COmputatioNal Imaging as a training network for Smart biomedical dEvices

COmputatioNal Imaging as training network for Smart biomedical dEvices

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Deliverable 2.1

Report on methods and processing: description and results

WP 2 – Advanced Fluorescence Imaging for Clinical Applications

Authors	Cosimo D'Andrea (POLIMI), Serban Cristian Tudosie (UCL), Shivaprasad Varakkoth (POLIMI), Simon Arridge (UCL)
Lead participant	UNISTRA
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Partner short names

POLIMI	Politecnico di Milano
CWI	Centrum Wiskunde & Informatica
UNISTRA	Université de Strasbourg
UJI	Universidad Jaume I
UCL	University College London
INTUITIVE	Intuitive Surgical
VIALUX	Vialux GmbH
accelCH	accelopment Schweiz AG

Abbreviations

CS	Compressive Sensing
D	Deliverable
DC	Doctoral Candidate
DMD	Digital Micromirror Device
DF	Data Fusion
EC	European Commission
EU	European Union
GT	Ground Truth
HEU	Horizon Europe
MS	Milestone
NN	Neural Network
SH	Scrambled Hadamard
SIM	Structured Light Illumination Microscopy
SPC	Single-Pixel Camera
TCSPC	Time-Correlated Single Photon Counting
WP	Work Package

Executive summary

Deliverable D2.1 is part of Work Package 2 (“Advanced Fluorescence Imaging for Clinical Applications”) of the CONcISE project. This document provides a report on the methods developed by the collaboration of UCL and POLIMI, and used to advance the field of computational multispectral lifetime fluorescence imaging and in particular microscopy. The report additionally provides insights into the developed optical system installed at POLIMI. These methods can also be applied in a plug-and-play fashion to endoscopy for clinical applications (in collaboration with UNISTRA and INTUITIVE) with minor modifications to the optical setup.

Objectives

The objective of this deliverable is to provide knowledge on the methods used, and show the achieved results of the developed computational techniques coupled within the optical system. This involves:

- Advancing compressive sensing (CS) techniques for the optical system.
- Development of data fusion (DF) algorithms for multidimensionality through optimization theory and machine learning (ML) tools.
- Development of ML architectures for highly compressed measurements at high reconstruction speed in fluorescence microscopy.

Methodology and implementation

The methodology follows the implementation of algorithms and software, alongside the physical realization of an optical system for acquiring volumetric multispectral fluorescence data, relying on two sensing devices, a CMOS camera and a single-pixel camera (SPC) coupled with a spectrometer and a time-correlated single photon counting (TCSPC) board. SPC setups require the usage of a digital micromirror device (DMD), mostly developed by VIALUX (part of CONcISE). We improve the SPC acquisition and reconstruction through multiple techniques. In particular, we use ML algorithms (autoencoder) to decrease acquisition and reconstruction time, increase compression, and maintain quality. We use optimal sampling algorithms in our optical system to retrive optimal patterns for the SPC given the CMOS camera adaptively. We develop a data fusion (DF) algorithm to merge our two acquisitions together to optain a high-resolution volumetric multispectral lifetime volume for fluorescence microscopy.

POLIMI leads the development of the optical setup and collaborates with UCL for the development of computational methods applied to the system. Lastly, POLIMI, UCL and UNISTRA, collaborate to develop and adapt the system for clinical applications.

In line with the concept of the CONcISE project, the 3 Doctoral Candidates (DCs) Gabriel Szydlo Shein (DC4 - UNISTRA), Shivaprasad Varakkoth (DC5 - POLIMI), and Serban Cristian Tudosie (DC11 - UCL) collaborated on this work. Initially, DC5 developed the optical system at POLIMI, DC11 developed the ML learning approach on simulated data at UCL and tested the ML architecture on the physical system at POLIMI during his secondment. Then DC5 and DC11 collaborated to develop the DF algorithm for optical system at POLIMI and UCL during both their secondments. Subsequently, DC5 and DC11 collaborated at UCL to build and adapt the optimal sampling strategy to the system. DC4 and DC5 discussed on the applicability of the microscopy system to endoscopy, and DC5 and DC11 plan to implement ML techniques on existent clinical data in collaboration with UNISTRA.

