MICROFLUIDIC TOOL KIT

OPERATING MANUAL

VERSION 3.30.00

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CHAPTER 1

GENERAL INFORMATION

Micralyne's µTK has been designed to provide researchers with a low cost tool that allows rapid entry into the field of microfluidics. Microfluidic chips allow small reagent and sample volumes to be manipulated and analyzed rapidly and efficiently. Additional benefits of micro-enabled processes such as rapid reactions, enhanced column efficiency and reduced dead volumes can also be exploited.

The Tool Kit has two main components: the optics unit and the electronics unit. The optics unit is supplied with a diode laser (either red or green), an epiluminescent confocal microscope and a chip stage with 4-8 HV probes. The electronics unit comprises a microprocessor and a variety of optional expansion boards. The expansion boards provide control signals and power to the optics unit by means of a control program (CP). The electronics unit is programmed via a PC using software compiled in LabView. Up to four HV expansion boards with two channels per board can be installed. Each channel can be set to voltages of 0 – 6 kV in 5 V intervals. While running, voltage and current are sampled at 50 Hz with 12 bit resolution. The quickswitch function allows for rise and fall times of up to 2 ms. Each channel has overload and short circuit protection.

The optical expansion board controls the laser and PMT. PMT gain is set by the user and the PMT signal is sampled at up to 200 Hz (selectable) with 16 bit resolution.

Safety Considerations

The following general safety precautions must be observed during all phases of operation, service and repair of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in the manual violates the safety standards of design, manufacture and intended use of the instrument and as such will null and void any warranty on the instrument. Micralyne Inc. assumes no liability for the user's failure to comply with these requirements.

DO NOT disconnect the serial cord or 120V AC power cord while the µTK is on. Doing so can cause damage to the microprocessor or to your PC.

NEVER run the µTK without a chip securely in place on the stage. In the absence of a chip, high voltage may arc from the electrode grid to the optical housing causing the laser or other components to be severely damaged.

Damage may occur to the PMT if it is run at a high gain, such that the PMT output signal exceeds the 5 V maximum displayed on-screen. In the event the PMT signal exceeds 5 V, abort the experiment, set the PMT gain to a lower value, and repeat the experiment.

The laser in the optical assembly may require a warm-up period to help stabilize the power output. Warming up the laser for 1-5 minutes immediately before collecting PMT data may enhance the laser power stability and hence fluorescence power stability. This can be achieved by either toggling the laser and LEDs on, or inserting a 'blank' step (in which no high voltage is applied to the chip), before beginning any microfluidic manipulations that will be recorded with the PMT.

Collecting data with the uTK is clock-intensive. Running other clock-intensive programs while collecting data may affect the performance of your PC.

In Quickswitch mode, the 'B' electrode is not grounded: the state of the 'B' electrode is unpredictable and the electrode should be considered to be live. The 'B' electrode should be disconnected at the HV board.

Technical Specifications

COMPUTER REQUIREMENTS:

Pentium 300MHz PII, 128MB RAM

Windows 95, 98, or NT

ELECTRICAL REQUIREMENTS:

100-220 V, 50-60 Hz

Repacement Fuses:	120V, 2.5A Slow Blow 3AG type
	220V, 1.5A Slow Blow 3AG type

LASER SPECIFICATIONS:

Laser Class:	3B
Input range:	0 to 5.0Vdc
Input resolution:	16 bits
Input time constant:	100µs ±10µs

(See appendix D for laser diagrams)

CHAPTER 2

INSTALLATION

Before you get started please ensure that you have everything on the packing list below. If you are missing anything please let your contact at Micralyne know immediately.

Packing List

When you receive your shipment it should contain the following items:

Full Assembly (Optical unit and electronics unit)**:**

- 1 optical assembly (black box)
- 1 electronics assembly (grey box)
- 1 four-pin mini din cable
- 1 six-pin mini din cable
- 1 RCA cable
- 1 DB9 (9-pin) RS-232 cable
- 1 Power cable
- 1 cable with a male DB9 jack on one end and a female on the other
- 4-8 white high voltage cables (2 per HV board)
- Replacement fuses

Electronics Only:

- 1 electronics assembly (grey box)
- 1 dongle (one ended four-pin mini din cable)
- 1 six-pin mini din cable
- 1 RCA cable
- 1 DB9 (9-pin) RS-232 cable
- 1 Power cable
- 1 cable with a male DB9 jack on one end and a female on the other
- 4-8 white high voltage cables (2 per HV board)
- Replacement fuses

1. Remove units from shipping boxes.

2. They should be carefully removed and unwrapped.

3. The protective wrap on the eyepiece and objective may now be removed. Also, temporarily unscrew the brass defocusing lens knob on the side of the microscope. (Figure 2 - 1)

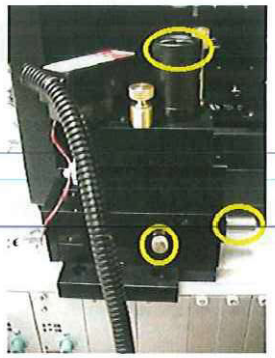


Figure 2 - 1

4. Remove the small black foam from the underneath the vertical stage. This is done by adjusting the stage down approximately 1 cm and then pushing upwards on the stage while removing the foam. The stage may then be gently allowed to return to its resting position. At this point, one should also loosen the two large silver-coloured coarse stage dovetail knobs and move the stage to a central position. Now would also be a good time to loosen the X and Y stage locks. Thay are the small black knobs located at the front of the X stage and the right of the Y stage. Simply turn several times counterclockwise. (Figure 2 - 2)

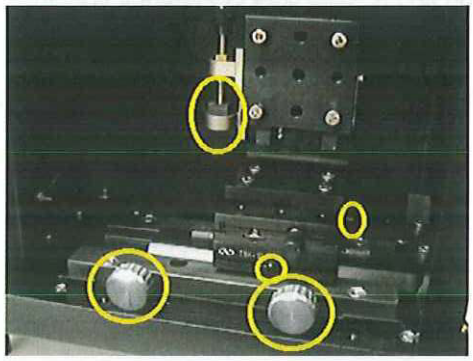


Figure 2 - 2

5. Remove the four bolts (red LED unit) or 3 bolts (green unit, the bottom left bolt is omitted) on the vertical stage. (Figure 2 - 3)

6. Now open the top lid. Unlock the electrode grid stage by twisting the shown lever upwards. (Figure 2 - 4) Raise the gride approximately 3 cm with the knob. (Figure 2 - 5)

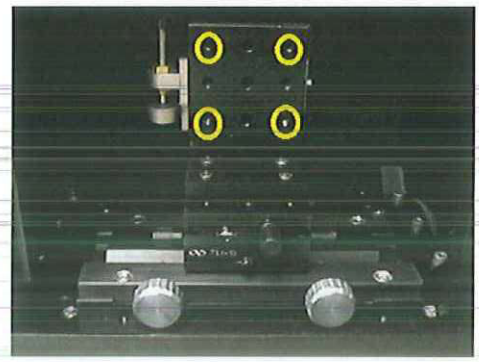


Figure 2 - 3

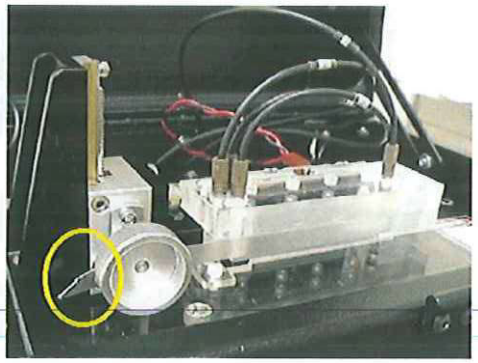


Figure 2 - 4

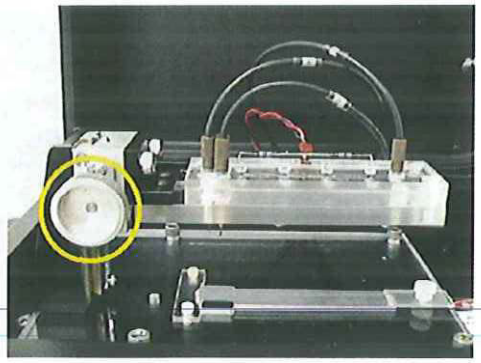


Figure 2 - 5

7. Carefully fasten the microscope to the vertical stage and secure with the bolts. (Figure 2 - 6) This is most easily done by inserting the microscope assembly diagonally and then twisting to the vertical. Care must be taken during this operation to not damage the objective lens.

8. After the upper right bolt is fastened you may replace the laser-defocusing knob. (Figure 2 - 7) Simply turn clockwise.

9. Finally, plug in the microscope cable into the DB9 plug on the lower right of the front stage opening. (Figure 2 - 8) Note that the cable can be routed either to the right or the left (around clockwise) of the microscope unit.

10. One can now insert a chip on the upper Plexiglas stage: remove the chip clamp on the right-hand side of the chip slot, slide the chip to the left in the fitted groove until it stops with about 1-2 mm of the chip underneath the hold-down on the left (you may have to lift the right end of the chip to get it under the hold-down), and re-secure the chip with the clamp on the right. When lowering the electrode grid the first time, confirm the electrodes still line up with the chip ports. (Figure 2 - 9)

Figure 2 - 6

Figure 2 - 7

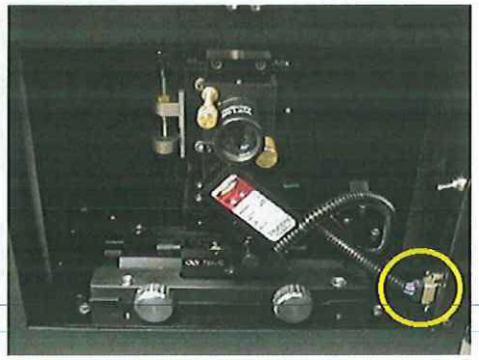


Figure 2 - 8

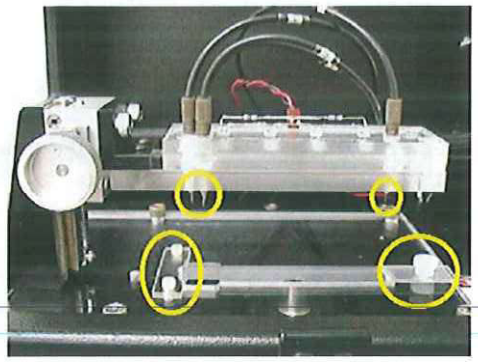


Figure 2 - 9

Connecting the Optics Unit to the Power Supply

We recommend that you arrange the boxes with the front of the power supply and the back of the optics unit facing you as shown below (Figure 2 - 10). Once connected, the optics assembly can be carefully turned 180 degrees for ease of operation.

