# **IGEM-HERC Full Proposal Cover Sheet**

Idaho State Board of Education

PROPOSAL NUMBER:

(to be assigned by HERC)

TOTAL AMOUNT REQUESTED: **\$ 127,800** 

Proposal Track (select one): Proof of Concept

TITLE OF PROPOSED PROJECT:

#### Improving Idaho Seed Crops with Cold Atmospheric Pressure Plasma (CAP) Technology

SPECIFIC PROJECT FOCUS:

This *proof of concept* project focuses on the development of a novel plasma (CAP) technology to treat seeds during processing to reduce the spread of plant diseases and improve germination. This project builds upon prior CAP technology work by the PI (Cornell) and co-PI (Browning) to develop plasma devices to sanitize hospital surfaces and treat chronic wounds. Initial plasma concepts have been patented by Cornell & Browning (US patent 11,871,978. 2024). Intellectual property that relates to the use of our CAP array technology to treat crop seeds is the topic of a recent (Jan 2025) patent disclosure to Boise State University. The focus of this project will be to (1) engineer arrays of 10 cm x 10 cm devices for treatment of seeds and surfaces during seed processing, and (2) to determine the CAP treatment parameters that will result in the elimination of >99% of pathogens from seeds, thus reducing the spread of plant diseases and foodborne illness.

PROJECT START DATE: 8/1/2025	PROJECT END DATE: 7/30/2026			
NAME OF INSTITUTION:	DEPARTMENT:			
Boise State University	Chemistry & Biochemistry			

#### ADDRESS: 1910 University Dr., Boise, ID 83725

E-MAIL ADDRESS: kencornell@boisestate.edu PHONE NUMBER: 208-426-5429 SIGNATURE: NAME: TITLE: PRINCIPAL INVESTIGATOR Dr. Kenneth A. Cornell Professor rethA/and/ CO-PRINCIPAL INVESTIGATOR Dr. Jim Browning Professor NAME OF PARTNERING COMPANY: COMPANY REPRESENTATIVE NAME: Mr. Roger Batt (IEOSA) Idaho – Eastern Oregon Seed Association (IEOSA) Crookham Company Mr. Dennis Demshar (Crookham Co) SIGNATURE: Junis Vinsh (IEOSA) (Crookham Company)

Authorized Organizational NAME: Ella Christiansen SIGNATURE:			
Office of Sponsored Programs	•	Assistant Director, Pre-Award	SIGNATURE:

# 2026 Idaho Global Entrepreneurial Mission Initiative - IGEM HERC

### 1. Idaho Public Institution: Boise State University

# 2. Title: Improving Idaho Seed Crops with Cold Atmospheric Pressure (CAP) Technology

# 3. PI(S)/Key Personnel/Institution:

# Principle Investigators Ken Cornell, Ph.D., (PI) Dept. Chemistry & Biochemistry, Boise State University Jim Browning, Ph.D.,(Co-PI) Dept. Electrical & Computer Engineering, BSU <u>Key Personnel</u> Marcus Pearlman, Ph.D., (Research Scientist), Dept. Electrical Engineering, BSU Bob Hay, Ph.D., (Emeritus faculty), Dept. Electrical & Computer Engineering, BSU Stephanie Rood, (Graduate Student), Biomolecular Sciences Program, BSU

#### 4. Total Amount Requested: \$127,800

# 5. Project Significance and Project Objectives:

# **Project Significance**

**The problem.** The contamination of seed crops with bacterial, fungal, and viral plant pathogens can severely reduce their germination rates and the overall yield of the next season's crop. This reduces the value of the seed crop, or requires post-harvest chemical treatments that may impact marketability. For seeds that are sprouted for consumption (alfalfa, mung bean, radish, etc.), seed contamination by human pathogens can be a source of foodborne illness. Ultimately, this has a negative impact on the crop seed industry. The combined SW Idaho and Malheur County Oregon area is one of the five major regions for crop seed production in the world, especially for sweet corn, popcorn, carrots, peas, onions, and green beans, as well as numerous other vegetable and root crops (broccoli, turnips, beets, sugarbeet, etc). The value of these seed crops in SW Idaho and Eastern Oregon is estimated at ~ \$750 million annually.<sup>1</sup> Because bacteria, fungi, and viruses impact the value and shelf-life of seeds and other fresh produce, new methods are needed for treatment to decrease the economic loss arising from these pathogens. Our CAP technology will benefit our important seed crop industry in Idaho, as well as the seed industry nationwide.

A viable solution. We have developed a cold atmospheric-pressure plasma (CAP) array system that could be useful for treating crop seeds during processing. CAP produces ionized reactive oxygen and nitrogen (RONS) gas species with antimicrobial activity that are useful for killing plant and human pathogens on seed surfaces and improving germination rates. Our prototype CAP device is a viable solution since it creates ionized gas using room air, thus it only uses electricity to operate and is amenable to incorporation into normal seed processing work flows. Since it does not require water or other chemicals to have its sanitizing activity, it can be engineered to operate while seeds are moving down a conveyor belt, or are getting sorted on a gravity separation table. We have toured industry partner seed processing facilities to examine the stages of seed preparation and identify where CAP would be most effective and require the least addition of process steps to accomplish.

**Innovation and alignment with Idaho HERC priorities.** The innovation of this proposal is the incorporation of CAP array systems into industrial machinery (conveyor belts, gravity separators) that are used during seed processing. CAP treatment is a novel advancement for this technology that reduces the use of toxic chemicals in seed processing. Seed production and processing for sale is a major industry in Idaho, so CAP technology will have a significant positive impact on the economy in the state. More broadly, the CAP technology could solve sanitization problems in the \$4 billion-dollar agricultural food processing industry in Idaho. This project aligns

closely with the HERC funding priorities in biotechnology/biomedicine because our technology will be useful in reducing the impact of plant and animal pathogens in seed crops.

**Technology Readiness Level (TRL).** As with most technology development, there are many arms to the process of prototype device projects. The technology development as a whole for our 10 cm x 10 cm CAP array to use in industrial settings is in <u>TRL stage 3</u> (experimental proof of concept). However, we have constructed our prototype based on a large volume of data collected on a smaller 2 cm x 2.4 array that was developed in our lab that demonstrates that CAP can deliver potent antimicrobial effects on surfaces and seeds. Thus, our technology is rapidly progressing through <u>TRL stage 4</u> (technology validated in lab).

**The purpose of this application** is to assemble our prototype 10 cm x 10 cm array system onto relevant equipment (conveyor, gravity separator table) to validate the antimicrobial technology in the lab, and prepare it for TRL stage 5 studies (technology validated in relevant environment).

#### Project Objectives.

**Objective 1:** Design and fabricate an array of 10 cm x 10 cm CAP devices that use air as the working gas and assemble a prototype onto a conveyor belt and gravity separator table.

**Objective 2:** Determine the operating parameters (exposure time, distance) required for the 10 cm x 10 cm CAP array system to inactivate >90-99% of pathogens on seeds (alfalfa, sweetcorn, carrot) and seed processing surfaces.

#### 6. Project Plan and Timeline:

**A. Background and Prior work.** "Cold atmosphericpressure plasmas" or CAPs are created when a voltage is discharged into a gas between electrodes. The process creates a low level of ionized gas at room temperature and pressure. The application of CAPs as a solution to contamination is gaining increasing attention, but researchers have yet to demonstrate large scale CAP treatment technology. As compared to typical disinfection methods<sup>2-4</sup> plasmas reduce



**Figure 1.** Photographs of 2.4 cm x 2 cm 8element plasma array (top), and 10 cm x 10 cm 43-element array (bottom).

material use and waste. Our lab<sup>5-9</sup> and others<sup>10-14</sup> have used CAP devices to generate plasma by ionizing gas mixtures (argon, water vapor, helium, and air).