Outcomes

The proposed approaches are key to advance the field of multispectral fluorescent lifetime imaging, and they can be adapted to volumetric acquisitions. ML approaches are essential to improve the speed and compression over classical inverse problem solutions, while optimal sampling techniques can aid the system to adapt patterns knowing the image structure in advance. Lastly, the optical system can easily be applied to endoscopic measurements which is fundamental towards clinical applications.

Next steps

DC5 and DC11 will send the work on DF to Optica for publishing, and they will continue working on it and collaborate with UJI to include a multi-exponential model in the fusion method. DC11 will finalize, and along with DC5, will send the ML work to ICCV 2025. DC5 and DC11 will work on acquiring automatically more high dimensionality data with the optical system to build a dataset. DC5 and DC4 will work on adapting the setup towards clinical application. A new collaboration in April between DC11 and DC9 will help in improving current methods in adaptive optimal sampling techniques through a short visit of DC9 in UCL, and the second secondment of DC11 in CWI.

1 Introduction

Medical and biological applications use fluorescence microscopy techniques extensively. The amount of information gathered and its acquisition speed are essential to follow dynamically variable phenomena. It is important to gather multidimensional information at high-resolution, in fact the spectral component can be crucial in identifiying different tissues, while lifetimes provide information about the microenvironment surrounding the fluorophore, crucial for understanding metabolic processes. However, measuring simultaneously spectra, time decays and volumetric data quickly is extremely difficult.

To address this problem, we have built a system that collects high-resolution multispectral lifetime values over a volume (see deliverable 2.2 for a deeper description). The system combines two acquisition schemes: (i) a low-resolution time-resoved multispectral imaging based on SPC; (ii) high-resolution volumetric fluorescence intensity based on structured illumination. Hereafter (Figure 1) the sketch of the system is shown.

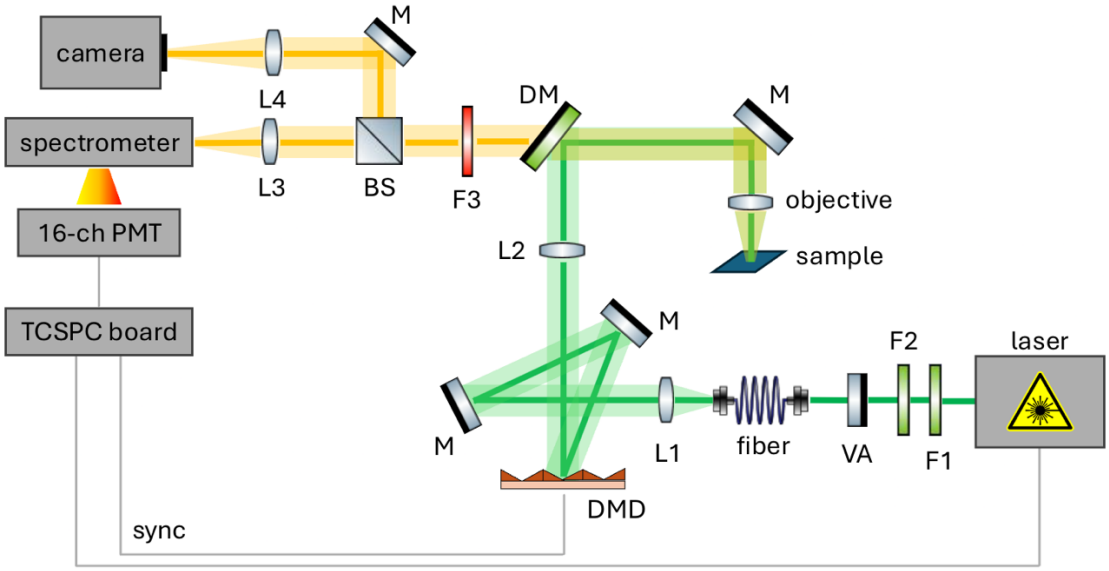

Figure 1. Optical Setup.

The system strongly relies on the synergy between optical and computational approaches and this multidisciplinarity is one of the key components of CONcISE. This connection between research fields is necessary towards the development of high perfomances biomedical optical imaging systems. In particular the developped system has allowed to develop novel algorithms within this computational imaging approach which can be used with the proposed microscopy but it can also extented to endoscopic and mesoscopic imaging.

