Remote Refocus Microscopes

Overview

In brief, remote refocus forms a 3D replica image of a sample (usually in air) with a carefully-constructed optical relay, and then a microscope looks at the replica 3D image instead of the physical one. Why go to all the trouble? Example applications are making fast Z stacks without disturbing the sample and implementing a light sheet microscope using a single objective for both illumination and detection (see Millett-Sikking and York, High NA single-objective light-sheet.

An excellent overview of building a remote refocus microscope can be found on another GitHub publication of Millett-Sikking and York Remote refocus enables class-leading spatiotemporal resolution in 4D optical microscopy, with many practical design details in the Appendix. The purpose of this page is not to rehash those details, but to give some ideas for building a remote refocus microscope using ASI-made components.

The required care in component selection and alignment depend strongly on the effective NA of the total remote refocus imaging system. Anything approaching NA 1.0 is going to require great care, whereas NA 0.3 has a lot more tolerance for imperfection simply because the resolution will be lower.

Designing the relay

The crux of designing a remote refocus microscope is designing the relay optics between the sample and the remote replica image. As described by Botcherby et al. in their 2008 publication, a faithful replica image in 3D requires the lateral and axial magnifications between the sample and the remote image are the same, and that condition occurs when the magnification is equal to the sample's refractive index n_{sample} (assuming the remote image is in air). This is the first of the “golden rules” noted in the Appendix of the GitHub publication. The steps to achieving the correct magnification are:

1. Select your O1 objective (nearest the sample) based on its properties such as field of view, working distance, and NA. Compute its focal length EFL_{O1}. (Remember that Nikon objectives assume 200 mm tube lenses and Olympus objectives assume 180 mm tube lenses, for example a Nikon 40x objective has focal length of 5 mm and an Olympus 60x objective has a focal length of 3 mm.)
2. Tentatively pick an objective for O2. It should have sufficient field of view to create the remote image with magnification n_{sample} and ideally NA greater than the NA of O1 divided by n_{sample}. Common choices for high-NA remote refocus are Nikon 40x/0.95 air, Olympus 40x/0.95 air, Nikon 20x/0.75, or Olympus 20x/0.8. Compute its focal length EFL_{O2}. You can revisit this choice later if you have difficulty finding a good tube lens pair.
3. Next find an appropriate pair of tube lenses which will give overall magnification n_{sample}. One approach is to use the “standard” tube lens for TL1 and then a special tube lens for TL2, which is required if you are using a commercial microscope stand. However, if you are designing the microscope from scratch (e.g. using ASI's modular parts) then you can use any pair of ASI's tube lenses. To find an appropriate pair of ASI's tube lenses you can search the table below for a ratio M_{TL} that closely matches EFL_{O2}/EFL_{O1}/n_{sample}.

Look for a match to within 1% for sure, ideally better for high-NA imaging (this preprint presents an analysis concluding that 50% loss in performance is expected for a 1% mismatch). For example, for a Nikon 40x water O1 and a Nikon 20x air O2, you require a ratio $M_{TL} = 10mm/5mm/1.333$ = 1.50 and the C60-Tube-133D and C60-Tube-B are a good combination. Another example is using an Olympus 60x silicone O1 and a Nikon 40x air O2, you can use a C60-Tube-300 with a C60-Tube-358. Another option is to construct a custom tube lens by combining Thorlabs parts as described here. Note that ASI's tube lenses have a "D" in the name when they are dual achromats. Tube-B has Nikon glass, Tube-180L is Olympus glass.

4. Double-check that the tube lenses selected have sufficient clear aperture for the application. This is especially important when the objectives have large back apertures and/or you are using a large FOV. See section below for details.

ASI PN	**C60-Tube-**	**80**	**100 or 100D**	**125D**	**133C**	**140D**	**160**	**180L**	**B or 200**	**250**	**265D**	**300**	**358**	**400**
C60-Tube-	**Focal length**	**80**	**100**	**125**	**133.3**	**140**	**160**	**180**	**200**	**250**	**265**	**300**	**358**	**400**
80	**80**	1.000	1.250	1.563	1.666	1.750	2.000	2.250	2.500	3.125	3.313	3.750	4.475	5.000
100 or 100D	**100**	1.250	1.000	1.250	1.333	1.400	1.600	1.800	2.000	2.500	2.650	3.000	3.580	4.000
125D	**125**	1.563	0.800	1.000	1.067	1.120	1.280	1.440	1.600	2.000	2.120	2.400	2.864	3.200
133C	**133.3**	1.666	0.750	0.938	1.000	1.050	1.200	1.350	1.500	1.875	1.988	2.250	2.685	3.000
140D	**140**	1.750	0.714	0.893	0.952	1.000	1.143	1.286	1.429	1.786	1.893	2.143	2.557	2.857
160	**160**	2.000	0.625	0.781	0.833	0.875	1.000	1.125	1.250	1.563	1.656	1.875	2.238	2.500
180L	**180**	2.250	0.556	0.694	0.741	0.778	0.889	1.000	1.111	1.389	1.472	1.667	1.989	2.222
B or 200	**200**	2.500	0.500	0.625	0.667	0.700	0.800	0.900	1.000	1.250	1.325	1.500	1.790	2.000
250	**250**	3.125	0.400	0.500	0.533	0.560	0.640	0.720	0.800	1.000	1.060	1.200	1.432	1.600
265D	**265**	3.313	0.377	0.472	0.503	0.528	0.604	0.679	0.755	0.943	1.000	1.132	1.351	1.509
300	**300**	3.750	0.333	0.417	0.444	0.467	0.533	0.600	0.667	0.833	0.883	1.000	1.193	1.333
358	**358**	4.475	0.279	0.349	0.372	0.391	0.447	0.503	0.559	0.698	0.740	0.838	1.000	1.117
400	**400**	5.000	0.250	0.313	0.333	0.350	0.400	0.450	0.500	0.625	0.663	0.750	0.895	1.000

