Building Bespoke Microscopes

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Bespoke Microscopes

- Why bother?

➡Specific applications

➡Flexibility

➡Cost

Bespoke Microscopes

- Why **NOT** to

➡Cost

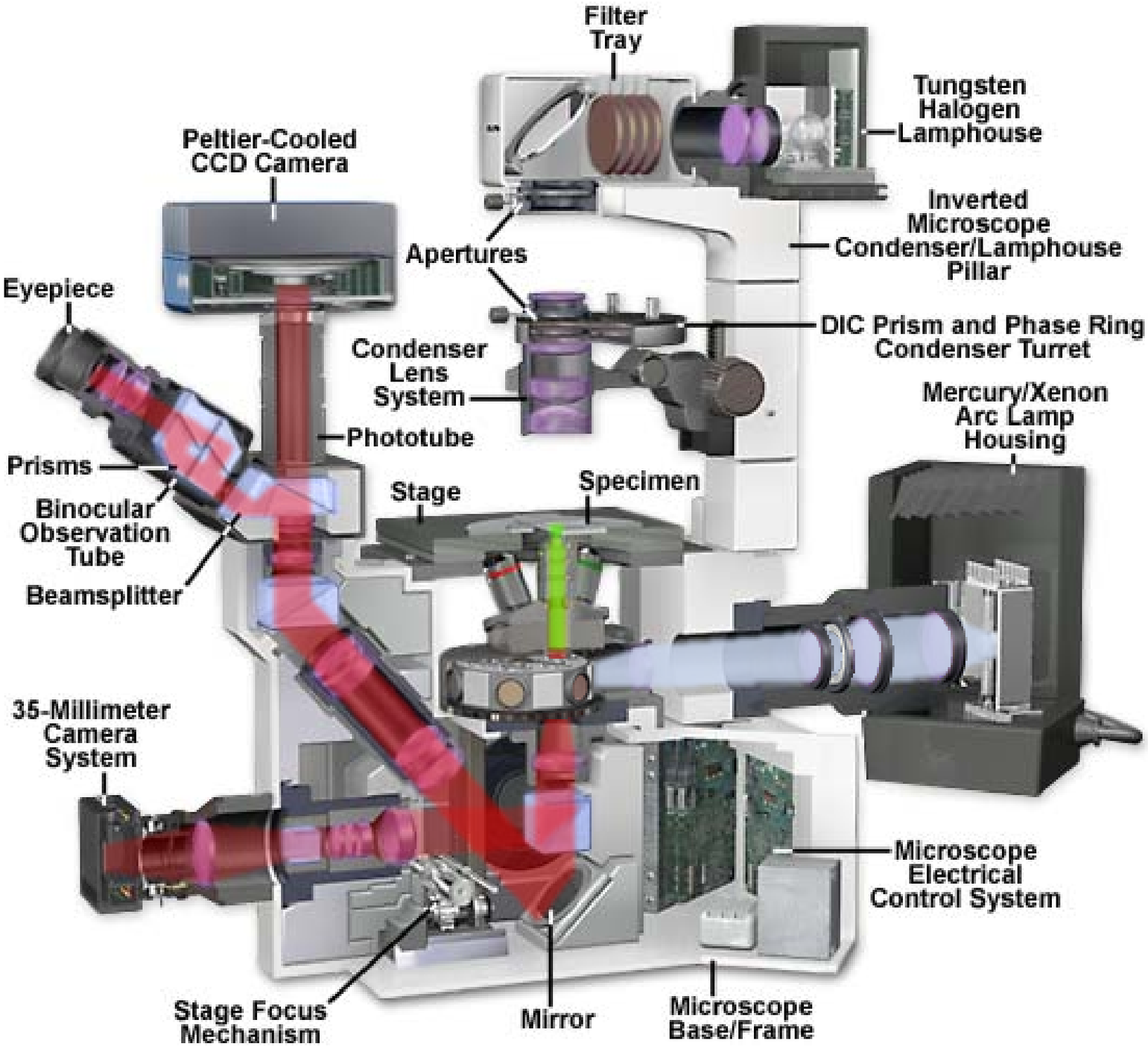
➡Time

➡Usability

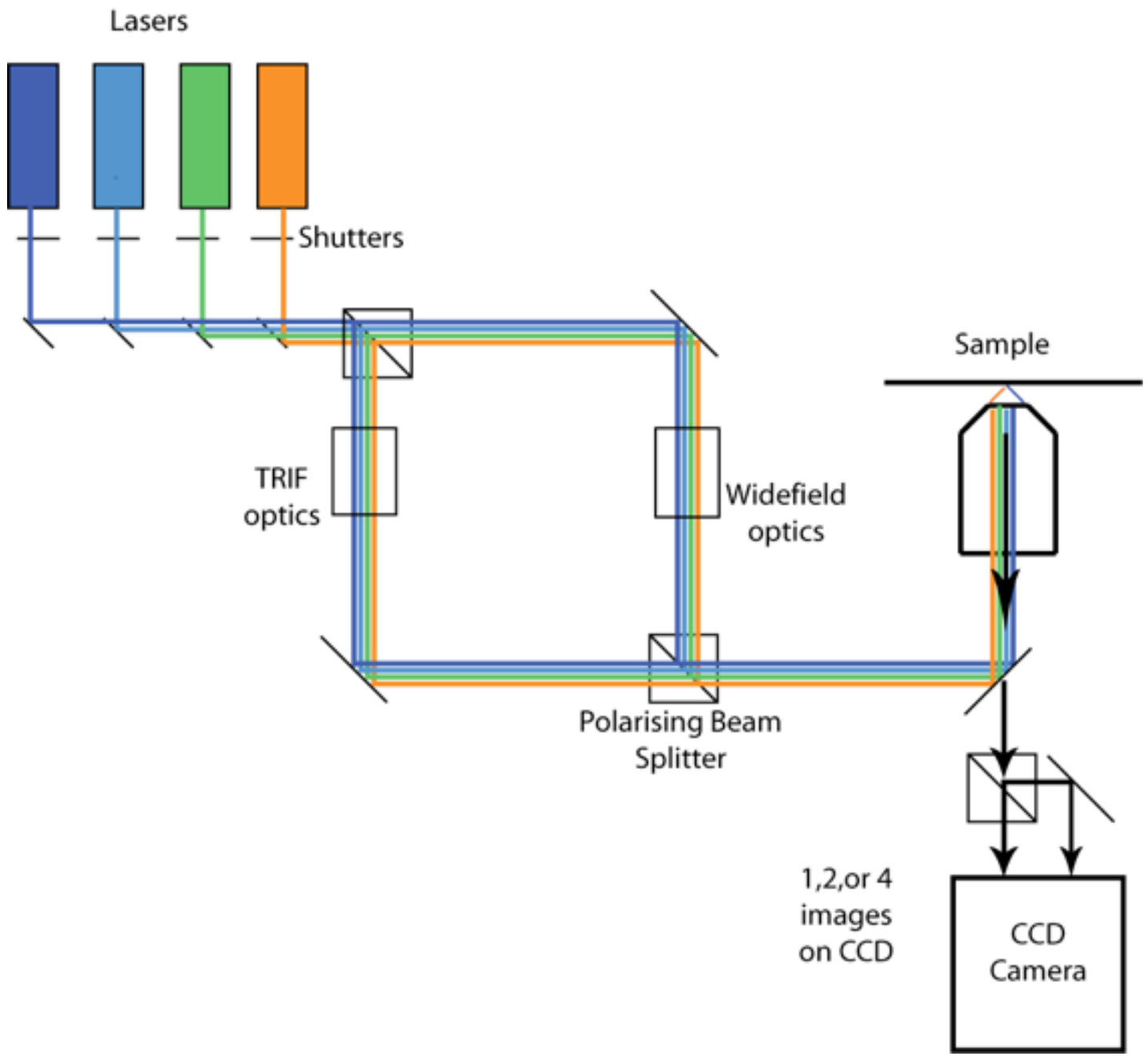
Example Bespoke Microscope

- TIRF - Slimfield Setup
- Built in the biochemistry department in collaboration with Mark Leake (physics).

