Neuronal Stimulation by Acoustic Fields

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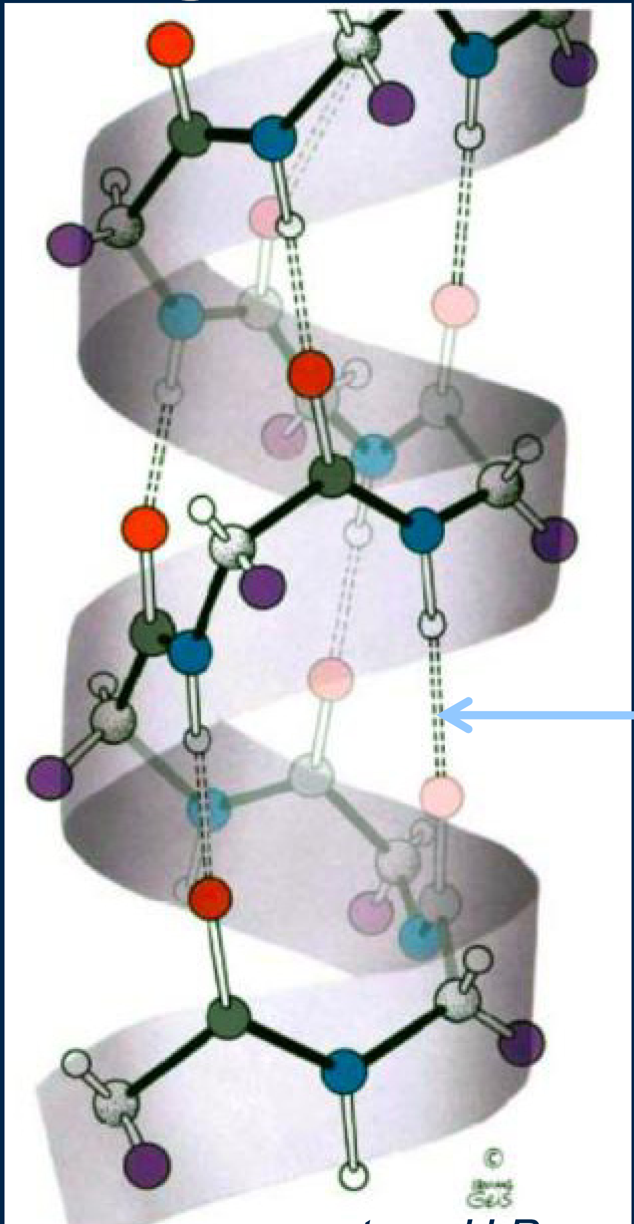
IEEE 2011-12-01 Melville NY USA

Introduction

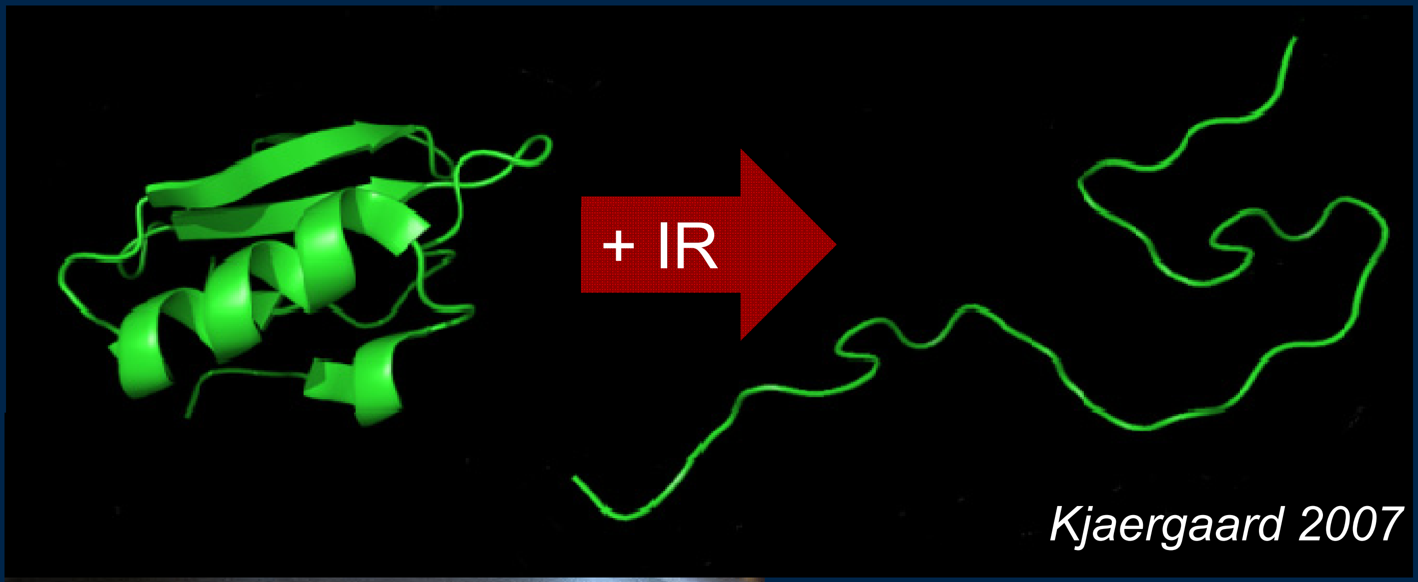
Hydrogen Bonds Form Protein Structures


courtesy U Penn

α Helix secondary structure

hydrogen (electrostatic) bond

Heat Denatures Protein



H bonds break at ½ eV, destroying 4th, 3rd, 2nd order structures.

Example:

ovalbumin in egg white turns from optically clear to opaque

Lossy Acoustic Beam Heats Tissue

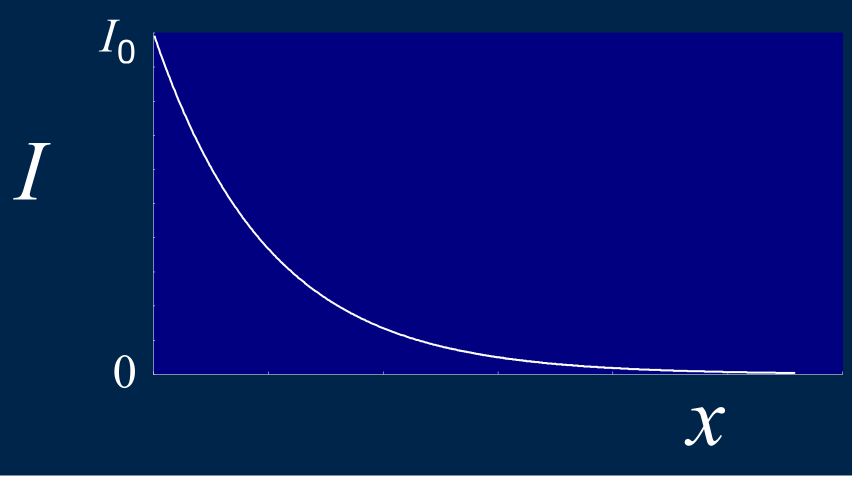
$$Q \sim I$$

$$I(x) = I_0 e^{-2\alpha f x}$$

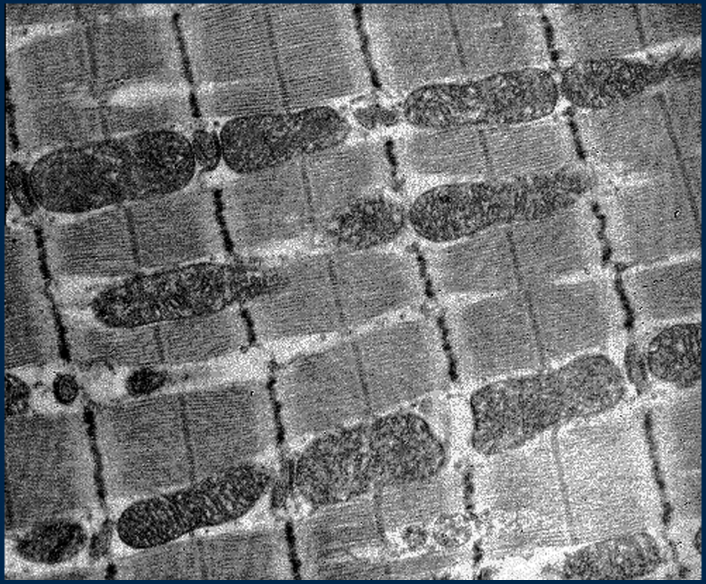
Q heat
I local beam intensity

transducer properties
I_0 incident intensity
f frequency

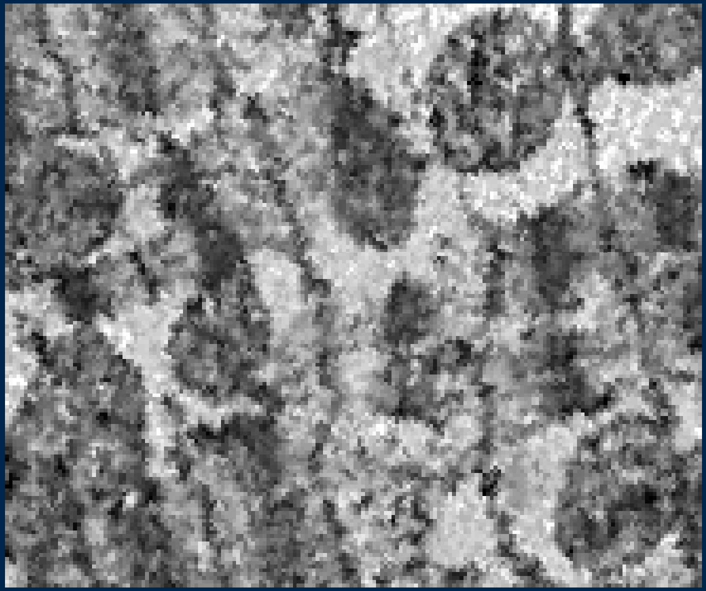
tissue properties
α attenuation coefficient
x depth



