Histological validation of neurite dispersion from diffusion MRI in MS

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January 31st, 2018

Workshop in Multiple Sclerosis

– Translating engineering innovation into the clinic –

Outline of the talk

- Background: quantitative imaging the spinal cord
- Neurite orientation dispersion: what is it?
- MRI-histopathology correlations
- Discussion and conclusions

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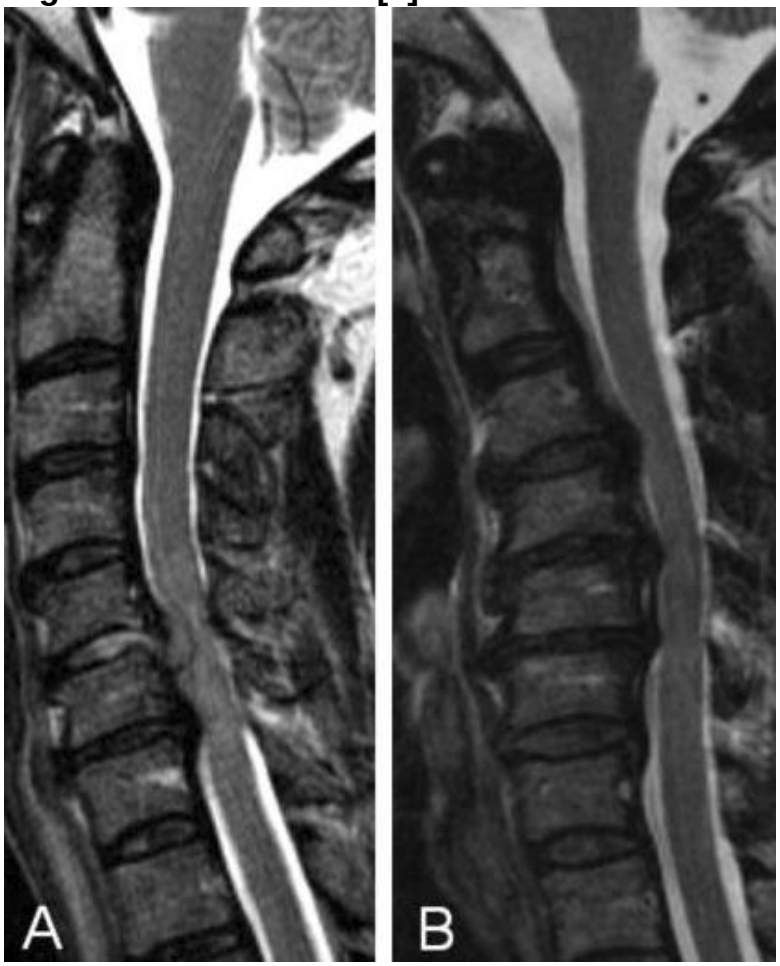
How could quantitative MRI be useful for spinal cord conditions?

Limits of conventional MRI

- Conventional readouts, i.e. signal hyper/hypointensities show certain value in prognosis or treatment/ surgery planning of conditions such as spondylosis, spinal cord injury, etc [1]

Figure from reference [2]



- Nonetheless, patients with similar radiological involvement often have strikingly different clinical outcomes.

[1] Wheeler-Kingshott et al, NeuroImage (2014), 84: 1082-93 [2] Salamon N et al, Spinal Cord (2013); 51(7): 558-563

Spinal cord and Multiple Sclerosis

- Spinal cord involvement in MS associated to high disability.

- Nevertheless, patients with similar number and locations of spinal lesions often exhibit different clinical syndromes.
- Prognosis is extremely complex, but necessary to choose the right treatment.

There is urgent need for more specific non-invasive indices of tissue damage that support more accurate prognoses and as outcome measures in clinical trials

Quantifying microstructural damage in MS

- Recent findings have shown that MS affects dendrite morphology, independently of other known pathological processes [1].

doi:10.1093/brain/awv353 BRAIN 2016: 139; 39–46 | 39

BRAIN
A JOURNAL OF NEUROLOGY

REPORT

Reconstruction of single cortical projection neurons reveals primary spine loss in multiple sclerosis

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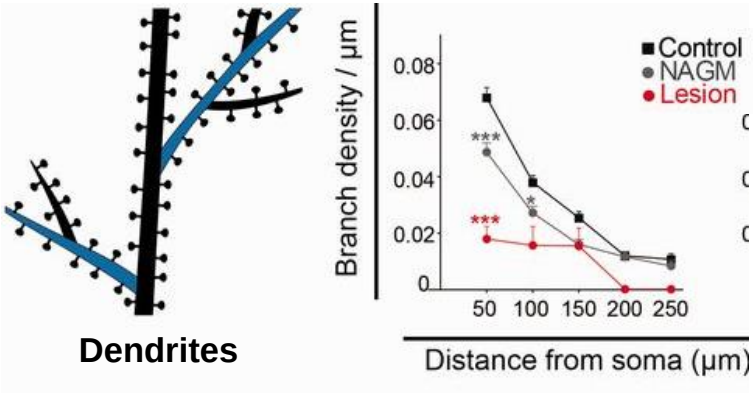

[1] Jürgens T et al, Brain (2016); 139(1), p39-46.

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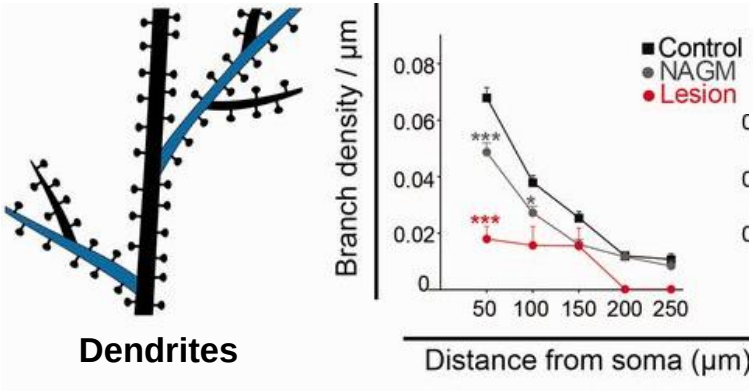

- There are layers of complexity that are not captured by the conventional way of looking at MS, via both histopathology and clinical MR imaging.

[1] Jürgens T et al, Brain (2016); 139(1), p39-46.

Neurite orientation dispersion: what is it?

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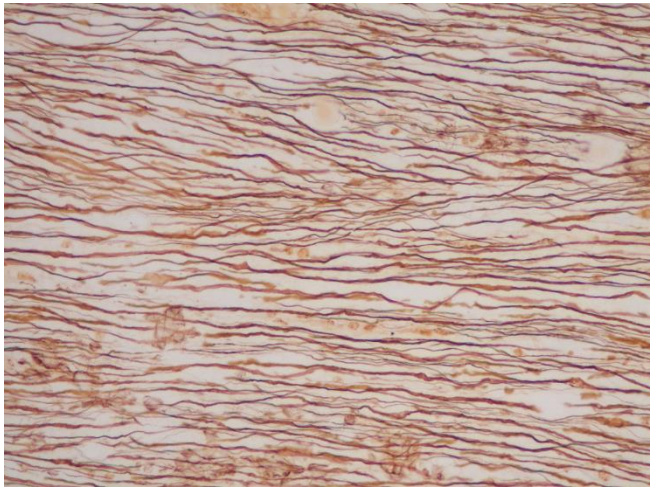
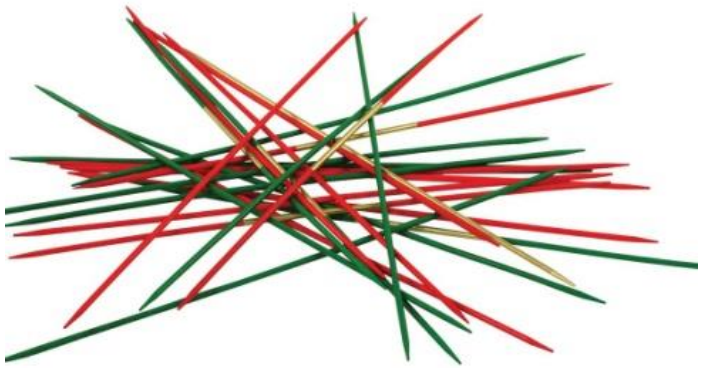
- *Neurite orientation dispersion* is a term employed to describe the **variability of axon and dendrite orientations within a volume**.

