Improved reproducibility of diffusion MRI of the human brain with a four-way blip-up and down phase-encoding acquisition approach

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Purpose: To assess the effects of blip-up and -down echo planar imaging (EPI) acquisition designs, with different choices of phase-encoding directions (PEDs) on the reproducibility of diffusion MRI (dMRI)-derived metrics in the human brain.

Methods: Diffusion MRI data in seven subjects were acquired five times, each with five different protocols. The base design included 64 diffusion directions acquired with anterior-posterior (AP) PED, the first and second protocols added reverse phase-encoded $b=0\,\text{s}/\text{mm}^2$ posterior-anterior (PA) PED images. The third one included 32 directions all with PED acquisitions with opposite polarity (AP and PA). The fourth protocol, also with 32 unique directions used four PEDs (AP, PA, right-left (RL), and left-right (LR)). The scan time was virtually identical for all protocols. The variability of diffusion MRI metrics for each subject and each protocol was computed across the different sessions.

Results: The highest reproducibility for all dMRI metrics was obtained with protocol four (AP/PA-RL/LR, ie, four-way PED). Protocols that used only $b=0\,\text{s}/\text{mm}^2$ for distortion correction, which are the most widely used designs, had the lowest reproducibility.

Conclusions: An acquisition design with four PEDs, including all DWIs in addition to $b=0\,\text{s}/\text{mm}^2$ images should be used to achieve high reproducibility in diffusion MRI studies.

KEYWORDS
artifacts, diffusion MRI, distortion correction, EPI, reproducibility

1 INTRODUCTION

Quantitative diffusion MRI (dMRI) acquisitions, including diffusion tensor imaging (DTI)¹² and high angular resolution diffusion imaging (HARDI)³⁻⁹ are used extensively to investigate architectural, microstructural, and compositional features of the human brain. However, obtaining reproducible and accurate dMRI results is challenging given that diffusion-weighted images (DWIs), which are collected using echo planar imaging (EPI), are susceptible to various
artifacts (see Ref. [10] for a review). DWI artifacts originate from ghosting, Gibbs ringing, signal drop outs, misalignment due to motion, eddy-currents distortions, as well as abnormal morphology due to distortions produced by static $B_0$ field inhomogeneities such as magnetic susceptibility variations, imperfect shimming, and concomitant fields.12

EPI distortions have been shown to have a significant impact on the accuracy of tensor-derived scalar maps13,14 and fiber tractography,15 and can negatively impact consistency of results in multicenter studies.16 Therefore, proper EPI distortion correction in preprocessing is likely to be relevant to improve dMRI reproducibility both for multi- and single-site studies.17,18 Correction of EPI distortions generally requires the acquisition of additional data, such as field-mapping11 or structural images for elastic registration.14,19,20 Earlier studies acquired dMRI using a single phase-encoding (PE) direction, most commonly anterior-posterior (AP) or posterior-anterior (PA) directions, and EPI distortion correction was either not performed (ADNI1) or performed using field mapping (ADNI3). More recently, reversed PE, or blip-up blip-down PE EPI distortion correction methods,17,21-24 have been adopted in diffusion MRI demonstrating generally superior performance to field-mapping in preprocessing. Although it has been shown that it is advantageous to use blip-up blip-down PE for the entire set of DWIs,17 the most commonly used approach is to acquire blip-up and -down data only for the non-diffusion-weighted, $b=0$ s/mm$^2$ volumes.22,24 Large imaging studies including the ABCD study25 and the UK Biobank26 performs blip-up and blip-down acquisitions only on the $b=0$ s/mm$^2$ volumes. The Human Connectome Project (HCP) protocol27 adopted blip-up and blip-down acquisitions for all DWIs (LR/RL directions). The Developing Human Connectome Project28 implemented a strategy with diffusion MRI acquisitions spread over all four PE directions (AP, PA, RL, and LR).

