Biological Detection Using Virus Lasers

ABSTRACT

Detecting biomolecules, such as proteins or cells, with precision and high sensitivity is labor intensive with traditional assay devices. Researchers from the United Kingdom have developed a virus laser for rapid biomolecular detection with the precision of commercial systems. The commercial feasibility and low manufacturing cost of a genetically-engineered Tobacco Mosaic virus-like particle (rTMV) is investigated for virus laser applications, and the detection limit and amplified lasing signal are quantified for a virus laser utilizing the rod-like virus, M13. The M13 virus laser enables greater than 10,000 times increase in signal from a 50% increase in probe concentration and is sensitive to 90 fmol mL^{-1} monoclonal antibody, while the rTMV probe promises commercial manufacturing and a low setup cost.

BIOLOGICAL DETECTION

With the COVID-19 pandemic affecting businesses, social life, and especially the health and life of everyone, advancing technology for biological detection is critical to prevent further spread of the COVID-19 virus and improve healthcare quality. The pandemic has exposed the shortcomings of current analytical technology for biological detection.[1]

Timely detection and diagnosis is vital to improve healthcare quality and is required to guide outbreak measures and infection control.[2] Analytical methods need to have sufficient accuracy, sensitivity (specifically for lower concentration measurements), low cost, high signal-to-noise ratio, low risk of human error, and minimal time consumption. High reliability in early stages of infection is essential to effectively detecting and tracking a virus and controlling the containment of infection. Recent experiments with virus lasers could meet these criteria for better healthcare quality.

EXISTING TECHNOLOGY

An Enzyme-Linked Immunosorbent Assay (ELISA) is the traditional method to determine quantitative concentration measurements of a ligand within a biological solution or to qualitatively determine the presence of a specified ligand. This gold standard method can be used to quantify biomarker concentrations in blood serum. The general principle of an ELISA measurement is the binding of an antibody to the specified ligand in the sample solution which creates a colorimetric, chemiluminescent, or fluorescent reaction which can be detected or measured.[1]

What are Virus Lasers and Detection Probes?

A bio-laser is a relatively new technology that utilizes biological material as part of the laser cavity or part of the gain/lasing medium.[3] A detection probe is used with a special tag to attach to the specific molecule for detection. Traditional probes have depended on antibodies and fluorescent dyes to make the binding detectable. Virus probes attached to the molecule will emit light as the virus is a part of the lasing system. **Figure 1** shows a virus probe consisting of the filamentous bacteriophage M13 virus. This virus laser will be discussed in detail.

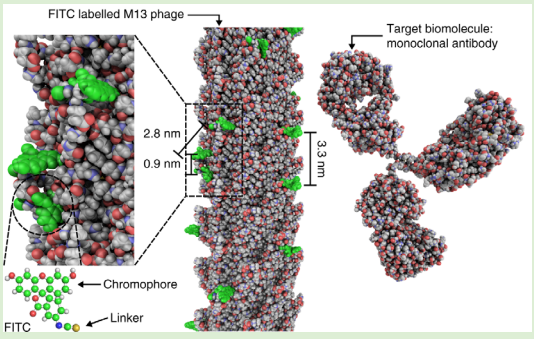

Figure 1. Virus probe design. Model of the atomic structure of a lasing detection probe composed of M13 bacteriophage covalently modified with fluorescein isothiocyanate isomer 1 (FITC) dyes.[3]

The downside to ELISAs is the signal created from the antibody fluorescent probe attaching to the ligand can be weak and hard to detect and distinguish from background noise.[3] Due to tedious liquid-handling and washing steps, these instruments are often slow and laborious. This can be problematic for time sensitive analytics and applications. In addition, although the instrument has a high price and is a labor-intensive investment, ELISAs can be imprecise, even with proper optimization. When dealing with life-threatening viruses, precision and accuracy are essential to quality detection and monitoring. High enough amounts of imprecision could lead to uncertainty in analysis results or monitoring situations.

