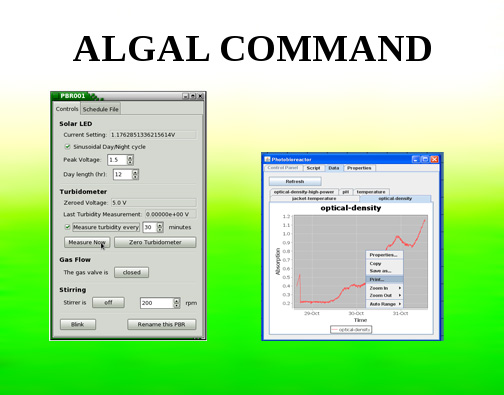
1. ******
2. ***Algal Command Software***
3. **User Guide**

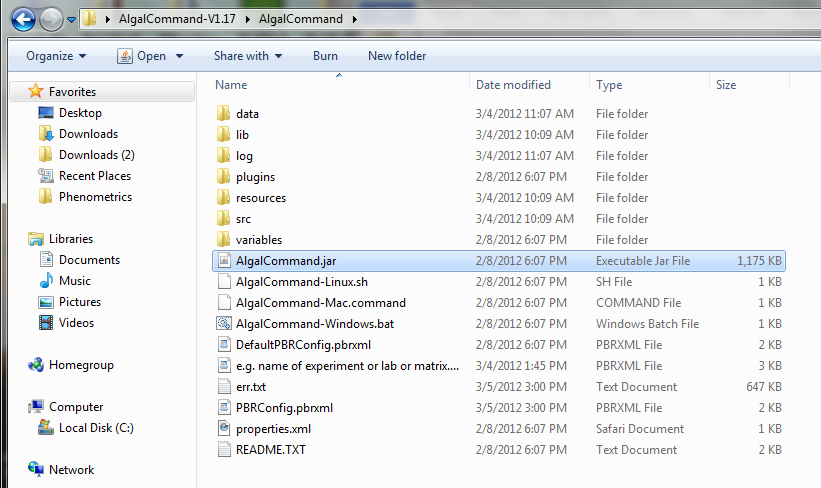
September 8, 2012

* 1. ***Algal CommandTM Software User Guide***

1. **Computer Requirements to run AlgalCommand software for the Bioreactor System:**
2. Processor: 1.7+GHz Multicore processor
3. Memory: 3+ GB RAM, 10 GB free hard-drive space (100+ GB recommended) Network card
4. HID: Mouse, keyboard, and monitor
5. OS: Windows XP or later (Windows 7 64bit recommended)
6. MAC OS X 10.6 or later
7. Linux (Ubuntu or Fedora with classic desktop recommended)
8. Software: Java SE 6 or later installed (Java SE 7 recommended), available at <http://java.oracle.com>

## Installation

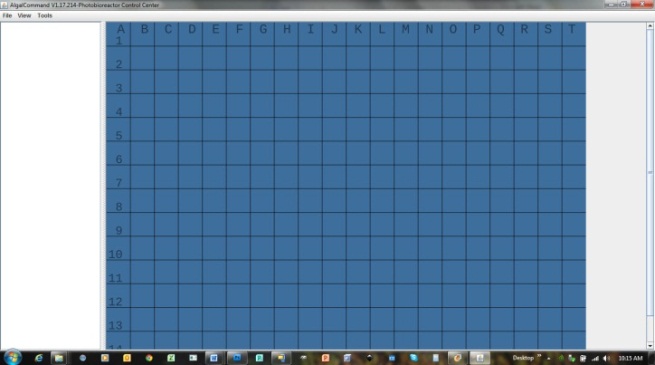
To install AlgalCommand, extract the Algal Command.zip file to a convenient location (such as in “My Document” or on the desktop).



Connect Ethernet cable from the ePBR to the router then another cable from the router to the computer. See ‘Installation Guide’ pg.\_\_\_

Connect the power cable and turn on power switch on the ePBR controller tower.

**Turn off WIFI on the computer – the WIFI will interfere with finding the ePBRs on its network.**

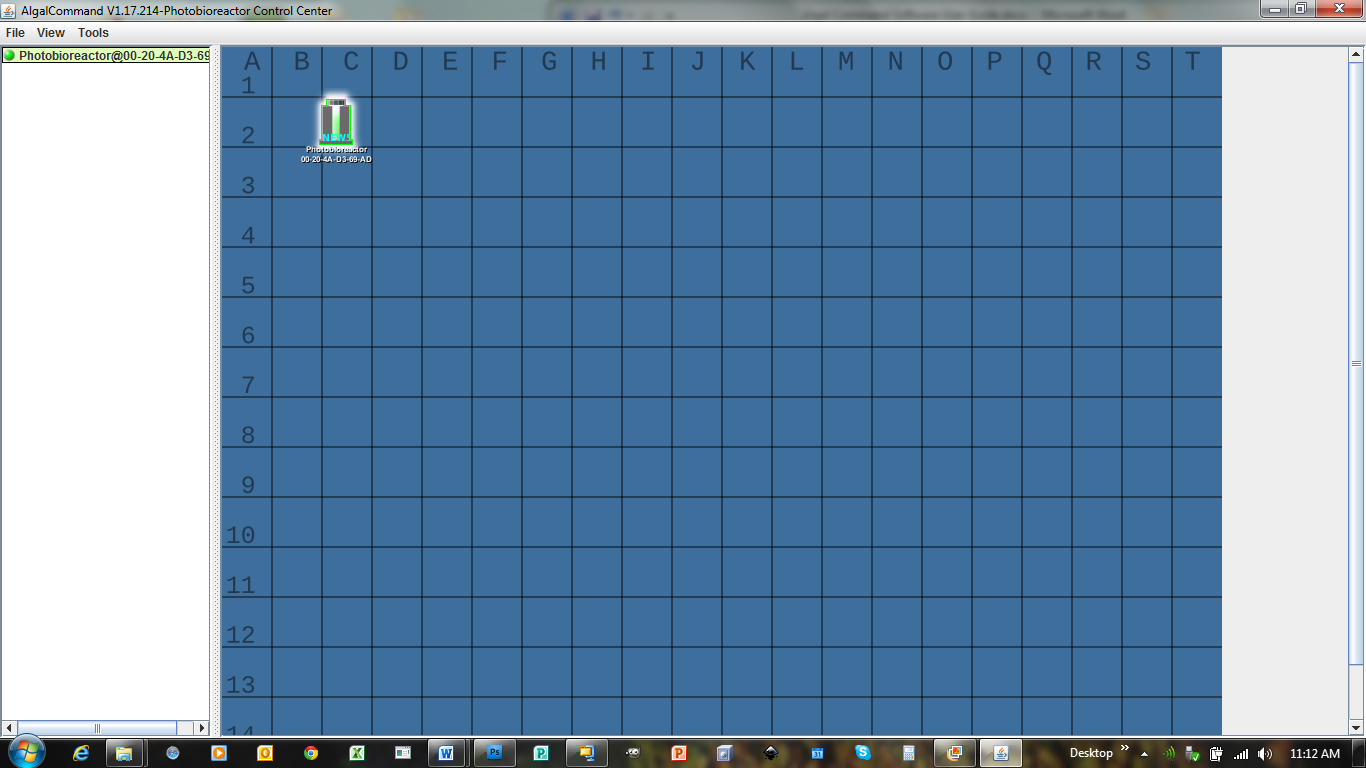
Open ‘AlgalCommand.jar’ file. You should see a grid of a floor plan.

## Using Algal Command Software

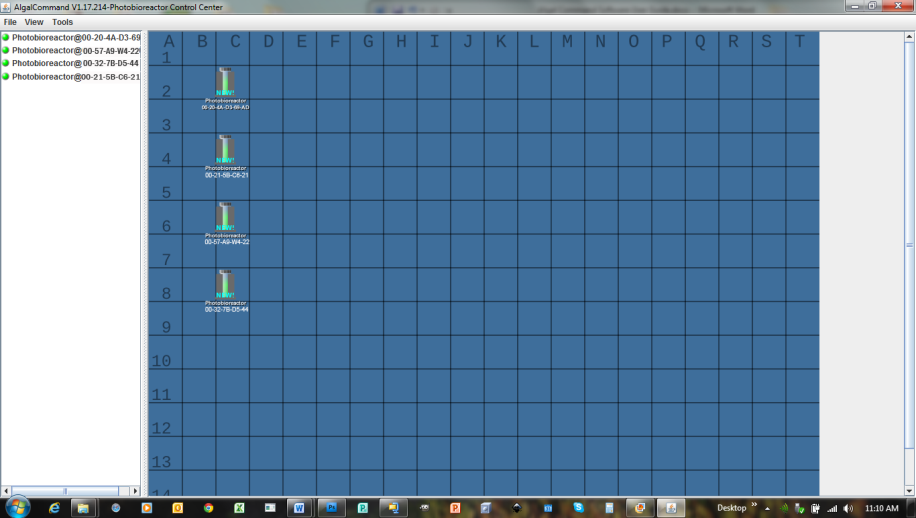
### FloorPlan Window

After opening the program, wait for a couple of minutes for the software to talk with the PBR, until you see an icon for the ePBR on the floor plan.

