<u>Supplemental information S1 – Model for double MLA in mercury based 10x epi fluorescence microscope</u>

For modeling the MLA system, the Fraunhofer approximation of the scalar Fresnel-Kirchoff diffraction theory is applied (S1). Lenses are approximated as infinitely thin. The system is modeled in one dimension, expanding the model to two dimensions is straightforward. Four major steps are taken to construct the double MLA model:

1. Wave passing through single lenslet (equations A1-A7, figure S1-1 panel A)
2. Expansion to wave passing through array of lenslets (equations A8,A9, figure S1-1 panel B/C) and imaged by field lens (Panel D)
3. Addition of a second array of lenslets identical to the first (equations A11,A12, figure S1-1 panels E/F)

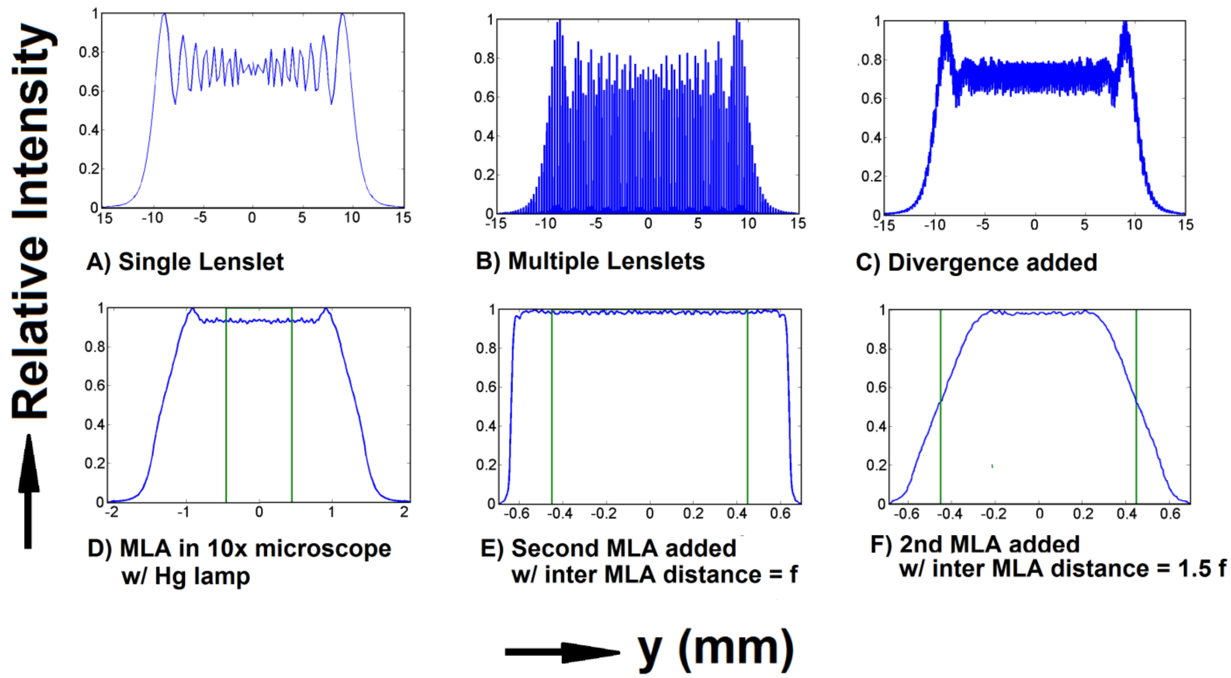

Figure S1-1: Model of an imaging MLA system. In panels D-F the pickup area is indicated with green lines. Panel A shows a monochromatic plane wave that has passed through a single lenslet. Panel B shows a monochromatic plane wave passing through an array of lenslets, an MLA. Panel C shows a monochromatic wave with 0.7° divergence passing through the MLA. Panel D shows the wave passing through an MLA and a 10x objective. The spectrum of a HBO lamp is taken into account. In panels E and F a second MLA is also added. By changing the distance between the MLAs from the MLA focal length (E) to 1.5 times the MLA focal length (F), the width of the flat top is changed, and the power within the green lines is changed from 70% of the total (E) to 90% of the total (F).