Conventional microscope



TIRF microscope with split polarisations - schematic

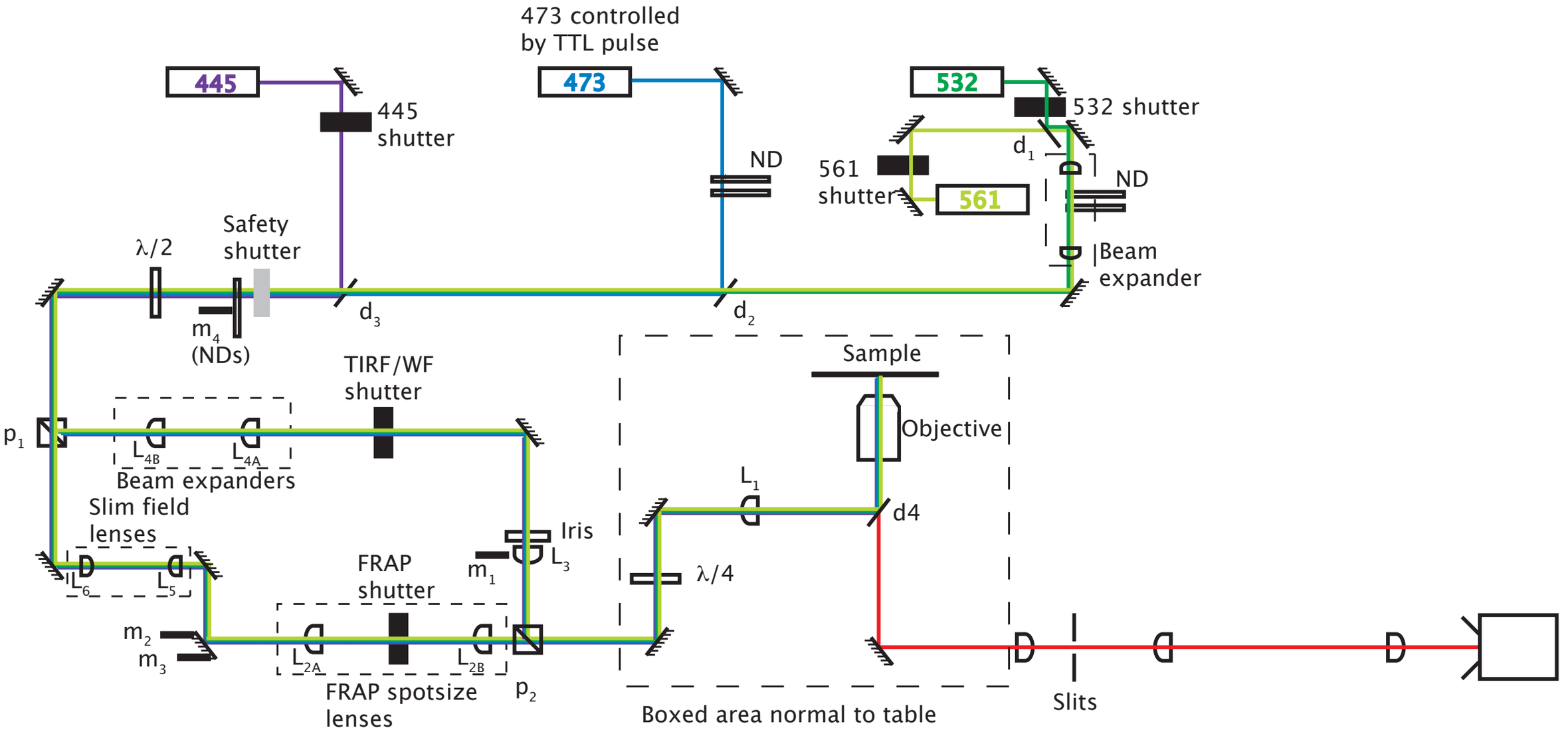


Setup for either

1. TIRF and Widefield
2. Polarisation imaging

Up to 4 images from dual colour in both polarisations

Updated Schematic



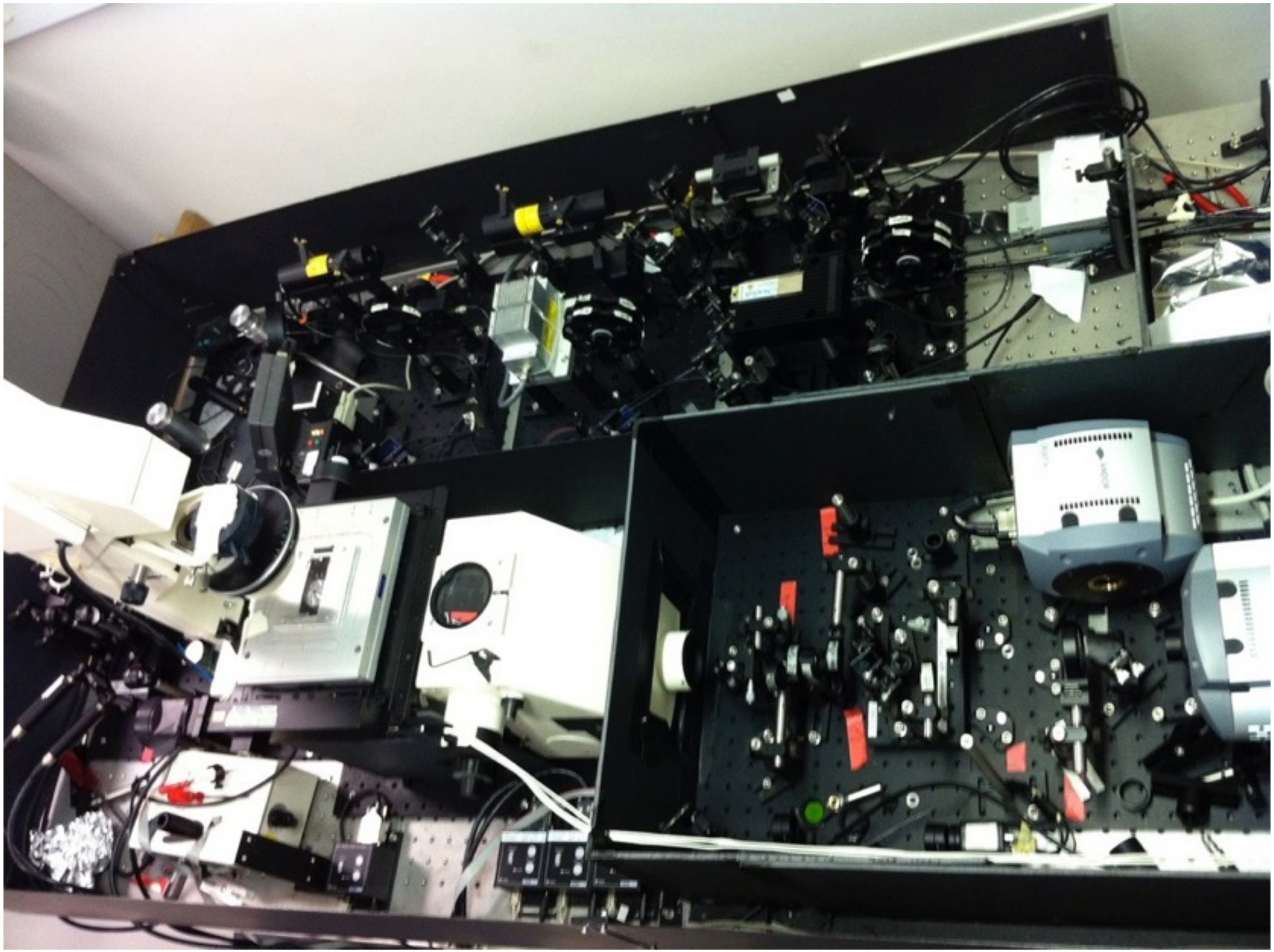
Lenses		Motors	Distances	Dichroics
L_1	f=250 mm	m_1 = TIRF angle	L_1–L_{2A} = 250 + 75	d_1 =
L_{2A}	f=75 mm	m_2 = FRAP spot X	etc....	
L_{2B}	f=200 mm	m_3 = FRAP spot Y		
L_3	f=75?? mm	m_4 = ND wheel		
L_{4A}	f=100?? mm			
L_{4B}	f=200?? mm			
L_5	f=150?? mm			
L_6	f=40?? mm			

