From Eye to Insight

Values of Microscope Objectives

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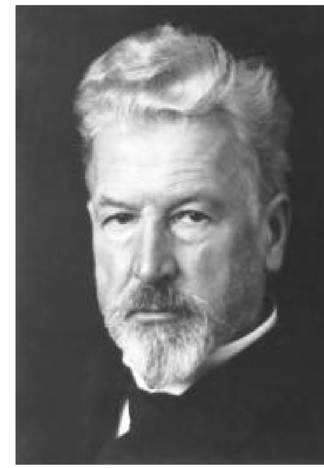
21.02.2017

Company History

- 1849 Carl Kellner's Optical Institute founded in Wetzlar, Germany
- 1869 Ernst Leitz takes over the company and changes its name to Ernst Leitz
- 1921 Establishment of Wild Heerbrugg in Switzerland
- 1972 Partnership between Leitz Wetzlar and Wild Heerbrugg
- 1986 Cambridge Instruments acquires Reichert-Jung
- 1986 Establishment of Wild Leitz Group
- 1990 Wild Leitz and Cambridge Instruments merge to form the Leica AG
- 1998 Leica Camera, Leica Microsystems, Leica Geosystems (previously Leica Group) become three independent companies
- 2005 Danaher Corporation acquires Leica Microsystems
- 2013 Leica Biosystems becomes independent company within Danaher Corporation
- today Leica Microsystems has ~2900 employees



Ernst Leitz
“With the user, for the user“

Customer Focus

Key Customer Groups

Major Product Groups

Life Science Research Laboratories & Universities

- Imaging solutions for life scientists researching in e.g. cancer, neuronal diseases or life-style-related diseases
- Specimen preparation for imaging at the nanoscale and correlative information between light and electron microscopy
- High throughput imaging, analysis and database management for connectomics and brain function research

Hospitals and Clinics

- Premium microscopes for neurosurgery, ophthalmology, plastic and reconstructive surgery, and ENT
- Digital imaging solutions including fluorescence, intrasurgical and handheld OCT, surgical guidance and 3D
- Routine clinic microscopes for dentistry and ENT

Industry, Material Research Centres, Scientific Institutions, Schools, Universities, Forensic Labs

- Imaging solutions for Industry & Education
- Leading ergonomic solutions for production facilities from automotive to watchmaking
- Stereo and Compound Microscopes for material and geosciences
- Forensic Microscopes
- State-of-the-art tools for quality control laboratories

Product Portfolio














Large portfolio of objectives

HC PL APO CS2 20x/0.75
HC APO L 20x/0.95 IMM
HC APO L 20x/1.00 W
HC FLUOTAR L 25x/0.95 W VISIR
HC FLUOTAR L 25x/1.00 IMM VISIR
HC IRAPO L 25x/1.00 W
N PLAN L 32x/0.40
HI PLAN I 40x/0.50
N PLAN L 40x/0.55
HC PL FLUOTAR L 40x/0.60
HI PLAN 40x/0.65
N PLAN 40x/0.65
HC PL APO 40x/0.75
HC PL FLUOTAR 40x/0.75
N PLAN 40x/0.75
N PLAN EPI 40x/0.75
HC APO L U-V-I 40x/0.80 W
HC PL APO 40x/0.85
HC PL APO CS2 40x/1.10 W
HC PL IRAPO 40x/1.10 W
HC PL APO 40x/1.10 W
ACS APO 40x/1.15 OIL
HC PL APO 40x/1.25 OIL
HC PL APO 40x/1.30 OIL
HC PL APO CS2 40x/1.30 OIL
HC PL FLUOTAR 40x/1.30 OIL
N PLAN H 50x/0.50
N PLAN L 50x/0.50
HC PL FLUOTAR L 50x/0.55
N PLAN EPI 50x/0.75
HC PL FLUOTAR 50x/0.80
HC PL APO 50x/0.85
N PLAN 50x/0.85
HC PL APO 50x/0.90
N PLAN 50x/0.90
HC PL FLUOTAR L 63x/0.70
HI PLAN 63x/0.75
N PLAN 63x/0.80
HC PL FLUOTAR 63x/0.90
HC APO L U-V-I 63x/0.90
HC APO L U-V-I CS2 63x/0.90
HC PL APO CS2 63x/1.20
HC PL APO UVIS CS2 63x/1.20
HC PL FLUOTAR 63x/1.25
ACS APO 63x/1.30

- Over **200** objective lenses
- About **100** individual optical designs

What is the value of a microscope objective?

Monetary value

Entry level

Price factor 20x

High end

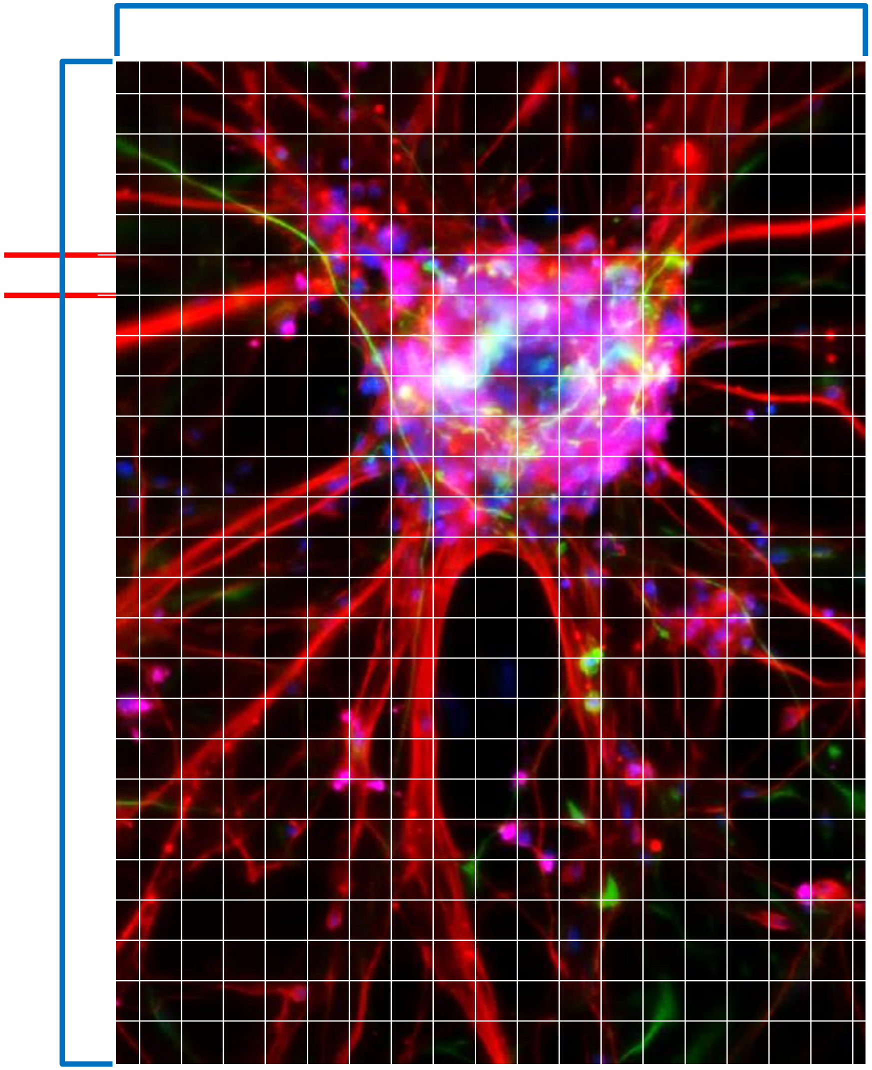
True value

- Interface between specimen and imaging system
- Basically, only an appropriate objective enables the experiment
- Can be quantified by a number of values
  - Magnification / field size, field correction / OFN, numerical aperture
  - Free working distance , chromatic correction range, transmittance, …
  - Increasing most of these numbers also increases the monetary value

What is the value of a microscope objective?