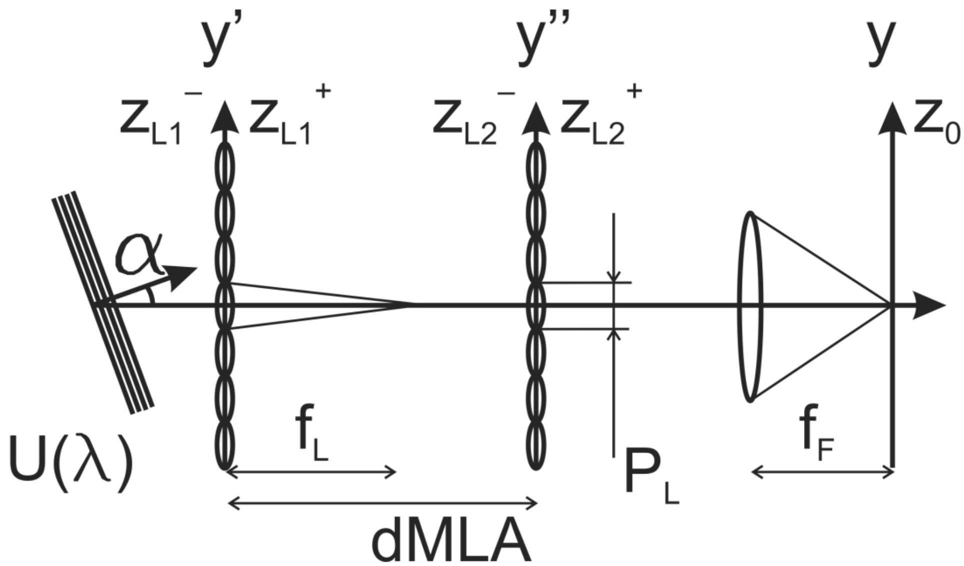

Figure S1-2: Diagram with model parameters for double MLA system

Notation is as follows; F is used for Fourier transformation, z is the distance along the optical axis, y the distance perpendicular to it. Superscript – and + signs denote whether a position before or after the lens is described, subscript L or L1 is used for the first MLA or part thereof, subscript L2 is used for the second MLA. Subscript F is used for the field lens or objective and subscript O denotes the observation plane, or sample plane. See figure S1-2 for a diagram with this notation. Symbols used are α the angle of incidence, λ the wavelength of the incoming wave, k the wavenumber (2π/λ), s the distance between two MLA, p the pitch of the MLA, f the focal length, R the radius of curvature and n the refractive index of a lens in the MLA.
In the Matlab simulations we started with a monochromatic plane wave of λ = 520nm, Micro lens array properties p_L 0.3mm, f_L 4.75mm and f_F 20 mm, α_{max} was 1.7° and for the polychromatic source the 520nm HBO arc line was used. We introduced divergence and polychromaticity after the contribution of an array of lenslets was derived. This nicely illustrates the contribution of each parameter to the final distribution. Figure S1-1, panels A-F illustrates the illumination field. All plots are normalized to the maximum value of the distribution.

If we assume the collimator lens is perfect and infinite in size, and our light source is monochromatic, the illumination impinging onto a lenslet can be expressed by a number of plane waves of the form:

$$U_L(\alpha, \lambda; y', z_L^-) = P(\lambda) \exp\,(iky' \sin\alpha) \qquad \text{(A1)}$$

With P(λ) the spectral distribution of the light source. The transmitted field immediately behind the lenslet is given by:

$$U_L(\alpha, \lambda; y', z_L^+) = U_L(\alpha, \lambda; y', z_L^-) T_L(y') \qquad \text{(A2)}$$

