Slide 1:
I would like to thank organizers for the invitation and I regret that I can not be personally here. I would like to talk today about several advanced MRI methods to study normal brain and cancer.

Slide 2:
I would like to start with a comparison between MRI and other experimental methods used in neuroscience regarding the spatial-temporal resolution and the invasiveness of each method. As you can see from the figure, MRI fills a large gap between different methods such as electrophysiology with surface electrodes (EEG, MEG) or single unit and patch clamp, optical microscopy, optical dyes, positron emission tomography (PET), and anatomical lesions. MRI can reach spatial resolutions from sub-millimeter to cm, and temporal resolution on the order of seconds to tens of milliseconds. This is relevant for the functional and anatomical organization of the brain since it can probe functional events for individual layers and columns of gray matter completely non-invasively.

Slide 3:
The advanced MRI methods that I would like to talk about today are perfusion, dynamic contrast enhancement, diffusion tensor imaging, spectroscopic imaging, and resting-state fMRI. I will show you results regarding the application of these methods to a novel antiangiogenic treatment of brain tumors.

Slide 4:
Perfusion weighted MRI uses a contrast agent that is injected as a bolus intravenously during dynamic acquistion of T2 weighted MR images. The passage of the bolus of the contrast agent through the capillary network shortens the T2 and T2* relaxation times which results in a drop in signal intensity in the T2 weighted images. You can see on the time course above on the right side image how the signal drops when the bolus arrives in the middle cerebral artery. By mathematically fitting the drop in signal intensity we can calculate important physiological parameters such as cerebral blood volume (CBV), cerebral blood flow (CBF) and mean transit time (MTT).

Good perfusion is crucial for normal functioning of brain or other organs. Changes in blood perfusion occur in many brain diseases, and measuring it allow us to understand pathology or monitor treatment, as I will show in the next slides for the case of brain tumors.

Slide 5:
You can see here an example of perfusion in a brain tumor. On the left side you can see the anatomical T2 weighted image. On the right you can see the calculated CBV and CBF maps. You can see that the tumor is quite heterogeneous and has some regions that are hypoperfused (red arrow) and other regions that are hyperperfused (white arrow) compared to the normal contralateral brain.

Slide 6:
Dynamic contrast enhancement is a technique used to measure the permeability of blood-brain-barrier (BBB). It is based also on a contrast agent that is injected intravenously as a bolus during dynamic acquisition of T1 weighted images. BBB of normal brain is intact and the contrast agent does not leak from capillaries into the brain, while the tumor microvasculature has a very leaky BBB and the contrast can leak from capillaries into the tumor where determines a shortening of the T1 relaxation time and an increase of signal intensity (!!! This is opposite of what happens in perfusion weighted imaging where T2 shortening causes a decrease in signal intensity). You can see in red (right side image) how the signal intensity increases after bolus injection.

Using a simple two compartment model and the mathematical relations that link signal intensity with the concentration of the contrast agent, we can calculate the permeability (Ktrans) of the blood-brain-barrier. As you will see in the next slides permeability is an important physiological parameter in the case of brain tumors, and can predict treatment outcome in combination with cerebral blood volume.

Slide 7:
Here you can see an example of Ktrans map in a brain tumor. On the left you can see a structural anatomical T1 weighed image acquired after the injection of the contrast agent. On the right you can see the Ktrans map calculated based on the DCE data acquired during the injection.
You can see that in the normal brain the Ktrans is zero, indicating an intact BBB that is not permeable to the contrast agent, while the tumor shows large Ktrans values which have a heterogeneous distribution.

Slide 8:
Diffusion tensor imaging is based on the restricted diffusion of the water molecules in an anisotropic medium such as the brain where the barriers formed by the myelin sheets of the axons favor the diffusion of water along axon dirrection. Mathematically the anisotropic diffusion is defined by a tensor that has three eigenvalues and three eigenvectors that define its size and orientation in space. This is opposite to the free diffusion in an isotropic medium which is characterized by a single diffusion constant which is scalar without any orientation.

