

1 ExploreASL: an image processing pipeline for multi-center ASL 2 perfusion MRI studies

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23 Word Count: abstract/article/references/Tables/Figures: 282/7588/158/1/5

24 Supplementary Count: body/reference/Table/Figure: 0/0/4/11

25

26

27 **Keywords**

28 arterial spin labeling; image processing; multi-center; cerebral perfusion; quality control

1 **Abstract**

2 Arterial spin labeling (ASL) has undergone significant development since its inception, with a focus
3 on improving standardization and reproducibility of its acquisition and quantification. In a
4 community-wide effort towards robust and reproducible clinical ASL image processing, we
5 developed the software package ExploreASL, allowing standardized analyses across centers and
6 scanners.

7 The procedures used in ExploreASL capitalize on published image processing advancements and
8 address the challenges of multi-center datasets with scanner-specific processing and artifact
9 reduction to limit patient exclusion. ExploreASL is self-contained, written in MATLAB and based
10 on Statistical Parameter Mapping (SPM) and runs on multiple operating systems. The toolbox
11 adheres to previously defined international standards for data structure, provenance, and best
12 analysis practice.

13 ExploreASL was iteratively refined and tested in the analysis of >10,000 ASL scans using different
14 pulse-sequences in a variety of clinical populations, resulting in four processing modules: Import,
15 Structural, ASL, and Population that perform tasks, respectively, for data curation, structural and
16 ASL image processing and quality control, and finally preparing the results for statistical analyses
17 on both single-subject and group level. We illustrate ExploreASL processing results from three
18 cohorts: perinatally HIV-infected children, healthy adults, and elderly at risk for
19 neurodegenerative disease. We show the reproducibility for each cohort when processed at
20 different centers with different operating systems and MATLAB versions, and its effects on the
21 quantification of gray matter cerebral blood flow.

22 ExploreASL facilitates the standardization of image processing and quality control, allowing the
23 pooling of cohorts to increase statistical power and discover between-group perfusion
24 differences. Ultimately, this workflow may advance ASL for wider adoption in clinical studies,
25 trials, and practice.

1 Introduction

2 Arterial spin labeling (ASL) is a non-invasive magnetic resonance imaging (MRI) technique with
3 the potential of providing absolute quantification of cerebral perfusion *in vivo*. Since its inception
4 almost three decades ago, ASL-based perfusion imaging has been increasingly used in basic
5 neuroscience and clinical studies. The first decade of research following the invention of ASL in
6 1990 ([Detre et al. 1992](#)) consisted mainly of technical developments, such as the prolongation of
7 the post-labeling delay in 1996 ([Alsop and Detre 1996](#)), background suppression in 1999 ([Alsop
8 and Detre 1999; Ye et al. 2000](#)), and pseudo-continuous labeling in 2005 ([Dai et al. 2008](#)) – all
9 features geared toward improving the signal-to-noise (SNR) ratio of ASL images. These advances
10 enabled proof-of-principle studies using small clinical datasets, such as patients with
11 cerebrovascular and neurodegenerative diseases ([Detre et al. 1998; Alsop et al. 2000](#)), epilepsy
12 ([Liu et al. 2001](#)), brain tumors ([Warmuth et al. 2003](#)), as well as pharmacological applications
13 ([Wang et al. 2011; MacIntosh et al. 2008](#)).

14
15 The second decade was primarily focused on the validation of the technique by way of clinical
16 implementation ([Deibler et al. 2008](#)), evaluation of multi-center reproducibility ([Petersen et al.
17 2010; Mutsaerts et al. 2015](#)), and comparison with [¹⁵O]-H₂O positron emission tomography (PET)
18 ([Heijtel et al. 2014](#)). Several reproducibility studies showed that conventional ASL techniques had
19 developed to the point where the intrinsic variance of the acquisition itself ([Chen et al. 2011;
20 Gevers et al. 2011; Heijtel et al. 2014; Mutsaerts et al. 2014](#)) was close to or below physiological
21 variance of perfusion ([Joris et al. 2018; Clement et al. 2017](#)). Another pivotal aspect for the use of
22 ASL were pharmaceutical studies ([Handley et al. 2013; MacIntosh et al. 2008](#)).

1
2 Currently in its third decade, following the consensus recommendations for the acquisition and
3 quantification of ASL images ([Alsop et al. 2015](#)), ASL is ready for large multi-center observational
4 studies and clinical trials ([Jack et al. 2010](#); [Almeida et al. 2018](#); [Blokhuis et al. 2017](#)). However,
5 despite the consensus in clinical implementation and image acquisition ([Alsop et al. 2015](#)), ASL
6 image processing ([Wang et al. 2008](#); [Shin et al. 2016](#); [Melbourne et al. 2016](#); [Chappell et al. 2010](#);
7 [Mato Abad et al. 2016](#); [Li et al. 2019](#); [Bron et al. 2014](#)) remains disparate among research
8 laboratories. In literature, detailed description of all processing steps is often lacking. Clinical
9 studies are often performed without proper quality control (QC) or with arbitrary QC metrics. This
10 hampers both the interpretation and reproducibility of individual studies as well as meta-analyses
11 of multiple studies. A consensus on the best practices to robustly process ASL data would facilitate
12 comparison of results across centers and studies, avoid duplicate development, and speed up the
13 translation into clinical practice, as is advocated by the Open Source Initiative for Perfusion
14 Imaging (OSIPI) (www.osipi.org).

15
16 For these reasons, the software package ExploreASL was initiated through the EU-funded ASL
17 workgroup COST-action BM1103 "ASL In Dementia" (www.aslindementia.org) with the aim of
18 developing a comprehensive pipeline for reproducible multi-center ASL image processing. To
19 date, ExploreASL has been used in more than 30 studies consisting of more than 10,000 ASL scans
20 from three MRI vendors - GE, Philips, Siemens, with pulsed ASL and pseudo-continuous ASL
21 (PCASL) sequences ([Mutsaerts et al. 2019](#)). The primary aims of ExploreASL are to increase the
22 comparability and enable pooling of multi-center ASL datasets, as well as to encourage and

1 facilitate cross-pollination between clinical investigators and image processing method
2 developers.

3

4 **Theory: Software Overview**

5 ExploreASL is developed in MATLAB (MathWorks, MA, USA, tested with versions 2011-2019) and
6 uses Statistical Parametric Mapping 12 routines (SPM12, version 7219) ([Ashburner et al. 2012](#);
7 [Flandin et al. 2008](#)). Here, we describe the implementation of ExploreASL version 1.0.0, which is
8 available as a compiled version with a manual on www.ExploreASL.org. ExploreASL provides a
9 fully automated pipeline that comprises all the necessary steps from data import and structural
10 image processing to cerebral blood flow (CBF) quantification and statistical analyses. Unique
11 features of ExploreASL include:

12

- 13 ● self-contained software suite: all third-party toolboxes are included in the installation,
14 compatible with Linux, macOS, and Windows and supporting multi-threading;
- 15 ● flexible data import from different formats including (enhanced) DICOM, Siemens'
16 MOSAIC variant, Philips PAR/REC, NIfTI and Brain Imaging Data Structure (BIDS)
17 ([Gorgolewski et al. 2016](#)), with automatic detection of control-label or label-control order;
- 18 ● data management: anonymization, compression of image files;
- 19 ● modular design: automatically iterates over all available subjects and scans, allows to
20 suspend and resume processing at any point, allows investigators to change/replace each
21 sub-module;

- 1 • image processing optimized for: multiple centers, different ASL implementations from
2 GE/Philips/Siemens ([Mutsaerts et al. 2018](#)), both native/standard-space analysis,
3 advanced ASL markers — e.g. spatial coefficient-of-variation (CoV) ([Mutsaerts et al. 2017](#)),
4 asymmetry index ([Kurth et al. 2015](#)), and partial volume correction (PVC) ([Asllani et al.](#)
5 [2008](#));
- 6 • extensive QC and data provenance: visual QC for all intermediate and final images,
7 comparison with perfusion templates from different ASL implementations, progress
8 report with processing history (provenance).

9

10 In the following sections, we review each processing step of the four ExploreASL modules as
11 outlined in Figure 1 and summarized in Table 1. Each section starts with a brief methodological
12 review including the rationale within the context of ASL processing, followed by a detailed
13 description of the ExploreASL implementation, and ending with a discussion of emerging
14 developments and potential future improvements.