Heating > 43 ° C Denatures Proteins



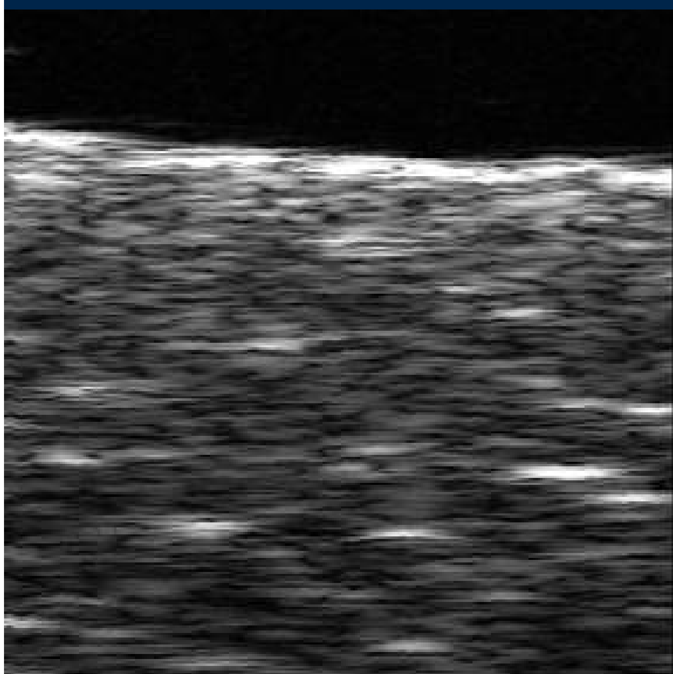
normal
myocardium



ablated

TEM
1 μm

Ablation Invisible to Ultrasound B-mode

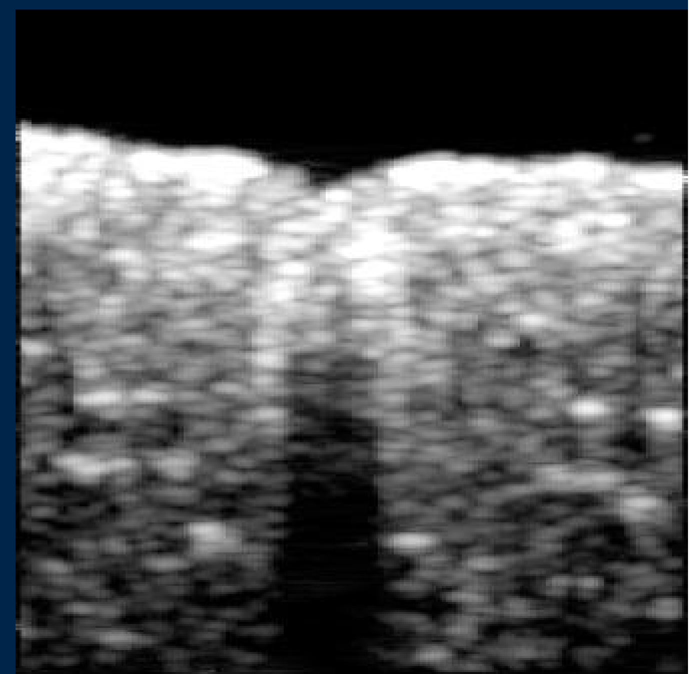


1 cm

9 MHz f/3.5
transducer

mechanical scan

ex vivo chicken
breast



conventional
B-mode image

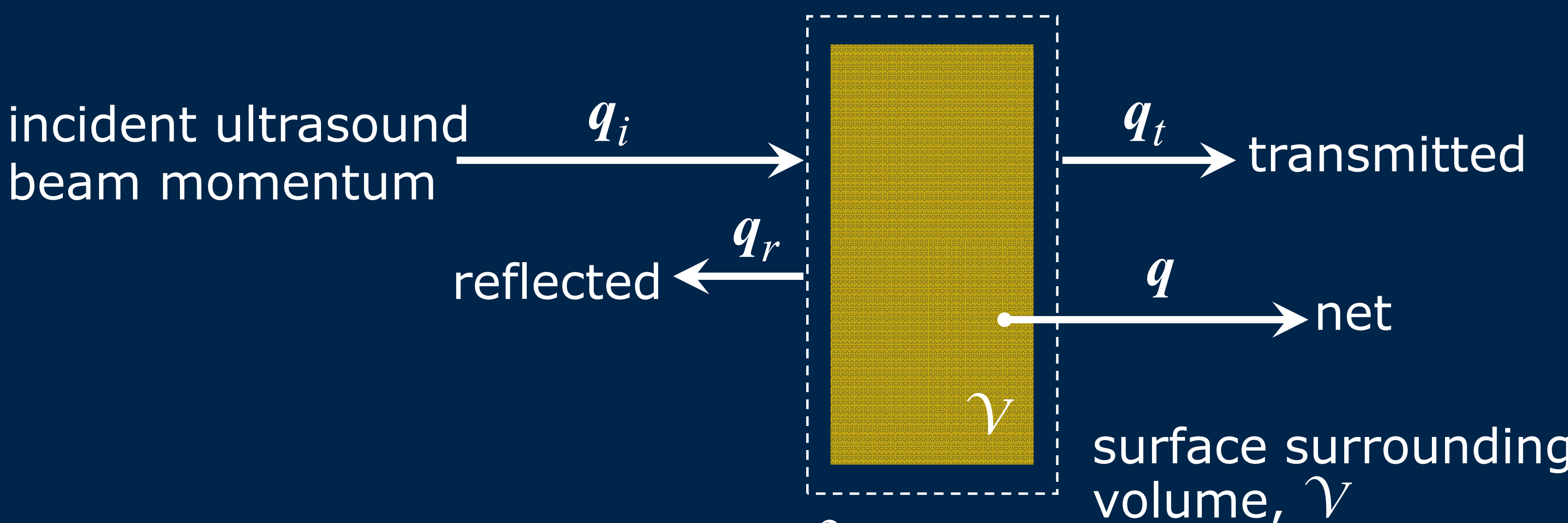
enhanced image
reveals
HIFU lesion

Acoustic Radiation Force for Lesion Palpation

$$F/\mathcal{V} \sim \alpha f I/c$$

F	acoustic radiation force
$\mathcal{V}$	volume
α	attenuation coefficient
f	frequency
I	local beam intensity
c	speed of sound

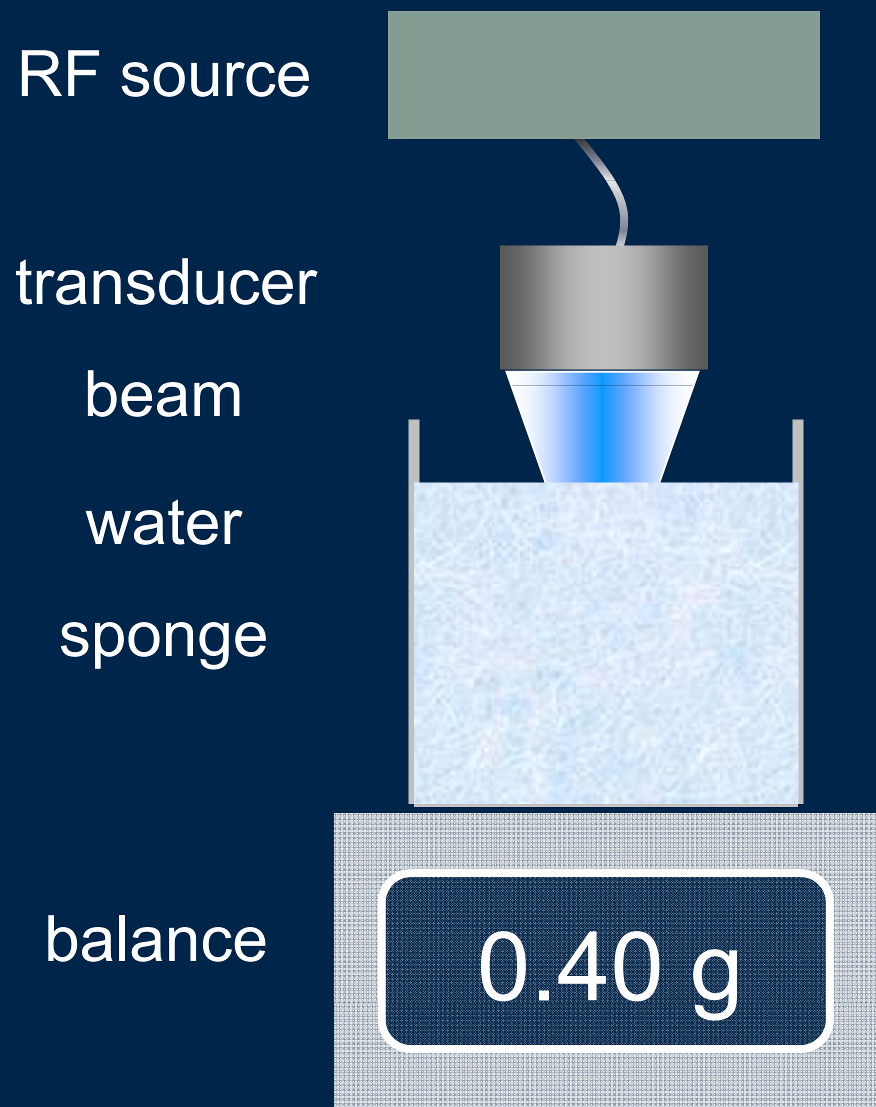
Acoustic Field Conserves Linear Momentum



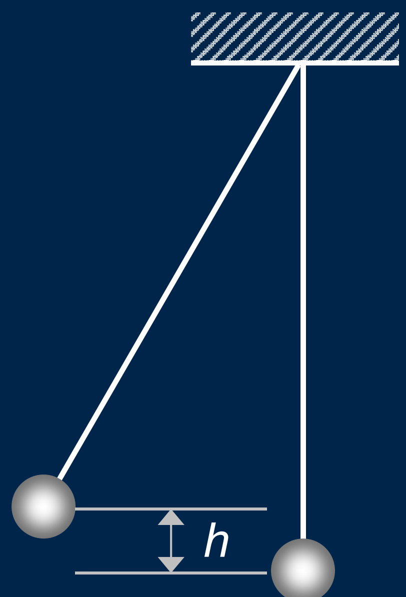
net momentum imparted to $\mathcal{V}$:

$$q = \underbrace{\underbrace{(q_i - q_t)}_{\text{absorption}} + \underbrace{\boldsymbol{q}_r}_{\text{reflection}}}_{\text{attenuation}}$$

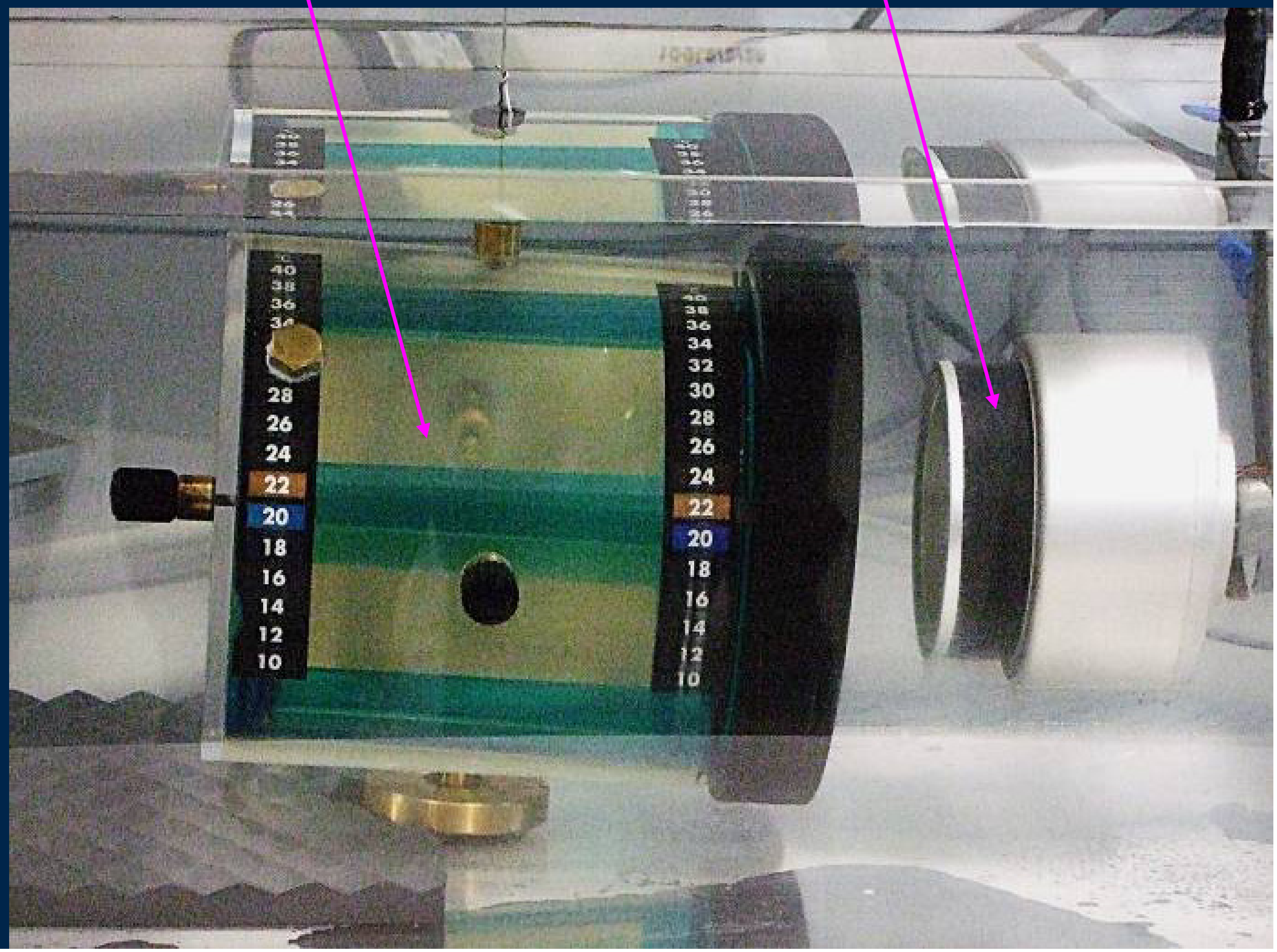
Flat Absorbing Target Wattmeter (FDA)



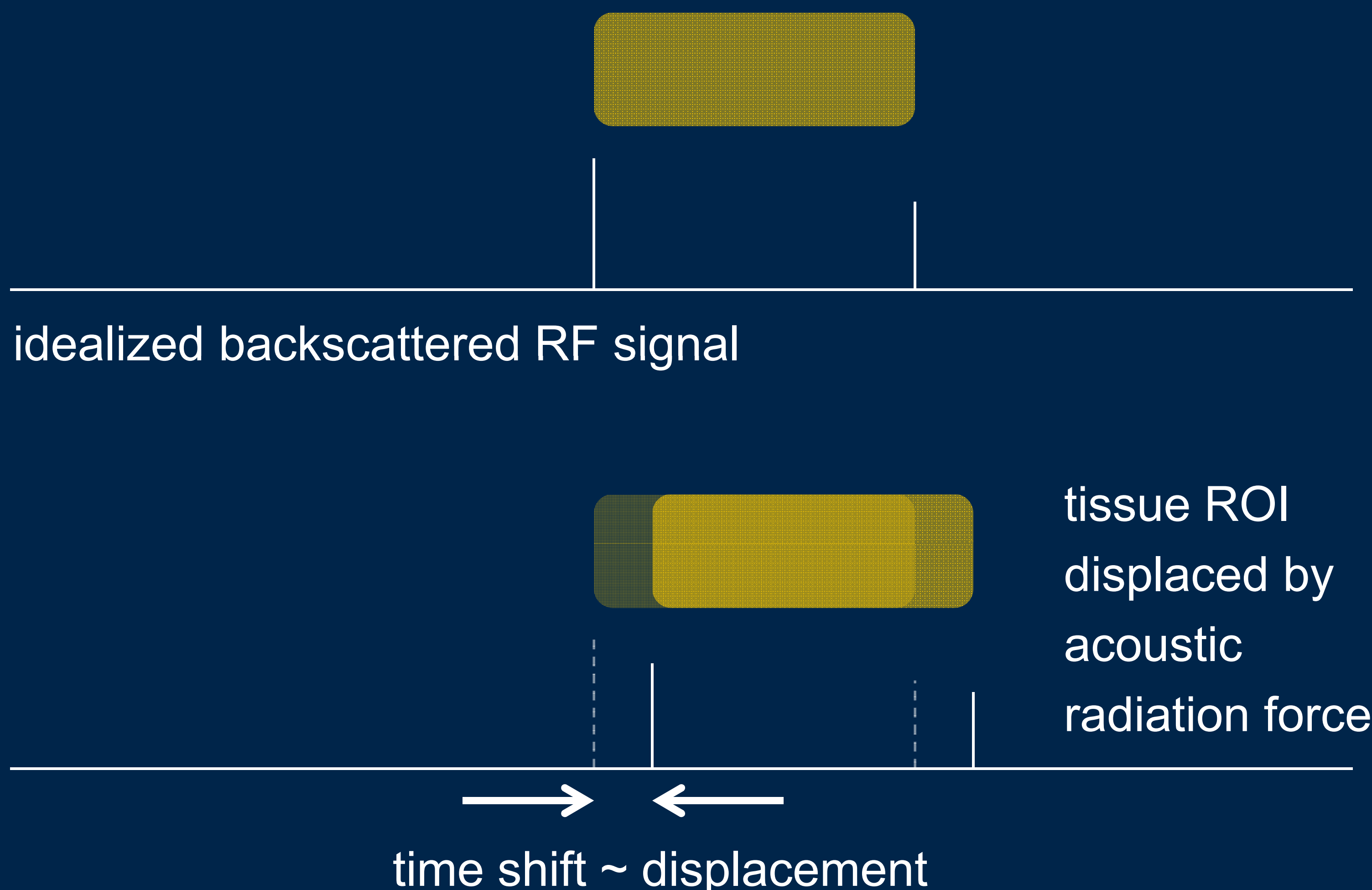
Ballistic Pendulum (NPL, UK)