In this work, we evaluate the effects of using different PE schemes for EPI distortion correction and artifact reduction on the reproducibility of diffusion MRI results. We analyze the reproducibility of dMRI-derived metrics across several scanning sessions in the human brain of healthy subjects. In each session, we acquired a base protocol with all volumes collected with a single direction of PE (eg, AP, blip-up only) in which distortion correction was not performed. In the remainder of this manuscript, we refer to this protocol as Protocol0. Then we added commonly used PE schemes for collecting blip-up blip-down datasets, namely: (a) all DWIs collected with a single PE direction adding a single $b=0$ s/mm$^2$ volume collected with reversed PE (eg, PA, blip-down), (b) same as (a) but adding six $b\approx0$ s/mm$^2$ volumes, instead of a single one, collected with reversed PE, (c) all volumes (diffusion gradients and b-values) collected as matching pairs between the blip-up and -down data, and (d) a “four-way” acquisition scheme that consists of volumes acquired with the same diffusion gradients and b-values of (c) but with the volumes partitioned equally in two perpendicular PE orientations with again matching pairs of both blip-up and -down volumes (ie, AP, PA, left-right (LR), and right-left (RL)). All protocols had virtually identical scan time.

Our working hypothesis was that the reproducibility of diffusion MRI results for the various AP-PA protocols would increase following the order in which we listed them above. In particular, we expect Protocol3 to outperform the other AP protocols given its previously demonstrated distortion correction robustness.17 The four-way PE protocol (AP, PA, RL, and LR) is also a promising candidate to manifest superior reproducibility, because it would combine the good distortion correction performance of using reverse phase-encoded data for all DWIs with potentially less imaging artifacts. Given that ghosting artifacts manifest themselves at different spatial positions on images acquired with different PE directions, the four-way PE protocol data would suffer from such artifacts to a lesser extent. For instance, images acquired with RL PE would be devoid of ghosting artifact originating from the eyes and other anatomical structures ventral to the brain. These differences in spatial positions of ghosting artifacts in the four-way PE protocol would also open the intriguing possibility (which we did not explore in this work) of performing a selective editing of ghosting artifacts when combining DWIs after distortion correction.

## Methods

### 2.1 Details of acquisition protocols

**Protocol 0:** The first acquisition protocol is possibly still the most commonly used, where the entire dataset is acquired with a single PE direction: AP. With such a dataset, motion and eddy-currents distortions can still be corrected; however, other EPI distortions can only be corrected through either image registration14,19,20 or fieldmapping11 if appropriate data are present, but not with blip-up blip-down approaches. In our experiments, no distortion correction was performed for this protocol, which constitutes our baseline.

**Protocol 1:** A single $b=0$ s/mm$^2$ is added to Prot0 with PA PE direction to enable blip-up blip-down distortion correction. **Protocol 2:** In addition to the single $b=0$ s/mm$^2$ of Prot1, five $b=50$ s/mm$^2$ images with PA PE direction are included in the blip-down dataset. This Protocol is conceptually identical to Prot1; however, EPI distortion correction should be more robust because it is less susceptible to the imperfections of a single image.

**Protocol 3:** The same diffusion gradients and b-values are used to acquire both the AP and PA datasets. After EPI distortion correction, the two datasets are merged into one by pairwise geometric averaging of corresponding volumes.17,29 To keep scan times identical to the previous protocols, the number of acquired volumes is halved for each PE direction compared to Prot0.
Protocol 4: This proposed design uses four PE directions: AP, PA, RL, and LR. AP and PA pairs have identical experimental designs to yield the APPA corrected dataset, as does RL and LR. However, the RL/LR pair is optimized to have different but complementary diffusion gradients to AP/PA. Both the AP/PA and RL/LR diffusion gradients are near-optimal in terms of electrostatic repulsion force by themselves; therefore, they can be used independently if desired, but when combined together, they yield the actual optimum set. Even though the AP dataset alone has half the number of volumes compared to Prot3, the effective number of gradient directions in the processed and combined APRL dataset is identical to the APPA data of Prot3.