Polymerase Chain Reaction (PCR) is often used for molecular diagnosis and many other applications. However, even PCR has limitations. It is effective on a narrow range of biomolecules, specifically nucleic acids and not proteins and cells, restricting its range of application.[1]

Recent experiments have investigated biological lasers using DNA scaffolds, fluorescent-protein-expressing cells, and the synthesis of plasmonic nanolasers. However, these are not adequate for lasing and binding a large variety of epitopes with the specificity of antibodies. Researchers from University College London, Bio Nano Consulting, and ETH Zürich have developed a virus laser to help create new methods of biological monitoring. The intense, monochromatic signals generated from the virus lasers are potentially a superior alternative to ELISAs and PCR methods of biological detection for rapid, highly sensitive, and more precise measurements.[3]

In the experiment, a new prototype laser photometer and a new generation of lasing-detection probes were researched for commercial manufacturing. The properties of a virus laser[3] and the commercial manufacturability of a virus laser[1] were examined for two distinct biological materials: filamentous bacteriophage M13 virus and Tobacco Mosaic virus-like particle (rTMV), respectively.

SPECIFIC VIRUS LASERS

M13 Virus

The M13 virus (**Figure 2**) is a filamentous bacteriophage, 7 x 900 nm rod-like virus, that infects F pilus (appendage on bacteria) expressing strains of *Escherichia coli* (*E. coli*). Not only can M13 be the key component in nanosystems (virus-based lithium-ion batteries and piezoelectric generators), but it can also be an effective substitute for antibody probes for biological detection.

Using this virus as a detection probe inside a resonator can generate a lasing emission signal to detect the binding of specific antibody molecules. This creates a stronger signal than standard fluorescence detection, and it can also be compatible with ordinary fluorimeters. When the probe is beginning laser emission, the photon flux from the probes increases by five orders of magnitude. It also narrows the spectral linewidth to below 5.0 nm, and the sensitivity of the output intensity is heightened to correlate to small changes in the probe concentration.[3] Utilizing a high performance laser driver with stable current helps maintain the narrow linewidth for better precision. Increasing the probe concentration will increase the lasing signal for greater responsivity.

