Basic concept

- Dual-view SPIM (like diSPIM) but with horizontal objective geometry
  - Sample is c. elegans worms in vertical tube with thin transparent walls and outer water chamber (customer's design)
  - Main application is GCaMP-type functional imaging
- Use Nikon 40x/0.8 W objectives for light-gathering and axial resolution even though intentionally will undersample laterally for speed
- Two dual-view SPIM (total 4 objectives**) where the pairs are displaced sightly vertically to look at top and bottom half of worm

Tricky things about the design

- Remote refocus requires alignment adjustment between every pair of lenses (O1-TL1-TL2-O2) for axial spacing, beam tip/tilt, and beam XY
- Remote refocus alignment requirement eliminates possibility of using ASI's standard SPIM head b/c space is at a premium and objectives need to be independently adjusted axially
- Plan: align each remote refocus microscope separately, and subsequently arrange the primary objectives to be co-focused and orthogonal using motorized XY stages and motorized** Z stages
- Using ASI beam scanners for illumination**

Remote refocus

- Require magnification of 1.333 to remote image
  - O1 = Nikon 40x/0.8 W (5mm EFL)
  - TL1 = ASI C60-Tube-133D dual achromat tube lens (133.3mm EFL)
  - TL2 = ASI C60-Tube-200 achromat tube lens in shorter body (200mm EFL)
  - O2 = Nikon 20x/0.75 air (10mm EFL)
- O3 will be on objective piezo for taking stacks
  - O3 = Olympus 20x/0.8 which is lightweight
  - Plan is for 150um travel piezo = 112um travel in sample space
- Total magnification options quoted per request:
  - With Olympus TL3 => M = 1.333*20 => 0.24um pixels, ~560um square FOV
  - With ASI Tube-100D TL3 => M = 1.333*100/9 => 0.44um pixels, ~1mm square FOV
  - Remember that objective's designed FOV is only 440um square = 625um diameter => expect vignetting and increased aberrations outside of that