2.2 | Dataset

Diffusion MRI data were collected from seven healthy subjects (four males and three females; mean age = 28 years, std age = 3.6), five or six times, over a period of 6 months using a Philips Achieva 3T MRI System. The study was carried out under Institutional Review Board (IRB) approved protocols and all volunteers provided informed consent prior to examination.

Acquisitions were performed using a 32-channel head coil and a SENSE factor of 2 with no simultaneous multislice. For each design, half of the diffusion gradients sampled the northern diffusion hemisphere and half the southern hemisphere to minimize the effects of imaging gradients and eddy-currents on b-matrices. For each protocol, two datasets were acquired: one with a maximum b-value of $1100 \text{s/mm}^2$ and one with a maximum b-value of $2700 \text{s/mm}^2$ to enable HARDI analysis. DWIs for the DTI regime were acquired with 2 mm isotropic resolution. To achieve identical echo and repetition times among all scans (TE/TR: 92/12875 ms), the HARDI data were acquired at 2.6 mm isotropic resolution. A square field of view was used for all acquisitions to have identical echo train length for both AP and RL PE.

For Prot0, the experimental design included 12 low-$b$ s/mm$^2$, 8 $b = 300 \text{s/mm}^2$, and 64 maximum b-value images (1100 and 2700) in the AP PE direction. For Prot3, which included full AP and PA acquisitions, the number of volumes were halved for each shell to achieve identical scan times. The low-$b$ s/mm$^2$ images of this PA dataset were used to realize the experiments of Prot1 and 2. For Protocol4, the same gradients and b-values used for Prot0 were near-optimally split between AP and RL sets. In addition to dMRI,

**Figure 1** Mean diffusivity maps for a single subject for all four phase-encoding directions. All data were corrected for motion and eddy-currents but not other EPI distortions. The region indicated by red arrows display artifactual MD values for AP and PA phase-encoded data. For the same regions, LR and RL phase-encoded data seem artifact-free. Similarly, the blue arrow displays the location of a ghost artifact that affect the MD values in RL-encoded data. For this region, the other three datasets do not exhibit this ghost artifact.
fat-suppressed T2W TSE and T1W MPRAGE images were also acquired as structural images. The total scan session for all protocols and the anatomical images took one hour and forty minutes.

To illustrate the level of distortions and artifacts in the data, Figure 1 displays the mean diffusivity (MD) images of one subject at two slice levels for all four PE directions. These datasets were corrected only for motion and eddy-currents distortions but not for other EPI distortions. In this figure, regions indicated by red arrows have artifactual MD values for AP and PA phase-encoded data, whereas LR and RL data do not exhibit this behavior. On the contrary, the blue arrow displays the location of a ghost artifact on the RL data and for this region, the other three phase-encoded data are not affected.

2.3 | DWI preprocessing

DWI preprocessing was performed separately using two pipelines, TORTOISE\(^{31}\) and FSL\(^{32}\) to verify that conclusions about the reproducibility of each design were not highly pipeline dependent. Both pipelines were used with their latest available feature sets at the time of writing. With the TORTOISE pipeline, the voxelwise B-matrices due to gradient nonlinearities were initially generated,\(^{33}\) then DWIs were corrected for Gibbs ringing artifacts\(^{34}\) and subsequently for motion and eddy-currents distortions\(^{35}\) and for other EPI distortions\(^{17}\) while reorienting the voxelwise B-matrices accordingly. For the FSL pipeline, susceptibility distortion correction was performed initially\(^{21}\) followed by motion and eddy-currents distortion correction\(^{36}\) including outlier rejection,\(^{37}\) slice-to-volume registration,\(^{38}\) and per-volume re-estimation of susceptibility.\(^{39}\)

For both pipelines, for protocols with unmatched blip-up and down-data (Prot1 and Prot2), the Jacobian-modulated signals were output in the corrected images. For protocols with matching blip-up and blip-down datasets (Prot3 and Prot4), these two datasets were combined into a single one by pairwise averaging of corresponding corrected volumes. For Prot4, the APPA and RLLR corrected data were simply concatenated.

