# Sequencer Scripts

4 May 2008

The Fta32 software includes the ability to run "user defined programs" on the instrument. These might be called *methods*, but scripts are more general in nature. The user program is called a *script* and the thing that runs or executes it is called the *sequencer*. It is entirely possible to run the instrument without using scripts and, in fact, scripts do nothing except push buttons and fill in text boxes the same way an operator can. Nevertheless the time investment in writing a script is often worthwhile because

- repetitive operations become easy
- samples may be manipulated so a large number of measurements are made
- the script becomes documentation of the details of the method.

## Three Levels of Automation

You can always run the instrument in *manual* mode. This means you specify each basic operation, like "move this axis 10mm to the right" or "analyze this image for contact angle". While the operation may be basic, it may also involve quite a bit of software work for one click of a button, as is the case with image analysis. Manual mode is the most basic and historically the oldest of the operating modes.

FTA provides a higher level of automation called *macros*. A macro is still a single click of a button, but now this work may take an indefinite amount of time because image analysis will be used to control stepper motor operation in a feedback loop. An example might be to hang a certain volume pendant drop on a tip. The pump will be run until the desired volume is achieved. This may take some time as, for example, there may not be any liquid in the line down near the tip at the start. Or perhaps there is too much liquid in a drop at the start and the pump has to retract volume. Macros are available for the basic operations necessary to make contact angle and surface tension measurements. You can think of manual mode as specifying *how to do* what you want done and macros as simply specifying *what you want done*.

The final and highest level of automation is the sequencer. The script is a program which can perform ordinary manual mode operations, start macros and wait for their completion, and has its own logic and counting structures to build a framework for complicated sequences.

## Script Attributes and Features

- A script is stored in a single file (it happens to be an Access database) which can be stored and loaded like any other file. You can have many scripts on disk.

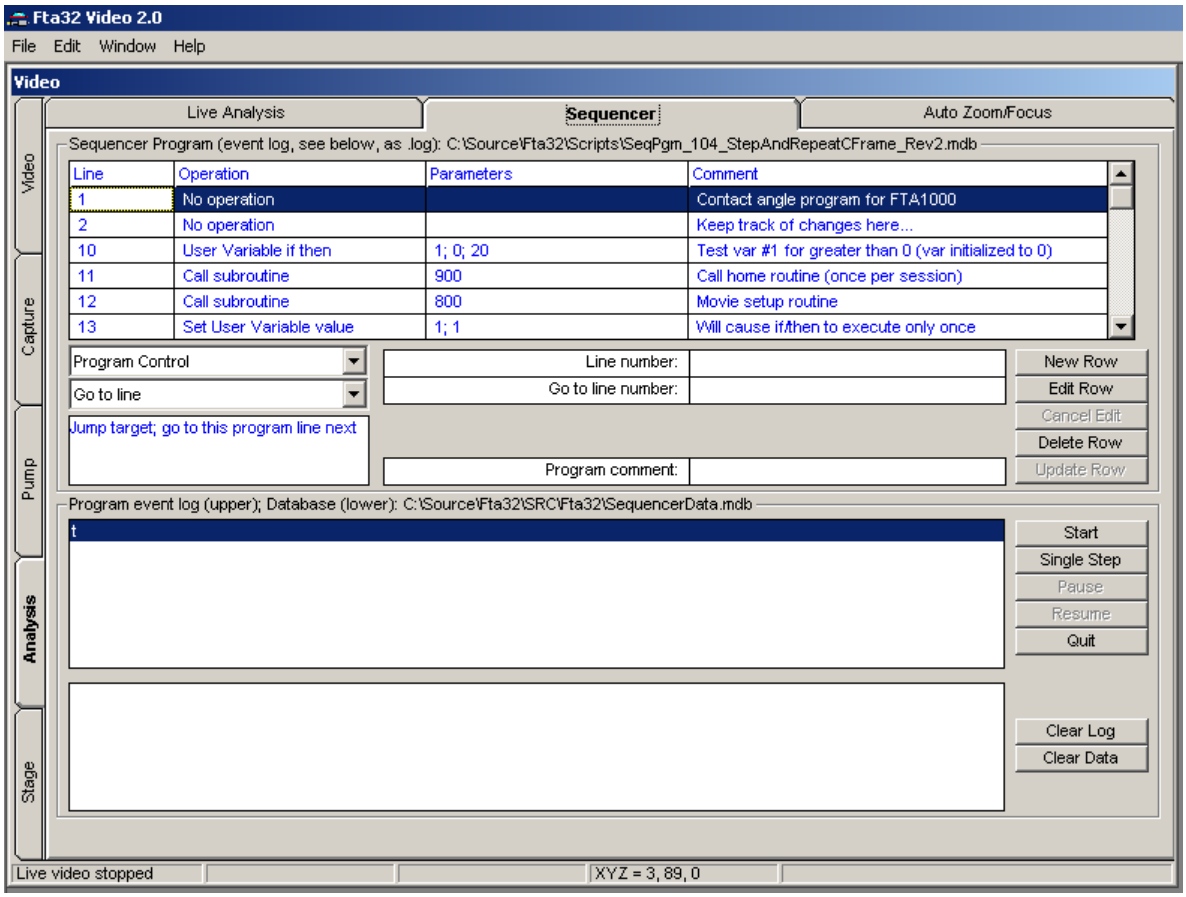
- It can be printed and it can be imported into a word processor. It is human readable.

- Scripts are created and edited inside the Fta32 software by point-and-click operations. It is not free-format writing as, say, with a word processor.

- If you are careful, it can be exported as a text file, edited as a text file, and re-imported as a script. When you edit externally, less error checking is available, but it is handy for "cut and paste."

- There is no limit as to the size of the script.

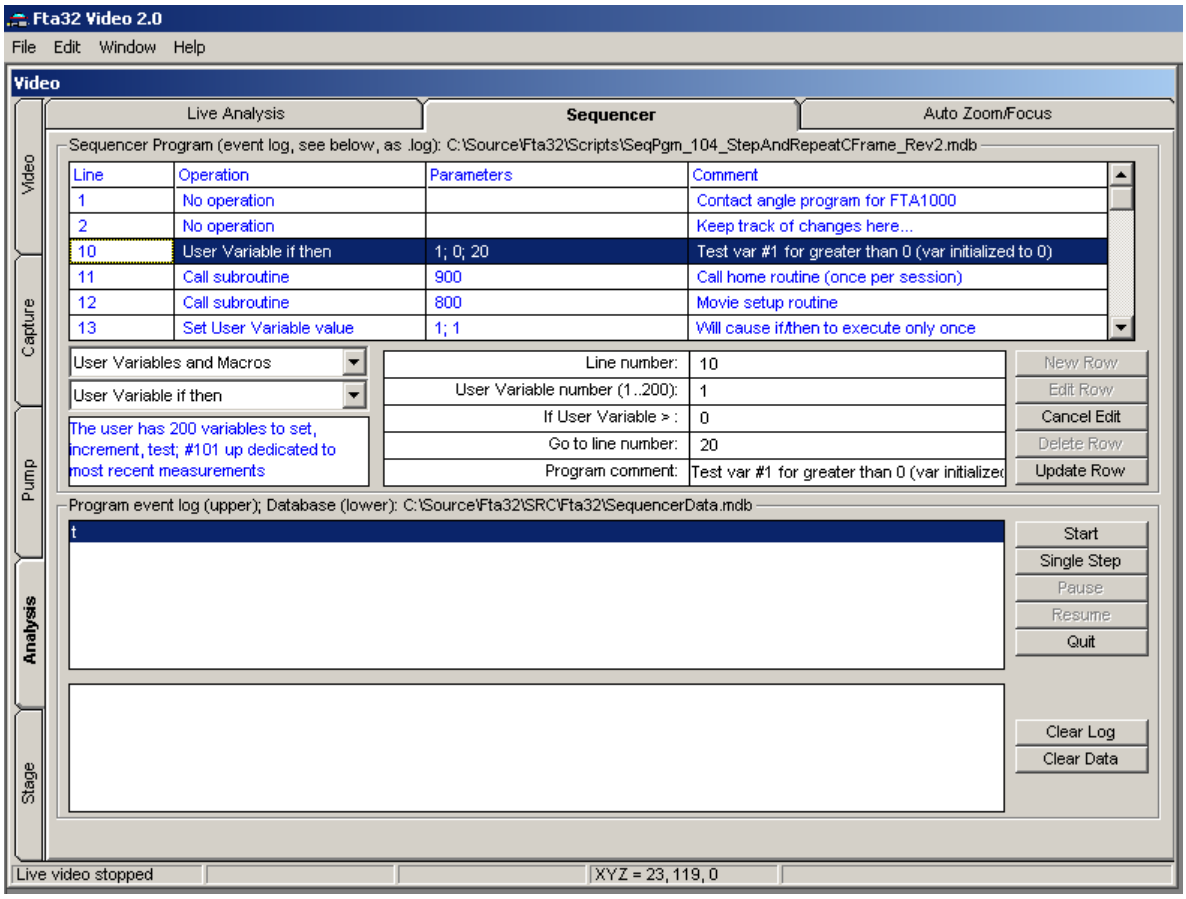
- Technically speaking, scripts are interpreted line by line as they execute. They are not compiled. This makes debugging easier and on-the-fly changes possible. This is discussed further below.




Basic Sequencer window with a script loaded.

