A Practical Guide to Lesion Symptom Mapping

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About this Guide:

This guide is intended to help anyone who would like to run a lesion symptom mapping analysis but isn't quite sure where (or how) to start. When I first started running lesion symptom mapping (LSM) analyses in my PhD, I spent several months meeting with different people who understood different bits of the processes and compiled all the information I learned into the first draft of this guide. I'm hoping that this guide can help other researchers design and run LSM analyses, without having to spend months learning (like I did).

Importantly, there are lots of different ways to run LSM analysis. This guide is meant to be a starting point for people who don't know where to start and will likely be updated and expanded over time. If there is anything you as a reader would like to be included/edited/expanded, please let me know and I will adjust the guide accordingly. I'm hoping that if we all work together to keep what we know in one place, we can help others towards designing and running higher quality, more informative LSM analyses.

All of my scripts mention in this guide are openly available on my Open Science Framework (Foster & Deardorff, 2017) profile (https://osf.io/mv2qf/). All other programmes/software are available via the links provided in Appendix I. I plan to incrementally improve and expand this guide over time. Feel free to get in contact if you have any suggestions for the next version.

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1 - A General Overview of the LSM Pipeline

Running any Lesion Symptom Mapping (LSM) analysis involves lots of little processing steps. Most of the steps themselves are fairly simple (especially if you're comfortable with MATLAB), but it can be complicated to understand the analysis pipeline as a whole. The purpose of this section is to provide a brief overview of each of the individual steps that are needed to complete a generic univariate LSM analysis. This section should be helpful if you don't really have a good idea of what practical steps you (or one of your students) will need to take to run a LSM analysis. I've provided practical details on what to think about and how to run each of these individual processes in later sections.

First, you need to **decide what question you're going to ask** your data. There are no stupid questions, but there are a lot of questions which won't yield informative LSM results. I recommend reading "A Hitchhiker's Guide to Lesion Symptom Mapping" (de Haan & Karnath, 2018) to get a good idea of what questions can and can't be appropriately addressed by different kinds of LSM analyses. In general, LSM tends to perform well when you're looking for localized, spatially contiguous neural correlates in a large sample of patients with good lesion coverage. LSM can produce false negatives when correlates are distributed (or bilateral) or when lesion coverage isn't good enough (Bates et al., 2003; Mah et al., 2014; Mirman et al., 2018). This guide will focus on univariate LSM which is the most common form of LSM, but there are many more advanced LSM (or Voxel Based Morphometry) variants which may be more appropriate for specific research questions (Geva et al., 2012; Hearne et al., Under Review; Sperber et al., 2019; Zhang et al., 2014).

Once you've settled on a question, the next step is to **collect some data**. All LSM analyses need both behavioural data and a brain scan showing a lesion, ideally collected at the same time point (de Haan & Karnath, 2018). In terms of behavioural data, this can be any measure, but measures yielding continuous variables generally do a better job of teasing out brain-behavior relationships (de Haan & Karnath, 2018). Any brain scan showing a lesion can be used. I generally use either CT or T2 MR, as these are often routinely collected and allow high-quality lesion segmentation (Moore et al., n.d.). Importantly, behavioural data and scans should be collected at the same timepoint, as cortical reorganization can complicate brain-behavior relationships. Ideally, both behavior and scans should be collected as soon as possible following stroke (de Haan & Karnath, 2018; Karnath & Rennig, 2017), but the importance of this practice depends on the exact questions being asked (more on this later).

Once you have your data, you generally need to **convert the scan data** from its raw format (often DICOM) to something which can be read by the established LSM programs (e.g. NIFTI). Once this is done, binarized lesion masks can be created by **lesion delineation** (manually outlining the boundaries of lesions on each slice of the native space scan). There's several different manual, automated, and

semi-automated ways to do this (e.g. Gillebert et al., 2014; Ito et al., 2019), but in this guide I will focus on manual delineation. Once lesion masks are created, native space scans and lesions need to **be normalized into standard space** to facilitate group-level comparisons. No informative group-level LSM conclusions can be drawn from looking at native space lesions alone, as factors such as head orientation, size, and location make native space lesions impossible to informatively compare. Next, it's important to **visually inspect all normalized scans** and data, as sometimes the programs performing these registrations make errors. Finally, you'll generally need to **reslice binarized lesion files** so that each file contains an identical number of voxels.

Next, it's time to **analyze**. There are several existing programs you can use to run a LSM analysis. I generally use NiiStat (https://www.nitrc.org/projects/niistat/), but you can use NPM (https://www.npmjs.com/package/download) or any number of in house toolkits (Rorden et al., 2007). All these programs pretty much need you to input each of your binarized lesion files and their associated behavioural data. You'll have to set a number of analysis parameters (e.g. corrections, minimum overlap, etc) and each of these choices can dramatically affect your output. I'll explain what you should think about when making each of these choices later on. Finally, you need to **figure out how you want to report** it. I've included descriptions of a number of different post-processing steps you can use to visualize, report, and interpret LSM output data.

2 - Asking the Right Questions

It's really important to understand that you can run a VLSM for pretty much any research question, but that doesn't mean you should ask any question. You can run through each of the analysis steps and get a result for pretty much any combination of behavioural and imaging data, but that doesn't mean your result is going be theoretically informative. For this reason, it's really important to have a good understanding of when and how LSM techniques should be used. In this section, I'm aiming to give readers a quick overview of the things they need to be thinking about when deciding whether or not LSM is suitable for their research question.

First, I'd recommend reading some basic papers about LSM methodologies to get an idea of what questions LSM analyses are appropriate for answering. Below, I've listed a series of questions you should think about and answer when deciding whether LSM is appropriate for your data:

Is the behaviour of interest something that could be captured by LSM?

LSM is a widely used technique, but it is now without its limitations. Univariate LSM (the method described in this guide) has several serious issues which can yield uninformative (if not misleading results) if used on the wrong data.

Mainly, in cases where underlying behaviour is supported by multiple, spatially distinct correlates (e.g. network-based or bilateral functions) LSM tends to report the spatial average of the related correlates (Gajardo-Vidal et al., 2018). This average may be in between the actual underlying correlates rather than at any of the areas actually involved in the behaviour of interest. For this reason, univariate LSM is better suited for investigating the anatomy of behaviours which are supported by single, spatially contiguous neural correlates. This doesn't mean you can't use LSM in cases where your behaviour of interest might involve multiple regions, it just means you need to be cautious whilst interpreting the results of your analyses.

Similarly, LSM is not good at dealing with cases in which behavioural deficits have bilateral correlates. This is due to the fact that having correlates which are far apart from one another will mean that there will likely be lots of patients with behavioural impairment who are included in the "spared" voxel-wise distribution for many voxels. This reduces the effect size of voxel-wise comparisons, reducing the probability that even significantly involved voxels will survive statistical testing. If you think your deficit might involve bilateral correlates, it's best to run separate LSM analyses for the right and left hemispheres.

Finally, LSM needs to have lots of patients with impairment in order to draw informative conclusions. If your behaviour of interest is something that is only observed in a few individuals, it's very unlikely LSM will yield informative results. For example, I do a lot of work with word-centred neglect dyslexia ((Moore & Demeyere, 2017, 2018, 2020) but I've never tried to do any LSM on this data. This is because I

only have lesion data from 2 patients with this condition, meaning that there's no way LSM would have enough power to detect the voxels related to this condition even if I have a massive control group.

Can scans/behavioural data be collected at the same timepoint?

This is a key issue. I recommend looking at this paper (Karnath & Rennig, 2017) for more details on what exactly goes wrong when data from different time points are combined.

Stroke lesions evolve very quickly over time and many post-stroke behavioural deficits can spontaneously recover very quickly (Karnath & Rennig, 2017; Stone et al., 1992). As cortical reorganisation occurs, and area which was not previously involved with a lost function can adapt to facilitate this function, introducing spatial variation. This means that scans taken at a later stage post-stroke (e.g. >1 month) shouldn't really be used to try to elucidate the functional structure of the brain (de Haan & Karnath, 2018). Chronic scans are great for studying the neural correlates of chronic impairment, but they can't really be used informatively investigate the functional architecture of the brain (see (de Haan & Karnath, 2018) for more details on this).

Conversely, it's also not really informative to combine acute scans with behavioural data taken at a later stage post-stroke. This again is because many patients who may have shown the deficit of interest during the acute phase (and shown associated lesions) may have recovered by the time of chronic assessment.

Ideally, LSM imaging and behavioural data should be collected at the same time point (e.g. within 1 week) as early as possible following stroke. If this is not feasible in your investigation, it may prove extremely difficult to draw informative conclusions from your result. It may seem a bit extreme to say that data collected at other time points shouldn't be used in LSM, but both past research and my own personal experience has convinced me that it is best practice to limit your sample to the patients who have both acute imaging and behavioural data.

Will you have sufficient statistical power?

As in any experiment, statistical power is extremely important in LSM. If your investigation is not sufficiently powered, it's impossible to say whether any of your reported non-significant results represent real null results (e.g. true negatives) or false negatives (Cohen, 1992; Maxwell, 2004). In behavioural experiments power is generally a function of the expected effect size and the number of patients included. However, in LSM power needs to be considered at both the group-level and the voxel-wise level.

In order to complete high-powered voxel-wise statistical tests, each voxel tested needs to be damaged in multiple patients. The number of lesions impacting each voxel isn't necessarily related to sample size, as you could include lesions from hundreds of patients, but they may not necessarily have any overlap. This is why it is

extremely important to use a minimum overlap inclusion threshold for all voxel-wise comparisons. I generally aim to only test voxels damaged in at least 8 patients (see my upcoming LSM simulation paper for details). If you test all voxels (e.g. with an overlay > 0), there's no way voxels impacted in such a low number of patients can yield a significant result and these underpowered voxels therefore have a high risk of being false negative results.

