Scaling Up Multiphoton Neural Scanning: the SSA algorithm

Renaud Schuck, Luca A. Annecchino and Simon R. Schultz *EMBS Member*

***Abstract*— In order to reverse-engineer the information processing capabilities of the cortical circuit, we need to densely sample neural circuit; it may be necessary to sample the activity of thousands of neurons simultaneously. Frame scanning techniques do not scale well in this regard, due to the time "wasted" scanning extracellular space. For scanners in which inertia can be neglected, path length minimization strategies enable large populations to be imaged at relatively high sampling rates. However, in a standard multiphoton microscope, the scanners responsible for beam deflection are inertial, indicating that an optimal solution should take rotor and mirror momentum into account. We therefore characterized the galvanometric scanners of a commercial multiphoton microscope, in order to develop and validate a MATLAB model of microscope scanning dynamics. We tested the model by simulating scan paths across pseudo-randomly positioned neuronal populations of differing neuronal density and field of view. This model motivated the development of a novel scanning algorithm, Adaptive Spiral Scanning (SSA), in which the radius of a circular trajectory is constantly updated such that it follows a spiral trajectory scanning all the cells. Due to the kinematic efficiency of near-circular trajectories, this algorithm achieves higher sampling rates than shortest path approaches, while retaining a relatively efficient coverage fraction in comparison to raster or resonance based frame-scanning approaches.**

I. INTRODUCTION

Understanding information processing at the neural circuit scale is currently a major bottleneck to progress in understanding and treating brain disorders. To advance our understanding of neural circuit function, we need to densely and near-simultaneously sample the activity of large populations of neurons under controlled conditions. Calcium imaging by multiphoton laser scanning microscopy (MPLSM) is one of the most promising contender technologies, as it has both the spatial resolution necessary to capture cellular processes *in vivo*, allows monitored cells to be precisely localised, and in some cases correlated with genetic markers, offers field of view sufficient to monitor hundreds or thousands of neurons, and has temporal resolution approaching that required to record individual action potential evoked calcium signals.

However, most MPLSM experiments currently utilise raster-based *frame scanning* strategies, which suffer from suboptimal sampling rates and poor photon counting efficiency, due to time wasted scanning extracellular space. This limits the number of cells from which spike trains can be accurately detected. Several improved scanning strategies have been developed, including inertialess scanning [1], and beam splitting approaches [2], [3]. These approaches both require substantial customisation of hardware. Most multiphoton microscopes are equipped with scanners based on galvanometer mounted mirrors. Thus, the development of efficient strategies for galvanometric scanning of large neural populations at high sampling rate would enable widely available multiphoton microscopes to be deployed for the study of neural circuitry. A significant advance in galvanometric MPLSM was the recent development of the Heuristically Optimal Path Scanning (HOPS) technique [4], in which the Travelling Salesman Algorithm (TSA) is applied to soma locations automatically extracted from a reference image, in order to minimize the scanning path length. The performance advances over frame-scanning with such an approach can be substantial (Sadovsky et al reporting 1000 neurons at 8.5 Hz). However, neither this nor other previously developed fast scanning strategies [5] [6] take into account the inertia of the galvanometric scanners themselves, and thus are likely to lead to suboptimal sampling rates.

In this paper, we propose a new scanning algorithm (Adaptive Spiral Scanning, SSA) which drives the beam in radially modulated circular motions, resulting in a spiral trajectory fitted to pass through each detected soma. Due to the kinematic efficiency of near-circular motions, this new scanning strategy scales well to large neural ensembles, and produces sampling frequencies higher than those previously achieved. We demonstrate the application of the algorithm through a MATLAB model of the galvanometric scan head in a commercial MPLSM (Scientifica SliceScope).

