A 128 × 1 SPAD-Camera for Protein Sequencing through Surface-Enhanced Raman Spectroscopy

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Nanopore sequencing is an established methodology for single-molecule analysis of nucleic acids. However, widely used readout methods based on variations of the ionic current through the nanopore lack detail about molecular structure of the analytes. This aspect makes the application of nanopore sequencing to proteins challenging, due to the large number of amino acids to be distinguished. An optical readout based on Surface-Enhanced Raman Spectroscopy (SERS) performed in plasmonic nanopores can provide rich structural information and expand the applicability of this sequencing technique. [1] In our SERS-based sequencing setup, proteins are unfolded and driven through the pore, where a laser stimulates Raman response for each amino acid, allowing their sequential identification (Fig. 1). Fast optical detectors with high sensitivity are needed to perform spectral acquisitions of the scattered signal in a short time period. SPADs represent an ideal choice, since they show single-photon sensitivity, good detection in the spectral range of interest (500-700 nm), are easily integrated in arrays providing spectral resolution, and can be coupled with time-gating or time-resolving architectures to remove the unwanted fluorescence signal from the spectral region of interest, exploiting the longer lifetime of fluorescence compared to Raman scattering. [2]

To enable SERS-based protein sequencing, we propose a camera based on a 128 × 1 SPAD array chip, which has been optimized for this application (Fig. 2). Additionally, we describe the design of a new chip based on a 128 × 4 SPAD array which should further improve detection performance, while still at an early characterization stage (Fig. 3).

The 128 × 1 SPAD camera is based on a chip previously designed and characterized by Politecnico di Milano. [3] We coupled the chip to a microlens array based on Segmented Refractive-Optics Elements [4] which improves the native Photon Detection Efficiency (PDE) of the chip by 4.5 times (Fig. 4a) resulting in a 28% peak PDE at 450 nm. We developed custom software for real time alignment and interfacing hardware able to reach a frame rate of 1 MHz, which includes two custom boards and a commercial FPGA board. It can generate a synchronization signal for laser excitation at the beginning of every frame and provide a trigger signal when a translocation is detected. The trigger signal can be used to vary the bias voltage driving the translocating molecule, slowing it down and enabling longer acquisition times for single amino acids. To temporally filter fluorescence, the camera has the possibility of *hard gating* (i.e., SPAD switch off outside the window of interest) with gating windows in the nanoseconds range. Preliminary acquisitions performed with this camera, showing the SERS spectrum of Glutamate, are reported in Fig. 4b and Fig. 4c.

To improve performance, we designed a new chip in a 3D-stacked technology by STMicroelectronics, with a SPAD dedicated top tier and a 40 nm CMOS bottom tier. It comprises an array of 128 × 4 SPADs presenting a 30 to 35% PDE in the spectral range of interest. The number of rows has been increased to maximize signal acquisition. While the hard gating capability shown by the 128 × 1 SPAD has the advantage of reducing afterpulsing events, it entails temporal limitations due to SPAD finite switch on/off time. Therefore, along with hard gating windows of 1 ns, the new ASIC includes *soft gating* structures to disable the readout logic, narrowing the detection window to hundreds of picoseconds. Finally, to increase the throughput of the sequential readout above 1 MHz, we developed a custom architecture to minimize the data transfer by reading only a user-selectable subset of spectral bands. At the end of the ongoing characterization and testing, this optimized SPAD array will represent an essential tool for the discrimination of single amino acids in protein sequencing.

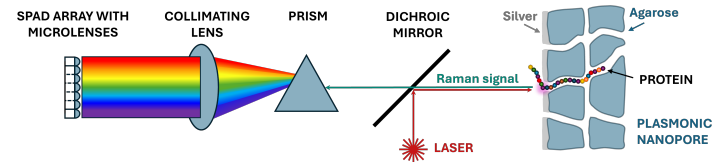

Fig. 1. Proposed setup for SERS-based protein sequencing in plasmonic nanopores

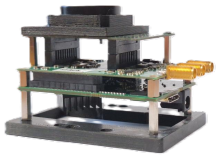

Fig. 2. 128 × 1 SPAD camera

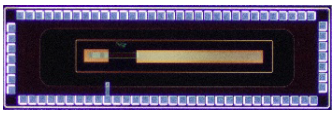

Fig. 3. 128 × 4 SPAD array: micrograph

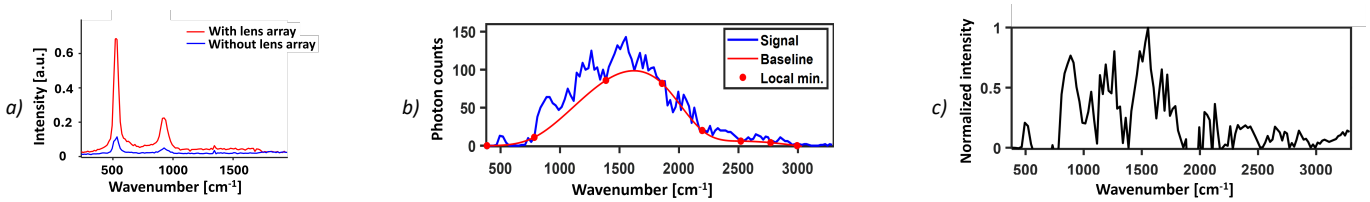

Fig. 4. Experimental measurements with the 128 × 1 SPAD camera. a) Raman spectrum of Silicon with and without the microlens array. b) Acquired spectrum of poly-Glutamate with 10-µs frames. c) SERS spectrum of Glutamate after post-processing baseline subtraction and normalization.

References

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