# Optimizing SRRF-Stream performance

It has been reported that the use SRRF stream implementation in MicroManager may result in two types of image artefacts appearing in images:

1) Shadow region in the close vicinity of observed cellular structures
2) Star-shaped pattern in the image when zoomed into it sufficiently

An example of Type 1 artefact can be seen in the pair of images below – the shadowing effect has been indicated by red arrows in a few areas along well-defined edges of tubulin ridges seen in the EM image. This is caused by the SRRF algorithm and the way it deals with intensity information contained within the original image. The algorithm looks at local neighbourhood of a visible feature, in this case tubulin fibres, to identify the presence of regions characterized by high likelihood of fluorophores located there and, in turn, by their high local radiality.

Whenever you transition locally between a region of high fluorophore presence and, consequently, of high radial fluctuations to low or no-fluorophore presence, SRRF algorithm uses the transition between such high and low regions to create contrast information. Or in other words, SRRF is more certain of fluorophores NOT being present when it is closely apposed to regions where fluorophores are present in larger number. This generates the shadowing effect you see close to structures. It is important to recognize that this shadowing effect will only ever manifest in true background, thus would not adversely affect the quantitative integrity of data. For example, if there were two side by side closely situated microtubules then one would not be affected by a contrast shadow of the other, as the SRRF algorithm would simply have not detected sufficient contrast information to produce the shadow effect in the first place.
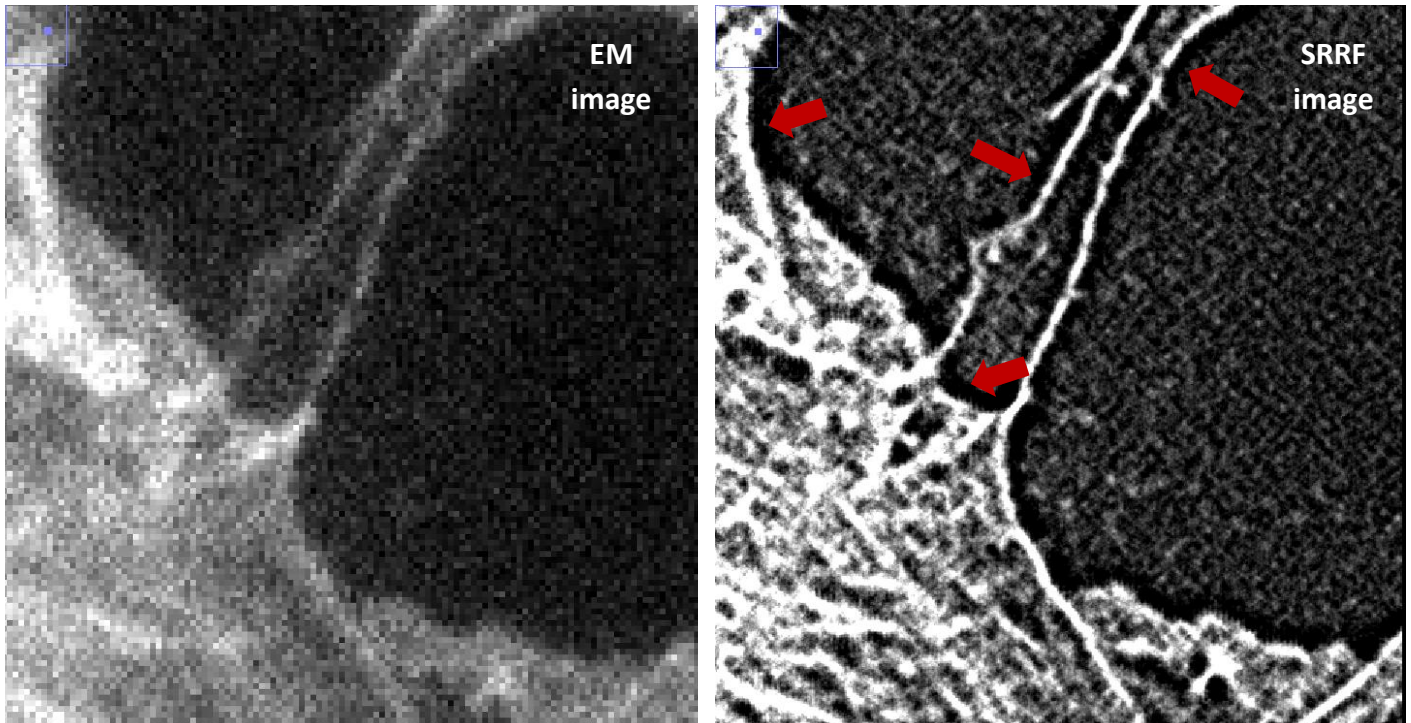
The Type 2 artefact is the star-shaped pattern appearing at the edges of features as shown below in the middle panel below. This becomes particularly apparent when your setup has not been optimized for lens magnification, its NA and camera's pixel size and therefore and is under-sampling. In other words - the further you are from fulfilling the Nyquist criterion the more visible this artefact becomes. A simple solution to counteract this is to increase the ring-radius in MicroManager SRRF settings box. However, this is a trade-off – it will suppress the star-shape artefact but at a slight

cost of resolution. You can see this in the RHS panel below where the ring radius has been increased from the default 0.5 to 1.0.
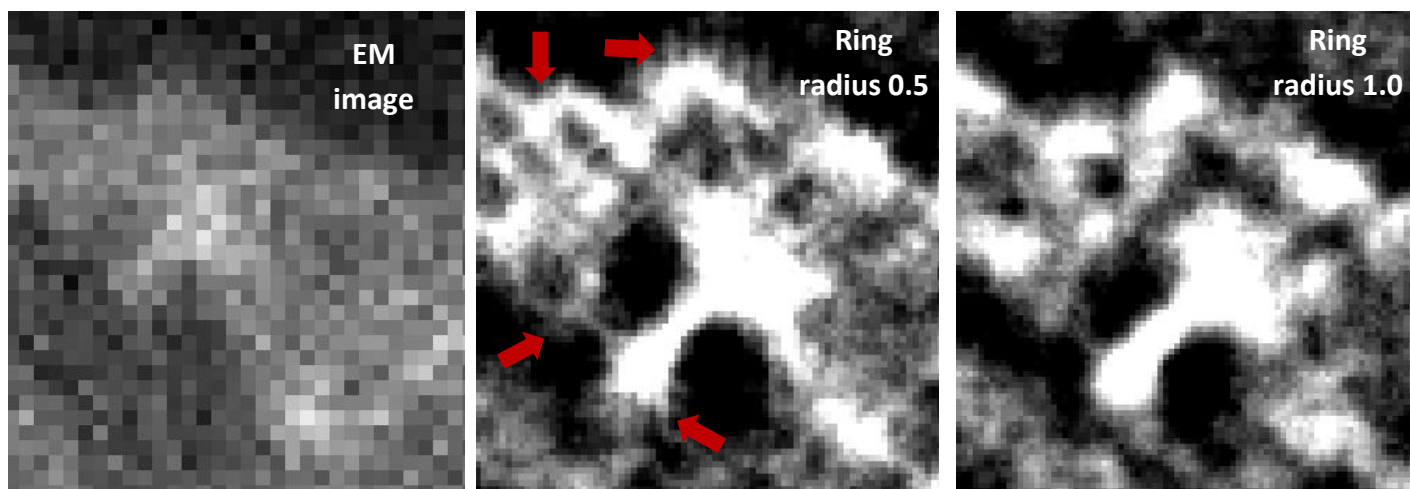
Should the pattern persist you can increase this value further to 1.5 at incremental detriment to resolution.