



Universitäts-Augenklinik  
Rostock



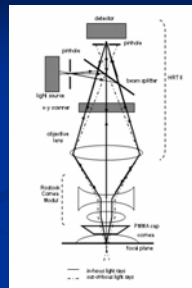
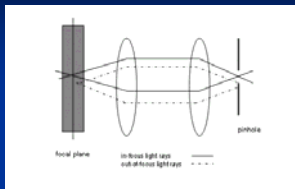
# Konfokale in-vivo Mikroskopie – auf dem Weg zur in-vivo Histologie? Bedeutung für die Hornhautchirurgie

Rudolf F. Guthoff

21. Kongress der DGII, Potsdam, 15.-17. März 2007

## Technik

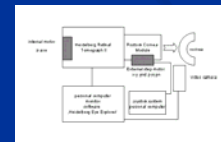
### In vivo konfokale Mikroskopie



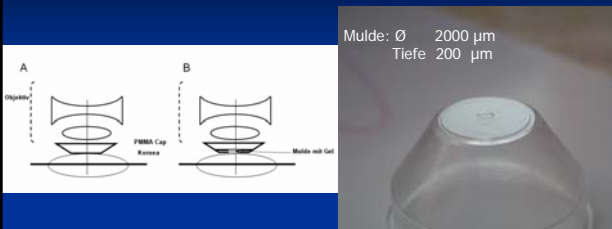
- Vorteile:
- In vivo Mikroskopie
  - schnelle und reproduzierbare Untersuchung
  - Zellzahl
  - Tiefenlokalisations- Pachymetrie

Guthoff R.F., Stave J ' In vivo micromorphology of the cornea-confocal microscopy principles and clinical applications' in Cornea and External Eye Diseases, Springer Verlag, 2006

### Heidelberg Retina Tomograph / Rostocker Cornea Modul



### Ankopplung des Rostocker Kornea Moduls

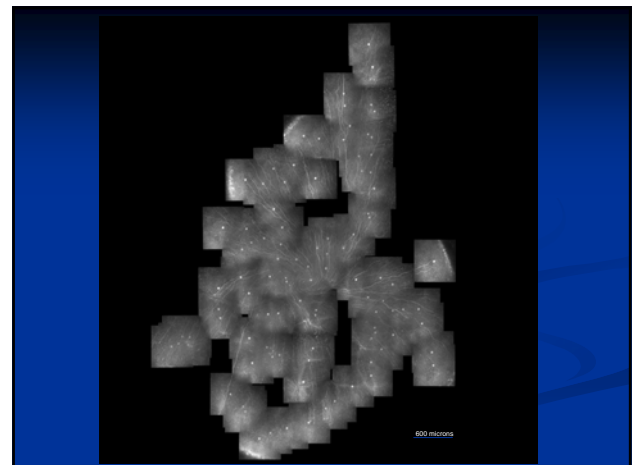
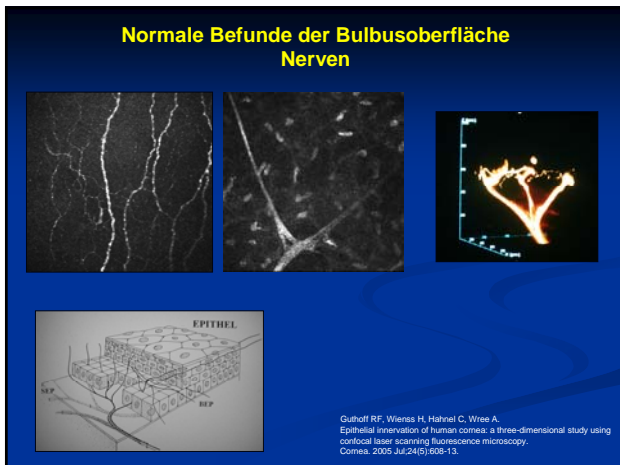
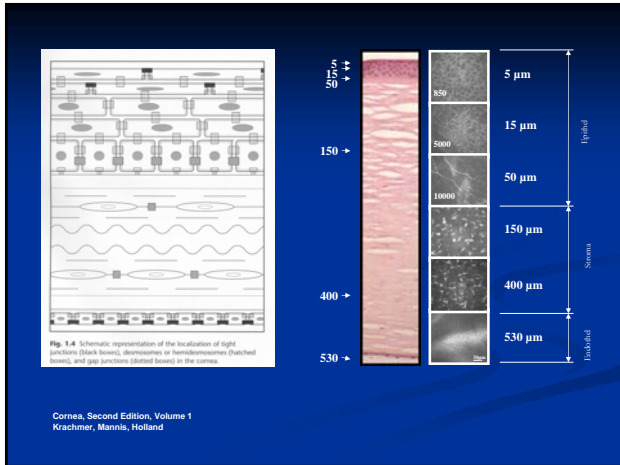


Mulde: Ø 2000 µm  
Tiefe 200 µm

- A Originale Ankopplung des Moduls durch PMMA – Kappe:  
es entsteht ein direkter Kontakt mit der Hornhaut.  
B Neue Kappe mit einer Mulde: Applanationsdruck wird minimiert.

