Signal reduction in fluorescence imaging using radio frequency-multiplexed excitation by compressed sensing

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ABSTRACT

Fluorescence imaging using radio frequency-multiplexed excitation (FIRE) has emerged to enable an order-of-magnitude higher frame rate than the current technologies. Similar to all high-speed real-time imaging modalities, FIRE inherently generates massive image data continuously. While this technology entails high-throughput data sampling, processing, and storage in real-time, strategies in data compression on the fly is also beneficial. We here report that it is feasible to exploit the radio frequency-multiplexed excitation scheme in FIRE for implementing compressed sensing (CS) without any modification of the FIRE system. We numerically demonstrate that CS-FIRE can reduce the effective data rate by 95% without severe degradation of image quality.

1. INTRODUCTION

Flow cytometry is an established technology for high-throughput cellular assay based on scattered light and multiplexed fluorescent light measurements. A large population of cells ($> 10,000$) is often required to produce statistically high-confident data for meaningful diagnostics. This technology comes short in niche applications where high sensitivity and high throughput are both required, such as rare cell detection for early cancer diagnostics as well as single-cell analysis. Hence, there is a rising interest in accessing the spatial information of individual cells at a high imaging throughput[2–4] with the aim of improved screening and diagnostic accuracy based on the sizable set of image data. So far, only a handful of technologies capable of delivering single-cell flow imaging at a speed comparable to the conventional flow cytometers, namely time-stretch imaging (also known as serial time-encoded amplified microscopy (STEAM)[5] and its second generation, asymmetric-detection time-stretch optical microscopy (ATOM),[3] and florescence imaging using radio frequency-multiplexed excitation (FIRE).[1] In contrast to STEAM and ATOM, which mainly capture the morphological information of the cells, FIRE is able to capture fluorescence cellular images and is thus particularly attractive for probing the functional information of the cells at high-throughput.[6]

Fluorescence imaging using radio frequency-multiplexed excitation (FIRE)[1] is one of the emerging high speed multiplexing imaging techniques to surpass the speed-noise tradeoff prevalent in fluorescence detection[6] by prolonging the effective dwell time without sacrificing the scan rate.[1,7,8] However, the inherently massive image data stream imposes stringent requirement on data processing and storage in real-time.[4] For example, existing digitizers at $\sim 250\,\mathrm{MSa/s}$ sample rate possesses limited memory depth up to 2Gpts which fills up within 8 seconds, which is inadequate for typical flow cytometry protocols spanning from minutes to hours. On-the-fly data compression is thus favourable to avoid

