Optotune

Microscopy

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Agenda

- Company presentation
- Why tunable lenses for microscopy?
- Tunable lens technology
- Integration of tunable lenses
- Application examples
- Conclusion

Optotune on one page

Established in 2008

Leader in tunable optics

27 sales partners in 30 countries

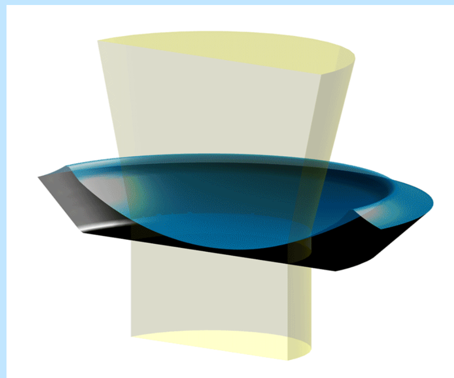
~80 employees in HQ in Zurich, Switzerland
~70 employees in Factory in Trnava, Slovakia

Two major businesses
- **Industrial**
- **Consumer**

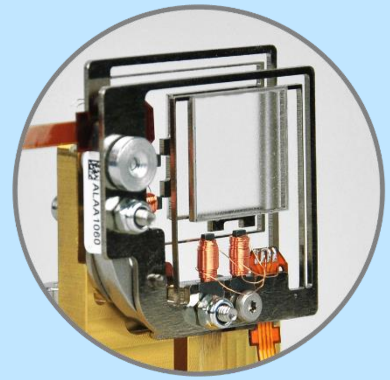
Privately owned

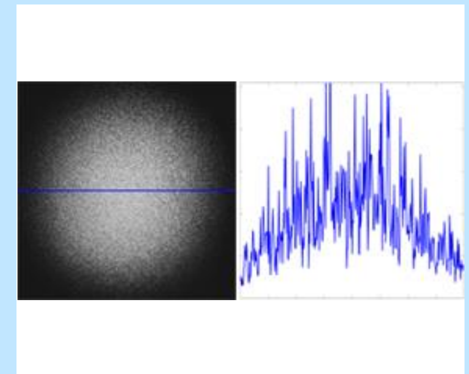
Optotune provides four core product lines

Focus tunable lenses

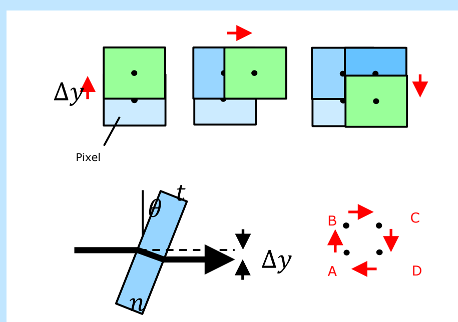


Laser speckle reducers

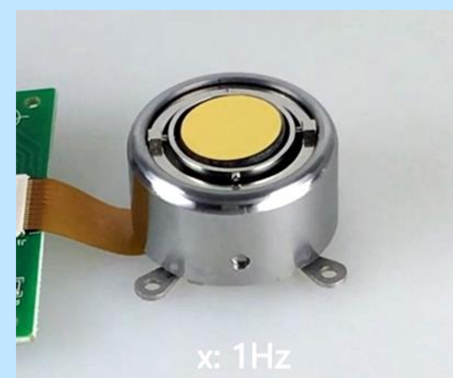




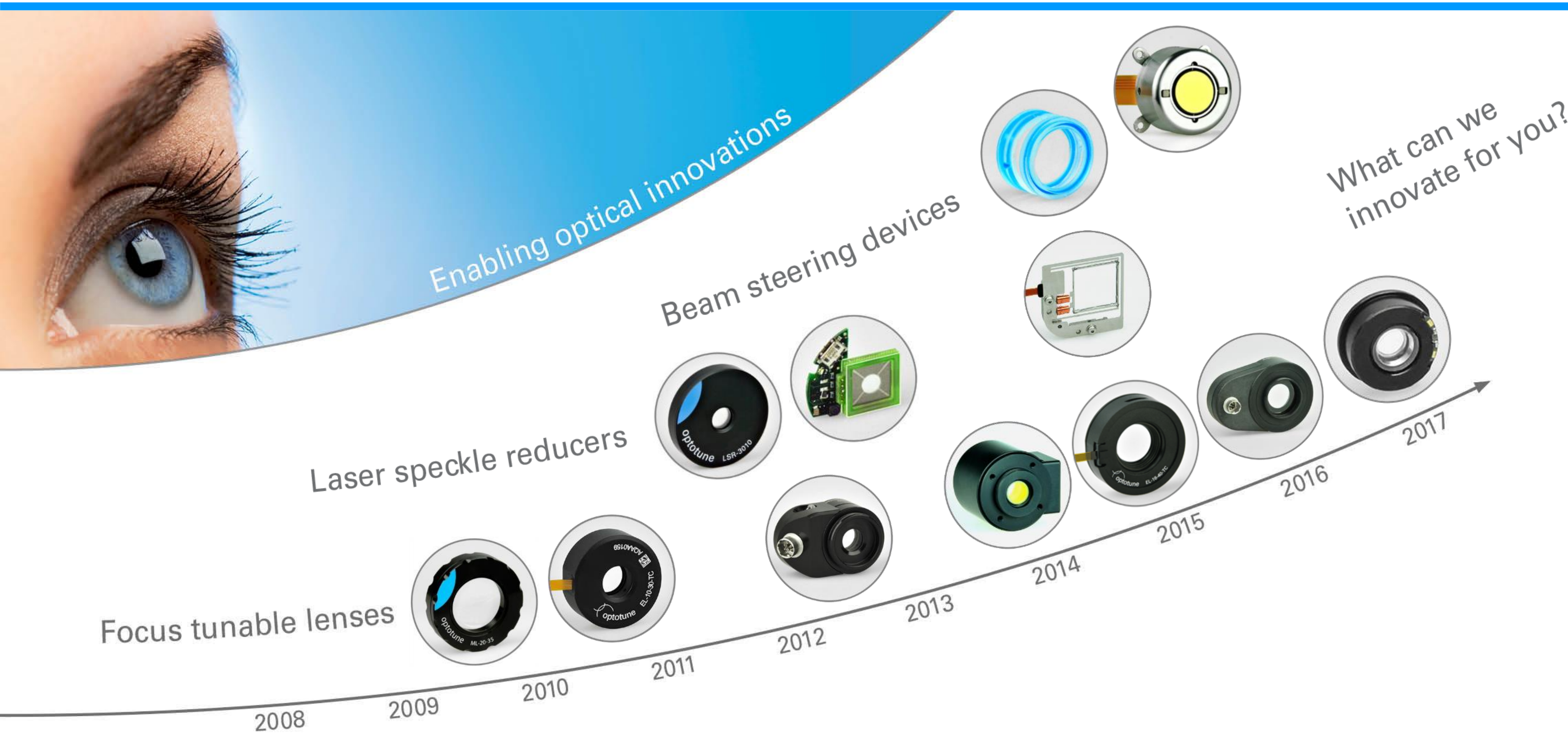
Extended pixel resolution actuators



2D mirrors



Expansion of product portfolio over the years



Our vision: Enable optical innovations

optotune

Enables product innovation

- Compact & fast autofocus
- 3D laser processing
- Laser-based cinema

By delivering key components

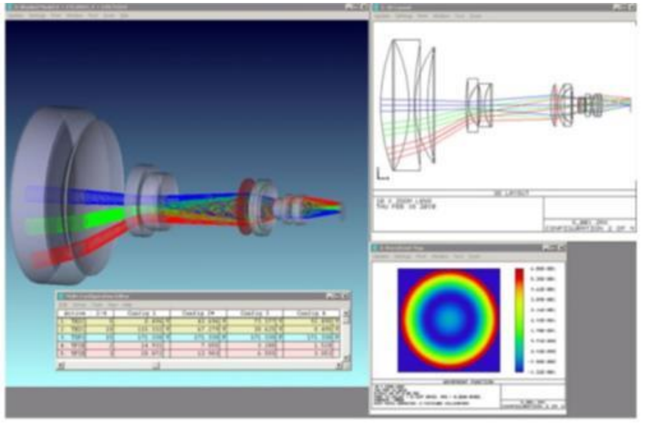
- Tunable lenses
- Laser speckle reducers
- Beam steering devices

Based on platform technologies

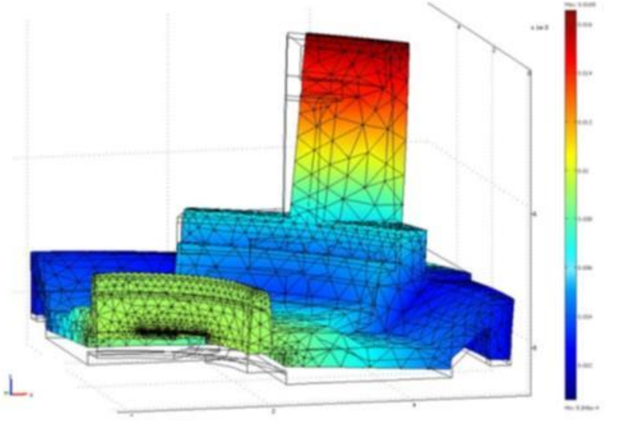
- Membranes & liquids
- Electroactive polymers
- Reluctance force actuators

Expertise in house from R&D to production

Materials Research



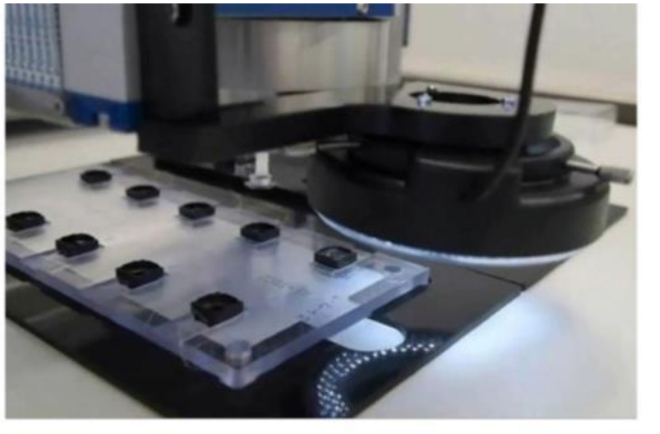
Optical Design



Mechanical Design

Prototyping

Testing



Production

Optotune's market focus

Laser projection

- ✓ High-resolution, speckle-free projections
- ✓ Ultra-compact solution with no mechanics
- ✓ Low power consumption

Machine vision

- ✓ Focus within milliseconds
- ✓ Working distances from infinity to 50mm
- ✓ Maximal flexibility

Laser processing

- ✓ Fast control of Z-axis
- ✓ Compact, reliable design with less mechanics
- ✓ Easy to integrate

Medical

- ✓ Compensation of visual defects
- ✓ Continuous adjustment in real-time
- ✓ +/- 20 diopters spherical, +/- 10 diopters cylindrical

Microscopy

- ✓ Axial focusing over several 100um within milliseconds
- ✓ Backward compatibility with several types of microscopes
- ✓ Speckle-free laser illumination

Custom design

- ✓ What is your application?

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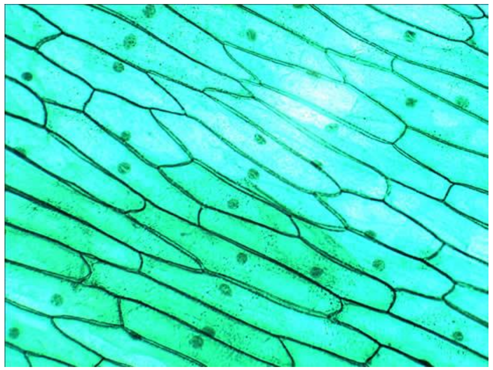
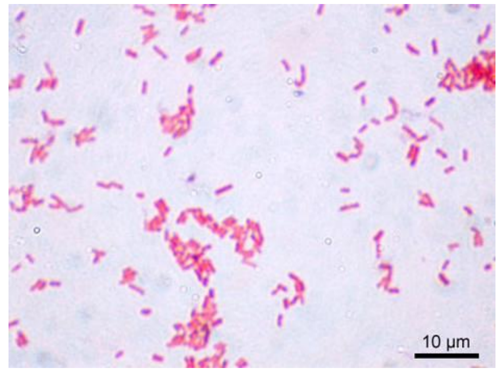
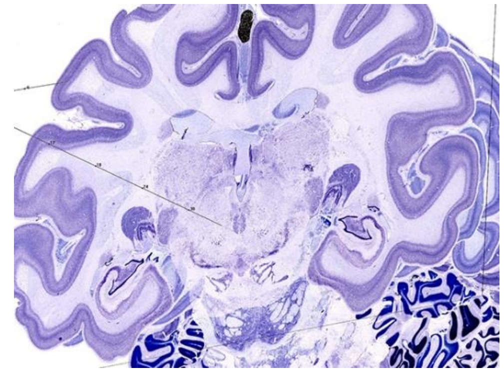
Starting point

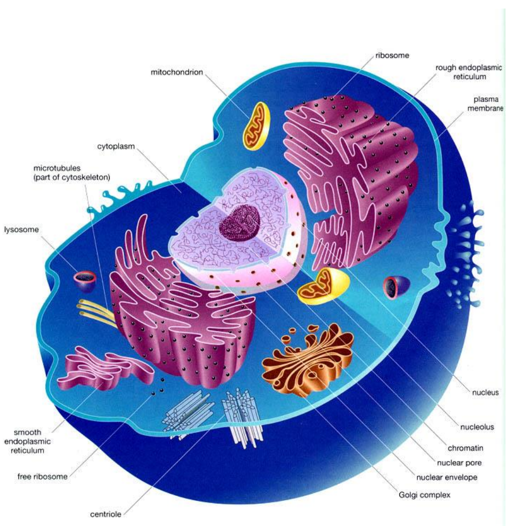
Today, most microscopes take 2D images, but …

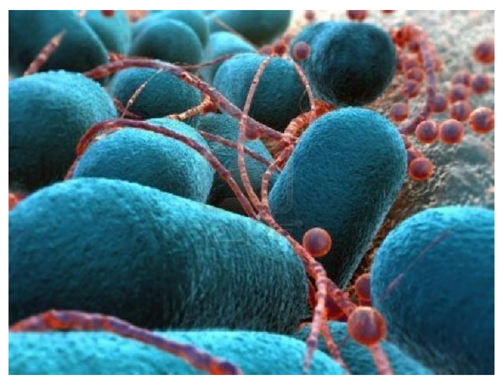






…Life is 3-dimensional !!





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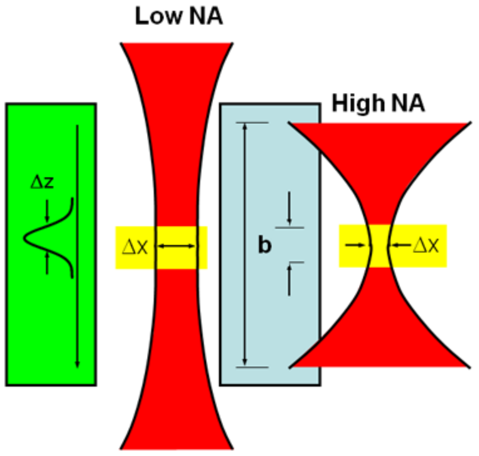
Starting point

Modern biology wants

- Imaging of 3D cell cultures
- Imaging of whole embryos
- In-vivo imaging in living animals

Issue:

- Microscopes have a limited "depth of field" (DOF)
- The higher the lateral resolution, the smaller the DOF

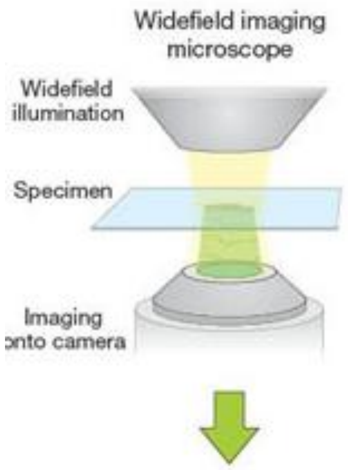


$$\mathrm{DOF} = \frac{n\lambda}{2\mathrm{NA}^2}$$

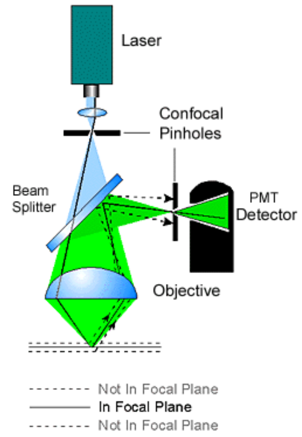
Solution:

- 3D microscope

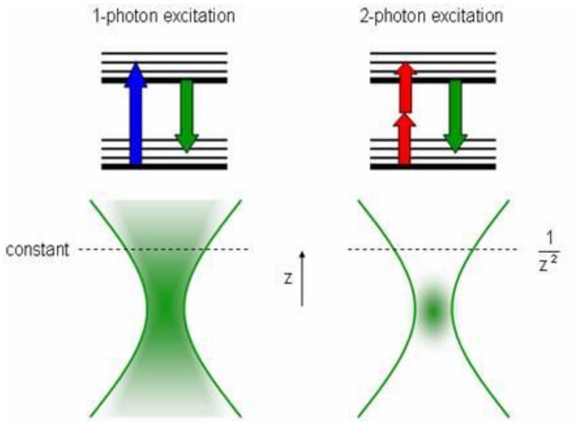
3D microscopy techniques



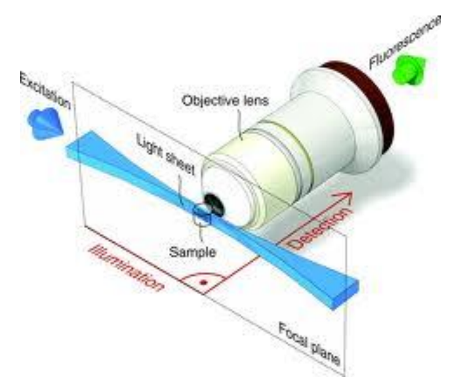
Wide-field microscopy



Confocal microscopy



Two-photon microscopy

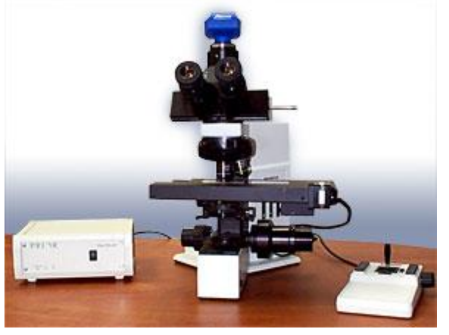


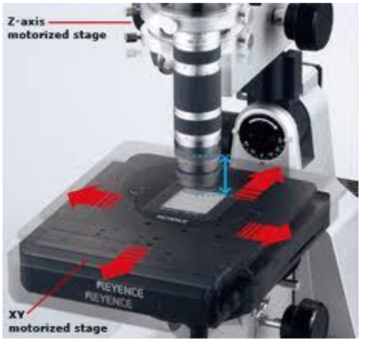
Light-sheet microscopy

Need to scan along z-axis

Solutions:

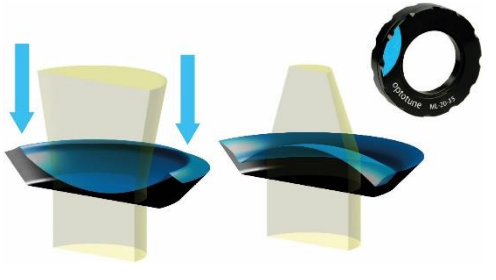
- Motorized stages
  - Slow
  - bulky

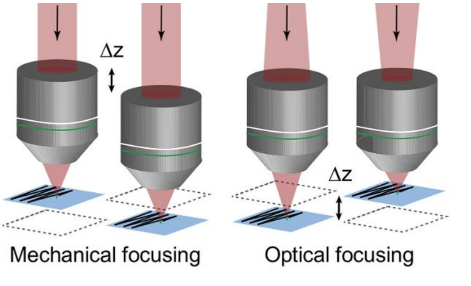




- Piezo-stages
  - small travel
  - expensive

- Focus tunable lenses
  - Fast
  - Compact
  - accurate





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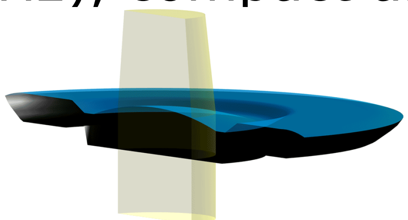
USP for 3D microscopy

- Fast (> 100 Hz), compact and accurate 3D scanning



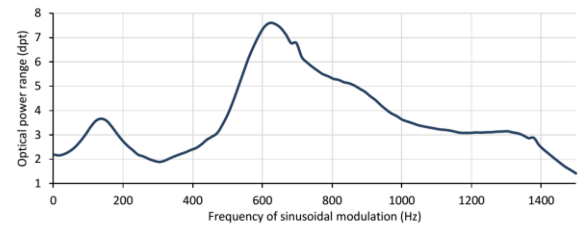


Figure 13: Typical frequency response of the EL-10-30-C with current oscillating from 50 to 150 mA.

- >100x Faster than motorized solutions

- >3x cheaper than piezo stages

- Larger z-range than with piezo stages (up to 600 µm with 40x objective)

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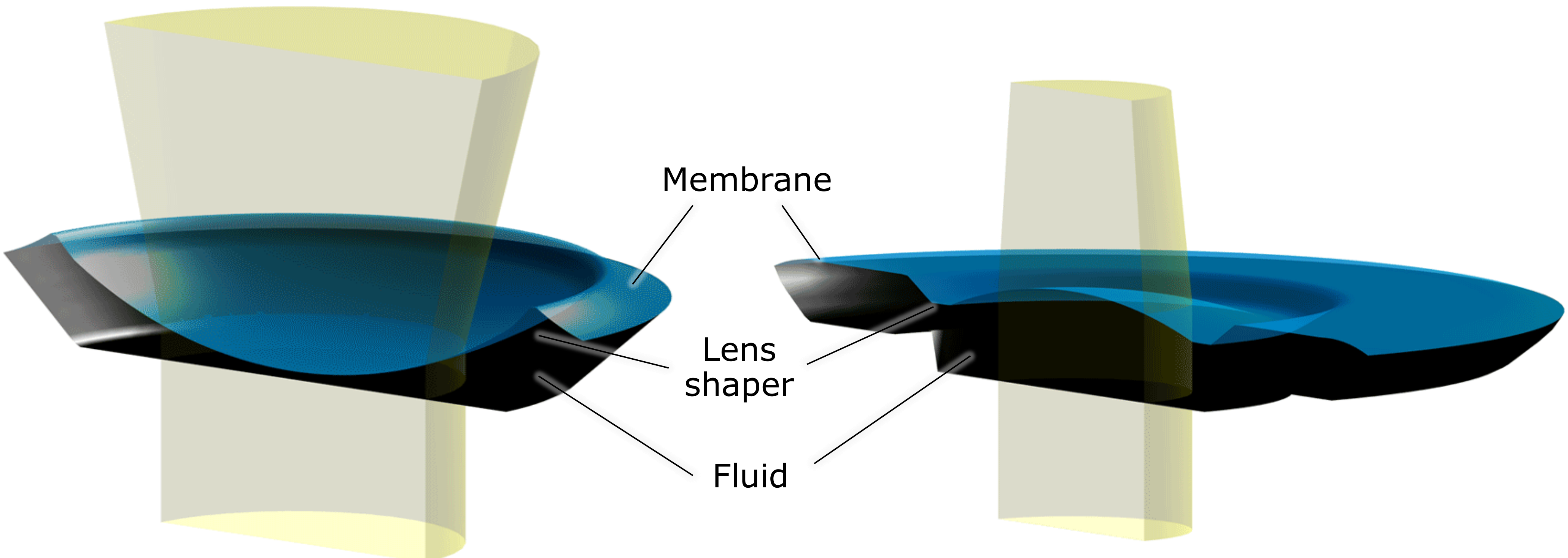
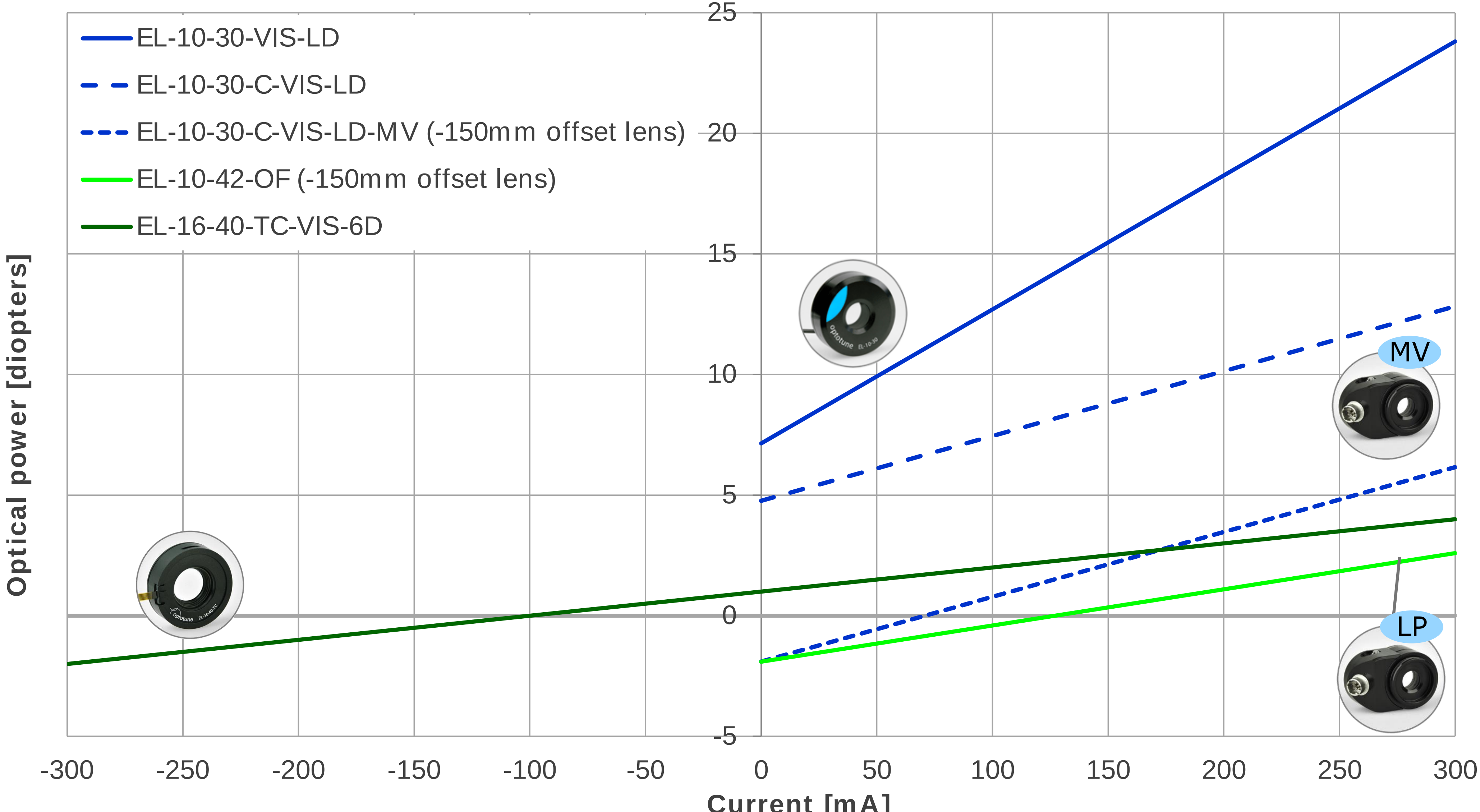
How does it work?



