

SCORED: Super-resolution Confocal Microscopy Enhanced by Deep-Learning

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ABSTRACT

We have developed a laser confocal microscope that is able to project on a sample programmable excitation patterns and filter and compose the final image entirely through digitally controlled solid-state devices. This fully digital laser microscope is orders of magnitude more flexible than current commercial microscopes and is naturally suited to integrate artificial neural networks at different stages of its imaging process. The envisaged "learning" microscope will be able to more easily adapt to the complex and changing needs of its users and show enhanced performance. Through ATTRACT-Phase I we have been able to demonstrate super-resolution imaging capacities beyond the diffraction limit of this ultra-flexible, totally digital, user-trainable microscopy concept.

Keywords: Laser microscopy; super-resolution microscopy; artificial neural networks; deep learning.

1. INTRODUCTION

- Laser confocal microscopes are the workhorse for sample visualization in all fields of cell biology and biomedical research as they allow targeted observation of cellular components with high contrast and resolution. Today, most universities and scientific institutions worldwide own laser confocal microscopes, jointly representing a huge market opportunity. However, despite the amazing technology feat that these microscopes represent, the scanning and filtering devices on which they rely remain disappointingly low-tech as these consist of motorized mirrors or spinning disks as well as physical apertures of fixed, predesigned sizes through which light needs to pass. This outdated electro-mechanical core of modern microscopes unnecessarily limits their flexibility and makes difficult the incorporation of technology developments from other fields, such as artificial intelligence (AI).
- During the last five years we have been developing the acousto-optic and electronic technology necessary to completely revamp the technological basis of laser microscopes. Our microscope is designed around two concepts:
 - a) a programmable laser illuminator based on acousto-optic technology. An ultra-fast acousto-optic deflector is driven by a computer-controlled waveform generator that holographically modulates the laser wavefronts

thus creating programmable excitation patterns.

- b) A custom camera with programmable pixel row readout and high-speed image processing electronics. This subsystem captures the emitted light from the sample, algorithmically filters out-of-focus photons and renders the final, user-viewable image in real time.

With these two key ideas we managed to completely digitize the operation of a laser confocal microscope. As a result, cross-fertilization with cutting-edge digital technologies, which was challenging before, now becomes all too natural. Our vision involves the incorporation of artificial neural networks (ANNs) that control the different stages of the imaging pipeline to create a "learning" optical instrument capable of tuning and improving its performance through interaction with the user. Such a fully digital, active-interrogation, sample-adapted, user-trainable microscope has simply no precedents in the market.

- Phase-I offered us a platform for a proof-of-concept demonstration of the capabilities of our system for merging with AI technologies. Toward that end, ANNs in charge of the final image integration were designed to obtain super-resolution information beyond the diffraction limit. Results obtained are commercially valuable (resolution below 100 nm at video rates) so a new patent application is planned.

2. STATE OF THE ART

As a proof of concept of the enhanced capabilities of our digital electro-optic laser microscope merged with AI techniques we focused on obtaining super-resolution. For many years, optical microscopy has been troubled by an apparently insurmountable obstacle: the diffraction limit imposed by the wave nature of light, which restricts its capacity to resolve sample details below 200 nm. It is then of little surprise that the knocking out of the diffraction resolution barrier by new techniques such as Stimulated Emission Depletion (STED) and Stochastic Optical Reconstruction Microscopy (PALM-STORM) has so quickly deserved worldwide recognition and a Nobel Prize (Chemistry 2014). Unfortunately, the stunningly resolved pictures produced by these instruments (down to a few tens of nm) come at a hefty price: a sophisticated and expensive technology or a very slow image acquisition. Therefore, the microscopy market eagerly seeks super-resolution alternatives with more balanced trade-offs [1].

