# Portable Scanless Microscope

## Two- and One-photon Imaging and Photostimulation with Spatial Light Modulators

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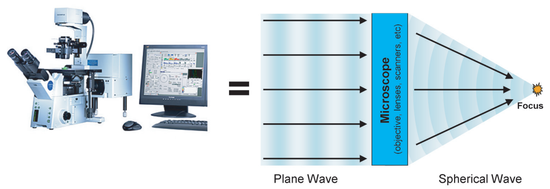
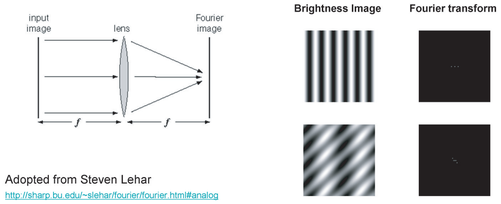
### Microscopes are essentially phase modulators

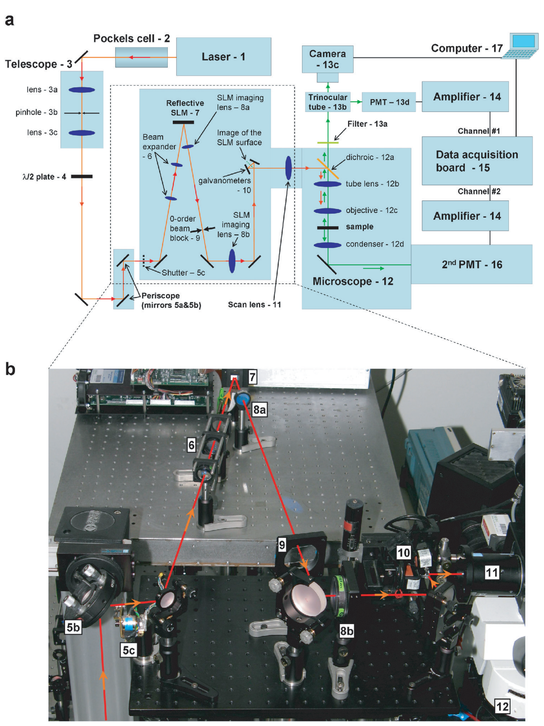



### Examples of Optical Fourier Transform

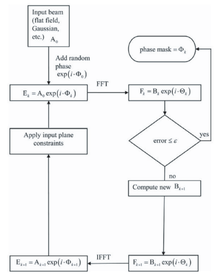



Adopted from Steven Lehar
http://sharp.bu.edu/~slehar/fourier/fourier.html#analog

### I[st] Generation two-photon SLM microscope

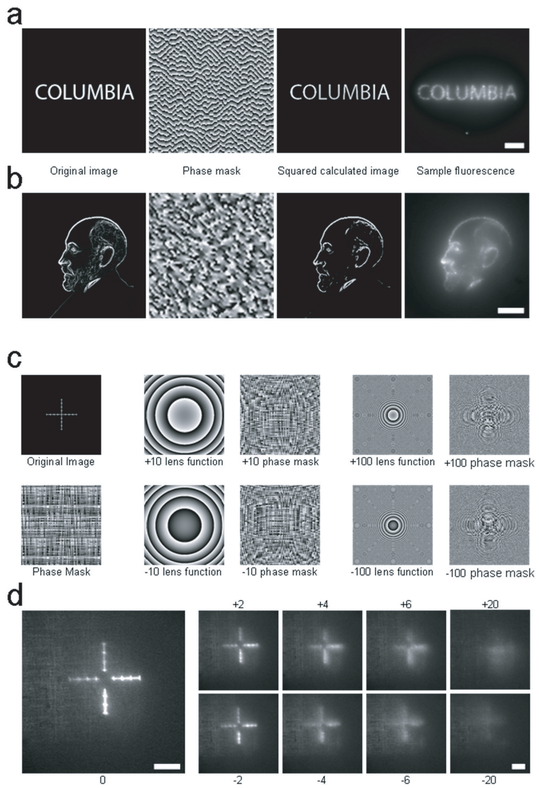



### SLM Phase Mask Formation




- Phase-only modulation has advantage of preserving total power
- It is crucially important for non-linear microscopy – two-photon imaging or photostimulation