1. Connect the sockets labeled INTERLOCK using the four-pin mini din cable.
2. Connect the sockets labeled OPTICS CONNECT using the six-pin mini din cable.
3. Connect the socket labeled PMT INPUT to the socket labeled PMT OUTPUT, using the cable with the RCA jack.
4. Plug in the female DB9 jack to a serial port on your computer (COM1), and then plug the male DB9 jack into the socket on the electronics box labeled TO COMPUTER.
5. Locate the bolt labeled SAFETY GROUND on the back of the electronics box. Locate the long green safety wire inside the optics box. Loosen the bolt connector on the electronics box and attach the green safety wire from the optics box, retighten the bolt connector.
6. Connect the power cord to the electronics box, plug into an outlet.

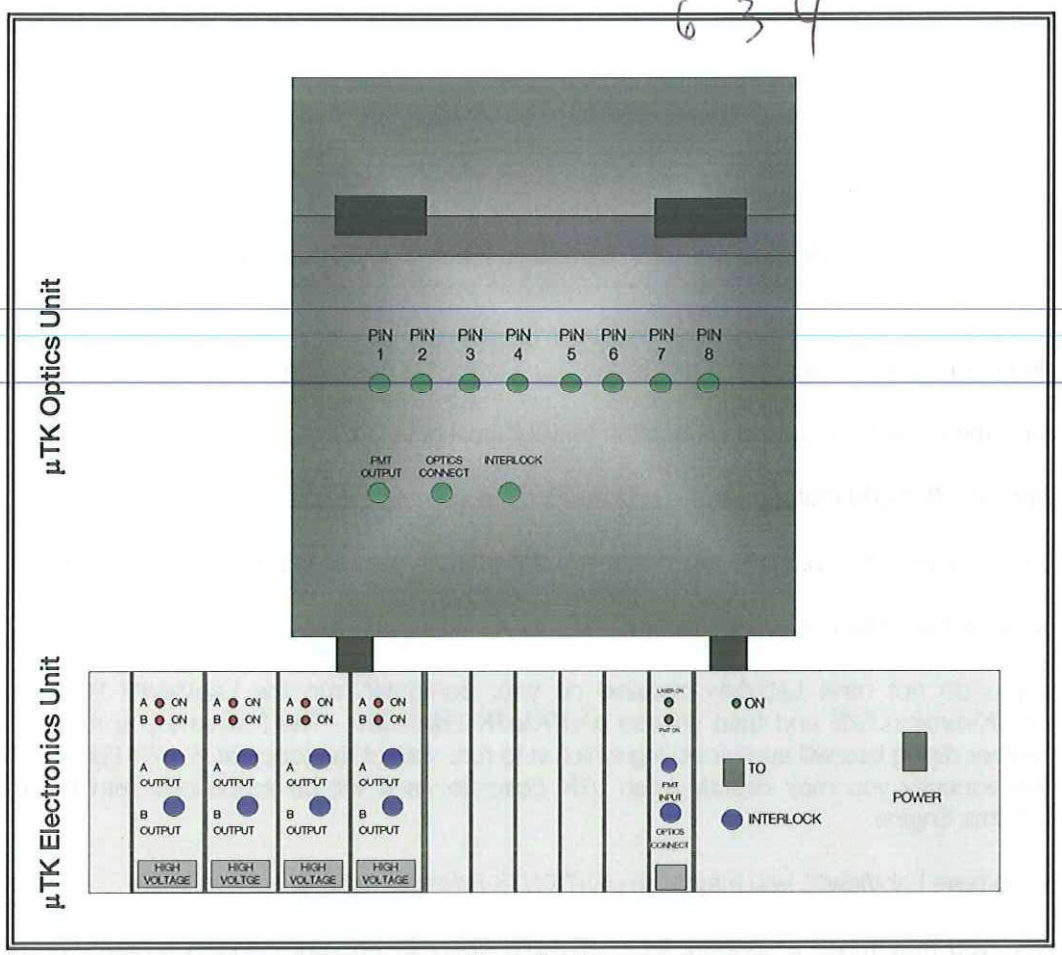


Figure 2 - 10

The base model electronics box will come with two to four high voltage boards. As a convention, these boards will be considered as 'HV 1', 'HV 2', etc. as can be read from left to right. This is important in the context of LabView, where you will be able to choose which power supplies are active at which times.

The white high voltage cables can be used in various configurations to connect the **high voltage outputs** (HV1A, HV1B, HV2A, ... HV4B) in the electronics box to the **pin sockets** (PIN 1, PIN 2, PIN 3, ... PIN 8) on the optics box.

The chip electrodes are set in the shape of a cross on the stage. The chip electrode numbers, as displayed in

Figure 2 - 11, correspond to the pin socket numbers on the back of the optics box.

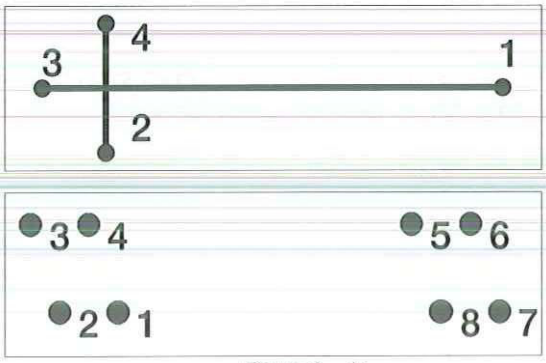


Figure 2 - 11

INSTALLING µTK CONTROL PROGRAM SOFTWARE

The control program software has been included on the CD provided with your µTK. It can also be downloaded from our FTP site at ftp://utk@ftp.micralyne.com/ the password is: "microchip".

1. Save the zip file to your desired location on your local drive (C:/).
2. Open the **3.30.00 dist.zip** file.
3. Extract all files to *C:\3.30.00 dist* ** It is very important to save to the top-level directory.
4. Rename the folder *3.30.00 dist* to *uTK*
5. If you do not have LabView installed on your computer, run the LabView™ Runtime Engine, *c:\uTK\runtime.EXE* and then choose *c:\uTK\uTK Editor.llb*. The first time you run the program another dialog box will appear asking which vi to run. Select the topmost vi, *uTK Editor.vi*. Note that subsequently you may directly open *uTK Editor.llb* as it will be associated with the LabView™ Runtime Engine.
6. If you have LabView™, you may open *c:\uTK\uTK Editor.llb* directly.
7. Now you may make a shortcut to *c:\uTK\uTK Editor.llb* on your desktop so that you can easily locate it in the future.

Comments:

Collecting data with the uTK is clock-intensive. Running other clock-intensive programs while collecting data may affect the performance of your PC

A 300 MHz machine with 128MB Ram is required to run the µTK software. We recommend Windows 95, 98 or NT for optimal performance.

CHAPTER 3

GETTING STARTED

This chapter contains a brief description of the μTK's controls and indicators and describes how to turn on the instrument and to check its operation.

Running μTK Control software in Lab View

Once you have installed the software you can open the program by running **uTK Editor.vi**.

Inserting a Chip

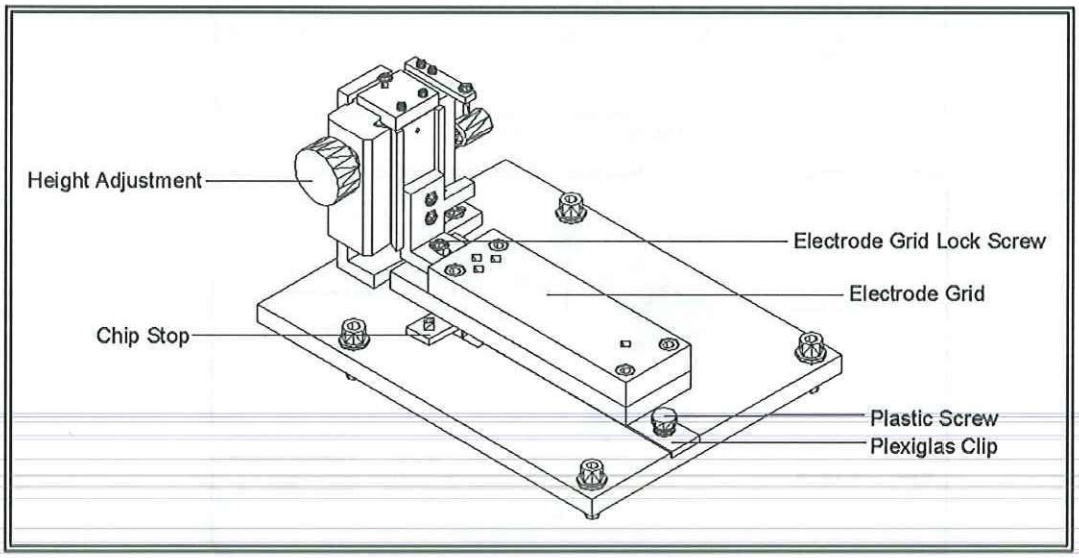


Figure 3 - 1 – Chip Stage

1. Load a standard microfluidic chip with the desired reagents.
2. Open the **top door** of the optical assembly to expose the chip mount and electrode grid.
3. Turn the **height adjustment** knob counter-clockwise to raise the **electrode grid** to its full extent.
4. Loosen the **plastic screw** and remove the **Plexiglas clip** that secures the chip.
5. Insert the loaded chip right side up into the grooves on the stage, slide to the left until it is flush with the **chip stop**.
6. Ensure that the wells in the chip mirror the **electrode grid** configuration suspended above.
7. Re-attach the **Plexiglas clip** to stabilize the chip against the **chip stop**.