3. BREAKTHROUGH CHARACTER OF THE PROJECT

Artificial neural networks have been previously applied to advanced microscopy, for example to accelerate the reconstruction of super-resolution information out of PALM-STORM images, as recently shown by W. Ouyang *et al.* [2] or to guess high-resolution details in low-resolution images based on prior experience, such as in Y. Rivenson *et al.* [3]. Although very probably these type of ANN applications will find its niche in future commercial products it should be noted that they work by analysing images produced through standard microscopy techniques a posteriori, they do not have any bearing on the image acquisition itself, so they more properly compete against image processing and reconstruction applications (such as deconvolution software, for example). An active-interrogation, capable of producing arbitrary fluorescence excitation patterns, ultrafast optical microscope controlled by an artificial neural network designed and trained to freely probe, extract and compose super-resolution images has simply no precedents, as we are the first to have devised or developed the unique combination of technologies that makes this possible.

1. PROJECT RESULTS

The microscope developed (Fig. 1) can generate through a smart illumination and a scanning protocol a series of different images from the same sample. The

ability to adapt lighting geometry, density and wavelengths is what allows us to define our proposal as programmable, in the sense of configurable, being able to adopt different identities of other types of microscopy and explore new configurations.

Therefore, each field of view becomes a stack of images that must be combined to reconstruct the final image. The way to combine the different partial views that are captured in each image, where the light has been strategically placed, gives a new dimension of improvement. From a typical confocal view (adding all the light) to the proposal developed in this project is based on Deep Learning, which strongly improves resolution.

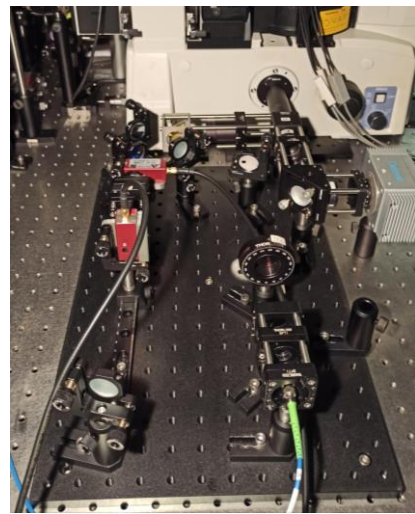


Fig. 1. Opto-mechanical prototype attached to a Nikon Eclipse TE-2000 epifluorescent microscope.

Of the different strategies used in DL, we have opted for the generation of simulated data. This strategy simplifies the generation of huge amounts of data and allows to obtain low (at diffraction limit) and high resolution (beyond diffraction limit) image pairs of the same field of view. We have developed a computational tool that mimics the physical principles in the real device: emission, illumination patterns, interference, cross talk, attenuations, Point Spread Function. It has been programmed using the same computation modules used in DL, which in the future could be inserted in the learning pipeline, directly combining and comparing real and simulated results.

In this initial stage of the proposal we have chosen to simulate large datasets composed of random spatially distributed nano-beads and straight lines. Followed by the addition of noise and background light, making our dataset as close as possible to the experimental conditions. Additionally, the ground truth to train the ANN (convolutional) is a blurred version of the sample used to simulate the microscopy images. Using perfect data as ground truth prevents the neural network from

generalizing and often finds solutions with good resolution but failing on localization or missing elements present in the sample. The blurring procedure allows to control the resolution achieved and avoids the above-mentioned problems.

To evaluate the super-resolution capabilities of our trained neural network, we used different synthetic resolution tests, see Fig. 2. The results show an isotropic lateral resolution improvement of more than 2-fold. The neural network can resolve structures of 80 nm with good localization, although with a slight decrease in the intensity, nevertheless this good localization is lost at 60 nm. The experiments have been performed simulating data coming from a 60 \times NA 1.2 objective using a 532 nm excitation laser. For this case the Rayleigh resolution limit is 270 nm.