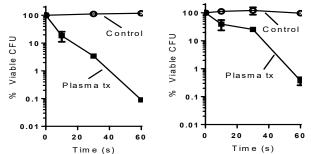
The plasma generated from our prototype devices has been shown to inactivate microbial pathogens, including viruses, bacteria, bacterial biofilms, and fungi on a variety of surfaces, on foodstuffs, and in wound lesions.<sup>5-9</sup> However, most CAP research has focused on CAP jet devices with limited spatial coverage. Therefore, we have developed CAP devices<sup>5-9</sup> capable of operating over larger areas. Our work has produced both a 2.4 cm x 2 cm CAP array<sup>6</sup> and a prototype 10 cm x 10 cm CAP single array consisting of a "stack" of 43 discharge elements (see Fig. 1). Preliminary results indicate that we can inactivate >90-99% of bacteria on variety of industrially relevant surfaces. In one experiment, 2-day *Ps. fluorescens* biofilm samples were grown on steel coupons and treated with our 2 cm x 2.4 cm 8-element CAP array<sup>6</sup> for up to 60 sec. Following plasma treatment, coupons were analyzed for viable bacterial cells by enumeration on nutrient agar. The results (Fig. 2, left) show a decrease of >99.9% in viable biofilm bacteria at 60 s plasma treatment, while the controls (gas only) show no effect. A similar approach showed the plasma system could decontaminate eggshells inoculated with *Salmonella* that causes salmonellosis

(**Fig. 2**, right). Chicken eggshells treated with the CAP array show a >99% reduction in viable *Salmonella* with 1 min plasma treatment.

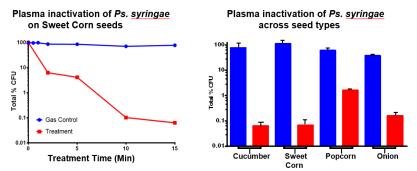
Pathogen Inactivation of Crop Seeds. New results with our 2.5 cm x 2 cm array show that plasma treatment can be used to inactivate plant pathogens such as *Pseudomonas syringae* on a variety of crop seeds. Results for plasma treatment of sweet corn seed are shown in Fig. 3 (left side), where >90% bacterial

>90% (left side), where inactivation was achieved with 1 min of treatment and >99.9% inactivation with 10 min treatment. Fig. 3 (right side) shows CAP inactivation of 99% - 99.9% inactivation of Ps. syringae achieved with 15 min plasma treatment of four different seed types (cucumber, sweet corn, popcorn, onion). Importantly, plasma treated seeds were found to be fully viable in germination studies (not

Ps. fluorescens biofilms Salmonella on eggshell



**Figure 2**. (Left) CAP treatment of 2-day *Ps. fluorescens* biofilms. (Right) CAP treated eggshells with *Salmonella enterica*.<sup>6</sup> CFU = colony forming units.



**Figure 3.** [left side] CAP inactivation of *Ps. syringae*. [right side] Total % inactivation of *Ps. syringae* across 4 seed types with CAP treatment (red) or gas treatment control (blue). CFU = colony forming units.

shown), indicating that the treatment was safe for the crop seed.

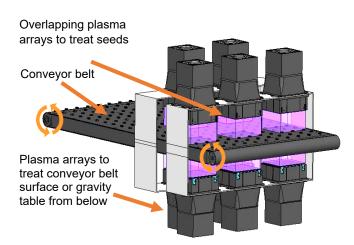
- These studies provide the rationale for the development of groups of 10 x 10 cm 43element plasma arrays, and their integration into seed processing equipment to reduce the carriage and transmission of plant pathogens and foodborne illness.
- This technology will be useful for the Idaho seed crop industry, and have broader impacts and applications in the Idaho food processing sector.
- B. Project Plan. For clarity, the project plan is presented in the context of the project objectives.

# <u>Objective 1:</u> Design and fabricate an array of 10 cm x 10 cm CAP devices that use air as the plasma gas and assemble a prototype onto a conveyor belt and gravity separator table.

In order to achieve our project goals, we must design, fabricate, and test a new generation of large CAP arrays based on our 10 cm x 10 cm prototype that can be grouped together to be economically deployed in food or seed processing facilities. Shown in **Fig. 4** is the concept drawing for using 5 CAP arrays combined to treat a conveyor belt or other separating/selection surface from the bottom of the surface as it rotates or vibrates. This orientation could provide a cleaning in place solution during operation, providing a constant sanitation that could prevent biofilm accumulation or pathogen transfer on a conveyor belt surface. Alternatively, if the surface is porous such as used in air-assisted vibrating gravity tables used in seed selection, placement of the arrays below the table would position the plasma in an airstream to accomplish both seed

sanitization and density separation. Also shown are staggered CAP arrays oriented above the conveyor belt that could be used to treat seeds prior to packaging as well as the conveyor surface.

New Generation of CAP Arrays. While a prototype single 10 cm x 10 cm CAP system has been developed, multiple 10 cm x 10 cm CAP arrays need to be developed for practical for practical use. These include (1) the ability to use room air as the working gas, (2) reduced plasma treatment times, (3) active device cooling, (4) new plasma drivers. For practical industrial use, it is expected that the working gas must be air for simplicity and cost. However, air has a low electron impact ionization cross section so it requires a high AC voltage to achieve uniform plasma. Preliminary work suggest operation of our arrays at 2.5 kV will be required, and power dissipation will be high (300-400 W/array). Therefore, a

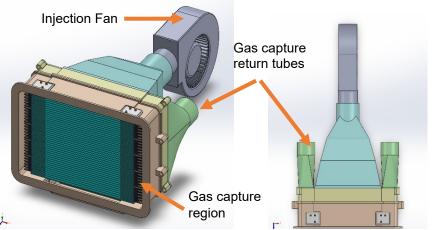


**Figure 4.** Concept drawing of a conveyor belt system with CAP arrays placed either above the conveyor surface to sanitize crop seed product as it passes underneath, or CAP discharges underneath the conveyor surface to sanitize the conveyor belt material after delivery of the product.

new plasma driver system and array cooling will be developed. This will be the focus of the work by Drs. Pearlman and Hay. The systems produce reactive oxygen and nitrogen species (RONS) needed to inactivate the pathogens. Our team has been developing a capture system where each CAP array has a gas return path to pull the RONS back to the array fan inlet and recirculate the RONS to enhance the CAP concentration available to treat the seed surface and reduce treatment times. A preliminary drawing showing the capture system with return pipes is shown in Fig. 5.

The current CAP array is driven using a very large transformer-based plasma driver. This lab power supply provides operation from 0-10 kV and up to 400 mA. In addition, this driver can support the CAP array capacitance of >200 pF. To improve the practical application of the CAP system for industrial applications, our team is developing a symmetric plasma driver specifically intended to support the high capacitance array in industrial settings. Unlike traditional plasma drivers, this drive system operates symmetrically. During a cycle there is a true sinusoidal signal with the system operates symmetrically.

with one electrode positive and the other negative. This driver has allowed the 10 cm x10 cm array to operate at lower total voltage (peak to peak) and is more efficient, thus approach making this more desirable for industrial settings. In order to operate with air as the working gas, а new generation of board must be designed and fabricated to achieve the required voltages of 2-2.5 kV and driver currents of



**Figure 5.** (Left) Preliminary pictorial drawings of an enclosure designed to recirculate gas in the plasma array to enhance plasma inactivation efficacy. (Right) A top down view of the structure.

200 mA. In an industrial setting, multi-arrays will be powered by one DC supply, improving setup simplicity and cost in device operation. In the new CAP array system, this driver board will be part of the CAP enclosure and connect directly to the plasma array. Dr. Hay will direct this work.

**Conveyor and Density Table Surface Treatment.** In the first case, the goal is to treat the conveyor belt surface to prevent pathogen transmission and biofilm build-up. In this example, 5 CAP arrays are positioned at the beginning (return) of the conveyor belt (**Fig. 4**). These arrays treat an approximately 35-40 cm wide conveyor belt in a staggered formation to ensure full surface treatment. As the belt surface passes through the continuously operating arrays, the plasma reactive species inactivates and removes pathogens from the surface. As our team has tested numerous surfaces (plastic, glass, stainless steel), this process should be effective. This 5 CAP array concept will be studied by positioning it over a simple commercially available conveyor belt system we have established in the lab that is 1.25-1.5 m in length. The impact of conveyor belt speed on the treatment effectiveness will be studied. In parallel studies, seeds are often processed by density table separation to remove higher density rocks and lower density chaff and defective seeds by agitating over airflow that travels from below through the angled table surface. In the process, seeds turn over so the whole surface can be exposed to plasma treatment. The 5 CAP array concept will be studied in the system by positioning over the density table to examine RONS antimicrobial delivery to the surface.

To accomplish this objective, the following methods and approaches will be used:

(a) <u>Cooling</u>: A high heat transfer, mechanically compliant polymer used in electronics will be forced against the edges of the CAP array elements to pull heat away from the array and ballast resistors. Spacers with high thermal conductivity will be used. Copper plates will be used to trap the polymer against the array, and extend back behind the array to convective cooling fins or a conductive water coolant system to maintain the array temperature below 75°C. The copper plates will be surrounded by the plastic 3D printed enclosure.