II. MATERIALS AND METHODS

A. Characterization of the Galvanometric Scanners

Galvanometric Scanners (GS) are defined by two main components: a moving magnet torque motor and a mirror rigidly attached to the rotor. Thus, it can be characterized by a mechanical system associated to an angular motion second order differential equation (see (1)).

$$J\frac{d^2\theta}{dt^2}+k\frac{d\theta}{dt}+c\theta=K_t I \tag{1}$$

where J is the inertia of the equivalent rotor-mirror system, k the frictional constant, c the restoring constant and K_t the driving torque constant. I and θ denote the current applied to the motor and the angular position of the mirror, respectively. In order to measure k and c, we recorded a step response of the Model 6215H Optical Scanner (Cambridge Technology; see Fig. 1). We used Labview (NI Ltd) to control the angle of the mirror and acquired the angular position output from the servo (MicroMax Model 671XX , Cambridge Technology).

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R. Schuck, L. A. Annecchino and S. R. Schultz are with the Department of Bioengineering, Imperial College London, South Kensington, London SW7 2AZ, UK. (e-mail: s.schultz@imperial.ac.uk).

TABLE I: Estimated galvanometer parameters

	k [kg.m$^2 s^{-1}$]	c [N.m.rad^{-1}]	z	ω_n [kHz]	$t_{r95\%}$[μs]
X	1.75×10^{-4}	2.04	0.82	19.1	161
Y	1.71×10^{-4}	2.26	0.76	20.1	152

The step responses Fig. 1 to a 1 Volt input show an angular deflection of 2 degrees (output voltage 0.5V/degree). Table I shows the parameters estimated for our system. In this table, z is the damping factor, ω_n the natural frequency, $t_{r95\%}$ the 95% rise time of the system, $J = 5.6 \times 10^{-9}$kg.m^{-2}, $K_t = 3.78 \times 10^{-3}$N.m.A^{-1} being the same for both GSs. The total inertia J is calculated for the worst case scenario, taking the higher value of the recommended load (the mirror) from the data sheet. Our final GS closed loop model is described in the block diagram shown in Fig. 2. The coefficients α_X and α_Y are estimated from an open loop simulation.

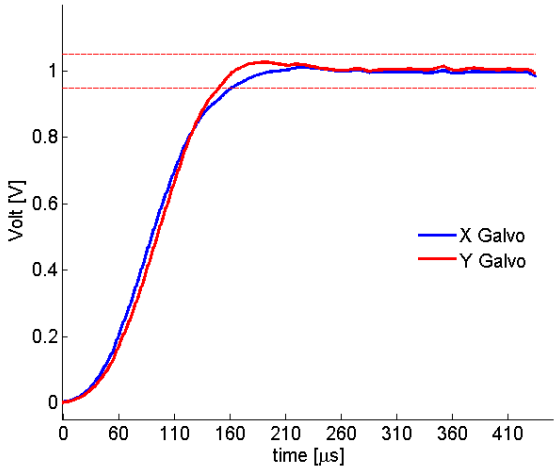


Fig. 1: Step response of the Cambridge Technology Model 6215H Galvanometric Scanners.

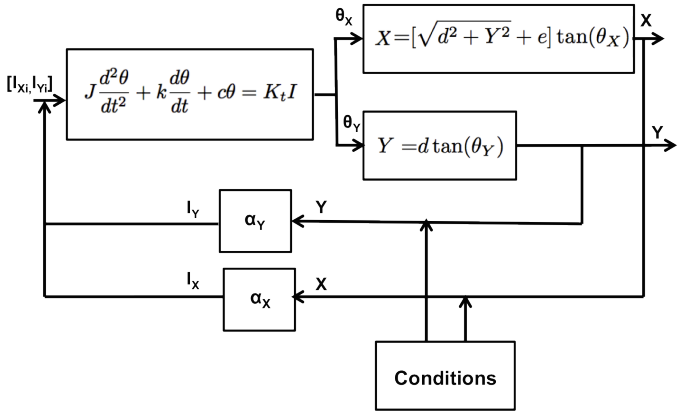


Fig. 2: The GS model block diagram. $d = 0.15$m denotes the distance from the centre of the Y mirror to the focal plane, and $e = 0.4$m the distance between the X and Y mirror centres [7].