Tirf setup

Ian Dobbie 4/1/2009

The real system

The real system II



Features of system

- Multi-colour illumination for widefield, TIRF or slimfield
  - 440, 473, 532, 561
- Dual camera - 512x512 and 128x128
- Simultaneous 2 or 3 colour imaging
- Dual polarisation on excitation and emission
- Dual excitation path for simultaneous photobleaching and imaging.

Advantages of TIRF Slimfield system

- TIRF - slimfield - widefield - FRAP
- More sensitive than commercial system.
- Speed
- Massively more flexible than commercial system.

Disadvantages of TIRF Slimfield system

- Custom written control software.
- Complication.
- Massively more flexible than commercial system.

TIRF-Slimfield system How expensive was it?

- Building costs ~ £100k (hardware)
- Time ~1 person year
- Total cost £150-200k
- Commercial TIRF system ~£150-200k

Some results:-

Signal-dependent turnover of the bacterial flagellar switch protein FliM.
Delalez NJ, Wadhams GH, Rosser G, Xue Q, Brown MT, Dobbie IM, Berry RM, Leake MC, Armitage JP.
Proc Natl Acad Sci U S A. 2010 Jun 22;107(25):11347-51. doi: 10.1073/pnas.1000284107. Epub 2010 May 24.

Positioning of chemosensory proteins and FtsZ through the Rhodobacter sphaeroides cell cycle.
Chiu SW, Roberts MA, Leake MC, Armitage JP.
Mol Microbiol. 2013 Oct;90(2):322-37. doi: 10.1111/mmi.12366. Epub 2013 Sep 9.

In vivo architecture and action of bacterial structural maintenance of chromosome proteins.
Badrinarayanan A, Reyes-Lamothe R, Uphoff S, Leake MC, Sherratt DJ.
Science. 2012 Oct 26;338(6106):528-31. doi: 10.1126/science.1227126.

Visualizing single molecular complexes in vivo using advanced fluorescence microscopy.
Dobbie IM, Robson A, Delalez N, Leake MC.
J Vis Exp. 2009 Sep 8;(31):1508. doi: 10.3791/1508.

Stoichiometry and architecture of active DNA replication machinery in Escherichia coli.
Reyes-Lamothe R, Sherratt DJ, Leake MC.
Science. 2010 Apr 23;328(5977):498-501. doi: 10.1126/science.1185757.

Example 2- DeepSIM

AIMS:

- Upright Structured Illumination System
- Multi-camera
- As fast as possible stripe generation and image collection for live SIM
- Imaging as deep as possible.

Example 2- DeepSIM

AIMS-2:

- Dual orientation, upright and inverted.
- Enable SIM and fast live imaging combined with neurophysiology.
- Fast Z imaging

DeepSIM

Results:

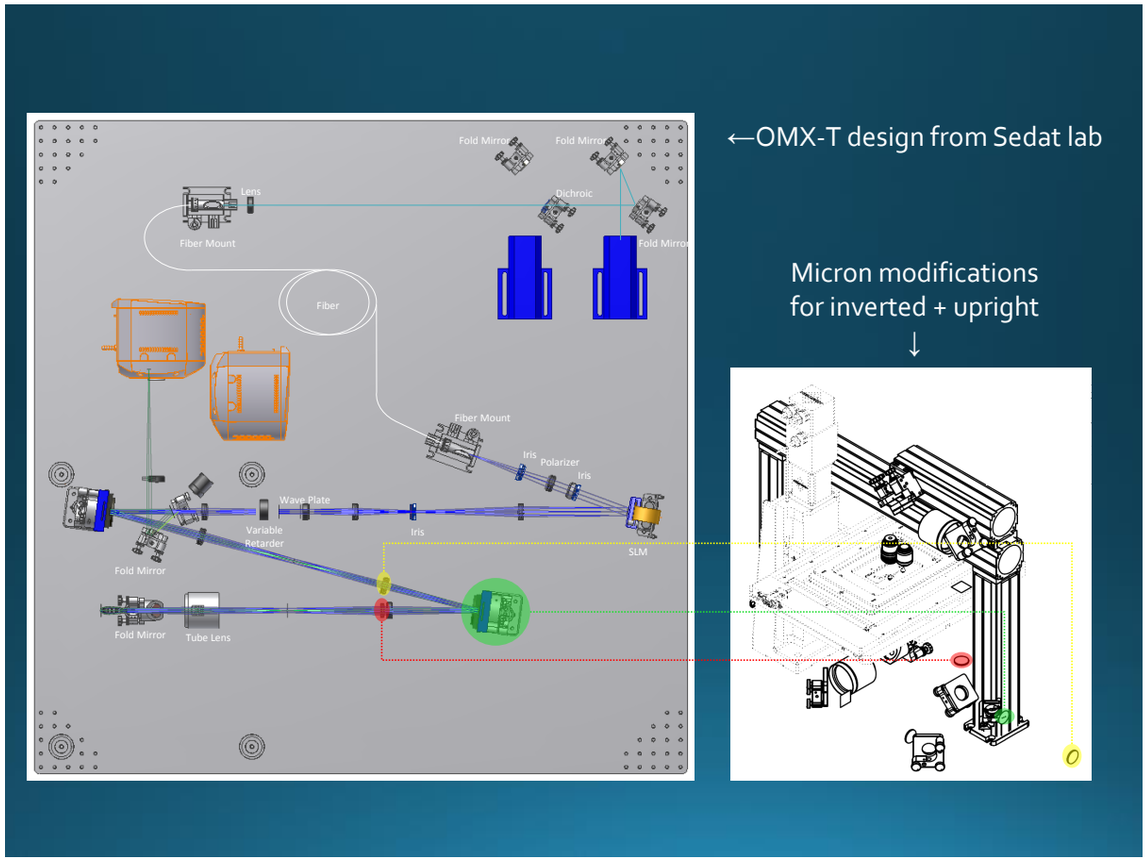
- Multi-laser (Deepstar lasers, fast switching, deep modulation)
- 4 cameras, 2 EMCCD - max sensitivity, 2-sCMOS - max speed
- SLM for pattern generation
- Flexible mirror for adaptive optics (AO) to go deeper.

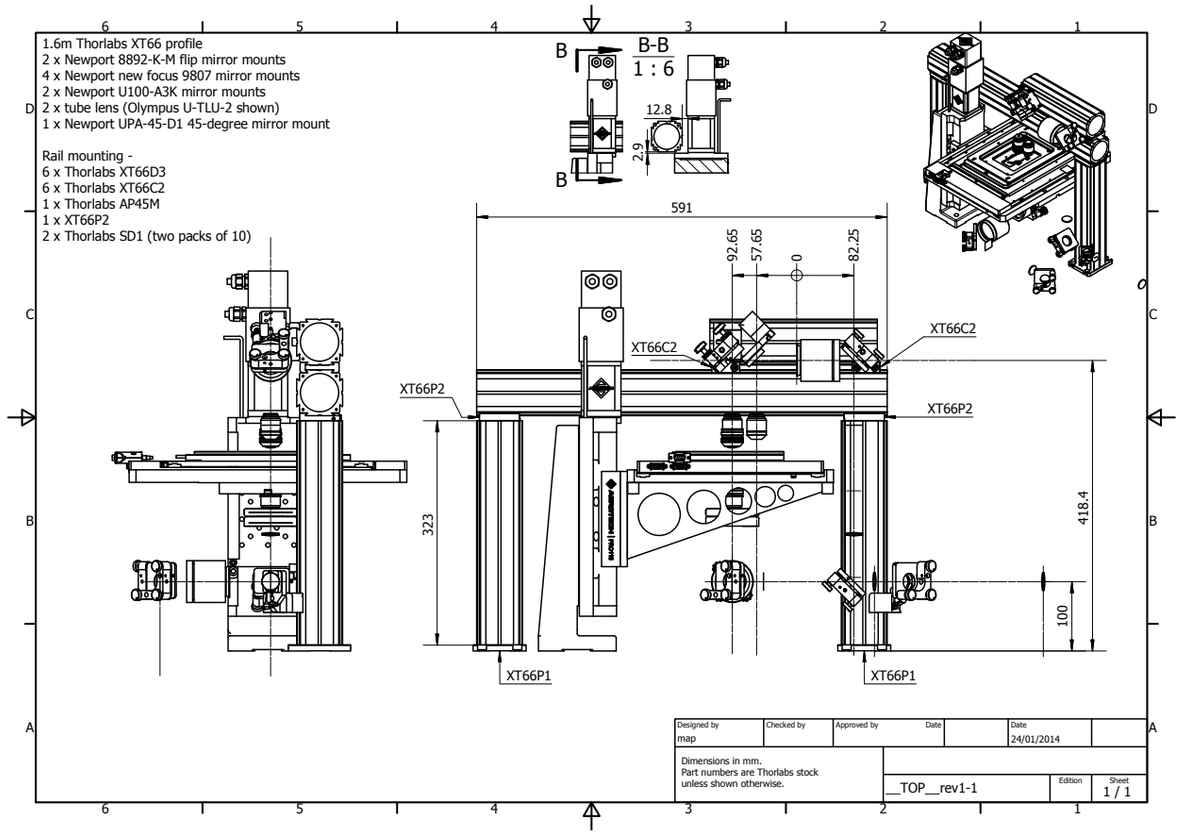
DeepSIM

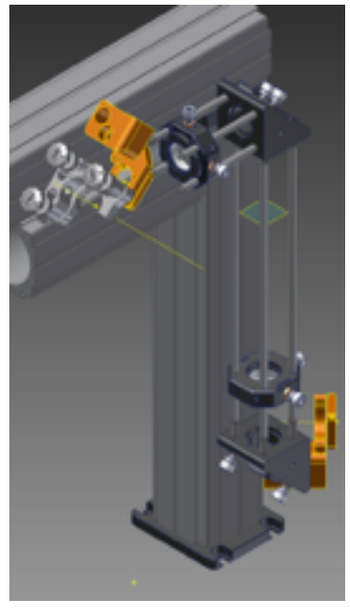
Results (2):