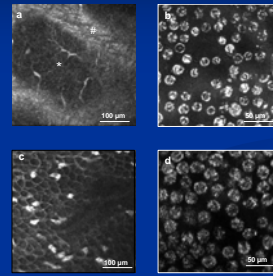
Als Kopplungsmedium wird ein Gel mit einem Brechungsindex von 1,350 verwendet (Vidisc, Dr. Mann Pharma, Berlin, Deutschland)

## Normale Befunde



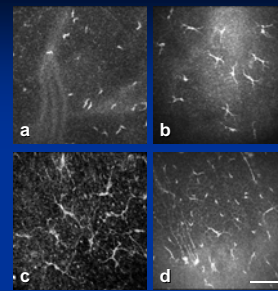
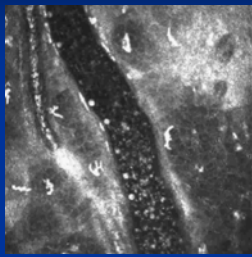
## Zelldifferenzierung

### Pathologische Befunde der Bulbusoberfläche In vivo konfokale Mikroskopie von verschiedenen Zelltypen



Hornhaut

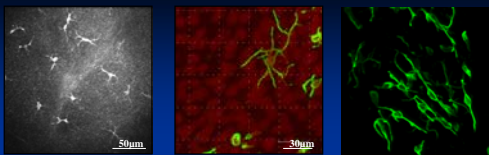
Blutastrich



- > kleine nichtdendritische Zellen (a)
- > große Zellen mit langen Dendriten (b)
- > Netz-Muster (c)
- > Kombination a+b (d)

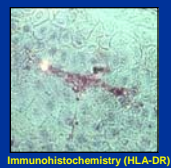
A. Zilber, J. Stave, B. Vollmar, R. Guthoff, In vivo confocal microscopic evaluation of lamellipodia cell density and distribution in the corneal epithelium of healthy volunteers and contact lens wearers. *Currus*, 2007 Jan;26(1):47-54.

Zhuova A, Stave J, Vollmar B, Guthoff R.F. In vivo confocal microscopic evaluation of Langerhans cell density and distribution in the normal human corneal epithelium. *Graefes Arch Clin Exp Ophthalmol*. 2005 Oct;43(10):1655-61.



confocal microscopy

immunohistochemistry (Vimentin)



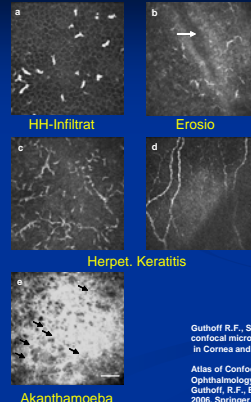
immunohistochemistry (HLA-DR)



phasen-contrast microscopy

- Durchmesser - bis 15µm
- LZ befinden sich in der Messtiefe von 35-60µm (Ebene von Superficial-, Flügel-, Basal-Zellen und SEP)

### Pathologische Befunde der Bulbusoberfläche Pathologische Hornhaut: Entzündungen



HH-Infiltrat

Erosio

Herpet. Keratitis

Akanthamoeba

Guthoff R.F., Stave J ' In vivo micromorphology of the cornea-confocal microscopy: principles and clinical applications' in Cornea and External Eye Diseases, Springer Verlag, 2006

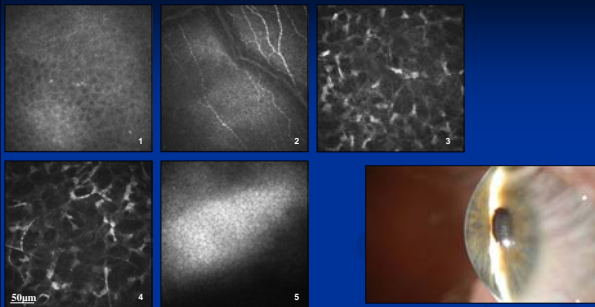
Atlas of Confocal Laser Scanning In-vivo Microscopy in Ophthalmology  
Guthoff, R.F., Baudouin, C., Stave, J.  
2006, Springer

## Hornhautpathologie - Beispiele

- Quervernetzung
- refraktive Chirurgie
- radiäre Keratotomie
- perforierende Keratoplastik

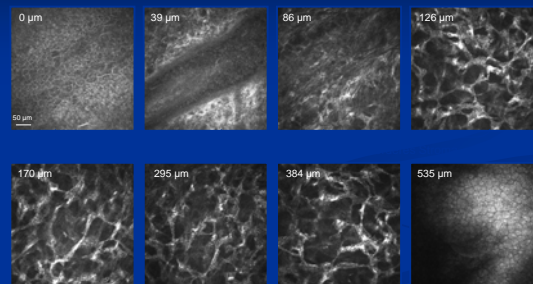
## Keratokonus und Quervernetzung

### Keratokonus



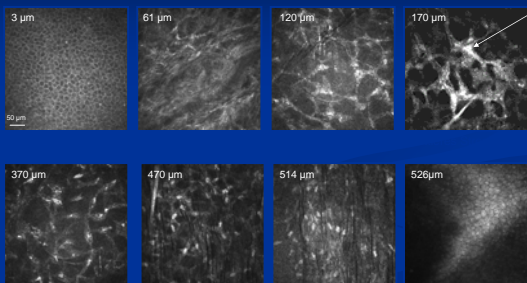
- (1) Feine Unregelmäßigkeiten der Intermediärzellen
- (2) Falten in der Ebene des subepithelialen Nervenplexus und der Bowmanschen Membran und im vorderen Stroma (3)
- (4) Kurvenförmige Nervenstrukturen des vorderen Stromas
- (5) normales Endothel