Taking this into account, you should only run LSM analyses when it is feasible to have sufficient lesion overlay throughout a good portion of the brain. There aren't really any set guidelines on what "good overlap" is. I generally just make a lesion overlay of all the patients I want to include and make sure that the main areas I'm interested in are damaged in at least 7-10 patients. This is particularly relevant when you've theorised that a specific ROI might be related to your deficit. If there is insufficient overlap in that ROI, you will get a null result no matter what the underlying relationship between this ROI and your behaviour is.

Mainly, if you're thinking about running LSM but your maximum overlay is <8 patients, I'd recommend recruiting more patients before attempting an analysis.

Is it feasible to include a representative sample of the target population?

It is generally difficult to recruit stroke survivors for neuropsychological research and many of the patients which end up in studies aren't exactly the average stroke survivor. In most data sets, it's much more likely that younger patients with smaller strokes meet standard inclusion criteria than patients with larger strokes.

It is also quite common to exclude patients with specific deficits (e.g. aphasia). This may seem to make sense for studies that aren't aiming to investigate aphasia, but this can ultimately result in dramatically reducing the number and location of voxels which can be tested. For example, if you end up excluding aphasiac patients, you're going to have to exclude more left hemisphere patients that right hemisphere stroke patients. This often creates an imbalance in the distribution of lesions across

3 - Collecting Data

In order to conduct LSM, you will need lesion masks and behavioural data from a set of patients. Behavioural data can be collected using pretty much any tool/ experiment, but there are a few things you should think about when deciding how (and when) to collect this data. Similarly, lesion masks can be made from a variety of imaging types, but choosing which imaging type to use can dramatically impact the quality and generalisability of your results. In this section, I will provide a quick overview of some things to keep in mind when collecting behavioural and imaging data for use in LSM.

Timing (For both Imaging and Behaviour)

As mentioned in the previous section, it's critically important to make sure that behavioural data and imaging data are collected at similar timepoints. Preferably, both data sources should be collected as soon as possible following stroke in order to facilitate inferences about the functional structure of the brain (de Haan & Karnath, 2018). If scans and behavioural data are collected at the same time point further after stroke (e.g. > 1 month), this data can still be used to investigate the correlates of chronic dysfunction. However, it is not advisable to combine neuroimaging and behavioural data collected at acute and chronic timepoints as this often confounds effective interpretation of results (de Haan & Karnath, 2018; Karnath & Rennig, 2017). I would say that this is the single most important thing to keep in mind when collecting data for LSM analyses.

Behavioural Data Distributions

LSM can be run on either continuous or binary data, but it's important to check the distributions of your data before analysis. Continuous data is generally considered best for LSM as these analyses generally have improved statistical power compared to binary comparisons (Cohen, 1992). However, if you're using continuous data, even if the group-level scores are normally distributed, it's very probable that the distributions used in voxel-wise testing may not always meet the assumptions of parametric testing (e.g. having a normal distribution). For this reason, it's often a good idea to use non-parametric testing or binarize your behavioural data before LSM analysis. Again, there's no one right way to do this. Chose whichever method seems best for your data.

Neuroimaging Modality

It's generally stated that MR modalities (e.g. FLAIR, T2, T1, DWI) are better for lesion delineation. However, this is not necessarily a valid claim (Moore et al., n.d.).

First, MR does a good job of visualizing lesions but is expensive and not everyone who has a stroke can actually have an MR (Singer et al., 2004). This reduces the number of people who can be included in MR LSM studies and generally mean that their results may not be generalisable. In comparison, CT scans have lower resolution but are more widely available and are routinely collected for every patient with a suspected stroke (Rabinstein & Resnick, 2009).

I'm working on publishing a very large simulation study which effectively shows that there's no major difference in LSM studies which employ CT- or MR-derived lesion masks (Moore et al., n.d.). Overall, this study shows that MR- and CT-derived lesion masks exhibit good agreement in location, overlap, and size. MR-based LSM was able to test more voxels than CT-based analyses, but CT-based analysis results were closer to the underlying target voxel. The results yielded by paired CT and MR LSM analyses demonstrated good agreement in terms of dice coefficient when systematic differences in cluster size and lesion overlay are taken

into account. Overall, this project shows that there's not actually that big of a difference between using lesion masks that were drawn on CT and MR in lesion mapping analyses.

This finding has some important implications. First, LSM analyses can and should use CT data (a point also made by de Haan & Karnath (2018) and Moore & Demeyere (Under Review)). CT data is cheaper and more widely available, meaning that using this data will likely dramatically increase the number of patients able to be included (versus MR only analyses). Lesion overlay is one of the most important factors underlying analysis accuracy (see my upcoming paper for details/evidence), so it's critically important to include as many patients as possible. CT is also collected for everyone with a suspected stroke, while a substantial non-random portion of the stroke population can't actually complete MR scans (Singer et al., 2004). For this reason, using CT may also help your LSM sample be more representative of your population of interest.

However, CT does have some disadvantages. It's generally much easier to delineate lesions on MR than CT (see the lesion delineation section for more info), meaning that you'll need a higher degree of expertise to use CT data versus MR (Bryan et al., 1991; Rabinstein & Resnick, 2009). Overall, the imaging you employ will depend on the exact data you have available to you. In summary, you can use either CT or MR in lesion mapping analysis, as long as the imaging was collected at a similar time point to the behavioural data.

4 - Converting Raw Scan Data

Overview: In order for scan files to be read by common analysis programmes such as SPM, NiiStat, and MRIcron, they generally need to be in nifty (.nii or .nii.gz) format. When downloaded from a scanner, most scans are in DICOM (.dcm) format instead. This section will walk you through how to convert scans from dicom/nifti using a MATLAB script I wrote. If you're comfortable with coding, you can write your own script or modify mine if you want it to be done differently. If not, follow the instructions below.

What you'll need: MATLAB, DICOM files, Statistical Parametric Mapping (SPM) software, my DICOM conversion code (DicomtoNifti_V2.m - https://osf.io/mv2qf/)
What does this do?: Converts all DICOMS to Nifti files and moves them to a new folder
Why this is important: Scans generally need to be in nifti format before they can be viewed by most programs used for manual delineation.

Basic Procedure:
1. Open the "DicomtoNifti_V2.m" in matlab
2. Change the file path in line 6 to the folder containing all your patient DICOMS
3. Change the file path in line 7 to the folder where you want the converted nifti files to go
4. Run the code and wait for the code to finish (usually 1-4 mins per scan)
5. Check the output folder to make sure it now contains the patient files

Notes:

Make sure the file containing your DICOM files either has each patient in a separate folder or all the DICOM in the same folder. These scripts do not deal well with directories within directories within directories.

If you want your output to be formatted differently (e.g. patient ID is wrong). You can adjust the arguments for the function `spm_dicom_convert` in line 32. Your options should all be explained thoroughly in the script documentation.

This step is pretty straight forward. If you run into any issues make sure the file paths (and directory structures) needed by the code match your data structures. If there is a different problem it will likely be with one of the SPM functions called by the script. There are plenty of resources for debugging these functions online, but NEVER make changes to the SPM script files (unless you really know what you're doing). Debug these files to find what's causing the error but always change your inputs to the function, never the actual function. The SPM functions are really versatile and a small change can cause lots of problems.

DICOM files generally contain information for multiple nifti scans. It's common for each scan session to contain between 3-10 separate scans. Most of these scans aren't relevant for lesion delineation. The next section will detail how to choose the right scans for analysis.

5 - Choosing Scans to Delineate

Overview: Once you've converted DICOM files to nifty, it's possible you'll end up with multiple scans for each patient. Most of this data will not be relevant. I've written a script (NAME) which automatically identifies most scans which won't be usable which makes checking through them a lot faster. Again, the pipeline I've described below is only one of many ways of doing this. It is, however, the method I've found to be most efficient.

What you'll need: MRIcron, raw nifti files, my nifti cleaning script (NiftiCleaner_V1_Moore.mat - https://osf.io/mv2qf/))
What does this do?: Finds all useful scan data and choose the right file to delineate
Why this is important: As you will see, each patient scan folder contains lots of different scans, but not all of them are relevant to us. We only want to keep data which we can use for research.

Basic Procedure:

1. Download and open the nifty cleaning script (NiftiCleaner_V1_Moore.mat) and set filepath (in line 4) to the directory containing your data
2. Run the script and wait for it to finish (usually < 5 mins)
3. Manually check through all the scans sorted into the "good scans" file in a nifty viewer such as MRIcron or FSLeyes (more details later)
4. Chose the best-resolution scan (more on this in notes)
5. Move or rename this usable scan to mark it for use in later analysis

How to View Scans

To open a .nii file in MRIcron (Rorden et al., 2007) , simply double click on it (assuming MRIcron is the default viewer). If MRIcron does not automatically open, right click on the file and set MRIcron as the default opening program. If the image is a CT scan you will need to manually set the contrasts to view the image in any detail. To do this, enter 0 and 70 in the contrast boxes (located just to the right of the "greyscale" menu). You can then click the up and down arrows on the "Z" box menu to scroll up and down through the axial view of the scan. To see the axial view in more detail, select "View>Display>Axial Only" in the MRIcron top bar menu.

To open a .nii file in FSLeyes, open FSLeyes and click the little "+" button in the bottom, left-had panel. You can then chose your file and it will open in the viewer. You can set the contrasts to 0 and 70 in the boxes in the top toolbar labelled "min" and "max". FSLeyes is more modern and user-friendly than MRIcron, but can't be used for lesion delineation.

It's fine to use either of these programs to visually inspect scans. I tend to prefer MRIcron for this, as opening multiple native-space scans in FSLeyes simultaneously can cause some strange visualisation errors which will look like

banded distortions in the image. These are caused by native space scans being in different sizes an orientations. If this happens, just close all open scans in FSLeyes and restart.

