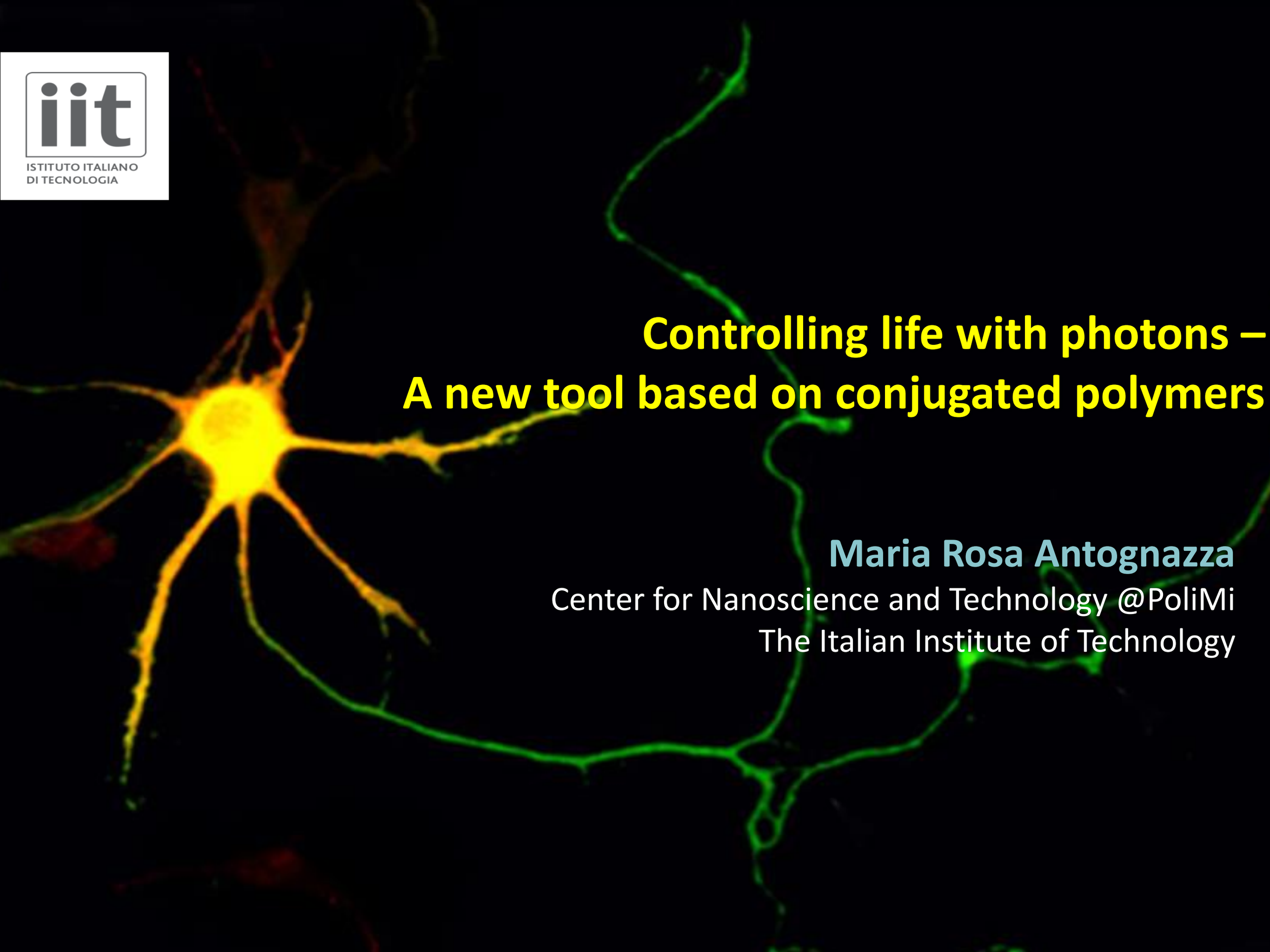

Controlling life with photons – A new tool based on conjugated polymers

Maria Rosa Antognazza
Center for Nanoscience and Technology @PoliMi
The Italian Institute of Technology

Controlling life with photons: not really a new idea

Arsonval AD. La fibre musculaire est directement excitable par la lumiere.
C R Soc Biol. 1891;43:318–320.

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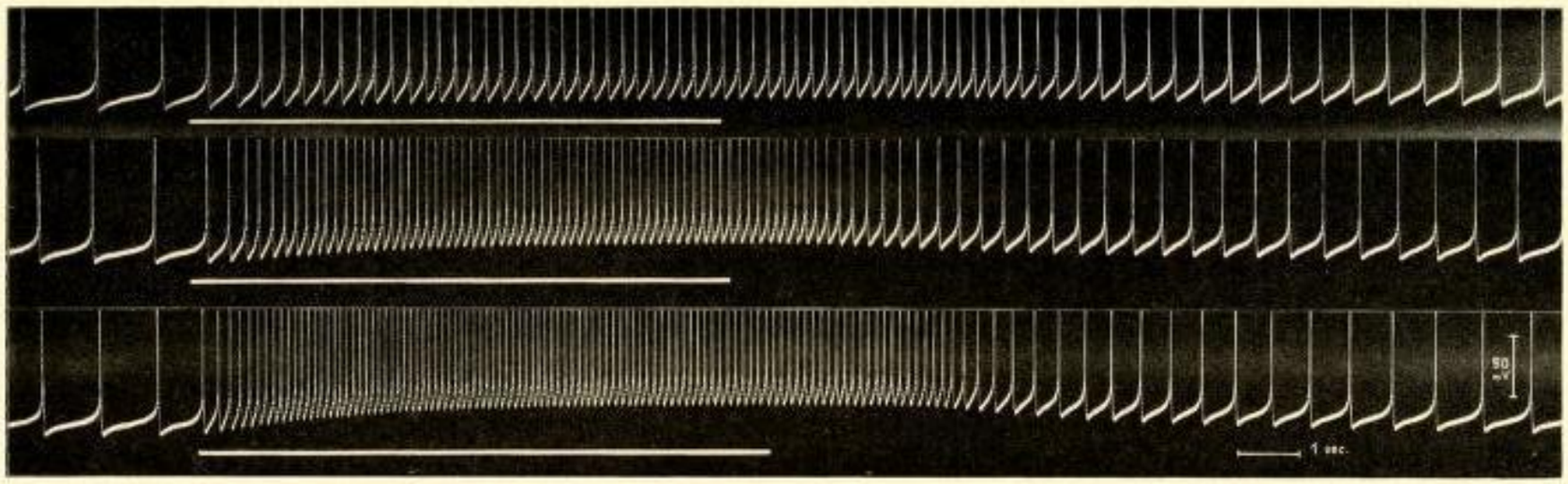


FIG. 8. Responses to light at different intensities in initially autoactive nerve cells. Nerve cell initially autoactive in the dark. Three steady monochromatic (579 mμ) activations, at increasing intensities, successively from top to bottom: 0·15, 0·6 and 1 × 10^{-6} cal g mm^{-2} sec^{-1}.

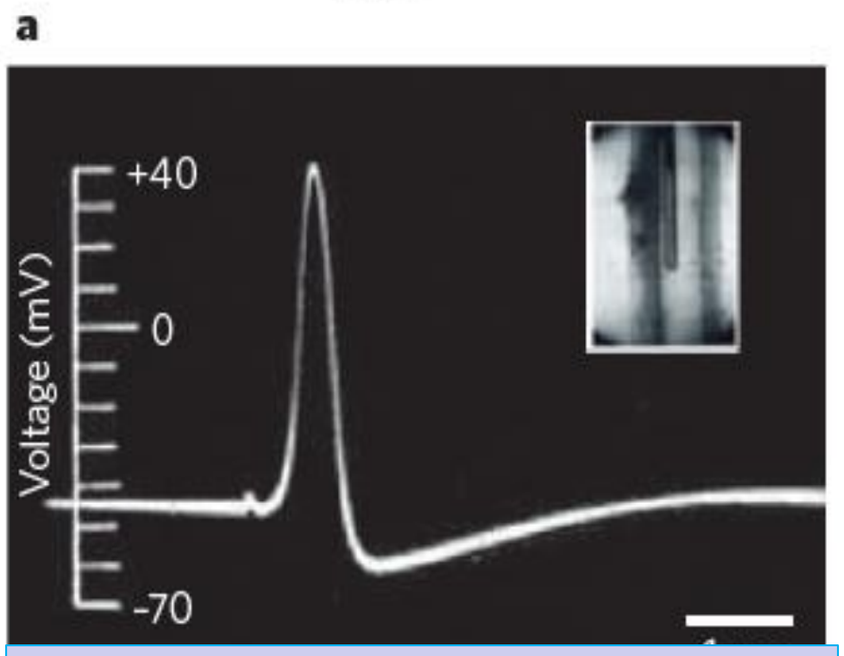
Duration of the illumination is slightly different in the three records as indicated by the underlying lines. The depolarization and the frequency of the spikes increase with the intensity of illumination. Note a slow adaptation as indicated by a slight lengthening in the period as the illumination proceeds. At the cessation of the light, the frequency of the spikes decrease towards the initial value in the dark. The upper portions of the spikes are not seen.

Recording and stimulation: past and present

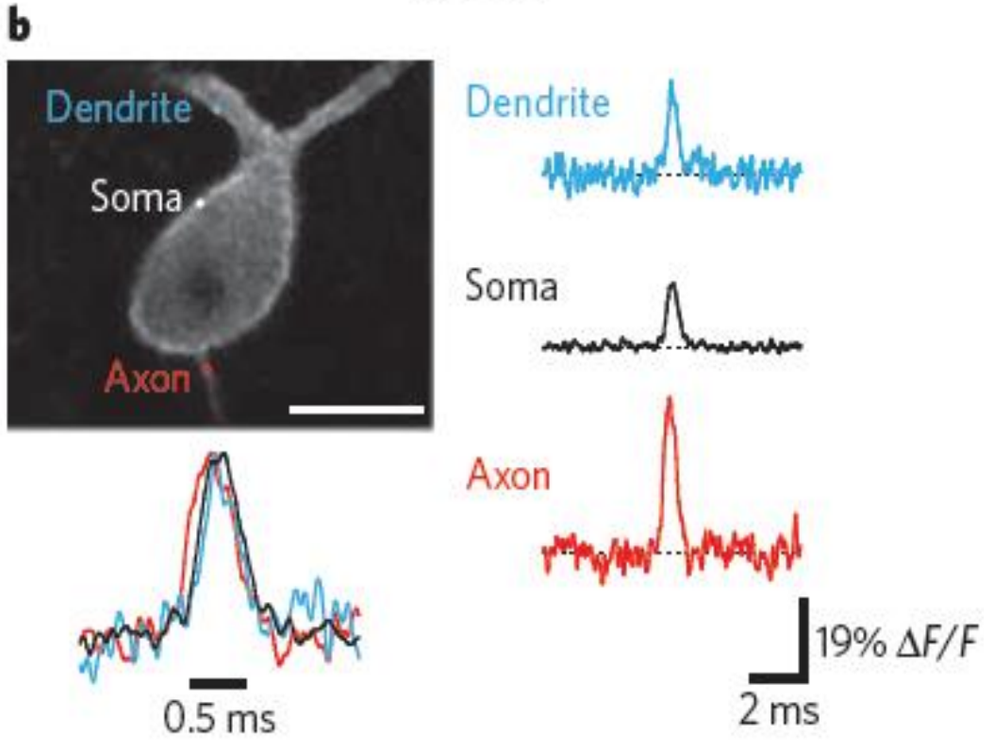
Image taken from *Nature*, **461**, 930 (2009)

Past **Present**

Measuring

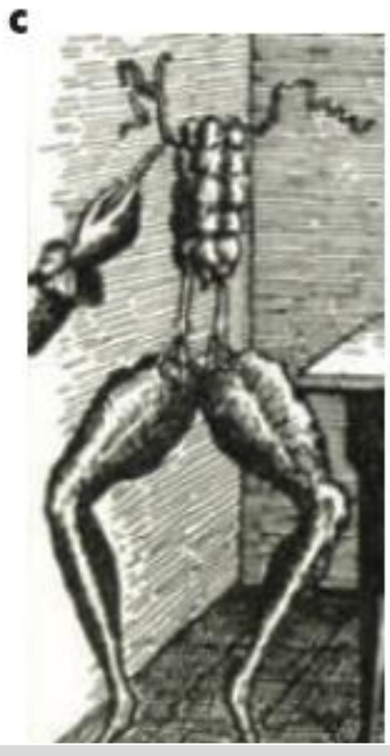


First recorded action potential (Hodgkin & Huxley, Nature 1939)

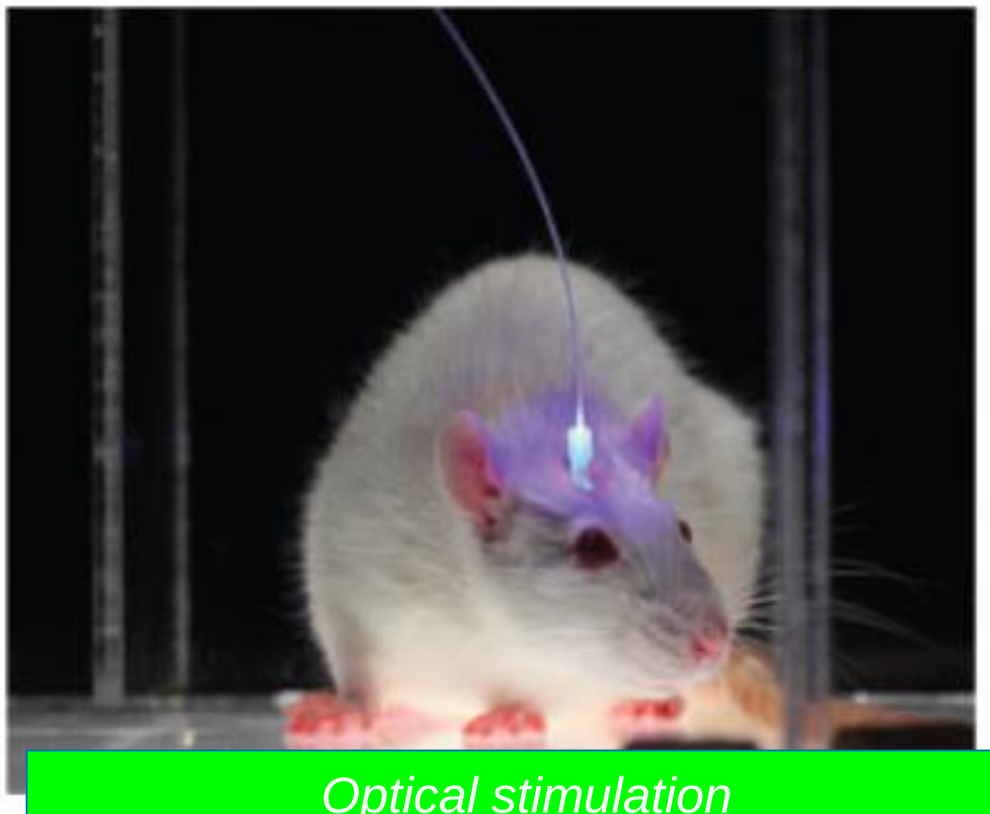


Multisite optical recording by voltage sensitive dyes

Manipulating



Electrical stimulation of a frog nerve (Galvani, 1791)

