Multi-Immersion Objectives

Summary specifications (click link to jump to details)

	57-12-19	54-10-12	54-12-8	57-12-5
Nominal Numerical Aperture	0.3	0.4	0.7	1.2
Approximate Magnification	10x	17x	24x	40x
Working distance [mm]	18.1	12.1	10.0	3.3
Nominal Refractive Index	1.53	1.45	1.45	1.52
Refractive Index Range	1.33 – 1.56			
Field of view [mm]	3.0	1.2	1.0	0.6
Parfocal length [mm]	75	62	83	83

History

Applied Scientific Instrumentation (ASI) worked with Special Optics to develop several dipping objective lenses for light sheet microscopy. They are intended for use with cleared tissue samples, but are useful for other applications where very long working distance or RI matching is important. These objectives work in any refractive index media without a correction collar, and so can be considered not just multi-immersion but “all-immersion” objectives.[1)] The first objective (54-10-12) has nominal NA 0.4 and was released October 2017. The second design (54-12-8) has nominal NA 0.7 and higher magnification and was released June 2019. The third objective (57-12-19) has nominal NA 0.3 and lower magnification and was released January 2023. A fourth design (57-12-5) with very high NA is available starting July 2025.

ASI is the sole distributor of these objectives but will sell them freely to anyone interested, including home builders and companies.

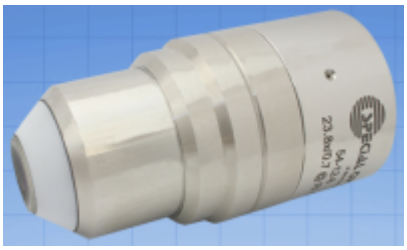
The original goal of the first objective was isotropic ~1 micron resolution at least 5 mm deep into slabs of cleared tissue. Doing this with a dSPIM/diSPIM geometry (two orthogonal identical objectives) requires a modest NA objective with extremely long working distance and tapered shape. We designed the objective lens accommodate a wide variety of imaging media, which is an important feature given the variety and rapid development of clearing protocols.

These are unique objectives because of the combination of multi-immersion capability, very long working distance, and mechanical profile amenable to light sheet imaging. [2)]

54-10-12 Specifications

Specification	Value	Comments
Numerical Aperture	0.40 @ RI 1.45	0.37 – 0.43 over RI range
Immersion Media RI	1.33 – 1.56	will also work in air or any media RI (16° optical angle)
Chemical Resistance	very high	Aqueous and organic solvents including DBE and more (see list)
Effective Focal Length	12 mm @ RI 1.45	15.3x – 17.9x over RI range w/ 200 mm TL
Working Distance	12 mm (for all RI)	> 5 mm imaging depth into flat sample (orthogonal co-focused; drawing)
Field of View	1.2 mm Ø	For all RI, check Zemax for specifics of edge degradation
Spherical Correction	480 – 1000 nm	Diffraction-limited for most media and λ
Chromatic Correction	480 – 720 nm	Performance varies by media, optimized for CLARITY and TDE
Transmission	~85-90%	Internal coatings 480 – 910 nm or -M variant with 400 – 1300 nm (extra cost)
Correction Collar	None	Designed for dipping (immersion w/o coverslip)
Form Factor	Nikon style (~CFI60)	61.6 mm parfocal distance, M25 x 0.75 threads, 38.5 mm OD, ~225 g
Mechanical Angle	22°	Measured from center of FOV

The mechanical drawing and corresponding 3D CAD file are available. [3]. Of interest is a drawing of how two of the 54-10-12 objectives co-focus which is posted on dispim.org. Zemax black box files are available too (forward, reverse).



54-12-8 Specifications

Specification	Value	Comments
Numerical Aperture	0.70 @ RI 1.45	0.64 – 0.75 over RI range
Immersion Media RI	1.33 – 1.56	will also work in air or any media RI (29° optical angle)
Chemical Resistance	very high	Aqueous and organic solvents including DBE and more (see list)
Effective Focal Length	8.4 mm @ RI 1.45	22x – 26x over RI range w/ 200 mm TL
Working Distance	10 mm (for all RI)	2 mm imaging depth into flat sample (orthogonal co-focused; drawing)
Field of View	1.0 mm Ø	For all RI, check Zemax for specifics of edge degradation
Spherical Correction	480 – 1000 nm	Diffraction-limited for most media and λ
Chromatic Correction	480 – 720 nm	Performance varies by media, optimized for CLARITY and TDE
Transmission	~85%	Internal coatings 480 – 1000 nm