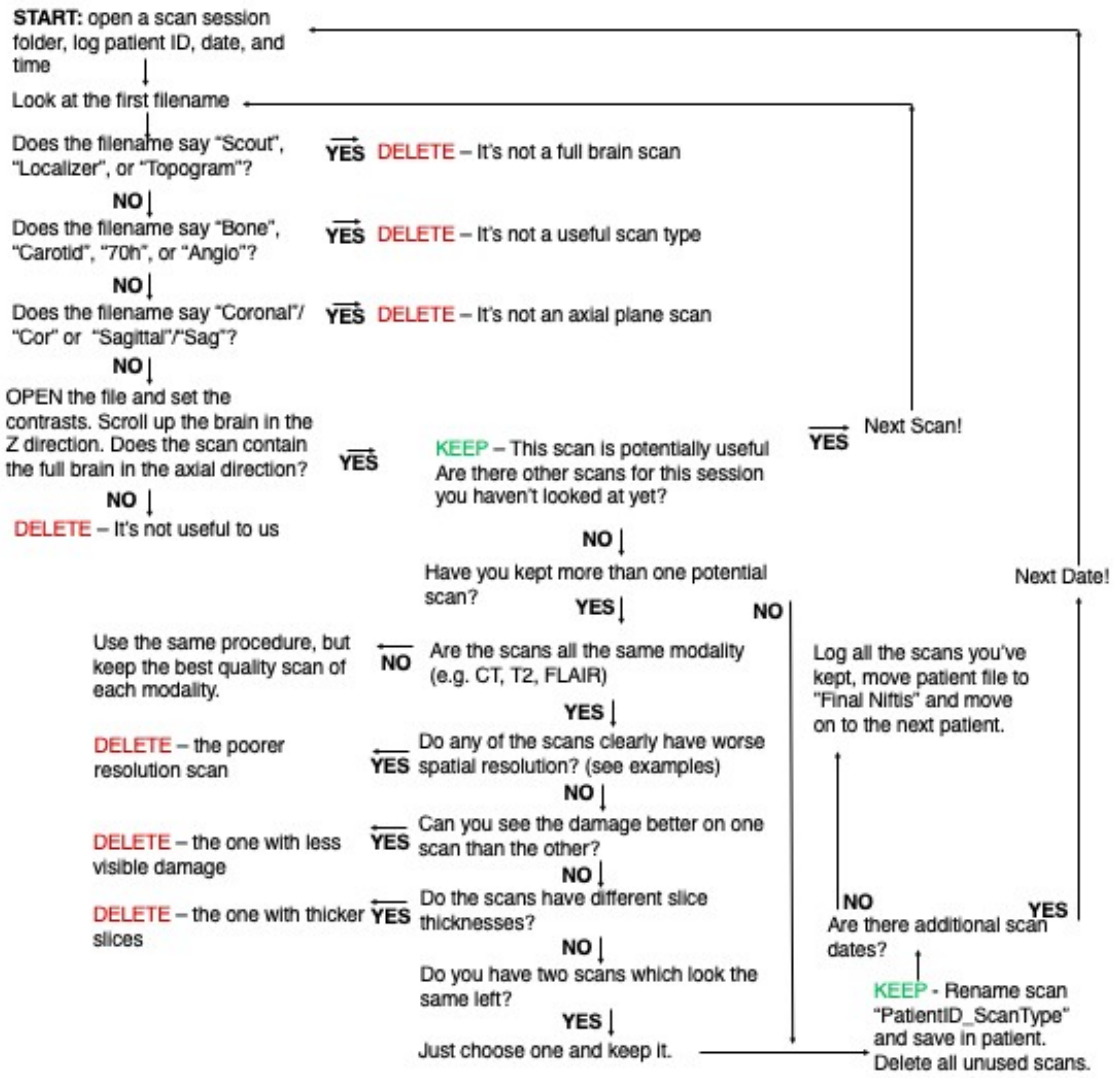
How to choose the correct scan:

In my own scan database, I keep the best quality scan of each modality on each testing date and discard all other scans. I'd recommend looking for scans which have the full brain in the Axial Plane and have good resolution. I generally delete all scans which do not contain the full brain or have worse resolution than the other existing scans, as these scans can always be remade if you keep the original DICOM files. If there are two scans from the same date of similar resolution, choose the one you can see the damage more clearly on.

My NifitCleaner script is written to automate some of this selection process. It works by checking the sizes of each nifti file. Scans which are too small are generally only a few slices (e.g. locator scans) and are not useful for analysis. Scans which are too big (eg > 400 z slices) are generally very small slice thicknesses (<1mm) and (in my data) tend to be very poor resolution and absolutely awful to delineate manually. If you're comfortable with MATLAB, you can change the parameters that this script uses to sort out data to match the data you're working with. Importantly, this script doesn't delete any scans, but simply moves most of the scans to a folder called "Bad Scans". If none of the scans flagged as being good are acutally usable, you can always use one of the scans flagged as bad. This script just aims to save the user some time.

Once the NiftiCleaner has been used, I recommend viewing each scan flagged as being "good" and choosing the scan which looks best for delineation. I made a flowchart for people in my lab, which helps them decide which scans are worth keeping (see below). In general, choose one scan from each date which does the best job of visualising any damage. There can be several very similar scans collected in each session, and it's ok just to chose which ever one looks best to you.

At the end of this process, you should have one usable scan for each patient. It's important to note that not all scans (especially acute CT scans) will show a lesion, even if the patient has a confirmed diagnosis of stroke. Unfortunately, scans with no lesion must be excluded from analysis.


START: open a scan session folder, log patient ID, date, and time
Look at the first filename
Does the filename say "Scout", "Localizer", or "Topogram"?
YES
DELETE – It's not a full brain scan
NO
Does the filename say "Bone", "Carotid", "70h", or "Angio"?
YES
DELETE – It's not a useful scan type
NO
Does the filename say "Coronal"/ "Cor" or "Sagittal"/"Sag"?
YES
DELETE – It's not an axial plane scan
NO
OPEN the file and set the contrasts. Scroll up the brain in the Z direction. Does the scan contain the full brain in the axial direction?
YES
KEEP – This scan is potentially useful
Are there other scans for this session you haven't looked at yet?
YES
Next Scan!
NO
DELETE – It's not useful to us
NO
Have you kept more than one potential scan?
YES
NO
Next Date!
Use the same procedure, but keep the best quality scan of each modality.
NO
Are the scans all the same modality (e.g. CT, T2, FLAIR)
YES
Log all the scans you've kept, move patient file to "Final Niftis" and move on to the next patient.
DELETE – the poorer resolution scan
YES
Do any of the scans clearly have worse spatial resolution? (see examples)
NO
DELETE – the one with less visible damage
YES
Can you see the damage better on one scan than the other?
NO
DELETE – the one with thicker slices
YES
Do the scans have different slice thicknesses?
NO
Do you have two scans which look the same left?
YES
Just choose one and keep it.
NO
YES
Are there additional scan dates?
KEEP - Rename scan "PatientID_ScanType" and save in patient. Delete all unused scans.

6 - Lesion Delineation

Overview: Manual lesion delineation is definitely the single hardest step in LSM analysis. It takes a lot of time to learn to "read" neuroimaging scans (especially acute CT scans) and it's not a skill that can be easily taught. I've included links to a number of resources which can help people learn to do this, but I think the best way to learn is just by practicing. I taught myself to do this by practicing delineating lesions and checking the resultant mask against existing delineations. The best way to learn is to get trained by someone who knows what they're doing, but if you don't have access to someone who's already trained, this guide should help you get started.

What you'll need: MRIcron, final CT scans
What does this do?: Create binarized lesion masks for each patient.
Why this is important: You can't run anatomy statistics or lesion comparisons if you don't have the extent of damage quantified.

Basic Procedure:
1. Open each scan in MRIcron
2. Visually scan each image and find any lesions
3. Outline and fill each lesion using the pen and fill tools
4. Smooth your lesion mask

Notes:
Lesion delineation isn't something that can be taught. I have attempted to train many students how to do this, and the only people who actually end up gaining the skill are those who are willing to put a lot of time in teaching themselves. In this guide, I'll walk you through how to quantify lesions, but if you want to learn to see lesions you'll need to do a lot of work on your own. The difficulty of this task will be determined by what kind of data you have. It's pretty simple to see lesions on most MR modalities and on chronic CT imaging. However, finding and quantifying lesions on acute CT scans can be a real challenge.

You can train yourself to find lesions by looking at the scans which have already delineated, practice finding lesions, practice delineating the lesions, then comparing your lesion masks to mine and adjusting your strategy accordingly. I can't make my lesion training set openly available due to potential patient anonymity issues, but feel free to get in contact with me if you need some data to learn on.

A good knowledge of neuroanatomy (key structures, vascular territories) will definitely make it easier to see lesions, but isn't strictly necessary. Most importantly, you need to know what acute/chronic ischemic and haemorrhagic strokes look like.

I'd also recommend becoming familiar with other age-related changes such as white matter hypo/hyper intensities, atrophy, and calcification. It's important to build up an idea of what a "normal" scan looks like in your population before you can find abnormalities. For example, I work with stroke survivors so it's quite common to have huge amounts of age-related atrophy and white matter changes. These changes can look like lesions to the untrained eye.

