

Advances in multiphoton microscopy for imaging embryos

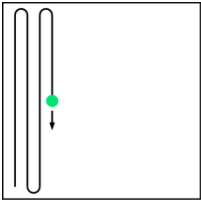
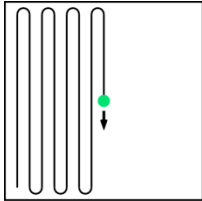
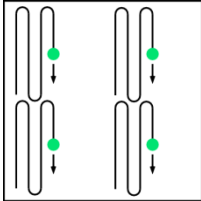
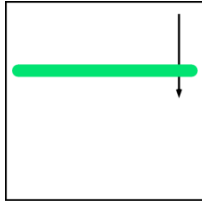
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Supplementary Material

Supplementary Table 1.

Strategies for fast imaging speed in multiphoton microscopy vs linear microscopy: theoretical scaling of illumination parameters.

SUPPLEMENTARY TABLE 1

| | Point-scanning 2p-microscopy | | Fast point- scanning 2p-microscopy (N -time faster scan) | | Multifocal multiphoton microscopy (N foci) | | Light-sheet 2p-microscopy | |
|---|---|-----|---|-----------------|--|------|---|-------------------------------|
| Scanning scheme |  | |  | |  | |  | |
| | Point scanning | | Fast point scanning | | Multiple point scanning | | Fast line scanning | |
| Fluorescence excitation mode | 1p | 2p | 1p | 2p | 1p | 2p | 1p | 2p |
| Acquisition speed (pixel rate) | r | r | Nr | Nr | Nr | Nr | Nr | Nr |
| Numerical aperture of illumination objective | NA | NA | NA | NA | NA | NA | $\frac{1}{\alpha}$ NA | $\frac{1}{\alpha}$ NA |
| Illumination time per excited volume | t | t | $\frac{1}{N} t$ | $\frac{1}{N} t$ | t | t | $\frac{\alpha^2}{N} t$ | $\frac{\alpha^4}{N} t$ |
| Illumination laser intensity per focus | I | I | NI | $\sqrt{N} I$ | I | I | $\frac{N}{\alpha^2} I$ | $\frac{\sqrt{N}}{\alpha^2} I$ |
| Illumination laser average power | P | P | NP | $\sqrt{N} P$ | NP | NP | NP | $\sqrt{N} P$ |

Supplementary Table 1. Strategies for fast imaging speed in multiphoton microscopy vs linear microscopy: theoretical scaling of illumination parameters. This table completes Table 1 with parameter scaling in the cases of 1p-microscopy techniques (see Table 1 caption for explanations). It shows that in linear microscopy, increasing the speed N -times always requires N -fold increase in laser

power to obtain images with the same signal level. In nonlinear microscopy, the square root of laser power is sufficient, except in the case of multifocal approach.