

# PCASL PREPROCESSING PIPELINE WITH MOTION SCRUBBING

Complete Documentation and Verification Guide

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Find all required files/ folders linked here,

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## 1. PIPELINE OVERVIEW

### PURPOSE:

Process pseudo-continuous arterial spin labeling (pCASL) data with motion correction and volume removal (SCRUB) to improve cerebral blood flow (CBF) quantification accuracy.

### PIPELINE STAGES:

Stage 1: DICOM → NIfTI conversion (MASTER\_SETUP\_CORRECTED.sh)

Stage 2: Realignment and initial preprocessing (batch\_run\_PART1\_clean.m)

Stage 3: Motion-corrupted volume removal and file modifications for r\_ASL.nii, mean\_ASL.nii and rp\_ASL.txt (removing volumes from subjects that exceed threshold frame displacement 5mm, and rotation 3 degree; using matlab\_step3\_removal\_AUTO\_NO\_BACKUPS.m)

Stage 4: Coregistration, smoothing, CBF quantification, normalization (batch\_run\_PART2\_clean.m)

This approach allows identification of motion-corrupted volumes AFTER realignment, when motion parameters are most accurate, then removes those volumes before coregistration and CBF quantification.

## EXPECTED OUTCOME:

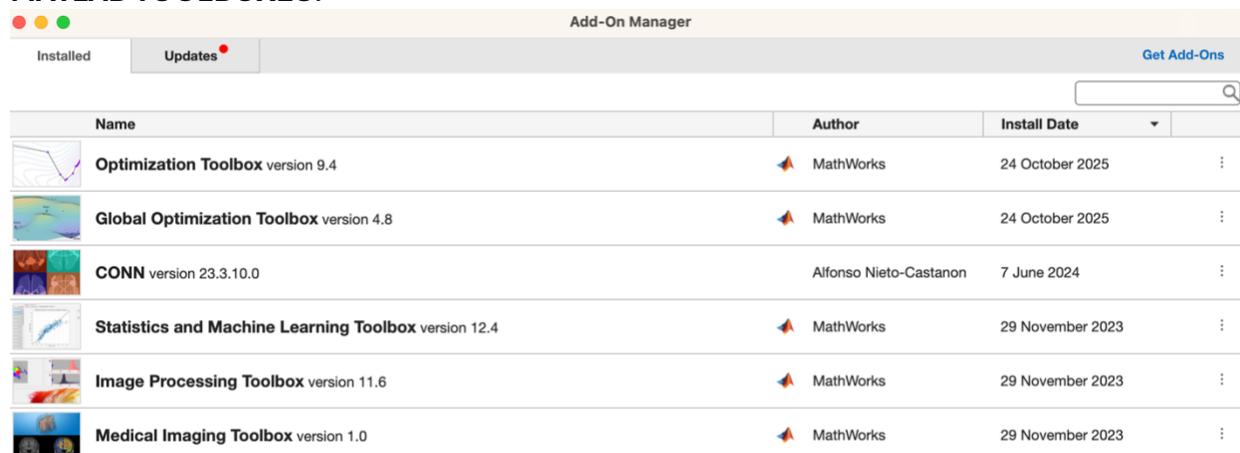
Improved CBF estimates by eliminating motion-corrupted label-control pairs that would otherwise contaminate the mean image and reduce CBF values.

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## 2. SOFTWARE REQUIREMENTS

- **SPM12**: <https://www.fil.ion.ucl.ac.uk/spm/doc/>
- **MATLAB** (tested with R2021b or later), <https://www.mathworks.com/downloads/>
- **FSL**: <https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>
- **dcm2niix**: <https://www.nitrc.org/plugins/mwiki/index.php/dcm2nii:MainPage#Introduction>
- Python 3.x with nibabel and numpy (for bash scripts, optional)

## MATLAB TOOLBOXES:



The screenshot shows the MATLAB Add-On Manager interface. The 'Updates' tab is selected, and a search bar is visible. The table below lists the installed toolboxes.

Name	Author	Install Date	
 <b>Optimization Toolbox</b> version 9.4	 MathWorks	24 October 2025	⋮
 <b>Global Optimization Toolbox</b> version 4.8	 MathWorks	24 October 2025	⋮
 <b>CONN</b> version 23.3.10.0	Alfonso Nieto-Castanon	7 June 2024	⋮
 <b>Statistics and Machine Learning Toolbox</b> version 12.4	 MathWorks	29 November 2023	⋮
 <b>Image Processing Toolbox</b> version 11.6	 MathWorks	29 November 2023	⋮
 <b>Medical Imaging Toolbox</b> version 1.0	 MathWorks	29 November 2023	⋮

## PATH SETUP:

SPM12 should be in MATLAB path

FSL should be properly configured in shell

---

### 3. DIRECTORY STRUCTURE TO REMEMBER AND APPLY

PROJECT ROOT here for me: /Users/vaidehipatel/Downloads/

Downloads

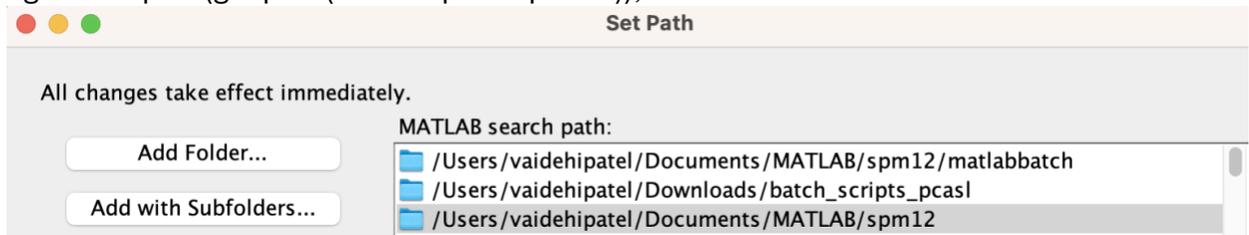
```
├─ data_dicom_archived/    # Original DICOM archives downloaded from Lab
Dropbox link below
├─ p033.tar.gz            # Example: sub86
├─ p061.tar.gz            # Example: sub135
├─ nifti_converted/       # Intermediate NIfTI files (generated from stage1, don't worry)
├─ sub86/
│  └─ ASL.nii.gz
│  └─ T1.nii.gz
├─ batch_scripts_pcasl/   # Main working directory (links for downloading
batchscripts and par.m as per lab on lab Dropbox linked below)
├─ par.m                  # Configuration file (linked below)
├─ batch_run_PART1_clean.m (linked below)
├─ batch_run_PART2_clean.m (linked below)
├─ matlab_step3_removal_AUTO_NO_BACKUPS.m (linked below)
├─ qc_outputs/           # Quality control outputs
├─ Master_Exclusion_List.txt # file that was created for deleting volume pairs from
outlier subjects linked below ;
    YOU HAVE TO SET PATH TO WHEREVER THIS FILE IS FOR YOU in the stage3
matlab script that removes volumes
├─ sub86/                 # Subject data (files are generated here)
│  └─ FUNC/
│     └─ ASL.nii          # Original 90 volumes
│     └─ rASL.nii         # Realigned, then cleaned to 62 vols
│     └─ rp_ASL.txt       # Motion parameters, 62 rows
│     └─ meanASL.nii      # Mean image for coregistration
│     └─ ...
│  └─ STRUC/
│     └─ T1.nii
├─ [other subjects...]
└─ MASTER_SETUP_CORRECTED.sh # Stage 1 script, linked below
```