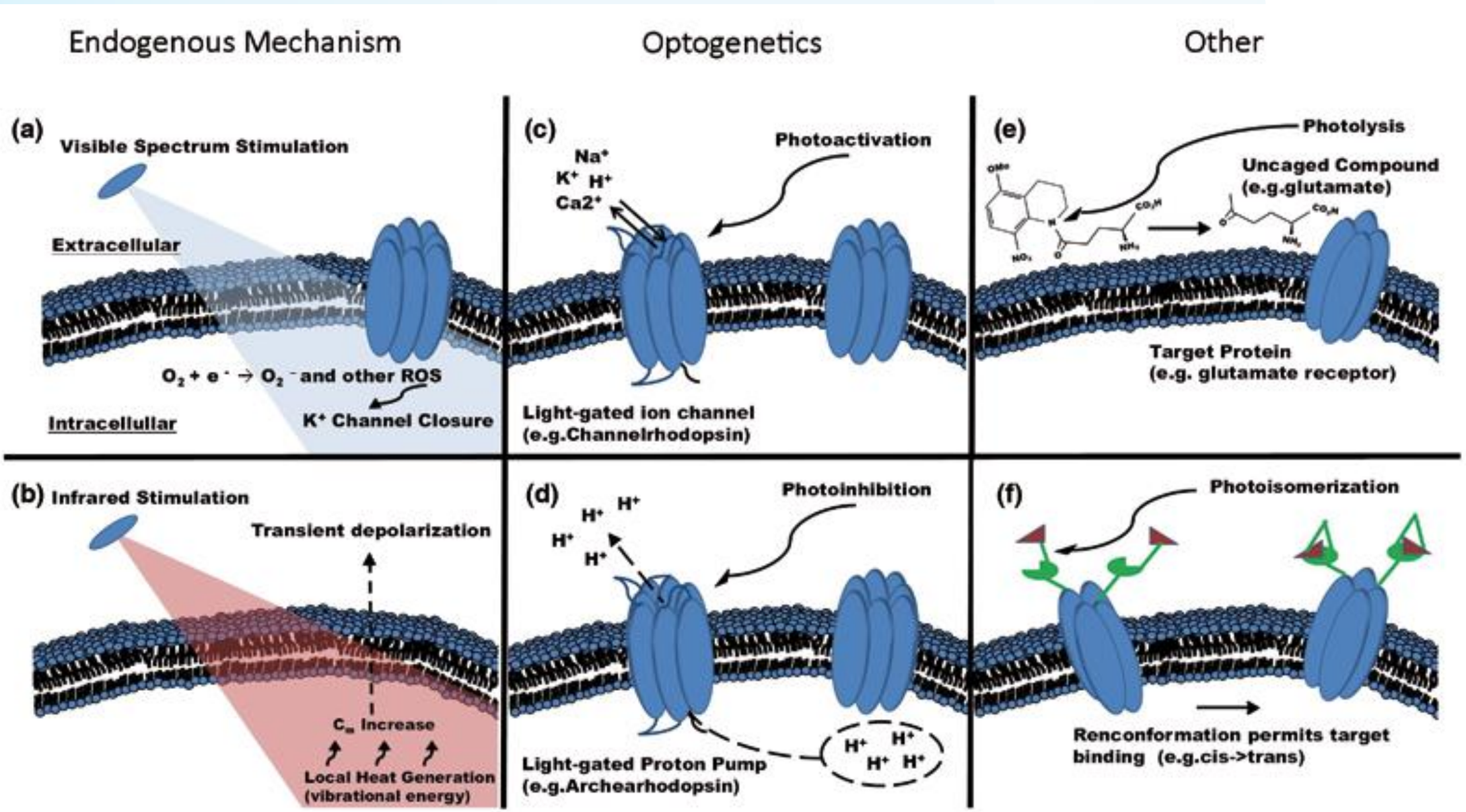


Optical stimulation

Looking for alternative techniques

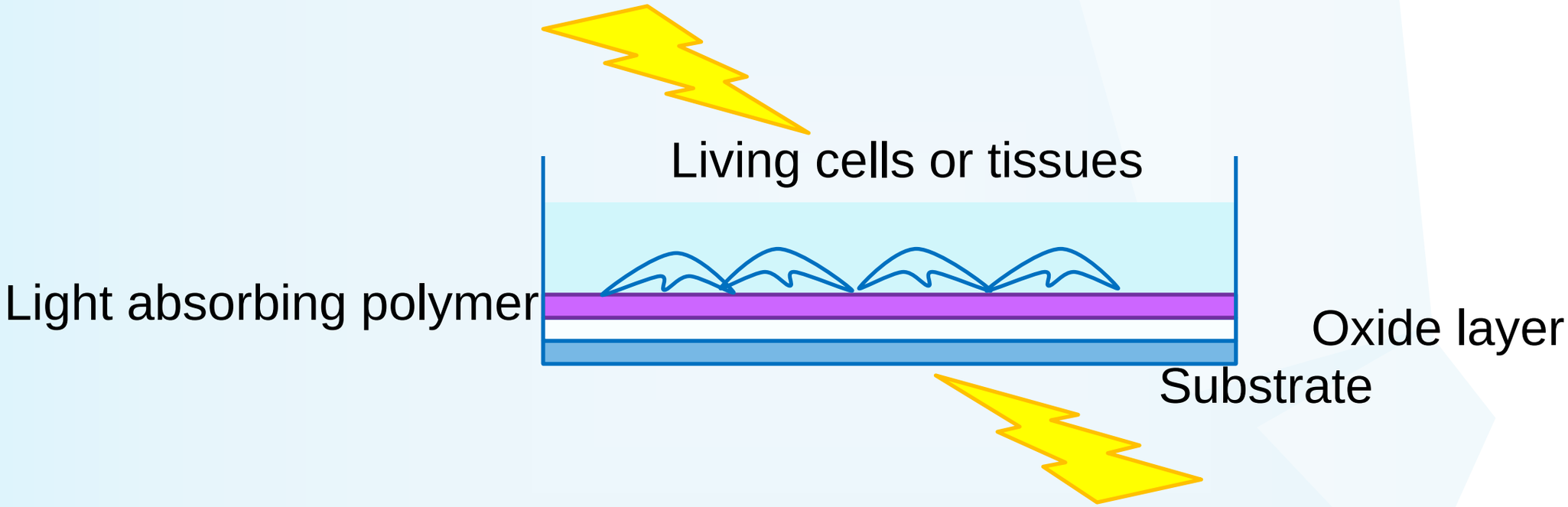
Cell optical stimulation: the techniques

1.Photochemical reaction through use of endo- or exo-genous fluorophores (Molecular switches; caged neurotransmitters)
2.Transgenic induction of light-gated ion channels (Optogenetics)
3.Use of extrinsic absorbers, mainly in the NIR
4.Infrared Neural Stimulation (water absorption, photo-termal effect)



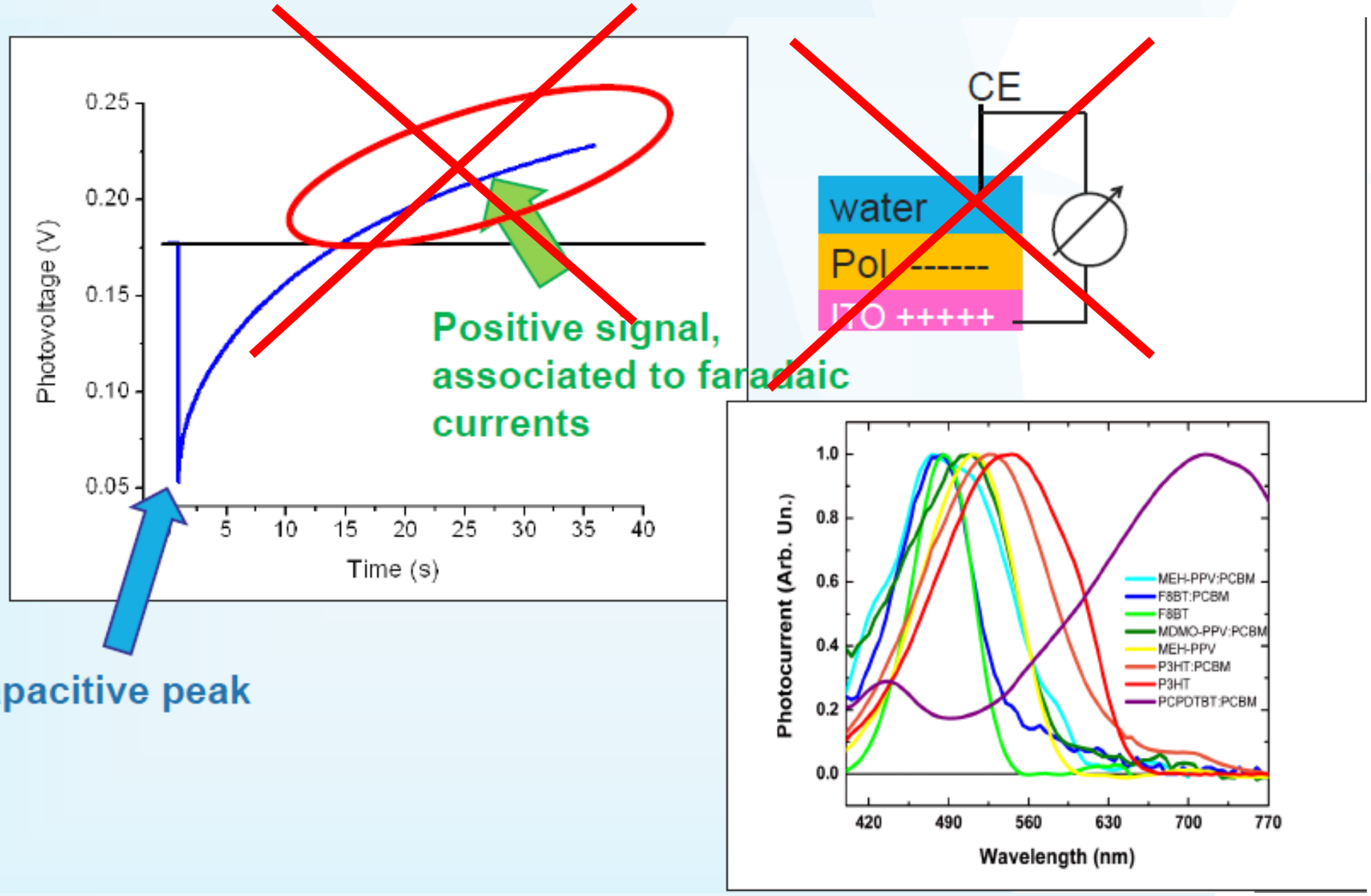
Our approach:

Use of light-sensitive conjugated polymers to optically excite electrical activity of cells or living tissues

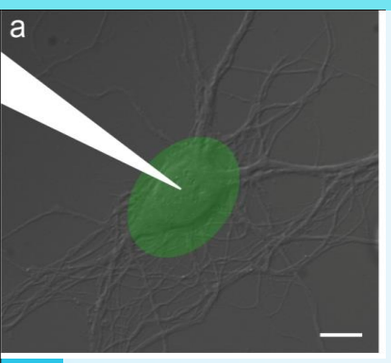


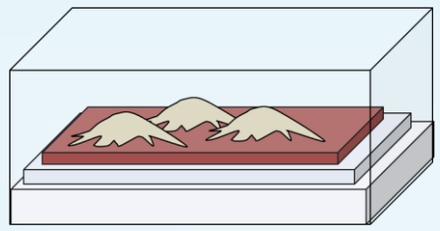
- ✓ Primary neurons
- ✓ Glial cells (astrocytes)
- ✓ Secondary cells (HEK-293)
- ✓ Explanted tissues (rat retinas; brain slices)
- ✓ Living animals, e.g. ‘implantable artificial retina’ (rats)

