OHBM 2016 Educational Course
MR Diffusion Imaging: From the Basics to Advanced Applications

Group comparison with diffusion imaging and application to brain plasticity

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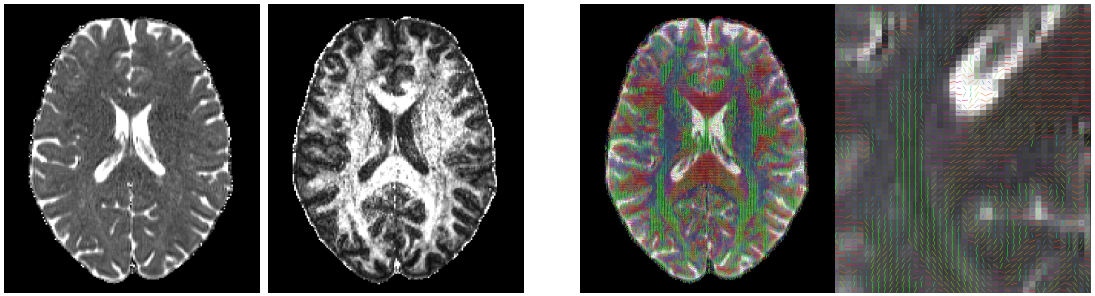
Diffusion-weighted imaging (DWI)

DWI is sensitive to the direction and strength of molecular diffusion (primarily water) in the brain. It reveals characteristics of the brain macro-structure and micro-structure *(e.g., Tournier et al., 2011, Magn Reson Med)*. It allows inferences about structural connectivity *(e.g., Fillard et al., 2011, Neuroimage)*.

Macro-structure

- gray matter (GM)
- white matter (WM)
- cerebrospinal fluid (CSF)
- ...

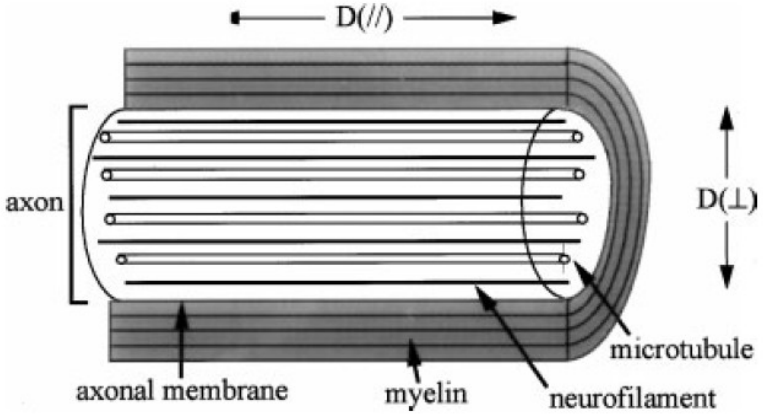
mean diffusivity *anisotropy* *major orientation*



Micro-structure

- cell integrity
- cell orientation
- cell density
- cell size
- ...

of neurons, astrocytes, gliacells, ...



(adapted from Beaulieu, 2002, NMR Biomed, Fig. 4)

Group comparison

Main research questions:

- **Is there a difference in brain micro-structure ...**
- **Is there a difference in structural connectivity ...**

... between two (or more) groups or two (or more) measurements or two (or more) brain areas.

Main steps and challenges:

- **Acquisition**
  Challenges: choice of b-values, gradient directions, voxel size, ...
- **Pre-processing**
  Challenges: (no) smoothing, correction for eddy currents and head motion, ...
- **Deriving diffusion metric / fiber tracks**
  Challenges: choice of diffusion model (DTI, DKI, ...) and parameters (e.g., FA, MD, ...) or choice of tracking algorithm and criteria for defining tracks
- **Normalization across brains**
  Challenges: WM is relatively homogenous on tissue-weighted MRI, but relatively diverse with regard to diffusion metrics. Volumetric (even non-linear) registrations do not account for WM diversity. Spatial smoothing across diverse WM regions produces false negatives / positives.
- **Inferential statistics**
  Challenges: choice of parametric vs. non-parametric tests and correction for multiple comparisons

Deriving diffusion-based parameters

Several models for quantifying voxel-wise diffusion were proposed based on:

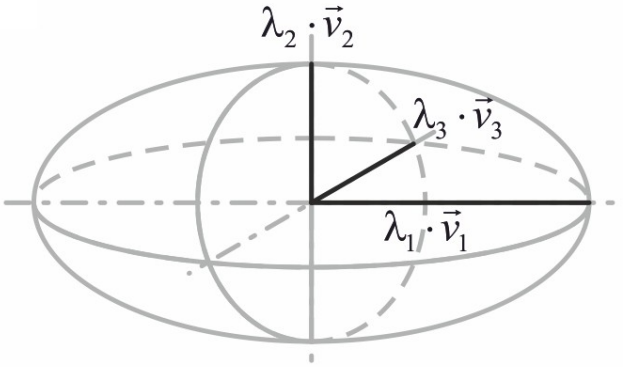
one b-value (plus b-zero):

- diffusion tensor (DT or DTI)
  (Basser et al., 1994, Biophys J)
- ball-and-stick
  (Behrens et al., 2003, Magn Reson Med)
- High angular resolution diffusion imaging (HARDI)
  (Tuch et al., 2002, Magn Reson Med)
- ...

multiple b-values:

- diffusion kurtosis (DK or DKI)
  (Jensen et al., 2005, Magn Reson Med)
- diffusion spectrum (DSI)
  (Lin et al., 2003, Neuroimage)
- ...

(see also previous speakers Flavio Dell'Acqua and Gary Zhang or reviews, e.g., Panagiotaki et al., 2012, Neuroimage)



$$\text{MD} = \bar{\lambda} = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} \qquad \text{RD} = D_\perp = \frac{\lambda_2 + \lambda_3}{2}$$

$$\text{FA} = \sqrt{\frac{3}{2}} \sqrt{\frac{\sum_{i=1}^{3} (\lambda_i - \bar{\lambda})^2}{\sum_{i=1}^{3} \lambda_i^2}} \qquad \text{AD} = D_\parallel = \lambda_1$$

Geometric illustration of the tensor model with eigenvalues (λ) and eigenvectors (v) and major diffusion parameters derived from eigenvalues: mean diffusivity (MD), fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity (AD).

Normalization across brains

Main approaches for group comparisons:

Region of interest (ROI) analysis

- **based on macrostructure**
  (e.g., Pfefferbaum et al., 2000, Magn Reson Med)
- **based on fiber tracks**
  (e.g., Yendiki et al., 2011, Front Neuroinform; Yeatman et al., 2012, PLoS One)

Whole-brain analysis

- **voxel-based analysis / morphometry (VBA / VBM)**
  (e.g.: Ashburner & Friston, 2000, Neuroimage; Good et al., 2001, Neuroimage)
  (e.g.: Rugg-Gunn et al., 2001, Brain; Büchel et al., 2004, Cereb Cortex)
- **tract (FA-skeleton) based spatial statistics (TBSS)**
  (e.g.: Smith et al., 2006, Neuroimage)
- **surface-based analysis**
  (e.g., Beer et al., 2011, Exp Brain Res; Turken et al., 2009, BMC Med Imag; Wu et al., 2014 Hum Brain Map)

ROI analysis based on macro-structure

Procedure:

- Pre-process DWI data
- Calculate parameters based on diffusion model (e.g., FA, MD, …)
- (Automatically) segment relevant brain structures based on T1-weighted (or T2-weighted) MRI as ROI
- Extract mean of diffusion measures (FA, MD, ...) per ROI and brain
- Compare across subjects and ROIs

Benefits:

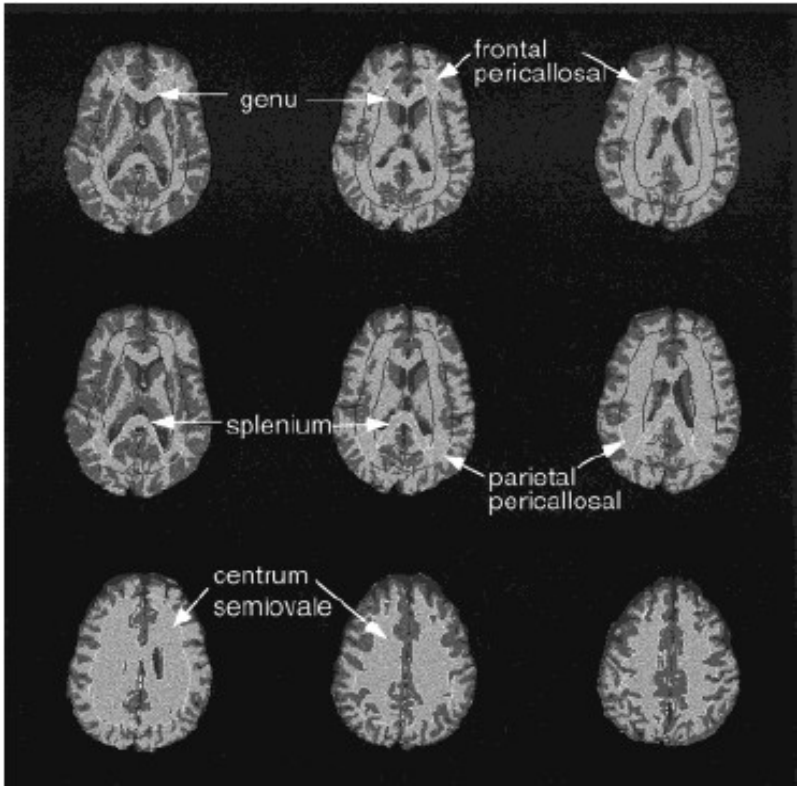
- correspondence between macro- and micro-structure
- straightforward analysis and interpretation

