# A tutorial for resting state fMRI analysis using ANTsR

## Introduction

### Overview

### fMRI issues

- Nuisance signal from CSF and WM [1]
- Bandpass filtering
- Motion correction [2, 3]
- Global signal[4]

### ANTsR implementation

The main fMRI-specific functions are:

- `fMRINormalization`
- `preprocessRestingBOLD` (supplants `preprocessfMRI`?)
- `antsBold`
- `antsMotionCalculation` (supplants `antsMotionCorr` and `antsMotionCorrStats`?)
- `antsSpatialICAfMRI`
- `filterfMRIforNetworkAnalysis`
- `frequencyFilterfMRI`
- `getfMRInuisanceVariables`

Helper functions include:

- `timeseries2matrix`
- `matrixToImages`
- `icaWhiten`

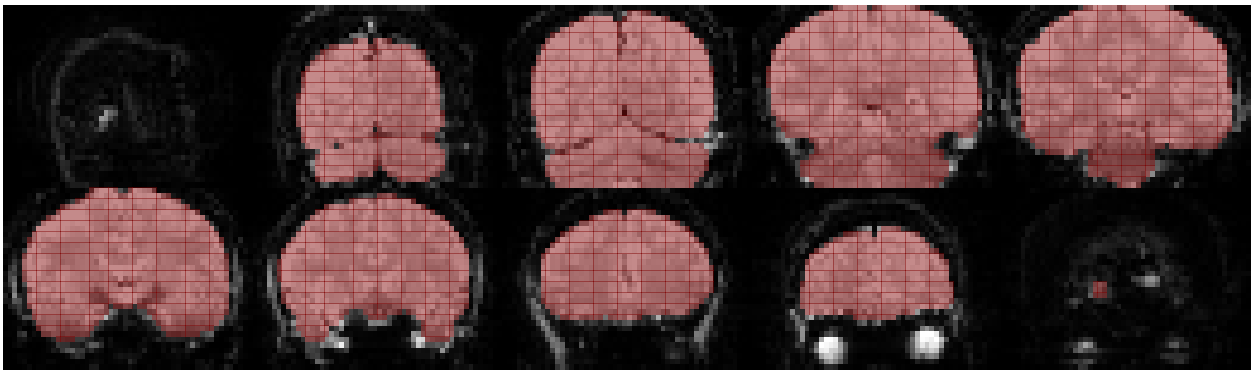
# Tutorial

## Initialization

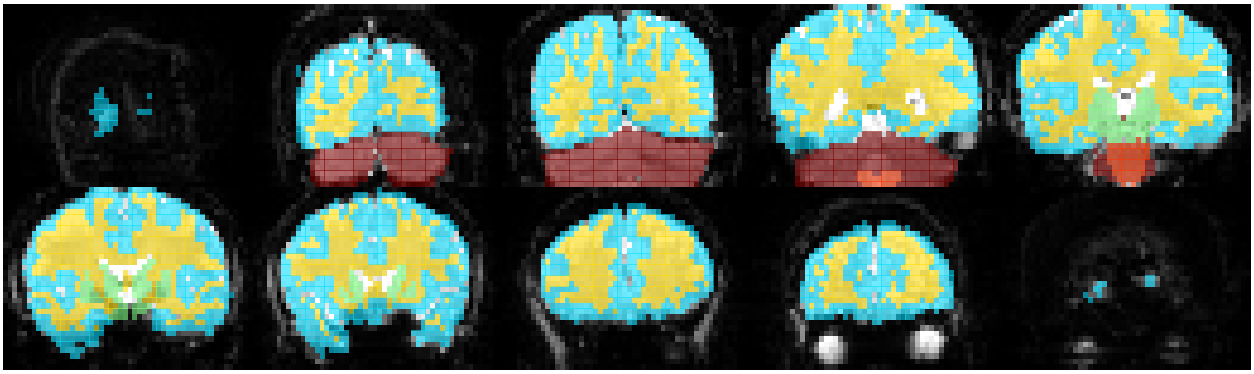
```
# We include all the necessary R package dependencies.  We assume that the user
# is running this script (stitchTutorialDocument.R) in the repo directory.

invisible( suppressMessages( library( ANTsR ) ) )
library( pander )
library( ggplot2 )
library( igraph )
library( psych )
library( corrplot )

rootDirectory <- "./"
knitr::opts_knit$set( root.dir = rootDirectory )
knitr::opts_chunk$set( comment = "" )

figuresDirectory <- paste0( rootDirectory, "Figures/" )
if( ! dir.exists( figuresDirectory ) )
  {
  dir.create( paste0( rootDirectory, "Figures/" ) )
  }
dataDirectory <- paste0( rootDirectory, "Data/" );
```

