Leica SP5 Tile Scan Protocol

1) **Open the LAS AF software**
   - Lower stage completely.
   - At the opening splash, click **OK**.
   - When asked to initialize the stage, select **Yes**.

2) **Focus your sample and locate the field of interest.**
   
   NOTE: Do not incorporate a field rotation when tiling!

3) **Select the desired image parameters.** (format, speed, line average, etc.)
4) **Set the sequential scan, if applicable.**
5) **Set the z-stack, if applicable.**
6) **Set the tile scan.**
   - Open the Tile Scan menu by clicking **.**
Open the Stage Configuration menu by clicking [Stage Configuration Icon]. Ensure the overlap size is at 10%, and the factory settings are enabled. Close the Stage Configuration window.

Turn on the [Live View Icon]. Move the stage to view the top-left corner of the sample. Make sure that part of the sample is visible within the field, and is in focus. Mark position by clicking [Click to Mark Position Icon].
Move the stage to view the bottom-right corner of the sample. Make sure that part of the sample is visible within the field, and is in focus. Mark position by clicking 🖼️.

Stop the live view, and click 🕳️ to start the tile scan.
The final image will be stored in a “New collection” folder, which will contain all of the individual images of the montage. A final merged image will appear below the collection, with the naming convention “New collection_Merging001.”

Convallaria rhizome test slide, Leica Microsystems