*If no icon shows up after a few minutes, then go to FILE>Check Network, and wait again. This should bring up the icons. If not, check to see that the ePBR is turned on and the Ethernet cable is connected appropriately.*

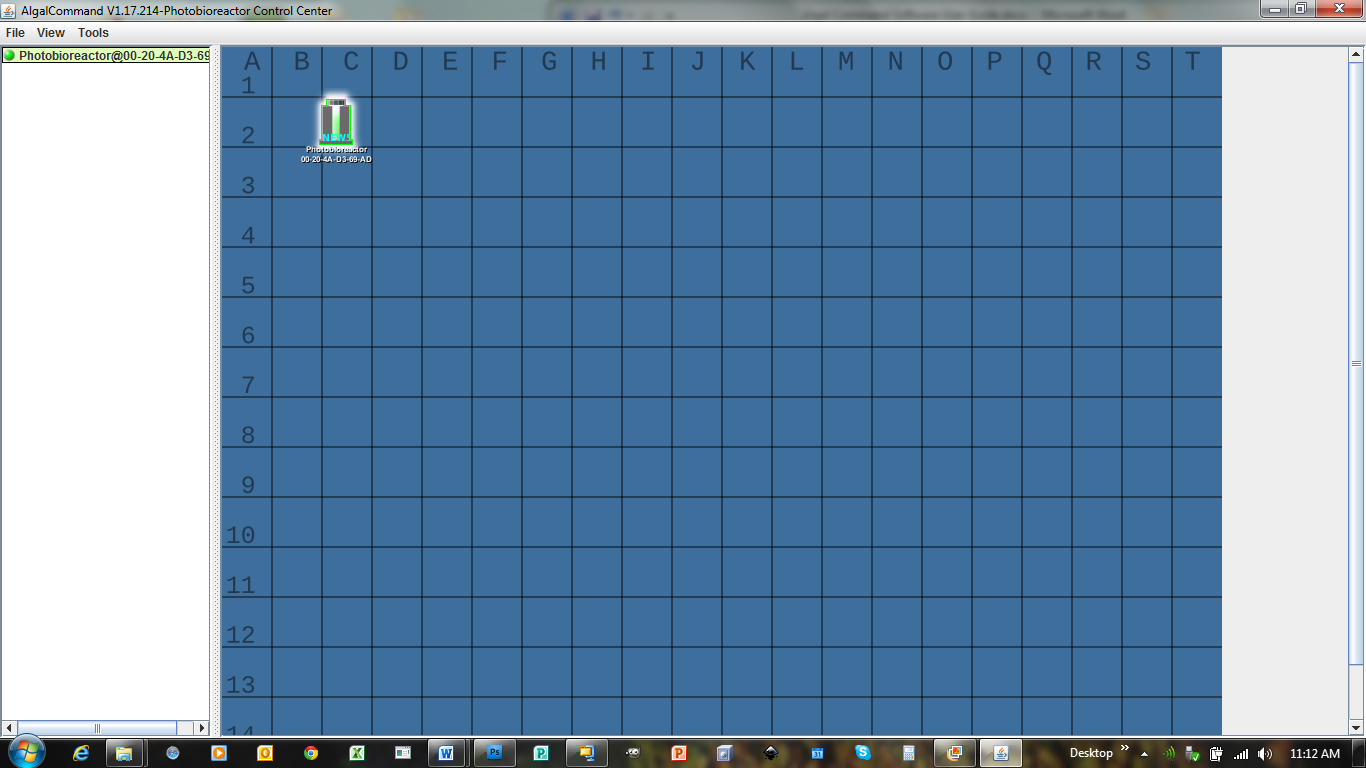
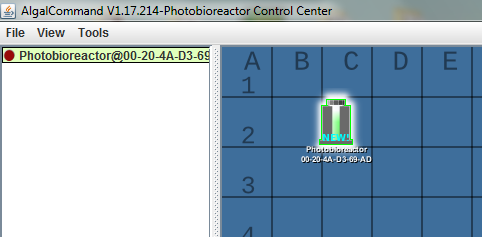
You can select/deselect PBR icons by shift-clicking on them. Selected ePBRs are also highlighted in the PBR list.

If you have more than one ePBR on the system you will see an icon for each of the units.



#### PBR List

The panel to the left of the floor plan area is the *PBR list*. Each ePBR in this list has a status icon followed by the name of the ePBR, followed by the '@' symbol and the unique hardware identifier (also known as the “Hardware Address”) for the ePBR. Selecting an ePBR in the list will also highlight the corresponding icon in the floor plan area. If you turn off the power of the particular ePBR the green dot in the ePBR list will turn red. This tells you when a unit is offline.

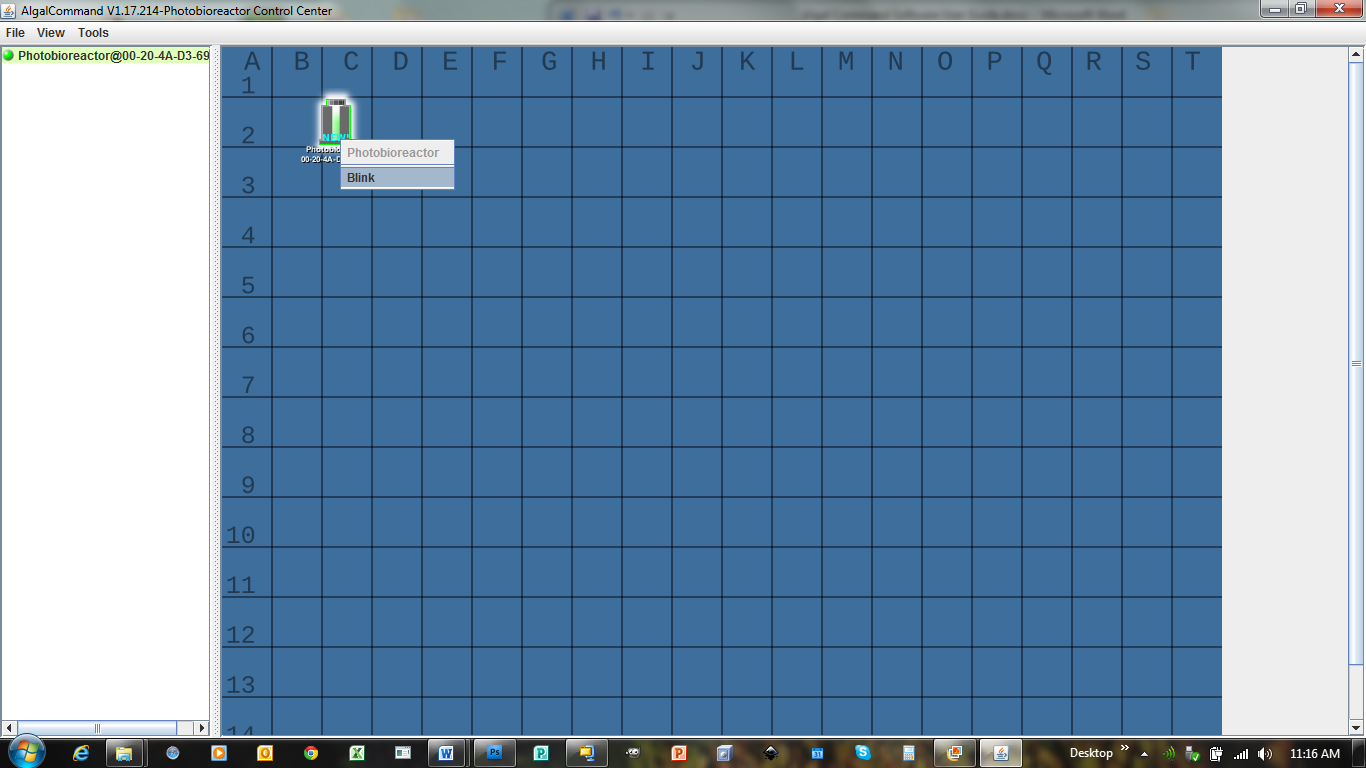
#### Floor Plan View and ePBR Icons

Each photo bioreactor is displayed as an icon in the floor plan area.

Sometimes when the multiple icons come in for the first time they are layered on top of each other in one spot. To automatically lie them out on the grid go to VIEW>Sort, and the icons will be distributed on the grid from top to bottom.

