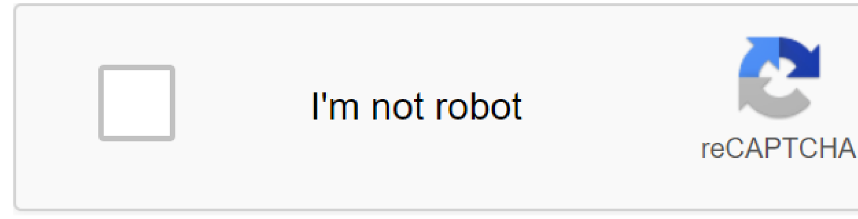
Spectronic 200 spectrophotometer manual



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This is what the Spectronic 200 looks like (or should look like) when it's just been received from where they're stored or when it's ready to be stored. All doors on the body are closed and the screen is closed. Here, the LCD screen has been tilted so that it can be easily viewed. The screen must be returned to a flat position before storage. In this photo, the sample camera cover was raised to show the location of the sample camera inside. Also, if you look closely, you should be able to see that the doors in the ditch-storage compartments on the right and left have been opened. At the top of the machine, in front of the screen, there is a number of control handles and buttons. The most left-handed one is the wavelength handle (or dial). Note that the symbol used to represent the wavelength is the Greek letter I (lambda). Thus, on this web page the wavelength handle will also be called a pen. You can rotate the wavelength dial to adjust the wavelength at 10-nm intervals until you are close to where you need to be. Tap down on the wavelength dial, turning it for a subtle wavelength adjustment at 1-nm intervals. Also note the photo above that the button to the right of the wavelength handle is the print button. Writing this web page, we haven't yet had a printer for our Spec 200s, but we're in the process of getting one. When this and/or computer interface software is installed, you will be able to print a copy of your results. To the right of the print button is the Empty button, or Auto-zero button (here's also called 0.00 to help you remember which button is being discussed). Since solvents such as water and ethanol, as well as the plastic from which the ditch are made, absorb some light, pressing the 0.00 button will zero the machine so that it ignores any light absorbed by the solvent and the thuvet, and displays only the amount of light that is absorbed by a particular substance being tested. To the right of the Home button is the Home button (it's also called ⌂). Pressing this button will send the Spec 200 back to your home display menu. On the right side of the top of the machine is a group of navigation buttons. The four arrow buttons (▲ up, right, down, and ◄ on the left) are used to navigate the different menu options displayed on the screen. CEnter ↵ is an entry button, used to select/execute different options, just like on a computer. Here's a close-up of Cuvette's sample camera. Spec 200 in Cuvettes are square plastic tubes that fit into the holder. Notice the photo detector below, the top edge of the ditch in place. The light source or lamp is on the right, and shines with its light through a sample in the ditch. To the left of the holder of the ditch, there is a detector that, as The name suggests detecting the amount of light that still remains after passing through the sample. The Spec 200 onboard computer compares this to the amount of light the lamp is sticking out to determine how much light was absorbed by the sample. Note that since the machine's calculations are based on comparing the amount of light put out and the amount of light left, it is necessary to keep the stray light while keeping the sample camera cover closed. The power switch is located on the back of the Spec 200. Once the machine is connected, power can be turned on. A couple of minutes of waiting will be needed for the Spec 200 onboard computer to load. In addition, the USB CONNECTOR and the printer port are located on the back if/when necessary (but the Spec 200 can only say one particular kind of small printer). Turning on the Spec 200 When the Spectronic 200 is on, it will take a couple of minutes for its onboard computer to load. After the machine's initial splash screen, it will display this initialization screen that asks you to check to make sure that someone has not left a cuvette in the sample compartment. Do check to make sure there is no one there and then make sure the lid is closed - the machine needs to properly calibrate itself to the boot. Once you're sure there's no cuvette in the camera and that the door is closed, click the Enter (↵) button. As the machine goes through its bootup sequence, it will show this initialization screen to show what it is doing. When this is done with its download, it will display its home screen. For this course, we'll use the Spec 200E Modern Interface Since it's highlighted by default, just click Enter (↵) to go there. After that, there will be a few options that you will need to do, depending on how the machine is used. Here's what the modern interface screen looks like. From this, you need to choose the right option to collect the data. Note that the default app is Live Display. Using the Spec 200 to obtain a beer law schedule for lab beer rights, there is one additional step that can be done first if desired. (If you're not taking this optional step, go to the instructions for setting up the app mode to quant.) Your protocol says use a wavelength of 450 nm for riboflavin, but you wonder where that number came from? It's really not just pulled out of the air. If you want to find this number for yourself, use the right (me) and/or left (◄) arrows to go from Live Display to Scan. The rest of what is displayed on the screen changes accordingly. If the Measurement Mode does not mean ABS (which means Absorbns), use the arrow down (I) to descend to the measurement line, and the right (I) and/or left (◄) to customize it to abs abs Vedomosti) - do not say %T. Then use the arrow down (I) for the cursor to a low level (remember, which means