$$mgh = \tfrac{1}{2} mv^2$$

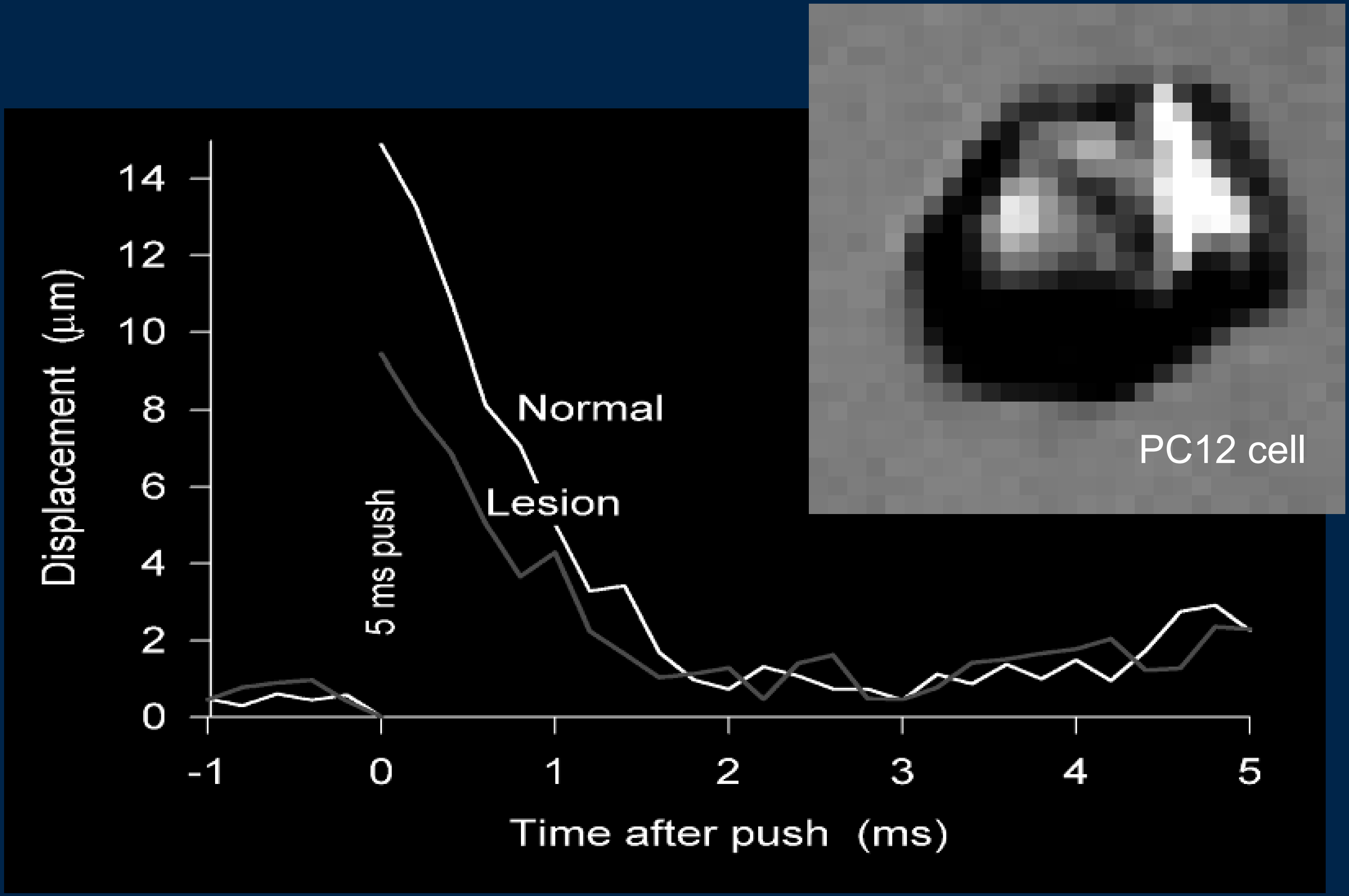


A-mode Imaging of Displacement



acoustic force field can be steady state (oscillatory) or transient (impulse)

Displacement < Cell Diameter



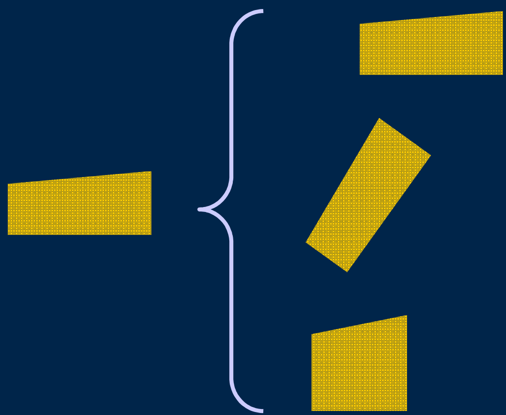
Lizzi, Muratore, Deng, et al. UMB 2003

Induced Cell Transformations

Acoustic field

↓

Stress ➔ Structural response ➔ Functional response



translation
rotation
} displacement ➔ cell-cell adhesive triggers

deformation ➔ membrane-bound & internal triggers

e.g.,
integrin-mediated response
cytoskeletal response

continuum mechanics view

Giancotti 1999 Science
Ingber 2003 J Cell Sci

Hypothesis

Acoustic field

↓

Stress ➔ Structural response ➔ Functional response

?

?

brightfield

& fluorescence

microscopy,

ultrasound

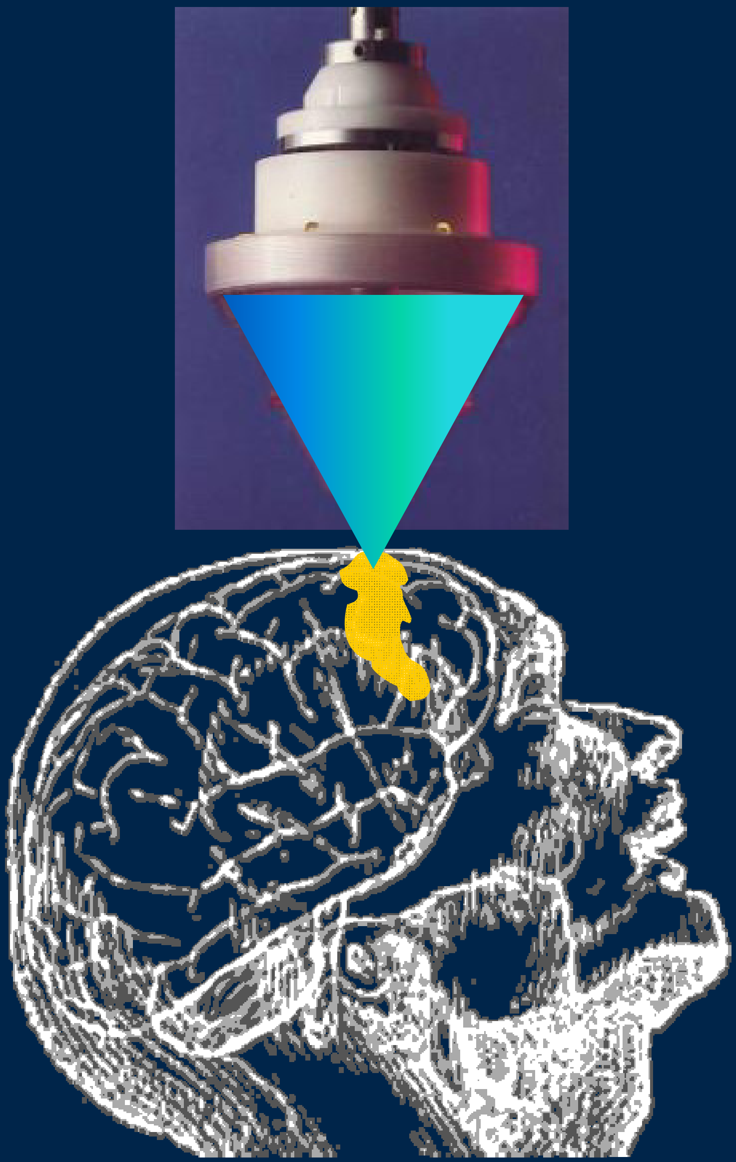
e.g., electrical activity

(neuronal tissue),

enhanced / disrupted

activity (many tissues)

Application: Non-invasive Brain Stimulus



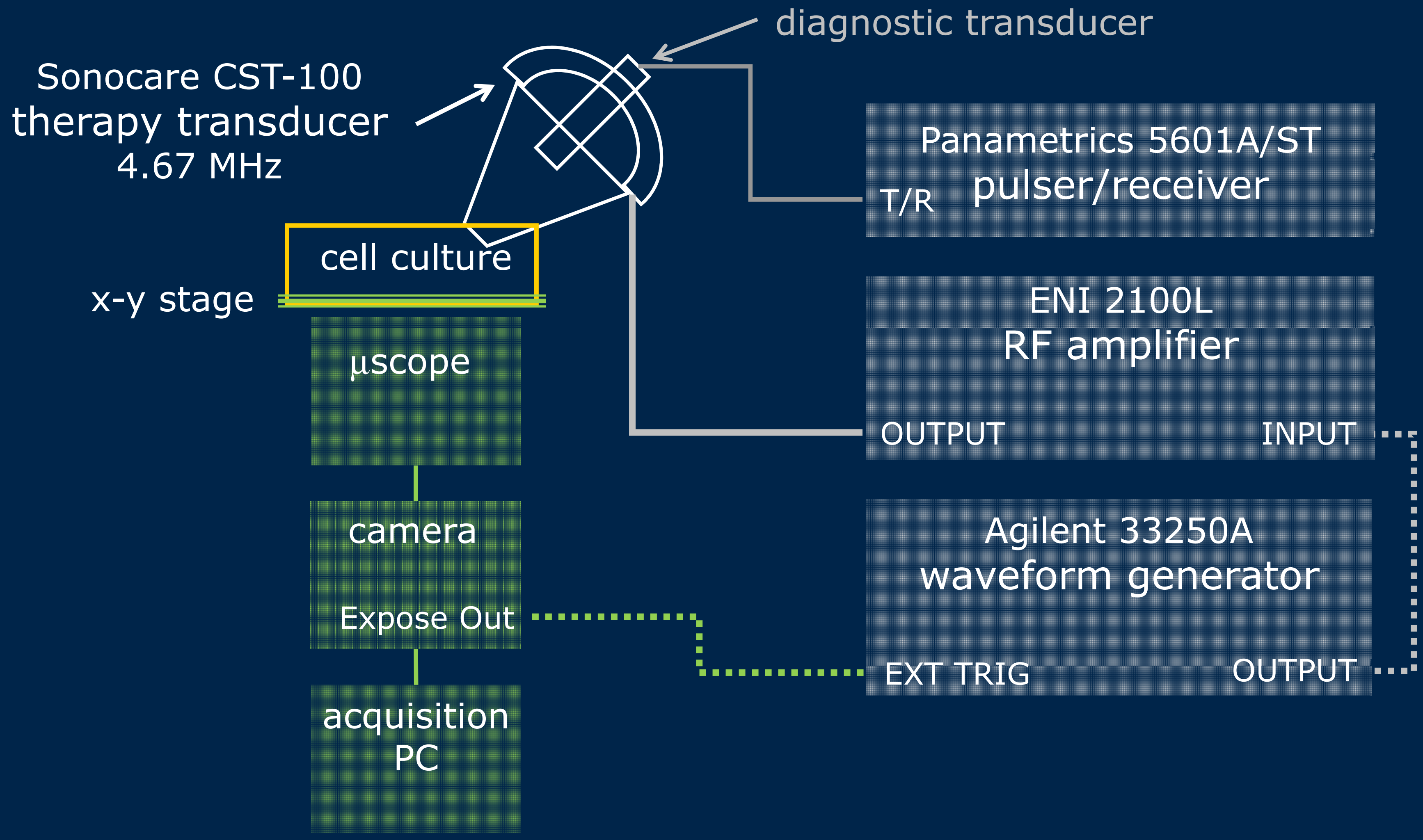
transducer

ultrasound beam

neuronal stimulus

Methods – Structural Studies

System


diagnostic transducer
Sonocare CST-100
therapy transducer
4.67 MHz
Panametrics 5601A/ST
pulser/receiver
T/R
cell culture
x-y stage
ENI 2100L
RF amplifier
μscope
OUTPUT
INPUT
camera
Agilent 33250A
waveform generator
Expose Out
EXT TRIG
OUTPUT
acquisition
PC

Therapy Transducer

Sonocare CST-100
PZT-4 spherical cap
80 mm diameter, 90 mm focal length
f/1.1, 19 mm diameter central hole
4.67 MHz