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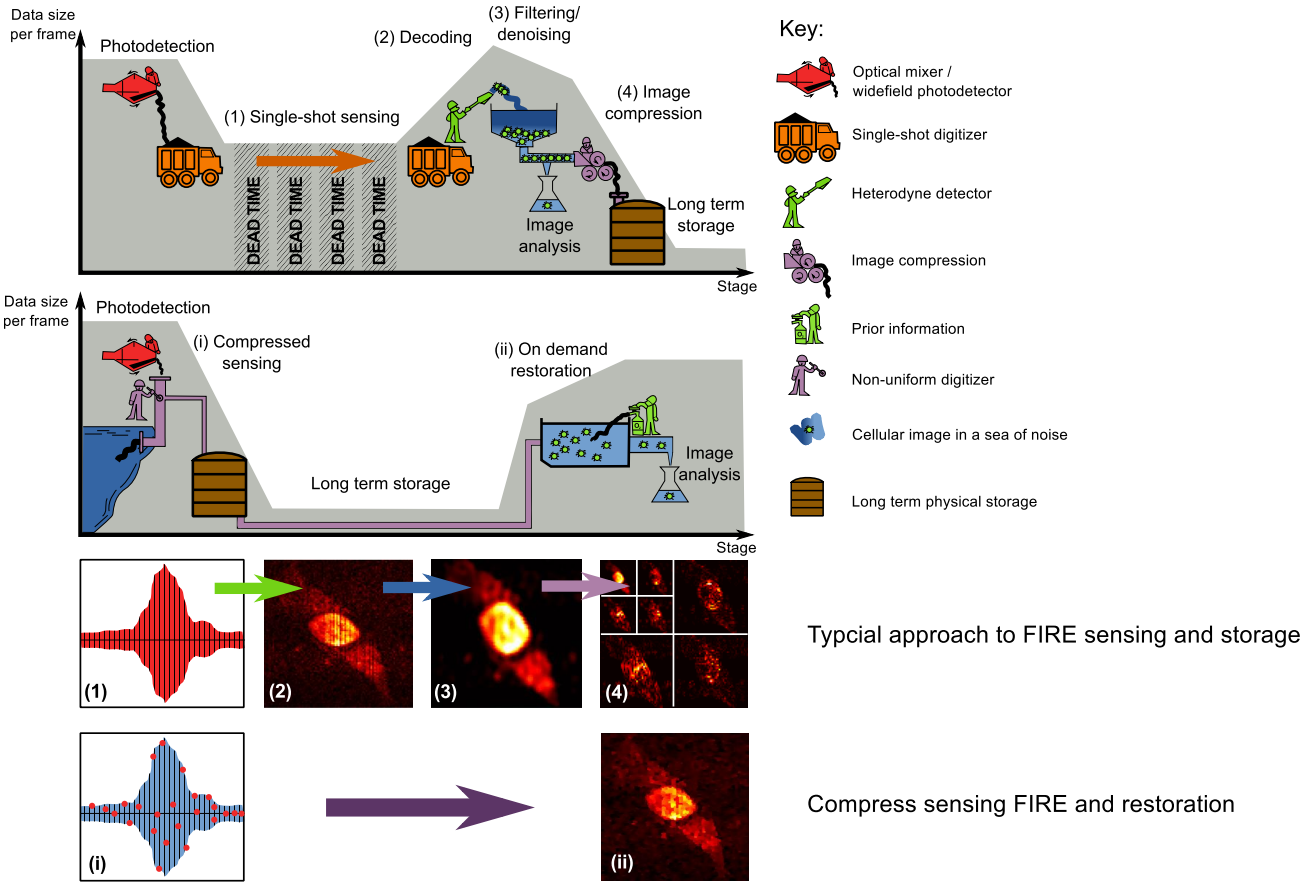

Figure 1. Sewage treatment analogy to FIRE imaging. (First row) Typical FIRE sensing requires the whole radio frequency (RF) response signal to be recorded into memory buffer, invariantly leading to intermediate dead time of the FIRE system. (Second row) Compressed sensing offers a way to systemically discard RF response signal on the sensor side to alleviate the storage requirement. It also enable on-demand restoration of compressed signal. (Third and fourth row) Typical FIRE decode-denoise-compress process,[1] compared to one-off restoration process enabled by compressed sensing.

system dead time due to memory overflow, albeit the increased computer load in image reconstruction as the raw data have to be (1) decoded, (2) denoised and then (3) compressed to the desired image format.

Compressed sensing (CS) offers a solution here for FIRE as it *undersamples* the radio frequency (RF) signal on the sensor side,[9,10] hence offloads both the storage and data processing requirement.[11,12] CS exploits three important properties of FIRE. First, the recorded image signal is *highly redundant* as FIRE multiplexes the pixel information in each line. Second, the cell images are of *low structural complexity* due to the choice of fluorescent dye for flow cytometry, i.e. dyes that either stains the cell nucleus or the cell membrane. Third, the frequency-tagging scheme employed in FIRE is only *weakly coherent* to the image structure. By systemically discarding digitized RF signals on the fly, the digital data rate can be reduced significantly without modifying the FIRE system, and without degradation of image quality. The speed improvement is also reflected on the reconstruction step, as the traditional decode-denoise-compress sequence becomes an one-off CS restoration process (Figure 1).