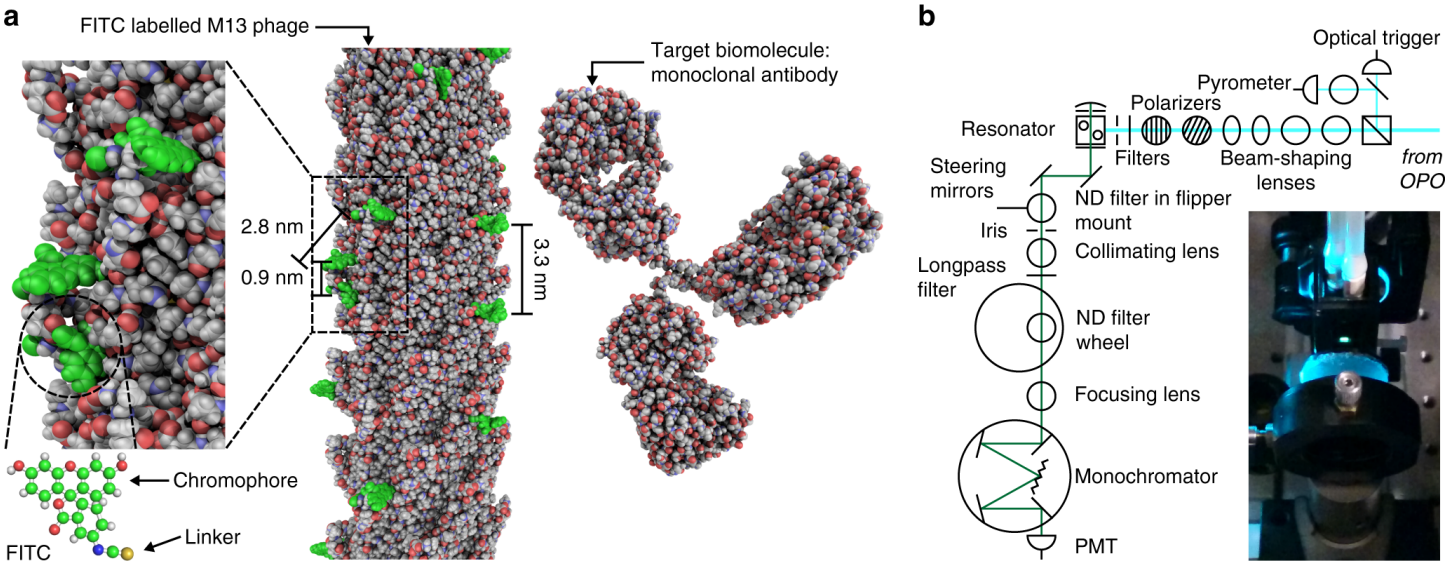

Figure 2. Design of the virus lasers. a) Model of the atomic structure of a lasing detection probe composed of M13 bacteriophage (PDB:2MJZ) covalently modified with fluorescein isothiocyanate isomer 1 (FITC) dyes (PubChem CID: 18730, green). b) Optical configuration for experiments conducted using resonant cavity. [3]

rTMV Virus-like Particle

The traits of a virus enable it to self-assemble into uniform nanoscale shapes and make the virus very attractive for a variety of applications. However, a problem arises when handling and using virus particles: viral replication and recombination that result in the loss of the desired functionalities.[4] To avoid these issues and to make the result more user friendly for widespread applications, the virus particle can be genetically modified to neutralize and stabilize the virus for small molecule binding. This is how a virus-like particle is assembled and created. Researchers have modified Tobacco Mosaic Virus (TMV) (**Figure 3**) to make it into rTMV, a virus-like particle.

The genetically engineered Tobacco Mosaic virus-like particle (rTMV) is not a virus, but can still be used as a detection probe with the classification of "virus laser." Because rTMV is not infectious, it can more easily be adapted for commercial use.[1] It is essential that this detection probe is not infectious, limiting problems and potential virus replication in the lab. This eliminates most health or safety concerns for worldwide application.

Although this virus-like detection probe was never tested for laser sustainability, the commercial construction of rTMV was investigated. Establishing commercial feasibility of virus laser technology opens up new potential applications at lower expenses. Further exploration of rTMV could improve its success of binding and selectivity as a detection probe.

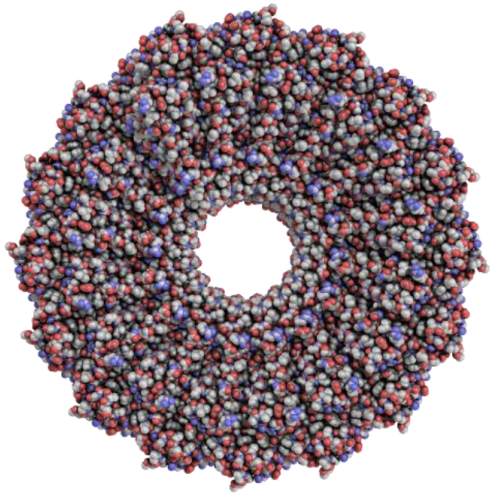

Figure 3. Model of the atomic structure of Tobacco mosaic virus. The rTMV lasing-detection probe would additionally have ligand-binding protein fusions on some coat proteins.[1]

Table 1 compares virus lasers and ELISAs with key parameters for biological detection.

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

	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 optimization 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.

RESULTS

The ability to commercially manufacture rTMV was proven successful for virus laser biological detection. With this development, virus lasers can continue to advance in detection markets for worldwide use. **Figure 4** shows the optical excitation setup and circuit assembly for the rTMV virus laser.

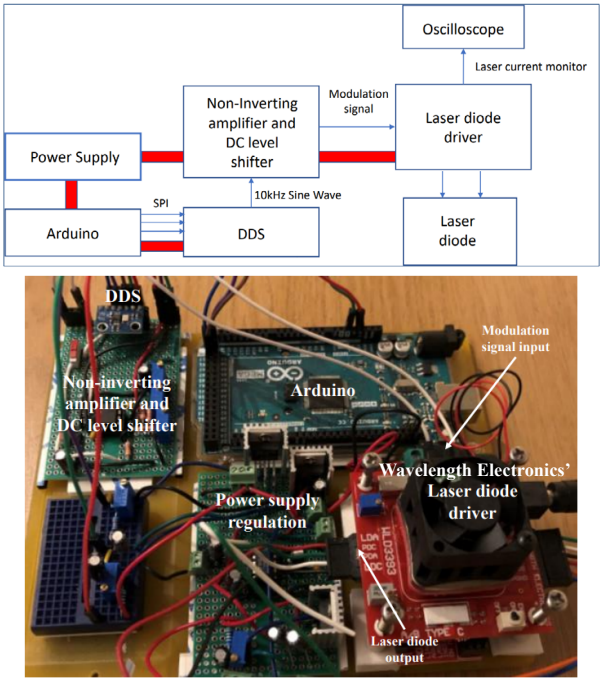

Figure 4. Schematic of the optical excitation setup (Upper) and circuit assembly (Lower) for the rTMV virus laser.[1]

