

Guidelines for Publications using Colocalization

Am J Physiol Cell Physiol 294: C1119-C1122, 2008. First published March 19, 2008;

doi:10.1152/ajpcell.00133.2008

0363-6143/08 \$8.00

PERSPECTIVES IN CELL PHYSIOLOGY

Image acquisition for colocalization using optical microscopy

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Submitted 29 February 2008 ; accepted in final form 17 March 2008

Guidelines for Publication

To enable readers to interpret images and to evaluate the meaning of colocalization from a given experiment, manuscripts should include the following information:

- The pixel sizes, the Z spacing between image planes, the refractive index of the medium, and how this relates to the Nyquist criteria for the experiment ([Table 1](#)).
- The objective's magnification and numerical aperture.
- For images obtained with a confocal microscope, either the pinhole size or the axial resolution should be reported.
- The emission wavelengths of the fluorophores and the filter sets used.
- Scalebars should be included in each image group.

In addition, the following conditions should be satisfied.

- Wide-field images must be deconvolved, and the methodology reported.
- Images in which the colocalization is primarily along the Z-axis should, where possible, be examined for edge artifacts.
- Bleed through should be evaluated and corrected for, if present. If corrections are made, details of the methodology used should be provided.

- Table 1. *Lateral and axial sampling distances determined by the Nyquist criteria*

Fluorophore	Emission Wavelength, nm	NA		
		1.2	1.3	1.4
DAPI/Alexa 350	450	97	89	83
		<i>394</i>	<i>316</i>	<i>248</i>
Cy2	505	105	97	90
		<i>426</i>	<i>341</i>	<i>269</i>
FITC/Alexa 488	520	108	100	93
		<i>441</i>	<i>353</i>	<i>278</i>
Cy3/Alexa 555	565	118	109	101
		<i>479</i>	<i>383</i>	<i>302</i>
TRITC/Alexa 546	575	120	111	103
		<i>487</i>	<i>390</i>	<i>307</i>
Texas red/Alexa 594	615	128	118	110
		<i>521</i>	<i>417</i>	<i>329</i>
Cy5/Alexa 647	670	140	129	120
		<i>568</i>	<i>455</i>	<i>360</i>

- The x and y sampling distances are calculated (in nm) using the formula $\lambda/(4NA)$ (4) where λ is the emission wavelength (in nm) and NA is the numerical aperture of lens. The axial (z) sampling distances (in italics) are for an oil ($n = 1.515$) immersion objective and are calculated with the formula, $z = \lambda/[2n(1 - \cos \alpha)]$, where $\alpha = \sin^{-1}(NA/n)$ and n is the refractive index of the medium (13). DAPI, 6-diamidino-2-phenylindole; TRITC, tetramethylrhodamine isothiocyanate, Cy2, Cy3, Cy5, different fluorescent cyanine dyes.