wavelength). The default setting for the lowest wavelength to be used is 400 nm. Use wavelength arrows and/or the right (me) and the left (◄) arrows to adjust it to 350 nm. Before following in a similar way, use down the (I) arrow cursor up high. The default option for the highest wavelength will be 900 nm. Use the wavelength arrow and/or right (I) and left (◄) arrows to adjust it to 800 nm. Before then, use the down (I) arrow cursor down to the next, and enter (↵) to move on to the next screen. The next screen that will come up is the empty spectrum that looks like this. The next step is an empty machine. Find a pair of plastic ditch in one of the side compartments and because our samples dissolve in water, put water in one of them. Only touch/keep cuvettes their top edges - fingerprints on the area through which light travels will spoil the readings. If necessary, the ditch can be carefully polished with a piece of paper for lenses. Note that the top of one side of each cuvette has an arrow (▽) on it. Make sure the cuvette is all the way down and the arrow is on the right side (to the light source). Then click zero or empty (0.00) so that the machine can automatically zero itself (also called empty by it). While the car performs its auto-zero, it will display the hourglass and please wait for the message. When the machine is done with its calibration, remove the ditch water, and as it was done with water, place some of the solution riboflavin from the tube of your most concentrated solution into the second ditch. Insert the ditch into the ditch holder, making sure the arrow points to the right. Make sure to cuvette all the way and close the lid. Then click Enter (↵) to determine at which wavelength riboflavin absorbs the most light. The Spec 200 will measure and display the spectrum for the riboflavin that should look like this. However, the green line that is the cursor may be more in the far left (or anywhere else on your chart). Use the wavelength arrows and/or right (to) and the left (◄) arrows to move the cursor's green line until it exactly matches the top of the highest peak. Note that when the cursor moves, the machine will display how long it is at and the corresponding absorption (ABS) for that wavelength. Note that the highest absorption of light is at (or very close to) 450 nm. That's why the instructions in the protocol say use 450 nm your data fits that). You can take a picture of this spectrum to include in your lab notepad (or if the new printer works, works, from a copy). Use the up arrow (▲) to return to the app screen. Change the app from Scan to quant. There are several options on the screen. No, no, no, no, no, no, no, STDs mean the number of standards, and the machine will allow no more than 4. If he hasn't said 4, cursor up this line and change it to 4. If the Measurement Mode doesn't yet speak ABS, cursor down to that line and change it to abs. If you can't already tell 450 in the Dimension (if you followed the previous instructions, it should remember any wavelength on which the green cursor line was when you left the Scan cursor), cursor up to that line, and change it to 450 nm. The way we express concentration (milliliter riboflavin solution added) doesn't really match the choice of Unit machine, so if it says something like C, you can just leave it there. Don't worry about the line that says: USB. When everything is set correctly, cursor down to Go, and press Enter (↵) to continue on to the next screen. Next, the Spec 200 asks you to insert a ditch blank solution (in this case, water) into the cuvette holder and press the Auto zero button. Check out the instructions above for the correct ditch insertion. When the machine is done with this, it will display a screen with spaces for the four standards to be tested. Use plain water (again) as the first standard (0 riboflavin must absorb 0 light) as well as 0.2 ml, 0.7 ml and 1.0 ml tubes for the remaining three standards. For each standard the cursor is up or down until you are on that line. Use the right (to) and left (◄) arrows to set the concentration for this sample (up to 0.0 or 0.2 or 0.7 or 1.0 as needed). Then click Enter (↵) to analyze this sample. The Spec 200 will put the consortium's readings in the last column. As you do each of your samples, write down the absorber reading received for each sample into your lab notebook. Then cursor until the next sample and repeat the process. In between samples, you can remove the cuvette, pour the sample that in the cuvette back into your test tube, and then pour the next sample into the ditch, insert it back into the sample chamber, close the lid, and take the reading. Do not rinse the ditch with water between the samples, as any remaining water droplets in the cuvette will dilute the sample that is in the cuvette, causing the reading absorption to be incorrect. When you have measured all four of these samples, the machine will give you the ability to click Enter (↵) to display the beer law curve. Note that it will calculate the most appropriate straight line from your data, but the object of the game here should be so good in the pipeline that all your data points fall exactly on the line rather than a little one way Another. Note that the dots on the graph in this photo don't all fall right on the line: some are higher and some are lower. How close to the straight line did you come from? You can take a picture (or print, if any) of your schedule to include in your lab notebook. To analyze your samples of 0.1 and 0.4 ml, one at a time, place each of them in a ditch and in spec 200. Note at the bottom of the display screen, it says: Measure sample ←. Click Enter (↵) to analyze this