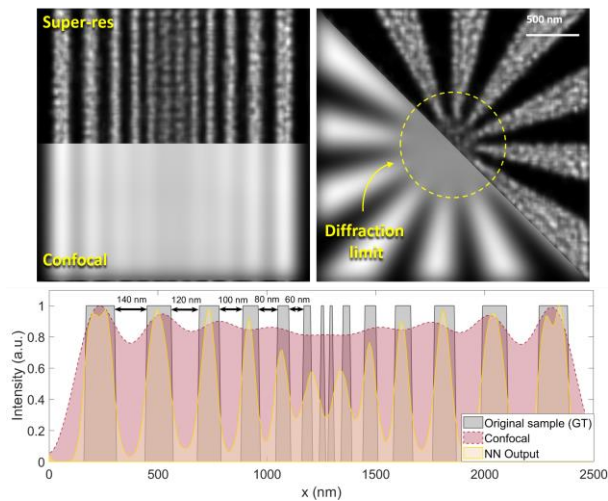


Fig. 2. (top) Comparison between confocal and super-resolution images. The yellow dotted ring denotes the physical resolution limit. (bottom) Average line profile (256 lines) from the top left sample.

Finally, to test the robustness of the network, it has been applied to several more realistic cases such as a simulated biological elements and finally in experimental samples. As seen in Fig 3a and 3b, simulated filamentous images (mimicking cytoskeletal structures) are also well reconstructed. The experimental samples are composed of randomly distributed sub-diffraction limit fluorescent beads deposited on a cover slip. Fig. 3c and 3d show low and medium density real images to test the performance in a real scenario. At low densities the scheme achieves better and remarkable results while in saturated areas in high density images some artifacts appear (grid structure). In the future, as the hardware part progresses in the prototype, the network training will be refined and improved, incorporating both synthetic and experimental training sets.

The results in both synthetic and experimental samples are surprisingly good. Network can generalize very

well, resolving much more complex structures, composed of lines, curves, constant zones and other arbitrary forms. Currently, the time required to compose a field using DL, assuming parallelism, give us 40 frames per second (fps), opening the possibilities of live cell imaging or real-time volumetric reconstructions. This time corresponds to the calculation time to process the data, but our prototype is able to acquire images up to 120 fps. This means that, if the image processing is done offline, biological processes can be observed at higher speeds.

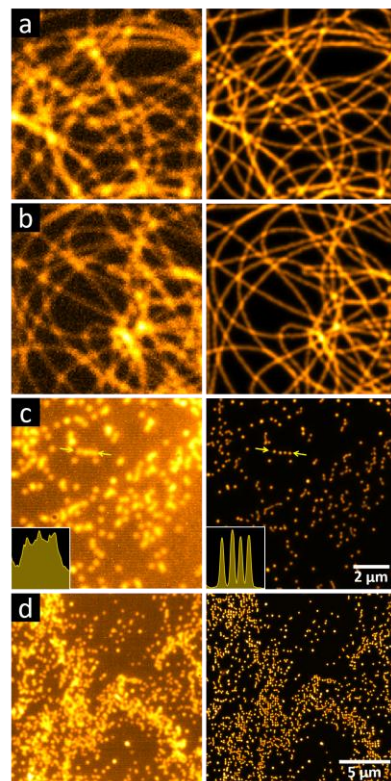


Fig. 3. Epifluorescence (left) and super-resolution (right) images. a) and b) Synthetic filaments, mimicking the cytoskeletal network of a cell [2]. c) and d) randomly distributed sub-diffraction limit fluorescent beads.

2. FUTURE PROJECT VISION

ATTRACT-Phase I has permitted us to show how our microscopy platform can gain commercially valuable new abilities (i.e. super-resolution in a little exploited performance niche) in a non-algorithm, non-predesigned way. However, the limited scope of phase I made us restrict the proof-of-concept to the easiest part within the imaging pipeline where an ANN can be embedded, that of the final image synthesis. The full vision of an advanced laser microscope that is totally managed by an user-trainable coordinated set of ANNs, which are in charge of both the sample exploration as

well as the information processing and image rendering steps, can only be attained in a more ambitious Phase II, with additional resources.

2.1. Technology Scaling

Following standard practice, our development plan towards a minimum viable product (MVP) will be arranged in four phases. Current status within the Technology Readiness Level (TRL) ladder is [TRL3](#).

1. **Alpha prototype** (TRL1 to 4): Proof of concept. Mostly off-the-shelf electronics; catalogue optics and prototyping opto-mechanics; software based on Matlab/Python; lab bench layout. Full ANN control of imaging pipeline & training devised.
2. **Beta prototype** (TRL5 to 6): Custom opto-mechanics; custom radiofrequency electronics; user-friendly basic software; deployable at core facility for feedback. Full ANN control of imaging pipeline implemented and pre-trained.
3. **Gamma prototype** (TRL7): Industrial design including external look & feel; fulfilment of CE marking and safety regulations; fabrication, alignment and installation documentation; full custom electronics; full user software
4. **MVP** (TRL8 to 9): Market ready. Final manufacturing materials, procedures and suppliers; Independent tests; technical/safety labelling; user manuals; CE certification for electromagnetic compatibility and laser safety.

2.2. Project Synergies and Outreach

In Phase II, according to the technology scaling in the previous section, we will need to liaise with:

- Manufacturers and developers of our core technology devices for further customization: e.g. AA Optoelectronic, France, Optronis, Germany.
- Electronic design partners to integrate current heterogeneous off-the-shelf electronic boards.
- Technology partners such as R&D organizations with expertise in random addressable active pixel image sensors, such as IMEC (Belgium) or CEA-Leti (France), and with expertise in application of ANNs to imaging problems, such as ElementAI (Canada) with whom we have a preliminary agreement.
- Advanced light microscopy core facilities for application development and testing, such as those in CNIO and CRG, Spain or EMBL Germany.

We have no plans to converge with other ATTRACT projects, as their originality makes this goal difficult without endangering each other's vision. Public dissemination in our field has proven very effective through Twitter, where many opinion leaders in microscopy are active. Regarding the mainstream public, the traditional media is still both effective and

interested in the development of advanced technologies, especially those related to Life Sciences [\[4\]](#).

2.3. Technology application and demonstration cases

A phase-II project will demonstrate an application in High-Content Screening (HCS). HCS is a microscopy-based phenotyping technique used in cell assays with a main application in drug discovery. The variety and complexity of HCS assays impose considerable demands on instrument manufacturers. For example, very high frame rates are needed to monitor calcium dynamics in cardiotoxicity assays. On the contrary, for analyzing fine structural elements, such as neurite outgrowth when testing neuroactive compounds, resolution is king. These are mutually exclusive goals that current technology cannot meet simultaneously. In our instrument the illumination patterns and matching confocal apertures are synthesized by digital means so that the main characteristics of the microscope can be tuned according to user's demands, potentially solving the issue. Screening clinically relevant drugs against patient derived cell lines has recently risen as a very promising alternative for personalized treatments in precision oncology [\[5\]](#). Personalized or precision medicine is one the hot-topics related to the health societal challenges of the future and is one of the top R&D priorities of the European Commission. Finally, as we mention in Sec. 2.2 we aim at partnering in Phase II with several research infrastructure organizations such as CE-Leti or the EMBL.

2.4. Technology commercialization

The technology assets can be transferred either by licensing the patents to a microscopy company or by launching a start-up. Regarding the first possibility, the points in favour are:

- The companies that dominate the microscopy market, the "big four": Nikon, Olympus, Zeiss and Leica, create "market-pull" type of innovations, which represent incremental enhancements over the state-of-the-art. Radically new technologies almost always come from research centres and universities, so these companies are used to license and develop technology that comes off site.
- We have a close contact with a C-suite executive in a national branch of one of the "big four", who has offered us help in getting the technology evaluated at the right decision level within the company.

Regarding launching a start-up, plus points are:

- The team has experience in starting up technology-based companies. Mario Montes is co-founder and major shareholder of Impetux Optics, a UB spinoff that markets laser-trapping instrumentation. Felipe Lumbreras, is a serial entrepreneur, having co-founded five spinoffs at the CVC-UAB.

- Starting a company captures considerably more value than merely licensing the technology. A company is an appropriate vehicle to encapsulate and leverage the know-how developed by the R&D team and an efficient way to finally transfer it at a larger profit.

Considering these pros and cons, and although we may still consider a license by a major corporation, we are working toward the incorporation of a company. As the technology is still in development in key areas, the contacts outside the university environment have been limited on purpose. However, an initial confidential inquiry has been launched with a microscope manufacturer in order to determine their potential interest in licensing our IP.

2.5. Envisioned risks

The following tables contain the risk probability matrix and mitigation plan:

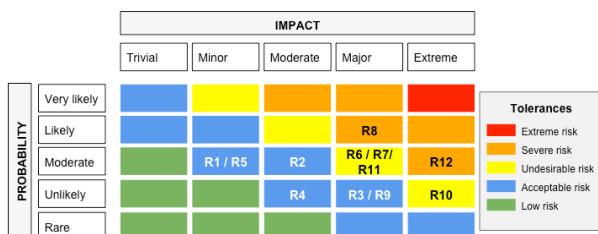
Tab. 1. a) Table with the identification and classification of risks. It also indicates the area they might impact. b) Mitigation strategy for the identified risks. c) Probability matrix and risk classification.

| RiskID | RiskName | Pos/Neg | RiskType |
|--------|--|----------|----------------|
| R1 | Limited color channels with a single device/need for more complexity | Negative | Technology |
| R2 | Underperforming image sensor | Negative | Technology |
| R3 | Only partial implementation of ANN approach is feasible | Negative | Technology |
| R4 | Lower acquisition/processing speed than expected | Negative | Technology |
| R5 | Technology useful outside the confocal field (e.g. super-resolution) | Positive | Technology |
| R6 | Less than desired first investment secured | Negative | Financial |
| R7 | Less than desired second investment, not finding an industrial partner | Negative | Financial |
| R8 | Not reaching mainstream markets/niche technology | Negative | Financial |
| R9 | High manufacturing costs | Negative | Business model |
| R10 | Competing optical technologies appearing in the market | Negative | Business model |
| R11 | Attraction of managerial/technical talent insufficient | Negative | Business model |
| R12 | Other business models more appropriate | Positive | Business model |

a)

| Response/plan |
|--|
| R1. Mitigate. Change the prototype desing, include two optical paths with 2 colors each. |
| R2. Mitigate adopting either alternatives already identified or fully custom device at a higher cost |
| R3. Accept it, if performance better than algorithmics. |
| R4. Mitigate. Enhance processing power at a higher cost. |
| R5. Exploit. Collaborate with experts/license IP and incorporate technology into own commercial product. |
| R6. Mitigate. Reduce specs for prototype and search for alternative funds |
| R7. Avoid. Find alternative funding. Find partners or VC abroad even as far as the US |
| R8. Mitigate. Bootstrap with revenues. Consider change of business model to Open Hardware |
| R9. Design and produce part of the electronics in-house. Negotiate with suppliers discounts based on volume. |
| R10. Accept. Deal with the competence, compete in value rather than in price. |
| R11. Avoid. Partner with venture builder if necessary. |
| R12. Exploit. Alternative business models: "Hardware as a Service" and "Open Hardware" approaches. |

b)



c)

2.6. Liaison with Student Teams and Socio-Economic Study

In project SCORED we are acutely aware, not only of the societal needs for advance training of MSc students

in entrepreneurial matters, but also of the capacity of these students to contribute with fresh and valuable ideas to R+D efforts. Prof. Mario Montes is instructor in the entrepreneurial course "Patents and Business in Photonics", belonging to the Barcelona Master in Photonics, in which these activities can find synergies within the current syllabus. Student activities regarding our possible open hardware business model as well as those related to section 5.3, applications with societal impact, could be used as the topic for exercises and the results actually be incorporated into the project.

Regarding the expert-driven socio-economic study of the ATTRACT initiative we are committed to participate in any activity that would foster ATTRACT goals. All data generated by our project regarding impact, links established with project's stakeholders, additional funding raised, commercial, scientific and academic outcomes, etc. we are willing to share with ATTRACT officials. We will also be happy to contribute with suggestions, initiatives and more active involvement.

3. ACKNOWLEDGEMENT

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