(b) <u>Gas Capture</u>: The array enclosure and plasma driver will be surrounded by a capture enclosure as shown in **Fig. 5**. A fan provides suction to pull the plasma products back into the outer enclosure to recirculate RONS through the device.

(c) <u>Recirculating System</u>: An additional innovation is to recirculate the plasma effluent through gas capture return tubes into the fan that pushes air into the CAP array. Recirculating the gas will be studied to see if it increases RONS delivery levels and reduces plasma treatment time.

(d) <u>Plasma Driver:</u> A new driver (power supply) system will be developed based on our preliminary design. This new driver will allow operation at higher voltages (~2500 V) and current (~200 mA) using air as the working gas. Early experiments show a shift in CAP array resonant frequency with air plasma (56 kHz to 28 kHz). The new system will allow programmed control to autotune the resonant frequency after plasma initiation. The driver system will include a controller, start and shut-down software, current and temperature sensors, and shut down circuitry.

(e) <u>RONS detection</u>. For this work to examine RONS delivery to the surfaces by the plasma array, we will focus on 2 RONS species: ozone and peroxide. <u>Ozone</u>. A Forensics Model FD-90A-03 ozone detector will be used to measure ozone (O<sub>3</sub>), either at the plasma source instantaneously, or over time by measuring the fill rate in the space confined by the gas recycling chamber. <u>Peroxide</u>. The production of reactive hydrogen peroxide ( $H_2O_2$ ) will be measured with an Amplex Red<sup>®</sup> Hydrogen Peroxide/Peroxidase kit (ThermoFisher) that detects fluorescent resorufin (ex/em 571nm/585nm) using a 96-well plate reader. Briefly, the plasma device is discharged into a petri dish placed on the conveyor or gravity table surface. The petri dish will contain 10mL of DI water placed 1-4 cm below the device. The liquid is sampled periodically and

the AmplexRed kit used to determine dissolved peroxide. A standard curve of known concentrations of peroxide is used to assign test sample concentrations.

# <u>Objective 2:</u> Determine the operating parameters (exposure time, distance) required for the 10 cm x 10 cm CAP array system to inactivate >90-99% of pathogens on seeds (alfalfa, sweetcorn, carrot) and seed processing surfaces.

**Crop Seed Treatment.** In the second approach, the same conveyor belt system will be used to treat crop seeds such as alfalfa, sweetcorn, and carrot. Our engagement with the local/regional crop seed industry makes this project highly relevant. Preliminary work has shown that the CAP can reduce pathogens on seed surfaces while not affecting germination. As shown in Fig. 3, the seeds can be placed on the conveyor and moved under a staggered array of 5 CAP devices. These array engineering features will be developed and implemented with planned experiments to determine the best operating parameters to inactivate planktonic bacterial or fungal pathogens on seed surfaces. The microbe contaminated seed samples will be placed on the conveyor or gravity table and colony forming unit (CFU) counts will be measured after plasma exposure. The effect of plasma array exposure time (0-15 min) with varying (1) current (100 to 300 mA), (2) air flow rate (by anemometer), (3) array to sample distance (1 to 4 cm), and (4) air recirculation will be measured using methods we have used previously with smaller arrays. The effect of recirculating RONS on the plasma efficacy will use a throttle valve to adjust the amount of recirculating gas with planned variation from 0% to 80% of total air flow depending upon the capture system capability. For antimicrobial assays, the following methods will be used:

(a) Antibacterial activity. Stocks of Ps. syringae (BAA-871), Xanthomonas hortorum carotae (a carrot seedborne pathogen), E. coli O157:H7 (43894), and Salmonella enterica (14028) are from the ATCC or supplied from the USDA ARS repository. All strains are maintained on Tryptic Soy agar (TSA) or Blood agar plates and cultured at 25-37°C. Sample preparation. 5 mL overnight cultures (25-37°C) are prepared in Trypticase Soy broth (TSB). Antimicrobial cell tests will be performed as described in our published work.<sup>5-9</sup> Briefly, overnight cultures of the above organisms are diluted in sterile PBS to 107-108 CFU/mL and small samples of culture placed on the surface of the seeds. Seeds will be placed under the plasma discharge for treatment. At least 3-6 replicate samples for every exposure or condition are prepared. The coupons are rinsed briefly in PBS to remove loosely bound planktonic cells prior to treatment. Sample treatment. The samples of seeds inoculated with planktonic cells will be placed 1-4 cm below the CAP array discharge and treated (0-15 min), then immersed in 10 mL sterile PBS and vortexed to liberate bacteria, serially diluted, plated on TSA to achieve 30-300 CFU/plate and incubated at 25-37°C until visible colonies appear. All treatments are performed in triplicate, and 3 independent experiments are performed. Controls consist of samples treated with air gas run through the device without plasma discharge (gas flow only). All work is performed in biosafety cabinets in a BSL-2 laboratory. Results are expressed as % total CFU vs. CAP exposure time.

(b) <u>Antifungal activity.</u> Small overnight cultures in 10 mL Yeast Potato Dextrose (YPD) broth of representative plant fungal pathogens (*Fusarium graminearum* ATCC MYA-4620, *Pythium ultimum* ATCC 200006) are grown from isolated colonies on YPD agar. Cultures are grown aerobically with shaking at 25–37°C. Following 1-2 days culture, samples are diluted in sterile phosphate buffered saline (PBS) to provide  $10^7$ - $10^8$  CFU/mL per milliliter. <u>Seed preparation</u>. Seeds (sweet corn, alfalfa, carrot) are obtained from Ferry-Morse or Crookham Seeds. For large seeds, a small volume of overnight inoculum will be applied directly to the surface. For small seeds (alfalfa, carrot), groups of 50 seeds will be immersed in 10 µL diluted overnight inoculum for 30 min with agitation. Seeds are dispersed on a PBS-dampened filter paper in a petri dish for plasma treatment. Seed samples (25 seeds/sample for large seeds; 50-100 seeds/sample for small seeds) in petri dishes are placed 1-4 cm below the plasma device. Plasma or control treatment will proceed for 0 – 15 minutes. Seed samples are then transferred to 50 mL tubes

containing sterile PBS, vortexed to liberate fungal cells, and dilutions in PBS plated onto YPD agar plates. CFUs are counted after 2-4 days at 25°C, Triplicate experiments are performed.

(c) <u>Seed germination.</u> Following 0-15 min CAP treatment, groups of 100 seeds (sweetcorn, alfalfa, carrot) will be placed between moistened germination papers in an illuminated incubator (e.g.16 hr light/8 hr dark at 22°C for alfalfa) using USDA ARS established guidelines. Seeds will be observed for germination and measurements of root and shoot growth will be measured at 7-21 days (depending on type). Germination capacity ([#germinated seeds/total # seeds]x100) will be determined. Results of 3 experiments will be analyzed.

# C. Timeline

Timeline of Project Objectives and Milestones schedule

	Project Months												
#	OBJECTIVE	1	2	3	4	5	6	7	8	9	10	11	12
1	Design and fabricate an array of 10 cm x 10 cm CAP devices that use air as the plasma gas and assemble a prototype onto a conveyor belt & gravity separator table.				A				В			С	
2	Determine the operating parameters (exposure time, distance) required for the 10 cm x 10 cm CAP array system to inactivate >90-99% of pathogens on seeds (alfalfa, sweetcorn, carrot) and seed processing surfaces.						D					E	F

# D. Milestones

(A) complete fabrication of the CAP array system using air with cooling and recirculation. Establish optimal operating conditions (voltage, current, etc).

- (B) incorporate the CAP array onto conveyor and gravity table devices and demonstrate parameters for optimal RONS delivery to surfaces.
- (C) assemble manuscript on plasma array fabrication and operation for RONS production.
- (D) complete antimicrobial and germination studies on seeds using single 10 cm x 10 cm plasma array. Demonstrate >90-99% pathogen inactivation, and no impact on seed germination.
- (E) complete antimicrobial and germination studies with plasma array on conveyor/gravity table. Demonstrate >90-99% pathogen inactivation, and insignificant impact on seed germination.
- (F) prepare 2nd manuscript on antimicrobial effects of plasma array on conveyors/gravity tables.

**7.** Potential Economic Impact: This proof of concept application will incorporate our CAP technology into seed processing machinery, and test the effectiveness of using the CAP technology in reducing the burden of pathogens by  $\geq$  90-99%. Importantly, our plasma array design is scalable and applicable to large surface areas that are found in the \$750M seed processing industry, with added potential use in the \$3.9B Idaho food processing sector. *Intellectual Property*. One patent (US Patent 11,871,978, 1/16/2024) has been awarded to BSU and the Cornell/Browning team for the plasma technology. We intend to pursue more patents on our novel large CAP arrays and their application to industrial surfaces. An additional patent disclosure (Jan 2025) has been made to Boise State University that covers the application of the 10 cm x 10 cm array configurations and power supplies to industrial and seed processing settings.