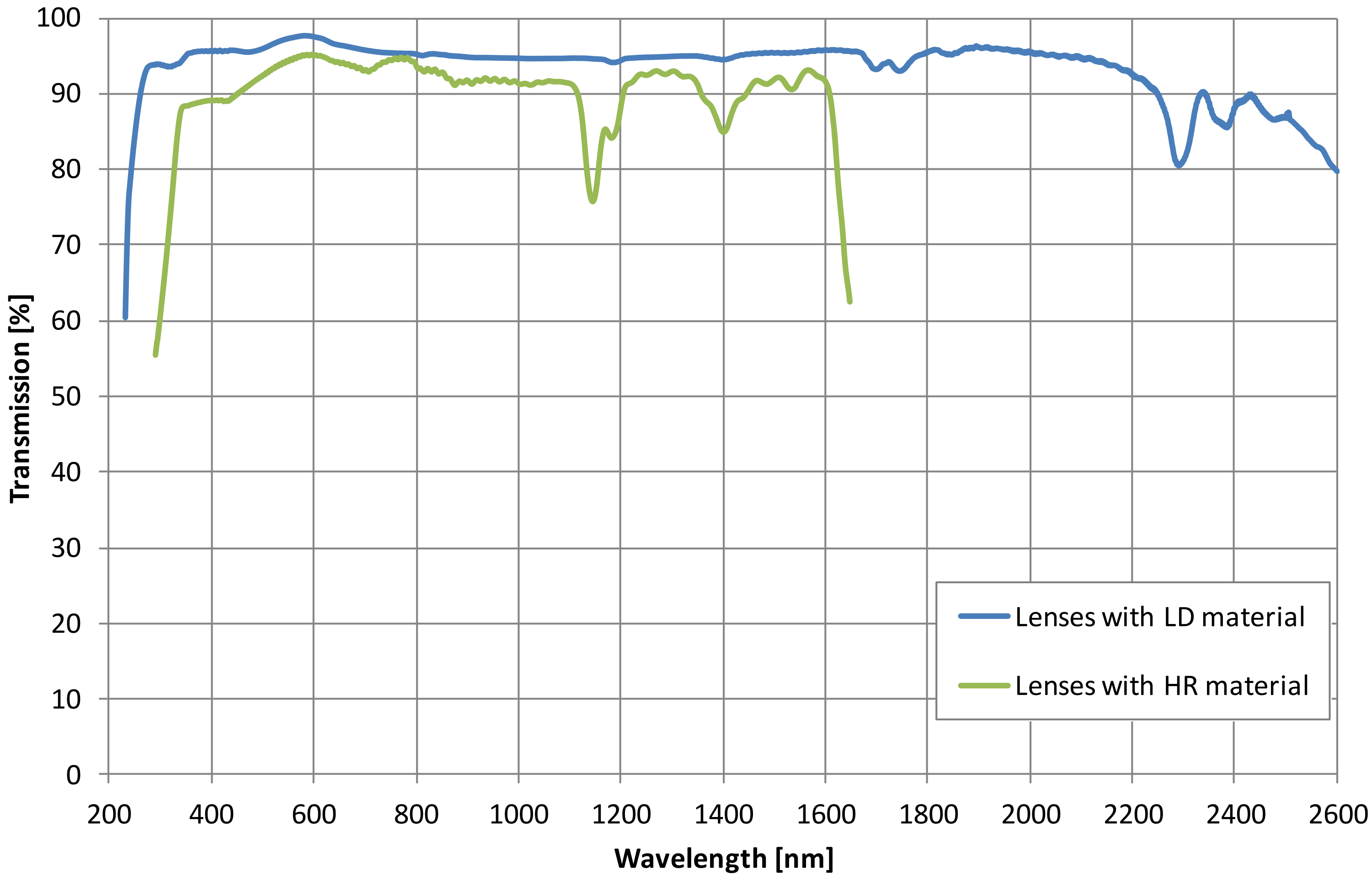
Videos available on www.optotune.com

The focal power (D = 1/f) of Optotune's lenses is controlled by current

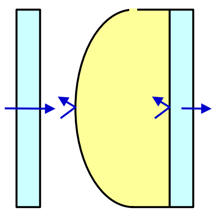


Note: This curve varies from lens to lens. However, it is reproducible once calibrated

Focus tunable polymer lenses for multi-spectral applications



Lens schematic



Transmission of the EL-10-30 assuming 100% transparent cover glasses.

Response time of ~10ms

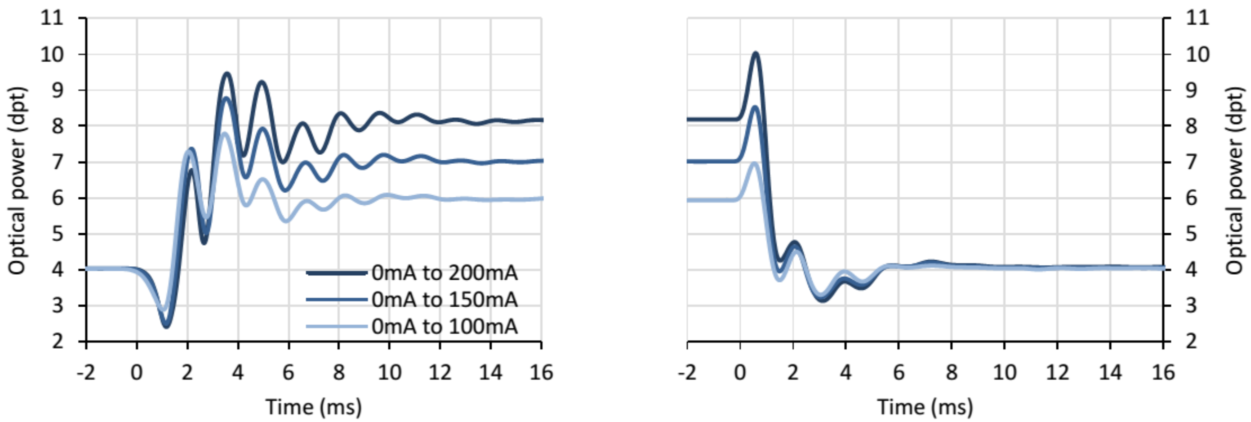
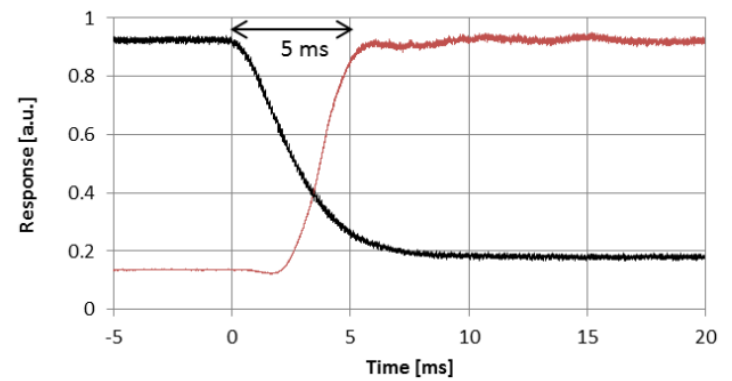


Figure 12: Typical optical response of the EL-10-30-C to a current step.

Low-pass filtered:



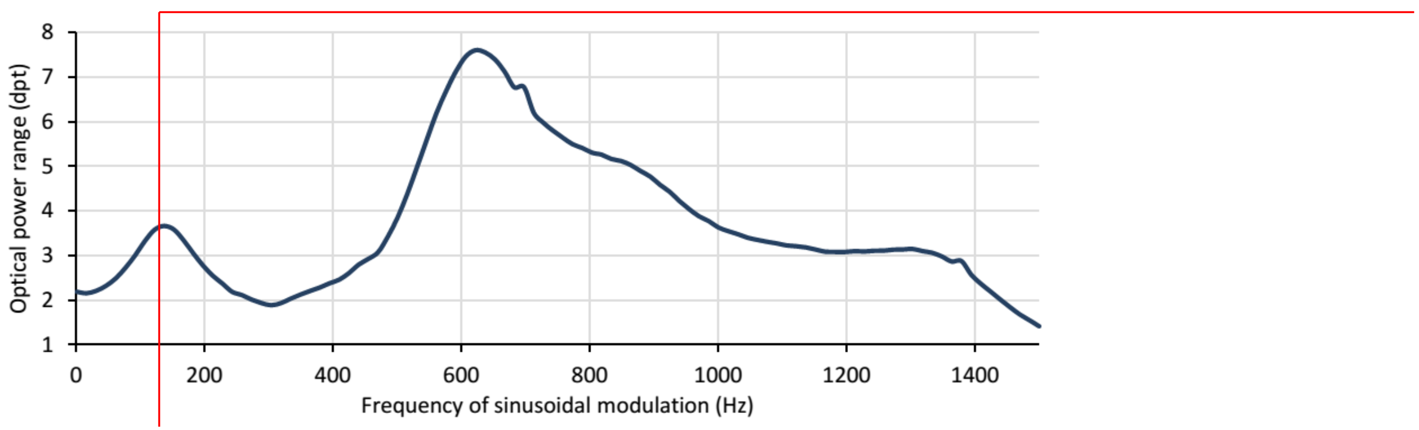
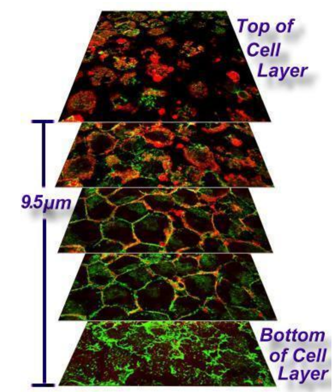


Figure 13: Typical frequency response of the EL-10-30-C with current oscillating from 50 to 150 mA.

Oscillation mode
→ fast image stacking



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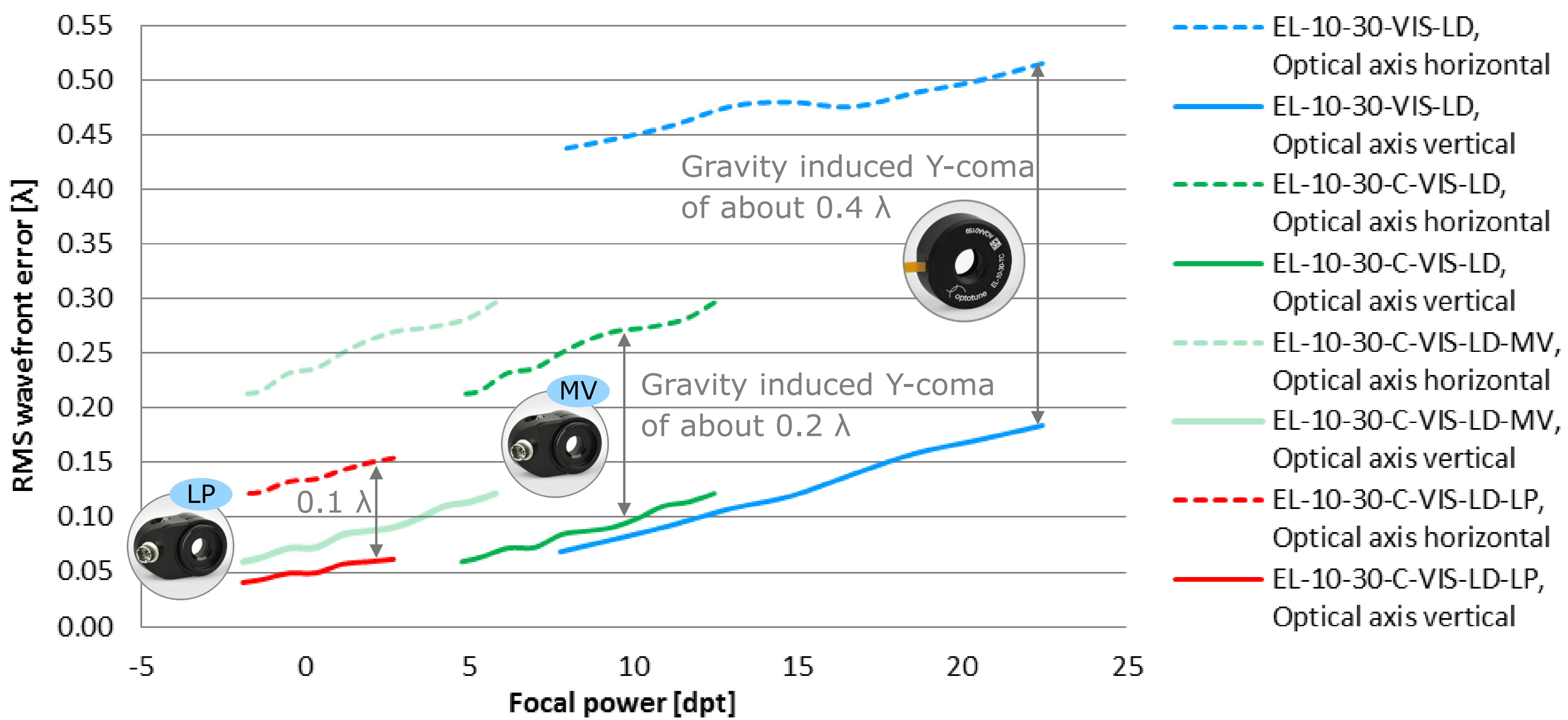
Typical wavefront quality of the EL-10-30



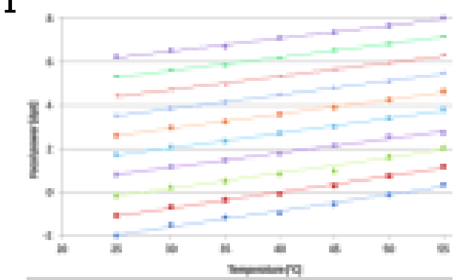
- Precision optics quality if optical axis is vertical
- Wavefront error in horizontal axis dominated by a Y-coma, due to gravity
  → This can be reduced using stronger membranes

Note: All measurements conducted at 80% of clear aperture; Wavelength is 525nm

Focal power mode for good reproducibility

- Why it is important:
  - The focal power of our lenses drifts with temperature by about 0.06 diopters / °C (depends on lens model)
- Typical accuracy achieved: +/- 0.1dpt

Lens characterization
f vs T vs I



Lens calibration curve stored on lens internal memory

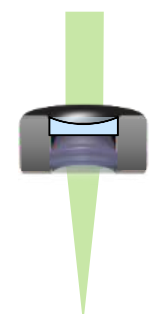
“I need a lens with f=125mm (8 diopters)”

Use focal power mode to set lens to 8 diopters

Temperature compensated control current to adjust lens to 8 diopters

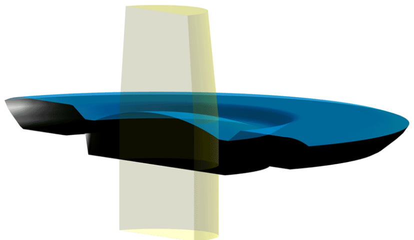
Lens calibration table and temperature read by lens driver

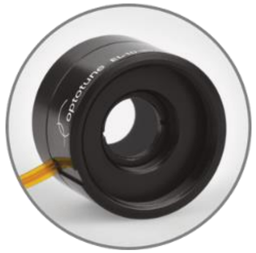
Temp sensor with EEPROM



f=125mm (8 diopters)

Optotune's electrically focus tunable lenses





	EL-3-10	EL-10-30-TC	EL-10-30-C(i)	EL-16-40	EL-10-42-OF
Focal power range*	-13 … 13 Dpt	8 … 22 Dpt	-1.5 … 3.5 Dpt	-2 … +3 Dpt	-10 … +10 Dpt
Clear aperture	3mm	10mm	10mm	16mm	16mm
Outer diameter	10mm	30mm	30mm	40mm	40mm
Wavefront quality RMS @525nm**	<0.15 / 0.15 λ	<0.25 / 0.5 λ	<0.15 / 0.25 λ	I: <0.15/ 0.5 λ II <0.25 / 0.5 λ	I: <0.25 / 2.5 λ II: <0.5 / 2.5 λ
Absolute focal power accuracy	N/A	< 0.1 Dpt	< 0.1 Dpt	< 0.1 dpt	< 0.1 dpt
Built-in sensors	None	Temperature	Temperature	Temp./Optical feedback	Temp./Optical feedback
Applications	Machine Vision Ophthalmology	Microscopy Ophthalmology	Machine vision	MV/Microscopy Ophthalmology	MV/Microscopy Ophthalmology

* Depends on selected optical fluid ** vertical / horizontal optical axis

Focus tunable polymer lenses are reliable

Test	Test conditions	Status
Mechanical cycling	40 million full-range cycles (0 to 300 mA rectangular, at 10 Hz) 5 billion sinusoidal cycles at resonant frequency	Passed
High temperature test	85±2°C; rel. hum. <6% for 168 hours, non-operational	Passed
Temperature cycling test	-40°C / +85°C for 30 min each, 3 min transition time, 100 cycles	Passed
Damp heat cycling test	25°C / 55°C at 90-100% relative humidity, 3 hour transition time, 24h per cycle (9h plus transition time each), 18 cycles	Passed
Shock test:	800g for 1ms duration, 5 pulses in each direction (30 pulses in total)	Passed
Solar radiation test:	1120 W per m2 (IEC 60068-2-5), 8 h irradiation & 16 h darkness, 10 cycles	Passed

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Digital microscopy configurations

Mag. change

- Cam
- Tube Lens
- ETL
- Obj (Inf)

- Large z-range

No Mag change

- Cam
- Relay lens
- ETL
- Relay lens
- Tube Lens
- Obj (Inf)

4f system (Relay lens – ETL – Relay lens)

Intermediate image plane (between Relay lens and Tube Lens)

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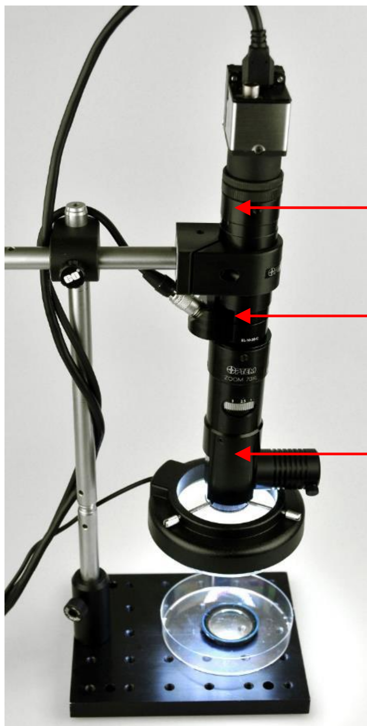
How to integrate the EL

Digital inspection microscope



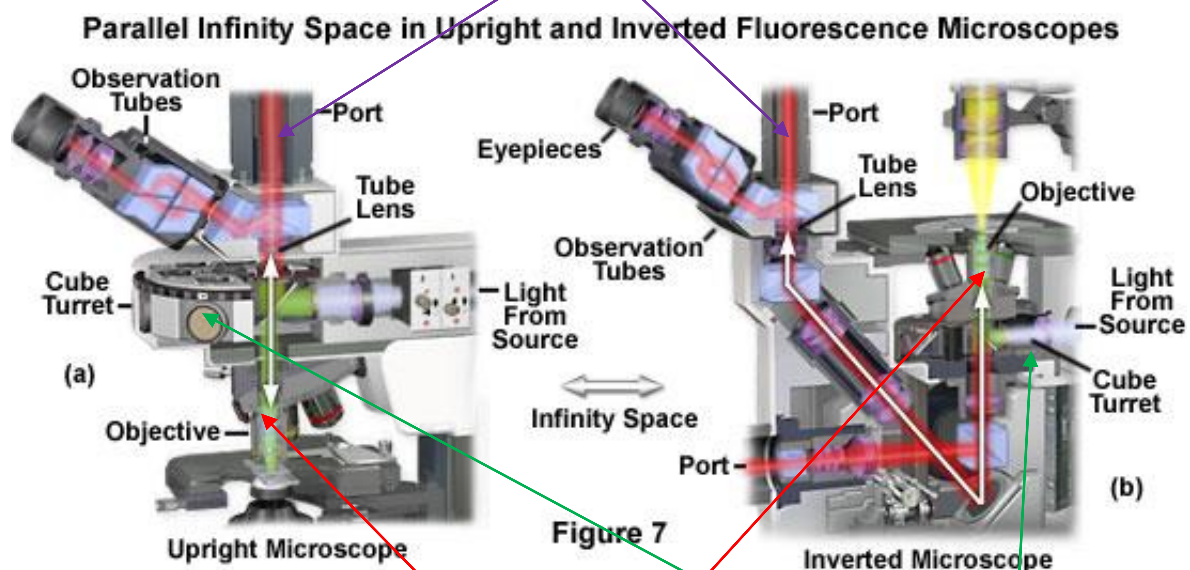
Tube lens

Optotune EL

Zoom lens

Scientific microscope

3. EL Pos.
(camera port)



1. EL Pos.
(after objective)

2. EL Pos.
(filter cube)

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Non-telecentric autofocus configuration: EL on top of objective

Zeiss Axioskop
http://labs.pbrc.edu/cellbiology/documents/AxioskopManual.pdf

- Zeiss Neofluar, 10x/0.3 Inf./0.17
- Zeiss LD Achroplan 20x/0.4 Korr Ph2 Inf./0-1.5
- Zeiss Plan-Neofluar 40x/0.75 Inf/0.17