Limitations:

- no whole-brain analysis
- requires strong a-priori hypothesis regarding relevant ROIs
- assumes homogenity within ROI
- objective / automatic segmentation of ROI preferred

ROI analysis based on macro-structure

Example:
Fractional anisotropy (FA) is reduced by age in several ROIs including anterior (but not posterior) corpus callosum. Inter-voxel coherence (IVC) increased in anterior callosum.
(Pfefferbaum et al., 2000, Magn Reson Med)



Five ROIs defined on anatomical images. Adapted from Pfefferbaum et al. (2000, Fig. 2)

Table 1
Correlations With Age in 31 Men

Brain region	FA		Coherence	
	r	*P*	r	*P*
Corpus callosum				
Genu	−0.55	0.002	0.47	0.01
Splenium	−0.27	n.s.	−0.01	n.s.
Frontal pericallosal regions				
Left	−0.63	0.0001	0.02	n.s.
Right	−0.71	0.0001	0.21	n.s.
Parietal pericallosal regions				
Left	−0.64	0.0001	−0.08	n.s.
Right	−0.51	0.004	0.05	n.s.
Centrum semiovale	−0.76	0.0001	0.08	n.s.

Correlations of fractional anisotropy (FA) and inter-voxel coherence (IVC) of the major eigenvectors with age. Adapted from Pfefferbaum et al. (2000, Table 1)

ROI analysis based on fiber tracking

Procedure:

- Pre-process DWI data
- Calculate parameters based on diffusion model (e.g., FA, MD, …)
- (Automatically) detect major tracts in all subjects by fiber tracking based on prior information on tract anatomy
- Quantify diffusion measures (FA, MD, ...) across and along tracts
- Compare across subjects and across tract sections

Benefits:

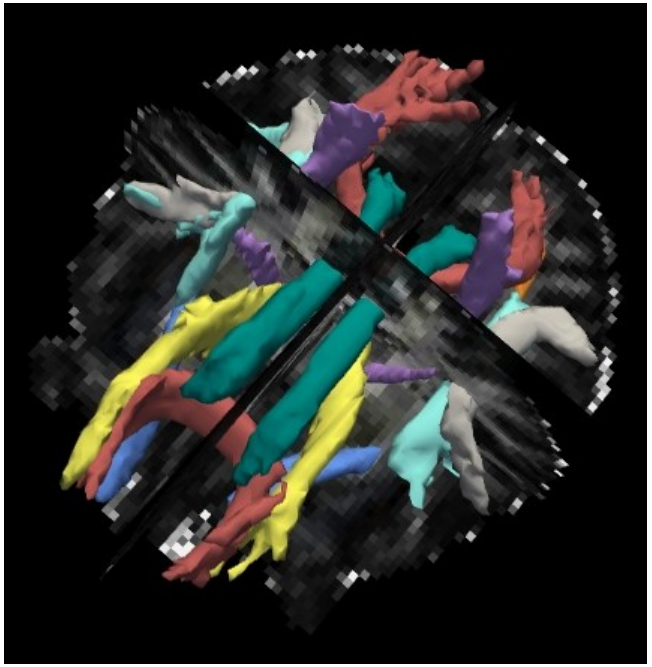
- good solution of the correspondence problem
- semi-automatic
- quantifies micro-structure along tracts

Limitations:

- no whole-brain analysis
- limited to established tracts with known trajectories and anatomical landmarks
- tract selection depends on quality of prior information
- requires substantial processing

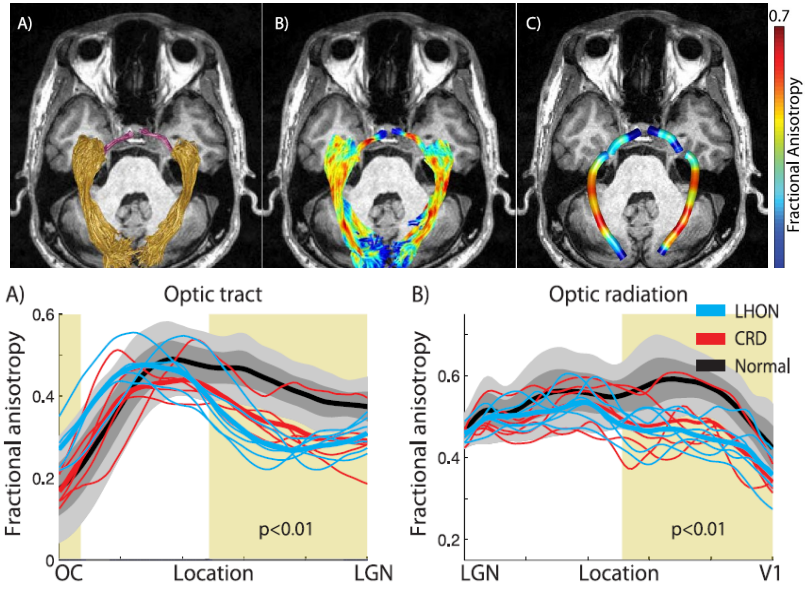
ROI analysis based on fiber tracking

Example 1:
TRActs Constrained by UnderLying Anatomy (TRACULA) of Freesurfer: Automatic parcellation of major white matter tracts by probabilistic tracking.
(Yendiki et al, 2011, Front Neuroinform)



Cortico- spinal tract (CST), inferior longitudinal fasciculus (ILF), uncinate fasciculus (UNC), anterior thalamic radiation (ATR), cingulum-cingulate gyrus (supracallosal) bundle (CCG), cingulum-angular (infracallosal) bundle (CAB), superior longitudinal fasciculus-parietal bundle (SLFP), superior longitudinal fasciculus-temporal bundle (SLFT), corpus callosum-forceps major (FMAJ), corpus callosum-forceps minor (FMIN). Adapted from http://freesurfer.net/fswiki/Tracula

Example 2:
Posterior sections of optic tract and optic radiation showed reduced FA in patients with cone-rod dystrophies (CRD) and Leber hereditary optic neuropathy (LHON).
(Ogawa et al., 2014, Invest Ophthalmol Vis Sci)

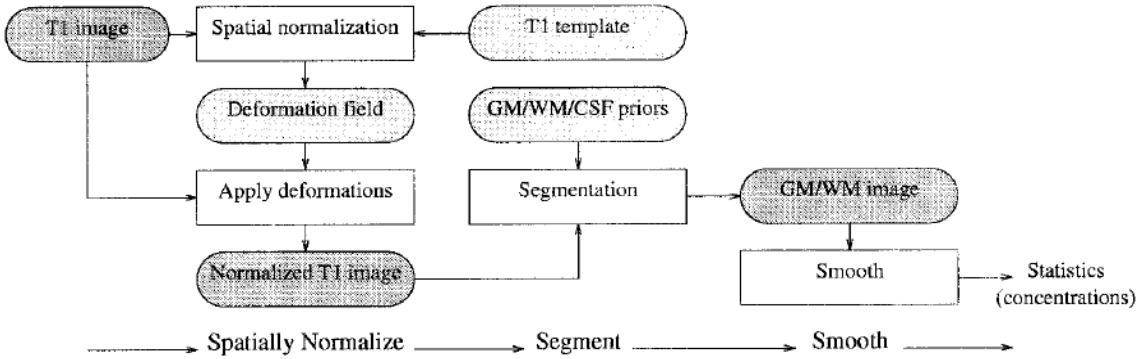


Top: Optic tract and optic radiation (and its core) in single brain. Bottom: FA values along tracts. Adapted from Ogawa et al. (2014, Fig. 4 and 5)

Voxel-based Analysis / Morphology (VBA / VBM)

Procedure:

- Pre-process DWI data
- Calculate parameters based on diffusion model (e.g., FA, MD, …)
- Non-linearly register (warp) T1 and b-zero DWI image to template space
- Segment major macroscopic structures (GM, WM, CSF)
- Warp subjects' diffusion parameters (e.g., FA, MD, ...) to template space
- Substantial smoothing of subjects' diffusion parameters
- Perform voxel-wise group statistics (parametric or non-parametric)



Adapted from Good et al. (2001, Fig. 1a). See also optimized procedure (Good, 2001, Fig. 2a)

Benefits:

- examines whole brain
- automatic
- fast
- well established (for T1)