## Editing Scripts

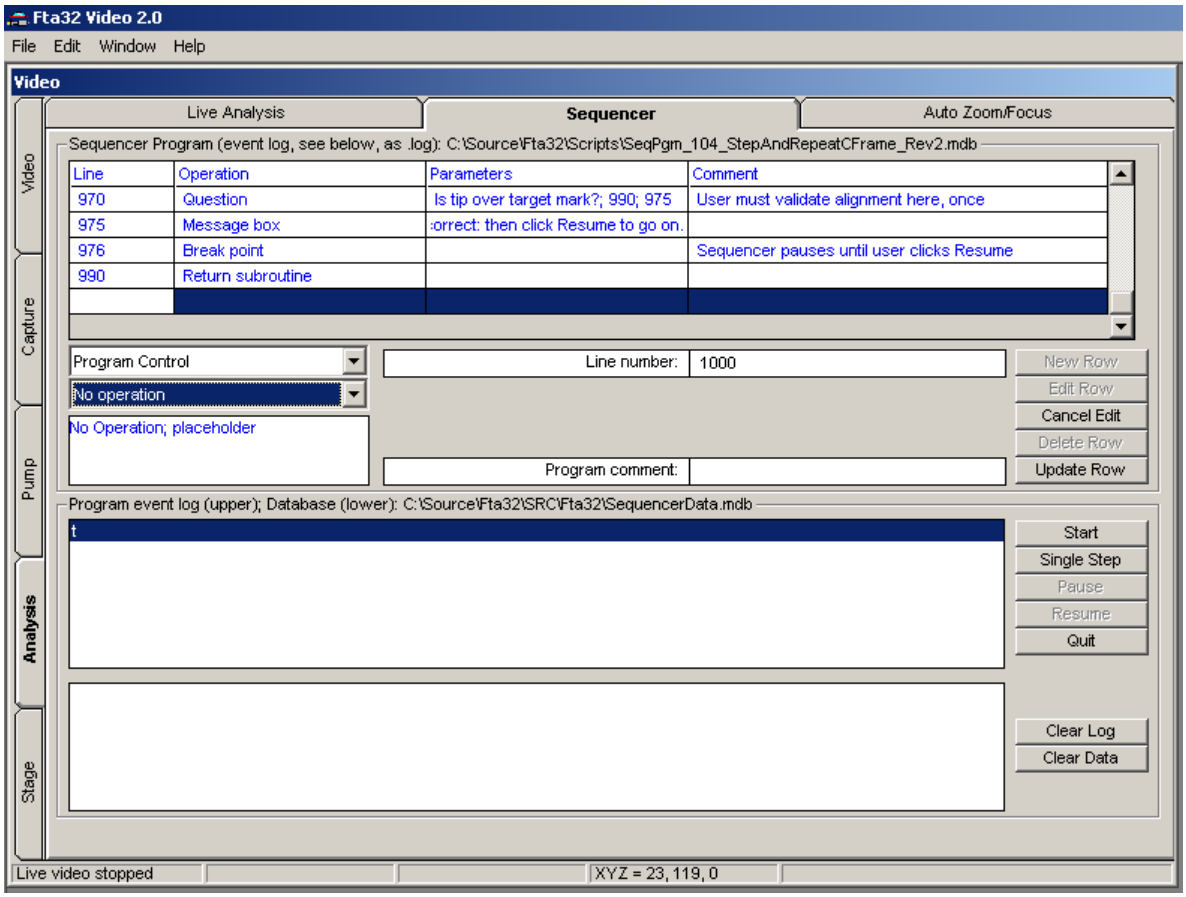
Scripts may be edited through simple commands. First we will illustrate editing an existing instruction, which is one row or line in the table. We will choose to edit the third line, which happens to be line number 10 in this example.




To edit: click in a row to select it, then click *Edit Row*. The details appear in the boxes in the middle.

When you *Edit Row* an existing row, all of the user changeable parameters appear in the text boxes. You could, for example, change the line number (10) or the user variable comparison value (0). The comment is your text description of what you are doing with this instruction. When you finish, you can *Cancel* or *Update Row* the script with your new values.

To create a new instruction within the script, click *New Row*. Now you must fill in all of the parameters before you *Update Row*. You can *Cancel* at any time. The main thing you must deal with is selecting the instruction. This is done from the two drop down (pick) list boxes on the left. The upper one is the instruction group or set. In the above figure, this is *User Variables and Macros*. Within the selected group, the current instruction is *User Variable if then*. There are many instructions, so they are grouped in sets to make finding them easier.




After clicking *New Row*. The line number is automatically set to the next available line number, in multiples of 10. You may well need a different line number, so you can edit this. The instruction set selected is *Program Control* and the current instruction is *No operation*. You can click in these lists and select any instruction you wish.

We will describe the instructions in more detail after we discuss running the Sequencer.

## Running the Sequencer

Once you have a script prepared, you can run it one of two ways:

- Start/full speed: normal operation executing each instruction in turn as fast as possible.

- Single step: execute the next instruction and then stop. While stopped, you can do anything you wish, including
  - performing manual motions or commands
  - executing macros
  - editing the script itself (*yes!*)

*Start* and *Single Step* are the ways you begin. You always begin at the first row, which will have the smallest (positive) line number.

If you are running full speed from a *Start*, you can *Pause* at the end of the current instruction. From *Pause*, you can *Resume* normal full speed or *Single Step* or, if you wish to quit now, *Quit*. After a *Quit*, you must start from the beginning next time.

From *Single Step*, you can again *Single Step*, *Resume* full speed, or *Quit*.

If you are in a paused or single step mode, you can edit the script at any line, but when you begin again (either by *Resume* or *Single Step*), you resume at the next instruction you would normally execute had you done no editing. You can not start again at any arbitrary instruction.

Single stepping is a good way to debug scripts because you can contemplate each instruction. Normal full speed operation is too fast to follow with the eye. To help matters, these sequencer run commands are available at the bottom of the drop down list on the Video tab. This lets you watch the image as the instructions execute. All of this makes what is called an interactive development environment.

## Debugging Aids

- The *Break* instruction will pause the script. This lets the script run full speed until it gets to the break. You can then inspect the system, edit the script, or do any other operation.

- A log is created of Sequencer events while running. All are date and time stamped. This tells you when important things happened. The log is a text file which can be saved or printed.

- There are instructions for writing your own message to the log file.

- There are instructions for stopping and asking the operator for a response. The script can branch (do an *if-then*) based on the response.

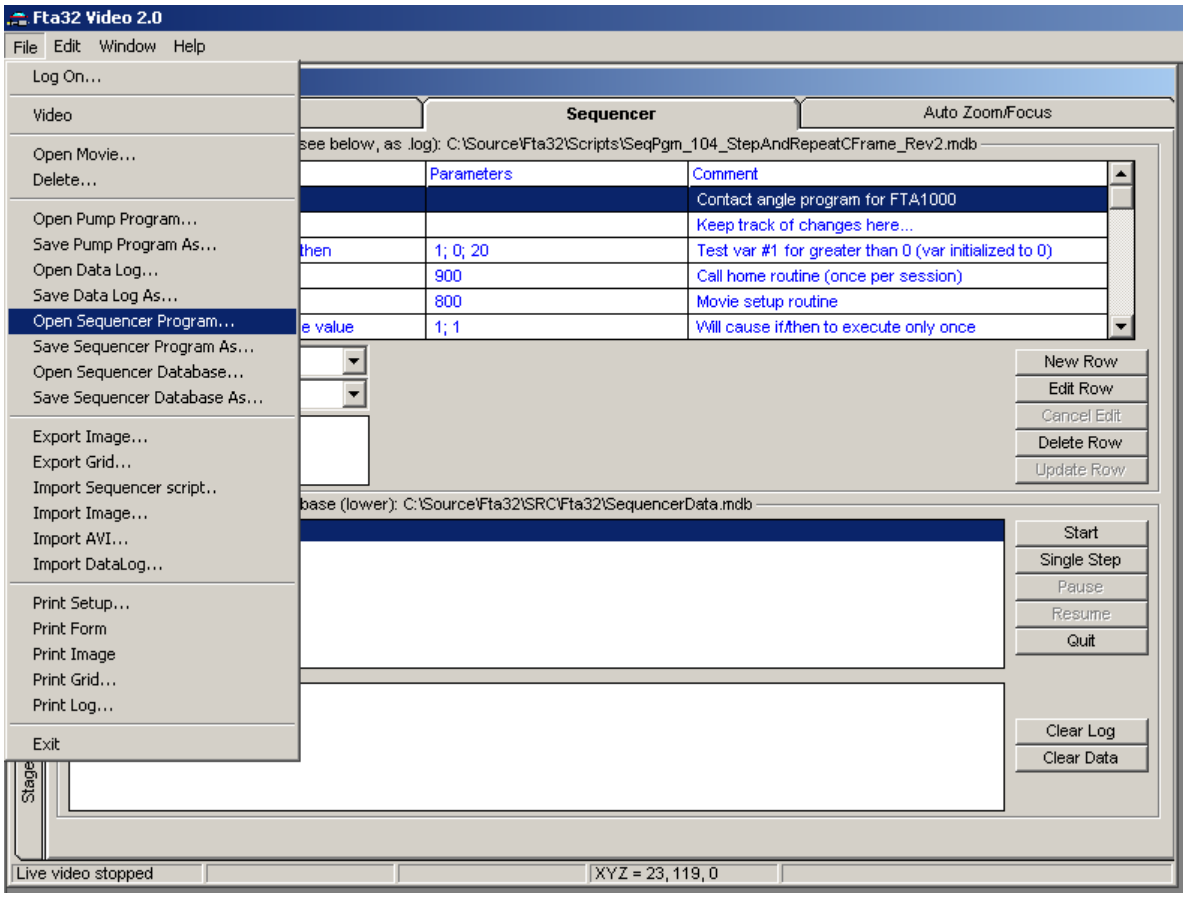
- You can email scripts to FTA engineers and we will analyze them (we can run them) and offer suggestions.

## Loading Scripts

Scripts are “mdb” files, as are FTA Movies. To help you keep track of which files are scripts, you may give them any valid file extension.

The Sequencer is available in all FTA1000 and FTA2000 configurations. These are selected on the LogOn screen in the hardware choices section.