sample. The machine will display its absorbent (please write that down in your lab notepad) and read concentration (as if this sample was unknown) based on the most suitable line that it is calculated (so if you didn't have a phenomenal pipette technique that wouldn't exactly match 0.1 or 0.4 ml - but hopefully would come close). Identify the absorption of these two samples with these tools. Using Spec 200 to produce spectrum (Photosynthesis Lab) Since there are usually about six samples to be tested in this exercise lab, one way to do this is to do that well worked in the past for a class to divide into six (or how many pigments there are) groups, and each group to adopt a pigment and spectrophotometer. Thus, the spectrum from each pigment can be displayed on its own spectrophotometer, and all students can thus go from machine to machine studying (and photographing) the spectrum. (However, if/when we get a printer and/or computer interface that/connected to just one Spec 200, that will change what works best.) Due to the way our old Spec 20s worked, the laboratory protocol for the Photosynthesis Laboratory includes instructions for receiving absorption readings for all pigments at 350 nm, then change to 375 nm, re-empty, and check all the pigments out there, then 400 nm, etc., but with these new Spec 200s, it's all no longer necessary, and following the instructions below, it's actually faster and easier to get the data needed to build the spectrum for each pigment, one by one. For the spectrum of photosynthetic pigments, start by tweaking the Spec 200 mode for scanning. This is the same mode described above to find peak absorption for riboflavin, so many of the destinations here are identical to the destinations given there. As mentioned above, use the right (me) and/or left (◄) arrows to change from Live Display to Scan. The rest of what is displayed on the screen changes accordingly. If you can't tell ABS (which means Absorbbans) in Measurement Mode, use the arrow down (me) to go down to the measurement line, and the right (I) and/or left (◄) arrows to adjust it to the ABS (it shouldn't say %T). Then use the arrow down (me) for the cursor to a low level. Configure The default for the lowest wavelength to be used is 400 nm. Use wavelength and/or right (I) and left (◄) arrows to Before following in a similar way, use down the (I) arrow cursor up high. The default option for the highest wavelength will be 900 nm. Use the wavelength arrow and/or right (I) and left (◄) arrows to adjust it to 800 nm. Before then, use the down (I) arrow cursor down to the next, and enter (↵) to move on to the next screen. Again, the next screen that will come looks like this. The next step is an empty machine. Find a pair of plastic ditch in one of the side compartments and put ETHANOL (do you know why you should use this and not water?) in one of them. Only touch/keep cuvettes their top edges - fingerprints on the area through which light travels will spoil the readings. If necessary, the ditch can be carefully polished with a piece of paper for lenses. Note that the top of one side of each cuvette has an arrow (▽) on it. Make sure the cuvette is all the way down and the arrow is on the right side. Then press the zero button (0.00) so that the machine can automatically zero itself. While the car performs its auto-zero, it will display the hourglass and please wait for the message. Then he's ready to analyze your samples, so place one inch for example, here's a sample of parsley extract, ready to be put in a sample chamber. As it was done with ethanol, pour some of the pigment solution you want to examine from the test tube into the second ditch. Insert the ditch into the ditch holder, making sure the arrow points to the right. Make sure to cuvette all the way and close the lid. Then click Enter (↵) to show the range of absorption of this pigment. The machine will display a message: Scan ... with an hourglass. That's the result of the spectrum for parsley extract used above. Please note that the cursor is at 350 nm. To move to another part of the spectrum, you can use the arrows of wavelength and/or right (me) and left (◄) arrows. Here the cursor is at 400 nm, and the machine says that at this wavelength the absorber is 1.63. Your protocol book instructs you to graph the data from the pigment (s) you checked. To do this, you will first need to chart the numbers that you will need to build a graph. Start with the list, going down the page, all the wavelengths to be used (350, 370, 400, 425 nm, etc.). You will then need another column (down page) for each pigment tested. Use the wavelength (i) handle and/or left (◄) and right arrow to move the green cursor line at 25 nm increments (350, 375, 400, 425 nm, etc.), to 800 nm. At each of these wavelengths, students must copy reported absorbrs into their lab notebooks. The protocol book explains Use these absorption numbers to build a graph. Your graph should look like a spectrum that On the Spec 200 screen, with the big difference that on your chart, you'll know what the actual numbers are. Here, on the left, the cursor has been moved to what appears to be a peak of 415 nm, and the machine says that at this wavelength, the absorption is 2.01. It's actually a little tricky because it's a parsley leaf extract that contains a mixture of pigments: chlorophyllS A and B, carotene, xanthophiles, etc., so here's a additive spectrum of all those put together (similar to the chart on the right). So while 415 nm seems to peak, this may be due to the additive effect of multiple pigments, peaks of which are actually at several different wavelengths: while their peaks are elsewhere, the sides of