



# **Ultrasound-enhanced Delivery of Antibiotics and Anti-inflammatory Drugs into the Eye**

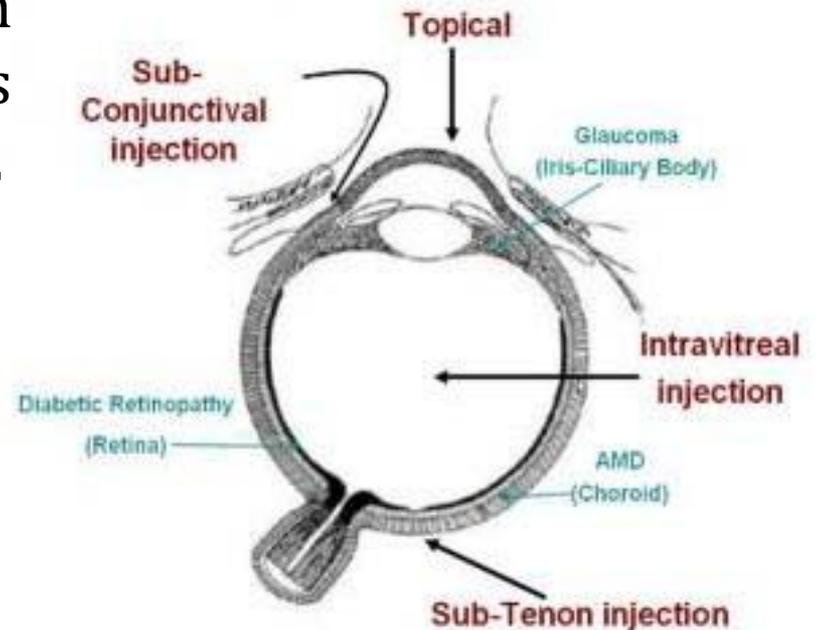
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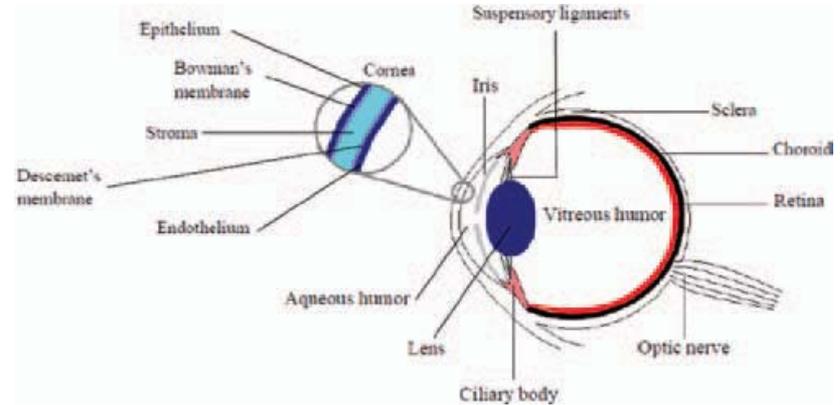
# Background

- Delivery of drugs at therapeutic levels is frequently a problem in the treatment of various ocular diseases.
- Topical administration of drugs to the cornea is a preferred route for delivery of ocular drugs.
- Achieving 2-3 times increase in the amount of delivered drugs is considered clinically significant.



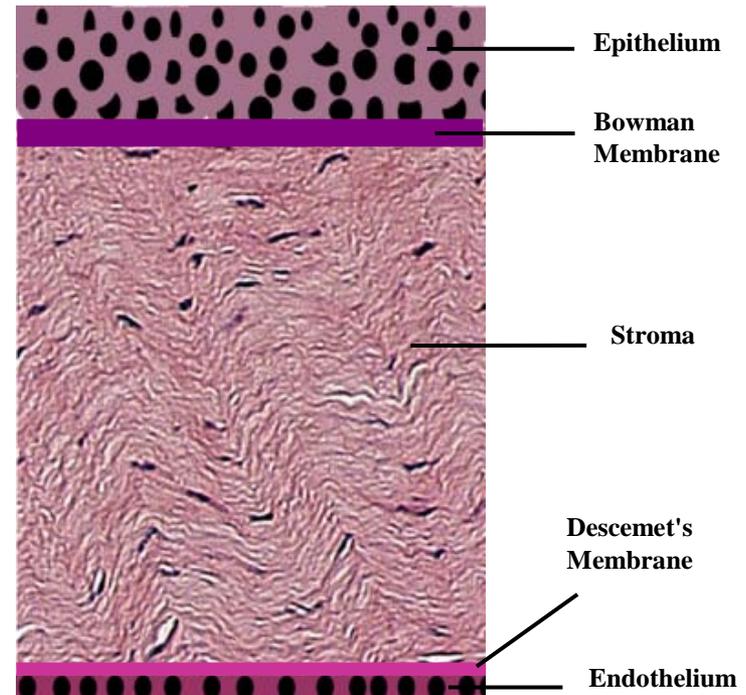
# Cornea

- Cornea represents 7% of surface area of the eye.
- Cornea has a lower permeability for hydrophilic drugs than for lipophilic drugs.
- Only 2-5% of an ophthalmic drug can penetrate through cornea.



The cornea is 0.5 mm thick with three primary layers:

- Epithelium with thickness of 50  $\mu\text{m}$  and 5-7 cell layers.
- Stroma is a 450  $\mu\text{m}$  thick, porous and hydrophilic tissue.
- Endothelium a single layer in inner surface of the cornea.



# Materials *In Vitro* Study

- Adult New Zealand white rabbit cornea: standard model for ocular drug delivery



- Unfocused custom-designed circular transducers (Sonic Concepts) with 15 mm active diameter at 400 kHz, 600 kHz, 800 kHz, and 1 MHz frequencies.
- The  $d_{ff}$  calculated for these transducers are 1.5, 2.25, 3, and 3.75 cm respectively.

