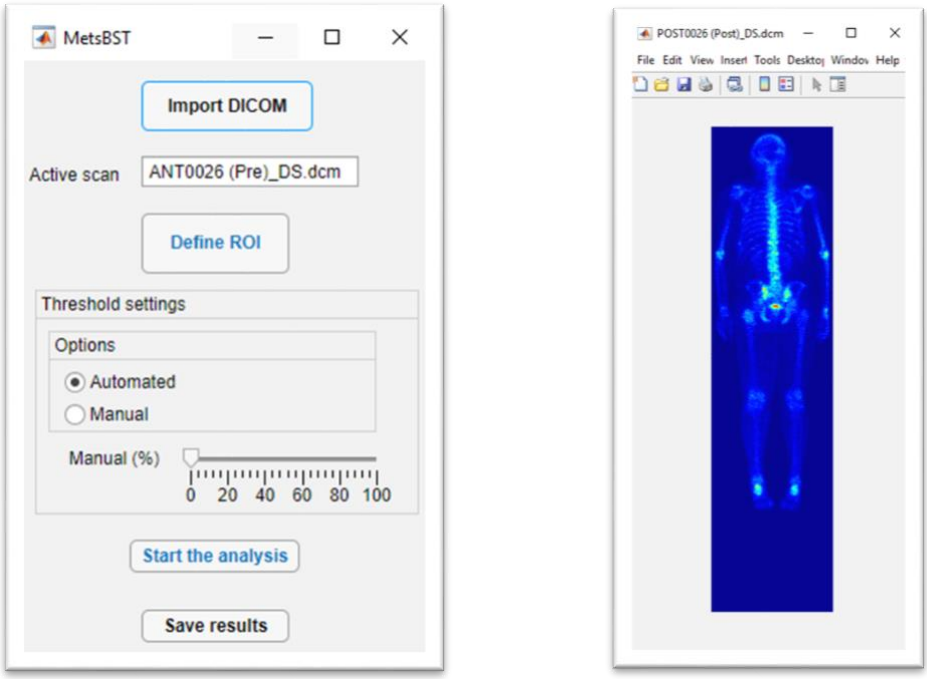
Appendix 1



(A) **(B)**

Figure 1. (A) The graphical user interface (GUI) for the MetsBST tool, built using the MATLAB Apps Designer. It is used to load the data define the region of interest (ROI) and to quantifying the extent of the tumour burden in the IBS using either automated pre-set thresholds or using thresholds set by the user (B) An example patient DICOM file loaded to MetsBST displayed using jet colormap.