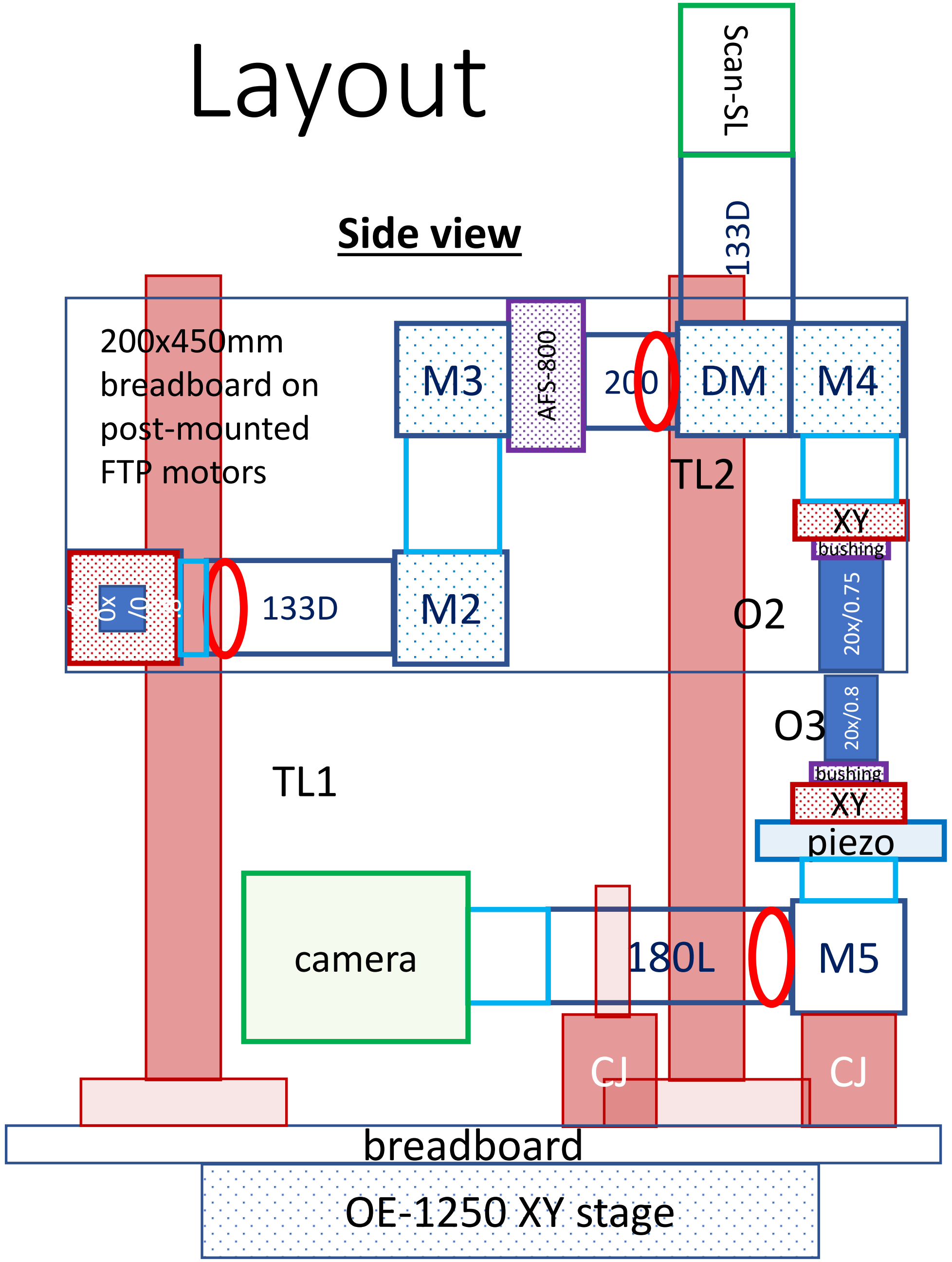
Light sheet illumination

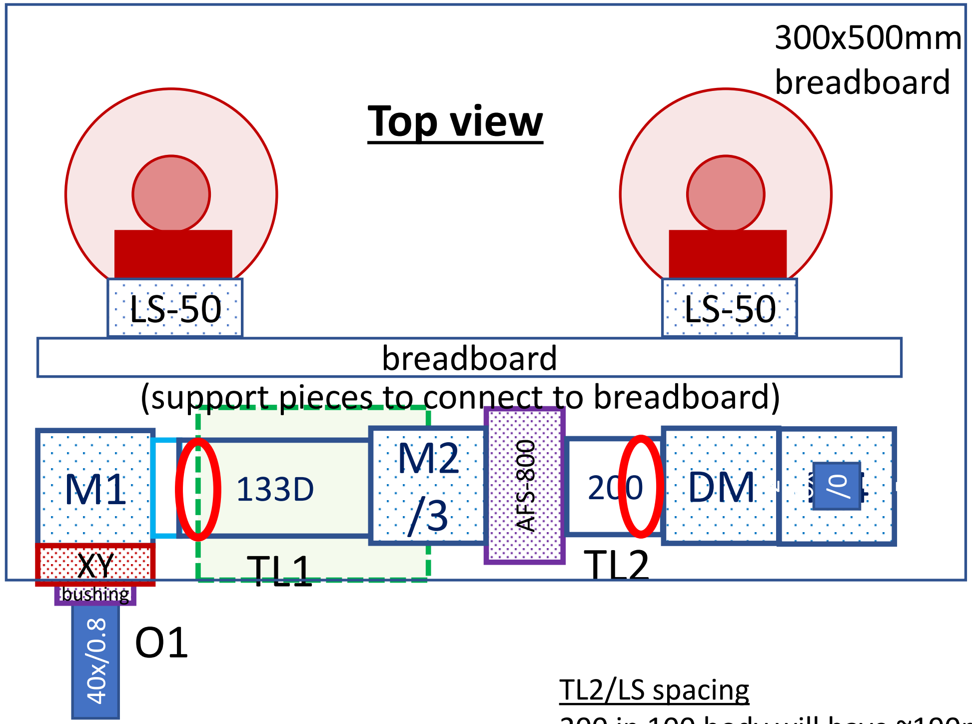
- To place MEMS at focal point of 200mm lens:
  - Scan-SL without scan lens
  - modified C60-SPACER-ADJ for axial positioning and adapted to Scan-SL
- max beam NA
  - 1.2mm*cos(22.5)/2 MEMS size
  - /200*133/5 = 0.073 max NA
  - 0.64*0.488um*1.333/.073^2 = 78um confocal length => plenty of NA
    - NA 0.029 gives 495um confocal length
    - => consider 7.5mm collimator
- want ~500um scan range to cover objective FOV
- Travel range at sample is OK
  - +/- 0.14 radian MEMS range (optical)
  - 200mm TL2
  - 133mm TL1
  - 5mm O1
  - => 1.05mm FOV or about 2x required