Here are some resources for learning about stroke anatomy and clinical imaging:

1) A tool neuroradiologists use to document lesions. This isn't delineation but it will help you learn about different kinds of lesions and how to read CT scans: https://www.ed.ac.uk/clinical-sciences/edinburgh-imaging/education-teaching/short-courses/training-tools/acute-cerebral-ct-evaluation-stroke-study-access

2) This textbook has lots of useful pictures and contains a lot of general information about stroke neuroimaging. It's in the library: https://www.amazon.com/Practical-Neuroimaging-Stroke-Case-Based-Approach/dp/0750675373

3) Resources for learning anatomy:
https://headneckbrainspine.com
https://apps.apple.com/gb/app/3d-brain/id331399332

Detailed Procedure:

First off, open your scan in MRIcron and set the contrasts. I recommend adjusting the contrast lower boundary between 0 and 20 while viewing the scan to help make lesion boundaries more clear.

Find your lesion. Look for key signs of stroke (e.g. obvious hypo-intensities or bleeding, hyperdense Middle Cerebral Artery (MCA), insular ribbon sign, loss of contrast, swelling, etc.). Make sure to consider information from multiple slices before making your decision. Always check the full brain in detail. Many scans show multiple strokes.

Once you've found your lesion. Select the autoclose pen tool and draw around your lesion boundaries on a single slice. I generally start on the slice where I can see the edges most clearly then work my way up/down the scan. Once you've delineated a lesion on one slice, use the fill tool to fill the area. This is only slightly more complicated than using the Paint programme. Repeat this procedure for every slice lesions are visible on. Use the wizard hat tool (I really don't know what this is actually called) to check to make sure your lesion masks match the scan underneath.

Adjust your lesion masks if necessary. You can hold shift and click to erase a filled area or press control z to undo (but this is only allowed once). This programme is not very user friendly.

Once you're happy with your masks, chose the Draw>Smooth VOI option in the toolbar to open the smoothing window. Set the smoothing value to 5mm, threshold to 0.5, and leave the other options as they are. You can adjust these settings if you have a good reason to, but otherwise this is what I use in all my LSM studies. This is really important to do properly as all data needs to be formatted the same. Once you are sure your options are correct, press OK. You can't undo smoothing or easily smooth a saved VOI so be sure to do this right the first try.

Once the VOI is smoothed, check through the scan to make sure the lesion isn't too distorted. Once you're happy with your lesion mask, save it as PatientID_Date_Lesion and close.

Note that the lesion will likely save in .voi format. You'll need to convert it to .nii before it can be read in by SPM, but this is easy to do.

7 - Creating Reorientation Matrices

Overview: Native space lesion masks aren't that helpful for analysis. To understand affected anatomy and make group-level comparisons we need to normalise each lesion into standard space. Creating reorientation matrices is a way to make normalising more accurate. SPM generally struggles to normalise scans/lesions without a normalisation matrix (and often fails to), so this is a tedious but necessary pre-processing step.

What you'll need: MATLAB, SPM
What does this do?: Manually checks to make sure scan origin coordinates are set in the appropriate location
Why this is important: Scans which are manually reoriented will have to undergo fewer transformations during normalisation. This means that the output of the normalisation program will be more accurate. Many scans can't be normalised with out a manually created reorientation matrix

Basic Procedure:
1. In the spm12 GUI select, "Display"
2. Select the raw CT scan file for each patient
3. Set contrasts
4. Locate and click on the anterior commissure
5. Select "Set Origin" then "reorient"
6. Save the reorientation matrix for future reference

Detailed Procedure:

Open MATLAB and enter `spm fmri` into the command line. If spm does not open correctly, make sure all spm folders and subfolders are on your path. This is almost always the problem. One spm is open, click on the "Display" button within the green GUI window and navigate to the directory containing your patient's lesion files. It is a bit challenging to navigate through directories in spm. You can make this process as easy as possible my making sure that the file containing all your patient scans is the working directory in MATLAB when you initiate spm. This will prevent unnecessary file switching. To move up one directory select the ".." option. Before opening a patient file, ensure that the file contains both a scan file and a lesion file. Spm can only read .nii files so if your lesion was saved as a .voi it won't show up here. If this is the case, you will have to convert each .voi file to a .nii file before creating reorientation matrices.

Converting .niis to .vois:

To convert a .nii file to a .voi file, open MRICRON then select Draw>Convert>VOI to NII and open the .voi file you'd like to convert. This will create an equivalent .nii file in the patient directory.

Viewing Scans in SPM:

Once you have .nii scans and lesions for each patient, the next step is to view these scans in spm. Do this by selecting the “Display” option in the green spm GUI and selecting the scan file you wish to view. Do not add in the lesion file.

If the scan is a CT scan, contrasts can be adjusted by selecting “Manual Window” in a drop down window located in the lower right box in the spm viewer. This window is set to “Automatic Window” by default. Once “Manual Window” is selected a dialog box will appear within the grey spm GUI which will allow you to set the contrasts. Enter 0 70 into this box to view the scans. You can navigate through the scans by clicking and dragging the blue crosshairs to the location you wish to view.

Sometimes the spm viewer GUI throws an error when you attempt to view a new scan. This can generally be corrected by closing the GUI and reopening it. If this does not work, try to close all of spm then reopen it to view the scan.

Finding the Anterior Commissure:

The anterior commissure is a small white matter tract which connects the two hemispheres across the midline. This tract serves as the origin point [0, 0, 0] in the program which warps patient scans to standard space. The anterior commissure is located at the anterior end of the fornix in the sagittal view, between the inferior end of the anterior lateral ventricles in the axial view, and in the “moustache” of white matter fibres between the lateral ventricles in the coronal view. This video provides a useful description of how to find this tract https://www.youtube.com/watch?v=AwNJAUKLhqY.

This tract can be a bit tricky to locate on poor quality CT scans. While it is important to be as exact as possible, it is perfectly fine if the coordinates you set are not perfectly aligned with this structure. The closer the origin is to the anterior commissure, the better the normalisation will be, but most scans can still be normalised even if the anterior commissure cannot be precisely identified.

Reorienting Scans:

Set the crosshairs to the anterior commissure in all three views. Press the “Set Origin” button and then the “Reorient” button. Both buttons are located in the lower left dialog box in the spm display GUI. A box will pop up asking if you would like to save the reorientation matrix for future use. Select “Yes” and save the file in the directory containing both your patient scan and lesion file. This will allow for the lesion file to be reoriented using the same matrix. Repeat this process for all patient files. Make sure that the reorientation matrix is saved in the same file as the patient native space scan and binarized lesion file.

8 - Normalising Scans and Lesion Masks

Overview: Normalising to standard space is essential for LSM. This section describes how to use a script I wrote to automate the normalising process which can be done manually in SPM. This isn't the only way to normalise data, but it has performed well for me in the past. Importantly, this process uses age-specific CT and MR templates made for use with stroke lesion studies. If your study isn't in stroke survivors, you can adjust the script to use a different template image.

What you'll need: MATLAB, spm12, my Scan Normaliser script (https://osf.io/mv2qf/)
What does this do?: Reorients native space scans to standard space
Why this is important: You can't run group-level or anatomical analyses on scan data until it is normalised.

Basic Procedure:
1. Open MATLAB and SPM
2. Run the script "ScanNormaliser.m"
3. Watch and wait (generally 1-2 minutes per scan)

Notes:

I've written a script that sets all the parameters and automates normalisation. If you're curious about how this works, have a look at the script and the spm functions it calls. It's a lot of math but it's pretty simple to use. You'll have to set the directory paths in lines 22 – 25 to match where you've put your data and toolboxes.

This script iteratively calls the spm base functions `clinical_ctnorm` or `clinical_mrnorm`. These functions takes the inputs V (filenames of scans to normalise), les (filenames of lesions to normalise), vox (voxel size), bb (bounding box), DeleteIntermediateImages (0/1 logical), and UseStrippedTemplate (0/1 logical). I've set these inputs to the standard values I use, but feel free to change them if needed. The spm functions documentation contains more details on what each input should be, You can run this function manually in the SPM GUI, but it's much faster to loop through data automatically using a scrips (like the one I've written).

In order to run this script, you'll have to make sure the scans you want to normalise are in the correct directory structure. If you haven't reoriented these scans, you'll likely get an error. If your lesions are in .voi format, you'll get an error. It will also be important for you to either format each scan name like I do (ID_DATE_MODALITY.nii) or adjust the code to recognise the modality and filename format of your own scans. The code I've written is quite specific to my way of naming things, so I'd recommend naming your scans like I do. It's easier than making the lots of little changes which would be needed to get the script to work with other filename structures.

9 - Checking Normalisations

Overview: Unfortunately, the output from spm's normalising functions isn't always usable. This generally is due to abnormalities in brain structure (e.g. enlarged ventricles, atrophy, midline shifts, very large lesions) that mean the native space scan isn't properly warped into standard space. These mistakes are very easy to catch by visal inspection, as the normalised scan will either not be aligned to standard space or the normalised lesion mask will fall outside the boundaries of the standard brain. Below, I've detailed the procedure I use to double check all my data is properly normalised before moving on to later stages of analysis.

What you'll need: Normalised Scans, scct.nii (or another standard brain template), FSLeyes
What does this do?: check to make sure your scans have normalised well
Why this is important: Not all scans normalise well and you can only use properly normalised scans in analysis

Basic Procedure:
1. Open scct.nii in FSLeyes
2. Overlay the wScan.nii and bswLesion.nii file
3. Check to make sure they match well
4. Upload Good scans to the scan database

Detailed Procedure:

I generally use FSLeyes to view and compare scans. You can use another NiftiViewer, but FSLeyes is best because you can save a lot of time by scripting the process in UNIX. If you're not familiar with FSLeyes, there's lots of good video walkthroughs showing you how to use the program (e.g. https://fsl.fmrib.ox.ac.uk/fslcourse/lectures/practicals/intro1/index.html).

To check each normalisation, you'll need to open a standard space template (e.g. scct.nii) in FSLeyes and overlay each normalised scan with it's accompanying normalised lesion. I recommend setting the contrasts for each of the scan files so you can see tissue differences clearly and changing the colour of the lesion overlay so that it stands out. Next, you can use the eye tool and crosshairs tool to check that the scans overlay well. I recommend placing the crosshair at key points within the standard space brain (e.g. skull/brain boundry, ventricle edges) and turning the normalised data overlay on/off to ensure that these points line up reasonably well between the standard brain and the normalised scan. I generally work my way through these steps:

1. Check to make sure the normalised scan relatively matches up with the template. I do this by placing the crosshairs on the normalised scan, then

turning the scan off to see whether the crosshair point matches the template. Check multiple places around the skull and lateral ventricles for alignment.

2. Repeat this process with the lesion. If the lesion extends into the template skull, doesn't match well with the anatomy impacted on the native space scan, or is too big/small it cannot be used. Only exclude lesions if you are confident they are bad. The most common problem will be that large lesions are in or outside the skull of the template.
3. If a lesion is good, move the normalised data folder to a "keep" location. If the scan is bad, move it to a "do not use" location. In most cases, scans which don't normalise well can't be corrected so there will be some inevitable data loss here.

You may notice that these steps will take a lot of time to run through (especially if you have lots of scans to normalise). I speed up this process by using a UNIX script to automatically open FSLeyes, add the relevant files, and set the contrasts/colours automatically. This is a simple process, but it saves a surprising amount of time. Here's an example of a script I use:

```
for i in *; do echo ${i};fsleyes /Applications/spm12/toolbox/Clinical/scct.nii -dr
2000 4500 ${i}/wp*CT*nii -dr 0 70 ${i}/bws*nii -a 40 -dr 0 70 -cm Blue; done
```

For people who aren't familiar with UNIX, UNIX is code run from your computer's terminal. It's really useful for automating processes that would normally take a lot of clicking, opening files, moving things around etc. FSLeyes (and FSL) plays well with UNIX, so you can automate most pipelines that require doing the same thing more than once. To open your terminal, find the terminal in your computer's applications and open. You can't use the mouse in the terminal (only arrow keys and keyboard commands). To start this script, you must first navigate to the directory containing your normalised scan files. To do this type cd DIRECTORY PATH into your terminal. If it works, nothing should appear to happen. Next, type ls. If you're in the right place, all the directories containing your normalised scans should be listed. To run my script you'll need to change "`Applications/spm12/toolbox/Clinical/scct.nii`" To whatever directory contains your standard space template file.

Here's a step-by-step translation of my code:

`for i in *;` - This is UNIX syntax for looping through all the files in the current directory

`echo ${i};` - Tells the terminal to print the name of the ith directory in the file

`fsleyes /Applications/spm12/toolbox/Clinical/scct.nii` – Opens the standard space template file in FSLeyes

`-dr 2000 4500 -` sets the standard template contrasts to 2000 and 4500. This will be different for different templates

`${i}/wp*CT*nii -` open the normalised scan file from the ith directory in FSLeyes. You may need to change the index (wp*CT*nii) to something that will catch all your scan files if you've formatted your names differently than I do

`-dr 0 70 -` set the normalised scan contrasts to 0 and 70
`${i}/bws*nii -` opens the binarized lesion file from the ith directory

`-a 40 -` sets the lesion file opacity to 40%

`-dr 0 70 -` sets the lesion file contrasts to 0 and 70

`-cm Blue; -` sets the lesion file colour map to Blue (pretty)

`Done -` ends the loop (this process will be repeated for every file you have, to move to the next directory, just close FSLeyes)

What to do with bad normalisations:

You'll notice that not all data normalises well. Sometimes the lesions are just a bit outside of the skull, and other times the scans are completely misaligned. Bad normalisations are generally completely unusable, so there's no point keeping them. You can try to fix normalisations by re-making re-orientation matrices, adjusting normalisation parameters, and re-running normalisation. This rarely makes a difference, but it can sometimes save a scan or two.

10 - Reslicing Lesions

Overview: Once you have successfully normalised your data, there are still a few steps you need to go through before analysing. First, it's generally important to ensure that all your lesion files are exactly the same dimensions. This can be done by reslicing lesions in SPM. This is a fairy quick and easy process which will prevent errors later on in the analysis pipeline. By now I'm assuming you know the basics of SPM, MRIcron, and MATLAB. These instructions will be a bit higher-level, but you should be able to follow them if you're familiar with these programmes

What you'll need: MATLAB, spm12, lesions to be resliced
What does this do?: Reslices your lesions so they all have the exact same number of slices.
Why this is important: You'll need to do this before you create any lesion overlays or calculate anatomy statistics

Instructions:

1. Open the spm GUI
2. Select the Corregister (reslice) option from the dropdown menu in the top left corner
3. Set "template defining space" to the ROI (.nii file) you want all yours to match. I generally use any area from the atlas ROI files in my lesion_toolbox (available on OSF) atlas directory (e.g "HOC_Angular_Gyrus_L.nii"). You can use any file you want if you just want to create an overlap, but if you want to run anatomy statistics you'll have to choose a ROI from one of the template atlas ROIs.
4. Set "images to reslice" too all the normalised lesions you want to reslice.
5. Press run
6. Check your output. All resliced files should now begin with the letter "r". Only use resliced files for VLSM/anatomy/overlay analyses

11 - Creating Lesion Overlays

Overview: It is surprisingly important to create lesion overlays at an early stage of the analysis pipeline. I generally use lesion overlays to evaluate whether my data is even good enough to think about running LSM on. It's also important to include a lesion overlay figure in any LSM paper, as your readers can use it to see which voxels you've actually included in your analysis and get an idea of your voxel-wise statistical power. There are several different ways to do this.

What you'll need: MRIcron (or SPM12)
What does this do?: Turns your lesions into overlap visualisations
Why this is important: Overlap visualisations help evaluate the quality of the analysis you're able to run and are important to include in LSM papers.

Instructions:
1. Open a standard space template file in MRIcron and set the contrasts.
2. Select Draw>Statistics>Overlay
3. Select all the .nii files you want to overlay and press ok
4. Name and save your file
5. Choose colour scheme. I recommend NIH.
6. Set contrasts for the overlap statistic (minimum above 0.1 and set your maximum so that no covered areas are left blank)
7. Use the Window>Multislice option to make visualisations.
    1. use the View>Slices option to choose which slices to display
    2. use the View>Overslice options to change the overlap

Notes:

You can also do this in SPM12 in the Batch>SPM>Util>ImageCalc GUI. Lesion overlaps are automatically generated by NiiStat during VLSM. However, I find it's generally best to look at an overlay before spending time running a LSM to decide if it's even worth analysing the data.

12 - Running VLSM

Overview: This step is probably the main reason that you're here. Running LSM itself is surprisingly simple. If this section doesn't provide you with enough information there's lots available on the NiiStat webpage.

What you'll need: MATLAB, NiiStat, Excel datafile, lesion directory of normalised scans
What does this do?: It runs VLSM
Why this is important: it is important if you'd like to run VLSM

VLSM is fairly complicated. It's important to understand exactly what each option is doing and why you've selected it. VLSM can be very problematic because it is comparatively easy to run, but rather difficult to fully understand. This means that lots of people make mistakes when designing their studies and organising their data that can cause very serious issues in their results, but don't know enough to notice these problems. A good rule of thumb is to never do/change anything unless you know exactly why you're doing it and why you're doing it that way. You can always look to previous VLSM studies to see what other people have done and why.

Before you run VLSM you should:

1. **Read papers:**
    1. https://www.sciencedirect.com/science/article/pii/S0028393217303962 (absolutely necessary to read and understand this one. Use it to determine whether your data is good for VLSM)
    2. VLSM versus VBM: https://www.sciencedirect.com/science/article/pii/S2213158212000046

2. **Ask yourself: Does my data suit a VLSM analysis?**
    1. **Do you have enough patients?** (I'd say there's no point running a VLSM analysis on less than 30 people. It's also important to think about what proportion of your sample is actually impaired. If you have 100 people but only 2 of them are impaired, you're not going to get an interesting results)
    2. **Is there sufficient overlap/coverage in your patient's lesion overlays?** (Always look at your overlays before deciding if a VLSM is appropriate. You may have enough patients but if your lesions are too spread out, you won't be able to conduct statistical tests with any power
    3. **Is there sufficient variance in your behavioural data?** (You should not analyse non-normal behavioural data using traditional LSM. Instead, use non-parametric analyses or binarise your data)

4. **Is the construct you're trying to map likely multifocal?** (VLSM has difficulty finding bilateral/multifocal rois, meaning that a null result may not be informative and a positive result may be incomplete)

3. **If you pass all these checks (or know enough to disagree with this general guidance), you need to clean up your data.**
    1. Set up data directory
        1. copy all relevant patient binarized normalised lesion files into a single directory
        2. You can use my script VLSMScanMatcher.m to help match scan file names to IDs.
        3. Make sure the number of patients in your patient list is the same as the number of scans in your directory

    2. Convert Nii files to Mat (optional)
        1. I generally do this as it allows NiiStat to regress out lesion volume (to control for stroke severity) in LSM analysis
        2. Use the NiiStat function `nii_nii2mat(niinames, modalityIndex, disknames, roi)` to convert a list of nii files to mat. Documentation for this function is available here: https://github.com/neurolabusc/NiiStat/blob/master/nii_nii2mat.m . I've also uploaded a short script I use for this on my OSF page in the Misc Scripts folder.
        3. Run the script and wait for the conversions to complete.

    3. Make an excel file
        1. Excel files must contain the patient lesion file name (exact match) and corresponding behavioural variable. Double check your file extensions match the format of lesions you want to input (e.g. .nii or .mat)
        2. NiiStat reads low scores as impairment, so keep this in mind
        3. Your excel sheet (not the workbook, the sheet) must be named NiiStat. If not the programme will fail.
        4. Unless you really know what you're doing, only use one behavioural variable
        5. Reslice all lesions before using. Make sure the lesion name listed in your excel file corresponds to the resliced lesion, not the native space one.

4. **Download and Run NiiStat**
    1. Link: https://www.nitrc.org/projects/niistat/

2. Download, open MATLAB, add the genpath to your path, and open the GUI by typing NiiStatGUI
3. If a GUI pops up, you've done it right

5. Set up VLSM Analysis

1. **Set data directory:** enter/select the filepath of the directory containing your binarized, resliced lesion masks
2. **Set output directory:** enter/select the filepath of wherever you'd like your results to be saved
3. **Select excel file:** enter/select the filepath of your excel file. This file must be inside your data directory and must contain a sheet named NiiStat
4. **Name for Results Folder:** change this to whatever you want your results folder to be named
5. **Minimum overlap:** set the minimum overlap of voxels to consider. Do your research to choose a specific value and know why you've chosen it. Smaller values lead to more comparisons but less power.
6. **Corrections:** Again do your research and know which one you're going to choose and why. FDR is commonly used and less conservative. Bonferroni is highly conservative (generally too conservative), but I usually use it because I generally include a lot more patients than normal (e.g. >400). Permutation based corrections are a powerful tool but take up a ton of computing power. Permutation corrections can take hours or days to complete so be careful. To use FDR you need to click another option then click back to the FDR option otherwise Bonferroni is used as default (fault in the GUI). I'd recommend reading what other studies have done and deciding accordingly.
7. **Corrected P:** use 0.05 unless you have a good reason not to
8. **ATLAS tab:** choose your atlas. I generally use Voxel-wise comparisons (upper right corner – will turn grey when selected). Unless you have a good reason not to, you should probably use this one too. You can use the anatomy toolbox codes to get atlas results later. I don't recommend ROI-based analyses as they effectively assume that each voxel within an ROI is involved in the same function (which generally isn't entirely correct).
9. **MODALITY tab:** choose LESION unless you're not doing lesion analysis (which would be weird, given that you're reading this guide).
10. **SPECIAL tab:** If you want to choose an of these options (and have a reason for doing so) go ahead. I generally regress for lesion volume, as it controls for stroke severity related effects. In my experience,

reviewers will ask for you to do this. Notably, your files must be in .mat format for this to work.

11. **SPECIAL + :** To be honest, I don't know why this tab exists. Best to ignore it.

6. Run VLSM

1. Once you've set all your presents, press the GO button and keep an eye on your command window. It will report how many voxels are being considered, the correction thresholds being used, z-thresholds, z-range and number of significant voxels
2. This can take quite a long time. 500 scans with 1 binary variable and FDR corrections takes about 15 minutes but the same analysis with permutation corrections (>2000) could take days.

7. Visualise/Interpret Results

1. Open the thresholded z output file over a template standard space scan in MRIcron and set contrasts. Use the z-threshold and maximum z value reported in the .txt results file to set the stat maximum/minimum values. Check your z-stat range to determine whether your threshold needs to be positive or negative
2. Do your results "look" right? Are they in the correct hemisphere? Do they match what you expected based on visual analysis of your lesion overlay?

13 - Using the Anatomy Toolkit

Overview: The anatomy toolkit is a tool I use to link voxels in LSM output or lesion files to anatomical names defined in standard atlases. These scripts were originally written by Celine Gillebert and Dante Mantini, but I was responsible for adapting these original scripts into their current versions available on my OSF page. If you do end up using them in any published analysis, be sure to give all of us a mention in your acknowledgements.

What you'll need: MATLAB, SPM12, lesion_toolbox scripts, atlas ROIs, binarized lesion maps (resliced)
What does this do?: Calculates anatomy statistics for binarized lesion masks
Why this is important: This is how you can actually find out which regions are damaged for VLSM results and for individual patient lesion masks

Basic Procedure:
1. download the lesion_toolbox folder from my OSF page (LINK)
2. Open the Atlas_Output_V2 script
3. Modify the file paths in lines 26-67 to match your data directories, atlas locations, and script folders
4. Enter the file paths for the atlases you want to use in lines 64-67 (see atlases folder for details)
5. Run the code
6. Get anatomy details from the Excel files which the code outputs. There should be individual files for each patient as well as summary fraction/extension* files

*Fraction = proportion of ROI damaged. Extension = proportion of lesion in ROI

Notes: Keep an eye on the command window. This code will let you know what it's doing and when it runs into issues.

This code will print out an excel sheet for each atlas used for each lesion mask included as well as summary excel sheets for each atlas used. I generally use this script to make tables summarising the anatomy impacted by each significant voxel cluster (see Moore et al., 2021; Moore & Demeyere, Under Review).

14 - Additional Tools to Help Interpret VLSM Results

Overview: While the lesion_toolbox scripts do yield most of the results worth reporting in any LSM manuscript, there's a few extra tools I often use to help report and summarise my results. I've included a brief description of these tools and several different approaches for secondary LSM results analysis below.

What you'll need: MATLAB, VLSM results, scripts available on OSF
What does this do?: Helps you make sense of VLSM results
Why this is important: Raw VLSM results are a bit difficult to interpret. Here are a couple ways you can make sense of them

Option One: Run Basic Anatomy Statistics

To determine which anatomical areas are significantly associated with your behavioural variable, run the thresholded z statistic results file (usually prefixed with "thresh". Through the lesion_toolbox scripts (described in part three). You can compare your lesion to both grey and white matter atlases, and report these statistics in your manuscript.

Option Two: Anatomical Regressions

VLSM anatomical analysis yields which areas are significantly associated with a behavioural impairment, but doesn't report how much variance is explained by each implicated ROI. You can determine this by conducting a regression with each implicated area's fraction and lesion volume as covariates. This will tell you how much variance is really explained by each ROI and will yield a nice equation for predicting impairment.

Be conscious that this is inherently "double dipping". However, you can apply regression models to new patients, or new behavioural variables to test if they're generalisable and specific to the deficit you're looking at.

Option Three: Peak Analysis

It's generally not helpful to say something like "impairment X was associated with damage to the left opercular cortex". The opercular cortex (as an example) is a large region with multiple different subdivisions. Different atlases define these areas in different ways and if you really want your readers to know exactly what you found, you need to report the MNI coordinates of your areas.

You can use my script MNIcoordreader to find the global z-value maximum and print out it's MNI cords. You can use the View>MNI coordinates function on a loaded template (File>Load Template) in MRIcron to check which areas these peaks fall into. See this paper for a really excellent example really detailed, useful peak info (https://www.frontiersin.org/articles/10.3389/fnhum.2012.00230) or any of my VLSM analyses to see simpler examples.

Option Four: Backwards Inferences

Not all data is well suited to VLSM and there are lots of other analyses you can do on lesion data. One cool option is evaluating other people's findings by seeing what proportion of patients with damage to a specific ROI actually have impairment. For example, a lot of past research has suggested that the right angular gyrus underlies neglect. I could evaluate this theory by taking all patients with damage to the R angular gyrus, seeing how many of them actually have neglect, or conducting a binary regression to see how R angular gyrus damage predicts neglect impairment.

This approach is not perfect. ROIs are not homogenous and effects driven by subregions of ROIs won't be picked up well in this analysis. However, it's something cool to play around with.

Option Five: Finding the "centre of mass" of lesion clusters

Sometimes it's helpful to identify the "centre of mass" of thresholded voxel clusters. I've written a couple scripts to do this (SCRIPT NAMES). Feel free to play around with these if this is something you think might help your analysis. Be careful when using these scripts with multifocal lesion masks as this will yield weird (and not very informative) results.

14 - How to write up LSM results

Overview: It's extremely important to communicate LSM findings clearly and concisely. If you're not sure how to do this, I'd recommend finding a couple past LSM papers that you think are well written, and mimic the methods/results structure in your own manuscript. I've included some checklists and examples from my recent LSM paper (Moore et al., 2021) to help you get an idea of how to report what you've done.

Key information to report in Participants Section:

1) All the basic patient info you would include in any study (demographics, consent, inclusion/exclusion, etc)
2) When neuroimaging and behavioural data was collected relative to stroke event
3) Basic stroke descriptives (Lesion hemisphere, stroke type, stroke territory, lesion size, etc.)

Sample Demographics Chart (Moore et al., 2021):

Table 1. A breakdown of the clinical characteristics of patients within each VLSM analysis group. Test Date reports the interval between stroke and behavioural testing in days. Scan Date reports the interval between stroke and neuroimaging data collection in days. Lesion sizes are reported in cm^3. Standard deviations are provided in parentheses. Visual field deficits as classified by the OCS are provided. L = Left, R = Right, B = Bilateral, Ego = Egocentric, Allo = Allocentric.

	Demographics						Lesion Details				Behaviour	
	N	Age	Female	L Handed	Test Data	Scan Date	Size	L	R	B	Total	Ego Score
Left Ego	58	72.9 (11.6)	55.2%	15.4%	6.2 (6.5)	1.4 (3.24)	5.9 (5.8)	6	48	4	26.8 (11.4)	0.72 (0.56)
Left Allo	27	72.0 (13.8)	48.1%	10.5%	4.5 (6.2)	2.6 (4.0)	5.5 (7.2)	8	17	2	32.4 (14.5)	0 (0.1)
Left Ego & Allo	42	74.5 (10.9)	38.1%	0.0%	5.6 (5.7)	1.4 (2.3)	10.9 (11.1)	2	39	1	17.4 (11.3)	1.30 (0.63)

Key information to report in Lesion Data section is:

1) Neuroimaging modality used (e.g. CT/MR)
2) How delineation was performed (including smoothing)
3) Reorientation procedure

Sample Lesion Data Section (from Moore et al., 2021):

The extent and location of patient lesions was quantified using clinical CT (n = 376) and MR (61 T2, 3 T1, 6 FLAIR) whole-brain scans obtained as a component of routine post-stroke clinical imaging. Patient lesions were manually delineated on native space scans using MRIcron (McCausland Centre for Brain Imaging, Columbia, SC, USA) by investigators who were blind to behavioural results (Varjacic et al., 2018). All lesion masks were smoothed at 5 mm full width at half maximum in the z-direction and binarized using a 0.5 threshold using built-in MRIcron smoothing functions. Smoothing is a standard lesion pre-processing step which helps prevent minor variations in delineation user input from impacting analysis (de Haan and Karnath, 2018). These scans and lesion masks were then reoriented to the anterior commissure and warped into 1 × 1 × 1 mm stereotaxic space using the Statistical Parametric Mapping 12 and Clinical Toolbox (Rorden et al., 2012) functions. All normalised scans and lesions were visually inspected for quality before conducting lesion mapping analyses. This lesion preparation process represents a standard analysis pathway which has been used in a number of previous VLSM investigations (e.g. Varjačić et al., 2018).

Key information to include in Statistical Analysis Section:

1) How many LSM analyses were conducted?
2) Was the analysis voxel-wise or ROI-based?
3) What statistical tests were used? (depends on whether data in binary or continuous)
4) Minimum overlap voxel inclusion threshold
5) How many voxels were tested
6) How statistical corrections were performed
7) Was lesion volume controlled for and if not, why?
8) Any post-processing (e.g. how was anatomy determined?)

Sample Statistical Analysis Section (from Moore et al., 2021):

Four VLSM analyses were conducted to determine the neural correlates egocentric and allocentric neglect within patients exhibiting left and right visuospatial neglect. These VLSM analyses were conducted on a theory-blind voxel-wise basis using the MATLAB package NiiStat (https://github.com/neurolabusc/NiiStat). Given that continuous impairment severity metrics (centre of cancellation or allocentric proportional scores) were employed, this software employed one-tailed pooled-variance t-tests to evaluate voxel significance. Only voxels which were lesioned in a minimum of 10 patients were considered (n = 589,216, Fig. 2). These analyses employed a highly conservative Bonferroni correction (corrected

alpha = 8.49×10^{-8}, z-cut = 5.23) and controlled for lesion volume. This conservative analysis approach was employed to harness this study's extremely large sample size and testing space to prioritize specificity over sensitivity (Sperber and Karnath, 2017). Specifically, this analysis aims to locate "core", highly significant lesion sites rather than peripheral areas which are less strongly associated with neglect impairment. Lesion anatomy was evaluated versus the Harvard-Oxford Cortical (Desikan et al., 2006) and John's Hopkins University White Matter (Mori et al., 2005; Wakana et al., 2007) atlases.

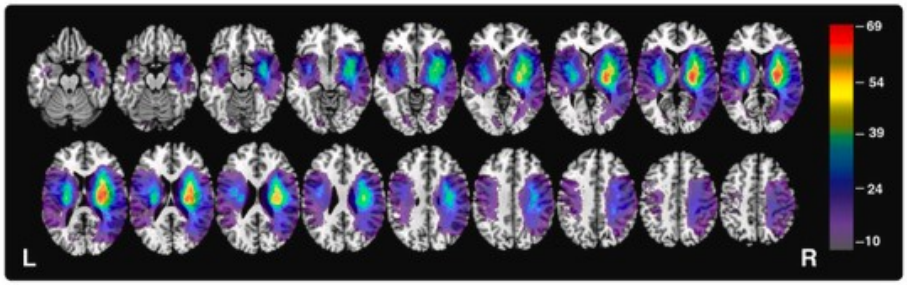
Finally, the voxel maps produced by each VLSM analysis were compared to analyse the degree of overlap between voxels associated with different neglect impairments. Specifically, voxel maps for similarly-lateralised egocentric and allocentric neglect were compared to confirm whether these conditions can be dissociated at an anatomical level. Next, the voxel maps associated with left hemisphere neglect impairments were inverted to overlay with their right-hemisphere homologues in order to quantify the degree of similarity between the correlates of right and left hemisphere neglect. All analysis output files and behavioural data are openly available on the Open Science Framework (Foster and Deardorff, 2017) (https://osf.io/vf9ew/). All additional data is available upon request.

Key information to include in Results Section:

1) Basic descriptive (lesion size, maximum overlay, behavioural score ranges/deviations)
2) Lesion overlay (to show readers where you could and couldn't test)
3) How many significant voxels (in each analysis)
4) Peak voxel-wise z-score value and location (e.g. MNI cords)
5) Descriptive anatomy of significant voxel cluster (summary in text, details in chart)

Sample Results Section (from Moore et al., 2021):

Fig. 2 presents the lesion overlay for all 446 participants within the voxels included in VLSM analysis. The highest lesion overlap (n = 69) was present within the MCA territory. Patients with neglect (defined as any impairment on the ego and/or allocentric measures, n = 197) were found to have significantly larger lesions than participants without neglect (mean volume 51.57 cm^3 versus 24.12 respectively, $t\ (445) = 14.793$, $p < 0.001$). A one-way ANOVA analysis revealed a significant relationship between neglect type and lesion volume ($F\ (5,191) = 4.026$, $p = 0.002$). (…)



Download : Download high-res image (724KB)
Download : Download full-size image

Fig. 2. Lesion overlay for the total sample of 446 participants. Colour represents number of patient lesions overlapping within each region. Only regions with a minimum overlap of 10 are visualised (MNI z coordinates -44 – 66). The voxels highlighted in this visualisation are tested in all reported VLSM analyses. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

VLSM analysis of left egocentric neglect yielded 11,526 significant voxels with the peak z-score (z = 7.392) centred within the left parietal operculum (MNI 39–34 19). This significant voxel cluster impacted a number of left temporo-pareital cortical areas including the supramarginal gyrus, and planum temporale, lateral occipital cortex (superior division). Voxels within underlying white mater tracts including the internal capsule (posterior and retrolenticular parts), tapetum, and posterior corona radiata were also significantly associated with left egocentric neglect impairment. Full anatomical statistics of significant voxels are reported in Table 2.

Table 2. Detailed anatomical descriptions of the significant voxel clusters identified in each individual VLSM analysis. Starred ROIs contain the peak z-values for each VLSM test. Full anatomical descriptions for each voxel cluster are available on the Open Science Framework. Fraction represents the proportion of each ROI covered by each significant z-statistic map. All anatomical areas are defined based on the Harvard-Oxford Cortical Atlas (HAROX) and the Johns Hopkins University White Matter Atlas (JHU). Hem = hemisphere (Left/Right). Nsig = number of significant voxels within each ROI.

Left Egocentric	Hem.	Nsig	Fraction	Atlas
Supramarginal Gyrus (Posterior Division)	R	1698	11.37%	HAROX
Planum Temporale	R	1087	20.43%	HAROX
Parietal Operculum Cortex*	R	972	17.94%	HAROX
Lateral Occipital Cortex (Superior Division)	R	946	2.01%	HAROX
Supramarginal Gyrus (Anterior Division)	R	696	8.77%	HAROX
Internal Capsule (Posterior Limb)	R	633	13.62%	JHU
Tapetum	R	552	62.80%	JHU
Internal Capsule (Retrolenticular Part)	R	408	12.42%	JHU
Heschl's Gyrus	R	257	7.90%	HAROX
Corona Radiata (Posterior)	R	237	4.69%	JHU
Angular Gyrus	R	209	1.44%	HAROX

Again, there's lots of different ways to run analysis and report data. These examples are included as a beginner's guide to what an informative (and publishable) LSM manuscript might look like.

15 – Conclusion

I hope you've found this guide to be helpful. If you have any questions or have an idea for what else might be helpful to include, feel free to get in contact. I hope to keep expanding and improving this guide over time as I learn more about LSM. I'm beginning to move towards network-based and multivariate LSM approaches in my own work, and will add instructions for these analyses to this guide sometime in the future. Don't hesitate to get in touch with me if you have any questions. I think the scientific community benefits from more open sharing of knowledge, so I'm happy to hear any suggestions for improvement and ideas for contributions to this guide.

Appendix 1: Software Download Links

Relevant Software:

1. MATLAB (https://uk.mathworks.com/downloads/)
2. MRIcron (https://www.nitrc.org/projects/mricron)
3. FSL / FSLeyes (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLeyes)
4. SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/download/)
5. Clinical Toolbox for SPM (https://www.nitrc.org/projects/clinicaltbx/)
6. NiiStat (https://www.nitrc.org/projects/niistat/)

Download and Setup Notes:

Make sure to carefully follow all the instructions on the relevant software download website. If any program is downloaded in the wrong location (or not included in the filepath), errors will occur. NiiStat is not great at letting you know what has gone wrong when something has gone wrong, including when it can't access spm functions.

Appendix 2: Common NiiStat MATLAB Errors

Overview: If you're not comfortable using MATLAB, working with the scripts and programmes described in this guide is probably going to be hard. To help out, I've listed some common MATLAB errors (in red) and described how to fix them below. This list isn't comprehensive, but it should help out with some of the most likely things to cause issues. If you're struggling with anything else, google is your friend. 99% of learning to code is just googling questions until you remember the answers. If things are going wrong, it's not because you're dumb it's because coding is hard and it takes a while to get used to the new way of thinking.

When I'm debugging code, I ask myself several questions to make things easier. First, I try to find the line that's causing the problem. This is generally listed in the error output. Once you find the line which is making the error, simple errors are easy to spot. I check whether it's a typo (e.g. wrong formula, extra comma, etc.) and whether all variables look like what I'm expecting them to (e.g. right data type, size, and content). If a variable is wrong, I try to figure out what line of code made this error and repeat the process described above. There's lots of different ways to do this. This is just what works for me.

This list is by no means comprehensive. I'm planning to expand it by new adding errors and fixes whenever I encounter them in analyses.