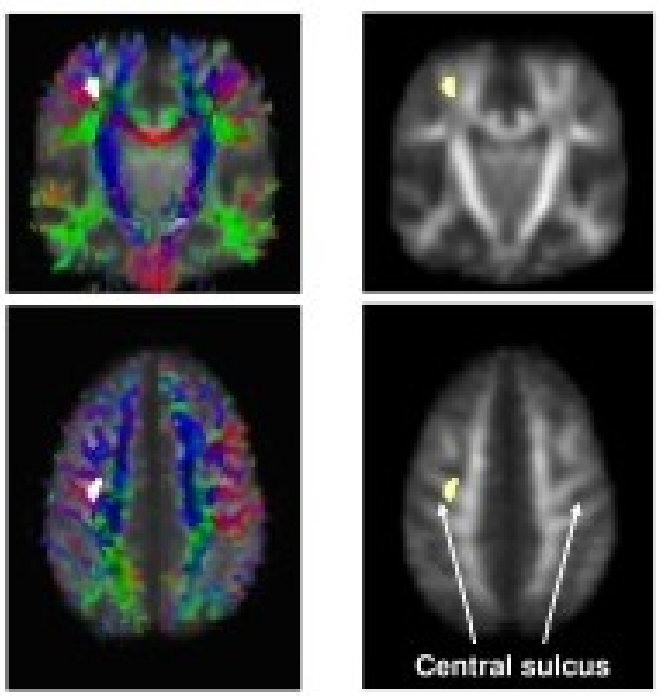
Limitations:

- Smoothing across heterogenous WM areas may result in false positives / negatives
- Typical smoothing kernel may not be appropriate for diffusion analysis

(e.g., Bookstein et al., 2001, Neuroimage; Jones et al., 2005, Neuroimage)

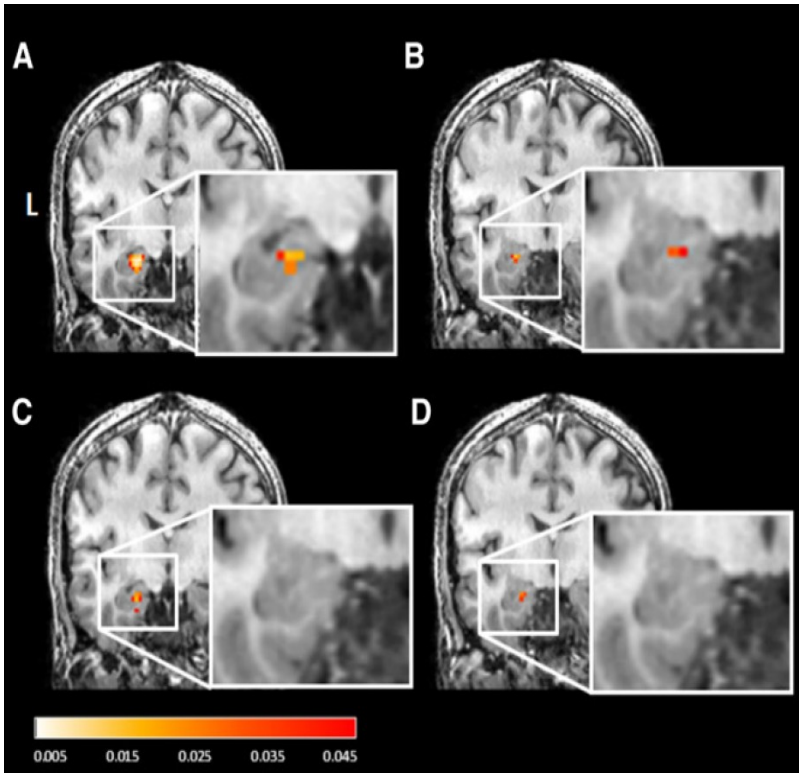
Voxel-based Analysis / Morphology (VBA / VBM)

Example 1:
FA values in white matter of the pre-central gyrus were enhanced contra-lateral to the dominant hand (right vs. left handers). First demonstration of sensitivity.
(Büchel et al., 2004, Cereb Cortex)



Adapted from Büchel et al. (2004, Fig. 2)

Example 2:
MD values in the hippocampus / para-hippocampus were reduced following two hours of a spatial learning task.
(Sagi et al., 2012, Neuron)

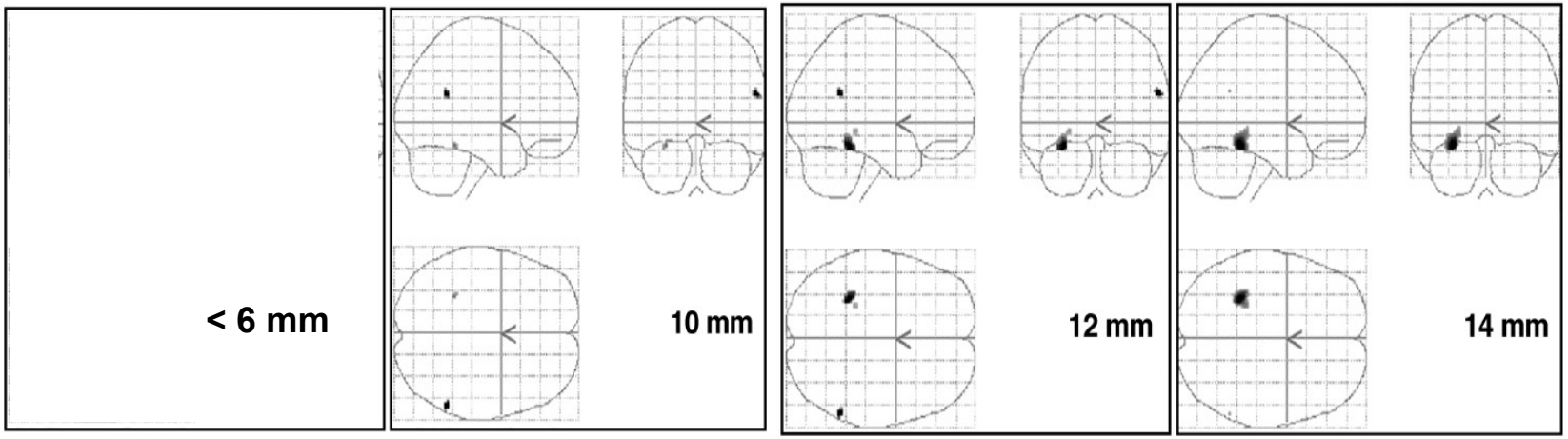


Adapted from Sagi et al. (2012, Fig. 2)

Voxel-based Analysis / Morphology (VBA / VBM)

Limitation:
VBM requires volumetric smoothing (typically 2 - 3 x voxel size). Detection of group differences in diffusion parameters (e.g., FA) between 14 schizophrenic patients and 14 controls depended on smoothing kernel. Assumptions for parametric tests are not met for all diffusion measures / brain areas.
(Jones et al., 2005, Neuroimage)

VBM-style comparison of FA (schizophrenic < controls) for various smoothing kernels (0 to 16 mm)
(adapted from Jones et al., 2005, Fig. 1)



Conclusion:
no group difference

Conclusion:
reduced FA in right superior temporal gyrus

Conclusion:
reduced FA in both right STG and left cerebellum

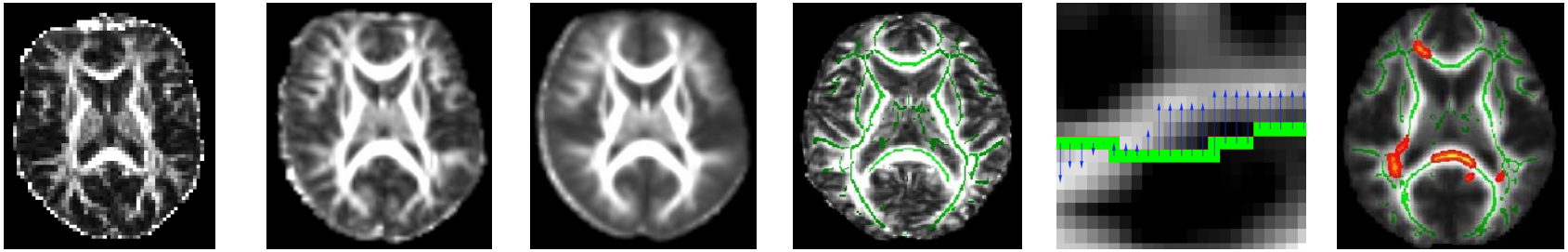
Conclusion:
reduced FA only in left cerebellum

Volumetric Analysis of FA-Skeleton (TBSS)

Procedure:

- Pre-process DWI data
- Calculate parameters based on diffusion model (e.g., FA, MD, …)
- Non-linear registration (warping) of FA images to standard space
- Create mean FA image, threshold and 'skeletonise' it
- Project subjects' (local maxima) diffusion parameters (e.g. FA) onto FA-skeleton
- Perform voxel-wise group (non-parametric) statistics on diffusion parameters

individual FA map | *standardized FA map* | *mean FA map (standardized)* | *thresh. mean FA (skeleton)* | *project nearest max. to skeleton* | *Voxel-wise statistic*



Adapted from http://fsl.fmrib.ox.ac.uk

Benefits:

- uses alignment invariant features (more robust than VBM style)
- limited number of comparisons (only voxels of FA-skeleton)