Camera

Teledyne Dalsa Genie TS-C1920

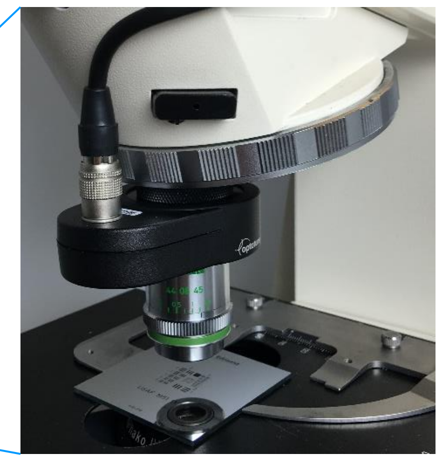
Optotune Lens

EL-16-40-TC-VIS-20D, ANAA0380

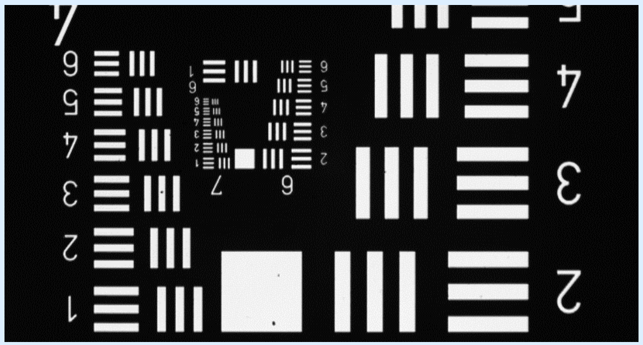
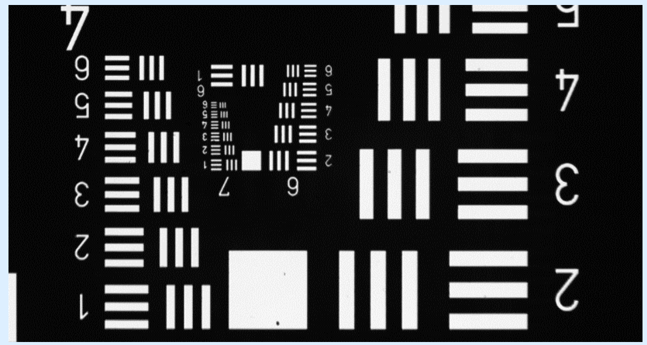
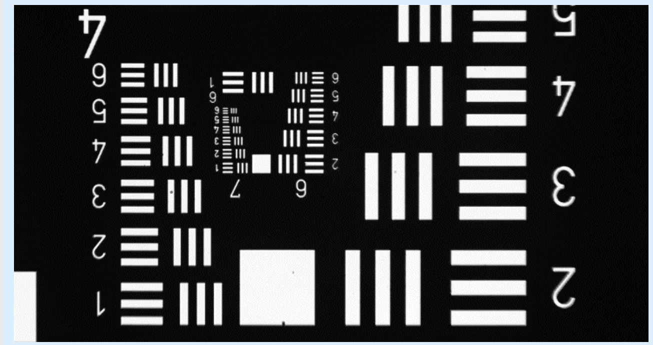
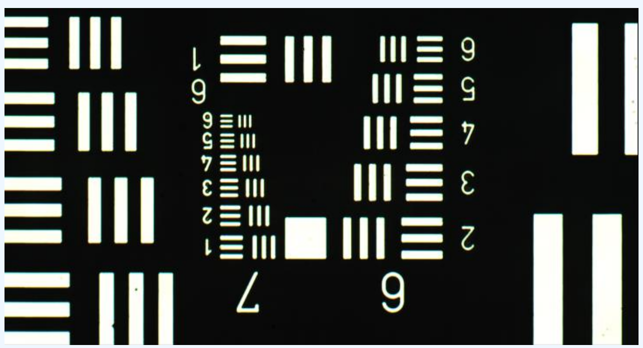
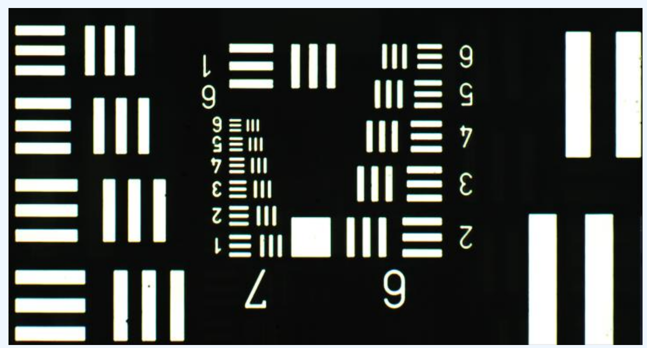
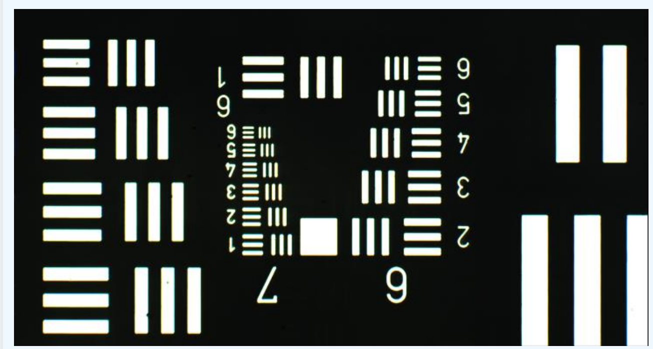
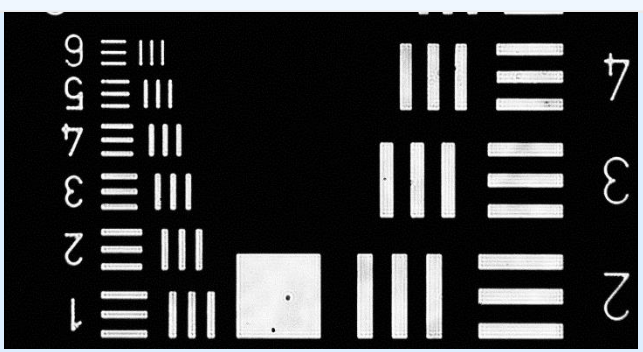
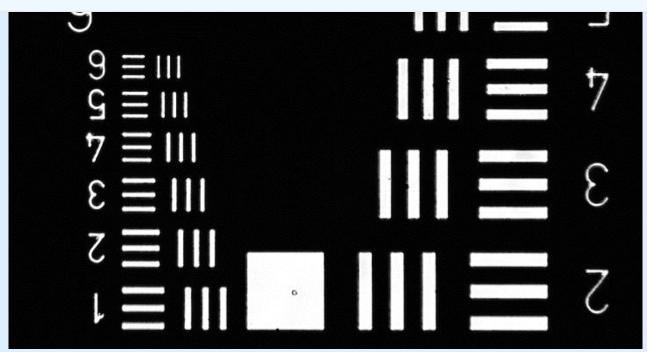
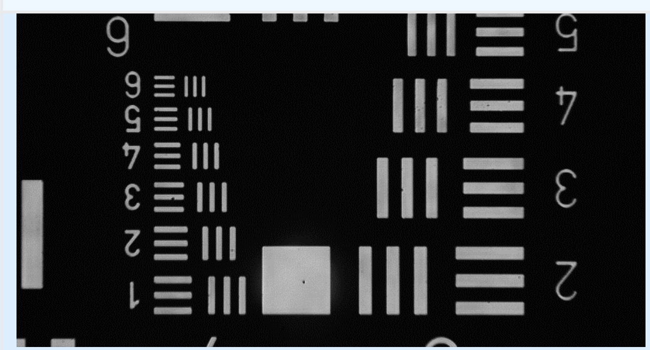
Mounted with C-mount RMS adapters from Thorlabs

- RMSA6 - Adapter with External RMS Threads and Internal C-Mount Threads

- RMSA5 - Adapter with External C-Mount Threads and Internal RMS Threads



Non-telecentric autofocus configuration: EL on top of objective

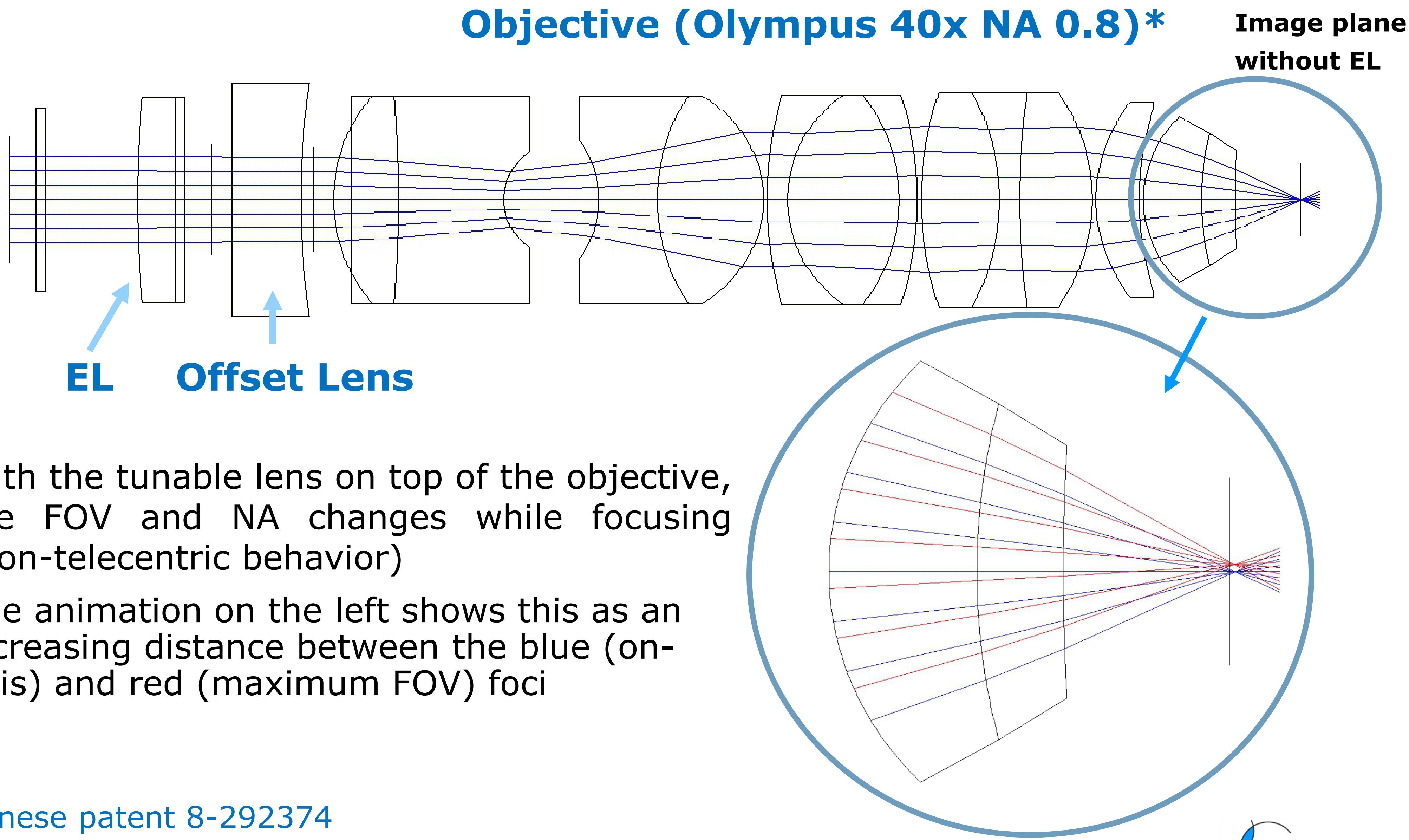
Mag	-2 dpt	0 dpt	+3 dpt
10x			
20x			
40x			

Non-telecentric autofocus configuration: EL on top of objective

- The tunable lens was operated between -2dpt and +3 dpt (nominal tuning range)
- Compact autofocus solution without the need of mechanical translation
- However, in such a configuration, the field-of-view (FOV) and numerical aperture (NA) changes while focusing (non-telecentric behavior)

	Z-range	Mag change
10x	2.56mm (20D: 10.24)	7.5 %
20x	0.64mm (20D: 2.56 mm)	12.2%
40x	0.16mm (20D: 0.64mm)	23.7%

Non-telecentric autofocus configuration: optical layout

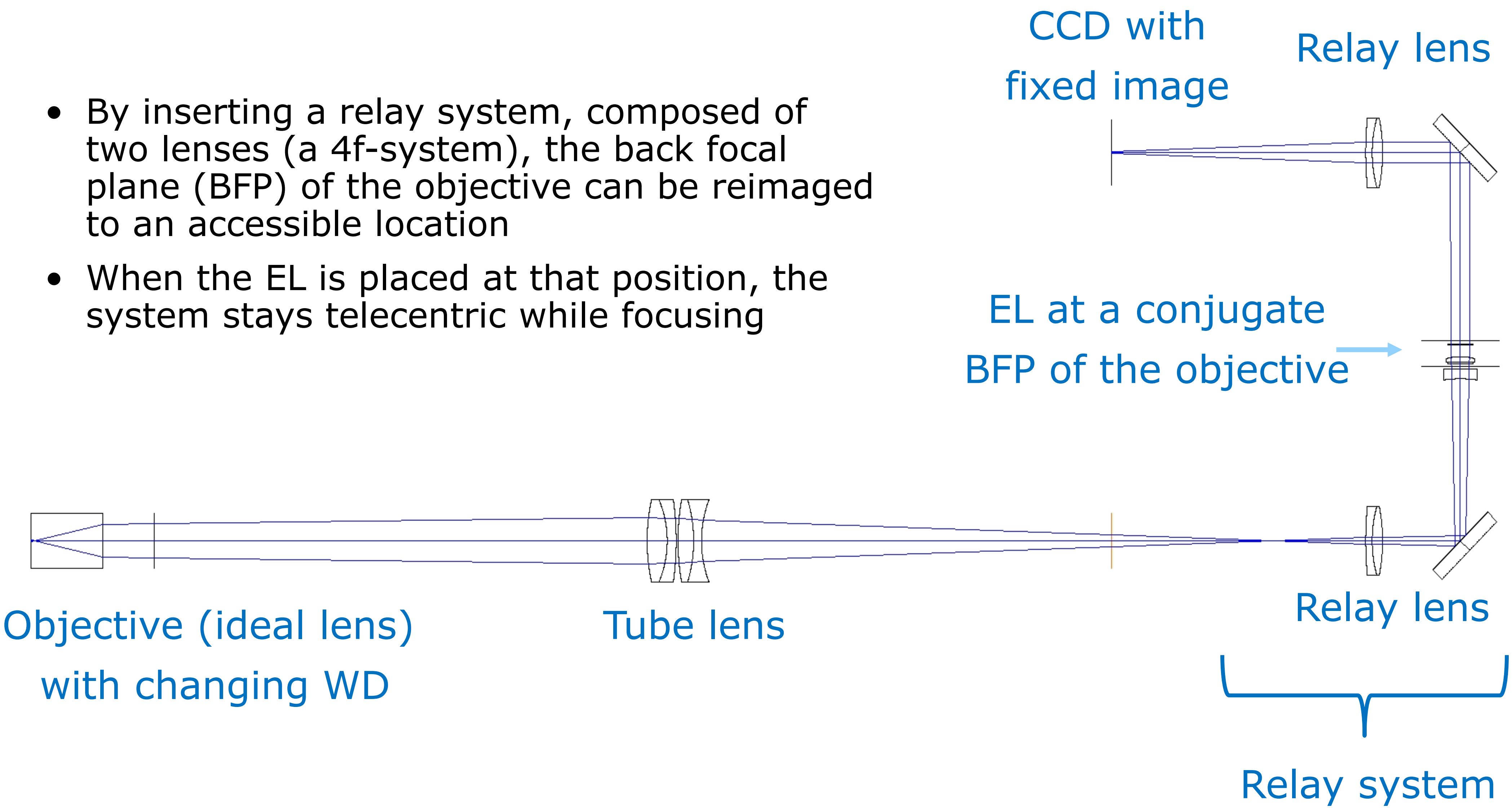


- With the tunable lens on top of the objective, the FOV and NA changes while focusing (non-telecentric behavior)
- The animation on the left shows this as an increasing distance between the blue (on-axis) and red (maximum FOV) foci

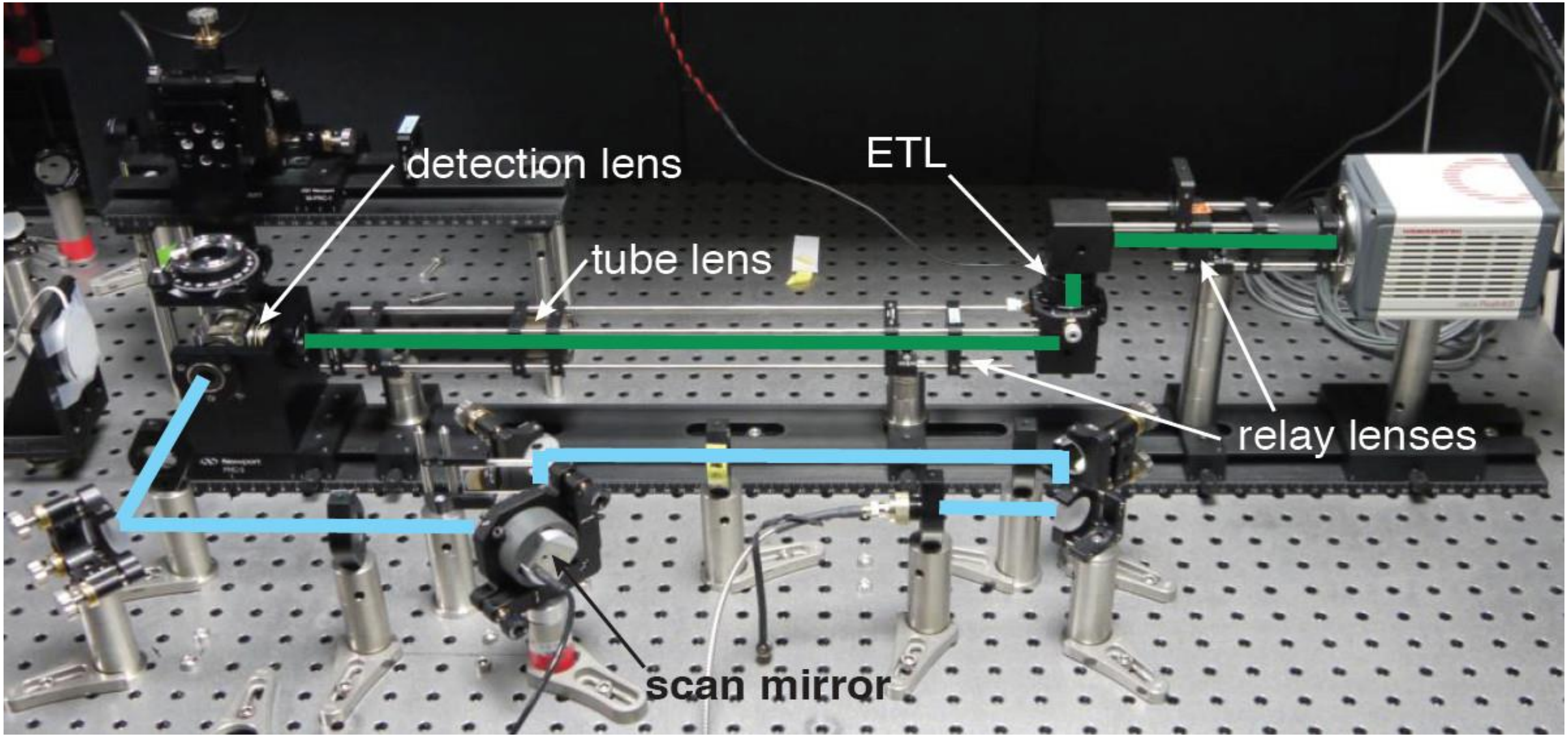
* Japanese patent 8-292374

Telecentric autofocus configuration: tunable lens EL with a relay system

- By inserting a relay system, composed of two lenses (a 4f-system), the back focal plane (BFP) of the objective can be reimaged to an accessible location
- When the EL is placed at that position, the system stays telecentric while focusing

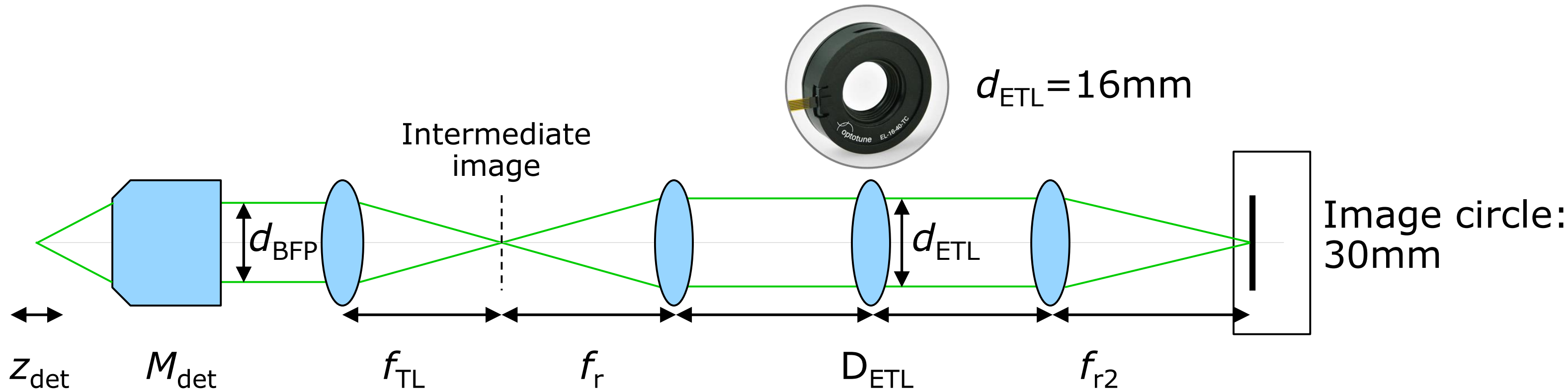


Exemplary setup of a telecentric microscope with a tunable lens autofocus solution



This design principle can be found in this EL-lightsheet microscope (Fahrbach et al., Optics Express 2013)

Axial scan range



- The displacement of the image plane is given by

$$\delta z_{\mathrm{det}} = -\frac{1}{M_{\mathrm{det}}^2} \cdot \frac{n f_{\mathrm{r}}^2}{f_{\mathrm{ETL,eff}}}.$$

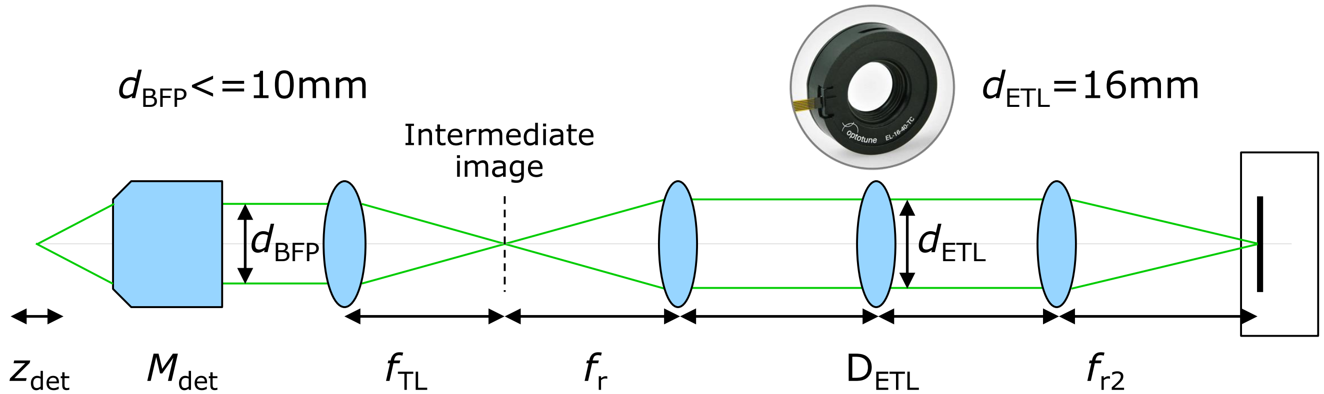
where n is the refractive index of the immersion medium, M_{det} is the magnification of the microscope objective, f_r is the focal length of the relay lens and $f_{ETL,eff}$ is the effective focal length of the Optotune lens ($1/f_{ETL,eff} \approx 1/f_{ETL} + 1/f_{OL}$) and f_{OL} is the focal length of a possible offset lens.