=====

## STEPS BEFORE WE MOVE TO STAGE 1,2: INITIAL PREPROCESSING

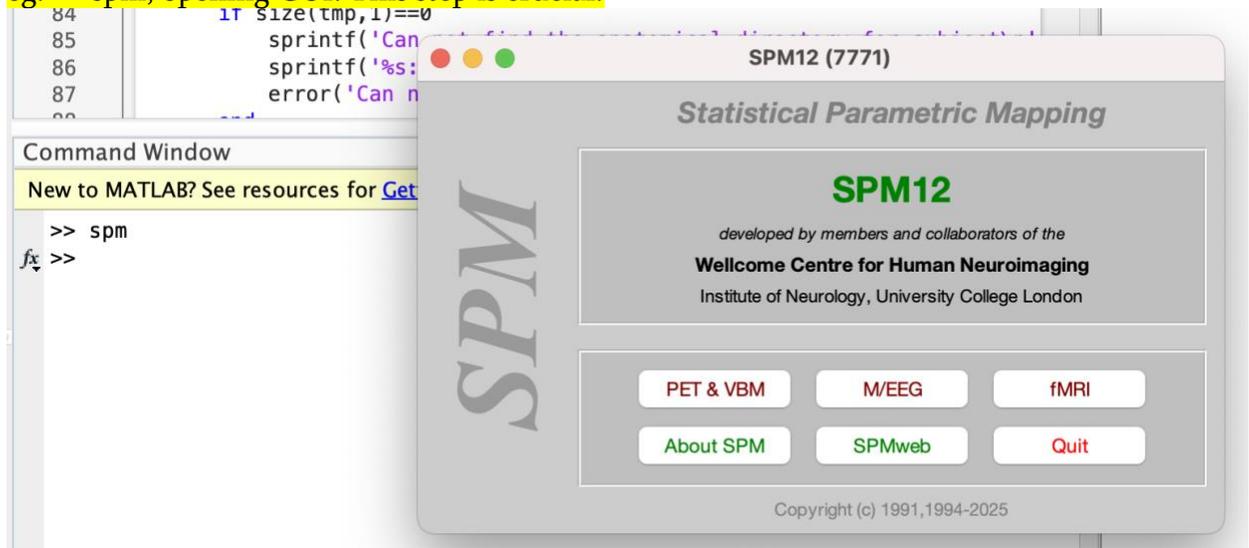
### Steps to initialize Matlab-SPM if not already before downloading batch\_scripts\_pcasl folder:

1. Open Matlab command window, Add path or set path to SPM12, eg. `>>addpath(genpath('/Users/path/spm12'))`,



2. Initialise and run SPM.

eg. `>> spm`, opening GUI. This step is crucial.

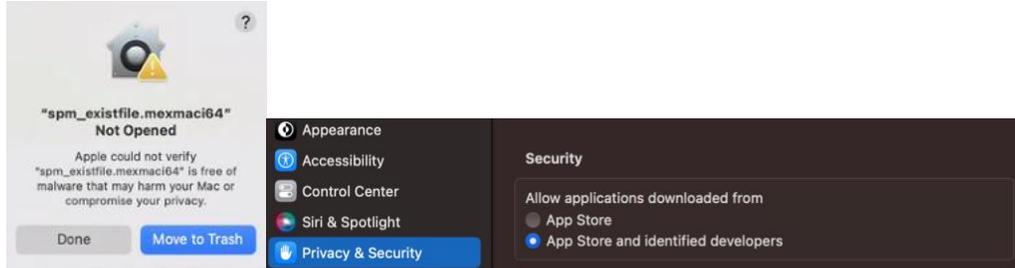




Make sure there's no multiple versions of SPM or Matlab that can cause confusion to you or the system. (Refer Error 1 below for problems in Mac for this step), then click quit spm, but don't exit Matlab.

## Errors usually encountered in the process of initializing SPM (Mac):

- 1) If while running par, an error relating to MEX files appears, you probably have one version of SPM/Matlab accessing or trying to compile using another older/newer version of the application present on your computer. If the error is relating to Mex files plus an apple pop up like below, it means you might need to goto Settings and enable downloading software/applications from sources besides AppStore.



Online refer: <https://www.mathworks.com/matlabcentral/answers/2154500-spm12-problem-in-macos>

- 2) If there's an error while intialising spm like below, you might have to check if you followed all instructions for installation properly. Here's a video channel many beginners prefer while installing SPM, it's a good resource channel for neuroimaging topics, <https://www.youtube.com/watch?v=qbcBLXJhzZg>

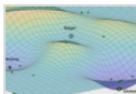
```
⌘ Explain Error
>> spm
Error using spm_check_installation>check_basic (line 140)
SPM uses a number of MEX files, which are compiled functions.
These need to be compiled for the various platforms on which SPM
is run. At the FIL, where SPM is developed, the number of
computer platforms is limited. It is therefore not possible to
release a version of SPM that will run on all computers. See
/Users/elikoukoulis/Documents/MATLAB/spm12-r6906/src/Makefile and
http://en.wikibooks.org/wiki/SPM#Installation
for information about how to compile mex files for MACA64
in MATLAB 25.1.0.2973910 (R2025a) Update 1.

Error in spm_check_installation (line 28)
    check_basic;
    ~~~~~

Error in spm (line 290)
    spm_check_installation('basic');
    ~~~~~
```

<https://www.fl.ion.ucl.ac.uk/spm/docs/installation/#macos>

- 3) Other error is about installing some toolboxes, this step needs to be done if and only if the errors indicate some function in batch\_run needs one of three toolboxes. Click one of the toolboxes like below and sign in your Matlab account when prompted, download and intall the toolbox like below and when Matlab terminates and restarts follow steps 4,5,6,7 from the steps to execute again.



