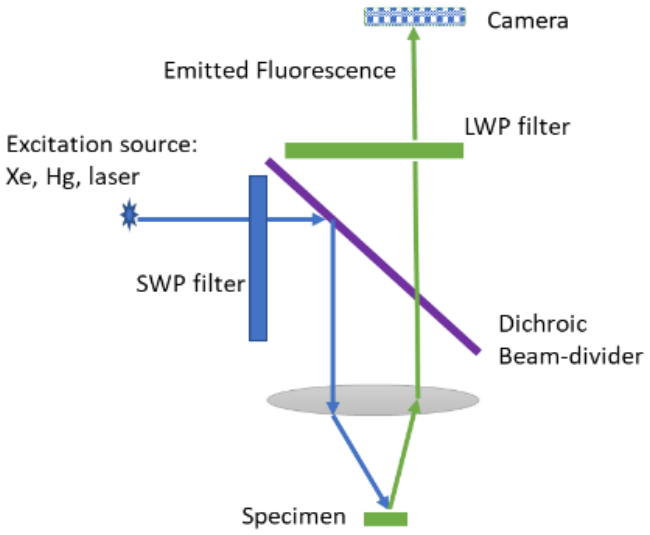

Figure 2. The essential components of fluorescent microscope optics showing the critical optical coatings.

The challenging tasks performed by filters are to separate the high intensity stimulating energy from the spectrally adjacent low fluorescent emission. To isolate this faint emission intensity, the long and short wave pass edge filters possess a steep transition slope between transmission and reflection where the separation

can be as small as 10 nm. The stimulating energy might be 10^6 times more intense than the fluorescent emission. The short wavepass (SWP) and LWP edge filters operate at 0º incidence, combined they provide out-of-band rejection OD >6. The layer count for these LWP and SWP edge filters exceeds 100. The dichroic beam divider operating at 45º incidence for parallel rays requires a more complex coating that creates minimum polarization separation to permit closely spaced wavelengths for efficient isolation. A Xenon (Xe) lamp emits a continuum, and consequently, filter rejection needs to cover a wider wavelength region. Mercury (Hg) lamps emit discrete lines, and therefore the rejection of out-of-band lines is easier. Laser stimuli are single line, but can be high power. Exposure of the marker dye to high intensity light causes a decrease in emission efficiency with time. Fluorescence Microscopy scopes employ an objective turret that permits the selection of cube assemblies specific to the specimen of interest. Images are collected to resolve otherwise invisible details of the subject material for the physician or researcher to use.

Flow Cytometry

In the case of CLIA flow cytometry, the analyte flows through a transparent tube where it is illuminated by several laser wavelengths, each with its own photomultiplier detector. This analysis system uses whole blood, plasma, or serum samples mixed with a known viral protein, buffer reagents, and specific enzyme-labeled antibodies. Antibodies in the patient sample react to form a complex. When bound with enzyme-labeled antibodies, a chemical reaction emits light. The intensity is proportional to the number of antibodies present. This test can look for multiple types of antibodies, including Immunoglobulin G (IgG), Immunoglobulin M (IgM), and Immunoglobulin A (IgA) (anti-bodies that are present in different concentrations at different times as a viral infection progresses).

Reactants added to the volume scatter signals, and stimulate signature fluorescent emissions. Sampling of the optical signals at several points along the tube permits data to be collected from a larger volume of cellular material. In operation, a series train of dichroic beam splitters reflect selected spectral passbands to individual photomultipliers and detectors. The flow tube detection optics resemble a multi-wavelength, dense wavelength division multiplexing (DWDM) system, where each channel is optimized for slightly different wavelengths to increase sensitivity. A series of fluorescence responses in increasingly complex digestions and tagging schemes critical for treatment formulation and validation is collected. The effectiveness of an anti-viral treatment, for example, is thereby tracked. The number and low emitting intensity of the sampled cells require high out-of-band blocking to provide a high signal-to-noise ratio. The dichroic filters also need to have high Laser-induced damage threshold (LiDT) since the intensity of the illumination laser is required to be high. The detection system relies on spectral filters with minimized scatter and wavefront error. As with fluorescence microscopy, satisfying those requirements dictate the high layer count and the optical quality of the coatings.

