EPR Spectroscopy

Spying on unpaired electrons - What information can we get?

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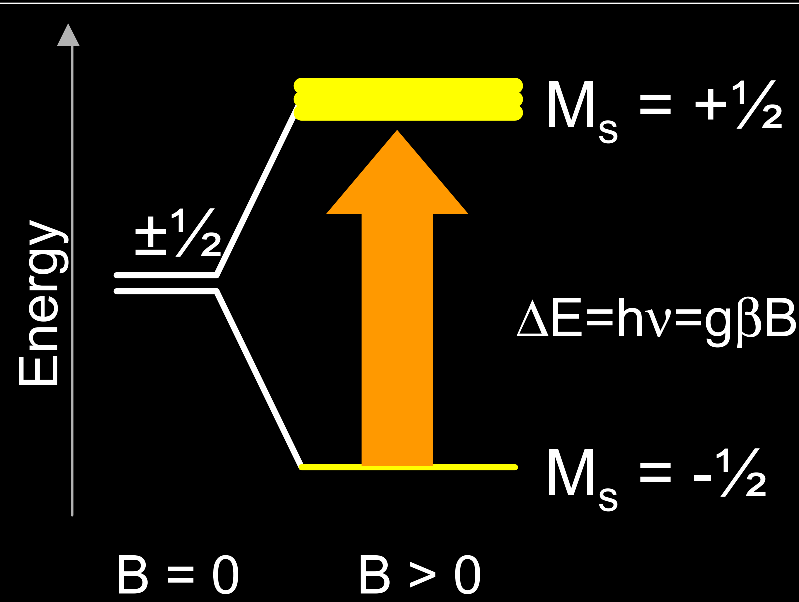
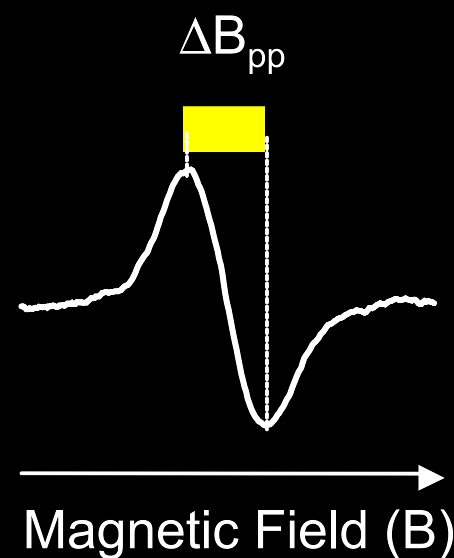
What is EPR?

Electron Paramagnetic Resonance (EPR)
Electron Spin Resonance (ESR)
Electron Magnetic Resonance (EMR)
EPR ~ ESR ~ EMR





h	Planck's constant 6.626196 x 10^{-27} erg.sec
ν	frequency (GHz or MHz)
g	g-factor (approximately 2.0)
β	Bohr magneton (9.2741 x 10-21 erg.Gauss^{-1})
B	magnetic field (Gauss or mT)

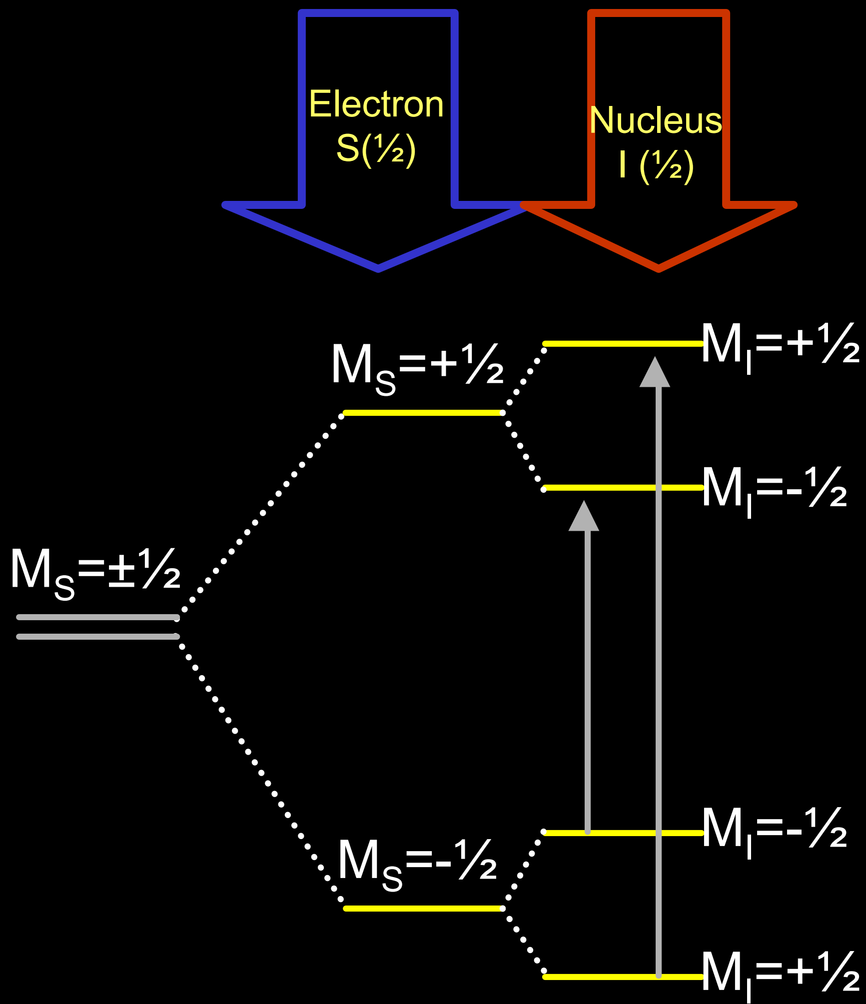
EPR is the resonant absorption of microwave radiation by paramagnetic systems in the presence of an applied magnetic field

$$h\nu = g\beta B$$

$\nu = (g\beta/h)B = 2.8024 \times B$ MHz

for B = 3480 G	ν = 9.75 GHz (X-band)
for B = 420 G	ν = 1.2 GHz (L-band)
for B = 110 G	ν = 300 MHz (Radiofrequency)

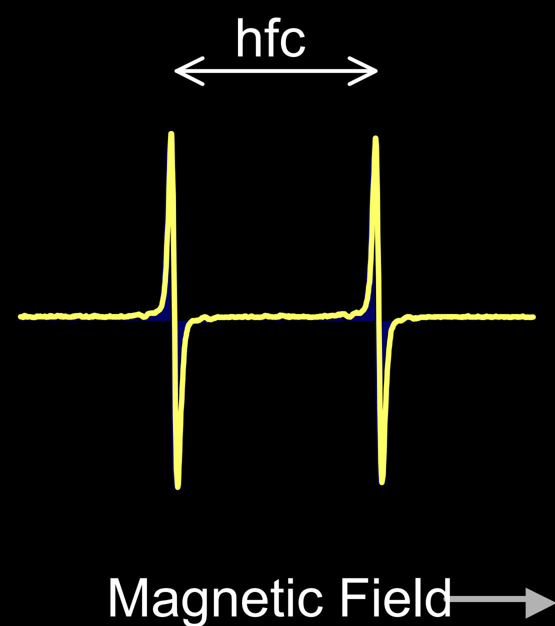
Hyperfine Coupling



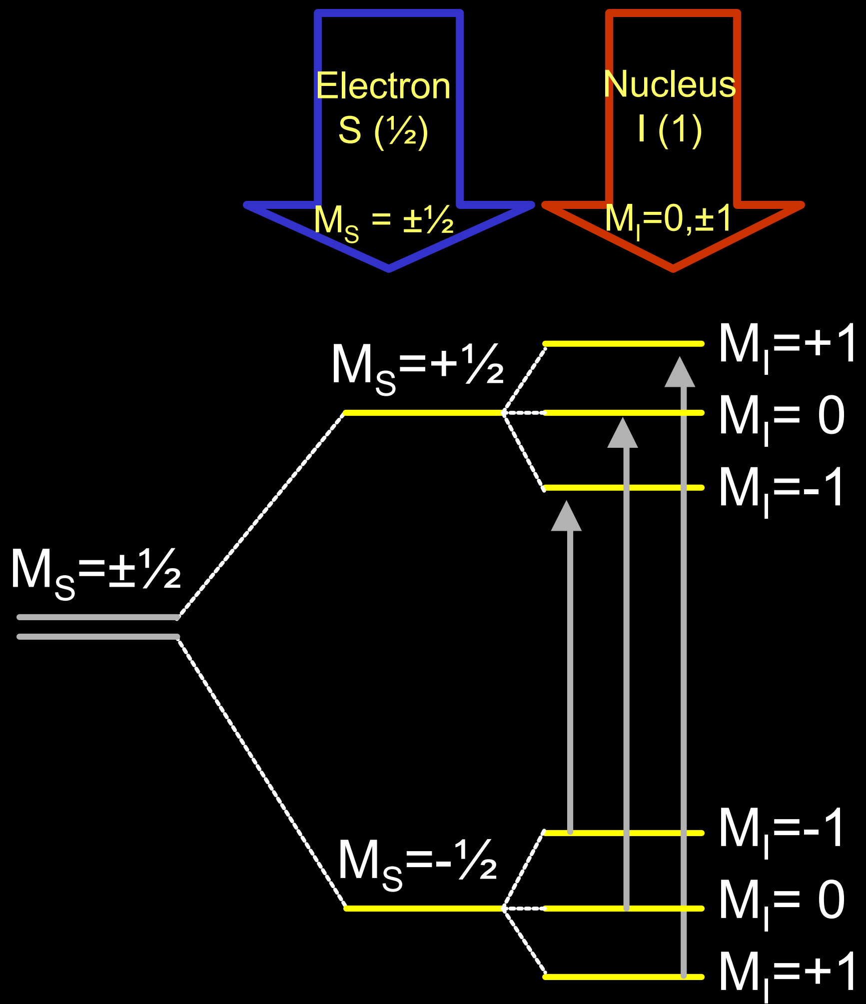
Selection Rule

$\Delta M_S = \pm1; \Delta M_I = 0$

S=½;
I=½
Doublet

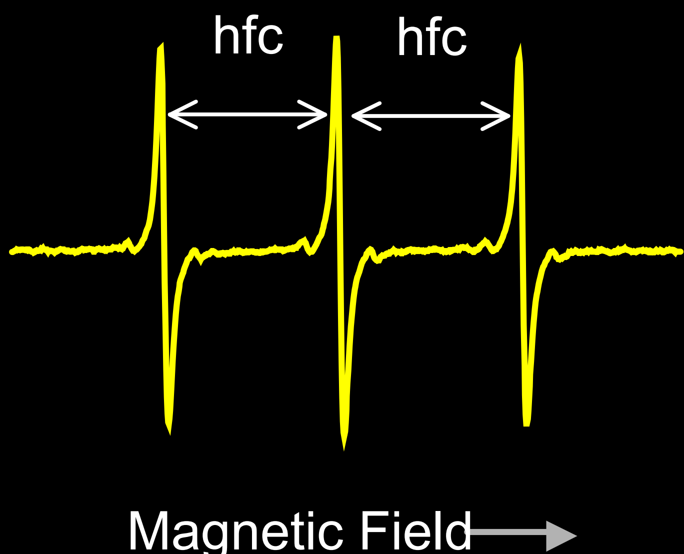


Hyperfine Coupling



Selection Rule
$\Delta M_S = \pm 1; \Delta M_I = 0$

S=½;
I=1
Triplet



What do we do with EPR?

We can detect & measure free radicals and paramagnetic species

- High sensitivity (nanomolar concentrations)
- No background
- Definitive & quantitative

Direct detection

e.g.: semiquinones, nitroxides, trityls

Indirect detection

Spin-trapping

Species: superoxide, hydroxyl, alkyl, NO

Spin-traps: DMPO, PBN, DEPMPO, Fe-DTCs

Chemical modifications

Spin-formation : hydroxylamines (Dikhalov et al)

Spin-change : nitronylnitroxides (Kalyanaraman et al)

Spin-loss : trityl radicals

Can we use EPR to measure free radicals from biological systems (*in vivo or ex vivo*)?

Yes! Intact tissues, organs or whole-body can be measured. But there is a catch!

Biological samples contain large proportion of water. They are aqueous and highly dielectric. Conventional EPR spectrometers operate at X-band ((9-10 GHz) frequencies, which result in (i) 'non-resonant' absorption (heating) of energy and (ii) poor penetration of samples. Hence the frequency of the instrumentation needs to be reduced.

What is the optimum frequency? - depends on sample size

Frequency	~300 MHz	~750 MHz	1-2 GHz	~3 GHz	9-10 GHz
Penetration Depth	> 10 cm	6-8 cm	1-1.5 cm	1-3 mm	1 mm
Objects	Mouse, rat	Mouse	Mouse, rat heart	Mouse tail Topical (skin)	In vitro samples (~100 uL vol.)
Pioneers	Halpern et al Krishna et al	Zweier et al	Hyde et al Swartz et al Zweier et al	Hyde et al Zweier et al	

What else can we do with EPR?

We can use free radicals as “spying probes” to obtain information from biological systems

- A known free radical probes is infused or injected into the animal
- The change in the EPR line-shape profile, which is correlated to some physiological function, is then monitored as a function of time or any other parameter.
- The measurements can be performed in *real-time* and *in vivo* to obtain ‘functional parameters’.

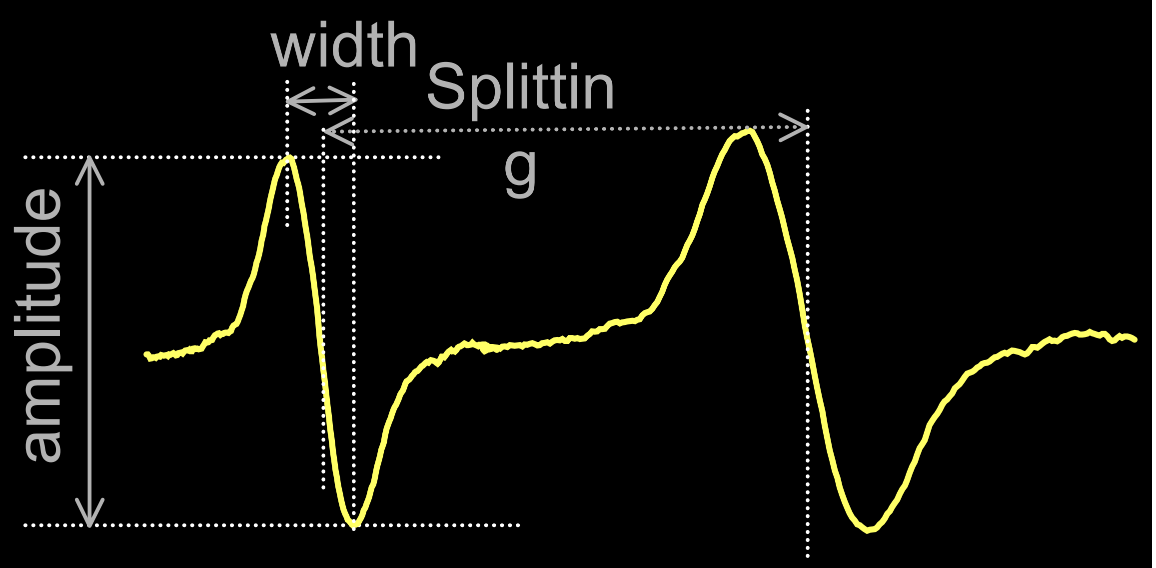
Functional parameters from an EPR spectrum

In vivo EPR spectroscopy is capable of providing useful physiologic and metabolic (functional) information from tissues

Oxygen, pO_2
Redox status
Acidosis, pH
Thiols (GSH)
Cell viability
Viscosity
Tissue perfusion
Molecular motion

Redox status
Cell viability
Tissue perfusion

Oxygen, pO_2
Acidosis, pH
Viscosity
Molecular motion



Can we image free radicals in biological systems?

Spatially-resolved information (mapping) can be obtained using EPR imaging (EPRI) techniques

Can we image free radicals in biological systems?