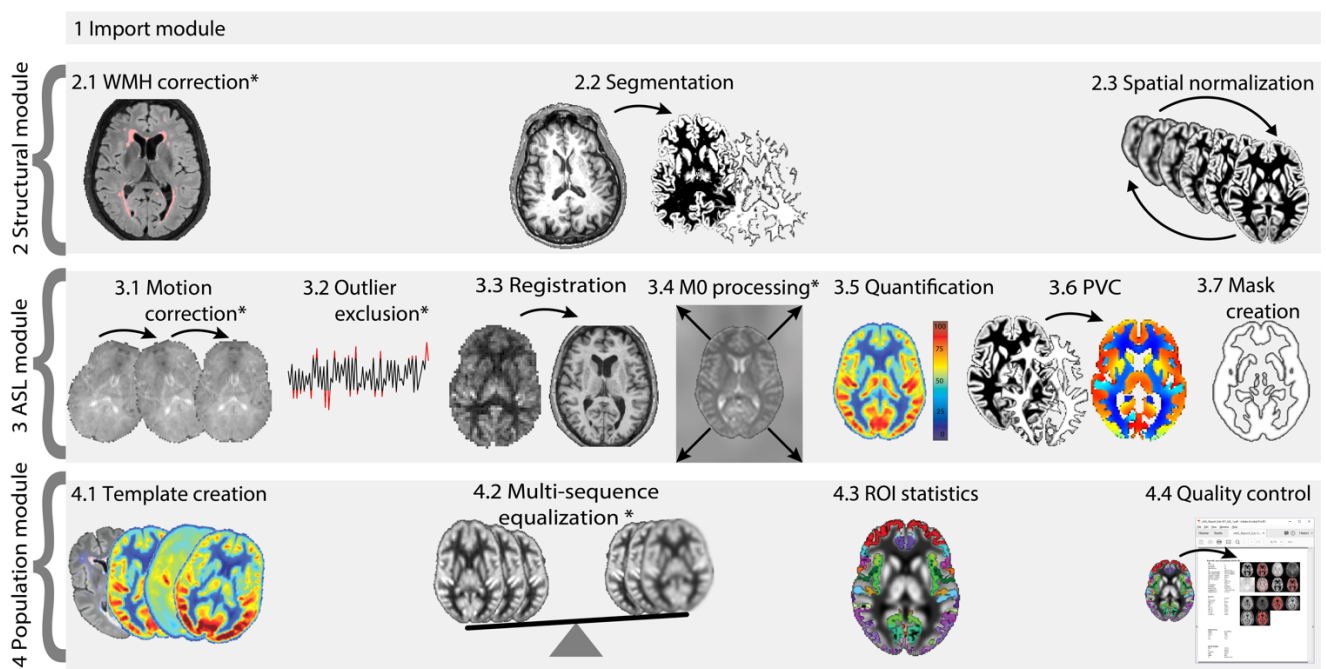
1

Processing step	ExploreASL implementation	Specifics, optional features
1. Import module		
1.1 Data import	dcm2niiX	Converts DICOM to NIFTI, supports MOSAIC, PAR/REC, BIDS
2. Structural module		
2.1 WMH correction	LST2 (r2.0.15) - LPA (LGA optional)	Segments WMH and fills lesions on T1w; improves T1w segmentation
2.2 Segmentation	CAT12 (r1363)	Outputs partial volume maps; supports lesion cost function masking
2.3 Spatial normalization	Geodesic Shooting	Uses CAT12 template, supports creation of study-specific templates
3. ASL module		
3.1 Motion correction	SPM12 realign	Realigns ASL control/label images to mean position, uses a zig-zag control-label regressor
3.2 Outlier exclusion	ENABLE	Removes motion peaks, uses tSNR optimization
3.3 Registration	SPM12 rigid-body	Registers ΔM -pGM or M0-T1w
3.4 M0 processing	M0 image	Masks, smooths, extrapolates M0 to avoid division artifacts
3.5 CBF quantification	Consensus paper model	Computes CBF based on the single compartment model, single PLD; supports dual compartment
3.6 PVC	Linear regression	Performs PVC on kernel or ROI basis, optionally estimates the effective spatial resolution/PSF of ASL
3.7 Analysis mask creation	Combine individual masks	Combines FoV, susceptibility artifacts and vascular artifacts, use $p > 0.95$ of population masks
4. Population module		
4.1 Template creation	Final images	Calculates population mean, SD, CoV, SNR, also for intermediate images
4.2 Multi-sequence equalization	Remove residual sequence-specific effects	Equalizes bias fields, spatial CoV, and smoothness, uses sequence-specific templates
4.3 ROI statistics	CBF and spatial CoV, with or without PVC	Uses MNI structural, Harvard-Oxford, Hammers, and custom atlases
4.4 Quality control	Single-subject PDF report	Performs QC of images, DICOM values, volumetrics, motion etc. Outputs population report in TSV files

2

3 Table 1. Overview of image processing steps and implementation in ExploreASL. ASL = arterial spin labelling, BIDS =
4 Brain Imaging Data Structure, CAT = Computational Anatomic Toolbox, CBF = cerebral blood flow, CoV = coefficient
5 of variation, ΔM = perfusion-weighted difference image, dcm2niiX ([Li et al. 2016](#)), DICOM = Digital Imaging and
6 COMmunications in Medicine, ENABLE = ENhancement of Automated BLOOD flow Estimates, FoV = field-of-view, LGA
7 = Lesion Growth Algorithm, LPA = Lesion Prediction Algorithm, LST = Lesion Segmentation Toolbox, MNI = Montreal
8 Neurological Institute, NiftI = NeuroImaging Informatics Technology Initiative, QC = quality control, pGM = gray
9 matter partial volume, PLD = post-labeling delay, PSF = point spread function, PVC = partial volume correction, r =
10 release, ROI = region of interest, SD = standard deviation, SNR = signal-to-noise ratio, SPM = Statistical Parametric

1 Mapping, tSNR = temporal SNR, tsv = tab-separated value, WMH = white matter hyperintensity, Zig-zag = “zig-zag”
2 regressor.
3
4



5
6 Figure 1. Schematic diagram of ExploreASL processing steps. Steps marked with a * are optional, e.g. when FLAIR,
7 ASL time-series, or M0 scans are available. PVC = partial volume correction, ROI = regions of interest, WMH = white
8 matter hyperintensity. The population module can be run on a single subject level, as well as on one or multiple
9 populations/centers/cohorts or other groups.

1 Theory: Implementation

2 1. Import module

3 To avoid manual restructuring of arbitrary data structures from the scanner or other sources
4 ([Fallis et al. 2013](#)), ExploreASL uses a flexible input data/directory description scheme based on
5 regular expressions and converts the data to a BIDS-compatible data structure ([Gorgolewski et al.](#)
6 [2016](#)); the full BIDS ASL extension is currently in development (bids.neuroimaging.io). The input
7 images can be NIfTI format, conventional or enhanced DICOM, Philips PAR/REC, or Siemens
8 mosaic format, which are then converted to NIfTI using dcm2niiX ([Li et al. 2016](#)). ASL images can
9 be provided as control-label time-series, a single perfusion-weighted image, or an already
10 quantified CBF image, from any 2D or 3D readout schemes, and from any MRI vendor. Before an
11 image is processed, ExploreASL first computes and aligns the center-of-mass of each image to the
12 origin of the world coordinates to deal with potentially incorrectly stored orientations.
13 Additionally, ExploreASL provides an overview of missing and unprocessed files, automatically
14 detects the order of control and label images from the image intensities, and checks the DICOM
15 tags of repetition and echo time and scale factors/slopes across individuals.

16

17 2. Structural module

18 This module process the structural images by the following steps: 2.1) segments the white matter
19 (WM) hyperintensities (WMH) on fluid-attenuated inversion recovery (FLAIR) images and uses
20 them to fill the corresponding WM hypointensities on the T1-weighted (T1w) images., 2.2) the
21 structural images are subsequently segmented into gray matter (GM), white matter, and

1 cerebrospinal fluid (CSF) maps, and 2.3) normalized to the MNI standard space ([Evans et al. 2012](#)).
2 The segmentations are used to obtain tissue partial volume (PV) fractions for computation of CBF
3 ([Asllani et al. 2008](#)). The registration transformations are used to bring ASL images acquired from
4 different sessions and/or different subjects in the same space and thus facilitate visual
5 comparison in the same space, automatic QC, as well as group analysis.

6

7 **2.1. WMH correction**

8 The presence of WMH can affect the GM/WM classification of T1w images in two ways: 1) WMH
9 themselves can be incorrectly segmented as GM; 2) image intensities of WMH bias global
10 modeling of GM and WM intensity distributions ([Pareto et al. 2016](#); [Battaglini et al. 2012](#)).
11 ExploreASL alleviates these complications by lesion-filling the T1w image before initiating the
12 segmentation ([Battaglini et al. 2012](#)): voxel intensities in the hypointense WMH regions on the
13 T1w images are replaced by bias field-corrected values from the surrounding, normal-appearing
14 WM ([Chard et al. 2010](#)) (Figure 1). The Lesion Segmentation Toolbox (LST, version 2.0.15) is used
15 because of its empirically proven robustness, scanner independence, and non-reliance on the
16 requirement of a training set ([de Sitter et al. 2017](#)). LST detects outliers in the FLAIR WM intensity
17 distribution and assesses their likelihood of being WMH ([Schmidt et al. 2012](#)). While ExploreASL
18 offers the option of both LST lesion growing and lesion prediction algorithms, the default is set to
19 the latter, which has been shown to be more robust ([de Sitter et al. 2017](#)). This WHM correction
20 described here is only performed when FLAIR images are available. Optionally, the WMH
21 segmentation can be skipped by providing an external WMH segmentation; then only the LST

1 clean-up procedure is performed to remove classification errors from this external input ([Schmidt](#)
2 [et al. 2012](#)).

3

4 **2.2. Segmentation**

5 To segment the 3 main tissue classes GM, WM, and CSF, ExploreASL uses the Computational
6 Anatomy Toolbox 12, release 1363 (CAT12, the successor of VBM8) ([Gaser et al. 2009](#)) for SPM12.
7 CAT12 allows local variations in the tissue intensity distributions, making it more robust to the
8 presence of pathology such as tumors, edema, and WM lesions ([Battaglini et al. 2012](#); [Petr et al.](#)
9 [2018](#)) (Supplementary Figure 1). CAT12 works on all operating systems (OS), and has been shown
10 to outperform other available methods such as FreeSurfer v5.3.0, FSL v5.0, and SPM12 ([Mendrik](#)
11 [et al. 2015](#)). The CAT12 segmentation algorithm is based on improvements of Unified
12 Segmentation ([Ashburner et al. 2005](#)), two essential improvements being that it allows spatially
13 varying GM-WM intensity distributions, and provides PV maps rather than posterior probability
14 maps ([Tohka et al. 2004](#)).

15

16 **2.3. Spatial normalization**

17 For non-linear registration to MNI space ExploreASL uses Geodesic Shooting ([Ashburner et al.](#)
18 [2011](#)) - the successor of Diffeomorphic Anatomical RegisTration using Exponentiated Lie algebra
19 (DARTEL) ([Ashburner et al. 2007](#)) - within the CAT12 toolbox ([Gaser et al. 2009](#)). The reason for
20 this choice is that CAT12 has a single subject implementation using the IXI adult template, [brain-](#)
21 [development.org/ixi-dataset](#). Optionally, new templates can be created by these SPM toolboxes
22 on a population level, e.g. for populations where an adult template is not sufficient. Although

1 alternative methods ([Klein et al. 2010](#)) may outperform DARTEL/GS in specific populations, the
2 default settings of DARTEL and Geodesic Shooting are sufficiently tested in clinical studies to
3 provide adequate performance across different populations and scanners ([Ripollés et al. 2012](#)).
4 We adapted the CAT12 segmentation algorithm to offer the possibility to input customized
5 segmentations of structural lesions such as space-occupying lesions or cerebral infarcts such that
6 the lesion region is ignored by the non-linear registration ([Crinion et al. 2007](#)) (Supplementary
7 Figure 2). To avoid unrealistic deformations within the lesion, the non-linear transformation is
8 only used outside the lesion; whereas the initial SPM transformation (low degree-of-freedom
9 non-linear) is used within the lesion. These two transformation fields are combined with a gradual
10 transition margin around the lesion amounting to 10% of the lesion volume.
11 ExploreASL offers the option to register longitudinal ASL studies with the SPM12 module for
12 longitudinal registration ([Ashburner and Ridgway 2012](#)), which takes the similarity between
13 structural images from the same subjects into account. The first time point is used as a reference
14 for both within- and between-subject registration. However, this requires further validation in
15 the presence of large brain deformations between sessions, such as tumors, resections, or infarcts
16 ([Petr et al. 2018](#)).

