Introduction to Population Receptive Field models

Sam Schwarzkopf

Before the start of the actual cookbook on using SamSrf for population receptive field (pRF) mapping, here is a basic introduction to how our method works. The procedure is largely based on Dumoulin & Wandell, 2008, NeuroImage, 39(2):647-60. You should read this article.

What is a pRF?

As any text book on neurophysiology will tell you, a neuron's receptive field is the region of space that can excite the neuron when we put a stimulus in it. "Space" can mean a lot of things in this context. Most frequently, people will mean *visual space*, that is, the retinotopic location. However, there are other stimulus spaces, such as maps of the frequency of a tone or locations on your skin. In fact, a visual receptive field is just a tuning function for position in visual space. So in essence what we want to do is map tuning functions for a particular stimulus space — and this could be anything, be it visual position, sound frequency, selectivity to particular objects, your location in the environment, or even things like language. So, don't let anyone tell you that pRF models are just a fancy form of retinotopic mapping. There is potential for so much more.

Naturally, we are not measuring single neurons but the activity of a large number of neurons combined (and, in fMRI, through an indirect, metabolic proxy measure of neuronal activity). This is why we call it a *population* receptive field. The properties of the pRF are not necessarily an upscaled version of the receptive fields of single neurons. The pRF will contain those parameters but is also dependent on the variability in the position of the individual receptive fields, how much visual space the receptive fields of all the neurons cover, and it also incorporates the extra-classical or contextual interactions between neurons within (and outside) the population.

Last but not least, the acronym is spelled with a lower case p for some arcane reasons. This is probably in reference to the fact that in the neurophysiology world there are such things as *classical* receptive fields that are abbreviated as cRF. For all I care you can spell it PRF.

How do we map pRFs?

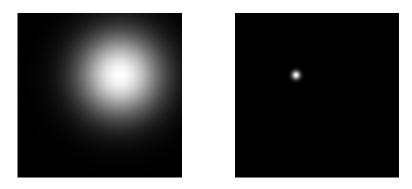
Simply put, for each population of neurons in the brain we want to estimate the parameters of the pRF from the fMRI (or other?) data we collected. The parameters of the pRF can be many things. In a simple case it would be the position (in Cartesian coordinates, x_0 and y_0) and the spatial spread of the pRF (since we use a 2D-Gaussian model, this is called σ , i.e. sigma). But more complex receptive field models are also possible, such as antagonistic centre-surround profiles (modelled as a difference-of-Gaussians function), or oriented and/or asymmetric profiles. Putting together all the parameters for neighbouring points on the cortical sheet gives us a map for this parameter.

In order to estimate these parameters we need to have two sets of information: the observed data (i.e. the fMRI time series) and the time series of the stimulus. In SamSrf the latter is called the **stimulus apertures**, but that's just a name for a small movie of the stimulus we presented to participants during the scan. In fact, it is usually a simplified version of the stimulus that just encodes whether or not a stimulus was present at each location in the visual field at a given time.

Armed with these two things we can now create a pRF model. What we need to do is find the pRF parameters (like position and size) that can best explain the observed time series. We can predict the time series a particular receptive field profile would produce if faced with the apertures of our stimulus sequence. At each moment in time (i.e. each TR of our fMRI scan) we calculate the overlap between the stimulus aperture and the receptive field profile. As already described, the apertures are just a movie. The receptive field profile is simply an image of the receptive field and we multiply this image, pixel-by-pixel, with each frame of the aperture movie in turn. Subsequently we calculate the sum of this pixel-wise overlap to estimate the neural response. You can try this for yourself using these functions that come with SamSrf:

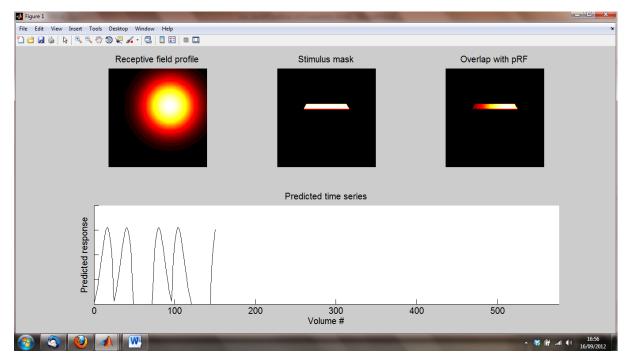
```
LargeRF = prf_gaussian_rf(0.5, 0.5, 1);
SmallRF = prf_gaussian_rf(-0.5, 0.5, .1);
```

Using imshow you can display these. You see one is in the upper right visual field (coordinates 0.5, 0.5) and very large, while the other is in the upper left visual field (-0.5, 0.5) and very small:



Next you want to load a stimulus aperture movie. You will have one if you run a pRF mapping experiment but if you don't, ask one of the people who have done the analysis already. This is stored in a variable called *ApFrm*. We can predict the time series each of these receptive field profiles would produce using the following function:

prf_predict_timecourse(LargeRF,ApFrm,1)



In the top row you see the receptive field profile on the left, the stimulus aperture at the current time point in the middle, and the overlap between the two on the right. In the bottom row we see the predicted response of this hypothetical receptive field.

Of course, because we are measuring BOLD signals with fMRI our time series does not reflect the underlying neural activity directly. Therefore, we have to convolve the predicted time series with the haemodynamic response function (HRF). For this we can either take a canonical HRF, or measure the HRF specifically for each subject and use this. The latter approach is probably better because it takes the individual differences in the HRF into account. In practice, this does not seem to make much of a difference though as Dumoulin & Wandell showed in their seminal 2008 study. Naturally, any individual HRF is only as good as the data that you used to model it. Using a bad HRF can completely screw up your pRF model. Another, more efficient approach is to first fit a pRF based on a canonical HRF, then use the predicted time series as predictor for fitting the individual HRF, and then refitting the pRF model with this individual HRF. An advantage of this approach is that it uses all the pRF mapping data you acquired to fit the HRF rather than spending extra time on measuring the HRF. This approach can also improve the goodness of fit of your pRF models but this is also quite circular and so not entirely kosher. Either way, usually the canonical HRF is fully acceptable and most people probably do not need to worry about this issue.

Fitting pRF models

The tricky thing we must do now is to find the receptive field profile (or more precisely its associated parameters) that can produce a time series that matches the observed time series. What we therefore need to do is fit a model with several free parameters (in this case at least three) to the observed data. Ideally we want to do this straight out of the box but this is tedious and difficult. So what we can do is apply a coarse-to-fine approach in which we first find a way to disambiguate the parameters and then refine them further.

The way this is done for most models in SamSrf is to run an extensive grid search, the *coarse fit*, where we first generate a large search space with a number of plausible combinations of the free parameters. For each point in this multidimensional search space we generate a predicted time series and correlate this with the actually observed data. The search parameters that produced the best correlation are then used in the *fine fit* where we run an optimization procedure to further hone the fitted parameters to achieve the (hopefully) optimal fit. The good thing about the coarse fit is that we can do this simultaneously for many vertices at the same time, which makes the procedure fairly fast. (Unfortunately, this appears to only work in MATLAB R2011b and higher. On older versions you must do it at a snails-pace one vertex at a time which takes ages...).

