#### Quick user manual

Mostafa Aakhte<sup>1</sup> Ehsan A. Akhlaghi <sup>2,3</sup>

H.-Arno J. Müller<sup>1</sup>

<sup>1</sup> Institute for Biology, Division of Developmental Genetics, University of Kassel, Heinrich-Plett Str. 40, 34132 Kassel, Germany

 $^{\rm 2}$  Department of Physics, Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan 45137-66731, Iran

<sup>3</sup> Optics Research Center, Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan 45137-66731, Iran



#### Introduction

The following document is a quick user manual of SPPIM toolbox which offers an open source toolbox for creating a hologram/SLM pattern to achieving appropriate optical beams for a wide range of light sheet-SPIM application. The toolbox is implemented in MATLAB's GUI environment and it can work standalone, as well. With SSPIM any users such as special and non-special are able to create a SLM pattern for a wide range of optical beams. The toolbox can be used for all type of the optical beams that have been used in light sheet-SPIM such as, single and array beam. In single beam category, SSPIM supports the static Gaussian, scanning Gaussian, 1D Airy beam, scanning 2D Airy and Bessel beam. In addition, incoherent and coherent optical beams like as Gaussian array beam, Bessel array beam and Lattice beam are supported with the SSPIM toolbox. A user is able to design an arbitrary hologram for two types of the SLM such as binary SLM and gray value SLM, then the far-field diffraction intensity of the hologram(SLM pattern) will be predicted as a feedback before implementing into the optical setup. It should be noted that the software can be used in a conventional setup of a light sheet-SPIM without need to changing the structure of the setup, especially for lattice light sheet.



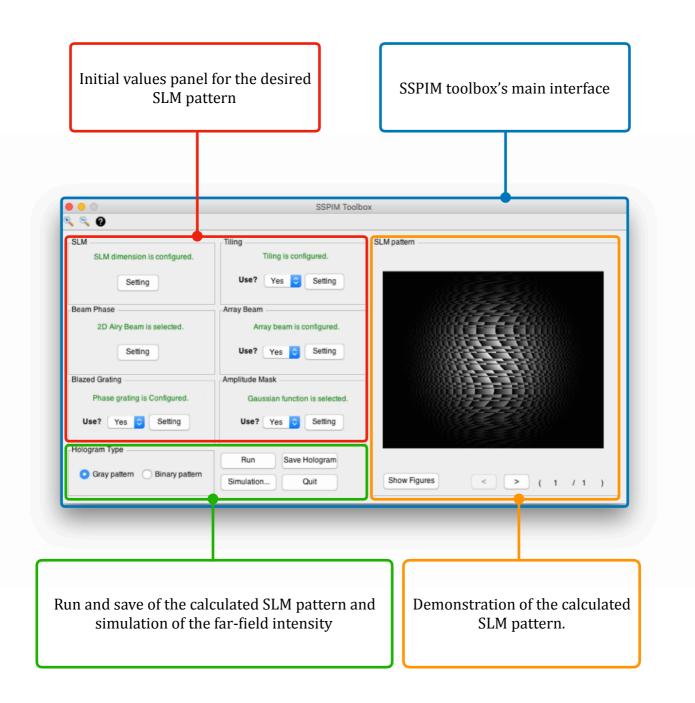
## Contents

1. SSPIM's main graphical user interface (GUI)	4
2. SSPIM's sub-GUIs	
2.1. SLM's panel	
2.2. Beam Phase panel	
2.3. 1D and 2D cubic phase mask (Airy beams)	
2.4. Annular mask (Bessel beam)	
2.5. Cylindrical phase mask	10
2.6. Spherical phase mask	11
<ul><li>2.6. Spherical phase mask</li><li>2.7. Lattice beam</li></ul>	12-14
2.8. Blazed Grating	15
2.9. Tiling	16
2.10. Array beam	17
2.11. Amplitude mask	
2.12. Save hologram	