17 18 **3. ASL module**

19 This module processes the ASL images by 3.1) correcting for motion, 3.2) removing outliers, 3.3)
20 registering with the structural data, and by 3.4) processing the M0 images. Then, 3.5) the CBF is
21 quantified with correction for hematocrit and vascular artifacts, after which 3.6) the PV effects
22 are corrected for. All image processing described below is performed in native space, unless

1 stated otherwise. All intermediate and final images are also transformed into standard space for
2 QC and group analyses.

3

4 **3.1. Motion correction**

5 The adverse effects of head motion can be partly alleviated by correcting for motion using image
6 processing ([Alsop et al. 2015](#)). Traditionally, head motion is estimated assuming a 3D rigid-body
7 transformation with a sum-of-squares cost function ([Wang et al. 2008](#); [Mato Abad et al. 2016](#)).
8 However, because the average control-label intensity difference can be partly interpreted by the
9 algorithm as motion, some investigators perform motion estimation separately for the control
10 and labeled images ([Wang et al. 2008](#)). Instead, in ExploreASL, an adaptation of the SPM12 motion
11 correction is used, which minimizes apparent motion attributable to the control-label intensity
12 difference from the estimated motion parameters using a “zig-zag” regressor ([Wang et al. 2012](#))
13 (Supplementary Figure 3).

14

15 **3.2. Outlier exclusion**

16 Despite motion correction, large motion spikes can still have a significant negative effect on the
17 ASL image quality, especially when they occur between control and label images ([Wang et al.](#)
18 [2008](#)). In fMRI literature, peak motion relative to mean individual motion is often excluded based
19 on a set threshold, e.g. RMS of 0.5 of the voxel size ([Power et al. 2012](#)). ExploreASL uses a
20 threshold-free method named Enhancement of Automated Blood flow Estimates (ENABLE)
21 ([Shirzadi et al. 2015](#)), which sorts control-label pairs by motion and cumulatively averages them
22 until the addition of further pairs significantly decreases the temporal voxel-wise signal stability

1 (Supplementary Figure 4). The ExploreASL implementation of ENABLE employs the median GM
2 voxel-wise temporal SNR (tSNR) as the criterion for signal stability ([Shirzadi et al. 2018](#)),
3 regularized by an empirically-defined minimum tSNR improvement of 5%. ENABLE can also
4 remove non-motion-related outliers, since other acquisition artifacts can be picked up by the
5 motion estimation algorithm (Supplementary Figure 4).

6
7 In addition to motion, ASL images can contain a variety of acquisition and physiological artifacts
8 including fat-shift artifacts, RF instability, gradient amplifier failure, labeling instability, and blood
9 pulsatility artifacts. Several correction algorithms using ASL time series have been proposed
10 which typically exclude voxels, slices or volumes as outliers, based on temporal and/or spatial
11 signal distribution of the individual pairwise subtracted images ([Bibic et al. 2010](#); [Spann et al.](#)
12 [2017](#); [Maumet et al. 2014](#); [Tan et al. 2009](#); [Dolui et al. 2017](#)). Their applicability needs validation,
13 however, as they base their correction criteria on the same parameter that is investigated (i.e.
14 CBF), and/or they do not account for GM-WM perfusion differences. ExploreASL currently relies
15 on the fact that ENABLE ([Shirzadi et al. 2018](#)) also partly removes outliers, as it operates relatively
16 independent of (patho-)physiological changes of the signal intensity in the pairwise subtracted
17 images ([Robertson et al. 2017](#); [Li et al. 2018](#)).

18

19 **3.3. Registration**

20 Accurate registration between the ASL and structural space is a critical step as registration errors
21 are propagated to subsequent stages and analyses of CBF data. Specifically, the relatively large

1 CBF differences between GM, WM, and CSF, mean that small misalignments can have a large
2 impact on the accuracy of tissue-specific CBF quantification ([Mutsaerts et al. 2018](#)).
3
4 The image registration steps implemented in ExploreASL are based on a previous study in which
5 the performance of several registration options were compared ([Mutsaerts et al. 2018](#)). Briefly,
6 the registration of ΔM to gray matter partial volume (pGM) outperformed the registration of M0
7 to T1w, except for cases where the ΔM contrast was dissimilar to the pGM contrast (e.g. vascular
8 artifacts, labeling artifacts, perfusion pathology). Rigid-body transformation proved to be a robust
9 default choice ([Mutsaerts et al. 2018](#)), especially in the presence of pathology ([Wang et al. 2008](#);
10 [Macintosh et al. 2010](#)). Therefore, ExploreASL performs a ΔM -pGM rigid-body registration by
11 default. The M0-T1w is used instead when macrovascular signal predominates tissue signal
12 (spatial CoV above 0.6) ([Mutsaerts et al. 2018](#)). Note that such images are typically excluded from
13 CBF statistics and only included when analyzing vascular parameters, such as the spatial CoV. For
14 future validation, ExploreASL offers the option to register to the atlas of spatially normalized
15 mean M0 images, CBF images, or CBF images with a high number of vascular artifacts created for
16 different vendors and ASL sequences from previously processed large ASL datasets.
17
18 The rigid-body transformation does not account for the geometric distortion typical for 2D echo-
19 planar imaging (EPI) or 3D Gradient And Spin Echo (3D GRASE) ASL images ([Gai et al. 2017](#)). Such
20 deformations can be partially corrected with B0 field maps or M0 images with reversed phase-
21 encoding direction ([Madai et al. 2016](#)) - which is implemented as option in ExploreASL by calling
22 FSL TopUp ([Andersson et al. 2003](#)). Affine and uniform non-linear transformations, such as FNIRT

1 or SPM's 'unified segmentation' ([Klein et al. 2009](#)) can outperform the rigid-body transformation
2 in the ΔM -pGM registration ([Petr et al. 2018](#)), although this remains to be validated in the
3 presence of pathology.

4

5 **3.4. M0 processing**

6 The measured control-label magnetization difference is proportional to the equilibrium
7 magnetization (M0) of blood. Ideally, blood M0 would be measured in voxels containing only
8 arterial blood, but that is not usually possible due to the relatively low spatial resolution of ASL
9 images. Instead, M0 is calculated from either the brain tissue or CSF signal intensity ([Çavuşoğlu
10 et al. 2009](#)). The use of the tissue-based M0 is recommended ([Alsop et al. 2015](#)) because of its
11 ability to account for acquisition-specific effects such as variations in receive coil inhomogeneity
12 or T2(*) weighting. For these reasons, ExploreASL by default processes an M0 image, and
13 optionally supports the use of a single CSF M0 value ([Çavuşoğlu et al. 2009; Pinto et al. 2019](#)).

14

15 Currently, no consensus exists on whether the M0 should be quantified separately for GM and
16 WM tissue types, especially for longer repetition time with a distinct GM-WM contrast. The M0
17 quantification can potentially be improved by using tissue specific quantification parameters -
18 such as blood-brain partition coefficients λ and tissue relaxation times ([Çavuşoğlu et al. 2009](#)),
19 and/or partial volume correction ([Ahlgren et al. 2018](#)). However, this can induce quantification
20 errors in cases of suboptimal ASL-M0 registration.

21 ExploreASL aims to deliver consistent M0 quantification for multi-center populations with M0-
22 scans acquired at different repetition time and different effective resolutions. ExploreASL

1 smooths the M0 image with a large kernel ([Beaumont 2015](#)) after it has been masked for WM
2 (Supplementary Figures 5-6) and rescaled to the mean GM M0 to account for B1 differences
3 between GM and WM. This approach reduces the M0 image into a smooth bias field with the
4 same smoothness/effective resolution for all ASL sequences and participants, and optimal SNR,
5 while still canceling out acquisition-specific B1-field related intensity inhomogeneity. This makes
6 the M0 image more robust and less sensitive to misalignment, and thus more consistent between
7 ASL sequences ([Mutsaerts et al. 2018](#)) and individuals ([Deibler et al. 2008](#)). ExploreASL has the
8 option to additionally mask the M0 bias-field for lesions that affect the M0 - e.g. brain tumors -
9 and interpolate the M0 signal from the relatively unaffected brain regions ([Croal et al. 2019](#)).

10

11 **3.5. CBF quantification**

12 An in-depth overview of ASL CBF quantification has been provided previously ([Alsop et al. 2015](#);
13 [Chappell et al. 2018](#)). ExploreASL uses the previously recommended single compartment
14 quantification approach for clinical studies ([Alsop et al. 2015](#)), with options to use the dual
15 compartment model, and/or to provide the hematocrit or blood T1 values. The previously
16 recommended single compartment model assumes that the label decays with arterial blood T1
17 only ([Alsop et al. 2015](#)). Although a two-compartment model can provide CBF values that are in
18 closer agreement with [¹⁵O]-H₂O PET ([Heijtel et al. 2014](#)), this is often not feasible when blood T1,
19 tissue T1, and micro-vascular arterial transit time are unknown, or would result in a constant
20 scaling factor when assuming literature values. For these reasons, ExploreASL uses the single
21 compartment model by default, and offers the two-compartment model as an optional feature.

1
2 The ASL label relaxes with the T1 of blood, a parameter that depends on hematocrit ([Hales et al.](#)
3 [2016](#)). Not taking hematocrit or blood T1 into account can lead up to 10-20% CBF overestimation
4 for hematocrit as low as 17% ([Vaclavu et al. 2016](#)). Accounting for hematocrit is particularly
5 relevant for between-group or longitudinal hematocrit changes e.g. due to treatment, which can
6 be expected in certain populations or diseases ([De Vis et al. 2014](#)). ExploreASL allows to adjust for
7 individual arterial blood T1 by either providing its value directly ([Li et al. 2017](#)) or by providing the
8 hematocrit value and computing the blood T1 ([Hales et al. 2016](#)). As hematocrit and blood T1
9 measurements can be noisy - especially when obtained at different laboratories - a pragmatic
10 approach is to apply the average blood T1 correction on a population rather than on an individual
11 level ([Elvsåshagen et al. 2018](#)). Additionally, hematocrit and blood T1 can be modeled based on
12 age and sex ([Hales et al. 2014](#)), but this requires validation. Note that after correcting the above-
13 mentioned methodological effect, hematocrit might be still associated with CBF physiologically:
14 hematocrit decreases or increases leading to compensatory hyper- or hypoperfusion.

