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Simple Formulae for Confocal Resolution Parameters: The Full Width Half Maximum (FWHM), the Ellipsoidal Observation Volume (OBSVOL) and the Root Mean Square Spatial Frequency (RMSF)

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INTRODUCTION

The need to summarize the overall resolution of an imaging system with a single indicator has led to a variety of resolution parameters. Traditionally measures such as the Rayleigh and Sparrow criteria have been used for the transverse resolution of an axially symmetric optical imaging system. With the advent of confocal microscopy the axial resolution became important too. Although the imaging performance of an isoplanatic system can be fully specified by the 3-D Point Spread Function (PSF), or equivalently, its transform; the 3-D Optical Transfer Function (OTF), there may be some advantage to using a single-valued resolution parameter. One particularly attractive option is the Full Width Half Maximum (FWHM). The idea of using the width of the PSF in one of three orthogonal directions is conceptually simple especially when the width is specified at a point where the system response drops monotonically to half its maximum value. A related parameter called the "observation volume" (OBSVOL) has been proposed for comparisons of multiple objective systems such as the 4Pi confocal theta microscope (Stelzer and Lindek 1994). The OBSVOL, like theFWHM is easily defined. It is, in fact, the volume of an ellipsoid which has each of its three principal axes equal to twice the corresponding FWHM. Unfortunately the analytic calculation of FWHM for systems with large apertures or multiple objectives (for illumination and detection) becomes rather awkward and the simplicity is sacrificed. This, in turn, compromises the apparent simplicity of the OBSVOL. There is, however, a parameter which is very closely related to both the FWHM and the OBSVOL which is easy calculated for all confocal systems and has a simple combination rule which allows elegant extension to more complex systems. This parameter is none other than the root mean square spatial frequency of the transfer function (RMSF). The following sections develop the elementary theory of the RMSF and its relation to the FWHM and OBSVOL.

MOMENT EXPANSION OF THE PSF

The PSF, $p(x, y, z)$, of an isoplanatic imaging system is related to the optical transfer function, $P(\xi, \eta, \zeta)$, by 3-D Fourier

$$p(x,y,z)=\iiint P(\xi,\eta,\zeta)\exp(-2\pi i[x\xi+y\eta+z\xi])\,d\xi\,d\eta\,d\zeta \tag{1}$$

where (x, y, z) are the Cartesian spatial coordinates in the focal region and (ξ, η, ζ) are the corresponding spatial frequencies (see, for example Gu and Shippard 1994). The PSF is typically the modulus squared of a amplitude PSF and consequently an even function which can be expanded as an even order Taylor series about the origin:

$$p(x,y,z)=p_0\Sigma_{l=0}^{\infty}\Sigma_{k=0}^{\infty}\Sigma_{j=0}^{\infty}a_{2j,2k,2l}x^{2j}y^{2k}z^{2l}. \tag{2}$$

The polynomial coefficient is given by

$$a_{2j,2k,2l}=\frac{1}{(2j)!(2k)!(2l)!}\frac{\partial^{2j}}{\partial x^{2j}}\frac{\partial^{2k}}{\partial y^{2k}}\frac{\partial^{2l}}{\partial x^{2l}}p(x,y,z)|_{x=y=z=0}. \tag{3}$$

Calculation of the FWHM from this expansion is accurate to better than 1% if $\{j \le 2,\ k \le 2,\ l \le 2\}$, which corresponds to a fourth order approximation. If only the second order terms of the above expansion are used, then the estimated FWHM can be as much as 12% in error (for typical forms of the PSF). Equation (2) can be rewritten in terms of the moments of $P(\xi, \eta, \zeta)$ using the Fourier moment-derivative theorem.

$$\frac{\partial^{2j}}{\partial x^{2j}} \frac{\partial^{2k}}{\partial y^{2k}} \frac{\partial^{2l}}{\partial x^{2l}} p(x,y,z)|_{x=y=z=0}$$
$$= (-2\pi i)^{2(j+k+l)} M_{2j,2k,2l} \tag{4}$$

The moment $M_{2j,\,2k,\,2l}$ is defined by

$$M_{2j,2k,2l} = \iiint \xi^{2j} \eta^{2k} \zeta^{2l} P(\xi,\eta,\zeta) d\xi d\eta d\zeta. \tag{5}$$

The benefit of this description is that the moments of the system pupil function are easily calculated analytically, even for systems with apodization. Transfer functions can be represented as correlations or convolutions of a few basic pupil functions. Under the operation of convolution or correlation moments add in a simple way. In particular, normalized second order moments are additive.

