The content of spots was estimated as follows. First, the brightest region of the image was used for cutting a 20x20 pixel region (ROI) of interest around this region. A more accurate spot location was then obtained by regular 2D cross-correlation of this ROI. The content of the spot was estimated using a circular clipping mask of radius 6 pixels around this spot location [1], a method that was shown to give best results for high-noise spot analysis [2].

[1] Llorente-Garcia, I., T. Lenn, ., M. C. Leake. 2014. Singlemolecule

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delocalized oxidative phosphorylation. Biochim. Biophys. Acta.

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[2] Moolman MC, Kerssemakers JW, Dekker NH.

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