**Global Optimization Toolbox** version 4.8

- 4) If there's an error like below it simply is indirectly indicating that spm wasn't initialized properly after installation. Refer Error 2) solution and execute steps 4,5,6,7 from steps to execute again after initializing spm.

```
Command Window
batch normalization for #1 - th subject...
Item matlabbatch: No repeat named
spm
Incorrect number or types of inputs or outputs for function 'list'.
Error in cfg_repeat/list (line 112)
    [id1, stop1] = list(citems(k), spec, tropts);
Error in cfg_util>local_getcjid2subs (line 1365)
    cjid2subsin = list(cjin, exspec, tropts);
Error in cfg_util>local_initjob (line 1555)
    [ucj, ucjid2subsin] = cellfun(@local_getcjid2subs, ucj, 'UniformOutput', false);
Error in cfg_util (line 815)
    [jobs(cjob), mod_job_idlist] = local_initjob(jobs(cjob), job, jobdedup);
Error in cfg_util (line 966)
    cjob = cfg_util('initjob',varargin{1});
Error in ASLtbx_spm12normest (line 18)
    cfg_util('run', matlabbatch);
Error in batch_norm_spm12 (line 33)
    ASLtbx_spm12normest(nP);
Error in batch_run (line 41)
    batch_norm_spm12;
```

=====

## BEFORE WE MOVE TO STAGE 1,2: INITIAL PREPROCESSING (PART1)

### Steps to organize ASLtbx2

1. Download a version of approved **batch\_scripts\_pcasl.zip** available on our lab Dropbox link .

You have to confirm if the par.m on dropbox match your requirements first, depending on the dataset you're working on, we are working on MOSS dataset here.

Alternatively, you can download Batch\_scripts folder named as ASLtbx2 from Upenn website, link, <https://www.cfn.upenn.edu/zewang/download.php>

But two scripts folders linked above, have scripts, namely par.m and batch\_norm\_spm12.m and batch\_perf\_subtract.m different in bounding box size and accepting PAR global parameters instead of local parameters. So its preferable to have atleast par.m from lab version and make minute change regardless of where you get batch\_scripts\_pcasl folder from. Differences explained in point 3.

- There is lab-specific version of script `par.m` that we have to replace in `batch_scripts_pcasl` folder (refer to Hoon for more details). Linked here, [par.m\(\)](#)

```

Editor - /Users/vaidehipatel/Downloads/batch_scripts_pcasl/par.m
par.m  batch_perf_subtract.m  batch_norm_spm12.m  asl_perf_subtract.m  +
141  PAR.ana_dir = 'glm';
142  PAR.subtractiontype=0;
143  PAR.glcbbfile=['globalsg_' num2str(PAR.subtractiontype) '.txt'];
144  PAR.img4analysis='cbf'; % or 'Perf'
145  PAR.ana_dir = ['glm_' PAR.img4analysis];
146  PAR.Filter='cbf_0_sr';
147  % parameters for cbf quantification
148  PAR.FirstimageType=0; % 0 means labeling first (images are acquired in an order of label
149  PAR.SubtractionType=0; % 0: simple subtraction, 1: surround subtraction, 2: sinc subtracti
150  PAR.SubtractionOrder=1; % 0: label - control, 1: control - label
151  PAR.MaskFlag=1; % Flag #1, 1 means masking out images using an implicit or explicit mask ima
152  PAR.MeanFlag=1; % Flag #2, 1 means creating mean images (for the non-subtracted raw data, AS
153  PAR.CBFFlag=1; % Flag #3, 1 means calculating CBF (this is the default value)
154  PAR.BOLDFlag=0; % Flag #4, 1 means extracting pseudo BOLD images (an obsolete option)
155  PAR.OutPerfFlag=0; % Flag #5, 1 means saving the perfusion difference images (the perfusion wei
156  PAR.OutCBFFlag=1; % Flag #6, 1 means saving CBF images rather than only the mean CBF map if Me
157  PAR.QuantFlag=0; % Flag #7, 1 means using a unique M0 value for the whole brain during CBF ca
158  PAR.ImgformatFlag=1; % Flag #8, 1 means saving images in NifTI format
159  PAR.D4Flag=1; % Flag #9, 1 means saving the image series in 4D format
160  PAR.M0wmcsfFlag=0; % Flag #10, 1 means using M0csf to estimate M0b, 0 means using M0wm
161  PAR.Flags=[PAR.MaskFlag PAR.MeanFlag PAR.CBFFlag PAR.BOLDFlag PAR.OutPerfFlag ...
162  PAR.OutCBFFlag PAR.QuantFlag PAR.ImgformatFlag PAR.D4Flag PAR.M0wmcsfFlag];
163  PAR.TimeShift = 0.5; % time shift for sinc interpolation. 0.5 means moving half of TR
164  PAR.ASLType = 1; % 1 means CASL or PCASL, 0 means PASL
165  PAR.Labeff = 0.8; % label efficiency. 0.85 for pcasl, 0.9 for pasl
166  PAR.MagType = 1; % 1 means 3T (please read the header in asl_perf_subtrac.m for more deta
167  PAR.Labeltime = 1.5; % labeling time in secs. For PASL, this parameter is for passing the T11.
168  PAR.Delaytime = 1.5; % post labeling delay time. For QUIPSS, this should be set to T12-T11.
169  PAR.slicetime = 27.5; % slice acquisition time in msec. Refer to the manual for how to calcu

