LECTURE 2

MRI AS A "BLACK BOX"

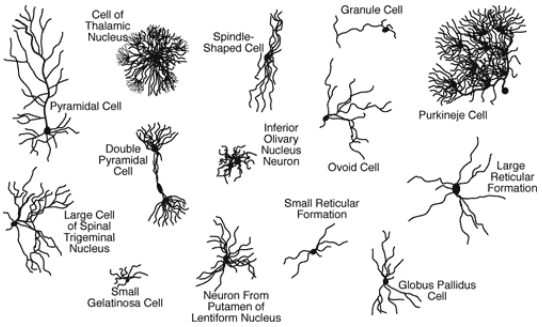
Lecture Notes by Assaf Tal

"BRAIN FACTS"

We will focus on the brain for most of this course, so it will be beneficial to discuss some elementary concepts about the brain before proceeding.

Gross appearance: The brain of an adult is about 1.3 L and weighs about 1.4 kg, giving it a density of about 1 gram/mL, very close to water.

Cellular composition: The brain is made out of two major families of cells: **neurons** and **glia**. Glia is a loose term used to join together several families of cells including **astrocytes**, **oligodendrocytes**, **microglia** and others. Neurons, the most famous cell type, conduct electricity and are supposedly responsible for our thought processes. There are many types of neurons:



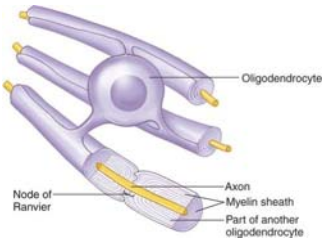
Different types of neurons.

We will usually just take a cartoon view of the neuron in which it has a simple linear structure:

(Dendrites) - (Cell body) - (Axon) - (Synapses)

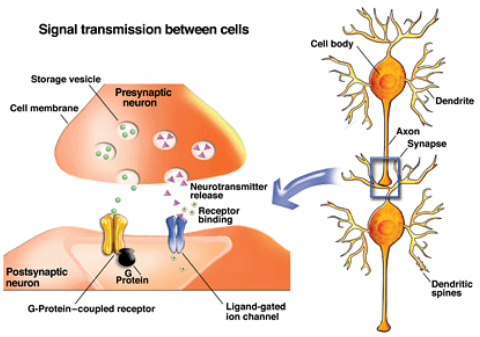
The dendrites of one neuron connect to the synapses of another neuron, where they "talk" to each other by secreting chemicals (neurons can have multiple dendrites and synapses). The axon of the neuron is usually wrapped in a fatty sheath called **myelin**, which helps it conduct electricity better.

The glial cells perform many supportive functions related to metabolism and looking after the well being of the central nervous system (**CNS** = brain + spinal cord). For example, the oligodendrocytes extend long arms like octopuses which wrap around the axos and myelinate it:

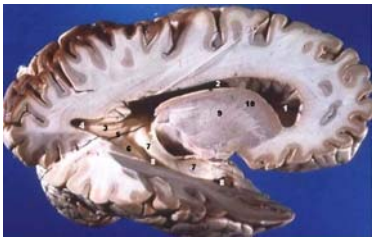


There are about 10^{11} neurons in the brain, and approximately 5 times as many glia cells. The ratio of glial to neuronal cells varies considerably between brain regions.

Neuronal signalling: neurons conduct electrical impulses, and the **synapse** of one connects to the **dendrite** of the next (multiple input neurons can feed into an output neuron). To communicate between themselves they secrete chemicals, known as **neurotransmitters** (or **neuromodulators**, if a cell sends these chemicals to large groups of neurons). There are multiple types of neurotransmitters/modulators, such as **glutamate**, γ-aminobutyric acid (**GABA**), acetylcholine, serotonin, dopamine and others. The neurotransmitters open or close ion channels, and the ions which enter the cell then continue the electrical propagation.

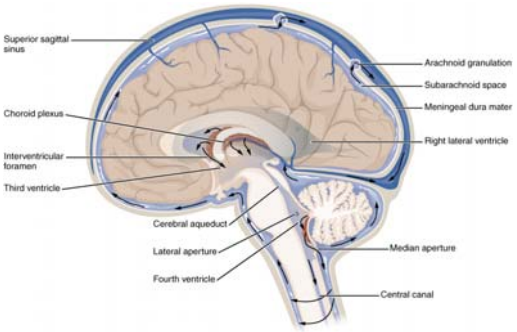


Tissue types: The brain is made out of two major tissue types, **gray matter** (GM) and **white matter**, which can be seen in the following dissected human brain:



You can think of the brain as a large switchboard of inter-connected neurons. The myelinated axons (the telephone lines) go to and fro inside the brain between aggregates of cell bodies (the switchboards). The axons constitute the white matter, which is white because fat (the major constituent of myelin) is white. The neuronal cell bodies give off a more grayish color, and are the gray matter. So, gray matter (GM) and white matter (WM) are really two parts of the same thing (neurons).

The brain floats in a fluid called the **cerebrospinal fluid** (CSF) white circulates around the brain and acts as a cushion and a source of immune agents. It also helps maintain homeostasis. It is mainly (99%) water.



"1317 CFS Circulation" by OpenStax College - Anatomy & Physiology, Connexions Web site.

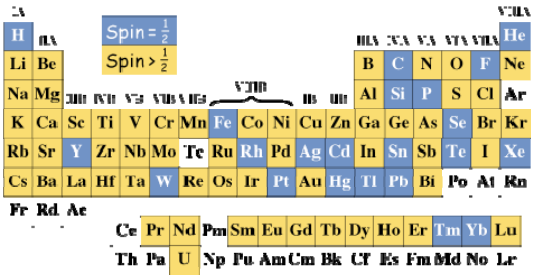
There is about 150 mL of CSF surrounding the brain and the spinal cord and it "pulses" at the same frequency as the blood in our body, albeit with a delay. CSF is continuously produced and reabsorbed. Most of you have heard of a lumbar puncture, also known as a spinal tap, where doctors insert a long needle into the subarachnoid space close to the base of the spinal cord in order to take a sample of the CSF in certain diseases.

What Can MRI Show Us?