**<u>8. Criteria for Success</u>: Success metric 1**: For the studies to meet **Objective 1** goals, the success metrics will include (1) completion of a 10 cm x 10 cm CAP Array optimized for using air with cooling and recirculation and new drivers, (2) demonstration that recirculation of the plasma improves RONS content, (3) determination of optimal conditions for use of the CAP system on conveyors and gravity tables. Success metric 2: For studies to meet **Objective 2** goals, the success metrics will include (1) <1% bacterial and fungal pathogen viability in treated samples with less than 60 s exposure time using a single 10 cm x 10 cm CAP device, (2) <1% bacterial

and fungal pathogen viability in treated samples with less than 60 s exposure time using the CAP array incorporated onto conveyors and gravity tables, and, (3) negligible effects on seed germination following plasma treatment conditions that eliminate 99% of microbial pathogens.

**9.** Anticipated Challenges/Barriers: The results of experiments used to meet our two objectives will demonstrate that engineered CAP arrays can successfully inactivate pathogens on surfaces and seed products in a reasonable time frame relevant to industrial applications. Development of large CAP arrays using air may have issues associated with plasma initiation, uniformity, and power consumption. Our preliminary results provide strong support that these problems can be overcome with proper design and implementation of active cooling systems and plasma drivers (power systems). Alternative approaches include decreasing the plasma discharge gap to make it easier to ionize the air gas and improving the effluent capture system to improve efficacy of treatment by increasing the RONS concentration. While the goal is to treat surfaces rapidly, the optimized arrays may require longer treatment times. Increased power and decreased treatment distances may be used to minimize required exposure times to achieve target killing of pathogens. Our results will validate the proof of concept that CAP arrays can inactivate pathogens rapidly on seed surfaces using only power and air.

**<u>10.</u>** Budget: We are requesting \$127,800 for the 1-yr project timeframe. See attached Form D for more detail. The budget is summarized below:

Line Item Request	Total Amount
Personnel (salary + fringe)	\$ 98,700
Equipment	\$ 0
Travel	\$ 0
Participant support	\$ 0
Other Direct Costs: Materials & Supplies	\$ 14,000
Other Direct Costs: Student tuition+insurance	\$ 15,100
Total	\$127,800

# 11. Budget Justification:

**Personnel:** A total of \$98,700 is requested to support salary (\$79,600) and fringe (\$19,100) for personnel working on this project during the grant-year. Additional descriptions of senior personnel can be found in Appendix C. Support is requested for:

**Dr. Ken Cornell** (PI). Salary and fringe costs (\$8,900 for Dr. Cornell for 0.5 month effort is requested. Additional effort on the project will be part of his workload (50% research). He will direct the project, including oversight of the Ph.D. student and undergraduates recruited to work on the project through IDeA internships. He will organize weekly lab meetings, plan experiments to meet milestones, and prep manuscripts/reports for industry partners and agency sponsor. He will direct IDeA Programs Office staff for grant management (ordering, hiring, reporting, etc).

**Dr. Jim Browning** (co-PI). Salary and fringe costs (\$12,500) to support Dr. Browning for 0.5 months effort are requested. Additional effort by Dr. Browning on the project will be part of his normal workload allocation (100% research). He will oversee the research scientist (Pearlman) and emeritus faculty (Hay), and work on design and troubleshooting of integrated plasma arrays. He will co-lead lab meetings, and work with Dr. Cornell on project reports and manuscripts.

**Dr. Marcus Pearlman** (Research Scientist). Salary and fringe costs (\$39,600) to support Dr. Pearlman for 5 months of effort on the project are requested. Dr. Pearlman has >10 years of experience modeling, designing, and fabricating plasma and other electronic devices. His primary role will be to design and fabricate arrays of 10 cm x 10 cm plasma devices and integrate them into seed processing machinery. This includes incorporating and testing cooling systems, array connectivity, and gas recirculation to improve RONS delivery. He will train undergraduate engineering students as part of senior design courses and undergraduate internship programs.

**Dr. Bob Hay** (Sr. personnel). Salary and fringe costs (\$6,000) to support Dr. Hay's efforts on the project are requested. Dr. Hay will devote ~ 100 hr to design improvements to the power supply drivers to improve plasma discharge uniformity and performance with air as the feedstock gas. He will work with Dr. Pearlman to troubleshoot plasma array problems and incorporation.

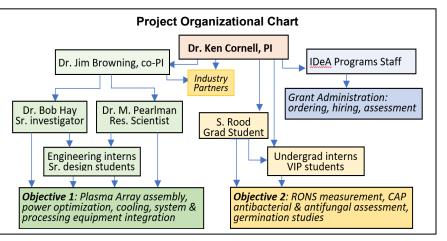
**Ms. Stephanie Rood** (Ph.D. student). Funds are requested for salary and fringe (\$31,700) for the graduate student to work on the project. Ms. Rood will design and perform experiments to demonstrate plasma device RONS production and antimicrobial activities against bacterial and fungal pathogens contaminating a variety of seed types. She will train and oversee undergraduate researchers working in the lab, assemble progress reports for lab meetings, analyze data, and write manuscripts. She will devote 100% of her efforts to this project.

<u>Equipment, Travel, Participant Support</u>. None requested. Necessary equipment is in place. Travel costs will be covered by other institutional sources available in the IDeA Programs office.

<u>Materials and Supplies</u>. Funds (\$14,000) are requested to purchase supplies (ceramic plates, conductive pastes, electrical wire, etc) for construct additional 10 cm x 10 cm plasma devices to assemble into multi-arrays of 5 devices for integration onto processing equipment. Supplies will also include plasticware (pipets, petri dishes, etc), fungal and bacterial media (broth, agar) for plasma antimicrobial studies, and chemicals to measure RONS production.

<u>Student Tuition and Fees</u>. Funds are requested to support the tuition (\$11,900) and student health insurance (\$3,200) for the graduate student (Rood) during this 1-year project.

**12. Project Management:** A chart is presented to the right to show the individuals on the project and their responsibilities to the objectives. The PI (Cornell) will meet weekly with the investigative team (Browning, Pearlman, Hay, Rood, 6-10 undergraduates) to review research progress, establish milestone goals, and brainstorm solutions to



experimental problems as they arise. The undergraduate researchers (6-10) at these meetings participate in the Cornell and Browning labs as part of (1) Vertically Integrated Projects (VIP) courses led by Cornell to develop plasma devices and engage in cross-disciplinary research, (2) engineering senior design courses, and (3) IDeA program summer internships. The students will work under the direction of the team to fabricate plasma devices and test their RONS production and antimicrobial activity to meet objectives 1 and 2. Quarterly, Drs. Cornell and Browning will arrange in-person or zoom meetings with industry representatives (IEOSA, Crookham, etc) to present project data and discuss seed crop industry needs. The PI and students will present their work at the annual IEOSA conference. General project management (ordering, hiring, accounting, reporting) will be handled by staff in the BSU IDeA Programs Office under Dr. Cornell's direction.

**13.** Additional Institutional and Other Sector Support: Industry Partnerships. We have partnered with both industry groups (Idaho Eastern Oregon Seed Association) and commercial seed growers (Crookham Co.) to understand the needs of the industry and determine where the CAP technology could fit into the processing stream. As part of these interactions, we have toured facilities, conducted joint experiments, and given talks at industry annual meetings. We have received numerous letters of support from industry for our grant proposals. We will expand our

connections with the agricultural and food processing industries as our technology continues to develop. This includes industry groups such as Dairy West, Idaho Crop Improvement Association, Food Producers of Idaho, and Food Northwest. Institutional Support. Additional **institutional support** is provided through the BSU **IDeA Programs Office** that supports (1) administrative staff (admin, accounting, assessment/reporting) to assist with grant management, and (2) sponsors a variety of paid undergraduate research internships to work in research labs.

**14. Future Funding:** The BSU team (Cornell, Browning) has a long history of pursuing grant funding to support various aspects of plasma research. This has included the development of plasma devices to treat infections and contaminated surfaces that were the topics of funded NIH R15, R21, and COBRE P20 pilot awards. An NIH R01 grant application to use our plasma scalpel device to treat chronic wounds is pending. USDA pilot and full grants to develop a radial plasma device to treat the inside of pipes in food processing were recently completed. An Idaho Specialty Crops grant is currently supporting preliminary work to demonstrate that a 2 cm x 2 cm plasma array can inactivate plant pathogens. Additional USDA and NSF applications to develop the plasma technology to treat processing surfaces to eliminate foodborne illness, and to treat water contaminated with polyfluorinated compounds (PFAS) are pending. The BSU team will continue to pursue extramural funding for plasma technology through NIH, NSF, and USDA sources.