2. WORKING PRINCIPLE

Compressed sensing FIRE (CS-FIRE) relies on *maximum likelihood estimation* of fluorescence image $I(x, y)$ from a series of undersampled RF response $I_{\mathrm{FIRE}}(t, y)|_{\mathrm{undersampled}}$. To fill in the missing signal in the undersampled RF data, *prior information* is provided to assist image restoration. In our case, the biological cells are *sparse* and exhibits clear edges, as nonspecific fluorescence dyes are often chosen to either stain the whole nucleus or the cell membrane in typical flow cytometry protocols. To this end, image restoration problem of CS-FIRE reduces to an edge-preserving minimization problem,[13,14]

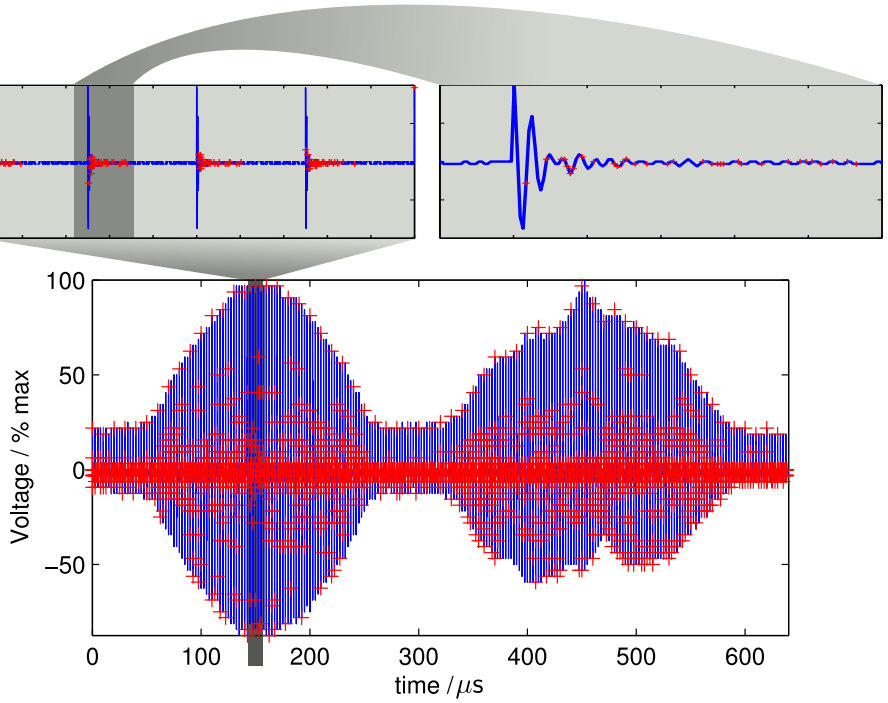

Figure 2. Simulated FIRE RF-multiplexed signal (blue line) where only 5% of the samples (red cross) are preserved.

i.e.

$$\min_{\hat{I}} \frac{1}{\lambda} \sum_{(t_m,y_j)\in S} \left[I_{\mathrm{FIRE}}(t_m, y_j)\Big|_{\mathrm{undersampled}} - \hat{I}_{\mathrm{FIRE}}(t_m, y_j) \right]^2 + \|\hat{I}(x,y)\|_{TV}, \tag{1}$$

where λ is the noise tolerance to the measured RF signal. A matching subsampling mask S defines a subset of coordinates (t_m, y_j) at which the RF signal is sampled; the estimated RF values at time points absent from S are not considered in the maximum likelihood estimation process in Eq. (1). The choice of S is discussed in Section 4. Here, we assume a Gaussian noise model validated by a combination of multiplexing gain and central limit theorem. For the sake of demonstration, we simplify the RF generation of FIRE to a discrete cosine transform (DCT), i.e.

