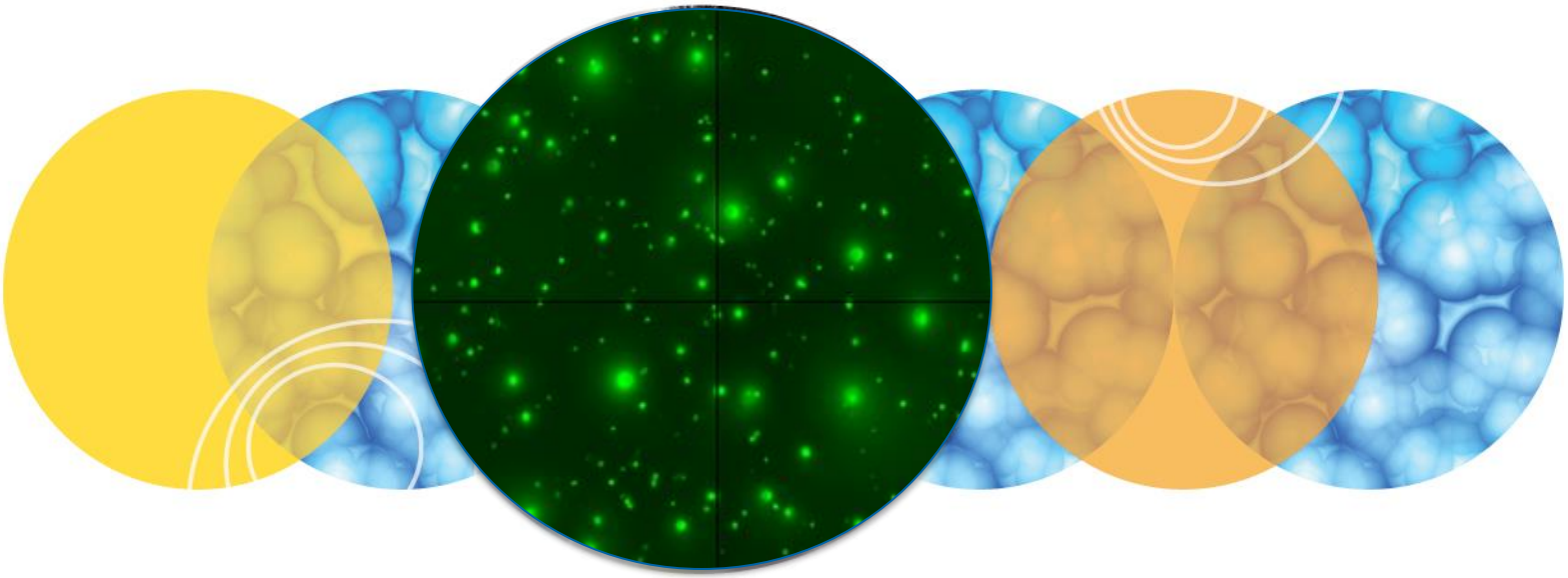

ClonePix 2 Training Guide

Prepare for Pick Run

Date Revised 2/27/2017 Version A

MOLECULAR DEVICES
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Index

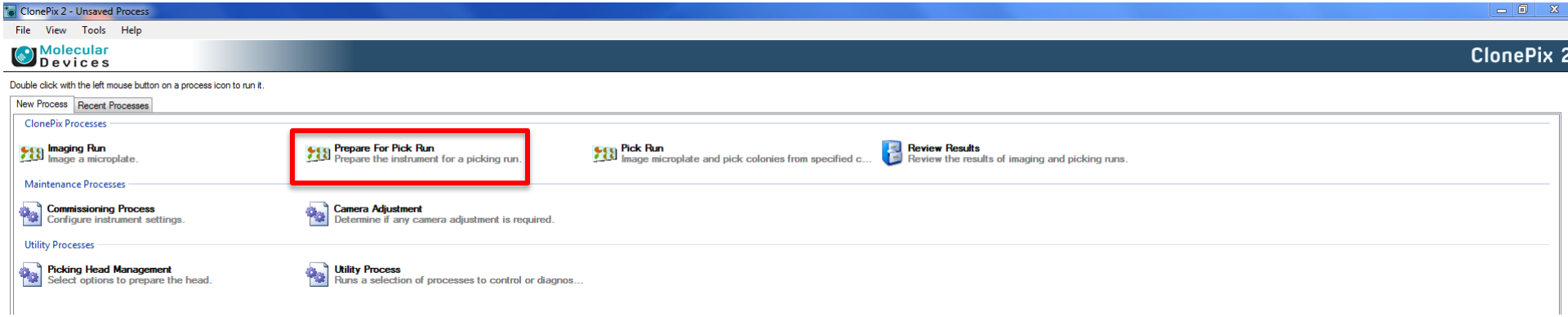
Chapter Purpose

The purpose of this chapter is to guide the user through the **Prepare for Pick Run** process on the **ClonePix 2**.

Pick head maintenance & basic pick run setup are not covered in this document. Please consult the dedicated training modules for detailed instructions on these other processes.

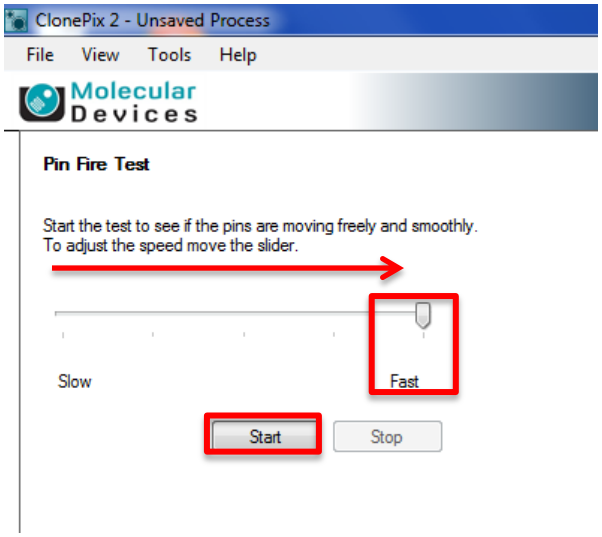
Launching Prepare for Pick Run

1. From the **Main Menu**, under **ClonePix Processes** select **Prepare for Pick Run**.



Pin Fire Test

2. The **Pin Fire Test** dialog now appears.
   a) **Click and drag** the **speed slider** to select the **Fast** setting, then click **Start**.



Pin Fire Test

2. The **Pin Fire Test** dialog now appears.
   a) **Click and drag** the **speed slider** to select the **Fast** setting, then click **Start**.
   b) You will now see that each **picking pin** in the **head** is fired **(extended)**, then **retracted sequentially**.

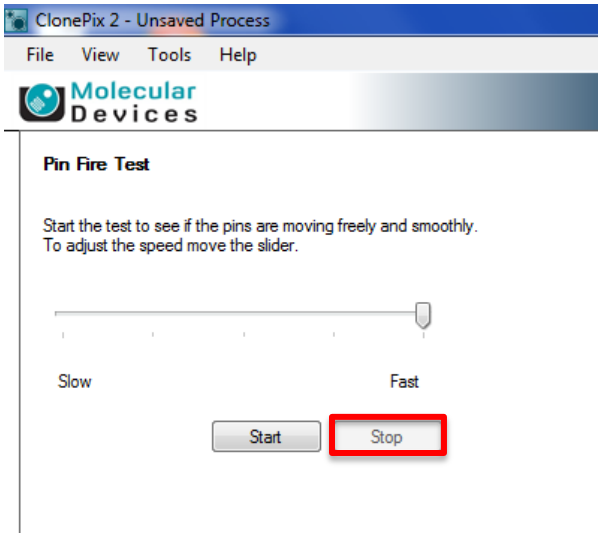
      Watch carefully for **3-4 full cycles** and ensure that **each of the 8 picking pins is moving smoothly**. If you note any **sticking** or **significant hesitation** of the **pin movement**, **contact Molecular Devices Technical support** for **further assistance** (**800-635-5577** or support@moldev.com)

Pin Fire Test

2. The **Pin Fire Test** dialog now appears.
   a) **Click and drag** the **speed slider** to select the **Fast** setting, then click **Start**.
   b) You will now see that each **picking pin** in the **head** is fired **(extended)**, then **retracted sequentially**.

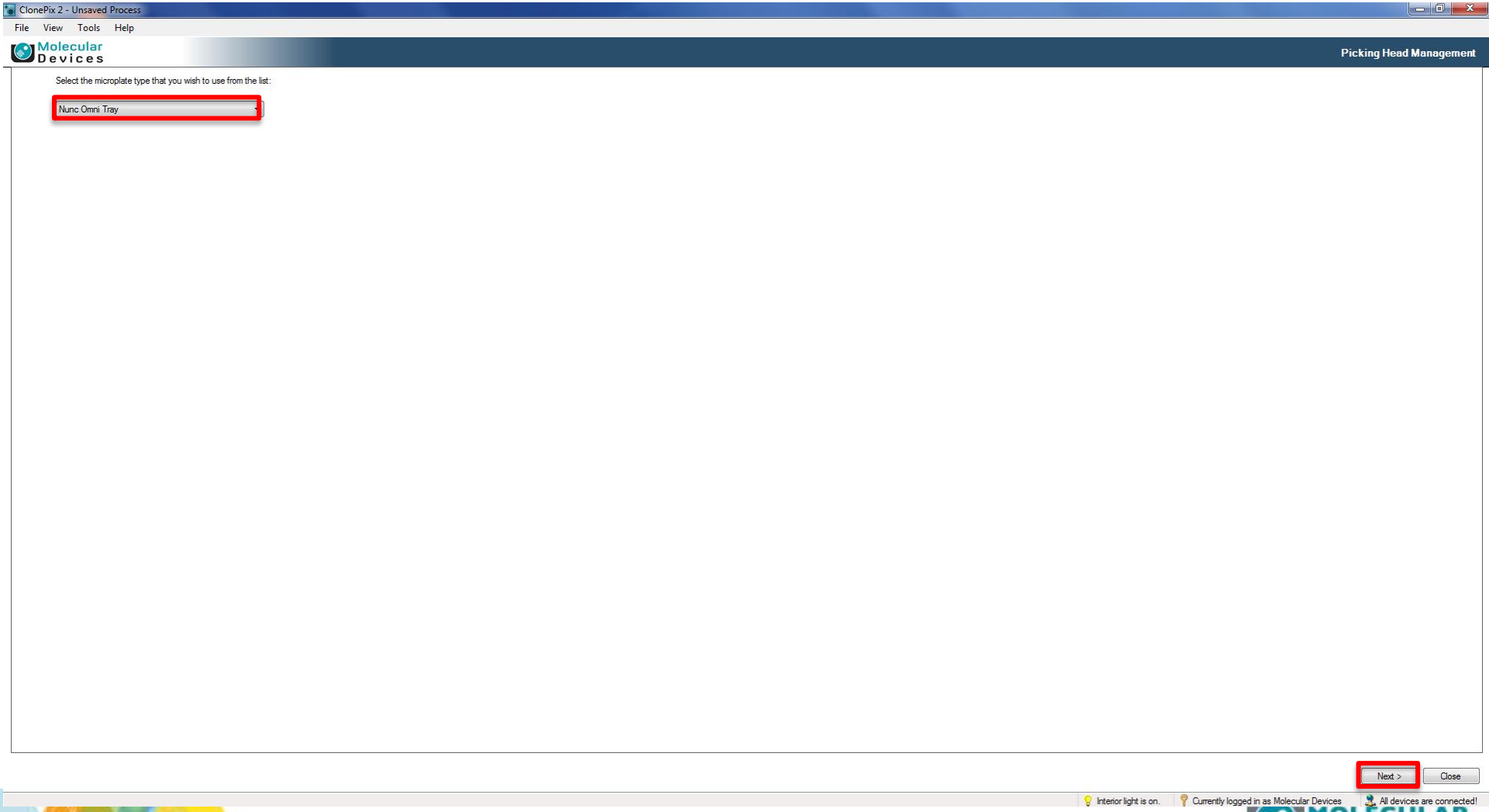
      Watch carefully for **3-4 full cycles** and ensure that **each of the 8 picking pins is moving smoothly**. If you note any **sticking or significant hesitation of the pin movement**, **contact Molecular Devices Technical support for further assistance** (800-635-5577 or support@moldev.com)

   c) Click the **Stop** button to conclude the test, then click **Next** to proceed.