Sagittal image of spinal cord axons, Palmgren silver stain method

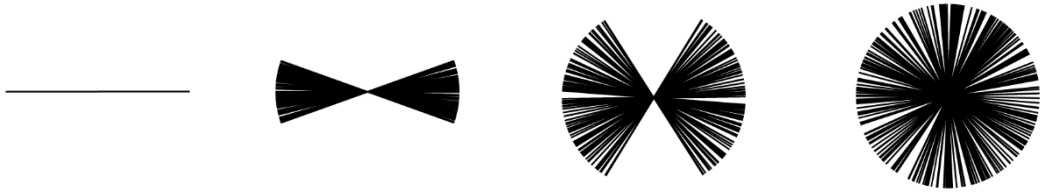


Mathematical model

Collection of sticks



Increasing orientation dispersion



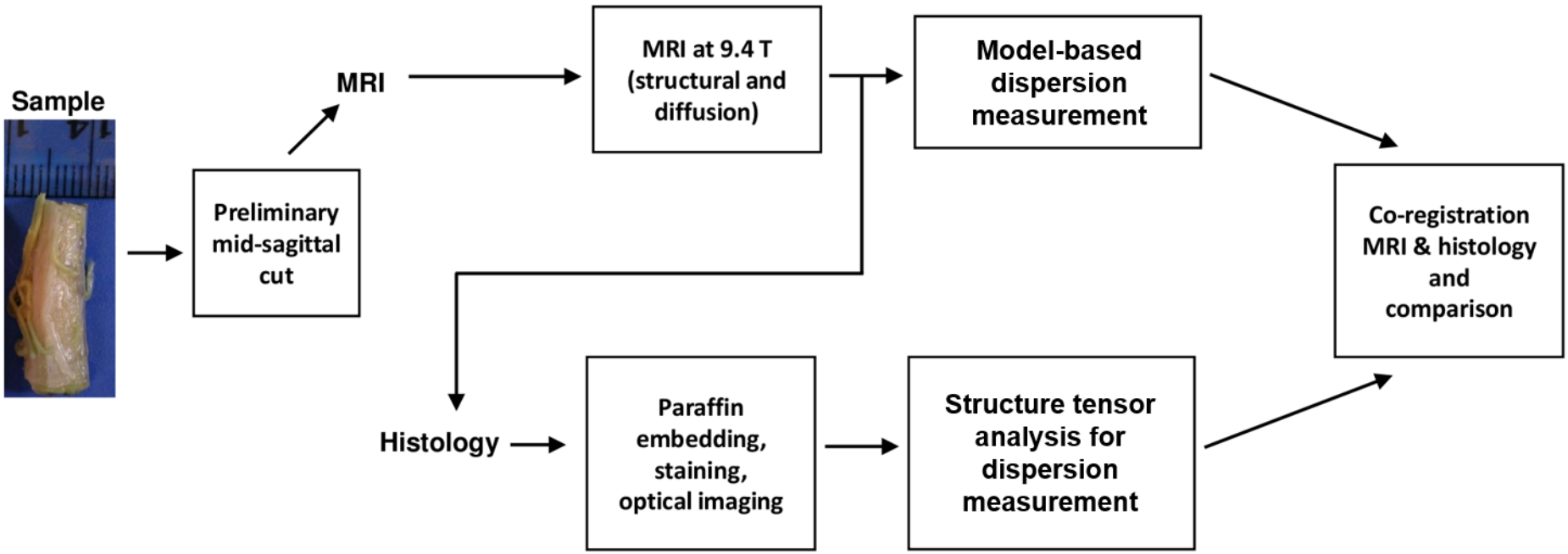
Neurite dispersion in MS

- We hypothesise that mapping the complexity of neurite architecture can provide new useful markers of MS pathology.

- We tested this hypothesis measuring directly variations in neurite configurations with histology and with MRI.
  - **Histology → ground truth**
  - **MRI → clinical potential**

MRI-histopathology correlations

Methods: MRI-histology pipeline



- Four fixed spinal cord specimens were analysed:
  - **two controls** (lumbar and thoracic levels);
  - **two MS cases** (PPMS; SPMS; lumbar and thoracic levels).
- Histology and MRI performed sagittally, as sagittal sectioning optimally shows the directions along which neural fibres run.

DW MRI Protocol

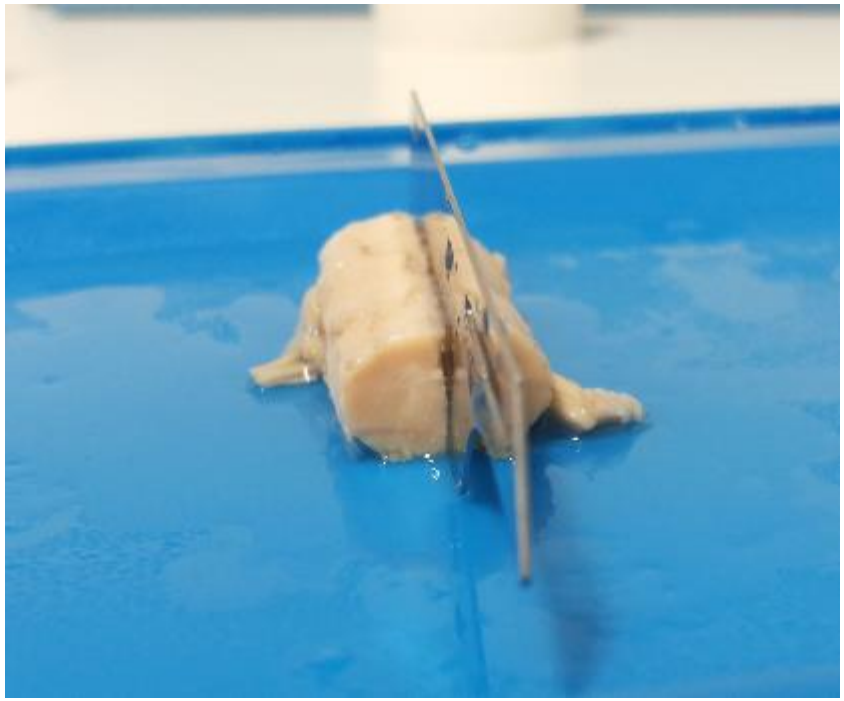
- Field strength: 9.4 T (Agilent system, small bore)
- b = {520, 2080, 4680, 8320, 13000, 18720} s mm^{-2}
- {6, 15, 24, 33, 42, 51} directions
- TE/TR = 39.5/2200 ms
- δ/Δ = 12/18 ms
- Gradient insert of 1T/m
- Resolution of 160 × 200 µm×µm; slice thickness of 800 µm

Histological protocol

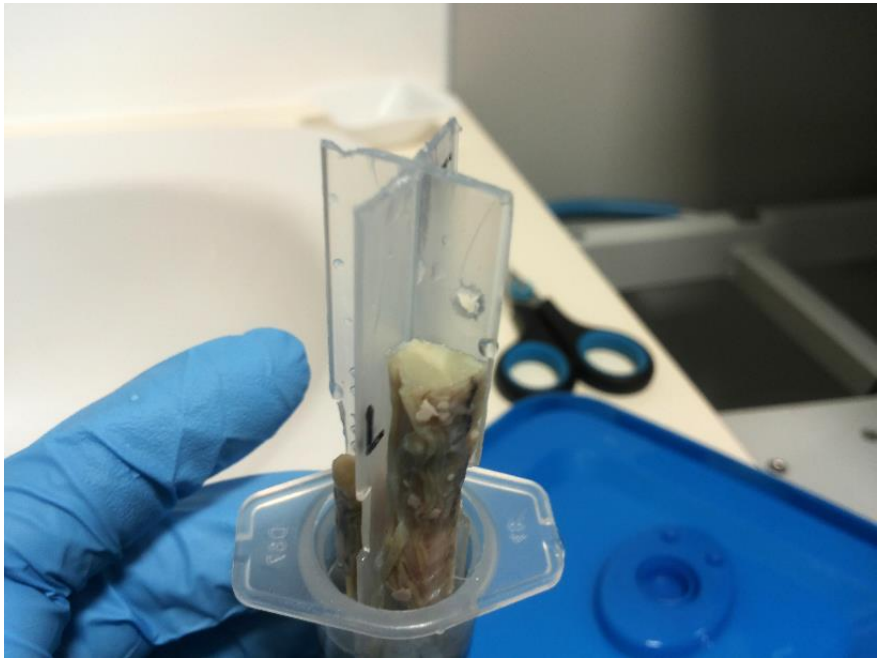
- 10 µm-thick sections, 2 per MRI slice, 200 µm apart, stained with:
  - **Palmgren Silver** →demonstrates neuros/axons
  - Phos/non-phos **neurofilament immunostain** → demonstrates neurons
  - **PLP immunostain** → demonstrates myelin
  - **GFAP immunostain** → demonstrates astrocytes
  - **Iba1+ immunostain** → demonstrates microglia
- Optical imaging of stained section performed with an Aperio slide scanner
- Resolution of 0.25×0.25 µm×µm (resampled at 1×1 µm×µm)

