Viral lasers for biological detection (VL4BD)

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ABSTRACT

The COVID-19 pandemic has highlighted a pressing need for new analytical technologies that provide more sensitive and quantitative measures of proteins and whole viruses Virus lasers are a new class of biological laser system for biological detection in which the detection probes produce an amplified optical – rather than chemical, as in the case of PCR – signal. Ligand-binding assays based on virus lasers promise to be more rapid and precise than competing technologies. In the long-term, virus lasers will substitute antibody-based techniques in a wide range of applications, including discovery research, bioprocess analytics, clinical diagnostics, and environmental monitoring. In this project, we demonstrated the feasibility of the technology by overcoming two major technical barriers.

Keywords: Virus laser; Analytical technology; Biological detection.

1. INTRODUCTION

The long-term objective is to establish virus lasers as a new analytical paradigm with commercial applications in discovery research, bioprocess analytics, clinical diagnostics, and environmental monitoring. The goal of this project was to overcome two of the main barriers to the commercial development of virus lasers and to demonstrate the commercial feasibility of the technology:

- The first barrier was the high cost of the optical excitation source in previous prototypes. This entailed the design a new version of the laser photometer that replaces a > €10,000 laser-based source with a < €100 alternative light source.
- The second barrier was the manufacturability of the lasing-detection probe. Our goal was to design a new lasing-detection probe which could be manufactured by a third-party contract manufacturing organisation.

Virus lasers are a new class of biological laser system and represent a major technological breakthrough at the interface of physics and synthetic biology.[1] Virus lasers enable new ligand-binding assay formats in which the detection probes generate ab amplified optical signal. This unique signal generation promises to unlock new approaches to metrology in the life sciences, including ultra-precise assays, and mix-and-measure ligand-binding assays. Within the timeframe of the next decade, virus lasers will replace the current gold-standard antibody-based assay formats, transforming discovery research, bioprocess analytics, clinical diagnostics, and environmental monitoring.

In this project, a new prototype laser photometer was designed which substitutes a <€100 high-powered laser diode for a > €10,000 laser system, greatly expanding the breadth of possible applications for this technology. We translated this design into an engineering schematic using computer-aided design software and subsequently manufactured a base and lid for the optical components and mounting clips. We designed and tested electronic circuits for the excitation light source and for the optical detection system.

In parallel, we developed a new generation of lasing-detection probes. Two new lasing-detection probes were designed: the first was based on fd bacteriophage; and the second design was based on a genetically-engineered recombinant Tobacco mosaic virus-like particle (rTMV). We focused on the rTMV design and developed a manufacturing process for this detection probe. After iterative improvement to the expression and purification protocols, a third-party contractor was able to manufacture rTMV at 1 L scale, yielding 4.8 mg/L at a cost of ~ €5000.

2. STATE OF THE ART

The COVID-19 pandemic has highlighted the shortcomings of current state-of-the-art analytical technologies for biological detection, underscoring the need for development of new and better analytical

technologies, a need which has become an immediate and pressing global concern.

Enzyme-linked immunosorbent assays (ELISAs) are ubiquitous in life science laboratories globally and are a ligand-binding platform for either qualitatively determining the presence of a target ligand or for quantitatively measuring the concentration of a ligand within a biological solution. For instance, ELISAs are used to quantify biomarker concentrations in blood serum in clinical settings. There are several ELISA formats, and in general their mechanism-of-action requires the binding of an antibody detection probe to the target ligand, followed by detection or quantification via a colorimetric, chemiluminescent, or fluorescent readout.

The technique is used to quantify ligand concentrations within complex mixtures at low concentrations. Antibody probes can be developed which have a high binding-specificity for their targets, enabling assays which are selective for the target. Some assays formats can achieve limits of detection as low as 0.1 amol mL^{-1}.[2] ELISA has several disadvantages which are especially problematic in certain scenarios. ELISA requires several liquid-handling steps which typically make the technique both slow and laborious and therefore ill-suited to time-sensitive applications. ELISAs for new ligands often require a substantial investment in development, and frequently an optimised ELISA will still be imprecise. The uncertainty created by a lack of precision is especially problematic in clinical settings requiring an unambiguous result.

The polymerase chain reaction (PCR) is an important technique used for molecular diagnostics. PCR is fundamentally limited to the detection of nucleic acids, and therefore cannot directly detect the full range of biomolecules, including proteins and cells.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

Virus lasers have the potential to replace antibody-based ligand-binding assays, including ELISA. Unlike techniques that rely on colorimetric, chemiluminescent, or fluorescent detection, virus-lasing detection probes generate a laser signal which opens up avenues towards new analytical paradigms (Tab. 1).