Select Source Plate Type

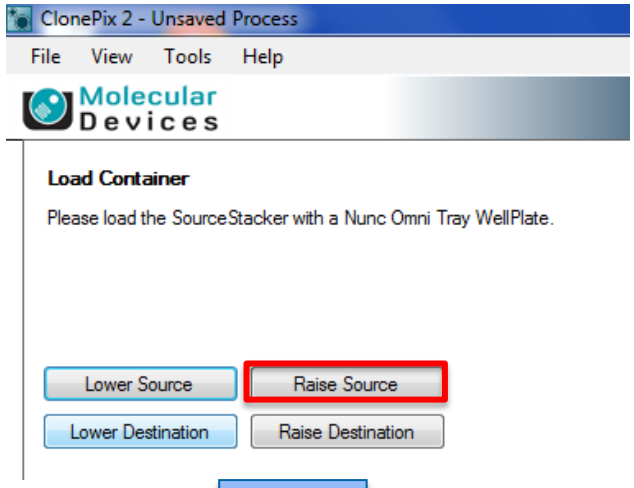
3. You will now see a screen where you are prompted to **select your source microplate type**. **Select** your **source microplate type** from the **dropdown list**, then click **Next**.

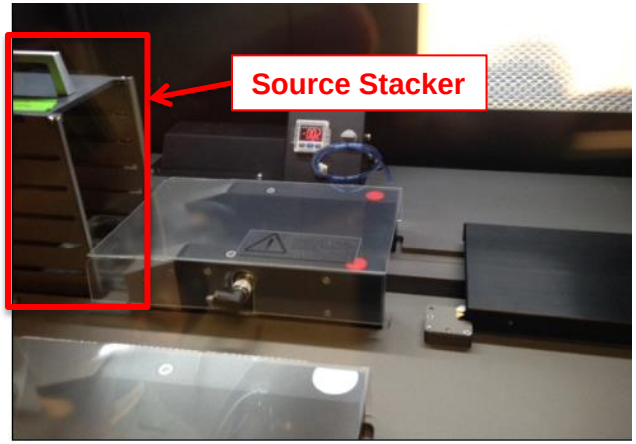


Load Source Plate Type

3. The Load Container dialog appears, where you are prompted to **load the source stacker** with your selected **source plate type**.

   a) Select **Raise Source** - the **Source Stacker** will now be **raised** within the **ClonePix 2 instrument.**



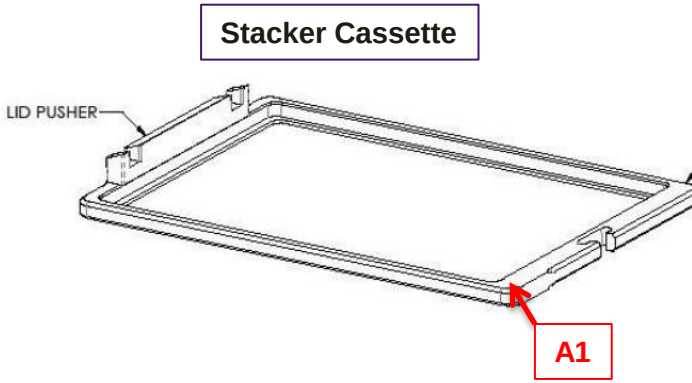


Load Source Plate Type

3. The **Load Container** dialog appears, where you are prompted to **load the source stacker** with your selected **source plate type**.

   a) Select **Raise Source** - the **Source Stacker** will now be **raised** within the **ClonePix 2 instrument.**

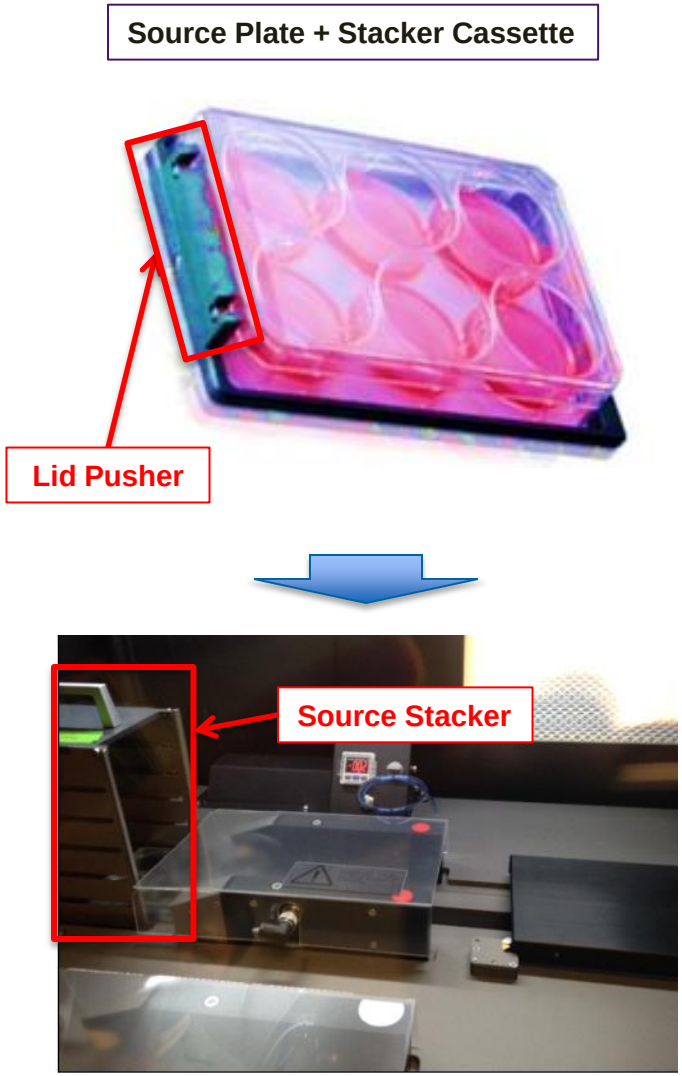
   b) Place an **empty** (no media, no cells) **source plate** in the **appropriate stacker cassette (see chart for details), with well A1 facing the lower right hand corner (away from the color-coded lid pusher).**



Source Plate	Lid Pusher Color	Lid Lifter Assembly
Greiner 6 well	Green	Red
Nunc Omni -	Blue	Silver
Nunc – 6 Well	Green	White

Load Source Plate Type

3. The **Load Container** dialog appears, where you are prompted to **load the source stacker** with your selected **source plate type**.
   a) Select **Raise Source** - the **Source Stacker** will now be **raised** within the **ClonePix 2 instrument.**
   b) Place an **empty** (no media, no cells) **source plate** in the **appropriate stacker cassette (see chart for details), with well A1 facing the lower right hand corner (away from the color-coded lid pusher).**
   c) Insert your **source plate + stacker cassette** into **any position** within the **source stacker** with the **lid pusher** facing the **back** of the **stacker** (until you hear the **magnet** engage), then click **Next.**



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   a) Select **Raise Source** - the **Source Stacker** will now be **raised** within the **ClonePix 2 instrument.**

   b) Place an **empty** (no media, no cells) **source plate** in the **appropriate stacker cassette (see chart for details), with well A1 facing the lower right hand corner (away from the color-coded lid pusher).**

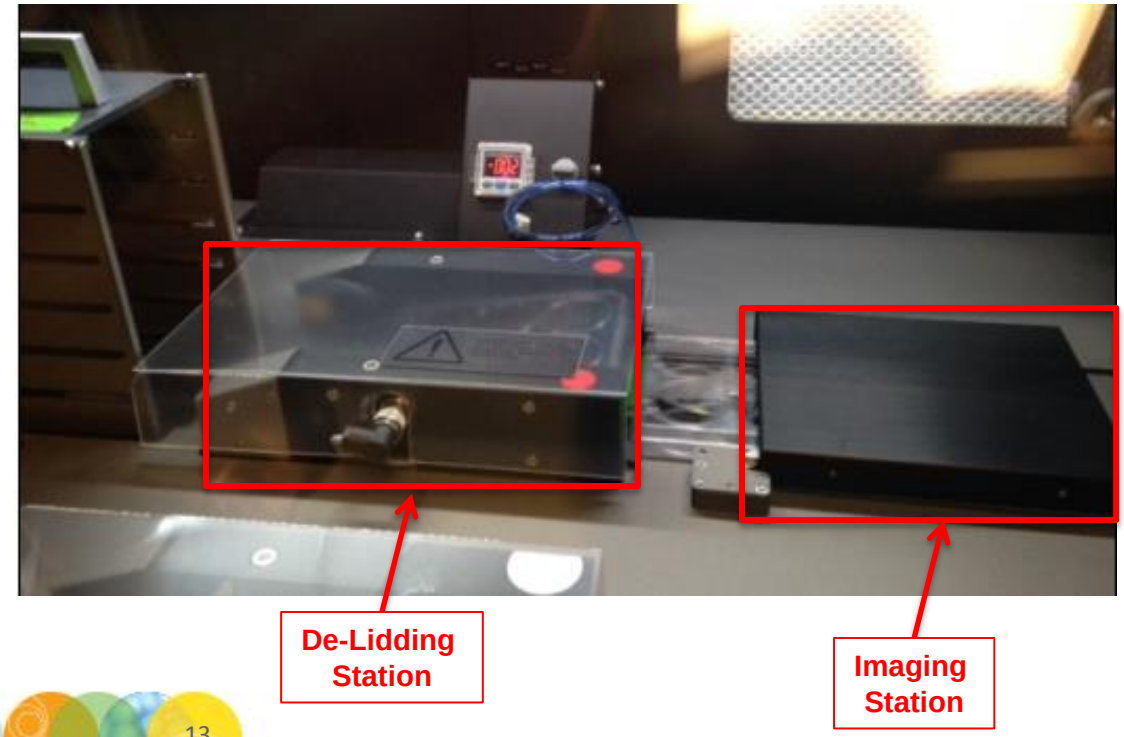
   c) Insert your **source plate + stacker cassette** into **any position** within the **source stacker** with the **lid pusher** facing the **back** of the **stacker** (until you hear the **magnet** engage), then click **Next.**

   d) The **stacker** will now **automatically** move through the stack and **detect the position of your loaded plate** (this could take up to a minute). You will see the **Loading holders** indicator on your computer screen during this process.

Load Source Plate Type

3. The **Load Container** dialog appears, where you are prompted to **load the source stacker** with your selected **source plate type**.