Matching histology and MRI (I)

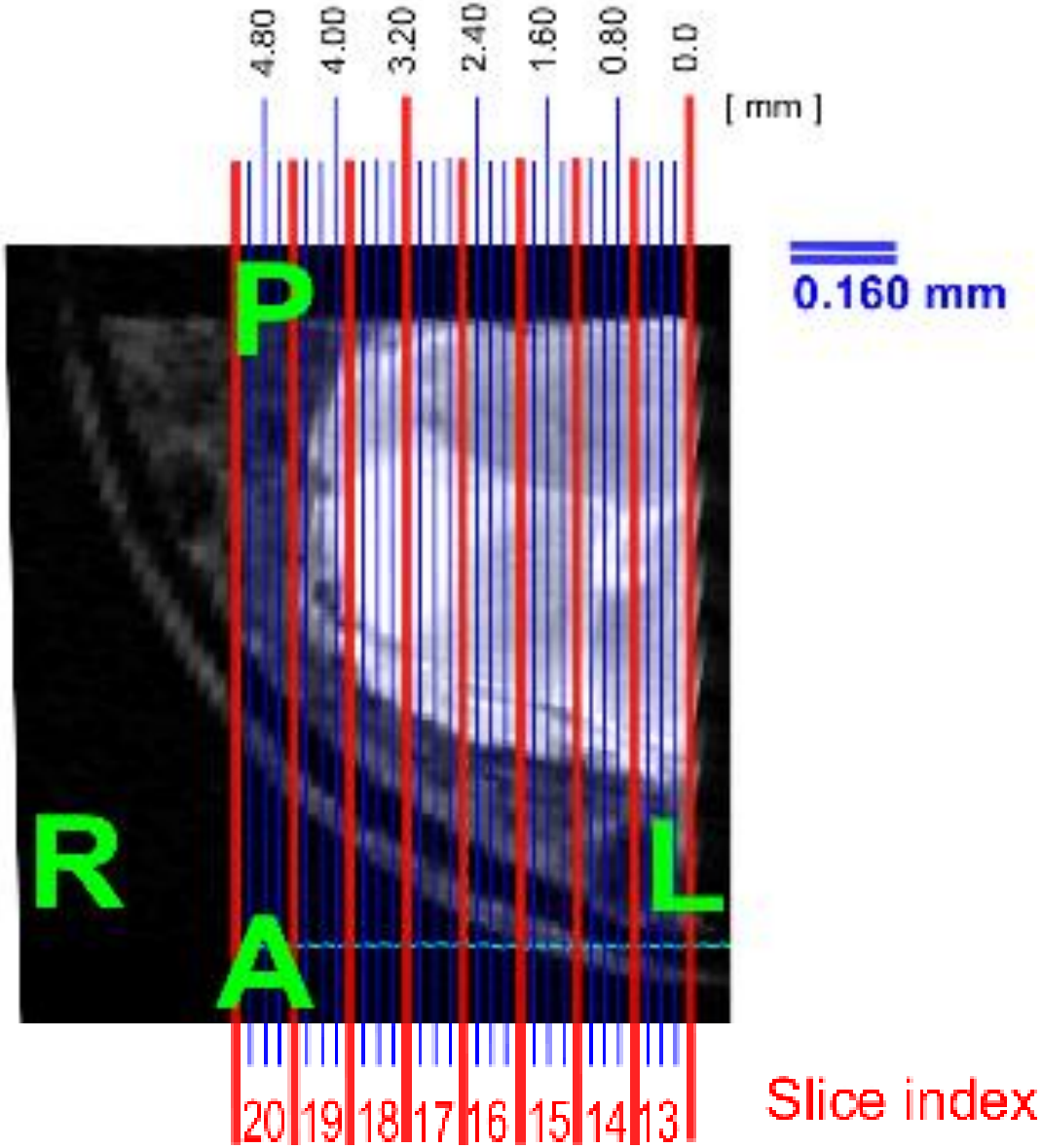
Preliminary mid-sagittal cut



Holder for MR imaging



Matching histology and MRI (II)