$$\hat{I}_{\mathrm{FIRE}}(t_m, y_j) = \sum_{k=0}^{K-1} I(x_k, y_j) \cos(2\pi f_k t_m + \phi_k) \tag{2}$$

$$= \sum_{k=0}^{K-1} I(x_k, y_j) \cos[2\pi(a + bk)m]. \tag{3}$$

This conversion is realized by phase locking all frequency channels ($\phi_k = 0$), and by substituting the hardware configurations of Diebold et al, where $K = 256$, $a = f_0/f_s = 10\mathrm{MHz}/250\mathrm{MHz} = 0.04$, and $b = \Delta f/f_s = 400\mathrm{kHz}/250\mathrm{MHz} = 0.0016$.

3. SIMULATION BASED ON FLUORESCENCE IMAGE SAMPLES

To demonstrate the power of CS-FIRE, we pick the widefield image of C6 astrocytes[1] as our test target. The highly-folded nuclei of the cells are stained with a nonspecific dye, so it serve as a indicator of edge preserving property of CS. Figure 2 demonstrates how the test target is scanned by 256 scan lines with 256 discrete beat-frequency channels in an acquisition time of 640 μs, from which 95% of the samples are discarded on the fly on the detector side. To further abide to actual scenario, we quantize the RF-multiplexed signal with a 10-bit digitizer. We find that the huge influx of data stream at 2.5 Gbits/s drops to 130 Mbits/s on average, that is sufficient throughput to offload the recorded data from memory buffer to secondary storage in real time to sustain flow cytometry process for hours.

We substitute the undersampled data $I_{\mathrm{FIRE}}(t,y)|_{\mathrm{undersampled}}$ to Eq. (1) to restore the image by a gradient-based optimization algorithm (TVAL3).[15] The noise tolerance λ is set to 0.03 to contain the quantization error. We find that the restored image matches well with the test target; the highly folded nuclei is visible at 5% undersampling ratio. Relaxing the undersampling ratio to 15% or 100% do not significantly improve the image quality, thus it validates our assumption on image sparsity. The restored RF-multiplexed signal is also found to match well with the analog RF signal before quantization.

To test the denoising ability of CS-FIRE for signals under ultrashort shutter time, we corrupt the test target with Poisson noise. To be specific, we assume 40 photons are emitted on average from the brightness pixels within the shutter time (2.5 μs). Meanwhile, we relax the noise tolerance λ to 0.06 to contain both the quantization error and the shot-noise. The image restoration result in Figure 4 shows an excellent noise rejection ability; the highly folded nuclei is revealed thanks to the edge-preserving property of Eq. (1). For fair comparison, a highly-denoised image from experimental FIRE data[1] are put side-by-side for comparison.

4. ENGINEERING THE UNDERSAMPLING SCHEME

As discussed in Section 2, the beat-frequency channels are phase-locked unlike the approach by Diebold et al. This results in fast decay of the RF-multiplexed signal for all line scans. If the RF signal is undersampled regardless of time, most of the acquired data will be clustered around the baseline (left of Figure 5). This results in waste of memory resources to null measurements. To encourage sampling at non-zero values, we modify the probability density function of the set S to that of a half-normal distribution, i.e.

$$P[(t,y) \in S] = \frac{\rho}{\sigma\sqrt{\pi/2}} \exp\left(\frac{-t^2}{2\sigma^2}\right) \quad \text{for all } t > 0, \tag{4}$$

where t is the local time of a single RF-multiplexed signal. We set the width $\sigma = 0.42\,\mu$s to capture mainly the first half of the trace, and tune the compression ratio ρ to match the overall undersampling ratio of 5%. Figure 5 demonstrates how the recorded samples are now less clustered around the zero baseline.