- To maintain the full NA of the detection lens, the ratio of the focal lengths of the relay lens f_r and the tube lens f_{TL} must not be larger than the ratio of the aperture of the ETL d_{ETL} and the diameter of the BFP of the detection lens d_{BFP}, i.e.

$$f_{\mathrm{r}} \leq f_{\mathrm{TL}} \cdot \frac{d_{\mathrm{ETL}}}{d_{\mathrm{BFP}}}.$$

optotune

Axial scan range examples



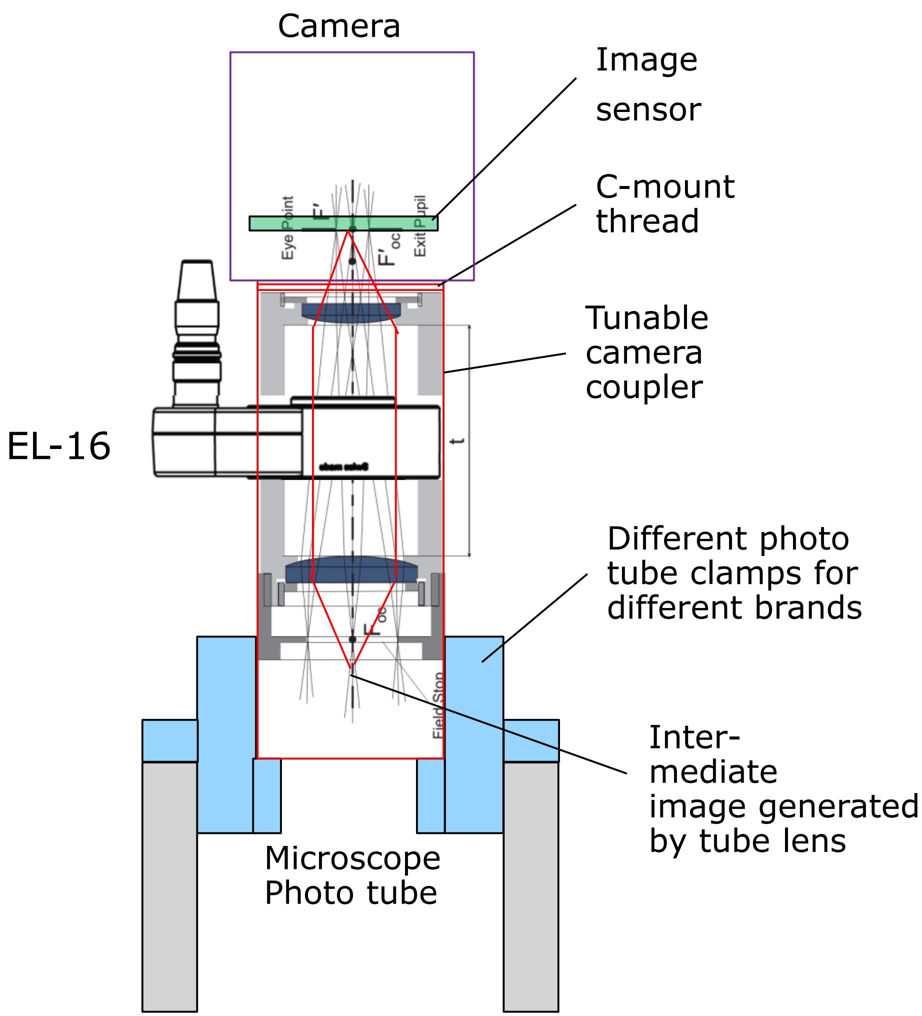
Scenarios:	z_{det}	M_{det}	f_{TL}	f_r	D_{ETL}	f_{r2}	Mag Relay	Image circle	Total length
10x, long relay, 5D:	2000um	10x	200mm	200mm	5D	200mm	1X	30mm	1100mm
Shorter relay lenses:	1000um	10x	200mm	141mm	5D	141mm	1X	30mm	864mm
20X, long relay, 5D:	500um	20x	200mm	200mm	5D	200mm	1X	30mm	1100mm
Shorter relay lenses:	250um	20x	200mm	141mm	5D	141mm	1X	30mm	864mm
10D lens:	500um	20x	200mm	141mm	10D	141mm	1X	30mm	864mm
1” sensor:	500um	20x	200mm	141mm	10D	70mm	0.5X	15mm	744mm
Microscopy adapter prototype (5D):	12um	40x	200mm	62mm	5D	31mm	0.5X	12mm	~450mm

$$\delta z_{det} = -\frac{1}{M_{det}^2} \cdot \frac{n f_r^2}{f_{ETL,eff}}.$$

$$f_r \leq f_{TL} \cdot \frac{d_{ETL}}{d_{BFP}}.$$

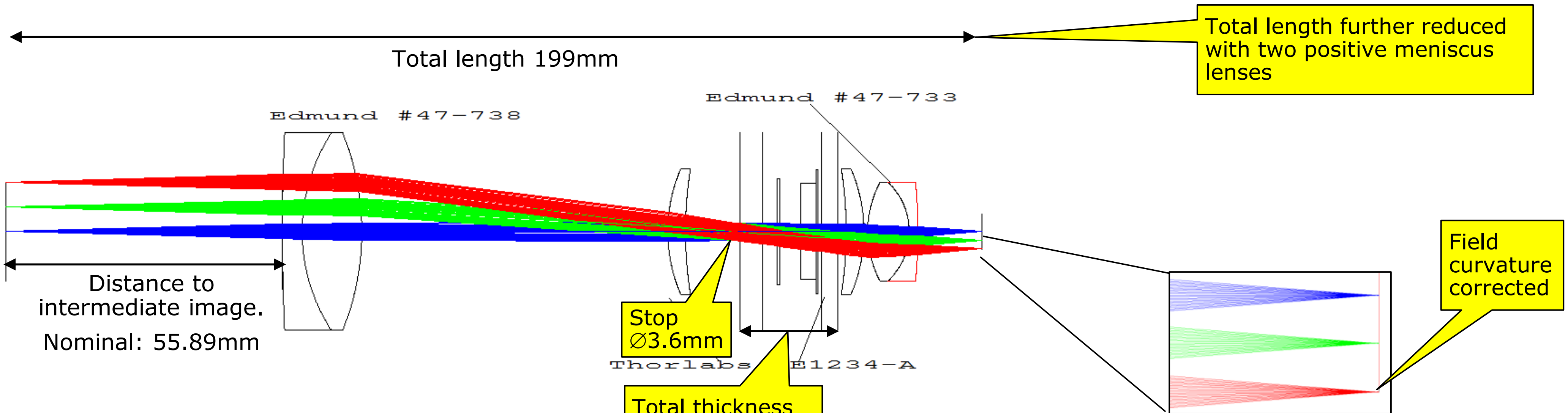
$D_{ETL} = 1/f_{ETL}$ $\quad Mag_{Relay} = f_r / f_{r2}$

Example: Tunable camera coupler retrofitted to microscope



- Retrofit to existing microscope possible
- Automatic user independent parfocality between eye and camera port
- Fast autofocus
- Focus on region of interest by clicking into image
- Wide-field 3D imaging (image stacking)

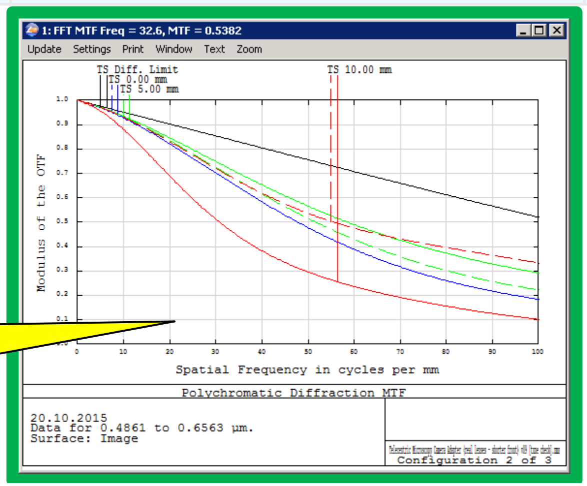
Optical design with off-the-shelf catalog lenses for ½” sensor

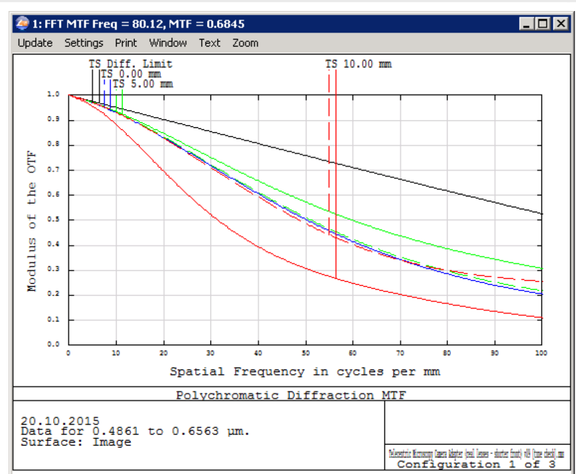


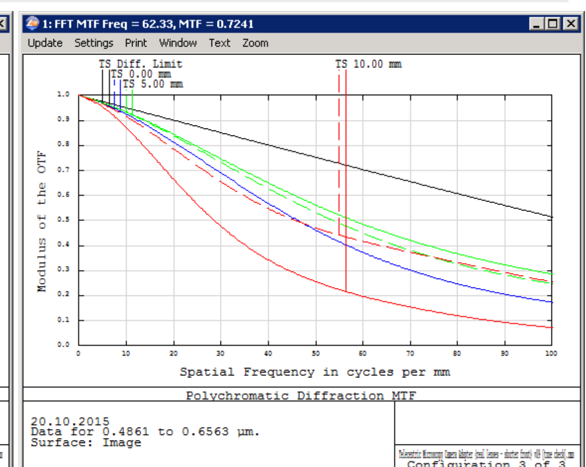
Distance to intermed image	Nominal	Nominal -4.0mm	Nominal +4.0mm
Distance to intermed image [mm]	55.89	51.89	59.89
Image magnification	0.35	0.35	0.35

Magnification stays the same when tuning EL-16

Image quality ok. Same over entire sensor.







See Telecentric Microscopy Camera Adapter (real lenses - shorter front) v09 [tune check].zmx

See Telecentric Microscopy Camera Adapter (real lenses - shorter front) v09.zmx

optotune

Microscopy adapter without magnification change

Zeiss Axioskop

- Zeiss Neofluar, 10x/0.3 Inf./0.17
- Zeiss LD Achroplan 20x/0.4 Korr Ph2 Inf./0-1.5
- Zeiss Plan-Neofluar 40x/0.75 Inf/0.17

Camera

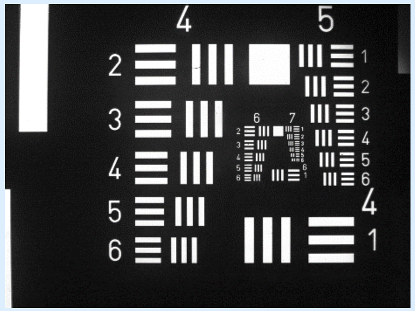
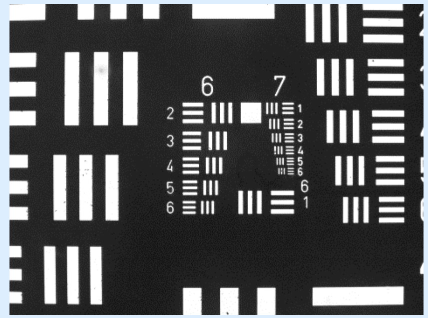
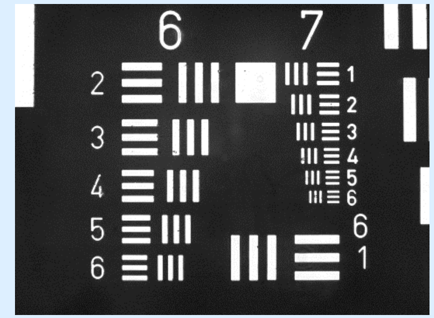
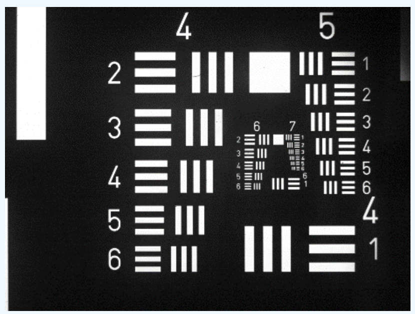
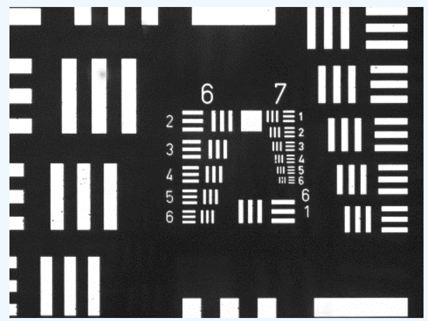
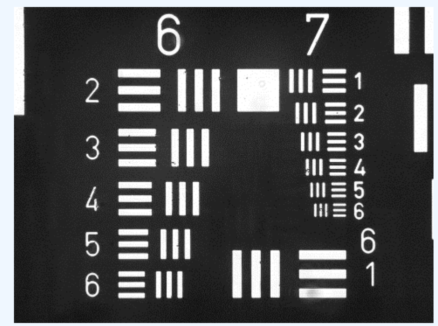
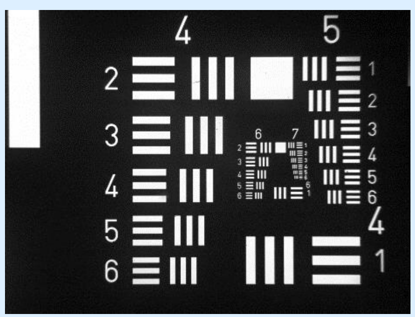
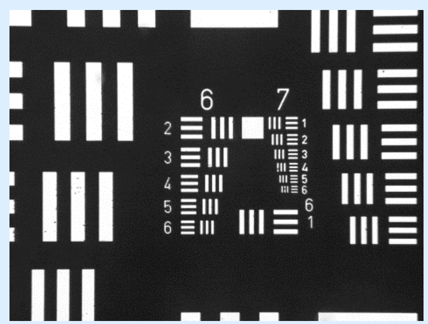
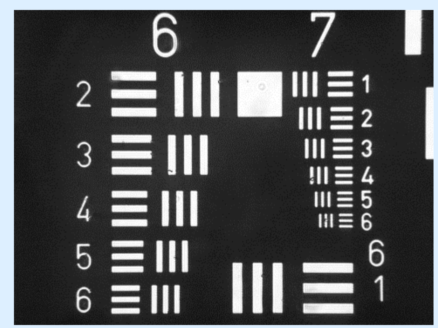
IDS UI-3580CP-C-HQ (1/2”, 5MP)

Optotune Lens

EL-16-40-TC-VIS-20D

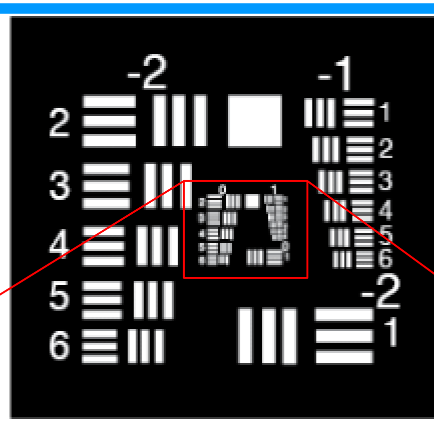
Mag	EL-16-40-TC-VIS-5D	EL-16-40-TC-VIS-20D
10x	262um	980um
20x	64um	254um
40x	12um	56um

Almost no magnification change

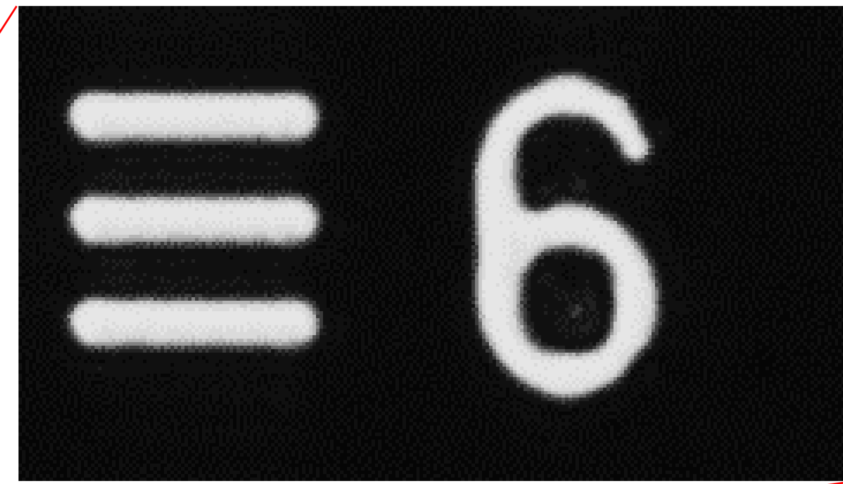
	10x	20x	40x
10 dpt			
0 dpt			
-10 dpt			

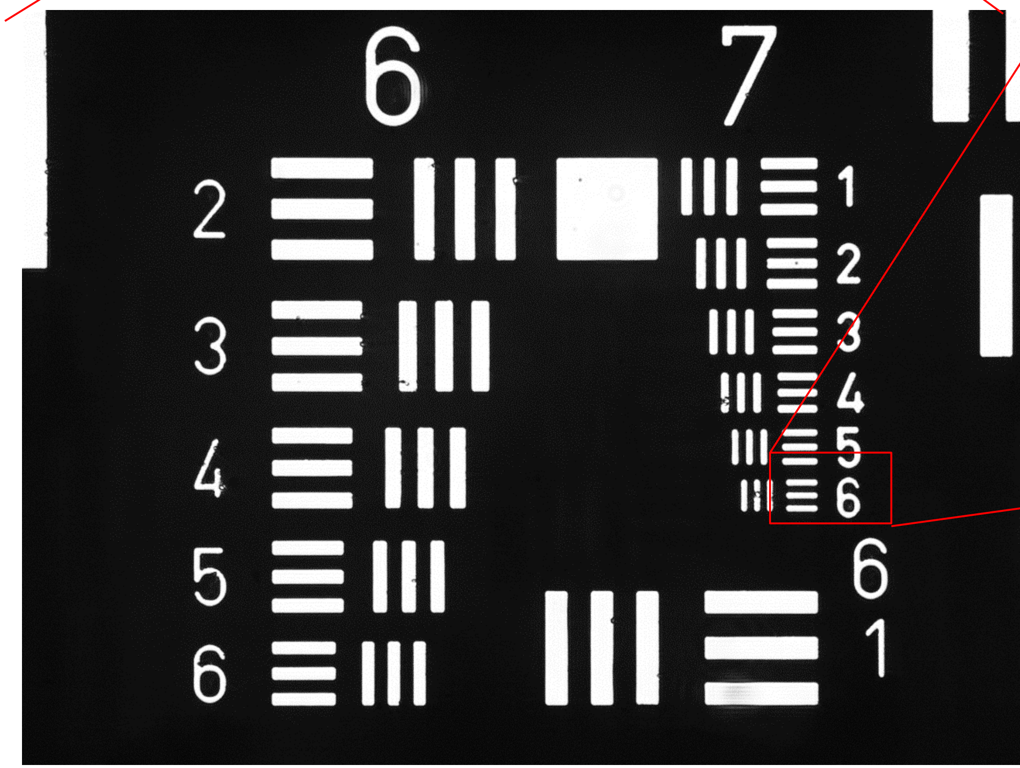
Optical quality is good!

1951 USAF target



Zoom-in

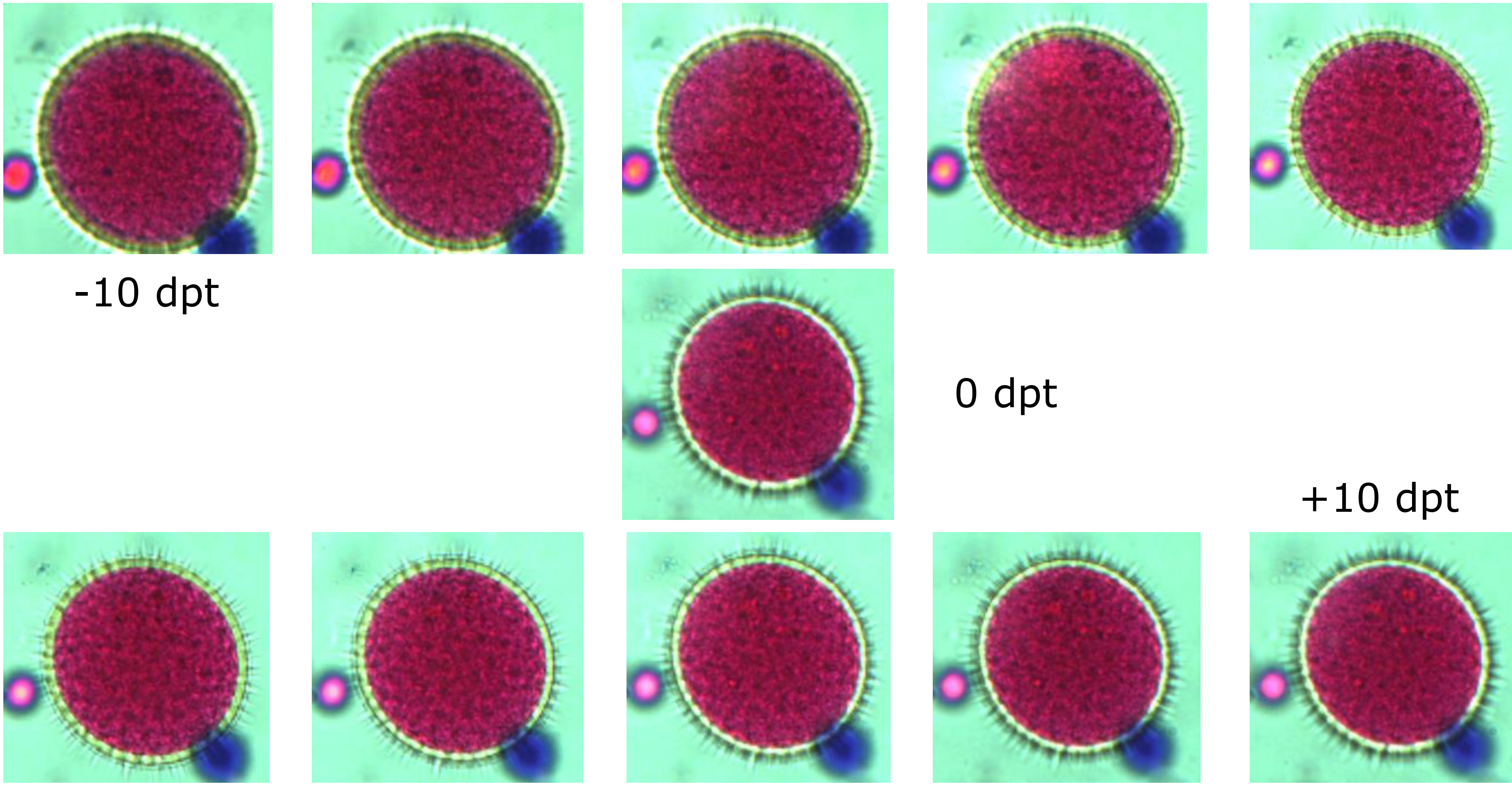




40x

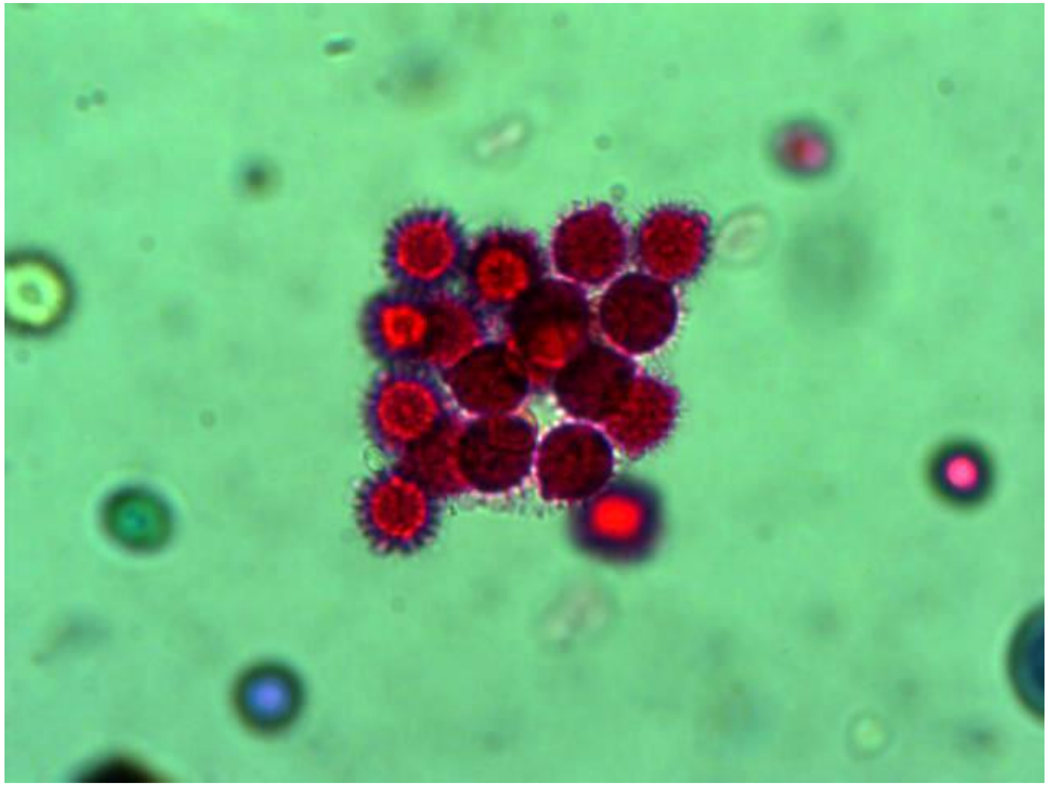
Stacking of pollen images

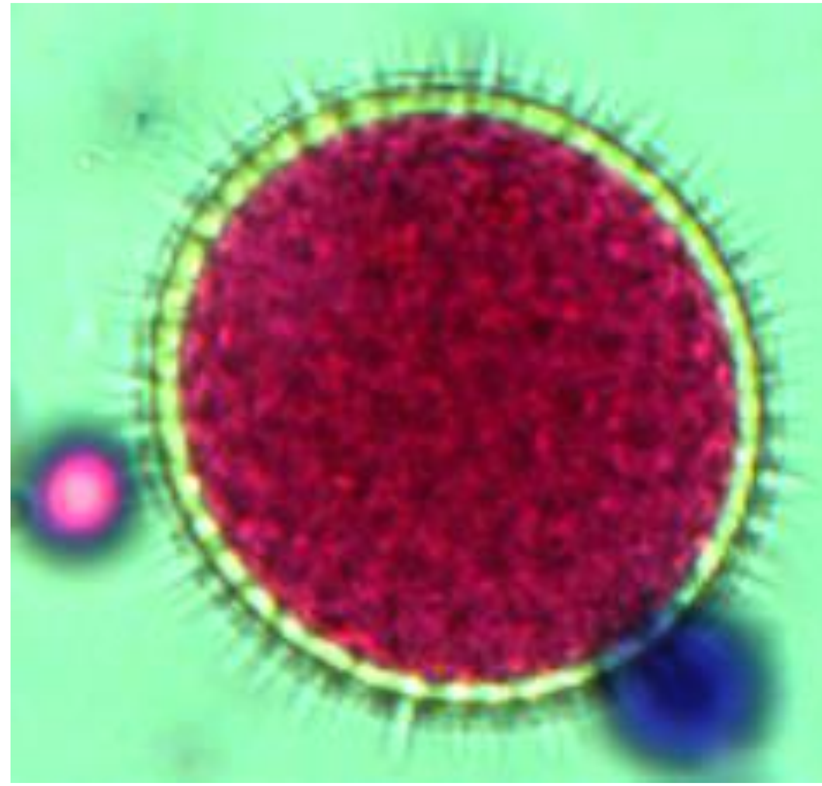
Images have been taken at 10x between -10dpt and 10dpt



Videos

40x





Agenda

- Company presentation
- Why tunable lenses for microscopy?
- Tunable lens technology
- Integration of tunable lenses
- Application examples
- Conclusion

18 publications using Optotune lenses for microscopy

Three-dimensional multiple-particle tracking with nanometric precision over tunable axial ranges
B. G. Sancataldo, L. Scipioni, T. Ravasenga, L. Lanzanò, A. Diaspro, A. Barberis, and M. Duocastella, Optica Vol. 4, Issue 3, pp. 367-373 (2017).

