10-AU Calibration for Rhodamine WT

3 Main Steps

1. Setting the basic operating level

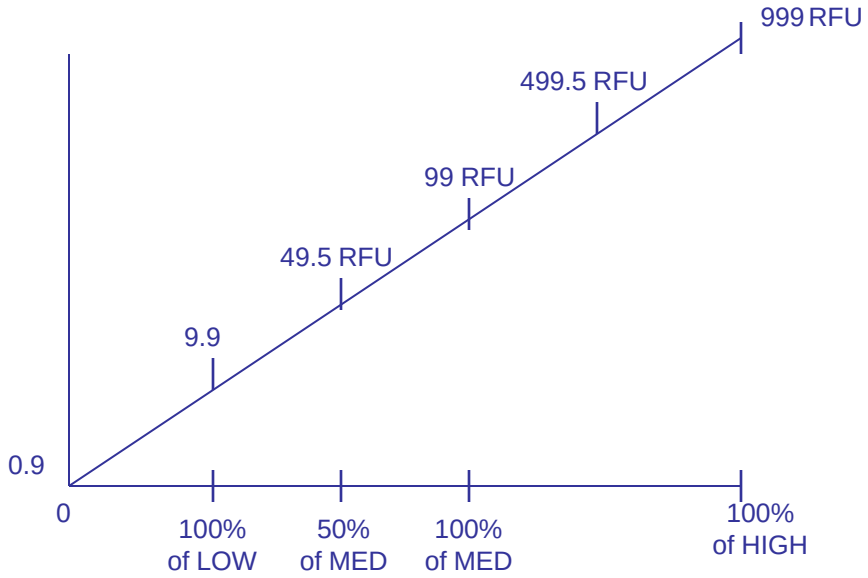
2. Blanking the instrument

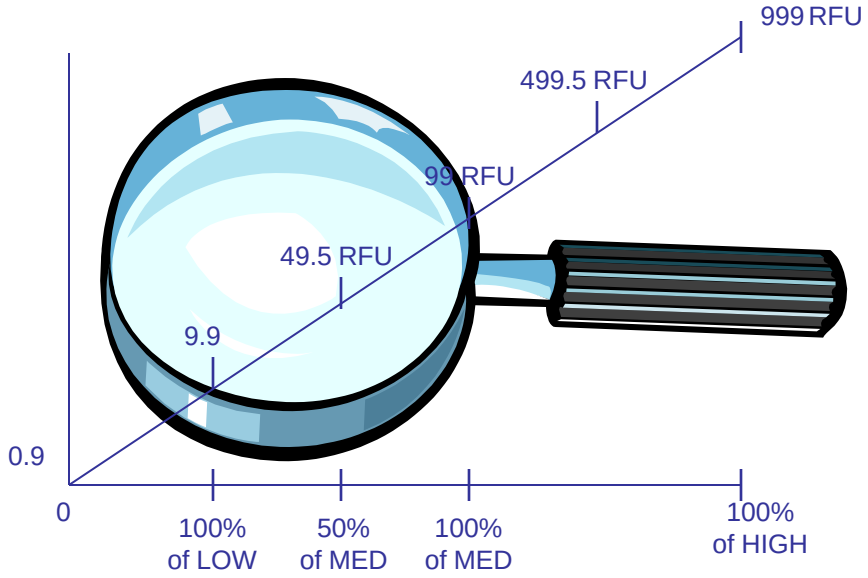
3. Calibration with a liquid primary standard that has a known concentration

#1 Setting the Basic Operating Level

This procedure should be performed the 1st time you use the instrument, or any time you switch to a different application, change filters or lamps.

1. **Turn on the 10AU and allow the instrument to warm up for at least 30 minutes.**





How scaling on the 10-AU works

The Full RFU (Relative Fluorescent Unit) Scale of the 10-AU is 0-999.

This is a simplistic way to think of it.
By increasing the sensitivity, you can think of it as using a magnifying glass. You are “focusing” the scale of the 10-AU to a smaller, more limited range, and therefore getting finer resolution.

The inverse is true when you decrease the sensitivity you get a broader range of RFU, but decreased resolution.

2. Table 1 is an example of how set the sensitivity of the 10-AU. Users should determine the appropriate range and %FS for their calibration standard.