Scripts are loaded from *File > Open Sequencer Program*, as shown in the next figure.




Preparing to load a script from disk. If you want a new, blank, script, you can give it a valid filename in the dialog box which follows.

You make a copy of an existing script and give it a new name with *File > Save Sequencer Program As*. This

- leaves the old script as it was, and
- makes a copy with the new name.

You then work on the new copy.

The "spreadsheet" like presentation of the script is called a *grid*. You can export the script to a text file using *File > Export Grid*. You can then, if you choose, work with it in an editor. You can import a text file back into the program as a script using *File > Import Sequencer script*. For this to work correctly, the formatting present in the original exported file must be preserved. You must have spaces in the right places and you must observe upper and lower case in text.

You can print the script, and any grid in the program, using *File > Print Grid*. Click in grid first.

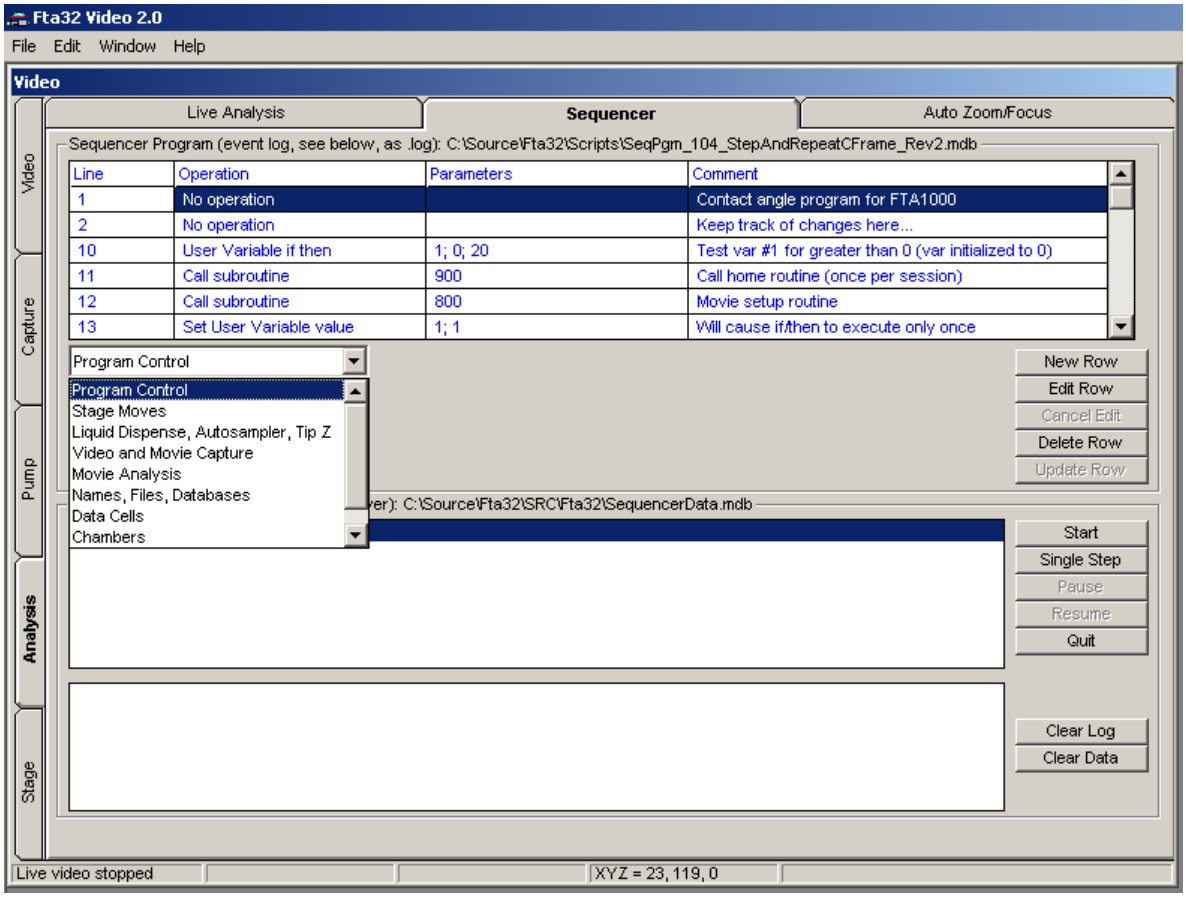
## Scripts and FTA Instrument Models

The same Sequencer is available for all FTA instruments. However, it accomplishes little if the instrument has no automation in the form of stepper motors.

A generic instruction like “Move X stage by 5mm” works on all instruments with a stepper motor driven X stage. Scripts written for one instrument need only the “obvious” modifications to reflect differences in generic hardware between the original and the newly targeted instrument.

## Instruction Groups

The instruction groups are static, in contrast to the instructions within them which grow in number in time as new features are added. The instruction group is selected by clicking in the upper list, as shown below.




Drop down list for instruction groups. Use the scroll bar to access the complete list.

Program Control: basic control over the script and what instruction is executed next are in this group. Specialty instructions such as *Break*, *No operation*, and *Quit* are here. *No operation* is used as a placeholder and is convenient for including extra comments. Various waiting statements are provided so the script will simply delay some time before proceeding. Calls to subroutines and loop setups are included here. Subroutines are separate pieces of code that you wish to execute at this time but which are located elsewhere for convenience. A simple example would be to have two separate subroutines for contact angle analysis and to choose which to use by calling the desired subroutine. Loops let you do some body of code repetitively. You can have up to 100 independent loops. You can have an indefinite number of subroutines. This group also includes messages to the operator while running and branches on their response.

Stage Moves: control over specimen stage and tilt stage movements are here. Manual stage movements are either a *Home* to a sensor at one excursion limit or an incremental *Move by* from the current position. Scripts also provide a *Move to* which is a move from wherever you are to the specified target. You need to keep in mind which type of move you are using. Incremental moves are particularly convenient within loops and subroutines: "move over 5 mm from where you are right now." Use these in step and repeat loops.

Liquid Dispense, Autosampler, Tip Z: liquid dispense operations are here. Tip Z and autosampler motions are also here. While they might seem like a "stage move", they are separated from the above specimen stage moves because these are concerned with getting liquid to some desired position.

Video and Movie Capture: camera and image capture control are in this group. Note you must use the *Open Movie* instruction to connect the Sequencer to a newly captured movie. This is because there could be other movies open on the screen and the new one must be identified. When you finish, you must have an explicit *Close Movie* to break the connection.

Movie Analysis: all analysis functions on the opened movie are in this group.

Names, Files, Databases: this is a catch-all group for assigning names and opening and closing files.

Data Cells: cells are sets of data which you wish to group together, typically for surface energy analysis, where a number of drops contribute to the surface energy of one "point." Think of that point as a cell.

Chambers: chambers and temperature and relative humidity measurements are in this group. The necessary sensors must be installed on your instrument.

User Variables and Macros: user variables can be assigned data by analysis functions or by the script itself. You can then branch on their value. Macros are real time feedback functions that use image analysis to drive stepper motors to some target.

## Example Script

The following script was *Edit > Copy Grid* and then pasted into this word processing program. To make it format nicely, it required changing the font and size and reseting tabs.

| Line | Operation | Parameters | Comment |
|---|---|---|---|
| 1 | No operation | | Contact angle program for FTA1000 |
| 2 | No operation | | Keep track of changes here... |
| 10 | User Variable if then | 1; 0; 20 | Test var #1 for greater than 0 (var initialized to 0) |
| 11 | Call subroutine | 900 | Call home routine (once per session) |
| 12 | Call subroutine | 800 | Movie setup routine |
| 13 | Set User Variable value | 1; 1 | Will cause if/then to execute only once |
| 20 | Home Kloehn pump | | Home pump each time you start --> plenty of liquid |
| 30 | Move Z to | 29.5 | Critical height: set as req'd (see also line 950) |
| 40 | Move tip Z to | -45 | May require adjustment |
| 100 | Begin loop | 3; 4 | Do 4 points |
| 105 | Call subroutine | 820 | Position stage at desired well |
| 110 | Call subroutine | 500 | Dispense and capture Snapshot |
| 111 | No operation | | Use subroutine at 700 for a full Movie |
| 120 | Call subroutine | 400 | SnapShot analysis |
| 121 | No operation | | Use 600 for full Movie analysis |
| 190 | End loop | 3 | |
| 200 | Home tip Z' | | Clean up at end |
| 210 | Home Z stage | | |
| 290 | Message box | All done! | Inform user |
| 299 | Quit | | All done |
| 400 | No operation | | SnapShot analysis |
| 410 | Open movie | 5 | Must have this... |
| 420 | Use previous baseline | 1 | Need this |
| 425 | Reflection image type baseline | 1 | This is a guess, but likely |
| 430 | Spherical mode analysis | 1 | Best choice for smaller drops |
| 435 | Contact angle baseline | | Leave parameters blank to use Macro's data |
| 440 | Do contact angle measurement | | Finally |
| 450 | Close movie | | Need this |
| 451 | Video | 1 | |
| 455 | No operation | | Do pickup here |
| 460 | Move Tip Z to target | .05 | Down to surface |
| 470 | Syringe pump volume, rate | -3; 3 | |
| 475 | Move tip Z by | 1.5 | Go up |
| 495 | Return subroutine | | |
| 500 | No operation | | Dispense liquid and capture SnapShot |
| 505 | Syringe pump volume, rate | -2; 1 | Clear tip of any liquid |
| 510 | Video | 1 | Ensure on |
| 515 | Position tip in image | 25 | Macro to put tip in top of image |
| 520 | Find potential baseline | | Macro to move tip down, then up, to establish baseline |
| 525 | Dispense volume | 3 | Macro to form fixed drop |
| 530 | Touch off drop | .05 | Macro to touch off drop; go extra 50um |
| 535 | Snap shot | | Grab it |
| 590 | Return subroutine | | |
| 600 | No operation | | Movie analysis |
| 610 | No operation | | Could use SnapShot here instead of earlier Run |
| 620 | Open movie | 15 | Must have Open/Close Movie pair |
| 630 | No operation | | Set analysis parameters |
| 631 | Use previous baseline | 1 | This default should be on (checked) |
| 633 | Reflection image type baseline | 1 | Normal case |
| 634 | Spherical mode analysis | 1 | You may prefer non-spherical mode |
| 640 | No operation | | Move to first valid image (depends on Capture values) |