## SSPIM's main graphical user interface (GUI)

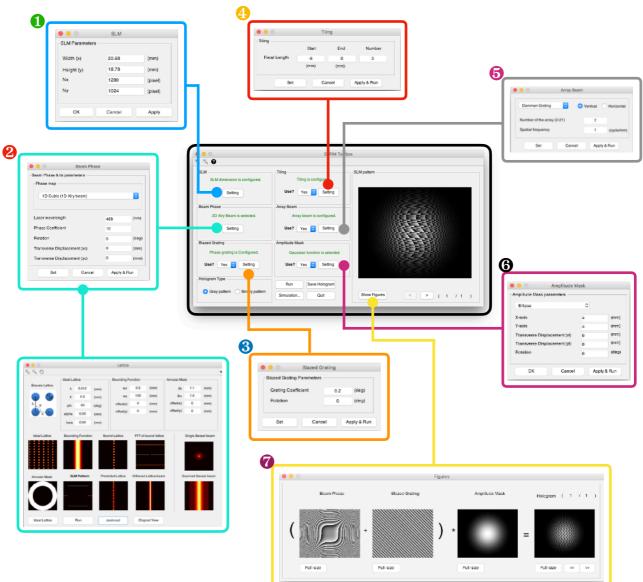
The main interface of the SSPIM toolbox is included three sections. The first section is designed for design a hologram or SLM pattern. The second part is for selection of the hologram type, running, simulation and saving the hologram(SLM pattern). The final section demonstrates the output of the SSPIM.





#### SSPIM's sub-GUIs

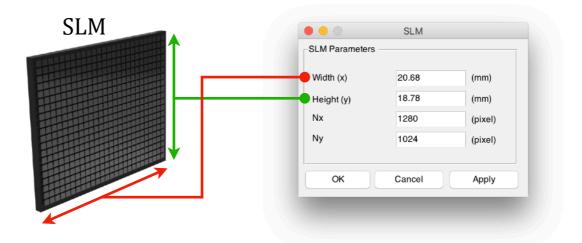
SSPIM offers a wide range of holograms for creating various beams which have arbitrary properties. All the parameters such as pixel number of SLM, the desired phase mask and its properties can be set with different sub-GIUs. SSPIM is included different sub-GUIs, such as SLM, Beam phases, Blazed Grating, Tiling, Amplitude mask and Array beam. All of the mentioned sub-GUIs can help the user to design an appropriate hologram for the specific application, axial resolution and field of view. In the first step, the dimension and number of the pixels of SLM can be set. In the second step, the parameters and position of the desired phase map or amplitude can be selected and adjusted manually. Then, a blazed or binary grating for separating the unwanted background due to SLM structure can be applied. The tiling method for increasing the field of view with keeping the axial resolution can be applied in the fourth step. If the user needs to create an array of the selected beam phase or amplitude the parameters of the fan-out elements can be set easily in the fifth step. Furthermore, the numerical aperture of the illumination beam can be adjusted with an amplitude mask (sixth step). In addition, the work-flow ion the SSPIM to creating a hologram is achievable with figures sub-GUIs.





## SLM panel

In SLM panel user is able to set the SLM parameters such as size of the width and height and the number of the active pixels in width and height, respectively.





#### Beam Phase panel

In beam phase panel, the user is able to select the six various phase or amplitude masks, such as 1D cubic (1D Airy beam), 2D cubic (2D Airy beam), annular (Bessel beam), Cylindrical lens, Spherical lens and Lattice. The parameters of the selected mask such as phase coefficient, rotation and transverse displacement can be adjusted with following sub-menu.

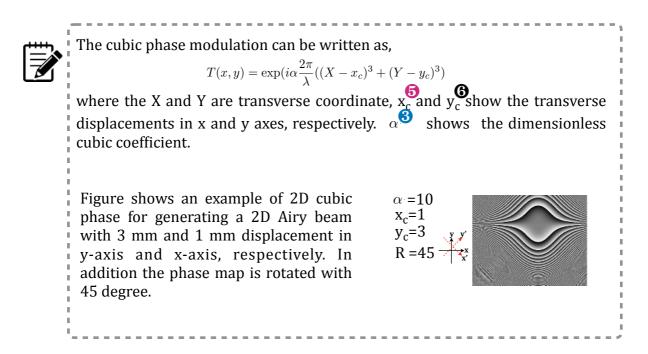
Phase map			Phase map			
1D Cubic (1D Airy beam)			✓ 1D Cubic (1D Airy beam)			
			2D Cubic (2D Airy beam) Annular mask (Bessel beam)			
			Cylindrical lens			
Laser wavelength	488	(nm)	Spherical lens Only Grating		(mm)	
Phase Coefficient	10	- 11	Lattice	10		
Rotation	0	(deg)	Rotation	0	(deg)	
Transverse Displacement (xc)	0	(mm)	Transverse Displacement (xc)	0	(mm)	
Transverse Displacement (yc)	0	(mm)	Transverse Displacement (yc)	0	(mm)	



## 1D and 2D cubic phase mask (Airy beams)

In order to produce a 1D or 2D Airy beam, the 1D or 2D cubic phase can be selected as phase maps, respectively. To creating a phase make for Airy beam, the wavelength of illumination laser? and coefficient of the cubic phase should be defined. The cubic phase coefficient controls the invariant propagation length and the axial resolution (see note). For high accuracy control of the position or orientation of the optical beam than to the optical axis, the phase map can be easily adjusted with rotation (R) and transverse displacements in x<sup>5</sup> and y-axis.