To provide a common space for longitudinal analysis, the T2W structural image of the first scan session for each subject was manually reoriented to ACPC orientation. The DWIs for all scan sessions for a subject were rigidly aligned to this ACPC-reoriented structural image, with the same registration method for both pipelines.

Even though all protocols that were analyzed used equal amount of data and identical scan times, as described in Section 2.1, the directional resolutions of Protocol3 and 4 were halved compared to the others, which might be undesirable for a HARDI analysis. To test the hypothesis with a HARDI model, we opted to use MAPMRI.\(^{40}\) DTI was computed with nonlinear regression and MAPMRI with constrained quadratic programming with no regularization. The scalar maps of choice for reproducibility analysis included: fractional anisotropy (FA) and trace (TR) from the tensor model (\(TR = 3^* \text{mean diffusivity (MD)}\)), as well as, return-to-origin-probability (RTOP), propagator anisotropy (PA), and non-gaussianity (NG) from the MAPMRI model. Principal eigenvector orientation dispersion (PEOD) was used as the metric to assess directional consistency of the diffusion tensors as described in Ref. [41], where \(\text{PEOD} = 0\) indicates that all primary eigenvectors are identical and a \(\text{PEOD} = 1\) indicates that eigenvectors span a uniform half-sphere.

2.4 | Variability analysis

As described in Section 2.3, each scan of a subject was rigidly aligned to the structural image of the first session; therefore, all the DWIs were in correspondence. For each subject, reproducibility between the five visits was assessed via voxelwise standard deviations (SD) and median absolute differences (MAD) for FA, TR, RTOP, PA, and NG maps. PEOD values were computed only within a WM mask. Statistics were computed separately for each design in the native space of the DWIs for both processing pipelines.

To produce a population summary map of reproducibility, a DTI atlas from the data of all seven subjects was created using the DRTAMAS software package\(^{42}\) and SD and MAD maps for each subject were warped onto the atlas space using the respective deformation fields, and then averaged.

3 | RESULTS

Figure 2 displays the standard deviation of TR maps computed over six scans of a representative subject for each protocol at three slice levels. Bright regions correspond to low TR reproducibility while dark regions correspond to high TR reproducibility. The protocol employing only one PE direction (AP), Prot0, results in the largest variability. Prot1 and Prot2, which used differing number of \(b = 0\) images for the PA direction, had slightly improved reproducibility, especially near tissue interfaces between WM/CSF and cortical gyri/sulci. Significant differences between these two protocols were not noticeable, indicating that the \(b = 0\) image chosen for Prot1 was of overall good quality. All these first three protocols suffered from the effects of artifacts that affected the AP acquisitions. TR values exhibited low reproducibility in regions indicated by red arrows, including the pons, a frontal white matter region and the centrum semiovale. A detectable improvement in overall reproducibility was achieved by Prot3;
however, the four-way encoding protocol, Prot4, virtually eliminated the poor reproducibility in these regions and clearly improved overall reproducibility throughout the brain. Overall, the reproducibility of TR maps computed using TORTOISE was slightly better than that of TR maps obtained with FSL; however, the trend of improvement in reproducibility across protocols was similar for the two pipelines.

Figure 4 provides a summary of the reproducibility of each protocol for the entire population (see Figure caption for the definition of the plotted quantities). For the tensor-derived maps (top row), these summary statistics showed little variation in reproducibility using the first three protocols. However, for both the FSL and TORTOISE pipelines, Prot3 and Prot4 significantly improved reproducibility compared to the first three protocols, with Prot4 producing the best reproducibility for all metrics. For the MAPMRI-derived metrics, TORTOISE processing produced results that followed a trend similar to that of the tensor-derived quantities. Curiously, for PA and NG, the FSL processing resulted in increased variability with Prot1 and Prot2 compared to the no distortion correction protocol, Prot0. Regardless of the analyzed metric

| Prot0 | Prot1 | Prot2 | Prot3 | Prot4 |
|-------|-------|-------|-------|-------|
| AP    | AP + 1PA | AP + 6PA | AP = PA | AP,PA,LR,RL |