In the earlier study (2019), a M13-based probe was used as the lasing media to generate the amplified signal. This allowed detection of the binding of a target antibody molecule. With this design, an increase of 50% in the probe concentration resulted in greater than 10,000 times increase in signal to detect. Sensitivity was as high as 90 fmol mL^{-1} allowing detection of clinically relevant concentrations of biomolecules without the time constraints of ELISAs.

Virus lasers can be just as effective as ELISAs or other detection probes from the amplified laser emission of the laser detection probe. This can increase the signal-to-noise ratio enabling easier detection of trace concentrations of specific molecules. With the reduced detection and analysis speeds and increasing precision and sensitivity, virus lasers show potential to replace current methods and devices for biological detection.

WAVELENGTH'S ROLE

Constant current and stable wavelength is a critical part of the laser emission with the virus. Modulation was also a key method to lower the cost of the experiment while avoiding expensive pulsed laser diodes. The modulation of the laser current was directed through Wavelength Electronics' WLD3343 Laser Diode Driver, and the modulated signal was output to the laser diode. **Figure 5** shows the WLD3343 Laser Driver component.

Figure 5. WLD3343 Laser Diode Driver

The WLD3343 provides up to 3 A of current to the laser diode using only a single supply of +5 V. The current can be remotely enabled or disabled, and there is zero leakage current. Multiple safety features and its compact size make the WLD3343 ideal for biomolecular detection applications.

Researchers also utilized the WLD3393 Evaluation Board for the WLD3343 Laser Driver. This allowed rapid prototyping for the virus laser design. On-board switches, connectors, and trimpots make configuration and operation simple. Connections from the power supply and to the laser diode are made simple using the input and output cables. Modulating the laser diode was easy to implement with the evaluation board for the experiment. **Figure 6** shows the WLD3393 Evaluation Board.

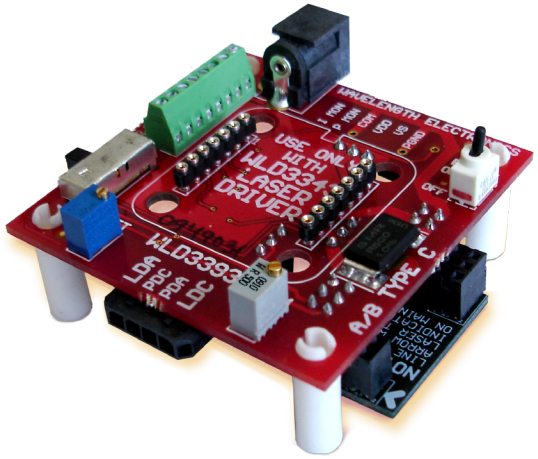

Figure 6. WLD3393 Evaluation Board for the WLD3343 Laser Diode Driver

SUMMARY

Researchers have designed and developed rTMV and M13 virus lasers for rapid, precise, and sensitive biological detection. With the virus material used as part of the lasing medium of the laser, detection of particular molecules in a sample is no longer difficult. The WLD3343 Laser Diode Driver and with the WLD3393 Evaluation Board aided the laser system in providing constant current and stable wavelength for accurate and repeatable experiments. The manufacturability of rTMV was proven a success, and the M13-based detection probe showed orders of magnitude increase for 50% increase in probe concentration. Virus lasers show great potential for biological detection for today's analytical needs.

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USEFUL LINKS

- WLD3343 Laser Driver Product Page
- WLD3393 Evaluation PCB Product Page

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The format of Table 1 was changed, but the content has remained unchanged.

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Figure 1 was cropped. No changes were made to the other images. They are presented here in their original form.

The captions for Figures 1 & 2 have been modified from their original form.

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PRODUCTS USED
WLD3343, WLD3393

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