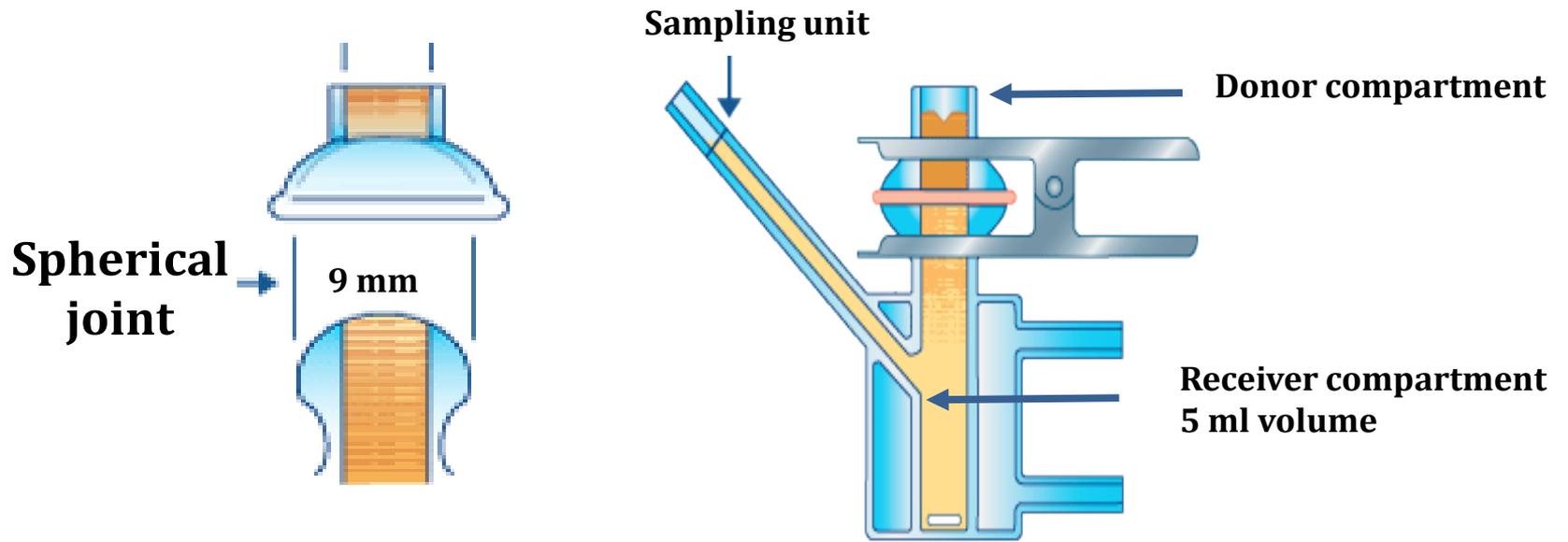




# Drug Solutions

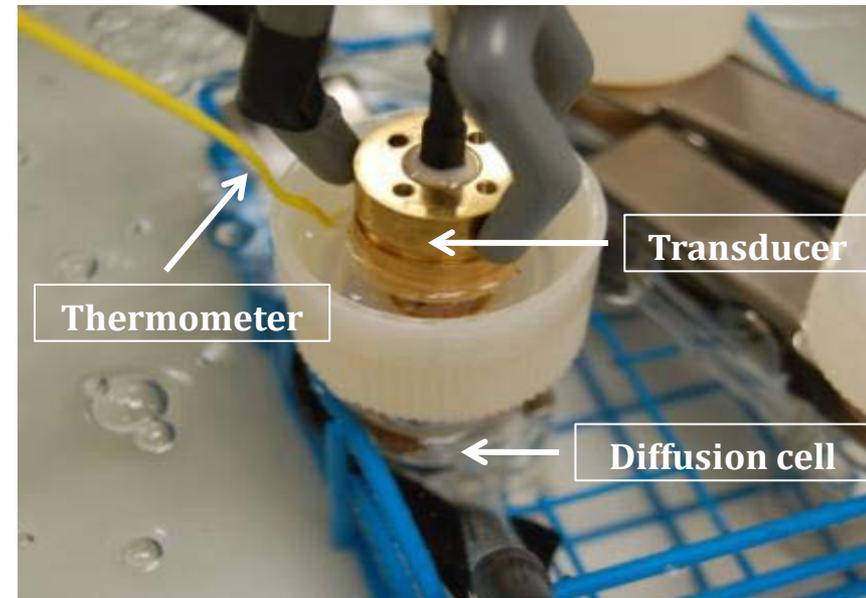
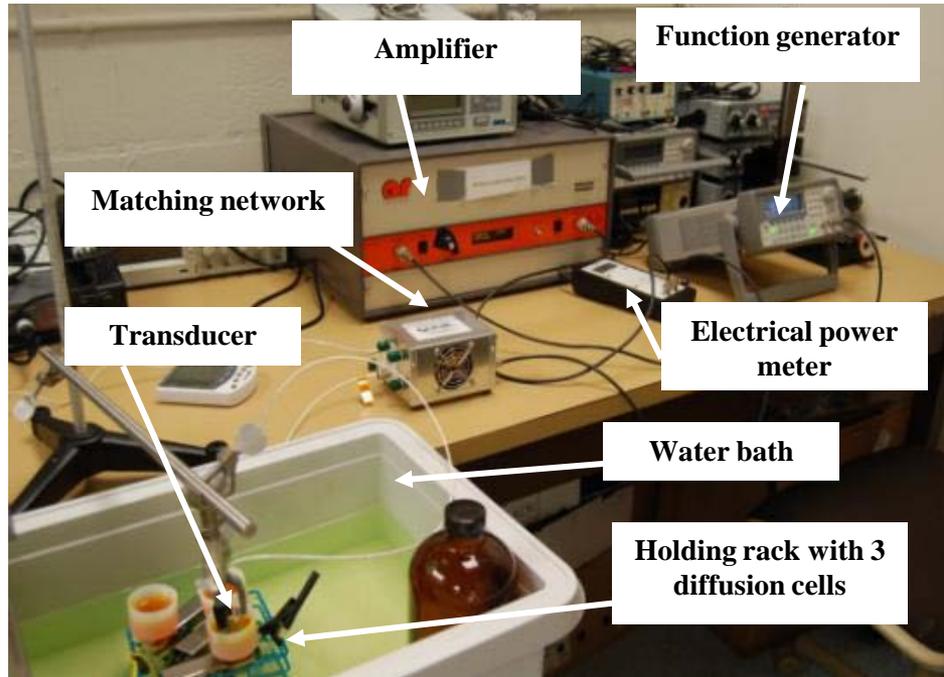
- **Sodium Fluorescein, 0.25%**
  - Used for diagnosis of corneal abrasions, corneal ulcers and infections
  - Hydrophilic
  - Maximum absorption @ 490 nm
- **Tobramycin, 0.3%**
  - Ophthalmic antibiotic formulation for tropical therapy of external infections
  - Hydrophilic
  - Maximum absorption @ 278 nm
- **Dexamethasone Sodium Phosphate, 0.1%**
  - Topical steroid solution used to suppress inflammatory response to different conditions
  - Hydrophilic
  - Maximum absorption @ 242 nm

# Spherical Diffusion Cell



# *In vitro* Setup

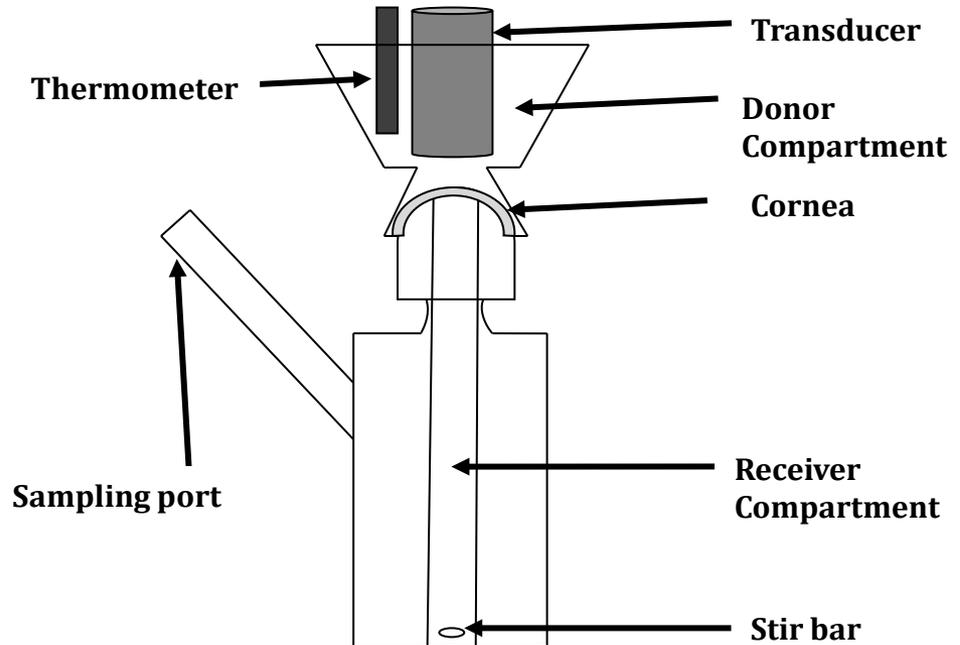
- Dissected cornea was placed over the spherical joint of diffusion cell, between donor and receiver compartments.



- Ultrasound was applied with intensities of  $0.3 \text{ W/cm}^2$  -  $1 \text{ W/cm}^2$  at different frequencies between 400 kHz- 1 MHz.
- The cornea was exposed to ultrasound for 5 min.
- Temperature was measured while applying ultrasound.

# *In vitro* Setup

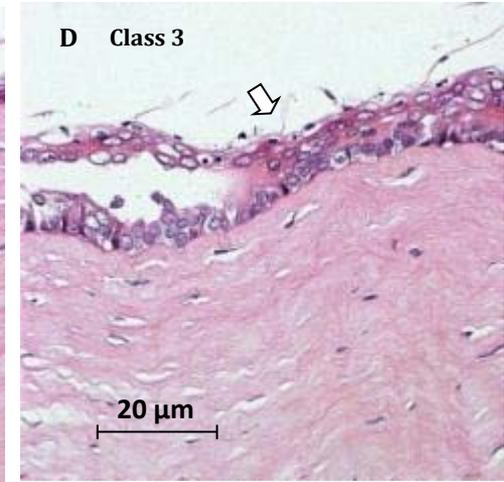
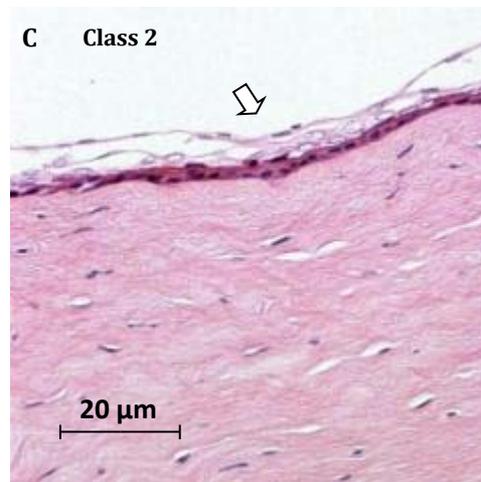
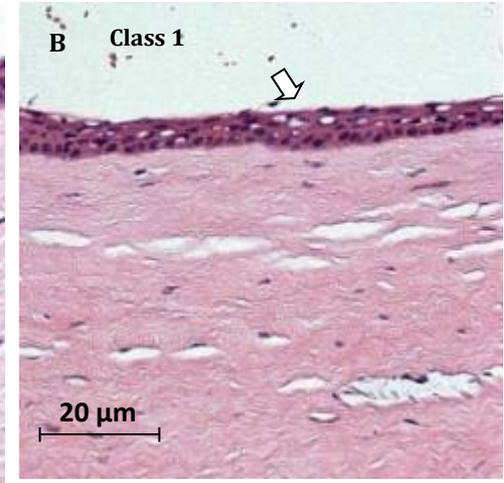
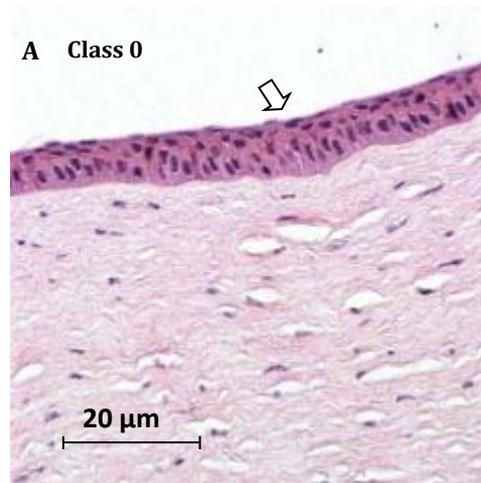
- The receiver compartment was stirred at 380 rpm using a magnetic stir bar.
- A 3 mL solution sample was collected through the sampling port of the receiver compartment after 60 min.
- The absorption of the sample was measured using spectrophotometer.
- Dissected cornea was placed in formalin after the experiment to be fixed and sent for histology.



# *In vitro* Histology

Different categories of histological damage are:

- A. Class 0: None of the layers are damaged or missing (0) .
- B. Class 1: Some cells are missing or the first layer of epithelium is removed (1/3).
- C. Class 2: Two layers are missing or damaged (2/3).
- D. Class 3: All three layers are missing or epithelium is severely damaged (1).

