Applied Plasmonics for the Cultivation and Study of Photosynthetic Microorganisms

by

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Abstract

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This thesis explores nano-scaled plasmonic solutions to the problem of light capture and distribution in microalgae cultivation schemes. An evaluation of the effect of light quality on productivity under different photobioreactor geometries shows a 2x increase in productivity under 630-nm red light for low density cultures under low light intensity, compared to 534-nm green light. The opposite was true however when high density cultures are exposed to high intensity light – 534-nm performed 4x better than strongly absorbed 630-nm red light. These results highlight the coupled influence of light quality and conventional photobioreactor design parameters. Wavelength specific, near-field plasmonic phenomena are then used as a means to couple light into cyanobacteria biofilms showing that plasmonic fields can be used to drive photosynthesis in living cells. 10-µm thick biofilms with maximum cell volume density of 20% vol/vol (2% more total accumulation than control experiments with direct light) were cultivated over three days. Far-field plasmonic phenomena were also applied to bulk cyanobacteria cultures to demonstrate a 6.5% enhancement in productivity. Resonant light is scattered back towards the culture increasing total absorption while off-resonance light is transmitted. Supporting technology that enables patterning of plasmonic nano-features with a commercial CO₂ laser system is also presented. Patterning speeds of 30
mm²/min and patterning costs of $0.10/mm² are demonstrated which are -30 fold faster and -400 fold less expensive than e-beam lithography. Finally, crude oil characterization based on surface plasmon resonance is demonstrated, with applicability to both conventional and alternative energy industries. Bitumen-toluene mixture tests show a measurement sensitivity of $74^\circ$ RIU⁻¹, and a limit of detection below 1% toluene. Seven crude oils from around the world are differentiated with refractive indices spanning 1.44 to 1.56, with a sensor limit of detection of 0.0006 RIU.
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Chapter 1.
Forward

1.1 Motivation

The world of micro- and nano-technology may seem like an unlikely place to look for solutions to the world’s energy related problems because the mismatch between length scales is so vast. The largest energy process on the planet however, is driven by machinery smaller than the eye can see and is quietly at work all around us, converting sunlight into chemical energy – photosynthesis. Nature has equipped plants and algae with the photosynthetic tools needed to terraform the globe and sustain life on earth, and it all happens through micro- and nano-scaled systems. Taking inspiration from these natural processes, this thesis explores how nano-scaled optical phenomena can transcend the gap between small-scale science and global need.

The need to develop a carbon neutral fuel source that is both energy dense and compatible with current infrastructure is increasing as climate change from CO₂ emissions continues to progress. Biofuels derived from microorganisms are a promising avenue to meet this objective. Commercially viable cultivation of these organisms however, is a challenge that has yet to be met in part because typical microalgal cultivation schemes suffer from low photosynthetic efficiency (<1%).¹ The low efficiency of photosynthesis is largely related to sub-optimal light intensity (either too high or too low). In one sense, having as many photons available for photosynthesis as possible seems like the right approach since more photons provide more energy which can be locked away as bioproducts. However, typical microalgal species grow best when the flux of photosynthetically active radiation - photons with wavelengths between 400 nm and 700 nm - is between 20 and 200 μmol photons/(m²s). This range is much lower than typical clear-day sun intensities which can be over 2000 μmol photons/(m²s) PAR. Beyond the photosynthetically active region of the spectrum, over half of the photons from the sun are unusable by photosynthetic organisms because they are not absorbed by the organism’s light harvesting pigments, again decreasing overall photosynthetic efficiency. Therefore, in order to maximize photosynthetic productivity and efficiency, one must address these root challenges,
a) Maximize the collection of available solar energy for photosynthesis and,

b) Distribute this energy to the microalgae at intensities not exceeding the organism’s ability to utilize it.

To date, most strategies to enhance light capture and distribution have been macro-scaled.\textsuperscript{1,2} Large transparent rods or plates have been used as light diffusers to dilute high intensity sunlight over larger surface areas.\textsuperscript{3,4} Open ponds with dilute cultures have been replaced with enclosed tubular photobioreactors that can support more dense cultures that are self-shading due to the optical density of the cell suspension. These strategies have consistently improved photosynthetic efficiency to \(-5\%\) compared to \(<1\%\) for open pond systems. This improvement however, is still far from the theoretical maximum efficiency of \(-12\%\) predicted for photosynthesis suggesting that there is still plenty of opportunity for improvement.

In spite of the success nanotechnology has had in other energy related fields such as photovoltaics, little attention has been given to how nanotechnology can be used to improve solar energy capture and distribution in photobioreactors. The objective of this thesis is therefore to explore nano-scaled solutions to the problems of light capture and distribution in microalgae cultivation schemes and demonstrate the potential such technologies have to enhance growth.

1.2 Thesis Overview

This thesis is divided into seven chapters which explore different aspects of how nano-technology, and in particular plasmonic phenomena, can be applied to energy related problems. In addition to the core contributions represented in each chapter, several other supplementary contributions have been made during the evolution of this work which are briefly enumerated at the end of this section. Two of these supplementary contributions which are particularly relevant to the work described in this thesis have been included in as appendices.

1.2.1 Core Contributions

The primary theme of this thesis represents a mechanical engineering contribution towards applying nanoscale phenomena to energy, particularly bioenergy, applications. Figure 1-1 summarizes these contributions graphically. The primary bio-energy theme of this work seeks to explore how 1) light capture and 2) light utilization can be enhanced in microalgal cultures. Contributions directly related to this theme
are highlighted blue in Figure 1-1. As a result of exploring this space, two other contributions have been made with impact to relevant neighboring fields, and are identified by the large arrows in Figure 1-1. Briefly, the chapters included in this thesis are:

Chapter 2 – Introduction, background and review.

The introduction of this thesis is composed of two parts. The first part reviews the current state of the art in managing light capture and distribution during microalgal cultivation. This review is currently under consideration at Nature Communications. The second part introduces the field of plasmonics and reviews the progress made in applying plasmonic phenomena to energy applications.

Chapter 3 – Spectral light dilution

This chapter demonstrates an approach to improving light distribution in high density cyanobacteria cultures under high light intensity by utilizing poorly absorbed green light. In studies and commercial applications which use LED illumination, the default wavelength is most often red or orange. However, as is shown in this work, higher productivity and photosynthetic efficiency can be achieved when high intensity green light is combined with high density cell cultures.
Chapter 2: Introduction
Microalgae cultivation: augmenting photosynthesis

Chapter 3: Spectral light dilution

Chapter 4: Plasmonic near-field enhanced growth:

Chapter 5: Plasmonic characterization of crude oil

Chapter 6: Plasmonic far-field enhanced growth:

Chapter 7: Rapid, large-area patterning of plasmonic structures

Figure 1-1 | Graphical table of contents outlining this thesis and its core contributions. Chapter 2.1 Photon Management for Augmented Photosynthesis is under review by Nature Communications, Chapter 4 has been published in Applied Physics Letters (2012), Chapter 5 in Energy and Fuels (2015), Chapter 6 in Applied Physics Letters (2015), Chapter 7 in Langmuir (2015). Inset under the label “Plasmonics for energy” has been adapted from ref. 9 by permission from Macmillan Publishers Ltd. © 2010.

Chapter 4 – Plasmonic near-field enhanced growth

This chapter demonstrates for the first time cultivation of photosynthetic microbes in surface plasmon enhanced evanescent fields. It is composed of work begun during my master’s degree and completed
during my Ph.D. The results indicate the ability to (1) excite surface-bound cells using plasmonic light fields, and (2) subsequently grow thick biofilms by coupling light from surface plasmons into living cyanobacteria cells. Plasmonic light delivery presents opportunities for high-density optofluidic photobioreactors for microalgal analysis and solar fuel production by providing a plasmonic mechanism of light delivery and distribution which confines light energy to surfaces for biofilm cultivation. This work has been published in Applied Physics Letters, and was ranked in the top 20 most read articles in Applied Physics Letters for Dec. 2012.

Chapter 5 – Plasmonic near-fields for crude oil characterization

This work utilizes plasmonic nearfields but applies them instead to the characterization of unrefined oil. A plasmonic sensor was developed to measure the refractive index of crude oil and differentiate between oil samples from around the world ranging in density from light crude oil to ultra-heavy Athabasca bitumen. The application of this sensing approach to crude oil characterization led to a publication in Energy & Fuels and a provisional U.S. patent.

Chapter 6 -Wavelength-selective plasmonics for enhanced cultivation of microalgae

This chapter demonstrates wavelength specific far-field scattering from plasmonic nano-patterned surfaces as a means of addressing the challenge of photon management in photobioreactors. Wavelength-specific reflection from plasmonic surfaces increases the flux of useful light to cultures without sacrificing the full spectrum. This work has been published in Applied Physics Letters.

Chapter 7 - Rapid and Inexpensive Large Area Patterning of Plasmonic Structures with Commercial CO₂ Laser Annealing

This chapter describes a fast and inexpensive method of creating nano-patterned plasmonic island films on glass substrates using a CO₂ laser to thermally anneal the substrate. This fabrication technique is amenable to large scale fabrication of patterned plasmonic films similar to those used in Chapter 5. This work has been published in Langmuir.

1.2.2 Supplementary Contributions

The following list includes projects which I contributed to as co-author. While these contributions are not discussed in detail as part of this thesis, interested readers are encouraged to refer to the respective articles in the appendices of this thesis or online for more details.
Biomass-to-biocrude on a chip via hydrothermal liquefaction of algae\textsuperscript{11}

Published in: Lab on a Chip
Authors: Xiang Cheng, Matthew D. Ooms, David Sinton

Evanescent cultivation of photosynthetic bacteria on thin waveguides\textsuperscript{12}

Published in: Journal of Micromechanics and Microengineering
Authors: Scott Pierobon, Matthew D. Ooms, David Sinton

A photosynthetic-plasmonic-voltaic cell: Excitation of photosynthetic bacteria and current collection through a plasmonic substrate\textsuperscript{13}

Published in: Applied Physics Letters
Authors: Scott Pierobon, Matthew D. Ooms, David Sinton
Chapter 2.

Introduction

2.1 Photon Management for Augmented Photosynthesis

Microalgae and cyanobacteria are some of nature’s finest examples of solar energy conversion systems, effortlessly transforming inorganic carbon into complex, energy-dense hydrocarbons through photosynthesis. Here, we focus on advances made in complementing nature’s processes for the synthesis of complex, energy-dense molecules. While opportunities to increase the efficiency of photon-to-fuel conversion exist at all stages of production they are predicated on efficient sunlight utilization during cultivation. We explore the latest advances that report how this can be done in microalgae cultivation augmented using innovative engineering and materials. We discuss how the innovative approaches to photon management in photosynthetic microorganism cultivation schemes offer a route to high-density liquid fuels and high-value products.

2.1.1 Introduction

Presently, photosynthesis is the model process for storing solar energy in complex chemical bonds.\textsuperscript{14} Annually it results in the fixation of 120 - 175 billion tons of carbon through terrestrial plants alone,\textsuperscript{15,16} and nearly as much again inside the world’s oceans.\textsuperscript{17} Humankind has sought over decades to mimic this process synthetically; however, to date, photosynthesis remains the only option for the sustainable production of many complex chemicals. In particular photosynthesis provides a sustainable path for the synthesis of high energy-density liquid fuels – an important priority in a world increasingly stressed by anthropogenic CO$_2$.\textsuperscript{18–20} For these reasons, cultivation of photosynthetic plants and microalgae for fuel production has attracted great interest. Achieving high biomass yields and productivities is necessary for cost-effective industrial applications. This in turn drives requirements for the efficiency with which solar energy is converted into stored chemical energy. The overall process (Figure 2-1) can be subdivided into light transit, capture and distribution within a photosynthetic microalgae cultivation scheme. Present-day operations typically exhibit photosynthetic energy conversion efficiencies of about 1%, far short of the theoretical maximum of ~8-12\%.\textsuperscript{21–24} As a result, industrial-scale cultivation of photosynthetic microorganisms has
yet to achieve economic viability, for today it does not lead to the production of alternative fuels at costs below the present-day market price of traditional fuels. Using microalgae as a solar-fuel feedstock remains compelling however, particularly when microalgae photosynthetic energy conversion is compared to terrestrial crop plants which have theoretical photosynthetic energy conversion efficiencies between 4-6%.21

Figure 2-1 | Sunlight to biomass conversion efficiency and strategies. Demand for energy including electrical and chemical represents the largest volume market for microalgal products and with few sustainable alternatives. However, from a value perspective these products are relatively inexpensive making uptake difficult to justify economically. Higher value products such as specialty chemicals, pharmaceuticals and nutraceuticals have substantially lower demand, but can be orders of magnitude more valuable. Technology maturation can be supported by including high-value products production to support low-value, high-volume production of energy. Approximate values based on refs. 25,26

To achieve major commercial impact, microalgal biofuels need to increase their use of the solar resource through effective photon management in industrial cultivation schemes – the theme of this review.

The products of photosynthesis can address a number of markets, with high-value, low-volume products such as pharmaceuticals and nutraceuticals attractive near-term targets.26,27 For example, the cyanobacteria *Arthrospira platensis* is a common health supplement sold in most drug stores and a common additive in cosmetics such as personal care products. Species of *Nannochloropsis* are an important source of eicosapentaenoic acid used in nutritional supplements and aquaculture,28 and *Haematococcus pluvialis* is a
Achieving efficient and productive photosynthesis is a multivariable problem intricately tied to environmental factors such as temperature, sunlight, water availability, land suitability; process parameters such as media type concentration, harvesting frequency, and species selection; and biological constraints such as pigment type, quantity, and absorption, enzyme kinetics, respiration, and metabolic maintenance costs. The growth of microalgae is amenable to engineering of the growth environment through the design of the containment systems used to cultivate the microalgae cultures, examples of which are shown in Figure 2-2a-f; these engineering options offer additional avenues for increasing source of the colorant and health supplement astaxanthin. For energy applications, cyanobacteria, green algae (principally chlorophytes), and diatoms have been used for biofuel production, and in electrical production via photovoltaic cells. The suite of useful products produced from microalgae, including high energy-density fuels, is an advantage photosynthesis demonstrates over other solar-fuel generation techniques. For instance, photovoltaic-powered electrolysis, which has a similar theoretical energy conversion efficiency of ~13 (20% photovoltaic energy conversion efficiency, 65% electrolyzer efficiency) is constrained to produce only hydrogen.

The relationship between market volume and product value can be presented as a pyramid (Figure 2-1): higher-value products with more modest market sizes can be pursued first, and their relatively high profit margins reinvested into making technological advancements, moving along the experience curve to larger markets with smaller margins. This cycle will increase the economic feasibility of producing large volumes of commodity products, with the ultimate high-volume target market being biofuels.

### INTRODUCTION

Achieving efficient and productive photosynthesis is a multivariable problem intricately tied to environmental factors including temperature, sunlight, water availability, land suitability; process parameters such as media type concentration, harvesting frequency, and species selection; and biological constraints such as pigment type, quantity, and absorption, enzyme kinetics, respiration, and metabolic maintenance costs. The growth of microalgae is amenable to engineering of the growth environment through the design of the containment systems used to cultivate the microalgae cultures, examples of which are shown in Figure 2-2a-f; these engineering options offer additional avenues for increasing...
photosynthetic efficiency and production. Excellent prior reviews have discussed these factors in depth in a variety of manners, including microalgal culturing techniques,\textsuperscript{1,35} economics,\textsuperscript{42} and applications.\textsuperscript{28,43}

Here we focus on advances made in complementing nature’s processes for the synthesis of complex, energy-dense molecules. Managing light efficiently is a prerequisite for realizing high efficiency in downstream processes. While opportunities to increase the efficiency of photon-to-fuel conversion exist at all stages of production they are predicated on efficient sunlight utilization during cultivation. We explore the latest advances that report how this can be done in microalgae cultivation augmented using innovative engineering and materials. We discuss how the innovative approaches to photon management in photosynthetic microorganism cultivation schemes offer a route to high-density liquid fuels and high-value products (Table 2). By following the fate of a solar photon from the sun to the cell, we identify opportunities to minimize loss via engineering and material science strategies.

2.1.2 Light transit
Solar photons begin their journey at the sun, travel approximately 150 million kilometers and arrive at the outer atmosphere of earth with a combined intensity of 1360 W/m\textsuperscript{2}. Their journey through the atmosphere reduces their numbers by a factor of 30% - 74% depending on weather conditions (Figure 2-1). In the context of commercial microorganism cultivation, incident photons can be intercepted by one of two primary types of microalgae cultivation schemes, (i) an open air microalgae pond, or (ii) an enclosed photobioreactor. Open ponds benefit from low capital and maintenance cost and are easily scaled to cover large areas. Enclosed reactors have higher productivity than ponds, greater control over the chemical and thermal environments, and provide protection from contamination and predation.\textsuperscript{35} Hybrid facilities consisting of both enclosed and open sections have been demonstrated and leverage the best of both categories.\textsuperscript{1} While these cultivation systems perform many roles including containment, mediating the physical and chemical environments (input and output), a central function is photon management: linking solar flux to the biology. Location, orientation, configuration and material considerations play key roles in affecting the efficiency of converting inbound photons into chemical energy.

2.1.3 Managing light capture - location
Solar resources vary by geographic region and are a principle determinant of the suitability of a location for microalgal culture. The simplest assessments of solar resources use clear sky irradiance models,\textsuperscript{14} but are limited in their predictive power because they overlook the impact of weather. Increasingly, site se-
lection studies make use of historic meteorological data to project the future solar resources available for a region and are now becoming common place. For example, in Western Australia, average daily solar fluxes ranging between 150 to 277 W/m² are reported with over 90% of the continent between a 208 and 277 W/m². This regional variation in sunlight can drive an increase in productivity from 3.6 g/(m²·day) to 7.7 g/(m²·day) based on historical productivity observed in Chrysotila criteria and Tetraselmis spp. Microalgae farming assessments in Chile identified primarily coastal desert regions as suitable locations for large scale culture facilities using 165 W/m² average annual irradiance as the threshold minimum. For decades, irradiance data has been collected for thousands of locations worldwide and resides in a variety of databases that can be referenced to establish weather trends and irradiances around the world to improve site selection assessments. Figure 2-2f shows a global map of potential lipid productivity based on global irradiance data. Owing to high annual irradiances (Figure 2-2), minimal cloud cover, and warm temperatures, equatorial locations have consistently shown the highest potential for microalgae cultivation, correlating with the global trend in primary photosynthetic production (Figure 2-2g). Based on growth models developed for Nannochloropsis sp., the global biomass production potential was estimated to be 9.4 g/m²·day, with the maximum occurring in Australia (18 g/m²·day).

2.1.4 Managing light capture - orientation

The form factor of the reactor surface presented to the sun affects the average intensity of sunlight seen by the reactor surface. The orientation determines the irradiation intensity providing a means of managing the light inside the culture.

Outdoor photobioreactors with typical cell density loading will have light path lengths on the order of cm. For flat plate designs (with large illuminated surface areas relative to their thickness) the direction in which they face is a significant factor in the amount of incident light they can intercept and varies by season. Models and tools for calculating the irradiance incident on a plane surface are now common in the solar energy field and can be used to calculate the irradiance potential for a given location and orientation. In general, vertical reactors oriented to face E-W intercept more light on average over the course of a year compared to both horizontal and north-south facing surfaces (Figure 2-2b). This trend has been observed in field tests involving flat plate photobioreactors in Spain (37° N Lat.) oriented with their illuminated surface facing north-south and east-west have shown that over the course of a year, an east-west facing reactor collected 4% more light compared to the north-south. During the summer, the east-west facing collected on average 62% more light. However, during the winter, because of the southern bias
in the sun’s trajectory (Figure 2-2g), the north-south facing reactors were most effective intercepting up to two times as much light.\textsuperscript{53} Compared to a horizontal reactor, the vertical east-west oriented reactor intercepted 5% more light annually. Similarly, using historical irradiance data from June from a location in Spain (37° N lat.), a north-south facing reactor collected 77% less light compared to a horizontal surface.\textsuperscript{54} Because of seasonal variance in both the sun’s trajectory, weather patterns, and atmospheric turbidity, periodic adjustments in the orientation of flat-plate reactors over a year can maximize the collected sunlight intercepted.\textsuperscript{55} For a site in Israel (31°N lat.), adjusting the tilt angle of a south facing flat plate reactor four times each year can achieve a 35% increase in productivity compared to a horizontally oriented reactor.\textsuperscript{55}
Continuous tracking of the sun ensures maximal collection of sunlight. Examples of plate photobioreactors equipped with continuous solar tracking (Figure 2-2e) showed 38% and 37% more intercepted sunlight can be intercepted compared to a horizontal surface averaged over the course of a year in France (47° N lat.), and Sudan (19° N lat.), respectively.49 In Germany (53° N lat.), solar tracked photobioreactors intercepted 45%-47% more sunlight compared to stationary horizontal surfaces over the course of a year, with the most significant enhancement (55%) during winter months.41,56

The light distribution within a facility with multiple photobioreactors can be influenced by shading. While vertically oriented reactors intercept more light, they also create shaded areas potentially limiting
the light available to adjacent reactors (Figure 2-2d). This shading can affect the productivity of large scale installations and makes the spacing and height of individual reactors an important design criteria. Limited shading may provide benefit in terms of photosynthetic efficiency by reducing the degree of photoinhibition under strong sunlight. For tubular photobioreactors greater orientation flexibility is permitted due to the radial symmetry of the vessel, and horizontally oriented tubular photobioreactors tend to exhibit higher areal productivity compared to vertically oriented cultures, particularly at midday when their curved surfaces help to distribute the high-intensity sunlight over a larger culture volume.

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site-selection</td>
<td>Solar irradiance and trajectory changes with latitude, with the highest irradiances near the equator. Weather patterns also greatly affect the amount of sunlight available to solar energy harvesting installations.</td>
<td>34,42,44-50</td>
</tr>
<tr>
<td>Orientation</td>
<td>E-W vertical facing surfaces intercept more light, but cast shadows limiting the advantage for large area installations</td>
<td>41,51-60</td>
</tr>
<tr>
<td>Light quality</td>
<td>Changing the spectral distribution of light can help to maximize growth by increasing the amount of photosynthetically active radiation and/or stimulate the expression of valuable metabolites.</td>
<td>61-69</td>
</tr>
<tr>
<td>Wavelength shifting</td>
<td>Converting light of low photosynthetic utility into light of high utility can make more energy available for photosynthesis.</td>
<td>70-74</td>
</tr>
<tr>
<td>Artificial light</td>
<td>Photovoltaic powered LEDs provide spectral, temporal and spatial control of light. High efficiency PV and LED systems are required for energy returns greater than outdoor cultivation schemes.</td>
<td>65-75-80</td>
</tr>
<tr>
<td>Flashing light</td>
<td>Alternating light and dark cycles of appropriate length can achieve higher overall photon efficiency compared to continuous light of the same maximum pulse intensity for intensities greater than saturation.</td>
<td>39,77,81-89</td>
</tr>
<tr>
<td>Light path management</td>
<td>Short path lengths coupled with high density cultures and mixing can cycle cells between light and dark zones, achieving the flashing light effect, increasing efficiency of outdoor cultures particularly when irradiances are above the saturation limit.</td>
<td>90-97</td>
</tr>
<tr>
<td>Light dilution</td>
<td>High intensity light can be distributed over larger surface areas using light guiding elements to dilute the light to an intensity below the saturation threshold of the microalgae.</td>
<td>3,12,91,98-104</td>
</tr>
<tr>
<td>Plasmonic scattering</td>
<td>Plasmonic scattering can be tuned to specific wavelengths, selectively enhancing the light-path of these wavelengths in the reactor.</td>
<td>5,7,105-107</td>
</tr>
</tbody>
</table>
2.1.5 Managing the light spectrum

Once photons are intercepted by the cultivation apparatus, there is an opportunity to influence the spectral distribution (quality) of the photons transmitted. Photoautotrophs have evolved an array of light harvesting pigments that can absorb energy across the visible spectrum, and the composition of these pigments determines the wavelengths that are absorbed for photosynthesis. While each species contains only a subset of these pigments, photosynthetically active radiation (PAR) is conventionally limited to wavelengths between 400 nm and 700 nm. This portion of the spectrum accounts for approximately 43% of the solar energy incident on earth and 28% of solar photons. Even though for convenience it is common to consider all PAR photons from the sun as equally adept at driving photosynthesis, the array of chromophores involved in light harvesting and phytochromes involved in light sensing suggest a more complicated picture.

The action spectrum of photosynthetic organisms describes the rate of photosynthesis in response to light at different wavelengths,\(^{108,109}\) and is typically determined by measuring autofluorescence, oxygen generation, or growth under low intensity monochromatic light and equivalent photon fluxes. Figure 2-3a-e shows examples of both the absorption and action spectra for several photosynthetic macroalgae and microalgae species. Energy conversion efficiency is typically higher for longer wavelengths (green to red) than for shorter wavelengths (blue), consistent with observations for some terrestrial plants.\(^{108}\) The microalgae *Nannochloropsis oculata* (Figure 2-3d) shows a similar trend, though shows a noticeable efficiency peak in the green and red regions.\(^{110}\) While these action spectra indicate wavelengths between 500 nm and 650 nm can be used efficiently, the absorption spectra indicate that the rate of photon absorption across the PAR spectrum varies substantially which impacts the effective rate of photosynthesis particularly for optically thin cultures. For *Nannochloropsis oculata*, because blue light is so strongly absorbed by the algae it resulted in the highest rate of photosynthesis in optically thin cultures at low light irradiances (60 µmol photons/m\(^2\)·s).\(^{110}\) For optically thick cultures, the productivity of *Spirulina platensis* has been shown to be greatest under red light.\(^{111}\) In unmixed cultures, green light outperformed other wavelengths in cultures of *S. elongatus* because green light was able to penetrate deeper into the culture increasing overall efficiency similar to the penetrating role green light plays in leaves and canopies of higher order plants.\(^{61,62}\) In summary the activity at any given wavelength is a complex coupling of the (i) the efficiency of transfer of the absorbed light energy by the light harvesting pigments to the photosynthetic reaction centers (action spectrum), (ii) the absorption spectrum of the organism which determines the fraction of...
the incident light harvested by the cell (absorption spectrum), and (iii) the local light intensity and quality and is related to culture density.

Many photosynthetic organisms can respond to changes in light quality through sophisticated sensory mechanisms to express desirable metabolites preferentially. Increased amounts of blue light have been shown to increase the expression of chlorophyll in *Chlorella sp.* and lipids in both *Tetraselmis sp.* and *Nannochloropsis sp.* Dynamically adjusting the wavelength distribution during cultivation can further enhance productivity. For *Haematococcus pluvialis*, red light promoted growth while blue light increased astaxanthin expression in the cells. By culturing first with red light and then switching to blue, high biomass productivity and high astaxanthin concentrations were achieved. Similarly, a 20% enhancement in growth of *Chlorella vulgaris* was observed when grown with blue light for two days which resulted in an increase in average cell size, followed by red light for 3 days which increased the rate of cell division (Figure 2-4a) resulting in an overall increase in productivity compared to control cultures.