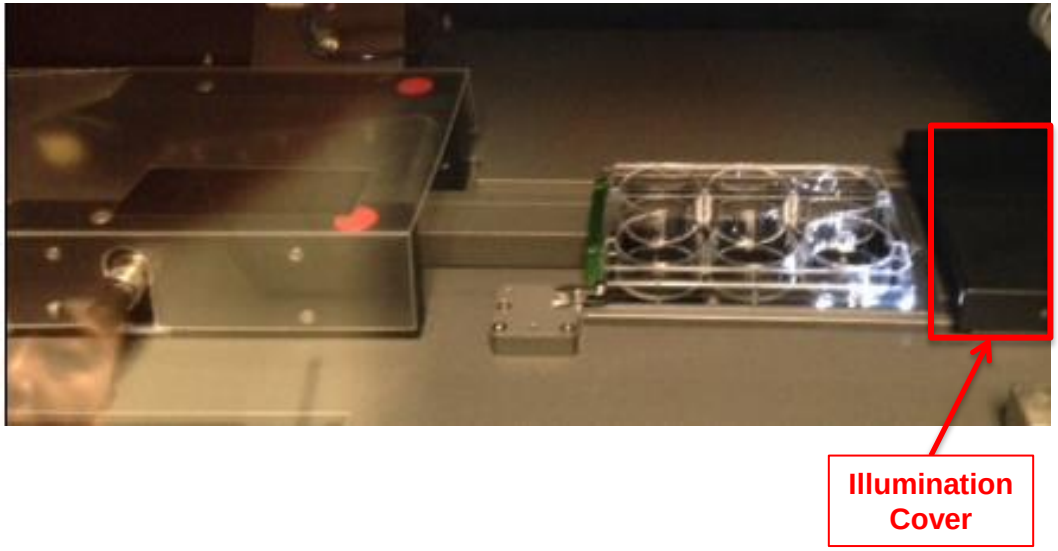
e) The **loaded plate** will next be pulled from the **stacker** into the **de-lidding station**, then to the **imaging station** for the next step. You will see the **Loading microplate** indicator on your computer screen during this process.



Adjusting Well Alignment

4. The **ClonePix 2 system** will now capture **white light images** of a **well** of your **source plate** and the software will enable any necessary **adjustment to alignment of the well** as follows:

a) The **illumination cover** will automatically **retract** and the system will capture **4 images** of your **well edges** (north, south, east, and west sides)**.** You will see the **Acquiring Alignment Images** indicator appear on your computer screen during this process**.**



Adjusting Well Alignment

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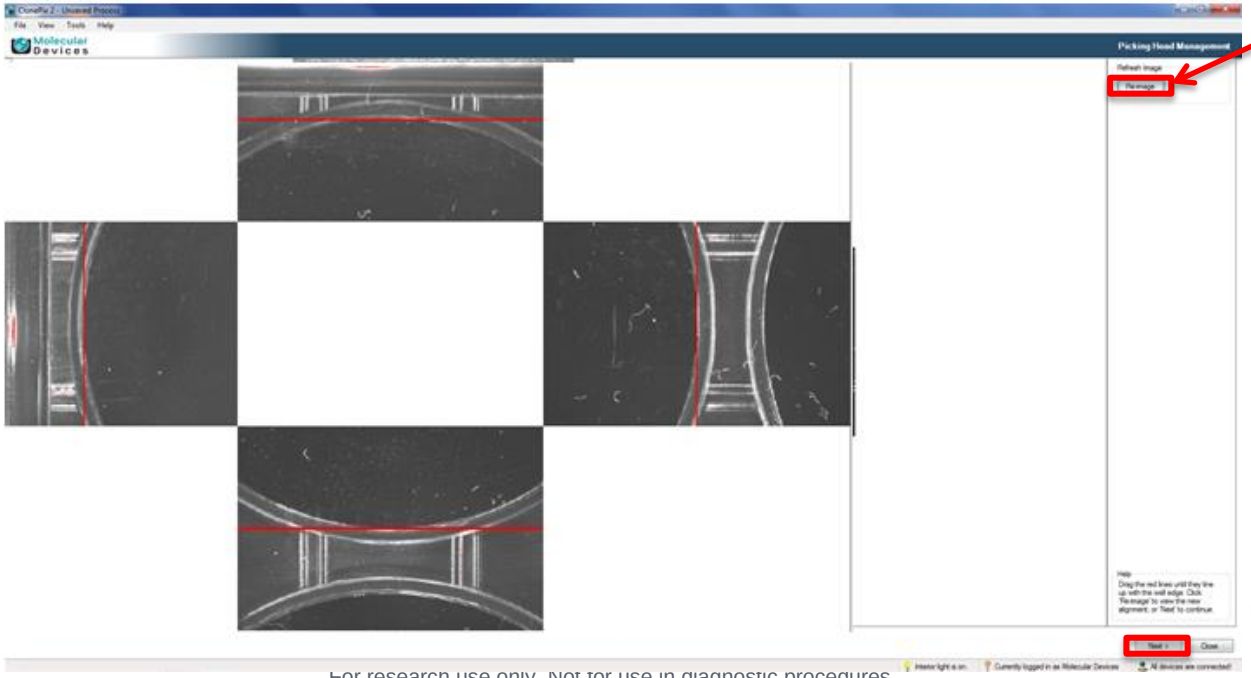
   a) The **illumination cover** will automatically **retract** and the system will capture **4 images** of your **well edges** (north, south, east, and west sides)**.** You will see the **Acquiring Alignment Images** indicator appear on your computer screen during this process**.**

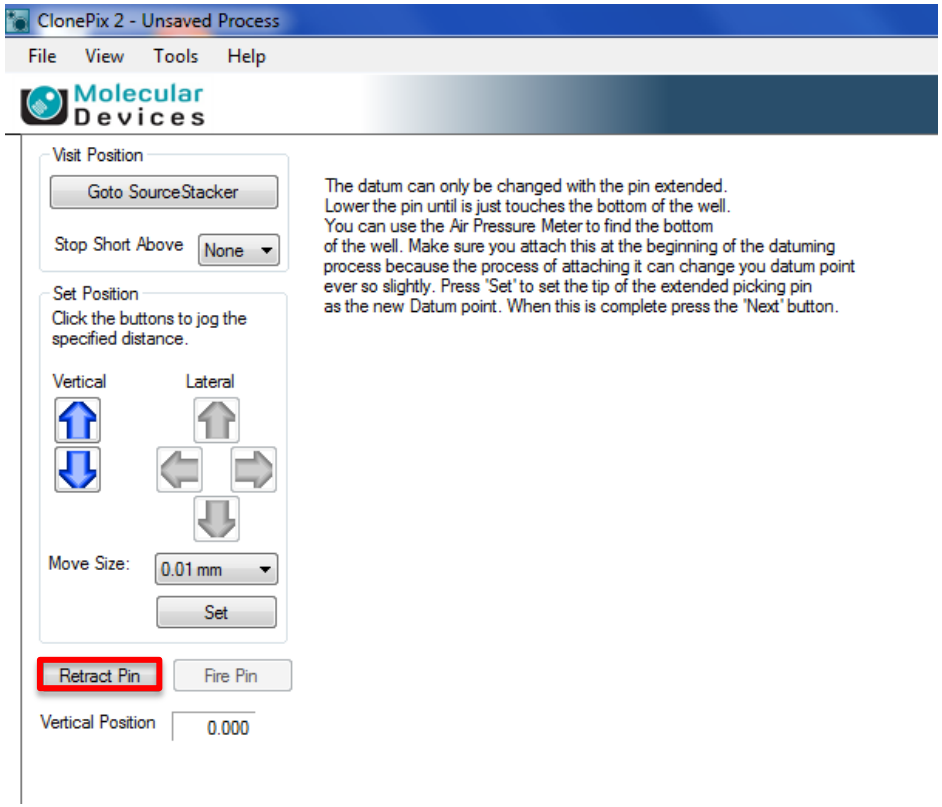
   b) You will now see the **4 well edge images** appear on your screen. The **red lines** superimposed on the image set should **align** closely with the **well edges. Adjust** the line positioning by **clicking and dragging** – note that the top/bottom (north/south) lines will move together, same for left/right (east/west). If you make an adjustment, click the **Re-image** button to confirm alignment. Click **Next** to proceed.



Adjusting Primary Picking Pin Z Datum Position

5. The **primary picking pin Z datum position** provides a **reference point** for the instrument to accurately pick your colonies while preventing collision with the plate bottom. This adjustment step ensures proper configuration of this position:

   a) The **illumination cover** will remain **retracted** and the **primary picking pin** (#1, closest to you as you face the instrument) will be **extended** to the center of a well in your loaded source plate. You will see the **adjustment dialog** appear (screenshot below) – click on **Retract Pin** before proceeding to the next step.

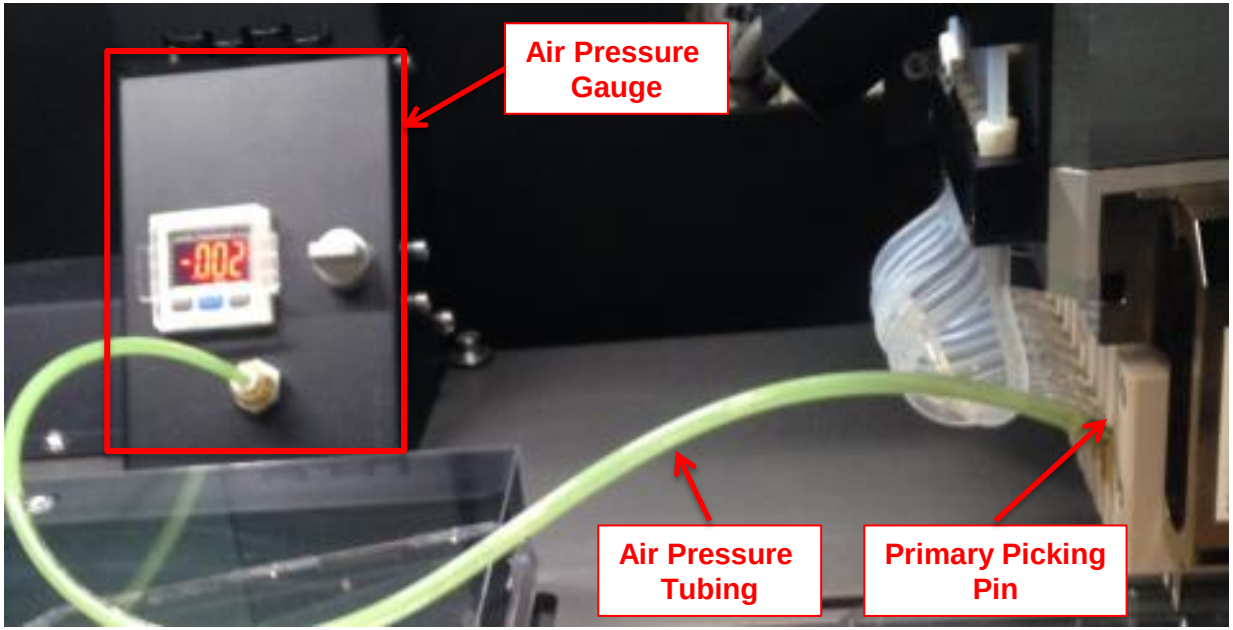


Adjusting Primary Picking Pin Z Datum Position

10. The **primary picking pin Z datum position** provides a **reference point** for the instrument to accurately pick your colonies while preventing collision with the plate bottom. This adjustment step ensures proper configuration of this position:

a) The **illumination cover** will remain **retracted** and the **primary picking pin** (#1, closest to you as you face the instrument) will be **extended** to the center of a well in your loaded source plate. You will see the **adjustment dialog** appear (screenshot below) – click on **Retract Pin** before proceeding to the next step.

b) Disconnect the **picking tubing** from the **primary picking pin**, then connect the **free end** of the **air pressure tubing** to the **primary picking pin** as shown below.