8. Gently lower the **electrode grid** assembly until resistance is felt, then stop. If grid pins and wells do not properly mate, fine adjustments can be made in any direction by loosening the **electrode grid lock screw.**

NEVER run the uTK without a chip securely in place on the stage. In the absence of a chip, high voltage may arc from the electrode grid to the optical housing causing the laser to be severely damaged.

ADJUSTING THE LASER AND PMT

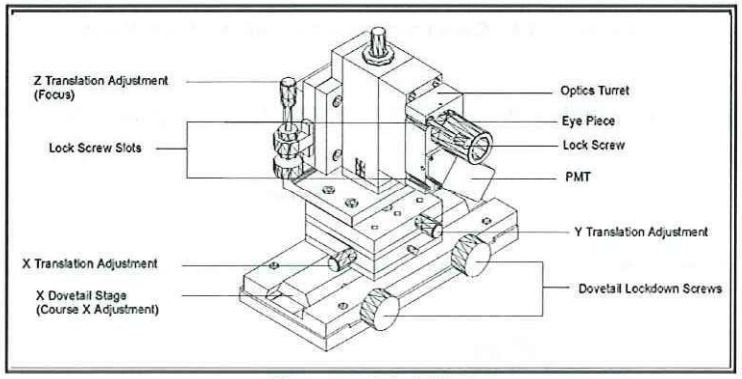


Figure 3 - 2 Left View

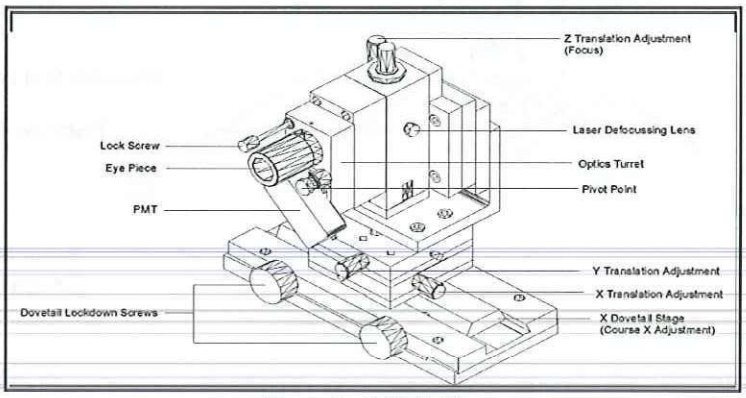


Figure 3 - 3 Right View

The **eyepiece** for the microscope and the **PMT** are set on the **optics turret**, which rotates about a **pivot point**. This support can be rotated to place either the **PMT** or the **eyepiece** in the optical train. Once rotated fully in either direction, the support should be secured using the **lock screw** on the optics turret.

1. Open the **front door** and **top door** of the optics assembly.
2. Supporting the **optics turret,** carefully loosen the **lock screw.**
3. Rotate the **optics turret** counter-clockwise to bring the microscope **eyepiece** into the optical train.
4. Mate the **lock screw slot** with the **lock screw** and tighten.
5. Loosen the **lockdown** knobs on the **X dovetail stage.**

6. Viewing the assembly from above, roughly adjust the **X dovetail stage** and then the **X and Y translation adjustments** to bring the objective approximately beneath the channel intersection.

7. Close the **top door** of the optics assembly.

8. Turn the **laser** on, using the laser button  on the PMT toolbar. This will turn on the LEDs above the chip as well as the laser, thus illuminating the chip surface (providing the box top is closed).

9. Looking through the **eyepiece**, use the **Z translation adjustment** to bring one of the channels in the chip into focus.

10. Looking through the eyepiece, finely tune the X and Y translation adjustments to bring the desired portion of the fluid channel into the field of view.

11. Push the brass **laser defocusing** knob inward to focus the laser into a small spot. Note also that the knob can also be pulled outward to defocus the laser and broadly illuminate a 200 um area of the chip.

12. Use the **X** and **Y translation adjustments** to move the laser spot into the center of the desired portion of the fluid channel.

13. At this point you can execute the control program while looking through the **eyepiece**, which will allow you to directly observe the movement of various florescent species applied to the ports of your chip. Manually turning off the LEDs, using the silver switch on the inside right panel of the optics box, will enhance the florescence contrast.

14. Turn the **laser** off, using the laser button on the PMT toolbar. Note that it is important to **shut off the laser and LEDs before rotating the PMT into the optical train** to avoid permanent damage to the PMT.

15. Rotate the **PMT** into the optical train and re-tighten the **lock screw**.

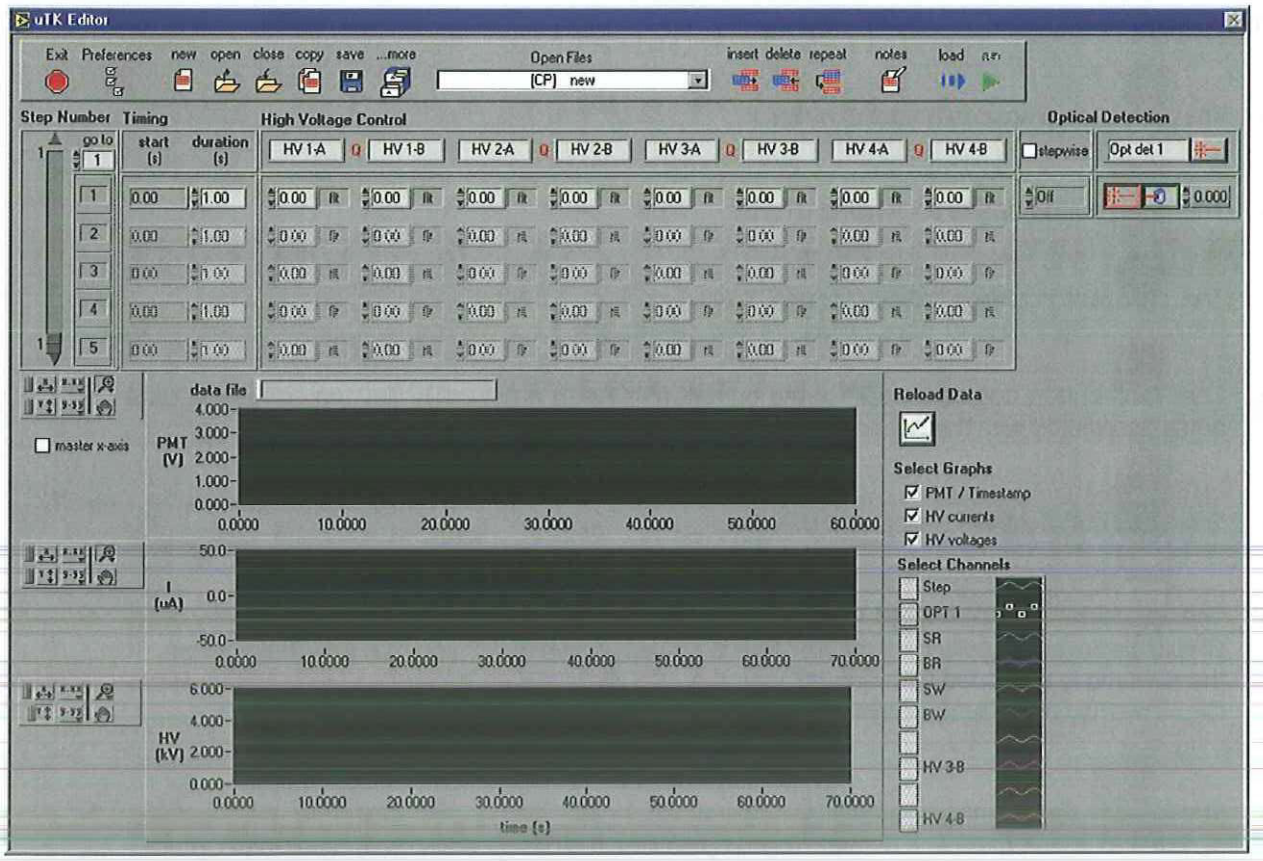
16. Close the **front door** of the optical assembly.

17. At this point, you can execute your control program and gather data using the **PMT**.

CHAPTER 4

OVERVIEW TO CONTROL PROGRAM INTERFACE

The uTK Program Editor has 3 main components: the Toolbar, the Control Program Display and the Data Display. This chapter gives an overview of the various controls. Operational details will be found in chapters 5 through 7.

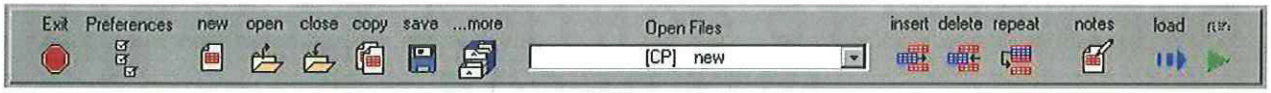


Toolbar

Control Program Display

Data Display

TOOLBAR

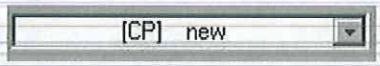


EXIT

- The  button **exits** the uTK Editor.

FILE

File management is discussed in detail in Chapter 7.

- The  button creates a **new** uTK file.
- The  button **opens** an existing uTK file.
- The  button **closes** the current uTK file.
- The  button **copies** the CP of the current uTK file to a new uTK file. Any data and data notes associated with the file (see Chapter 7) will NOT be copied using this function.
- The  button **saves** the current uTK file.
- The 

CP drop down menu selects which of the currently open CPs is displayed for editing. If the filename is preceded with "[CP]" you may edit the program. If the filename is preceded with "[data]" then the .uTK file is a data file and you may NOT edit the CP. You may however still **download** and **run** the CP from the [data] file.
- The  **file manager** allows file operations (save, export and close) to be executed simultaneously on multiple uTK files. These operations are described in more detail in Chapter 7.