General Errors:

```
Unrecognized function or variable NIISTAT/SPM FUNCTION NAME …
```

This is generally caused by one or more of the needed packages or directories not being added to the path. Double check the file paths you've entered and re-add all niistat and/or spm files (and sub-files) to the path and try again.

```
'spm' is not found in the current folder or on the MATLAB path, but exists
in:
    /Applications/spm12

Change the MATLAB current folder or add its folder to the MATLAB path.
```

This one is pretty self-explanatory. Add SPM (folders and subfolders) to your path either by using the function addpath() or using MATLAB GUI.

```
ANY ERROR WHILST RUNNING SPM NORMALISATION FUNCTIONS
```

Not all scans can be normalised, but most normalising errors can be corrected. Make sure your scan/lesion files match up in a nifti viewer (e.g. MRIcron) and that the

reorientation matrix file is saved in the correct directory (e.g. same folder as the scan/lesion files). Check to make sure you're using the right SPM clinical toolbox template for your imaging modality. This should automatically be set by my scripts, but it won't work if you don't have the modality in your file name.

If this doesn't fix it. Try normalising a different scan/lesion pair. If the error you had before isn't happening with new data, the original scan/lesion pair proabably can't be normalised. A real normalisation error should only impact a few scans (e.g. about 10 of my 500 couldn't be normalised). If it's happening for every scan, there's something wrong with your code, data, or analysis pipeline. SPM errors are generally pretty good at telling you what's gone wrong so read them carefully.

NiiStat Errors:

```
Unable to perform assignment because the left and right sides have a
different number of elements.

Error in nii_stat_core (line 105)
    sumImg(good_idx(:)) = sum (les, 1);

Error in NiiStat>processExcelSub (line 1075)
            nii_stat_core(les, beh, beh_names,hdr, pThresh, numPermute,
logicalMask,statname, les_names, hdrTFCE, nuisance);

Error in NiiStat (line 261)
           processExcelSub(designMat, roiIndex, modalityIndex,numPermute,
pThresh, minOverlap, regressBehav, maskName,
                GrayMatterConnectivityOnly, deSkew, customROI, doTFCE,
reportROIvalues, xlsname, kROIs, doSVM, doVoxReduce, hemiKey,
        interhemi, statname,GUI, nuisanceMat); %%GY

Error in NiiStatGUI/GOButtonPushed (line 448)
            NiiStat;

Error                                                              using
matlab.ui.control.internal.controller.ComponentController/executeUserCallba
ck (line 410)
Error while evaluating Button PrivateButtonPushedFcn.
```

If you get this error whilst running NiiStat, there's usually an issue with how you've resliced your lesion files. The lesion files you are trying to analyse are usually a) not all the same sizes or b) not binarized. To correct this, double check that all the files are the same dimensions and that the .nii files only contain 0s and 1s. If more numbers are present, you'll need to threshold the images so that they are binary.

Appendix 3: Sample Procedure for Downloading DICOM files from an NHS Database

Note from Margaret: This is the guide I wrote up to help my lab members download raw scan data from the John Radcliffe Hospital in Oxford UK. I'm not sure if this will be helpful to anyone outside of Oxford, but I wrote it so I'm including it here. It is in its rough draft (very informal) form.

What you'll need: Access to the Research Office in the John Radcliffe, PACS login information, Lots and lots of patience.
What does this do?: Pulls patient data from the hospital database, anonymises, and downloads so that we can use it for research
Why this is important: We need brain data to learn about brain things.
Basic Procedure:

1. Login to both the PACS and research computer
2. Find patients in PACS
3. Send relevant data to ClearCanvas workstation
4. Delete dose files and anonymise
5. Upload scans to a removable hard drive

Note: This section is adapted and reformatted from Jacob Levenstein's (jacob.levenstein@ndm.ox.ac.uk) guide which was adapted from an original guide written by Kevin Bowden (no idea who this is, but credit where credit is due).

Logging Into Computers

This process involves using two computers, both are located in the Stroke office on the 7th floor. The computer on the left is currently a university computer (donated by CNC) and the computer to the right is an OUH machine. First, make sure the (currently red) ethernet cable from the research computer is plugged into the wall to the right. These two ethernet ports are on the hospital IP. If needed, please unplug the grey cable that might be on the right slot and is connected to a phone. Do not unplug the OUH computer's ethernet cable, as both the Research and OUH computer need to be plugged in the OUH IP.

To log into the hospital computer (Right) use your OUH login credentials or ask Rachel Teal to log you in. To login to the university computer (Left) use the user profile "Research" and password "research".

Locating Clinical Imaging Series

Using the hospital computer, Launch Insight Web by selecting the icon on the desktop and sign in using your PACS credentials. Open the Patient Explorer by selecting the icon shown below in the top left hand side of the screen.

The patient explorer window (shown above) will open. Select the “patient search” option in the top left hand corner and enter your patient’s first and last name in the “surname / forename” boxes and press search. When you search a patient name, all patients with that name will be displayed in a list below the search box. Use your patient’s date of birth to check that you have selected the right patient. Be sure only to open patient records of patients who have consented to be in our study as this database contains confidential, personal, and anonymised medical data.

When you have found the correct patients, scroll through the scan list (marked in a green box) to select relevant files. As of now, we’re interested in all CT Head and MR Head files for each patient. Once you have found a relevant file, drag it to the “TABLET COGNITIVE” folder which will be displayed in the menu containing the “patient search” folder (marked in red).

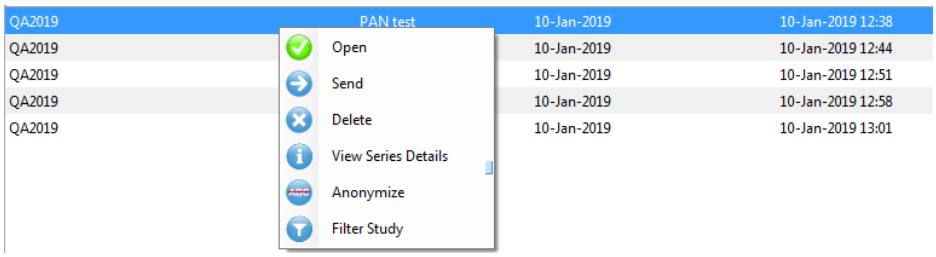
You will then be prompted to enter patient info (see below). Enter the patient’s study ID underscore temp (e.g. P5211_temp) in both the forename and surname box. Once you have dragged files into this folder, the system will automatically move them into the ClearCanvas workspace.

Anonymising and deleting Dose Pages

You will now switch over to the other computer (the university computer) and launch ClearCanvas by selecting the program’s desktop icon.

In ClearCanvas, select “My Studies”. Patient Data which you have downloaded to the TABLET COGNITIVE file will appear in the window marked in blue. This can take some time, so be patient.

When a relevant study appears, right hand click on it and select “view series details”.



We will now delete the DOSE page. Note that not every scan (especially MR scans) will have a dose page, but if there is one it is extremely important for us to delete it before taking this data. The DOSE page contains each patient's name and NHS info. It is a breach of our ethics to take this data out of the hospital. The DOSE page will generally be labelled "999" but it can be labelled other things as well. You can open up each scan session to check whether it contains patient information. Once you have found the DOSE page, right click it and select "delete".

Next, we will need to anonymise each scan. To do this right click on each examination, select "Anonymize" and fill in the following:

Patient ID: CNC ID (e.g., P5001)
Patient's Name: CNC ID (e.g., P5001)
Date of Birth: This will auto change day and month, but keep original year. Change this to today's date to avoid future error…
Study Description: Leave this as original
Accession Number: Make this a random number, try to not reproduce the same pattern (e.g., 78324923721)
Study Date: IMPORTANT, make sure you change this back to the original Study Date. This can be found under the study date column in the viewer. You can also check these two match once you select okay on the Anonymize Study GUI, as each will be in the clearcanvas viewer.

Transferring Anonymised Files to a Hard-Drive

To find where the data on clearcanvas is located, you need to first right-click on a series and select open. Then you can go to Tools, Utilities, Locate on Disk. This should point you to the filestore.

You can now transfer the scans (which are back one directory in filestore) to an encrypted hard-drive. The total amount of files in filestore should be the same amount of exams on my studies in Clear Canvas. Please note, for CNC scans where we generate a anonymized version, we will need to delete the originally pulled scan before transferring (please see this process below in the following step).

If you are moving the location of these filestore file to somewhere else on the computer, please *copy paste* them instead of dragging them. There is a chance that dragging the files might cause an error with the link between the file locations and the data in clearcanvas.

NOTE: after you transfer (or copy-paste) the files from the filestore, PLEASE DO NOT delete them from filestore.

Deleting files from OUH computer and Exit procedure

Deleting files on the university computer (ClearCanvas):

From ClearCanvas, you should only ever remove files using the right click, delete, or using the X button.

On the Hospital computer (Insight):

Within Insight, you can remove files from your examination directory by using right click, delete. Only do this after the data has been sent to ClearCanvas.

Once you have finished, please ensure that you have logged off of both computers and shut them down. Please also unplug the OUH computer's ethernet cable from the wall socket and put back the cable that was originally plugged in.

Other potentially helpful notes:

1. If you accidently deleted a file from the filestore or move them out, you might need to run the following from clearcanvas: Tools, Re-index FileStore. This will update all of the files saved in clearcanvas to the file store.
2. The computer on the left is currently a university computer (donated by CNC) and will eventually need to be updated with Windows 10 by someone at on the University side, who will then be responsible for looking after this. For ClearCanvas, the computer must be a static IP and at the moment, the university computer is satisfying this criterion. It is possible to make the OUH computer (on the right) static, but that will require everyone to log in using OUH credentials.

Conflicts of Interest:

The authors report no conflicts of interest

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