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- 6. Cornell KA et al. (2021) Design and fabrication of a multi-discharge cold atmospheric pressure plasma array. *IEEE Transactions Plasma Science*, 49(4), 1388-1395.
- 7. Croteau A, White A, Cornell KA, Browning J (2022) Cold atmospheric pressure plasma device exhibits etching effects on bacterial biofilms. *IEEE Trans Rad Plasma Med Sci.* 6(5): 619-625.
- 8. Okebiorun M et al. Autofluorescence-Guided Removal of Bacterial Biofilms from Tissues using Cold Atmospheric Pressure Plasma. *IEEE Trans Rad Plasma Med Sci.* 2024 Feb; 8(8): 990-996.
- 9. Okebiorun M, Oberbeck C, Waite C, Clark S, Miller D, Barney Smith EH, Cornell KA, Browning J (2023) Selective optical imaging for detection of bacterial biofilms in tissues. *J Imaging*. 9(8): 160.
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# **Appendix A – Facilities and Equipment**

# **PI CORNELL FACILITIES AND RESOURCES**

### LABORATORY

Dr. Cornell's lab consists of approximately 1200 sq. ft. of laboratory space in three contiguous rooms. Designated within this space is a 1) BSL-2 microbiology facility; 2) mammalian cell culture room; and 3) general wet lab space for plasma treatments and sample preparation. The space is outfitted with two chemical fume hoods, ultrapure water source, storage areas for chemical and biological wastes, and autoclave.

# COMPUTERS

The Cornell research group has five workstation computers (Windows 11 desktops) outfitted with word processing suites and GraphPad Prism for analyzing data and preparing manuscripts. The computers are connected to a lab specific drive (Alkali Creek) and automatically backed up on a daily basis.

#### Office

The PI has approximately 150 sq. ft. of office space located on the second floor of the Math building in the IDeA Programs office. An adjacent 100 sq. ft. conference room is available for meetings with small groups of students, and has an extended monitor display for remote videoconferencing.

# MAJOR EQUIPMENT

Relevant equipment in the Cornell lab includes five Type II Biosafety hoods, shaking and static incubators, two refrigerated tabletop centrifuges with biocontainment rotors, and two BioTek 96 well UV/Vis/fluorescence plate readers with temperature and agitation control. Also present are: Eppendorf RealPlex2 thermocycler, BioRad horizontal and vertical gel electrophoresis tanks and power supplies, ECM electroporator, FPLC chromatography system, chromatography refrigerator, -20°C and -80°C freezers, liquid nitrogen tank, 3 Nuaire CO2 incubators, sonicators, standard light and inverted microscopes, EVOS fluorescence imaging microscope, refrigerated microcentrifuges, ultracentrifuge, and various spectrophotometers (Varian Cary 50, Cary 100, Thermo nanodrop). A CAP test setup containing a high voltage AC power supply, gas canisters with regulators, mass flow controllers, oscilloscope and octamer plasma array is established in Dr. Cornell's lab to treat PFAS samples.

Other equipment and facilities to measure effects by the CAP device are found in the Biomolecular Research Center, and Depts. of Biological Sciences and Chemistry & Biochemistry in common use facilities that have been established with aid from the Idaho State Board of Education, Murdock Charitable Trust, and NSF Major Research Infrastructure and CRIF grants. These resources comprise approximately 4000 ft2 total and are located on four floors of the Science building and the Mathematics Building at Boise State University.

<u>Spectrometry:</u> A 750 ft2 center in the Dept. of Chemistry & Biochemistry houses a 600MHz NMR and EPR spectrometers, and is maintained by a full-time NMR facility

manager. A Bruker Thermo Orbitrap mass spectrometer outfitted with a Dionex UPLC and MALDI AutoFlex are operated on a fee-for-service basis and maintained by a full-time mass spectrometry facility manager (Dr. Shin Pu) within the Biomolecular Research Institute. Additional common-use analytical facilities include UV/Vis spectrophotometers, stopped flow fluorimeter, FT-IR, Flame AA, Thermo GC/MS, GC, HPLC with diode array and fluorescence detector, and Bruker HCT ETD ion trap MS.

<u>Confocal Microscopy and Imaging facilities:</u> The Biomolecular Research Center provides essential equipment for histology and fluorescence microscopy, including dedicated space for sample preparation and examination by confocal microscopy (Leica Stellaris scope). The confocal microscopy center is run on a fee for service basis and managed by a full-time technician. In addition, a 600 ft2 histology core center containing tissue processors, cryostats, and automated slide staining is managed by a part-time Ph.D. level scientist in the BRC, with access and services available on a fee-for-service basis. Scanning probe microscopy and Electron Microscopy facilities are available through the Dept. of Materials Sciences at BSU (Veeco Nanoscope IV Scanning Probe Microscope).

<u>Other facilities at Boise State University.</u> In addition, the Colleges of Arts and Sciences and Engineering have complete electronics and machine shop available for repair of equipment and instrumentation and for fabrication of items necessary in research. Administrative support for grant preparation and grant management is provided at the department, college, and university level. Institutional support for the proposed project is demonstrated by commitments of newly remodeled space for the Biomolecular Research Core and the Biomedical Research Vivarium (6,000 square feet with the potential for future expansion to 11,000 square feet). The University Cyberinfrastructure and Office of Information Technology support advanced data acquisition, data storage, data management, data integration, data mining, data visualization and other computing and information processing services distributed over the Internet, efficiently connecting laboratories, data, computers, and people with the goal of enabling novel scientific theories and knowledge. The Office of Information Technology occupies 38,000 square feet of space in multiple locations on campus to provide academic, administrative and research support to the university.

# COPI BROWNING FACILITIES AND RESOURCES

# LABORATORY

Dr. Browning's lab consists of 950 sq. ft. of space in a single room. The Plasma and Vacuum Electron Devices lab is facilitated with a pump exhaust system, a laminar flow hood, and gas delivery systems. The lab has three high vacuum chamber tests systems. A long working distance (8") microscope with camera capabilities is available for imaging of devices under test. The lab contains numerous high voltage power supplies (up to 25 kV), signal generators, RF amplifiers, oscilloscopes, a spectrum analyzer, and a network analyzer. The lab also has two high voltage (10 kV), low frequency (20 kHz-60 kHz) plasma source AC voltage supplies. A Gas Detector (IS Model MX6) is available along with various gas sources including nitrogen, oxygen, and argon. Three different data

acquisition systems are available in the lab. A National Instruments Data Acquisition and Control system is available for use on the experiment. Two CAP research systems are available including one with an XY-stage enclosed in a box to allow for gas venting and a second system also in a vented enclosure. Compressed air, argon, nitrogen, and oxygen are available in the lab for CAP testing. One computer system is dedicated to the National Instruments data acquisition hardware for this experiment. The lab also has a substantial tool set including drills, saws, and punches.

Plasma Array Test Systems: The Plasma and Vacuum Electronic Devices laboratory has two test systems. Specifically, the systems use an AC (8 kV, 20 kHz) power supply. A new power supply (PVM2000) for higher power has been purchased. It is capable of 10 kV, 200 mA from 20-60 kHz and it able to drive a load capacitance of >200 pF which is need for the HIPA. These setups will be used for the array testing. One configuration consists of an XY-stage enclosed in a box for exhaust purposes. A mass flow controller is used to measure the air flow rate into the array. An Agilent DSO5014A oscilloscope will be used to measure the AC voltage and current transformer signals and current across ballast resistors. Additional supporting equipment includes a National Instruments (NI) PXI-1033 crate and a NI PXI-6229 data acquisition system with the needed computer to operate the system. This system will control the plasma array operation including the AC and DC power supplies, the gas flow controllers, and the positioning stage. A new optical spectrometer (Flame Brand) will be purchased and mounted on the plasma system. The Model Flame-T-UV-VIS-ES spectrometer which operates from 200 nm - 850 nm (1.34 nm resolution) will be spectrometer will be integrated into the LabView system. A General Model UV512C UV meter is used to measure the UV intensity from 220 to 275 nm. A FLIR systems ThermoVision A320 Camera is used to measure the temperature of the treated samples and the CAP structures.