B. Simulation of populations of neuronal calcium signals

In our simulation we generated surrogate data using the Izhikevich point neuron model [8], driving the dynamics of a simulated genetically encoded calcium indicator with the dynamics of GCaMP6 [9] (see Fig. 3). The population consist of 80% excitatory and 20% inhibitory neurons. All of them were randomly interconnected with a synaptic current within [0, 0.5pA] for the excitatory neurons and within [-1, 0pA] for the inhibitory neurons. Each simulated neuron also received Gaussian synaptic noise ($\mu = 0$, $\sigma = 5$ for excitatory neurons; $\mu = 0$, $\sigma = 2$ for inhibitory neurons) representing the external thalamic input.

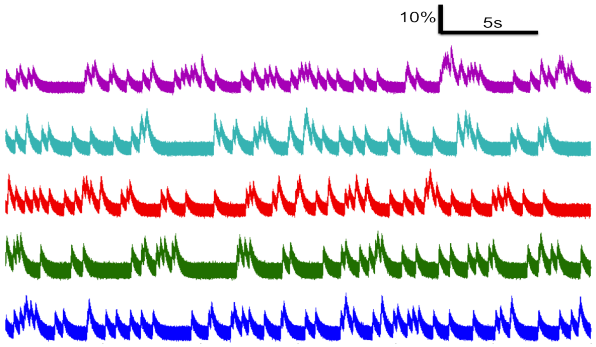


Fig. 3: Neural ensemble simulation underlying the system model. Calcium dynamics of five simulated neurons (Izhikevich neuron model). Four of the neurons are excitatory regular spiking neurons and one is a fast spiking inhibitory neuron. Calcium transients are modelled as an AP-evoked ramp (45 ms) followed by a single exponential decay (150 ms), following GCamp6-like kinetics and coupled to a white Gaussian noise.

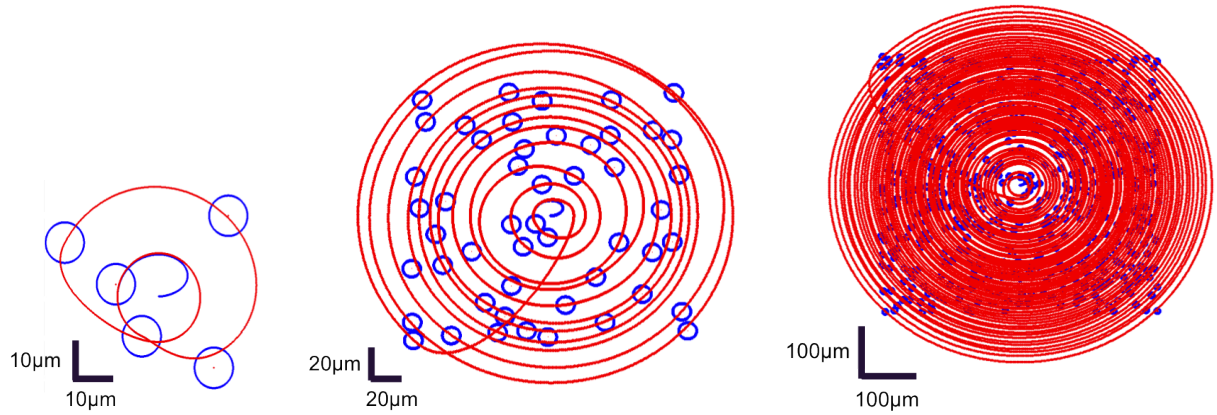
III. RESULTS

We incorporated the two GS model described in Fig. 2 into the neuronal population simulation code, in order to test algorithms for finding a rapid laser path through randomly generated, pre-selected cell body locations.