15

16 **3.6. Partial volume correction**

17 Since the spatial resolution of ASL is relatively low, a typical ASL voxel contains a mixture of GM,
18 WM, and CSF signal, which is referred to as the partial volume effects. As the GM-WM CBF ratio
19 is reported to lie between 2 and 7 ([Asllani et al. 2008](#); [Pohmann 2010](#); [Zhang et al. 2014](#); [Law et](#)
20 [al. 2000](#)), the tissue partial volume in each voxel has a large influence on the ASL measurement
21 (Supplementary Figure 7). For these reasons, PVC ([Asllani et al. 2009](#)) is essential in studies that
22 aim to differentiate structural changes (e.g. atrophy) from perfusion changes (e.g. related to

1 neurovascular coupling) ([Steketee et al. 2016](#)). Several PVC algorithms have been proposed
2 ([Chappell et al. 2010](#); [Zhao et al. 2017](#); [Asllani et al. 2008](#); [Liang et al. 2013](#)), which assume locally
3 homogeneous GM and WM CBF. Instead in some studies, GM volume is used as a covariate in the
4 statistical analysis ([Chen et al. 2011](#)). Note that while PVC, in theory, corrects only for the PV
5 effects and takes into account the intra- and inter-subject variability of the GM-WM CBF ratio,
6 GM covariation can additionally affect the estimated physiological correlation between GM CBF
7 and GM volume ([Petr et al. 2018](#)).

8
9 ExploreASL employs two versions of PVC, both based upon the most frequently used PVC, i.e.
10 linear regression ([Asllani et al. 2008](#)): 1) a 3D Gaussian instead of a 2D flat kernel (default, referred
11 to as “voxel-wise”) ([Oliver 2015](#)), or 2) computing PV-corrected CBF within each anatomical or
12 functional region of interest (ROI) separately instead of using a kernel. Whereas the voxel-wise
13 option allows further voxel-based analysis (VBA), the ROI-based PVC is in theory beneficial for a
14 ROI-based analysis as effectively the kernel-size is selected based upon the anatomical ROI, which
15 should be less sensitive to local segmentation errors. Moreover, it avoids cross-talk between ROIs.
16 It still needs to be investigated how to define regions of optimal shape with respect to PVC
17 performance, which depends on the spatial uniformity and SNR of the GM and WM CBF, and
18 partial volume distributions within the ROI. To evaluate the effects of PVC, ExploreASL exports
19 CBF maps and ROI values both with and without PVC.

20
21 For proper PVC or ROI definition, the true acquisition resolution - which often differs from the
22 reconstructed voxel size - needs to be taken into account ([Petr et al. 2018](#)). This is especially

1 important for 3D readouts, where the through-plane PSF can be up to 1.9 times the nominal
2 voxel-size ([Vidorreta et al. 2013](#); [Vidorreta et al. 2014](#)). Effects such as motion ([Petr et al. 2016](#))
3 and scanner reconstruction filters can contribute to further widening of the PSF of the final image.
4 ExploreASL by default uses previously estimated true acquisition resolutions ([Vidorreta et al.](#)
5 [2013](#); [Vidorreta et al. 2014](#); [Petr et al. 2018](#)) and can optionally perform a data-driven spatial
6 resolution estimation ([Petr et al. 2018](#)) that is generalizable to all ASL acquisitions. Contrary to
7 alternative PSF estimations based on temporal noise autocorrelation ([Cox 2012](#)) or simulations of
8 the acquisition PSF ([Vidorreta et al. 2013](#); [Vidorreta et al. 2014](#)), this method does not require
9 time series and inherently accounts for other sources of blurring (e.g. smoothing by motion
10 and/or image processing) and is applicable without having detailed information about the
11 sequence parameters needed to calculate the resolution from the k-space trajectory. However,
12 this method requires further validation, especially in the presence of ASL image artifacts.

13
14 Lastly, the GM/WM maps obtained from the high-resolution structural images need to be
15 downsampled to the ASL resolution before they are used for PVC or for ROI delineation in native
16 space. A trivial interpolation to lower resolution may introduce aliasing, which can be addressed
17 by applying a Gaussian filter - or a convolution with the PSF, if the PSF is known - prior to
18 downsampling ([Cardoso et al. 2015](#)). It is important to note that the ASL image often has an
19 anisotropic resolution and may be acquired at a different orientation compared to the structural
20 image. To correct for this effect, ExploreASL pre-smooths the structural images with a Gaussian
21 kernel of which the covariance matrix takes the orientation and PSF differences between the ASL
22 and structural images into account ([Cardoso et al. 2015](#)).

1

2 **3.7. Analysis mask creation**

3 For the statistics performed in section 4.3 - as well as for any voxel-based group statistics - an
4 analysis mask aims to exclude voxels outside the brain or voxels with artifactual signal (e.g. macro-

5 vascular, signal dropout) and restrict the analysis to regions with sufficient SNR and/or statistical

6 power. This also avoids over-penalizing statistical power by family-wise error corrections. The

7 susceptibility and field-of-view (FoV) masks are combined in section 4.3 into a group mask. The

8 vascular masks are applied subject-wise to reflect the individual differences in vascular anatomy.

9

10 First, regions outside of the ASL FoV are identified, as whole brain coverage is not always achieved

11 (Supplementary Figure 8). Second, a mask is created to remove voxels with intravascular signal.

12 While intra-vascular signal - resulting from an incomplete tissue arrival of labeled spins - can be

13 clinically useful ([Mutsaerts et al. 2017](#); [Mulhollan et al. 2018](#)), it biases regional CBF estimates.

14 The relatively large local temporal variability of such vascular artifacts can be detected in time

15 series, in multi-post-labeling delay (PLD) acquisitions ([Chappell et al. 2010](#)) or by an independent

16 component analysis (ICA) ([Hao et al. 2018](#)). ExploreASL uses a vascular artifact detection approach

17 that is suitable for both single and multi-PLD ASL images. It identifies clusters of negative apparent

18 CBF ([Maumet et al. 2012](#)) and voxels with extreme positive apparent CBF (Supplementary Figure

19 9). One potential caveat of masking out vascular voxels is the violation of the stationarity criterion

20 of parametric voxel-wise statistics. While excluding voxels with high signal can violate the

21 stationarity criterion of the ASL signal, there is currently no validated method that would be able

1 to reliable estimate the perfusion and vascular signal contribution in such voxels from single-PLD
2 data.
3
4 Second, regions with susceptibility signal-dropout artifacts are removed. Regions frequently
5 having low SNR for 2D EPI and 3D GRASE ASL include the orbitofrontal cortex near the nasal sinus
6 and the inferior-medial temporal gyrus near the mastoid air cavities. There are several methods
7 that we decided not to include in ExploreASL: thresholding the M0 or the mean control image to
8 identify signal dropout ([Wang 2014](#)) or masking them with FSL BET ([Smith 2002](#)), as this may fail
9 with background suppression, blurred 3D acquisitions, poor ASL-M0 registration, or a strong bias
10 field ([Mutsaerts et al. 2018](#)). If multiple individual unsubtracted control-label images are
11 available, a mask could be created based on the tSNR of the control or label images. However,
12 time series are not always available, and the tSNR may be biased by the presence of (patho-
13)physiological signal changes and head motion. Therefore, the option implemented in ExploreASL
14 is to use sequence-specific template masks obtained from previous population analyses, after
15 which individual masks are restricted to $(pGM+pWM)>0.5$ to remove voxels outside the brain.
16 Further development is needed to create masks that take individual anatomical differences in
17 skull and air cavities into account. Noteworthy, ExploreASL applies this analysis mask only for
18 analyses, not for visual QC.

19

20 **4. Population module**

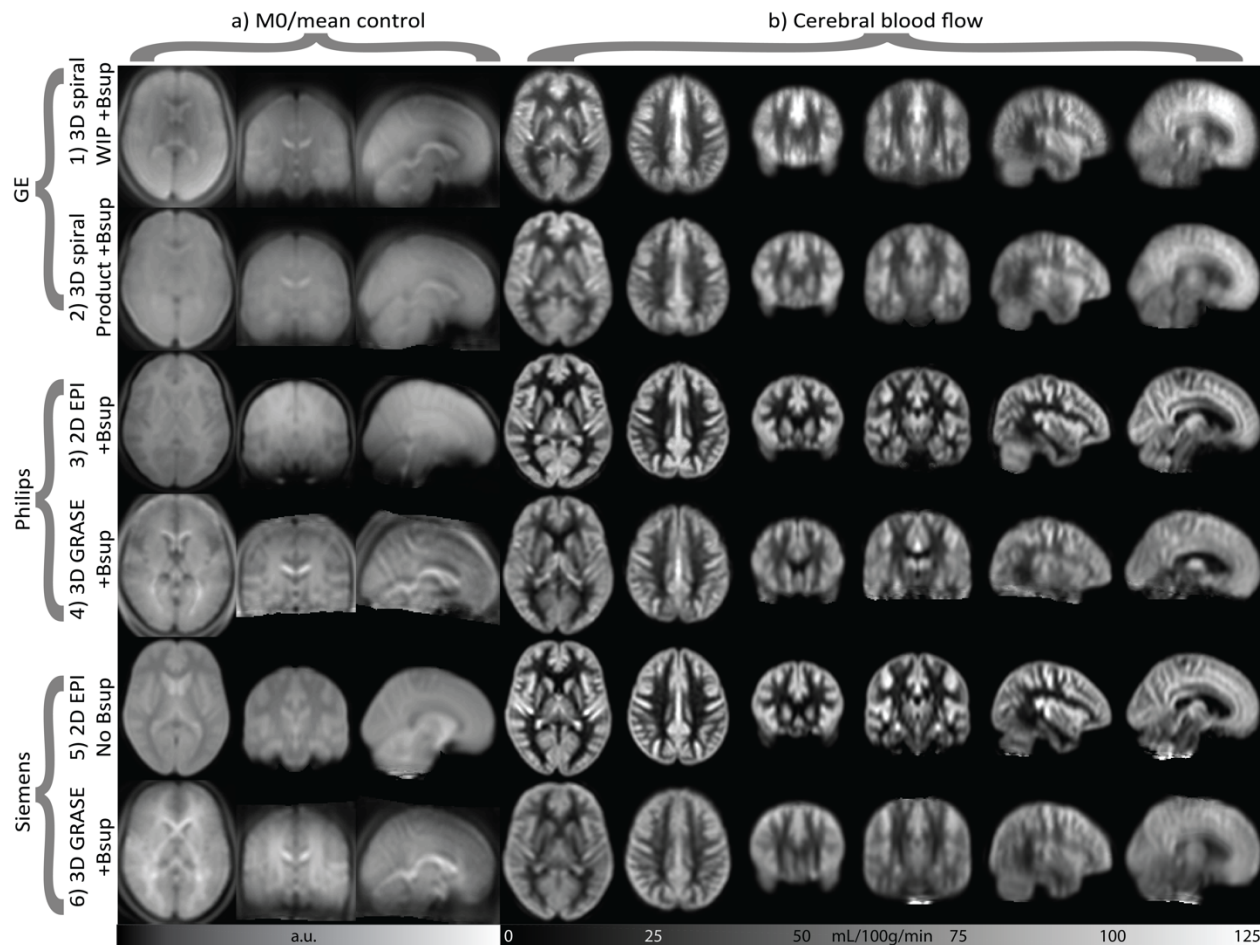
21 This module performs group-level QC and creates group-level results for statistical analyses.
22 Whereas the above-described Structural and ASL modules perform image processing on the

1 individual level, this module performs its analysis on multiple-subjects and/or multi time-point
2 level. For this purpose, ASL images are transformed into standard space using the T1w
3 transformation fields smoothed to the effective spatial resolution of ASL. For transformation of
4 all intermediate and final images, all previous spatial transformations are merged into a single
5 combined transformation to minimize accumulation of interpolation artifacts through the
6 pipeline. Partial volumes of GM and WM obtained from anatomical images are multiplied by the
7 Jacobian determinants of the deformation fields - a.k.a. modulation - to account for voxel-volume
8 changes when transforming to standard space ([Ashburner and Friston 1999](#)).