Taking advantage of the benefits of spectral tuning in photobioreactors using sunlight typically involves one of three approaches, (i) converting photons to electrons using photovoltaic cells to power artificial light sources, in particular light emitting diodes (LEDs), or (ii) converting or augmenting the solar flux using wavelength-shifting materials or wavelength-specific scattering.

### 2.1.6 Light-emitting diodes

High efficiency LED emitters (25-55% W/W) are now readily available and emit at wavelengths spanning the visible spectrum (Figure 2-3f). Although other types of artificial illumination have been used for photosynthesis, including high-intensity discharge and fluorescent lamps, LEDs are expected to become dominant due to their long lifetimes, stability, low operating temperature, size, and efficiency. Furthermore, almost limitless customization of the emitted spectrum can be achieved when multiple LEDs are combined. Artificial light sources also allow culture vessels to be decoupled from the sunlight collection apparatus, simplifying temperature control, reactor geometry and making reactor positioning more manageable. Control of temperature is particularly important since the reaction rate and regulation of genes involved in the photosynthetic dark-reactions are strongly temperature dependant. In temperate climates outdoor temperatures are often too low to support growth, and the costs of active temperature control are energetically and economically prohibitive.
LEDs have become ubiquitous in lab-scale microalgal research. Extrapolation of results to outdoor reactors can be difficult however, as artificial light sources have spectral distributions and irradiance profiles that differ from solar irradiance. In addition, measured light intensity for artificial sources can vary widely owing to the distance dependence of LED lights which are typically point sources, intensity scaling inversely with distance cubed. Using arrays of LEDs, collimating optics, or diffusers can help homogenize the light field, but the detailed optics of these configurations are not often reported. Control experiments typically involve white LEDs or fluorescent lamps which can have a range of emission spectra, most of which are poor approximations of sunlight, as shown in Figure 2-3g.
Deploying LED technology in commercial applications coupled to photovoltaic energy generation can provide the benefits noted above while remaining carbon neutral. The energy balance of such schemes is
largely dependent on the efficiency of solar-to-electricity conversion and the efficiency of the light emitter. Presently, the efficiency record for concentrating photovoltaics is 43.5%, and commercially available silicon photovoltaic devices are between 15% and 20%. Using the state-of-the-art photovoltaic technology with best-case efficiencies of ~40% connected to 60% efficient LEDs, PV-LED-PBR configurations could be energetically competitive with photobioreactors illuminated with sunlight. Economic and energy assessments of a PV-LED-PBR (photovoltaic light-emitting diode photobioreactor) systems using commercially available technology, however, suggest that artificial illumination for photobioreactors is likely to lose on cost and energy perspective, remaining viable only in the production of the highest-value products. Energy conversion efficiencies using photovoltaic cells to power high efficiency LEDs were calculated to be approximately 1% compared to an estimate of ~4% for outdoor enclosed photobioreactors under similar conditions.

While strictly speaking the overall efficiency benefit of PV-LED-PBR systems is marginal, the additional benefits of artificial illumination may yet increase the practicality and conversion efficiency beyond this point in certain contexts. For example, when combined with energy storage, artificial lighting can provide levels of spatial and temporal control unachievable under direct sunlight, compensating for the intensity and intermittency of sunlight and reducing the effects of nighttime biomass loss to respiration. For smaller installations, the ability to locate reactors indoors allows for enhanced temperature control and containment, mitigating the negative effects of seasonal temperature changes and allowing sustained productivity throughout the year in temperate locations. With the further development of enabling technologies, efficient and inexpensive LEDs will present exciting future opportunities for photon management, particularly for the production of high value compounds.

2.1.7 Wavelength shifting materials

Converting photons from one wavelength to another using fluorescent or phosphorescent materials can produce a spectrum amenable to increased growth or metabolite expression. Organic and inorganic dyes, phosphors, and quantum dots are promising candidates for converting light with little or no photosynthetic potential into light with higher photosynthetic potential. Ideally, materials should be both highly absorbing in order to harvest a meaningful amount of light, exhibit high conversion efficiencies and have emission spectra sufficiently separate from their absorption spectra so as to avoid reabsorption of converted light.
Down-conversion of light incurs an energy penalty. Nevertheless, the opportunity to harvest light that would otherwise not be available for photosynthesis is compelling. Most notably, ultraviolet light which is generally detrimental to growth can be converted to visible light usable for photosynthesis. For low density cultures, converting poorly-absorbed light to highly-absorbed light (i.e. green to red) also improves light capture efficiency. For high density cultures, converting blue light to green can provide greater light penetration into the culture by increasing the fraction of highly penetrating wavelengths, and mitigating the effects of light saturation under high intensity illumination.

In early demonstrations of spectral tuning, *Chlamydomonas reinhardii* was cultured under broadband light passed through down-converting layers of Rhodamine dyes. Dye solutions served as filter layers, separate from the microalgal culture resulting in a growth enhancement of 16%. Mixing the spectral tuning dye directly into the culture media would result in the most efficient light distribution by reducing the amount of light re-emitted to the atmosphere, however dye toxicity overwhelms the optical benefit.

Front-filtered photobioreactors made from luminescent acrylic have also enhanced growth in green algae *Chlorella vulgaris* and cyanobacteria *Gloeothecae membranacea*. Under unmixed conditions and continuous illumination, the growth rate of *C. vulgaris* was maximized for orange filtered light and was 2.2-fold the maximum growth rate observed for an unfiltered control. For *G. membranacea*, the growth rate of filtered light was similar to controls, but violet filters maintained productivity over a longer period than
the unfiltered control resulting in ~12% additional biomass. With the addition of mixing, red luminescent reactors showed the greatest performance in the same species. Pigment ratios were also influenced by the spectral tuning, both through an increase in useful light and reduction in detrimental high-energy wavelengths.73

Harmful UV light is often attenuated by the materials used in photobioreactor construction, such as polycarbonate and many types of glass. By entraining UV converting dyes into these materials, a portion of the absorbed UV light can be converted to visible light and transmitted to the culture.112,118 Similarly, for materials transparent to UV light such as acrylic, incorporating UV absorbing dyes can both block harmful UV radiation and increase the number of photosynthetically active photons. Examples of this approach have shown a 74% increase in biomass productivity when polycarbonate front-side UV converts were used and illuminated with UV light, and 45% increase when acrylic converters were used.112 When full spectrum light was used,118 the added effect of down-converted photons was masked by the overall attenuation of the wavelength-shifting layer. Specifically, the total PAR intensity decreased from 11.4 a.u. to 9.9 a.u. Nevertheless, by eliminating the UV-A radiation, a net increase in productivity of 10% was realized.118

To reduce cost, commercially available fluorescent paints have been used to alter the spectrum of incident light. Flat plate photobioreactors were equipped with a top mounted layer of liquid paints that absorbed ~90% of incident solar energy. The quantum efficiencies of these converting layers were between 11.3% for a filter fluorescing blue, and 28% for a filter fluorescing red when excited at 325 nm. This approach suffers from low conversion efficiencies, but is the most inexpensive approach.

For low density cultures which allow significant amounts of light to be transmitted, a backside conversion layer can improve the average light intensity in the culture.74 An example of this approach is highlighted in Figure 2-4b, where a photoluminescent phosphor coated mirror back-plate resulted in 36% more biomass.

A common challenge in evaluating spectral tuning strategies from literature is control and reporting of light variables (intensity, spectrum, directionality and quantum conversion efficiency). Of particular importance is the efficiency of the spectral tuning layer with respect to forward scattered light (for front mounted layers) or backscattered light (for rear mounted layers). Unless sub-saturating light is used, it is difficult to disambiguate the impact of light attenuation (which alone will have a positive effect on photo-
2.1.8 Plasmonic scattering

When photons interact with the conduction electrons in metals or metallic nano-particles, collective oscillations of the electrons can result, called surface plasmons. Exciting surface plasmons in metals significantly enhances the electro-magnetic field in the vicinity of the metal surface or particle leading to enhanced absorption or scattering at specific resonant wavelengths. The precise nature and magnitude of these effects is a function of the material type, size, shape, and the surrounding media. These optical effects have previously been used to enhance photo-conversion in other areas, particularly photovoltaics. Various enhancement approaches have been proposed and demonstrated with parallels emerging for augmenting photosynthesis. Current strategies leverage (i) far-field scattering of light and/or (ii) near-field confinement of light. Both approaches encourage photosynthetically-useful wavelengths to be redirected into, or confined within, the culture space.

Plasmon enhanced fluorescence has been the subject of intensive research. The intensity of the electromagnetic field in the presence of plasmons can be several orders of magnitude greater than the intensity of the excitation light field. Fluorescent molecules in the vicinity of these enhanced fields can be excited through energy transfer with the excited plasmon. For instance, the fluorescence intensity of photosynthetic light harvesting complexes has been enhanced by locating them near the surface of silver island films. The magnitude of the enhancement has been shown to depend on the morphology of the silver island films, the orientation of the light harvesting complex relative to the nano-particles and the polarization of the light. Similarly, Live cyanobacteria (Synechococcus elongatus) have been cultivated in the presence of plasmonically enhanced electromagnetic fields. High density biofilms were cultivated by coupling light into thin gold films using a prism. Biofilms grown in the presence of the plasmon enhanced evanescent fields grew 2% denser than those formed under direct illumination. Confining both the light and the cells to the substrate surface is as a key factor particularly for supporting surface-attached biofilms.

In addition to near-field confinement of light, far-field scattering of light can assist in containing specific wavelengths within photobioreactors. Chlamydomonas reinhardtii and Cyanothece 51142 showed 30%
increased biomass production when plasmonic layers made of silver nanoparticle suspensions backscattered blue light back towards cells.\textsuperscript{106} Combinations of nanoparticles with different geometries and materials can produce more complicated resonant wavelength spectra.\textsuperscript{107} Composite spectra resembling the absorption of \textit{Chlorella vulgaris} induced a higher degree of chlorophyll A and carotenoid expression.\textsuperscript{107} Nanoparticles adhered to a reactor surface instead of suspended in a solvent can also provide similar spectral effects but with greater mechanical stability. For instance, arrays of gold nanodisks enhanced the growth of cyanobacterium \textit{S. elongatus} (Figure 2-4c).\textsuperscript{7} The plasmonic substrates reflected 35\% of the red light transmitted through a dilute culture back into the reactor while allowing other wavelengths to be transmitted. This approach allowed for photosynthetically useful light to be returned to the culture while permitting shorter wavelengths to be transmitted for use in photovoltaics.\textsuperscript{7} A drawback of all plasmonic approaches is energy loss. Because the metals are imperfect conductors, the electron oscillations result in ohmic losses, converting input light energy to heat. For low density cultures, however, plasmonic redirection of useful light resulted in a 52\% increase in power efficiency overall compared to a broad spectrum reflector.\textsuperscript{7}

\textbf{2.1.9 Light dilution, path length, and culture density}

Photosynthetic organisms are limited in the rate at which they utilize absorbed light – becoming saturated if the photon absorption rate exceeds the capacity of metabolic processes. Saturation intensities vary widely in literature but are typically around 150-400 \textit{mmol photons}/(m\textsuperscript{2}s).\textsuperscript{23,81,119} When the saturation limit is reached, excess energy is dissipated as heat and fluorescence,\textsuperscript{120} and many species will initiate photoprotection mechanisms to prevent a buildup of excess energy and reactive oxygen species produced by photosynthesis.\textsuperscript{39,120} Similarly, when light intensity drops below a certain compensation intensity, photosynthesis is outpaced by respiration and the organism will begin to consume oxygen and high energy compounds to maintain metabolism through the dark period. This dark respiration can have a significant impact on culture productivity particularly at night, leading to a decrease in productivity of ~50\%.\textsuperscript{22,35}

Inside a reactor, the light-path length, and optical density of the suspension are critical to productivity and overall efficiency. Because most microalgal species become saturated at light intensities close to -10\% of peak sunlight, during much of the day they operate at low photosynthetic efficiency – absorbing but not effectively using incoming radiation. To manage high sunlight intensities, high biomass density can be employed such that mutual shading between cells attenuates the light to a suitable volume-averaged intensity.
In high density cultures the photic zone may be only a few millimeters deep due to absorption and scattering. Below this depth, cells do not receive sufficient light. Consequently, the path length of light through the culture should be chosen such that the ratio of the photic zone to dark zone is optimized. While no universal rules for optimal path length and density apply across all species, there are general trends. Under high intensity sunlight, the highest productivity results from a combination of path-length, density, and mixing rate such that the frequency of cycling between the light and dark regions is on the same timescale as the turnover rate of the photosynthetic machinery. Path lengths on the order of millimeters have been suggested as the most productive, particularly under high intensity light in order to achieve this light-dark cycle frequency. The flashing light effect has been shown to mitigate the negative effects of light saturation by providing a dark period of sufficient length to allow the electron transporters involved in shuttling electrons between reaction centers time to reoxidize, and by avoiding the need to exhaust energy through non-photosynthetic quenching pathways. Efficiency is greater for higher frequency pulses of 10-50 Hz (Figure 2-5a). Similarly for saturating light, pulse times on the order of 1-10 ms followed by dark periods on the order of tens of ms tend to be optimal, and correspond to the turnover rate of photosystem II (PSII) and replenishment of the electron transporters. In this way, photosynthetic productivity approaching the productivity of continuous light at the same time-averaged intensity can be achieved, even when the absolute intensity of the pulsed light is beyond the saturating intensity of the organism. For high density mixed cultures in which cells cycle between light and dark zones, the rate of photosynthesis of the whole culture approaches that of an optically-thin culture with an equivalent volume-average irradiance. Selecting the path length, mixing speed, and optical density such that the volume-averaged light intensity is close to but not exceeding the saturating intensity typically results in optimal productivity. Short path lengths can thus enable higher density cultures to be maintained at no additional cost to efficiency, which has advantages for downstream processing. For instance, a 32-fold increase in areal productivity for *Spirulina platensis* resulted from reducing the path length from 200 mm to 7.5 mm in a flat plate reactor. Without careful balancing of intensity, mixing rate and optical density, contrary results may occur. For instance, in a study using the green algae *Nannochloropsis* where the light-path was similarly reduced, areal productivity decreased markedly. Although volumetric productivity increased, it was insufficient to compensate for the corresponding decrease in reactor volume.
Figure 2-5b shows how optimal productivity depends on culture density for different light intensities. As shown, peak productivity increases with both irradiance and culture density. Even at intensities of 4 suns, continuous cultures of *Spirulina Platensis* in short light path reactors (7.5mm) were productive with ultra-high biomass densities (~30 g/dw/L) that were vigorously mixed (Figure 2-5b). In contrast, for *Chlorella sorokiniana* under continuous cultivation, a flat plate reactor with a path length of 14 mm achieved maximal productivity with high dilution rates sustaining a stable density of 2.1 g/dw/L at ~1 sun intensity supplied by red LED’s. Under these conditions, the photosynthetic efficiency remained relatively constant regardless of the culture density determined by the dilution rate. Nevertheless, when applied to high-density outdoor cultivation in open, short light-path, cascade reactors similar to the one shown in Figure 2-5c, the strategies discussed here have increased photosynthetic efficiency to ~3%. These systems use culture densities up to two orders of magnitude greater than typical raceway ponds (up to 50 g/dw/L) with depths between 6 – 45 mm. In general, net productivity is a complex function of culture density, light intensity, mixing, and path length. Record productivities result from high density, intensity, and mixing with low path lengths.
Physiological changes in high density cultures can further contribute to more effective light regimes and specific light supply rates. For instance, cells immobilized in high density biofilms and exposed to high light showed a reduction in chlorophyll concentration, reducing absorption in the biofilm and allowing light to penetrate deeper than it normally would (Figure 2-5d).  

Alternatively, an optical approach is to dilute the high-intensity incident light over a larger surface area, thus reducing the local light intensity to productive levels and increasing light distribution. The curved surfaces of tubular photobioreactors are the simplest approach to achieve a degree of spatial dilution and can dilute incident light by a factor of 1.57. Incorporation of transparent panels or waveguides can further dilute incident light to sub-saturating intensities (Figure 2-5e). Biofilm photobioreactors (in which cells are cultivated as surface attached colonies rather than in suspension, (Figure 2-5f)) can partic-
ularly benefit from larger surface areas since more illuminated area is available for attachment and cultivation. Roughened optical fibers have also been used to distribute light to biofilms attached to the fiber surfaces, and achieved light energy conversion efficiencies of 9.3% compared to 3.9% for cultures in suspension and 6.8% for smooth fibers when producing hydrogen. Similarly, stacks of optical waveguides have been used to distribute light to ultra-high density cultures with path lengths of 2 mm and achieved an 8-fold improvement in productivity over bulk cultures with a path length of 30 mm.

An innovative hybrid approach to reducing overall light intensity used a photovoltaic cell to partially shade the culture. The average irradiance was reduced resulting in higher photosynthetic efficiencies while capturing the energy of the unused photons. A combined conversion efficiency of light into both electric current and biomass was as high as 15% when 20% efficient photovoltaic cells were used, exceeding the theoretical limit of biomass growth alone (12%).

Alternative avenues of solar conversion via photosynthesis are emerging, many which draw inspiration from nature in an engineering-biology-materials partnership. For example, charge-carriers generated through microalgal photosynthesis have been captured and used directly to power electrical devices. The efficiency of these bio-photovoltaic cells, while still relatively low, is increasing, and may approach that of other photo-biological and photovoltaic technologies. Other benefits of bio-photovoltaic cells and similar bioelectrical systems are that they do not require complex manufacturing and are relatively inexpensive since much of the heavy lifting is performed by the biology which is inexpensive, self-replicating, and self-healing. Another biology-material hybrid system combines silicon nanowire arrays with bacteria to generating acetic acid. Reducing equivalents generated by solar energy incident on the silicon nanowires are used by surface adhered bacteria to metabolize CO₂. Future embodiments involving multiple, genetically-tailored, bacteria could then use this acetic acid as a substrate to produce a range of useful polymers, fuels, and pharmaceutical precursors.

### 2.1.10 Managing cellular light harvesting

After light reaches the cell, the solar photons are absorbed by the light harvesting pigments of either photosystem I or II (PSI, PSII). The excitation energy is rapidly transferred to the photosystem’s respective reaction center, initiating a cascade of reactions. The energy in activated PSII is first used to oxidize water generating oxygen, protons, and electrons that are used to form ATP through a series of electron transport reactions before being transported to PSI. The remaining excitation energy in these transferred
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electrons is combined with additional energy from the PSI light capture reactions and used to energize NADP+ to NADPH which is ultimately used to power the carbon-fixing Calvin cycle. The overall efficiency of this conversion from excited charge-carrier to the most basic form of fixed carbon is about 27%. Additional losses are incurred from subsequent non-biomass forming metabolic processes necessary for cell maintenance which account for another 10-90% efficiency factor (Fig. 1). Reducing internal losses inherent in the biology of photosynthetic organisms has been the focus of extensive biological and genetic engineering.

Engineering the molecular structure of the light harvesting apparatus presents particular opportunities for efficiency improvement. Excitation energy transfer to the reaction centers happens within 100 ps with efficiencies of 80-100%, however, the slower enzyme-mediate reactions involved with water oxidation, electron transport, and carbon fixation, can introduce bottlenecks particularly under high light. These bottlenecks can be avoided by reducing the photon absorption rate, which can be accomplished by reducing the size and absorption cross-section of the light harvesting complex.

For sub-saturating intensities, photosynthetic efficiency can be improved by increasing the spectral sensitivity of the light harvesting apparatus to utilize a larger portion of the spectrum. This can be accomplished by engineering the pigment composition of the light harvesting complexes making more wavelengths available for photosynthesis. Control over spectral sensitivity as well as photo-protective mechanisms (which tend to direct energy towards non-photosynthetic channels) can be achieved by synthetically accessorizing light harvesting antennae with additional carotenes and xanthophylls. This approach represents a synthetic variation of the natural photo-adaptive responses of plants and microalgae in which pigments re-organize in response to changing light conditions. Making more photons available for photosynthesis is advantageous provided photoinhibition by light saturation is avoided; strategies that rely on increasing absorption must simultaneously manage the incident photon flux.

Light harvesting and energy transduction at the cellular level can also leverage recent advances in nanomaterials. For example, single-walled carbon nanotubes have been inserted and irreversibly localized within the lipid envelope of isolated plant chloroplasts to assist with electron transport. The result was a 3-fold increase in photosynthetic activity compared to unmodified chloroplasts. Light-harvesting nanocrystals have been grown on the surface of non-photosynthetic bacteria to generate electron-hole pairs and transfer these excited carriers directly to the cell for use in acetate synthesis. Conversion efficiencies of
~80\% were achieved under low light intensity by utilizing highly absorbing nanocrystals that feed charge carriers to the high efficiency Wood-Ljungdahl acetic acid synthesis pathway. These studies, while still at the proof-of-concept stage, point towards the emerging opportunities for nanotechnology-augmented photosynthesis.

2.1.11 Conclusions

Efficiently navigating the conversion of sunlight into value added products is a key challenge for commercially viable bio-products from microalgae, in particular for fuels where margins are low. Incumbent global and regional energy systems are entrenched with decades and even centuries of experience, innovation, investment and infrastructure. The unprecedented success of fossil fuels puts a tremendous burden on emerging technologies in a market that is not fully motivated to seek alternatives. The strategies outlined in this review provide an inventory of the options available to maximize the conversion efficiency of solar photons into useful products by managing design choices related to light transit, capture and distribution in photobioreactors. Latitude and weather determines the solar resources available and consequently the maximum productivity. The quality and intensity of light can be tuned using modern materials, reactor configurations, and light sources, while mixing, culture density, and light path-lengths can be adjusted to further optimize the light environment achieving high conversion efficiencies, productivity, and the expression of useful metabolites. Augmenting biological systems in these ways with modern materials and engineering can provide elegant solutions to the problems faced by entirely synthetic devices.

2.2 Plasmonics for Energy

This section reviews recent work in the field of plasmonics, and in particular applications of plasmonics for solar energy capture in photovoltaic devices. It is from this field that much of the inspiration for the work in this thesis is drawn. Many of the same issues associated with light capture and distribution in thin film photovoltaics are applicable also to microalgal cultivation, including light distribution, path-length considerations, visible spectrum sensitivity, and absorption. The synergy between the length scale of microalgae, plasmonic nearfields, and typical plasmonic structures, presents exciting and unexplored opportunities to apply plasmonics to bioenergy production.
2.2.1 The diffraction limit

Our ability to convert solar energy into different forms of energy and products is fundamentally driven by our ability to manage and control light. The fields of imaging and sensing have historically been known for pushing the frontiers of our applied knowledge and allowed us to work with light in extraordinary ways. With the development of modern manufacturing techniques the production of precision optical elements and systems has largely been removed as a barrier to achieving high resolving power in both sensing and imaging applications. Having overcome these practical concerns, researchers are now turning their attention to overcoming more fundamental limitations inherent in optical systems which limit their resolution.

The diffraction limit, a consequence of the wave nature of light, has for years dictated the smallest discrete objects that can be resolved in an optical application. When an electromagnetic wave is passed through an aperture, it diffracts, analogous to waves in water passing through a gap in a wall or how sound waves spread out to fill a room. A consequence of diffraction is that it is not possible to perfectly collimate light since all optical systems require an aperture of some sort through which the light is passed. Even laser light sources have a degree of diffraction resulting from the beam passing out of the laser cavity through the laser aperture. The degree of diffraction can be further aggravated through imperfect alignment of optical elements and imperfect manufacturing. In the absence of these additional factors however, an optical system is said to be diffraction limited. Ernst Abbe was the first to define this theoretical limit which can be expressed as the diameter of the smallest spot that light can be focused into:

\[
d = \frac{\lambda}{2 n \cdot \sin(\theta)}
\]

where \(d\) is the diameter of the spot, \(\lambda\) is the wavelength of light, and \(\theta\) is the angle of incoming light relative to the surface normal. The denominator \(2 n \cdot \sin(\theta)\) is known as the numerical aperture and is a convenient parameter to use when characterizing the resolving power of optical systems. In optical microscopy for instance, microscope objectives with very high numeric apertures are designed such that they accept incoming light at much higher angles than objectives with lower numerical apertures. This affords them greater resolving power but also reduces their working distance. Similarly, long working distance lenses often have lower numerical apertures and lower resolving power.
The diffraction limit dependence on wavelength also suggests why the performance of systems like electron or x-ray microscopes is far greater than optical microscopes. Smaller features can be resolved at the diffraction limit of electron and x-ray microscopes because the wavelength of electron beams and x-rays is orders of magnitude smaller than visible light. Cost and potentially harmful exposure to these radiation sources however add additional practical limits to their applicability.

Researchers have spent significant effort searching for ways to overcome the diffraction limit in optical microscopy applications. Assisted by advances in nanoscale fabrication and trends towards miniaturization, the next generation of nano-optical devices will require control of light on scales below the diffraction limit and require clever new approaches to how light is managed.

2.2.2 The emergence of plasmonics

The field of plasmonics has evolved over the past decade and a half motivated by interest in overcoming the diffraction limit and by technological advances. The field of plasmonics deals with the science and technology of coupling light at or near visible wavelengths into plasmon resonances at the nano-scale. Plasmons are localized charge densities formed within the free electron gas of a conducting material. Plasmons can be excited optically when the energy and momentum of both the plasmon and incident light are matched, allowing for the coupling of light into electron oscillations and vice-versa by wave matching. Once light is coupled into plasmonic modes, the light is no longer restricted spatially in the same manner as it is when it is in its free-space propagating mode. In the case of surface plasmons, the charge oscillations are restricted to the interface between the conductor and dielectric. The intensity of the electric field of the plasmon decays exponentially away from the surface on which it is excited and so does not propagate. The confinement of the evanescent electric field to the surface of the conductor allows for light to be manipulated at sub-wavelength length scales. This flexibility has opened the door to new areas of optical computing, lasing, particle manipulation as well as imaging that are able to overcome the diffraction limit.

In the early 2000's interest in plasmonic research was accelerating. In the introduction to his early book chronicling the progress made in the field,\textsuperscript{135} Pohl discusses the history of surface plasmon resonance and near-field optics form its theoretical discovery during a time when micro- and nano-scale phenomena were of little interest. He highlights the work of early efforts towards understanding the behavior of small particles and fluorescent molecules in the presence of plasmonic structures and media, and shows the progress
made towards using these interactions to develop advanced imaging techniques such as scanning near-field optical microscopy (SNOM), or near-field scanning optical microscopy (NSOM).