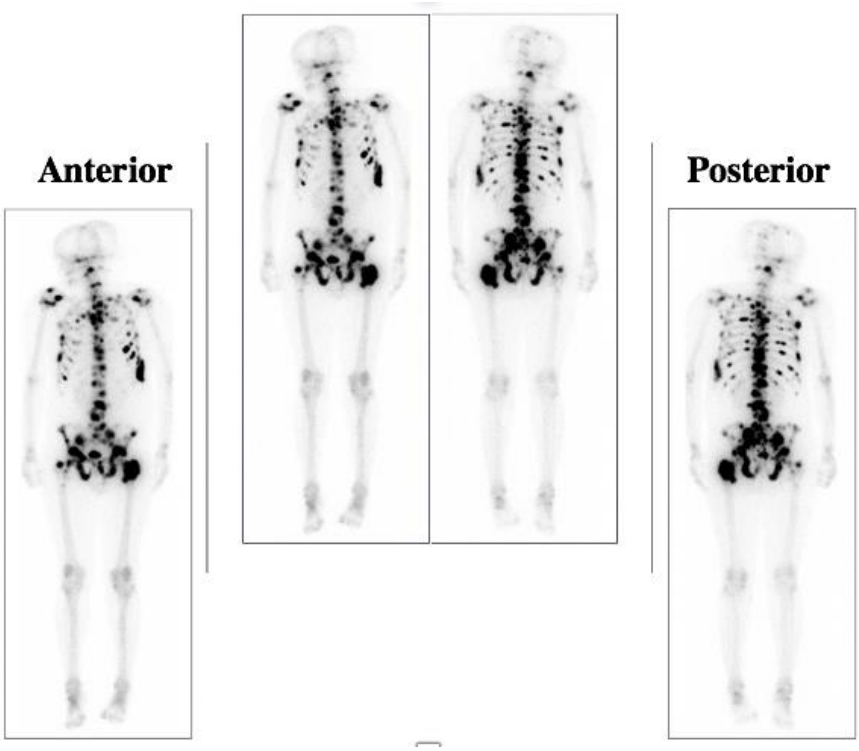


Figure 2. The whole-body planar images (anterior and posterior views) in a DICOM format (greyscale) were imported to the MATLAB workspace and displayed using the image display function as shown in Figure 3.

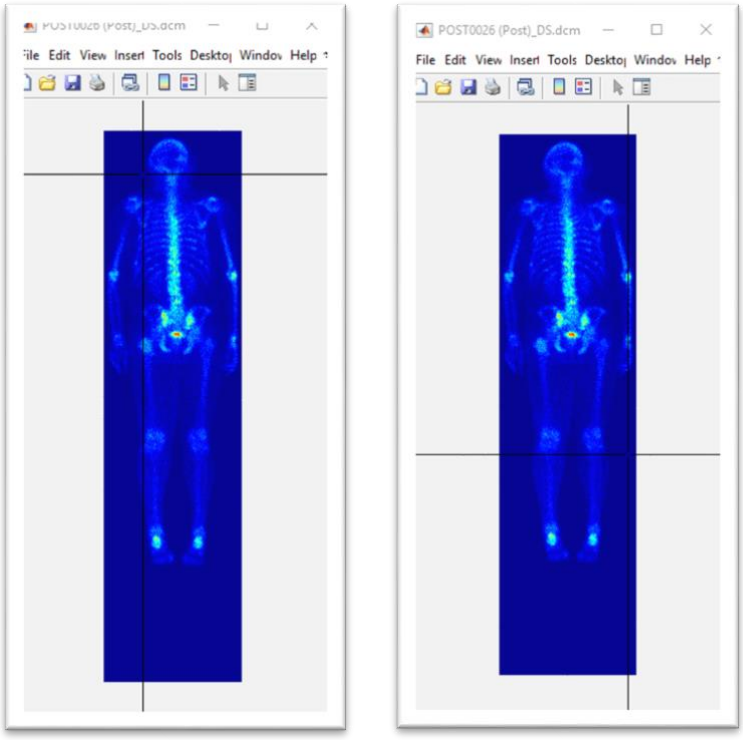


Figure 3. Users are giving a window displaying a whole-body image as shown here. They are then are prompted to choose two points to define the area of interest.

Bone scans from three cohorts of prostate cancer patients were used in the developmental phase of MetsBST, and different thresholds, as shown in Figure 4, were tested using the maximum intensity in the IBS to collect consistent readouts and to obtain relevant values for IBS tested in aBSI. The results from the aBSI were used as a reference to fine-tune MetsBST. The intensity threshold chosen (highlighted in red in **Figure 4** [fixed threshold (300)]) for analysing IBS using MetsBST provided consistent measurement of bone metastases, allowing for differentiation between prostate cancer groups and between multiple bone areas in the same patients. Fixed threshold (300) is defined as that maximum intensity value 300 is used by setting all pixel values >300 to 300. Pixels with intensity >100 identified as areas with metastatic disease. The pixels with intensity value between 5 and 100 are considered as normal bone. Pixel values below 5 were counted as background and removed from the analysis.