Phase map		
1D Cubic (1D Airy beam)		٢
Laser wavelength	488	(nm)
Phase Coefficient	10	
Rotation	0	(deg)
Transverse Displacement (xc)	0	(mm)
Transverse Displacement (yc)	0	(mm)
Set Cancel	Apply	& Run

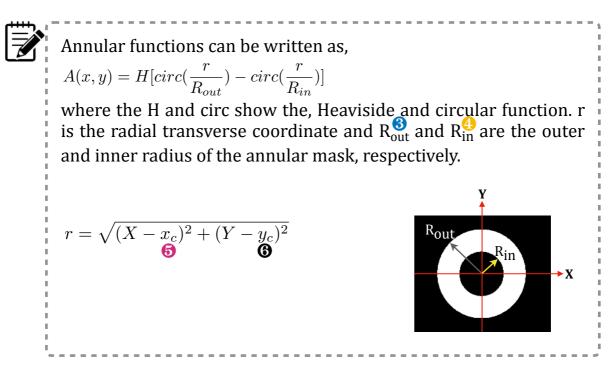




### Annular mask (Bessel beam)

Fourier transformation of a ring is Bessel function. Hence, in order to create a Bessel beam, an annular mask  $\bullet$  can be used for creating a light ring, then the far-field intensity of the light ring can give a Bessel beam. An annular mask can be created with combination of the two circular functions with different radius(Rin<sup>3</sup>, Ro<sup>4</sup>) (see note). For high accuracy control of the mask than the incident beam the transverse coordinate of the mask can be controlled with transverse displacement parameters (xc<sup>5</sup>, yc<sup>6</sup>).

0		_
Annular mask (Bessel beam)		$\bigcirc$
aser wavelength	488	(nm)
R_in (radius)	3	(mm)
R_out (radius)	4	(mm)
Fransverse Displacement (xc)	0	(mm)
Fransverse Displacement (yc)	0	(mm)

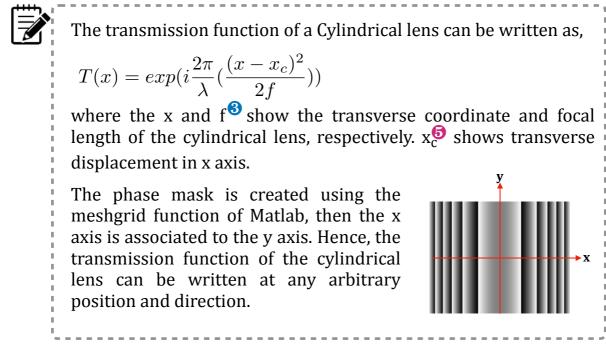




## Cylindrical phase mask

The conventional static light sheet is achievable by using a simple Cylindrical lens. The transmission function of a Cylindrical lens can be written as a quadratic function in one dimension (see note). The focal length, orientation and position of the Cylindrical phase mask than to the optical axis can be easily controlled with this sub-menu.

Phase map			
Cylindrical lens	<b>\$</b>		
Laser wavelength	488	(nm)	
Focal Length	20	(mm)	
Rotation	0	(deg)	
Transverse Displacement (xc)	0	(mm)	
Transverse Displacement (yc)	0	(mm)	
Set Cancel		& Run	





## Spherical phase mask

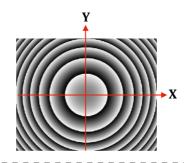
In order to compensation of the spherical aberration or changing the position of the focused light a quadratic phase mask (spherical lens) can be added to the initial illumination beam. In phase map sub-menu the focal length and transverse displacement can be adjusted.