Primary features:

- Field size (Magnification, OFN)
- Resolution (Numerical aperture)
- Chromatic correction



Secondary features:

- Free working distance
- Correction collar
- Transmittance

Customer demands

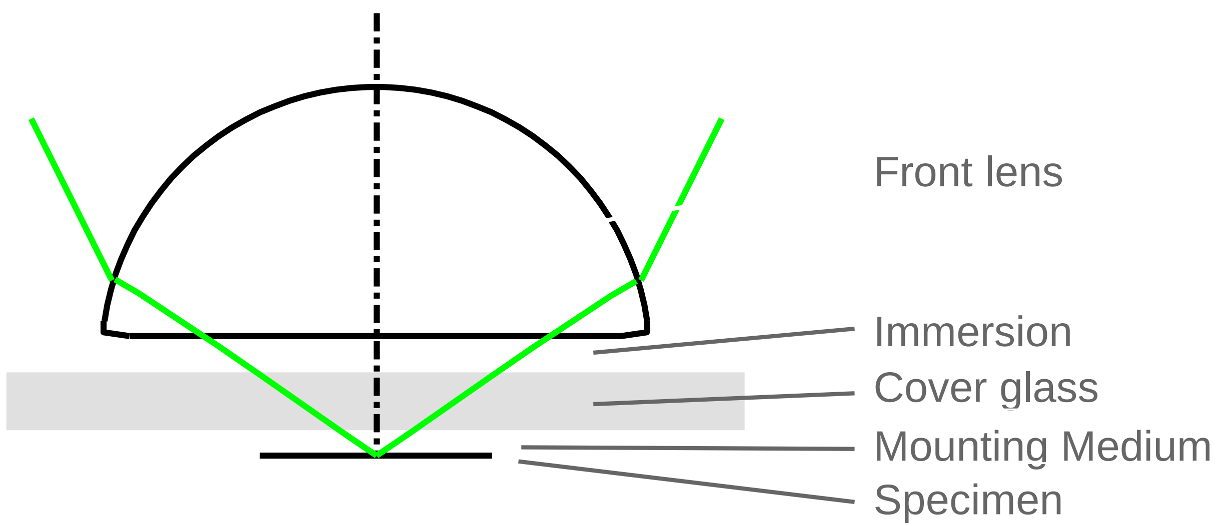
Example: HCX APO L 20x/1.00 W

- Live cell imaging, investigate functional dependencies
  - large field of view: 1.25 mm (eyepieces), 0.95 mm (on camera)
  - large numerical aperture → Airy radius: 333 nm
    → large pupil size (20 mm)
- Sample in aqueous solution held in dishes
  - electrophysiology → ceramic front lens mount
  - large free working distance: 1.95 mm
- High signal to noise for fluorescence imaging
  - high transmittance across range 400-700 nm
  - low autofluorescence of used glasses and optical adhesives

Interface to the object

The properties of object space have a serious impact on the imaging quality and can result in loss of contrast. Especially high-NA objectives are very sensitive.

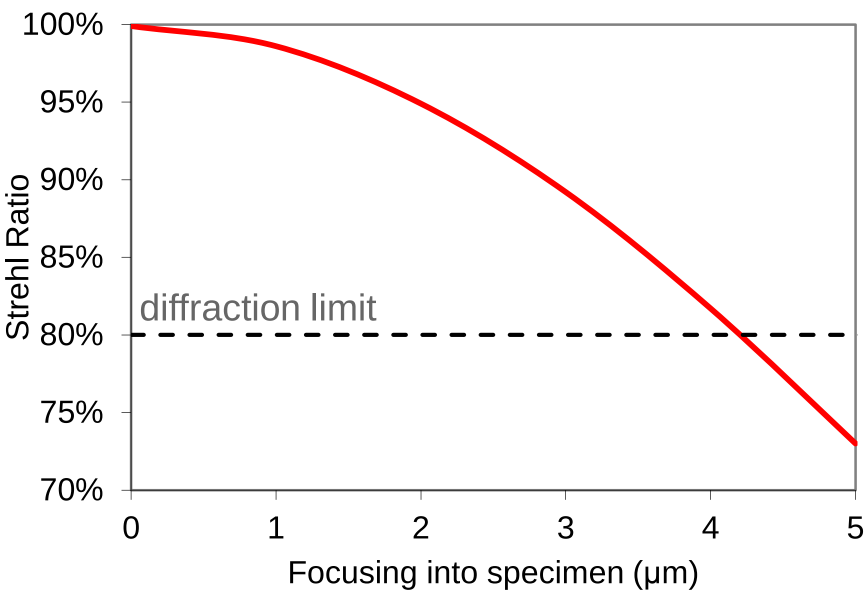
How sensitive are they?



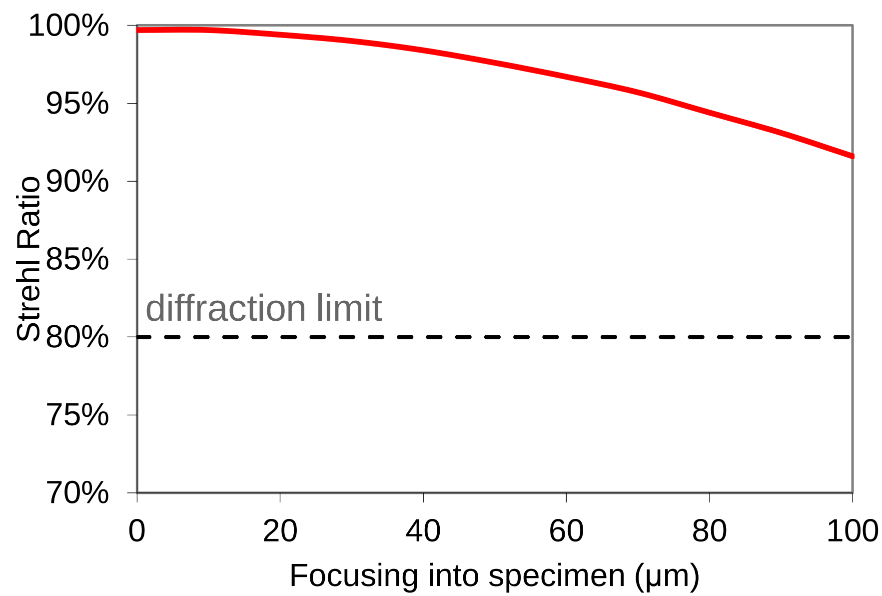
Application tolerances

Mismatch of immersion and mounting medium

Focusing an oil objective into glycerol
(NA = 1.40)



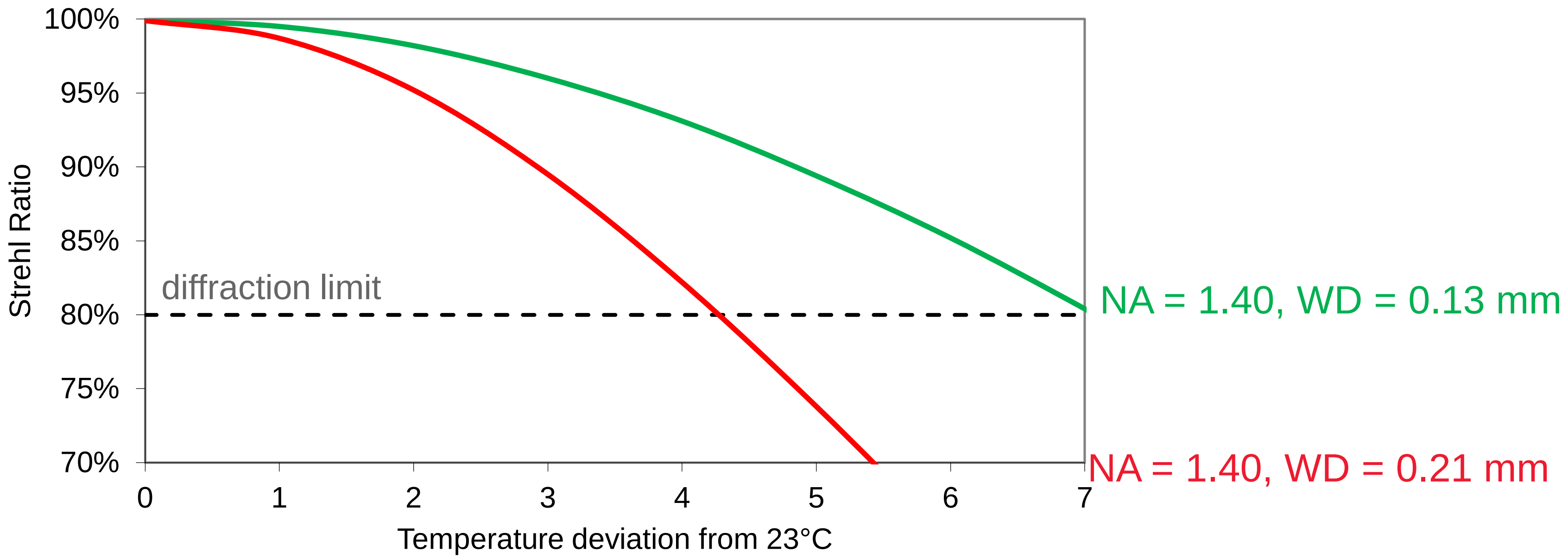
Focusing a dry objective into water
(NA = 0.50)



Application tolerances

Immersion temperature

The refractive index of immersion oil is very sensitive to changes in temperature.