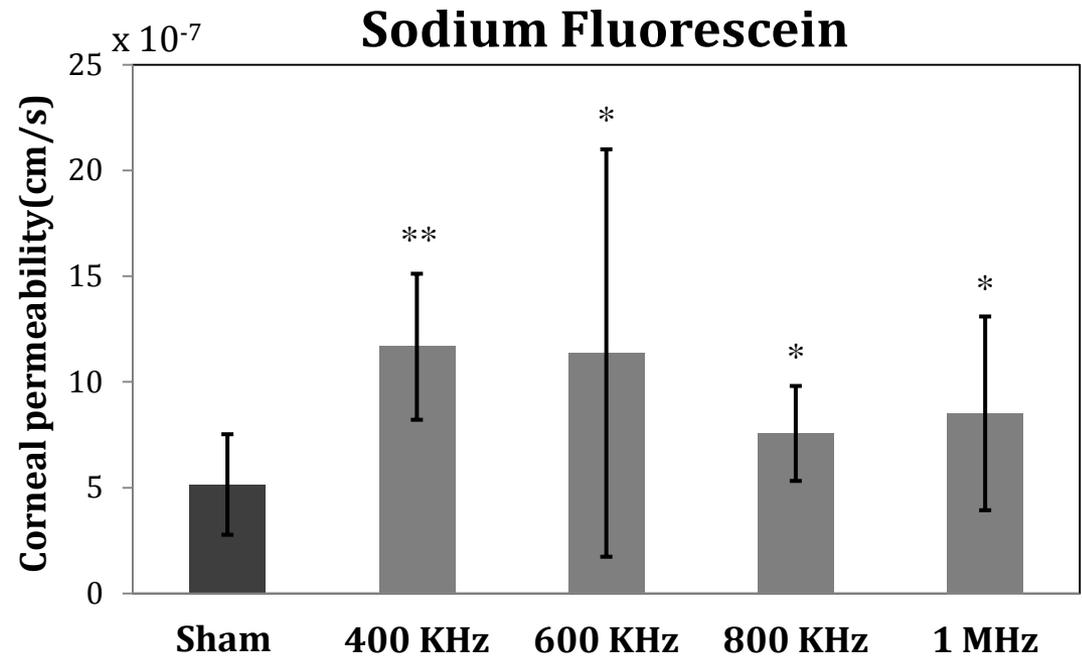


# *In vitro* Results

Ultrasound application for 5 min at 1.0 W/cm<sup>2</sup> produced permeability increase of :

- 126% at 400 kHz (n=9),
- 121% at 600 kHz (n=13),
- 47% at 800 kHz (n=9),
- 65% at 1 MHz (n=12)

as compared to sham treated cases (n=9).

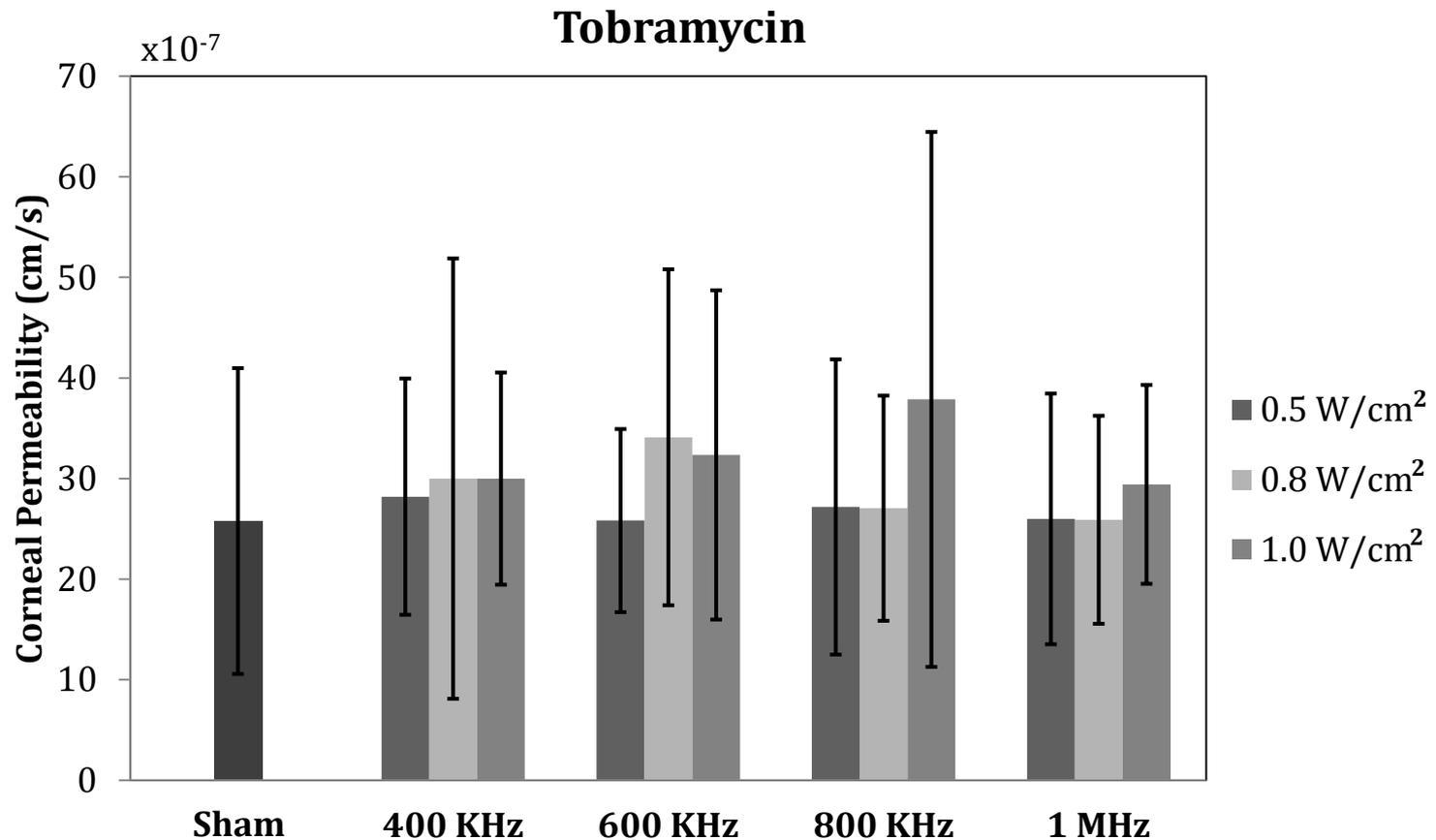


\* Indicates p-value < 0.05

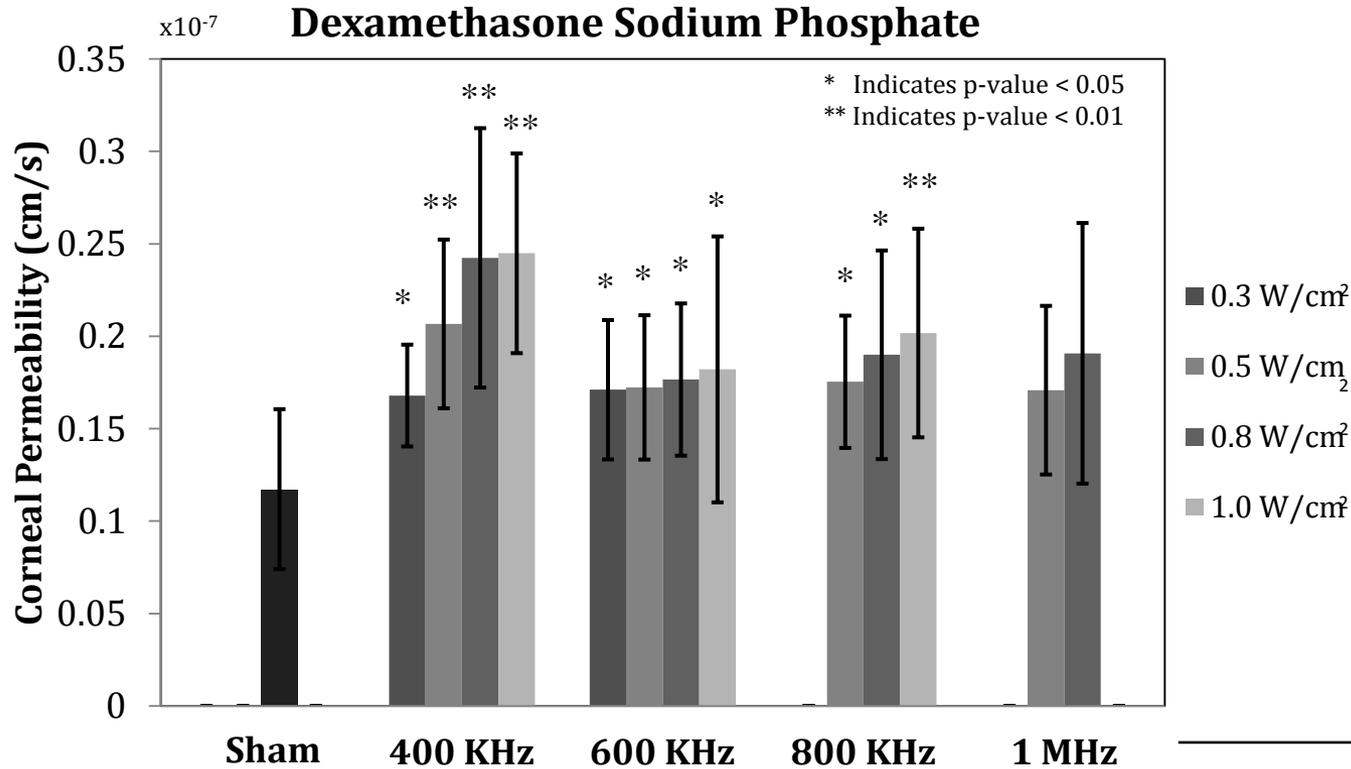
\*\* Indicates p-value < 0.001

# *In vitro* Results

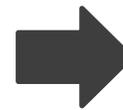
The increase in corneal permeability ranged from 14% to 46.9% depending on ultrasound parameter combination, with no statistical significance achieved in all cases.



# In vitro Results



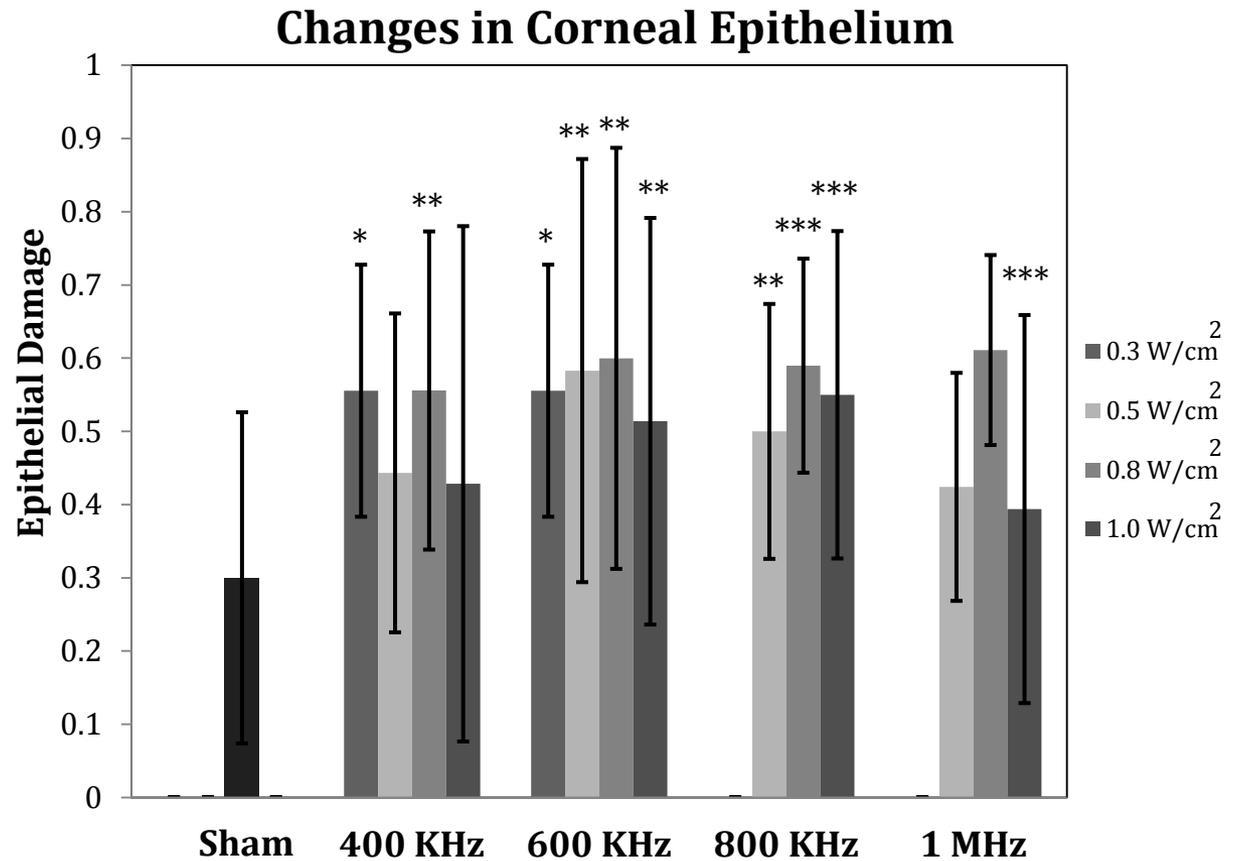
The percentage increase in corneal permeability to Dexamethasone Sodium Phosphate as compared to sham treated samples are shown in this table.



	400 KHz	600 KHz	800 KHz	1 MHz
<b>0.3 W/cm<sup>2</sup></b>	43% (n=6)	46% (n=6)	--	--
<b>0.5 W/cm<sup>2</sup></b>	76% (n=6)	47% (n=8)	50% (n=6)	46% (n=5)
<b>0.8 W/cm<sup>2</sup></b>	107% (n=6)	51% (n=8)	62% (n=6)	63% (n=6)
<b>1.0 W/cm<sup>2</sup></b>	109% (n=6)	55% (n=9)	72% (n=8)	--

# Changes in Corneal Epithelium *In vitro*

- Sham shows the corneal changes with no ultrasound treatment; different shades of gray represents the corneal damage due to ultrasound application.
- Data are shown as mean  $\pm$  standard deviation.



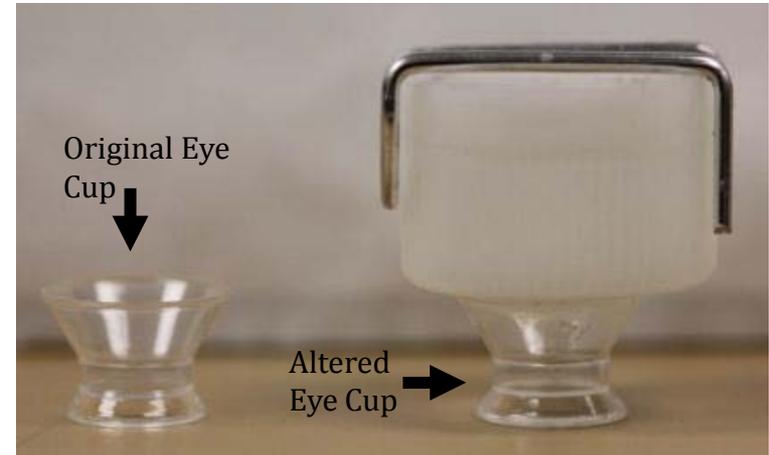
\* Indicates p-value < 0.05

\*\* Indicates p-value < 0.01

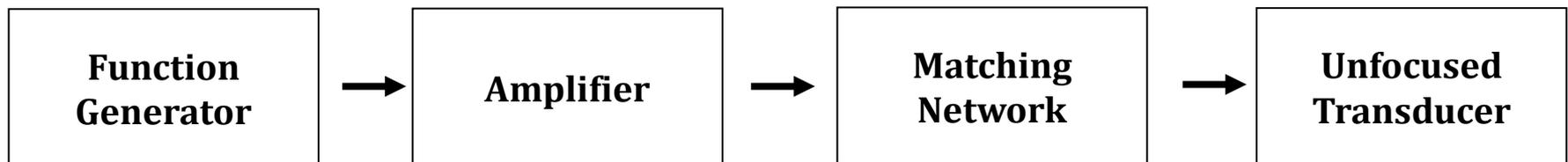
\*\*\* Indicates p-value < 0.001

# Experimental Preparation

- The most effective parameters used *in vitro* study
  - $f = 400$  kHz and  $600$  kHz
  - Intensity =  $0.8$  W/cm<sup>2</sup>
  - Exposure time =  $5$  min
  - Total study time =  $60$  min
- Dexamethasone sodium phosphate

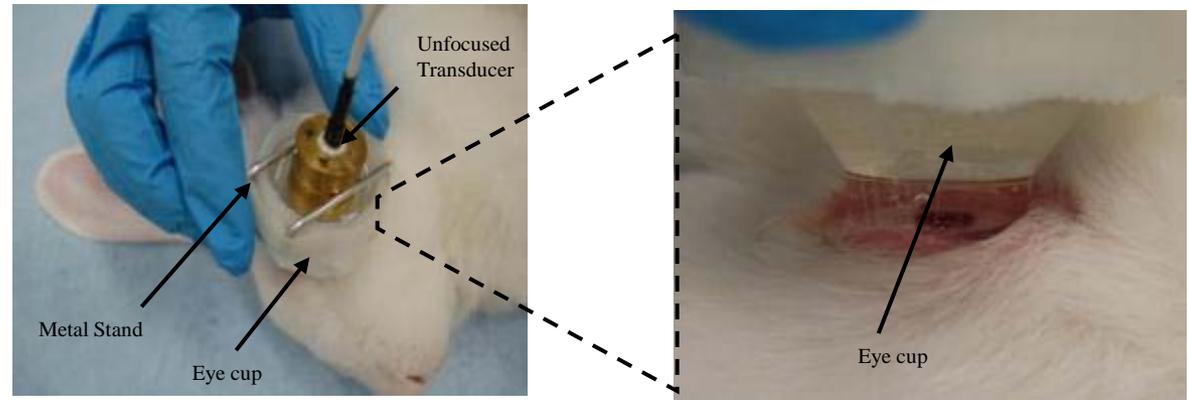


## Driving unit of transducers

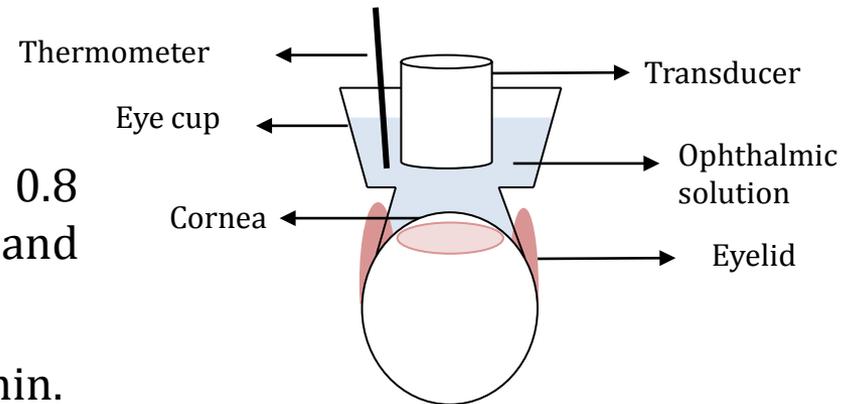


# *In vivo* Setup

- The eye cup was placed on the eye filled with drug solution.
- Transducer was placed on a metal stand and submerged inside the solution.



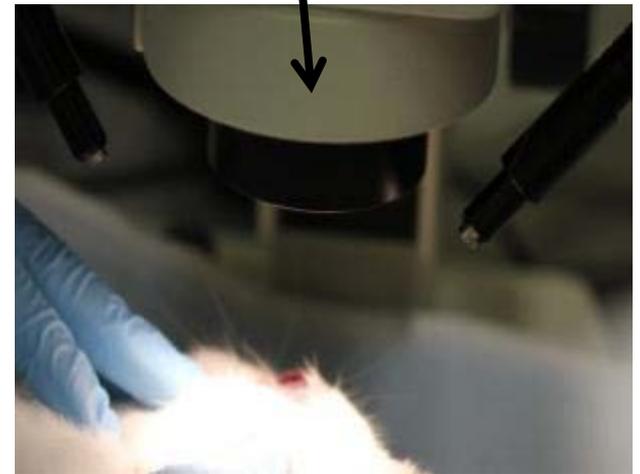
- Ultrasound was applied with intensity of  $0.8 \text{ W/cm}^2$  at different frequencies of 400 kHz and 600 kHz.
- The cornea was exposed to ultrasound for 5 min.
- Temperature was measured 3 times while applying ultrasound ( $t = 0, 2.5, \text{ and } 5 \text{ min}$ ).



# Methods

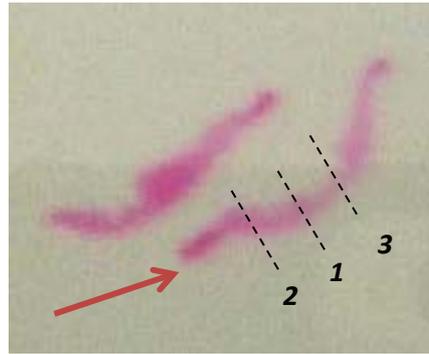
- After ultrasound application and also before euthanasia, *in vivo* gross observation of the cornea was performed using a high magnification stereomicroscope.
- About 0.3 mL sample of aqueous humor was collected using 27 G  $\times$  1/2" needle (12.7 mm length) approximately 60 min after the ultrasound treatment and immediately after the animal was euthanized.
- These samples were sent for chromatography.

Stereomicroscope



# Histological Analysis

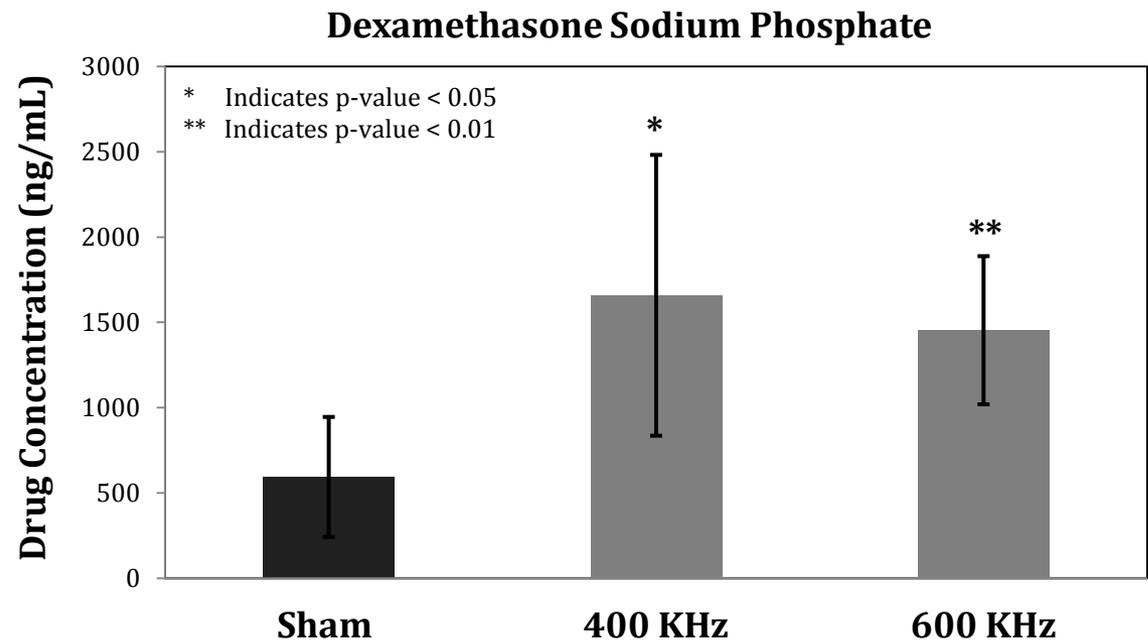
- Thickness of different layers of cornea (epithelium, stroma, and endothelium).



- Zeiss Axiolmager light microscope at 5-20X magnification
- Investigating the structural changes in cornea using histology slides.
  - Same criteria used in *in vitro* study

# Drug Concentration in Aqueous Humor

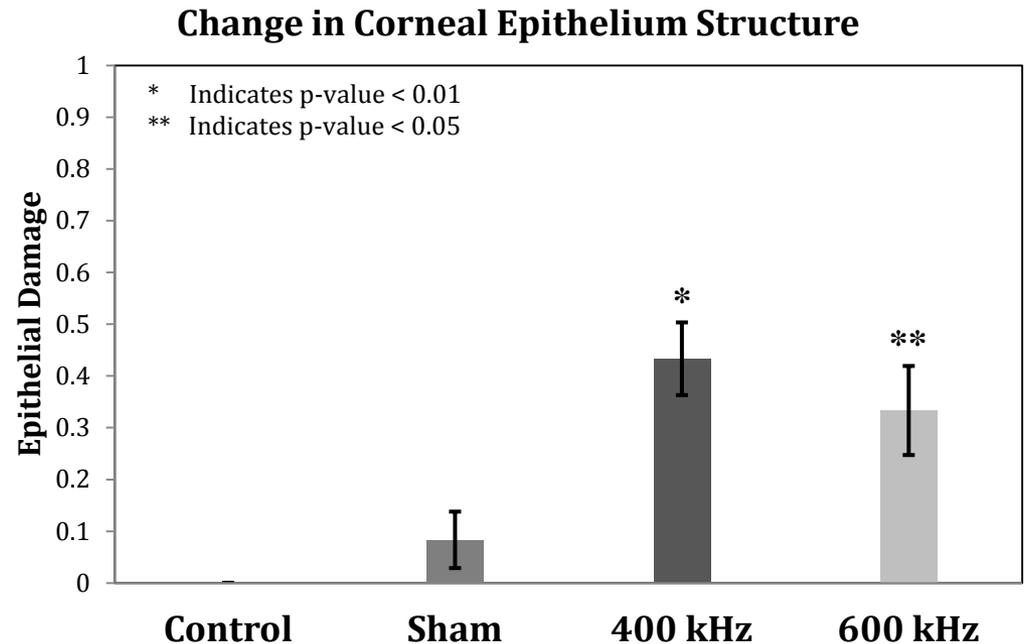
- Drug concentration in aqueous humor samples as compared to sham treated samples increased by:
  - 2.8 times using 400 kHz
  - 2.4 times using 600 kHz



- For sham treatments n=7, using 400 kHz frequency n=5, and n=6 using frequency of 600 kHz.

# *In vivo* Epithelial Change Comparison

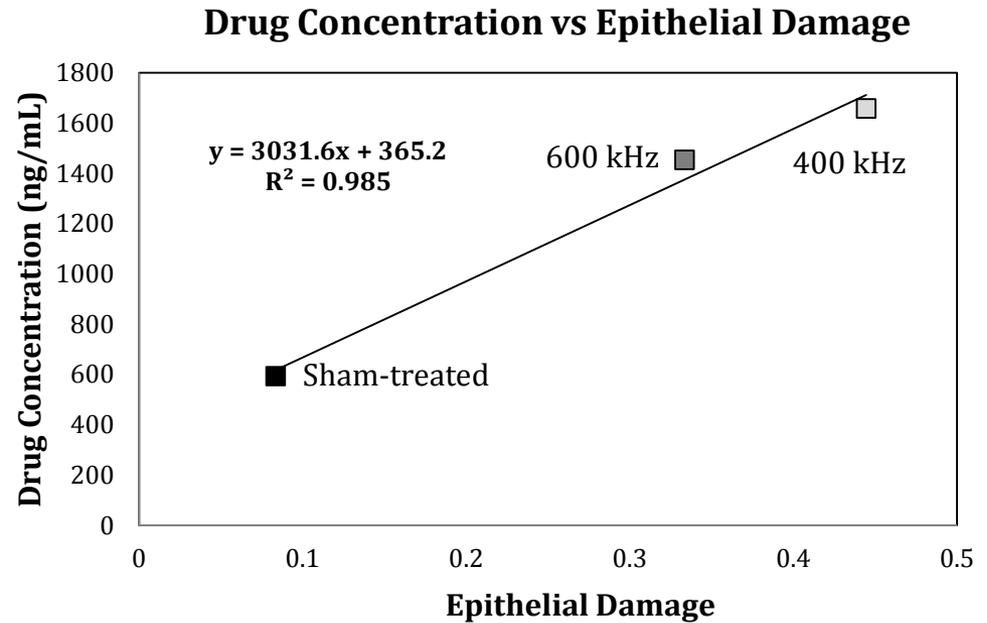
- The epithelial structural changes, observed in histological analysis, showed an increase of:
  - 4 times using 400 kHz
  - 3 times using 600 kHz



- For sham treatments n=8, using frequency of 400 kHz n=6, and n=6 using frequency of 600 kHz.

# Drug Concentration vs Epithelial Damage

There is a direct relation between the drug concentration in aqueous humor and epithelial damage.



# Temperature Changes

- The change in temperature from  $t = 0$  to  $t = 5$  min:
  - In ultrasound-treated cases was
    - 3 - 6 °C ( $4.0 \pm 1.1$  °C) for 400 kHz
    - 4 - 5 °C ( $4.8 \text{ °C} \pm 0.4\text{°C}$ ) for 600 kHz
- Temperature recorded at different time intervals:

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	Temp (t=0 min)	Temp (t=2.5min)	Temp (t=5min)
<b>Sham-treated</b>	25.3±0.7	25.6±1.0	26.6±1.3
<b>Ultrasound-treated</b>	25.3±1.0	27.8±1.3	29.7±0.1

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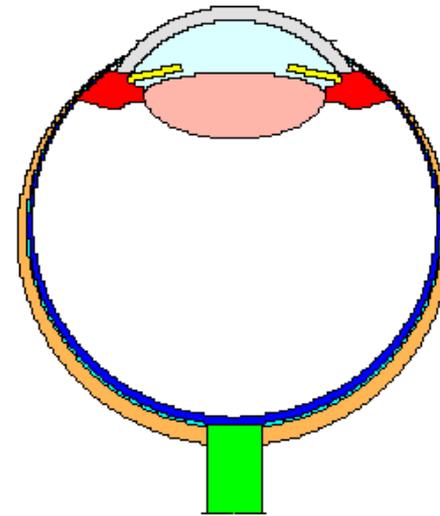
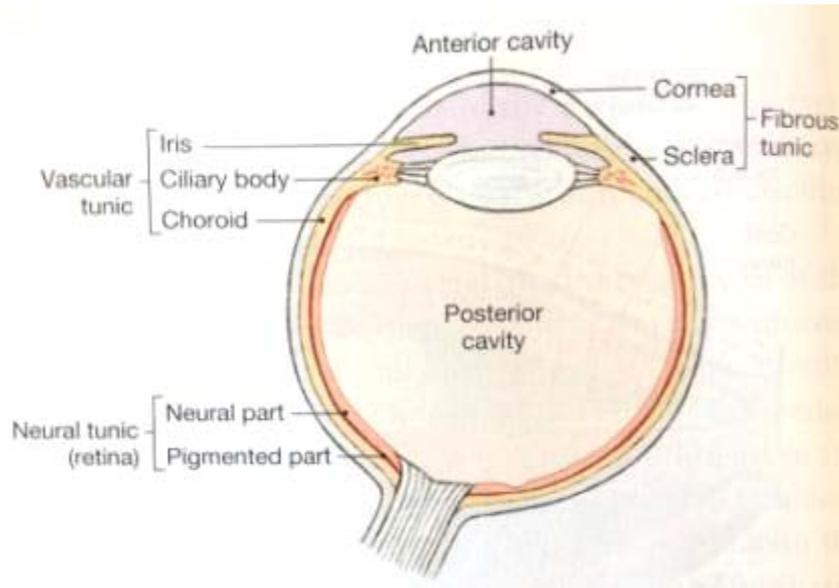
Values are shown as mean  $\pm$  standard deviation



# Modeling Objectives

- Thermal effects in different parts of the eye
- Temperature increase at different parameters
- Validating *in vitro* and *in vivo* results for temperature increase in cornea
- Limitation: no perfusion
  - No blood flow in cornea and lens
- PZFlex

# Geometric Eye Model

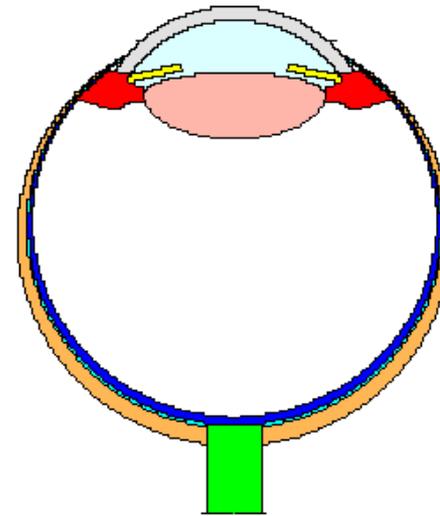
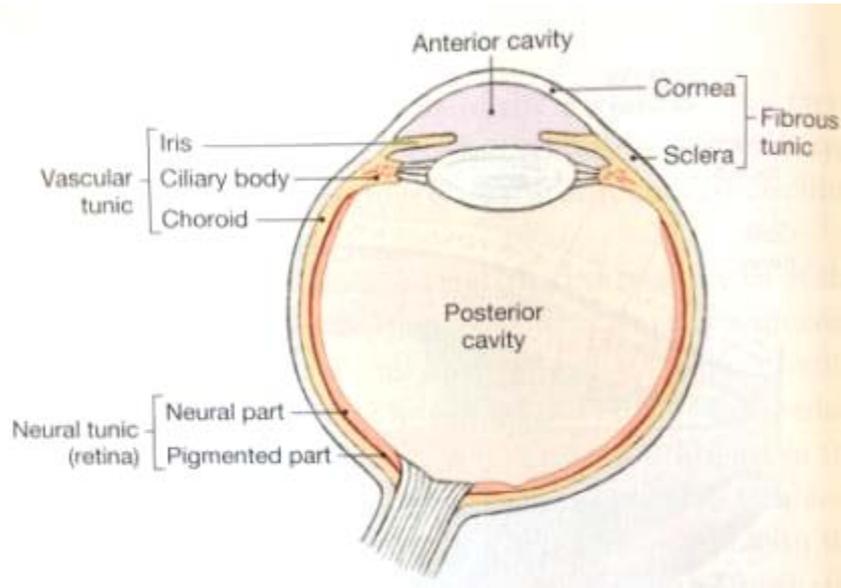


**Dimensions for rabbit and human eyeball structures in mm.**



	<b>Rabbit</b>	<b>Human</b>
<b>Antero-posterior length</b>	16-19	23-25
<b>Anterior chamber depth</b>	2.9	3.5
<b>Thickness of Cornea in center</b>	0.3-0.4	0.5
<b>Diameter of Cornea</b>	13.5-14	10.6
<b>Thickness of Cornea in periphery</b>	0.45	0.7
<b>Thickness of Lens</b>	6.36	3.5-4.3
<b>Thickness of Sclera</b>	0.328	0.5-1.0
<b>Thickness of Choroid</b>	0.068	0.1-0.5
<b>Thickness of Retina</b>	0.051	0.1-0.5

# Geometric Eye Model

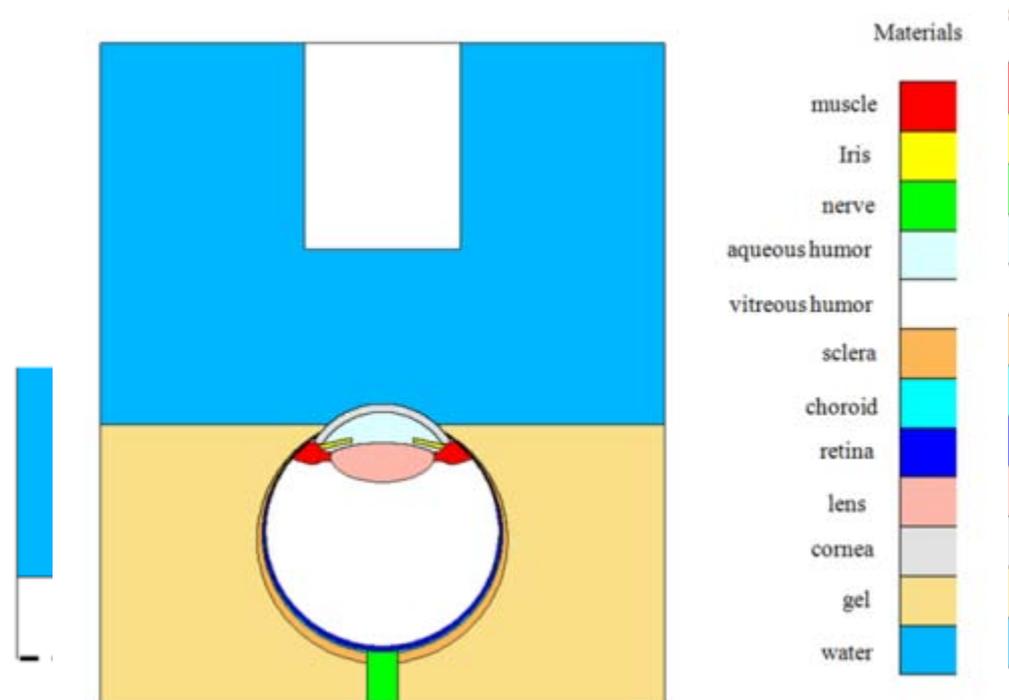


Acoustic and thermal characteristic of different eye structures

	Speed of Sound (m/s)	Acoustic Attenuation (dB/cm/MHz)	Specific Heat (J/kgK)	Thermal Conductivity (W/mK)
<b>Cornea</b>	1586	0.78	4178	0.58
<b>Sclera</b>	1647	0.97	4178	0.58
<b>Aqueous humor</b>	1497	0.01	3997	0.59
<b>Choroid</b>	1527	0.95	3840	0.60
<b>Lens</b>	1647	1.19	3000	0.40
<b>Vitreous humor</b>	1532	0.01	3999	0.60
<b>Retina</b>	1538	1.15	3680	0.57
<b>Optical Nerve*</b>	1644	0.7	3750	0.53

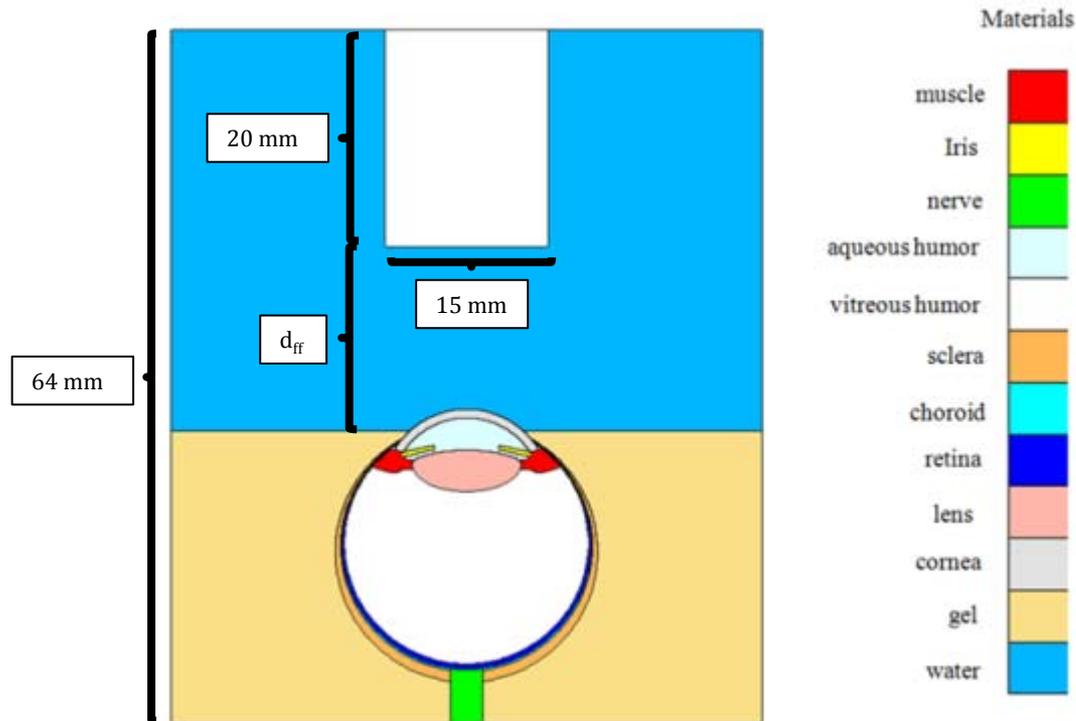
# Modeling Setup

- Theoretical model of whole eye based on accurate geometrical measurements, and acoustic and thermal characteristics of eye structures.
- An unfocused continuous ultrasound beam at frequency of 400 KHz - 1 MHz and  $0.3-1.0 \text{ W/cm}^2$  intensities.
- Axi-symmetric modeling
- Base temperature =  $37^\circ\text{C}$

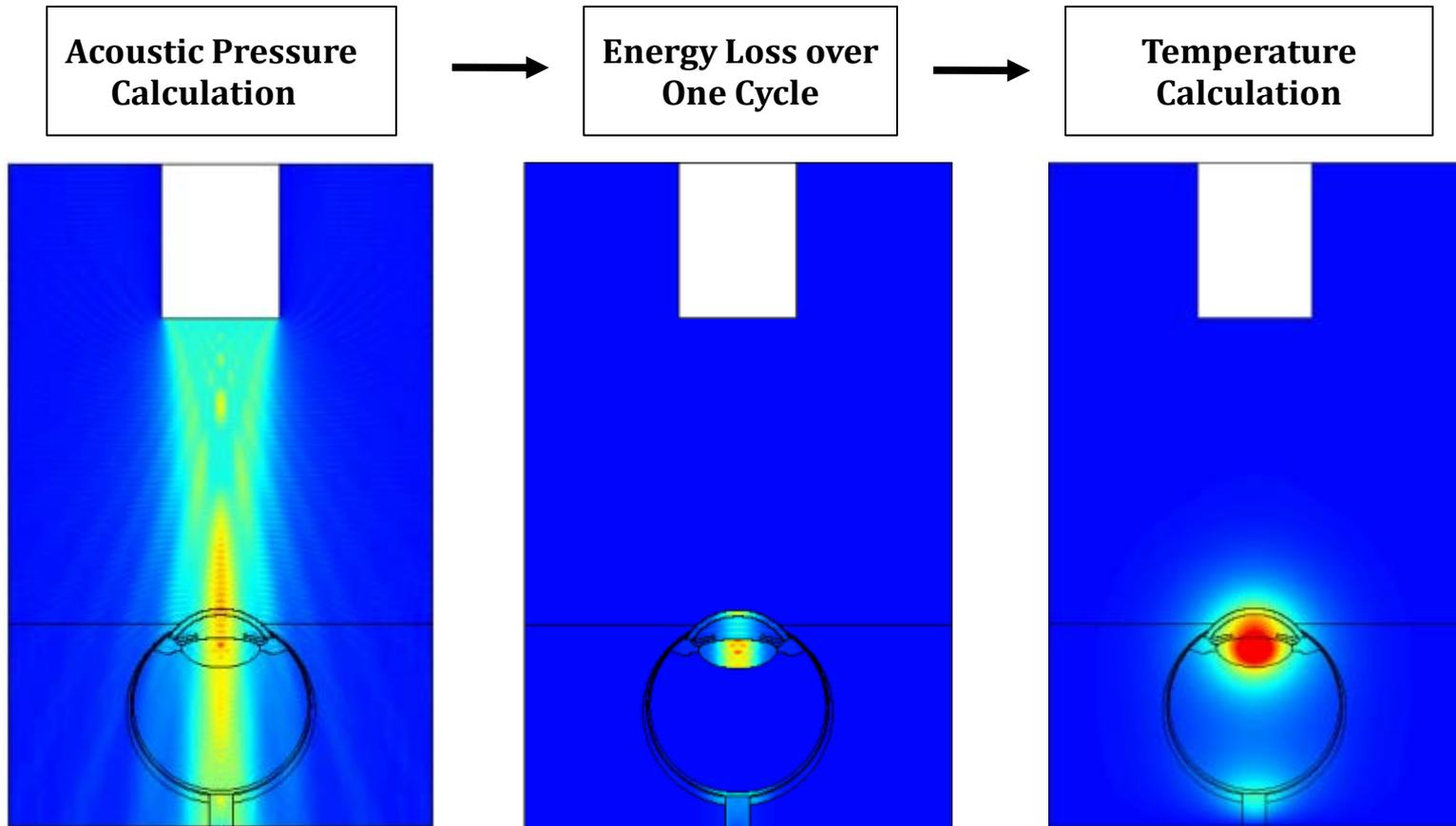


# Modeling Setup

- Unfocused transducer with 15 mm active diameter was placed at  $d_{ff}$ .
- The entire eye, other than cornea, was placed inside tissue mimicking gel.
- The material between eye and transducer was water.



# Modeling Ultrasound Wave Propagation into Eye

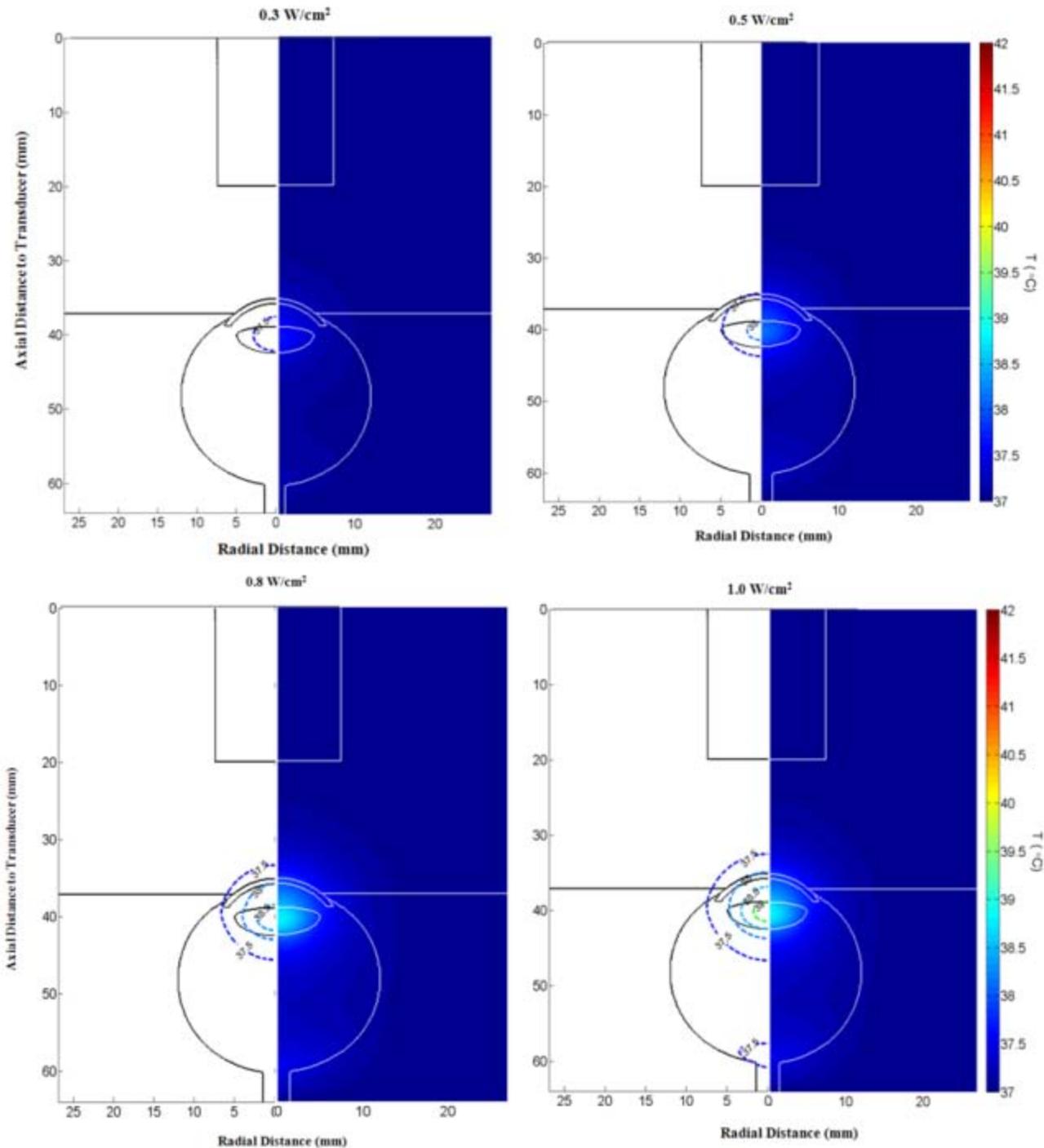


$$I = \frac{p_0^2}{2\rho c} \rightarrow p_0 = \sqrt{I \times 2\rho c}$$

$I$  = intensity  $W/m^2$  ,  $p_0$  is the pressure amplitude in  $kg/s^2m$ ,  $\rho$  is density in  $kg/cm^3$ , and  $c$  is speed of sound in  $m/s$ .

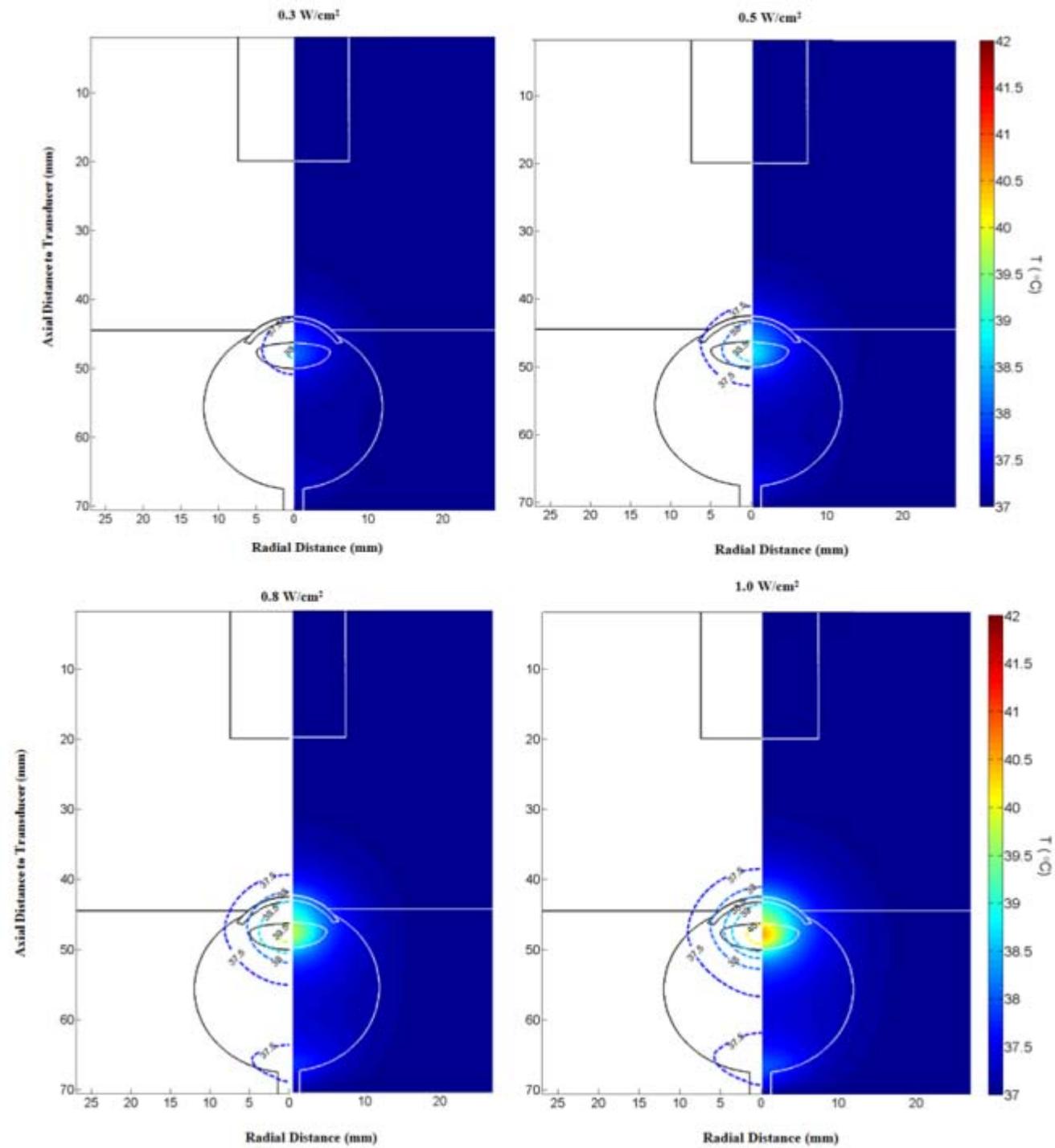
# Results at 400 kHz

$T_{MAX} \sim 39^{\circ}\text{C}$  in the lens at frequency of 400 kHz and intensity of  $1.0 \text{ W/cm}^2$ .



