Standard Operating Procedures, Frostad Research Group

Contributors: Abner Bogan, Emilie Espitalie, Emily Gubski, Beiduo Hu, Yun-Han Huang, Xiran Li, Diane Nguyen, Dan Walls, Minghuan Xu, Mengxiao Yang, Charlotte Yu

Last updated: **May 2019**

Contents

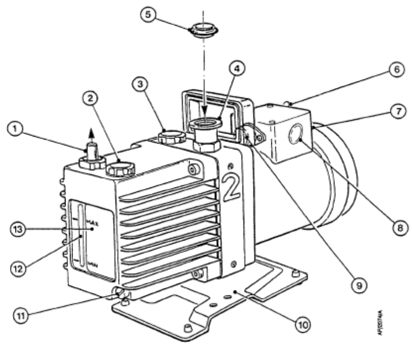
1 Lab Equipment

Edwards Direct-Drive Rotary Vane Vacuum Pump (E2M8)

Hazards: These pumps are not recommended for pumping hazardous substances. Furthermore, if you use a hydrocarbon oil in this pump, you cannot pump oxygen in concentrations greater than 25 percent in volume (explosion hazard). Users must ensure that the electrical supply is suitable for operation at 220-240 V and 50 Hz.

Personal Protective Equipment: It is required that users wear standard lab PPE, however gloves are not required unless you will be working with hazardous solvents (see MSDS for further PPE guidelines if this is the case).

View of Parts:



1. Outlet nozzle
2. Oil filler-plug
3. Gas-ballast control
4. NW25 inlet-port (adaptor flange)
5. NW25 centring ring and 'O' ring (supplied)
6. On/Off switch (single-phase motors only)
7. Motor terminal box
8. Cable gland
9. Overload reset button (single-phase motors, where applicable)
10. Baseplate
11. Oil drain-plug
12. Oil sight-glass
13. Pump identification label

Figure 1 - The E2M2 pump (single-phase motor shown)

Operation: Before operating the vacuum, ensure that the oil mist filter is attached to the outlet of the pump, and the threads are sealed with teflon tape. The user should also ensure that the oil has been replaced within the past 6 months. During operation, no more than 0.1 kg/hr water vapor should enter the pump. The control of the gas ballast is based on the nature of the inlet flow. If the air is completely dry, the gas ballast should be completely closed during operation so that the pump can reach its ultimate vacuum. If the inlet stream is suspected to contain some level of solvent vapor, then the gas ballast should remain open during operation to assist in purging some of these contaminants from the vacuum pump oil.

Maintenance: The vacuum oil must be replaced every 6 months with only high mineral oil (Ultragrade 19 is recommended but not required). When refilling the oil tank, fill the oil level up to the MAX line (about 0.6 liters oil); the oil level should always reside between the MAX and MIN level marks on the bezel of the oil sight glass. Lastly, users should regularly check the inlet and gas ballast filters through visual inspection and clean them with a suitable cleaning solution if necessary.

2 Experimental Setups

Pendant Drop Tensiometer

Hardware: A diagram of the current setup is shown in the Figure 1. To manually adjust the setup prior to testing, you will need a 5/32" and 3/16" Allen key. The camera mount and back wall parts can be adjusted through the 5/32" Allen key. All the linear stages are adjusted through the 3/16" Allen key. NOTE - the syringe fastener should only be hand tightened and additional force will deform or break the syringe barrel.