EDIT

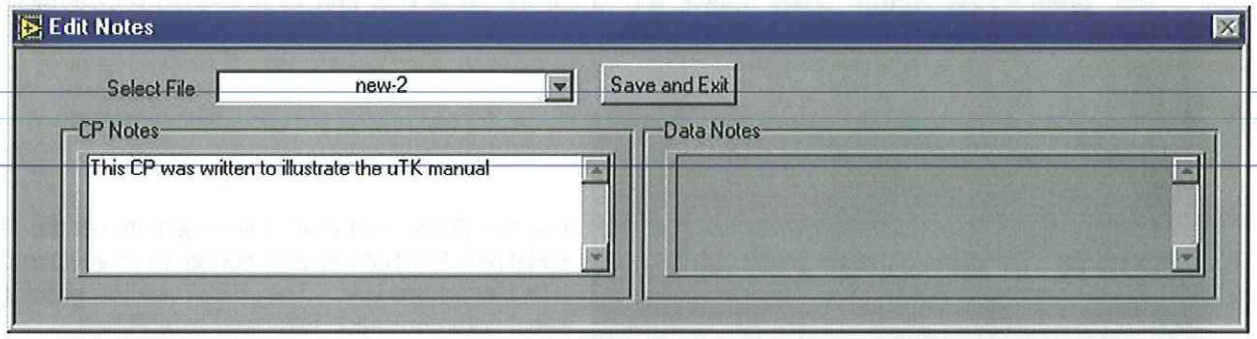
Editing a CP is described in more detail in Chapter 5

- The **insert**  , **delete**  , and **repeat**  steps buttons help you to **edit** a control program.

USER NOTES

The  **edit notes** button allows you to add Control Program **(CP) Notes** or **Data Notes** to a file.

CP notes can be edited for CP files. In the example below, a CP note is added to the file *new-2.uTK*:



When the CP from *new-2.uTK* is run, the CP notes will be automatically transferred to the data file as shown below for the data file *02_06_27_11h56m.uTK*.

Also added automatically to the data file are the following data notes:

- The uTK program from which the CP was downloaded
- The date and time at which the run was started
- Any use of the **manual advance** feature (see chapter 6)
- Any use of the **abort run** feature (see chapter 6)

OPERATE

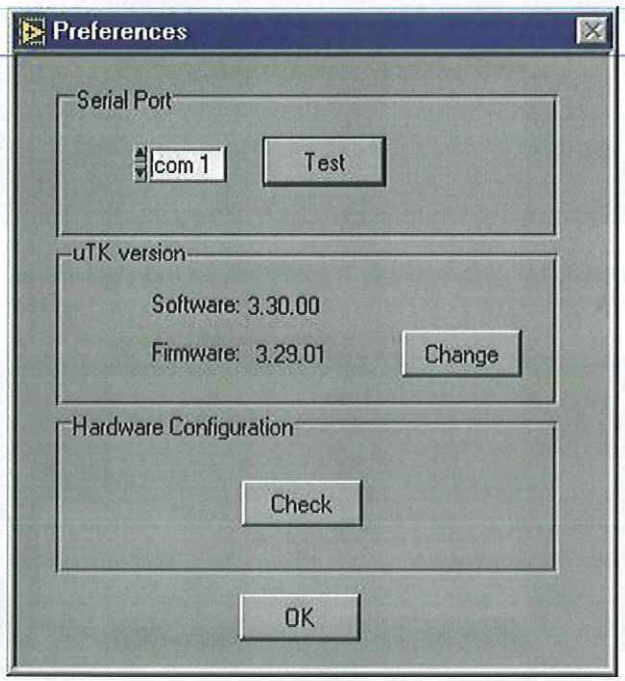
Operation of the uTK is described in detail in Chapter 6

- The **download** button  allows you to program the uTK electronics unit with the currently displayed CP.

- The **run** button  allows you to run the downloaded program.

PREFERENCES

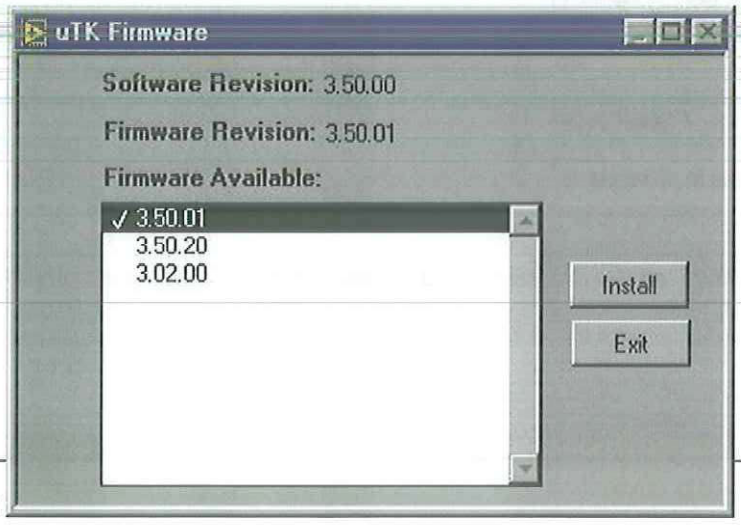
The **preferences button**  allows you to set the serial port and to download new firmware if appropriate. Clicking the preferences button will bring up the following dialog box:



Use the **Serial Port** drop down menu to set the serial port to whatever port connects your PC to the uTK Electronics Unit. The 'TEST' button checks to see if communication has been established.

When the software is started it automatically checks the **hardware configuration**: that is, which expansion boards are installed in which slots. Pressing the **check** button repeats this operation.

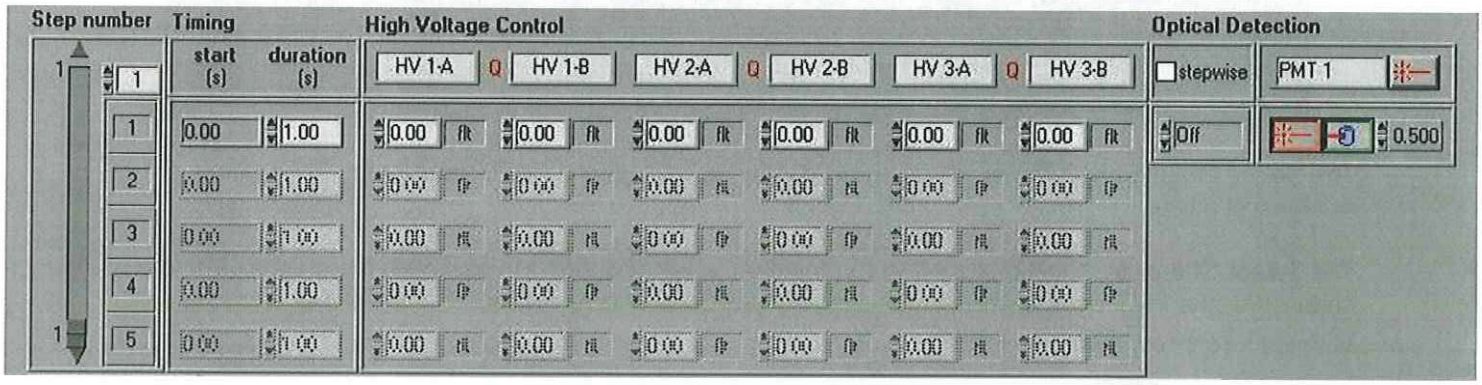
From time to time firmware upgrades may be available from Micralyne via the Micralyne FTP site. After downloading the firmware from the FTP site to your PC, place it in the *C:\uTK\FW* directory. You may then install the firmware in the uTK electronics unit. Clicking on the **Change** button will bring up the following dialog box:



Select the firmware you wish to download and click **Install** to proceed. You will be prompted to restart the uTK after the installation. Please do so by turning the uTK Electronics Unit off, then on. You may then **Exit** from the installation window.

CONTROL PROGRAM (CP) DISPLAY

The CP display shows the Control Program for the current uTK file. Display a different file by selecting a previously opened file from the **CP drop down menu** in the toolbar, by opening a previously saved file using the **open** button or by creating a new file using the **new** button.



The display shows a 5-step segment of the CP program. It also reflects the number and type of expansion boards for which the program was written: the example above shows a one step program for two HV expansion boards and one optical detection board. When creating a new file, the number and type of expansion boards currently installed in the uTK will be shown.

If the uTK file is a Control Program file, you can use the Control Program Display to edit the number of steps, the duration of each step and the state of each **expansion board** for each step.

If the uTK file is a data file, you cannot edit the Control Program. You may however download and run the program. You may also make an editable copy of the control program using the **copy** button in the toolbar.

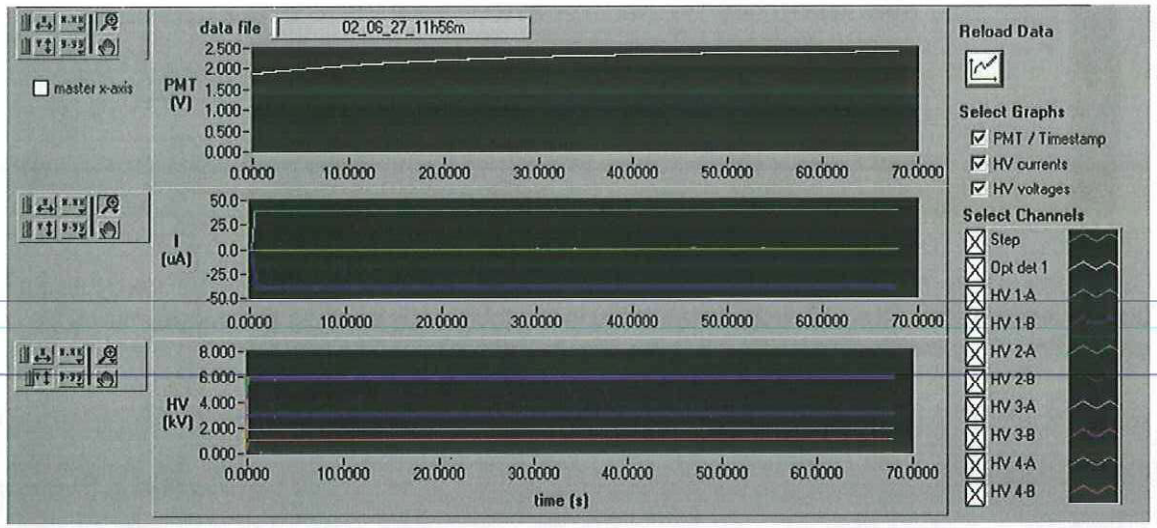
Currently available expansion boards are High Voltage boards and Optical Detection boards.

