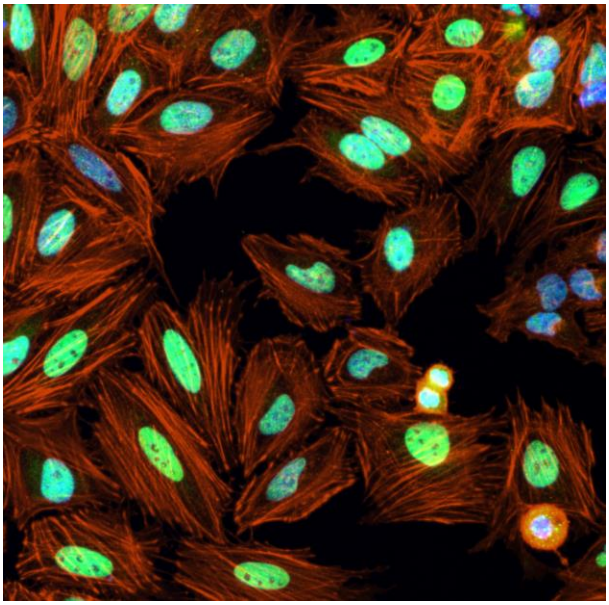
Choosing the Right Microwell Plates for High-Quality Cell Imaging Assays

Introduction

Microwell plates, also known as microplates and microtiter plates, are versatile tools in the life sciences, with a broad range of formats to fit any application. From 6-well to 1536-well formats, these plates are essential for high-throughput experiments. In this white paper, we delve into the world of automated microscopy and explore how to choose the best microwell plate for your cell-based imaging assays, particularly those employing fluorescence imaging. We'll focus primarily on selection of 96-well and 384-well plates used in high-throughput and high-content screening, providing you with the knowledge you need to make informed decisions.



If you're new to using microwell plates for cell-based imaging assays, it's important to note that they are used with inverted microscopes. This means that the objective lens is positioned below the plate bottom, allowing it to image the specimen through the plate-bottom material, either glass or plastic. Upright microscopes, on the other hand, position the objective lens above the sample. However, due to the well depth of microwell plates, it normally exceeds the working distance required by objective lenses to focus on cells adhering to the well floor.

Optical Properties

The optical properties of the microwell plate can affect the imaging quality and the signal-to-noise ratio of cell-based assays. The most important optical properties of the microwell plate are the following:

1. Transparency
2. Autofluorescence
3. Refractive Index

Microwell plates with high transparency and low autofluorescence are recommended for imaging applications.

Transparency of a microwell plate bottom plays a crucial role in cell-based imaging as it affects the amount of light transmitted through the well. A relatively thinner plate bottom is more transparent and therefore preferable for imaging. The chemical composition of the plate-bottom material is also an important factor in determining its transparency. We explore plate-bottom material and thickness in further detail below.

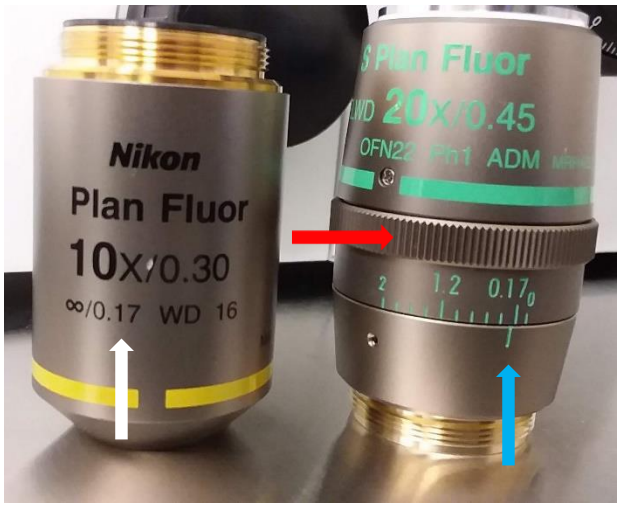
Autofluorescence is the light emitted by a material when it is excited by light of a specific wavelength. This can cause background noise in fluorescence imaging and interfere with detecting and measuring fluorescent signals. The chemical composition of the plate material, not its thickness, primarily determines autofluorescence. Glass and cyclic olefin copolymer (COC) have lower autofluorescence than polystyrene, which is the most common plastic used in microwell plates.

Refractive index is a fundamental property of materials that determines how light travels through them. It describes the extent to which a material can bend or refract light. When light passes from one medium to another with a different refractive index, it changes direction or bends, causing image distortion and aberrations. To minimize such distortions when imaging biological samples, cells were traditionally plated on a standard #1.5 cover glass (0.17 mm thickness) and a drop of oil was placed between the glass coverslip and the objective lens to match their refractive indices. Each of these optical components was designed to have a refractive index of approximately 1.5 to minimize image distortions and produce high-quality images.

Microwell plates with plate bottoms made of #1.5 cover glass offer several advantages for cell-based imaging. These glass-bottom plates have more uniform thickness, greater transparency, and less autofluorescence than plastic-bottom plates. Additionally, their refractive index matches that of high-resolution objective lenses (i.e., high numerical aperture objective lenses). While glass would seem to be the ideal material for imaging cells based on its optical properties alone, plastic is often preferred due to considerations of cell health. Also, most scientists consider it impractical to use oil-immersion objective lenses for automated imaging of microwell plates. However, water-immersion objective lenses are increasingly used for 3D imaging. In this case, it is important to match the refractive indices of the objective lens, water (1.33), and plate bottom to avoid image distortions.

Plate-Bottom Thickness and Correction Collars

The photograph below shows an objective lens designed for imaging through a 0.17 mm thick coverslip, as indicated by the white arrow. However, some objective lenses have correction collars that allow them to focus on objects through glass coverslips or microwell plate bottoms of variable thickness while minimizing optical distortions. In the photo below, the red arrow points to the correction collar, which can be adjusted for plate bottom thicknesses ranging from slightly less than 0.17 mm to 2 mm (blue arrow indicates mark for adjusting correction collar). If you plan to use microwell plates with plate-bottom thickness greater than 0.17 mm, you'll need objective lenses with appropriately adjusted correction collars for the best image quality. We recommend purchasing microwell plates with plate-bottom thickness close to 0.17 mm for microscopy applications.



Microwell Plate Materials

Many cell-based imaging applications have transitioned from glass to plastic plate bottoms for the sake of cell health. Plastic polymers provide a more suitable surface for cell attachment, growth, and proliferation, particularly after undergoing "tissue-culture treatment." Polystyrene is a widely used polymer for microwell plates due to its transparency, low cost, ease of manufacturing, and compatibility with various cell types. While polystyrene plates are often suitable for routine imaging applications, their optical properties are inferior to glass.

Cyclic olefin copolymer (COC) and cyclic olefin polymer (COP) are two materials that provide superior cell-culture conditions compared to glass while maintaining similar optical properties. COC plates are more transparent than polystyrene, exhibit high resistance to solvents, and have low autofluorescence. This makes them ideal for high-throughput screening applications and imaging experiments that require low background fluorescence, high resolution, and low light scattering. COP is a more expensive alternative to COC but has similar properties.

Another common polymer used for microwell plates is polypropylene. Polypropylene has limited transparency and is not typically used for cell-based imaging. However, it is more resistant to common chemicals/solvents (e.g., DMSO) and temperature changes than polystyrene, making it ideal for storing compounds or preparing serial dilutions.

The choice of material for microwell plates depends on specific application requirements such as cell type, imaging quality, chemical resistance, and cost. COC has become a popular material for cell-based imaging applications due to its unique properties.

Surface Treatment

Tissue-culture treatment is a process used to modify the surface properties of microwell plate polymers to make them suitable for cell-culture applications. The treatment involves exposing the plastic surface to certain chemicals or physical treatments that render it hydrophilic and typically negatively charged, promoting cell attachment, growth, and proliferation. Tissue-culture treated plastic provides a more suitable substrate for cell attachment, growth, and proliferation than glass surfaces for multiple reasons:

1. Tissue culture-treated plastic surfaces are modified to be more hydrophilic than regular plastic or glass surfaces. This promotes cell adhesion and spreading, allowing cells to attach and grow more easily.
2. Tissue culture-treated plastic surfaces have consistent surface chemistry and topography, allowing for reproducible cell-culture experiments. In contrast, glass surfaces may have variability in surface charge, roughness, and composition, which can affect cell adhesion and growth.
3. Tissue culture-treated plastic surfaces are easy to sterilize using standard methods such as autoclaving, gamma irradiation, or ethylene oxide gas. Glass surfaces may be more difficult to sterilize due to the risk of breakage and potential thermal shock during autoclaving.

Tissue culture-treated surfaces may be coated with a thin layer of polypeptides, polymers, or extracellular matrix (ECM) proteins to enhance cell attachment and growth. These surface coatings adhere better to tissue-culture treated plastic than to glass. Positively charged peptides and polymers such as poly-D-lysine (PDL), poly-L-lysine (PLL), polyethyleneimine (PEI), poly-D-ornithine (PDO), and poly-L-ornithine (PLO) bind to negative charges on the surface of tissue-culture treated plastic, creating a more favorable environment for cell attachment and growth. ECM proteins such as collagen, laminin, and fibronectin can also be used to coat surfaces for cell culture. These proteins provide a more natural substrate for cell adhesion and growth and are major components of commercial ECM products used for coating cell-culture vessels, including Matrigel® from Corning and Geltrex® from ThermoFisher. By mimicking the *in vivo* microenvironment of cells, ECM proteins can promote cell adhesion, migration, and differentiation.

When choosing a polystyrene or cyclic olefin plate for culturing and imaging cells, it's important to ensure that the plates have undergone tissue-culture treatment during the manufacturing process. It's also crucial to know which cell types require additional surface coatings, such as positively charged peptides like PDL or ECM proteins like laminin. For example, neurons are typically cultured on microwell plates pre-coated with a positively charged polypeptide, followed by an additional coating with one or more ECM proteins.

Suspension Cells

While this white paper has primarily focused on the selection of microwell plates for imaging adherent cells, it is also possible to use microwell plates for imaging suspension cells. One simple method is to seed suspension cells at high density in the wells of 96-well or 384-well plates for live-cell imaging applications. When seeded at high density, suspension cells will usually settle to the bottom of the well without adhering. To enhance the adherence of suspension cells, some plate coatings or gentle centrifugation can be used. Suspension cells can also be fixed and immunostained in microwell plates, but this requires several wash steps performed by centrifugation.

Well Volume and Shape

The well volume in microwell plates plays an important role in cell growth and imaging quality. For instance, 384-well plates used for cell culture and imaging have a maximum well-volume range of about 70 µL - 140 µL (see examples of variable well volumes in the photo below). For 384-well plates, a range of 40 - 80 µL cell-culture media per well is typically recommended. The lower end of that range can be used for short-term assays (e.g. 24 hours) while the higher end should be used for long-term assays (> 48 hours) or when media exchanges are required. Assay volumes should be optimized for each application but may be less than 20 µL in some low-volume wells. While low-volume wells can save money on assay reagents and facilitate visibility into the well for manual pipetting, low-volume cell-culture media may compromise cell health and low-volume assays may result in image distortions and low signal-to-noise ratio from variable volumes and evaporation, with the meniscus of the assay solution at different levels across wells. It is important to carefully consider the benefits and drawbacks of reducing culture-media volumes and assay volumes in 384-well format.

Low Volume High Volume

The shape of the wells refers to the shape created by the well walls, not the well-bottom. The most common well shapes are round and square. Round wells are typically found in 6-well to 96-well plates, while square wells are often used in 384-well plates because they provide a larger surface area for the well-bottom. However, round wells are sometimes used in 384-well format to minimize assay volume.

The well bottoms of microwell plates can be either flat or curved for cell-based imaging applications. Flat-bottom (F-bottom) wells are most commonly used for high-resolution imaging because they minimize light scattering and distortion. They are typically used with 2D cell culture. Rounded (U-bottom) plates are often used for culturing and imaging 3D spheroids and organoids. Some polypropylene microwell plates have conical well bottoms (V-bottom), which are not commonly used for imaging but for ease in pipetting; for example, to extract nearly all of the volume from a polypropylene compound plate or dilution plate.

3D Imaging

The shape of the well bottom is an important consideration when selecting a microwell plate for imaging 3D spheroids and organoids. Ultralow attachment U-bottom plates are often used to direct cells to the center of the well bottom to form 3D spheroids. This also makes it easier to image the entire structure. Other well shapes may also be used depending on the needs of the experiment.

Extracellular matrix proteins, such as collagen and laminin, can be used to promote the formation of 3D spheroids and organoids by providing a scaffold for cell attachment and organization into 3D structures. These protein-rich matrices also provide additional microenvironmental cues, such as growth factors and hormones, that help define the growth and organization of the 3D tissue. This can promote the formation of more complex and realistic 3D structures.

While most of the advice provided above focuses on selecting flat-bottom microwell plates for imaging 2D cell cultures, there is a growing trend towards using 3D cell cultures. This shift is driven by multiple factors: 1) There is a profound need to increase the physiological and disease relevance of preclinical models because drug-discovery assays and preclinical drug development have not been highly predictive of outcomes in human clinical trials. 2) Restrictions on animal testing are increasing due to limited translational value and a desire for a more

humane approach to drug development. 3) There is presently a convergence of technologies enabling 3D organoids, including human iPSC-derived cell types, microfluidics, improved cell-culture conditions for mixed cell populations, organ-on-a-chip, and better image-analysis tools using artificial intelligence. At Scintillant Bioscience, we firmly believe that imaging is the best way to interrogate these emerging 3D models.

Practical Considerations

Beyond the technical specifications of microwell plates, there are several practical considerations to keep in mind when selecting plates to purchase.

Cost: The cost of microwell plates is an essential factor to consider when selecting the right plate for your needs. Cost can vary significantly depending on the plate type, quality, and quantity required. When comparing costs, it is essential to consider the overall value of the plate, including the manufacturing quality and features that may be necessary for your specific application.

Availability: The COVID-19 pandemic made us all keenly aware of the necessity of navigating supply chain problems. Some microwell plates used for cell-based imaging are still experiencing backorders. It is essential to consider the availability of a particular plate before selecting it for your application, especially if you need a large quantity or have a tight deadline.

Manufacturing Quality: The manufacturing quality of microwell plates is essential to ensure consistent and reliable results. During the pandemic, we received many plates with scratches on the plate bottom, cracks in the lid or plate, and warped plate bottoms. It appears that most of these manufacturing issues have been addressed, but it is important to consider the impact that plate quality can have on your project. Even if plate quality is generally good, plate-to-plate variability can cause significant problems for assay development or screening.

Plate Dimensions: Modern microwell plates have a standard footprint and well positions. However, when using automated microscopes (high-content imaging instruments) to capture images in 96-well, 384-well or 1536-well plates, it may be necessary to input precise plate dimensions into the instrument settings to enable the microscope to focus on the cells properly in the desired well region. Technical drawings of the plate design are usually available on the manufacturer's website.

Skirt Height and Edge Wells: Microwell plates are manufactured with a skirt that extends below the microwell plate bottom. This can make edge wells inaccessible to objective lenses with short working distance, i.e., the distance required by the objective lens to focus on a specimen. In 384-well format, multiple rows or columns of edge wells may be inaccessible. This may or may not be a limiting factor for certain applications because edge wells are often avoided in microwell assays due to well-known edge effects attributed primarily to greater evaporation in edge wells than middle wells. Edge effects can be mitigated by filling edge wells with sterile water or PBS.