Reduction of coherent artefacts in super-resolution fluorescence localisation microscopy
A. P. Georgiades, V. J. Allan, M. Dickinson, T. A. Waight, Journal of Microscopy (2016); doi: 10.1111/jmi.12453

Correction-free remotely scanned two-photon *in vivo* mouse retinal imaging
A. Schejter Bar-Noam, N. Farah & S. Shoham, Light: Science & Applications (2016) 5, e16007; doi:10.1038/lsa.2016.7

High-speed microscopy with an electrically tunable lens to image the dynamics of in vivo molecular complexes
Y. Nakai, M. Ozeki, T. Hiraiwa, R. Tanimoto, A. Funahashi, N. Hiroi, A. Taniguchi, S. Nonaka, V. Boilot, R. Shrestha, J. Clark, N. Tamura, V. M. Draviam and H. Oku, Rev. Sci. Instrum. 86, 013707 (2015).

Multi-depth photoacoustic microscopy with a focus tunable lens
Kiri Lee, Euiheon Chung, Tae Joong Eom, Proc. of SPIE Vol. 9323 93233O-1 (2015)

Calcium transient prevalence across the dendritic arbour predicts place field properties
M. E. J. Sheffield, D. A. Dombeck, Nature 517, 200–204 (2015).

3d high- and superresolution imaging using single-objective SPIM
Remi Galland et al., Nature Methods 3402, 1-4 (2015)

Fast imaging of live organisms with sculpted light sheets
A. K. Chmielewski, A. Kyrsting, P. Mahou, M. T. Wayland, L. Muresan, J. F. Evers & C. F. Kaminski, Scientific Reports 5, Article number: 9385 doi:10.1038/srep09385 (2015).

A rapid image acquisition method for focus stacking in microscopy
D. Clark, B. Brown, Microscopy Today, Volume 23, Issue 04, pp 18-25 (2015)

Rapid quantitative phase imaging for partially coherent light microscopy
B. José A. Rodrigo and Tatiana Alieva, Optics Express, Vol. 22, Issue 11, pp. 13472-13483 (2014).

Investigation of diffraction-based measurement errors in optical testing of aspheric optics with digital micromirror devices
Stephan Stuerwald, Robert Schmitt, J. Micro/Nanolith. MEMS MOEMS 13(1), 1-8, (2014)

Technical improvements applied to a double-pass setup for performance and cost optimization
Ferran Sanabria et al., Optical Engineering 53(6), 061710 (2014)

Improved quantitative phase contrast in self-interference digital holographic microscopy and sensing dynamic refractive index changes of the cytoplasm using internalized microspheres as probes
B. Kemper, R. Schubert, S. Dartmann, A. Vollmer, S. Ketelhut, G. von Bally, SPIE Three Dimensional and Multidimensional Microscopy: Image Acquisition and Processing XX, Proceedings Vol. 8589 (2013).

Rapid 3D light-sheet microscopy with a tunable lens
F. O. Fahrbach, F. F. Voigt, B. Schmid, F. Helmchen, J. Huisken, Optics Express, Vol. 21, Issue 18, pp. 21010-21026 (2013).

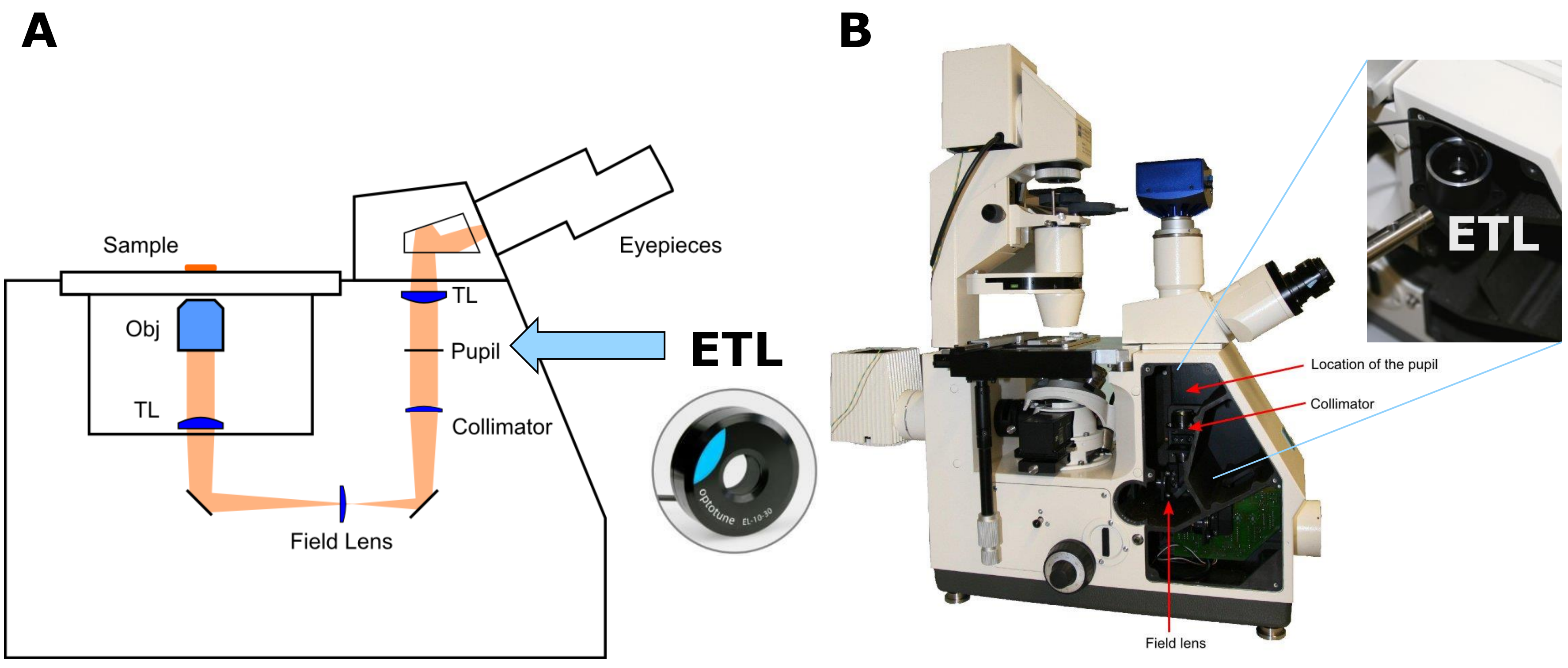
Online correction of licking-induced brain motion during two-photon imaging with a tunable lens
J. L. Chen, O. A. Pfäffli, F. F. Voigt, D. J. Margolis, F. Helmchen, Journal of Physiology, 00.00, pp. 1-10 (2013).

High-speed transport-of-intensity phase microscopy with an electrically tunable lens
C. Zuo, Q. Chen, W. Qu, and A. Asundi, Optics Express, Vol. 21, Issue 20, pp. 24060-24075 (2013).

Notch spatial filtering of image artifacts for structured illumination microscopy of cell-based assays
Jong-ryul Choi, Donghyun Kim, Optics Communications 308 (2013) 142–146 (2013)

Fast two-layer two-photon imaging of neuronal cell populations using an electrically tunable lens
B. F. Grewe, F. F. Voigt, M. van't Hoff, F. Helmchen, Biomedical Optics Express, Vol. 2, Issue 7, pp. 2035-2046 (2011).

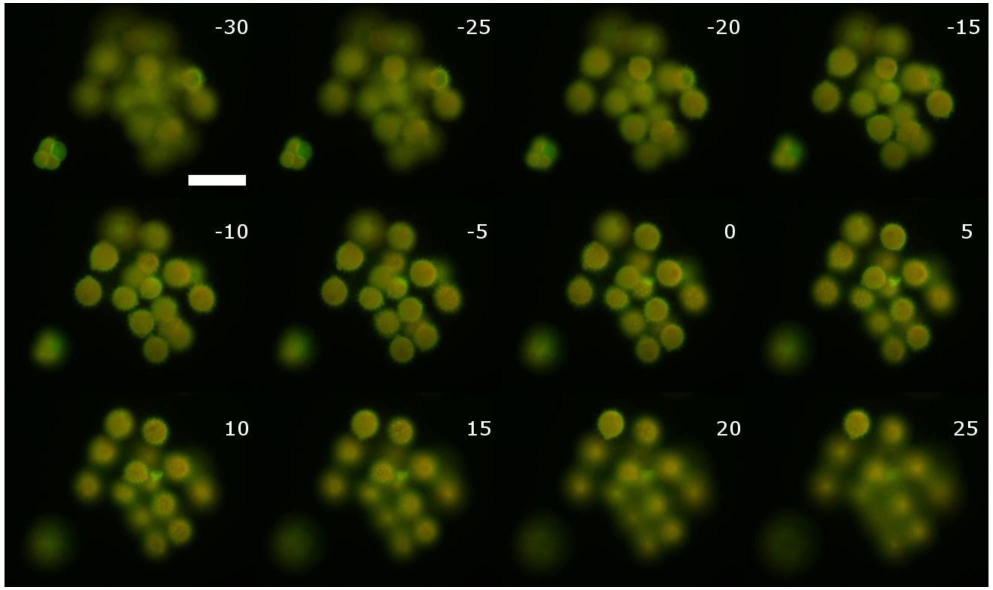
Wide-field microscopy

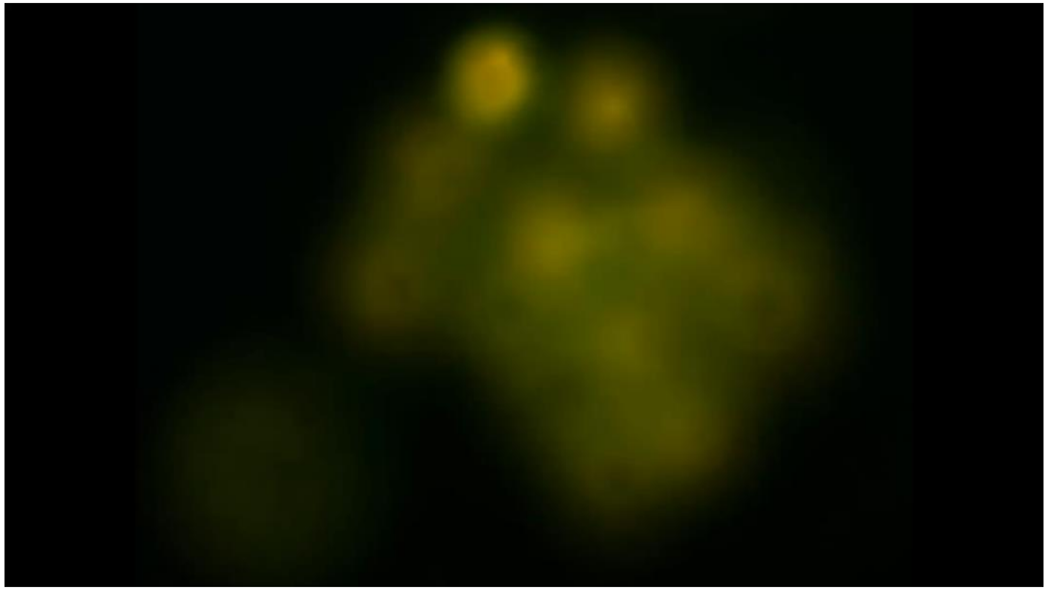


Optical path of the Axiovert 35 microscope. The ETL/OL assembly can be placed at the pupil without inserting an additional relay system. TL: Tube lens.

Images courtesy of F. F. Voigt, Department of Neurophysiology, Brain Research Institute, University of Zurich

Wide-field microscopy



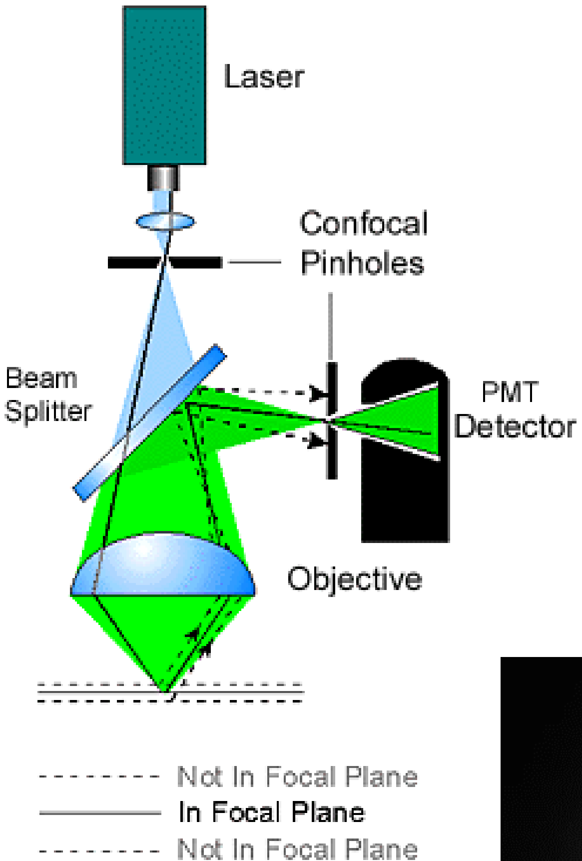


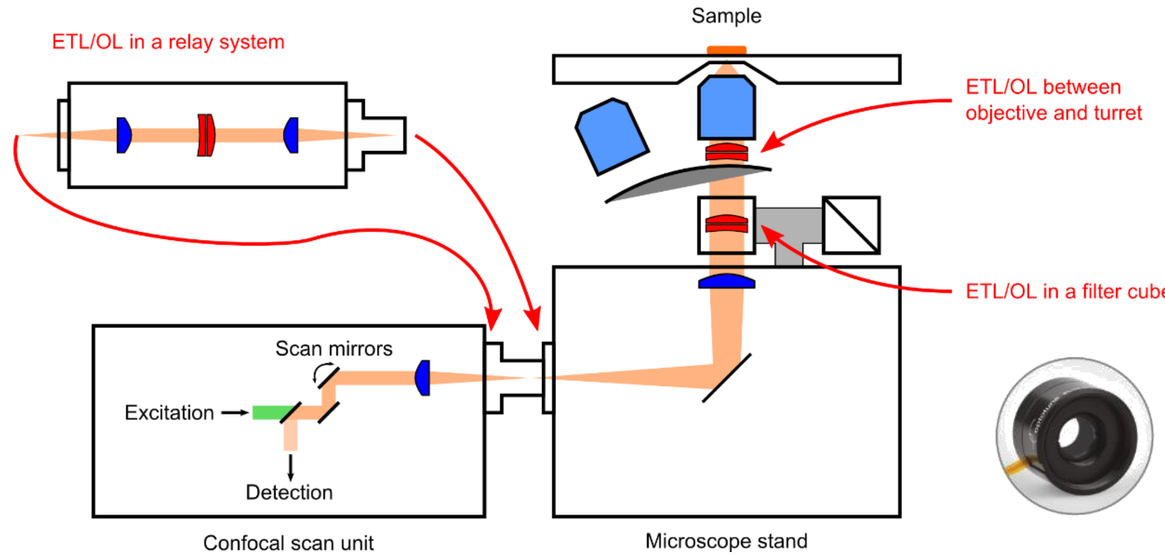
Video

ETL-based focusing through a group of pollen grains.

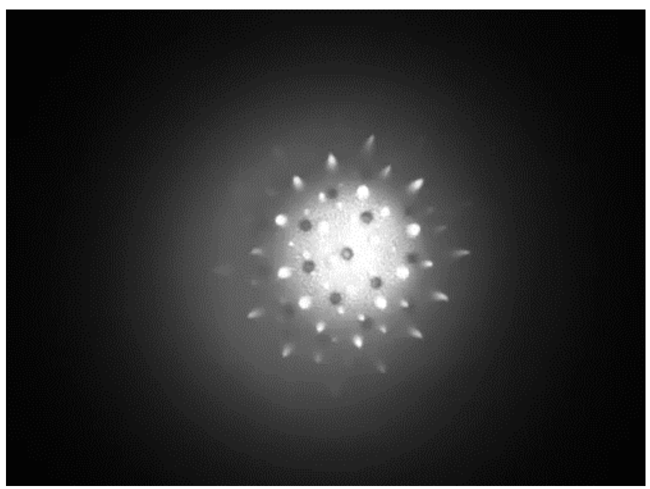
Images courtesy of F. F. Voigt, Department of Neurophysiology, Brain Research Institute, University of Zurich

Confocal microscopy



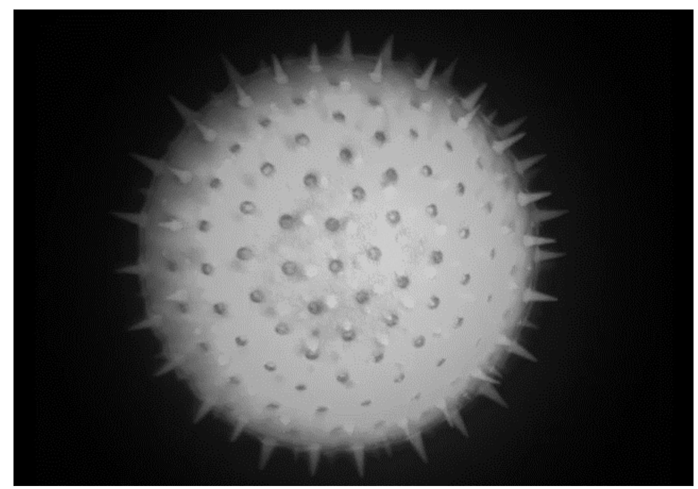


Images courtesy of
F. F. Voigt, Department
of Neurophysiology,
Brain Research Institute,
University of Zurich



Video

Max. intensity projection of a pollen corn



Video

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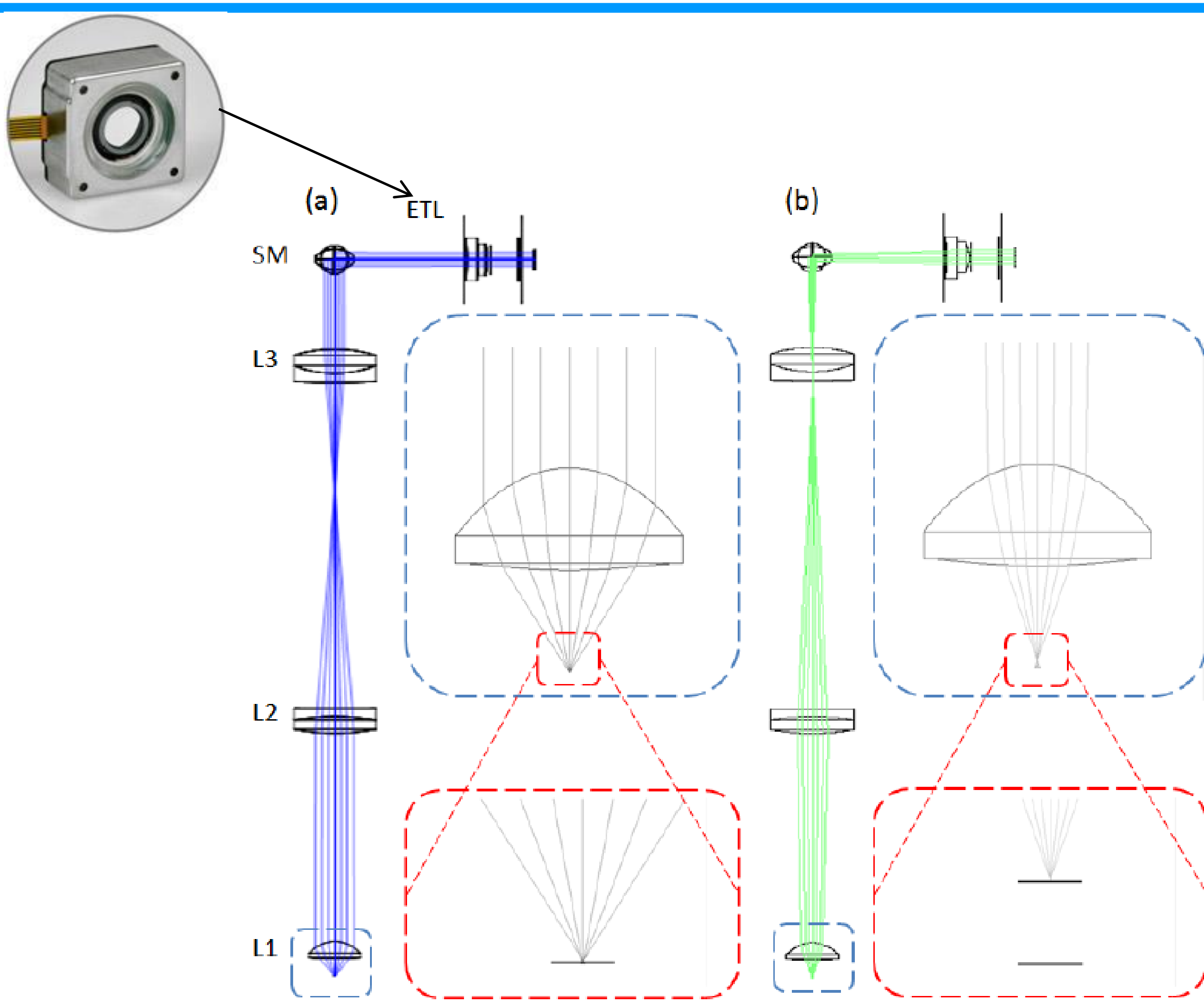
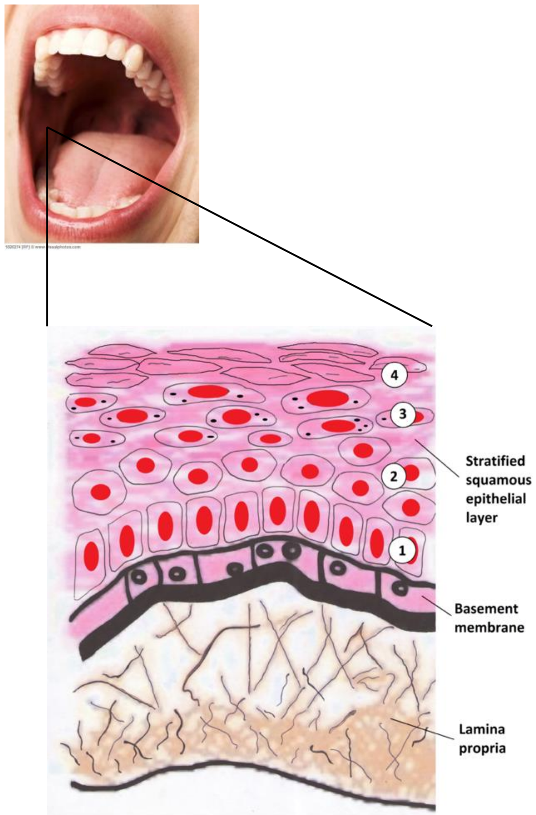
Confocal endomicroscopy