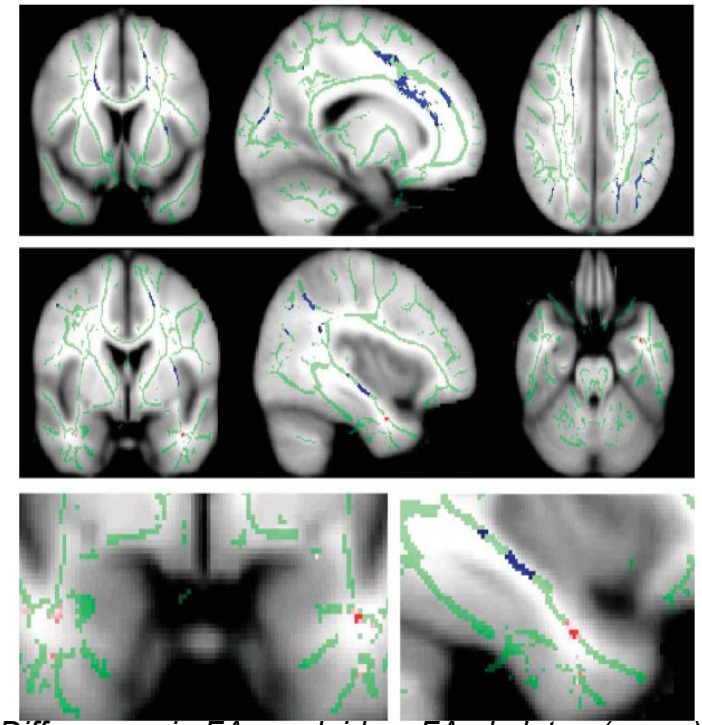
Limitations:

- Systematic alignment errors may still result in false negatives / positives
- FA-skeleton does not necessarily correspond to 'real' fiber tracts
- Analysis is limited to local maxima

(e.g., Bach et al., 2014, Neuroimage)

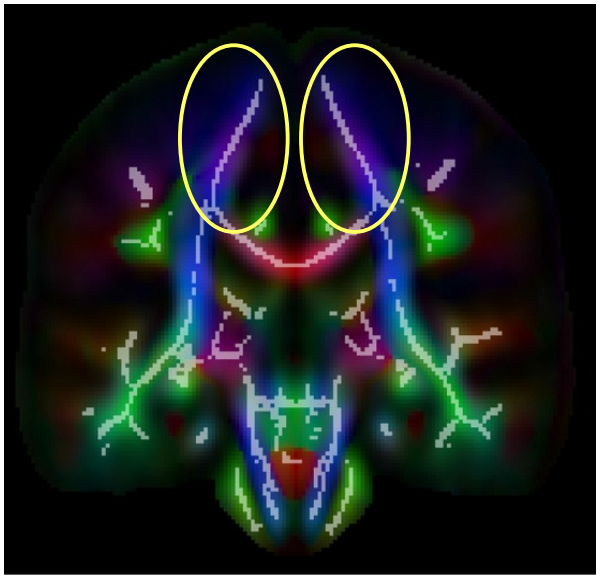
Volumetric Analysis of FA-Skeleton (TBSS)

Example:
Reduced FA in healthy older people: frontal, parietal, and subcortical areas
Reduced FA in patients (Alzheimer): left anterior temporal lobe
(Damoiseaux et al., 2009, Hum Brain Map)



Differences in FA overlaid on FA-skeleton (green) between older vs. younger healthy subjects (blue) and Alzheimer patients vs. older healthy subjects (red). Adapted from Damoiseaux et al., 2009, Fig. 2)

Limitation:
FA-skeleton collapses across different white matter tracts.
(Bach et al., 2014)



Adapted from Bach et al. (2014, Fig. 1)

Improvements:

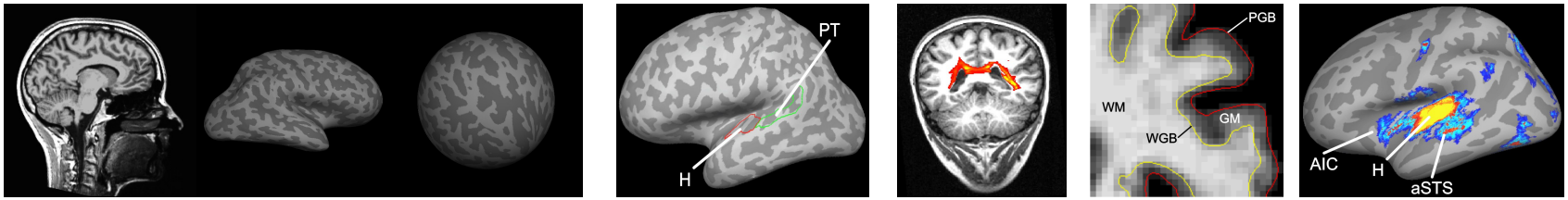
- *Crossing fibers TBSS*
  (Jbabdi et al., 2010, Neuroimage)
- Skeletonization based on directional information
  (Yushkevich et al., 2008, Neuroimage)

Surface-based Analysis of Fiber Tracking

Procedure:

- Reconstruct cortical surface based on T1-weighted images (e.g., with Freesurfer)
- Pre-process DWI data (including registration to individual T1)
- Fiber tracking constrained by seeds and masks (e.g., probabilistic with FSL)
- Convert into track probabilities
- Project track terminations to cortical surface
- Spherically register to average surface
- Extract vertex-wise group statistics

reconstruction of cortical surface including spherical registration | *Defining seeds and masks* | *(probabilistic) fiber tracking* | *project to surface* | *group statistics*



Beer et al. (2011, Exp Brain Res)

Benefits:

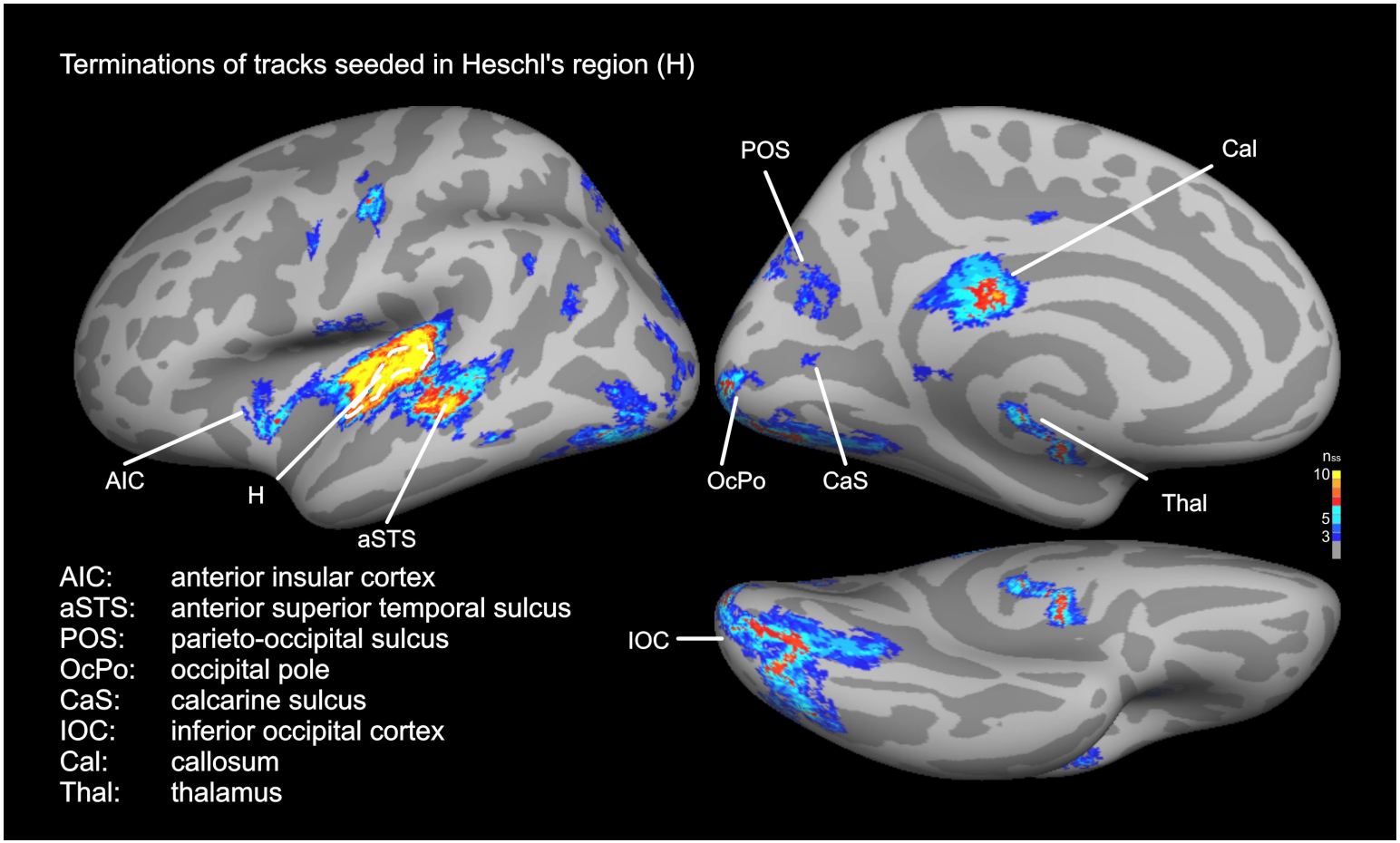
- Normalization based on major gyri and sulci
- Close correspondence to cortical brain areas

Limitations:

- Analysis is limited to cortical surface (no trajectories or sub-cortical tracks)
- Requires multiple analysis tools
- Superficial white matter might impede long-range tracking
  (Reveley et al., 2015, Proc Natl Acad Sci USA)

Surface-based Analysis of Fiber Tracking

Example 1:
Cortical track terminations of white matter tracks seeded in Heschl's gyrus (H)
(Beer et al., 2013, Front. Int. Neurosci.; see also Beer et al., 2011, Exp Brain Res.)



