Purpose: Based on the 3D-3D image registration and mapping the imaging space to the stereotactic frame coordinate space, an online software was developed to automatically update the target isocenter position prior to each non-invasive SRS/SRT treatment. The prescription dose can then be delivered to the entire target volume according to the treatment plan. Method and Materials: The key concept of SRS/SRT technique is the use of stereotactic coordinates to define the target volume and all other structures within the stereotactic frame space. This software will automatically setup stereotactic coordinate system through localize the 9-rod positions of the stereotactic frame in computed tomography (CT) images. The CT imaging spaces of both planning and pretreatment are then mapped to the stereotactic coordinate spaces using transformation matrices. The correct target isocenter are determined by fusing pretreatment CT with the planning CT based on the mutual information derived from 3D volume CT images. The final visual verification of the treatment isocenter is coincided with planning isocenter through the registration of a pair of orthogonal digitally reconstructed radiographs (DRRs) generated from pretreatment and planning CT images. Results: A sphere and a rectangle structures within a SRS head phantom were chosen as the targets to evaluate the accuracy of our software. Within the head phantom, the accuracy for repositioned isocenter can be achieved within 0.1 mm for displacements and 0.2 degree for rotations. Also three non-invasive SRS/SRT patient data sets were used to evaluate the software, and the results were verified with Radionics XKnife RT4 system, the translational discrepancies between reconstructed and optimized isocenters are within 0.5 mm. Conclusion: Using this software for the non-invasive SRS/SRT treatment, patient setup accuracy within 0.5 mm can be achieved without the discomfort from the pin fixed to the patient’s skull.