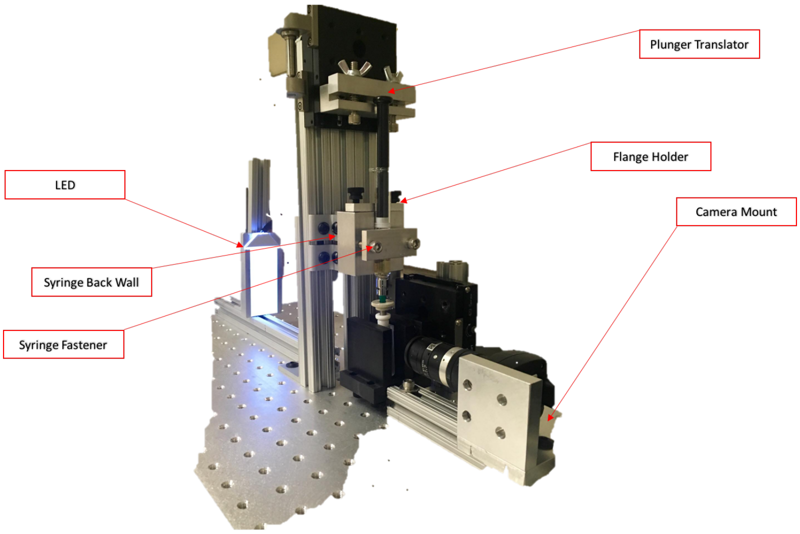


Figure 1: Current Iteration of Pendant Drop

1. For droplet time lapses, ensure syringe is first properly assembled with the Teflon plug piece
    (a) Hand-tighten knob (pictured in Figure 2 below) after insertion of needle to compress the o-rings to get a good seal

Figure 2: Knob for Needle Securing

2. Turn on the camera through the IDS Camera Manager Application
    (a) Turn on live video so you can see the area of focus, depth of field, etc. for positioning object of focus
3. Adjust the two linear stages and the syringe back wall piece for proper positioning of the object of focus (if necessary)
    (a) Ensure that the linear stage attached to the plunger translator is adjusted such that it has enough vertical range of motion
4. (For droplet time lapses) Put 1mL of working liquid into cuvette for humidity control purposes
5. (For droplet time lapses) Coat Teflon seal with vacuum grease with a clean cue tip, and gently twist into cuvette

6. Bring syringe and cuvette to setup, position at center of the back wall piece and hand-tighten with the syringe fastener.
   (a) Finely position plunger translator with micrometer and thumb screws to lock system into place
7. Turn on backlight LED
8. Adjust camera as needed for proper focal length
   (a) Coarse adjustments done by moving camera mount, fine adjustments done performed on camera

Software: For the current design, MATLAB and Python are used to capture pictures and process images respectively. NOTE - this is a temporary solution as this method does not allow for real-time data processing during testing.

1. MATLAB
   (a) Open file called Droplet Time Lapse (Research - Pendant Drop - Code)
   (b) Ensure that the IDS Camera software is closed before starting - you will receive an error in the MATLAB script (from videoinput function) if the camera is not available
   (c) Change working directory to the desired folder for storing data
   (d) Run script
      i. The command line prompt will ask you to take an image of ONLY the capillary tip, which is critical for magnification ratio and droplet isolation. For this reason, try to ensure that there is no residual fluid/debris on the needle that would significantly distort its rigid shape. Press Enter in command window to take capillary image
      ii. After taking capillary image, the command line prompt will ask you to take an image of the droplet. Create the droplet by using the linear stage micrometer (attached to plunger translator) to a desired (or standard) size. Press Enter to take series of droplet photos
2. Python
   (a) Open file called Time Lapse Script.py (File under Research - Pendant Drop - Code)
   (b) Adjust input parameters in the first section of script.
      i. Make sure you input the full path name to the folder containing the images for analysis (variable name = folderName, type = string) and properly delineate the extension type of the images
      ii. To plot and/or store the data, use True/False to toggle or suppress these commands (this executes flagged code blocks when user runs script explicitly)