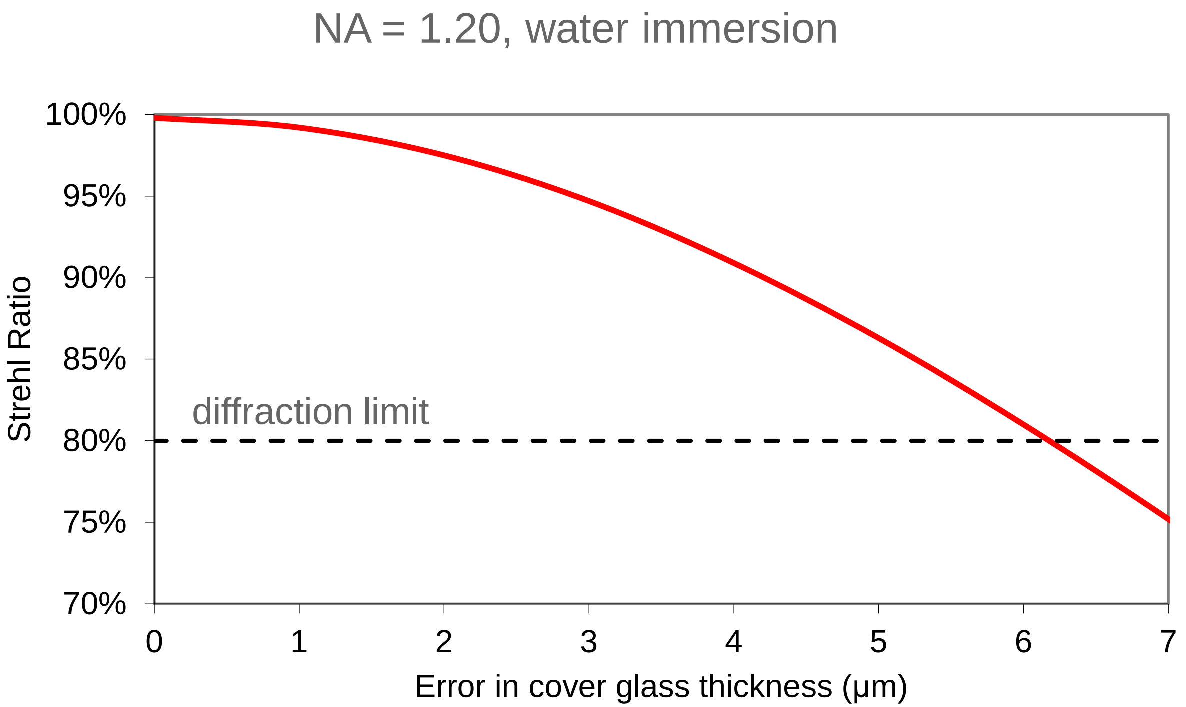


Application tolerances

Cover glass thickness

Sensitivity to cover glass thickness in descending order: Dry, water, glycerol objectives

→ Use objectives with correction collar.



Application tolerances (summary)

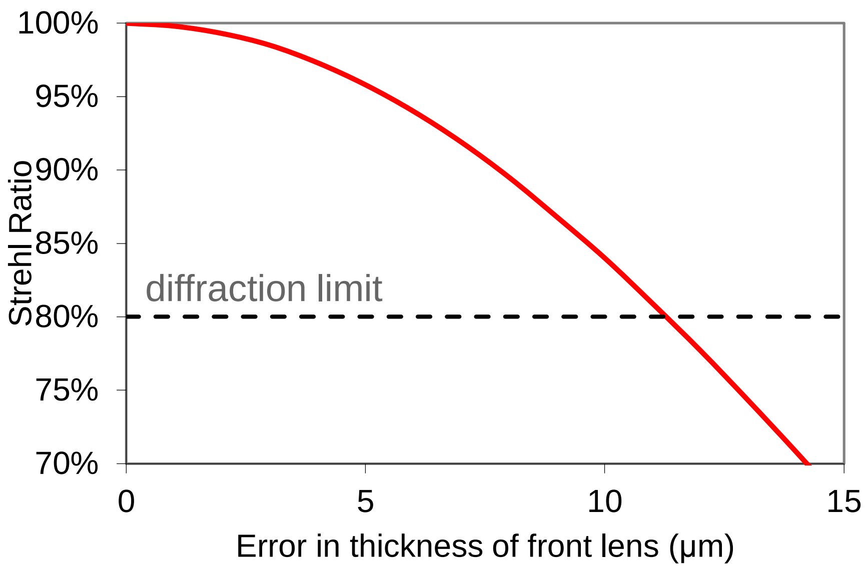
- Imaging performance depends strongly on the properties of object space

- Recommendations for users
  - Use the appropriate immersion and/or mounting medium, or a multi-immersion objective
  - If the above is impossible to achieve, use a low-NA objective
  - A large working distance is not always a good performance indicator when selecting an oil-immersion objective
  - Use the highest cover glass quality: No. 1.5H (0.170mm ±0.005mm)
  - Use objectives with correction collar

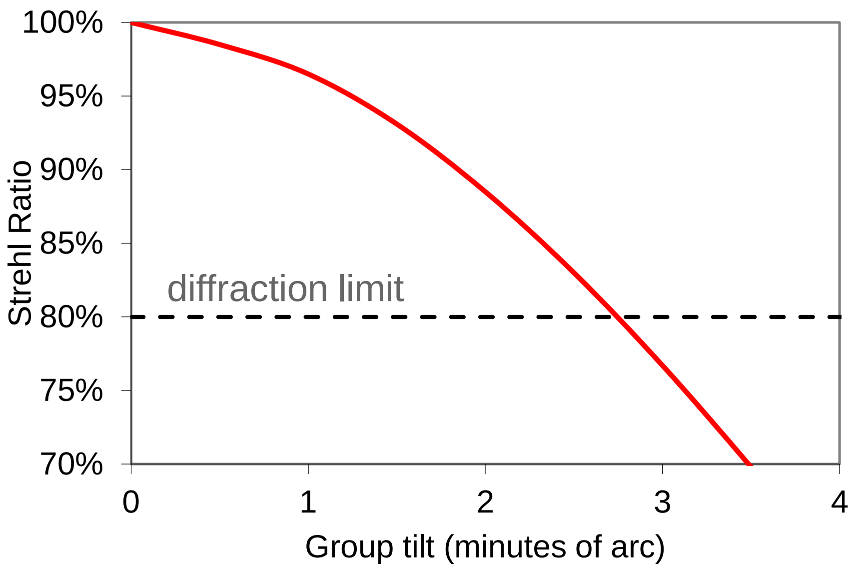
Design tolerances

Manufacturing imperfections

Wrong lens thickness
(PL APO 63x/1.40 OIL)



Lens group tilt
(PL APO 63x/1.40 OIL)