Adjusting Primary Picking Pin Z Datum Position

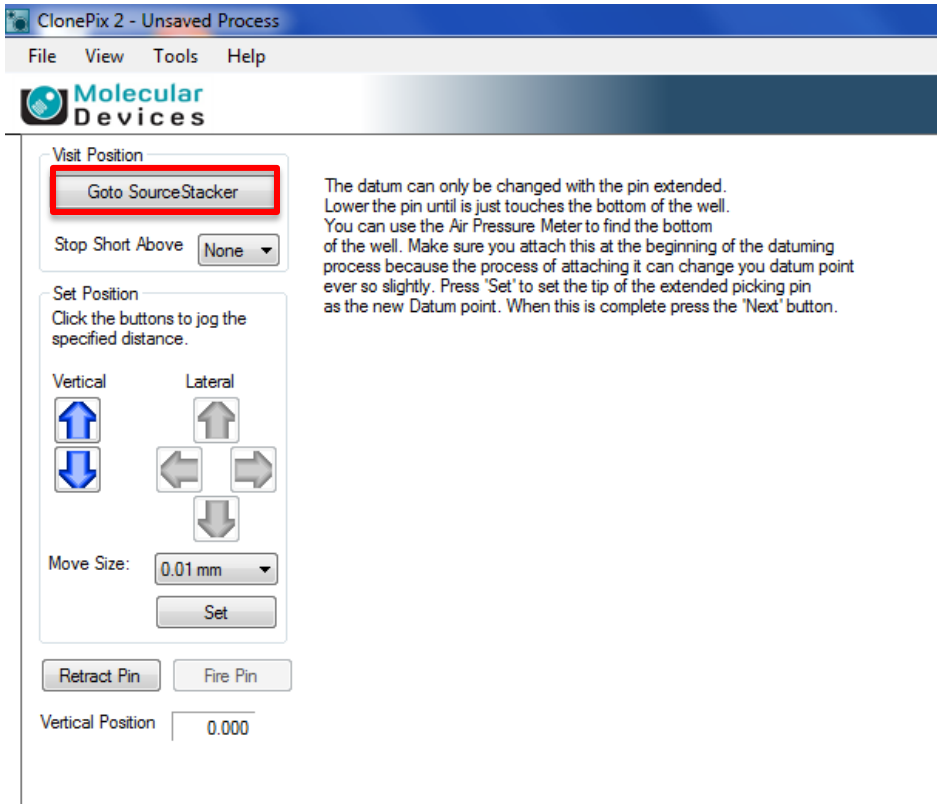
10. The **primary picking pin Z datum position** provides a **reference point** for the instrument to accurately pick your colonies while preventing collision with the plate bottom. This adjustment step ensures proper configuration of this position:

c) Switch on the **air pressure gauge** by twisting the **grey knob counterclockwise** so that the **handle** is **vertical** as pictured**.**

Adjusting Primary Picking Pin Z Datum Position

5. The **primary picking pin Z datum position** provides a **reference point** for the instrument to accurately pick your colonies while preventing collision with the plate bottom. This adjustment step ensures proper configuration of this position:

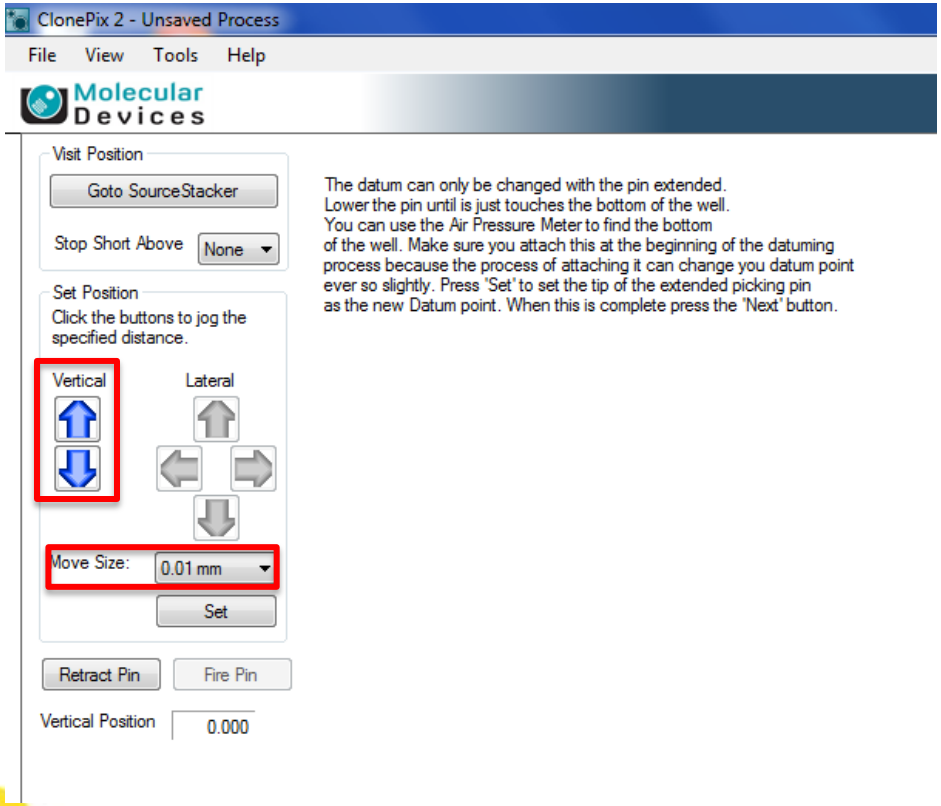
d) Click the **Goto SourceStacker** button - you will now see the **primary picking pin extend** to the bottom of the well and the **air pressure gauge readout value** will **increase (>0.25).**



Adjusting Primary Picking Pin Z Datum Position

5. The **primary picking pin Z datum position** provides a **reference point** for the instrument to accurately pick your colonies while preventing collision with the plate bottom. This adjustment step ensures proper configuration of this position:

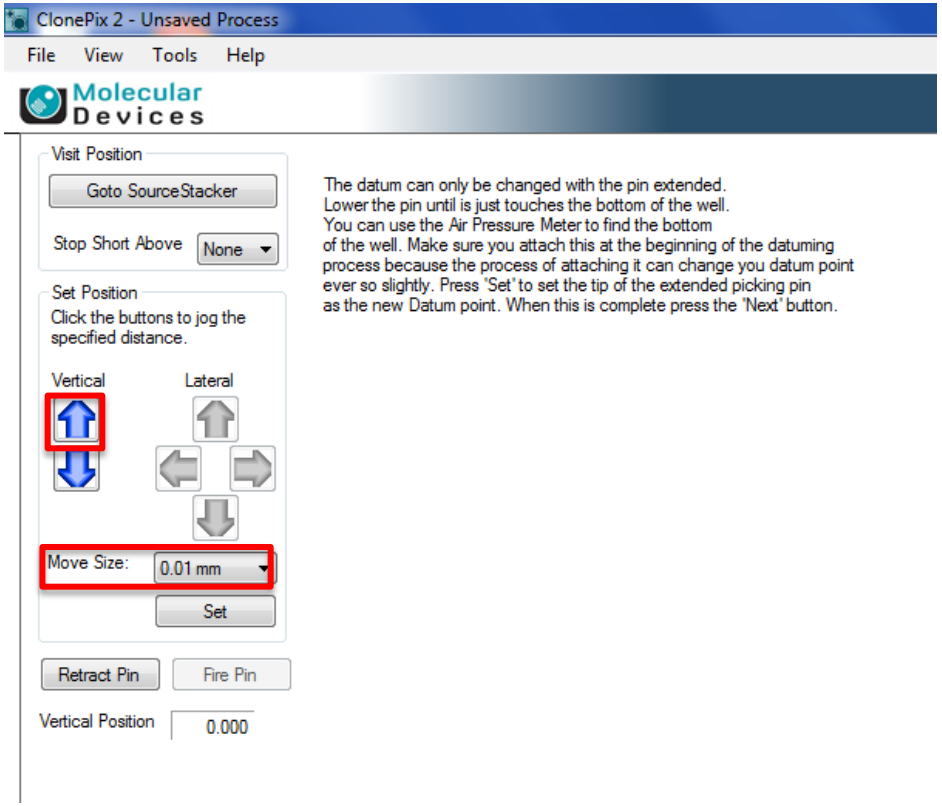
e) Use the **vertical arrow keys** to adjust the **Z position** of the **primary picking pin** until the **air pressure gauge readout color** changes to **green**. Select **move size increments** from the **dropdown** to refine the positioning.



Adjusting Primary Picking Pin Z Datum Position

5. The **primary picking pin Z datum position** provides a **reference point** for the instrument to accurately pick your colonies while preventing collision with the plate bottom. This adjustment step ensures proper configuration of this position:

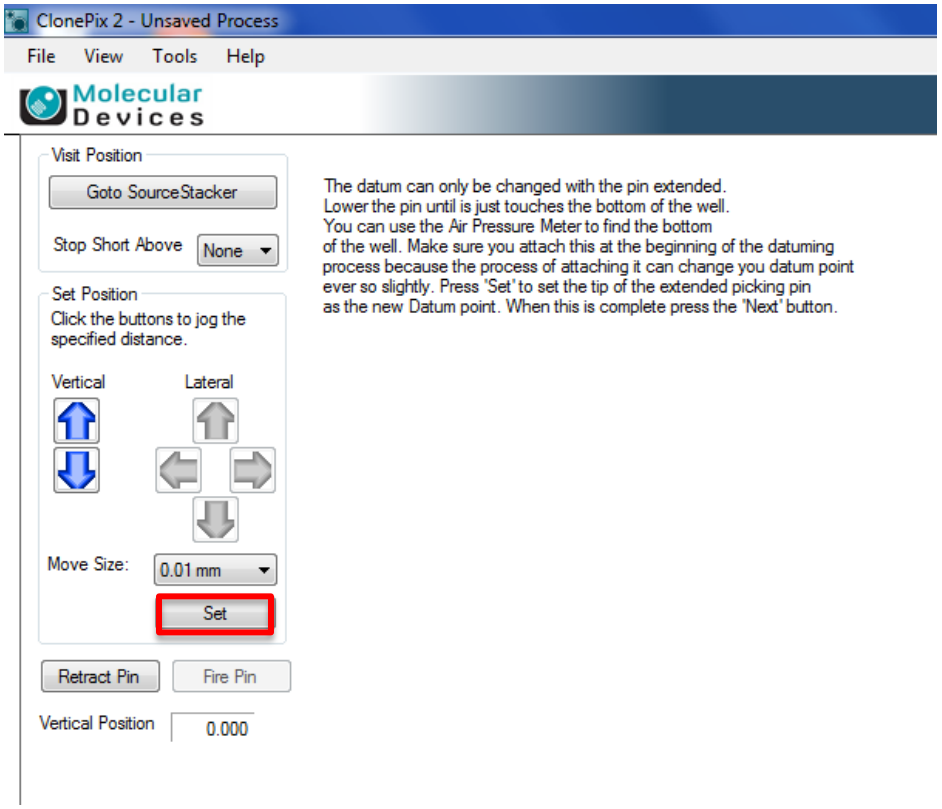
f) Once you have refined the position such that the indicator just turns **green** at the *smallest move increment (0.01 mm in the dropdown*), **move the pin up** by selecting the **1 mm move size setting** and clicking the **up key once** (the **air pressure gauge readout** will now change back to **red**).