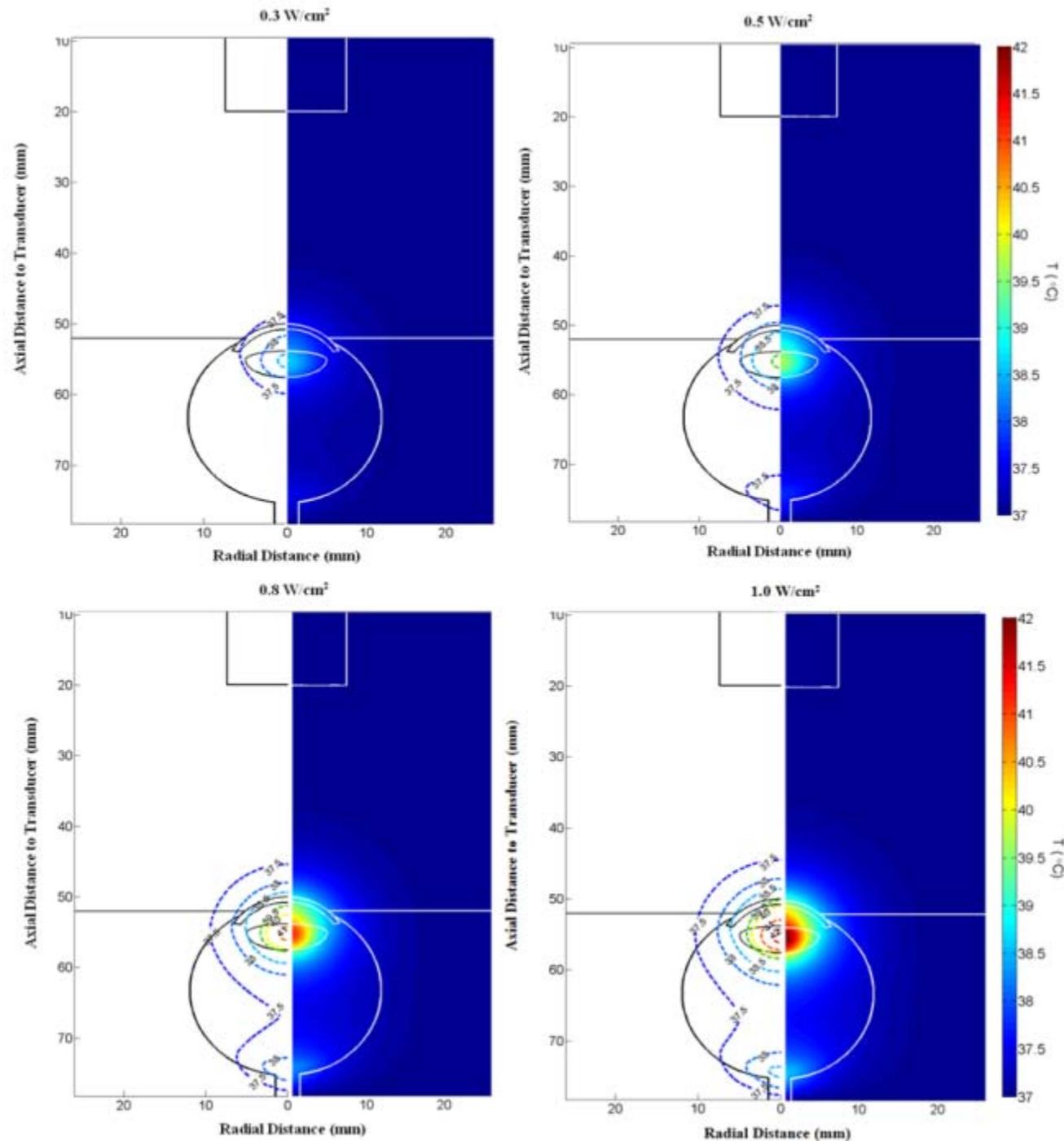
# Results at 600 kHz

$T_{MAX} \sim 40.5^{\circ}\text{C}$  in the lens at frequency of 600 kHz and intensity of  $1.0 \text{ W/cm}^2$ .



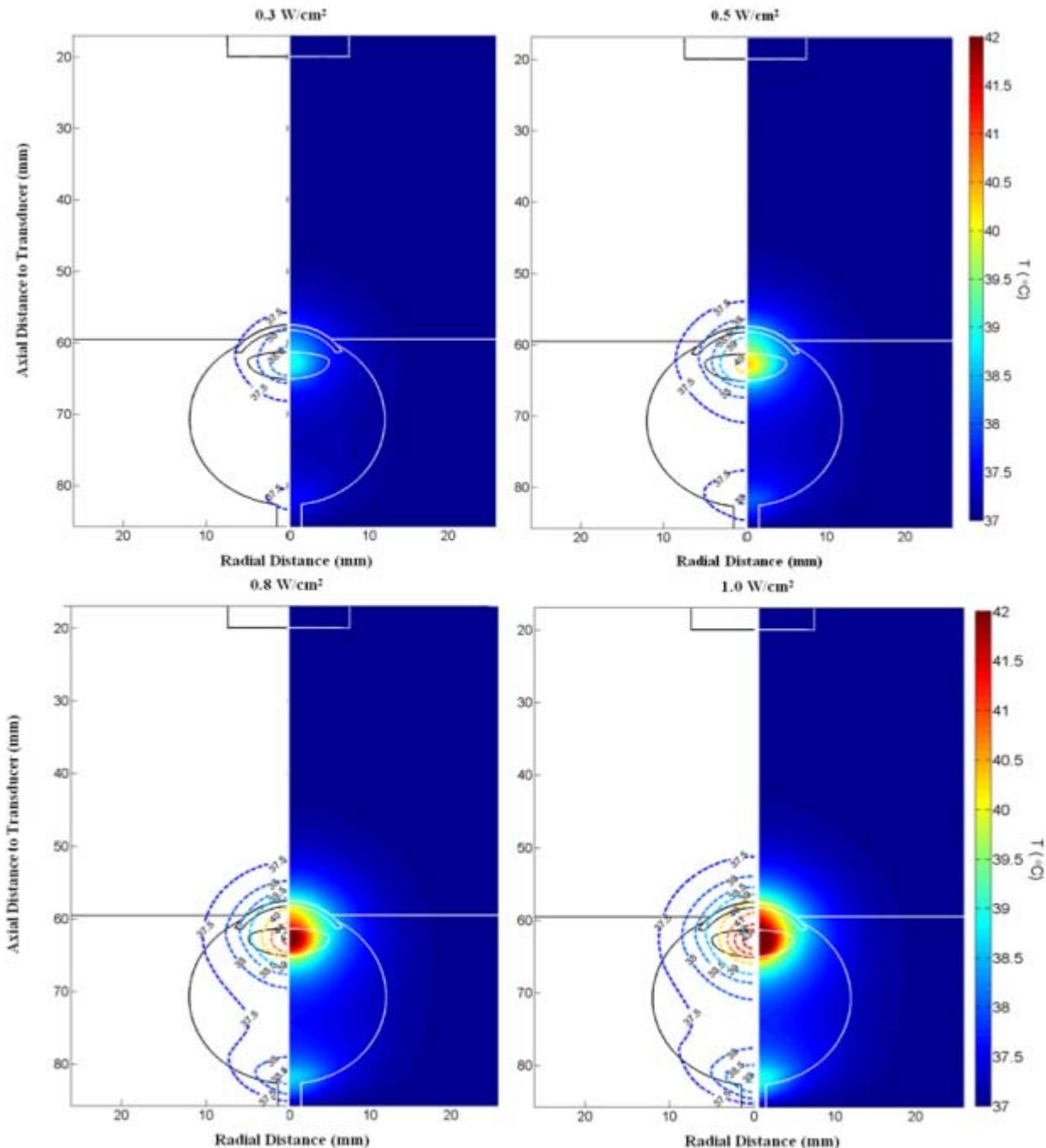
# Results at 800 kHz

$T_{MAX} \sim 42.5^{\circ}\text{C}$  in the lens at frequency of 800 kHz and intensity of  $1.0 \text{ W}/\text{cm}^2$ .



# Results at 1 MHz

$T_{MAX} \sim 43.5^{\circ}\text{C}$  at proximity  
of the lens at frequency of 1  
MHz and intensity of  $1.0$   
 $\text{W}/\text{cm}^2$ .





# Future Work

- Investigating safety factors of ultrasound application in the proximity of the bone (for example optical nerve) and also bone itself.
- Using pulsing method may increase the treatment time but would result in lower temperature increase.
- Drug delivery into the back of the eye.
- Ocular delivery of macromolecules.

# Conclusions

- Confirmed the use of ultrasound *in vitro* and *in vivo*, increased ocular drug delivery.
- Skills in tissue processing, animal handling, and image analysis using microscope were developed.
- A set up demonstrated the feasibility of mechanical and thermal effect of ultrasound in enhancement of corneal permeability.
- A model was established for safety of this application.

## Acknowledgments

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