Group map of cortical track terminations. n_{ss} = number of brains showing supra-threshold track probabilities

Surface-based Analysis of Fiber Tracking

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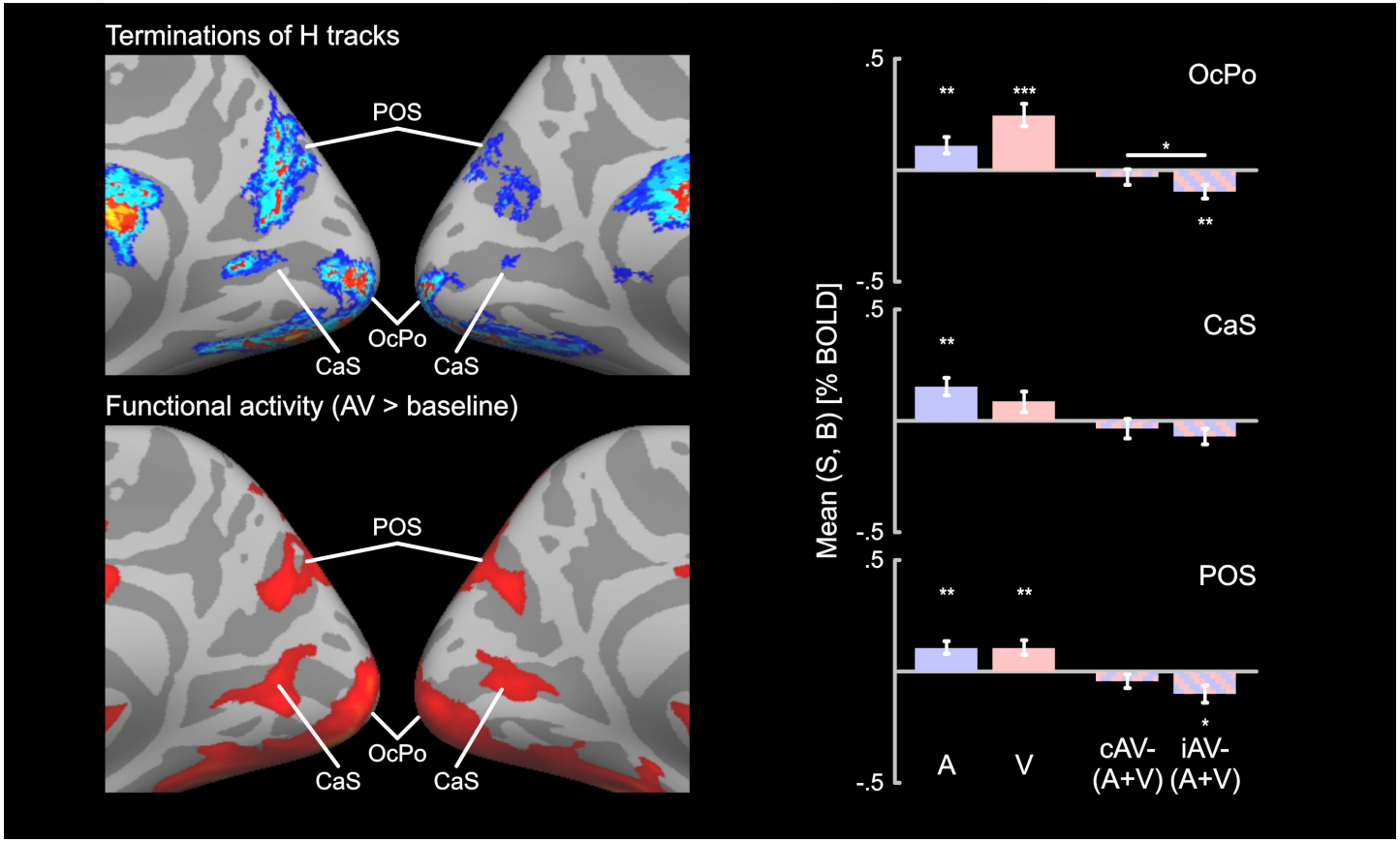
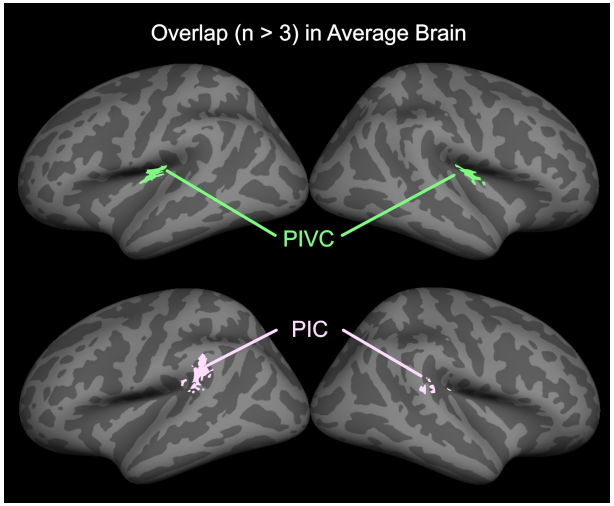


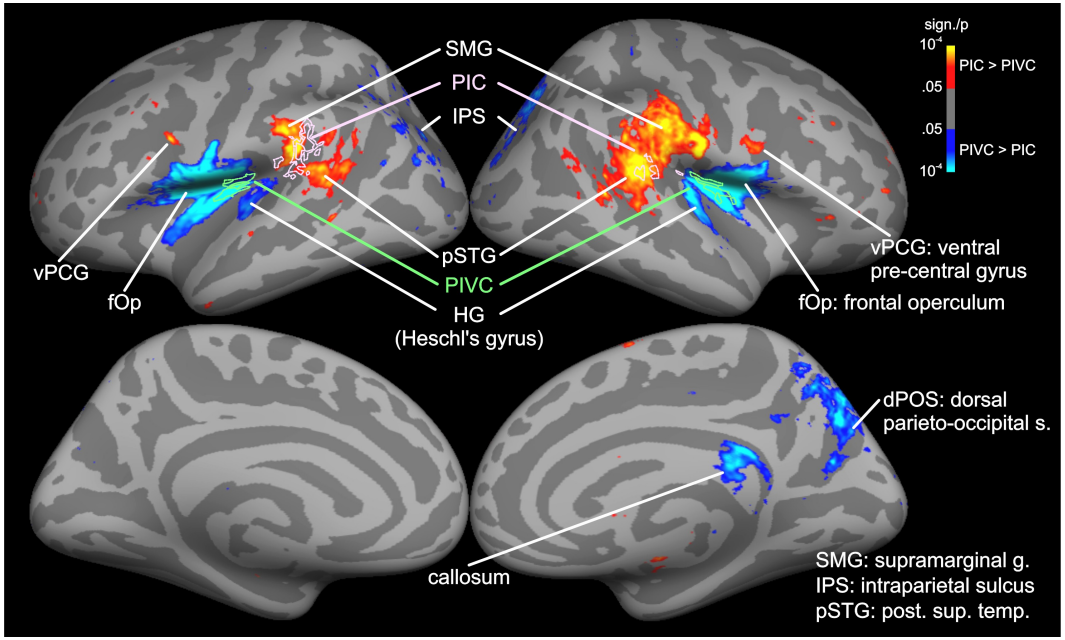
Spatial correspondence between terminations of H tracks (top left) and functional response (bottom left) to congruent (c) or incongruent (i) auditory-visual (AV) stimulation in the medial occipital cortex. Region-of-interest (right) analysis showed that parts of the occipital cortex with track terminations respond to both auditory and visual stimuli.

Surface-based Analysis of Fiber Tracking

Example 2:
Differences in cortical track terminations of the parieto-insular vestibular cortex (PIVC) and the posterior insular cortex (PIC)
(Wirth, Frank, Beer, & Greenlee, in prep.)



Location of PIVC and PIC in average brain. PIVC defined by functional localizer with caloric stimulation (e.g., Frank & Greenlee, 2014). PIC defined by visual motion stimulation (e.g., Beer et al., 2009).

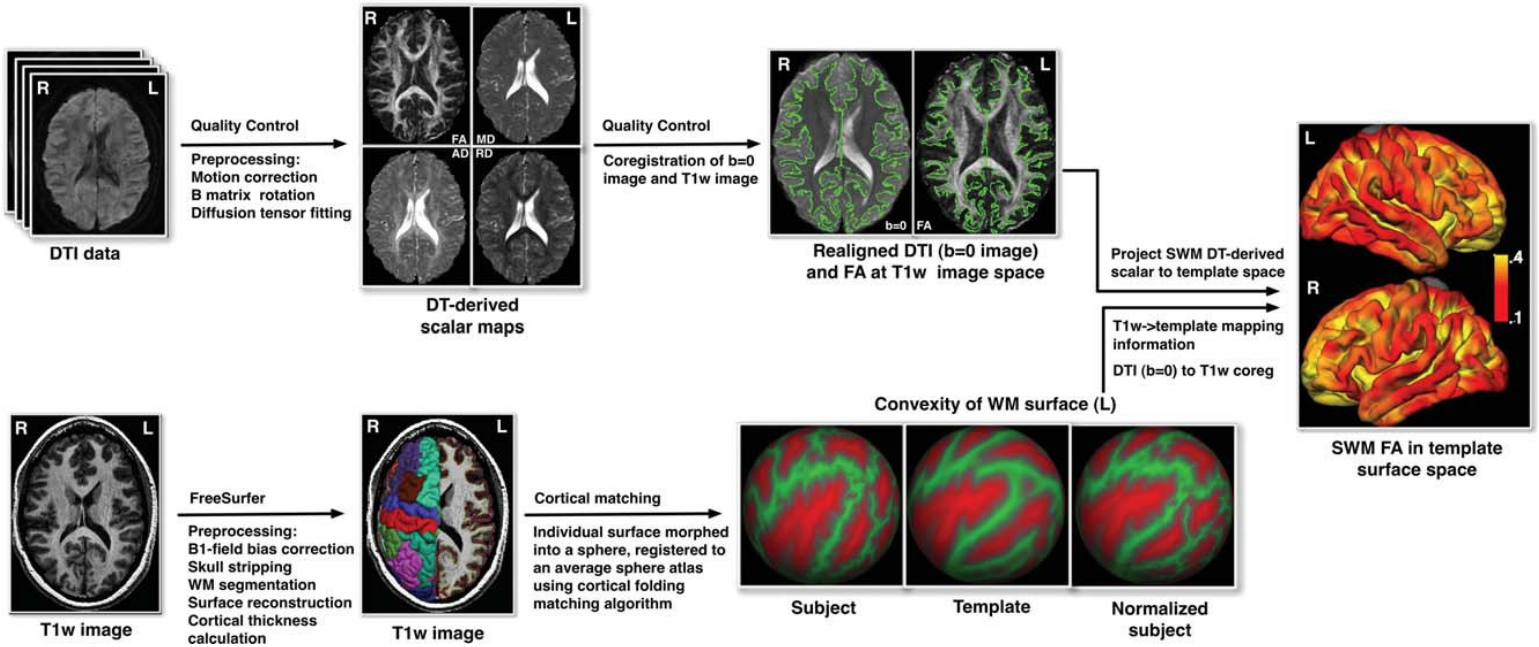


Significant group differences in the cortical track termination patterns of PIVC and PIC.

Surface-based Analysis of Microstructure

Procedure:

- Reconstruct cortical surface based on T1-weighted images (e.g., with Freesurfer)
- Pre-process DWI data (including registration to individual T1)
- Calculate parameters based on diffusion model (e.g., FA, MD, …)
- Project diffusion parameters of the superficial white matter (SWM) or gray matter (GM) to cortical surface
- Spherically register to average surface
- Perform vertex-wise group statistics



Analysis pipeline adapted from Wu et al. (2014, Fig. 1)

Surface-based Analysis of Microstructure

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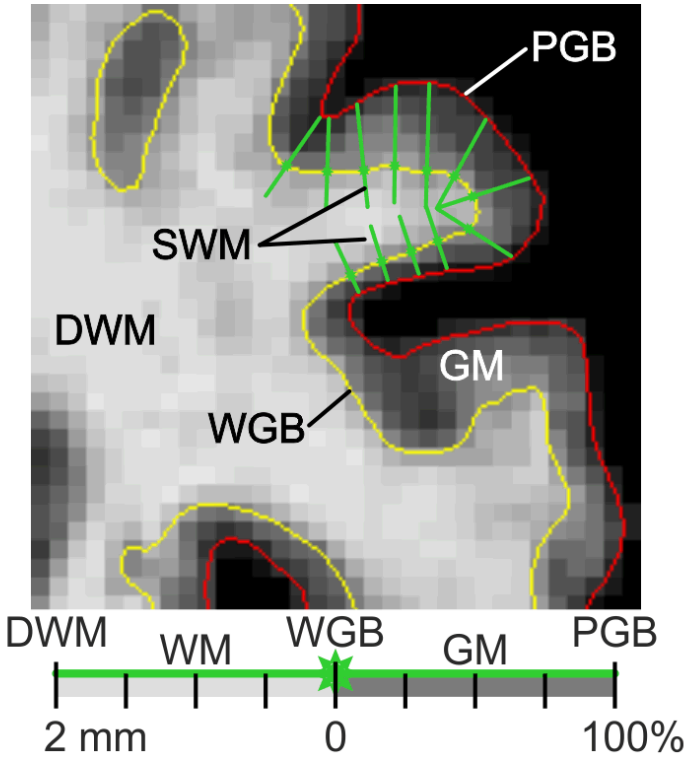
- Spherically register to average surface
- Perform vertex-wise group statistics

Benefits:
- Analysis of superficial white matter (SWM) and gray matter (GM)
- Normalization based on major gyri and sulci
- Close correspondence to cortical brain areas
- Option for laminar analysis

Limitations:
- No analysis of deep white matter (DWM)
- Requires multiple analysis tools

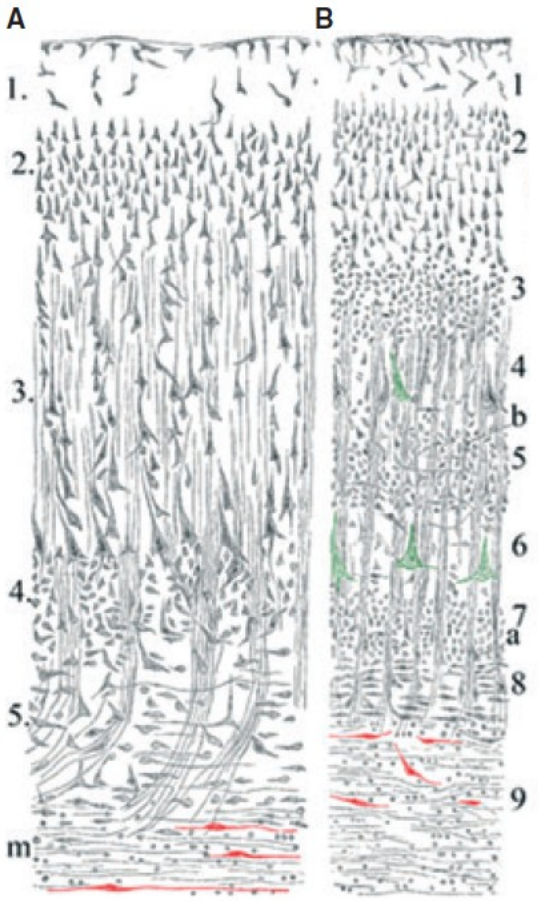


Surface-based Analysis of Microstructure

Superficial white matter (SWM):

- contains local association fibers
  (as opposed to long-range fibers)
  also called 'U'-fibers (Meynert, 1872)
  (e.g., Oishi et al., 2008, Neuroimage)

- high density of 'interstitial neurons' (IN)
  IN are associated with neurological and
  psychiatric disorders (e.g. schizophrenia)
  (e.g., DeFelipe et al., 2010, Front. Neuroanat.)



Interstitial neurons in superficial white matter of human frontal (A) and primary visual cortex (B). Adapted from Meynert (1884, as cited in Judas et al, 2010, J Anat).

Surface-based Analysis of Microstructure

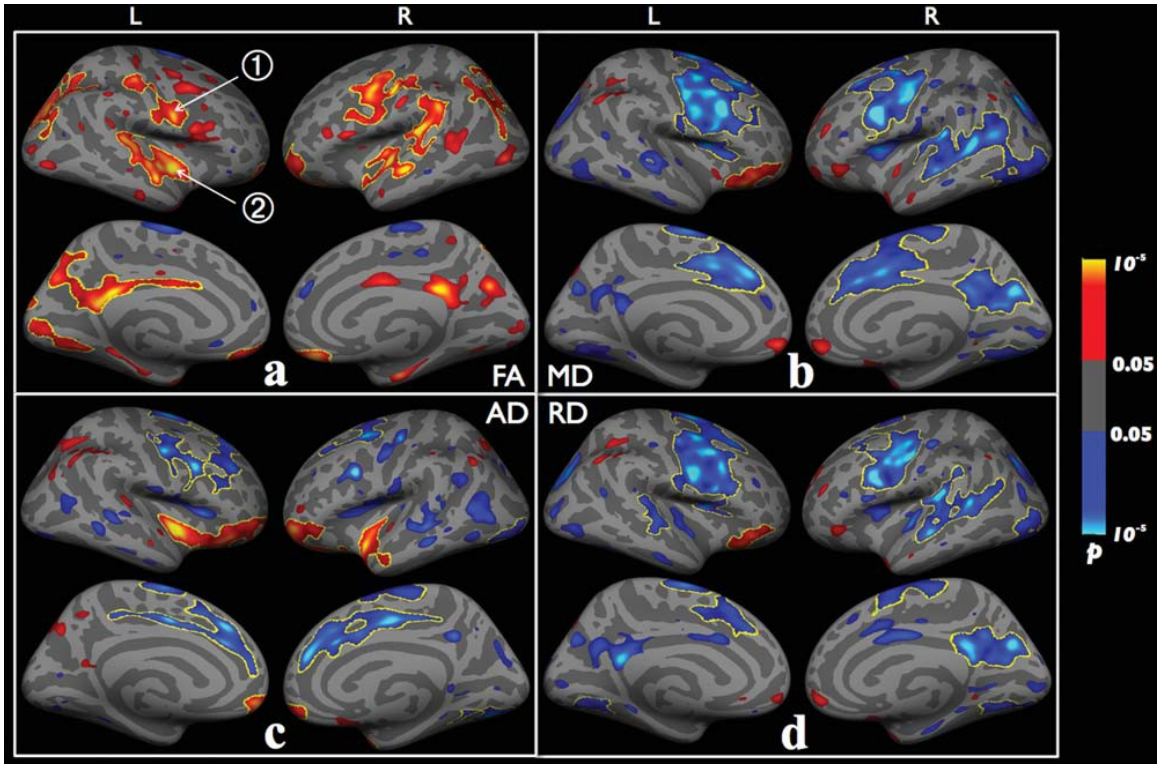
Example 1:
Development of Superficial White Matter (SWM) in healthy children and adolescents
(Wu et al., 2014, Hum Brain Map)

Sample: n = 144, 10 - 18 y.
SWM projection:
5 mm into WM

At least 2 patterns:

- motor cortex and superior temporal cortex (e.g., see 1)
  - increased FA
  - decreased MD/RD suggests increased myelination

- orbitofrontal and insula (e.g., see 2)
  - increased FA/AD suggests enhanced axonal coherence



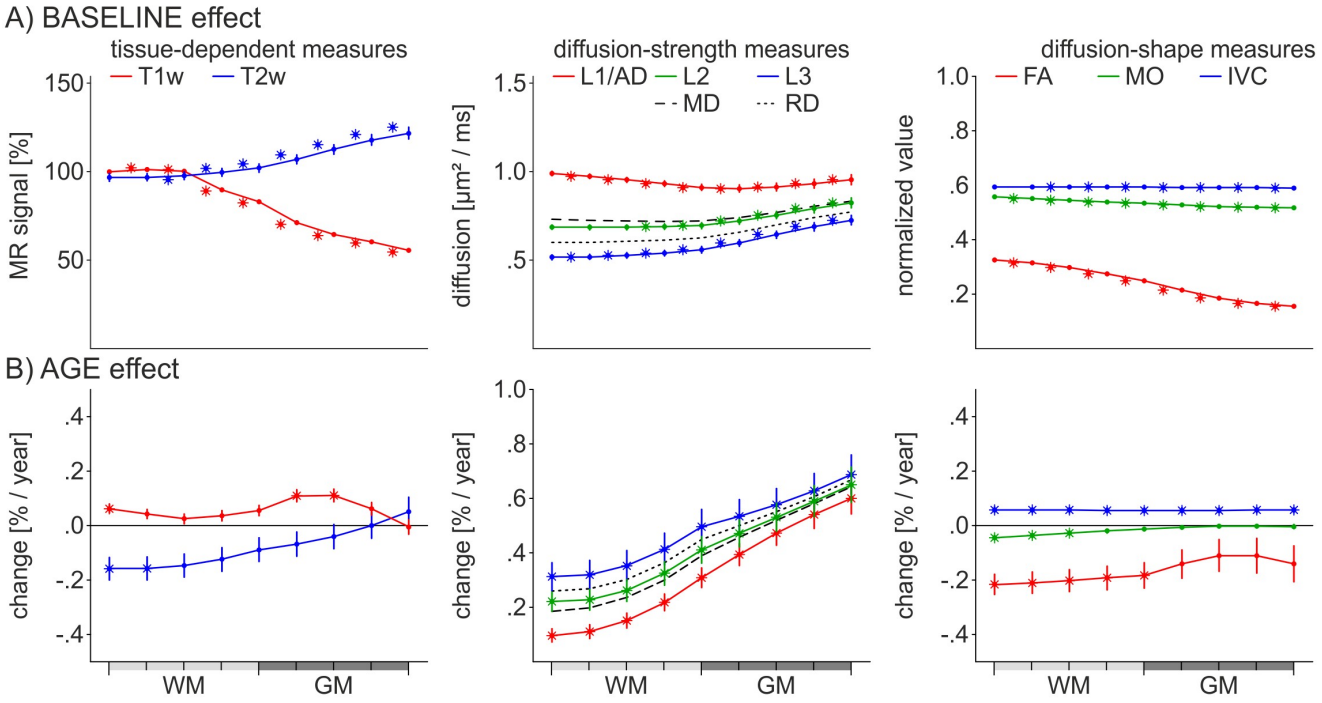
Linear age effects: red/yellow = increase with age, blue = decrease with age. Adapted from Wu et al. (2014, Fig. 2)

Surface-based Analysis of Microstructure

Example 2:
Development of superficial white matter (SWM) and gray matter in adults and elderly healthy people and patients with macular degeneration
(Beer et al., in prep., see also OHBM poster 3520, Wednesday)

Sample: n = 38 + 38
Projection: from GM to 2 mm into WM

Across whole surface:
- layer-sensitive change in tissue- and diffusion-weighted values
- layer-specific change by age



Surface-based Analysis of Microstructure

Example 2:
Whole-surface age effect

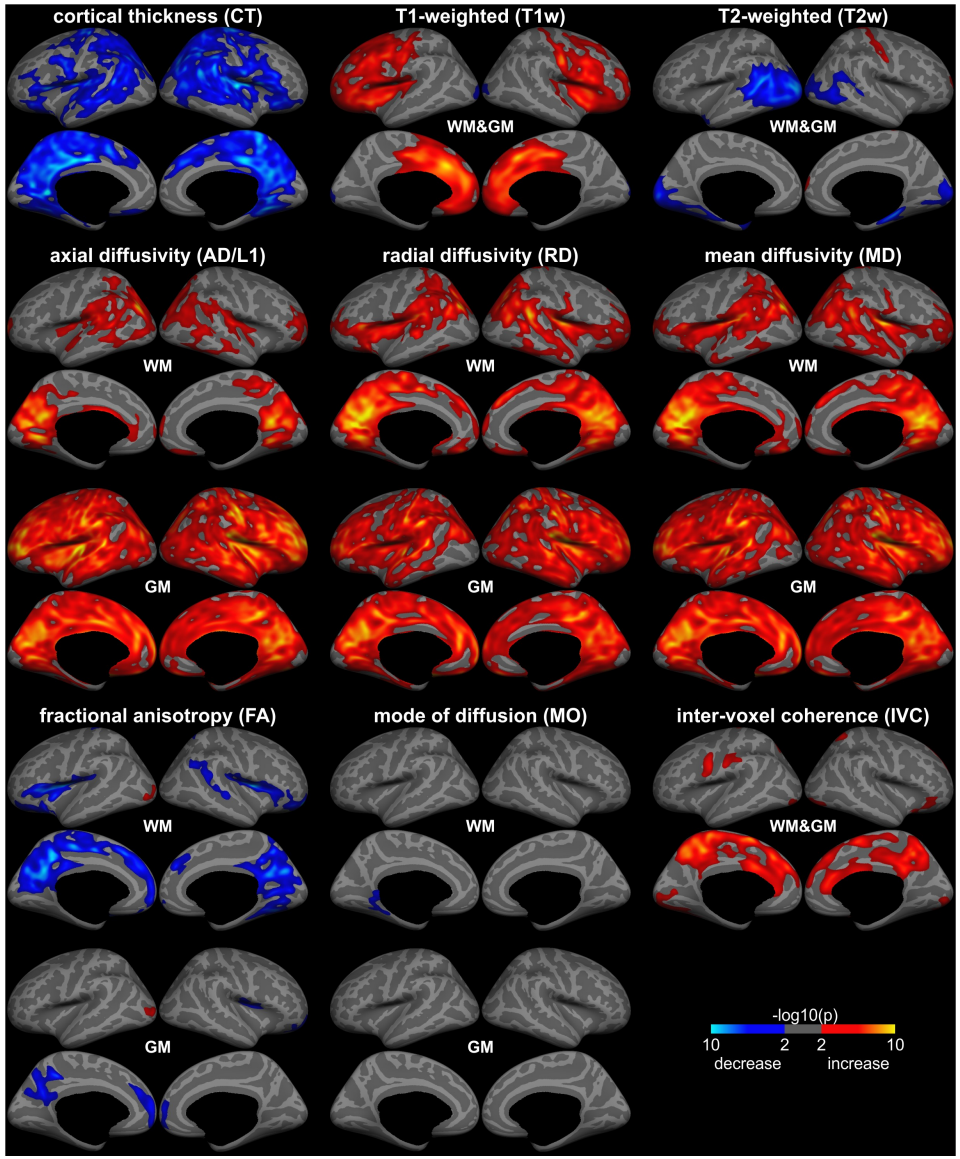
Linear decrease / increase by age was observed in

Tissue-weighted
- decrease in cortical thickness
- increase in T1w values
- decrease in T2w values

Diffusion-strength
- increase in AD
- increase in RD/MD

Diffusion-shape
- decrease in FA
- increase in IVC

Age-related changes in tissue-weighted and diffusion-weighted measures were regionally specific.

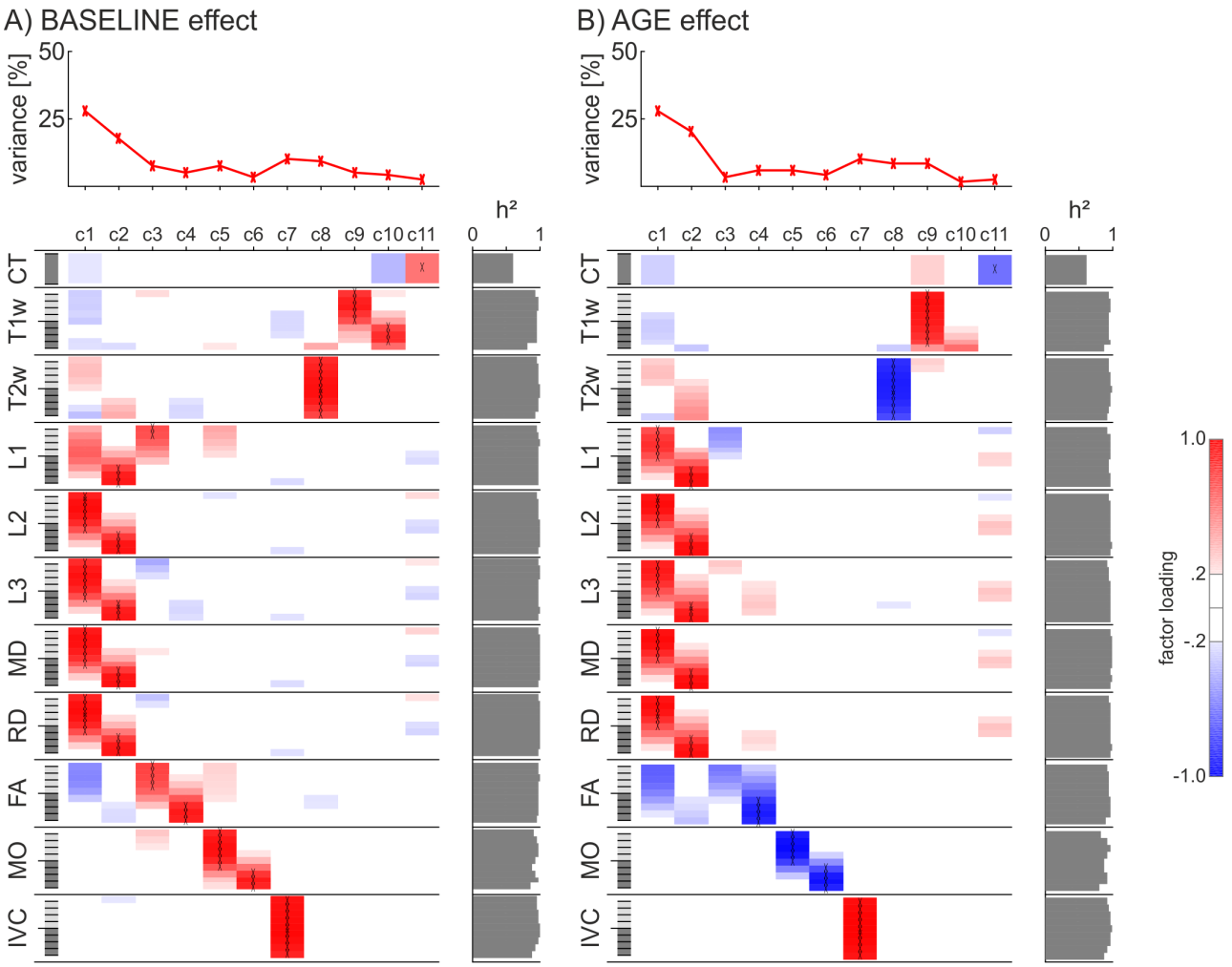


Surface-based Analysis of Microstructure

Example 2:
Independent measures

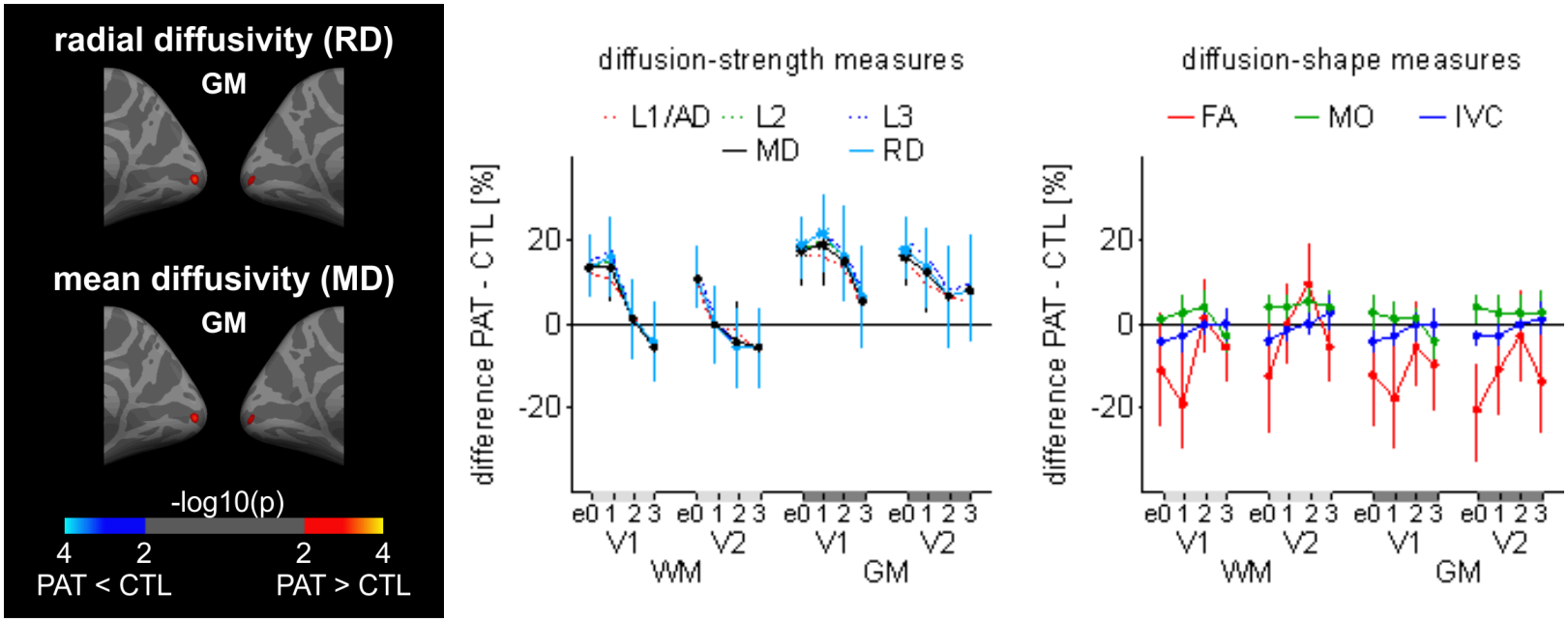
Factor analysis showed 11 components

- difference between SWM and GM
- difference between measures of diffusion-shape (e.g., FA, MO, IVC) and diffusion-strength (e.g., AD, RD, MD)
- cortical thickness (CT) not (only loosely) correlated with diffusion measures



Surface-based Analysis of Microstructure

Example 2:
Difference between patients (PAT) with macular degeneration and healthy controls (CTL)



- Reduced radial (RD) and mean diffusivity (MD) were observed in gray matter of posterior sections of the calcarine sulcus.

- Surface-based ROI analysis of primary (V1) and secondary (V2) visual cortex showed increased diffusion in representations of the central (e0, e1) but not peripheral (e2, e3) visual field.

Summary

- **Long-term and short-term brain plasticity**
  Diffusion-weighted imaging is sensitive to long-term (e.g., aging) and short-term (e.g., deprivation, learning) brain plasticity.
- **Various diffusion-based parameters**
  The various diffusion-based parameters are sensitive to different aspects of brain plasticity. Multivariate analysis separates measures that are correlated from those that are not.
- **Sensitivity of DWI versus conventional MRI**
  Diffusion-based parameters are more sensitive to various aspects of brain plasticity (distinguishing across brain regions and mechanisms) than conventional parameters of macro-structure (e.g., cortical thickness).

- **Several procedures for group comparisons**
  The different approaches for group comparisons of brain micro-structure and fiber tracking are each associated with strengths and limitations. Limitations need to be considered in order to find consistent and / or complimentary results.
- **Surface-based analysis**
  Surface-based analysis is a valuable alternative to conventional ROI-based, tract-based, or volumetric approaches.

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