Adjusting Primary Picking Pin Z Datum Position

5. The **primary picking pin Z datum position** provides a **reference point** for the instrument to accurately pick your colonies while preventing collision with the plate bottom. This adjustment step ensures proper configuration of this position:

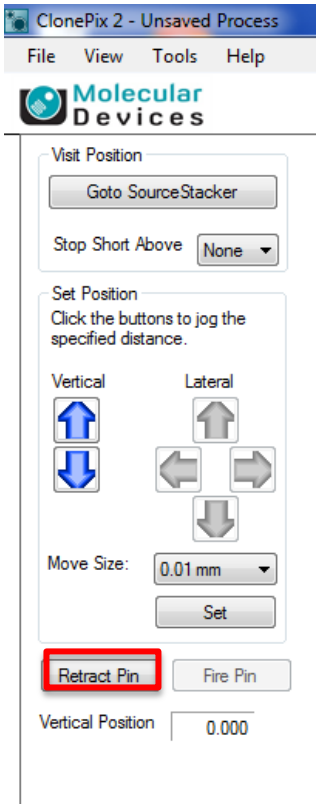
g) Click the **Set** button to save the current **Z datum position**. You will be prompted to confirm your choice to save the position – click **Yes**.

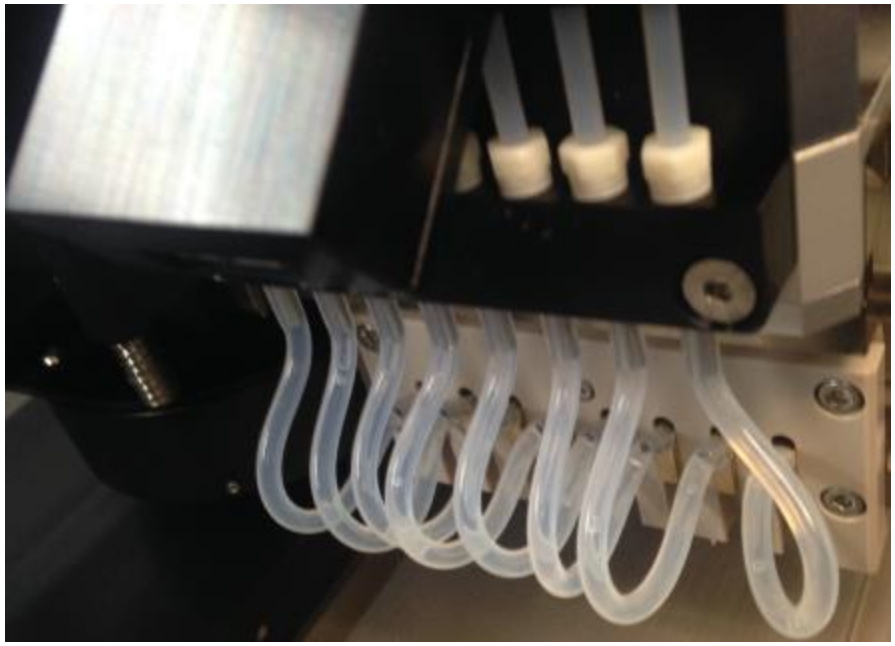


Adjusting Primary Picking Pin Z Datum Position

5. The **primary picking pin Z datum position** provides a **reference point** for the instrument to accurately pick your colonies while preventing collision with the plate bottom. This adjustment step ensures proper configuration of this position:

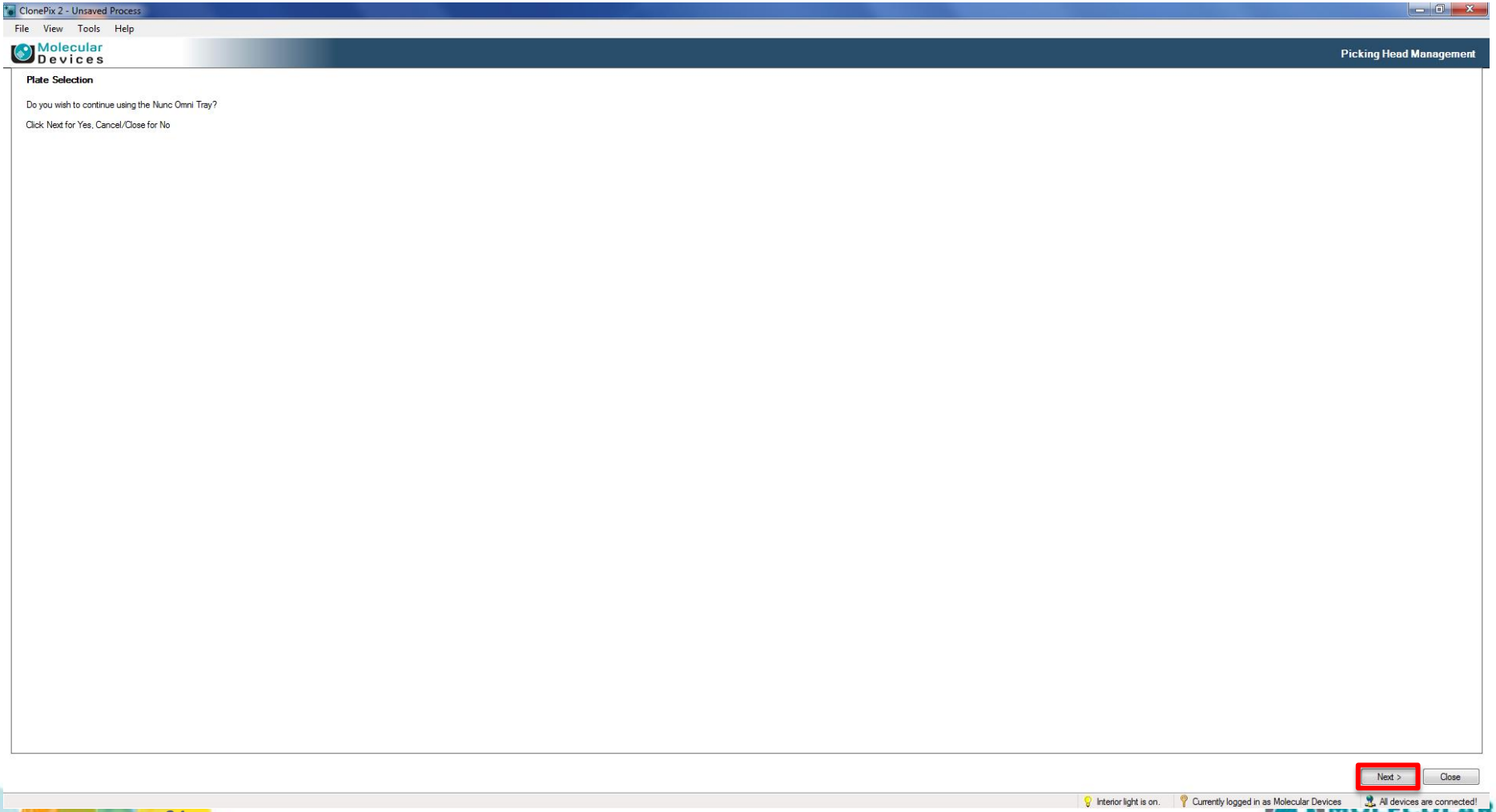
h) Click on **Retract Pin. Disconnect** the **air pressure tubing** from the **primary picking pin**, then **reconnect** the **picking tubing** to the pin. Click **Next** to proceed.





Confirm Plate Type

6. The **Plate Selection** dialog now appears. Click the **Next** button to **confirm** that you want to **continue** to the next step using the **same source plate type** that you have been working with up to this point in the process.

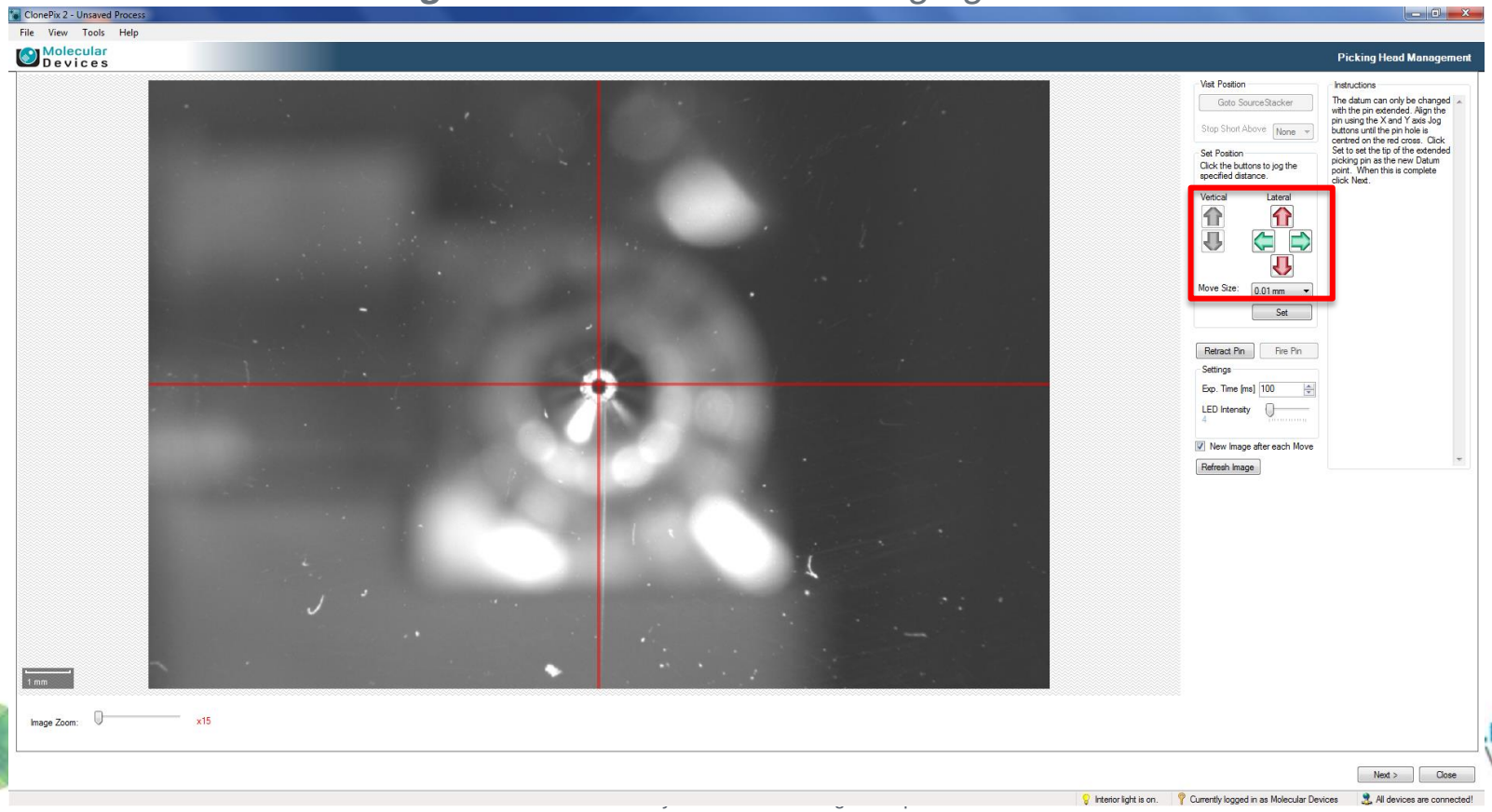


Adjusting Primary Picking Pin XY Alignment

7. This next step allows for **adjustment** of the **primary picking pin XY alignment**. This step is important to ensure accurate colony picking. Follow these steps to adjust the alignment:

a) Click on the **lateral arrow keys** to adjust the position of the **primary picking pin** so that the **center** of the **pin** aligns as closely as possible with the **intersection** of the **red crosshairs**. Change the **Move Size** via the **dropdown** as needed to refine positioning.