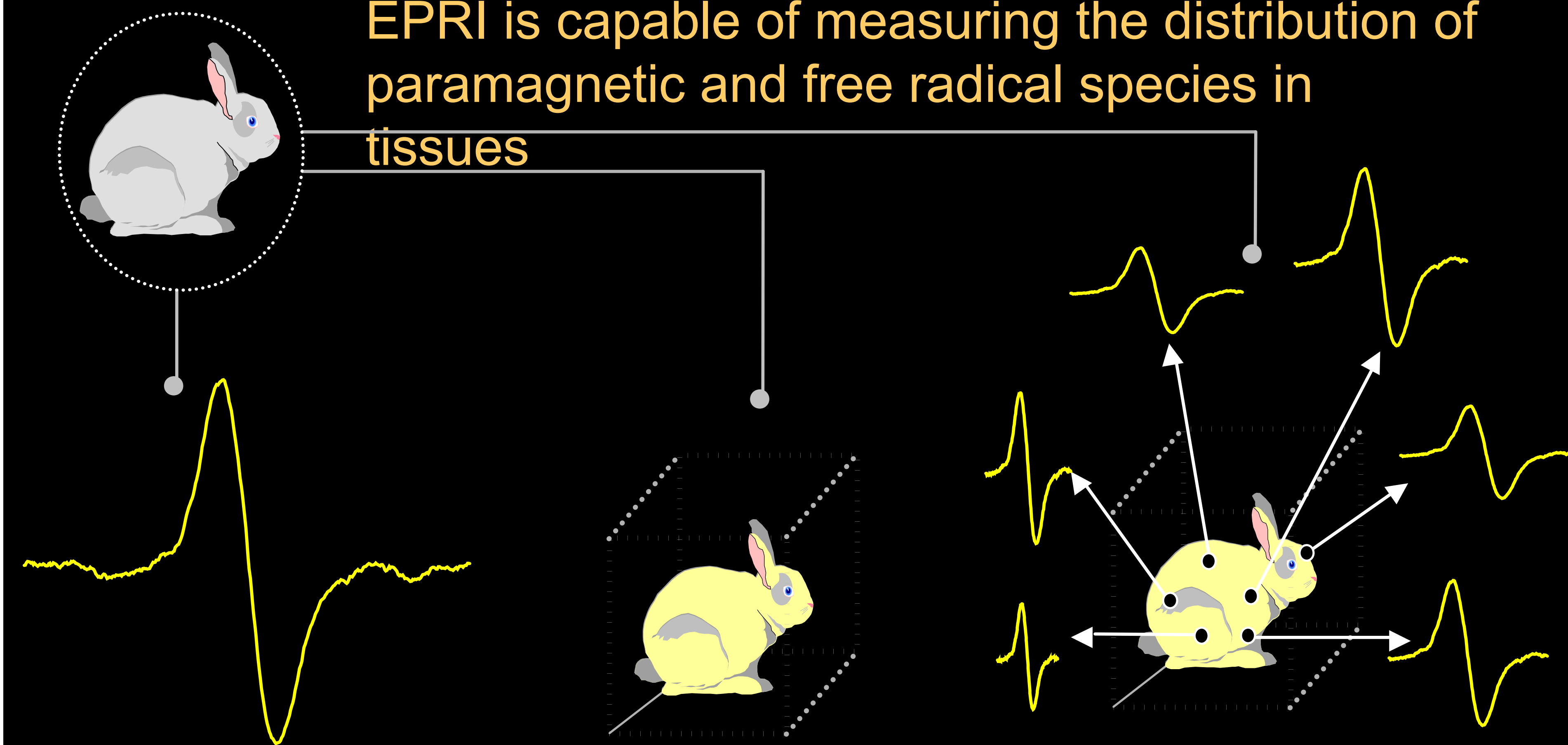
	NMR	EPR
Spin Probes	Tissue protons	Free
radicals		
(endogenous)	(>50 M)	(<< nM)
Probe Stability	Ideal	<
nanoseconds		
Relaxation time (LW)	m sec	< μ sec
(LW: 1 G)		

No suitable endogenous spin probes for EPRI Nothing to image

Fast electronics needed for EPRI. No way to image

Eaton & Eaton (1989)

EPRI is capable of measuring the distribution of paramagnetic and free radical species in tissues

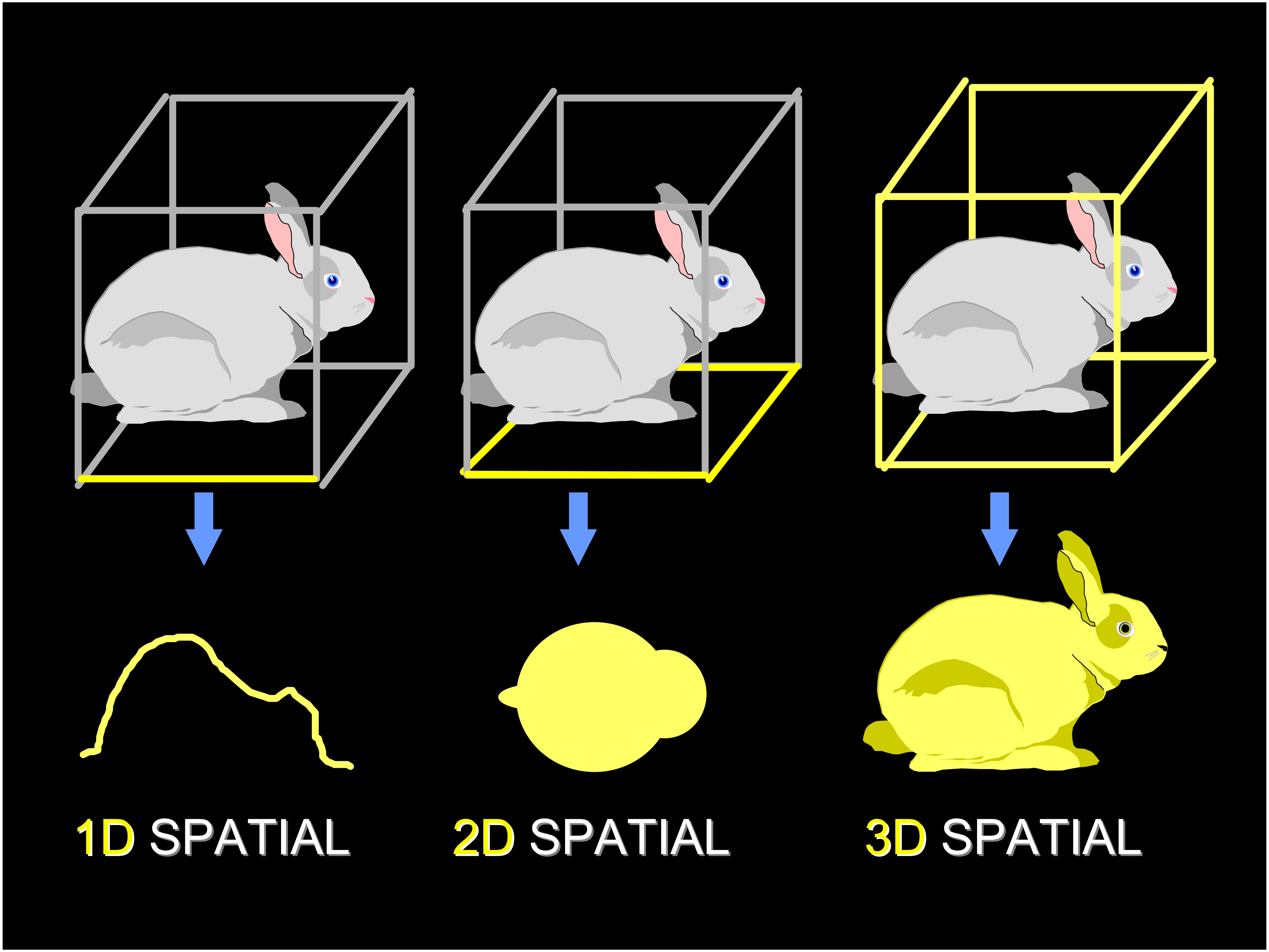


EPR Spectroscopy
Spatially-unresolved

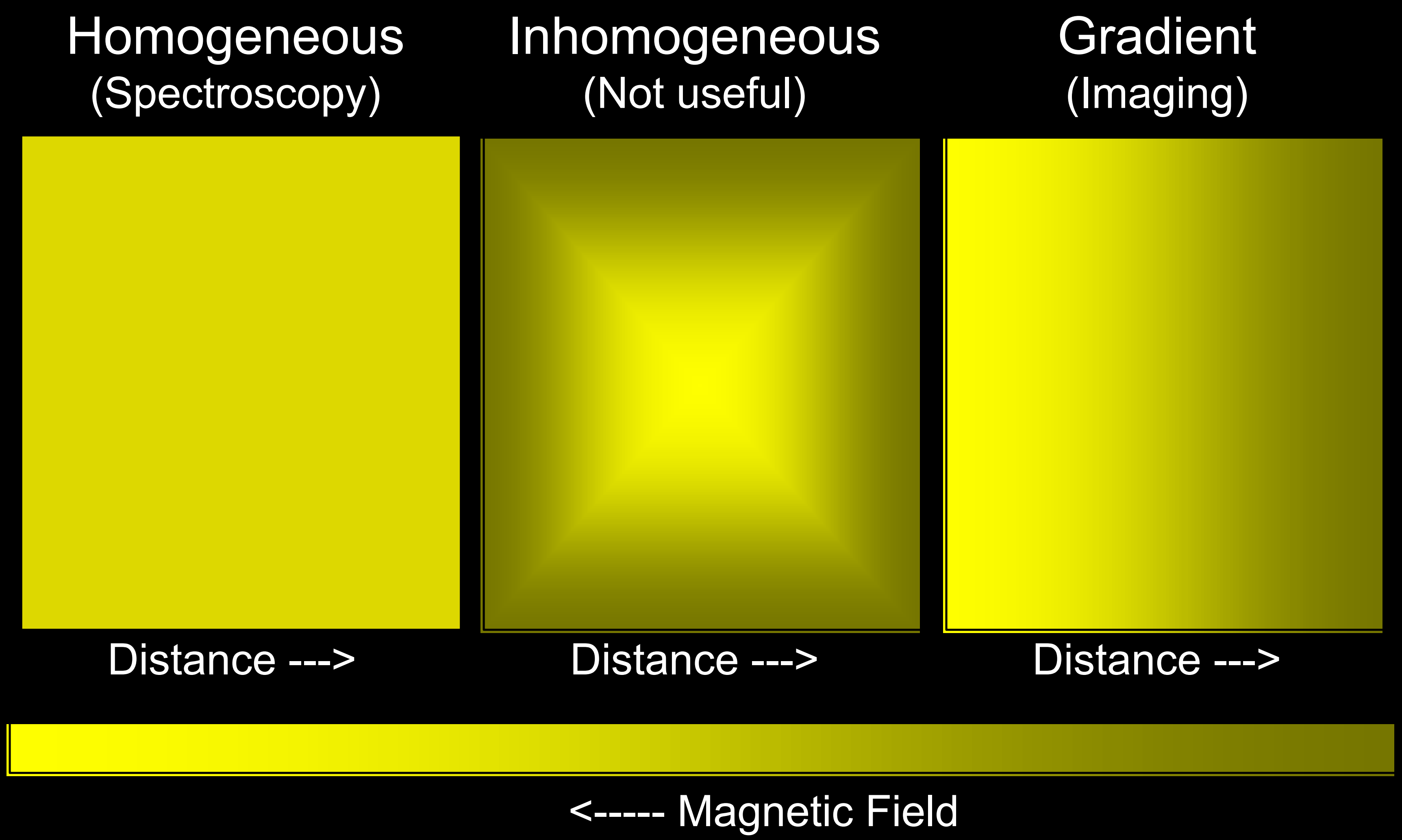
0 + 1 dimensional

Spatial Imaging
Spatially-resolved
Spin density
3 + 0 dimensional

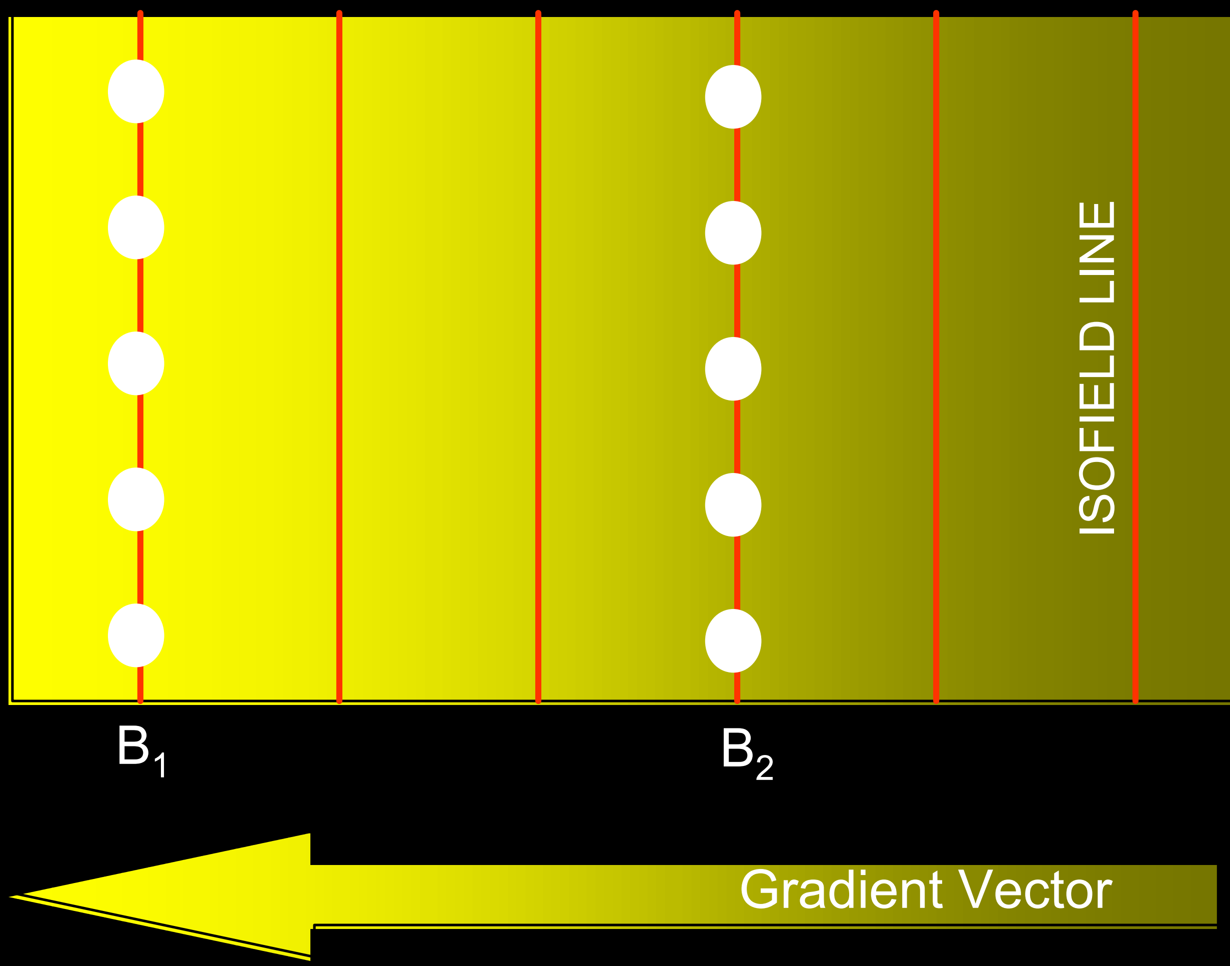
Spectral-spatial Imaging
Spatially-resolved
Spectral shape
3 + 1 dimensional


1D SPATIAL
2D SPATIAL
3D SPATIAL

Gradient Magnetic Field



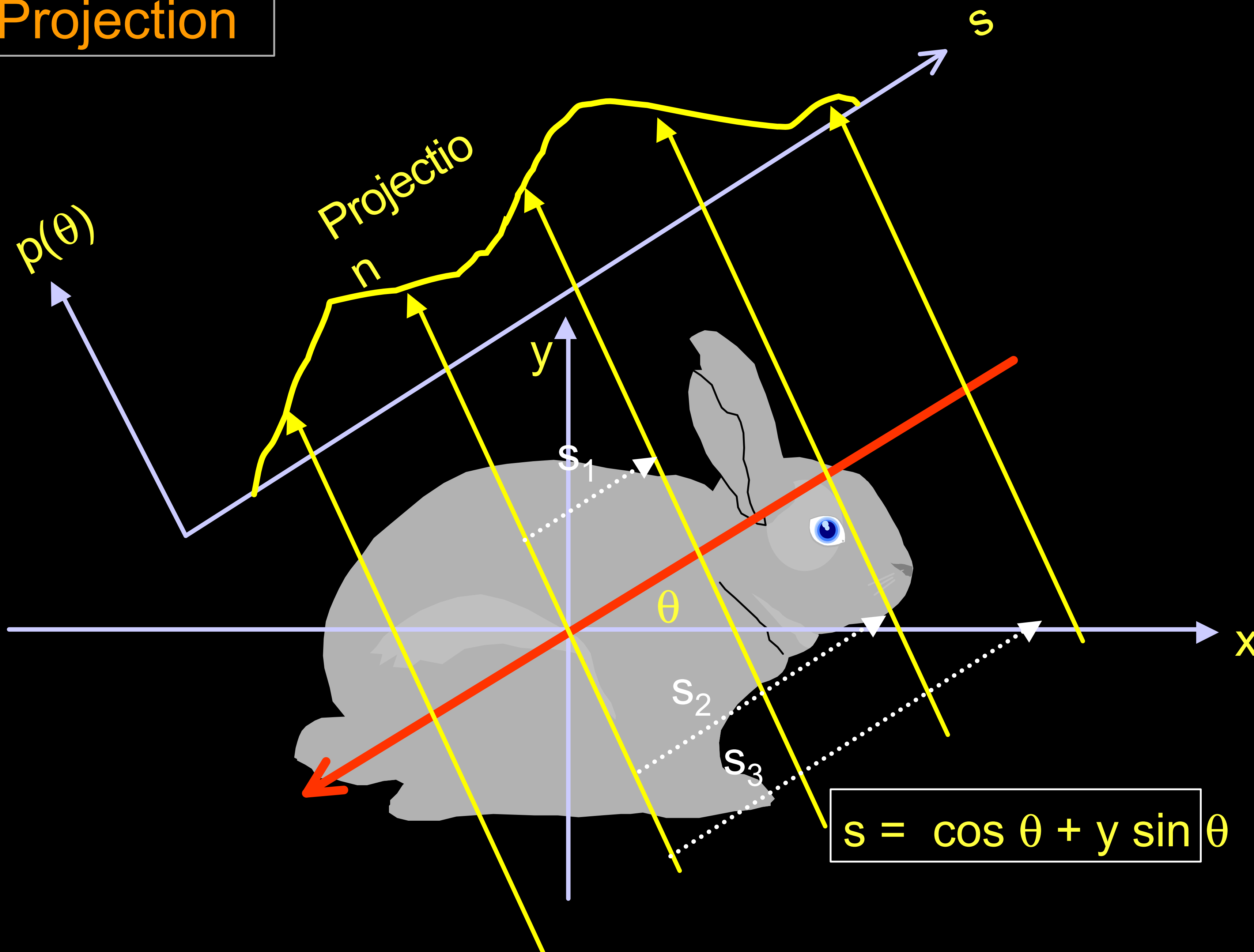
Gradient Magnetic Field



$B_1 > B_2$

In the conventional CW EPR sweep mode, spins at B_1 will come into resonance first.