Surface Plasmon Resonance (SPR)

SPR analysis is based on the optical principle that the very small changes in the optical properties (refractive index) of a material can be detected by frustrated total internal reflection. The instrument consists of a 45 deg prism on whose hypotenuse is deposited a very thin semi-transparent layer of metal. Light reflecting off the hypotenuse is no longer totally internal reflected because the wave penetrates the metal. Reflection will reach a minimum at a specific incidence angle at the glass-metal interface due to the excitation of a surface plasmon. The addition of a layer of cellular material, for example, will cause a measurable shift in the reflected angle. This technique is very sensitive to the material contaminate and is used less widely in the clinical environment than the other analysis methods discussed above.

Challenges of Filter Coating Requirements for Immunoassay Instruments

Low absorption and low scatter are required properties of all coated optical components in the analysis chains, and are crucial to the success of the preeminent biotechnology tools used for diagnostics and research. The advanced deposition technologies capable of satisfying these rigid requirements include ion beam sputtering (IBS) [1], plasma ion assisted deposition (PIAD) and plasma assisted reactive magnetron sputtering (PARMS). These processes produce the required high density, amorphous, low scatter, environmentally stable and high LIDT properties in the visible spectrum (VIS) and near-infrared (NIR) regions. Low absorption coatings for high power UV lasers are made by E-beam deposition where the lower film density of the oxide stacks is accounted for with PIAD, or when fluorides are used due to their transparency at short wavelengths. The oxide technologies benefited from advances made in the 1990's for depositing DWDM filters where extremely precise and stable narrow band filters were routinely produced [1].

Construction of the separation filters used in the immune-assay instruments benefit from advanced deposition monitoring technology and require layer thickness control of better than 0.5%. Since the optical immuno-assay techniques described are done in the UV-visible region, metal oxide compounds are deposited using ion beam sputtering. Typical high-index materials that are combined with low-index Silicon dioxide (SiO_2) or Aluminum oxide (Al_2O_3) are Tantalum pentoxide (Ta_2O_5), Niobium pentoxide (Nb_2O_5) and Hafnium dioxide (HfO_2). We have previously discussed the qualities and advantages of IBS [2].

PIAD energies are lower than directed ion beams in IBS. In the PIAD process, a reactive plasma fills the coating chamber, overcoming the geometry dependence that IBS systems can impose on uniform composition (oxidation level) and density distribution. Intrinsic stress is also generally lower.

In the PARMS process, thin metal layers are magnetron sputter deposited from each target and subsequently oxidized in a radio frequency (RF) reactive plasma to produce dense stoichiometric layers [2].

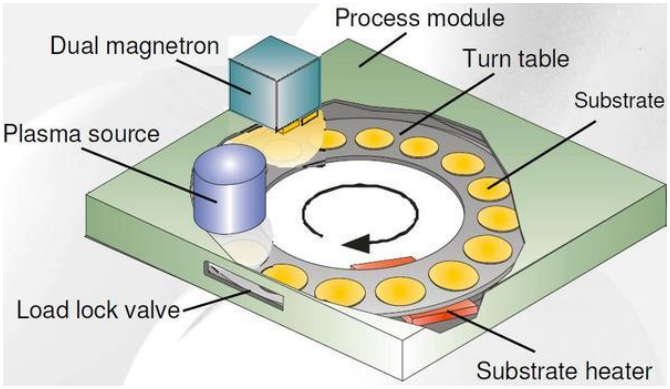

Figure 3. Functional components of a PARMS system (from the website, coos.iglos.com)

The oxidation of individual nanometer thick layers is complete, thus eliminating index and density non-uniform depth profiles. This results in precisely controlled high-quality film layers.

As illustrated in Figure 3, the substrates are rotated under the sources on a turntable and can be preloaded or fed from a wafer/substrate cassette through a load lock interface. Other variants of this approach include rotation of the substrate on the platen, multiple sources with different metals, or with the source off-axis to increase uniformity yield or enhance dielectric properties. Because the film is grown from the substrate up with metal that is then oxidized, PARMS and similar approaches offer a lower stress profile than IBS. The ability to tune the rate of growth based on metal mode conversion also permits low temperature materials,

such as polymers, to be coated with adherent depositions. In all these reactive processes, the heat load tolerance of the substrates and targets has driven innovation at both the consumable providers and the deposition system designers.

Conclusion

Precision filters are required in many disciplines ranging from aerospace to medical and clinical settings. Realization of environmentally stable coatings is dependent on the application of advanced material and deposition technology. As analysis techniques advance to respond to challenging biochemical research, materials and deposition processes will similarly be advanced. We intend to report on these innovative advancements in a future Coating Materials News issue.

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