| | | | |
|---|---|---|---|
| 641 | Move to first image in movie | | |
| 642 | Begin loop | 1; 8 | Loop #1 will skip to image #9 |
| 643 | Move to next image in movie | | |
| 644 | End loop | 1 | |
| 645 | Contact angle baseline | | Leave parameters blank to now use Macros' baseline |
| 646 | No operation | | 'C.A. baseline' must be set with target image present |
| 647 | No operation | | therefore move to desired Movie image first |
| 650 | Begin loop | 2; 16 | Loop #2 do rest of images in Movie (see Capture) |
| 651 | Do contact angle measurement | | Makes actual measurement |
| 652 | Move to next image in movie | | |
| 654 | Wait time | 2 | So user can inspect (could eliminate) |
| 655 | End loop | 2 | |
| 670 | Close movie | | |
| 680 | Status info | All done! | Could write something to log... |
| 690 | Return subroutine | | |
| 700 | No operation | | Dispense liquid and capture multiple image Movie |
| 710 | Syringe pump volume, rate | -2; 1 | Pull in 2ul at 1ul/s to clear tip |
| 720 | Video | 1 | Setup Movie capture (you may prefer other values) |
| 721 | Pretrigger images | 5; .033 | To show touch-off |
| 722 | Posttrigger images | 20; .1; 1.05 | Total time 3.3s (you can change values) |
| 723 | No operation | | Trigger will be provided by Touch-Off Macro |
| 730 | Position tip in image | 25 | Macro to put tip in top of image |
| 740 | Find potential baseline | | Macro to find baseline from image |
| 750 | Dispense volume | 5 | Macro to form 5ul pendant drop (other volumes OK) |
| 751 | Start pump on run | 0 | No additional pump run |
| 760 | Run | | Starts Movie acquisition; could also use SnapShot later |
| 770 | Touch off drop | -0.05 | Actual touch off; go down extra 50um |
| 790 | Return subroutine | | |
| 800 | No operation | | Setup saving movies; directory must make sense |
| 801 | No operation | | Enter folder and basic file name you want |
| 805 | Save movies as | C:\Temp\CA_DataMovie.mdb; | 0 or no suffix = autoincrement within session |
| 810 | Return subroutine | | |
| 820 | No operation | | Table to go to 4 random locations |
| 830 | Loop if then | 3; 3; 870 | Go to point 4 |
| 831 | Loop if then | 3; 2; 860 | Go to point 3 |
| 832 | Loop if then | 3; 1; 850 | Go to point 2 |
| 833 | Loop if then | 3; 0; 840 | Go to point 1 |
| 834 | Return subroutine | | Should not get here; this is for safety |
| 840 | No operation | | Absolute move to first point |
| 841 | Move X to | 23.629 | |
| 842 | Move Y to | -35.364 | |
| 843 | Return subroutine | | |
| 850 | No operation | | Absolute move to second point |
| 851 | Move X to | 23.629 | |
| 852 | Move Y to | 35.364 | |
| 853 | Return subroutine | | |
| 860 | No operation | | Absolute move to third point |
| 861 | Move X to | -23.355 | |
| 862 | Move Y to | -35.364 | |
| 863 | Return subroutine | | |
| 870 | No operation | | Absolute move to fourth point |
| 871 | Move X to | -27.128 | |
| 872 | Move Y to | 27.128 | |
| 873 | Return subroutine | | |
| 900 | No operation | | Optional homing routine |

| | | | |
|---|---|---|---|
| 901 | No operation | | We use home offsets to "zero" X and Y |
| 910 | Home Z stage | | Do before Y to get Z low |
| 920 | Home Y stage | | |
| 930 | Home X stage | | |
| 940 | Home tip rack | | |
| 941 | Move tip to video position | | You must have correct values on Pump tab |
| 942 | No operation | | Now have positioned tip over fiduciary mark |
| 943 | No operation | | Assume it is center of wafer |
| 944 | No operation | | We will measure from this "origin" |
| 950 | Move Z by | 30 | Move stage up near needle; adjust as req'd |
| 951 | No operation | | You must get sample to reasonable Z position here! |
| 970 | Question | Is tip over target mark?; 990; 975 | |
| 975 | Message box | Click OK to close this box: then move specimen (using Stage Move commands and set X=0: Y=0) till correct: then click Resume to go on. | User must validate alignment here, once |
| 976 | Break point | | Sequencer pauses until user clicks Resume |
| 990 | Return subroutine | | |

What does this script do?

●Line 10 is a test of a user variable (#0 in this case) to see if it has been set to a value greater than 0. By convention, all variables start out with value zero. The purpose of this is to see if this is the first time the script has run in this Fta32 session. User variables are "static" meaning they are remembered for as long as the program is running. Being the first time, the test fails and the script moves to the next instruction, which happens to be line 11. It will then go and home the instrument by a call to subroutine 900. When it gets to line 13, it sets this #0 user variable to the value "1" so the next time the test will be satisfied. When satisfied, the test jumps to the specified line number, which happens to be "20".

●Lines 900 through 990 take care of homing the stages for this application. You may well wish to change some aspects of this routine. The message box at line 975 asks the operator whether the tip is positioned correctly. *If not, the operator could move the stage manually at this point since the Sequencer is in a break.* When all is well, the operator clicks *Resume* and the script continues.

●Lines 800 through 810 are a short routine to set the base name for saved movies. Each movie will have this base name plus a numeric suffix which indexes by 1 each movie. The suffix number is static so it can continue to increment as long as the Fta32 program is running (not just for one execution of the script).

●Lines 20 through 40 take care of some housekeeping we want to do *every time* the script executes, unlike the homing which we do just once.

●Lines 100 through 190 are the fundamental loop for this script. This loop is given the name *3*. It has a *Begin loop* and an *End loop* which act as "bookends" for the loop. The script will cycle back from line 190 to 100 four times because of the loop count parameter *4*. This script will simply put down four drops and analyze them, but you can have loops inside of

loops in order to create complicated patterns of drops.

●Within the fundamental loop, there is a call to subroutine lines 820 through 834 which move the specimen stage to four arbitrary positions. A simple "move over X" in this routine would give you a row of drops. Lines 830 through 833 have *Loop if then* instructions. These are setup, in this case, to test the loop count of the loop called *3*. If this is the first time through this loop, only line 833 will be satisfied and the script will call the subroutine at line 840 to do the actual X-Y move. Since the parameters for these moves are explicit and arbitrary, the stage can be sent anywhere.

●After the stage is positioned, we call the subroutine at lines 500 through 590 to form a drop, touch it off on the sample and take a *SnapShot* of the drop. Starting with line 515, we use four macros in a row:

○*Position tip in image* will move the Tip Z axis so the tip is, in this case, 25 pixels down from the top. This is about 5% of the 640x480 pixel image.

○*Find potential baseline* will probe the surface by moving the dry tip down close and observing the image to figure out where the surface is. This location may be used later by the contact angle analysis to set the baseline.

○*Dispense volume* runs the pump to form, in this case, a pendant drop of 3 microliters.

○*Touch off drop* lowers the Tip Z in such a fashion that the bottom of the liquid drop just touches the surface. The tip is then raised and the drop will normally break free from the tip. The *0.05mm* parameter is extra down motion to make sure the drop adheres to the specimen.

●After capturing an image of the sessile drop on the surface, subroutine lines 400 through 495 perform a contact angle analysis. Notice the *Open movie* (line 410) and *Close movie* (450) pair. This routine also attempts to pickup the liquid on the surface. It is not possible to remove 100% of the liquid, but most can be removed. This is handy if you are placing drops close to one another and you worry about the old ones appearing in the newer one's images.

●Lines 600 through 690 alternatively analyze a full movie rather than just a snap shot.

●Lines 700 through 790 alternatively deposit drops and capture a full movie rather than just a snap shot.

●To use the alternative formats, you would change the calls at lines 110 and 120.