Imaging speed

- Application calls for rapid volumetric imaging
- Camera readout is fundamental speed limit with readout requiring ~5um/row
- Large pixels mostly to have smaller images for faster readout
- Hamamatsu Fusion selected for good speed and excellent all-around performance and versatility
- 1.33x mag to remote image, additional mag of 20x or 11x to sensor => total mag will be 26.7x or 14.8x
- Objective-limited FOV of ~440um square = ~625um diagonal
- 440um square = ~1800 pixels square at 26.7x; ~1000 pixels square at 14.8x

Layout





<u>TL2/LS spacing</u>

200 in 100 body will have ~190mm free focal length towards collimated space
60 Cube-III
87 Spacer-Adj body
0-30 travel
30 minimum Scan-SL body
13 extra space

<u>O1/TL1 spacing</u>

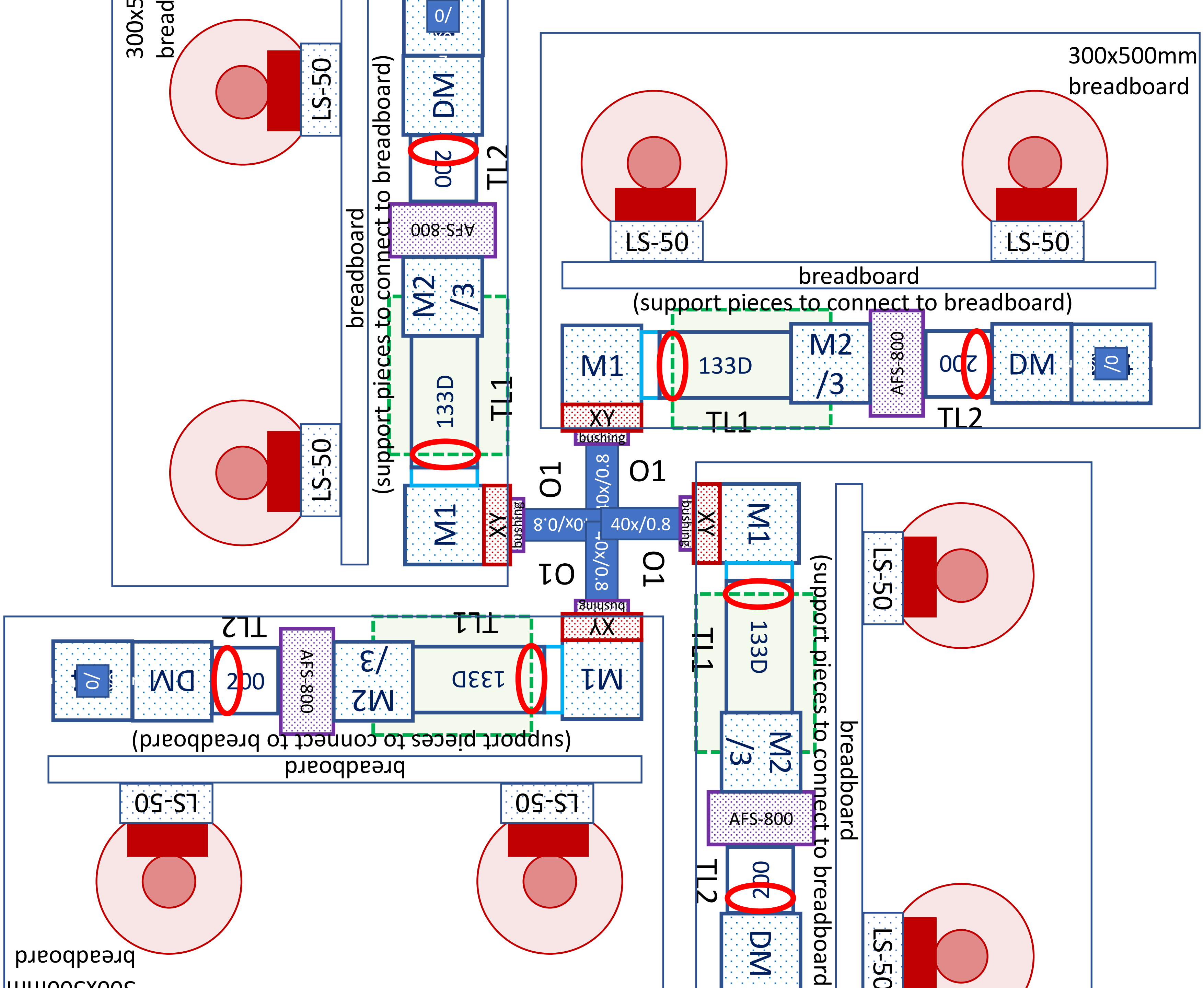
133D will have ~111mm free focal length towards collimated space
13 inside objective
8 bushing
0-4 bushing gap (adjustable)
14? XY adjuster
60 Cube-III w/ mirror
~14 extra space => C60-EXT-15