NOTE: *The **arrow keys** move the **pin** itself (NOT the crosshairs). Please click **once**, then **wait** for the **image** to **refresh before** clicking again.*

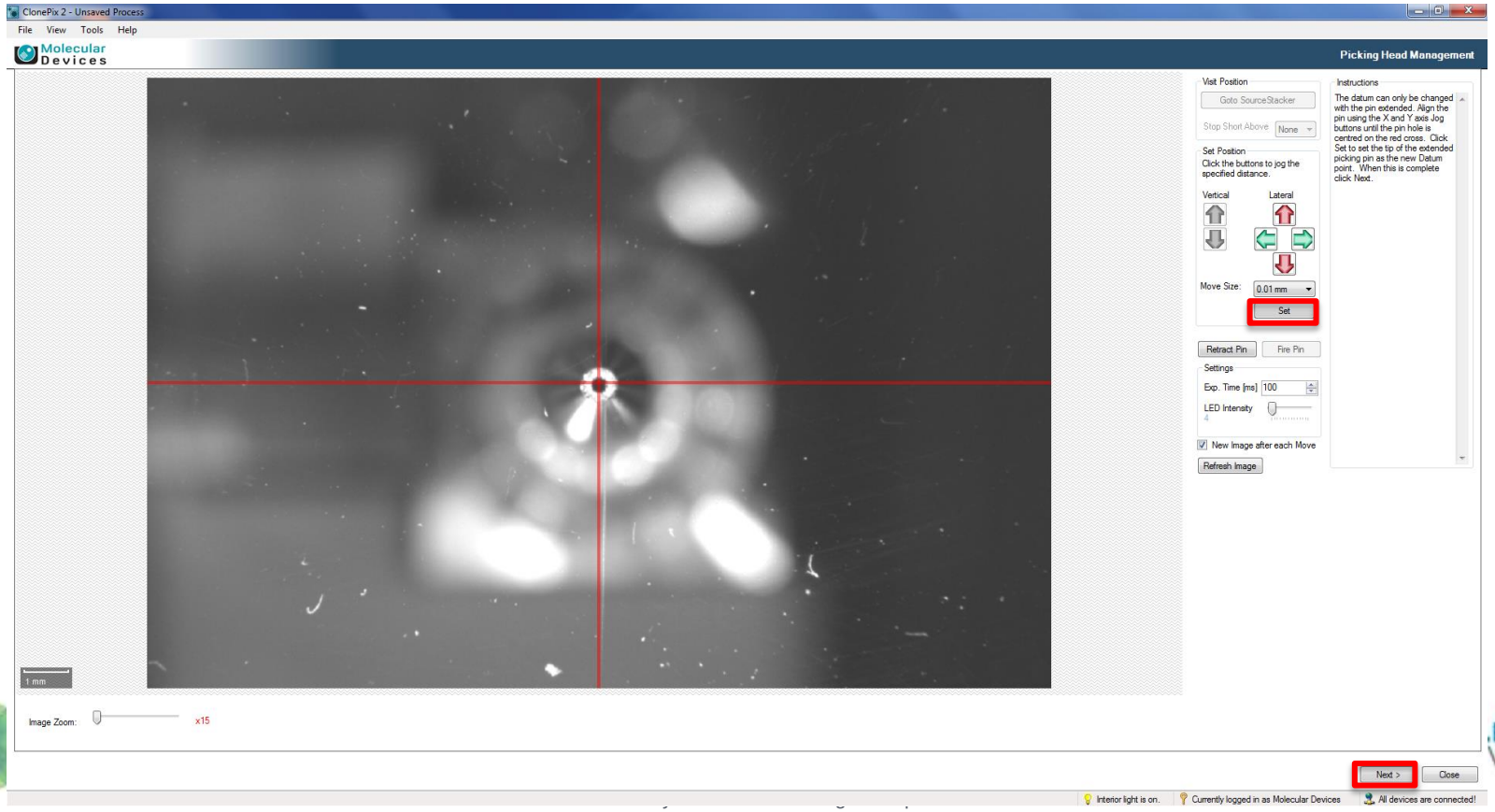


Adjusting Primary Picking Pin XY Alignment

7. This next step allows for **adjustment** of the **primary picking pin XY alignment**. This step is important to ensure accurate colony picking. Follow these steps to adjust the alignment:

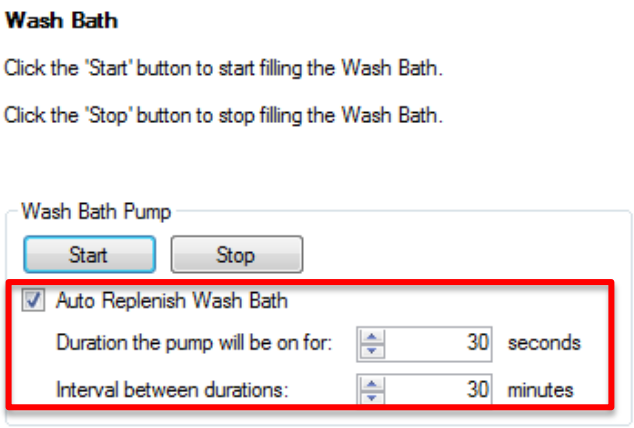
b) Once the positioning is correct, click the **Set** button. You will then see a **dialog** that will ask you to **confirm save** of the **datum point** – click **Yes**.

c) Click the **Next** button to proceed to the next step.



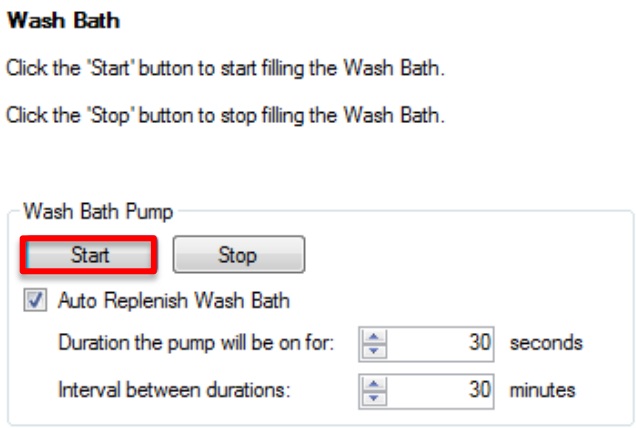
Filling the Ethanol Wash Bath

8. The **Wash Bath** dialog now appears.
   a) Ensure that your **Wash Bottle** is at least ¾ full with **70% Ethanol** (**second** bottle from **left front** side of instrument).
   b) Ensure that the **Auto Replenish Wash Bath checkbox** is **checked**, and **keep** the **default settings** (**30 seconds for pump duration, 30 minutes for intervals between durations**)



Filling the Ethanol Wash Bath

8. The **Wash Bath** dialog now appears.
   a) Ensure that your Wash Bottle is at least ¾ full with 70% Ethanol (second bottle from left front side of instrument).
   b) Ensure that the **Auto Replenish Wash Bath checkbox** is **checked**, and **keep** the **default settings** (**30 seconds for pump duration, 30 minutes for intervals between durations**) for the **Wash Bath Pump**.
   c) Click the **Start** button to **initiate filling** of the **Ethanol Wash Bath.**



Filling the Ethanol Wash Bath

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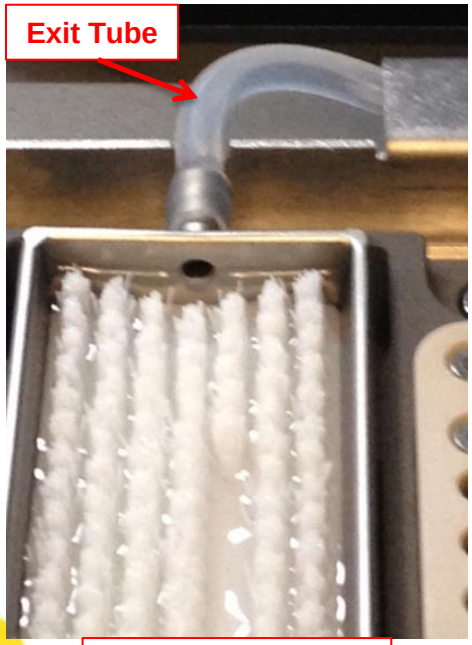
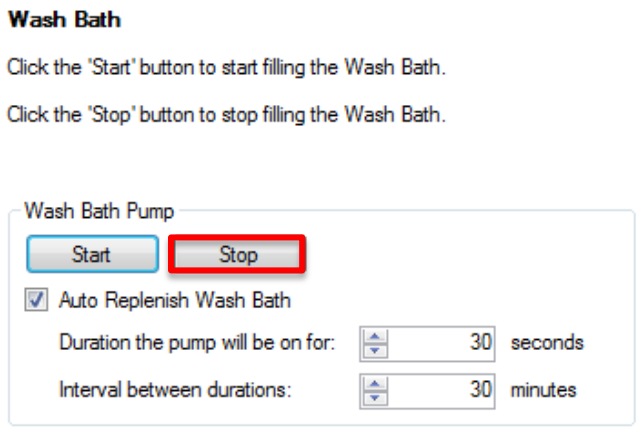
   a) Ensure that your Wash Bottle is at least ¾ full with 70% Ethanol (second bottle from left front side of instrument).
   b) Ensure that the **Auto Replenish Wash Bath checkbox** is **checked**, and **keep** the **default settings** (**30 seconds for pump duration, 30 minutes for intervals between durations**) for the **Wash Bath Pump**.
   c) Click the **Start** button to **initiate filling** of the **Ethanol Wash Bath.**
   d) Observe the **filling** of the **ethanol wash bath** in the **ClonePix 2** – allow the fill to **continue** until the **fluid reaches the exit tube** at the **back** of the bath (it is okay if you still see bubbles in the exit tube). Click the **Stop** button, then click **Next** to proceed.



