Crossbill Design

A GUI to design your own single objective light-sheet microscope

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Introduction

Single (front facing) objective based light-sheet microscope [Fig. 1] is a complicated optical instrument to design and assemble [1-2]. The microscope design is involved as there are multiple optical parts to be chosen, multiple physical conditions to be satisfied, and multiple parameters to be calculated [Fig. 1]. Here, we present a simple GUI (graphical user interface) to empower the users to design their own microscope configuration and evaluate their theoretical feasibility before investing in buying parts and assembling the microscope.
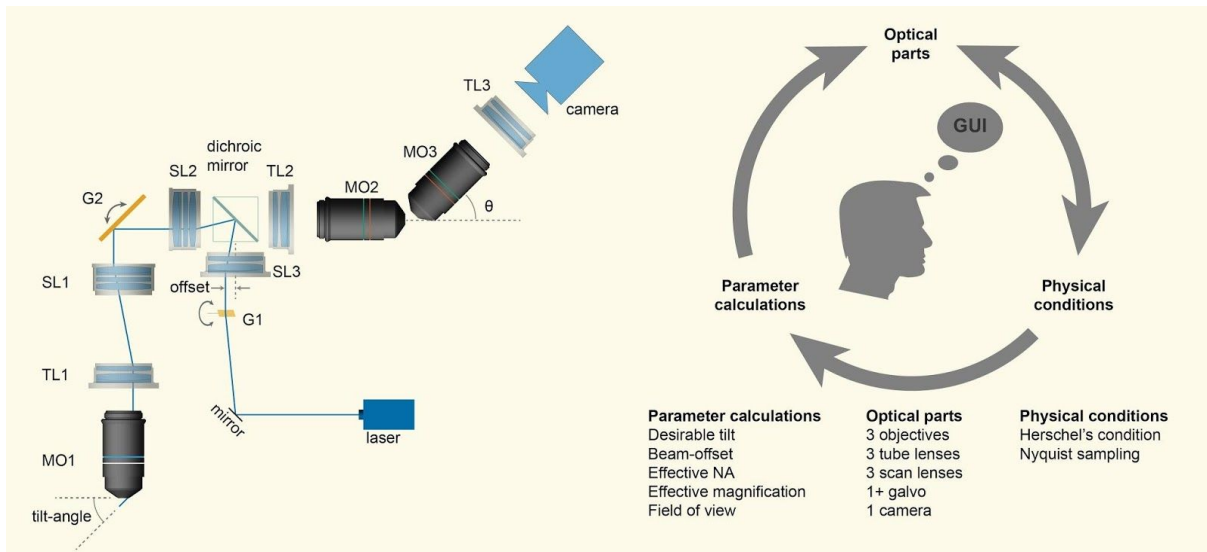

Fig. 1: Typical configuration of a single objective light-sheet microscope (left). A GUI may simplify the microscope design process (right).

About the GUI

The GUI provides a logical flow to design a single objective light-sheet microscope. It helps a user to choose the components in the correct order, with the implications of their selection visible in the corresponding text boxes for immediate re-evaluation of the selection. This helps optimize the whole design process and helps the user recognize the limitations of each

parameter. The default starting values in the GUI are based on the published SOPi setup [2]. A user can modify the values as detailed below.

1. Choose/enter MO1 and MO2 parameters and click the top most button (near the top right corner) to register these values. The text output box below the button states the registered values and gives additional information about how you can meet the Herschel condition [3] in the system. The choice of these two objectives also decides the maximum tilt of the light-sheet. If unhappy with the results, modify the MO1 and MO2 parameters and click the button again to register new values.

2. Enter tube-lenses TL1 and TL2 and scan-lenses SL1 and SL2 focal length values and click the 2nd button to register these values. Figure 1 specifies the position of each of these components. They determine whether Herschel condition is satisfied to meet minimal aberration imaging. The GUI will flag the condition as unsatisfied when the error in TL and SL focal length selection is more than 5%. Modify the TL and SL focal lengths to satisfy the Herschel condition. If it is not possible to satisfy this condition, one may proceed further with the selection but many of the calculated parameters would become unreliable.

3. Choose/enter MO3, TL3, and Camera parameters and click the 3rd button to register their values. They determine the maximum system NA limit, overall magnification, and whether the Nyquist sampling condition is satisfied. If unhappy with any of these outcomes, keep modifying the parameters and clicking the button to check their effect on the outcomes.

4. Enter SL3, light-sheet tilt, FN1, FN2, and FN3 parameters and click the 4th button to register them. They determine the beam-offset, actual numerical aperture, and maximum field of view of the system as displayed in the corresponding text output box. If maximum allowed field of view is larger than the camera limited field of view (as displayed in the previous text output box), one can modify the camera/MO3/TL3 focal length parameters to make use of the available field of view effectively.

As is clear from the above description, every button click makes the GUI register the parameters and provides useful feedback in the corresponding text output boxes. The buttons follow top-down hierarchy i.e. pressing any button also simulates all the previous button presses. Whenever the last (4th) button is pressed, a summary log of all the entered parameters along with their implications is appended in a text file. The text file is written in the same parent directory/folder and provides a valuable log for future reference and analysis.

Definitions of the parameters in the GUI

1. **Make/design tubelens FL:** Every infinity corrected microscope objective is designed for a particular tube lens focal length. The printed magnification value on an objective is valid only when used with the corresponding focal length tube lens. Select the correct parameter from the dropdown menu. If using a custom objective, make the selection corresponding to its design tube lens focal length.