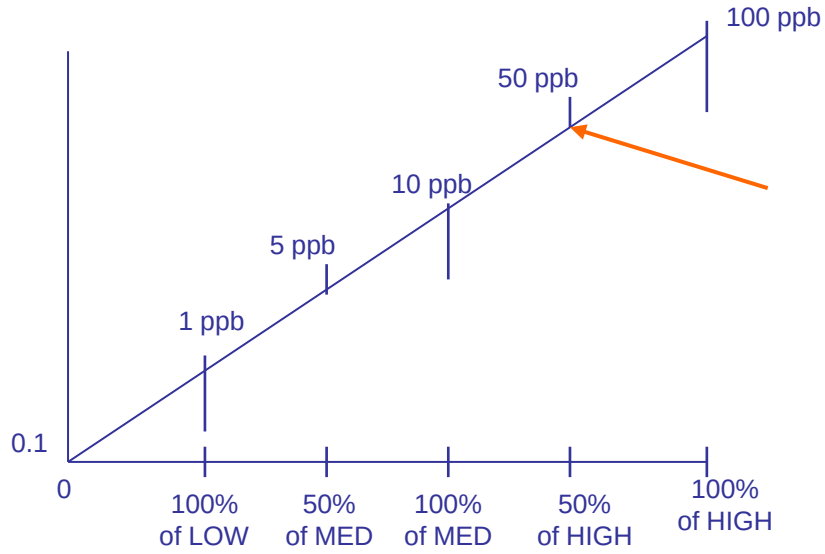
Chlorophyll (ug/l)	Rhodamine WT (ppb)	Range	% FS (+/-5)
240 - 180	100 - 75	High	80%
180 - 160	75 - 65	High	70%
160 - 130	65 - 55	High	60%
130 - 110	55 - 45	High	50%
110 - 80	45 - 35	High	40%
80 - 60	35 - 25	High	30%
60 - 40	25 - 15	High	20%
24 - 18	10 - 7.5	Medium	80%
18 - 16	7.5 - 6.5	Medium	70%
16 - 13	6.5 - 5.5	Medium	60%
13 - 11	5.5 - 4.5	Medium	50%
11 - 8.0	4.5 - 3.5	Medium	40%
8.0 - 6.0	3.5 - 2.5	Medium	30%
6.0 - 4.0	2.5 - 1.5	Medium	20%
2.4 - 1.8	1.0 - 0.75	Low	80%
1.8 - 1.6	0.75 - 0.65	Low	70%
1.6 - 1.3	0.65 - 0.55	Low	60%
1.3 - 1.1	0.55 - 0.45	Low	50%
1.1 - 0.8	0.45 - 0.35	Low	40%
0.8 - 0.6	0.35 - 0.25	Low	30%
0.6 - 0.4	0.25 - 0.15	Low	20%

Table 1 is an example of how to set the scale for a given linear range of the 10-AU.

Refer to page 4 and 6 for examples of how the scaling works.

It is a good idea to set the range near where you expect your samples to be.

For this example we are using a **50ppb** standard.

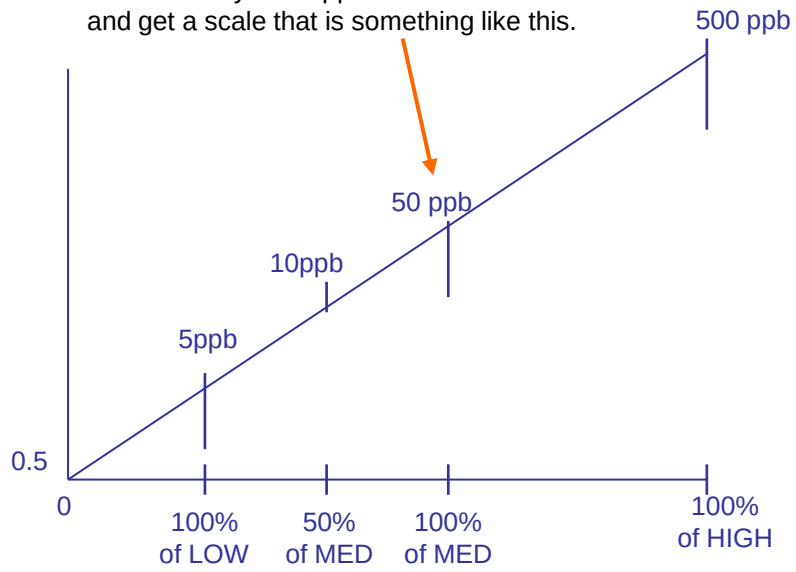


For our example, we set the 50 ppb to 50% of HIGH and get a scale that is something like this.