## Instruction Listing: Program Control

A <...> indicates you fill in a number or some text in the edit box. Instructions are shown in the approximate order they appear in the drop down list on the Sequencer tab.

| Program Control | | |
|---|---|---|
| Instruction | Parameters | Description |
| Go to line | Go to line number: <line number> | Jump to the line number in the program. An invalid line number will create an error. Line numbers are always positive integers. You can think of line numbers as labels for instructions. |
| No operation | | This instruction does nothing. Instead its place in the program allows the user to add comments or save a line number. |
| Break point | | Ordinary Sequencer operation will stop at this point. This is useful for debugging. You can start the Sequencer again by clicking Resume. |
| Quit | | Conclude Sequencer operation with this instruction. You will then Start over later. |
| Wait time | Wait time: <seconds> | Wait here for the indicated number of seconds. You can wait any positive length of time measured in seconds. A fractional second is OK. |
| Wait for external device | Wait time (0…60s): <seconds><br><br>On timeout or error go to: <line number> | Wait here for an "OK" from an external controller or robot. If a non-zero time is provided, wait up to this time, otherwise wait forever.<br><br>If there is a timeout, or the external device sends an error message (for example, it has stalled), go to the line provided. If no line, then do not go anywhere. |
| Begin loop | Loop number (1…100): <index><br><br>Loop count: <count> | Starts a loop. Up to 100 loops can be defined. Each is given a unique index number by user. There will be a mating End loop for each Begin loop.<br><br>How many loops to do. An integer greater than zero. Upper limit is $2^{31.}$ |

| Program Control | | |
|---|---|---|
| Instruction | Parameters | Description |
| Loop if then | Loop number (1…100): <index> | The if/then test refers to this loop. This lets you branch on the loop iteration count. The loop count starts at 0 and is incremented (say to 1) by the End Loop instruction after the loop is completed. |
| | If loop count: <count) | If the loop count of the selected loop is equal to the user set target, then do the go to. |
| | Go to line number: <line number> | Jump to this line if test satisfied. |
| Loop if then subroutine | Loop number (1…100): <index> | The if/then test refers to this loop. This lets you call a subroutine based on the loop iteration count. |
| | If loop count: <count) | If the loop count of the selected loop is equal to the user set target, then call the subroutine. |
| | Subroutine line number: <line number> | Jump to this line if test satisfied. |
| End loop | Loop number (1..100): <index> | This line ends the selected loop. It increments the loop counter. |
| Call subroutine | Subroutine line number: <line number> | Jump to the indicated line number to start a subroutine. When you return from the subroutine, you will go to the line following this call. There must be a mating return subroutine instruction for each call. |
| Return subroutine | | Program flow returns to the instruction following the original call instruction. |
| Status info | Status message: <user text> | Message to output to event log. |
| Sequencer run log | On=1, Off=0: <0 or 1> | Turns run time log on or off. |
| Question | Question message: <user text> | This question allows the user to direct the program flow in one direction or another at some critical point. This text is the question to appear in a dialog box. |
| | If “Yes” GoTo line” <line number> | Jump to this line if user answers yes to the question. |
| | If “No” GoTo line: <line number> | Jump to this line if the user answers no to the question. |

| Program Control | | |
|---|---|---|
| Instruction | Parameters | Description |
| Message box | User message: <user text> | This is a simple message presented to the user in a dialog box. The only response is OK. |
| Message user variable | User variable number: <index> | The value of the chosen user variable (index typically 1...200) will be given to operator in a dialog box. The only response is OK. |
| Show video | | Switch to the Video tab. Useful to force the image to be visible to operator. |
| Show sequencer | | Switch to the Sequencer tab. |
| Begin no waiting for motion end | | Normally each motor motion is completed before the Sequencer goes to the next instruction. This instruction starts a period when the Sequencer will continue with its next instruction before the motion started with this or a previous instruction is complete. The intended use is with tilt axis motions for tilting plate contact angle measurements. |
| End no waiting for motion end | | Return to the normal mode of waiting for each motor motion to complete before moving to the next instruction. |

## Instruction Listing: Stage Moves

Not all instructions will be available for any particular instrument. You will have the instructions appropriate to your configuration.

| Stage Moves | | |
|---|---|---|
| Instruction | Parameters | Description |
| Move tilt by | Move by (deg): <angle to increment><br><br>Rate (deg/s): <tilt rate> | Move the motor by the specified increment at the rate. This is a relative move from the current position. |
| Move tilt to | Move to (deg): <angle><br><br>Rate (deg/s): <tilt rate> | Move the motor to a specific target location at the rate. It is OK if the motor is already there. |
| Move tilt by, no wait | Move by (deg): <angle to increment><br><br>Rate (deg/s): <tilt rate> | Move the motor by the specified increment at the rate. Similar to the above except that the Sequencer will not wait until the move is finished, but will go on to the next instruction. |
| Move tilt to, no wait | Move to (deg): <angle><br><br>Rate (deg/s): <tilt rate> | Move the motor to a specific target location at the rate. Similar to the above except that the Sequencer will not wait until the move is finished, but will go on to the next instruction |
| Home tilt stage | | Home the tilt stage. |
| Move Z to | Move to (mm): <Z target mm> | Move the motor to a specific target location. It is OK if the motor is already there. |
| Move Z by | Move by (mm): <Z increment mm> | Move the motor by the specified increment. This is a relative move from the current position. |
| Random (1mm) stage Z move | | Make a random Z move. Limited to +/-1mm from current position. Useful for testing. |
| Move X to | Move to (mm): <X target mm> | Move the motor to a specific target location. It is OK if the motor is already there. |
| Move X by | Move by (mm): <X increment mm> | Move the motor by the specified increment. This is a relative move from the current position. |
| Move Y to | Move to (mm): <Y target mm> | Move the motor to a specific target location. It is OK if the motor is already there. |
| Move Y by | Move by (mm): <Y increment mm> | Move the motor by the specified increment. This is a relative move from the current position. |
| Home X stage | | Home the X axis stage. |

| Stage Moves | | |
|---|---|---|
| Instruction | Parameters | Description |
| Home Y stage | | Home the Y axis stage. |
| Home Z stage | | Home the Z axis stage. |
| Move R (radial) to | Move to (mm): <R target mm> | Move the motor to a specific target location. It is OK if the motor is already there. |
| Move R (radial) by | Move by (mm): <R increment mm> | Move the motor by the specified increment. This is a relative move from the current position. |
| Move Q (theta) to | Move to (deg): <Q target degrees> | Move the motor to a specific target location. It is OK if the motor is already there. |
| Move Q (theta) by | Move by (deg): <Q increment degrees> | Move the motor by the specified increment. This is a relative move from the current position. |
| Move XY by | Move X by (mm): <X increment mm><br><br>Move Y by (mm): <Y increment mm> | This instruction allows the R-theta stage to behave like an X-Y stage. The motions must be increments from the current position. |
| Home R (radial) stage | | Home the radial axis stage. |
| Home Q (theta) stage | | Home the theta (rotation) axis stage. |
| Home Z stage | | Home the Z axis stage. |
| Move external device | Parameter 0: <user text><br>Parameter 1: <user text><br>Parameter 2: <user text> | This instruction will send up to three user text strings to the external robot. The text has no meaning to the Sequencer but does have meaning presumably to the robot. |
| Home cylindrical X stage | | Home the X axis of the cylindrical stage (coffee can stage). |
| Home cylindrical rotation stage | | Home the rotation axis of the cylindrical stage |
| Move cylindrical X by | Move by (mm): <X increment mm> | Move the motor by the specified increment. This is a relative move from the current position. |
| Move cylindrical rotation by | Move by (deg): <rotation increment degrees> | Move the motor by the specified increment. This is a relative move from the current position. |

## Instruction Listing: Liquid Dispense, Autosampler, Tip Z

Not all instructions will be available for any particular instrument.

| Liquid Dispense, Autosampler, Tip Z | | |
|---|---|---|
| Instruction | Parameters | Description |
| Home autosampler | | FTA2000: Home autosampler; this is a “home all”. |
| Home tip Z’s | | Bring all tip Z axes up to top. |
| Liquid index | Set test liquid index: <index number> | Set the index number for the test liquid. This refers to the row number in the current Liquids database. This will provide specific gravity information to IFT measurements. |
| Inlet solenoid valve | Open=1, Closed=0: <0 or 1> | FTA2000: Manually force the inlet valve to the pump to be open (1) or closed (0). The inlet valve sits between the system liquid bottle and the pump. For most operation, this valve is closed. This instruction checks or unchecks the checkbox of the same name on the Pump \| Autosampler tab. |
| Outlet solenoid valve | Open=1, Closed=0: <0 or 1> | FTA2000: Manually force the outlet valve to the pump to be open (1) or closed (0). The outlet valve sits between the pump and the dispense tips. For most operation, this valve is closed. This instruction checks or unchecks the checkbox of the same name on the Pump \| Autosampler tab. |
| Valve position | Tip=1, Vial=0: <0 or 1> | FTA1000: Send Kloehn valve to indicated position. |
| Valve to tip | Connect pump to tip: <1, 2… 6> | FTA2000: This instruction moves the Hamilton distribution valve to one of the six tips. The pump is then connected to a specific tip. |
| Pump volume, rate | Pump volume (ul): <dispense volume><br><br>Pump rate (ul/s): <dispense rate> | FTA2000: Move the pump so as to dispense (towards a more positive volume) or aspirate (towards a more negative volume).<br>Excessive rates, above 20 microliters per second, will cause air bubbles to form in the system liquid. |

| Liquid Dispense, Autosampler, Tip Z | | |
|---|---|---|
| Instruction | Parameters | Description |
| Syringe pump volume, rate | Pump volume (ul): <dispense volume><br><br>Pump rate (ul/s): <dispense rate> | FTA1000: Move the pump so as to dispense (towards a more positive volume) or aspirate (towards a more negative volume). Excessive rates, above 20 microliters per second, will cause air bubbles to form in the system liquid. |
| Select tip for move | Set tip to use: <selected tip> | Selected tip will be used for future pump or tip Z moves. |
| Home Kloehn pump | | FTA1000: Home Kloehn pump. Required before first move in a session. |
| Tip to vial | Set vial: <target vial> | FTA2000: Move the selected tip to the specified vial. The tip is then the one which will be moved up or down with subsequent Tip Z moves. If you want to change the selected, or current, tip, do a Tip to vial or a Tip to video move, even if the tip in question is already at that location. this will make it the current tip. |
| Tip to video | Set tip to use: <selected tip> | Move the selected tip to the video position. The tip is then the one which will be moved up or down with subsequent Tip Z moves. |
| Move tip Z to | Move tip Z to (mm): <Z target mm> | Move the current tip to a specific Z height. The upper most position is 0. All target positions are below this, so they are negative. |
| Move tip Z by | Move tip Z by (mm): <Z increment mm> | Move the current tip by the specified Z increment. This moves an additional amount rather than to a specific position. |
| Move tip rack X by | Increment (mm): <X distance mm> | FTA1000: Move tip rack by increment. |
| Move tip to video position | | FTA1000: Move current selected tip to video position. |
| Move tips up | | FTA1000: Move all tips high. Same action as Home Tip Z's. |
| Home tip rack | | FTA1000: Home tip rack. Also homes tip Z's. |
| Tip Z slow | Normal=0, Slow=1 <0 or 1> | FTA2000: Vary tip Z speed for touch-offs with less drop vibration. |