Specification	Value	Comments
Correction Collar	None	Designed for dipping (immersion w/o coverslip)
Form Factor	long Nikon style	83 mm parfocal distance, M25 x 0.75 threads, 38.5 mm OD, ~383 g
Mechanical Angle	36°	Measured from center of FOV

The mechanical drawing and corresponding 3D CAD file are available. Of interest is a drawing of how two of the 54-12-8 objectives co-focus which is posted on dispim.org. Zemax black box files are available too (forward, reverse).

57-12-19 Specifications

Specification	Value	Comments
Numerical Aperture	0.30 @ RI 1.53	0.26 – 0.31 over RI range
Immersion Media RI	1.33 – 1.56	will also work in air or any media RI (11.4° optical angle)
Chemical Resistance	very high	Aqueous and organic solvents including DBE and more (see list)
Effective Focal Length	19.6 mm @ RI 1.53	9x – 10.6x over RI range w/ 200 mm TL
Working Distance	18.1 mm (for all RI)	8.2 mm imaging depth into flat sample (orthogonal co-focused)
Field of View	3.0 mm Ø	For all RI, check Zemax for specifics of edge degradation
Spherical Correction	480 – 900 nm	Diffraction-limited for most media and λ
Chromatic Correction	480 – 720 nm	Performance varies by media: optimized for ECi/BABB/EasyIndex [4])
Transmission	~85%	Internal coatings 480 – 900 nm or -M variant with 400 – 1300 nm (extra cost)
Correction Collar	None	Designed for dipping (immersion w/o coverslip)
Form Factor	Nikon style	75 mm parfocal distance, M25 x 0.75 threads, 38.5 mm OD, ~307 g
Mechanical Angle	28°	Measured from center of FOV to body, to glass is 22°

The mechanical drawing and corresponding 3D CAD file are available. Zemax black box files are available too (forward, reverse).

57-12-5 Specifications

The first lenses are made! Here are the specifications.

Specification	Value	Comments
Numerical Aperture	1.20 @ RI 1.52	NA = 0.79 * medium RI
Immersion Media RI	1.33 – 1.56	Will also work in air or any media RI (52° optical angle)
Chemical Resistance	very high	Tentatively same as others but needs to be tested (see list)
Effective Focal Length	4.93 mm @ RI 1.52	36x in water, 42x in ECi w/ 200 mm TL
Working Distance	3.3 mm (for all RI)	able to co-focus with 54-10-12
Field of View	0.3 - 0.6 mm Ø	FOV 0.6 mm in RC1.52 and similar, ~0.3mm in water, simulate if this is critical
Spherical Correction	480 – 1300 nm	Diffraction-limited for most media to 910 nm full NA, NA 0.8 from 910 – 1300 nm
Chromatic Correction	480 – 720 nm	Performance varies by media, optimized for RC1.52 and organic solvents in general
Transmission	Expected ~75%	Internal coatings 400 – 1300 nm
Correction Collar	None	Designed for dipping (immersion w/o coverslip)
Form Factor	long Nikon style	83 mm parfocal distance, M25 x 0.75 threads, 38.5 mm OD
Mechanical Angle	59°	Measured from center of FOV

The mechanical drawing and corresponding 3D CAD file are available. Zemax black box files are available too (forward, reverse).

Magnification

All these objectives are infinity-corrected and designed to be used with an infinity-corrected tube lens as is common practice in modern microscopes. The magnification is given by the ratio of the effective focal lengths of the tube lens and that of the objective lens. The effective focal length of the multi immersion objectives (EFL_{obj}) depends on the medium refractive index (RI) according to the following formula:

54-10-12: EFL_{obj} = 17.4 mm / RI

54-12-8: EFL_{obj} = 12.19 mm / RI

57-12-19: EFL_{obj} = 30.0 mm / RI

57-12-5: EFL_{obj} = 7.50 mm / RI

For the 54-10-12 and 54-12-8 the nominal RI is 1.45 (FocusClear/CLARITY), so for example the focal length is 12.0 mm for the 54-10-12 in FocusClear. Taking a 200 mm focal length tube lens, which ASI uses by default on SPIM systems, this results in a magnification of 16.7x. However, if a different magnification is needed then a different tube lens can be used. ASI offers a variety of suitable tube lenses as do others.