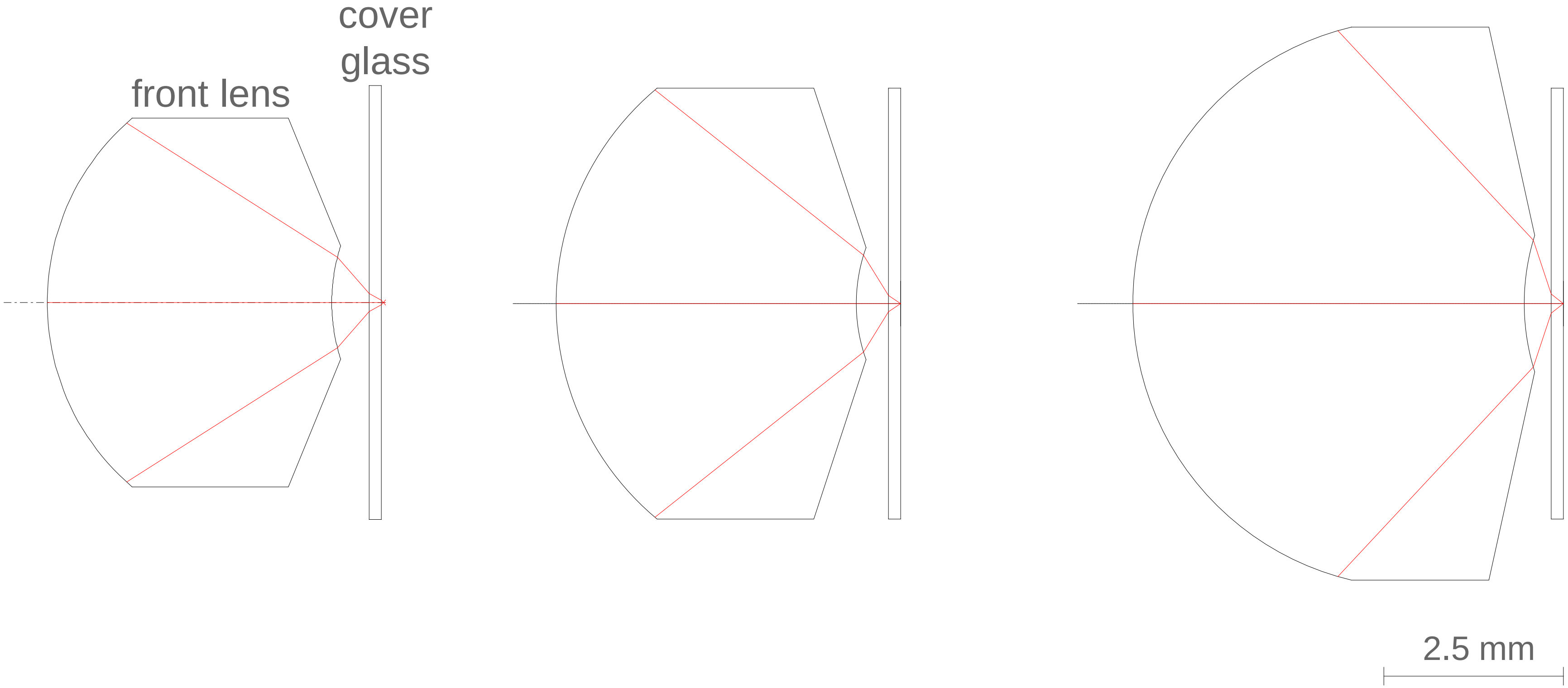


Design Challenge: high NA optics

NA = 0.75 (f/0.67)
Marginal ray angle 49°

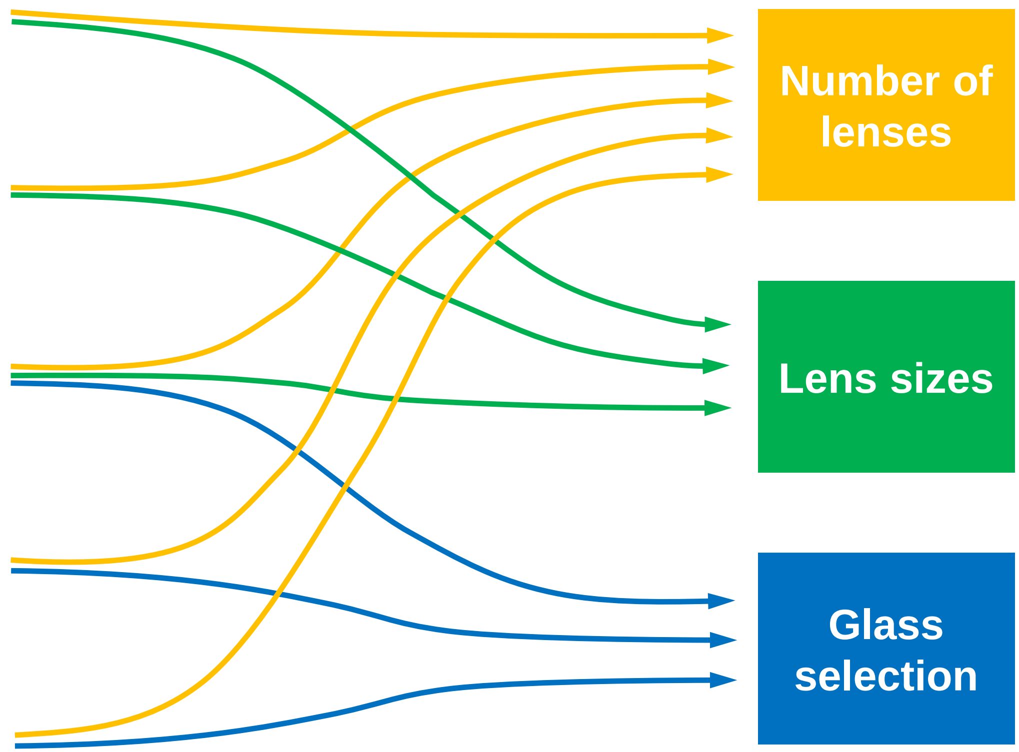
0.85 (f/0.59)
58°

0.95 (f/0.53)
72°



Design targets and implications

- Numerical aperture
  - → Resolution
  - → Light gathering power
- Free working distance
  - → Manipulation of specimen
  - → Focusing into specimen
- Field size and field performance
  - → Size of specimen
  - → Nature of specimen
- Chromatic correction range
  - → Polychromatic imaging
  - → Fluorescence imaging
- Transmittance
  - → UV Fluorescence excitation



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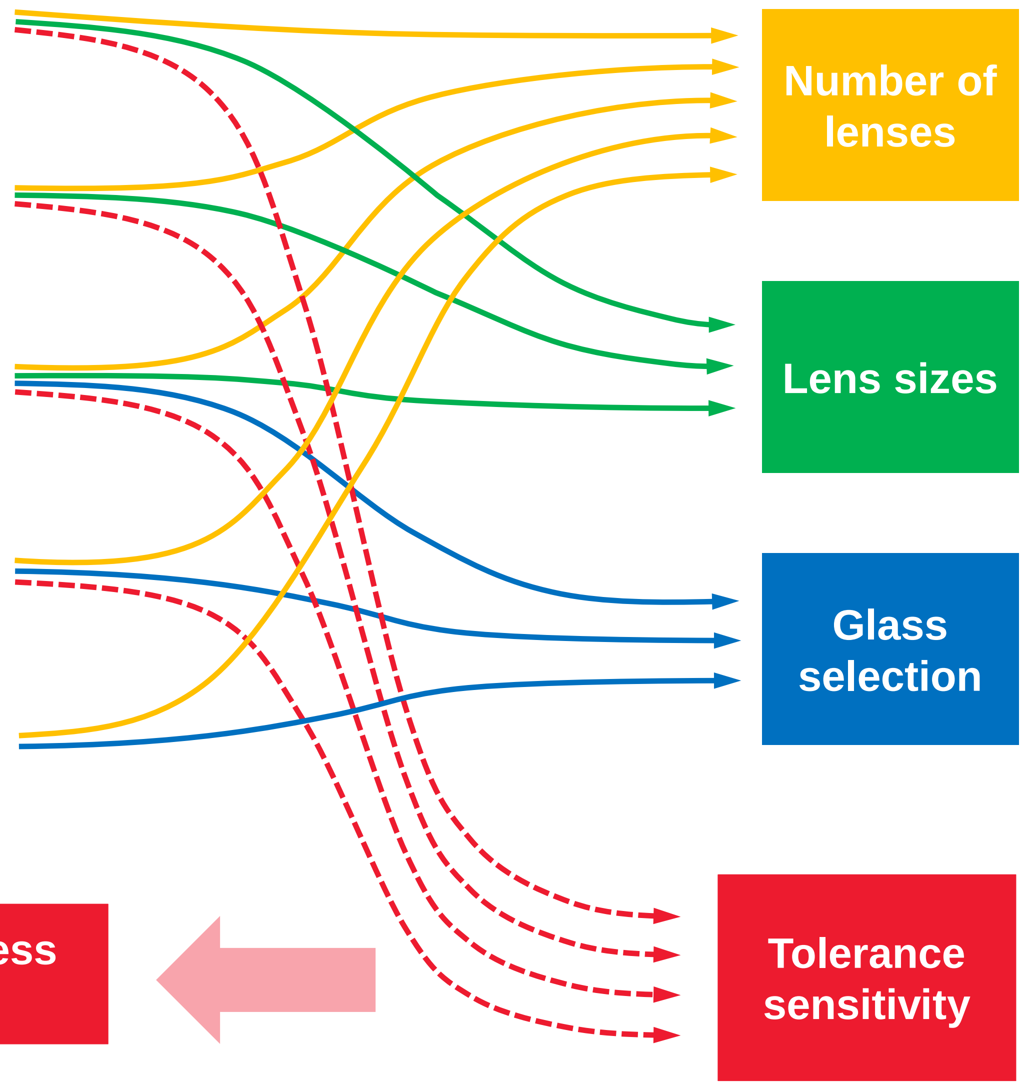