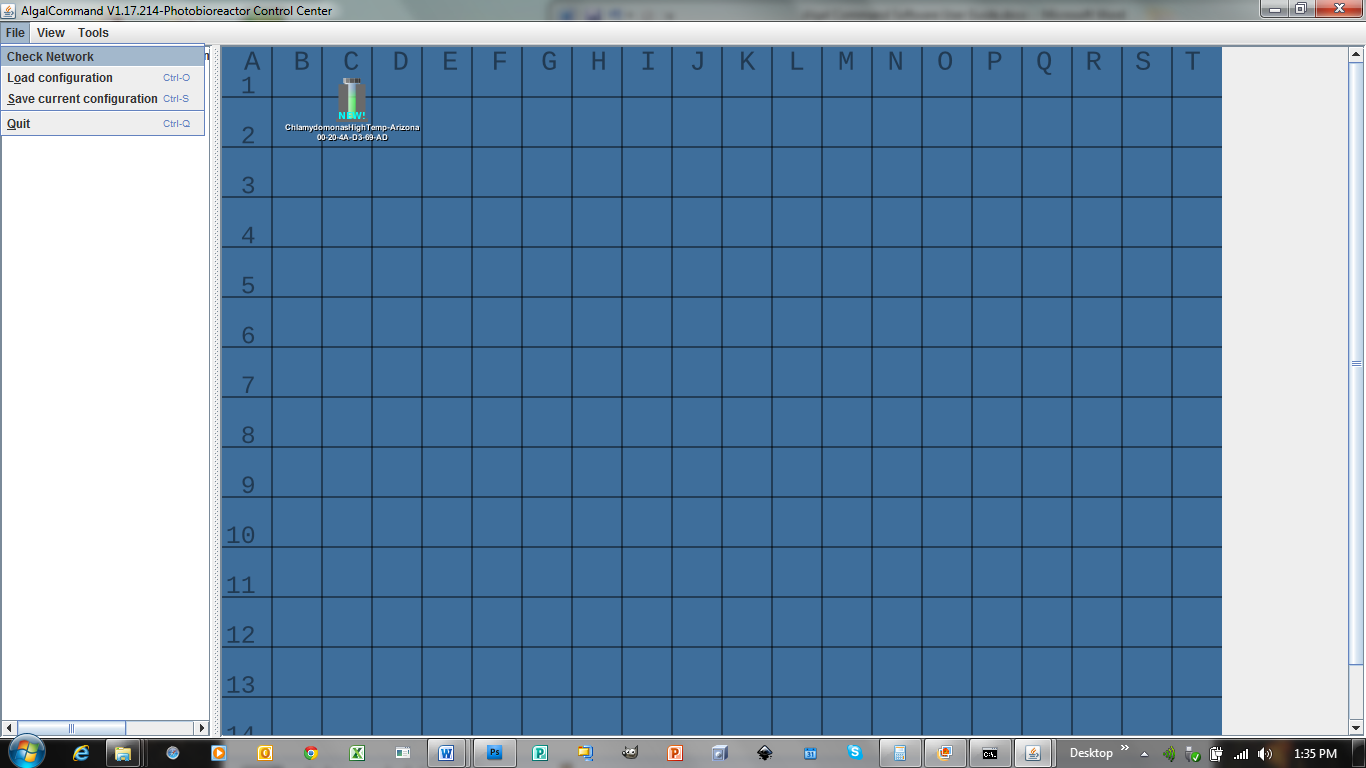
You can move the ePBR icons around the floor plan by holding down the left-button of the mouse on top of the icon and dragging it to the desired location. Dragging the icon off the floor plan image may cause the icon to jump back to its original position before you dragged it.

Right-clicking the mouse on a PBR icon will bring up a pop-up menu. If you select “Blink” from this menu, the ePBR instrument will rapidly flash its lights for 10 seconds. This feature is useful for determining which piece of hardware you are controlling when there are more than one ePBR on the network.