Chapter 5 discusses writing and editing CPs in greater detail.

DATA DISPLAY

Data and data manipulation are discussed in detail in Chapter 6.

The data for the currently selected data file (02_06_27_11h56m.uTK in the case below) is shown in the data display area. Use the **CP drop down menu** in the toolbar to view data from a different file.



The **Select Graphs** checkboxes allow you do display up to 3 graphs (PMT/Timestamp , HV currents or HV voltages) at a time.

The **Select Channels** checkboxes allow you to select which channels to view within the graphs. There is a channel for the PMT data, a channel for each HV electrode and an additional channel that shows a spike whenever a new CP step has started.

The **reload data**  button updates the display to reflect a new selection of graphs and channels.

CHAPTER 5

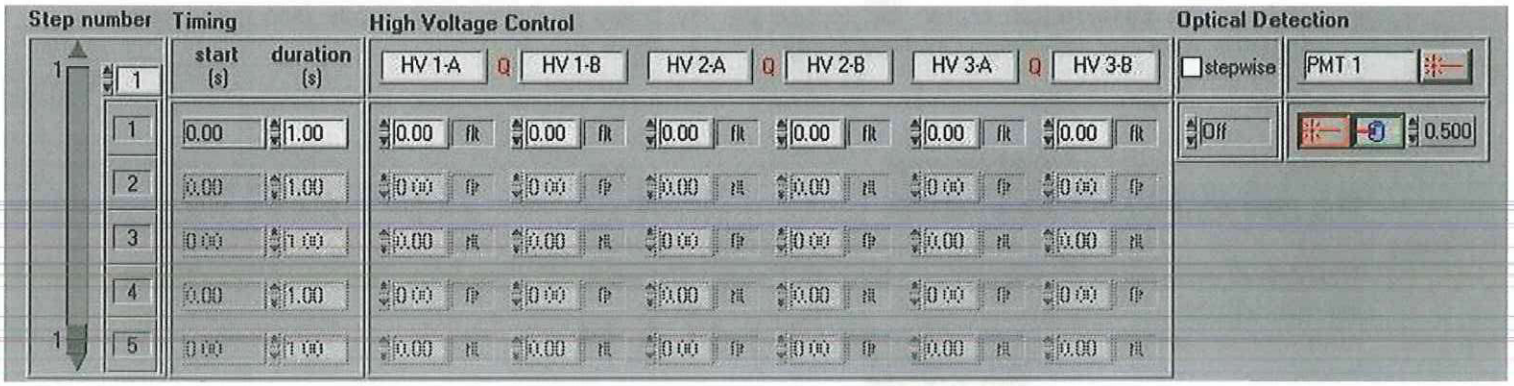
WRITING A CONTROL PROGRAM

The Control Program (CP) Display allows you to view and edit the CP for the currently selected uTK file. A CP comprises a series of steps (from 1 to 100) executed by the uTK. Within each step the states of the various expansion boards can be set.

The **insert**  , **delete**  , and **repeat**  steps buttons allow you to add, delete or copy steps within the CP.

If you wish to make notes concerning the program or the data, use the  **edit notes** button. A uTK file can have two distinct sets of notes: the CP notes and the Data notes. When a CP is downloaded and run, the CP notes are added to each data file created. After a data file is created, its Data notes (and its CP notes) may be altered.

There are four main parts to this display: Step Number, Timing, High Voltage Control and Optical Control.



STEP NUMBER

The display shows a 5-step section of the control program (maximum length is 100 steps). Use the slider or the **go to** control 

to view various sections of the CP.

TIMING

Start: the time at which the step starts (in seconds)

Duration: the length of time (in seconds) that elapses before the next step begins. The minimum step duration is 20 ms. The maximum is 83,886 seconds (approximately 23.3 hours)

HIGH VOLTAGE (HV) CONTROL

Up to 4 HV boards can be installed in the uTK. Each board has two electrodes (A and B).

The HV Header

The HV header indicates the electrode names. The default name reflects the position of the board in the uTK (#1 is in the leftmost slot). The top electrode is labeled 'A', the bottom 'B'. These names will be used as column headers when exporting data.

- You may change the names by clicking on the text and typing: 

- Click on the **Quickswitch** button  to use the HV board in Quickswitch mode (see below and Appendix A for discussions on Quickswitching).

The Electrode State

The state of each HV electrode can be set for each step. The electrode state comprises the HV supply and the relay setting.

- To adjust the **HV supply,** 

directly type in a new voltage or use the toggle arrows to the left of the number. The electrodes can be set to any voltage in the range of the available supplies (the initial HV boards have 0-6 kV supplies), to a resolution of 10 V. The default voltage is 0 kV.

- The relay state 

can be set using the drop down menu. There are three states **'gnd'** (ground), **'kV'** (live), and **'flt'** (floating). If **'gnd'** or **'flt'** is selected, the voltage settings have no meaning, and changing them has no effect on the execution of the control program. The default electrode state is 'float'.

Quickswitch

The rise and fall time of a HV supply, and thus of the electrode connected to it, depends on the change in the supply voltage, the electrode state and, if live, the electrical resistances between electrodes. The nominal rise time is approximately 150 ms, and varies with the final voltage, being faster for higher voltages. The

nominal fall time is in a worst-case scenario is 600 ms. The uTK has two built-in strategies to minimize this effect: Quickswitch and Lookahead.

Quickswitch

Clicking on the red 'Q' situated in the electrode column headings 

in the program editor activates the Quickswitch feature. Quickswitch allows you to apply both HV power supplies in one board to the 'A' electrode. This allows one HV supply to drive the electrode while the other charges for the next program step. Once Quickswitch has been turned on, only electrode 'A' will remain functional. The selection of the 'B' electrode is disallowed.

In Quickswitch mode, the 'B' electrode is not grounded: the state of the 'B' electrode is unpredictable and the electrode should be considered to be live. The 'B' electrode should be disconnected at the HV board.

Lookahead

If 'Quickswitch' is off, then each electrode is associated with a single HV supply. When the electrode is not live (and so the HV supply is not connected to the electrode), the HV supply is set to the voltage of the next step. This allows the HV supply to be at the correct voltage when the electrode becomes live.

Optical Detection

If an Optical Detection expansion board is installed, the following controls will appear in the CP Display:

The Optical Board Header 

- The **laser** button  toggles the laser (and the overhead lights) on and off while the CP is not running. Use this feature to align the optics with the microfluidic chip. The laser is disabled if the top door of the uTK optics unit is open. The overhead lights are disabled using the silver switch located just inside the front door of the optics unit. Enable the overhead lights to align the chip, but disable them before collecting data.

- If checked, the **stepwise** checkbox 

allows you to set the optical board state (see below) for every step. If unchecked a single setting will be used for all steps.

The Optical Board State

The state of the board is set for each CP step. If the stepwise checkbox in the header is unchecked, a single state is used for all CP steps.

- Use 

to select the **acquisition frequency** of the PMT. You may reduce your file sizes by only using high frequency acquisition during critical steps.
- The  button turns the **laser** on or off for the duration of a CP step. Note that your laser may require some warm-up time before quantitative data may be acquired. See appendix E
- The  button turns the **PMT** on or off for the duration of a CP step.
- The 

control sets the **PMT gain**. Limits are 0.000 to 0.800.

Damage may occur to the PMT if it is run at a high gain, such that the PMT output signal exceeds the 5 V maximum displayed on-screen. In the event the PMT signal exceeds 5 V, abort the experiment, set the PMT gain to a lower value, and repeat the experiment.

If unsure of an appropriate PMT gain start with a low value, e.g. 0.3, and repeat the experiment with small increases in this value until sufficient signal is obtained.

Operating Note

The green or red laser in the optical assembly may require a warm-up period to help stabilize the power output. Laser power (and hence fluorescence) stability may be enhanced by warming up the laser for 1-5 minutes immediately before collecting PMT data. This can be achieved by either toggling the laser button in the header prior to running the CP or by inserting a 'blank' step (in which no high voltage is applied to the chip) of suitable length as the first step of the CP.

CHAPTER 6

RUNNING A CONTROL PROGRAM

DOWNLOADING AND RUNNING A CP

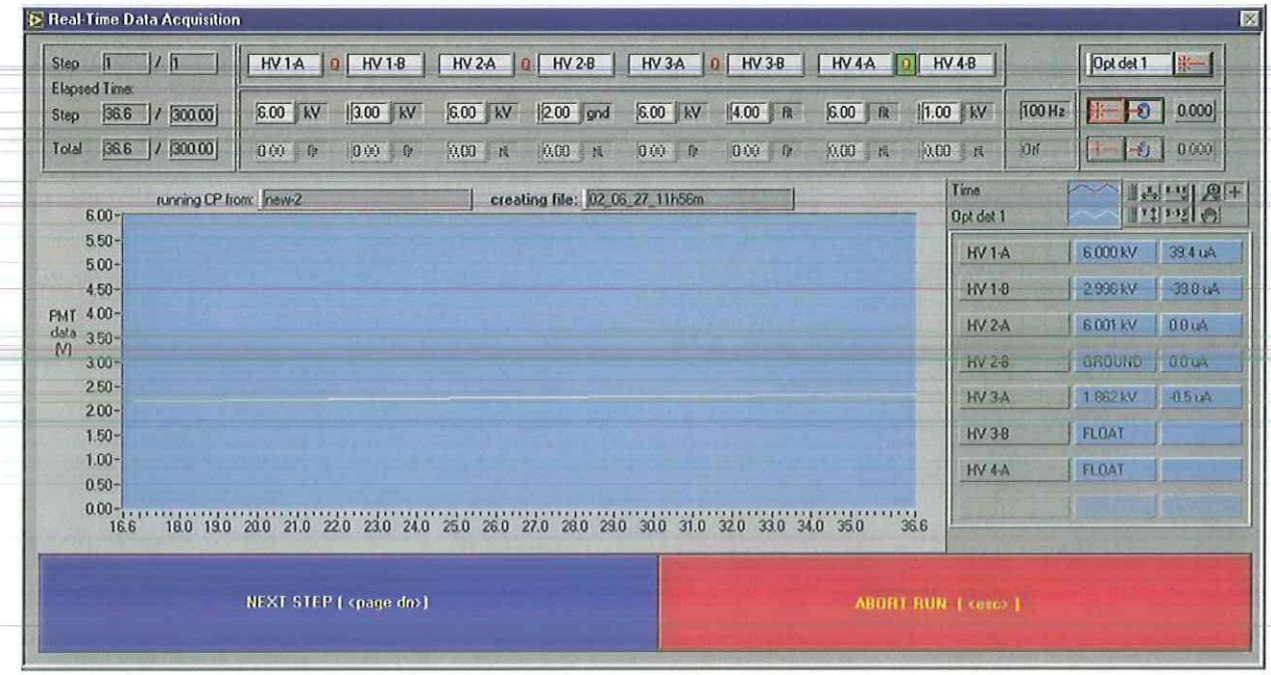
Before a CP may be run it must be downloaded to the uTK Electronics Unit.