### Quervernetzung – 6. postoperativer Tag



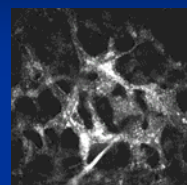
Knappe et al 2007

### Quervernetzung – 3 Monate postoperativ

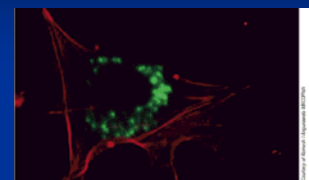


Knappe et al 2007

### Aktivierte Keratozyten ?

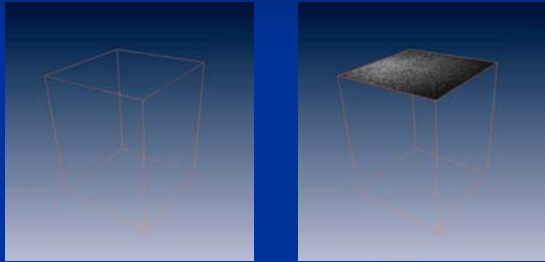


Z.n. Quervernetzung



In vitro Modell, Kaninchen, post.op

In vivo konfokale Mikroskopie und 3-dimensionale Rekonstruktion  
Z.n. Quervernetzung

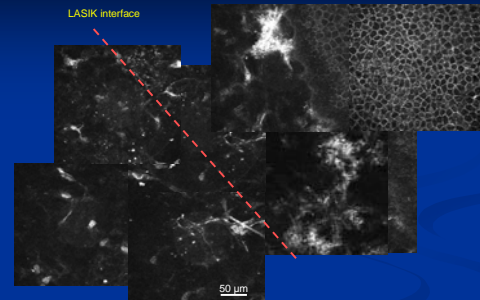


• Nach Quervernetzung:

- rasche Regeneration des Epithels
- subepithelialer Nervenplexus nicht mehr darstellbar
- wabenartige Stromaarchitektur direkt postoperativ sichtbar;
- ca. 3 Monate postoperativ Keratozytenkerne wieder erkennbar
- Endothel bleibt unbeeinflusst

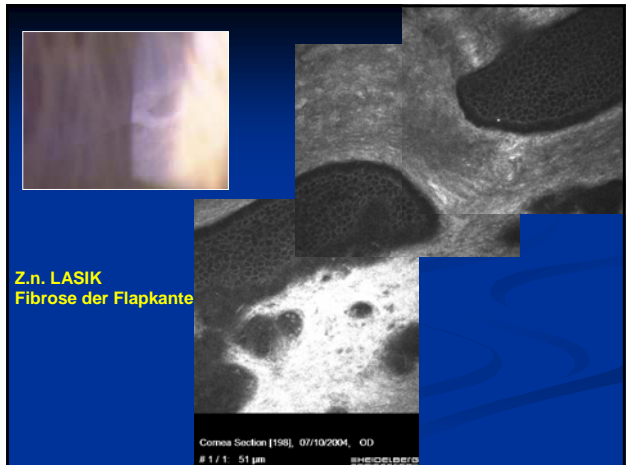
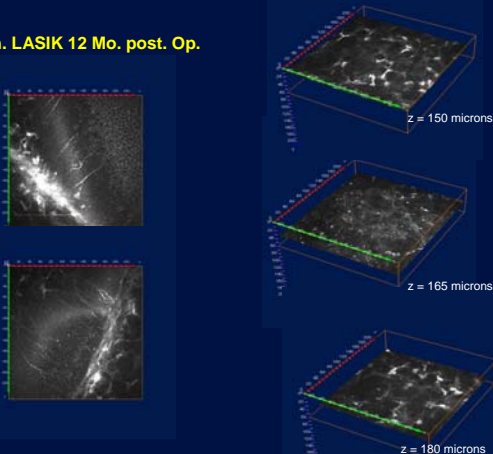
Refraktive Chirurgie

Z.n. LASIK



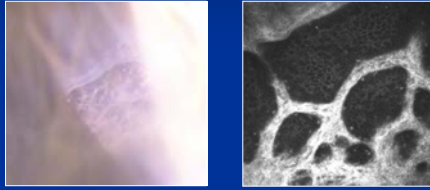
Epithelium and keratocytes  
5 month after LASIK

Z.n. LASIK 12 Mo. post. Op.

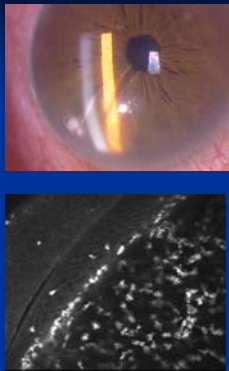
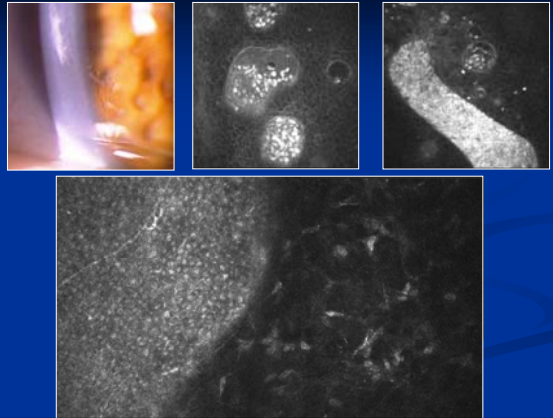