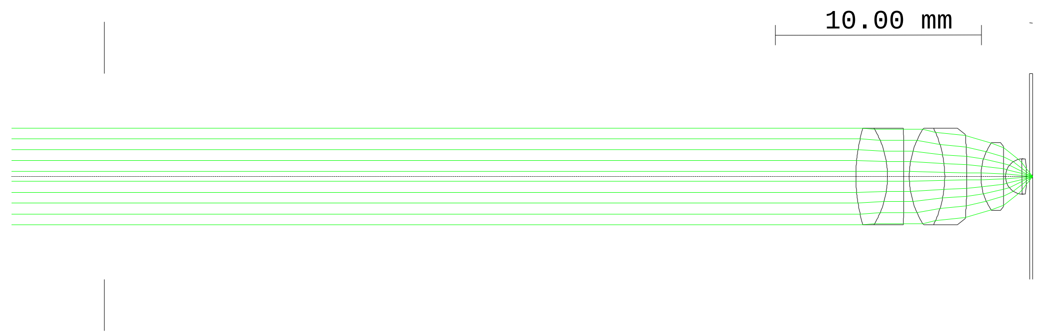
Sustainable production process and product quality

Example: Flatness of field

- More and larger lenses
- Tighter tolerances

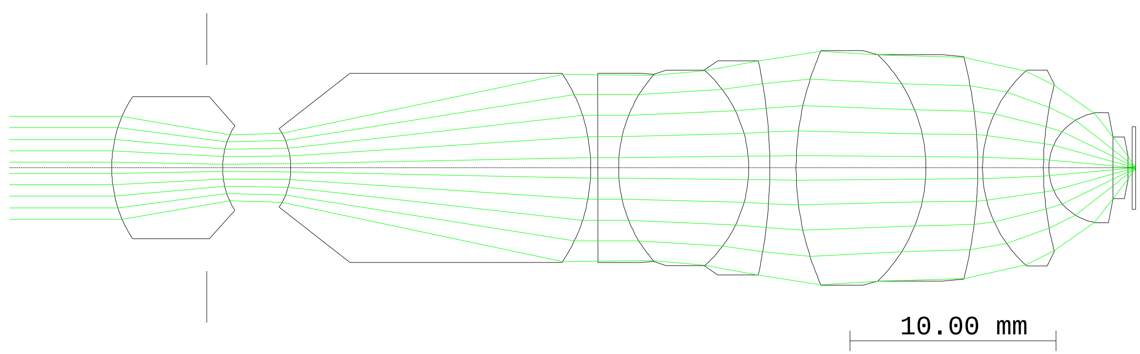
EF 100/1.25 OIL

Flat to OFN7



N PLAN 100x/1.25 OIL

Flat to OFN20

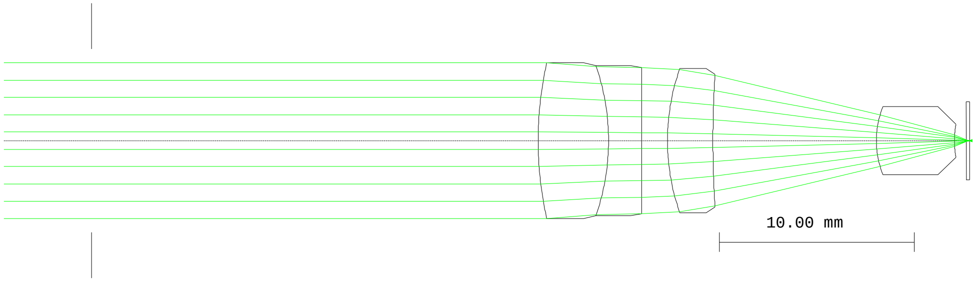


Example: Working distance

- More and larger lenses

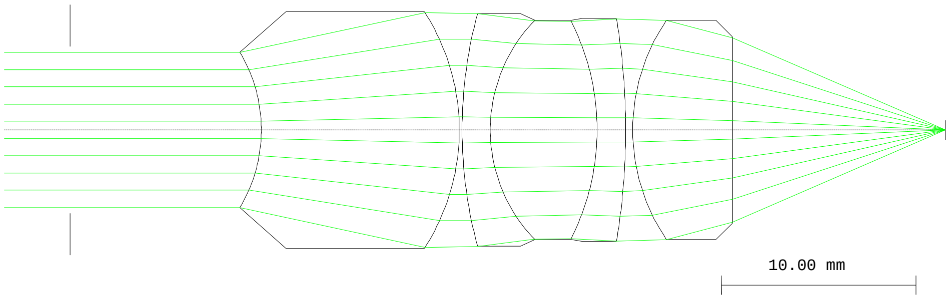
N PLAN 20/0.40

WD of 0.5 mm



N PLAN **L** 20/0.40

WD of 11 mm



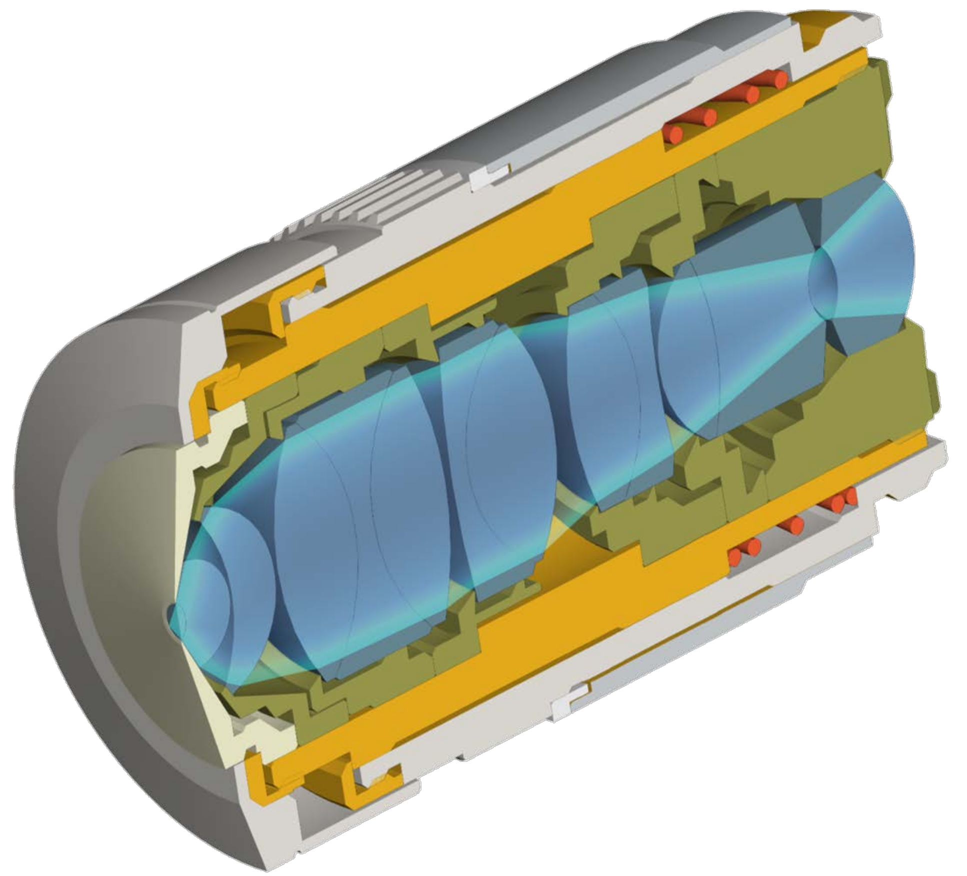
Design targets and implications

Technical requirements:

- Up to 15 lens elements in 50 mm length
- Lens surface deviation from ideal shape less than 30 nm
- Lens thickness tolerances down to 5 µm
- Air space tolerances down to 10 µm
- Glass tolerances
  - absolute error in index of refraction 10^{-4}
  - relative error in index of refraction 10^{-5}

- Maximum element decenter of 3 µm
- Maximum element tilt of 1’

- Even such tight tolerances require adjustment steps in final assembly



PL APO 100x/1.40 OIL

The microscope objective production process

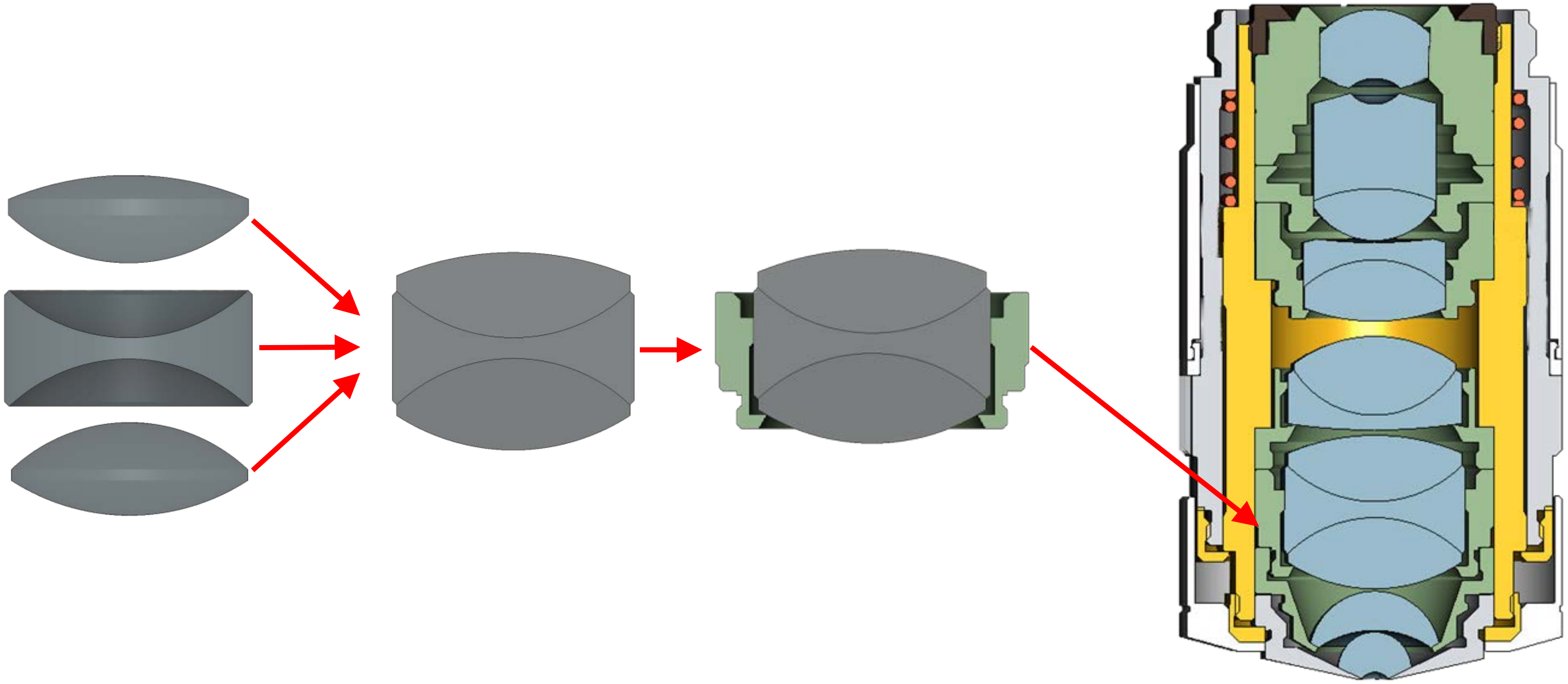
Leica MICROSYSTEMS

Single lens manufacturing

Multiplet

Mounting

Final assembly and adjustments



Leica MICROSYSTEMS

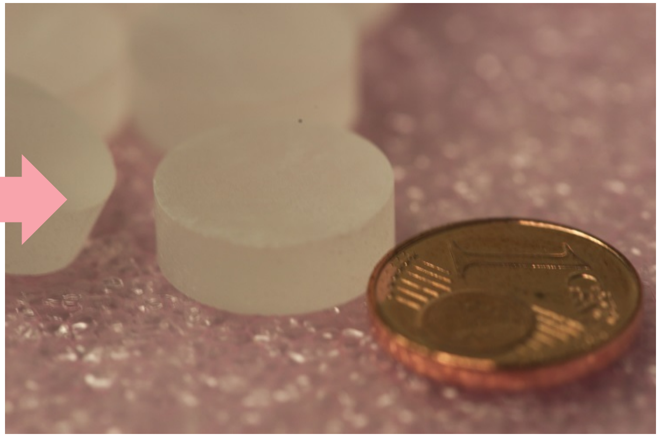
Cutting, Grinding and Polishing

- Starting material:
  Raw glass block

- Targets (single lens):
  - Surface deviation within tolerance
  - Lens thickness within tolerance

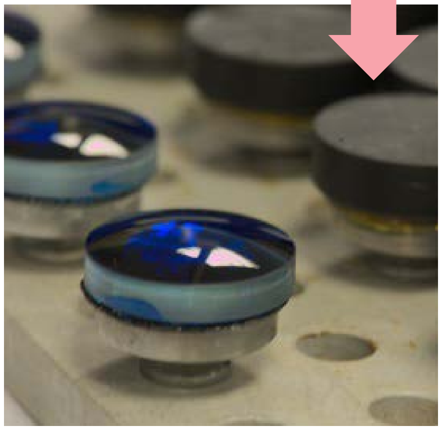
Hollow drill

Cutting, Grinding and Polishing



Diamond saw

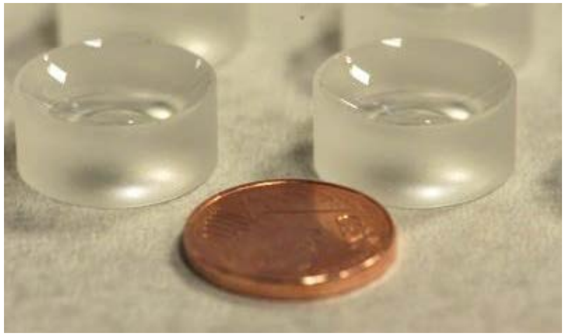
Grinding and polishing machines, occasionally finishing by hand necessary