3 Experimental Methods and Procedures

Biofilm Characterization - Food Safety Lab

1. **Bacteria Preparation and Inoculation**
2. **Sterilization of Parts**
   (a) Autoclave a folded up piece of aluminum foil. The inside will serve as the sterilized surface for drying and subsequent transportation of parts
   (b) In an appropriately sized graduated cylinder, place syringe barrel, plunger, luer lock tip, plug assembly and cuvette and fill up with 70% Ethanol (fill up until parts are completely submerged). Allow parts to sit in solution for 15 minutes
      i. NOTE - the plunger and syringe barrel must be disassembled. The syringe barrel, luer lock tip and plug assembly should remain assembled
   (c) Transfer foil and parts (parts still in EtOH solution) to biosafety cabinet or laminar flow hood. Unfold autoclaved aluminum foil wrapping, take parts out of solution and allow them to rest on sterilized surface for 15 minutes, where they can then be packaged up to transport back to working area
3. **Loading into Setup**
   (a) Place vials of broth into sonicator on the degas setting for 5 minutes (do not exceed this time frame)
   (b) Clean working area with 70% Ethanol and turn on Bunsen burner. All steps involving any exposure to open environment must be performed in direct vicinity of the Bunsen burner
   (c) Take out parts and assemble the plunger into the syringe barrel, and squeeze out any residual EtOH from syringe.
   (d) Via a cue tip, apply vaccuum grease onto the edges of the Teflon plug piece (important for air-tight seal)
   (e) Load the syringe with 1mL media and gently deposit into the cuvette.
   (f) Load the syringe with another 1mL media and remove any air bubbles (flipping the syringe and flicking the barrel works best for dislodging bubbles)
   (g) Gently twist Teflon plug piece into cuvette, and then follow standard pendant drop procedure outlined in Section 2 for standard experimental setup protocol
4. **Taking Tests**
   (a) Initial droplet formation should be made by rotating the calibrated screw counterclockwise by 35 degrees.
   (b) After 1 hour, compress droplet by rotating the calibrated screw clockwise by 20 degrees. This will ensure that the droplet will not fall off during operation due to decreases in surface tension over time
      i. Ensure that syringe flange is securely held to allow for volume reduction using micrometer

4 Software Development

4.1 Guide to GUIs (for Windows)

This guide is written as a basic step-by-step walkthrough on how to create a GUI on QT Designer and attach the GUI to a Python script. This guide assumes the user has installed PythonXY on their Windows system with all the required modules installed. The guide will be split into 2 sections: creating a GUI and attaching the GUI to a Python script.

Prerequisites

Make sure to have these programs installed before moving on. Note that PythonXY should install of these when it is being installed but double-check nonetheless.

1. Python 2.7
2. Qt Designer (make sure it has MatplotlibWidget in the Widget Box)
3. Enhanced console (right click on file explorer to check)
4. Spyder interactive development environment

Creating the GUI

1. Open QT Designer. The first thing you see will be 3 boxes: the widget box, the object inspector, and the signal slot editor / action editor / resource browser box (referred to as 'miscellaneous' from now on).

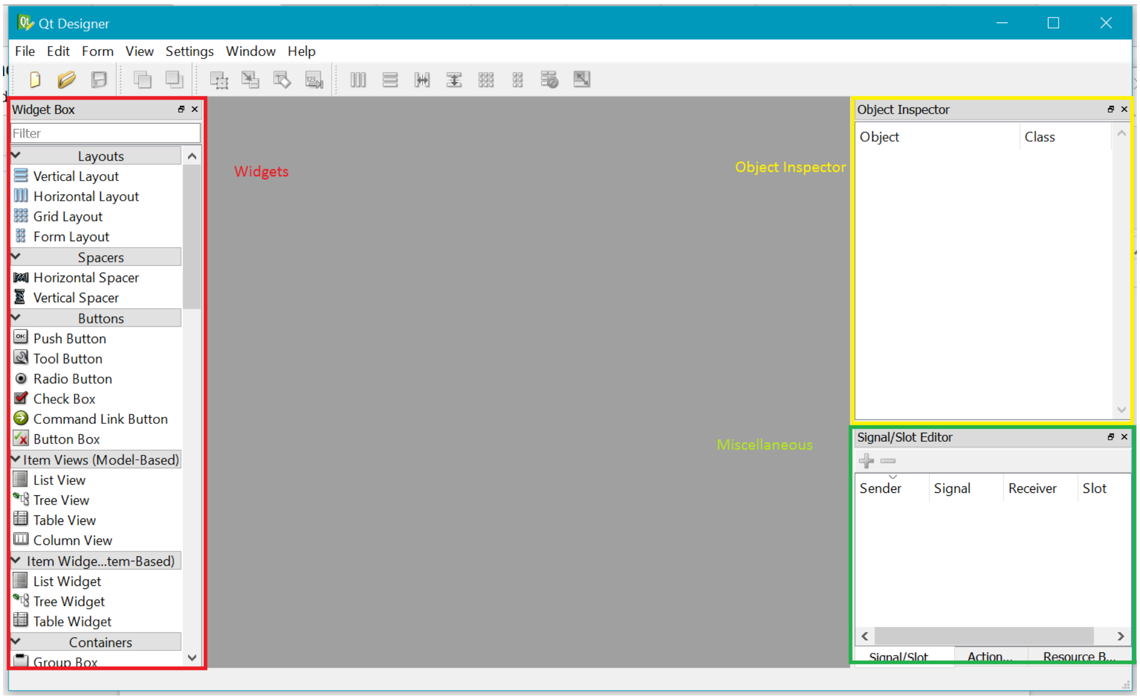


Figure 1: Creating the GUI (Step 1)