Projection



Projection Acquisition

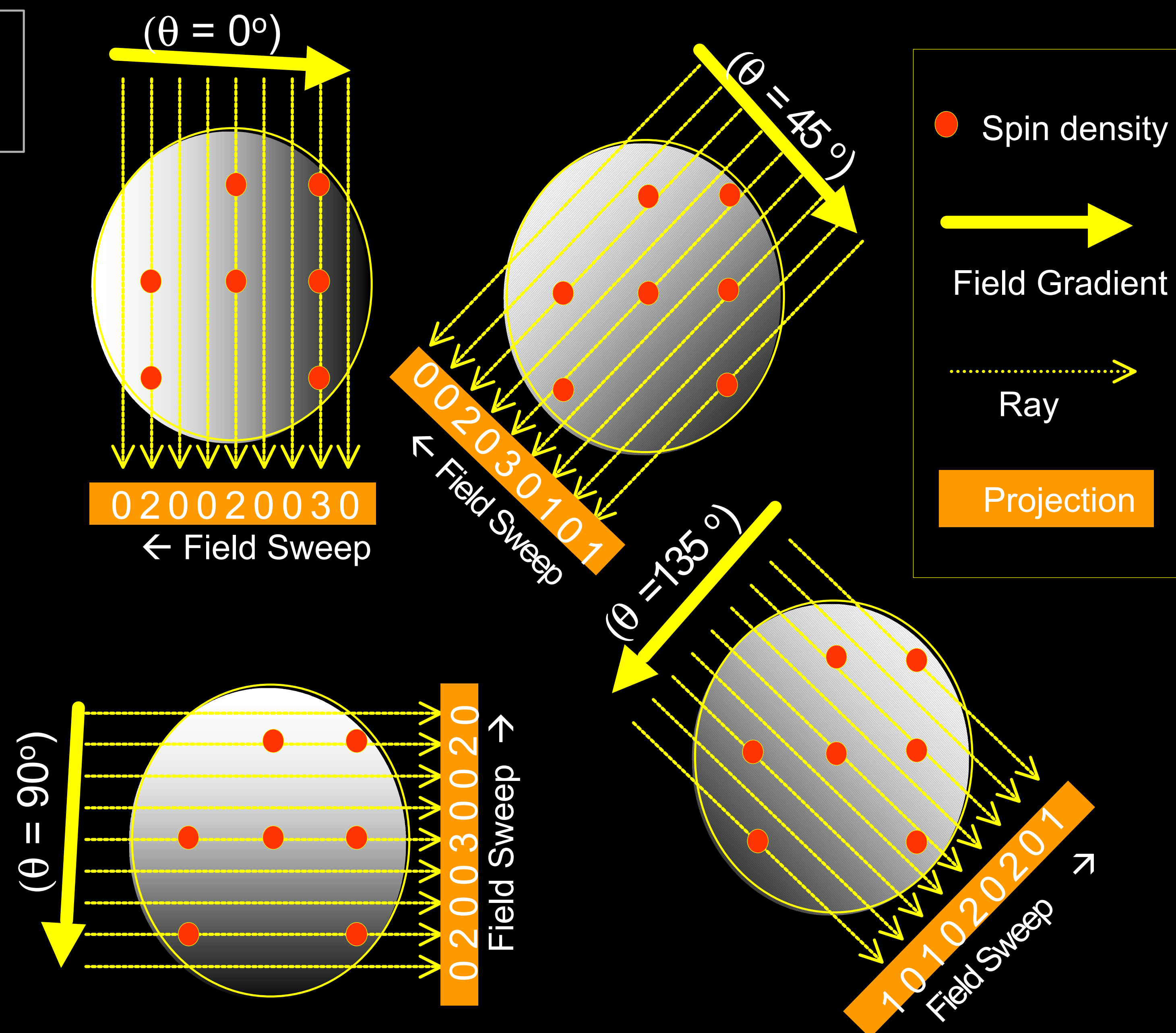
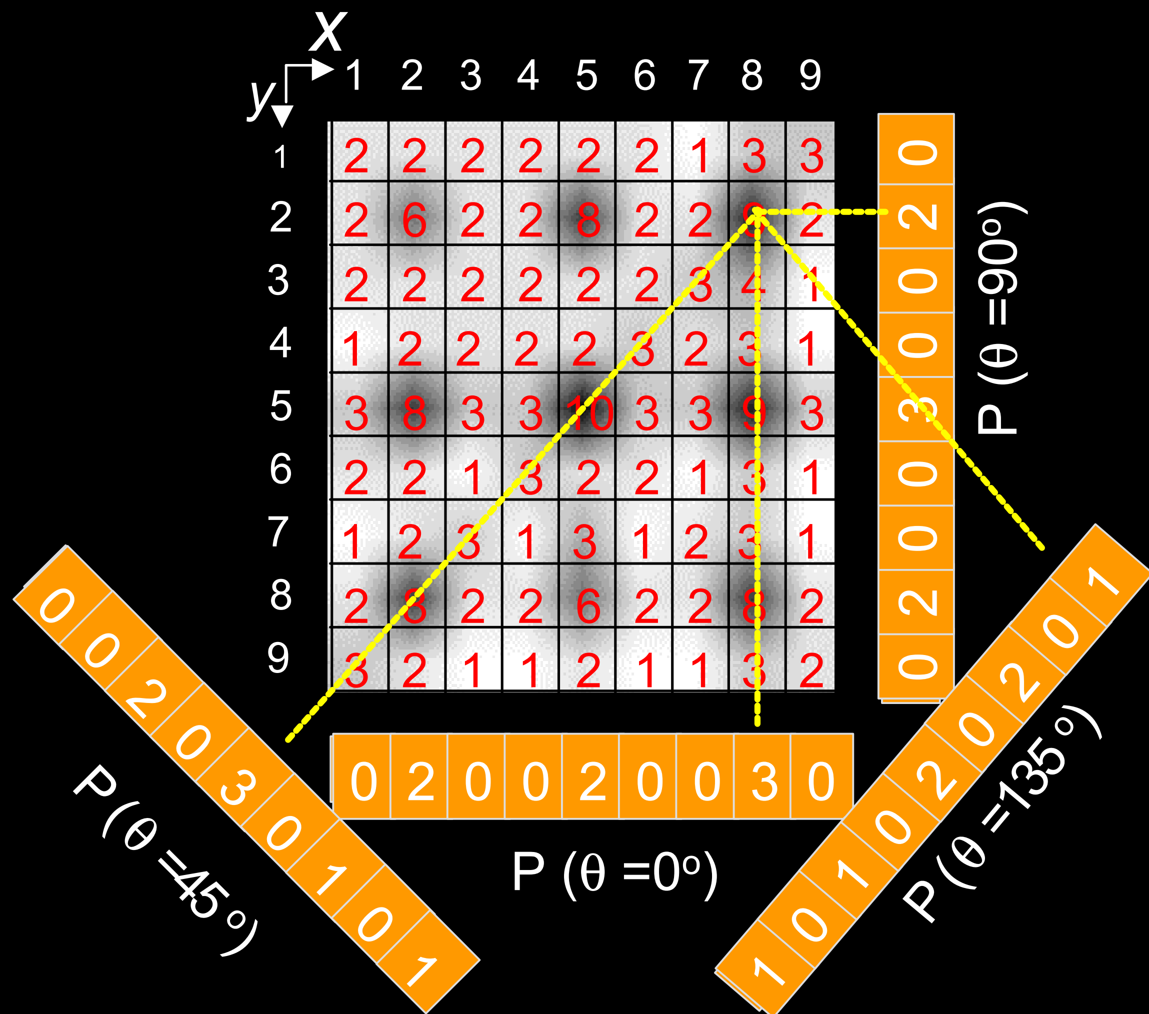
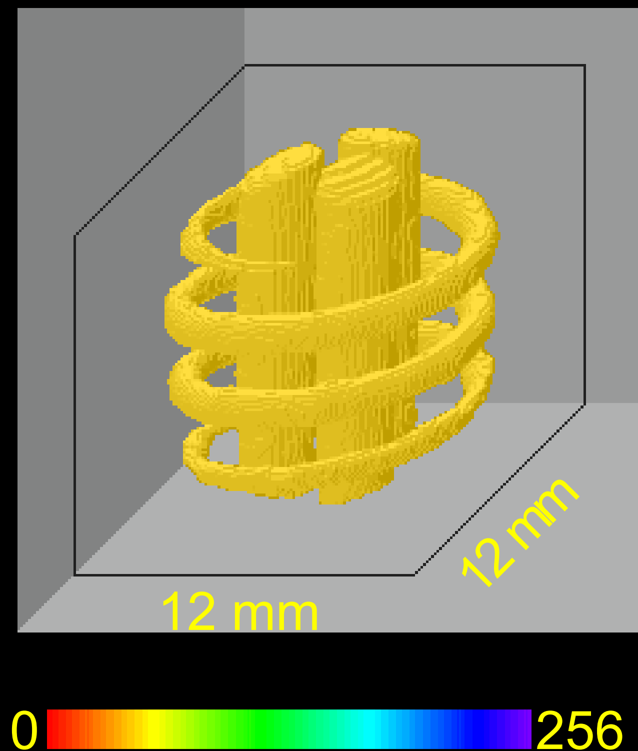


Image Reconstruction by Backprojection

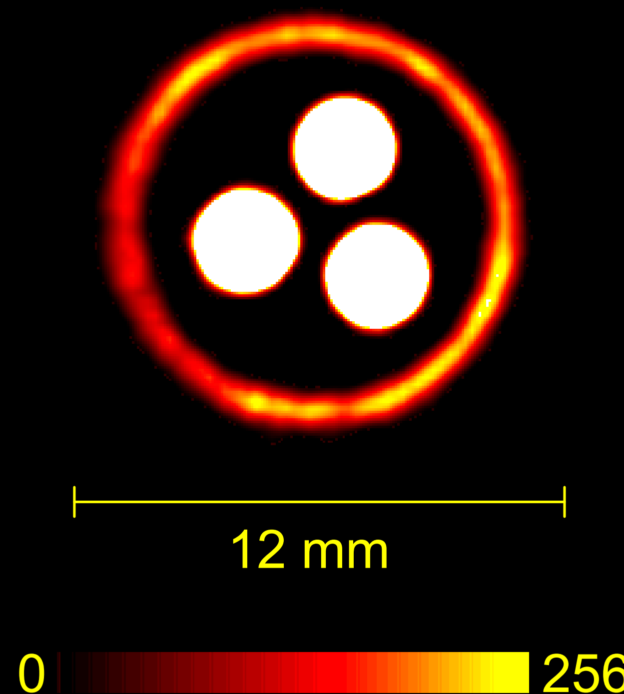


3D IMAGING OF A SPIRAL PHANTOM

A pack of three identical tubes (i.d.: 3 mm) and a polyethylene tubing (id: 1.1 mm) wound around the pack. The tubes were filled with 0.5 mM solution of TAM.



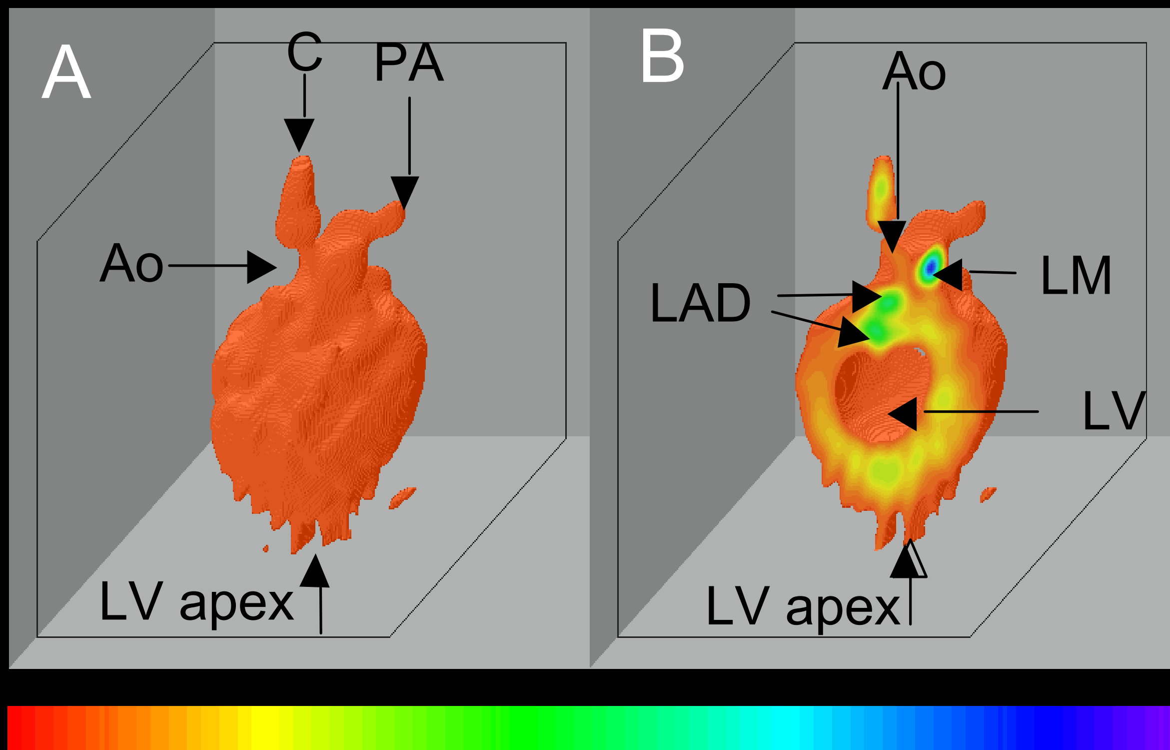
3D composite view



A 2D projection of the image

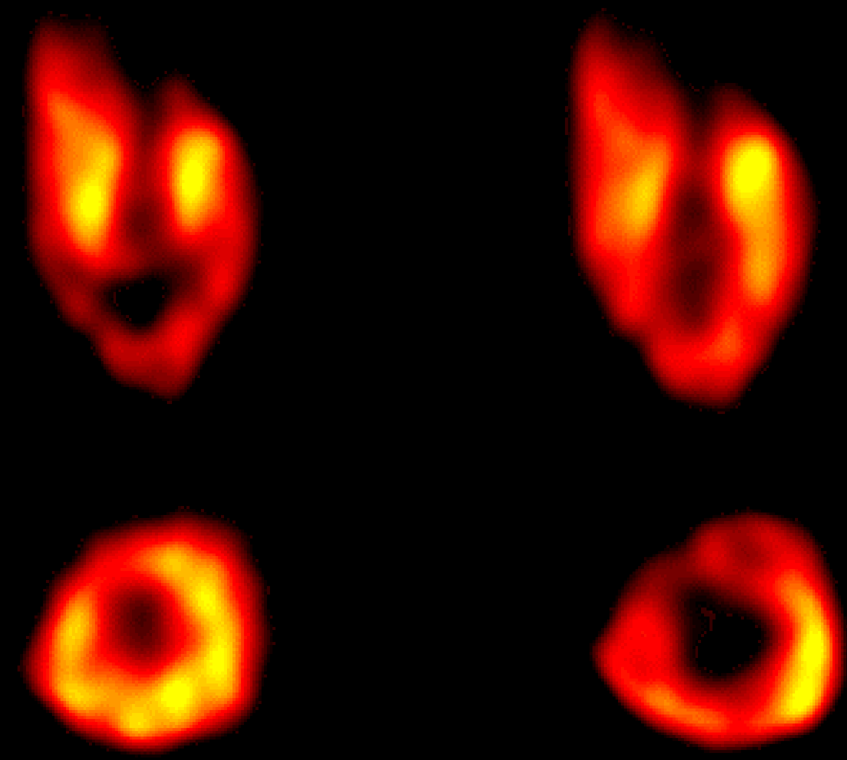
Proj., 1024; gradient, 10 G/cm; acq. time, 51.6 min; resolution, 100 mm.