A. Fitting spiral trajectories through a random population of neurons increases the sampling frequency

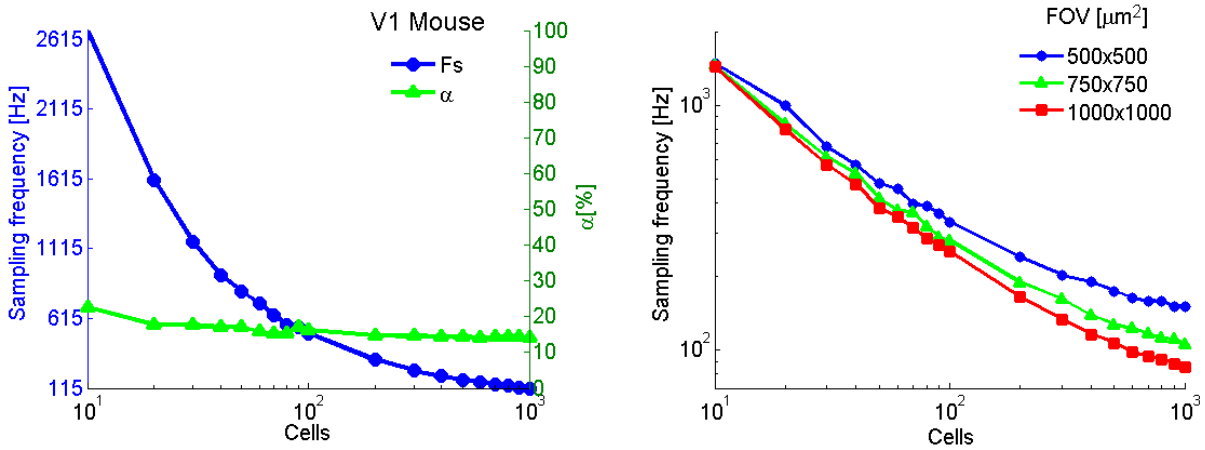
For inertial systems, smooth patterns (with moderate curvature) and continuous movements are well suited to provide acceleration-free travel [11]. The spiral scanning strategy is well known for imaging arrays and has already been applied to two-photon microscopy experiments [12], [13]. We propose here an extension: fitting a spiral trajectory to a specific set of points, after an initial frame-scan to determine soma locations. In order to reduce the impact of inertia (i.e the transient acceleration period, see Fig. 1) in our system, both GS are driven at constant angular speed. This results in circular movements. We are then able to sample all the cells through an algorithm that modulates the radius of the circular trajectories resulting in a distorted spiral pattern.

Algorithm description (pseudo-code):



(a) *(b)* *(c)*

Fig. 4: Improved fit of spiral trajectories through a pseudo-random population of neurons. (a), (b) and (c) represent five, fifty and five hundred cells, respectively, with the spatial density of active neurons in mouse V1 [10]. The blue trace represents the laser path of the first cycle (starting in the centre of the FOV) and the red trace the laser path for all other cycles.



(a) *(b)*

Fig. 5: Sampling frequencies and useful signal ratio α achieved by the SSA algorithm. α is defined as the fraction of time the laser is focused on active neurons during each cycle (the laser scanned n_1 to n_N). (a) Sampling frequency achieved for spatial density of active neurons in mouse V1 (in blue) and α associated (in green). (b) Three different FOVs: 500×500, 750×750 and 1000×1000 μm^2. For each graph we have averaged 5 different cell location configurations.

For a randomly distributed neuron population $\{n_1, n_2, ..., n_N\}$, $N \in \mathbb{N}^*$ in a certain Field Of View (FOV), $\forall (i, j) \in N$:

(i) Sort the neurons by their ascending radius r_i to the centre of the FOV.

(ii) For neuron n_i apply sinusoidal current (I_{Xi}, I_{Yi}) to both GS to drive the laser beam in a circular motion with radius r_i

(iii) While scanning circle r_i,
for all the n_j with $r_j > r_i$
if laser scans with sufficient sample n_j
indx = indx+1
if laser scanned n_i
i = i+indx

(iv) back to (ii)

(v) if scan n_N $->$ go back to n_1

The geometrical conditions used in this algorithm are fed into the model (Fig. 2). At each instant, radius of the circular trajectories is modulated. Although the impact of inertia is still present, it is reduced by allowing multiple cells to be scanned in a single period. For one cycle (the laser scanned n_1 to n_N), the number of periods (360 degree rotations) is less than the number of cells. In (iii), the number of sufficient samples can be defined by the user for the neurons that are traversed en passant. Using a driving frequency of $\omega_I = 10kHz$ and a FOV of 750×750 μm^2 we performed several simulations with different cell densities (see Fig. 5(b)). With these parameters we achieved sampling frequencies as high as $475Hz$ for 50 Cells and $105Hz$ for 1000 cells. This suggests that the SSA approach could potentially achieve sampling frequencies higher than those achieved by previously documented scanning approaches.