Phase map		
Spherical lens		<b></b>
Laser wavelength	488	(nm)
Focal Length	40	(mm)
Rotation	0	(deg) (mm)
Transverse Displacement (xc)	0	
Transverse Displacement (yc)	0	(mm)

The transmission function of spherical lens can be written as,

$$T(x,y) = \exp(i\frac{2\pi}{\lambda}(\frac{(x-x_{c})^{2} + (y-y_{c})^{2}}{2f}))$$

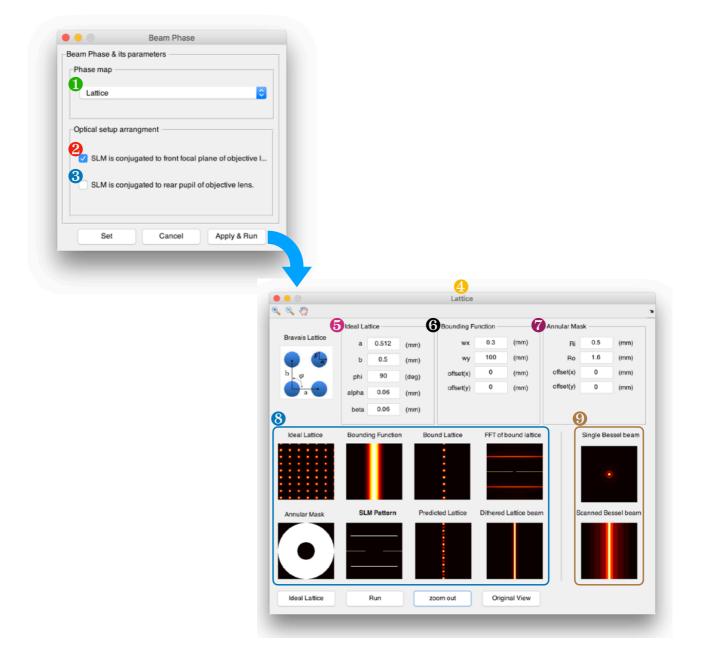
where the x, y and  $f^{\textcircled{3}}$ show the transverse coordinate and focal length of the spherical lens, respectively.  $x_{c}^{\textcircled{5}}$  and  $y_{c}^{\textcircled{6}}$  shows transverse displacement in x axis.





#### Lattice beam

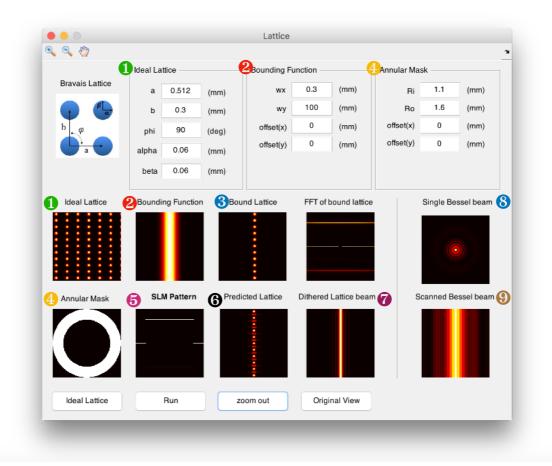
Another type of interesting and well-known illumination beam with a wide range application in light sheet microscopy is lattice beam. To optimize of a lattice light sheet, the SLM pattern should be correctly adjusted. The tool for this propose can be found in lattice sub-GUI<sup>4</sup> in SSPIM toolbox. In beam phase map panel with choosing the lattice, <sup>1</sup> user can select one of the setup arrangement. It means, the position of the SLM than to rear pupil of illumination objective lens can be selected in optical setup arrangement panel.<sup>23</sup> Then, the lattice panel related to the demanded setup arrangement will be appeared.<sup>4</sup> The lattice panel is included several parts such as ideal lattice,<sup>5</sup> bounding function,<sup>6</sup> annular mask<sup>7</sup> and the work-flow<sup>8</sup> of producing of a SLM pattern for desired lattice beam. In final the predicted lattice sheet can be compared with the equivalent Bessel sheet.<sup>9</sup>





