Modified procedure for 405 nm laser for FLIM

Startup: Follow the regular procedure steps 1-19, but
First ask Dorus to replace the ND filter in the reference cube to an ND filter that transmits 405 nm light, and ask Dorus to transfer the BNC cable from the AOM-modulator to the MDL300 unit (into RF input).

A) Skip step 1) and 2)
B) Step 3: turn on power supply of MDL300 of the modulated diode (405) laser (button on the rear of the unit). Turn key to ‘on’ on MDL300 (front side left), wait 10 and then turn the ‘SOURCE’ switch on the middle ‘modulation’ panel to ‘ext’ (turn to the left). The laser should now be on immediately.
C) steps 4-12 are the same
D) step 12 set MCP to 200 gain, but use 110 for the cathode DC (instead of 90).
E) steps 13-19 are the same, except for step 17 use 12 phase steps or in case you want high precision use 36 phase steps, and use 2x2 binning.
F) <u>addition</u>: set the frequency of both synthesizers to 80 MHz, ignore the red warning.
G) <u>addition:</u> For reference measurements: check that the intensity in the reference measurements is approximately 3000 counts. If it is lower, then adjust the reference measurement duration by typing in the command window ‘edit calibrate.m’. Change under the fourth laser option the exposure time. Default is 0.1x10^7.

H) Shutdown procedure: follow regular procedure: **Only change**: Step 3: turn off 405 laser MDL300: turn ‘SOURCE’ switch on ‘modulation’ panel to ‘off’ (middle position). Turn key to ‘STBY’. Wait 5 minutes and turn off power supply MDL300. **Ask Dorus to replace the ND filter in the reference cube and change the BNC cable back.**

I) Calibration procedure (for specialists).
The reference cube values should be calibrated at the end of each session. For this move the ND slider (at the back of the microscope) into the middle position, the laser light should be 10x reduced. Increase the reference exposure time in the ‘calibrate.m’ 10x (see procedure F), the default for calibration is 1x10^7.
Then image the <u>blue plastic</u> using the CFP cube. Exposure times of approximately 30 ms/frame are ok. Save the blue plastic images, and note the reference cube phase (φ_R) and the reference cube modulation (M_R)
After finishing calibration measurements, return reference exposure time to default values in the ‘calibrate.m’ macro (0.1x10^7), and move ND filter (back microscope) to open pos.
Save images and calculate a phase image of the blue plastic (in the calculate lifetimes command), and determine the average phase using the histogram command (φ_P).
Also calculate a modulation image of the blue plastic (in the calculate lifetimes command), and determine the average modulation using the histogram command (M_P).
The lifetime of the blue plastic is 0.9113 ns (single exponential).
If you have a frequency of y MHz, then the phase shift relative to the calibration cube should be:

$\Delta\varphi_C = \tan^{-1}(z)$ degrees and the demodulation is: $\Delta M_C = \frac{1}{\sqrt{z^2+1}}$ both with

$z = 5.7259 \cdot 10^{-3} y$

For 80 MHz $\Delta\varphi_c = 24.61°$ and $\Delta M_C = 90.916$ %. Now calculate $\Delta\varphi_O = \varphi_P - \varphi_R$ and $\Delta M_O = M_p / M_R$. Compare these with the predicted values. If the deviation is more than 0.5º or more than 0.5% then you can correct it in the export_to_ij.m macro using: phase_correction=$\Delta\varphi_O - \Delta\varphi_C$ (in degrees) and mod_correction=$\Delta M_O / \Delta M_R$.