<u>TL1/TL2 spacing</u>

133D will have 60mm free focal length towards focus space
200 in 100 body will have ~163mm free focal length towards focus space
37 AFS body minimum
0-8 AFS travel (adjustable)
60 Cube-III with mirror
60 Cube-III with mirror
~62 extra space => EXT-37 + EXT-25

<u>TL2/O2 spacing</u>

200 in 100 body will have ~190mm free focal length towards collimated space
10 inside objective
8 bushing
0-4 bushing gap (adjustable)
14? XY adjuster
60 Cube-III w/ mirror
60 Cube-III w/ dichroic
~36 extra space => EXT-25 + EXT-10



300x500mm
breadboard
LS-50
LS-50
breadboard
(support pieces to connect to breadboard)
M1
133D
M2
/3
AFS-800
200
DM
0/
TL1
TL2
XY
bushing
O1
40x/0.8

Estimated mass

- Estimate 10 kg for assembly to be raised/lowered
- LS-50 limit is 5 kg each
- => right near limit but should be OK if we take steps to e.g. trim breadboard

Item	Mass [g]	Qty	Total	Comments
breadboard	3000	1	3000	10 g/mm with 300mm width, could trim
Cube-III	400	5	2000	including mirror, includes spacer-ADJ cube
spacer-ADJ	600	1	600	
scanner	700	1	700	
AFS	600	1	600	
O1	120	1	120	
O2	250	1	250	
OBLPA	150	2	300	stand-in for XY positioner
Ext-75	225	2	450	Stand-in for tubes
cube jack	75	2	150	
Tube 133D	420	2	840	
mounting	300	3	900	guess
total			9910	

Remote refocus alignment procedure Part 1

- Requires collimated light source (beam size options ~5mm and ~20mm) and shear plate
  - not included in quote but can be provided by ASI if desired
  - Procedure can be tedious but it is algorithmic
- O1/TL1
  - Shine collimated light through TL1 towards O1 and check back-reflection
  - Use O1 bushing to get collimation
  - Use M1 and O1’s XY to get non-deflection when O1 added/removed
- TL1/TL2
  - Shine collimated light through TL1 towards TL2 and check back-reflection
  - Use AFS-800 to get collimation
  - Use M2 and M3 to get non-deflection when TL2 added/removed

Remote refocus alignment procedure Part 2

- TL2/O2
  - Shine collimated light through TL2 towards O2 and check back-reflection
  - Use O2 bushing to get collimation
  - Use M4 and O2’s XY to get non-deflection when O2 added/removed
  - (do this with dichroic in place, but dichroic doesn’t need to be aligned yet)
- O2/O3
  - Shine collimated light through TL1 through system and check back-reflection
  - Move O3 in XYZ to find focus of that light