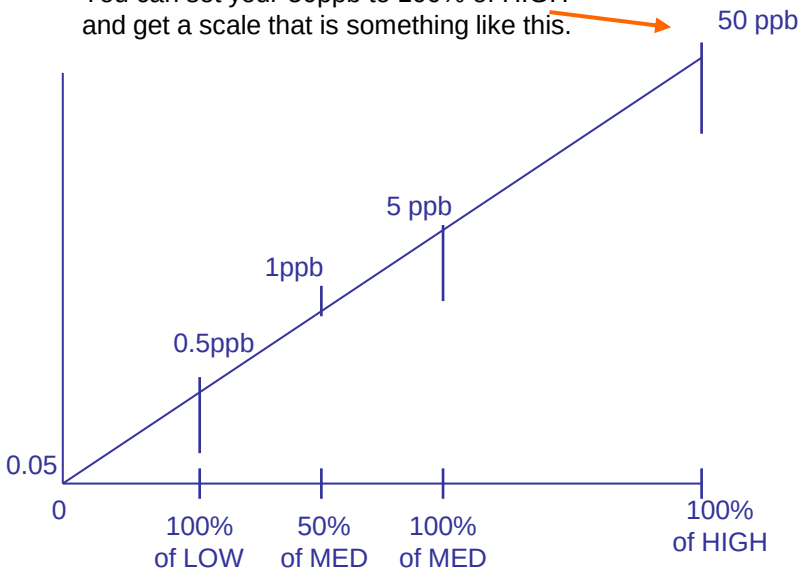
<u>You can skew the scale however you want to meet your needs.</u>

You need to remember that if you limit the scale too far on one end or the other you will lose resolution on the other end. For example if you look at the full scale 0-150ppb you will not have the ability to get fine resolution it is simply too broad of a range to expect to see a concentration like 0.05 ppb

You can set your 50ppb to 100% of MED and get a scale that is something like this.

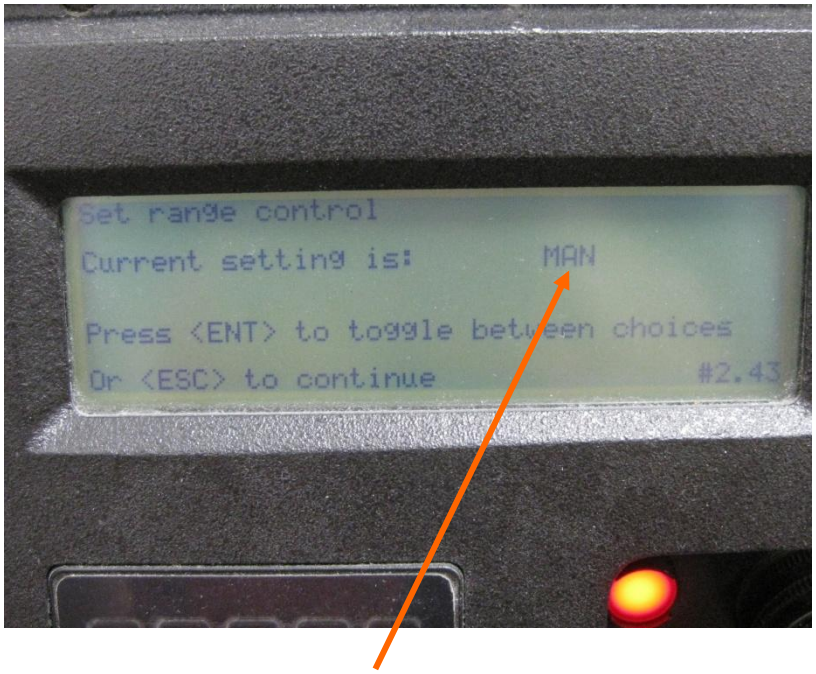


You can set your 50ppb to 100% of HIGH and get a scale that is something like this.