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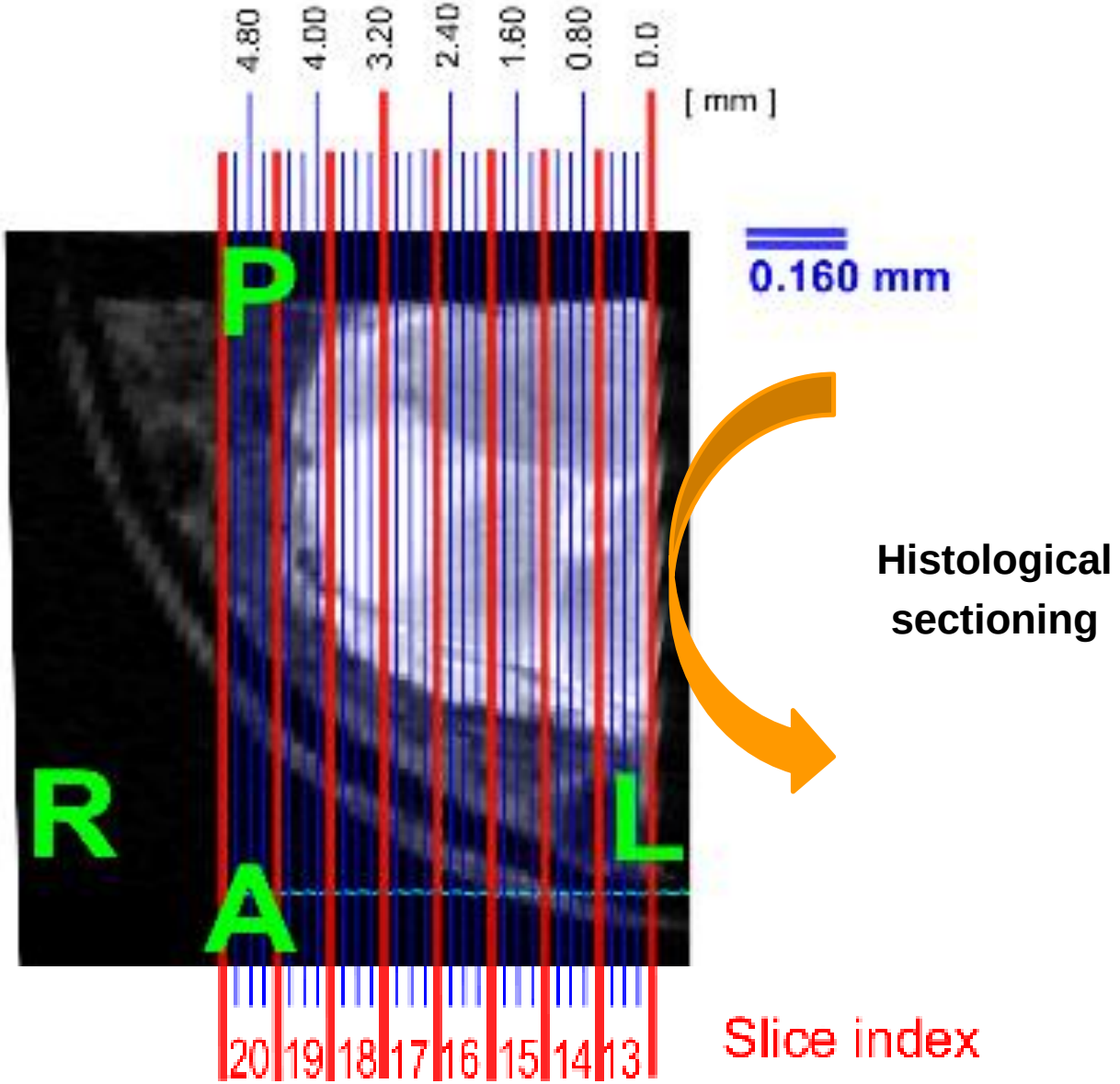
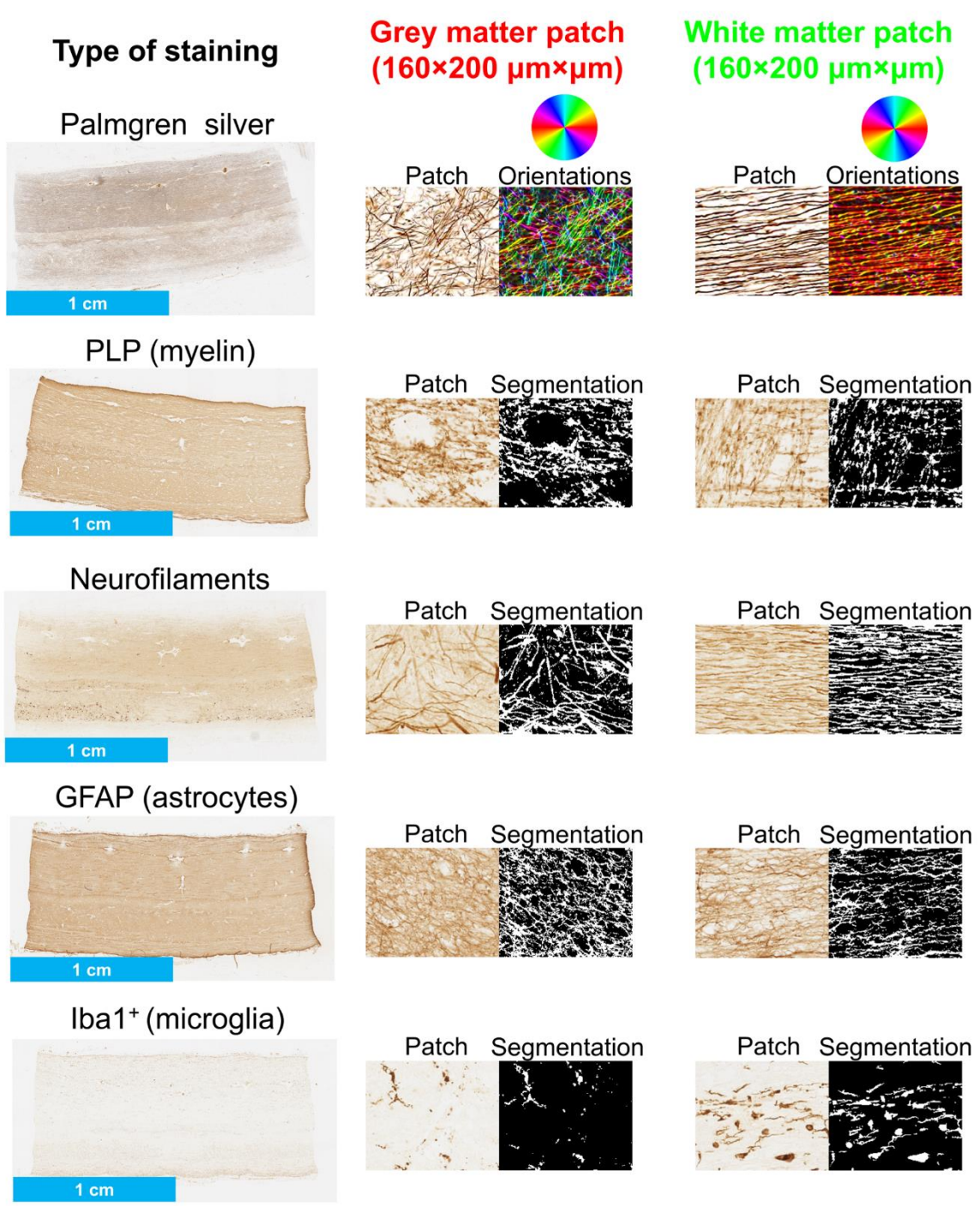




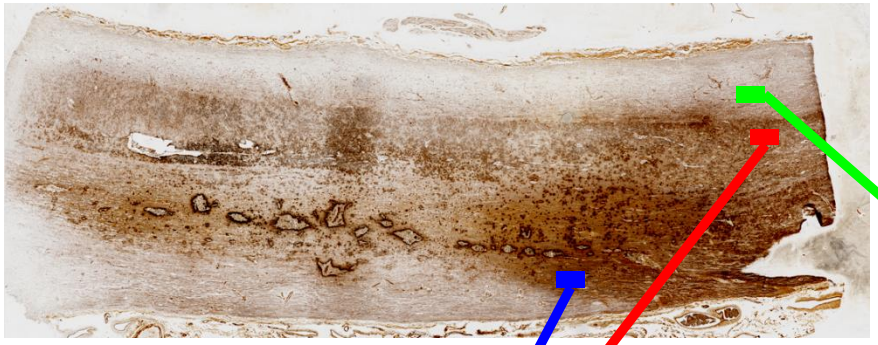
Histological features

- Histological features were evaluated within patches matching the MRI in-plane resolution

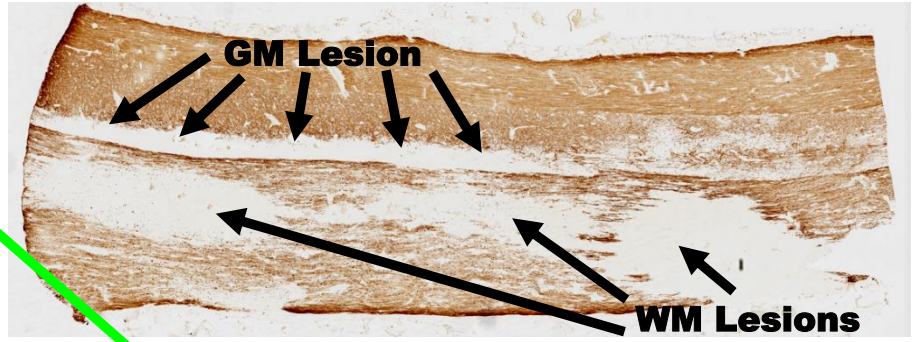
- Quantitative histological maps co-registered to MRI via manual non-linear registration.

Results: details from lumbar case, sagittal histology

Silver staining

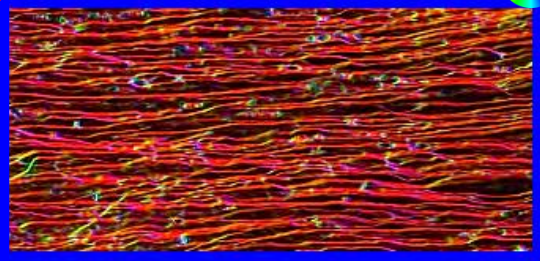


Myelin staining (PLP immunohistochemistry)