In the following report, we will discuss three computational approaches for multispectral fluorescence lifetime imaging fluorescence microscopy based on SPC approach.

1. **Compressive Sensing Algorithms**. These algorithms allow to exploit a number (M) of illumination patterns lower than the number (N) of pixels of the reconstructed image.

2. **Data Fusion Algorithm**. The idea of DF is to “fuse” datasets acquired with different systems measuring the same sample in order to improve the performances (e.g. spatial or spectral resolution, acquisition time) of the reconstrcucted image which could not be reached by a single system.

3. **Machine Learning** (ML) approach for SPC based imaging system. Exploiting ML allows to optimize the patterns and reconstruction algorithm based on data. This can improve compression and recontruction quality with respect to classical CS algorithms.

2 Compressive Sensing Algorithms

Linear inverse problems deal with the reconstruction of a signal x from acquired measurements m. We can write the forward problem as $m = W\mathrm{x}$. $W \in R^{MxN}$ is the measurement matrix. If the matrix is invertible and there is no noise, retrieving the signal involves the inversion of the matrix. However, in the presence of noisy measurements, the problem requires more attention and involves the usage of regularization techniques. Additionally, if the system is underdetermined we step into the field of compressive sensing (CS).

CS deals with reconstructing signals from a reduced number of measurements ($M < N$). CS states that it is possible to reconstruct a signal by randomly sampling it, even at sub-Nyquist frequency, if that signal is sparse in some domain. This allows certain applications today, like the SPC, to be effective and widespread. In particular, in CS we deal with the problem of retrieving a signal x from a reduced set of measurements $m = W\mathrm{x}$. If the signal is sparse in some domain (like Fourier), then x can be retrieved by solving $\tilde{x} = argmin_x \,||\, x \,||_{l1}\, s.t. Wx = m$ (also called basis pursuit). The requirement is that the measurement matrix W has to follow the restricted isometry property and be incoherent with respect to the basis in which the signal is sparse (Tao 2009). For example, a good measurement matrix is one where each element is drawn from a Gaussian distribution.

This can be applied to SPC systems directly since we are dealing with the same problem $m = W\mathrm{x}$; the image x can be seen as a flattened version of the 2D original image, each row in W represents a pattern, and each value in the measurement vector m is the dot product between the image and the corresponding pattern. Over the years, more advanced algorithms have been developed to retrieve a solution efficiently in the field of inverse problems for SPC. In particular, the TVAL3 algorithms (Li 2013) is widely used in the community since it can efficiently retrieve signals from compressed measurements corrupted by noise.

Since our system comprises both a CMOS camera and a SPC one, it is possible to gather structural information about the sample with the CMOS camera before acquiring the SPC multispectral lifetime image. This opens the path to optimal sampling techniques that have been used on magnetic resonance imaging to sample patterns (Benning 2024). We have adapted this approach for Hadamard and Scrambled Hadamard (SH) matrices. It allows us to select the best patterns given the CMOS camera and reconstruct a high quality image at high compression.

3 Data Fusion 3D Multispectral Lifetime Fluorescence Microscopy

Current approaches for multispectral Lifetime Fluorescence Microscopy are based on scanning methodologies, that is for each illumination point time-resolved emission spectrum is acquired. Axial sectioning is normally obtained by exploiting confocality on the detection or multiphoton excitation of the sample. This approach provides an extremely accurate multidimensional data set with the major drawback of the acquisition speed. The possibility of having a fully wide-field system that preserves multidimensional data and enables 3D mapping offers significant advantages in terms of acquisition speed and information content. The developped system combines structured light illumination microscopy (SIM) which allows axial sectioning with multispectral Fluorescence Lifetime Imaging (FLIM) based on Single Pixel Camera (SPC). In particular, SIM allows volumetric fluorescence imaging, while SPC-FLIM allows low spatial resolution multispectral time resolved imaging In this application the same sample is measured in two different ways and it is possible to exploit the computational approach of data fusion (DF) to achieve the desired result of a

high-resolution output (spatial, spectral and temporarly). We provide two implementations of DF, one achieved with a linear conjugate gradient approach, and one where we optimize with ADAM.