Panametrics MD3657
A-mode diagnostic transducer, 7.5 MHz

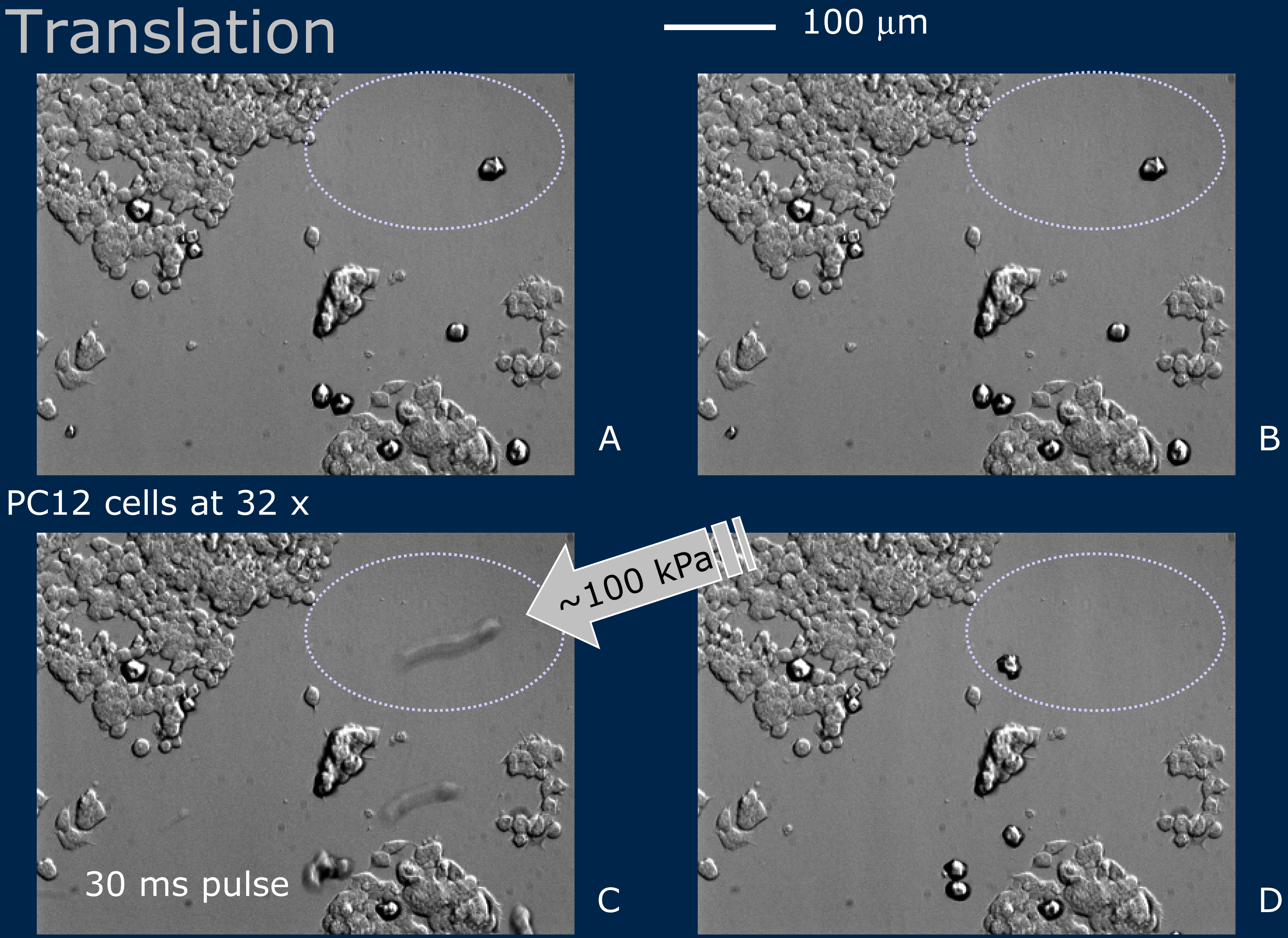
water-filled coupling cone

Muratore 2005 ISTU

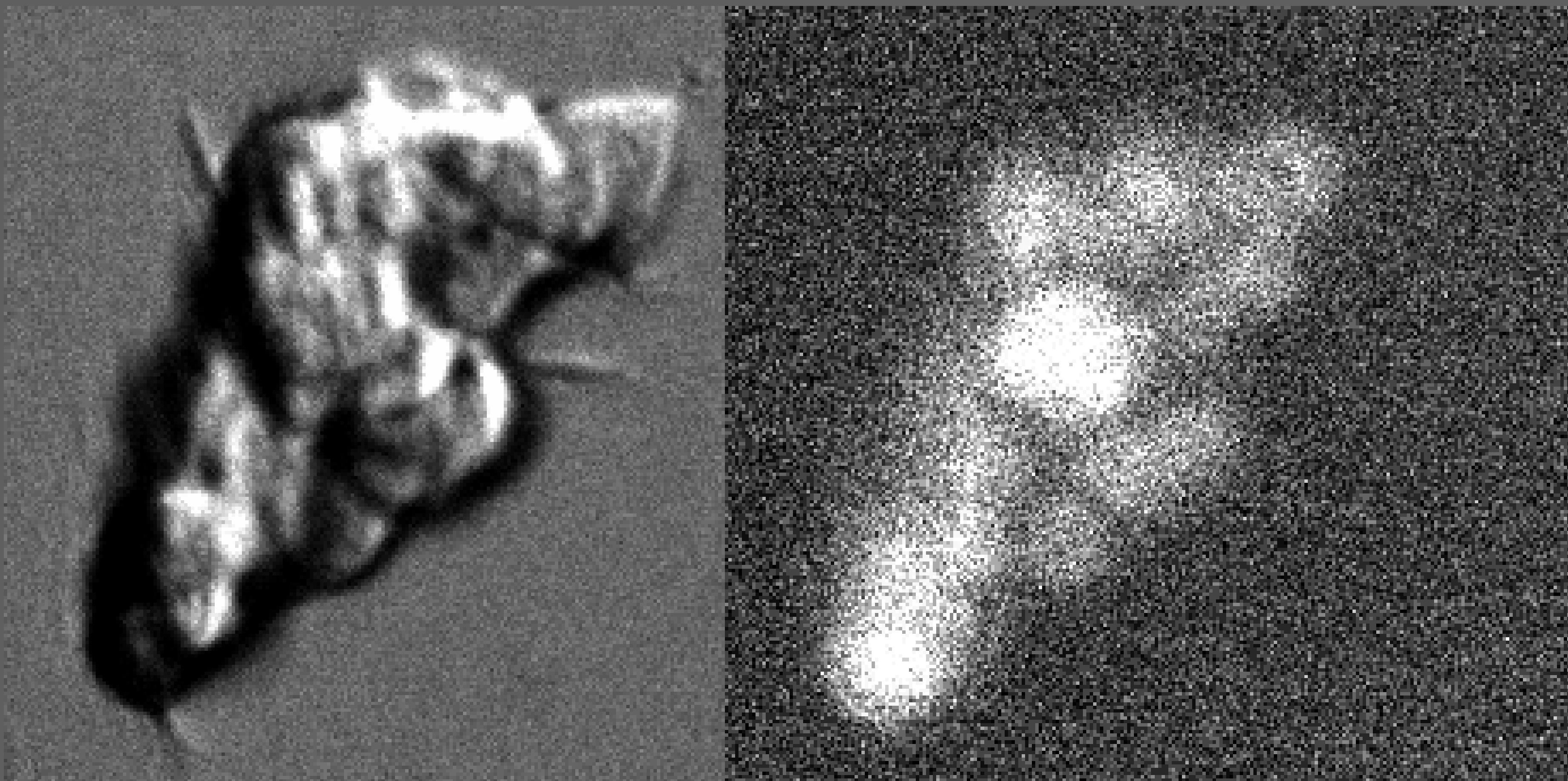
-3 dB focal region ≈ 0.4 mm x 1.2 mm

Results – Structural Studies

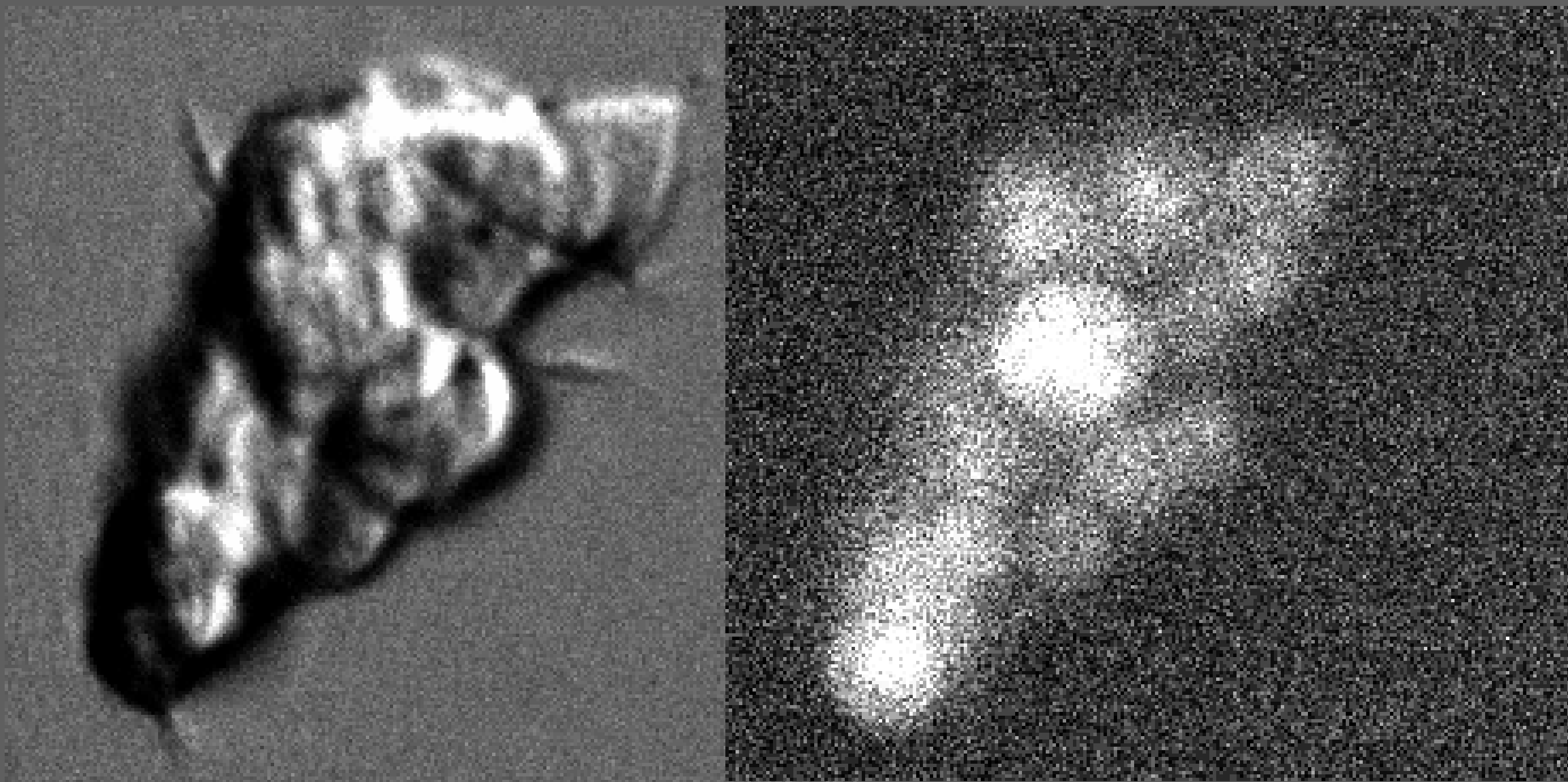
Translation



Deformation



PC12 cells adhered to polystyrene with poly-L-lysine



optical

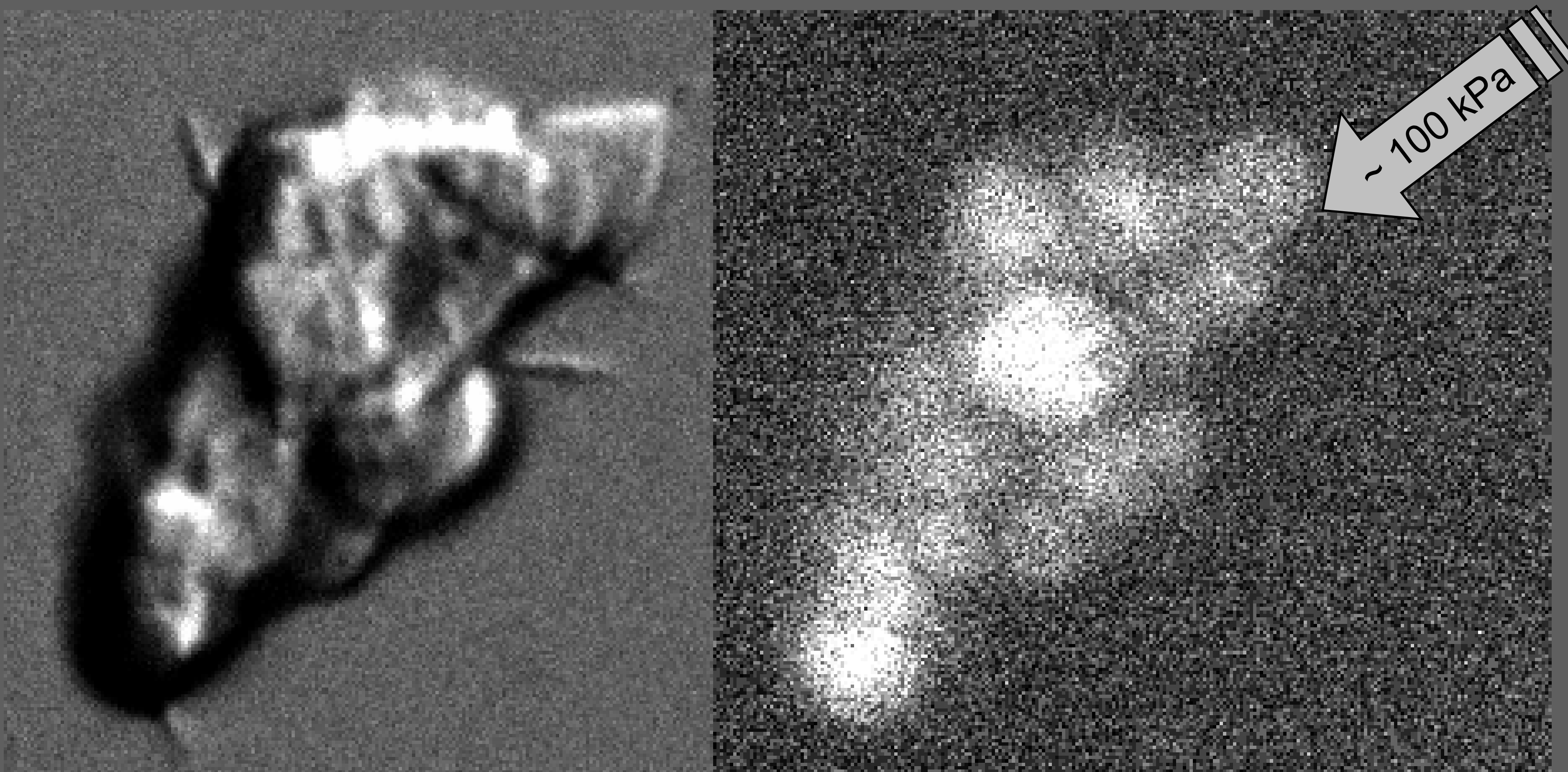
DAPI

acoustic
radiation
force
4.67 MHz
30 ms

10 μm

B

PC12 cells adhered to polystyrene with poly-L-lysine



optical