ESTIMATION OF THE FWHM

The second order approximation to the PSF is

$$p(x,y,z) = p_0 [\, 1 - \mu_{200} x^2 - \mu_{020} y^2 - \mu_{002} z^2 \,]. \tag{6}$$

The normalized moments are

$$\mu_{2j,2k,2l} = \frac{M_{2j,2k,2l}}{M_{0,0,0}} \tag{7}$$

In equation (6) the second order normalized moments are equivalent to the mean square spatial frequencies of the transfer function measured along the three axes. Equation (6) represents a paraboloidal distribution which has ellipsoidal surface contours of constant value (iso-surfaces). Hence an ellipsoid can be defined which contains all values of p greater than some specified proportion of p_0. Generally the proportion specified is one half and the FWHM along the x, y, and z axes arise from the solution of equation (6). As mentioned in section 2, the second order approximation is prone to systematic errors in the predicted FWHM. A fourth order approximation gives an accurate FWHM and a more realistic form of the iso-furface contours. It is worth noting that the iso-surface is no longer (in general) ellipsoidal. This is the source of an inconsistency in the use of an ellipsoidal OBSVOL for a non-ellipsoidal iso-surface. One possible conclusion is that the simple physical ideal of an ellipsoidal volume is incompatible with the precise representation of the PSF. Clearly the ellipsoid no longer has any real physical meaning - it is just a mathematical construct. Such a construct can still be useful if it has some other redeeming feature(s). It can be argued convincingly that the ellipsoid is still a good indicator of the effective OBSVOL. However, a similar argument can be formulated for the volume represented by the second order approximation which has the added advantage that it is easily calculated and extended.

FEATURES OF THE SECOND ORDER APPROXIMATION

Before examining some details of the second order approach it is instructive to consider some general aspects of a second order theory:

Firstly, for any particular PSF form the second order approximation to the FWHM exihibits a fixed error, for example:

sinc()	-5%
sinc () squared	-12%
Airy amplitude	-6%
Gaussian	-17%

The Gaussian is significant because it represents the limit of a PSF which is the product of many individual PSFs as can be shown by the Central Limit Theorem. The peak errors above can be reduced by a consistent rescaling of the estimate, so, for example, the new error could be better than +/-9% in all cases.

Secondly, the concept of an ellipsoidal OBSVOL can be considered to be implicitly second order.

Lastly, the second order approach resembles second order statistics and its familiar concepts of mean and variance which, again, are additive under convolution.

Returning to the main discussion, the most important factor to consider in this section is the relation between the normalized moments and the estimated FWHM. The second order FWHM, w_x, w_y, w_z, are determined from equation

(6) as follows:

$$\frac{1}{2}=\mu_{200}\left(\frac{w'_x}{2}\right)^2,\quad \frac{1}{2}=\mu_{020}\left(\frac{w'_y}{2}\right)^2,\quad \frac{1}{2}=\mu_{002}\left(\frac{w_z}{2}\right)^2. \tag{8}$$

The normalized moments μ_{200}, μ_{020} and μ_{002} are the mean square spatial frequencies of the transfer function measured along the three principal axes. Consequently:

$$w_x=\frac{\sqrt{2}}{\sqrt{\mu_{200}}} \text{ and similarly for } w_y, \text{ and } w_z \tag{9}$$

Simply stated the FWHM (second order) is inversely proportional to the root mean square spatial frequency (or RMSF) of the transfer function.

The OBSVOL can now be defined in terms of these FWHM. The treatment given here is purely second order so that all confocal systems can be compared in a consistent manner. The usual definition of OBSVOL, V, is as follows:

$$V=\frac{4}{3}\pi w_x w_y w_z \tag{10}$$

In the limit, as the PSF approaches an ellipsoidal Gaussian distribution such a volume encloses almost 85% of the PSF.

The rule for combining FWHM which result from the product of multiple PSFs can be shown to be:

$$\frac{1}{w_x^2}=\sum_n \frac{1}{w_{n,x}^2} \text{ and similarly for } w_y \text{ and } w_z. \tag{11}$$

Equation (11) is merely the re-expression of the additive property of variances. The overall OBSVOL is therefore such that:

$$V^2=\frac{\left(\frac{4}{3}\right)^2\pi^2}{\left(\sum_n \frac{1}{w_{n\,x}^2}\right)\left(\sum_n \frac{1}{w_{n\,y}^2}\right)\left(\sum_n \frac{1}{w_{n\,z}^2}\right)}$$

$$=\frac{128\,\pi^2}{9\,\mu_{200}\,\mu_{200}\,\mu_{200}} \tag{12}$$

020 002

The product of three orthogonal RMSFs is inversely related to the OBSVOL.

It has been suggested that in confocal systems of the 4Pi type that the FWHM is not a useful parameter because it completely omits the effects of large subsidiary maxima. Instead the variance of the axial PSF has been mooted as a preferred indicator. It is not difficult to show that (for any semi-aperture less than 70 degrees) the variance of the PSF is equal to the variance of the envelope of the axial PSF. The implication is therefore that no improvement is to be inferred from the interference of two juxtaposed objectives. There is also the more serious defiency of real space variances that they are often infinite (especially in conventional imaging systems) so they must be used with great caution, if at all. The simplified methods proposed here only work with variances defined in the spatial frequency domain and FWHM in the space domain. Such variances are always finite because of the limitation upon maximum spatial frequency. With this in mind it is expedient, in 4Pi configurations, to utilize the FWHM of the envelope of the axial PSF. This corresponds to using the transfer function variance of either opposing objective, not both.

DISCUSSION

The three equations (9), (11) and (12) summarize the simple formulae for FWHM, OBSVOL, and RMSF. Obviously a more detailed analysis of the consequences of an explicit versus an implicit second order theory of OBSVOL is needed before either procedure is adopted as a metric by practitioners of confocal microscopy. Ultimately judgement will depend upon demonstration of a one-to-one, monotonic relation between an approximate formulation and the exact formulation. Assuming (presuming!) this to be the case, then the simple formulation presented here could become an attractive alternative to the other currently in use.

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