The granularity and dimensions of this search space for the coarse fit you can define when you specify your pRF model. The more predictions this coarse fit contains, the longer it will take to generate predictions and how much disc space and memory the search space requires. There is probably a sweet spot between having a well-sampled search space and unnecessarily wasting too much time and memory on the coarse fit. Obviously, a pRF model with more free parameters also requires more resources and will put limits on how accurately you can sample the search space. Importantly, you only really need to generate this search space once provided you collect the same amount of data in the same sequence for each subject. If the stimulation paradigm differed between subjects, then unfortunately you also need individual apertures and thus an individual search space.

For both the coarse and fine fit, the best fitting model is determined by the Pearson correlation between the observed and predicted time series. It is quantified by the coefficient of determination (the "goodness of fit"), which is the square of the correlation coefficient. Once the parameter fitting is complete, SamSrf subsequently runs a regression analysis to fit a *beta* parameter to estimate the response amplitude, and a baseline (intercept) parameter to estimate the baseline. Since we typically z-standardize the time series, the baseline will typically be close to zero but it can vary. More importantly, the beta can be positive or negative. Negative betas are often a sign of some artifactual data.

The following pRF methods are currently available in SamSrf:

- 1. Simple Gaussian: This is the most basic pRF model that is most widely used in the literature. It models the pRF as a two-dimensional Gaussian. It is defined by its position (x_0 and y_0) and its size (sigma).
- 2. Difference-of-Gaussians: This procedure models centre-surround antagonistic receptive fields. In addition to position (x₀ and y₀), we have two sigmas (one for the centre, one for the surround) and a parameter for the ratio of the amplitudes (delta) of the two components. The second surrounding Gaussian is subtracted from the first, the centre Gaussian. This function produces a Mexican hat shape. Importantly, you cannot directly translate the centre sigma into a classical sigma from the standard simple Gaussian pRF. Rather, you need to quantify the full-width at half-maximum of this function to determine the pRF size.
- 3. Multivariate Gaussian: This is an oriented elliptical Gaussian so this has two pRF size parameters (sigma1 and sigma2, for the horizontal and vertical axis, respectively) and an orientation parameter (phi). There is some ambiguity in this model because the model is rotationally symmetric. You need to account for this in your post-modelling analysis.
- 4. Gaussian Curve: This procedure is very similar to the standard pRF approach except that it is fitting a one-dimensional tuning curve rather than a two-dimensional receptive field. The apertures in this case should contain bars traversing the stimulus space in only one direction. This direction can be either horizontal or vertical. The tuning curve is modelled as a Gaussian pRF that is fixed in the orthogonal dimension (so if the stimulus moves horizontally, the pRF's vertical position is fixed at 0. The assumption here is that the stimulus space is linear. So if your stimulus dimension is non-linear (e.g. in tonotopy) you must log-transform it first.
- 5. Polar Angle Curve: This is the same as the Gaussian Curve model except that this assumes a circular stimulus space (e.g. orientation and direction) and therefore fixes the position of the Gaussian pRF to be at a constant distance from the origin.
- 6. Reverse Correlation: This procedure uses a model-free approach to estimate the pRF profile. It convolves the stimulus apertures with the HRF, then multiples the activity measured in each fMRI volume with these apertures, and finally averages the frames for all the volumes together. This gives an image that reflects at which location the voxel showed the strongest response. Because our stimuli are usually temporally ordered and predictable designs there will be a lot of correlation throughout the image (e.g. with a bar stimulus you will see a set of bars intersecting at the pRF location). Some filtering is therefore applied to minimise these artifacts whilst retaining the (presumably) true pRF. These pRF profiles are then saved and the x and y coordinates of the pRF peak estimated from that. No further modelling is applied so you cannot quantify pRF size or shape without further processing. The lack of model fitting makes this procedure comparably fast.

7. Connective Field Reverse Correlation: This method is vaguely based on the ideas first presented by Haak et al. 2013 in NeuroImage. It estimates the vertex-wise functional connectivity between each vertex in your region of interest, and all of the vertices in a predefined seed region. In a retinotopic paradigm, this seed region could be V1 for example. For each vertex, we calculate the correlation between its time series and all the time series in the seed region. This produces a correlation profile across the whole seed region. The connective field is the region where you observe the strongest correlation. The procedure estimates the peak location and spread of this connective field. Finally, it uses a template map of the seed region to assign a position to each connective field. This template could be the probabilistic retinotopic map predictions described by Benson et al. 2012 in Current Biology or it could be an anatomical map created using samsrf_anatomy_srf.

Surface projection

One last thing you should know about how the analysis in SamSrf works is surface projection. FreeSurfer's automatic cortical reconstruction creates a 3D model of the boundary between grey and white matter. This is a mesh consisting of over 100,000 little points (vertices) that are connected to one another in an even greater number of tiny little triangles (faces). FreeSurfer also expands this surface until it hits the pial boundary between grey matter and cerebrospinal fluid. Thus, for each vertex on the grey-white mesh there is a corresponding vertex in the pial mesh. We can use this to find points that lie within the grey matter and locate which voxels those correspond to in our functional MRI scans. This is what we do when we project the fMRI data onto the cortical surface. All of SamSrf's analyses take place on the surface.

FreeSurfer further inflates the cortical meshes to smooth out the cortical folding structure. This is helpful for displaying the data on the cortical sheet and delineating maps. Finally, it also expands these inflated meshes to a spherical mesh. This can be used to combine data from different individuals. It is also extremely useful for surface-based (geodesic) operations that we might want to do later (e.g. smoothing, field sign calculation, cortical magnification, etc.).

Overview of pRF pipeline

Before delving into SamSrf analysis proper, let's look at the overall plan for data analysis in pRF modelling. The analysis takes place in three component streams: pre-processing in an fMRI package of your choice (e.g. SPM, FSL), surface reconstruction in Freesurfer, and pRF modelling in SamSrf. Between these streams, the whole analysis can be broken down into 5 main steps:

- 1. fMRI pre-processing (in any package)
- 2. Anatomical reconstruction (FreeSurfer)
- 3. Surface-projection of functional data (SamSrf)
- 4. pRF modelling with inputs from all previous steps (SamSrf)
- 5. Displaying results, area delineation, and quantitative analysis (SamSrf)

SamSrf 6 for pRF models

This cookbook is a generic manual indicating how to conduct a simple population receptive field (pRF) experiment using SPM, Freesurfer and SamSrf for data analysis. Currently, SamSrf stands for **Seriously Annoying Matlab Surfer**. If you come up with a better acronym that meets Sam's approval, you will win a prize and the acronym will be changed in your honour.

Note that this guide has been written in part from the perspective of a UNIX-based system user (e.g. Linux, MacOS). If you are working in Windows, working through a virtual machine may be necessary although as of Windows X it is possible to run the UNIX terminal scripts directly in Windows. This guide assumes basic knowledge of MATLAB, SPM, and of the UNIX command line.