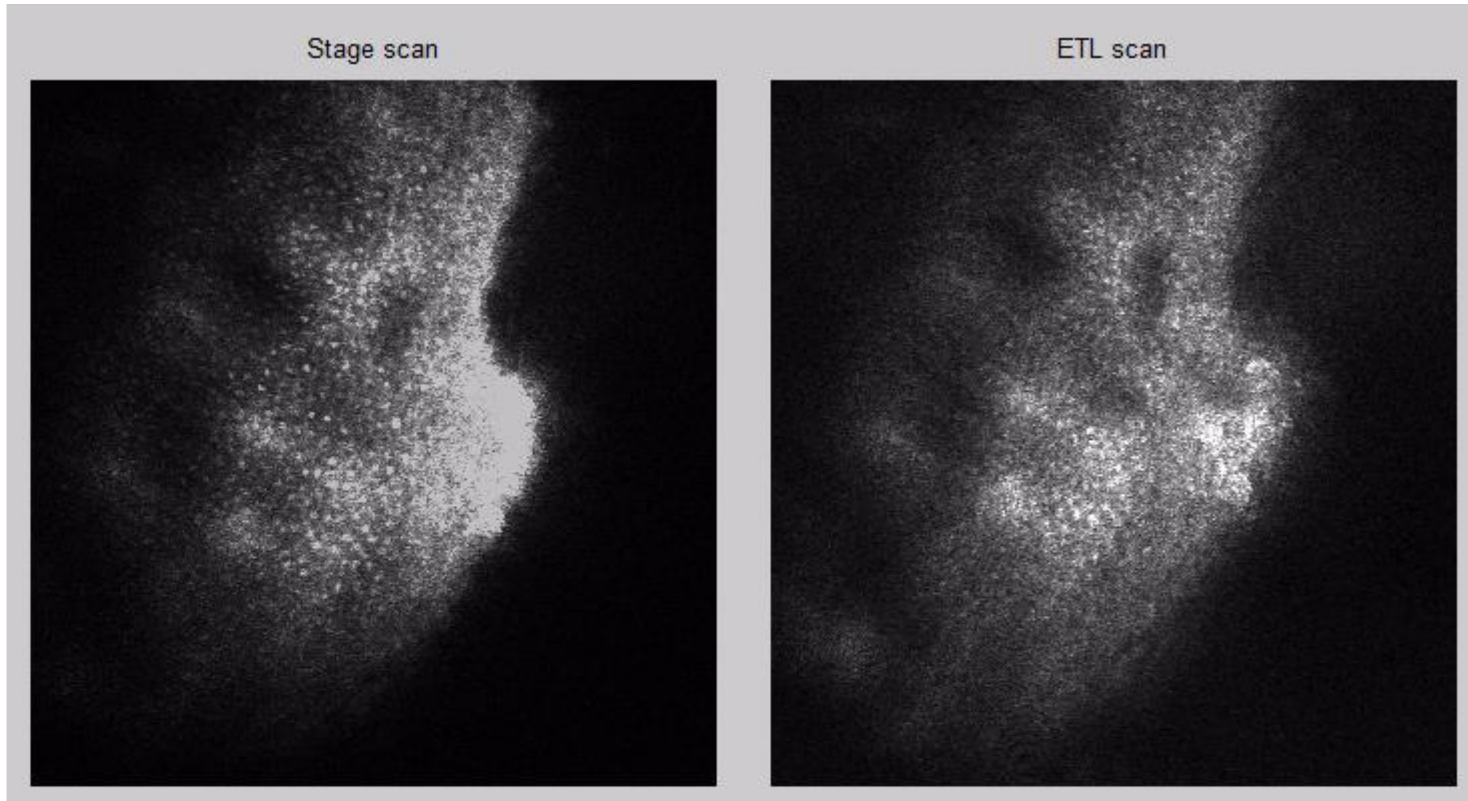
Focal power range	-127 mm to +44.3 mm
Axial scan range @ sample	700µm

Ref: J.M. Jabbour et al., BIOMEDICAL OPTICS EXPRESS 2014, **5**, (2), pp. 645, 2014, “Optical axial scanning in confocal microscopy using an electrically tunable lens”

Confocal endomicroscopy



Traditional approach | **Optotune approach**

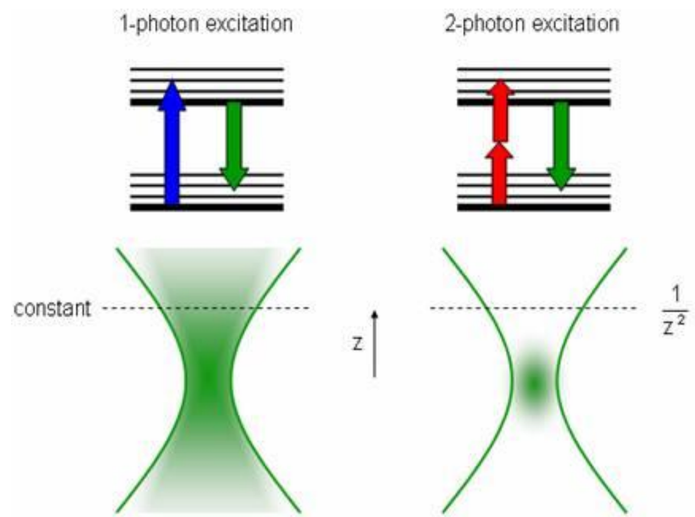


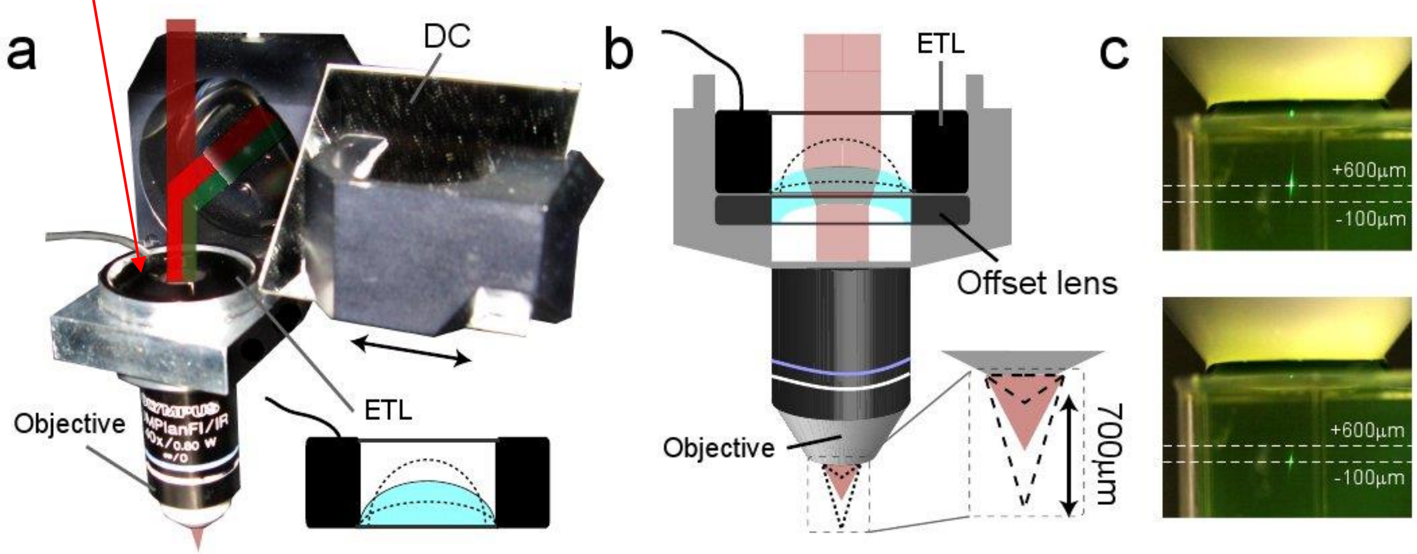
Scan through oral mucosa *ex vivo*

[Video]

Ref: J.M. Jabbour et al., BIOMEDICAL OPTICS EXPRESS 2014, **5**, (2), pp. 645, 2014, "Optical axial scanning in confocal microscopy using an electrically tunable lens"

Two-photon microscopy

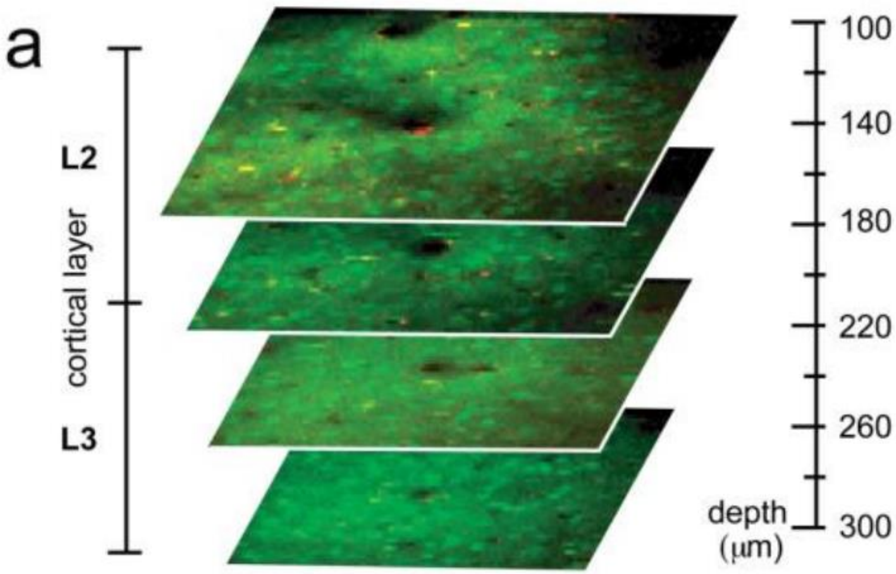


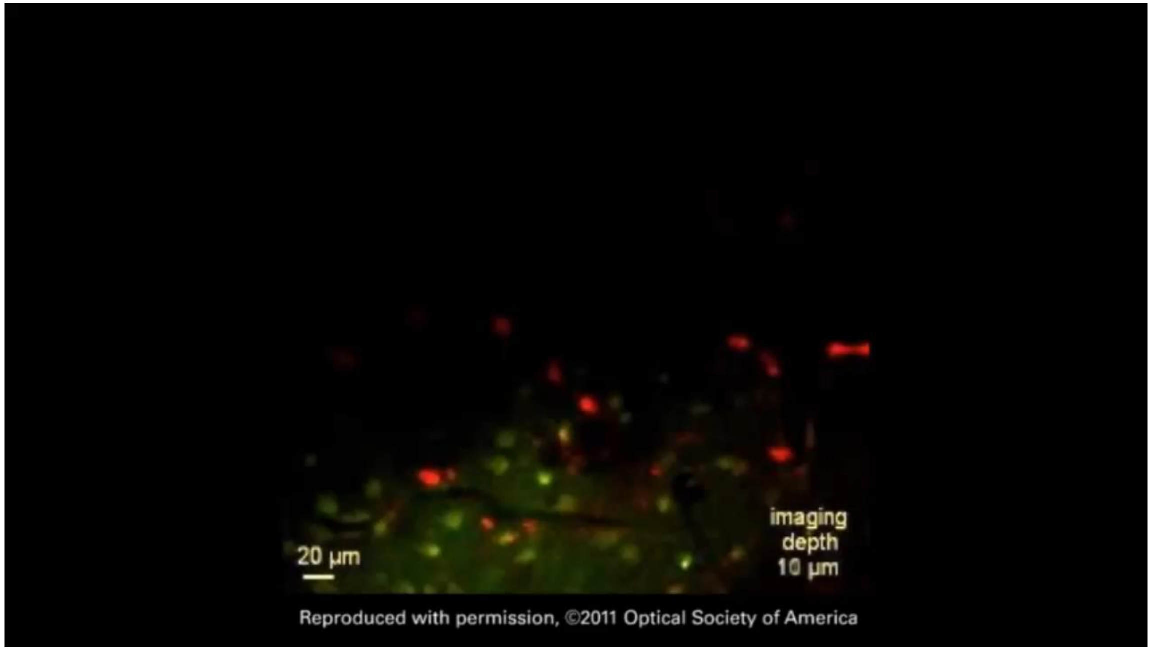


B.F Grewe *et al*., Biomedical Express (2011), **2**, (7), pp.2035

Two-photon microscopy

Example: Two-photon two-layer calcium imaging in mouse neocortex



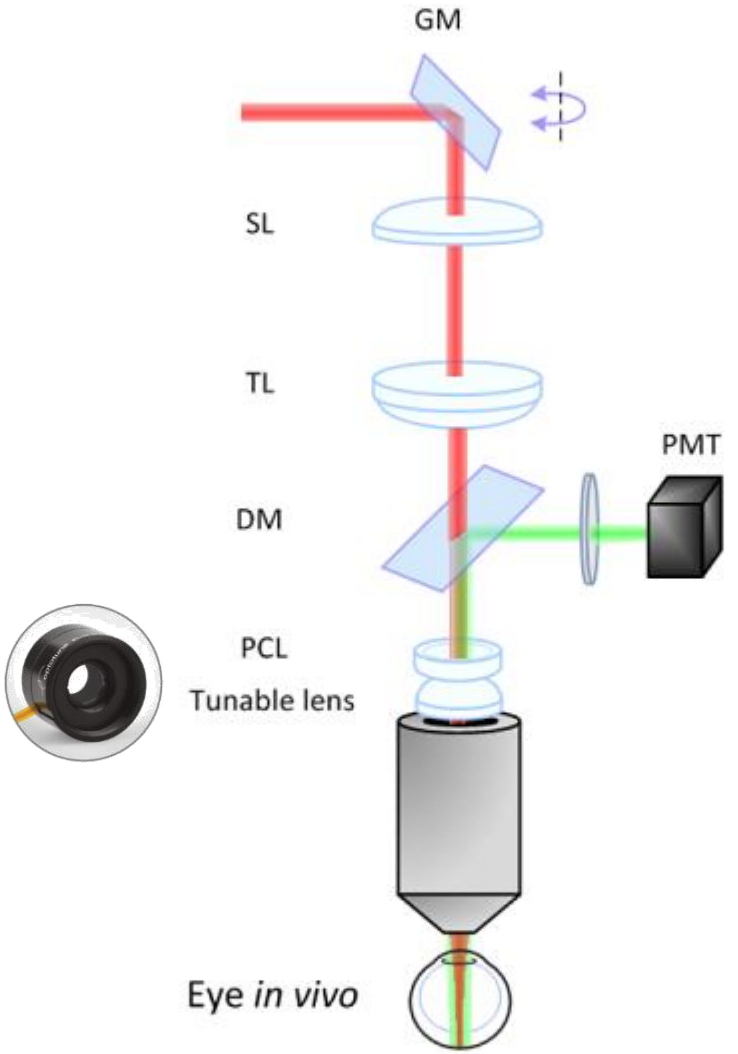


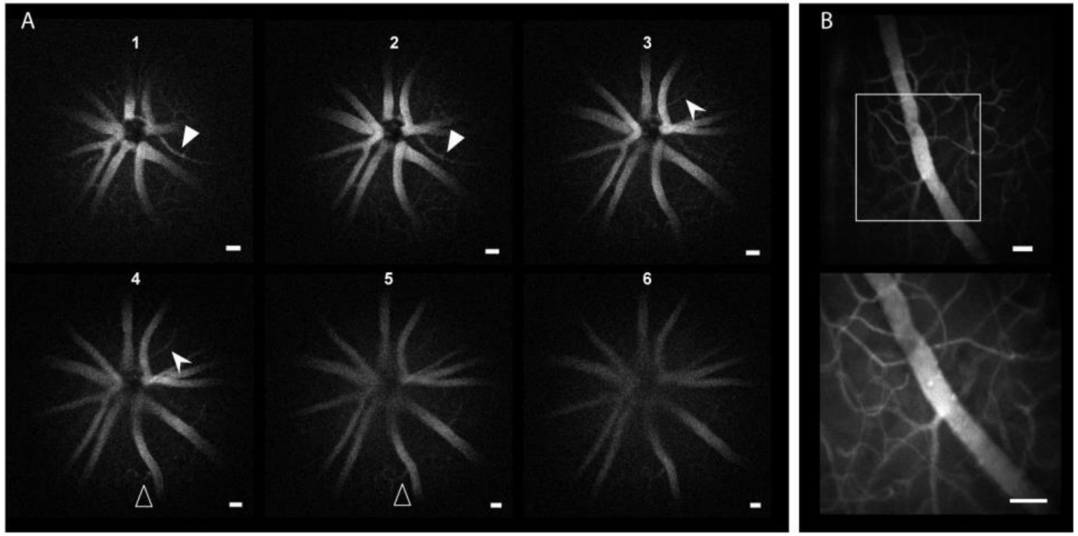
Two-photon images of a stained neuronal cell population (green)

Video

Benjamin F. Grewe, BIOMEDICAL OPTICS EXPRESS (2011), **2**, (7), pp. 2035

Two-photon in-vivo imaging of retinal microstructures

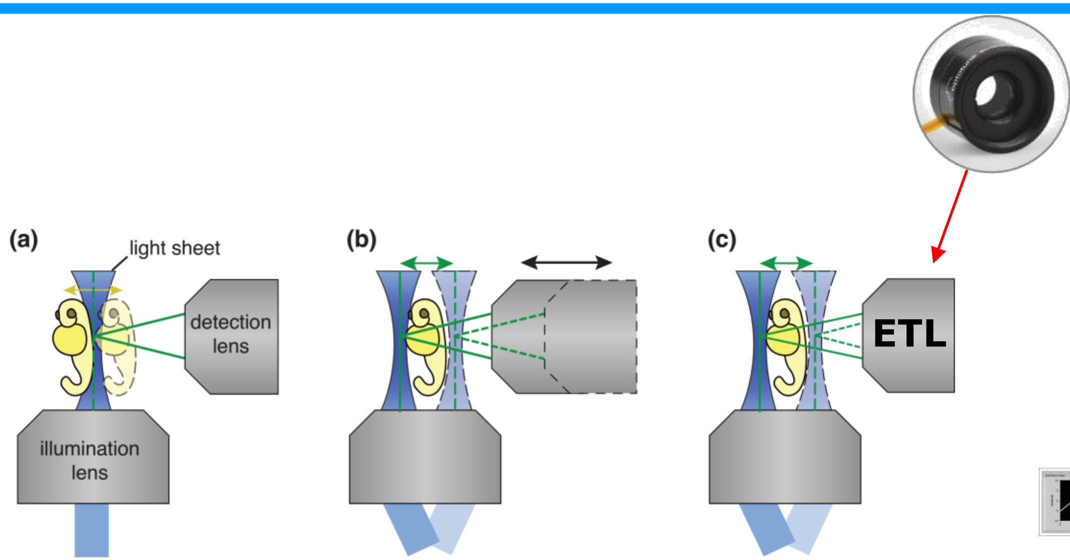


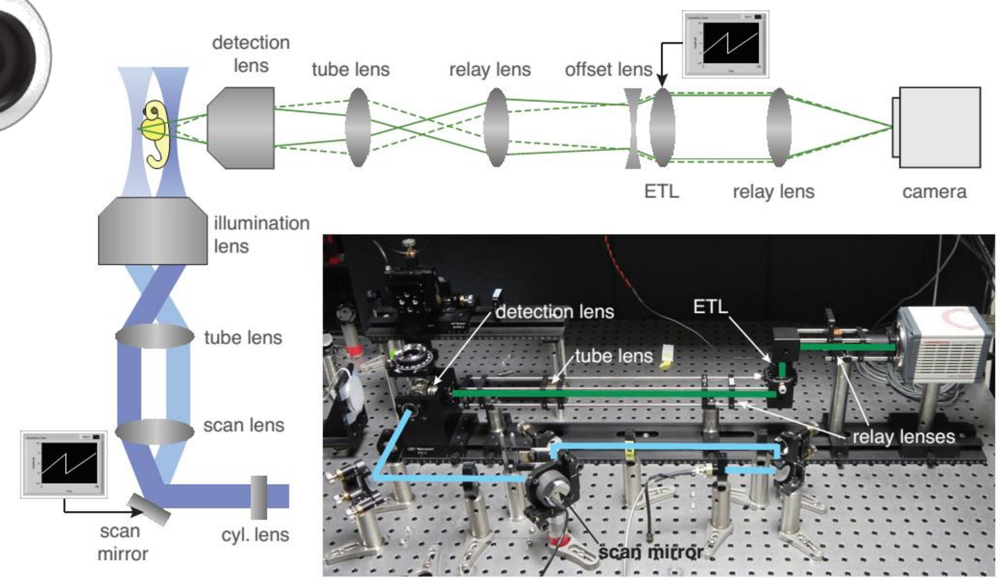


Optical sectioning in mouse 2P fluorescence angiography. A. Two-photon images of the optic disc. The microscope objective lens and mouse were held in place, and each image was acquired at different ETL currents (10mA interval between successive images; each image is an average over 30 frames acquired at 1 fps). Arrowheads point to blood vessels visible in only a few images, but not in others. B. Images of blood vessels outside the optic disc, acquired at different scan zooms (average over 100 and 200 frames; different animal than A). The FOV of the lower image is marked by a white box. Scale bars = 50 µm.