3.1 Results

With this system we are able to retrieve a final dataset up to 512x512x20 for the spatial dimensions, 16 spectral channels and 100 temporal points for the time decay in matters of seconds on an NVIDIA RTX A6000. In Figure 2. High-resolution output from our system. In this multidimensional setting, we first show the intensity images in the first row, the multispectral component in the second, and the related FLIM in the third. At the same time each column represents a different axial position in our five dimensional output. we show the output of our system for all dimensions on a biological sample (mouse kidney cells). From left to right we scan through the volume axially to show different slices. In this case we show the output at a resolution of 256x256 performed with the ADAM approach. The different sections highlight the 3D structure, lifetime and emission spectrum of the glomerulus in the top left, the convoluted tubule in the centre, and the proximal tubules elsewhere.

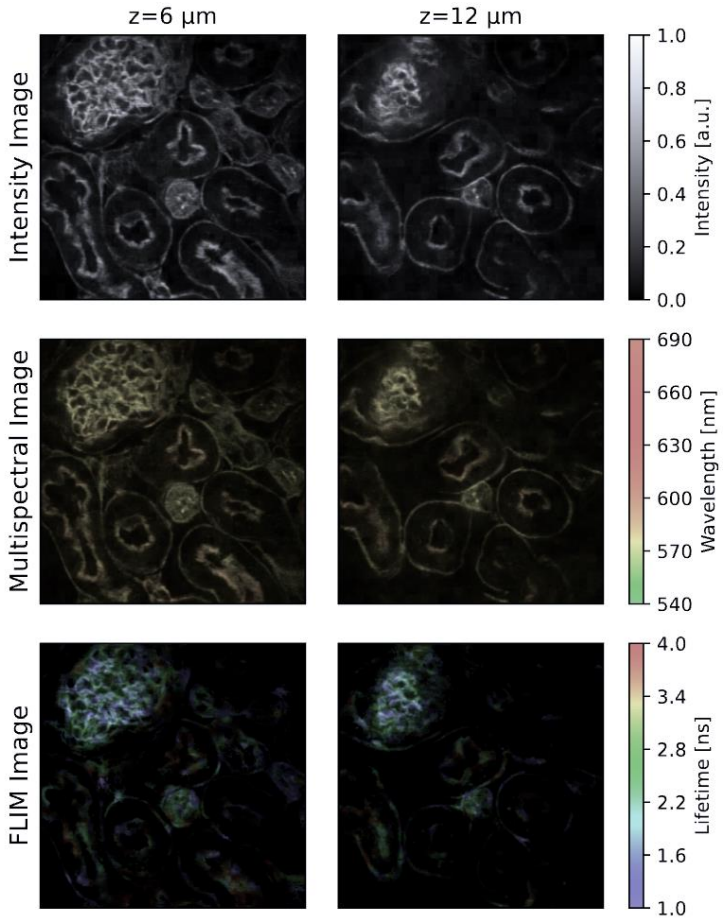

Figure 2. High-resolution output from our system. In this multidimensional setting, we first show the intensity images in the first row, the multispectral component in the second, and the related FLIM in the third. At the same time each column represents a different axial position in our five dimensional output.

We then further assess the capability of the system, and make sure that the outputs are consistent with an experimental measurement at the same resolution. Thus, we perform a hardware zoom with the SPC and compare that to the result of the DF algorithm (Figure 5). We consider the hardware zoom a physical ground truth (GT). The results show that the spectral dimension of DF closely matches the GT (quantitatively with a spectral angle mapper of 0.19 rad). Similarly, the lifetime of the DF's output closely follows the one of the GT (with a difference of 0.2 ns).