**MENU FUNCTIONALITY**

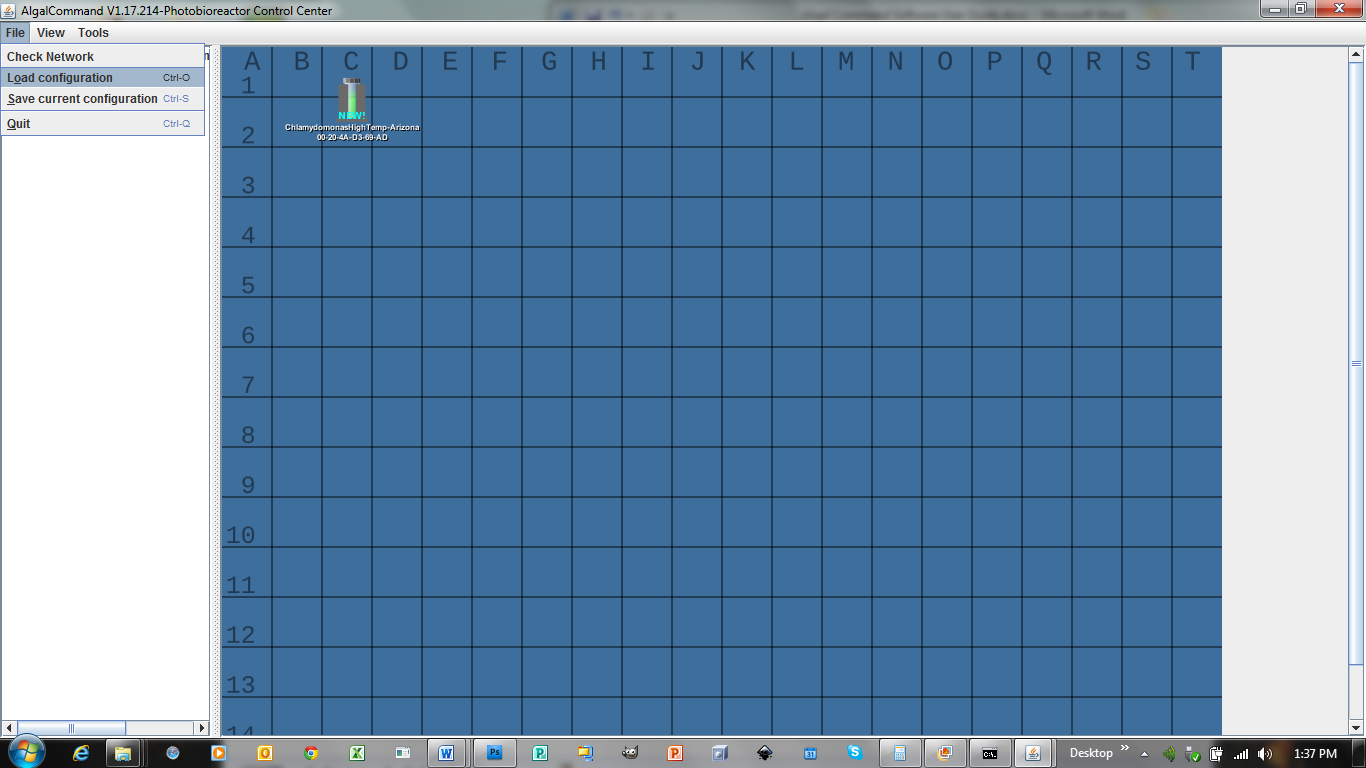
**The File Menu**

FILE>Check Network

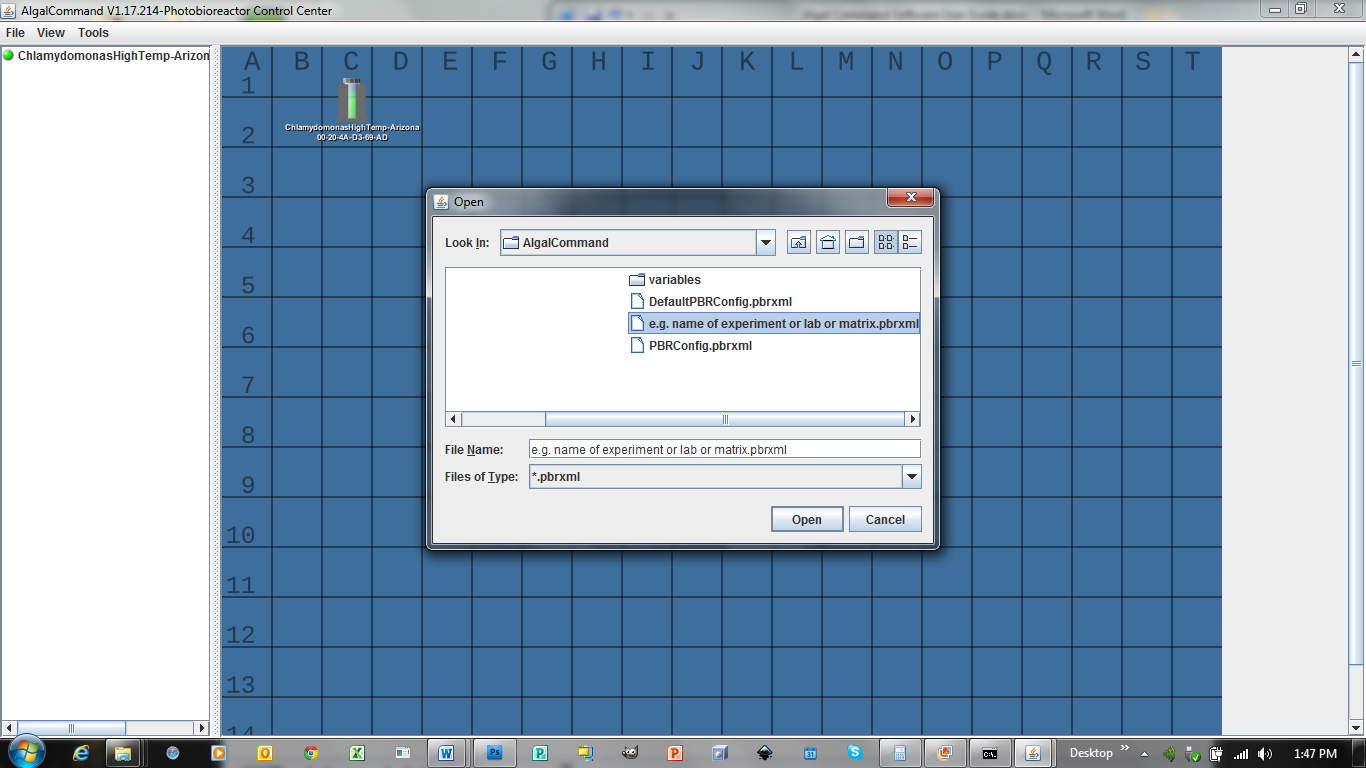
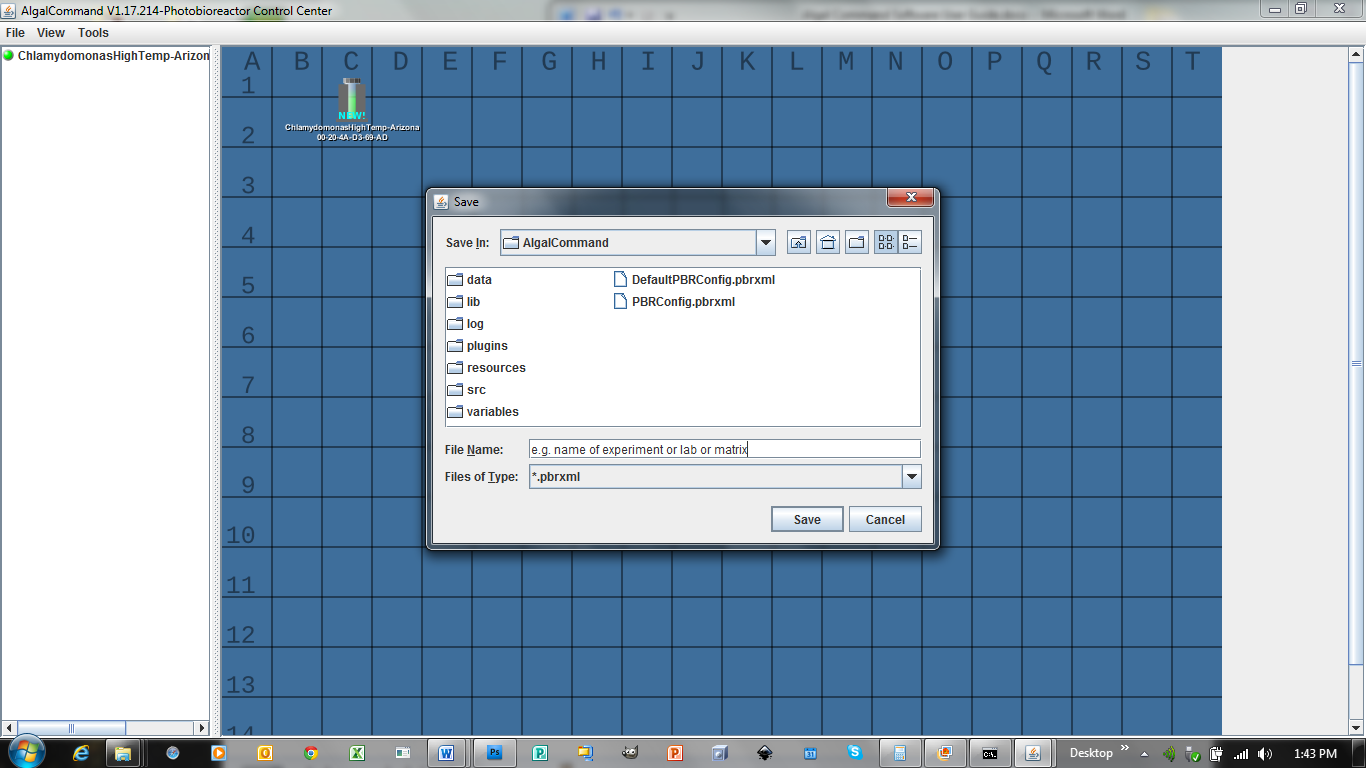
If the icons do not come up on the floor plan you can ‘Check the Network’ and try again.

**FILE>Load Configuration and FILE>Save Current Configuration**

It is possible to load different configurations for the different conditions programmed in each ePBR.

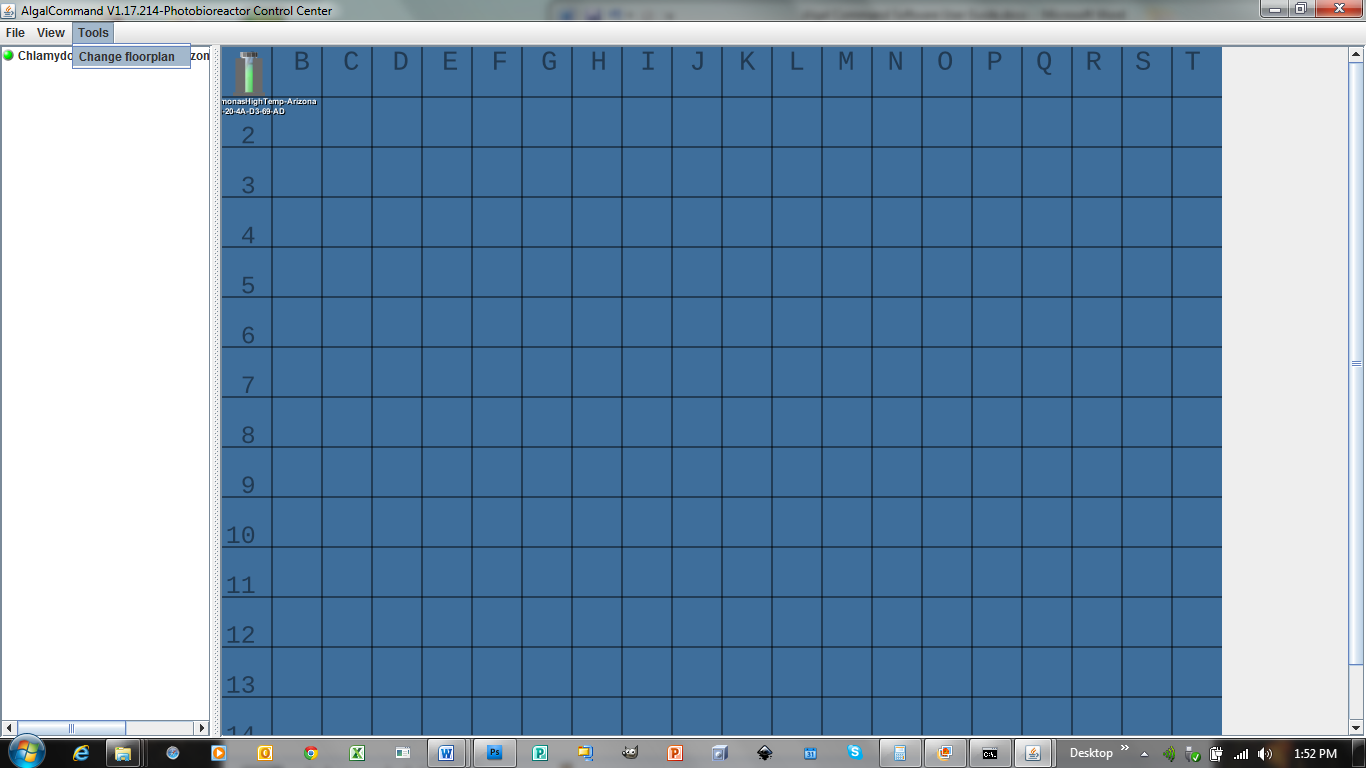


After the user has set the light intensity, temperature, stirring and bubbling parameters in the *Control Panel* (see Control Panel Section), it is possible to save these configurations by using FILE>Save Current Configuration.



