Standardized measurement of areas in ImageJ

1. Central scan to be dealt with is opened in standard size window of the Heidelberg Eye Explorer.
2. Screenshot of standardized size is taken from the window and saved. The layer to be assessed (here: inner nuclear layer) has been selected previously and the corresponding thickness profile is displayed below the OCT scan.

3. A copy of the screenshot is opened in ImageJ.
4. Continuous vertical lines (red arrows) covering both OCT scan and layer thickness plot are drawn, delineating the zone of atrophy (between lines 1 & 2). Yellow arrows highlight area of hyper-reflectivity due to geographic atrophy. Line 3 is drawn 1.5 mm distal from the temporal edge of the zone of atrophy (AZ) using the scale on the x-axis from the screenshot (green arrows). The copy of the screenshot containing the lines drawn is saved.

[Arrows and line numbering are for illustrative purpose only]
6. The image saved in step 5 is cropped to the size of the layer thickness plot.

7. The areas under the curve corresponding to the AZ (black) and temporal zone (TZ; grey), respectively, are measured (number of pixels) using ImageJ and measurements recorded.

8. The average thickness of the layer (in pixels) in the zone of interest can be calculated by dividing the area by the length of the x-axis (in pixels) of the relevant area.

9. The average layer thickness in pixels is converted to µm for further processing and analysis.

**Important:** Screenshots need to be taken in standardized fashion to assure that the constant OCT scan length corresponds to a fixed number of pixels. Similarly, the y-axis (thickness) of constant length needs to correspond to a constant number of pixels on the screenshot.