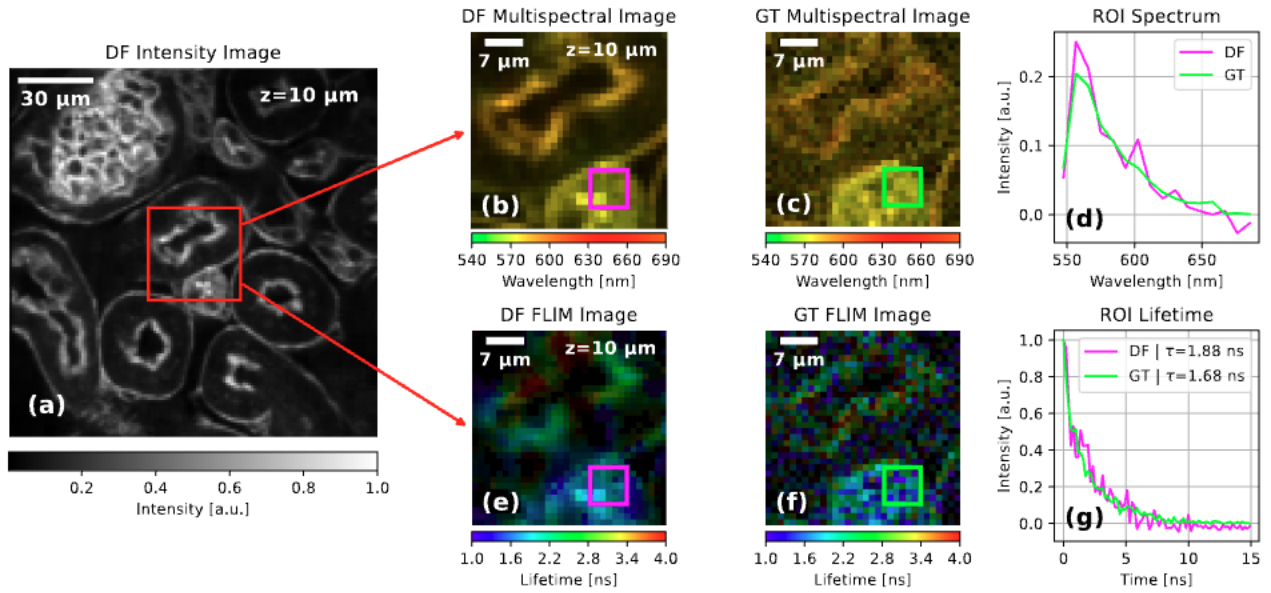

Figure 3. Comparison between a slice's region of the fused tensor and the matching hardware zoomed SPC spectrum and lifetime. (a) DF intensity image, (b) DF multispectral image, (c) GT multispectral image, (d) multispectral comparison on the region of interest, (e) DF FLIM image, (f) GT FLIM image, (g) time decay and lifetime comparison on the region of interest.

4 Machine Learning for Single-Pixel Microscopy

Single-pixel imaging has emerged as a key technique in fluorescence microscopy, where fast acquisition and reconstruction are crucial. However, current methods solve an inverse problem for each acquisition. We develop an approach for acquiring compressed measurements and reconstructing images for fluorescent microscopy by first learning a reduced optimal measurement matrix (encoder) and its reconstruction (decoder). This advances the applicability of the SPC across multiple fields as its reconstruction can now be instantaneous (few milliseconds) and the number of measurements can be reduced without compromising the quality.

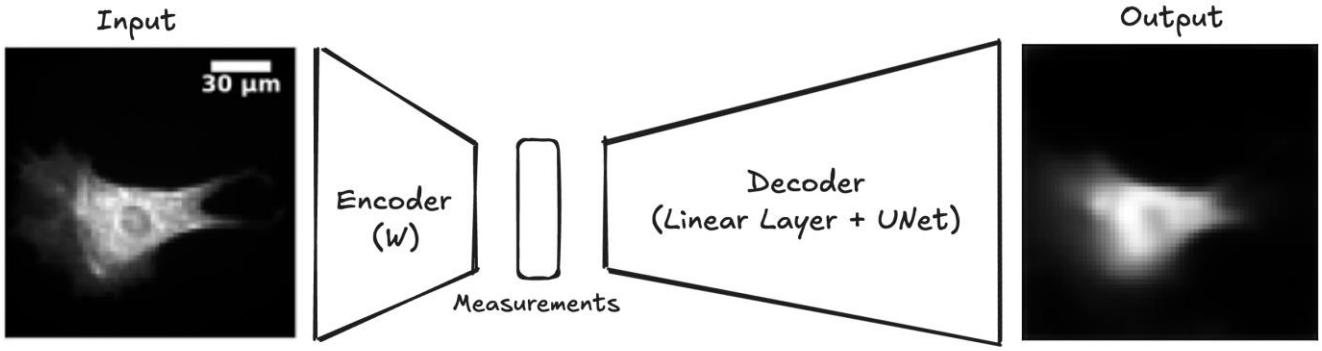

Figure 4. Schematic of the neural network at training time.

We achieve this by using a neural network (NN) called autoencoder (Figure 4.). This network is trained to learn how to compress and then reconstruct the same data. At the same time we impose a series of constraints, such that the first part of the network (encoder) resembles patterns than can be used in a plug-and-play fashion in the physical device. A similar type of NN has been previously applied to natural images on a SPC (Higham 2018).

In more details, the architecture of the network is comprised of a linear encoder, which will be our matrix W. This matrix then generates measurements when applied to an image. After this simulative step, we normalize the measurement and apply a Gaussian noise to them (with a standard deviation of 0.1). Then, the decoder reconstructs the image, first by projecting back into the image space the measurements through a linear layer; and finally, a two-dimensional UNet (2015 Ronneberger) completes the decoder to help denoise the corrupted measurements. To create a loss function to update the NN, the output of the network is compared to the original image with two metrics: MSE and SSIM. To the loss we add a binarization penalty based on the linear encoder W to drive the values of W to be 0 or 1 (and thus directly applicable to the DMD in our system). All the weights of the NN are then updated through ADAM using this combined loss. We program the NN in Python with Pytorch.

4.1 Training

The network is initially trained on an existing dataset of 100000 images of cells. In this way the network learns two different tasks: how to encode with a compressed number of binary patterns, and how to decode from the measurements to reconstruct the input image. The dataset is a reduced version of the open source CytoImageNet widely available (Hua 2021). We notice that the dataset contains images at different resolutions, and our desire is to train an NN that works at 32x32 and 64x64. These resolutions are best suited for our optical setup, as going at higher resolution highly increases the acquisition time and requires a decrease in the number of mirrors used in the DMD (increasing the noise). However, most images at 64x64 of CytoImageNet are corrupted, so we use 100000 images from the dataset at 128x128 resolution and resize them to 64x64 through bilinear interpolation. We split this reduced CytoImageNet into a training set (90%) and a validation set (10%). The validation set is used to choose hyperparameters for training, like the batch size, number of epochs, and the internal structure of the UNet. We stop training at 300 epochs using a batch size of 128 on an Nvidia RTX A6000.

We are additionally creating our own training dataset, by improving the optical system with scanning techniques in the x-y plane. An automated scan of the slides could provide big quantities of data for multispectral lifetime fluorescence microscopy, allowing us to train for the spectral and time decay dimensions. This could advance the currently available datasets that do not include this multidimensionality simultaneously.

4.2 Testing

At testing time, the learned patterns used in the SPC microscope to create the measurements from a sample and then the image is computationally reconstructed with the decoder. Until now these measurements where achieved through the usage of SH patterns and the reconstruction with an algorithm called TVAL3 which is a model based approach for solving this type of inverse problems.

4.3 Results

We show how our machine learning approach compares with the model based one in Figure 5. The first column shows the ground truth data acquired with the CMOS camera. The second column shows the output of a model-based approach, SH patterns and TVAL3 reconstrution. The third is a mixture of learned patterns through ML and TVAL3 reconstruction. The last, is our learned network, and it outperforms for two metrics (PSNR and SSIM) the previous model-based approach. In particular, the fully learned approach maintains more of the higher frequencies in the reconstructed images. For example, in Figure 5, on TEST CELL #2 SH measurements with TVAL3 reconstruction provides a solution in which a second cell is missing, while the fully learned approach maintains it. Similarly in TEST CELL #1, the fully learned solution maintains a more rigorous structure of the cell's nucleus and

shape compared to the model-based approach, with a difference of 1.68 in PSNR and 14 in SSIM. Ultimately this shows that we can compress more, reducing acquisition time, without loss of reconstruction quality.