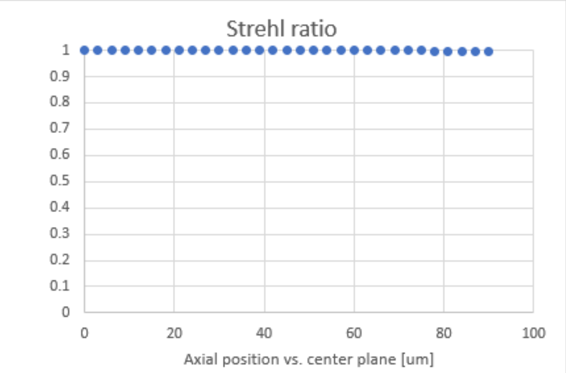
Objective alignment procedure

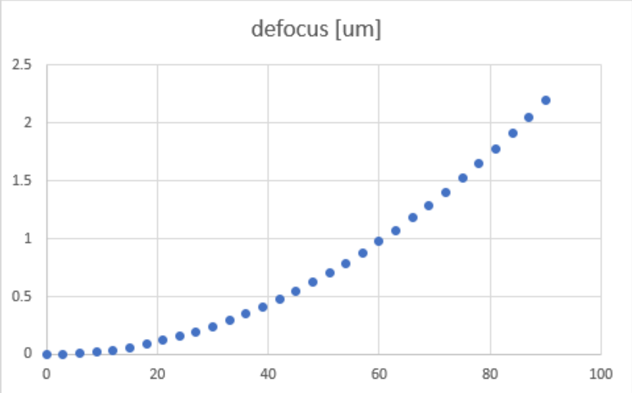
- Adjust height of objectives and lateral positions by eye, then turn on cameras with dye solution in chamber
- Use XY and Z stages to adjust 3D position of each objective

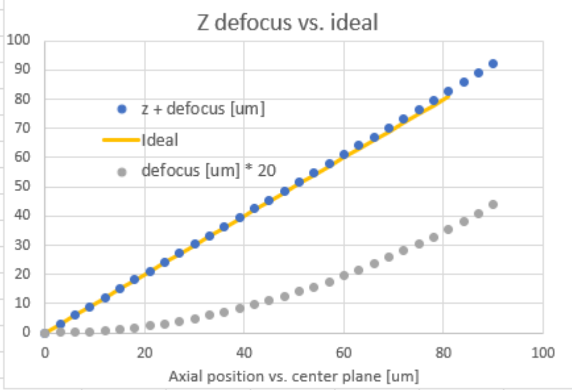
Remote refocus aberrations seem tolerable

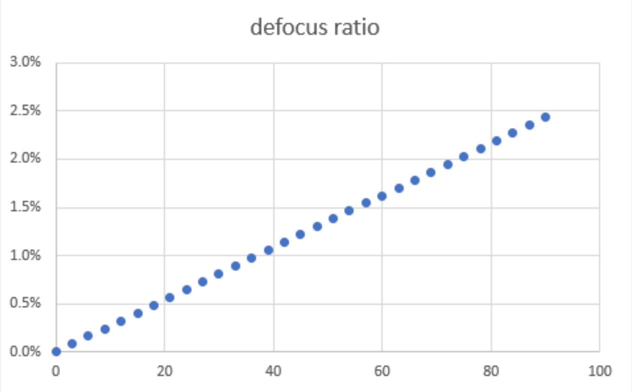
Objective NA	0.8	
Medium RI	1.333	
Objective EFL	5	mm
wavelength	510	nm
plot increment	3	um
Strehl target	0.9	
defocus target	1	um
Axial position @ Strehl target	#REF!	
Axial position @ displacement target	60.8	
alpha	0.644	rad
f	0.00667	m
k	12319971	1/m

z [um]	Strehl	Streh	defocu	defocus	z + defo	defocus [um] * 20
0	1	0%	0	0.0%	0	0
3	1	0%	0.002	0.1%	3.0024	0.05
6	1	0%	0.01	0.2%	6.0097	0.19
9	1	0%	0.022	0.2%	9.0219	0.44
12	1	0%	0.039	0.3%	12.039	0.78
15	1	0%	0.061	0.4%	15.061	1.22
18	1	0%	0.088	0.5%	18.088	1.75
21	1	0%	0.119	0.6%	21.119	2.39
24	1	0%	0.156	0.6%	24.156	3.12
27	1	0%	0.197	0.7%	27.197	3.94
30	1	0%	0.243	0.8%	30.243	4.87
33	1	0%	0.295	0.9%	33.295	5.89
36	1	0%	0.351	1.0%	36.351	7.01
39	1	0%	0.411	1.1%	39.411	8.23
42	1	0%	0.477	1.1%	42.477	9.54
					45.548	11
					48.623	12.5
					51.704	14.1
					54.789	15.8
					57.879	17.6
					60.974	19.5
					64.074	21.5
					67.178	23.6
					70.288	25.8
					73.402	28
					76.522	30.4
					79.646	32.9
					82.775	35.5
					85.909	38.2
					89.047	40.9