MRI "Sees Atomic Nuclei"

Molecules are made out of atoms, which are made out of electrons orbiting a nucleus. In some atoms, the nuclei have an intrinsic property called **spin**. This is the intrinsic angular momentum of the particle, *as if* it were a spinning ball of charge (although it isn't). It is a fundamental property of nature just like charge and mass. Those nuclei with non-zero spin give off detectable MRI signals, and are called **MRI-active** or **NMR-active**.

Spin is measured in units of angular momentum (Joule·sec) and appears only in half-integer multiples of $\hbar \approx 1.05 \times 10^{-34}$ Joule $\cdot$ sec, **Planck's constant**. When one says a nucleus has spin-5/2 they really mean the nuclear spin is $\frac{5}{2}\hbar$. There are plenty of online tables of elements that show you which atoms have nuclei with non-zero spin:

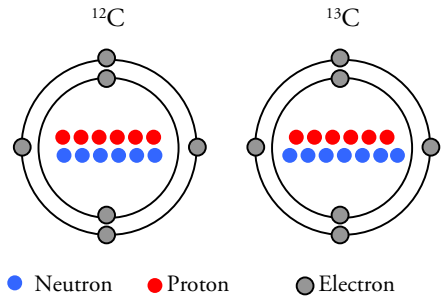


There is a fundamental difference between spin-1/2 those with larger spin in that spin>(1/2) their nuclei have an non-spherical charge distribution, which in turn makes them much more difficult (but not impossible) to see for reasons we won't go into now.

An **isotope** of an element is one having the same number of protons but a different number of neutrons in its nucleus. One usually write the total number of protons and neutrons on the top-left corner of the element's letter when discussing isotopes:

$$^{\text{protons + neutrons}}\text{X}$$

As an example, consider to isotopes of carbon:



Two cartoon representations of ^{12}C (left), which has no nuclear spin, and ^{13}C (right), which has a nuclear spin of 1/2.

The **natural abundance** of an isotope expresses its relative abundance on Earth. For example, carbon comes in two flavors in nature: ^{12}C (~99% natural abundance) and ^{13}C (~1 %). It is important to realize that not all isotopes of a given element have nuclear spin: ^{12}C has none, while ^{13}C has a spin-1/2 nucleus.

If a nucleus has a nuclear spin it can be picked up with MRI. So one can speak of "^{13}C-imaging" or "^{1}H-imaging" or "^{31}P-imaging", but not "^{12}C-imaging". Sometimes one speaks informally of "carbon imaging", taking for granted the audience understands they're referring to the only carbon isotope that can be picked up with MRI. When one speaks of "proton imaging" they mean ^{1}H imaging.

Two nuclei give off a signal twice as large as one nucleus, so the MRI signal is proportional to the number of nuclei per unit volume:

$$\text{MRI Signal} \propto \text{Nuclear Density}$$

Element	Fractional mass of Body	Atomic percent in Body	Isotopes (Natural Abundance, %)	Isotopic Percent in Body	Spin ($\hbar$)	Gyromagnetic Ratio (kHz/mT)
Hydrogen	0.1	62	^{1}H (99.99)	61.99	½	42.576
			^{2}H (0.01)	0.62	1	6.536
Oxygen	0.65	24	^{16}O (99.76)	23.94	0	N/A
			^{17}O (0.04)	0.96	-5/2	-5.772
			^{18}O (0.2)	4.8×10^{-2}	0	N/A
Carbon	0.18	12	^{12}C (98.93)	11.87	0	N/A
			^{13}C (1.07)	0.13	½	10.705
Nitrogen	0.03	1.1	^{14}N (99.63)	1.096	1	3.077
			^{15}N (0.37)	4×10^{-3}	-½	-4.316
Calcium	0.014	0.22	^{40}Ca (96.94)		0	N/A
			^{42}Ca (0.647)		0	N/A
			^{43}Ca (0.135)	3×10^{-4}	-7/2	2.867
			^{44}Ca (2.086)		0	N/A
			^{46}Ca (0.004)		0	N/A
			^{48}Ca (0.187)		0	N/A
Phosphorous	0.011	0.22	^{31}P (100)	0.22	½	17.235
Sulfur	0.0025	0.038	^{32}S (94.93)		0	N/A
			^{33}S (0.76)		3/2	3.266
			^{34}S (4.29)		0	N/A
			^{36}S (0.02)		0	N/A
Sodium	0.0015	0.037	^{23}Na (100)	0.037	3/2	11.262
...	...	...	...		..	...

The most common nuclei in the human body (by atomic percent). Fractional mass refers to what fraction of the body's weight (mass) is made out of the element (between 0 and 1). Atomic percent indicates what percentage of the nuclei in the body are made of the nucleus (e.g. 62% of the body's nuclei are hydrogen). Isotopic percent in the body merely multiplies the atomic percent by the isotopic percent for each isotope. Also listed are the spin and gyromagnetic ratio.

This is an extremely important conceptual relation. Remember this is **nuclear density** and not **molecular density** or any other quantity! For example, in proton imaging, 1000 molecules of H_2O would give off twice the signal as 1000 atoms of H (all other things being equal).

Finally, an MRI-active nucleus is characterized by a **gyromagnetic ratio**, γ, which is the proportionality constant between its spin angular momentum and magnetic moment:

$$m = \gamma S .$$

where, e.g., $S = \frac{\hbar}{2}$ for protons. One sometimes denotes

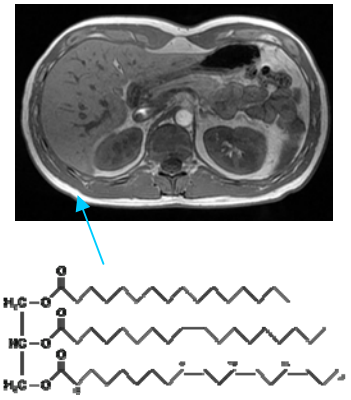
$$\not\gamma = \frac{\gamma}{2\pi} .$$

$\not\gamma$ is given in the table above in units of frequency per field strength (e.g. kHz/mT) and determines the signal strength from a particular nucleus, as well as its resonant frequency, which is also proportional to the magnetic field:

$$\nu = \not\gamma B_0 .$$

Proton MRI Mostly "Sees" Water

Most MRI images you'll see measure the signal from the hydrogen nuclei in the body. Since our body is mostly made out of water, most of the hydrogens' signal comes from H's in water (H_2O), and what we end up seeing is by far the signal of the water molecules in our body. However, while this is a good rule of thumb, it is not 100% accurate. The biggest exception is lipids. The abundance of hydrogen nuclei and the high concentration of fat molecules such as triglycerides throughout the body can be easily seen on MRI scans throughout the body. For example, the bright white strip on the exterior part of the abdominal MRI shown below is mostly subcutaneous fat:



Although it might seem at first that we are unable to resolve fat from water, there are in fact methods which we'll encounter later on for telling the two (and several other molecules) apart.