Under the assumption that the lenslets are parabolic in shape and the fill factor of each lenslet is unity, the transmission function can be described as:

$$T_L(y') = \exp\left(\frac{i\pi y'^2}{\lambda f_L}\right) \mathrm{rect}\left(\frac{y'}{p_L}\right) \qquad \text{(A3)}$$

$$\mathrm{rect}\left(\frac{y'}{p_L}\right) = 1 \text{ for } |y'| \le \frac{p_L}{2} \;\&\; rect\left(\frac{y'}{p_L}\right) = 0 \text{ for } |y'| > \frac{p_L}{2} \qquad \text{(A4)}$$

The focal distance of a single lenslet is given by $f_L=R_L/(n(\lambda)-1)$. The amplitude distribution in the observation plane from a single lenslet is obtained by Fourier transformation:

$$U_L(\alpha,\lambda;y,z_O) = \mathfrak{F}_{f_F}\{U_L(\alpha,\lambda;y',z_L^+)\} = \int_{-\infty}^{\infty} U_L(\alpha,\lambda;y',z_L^+)\exp\left(\frac{-ikyy'}{f_F}\right)dy' \qquad \text{(A5)}$$

The intensity distribution is proportional to the square of the wave amplitude:

$$I_L(\alpha,\lambda;y,z_O) \propto |U_L(\alpha,\lambda;y,z_O)|^2 \qquad \text{(A6)}$$

By integrating over all wavelengths and angles of incidence this is further simplified and we obtain the contribution of a single lenslet:

$$I_L(y,z_O) \propto \int_{\lambda_{min}}^{\lambda_{max}}\int_{\alpha_{min}}^{\alpha_{max}} |U_L(\alpha,\lambda;y,z_O)|^2 \, d\alpha d\lambda \qquad \text{(A7)}$$

The contribution of all lenses in the MLA is expressed by:

$$I_L(y,z_O) \propto \int_{\lambda_{min}}^{\lambda_{max}}\int_{\alpha_{min}}^{\alpha_{max}} \left|U_L(\alpha,\lambda;y,z_O)S_{N_0}(\alpha,\lambda;y)\right|^2 d\alpha d\lambda \qquad \text{(A8)}$$

With S_{N0} derived from equations 11 thru 14 in Buttner and Zeitner [14] as follows: The transmission function of the lens array is a summation over the transmission functions of the individual lenses, which is substituted by a finite shah function and, indeed, a convolution of the transmission function of a single lens located at y' (eq. 11). After applying the shift and convolution theorem, the modulation function is given by:

$$S_{N_0}(\alpha,\lambda;y) = \mathfrak{F}_{f_F}\left\{\sum_{l=-N}^{N}\delta(y'-lp_L)\right\} = \int_{-\infty}^{\infty}\sum_{l=-N}^{N}\delta(y'-lp_L)e^{\frac{-2\pi iyy'}{\lambda f_F}}dy' = \sum_{l=-N}^{N}e^{\frac{-2\pi iylp_L}{\lambda f_F}}$$

We can write

$$S_{N_0}(\alpha,\lambda;y) = \sum_{l=-N}^{N} e^{\frac{-2\pi iyl\,p_L}{\lambda f_F}} = \sum_{l=-N}^{N} w^l$$

Where

$$w = e^{\frac{-2\pi iy\,p_L}{\lambda f_F}}$$

Multiplying every term in the sum by w increases each power of w in the sum by one, giving

$$wS_{N_0}(\alpha,\lambda;y) = \sum_{l=-N+1}^{N+1} w^l = S_{N_0}(\alpha,\lambda;y) + w^{N+1} - w^{-N}$$

Resulting in

$$S_{N_0}(\alpha,\lambda;y) = \frac{w^{N+1}-w^{-N}}{w-1} = \frac{e^{\frac{-2\pi i y p_L (N+1)}{\lambda f_F}} - e^{\frac{2\pi i y l p_L N}{\lambda f_F}}}{e^{\frac{-2\pi i y p_L}{\lambda f_F}} - 1} = \frac{e^{\frac{-2\pi i y p_L (N+1/2)}{\lambda f_F}} - e^{\frac{2\pi i y l p_L (N+1/2)}{\lambda f_F}}}{e^{\frac{-\pi i y p_L}{\lambda f_F}} - e^{\frac{\pi i y p_L}{\lambda f_F}}}$$

$$= \frac{\sin\left(\frac{N_0 \pi p_L y}{\lambda f_F}\right)}{\sin\left(\frac{\pi p_L y}{\lambda f_F}\right)} \qquad \text{(A9)}$$

by applying Euler's formula and using $N_0 = 2N + 1$. N is the number of lenslets that are being illuminated. From our simulations, N needs to be at least 10 to realize a uniform illumination.