The  **download** button will download a CP from the uTK Editor to the uTK Electronics Unit. You will be prompted if the download fails. There are four common reasons for a failure. First, a communications error may have occurred: try downloaded again. Second, ensure that the µTK Electronics Unit is switched on. Third, check that the serial cable is securely connected at the computer and uTK serial ports. Finally, ensure that the correct computer port has been selected (see 'Preferences' in Chapter 4).

The  **run** button will execute the downloaded CP.

NEVER run the uTK without a chip securely in place on the stage. In the absence of a chip, high voltage may arc from the electrode grid to the optical housing causing the laser to be severely damaged.

After hitting the run button, the **'Real-Time Data Acquisition'** window will appear as shown below.



The current step number, the total elapsed time and the time elapsed for the current step are shown at the upper left of the screen. The upper right of the screen shows the settings for the current and upcoming CP steps.

The most recent 20-second span of PMT data is shown on the graph. A spike will appear on this graph at the start of a new CP step . The current HV Supply Voltages are displayed to the right of the graph. If available, the HV Electrode Currents are also indicated.

Pressing the large blue **Next Step** button (or the <Page Down> button on the keyboard) causes the uTK to advance immediately to the next CP step. Each use of the next step button is itemized in the **data notes**.

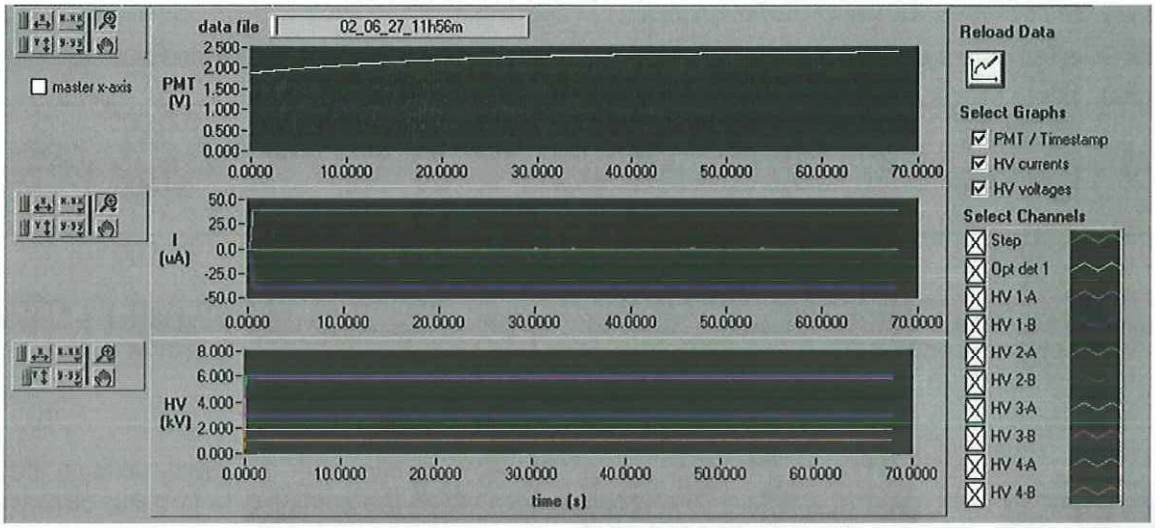
Execution of the Control Program can be aborted at any time by either clicking on the large red **Abort Run** button or by hitting the **<Esc>** key on the keyboard. When aborting, the uTK automatically sets all electrodes to 'float'. The use of the abort run button is noted in the **data notes**.

When a CP run has finished the data is displayed in the data display of the uTK Editor. See Chapter 7 for details on viewing data and manipulating the data display.

CHAPTER 7

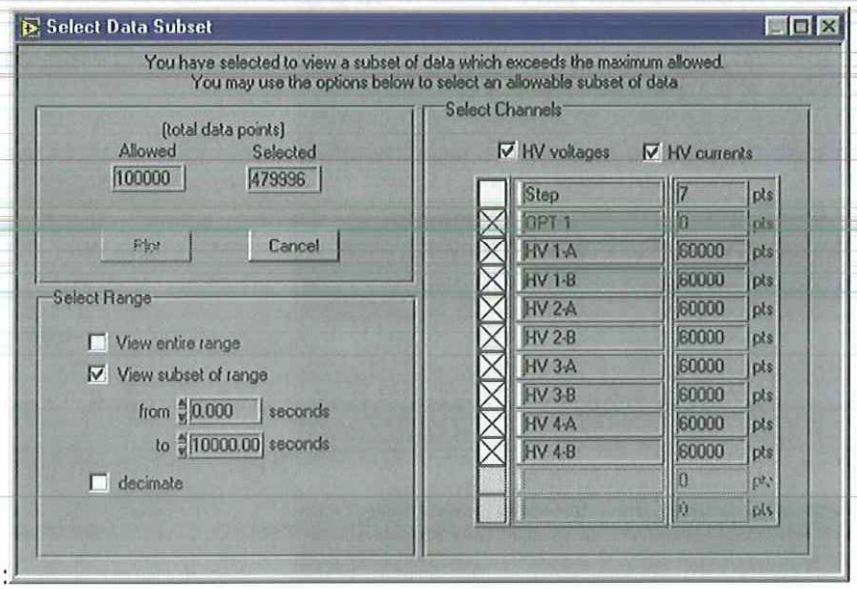
VIEWING DATA

The data for the currently selected data file (02_06_27_11h56m.uTK in the case below) is shown in the data display area. Use the **CP drop down menu** in the toolbar to view data from an open file, or use the **open** button on the toolbar to view data from a previously saved file.



LIMITS ON VIEWING DATA

The software limits the number of data points that may be viewed at a given time. If the selected data exceeds this limit the **Select Data Subset** dialog box will appear



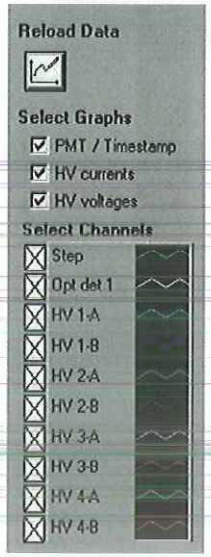
The number of allowed points is shown alongside the number of points you have selected. The number of points for each channel is shown alongside the channel name. You may reduce the number of data points selected by any combination of the following methods:

- Deselect channels (e.g. HV 1-B)
- Deselect HV currents or voltages (This applies to all HV channels)
- Select a subset of the range (e.g. 200 s to 400 s)
- **Decimate** the data. Decimating the data divides the number of points by a power of two (2, 4, 8, etc) so that the resulting number of points left is less than the number allowed. The following table shows some examples of how decimating works with 100,000 points allowed:

Points Selected before decimating	Decimating Factor	Points Selected after decimating
100,000	$2^0=1$	100,000
120,000	$2^1=2$	50,000
700,000	$2^3=8$	87,500

Decimating the data does NOT discard or destroy data. Rather, if the decimating factor is 2, every second data point is displayed. If the decimating factor is 8, every eighth data point is displayed.

LEGEND



Up to 3 graphs (PMT/Timestamp, HV electrode current or HV supply voltage) may be displayed at a time. The program from which the current data is being displayed is indicated above the topmost graph.

The **Reload Data** button reflects changes made to the graphs or to the channels selected.

The **Select Graphs** checkboxes allow you to select which graphs you wish to view. The display points on the blue background, such as the red circles shown here, allow you to change the way the data points are displayed. Right-clicking on a set of points will open a pop-up window that offers a variety of graphing styles.

The **Select Channels** checkboxes allow you to select which channels to view within the graphs. There is a channel for the PMT data, a channel for each HV electrode and an additional channel that shows a spike whenever a new CP step has started. The style (e.g. color) of the display for each channel may be altered by right clicking over the indicator to the right of the channel name.

DATA TOOLBAR

The data toolbar  allows you to zoom in on the loaded data.

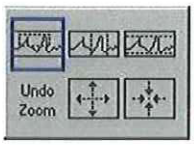
- The x-axis **auto-scale** button  scales the x-axis to fit all of the loaded data. Likewise for the y-axis auto-scale button.

- A graph may also be rescaled by double-clicking on the maximum or minimum value on an axis and typing in the desired value.

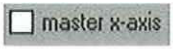
- The **format scale** drop down menus   can be used to adjust the precision and format of the x- and y -axis scales. Left-clicking over these boxes will reveal these options.

- Use the **pan** button  to pan through the loaded data by clicking on the graph and dragging the mouse.

- The **auto-zoom** drop down menu  allows you to focus in on a particular section of interest in the graphical display. When selected, the following pop-up window appears.



The top three boxes allow you to zoom in on data selected by dragging a rectangle in the graph. The two boxes in the bottom right corner allow you to zoom into, or away from, a particular section of the graph. To return to the previous screen magnification, select 'Undo Zoom'.