Z.n. LASIK  
Fibrose der Flapkante

### Z.n. LASIK - Interface-Fibrose

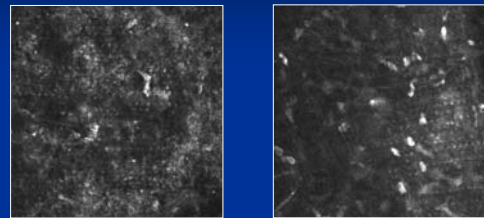


### Epithelwachstum im Interface-Bereich

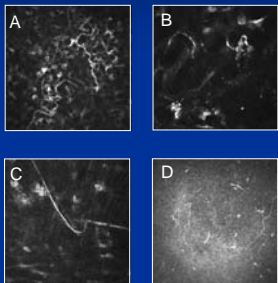


Z.n. Radiärer Keratotomie

### Z.n. Femtolaser



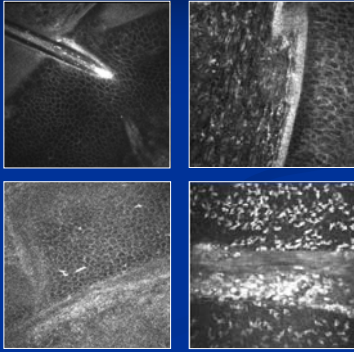
### Representative in vivo confocal images of abnormal nerve configurations after LASIK



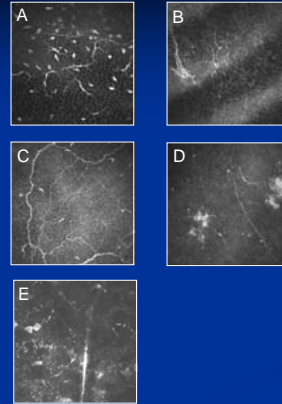
- A 16 months post. op post. op, 32 yrs. female subject, corneal sensation 3.5 mm, anterior stroma, very thin and curved nerve fibres
- B 12 months post. op, corneal sensation 4.0 mm, near interface zone, thin and curved nerve fibres
- C 18 months post. op, corneal sensation 2.5 mm, thin stromal nerve
- D 4 months post. op, corneal sensation 1.0 mm, very thin and curved subepithelial nerve fibres without preferred direction

### Keratoplastik

### In vivo konfokale Mikroskopie bei Z.n. PKP

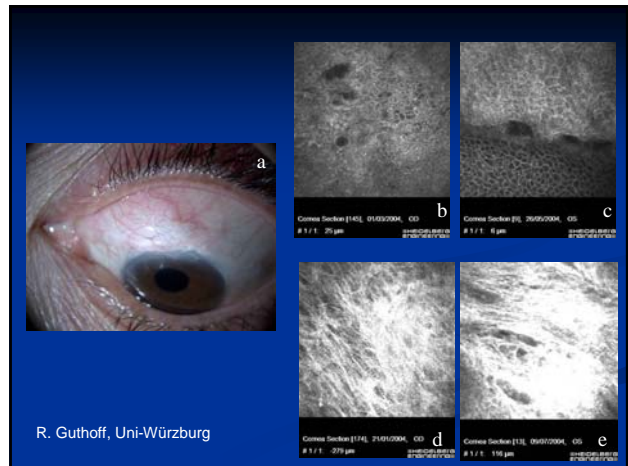


### Representative in vivo confocal images of SEP after penetrating keratoplasty



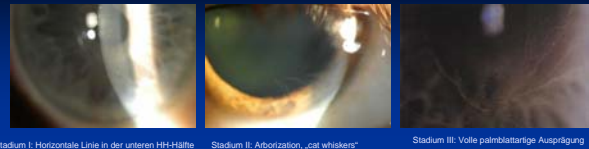
- A graft center, 12 month after penetrating keratoplasty, no sensation in central cornea, peripheral cornea 2,0mm
- B graft center, 24 month after penetrating keratoplasty, no sensation in central cornea, peripheral cornea 1,0mm
- C graft center, 54 month after penetrating keratoplasty, sensation in central cornea 2,5mm, peripheral cornea 3,0mm
- D graft center, 12 month after penetrating keratoplasty, sensation in central cornea 0,5mm, peripheral cornea 1,5mm
- E graft center, nerve trunk in middle stroma, 24 month after penetrating keratoplasty

### Glaukomchirurgie Sickerkissen

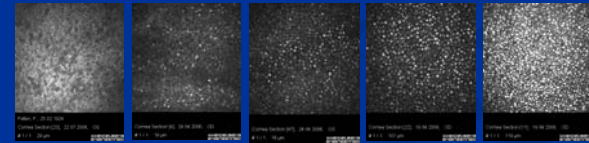


### Evaluierung von Systemerkrankungen (M. Fabry)

### Die 3 Stadien der Cornea verticillata nach Orlando



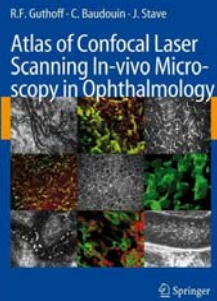
### Einteilung der KFM-Veränderungen bei Cornea verticillata nach Schweregraden