MRI Is (Almost Always) Sensitivity-Limited

The MRI signals are very weak, meaning we need a large amount of nuclei to give off a detectable signal. How many? Let's do a calculation. A typical MRI image of protons has a spatial resolution of about 1 mm^3 in the brain. The most abundant source of protons in the body is by far water (H_2O), so let's neglect all other sources of signal. The density of brain tissue is similar to that of water (~1 gram/mL), and we are made out of approximately 65% water by weight, meaning the brain is about 0.65 gram water/mL, or 0.65×10^{-3} gram water/mm^3. The molar mass of water is about 18 gram/mole, where 1 mole is about 6×10^{23} molecules. Therefore, there are about

$$\begin{pmatrix}\#\ H_2O\ \text{molecules}\\ \text{in a 1 mm}^3\ \text{voxel}\end{pmatrix} = \begin{pmatrix}\text{gram } H_2O\\ \text{per mm}^3\end{pmatrix} \div \begin{pmatrix}\text{moles } H_2O\\ \text{per gram}\end{pmatrix} \times \begin{pmatrix}\text{molecules}\\ \text{per mole}\end{pmatrix} \sim 10^{19}$$

This is quite a large number! Another interesting quantity is *how many neurons are there in a 1 mm^3 voxel?* There are about 10^{11} neurons in the brain, which has a volume of about 1300 mL, meaning there are about 10^8 neurons/mL, or ~10^5 neurons per voxel. Even with this large number of molecules or cells the MRI signal is still quite

weak. Smaller voxels will simply lack the required number of nuclei to yield adequate signal. The main issue with better spatial and temporal resolutions is sensitivity, and we therefore say that MRI is **sensitivity-limited** (as a counter-example, optical detection usually has excellent sensitivity but might be diffraction-limited due to blurring caused by diffraction of light).

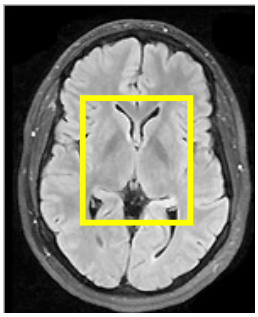
The situation is even worse for other nuclei. Let's take ^{13}C imaging as an example. Here we need larger voxels for three reasons:

1. The natural abundance of ^{13}C is only 1%, meaning our voxels will have to be ×100 as large, all other things being equal.
2. All other things are not equal! The signal a ^{13}C nucleus gives off is about 1/64 of that of ^{1}H. This has to do with its gyromagnetic ratio, which we'll talk about later, but **different nuclei give off different signal strengths**. So we need to make our voxel about 64 time larger.
3. Finally, there are about 1/5 carbons in a given volume in the human body compared to hydrogen nuclei, so we get another factor of 5.

Putting it all together, we find that our ^{13}C voxel needs to be

$$100 \times 64 \times 5 \sim 3 \times 10^5$$

larger. That's about 100 mL, or on the order of $5\text{ cm} \times 5\text{ cm} \times 5\text{ cm}$. That's quite huge!

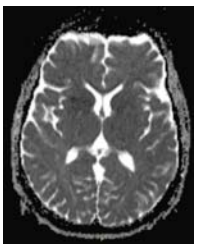


Spatial resolution of natural abundance ^{13}C imaging, drawn on top of a high resolution ^{1}H image.

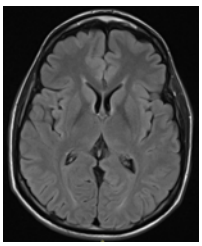
Not all nuclei are that bad, and our estimation is a bit crude, but this explains why most MRI focuses on hydrogen atoms, and specifically water, which yield the highest spatial resolution by far. **For almost all of this course we will assume we're talking about proton MRI**.

MRI Can Image More Than Just Nuclear Density By Shaping The Magnetic Fields Inside The Scanner

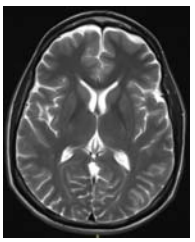
We've remarked that the MR signal is proportional to the nuclear density of the observed nucleus. However, this is only a part of the story, as the following four images show:



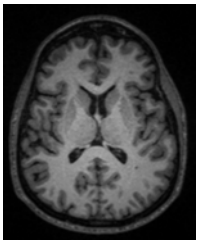
PG-GRE



FLAIR



TSE



MPRAGE

At first it seems as if the four images are alike and some software image manipulation was used to generate all four from the same source. **This is not the case**. The four were obtained by modulating the magnetic fields in the MRI scanner in a different way. Each modulation is unique and is called a **pulse sequence**, and TSE, MPRAGE, and FLAIR are the names of different pulse sequences. Pulse sequences are like a "program" that, instead of modifying bits in a computer memory, affects the physical evolution of the nuclear spins in such a way so as to bring out a particular and unique property. That is, our signal equation is:

$$S(\mathbf{r}) \propto ND(\mathbf{r}) \cdot f(A(\mathbf{r}), B(\mathbf{r}), C(\mathbf{r}), \ldots)$$

where ND is the nuclear density at point **r** and A, B, C, ... are the different microscopic properties of the spins which we'll take a closer look at in a moment. All quantities are a function of position.

By changing the pulse sequence we can affect the shape of f, the signal equation.

Definition: We say a scan is "X-weighted", where X is some imaged quantity, if f depends strongly on X, so changes in X bring out large changes in the signal.

T_1 and T_2 Imaging

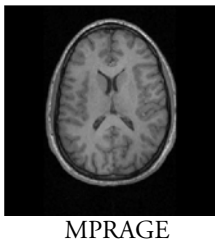
T_2 is a microscopic physical parameter of the spins that tells you how fast your signal decays due to noise introduced by thermal molecular motion. T_1 is a microscopic physical parameter that tells you have fast the nuclear spins assume thermal equilibrium. Both parameters are difficult to interpret microscopically or visually, but have to do with how "crowded" the microscopic environment is. We will delve into them in greater detail down the road.