```

These parameters are lab-specific in `par.m()`

- Now regardless of what version of `Batch_scripts_pcasl` folder you have, make sure at least `par.m` is lab-version. And check the following lines in following scripts and re-confirm with Hoon if some or all of the changes should apply to your scripts.
  - Confirm Which subjects should be part of line `PAR.subjects` in `par.m`, mapping of subject id details in next stage.

```

Editor - /Users/vaidehipatel/Downloads/batch_scripts_pcasl/par.m
par.m  batch_perf_subtract.m  batch_norm_spm12.m  asl_per
37  cd(PAR.batchcode_which);
38  %cd ../
39  data_root=pwd;
40  cd(old_pwd);
41
42
43  PAR.root=data_root;
44
45  % Subjects' directories
46  %One subject
47  PAR.subjects = {'sub10'};
48  %Subset of subjects
49  % PAR.subjects = {'sub3' 'sub4' 'sub5' 'sub6' 'sub7' '
50  % 'sub11' 'sub12' 'sub13' 'sub14' 'sub15' 'sub16'
51  % 'sub21' 'sub22' 'sub23' 'sub24' 'sub25' 'sub26'

```

- b. In batch\_norm\_spm\_12.m check the line highlighted in screenshot below it should have bounding box smaller than [-90 -126 -72; 90 90 108]<- which we have to comment % in case its uncommented . And make sure the bounding box size is either hardcoded [-78 -112 -70; 78 76 85] or accepting PAR.bb; as input from par.m (explained below)

```

25  r=spm_select('EXTFPList',PAR.structdir{sb},[PAR.structprefs '.*.nii']);
26  matname = fullfile(PAR.structdir{sb}, ['y_' spm_str_manip(P,'dst') '.nii']);
27  if exist(matname, 'file')==0
28      nP=strvcat(nP, P);
29  end
30  end
31  if ~isempty(nP)
32      ASLtbx_spm12normest(nP);
33  end
34  matlabbatch{1}.spm.spatial.normalise.write.woptions.bb = [-78 -112 -70; 78 76 85];%[-90 -126 -72; 90 90 108];PAR.bb;
35  matlabbatch{1}.spm.spatial.normalise.write.woptions.vox = [2 2 2];
36  matlabbatch{1}.spm.spatial.normalise.write.woptions.interp = 4;
37  for sb = 1:PAR.nsubs
38      P = spm_select('FPList',PAR.structdir{sb}, ['^' PAR.structprefs '.*.nii$']);
39      P = P(1,:);
40
41      imgs{1,1}=spm_select('FPList', char(PAR.condirs{sb,c}), ['^meanCBF.*.nii$']);
42      %% if you want to normalize the cbf image series, you can enable the
43      %% following lines
44      %   cbfimgs=spm_select('EXTFPList', char(PAR.condirs{sb,c}), ['^cbf.*.nii$'], 1:1000);
45      %   for i=1:size(cbfimgs,1)
46      %       imgs{1+i,1}=deblank(cbfimgs(i,:));
47      %   end
48
49      % Make the default normalization parameters file name
50      matname = fullfile(PAR.structdir{sb}, ['y_' spm_str_manip(P,'dst') '.nii']);
51
52  matlabbatch{1}.spm.spatial.normalise.write.subj.def{1} =matname;

```

- c. In par.m the bounding box size is defined in PAR.bb, in the lab version of par.m the bounding box is originally [-90 -126 -72; 90 90 108], we have to comment it %, and specify the bounding box size to [-78 -112 -70; 78 76 85] like below, especially if using the Lab batch\_scripts\_pcasl.zip folder and not the scripts folder from ASLtbx2 directly. If in case you're not accepting bounding box variables in batch\_norm\_spm12.m **from Par.bb**; then making change in Par.bb line hardcoding [-78 -112 -70; 78 76 85] is optional.

```

Editor - /Users/vaidehipatel/Downloads/batch_scripts_pcasl/par.m
par.m  batch_perf_subtract.m  batch_norm_spm12.m  asl_perf_subtract.m  +
116
117     if size(tmp,1)>1
118         sprintf('Panic! subject %s has more than 1 directories!\n', [PAR.subjects{sb}])
119         error('Panic! condition has more than 1 directories!')
120         %return;
121     end
122     PAR.condirs{sb,c}=fullfile(PAR.root,PAR.subjects{sb},spm_str_manip(char(tmp(1).name),'d
123     PAR.M0dirs{sb,c}=fullfile(PAR.root,PAR.subjects{sb},spm_str_manip(char(tmp(1).name),'d
124 end
125 end
126
127 % Smoothing kernel size
128 PAR.FWHM = [6];
129 PAR.bb = [-78 -112 -70; 78 76 85];%[-90 -126 -72; 90 90 108];%[-78 -112 -70; 78 76 85];
130 % % TR for each subject. As one experiment was carried out in one Hospital (with one machine)
131 % % and the other in another hospital (different machine), TRs are slightly different
132 % %PAR.TRs = [2.4696 2];
133 PAR.TRs = ones(1,PAR.nsubs)*6; % It is not used anymore; it was used for generating pseudo-BOLD
134 % PAR.mp='no';
135
136 %
137 PAR.mp='no';
138 %
139 PAR.groupdir = ['STAT'];
140
141 PAR.ana_dir = ['glm'];
142 PAR.subtractiontype=0;
143 PAR.glcbffile=['globalsg_' num2str(PAR.subtractiontype) '.txt'];
144 PAR.imganalysis='chfl'; % as 'Perf'

```

- d. Lastly, especially if you're not using the Lab batch\_scripts\_pcasl.zip folder, and using the scripts folder from ASLtbx2 directly, then make sure the batch\_perf\_subtract.m calls the asl\_perf\_subtract.m script passing labeling and other parameters during it defined in PAR. variables in par.m. Make sure the parameters are not hardcoded in the asl\_perf\_subtract() call,

```
asl_perf_subtract(P, PAR.FirstimageType, PAR.SubtractionType, ...
```

```
PAR.SubtractionOrder, PAR.Flags,...
```

```
PAR.TimeShift, PAR.ASLType, PAR.Labeff, PAR.MagType,...
```

```
PAR.Labeltime, PAR.Delaytime, PAR.slicetime, PAR.TE, [], [], maskimg);
```

