— Selecting the Right Objectives — Bright, Sharp Imaging of Structures down to Deep Areas

Spherical aberrations caused by a mismatch of refractive indices may lead to a reduction in image resolution and brightness, and are one of the key problems in imaging. In this application note, we will demonstrate the effects of spherical aberration using 3D imaging of an enteroid, which is a 3D culture system for small intestinal epithelial cells, as an example, and show how to select the appropriate immersion liquid and objective.

What is spherical aberration?

When the refractive index changes while an objective is being focused, light rays passing through the optical axis and those passing away from the optical axis are not focused on one point (Figs. 1A and 1B). This phenomenon is called "spherical aberration".
When observing beads in a medium with a refractive index of 1.49, light passes through the medium (n = 1.49), the cover glass (n = 1.55), and the immersion liquid between the cover glass and the objective. The amount of spherical aberration generated varies depending on the immersion liquid type, and the aberration effect increases as the observation plane reaches the deeper regions further from the cover glass. For example, when silicone oil (n = 1.40) having a refractive index close to that of the medium is used as the immersion liquid, the influence of spherical aberration can be reduced in comparison with a case where water (n = 1.33) is used as the immersion liquid (Fig. 1C).

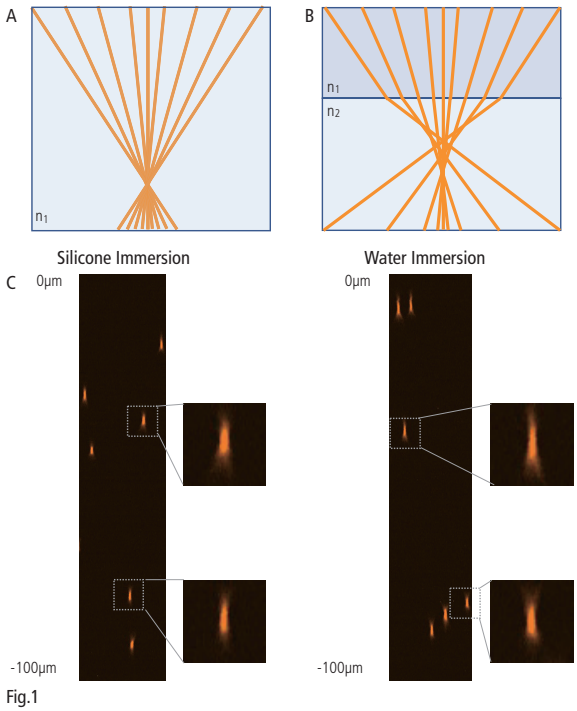


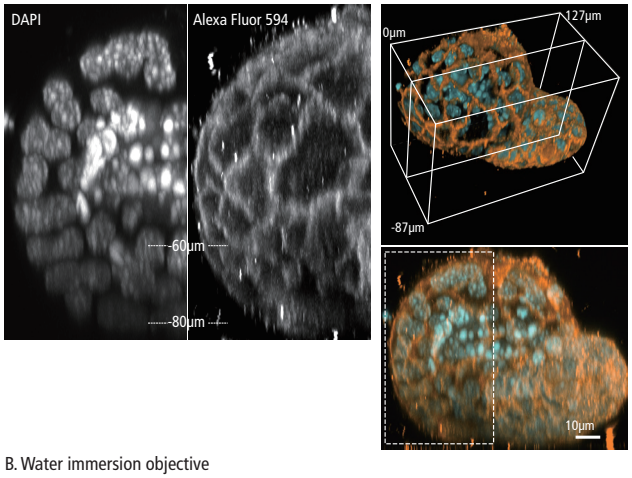
Fig.1
(A) In a medium with a uniform refractive index (n1), light is focused on one point.
(B) When light passes through a medium (n1> n2) with different refractive indices, it is not focused on one point.
(C) PSF images of fluorescent beads in a medium with a refractive index of 1.49. When using water as the immersion liquid, the bead images become longer in the deeper regions, but if silicone oil is used, this effect is smaller.
TetraSpeck fluorescent beads with 200 nm diameter were excited with a wavelength of 561 nm and the Z-stack images were captured.
XY resolution: 0.14 μm/pixel, Z step: 0.2 μm

Experiment overview

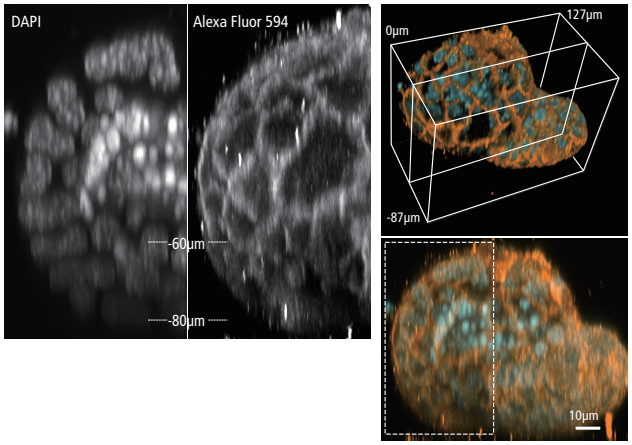
Enteroids, with their membrane and nucleus stained, were treated with clearing reagents having different refractive indices (RapiClear (SunJin Lab) with refractive indices of 1.45, 1.47 and 1.49) were observed with silicone or water immersion objectives. The effect of spherical aberrations due to refractive index mismatches was evaluated.

Fig. 2: Observation of enteroids cleared with RapiClear with a refractive index of 1.49

A. Silicone immersion objective
XY resolution: 0.12 μm/pixel, Z step: 0.5 μm



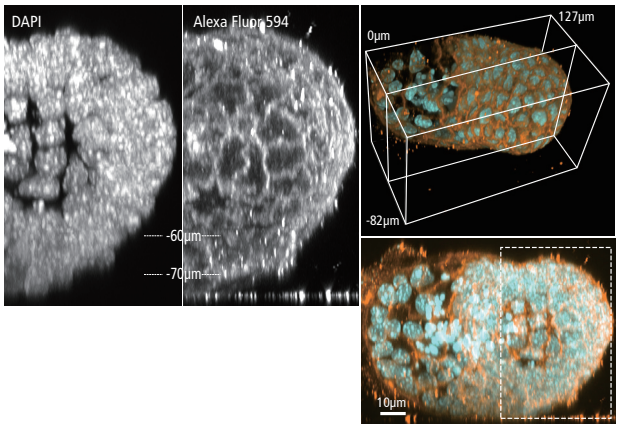
B. Water immersion objective
XY resolution: 0.12 μm/pixel, Z step: 0.7 μm



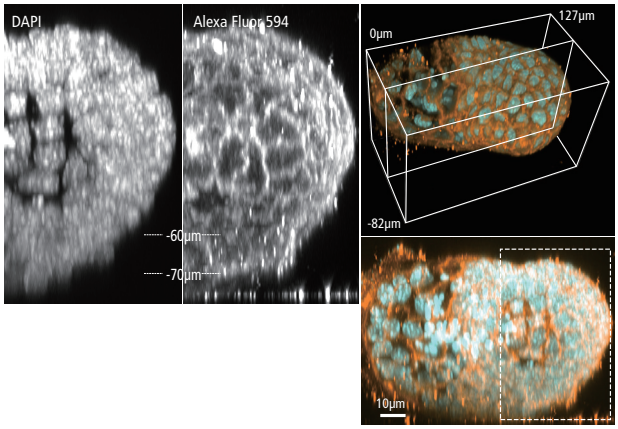
Cyan: DAPI (cell nuclei), Orange: Alexa 594 (E-cadherin)

Fig. 3: Observation of enteroids cleared with RapiClear with a refractive index of 1.47

A. Silicone immersion objective
XY resolution: 0.12 µm/pixel, Z step: 0.5 µm



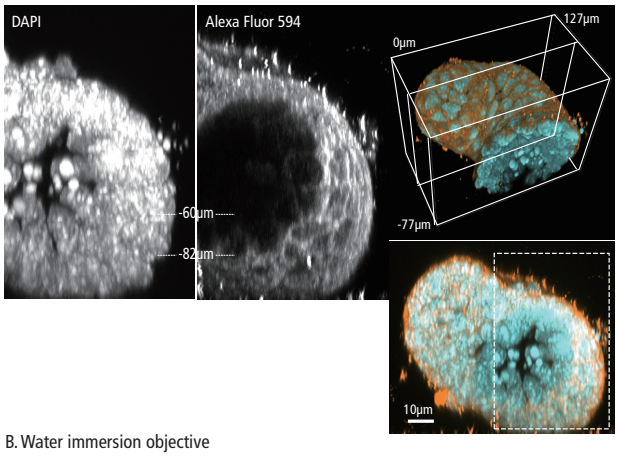
B. Water immersion objective
XY resolution: 0.12 µm/pixel, Z step: 0.7 µm



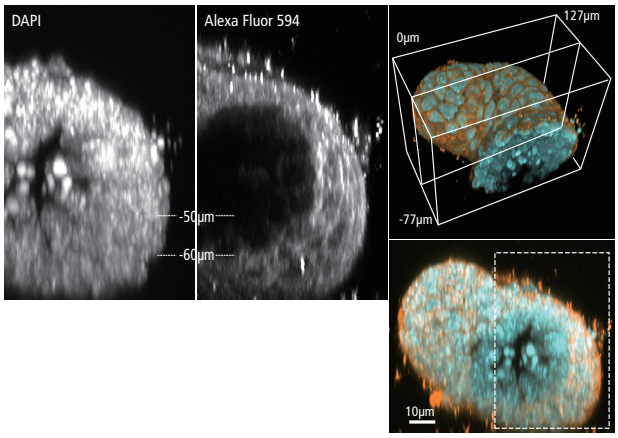
Cyan: DAPI (cell nuclei), Orange: Alexa 594 (E-cadherin)

Fig. 4: Observation of enteroids cleared with RapiClear with a refractive index of 1.45

A. Silicone immersion objective
XY resolution: 0.12 µm/pixel, Z step: 0.5 µm



B. Water immersion objective
XY resolution: 0.12 µm/pixel, Z step: 0.7 µm



Cyan: DAPI (cell nuclei), Orange: Alexa 594 (E-cadherin)

Results and summary

In this observation of enteroids with three different refractive indices, it was shown that a silicone immersion objective causes less image elongation than the water immersion objective, even in the deeper areas of the sample, and is suitable for observation of cell structures.
In order to obtain bright, high-definition images even in the deeper areas of a sample, it is important to select an appropriate objective while considering not only the magnification and working distance but also the refractive index of the immersion liquid to be used.

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Reference

Nikon's MicroscopyU
https://www.microscopyu.com/microscopy-basics/water-immersion-objectives

Product Information

CFI Plan Apochromat Lambda S 25XC Sil

By using silicone oil, which has a similar refractive index to biological samples, bright, high-definition 3D structures of tissues can be obtained even from deeper regions. The wide field of view of this lens makes it possible to efficiently capture images of the entire tissue.

A1R HD25 Confocal Microscope

This system has achieved the industry's largest field of view of 25 mm, achieving both high throughput and high-resolution imaging. It is ideal for observing large samples such as living tissues and model organisms.