9

10 **4.1. Template creation**

11 Population templates can reveal population- or sequence-specific perfusion patterns that are not
12 visible on the individual level. ExploreASL generates between-subject mean, standard deviation
13 (SD), CoV, and SNR images for the total study population and, optionally, for different sets (e.g.
14 different centers/sequences/cohorts) within the study (Figure 2). In addition to CBF itself,
15 auxiliary images (e.g. M0), intermediate images (e.g. mean control images), or QC images (e.g.
16 temporal SD) can provide a valuable overview of the data, for example when comparing data
17 originating from different centers.



1
2 Figure 2. Templates (population-averages from previous studies) are shown for source images (a) and CBF maps (b),
3 for several arterial spin labeling (ASL) acquisitions with/without background suppression (Bsup) from different
4 vendors. The average CBF images are intensity normalized to a mean total GM CBF of 60 mL/min/100g (see Suppl Fig
5 10 for the unscaled CBF images). Source images are mean control images for Philips and Siemens and M0 images for
6 GE, which does not output control images. Note that the images differ mostly in their effective spatial resolution,
7 orbitofrontal signal dropout, and the amount of macro-vascular artifacts. The differences in geometric distortion are
8 mostly too subtle to be noted on these population-averages images. Note the inferior-superior gradient in the source
9 images in the 2D EPI sequence with background suppression. a.u. = arbitrary units, Bsup = background suppression,
10 WIP = work-in-progress pre-release version. See sequence details in Supplementary Table 1.

1 **4.2. Multi-sequence equalization**

2 Quantitative CBF images can differ between centers because of a number of hardware, labeling,
3 and readout choices implemented by different MRI vendors and/or laboratories ([Deibler et al.](#)
4 [2008](#); [Heijtel et al. 2014](#); [Alsop et al. 2015](#); [Jack et al. 2010](#)). Some of these differences can be
5 accounted for, as detailed in the previous sections. However, until a more robust procedure is
6 devised - e.g. the use of a flow phantom ([Oliver-Taylor et al. 2017](#)) - a pragmatic approach is
7 required to remove the remaining CBF quantification differences between sequences, scanner
8 types, and centers ([Mutsaerts et al. 2019](#)). ExploreASL optionally performs a spatially varying
9 intensity normalization by computing a smooth average CBF bias field for each ASL sequence
10 (Supplementary Figure 10). This step assumes that the studied physiological effects are equally
11 distributed across the subjects scanned with each sequence, scanner and/or site. The final CBF
12 images in the standard space are smoothed with an 8x8x8 mm full-width at half-maximum
13 Gaussian, and averaged to create a sequence/scanner type/site-specific mean CBF image. These
14 sequence-mean CBF images are intensity normalized to GM CBF of 60 mL/100g/min and averaged
15 to create a general mean CBF image. The sequence bias field is calculated by dividing the general
16 mean with the sequence-mean CBF image. The individual CBF images are multiplied by their
17 sequence bias to normalize the intensities across sequences ([Mutsaerts et al. 2018](#)).

18

19 **4.3. ROI statistics**

20 In ExploreASL, ROI masks are created by combining existing atlases with individual GM and WM
21 masks. The GM atlases currently implemented are: (i) MNI structural ([Mazziotta et al. 2001](#)), (ii)
22 Harvard-Oxford ([Desikan et al. 2006](#)), and (iii) Hammers ([Hammers et al. 2002](#)). A deep WM atlas

1 is created by eroding the SPM12 WM tissue class by a 4 voxel sphere (i.e. 6 mm), to avoid signal
2 contamination from the GM ([Mutsaerts et al. 2014](#)). Other existing, or custom, atlases can be
3 easily applied. The Online Brain Atlas Reconciliation Tool (OBART) at obart.brainarchitecture.org
4 ([Bohland et al. 2009](#)) provides an overview of the overlap and differences between atlases. For
5 each ROI, statistics are calculated separately within the left and right hemisphere, as well as for
6 the full ROI; both with and without PVC. The same CBF statistics are also calculated for user-
7 provided ROIs and lesion masks, as well as for the 25 mm margin around the ROI/lesion
8 ([Moghaddasi et al. 2015](#)), for the ipsilateral hemisphere excluding the lesion, and for the same
9 three masks at the contralateral side. Subject-specific ROI and lesion masks are treated the same,
10 except for the fact that lesion masks are also used for the cost function masking (see section 1.3).
11 Individual vascular masks are used to exclude regions with intra-vascular signal (see section 3.7)
12 from CBF statistics, but not from spatial CoV statistics.

13
14 Finally, all masks are intersected with a group-level analysis mask, created from the individual
15 analysis masks created in section 3.7. Individual differences of these analysis masks can be caused
16 by differences in head position, FoV, and nasal sinus size. To limit the effects of this mask
17 heterogeneity on statistical analyses, ExploreASL creates a group-level analysis mask from
18 standard-space voxels present in at least 95% of the individuals masks (Supplementary Figure 8).

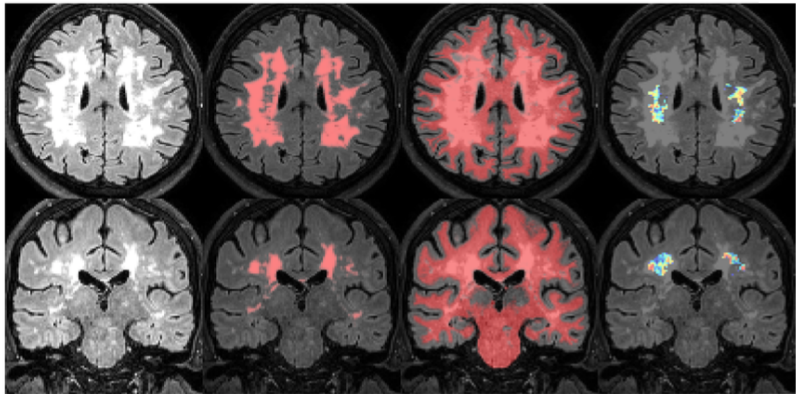
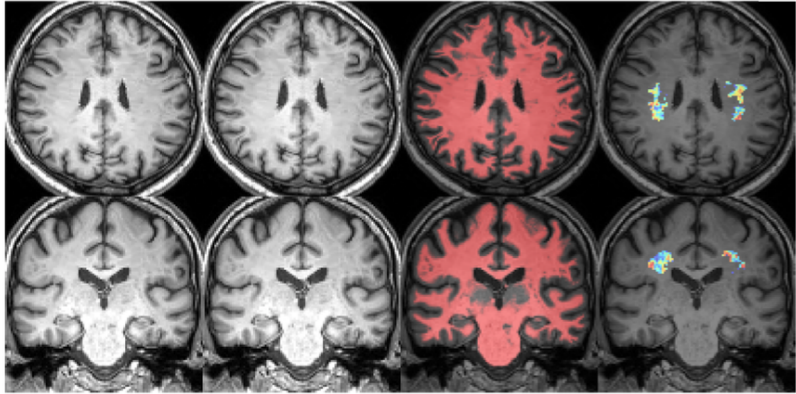
19
20 **4.4. Quality Control**
21 On a participant level, ExploreASL outputs QC parameters in a JSON file and provides unmasked
22 images in standard space for visual QC, for both intermediate and final images (Supplementary

1 Figure 11) to detect technical failure, outliers and artifacts. QC parameters are also obtained by
2 comparing individual ASL images with an atlas, a group average, or an average from a previous
3 study. Whole-brain and regional differences larger than 2-3 SD are indicated and should be
4 visually inspected. Deviations can hint to software updates or different scanners and, if not
5 accounted for, can lead to low power of the statistical analyses ([Chenevert et al. 2014](#)). All QC
6 parameters and images are also collected in a PDF file (Figure 3, Supplementary Table 2). While
7 these QC parameters can be helpful in detecting artifacts and/or protocol deviations, their use
8 has not yet been validated, and the normal and abnormal range for each of the parameters still
9 need to be determined.