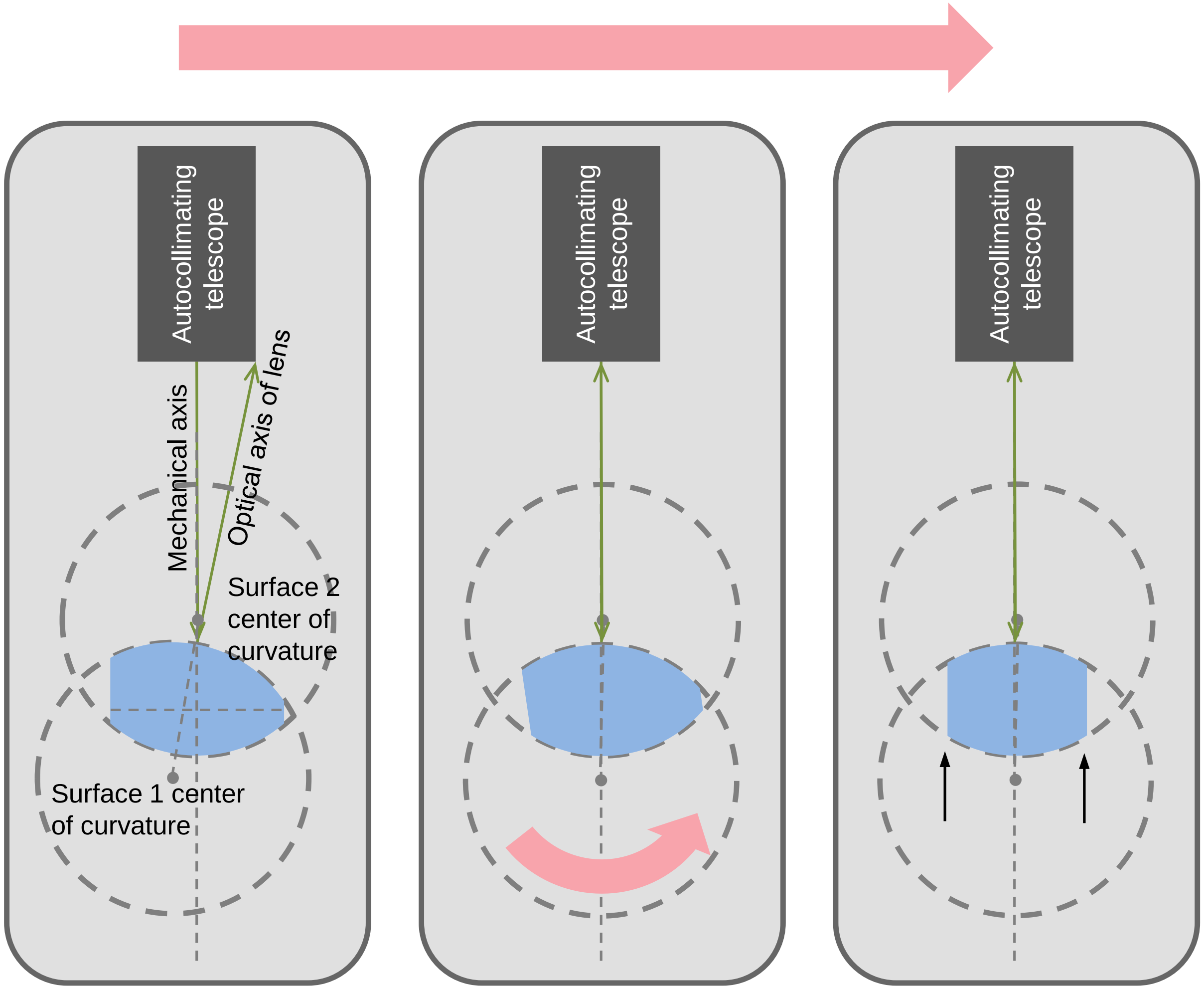


Front lenses in high-aperture objectives

Single lens centering

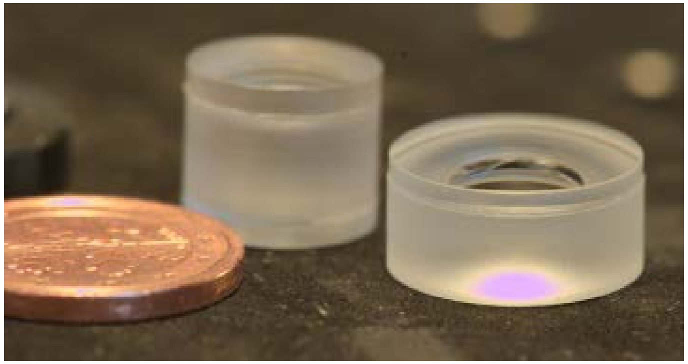
- Starting material:
  Unmounted single lenses
- Target: Max. surface tilt: 1’
- Methods & Tools:
  - Autocollimating telescope
  - Computer-aided analysis
  - Pivot mounting system and grinding machine

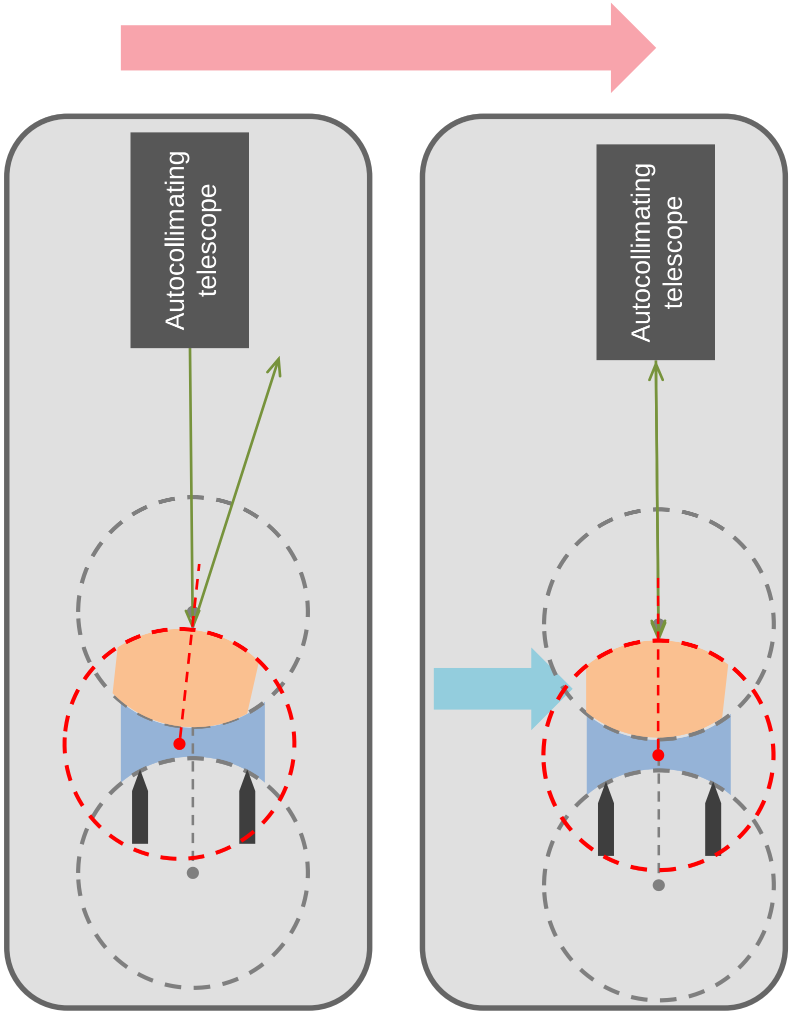




Cementing

- Starting material:
  Unmounted single lenses (at least one centered)
- Target: Max. surface tilt: 1’
- Methods & Tools:
  - Autocollimating telescope
  - Computer-aided analysis
  - Fine-adjustment manipulation system