<u>LTCC Fabrication</u>: This project will require the fabrication of Low Temperature Co-Fired Ceramic devices, which is currently available in the Ceramic MEMS laboratory. All the fabrication equipment required for this project is available including a Lindberg box furnace, an nScrypt direct write system, an MPM speedline screen printer, a PHI uniaxial laminator and a KEKO isostatic laminator, a 30W Universal LASER router, and a Bungard PCB milling machine.

# SHARED FACILITIES RESOURCES

**Engineering & Material Sciences:**\_Students have access to the user facilities at the Surface Science Lab and the Center for Advanced Energy Studies (CAES), both of which are located within the MCMR building. The facilities house state-of-the-art instrumentation for characterization, including a transmission electron microscopy, dynamic light scattering, atomic force microscopes, UV-vis spectrometers, spin coaters, infrared spectrometers, etc. The UV-vis and infrared spectrometers will be used to determine the bulk adsorption of the organic molecules of interest.

# Appendix B – Biographical Sketches

Effective 05/20/2024

NSF BIOGRAPHICAL SKETCH

OMB-3145-0279

### **IDENTIFYING INFORMATION:**

#### NAME: Cornell, Kenneth A

ORCID iD: https://orcid.org/0000-0003-3959-2499

POSITION TITLE: Professor

PRIMARY ORGANIZATION AND LOCATION: Dept. Chemistry & Biochemistry, Boise State University, Boise, ID, USA 83725

#### Professional Preparation:

ORGANIZATION AND LOCATION	DEGREE	RECEIPT DATE	FIELD OF STUDY
Oregon Health Sci. Univ, Portland, OR, USA	Ph.D.	02/1997	Biochemistry
Wichita State University, Wichita, KS, USA	M.S.	12/1985	<b>Biological Sciences</b>
Oregon State University, Corvallis, OR, USA	B.S.	05/1983	Microbiology

#### Appointments and Positions

2023 – pres	Director, IDeA Programs Office, Boise State University, Boise, Idaho, USA
2023 – pres	Assoc. Director, Center Biomedical Res. Excellence, BSU, Boise, Idaho, USA
2019 – pres	Professor, Dept. Chemistry & Biochemistry, Boise State Univ., Boise, ID, USA
2017 – pres	Director, Data Science Core, Idaho INBRE program, BSU, Boise, ID, USA
2012 – 2020	Associate Director, Biomolecular Research Center, BSU, Boise, ID, USA
2009 – 2019	Associate Professor, Dept. Chemistry & Biochemistry, BSU, Boise, ID, USA
2004 – 2009	Assistant Professor, Dept. Chemistry & Biochemistry, BSU, Boise, ID, USA
2002 – 2004	Senior Scientist, ACRYMED, Inc., Portland, OR, USA
2002 – 2004	Clinical Res. Asst Professor, Vascular Surgery, OHSU, Portland, OR, USA
1999 – 2002	Staff Scientist, Molecular Group Leader, INTERLAB, Inc., Portland, OR, USA
1997 – 1999	Postdoctoral Fellow, Portland VA Medical Center, Portland, OR, USA
1988 – 1991	Research Associate, Arthritis & Rheumatic Dis., OHSU, Portland, OR, USA
1985 – 1987	Science Teacher, U.S. Peace Corps, Sokoke Harambee School, Kilifi, Kenya
1983	Microbiologist, Stayton Canning Company, Stayton, OR, USA

#### **Products**

Products Most Closely Related to the Proposed Project

- Cornell KA, Benfield K, Berntsen T, Clingerman J, Croteau A, Goering S, Moyer D, Provost M, White A, Plumlee D, Oxford JT, Browning J. A Cold Atmospheric Pressure Plasma Discharge Device Exerts Antimicrobial Effects. *Int J Latest Trends Eng Technol.* 2020 Jan; 15(3): 36-41. PMCID: <u>PMC7098701</u>.
- 2. **Cornell KA**, White A, Croteau A, Carlson J, Kennedy Z, Miller D, Provost M, Goering S, Plumlee D, Browning J. Fabrication and Performance of a Multi-Discharge Cold

Atmospheric Pressure Plasma Array. *IEEE Trans Plasma Sci.* **2021** Apr; 49(4): 1388-1395. PMCID: <u>PMC8132946</u>.

- Croteau A, White A, Cornell KA, Browning J. Cold Atmospheric Pressure Plasma Device Exhibits Etching Effects on Bacterial Biofilms. *IEEE Trans Radiat Plasma Med Sci.* 2022 May; 6(5): 619-625. PMCID: <u>PMC9629775</u>.
- Okebiorun M, Oberbeck C, Waite C, Clark S, Miller D, Barney-Smith EH, Cornell KA, Browning J. Selective Optical Imaging for Detection of Bacterial Biofilms in Tissues. J Imaging. 2023 Aug 15; 9(8): 160. PMCID: <u>PMC10455256</u>.
- Browning J, Cornell KA. Inventors. Boise State University, assignee. Plasma Scalpel for Selective Removal of Microbes and Microbial Biofilms. *United States Patent* 11,871,978.
  2024 Jan 16.

#### Other Significant Products, Whether or Not Related to the Proposed Project

- Cornell KA, Knippel RJ, Cortright GR, Fonken M, Guerrero C, Hall AR, Mitchell KA, Thurston JH, Erstad P, Tao A, Xu D, Parveen N. Characterization of 5'methylthioadenosine/S-adenosylhomocysteine nucleosidases from *Borrelia burgdorferi*: Antibiotic targets for Lyme disease. *Biochim Biophys Acta Gen Subj.* 2020 Jan;1864(1): 129455. PMCID: <u>PMC6881558</u>.
- Chakraborti M, Schlachter S, Primus S, Wagner J, Sweet B, Carr Z, Cornell KA, Parveen N. Evaluation of Nucleoside Analogs as Antimicrobials Targeting Unique Enzymes in *Borrelia burgdorferi*. *Pathogens*. 2020 Aug 20; 9(9): 678. PMCID: <u>PMC7557402</u>.
- Thurston JH, Clifford AJ, Henderson BS, Smith TR, Quintana D, Cudworth KF, Lujan TJ, Cornell KA. Development of Photoactive g-C(3)N(4)/Poly(vinyl alcohol) Composite Hydrogel Films with Antimicrobial and Antibiofilm Activity. ACS Appl Bio Mater. 2020 Mar 16; 3(3): 1681-1689. PMCID: <u>PMC7968866</u>.
- Thurston JH, Vitale-Sullivan M, Koshkimbayeva A, Smith TR, Cornell KA. 1,4,5,8-Naphthalene tetracarboxylate dianhydride/g-C(3)N(4) van der Waals heterojunctions exhibit enhanced photochemical H(2)O(2) production and antimicrobial activity. *RSC Adv.* 2021 Oct 28; 11(56): 35425-35435. PMCID: <u>PMC9043264</u>.
- Pradhan R, Tiwari L, Groner VM, Leach C, Lusk K, Harrison NS, Cornell KA, Waynant KV. Evaluation of azothioformamides and their copper(I) and silver(I) complexes for biological activity. *J Inorg Biochem.* 2023 Sep; 246: 112294. PMCID: <u>PMC10681367</u>.

#### **Certification:**

I certify that the information provided is current, accurate, and complete. This includes but is not limited to current, pending, and other support (both foreign and domestic) as defined in 42 U.S.C. §6605. I also certify that, at the time of submission, I am not a party to a malign foreign talent recruitment program.

Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§ 287, 1001, 1031 and 31 U.S.C. §§ 3729-3733 and 3802.