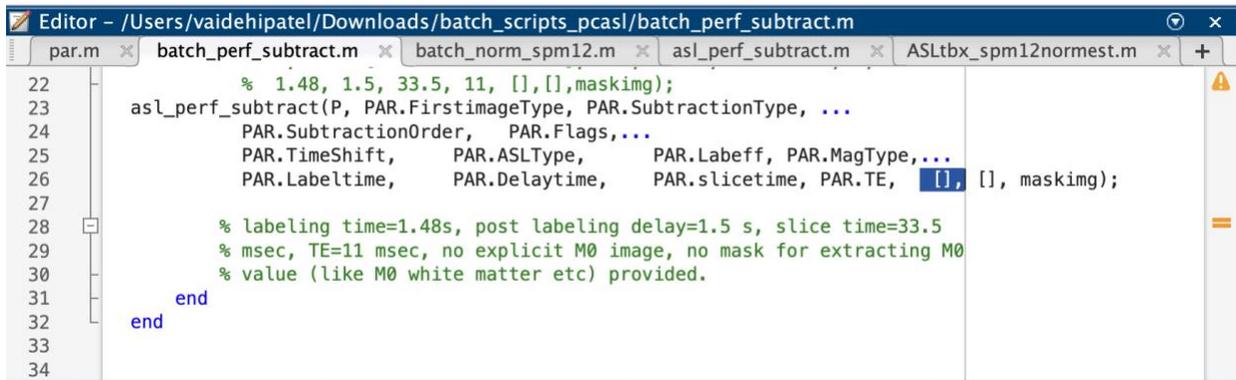
```

Editor - /Users/vaidehipatel/Downloads/batch_scripts_pcasl/batch_perf_subtract.m
par.m  batch_perf_subtract.m  batch_norm_spm12.m  +
23     M0img = spm_select('FPList', PAR.M0dirs{sb,c}, ['^' PAR.M0filters '.*\.nii']);
24 end
25 end
26 %     spm_perf_subtract(Filename,FirstimageType, SubtractionType,...
27 %     SubtractionOrder,Flag,Timeshift,AslType,MagType,Labeltime,Delaytime,Slicetime,M0img,M0
28 asl_perf_subtract(P, PAR.FirstimageType, PAR.SubtractionType, ...
29     PAR.SubtractionOrder, PAR.Flags,...
30     PAR.TimeShift, PAR.ASLType, PAR.Labeff, PAR.MagType,...
31     PAR.Labeltime, PAR.Delaytime, PAR.slicetime, PAR.TE, M0img, [], maskimg);
32
33 % labeling time=1.48s, post labeling delay=1.5 s, slice time=33.5
34 % msec, TE=11 msec, no explicit M0 image, no mask for extracting M0
35 % value (like M0 white matter etc) provided.
36
37 end

```

Command Window

M0img mostly needs be empty if in case if it gives error make it empty like highlighted line below.



```
22 % 1.48, 1.5, 33.5, 11, [], [], masking);
23 asl_perf_subtract(P, PAR.FirstimageType, PAR.SubtractionType, ...
24 PAR.SubtractionOrder, PAR.Flags, ...
25 PAR.TimeShift, PAR.ASLType, PAR.Labeff, PAR.MagType, ...
26 PAR.Labtime, PAR.Delaytime, PAR.slicetime, PAR.TE, [], [], masking);
27
28 % labeling time=1.48s, post labeling delay=1.5 s, slice time=33.5
29 % msec, TE=11 msec, no explicit M0 image, no mask for extracting M0
30 % value (like M0 white matter etc) provided.
31
32 end
33
34
```

---

#### 4. STAGE 1: DICOM TO NIFTI CONVERSION

SCRIPT TO USE: MASTER\_SETUP\_CORRECTED.sh  
Download MASTER\_SETUP\_CORRECTED.sh at link

INPUT FOLDER NEEDED FOR IT: Download data\_dicom\_archived folder that has all the Moss raw data from Lab Dropbox linked here

The subject mapping excel sheet example, p033\_s1=sub86, uploaded on Dropbox, for reference

#### PURPOSE:

Extract DICOM files from archives, convert to NiftI format, and setup directory structure for preprocessing.

#### BEFORE EXECUTION:

CHANGE PATH TO your 'data\_dicom\_archived (input) folder'  
CHANGE PATH to your 'batch\_scripts\_pcasl (output)'

## USAGE:

In `./MASTER_SETUP_CORRECTED.sh` set path to your `data_dicom_archived` folder. And set output folder to your specific output folder.

Execution command example(bash): `./MASTER_SETUP_CORRECTED.sh sub86`

## WHAT IT DOES:

---

1. Maps subject ID to archive name and session (if multi-session) `sub86 | p033 | s1` IS NOW EXCLUDED SO MAKE SURE TO NOT PROCESS IT ANYMORE with any steps.

2. Extracts DICOM from compressed archive
3. Locates ASL (pCASL) and T1 (MPRAGE) series
4. Converts to NIfTI using `dcm2niix`
5. Creates `batch_scripts_pcasl/[subject]/` directory structure
6. Copies ASL.nii to `FUNC/` and T1.nii to `STRUC/`, as required as input for ASLtbx

It does OUTLIER SUBJECT MAPPING in `data_dicom_archived` folder from Dropbox:

---

```
sub86 → p033.tar.gz, session s1
sub135 → p061.tar.gz, session s1
sub123 → p053.tar.gz, session s3
sub122 → p053.tar.gz, session s2
[...additional subjects in script...]
```

## OUTPUT:

---

```
batch_scripts_pcasl/sub86/FUNC/ASL.nii (90 volumes, 64x64x18)
batch_scripts_pcasl/sub86/STRUC/T1.nii
```

## VERIFICATION:

---

Check volume count:  
`fsinfo batch_scripts_pcasl/sub86/FUNC/ASL.nii | grep dim4`  
Expected: `dim4 = 90`

## CRITICAL DECISION MADE #1:

---

Multi-session subjects (e.g., p053 archive contains s1, s2, s3) require session specification. The script navigates to the correct session subfolder before searching for ASL and T1 series.

Rationale: Some archives contain multiple scanning sessions. Without session

specification, the script would find all sessions and fail or select the wrong data.

---

## 5. STAGE 2: INITIAL PREPROCESSING (PART1)

If you're running just batch\_run.m once with the whole pipeline, you don't need to see the next steps but if you're preprocessing batchrun in two parts where first you rest orientation, realign and then remove volumes and complete rest of batch run with removed volumes , continue.

### **FOR SCRIPT: batch\_run\_PART1\_clean.m , run in Matlab**

---

I divided batch\_run.m into two parts, linked here, in case you aren't running batch\_run.m all at once for pre-processing, below is link for batchrun\_PART1,

PATH SETTING IN par.m: adjust path between line 30-45 in case your subjects aren't being searched at the folder where you have your subjects

SUBJECT SPECIFICATION IN Line ~47 in par.m:

```
PAR.subjects = {'sub86'};
```

Change this to process different subjects as per the mapping of subjects eg p033\_s1 is sub86, p033\_s2 becomes sub87 and so on.