3D Image of a rat heart perfused with glucose char



3D EPR image of an ischemic rat heart infused with glucose char suspension oximetry label. **A**: Full view **B**: A longitudinal cutout showing the internal structure of the heart. Ao, aortic root; C, cannula; PA, pulmonary artery; LM, left main coronary artery; LAD, left anterior descending artery; LV, left ventricular cavity.

Gated Imaging of Rat Heart



Transverse slices

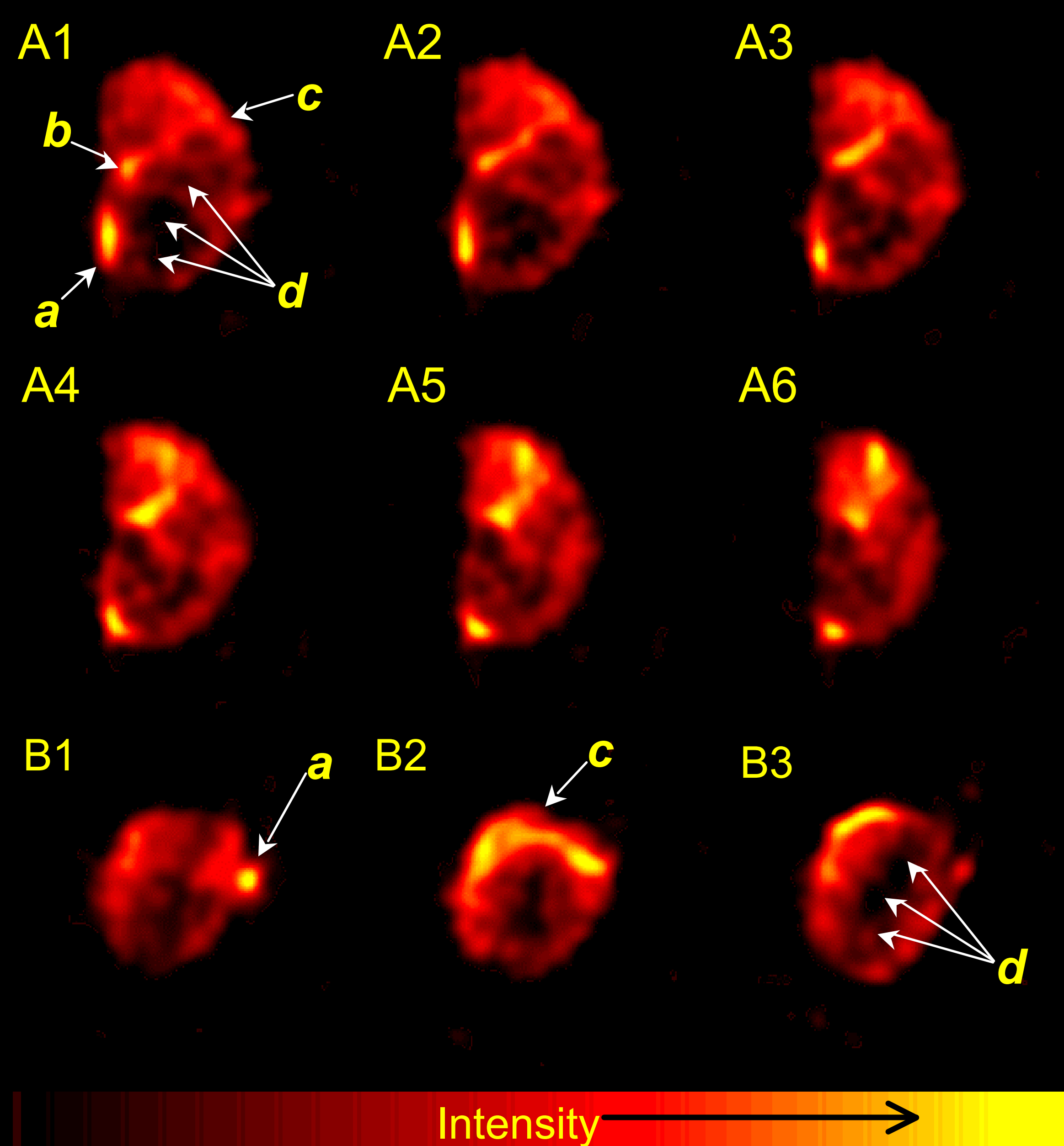
Longitudinal slices

systolic

diastolic

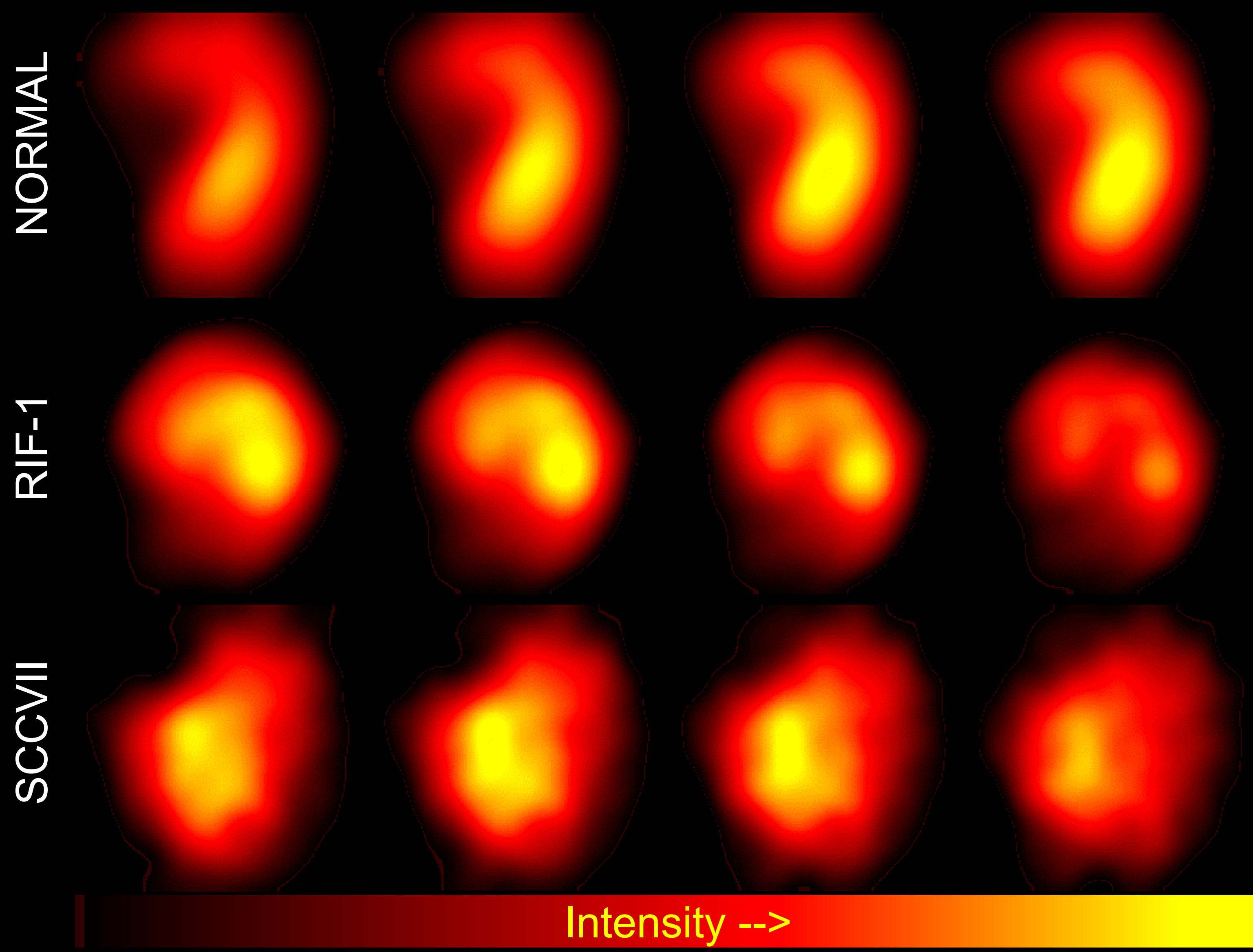
IMAGING OF RAT KIDNEY PERFUSED WITH TAM

Representative slices ($24x24\ mm^2$, thickness, 0.19 mm) obtained from a 3D spatial image.
A1-A6: Vertical slices
B1-B3: Transverse slices
a - cannula
b - renal artery
c - cortex
d - calyces
Proj, 1024;
grad, 25.0 G/cm;
acq. time, 76.8 min;
resolution, 200 um



3D IMAGING OF NITROXIDE DISTRIBUTION IN TUMORS

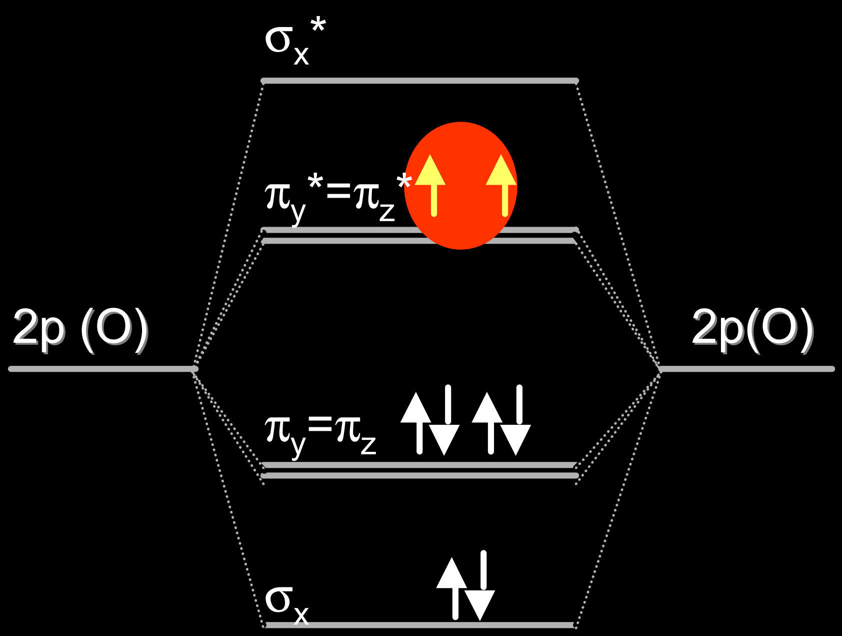
SLICES (0.3 MM) FROM 3D IMAGES OF MURINE TUMORS (3-CP; 100 mg/kg)



C3H mice; gradient 20 G/cm;144 projections;10x10 mm^2

Kuppusamy, P., et al. Cancer Research, 58, 1562-1568 (1998)

Molecular oxygen is paramagnetic



Molecular oxygen has two unpaired electrons

- Oxygen gives strong EPR signals in the gas phase
- However, no EPR spectrum has been reported for oxygen dissolved in fluids. (too broad!)
- Thus, there seems to be no possibility for direct detection of oxygen in biological systems using EPR
- However, molecular oxygen can be measured and quantified indirectly using spin-label EPR oximetry

EPR oximetry probes

Particulate (Solid) probes

Lithium phthalocyanine (LiPc)
Sugar chars
Fusinite
Coal
India ink

What is reported?

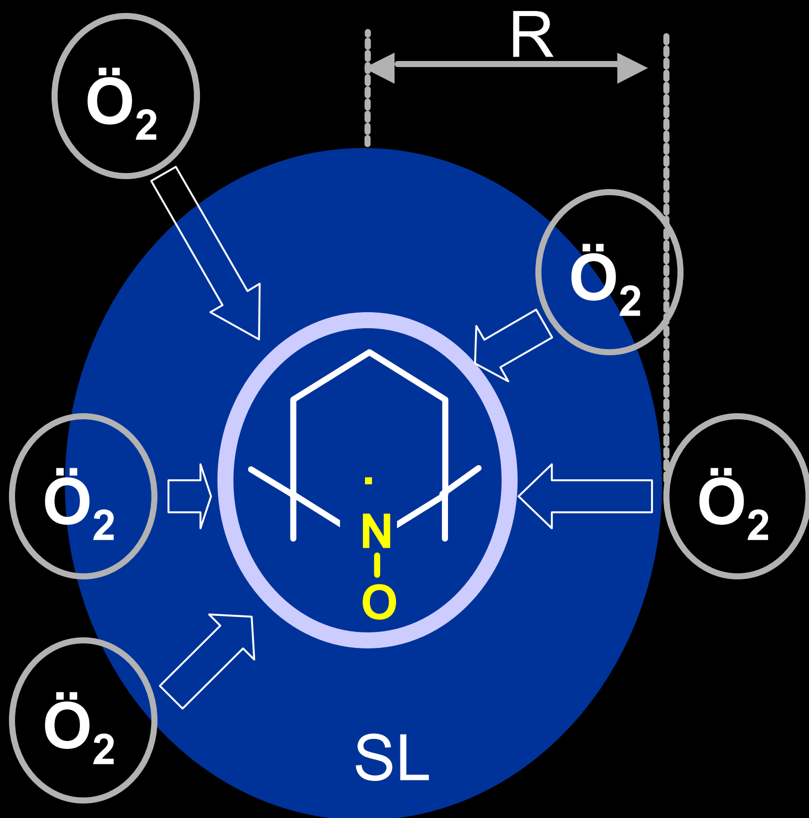
- pO_2 (mmHg/Torr)
- Localized Measurement
- Resolution < 0.2 mmHg
- Repeated Measurements
- Stable for days to weeks
- Independent of medium

Soluble probes

Nitroxides
Trityl radicals

- Concentration (μM) of dissolved oxygen in the bulk volume
- Resolution 2-10 mmHg

Principle of EPR oximetry



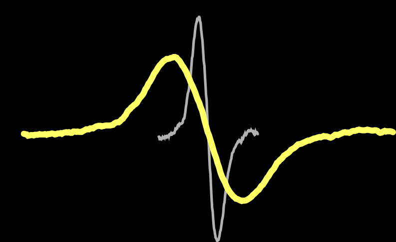
Bimolecular collision between SL and oxygen leads to Heisenberg spin exchange