Certified by Cornell, Kenneth A in SciENcv on 2024-07-28 22:01:23

Effective 05/20/2024

#### **IDENTIFYING INFORMATION:**

#### NAME: Browning, Jim

ORCID iD: <u>https://orcid.org/0000-0002-2768-1817</u>

POSITION TITLE: Professor

PRIMARY ORGANIZATION AND LOCATION: Dept. Electrical & Computer Engineering, Boise State University, Boise, ID, USA 83725

#### Professional Preparation:

ORGANIZATION AND LOCATION	DEGREE	RECEIPT DATE	FIELD OF STUDY
University of Wisconsin, Madison, WI, USA	Ph.D.	07/1988	Nuclear Engineering & Engineering Physics
Missouri University of Science & Technology, Rolla, MO, USA	M.S.	01/1985	Nuclear Engineering
Missouri University of Science & Technology, Rolla, MO, USA	B.S.	05/1983	Nuclear Engineering

#### **Appointments and Positions**

2006 – present	Professor, Electrical & Computer Engineering, BSU, Boise, ID, USA
2021 – 2024	Associate Dean of Research, College of Engineering, BSU, ID, USA
2001 – 2006	Consultant, WatBro Consulting, Boise, ID, USA
1998 – 2001	Research & Development Manager, PixTech Inc., Boise, ID, USA
1992 – 1998	Research & Development Manager, Micron Technology, Field Emission
	Display Division, Boise, ID, USA
1988 – 1992	Senior Research Associate & Visiting Professor, Northeastern University, Electrical Engineering, Boston, MA, USA

#### **Products**

#### Products Most Closely Related to the Proposed Project

- Cornell KA, Benfield K, Berntsen T, Clingerman J, Croteau A, Goering S, Moyer D, Provost M, White A, Plumlee D, Oxford JT, **Browning J**. A Cold Atmospheric Pressure Plasma Discharge Device Exerts Antimicrobial Effects. *Int J Latest Trends Eng Technol.* 2020 Jan; 15(3): 36-41. PMCID: PMC7098701.
- Cornell KA, White A, Croteau A, Carlson J, Kennedy Z, Miller D, Provost M, Goering S, Plumlee D, Browning J. Fabrication and Performance of a Multi-Discharge Cold Atmospheric Pressure Plasma Array. *IEEE Trans Plasma Sci.* 2021 Apr; 49(4): 1388-1395. PMCID: <u>PMC8132946</u>.
- Croteau A, White A, Cornell KA, Browning J. Cold Atmospheric Pressure Plasma Device Exhibits Etching Effects on Bacterial Biofilms. *IEEE Trans Radiat Plasma Med Sci.* 2022 May; 6(5): 619-625. PMCID: <u>PMC9629775</u>.
- 4. Okebiorun M, Oberbeck C, Waite C, Clark S, Miller D, Barney-Smith EH, Cornell KA, **Browning J**. Selective Optical Imaging for Detection of Bacterial Biofilms in Tissues. *J*

Imaging. 2023 Aug 15; 9(8): 160. PMCID: PMC10455256.

 Okebiorun M, Oberbeck C, Waite C, Clark S, Alomar Z, Miller D, Cornell KA, Browning J. Autofluorescence-Guided Removal of Bacterial Biofilms from Tissues using Cold Atmospheric Pressure Plasma (CAP). *IEEE Trans Radiat Plasma Med Sci.* 2024 Feb; 8(8): 990-996. <u>doi: 10.1109/TRPMS.2024.3370503</u>.

### Other Significant Products, Whether or Not Related to the Proposed Project

- Yue A, Browning J. Electron population analysis techniques for understanding fundamental cross-field electron device physics. *IEEE Transactions on Plasma Science*. 2022; 50(6):1775-1780. <u>issn: 0093-3813</u>.
- Pearlman M, Smithe D, Roark C, Worthington M, Watrous J, Garner A, Browning J. Simulation of a Pulsed 4.7 MW L-Band Crossed-Field Amplifier. *IEEE Transactions on Electron Devices*. 2022; 69(12):7053-7058. <u>issn: 0018-9383</u>.
- Browning J, Cornell KA. Inventors. Boise State University, assignee. Plasma Scalpel for Selective Removal of Microbes and Microbial Biofilms. *United States Patent* 11,871,978.
  2024 Jan 16.

# **Certification:**

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Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§ 287, 1001, 1031 and 31 U.S.C. §§ 3729-3733 and 3802.

Certified by Browning, Jim in SciENcv on 2024-11-13 20:42:21

#### **IDENTIFYING INFORMATION:**

#### NAME: Pearlman, Marcus

ORCID iD: https://orcid.org/0000-0003-1792-179X

POSITION TITLE: Research Scientist

PRIMARY ORGANIZATION AND LOCATION: Dept. Electrical & Computer Engineering, Boise State University, Boise, ID, USA 83725

#### Professional Preparation:

r rolessional r reparation.			
ORGANIZATION AND LOCATION	DEGREE	RECEIPT DATE	FIELD OF STUDY
Boise State University, Boise, ID, USA	Ph.D.	05/2017	Electrical & Computer Engineering
Boise State University, Boise, ID, USA	M.S.	05/2012	Electrical & Computer Engineering
University of Colorado, Boulder, CO, USA	B.S.	05/2005	Electrical Engineering

#### Appointments and Positions

2020 – present Research Scientist, Electrical & Computer Engineering, Boise, ID, USA
2019 – 2020 Lab Manager, Electrical & Computer Engineering, BSU, Boise, ID, USA
2017 – 2018 Post-Doctoral Researcher, Electrical & Computer Engineering, BSU, Boise, ID, USA

#### Products

Products Most Closely Related to the Proposed Project

- Pearlman M, Browning J. Simulation of a Time-Varying Distributed Cathode in a Linear Format Crossed-Field Amplifier. *IEEE Transactions on Plasma Science*. 2019 July; 46(7): 1-7. DOI: <u>10.1109/TPS.2019.2924376</u>
- Pearlman M, Smithe D, Roark C, Worthington M, Watrous J, Garner A, Browning J. Simulation of a Pulsed 4.7 MW L-Band Crossed-Field Amplifier. *IEEE Transactions on Electron Devices*. 2022; 69(12): 7053-7058. DOI: <u>10.1109/TED.2022.3218267</u>
- 3. **Pearlman M**, Okebiorun M, Waite C, Miller D, Koch T, Escoba J, Tenorio J, Plumlee D, Cornell K, Browning J. Biofilm Remediation Using Cold Atmospheric Pressure Plasma Planar and Radial Arrays; *2022 IEEE International Conference on Plasma Science* (*ICOPS*), Seattle, WA, USA, **2022**, pp. 1-2, doi: <u>10.1109/ICOPS45751.2022.9813128</u>.
- Pearlman M, Lupercio A, Rektor A, Lamb J, Fleming A, Jaques B, Subbaraman H, Kandadai N. Infrared Thermography Method to Detect Cracking of Nuclear Fuels in Real-Time. *Nuclear Engineering and Design*. 2023; 405:112196. https://doi.org/10.1016/j.nucengdes.2023.112196
- Pearlman M, Smithe D, Worthington M, Watrous J, Garner AL, Browning J. Spoke Characterization of Re-Entrant Backward Wave Crossed-Field Amplifiers via Simulation. *IEEE Transactions on Electron Devices*. 2024; 71: 520-527. DOI:10.1109/TED.2024.3414379

#### Other Significant Products, Whether or Not Related to the Proposed Project

- 1. **Pearlman M**. Investigation of the Current Transmission Hysteresis in Electron Hop Funnels. M.S. Thesis. Boise State University; 2012.
- Pearlman M, Rowe T, Browning J. Simulation of Electron Hop Funnel Hysteresis. *IEEE Transactions on Plasma Science*. 2013 August; 41(8): 2291-2298. DOI: <u>10.1109/TPS.2013.2271998</u>
- Rowe T, Pearlman M, Browning J. Hysteresis in Experimental I-V Curves of Electron Hop Funnels. *Journal of Vacuum Science and Technology B*: Microelectronics and Nanometer Structures. 2013 July; 31(4): 042204. <u>https://doi.org/10.1116/1.4813779</u>
- 4. **Pearlman M**. Simulation of a Crossed-Field Amplifier Using a Modulated Distributed Cathode. Ph.D. Dissertation. Boise State University; **2017**.
- Pearlman M, Browning J. Simulation of a Distributed Cathode in a Linear-Format Crossed-Field Amplifier. *IEEE Transactions on Plasma Science*. 2018 June; 46(7): 2497-2504. DOI: <u>10.1109/tps.2018.2844732</u>

#### **Certification:**

I certify that the information provided is current, accurate, and complete. This includes but is not limited to current, pending, and other support (both foreign and domestic) as defined in 42 U.S.C. §6605. I also certify that, at the time of submission, I am not a party to a malign foreign talent recruitment program.

Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§ 287, 1001, 1031 and 31 U.S.C. §§ 3729-3733 and 3802.

Certified by Pearlman, Marcus in SciENcv on 2024-07-19 13:56:39

# Appendix C – Senior Personnel

**Senior Personnel** includes the Principle investigator, Co-PI, Research Scientist, Consultant, and Ph.D. Graduate Student.