Which for a monochromatic plane wave results in interference fringes corresponding to the diffraction orders in the Fourier plane:

$$y_m = \frac{\lambda m f_F}{p_L} - \alpha f_F \qquad \text{(A10)}$$

Both wavelength and angle of incidence contribute to the position of the diffraction orders.

The derivation for the double MLA is analogous to the derivation above. Neglecting constant phase factors, the field impinging on the second MLA is given by (S3):

$$U_L(\alpha,\lambda;y'',z_{L2}^-) = d\,\exp\left(\frac{iky''^2}{f_L}\right)\int_{-\infty}^{\infty} U_L(\alpha,\lambda;y',z_L^+)\,\exp\left(\frac{-iky''y'}{f_L}\right)dy' \qquad \text{(A11)}$$

With d a quadratic phase factor compensating for the distance between the MLA, provided $f_L < s \leq 2 f_L$

$$d = \exp\left(\frac{ik}{f_L}\left(1 - \frac{s}{f_L}\right)y''^2\right) \qquad \text{(A12)}$$

Inserting equation A2 into A11, dropping constant phase coefficients and cancelling the parabolic phase curvatures inside the integrand due to opposite signs in the exponent gives a Fourier transformation:

$$U_L(\alpha,\lambda;y'',z_{L2}^-) = d\,\exp\left(\frac{iky''^2}{2f_L}\right)\int_{-\infty}^{\infty} |U|\,\exp(iky'\sin\alpha)\,\mathrm{rect}\left(\frac{y'}{p_L}\right)\,\exp\left(\frac{-iky''y'}{f_L}\right)dy''$$

$$= d\exp\left(\frac{iky''^2}{2f_L}\right)\mathfrak{F}_{f_L}\left\{|U|\exp(iky'\sin\alpha)\,\mathrm{rect}\left(\frac{y'}{p_L}\right)\right\}$$

$$= d\exp\left(\frac{iky''^2}{2f_L}\right)\delta(y''-f_L\sin\alpha)\times\mathfrak{F}_{f_L}\left\{|U|\mathrm{rect}\left(\frac{y'}{p_L}\right)\right\} \qquad \text{(A13)}$$

Applying A2 to find the field after the second MLA, cancelling the opposing parabolic phase curvatures:

$$U_L(\alpha,\lambda;y'',z_{L2}^-) = d\,\mathrm{rect}\left(\frac{y''}{p_L}\right)\,\delta(y''-f_L\sin\alpha)\times\mathfrak{F}_{f_L}\left\{|U|\mathrm{rect}\left(\frac{y'}{p_L}\right)\right\} \qquad \text{(A14)}$$

Which is Fourier transformed by the field lens to yield the amplitude distribution in the illumination plane:

$$U_L(y,z_O) = \exp\left(\frac{ikf_L}{f_F}y\sin\alpha\right)\mathfrak{F}_{f_F}\left\{\mathrm{rect}\left(\frac{y''}{p_L}\right)d\mathfrak{F}_{f_L}\left\{U_L(y',z_L^+)\mathrm{rect}\left(\frac{y'}{p_L}\right)\right\}\right\} \qquad \text{(A15)}$$

References:

S1 GR Fowles, Introduction to modern optics, 2nd edition, 1975, Dover Publications, Mineola, NY, USA, p8,22

S2 F. Schreuder, Laser image cytometer for analysis of circulating tumor cells, 2008, PhD thesis, Universiteit Twente, Enschede, The Netherlands, p15,48

S3 M. Bass, Handbook of optics, Volume I, 2nd edition, 1995, McGraw-Hill, NY, USA, p 30.6