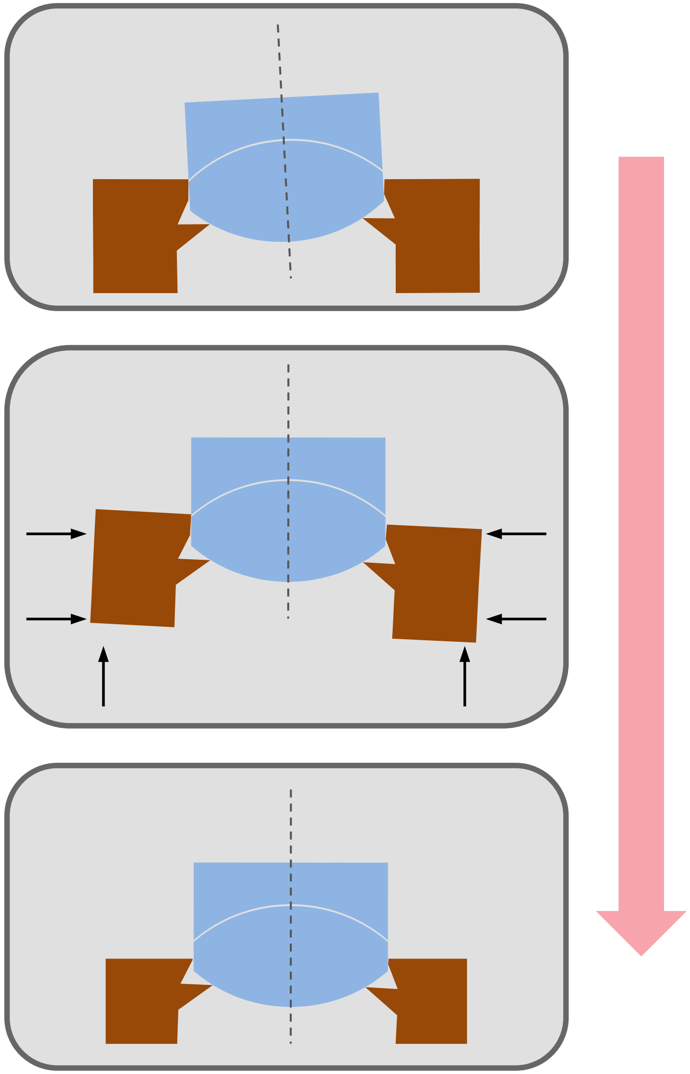


Mounting & Centering

- Starting material:
  Unmounted single lenses or cemented multiplets

- Targets:
  - Max. lateral element displacement: 3 µm
  - Max. element tilt: 1’

- Methods & Tools:
  - Autocollimation telescope
  - Computer-aided analysis
  - Pivot mounting system with integrated lathing equipment



Machinery

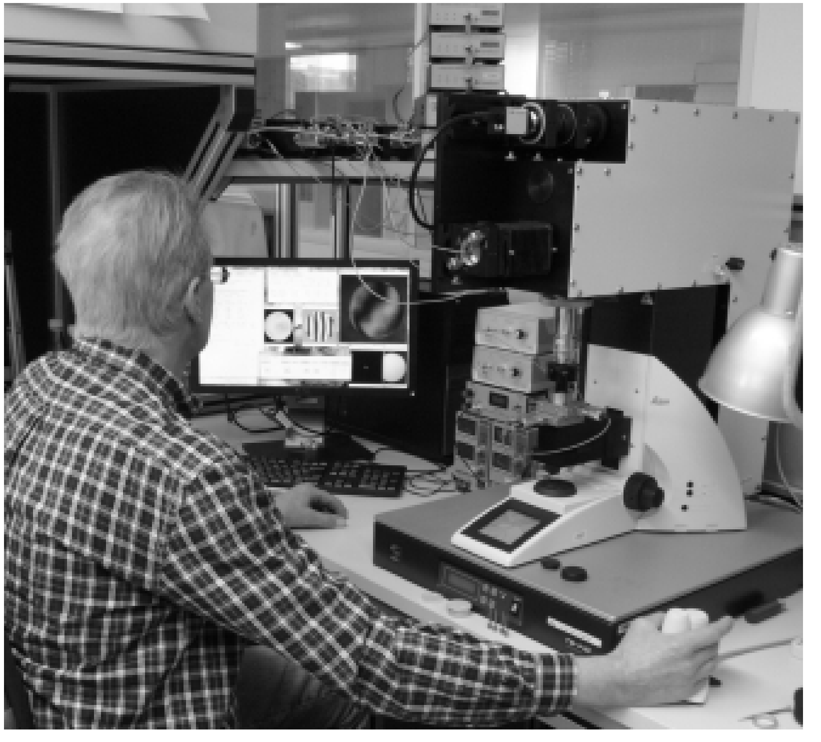
- Alignment lathe turning machine for fully automatic alignment and turning of the mount of mounted lenses
- Developed by third-party company in cooperation with Leica
- Mass approx. 6000 kg

Final assembly and quality control

Final assembly and adjustment of correction elements

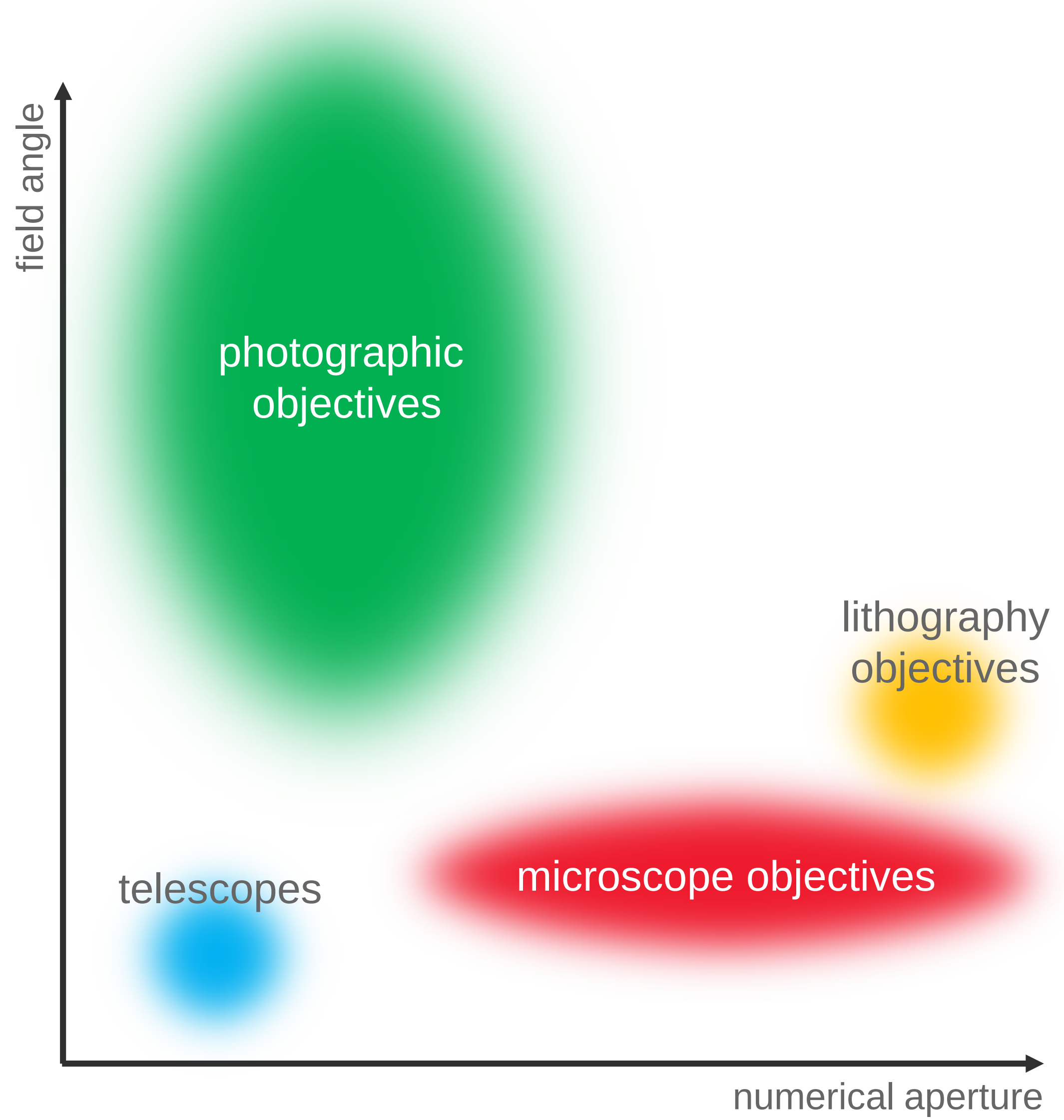
Interferometric quality control of assembled objective with Twyman-Green interferometer

Application-specific criteria on different types of final wave front error



Summary

- Microscope objectives are large NA optical systems
- User must take care to achieve best optical performance
- Manufacturing an objective consists of many detailed and specialized steps



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