### SLM-microscope Patterns Examples: Two-Photon Fluorescence

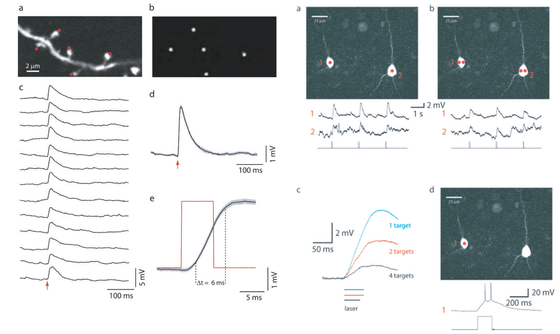



**Two-photon fluorescence images generated by SLM on a gel infused with fluorescence dye**
60x 0.8 NA objective.

**A-B.** Sample patterns are on the left, and the obtained phase mask is shown in the second panel. Grayscale corresponds to phase shift from 0 to 2π. The resulting two-photon fluorescence image of acquired with microscope CCD camera from the sample is on the right. For comparison, the output of the phase mask was also used to calculate the projected pattern, and was squared to better resemble a two-photon process (third panel). Scale 20 μm.
A. A simple Binary bitmap pattern ("COLUMBIA"),
B. Complex Gray-Scale patterns (Santiago Ramón y Cajal)

**C-D.** Focusing with an SLM.
C. A lens function to shift the focus of excitation in axial dimension. The panel shows the original image and corresponding phase mask, as well as lens phase function alone and added to original phase mask. -10, -100, +10 & +100 are arbitrary units to indicate correspondingly negative/positive lens and relative optical strength.
D. Two-photon fluorescence image of the test pattern. The virtual focus plane is moved away in both directions from the original plane using a lens function of corresponding strength. Scale 50 μm.
**These data illustrate that SLMs can be used as a "universal scanners" that do not require physically moving parts.**

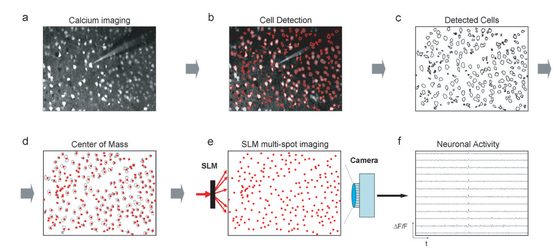
### Applications of the SLM-microscope: Two-Photon Photostimulation




**Micro-objects – dendritic spines**
Twophoton glutomate uncaging elicits EPSP like events

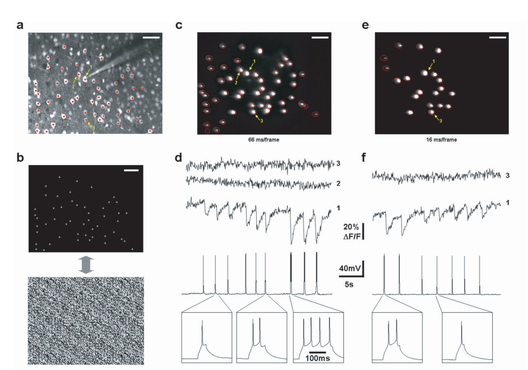
**Circuits - glutomate uncaging to photostimulate triggering action potentials**