| Liquid Dispense, Autosampler, Tip Z | | |
|---|---|---|
| Instruction | Parameters | Description |
| Prime towards tips | Prime in volume (ul): <volume> | FTA2000: Carry out a prime in sequence from the system vial. This will move liquid towards the dispense tips. |
| Prime from tips | Prime out volume (ul): <volume> | FTA2000: Carry out a prime out sequence away from the tips This will move liquid towards the dispense tips. |
| Drop tip | | FTA2000: For systems with the automatic tip changer, discard the dispense tip from the active tip. |
| Pickup new tip | | FTA2000: For systems with the automatic tip changer, pickup a new dispense tip on the active tip. The tip storage wheel will advance automatically to the next position. |
| Require tips high before rotate | | FTA2000: Require that tip Z's be high before carousel move. Tips will be moved if necessary. |
| Plate 1 first active alpha row | First alpha row: <A, B....> | FTA1000: First active alphabetic row on plate 1. Allows you to ignore some initial rows. |
| Plate 1 last active alpha row | Last alpha row: <...G, H> | FTA1000: Last active alphabetic row on plate 1. Allows you to ignore some final rows. Available rows depend on pitch entered on tab (e.g., 9mm). |
| Plate 1 first active numeric col | First numeric column: <1, 2...> | FTA1000: First active numeric column on plate 1. Allows you to ignore some initial columns. |
| Plate 1 last active numeric col | Last numeric column: <...11, 12> | FTA1000: Last active numeric column on plate 1. Allows you to ignore some final columns. Available columns depend on pitch entered on tab (e.g., 9mm). |
| Plate 2 first active alpha row | First alpha row: <A, B....> | FTA1000: First active alphabetic row on plate 2. Allows you to ignore some initial rows. |
| Plate 2 last active alpha row | Last alpha row: <...G, H> | FTA1000: Last active alphabetic row on plate 2. Allows you to ignore some final rows. Available rows depend on pitch entered on tab (e.g., 9mm). |

| Liquid Dispense, Autosampler, Tip Z | | |
|---|---|---|
| Instruction | Parameters | Description |
| Plate 2 first active numeric col | First numeric column: <1, 2...> | FTA1000: First active numeric column on plate 2. Allows you to ignore some initial columns. |
| Plate 2 last active numeric col | Last numeric column: <...11, 12> | FTA1000: Last active numeric column on plate 2. Allows you to ignore some final columns. Available columns depend on pitch entered on tab (e.g., 9mm). |
| Move autosampler Y by | Increment (mm): <X increment mm> | FTA1000: Move autosampler by increment. The vial tray moves in the Y direction, parallel to the microscope's optical axis. |
| Home autosampler tray | | FTA1000: Home the autosampler vial tray. |
| Move tip to P1 XY | | FTA1000: Move current tip to target XY position over plate 1. No tip Z motion. |
| Move tip to P1 XYZ | | FTA1000: Move current tip to target XYZ position over plate 1. |
| Move tip to P2 XY | | FTA1000: Move current tip to target XY position over plate 2. No tip Z motion. |
| Move tip to P2 XYZ | | FTA1000: Move current tip to target XYZ position over plate 2. |
| Move tip to waste XY | | FTA1000: Move current tip to waste XY position. |
| Move tip to waste XYZ | | FTA1000: Move current tip to waste XYZ position. You can discharge waste liquid here. |
| Move tip to tip hook XY | | FTA1000: Move current tip to detachment XY position. |
| Move tip to tip hook XYZ | | FTA1000: Move current tip to detachment XYZ position. This instruction is mainly for setup purposes to test the XYZ values entered on the autosampler tab. |
| Detach tip | | FTA1000: Performs the complete XYZ move sequence to detach a tip. Tips are attached by driving the bare Luer hub down into an available tip in the plate's XY matrix, The vial tray must be setup to hold new tips, as opposed to vials or a plate. |
| Set P1 target to first position | | FTA1000: Set the current autosampler target for plate 1 to the first active position. |

| Liquid Dispense, Autosampler, Tip Z | | |
|---|---|---|
| Instruction | Parameters | Description |
| Increment P1 target | | FTA1000: Increment the current autosampler to the next plate 1 available position. This will wrap around to the first position after the last position is reached. |
| Set P2 target to first position | | FTA1000: Set the current autosampler target for plate 2 to the first active position. |
| Increment P2 target | | FTA1000: Increment the current autosampler to the next plate 2 available position. This will wrap around to the first position after the last position is reached. |
| Home current tip Z | | FTA1000: Home only the current selected tip Z. Note that “current pump” and “current tip” are the same and are chosen by checkboxes. |
| Home tip X rack only | | FTA1000: Home the tip X rack only. You should make sure the tips are high enough that they will not hit anything. |

# Instruction Listing: Video and Movie Capture

| Video and Movie Capture | | |
|---|---|---|
| Instruction | Parameters | Description |
| Snap shot | | Capture a single-image Movie now. |
| Run | | Start the capture of a multiple image movie. This Movie must have a Trigger event to complete. |
| Trigger on Run start | On=1, Off=0: <0 or 1> | Automatically generate a Trigger as soon as Run starts to capture a Movie. |
| Tilt on Run start | On=1, Off=0: <0 or 1> | Start a tilt motion when the Run starts (if a stage is present and a tilt increment specified). |
| Restart Video after Movie | True=1, False=0: <0 or 1> | If set = 0, the Video will not be restarted automatically when the captured Movie is closed. If this instruction is not used, Video will be restarted after a Movie. If set = 1, it will also be restarted. |
| Video | On=1, Off=0: <0 or 1> | Turn on or off the Video checkbox on the Live Video tab. Video must be turned on to get a live image. |
| Back light | On=1, Off=0: <0 or 1> | Turn on or off the back light. The back light will normally be turned on automatically when Video is started. The back light is the one used to illuminate normal analysis images. |
| Front light | On=1, Off=0: <0 or 1> | Turn on or off the front light (if it exists). This is used for observing the general nature of the sample better. |
| Increment Live video brightness | Increment brightness by: <value> | Brightness can be vary from 0 to 100. This will change the current brightness setting, but will stay within 0...100. |
| Increment Live video contrast | Increment contrast by: <value> | Contrast can be vary from 0 to 100. This will change the current contrast setting, but will stay within 0...100. |
| Pretrigger images | Pretrigger images: <image count><br>Pretrigger image period (s): <period> | Setup the number and period of images before the Trigger in a multi-image Movie. These entries are just like those on the Capture tab. |

| Video and Movie Capture | | |
|---|---|---|
| Instruction | Parameters | Description |
| Posttrigger images | Posttrigger images: <image count><br><br>Posttrigger image period (s): <period><br><br>Posttrigger period multiplier: <factor> | Setup the number and period of images after the Trigger in a multi-image Movie. These entries are just like those on the Capture tab. The post-trigger multiplier should be 1 unless you want the period to gradually increase after each image, in which case it should be slightly greater than 1, say 1.05. |
| Video trigger by gray level | On=1, Off=0: <0 or 1><br><br>X coordinate (pixels): <position><br><br>Y coordinate (pixels): <position> | Turn on or off the Video Trigger by Z < 120 checkbox on the Capture tab. If enabled, a cross-hairs will be placed in the image. If the gray scale of the image is darker than mid-gray, it will cause a Trigger. |
| Video trigger by gray change | On=1, Off=0: <0 or 1><br><br>X coordinate (pixels): <position><br><br>Y coordinate (pixels): <position> | Turn on or off the Video Trigger by Z < 120 checkbox on the Capture tab. If enabled, a cross-hairs will be placed in the image. If the gray scale of the image is darker than mid-gray, it will cause a Trigger. |
| Trigger on pump done | On=1, Off=0: <0 or 1> | Turn on or off the Trigger at Pump Completion checkbox on the Capture tab. If enabled, a Trigger will be generated when the pump finishes its motion. |
| Start pump on run | On=1, Off=0: <0 or 1> | If 1, start the pump when a Run is started. This will set the similarly named checkbox on the Pump \| Syringe tab, if this tab is visible to the user. |
| Stop pump on trigger | On=1, Off=0: <0 or 1> | If 1, stop the pump whenever a Trigger occurs. This will set the similarly named checkbox on the Pump \| Syringe tab, if this tab is visible to the user. |
| Do pump program on run | On=1, Off=0: <0 or 1> | If 1, start the pump program when a run starts. This will set the similarly named checkbox on the Pump \| Syringe tab, if this tab is visible to the user. |
| Trigger | | Trigger the Movie now, without any condition. |

| Video and Movie Capture | | |
|---|---|---|
| Instruction | Parameters | Description |
| Open movie | Max time to wait (s): <seconds> | You must Open Movie after the movie is captured and before any analysis can be performed or reference to the Movie made. This instruction connects the two screens in Windows. The timeout parameter lets you establish a maximum time to wait for the Movie to be ready for opening. If you specify no time, then the instruction will wait as long as necessary for the Movie to be captured and ready. If, for example, no trigger ever occurs, then the Open Movie instruction (without a timeout) will never complete and the Sequencer program will hang. |
| Close movie | | All Movies, including SnapShots, must be Closed before you can move on to capturing another Movie. The Movie is saved to a disk file at this time. |
| Open last movie | | There are times it is useful to open the previous Movie for analysis. This instruction does that. |
| Close last movie | | This instruction Closes any previous Movie you opened. |
| Autofocus | | This instruction carries out an Autofocus sweep. You must setup the details of the sweep on the Analysis \| Auto Zoom/Focus tab. The Autofocus routine takes a minute or so to execute. |
| Zoom position | Zoom position (steps): <position> | Move the zoom (magnification) motor to the specified position. Zero steps is the position of lowest magnification. |
| Zoom step by | Step Zoom by: <increment> | Move the zoom motor by the increment from its current position. |
| Focus position | Focus position (steps): <position> | Move the focus motor to the specified position. |
| Focus step by | Focus index: <increment> | Move the focus motor by the increment from its current position. |

| Video and Movie Capture | | |
|---|---|---|
| Instruction | Parameters | Description |
| Home Zoom | | Home zoom motor. Leaves zoom at minimum magnification (largest field of view). |
| Home Focus | | Home focus motor. Leaves focus at farthest focal plane from microscope. |