**Principle investigator.** Dr. Ken Cornell is a Professor in the Dept. of Chemistry & Biochemistry, and the Director of the BSU IDeA Programs office that manages INBRE and COBRE programs at Boise State. Dr. Cornell earned his Ph.D. in Biochemistry & Molecular Biology from Oregon Health Sciences University in 1997. He will design antimicrobial and mechanistic experiments, analyze results, write reports, and assemble manuscripts of the research. He will supervise the Ph.D. graduate student (S. Rood) and co-supervise the Research scientist (M. Pearlman) on the proposed project. Dr. Cornell will be the primary contact with industry collaborators and advisors (IEOSA, Crookham Farms) and work to develop new contacts in the seed growing industry during the course of the project. Dr. Cornell requests 0.5 months of salary support for his efforts directing the project. Dr. Cornell's workload at BSU includes 50% release time for research that he will devote to this project.

**Co-Principle investigator.** Dr. Jim Browning is a Professor in the Dept. of Electrical & Computer Engineering, and the PI of the Center of Biomedical Research Excellence in Convergent Engineering & Biomolecular Sciences. Dr. Browning earned his Ph.D. in Nuclear Engineering & Engineering Physics from the University of Wisconsin – Madison in 1988. He will work on the design of 10 cm x 10 cm plasma arrays and their integration into machinery (conveyor belts, gravity tables) that are used in seed processing. He will supervise the efforts of the Research scientist (M. Pearlman) and work closely with the consultant (Dr. Hay) on the implementation of the plasma device arrays. Dr. Browning's workload at BSU allows him to devote 100% of his efforts to research. We request 0.5 months of salary support for his efforts on the project.

**Research scientist.** Dr. Marcus Pearlman is a Research Scientist in the Dept. of Electrical & Computer Engineering. Dr. Pearlman earned his Ph.D. in Electrical Engineering at Boise State University in 2017 with expertise in computer simulation, electrical device design and fabrication. He will oversee the fabrication and assembly of the 10 cm x 10 cm plasma devices and enclosures that are configured into arrays to cover large areas of seed processing equipment. He will perform testing and troubleshooting of the full array, measure the current-voltage characteristics and plasma uniformity, implement the gas injection and capture systems, and develop the complete plasma power driver system in collaboration with Dr. Hay (consultant) and Dr. Browning (co-PI). The completed "turn-key" system will be installed in the Cornell lab for plasma treatment of pathogen contaminated seeds. Dr. Pearlman will continue to update and troubleshoot the system and support treatment experiments during the entire project year. Salary support is requested for 5 mo. of effort that Dr. Pearlman will devote to the project.

**Emeritus faculty/Sr. investigator.** Dr. Bob Hay is a Professor Emeritus in the Dept. of Electrical & Computer Engineering at Boise State University. Dr. Bob Hay will further develop the plasma driver system for the plasma array device. <u>His recent work on the plasma driver system is part of a patent disclosure to Boise State University</u>. This plasma driver system consists of an H-bridge driver, a resonant frequency tuning controller, and a 600 V DC-DC converter. The new work will include making the system capable of initiating and tuning the driver to the resonant frequency of the CAP array when room air is used for the plasma. Development will also include reducing size and making the entire system more compatible with

industrial settings. We request ~ \$6,000 (\$60/hr; 100 hr) for this development to support Dr. Hay's efforts on the project.

**Ph.D. graduate student.** Stephanie Rood is a student in the BSU Biomolecular Ph.D. program. Her dissertation research is focused on examining the antimicrobial effects of cold atmospheric plasma treatment on plant and animal bacterial and fungal pathogens, and elucidating molecular mechanisms of plasma action. She will devote 12 months of effort to the project to test the activity of the 10 cm x 10 cm array devices on contaminated seeds on conveyor and gravity table processing equipment. Support is requested to cover the costs of Ms. Rood's graduate tuition, fees, stipend, and health insurance.

# Appendix D – Letters of Support

While this is a "Proof of Concept" application and letters of support are not required, our collaborators/advisors in the seed crop industry have graciously provided letters supporting the continued development of our plasma technology.

Letters from the following industry representatives are included in this appendix:

- Mr. Roger Batt, Executive Director, Idaho-Eastern Oregon Seed Association (IEOSA). The IEOSA is an industry association that represents over 100 seedgrowers and related businesses in the region.
- Mr. Dennis Demshar, Popcorn breeder and seed quality researcher, Crookham Company. The Crookham Company is one of the largest seed producers in the SW Idaho – Eastern Oregon region, and specializes in sweet corn, popcorn, and onion seed.



# **IDAHO - EASTERN OREGON SEED.ASSOCIATION**

55 S.W. 5th Ave. #100 • Meridian, ID 83642 208-888-0988 Fax: 208-888-4586

February 18, 2025

IDAHO - EASTERN OREGON SEED ASSOCIATION

Ken Cornell, Ph.D. Dept. Chemistry & Biochemistry Boise State University 1910 University Dr. Boise, ID 83725-1520

RE: Improving Idaho Seed Crops with Cold Atmospheric Pressure Plasma (CAP) Technology

#### Dear Dr. Cornell,

I am happy to supply this letter of support for your proposal entitled "Improving Idaho Seed Crops with Cold Atmospheric Pressure Plasma (CAP) Technology" to the Idaho HERC program reduce the presence of plant pathogens on seeds.

As you are aware the IEOSA represents the interests of the seed industry in Idaho and Eastern Oregon, which is made up of over 120 seed producers and related businesses. Our region has an ideal climate for seed production, and is in among the top 5 seed producing areas in the world. Seeds grown here are responsible for a huge proportion of the nation's and world's crop production and are shipped to over 120 countries across the globe. This includes producing and shipping 65% of the world's hybrid sweet corn seed. Our region is also renowned for its production of seeds for alfalfa, carrots, green beans, onions, peas, radishes, and turnips. Increasingly, we are also producing more oil seeds, such as canola, safflower and sunflower.

Technologies that reduce the burden of plant pathogens on seeds would improve the value of our seed crops, and reduce the spread of bacterial, fungal and viral plant diseases that impact germination rates and crop productivity for our consumers in the agricultural industries. Your plasma technology could have a great benefit for our industry and improve our competitiveness in the seed production industry as a whole.

We wish you every success in your grant application and your research, and look forward to hearing more about your project in the future.

Sincerely,

Roger Batt Executive Director Idaho – Eastern Oregon Seed Association

Appendix D Letters of Support



Ken Cornell, Ph.D. Professor, Department of Chemistry & Biochemistry Director, IDeA Programs Office Boise State University Boise, ID 83725

February 18, 2025

RE: Improving Idaho Seed Crops with Cold Atmospheric Pressure Plasma (CAP) Technology

Dear Dr. Cornell,

It was a pleasure to meet with you last Fall and discuss your project to develop cold atmospheric pressure technology to treat seeds and reduce the spread of plant diseases. I fully support your HERC proposal to engineer plasma arrays to treat seeds during processing. As we discussed during you and your student's visit to our seed processing plant, there are several steps in seed processing and preparation for packaging that may be amenable to the CAP technology. I look forward to our interactions on how CAP technology can be used to treat our crop seeds.

The Crookham Company is a family owned company founded in 1911, with headquarters in Caldwell, Idaho. It is a leading producer of sweet com, popcorn, and onion seed crops that are marketed around the globe. We have- 125 employees and have an annual revenue of \$36M. As you may be aware, this region of SW Idaho among the top 5 producers of crop seeds on the planet due to its high desert climate and abundant water. It is one of the primary regions for growth sweet corn seeds, particularly SH2 and SU varieties that are specialties of Crookham Co.

To ensure high germination properties of our seeds, they are processed to remove undesirables (pieces of husk, cob, non-uniform seeds, etc) and select for denser seeds that have the highest germination efficiency. Depending on the market, the seeds may be further treated with a fungicidal cocktail to improve seed survival during the next seasons planting. This is particularly important when the seed is planted in cool, wet spring ground that favors fungal growth and negatively impacts germination.

Unfortunately, many of our international markets (Japan, Canada, European Union) have banned a number of the active ingredients in these treatments. In addition, for farmers specializing in organic produce, these treated seeds are often not deemed suitable. To meet the needs of these markets, we have untreated seed products, but their germination efficiency can drop to half of the 90% germination that we expect with our fungicide treated seeds. This means higher costs for the farmer, since they must plant more seed in order to get the same level of crop. Obviously, this raises their costs. Treatment that reduce the carriage of fungal and bacterial diseases on seeds (without reducing germination rates) could be highly beneficial to the seed crop industry, and improve the sale of our products to both here in the U.S. as well as abroad. Ultimately, this would be a benefit to our company, as well as the many other seed producers in the SW Idaho region.

Good luck with your application to the Idaho HERC-IGEM program.

meh mis

Dennis Demshar Popcorn Breeder and Quality Researcher Crookham Company PO Box 520 Caldwell, ID 83606-0520 dennisd@crookham.com