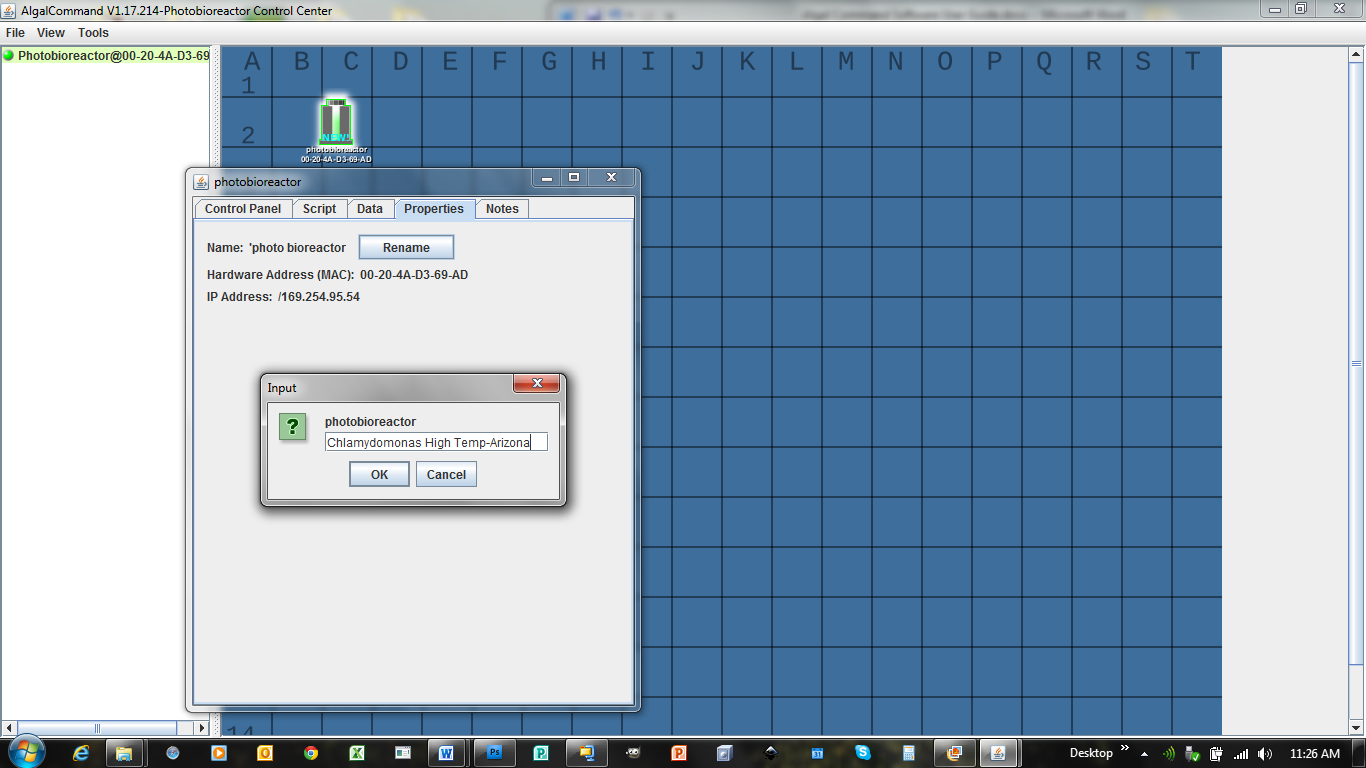
If you have lost some data or your computer crashes, and the user has saved the configuration, it can be reloaded again. **IT IS HIGHLY RECOMMENDED THAT YOU SAVE YOUR SETTINGS CONFIGURATIONS anytime you set or change them.**

**TOOLS>Change Floor Plan**

You can change the floor plan view by going to TOOLS>Change Floor Plan. Choose an image from a folder on your computer.

#### Naming Each Bioreactor

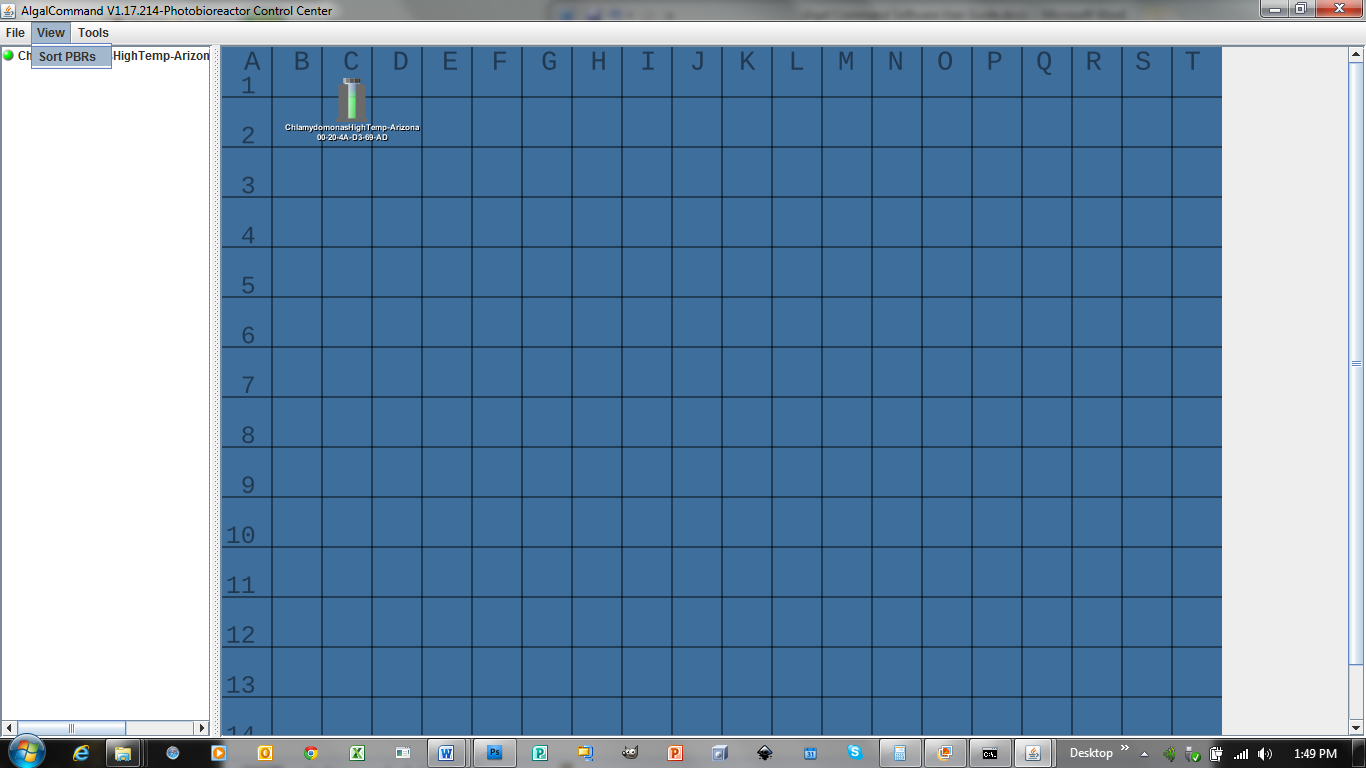
Double-clicking on an icon will open the *Control Window* for that bioreactor. Clicking on the **Properties Tab** will give you an opportunity to name the ePBR. Click on the Rename button and fill in what you want to call it to identify it. Some scientists call the ePBR based on the experiment they are doing such as ‘Chlamydomonas High Temp – Arizona Pond’. Names must be shorter than 64 characters.



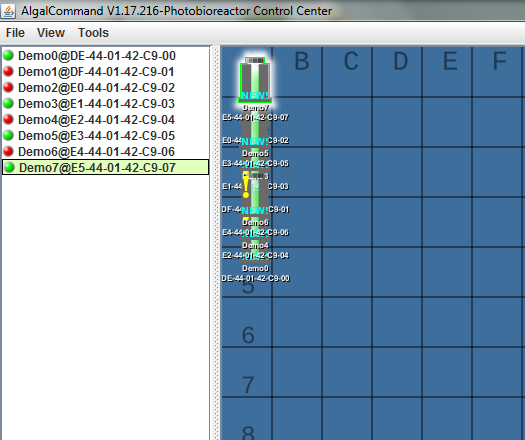
#### The View Menu

#### Sort PBRs

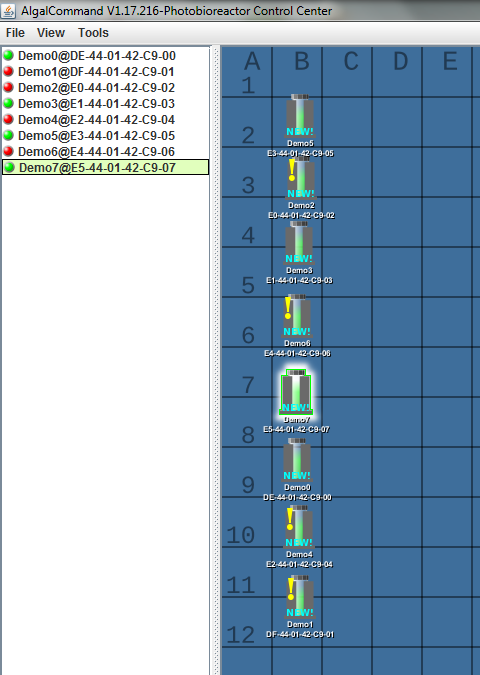
Clicking on *Sort PBRs* will cause all of the PBR icons to be rearranged into a grid pattern.