The collision frequency ω, according to the hard sphere theory of Smoluchowski is

$$\omega = 4\pi R p(D_{SL} + D_{O_2})\,[O_2]$$

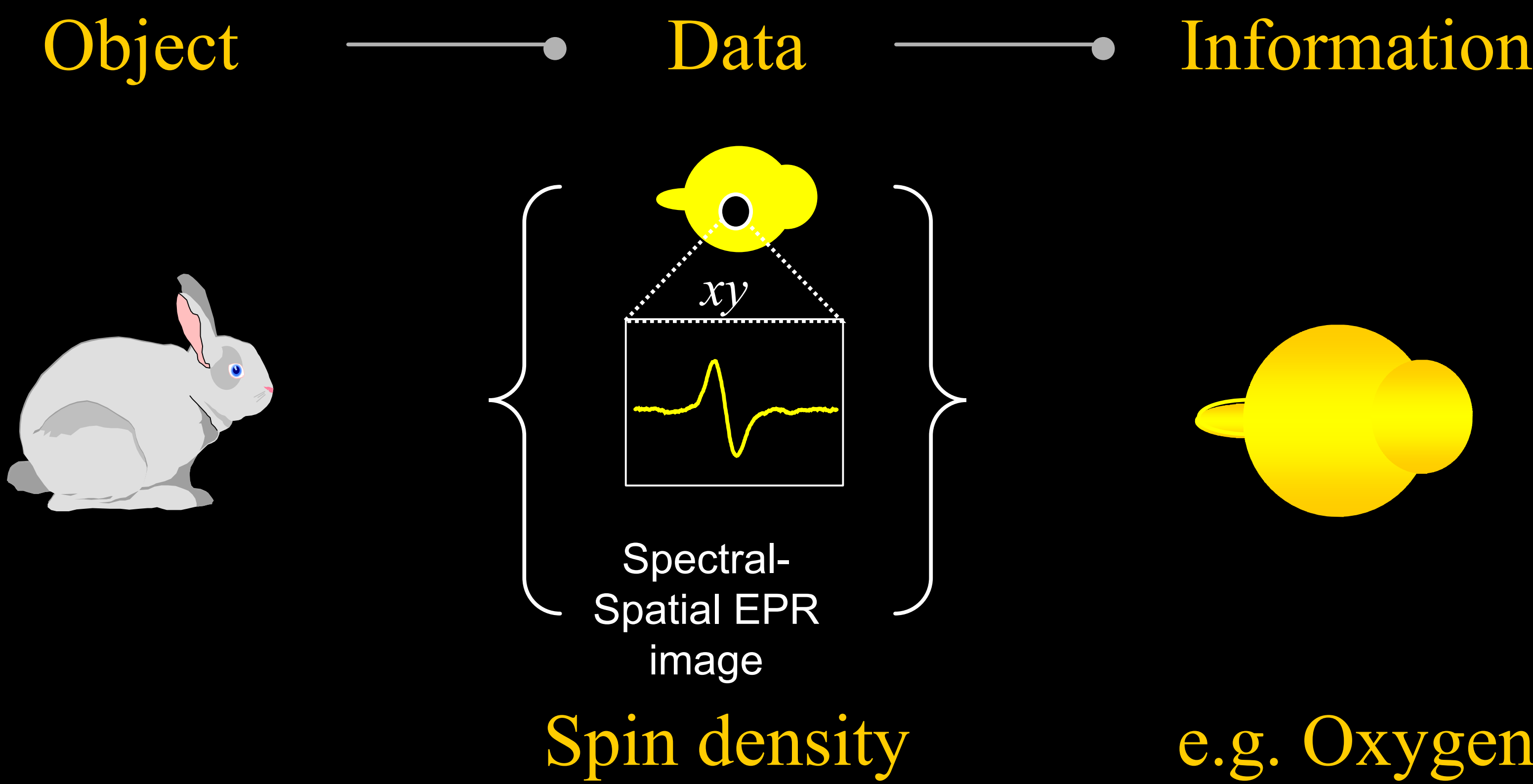
which translates to EPR line-broadening as

$$\Delta w = k\, D_{O_2}\,[O_2]$$



Mapping of Oxygen

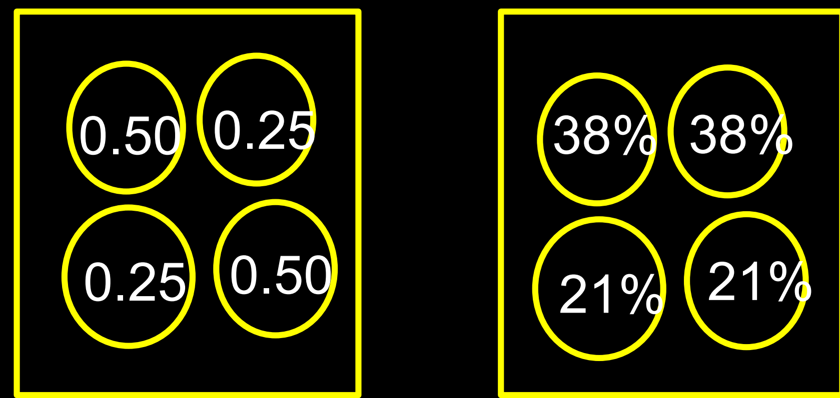
Mapping of oxygen in biological tissues is possible by spectral-spatial or ‘spectroscopic’ EPR imaging.



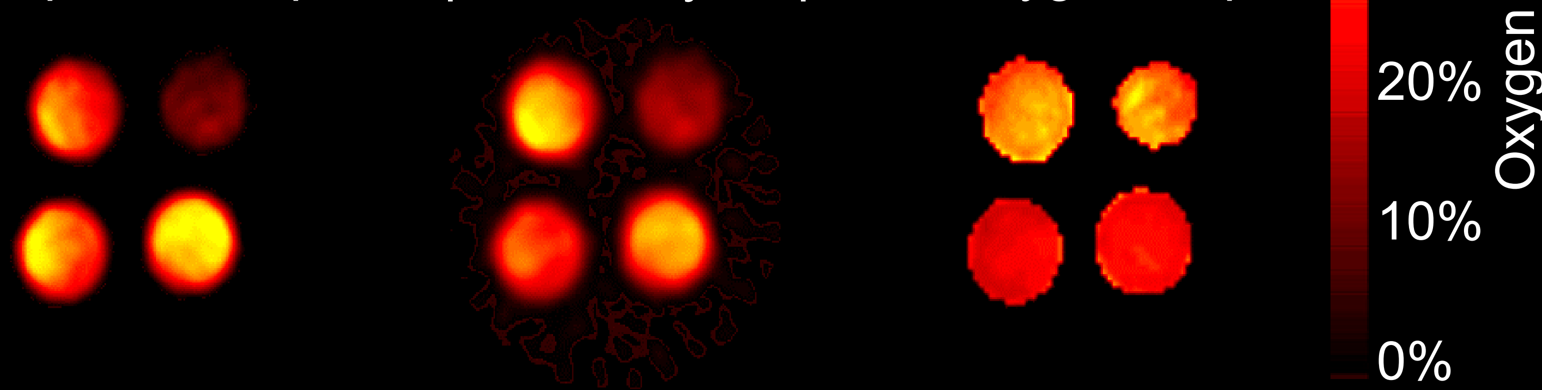
EPR Oxegen Mapping (EPROM)

Phantom
of tubes with
^{15}N-PDT

[PDT, mM] [Oxygen]

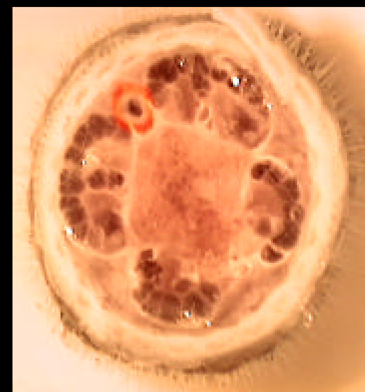


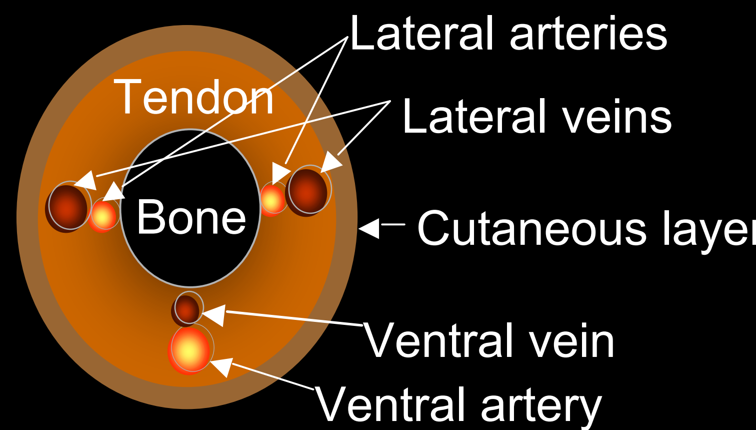
Amplitude Map Spin Density Map Oxygen Map

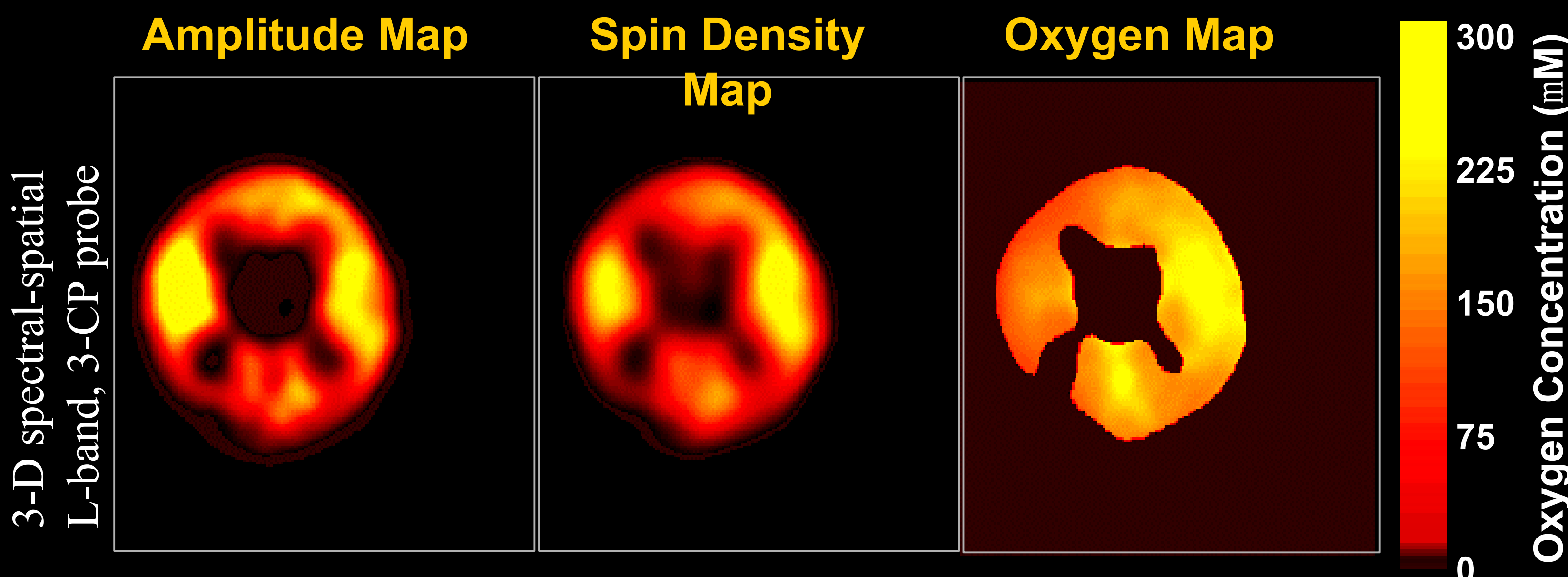


S. Sendhil Velan, R. G. S. Spencer,J. L. Zweier & P. Kuppusamy, Magn. Reson. Med. 43, 804-809 (2000)

Mapping of arterio-venous oxygenation in a rat tail, *in vivo*



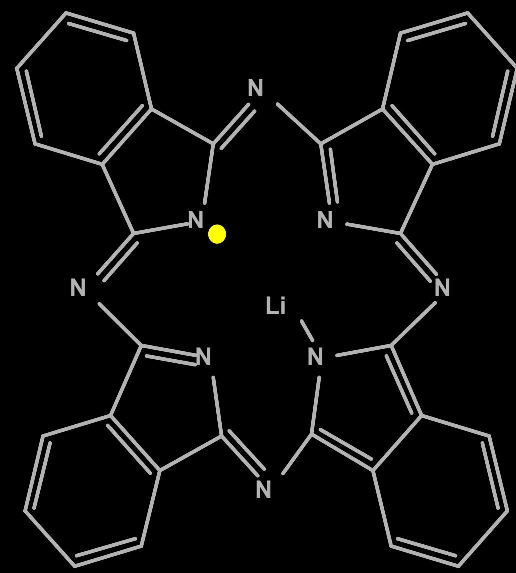


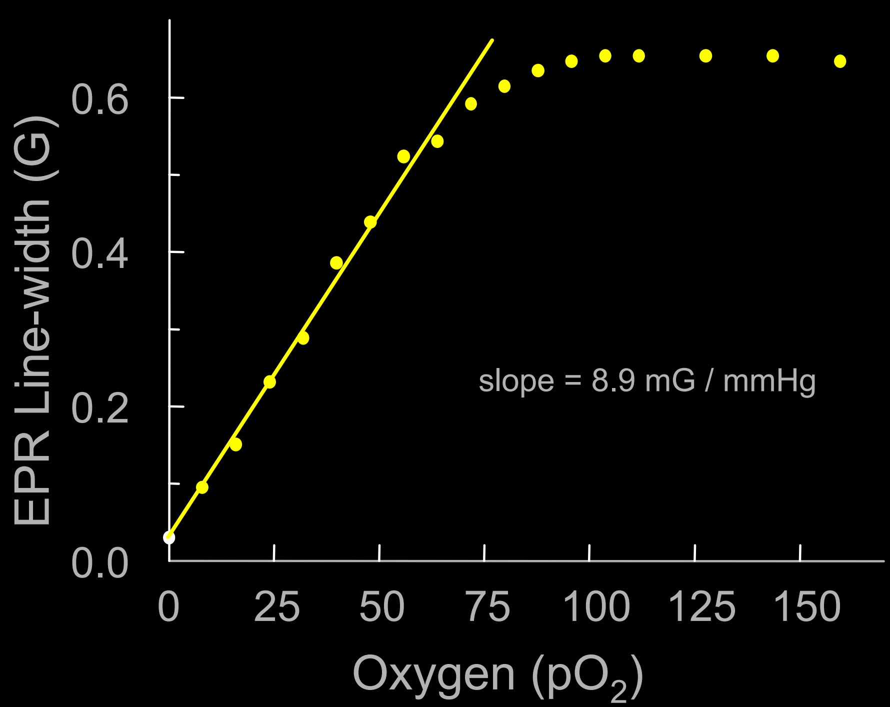


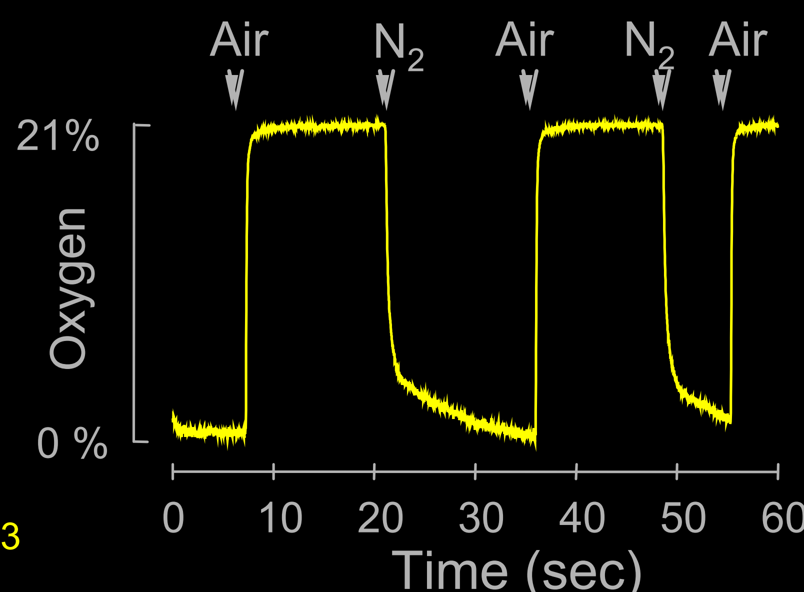
Sendhil Velan, S., Spencer, R.G.S., Zweier, J. L. & Kuppusamy P. Magn. Reson. Med. 43, 804-809 (2000)

LiPc (Lithium Phthalocyanine)