- AO used for fast focusing
- 3 objectives, two above, one below
- Complex control and acquisition software/ hardware.

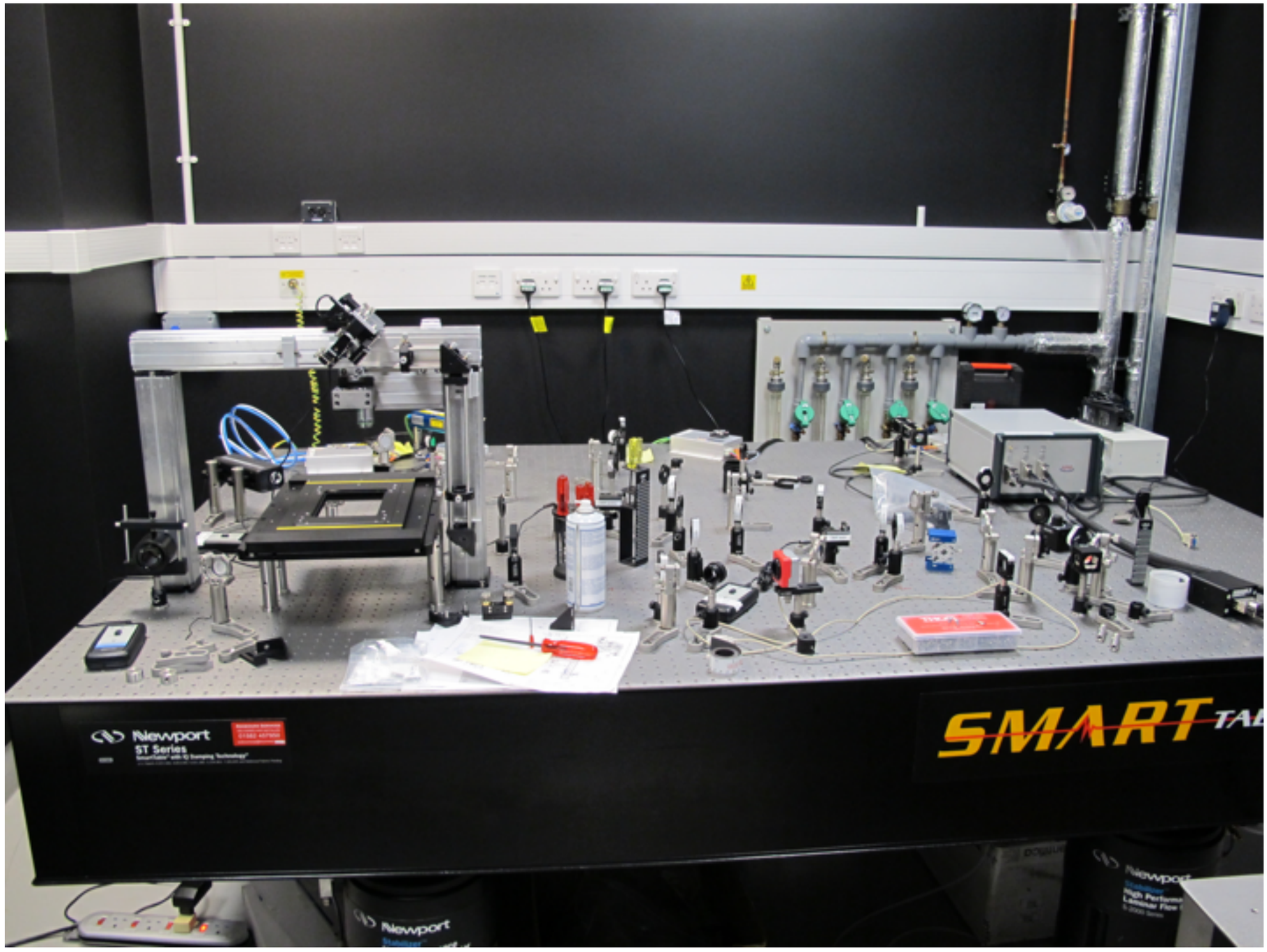
DeepSIM Design



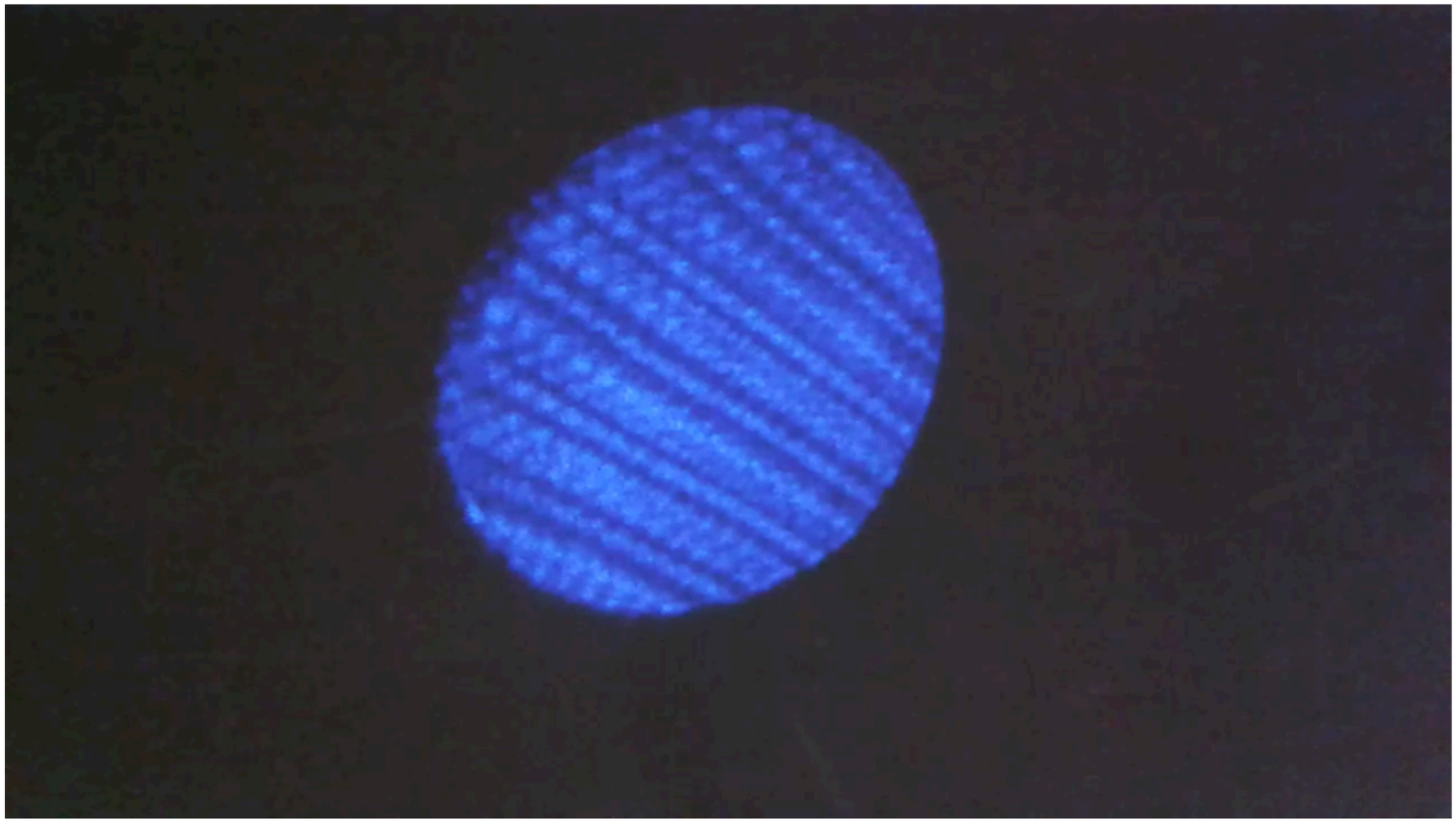




DeepSIM - the reality



SLM stripe tests



Should you build a bespoke system?

Yes!

and NO

Justification for Bespoke Systems

- Often necessary for specific specialised problems.
- Easily optimised for several parameters, speed, sensitivity etc...
- Can provide extremely flexible systems

BUT think hard as it is likely to be harder, longer and more expensive than at first thought.

So How do you do it?

- Light path in software - Zemax
- Mechanical design in CAD - AutoCad

Lenses

100mm	ThorLabs	AC254-100
150mm	ThorLabs	AC254-150
175mm	Newport	PAC061
200mm	ThorLabs	AC254-200