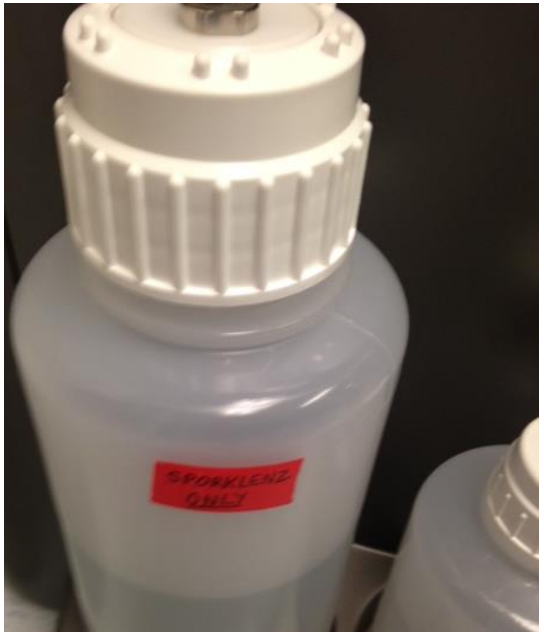
Ethanol Wash Bath



Sanitizing Picking Pins

9. This next step ensures that the **picking pins & fluidics** are **flushed** with **sanitizing solution** (important for maintaining sterility during picking):

a) Ensure that the **5L wash bottle** containing approximately **4L of SporKlenz solution** is attached to the **leftmost fluidics connection** on the ClonePix 2 instrument.



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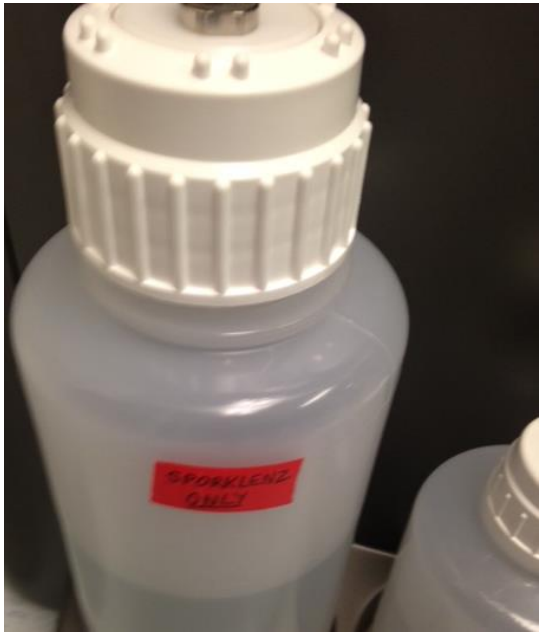
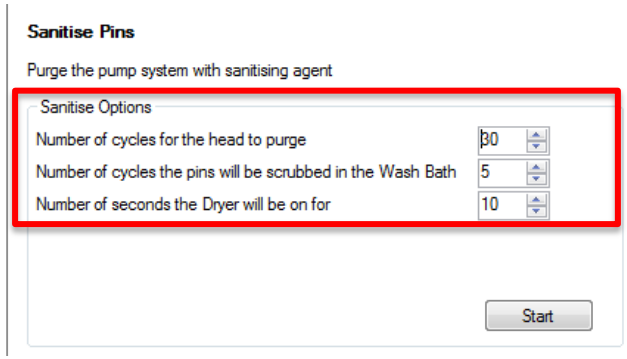
a) Ensure that the **5L wash bottle** containing approximately **4L of SporKlenz solution** is attached to the **leftmost fluidics connection** on the ClonePix 2 instrument.

b) Leave the **default** settings in the **Sanitise Options** dialog (30/5/10 as pictured).





Sanitizing Picking Pins

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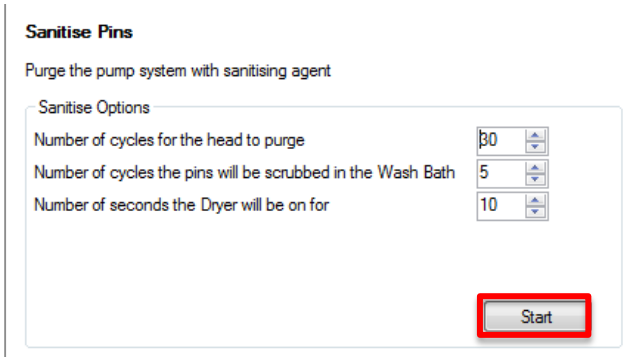
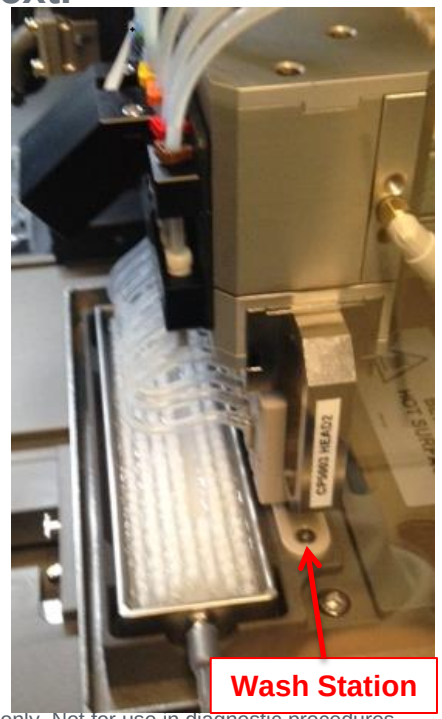
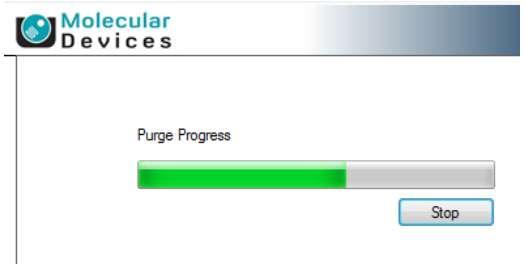
a) Ensure that the **5L wash bottle** containing approximately **4L of SporKlenz solution** is attached to the **leftmost fluidics connection** on the ClonePix 2 instrument.

b) Leave the **default** settings in the **Sanitise Options** dialog (30/5/10 as pictured).

c) Click the **Start** button in the **Sanitise Pins** dialog to begin **flushing** the ClonePix 2 with **SporKlenz**. You will see the **picking head** move to the **wash station** and the **pins** inserted into the **8 ports** within. You will see the **Purge Progress** dialog appear as well. Once this step is complete, click **Next.**



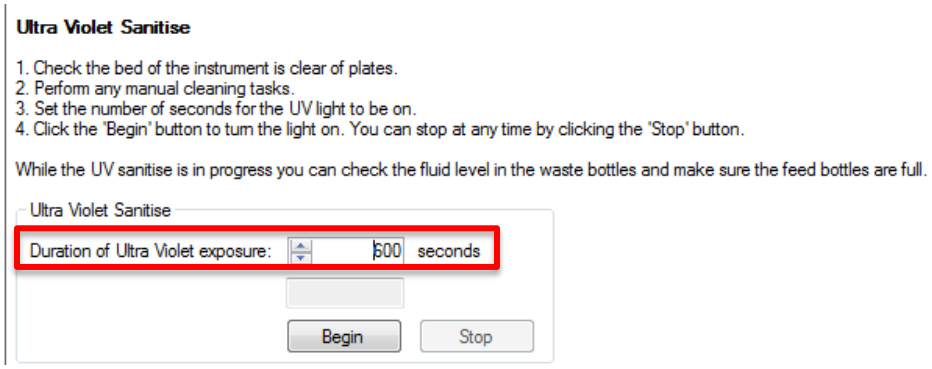




UV Sanitizing ClonePix 2 Deck

10. This next step ensures that the **ClonePix 2 instrument deck** is **sterilized** with **UV light** (important for maintaining sterility during picking):

a) The **Ultra Violet Sanitise** dialog appears. Leave the **Duration of Ultra Violet exposure** setting at the **default** (600 seconds).

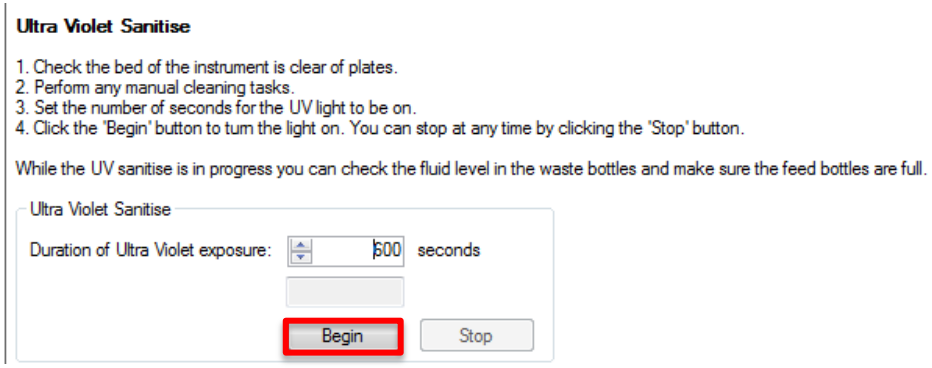


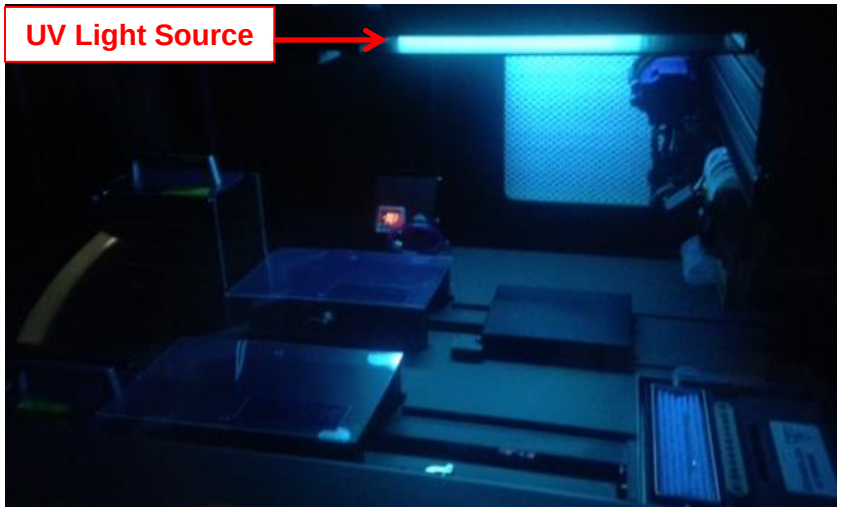
UV Sanitizing ClonePix 2 Deck

10. This next step ensures that the **ClonePix 2 instrument deck** is **sterilized** with **UV light** (important for maintaining sterility during picking):

a) The **Ultra Violet Sanitise** dialog appears. Leave the **Duration of Ultra Violet exposure** setting at the **default** (600 seconds).

b) Click **Begin** to start **UV sterilization** – this step takes **10 minutes**. You will see the **UV light illuminate the deck**, as well as the **UV light indicator** on the instrument **front panel** illuminate.