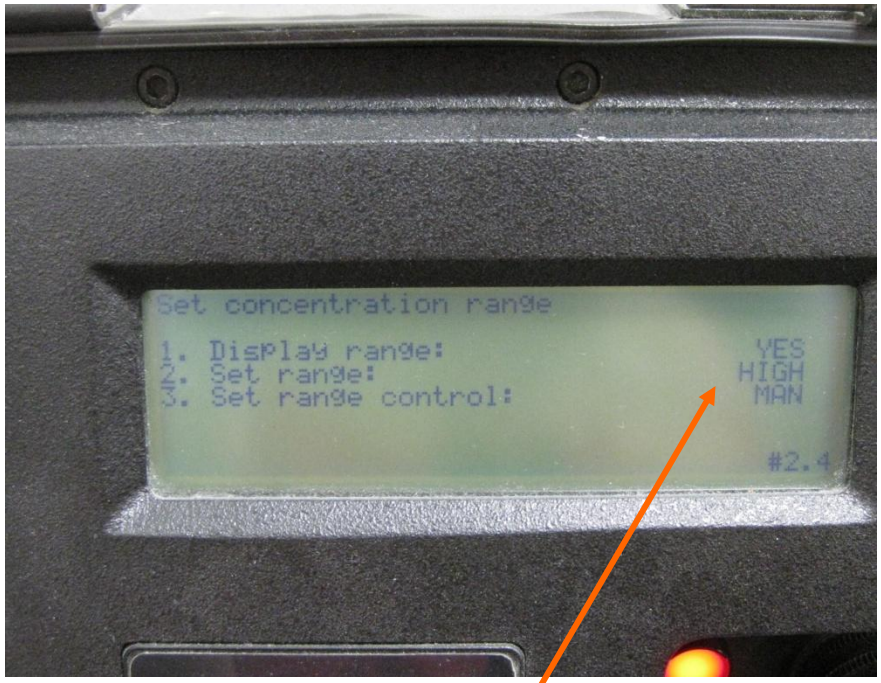


3. Prepare your standards and deionized water in test tubes so you are ready to set the sensitivity and calibrate the instrument.

4. **Access screen 2.43 and set range control to <u>manual</u> and range to the appropriate level for your standard. For our example the range should be set to HIGH on Screen 2.42.**

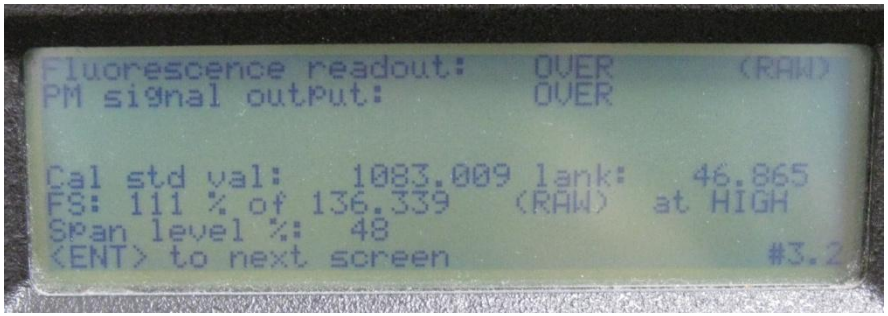


Change to MAN



Screen should look like this when you are finished

4. Access Screen 3.2



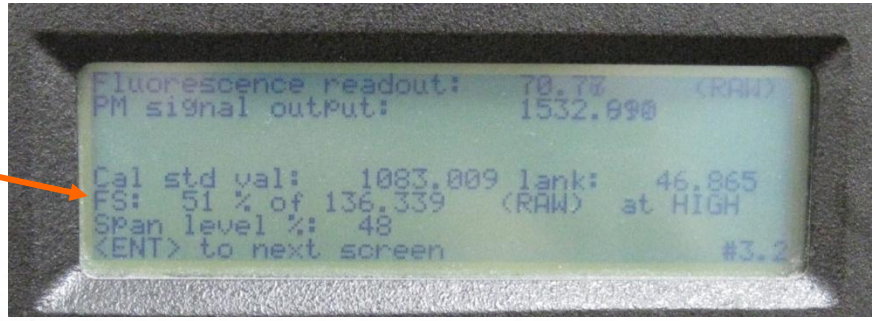
5. Loosen the sensitivity locking screw using allen wrench.