Since that time, the field has expanded rapidly. In 2005, Maier and Atwater summarized a portion of the research that had emerged and its applications to new technologies. In particular, they discuss developments in particle plasmonic resonance (i.e. localized plasmon) and plasmonic waveguides which are able to direct and control electromagnetic excitations on scales that overcome the diffraction limit. Particles located within each other's near-field such that the spacing between them is much smaller than the wavelength of exciting light are able to couple electromagnetically when excited, passing between them. Because excitations occur through near-field interactions, the resolution of the waveguide is smaller than the wavelength of light. Asymmetrical particle geometries have also been shown to enhanced the local electric field at sharp points. At these focal points, the electric field lines are essentially crammed together as the surface charge density in the narrow point of the structure increases resulting in field enhancements of 3-4 orders of magnitude. Plasmonic modes on metal surfaces sandwiched between two identical dielectrics can give rise to "long ranging surface plasmon polariton" with propagation distances on the order of centimeters. In this geometry, surface plasmons excited on one surface induce an evanescent field both into the surrounding media and into the conductor. If the material is thin enough, the tail of the evanescent field extending back into the conductor can excite surface plasmons on the opposite surface as well, enhancing the electric field further.

2.2.3 Energy applications

Photovoltaic technology is one promising avenue to sustainable energy production. A key challenge in reducing the cost of photovoltaic technologies lies is reducing the amount of costly materials required to manufacture them. In 2008, 90% of solar cells sold contained silicon as a key component which accounted for nearly 40% of the total cost per panel. The most straightforward approach to reducing the amount of material used in the active layers of a photovoltaic cell is to reduce the cell thickness. Reducing cell thickness has the adverse effect however, of also reducing efficiency since the path-length of light within the photovoltaic cell is also reduced, reducing light absorption. Strategies to improve the efficiency of thin film solar cells have therefore been focused on increasing the path length of light within the active media which may be as thin as several microns or less.
Since the mid 2000's much work has been done in this area with review articles summarizing the progress published annually if not more frequently. Over these years, three primary strategies have been implemented to trap light more effectively within the active layer of solar cells:\textsuperscript{138}

- Geometric Light Trapping
- Photonic Crystal Light Trapping
- Plasmonic Light Trapping

In each case, light is controlled through interaction with different micro and nano structures designed to redirect it and contain it to the active medium such that the likelihood of it being absorbed by the active medium increases. Geometric light trapping relies on altering the direction of freely propagating light and as such is amenable more thicker photovoltaic devices. However, when film thicknesses approach or drop below the wavelength of light, geometric patterning becomes impractical and ineffective. Photonic crystals have allowed new approaches to light filtering and selection. A feature of photonic crystals is their ability to reflect nearly 100\% of light within a certain wavelength band determined by their crystal structure.\textsuperscript{138} In this way, light of certain frequencies can be selected and directed to where it can be utilized most effectively. The final approach involves the use of plasmonic excitations to enhance and focus the electric field of incident light within the absorbing media and will be the focus of the following sections.

\textbf{2.2.4 Plasmonic light trapping}

\textbf{2.2.4.1 Near-field enhancements}

The application of plasmonics to light trapping for solar energy production can take several forms. A common approach is to leverage plasmonic resonances within metallic nano-particles to either concentrate light within an active medium or scatter light into guided modes of the active medium, as shown schematically by Atwater in Figure 2-6.\textsuperscript{9}
Nano particles, because of their small size, are able to sustain highly localized plasmonic resonances. The free electron path for the conduction band electrons within conducting metals is on the order of tens of nanometers. Consequently, for nano-particles of similar dimensions, almost all of the free electrons are effectively surface electrons, with almost no contribution from those contained within the bulk material.

When light of a wavelength much greater than the particle's size is incident upon them, the electric field across the particle at any point in time is essentially uniform, and oscillates in time. This oscillation acts as a driving force on the free electrons. Resonance occurs when this oscillating driving force matches the natural frequency, or plasma frequency, of the electrons. This requires that both the frequency (energy) and the momentum (wave-vector) of the exciting light and the electrons match. The dispersion relationship (here the dependence between wavelength or wavenumber and frequency) for surface plasmons on a plane surface is:

\[ k_{sp} = \omega c \frac{\varepsilon_1 \varepsilon_2}{\sqrt{\varepsilon_1 + \varepsilon_2}} \]

where \( k_{sp} \) is the surface plasmon wave vector, \( \omega \) is the frequency of exciting light, \( c \) is the speed of light in a vacuum and \( \varepsilon_1 \) and \( \varepsilon_2 \) are the permittivity of the metal and surrounding dielectric respectively. For many cases, this can be used as an approximation for particle plasmons.

Because the conduction band electrons are all surface electrons, the plasma frequency depends on the permittivity of both the metal and the surrounding dielectric, resulting in a specific band gap for which light is strongly absorbed by the particle. As a reminder, permittivity is a measure of the degree to which
a material allows an electric field to penetrate through it, and is related to the refractive index of a material, n, by:

\[ \epsilon = n^2 \]

Under resonant conditions, incident light is coupled into the free electron oscillations and strongly absorbed by the particle. By adjusting both the size and geometry of the nano-particles, this absorption band-gap can be tuned. For many metals, this absorption band gap exists at visible wavelengths. An interesting point to note is that this type of resonance does not scale. In other words, there is no similar scenario for long wavelength light such as microwaves or radio-waves where plasmonic resonance could arise. At the nano scale, a unique synergy exists between particle size and the wavelength of light which ensures that surface charges are the dominant form of conduction band electrons with oscillation frequencies that match the wavelength of visible light. As the scale of the system increases, the properties of matter do not likewise scale, and plasmonic resonance does not occur due to the dominance of other factors, such as interactions with bulk electrons. Consequently, the study of plasmonics is one unique to the nano-scale with wavelengths of light in and around the visible spectrum.

In terms of their application to photovoltaic technology, metallic nano particles (10's of nanometers in diameter) can be useful in two ways. They can either be used as plasmonic antennas embedded within the active media, focusing harvested light into strongly enhanced electric nearfields. The energy within these fields can then be absorbed to generate current. Alternatively, nanoparticles can be used the scatter light into waveguide modes of a nearby substrate.

The nano antenna approach has been explored by Hagglund et al. in their investigation of the effect that gold nano-ellipses (92-nm major axis and 40-nm minor axis and 30-nm high) had on the photocurrent generated at a silicon p-n junction. While for some incident angles of excitation light they found enhanced photocurrent generation, at peak resonant conditions they actually noticed a photocurrent decrease. They attributed this to the interaction and phase shifting induced by the different dielectric materials in which the gold nanodisks were placed. At peak resonance, a greater portion of the incident light was back-scattered from the particles, rather than being coupled into the active layer. At wavelengths off resonance however, forward scattering into the substrate dominated and enhanced currents were observed.
More recently, Hagglund developed a model to better understand the absorption of light in subwavelength thin-film systems laced with metallic nano-spheroids. In a system where the dimensions of the constituent parts are all smaller than the wavelength of light, the system is dominated by near-field effects. He was able to show that in such circumstances, the maximum absorption ($A$) of incident light by the plasmonic thin film was related to the refractive indices of the particle ($n_1$) surrounding material ($n_2$) and defined by:

$$A = \frac{n_1}{n_1 + n_2} + O \left( \frac{d^2}{\lambda^2} \right)$$

Similarly, Kim et al. used 13 nm silver nano-particles to increase the photo-current harvested from an organic thin film cell. He reports an increase in power conversion efficiency from 3.05% to 3.69%, again citing the increased absorption due to the localization of light into concentrated electric fields.

Higher absorption coefficients and necessarily shorter carrier diffusion lengths (~10 nm) make organic solar cells particularly well suited to enhanced absorption through near-field concentration techniques. To this end, Lindquist used plasmonic cavities instead of nano-particles. A patterned silver grating was applied over top of an organic active layer backed with aluminum. The silver grating served both as a plasmonic light concentrator as well as an anode to collect current. Nearfield coupling between the plasmonic modes excited in the aluminum substrate focused the nearfield enhancement within the semiconductor, as shown in Figure 2-7, and demonstrated a 3.2 fold increase in power conversion efficiency.
2.2.4.2 Enhancements from light scattering

An alternative to utilizing nearfield enhancement is to leverage the unique scattering effects of nanoparticles. Ordinarily, a spherical nanoparticle can be approximated as a dipole and will scatter light evenly in dipolar fashion – both forward and back. When placed near a dielectric surface with greater permittivity than the surrounding media however, the plasmonic nearfield of the excited particle will couple energy out of the plasmonic modes and into the propagating regime inside the adjacent substrate preferentially directing light in that direction. Atwater and Polman discuss this approach with respect to enhancing light trapping in photovoltaic cells. Arrays of nanoparticles placed adjacent to the active media layer can harvest incident light, re-emitting it into the active media. When the light is re-emitted it is done so preferentially towards the material with higher refractive index, with almost no backscattering.

Of most importance for increasing light absorption in photovoltaics is the tendency for scattering from nanoparticles to be preferentially directed towards the dielectric with the greatest permittivity. Because the radiation is scattered along a spectrum of angles, the path length of light through the active media is enhanced, either through coupling into waveguide modes within the substrate or by repeated scattering by other nanoparticles as the light attempts to escape.

Early work performed by Stuart and Hall used silver nanoparticle aggregates deposited onto a 165 nm thick silicon photodetector as shown in Figure 2-8a. Due to the enhanced in-coupling from scattering, the photocurrent was increased by a factor of 20 for mean particle diameters of 108 nm (Figure 2-8b).
Similarly, Pillai et al. worked with silver nanoparticles to increase the absorption of silicon photocells.\textsuperscript{148} Figure 2-9 shows the absorption enhancement they achieved by varying the thickness of silicon. A significant increase in absorption enhancement across the spectrum for the 1.5 $\mu$m cell is observed but only marginal enhancement (except in the infrared) for the thicker 300 $\mu$m cell. In the latter case, because silicon is already such a strong absorber in the visible spectrum, the nanoparticle scattering offers little additional value. For very thin films however, the contribution of the additional light scattering becomes significant.
Nakayama, Tanabe and Atwater used silver nano particles to demonstrate photocurrent enhancement in GaAs absorbing media instead of silicon. A range of particle geometries were tested with effective average radii of 1 nm and heights between 55 nm and 220 nm, as shown in Figure 2-10. They note that for wavelengths below the resonant wavelength, enhanced scattering was observed and resulted in an 8% increase in short circuit current density.

Figure 2-10 | Particle geometry variation for enhanced solar cells. SEM images of the plasmonic nanostructures used by Nakayama to enhance performance in GaAs solar cells. Reprinted from ref. 149 with permission. © 2008, AIP Publishing LLC.

Work has also been conducted comparing the effects of placing nanoparticles on the front and back of the photoactive layer. In work done by Beck et al. silver nanoparticles were found to enhance absorption. For back loaded particles, ultra-thin spacer layers between the particle and silicon enhanced the scattering cross-section most significantly (Figure 2-11) up to four times.
Figure 2-11 | Front and back loaded nanoparticles for plasmonic solar cell enhancement. Graphs showing the calculated scattering cross sections for silver nano particles distributed on the front and back of a silicon photovoltaic device. Enhancements to the scattering cross sections can be observed at resonant wavelengths, and are most significant for front loaded nanoparticles. Reprinted from ref. \textsuperscript{150} with permission. © 2010, AIP Publishing LLC.

Gold nano particles have also been used as scattering dipoles such as in the work by Lim et al.\textsuperscript{151} Gold spheres 100 nm in diameter were analyzed experimentally and numerically to assess the effects they had on photocurrent in a silicon based solar cell. They found that for their configuration, increased scattering above the plasmonic resonance wavelength led to an increased photocurrent. However, at sub-resonant wavelengths, the polarizability of the gold nano-particle (a measure of the response of the particle's electrons to redistribute in the presence of an electric field) has a very large phase shift. This leads to destructive interference of light scattered into the silicon substrate by the nano-particle with transmitted light, leading to an overall reduction in photocurrent as shown in Figure 2-12.

Figure 2-12 | Performance of Au enhanced silicon photodiode. The graph shows the ratio of between the spectral photo-responses of a Si photodiode coated with Au nanospheres and the uncoated device. At sub-resonant wavelengths, destructive interference results in a decline in photocurrent. Reprinted from ref. \textsuperscript{151} with permission. © 2010, AIP Publishing LLC.
In an effort to explore lower cost metals, Souza, Corio, and Brolo from the University of Victoria immobilized copper nanospheres to the surface of a silicon photovoltaic device using an inexpensive surface treatment. While oxides formed around the surface of the nanoparticles, notable enhancements in scattering and photocurrent were still observed. Increased power productions of 16% were observed and 2% increase in short circuit current density for light in the visible range and 8-12% for light in the near infrared.

Chang et al. used Ag$_2$S-encapsulated Au nanorods to enhance the efficiency of dye sensitized photovoltaic cells. He notes the importance of coating the plasmonic nano-particles with a non-conducting layer in order to prevent carrier recombination due to the high conductivity of typical metals used for plasmonic applications. This recombination of charge carriers ultimately leads to a reduction of harvestable photocurrent and decreased performance. In their study, they achieved a 37.6% improvement in photocurrent generation over the 600-720 nm spectral range. For die sensitized solar cells, this is particularly advantageous since most dyes absorb well below this range. By enhancing absorption where typical dyes perform poorly, the benefits of the plasmonic nanoparticles can be maximized. The researchers again describe the impact that the geometry of the particles have on their optical response. Tuning of the peak scattering and absorption wavelengths can be achieved by varying the aspect ratio of the rods.

(Stuart and Hall describe the thermodynamic limits to light trapping in thin planar structures and provides a rigorous mathematical model for what has been discussed here. Work by Catchpole and Pillai in 2006 also presents a mathematical model describing light scattering in silicon waveguides by nanoparticles and is found to be in strong agreement with their experimental results. From a design perspective, Mokkapati summarizes many of the learnings of the work done by Catchpole in some convenient design parameters for maximizing the effectiveness of nanoparticle light trapping. Already referenced here, but worth repeating, are several review articles which are helpful summaries of the work done in the field of plasmonic photovoltaics. 9,136–138,141,142)

### 2.2.4.3 Surface plasmon based enhancements

In addition to using nanoparticles as either sub-wavelength antennas to concentrate incident light into amplified evanescent fields or as scattering elements, patterned metallic surfaces can be used to generate surface plasmon polaritons which have been shown to also enhance light absorption in thin film photovoltaics.
Atwater and Poleman discussed how this approach is a balancing act between reducing cell thickness and ohmic losses in the metal. While energy is indeed lost for very thin cells, the net benefit may still outweigh the loss incurred by light simply passing through the very thin absorbing layer and being lost anyway. Efficient solar cell design requires the greatest fraction of absorption to occur in the dielectric/active medium, as opposed to the metallic layer. In their review paper, Atwater and Poleman charted this fractional absorption between the metal surface and the dielectric for several types of photovoltaic cells (Figure 2-13). For wavelengths below the plasmonic resonant wavelength, absorption is dominated by the dielectric semiconductor, but drops dramatically for longer wavelengths, owing to increased plasmonic losses and decreased absorption by the dielectric. Green provided a relationship between the losses in the dielectric and conductor in his 2012 review paper stating that:

\[ f_s = \frac{k_s k_m^3}{n_s^2 n_m + k_s k_m^3} \]

Where \( n \) and \( k \) are the real and imaginary parts of the refractive indices respectively for the metal (subscript \( m \)) and semiconductor (subscript \( s \)). The authors noted that when \( \frac{n_m}{k_m} \) is very small for a metal, absorption of the plasmonic energy within the dielectric is maximized.

Ferry et al. demonstrated through numerical modeling and experimentation that performance can be enhanced by adding grooved metallic liners to the back of silicon substrates. Several plasmonic modes within the structure significantly enhanced light containment of propagating modes in the metal and sili-
con. Grooves with subwavelength widths (~100 nm) were embedded within an aluminum film and used as a backing to a 200 nm thick silicon layer, enhancing absorption by a factor of 2.5.

Mapel, Singh and Baldo coupled an organic photovoltaic device to a prism in the Kretschmann configuration to detect the impact of surface plasmon polariton absorption as shown in Figure 2-14.\textsuperscript{158} They note that the propagation length of the surface plasmon polariton is nearly 30 μm for 23 nm thick silver films, which is already much greater than the 0.05 μm thickness of the photovoltaic active layer. They report a 200% increase in photocurrent at resonance angles using λ = 532 nm laser light. While this increase in photocurrent is not representative of broad spectrum photovoltaic performance due to the monochromatic illumination scheme used, this work demonstrates the advantages to using surface plasmon coupling to increase path lengths in the photovoltaic semiconductor, in spite of concomitant ohmic losses.

![Figure 2-14 | Silver film enhanced organic solar cell. Schematic of the photodetector incorporated onto a prism for plasmonic excitation via the Kretschmann configuration. Reprinted from ref. \textsuperscript{158} with permission. © 2010, AIP Publishing LLC](image)

While the Kretschmann configuration provides a convenient coupling method for analysis, practical devices must rely on other methods of coupling incident light into the plasmonic modes. Gratings and photonic crystals are the most common methods of accomplishing this. Ferry et al. used an array of nanoholes fabricated using substrate conformal imprint lithography.\textsuperscript{159} Each hole was 350 nm in diameter and 200 nm deep with an array pitch of 513 nm. Compared with a similar cell with a flat backing, the nanohole array resulted in an increase from 4.5% to 6.2% in overall cell efficiency, with gains primarily made in the red and far red regions of the spectrum (600-800 nm).
Heidel used an antenna geometry to induce surface plasmon generation in a phthalocyanine-based photo-voltaic cell.\textsuperscript{160} Using two silver layers separated by a Rubrene layer, surface plasmon resonance was achieved. Peak efficiency was measured to be $51\pm 10\%$.

Tvingstedt et al. demonstrated an aluminum grating architecture for coupling light into plasmonic modes.\textsuperscript{161} They used soft lithography to create their gratings with a period of 277 nm and ridge height of 50 nm. While the impact of this particular grating structure did not have a major effect on efficiency, the authors noted the presence of plasmonic coupling by comparing the difference in quantum efficiency for $p$ and $s$ polarized light which indicates the presence of plasmonic interactions. An important note worth mentioning is that enhanced photocurrent was only observed when the plasmonic resonance wavelength was lower than the band gap of the photoactive media.

An interesting evolution of the grating method for light trapping could involve a multi-layered cell as suggested by Atwater and Poleman.\textsuperscript{9} Since plasmonic modes are wavelength dependent, achieving broad spectrum absorption can be difficult. However, a multi-layered device with gratings tuned for different wavelengths could result in significant broad spectrum enhancement of light collection, as shown in Figure 2-15.

Figure 2-15 | Multi-junction plasmon-enhanced solar cell. Schematic showing a proposed design for high efficiency plasmonic solar cell with each cell layer tuned to a different wavelength in order to achieve broadband absorption. Reprinted from ref. \textsuperscript{9} by permission from Macmillan Publishers Ltd. © 2010.

2.2.5 Plasmonic antennas

As discussed, the key advantages that plasmonics offer to energy production have been in photovoltaic cell design. By focusing and scattering light, plasmonic architectures can increase the effective path
length of light through the device allowing for greater overall absorption. In the cases where the plasmonic elements, usually nanoparticles, enhance absorption through nearfield interactions, they can be thought of as plasmonic antennas.

Plasmonic antennas have received significant attention in their own right given their applicability to other fields of study, particularly microscopy and single particle analytical techniques such as Surface Enhanced Raman Spectroscopy (SERS). Field enhancements many orders of magnitude have been achieved using antenna plasmonic resonators which enable concentrated and confined excitation and detection of single molecules. For nonlinear applications such as two photon fluorescence and for Raman microscopy, these benefits are what allow the massive signal enhancement which make these techniques extremely sensitive.

In previous decades, it has been observed experimentally and predicted theoretically that resonating nanoparticles located near each other can achieve additional enhancement through the coupling of their nearfields. This is analogous to the classical dipole radio antenna. The direction of oscillation of charges in each of the two particles is in the same direction, and provided that the particles are not in direct electrical contact, the electric fields in the gap reinforce the oscillations in each arm of the antenna and become concentrated in the small gap between them, leading to a hot spot. For traditional dipole antennas, which use a pair of linear conductors separated by a small gap, the combined length of both conductors is $\lambda/2$. In plasmonic antennas however, the characteristic length can be much smaller.

A significant body of work with respect to plasmonic antennas has accumulated and is evidence of the growing interest in the field. Several recent reviews provide good overviews of the work today including the work by Giannini looking at nanoantennas developed for infrared wavelengths, Berkovitch who reviewed both visible and infrared wavelength antennas, as well as Biagioni who provided a general review of the field with specific attention given to the fundamental principles governing the behavior of nanoantennas. Here, several illustrative examples will be noted as a sample of the types of work being undertaken and the methods employed.

In 2005, Schuck et al. published work exploring the relationship between nano-feature size and the wavelength of light used to excite resonance within them. The author notes that previous experiments in leveraging electric field enhancements from coupled nanostructures used randomly deposited and uncontrolled geometries that did not give a clear understanding of the effect that particle size and shape have
on electric field enhancement. Schuck used e-beam lithography to fabricate a series of bowtie antennas out of silver deposited on a fused silica cover slip. The bowtie architecture allowed collection of incident light from a larger area but focused the resonating plasmons into their narrow tips, which essentially behave as nanoparticles. The triangles making up the bowties were 75 nm long with curved tips having a radius of 18 nm and were ~18 nm high. The detection method used to estimate the field enhancement made use of two photon excited photoluminescence, a non-linear absorption processes that can occur in silver. Using this phenomena as a proxy for direct measurement of the E-field, they experimentally determined the effect that gap distance had on the field enhancement, as shown in Figure 2-16. Field enhancements were observed for all gap widths, however for bowties with gaps of 400 nm it was hypothesized that there was no coupling between the triangles, and each was behaving as an independent [article where enhancement is expected but to a lesser extent. Separations on the order of tens of nanometers provided the most significant enhancement, increasing dramatically as the gap narrowed.

Similarly, Mhlschlegel and colleagues fabricated simple dipole antennas using focused ion beam lithography out of gold on ITO coated glass. The gap between antenna bars was 20 nm. The field enhancement in the gap region induced white-light super-continuum generation, a non-linear combination of processes that produces broadband emissions from materials such as water or glass. By detecting these emissions, shown in Figure 2-17, they were able to evaluate the electric field strength in the antenna gap. Their findings provide experimental evidence of peak resonance for characteristic antenna lengths much
smaller than the wavelength of light which is an advantage plasmonic antennas have over the traditional dipole antenna.

Novotny looked further into the questions of why the characteristic length of visible spectrum antennas is much smaller than half the wavelength, in disagreement with classical antenna theory. He notes that at high wavelengths, metals reflect all the incident radiation, but at visible wavelengths, penetration of the metal occurs exciting the free electron gas. This is related to the metal band gap being located within or very near visible spectrum.

Taminiau, Moerland, Segerink, Kuipers, and van Hulst demonstrated resonance of a monopole plasmonic antenna at the tip of a glass fiber for use as a probe as shown in Figure 2-18. They used fluorescent molecules to measure the field enhancement around the antenna for various monopole lengths. In order to overcome background noise from the excitation light, the light was instead passed through the fiber where it encountered a sub-wavelength aperture near the monopole antenna. The local field induced at this aperture is what was used to drive the antenna and eliminated the effects of parasitic radiation on fluorescence detection.
Volpe, Cherukulappurath, Parramon, Terriza, and Quidant used 500 x 400 x 40 nm gold bars on ITO coated glass to explore the effects of spatial distribution of arrays of nano-antennas.\textsuperscript{169,170} They used two-photon fluorescence to image the field enhancement achieved in their designs. Two photon fluorescence is a viable technique in this instance because the probability of two photons hitting the same fluorophore at the same time are usually very small. The high amplification of the field however makes the likelihood orders of magnitude greater and so it is a useful method for detecting localized enhancements. The purpose of their study was to show how the plasmonic distribution and resulting electric field hot-spots could be effected by using higher order beam profiles as excitation sources.

Further work by this same group characterized the modal distributions of a variety of dipole antenna structure for a variety of wavelengths and resonant conditions, again using two photon fluorescence microscopy.\textsuperscript{171} By systematically mapping the modes and resonances for a variety of antenna sizes and gap dimensions, they were able to validate existing models and predictions in a comprehensive manner for the first time.

More recently, Mivelle, van Zanten, Neumann, van Hulst, and Garcia-Parajo demonstrated a probe constructed from an aluminum coated optical fiber with a bowtie shaped aperture at the tip.\textsuperscript{172} Resonances within the periphery of the aperture induced enhanced electric fields which were used to probe for fluorescent markers in a sample. Because the spatial resolution of the probe was subwavelength, superior quality could be obtained compared to diffraction limited optical confocal microscopy, as shown in the image sequence in Figure 2-19.
2.2.6 Conclusions

This concludes the review of previous work in the area of plasmonics with attention given to plasmonics in photovoltaic cell design and plasmonic nano-antennas. The field of plasmonics has opened new areas in sub-wavelength ultra-high resolution manipulation of light. This review has focused on the emerging applications of this technology on energy generation, particularly photovoltaic cell design and the related area of plasmonic antennas useful in the design of truly nanoscale photonic devices.

Future work will extend this knowledge and apply it to other energy capture technologies. In particular, opportunities exist to apply the plasmonic techniques discussed here to the cultivation of photosynthetic microorganisms. Many of the same issues associated with light capture and distribution in thin film photovoltaics are applicable to microalgal cultivation, including light distribution, path-length considerations, visible spectrum sensitivity, and absorption. Photosynthetic biofilms in particular have thicknesses on the order of tens of microns and require light to grow and maintain cohesion. As in photovoltaics, unabsorbed photons are wasted photons and consequently photosynthetic efficiency within biofilm photobioreactors can benefit from the light capture and scattering enhancements developed for photovoltaics. Furthermore, it has been shown that textured surfaces often promote the formation and strength of biofilm colonies, potentially providing an ancillary benefit to biofilm photobioreactors in addition to enhanced light utilization. The synergy between the length scale of microalage and plasmonic nearfields, as well as the typical size of plasmonic structures present exciting and unexplored opportunities for applied plasmonics for bioenergy.
Chapter 3.

Spectral Light Dilution

3.1 Introduction

The productivity of a microalgal cultivation enterprise is what ultimately determines its profitability. Ideally, high productivity is paired with high-efficiency conversion of light into bio-products, though this is rarely the case. High productivity requires high intensity light which is known to have a negative impact on photosynthetic efficiency since photoinhibition will occur when the light intensity is near or above the saturation limit of the organism. Because full sunlight can be many times greater than the saturation limit, strategies to reduce photoinhibition and increase photosynthetic efficiency without compromising productivity need to be considered. Large scale photobioreactor design has therefore focused on optimizing the combination of culture depth, optical density, and mixing rate in order to maintain an optimal light environment inside the photobioreactor. Specific examples of approaches to maximize productivity include using light diluting elements to reduce the average intensity of light seen by the culture and genetically modifying the microalgae to reduce the light harvesting antenna size and overall absorption cross section enabling increased efficiency in strong light.

