

**FLFM Performance calculator manual****I. Prepare configuration file:**

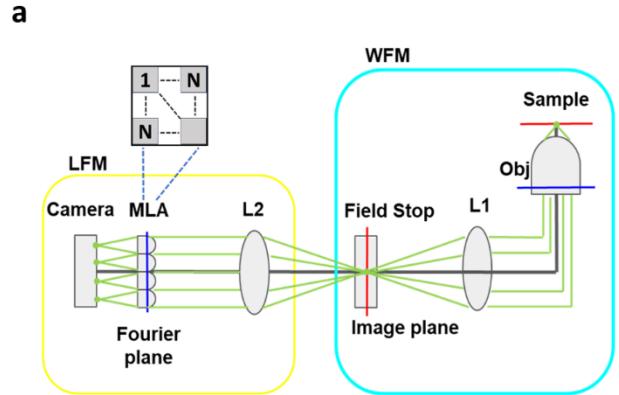
Input hardware parameters into the excel sheet below:

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
gridType square	gridDim 8	Wavelength 0.516	M 2	n 1	NA_real 0.08	NA 0.08	Field_Num 26.5	f1 180	f2 180	fm 29.9	mla2sensor 29.9	lensPitch 1.5	pixelPitch 6.5	spacingPixels 230	MLAump_d 221	Dcam 13

The hardware parameters are considered for our versatile FLM platform [soon to be submitted]. Here, I am showing the parameter description and the schematics of the setup (Figure 1a in the main text).

**Parameter description:**

- gridType: shape of the MLA (does not really matter for this pipeline)
- gridDim: Linear dimension of the MLA (ex: 8 means 8 by 8 square MLA)
- WaveLength: emission light wavelength in  $\mu\text{m}$
- M: Magnification of the primary objective
- n: refractive index of the objective immersion medium
- NA\_real: NA of the primary objective
- NA: NA\_real/n
- Field\_Num: Field number of the objective
- f1: focal length of the tube lens in the IX83
- f2: focal length of L2
- fm: focal length of each len-slet
- mla2sensor: distance between MLA and the camera
- lensPitch: diameter of each lens-let
- pixelPitch: diameter of each pixel
- spacingPixels: distance between adjacent lens-lets in # of pixels
- MLAump\_d: diameter of each lens-let in # of pixels
- Dcam: diameter of the camera in mm.



**MLAump\_d** is updated by the code. Therefore, no need to fill in by yourself.

For our system, once the hardware is built, the only parameters that require changes are the one related to primary objectives (in the red box). For example, if we are imaging with a primary objective of 30x 1.05NA Silicone oil, we just need to type in: M=30, n=1.45, NA\_real:1.05. Then save the file and close it before running the code.

**II. Running calculation code:**

Open *FLFM\_Performance\_calculator.m*. Set your configuration file (configFile.xlsx) in the first section.

**%% set path...**

```
datasetName='C:\Users\yangyanb\Documents\MATLAB\FLFM Design\Doc';
configFile ='configFile.xlsx';
Camera = table2struct(rmmissing(readtable([datasetName,configFile]),2));
```

Run the code and you will see the performance printed on the command window:

```
d_mla= 1500.0um, f_mla= 29900.0um
sampling rate: 1.58 , Number of mla: 5
Ap_DiaRelay= 8689.7 um, Ap_Diaobj= 8689.7 um
DOF:86.9 um, FOV: 288.3 um
Rxy:2.6 um, Rz:4.4 um
Obj: NA_0.72, Mag_30.0 -----
```

Description of the output result:

- d\_mla: diameter of each lens-let
- f\_mla: focal length of each lens-let
- sampling rate: # of pixel per FWHM of the optical diffraction limit defined as  $\frac{\lambda}{2NA}$
- Number of mla: number of lens-let completely illuminated by the relayed aperture at MLA
- Ap\_DiaRelay: Diameter of the Relayed aperture arrived at MLA
- Ap\_Diaobj: Diameter of the primary objective aperture
- DOF: Depth of f View (Field)
- FOV: Field of View
- Rxy: lateral resolution
- Rz: axial resolution
- Obj NA: converted NA using NA\_real/n (same as the NA in the excel sheet, see the Part I)
- Obj Mag: Magnification of the primary objective.

That's all! Contact me if you have any questions. 😊