Virus lasers are especially well-suited to applications requiring rapid feedback, or applications that need high levels of precision. For instance, virus lasers could be used to create rapid and precise clinical diagnostics.

Tab. 1. Comparative table highlighting the breakthrough character of virus-laser ligand-binding assays versus ELISA.

	Virus lasers	ELISA
Mode of signal generation	*Laser emission from the lasing-detection probes in a laser photometer.*	Colorimetric, chemiluminescent, or fluorescent signals from enzyme-conjugate or dye-conjugated antibodies.
Precision	*Ultra-precise, digital measurements at critical concentrations*	Extensive optimisation required to reduce the coefficient of variation to less than 10 %.
Speed	*Mix-and-measure ligand-binding mode yields a readout as soon as the ligands start to be bound (minutes).*	Several liquid-handling steps make ELISA slow (hours).
Sensitivity	*30 fmol mL^{-1} is the current limit of detection, but this is likely to be reduced with further development.*	0.1 amol mL^{-1} is feasible in some assay formats, but depends on the binding affinity of the antibody.

4. PROJECT RESULTS

Photometer

A laser photometer was designed that incorporated a continuous-wave laser diode as the optical excitation source. The design of the resonator geometry was iterated in response to a theoretical investigation of the optical configuration. Computer-aided design software was subsequently used to design a base and lid to house the optical components, and to design mounting clips to hold the components in position. The designs for the base and the lid were optimised to reduce the cost-of-manufacture.

A supply chain for manufacturing the base and the lid for the optical configuration was established: both parts were computer-numerical-control (CNC) milled from aluminium by one contract manufacturing organisation (CMO), and then transferred to another CMO for anodization (Fig. 1). A separate facility was used to 3D print the mounting clips using both stereolithography and selective laser sintering.

Electronics

Electronic circuits for the optical excitation and for the optical detection were designed, assembled, and tested. Optical excitation electronics include a 1.2A current driver circuit assembly using the laser diode driver from Wavelength Electronics. The modulation signal, sinusoidal modulation, is generated using Direct Digital Synthesis

Fig. 1. Photographs of the laser photometer base plate (upper), and lid (lower). The parts were CNC milled from aluminium, and then anodized, by separate contract manufacturing organisations.

(DDS) to modulate the baseline output current, which itself is set via an onboard trimmer according to laser diode specifications. This can generate sinusoidal waveforms up to 12.5MHz (using a 25MHZ reference clock) with a 0.1Hz resolution and is programmed through a 3-wire Serial Parallel Interface (SPI) using an Arduino. Prior to input onto the laser diode driver, the generated modulation signal is input to a custom-made, non-inverting amplifier and DC level shifter assembly to regulate amplitude and DC level as dictated by the laser diode driver transconductance factor and output the anticipated laser diode current. A PC-based oscilloscope monitors the modulated output current. A schematic of the optical excitation electronics setup and circuit assembly is shown in Fig. 2.

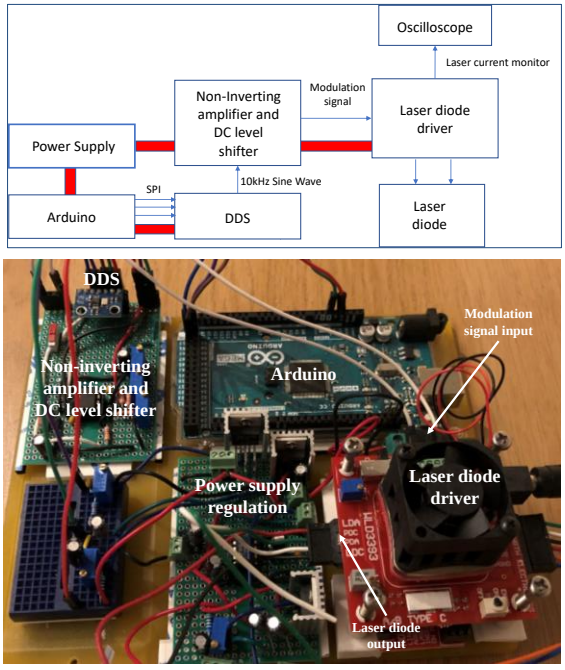

Fig. 2. Schematic of the optical excitation setup (Upper) and circuit assembly (Lower).

Optical detection electronics include a transimpedance amplifier stage which interfaces with an S2281 silicon photodiode from Hamamatsu via a coaxial BNC connector. The generated photo-current is amplified and converted to a voltage through the transimpedance stage which includes an CMOS operational amplifier with two gain settings, 15.8kV/A and 158kV/A respectively, over a bandwidth of 100kHz. The gain is set through an CMOS SPDT switch and the output voltage is then input to a voltage buffer assembled using a precision rail-to-rail output amplifier. Output to the buffer is fed to a PC-based oscilloscope. The optical detection setup and circuit assembly is shown in Fig. 3.