Modifying the spectral quality of light also presents an opportunity to spectrally dilute light to compatible intensities. In this context, illuminating cultures with a wavelength of light that is poorly absorbed yet still capable of driving photosynthesis can achieve the goal of high productivity with increased efficiency. The action spectrum based on absorbed photons of many microalgal species suggests that light at most visible wavelengths is useful for photosynthesis, though is not equally absorbed. Because absorption is determined by the particular compliment of photosynthetic pigments in the cell, microalgae will respond differently to different wavelengths of light depending on whether or not the culture suspension is optically thin or thick. For example, in an optically thin culture, significant portions of green light which is poorly absorbed will escape the reactor without being absorbed leading to lower efficiency and productivity compared to red light which is strongly absorbed. In optically thick cultures however, nearly all
photons of both colors are absorbed within the first few millimeters, but green light will penetrate deeper than other wavelengths leading to a larger illuminated volume.

This spectral dependency is becoming particularly relevant now that monochromatic LEDs have become ubiquitous in lab scale studies of microalgal growth and also gaining popularity in commercial applications. The majority of research investigating photobioreactor effectiveness defaults to using red illumination owing to its demonstrated effectiveness at low density and intensity. A limited number of studies however, have investigated the effects of light quality and monochromatic light sources on growth. For example Chlamydomonas reinhardtii grown under moderate light intensity (100 µmol photons /m²·s ) and low culture density showed peak productivity when primarily red light was used, compared to green and blue. Green alga Scenedesmus bijuga performed best under green light at relatively high light intensity (500 µmol photons /m²·s) and high culture density > 2 g dry/L. In contrast cultures of the cyanobacteria Spirulina platensis performed best under red light at high light intensity (3000 µmol photons / m²·s) and for low culture density (OD < ~1). These results all appear somewhat contradictory. What is lacking is consideration of how culture density, light intensity, and path length, all simultaneously affect productivity. All these parameters affect the light regime within the photobioreactor, and therefore all these factors influence the optimal growth conditions. Studies which draw conclusions on the efficacy of different wavelengths based only on a subset of these parameters can lead to incomplete conclusions. As the study of Scenedesmus bijuga hints at, high intensity green light with high density cultures may lead to optimal conditions owing to the low absorption and concomitantly high penetration of green light in these scenarios.

In this work, we show the effects of spectral quality and light dilution on the photosynthetic efficiency and productivity of cyanobacteria, Synechococcus elongatus, under high and low light intensity, high and low initial culture density, and long and short culture depths. In addition to confirming higher performance with red light at low density and low intensity, it is also illustrated how this trend is reversed when the culture conditions are changed to high intensity and high density, with green light significantly outperforming other wavelengths. Similar to higher order terrestrial plants, green light clearly plays a role in achieving increasing productivity under high irradiance. Furthermore, significant changes in cell pigmentation were observed for longer wavelength light.
3.2 Materials and methods

3.2.1 Cyanobacteria cultures

Bulk cultures of *Synechococcus elongatus* T2SE provided by professor Rakefet Schwarz of Bar-Ilan University, Israel, were cultured under 30 µmol photons/(m²·s) warm white fluorescent illumination with constant agitation, at 30°C. This biofilm producing cell line has been modified previously to be resistant the antibiotic kanamycin, providing a means of preventing contamination. Cells were kept in exponential growth phase through periodic dilution with fresh media. 2x BG11 cyanobacteria growth media (Sigma Aldrich, 73816) was used for all experiments and culture maintenance to ensure cells were not nutrient limited in any context. Media was inoculated with 50 mg/L kanamycin and buffered with 40 mM HEPES to pH 8 by adding NaOH. Cells were centrifuged and re-suspended in fresh media to the required density prior to experiments.

3.2.2 Monochromatic LED photobioreactors

Monochromatic LED photobioreactors, shown schematically in Figure 3-1a, were constructed from 1” diameter acrylic tubing. The exterior of each reactor was coated in a sheet of aluminum foil to reflect light incident on the sidewall back towards the culture and to prevent parasitic radiation from entering the reactor from outside. The bottom of the acrylic tube was composed of a fused silica glass window mounted inside a 1” aluminum lens tube (Thorlabs, SM1L03) which both sealed the reactor and provided a convenient interface with the illumination system. The reactors were sealed on top with an acrylic cap with a port for a 3mm diameter aeration tube to be inserted into the reactor from above. A constant flow of 5% CO₂ in air was filtered and humidified before being bubbled into each reactor at a rate of ~1.5 m³/hr. CO₂ concentration was monitored with an inline CO₂ meter. Evaporation losses were monitored by measuring the mass of each reactor daily and replacing the difference with deionized water. Daily evaporative losses were typically less than ~1% of total volume.

For monochromatic experiments, light was supplied from below using high power LEDs controlled with a custom made constant-current power supply. 5 different wavelengths were tested, with center wavelengths at 454 nm, 534 nm, 593 nm, 630 nm, and 660 nm. Normalized emission spectra for the LEDs are shown in Figure 3-1c, measured with a fiber coupled spectrometer (Oceanoptics USB2000+). Light from the LEDs was collimated and directed into the photobioreactor using an aspheric condenser lens (Thorlabs ACL25416U) to mimic the direct beam radiation received by outdoor photobioreactors from
the sun. The intensity of each LED was monitored using a photodiode power sensor calibrated at each respective wavelength.

During each experiment, three photobioreactors of each colour were operated inside a temperature controlled enclosure maintained at 30°C. Each LED was mounted onto an aluminum heat-sink to prevent direct heating of the culture by the LED. During high-light experiments, additional heat transfer away from the LEDs was achieved through forced convection with fans, ensuring culture temperatures were maintained at 30°C ± 0.5°C. At lower intensities, forced convection cooling was not required.

Figure 3-1 | LED Photobioreactors. (a) Schematic of the photobioreactors used in this study showing light from an LED emitter being collimated into the reactor vessel to achieve uniform, direct irradiation. (b) LEDs of different wavelengths each have a different penetration depth into the culture which is determined by (c) the emission spectra of the LED emitter and the absorption spectra of the cyanobacteria. The absorption spectrum of *S. elongatus* is primarily determined by the absorption of its two primary light harvesting pigments, chlorophyll A with peaks near 430 nm and 673 nm, and phycocyanin with a peak near 623 nm.

### 3.2.3 Biomass growth measurements

Biomass accumulation was determined through optical density measurements. Samples were withdrawn from each photobioreactor and diluted below $\text{OD}_{750\text{-nm}} = 1.0$. A linear relationship (eq. 3-1) between optical density and dry mass ($M \text{[g/L]}$) was established through dry mass measurements of cell suspensions of various densities according to the method described by Moheimani et al. 175
Optical density was measured using a halogen light source and spectrometer (Oceanoptics USB2000+) coupled to a 10 mm cuvette holder. Biomass can be used as a first order indicator of photosynthetic productivity. Since photosynthesis is the primary mechanism for energy conversion in photoautotrophic species, all metabolism within the cell depends on the efficiency of light conversion. Applications that prioritize bio-products over cell division still require efficient photosynthesis. Which metabolic pathways are prioritized (biomass production, lipid production, pigment expression, etc.) depends on a variety of external factors including nutrient availability and light quality.

For productivity calculations, it was assumed that the cells were growing linearly owing to light limitation in dense cultures. Productivity was therefore determined by measuring the daily change in biomass and dividing by the corresponding change in time. For low density cultures, exponential growth is possible, particularly at early time points. Nevertheless, because the doubling time is on the order of 12 hours to several days, growth and productivity can be reasonably approximated as a linear function.

### 3.2.4 Photosynthetic efficiency

Photosynthetic efficiency measures the efficiency of energy conversion from incident light to stored biomass, according to eq. 3-2.

\[
PCE_\lambda = \frac{P_\lambda \cdot G_{\text{biomass}}}{\hbar \cdot c \cdot I_{\text{PPFD}}} \quad \text{eq. 3-2}
\]

Where \( P_\lambda \) is the areal productivity of the culture at a given wavelength, \( G_{\text{biomass}} \) is the biomass energy content, \( \hbar \) is planks constant, \( c \) is the speed of light, \( \lambda \) is the wavelength of light, and \( I_{\text{PPFD}} \) is the quantum intensity of light. The value for the biomass energy content used in this study was approximated from literature to be \( 21 \text{ kJ/gdw} \).\(^{24,176}\)

The maximum theoretical productivity for outdoor microalgal photosynthesis has been estimated to be \(-8-12\%\) based on full spectrum sunlight.\(^{21-24}\) These values account for the \(-57\%\) of sunlight which falls outside the visible spectrum and is of little use for photosynthesis,\(^{24}\) and for losses internal to the photosynthetic energy conversion process. These internal losses are related to the quantum requirement of \( \text{CO}_2 \) fixation which is the number of photons required to fix one \( \text{CO}_2 \) molecule as described in the simplified
photosynthesis equation, eq. 3-3. Estimates for the quantum requirement range between 6 and 13, with consensus suggesting 8 as a reasonable middle ground. The product in eq. 3-3, \( CH_2O \), represents the simplest form of carbohydrate and the starting point from which more complex molecules are assimilated. The equation for the efficiency of photosynthesis (\( \varepsilon_{\text{internal}} \), eq. 3-4, is simply the ratio of the energy contained in this product (\( E_{CH_2O} \)) relative to the energy of absorbed photons (\( E_{\text{photons}} \)), which is wavelength dependent.

\[
\text{CO}_2 + H_2O + 8 \text{ Photons} \rightarrow CH_2O + O_2 \quad \text{eq. 3-3}
\]

\[
\varepsilon_{\text{internal}} = \frac{E_{CH_2O}}{8 * E_{\text{photons}}} \quad \text{eq. 3-4}
\]

Since practically all the light emitted from a monochromatic LED is in the visible range and is therefore useful for photosynthesis, the maximum photosynthetic conversion efficiency can be calculated from eq. 3-4 alone and is ~27%, assuming the energy content of \( CH_2O \) to be 483 kJ/mol. For full spectrum sunlight, an additional factor of 43% accounts for the photosynthetically active portion of the spectrum and leads to sun-lit maximum efficiencies of ~12%. Comparing photosynthetic efficiencies between sun-lit cultures and LED-lit cultures must therefore take into account the difference in illumination spectrum. It is a common mistake to compare the photosynthetic efficiency of LED-lit cultures to the theoretical maximum for sun-lit cultures, and vice-versa. Calculating sun-lit efficiencies based only on photosynthetically active radiation is one way to mitigate the discrepancies caused by different illumination spectra, but can lead to additional confusion when comparing microalgal solar energy conversion to other solar energy conversion technologies, such as photovoltaics.

### 3.3 Results and Discussion

#### 3.3.1 Illumination profile

As the biomass density in a culture increases, inter-cell shading results in attenuation of light in the direction of propagation leading to heterogeneous light intensity in the reactor, and is different for each wavelength of light as seen in Figure 3-2(a-b). This attenuation has most often been approximated using the Beer-Lambert law which describes a simple exponential decay of light due to absorption. The beer-lambert law however presupposes a non-scattering media which, for microalgal suspensions, is only approximated when the culture density is very low. Alternatively, models based on the radiative transfer equation have been used to describe the effects of both absorption and scattering on the propagation of
light through microalgae suspensions with a greater degree of accuracy. Cornet’s model of 1-dimensional transfer using Schuster’s approximation is a solution to the radiative transfer equation applicable for photobioreactor architectures where light is propagating and is scattered along a single axis in both forward and backward directions. Simplifying the radiative transfer equations to a single axis allows for analytical expressions for the intensity of the light with respect to depth to be derived. Figure 3-2c compares the results of this model against direct measurements of light transmission for an optical density of OD\textsubscript{750,10mm} = 0.5, and clearly shows the superiority of Cornet’s model over the Beer-lambert equation. The model requires the absorption and scattering cross-section spectra of the cells to be known in advance. Here, approximations based on the measured attenuation spectra of S. elongatus and the scattering profile described by Stramski have been used. Figure 3-2d shows these absorption and scattering profiles along with the single-scattering albedo. Using this model, the volume fraction of the culture exposed to actinic light can be approximated for each scenario explored in this study, as shown in Figure 3-2e.
Figure 3-2 | Attenuation of light in a cyanobacteria suspension. (a-b) Photographs taken of columns of cyanobacteria under two different lighting schemes. When illuminated from above by collimated LEDs, the penetration depth of each wavelength is clearly visualized. (c) The normalized intensity of forward propagating light for five different wavelengths are shown as measured (circles), and as predicted by Cornet’s model of 1-dimensional radiation transfer using Schuster’s approximation\(^{178,179}\) (solid line) and by the Beer-Lambert equation (dashed line). The radiative transfer model gives a closer approximation to the measured data since it accounts for wavelength dependent scattering by the cells. The measured optical densities for the culture across a 10 mm path-length are 1.2, 0.85, 0.75, 0.85, 0.71, 0.49 for 454-nm, 534-nm, 593-nm, 630-nm, 660-nm, and 750-nm light respectively. (d) Graphs showing the scattering and absorption cross sections and the single-scattering albedo for the cells used in the radiative transfer model for light attenuation through the microalgal suspension. (e) Based on the radiative transfer model, the attenuation of light gives rise to photic zones of different relative size in each culture scenario. For cultures with initial cell concentration of 0.58 g/L (OD\(_{750,10\text{mm}}\) = 2.6), dark regions exist in all cases, whereas for initial cell concentrations of 0.04 g/L (OD\(_{750,10\text{mm}}\) = 0.2), the entire volume is illuminated.

3.3.2 Low light - effects of path-length and culture density

Four different sets of conditions were explored to study the combined effects of path-length, culture density, and wavelength, for both high and low light intensity. For each wavelength, both short (2 cm) and long (6 cm) culture depths were tested with both low (0.04 g/L) and high (0.58 g/L) initial culture density. These combinations were repeated for both low (50 \(\mu\text{molphotons}/(\text{m}^2\cdot\text{s})\)) and high 2000 \(\mu\text{molphotons}/(\text{m}^2\cdot\text{s})\) light intensities.

Figure 3-3 shows the productivity and photosynthetic efficiency of cultures cultivated under these four conditions with low light intensity. At low intensity the culture was light limited. There was no portion of the culture where the local intensity was above the saturation limit, and the photosynthetic efficiency was virtually unaffected by photoinhibition. At high initial culture density, light gradients resulted in light and dark zones within the reactor volume, as shown in Figure 3-2e, with cells continually cycling between them. Because of the high initial culture density, transmission losses were negligible since virtually all the light of each wavelength was absorbed by the culture. However, the frequency in which cells passed through the illuminated fraction of the reactor was quite different between the short and long light-path scenarios. For cells in the long light-path reactors, the fraction of time spent illuminated was far smaller than the time spent in the dark, owing to the larger dark zone. It has been well documented that the frequency and duty cycle of pulsed light can significantly alter the productivity of microalgae.\(^{83,182}\) The relatively high productivity and photosynthetic efficiency observed in the short light-path reactor compared to the long light-path reactor (Figure 3-3(a-b)), suggests that the fraction of illuminated volume in the reactor and therefore the frequency with which cells are exposed to light was a significant driver of the observed difference in performance. The difference in performance between wavelengths provided further evidence of this dependency. The most strongly absorbed wavelength, 630-nm
red light, had a shallower photic zone compared to the other wavelengths and also has Figure 3-3(a-b) to have the lowest productivity.

When the initial culture density was reduced, Figure 3-3(c-d), the effects of light gradients were minimized and transmission loss became an increasingly important factor affecting productivity. In both short and long light-path reactors, poorly absorbed green light showed significantly lower productivity than longer wavelengths, owing to higher transmission losses for green light. Indeed, the productivity and efficiency of all wavelengths in the long light-path reactors (Figure 3-3c) improved over the short light-path reactors due to increased absorption in the deeper culture, with green light showing a 128% increase in productivity and a 2.3 fold increase in photosynthetic efficiency. In spite of this improvement however, under low-light and with low-density cultures, longer wavelength light outperformed green light, with the highest productivity and photosynthetic efficiency resulting from 630-nm red light.
In all cases, 454-nm blue light underperformed compared with other wavelengths at the same intensity. Due to their complement of pigments, cyanobacteria are known to respond poorly to shorter wavelength light, and so this result is not unexpected, and consistent with those previously published. In spite of the strong absorption of light at 454 nm, most of the energy is diverted to other pathways. This non-photochemical quenching of absorbed light energy in cyanobacteria is typically induced through a series of reconfigurations triggered by blue-light absorbing proteins, and is the likely cause for the low performance at this wavelength. The results from monochromatic blue light are therefore an anomaly among the wavelengths tested here since blue light is particularly well suited to triggering photo-protection mechanisms in cyanobacteria, which are not triggered by other wavelengths.

It is clear that in the context of low culture density and low light intensity cultivation, red light is the preferred wavelength to maximize productivity and photosynthetic efficiency. Increasing culture density increases the effects of light gradients on culture performance which are also wavelength dependent. Large dark volumes result in slow cycling of cells between light and dark zones, damping overall performance. The ability for green light to penetrate deeper into high-density cultures compared to other wavelengths makes it competitive with other wavelengths at higher culture density. Optimal performance at low light however occurred with low culture density, long light-path, and 630-nm red light. The highest productivity and photosynthetic efficiency for each wavelength under low light conditions are summarized in Table 3-1.

<table>
<thead>
<tr>
<th>Wavelength [nm]</th>
<th>Low Light Max Prod [g/(m²day)]</th>
<th>Max PCE [%]</th>
<th>High Light Max Prod [g/(m²day)]</th>
<th>Max PCE [%]</th>
<th>ΔPCE [%] (PCELL – PCEHL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>454</td>
<td>0.9</td>
<td>1.7</td>
<td>6.8</td>
<td>0.3</td>
<td>-1.4</td>
</tr>
<tr>
<td>534</td>
<td>3.4</td>
<td>7.3</td>
<td>28</td>
<td>1.5</td>
<td>-3.2</td>
</tr>
<tr>
<td>593</td>
<td>3.7</td>
<td>9.0</td>
<td>19</td>
<td>1.1</td>
<td>-7.7</td>
</tr>
<tr>
<td>630</td>
<td>4.3</td>
<td>11</td>
<td>13</td>
<td>0.8</td>
<td>-10</td>
</tr>
<tr>
<td>660</td>
<td>3.7</td>
<td>9.9</td>
<td>18</td>
<td>1.2</td>
<td>-8.7</td>
</tr>
</tbody>
</table>

### 3.3.3 High light - effects of path-length and culture density

When the light intensity was increased far above the saturation limit of the cells, the trends observed in the low light trials no longer applied, as is evident in Figure 3-4. Notably, 534-nm green light demon-
SPECTRAL LIGHT DILUTION

...strated the highest productivity and photosynthetic efficiency under high light conditions in all cases, with 630-nm red light showing the lowest performance. This is particularly significant for the low-density culture scenario since it represents a direct reversal of the trends observed in the corresponding low-light scenario in which 630-nm red light performed the best.

Figure 3-4 | High-light areal productivity and photosynthetic efficiency. Productivity and efficiency after 1 day of cultivation under 2000 µmol photons/(m²·s) light for cultures with initial conditions corresponding to (a) cell concentration = 0.58 g/L (OD 750,10mm = 2.6), light path length = 2 cm, (b) cell concentration = 0.58 g/L (OD 750,10mm = 2.6), light path length = 6 cm, (c) cell concentration = 0.04 g/L (OD 750,10mm = 0.2), light path length = 2 cm, (d) 0.04 g/L cell concentration (OD 750,10mm = 0.2), light path length = 6 cm. Error bars represent the standard deviation from 3 independent trials.

As expected, the photosynthetic efficiency in the high-light scenarios (Figure 3-4) was dramatically lower than the low light scenarios (Figure 3-3). At high intensity, light was absorbed at rates greater than the cell’s photosynthetic apparatus could utilize it, forcing the excess energy to be converted to waste heat and fluorescence, reducing efficiency. Productivity however was at its highest since the cell’s photosynthetic machinery was as full capacity.

While the spectral performance between high and low light scenarios is very different, the relative performance between low- and high-density, and short and long path-length scenarios was similar for high
light as it was in low light. In particular, for high density cultures, transitioning from a short to long path-length (Figure 3-4(a-b)) resulted in a decrease in productivity and photosynthetic efficiency owing to an increase in the ratio of dark to light volume in the reactor due to the large light gradients. In addition, for low density cultures, transitioning from a short to long path-length (Figure 3-4(c-d)) resulted in increased productivity and efficiency, owing again to reduced transmission losses.

![Figure 3-5 | Peak performance trends.](image)

The maximum photosynthetic efficiency and productivity for each wavelength under high and low light conditions are reported in Table 3-1, and Figure 3-5 summarizes the peak performance scenarios in terms of photosynthetic conversion efficiency and productivity for each wavelength investigated. Green light achieved the highest productivity, outperforming 454-nm blue 4.1 fold, 593-nm orange by 1.5 fold, 630-nm red by 2.2 fold and 660-nm red by 1.6 fold. For all wavelengths peak productivity is achieved with high light, high density, and short path-length. Peak efficiency is achieved for all wavelengths with low light, and low density, and long path-length for 454-nm, 630-nm and 660-nm light, and high density and short path-length for 534-nm and 593-nm light.

### 3.3.4 Batch cultivation

The reactors were operated in batch mode for four days. The accumulated biomass relative to each experiment’s starting condition is plotted in Figure 3-6. Low light experiments demonstrated consistent growth over the duration of the experiment. Under high light conditions however, growth rates slowed and for
many cases became negative after ~3 days. This decline was most notable for longer wavelengths (593 nm, 630 nm, and 660 nm) and least pronounced for 534-nm green light. The most likely cause for the reduction in productivity and loss of biomass relates to accumulated photo-damage due to high irradiance, particularly for the highly absorbed wavelengths.

Figure 3-6 | Accumulated biomass. Error bars represent the standard deviation from 3 independent trials.

Significant changes in cell pigmentation also occurred for 593-nm, 630-nm, and 660-nm light at high intensity, as shown in Figure 3-7, and is indicative of chromatic adaptation in response to the high light conditions. Chromatic adaptation in cyanobacteria allows for the customization of light harvesting pigment composition and expression within the cell in response to changing light intensity and quality. While indication of pigmentation change was evident in all high light scenarios, for cultures that began with high cell density (Figure 3-6(e-f)) the adaptation response appeared to be slower. For these cultures, the biomass concentration plateaued after ~3 days (Figure 3-6(e-f)) and in many cases began to decrease for 593-nm, 630-nm, and 660-nm light, with only a moderate shift in the pigmentation of the cells (Figure 3-7(a-b)). This trend contrasts with that observed for cultures that began with low initial cell densities.
(Figure 3-6(g-h)) for which an obvious change in cell colour and attenuation spectrum occurred (Figure 3-7(c-d)). Notably, this shift in pigmentation was accompanied by a much smaller decrease in culture density compared to the high initial culture density reactors, suggesting that the deleterious effects of high light intensity over time were in part mitigated by the more rapid shift in pigment content. The low initial culture density allowed more cells to be exposed to high light conditions for a larger amount of time, inducing more rapid acclimation to the light conditions.

Figure 3-7 | Pigmentation shift under high light. After four days of growth cells grown under high light with (a) high initial culture density showed a moderate shift in pigmentation and (b) attenuation spectra. (c) Cells exposed to high light with a low initial density showed a much more dramatic shift in pigmentation and (d) attenuation spectra.

3.4 Conclusions

To maximize the cost effectiveness of microalgae cultivation, high productivity and high efficiency must be achieved. Here we have demonstrated that contrary to conventional wisdom, highly absorbed red light is not always the wavelength of choice for monochromatic cultivation. Instead, depending on culture density, depth and irradiance, poorly absorbed green light may provide the best option for achieving high
productivity with increased efficiency because it is able to spectrally dilute the light over a larger portion of the reactor. In low light scenarios, red light indeed outperformed other wavelengths but was unable to achieve the productivities that green light achieved under higher irradiances. Further work to explore the optimal combinations of wavelength, density, irradiance, and path-length will provide further direction to microalgal cultivation efforts, particularly those which make use of artificial light sources.
Chapter 4.

Plasmonic Near-Field Enhanced Growth

*This chapter was originally published in Applied Physics Letters, and has been adapted from ref. 5 with permission. © 2012, AIP Publishing LLC. The applicant was the primary author for this work and played the primary role in experiment design, execution, data collection, data analysis, and paper writing. The efforts of all other authors are gratefully recognized, they are: Lauren Bajin and David Sinton.

4.1 Introduction

Photosynthetic microorganisms are an increasingly important feedstock for products such as nutritional supplements, colorants, high value compounds, and as a feedstock for biofuel production.\(^\text{28,43,192}\) In response to the environmental urgency to reduce carbon emissions, researchers have shown that microalgae as a biofuel feedstock is able to compete with fossil fuels from an energy and volume perspective.\(^\text{193–196}\) This is because microalgae, in particular cyanobacteria, boast high growth rates, low nutritional requirements, and can be cultivated in areas not presently used for agriculture. Consequently, significant attention is being focused on more effective bulk cultivation approaches and on the genetic engineering of more efficient fuel producing species.\(^\text{197,198}\) With these advances comes the need for innovative methods to study, and ultimately cultivate, photosynthetic microorganisms in order to maximize the effectiveness of engineered systems and organisms.

Optofluidic technologies – the marriage of optics and fluidics - enable precise control of each cell’s micro-environment including chemical, mechanical and optical aspects. Microfluidics has been applied to study the proliferation of bacterial colonies as biofilms on channel surfaces, though largely focusing on non-photosynthetic species.\(^\text{199–201}\) To complement these techniques, evanescent light based methods have emerged as an optofluidic approach to deliver light to photosynthetic organisms within microfluidic devices. Evanescent light fields were first employed to excite photosynthetic bacteria using total internal reflection off a glass prism.\(^\text{202}\) Later, Jung et al. demonstrated evanescent growth of cyanobacteria on a glass slab waveguide and quantified this growth in terms of photosynthetic output compared to direct
illuminaton. Other work has leveraged this approach to grow high-density biofilms toward lab-scaled photobioreactors. A related application for microalgal culturing was presented by Torkamani where he showed enhanced microalgal growth in miniphotobioreactors from backscattering due to localized surface plasmon resonance in suspended gold nano-particles. Despite advantages of high intensity and localization offered by surface plasmons, direct growth of photosynthetic microorganisms in a surface plasmon resonant light field has not been demonstrated to date.

While applications of surface plasmon resonance are rapidly expanding, in the past they have almost exclusively been in sensing and imaging applications that leverage the high intensity, surface confined, plasmonic light field. In this work, it is shown that surface plasmon resonance on a planar surface can also be used as a means of achieving targeted light delivery to a photosynthetic biofilm. Biofilm density and morphology are shown to vary with field intensity reaching maximum cell volume concentration of 20% v/v comparable to 26% v/v for films grown by direct irradiation in an incubator. Maximum biofilm thickness is measured at 10 µm compared to 7 µm for incubated cultures. Plasmon enhanced evanescent fields afford the advantages of focused delivery of high intensity light, which in the past have been reserved for sensing and analysis applications, to the direct optofluidic cultivation of photosynthetic bacteria for solar fuels and other products.