## Read in input data

```
# Load the AAL (Automated Anatomical Labeling) data table and the AAL label image.
# Also load the individual subject resting state BOLD images:  4-D bold, 3-D bold
# mask image, and 3-D segmentation (csf, gm, wm, etc.) image.

data( aal, package = 'ANTsR' )
aalLabelTable <- aal
aalFileName <- paste0( dataDirectory, "aal.nii.gz" )
aalImage <- antsImageRead( filename = aalFileName, dimension = 3 )

restingStateBoldFile <- paste0( dataDirectory, "rsbold.nii.gz" )
restingStateBoldImage <- antsImageRead( restingStateBoldFile, dimension = 4 )

restingStateBoldMaskFile <- paste0( dataDirectory, "rsboldmask.nii.gz" )
restingStateBoldMaskImage <- antsImageRead( restingStateBoldMaskFile, dimension = 3 )

restingStateBoldSegFile <- paste0( dataDirectory, "rsboldseg.nii.gz" )
restingStateBoldSegImage <- antsImageRead( restingStateBoldSegFile, dimension = 3 )

# Let's look at the images to make sure things make sense, e.g. masks are aligned.
#  Average of 4-D bold with mask superimposed

restingStateBoldAverage <- getAverageOfTimeSeries( restingStateBoldImage )
invisible( plot.antsImage( restingStateBoldAverage, restingStateBoldMaskImage,
   alpha = 0.75, ncolumns = 5 ) )
```

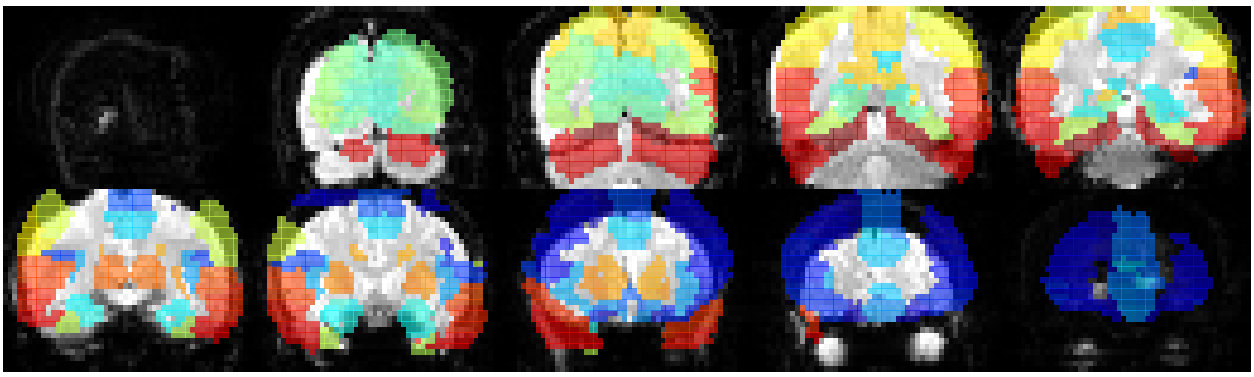


```
#  Average of 4-D bold with segmentation mask superimposed
invisible( plot.antsImage( restingStateBoldAverage, restingStateBoldSegImage,
   alpha = 0.9, ncolumns = 5 ) )
```

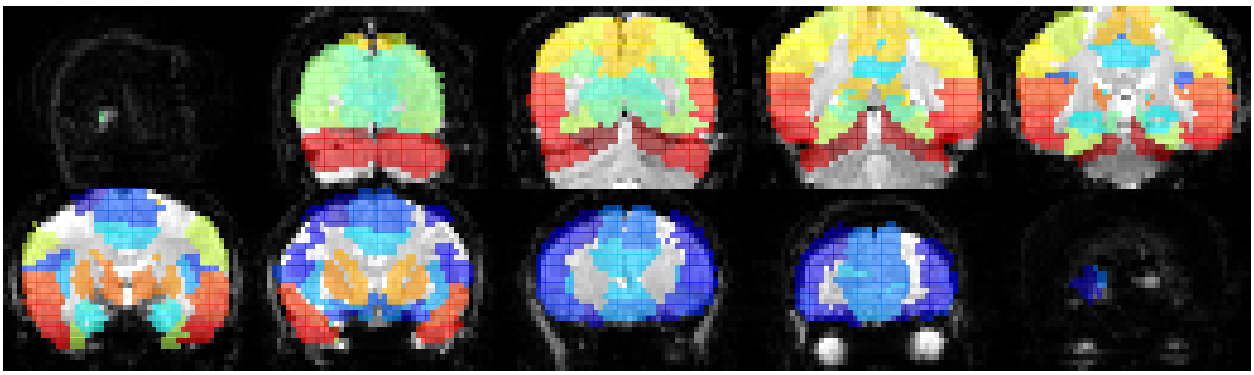


### Spatially normalize AAL image

```
# The AAL image (which will be used later in the tutorial) is not in the space of the
# BOLD image so we do a quick registration of the AAL labels to the bold mask.  We
# first do an "AffineFast" transform to see if that has sufficient degrees of freedom.

aalRegistration <- antsRegistration( fixed = restingStateBoldMaskImage,
                     moving = aalImage, typeofTransform = "AffineFast",
                     outprefix = paste0( dataDirectory, "rsboldxaal" ) )

aalWarpedImage <- antsApplyTransforms( fixed = restingStateBoldMaskImage,
                     moving = aalImage, interpolator = 'genericLabel',
                     transformlist = aalRegistration$fwdtransforms )

invisible( plot.antsImage( restingStateBoldAverage, aalWarpedImage,
   alpha = 0.9, ncolumns = 5 ) )
```



```
# Clearly the alignment is not as good as we would like so we redo the registration
# using an "ElasticSyN" transform which looks much better.

aalRegistration <- antsRegistration( fixed = restingStateBoldMaskImage,
                     moving = aalImage, typeofTransform = "ElasticSyN",
                     outprefix = paste0( dataDirectory, "rsboldxaal" ) )

aalWarpedImage <- antsApplyTransforms( fixed = restingStateBoldMaskImage,
                     moving = aalImage, interpolator = 'genericLabel',
                     transformlist = aalRegistration$fwdtransforms )

invisible( plot.antsImage( restingStateBoldAverage, aalWarpedImage,
   alpha = 0.9, ncolumns = 5 ) )
```



## Preprocessing the resting state fMRI data

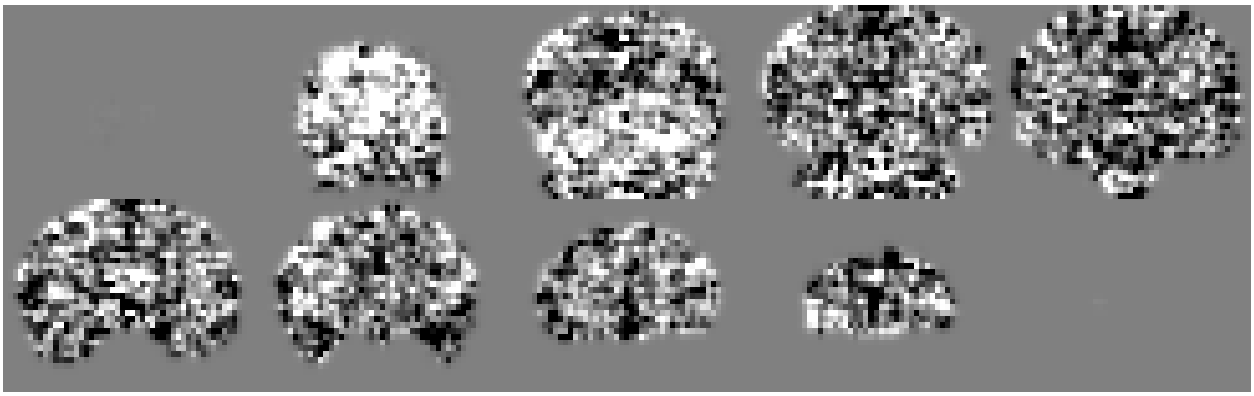
```
# The evolution of fMRI functionality in ANTsR is still ongoing.  It began with
# various utility functions to perform different aspects of fMRI preprocessing
# (e.g., motion correction, band-pass filtering).  The function ``preprocessfMRI``
# was created to join all these components into a single function with slight
# enhancements made to create the function ``preprocessRestingBOLD``.  We should
# probably deprecate the former..  Although this basic functionality should suffice
# for most users, Brian has recently created the function ``fmriNormalization`` to
# take advantage of fMRI with simultaneous structural T1-weighted acquisitions that
# have been processed through the ``antsCorticalThickness.sh`` script.
```

```
# We process our current subject with ``preprocessRestingBOLD`` and plot the average
# of the resulting processed fMRI.  Note that we're doing motion correction on the
# lowest accuracy level for tutorial purposes.  For actual data, one would probably
# want to increase the accuracy level.

preprocessedRestingState <-
  preprocessRestingBOLD( restingStateBoldImage,
                         maskImage = restingStateBoldMaskImage,
                         denseFramewise = FALSE, numberOfCompCorComponents = 6,
                         doMotionCorrection = TRUE, motionCorrectionAccuracyLevel = 0,
                         motionCorrectionIterations = 1, frequencyLowThreshold = 0.01,
                         frequencyHighThreshold = 0.1,
                         spatialSmoothingType = "gaussian",
                         spatialSmoothingParameters = 2 )

pander( summary( preprocessedRestingState ), style = "rmarkdown",
  caption = "Returned values from the function preprocessRestingBOLD." )
```

Table 1: Returned values from the function preprocessRestingBOLD.

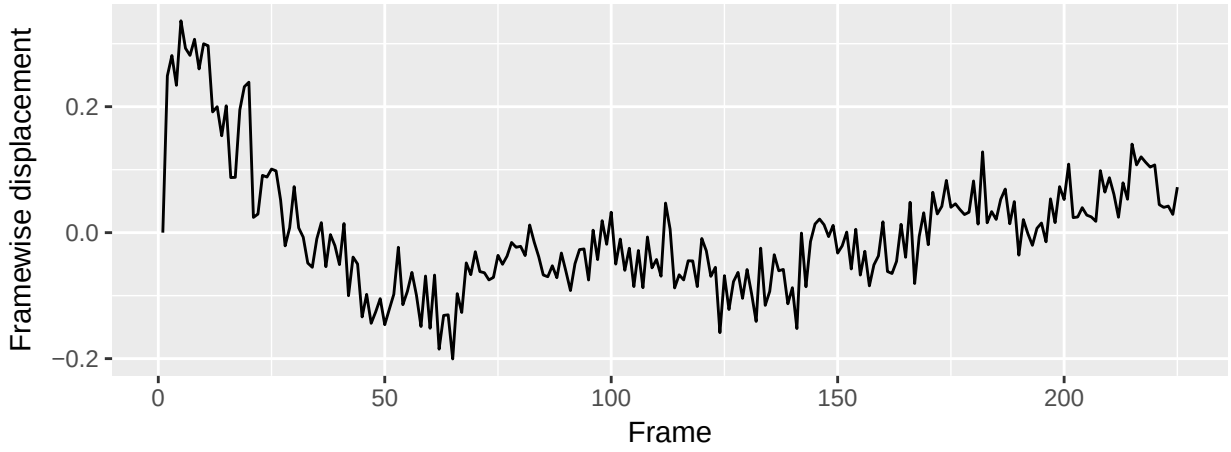
| | Length | Class | Mode |
|---|---|---|---|
| **cleanBoldImage** | 1 | antsImage | S4 |
| **maskImage** | 1 | antsImage | S4 |
| **DVARS** | 225 | -none- | numeric |
| **DVARSpostCleaning** | 225 | -none- | numeric |
| **FD** | 225 | -none- | numeric |
| **globalSignal** | 225 | -none- | numeric |
| **nuisanceVariables** | 1350 | -none- | numeric |

```
invisible( plot.antsImage(
   getAverageOfTimeSeries( preprocessedRestingState$cleanBoldImage ), ncolumns = 5 ) )
```

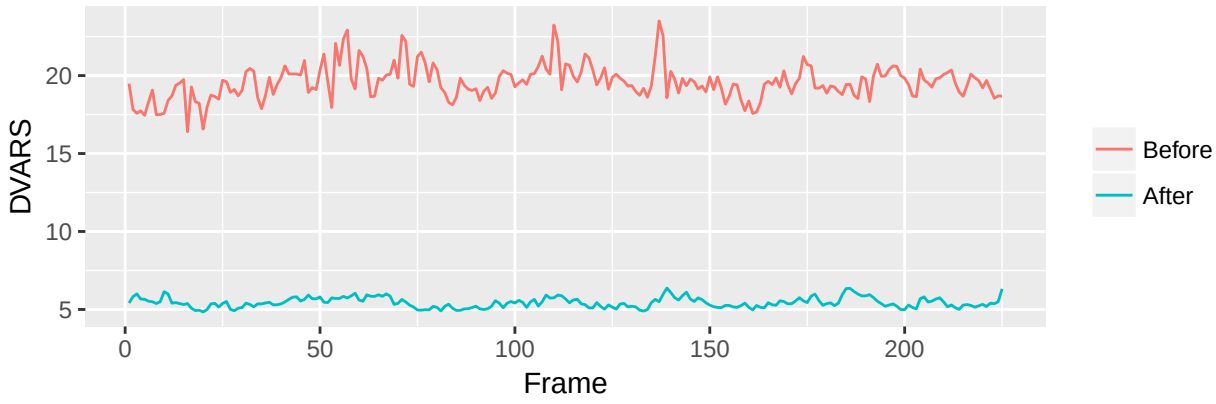


```
# We continue to check the preprocessing by plotting:
#   1. the framewise displacement (FD)
#   2. the global signal before and after regression (globalSignal)
#   3. comparing the DVARS of the original data (DVARS) and the processed
#        data (DVARSpostCleaning)
```

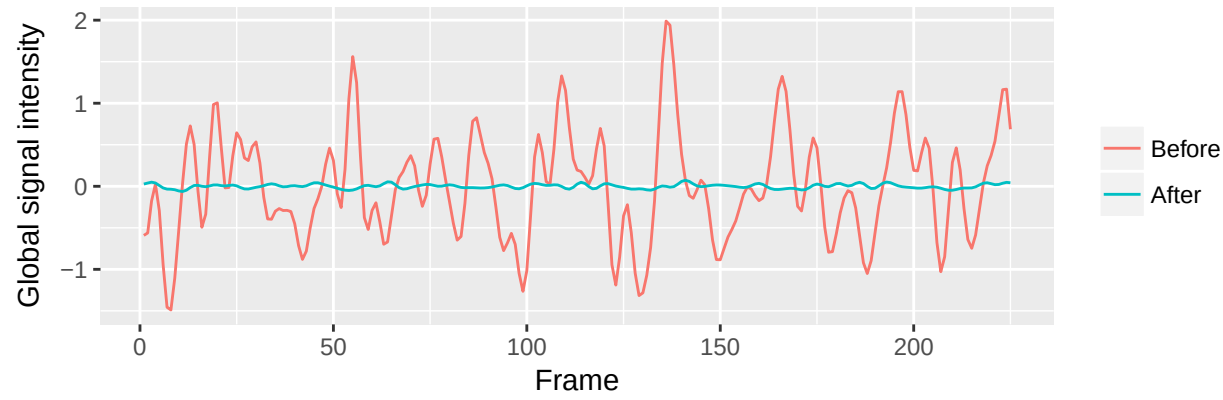
```
#   4. Plot the CompCor nuisance variables

numberOfTimeFrames <- dim( restingStateBoldImage )[4]

# Plot the framewise displacement.

fdDataFrame <- data.frame( Frame = 1:numberOfTimeFrames,
   FD = preprocessedRestingState$FD - mean( preprocessedRestingState$FD ) )

ggplot( fdDataFrame ) +
   geom_line( aes( x = Frame, y = FD ), size = 0.5 ) +
     xlab( "Frame" ) + ylab( "Framewise displacement" ) +
     theme( legend.title = element_blank() ) + theme( aspect.ratio=1/3 )
```




```
# Plot the DVARS.  Defined as the framewise backwards RMS voxelwise difference averaged
# over each time frame.

dvarsDataFrame <- data.frame( Frame = rep( 1:numberOfTimeFrames, 2 ),
       DVARS = c( preprocessedRestingState$DVARS,
                  preprocessedRestingState$DVARSpostCleaning ),
       Type = factor( c( rep( "Before", numberOfTimeFrames ),
              rep( "After", numberOfTimeFrames ) ), levels = c( "Before", "After" ) ) )

ggplot( dvarsDataFrame ) +
   geom_line( aes( x = Frame, y = DVARS, colour = Type ), size = 0.5 ) +
     xlab( "Frame" ) + ylab( "DVARS" ) +
     theme( legend.title = element_blank() ) + theme( aspect.ratio = 1/3 )
```

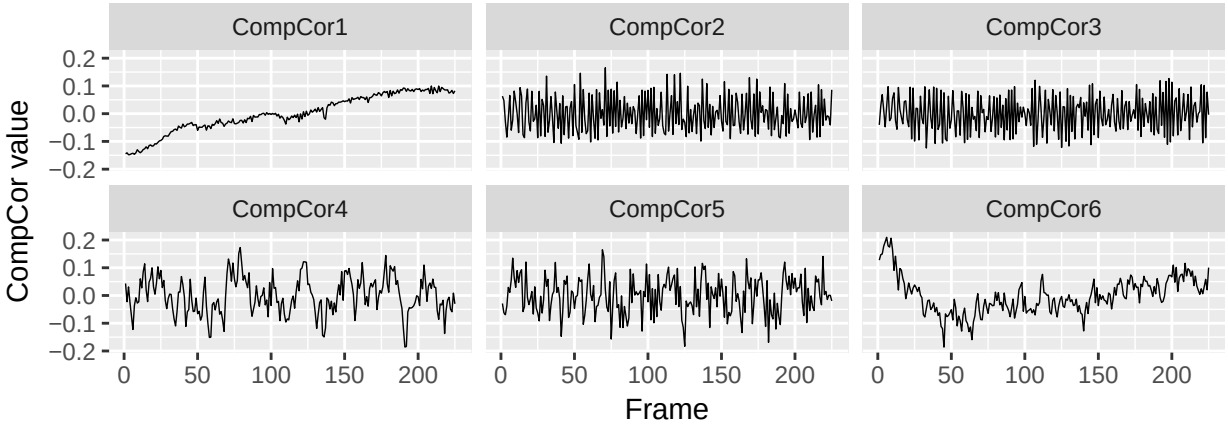



```
# Plot the global signal.  Do we regress out the global signal? Still an open issue.
# Let's just explore the approach to regressing it out afterwards.  A better way would
# be to include it as an ``initialNuisanceVariable`` in ``preprocessRestingBOLD()``.

boldMatrix <- timeseries2matrix(
  preprocessedRestingState$cleanBoldImage, restingStateBoldMaskImage )
boldMatrixGlobalSignalRegressedOut <-
  residuals( lm( boldMatrix ~ scale( preprocessedRestingState$globalSignal ) ) )

globalSignalDataFrame <- data.frame( Frame = rep( 1:numberOfTimeFrames, 2 ),
       GlobalSignal = c( preprocessedRestingState$globalSignal,
          apply( boldMatrixGlobalSignalRegressedOut, mean, MARGIN = 1 ) ),
       Type = factor( c( rep( "Before", numberOfTimeFrames ),
              rep( "After", numberOfTimeFrames ) ), levels = c( "Before", "After" ) ) )

ggplot( globalSignalDataFrame ) +
   geom_line( aes( x = Frame, y = GlobalSignal, colour = Type ), size = 0.5 ) +
     xlab( "Frame" ) + ylab( "Global signal intensity" ) +
     theme( legend.title = element_blank() ) + theme( aspect.ratio = 1/3 )
```



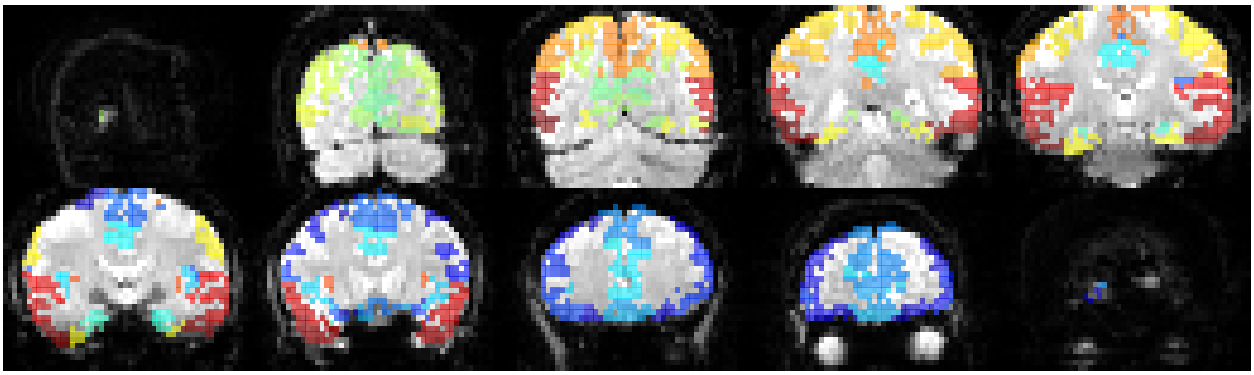

```
# Plot the CompCor nuisance signals.  Defined in terms of the PCA decomposition of
# the high frequency components of the BOLD signal.

numberOfCompCorComponents <- ncol( preprocessedRestingState$nuisanceVariables )
whichComponentLevels <- paste0( "CompCor", 1:numberOfCompCorComponents )
whichComponent <- factor( as.vector(
  matrix( rep( whichComponentLevels, numberOfTimeFrames ),
```

```
    nrow = numberOfTimeFrames, byrow = TRUE ) ), levels = whichComponentLevels )

compCorDataFrame <- data.frame(
  Frame = rep( 1:numberOfTimeFrames, numberOfCompCorComponents ),
  WhichComponent = whichComponent,
  Values = as.vector( preprocessedRestingState$nuisanceVariables ) )

ggplot( compCorDataFrame ) +
   geom_line( aes( x = Frame, y = Values ), size = 0.25 ) +
     facet_wrap( ~ WhichComponent, ncol = 3 ) +
     xlab( "Frame" ) + ylab( "CompCor value" ) +
     theme( legend.title = element_blank() ) + theme( aspect.ratio = 1/3 )
```