Of the images shown above, MPRAGE is a T_1-weighted sequence and TSE is a T_2-weighted sequence. FLAIR is a T_2-weighted sequence with CSF suppression. So, while all images are proportional to the proton density, each is in addition heavily weighted by a particular microscopic parameter of the water molecules:

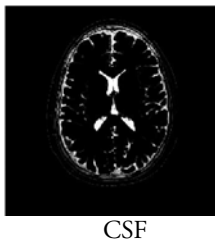
Sequence	Weighting	WM	GM	CSF
MPRAGE	T_1	"White"	"Gray"	Black
TSE	T_2	"Gray"	"White"	Bright
FLAIR	T_2 w/ CSF sup.	"Gray"	"White"	Dark

T_2 and T_1 Contrast Can Be Used For Tissue Segmentation

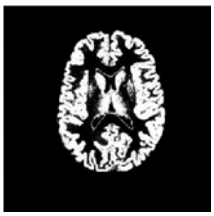
The excellent tissue contrast obtained in T_1 and T_2 weighted MRI images can be used to segment out the different tissue types: GM+WM+CSF. For example, shown below is such a segmentation based on a T_1-weighted MPRAGE:



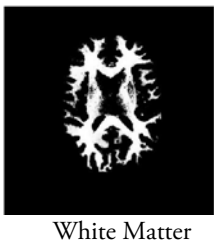
MPRAGE (T_1-weighted)



CSF



Gray Matter



White Matter

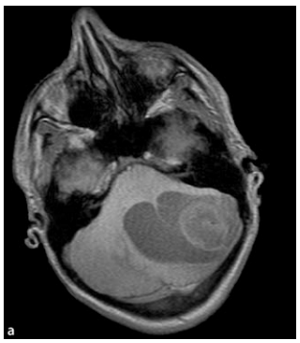
Some pathologies – Alzheimer, for example – that are characterized by degeneration of different tissue types (mostly GM for Alzheimer). Using such segmentation one can track gray matter volume over time.

T_2 and T_1 Can Vary Considerably In Pathology

The great strength of MRI comes not from its high spatial or temporal resolution but from two other sources:

1. It is capable of visualizing other microscopic parameters of the nuclei.
2. These parameters tend to vary considerably between different tissue types and under various physiological conditions, yielding excellent CNR.

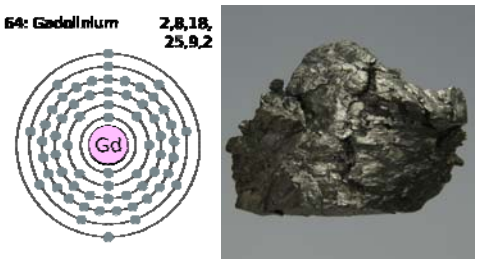
In particular, T_1 and T_2 often vary between healthy and pathological tissue, as can be seen below in an astrocytoma, a type of malignant and lethal brain tumor affecting astrocytes (astro = astrocytes, cytosis = cells, oma = tumor or mass). One does not need to be a trained radiologist to know something is not quite right in the image, since the tumor stands out so clearly:



An axial T_1-weighted brain scan
of a patient with an astrocytoma.
Taken from: Reiser, Magnetic Resonance
Tomography (2008), p.249

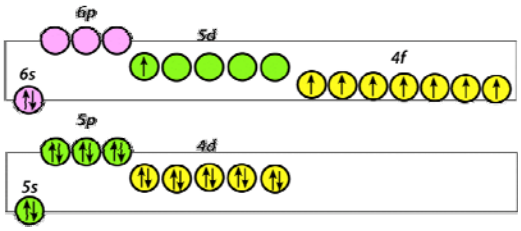
Contrast Agents Can Be Used To Shorten T_1 And/Or T_2

The T_1 and/or T_2 of tissue can be considerably shortened by injecting paramagnetic **contrast agents** into the body. **Gadolinium** (Gd), a rare earth element, is such a substance:



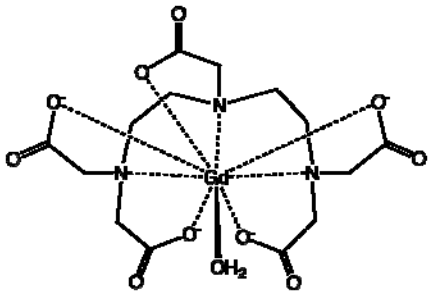
Left: Gd atom. Right: Gd, as found in nature.

Gadolinium as an atom is paramagnetic, meaning it has seven electrons with unpaired spin magnetic moments in its ground state configuration in the 4f shell:

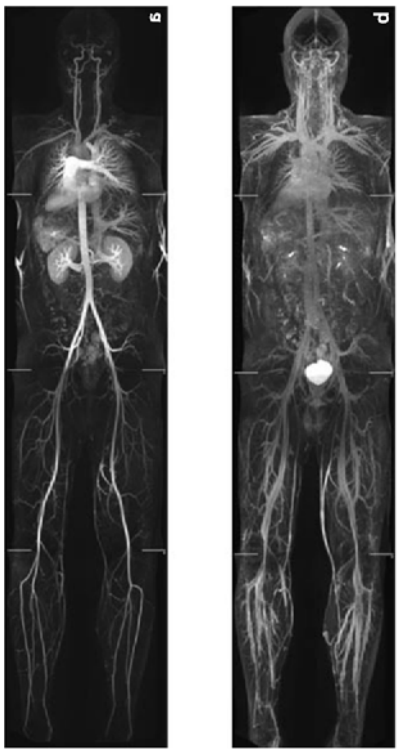


This means it gives off a very strong magnetic field when compared to the magnetic field created by the nuclear spins. Even when it forms a chemical bond it does so with electrons from $6s^2$ and $5d^1$, meaning its $4f^7$ electrons remain intact.