Slide 9:
Thus, measuring the diffusion tensor of the water molecules in the brain together with tracking algorithms allow us to determine the direction of the white matter fiber tracts. This is done completely non-invasively and MRI is the only imaging technique that can do this. Previously the only possibility to do this was in post-mortem brains by injecting optical dyes in a region of the cortex and see where they end up. You can see that the in-vivo DTI tractography compares very well with the classical neuroanatomy atlases. The different colors in the right image represent: red fibers that go from right to left (corpus calosum), green fibers that go from anterior to posterior (superior longitudinal

fasciculus), blue fibers that go from superior to inferior (cortico-spinal or pyramidal tracts).

Slide 10:
I will switch now to the application of these techniques in the case of antiangiogenic treatment in glioblastoma multiforme (GBM) patients. GBM is the most aggressive brain tumor that has an average survival of less than 1 year with the current treatment based on surgery, chemo- and radio-therapy.

Antiaangiogenic treatment is a new type of treatment which is still under investigation and is not approved yet for clinical use in newly diagnosed brain tumors. Antiangiogenic treatment aims to normalize the dysfunctional microvasculature of tumors characterized by dylated, tortuous, and leaky blood vessels. This happens due to the inbalance between pro-angiogenic and anti-angiogenic factors. Antiangiogenic treatment tries to restore this balance by targeting one or more pathways involved in blood vessel formation. In particular I will present results obtained with the drug cediranib that blocks primarily the VEGF pathway.

Slide 11:
You can see here data from a longitudinal study on 30 patients with glioblastoma receiving antiangiogenic treatment over the course of one year (actually till death occurs). There are 6 time points shown, the first two are baselines before the start of the treatment (within one week), and the other after the treatment started: after 1 day, 1 month, 2 months, and 4 months. You can see quite a rapid effect in normalizing Ktrans after 1 day, while CBV and DTI normalize gradually towards two months of treatment.

Slide 12:
The average values for Ktrans, CBV and the apparent diffusion constant over the entire cohort of 30 patients during the entire treatment are shown here. On the left side it can be seen that there is a window of normalization for most of the parameters during the first two months, however towards the end of the treatment the CBV and the tumor volume seem to increase again. On the right side the data measuring cerebral edema show a constant improvement, and this is supported also by the fact that the patients did not need corticosteroids to treat edema.

Slide 13:
The change in imaging biomarkers measured by MRI can be used to predict tumor response to antiangiogenic treatment. A combined parametric index called “vascular normalization index“ (VNI) can be calculated using the values of Ktrans, CBV and collagen IV from the blood using the values before the treatment (Day -1) and after 1 month of treatment. It can be seen that an increase of the VNI during the first month is associated with an increase in the progression free survival (or time to progression, left side) and the overall survival (right side) indicated in days.

Slide 14:
MR spectroscopy (MRS) is a method that measures concentrations of various metabolites in the brain. This is different from MRI that measures the signal of water in the brain. On the right side you can see an example of a typical brain spectrum which is a combination of several metabolites.

Slide 15:
There are described more than 30 metabolites that can be detected by MRS in the brain. However the most concentrated in the healthy brain are represented by NAA, choline, creatine, glutamate, GABA and myoinositol. Their concentration is in the mili-molar (mM) range.

Slide 16:
MRS is very sensitive to the change of the metabolic profile. Tumor metabolic profile is very different compared to the healthy brain, almost like in a mirror, typically the ratio of NAA over Choline changes from greater than one to less than one. Extra metabolites that are not visible in the healthy brain appear in tumors, such as lipids and lactate.

Slide 17:
In this slide you can see an example from a glioblastoma patient receiving antiangiogenic treatment. You can see in the upper raw spectra measured in the tumor before treatment and after 1 day and 1 month since the start. Before treatment a large lipid peak indicating tumor necrosis is evident, after 1 month the spectrum changes considerably towards the normal metabolic profile. Bellow the control spectra from the healthy brain show very little change.

Slide 18:
By doing spectroscopic imaging we can obtain images of different metabolites. In the case of brain tumors, the most active parts of the tumors are indicated by a strong choline signal. The choline map is quite different from the T1 weighted post contrast image, and shows two clear active sites.

Slide 19:
Spectroscopic imaging is useful for other diseases as well. Here an example from a stroke patient shows a buildup of lactate in the ischemic region.