WM lesion: silver staining

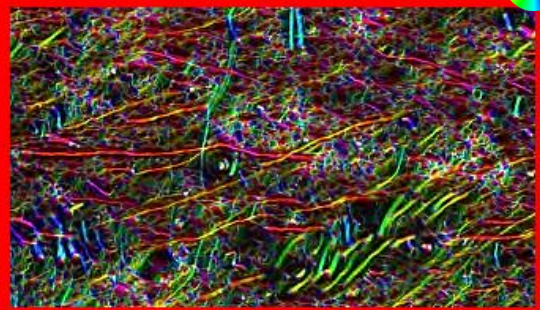
WM lesion: axonal orientations



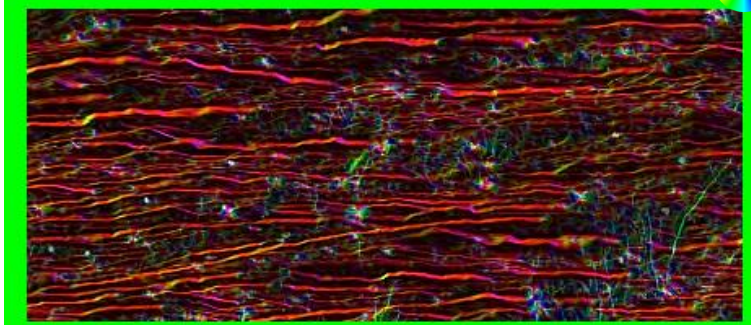
WM: silver staining

GM: silver staining

GM: dendritic orientations



WM: axonal orientations



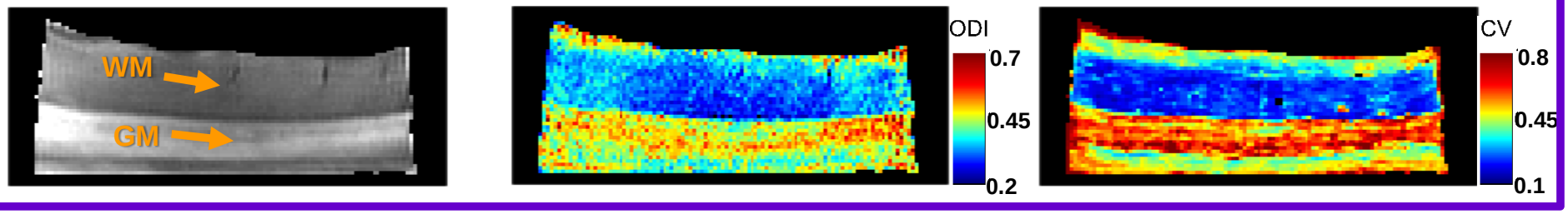
Summary: complete set of metrics

Metric	Abbreviation	Modality	Description
Orientation dispersion index	ODI	NODDI MRI	Variability of neurite orientations
Neurite density index	NDI	NODDI MRI	Amount of neurites
Isotropic volume fraction	IVF	NODDI MRI	Amount of free water
Circular variance	CV	Histology	Variability of neurite orientations
Myelin staining fraction	MSF	Histology	Amount of myelin
Neurofilament staining fraction	NSF	Histology	Amount of neurofilaments
Astrocyte staining fraction	ASF	Histology	Amount of astrocytes
Microglia staining fraction	μGSF	Histology	Amount of microglia

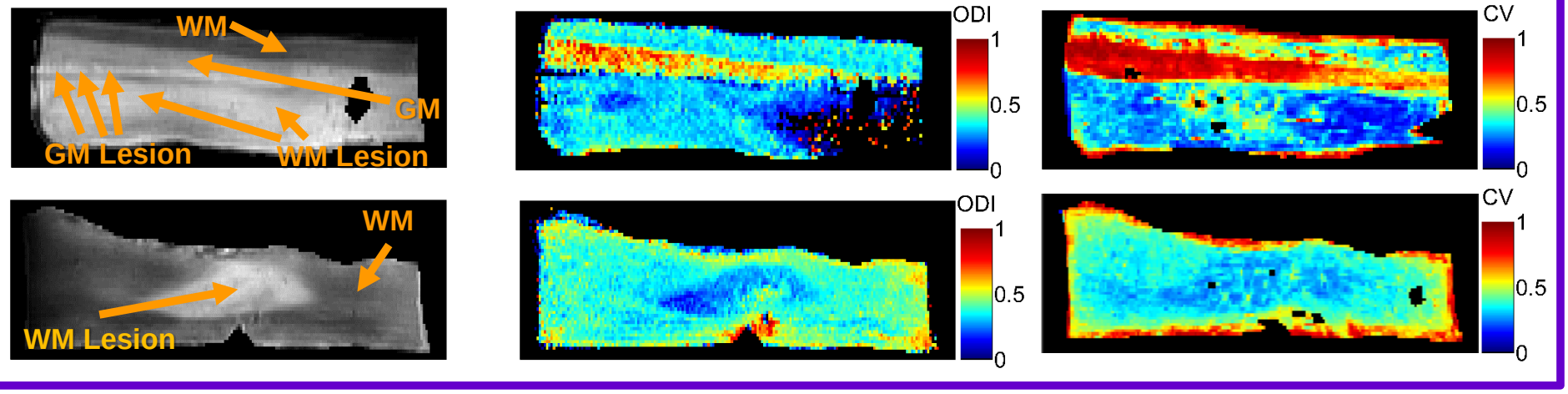
Results: NODDI ODI and histological CV

MRI *b* = 0 image (T2-w) **NODDI MRI dispersion (ODI)** **Histological dispersion (CV)**

Example in upper lumbar control

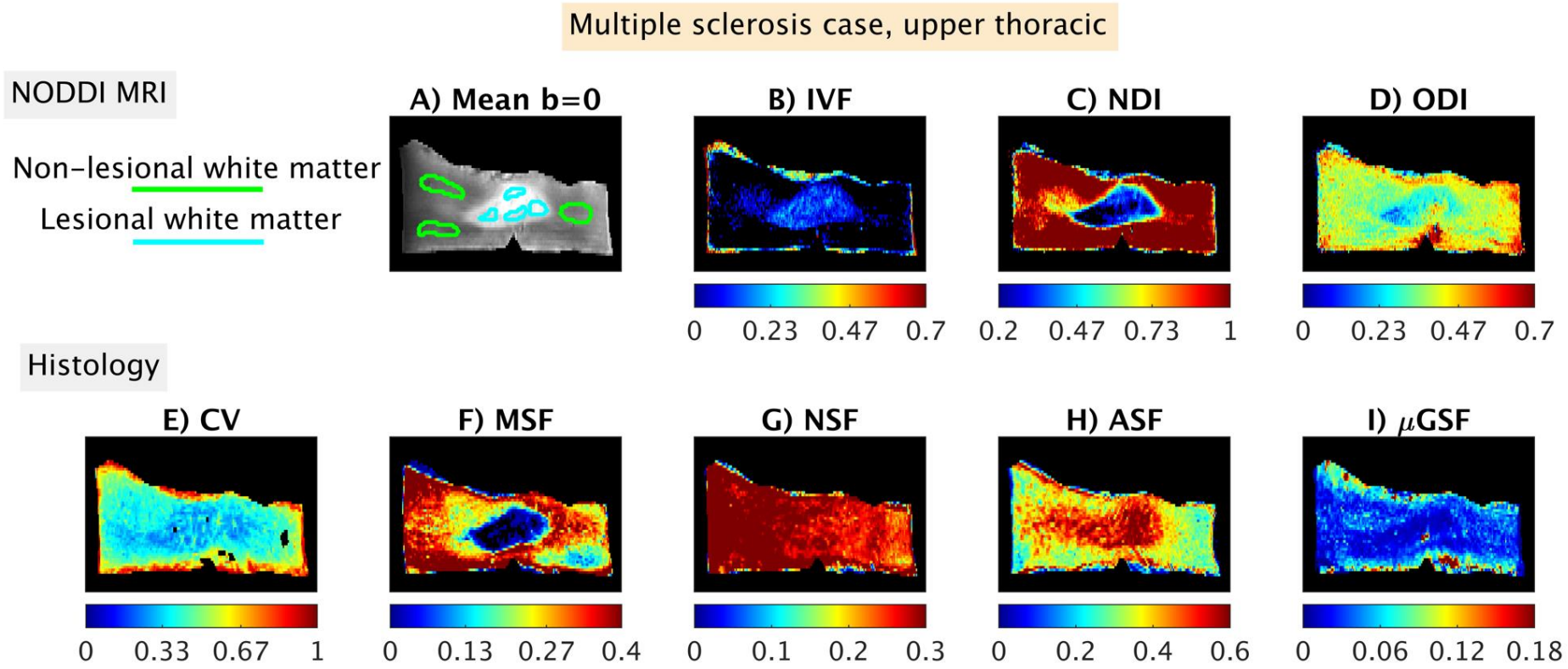


Examples in the two Multiple Sclerosis specimens



- Reduced ODI and CV in lesions → reduced neurite complexity.
- MRI-/histology-derived dispersion show very similar contrasts.

Results: all maps, multiple sclerosis case

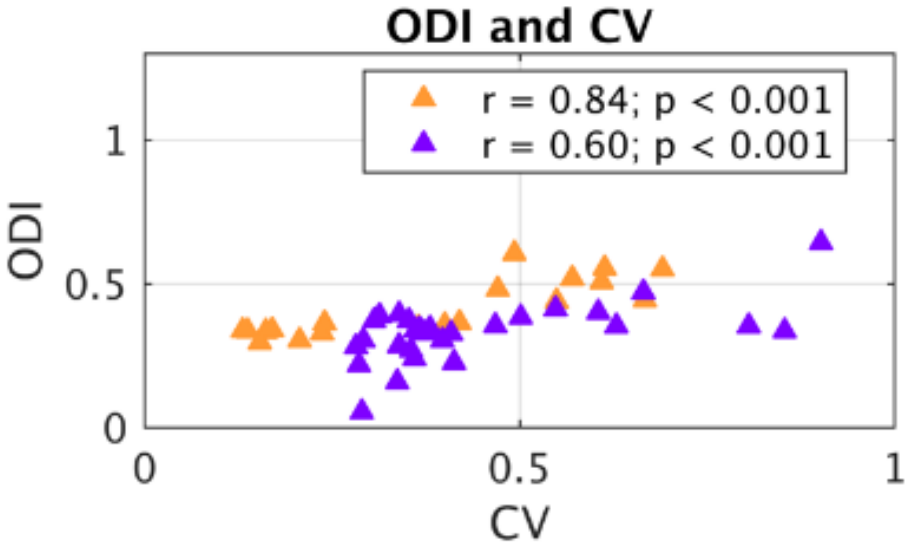


- Reduced dispersion in focal lesion, highlighted by both MRI/histology.
- Axons preserved in demyelinated lesion

  → NDI strongly influenced by changes in myelination.

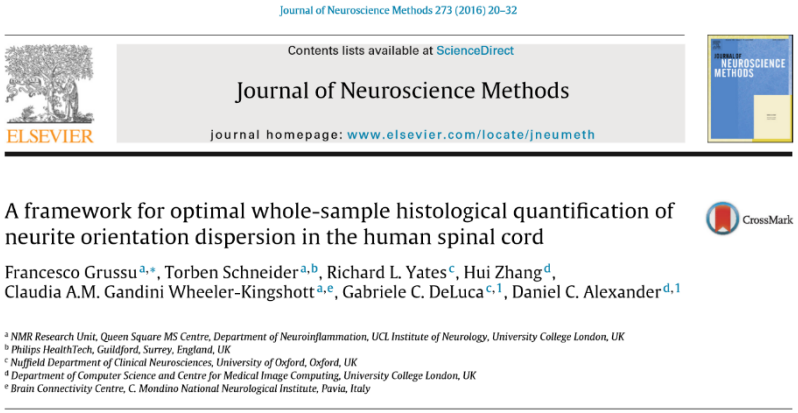
Correlations: neurite dispersion

- ODI sensitive and specific to histological dispersion in both controls/MS



Dissemination

- The Methods of our histological approach can be found here:



Journal of Neuroscience Methods 273 (2016) 20–32

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[e] Brain Connectivity Centre, C. Mondino National Neurological Institute, Pavia, Italy

- Full MRI-histology comparison available here:

ANNALS of Clinical and Translational Neurology

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RESEARCH ARTICLE

Neurite dispersion: a new marker of multiple sclerosis spinal cord pathology?

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[7]Brain MRI 3T Mondino Research Centre, C. Mondino National Neurological Institute, Pavia, Italy
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Research Highlights of Nature Reviews Neurology, Oct 2017

RESEARCH HIGHLIGHTS

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Microstructural pathology imaged in MS

> the complexity of axon and dendrite architecture carries a signature of MS pathology

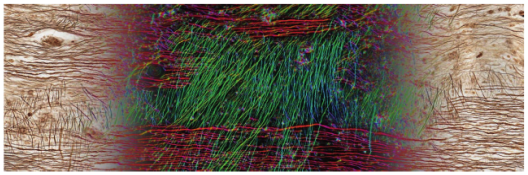
The variability of axon and dendrite orientations — termed neurite orientation dispersion — is reduced in the spinal cords of patients with multiple sclerosis (MS), according to new research. This finding could provide a new biomarker for MS prognosis and therapeutic efficacy.

The clinical manifestation, disease course, and severity of MS are highly variable, making the search for new biomarkers crucial. Although inflammation is a central hallmark in MS, other pathological mechanisms exist. "Recent work has demonstrated that the complexity of axon and dendrite architecture carries a signature of MS pathology. We therefore decided to test whether neurite orientation dispersion is a useful biomarker in MS, as to date, conventional MRI readouts provide limited information about clinical prognosis", explains Gabriele De Luca, co-senior author of the study with Gandini Wheeler-Kingshott.

The researchers used a state-of-the-art, clinically viable, MRI technique, called neurite orientation dispersion and density imaging (NODDI), and histological investigations, to study neurite architecture, in progressive MS and two from healthy controls. "Spinal cord tissue was analysed in a completely novel way, by looking at key neuropathological elements — astrocyte, microglia, myelin and neurofilament density — from a sagittal view, to get a better idea of the architecture of nerve cells", explains De Luca. Analyses showed reduced orientation variability of neurites within focal areas of demyelination in MS, with NODDI and histology, indicating reduced branching or morphological alterations of individual axons in white matter, and reduced complexity of dendritic arborisations in grey matter, compared to non-lesional tissue.

"NODDI-derived dispersion was sensitive and specific to its histological counterpart, which sets the stage for understanding clinically relevant nerve fibre-related changes in MS that could only be detected previously post-mortem" De Luca concludes. The research team now intends to apply these imaging techniques to people with varying degrees of MS, to determine if they have a clinical correlate.

Mitesh Patel



A sagittal spinal cord section (top of spinal cord to left) stained with the Palmgren's silver method, which labels axons dark brown. In the centre, axons have been coloured according to their orientation, calculated by Structure Tensor Analysis (STA). The local variability of these orientations at the MRI voxel scale represents orientation dispersion. Image courtesy of F. Grussu.

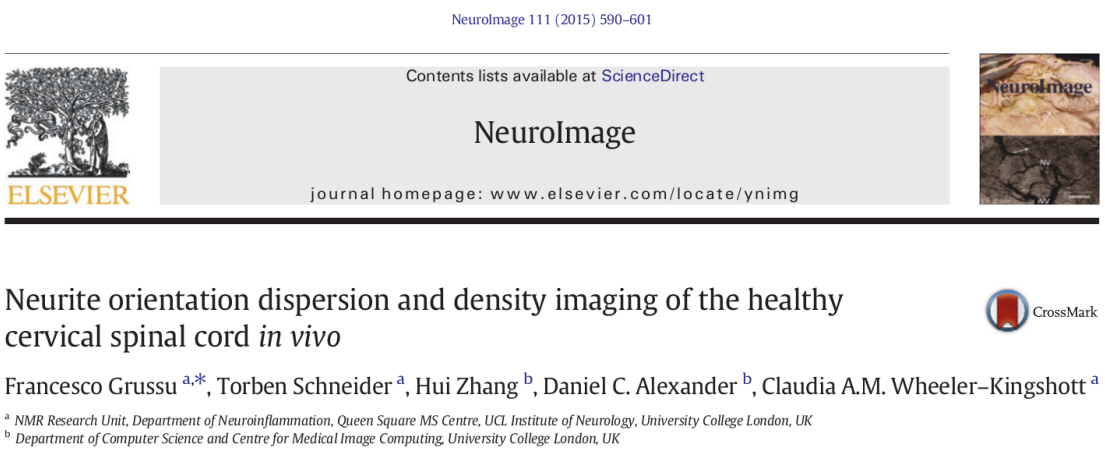
ORIGINAL ARTICLE Grussu, F. *et al.* Neurite dispersion: a new marker of multiple sclerosis spinal cord pathology? *Annals of Clinical and Translational Neurology*. http://onlinelibrary.wiley.com/doi/10.1002/acn3.445/full (2017)

Discussion and conclusions

Discussion

- For the first time, we have measured with state-of-the-art histology changes in neurite architecture caused by MS spinal cord pathology
  - MRI is a sensitive and specific tool to measure these changes non-invasively

- Neurite dispersion measurements are feasible in vivo → a new powerful biomarker of microstructural damage?



NeuroImage 111 (2015) 590–601

Contents lists available at ScienceDirect

NeuroImage

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Neurite orientation dispersion and density imaging of the healthy cervical spinal cord *in vivo*

Francesco Grussu [a,*], Torben Schneider [a], Hui Zhang [b], Daniel C. Alexander [b], Claudia A.M. Wheeler–Kingshott [a]

[a] NMR Research Unit, Department of Neuroinflammation, Queen Square MS Centre, UCL Institute of Neurology, University College London, UK
[b] Department of Computer Science and Centre for Medical Image Computing, University College London, UK

- Techniques such as NODDI only provide a partial picture
  - it cannot disentangle between demyelination and axonal loss

Conclusions

- Quantifying microstructure in the spinal cord with MRI is difficult
  - acquisition
  - image analysis
- Nonetheless, it could lead to paradigm shifts in current clinical practice
  - research must continue, and hopefully the spinal cord community will keep expanding
- New exciting developments will follow in the years to come

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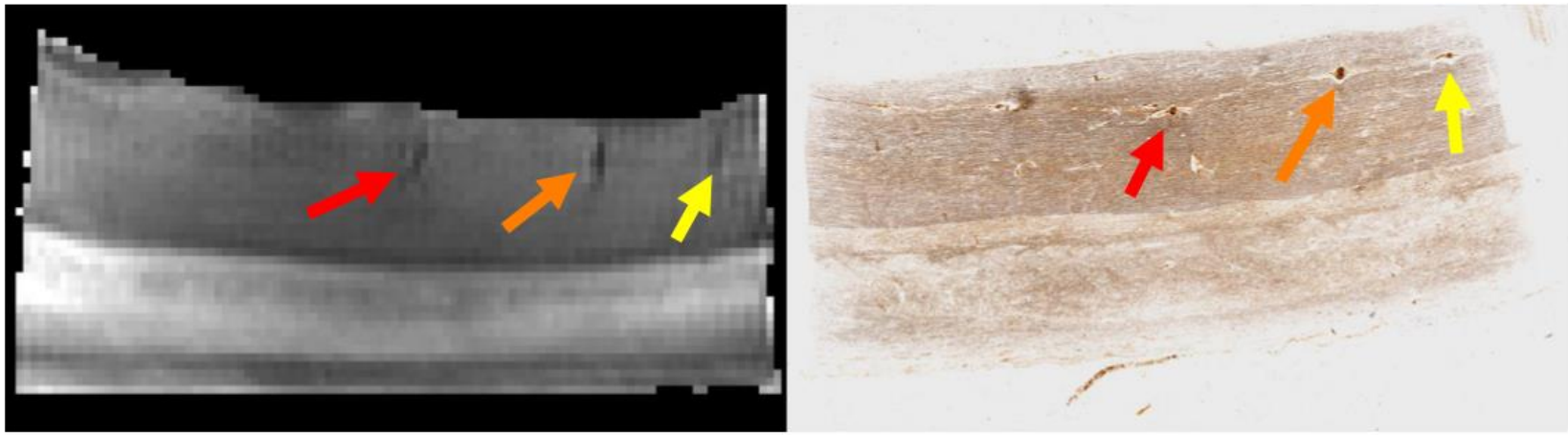
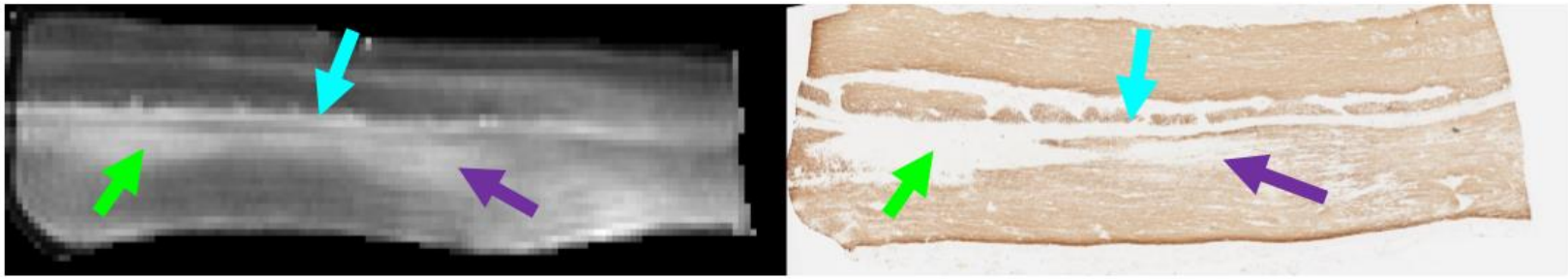
Thank you!

Questions?

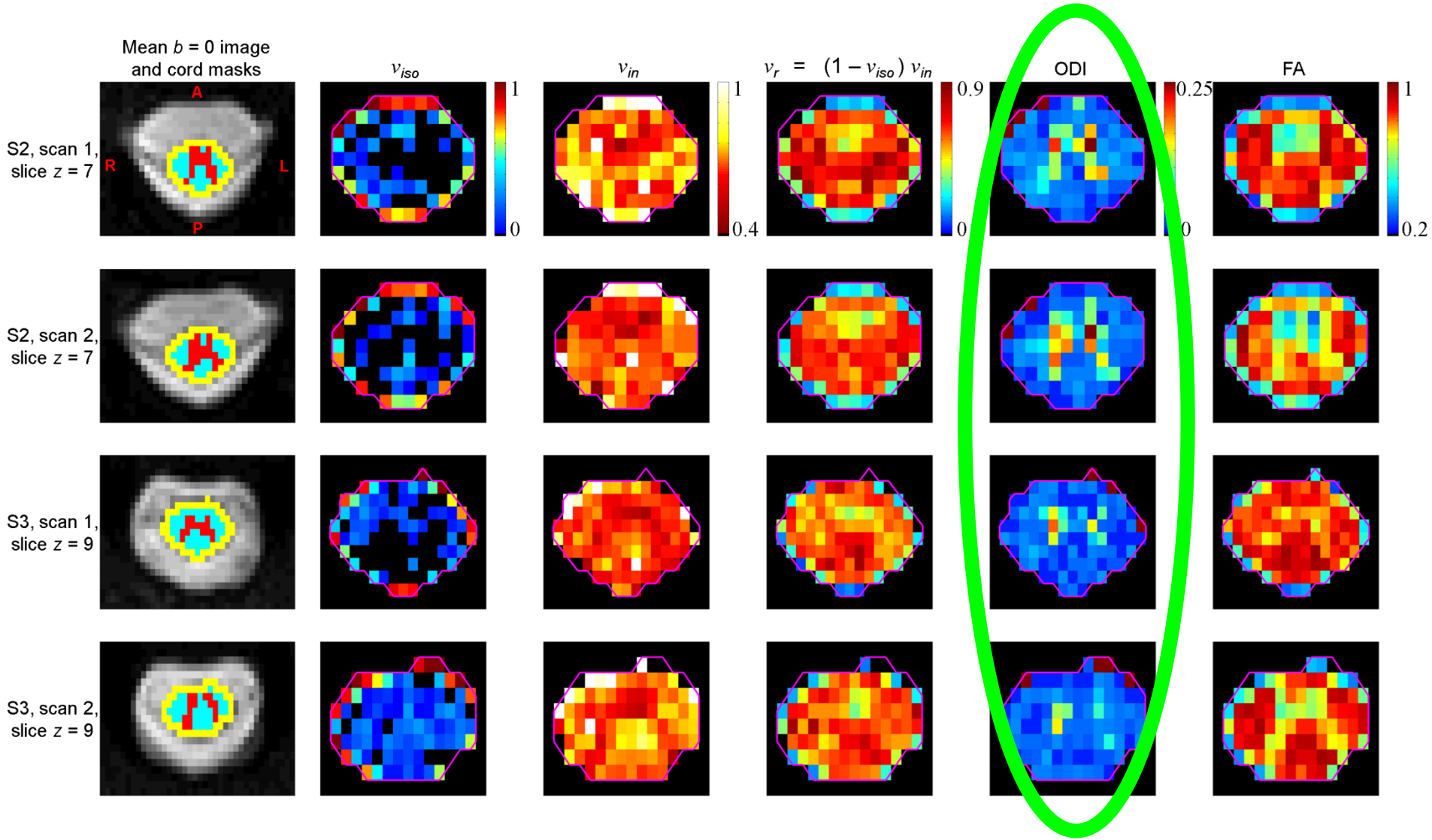
Supplementary material

Spatial correspondences MRI-histology

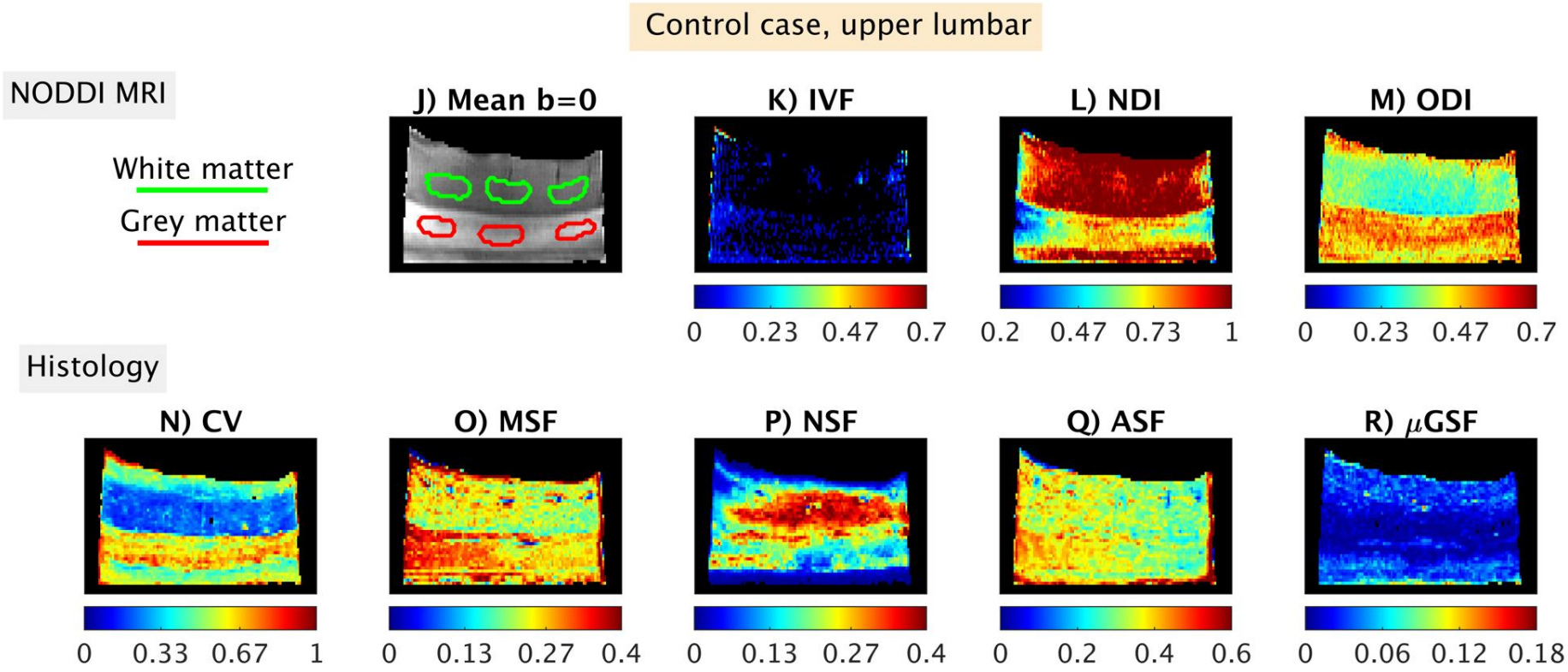




NODDI metrics in the healthy cord in vivo



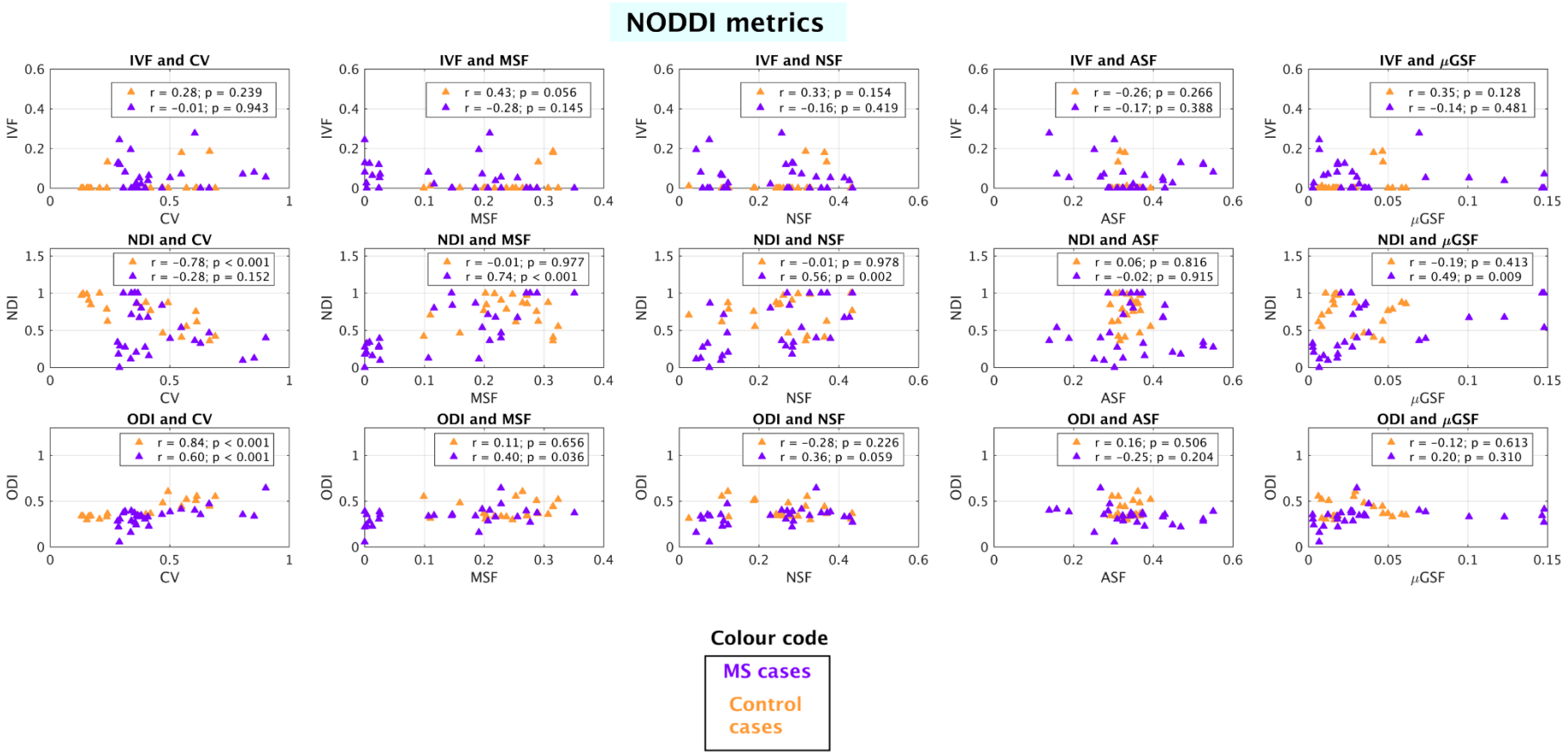
NODDI metrics in the healthy cord ex vivo



- NODDI metrics show known trends.
- All histological indices but CV are relatively flat between GM/WM.

Correlations: NODDI metrics

- ODI sensitive and specific to histological dispersion in both controls/MS
- NDI sensitive to neurofilament density, but also influenced by the myelination level



Correlations: conventional DTI metrics

- DTI metrics sensitive but rather non-specific
- DTI metrics fail to correlate with histological dispersion in MS

Colour code
MS cases
Control cases