6. Insert and cover your standard.

7. Using the sensitivity knob slowly turn the knob to adjust the %FS to the value appropriate for your standard's concentration. A deviation ± 5% is acceptable.

Sensitivity Knob

8. When the %FS reaches the designated value, tighten the sensitivity locking screw. For our example this is ≈ 50%



The Basic Operating Level is now set, and you can remove the standard for the 10-AU.

Setting the Basic Operating Level (aka Sensitivity) of your 10-AU is now complete.

You can access Screen 2.43 again and return range control to AUTO and begin calibration.

#2 Blanking the Instrument

Now that the sensitivity is set, you should blank the instrument.

1. Access screen 2.1

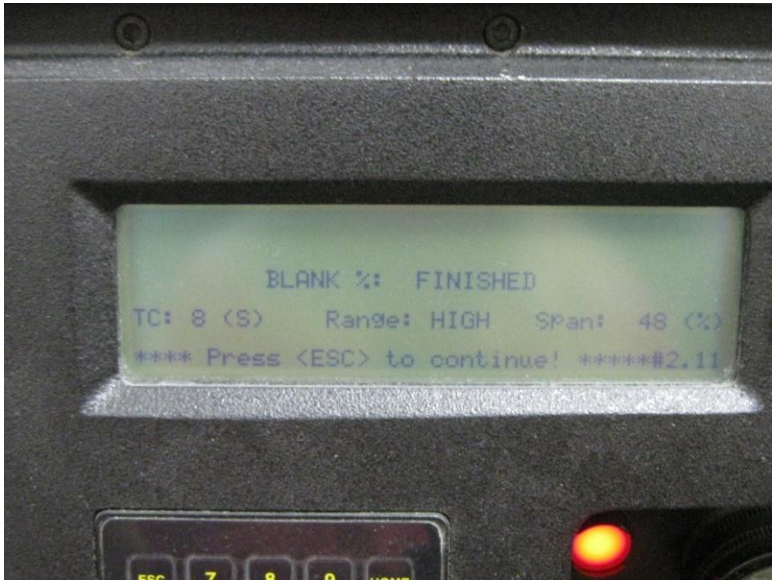
2. Insert and cover your blank (deionized water). Press 1 to Run blank.

***You can also choose to have the 10-AU subtract the blank signal from the sample readings on screen 2.12.**

Wait for the readings to stabilize and press “0”.

Wait for 15 seconds for the instrument to blank.

If Blanking is successful you will receive a “Blank Finished” message



#3 Calibration with Your Standard

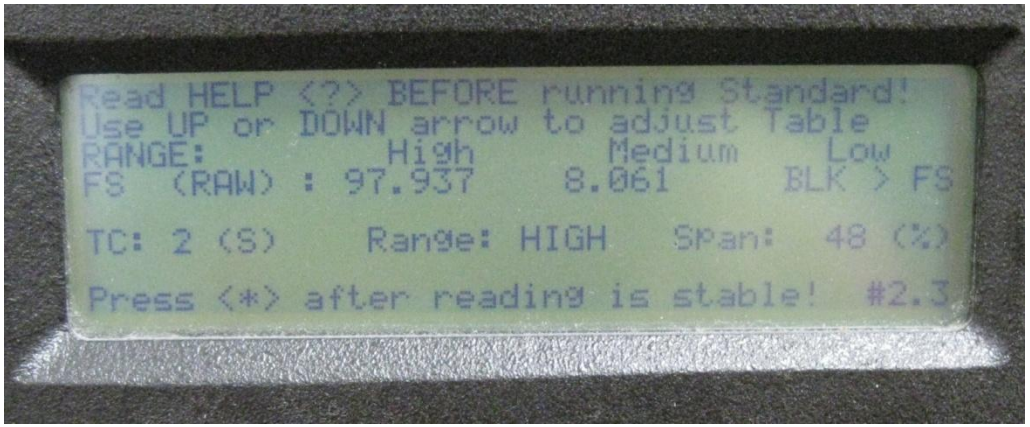
Now that the blank is set, you should calibrate with your standard.