**FIGURE 2** Standard deviation of Trace (3x MD) for a single subject over six scans for each protocol using TORTOISE and FSL. Brightness indicates increased variability among scans after processing; therefore darker regions are more reproducible. Pure white corresponds to 700 μm²/s. Red arrows point to the locations of high variability due to imaging artifacts such as ghosts. These regions include but are not limited to the pons and regions lateral to the ventricles at mid-brain level. Reproducibility improves with each protocol with Protocol4, that is, the four-way encoded design, yielding the best quality with both processing pipelines.
or the processing pipeline, the four-way PE approach provided the best reproducibility even in this population-wide analysis.

To allow an evaluation of the topological patterns of reproducibility at the population level, we report in Figure 5, the population level SD maps of tensor-derived metrics generated with the TORTOISE pipeline as described in Section 2.4, for two slice levels. The slice including the brainstem and the cerebellum (top panel) shows that the variability of FA in the pons (green arrows) is high for all protocols that did not include $RL$ and $LR$ data. For TR and PEOD, some improvement in variability can be appreciated in Prot3. However, the protocol that had the lowest variability for all metrics was again Prot4. It is also interesting to notice that some high variability in the temporal lobes (red arrows) can be noticed in all $AP$ and $PA$ protocols, for TR in particular. This high variability, which is due to ghosting from the eye signal into the temporal lobes, is barely detectable in Prot4. The slice including the internal capsule (bottom panel) shows a similar variability improvement with Prot4; however, for all metrics, the protocol with no EPI distortion correction performed (Prot0) shows clearly higher variability compared to the other protocols that included EPI distortion correction. The light purple arrows highlight the variability at the boundaries of the corpus callosum.

Figure 6 displays the population level variability maps for PA, NG, and RTOP computed from the MAPMRI model. MAPMRI-derived variability maps showed a similar overall pattern to their tensor-derived counterparts with some exceptions. For instance, reproducibility of RTOP seemed to be worse with Prot1 and Prot2 compared to the no-correction case (Prot0) in the pons and the internal capsule regions. Additionally, at the level of the putamen and thalamus, Prot1 and Prot2 seemed to perform worse for all MAPMRI-derived...
metrics. Despite these exceptions with Prot1 and Prot2, Prot3 again significantly improved reproducibility and Prot4 was confirmed to produce the lowest variability for all metrics among all the approaches.

Table 1 displays the relative variability of each protocol for all metrics compared to the case where no distortion correction was performed (Prot0), which was set as reference with a value of 100%. For nearly all DTI-derived metrics, the variability was reduced with all successive protocol number, with Prot1 and Prot2 generally displaying a similar behavior. MAPMRI-derived measures also exhibited similar patterns, with Prot1-2 performing slightly worse than the baseline, but Protocol3 and Protocol4 performing significantly better. The four-way PE scheme, that is, Protocol4 was the least variable, that is, most reproducible with all metrics.