Often, contrast agents are toxic so they have to be **chelated** – that is, put in a molecular cage-like structure that prevents them from interacting chemically (but not magnetically!) with their environment. One such contrast agent puts a gadolinium salt inside a pentetic acid (DTPA) chelate:

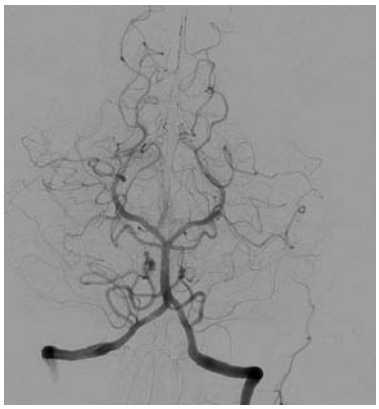


Once injected, often at about 0.1 mmol/kg, it causes significant changes in T_1 and T_2 wherever it travels, although it stays in the extracellular and intravascular space and does not permeate the intracellular space. Thus, regions with contrast agents either "light up" or "turn dark" compared to regions where these contrast agents do not permeate. A very striking effect of many of these contrast agents is to "light up" the intravascular space, yielding an image of the arteries and (after sufficient time) veins:



Left: T_1-weighted image acquired immediate after injection of 0.025 mmol/kg of Gd-DTPA, showing arteries. Right: after 10 minutes, showing arteries+veins. (From: Reiser, 2008, Magnetic Resonance Tomography)

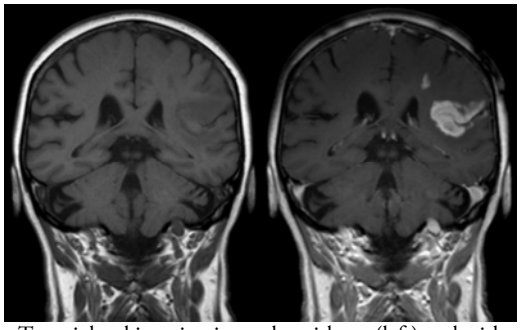
An image of the arteries is known as an **angiography**. Other modalities are also capable of acquiring angiographic images, most notably x-ray:



Angiographic x-ray image of the posterior cerebral circulation

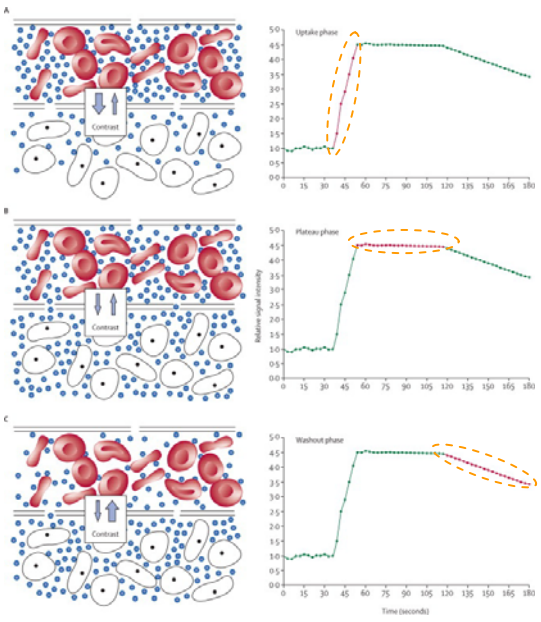
X-ray angiographies are obtained by injecting a contrast agent which are iodine based and can have serious side effects. MRI based contrast agents are somewhat safer.

One very prominent application of such contrast agents is in the imaging of stroke. Gd-DTPA is sensitive to even subtle disruptions in the **blood brain barrier**, a barrier between the brain and the rest of the body which blocks most molecules from entering the brain directly. This means that brain injuries or pathologies that cause such disruptions – notably stroke – tend to "light up" upon contrast injection.



T_1-weighted imaging in stroke without (left) and with (right) contrast administration.

There are also many studies that examine the time evolution of signal under contrast injection. These **dynamic contrast-enhanced MRI** studies (DCE-MRI) then attempt to extract physiological parameters, such as how fast a tumor might take up the contrast and release it, and reason about its malignancy and invasiveness.

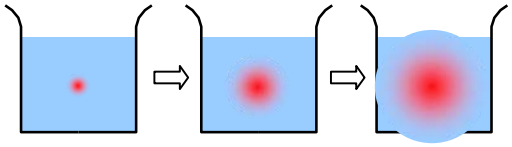


The time-dynamics of signal as contrast is taken up in the extracellular space (from: Lancet 8:63-74 (2007))

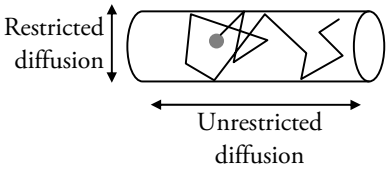
Imaging Molecular Motion: Diffusion and Tractography

The protons observed in proton-MRI belong to water molecules, and these water molecules have both **coherent motion**– e.g. the flow of blood in vessels - and **incoherent motion**, such as the **diffusion** of water molecules inside the cells in our body.

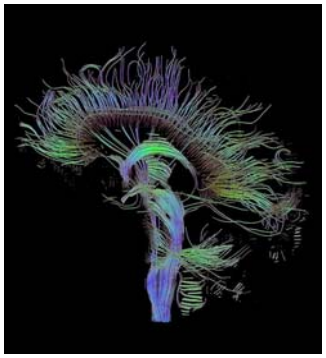
If we put a drop of ink inside a vessel containing water the ink will slowly spread out. How fast this will happen will depend on the **diffusion coefficient** (D) of the ink, and the radius of the sphere of ink will equal approximately $\sqrt{6Dt}$:



If we want to be more exact, we need to note that diffusion is a directional quantity. This means that water molecules diffusion in one direction - say, along an axon - will diffuse more freely than water molecules diffusing perpendicular to the axon:



Directional quantities are called tensors, and there are MRI pulse sequences that can be used to map the **diffusion tensor**. It is widely believed this reflects the directionality of bundles of axons in the brain. One can then deduce the directionality of the axon bundles in each point in space, in a process known as **diffusion tensor imaging** (DTI):

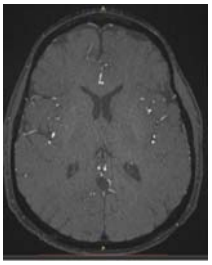


DTI

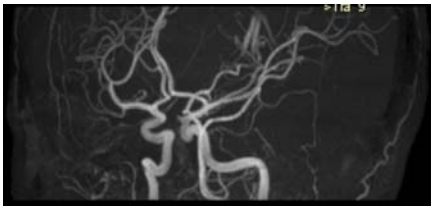
(From: Wikipedia, by Thomas Schultz)

Imaging Molecular Motion: Velocity And Angiography

Coherent motion can also be imaged: the following image has high intensity for water molecules having large velocities, in what's known as a time-of-flight image:



By acquiring axial slices from the entire brain, one can construct a 3D image (using image processing tricks) of all the arteries in the brain:



3D TOF

This is an angiogram of the brain that does not use any contrast agent.