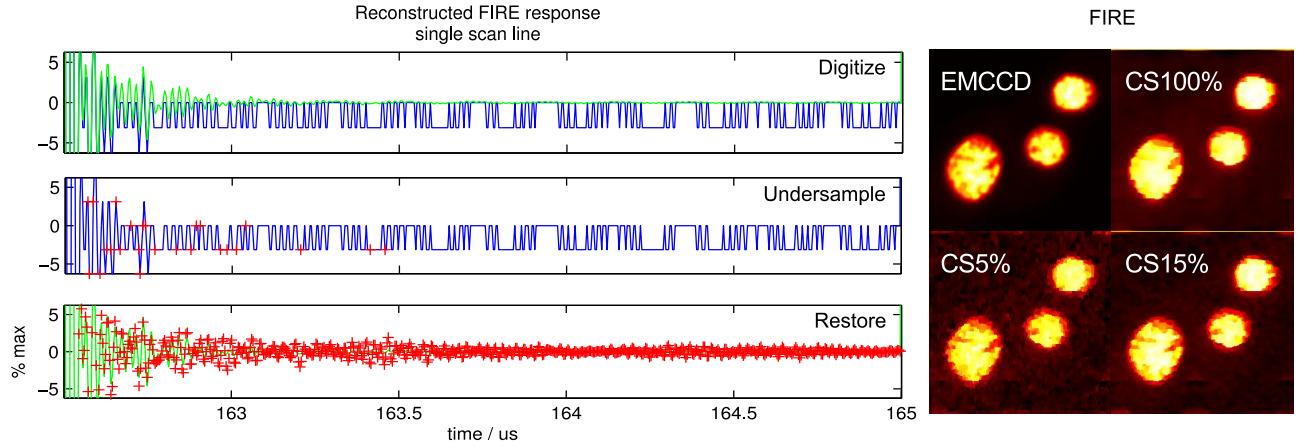

Figure 3. Simulated reconstruction of CS-FIRE with noiseless specimen. The RF temporal response (green) is first quantized by a 10-bit digitizer (blue). Then, 95% of the voltage data are discarded on the fly. The whole RF signal is restored with high accuracy from the remaining 5% data (red cross). Relaxing the undersampling ratio to 15% or 100% (right) do not significantly improve the image quality, thus implying limited structural complexity of the image.

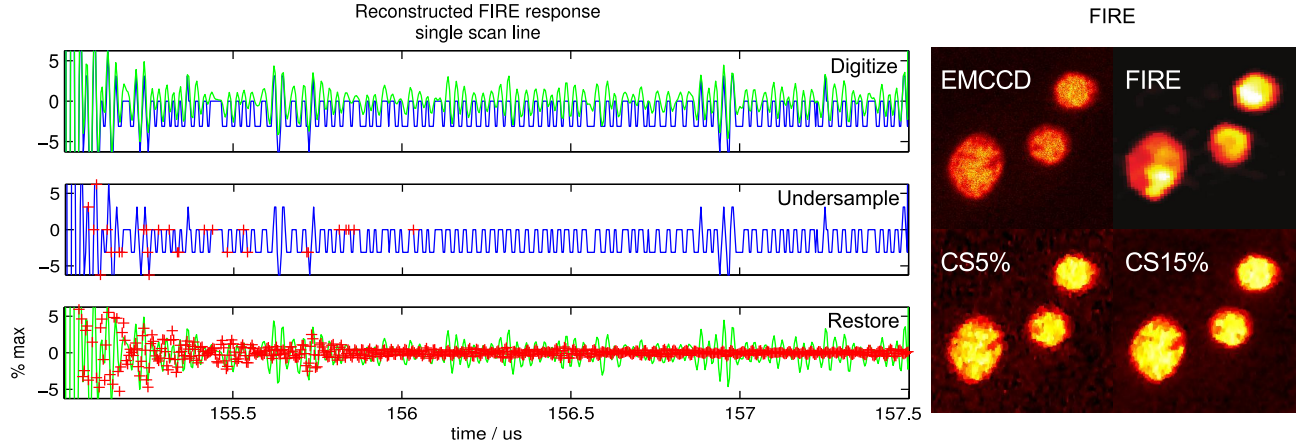

Figure 4. Edge preserving noise removal by compressed sensing (CS). We assume a shot-noise scenario from which at most 40 photons are emitted from each frequency channel during the shutter time of 2.5μs.