#### Work-flow of the Lattice beam sub-GUI

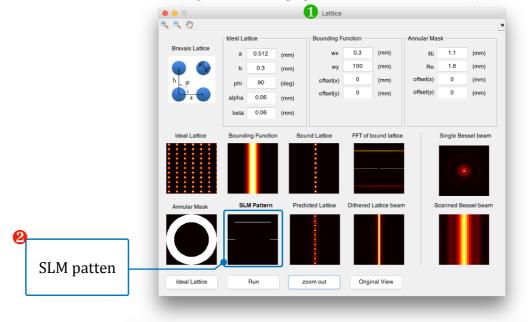
The first step of creating a SLM pattern for a lattice beam is defining a two dimensional ideal lattice. An ideal 2D lattice is a periodic structure that is included an array of infinite unit spot cells. The size and spatial coordinate of an unit spot cells can be defined by user in ideal lattice panel. Then, a Gaussian function can be set to creating a 1D-like lattice in Bounding function panel. In this panel, the width (wx and wy) and spatial position (offset(x) and offset(y)) of the Gaussian function in x and y-axis can be defined. For creating an coherent array of the Bessel beam and selecting the arbitrary spatial frequencies of bound lattice, an annular mask can be used. The size of the outer and inner ring of the mask can be easily adjusted by user in annular mask panel. Then, the SLM pattern (or intensity at rear pupil) and cross section of the predicted lattice beam and dithered lattice beam will be calculated immediately. The predicted lattice sheet will be compared with equivalent Bessel sheet. Is should be noted that the Bessel beam is resulted using the defined annular mask.





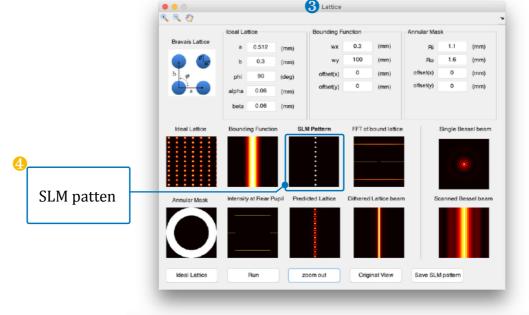
#### Lattice beam

SLM pattern for generating a lattice beam depends on the position of the SLM into the microscopic setup. If the SLM is conjugated to the rear pupil of the illumination objective lens, the SLM pattern is the binarized  $(0, \pi)$  diffraction pattern of the bound lattice that filtered by an annular mask. Otherwise, if the SLM is conjugated to the front focal plane of illumination objective **3**lens the SLM pattern is binarized  $(0, \pi)$  bound lattice.



SLM is conjugated to rear pupil of the illumination objective lens

SLM is conjugated to the front focal plane of illumination objective lens





#### **Blazed Grating**

A spatial light modulator (SLM) has a pixelated structure. Hence, the diffracted light from phase mask is added to the undesired pattern due to pixelated structure of SLM. In order to separation of the desired pattern, an asymmetry can be introduced into the phase mask. A Blaze grating can be added to the phase mask. On another hand, a Blazed gating is a linear phase map with a specific slope can shift the center of the diffracted light (see note).

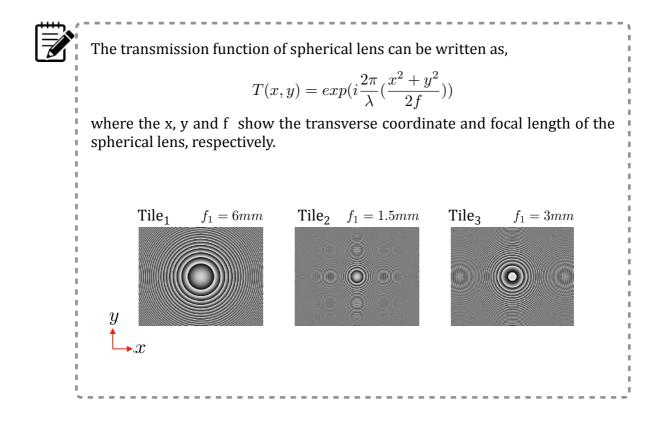
	🔴 😑 💿 🛛 Blazed	Grating	
	Blazed Grating Paremete	rs	
	Crating Coefficient	0.2 (deg	
	3 <sub>Rotation</sub>	0 (deg	
	Set Ca	Apply & F	Run
A linear phase map	can be written as	,	
	$\phi(x) = a$	x	
	anel the angle of t		coordinate, respectively. e can set that is related to
$\phi(x)$			y 3 Rotation
a =	$\phi(x) = ax$ $x$ $= tan(\theta)$		
Fig1			Fig2
this sub-GUI. In add	ition, the orienta It should be not	tion of this gra	matically calculated with ating can be controlled by nal blazed grating will be



#### Tiling method

High resolution imaging in light sheet-SPIM demands a narrow illumination light sheet. While with minimizing the illumination beam waist the field of view will be minimized. For compensation the field of view, the minimized illumination beam can be swept in beam propagation axis. For this purpose, the center position of the focused beam should beam changed with using several quadratic phase maps. The quadratic phase map is transmission function of a spherical lens that can be defined with a focal length (see note). The start<sup>1</sup> and end focal lengths<sup>2</sup> and the number<sup>3</sup> of the tiling beams can be inserted in tiling panel. For example, if the start value is -6 and end value is 3 for 3 number of tiling phase map we will have three quadratic phase maps with 6, 1.5 and 3 focal length. The minus and plus sign demonstrate the concave and convex lens, respectively.