Since SamSrf version 5.69 a basic example data set has been available here: https://zenodo.org/record/163582

Important note to users of SamSrf versions prior to 6: A lot of things have changed in SamSrf 6. In particular, the GUI has been removed. Most people will use scripting sooner or later for most of their analysis pipeline and the GUI started to become increasingly complex and it was not worth the effort supporting it any longer. There are now a few GUI tools available for displaying maps and delineating ROIs.

Required dependencies:

- MATLAB 64-bit (version R2011b 7.13 or later) 32-bit versions have not been tested
- Statistics and Optimization toolboxes from MATLAB
- **SPM8** or **SPM12** (later versions not tested)
- FreeSurfer 5.3.0 is what we currently use

Note that SamSrf saves data MAT files in format 7.3. This should not be a problem for most people but **if your Matlab cannot read the MAT files** generated by SamSrf you need to change the default setting in *File->Preferences->General->MAT-Files*

CHAPTER 1: ACQUIRING DATA

In order to perform a pRF experiment you will need to acquire the following MRI scans:

Echo-planar imaging (EPI)

- One or more runs of a visual mapping stimulus
 - In this example, we use 2 runs of geometric bars in the following configuration:
 - 1. Bars traversing the visual field in four different directions
 - 2. The same bars but sweeping in a different order
- (Optional) You may collect one run of sparse visual stimulation for estimating hemodynamic response functions (HRF) but we will normally use the canonical HRF
- (Optional) You may want to collect B0 field maps to correct distortion in your EPI scans (probably more important for 3T and 7T data than 1.5T)

Structural images

- A high-resolution T1-weighted anatomical image with the full 32-channel head-coil on.
- (Optional) If acquiring EPI scans with the top part of the head-coil off, a short T1-weighted anatomical image also with the top coil section off. Generally, when reusing an older anatomical scan for your participant, it is advisable to acquire a fast structural to aid coregistration with the old scan on which your FreeSurfer reconstruction is based.

CHAPTER 2: DIRECTORY STRUCTURE

For each of your subjects, you will need to create an organised directory structure to perform the analysis. See Figure 1:

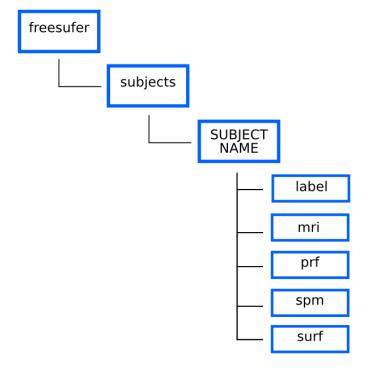


Figure 1

The analysis for each subject is contained within a directory specific to that subject. Within the subject directory you will find:

label contains the surface labels are saved, including the maps you draw mri contains the volume information and the original T1-weighted image reconstructed surf contains the surface reconstruction information from Freesurfer spm contains the files for the pre-processing steps will reside (you can choose this name) prf is the population receptive field analysis directory (you can choose this name) anatomy is the folder where the anatomical meshes for the subject will be stored

Note that most of your directory structure will be created when you run recon-all in Freesurfer (step 3), except the folders *spm*, *anatomy*, and *prf*. The folders *spm* and *prf* will need to be created manually and you can choose the names for these. (In theory, you can also keep those folders somewhere else if you so desire, but this cookbook assumes you leave them with the FreeSurfer data). The folder *anatomy* is created when you surface-project data.

If you wish to carry out more than one pRF experiment in a single subject (e.g. two conditions), then a sensible step would be to create two folders (prf_1 and prf_2) instead of one, and treat them independently from chapter 4 onwards.

Note that the subjects folder shown here is inside the Freesurfer installation. However, you can use any arbitrary folder as your subjects folder, e.g. when you have different projects and you don't want the data to be mixed. To change the subjects folder you need edit SetUpFreeSurfer.csh, which is located inside your main Freesurfer folder. You will find a line that says something like:

setenv SUBJECTS_DIR /usr/local/freesurfer/subjects

You can change the path to wherever you want to keep your data. However, you have to make sure that various folders FreeSurfer wants (from the original *freesurfer/subjects* folder) are present.

CHAPTER 3: PREPROCESSING

1. fMRI pre-processing

Copy your data to SUBJECT/spm and perform your standard pre-processing for fMRI images in your software of choice.

In the case of data acquired at the FIL, we recommend the following steps in SPM8:

- DICOM import*
- Bias correction (only when using 32-channel head coil)
- Remove dummies
- Optional: Create B0 field maps
- Realign & unwarp (with or without B0 field maps)
- Optional: Slice-timing correction (Karl Friston will tell you it's useless)
- Co-registration to the structural
- Optional: Smoothing (pRF estimates will be poorer)
- 4D merge (Sam actually does this before anything else and in some centres this is the normal output from the scanner)

An expanded explanation of these steps is provided in Appendix A.

From your pre-processing, you should end up with a single 4D file for each stimulation run in NiFTI format. In this example, we have 3 files: 1st run of fMRI (bars_1), 2nd run of fMRI (bars_2) and the HRF scan.

You also need to convert the T1-weighted structural from .hdr/.img to .nii.

2. Run recon-all

Locate the T1-weighted file and run the automatic reconstruction:

recon-all -i /path/to/T1.nii -s SUBJECT -all

Note SUBJECT is going to be the subject name in the *freesurfer/subjects* directory.

Additionally you can use the <code>-label_v1</code> option in this command to derive an anatomical prediction of V1 (based on Hinds et al., 2008, NeuroImage). This does not work on all versions of FreeSurfer (there is a bug in some versions newer than 5.0.0. The latest version at the moment is 5.3.0 and we recommend you use this).

3. Inspect surface

Check that the reconstruction worked in each hemisphere with tksurfer. Example:

tksurfer SUBJECT Ih inflated

4. Create occipital labels

This is optional but advised for saving time. There are two ways to create the occipital ROI.

- 1. The default in SamSrf is now to do this automatically in MATLAB using the MakeOccRoi command (in SamSrf/Utils). This reads in the surface data created by recon-all and restricts the occipital ROI label to the posterior vertices.
- 2. Originally, we created these labels manually in *tksurfer*. For the sake of completeness we leave these instructions in: Open the surface file as in step 3 and draw a wide region encompassing the occipital lobe.