1. Access screen 2.0

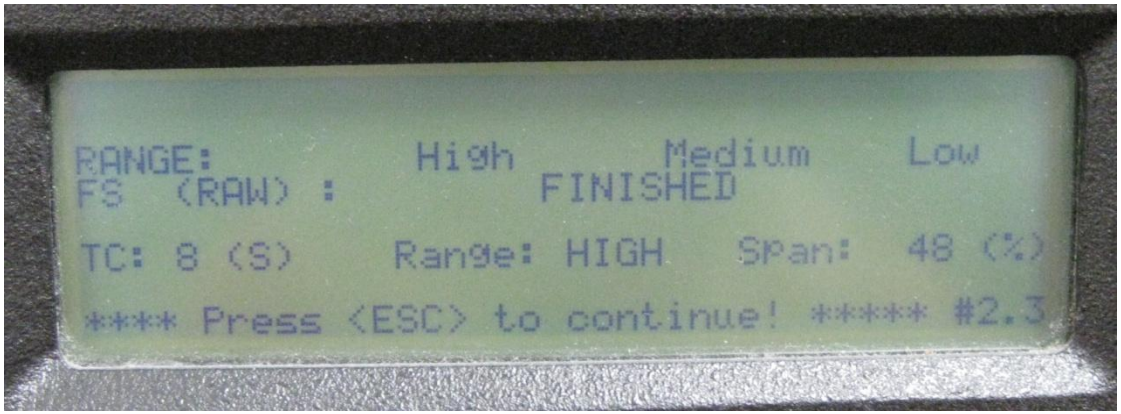
2. Press <2> to access screen 2.2 and input the actual concentration of your standard. Return to screen 2.0

3. Insert and cover your standard.

4. Press 3 to run standard. Wait for the reading to stabilize.

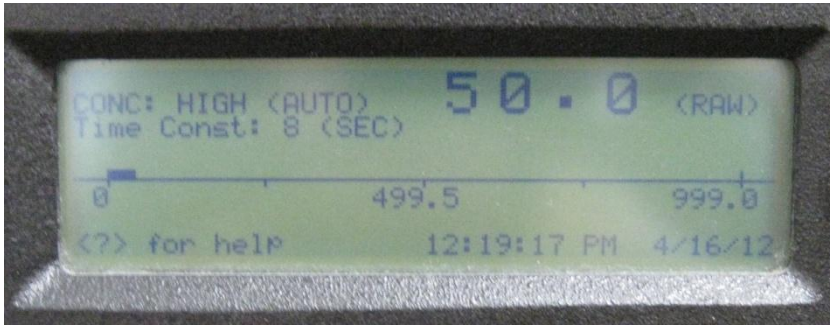


Press the <*> key and the calibration point is set.
Wait 15 seconds and you should receive a "FINISHED" message.

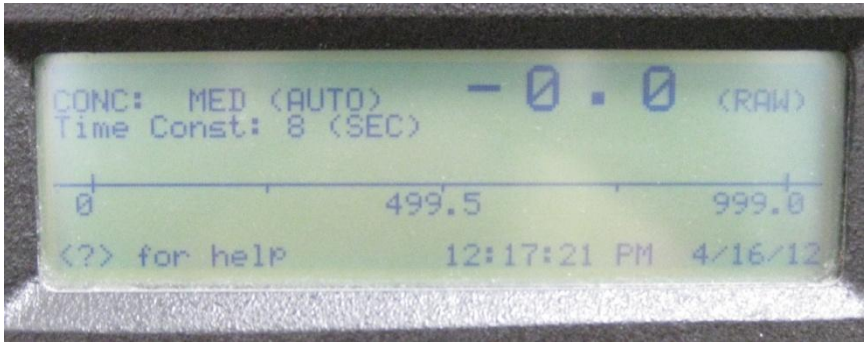


You can test/check your calibration by running your standards and other samples or standards.

I ran the 50ppb and got:



I ran the blank and got:



I ran a 10ppb standard and got: