Let's talk about live-cell imaging

By Alex & Gaëtan

Light sources can induce photoxicity and photobleaching, which can have negative effects on live samples and their quantification. The correct configuration of microscopes and the choice of appropriate technology are prerequisites for obtaining reproducible quantitative data on biological processes.

This Tips and Tricks will help you to :

- ☑ Optimize sample preparation
- ☑ Optimize imaging conditions
- ☑ choose the appropriate microscope
- ☑ Select microscopy techniques

Best Practices in live-cell microscopy

Photobleaching: The total number of photons impacting the sample determines the degree of photobleaching, rather than how light is delivered. In addition, fluorophore with high photosability should be prioritized.

Phototoxicity (ROS): Exposure time doesn't affect a lot the ROS production when the light power is constant. However, if the light source power increases, the amount of ROS production increases too.

Staining spectra: Illumination with longer wavelengths decreases phototoxicity. Avoid 405 nm excitation for live cells. Opt for green, orange, red or NIR molecules to stain your sample.

Staining brightness: brighter fluorophores require less intensity illumination and that will reduce phototoxicity.

Optimal acquisition speed: Increasing exposure time or decreasing scan speed improves the quality of S/N ratio. Unfortunately, longer exposure times can also cause object blurring when capturing dynamic process.

ALWAYS KEEP IN MIND :

When conducting time-lapse images, prioritize night and week-end acquisitions

Widefield

Advantages :

High Speed: Widefield microscopy is fast, making it suitable for observing dynamic processes in live cells.

Low Photobleaching & Phototoxicity: It can work with weak fluorescence signals, reducing the risk of photobleaching and phototoxicity, making it suitable for long-term live cell imaging.

Label-Free Imaging: You can perform label-free imaging of live cells when DIC or phase contrast techniques are used.

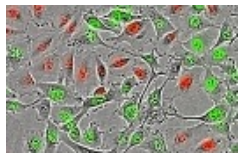
Disadvantages :

Lack of Optical Sectioning: It is not possible to selectively focus on a specific depth in the sample, leading to limited 3D information.

Reduced Image Contrast: Due to the presence of out-of-focus light, widefield microscopy often results in lower image contrast.

Microscopes available :

Incucyte S3 and Incucyte SX5 (automated), Echo Revolve and Olympus CKX 41, Zeiss Observer 7 + ApoTome



Confocal / Airyscan

Advantages :

Optical Sectioning: Confocal microscopy provides excellent optical sectioning, eliminating out-of-focus light and allowing for 3D reconstructions of live cells.

Improved Image Contrast: It offers high contrast images with reduced background noise, making it ideal for detailed live cell imaging.

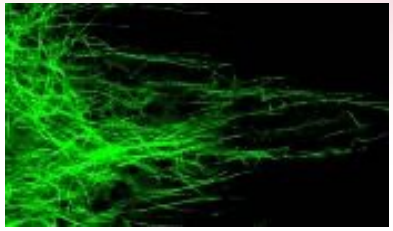
Higher resolution: Confocal microscopy employs high numerical aperture objectives and pinhole to enhance spatial resolution. It allows the observation of subcellular structures.

Disadvantages :

Slower Scanning: Confocal microscopy is generally slower than widefield microscopy, limiting its application for rapid cellular processes.

Microscopes available :

Nikon A1R , Leica Stellaris 8, Zeiss LSM 880 and Zeiss LSM 980



Holotomography

Advantages :

Label-Free Imaging: Holotomography is label-free, making it ideal for live cell imaging without the need for fluorescent labels.

- 3D Refractive Index Information: It provides quantitative 3D information about the refractive index of cells and their internal structures, enabling detailed live cell visualization.

- High Resolution: Holotomography can achieve high-resolution imaging, allowing for the observation of subcellular structures and fine cellular details.

Disadvantages :

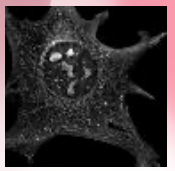
Slower Imaging: Holotomography typically has slower acquisition times, which can limit its application for high-speed imaging of dynamic cellular processes.

Complex Data Analysis: Processing and interpreting holotomography data can be more complex than traditional fluorescence microscopy techniques.

Microscope available :

Nanolive CXA

(@ CIRM Platform, ask Gaëtan if needed)



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