### 



Go to **VIEW>Sort PBRs**  to sort them in rows top to bottom of the grid.



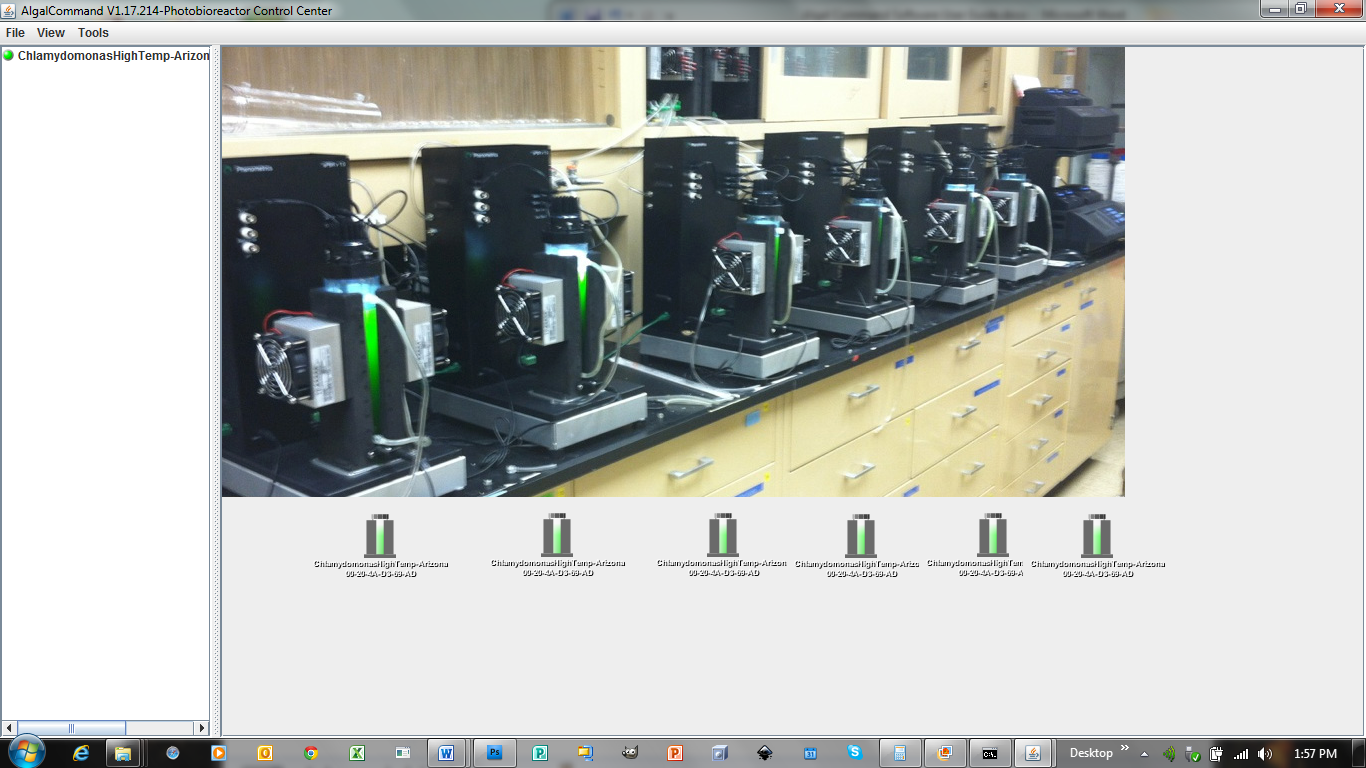
### The Tools Menu

### TOOLS>Change Floor plan.

#### Change Floor Plan

The floorplan image behind the PBR icons can be changed to any image in the .png, .jpg, and .tif formats. One common usage of this feature is to load a diagram or photo of the laboratory space so that the PBR icons can be dragged to locations on the image corresponding to their physical location in the lab.

### Here is an example of a floor plan using the photo of a matrix in a lab setting.



### The Control Window

Each ePBR has its own *control window*, which can be accessed by double-clicking on the ePBR's icon in the floor plan area or the list name.

#### CONTROL PANEL TAB

The *Control Panel Tab* allows for direct control over the ePBR and provides some common environmental simulation controls, such as light intensity, temperature, bubbling, and stirring.

**Control Panel**

##### 

##### Solar LED Settings

The *Solar LED* controls the illumination LED at the top of the culture vessel.

**Calibrate Solar LED:** You can recalibrate the *solar LED* to account for variations in LED manufacturing and/or different experimental conditions (such as column height). To do so, you will need a PAR light meter.

**Sinusoidal Day/Night Cycle:** If checked, then the light intensity will be varied over the course of the day to simulate the intensity of the sun as it rises and sets. The intensity at noon is determined by the peak intensity setting. If not checked, then the light will be set to the peak intensity setting during the day and 0 at night.

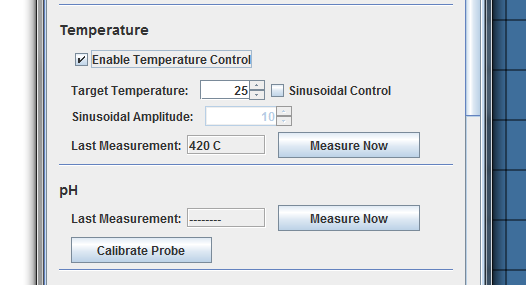
**Peak Intensity:** This is the maximum light intensity setting for day light.

**Day Length:** The length of the day portion of the day/night cycle in hours. The system clock is used for time calculations, so the days will be centered on 12:00pm noon.

##### Temperature Settings

To activate temperature control put a check mark in the ‘Enable Temperature Control’, then put in the target temperature and choose the sinusoidal control to determine the degree variation parameters.

**Do not turn on temperature control unless both temperature controls are plugged in and the thermistor is inserted into the culture vessel. The thermistor should be lightly screwed through a hole in the LED cap (remove screw to insert probe). Please read set-up instruction in chapter one.**



Click on ‘Measure Now’ button to establish baseline starting temperature. This information will be visible in the data tab section.

**Proper Care and Calibration of Phenometrics pH Probe And Reference Electrode**

**Operating Instructions**

**101-PHM –pH Probe**

**Use of the Probe**

The Probe is ready to use. Carefully unwind the tape and remove the probe from the protective glass tube.

***Use of separate Reference Probe is required***

**Calibration**

The Probe can be held by hand when measuring pH. The 101-PHM is standardized in two (2) pH buffers. Follow the procedure recommended by the manufacturer of your pH meter for calibrating your pH meter with our 101-PHM.

*Optimum Response Time:* Optimum response time will be obtained after the probe has been exercised in two (2) buffer solutions. Place a pH 4 buffer or equivalent in a 50 ml beaker and a pH 7 buffer or equivalent in a second 50 ml beaker (other beakers can also be used). Hold the 101-PHM and reference Probe together and touch the pH 4 buffer surface, allowing 15-20 seconds for equilibration. Rinse the two Probes with distilled water and then touch the pH 7 buffer surface in the same manner. Do this several times.

**Handling, Cleaning, and Storing the Probe**

***Be careful not to apply pressure against, or to shock, the inner glass capillary tube.***

***Cleaning:*** When using the Probe in solutions containing protein, the 101-PHM and reference Probe should be soaked in an enzyme cleaning solution such as Terg-a-zyme, by Alconox Inc, or a chromic/sulfuric acid glass cleaning solution after each use for a few minutes to remove the protein from the glass and the reference junction. This will prolong the useful life of the Probe.

***Storing:* Always clean the Probe before storing**.

*Long-term (over 2 weeks):* Return the Probe to its original container and prepare it in the same condition in which you received it. Usually this means moistening the blue sponge located in the bottom of the protective glass tube with distilled water.

*Short-term:* The Probe can be left in an acid pH buffer solution, e.g. pH 4.

**Troubleshooting**

**A. *Little or No Response***

Inspect the Probe for visible cracks (usually occurring around the tip of the Probe). If any exist, the Probe cannot be repaired and must be replaced. The slightest crack in or around the glass will cause the Probe to give similar readings in all solutions.

**B. *Response Pegs Off Scale***

**1.** Visually inspect the Probe for broken bulb.

**2.** Blocked or clogged liquid junction (reference) – soak the tip of the Probe in warm (50°C) distilled water for 5 to 10 minutes. If still clogged, soak overnight in distilled water.