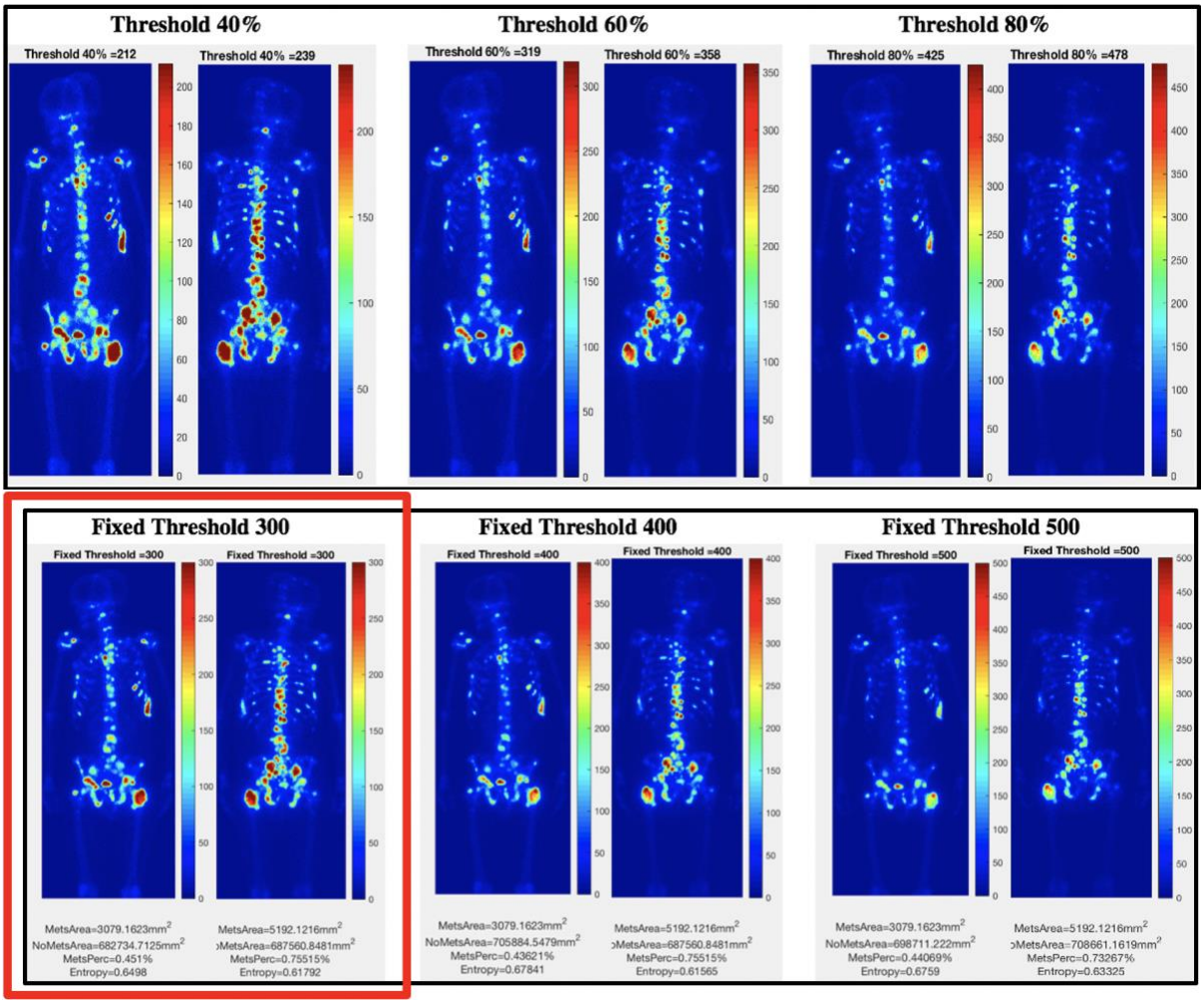


Figure 4. Different thresholds (10% to 90% of maximum intensity) and different fixed thresholds are tested to find the best threshold to identify the extent of bone metastases in the bone scan.