xASL report: Philips2DEPI, EPAD-001_AS_L_1

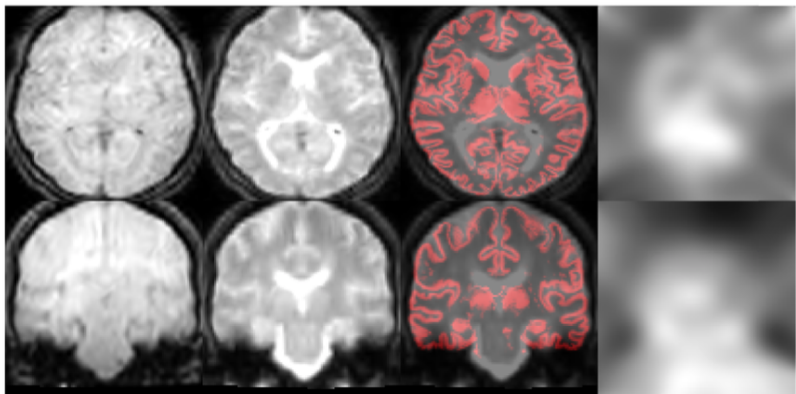
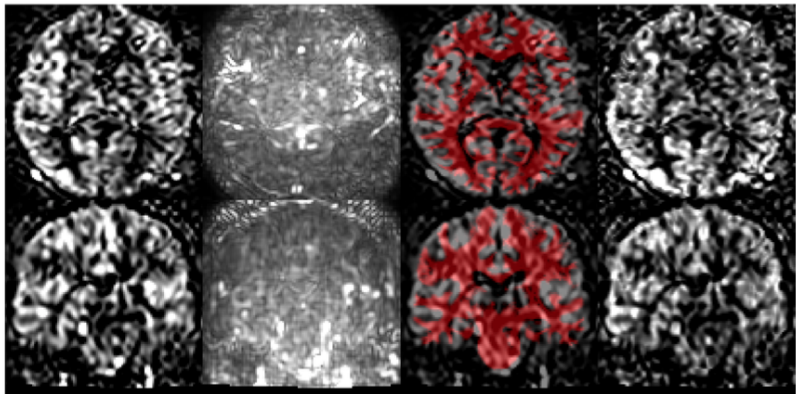
Structural

FLAIR_CNR_GM_WM_Ratio	0.002365
FLAIR_EFC_bits	1.866e+04
FLAIR_FBER_WMref_Ratio	5.876
FLAIR_Mean_AI_Perc	7.012
FLAIR_SD_AI_Perc	5.699
FLAIR_SD_WMref	147.7
FLAIR_SNR_GM_Ratio	6.526
FLAIR_WMH_n	26
FLAIR_WMH_vol_mL	94.406
FLAIR_WMref_vol_mL	10.58
ID	EPAD-001
T1w_CNR_GM_WM_Ratio	0.2872
T1w_CSF_vol_mL	603.8
T1w_EFC_bits	1.37e+04
T1w_FBER_WMref_Ratio	178.3
T1w_GM_ICV_Ratio	0.335
T1w_GM_vol_mL	604
T1w_ICV_vol_mL	1805
T1w_IQR_Perc	83.57
T1w_LR_flip_YesNo	0
T1w_Mean_AI_Perc	7.012
T1w_SD_AI_Perc	5.699
T1w_SD_WMref	31.64
T1w_SNR_GM_Ratio	25.06
T1w_WM_vol_mL	597
T1w_WMref_vol_mL	15.09
Version_CAT12	1363
Version_ExploreASL	0.2.2
Version_LST	2.0.15
Version_Matlab	8.5_R2015a
Version_SPM12	7219



ASL

AI_Perc	40.42
ASL_CoveragePerc	92.02
ASL_tSNR_CSF_Ratio	0.04633
ASL_tSNR_GMWM_Ratio	0.1819
ASL_tSNR_GMWM_WMref_Ratio	0.2626
ASL_tSNR_GM_Ratio	0.3236
ASL_tSNR_Physio2Thermal_Ratio	0.7212
ASL_tSNR_Slope_Corr	0.0002348
ASL_tSNR_WM_Ratio	0.1745
ASL_tSNR_WMref_Ratio	0.1205
AcquisitionTime_hhmmss	140449.29
BackGrSupprPulses	2
CBF_GM_Median_mL100gmin	32.05
CBF_GM_PVC2_mL100gmin	51.24
CBF_GM_WM_Ratio	4.751
CBF_WM_PVC2_mL100gmin	10.79
EchoTime_ms	10.49
ID	EPAD-001_AS_L_1
Initial_PLD_ms	2025
LR_flip_YesNo	0
LabelingDuration_ms	1650
LabelingType	CASL
MRSscaleSlope	0.0007
Matrix	64 64 36 60
Mean_SSIM_Perc	69.13
MotionExcl_Perc	0
MotionMean_mm	0.1479
PeakSNR_Ratio	674.7
RMSE_Perc	59.37
RepetitionTime_ms	4800
RescaleIntercept	0
RescaleSlope	2.712
RescaleSlopeOriginal	2.712
RigidBody2Anat_mm	100.7
SliceReadoutTime_ms	36.53
SpatialCoV_GM_Perc	96.69
Vendor	Philips
Version_FSL	6.0.1
VoxelSize_mm	3.4375 3.4375 4.5
nRMSE_Perc	23.25



1 Figure 3. Example PDF report for a single subject. This provenance and QC report includes information collected
2 from each image processing step across the pipeline and assembled in the population module. It is stored in a key-
3 <value> format, facilitating inclusion of plugin or new parameters. Keys and values are grouped into the structural
4 and ASL modules, and the software versions (see Supplementary Table 2). Figures represent transversal and
5 coronal slices in MNI standard space: 1-4) T1w before and after lesion filling, pWM projected over T1w, WMref
6 projected over T1w, 5-8) FLAIR, WMH mask projected over FLAIR, pWM projected over FLAIR, WMref projected
7 over FLAIR, 9-12) CBF, temporal SD, pWM projected over CBF, temporal SNR, 13-16) mean control, M0 before
8 processing, pGM projected over M0, M0 after processing. The pWM/pGM projections in the third column allow a
9 visual assessment of registration performance. CBF = cerebral blood flow, FLAIR = FLuid Attenuated Inversion
10 Recovery, GM = gray matter, pGM = GM partial volume, pWM = WM partial volume, SNR = signal-to-noise ratio,
11 WMref = WM noise reference region, WM = white matter. Example data are from the EPAD study ([Ritchie et al.](#)
12 [2016](#)).
13

14 **Methods**

15 We illustrate the ExploreASL image processing results and reproducibility for three populations
16 with similar 2D-EPI PCASL protocols: perinatally-infected HIV children, healthy adults, and elderly
17 with mild cognitive complaints, from the NOVICE ([Blokhuys et al. 2017](#)), the Sleep ([Elvsåshagen et](#)
18 [al. 2018](#)), and the European Prevention of Alzheimer's Dementia (EPAD) studies ([Ritchie et al.](#)
19 [2016](#)), respectively (Supplementary Table 3). All three studies adhered to the Declaration of
20 Helsinki and were approved by the local ethics committees (Academic Medical Center (AMC) in
21 Amsterdam, Norwegian South East Regional Ethics Committee, and VU Medical Center
22 Amsterdam and University of Edinburgh, respectively). Written informed consent was obtained
23 from all participants (or parents of children younger than 12 years for NOVICE). Each participant
24 of the Sleep study received NOK 500 for participation.

25
26 The performance of image processing should be comparable between different centers,
27 independent of used hardware and OSes, to allow data pooling and comparison between studies.
28 Here, we investigated the between-center reproducibility of the intermediate and final pipeline
29 results without and with the ExploreASL-specific modifications of the SPM12, CAT12, and LST

1 source code (modifications described below). To this end, a single participant from each study
2 was analyzed: with the lowest GM volume from NOVICE and EPAD (GM/ICV ratio 0.41 and 0.33,
3 respectively), and the highest GM volume (GM/ICV ratio 0.55) from the Sleep study. These three
4 datasets were processed at two centers with the following combinations of OS and MATLAB
5 version: Linux-2018b (HZDR, Dresden, Germany; Linux server, 2.1 GHz Intel Xeon 6130, Ubuntu
6 5), Windows-2015a and 2018b (Amsterdam UMC, The Netherlands; Dell Alienware laptop, 2.9-
7 4.3 GHz Intel i7-7820HK, Windows 10 Version 1903). After each pipeline step, the between-center
8 - or between-system - reproducibility was obtained as difference of the image intensities and
9 orientation between the NIfTIs of the two compared systems. The image intensity reproducibility
10 was calculated as the median voxel-wise relative intensity difference ([Kurth et al. 2015](#)), whereas
11 the image orientation reproducibility was calculated as the mean voxel-wise net displacement
12 vector in real-world coordinates ([Power et al. 2012](#)). These were calculated for T1w with GM
13 segmentation, FLAIR with WMH segmentation, M0, quantified CBF, GM partial volume in ASL
14 native space (pGM_{ASL}), and PV-corrected GM CBF.

15
16 More complex calculations involving floating-point arithmetic operations, e.g. matrix inversions,
17 can produce different results between OSes and MATLAB versions in the last digits. These minimal
18 differences can accumulate in iterative algorithms such as segmentation and registration, and
19 propagate across the pipeline. To mitigate these effects, during the process of implementing and
20 using the pipeline for previous clinical studies, we modified parts of the SPM12, CAT12, and LST
21 toolboxes: e.g. using the MATLAB '\ ' operator for solving a system of linear equations instead of

- 1 calculating a matrix inversion, providing a separate C++ implementation for convolutions, and/or
- 2 rounding some calculations to 15 significant digits.