these peaks may add to give an obvious peak here. Here the cursor was moved to another peak of 665 nm. The machine says that at this wavelength the absorption is 1.13. Use the wavelength (I) handle and/or left (◄) and right arrow to move the green cursor line to the highest point at each of the absorption peaks (hint: watch out for the highest ABS as the green cursor line moves back and forth). Notice each of these wavelengths and the absorber readings in your lab pad. These figures can be compared to published max/min numbers. For example, here the cursor has been moved to 525 nm, where it seems to be the minimum. The machine reports that the absorption is only 0.02 at the moment. However, it is difficult to tell whether the cursor is at the absolute lowest point. One way to determine is that it is slowly moving the cursor back and forth while browsing for the smallest absorbent (ABS) reading. The Spec 200 has another feature that can help in this situation. Note the photo, above, at the bottom of the screen, it is said to push down the (I) arrow to zoom in to a higher zoom. Here, with a cursor at 525 nm, the down (I) arrow has been pressed, resulting in the machine displaying only a section of 400 to 550 nm. The downside of using zoom is that there is no un-zoom function, and the only way to get out of a large-scale view is to click the arrow up (▲) to go all the way back to the Scan menu and then ask the machine to regenerate the spectrum. The Spec 200 also gives us a means to see which color of light corresponds to a given wavelength. Here, with a cursor left at 525 nm (or any wavelength you want to explore), arrow up (▲) has been pressed to return to the scan menu, and back from that in the app menu. There Scan can be changed to Live Display as shown here. Then use the arrow key down to the cursor to where it says: GO and press enter (↵) to do so. Here's to for 525 nm. It is still said that the takeover at 525 nm is 0.20. Notice the rainbow (spectrum) at the bottom of the screen, and especially notice where the arrow (I) points. The color of the spectrum at that time indicates (as best as possible on the LCD screen) the color of light that corresponds to this wavelength: 525 nm green light, which plants do not absorb (which is why they look green for us). You can also check the color that corresponds to other areas of the spectrum, especially including peaks. If you record the wavelength (s) of the peak (s), you can use the wavelength (I) dial and/or right (i) and left (◄) arrow keys to go to that wavelength. If you don't know or remember the wavelength, the color you want to determine, you'll need to go back to the app menu, go back to the scan, re-scan the pigment (re-make the spectrum), move the cursor over the desired wavelength, and then go back to Live Display. 428 nm 665 nm Here are displays (for the same parsley solution) for 428 nm (which your lecture textbook says is an absorption max for chlorophyll) and 665 nm (location is smaller than peak. Again, since at the moment we don't have available printers, print out a copy of the spectrum graphics displayed by the machine is not possible. to bypass all spectrophotometers and photograph the spectrum for each of the six pigments, so that everyone can have the whole set), and include a printout of his/all of them in their lab laptop. However, this is not a substitute instead of learning the proper method for building the schedule, so the right done, hand-drawn graph should be built. If the second (or more) pigment needs to be tested, simply remove the first ditch pigment and insert a new ditch/pigment for testing, then press the Enter button (↵) to create a spectrum for the new pigment. As stated above, these absorption readings can be added as another column on the chart/table students have created in their lab notebooks and subsequently used to create a graph for this pigment. Depending on how the chromatography turned out, your class should have test tubes made of red insoles, individual pigments: carotene, xanthophyll 1, xanthophyll 2, chlorophyll A, chlorophyll B and the central place, which is not Get a range of absorption for each of these pigments and any others that your class may be testing (and photos to be included in your lab notebook or print out if the printer is available). In your lab notebook, bequeath wavelength (nm) of absorption maxim (peak tips) and minim (valley bottom) for each of the pigments. Be very careful in your observations - for example, Chlorophyll A will probably show a peak of about 425 to 428 nm, and this is actually significantly different from the peak of carotene around 450 nm. Things to include in your notebook refer to the lists at the end of the beer law and the laboratory's photosynthetic pigments web pages for lists of items that need to be included for each of these laboratories. all the notes you take during the introductory mini-lecture are all notes and data that you collect as you perform these experiments all the requested calculations and graphs based on this data print class data for each of these labs (available online) drawing (your!) from Spectronic 200 with tagged parts of additional printouts of photos of data/results, as shown on the Spec 200 screen (or when we get a printer, printouts of data answers to all discussion questions, resumes/conclusion in your own words, and any suggestions you can have for each of these labs any returned, graded pop quiz for each of these copyright labs © 2015 J. Stein Carter. All rights are reserved. Chickadee photo ©, which David B. Fankhauser This page has been available once since August 17, 2015. 2015.

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