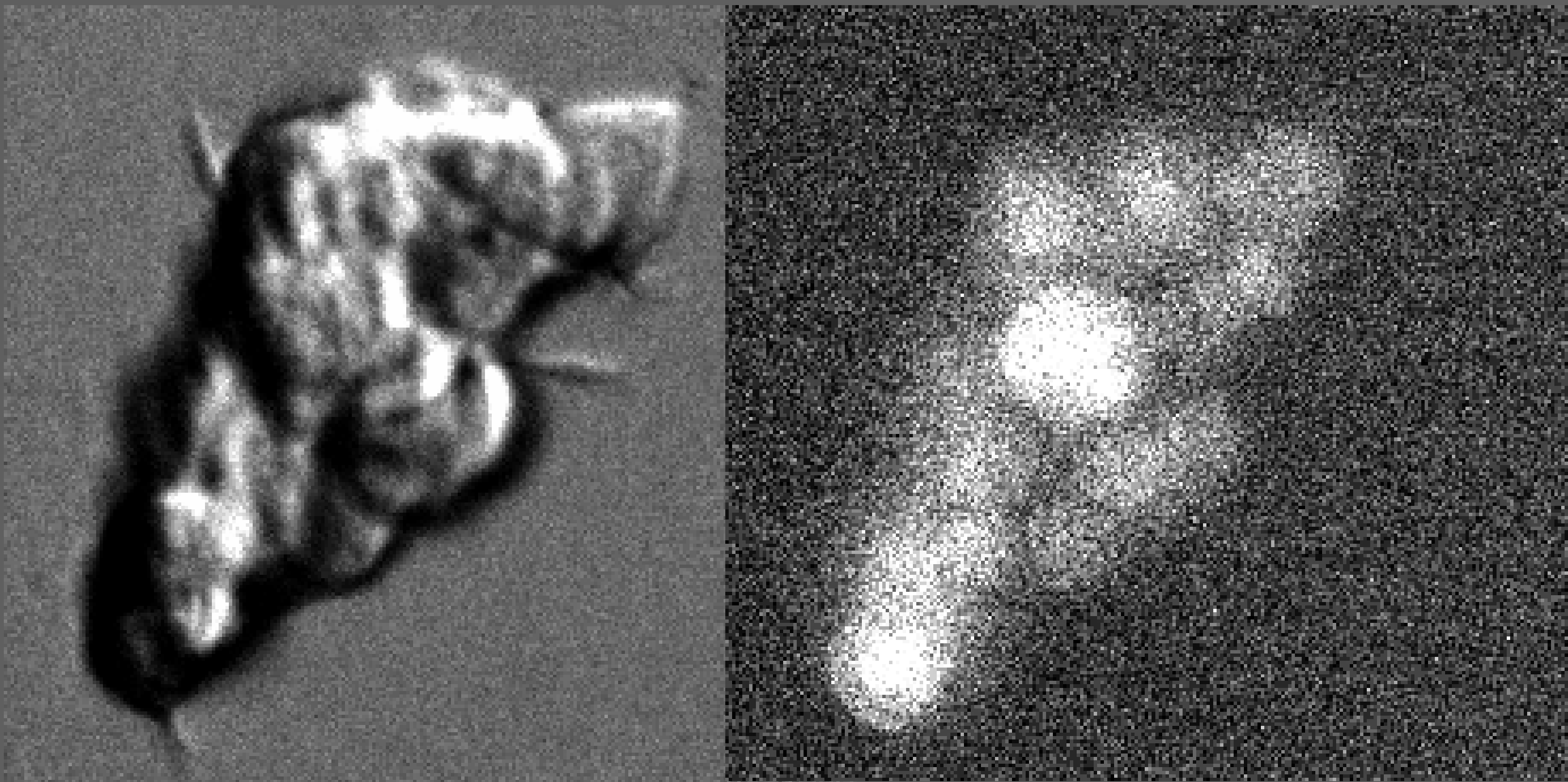
DAPI

acoustic
radiation
force
4.67 MHz
30 ms

10 μm

C

PC12 cells adhered to polystyrene with poly-L-lysine



optical

DAPI

10 μm

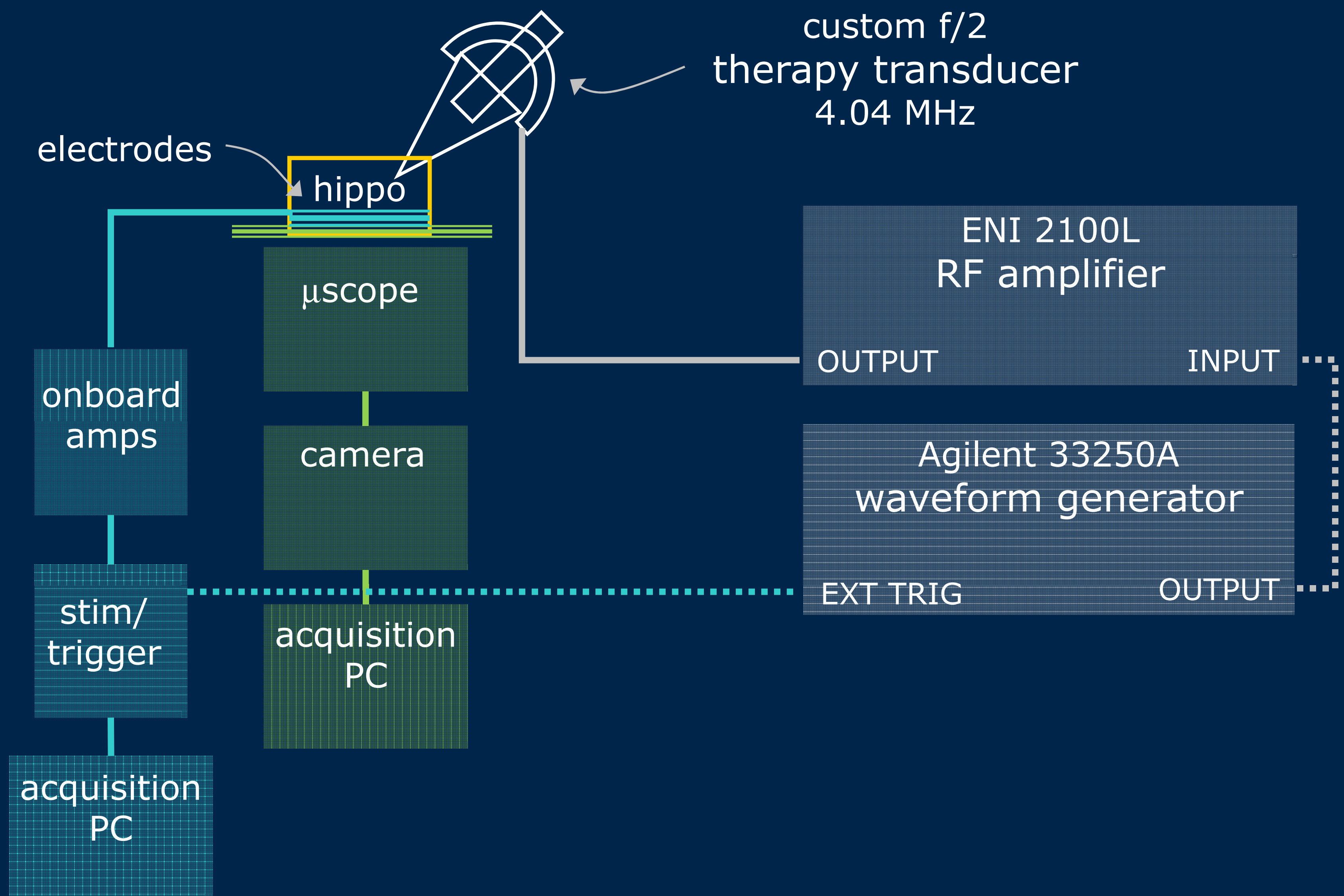
acoustic
radiation
force
4.67 MHz
30 ms

D

PC12 cells adhered to polystyrene with poly-L-lysine

Methods – Functional Studies

System


custom f/2
therapy transducer
4.04 MHz
electrodes
hippo
µscope
camera
acquisition PC
onboard amps
stim/ trigger
acquisition PC
ENI 2100L
RF amplifier
OUTPUT
INPUT
Agilent 33250A
waveform generator
EXT TRIG
OUTPUT