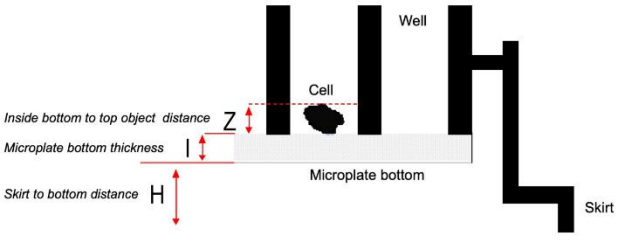


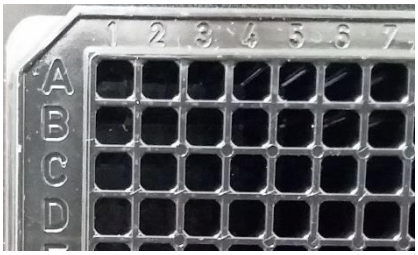
Image from NIH's Assay Guidance Manual
https://www.ncbi.nlm.nih.gov/books/NBK558077/

Lids and Evaporation: Microwell plate lids are an essential component of microwell plates, as they help prevent contamination and evaporation. The primary consideration for plate lids is the effect on evaporation. Some microwell plates are manufactured with lids that reduce evaporation, which helps to minimize edge effects. It is also important to ensure that plate lids are compatible with any plate handling automation you may employ. Fluorescence imaging usually can be done with an inverted microscope without interference from the plate lid.

For fixed cells that may not be imaged the same day the assay is completed, it is good practice to apply a plate seal to prevent evaporation and store the plate in the dark, preferably in a refrigerator.

Pigmented/Colored Plate Frame & Well Walls: Black-walled plates are normally used for fluorescence imaging to prevent crosstalk between adjacent wells and to reduce background fluorescence. White-walled plates are often used for luminescence assays because they amplify the signal. However, white-walled plates are typically not used for fluorescence imaging of cells because they also amplify background fluorescence.

Numbers and Letters for Manual Pipetting: Some 96-well and 384-well plates are manufactured with numbers displayed above each column and letters next to each row. If manual pipetting is required, this feature is especially important for 384-well plates, where the wells can be challenging to see and identify. It is essential to select plates with clear and visible numbers and letters to ensure accurate and efficient pipetting.



Sterility and Tissue-Culture Treatment: When selecting microwell plates for cell culture and imaging, it is important to recognize that many microwell plates have not been tissue-culture treated or sterilized in the manufacturing process. Cell-based imaging applications require sterile plates for good cell health. Plastic plates should be tissue-culture treated. Glass-bottom plates should be coated with positively charged peptides or polymers, such as PDL, and/or ECM proteins to promote cell adherence and health. Even tissue-culture treated plates should be further coated with ECM proteins for certain cell types.

Conclusion

In this white paper, we have provided guidance on selecting the best microwell plate for imaging applications. We have focused on the essential parameters of 96-well and 384-well plates for cell-based imaging, as these plates are commonly used in high-throughput and high-content assays for drug discovery in biotechnology companies, academic research labs, and large pharmaceutical companies. Notably, 1536-well plates are not suitable for manual pipetting and are only useful for labs with a high degree of automation and expertise in plate selection. For these reasons, we have not addressed the selection of 1536-well plates.

In conclusion, selecting the right microwell plate type is crucial for successful automated imaging of cells. The plate's optical properties and impact on cell health are key considerations. Factors that determine the plate's suitability for a specific application include the plate-bottom material and thickness, well shape and well-bottom shape, among other technical specifications. To ensure optimal cell health, polystyrene and cyclic olefin plates should be tissue-culture treated, and additional plate coatings may be necessary or desirable for certain cell types and applications. Given the many practical considerations that can impact plate selection, we recommend testing 2 or 3 different plates from different manufacturers during assay development. Technical specifications are a valuable guide, but empirical testing may uncover significant differences in the performance of specific assays. By choosing the best plate for your application and instrumentation, you can significantly improve the quality of your cell-based imaging assay.