NanoJ-SQUIRREL – Baby UNIQORN! release note

20/03/2019

The most recent update of SQUIRREL has a beta version of the new optimiser, ‘UNIQORN!’ included.

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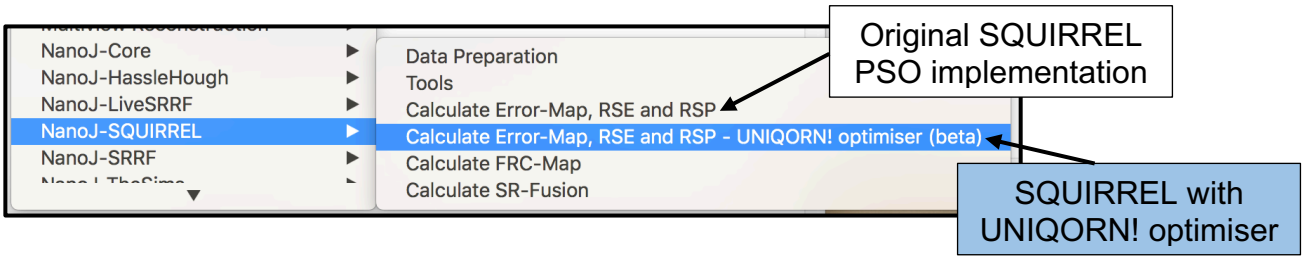
Overview

The core mathematical operation happening inside SQUIRREL is finding parameters for the Resolution Scaling Function (RSF). The RSF is the convolution kernel that is applied to the super-resolution image(s) to try and match their intensity and resolution to that of the provided reference image. In the original implementation of SQUIRREL, the RSF parameters (α, β, σ) were found using a particle swarm optimiser (PSO).

The beta version of SQUIRREL provided in this update has a new method for optimising (α, β, σ) called UNIQORN!. This involves a closed-form solution for the two intensity scaling parameters (α, β) and then a 1-dimensional optimisation to find σ. The benefit of the UNIQORN! optimiser is that it is 6-7 times faster than the original implementation of SQUIRREL.

The update still contains the original PSO version of SQUIRREL as documented in the Nature Methods publication and manual ('Calculate Error-Map, RSE and RSP' in the NanoJ-SQUIRREL menu), but also the new version.



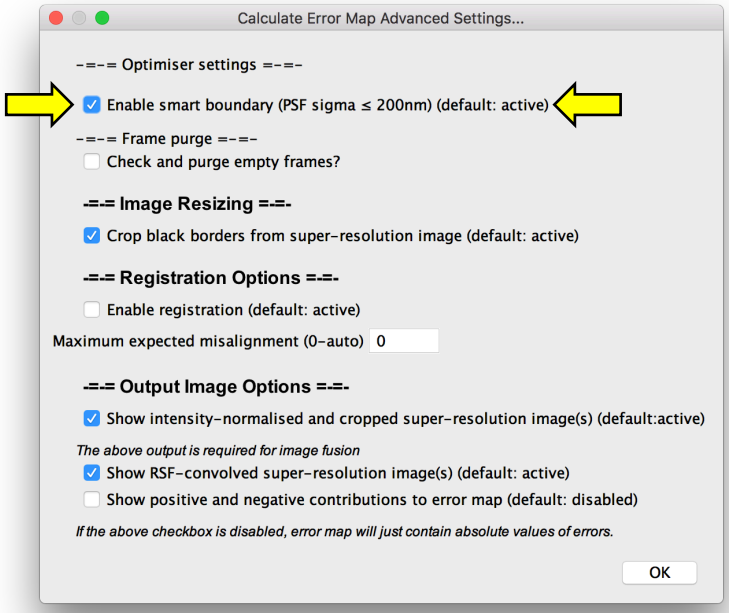
Performance comparison of PSO and UNIQORN!

To evaluate the relative performance of the PSO and UNIQORN! optimisers, super-resolution and reference image pairs were created with randomly assigned values of (α, β, σ). The values of the parameters retrieved using the PSO and UNIQORN! were then compared with the known values applied during simulation. The Pearson's correlation between the true values of (α, β, σ) and the values estimated by the two optimisers are shown below (N=500 independent simulations, 95% confidence intervals indicated).

	PSO	UNIQORN!
α	0.9988 (0.9985, 0.9990)	0.9997 (0.9997, 0.9998)
β	0.9905 (0.9887, 0.9920)	0.9964 (0.9957, 0.9970)
σ	0.9939 (0.9927, 0.9949)	0.9991 (0.9989, 0.9992)

Option for ‘smart boundary’

In the UNIQORN! version of SQUIRREL, there is an option to truncate the maximum boundary of the RSF σ to a ‘physically realistic’ value. This is found in the ‘Advanced settings’.



The rationale underlying this is as follows; if the super-resolution image has infinitely high resolution, then the correct σ of the RSF will be equal to that of the PSF. If the super-resolution image had finite resolution, then the correct σ of the RSF will be smaller than that of the PSF to account for the corresponding imperfect resolution. There is thus no physically realistic scenario where the σ of the RSF should be larger than that of the PSF.

By enabling RSF truncation, UNIQORN! will set the maximum boundary of the σ optimiser to 200nm (corresponding to a generous PSF FWHM of 470nm to account for aberrations). **In order for this to be accurate, the pixel size of the reference image should be correctly calibrated. If there is no calibration data associated with the reference image, a pixel size of 100nm will be assumed.**

If the smart boundary option is not enabled, then the σ value found by UNIQORN! (and thus the subsequent RSE, RSP, and error maps) will match the PSO outputs perfectly.

Outputs

The outputs of SQUIRREL (UNIQORN!) are the same as for the PSO implementation. The only difference is that the results table containing the RSE and RSP values will

have an additional column labelled ‘Overblur warning’. Regardless of whether the smart boundary was enabled or not, this column will say ‘True’ if the estimated $\sigma \geq$ 200nm.

Note that σ values greater than the PSF sometimes provide better mathematical solutions to the optimisation matching the super-resolution image to the reference image than physically realistic values do. This is because of, among other things, inhomogeneous background in the reference image and non-linear intensities in the super-resolution image(s). I’ll discuss this further in a forthcoming SQUIRREL version 2 paper ☺.

Acknowledgements

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