Adi Schejter, Proc. SPIE 8948, Multiphoton Microscopy in the Biomedical Sciences XIV, 894824 (February 28, 2014); doi:10.1117/12.2039375

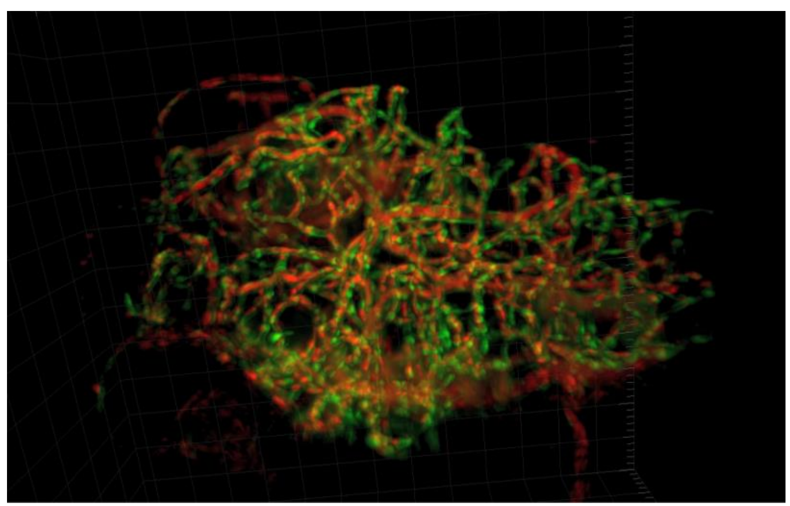
Light-sheet microscopy

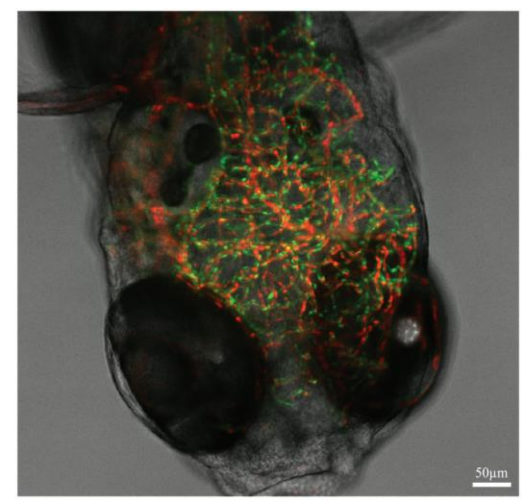




Vascular system in the brain of a zebrafish

Video



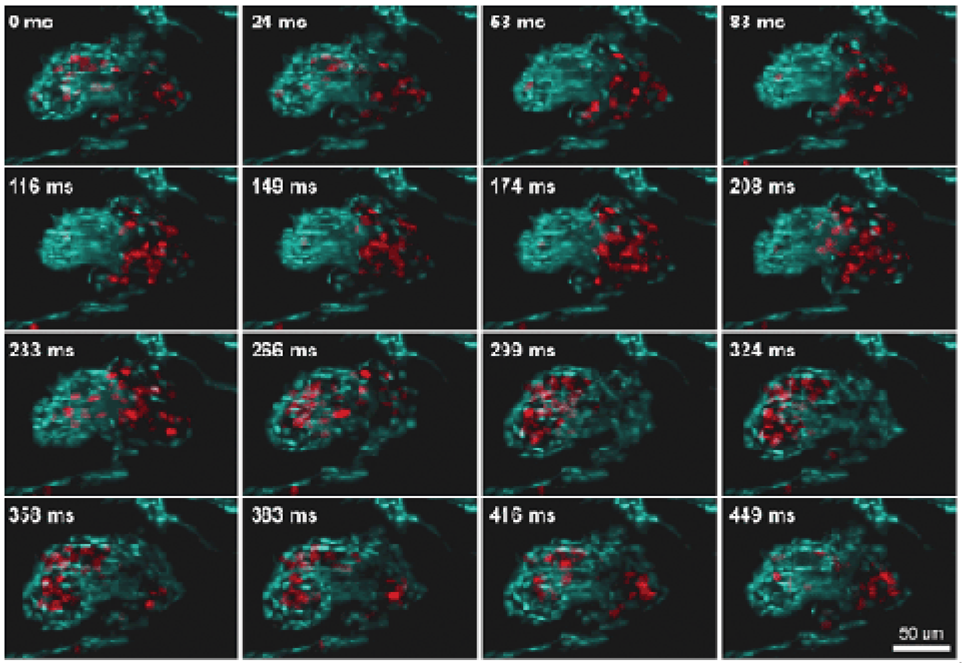


F. O. Fahrbach *et al.,* Opt. EXPRESS (2013), **21**, (18), pp. 21010.

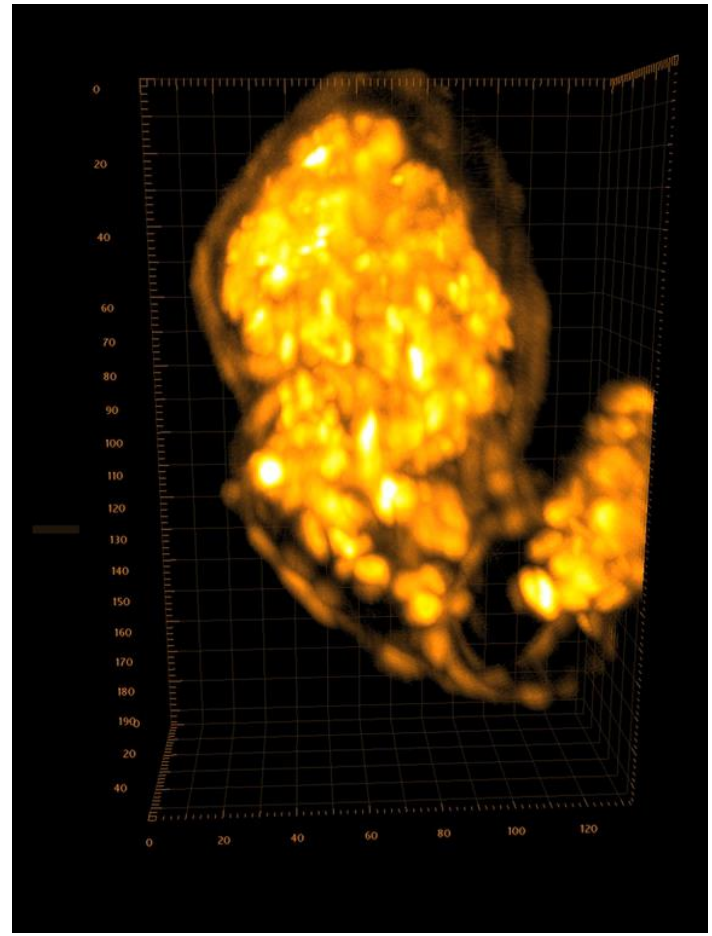
optotune

Light-sheet microscopy

Large volume scan with an ETL through the heart of a zebrafish (10x magn.)



Courtesy of Florian Fahrbach, Michaela Mickoleit and Jan Huisken.

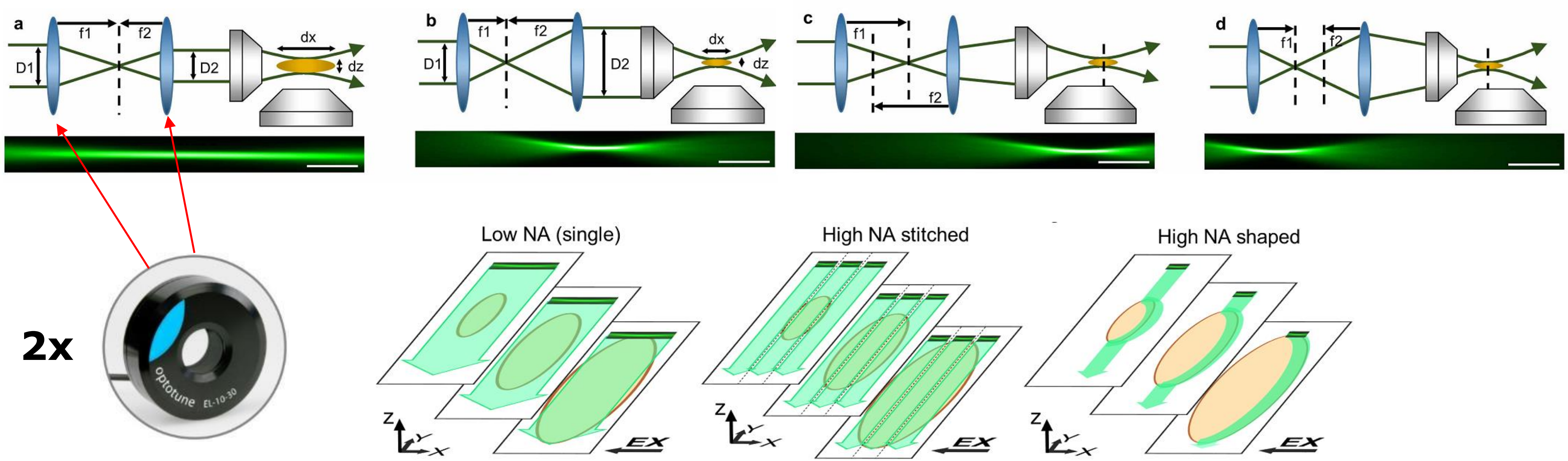


[Video](#)

F. O. Fahrbach *et al.,* Opt. EXPRESS (2013), **21**, (18), pp. 21010.

Light sheet microscopy

- Goal: *Optimize with help of tunable lenses the illumination light-sheet to the requirement at hand.*
- A telescope composed of two electrically tuneable lenses enable to define thickness and position of the light-sheet independently but accurately within milliseconds, and therefore optimize image quality of the features of interest interactively.
- This technique proved compatible with confocal line scanning detection, further improving image contrast.



A. K. Chmielewski et al., Nature Scientific Reports 5, Article number: 9385 doi:10.1038/srep09385 (2015).

3D High- and super-resolution imaging using single-objective SPIM

- Single-objective selective-plane illumination microscopy (soSPIM) is achieved with micro-mirrored cavities combined with a laser beam–steering unit installed on a standard inverted microscope.
- Based on custom EL-C-10-30 focus-tunable lens (TL) from −80 mm to +1,000 mm.

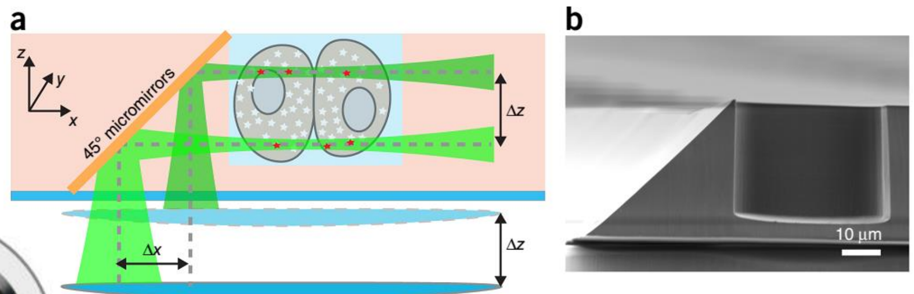


Figure 1 | Principle and 3D high-resolution capabilities of the soSPIM method. (**a**) Schematic representation of soSPIM. A light sheet is created by reflection from a 45° mirror. The excitation-beam displacement (Δ*x*) along the mirror combined with the axial positioning of the objective (Δ*z*) enables 3D-volume imaging.

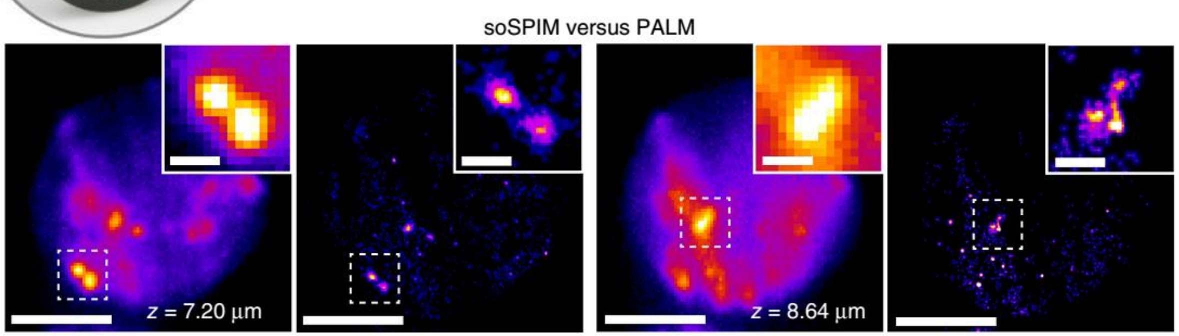


Figure 2 | 3D super-resolution capabilities of the soSPIM method. (**a**) High-resolution (two leftmost panels) and PALM super-resolution (two rightmost panels) images of a U2-OS cell nucleus expressing the nucleolus protein fibrillarin-Dendra2 at two different planes 1.44 µm apart (representative images; $n = 15$).

Remi Galland, Nature Methods, published online 11 May 2015DOI:10.1038/NMETH.3402

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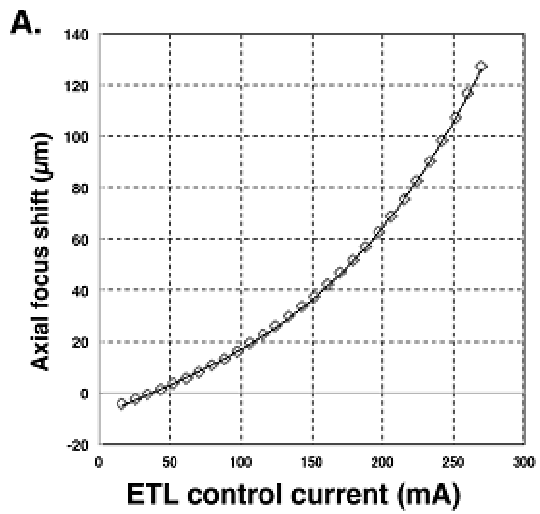
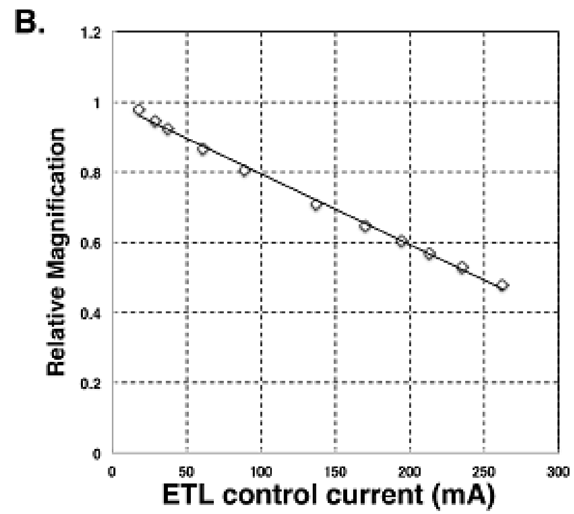
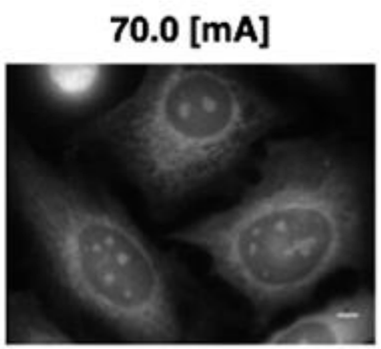
Z-stacking with inverted microscope, 100x mag

B.

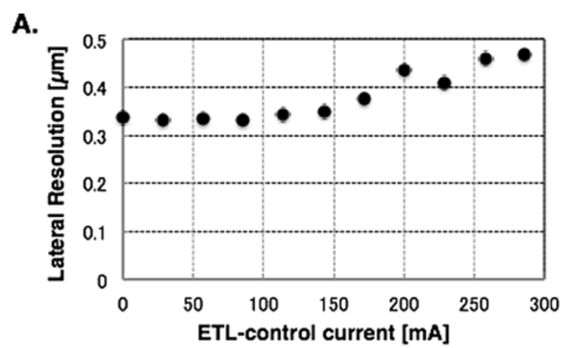
Optotune
EL-10-30

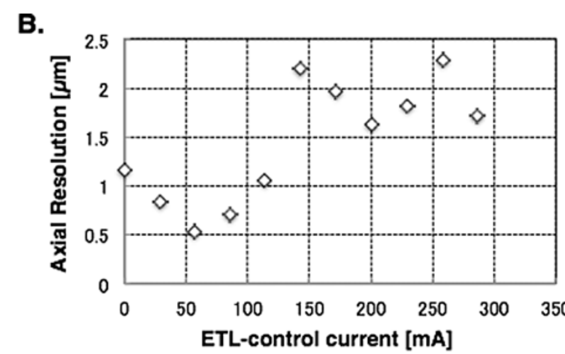






microtubules in HeLa cells





http://scitation.aip.org/content/aip/journal/rsi/86/1/10.1063/1.4905330

optotune

A Rapid Image Acquisition Method for Focus Stacking in Microscopy

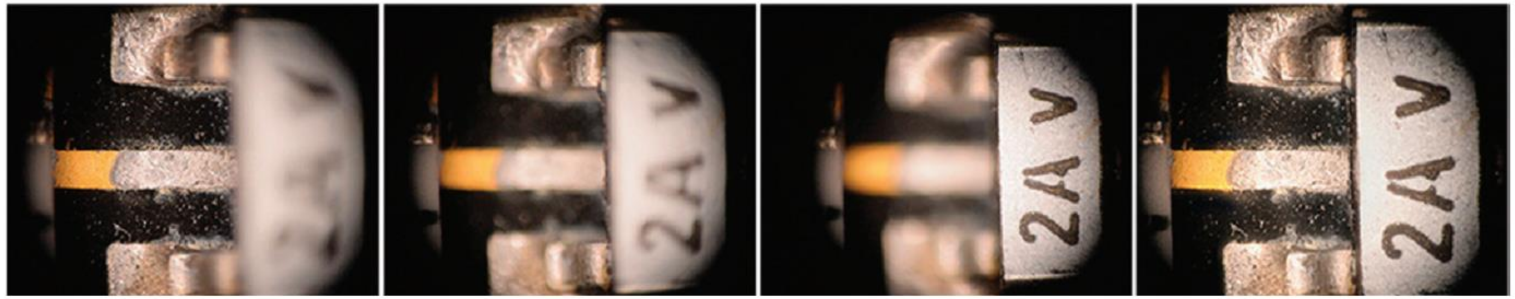
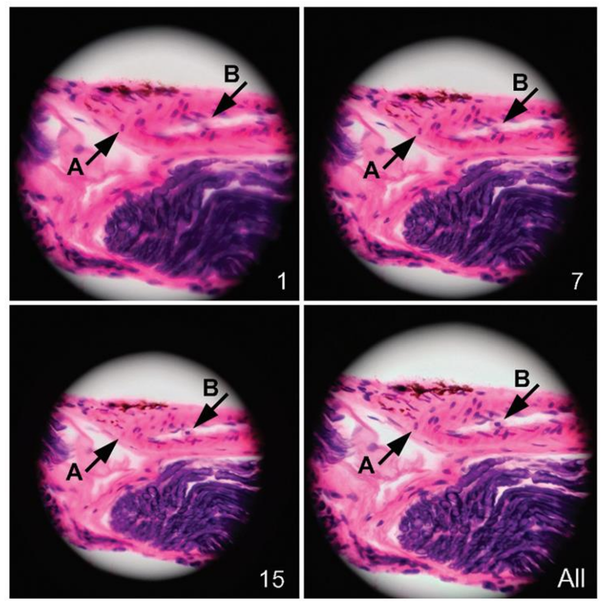


Figure 9: The circuit board. Left to right: the circuit board in focus, halfway between the circuit board and the top of the component, the top of the component, and the processed image completely in focus. 5× objective, stack of 27 images, final image diameter ≅3.0 mm, acquisition time = 0.45 sec.



D. Clark, Microscopy Today / Volume 23 / Issue 04 / July 2015, pp 18-25Copyright

DOI: http://dx.doi.org/10.1017/S1551929515000577

Figure 12: Mammalian tissue specimen. Image 1 is focused at the lowest level where feature A is in focus. Image 7 is focused near the center of the specimen. Image 15 is focused at the top where feature B is in focus. Image All is a processed image showing all features in focus. 50× objective, stack of 15 images, final image diameter ≅0.3 mm, acquisition time = 0.25 sec.

Image Stacking example (Python + Helicon Focus)

- C-mount camera
- Empty C-mount tube, 40-60mm long
- **Optotune lens EL-10-30-Ci-VIS-LD**
- M22 to C-mount adapter
- 25mm lens (reversed!) Edmund Optics 85358

Working distance: ~20mm

Python Script

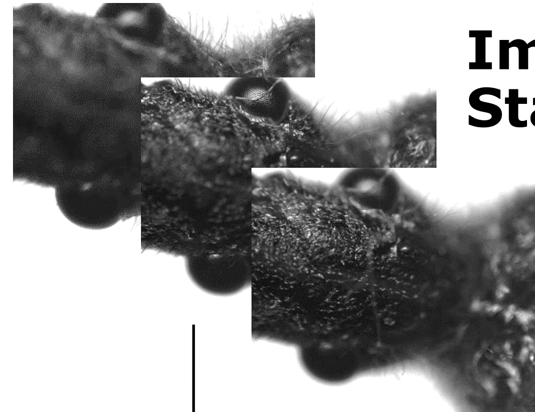


Image Stack

Helicon focus

Hyperfocus Image

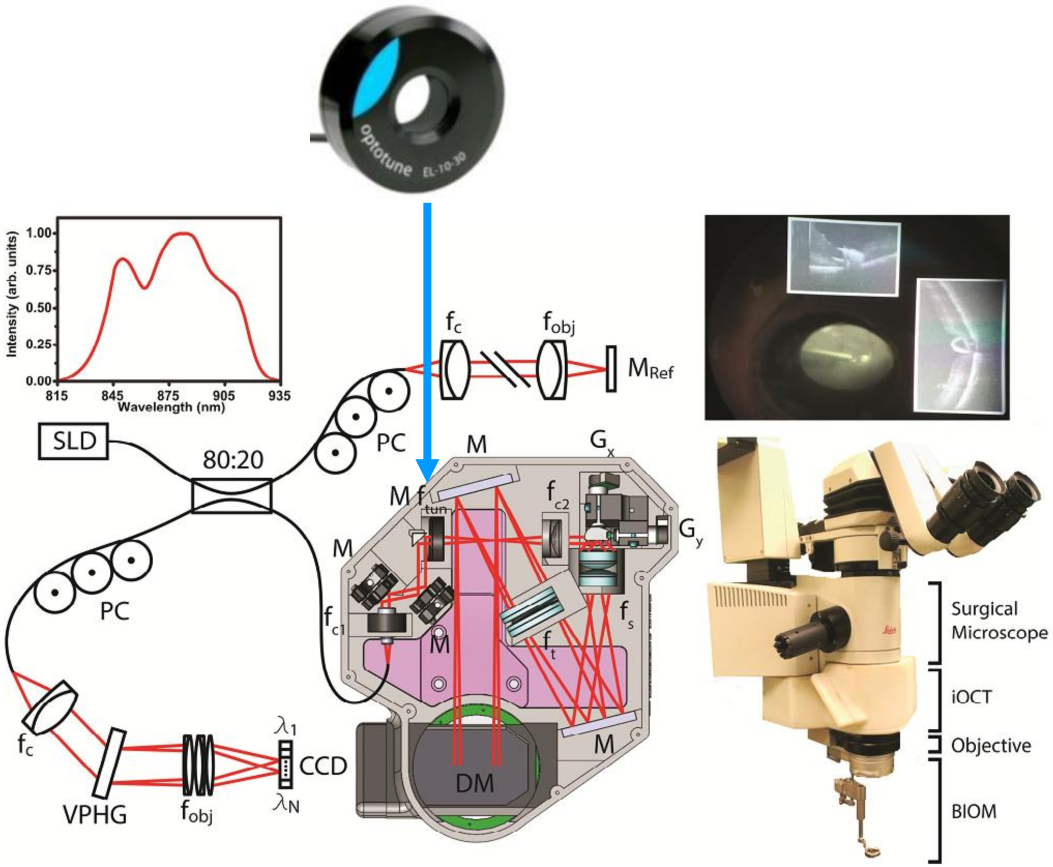
optotune

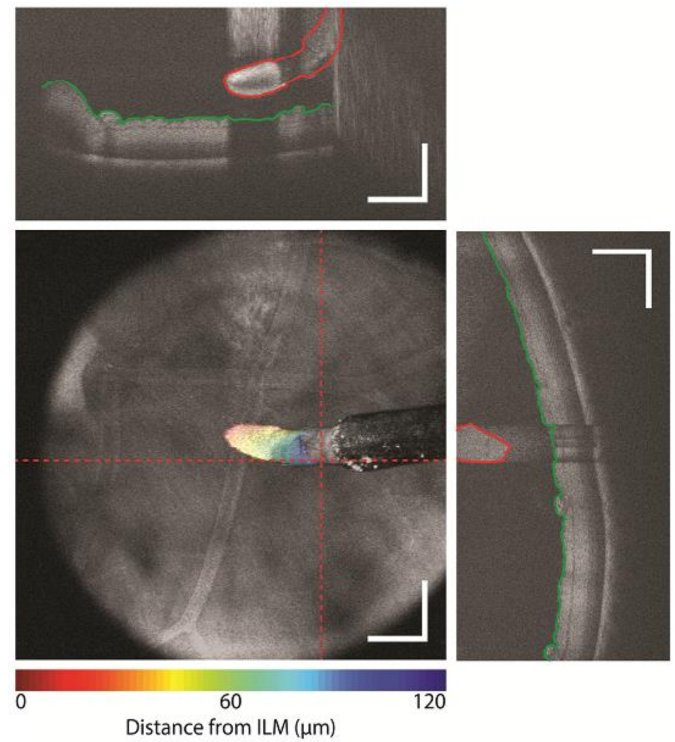
Sanxo scope: Inspection at HD

- Inspection station with 10MP camera
- EL-10-30-Ci in front lens configuration with 25mm lens
- Driver integrated in machine vision software “Modular X”
- Features:
  - Click to autofocus
  - Focal sweep with 3D rendering

Microscope-integrated intraoperative OCT

- Optotune's electrically tunable lens EL-10-30-NIR-LD allowed real-time adjustment of the OCT focal plane to maintain parfocality with the microscope view.
- Potential for iOCT-guided maneuvers and clinical decision-making in ophthalmic surgery