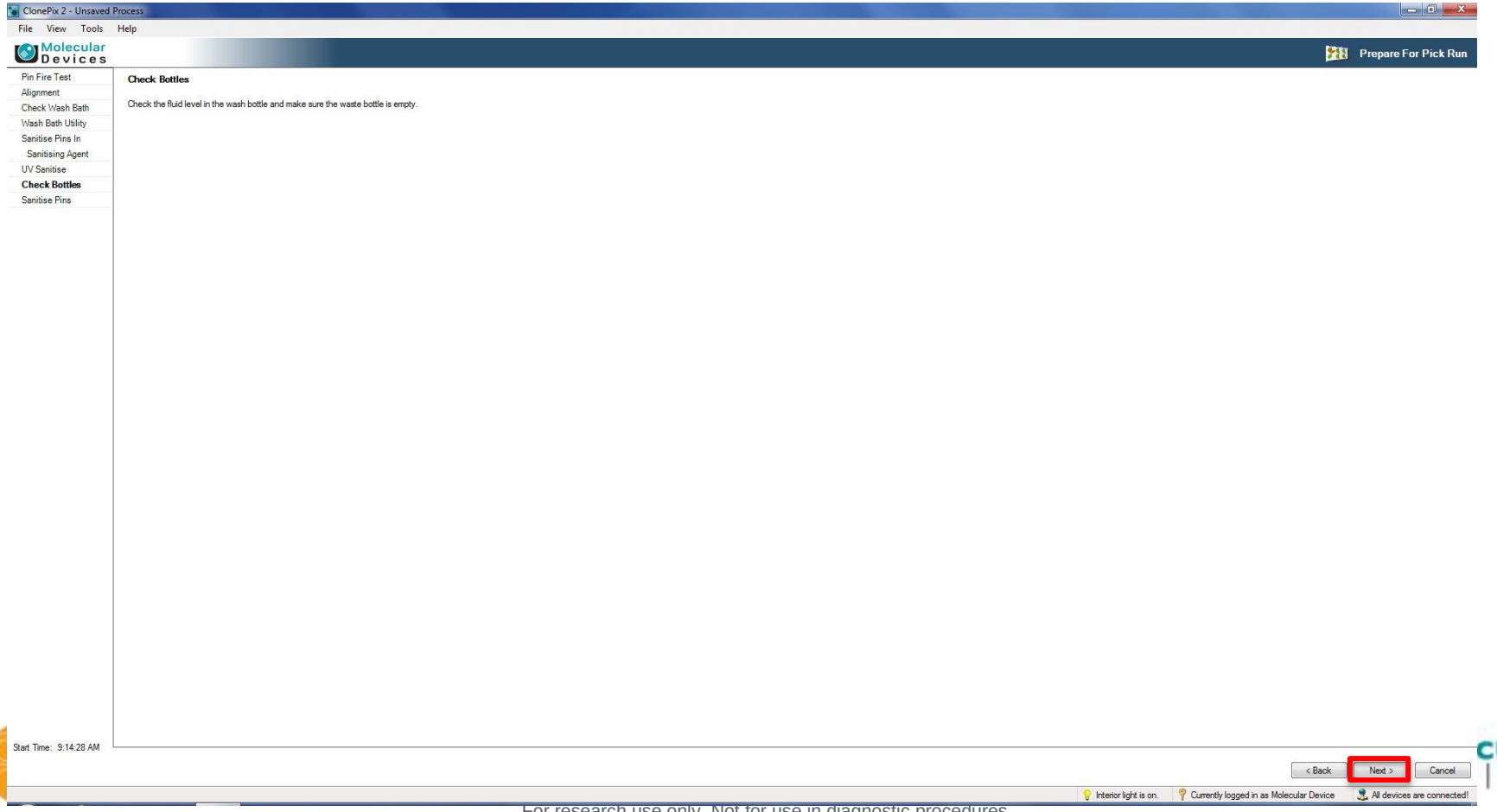


Check Wash & Waste Bottles

11. This next step is a reminder to check that the **wash bottles** are **full** and that the **waste bottles** are **empty.**

a) Ensure that the **wash bottles** (**SporKlenz 5L, Sterile Water 5L, 70% Ethanol 2L**) are at least **75% full** and that the **waste bottles** (wash & picking waste) are **empty**.

b) Click the **Next** button to proceed.

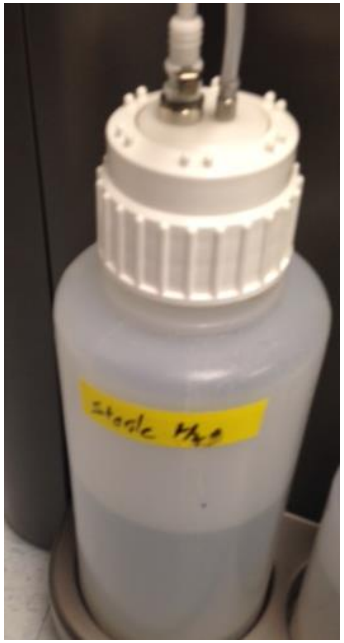


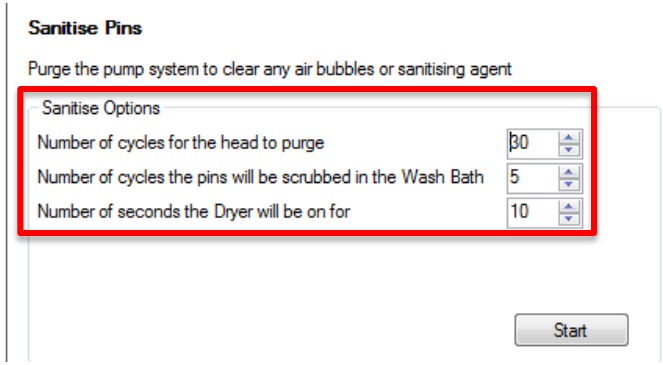
Rinsing Picking Pins

12. This next step ensures that the **picking pins & fluidics** are **flushed** with **sterile water** (important for removing sanitizing solution & maintaining sterility during picking):

a) **Disconnect** the **5L wash bottle** containing **SporKlenz** and then **connect** the **5L wash bottle containing** approximately **4L of Sterile Water** is **attached** to the **leftmost fluidics connection** on the ClonePix 2 instrument.

b) Leave the **default** settings in the **Sanitise Options** dialog (30/5/10 as pictured).





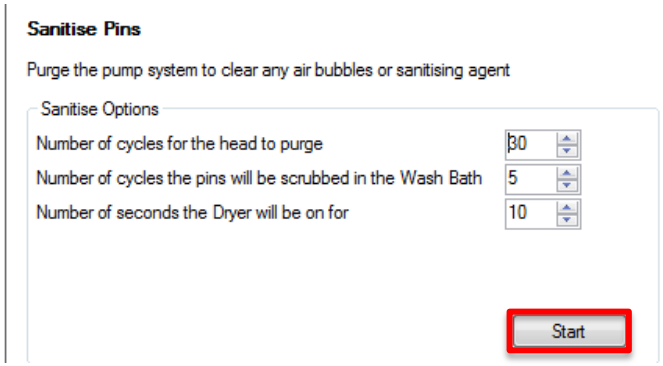
Rinsing Picking Pins

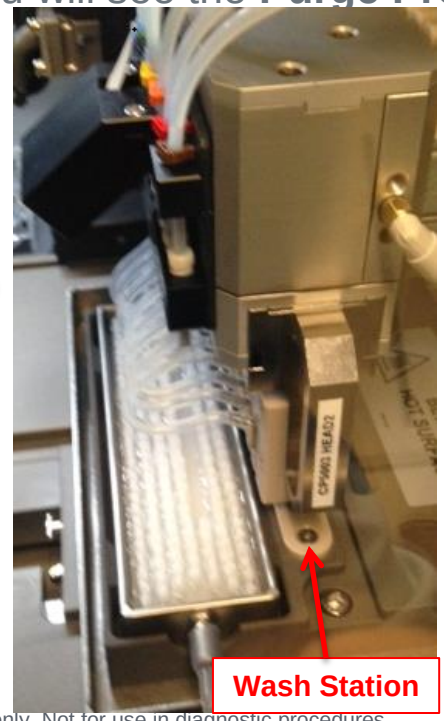
12. This next step ensures that the **picking pins & fluidics** are **flushed** with **sterile water** (important for removing sanitizing solution & maintaining sterility during picking):

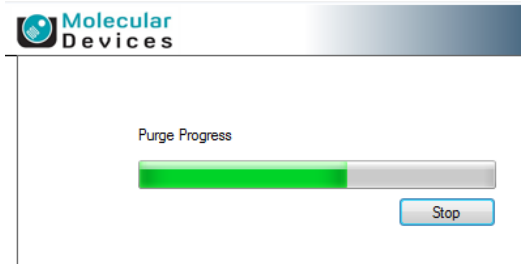
a) **Disconnect** the **5L wash bottle** containing **SporKlenz** and then connect the **5L wash bottle** containing approximately **4L of Sterile Water** is **connected** to the **leftmost fluidics connection** on the ClonePix 2 instrument.

b) Leave the **default** settings in the **Sanitise Options** dialog (30/5/10 as pictured).

c) Click the **Start** button in the **Sanitise Pins** dialog to begin **flushing** the ClonePix 2 with **Sterile Water**. You will see the **picking head** move to the **wash station** and the **pins** inserted into the **8 ports** within. You will see the **Purge Progress** dialog appear as well.







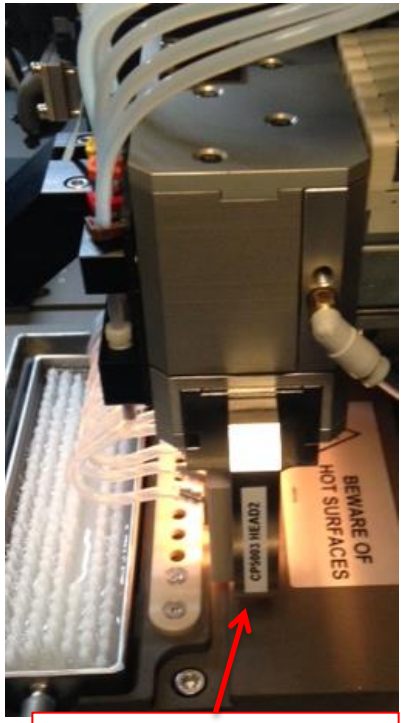
Rinsing Picking Pins

12. This next step ensures that the **picking pins & fluidics** are **flushed** with **sterile water** (important for removing sanitizing solution & maintaining sterility during picking):

d) The **picking head** will now **automatically** move to the **ethanol wash bath** and **scrub** the **picking pins**, followed by **drying** of the pins in the **halogen drying station**. You will see the **Washing Pins indicator** followed by the **Drying Pins indicator** on your **computer screen** during these steps. At the conclusion of the drying step, click **Next** to continue.

Ethanol Wash Bath

Washing Pins



Halogen Drying Station

Drying Pins

Conclusion of Process

13. This final step will result in **completion** of the **Replace Head** process:

a) You will see the **Process Completed** screen appear. Click the **Finish** button to return to the **main menu.** Click the **Finish** button.

Process Completed

Click the Finish button to return to the process properties.

b) Within the instrument, the following will occur **automatically**:

- **Re-homing** of the **picking head**
- **Re-lidding and return** of the **source plate** to the **source stacker**
- **Closing** of the **illumination cover** (at the imaging station).

c) Be sure to **remove** your **empty source plate** from the **source stacker** before proceeding with setting up a **Pick Run**.

Support Resources

- Go to the HELP menu within ClonePix 2 Software
- Support and Knowledge Base: http://mdc.custhelp.com/
- Request Support: http://mdc.custhelp.com/app/ask or via email support@moldev.com
- Technical Support can also be reached by telephone:
  - 1 (800) 635-5577
  - Select options for Tech Support → Biotherapeutics Products → ClonePix Instruments

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