**C. *Sluggish Response***

If the Probe becomes sluggish in responding to changes in pH, the response time can be improved using the following procedure:

**First:** Clean the Probe as described earlier. **Second:** Soak the Probe in 0.1 N HCl for 5 minutes, followed by soaking in 0.1 N NaOH for 5 minutes. After repeating several times, rinse the Probe thoroughly with distilled water. The Probe can then be calibrated in the usual manner.

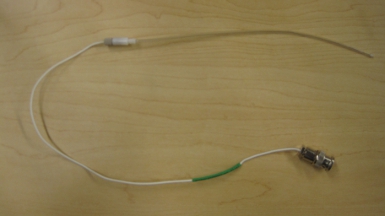
For additional assistance, call our Customer Service Department at 1-(517)884-4362.

***For Faster Service When Calling!***

Please have the following information available:

1. Model Number of the Probe (Ex. 101-PHM)

**101-REF Micro-Reference Electrode With Flexible Barrel for Filling**

**Contents and Specifications**

This Reference Electrode Kit contains the following:

1 101-REF Electrode (assembled)

1 Bottle of electrolyte

1 Replaceable barrel

1 Filling tube

1 Set of instructions

***Specifications:***

Internal Element……………………………………………..Ag-AgCl

Electrolyte ……………………………………………………….3M KCl

Junction …………………………………………………..Ceramic Frit

Electrode Length ………………………………………………9.5 cm

Dia. of Reference Barrel ………………………………….1.9 mm

Barrel Material ………………………………………….PVC Tubing

Electrode Cap…………………………………………………..2 Parts

Cap Diameter …………………………………………………0.64 cm

Lead Length ………………………………………………………….2 m

Depth of Immersion …………………………..Surface Contact

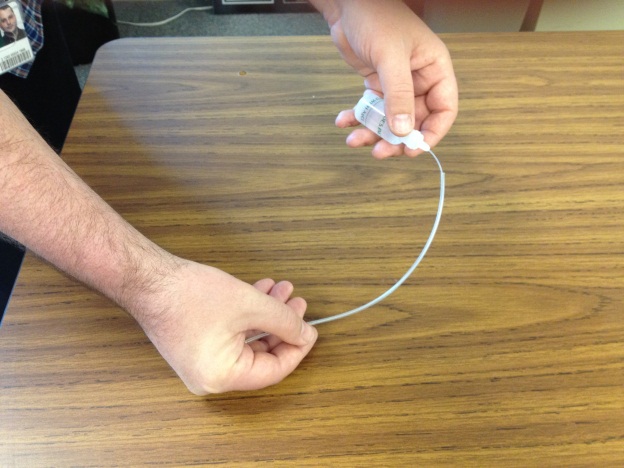
**Reference electrode Use**

The 101-REF Micro-Reference Electrode can be used with any pH, ion-selective, or redox electrode. The reference electrode is composed of an internal silver-silver chloride electrode with an internal filling solution of 3M KCl saturated with AgCl.

Before the reference electrode can be placed into operation, the PVC reference barrel must be filled with the provided internal reference solution.

**Filling the Reference Barrel**

Fill the PVC reference barrel in the following manner:

**1.** Remove the PVC barrel from the reference electrode cap by grasping each end of the cap and twisting the lower body counter-clockwise.

**2.** Add the internal reference solution to the PVC barrel using the polyethylene tubing (filling fiber) provided. Insert one end of the tubing into the opening of the electrolyte bottle firmly. Ensure that all air bubbles are free from the tubing before filling. Gently squeeze electrolyte bottle to begin filling. *Fill reference barrel to within ½ in. of top.*

**3.** After filling the PVC barrel with the reference electrolyte, insert the silver wire into the PVC barrel and re-assemble the electrode cap.

**Cleaning and Storing the Reference electrode**

***Cleaning:*** When using the reference electrode in solutions containing protein, the electrode should be soaked in an enzyme cleaning solution such as Terg-a-zyme, by Alconox Inc, or a chromic/sulfuric acid glass cleaning solution after each use for a few minutes to remove the protein from the PVC and the reference junction. This will prolong the useful life of the electrode.

***Storing:*** Always clean the reference electrode before storing.

*Long-term (over 4 weeks):* Remove the PVC barrel containing the probe and store the entire PVC barrel in a stoppered test tube or other suitable container filled with reference probe. Rinse the silver wire and reference electrode cap with distilled water to remove the salt solution. Dry gently with an absorbent towel. Store the reference electrode in its original box or any closed container to keep dust from the electrode.

*Short-term:* Place the tip of the reference electrode into a test tube or beaker containing reference electrolyte.

**Troubleshooting**

**A. *Unstable Response***

**1.** Visually inspect the reference electrode for broken or dissolving internal elements or for inadequate volume of reference electrolyte. Electrolyte level should be above the Ag-AgCl element.

**2.** Blocked or clogged liquid junction – clean reference electrode tip, then soak tip in warm (50°C) distilled water for 5 to 10 minutes. If still clogged, soak overnight in distilled water or replace reference barrel with provided extra barrel.

**101-REF Replacement Barrels are available.**

For additional assistance, call Phenometrics Customer Service Department at 1-(517)884-4362.

**Operation of pH and Reference Probe**

##### *To calibrate pH probe open the Algal Command software and follow the instructions below.*

##### pH Settings and Calibration

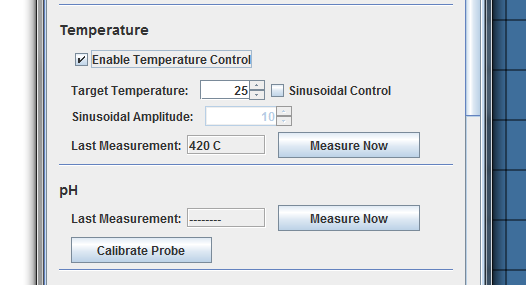
pH Calibration requires purchasing a pH calibration kit with pH4, pH7, and pH10 buffers. To check the calibration of the probes first clean them by rinsing with de-ionized water, then put the both pH and reference probes in the pH4 buffer together making sure that the pH and Reference probes do not touch in the buffer, (see picture next page) and click on ‘Calibrate Probe’. Then clean both pH probe and reference probe with de-ionized water, then put both probes into the pH7 buffer and click on ‘Calibrate Probe’. Then repeat the same procedure for pH 10 to set the range of pH that will be measured. The ‘Calibrate Probe’ button will display a pop-up with further instructions to walk you through the calibration protocol.

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When calibration procedure is finished, put both probes in the pH4 base and click on MEASURE NOW button. Sometimes the probes take time to stabilize before they can read the measurement. Wait 20 seconds and click on MEASURE NOW a few more times to get it to read pH4. It should show approximately 4 pH, if it shows a ‘  pH’ next to the Last Measurement box, it means that the calibration did not succeed. Repeat procedure. Make sure that the media was not made with de-ionized water as this would keep the pH probe from reading the correct measurement.

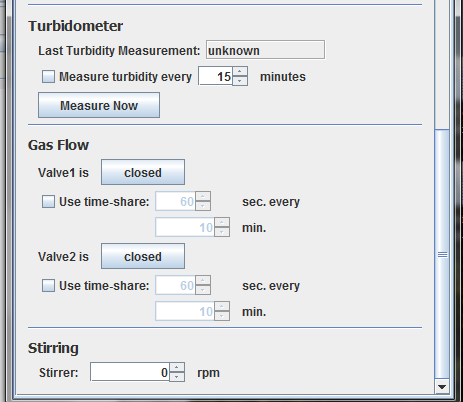
Please read the section o ‘Care, Storage and Maintenance’ of the pH probes in the hardware user guide.

**Note: Sometimes it is necessary to calibrate the pH probe multiple times to get it calibrated correctly.**



##### Turbidometer Settings

Enter the frequency of the measurements desired for the experiment. Check the ‘Measure Turbidity’ box to turn on the function. Click on ‘Measure Now’ to set baseline for data collection.

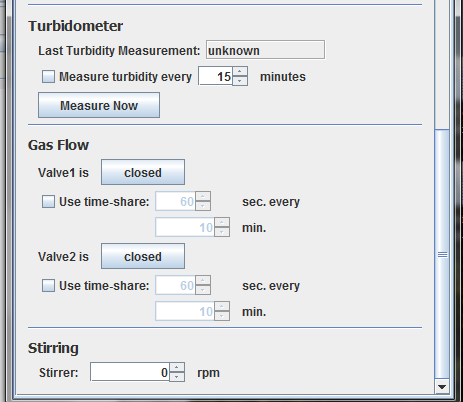


##### Gas Valve Settings

To activate gas flow click on ‘Closed’ button to Open. This will have a continuous flow. If the user wants to have specific gas flow times check ‘Use time-share’ and then set the intervals needed. The first box is for the desired amount of time the gas will flow and the second box sets how often. The default settings are for straight air with low CO2.