4 | DISCUSSION

Low reproducibility of diffusion-derived metrics has been a major obstacle to the adoption of quantitative diffusion MRI in the clinical setting. The main goal of this work was to examine the effects on diffusion MRI reproducibility of different strategies for the acquisitions of DWIs that are suitable for reversed PE (or blip-up blip-down) based EPI distortion correction techniques.17,21,22,24 Traditionally, in a clinical setting, DWIs are acquired with a single PE direction, either AP or PA and no EPI distortion correction is performed. Our first hypothesis was that correcting for EPI distortions in the DWIs would have resulted in better reproducibility of results in comparison to the clinical default of no correction (Prot0). Somewhat surprisingly, the protocols employing the popular EPI distortion correction strategies used in most large diffusion MRI multicenter studies: Prot1 and Prot2 (ie, using only $b = 0$ images in the PA direction for the correction) did not result in an appreciable improvement of reproducibility compared to performing no distortion correction, Prot0. This surprising outcome might be attributed to the following: as observed in Ref. [17], an EPI distortion correction strategy...
FIGURE 5  Population level tensor-derived average standard deviation maps from the TORTOISE pipeline at two slice levels. Columns represent the different protocols and the rows contain different tensor-derived modalities: FA, TR, and PEOD, respectively. The separation of data along more phase encoding directions improves the reproducibility for all metrics. The green arrows indicate the reproducibility improvements achieved in the pons region with Prot4 data compared to Prot0. The red arrows point to the temporal lobes which suffer from high variability in all protocols except Prot4 due to the ghosts of the eyes manifesting in this region. Light purple arrows point to the genu of corpus callosum which suffers from high variability in the non-distortion corrected data due to misalignments caused by EPI distortions.
FIGURE 6  Population level MAPMRI-derived average standard deviation maps from the TORTOISE pipeline at two slice levels. Columns represent the different protocols and the rows contain different MAPMRI modalities: propagator anisotropy (PA), non-gaussianity (NG), and return-to-origin-probability (RTOP), respectively. Green arrows point to the high variability in the temporal lobes. Red arrows indicate the improvements achieved in reproducibility in the pons region with all protocols that included an EPI distortion correction step compared to the data which did not (Prot0). Blue arrows indicate the reproducibility improvements in the internal capsule region again with distortion correction. The reader should note that even though the reproducibility significantly improved for the internal capsule with all protocols compared to Prot0, with Protocols 1 and 2 the reproducibility worsened for the caudate region.
that only uses $b = 0$ s/mm$^2$ images can only correct the distortion of the contour of regions that are homogeneous in the $b = 0$ s/mm$^2$ images, as no information is present to guide the correction within the structure. This may lead to spurious deformations within the structures that in turn may lead to poor alignment of anatomical features and consequently lead to higher variability than performing no distortion correction at all.

The dataset which had blip-up and blip-down images for all the DWIs in the AP and PA directions, that is, Prot3, showed a significant improvement in reproducibility compared to the previously mentioned protocols. Reproducibility of all imaging metrics was further improved with the four-way protocol (Prot4), which also used the LR and RL PE data. Overall, a small systematic difference was observed between TORTOISE and FSL pipelines with TORTOISE data variability being generally lower than that of FSL; however, the overall reproducibility trend across different protocols is present in data processed with either pipeline.

The magnitude of reduction in variability with the four-way protocol compared to the baseline was spatially varying, with some regions exhibiting more significant improvements than others but nearly all brain voxels showed improvements. The most remarkable reproducibility improvements were found both in regions susceptible to severe EPI distortions and in regions that suffered from ghosting artifacts. For the former category, the TR in the genu of the corpus callosum showed a reduction of 30% in variability ($1 - \frac{\sigma_{prot4}}{\sigma_{prot0}}$). For the latter category, the TR variability in the temporal lobes, which contained a ghost of the eyes in AP encoded data, exhibited a reduction in variability of 50%. Also, the pons, which suffered from ghosting of the surrounding CSF regions, showed a decrease in variability of 30% for TR and 18% for FA. In addition to the improvements in regions where obvious artifacts were present, the four-way PE protocol also provided the lowest overall whole-brain variability (Table 1), which has the obvious benefit of improving the statistical power, when, for instance, exploring the differences between healthy and patient populations. Moreover, Prot4 showed the most homogeneous variability across brain regions (Figure 5), which is a desirable feature because it achieves the goal of having the same statistical power across brain regions for a given number of recruited subjects.