## Calculate functional connectivity measures

```
# We use the AAL labels to calculate various functional connectivity measures for
# this subject.

# Mask out non-gray matter pixels from aalImage and plot image to ensure things look
# correct.

aalWarpedImage[restingStateBoldSegImage != 2] <- 0

invisible( plot.antsImage( restingStateBoldAverage, aalWarpedImage,
   alpha = 0.9, ncolumns = 5 ) )
```



```
# Determine the unique AAL labels and construct the correlation matrix

aalRoiLabelVector <- as.vector( as.array( aalWarpedImage[aalWarpedImage > 0] ) )
aalUniqueLabels <- sort( unique( aalRoiLabelVector ) )

boldMatrix <- timeseries2matrix(
  preprocessedRestingState$cleanBoldImage, restingStateBoldMaskImage )

boldLabelMatrix <- matrix( NA, nrow = nrow( boldMatrix ), ncol = length( aalUniqueLabels ) )
for( j in 1:length( aalUniqueLabels ) )
  {
  currentLabelIndices <- which( aalRoiLabelVector == aalUniqueLabels[j] )
  if( length( currentLabelIndices ) > 1 )
    {
    boldLabelMatrix[, j] <- rowMeans( boldMatrix[, currentLabelIndices] )
    }
  else
    {
    boldLabelMatrix[, j] <- mean( boldMatrix[, currentLabelIndices] )
    }
  }
correlationMatrix <- cor( boldLabelMatrix, boldLabelMatrix )
correlationMatrix[which( is.na( correlationMatrix ) )] <- 0
rownames( correlationMatrix ) <- colnames( correlationMatrix ) <-
  aalLabelTable$label_name[aalUniqueLabels]

# We calculate the significance for each entry.

cor.mtest <- function( mat, ... )
  {
  mat <- as.matrix( mat )
  n <- ncol( mat )
  p.mat <- matrix( NA, n, n )
  diag( p.mat ) <- 0

  for( i in 1:( n - 1 ) )
    {
    for( j in ( i + 1 ):n )
      {
      tmp <- cor.test( mat[, i], mat[, j], ... )
      p.mat[i, j] <- p.mat[j, i] <- tmp$p.value
      }
    }
  colnames( p.mat ) <- rownames( p.mat ) <- colnames( mat )
  p.mat
  }

p.mat <- cor.mtest( correlationMatrix )

uvaColors <- colorRampPalette( c( "#F59A2C", "#F1E5C7", "#E6E7E8", "#46A8C2", "#0D3268" ) )
correlationPlotFile <- paste0( figuresDirectory, '/CorrelationMatrix.pdf' );
pdf( height=10, width=10, file = correlationPlotFile )
corrplot( correlationMatrix, method = "circle", diag = FALSE, type = "upper",
```

```
  tl.col = "black", tl.cex = 0.48, tl.srt = 45, col = uvaColors( 200 ),
  p.mat = p.mat, sig.level = 0.01, insig = "blank" )
invisible( dev.off() )
```