Ultrasonic Subsystem

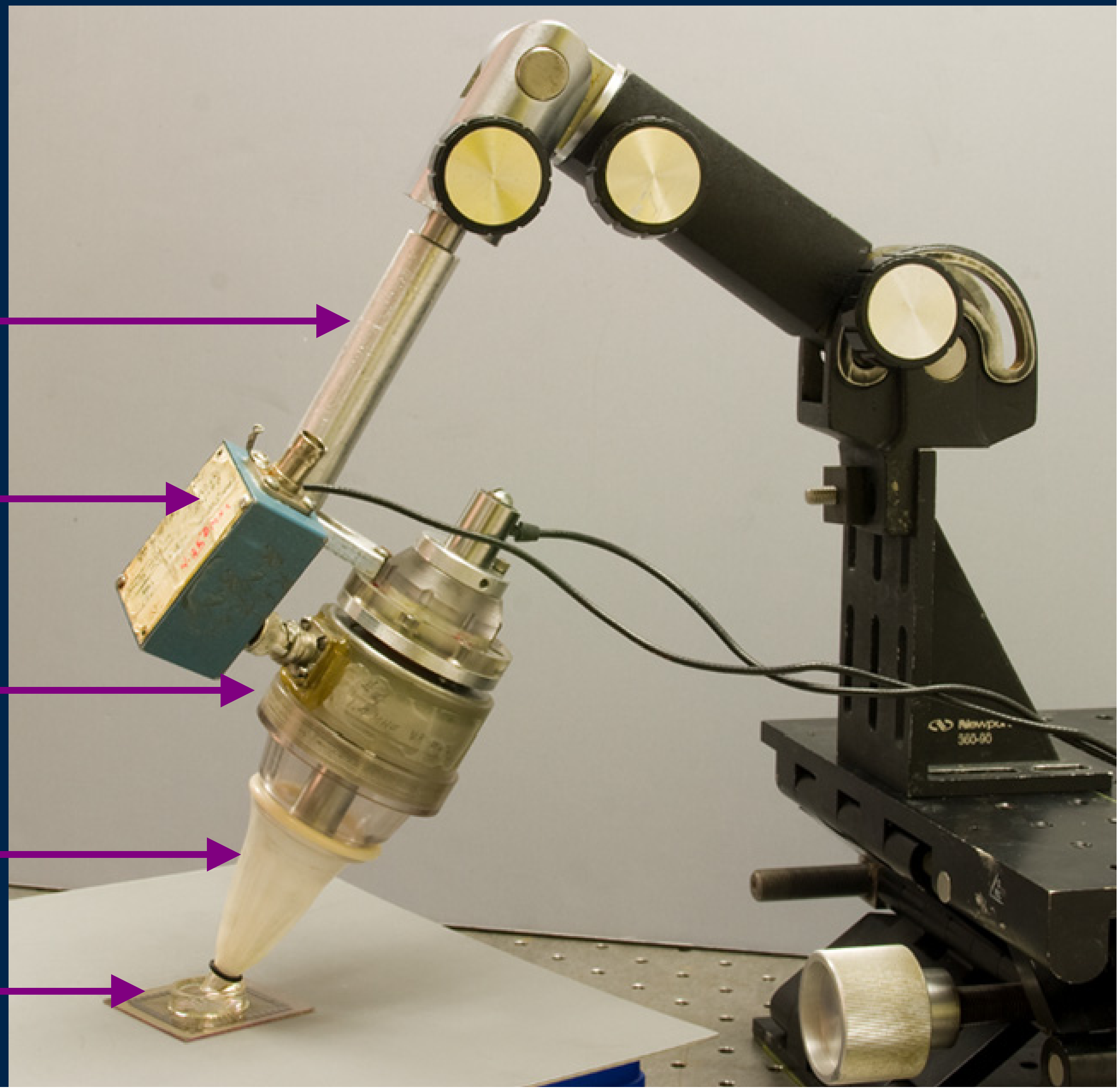
multi-axis support armature

matching network

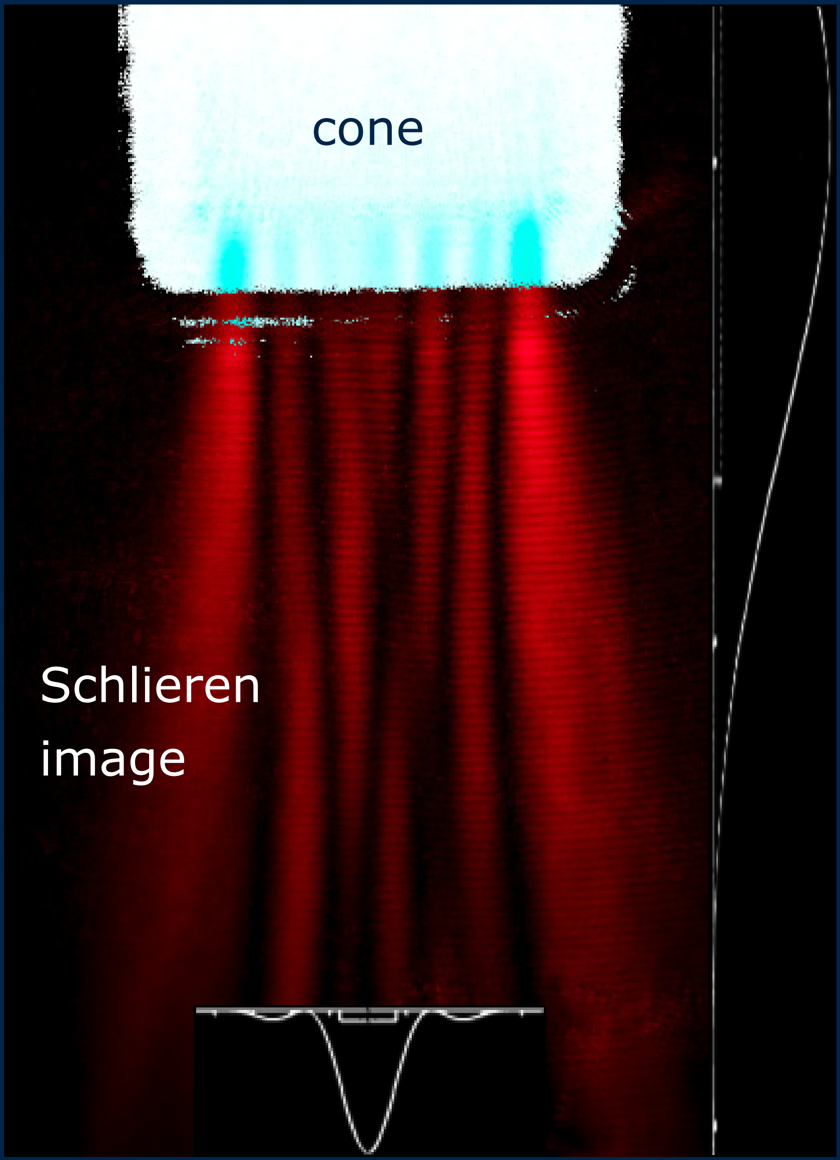
PZT spherical cap transducer f/2 4MHz

water-filled coupling cone

cell culture dish

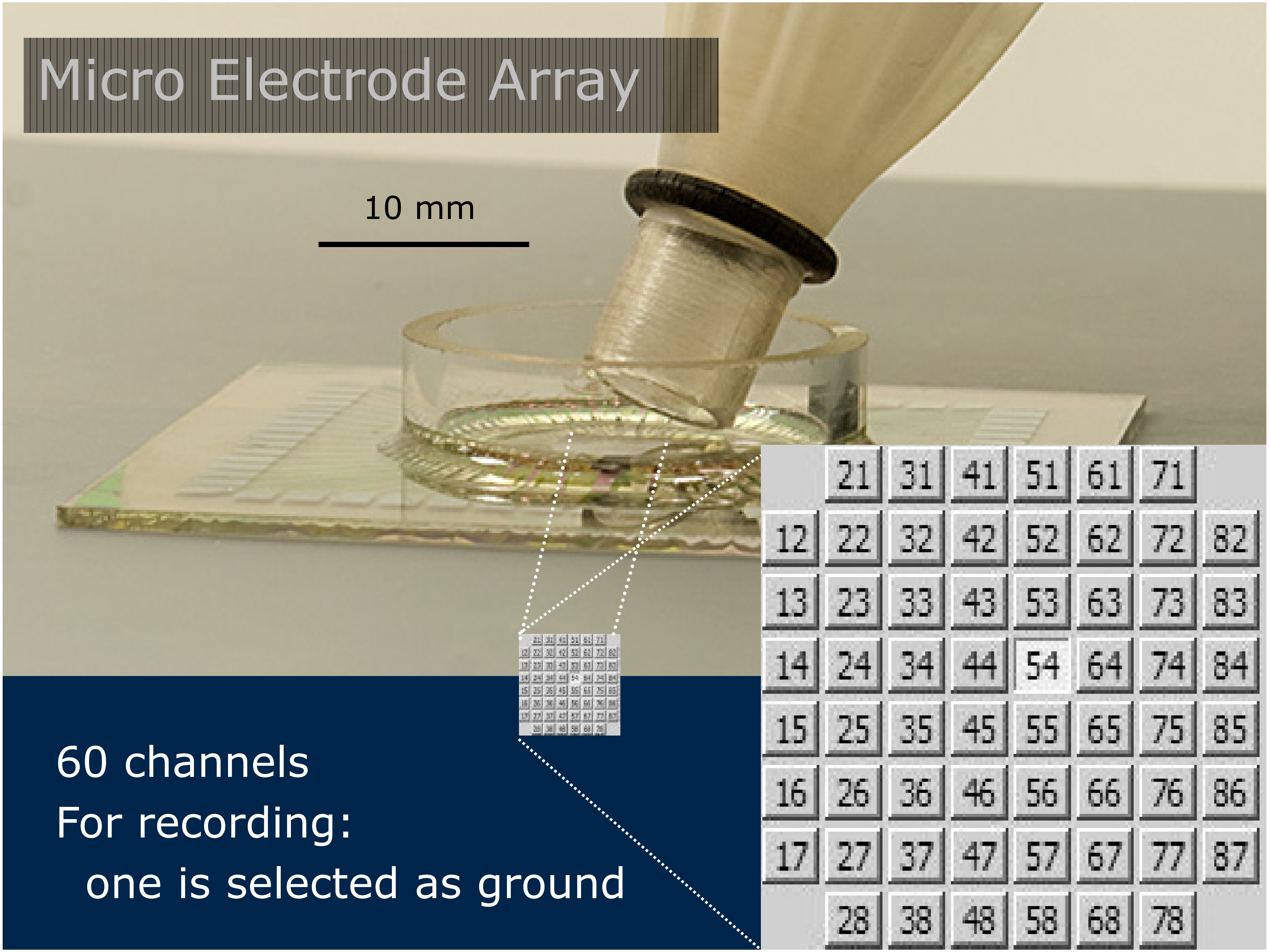


Ultrasound Stimulus

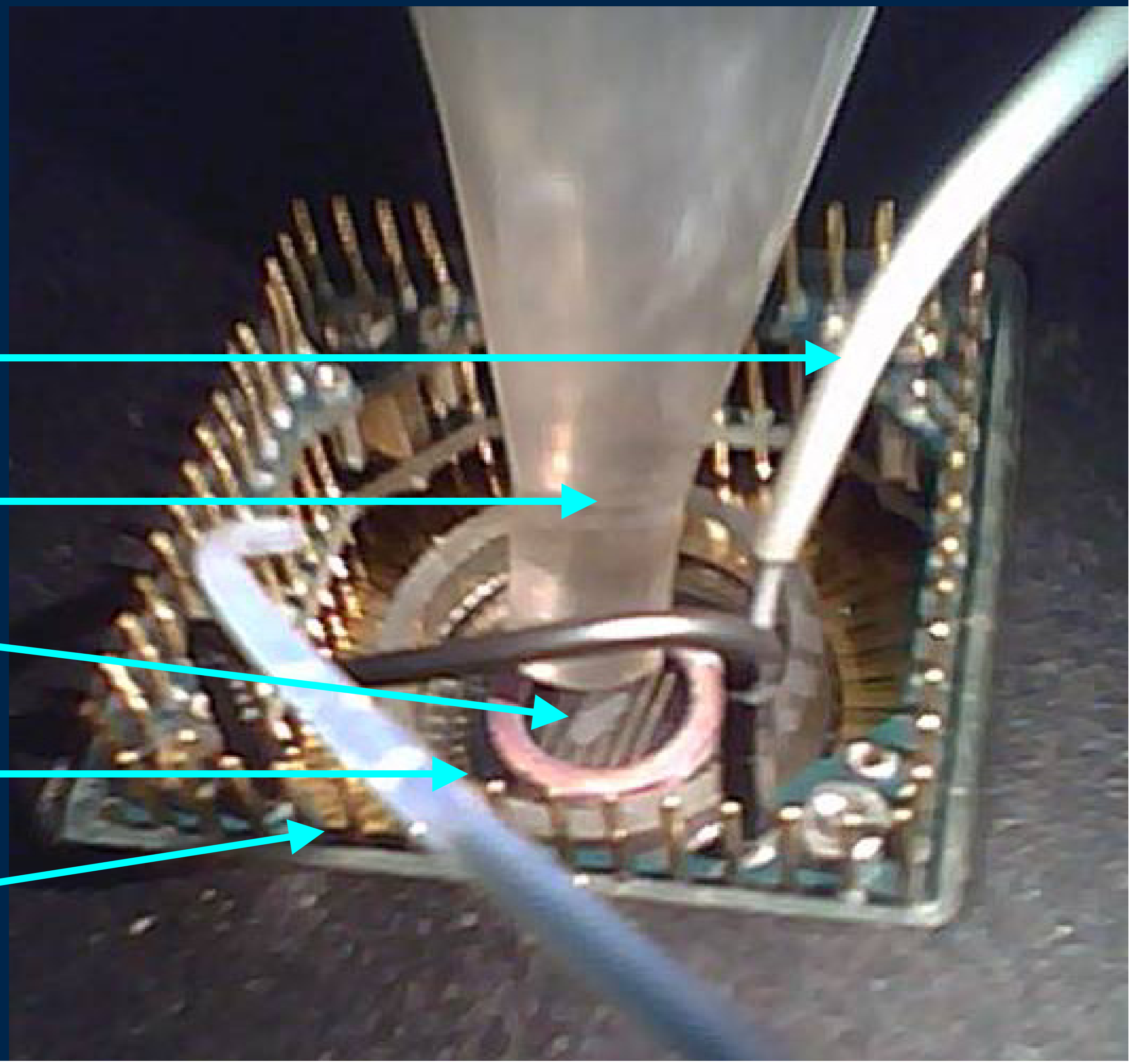


peak pressure ~ 0.1 MPa
per Onda HNA-040
hydrophone

calculated
beam profiles


Micro Electrode Array
10 mm
60 channels
For recording:
one is selected as ground
21 31 41 51 61 71
12 22 32 42 52 62 72 82
13 23 33 43 53 63 73 83
14 24 34 44 54 64 74 84
15 25 35 45 55 65 75 85
16 26 36 46 56 66 76 86
17 27 37 47 57 67 77 87
28 38 48 58 68 78