Oxygen sensitive (T_2) EPR probe

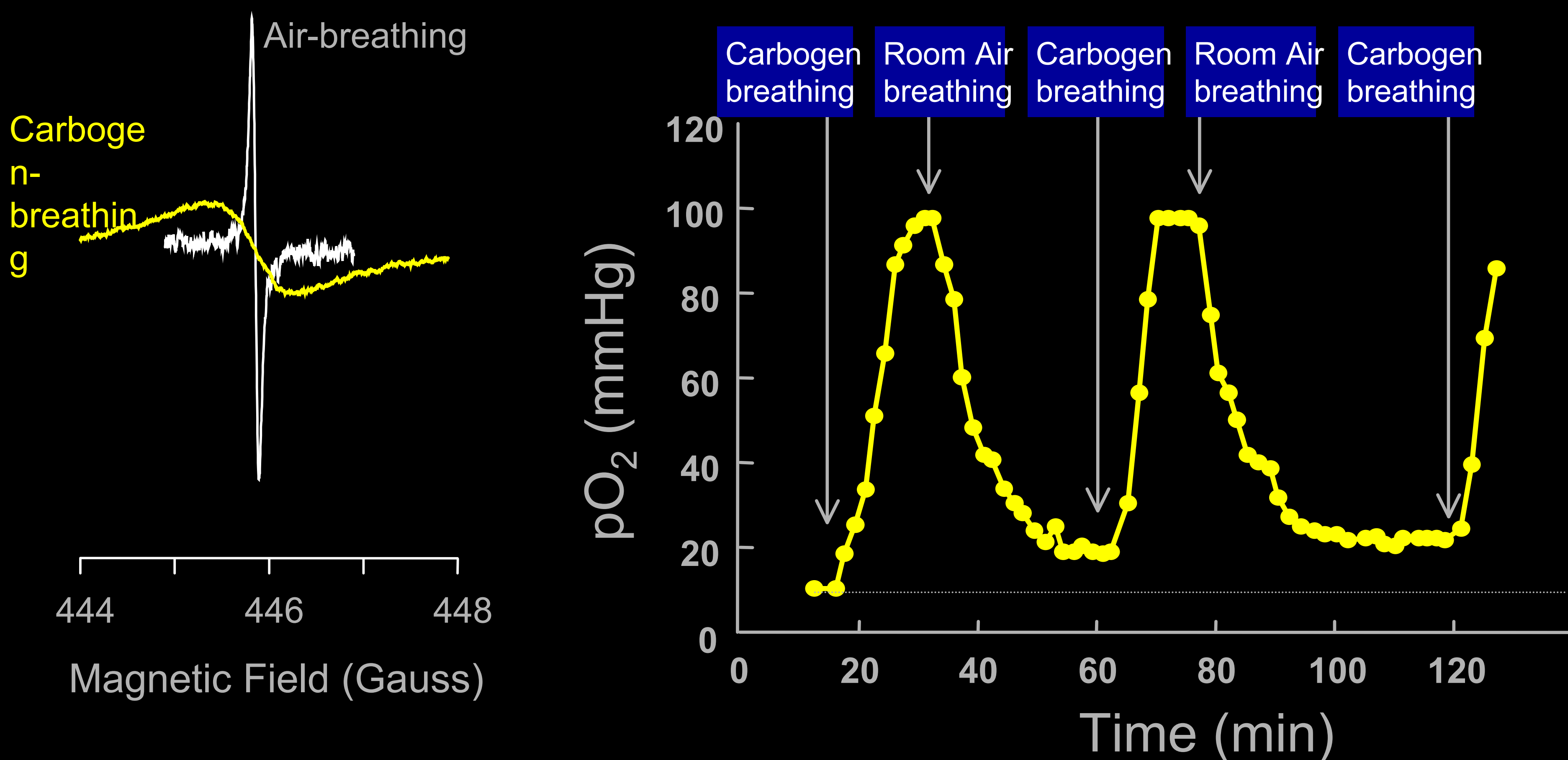




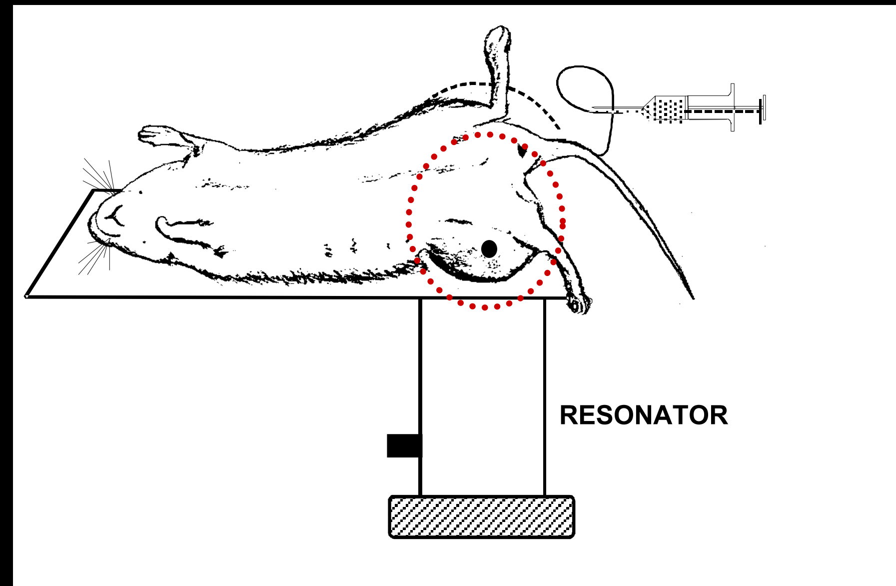


Ilangovan, G., Li, H., Zweier, J.L., Kuppusamy, P.
J. Phys. Chem. B 104, 4047 (2000); 104, 9404 (2000); 105, 5323 (2001)

Oxygenation of RIF-1 Tumor
(Carbogen-breathing)



Oxygen Measurements using LiPc, a Particulate EPR Probe



Co-imaging of the oximetry probe in the tumor tissue

Perfusion image of a nitroxide

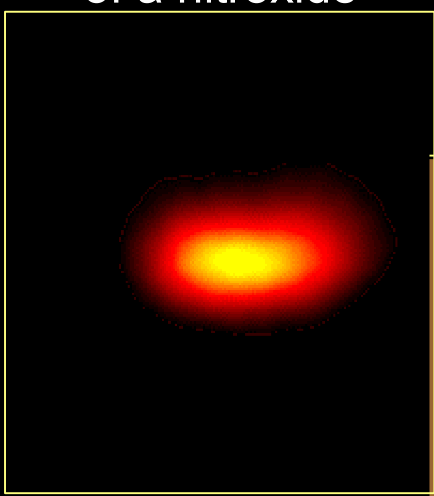
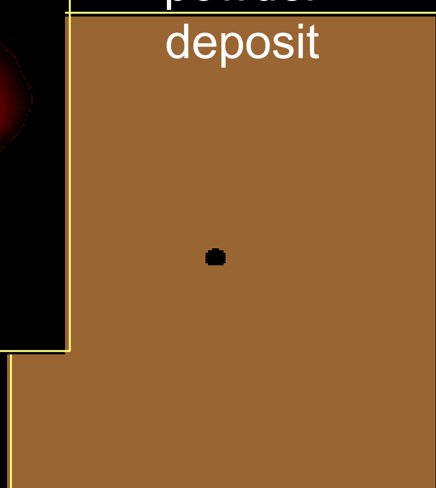
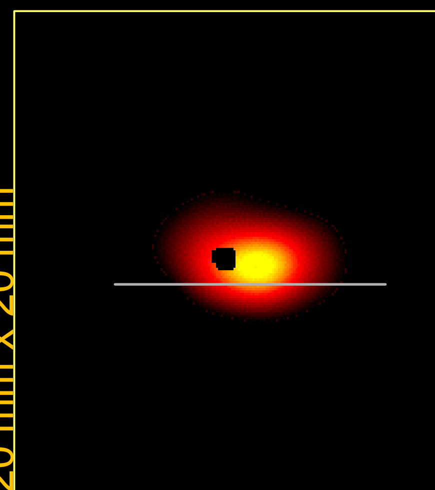


Image of LiPc powder deposit

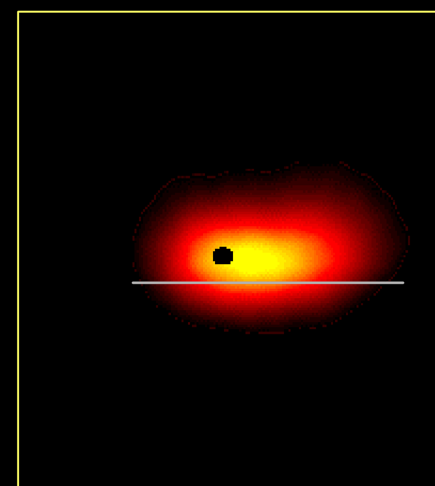


20 mm x 20 mm



Normal Leg Tissue

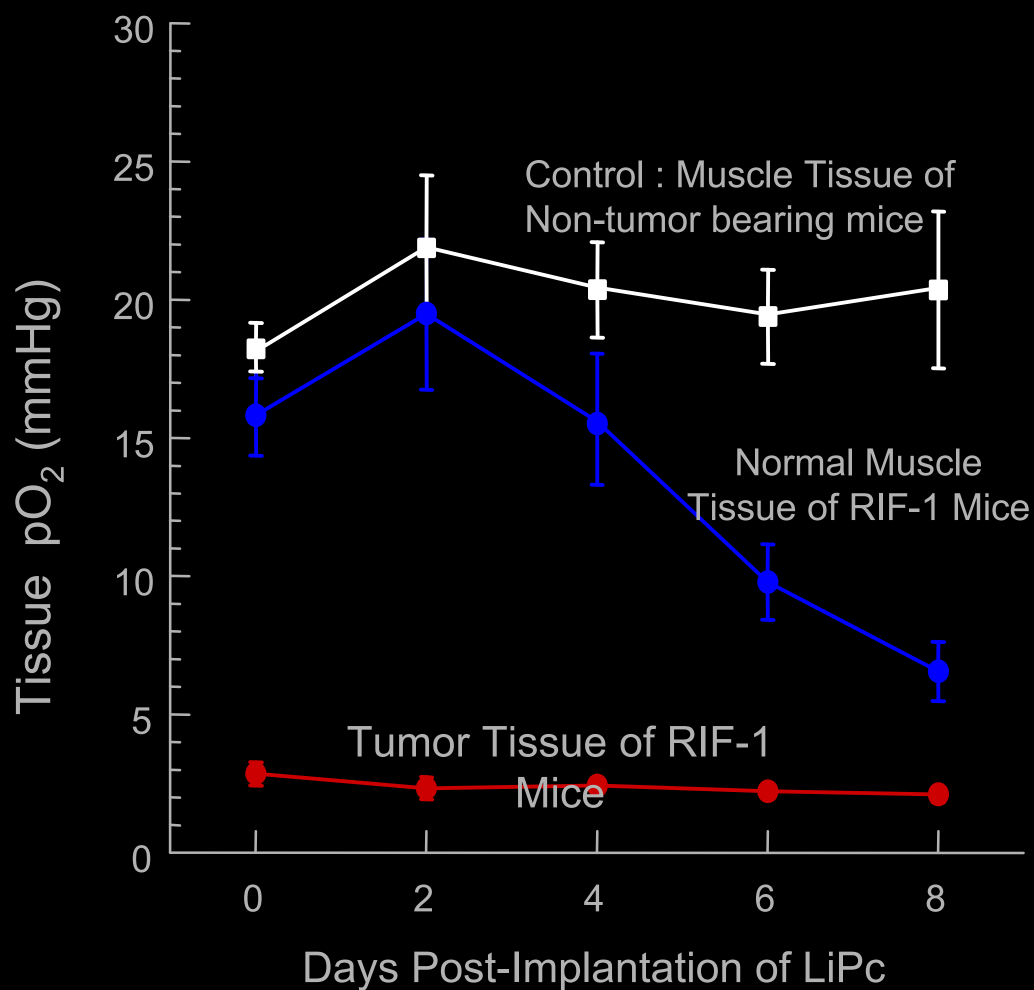
pO_2 = 17.6 mmHg



RIF-1 Tumor (14x 16 mm)

pO_2 = 4.6 mmHg

In vivo measurements of pO_2 from tumor and normal gastrocnemius muscle tissues of RIF-1 tumor-bearing mice.



LiPc particles were implanted in the tumor on the right leg and normal muscle on the left leg and tissue pO_2 values were repeatedly measured on the same animals for up to 8 days using EPR oximetry. (N = 5)

Redox Status

Redox State describe the ratio of the interconvertible oxidized and reduced form of a specific redox couple

GSSG/2GSH
$GSSG + 2H^+ + 2e^- \rightarrow 2GSH$
$E_{hc} = E_0 - (RT/nF)\log([GSH]^2/[GSSG])$
Redox State = Reduction potential *x* concentration

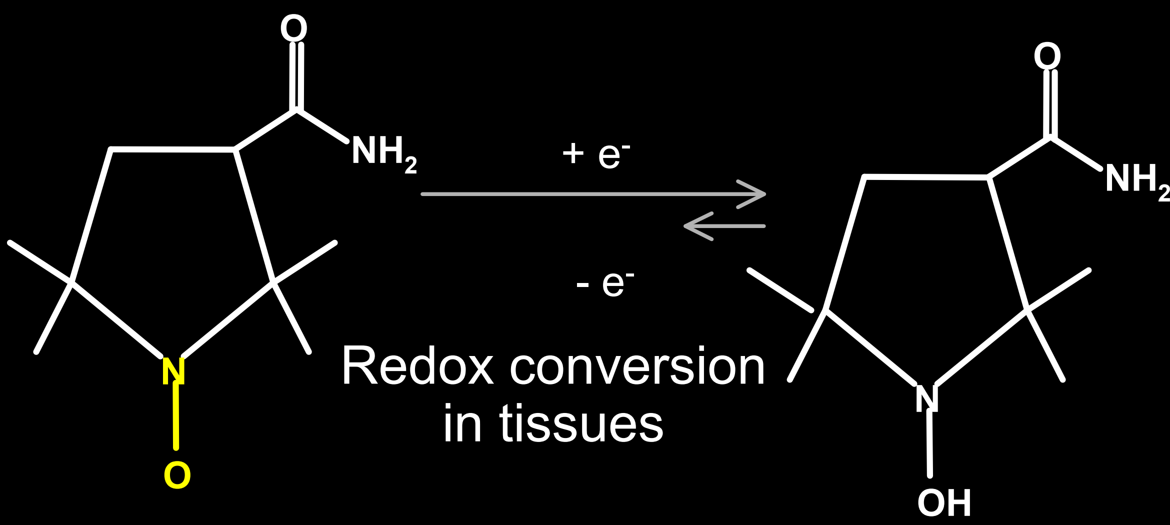
Redox Status applies to a set of redox couples.
It is the summation of the products of the reduction potential and reducing capacity of all the redox couples present

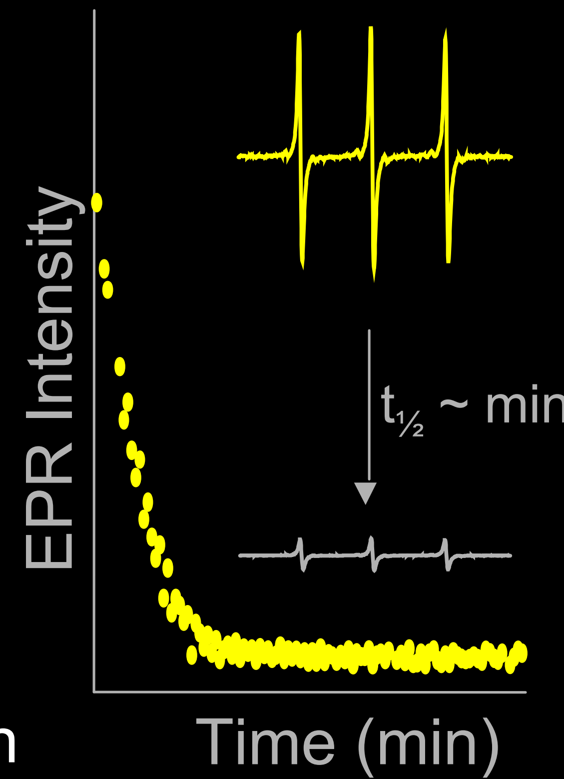
GSSG/2GSH
$NADP^+/NADPH$
$TrxSS/Trx(SH)_2$

Redox Status = Σ [Reduction potential *x* concentration]$_i$

Schaefer, F. Q. & Buettner, G. R. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic. Biol. Med. 30, 1191-1212 (2001)

Nitroxides as probes of tissue redox status



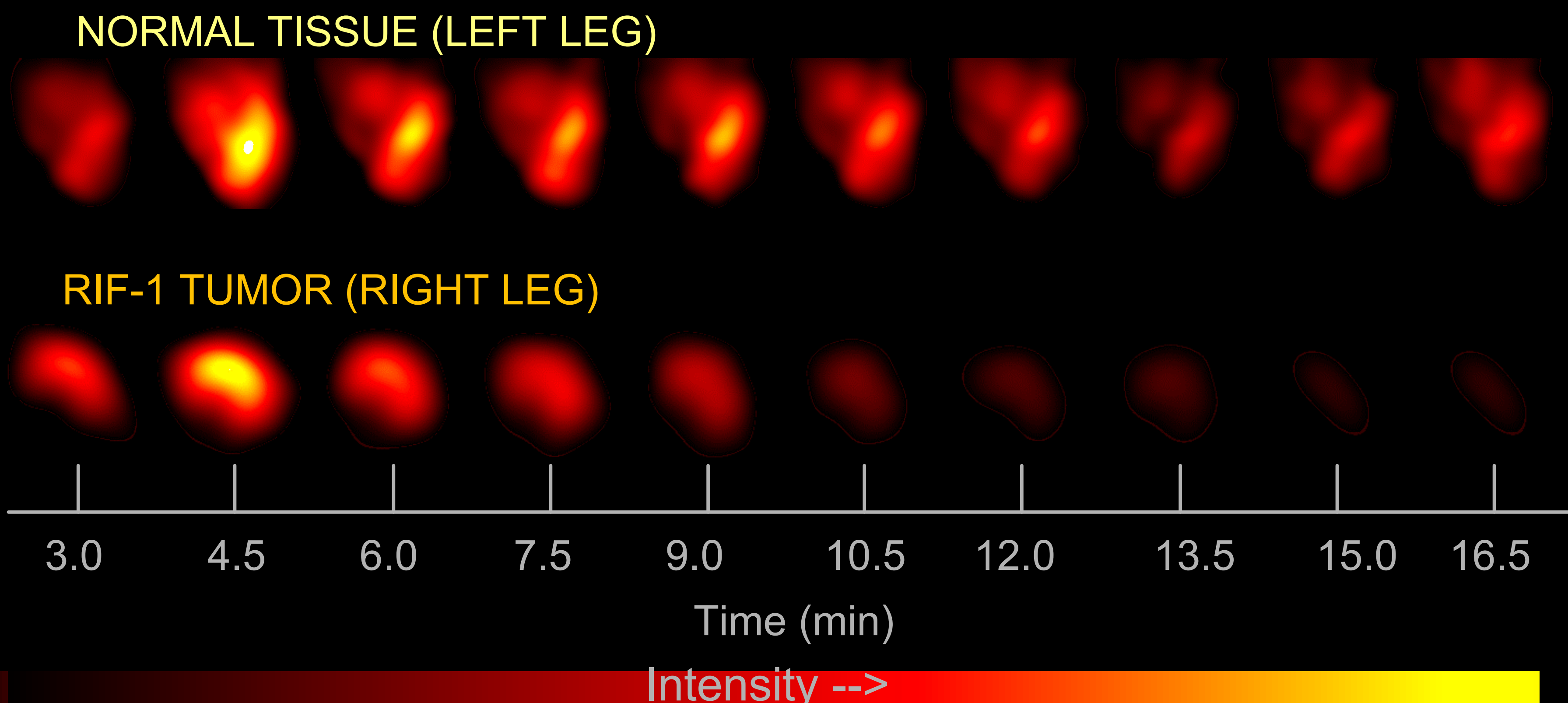


Nitroxide
EPR 'active'