Save label with meaningful names, e.g. 'lh occ' and 'rh occ' in the prf directory

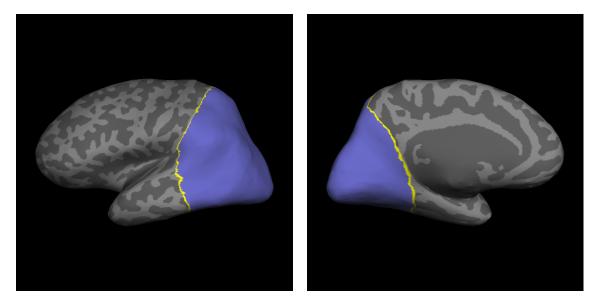


Figure 2

CHAPTER 4: SAMSRF PROCESSING

5. Check structural registration

In MATLAB, navigate to where you want the pRF data to live (usually in SUBJECT_NAME/prf) Now launch SurfaceProjection

Select the main folder for your FreeSurfer subject (labelled SUBJECT NAME in Figure 1) After a moment of loading the files a SPM check registration window will open A red line indicating the grey-white matter boundary will appear in both hemispheres

Look at the match-up between the anatomy and the red overlay as shown in Figure 3. It should define a boundary between the white and grey matter that follows the sulcal pattern of the cortex:

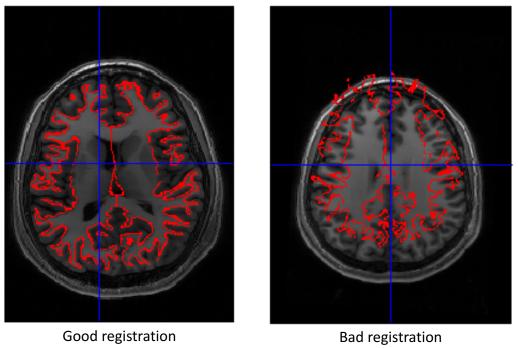


Figure 3A

There are various reasons why the link between FreeSurfer's coordinate system and SPM may fail. For example, if you fiddled with the affine transformation of the structural scan you used for FreeSurfer reconstruction (or if you resliced it or used it as source image in coregistration), then the alignment will be broken.

The example in Figure 3A should be pretty obvious but note that things can be a lot more subtle, as in Figure 3B. Note that the surface is only off by a few millimetres:

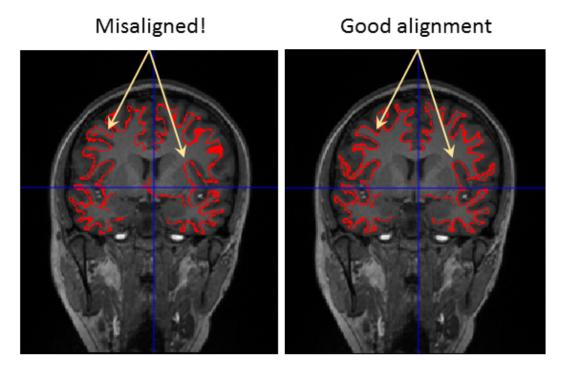


Figure 3B

This tool is also helpful for inspecting the surface reconstruction has worked properly (instead of step 3 above). If the red surface doesn't cover the whole cortex but parts of the cortex are outside of it, then you will have to fix the reconstruction. This may be due to too sharply defined structural images. Subtly smoothing (~1mm kernel) the structural in SPM can help in this situation.

What to do if registration is bad

If the registration is bad you can follow these quick extra steps to bring them in line: Cancel out of SurfaceProjection

In the terminal, go to the freesurfer/subjects/SUBJECT/mri (or wherever the nifti of your T1 is being kept) and run:

tkregister2 --mov NAME_OF_T1.nii --s SUBJECT --regheader --noedit --reg register.dat

This will produce an output with several affine transformation matrices (4x4 matrix). You will need to copy and paste two of these into a text file called <code>coregistration.txt</code> inside your freesurfer/subjects/SUBJECT/surf folder. The two matrices are the one entitled **Tmov** (2nd from top) and **RegMat** (5th). (Note that copy&paste is annoying in the Linux terminal in that it only works via right-clicking and using the context menu – keyboard combos don't work). You will need to place the RegMat matrix underneath the Tmov matrix so that in the file there will be a matrix with 8 rows and 4 columns. Finally, you must remove the semicolons at the end of the lines.

Now, if you run the check registration tool again it should automatically locate this coregistration info and use this to correctly align FreeSurfer space to native brain space.

As of SamSrf 6, samsrf_vol2srf throws up a warning when Coregistration.txt hasn't been defined. Not everybody needs this file, but it doesn't hurt to have it. You only need to do this once after you did your FreeSurfer reconstruction so it doesn't cost you much time.

6. Create surface data files

SurfaceProjection will then automatically ask you for the other required files:

Select FreeSurfer subject folder (e.g. freesurfer/subjects/SUBJECT/)

This will look for the .nii file from freesurfer that was used for reconstruction in freesurfer/subjects/SUBJECT/mri/NAME_OF_T1.nii or its orig subfolder.

Select functionals: choose all the pre-processed 4D NiFTI images, e.g. bars_1.nii, bars_2.nii

You can also provide the *Cortical sampling steps* as a vector. The number is in proportions of cortical thickness. If you have more than one value here, then the activity of different steps will be averaged. If you don't enter anything, a default of 0.5 is used.

Next there is a dialog asking you whether you want to average, concatenate or separate runs. Obviously, for averaging the stimulus protocol must then be identical in all those runs. For concatenation you may run into problems if there are too many runs to put into one matrix which will depend on the amount of RAM in your machine. Finally, you can keep individual runs separate (the projection will be done separately for each then and thus take much longer). For most purposes, averaging or concatenating are more useful but we left this function in for completeness' sake.

Next there is a dialog asking whether you want to use temporal normalisation. For functional data analysis, normalisation is necessary but you may also want to project all sorts of other data (e.g. structural data, GLM betas, t-statistics, etc) so then you will not want to use it.

Finally, there is also menu allowing you to specify the projection method. This menu only appears if you defined more than one cortical sampling step. By default, this is the mean across cortical sampling steps but it can also be the median, minimum, maximum, or you can turn it off so all sampling steps are saved (this is useful for plotting the data along the vertex normal – currently the main analysis functions in SamSrf however do not support such data).

This will take a few minutes (or longer if you have many cortical sampling steps). Once it's

done, SamSrf will output all the files you gave it in .mat format with the prefix 'lh_' or 'rh_' appended to indicate which hemisphere it belongs to. The SurfaceProjection tool does this automatically for both hemispheres.

Remember to do this for both the functional runs from pRF mapping <u>as well as</u> the HRF measurement run (if you have one). The functions used to extract the HRF also work in surface space so they need this data.

Note: as of SamSrf 6.1 the surface projection stage also calculates the noise ceiling of your data – but only when using averaged runs. This is based on splitting the data into odd and even runs and so works best when having an even number of runs. The noise ceiling gives you an upper bound of what goodness-of-fit your pRF model can possibly achieve with these data. It is stored in Srf.Noise_Ceiling in raw data files but after model fitting it is moved into Srf.Data.

7. Fit HRF

This is optional: you can simply use a canonical HRF or fit the HRF using your pRF data

In MATLAB, run this function to fit an HRF to the data from your HRF run. Assuming your HRF run is called lh_uabfHrf.mat and the TR of that run was 2.55 seconds you run:

samsrf_extract_hrf('lh_uabfHrf', 2.55)

Check the curve-fit is sensible, for example (Figure 4):

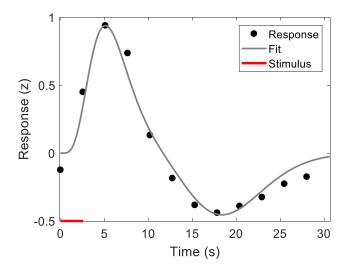


Figure 4

Fix bad HRF:

This step is rarely ever necessary. If your HRF data contain outliers that skew the HRF fit, you can fix this using the samsrf_fix_broken_hrf function.