- Stadium 0: Keine Cornea verticillata, Normale Basalzellen
- Stadium 1: Vereinzelte Hyperreflektivitäten in der Basalzellschicht
- Stadium 2: Vermehrt Hyperreflektivitäten in der Basalzellschicht
- Stadium 4: Starke Ausprägung der Hyperreflektivitäten in der Basalzellschicht
- Stadium 5: Massive Ausprägung. Fast jede Basalzelle hyperreflektiv

## Schlussfolgerungen

- Schnelle und reproduzierbare in vivo Untersuchung: Zellzahl, Pachymetrie
- Nachweis von entzündlichen Zellen (Langerhans- Zellen, Leukozyten) sowie Mikroorganismen (Akanthamoeba)
- nach LASIK: Flapdicke, Reinnervation, Mikromorphologie des Interfaces, aktivierte Keratozyten
- nach KP: Hornhautdicke, Endothelzellzahl, Transplantatrand, Interfacezone nach lamellierender KP, Reinnervation
- nach Quervernetzung: Pachymetrie, Epithelisation, Stromaarchitektur, Endothelsituation
- 3D Darstellung der Hornhaut
- Kontrolle der Wundheilung nach fistulierender Glaukomchirurgie
- Evaluierung von Systemerkrankungen (M. Fabry)



Atlas of Confocal Laser Scanning In-vivo Microscopy in Ophthalmology  
Guthoff, R.F., Baudouin, C., Stave, J.  
2006, Springer  
ISBN-10: 3-540-32705-3

Vielen Dank  
für Ihre Aufmerksamkeit !

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Prof. Dr. B. Vohnat  
Dr. A. Eckhard  
Dr. S. Knappe  
R. Kozak  
Dr. O. Stachs  
Dr. A. Zährcke



## Neue Diagnostische Verfahren: 3D Ultraschallbiomikroskopie

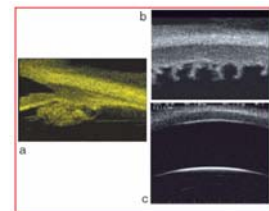
O. Stachs

Department of Ophthalmology  
University of Rostock  
Germany

DGH 2007, 16.-17.03.2007, Potsdam

## High-resolution ultrasound

Ultrasound Biomicroscope Model 840 (Humphrey Instruments, Carl Zeiss Group)



- linear scan
- kommerziell nicht mehr erhältlich
- begrenzte Eindringtiefe
- Ziliarkörper und Vorderkammer, keine posteriorer Pol



## High-resolution ultrasound

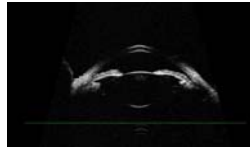
VuMAX UBM 35/50, Sonomed, Inc.



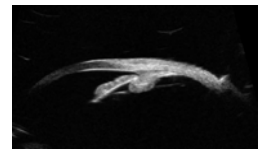
- Hand piece with 35Mhz and/or 50Mhz transducer(s)
- Sector scan
- Predefined settings

## Standard features

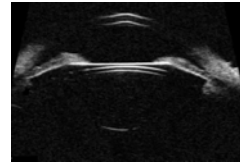
VuMAX UBM 35/50, Sonomed, Inc.



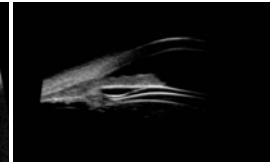
Anterior segment with sulcus to sulcus, angle to angle, posterior pole



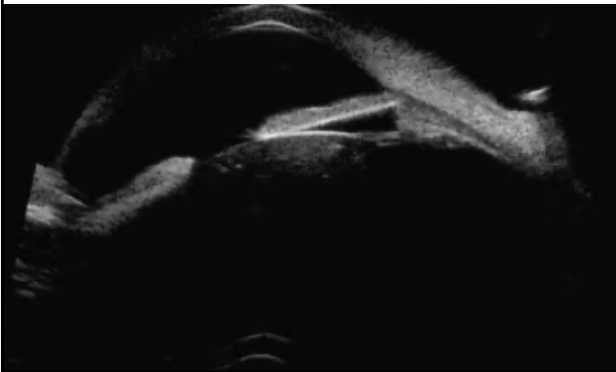
Ciliary body



ICL with haptic placement



## Dynamic investigations



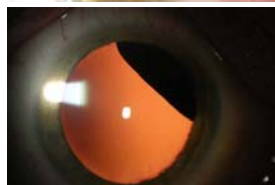
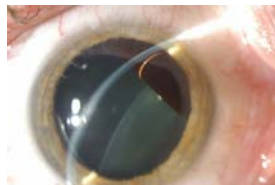
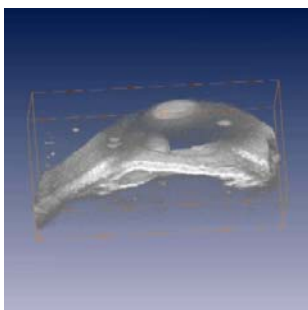
## 3D high-resolution ultrasound

VuMAX UBM 35/50, Sonomed, Inc.