Checking the **master x-axis** checkbox 

forces the x-axis of all of the graphs to conform to that of the top graph.

CHAPTER 8

FILES AND FILE MANAGEMENT

File Types

Only files with a .uTK extension can be opened and operated on by the uTK Editor. You may export your data to a spreadsheet-readable text file if you wish to use your data in another setting.

UTK FILES

A .uTK file comprises the following information

- Configuration: the physical configuration of the uTK Electronics Unit, including the firmware revision and the location and serial numbers of the installed expansion boards.
- Control Program: The series of operations you wish to perform with your uTK.
- Control Program notes: user added notes.
- Data: all of the data collected during a CP run.
- Data notes: user added notes.

EXPORTED (.TXT) FILES

You may export any of the following elements to a spreadsheet-readable text file:

- uTK configuration
- Control Program
- Control Program Notes
- Data:
  - Step changes
  - PMT data
  - HV electrode currents
  - HV supply currents
- Data notes

Exported files cannot be read by the uTK software.

BASIC FILE OPERATIONS

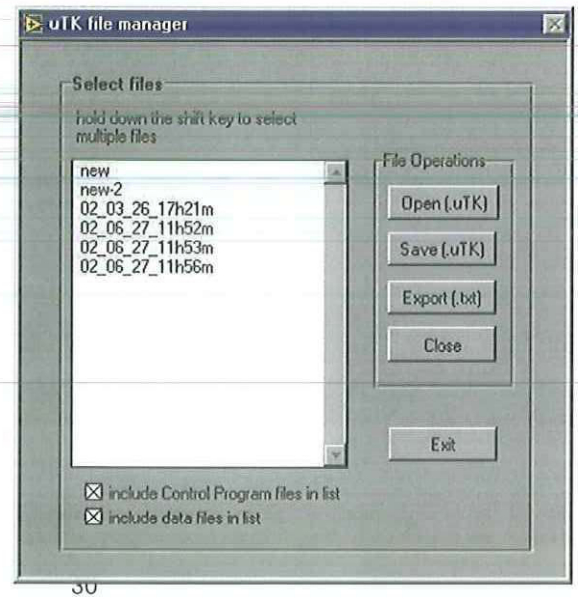
The basic file operations can be accessed via the toolbar.

- The  button creates a **new** Control Program (CP)
- The  button **opens** an existing CP from a .uTK file.
- The  button **closes** the current CP
- The  button **copies** the CP and the CP notes of the current file to a new file, which is then opened.
- The  button **saves** the current CP and any associated notes and data.
- The 

CP drop down menu selects which of the currently open CPs is displayed for editing. If the filename is preceded with "[CP]" you may edit the program. If the filename is preceded with "[data]" then the .uTK file is a data file and you may NOT edit the CP. You may however still **download** and **run** the CP from the [data] file.
- The  **file manager** button allows you to perform advanced file operations

ADVANCED FILE OPERATIONS

Basic file operations (open, save, and close) may be executed simultaneously on multiple files. An export option is available to save your data in a spreadsheet-readable text file. Advanced options are accessed via the following dialog box by pressing the file manager button on the toolbar:



The **Select Files** area shows the currently opened .uTK files. You may select multiple files by holding down the 'shift' key and clicking on the file names. The checkboxes at the bottom restricts the view to just CP files, just data files, or both.

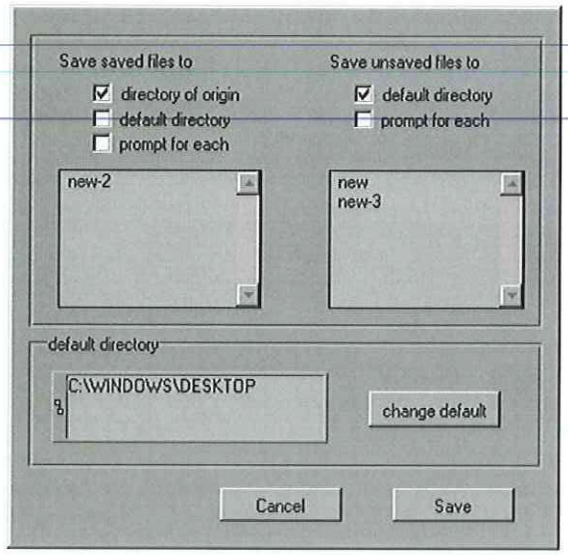
Use **open** to open a previously saved uTK file.

Use **close** to cleanly close the selected files.

Otherwise, selected files may be saved or exported.

BATCH OPERATIONS

If more than one file has been selected for save or export, the **batch file dialog** determines how the files will be directed.



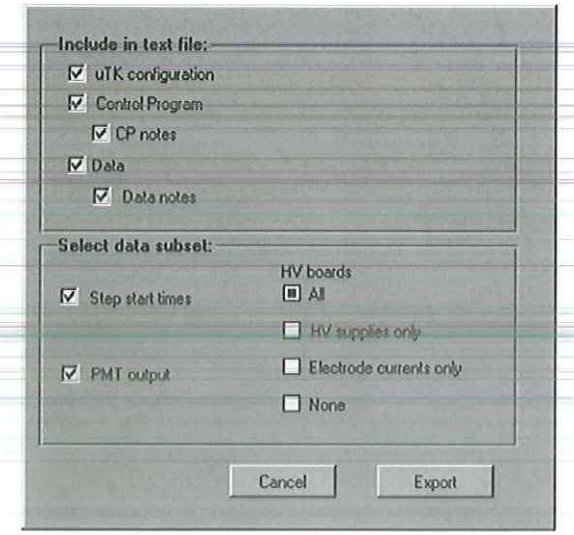
The **prompt for each** checkbox will allow you to choose the filename and target location for each individual file.

The **default directory** checkbox will write the files to the default directory as displayed. Use the **change default** button to select a different default directory. The files will be saved using their current filename with the appropriate extension.

The **directory of origin** checkbox is only available for programs that have already been saved. If the file operation is "export" the exported text files will be saved in the directory containing the original .uTK files. If the file operation is "save" the original files will be replaced by the new files. The files will be saved using their current name with the appropriate extension.

EXPORTING DATA

If **export** is selected, the Export dialog box will appear. Check the boxes to select which file elements you wish to have included in the exported file.



APPENDIX A

QUICK SWITCH APPLICATION

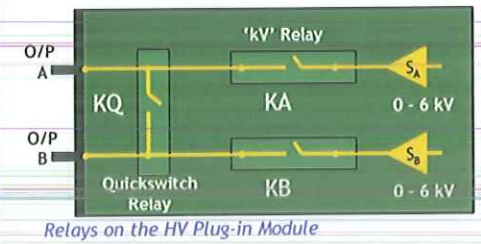
micralyne

Created November 23, 2001

µTK Application: High Voltage Plug-in Modules and the Quickswitch Function

The Microfluidics ToolKit (µTK) Base Electronics can accommodate up to four High Voltage Plug-in Modules (P/N TK-0002-01). Each HV Module contains two independent high voltage supplies that can be programmed individually or used together with the Quick Switch Function.

Normal Operation of the HV Modules:
Each HV output can be programmed in 10V increments over a range from 0V to 6000V. These outputs are controlled by relays which connect the high voltage electronics to a 15kV-rated connector on the front panel of the HV Plug-in Module. The relay function for each HV channel has three states: 'kV' state, 'Gnd' state, and 'Flt' State. The 'kV' state connects the HV electronics directly to the output connector. Up to 6000V is available in this state. The 'Gnd' state connects an internal ground plane in the HV Plug-in Module to the output connector allowing energy to discharge into the ground plane. The 'Flt" state provides an open circuit at the connector; this is the normal state when a the ToolKit is not executing a Control Program.

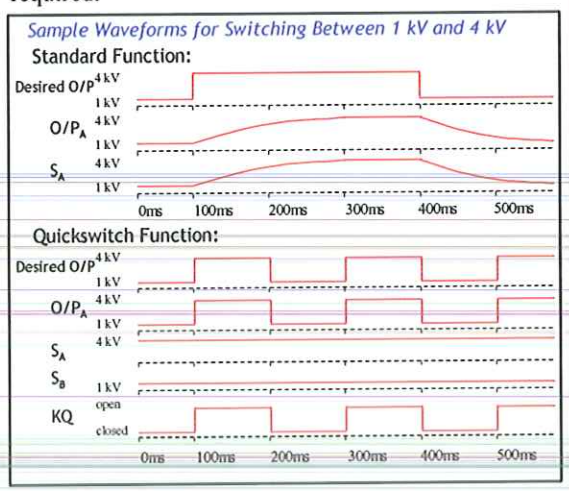


Relays on the HV Plug-in Module

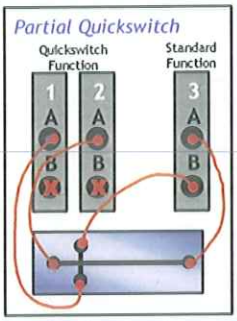
The Microfluidics ToolKit LabView Interface permits the user to write Control Programs to define the output voltage levels and relay states of each available HV channel. These programs can be up to 100 steps in length.

The standard voltage function uses one HV channel per electrode. To perform an experiment requiring four electrodes, two HV Plug-in Modules are required. Due to the high output impedance of the high voltage electronics, rise times are typically 150 ms to 600 ms and fall times are typically 300 ms to 600 ms. If this is too slow, then the Quickswitch function should be used.

Quickswitch Function:
The advantage of the Quickswitch function is speed of response when changing voltage levels. Rise and fall time of 2 ms to 5 ms can be achieved using this method. The Quickswitch function alternately connects the Channel A and Channel B electronics to the Channel A output through a relay. The Channel B output is not used with the Quickswitch. Each voltage level is set one step in advance of its connection to the output, so that it has already settled to its final value before the relay is switched. To perform an experiment requiring four electrodes, four HV Plug-in Modules are required.



Partial Quickswitch:
In some applications, not all of the outputs are required to have the quicker response. In these cases a combination of voltage functions can be used in a single Control Program. For example, a four electrode experiment with two electrodes controlled by the Quickswitch function and two electrodes controlled by the standard voltage function would require three HV Plug-in Modules.