8. Create apertures

Next you need the apertures to tell the model where the stimulus was in each volume.

If you used a standard retinotopy design using some of the SamPenDu lab's scripts, you

should be able to do this using the CreateApertures function and load the results files containing the apertures. If you used your own stimulus protocol, you will have to generate them in your own way.

Critically, the apertures should be stored in a .mat file in a variable called *ApFrm*.

This is a 3D matrix where columns and rows correspond to the y and x coordinates of pixels, and the third dimension is time. There is a frame for every volume. Usually these are binary images (1 for stimulus, 0 for background) but that is not essential.

9. Inspect apertures

Once generated/saved, you may want to check that the apertures look as they should. Run the ViewApertures tool.

Select the aperture file (should be named something like aps *.mat).

Click through the display to make sure everything is correct.

You can also click Play Movie to play it automatically.

CHAPTER 5: pRF MODEL ANALYSIS

Unlike in previous versions, in SamSrf 6 there is no longer a GUI for running the pRF analysis. More people scripted their analysis pipeline anyway and the GUI wasn't really that convenient. We now have wrapper scripts that define the parameters of the analysis. You can either run these as a GUI, or you can use them in your batch scripts to completely automate the process.

Example scripts are kept in the SamSrf/Models folder. There are scripts for the basic pRF models you might want to use, such as the standard 2D Gaussian, the Difference-of-Gaussians, various asymmetric pRFs, as well as 1D tuning curve models. You can also create your own by adding new kinds of pRF functions in the SamSrf/pRF folder – but this is way outside the scope of this cookbook. Here we will describe what you need to do to run a standard 2D Gaussian model defined in the script Standard_2d_Gaussian_pRF.m

The idea is that you take a copy of these example scripts, put that in a place related to your analysis, such as the parent folder where you keep all your subject data or a folder where you keep your other analysis scripts. You then modify the script to define the parameters of your analysis. It might also be advisable to give your script a unique name that reflects the project. For instance, in a study on pRFs in aging we might want to call this function Aging_Study_2d_pRF.m

In the script, you define a structure called *Model* which contains various parameters. If that seems scary, don't fret because most of these you won't need to worry about unless you define your own pRF model. You can simply skip forward to step **15-18** and then on to **20**.

10. Define the pRF model

Model.Prf_Function is a MATLAB functional handle to the pRF model function. For the standard 2D Gaussian pRF model this is defined like this:

```
@(P,ApWidth) prf_gaussian_rf(P(1), P(2), P(3), ApWidth)
```

The handle has two inputs: *P*, which contains the actual model parameters to be fitted, and *ApWidth*, which defines the size of the apertures (vertical and horizontal length in pixels).

The maximum number of parameters is five. You can see how other models are defined in the other example scripts in *SamSrf/Models*. You don't need to worry about this part unless you want to define your own pRF models.

11. Model name

Model.Name defines the name of the pRF model, which is the name will be called. In our example script, this is 'pRF_Gaussian' so the final map file for the left hemisphere will be called *lh_pRF_Gaussian.mat*. You can change this if you want.

12. Parameter names

Model.Param_Names is a cell array defining the names of the parameters. In a retinotopy model, the first two will be the horizontal and vertical coordinate of the pRF centre, so we call them x0 and y0. For the pRF size (standard deviation of the Gaussian), we define the name as Sigma. The standard 2D Gaussian model only has three parameters so we can stop there. If we fit more parameters, they need to be named here.

Note that unlike in previous versions, SamSrf 6 fits the beta (amplitude) and intercept separately, after it has estimated all the parameters of the pRF.

13. Determine scaled parameters

Depending on the receptive field model you use, some or all of the parameters need to be scaled to the eccentricity of your mapping stimulus. The apertures are defined such that the centre corresponds to coordinate 0,0 and the outer edges are -1 and +1. Therefore, any parameter that refers to a measure in this space, such as the pRF centre coordinates and pRF size, must be scaled to the actual eccentricity of the stimulus to make sense.

Model.Scaled_Param is a Boolean vector and it toggles whether a parameter is scaled or not. In the case of the standard 2D Gaussian model, all three parameters are scaled so it is [1 1 1]. But in the case of some other models, such as the oriented asymmetric 2D pRF, one of the parameters is the orientation of the pRF and this is obviously *not* scaled. **Critically, this vector must be of equal length as the cell array in step 12.**

14. Determine positive-only parameters

The model fitting function will look at the fitted (optimised) parameters in turn and reject those with absurd estimates. Any parameters that are scaled (see 13. Model_Scaled_Param) will automatically be rejected if the estimate is greater than 3 times the scaling factor. However, some parameters should also only be positive, e.g. the pRF size or tuning width cannot be negative.

Model.Only_Positive is a Boolean vector that toggles whether a parameter must be positive. In the case of the standard 2D Gaussian model, this is only the pRF size (sigma).

15. Define scaling factor

Once we know which parameters will be scaled, we also need to define by how much it will be scaled. In two-dimensional retinotopic models, the scaling factor is the maximal eccentricity of the mapping stimulus (usually the radius of the mapped area). In one-dimensional tuning curve models (for example, tonotopy) this would be the half-width of the stimulus space because the middle is defined as 0.

You must define this critical parameter because this will inevitably differ for your setup!

16. Define the TR

You will also need to define the TR of your pulse sequence in seconds. This is critical because the convolution of the predicted time series with the HRF depends on the TR.

This is also a critical parameter that depends on your experimental setup!

17. Specify the HRF

In *Model.Hrf* you will also need to specify which HRF to use. We typically use the canonical HRF in which case we leave this empty []. If you estimated the HRF as described earlier in step 7, you can provide here the file name of the estimated HRF. Or you can provide the HRF directly as a vector, where each component corresponds to one TR. Finally, if you don't want any HRF to be used (as you might in some situations) you must set this to 1. Whatever you do, you need to specify this, even if it just [] to use the canonical.

18. Specify aperture file

Finally, in *Model.Aperture_File* you will need to specify the file containing the apertures. Since this will typically depend on your experimental setup, **you must define this**. If you used the exact same stimulus design for each participant, you could just keep the aperture file in a common folder (with your model script) and provide the full path name here.

19. Define search space

You can also define the parameters of the search space used for the coarse fitting (extensive grid search) stage. For each of the five parameters you define a vector of the points of the search grid for that parameter, so this vector determines both the range and the granularity of the search space.

For most people, there probably isn't much of a reason to change these parameters. If you make the granularity too fine the search space will become unwieldy and you may even run out of memory.

The following parameters must be defined. These are just names for the model engine. The actual names in the final map you already defined above in step 12. The parameters can mean a number of different things, depending on the pRF model you use. In models that don't require all five parameters you simply set that one to 0.