B. Signal Acquisition

During signal acquisition, a matrix is collected, with columns containing the photomultiplier output binned throughout each cycle (see Fig. 6). To demonstrate the system working, we applied it to the Izhikevich neuron simulation (with calcium dynamics) described in Fig. 3. In this example, the FOV was 500×500 μm^2 for five neurons, allowing a sampling rate of 5 kHz, well above the level required to faithfully reconstruct calcium dynamics.

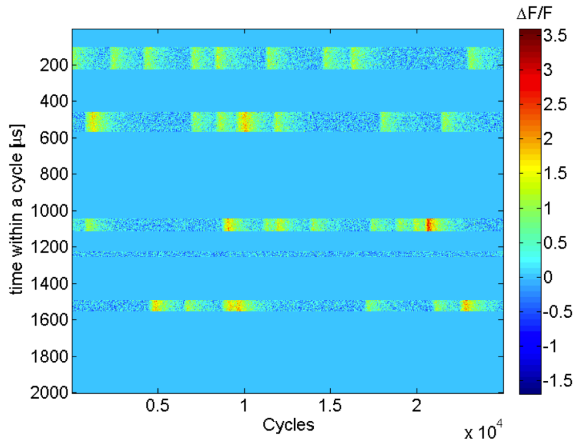


Fig. 6: Acquisition matrix for a 5 neuron population. Time series are extracted by collecting and averaging rows in which the laser is over the same neuron.

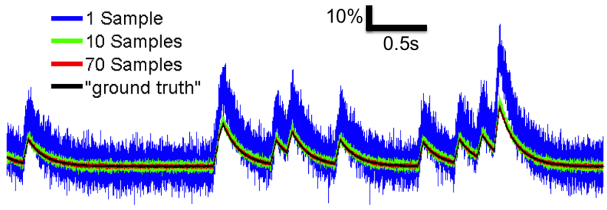


Fig. 7: Recorded fluorescence ($\Delta F/F$) from a simulated excitatory neuron, demonstrating that, at least in simulation, the system is capable of faithfully reproducing calcium dynamics.

IV. Discussion

We have developed a novel galvanometric scanning algorithm for two photon laser scanning microscopes, Adaptive Spiral Scanning (SSA). Our algorithm has a number of advantages over traditional frame-scanning approaches to galvanometric scanning, yielding both higher sampling rates and a higher proportion of time spent counting photons from structures of interest. This indicates that it should allow spike trains to be reconstructed from a larger population of neurons (for a given detection accuracy), or with better spike detection accuracy (for a given number of neurone) than would be possible with frame-scanning. It also achieves higher sampling rates than path minimisation approaches such as HOPS [4], although at the expense of fraction of time spent over neurons, as the latter method uses a "stop start" approach that allows dwelling over individual neurons in order to increase photon count. The effect of this compromise on spike train detection accuracy [14] remains to be studied.

One disadvantage of linescanning techniques including the approach presented here is that they may be quite vulnerable to movement artefact, being unable to take advantage of image registration techniques to correct for motion artefacts. This may prove to limit the number of cells to which the algorithm can be scaled *in vivo*. We tested our algorithm by simulating the galvanometric scanners in a commercial multiphoton microscope, with parameters measured experimentally from galvanometric step responses. Validation of the algorithm *in vitro* and *in vivo* is the focus of ongoing work. If validated experimentally, our algorithm could be expected to enhance the number of cells that can be recorded, and the temporal resolution of functional imaging, using standard, off-the-shelf two photon microscope hardware.

V. Acknowledgements

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