With Prot1 and Prot2, MAPMRI-derived metric reproducibility exhibited a different behavior than their DTI counterparts, even worsening the reproducibility in some regions compared to the protocol where no distortion correction was performed. This can be attributed to the following: MAPMRI is a more complex and flexible model than DTI. Therefore, it is more sensitive to the imperfections of the data during the fitting process. When performing EPI distortion correction with a full AP dataset using only the $b = 0$ images for the PA PE (Prot1-2), any imaging artifacts such as ghosts in these PA $b = 0$ images would cause the estimated deformation fields to be inaccurate. These variations in the deformation fields would in turn cause the signals to be different for the same anatomical location in the longitudinal scans. Therefore, the highly flexible MAPMRI fitting then would assign these spurious signals as features of the apparent diffusion propagator causing the low reproducibility of the derived metrics. Another interesting observation regarding MAPMRI is that the reductions in reproducibility for MAPMRI with Prot1-2 compared to Prot0 were not systematic but they were regionally dependent. As can be observed in Figure 6, the reproducibility actually improved with Prot1 in the internal capsule but at the same time worsened in the basal ganglia region, in particular in the globus pallidum, which typically has lower SNR in the $b = 0$ images, resulting in unstable deformation fields.

Combining AP phase-encoded data and RL phase-encoded data would not be feasible in case the distortions are not fully corrected and the PE bandwidth, TE, and TR are not the same for the two datasets. For our experiments, we ensured that AP and RL acquisition parameters were identical and that we verified that EPI distortion correction was adequate for combining the two datasets. Several

| TABLE 1 | Absolute (left part of cells) and relative (right part of cells) variability of each protocol |
|---------|---------------------------------------------------------------|
| Protocol0 | Protocol1 | Protocol2 | Protocol3 | Protocol4 |
| Absolute Median $\sigma/Relative Median \sigma$ | | | | |
| TR 143/100% | 134/94% | 136/95% | 116/81% | 97/68% |
| FA 0.025/100% | 0.025/100% | 0.026/102% | 0.024/96% | 0.023/92% |
| PEOD 0.031/100% | 0.030/97% | 0.030/97% | 0.029/92% | 0.028/88% |
| PA 0.018/100% | 0.019/102% | 0.018/100% | 0.016/88% | 0.015/83% |
| NG 0.031/100% | 0.033/105% | 0.032/101% | 0.026/82% | 0.023/72% |
| RTOP 1.4E-5/100% | 1.4E-5/100% | 1.4E-5/99% | 1.1E-5/82% | 1.5E-5/71% |

Notes: Relative variability was computed with respect to Prot0, that is, the protocol where no distortion correction was performed ($\sigma_{prot}/\sigma_{prot0}$). The unit for TR is μm²/s and the other metrics are unitless. The statistics were computed at the population level. Prot4 is the best performing protocol with the most reduction in variability for all metrics.
strategies to combine the AP/PA and RL/LR data could be considered. The simplest approach would be the simple concatenation of the two datasets. Given that the locations of ghosting artifacts are different in the AP and RL datasets, more sophisticated combination approaches would be aimed at identifying these artifacts and favoring the dataset that is artifact-free. For artifact identification, several strategies can be considered including: (a) Iterative re-weighting–based fitting approaches similar to RESTORE\textsuperscript{43} or REKINDLE\textsuperscript{44} which can be retrofitted to handle AP and RL data separately for voxelwise outlier identification, (b) Postprocessing registration approaches which handle the APPA and RLLR data separately without any combination but which perform an additional successive diffusion MRI based registration,\textsuperscript{42,45,46} and (c) Computer vision-based artifact identification approaches which detect artifacts using image processing or machine learning techniques and pass this information as weights to a tailored fitting process. In this work, we chose the simplest approach that is concatenating the two datasets. We reasoned that if reproducibility is improved with simple concatenation, any future, smarter way of combining the two datasets could only improve the results.

4.1 Limitations of the current study and future directions

In this work, the duration of a single scanning session was close to 2 hours, which was prohibitively long to acquire data for any additional experiments that we would like to have conducted. Such experiments and other limitations of the current study are discussed below.