3

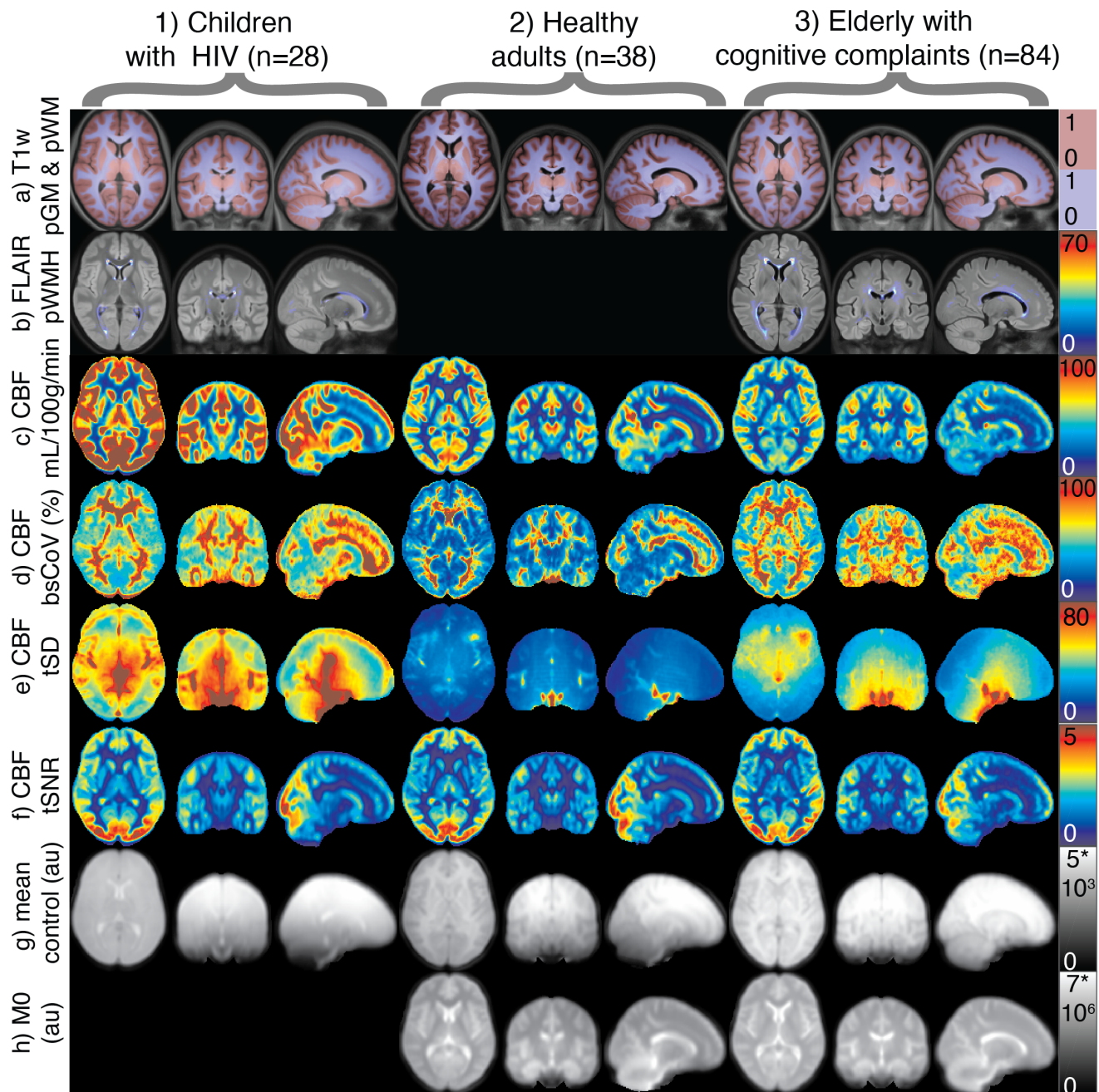
4 **Results**

5 Running time for a single EPAD participant took 22:11, 4:34, and 0:40 min for the Structural, ASL,
6 and Population modules, respectively (27:25 min in total). On low quality, the same processing
7 took 7:30 min, 2:24, and 0:34 respectively (10:28 min in total) (Windows-2018b). Figure 4 shows
8 differences between populations or sequences on the ExploreASL population-specific parametric
9 maps. While the GM CBF was highest in the pediatric and lowest in the geriatric population (Figure
10 4c), both the between-subject CoV and within-scan temporal SD were comparable in these
11 populations and lowest in the healthy adults (Figure 4d-e). The temporal SD (Figure 4e) was high
12 in vascular regions and highest around the ventricles in the pediatric dataset, due to a 2D EPI fat-
13 saturation related artefact. Despite these differences, the temporal SNR appeared relatively
14 comparable (Figure 4f), albeit slightly higher for the pediatric population. The average mean
15 control images (Figure 4g) showed subtle differences in background suppression efficacy, as
16 different tissue contrast and inferior-superior background suppression efficiency gradient. Only
17 slight differences in ventricle and sulci size were visible between the pediatric and geriatric
18 population (Figure 4a) confirming satisfactory performance of spatial normalization.

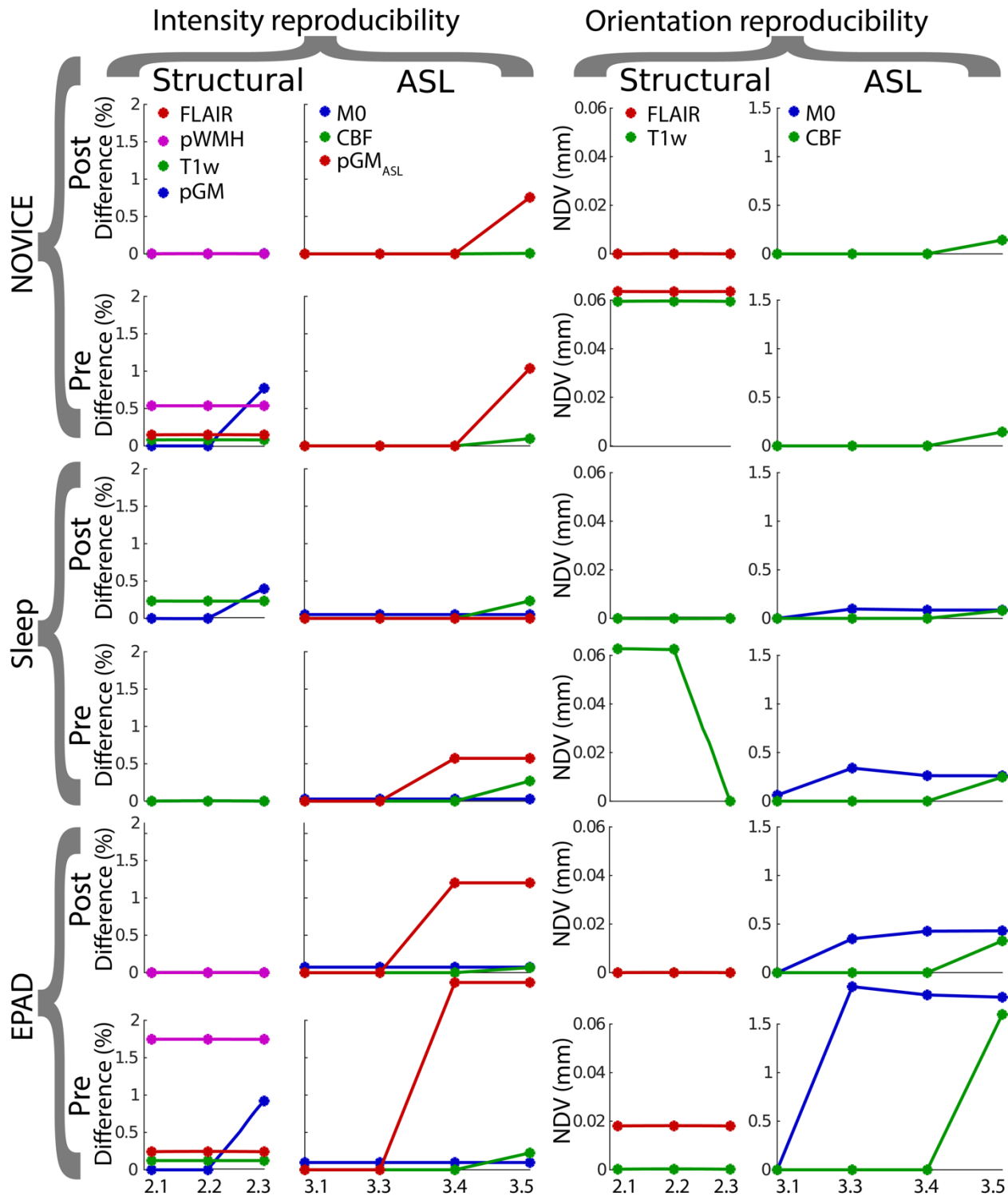
19

20 All three datasets showed zero difference when the pipeline was repeated twice on the same
21 system. When comparing OSes only - Linux-2018b vs Windows-2018b - the structural module
22 showed final voxel-wise differences of 0.77% pGM in NOVICE and 1.74% WMH in EPAD that

1 became negligible after our code modifications (Figure 5). The ASL module differences were
2 smaller than 0.5%, except for the pGM_{ASL} (0.57-2.5%) and PV-corrected GM CBF (0.61-5.76%).
3 Both improved after modifications to 0-1.2% and 0.32-1.5% for pGM_{ASL} and PV-corrected GM CBF,
4 respectively, showing the impact of our modifications. The reproducibility between OS and
5 MATLAB versions - Linux-2018b vs Windows-2015a - showed satisfactory post-modification
6 reproducibility, e.g. pGM_{ASL} (0.47-1.79%) and GM CBF (0.57-1.77%) (Supplementary Table 4).
7 Compared with the above-mentioned Linux-2018b vs Windows 2018b results this shows an
8 additional decrease in reproducibility when a different MATLAB version is used on top of different
9 OSes and/or systems.



1
2 Figure 4. Transversal, coronal, and sagittal population average images for the three example populations: 1) NOVICE,
3 2) Sleep study, 3) EPAD (see Supplementary Table 3): a) T1w anatomical image with pGM and pWM overlay , b) FLAIR
4 anatomical image overlaid with probability of WMH presence across the whole population, c) Mean CBF, d) between-
5 subject CBF variation (SD CBF/mean CBF per voxel across all subjects), e) temporal SD of CBF, mean over all subjects
6 is shown, f) temporal SNR of CBF (mean CBF/tSD CBF), g) mean control image (note the background suppression
7 gradient), h) M0 calibration image. Note that the FLAIR and M0 were not acquired in the Sleep and NOVICE studies,
8 respectively. CBF = cerebral blood flow, tSNR = temporal signal-to-noise ratio, tSD = temporal standard deviation, au
9 = arbitrary units, bs = between-subject, CoV = coefficient of variance, p = probability, GM = gray matter, WM = white
10 matter, WMH = white matter hyperintensity.



1
2 Figure 5. Reproducibility between Linux-2018b and Windows-2018b and for the three datasets NOVICE, Sleep, and
3 EPAD. Results are shown before (pre) and after (post) the ExploreASL-specific modifications of MATLAB and SPM12
4 code. The median relative intensity difference is shown in the two columns on the left (referred to as difference) and
5 the mean voxel-wise net displacement vector (NDV) is shown in the two columns on the right. Labels on the x-axis
6 describe the processing steps in the Structural (2.1 = WMH correction, 2.2 = Segmentation, 2.3 = Spatial
7 normalization) and ASL module (3.1 = Motion correction, 3.3 = T1w-ASL registration, 3.4 = M0 processing, 3.5 = CBF
8 quantification) as described in Table 1. pGMASL = gray matter partial volume map in ASL space.

1 Discussion and future directions

2 In this manuscript, we reviewed many of the most salient ASL image processing choices, and their
3 implementation in ExploreASL version 1.0.0. We demonstrated the software's functionality to
4 review individual cases as well as population-average images for quality control. Our findings
5 show that between-center computing differences can lead to voxel-wise CBF quantification
6 differences of up to 5.7% on average for the total GM, which were reduced to 1.7% by addressing
7 implementation differences of complex floating-point operations between MATLAB versions and
8 OSes. This may especially be beneficial for multi-center studies or for pooling multiple ASL studies
9 to attain sample sizes required for the discovery of subtle (patho-)physiological perfusion
10 patterns.