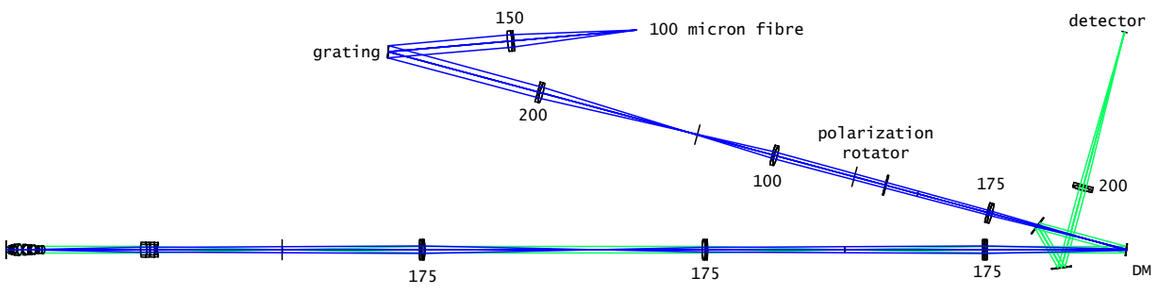
Notes

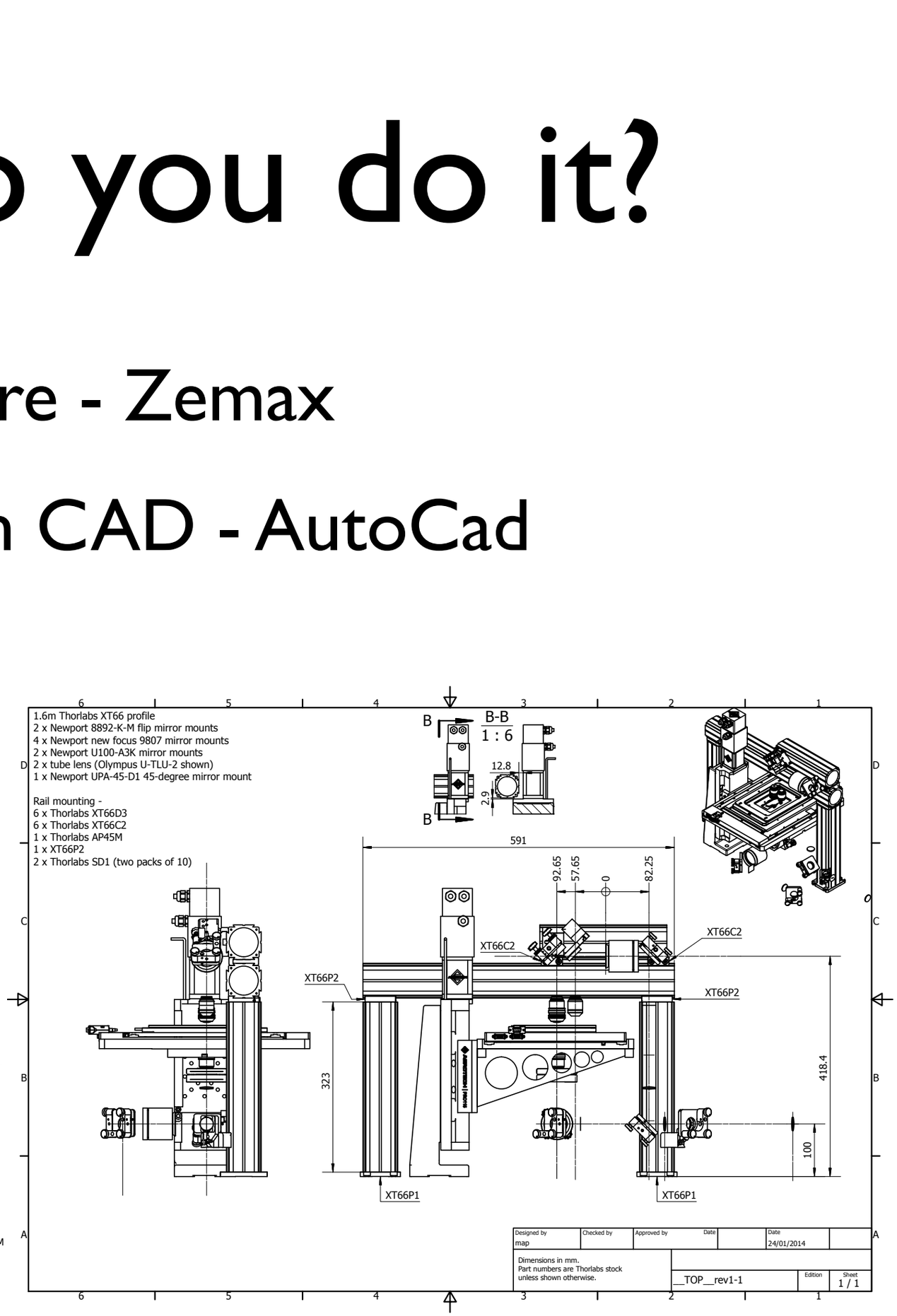
The design shows defocussing of light from a 100 micron fibre by a 150mm lens. For direct launch, the 150mm lens may be replaced with a telescope to expand the incident beam. Prior to this telescope, each beam should be treated so that they all share a similar diameter. (The Zemax design shows semi-diameters of around 7.5mm at both the 150mm achromat and the grating.)

Immediately prior to the grating, the beam is focused at infinity. This region can be extended to allow more space for flip mirrors to auxilliary paths.

The 0th-order is also infinity focused in several regions after the grating, but component separations can not be adjusted here, as changes to the path of the 1st-order beams will affect the formation of the SIM pattern.

Auxilliary paths can merge between the polarisation rotator and the first 175mm achromat. A 7mm diameter parallel beam inserted here will yield a ~45 micron spot in the object plane.





The Reality

- Rules of thumb
- Many changes as we go along
- Takes longer and costs more than expected.

A few rules of thumb

- Try and keep beam height constant
- Keep beams on X or Y axis only
- Use lenses with medium focal distances, say 25-400 mm.
- Use achromatic doublets if possible
- Setup lenses in a 4f arrangement.

A few rules of thumb

- For alignment have 2 mirrors for every path.
- leave space for irises/pinholes