In automated scanning microscopy, autofocus is critical to obtain reliable measurements from biological samples. We propose an algorithm to optimally sample focus positions and reduce the total time needed for autofocusing a large region of the slide.

When scanning multiple fields-of-view (FoVs), autofocus can be performed at each FoV just before the acquisition of a single FoV or pre-computed before starting the scan (pre-focusing). The latter involves choosing a subset of positions where to perform autofocus and recovering the remaining by interpolation. A typical pre-focusing algorithm can be divided into three components: the contrast function, the optimization algorithm and a sampling strategy. The first measures the contrast of an image, which is related to the degree to which the image is in-focus. The optimization algorithm tries to find the point along the optical axis where the contrast is highest. The sampling algorithm tries to minimize the amount of positions where to perform autofocus while keeping the interpolation error to a minimum.

One way to perform sampling is to refocus every \( n \) FoVs in both directions. This method results in points arranged in a raster-like pattern (equidistant grid sampling). We propose a new sampling strategy based on the Halton point set (see Figure 1), which has a lower discrepancy than the equidistant grid and thus provides better uniformity. We hypothesize that a higher degree of uniformity in the sampling set will result in a lower error after interpolation.

We test our assumption by comparing the three sampling strategies: equidistant grid sampling, hexagonal lattice sampling and Halton sampling. We sample a total of nine surfaces – three generated synthetically, three sampled from slides containing fluorescent beads and three sampled from slides containing cells stained with DAPI – under 24 different combinations of simulated precision and sampling rates. Our results show that in 88% of the cases, Halton sampling presents a lower interpolation error and therefore results in better focused images.