4.2 Methods and discussion
Cells of the wild type S. elongatus (ATCC 33912) cyanobacteria were used for all experiments. Seed cultures were incubated at 30 °C under continuous fluorescent lamp irradiation of 50-75 µmol photons·m⁻²·s⁻¹, as described previously, to achieve a controlled initial condition for the plasmonic growth experiments. Samples were drawn from this controlled culture for use in each of the experiments.
The growth chambers used in this study were molded from PDMS (Sylgard(R) 184 Elastomer Kit, Dow Corning) cast from a poly(methylmethacrylate) (PMMA) master. Oblong cylindrical growth cavities (~0.6 mL) were formed and contact bonded to the gold surface of a gold coated microscope slide (50 nm Au, 2.5 nm Ti, 0.7 mm aluminosilicate glass - Platypus Technologies AU.0500.ALSI).

Surface plasmons were excited using the so-called Kretschmann configuration,\textsuperscript{209} as shown in Figure 4-1. To achieve this, the cavity and gold coated slide were placed in optical contact with the top face of a right angle BK7 prism (Thorlabs PS908L-A) using an index matched immersion oil (Leica 11513 859). Light was coupled into the gold film from a helium neon laser ($\lambda$=633 nm, Thorlabs HRR020). The unpolarized beam from the laser passed through a polarizing beam splitter (Thorlabs CM1-BS013) mounted to a precision rotation mount (Thorlabs CRM1P). The p-polarized component was then passed through the prism, exciting the sample, and subsequently to a photodiode power sensor (Thorlabs S120C). The s-polarized beam was passed directly to a similar photodiode power sensor as an input power reference. The incident angle of the beam on the glass/gold/media interface was tuned by rotating the beam splitter held in the rotation mount.

Growth experiments were initiated by dead-end filling of the growth chamber with cells from a highly dilute suspension (OD$_{750}$ < 0.02). A dilute suspension was used to keep the cell coverage on the gold surface low at the onset of the experiment. A period of 20 minutes was allowed for initial cell settling and adhesion to the gold surface before the culture cavity was purged with fresh BG11 growth media to remove any remaining, free-floating cells. The sample was then left in darkness for a minimum of 12 hours to ensure all cells were settled prior to light exposure.
During the initial setup, the ratio of the input and output beam powers was monitored until a minimum reflectance was observed, indicating maximum coupling of input laser light into the gold film plasmonic modes. Specifically, for the prism/slide/gold/media interface with refractive indices $n_{\text{prism}} = 1.513$, $n_{\text{slide}}=1.505$, $n_{\text{gold}}= 3.321+0.22i$, $n_{\text{media}}=1.33$ and a gold film thickness of 50 nm the resonance occurred at an angle of $\theta_{spr} = 73^\circ$ to the interface normal. This resonance angle is supported by analytical solutions to the electric field for this geometry found in literature. Because this angle is greater than the critical angle for total internal reflection ($\theta_c = 61.5^\circ$), all other light is either reflected back into the prism or absorbed by the gold. Any initial scattering of light due to surface imperfections is considered negligible compared to the intensity of the evanescent field strength induced through surface plasmon resonance near the surface. When this evanescent field is unperturbed, no net energy transfer into the media occurs. However when absorbing/refracting species such as cells are placed in the vicinity of the surface, the energy contained in the evanescent field can be harvested/scattered. The entire apparatus was optically isolated in a black hardboard enclosure to prevent ambient radiation from affecting cell growth and kept at a constant temperature of -30 °C for the duration of the experiments.

Figure 4-2 shows the early cell proliferation in the region excited by surface plasmons, as imaged through epifluorescent microscopy (Leica DML fluorescence microscope). The autofluorescence of phycocyanin, a primary photosynthetic pigment in *S. elongatus* contained in the cell’s peripheral thylakoid membrane, served as the fluorescent marker. Because of the location of these pigments near the outer edge of the bacterium, the signal obtained from the autofluorescence of these pigments provided a clear indication of the extent of the cells. Figure 4-2a-d shows the accumulation of cells over time near the center of where the laser was incident on the gold film. At time $t = 0$ (Figure 4-2a), prior to application of the plasmonic light field, only a sparse layer of independent cells was present. Over the course of one day these surface-bound cells developed rapidly as shown in Figure 4-2e and image progression (Figure 4-2a-d). In general, accumulation in the light field is expected to be both a product of reproduction under the favorable (illuminated) condition as well as relocation of cells via swimming motility from adjacent areas to the illuminated region – both effects indicating favorable excitation conditions. Based on a curve fit of the surface cell coverage to the exponential growth equation, total accumulation corresponds to a doubling time of 11 hrs – a value in keeping with the range expected for this bacterium (<24 hrs). During the early stages of biofilm development, the cultures were expected to not be nutrient limited given the low den-
sity of the biofilm and nutrient replete media used. Most importantly, these results demonstrate successful excitation of photosynthetic microorganisms using plasmonic light fields.

Trials conducted over the course of three days using this same configuration showed continued cell growth and the development of surface bound colonies forming biofilms. These biofilms developed in the characteristic elliptical shape matching the pattern created by the circular laser beam when incident on the interface at the resonance angle, as shown in Figure 4-3a. Surface plasmon propagation along the surface is expected on the order of μm$^{-2}$ for the conditions here, and thus additional extension of the light field beyond the illuminated ellipse through surface plasmon propagation is minimal in the present experiments. The calculated intensity of the evanescent field at the central point is also shown in Figure 4-3b as a function of distance away from the interface.

Figure 4-2 | Early proliferation of cells when exposed to the evanescent field excited by surface plasmon resonance. (a-d) Time series fluorescence images of cell accumulation at 8 hour intervals. Scale = 25 μm. (e) Graph showing the increase in cell coverage on the surface at 1 hr intervals.
Figure 4-3 | Plasmonically grown biofilm growth pattern. (a) Fluorescence image of a maturing biofilm after three days of growth under evanescent illumination conditions. The dashed red outline represents the shape of the laser beam and is positioned where the laser intensity reaches 1/e² of its maximum intensity based on a Gaussian distribution. (b) Plot showing the concentration of cells as a function of radial distance from the center of the spot compared to the evanescent field strength at distances from the surface on the order of typical cell dimensions.

The optimal field intensity for growth of *S. elongatus* has been found previously to be between 66 W·m⁻² and 12 W·m⁻². From Figure 4-3b, it can be seen that at a distance less than 500 nm away from the gold surface (approximately 50% the thickness of a cell's minor dimension), these intensities are greatly exceeded. Despite the high intensity of the light field, cell growth is not inhibited, and maximum growth is observed at and around the central axis of the reflection spot. Cell health in the highest intensity region is attributed to the small percentage (< 50%) of the cell that is exposed to these growth-inhibiting intensities, which leaves the majority of a surface-located cell exposed to lower evanescent field intensities or able to benefit from light coupled out of the waveguide by scattering from cell components closer to the surface. In previous studies using similar (~1 mW) light at the same wavelength without the addition of the plasmon enhancement, substantial photobleaching (high light induced damage) was observed.

With the plasmon enhanced fields used here, it is hypothesized that cells are protected from the damaging
effects of these high intensities because the field is localized to a greater degree - focused only on the lower extremities of the cell. Light tripped back into the propagating regime by cell components exposed to the high intensity fields can be leveraged by the rest of the cell and adjacent ones to drive photosynthesis.

Quantification of biomass volume fraction and film thickness was performed for films grown under plasmon enhanced evanescent illumination as well as direct light illumination, for comparison. For the samples grown in direct light, identical culture chambers were placed in an incubator maintained at 30-32 °C and supplied with direct broadband light from a fluorescent lamp. After three days of biofilm development, the PDMS chambers were removed from the gold film slides and the gold surfaces gently rinsed with fresh BG11 cyanobacteria growth media to remove non-adhered cells. These samples were then imaged in a petri dish containing BG11 growth media. Z-stack images of the biofilm were generated using a Zeiss LSM-510 scanning confocal microscope. A 63x water immersion lens (Zeiss 440067-9901) was used and each z-slice had an optical depth of 1.5 µm with a vertical spacing of 1.5 µm between them. A custom image analysis Matlab code was used to identify the location of cells based on their autofluorescence and used to calculate the volume fraction of cells for each optical slice.

Imaging results in Figure 4-4 quantify the biofilm thickness and density for each illumination scheme. For the biofilm grown with surface plasmon resonance, Figure 4-4a, the film was measured to be 10 µm in depth with a maximum cell volume fraction of 20% in the center of the growth spot. The presence of a surface attached biofilm was evident by the lack of cell movement within the biofilm and the film’s tolerance to agitation of the culture enclosure. Long term qualitative observation showed little detachment from the surface even with extensive handling and indicated that cells were entrapped within an extracellular matrix anchored to the surface, characteristic of biofilms. The total volume fraction of biomass in the 10.5 µm space above the surface was 11%. The biofilm grown under direct illumination, Figure 4-4b, was uniform across the surface due to the even lighting conditions in the incubated culture. At a representative location in this film, the average thickness was measured to be 7 µm and cell volume fractions reached a maximum of 26%. For the space extending 10.5 µm from the surface, the total volume occupied by biomass was found to be 9% which was 2% less than the plasmon-excited film. Figure 4-4c charts the volume fraction of cells with respect to height from the growth surface for both illumination schemes highlighting the similarity in cell volume and thickness. These results suggest that biofilms grown under plasmon enhanced evanescent fields are viable in terms of key biomass metrics. An advantage to growing photosynthetic cells using surface plasmon enhanced evanescent fields however is that
the surface confined nature of the field prevents excessive illumination of the bulk volume while still providing sufficient energy to sustain cell development near the surface. This approach allows for guided and focused delivery of light where it is needed, the biofilm, without compromising growth and represents a uniquely optofluidic approach to cellular illumination of photosynthetic cyanobacteria.

Figure 4-4 | Laser scanning confocal microscope cross-sections of biofilms grown using (a) evanescent illumination excited through surface plasmon resonance and (b) direct broadband illumination. (c) Graph showing the cell volume fraction occupying each optical slice with respect to distance from the growth surface. Representative error bars are provided once for each case. It was possible to collect depth-wise cell density data up to 1.5 µm from the gold surface. The total cell volumes reported represent the total volume percentage of cells in the space extending from the gold surface to a height of 10.5 µm.

4.3 Conclusions

In conclusion, we have demonstrated the cultivation of cyanobacteria cells and biofilms using plasmon enhanced evanescent fields to direct radiant energy from a waveguide into the photosynthetic cells. Cultures of *S. elongatus* were exposed to evanescent fields excited by red laser light totally internally reflected off the interface between a glass prism, 50 nm gold coated slide, and a cyanobacteria suspension in the Kretschmann configuration. Biofilm proliferation was observed and corresponded to the location of surface plasmon excitation. Biofilm thickness was found to be 10.5 µm in the center of the growth area and
with a total biomass volume density of 11% representing 2% more accumulation than control experiments with direct light over the same period. Total biomass contained within the biofilms grown under the two illumination schemes showed that plasmon enhanced evanescent growth is a viable approach to biofilm illumination. The high intensity enhanced evanescent field penetrates less than 1 µm into the media accounting for partial illumination of adjacent cells only. Scattering of light by cells and materials interacting with this field enabled targeted illumination of the film from below. Collectively, these results indicate the ability to (1) excite surface-bound cells using plasmonic light fields, and (2) subsequently grow thick biofilms by coupling light from the surface. This approach has the benefit of providing focused light delivery with spatial control in environments where direct illumination may be challenging such as integrated optofluidic devices, or dense photobioreactors.
Chapter 5.

Plasmonic Characterization of Crude Oil

*This chapter was originally published in Energy and Fuels, and has been adapted here from ref. 6 with permission. © 2015, American Chemical Society. The applicant was co-first author with Dr. Hossein Fadaei and contributed equally in experiment design, execution, data collection, data analysis, and paper writing. The efforts of all other authors are gratefully recognized, they are: David Sinton.

5.1 Introduction

Oil and gas constitute 57% of current primary world energy use.\textsuperscript{217} The measurement of the physico-chemical and thermophysical properties of crude oil and related hydrocarbons (fractions) are critically important to the industry and global energy supply.\textsuperscript{218} Specifically, fluid properties determine the ultimate economic and environmental feasibility of operations, and play an important role in facility design, well completion, surface production facilities, managing reservoir recovery, production forecasting, and downstream refining and processing.\textsuperscript{219} Analysis of hydrocarbon fluids, however, is associated with many challenges including high pressure and temperature conditions, complex mixtures, variable solvent-dilution ratios, and opaqueness of the sample.\textsuperscript{219} The opaqueness of crude oils, specially medium and heavy oils, is primarily an issue for optical characterization (i.e absorption). In addition, for sampling/analysis in wells and surface pipelines, the multiphase (water, oil and gas) nature of the flow adds further complications. Reliable measurement of fluid properties therefore requires measurement methods that are applicable to complex opaque fluids and robust in extreme conditions.

A crude oil’s refractive index (RI) can be used to characterize and estimate its key thermo-physical properties such as density, critical constants, and heat capacity\textsuperscript{220} and thus refractive index is a property of significant interest to reservoir engineers. A conventional refractometer, based on light transmission, is the most established RI measurement device used for transparent and semi-transparent fluids. Refractometers however, are not suitable for opaque liquids such as heavy and extra heavy crude oils.\textsuperscript{221} One approach to
deal with this is to dilute the crude oil with solvents (i.e. toluene), measure the RI of the diluted mix-
tures, and then extrapolate the RI for the undiluted sample using mixing rules.\textsuperscript{222,223} This approach is not practical, however, for continuous RI measurement (i.e. inline) and dilution can have unintended effects on the properties of complex natural fluids that contain a range of light and heavy fractions.\textsuperscript{224} To accommodate dark crude oils, reflection based techniques using a fiber optic\textsuperscript{225,226} or critical angle measurement have been used.\textsuperscript{222} In both approaches, the absorption of the fluid remains an issue when measuring heavy oil samples with high absorption at typical wavelengths, requiring an additional correction.

Surface plasmon resonance (SPR) is an optical phenomenon previously employed in a variety of fields including bio-sensing,\textsuperscript{227–229} imaging,\textsuperscript{230} photovoltaics,\textsuperscript{231} lasing,\textsuperscript{232} advanced disease treatments,\textsuperscript{233,234} and energy.\textsuperscript{5,13,106} Under resonant conditions, a large majority of the incident energy is coupled into these electron oscillations and a sharp decrease in the amount of reflected light can be observed.\textsuperscript{235} The point of maximum resonance is highly sensitive to the geometry of the metal thin-films and the refractive index of the surrounding materials. This allows for the detection of even slight changes in material properties by monitoring where the peak resonance occurs.\textsuperscript{236} Because it is an optical phenomenon it is inherently non-intrusive and amenable to in-line measurements. Applications of surface plasmon resonance are rapidly expanding and there is already some precedent for analysis of light hydrocarbon gas mixtures.\textsuperscript{237,238} Due in part to the challenges noted above, however, SPR-based crude oil characterization has not been presented to date.

In this work, we apply surface plasmon resonance on a planar surface to differentiate crude oils across the spectrum of oil types (from light oil to extra heavy oil and bitumen), and crude oil-solvent mixtures by measuring the incident angle at which resonance occurs. The application of SPR to these opaque, high index, fluids is made possible using infrared light to which the crude is largely transparent, coupled with a high-index prism. This method leverages the high sensitivity inherent to the SPR technique, and does not require any dilution for heavy and extra-heavy oils. This approach is non-intrusive, robust, and well suited to continuous monitoring in reservoirs, surface facilities and pipelines.

5.2 Experimental Section

5.2.1 Oil samples

Crude oil samples with specific gravities ranging from 0.825 to 1.007 (40 to 90°API) were obtained from ONTA Inc. (USA) and were used without further treatment. Athabasca bitumen (Alberta, Canada) was
obtained from Syncrude Canada, Ltd. These samples had previously undergone two extraction steps:
warm-water extraction and naphtha dilution (with the naphtha recovered via distillation). Mixtures of
Athabasca bitumen and toluene were prepared by dissolving the appropriate ratio (volume based) of the
bitumen in reagent grade toluene (99.5% purity, Sigma Aldrich). The mixtures were kept in sealed vials
at room temperature to prevent any evaporation prior to testing. Absorption at 1550 nm for each oil
sample was determined using the Beer-Lambert law by measuring the intensity of light transmitted
through oil samples held polystyrene micro-cuvettes, which had an optical path-length of 10 mm.

Figure 5-1 | Surface plasmon resonance based crude oil characterization. (a) The experimental setup based on
incident angle interrogation (Kretschmann configuration). The hemispherical prism (SF11 glass, refractive in-
dex 1.74) ensures that the incident light hits the sample at the same location for different angles. (b) Graph
showing the measured absorbance of water and the crude oils used in this study at the interrogation wave-
length of 1550 nm (ordered by increasing density, left-to-right), with an optical path length of 10 mm.

5.2.2 Sample holder
The sample holder and sensor consisted of a rectangular glass substrate (SF11, $n_{1550\text{nm}}=1.745$, 25 mm×25
mm×1 mm, Newlight Photonics) attached to an aluminum cavity with a screw-top cap to prevent sample
evaporation during testing. The plasmonic substrate was prepared by thermal evaporation of a 2.5 nm
chromium adhesion layer followed by 30 nm of gold onto the glass substrate. A 30 nm thick gold film
was chosen in order to achieve maximum coupling between the 1550 nm laser and surface plasmons re-
sulting in a stronger signal as calculated using established theory.\textsuperscript{235} At this thickness, surface plasmon
coupling is also least sensitive to minor variations in the film thickness that may result during fabrication.
In the experiment, ~2 mL crude oil (oil-solvent mixture) was placed in the sample holder, in contact with
the gold side of the glass and sealed.
Table 5-1 Density and type of crude oils analyzed

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Oil Name and Region</th>
<th>Density [g/mL]</th>
<th>API*</th>
<th>n**</th>
<th>n***</th>
</tr>
</thead>
<tbody>
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<td>Oil 1</td>
<td>Appalachian - East NA</td>
<td>0.852</td>
<td>40.0</td>
<td>1.445</td>
<td>1.44</td>
</tr>
<tr>
<td>Oil 2</td>
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<td>0.839</td>
<td>37.0</td>
<td>1.465</td>
<td>1.46</td>
</tr>
<tr>
<td>Oil 3</td>
<td>Qua Iboe - Nigeria</td>
<td>0.855</td>
<td>34.0</td>
<td>1.467</td>
<td>1.47</td>
</tr>
<tr>
<td>Oil 4</td>
<td>Hoops - Texas</td>
<td>0.869</td>
<td>31.4</td>
<td>1.474</td>
<td>1.47</td>
</tr>
<tr>
<td>Oil 5</td>
<td>Oriente - Ecuador</td>
<td>0.910</td>
<td>24.0</td>
<td>1.505</td>
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</tr>
<tr>
<td>Oil 6</td>
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<td>0.968</td>
<td>14.7</td>
<td>1.528</td>
<td>1.53</td>
</tr>
<tr>
<td>Oil 7</td>
<td>Bitumen - Canada</td>
<td>1.026</td>
<td>6.4</td>
<td>1.556</td>
<td>1.55</td>
</tr>
</tbody>
</table>

*API is a measure of how heavy or light an oil is compared to water. It is defined by the American Petroleum Institute as: API=141.5/SG – 131.5, where SG is the specific gravity of the oil.

** Calculated from the measured SPR angle using a 3-Layer Fresnel model.

*** Calculated from the measured critical angle of a 1550 nm wavelength beam off a SF11 glass/oil interface according to Snell’s law.

5.2.3 Optical setup

Light was coupled into surface plasmon modes using the so-called Kretschmann configuration as shown in Figure 5-1. The apparatus was placed in a shrouded room to limit external radiation. The sample holder cavity was placed in optical contact with the top face of a SF11 glass hemispherical prism (radius 35mm thickness 20mm, n_{1550nm} = 1.74, Newlight Photonics) using an index matched immersion fluid (Cargille Labs, 1815X). The light source used for interrogation was a polarized 4.5mW laser diode module (Thorlabs, LDM1550) with λ=1550nm. This wavelength was selected because of the low absorption of oil at this wavelength. The beam from this laser was passed through an aperture to select only the central region of the beam and reduce beam divergence. After reflecting off the glass/gold/oil interface, the reflected beam power was measured using a photodiode detector (Thorlabs, S122C). The laser source and photodiode detector (Thorlabs, S122C) were mounted on micrometer adjusted rotation arms and focused on the radial center of the hemispherical prism, allowing for angle interrogation of the sample. As the laser source arm was rotated, the detector arm was similarly rotated to intercept the reflected beam until the maximum reflected power reading for that particular input angle was obtained. Interrogation pro-
ceeded in this way for all angles of interest. The angle sweep was conducted three times for each oil sample studied. Before each test, the cavity was cleaned using toluene to remove the oil from the previous test, and then the cavity was rinsed with toluene/isopropanol/water in different cycles to ensure a clean gold surface. For each sample, the reflectance was measured at angle increments of 0.2 degrees. Near the angle of resonance, the reflectance was measured three more times with increments of 0.04 degrees for greater resolution in this region of interest.

5.3 Results and discussion

Determination of the refractive index of oil samples was conducted by exciting surface plasmon polaritons at the interface between a thin gold film and the oil sample. The Kretschmann configuration was used as shown in Figure 5-1a. In order for coupling of light into surface plasmons to occur, the refractive index of the coupling prism must be greater than the refractive index of the sample. Typical SPR applications which involve biological, water-based samples (n\text{visible} \sim 1.33), can make use of coupling prisms made of standard optical glass (n\text{visible} \sim 1.49-1.51). In contrast, oil samples typically have refractive indices between 1.45 and 1.56, requiring a coupling prism with refractive index greater than 1.56. Here, SF11 glass (n\text{1550nm} = 1.74) was used, with a 30 nm thick of gold layer. Likewise, SPR analysis of aqueous samples typically employs light in the visible spectrum. In contrast, crude oil samples are opaque in the visible (largely due to the presence of asphaltenes), attenuating the signal which makes visible light a poor choice for optical analysis. Here, infra-red light with a wavelength of 1550 nm was used in order to mitigate the effects of light attenuation. At this wavelength, oils and their constituent components exhibit relatively little absorption, as quantified for all samples in Fig 1b, with oils arranged light to heavy (left to right). Based on theoretical calculations using a three layer Fresnel model, using the refractive index of gold determined by Palik, the effect of oil absorption at 1550 nm is only expected to attenuate the SPR signal by < 1% with no measurable impact to the resonance angle.
PLASMONIC CHARACTERIZATION OF CRUDE OIL

Figure 5-2 | Surface plasmon resonance measurement of diluted bitumen. (a) Resonance angle measurement for Athabasca bitumen and dilutions with toluene, by volume. (b) Measured peak SPR absorption angle as a function of calculated RI, for all mixtures and the theoretical curve based on a 3-layer Fresnel reflection model.

The method was first applied to measure solvent concentration in heavy oil. Solvent addition is a common strategy to dilute crude oils for pipeline transport, as well as in the recovery of particularly heavy oils. Samples of Athabasca bitumen were diluted with toluene in ratios from 10 to 90% (v/v). The refractive index of these dilutions is expected to be a linear combination of the refractive index of toluene (1.47 – supplier reference) and bitumen (1.55 – measured) weighted by their volume proportion in the dilution. The refractive index for bitumen was measured using a classical total internal reflection technique. Figure 5-2a shows the resulting reflectance curves for each of the dilutions showing a clear resonance profile that red-shifts with increasing oil content. The angle at which the minimum reflectance occurs (θ_{spr}) indicates maximum coupling of input laser light into surface plasmon modes of the gold film. Curve fitting of the measured reflectance was performed following the approach described by Kurihara et al. to determine θ_{spr}. Notably the quality of the SPR resonance curve remains consistent over the full test range from pure toluene to pure bitumen, indicating little impact on the signal due to attenuation by the oil. Figure 5-2b shows θ_{spr} for each dilution with respect to refractive index and shows a near linear response (R²=0.9939) with a slope (∆θ/∆n) of 74. Figure 5-2b also shows the expected response based on a 3 layer Fresnel model. The model is also in close agreement with the measured values (R²=0.9932) and is therefore suitable for calculating the refractive indices of unknown samples.
Figure 5-3 | Surface plasmon resonance measurement of crude oil samples. (a) Reflectance spectra for different crude oils from around the world. Oil names and their respective API gravities are given in Table 5-1. (b) Measured peak SPR absorption angle as a function of calculated RI, for all the different crude oil types.

Figure 5-3a shows the experimental data of incident angle versus the reflectance for a variety of crude oil samples from around the world, with densities ranging from 0.825 g/cm³ to 1.026 g/cm³ for Athabasca bitumen. A full list of the oils used and their densities is given in Table 1. Classifying different types of oil by measuring refractive index is critical to assessing quality and composition changes during different stages of the recovery process. As before, curves were fit to the data in order to determine the $\theta_{spr}$ for each oil sample. The resonance angle for each sample is plotted against the refractive index of the oil in Figure 5-3b. In this case, because the refractive indices for the oils were unknown, they were calculated directly from the theoretical model. Over the range of refractive indices represented by the oil samples, a nearly linear ($R^2=0.9989$) response of $\theta_{spr}$ with respect to refractive index was observed with a slope ($\Delta\theta/\Delta n$) of 69. For comparison, the refractive indices of the oils were measured using a classical total internal reflection method in which the critical angle at 1550 nm for each oil sample was measured and the refractive index determined using Snell’s law. These values are reported for each oil in Table 1, and are all within $\pm 0.003$ RIU of the values measured using the SPR approach.
Figure 5-4 | The relationship between $\theta_{spr}$ and refractive index for both experimental and theoretical data. Insets are photographs of representative oil samples illustrating the range of fluids that can were analyzed, from toluene to bitumen.

Figure 5-4 summarizes the relationship between $\theta_{spr}$ and refractive index with an ensemble of all samples tested here. Also shown is the theoretical curve based on a three layer Fresnel model using the refractive index of gold determined by Palik. As expected, there is strong agreement between the theoretical model and the experimental data. In practice, a polynomial fit could be applied in lieu of the theoretical model. A second order polynomial fit to the experimental data gives an $R^2$ value of 0.9985.