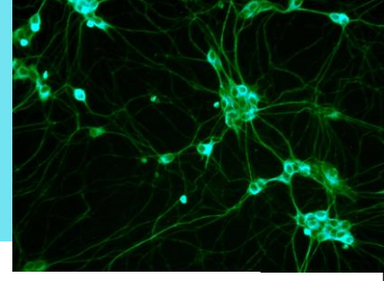
Same device, different exploitation

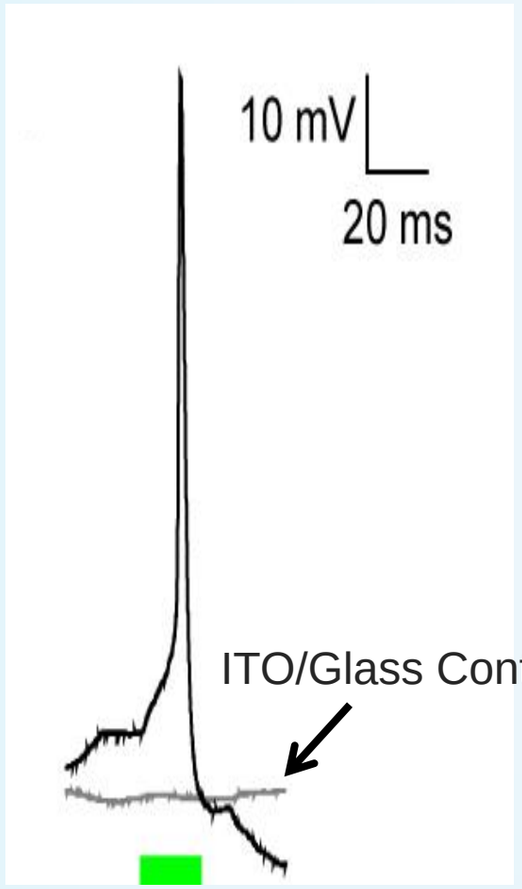


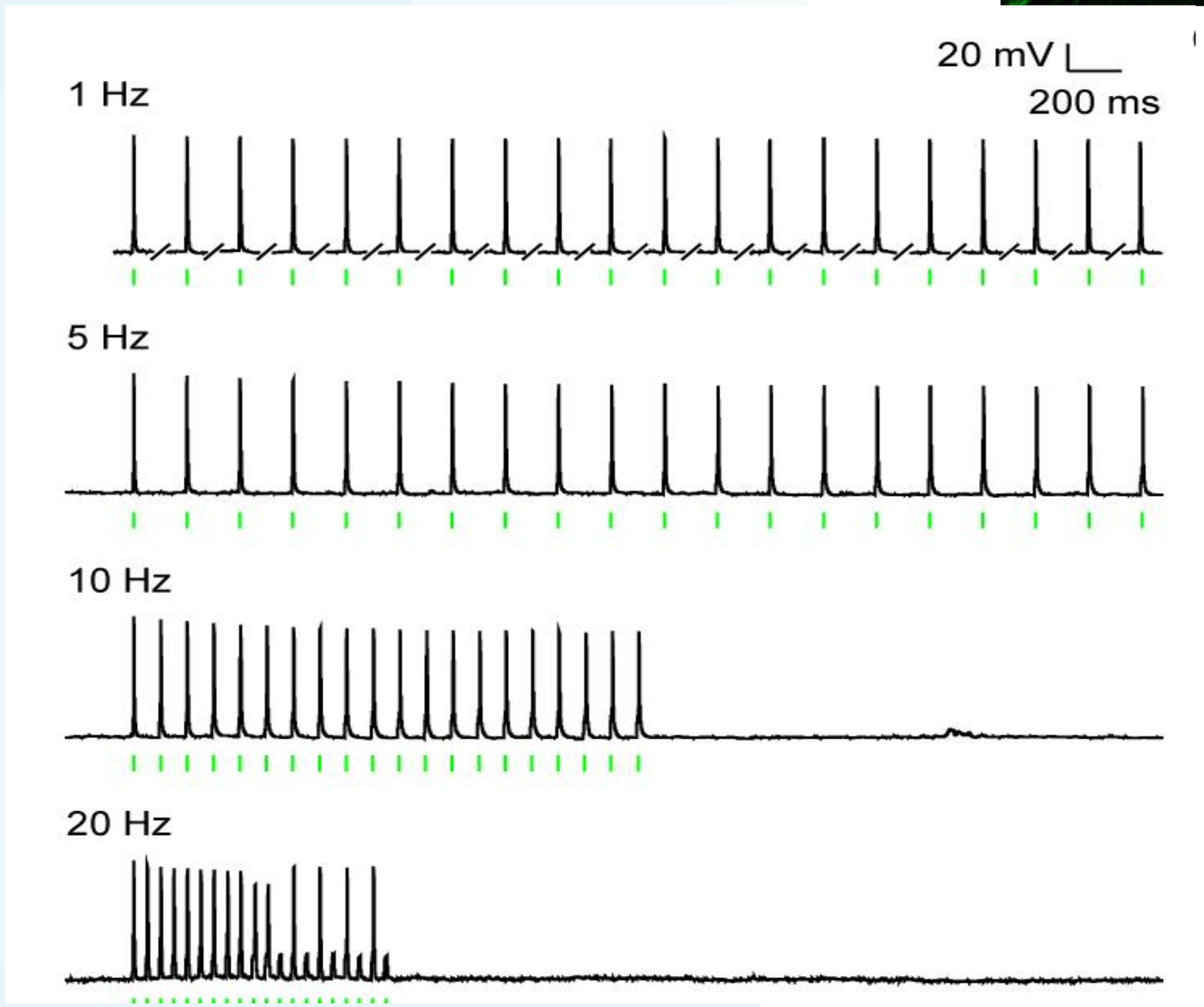
1st Study case: primary neuronal networks







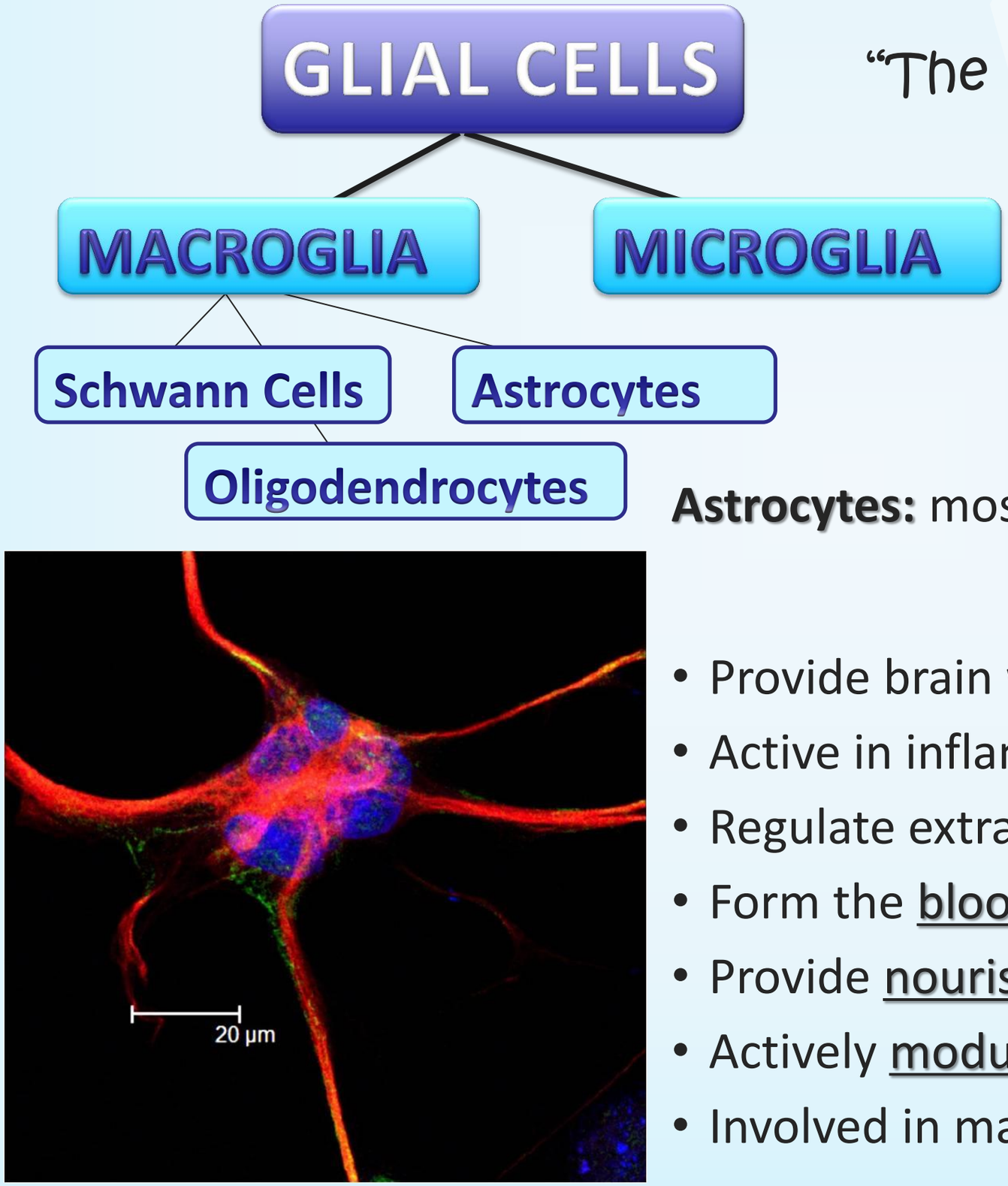




- Light pulse:20 ms, 15 mW/mm²
- Neural Stimulation at various stimulus frequencies

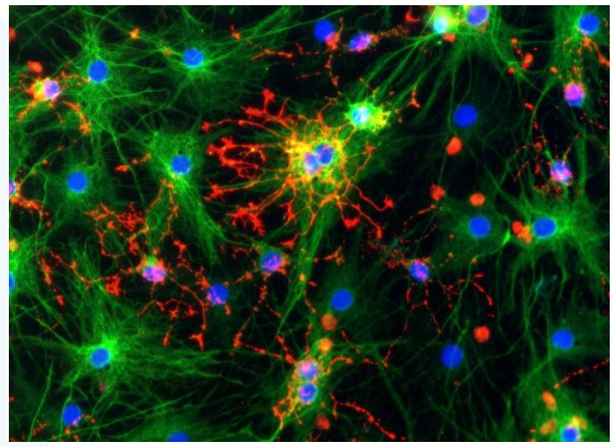
D. Ghezzi, M.R. Antognazza et al., Nature Photonics 2013
D. Ghezzi, M.R. Antognazza et al., Nature Communications 2011

2nd Study case: Astrocytes



"The 

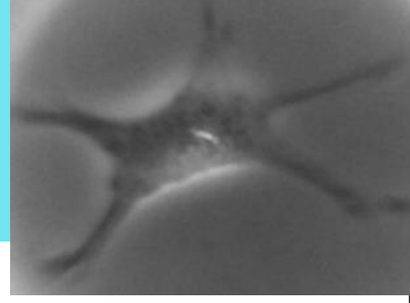
of the brain"



Astrocytes: most numerous glial cells

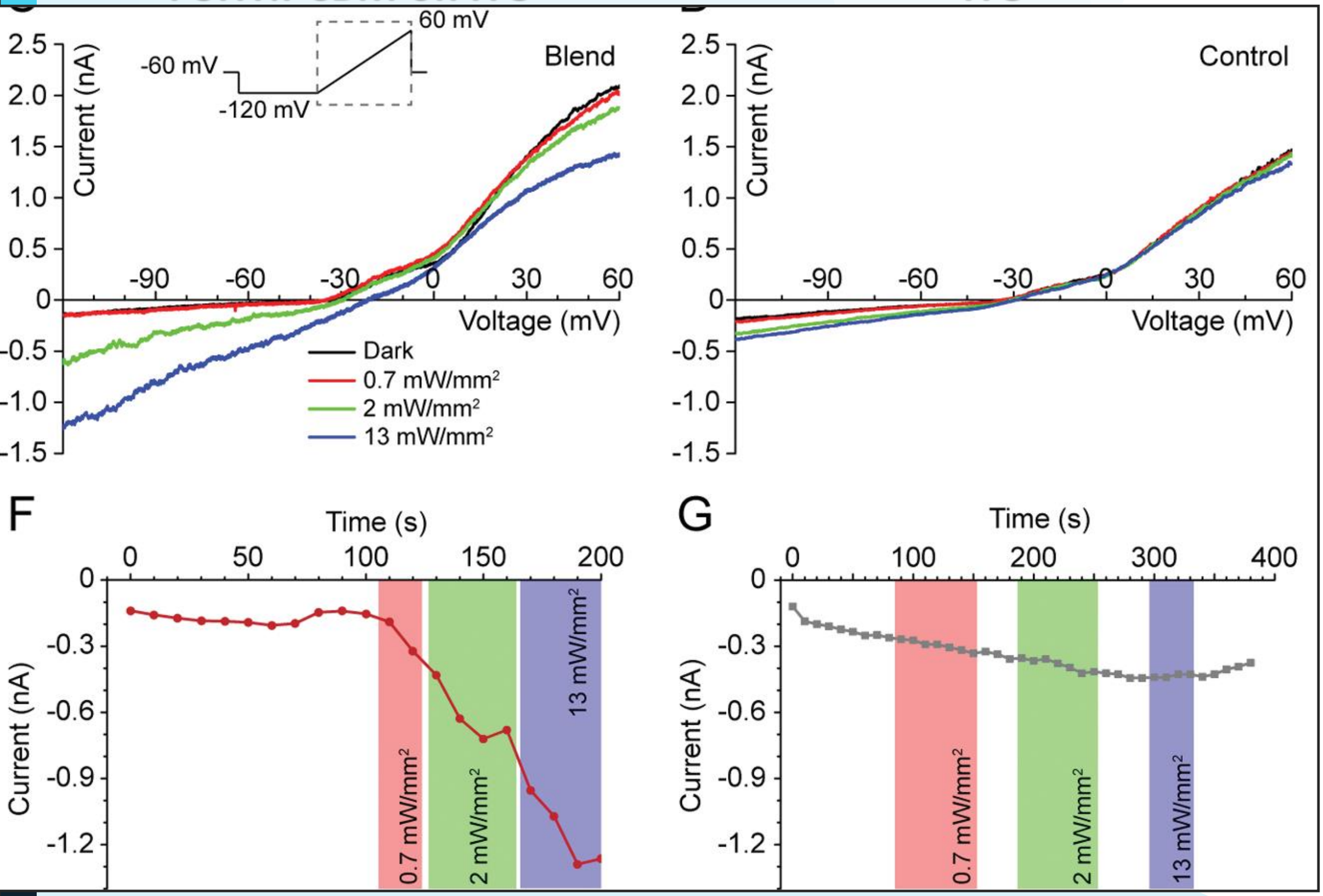
- Provide brain with structure
- Active in inflammatory reaction forming glial scar
- Regulate extracellular ionic concentration
- Form the blood-brain barrier
- Provide nourishment to nerve cells
- Actively modulate neuronal signalling
- Involved in many neurodegenrative diseases

2nd Study case: Astrocytes



P3HT:PCBM on ITO

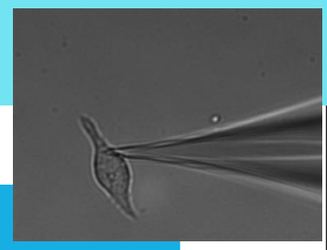
ITO



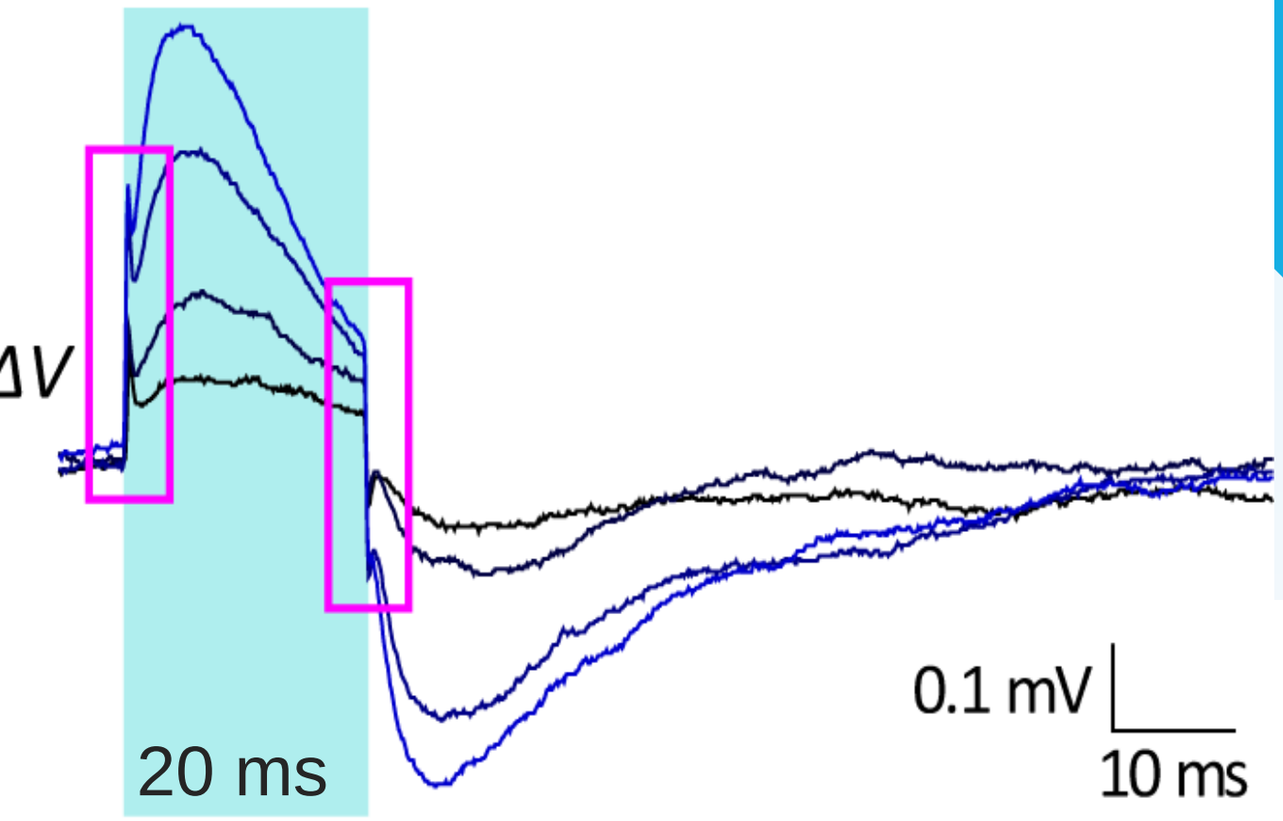
Current at -120 mV

Benfenati, Antognazza et al., Adv. Healthc. Mater. 2013

3rd Study case: non electrogenic cells (HEK-293)