### Applications of the SLM-microscope: Fast Multi-Beam Imaging




**The strategy for SLM multispot imaging**

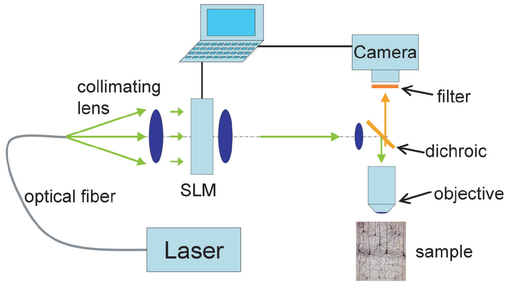
**A.** An image of the objects (for example a two-photon fluorescence image of neurons bulk-loaded with a calcium indicator or transgenic neurons labeled with fluorescent proteins) is acquired.
**B.** Contours of objects of interest are detected
**C.** Pixel centers of mass of each contour are calculated, and their coordinates (**D**) are used as a command image uploaded to SLM.
**E.** SLM works as an intelligent beamsplitter to illuminate all or as subset of objects of interest. A wide-field detector (a CCD camera / photodiode arrays or multi-anode PMTs), with spatial resolution suitable to resolve individually illuminated objects, is used to record time lapsed signals from all illuminated spots simultaneously.
**F.** Optical signals from the objects, acquired simultaneously. If more than one pixel of the detector is allocated to each illuminated spot, the integration step is necessary, in order to extract time lapsed signals corresponding to different objects. Contours detected during steps B-C could be used for this integration.



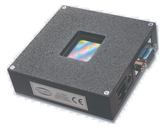

**Practical SLM multispot imaging of neurons**

**A.** A neocortical slice was bulk-loaded with a Ca indicator (Fura-2AM/mag-Indo-1AM mix). This image was taken using standard 2-photon raster imaging mode. Fifty neurons were targeted for imaging using diffractive SLM. One of the neurons was targeted for patch-clamp recording in order to trigger action potentials using current injection.
**B.** Command image file for SLM software and corresponding phase mask. Scale 50 μm
**C.** The image of two-photon fluorescence from multiple locations obtained with the camera. Diffractive SLM splits laser beam in order to continuously illuminate spatially different locations with a static pattern (~4.4 mW per spot).
**D.** Calcium signals recorded from stimulated cell (1) corresponding to different number of elicited action potentials. Even individual spikes can be detected with good signal to noise ratio. Neurons 2 and 3 were not stimulated. Imaging was performed with ~15 frames/second temporal resolution (66 ms/frame).
**E-F.** Similar results were obtained with ~60 frames/second (16 ms/frame). No noticeable photobleaching or photodamage was observed over the course of the experiment (several minutes of continuous illumination).

### II[nd] Generation two-photon SLM microscope – keep things simple

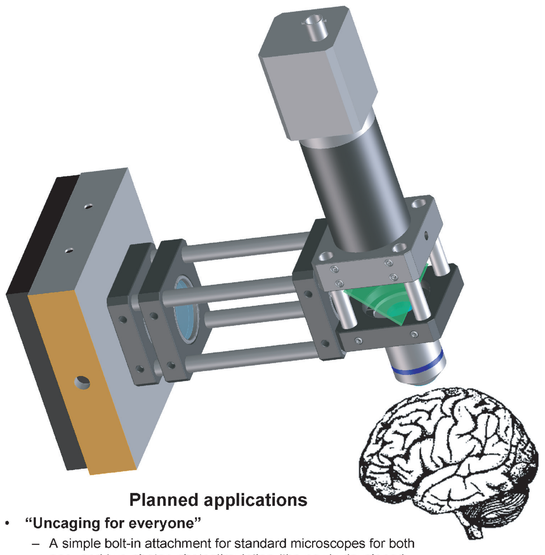



No mechanically moving parts !
relatively simple hardware and all optical control can happen in software



- Transmissive Liquid-Crystal SLM, Holoeye LC 2002
- 2-π modulation, 8-bit addressing
- 800x600 pixels
- Standard VGA interface
- 60 Hz Image Frame Rate
- 32 um pixel pitch => ~1.5-2 degrees effective angle of modulation for the 1[st] order

## The "PocketScope"



### Planned applications

- **"Uncaging for everyone"**
  - A simple bolt-in attachment for standard microscopes for both one- and two-photon photostimulation (the newly developed RuBi-family of caged compounds or genetic methods)
- **Portable version**
  - Further miniaturization for possible in-vivo / medical uses

**Acknowledgments**
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**References**

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