11
12 Several other ASL image processing pipelines are publicly available and free for academic use,
13 each providing specific features. The first publicly available pipeline ASLtbx quantifies CBF of
14 various ASL sequences ([Wang et al. 2008](#)) and features customized motion correction and
15 advanced outlier detection ([Dolui et al. 2017](#)); ASAP contains a graphical user interface (GUI) with
16 an interface for population analyses, and generates statistical reports ([Mato Abad et al. 2016](#));
17 the ASLM toolbox is a MATLAB- and SPM-based command-line tool ([Homan et al. 2012](#)), ASL-
18 MRICloud features a web interface with an automated cloud solution ([Li et al. 2018](#)); ASL-QC
19 handles multiple vendors and provides QC metrics (not published); BASIL uses a Bayesian
20 approach for the quantification and PVC of multi-TI, QUASAR, and time-encoded ASL data, thus
21 offering the most comprehensive quantification ([Chappell et al. 2010](#)); CBFIRN offers an online
22 data repository with online image processing ([Shin et al. 2016](#)); Functional ASL (FASL) ([FASL](#)

1 [webpage](#)) and fMRI Grocer ([Zhu Grocer](#)) are SPM toolboxes that process both functional ASL and
2 BOLD MRI; GIN fMRI performs separate control and label realignment and automatically excludes
3 outliers and volumes with strong motion (unpublished); MilxASL features spatial and temporal
4 denoising ([Fazlollahi et al. 2015](#)); MJD-ASL is implemented into 'cranial cloud', addresses noise
5 concerns and processes cerebral blood volume (CBV) ([MJD-ASL Webpage](#)); NiftyFit supports
6 quantification of other MRI sequences as IVIM, NODDI, and relaxometry ([Melbourne et al. 2016](#));
7 VANDPIRE is Python-based, has a scanner console plugin and allows flow territory mapping from
8 vessel-encoded ASL ([Arteaga et al. 2017](#)).

9
10 The main advantage of ExploreASL is the use of image processing that is optimized for clinical
11 studies, to address the diversity of the clinical populations, hardware, and sequences used. Other
12 strengths include its between-center reproducibility of image processing, and its flexible open
13 source development through GitHub with a growing team of international scientists. This
14 improves the likelihood of rapid debugging, encourages inter-institutional cross-pollination in
15 validation of new techniques, and allows quick adaptation of the software to new regulations
16 regarding e.g. best practice ([Nichols et al. 2017](#)) and data transfer ([Regulation 2016](#)). Moreover,
17 the use of a common validated pipeline rather than in-house software increases reliability and
18 reproducibility in neuroscience research ([Poldrack et al. 2017](#)). The ExploreASL team actively
19 focuses on keeping the pipeline user-friendly by allowing download on demand during the
20 development phase and by being in close-contact with all the users. ExploreASL was built upon
21 freely available software that performs well in a wide array of cases, rather than opting for
22 solutions with optimal performance in specific cases but not applicable in general. Still, the

1 modular structure of ExploreASL allows the replacement of some steps by solutions tailored for
2 specific datasets.

3
4 Although ExploreASL allows custom labeling efficiency values and global CBF calibration, it does
5 not estimate labeling efficiency. While literature values for labeling efficiency ([Dai et al. 2008](#))
6 may suffice in many clinical cases ([Heijtel et al. 2014](#)), individual correction can be beneficial for
7 specific populations ([Václavů et al. 2018](#)). For these, phase-contrast MRI can improve the CBF
8 quantification by a) calibrating CBF based on total flow through the brain-feeding arteries ([Aslan
9 and Lu 2010; Ambarki et al. 2015](#)), or b) modeling the labeling efficiency based on the velocity in
10 the labeling plane ([Václavů et al. 2019](#)). Compared to ASL, drawbacks of the phase-contrast MRI
11 include its lower reproducibility for whole brain CBF estimates ([Dolui et al. 2016](#)), and its lower
12 agreement with PET ([Puig et al. 2018](#)). Moreover, an automatic implementation requires good
13 data quality, perpendicular placement of the labeling plane to the vessels, and the absence of
14 vessel tortuosity, conditions that are rarely met in clinical datasets. Future solutions may be
15 provided by new sequences under development, which allow direct labeling efficiency
16 measurements during the ASL acquisition ([Chen et al. 2018; Lorenz et al. 2018](#)).

17
18 Several additional features are scheduled for future releases, after being tested first through the
19 GitHub beta versions of ExploreASL. These include full BIDS support ([Gorgolewski et al. 2016](#));
20 support for Hitachi and Canon datasets; unit testing to ensure stability of the pipeline through the
21 continuous development; inclusion of WM atlases for extended WM analysis; a GUI for easier
22 configuration and execution; quantification of advanced ASL schemes such as velocity- and

1 acceleration-selective ASL ([Schmid et al. 2015](#)) and integration of the BASIL toolbox to allow multi-
2 PLD and time-encoded sequence quantification ([Chappell et al. 2009](#)); and support for individual-
3 center calibration, e.g. using the recently introduced Quantitative ASL Perfusion Reference
4 (QASPER) ([Oliver-Taylor et al. 2017](#)) phantom ([Gold Standard Phantoms, London, UK](#)). Although
5 ExploreASL's computation times are moderate for research purposes, a clinical scanner
6 implementation would benefit from parallelization on graphical processing units (GPUs) to
7 provide robust automatic QC within clinical scanning time (e.g. <5 min). Another improvement
8 would be the investigation of the effect of image processing choices, as well as the availability of
9 physiological and quantification parameters for different populations ([Fazlollahi et al. 2015](#)). This
10 would allow for the incorporation of quantification confidence intervals in the output of
11 ExploreASL. For anonymization purposes, the face can be removed from the structural scans
12 ([Nichols et al. 2017](#); [Leung et al. 2015](#)) using a defacing algorithm such as the one implemented
13 in SPM12, but further testing is required to verify that the analysis is not affected ([de Sitter et al.](#)
14 [2017](#)). Statistical analyses can be biased for populations with large inter-subject differences in
15 their deformations, e.g., developing brains or a wide range of atrophy. The CerebroMatic toolbox
16 ([Wilke et al. 2017](#)) is a tool that accounts for this bias and will be incorporated in future releases
17 of ExploreASL. Finally, we intend to implement ExploreASL as a cloud solution and scanner
18 console plugin.

19
20 Image processing techniques that require validation include: using the UNWARP toolbox for
21 simultaneous motion and susceptibility deformation correction ([Andersson et al. 2001](#)), using
22 temporal information for artifact removal through the application of an independent-component

1 analysis (ICA) ([Wells et al. 2010](#); [Hao et al. 2018](#); [Zhu et al. 2018](#)) or using respiratory and cardiac
2 signal ([Restom et al. 2006](#)), spatial denoising – once validated under realistic conditions ([Spann et](#)
3 [al. 2017](#); [Wells et al. 2010](#); [Bibic et al. 2010](#); [Liang et al. 2015](#)), obtaining GM-WM segmentations
4 from fractional signal modeling of the magnetization recovery profile acquired with a Look-Locker
5 readout ([Petr et al. 2013](#); [Ahlgren et al. 2014](#)), and using the BBR method for motion correction
6 or registration ([Greve and Fischl 2009](#)). Finally, we aim to improve the inter-center reproducibility
7 even further.

8

9 **Conclusion**

10 ExploreASL is a versatile pipeline that performs well on a wide-range of diseases, including
11 datasets with lesions, allows flexible parameter definition, and a quick exploration of datasets
12 and QC images of each pipeline step in the same space. We made the pipeline available at
13 www.ExploreASL.com. We anticipate that ExploreASL will allow for more flexible collaboration
14 amongst clinicians and scientists, help to achieve the consensus standards for ASL processing
15 sought by the OSUPI, facilitate validation of ASL image processing approaches, and accelerate
16 translation to clinical research and practice.

17

18 **Acknowledgments**

19 This project has received support from the following EU/EFPIA Innovative Medicines Initiatives (1
20 and 2) Joint Undertakings: EPAD grant no. 115736, AMYPAD grant no. 115952. Additionally, this
21 work received support from the EU-EFPIA Innovative Medicines Initiatives Joint Undertaking

1 (grant No 115952). HM is supported by Amsterdam Neuroscience funding. FB and XG are
2 supported by NIHR funding through the UCLH Biomedical Research Centre. DLT is supported by
3 the UCL Leonard Wolfson Experimental Neurology Centre (PR/ylr/18575). EDV is supported by
4 the Wellcome/EPSRC Centre for Medical Engineering [WT 203148/Z/16/Z]. IA is supported by The
5 Gleason Foundation. MJPvO receives research support from Philips, the EU under the Horizon
6 2020 program (project: CDS-QUAMRI, project number 634541), and the research program
7 Innovational Research Incentives Scheme Vici with project number 016.160.351, which is
8 financed by the Netherlands Organization for Scientific Research (NWO). MC received funding
9 from the Engineering and Physical Sciences Research Council UK (EP/P012361/1). The Wellcome
10 Centre for Integrative Neuroimaging is supported by core funding from the Wellcome Trust
11 (203139/Z/16/Z). The authors wish to thank the COST-AID (European Cooperation in Science and
12 Technology - Arterial spin labeling Initiative in Dementia) Action BM1103 and the Open Source
13 Initiative for Perfusion Imaging (OSIPI) and the ISMRM Perfusion Study groups for facilitating
14 meetings for researchers to discuss the implementation of ExploreASL. The authors acknowledge
15 Guillaume Flandin, Robert Dahnke, and Paul Schmidt for reviewing the structural module for its
16 implementation of SPM12, CAT12, and LST, respectively; Krzysztof Gorgolewski for his advice on
17 the BIDS implementation; Jens Maus for help with MEX compilation; Cyril Pernet for providing
18 the SPM Univariate Plus scripts; and Koen Baas for curating the Philips 3D GRASE data. The
19 authors acknowledge the following researchers and teams: Yannis Paloyelis from King's College
20 London, for providing the data of the INtranasal OxyTocin trial, Torbjørn Elvsåshagen from Oslo
21 University Hospital for providing the Sleep study dataset; the EPAD investigators for providing the
22 Amsterdam site elderly dataset; Kim van de Ven from Philips Healthcare for providing the 3D

1 GRASE dataset; Philip de Witt Hamer from Amsterdam UMC for providing the PICTURE dataset,
2 and Chris Chen from the National University of Singapore for providing the Singapore Memory
3 Clinical dataset.

4

5 Declaration of interest

6 None.

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