Hydroxylamin
EPR 'inactive'

Swartz et al, Free Radic. Res. Commun., 9, 399-405 (1990)
Kuppusamy et al, Cancer Research, 58, 1562-1568 (1998)
Krishna et al, Breast Disease, 10, 209-220 (1998)

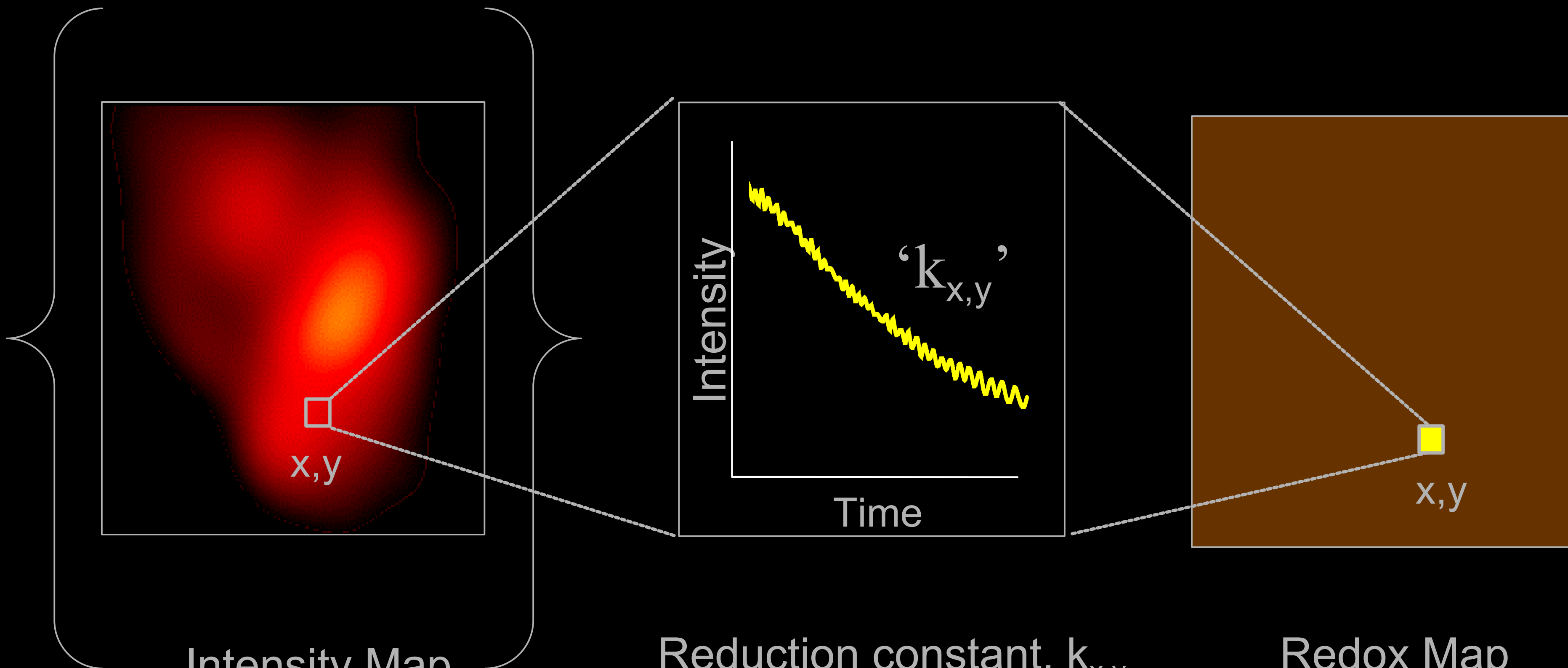
Reduction of 3-CP in the Normal & Tumor Tissue



C3H mice with RIF-1 tumor; ~30 g bw; dose: 100 mg/kg, iv; Measured *in vivo* using surface resonator at L-band (1.25 GHz); Images: 10x10 mm^2

Kuppusamy, P., et al. Cancer Research, 58, 1562-1568 (1998).

Reconstruction of Tissue ‘Redox Status’ Image



Intensity Map

[64x64]

Reduction constant, $k_{x,y}$

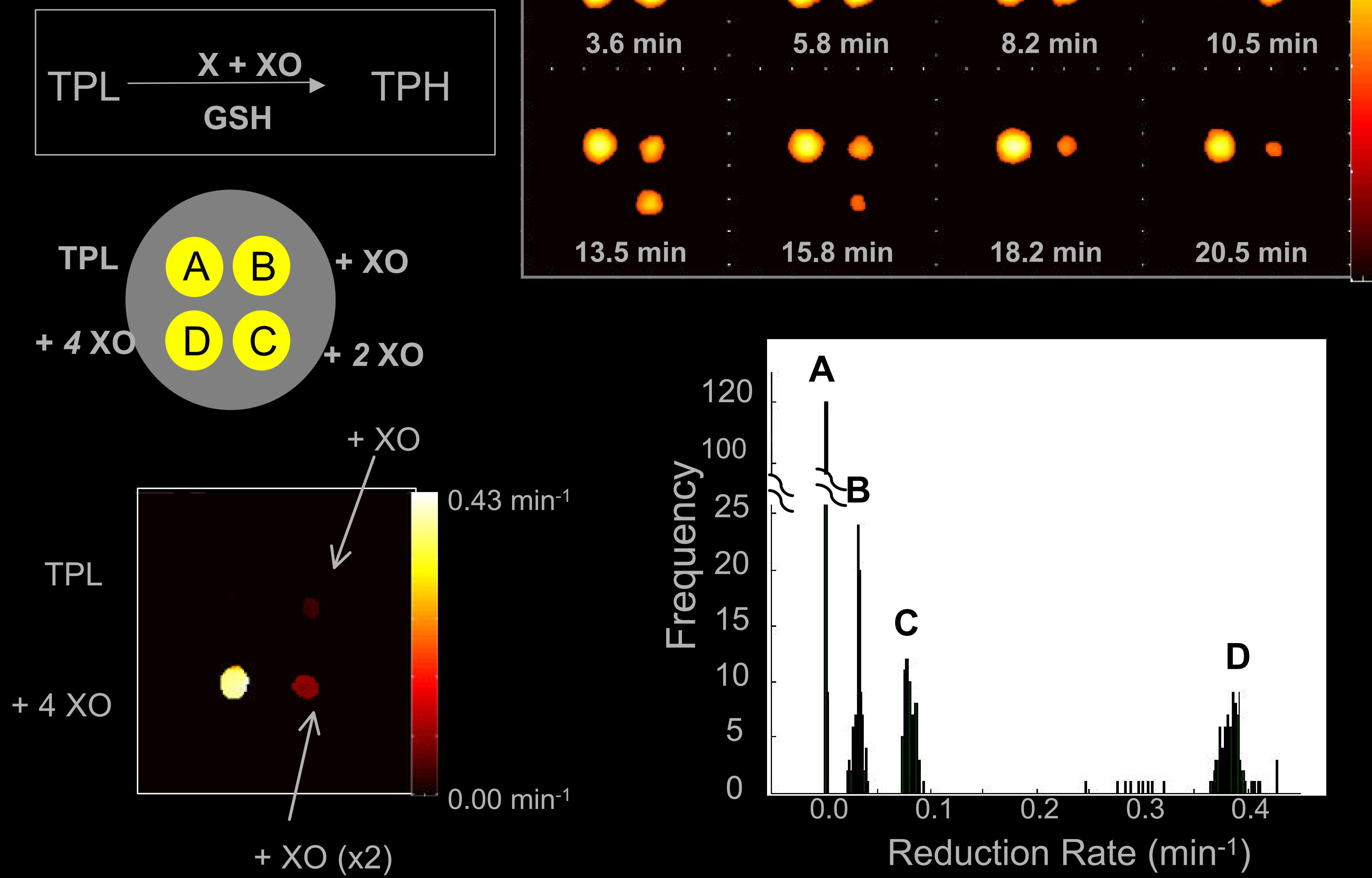
8-12 points, first order

Redox Map

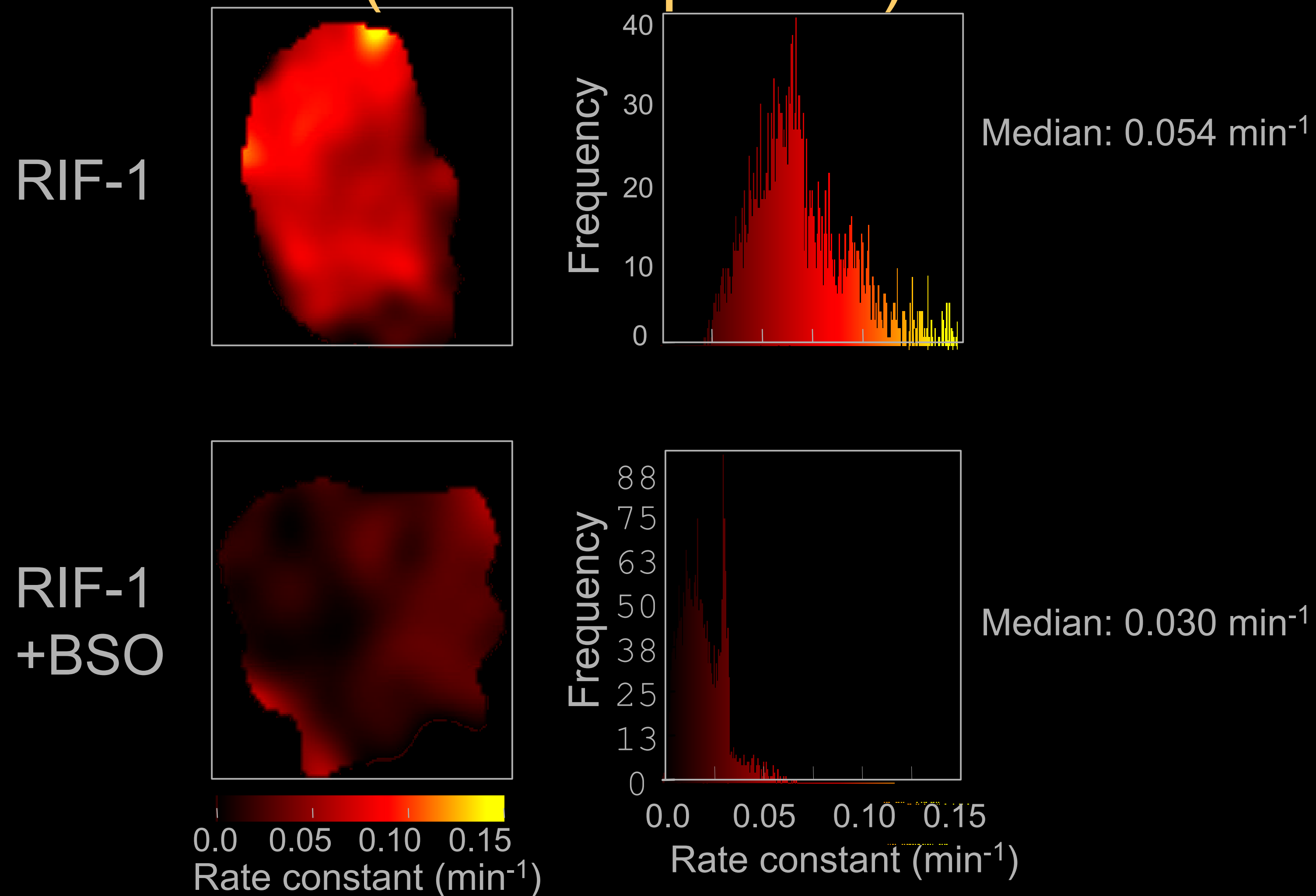
[64x64]

Kuppusamy, P., & Krishna, MC., Curr. Topics in Biophys. (2002)

REDOX MAPPING BY EPR IMAGING
(VALIDATION EXPERIMENT)

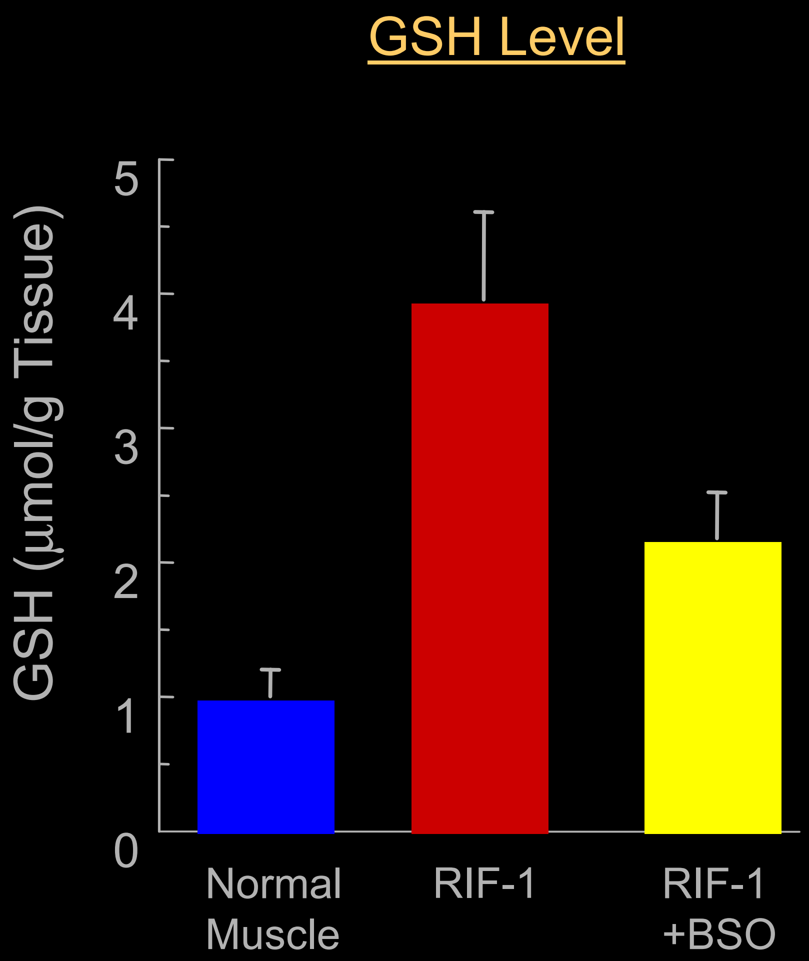


Redox Mapping of Tumor: Effect of BSO (GSH Depletion)