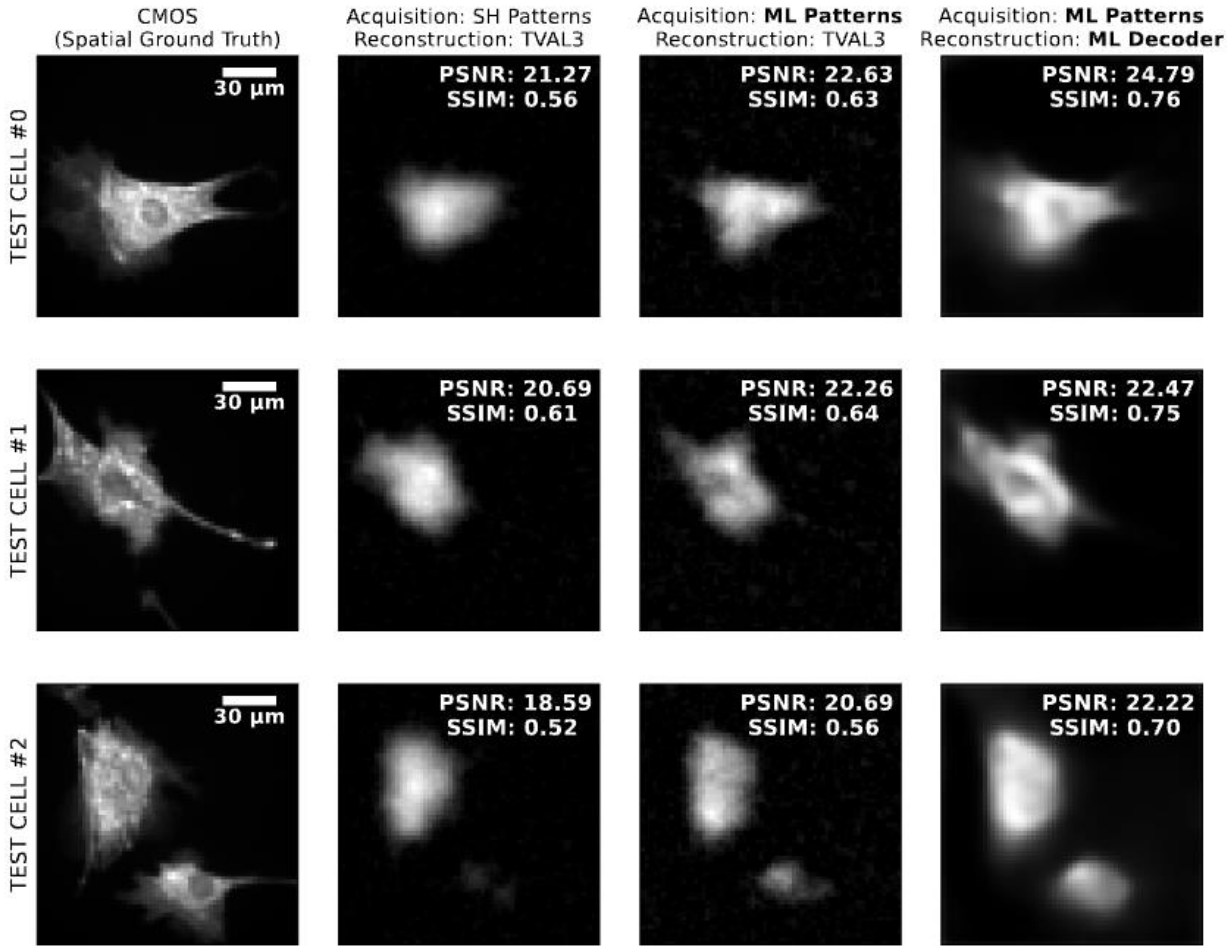

Figure 5. Comparison of different approaches on experimentally acquired data at 87.5% compression.

5 Conclusions

We have developed three computational approaches that can be used alongside. An optimal sampling technique from for CS and the SPC with aid from the CMOS. A data fusion approach to retrieve 3D multispectral lifetime fluorescent images from a microscope. An finally, a ML approach that can be used to acquire the SPC data and reconstruct the multispectral images from the measurements. These approaches advance the field in terms of speed and multidimensionality of acquisition and reconstruction.

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