

Fast 3D multiphoton laser scanning microscopy by remote z-scanning with a macroscopic voice coil motor

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This is an accompanying documentation provided together with the Mathematica code used for the calculations.

1 | Linear optics analysis of configuration 1 (single-lens ZSU)

We introduce the Rayleigh length $z_R = \frac{\pi w^2}{\lambda}$, the complex beam parameter $q(z) = z + iz_R$, which can also be written as $\frac{1}{q(z)} = \frac{1}{R(z)} - i \frac{\lambda}{\pi w^2(z)}$, the propagation scheme for Gaussian beams $q_1 = \frac{Aq_0+B}{Cq_0+D}$ with the complex beam parameters of the incoming (q_0) and outgoing (q_1) beams, and the linear propagation matrix of the system, $\begin{pmatrix} A & B \\ C & D \end{pmatrix}$. The input beam is assumed to be collimated, i.e. $q_0 = iz_{R0}$, with z_{R0} depending on the beam width w_0 via $z_{R0} = \frac{\pi w_0^2}{\lambda}$. The optical linear ABCD transfer matrix system of the single-lens configuration can be described as follows, starting from the beam entering the z-scan lens (right) to the focus position after the objective (left):

$$\begin{pmatrix} A & B \\ C & D \end{pmatrix} = \begin{pmatrix} 1 & f_0+d \\ 0 & 1 \end{pmatrix} \cdot \begin{pmatrix} 1 & 0 \\ -1/f_0 & 1 \end{pmatrix} \cdot \begin{pmatrix} 1 & f_1+f_0 \\ 0 & 1 \end{pmatrix} \cdot \begin{pmatrix} 1 & 0 \\ -1/f_1 & 1 \end{pmatrix} \cdot \begin{pmatrix} 1 & f_2+f_1 \\ 0 & 1 \end{pmatrix} \cdot \begin{pmatrix} 1 & 0 \\ -1/f_2 & 1 \end{pmatrix} \cdot \begin{pmatrix} 1 & f_z+f_2+x_z \\ 0 & 1 \end{pmatrix} \cdot \begin{pmatrix} 1 & 0 \\ -1/f_z & 1 \end{pmatrix} \cdot \begin{pmatrix} 1 & 2f_z+2D \\ 0 & 1 \end{pmatrix} \cdot \begin{pmatrix} 1 & 0 \\ -1/f_z & 1 \end{pmatrix} =$$
$$= \begin{pmatrix} -\frac{2Df_0^2f_z^2 + df_1^2f_z^2 - 2dDf_1^2x_z}{f_0f_2f_1f_z^2} & \frac{f_z(f_0^2f_z^2 + df_1^2(f_z - x_z)) + 2D(f_0^2f_z^2 - df_1^2x_z)}{f_0f_2f_1f_z} \\ \frac{f_1(-f_z^2 + 2Dx_z)}{f_0f_2f_z^2} & \frac{f_1(f_z^2 - 2Dx_z - f_zx_z)}{f_0f_2f_z} \end{pmatrix}$$

Using Gaussian beam optics and the ABCD propagation scheme, the displacement in the sample can be calculated. We take advantage of the fact that the beam curvature is infinite at the minimum of the beam waist, i.e., at the focal position. The condition for the focal position is therefore that the real part of the inverse complex beam parameter, $\Re(1/q_1)$, be zero, which yields a functional relation $d(D)$. For the initial beam parameter $q_1 = iz_R$ as introduced above, it can be assumed that z_R is larger than any focal length, mirroring the assumption of a collimated Gaussian beam as input to the system.

$$d = \frac{-2Df_0^2f_2^2}{f_1^2(f_z^2 - 2Dx_z)}$$

If the ideal condition $x_z = 0$ is not met, the relation $d(D)$ is non-linear.

For this single-lens system, axial scanning can lead to prominent over- or underfilling of some optical apertures, namely the back aperture of the objective. Using the respective part of the ABCD transfer matrix, the beam size at the BFA can be calculated:

$$R_{BFA} = R \left(\frac{f_1}{f_2} - \frac{2Df_0f_2}{f_1f_z^2} - \frac{2Df_1x_z}{f_z^2f_2} \right)$$

with the beam diameter entering the system, R . The first term is the simple beam expansion without axial scanning. The second term, independent of x_z , introduces a slight dependence of the BFA filling on the scanning position, whereas the third term, which vanishes for $x_z = 0$, shows a much stronger dependence for a typical configurations.

An overview of the beam path for different focal shifts is given in Supplementary Fig. 2.

2 | Linear optics analysis of configuration 2 (ZSU with telescope)

The optical linear ABCD transfer matrix of the telescope system can be written in closed form:

$$\begin{pmatrix} A & B \\ C & D \end{pmatrix} = \begin{pmatrix} -\frac{f_1d}{f_0f_2} - \frac{2Df_1'^2f_0f_2}{f_2'^2f_1f_z^2} & \frac{f_2'f_1d}{f_0f_2} + \frac{f_0f_2(2Df_1'^2 + f_2'f_z^2)}{f_2'f_1f_z^2} \\ -\frac{f_1}{f_0f_2} & \frac{f_2'f_1}{f_0f_2} \end{pmatrix}$$

Using Gaussian beam optics and the ABCD propagation scheme, the displacement in the sample can be calculated as above:

$$d = -2 \frac{f_0^2f_2^2f_1'^2}{f_1^2f_z^2f_2'^2} D$$

This solution is valid if the Rayleigh length z_R of the input beam is much longer (order of many meters) than the focal length of any of the used lenses. This is typically the case.

The beam diameter in the back aperture of the objective can now be computed:

$$R_{BA} = R \left(\frac{f_1}{f_2} - \frac{2Df_1'^2f_0f_2}{f_2'^2f_1f_z^2} \right)$$

The first term represents the beam expansion by the telescope in the microscope and the second term is small. Hence, displacements of the z-scan mirror have only minimal effects on beam diameter, as illustrated in Supplementary Fig. 2. The telecentric lens system therefore linearizes the dependence $d(D)$, prevents underfilling of the objective, and magnifies the scan range.

3 | Modulation of the field of view

The dependence of FOV size on D can be computed and depends on the distance of the xy-scan mirrors from the focal point of xy-scan lens and relay lens 2 (see paper). Using the linear ABCD scheme, one can calculate the expected size of the FOV based on the deflection angle β at the xy scanners, taking into account the distance w between each scan mirror and the optimal position at the focal points of the relay lens and the xy-scan lens:

$$FOV \approx -\frac{f_o f_2}{f_1} R \left(1 + \frac{2f_1'^2}{f_2'^2 f_z^2} Dw \right) \beta$$

The first term for the expression of the FOV size arises from xy scanning. The second term includes a linear contribution of z-scanning (D) and a linear contribution of the non-ideal distance between the scan-mirrors and the xy-scan lens (w). Due to the separation of the scan mirrors, w is different for x- and y-direction. Using the distance of resonant and galvo scanners in the LSM employed ($\Delta w \approx 7$ mm), one can predict the difference of the slopes of the relationship FOV(w) in x and y directions ($\frac{2f_1'^2 w}{f_2'^2 f_z^2} \approx 0.060 \text{ mm}^{-1}$), which was found to be in agreement with experimental observations (see paper, Fig. 2d,e). Hence, the asymmetric modulation of FOV size during z-scanning can be explained entirely by the fact that the x and y scanners cannot be placed at the same position.