2. **Magnification:** Select from the dropdown menu based on the printed value on the microscope objective.

3. **NA:** Enter the numerical aperture of the microscope objective in this box.

4. **Immersion media R.I.:** Enter the immersion media of the objective as per design. Do not use an objective with any other immersion media, as it would lead to additional optical aberrations.

5. **TL:** Tube lens. These are the lenses which immediately follow microscope objectives in the optical path. TL focal lengths, in conjunction with the printed magnification and design tube lens focal length of microscope objective, determine the effective magnification. Although called as tube lens, an achromatic doublet (or another) lens can be used with some compromise on image quality.

6. **SL:** Scan lens. These lenses help relay the back focal plane of the first two objectives to the rotation axis of the galvo scanner. The third scan lens determines the relationship between beam offset and desirable light-sheet tilt. Although called as scan lens, an achromatic doublet (or another) lens can be used with some compromise on image quality.

7. **Camera pixel size:** Enter the size of individual camera pixel in µm. It assumes square pixels so only one side length needs to be entered.

8. **Pixels along width/pixels along height:** Enter the corresponding counts of pixels along the width and height of the camera sensor.

9. **Light-sheet tilt:** Enter the desired light-sheet tilt angle. The GUI uses this parameter to calculate the overall numerical aperture of the microscope system and the corresponding beam offset.

10. **FN:** Field number. Field number of an objective specifies its effective field of view. FOV = FN/magnification (in mm).

11. **Beam offset:** This is an output parameter from the GUI. Beam offset is the amount of required lateral offset in the illumination beam to obtain the desired light-sheet tilt. This parameter is important for the experimental setup and also constrains the smallest possible mirror size of the galvo scanner.

12. **Clearance angle:** This is a parameter to consider for mechanical compatibility of two objectives [MO2 and MO3]. Clearance angle, as the name suggests, specifies the maximum angle at which a sample plane can be approached without clashing with the objective.
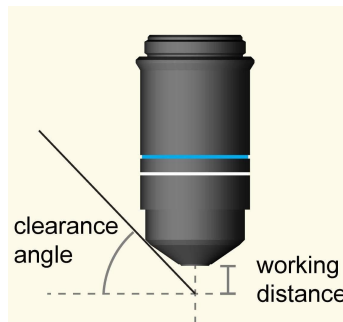

Fig. 2: Clearance angle of a microscope objective

How to run the GUI

The GUI is written as a jupyter notebook in Python. It depends on PyQt5, which is a set of Python bindings for Qt v5. The easiest way to run the GUI is by installing anaconda distribution (https://www.anaconda.com/distribution/#download-section) on your machine. The latest anaconda distribution (python 3.7 at the time of this writing) assembles all the required packages to run the provided jupyter notebook.

If you do not wish to use the anaconda distribution and prefer to build your own environment from scratch, then you will need the following packages/modules in your environment:

- Python 3.7+ (older 3.X versions should also work)
- Jupyter lab
- Nbconvert (not required if not running from terminal or not converting to .py executable)
- PyQt5

Once you have the proper installation:

1. Download the provided .ipynb file on your local machine.
2. Run the file in your preferred way of running any jupyter notebook.
   If you prefer command line control, then open a terminal and run the following command:
   ```
   jupyter nbconvert --to script --execute CrossbillDesign.ipynb
   ```

Some examples to run

Following table provides some examples (all from published papers) to explore in the GUI. Dig in the provided ipynb file to see the correct way to calculate effective NA of an oblique light-sheet system.

	MO1 (Make,Mag,NA, R.I.)	MO2 (Make,Mag, NA,R.I.)	MO3 (Make,Mag, NA,R.I.)	TL1, TL2, TL3 (FL in mm)	SL1, SL2,SL3 (focal lengths in mm)	Camera pixel size, (W x H)	Tilt
SOPi	Olympus, 20x, 1.0, water (1.333)	Olympus, 20x, 0.75, air (1.0)	Olympus, 20x, 0.45, air (1.0)	200, 150, 80	100, 100, 100	5.86 µm, 1920x1200	45
OPM	Olympus, 60x, 1.35, oil (1.5?)	Olympus, 40x, 0.85, air	Olympus, 10x, 0.3, air	100, 100, 350			60
SCAPE	Olympus,20x, 1.0, water	Olympus, 20x, 0.75, air	Olympus, 20x, 0.40, air	100, 100, 75	50,50, 50 (cylindrical)	6.5 µm, 2560x2160	20-40
eSPIM	Nikon, 60x, 1.27, water	Nikon, 100x, 0.9, air	Nikon, 60x, 1.0, water	200, 150, 125	75, 125, (unknown)	6.5 µm, 2048x2048	60

You now have all the power in your hands to design a new working configuration of the microscope!

Limitations

1. The GUI does not estimate all optical aberrations. If you use lenses with significant aberrations, the results will not match the theoretical predictions of the GUI.
2. The GUI does not estimate mechanical compatibility of various components. For example, using a lens with 20 mm focal length may be feasible as per the GUI but may not be possible in the actual setup. A detailed mechanical drawing of the objectives and other components is required to evaluate their mechanical fit.

3. The GUI estimated field of view does not take account of lens aperture size. So a small aperture scan or tube lens may further limit the field of view.

References

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2. M. Kumar, and Y. Kozorovitskiy, "Tilt-invariant scanned oblique plane illumination microscopy for large-scale volumetric imaging," *Optics Letters*, **44** (7), 1706-1709 (2019) https://doi.org/10.1364/OL.44.001706
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