2. Select File -> New. A 'New Form' window will appear. Go with the default option and press 'Create'.
3. An empty MainWindow will appear. This is the blank canvas on which you can drag and drop widgets to form the GUI. The widgets and other objects you put will also have its name shown on the object inspector.

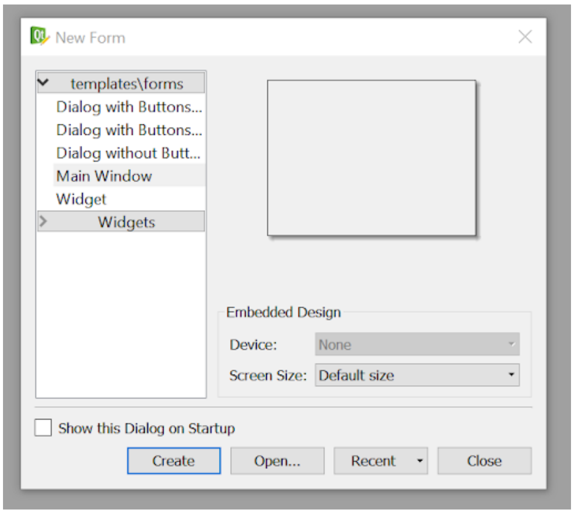


Figure 2: Creating the GUI (Step 2)

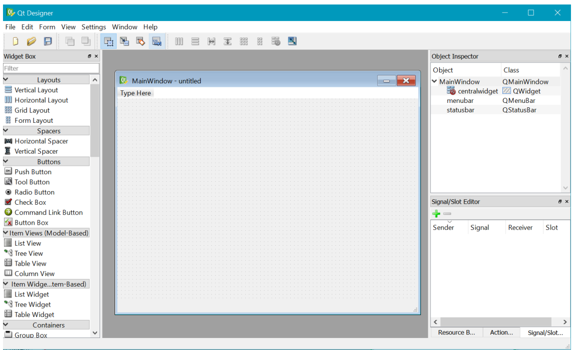


Figure 3: Creating the GUI (Step 3)

4. Using the widget box, select then drag and drop the widgets onto the MainWindow to the desired layout. The object names will be shown on the object inspector. In the 'Form' menu you can select the type of layout for the objects so that they stay to their configuration when you resize the MainWindow. You can also change the name of the objects in the object inspector. Once you're done with the layout, save the file. The file will have a .ui extension.

Attaching the GUI to a Python Script

1. Make sure the GUI file and the Python script that's going to be attached to the GUI are on the same folder. On file explorer, right click and select 'Open enhanced console here'.

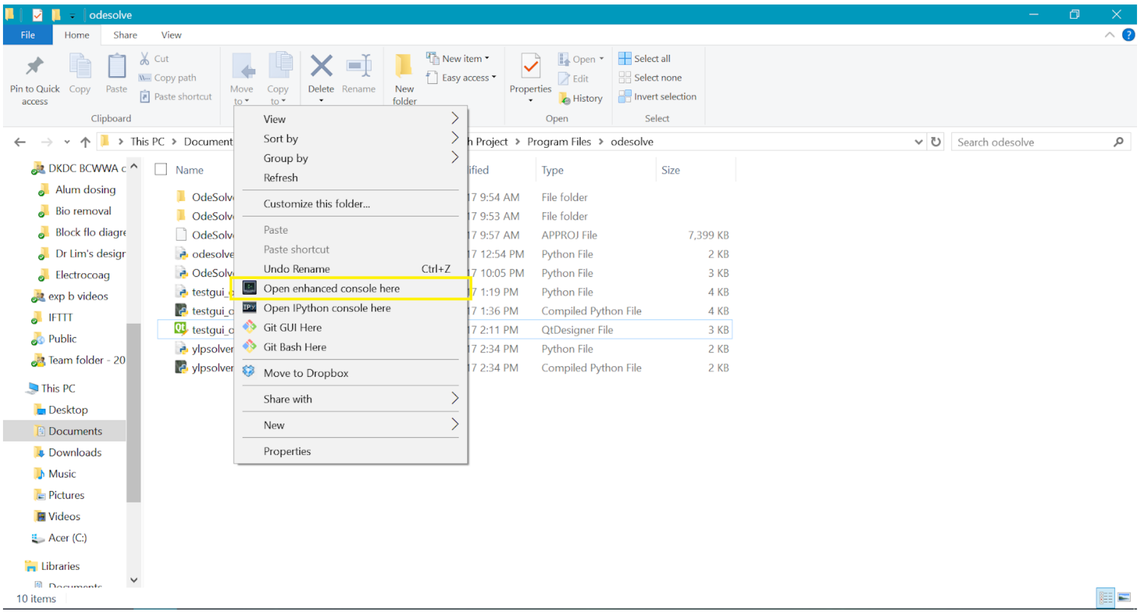


Figure 4: Attaching the GUI to a Python Script (Step 1)

2. On the enhanced console, write 'pyuic4 -x nameofgui.ui -o nameofgui.py' and press enter. This will generate a Python file equivalent of the .ui file. The gui python file should be in the same folder the backend python script file will be in.

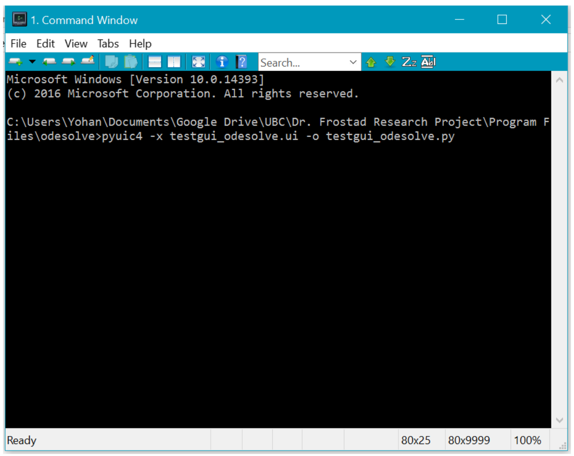
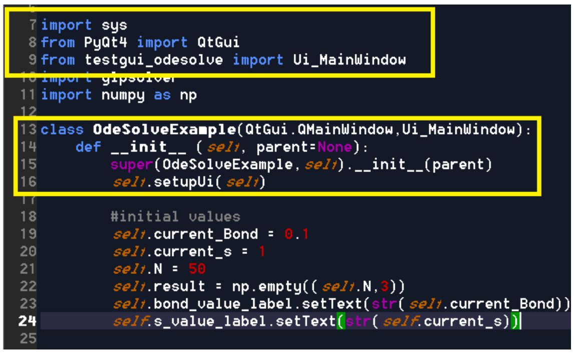


Figure 5: Attaching the GUI to a Python Script (Step 2)

3. Open the backend Python script in Spyder. On the Python script that's going to be linked to the GUI, make sure that 'Ui_MainWindow' is imported from the GUI .py file. A class also needs to be linked to the GUI file. An example is shown in the boxes below. Have everything in the boxes available on the script.

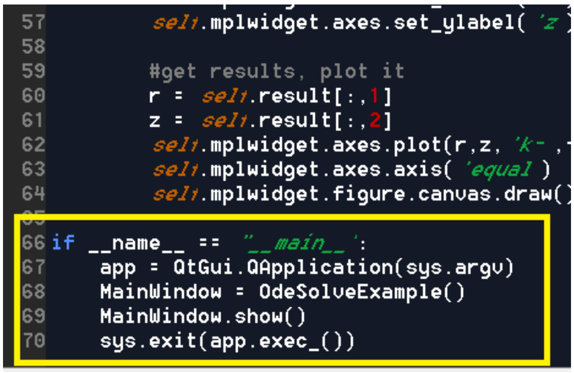

```
import sys
from PyQt4 import QtGui
from testgui_odesolve import Ui_MainWindow
import gipsolver
import numpy as np

class OdeSolveExample(QtGui.QMainWindow,Ui_MainWindow):
    def __init__ (self, parent=None):
        super(OdeSolveExample,self).__init__(parent)
        self.setupUi(self)

        #initial values
        self.current_Bond = 0.1
        self.current_s = 1
        self.N = 50
        self.result = np.empty((self.N,3))
        self.bond_value_label.setText(str(self.current_Bond))
        self.s_value_label.setText(str(self.current_s))
```

Figure 6: Attaching the GUI to a Python Script (Step 3)

4. At the end of the script, also make sure that the following is written. Make sure that the class linking the GUI file is assigned to 'MainWindow'.


```
        self.mplwidget.axes.set_ylabel('z')

        #get results, plot it
        r = self.result[:,1]
        z = self.result[:,2]
        self.mplwidget.axes.plot(r,z,'k-',
        self.mplwidget.axes.axis('equal')
        self.mplwidget.figure.canvas.draw()

if __name__ == "__main__":
    app = QtGui.QApplication(sys.argv)
    MainWindow = OdeSolveExample()
    MainWindow.show()
    sys.exit(app.exec_())
```

Figure 7: Attaching the GUI to a Python Script (Step 4)

5. In Spyder (or the IDE you're working on), run the program

For further tutorials go to: https://www.tutorialspoint.com/pyqt/

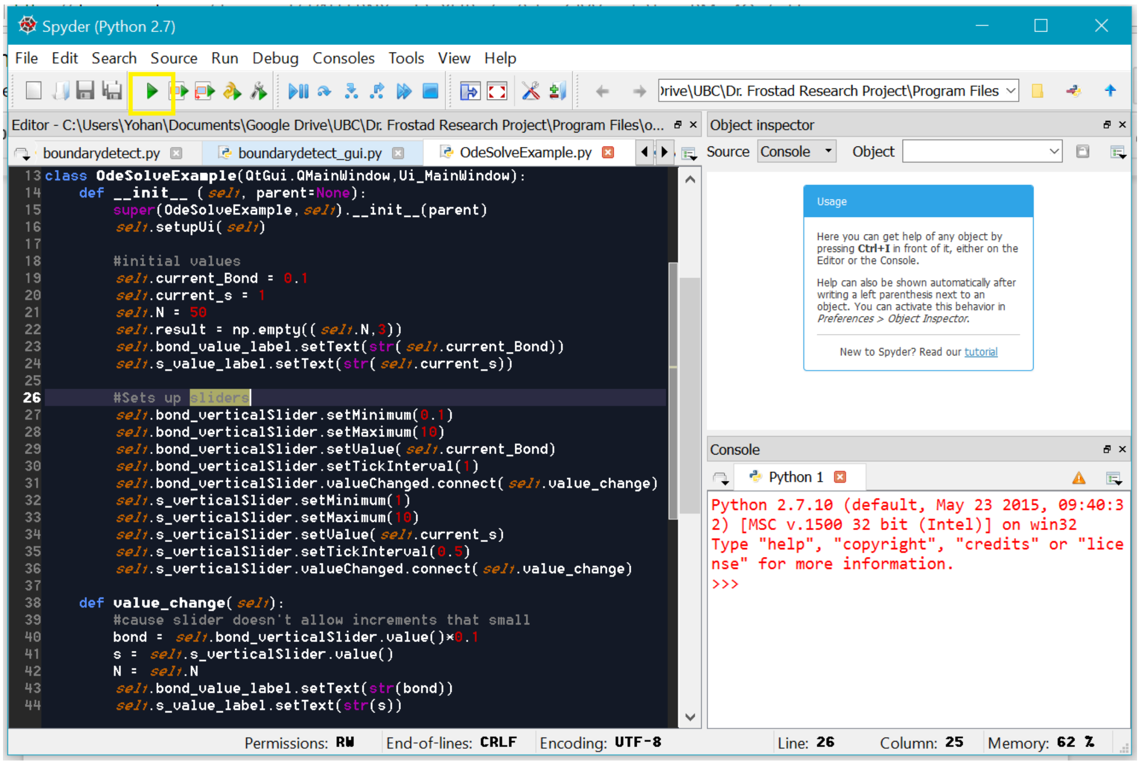


Figure 8: Attaching the GUI to a Python Script (Step 5)