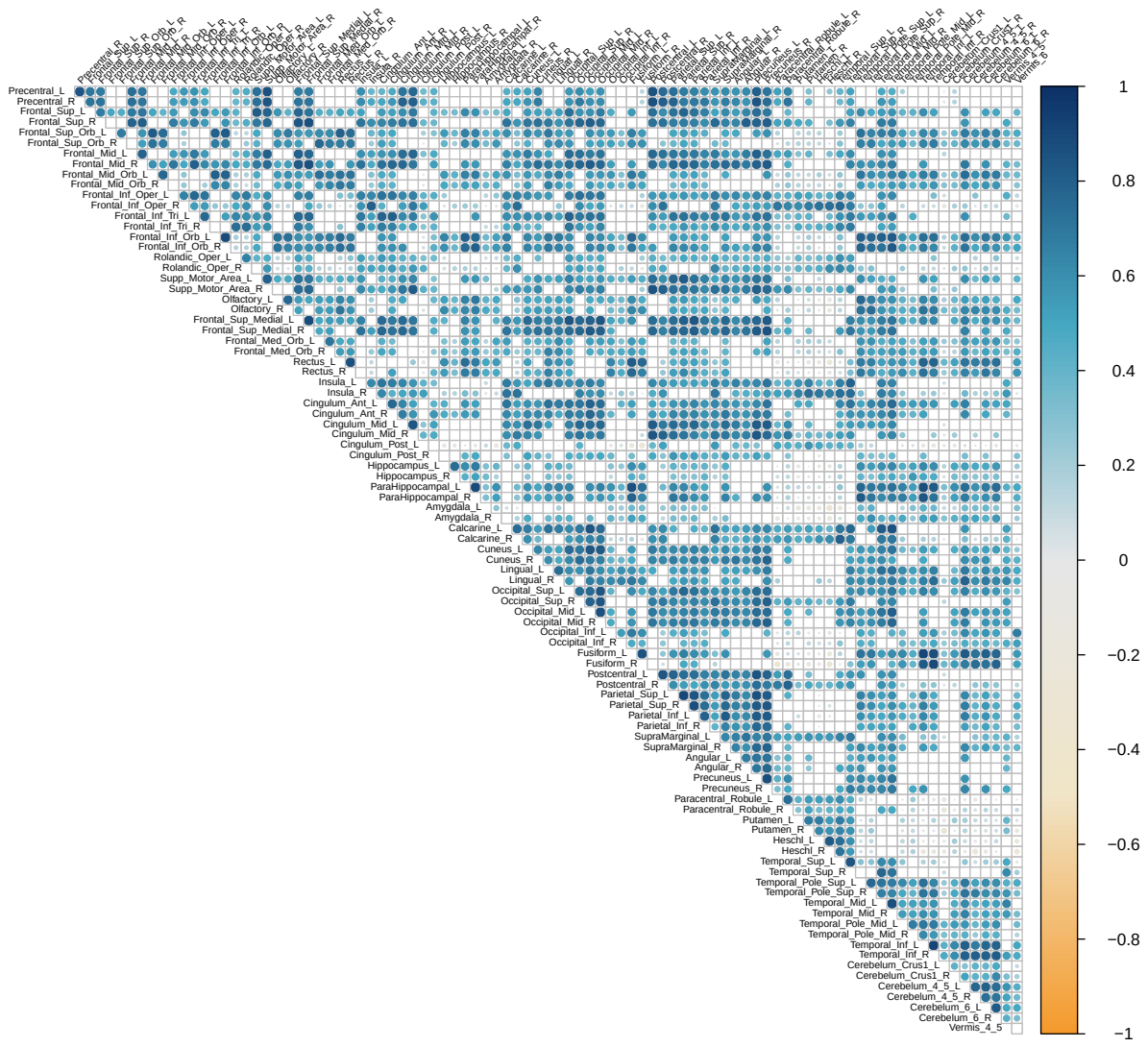



Figure 1: Correlation plot illustrating the functional connectivity relationships between AAL-defined regions.

```
# Now we calculate various graph-based measures from the connectivity relationships.

networkGraph <- makeGraph( correlationMatrix, graphdensity = 0.25, getEfficiency = TRUE )

pander( summary( networkGraph ), style = "rmarkdown",
  caption = "Graph-based connectivity measures." )
```

Table 2: Graph-based connectivity measures.

| | Length | Class | Mode |
|---|---|---|---|
| **mygraph** | 10 | igraph | list |
| **centrality** | 92 | -none- | numeric |
| **closeness** | 92 | -none- | numeric |
| **pagerank** | 92 | -none- | numeric |
| **degree** | 92 | -none- | numeric |
| **betweeness** | 92 | -none- | numeric |
| **localtransitivity** | 92 | -none- | numeric |
| **globalTransitivity** | 1 | -none- | numeric |
| **strength** | 92 | -none- | numeric |
| **degcent** | 92 | -none- | numeric |
| **hubScore** | 92 | -none- | numeric |
| **effinv** | 92 | -none- | numeric |
| **community** | 1 | -none- | logical |
| **walktrapcomm** | 7 | communities | list |
| **adjacencyMatrix** | 8464 | -none- | numeric |

# References

1. Behzadi, Y., Restom, K., Liau, J., and Liu, T. T. "**A Component Based Noise Correction Method (CompCor) for BOLD and Perfusion Based FMRI**" *Neuroimage* 37, no. 1 (2007): 90–101. doi:10.1016/j.neuroimage.2007.04.042

2. Power, J. D., Barnes, K. A., Snyder, A. Z., Schlaggar, B. L., and Petersen, S. E. "**Spurious but Systematic Correlations in Functional Connectivity MRI Networks Arise from Subject Motion**" *Neuroimage* 59, no. 3 (2012): 2142–54. doi:10.1016/j.neuroimage.2011.10.018

3. Power, J. D., Mitra, A., Laumann, T. O., Snyder, A. Z., Schlaggar, B. L., and Petersen, S. E. "**Methods to Detect, Characterize, and Remove Motion Artifact in Resting State FMRI**" *Neuroimage* 84, (2014): 320–41. doi:10.1016/j.neuroimage.2013.08.048

4. Liu, T. T., Nalci, A., and Falahpour, M. "**The Global Signal in FMRI: Nuisance or Information?**" *Neuroimage* 150, (2017): 213–229. doi:10.1016/j.neuroimage.2017.02.036