NOTE: Make sure you run clear all command on matlab console after each subject is fully processed after completion of stages, I processed all steps one subject at a time. Clear all makes sure the workspace variables for one subject does not get previous subjects workspace variable while processing new subject, as both have individual differences.

WHAT batch\_run PART1 DOES:

---

1. Resets orientation matrices to standard space
2. Realigns all 90 ASL volumes using spm\_realign\_asl.m
3. Generates motion parameters (rp\_ASL.txt, 90 rows x 12 columns)
4. Creates rASL.nii (90 realigned volumes)
5. Creates meanASL.nii (mean of all 90 volumes)

REALIGNMENT ALGORITHM:

---

Uses SPM's two-pass realignment with mean image as reference:

Pass 1: Realign to first volume, create initial mean

Pass 2: Realign to mean from Pass 1, create final mean

For a detailed flowchart of these two passes that happens automatically within realign, refer [SPM\\_TwoPass\\_Flowchart.html](#) u loaded on dro box, link



(NOTE: the key insight section at the botton isn't accurate but the Flowchart is)

Motion parameters (rp\_ASL.txt) contain 12 columns:

Columns 1-6: Translation (x,y,z) and rotation (pitch,roll,yaw)

Columns 7-12: "zigzag pattern cleaned" motion timecourses.

#### INPUT FILE:

batch\_scripts\_pcasl/sub86/FUNC/ASL.nii (90 volumes) from stage1 program for dicom to nifti conversion

#### OUTPUT FILES:

---

batch\_scripts\_pcasl/sub86/FUNC/rASL.nii (90 volumes, realigned)  
batch\_scripts\_pcasl/sub86/FUNC/rp\_ASL.txt (90 rows, motion parameters)  
batch\_scripts\_pcasl/sub86/FUNC/meanASL.nii (1 volume, mean of 90)

#### VERIFICATION:

---

Check files created:

```
ls -lh batch_scripts_pcasl/sub86/FUNC/rASL.nii
```

```
ls -lh batch_scripts_pcasl/sub86/FUNC/rp_ASL.txt
```

Check volume counts:

```
fsinfo batch_scripts_pcasl/sub86/FUNC/rASL.nii | grep dim4
```

Expected: dim4 = 90

```
wc -l batch_scripts_pcasl/sub86/FUNC/rp_ASL.txt
```

Expected: 90 rows

CRITICAL DESIGN DECISION #2:

---

Volume removal is performed AFTER realignment, not before.

Rationale: Motion parameters are most accurate after realignment. Attempting to identify bad volumes before realignment would require using unreliable motion estimates. Additionally, realignment itself can reveal motion through the creation of a blurry mean image when motion-corrupted volumes are included.

=====

## 6. STAGE 3: VOLUME REMOVAL AND MODIFICATIONS

**INPUT: Master\_Exclusion\_List.txt**

---

**Masterlist for outlier subject with info on volume pairs to remove**, its uploaded on Dro box link here

**The Severe Motion report where the outlier volumes and values highlighted are uploaded here, just for reference and big picture understanding,**

FORMAT:

---

Subject: sub86

Pairs to remove: 14

24-25

26-27

28-29

30-31

42-43

44-45

46-47

48-49

62-63

64-65

70-71

84-85

86-87

88-89

## CRITICAL: INDEXING CONVENTION

---

Master\_Exclusion\_List.txt uses 0-BASED INDEXING (like FSL, Python)

- First volume = 0
- Last volume = 89
- Total volumes = 90 (indices 0-89)

Pair "24-25" means:

- Volume at index 24 (0-based) = 25th volume physically
- Volume at index 25 (0-based) = 26th volume physically

MATLAB uses 1-BASED INDEXING:

- First volume = 1
- Last volume = 90
- Total volumes = 90 (indices 1-90)

CONVERSION REQUIRED: Add 1 to all Master List indices for MATLAB. For removal program is in matlab we switch indexing, if it's a shell script we don't have to switch indexing.

Example:

Master List (0-based): 24-25

MATLAB (1-based): 25-26

### **SCRIPT: matlab\_step3\_removal\_AUTO\_NO\_BACKUPS.m**

---

Download matlab ste 3 removal AUTO NO BACKUPS.m sta e 3 matlab scri t link



#### PURPOSE:

Read Master\_Exclusion\_List.txt, convert indexing, remove volumes from rASL.nii and rows from rp\_ASL.txt, recreate meanASL.nii from clean data.

#### USAGE:

---

In MATLAB:

```
>> cd /Users/vaidehipatel/Downloads/batch_scripts_pcasl
>> matlab_step3_removal_AUTO_NO_BACKUPS('sub86')
```

#### WHAT IT DOES:

- 
1. Reads Master\_Exclusion\_List.txt
  2. Locates subject section
  3. Extracts volume pairs (0-based indices)
  4. Converts to 1-based by adding 1
  5. Calculates volumes to keep using setdiff()
  6. Loads rASL.nii (90 volumes)
  7. Extracts only good volumes
  8. Overwrites rASL.nii with cleaned data (62 volumes for sub86)
  9. Loads rp\_ASL.txt (90 rows)
  10. Extracts only good rows
  11. Overwrites rp\_ASL.txt with cleaned data (62 rows)
  12. Deletes old meanASL.nii
  13. Loads cleaned rASL.nii
  14. Computes mean across cleaned volumes
  15. Writes new meanASL.nii (mean of 62 clean volumes)

#### INDEXING CONVERSION EXAMPLE (example: sub86):

---

Master List pairs (0-based):

24-25, 26-27, 28-29, 30-31, 42-43, 44-45, 46-47, 48-49,  
62-63, 64-65, 70-71, 84-85, 86-87, 88-89

Convert to MATLAB 1-based (add 1):

25-26, 27-28, 29-30, 31-32, 43-44, 45-46, 47-48, 49-50,  
63-64, 65-66, 71-72, 85-86, 87-88, 89-90

Flatten to individual volumes to remove:

25, 26, 27, 28, 29, 30, 31, 32,  
43, 44, 45, 46, 47, 48, 49, 50,  
63, 64, 65, 66,  
71, 72,  
85, 86, 87, 88, 89, 90

Total: 28 volumes to remove

Calculate volumes to keep:

all\_volumes = 1:90

volumes\_to\_remove = [25, 26, ..., 90]

volumes\_to\_keep = setdiff(all\_volumes, volumes\_to\_remove)

Result: [1:24, 33:42, 51:62, 67:70, 73:84]

Count: 24 + 10 + 12 + 4 + 12 = 62 volumes

## OUTPUT:

---

batch\_scripts\_pcasl/sub86/FUNC/rASL.nii (62 volumes, cleaned)  
batch\_scripts\_pcasl/sub86/FUNC/rp\_ASL.txt (62 rows, cleaned)  
batch\_scripts\_pcasl/sub86/FUNC/meanASL.nii (1 volume, clean mean)

Note: ASL.nii (original 90 volumes) is NEVER modified.