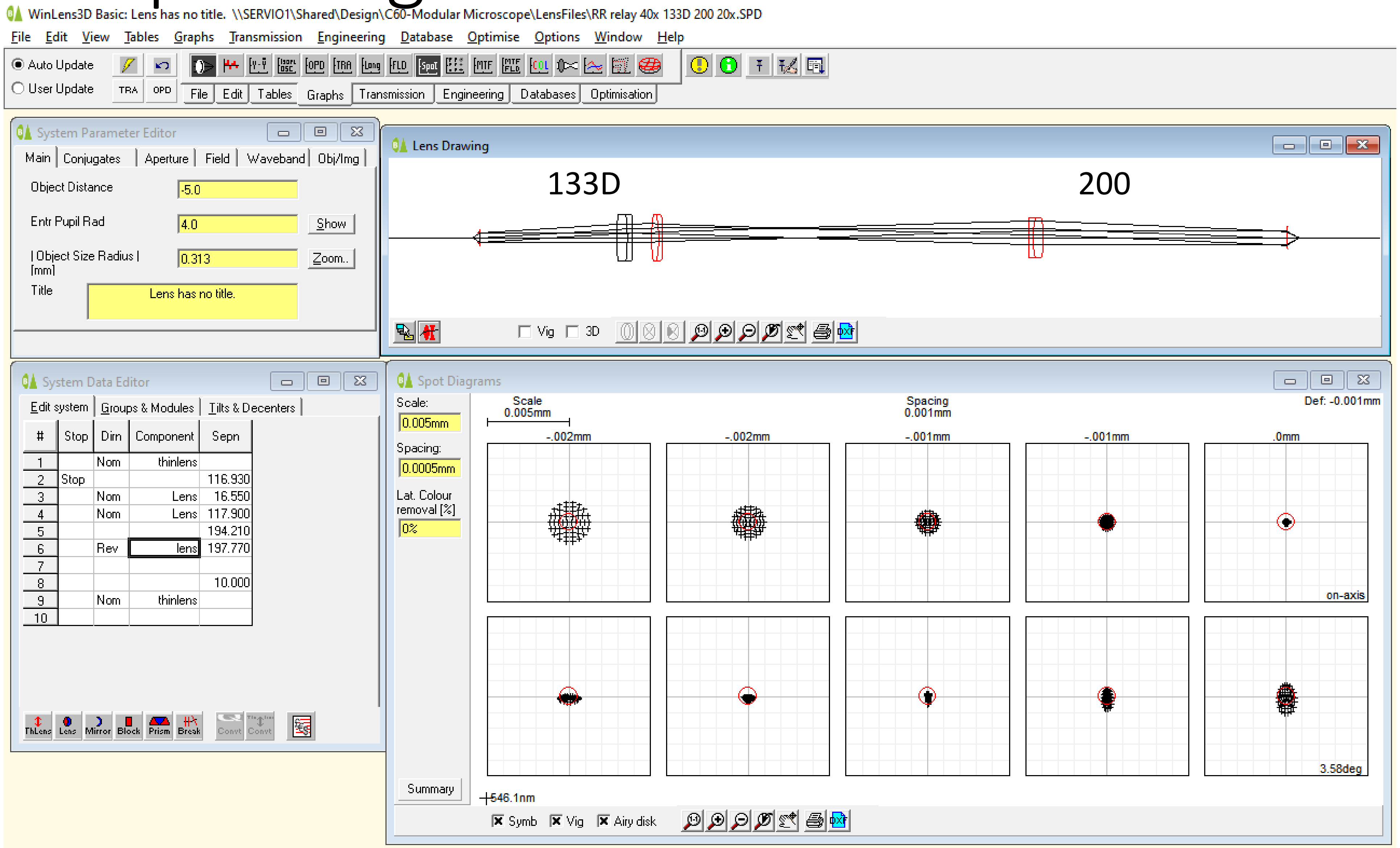






Botcherby-derived fundamental abberations with remote refocus for this situation (+/- 56um travel): Strehl ratio is excellent, ~1um axial defocus at edge of Z travel which means axial stretching varies from 0 to 1.5% in the range

Spot diagram of remote refocus



Aberrations are quite minimal, just start to vignette at objective full field of 625um diameter

New parts for ASI

- APZOBJ-150-S
  - Extension of existing product
  - Parts are already being made and should be ready to go shortly
- Tube-133D
  - Optic design done, will be ~1 month from order to make parts
- XY adjuster
  - Require adjuster akin to Thorlabs CXY1 but compatible with ASI's bushings
  - Will probably be designed for other purposes anyway
  - Have sketch, need to do full mechanical design and machine
  - Ready 6-8 weeks from when we get serious about it (an order makes it serious)