Model.Param1: The horizontal coordinates of 2D pRF centres

or the peak location of a 1D tuning curve mode

Model.Param2: The vertical coordinates of 2D pRF centres

or the tuning width of a 1D tuning curve model

Model.Param3: pRF size, usually the standard deviation of a Gaussian pRF

Model.Param4: Second pRF size in the DoG pRF or elliptical pRFs

Model.Param5: The rare fifth parameter. In the DoG model the amplitude

ratio, in oriented model this is the orientation, etc...

It may have already dawned on you now that SamSrf does not allow more than five parameters. While we may change that in the future, five parameters are more than enough. This way madness lies, my young apprentice.

Last edited: Monday, 27 May 2019

20. Run model fit

Now we are finally ready to run the actual model fitting. This is done using the generic samsrf_fit_prf function which takes the following inputs: the Model structure you specified above, a cell array with the SamSrf data files we created in Chapter 4, and (optionally) the region of interest label we created in step 4. The example scripts included in <code>SamSrf/Models</code> will open a GUI to select these files if you don't provide them as inputs. Therefore, you can simply run the standard 2D Gaussian pRF analysis thusly:

Standard_2d_Gaussian_Prf

Other, more complex pRF models or 1D tuning curve models are run in exactly the same way and defined similarly. You can hopefully deduct a lot of that from the example scripts.

OPTIONAL MODEL PARAMETERS

In addition to these standard parameters in Model there a few optional flags have been added in later versions. You can omit them and they are turned off (whatever off means) by default but for the sake of completeness we describe them here:

Model.Replace_Bad_Fits: Boolean flag. If this is true, then any fine fits that do not meet

the criteria for a good fit will be replaced by the original

coarse fit parameters.

Model.Smoothed_Coarse_Fit: If defined and greater than zero, the data are first smoothed

with the kernel defined here. This makes the process more compatible with data estimated by versions prior to SamSrf 6.

A kernel of 5 is what was used in older versions.

Model.Coarse_Fit_Only: Boolean flag. If this is true, only the coarse fit is conducted.

Betas are estimated by linear regression afterwards as would

normally be done after the fine fit.

21. Post-processing pRF map

The pRF fitting function does not use any smoothed data (this is one of the major differences from previous SamSrf versions). It saves the final map file with only unsmoothed data.

However, for delineation or even just making figures you may wish to have smoother maps. You may also want to calculate field sign maps or the cortical magnification factor. To do all of that we can load the pRF map file and run the function <code>samsrf_surfcalcs</code>. The example scripts already contain that to show you how to use this.

In addition, for more complex models you may need to do other post-processing, for example calculating the FWHM of the DoG model or ordering orientation parameters. This can also be done at this point and some of the example scripts do that already.

RECAP: WHAT FILES DO I NEED TO RUN MODEL FITTING?

Here is you need to have in the *prf* folder (change *lh* to *rh* for right hemisphere):

Apertures file with same length as the whole time series: (e.g. aps_Bars.mat) Surface data files for **pRF mapping runs** (e.g. lh_Bars1.mat, lh_Bars2.mat, etc)

(Optional) HRF parameter file from an **HRF run** created in step 7 (e.g. *hrf_lh_Hrf.mat*) (Optional) **ROI label** file to restrict analysis created in step 4 (e.h. *lh_occ.label*)

INTERMISSION: WHAT HAPPENS DURING MODEL FITTING?

First the surface data files are loaded into memory. If a region-of-interest was chosen the mask is loaded. Next, the search space is generated (see the *Introduction to pRF models* section). This used to take a long time on earlier machines but nowadays this is probably fine for most people. For more complex models (e.g. the Difference-of-Gaussians pRF) this takes longer because the search space is more complex. If you have the exact same order of conditions for each subject you can reuse the same search space (src_*.mat files) for each though and thus save time.

Next, the search space predictions are convolved with the haemodynamic response function. Then the coarse fitting starts. On modern computers and MATLAB versions this should take no more than a few minutes. Then the fine fitting commences and this can take from a half hour to a day or even a week, depending on what model you're fitting, which procedure you chose, and which MATLAB version you are running. Finally, the maps are saved in a .mat file. Note that the Model variable in which you defined the model parameters (such as the search space dimensions and the HRF) is also saved in that file! Be aware of this if you are reloading the .mat file!

You may wish to perform some additional analysis steps after the main model fitting is complete. For example, all the analysis is performed on unsmoothed data. If you want smoothed maps, or calculate cortical magnification or field signs etc., you will need to do this now. You can add those steps to the pRF model script. In the examples scrips in SamSrf/Models this has already been done.

CHAPTER 6: RENDERING MAPS

22. Displaying maps

To inspect maps and save figures you can use the DisplayMaps tool. This will ask you for a pRF map file in SamSrf .mat format and then load this up. By default this limits the view to the occipital ROI called *lh_occ.label* or *rh_occ.label*.

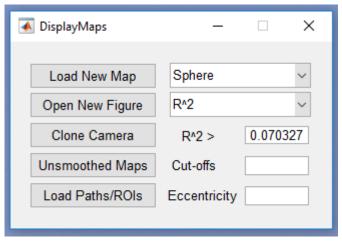


Figure 5

You can load any of the maps stored in your SamSrf file. The Polar Angle and Eccentricity maps need to be calculated as the file only stores Cartesian X and Y variables.

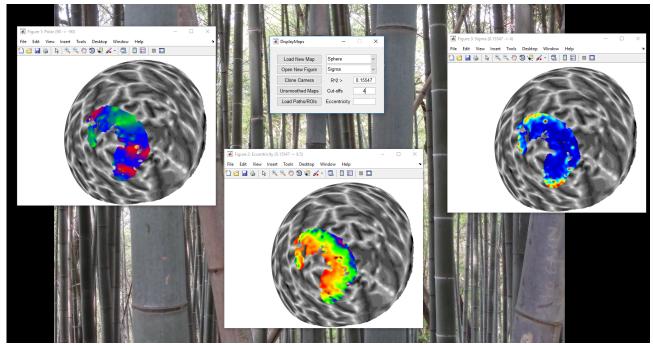


Figure 6

You can also load labels or paths previously saved. These can be in FreeSurfer *.label* format (ASCII files with a list of vertices) or paths in *.mat* files. You can also load paths stored in a delineation file saved by the DelineationTool

You can select any of the following cortical meshes to display: *Inflated, Pial, White*, and *Sphere*. The spherical mesh is the default.

The R^2 threshold is automatically adjusted to give you a pre-defined p-value. You can manually adjust this to your liking. Similarly, you can adjust the *Eccentricity Range* as a two-element vector (e.g. 1 8 to only show eccentricities between 1 and 8 degrees).

The *Cut-off* determines how the maps are clipped. This depends on the kind of map you display. For R² maps this value would determine at which level the hottest colours are displayed. For eccentricity maps, this value determines beyond which eccentricity the maximum colour is used.