Rat Hippocampal Slice Culture



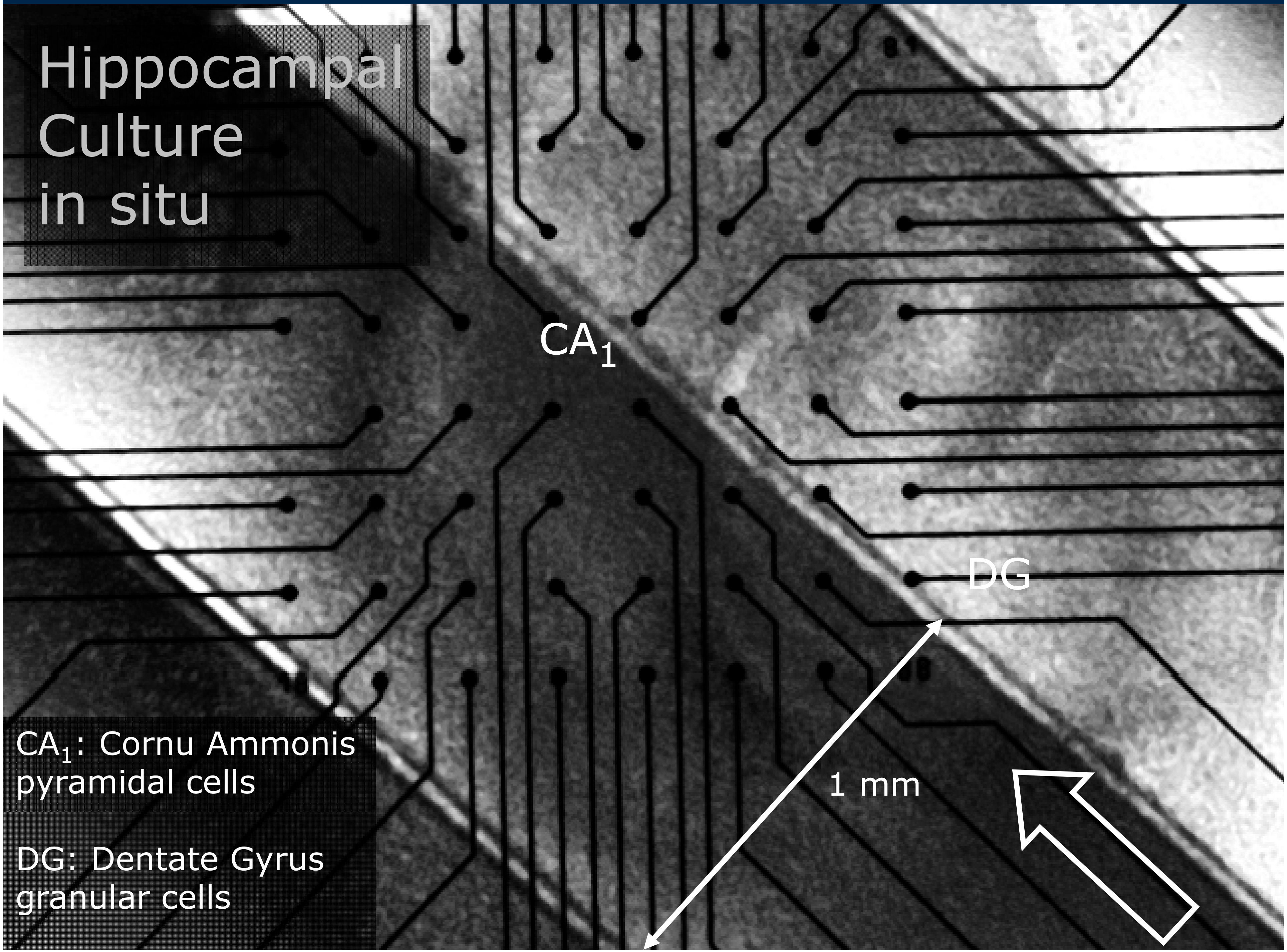
O_2-saturated artificial cerebro-spinal fluid control

coupling cone

hippocampal slice

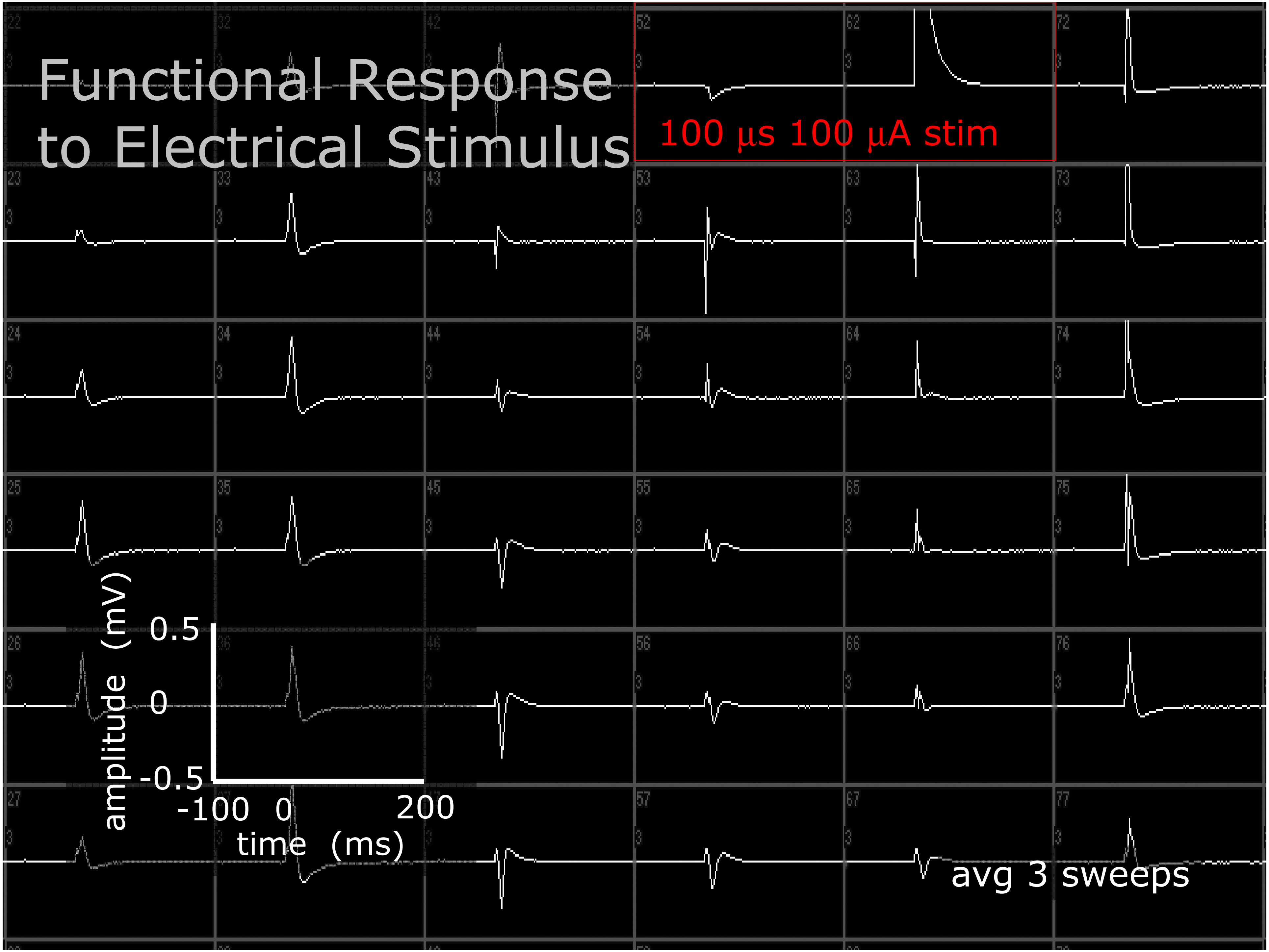
60 electrode array

multi-electrode array cassette



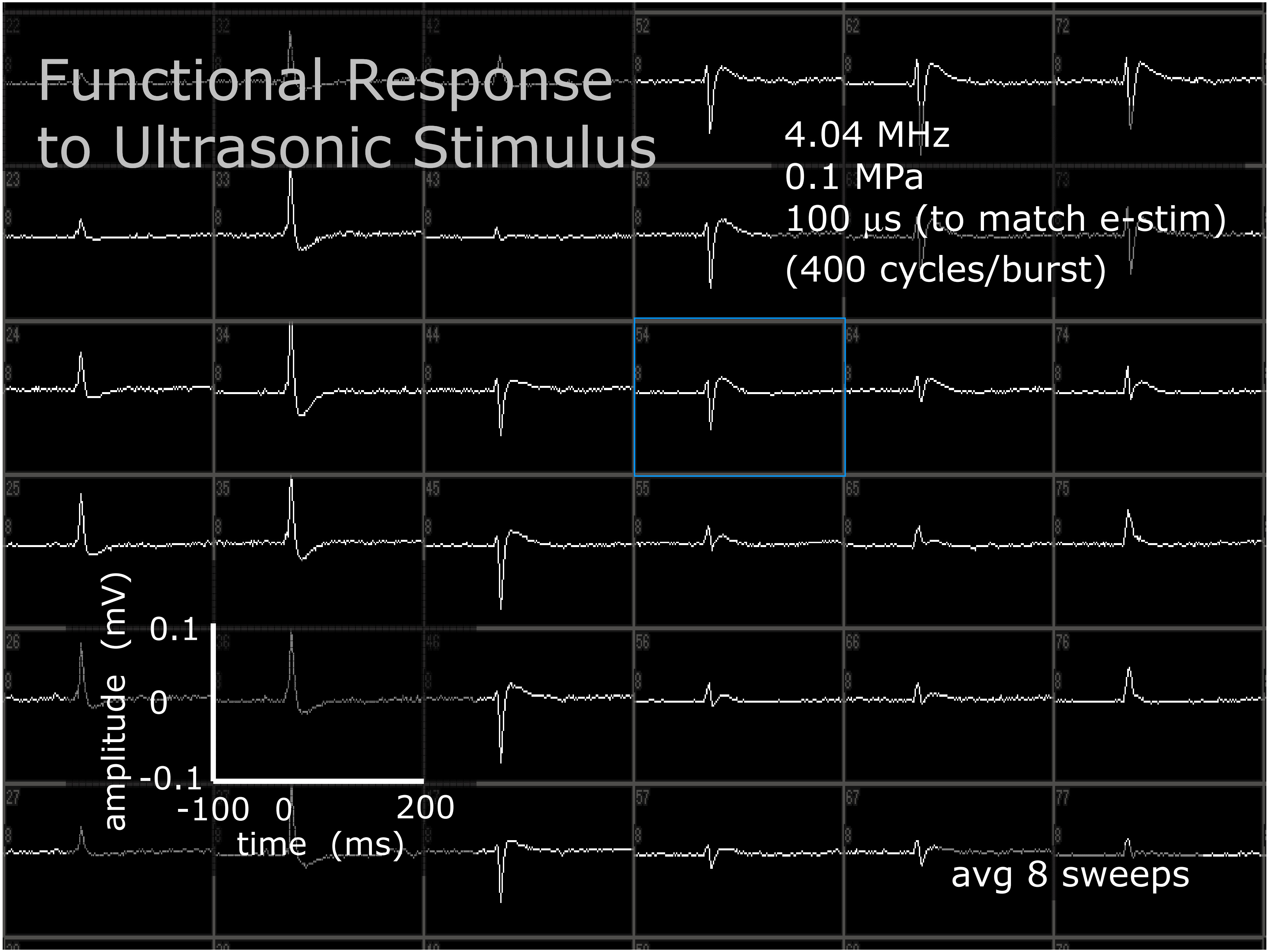
Hippocampal Culture in situ
CA_1
DG
1 mm
CA_1: Cornu Ammonis pyramidal cells
DG: Dentate Gyrus granular cells