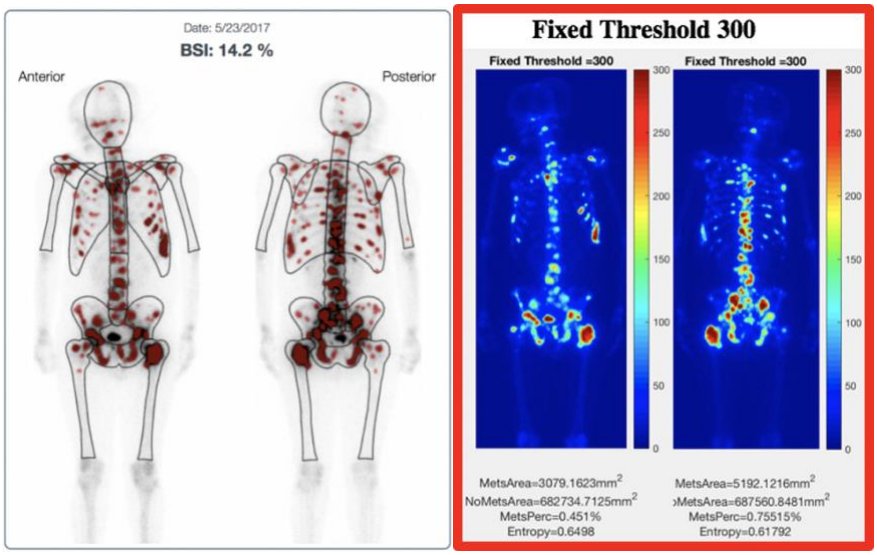


Figure 5. The fixed threshold (300) and cut-off value of 100 for identifying metastases yields consistent readings that correlated with the extent of bone metastases and gives the best comparable readouts in comparison to aBSI.

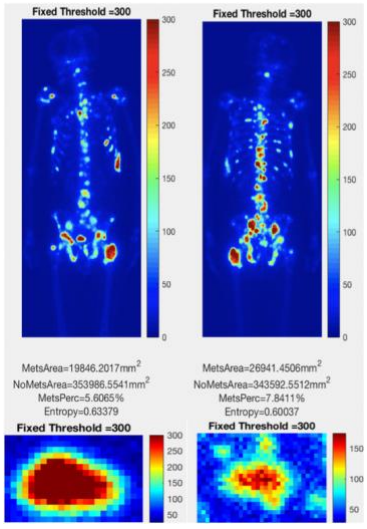


Figure 6. Users are given a window displaying the appearance of bone metastases in the IBS with the background signal removed. They are prompted to extract data that corresponded to the bladder based on size and location by defining the ROI around the uptake shown in the bladder.

For a locally led clinical trial investigating the combination of EBRT and radium-223 in our institution, we identified two separate areas: (1) in-field regions: the prostate and pelvic areas that received combinated EBRT and radium-223; and (2) out-of-field regions - the area of the spine that received only radium-223 and no EBRT, as shown in **Figure 7**.

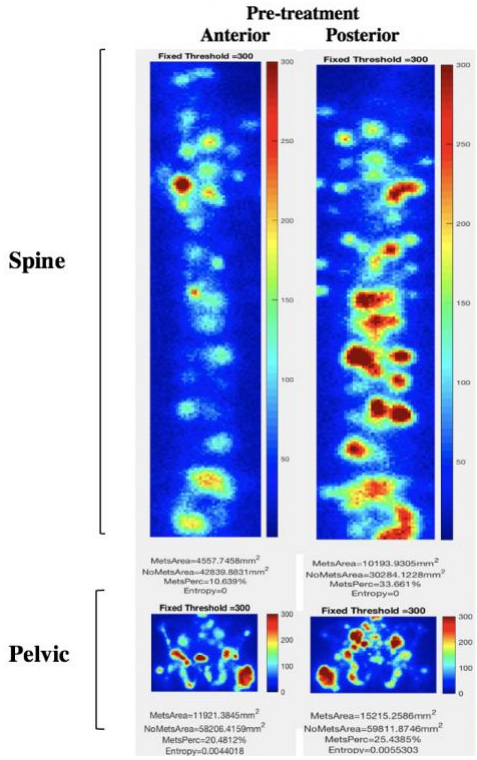


Figure 8. To aid the interpretation of responses in specific anatomical regions (i.e., prostate and pelvic regions) that have received a combined treatments (radium-223 and EBRT) compared to regions (i.e., spine) that have received a single treatment (radium-223), users can follow the same steps as in Figures 2 and 2. Here, two points are chosen to cover the spine and pelvic regions, separately.