You can use the *Open New Figure* button to create a new main figure. This is the only figure that is updated by anything you do in the tool. So, you should first load the maps you want and then open a new figure for any further maps you want to look at simultaneously. If you have several figures open (e.g. the polar, eccentricity, sigma and R² maps) and want them all to show the same view, you click the *Clone Camera* button. This means all the other plots will adopt the same view as your main figure.

You can load a completely new map using the *Load New Map* button.

23. Renderer lighting

The pretty lighting can be nice for figures but does not seem to work on all computers and may result in a display bug. On some computers, the display is also very slow when the lighting is turned on. You can turn the lighting in the figures on and off with this command:

```
samsrf_lighting('off')
samsrf_lighting('on')
```

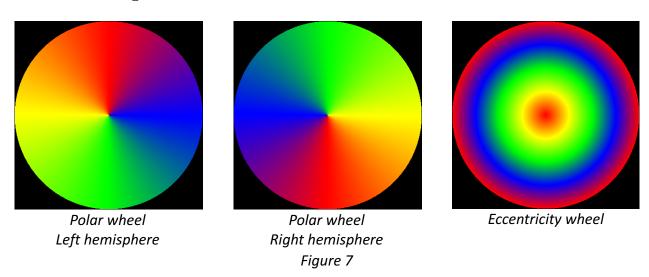
Lighting is turned off by default. For pretty figures, you may want to **first** adjust the camera angle and then turn on lighting.

24. How to delineate regions

A detailed description of how to delineate visual regions (including the higher extra-striate cortex) is the subject of a separate cookbook. In short, visual regions are bounded by the reversals in the polar and eccentricity maps. For the early regions (V1-V3) the polar map is fully sufficient. For higher regions (e.g. V4 and in particular the V3A/B complex and VO-1/2) it helps to also look at the eccentricity map because most of these regions have foveal representations separate from the early visual areas. A field sign map (showing whether or not a map is a mirror image of visual space) can also be useful, although the clarity of this map can vary a lot. Finally, the beta (amplitude) map can be very helpful in identifying bad data, in particular off-the-edge artifacts in parts of the visual field that were not stimulated.

To help you with the delineation Figure 6 shows the colour wheels. They can also be found in the *SamSrf/Colours* folder in your SamSrf installation or you can display them in MATLAB using the function

samsrf_colourcode



Important note: there was a bug in SamSrf versions prior to 6.05 that meant the eccentricity wheel was wrapping around as in FreeSurfer (which is what SamSrf also did originally). This meant that there was a bit of purple in the fovea, which is not correct.

25. Exploring the results

To save the variables that generate the above graphs, you need to run these command line functions and repeat for each ROI:

```
load Ih_pRF_Gaussian
Srf = samsrf_expand_srf(Srf)
```

This loads the pRF data into the workspace as a variable Srf. The second line is critical to expand the data set and add in the anatomical meshes! Without that, none of the other functions may run properly, although all native functions in theory should do that automatically (see also Appendix B).

To then plot summary data for a particular region of interest you can run:

```
samsrf_plot(Srf, 'Sigma', Srf, 'Eccentricity', 0:9, 'lh_V1', 0.15)
```

This produces a binned plot for sigma in Srf against the eccentricity in Srf. The vector 0:9 determines the eccentricity bins to be plotted. You can provide two different Srf variables here, e.g. when you want to compare different conditions based on a common eccentricity estimate. Here 'lh_V1' is the name of a ROI label for V1 in the left hemisphere. The 0.15 refers to the R² threshold to be used.

There are many other functions available to analysing and quantifying the pRF results. For instance, you may want to plot the visual field coverage of V1 up to an eccentricity of 8.5 and clipped to >1% of pRF coverage.

```
samsrf_vfcoverage(Srf, 8.5, 'lh_v1', 0.15, 0.01)
```

All functions contain help information that should explain how they work.

APPENDIX A: FMRI PRE-PROCESSING

Sample pre-processing steps for fMRI data carried out in SPM8. Note that these instructions are specific to data acquired in the FIL on a 3T TIM Trio scanner with a 32-channel coil. If you didn't acquire your data there under these conditions, the procedure for you will most likely differ.

DICOM import

SPM > DICOM import

Convert all your raw files to 3D NiFTI format, conserving the original filenames prefix added: [f]

Bias correction

Perform bias correction on all the EPI images using spm_biascorrect prefix added: [b]

Remove dummies

Place the first four volumes in a separate folder

Create fieldmap vdm * files

After DICOM import, fielmaps are divided into two folders; the first has two files (magnitude) and the second has a single file (phase).

Batch script

SPM > Tools > Fieldmap > Presubstracted phase and magnitude data

Phase image: second field map folder (single file)
Magnitude image: first file in first field map folder

Blip direction: -1

EPI-based field map?: no Jacobian modulation?: no

Brain mask = no

Match to the first volume in each EPI run

Realign and unwarp

Batch script

SPM > Spatial > Realign & Unwarp

1 subject = 1 module, each run in a separate session

for each run, use the single session-matching vdm_file

Masking = yes

prefix added: [u]

Slice-timing correction

Batch script

SPM > temporal > Slice timing

On all bias-corrected, realigned and unwarped functionals

Insert scan details (TR, TA, number of slices, slice order)

Reference slice is middle one (15 of 30)

prefix added: [a]

If you acquired a single structural, do a one-step coregistration. If you acquired a short and a long structural, do a two-step coregistration. Note that it is important to select only 'estimate' and not 'estimate and reslice' in order to preserve the spatial origin from the fMRI images throughout.

One-step coregistration

Batch script - Estimate

All functionals to high-resolution structural

Reference (target): high-resolution structural from Freesurfer folder

Source: 1st aubf_* image of 1st run

Other images: all other aubf_* volumes for all runs

One module per participant

Two-step coregistration

Batch script

SPM>Spatial>Coreg>Estimate

All functionals to short structural with coil off

Reference (target): short structural Source: 1st aubf * image of 1st run

Other images: all other aubf_* volumes for all runs

SPM>Spatial>Coreg>Estimate

All functionals to high-resolution structural with coil on

Reference (target): high-resolution structural from Freesurfer folder

Source: short structural

Other images: dependency on results from step above

One module per participant

Check coregistration

SPM>CheckReg

Optional: Smoothing (use only with the pre-smoothed receptive field model!)

All the coregistered, slice-timing corrected, unwarped, and bias-corrected volumes Kernel size: 4mm

prefix added: [s]

4D merge

Combine smoothed images for <u>each run</u> using spm_file_merge Good idea to rename them here into the correct run, for example:

- bars 1
- bars_2
- HRF

APPENDIX B: STRUCTURE OF SAMSRF DATA FILES

Introduction

You carefully designed your study, pre-processed your data and ran a model fit with SamSrf. Now, you can use the buttons in the SamSrf graphic interface to get some results plotted, but what if you want to get your hands on the data?

This guide covers the contents of the SamSrf results files and their potential uses. It assumes basic knowledge of the MATLAB environment and programming language. If you wish to learn MATLAB, you can head to MathWorks¹ or the excellent guide of MATLAB for Psychologists².