# Instruction Listing: Movie Analysis

| Movie Analysis | | |
|---|---|---|
| **Instruction** | **Parameters** | **Description** |
| ROI upper left point | Upper left X (pixels): <position><br><br>Upper left Y (pixels): <position> | You may specify a Region of Interest box for analysis. The analysis will ignore anything outside the box. Move the cursor over the image and read the X and Y values in the lower status bar. The origin (0, 0) is in the upper left hand corner of the image. |
| ROI lower right | Lower right X (pixels): <position><br>Lower right Y (pixels): <position> | This is the lower right hand corner for the ROI box. |
| Contact angle baseline | Left X (pixels); <position><br><br>Right X (pixels): <position><br><br>Y (pixels): <position> | You may specify a starting baseline for contact angle measurements. The final line will be an extension of this line. The specified line must be horizontal, hence there is one common Y value. Move the cursor over the image and read the X and Y values in the lower status bar. The origin (0, 0) is in the upper left hand corner of the image.<br><br>If you use the Find Potential Baseline macro, include this instruction in your script but leave the parameters blank to indicate you want to use the macro's data. |
| Contact angle measurement set | Contact angle set number (1..6): <index> | Contact angle measurements can be grouped into sets. Up to 6 sets can be defined. This sets the index (numeric label) of the current set. |
| Index contact angle set | | Increment the contact angle set index (ID number) by one. |
| Do contact angle measurement | | Make a contact angle measurement on the current image in the Movie. |
| Data limits | Lower limit: <value><br><br>Upper limit: <value> | Contact Angle and IFT measurements may be checked by applying limits to their value. Data that fail are not included in the output set. |

| Movie Analysis | | |
|---|---|---|
| Instruction | Parameters | Description |
| Baseline tilt limit | Limit: <degrees> | Contact angle data taken with tilting baselines allowed may be checked by applying a limit to the tilt. A reasonable value is 5 degrees. Angles that fail are not included in the output data set. |
| Left-right angle limit | Limit: <degrees> | Contact angle data taken in non-spherical mode may be checked by applying a limit to the difference between the left and right angles. A reasonable value would be 2 or 3 degrees. Angles that fail are not included in the output data set. |
| Edge sensitivity | Edge sensitivity (0..100%): <sensitivity> | Edge Sensitivity is a user parameter to adjust how the analysis handles imperfect drop profiles. It is normally left at the default 50%. A lower number will make the analysis more tolerant of imperfections or poor image quality. A higher number will make it less tolerant. |
| Simulation data | Mean value data: <value><br><br>Variance: <value> | You can simulate data to generate output files when no instrument is connected. This instruction will generate data in place of a Contact Angle or IFT measurement. The data can be pseudo-random by adding a varying amount according to the variance specified. Zero variance makes the data precisely equal to the mean value specified. You enable the simulation mode by having unchecking the Enable Camera box on the LogOn screen. |
| IFT measurement set | IFT set number (1..6): <index> | IFT measurements can be grouped into sets. Up to 6 sets can be defined. This sets the index number of the current set. |
| Index IFT set | | Increment the IFT set index (ID number) by one. |
| Do IFT measurement | | Make an interfacial tension measurement on the current image in the Movie. |

| Movie Analysis | | |
|---|---|---|
| Instruction | Parameters | Description |
| Spherical mode analysis | On=1, Off=0: <0 or 1> | Turn on or off the Spherical checkbox on the Contact Angle tab. When checked, the analysis will fit a portion of a circle to the liquid-vapor profile. This is the better fit for smaller drops which are not distorted by gravity. |
| Non-spherical mode analysis | On=1, Off=0: <0 or 1> | Turn on or off the Non-spherical checkbox on the Contact Angle tab. When checked, the analysis will fit polynomial curves to the liquid-vapor interface, on to each side. |
| Reflection image type baseline | On=1, Off=0: <0 or 1> | Turn on or off the Reflection Image Present checkbox on the Contact Angle tab. A reflection image is when you can see the sample surface in front of the drop and you can also see the drop's shadow or reflection, like the reflection of the shoreline on the surface of a small lake. |
| Touch-off reflection baseline | On=1, Off=0: <0 or 1> | Turn on or off the Touch-Off Reflection checkbox on the Contact Angle tab. If checked, the algorithm requires a full Movie with the first frames having the pendant drop above the sample. The algorithm determines the baseline location by watching the reflection image of the pendant drop as it comes down to the surface. |
| Horizon image type baseline | On=1, Off=0: <0 or 1> | Turn on or off the Use Horizon checkbox on the Contact Angle tab. A horizon image is when the sample edge and the liquid-solid baseline are coincident, or one on top of the other. |
| Tilting baseline OK | On=1, Off=0: <0 or 1> | Turn on or off the Tilting Baseline OK checkbox on the Contact Angle tab. Most baselines are horizontal and the analysis will assume that unless this box is checked. |

| Movie Analysis | | |
|---|---|---|
| Instruction | Parameters | Description |
| Dispense tip in sessile drop | On=1, Off=0: <0 or 1> | Turn on or off the Dispense Tip in Drop checkbox on the Contact Angle tab. If the tip stays in the sessile drop, this must be checked for the analysis to find the profile correctly. |
| Upside down drop | On=1, Off=0: <0 or 1> | Turn on or off the Upside Down Drop checkbox on the Contact Angle tab. Normally off. |
| Complement measured angle | On=1, Off=0: <0 or 1> | Turn on or off the Complement Angle checkbox on the Contact Angle tab. Typical use of this is with air bubbles inside a liquid. |
| IF tension by pendant drop | On=1, Off=0: <0 or 1> | Turn on or off the IFT by Pendant Drop checkbox on the IFT tab. You must select one IFT method from the list. This is the normal choice. |
| IF tension by sessile drop | On=1, Off=0: <0 or 1> | Turn on or off the IFT by Sessile Drop checkbox on the IFT tab. You must select one IFT method from the list. |
| Dispense tip is tapered | On=1, Off=0: <0 or 1> | Turn on or off the Tapered Tip checkbox on the IFT tab. This helps the analysis find the tip to drop interfacial line. |
| Move to first image in movie | | Move to the first image in the current Movie. This is the normal starting point in a movie. .This is necessary only if you want to go back to the start after moving forward. |
| Move to next image in movie | | Move to the next image in the current Movie. If you are already at the last image, you will simply stay there. |
| Surface energy set | Surface energy set number (1..3): <1, 2, or 3) | Select one of three possible surface energy calculations for the same data. This lets you use more than one model for the same data. |
| Index surface energy set | | Increment the surface energy set number by one. |
| C.A. data to new angles table row | | Transfer the current contact angle data to a new row in the angles table. |
| Angles row to surface energy set | | Transfer the current angles table row to the current energies table row for a subsequent surface energy calculation. |

| Movie Analysis | | |
|---|---|---|
| Instruction | Parameters | Description |
| Do surface energy calculation | Index of surface energy method: <index> | Select the desired method (model) by its index number. You can obtain this from the drop down (pick) list on the Surface Energy tab. Then click the Calc button on the Surface Energy tab. This makes the calculation. |
| Delete image | | Delete the current image from the Movie. You may select any image by using Move First and then Move Next. |
| Do Function on Image | | Execute the previously selected image processing function(s) on the current image in the Movie. |
| Save Image as Reference | On=1, Off=0: <0 or 1> | If 1, save the current image as a reference for a subsequent image subtraction operation. |
| Subtract Reference function | On=1, Off=0: <0 or 1> | Enable the subtract reference image function. |
| Sharpen image function | On=1, Off=0: <0 or 1> | Enable the sharpen image function. This makes edges stand out better. |
| Threshold image function | On=1, Off=0: <0 or 1> | Enable the threshold image (make it a black and white silhouette) function. |
| Smooth image function | On=1, Off=0: <0 or 1> | Enable the smooth image function. This reduces noise. |
| Meniscus image function | On=1, Off=0: <0 or 1><br><br>Direction (0...3): <0, 1, 2, or 3> | Enable the meniscus finder function. The directions are defined on the image processing function tab: 0=down, 1-up, 2=left, 3=right. |
| Aspect ratio function | | Enable the aspect ratio function. |
| Save Live image as reference | | Save the current live image as the reference image for a subsequent subtraction. This is an alternative to an image in the Movie. |