The accurate determination of the refractive index of an oil sample is of significant value since it can be correlated to many other thermophysical values, which give insight into oil type and quality as discussed by Riazi and Roomi. Using surface plasmon resonance as the tool to measure refractive index provides greater sensitivity than traditional approaches of RI determination in a purely optical fashion. This high degree of sensitivity has already been recognized in other fields where the proliferation of surface plasmon based sensors has occurred, such as in biosensing. Even given the relatively simple apparatus used in this study, the limit of detection was 0.0006 refractive index units, limited here by the resolution of the angle adjustment micrometer which was 0.04 degrees. For the toluene dilutions shown here, this limit of detection corresponds to a concentrations of toluene in bitumen of 0.7%. Furthermore, a key advantage of this approach, particularly in the context of crude oil measurements, is the surface confined nature of the measurement. Since the resonance is sensitive only to the oil within a few hundred nanometers of the sensor surface, impurities and sediments that exist in the bulk are less likely to impact the signal once the surface of the sensor is wetted with oil. In addition, the approach developed here could be useful in detecting adsorbed and precipitated species. Since the formation of precipitates would presumably result in a change in the local refractive index near the sensor surface, the occurrence of precipitation could be de-
tected. Similarly, as in other forms of surface plasmon based sensors, adsorption of chemical species can be detected through surface functionalization resulting in a change in refractive index at the surface as adsorption occurs. The precipitation and/or adsorption of asphaltenes, particularly from heavy oils, is of great concern due to pore-blocking in reservoirs and contamination of surface facilities.

5.4 Conclusions

In conclusion, we have demonstrated the characterization of different crude oils and crude oil dilutions using surface plasmon resonance. The SPR technique was enabled through the use of a high index prism to detect oils with refractive indices between 1.44 and 1.56 with a sensitivity of up to 74° RIU\(^{-1}\). Importantly, infrared light was used to avoid attenuation of the signal by the oil, which is highly absorbing at the visible wavelengths of light traditionally used in other SPR sensors. Using this approach, a high degree of specificity between oils and oil dilutions can be achieved. The results presented here indicate a significant potential for surface plasmon resonance, as a nonintrusive, fast and simple technique for oil characterization applicable to reservoirs, surface facilities and pipelines.
Chapter 6.

Plasmonic Far-field Enhanced Growth

*This chapter was originally published in Applied Physics Letters, and has been adapted here from ref. 7 with permission. © 2012, AIP Publishing LLC. The applicant was the primary author for this work and played the primary role in experiment design, execution, data collection, data analysis, and paper writing. The efforts of all other authors are gratefully recognized, they are: Yogesh Jeyaram and David Sinton.

6.1 Introduction

In recent years, interest in mass cultivation of photosynthetic algae and bacteria has increased due to their potential usefulness in the production of biofuel, and other commercial products. Traditionally, cultivation of these organisms has been done in large outdoor ponds in order to keep costs low. One problem posed by this approach however, is that the density of suspended cells needs to be kept low to avoid large light gradients developing between the top surface, which would be over illuminated, and the bottom surface, which would be under illuminated. Uneven light distribution means only a small volume fraction of cells receive optimal light levels. Furthermore, only photons of certain visible wavelengths are absorbed for photosynthesis with the rest of the solar spectrum (UV, NIR) going unused. Consequently, typical pond facilities are limited to yields of around 35 g m$^{-2}$ day$^{-1}$ of dry biomass. While there has been significant efforts made to improve the yields of photobioreactor technology, commercial viability remains elusive and continued effort is still required. Improvements to light management in photobioreactors have followed a few themes which include new screening techniques and genetic modification to improve strain efficiency, spectral shifting to make a larger portion of the solar flux available for photosynthesis, and engineering of enclosed photobioreactors which provide avenues for tailored optics to improve the spatial distribution of broadband light.

An emerging approach to light management involves using the phenomena of surface plasmon resonance. Surface plasmon resonance of nanostructured metals has been shown to be an effective tool at controlling
the absorption, transmission and scattering of light. For instance, surface plasmon based light management has shown promise in improving light absorption by thin film photovoltaic devices.\(^9\) Recently, researchers have also begun to exploit surface plasmon resonance to enhance light collection in photobioreactors. One study has used suspensions of silver nanoparticles to scatter light back into suspensions of micro-algae and cyanobacteria.\(^{106}\) Additionally, we have demonstrated surface plasmon resonance on thin Au films as a means of coupling light energy into photosynthetic biofilms, enabling the growth of thicker and denser biofilms.\(^{5,13}\) These emerging photobioreactor designs growing dense cultures,\(^{12,104,124}\) are particularly well suited to leverage the spectral selectivity of plasmonics.
In this letter we show increased growth rate in photobioreactors by incorporating plasmonic substrates as wavelength specific reflectors as shown in Figure 6-1a. The plasmon resonance of these structures are tuned to the absorption peaks of the primary light harvesting pigments of the bacteria, *Synechococcus elongatus*, resulting in substrates that preferentially reflect light at wavelengths that match the absorption of the bacteria, and transmit light which is not (Figure 6-1b). By enhancing light scattering tuned to
the bacterial light absorption bands, we report a 6.5% increase in growth rate over untreated surfaces and 52% increase in power efficiency when compared with broadband mirrors.

### 6.2 Methods and discussion

In metal nanoparticles, incident light induces a dipole moment and at resonant frequencies the polarizability of the particle is enhanced.\(^{136}\) This enhanced dipole polarizability results in increased scattering and concentration of electric fields near the nanoparticle. The resonance wavelength of plasmonic nanoparticles can be tuned via material properties, particle geometry, particle size, and periodicity.\(^{136,250}\) Figure 6-1c shows the theoretical resonances, determined using finite difference time domain simulations, as a function of nanodisk diameter (see Section 6.4 - Supplementary Information, for finite difference time domain simulation details). In the context of photobioreactors, the wavelength at which plasmonic resonance occurs should be chosen to match the absorption spectra of the light harvesting pigments of the micro-organisms being grown. Light at other non-resonant wavelengths can then be transmitted for use by, for example, microorganisms that absorb in a different range, or photovoltaics.

![Figure 6-2](image_url)  

**Figure 6-2** | Spectral properties of plasmonic nanodisk arrays and cyanobacteria pigments.  

- **(a)** Absorption spectra for the primary light harvesting pigments in S. elongatus and the nanodisk array reflection spectrum showing good alignment between the peak reflectivity and the absorption of the pigments. Inset shows both the reflection and transmission intensity spectra of the nanodisk array normalized to incident light intensity (I<sub>0</sub>).  
- **(b)** SEM image of the nanodisk arrays and photographs of the completed array  
- **(c)** transmitting and **(d)** reflecting broadband light.
Light harvesting in the cyanobacteria used in this study, *Synechococcus elongatus*, is accomplished primarily by two chromophores, chlorophyll A and phycocyanin. The normalized absorption spectra for these two pigments are shown in Figure 6-2a. Nanodisk arrays that resonated at or near these absorption maxima were fabricated to specifically reflect photosynthetically useful light to these organisms. Based on the models shown in Figure 6-1c, square lattice arrays of nanodisks (15mm x 15mm) were fabricated with a disk diameter of ~90 nm and a period of 180nm (Figure 6-2b). Fabrication was accomplished using e-beam lithography (e-beam resist used was ZEP 520A diluted with Anisol, 1:1) on glass substrates (refractive index = 1.51, Newlight Photonics). To facilitate electron beam patterning, the glass substrates were made conductive by depositing a thin layer (~20 nm) of indium tin oxide by sputter deposition (ATC Orion 8, AJA International) and annealed at 550°C for two hours in a temperature controlled furnace. Once developed, a 30 nm Au layer was thermally evaporated with a 4 nm Cr adhesion layer (TES12D, Datacomp Electronics). Lift-off was then performed in a N,N-Dimethylacetamide bath overnight with mild sonication. The plasmon resonance wavelength of the fabricated Au nanodisk array was 623 nm as shown in Figure 6-2a, making it appear blue/green when transmitting light (Figure 6-2c) and red when reflecting light (Figure 6-2d). Reflection and transmission spectra were measured with a spectrometer (USB2000+, Ocean Optics) coupled with a tungsten-halogen source (SLS201, Thorlabs). Tuning the resonance of the plasmonic coatings to the biology in this way allows for species specific design of growth enhancing substrates.
Mei theory provides the basic understanding for how light interacts with small particles in homogenous media; how it is scattered and how it is absorbed.

Briefly, a trade-off exists between plasmonic light scattering and absorption by metallic nano-particles. It is well known that the scattering and absorption cross sections of metallic nanoparticles under resonant conditions are enhanced and in an isotropic media can be described by the equations:\textsuperscript{251}

\[
C_s = \frac{1}{6\pi} \times \left(\frac{2 \times \pi}{\lambda}\right)^2 \times |\alpha|^2
\]

\[
C_a = \frac{2\pi}{\lambda} \times \text{Im}(\alpha)
\]

\[
\alpha = 3V \times \left[\frac{\varepsilon_p/\varepsilon_m - 1}{\varepsilon_p/\varepsilon_m + 2}\right]
\]

Where \(C_s\) and \(C_a\) are the scattering and absorption cross sections respectively, \(\lambda\) is the wavelength of exciting light, \(\alpha\) is the effective polarizability, \(V\) is the particle volume, and \(\varepsilon_p\) and \(\varepsilon_m\) are the dielectric functions of the particle and surrounding media respectively. To maximize the albedo of the particles (the ratio of scattered light to the sum of scattered and absorbed light), it is advantageous to work with...
particles with effective diameters larger than 90 nm, large enough to result in significant scattering as shown in Figure 6-4, yet still much smaller than the wavelength of the exciting light avoiding losses due to dynamic depolarization.\textsuperscript{137}

![Figure 6-4](image-url)  
**Figure 6-4 | Scattering and absorption by nano-particles.** Graphs showing the scattering and absorption of light by small gold spheres in air. As particle diameter increases, the proportion of scattered light increases compared to absorbed light.

To test the effectiveness of the plasmonic reflectors, photobioreactors were constructed out of modular aluminum lens tubes (Thorlabs) which could be stacked and fastened together to facilitate integration of the required optical elements. A schematic of the modular design is shown in Figure 6-3. An array of red LEDs ($\lambda=627$ nm, FWHM 18 nm) was mounted into the top most lens tube (Figure 6-3a) and connected to an LED controller to allow independent light intensity adjustments for each photobioreactor. The LED light was directed through a collimating lens mounted in a second tube. A third tube had ports connected to a source of humidified air with 5% CO$_2$ (v/v) which was continuously flowed through the headspace. The flow rate of the CO$_2$/air mixture was set at 8 L/hr to ensure an ample supply of CO$_2$ to support photosynthesis and assist in the removal of the photosynthetically evolved O$_2$. The fourth tube provided space for the culture suspension and had the reflective substrate mounted in its base. Reflective substrates included untreated glass, nanodisk arrays, or Al mirrors. In each case, the reflective substrate was separated from the culture by a layer of protective glass. The final layer contained a piece of black cardboard to absorb any transmitted light. This modular design, (photo shown in Figure 6-3b), allowed for easy swapping and control of the substrate type, the light source and intensity, and the CO$_2$ environment. Eight such photobioreactors were constructed and mounted to a shaker table to assist in mixing and gas transport, and then placed in a temperature controlled enclosure maintained at 26°C (recommended incubation temperature for *S. elongatus*). Heating of the culture resulting from absorption and ohmic losses in the nanodisk arrays was estimated to be less than 0.5°C according to a lumped heat trans-
fer analysis described in Section 6.4 - Supplementary Information, and therefore considered to have a trivial effect on growth dynamics.

Under normal conditions, growth rate is dependent on light intensity. In these experiments, red light from an LED light source ($\lambda = 627 \text{ nm } \pm 9 \text{ nm}$) was chosen to illuminate the cultures in order to align with the absorption peaks of phycocyanin, and to a lesser degree, chlorophyll A as well as the resonance wavelength of the plasmonic structures (Figure 6-2a). By using monochromatic light instead of broadband light, the impact of the wavelength tuned plasmonic reflectors could be isolated from the impact that other wavelengths of light may have on cell growth.

![Graph showing the relationship between red LED intensity and growth culture growth rate.](image)

![Bar graph showing the impact of using both plasmonic nanodisk and reflective substrates on growth, along with the growth rates predicted by the model shown in a).](image)

Figure 6-5 | Growth enhancement from wavelength specific back-scattering. a) Graph showing the relationship between red LED intensity and growth culture growth rate. The black line is shows the fit of the light inhibition model to this growth data. b) Bar graph showing the impact of using both plasmonic nanodisk and reflective substrates on growth, along with the growth rates predicted by the model shown in a).

The growth rate of *S. elongatus* under continuous illumination with red LED’s in the experimental photobioreactors is shown in Figure 6-5a (see Section 6.4 - Supplementary Information, for stock culture
growth protocol). All data in Figure 6-5a was collected using photobioreactors equipped with glass substrates only, and each point corresponds to a separate trial. Cells were cultivated for two days. The growth rate of the cyanobacteria was calculated based on the change in optical density over the course of the experiment and represents the bulk average growth rate for the culture in the reactor. Because low cell concentrations were used, light limitation from cell-to-cell shading was not expected to be a factor. Consequently, exponential growth was assumed and the growth rate ($\mu$) calculated according to:

$$\mu = \frac{\ln \left( \frac{C_f}{C_0} \right)}{t_f - t_0}$$

Where $C_0$ and $C_f$ are the initial and final cell concentrations, and $t_0$ and $t_f$ are the initial and final times. Under low to moderate illumination, the cells are able to accommodate all the energy they absorb. For low and moderate light, increasing the intensity results in a corresponding increase in growth rate. As the light intensity continues to increase, the cell’s photosynthetic machinery becomes saturated and the benefit of additional light is diminished (Figure 6-5a). The line plotted in Figure 6-5a is from a photosynthetic growth model (see Section 6.4 - Supplementary Information, for growth model details), fit to the experimental data. As shown, the curvature indicates growth under light-limited conditions (pre-inhibition).

The same photobioreactors were equipped with substrates including untreated glass, a plasmonic nanodisk array, and an aluminum mirror. *S. elongatus* was cultivated in these photobioreactors as before under a light intensity of 36 pmolphotons m$^{-2}$ s$^{-1}$. This intensity was chosen to provide suitably high and predictable growth rates while avoiding the effects of light saturation (Figure 6-5a). Both the theoretical and experimental growth rates of the bacterial cultures are shown in Figure 6-5b with reasonable agreement between the experimentally observed growth rates and the model (see Section 6.4 - Supplementary Information, for growth model details). Plasmonic nanodisk substrates increased the growth rate by 6.5%. Predictably, the largest increase in growth rate came from introducing a broadband reflector which resulted in a 15.7% growth rate enhancement – the benchmark case for 100% reflecting surface coverage and full reflection. Notably, the plasmonic nanodisk substrate achieved 6.5% enhancement while having only 9% of its surface covered with material. Furthermore, the nanodisk substrate transmitted much light at other wavelengths (Figure 6-2a), leaving substantial energy available for other uses, such as, successive layers of photosynthetic bacteria (with different absorption spectra), or photovoltaics. In contrast,
the broadband reflector required complete surface coverage and reflected all incident light back towards the culture, sacrificing the whole light spectrum and losing a large proportion of the light out the top. By measuring the power of the transmitted light through the photobioreactors, we found that the power consumption factor between the plasmonic and broadband reflectors was 60% (\( F_P = \Delta P_{\text{nanodisk}} / \Delta P_{\text{broadband}} = 2.1 \text{mW} / 3.5 \text{mW} \)). Similarly, the growth rate enhancement factor was 92% (\( F_\mu = \mu_{\text{nanodisk}} / \mu_{\text{broadband}} = 1.6 \text{day}^{-1} / 1.7 \text{day}^{-1} \)). Normalizing the growth rate enhancement by the power consumption factors (\( F_\mu / F_P \)) shows a normalized improvement of 52%. In other words, the plasmonic reflectors use less energy than the broadband reflectors to achieve the same increase in growth. These results demonstrate that with comparatively little material (9% surface coverage) plasmonic reflectors can be constructed to enhance the growth of photosynthetic microorganisms with power efficiencies lower than broadband reflectors.

### 6.3 Conclusions

In conclusion, this work has shown that by employing plasmonic nanodisk arrays and tuning their resonant wavelength to the absorption peaks of photosynthetic organisms, photosynthesis can be enhanced. Here, a 6.5% increase in growth rate was achieved with only 9% surface coverage of Au under low light illumination and a 52% normalized power efficiency improvement over broadband reflectors. These results represent an effective photon management approach within photobioreactors.

### 6.4 Supplementary Information

#### 6.4.1 Stock Culture Maintenance Protocol

Cultures of *Synechococcus elongatus* T2SE\(\Omega\) were supplied by Professor Rakefet Schwarz of Bar-Ilan University, Israel, and incubated at 30 °C under constant illumination from fluorescent lamps. Samples were drawn from stock suspensions and diluted to an optical density of 0.02 with fresh media at the start of each experiment. The media was modified BG-11, containing solutions of: NaNO\(_3\) 1.5 g, MgSO\(_4\)\(\cdot\)7H\(_2\)O 65 mg, CaCl\(_2\)\(\cdot\)2H\(_2\)O 36 mg, K\(_2\)HPO\(_4\) 306 mg, Na\(_2\)EDTA\(\cdot\)2H\(_2\)O 1 mg, Iron (III) ammonium citrate 6 mg, Citric acid 6 mg L\(^{-1}\) sterilized by autoclave and Trace Metal Mix A5 1 mL L\(^{-1}\) sterilized by filtration, buffered to pH 8.0 with NaOH. The T2SE\(\Omega\) strain is resistant to the antibiotic kanamycin, which was added (50mg/L) to prevent growth of competitive bacteria species.
6.4.2 Surface Plasmon Resonance and Finite Difference Time Domain Simulations

In metal nanoparticles, incident light induces a dipole moment and at resonant frequencies the polarizability of the particle is enhanced. This enhanced dipole polarizability results in increased scattering, concentration of electric fields near the nanoparticle, and enhanced absorption. In the quasi-static case, for particle sizes that are much smaller than the wavelength of light, retardation can be ignored and it can be assumed that the entire particle responds simultaneously to the incident electric field. In this case, the polarizability of the nanoparticle can be written as

$$\alpha = 4\pi r^3 \frac{\varepsilon_m - \varepsilon_d}{\varepsilon_m + 2\varepsilon_d}$$

(S1)

Where $\alpha$ is the polarizability, $r$ is the radius of the particle and $\varepsilon_m, \varepsilon_d$ are the frequency dependent dielectric properties of the metal and the surrounding media. One can observe from Eq. 1, that the polarizability is dependent on the particle dimensions and frequency dependent dielectric constants of both metal and the surrounding media. When $\varepsilon_m = -2\varepsilon_d$ the polarizability approaches infinity and the nanoparticle exhibits a dipolar surface plasmon resonance. Enhanced scattering at the resonance frequency is expected since the scattering cross section of the particle is proportional to its polarizability according to:

$$\sigma_{\text{scat}} = \frac{1}{6\pi} \left( \frac{2\pi}{\lambda} \right)^4 |\alpha|^2$$

(S2)

By changing material type, substrate, particle geometry, particle size, and periodicity, the resonance wavelength of plasmonic nanoparticles can be tuned across the visible spectrum and beyond. In the context of photobioreactors, plasmonic reflectors can be used by tuning them to preferentially reflect photosynthetically useful light back towards the cell culture, increasing absorption of that light. The wavelength at which plasmonic resonance occurs should be chosen to match the absorption spectra of the light harvesting pigments of the micro-organisms being grown. Light at other non-resonant wavelengths will be transmitted and can be collected and used for other applications such as photovoltaic electricity generation or potentially even to photobioreactors growing microorganisms with a different combination of light harvesting pigments better suited to absorb the transmitted light.

Finite difference time domain simulations were performed to simulate the resonance achieved under specific geometries. The model consisted of a single nanodisk in contact with a semi-infinite substrate representing the glass. The refractive index of the substrate was set to 1.513, neglecting dispersion in the visi-
ble spectrum. The refractive index of the Au disk was set to that defined by Palik.\textsuperscript{241} Periodic boundary conditions were selected to simulate the complete array of nanodisks. For these simulations, the presence of the thin ITO layer applied during e-beam patterning is neglected. A pitch to diameter ratio of 2 was used in all simulations and held constant. Both disk diameter and thickness were varied, and the normalized reflection spectra are shown in Figure 6-1(c).

6.4.3 Photosynthetic Growth Model

Using the data of Fig. 4(a), the growth rate dependence on the LED light intensity can be approximated by a photosynthetic growth model accounting for inhibition at higher intensities\textsuperscript{253} such that:

$$\mu = \mu_{\text{max}} \times \frac{I_{\text{eff}}}{I_{\text{eff}} + \frac{I_{\text{eff}}^2}{K_1 + \frac{I_{\text{eff}}}{K_2}}}$$ (S3)

Where $\mu$ is the growth rate, $\mu_{\text{max}}$ is the maximum growth rate without inhibition, $I_{\text{eff}}$ is the effective light intensity, and $K_1$ and $K_2$ are parameters representing the lowest and highest intensity respectively at which the growth rate is equal to $\mu_{\text{max}}/2$. By finding the best fit of this function to the experimental data of Fig. 4(a), values for the parameters $\mu_{\text{max}}$, $K_1$, and $K_2$ were determined to be 4.5 day\textsuperscript{-1}, 71.4 µE m\textsuperscript{-2} s\textsuperscript{-1}, and 8374 µE m\textsuperscript{-2} s\textsuperscript{-1} respectively. The resulting function is shown in Fig. 4(a). For photobioreactors equipped with reflective bottom surfaces, the effective intensity ($I_{\text{eff}}$) can be approximated by:

$$I_{\text{eff}} = I_0 \times (1 + R \times 10^{-OD_\lambda L})$$ (S4)

Where $I_0$ is in the intensity of the LED light source, $R$ is the increase in reflectivity associated with the reflective surface, $OD_\lambda$ is the optical density of the culture at the illumination wavelength and $L$ is the depth of the culture. By combining equation 4 and 5, the growth rate enhancement for different back reflecting substrates can be estimated.
6.4.4 Plasmonic Heating Analysis

Figure S1. Lumped heat transfer model of a simplified photobioreactor.

In order to estimate the degree of heating due to absorption by the plasmonic nanodisk array, a simplified lumped model analysis was conducted, shown schematically in Fig. S1. The model assumes that the plasmonic nanodisk arrays are in direct contact with the media in the reactor and that convective heat transfer occurs directly between the water and the surrounding environment from all surfaces. With these simplifying assumptions, the change in temperature of the media can be estimated according to:

\[
\Delta T = \frac{Q_{in}}{h_c A_{surface}} \quad (S5)
\]

\[
A_{surface} = 2\left(\frac{\pi}{4} D^2\right) + \pi Dh \quad (S6)
\]

Where \( Q_{in} \) is the heat flux due to plasmonic heating, \( h_c \) is the free convective heat transfer coefficient of air, \( A_{surface} \) is the surface area of the reactor, \( D \) is the diameter of the reactor and \( h \) is the height of media in the reactor. The plasmonic heat flux is determined based on the absorption of the nanodisk array calculated from the reflection and transmission spectra according to:

\[
A = 1 - T - R \quad (S7)
\]

Where \( A \) is the absorptivity, \( T \) is the transmissivity and \( R \) is the reflectivity. Based on the spectra in figure 2a, the average absorptivity is 0.17 across the spectrum range of 400nm – 900nm. By assuming an incident solar irradiance of 625 W m\(^{-2}\) (approximately 1.25 W m\(^{-2}\) nm\(^{-1}\)) in the spectral region of 400-900 nm), the heat flux from the film due to absorption is found to be 106 W m\(^{-2}\). Using a convective heat transfer coefficient for air of 25 W m\(^{-2}\) K\(^{-1}\), a reactor diameter of 25.5 cm, and reactor height of 1cm, the steady state temperature is expected to rise by 0.2 degrees from equation S5 and S6.
Chapter 7.

Rapid, Large Area Patterning of Plasmonic Structures

*This chapter was originally published in Langmuir, and has been adapted here from ref. 7 with permission. © 2015, American Chemical Society. The applicant was the primary author for this work and played the primary role in experiment design, execution, data collection, data analysis, and paper writing. The efforts of all other authors are gratefully recognized, they are: Yogesh Jeyaram and David Sinton.

7.1 Introduction

Plasmonics is an evolving field which uses the coupling between light and free electrons in metals\textsuperscript{254,255} and 2D materials\textsuperscript{256,257} to confine light into sub-wavelength dimensions, overcoming the diffraction limit and substantially enhancing the intensity of the local electric field.\textsuperscript{258} Plasmonic nanostructures can be fabricated through a number of techniques, including electron beam lithography, laser interference lithography, focused ion beam milling, wet chemical synthesis, embossing, and annealing of thin films.\textsuperscript{259} Each technique has strengths and limitations; for instance, e-beam lithography offers unrivaled resolution but is cost- and time-intensive and therefore limited to small surface areas.\textsuperscript{259} Laser interference lithography\textsuperscript{260,261} can cover large areas quickly and inexpensively for applications including antireflective coatings and surface enhanced raman scattering detection,\textsuperscript{262,263} but is typically limited to uniform arrays of structures. Thin-film deposition and bulk annealing can also cover large surface areas with nanoparticles but provides little control over where these particles are located and how they are distributed.\textsuperscript{264} Selection of a given fabrication technique will depend on the application which can be as diverse as imaging,\textsuperscript{230,265} sensing,\textsuperscript{266,267} or energy systems.\textsuperscript{5,7,142} For many applications, the added resolution of techniques such as e-beam lithography or focused ion beam milling does not justify the added cost, particularly if large areas are to be patterned. The widespread application of nanofabricated plasmonics in, for instance disposable diagnostics, will require high throughput low cost fabrication methods.\textsuperscript{268} Methods for rapidly creating patterned plasmonic architectures that are inexpensive, fast, and do not require highly trained personnel would make plasmonic technologies accessible to a broad range of fields and users.
One simple approach to large scale and low cost fabrication of plasmonic nanostructures is to deposit a thin metal film, usually Au or Ag, onto a substrate with a low affinity for the metal such as glass or silicon.\(^{269, 270}\) With sufficiently thin films or through the application of heat, the metal film aggregates into nanostructured islands.\(^{264}\) This approach to nano-particle formation has been studied extensively and applied to larger areas, for instance, in plasmon enhanced thin film photovoltaics.\(^{271}\) Typically, the annealing process is performed through bulk heating of the substrate with a hot plate or furnace\(^ {264}\) and consequently provides no control over where on the substrate the plasmonic islands form and the degree of local aggregation. In order to achieve greater spatial control over nano-particle formation, direct heating of metal films with focused ultraviolet or visible lasers has been employed.\(^{272-275}\) Ultraviolet and visible light from YAG or eximer lasers are absorbed by the metal causing localized heating and inducing aggregation of the metal. These methods provide localized control over what part of the metal film is transformed and can achieve near diffraction limited resolution. Because of the laser systems used in these methods however, they require specialized optics and purpose built translation equipment to achieve basic levels of patternability. Such specialized hardware is not available in typical fabrication user facilities, limiting the application of this method to niche lab settings. By using the methods described here, these challenges are overcome.

