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Guidance for quantitative confocal microscopy

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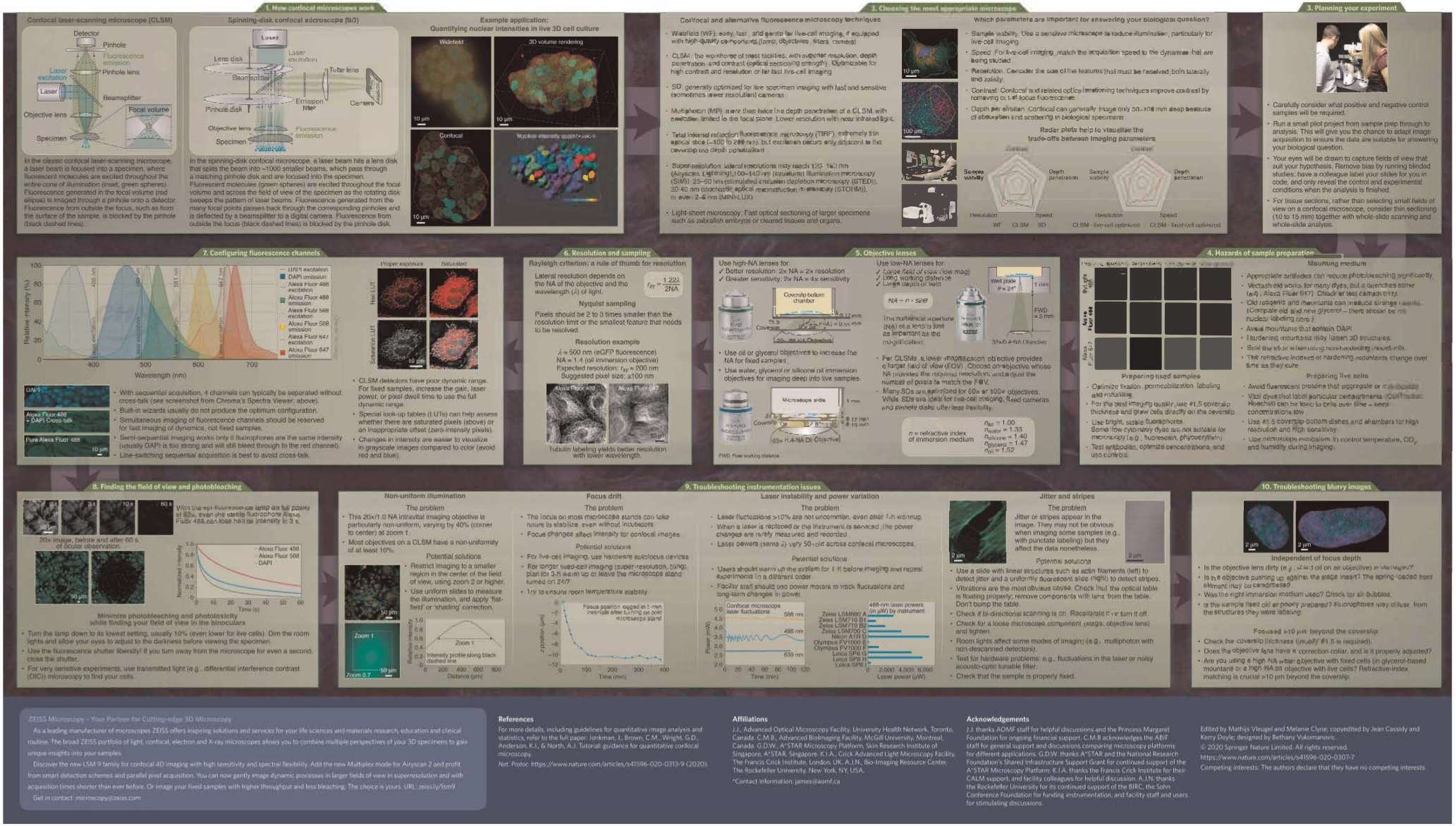
When used appropriately, a confocal fluorescence microscope is an excellent tool for making quantitative measurements in cells and tissues. The confocal microscope's ability to block out-of-focus light and thereby perform optical sectioning through a specimen allows the researcher to quantify fluorescence with very high precision. However, generating meaningful data using confocal microscopy requires careful planning and a thorough understanding of the technique. Here we guide the researcher through all aspects of acquiring quantitative confocal microscopy images, including optimizing sample preparation for fixed and live cells, choosing the most suitable microscope for a given application, and configuring the microscope parameters. Common pitfalls such as photobleaching and crosstalk are addressed, as well as several troubling instrumentation problems that may prevent the acquisition of quantitative data. The accompanying *Nature Protocols* paper includes guidelines for analyzing and presenting confocal images and performing statistical analysis.



References

For more details, including guidelines for quantitative image analysis and statistics, refer to the full paper: Jonkman, J., Brown, C.M., Wright, G.D., Anderson, K.I. & North, A.J. Tutorial: guidance for quantitative confocal microscopy. *Nat. Protoc.* https://www.nature.com/articles/s41596-020-0313-9 (2020).

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