Although it has been clearly shown that dual PE both $b = 0$ images and DWIs is advantageous,$^{17}$ this approach we have evaluated in Prot3 has not gained wide acceptance. The main concern in adapting such a strategy is that by reverse PE all DWIs, the number of unique diffusion gradient directions in the dataset is cut in half. In other words, the directional resolution of the diffusion sampling is compromised. In this work, we followed this design for Prot3; therefore the directional resolution of our gradient scheme was half of that of the protocols that reversed the PE of the $b = 0$ images only (Prot1 Prot2). However, prospective studies do not need to follow this sampling scheme. For applications that are sensitive to gradient direction resolution, such as fiber tractography, the gradient directions of the baseline protocol can simply be split among the four PE directions, without any penalty in directional sampling resolution. A similar approach has been adopted in the UK dHCP project.$^{28}$

In this study, we analyzed the diffusion MRI reproducibility using only one scanner. Ideally, this study should be extended to other vendors and scanner models to generalize the superiority of the four-way PE and to encourage its clinical adaptation. It should be noted that the implementation of the four-way PE protocol might not be straightforward in some scanners. Additional research licenses might be required for such acquisitions in clinical settings. Even with these licenses, special attention has to be paid to keeping all four acquisitions identical in parameters, such as FoV, TE, TR, and diffusion times. Additionally, the minimum achievable TE might be slightly penalized for RL and LR PE directions due to peripheral nerve stimulation related restrictions. For instance, in this work, the minimum TEs for RL and LR was about 7 ms longer than those of AP and PA. At the expense of this small reduction in SNR for AP and PA, all four TEs were individually set to the longer of these two values. For clinical settings, the cooperation from scanner manufacturers for an easy implementation of such a four-way protocol with a user friendly parameter optimization would be very beneficial.

Given scan time limitations, one protocol that is missing from the set of our tested protocols is the unique RL and LR protocol acquired with the same directional sampling resolution of AP and PA protocols. Therefore, we could not assess whether a purely RL and LR protocol would have achieved even higher reproducibility than the hybrid four-way protocol that was tested.

4.2 Conclusions and practical recommendations

In summary, the four-way PE protocol that we have proposed was shown to provide a very relevant improvement in reproducibility, for both diffusion tensor and higher order diffusion metrics, compared to performing no distortion correction or using current blip-up blip-down acquisition and distortion correction approaches used in large quantitative dMRI studies. It is important to remind readers that these comparisons were performed using datasets collected using identical scan times.

In order to improve the reproducibility and provide a robust acquisition of dMRI data, we suggest to adopt some of the strategies that we have used in this study that are not commonly employed in clinical acquisitions, namely:

1. Gradient distribution over PEs: In case maintaining high directional resolution of diffusion sampling is desired, the gradient sampling scheme should be partitioned across the four PE directions. Each of these subsets should still maintain a near optimal sampling distribution in three-dimensional gradient vector space\textsuperscript{30} so that each PE direction dataset would be intrinsically balanced and potentially analyzed independently.
2. Imaging gradients: The diffusion gradients for each PE direction should span the entire sphere instead of a half-sphere to balance the potential effects of imaging gradients.47,48

3. Parameter harmonization: Once the diffusion gradient sampling over PEs is finalized, the other acquisition parameters such as TE/TR have to be harmonized over all acquisitions. Given that typically RL and LR acquisitions have more stringent TE limitations, we advise to first investigate the minimum achievable TE and TR for RL and LR acquisitions and then set the same values for AP and PA acquisitions.

4. Scanner considerations: As stated above, implementations of the four-way protocol is not straightforward in some scanners. For implementations on different scanner platforms, the website https://tortoise.nibib.nih.gov/tortoise/data_acquisition contains specific instructions and pointers.

5. Preprocessing pipeline considerations: The preprocessing pipeline that is intended to be used might have specific acquisition requirements. For instance, the TORTOISE pipeline used in this work is designed, in a user-friendly way, to handle four-way PE datasets; however, it requires a distortion-free T2W fat-suppressed anatomical image at several steps of the processing. Other pipelines might have different requirements.

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