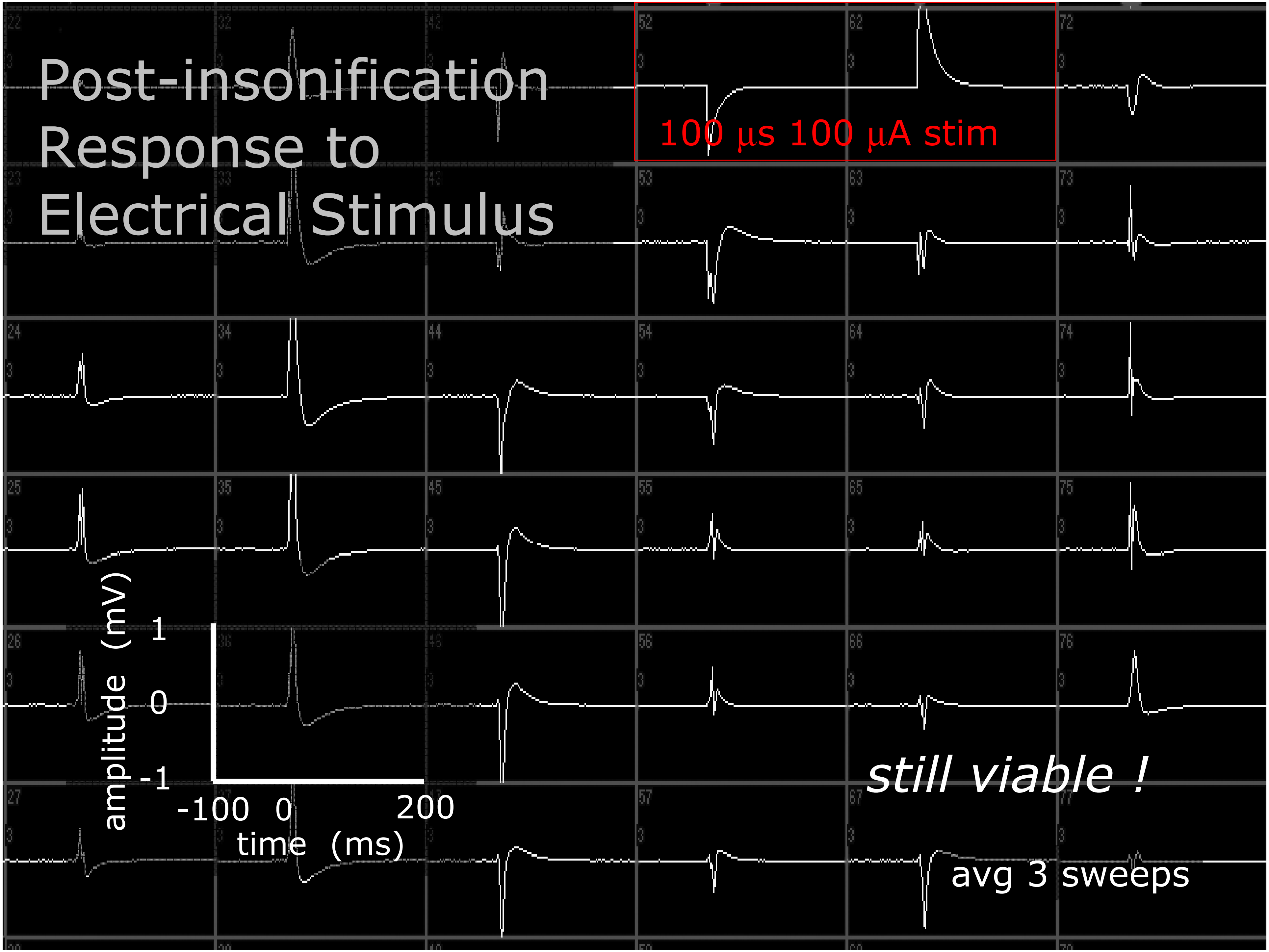
Results – Functional Studies



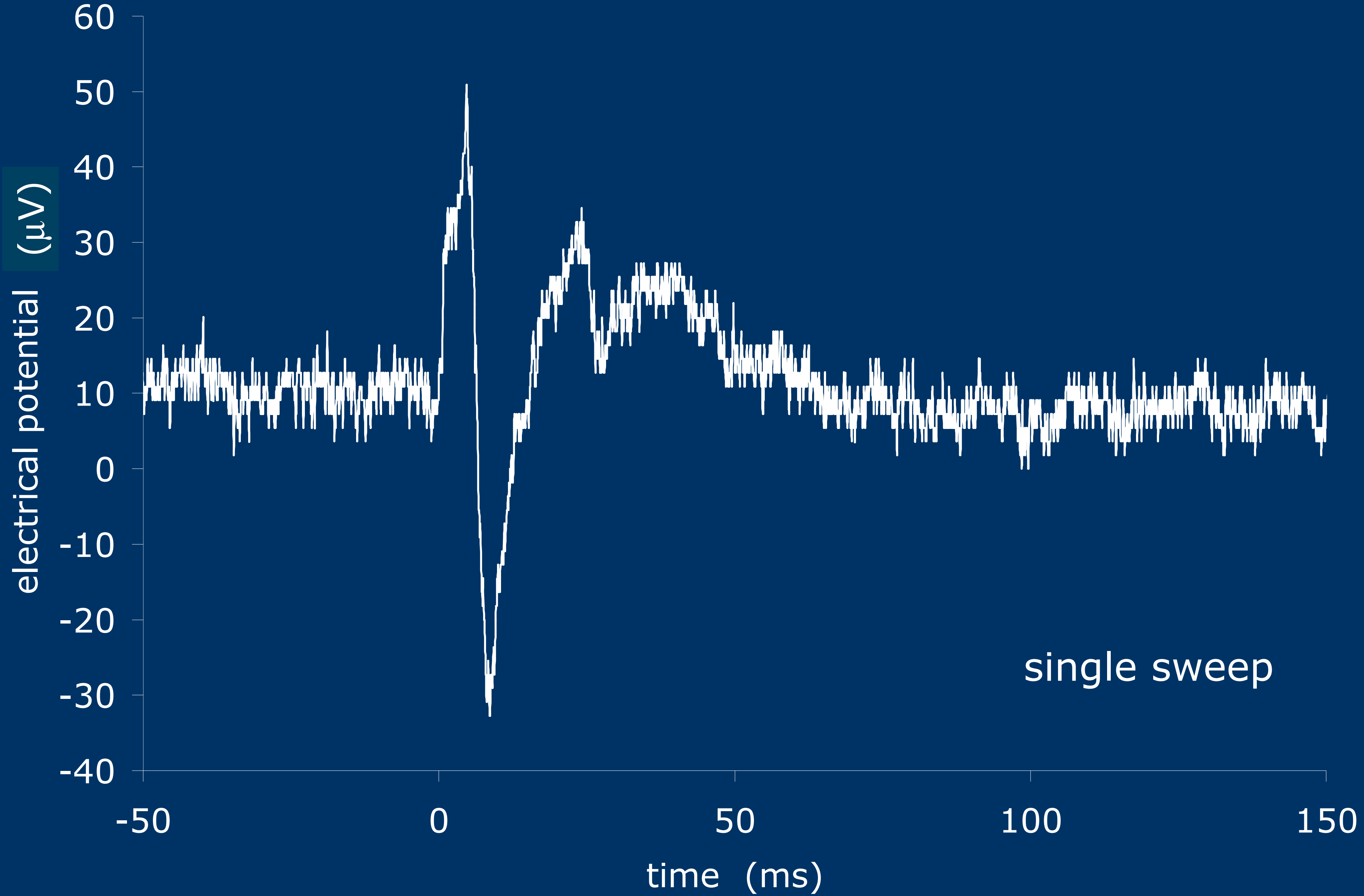
Functional Response
to Electrical Stimulus
100 µs 100 µA stim
amplitude (mV)
0.5
0
-0.5
-100 0 200
time (ms)
avg 3 sweeps

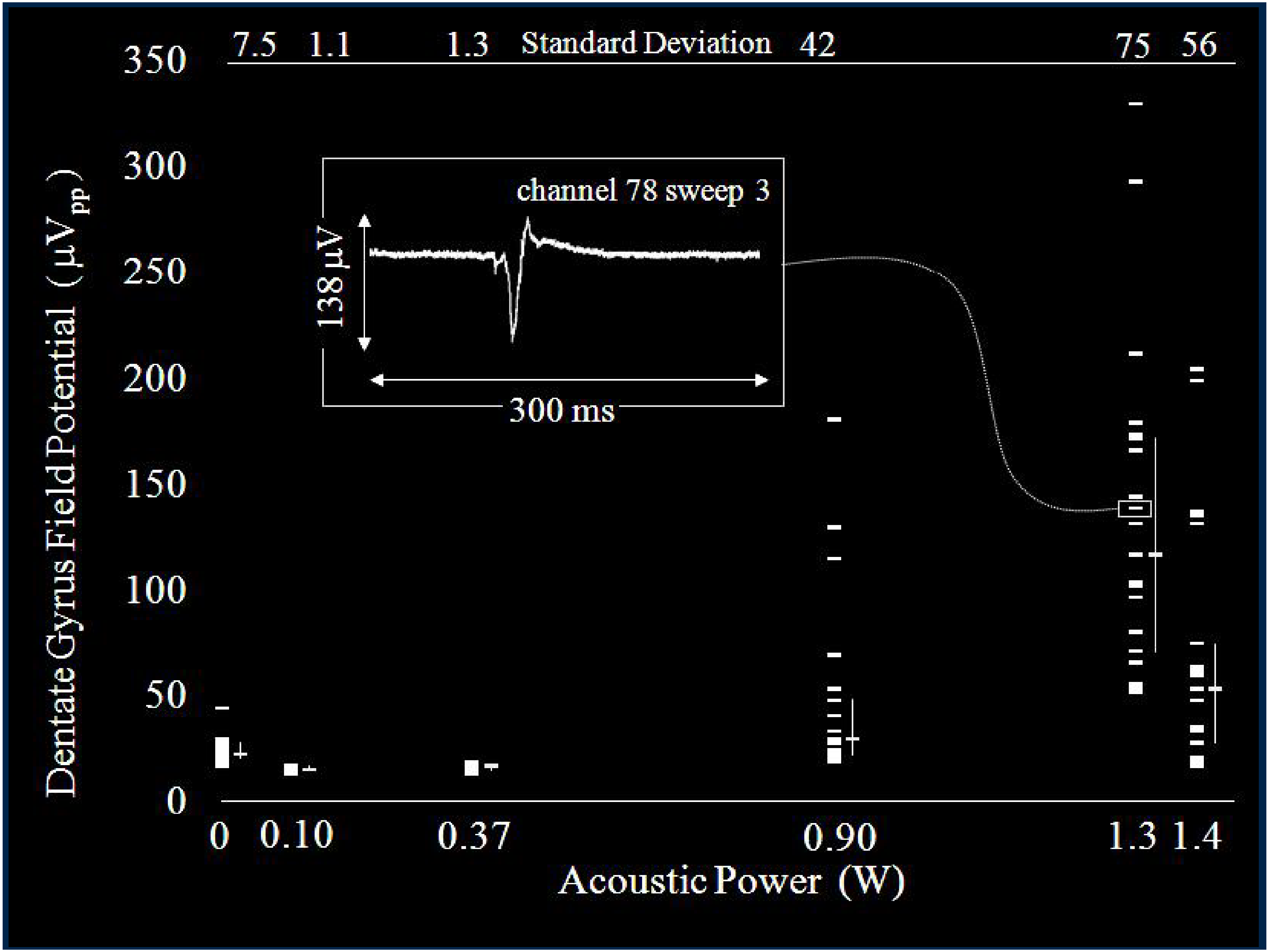
Functional Response to Ultrasonic Stimulus



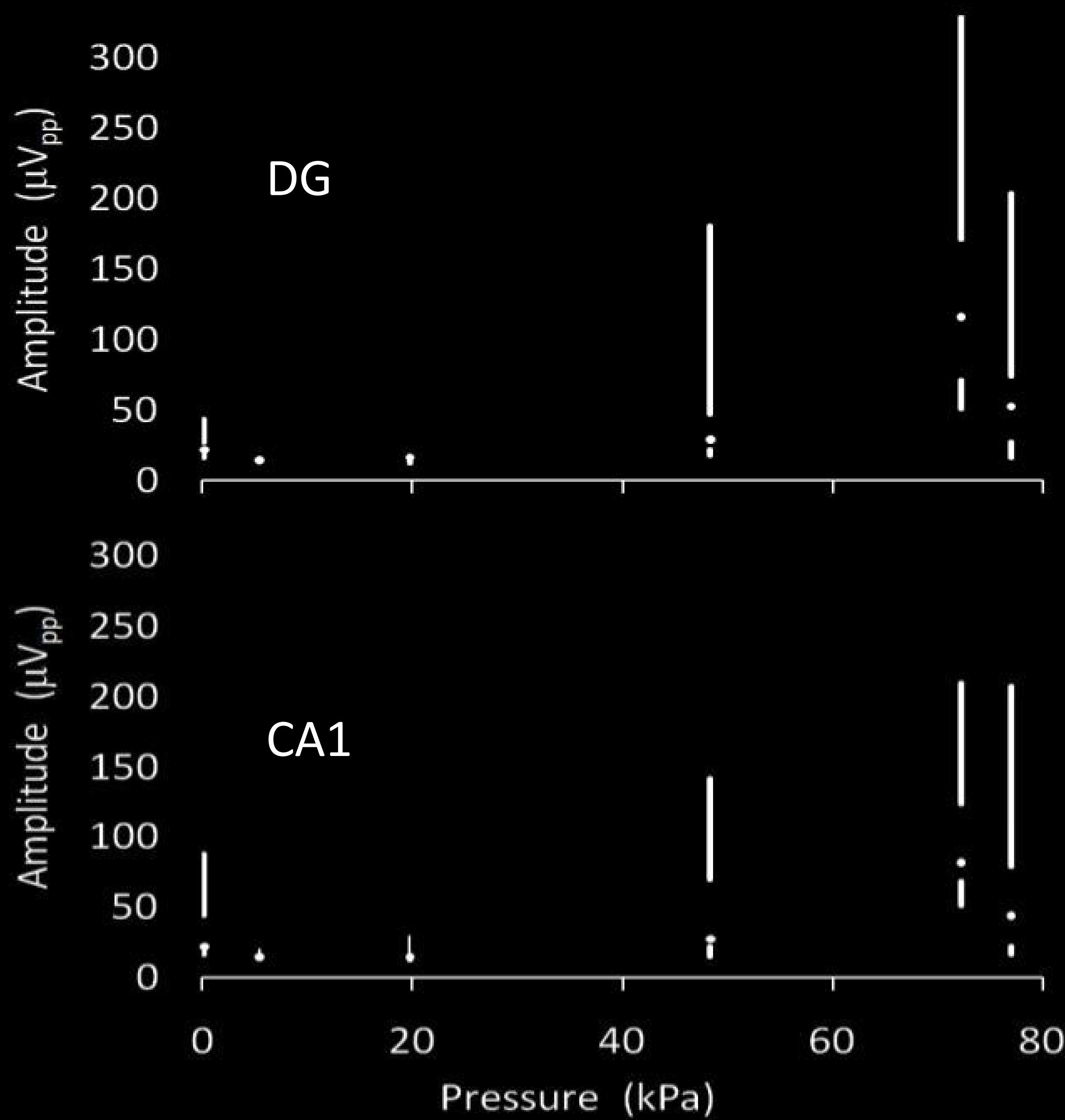

Post-insonification
Response to
Electrical Stimulus
100 µs 100 µA stim
amplitude (mV)
1
0
-1
-100 0 200
time (ms)
still viable !
avg 3 sweeps

Detail of Aggregate Response





7.5
1.1
1.3
Standard Deviation
42
75
56
350
300
250
200
150
100
50
0
Dentate Gyrus Field Potential (μV$_{pp}$)
channel 78 sweep 3
138 μV
300 ms
0
0.10
0.37
0.90
1.3
1.4
Acoustic Power (W)



Dose-Response Quartiles:

similar behavior

apparent threshold

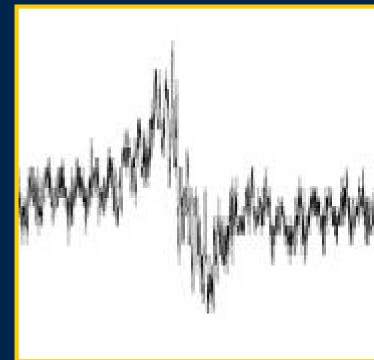
low dose “quiets” system

possible fatigue

Discussion

Functional Response Mechanism

✓ultrasonic device stimulus



electrical leakage

$R > 50\ M\Omega$;
no measured pulse with agarose;
no response with air block

acoustic effect

$\Delta T_{est} << 1\,^{\circ}C$ heat

radiation force

cavitation *possible; less likely at high f*

hypothesis; observe mechanical deformation under similar conditions direct

streaming *blocked*

Conclusions

Ultrasonic stimulus
acting on rat hippocampal culture:

- elicits response similar to electrical stimulus
- does not apparently harm culture
- radiation force is possible mechanism
- exhibits threshold behavior