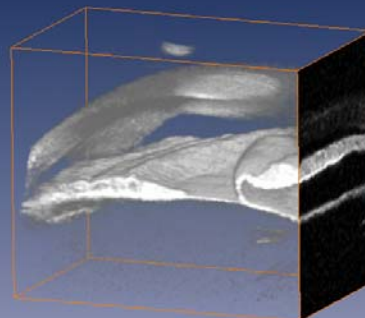


- 3D - UBM with VuMAX UBM 35/50
- Development University of Rostock in cooperation with Hasotek (Germany)
- Commercial available: ASCRS 2007

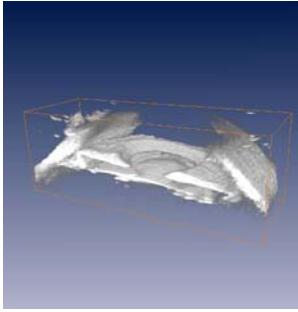
## 3D with VuMAX, in vivo - case 1



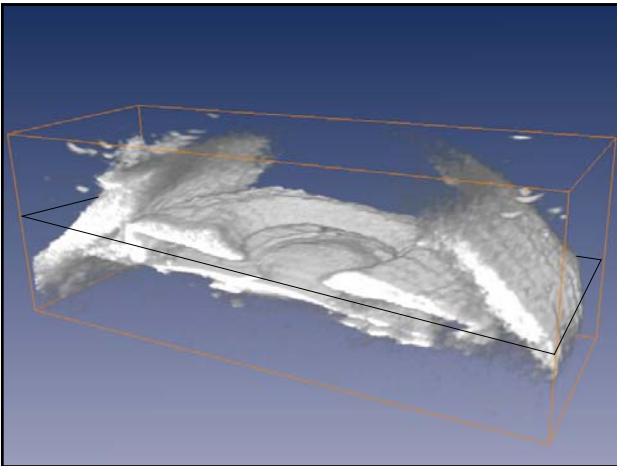
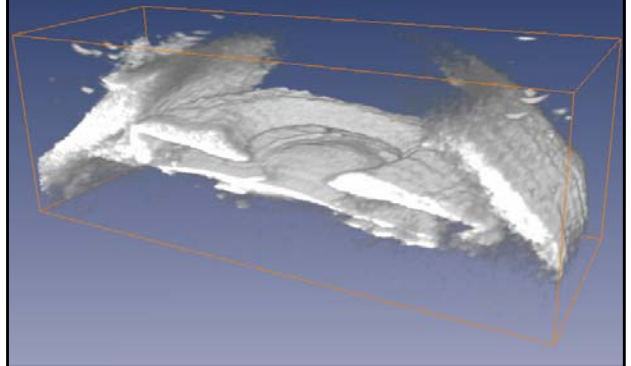
## 3D with VuMAX, in vivo - case 1



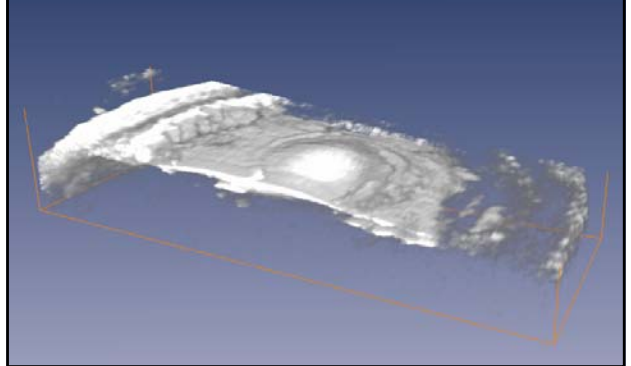
3D with VuMAX, in vivo - case 2  
acrylic intraocular lens