Virus-lasing detection probes

Two virus-lasing detection probes were designed for commercial manufacture. The first design consisted of a fd bacteriophage displaying a ligand-binding protein fusion on ~150 copies of the major coat protein. The second design consisted of a genetically engineered Tobacco mosaic virus-like particle (rTMV) (Fig. 4.). Recombinant expression of a similar virus-like particle has been demonstrated previously[3]. Our novel design additionally included a method for displaying a ligand-binding protein fusion on some of the coat proteins. After evaluating the merits of both designs, the project focused on rTMV, as this probe is not a virus and is therefore not infectious, which will present fewer hurdles to widespread customer adoption.

A synthetic gene for rTMV was synthesised, cloned into an expression vector, and transformed into an appropriate strain of *E. coli*. Initial expression tests were performed based on literature precedence[3] and showed that the coat proteins with and without the protein fusion could be co-expressed (Fig. 5). However, the purification processes recommended in the literature[3] were not successful for rTMV, suggesting that the fusion proteins substantially affected the biophysical properties of the virus-like particle. We tested different purification strategies at small scale and discovered that an affinity purification technique increased the yield by a factor of 15 and resulted in an acceptable level of purity.

We performed additional small-scale expression tests to evaluate the performance of three further culture media.

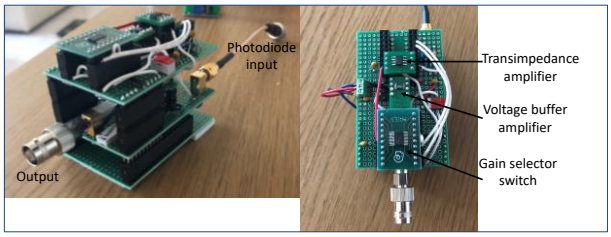

Fig. 3. Optical detection setup and circuit assembly (plan view).

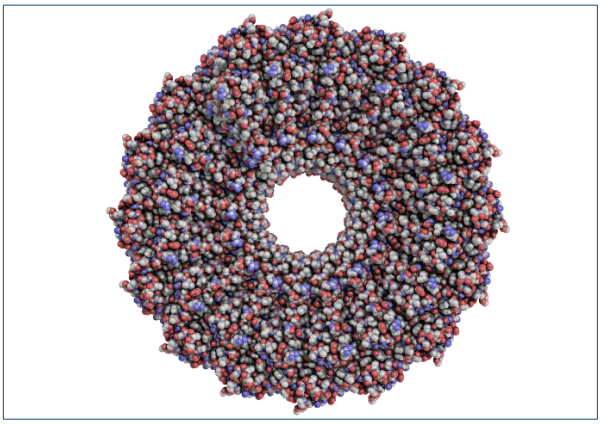

Fig. 4. Model of the atomic structure of Tobacco mosaic virus viewed along its long axis (PDB: 6R7M).[4,5] The rTMV lasing-detection probe would additionally have ligand-binding protein fusions on some coat proteins.

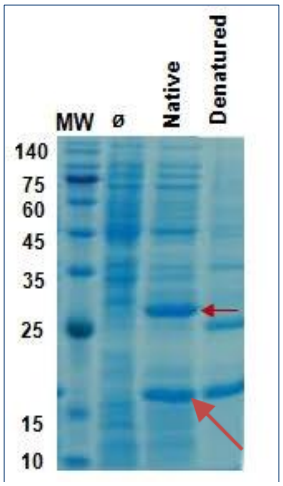

Fig. 5. Reduced PAGE showing the results of the initial expression test. Protein bands corresponding to the coat protein with and without the protein fusion are present, as indicated by the red arrows.

We discovered that the production yield was 43% higher in one of the culture media versus the original choice of culture medium.

The process was scaled up to 1 L from 200 mL scale using the improved selection of culture medium, and the improved purification strategy. The purity was comparable to the small-scale tests, but the yield was only 33 % of the anticipated yield, which was likely due to a low-quality batch of the affinity resin. A process was devised for labelling rTMV with Alexa Fluor™ (Thermo Fisher Scientific) dyes, and for small-scale purification of the dye-conjugated rTMV.

Whilst the design of rTMV was performed at UCL, the small-scale expression and purification testing, and the one-litre scale-up were performed by a contract development and manufacturing organisation (CDMO).

By assigning the work to a CDMO, we have shown that it is possible to commercially manufacture rTMV.