Navigation

To access the SamSrf results file, open MATLAB and go into the subject folder where you ran the pRF fit. Here you will find a file called *lh_prf_Gaussian.mat* or similar.

- 'lh' or 'rh' for left and right hemisphere, respectively
- Or any arbitrary name for the actual data prior to any analysis (e.g. 'lh_Bars1')

Open the file by double clicking or typing:

load('lh_prf_Gaussian.mat')

Once loaded, you will see 2 variables in workspace (some formats may contain more):

Model: The pRF model specified as described in Chapter 5

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¹ http://www.mathworks.co.uk/academia/student center/tutorials/launchpad.html

http://antoniahamilton.com/matlab.html

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Srf: The main structure with all the data

By default, Srf variables are compressed and have their anatomical meshes removed. So before you can use them you must expand them (but all of the native SamSrf functions should in theory do that automatically). To do so run:

Finally, before saving .mat files with a Srf you may wish to compress them again:

This will remove the anatomical meshes from the Srf. You can also restrict the Srf to a region of interest (such as the occipital region). See samsrf_compress_srf for details.

Contents of 'Srf'

The variable Srf is a structure containing a number of sub-variables:

Srf.Version: [scalar] Contains the version number used by the analysis. As of SamSrf 6, this is first added during surface projection but then updated again during pRF model fitting. Also, this has only been introduced in version 4.0 so if an analysed map file doesn't have this field it was analysed with an older version. This field is important to ensure data are comparable (e.g. Sigmas before version 4 were measured incorrectly). But ideally you shouldn't have to use any files created by versions prior to 6...

Srf.Structural: [string] Full path location of the structural image used as reference.

Srf.Functional: [string or cell array] If this is a pRF map, this contains a description of analysis carried out (e.g. which pRF model) and details of smoothing (if any) and name of ROI restricting the analysis (if any). If this is a data file, this contains the name of the functional NII file the data came from. If this is an average, it is a cell array of all the file names that were averaged.

Srf.Hemisphere: [string] Hemisphere contained in this file. This should match the filename.

Srf.Cortex_Steps: [scalar] Cortical sampling steps, as defined in the SamSrf GUI. This defines the voxel positions and number that each vertex is sampled from in volumetric space and is in units of cortical thickness. Therefore, the default 0.5 would mean that each vertex contains the data from that voxel that is located 50% along the vector from the vertex on the grey-white matter surface and the corresponding vertex on the pial surface. You could also choose more voxels though by setting this to a vector, e.g. [0:.2:1] would sample six voxels along this vector between grey-white and pial surface in steps of 20% of the cortical thickness. Taking more than one voxel probably only makes sense for relatively high-resolution data.

Srf.Meshes: [char] Contains a link to refer to the folder and file name where the anatomical mesh data are stored. This is not mandatory but is used by default when analysing data in SamSrf 6. It means that when you have more than one file with functional data projected to the surface, you only need to have one file for the anatomical meshes rather than duplicating this across files.

Srf.Vertices: [*m x 3* matrix] An index list of every vertex. Each row corresponds to the X, Y and Z coordinates of a single point in native space using FreeSurfer's internal coordinate system. The vertex coordinates correspond to the grey-white matter surface, so gyri and sulci are folded. May be stored in a separate file for anatomical meshes (see *Srf.Meshes*).

Srf.Faces: [*m x 3* matrix] Face values for every vertex. The surface meshes are constructed from thousands of tiny triangles, called *faces*. Each face is defined by three vertices and they are defined by the vertex indices in this list, one face per row. May be stored in a separate file for anatomical meshes (see *Srf.Meshes*).

Srf.Normals: [*m x 3* matrix] Each rows contains the vector pointing from a vertex on the grey-white matter surface to the corresponding vertex on the pial surface. This is used by surface projection to determine the voxels to sample from. May be stored in a separate file for anatomical meshes (see *Srf.Meshes*).

Srf.Inflated/Sphere/Pial: [*m x 3* matrix] Like Srf.Vertices but contains the coordinates model for the inflated, sphere, or pial surface mesh instead of the white-grey matter boundary. May be stored in a separate file for anatomical meshes (see *Srf.Meshes*).

Srf.Curvature/Area/Thickness: [1 x m vector] Contains the curvature, surface area, or thickness of the cortical surface model for each vertex. May be stored in a separate file for anatomical meshes (see *Srf.Meshes*).

Srf.Data: [$k \times m$ matrix] This contains all the resulting values of the analysis. If this field Srf.Values (see below) is present, the rows correspond to the k value descriptions in Srf.Values (e.g. R^2 , X_0 , Y_0 , Sigma, etc..) and each column corresponds to a single vertex. So, if you are interested in, say, the R^2 values of every single vertex, you can index the first row and every column of Srf.Data. If Srf.Values is not present this is probably a file containing the data prior to analysis and each row of Srf.Data contains one volume. If you applied surface smoothing post-analysis, this variable contains the smoothed data. The un-smoothed data is stored in $Srf.Raw_Data$.

Srf.Rule: [char] Defines how the voxels in *Srf.Voxels* relate to the data for each vertex in *Srf.Data*. This only makes a difference if you have more than one cortical sampling step. The default is 'Mean' which means that each vertex is the arithmetic mean of the sampled voxels. Other options are 'Sum' for the sum, or 'Median' for the median (see *samsrf_vol2srf* for details). This is obviously only relevant for the actual surface projection. The analysed data files inherit this field as this tells you how surface projection was done.

Srf.Noise_Ceiling: [1 x m vector] Contains the noise ceiling if it has been calculated. This is only the case for raw data files. If the noise ceiling exists, the model fitting procedure will add this to Srf.Data and so there won't be a separate field in Srf for that in those files.

Srf.Y: [*n* x *m* matrix] The preprocessed time series for each vertex after z-normalizing each run and concatenating them. Each column is for a vertex, each row is for a volume (time point). This only exists for analysed data files.

Srf.X: [n x m matrix] The neural (rather than metabolic, so it is not convolved with the HRF!) time series for each vertex predicted by the pRF model. Therefore, R² can be calculated by comparing Srf.X to Srf.Y, after convolving Srf.X with the HRF. All other conventions are the same as for Srf.Y.

Srf.Values: [k x 1 cell array] Description of the values stored in *Srf.Data*. Each row in this variable corresponds to the matching row in *Srf.Data*, so if the 4th row in 'Sigma', all the values along the 4th row in *Srf.Data* are Sigma values. Used for indexing. As of SamSrf 6, this is mandatory (and automatically added by *samsrf_expand_srf*, if it doesn't exist).

Srf.Raw_Data: [p x m matrix] If you applied surface smoothing, this variable will contain the <u>non-smoothed</u> data. If no smoothing is applied, this variable will not exist. Because values like the CMF are calculated after smoothing, they are not contained in this field.

Srf.Roi: [vector] A list of vertex indices that indicate whether any given vertex is inside the ROI used to limit the analysis. If used, the number of columns in *Srf.Data* should match the length of *Srf.Roi*. If this is the case, use *samsrf_expand_srf* to expand the data. This is essential before running any further analysis scripts on the data.