Here, we present a method of direct patterning of plasmonic nano-features on glass substrates that is fast, scalable, tunable, and accessible to a wide range of users – a unique combination in the context of current nanofabrication options for plasmonic devices.\(^ {259}\) These benefits are made possible by the localized heating and subsequent annealing of thin metal films using infrared light from a commercial CO\(_2\) laser system. CO\(_2\) laser systems are common in machine shops as well as industrial and commercial facilities and are used for cutting, engraving and texturing a variety of materials. Furthermore, this type of laser has found a place in many microfluidics and optofluidics laboratories as they are useful for cutting micro-channels in a variety of polymers, and for rapid prototyping. This approach results in a patterning times of 30 mm\(^2\)/min with an average cost of $0.10/mm\(^2\). Colloidal Au nanoparticles with diameters between 15 and 40 nm can be formed on glass surfaces with x-y patterning resolutions of -180 \(\mu\)m.

### 7.2 Experimental

B33 glass substrates are cleaned in a Piranha solution (30% H\(_2\)O\(_2\) in H\(_2\)SO\(_4\), 1:3) and rinsed with copious amounts of deionized water. Au is then deposited using a table top magnetron sputter coater (Quorum Technologies, SC7640) operated at 2 kV and 20 mA with deposition times ranging from 20s-50s to control
the initial film thickness. The films are then patterned using the CO₂ laser system without further treatment.

Laser patterning is conducted using a professional 60W CO₂ laser system (Universal, M-360). Laser exposure is controlled through several parameters accessible through the included printer drivers. These parameters include laser power, laser scan speed, points-per-inch, and image density. For this study, points-per-inch was set to its maximum value of 1000, and image density set to 5 for all trials. Calibration of both the laser power and laser speed was conducted to convert between the arbitrary laser power and scan speed settings and the actual translation speeds and laser intensities respectively. Calibration curves are shown in Section 7.5 - Supplementary Information.

SEM images were taken using a FEI Quanta FEG 250 SEM operated at a pressure of 100 Pa, and 20kV accelerating voltage. SEM Images were processed using custom image analysis code to determine Au island size distribution.

AFM images were taken using a Bruker Bioscope Catalyst AFM. The probe had a tip height of 2.5-8.0 microns and a nominal radius of 2 nm according to the manufacturer’s specifications (Bruker, Scanasyst-Air). Images were captured at a rate of 0.4-0.5 Hz, with 512 samples/line over an area of 500nm x 500nm.

Extinction spectra were obtained using transmission spectroscopy with an inverted microscope equipped with spectrometer. Spectra for all pallets shown in Figure 7-2(a-d) are shown in Section 7.5 - Supplementary Information, and are reported relative to uncoated glass.

7.3 Results and discussion

Thin Au films were deposited onto B33 glass substrates via physical vapor deposition using a tabletop sputter coater. Evolution of the Au thin films on the glass surfaces proceeds according to the established Volmer-Weber mode of thin film formation, as shown in Figure 7-2a-c. During initial film formation, the Au atoms arriving at the substrate diffuse across the surface until they are trapped by a discontinuity or encounter another Au atom and bind together. This process occurs because the Au affinity for the glass substrate is lower than its affinity for other Au atoms. In this way, nucleation occurs resulting in the formation of isolated islands. As material deposition continues, these islands grow in size and begin to impinge on each other. Full coalescence will typically occur if the impinging particles are small, but as the island size increases, only partial coalesce occurs. In such cases the merging particles form iso-
lated worm-like structures in a process described by the “interrupted coalescence model”. As more material is added, these worm-like structures merge to form a percolated film morphology where all the previously isolated structures have partially merged leaving a series of isolated gaps. Eventually, the voids in the percolated structure fill as more material condenses to form a continuous film. Figure 7-1b-e shows this evolution occurring for film thicknesses up to 12 nm used in this study. AFM analysis indicates that for average deposition thicknesses starting at 7 nm, (as measured via atomic force microscopy), interrupted coalescence can be observed (Figure 7-1b-c). As the thickness increases, larger worm-like island structures form and eventually evolve into a percolated film at 12 nm (Figure 7-1e).

A commercial CO₂ laser system with a wavelength of 10 µm was applied to thin films of various thicknesses, with initial conditions shown in Figure 7-1. When the laser is focused on the sample, the laser’s energy is partially reflected by the Au thin-film and partially transmitted to the glass substrate. Because the laser frequency is well below the plasma frequency of Au, and the lowest interband transition frequency, absorption by the Au is expected to be low. Energy that is incident directly on the glass or transmitted by the Au is absorbed primarily by the glass substrate causing it to heat up and allowing for further aggregation of the Au structures beyond the room temperature equilibrium point, as shown schematically in Figure 7-1f. While previous methods of laser patterning using UV lasers rely heavily on the absorption of Au at those shorter wavelengths (owing to the interband transitions that occur around 330 nm), this method relies instead on absorption by the substrate. Using a commercial laser system also enables sim-
plified control over the amount of thermal energy delivered to the substrate. In these experiments, the beam power is held constant and the scan rate adjusted directly through the off-the-shelf printer drivers. The local laser energy flux on the material, the fluence, is controlled by varying the scan speed of the laser over the sample while keeping the laser power constant. Thus the dwell time at a given location on the sample decreases with increasing scan speed resulting in lower fluence and less overall heating. The laser beam diameter is \(~30 \mu m\), and is operated with translational speeds from 6 to 26 mm/s at a measured power of 750 mW for the 7, 9, and 11 nm films and 1500 mW for the 12 nm film. A higher power is used for the 12 nm film because its percolated structure is more robust to changes than the other films which have island morphologies, requiring additional heating to impart morphological changes.

In this study, B33 glass substrates were used because of their strong absorption of the 10 µm CO₂ laser light and their low affinity for Au which allows for the thermal annealing and dewetting of the metallic structures described above. Other combinations of substrates and metals which have similar properties (low affinity for each other and a substrate with strong absorption at 10 µm) are also potentially amenable to this method. This could include other metals such as Ag which is commonly used to form island features, or combinations of Au and Ag, and substrates such as silicon.

Figure 7-2a-d show the pallets that form by irradiating the thin films with the CO₂ laser and adjusting the scan rate to achieve a range of fluences and heating conditions. In each pallet, energy flux is increased from left to right and bottom to top by varying the scan rate. The exposure conditions were identical for each pallet, with the exception of the 12 nm percolated film which was exposed to twice the power of the other three films (1500 mW). The variation in the amount of heating imparts observable changes to the optical properties of the films, resulting in various shades of color. Figure 7-2e-p shows SEM images of the produced nanostructures, grouped here into three categories: 1) partial coalescence resulting in worm-like islands resembling the as-deposited state, 2) complete coalescence of distinct Au colloids, and 3) thermal damage to the substrate resulting in Au removal. These three regimes are mapped back to the corresponding pallets via the colored dashed lines (Figure 7-2a-d).
Figure 7-2 | Plasmonic color pallets showing the optical changes imparted to Au films of different thicknesses under CO₂ laser heating at different scan rates. For each pallet (a-d), energy flux, fluence, was increased from left to right and bottom to top by controlling the scan rate which starts at 26.9 mm/s and decreases in increments of 1.8 mm/s, while keeping beam power constant (P = 1500 mW was used for the 12 nm sample, and P = 750 mW for all others). (e-p) SEM images showing the Au island structures typical of the three different patterning regimes observed for the four different initial film thicknesses. The fastest scan rates and therefore the lowest fluences result in only partially coalesced, worm-like clusters (e-h), while increased fluence results in distinct colloids (i-k), and the thickest film did not show complete coalescence (l). At further increased fluences, thermal damage to the glass substrate is evident (m-p).

Laser processing of the 12 nm film results only in an increase in the void size (lower row in Figure 7-2) compared to the as-deposited film (Figure 7-1g-h) with no coalescence into discrete islands. Furthermore, the pallet created from the 12 nm film shows only slight plasmonic resonances (Section 7.5 - Supplementary Information) and behaves optically much like a continuous film. These results indicate that the energy required to break apart the 12 nm thick continuous network of Au into distinct islands cannot be reached without exceeding the damage threshold of the glass. Control over island size and shape therefore requires deposition thicknesses that are sufficiently small to avoid a percolated film or substrates with higher damage thresholds. Consequently, the 12 nm film thickness represents the limit here in terms of patternability and plasmonic device fabrication.
Figure 7-3 quantifies the optical and geometric properties of the 7, 9, and 11 nm thick films, as a function of scan rate, and corresponding fluence level. At the lowest exposure levels (scan rates > 20 mm/s, fluences < 77 J/m²) the films show only partial coalescence, as shown inset (and Figure 7-2e-h). That is, the films are modified from the as-deposited state but still exhibit a mixture of elongated, partially coalesced Au islands, decreasing in size as the fluence increases, as quantified in Figure 7-3a. The fluence and corresponding heating are sufficient to cause some coalescence beyond the room temperature equilibrium but are insufficient to enable complete coalescence of distinct colloids. These films nonetheless exhibit plasmonic properties, as evident in the extinction spectra (Section 7.5 -Supplementary Information) and the change in film color evident in Figure 7-2. As the scan rate decreases (or fluence increases), the geometry of the nanoparticles becomes increasingly uniform and the plasmonic resonance peaks narrow, as quantified by the FWHM in Figure 7-3b. This transition is accompanied by a corresponding decrease in the resonance wavelength in the partial coalescence regime (Figure 7-3c).
At intermediate exposure levels (fluence 77-153 J/cm\(^2\), scan rate 10-20 mm/s), complete coalescence occurs, as quantified in the middle sections of Figure 7-3(a-d). In this regime, the Au films aggregate into dispersions of fully coalesced colloidal nanoparticles and exhibit the strongest observed plasmonic effects and the most dramatic change in color (Figure 7-2). The size of the colloids produced is strongly influenced by the initial film thickness as evidenced by the average area of the nanoparticles plotted in middle region of Figure 7-3a. 7 nm, 9 nm and 11 nm Au films aggregate into particles with diameters averaging...
18 nm, 23 nm, and 32 nm respectively. Additionally, Figure 7-3a shows that for each film the average diameter of the colloids increases slightly with decreasing scan rate (increasing fluence) consistent with previous observations for annealed Au films.\textsuperscript{274,278} This change in diameter results from the higher fluences received at lower scan rates which leads to higher surface temperatures increasing the rate of surface diffusion-driven aggregation; smaller particles coalesce to form larger ones when more energy is applied.\textsuperscript{279} The change in particle size also contributes to the red-shift in resonance wavelength (Figure 7-3c – see also Section 7.5 -Supplementary Information, for complete resonance curves), consistent with observations of Au nanoparticle suspensions.\textsuperscript{280} This mechanism provides a convenient handle to tune the resonance wavelength of the film within the complete coalescence regime. Specifically, by controlling the film thickness and laser write speed in this regime, the resonance wavelength of the film can be tuned between 515 nm to 540 nm.

At higher exposure levels (fluence > 153 J/cm\textsuperscript{2}, scan rate < 10 mm/s), the films exhibit thermal damage. The glass surface temperature rises above the glass transition temperature (~ 560°C) resulting in surface deformation (Figure 7-2(n-o)), and significant fractures at the highest exposure level (Figure 7-2p). Because glass is highly absorbing at wavelengths of 10 µm, the penetration depth of the beam into the substrate is less than 50 µm causing only the surface to heat. The resulting localized thermal expansion and cooling causes permanent structural damage to the glass.\textsuperscript{281} This phenomenon is commonly exploited by CO\textsubscript{2} laser systems or fiber lasers for engraving and etching,\textsuperscript{281–284} in various materials, including glass and silicon.\textsuperscript{263} In the present context, these high exposure levels represent the upper bound for patterning, removing the Au film completely. The ability to easily select the desired patterning regime by adjusting the scan rate and fluence has implications for fabrication of plasmonic devices. For instance, for plasmonic sensing applications, narrow resonance peaks are desirable as they help to increase the precision of the instrument and determine the figure of merit (for a refractive index sensor, the figure of merit is defined as (δλ/δn)/FWHM).\textsuperscript{285} Operating within the complete coalescence regime with initial film thicknesses low enough to avoid a percolated morphology is therefore beneficial for sensing applications where narrow resonance peaks are required.

For other types of plasmonic applications, including filtering and imaging, the amplitude of the resonance is also a key parameter in addition to the width of resonance. Figure 7-3d shows the amplitude of the resonance peaks and indicates that for slower scan rates stronger coupling occurs between the incident
light and the plasmonic modes which results in greater color saturation as seen in Figure 7-2. The control of resonance peak amplitude, width, and wavelength allows for a range of colors to be patterned.

Figure 7-4 | Resolution analysis. An 11-nm thick Au film with single lines written at speeds ranging from 7.1 mm/s to 26.7 mm/s at even increments (labels for intermediate increments omitted for clarity). Thermal diffusion in the substrate leads to a widening of the affected area as shown in the inset.

The patterning resolution of this method is determined both from the beam diameter and thermal diffusion in the substrate. Because this method of patterning is based on absorption and heating of the substrate rather than the Au, thermal diffusion in the substrate causes a widening of the affected area beyond the extent of the beam spot. Figure 7-4 shows the results of writing single lines into an 11 nm thick film. Even though the diffraction limited laser spot is ~30 µm, thermal diffusion causes the average line thickness to be closer to 180 µm. The effects of the thermal gradient can be seen at the edges of the line shown inset in Figure 7-4 as the morphology of the Au film changes with temperature. Strategies for improving the resolution of the process include actively cooling the substrate or selecting a substrate with lower thermal conductivity.

To demonstrate the benefits of this method with respect to ease of processing, speed, and tunability, a photograph of the skyline of Toronto, Canada (Figure 7-5a),\textsuperscript{286} was imported into a conventional photo-editing software package and reduced to a 6 color image (Figure 7-5b). Each color was then mapped to a scan rate using the printer driver that comes standard with the CO\textsubscript{2} laser system. The image was printed directly onto a glass substrate which had been coated with an 9 nm thick Au layer. The resulting plasmonic print (Figure 7-5c) took less than 20 minutes to write and covers an area of 25 mm x 25 mm, equating to a write speed of ~30 mm\textsuperscript{2}/min.
Figure 7-5 | Demonstration of the large-area laser annealing of Au films on glass. a) A photograph of the skyline of Toronto, Canada was converted into b) a six color image file and each color was mapped to a different scan rate of the laser system. c) The final plasmonic print was written in under 20 minutes and covers an area greater than 6 cm² (30 mm²/min).

With respect to patterning expense, to produce Figure 7-5c costs ~ $20 based on typical facility fees. The total processing time from a clean substrate to the finished image is less than 45 minutes using a tabletop Au sputter coater to deposit the film, with a total fabrication cost of $65.33 ($45 Au deposition, $20 patterning, $0.33 substrate cost). By area, this total fabrication cost corresponds to $0.10/mm². The process is entirely solvent free, does not require clean-room facilities, and uses equipment that required no specialized skill to operate, making it accessible to users of all levels. These costs can be compared with the cost of other methods in research and industry. In a research context, to pattern the same area using e-beam lithography to create simple circular structures could take upwards of 25 hours for patterning alone. At typical research facility rates of $120/hr, the cost is prohibitive (on the order of $10,000 for a sample area equivalent to Figure 7-5.). In a commercial context, it is the capital equipment cost that is most relevant and the commercial laser system cost ($15,000) is ~400-fold less than that of an e-beam system. Thus for this large area patterning example, the CO₂ laser method is ~30-fold faster and ~400-fold less expensive. Other approaches such as nano-imprint lithography have been suggested as options for larger scale fabrication but still require complex and potentially expensive fabrication of the original master stamp, in addition to highly skilled personnel, specialized equipment, and cleanroom facilities. The resolution provided by e-beam and nano-imprint lithography is essential in some applications, but where the spatial patterning resolution threshold is lower, commercial laser system processing as demonstrated here provides a fast and cost-effective alternative. Furthermore, scale-up is easily accommodated since turn-key CO₂ laser solutions are widely available and customizable to all kinds of industrial applications.
Plasmonic nanotechnology has been identified as an avenue for achieving the World Health Organizations criteria for the ideal diagnostic test,\textsuperscript{268,289} which lists affordability as the first criteria. To this end, early examples of disposable paper based devices using plasmonic nanoparticles for surface enhanced Raman based assays have been demonstrated.\textsuperscript{290–292} In the absence of cost effective and rapid fabrication alternatives, these methods have instead used nanoparticle dispersions supported by a paper matrix to enable low cost fabrication. By using the method described in this work to fabricate plasmonic patterns for devices with an active area of -5-10 mm\(^2\), over 65 devices could have been fabricated for the time and cost spent to print Figure 7-5. The cost for the plasmonic components would be less than $1 per device, enabling low-cost, mass produced, disposable plasmonic devices. It should be noted that in the absence of an adhesion layer the Au films can be susceptible to damage. Strategies to prevent Au removal and increase device stability have been suggested in literature including pre-treatment of the glass surface with silanizing agents to promote adhesion,\textsuperscript{293} or the addition of an ultrathin layer of protective silica.\textsuperscript{294}

### 7.4 Conclusions

In conclusion, we demonstrated an approach to creating plasmonic patterns from Au thin films using a commonly available CO\(_2\) laser system. This method fills a gap in fabrication techniques for applications requiring fast and low cost patterning of plasmonic nanostructures. Patterning speeds of 30 mm\(^2\)/min and patterning costs of $0.10/mm\(^2\) are demonstrated which are ~30 fold faster and ~400 fold less expensive than e-beam lithography. While the spatial resolution is determined by the diffraction limited beam spot diameter (~30 µm), the translation hardware of the laser system (~50 µm /steps), and thermal diffusion in the substrate (~180 µm), it is more than sufficient for many plasmonic applications that require active areas on the scale of microns to millimeters such as handheld diagnostics and optofluidics. Lastly, the method presented here is amenable to a wide range of users and skill levels owing to the simple processing steps and out-of-the-box patterning functionality of the widely available laser system. These strengths set this approach apart from previous methods of laser patterning making the production of island film plasmonic devices fast, simple, and accessible.
7.5 Supplementary Information

Figure S1: Extinction spectra for plasmonic color pallets of Figure 2 (shown as insets) for the a) 7nm, b) 9nm, c) 11 nm and d) 12 nm films. The color legend represents laser scan rates from 0.4% to 1.5% in steps of 0.1%. The actual translation speed for the minimum and maximum speeds is given in brackets (mm/s) in each legend as determined from the calibration curve in Figure S2a. The intermediate speeds can be similarly determined but have been omitted for clarity.
Figure S2: Calibration curves for a) the laser scan speed setting and b) the laser power setting.

Table S1: Standard deviation of particle areas

<table>
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<th>Average particle area [nm²]</th>
<th>Standard deviation of particle areas [nm²]</th>
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<td></td>
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<td>9-nm film</td>
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Chapter 8.

Conclusions

8.1 Summary
Achieving greater productivity from the light available to microalgae cultures requires creative approaches to address the central challenges of light distribution and utilization – i.e. how to make as many photons available for photosynthesis as possible and supplying these photons to cells at an optimal. This thesis has examined the role that light wavelength plays in concert with conventional photobioreactor design parameters (culture density, path length, light intensity) in distributing light within a photobioreactor. By using wavelength specific phenomena such as plasmonic resonances, new methods for manipulating light within photobioreactors are made possible. Two such approaches have been described. The first utilizes near-field phenomena to confine light to the surface of a metallic film to cultivate high-density biofilms. Co-locating the light source and the biology allows for cell-scaled confinement and control over light delivery. The second productivity enhancing approach uses far-field plasmonic scattering from nanodisk arrays to scatter photosynthetically useful light back towards low-density suspended cultures, increasing the light-path length in the reactor and consequently increasing absorption.

These methods demonstrate wavelength sensitive and plasmonic approaches to improving productivity in microalgal cultures, but cost-effective manufacturing is also a requirement for plasmonic technology to be viable. With this in mind, a method to rapidly and inexpensively fabricate patterned 2D plasmonic surfaces was presented which utilized a scalable, user-friendly, and commonplace CO₂ laser. In addition, an approach to classify and characterize complex samples using plasmonic phenomena was presented which enables convenient analysis of difficult to manage products. The results presented here demonstrated this sensing approach to conventional crude oil and crude-oil dilutions. Nevertheless, the applicability of this characterization approach to algal products and bio-crude oil is evident and allows for highly sensitive, all-optical characterization – a critical component in any production process.
8.2 Future Outlook

As discussed in Chapter 2 - Photon Management for Augmented Photosynthesis, efficiently converting sunlight into chemical energy is a key challenge for the production of commercially viable bio-products from microalgae, in particular for low margin fuels. Incumbent global and regional energy systems are entrenched with decades and even centuries of experience, innovation, investment and infrastructure. The unprecedented success of fossil fuels puts a tremendous burden on emerging technologies in a market that is not fully motivated to seek alternatives.

Looking forward I see three major opportunities for integrating the best photon management strategies in developing photobioreactor operations.

First, advanced materials for customizing the spectrum of light incident on microalgal cultures will enable production of specific bio-products to be preferentially expressed. Plasmonic phenomena offer a variety of compelling benefits to such operations, allowing for wavelength level management of photons as discussed in Chapter 4 - Plasmonic Near-Field Enhanced Growth, and Chapter 6 - Plasmonic Far-field Enhanced Growth. So far, these effects have been studied in a relatively small number of organisms with additional work needed to understand the effects of spectral quality in a wider range of species. Scale-up of these proof-of-concepts will require further study and optimization of light quality on growth and metabolite expression. Work such as that presented in Chapter 3 - Spectral Light Dilution, will help provide vital insight towards maximizing the output of commercial facilities, particularly in cases where light quality can be managed. Additionally, further attention to cost and energy losses within photobioreactor systems need to be quantified, at both lab and commercial scales. Plasmonics has not traditionally excelled in the areas of cost and loss, owing to material and fabrication expenses and inherent ohmic losses. However, fabrication methods such as the one presented in Chapter 7 - Rapid, Large Area Patterning of Plasmonic Structures, can provide routes to precise large-scale device fabrication which will reduce cost and make fabrication accessible to a larger cross-section of users and manufacturers.

Second, a clearer understanding of the economics of microalgal cultivation will become available by integrating the design of cultivation schemes with enabling infrastructure including sources of carbon and nutrients such as flue gas or municipal waste systems. This type of integration and context-specific assessment will quantify the ancillary benefits of mitigating and utilizing waste streams, adding value to the microalgal cultivation process. Further integration and development of on-site biomass processing tech-
nology such as hydrothermal liquefaction (see Appendix A1 - Biomass-to-biocrude on a chip via hydrothermal liquefaction of algae) can provide insight into the full life-cycle efficiency of microalgal biofuels.

Third, better coordination and partnerships between pilot scale plant developers/operators and academia will avail the latest developments to industry, provide for more meaningful data collection and reporting, and focus the efforts of academia on the specific constraints affecting the success of early pilots. This will stimulate cross-disciplinary innovation resulting in ancillary technology development, such as the sensor described in Chapter 5 - Plasmonic Characterization of Crude Oil. This sensor can be applied both to conventional energy applications with established markets and to emerging alternative fuel applications which will benefit from the accelerated infrastructure development due to academic collaboration with established industries.

Finally, many exciting options exist for increasing photosynthetic efficiency and productivity at the front end of the photosynthetic process using nano-technology, a critical first step toward large scale industrial processing. The markets open to microalgal products are vast and diverse. Low-volume high-value bio-product markets are the best initial target with biofuels being the ultimate long term high-volume market. Collectively these opportunities motivate near-term advances in all aspects of cultivation with efficient photon management being a priority for augmenting photosynthesis.
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Appendices

The following two appendices are reprints of papers for which the applicant was co-author and that have particular relevance to this thesis. While they are not to be considered part of the core contributions of this thesis, they are nevertheless worthwhile considering since they provide additional breadth to the discussion of microalgal bioenergy.
A1. Biomass-to-biocrude on a chip via hydrothermal liquefaction of algae

Authors: Xiang Cheng, Matthew D. Ooms and David Sinton

*This appendix was originally published in Lab-on-a-chip, and has been adapted here from ref. 11 with permission from The Royal Society of Chemistry. The applicant was a co-author on this work and played the supporting role in experiment design, execution, data collection, data analysis, and paper writing. The efforts of the first author, Xiang Cheng are particularly noted, as are the contributions of co-author and principal investigator, David Sinton.

Hydrothermal liquefaction uses high temperatures and pressures to break organic compounds into smaller fractions, and is considered the most promising method to convert wet microalgae feedstock to biofuel. Although, hydrothermal liquefaction of microalgae has received much attention, the specific roles of temperature, pressure, heating rate and reaction time remain unclear. A microfluidic screening platform to precisely control and observe reaction conditions at high temperature and pressure. In-situ observation using fluorescence enables direct, real-time monitoring of this process. A strong shift in the fluorescence signature from the algal slurry at 675 nm (chlorophyll peak) to a post-HTL stream at 510 nm is observed for reaction temperatures at 260°C, 280°C, 300°C and 320°C (P = 12 MPa), and occurs over a timescale on the order of 10 min. Biocrude formation and separation from the aqueous phase into immiscible droplets is directly observed and occurs over the same timescale. The higher heating values for the sample are observed to increase over shorter timescales on the order of minutes. After only 1 minute at 300°C, the higher heating value increases from an initial value of 21.97 MJ/kg to 33.63 MJ/kg. The microfluidic platform provides unprecedented control and insight into this otherwise opaque process, with resolution that will guide the design of large scale reactors and processes.

Microalgal biomass is an attractive feedstock for the generation of carbon neutral biofuels because of the high growth rate and lipid content of many microalgae species.  Conversion of the raw algal biomass
into biocrude however, remains an energy intensive\textsuperscript{295} and costly process\textsuperscript{296} which is in part why algal biofuels have not yet achieved commercial success.

To produce biodiesel from microalgae using conventional methods, the fatty components of the cells (lipids), must first be extracted and then converted to biodiesel via transesterification. Traditionally this is accomplished through mechanical pressing or solvent extraction. A challenge for both these approaches is that the biomass must first be dried, which significantly increases the carbon intensity of the final fuel as well as its cost.\textsuperscript{297} From a life-cycle assessment perspective, drying of the biomass can consume more than 90\% of the energy content of the final algal oil.\textsuperscript{298} Consequently, the potential benefit of microalgal biofuels is severely undermined by the high energy and financial cost associated with biomass-to-biofuel conversion.

An emerging conversion approach that does not require pre-drying is hydrothermal liquefaction (HTL).\textsuperscript{299–301} HTL uses high temperatures and pressures to break organic compounds into smaller fractions to produce biocrude which can be further upgraded into a variety of fuels. Optimizing the many parameters involved in HTL processing, however, remains a challenge. To date, HTL experiments have been conducted in laboratory scale batch reactors using sand-baths, ovens or heating coils for temperature control.