5. FUTURE PROJECT VISION

5.1. Technology Scaling

The first step towards scaling the technology involves building a benchtop laser photometer which could be manufactured by CMOs. The work completed in this ATTRACT phase 1 project represents the starting point for this technology scaling process. The existing designs will be used to construct a laser photometer, including the optics and the electronics, which would then be tested using appropriate dye solutions to establish whether laser action can be sustained. These results would be used to fine-tune the optical configuration, and the electrical circuits. The next step would be scaling down the electrical circuits by designing printed circuit boards (PCBs) which could be readily manufactured. In parallel, a case for the instrument would be designed.

Technical development of the lasing-detection probes would occur concurrently with the development of the laser photometer. The first step is the optimisation of a dye-labelling process for rTMV, which would require characterisation of the detection probe by mass spectrometry, surface plasmon resonance (SPR), and electron microscopy. Mass spectrometry will determine the percentage of the coat proteins with a fusion protein, and the percentage with a covalently attached dye molecule. SPR will quantify the binding kinetics of the detection probe, which will allow direct comparison of rTMV to the antibody gold standard. Electron microscopy will enable visualisation of the detection probes for assessment of their length and morphology.

Once a benchtop instrument has been constructed, it will be subjected to side-by-side testing against the current standard techniques, including ELISA. The output would be a specification sheet detailing the limit of detection, precision, and robustness compared to other relevant techniques. This testing would take place in a laboratory setting similar to where the final product would be used, increasing the Technology Readiness Level (TRL) to 5.

Beyond this, the technology would be subjected to iterative developments to improve its performance and adapt it for healthcare applications.

5.2. Project Synergies and Outreach

Expanding the consortium to include 5 to 9 partners from relevant industries will be critical to realising the vision, and building momentum. We would seek out partners who could independently test the prototypes in their laboratories, and provide feedback on its performance and usability. For instance, we would seek a partnership with the European Molecular Biology Laboratory (EMBL), an ATTRACT consortium member. In addition, we will connect with other ATTRACT Phase 1 cohort members to look for opportunities and synergies.

We plan to publicly disseminate results through peer-reviewed articles published in international journals to educate the academic community on the technology. We will keep the public up to date on our progress during Phase 2 through a new website which will host webinars and other interactive sessions. The benefits of the technology will be shared with potential early adopters of the technology at industry-focused conferences.

5.3. Technology application and demonstration cases

Virus lasers are a major technological advance on the current state-of-the-art and have a diverse range of possible application in areas such as discovery research, bioprocess analytics, clinical diagnostics, and environmental monitoring.

The COVID-19 pandemic has highlighted the inadequacy of current analytical techniques, and virus lasers offer a compelling alternative. In ATTRACT Phase 2, we plan to raise the TRL so that the technology can be deployed to solve some of the most pressing analytical challenges in *healthcare*. For instance, virus lasers could form the foundation of a new analytical platform for rapid, ultra-precise clinical measurements of virus-neutralising antibodies in blood serum.

The technology will benefit the European research community by providing access to a more-precise, less-ambiguous ligand-binding assay format better suited to high-throughput studies.

5.4. Technology commercialization

The focus of this ATTRACT project was to establish commercial feasibility of the technology. We have successfully achieved this goal by showing that both the laser photometer and the lasing-detection probes could be manufactured by CMOs at modest cost, opening up a broad range of potential application spaces. The next phase requires further technical development to raise the technology readiness level to commercial levels and the expansion of the consortium to include industrial partners. At this stage, the technology would be well positioned to generate interest from private investors.

5.5. Envisioned risks

There are risks associated with the development of both the laser photometer and the lasing-detection probes.

The prototype laser photometer might not be able to sustain continuous laser emission, in which case the continuous-wave laser diode would be replaced by a nanosecond pulsed laser module. This would increase the total cost of goods, impacting which arenas the technology could be economically deployed in.

The lasing-detection probe might not bind the target-ligand with an affinity comparable to the gold-standard antibodies. In that case, rTMV would be subjected to further biological engineering to improve its binding affinity and selectivity.

Travel disruption in Europe will make it more challenging to establish an international consortium, which will be mitigated by taking the opportunity to meet other ATTRACT teams through the network.

5.6. Liaison with Student Teams and Socio-Economic Study

An experienced person would be nominated to facilitate an MSc. level explanation of the projects and its long-term vision. We would prepare videos and teaching material for online classes and discussion groups. We would contribute to the socio-economic study of the ATTRACT initiative and ecosystem by providing opportunities for monthly interviews with key personnel in the consortium and by providing case studies on different applications of the virus-laser technology.

6. ACKNOWLEDGEMENT

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7. REFERENCES

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