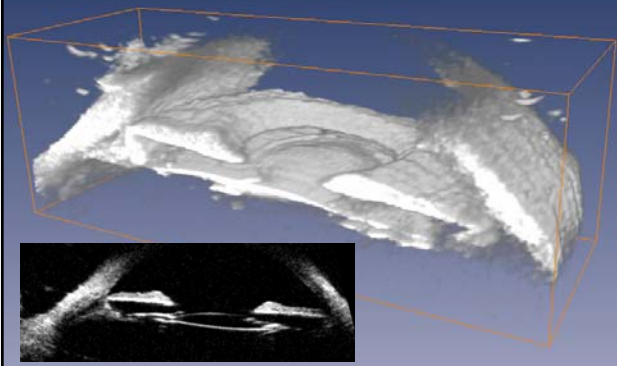
3D with VuMAX, in vivo - case 2



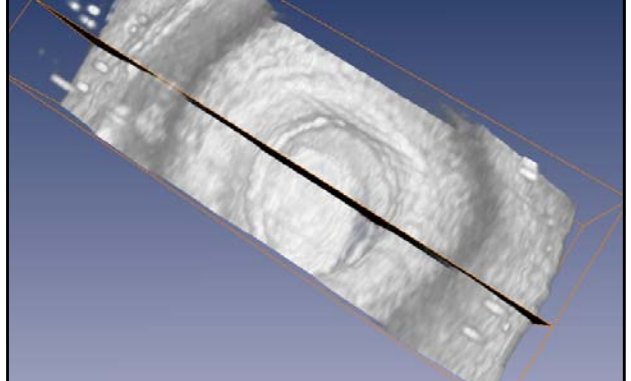
3D with VuMAX, in vivo - case 2

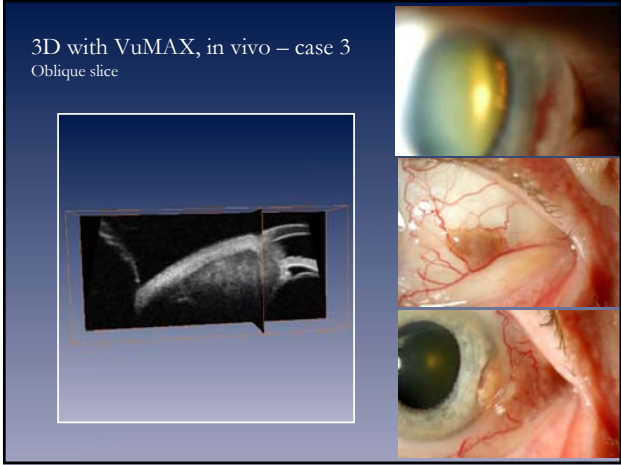
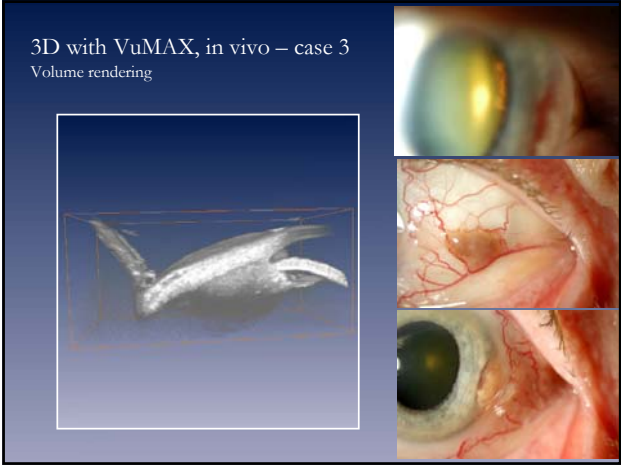
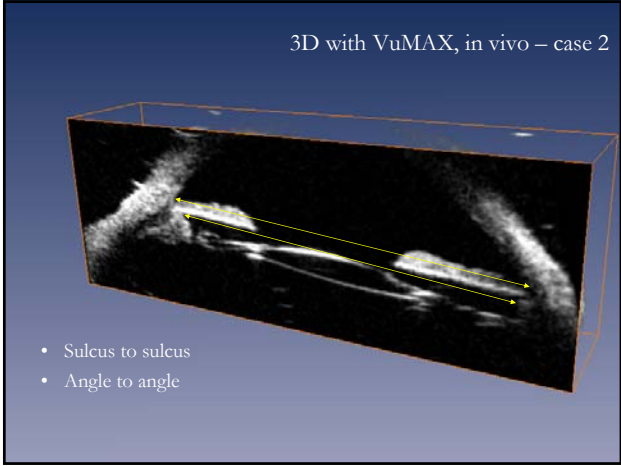
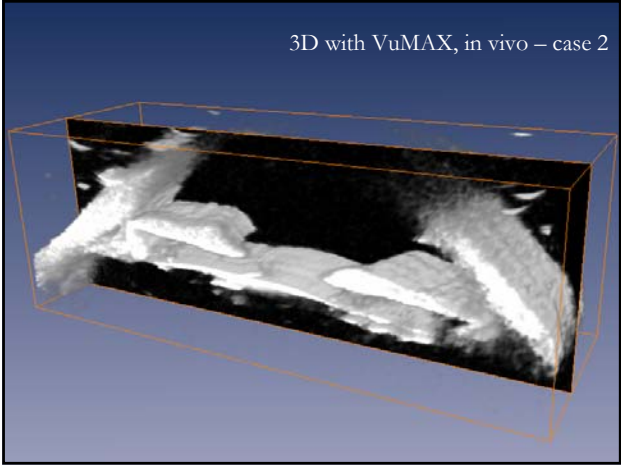
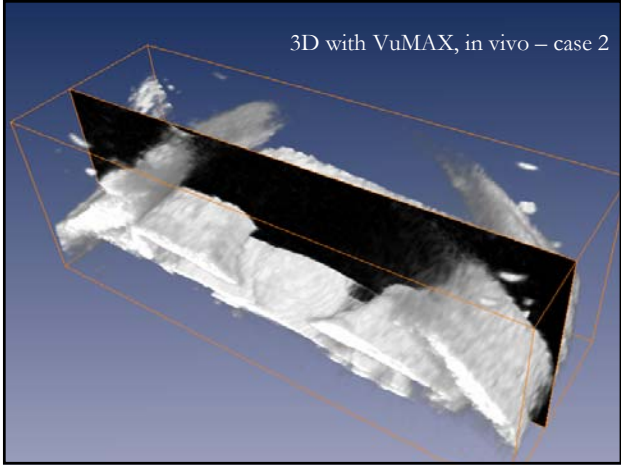