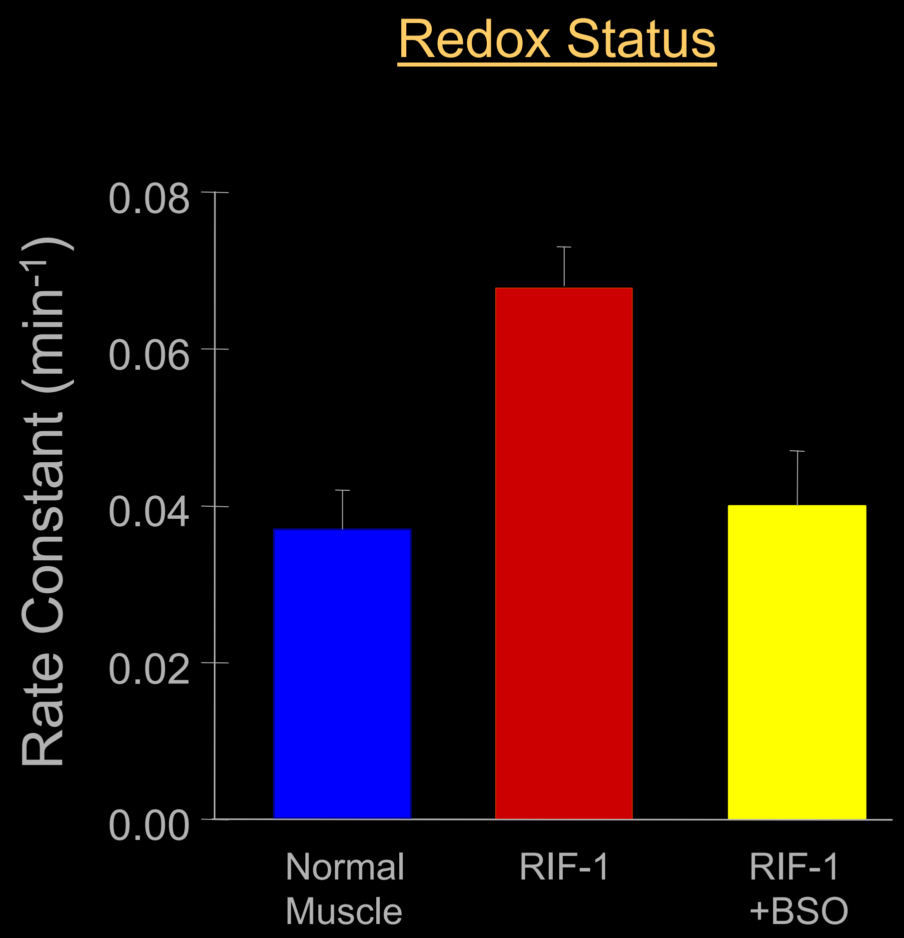


Kuppusamy et al Cancer Research (2002

REDOX STATUS & GSH LEVELS IN RIF-1 TUMOR

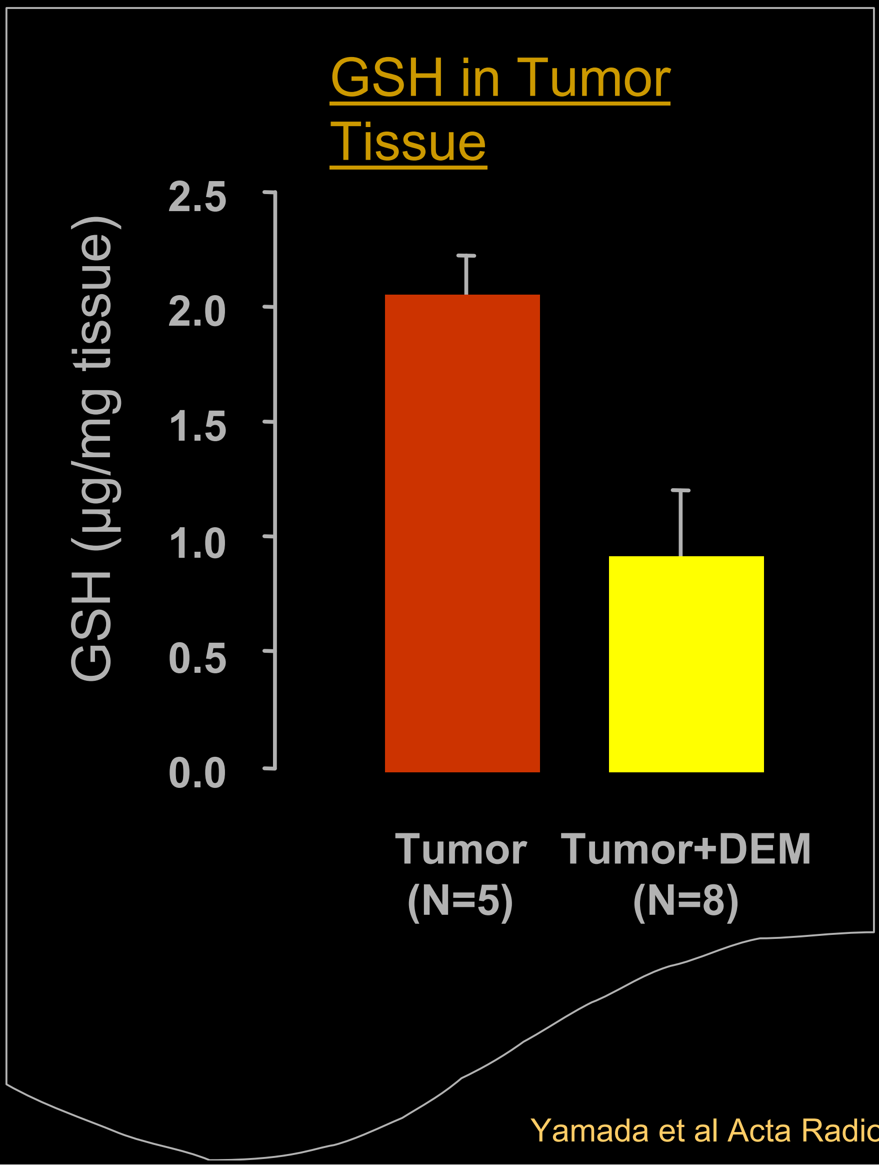


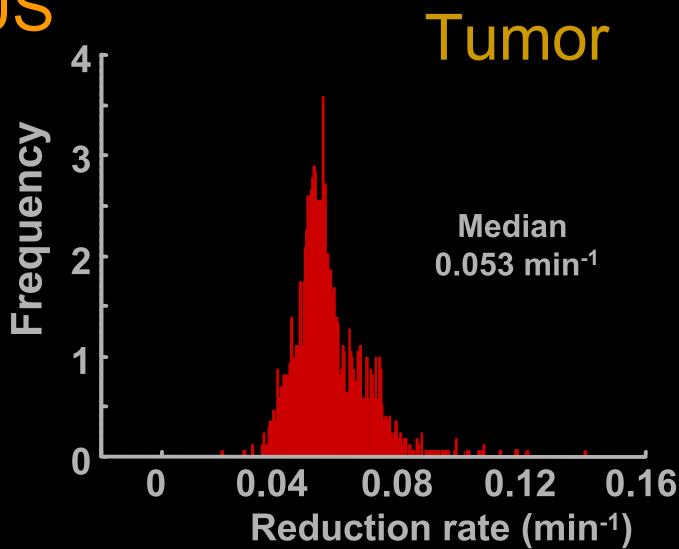
GSH levels in leg muscle (Normal) and RIF-1 tumors of untreated and BSO-treated (6-hrs post-treatment of 2.25 mmol/kg of BSO, *ip*) tumor-bearing mice. (N=7)

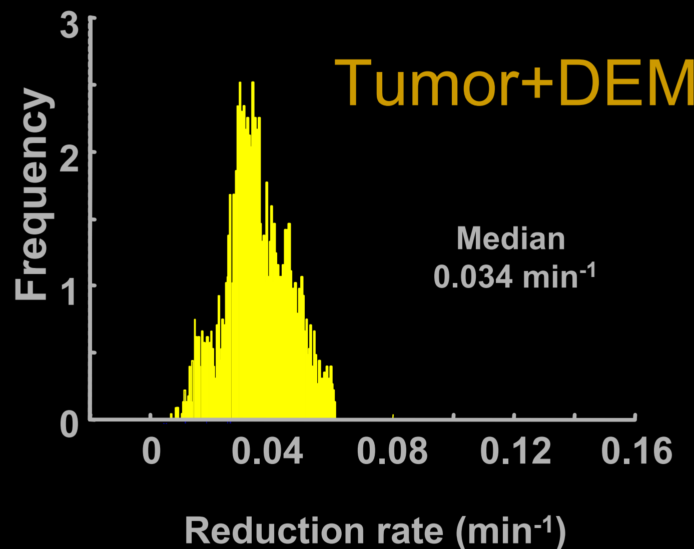


Rate constants of nitroxide clearance in leg muscle (Normal) and RIF-1 tumors of untreated and BSO-treated (6-hrs post-treatment of 2.25 mmol/kg of BSO, *ip*) tumor-bearing mice. (N=5)

EFFECT OF DIETHYLMALEATE (DEM) ON TUMOR REDOX STATUS

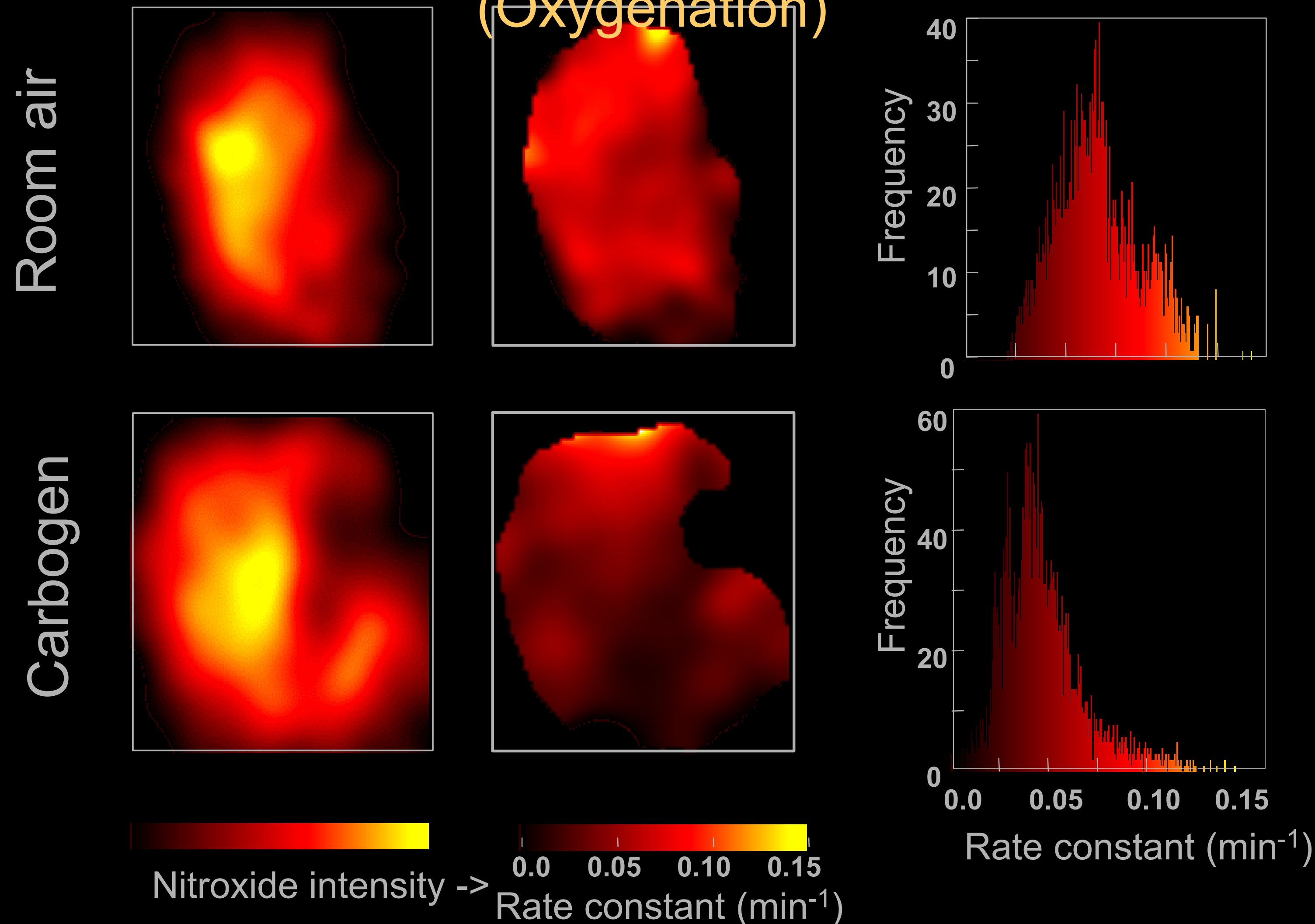






Yamada et al Acta Radiol (2002)

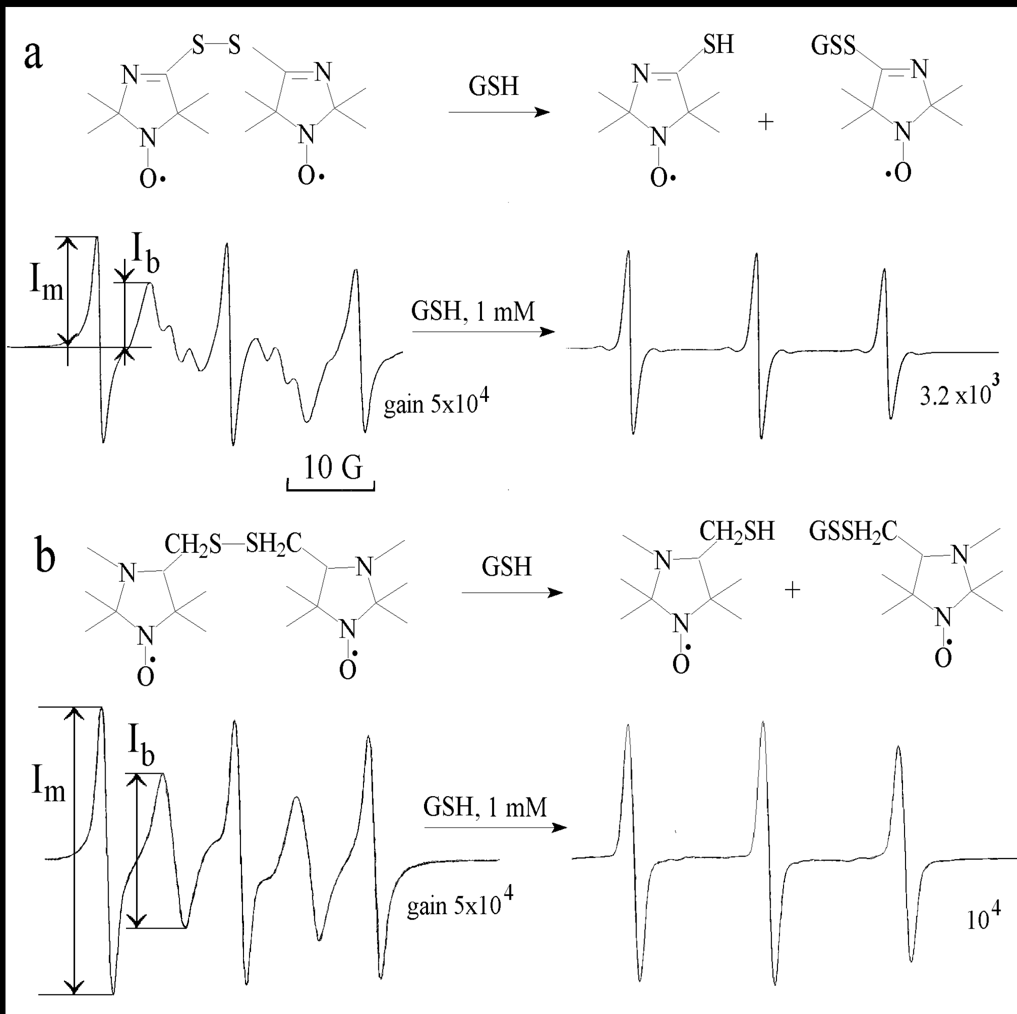
Redox mapping of tumor: Effect of Carbogen-Breathing (Oxygenation)

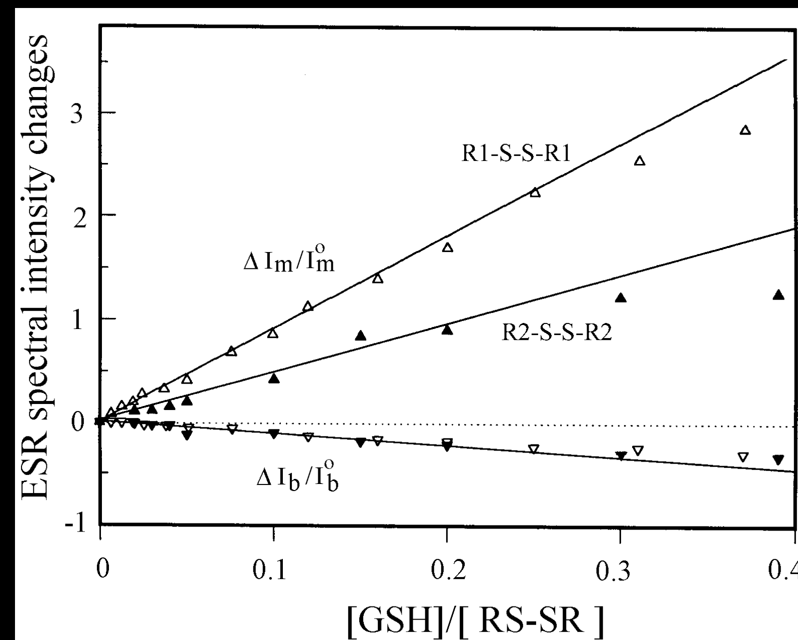


Ilangovan G., Li, H., Zweier, J. L., Krishna M. C., Mitchell J. B. Kuppusamy, P. Magn. Reson. Med. (2002))

EPR detection of SH-groups
(ESR analogs of Ellman's reagent)

Reaction with GSH





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Summary

1. EPR spectroscopy is a direct & definitive technique for detection and quantitation of free radicals and paramagnetic species.
2. Low-frequency EPR spectroscopy enables measurement of free radicals (endogenous/exogenous) in biological systems including intact tissues, isolated organs and small animals.
3. *In vivo* EPR spectroscopy and imaging methods enable noninvasive measurement and mapping of tissue pO_2, redox status and pH.

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The Illinois EPR Research Center: http://ierc.scs.uiuc.edu/

The National Biomedical EPR Center at the Medical College of Wisconsin : http://www.biophysics.mcw.edu/bri-epr/bri-epr.html

NIEHS - Spin Trap Database: http://epr.niehs.nih.gov/stdb.html