Magnetic Susceptibility Imaging (T_2*)

When different objects are placed in an external magnetic field they tend to distort it by different amounts. The geometry of the distortion will depend on the object's shape, and its magnitude

will depend on a property of the material called its **magnetic susceptibility**. This is a dimensionless quantity, and some typical magnitudes are given in the table below:

Material	Approximate Susceptibility	Type
O_2	$+10^{-6}$	Para
Oxygenated Blood	$1.6 \cdot 10^{-6}$	Para
Deoxygenated Blood	$-4.46 \cdot 10^{-6}$	Dia
Water	-10^{-5}	Dia
Human tissues	-10^{-5}	Dia
Copper	-10^{-5}	Dia
Stainless steel	$+10^{-3}$ to $+10^{-2}$	Para
Ferromagnetic iron	$+10^{5}$	Ferro

The sign of the susceptibility usually indicates whether the material is **diamagnetic** (repelled by magnetic field) or **paramagnetic** (attracted to magnetic field).

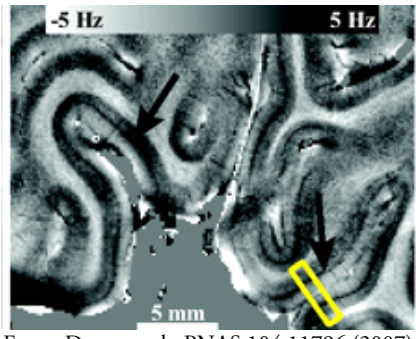
The distribution of magnetic fields within each voxel has a direct effect on another MRI parameter known as T_2^*, which can also be imaged:

$$\begin{pmatrix}\text{greater}\\ \text{susceptibility}\end{pmatrix} \rightarrow \begin{pmatrix}\text{greater}\\ \text{inhomogeneity}\end{pmatrix} \rightarrow \begin{pmatrix}\text{Shorter}\\ T_2^*\end{pmatrix} \rightarrow \begin{pmatrix}\text{Less}\\ \text{signal}\end{pmatrix}$$

Susceptibility is, in fact, not just a number but a tensor, just like the diffusion tensor, because the deformation of the magnetic field will depend on the orientation of the foreign object within the field. More advanced sequences can be used to map the susceptibility tensor as well.

T_2^* Can Be Used To Image Brain Microstructure

Magnetic susceptibility can provide excellent contrast depending on the different microstructure in different tissue types. For example, the following image of the primary visual cortex shows such T_2^* weighted images acquired at 7 Tesla with a nominal spatial resolution of $0.24 \times 0.24 \times 1.0$ mm^3 = 58 nL, showing a CNR of about 3-20 between GM and WM, almost 10-fold better than typical T_1 and T_2 weighted contrast:

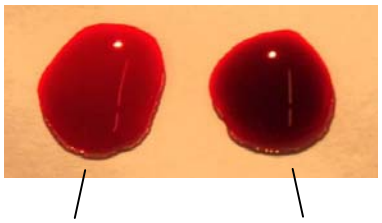


From: Duyn et. al., PNAS 104:11796 (2007)

Further contrast can be seen within GM and WM, perhaps due to vascular density, hemoglobin content, myelin content and iron concentration, factors which affect susceptibility and hence T_2^*.

T_2^* Can Be Used To Image Brain Function (BOLD-fMRI)

One of the most important effects in in-vivo magnetic resonance pertains to oxygenated vs. deoxygenated blood.



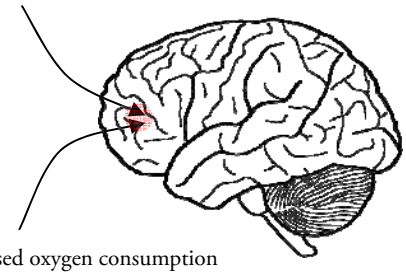
Oxygenated (arterial) blood drop: smaller susceptibility, longer T_2^*

Deoxygenated (venous) blood drop: greater susceptibility, shorter T_2^*

You can see that oxygenated blood is a brighter red (veins appear blue due to absorption by the skin, unrelated to blood. If you ever donated blood the blood in the bags appears very dark because it is extracted from the veins, not the arteries). A blood cell is about 6 μm in diameter, and on its surface are many hemoglobin molecules, which are about 6 nm in diameter. This hemoglobin binds the O_2 oxygen molecules absorbed from the lungs. Depending on whether it is bound (**oxyhemoglobin**) or unbound (**deoxyhemoglobin**) it changes its color and its magnetic susceptibility.

This change can be observed – in bulk – using MRI. This is the basis for an effect known as **Blood Oxygenation Level Dependence**, or **BOLD**, which is used to image brain function by acquiring T_2*-weighted images. When we think or perform a task that activates a particular brain region, the region consumes oxygen on the one hand, and more oxygenated blood flows in on the other hand. This changes the ratio of oxygenated vs deoxygenated blood, and as a result the signal intensity in the region.

Increased oxygen & glucose demands trigger increased cerebral blood flow (CBF) and volume (CBV) → more oxygenated blood → Longer T_2*

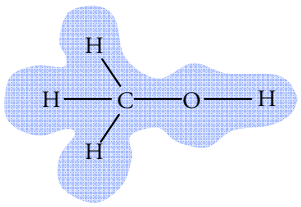


Increased oxygen consumption → less oxygenated blood → shorter T_2*

In short, there are two competing effects – oxygen is used up, but also supplied at a higher rate – the end result of which is more oxygenated blood, a longer T_2*. This change can be measured using T_2*-weighted MRI techniques, and the region itself identified. This sort of imaging is called **BOLD functional imaging** and is of great interest in neuroscience.