## VERIFICATION:

---

Check volume counts:

```
fsinfo batch_scripts_pcasl/sub86/FUNC/rASL.nii | grep dim4
```

Expected: dim4 = 62

Check row counts:

```
wc -l batch_scripts_pcasl/sub86/FUNC/rp_ASL.txt
```

Expected: 62 rows

Check meanASL quality:

```
fsleyes batch_scripts_pcasl/sub86/FUNC/meanASL.nii &
```

Expected: Sharp image, no motion blur

## CRITICAL DESIGN DECISION #3:

---

No backup files are created during volume removal.

Rationale: SPM batch scripts fail if unexpected backup files (e.g., rASL\_BEFORE\_SCRUB.nii) exist in the FUNC/ directory. The original ASL.nii remains untouched and serves as the permanent backup. All modifications are done to rASL.nii, rp\_ASL.txt, and meanASL.nii only.

## CRITICAL DESIGN DECISION #4:

---

meanASL.nii is recreated from the cleaned volumes rather than simply removing bad volumes from the original mean.

Rationale: The mean image is used for coregistration to the structural image. A blurry mean (from 90 volumes including motion artifacts) will result in Slightly poor coregistration. Recreating the mean from only the 62 clean volumes produces a sharp reference image, improving coregistration accuracy and ultimately CBF quantification.

## CRITICAL DESIGN DECISION #5:

---

Both rASL.nii and rp\_ASL.txt must be modified to have matching counts.

Rationale: PART2 preprocessing expects rASL.nii volume count to match rp\_ASL.txt row count. Mismatch causes errors. Both files must be updated to maintain 1-to-1 correspondence between volumes and motion parameters.

=====

## 7. STAGE 4: FINAL PREPROCESSING (PART2)

**SCRIPT: batch\_run\_PART2\_clean.m**

---

Uploaded code on lab Dropbox, batch\_run\_PART2,



## WHAT PART2 DOES:

---

1. Coregisters structural T1 to functional meanASL
2. Smooths functional images with 6mm FWHM Gaussian kernel
3. Performs CBF quantification (label-control subtraction)
4. Segments structural T1 (gray matter, white matter, CSF)
5. Normalizes to MNI space

## CBF QUANTIFICATION PARAMETERS (from our lab specific par.m):

---

PAR.FirstimageType = 0 (labeling first)  
PAR.SubtractionType = 0 (simple subtraction)  
PAR.SubtractionOrder = 1 (control - label)  
PAR.ASLType = 1 (CASL/pCASL)  
PAR.Labeff = 0.8 (label efficiency for pCASL)  
PAR.MagType = 1 (3T scanner)  
PAR.Labeltime = 1.5 (labeling duration, seconds)  
PAR.Delaytime = 1.5 (post-label delay, seconds)  
PAR.slicetime = 27.5 (slice acquisition time, msec)

## OUTPUT:

globalsg.txt in later version of asl\_perf\_subtract.m.

filename	descriptions
ASL.nii	The 4D raw data saved in an order of label-control-label ...
rASL.nii	Output of motion correction.
meanASL.nii	Output of motion correction. The average of all images.
rp_AS_L.txt	Output of motion correction. Motion timecourses. There are 12 columns. The first 6 ones are the output of SPM motion correction: x,y,z translations and 3 rotations; the last 6 are the zigzag pattern cleaned motion timecourses.
ASLfit_rASL.nii	Output of temporal filtering.
sASLfit_rASL.nii	Output of smoothing.
meanCBF_0_srASLfit_rASL.nii	Output of CBF quantification. The mean CBF map. "_0_" means simple subtraction.
cmeanCBF_0....	Outlier cleaned mean CBF map.
meanPERF_0_srASLfit_rASL.nii	Output of CBF quantification. The mean perfusion difference map (nonquantitative).
wmeanCBF_0_srASLfit_rASL.nii	Mean CBF map warped into the MNI space.
cbf_0_srASLfit_rASL.nii	Output of CBF quantification. The CBF map time series. Each control/label image pair has a corresponding CBF map saved in this

27

	file.
brainmask.nii	A rough brain mask by hard thresholding if FSL BET is not available.
globalsg_0.txt	The first and second column are the whole brain mean of the perfusion difference image and the CBF map at each timepoint.

## CONSOLE OUTPUT EXAMPLE (sub86):

CBF quantification for L/C pair: # 31 / 31: ...done  
The mean CBF is 52.457554 (44.345489).

Interpretation:

52.46 mL/100g/min = whole brain mean CBF

44.35 mL/100g/min = likely gray matter only

VERIFICATION:

---

Visual inspection:

fsleyes batch\_scripts\_pcasl/sub86/FUNC/Meancbf\_0\_sr\_rASL.nii &

Expected:

- Higher CBF in gray matter (cortex, deep gray nuclei)
- Lower CBF in white matter
- Clear anatomical structure
- No extreme outliers or artifacts

=====

## 9. DESIGN DECISIONS AND RATIONALE

DECISION 1: Split Preprocessing into PART1 (before removal) and PART2 (after)

---

RATIONALE:

SPM realignment generates most accurate motion parameters. Identifying motion-corrupted volumes before realignment would require unreliable motion estimates. Splitting allows:

1. Accurate realignment of all volumes
2. Identification of bad volumes using reliable motion parameters
3. Removal of bad volumes
4. Final preprocessing with clean data

ALTERNATIVE REJECTED:

Removing volumes before any preprocessing. This would require identifying bad volumes from raw, unrealigned data with inaccurate motion estimates.