For the 54-12-8 at RI 1.45, the focal length is 8.4 mm and thus nominal magnification is 24x (assuming 200mm tube lens).

The 57-12-19 has a nominal RI of 1.53 and is specifically intended for ECi/BABB/EasyIndex media.

The 57-12-5 has a nominal RI of 1.52 and is specifically intended for organic media such as RapidClear 1.52 and EasyIndex. The diffraction-limited FOV is reduced in water.

Because the magnification depends on the imaging medium, magnification ideally would be measured empirically. However, for most situations estimating the magnification based on the presumed RI of the imaging medium is probably sufficient.

The reason that the effective focal length (and hence magnification) depends on the refractive index of the medium can be understood in a few different ways. Perhaps the most simple way to understand is to consider the “back aperture” of the objective.[5] The NA, the EFL, and the size of the back aperture are related by the following well-known equation $BA_{ø} = 2 * NA * EFL$. The NA of the objective is proportional to the RI of the media; this is essentially definitional. The back aperture has a fixed size. For the equation to hold then the EFL must be inversely proportional to the RI, meaning the magnification is proportional to the RI. An alternative way of understanding is to consider an off-axis ray which passes through the center of the surface between the first objective element and the media. The ray will be refracted off of the glass/media surface with an angle given by Snell's law which says the ratio of sin θ to RI is constant. For relatively small angles, $\sin \theta \approx \theta$, which means that the outgoing angle of the ray is is inversely proportional to the medium's RI. Hence the the displacement from center of that ray at the image plane is inversely proportional to the RI, which is equivalent to saying that the magnification is changed proportionally by the medium's RI. Incidentally, the same argument suggests that the magnification varies with RI for **all** objective lenses.

Numerical Aperture and Resolution

The numerical aperture (NA) of the objectives depends on the medium refractive index (RI) according to the following formula:

54-10-12: NA = 0.276 * RI

54-12-8: NA = 0.483 * RI

57-12-19: NA = 0.196 * RI

57-12-5: NA = 0.790 * RI

The diffraction-limited resolution is a function of the lens NA which depends on the RI of the immersion medium as described above. The pre-factors can differ depending on the criteria used to define resolution, but common expressions are as follows for the resolution in the lateral (x,y) and axial (z) directions where λ is the wavelength of light and RI is the refractive index of the mounting medium:

$Res_{x,y} = 0.61 * \lambda / NA$

$Res_{z} = 2 * \lambda * RI / NA^2$

From these expressions it is clear that axial resolution is worse than lateral resolution. For 500 nm

light at RI 1.45, the lateral resolution is 0.76 µm and the axial resolution (depth of field) is 9.1 µm for the NA 0.4 lens (for the NA 0.7 lens, the numbers are 0.44 µm and 3.0 µm). The dSPIM/diSPIM geometry provides the opportunity to overcome poor axial resolution by combining two views of the same object from orthogonal directions so that each feature is seen from at least one high-resolution vantage point, all without needing to move the sample. Some home-built imaging systems use the similar concept but rotate the sample to be imaged from different directions.

In light sheet microscopy, commonly only a small fraction of the objective's NA is used for light sheet generation. The baseline axial resolution is the depth of field of the detection objective, but can be improved if the light sheet is thinner than the depth of field. Whether or not this happens depends on the illumination NA and hence imaging FOV. In many cases light sheet provides no true resolution benefit even though out of focus florescence will be reduced (“optical sectioning”) which improves SNR and image quality. There are ways of creating very thin light sheets to increase axial resolution (e.g. Bessel beams) but they generally have other undesirable properties including extra complexity/cost, large amounts of out of focus light, and/or being extremely sensitive to scattering or sample inhomogeneity.

Working Distance

All of these objective lenses have been designed with large working distance. A unique feature is that the working distance is exactly the same for all media.

For most objective lenses, the WD depends on the medium's RI due to refraction at the objective/media surface. Realizing this was going to be a practical issue we specifically designed both objectives to have uniform WD for all RI. The optical implementation of this feature is straightforward: the first element of the objective is concave with radius of curvature centered on the focal plane. Thus to first order there is no refraction at that surface and hence the WD is constant for all RI. For the same reason, there is no intrinsic spherical aberration as the medium RI changes, unlike objective lenses with a flat first surface.

Location of Back Focal Plane

For some applications (e.g. light sheet illumination) it is important to maintain “4f” or telecentric spacing between optical elements, i.e. space adjacent lenses so that their focal planes line up. If an external lens is positioned with focal position at this plane, parallel rays into the lens pair will emerge from the objective parallel. Although the working distance of these objectives does not depend on the refractive index of the medium, the location of the back focal plane does depend on it slightly.

Per optical simulations for the 57-12-19 objective lens, the location of the back focal plane referenced to flange is approximately 40.07 mm - 78.77 mm * RI + 19.77 mm * RI^2 (negative inside flange). Specifically, this is ~29.8 mm inside the flange for water, ~32.7 mm inside the flange for FocusClear, and ~35.0 mm inside the flange for ethyl cinnamate (RI 1.56). To a rough approximation the back focal plane location is about 14mm closer to the sample compared to the 54-10-12 objective and about 9mm closer to the sample compared to the 54-12-8.

Per optical simulations for the 54-10-12 objective lens, the location of the back focal plane referenced to flange is approximately 31.07 mm - 38.80 mm * RI + 9.42 mm * RI^2 (negative inside flange). Specifically, this is ~4 mm inside the flange for water, ~5.4 mm inside the flange for FocusClear, and

~6.5 mm inside the flange for ethyl cinnamate (RI 1.56).

Per optical simulations for the 54-12-8 objective lens, the location of the back focal plane referenced to flange is approximately -11.75 mm - 22.90 mm * RI + 5.87 mm * RI^2 (negative inside flange). Specifically, this is ~31.8 mm inside the flange for water, ~32.5 mm inside the flange for FocusClear, and ~33.2 mm inside the flange for ethyl cinnamate (RI 1.56). The difference in back focal plane position between the two objectives depends on RI, but is to a good approximation the location is 27 mm deeper inside the objective for the 54-12-8 as measured from the flange, or 5 mm closer to the sample.

Per optical simulations for the 57-12-5 objective lens, the location of the back focal plane referenced to flange is ~44.0 mm inside the flange for water and ~44.9 mm inside the flange for the design medium which is RC1.52.

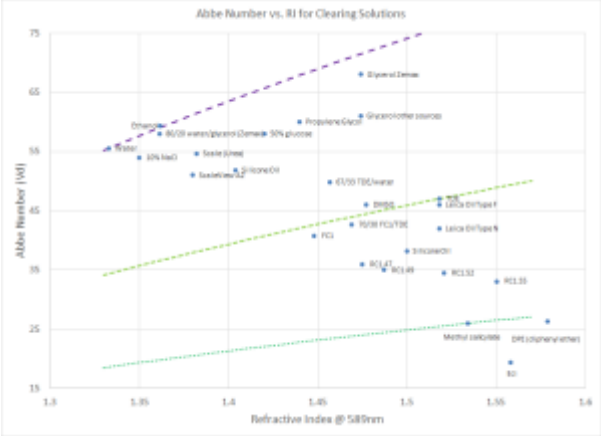
Spherical and Chromatic Aberrations

Correction collars are commonly used to correct high-NA objectives for spherical aberrations when imaging through a variable-thickness coverslip and/or at different temperatures. Other multi-immersion objectives have a correction collar for different media RI. Even though our objectives have no correction collar, spherical aberrations are still within the diffraction limit for all media and wavelengths simulated due to design features discussed below, most notably the curved first surface.

The objectives have minimal spherical aberration in the NIR spectrum to allow for multi-photon excitation, though they are corrected chromatically only in the visible. Internal anti-reflective coatings are optimized for visible, but it is possible to make a batch with coatings optimized for NIR (e.g. 800-1600 nm); contact ASI if you are interested in this possibility.

Chromatic aberrations are rooted in dispersion, which describes how the exact RI changes with wavelength (commonly reported as the Abbe number). For a specific medium the dispersion can be corrected, but since these objectives are designed to work in many media it cannot be perfectly corrected for all of them. Chromatic correction could have been improved with a correction collar; this was deemed to add too much complexity for the corresponding benefit. Hence, during the design we can only optimize the chromatic correction for one media, but TDE and CLARITY/Focus Clear are very well corrected for simultaneously. The middle dotted line on the plot below shows the approximate correction line used for 54-10-12 and 54-12-8, whereas the bottom line going through methyl salicylate was chosen for the 57-12-19 (i.e. emphasizing organic solvents).

Chromatic aberrations can be categorized as lateral color, meaning different wavelengths have slightly different magnification, and axial color, meaning that the focus point is shifted slightly. These chromatic effects scale with the distance from the “perfect” correction line in the plot. In the 54-10-12, the lateral color for water, (a rather extreme case as seen from the plot). amounts to ~0.3% change in magnification between 480 nm light and 640nm light. We expect that the lateral color can be corrected in post-processing if needed. The axial color generally remains within the diffraction limit for all media for all the objectives but should be simulated if this is important.



The lateral color is somewhat worse for 57-12-19 due to the significantly larger field.

Immersion Media

RI Range

A common question is how these objective lenses can accommodate such a wide range of media refractive indexes without a correction collar. There are at least two reasons:

1. The first surface is concave with radius of curvature at the focal plane. This completely eliminates refraction of rays coming from the center of the FOV. Light coming from off-axis points in the focal plane will undergo some refraction, but the refraction is both small (compared to the case of a flat first surface) and predictable so that refraction helps in the first step of the lens' function of converting positions (at the sample focal plane) to angles (at the back focal plane). The intentionally-chosen concave first surface is unique among objective lenses to our knowledge.
2. They are dipping objective lenses (no coverslip; assumed constant RI from objective to sample). [6)]

Chemical Compatibility

Our objective lenses are designed for direct immersion in index-matching medium. Even though some media used for tissue clearing are corrosive, these objectives can be immersed in most all of them (see list below). The main damage mechanism is media dissolving the glue holding the lens elements in place. This objective was designed to be maximally resistant to media.

ASI maintains a list of known safe media (below). Exposing the objectives to the safe media is covered by the warranty on the objectives. **If you want to use a media not on the known safe list you can either proceed at your own risk or take the time to arrange for a chemical compatibility test using some special dummy objectives.** If the test passes then the media will be added to the known safe list so that others won't have to repeat the test. Contact ASI to arrange a loan of these dummy objectives and instructions for performing the test.

At Society for Neuroscience meetings in 2016 and 2017 there were at least three research groups with preliminary results transferring solvent-cleared tissues to more gentle media for imaging; such protocols could allow e.g. DISCO-cleared samples to be imaged with this cleared tissue objective lens.

We expect further developments in this general approach, but we are pleased that tests show that the ASI multi-immersion objectives are compatible with DBE, BABB, and other harsh organic solvents.

There is a groove 51.6mm from the focal plane that can be used for an o-ring seal. It is fine to dip the objectives to at least that depth, though be aware that the engraving (on some objectives below that groove) can become discolored or slightly corrode without affecting the integrity of the objective.

Known Safe Media

- water with salt, sugars, and/or other non-aggressive solutes including routine-use biological buffers
- FocusClear (CLARITY)
- glycerol
- CUBIC-1, CUBIC-2, CUBIC-R1, CUBIC-R2
- mineral oil
- silicone oil
- TDE (2,2-thiodiethanol)
- ECi (ethyl cinnamate)
- benzyl benzoate and BB-PEG (PEGASOS)
- BABB
- DBE (dibenzyl ether)
- 100% ethanol
- other proprietary organic media

[1)]
they are actually dipping lenses, as they are not correcting for imaging through a coverslip
[2)]
Other objectives being used for light sheet imaging of cleared tissue were designed for confocal imaging, with relatively large NA and “fat” form factor so they can't be used in symmetric configuration.
[3)]
There is a difference in the profile of the step position in objectives with SN less than 58 (roughly May 2019 transition), the drawing for it is at here. Very early objectives made in 2017 had further slight differences in the nose profile but those were all later retrofitted.
[4)]
modest lateral color for FocusClear/CLARITY/TDE and significant lateral color for water and Scale (~0.9% difference in magnification in water between 500nm and 700nm
[5)]
The back aperture or pupil is closely related but not identical to the back focal plane
[6)]
Most commonly correction collars are to compensate for different coverslip thicknesses because the coverslip has a different RI from the immersion medium

From:
http://www.asiimaging.com/docs/ - **Applied Scientific Instrumentation**

Permanent link:
http://www.asiimaging.com/docs/multi-immersion_objectives

Last update: **2025/09/29 20:38**