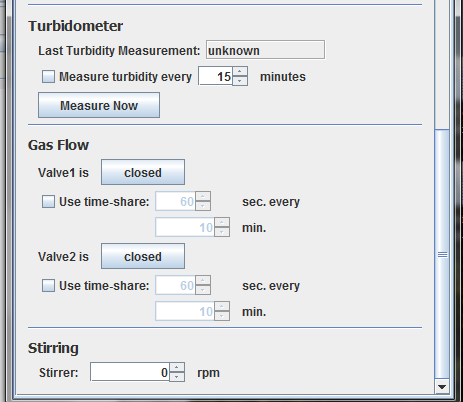
If you have multiple ePBRs in your matrix, the time-share function will share the gas source by activating the valve on only one ePBR at a time, each one in turn. This means that there will only be one ePBR bubbling at a time, but each individual ePBR will receive the specified frequency of gas bubbling. This way, a single gas flow controller can service the entire matrix.

Note: It is recommended if using high CO2 to set the interval for twice a day (570 minutes) for 2 minutes.



##### Stirring Settings

Stirring



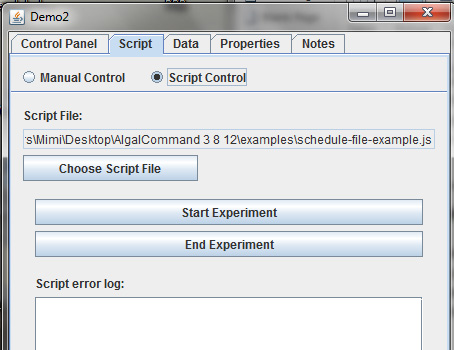
Stirring setting controls the stir motor below the vessel, which controls the magnetic stir bar. The Stir bar is autoclavable. It is recommended to put the stir bar in the vessel before autoclaving to reduce the likelihood of contamination.

Typically **rpm 200** is recommended to mix the culture evenly, but not so fast as to create a vortex.

**SCRIPT TAB**

The script tab allows the user to program the photo bioreactor for a wider range of possible experiments using a more complex set of commands. For example, perform specific actions in response to the pH (if measurement is over at certain level, reduce CO2). The current software release provides 2 example scripts that can be immediately implemented.

Click on ‘Choose Script File’.



Choose Example Script folder. The program comes with a couple of preset scripts.

Click on one of the following scripts:

**pH-stat\_day-night-cycle \_constant-temperature.js** or

**pH-stat\_day-night-cycle \_fluctuating-temperature.js**

Description of the script files and functionality:

**pH-stat\_day-night-cycle \_constant-temperature.js**

This script takes measurements every 10 minutes. When the pH is found to be above pH upper limit, it turns on the gas valve until the pH drops below the value of pH lower limit. Temperature is maintained at the value of temperature. Average and a sinusoidal day-night cycle is provided with a peak light intensity of peak Solar Intensity in micro Einstein’s PAR.

===========================================================================

**pH-stat\_day-night-cycle \_fluctuating-temperature.js**

This script takes measurements every 10 minutes. When the pH is found to be above the pH upper, it turns on the gas valve until the pH drops below the value of pH lower. A sinusoidal day-night cycle is provided with a peak light intensity of peak Solar Intensity in micro Einsteins PAR. The temperature fluctuates as a sin wave such that the temperature is warmest at 18:00 and coldest at 6:00.

===========================================================================

Control Panel is partially disabled if using a script. Check ‘Script Control’ circle. It is not necessary to click on ‘Start Experiment’ button unless the user has written a customized script.

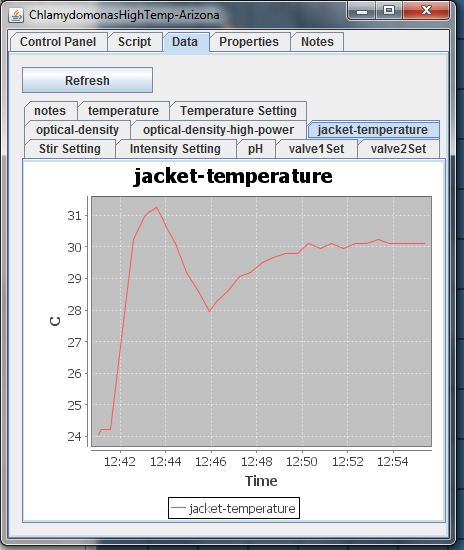
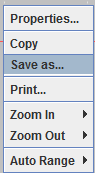
**Note:** Script based control is still under ongoing development and therefore subject to change in future releases of the software. If the user wants to write their own scripts, they need to know how to program in JavaScript and refer to Phenometrics script manual, which is due for release at the end of October 2012.

**DATA TAB**

The Data Tab show graphs of data generated by the ePBR. As the user sets the parameters in the Control Panel tab and begins operation of the photo bioreactor, multiple tabs will appear showing all the programmed functions and data. To update graphs click on the ‘Refresh’ button

To get current data it is important to initially click on the ‘Measure Now’ buttons when first setting up the Temperature, pH and Turbidometer in the Control Panel.

**REMEMBER: Click on ‘Refresh’ button to update data after closing and reopening AlgalCommand.**



Right-click on graph

To *copy, save, or print* the graphs right-click on the window of each graph.

Note that the X-axis (horizontal axis) is referred to as the “Domain” and the Y-axis (vertical axis) is referred as the “Range”

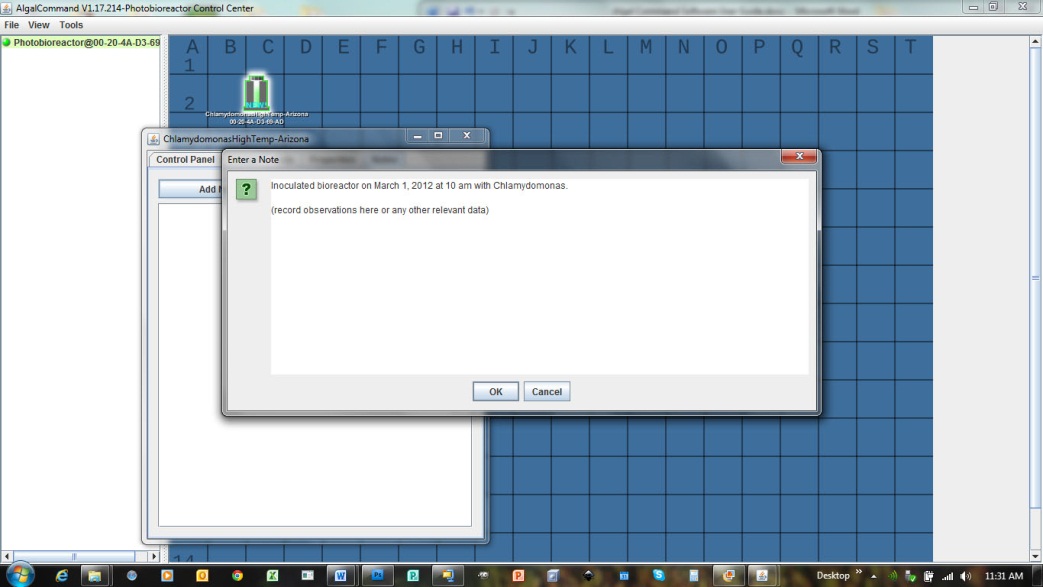
The following tabs will appear when the unit is operating with full functionality:

* Notes – allows the user to make notes on the particular ePBR. Particularly useful if more than one operator is using the ePBR, to record changes or dates of operation.
* Temperature – shows the recorded temperature data of the culture temperature
* Temperature Setting – shows control panel settings
* Optical-Density – measures turbidity based on infrared light absorption for **low density culture**
* Optical-Density-High-Power - measures turbidity based on light absorption for **high density culture**
* Jacket Temperature- is record of the jacket unit temperature variations
* Stir Setting – shows when the stirring rpm is changed
* Intensity Setting – shows record of the intensity of the LED light source over time.
* pH – Show pH measurement over specific time frame
* Valve 1 Set – Shows record of when gas valve was open and when it was off
* Valve 2 Set – Not currently installed or operational. Can be upgraded in future upgrades.

**NOTES TAB**

The Notes Tab is used to keep a journal of the experiment and settings, as well as pertinent information.

Click on ‘Add Note’ then enter text and click OK.



**General Precautions**

Make sure the ePBR is in a dry clean area near accessible power and gas.

Please have space for a computer to hook up to the router.

Take special care to not leave it unattended for more than 24 hours.

Always use appropriate procedures for cleaning probes and sensors. Never use harsh chemicals.

**Customer Support**

For customer support please contact:

[support@phenometricsinc.com](mailto:support@phenometricsinc.com)  +1 517 884-4362

Thank you for reading this user guide. We welcome any suggestions or improvements to this guide.

**UPGRADING TO NEW VERSION SOFTWARE PROCEDURE**