Y. K. Tao *et al.,* BIOMEDICAL OPTICS EXPRESS (2014), **5**, (6), pp. 1877.

Autofocus for high magnification with EL-10-30-C and Optem® 70XL by Qioptiq

C-mount camera
1/2.5" 5MP sensor

1.5x mini tube lens
P/N 29-90-28-000

Optotune lens
EL-10-30-Ci-VIS-LD-MV

Optem 70XL zoom (0.75x-5.25x)
P/N 399510-309

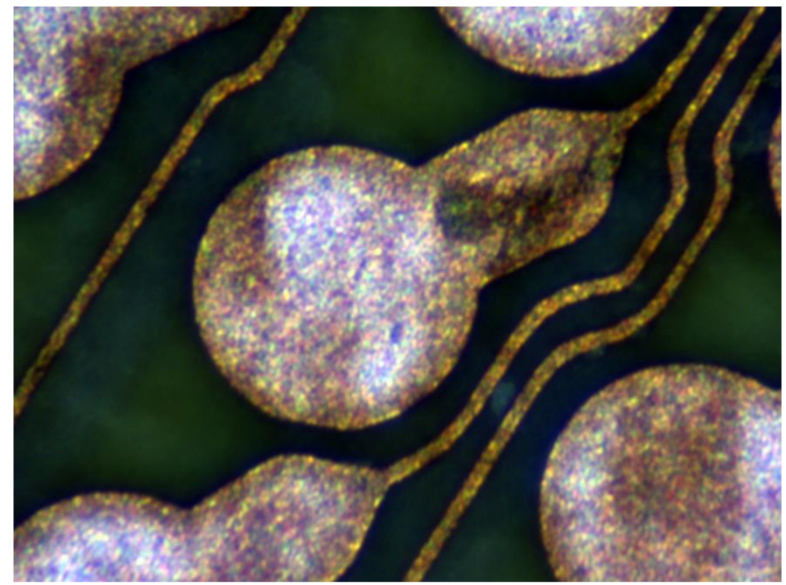
Coaxial lighting unit with lens
P/N 296515-310

LED ring light (used instead)

Working distance: ~90mm

Results:

Magnification	1.1x	3.5x	7.9x
Z range	400mm	40mm	8mm
Z resolution	100µm	10µm	2µm
DOF (approx.)	1mm	0.3mm	0.1mm
HFOV	4.5mm	1.4mm	0.65mm

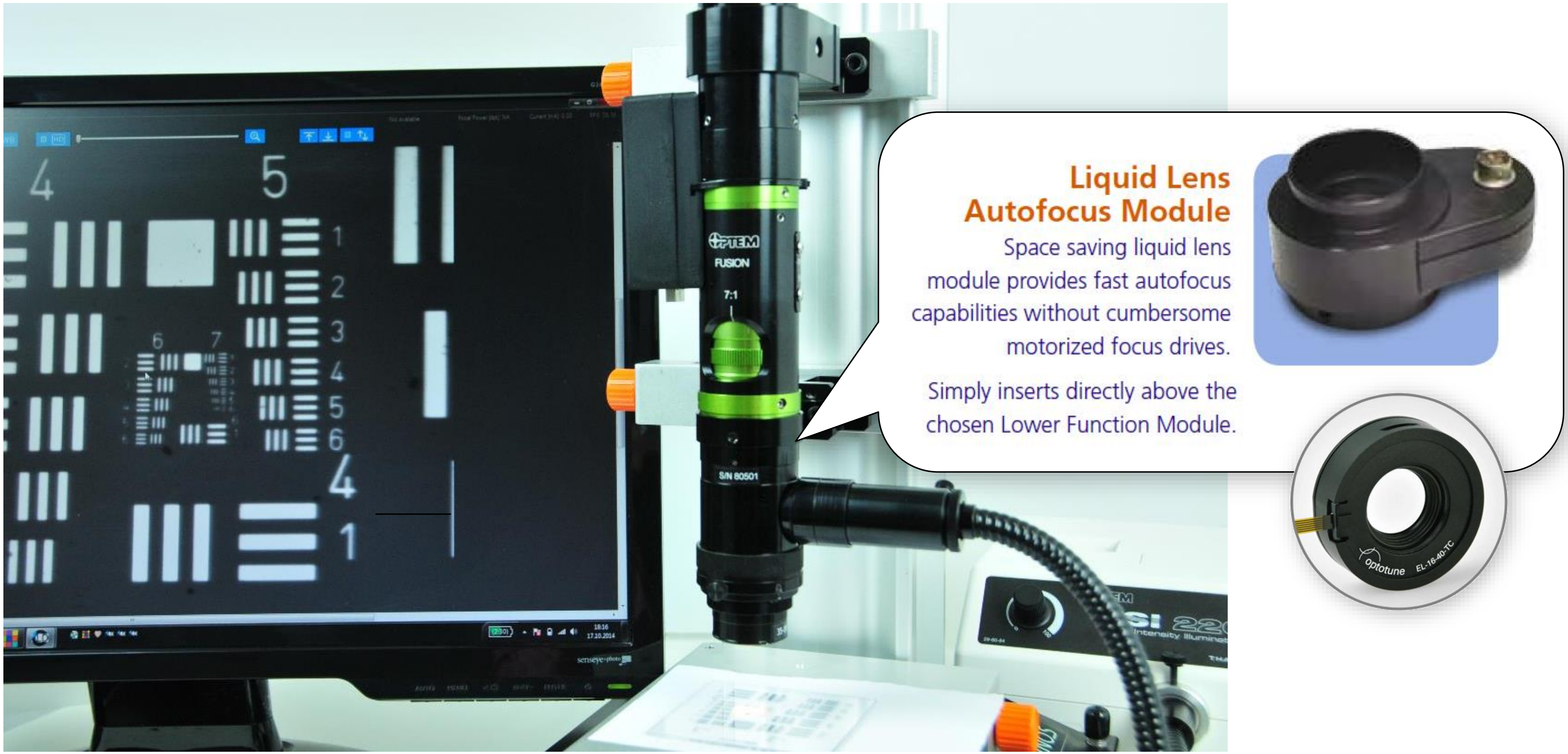


- No vignetting
- Off-the-shelf components

Optem® is a registered trademark of Qioptiq, Inc

Qioptiq Optem Fusion industrial microscope with EL-16-40-TC autofocus module

- The zoom is parfocal as the EL is placed BELOW the zoom



http://www.qioptiq.com/optem-fusion-lens, Optem® is a registered trademark of Qioptiq, Inc

optotune

Low cost AF microscope with fixed mag

C-mount camera

Empty C-mount tube,
40-60mm long

Optotune lens
EL-10-30-Ci-VIS-LD

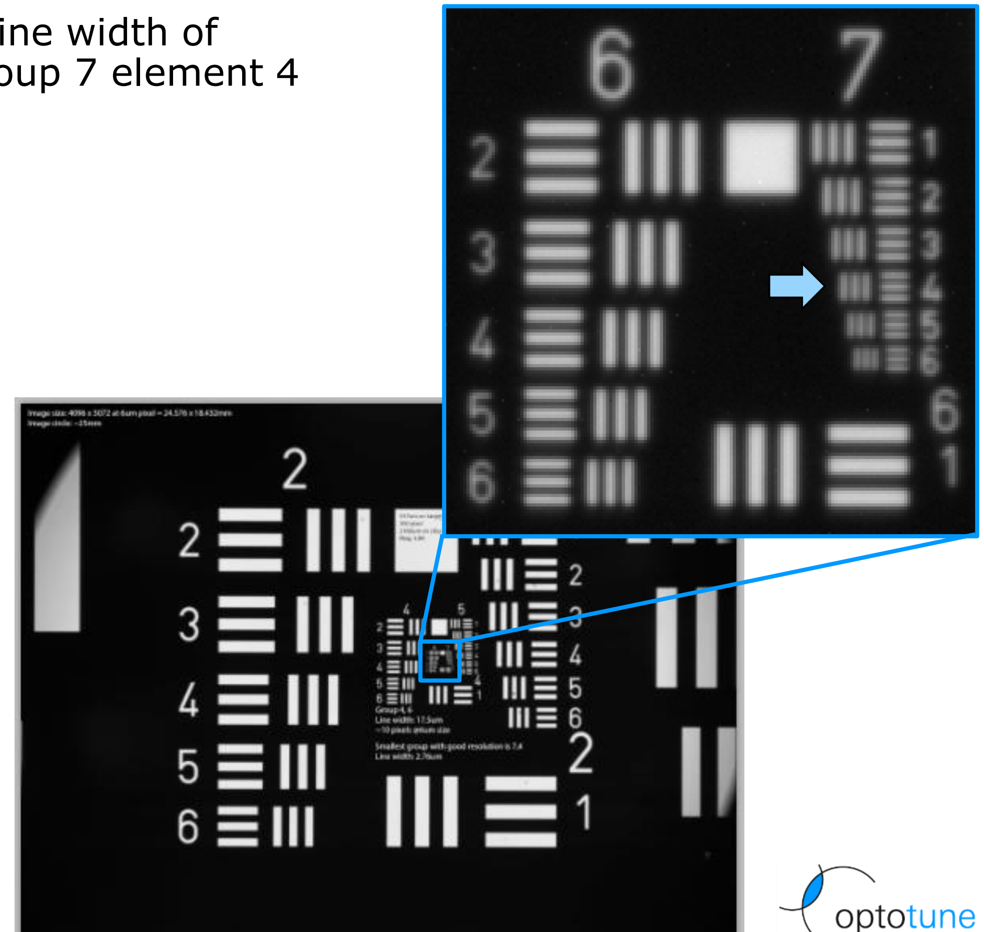
M22 to C-mount adapter

25mm lens (reversed!)
Edmund Optics 85358

Working distance: ~20mm

Tube length	40mm	60mm
Magnification	3X	4X
Z-range:	~3mm	~2mm
Resolution*:	3.7um	2.8um
Image circle	25mm	25mm

*Line width of
group 7 element 4



Z-stepping solutions for microscopes and industry based on EL-10-30

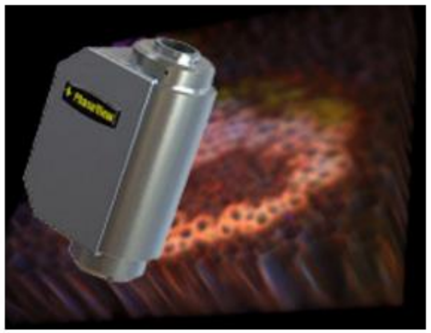
PhaseView®

Life Sciences & Scientific Imaging

Microscopy Volume Imaging Solutions

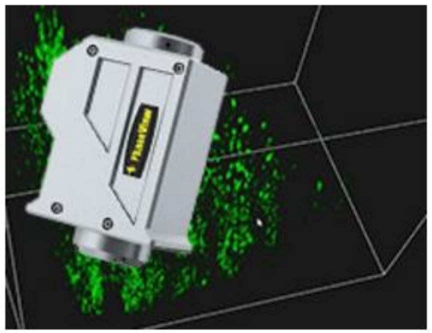
Industries & Quality Control

3D Solutions For Microscopes And Automated Vision Systems



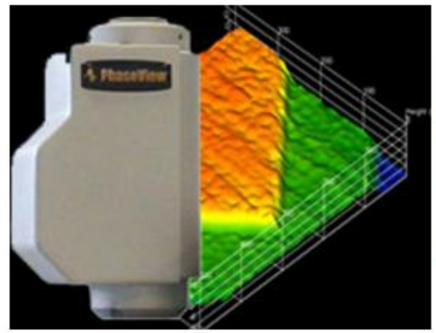
NeoScan

Fast Volume Scanning



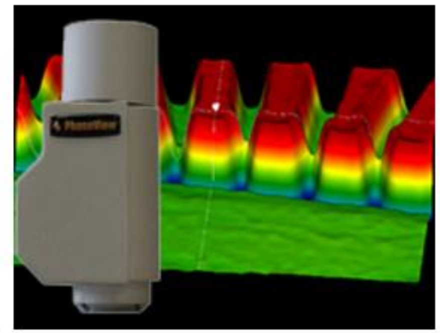
ThunderScan

Ultra High Speed Scanning



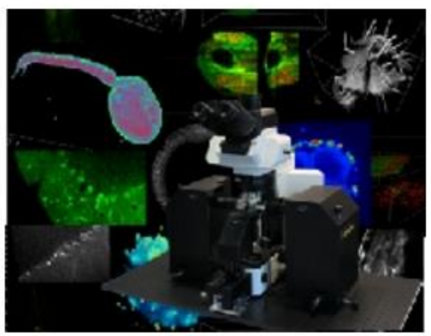
ZeeScan

3D Add-On for microscopes



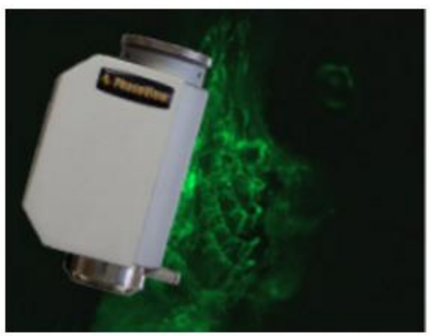
ZeeCam

3d microscope camera



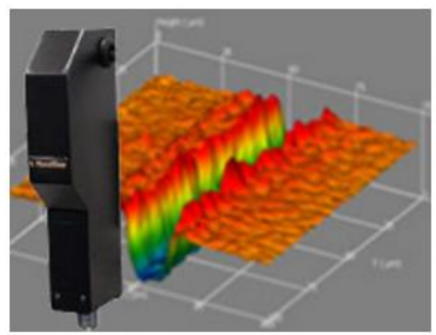
Alpha^3

Light Sheet Microscope



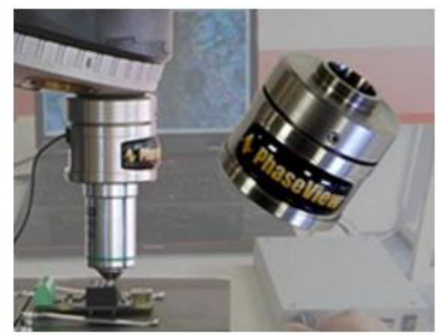
InSight

Real Time 3D Acquisition



ZeeScope

3d measurement microscope



SmartScan

Motorless focus control

www.phaseview.com

Edmund optics dynamic focus VZM with the EL-10-30-Ci-VIS-LD-MV integrated

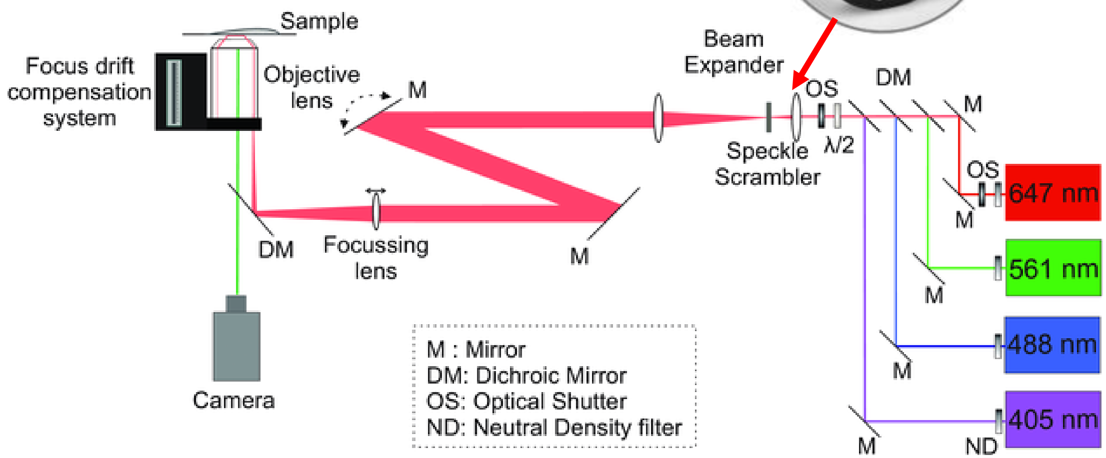
- Very large focus range as EL is placed close to aperture stop
- The zoom is NOT parfocal, however, as the EL is placed above the zoom

Magnification setting	0.75X	1X	2X	3X	4X	4.5X
Magnification range	0.65X - 1.15X	0.9X - 1.2X	1.5X - 2.0X	2.4X - 3.0X	3.2X - 4.0X	3.7X - 4.6X
Working distance (mm)	20 - 101	20 - 100	54 - 90	75 - 90	82 - 90	84 - 90
Horiz. FOV (1/2" sensor)	9.8 - 5.6	7.1 - 5.3	4.3 - 3.2	2.7 – 2.1	2.0 - 1.6	1.7 - 1.4

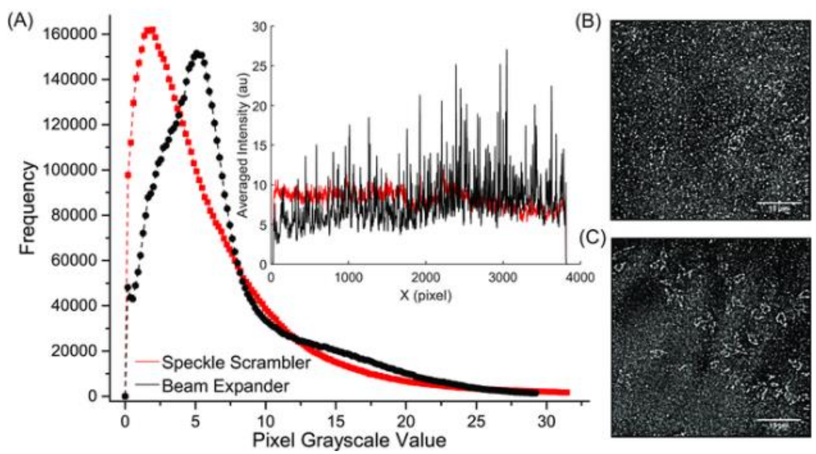
Optotune's LSR boosts image quality in super-resolution fluorescence microscope (STORM)

Setup:

LSR-3005-17S-VIS

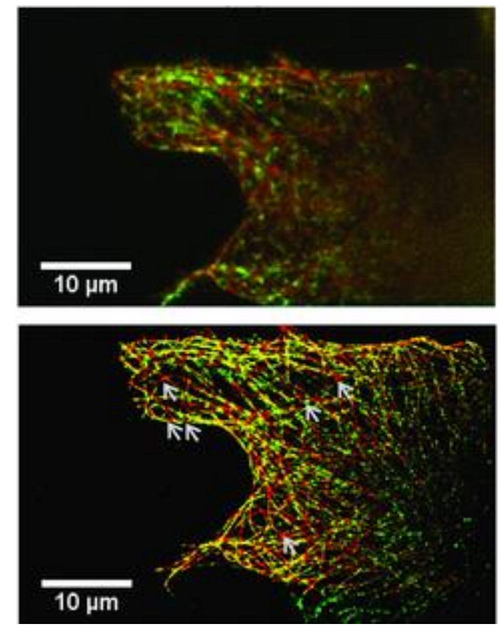


Distribution of pixel greyscale values:



LSR off

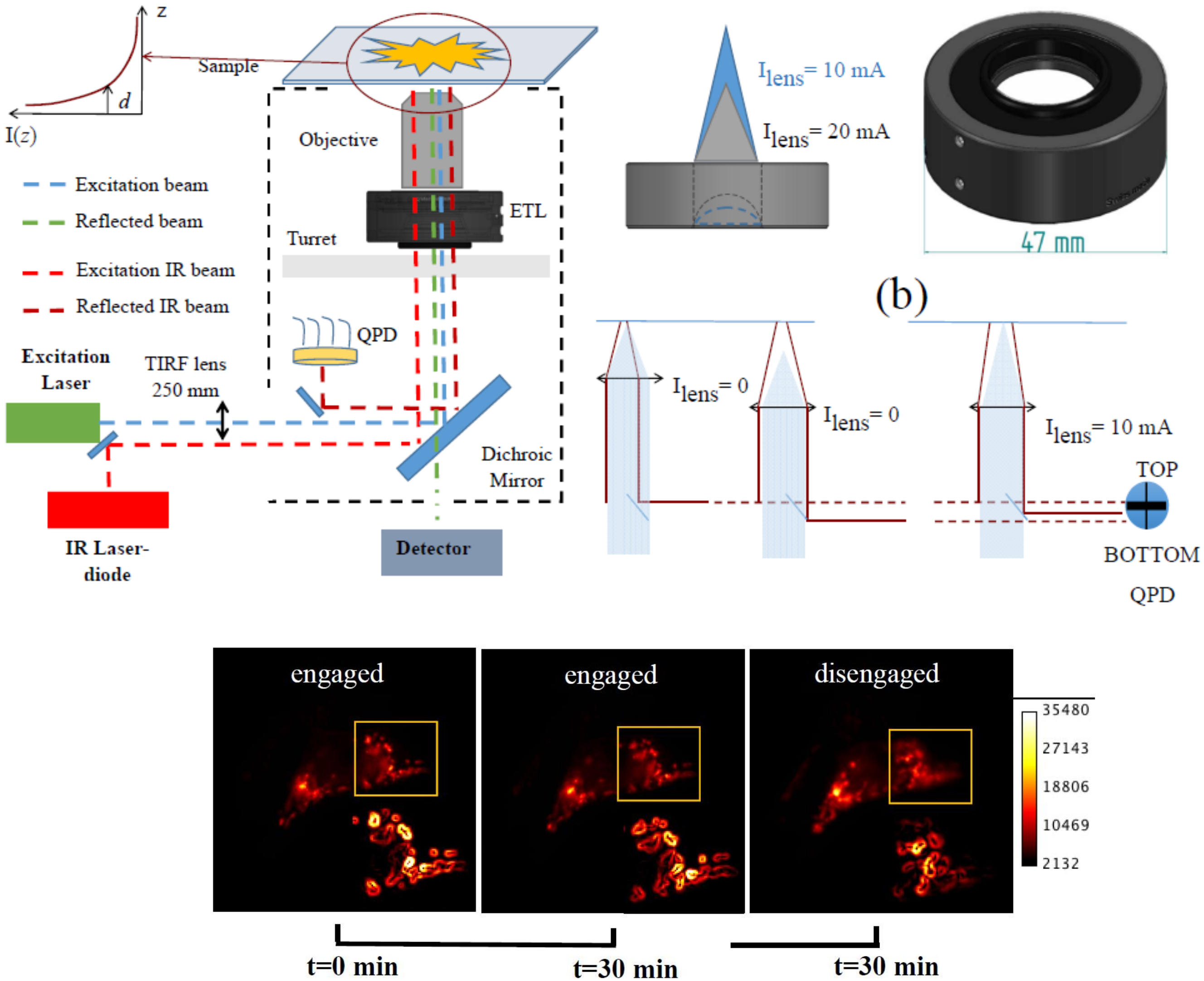
LSR on



MRC5 cells stained with Alexa Fluor 647

Ref: P. Georgiades et al., Journal of Microscopy (2016), http://onlinelibrary.wiley.com/doi/10.1111/jmi.12453/full

All-optical microscope autofocus based on an ETL and a totally internally reflected IR laser



https://www.osapublishing.org/oe/abstract.cfm?uri=oe-26-3-2359

optotune

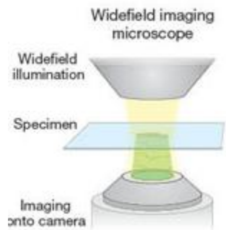
Agenda

- Company presentation
- Why tunable lenses for microscopy?
- Tunable lens technology
- Integration of tunable lenses
- Application examples
- Conclusion

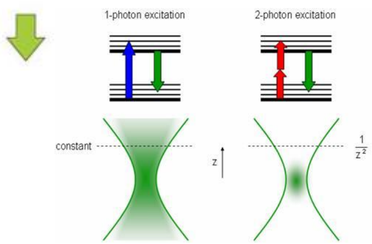
Microscopy application overview

3D microscopy

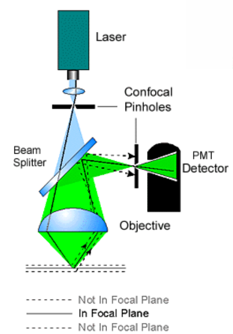
- Wide-field microscopy



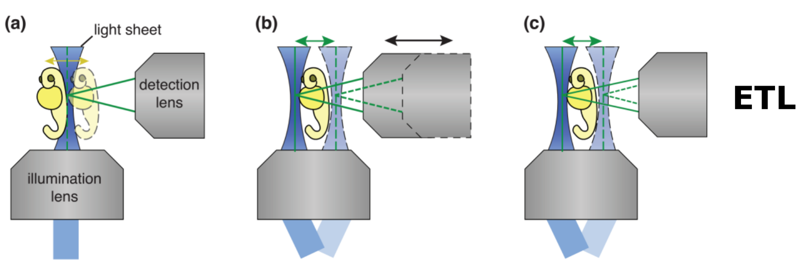
- Two-photon microscopy



- Confocal microscopy



- Light-sheet microscopy



2D microscopy

- Digital microscopy

- Adapter for video port

+

Conclusion

- Trend towards 3D biomedical imaging

- Focus tunable polymer lenses are compatible with
  - Wide-field microscopy
  - Confocal microscopy
  - Two-photon microscopy
  - Light-sheet microscopy

- Tunable lenses:
  - Fast
  - Compact
  - Large tuning range
  - Vibration-free
  - Broadband

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shaping the future of optics

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