EVIDENCE:

Motion parameters differ between before and after realignment by 0.2-1.4mm due to different reference frames. Only post-realignment parameters are reliable for identifying outliers.

## DECISION 2: Use 0-based indexing in Master\_Exclusion\_List.txt

---

### RATIONALE:

FSL and Python (primary QC tools) use 0-based indexing. Using the same convention in the Master List avoids confusion during QC when visualizing volumes in FSLeys or analyzing motion with Python scripts.

### CONVERSION STRATEGY:

MATLAB removal script explicitly converts by adding 1, with clear documentation and verification output showing the conversion:

"0-based: 24-25 → 1-based: 25-26"

### ALTERNATIVE REJECTED:

Using 1-based indexing in Master List to match MATLAB. This would cause confusion during QC in FSLeys (0-based) and increase error risk.

## DECISION 3: Remove label-control pairs, not individual volumes

---

### RATIONALE:

CBF is calculated by subtracting label from control images:

$CBF \propto (\text{Control} - \text{Label})$

If only one volume of a pair is corrupted, the subtraction still produces an unreliable CBF value. Both volumes must be removed to maintain data integrity.

### IMPLICATION:

If 15% of individual volumes are corrupted, removing pairs means ~30% of data is removed (each bad volume necessitates removing its pair).

For sub86: 14 corrupted pairs = 28 volumes removed.

## DECISION 4: Use setdiff() to calculate volumes to keep

---

### RATIONALE:

Manually specifying ranges (e.g., [1:24, 33:42, ...]) is error-prone and subject-specific. Using:

```
volumes_to_remove = [25, 26, 27, ..., 90];  
volumes_to_keep = setdiff(1:90, volumes_to_remove);
```

...is foolproof, generalizable, and automatically handles any removal pattern.

#### ALTERNATIVE REJECTED:

Hard-coding volumes\_to\_keep ranges for each subject. This approach led to off-by-one errors in initial testing.

#### DECISION 5: Recreate meanASL.nii from cleaned volumes

---

##### RATIONALE:

meanASL.nii is the reference for coregistration to structural T1. A blurry mean (from 90 volumes including motion) causes poor coregistration, which propagates to CBF quantification errors.

##### EVIDENCE FROM sub86:

Before SCRUB: meanASL.nii appeared blurry  
CBF = 38.51 mL/100g/min (underestimated)

After SCRUB: meanASL.nii sharp and clear  
CBF = 52.46 mL/100g/min (+36% improvement)

The improvement demonstrates that motion-corrupted volumes were significantly contaminating the mean image and reducing CBF estimates.

#### DECISION 6: No backup files created during removal

---

##### RATIONALE:

SPM batch scripts in PART2 search for files matching patterns like \*ASL.nii. Creating backups like rASL\_BEFORE\_SCRUB.nii causes SPM to find multiple matching files, leading to errors or processing wrong files.

##### SAFETY:

ASL.nii (original 90 volumes) is never modified and serves as permanent backup.

#### DECISION 7: Use explicit confirmation prompt before removal

---

##### RATIONALE:

Volume removal is irreversible (within the workflow). Showing the user exactly what will be removed and asking for confirmation prevents accidental data loss.

##### IMPLEMENTATION:

Script displays:

- Volumes to remove (1-based MATLAB indexing)
- Volumes to keep (ranges)
- Retention rate
- Prompt: "Continue? (y/n):"

DECISION 8: Place all subjects in batch\_scripts\_pcasl/ directory

---

**RATIONALE:**

par.m uses relative path navigation (cd ../) to find data\_root. To make par.m work without modification:

**ORIGINAL INCORRECT:**

```
par.m location: /Downloads/batch_scripts_pcasl/par.m
cd ../ → /Downloads/
Looks for: /Downloads/sub86/FUNC/ ← WRONG
```

**CORRECTED:**

```
par.m location: /Downloads/batch_scripts_pcasl/par.m
% cd ../ ← COMMENTED OUT
Looks for: /Downloads/batch_scripts_pcasl/sub86/FUNC/ ← CORRECT
```

This avoids modifying DICOM conversion script, removal script, and multiple paths throughout the codebase.

DECISION 9: Process subjects one at a time

---

**RATIONALE:**

While batch processing all subjects is possible, processing one at a time:

- Allows verification at each stage before proceeding
- Prevents propagation of errors to all subjects
- Facilitates troubleshooting
- Enables quality control between stages

**IMPLEMENTATION:**

```
par.m: PAR.subjects = {'sub86'}; % Single subject
Change to {'sub86', 'sub135', ...} for batch processing
```

DECISION 10: Verify indexing with explicit console output

---

**RATIONALE:**

Indexing errors (off-by-one) are common and catastrophic. Removal script

explicitly prints conversion for verification:

Converted pairs (1-based MATLAB indexing):

0-based: 24-25 → 1-based: 25-26

0-based: 26-27 → 1-based: 27-28

...

This allows visual verification that conversion is correct before proceeding.

=====

---

PROBLEM 8: Timeseries verification shows mismatch

SYMPTOM:

Cleaned vol 25 ≠ Original vol 33

CAUSE:

Indexing error in volume removal

FIX:

1. Verify Master List uses 0-based indexing
2. Check conversion in script output:  
0-based: 24-25 → 1-based: 25-26
3. If indexing is wrong, adjust script or Master List
4. Re-run Stage 3 after correction

APPENDIX : SUBJECT MAPPING TABLE example (refer masterlist.txt)

---

Subject ID	Archive	Session	Volumes	Pairs Removed	Retention
-----	-----	-----	-----	-----	-----
sub86	p033	s1	90	14	68.9%
p033 IS NOW EXCLUDED SO MAKE SURE TO NOT PROCESS IT ANYMORE					
sub135	p061	s1	90	TBD	TBD
sub30	c033	s1	90	TBD	TBD
sub10	c013	s1	90	TBD	TBD
sub123	p053	s3	90	TBD	TBD
sub122	p053	s2	90	TBD	TBD
sub12	c015	s1	90	TBD	TBD
sub66	p019	s3	90	TBD	TBD
sub40	p006	s3	90	TBD	TBD
sub127	p056	s1	90	TBD	TBD
sub141	p064	s1	90	TBD	TBD
sub20	c023	s1	90	TBD	TBD
sub98	p039	s1	90	TBD	TBD

