**Flattening**

The nth order cross-cumulant can be described by the following equation:

This equation describes the nth order cross-cumulant computed and shown in the previous step (Raw Cumulants). The sum in equation (1) is weighted by the product of *n* PSF-shaped weight factors, called the ***distance factor****,* which depends on the distances ***djl*** between **r**j and **r**l, *j* and *l* spanning the pixel set used for cross-cumulant computation (see Raw Cumulants). As ***d*** increases, rj and rl become further apart reducing the value of the distance up to a certain point when the cross-cumulant approaches zero. As a consequence, only nearby pixels (i.e. a small distance ***d*** between **r**j and **r**l) can be used to compute cross cumulants so as to reduce as much as possible the error introduced by this weighting/distance factor. However, as the order of SOFI grows, so does the error introduced by the weighting factor.

Indeed, Figure 1 depicts a 4th order 1D SOFI grid where light gray squares with single letters correspond to the physical pixels of the camera and light blue squares with four letters to the inter-pixels computed with cross-cumulants. The error introduced in this case by the distance factor can be expressed in the following terms: the inter-pixel exactly between physical pixel A and B (pixel AABB) will have a different weighting factor than the one closer to A (pixel AAAB) since the distance between them and the physical pixels is different. Therefore, both inter-pixels (AAAB and AABB) have distinct weighting factor.

An elegant way to resolve this issue is to multiply each cross-cumulant pixel by the inverse of its corresponding distance factor (which we can compute by estimating the PSF of the optical system). The cross-cumulants after flattening become:

Nevertheless, the estimation of can quite often be cumbersome and based on large approximations. Therefore we have developed a simple and robust method in order to correct for the weighting factor which gives either a better or similar result as compared to estimating the inverse of the distance factor.

Figure 2.a. depicts a 2nd order 3x3 SOFI grid where light gray squares with single letters again correspond to the physical pixels on the camera and light blue squares with two letters to the inter-pixels (or virtual pixels) computed with cross-cumulants. This grid can be decomposed into two separate grids, a grid containing all the physical pixels and another containing all the inter-pixels, shown in Figure 2.b and 2.c respectively. Both grids describe the same confined region in the sample and should hence share the same variance. Therefore, by multiplying the inter-pixel grid (Figure 2.c.) with the ratio between the variance of the physical pixels and the variance of the virtual pixels, the inter-pixels values are brought in the same range of the physical pixel values removing the weighting factor’s effect.