Slide 20:
The acquisition of spectroscopic imaging data is more challenging due to the much smaller concentration of metabolites compared to the water signal. Hence, long acquisition times (10 minutes or more) and low spatial resolution (1 cm voxels) are necessary with typical methods. Using optimized pulse sequences the acquisition times and spatial resolution can be improved. Here I demonstrate that acquisition time can be shortened four times, or the resolution doubled. At higher resolution the heterogeneous features of the tumors can be better visualized, such as the necrotic core and the active regions.

Slide 21:
Another challenge for spectroscopy is represented by the fact that spectra of different metabolites overlap, so precise quantification of some metabolites is difficult even when using advanced fitting methods.

Slide 22:
However, acquisition of data can be modified by introducing additional spectral dimensions in order to separate overlapped signals. You can see here in the case of brain biopsies multidimensional correlation spectra. Each signal in these spectra corresponds to a unique metabolite and quantification can be done precisely.

Slide 23:
We demonstrated for the first time that correlation MR spectroscopic imaging can be done in-vivo on human subjects with clinical scanners. A zoom on a spectrum from a central voxel shows several well separated signals. In particular GABA, which is difficult to be quantified with conventional methods due to overlap with stronger NAA signal, can be identified unambiguously in multidimensional correlation spectra.

Slide 24:
A similar situation happens in the case of brain tumors. Lactate and lipids overlap, and they can be separated in multidimensional spectra.

Slide 25:
I will switch gears now for the last subject.

Traditional fMRI is done in relation with a task that the subjects perform during the measurement, like many of the previous presenters showed. Experimental paradigms alternate periods when the subject is active and executes the task with periods when the subject is inactive or at rest. By comparing what happens during active periods versus what happens in the rest periods an activation map of the cortex can be calculated. This paradigm implies that a true resting state of the brain exists.

However, a meta-analysis involving thousands of subjects for which only the rest periods from task-related fMRI or PET data were analyzed have shown that there isn‘t a so called resting-state of the brain, as it was hypothesized, but rather as would seem more naturally the brain always does something. Moreover, it was discovered that for all healthy volunteers the same regions of the brain are active during rest. These regions were proposed to form a so called brain‘s default network which is active at rest (although this two words together seem to form a contradiction).
Since then the paradigm of the resting-state fMRI has developed into an established method. Resting-state could be more valuable than task related fMRI especially in clinical cases of neuropsychiatric diseases or other diseases where the subject can not be trained or does not cooperate. Task-related fMRI requires training of a subject and its full cooperation, and with many patients this can not be achieved at all or only partially, thereby compromising the conclusions. On the other hand, resting-state fMRI studies

begin to show that the brain‘s default network is disrupted in different ways in different diseases.

Slide 26:
In this figure you can see the cortical areas involved in the brain‘s default network and the type of thinking that activates them mostly. This is to say that the brain always does something, there are always thoughts going through our minds the so called day-dreaming. Especially introspection thinking seems to activate the areas belonging to the brain‘s default network. Introspection thinking can be classified in four categories such as, autobiographycal memory, envisioning future, theory of mind and moral decision making. Although the same core areas are activated, the pattern of activation is different as you can see.

On the left side you can see the regions involved on the lateral side of the brain.
Three lateral regions are important:

- inferior parietal lobule (IPL) shows the largest activation
- Lateral temporal cortex (LTC)
- Dorsal medial prefrontal cortex (dMPFC)

On the right side you can see the regions involved on the medial side of the brain
Three medial regions are important:

- Posterior cingulate / retrosplenial cortex (PCC/rsp)
- Ventral medial prefrontal cortex (vMPFC)
- Hippocampal formation (HF)

Slide 27:
In this figure you can see the interconnection between different regions of the brain’s default network. The same color means that the regions involved are correlated. The most important observation is that PCC/Rsp, IPL, and vMPFC represent anatomic hubs in the default network to which all other regions are correlated. The second observation is that dMPFC and HF+, which are both strongly correlated with the hub regions, are not correlated with each other, indicating that they are part of distinct subsystems.

Slide 28:
Finally, I would like to thank you for listening to my presentation and to acknowledge the people involved. I regret that I can not personally questions and I wish you to have a wonderful meeting.