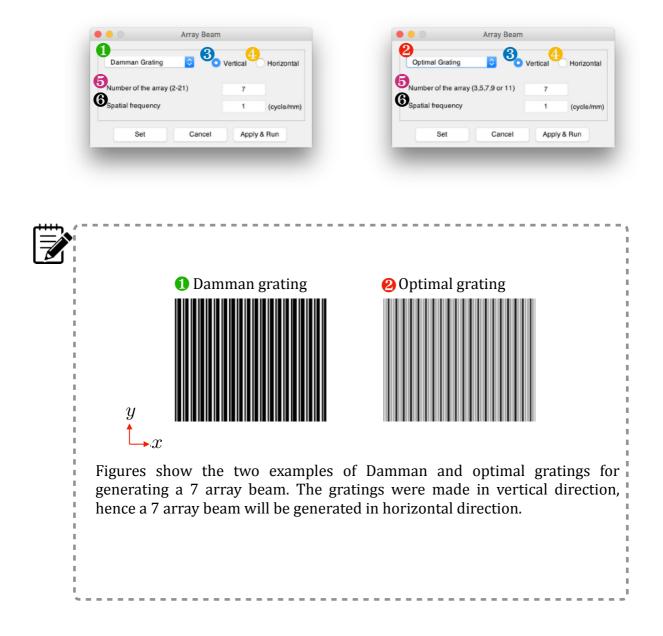






#### Array beam

A well-known light sheet-SPIM mode to improvement of imaging resolution and contrast is structured illumination that is called SIM mode. In contrast to conventional light sheet mode, in this mode, an array beam illuminates a sheet of sample several times. An array beam can be produced using a fan-out element. Damman<sup>1</sup> and optimal<sup>2</sup> grating are known diffractive optical elements that can make n copies of the incident beam. The Damman grating is a binary pattern (0 or  $\pi$ ) which can make an incoherent array beam in vertical<sup>3</sup> or horizontal direction from 2 to 21 beams. In addition, SSPIM supports the optimal grating for creating a gray value pattern (0-2 $\pi$ ) for uniform array beam for 3, 5, 7,9 and 11 beams. The parameters (number of spots<sup>6</sup> and spatial frequency<sup>6</sup>) of these fan-out elements can be set in Array beam sub-GUI manually by the user.

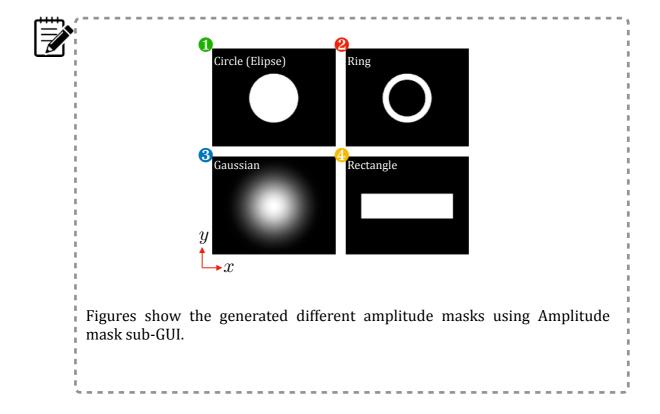




#### Amplitude mask

The numerical aperture of the illumination beam can be easily adjusted with a truncation function or an amplitude mask. Considering the desired numerical aperture and spatial shape of the illumination beam the function of the amplitude mask can be chosen. For this purpose, SSPIM supports different amplitude function such as ellipse, ring, Gaussian and rectangle<sup>4</sup> function.

Ellipse	0		✓ Ellipse		
			Ring Gaussian		
X-axis	4	(mm)	Rectangle	4	(mm)
Y-axis	4	(mm)	T-dxis	4	(mm)
Transverse Displacement (xt)	0	(mm)	Transverse Displacement (x	t) O	(mm)
Transverse Displacement (yt)	0	(mm)	Transverse Displacement (y	i) 0	(mm)
Rotation	0	(deg)	Rotation	0	(deg)





#### Save Hologram

In final step, the generated hologram or SLM pattern<sup>1</sup> can be exported for any type of the SLM such as binary<sup>2</sup> and gray value<sup>3</sup>