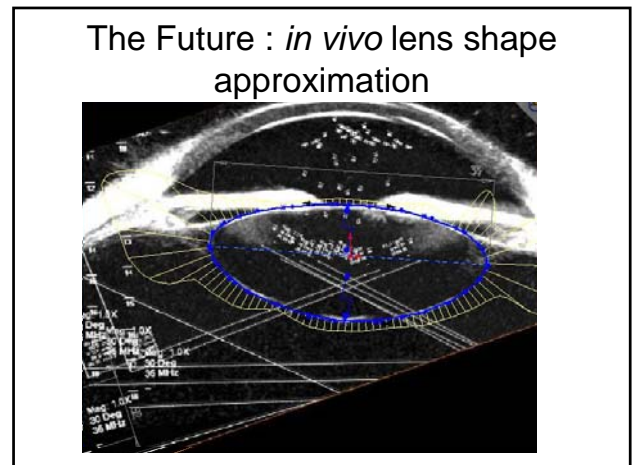
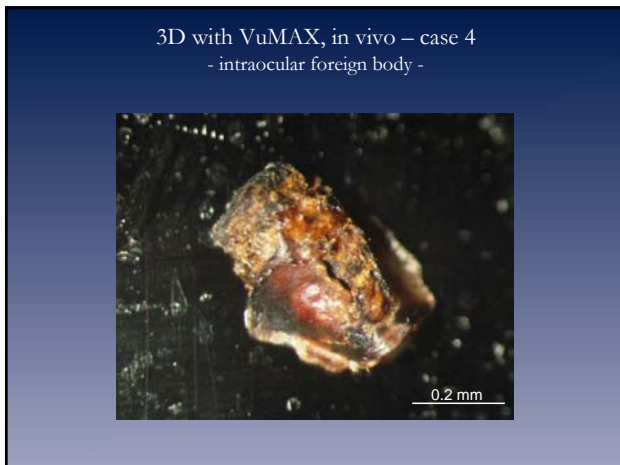
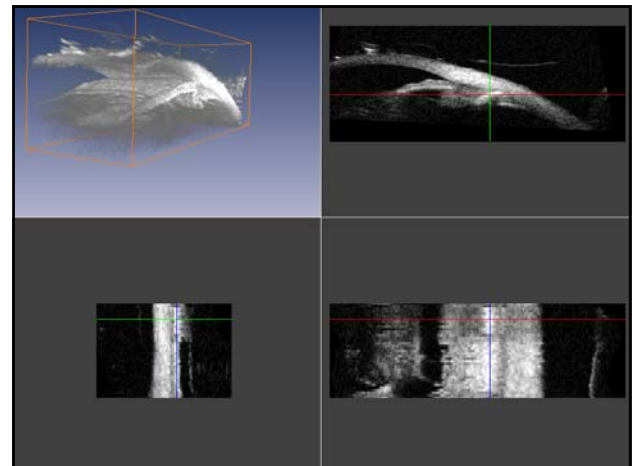
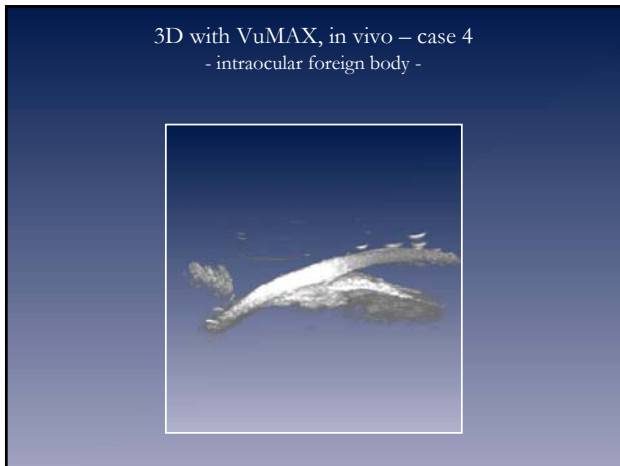
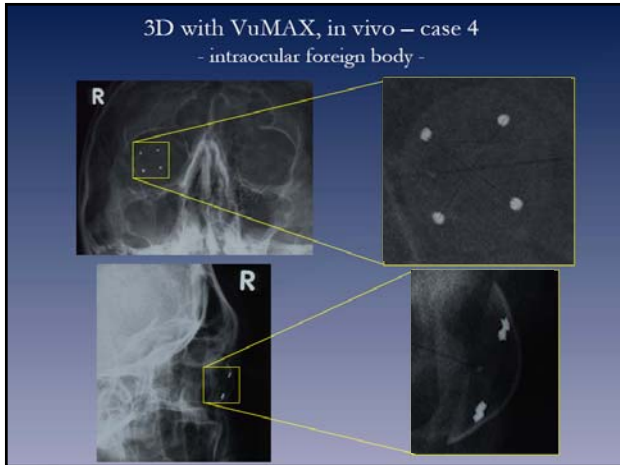
3D with VuMAX, in vivo - case 2



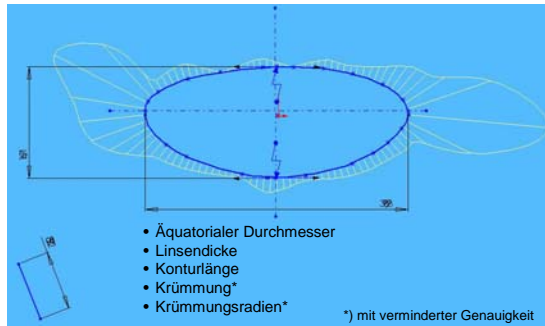
3D with VuMAX, in vivo - case 2







## The Future : *in vivo* lens shape approximation



## Conclusions

- The three-dimensional ultrasound biomicroscopy yields extended diagnostic findings regarding iris and ciliary body pathology.
- Potentialities
  - 3D anterior segment imaging
  - Sulcus to sulcus measurements in consideration of the ciliary processes
  - Angle to angle measurements
  - Assessment of the accommodation dynamics in consideration of the ciliary processes
  - Analysis of accommodative IOL's (haptic geometry)
  - ...

- R.F. Guthoff
- K.P. Schmitz
- J. Stave
- O. Stachs
- S. Knappe
- P. Enzenross
- C. Schlüter
- G. Grümmer (Hasotec)