Simulating the relay

If you have optical models for the tube lenses you can simulate them using Zemax (paid), WinLens 3D (free version), or similar software. ASI can provide optical models for their tube lenses on request. It is very difficult to come by optical models for objective lenses, but it usually suffices to simulate them as ideal lenses with the appropriate focal length.

Particularly with high-NA imaging with short focal length tube lenses, it is recommended to simulate the pair of tube lenses to to make sure that they don't add intolerable aberrations. For example, to achieve a tube lens ratio of 1.5 there are significant aberrations (especially at the edge of the field) using a ASI's C60-Tube-100D and a stand-alone 150 mm achromat lens, but much better to use ASI's C60-Tube-133D and C60-Tube-B (200 mm focal length, Nikon glass).

Ensuring sufficient clear aperture

Ensuring sufficient clear aperture of optics downstream of the primary objective is the second of the "golden rules" noted in the Appendix of the GitHub publication on remote refocus.

It is relatively straightforward to ensure that O2's back aperture $Ø_{O2}$ is large enough to fit the image of O1's back aperture when magnified by the ratio $M_{TL} = {EFL}_{TL2}/{EFL}_{TL1}$ as described above. Recall that the back aperture of an objective is given by $2*{NA}*{EFL}$

It can be shown that the clear aperture condition for TL1 is that ${CA}_{TL1} > Ø_{FOV}(EFL_{TL1}/EFL_{O1} + 1) + Ø_{O1} $. From there it is straightforward to see that the condition for TL2 is ${CA}_{TL2} > M_{TL} * (Ø_{FOV}(EFL_{TL1}/EFL_{O1} + 1) + Ø_{O1}) $.

Intrinsic aberrations

Botcherby et al. derived closed form approximations for residual aberrations 2008 publication. There are two such aberrations intrinsic in the remote refocus mechanism, non-unity Strehl ratio (what fraction of light ends up in a diffraction-limited spot) and also a slight axial stretching. In both cases the aberrations get worse and worse away from the native focal plane, so the question is whether the aberrations are tolerable for the Z dimension required (note in single-objective light sheet each image spans various Z positions). In practice there might be further aberrations due to imperfections in the lenses or objectives. The axial stretching can be compensated for in post-processing, at least in theory.

An Excel spreadsheet to make it easy to estimate these intrinsic aberrations (based on the Botcherby-derived equations) is available here for

download here

. Fill out the cells in green and the spreadsheet will update plots and the values in yellow corresponding to the thresholds set.

Alignment considerations

For remote refocus with highest fidelity, the objectives and tube lenses should be telecentric, where the back aperture of one objective lens is exactly imaged into the back aperture of another objective lens with both positions and angles (or amplitude and phase) preserved. Each successive element ideally will be aligned with respect to the others in 5 dimensions: axially, in X/Y, and in tip/tilt. These adjustments are possible on a breadboard implementation of course, but they are also possible to do with an enclosed beam path using ASI components. The higher the NA, the more precise the alignment needs to be to avoid aberrations. For effective NA < 0.3 alignment can be relatively coarse but with effective NA > 1.0 then very careful alignment is required.

Some excellent suggestions for optical alignment are the 2013 guide “A Appendix: Practical Guide to Optical Alignment”.

Telecentricity

To achieve telecentric or “4f” spacing it is important to know the focal positions of the tube lenses in both directions as well as the location of the objectives' back focal plane inside of its flange (Olympus specifies the BFP position, and Nikon might tell it to you if you ask nicely or you can measure it). Start by designing approximately correct spacing and allow adjustment between every pair of elements, e.g. on both objectives and between the tube lenses.

Axial adjustments can be achieved in ASI's modular system using any of the following:

- for objectives, RAO-series objective bushings with teflon (+/- 2 mm or a bit more), e.g. RAO-0004L has 8mm shoulder height
- for collimated space: C60-AFS-800 has 30 mm clear aperture and +/- 4 mm space with C60-RING connections on both sides; C60-SPACER-ADJ for some tube lenses (including C60-Tube-B and most shorter focal length; inquire for more details)

C60-EXT-xxx tubes are available to fill fixed spaced requirements and it is always possible to find a 1.5mm of any target by using combinations. Available lengths include (all in millimeters): 7.5, 10, 12, 15, 25, 37.5, 50, and 75.

X/Y and Tip/Tilt

If a pair of mirrors is placed between two lenses then that pair of mirrors can be used to “walk the beam” which can adjust both the X/Y and Tip/Tilt of the beam, which is all the degrees of freedom that are required. ASI's MIM-CUBE-III-KM is a 25 mm right angle mirror with a kinematic adjuster inside an outer cube measuring 60 mm on each side and 60 mm optical path length.

If you have a separate X/Y adjuster (e.g. the position of an objective lens with ASI's motorized objective slider) then you only require a single mirror for Tip/Tilt alignment.

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