![Figure A1-1](image-url)  
**Figure A1-1** | a) Schematic of hydrothermal liquefaction of microalgae in the microfluidic chip with \textit{in-situ} observation of biocrude production using fluorescence microscopy. b) Distinct fluorescence signatures of algae slurry at the inlet and biocrude at the outlet.
control. These approaches have two critical issues. First, due to the large fluid volumes and large physical size of the apparatus, substantial heating times are required, which are often ignored in the subsequent analyses. These long heating times lead to ambiguity in the reported optimal values for temperature, heating rate, and reaction time since the heating and pressurization delays blur the results. Second, in-situ monitoring of the reaction process is not possible in current reactors, precluding real-time quantification of the reaction process.

Microfluidic and lab-on-a-chip methods have recently been applied to bioenergy generation, particularly with respect to microalgae. The cellular scale of microalgae makes them well suited to manipulation and analysis using microfluidic platforms. Ensuring cells receive both light and fluids is an optofluidic challenge that has been addressed, for instance, with integrated waveguides to deliver light to cultures and micro-reactor arrays integrated onto individual LCD displays for parallelized illumination and growth studies. These efforts have focused primarily on cultivation of microalgae, but have not addressed the downstream challenge of converting biomass into useful products.

In this work, we demonstrate a high temperature and high pressure, continuous flow, microfluidic reactor to perform controlled HTL on a glass and silicon chip (Figure A1-1a). The small length-scales of the microfluidic chip enable effectively immediate heating of the algal slurry eliminating the ambiguity associated with conventional reactors. This work leverages established advantages of using high-pressure high-temperature silicon-glass microfluidic reactors, for bioenergy applications. Our microfluidic chip makes possible real-time in-situ observation including fluorescence imaging and analysis as shown in Figure A1-1. The fluorescence signatures from both chlorophyll in algae and aromatics in the produced oil provide direct indicators of chemical composition during the reaction.

The chip was fabricated out of glass and silicon because common chip materials such as polydimethylsiloxane (PDMS) and polymethacrylate (PMMA) cannot support the high temperatures and pressures used in HTL and metal-based chips do not permit in-situ observation. The combination of silicon and glass allowed for (i) optical access through the glass, (ii) high thermal conductivity through the silicon, and (iii) both high pressure and temperature (over 10 MPa and 300°C respectively).

The biomass injected into the chip consisted of Nannochloropsis oculata with an ash content of 5.9% (Reed Mariculture Inc.). The as-received biomass was cleaned through centrifugation and suspended in DI water prior to use. Algal slurry at a concentration of 2 wt% was then injected into the chip using a high
pressure pump with constant flow rate. This concentration was chosen in order to minimize clogging and fouling issues while still providing enough material to allow real-time observation of the chemical reactions on-chip and for subsequent off-chip analysis. An ultra-low flow, back pressure regulator (Equilibar Inc.) was used at the outlet to maintain steady flow at a constant pressure. It was essential to minimize dead volume downstream of the chip in order to collect representative samples for off-chip product analysis. Here, a separate fixed-volume sample collector loop (300μL) was incorporated into the outlet stream, immediately downstream of the chip and upstream of the backpressure regulator. The outlet line was switched using a 6-port valve with minimal dead volume (Rheodyne®7030). When isolated, the contents of the collector loop could be dispensed into a collection vial without depressurizing the entire chip. This enabled inline sampling directly from the output stream (see full system schematic in the ESI†).

![Schematic representation of the assembly of the water-cooled manifold, temperature controlled heating chuck and the microfluidic chip using a separation glass and double seal O-ring to prevent cracking from hard contact.](image)

Figure A1-2 | Schematic representation of the assembly of the water-cooled manifold, temperature controlled heating chuck and the microfluidic chip using a separation glass and double seal O-ring to prevent cracking from hard contact.

The heated region of the chip was mounted in a temperature controlled stainless steel heating chuck, as shown in Figure A1-2. Precise temperature control was accomplished through a proportional-integral-derivative temperature controller with three cartridge heaters maintaining a constant temperature over the entire heating region of the chip. A thermocouple inserted in the heating chuck provided closed-loop feedback and kept the temperature variation to less than +/- 1°C during steady state operation at a reaction temperature of 300°C. The heating chuck was also equipped with a borosilicate glass viewing window which enabled in-situ observation of the HTL reactions that occurred in the channel. The chip manifold was maintained at a lower temperature (~ 50°C) to (i) prevent O-ring material failure and leakage at the
ports and (ii) to allow rapid and controlled on-chip heating as the fluid transitioned from the cold to the hot zone (Figure A1-2).

The square cross-section channel dimensions were 200 µm x 200 µm with a total length of 1320 mm, 1250-mm of which were located in a serpentine heating region as shown in Figure A1-1a. Raw algae slurry was continuously pumped into the chip at a flow rate of 5 µL/min at 12 MPa. At this rate, the slurry required 10 min to flow through the length of the heated region. Under steady state flow, the reaction time (time spent in the heated region) at a given location along the channel could be calculated. The heating rate of 15°C/s, the fastest reported heating rate for HTL experiments in literature, was calculated based on the 35 mm length of channel between the inlet at 50°C and the beginning of the heating region at 300°C.
During operation, the chip was monitored with a fluorescence microscope for changes in the fluorescence signature of the algal slurry. The sample was excited with ultraviolet light ($\lambda = 375-400$ nm) and fluorescence detected through a long-pass filter ($\lambda > 405$ nm) using a spectrofluorometer (Ocean Optics USB2000) attached to the microscope. Algae slurry was continuously pumped through the chip for 15 min to achieve steady state. By observing the fluorescence signature at different points along the channel (Figure A1-3a), the progression of the HTL process as a function of time spent in the reactions chamber was quantified.
Here, a series of viewing points along the channel were chosen to correspond to reaction times at 0.4-min intervals, ranging from 0 to 10 min. Near the inlet, raw algal slurry mainly showed chlorophyll fluorescence with a peak at 675 nm (Figure A1-1b). By 1.2 min, the chlorophyll peak had significantly dropped and an emerging peak at 510 nm began to rise. Over the next 2 min, the 510 nm peak became dominant and continued to grow. After 10 min, the original chlorophyll peak was no longer visible and the normalized peak intensity at 510 nm approached a saturation point as shown in Figure A1-3b. The evolution of the peak at 510 nm indicated the formation of aromatic compounds which are a characteristic component of crude oils as well as other processed plant based oils. The progression of the fluorescence signature from one dominated by chlorophyll fluorescence to one resembling conventional crude oils tracked the progression of the HTL conversion process.

The effect of reaction temperature was investigated by performing identical experiments at 260°C, 280°C, 300°C and 320°C. For each temperature, the normalized peak intensity at 510 nm over the course of the reaction is shown in Figure A1-3b. The fluorescence signals were normalized to the projected saturation intensity based on a 1st order exponential curve fit to the experimental data. The results in Figure A1-3b clearly show that higher reaction temperatures resulted in higher reaction rates. Specifically, the characteristic times (time required for the fluorescence intensity at 510 nm to reach 63% of its maximum value) were: 6.0 min, 4.6 min, 3.4 min, and 1.9 min for the reactions run at 260°C, 280°C, 300°C, and 320°C respectively.

Produced samples were collected off-chip for analysis of their higher heating values (HHV) by isolating the reaction products in the sample collector and eluting them to small glass vials. The product at the outlet had a variety of components including water-soluble compounds and biocrude, with other smaller amounts of solid particulates and gas. To isolate the biocrude, 2 mL of dichloromethane was added to the recovery vial followed by vigorous shaking for several minutes, completely dissolving the biocrude into the dichloromethane. The vials were then set aside to allow complete phase separation. Once separated the dichloromethane layer was withdrawn using a glass syringe and stored in a separate vial. This extraction process was performed multiple times to ensure more than 95% of biocrude was recovered from the sample. The extracted biocrude was then heated in an oven at 40°C for 8 hours to remove the solvent.

The higher heating values of each sample were calculated using the modified Dulong’s formula as follow:

\[
HHV \ (MJ/kg) = 0.335C + 1.423H - 0.154O - 0.145N
\]
A carbon-hydrogen-nitrogen elemental analyser was used to measure the carbon (C), hydrogen (H), nitrogen (N) composition of the initial dry algae sample and the produced biocrude. The oxygen (O) content was estimated according to:

\[ \%O = 100\% - \%C - \%H - \%N - \%Ash \]

where the sulphur content is assumed to be negligible, as is typical for microalgae.\textsuperscript{314,315} Prior to elemental analysis, the raw algae was dried in an oven at 105°C for an hour.

The measured HHVs of dry algae and biocrude are shown in Table 1 and correspond well with previously reported values\textsuperscript{316–318} using similar microalgae species and reaction conditions. As shown, the most significant increase in HHV occurred within the first few minutes of the reactions. Beyond reaction times of 1 minute, the variation of HHV was less than 5%.

<table>
<thead>
<tr>
<th>Table A1-1: Elemental composition and Higher Heating Value of dry algae and biocrude from 1 min, 5 min and 10 min reaction times.</th>
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</thead>
<tbody>
<tr>
<td>Element</td>
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<tr>
<td>C [%]</td>
</tr>
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<td>H [%]</td>
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<tr>
<td>N [%]</td>
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<tr>
<td>O [%]</td>
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<tr>
<td>HHV [MJ/kg]</td>
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</tbody>
</table>

Combined with the fluorescence data collected, this analysis of HHV suggests that while the energy content of the biocrude approached saturation at very early times (~ 1 min), other reactions continued to occur that were not accounted for by the HHV alone. The sharp increase in HHV during the first minute of the reaction was likely due to rupturing of the cells which made lipid extraction by dichloromethane more efficient.\textsuperscript{319} As such, the increase in HHV should be used as an indicator of mechanical disruption rather than chemical conversion which can be observed more directly with the fluorescence signal.
Direct observation of the channel during operation provided additional insight into this process. Most notably, non-fluorescent droplets were observed forming and adhering along the length of the channel and increased in size towards the outlet (Figure A1-4a). These droplets are expected to be comprised of aliphatic compounds which are immiscible with water and do not fluoresce. The formation of these droplets progressed along the length of the channel which suggests a correlation between the formation of immiscible oil droplets and the parallel change in fluorescence signature resulting from the formation of aromatic compounds in the aqueous phase. Additionally, the degradation of the microalgae could be observed between the inlet where whole cells were clearly visible and the outlet where individual cells were no longer discernable (Figure A1-4b).

It was also observed that a significant amount of cell debris and solid particulates (and in some cases, clogging) resulted from operating with short reaction times. Specifically, we observed that at 320°C when the flow rate was increased such that the maximum reaction time was only two minutes, a significant amount of solid debris remained in the effluent. In contrast, less solid debris in the output was observed for longer reaction times achieved with lower flow rates and otherwise similar conditions. This finding is
likely a result of more complete disruption of the biomass during the first few minutes of the reaction (also indicated by the increase in HHV described earlier). Furthermore, clogging at the outlet was exacerbated by the rapid cooling of the effluent which promoted the separation of the oil phase from the aqueous phase, which was directly observed as an increase in the number and size of oil droplets in the cooled outlet line (Figure A1-4a). Lastly, the multiphase nature of the generated products, visible as channel-adhered droplets in Figure A1-4, will influence to some extent the residence time, accumulation and ultimate production of different components. The HHV analysis here, however, is largely unaffected by this issue as the oil is separated from the produced fluid and based on the relative elemental composition of the biocrude. These observations, which were made possible only through the direct visualization afforded by our chip design, have implications for the optimum processing parameters of continuous flow HTL reactors. Specifically, these findings indicate optimal reaction times between 2 and 10 minutes to both maximize the conversion of biomass to biocrude, and minimize the amount of debris in the effluent to prevent fouling. Gradual cooling of outlet stream is also recommended to avoid clogging.

In summary, our microfluidic reactor provides unprecedented insight and control over the high temperature and high pressure cracking of biomass via hydrothermal liquefaction. It allows for in-situ observation of hydrothermal liquefaction reactions using fluorescence microscopy and convenient and precise control of reaction temperature, pressure and reaction time in a continuous flow reactor. These advantages enable the study of high temperature and pressure cracking of biomass on a platform with a high degree of control which will allow improved understanding of the reactions taking place during hydrothermal liquefaction. The significant change of fluorescence signature between the algal slurry (peak at 675 nm) and converted biomass (peak at 510 nm) was observed as an indicator of the progression of hydrothermal liquefaction. Biocrude formation and separation from the aqueous phase into immiscible droplets was directly observed and occurred over timescales of ~10 min. The rapid increase of higher heating values was observed over the timescales of ~1 min and was correlated to observations of particulate matter in the effluent which manifested as partially clogged channels. These results and the microfluidic platform on which they were collected represent the first of their kind in the field of hydrothermal liquefaction research. Lab-on-a-chip methods offer a unique toolset to probe high temperature and high pressure reaction dynamics and inform large scale reactor design.

This work was supported through a Strategic Grant from the Natural Science and Engineering Research Council of Canada, the University of Toronto Connaught Global Scholars Program in Bio-Inspired Ideas
for Sustainable Energy, the University of Toronto McLean Senior Fellowship (DS), and the Vanier Canada Graduate Scholarship (MO). CHN analysis was performed by the Analest facility at the University of Toronto. Fabrication was performed at the Toronto Nanofabrication Centre and the Centre for Microfluidic Systems in Chemistry and Biology at the University of Toronto. Ongoing infrastructure support from the Canadian Foundation for Innovation is also gratefully acknowledged.
A2. A photosynthetic-plasmonic-voltaic cell: Excitation of photosynthetic bacteria and current collection through a plasmonic substrate

Authors: Nathan Samsonoff, Matthew D. Ooms and David Sinton

*This appendix was originally published in Applied Physics Letters, and has been adapted here from ref. 13 with permission, © AIP Publishing LLC. The applicant was a co-author on this work and played the supporting role in experiment design, execution, data collection, data analysis, and paper writing. The efforts of the first author, Nathan Samsonoff are particularly noted, as are the contributions of co-author and principal investigator, David Sinton.

Excitation of photosynthetic biofilms using surface-confined evanescent light fields enables energy dense photobioreactors, while electrode-adhered biofilms can provide electricity directly. Here we demonstrate concurrent light delivery and electron transport through a plasmonically-excited metal film. Biofilms of cyanobacterium Synechococcus bacillaris on 50-nm gold films are excited via the Kretschmann configuration at $\lambda = 670$ nm. Cells show light/dark response to plasmonic excitation and grow denser biofilms, closer to the electrode surface, as compared to the direct irradiated case. Directly irradiated biofilms produced average electrical powers of $5.7 \mu W/m^2$, and plasmonically excited biofilms produced average electrical powers of $5.8 \mu W/m^2$, with individual biofilms producing as much as $12 \mu W/m^2$.

Microalgae show promise as a renewable feedstock for biofuel production due to their high growth rates, simple nutrient requirements, low demand for arable land and amenability to genetic manipulation.1-4 In spite of this potential, the commercial development of microalgal biofuels has been limited by low operational density and associated high facility costs.5 Enclosed photobioreactor systems have achieved productivity increases over pond systems, though with additional capital and maintenance costs.1,6 Growing dense biofilms, as opposed to cells in suspension, is one route to increased system density.7-9 Evanescent light fields at the surface of waveguides have been recently employed to deliver light directly to waveguide-absorbed biofilms,10,11 providing a route to greatly increased photobioreactor density.12 In ad-
dition, excitation of a biofilm on a metal film has been achieved using surface plasmon resonance\textsuperscript{13} – an enhancement of the evanescent field at a metal/dielectric interface.

A complementary approach to microalgal biofuel production is direct electricity production using the charge transport system inherent to the photosynthetic process.\textsuperscript{14} This approach has its roots in microbial fuel cells and has led to the field of biophotovoltaics (BPV).\textsuperscript{15,16} The key difference between microbial fuel cells and BPVs is that the photosynthetic organisms used in a BPV device harvest energy from light as opposed to chemical sources. A BPV operates by using photosynthetic organisms as biocatalysts to split water through routine photosynthesis producing hydrogen ions, molecular oxygen and high energy electrons. By inserting an anode and cathode nearby, these electrons can be harvested.\textsuperscript{17} Recently BPVs have been demonstrated using prokaryotes\textsuperscript{18–20} and eukaryotes\textsuperscript{16} employing cell components,\textsuperscript{18,21} whole cells,\textsuperscript{15,18,20,22,23,24} and entire plant systems;\textsuperscript{25–28} both with\textsuperscript{18,20} and without\textsuperscript{15,20,22} electron mediators. Photosynthetic biofilms cultured directly on electrodes enable rapid electron transport in the absence of electron mediators\textsuperscript{15,20,22} – a preferred approach due to mediator toxicity and cost.\textsuperscript{29,30} A challenge in developing/scaling these BPV technologies is achieving effective transmission of both light and electrons at the cellular level. Ideally, light energy would be delivered precisely where current is collected.

In this work we demonstrate an approach to bio-electricity production using a metal film for both photosynthetic excitation through plasmonic light delivery and current collection – a photosynthetic-plasmonic-voltaic (PPV) cell. Light energy was coupled into surface plasmons in a 50-nm thin gold film which also supported the biofilm in a single-chamber, mediator-less cell. The same gold layer was thereby used to confine light energy through surface plasmon excitation to the biofilm growth surface and was also used for current collection.
Figure A2-1 | The photosynthetic-plasmonic-voltaic (PPV) cell (a) Schematic showing the combined light delivery and current collection through a plasmonic metal film (b) Diagram of PPV device and plasmonic excitation scheme. (c) PPV device-level function showing electron generating reactions.

The concept of the photosynthetic-plasmonic-voltaic (PPV) cell is illustrated in Figure A2-1. As shown in Figure A2-1a, the collection of electrons is accomplished through the same metal film used to excite the plasmonic resonances, collocating photosynthetic excitation and electron collection. Figure A2-1b shows a schematic of the device. Surface plasmons are excited on a 50-nm thick gold film via prism coupling of a beam from a laser diode module ($\lambda=670\text{nm}$). The resulting enhanced evanescent field extends into the culture media. Cyanobacteria cells adjacent to the film are photosynthetically excited by nearfield coupling with the evanescent light field and associated farfield scattering. These cells use the light to energize electrons which are passed along the electron transport chain driving the photosynthetic machinery of the cell. Some electrons are intercepted and redirected through an external circuit for power production, as shown in Figure A2-1c. While the phenomena of direct electron transport out of the cell is well established, the exact mechanism by which it occurs is a topic of ongoing investigation. It has been reported that direct electron transport occurs through surface bound redox proteins and/or naturally present electron mediators.
To demonstrate the feasibility of this approach, plasmonically excited biofilms were cultivated in the PPV device pictured in Error! Reference source not found.. All devices consisted of a platinum coated carbon paper cathode (0.5 mg/cm², FuelCellStore, area=75 mm²) and a 50-nm gold-on-glass microscope slide (AU.0500.ALSI, Platypus Technologies, area=225 mm²) anode in a polymethylmethacrylate chamber (volume=1125 μL). They were inoculated with a high density suspension of Synechococcus bacillaris (CCAP WH5701) (OD750=0.54). The inoculated devices were then irradiated by prism coupling of a 4.5 mW laser diode light source (λ=670 nm, CPS198, Thorlabs), into resonant surface plasmon modes from below (Kretschmann configuration) using a range of intensities, and placed in a dark enclosure. The resulting 100 mm² irradiated area is shown in Figure A2-2a. The PPV devices were then allowed 24 hours
for cell settling and biofilm initiation. Figure A2-2b plots the resulting voltage response due to photosynthetic electron generation for different field intensities measured across a 1MΩ external load. This resistance was chosen such that the device was operating near its peak power density. Voltages were recorded using a USB compatible digital multimeter (5492B, B&K Precision, internal resistance > 10 GΩ) at a sampling rate of 10 seconds per reading. The cells show rapid light/dark response to plasmonic excitation with an average step increase of 60 µV/(W/m²) (anode surface area and total absorbed light power). At maximum irradiance, the output voltage was 13.7 mV, 10% higher than the unirradiated PPV device. Importantly, these results show the viability of simultaneous light delivery to cyanobacteria and current collection via a plasmonic metal film.

Figure A2-3 | (a) Time-domain light response of S. bacillaris and culture media control under direct irradiation showing positive voltage response with increased light intensity. Near-instantaneous drops in voltage output are observed when the light source is turned off. Voltage was sampled every ten seconds. (b) Steady-state voltage output with zero light intensity set to zero volts of S. bacillaris and cell culture media under a range of direct light intensities. Error bars are 95% confidence intervals.
For comparison with the plasmonically excited PPV device performance, light response for direct irradiation are shown in Figure A2-3a, using the same configuration as before but with light from a tungsten-halogen bulb (60 W/m²). Figure A2-3a shows a consistent increase in voltage output of approximately 67 µV/(W/m²) (anode surface area) each time the light is turned on with a corresponding decrease when the light is turned off. Although small, the culture media (50% v/v BG11 (C3061, Sigma-Aldrich) and ASW (Instant Ocean®, Spectrum Brand)) also shows a small and repeatable light response. While there may be a number of weak electrochemical and/or transport aspects involved in this media response, we expect the main contributor is optically-induced thermal mixing of the near-electrode solution resulting in a small net increase in electrochemical activity. It is noteworthy that the timescale for the response to light and dark ( ~3 min and ~1 min, respectively) is similar in both the plasmonic and direct cases.

To ensure that the observed light response was a result of photosynthesis, the average steady-state voltages achieved by a bacteria suspension under different light intensities were compared to that of sterile growth media, as shown in Figure A2-3b. The steady-state exoelectrogenic activity of *S. bacillaris* and sterile culture media are plotted against incident light intensity and normalized to voltage output under no irradiation. The increasing photosynthetic activity of *S. bacillaris* with increasing light intensity results in an increased voltage output as the rate of electron production from photosynthesis increases. At the maximum irradiance provided, the change in voltage output was approximately five times greater than the voltage change in the unirradiated culture. Collectively these results affirm (1) the voltage responses observed are attributable to increased photosynthetic activity, and (2) the efficacy of concurrent plasmonic excitation and electron collection through a metal film.
Figure A2-4 shows a plot of cell coverage density as a function of distance from the anode surface for the four different growth conditions: unirradiated (dark), directly irradiated (direct), directly irradiated without a connection between the electrodes (direct open circuit) and plasmonically irradiated (plasmonic). Biofilm density was determined via confocal microscopy and subsequent z-stack image analysis. All experimental conditions produced thicker biofilms compared to the dark control experiment where biofilm thickness was due primarily to cell settling. The biofilm located within the evanescent field in the plasmonic irradiation test had a cell density of 22% v/v compared with 13-15% v/v of the other experimental cases. In addition, the peak cell density, as defined by full-width-half-maximum is 8 µm from the surface in the plasmonic case, as compared to 9.5 µm in the comparable direct irradiation case. While the shift is small, it is indicative of a bias based on lighting direction, with plasmonic excitation from below favouring dense films close to the electrode, and direct irradiation favouring growth away from the electrode. Most importantly these results demonstrate dense biofilm formation at the electrode surface promoted by plasmonic excitation.
Figure A2-5 | Polarization curve showing PPV output voltage across a variety of resistance values (4.9 kΩ to 40 MΩ) under different lighting conditions (Dark ~1 µW/m²; Direct irradiation 60 W/m² of white light; Plasmonic 30 W/m² of red light, λ=670 nm, including both Ohmic losses and light available to the biofilm). Plots are shown of (a) calculated power density and (b) voltage output versus current density. Both direct light and plasmonic excitation of cyanobacteria produce significantly more power than cells without irradiation.

Figure A2-5a shows the polarization diagrams for the sterile culture media control case and three other experimental conditions: dark, directly irradiated and plasmonically excited. The exoelectrogenic power generating capacity of *S. bacillaris* was characterized in a PPV device under a range of external resistive loads (4.9 kΩ to 40 MΩ). 15 minutes were allowed between each measurement for the system to reach steady state before readings were taken. The results shown in Error! Reference source not found.a are the averages of measurements taken each day for three days of at least two devices. The control case of sterile culture media had a negligible power output and was significantly outperformed by all cases involving live cells. The significant power output generated by unirradiated cultures is noteworthy.
This dark power output is presumed to be due to heterotrophic consumption of intracellular carbon stores in the same manner as MFC devices that produce power through biological respiration.\textsuperscript{15,18,23} Even so, both cases, direct irradiation and plasmonic, outperformed the dark case and had comparable power densities with a maximum of 6 µW/m\textsuperscript{2} (anode surface area). This increase in power output under direct light and plasmonic excitation is due to the increased rate of photosynthetically driven electron generation.\textsuperscript{15,18} Using \textit{S. bacillaris}, the PPV device reliably produced power under both direct light irradiation and plasmonic excitation schemes.

The maximum power output achieved by a PPV device under plasmonic excitation is shown in Figure A2-5b. As is typical for biofilm systems, there was appreciable variation between devices.\textsuperscript{15,32} The trend of direct irradiation and plasmonic excitation outperforming the dark conditions, however, held true in all cases. In the plasmonic tests, the highest power density output was 12 µW/m\textsuperscript{2} (anode surface area). Further optimization of the device is possible by adjusting platinum concentrations on the cathode, electrode spacing, media pH and device temperature. Most notably, these results demonstrate simultaneous photosynthetic excitation of a microbe with plasmonically-enhanced evanescent fields with practical voltage outputs, while simultaneously using the plasmonic metal film to collect photosynthetically generated current.

Direct comparison of power densities is complicated by the unique nature of plasmonic excitation as compared to direct irradiation. Specifically, Ohmic losses within the gold film reduce the light available to the bacteria and are generally difficult to quantify.\textsuperscript{33} To estimate available light energy, the total coupled intensity (including losses) was determined from the difference between the incident and reflected light powers (30 W/m\textsuperscript{2}), for a rough estimation of usable light energy of 15 W/m\textsuperscript{2}. For direct irradiation cases plotted in Figure A2-5, 60 W/m\textsuperscript{2} was provided, however, leaving effectively \textasciitilde 15-20 W/m\textsuperscript{2} usable red light. Thus while the cases were designed to be comparable, there are subtleties on both sides that would be interesting to explore in future studies. Here, the full light intensities are provided for completeness.

In conclusion, We have demonstrated a PPV device with a dual-purpose plasmonic gold film used for both electron harvesting and light confinement/delivery to the biofilm, advancing the current state-of-the-art. Plasmonically excited biofilms enable combined light delivery and current collection with practical output levels. Cells showed light/dark response to plasmonic excitation and grew denser near the
anode surface. Directly irradiated biofilms produced average electrical powers of 5.7 \( \mu \text{W/m}^2 \), and plasmonically excited biofilms produced average electrical powers of 5.8 \( \mu \text{W/m}^2 \), with individual cells producing as much as 12 \( \mu \text{W/m}^2 \). This approach achieves plasmonic light delivery and current collection from a single metal film with important implications for the design, and ultimate achievable power density, of biophotovoltaics.

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