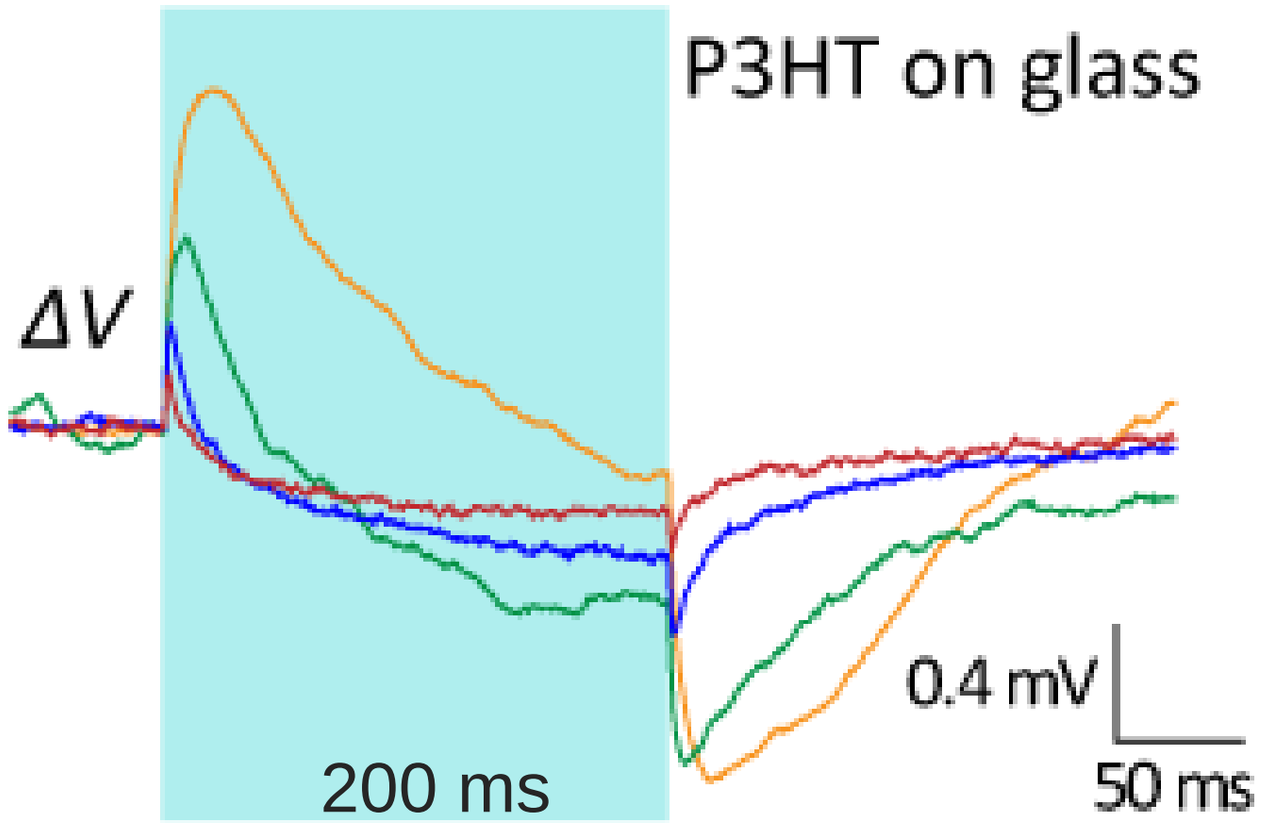
Short pulses >> cell membrane depolarization



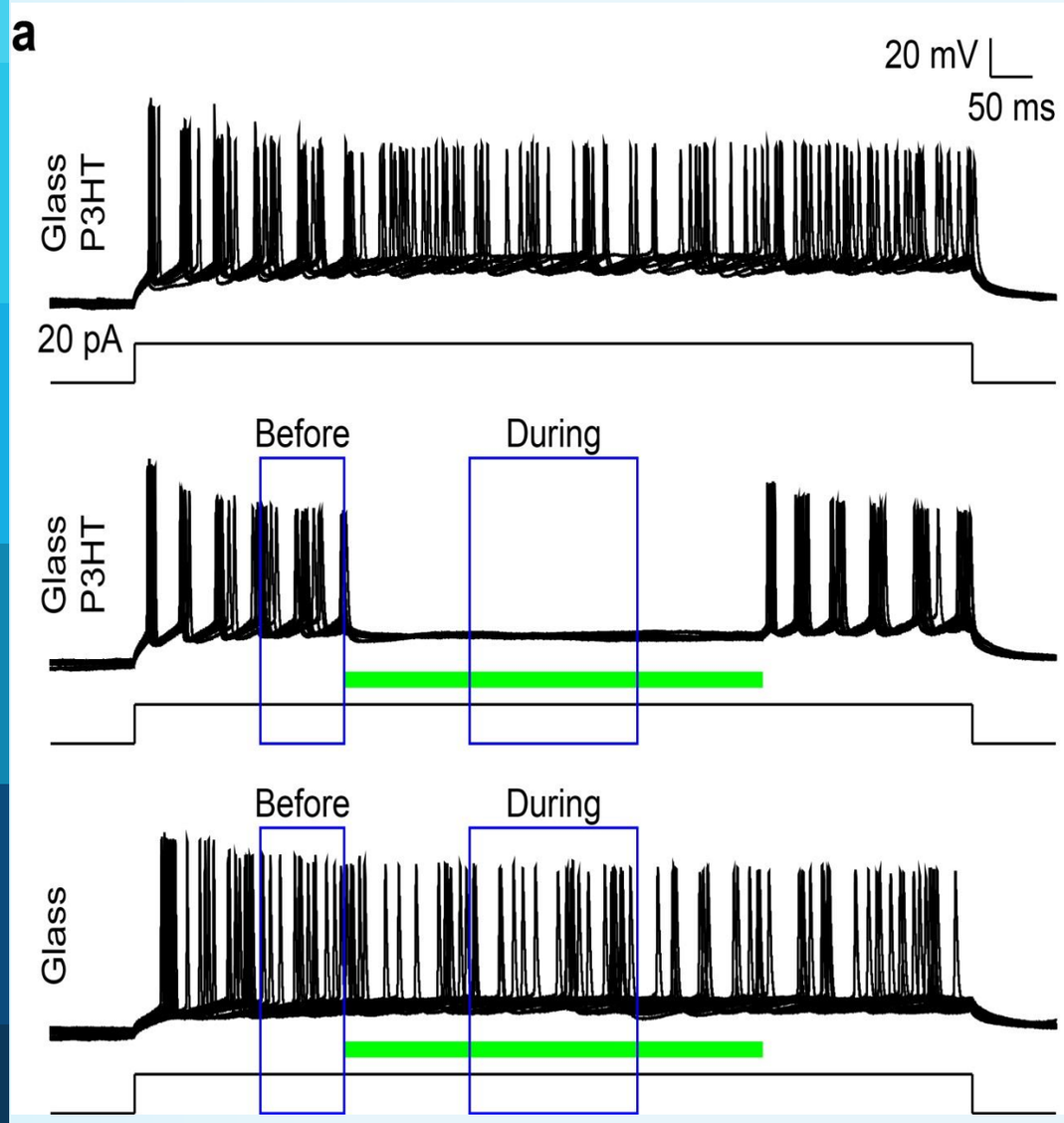
Long pulses (200 ms) >> cell membrane hyperpolarization

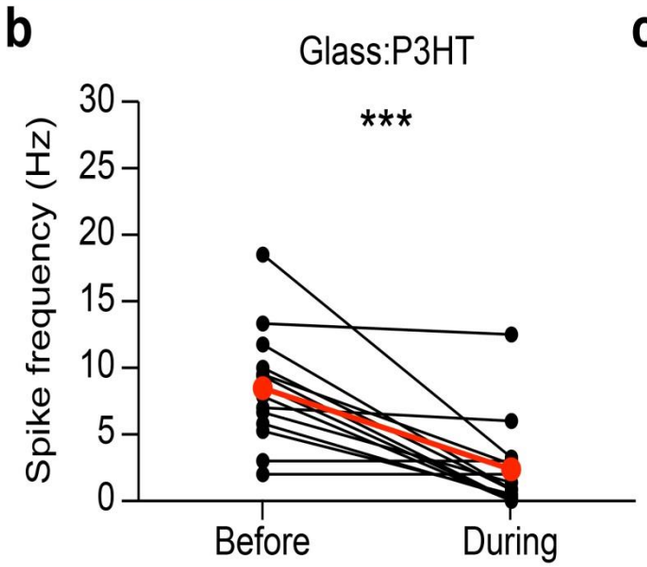
Dual control of cellular activity

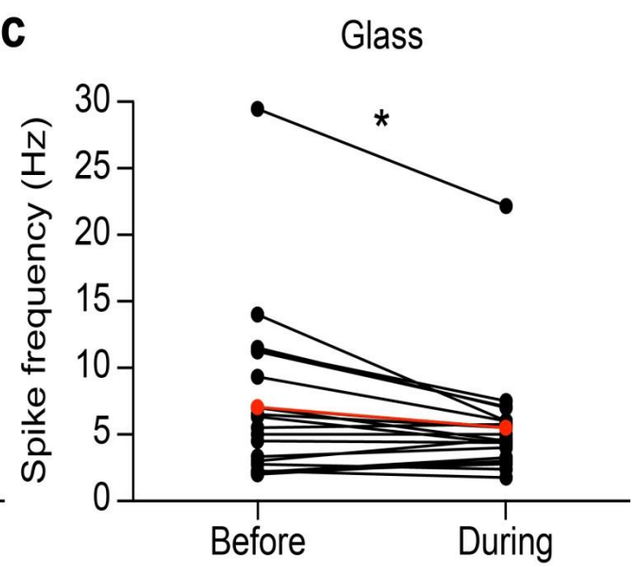
N. Martino et al., Sci. Rep. 2015

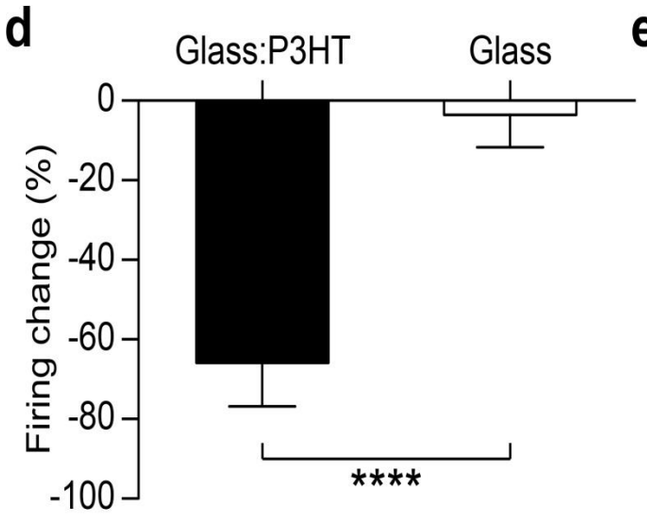


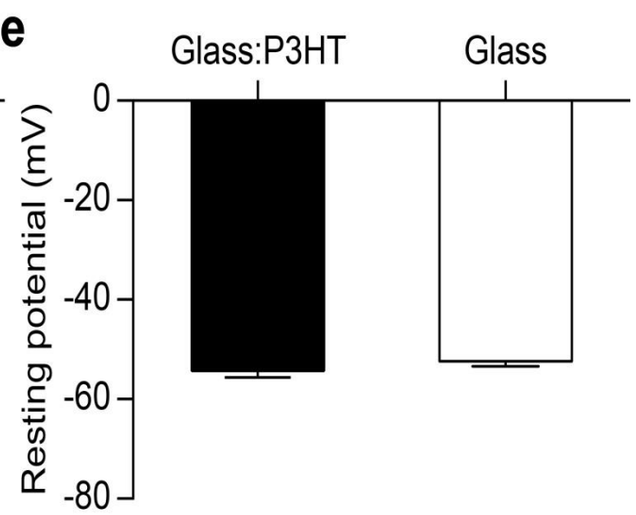
Inhibiting activity: Reduction of evoked spiking in primary neurons





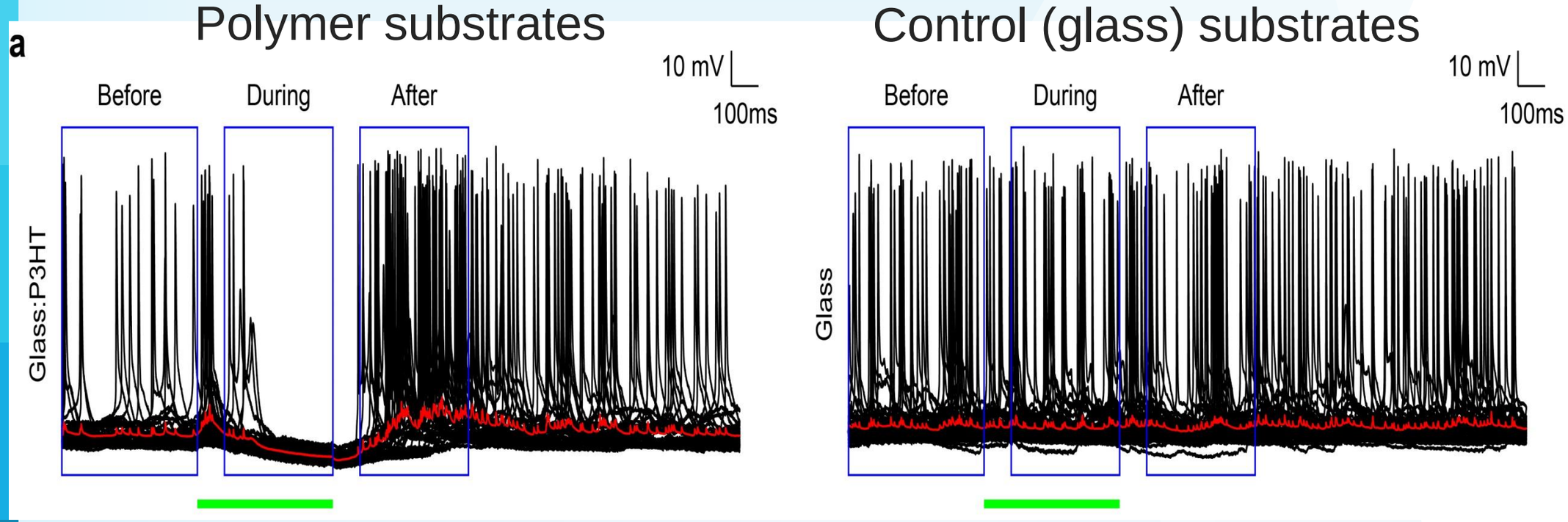


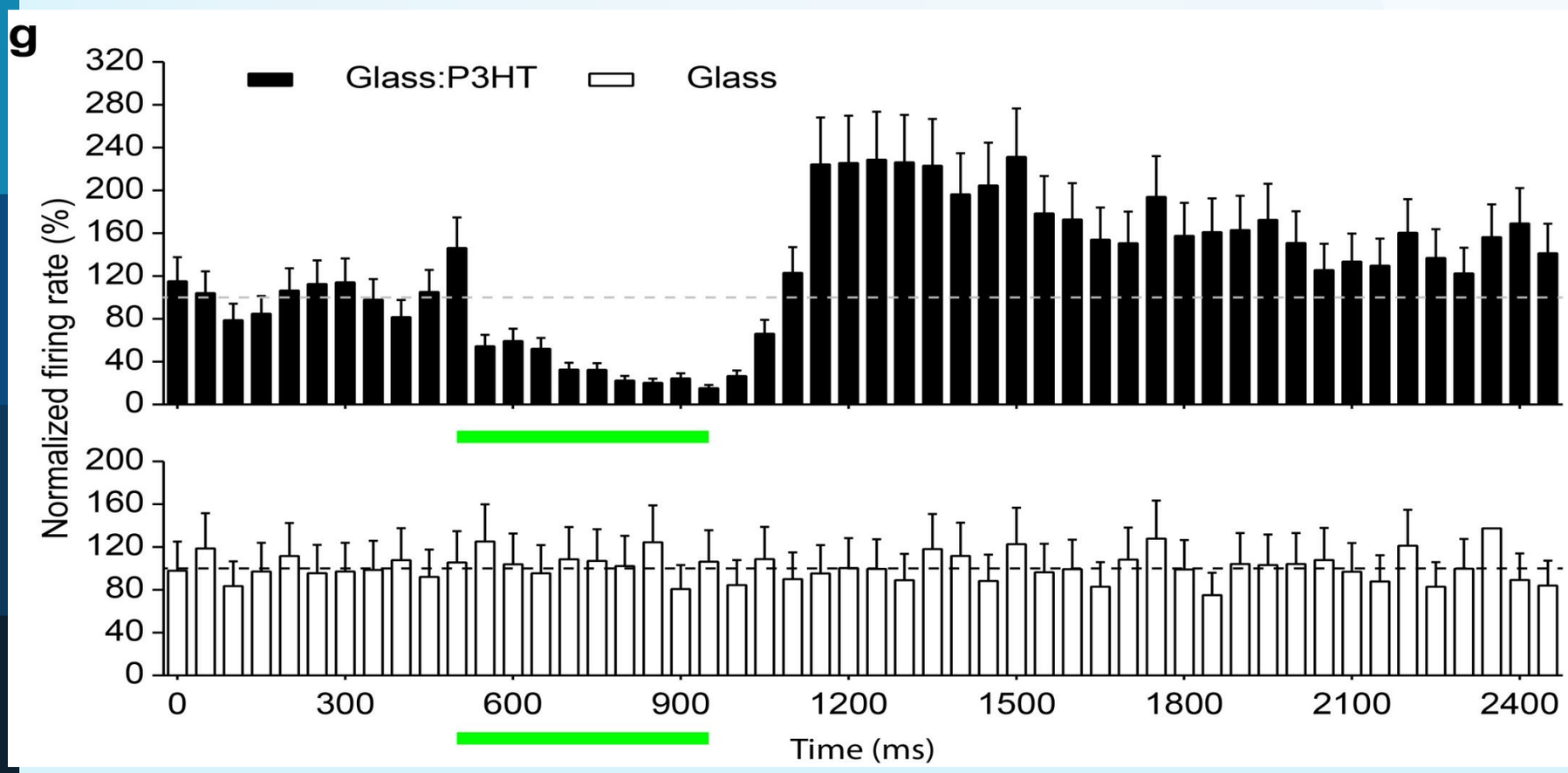


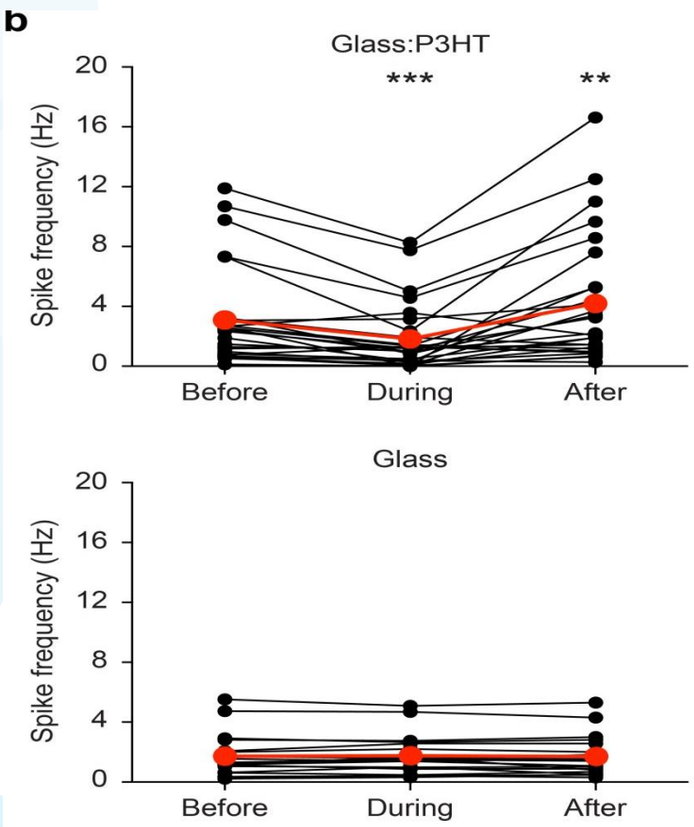


P. Feyen et al., under review

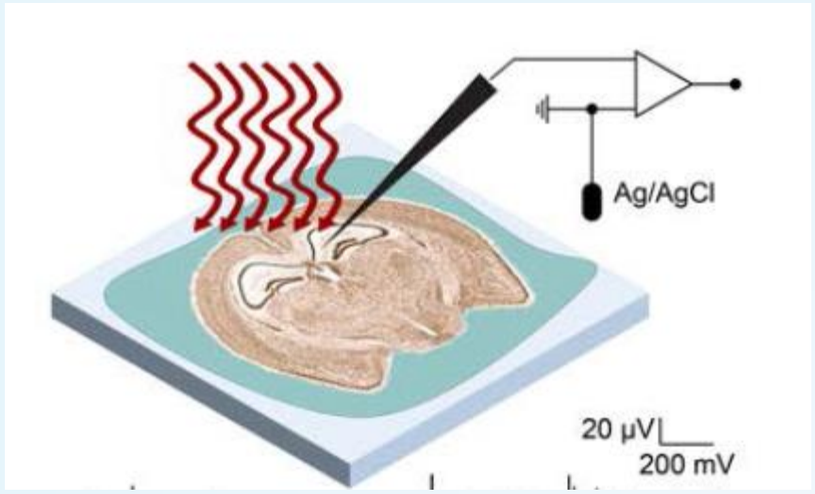
Modulation of *spontaneous* activity in primary neurons

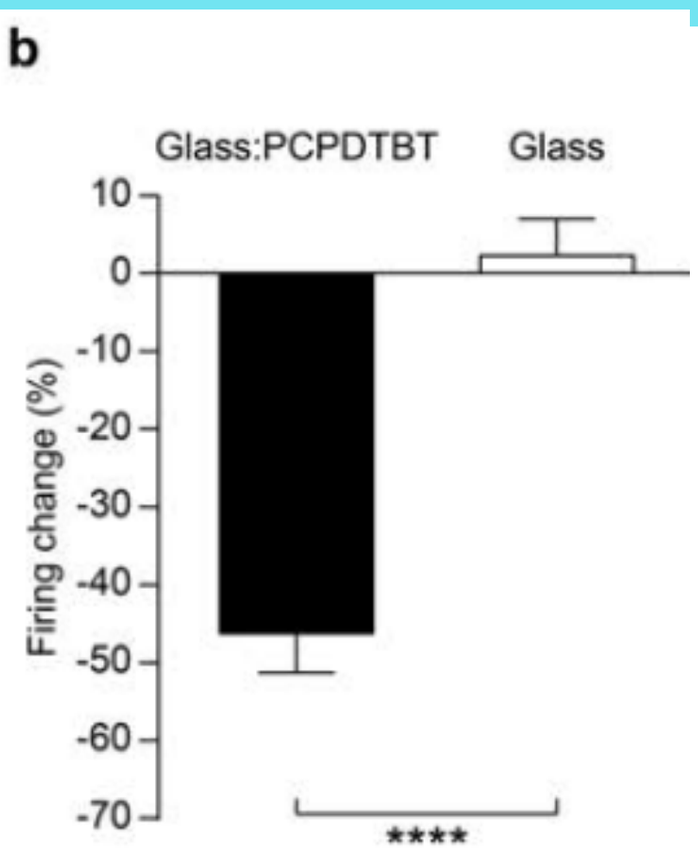


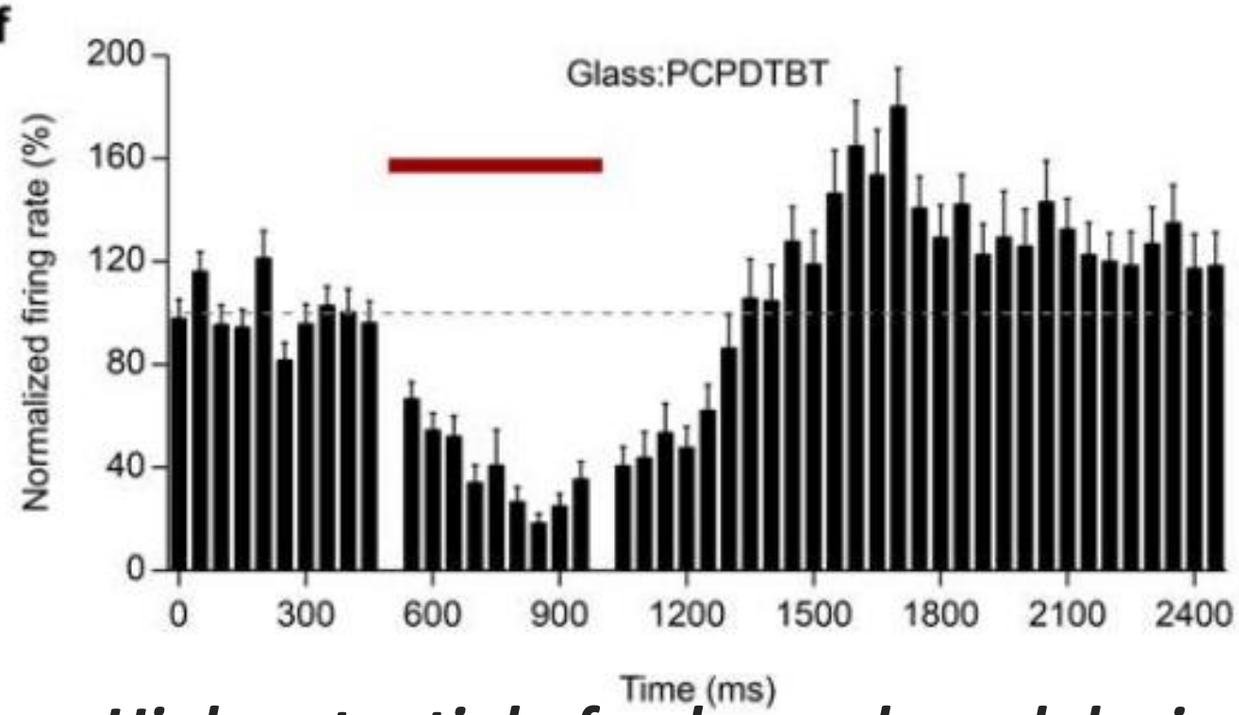


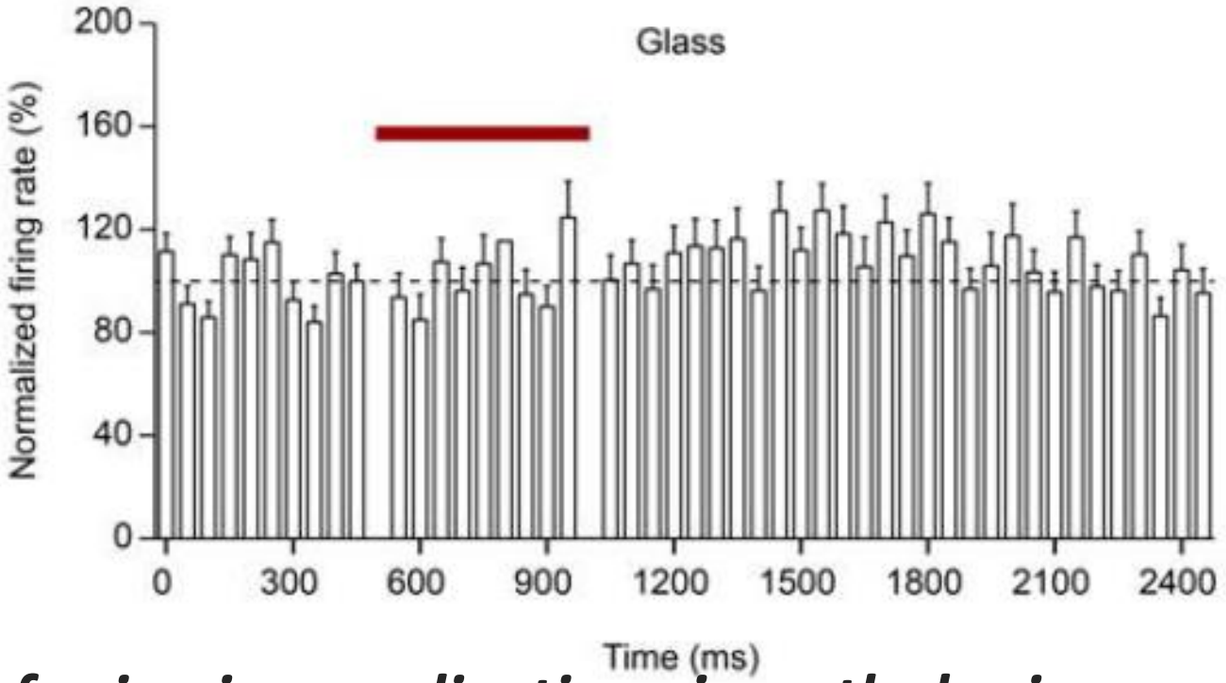


Inhibition of activity in epileptic brain slices



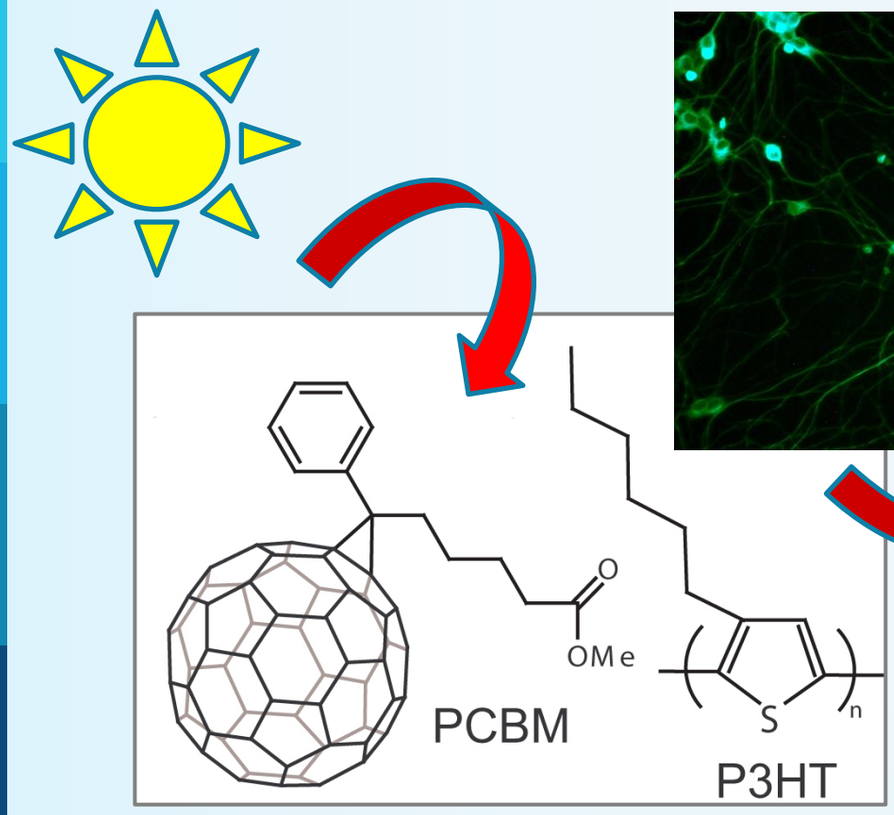


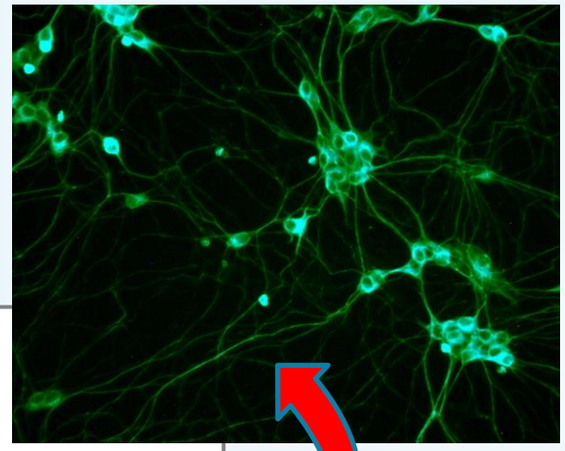


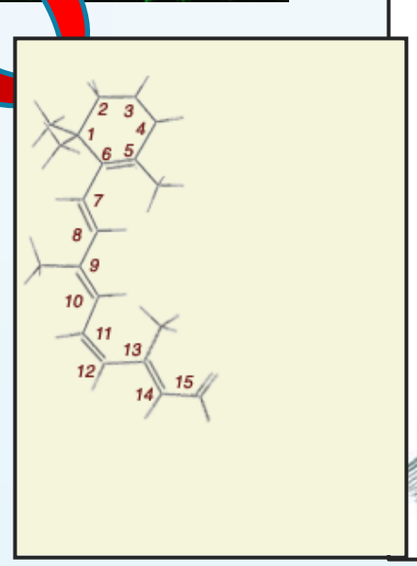


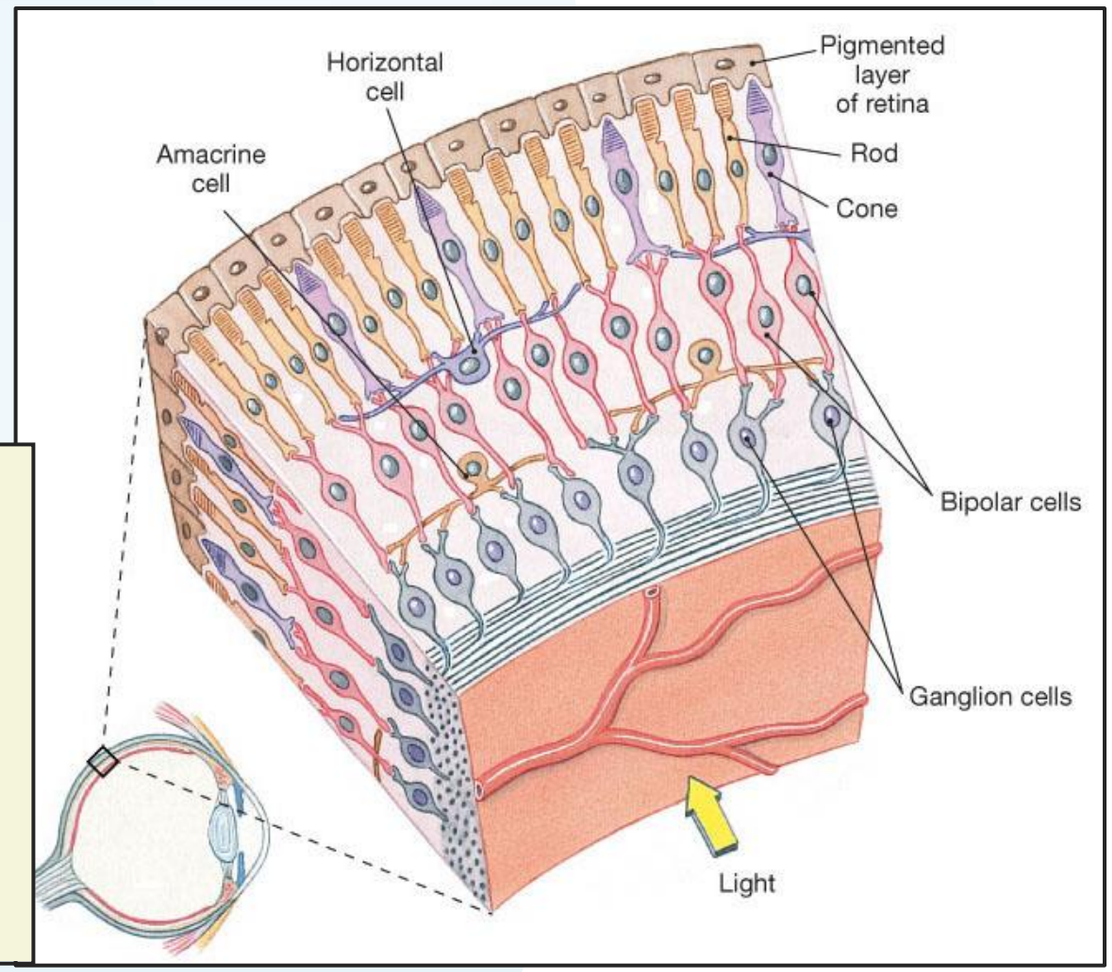
High potential of polymer-based devices for in vivo applications in pathologies characterized by neural iperactivity

Possible application as an artificial photoreceptor?









Retinal diseases

Retinitis Pigmentosa *(RP;* prevalence 1:3400)

Macular Degeneration (*MD*, (~30 million people affected)

- Stargardt's disease (juvenile MD)

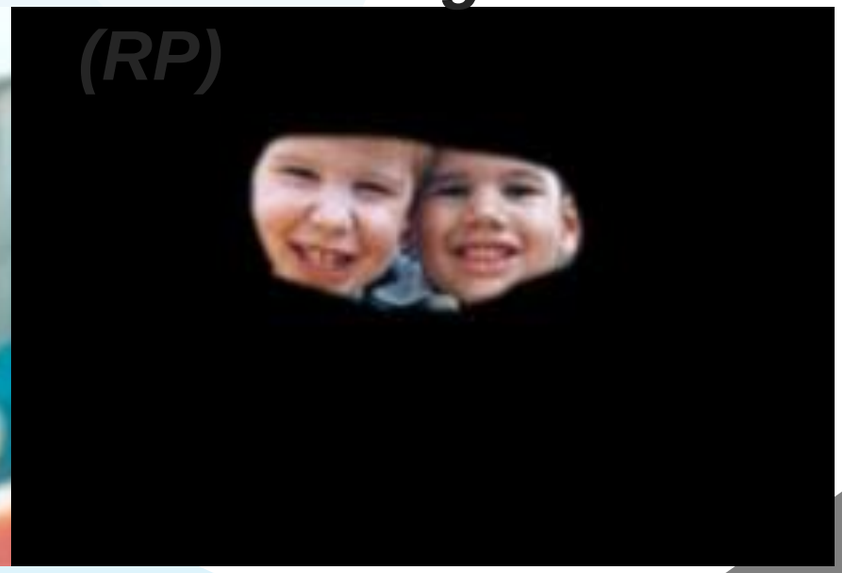
-age-related MD (AMD)

- dry age-related MD (dAMD; RPE degeneration)

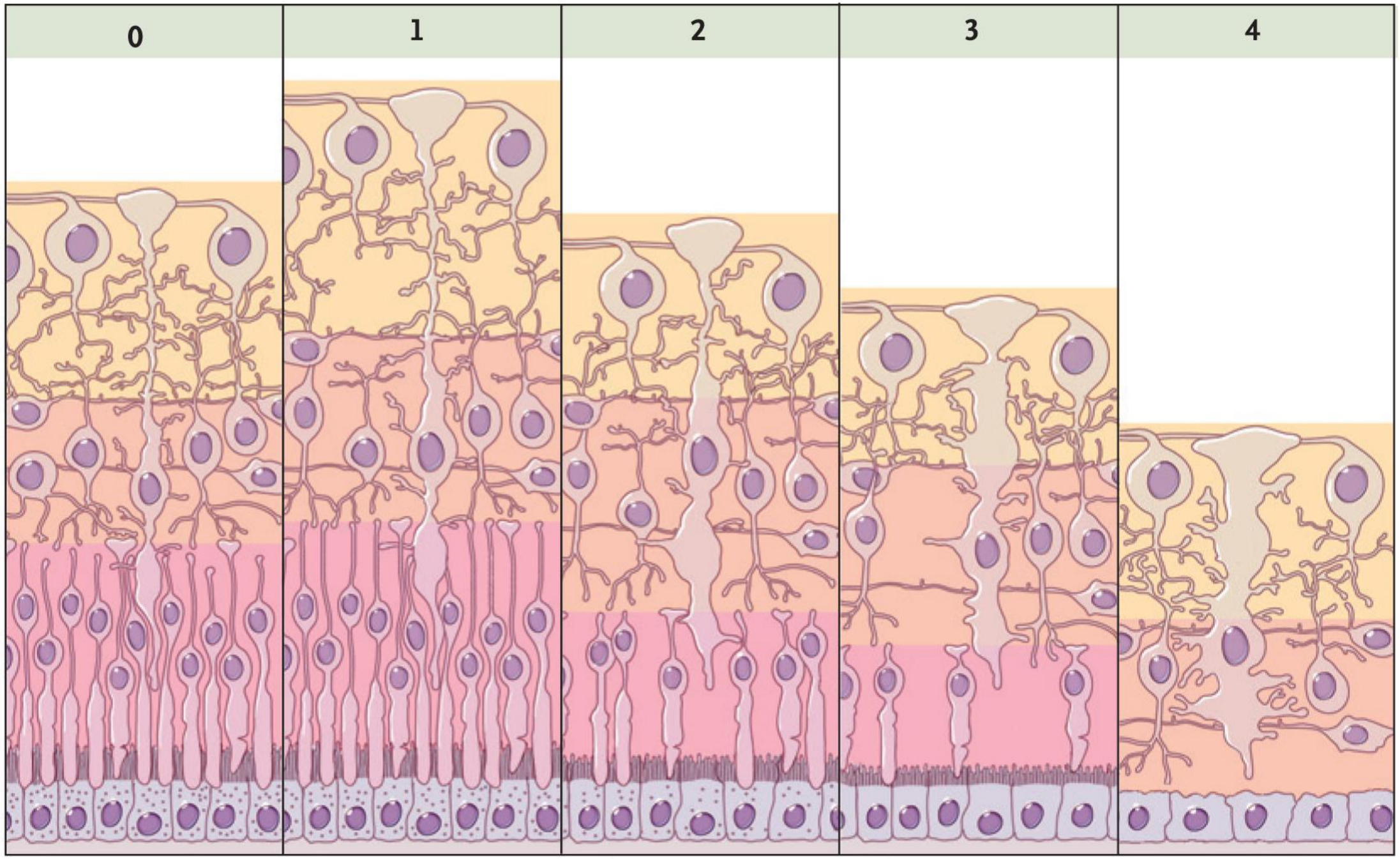
Normal

Macular degeneration

Retinitis Pigmentosa (RP)



RP



	0	1	2	3	4
Correct the dysfunction	✓	✓	✓	✓	
Slow cell death	✓	✓	✓	✓	
Regenerate photoreceptors					✓
Introduce substitute for photoreceptors				✤	✓

Phase 4: Cell/Tissue transplant; artificial prosthesis

Phase 1-3 : Gene therapy and Medication

The optogenetic approach:

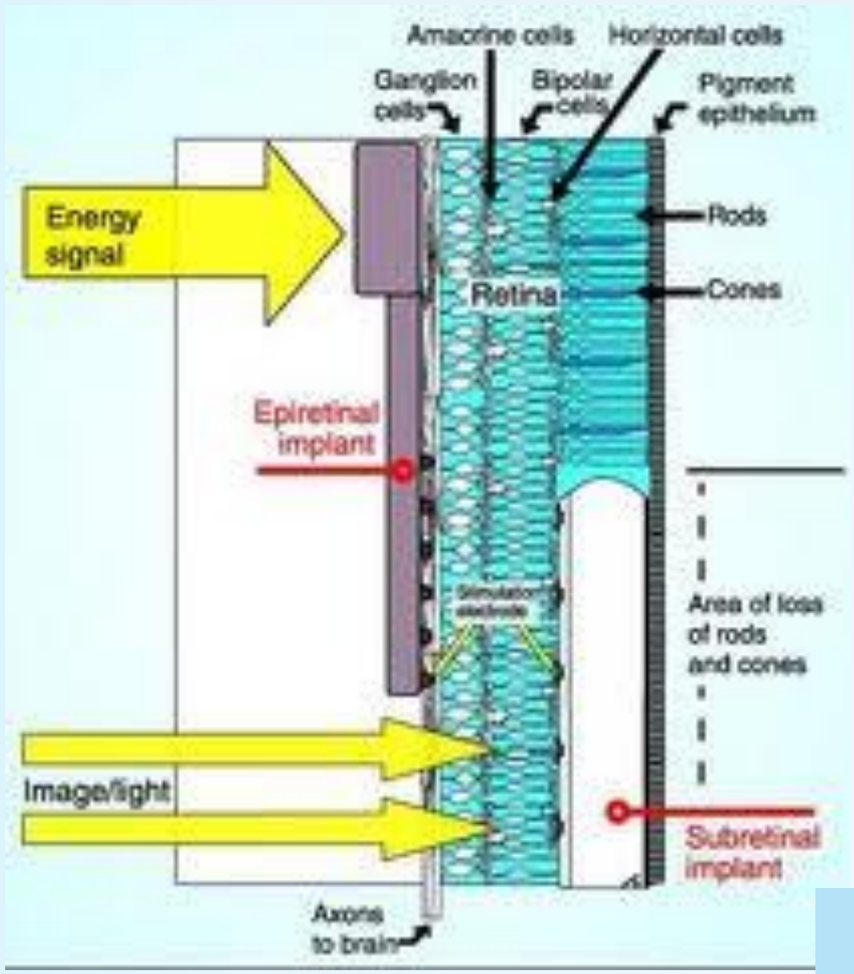
Rifunctionalization of photoreceptors which have lost light sensitivity

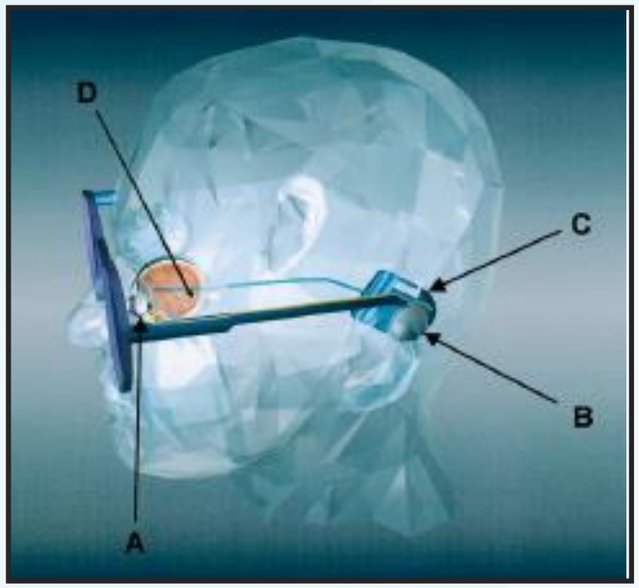
Busskamp et al., Science 329, 413-417 (2010)

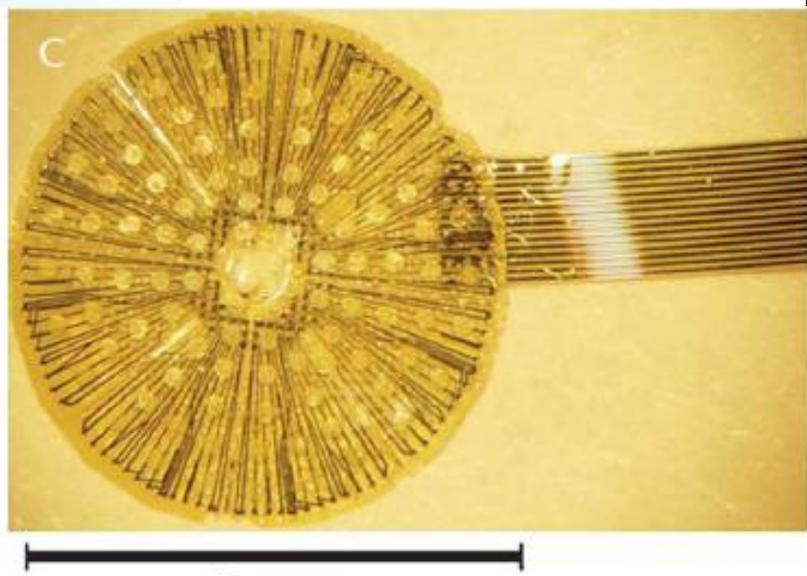
Disease Category	*Mapped Loci (not Identified)*	*Mapped and Identified Genes*
Bardet–Biedl syndrome, autosomal recessive	none	ARL6, BBS1, BBS2, BBS4, BBS5, BBS7, BBS10, BBS12, MKKS, PTHB1, TRIM32, TTC8
Chorioretinal atrophy or degeneration, autosomal dominant	MCDR1	RGR, TEAD1
Cone or cone–rod dystrophy, autosomal dominant	CORD4, RCD1	AIPL1, CRX, GUCA1A, GUCY2D, PITPNM3, RIMS1, SEMA4A, UNC119
Cone or cone–rod dystrophy, autosomal recessive	CORD8, CORD9	ABCA4, CACNA2D4, CNGB3, KCNV2, RDH5
Cone or cone–rod dystrophy, X–linked	COD2, COD4	RPGR
Congenital stationary night blindness, autosomal dominant	none	GNAT1, PDE6B, RHO
Congenital stationary night blindness, autosomal recessive	none	CABP4, GRK1, GRM6, RDH5, SAG
Congenital stationary night blindness, X–linked	none	CACNA1F, NYX
Deafness alone or syndromic, autosomal dominant	none	WFS1
Deafness alone or syndromic, autosomal recessive	USH2B	CDH23, DFNB31, MYO7A, PCDH15, USH1C
Leber congenital amaurosis, autosomal dominant	none	CRX, IMPDH1
Leber congenital amaurosis, autosomal recessive	LCA3, LCA9	AIPL1, CEP290, CRB1, CRX, GUCY2D, LCA5, LRAT, RD3, RDH12, RPE65, RPGRIP1, TULP1
Macular degeneration, autosomal dominant	BCMAD, BSMD, MCDR1, MCDR2, MCDR3, MCDR4, MCDR5, MDDC, STGD4	ARMD1, C1QTNF5, EFEMP1, ELOVL4, FSCN2, GUCA1B, RDS, TIMP3, VMD2
Macular degeneration, autosomal recessive	none	ABCA4
Macular degeneration, X–linked	none	RPGR
Ocular–retinal developmental disease, autosomal dominant	none	CSPG2
Optic atrophy, autosomal dominant	OPA4, OPA5	OPA1
Optic atrophy, autosomal recessive	ROA1	none
Optic atrophy, X–linked	OPA2	TIMM8A
Retinitis pigmentosa, autosomal dominant	RP33	CA4, CRX, FSCN2, GUCA1B, IMPDH1, NR2E3, NRL, PRPF3, PRPF8, PRPF31, RDS, RHO, ROM1, RP1, RP9, SEMA4A, TOPORS
Retinitis pigmentosa, autosomal recessive	RP22, RP25, RP28, RP29, RP32	ABCA4, CERKL, CNGA1, CNGB1, CRB1, LRAT, MERTK, NR2E3, NRL, PDE6A, PDE6B, PRCD, PROML1, RGR, RHO, RLBP1, RP1, RPE65, SAG, TULP1, USH2A
Retinitis pigmentosa, X–linked	RP6, RP23, RP24, RP34	RP2, RPGR
Syndromic/systemic diseases with retinopathy, autosomal dominant	CORD1, CRV	ABCC6, ATXN7, COL11A1, COL2A1, CSPG2, JAG1, PAX2
Syndromic/systemic diseases with retinopathy, autosomal recessive	AXPC1, CORS2, FHASD, JBTS1, LOC619531, MRST, WFS2	ABCC6, AHI1, ALMS1, CEP290, CLN3, COL9A1, INVS, IQCB1, LRP5, MTP, NPHP1, NPHP3, NPHP4, OPA3, PANK2, PEX1, PEX7, PHYH, PXMP3, RPGRIP1L, TTPA, WFS1
Syndromic/systemic diseases with retinopathy, X–linked	(– – –)	TIMM8A
Usher syndrome, autosomal recessive	USH1E, USH2B	CDH23, DFNB31, MASS1, MYO7A, PCDH15, USH1C, USH1G, USH2A, USH3A
Other retinopathy, autosomal dominant	CACD, CODA1, EVR3, MCDR4, SVD, VRNI	CRB1, FZD4, LRP5, OPN1SW, RB1, VMD2
Other retinopathy, autosomal recessive	ACHM1, RNANC	CDH3, CNGA3, CNGB3, CYP4V2, GNAT2, LRP5, MFRP, NR2E3, OAT, PROML1, R9AP, RBP4, RGS9, RLBP1
Other retinopathy, mitochondrial	none	KSS, LHON, MTATP6, MTTH, MTTL1, MTTS2
Other retinopathy, X–linked	AIED, PRD	CHM, DMD, NDP, OPN1LW, OPN1MW, PGK1, RS1

Looking for alternative techniques...

Artificial retinal prosthesis: state of the art

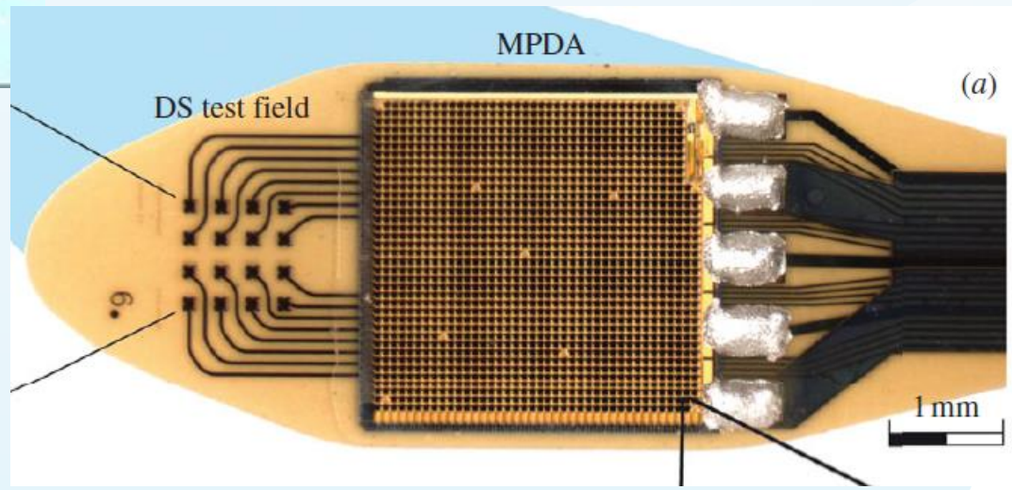


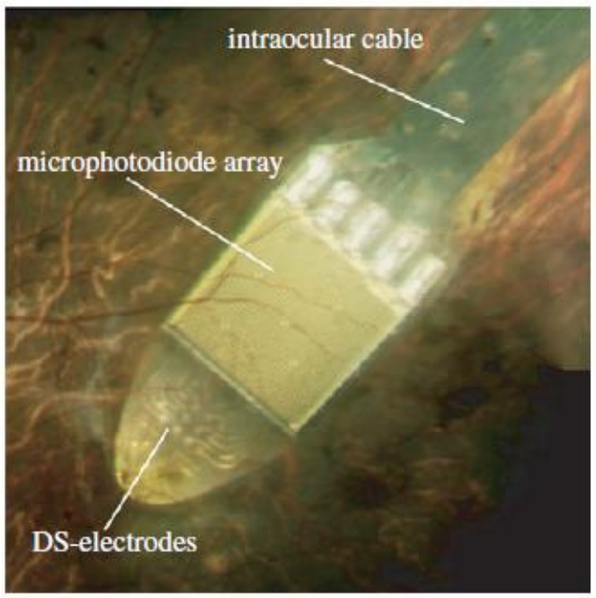




Rizzo et al., 2011

(A) Camera (B) wireless transmitter (C) extraocular electronic receiver (D) intraocular implant (electrodes array)

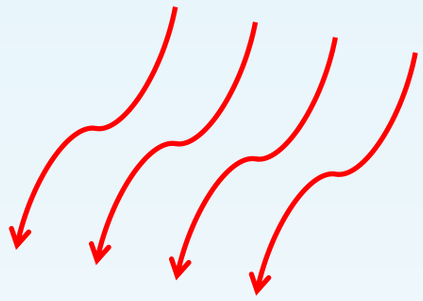




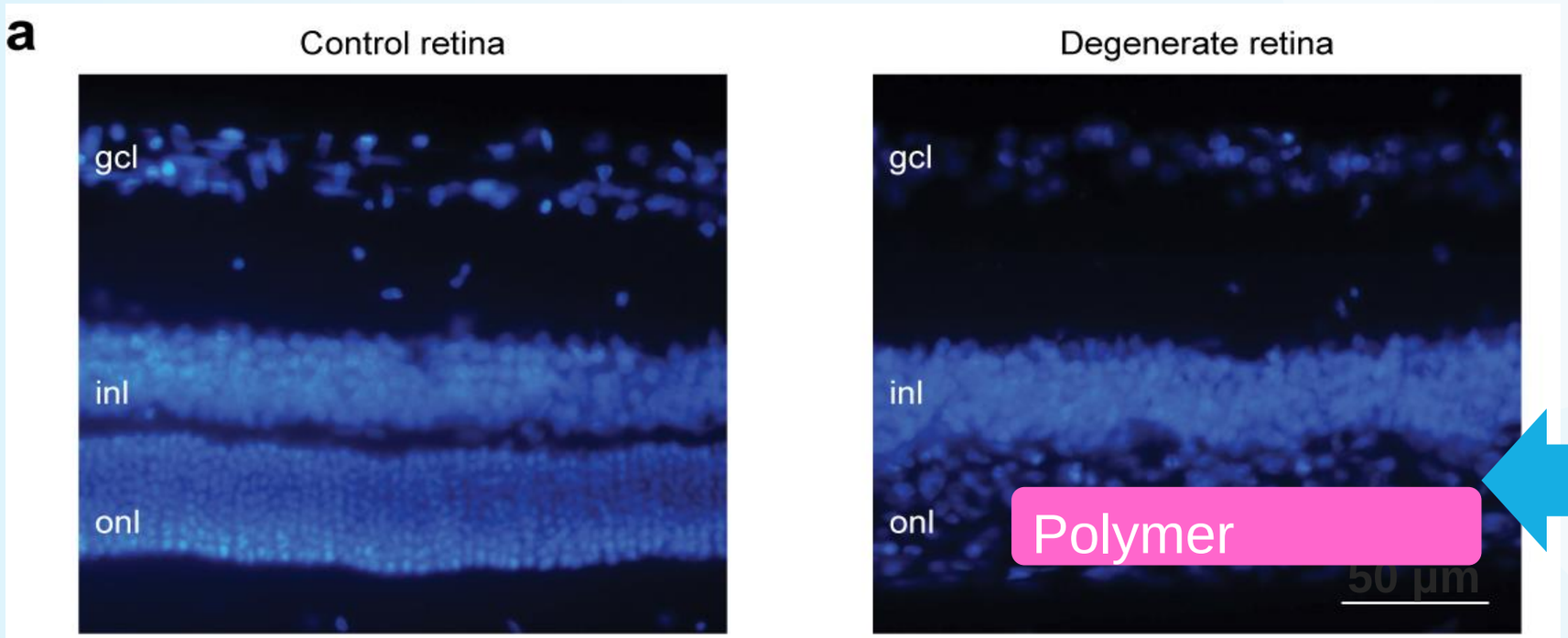
Zrenner et al., 2010

Major drawbacks: poor resolution, due to the limited number of electrodes; biocompatibility; durability; connection to the existing retinal wiring

Ex-vivo studies



Light impinging from the inner layer



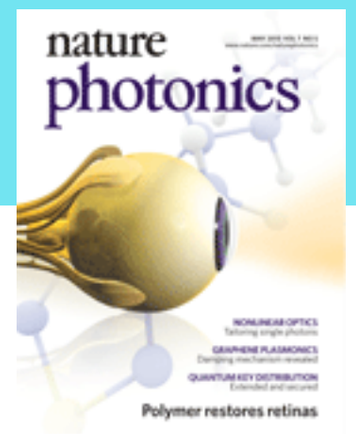
Photoreceptors Degeneration

Sub-retinal Configuration

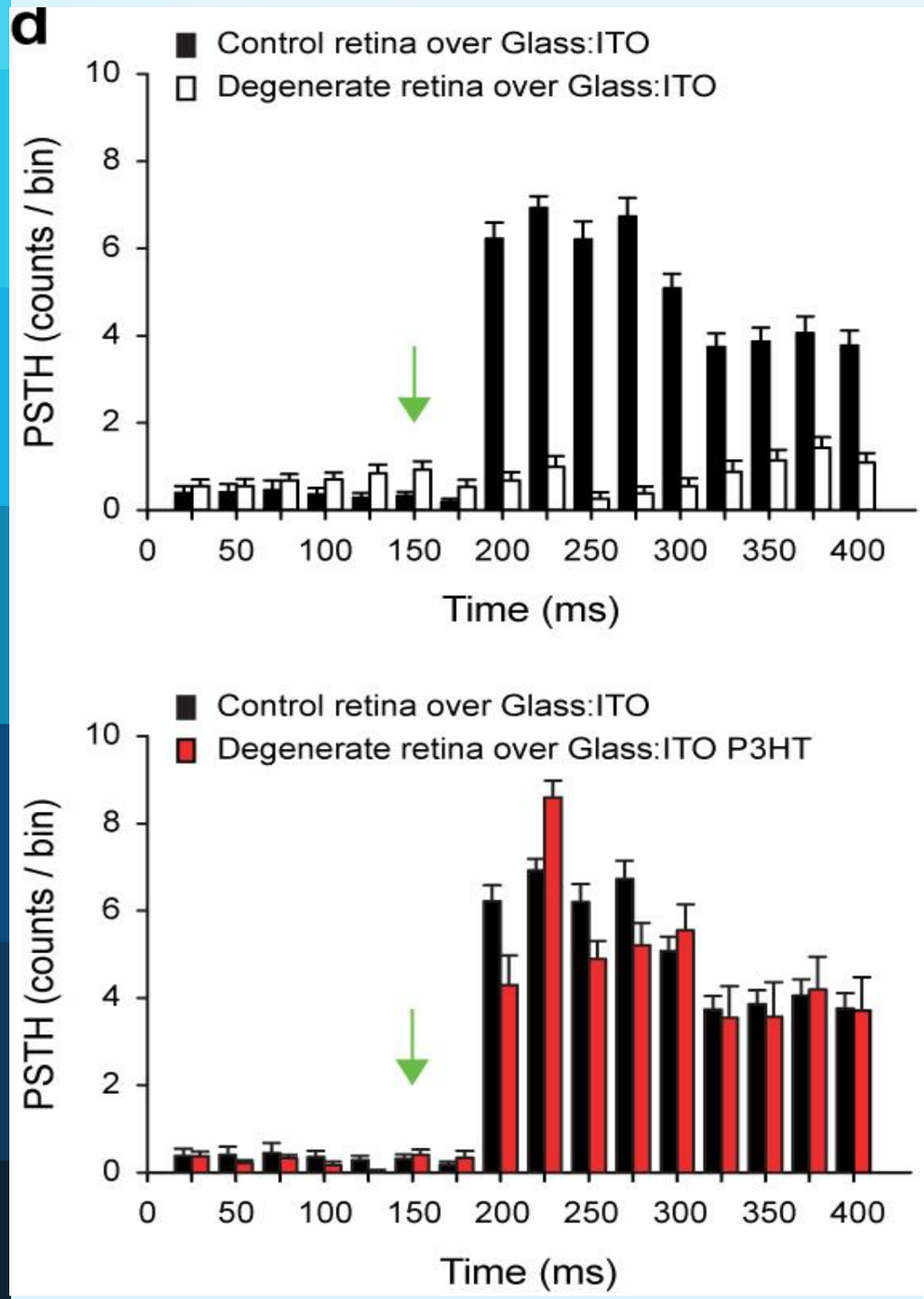
- Sprague-Dawley albino rats
- 4-6 weeks photoreceptors damage

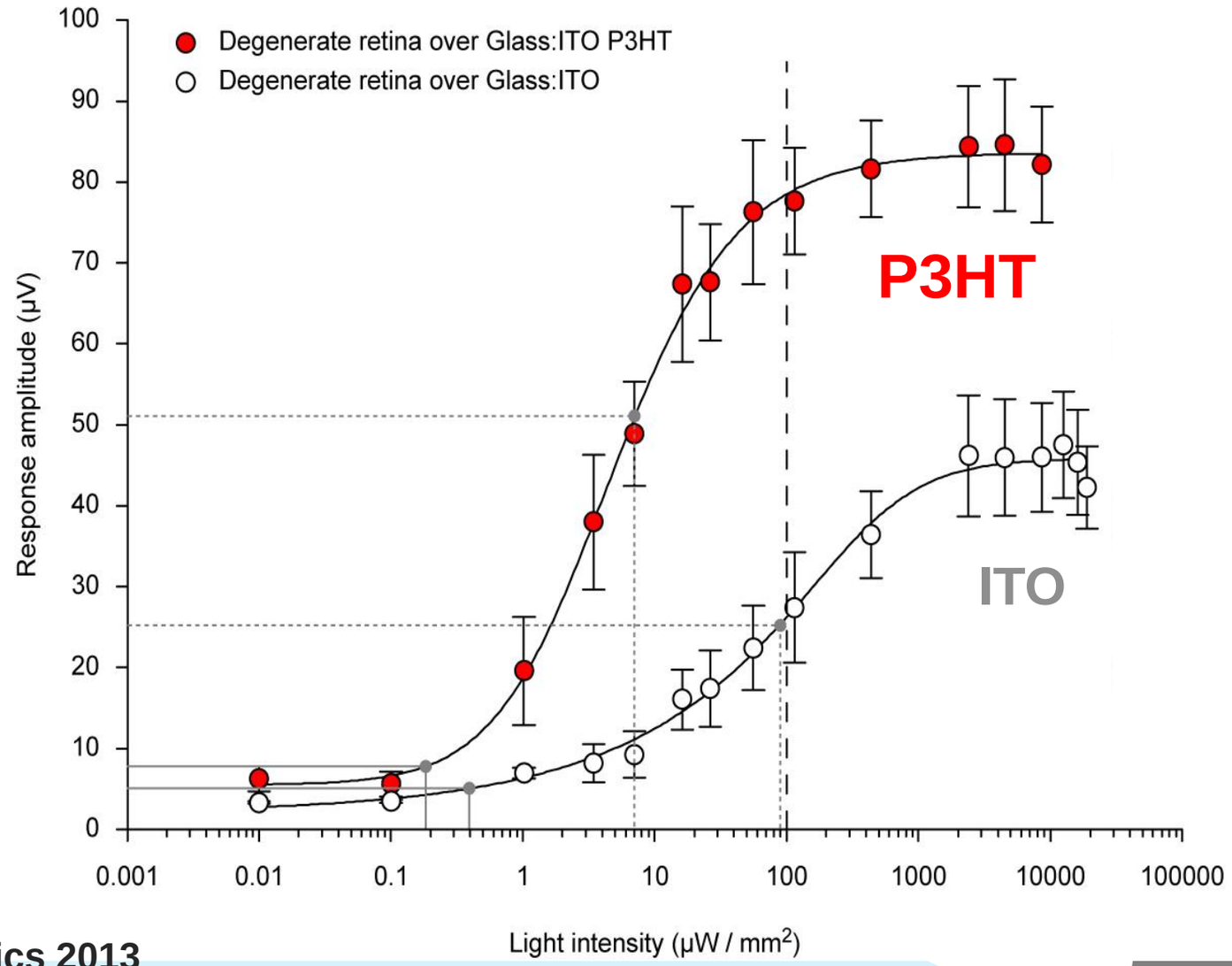
D. Ghezzi, M.R. Antognazza, et al., Nature Photonics 2013

Results on rats' explanted retinas



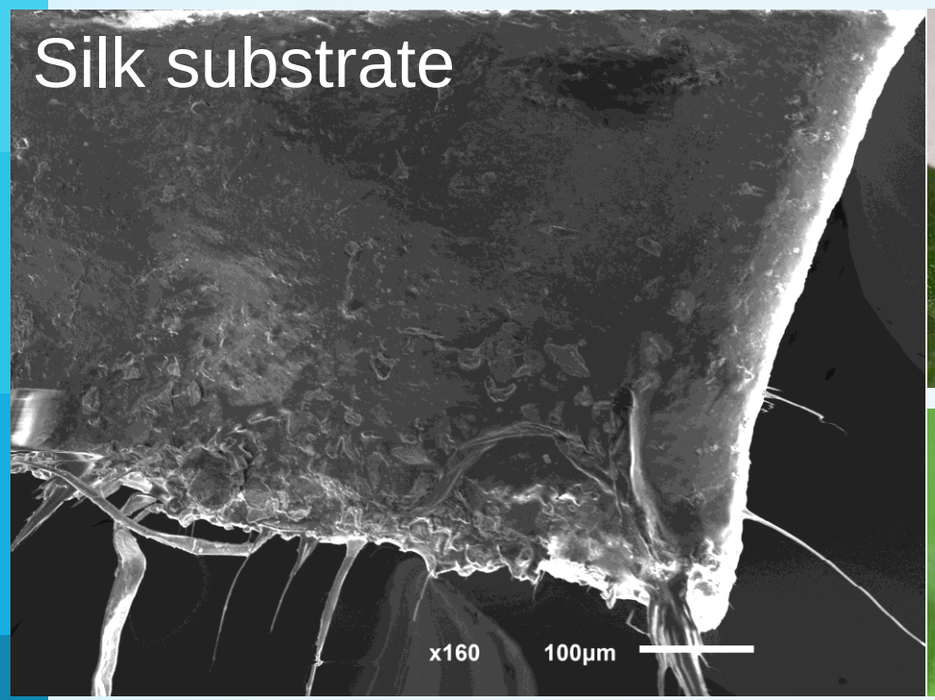
Light pulse: 10 ms, 4 mW/mm²

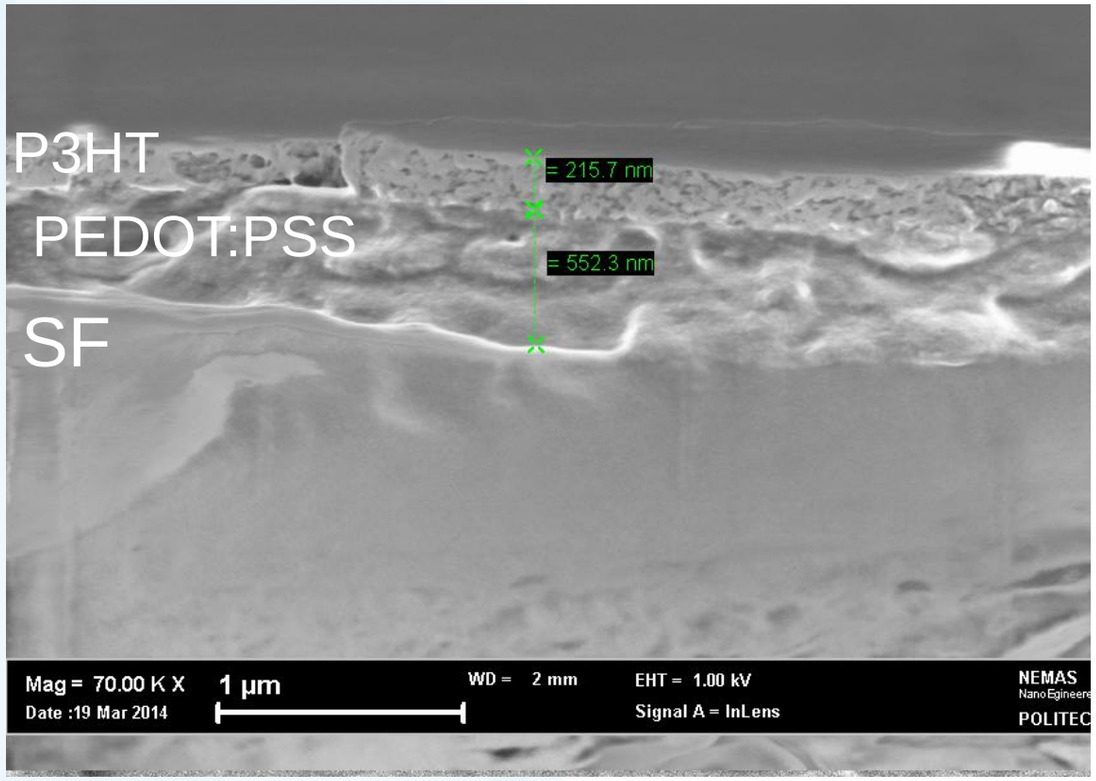


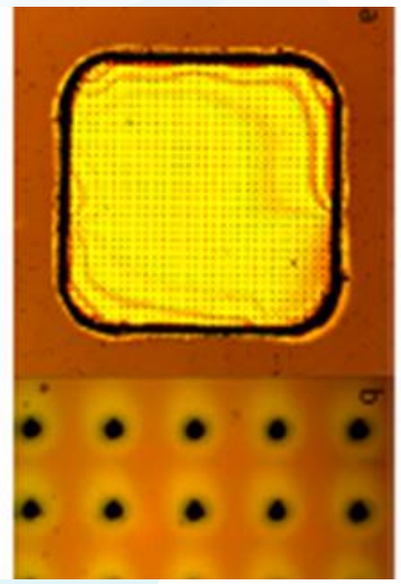


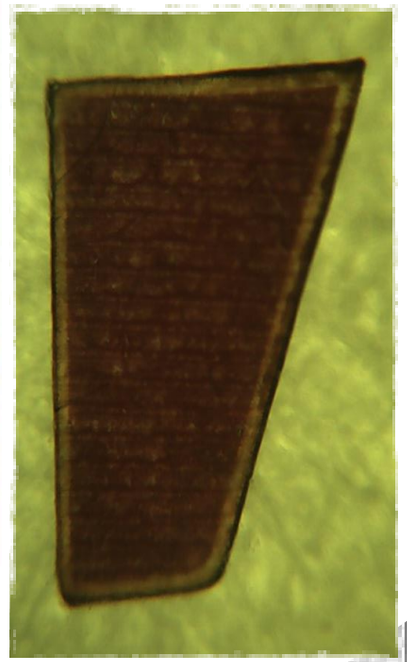
D. Ghezzi, M.R. Antognazza, *et al*., Nature Photonics 2013

Realization of the prosthetic device: the substrate









Still……many open issues:

HEK cells: 7 – 50 mW/mm^2 >> thermal effects;

Neurons: ca. 10 mW/mm^2 >> thermal effects?

Explanted retinas: ca. 1 – 100 μW/mm^2 >> ???

In vivo: ca. 0.1 - 10 μW/mm^2 >> ???