

Supplemental information S3 – Guideline for modifying an epi fluorescence microscope with double MLA

1. Determine the MLA pairs that are suitable for your microscope:
 - A. Area of illumination should be larger than the field of view of the CCD, keeping in mind that the larger the excess size is, the lower the intensity you will achieve in the field of view. For equal MLA arrays use equation 1 (with $s=f_L$) to determine size of flat top. For combination of different MLA arrays use m-file in supplemental 2 (vary s from smallest of f_{L1} , f_{L2} to $f_{L1}+f_{L2}$). When using different MLA arrays the pitch must be the same for both.
 - B. **Maximum divergence angle should not be larger than what is achievable with a collimator**, see figure 2. Higher maximum angle increases alignment stability. If you use a light source that is significantly different in source size from a 100W Hg arc lamp, you need to repeat the measurements for fig 2. The maximum divergence angle is typically given for MLA distance $2f_L$ (worst case) and can easily be determined for other inter MLA-distances using the simulation program.
2. Modify your epi fluorescence module
 - A. **Distance between second MLA and back pupil of objective is limited**. We determined this relationship on an optical bench using the Meade CCD camera. For the MLA in this paper, the achievable CV starts to increase at 80mm distance from 2.5%, to 10% at 200mm distance. Power is highest at 0 distance, ~90% of peak at 80mm and ~80% at 200mm. If you choose another MLA pair, we strongly recommend you determine this relationship.
 - B. Modify the epi fluorescence module to achieve the distance between objective and the MLA. This will require some precision machining. Figure S3-1 shows pictures of a Nikon epi fluorescence module before and after modification.

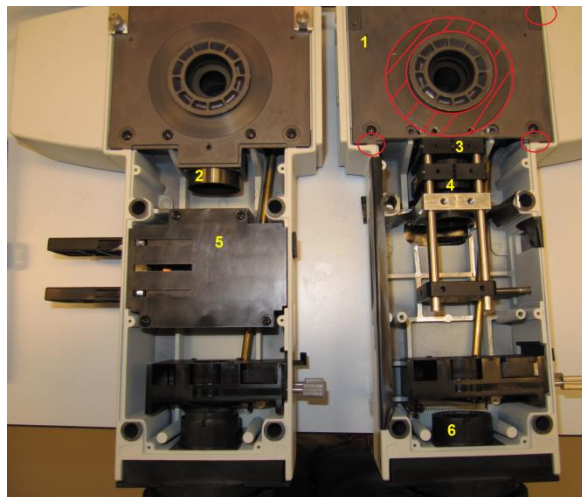


Figure S3-1. Modification of Nikon Eclipse 400 epi fluorescence module. Steps needed for modification of this module shown in yellow: Plate (1) machined to mount MLA close to filter cube (the surfaced used to mount this piece in the machining vice are shown in

red). Lens removed (2/6). MLA (3) and relay lens (4) mounted in cage system. ND filter assembly removed (5).

3. Align the system. We used a cage system with premounted MLA from SUSS, one of the two MLA was adjustable perpendicular to the optical axis (xy) and rotationally. The final adjustments are quite precise ($<10\ \mu\text{m}$), we used long levers to achieve the required precision.
 - A. Rotate the adjustable MLA so that the micro lenses are perpendicular with the optical base plate (by eye).
 - B. Align the the mutual xy orientation or the MLAs (by eye).
 - C. Place the MLAs in the setup.
 - D. Turn on the mercury lamp and use a reflective ND3 filter to attenuate the light. Align the beam on the MLAs by adjusting the xy position of the arc (by eye, that is important!). **Wear UV protective goggles while you do this!**
 - E. Place a power meter under the objective. Adjust the z position of the arc to maximize the power throughput.
 - F. Turn the x set screw of the adjustable MLA clockwise till they can't go further. As you turn counterclockwise, the power will fluctuate. Within a displacement equal to the pitch of the MLA there will be a minimum (lenslets misaligned) and a maximum (lenslets aligned). Determine the power at each (local) maximum. The position with the global maximum is where all lenslets of the first MLA have a mate on the second MLA. Set the x alignment to this setting. In subsequent steps, don't move the MLA by more than half a pitch from this setting.
 - G. Repeat step F for the y direction.
 - H. Remove the power meter and place a CCD camera in the focal plane of the objective (we used the Meade DSI II).
 - I. Rotate the adjustable MLA so that you see a rectangular or squared pattern with crisp borders. Take your time to get this as good as possible, this is critical. Smaller pitch MLAs are significantly more difficult to align for this step.
 - J. You should see some ghost images. If they are more than 50% in intensity of the central illumination area, the divergence of the collimated bundle is too large. If you see no ghost images, move the collimator lens a little along the optical axis to generate them.
 - K. Use the set screws on the adjustable MLA to balance the cross talk illumination between left/right and top/bottom. The Meade DSI II software application is horrendous to use here. We saved images, analysed line profiles in x/y direction in Imagej (<http://rsbweb.nih.gov/ij/>). Then we adjusted the MLA and checked the alignment again. This will get the lenslets aligned perfectly. A real time feedback system could easily be built in Labview, to make this less cumbersome.
 - L. The flat top may still be a little Adjust the xy position of the arc to balance the flat-top. Step K and L have to be repeated till you are happy with the result. The top should now be nearly flat and most non-flatness is symmetrical around the middle of the illumination area, like figure S3-2.
 - M. Repeat steps I,K and L. Due to the mechanical design of the rotation adjustment, it can be affected by the xy adjustments.
 - N. Adjust the lamp along the optical axis to maximize power throughput, this is also the point with maximum flatness.
 - O. Congratulations, you have successfully aligned your first imaging MLA homogenizer!
 - P. When replacing a bulb, just perform steps L and N

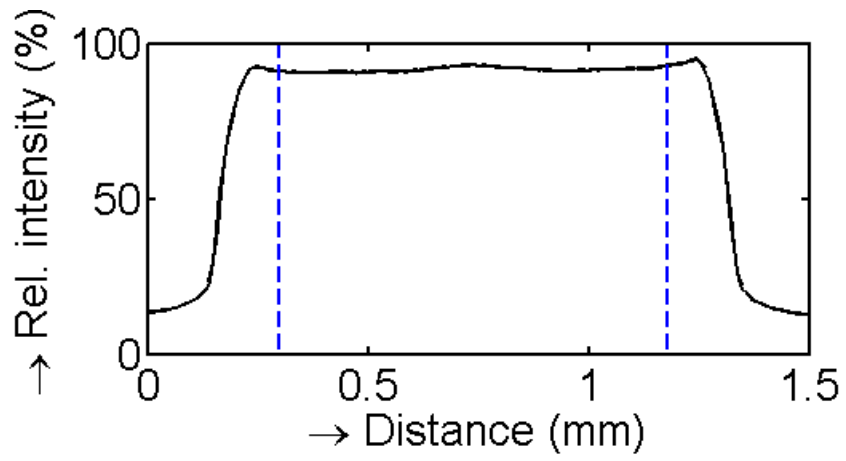


Figure S3-2. Line profile of a well aligned MLA system, with correctly xy aligned arc lamp. The profile is shown in the long direction of the arc, some residual asymmetry is due to the shape of the arc.