APPENDIX B

USING THE OPTICS BOARD FOR DATA ACQUISITION

micralyne

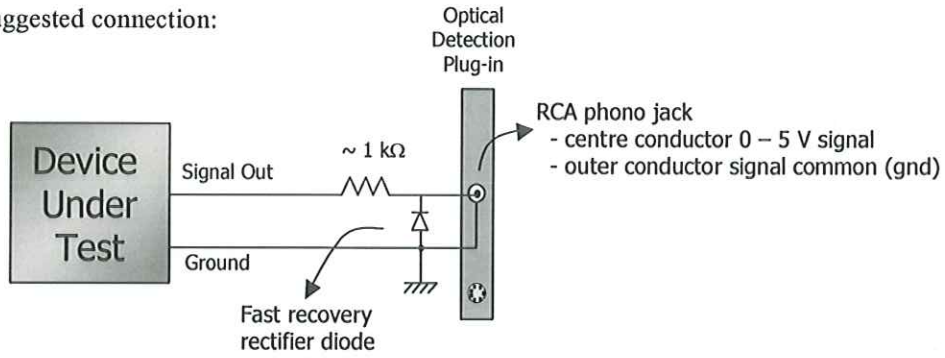
Created November 14, 2001

µTK Application: Using the Optical Detection Plug-in Module as an ADC Input

Input range: 0 – 5 Vdc
Input Resolution: 16 bits (~75µV / step)
Sample Rate: 200 Hz

Input filter – low pass, with f_{CUTOFF} = 10 kHz
(function is noise reduction and input protection)
Input protected to > 4 kV electrostatic discharge

Suggested connection:



1) Solder the resistor and diode close to the RCA phono plug that is connected to the O.D. module.

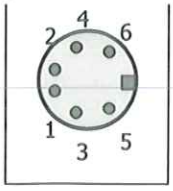
2) Use of a coaxial cable is recommended (e.g.. The rest of a cannibalized RCA cable). At least use a (tightly) twisted pair of 24AWG or larger.

3) Cable length less that two meters is recommended (the shorter the better)

4) Eliminate any ground loops, as these will cause measurement errors.

If required, a 5 Vdc/100mA voltage supply is available. It is relay-controlled, and is switched on at the beginning of a control program if the laser interlock is closed.

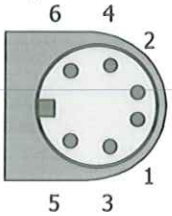
Plug-in Power Connector



1. Gnd
2. Gnd
3. -15 Vdc O/P (20mA max)
4. 5 Vdc O/P (150mA)
5. +15 Vdc O/P (20mA max)
6. PMT control (0-1V)

Shell Gnd

PS/2 DIN Cable Wire



1. Black
2. Brown
3. Red
4. Orange
5. Yellow
6. Green

APPENDIX C

USING MICRALYNE MICROFLUIDIC CHIPS

We have published a paper in Analytical Chemistry that describes our recommended chip handling procedure. You may download the paper form the Analytical Chemistry web page paper: AC 73, 4079-4086 (2001).

GETTING STARTED

When you first receive your chip be sure to remove the blue tape. Prior to shipping the chips undergo a final rinse consisting of soap and water and then just water so the final chip that you receive should have bare glass surfaces. Before using the chip for the first time we highly recommend that you clean it according to the following cleaning procedures.

CLEANING THE CHIP

How often you clean the chip is completely up to you. It is dependant on the type of solution you are using and what you are trying to accomplish.

To clean the chip we usually flush ~1M HNO3, 1M NaOH and then the running buffer through the chip, in that order.

We recommend the following general cleaning procedure:

For most any aqueous solution, with buffers/reagents up to 1M concentrations, capillary forces will draw the solution through the channels; for continuous flushing, vacuum (e.g. 1atm) will suffice. Be sure to make a good connection to the port on the chip; do so by cutting a pipet tip at the right diameter for a snug fit. Mate a syringe directly to this tip, and draw the plunger to create a vacuum; lock the plunger in position. You should be able to maintain vacuum for a couple of weeks, and draw through 10-20mL of water. If you are using a solution with significantly higher viscosity, draw ~100mL of that solution in the syringe with cut pipet tip attached, mate to chip, and force through (you'll get 5-10atm with a 1mL syringe). Be sure you push on the plunger and not on the syringe and/or chip, or you will break the chip (the chip can easily handle the pressure).

STORING THE CHIP

We recommend storing the channels wet, flushing with water.

TROUBLESHOOTING

Occasionally the channels of the chip get clogged (this is generally caused by solutions drying and attaching to the glass). When this happens it is very difficult to unclog the chip. The method that works best is to determine the cause of the blockage and to wash the chip with an appropriate solvent. Please take care when doing this as certain solvents may eat away at the glass or affect the bonding.

APPENDIX D

LASER DIAGRAMS

SPECIFICATION SHEET: OPTICAL DETECTION MODULE

The μTK optical detection PCB has been designed to provide power for a photomultiplier (PMT) assembly and to sample the PMT analog output signal at a fixed rate of 50 samples per second.

The PMT input on the optical detection PCB accepts a 0 to 5 V analog signal. The signal is sampled by a 16 bit analog-to-digital converter (ADC); the resulting data may be displayed on a computer monitor in real time or stored for future analysis. The signal input is protected against electrostatic discharge (ESD) by a device rated for IEC 1000-4-2 standard discharges of up to 8 kV.

The ADC generates very low noise levels. When the PMT input connector is driven by a low impedance, low noise dc voltage source or is shorted to analog ground, approximately 96% of the readings will have the same value, approximately 4% will deviate by 1 ± bit, and fewer than 0.2% will differ by more than ±1 bit.

Specifications:

PMT SIGNAL INPUT

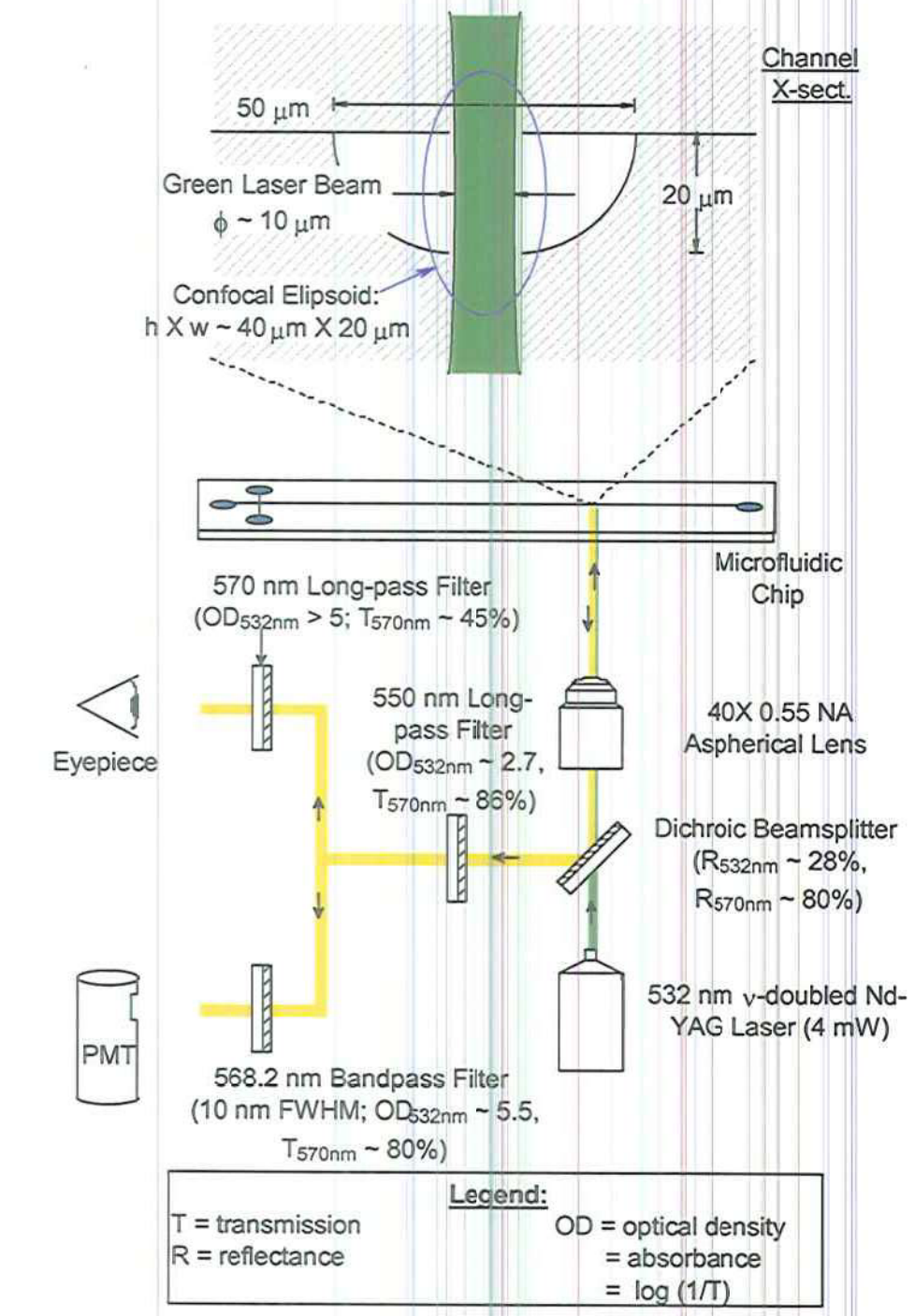
Connector	RCA phono jack, panel mount
Input range	0 to 5.0Vdc
Input resolution	16 bits
Input time constant	100μs ±10μs

PMT POWER SUPPLY (OPTICS) OUTPUT

Six Position Shielded Circular Mini-Din Connector

Pin	Function
1	Ground
2	Ground
3	-15Vdc / 20mA unregulated voltage output
4	+5Vdc / 150mA regulated voltage output
5	+15Vdc / 20mA unregulated voltage output
6	12 bit DAC output, 0 - 5Vdc / 5mA

Optics Diagram: Green Laser



Optics Diagram: Red Laser