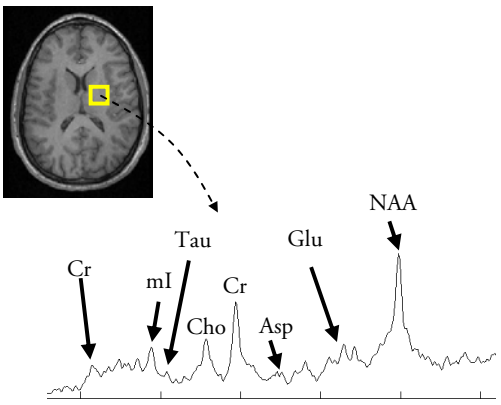
Proton-MRI Can Also See Small Metabolites: Proton Magnetic Resonance Spectroscopy (^{1}H-MRS)

We have so far assumed that proton MRI sees the protons in the water molecules. However, we can see protons in other molecules based on an effect called the **chemical shift**: The electron cloud surrounding the nucleus can be thought of as having some susceptibility and shielding the nucleus – that is, reducing the magnetic field seen by the nucleus by a tiny amount. This amount is dependent on the shape and density of the electron cloud. In other words, this depends on the particular site in the particular molecule under investigation. For example, in methanol (CH_3OH), the electron cloud around the OH proton is different from that around CH_3:



A cartoon drawing of methanol showing a different electron cloud around the different hydrogens in the molecule.

The chemical shift effect can be used to distinguish different nuclei in different molecules. This is a **magnetic resonance spectroscopy** (MRS) experiment, in which one acquires a **spectrum** from a localized voxel in the brain:

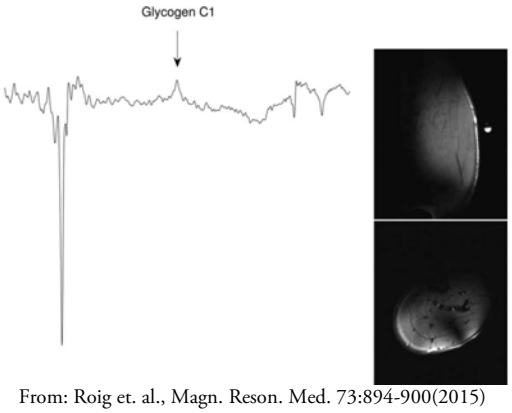


The acquired spectrum from the ~ 1 cc voxel (shown in yellow) enables us to detect several metabolites – small molecules – in the brain. These include the brain's major inhibitory and excitatory neurotransmitters, gamma-aminobutyric acid and glutamate, metabolites having to do with energy metabolism such as creatine, and markers of neuronal and astrocytic cell densities such as n-acetyl-asparate and myo-inositol.

Spectroscopic acquisitions can also include multiple voxels simultaneously, which would then be called a magnetic resonance **spectroscopic imaging** experiment (MRSI, as opposed to MRS).

Carbon-MRI Can Follow Cellular Energetics

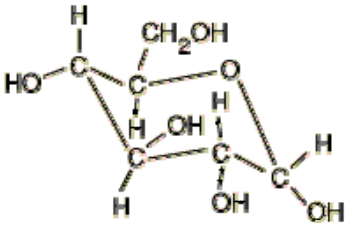
Spectroscopy is not confined to hydrogen nuclei and can be also applied to other nuclei. We bring as an example carbon imaging. Remember we've remarked that carbon voxels must be quite large, and this is indeed the case, but often we are less interested in the spatial variation of a quantity and more in its biochemistry over an entire organ/region. Such is the case in natural abundance ^{13}C-MRS of the calf muscle, shown below using a **surface coil**, which is basically a loop several cm in diameter placed on the calf, picking up a signal from the entire organ:



From: Roig et. al., Magn. Reson. Med. 73:894-900(2015)

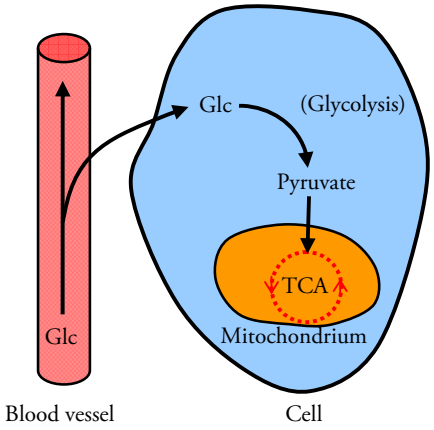
The above spectrum shows a peak corresponding to muscle glycogen, which plays an important part in muscle physiology and exercise.

The low natural abundance of ^{13}C is both a curse and a boon: on the one hand, the low steady state levels yield very weak signals. On the other hand, injection of ^{13}C in high concentrations creates excellent contrast and lets us say with confidence that the signal we are observing originated from the injected material. It is customary to infuse labelled glucose (shown below is α-glucose):

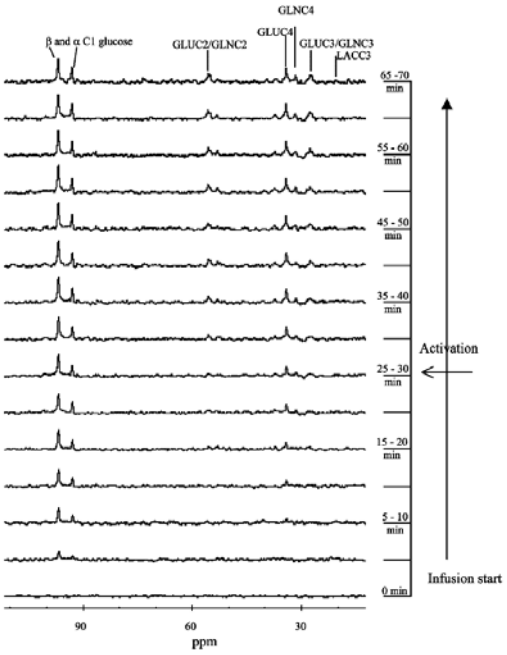


α-glucose (Glc)

Using labeling techniques, each of the six ^{12}C nuclei can be replaced with ^{13}C nuclei. Once injected, glucose gets taken up by cell and metabolized into pyruvate, which then gets taken up by the mitochondrium and used in Kreb's cycle (red circle in image below). This means the labeled carbons end up hopping from one molecule to the next. We can follow them as they do so and even determine the rate at which Kreb's cycle operates (red circle in cartoon below):



For example, Morris and Bachelard (NMR in Biomed, 16:303 (2003)) have used 1-^{13}C labeled glucose while acquiring ^{13}C spectra from the primary visual cortex (V1). 25 minutes into the infusion, a visual stimulation is applied:



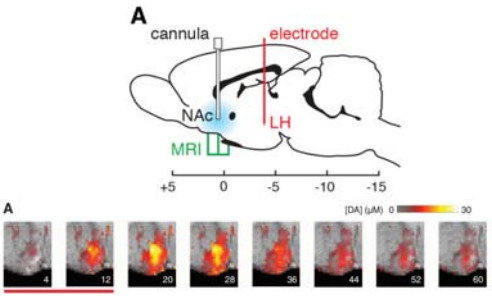
Several interesting things can be seen: before activation, peaks start building slowly up. Each peak corresponds to a different molecule because of the chemical shift effect. We see glucose levels in the brain building up, and glutamate and glutamine appear approximately 15 minutes after commencing infusion. Glutamate, glucose, glutamine and lactate all take part in some way in the TCA cycle in-vivo, and the rate at which they're synthesized – as gleaned from the ^{13}C spectra – can be used to estimate the rate at which Kreb's cycle operates. The researchers found an increase in TCA cycle rates of about 60% upon visual activation, which indicated that cerebral glucose is metabolized oxidatively even during visual activation. Such experiments have helped determine that energy supply to the brain during functional activation is oxidative (i.e. in the mitochondria) as opposed to non-oxidative (i.e. via glycolysis).

Metabolic Imaging

Proton MRI sees mostly water molecules which are not biochemically interesting. Spectroscopy can be used to observe other molecules but at the cost of severely degraded spatial and temporal resolutions. Is there a way to combine both of their advantages? The answer is sometimes yes, and it comes in the form of exogenous (administered) contrast agents.

We've previously described the use of contrast agents which alter T_1 by virtue of their presence in the intravascular and extracellular spaces. Some researchers work on creating contrast agents with a particular affinity to a certain molecule or class of molecules, we can assure that greater concentrations of contrast agent – and therefore, greater signal variations – will occur wherever these molecules exist at a high enough concentration. One such application, demonstrated by Lee et. al. (Nature 344:533, 2014), uses the BM3 protein, which is featured in intracellular electron transfer reactions, to image dopamine concentrations. Dopamine is a neurotransmitter having to do with the brain's reward system and is associated with a "good feeling" and addiction.

By using protein engineering the authors have increased the protein's affinity to dopamine, such that higher concentrations of dopamine would change the signal intensity in T_1-weighted images. The modified protein was injected intracranially into mice brains in the ventral striatum, a brain region that has to do with the brain's addiction and reward mechanisms, and images were acquired while stimulating the medial forebrain bundle using implanted electrodes, showing increased dopamine release in the extracellular space and implicating the hypothalamic circuitry in modulating dopamine responses.



From: Lee et. al., Nature 344:533, 2014

There are other similar examples, such as contrast agents that bind to fibrosis-inducing agents in the liver, to glucose in the brain and more.

What MRI Can't Show Us

We've so far discussed some things we can see with MRI, but it is equally important to stress what can't be seen.

MRI Can't See Atoms Without Nuclear Spin

We've made this point quite clearly, but it is worth stressing again: the most fundamental requirement for an atom to be visible on an MRI scan is for it to have nuclear spin.

MRI Can't See Low-Concentration Molecules

Water exists at a concentration of about 50 Molars in the brain, and we still can't get a spatial resolution better than ~ 1 mm^3 in reasonable scan times. If we want to observe lower concentration molecules we need to worsen the spatial resolution. Glutamate, for example, can be seen with proton MRI using something called spectroscopic imaging which tells apart the glutamate protons from the protons in water and other molecules. The concentration of glutamate, however, is 10 mM, roughly 1000 times smaller than water. Consequently, the spatial resolution in glutamate imaging is about 1 cm^3 (1 cm^3 = 1000 mm^3). In general, it is difficult to impossible to see anything with a concentration smaller than 1 mM using proton-MRI.

MRI Can't See Immobile Molecules

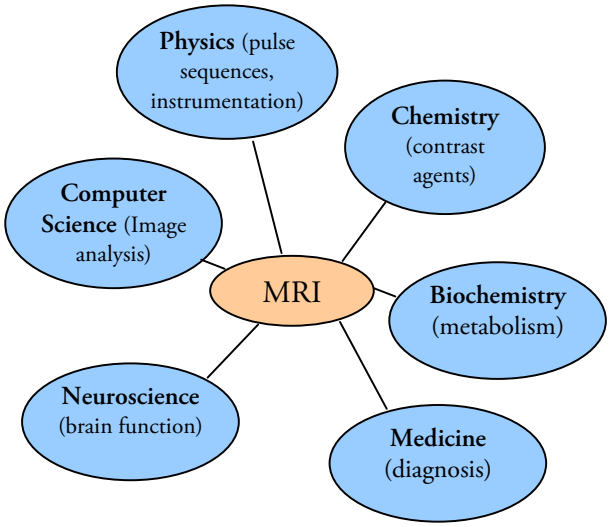
Immobile molecules, such as those trapped inside membranes or even tied to membranes, or part of solid structures such as bones, have reduced or no visibility. Small molecules tend to rotate very fast and average out many interactions which lead to decoherence and signal loss, but immobile molecules lack these averaging mechanisms and have very short T_2 values which lead to very fast signal decays (T_2 ~ ms to µs and less). The MRI electronics are often too slow to detect them and they decay below the noise levels before we can acquire enough signal.

MRI Can't See Large Molecules

Large molecules are by virtue of their size less mobile than small molecules, which is directly related to our statement about immobile molecules. But it's not only their slow or restricted immobility that is an issue: intra-molecular interactions between nuclei in the same molecule tend to dephase the signal as well. Molecules larger than ~ 1 kDa are already difficult to detect, and large proteins are pretty much invisible (e.g. prolactin proteins secreted by the pituitary gland, ca. 22 kDa).

MRI Is Interdisciplinary

We have so far seen a long – but incomplete – list of things we can visualize in the brain non-invasively in-vivo using MRI. The examples should illustrate how diverse the field of magnetic resonance imaging is:



The current course will focus on the basic physics of MRI, while keeping applications closely in sight. We are going to learn:

- How an MRI machine works.
- What a pulse sequence is, and how to design it to bring out some ofthe contrasts we've seen so far.
- How to set the parameters in some famous pulse sequences.
- Why some sequences fail and what sort of artifacts they create.
- What are the biophysical/physiological origins of some of the contrast parameters seen in MRI (T_1, T_2, T_2*, diffusion and so forth).