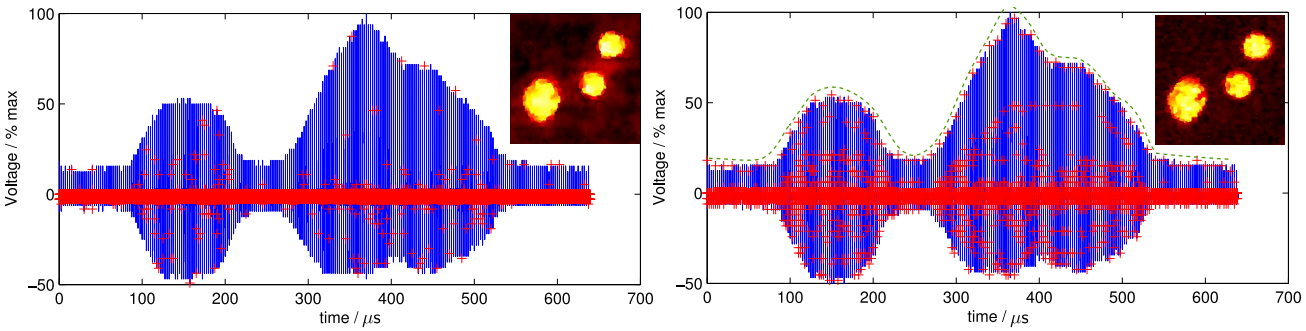

Figure 5. Simulated FIRE RF-multiplexed signal (blue line) where only 5% of the samples (red cross) are preserved. (Left) random sampling regardless of the signal structure results in loss of forward scattering signal (FSC); (Right) alternative random sampling scheme to preserve the RF envelope so that FSC information can be extracted without image restoration (green dotted line). The new sampling scheme is also designed to be less clustered around the zero baseline for improving image quality (inset).

Another modification to the undersampling scheme is the envelope preservation of the RF-multiplexed signal for cell screening. The area (FSC-A) and the width (FSC-W) of the forward scattering signal are the traditional metrics cellular volume and cell doublets indicator respectively. These can be directly measured from the RF envelope without intensive image restoration process. If the fluorescence emission signal is compressed on the sensor side regardless of the signal structure, the envelope shape would have to be reconstructed along with fluorescence image restoration (left of Figure 5). To preserve the FSC signal, we record the sample at which all frequency channels are in phase, i.e. at local time $t = 0$. The slow varying RF envelope can be further undersampled to reduce the sampling requirement. In this paper, we subselect sample once in every four consecutive scan lines:

$$P[(0, y_j) \in S] = \begin{cases} 1 & \text{if } j = 4n, \\ 0 & \text{otherwise,} \end{cases} \tag{5}$$

where n is a non-negative integer. These samples can be subsequently selected on the fly to obtain first-hand statistics about FSC-A and FSC-W before image restoration. This practice also aligns with our advocate about on-demand image restoration, where the FIRE signals are pre-screened according to their FSC metrics so only the image of biological cells of interest are restored upon request.

5. CONCLUSION

The inherently massive data rate of FIRE poses a challenging image processing and storage requirement for the continuous real-time operation in many flow cytometry protocols. We present a compressed sensing approach to alleviate the memory overflow problem by discarding redundant RF-multiplexed signal on the sensor side in real time. Under shot-noise limited scenario, we demonstrate that 95% of the temporal RF signal can discarded without degradation to image quality. Compressed sensing also enable one-off step to FIRE decoding, denoising and image compression for visual inspection. By systematically samples the RF trace at non-zero values while preserving the forward scattering metrics of the biological cells, we can realize screening of CS-FIRE data for on-demand image restoration.

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