## Instruction Listing: Names, Files, Databases

| Names, Files, Databases | | |
|---|---|---|
| Instruction | Parameters | Description |
| Run ID | | An input box will be presented to operator into which a Run ID text string can be entered. This will be used to identify the set of Movies captured by this execution of the script. |
| Set Database name = RunID | | Set Data Movie database name = RunID + Sequencer program name. This is a database, with mdb extension, that holds multiple Movie data. It holds no images. It has been largely replaced by cumulative XML files which are enabled by a checkbox on the LogOn screen. |
| Save movies as | Save movies as: <file name><br><br>First suffix number: <index number> | Establish a base name and a starting index for Movies to be saved to disk upon their close. |
| Save BMP images | JPG=2, BMP=1, Off=0: <0,1 or 2> | If 1, save bmp images with the same name as the Save Movies As provides. Images are indexed, independently of Movies, with a suffix number. Images are saved when the Contact Angle measurement is made. Each image in the Movie will becomes a separate file. If 2, save as a jpg image. |
| Name in movie | Sample name: <user text> | This sample name is stored in the Comments field of the Solids database entry for this drop. If the Solids database is not used, this sample name has no effect. |
| Make directory | Results directory: <path> | Make a specific folder for data results. This instruction can also be executed by an external robot over the serial line. |
| Make drive path | Results drive path: <path> | Make a specific path for data results. This instruction can also be executed by an external robot over the serial line. |
| Liquids database | Liquids database: <path and filename> | Complete path for an alternative liquids database to the current default database. Normally the default is satisfactory. The liquids database holds surface tension data for standard test liquids. |

| Names, Files, Databases | | |
|---|---|---|
| Instruction | Parameters | Description |
| Solids database | Solids database: <path and filename> | Complete path for an alternative solids database to the current default database. Normally the default is satisfactory. The solids database holds contact angle data for surface energy calculations. |
| Kill SolidsDB | | Delete the current solids database file if it exists. This is used when you want to repeatedly use one solids database name (for contact angle data), but want to start over with no data. |
| Open file Movies as | Open file movies as: <file name><br><br>First suffix number: <number> | Establish a base name and a starting index for subsequent opening of Movies on disk. These Movies may be analyzed by the script. |
| Open file Movie | | Open the next Movie from disk. |
| Close file Movie | | Close the existing open Movie. |

## Instruction Listing: Data Cells

Cells are groupings of contact angles, perhaps of drops of several liquids, that you wish to be associated with a single spatial point on the sample, typically for surface energy analysis.

| Data Cells | | |
|---|---|---|
| Instruction | Parameters | Description |
| Begin cell | | Begin a new cell. A cell is a group of drops to be considered in one surface energy equation. Typically each drop will be a different test liquid. |
| Cell number | Cell number: <index> | The index (name) for this cell. |
| Index cell number | | Increment the current cell index by one. |
| End cell | | Mates with a *Begin cell* instruction to set the range of measurements for this cell. |
| Plot R/X | Set R or X value for graph: <position> | Cell data can be plotted as multi-dimensional point in an external graph. This instruction sets the radial (for cylindrical co-ordinates) or X (for Cartesian coordinates) axis value for this cell in the plot. |
| Plot Q/Y | Set Q or Y value for graph: <position> | Cell data can be plotted as multi-dimensional point in an external graph. This instruction sets the theta (for cylindrical coordinates) or Y (for Cartesian coordinates) axis value for this cell in the plot. |
| Index plot R/X | | Increment the plot R/X value by one. |
| Index plot Q/Y | | Increment the plot Q/Y value by one. |

## Instruction Listing: Chambers

These will be present only if you have a controlled enclosure or a chamber.

| Chambers | | |
|---|---|---|
| Instruction | Parameters | Description |
| Temperature measurement | On=1, Off=0: <0 or 1> | Turn on or off the Temperature Measurements checkbox. This enables making temperature measurements. |
| Humidity measurement | On=1, Off=0: <0 or 1> | Turn on or off the Humidity Measurements checkbox. This enables making humidity measurements. |
| Temperature controller setpoint | Temperature control setpoint (deg): <degrees> | Enter the temperature you wish for the enclosure. Must be above the ambient. |
| Temperature control by heater | On=1, Off=0: <0 or 1> | Turn on or off the Enable Controller checkbox. This enables power to the enclosure heater. |
| Chamber temp setpoint | Temperature control setpoint (deg): <degrees> | FTA1000: Enter the setpoint you wish for the main chamber temperature. You can read the actual temperature via User Variable 120. |

# Instruction Listing: User Variables and Macros

| User Variables and Macros | | |
|---|---|---|
| Instruction | Parameters | Description |
| Set User Variable value | User Variable number (1...200): <index><br><br>Assign this value to User Variable: <value> | User Variables are independent variables that may be manipulated and tested by the script (i.e., the "user"). The name of the variable is a number specified by index. You may assign any numeric value to the variable. User Variables are enumerated in a table at the end of this section. |
| Increment user variable | User Variable number (1...200): <index><br><br>Increment : <value> | User Variables may be incremented (changed) by any amount. |
| User Variable if then | User Variable number (1...200): <index><br><br>If User Variable > : <test value><br><br>Go to line number: <line number> | Test a User Variable and branch if the test is satisfied. The User Variable must be greater than (more positive than) the test value. If this is true, branch to the line number. |
| Get Live video black level | | Get the black level (blackest black) from the current Live image and store in Last Data. It will typically be a value between 0 and 100. Last Data is a special User Variable that can be tested with if/then statements. Last Data can be store many values, so it should be tested immediately after being updated.<br><br>Use the I*ncrement Live video brightness* instruction in *Video and Movie Capture* to change the video black level. |
| Get Live video white level | | Get the white level (whitest white) from the current Live image and store in Last Data. It will typically be a value between 150 and 255.<br><br>Use the I*ncrement Live video contrast* instruction in *Video and Movie Capture* to change the video white level. |

| User Variables and Macros | | |
|---|---|---|
| Instruction | Parameters | Description |
| Get Live video sharpness | | Get sharpness from current Live image. This will be a value between 0 and 255, typically in the 50 to 75 range. Sharpness is primarily a function of focus, but overall black to white level range affects it also. |
| Last Data if then | If Last Data > : <test value><br><br>Go to line number: <line number> | Test current value of Last Data and, if test is satisfied, branch to line number. |
| Position tip in image | Tip Z below image top (pixels): <value> | Run macro to position tip bottom in image. Tip does not have to be visible to start. Pixels are measured down from top edge. There are typically about 480 vertical pixels in image, so 50 pixels would be about 10%. |
| Position stage in image | Stage Z above image bottom (pixels): <value> | Run macro to position stagein image. Stage does not have to be visible to start. Final position is measured in pixels up from image bottom. A typical value might be 50 to 100, or about 10 to 20% of image height. |
| Dispense volume | Pendant volume (ul): <value> | Run macro to hang a pendant drop of specified volume on tip. Pump will either dispense of aspirate to achieve target. A tolerance is allowed so pump does not oscillate back and forth trying to correct a very small amount. |
| Dispense to shape (beta) | Pendant shape (beta): <value> | Run macro to dispense a pendant drop so that a specific shape is reached. Beta refers to a Laplace-Young analysis parameter. A beta of 0 is a perfect circle. A typical pendant drop will have a beta of -0.20. |
| Find potential baseline | | Run macro to lower tip close to surface to ascertain surface location using image analysis. Surface location is stored as a potential baseline location, assuming drop is deposited below tip. You can use this proposed baseline with the *Contact angle baseline* instruction and no values given (see this instruction in *Movie Analysis*). |

| User Variables and Macros | | |
|---|---|---|
| Instruction | Parameters | Description |
| Touch off drop | Extra down motion (mm): <value> | Run macro to deposit a hanging drop on the surface. Tip will be lowered until pendant drop touches surface. Extra distance is the further motion allowed to ensure drop attaches to surface. |
| Move Tip Z to target | Bias above image target (mm): <value> | Move the tip until its bottom is at the surface + any bias value specified. This is useful for aspirating liquid back off the surface. You must have determined the "target" value earlier using *Find potential baseline*. |
| Center tip in image | | Run macro to center the tip in the image. Tip must be visible. |
| Capture reference image | | This is used when the Live image is present. It will capture and hold the current view for later image background subtraction within a macro. To be useful, the Live image will typically not show the tip and drop – instead it will show background objects or artifacts that you wish to have removed from consideration by the subsequent image processing. |
| Macros use ref image | On=1, Off=0: <0 or 1> | When "1" and when a reference image is available, the reference image will be subtracted from the current image before use by the macro. |

## Special Note on Macros

With the exception of *Position tip in image* and *Position stage in image*, macros require the tip to be present in the image when the macro starts. If there is no tip, the macro will end immediately and report an error in Last Data. This is necessary because these other macros need to move the tip and they would not know where to start with the tip visible.

## Last Data Assignments

The following values will be assigned to the User Variable *Last Data*.
There is only <u>one</u> location for *Last Data*, so it will contain the most recent assignment.
Example: if the last instruction was a macro, *Last Data* will contain the macro's return code, but if the last instruction was a contact angle measurement, then *Last Data* will be an angle.

Error return code from any macro. 0=success. Other values encode possible failures. Non-zero codes are normally output to the Sequencer event log. Some common codes:
0 = success
1 = no image
2 = stopped by user
3 = general image error, typically low contrast or interference
4 = no tip could be found in image
5 = spare
6 = drop fell off tip
7 = too many pump moves for small drop (<2ul)
8 = too many pump moves for large drop (>2ul)
9 = too many tip Z moves
10 = too many stage Z moves

Contact angle (last angle measured)

Interfacial tension (last IFT measured)

Black level of Live image (from contrast measurement)

White level of Live image (from contrast measurement)

Sharpness of Live image (from contrast measurement)

## How to Test Last Data and User Variables

You can test and branch in your script based on the current value of *Last Data* or any *User Variable*.

Use *Last data if then* to test *Last Data*. If *Last D*ata is greater than the comparison value you furnish, the instruction will branch (go to) the line number you specify. If the test is not true (*Last Data* is equal or smaller than your test value), the script will continue on to the next instruction.

Use *User variable if then* to similarly test the current value of a user variable you specify against your test value. If the test is satisfied, the script will branch to the line number you specify.

You can build a compound test to find out if the test variable (*Last Data* or your chosen user variable) is within some range. You will need two sequential if then tests. As an example, say you want to find out if *Last Data* is between 3 and 5. Assume *Last Data* is an integer. The script segment will look like

```
1000  Last data if then    5, 1100     if greater than 5, goto 1100 and continue script
1010  Last data if then    2, 1050     if greater than 2, as 3 would be, you are between 3 and 5
1020  Go to line           1200        got here because 2 or less
1050   your instruction                case of between 3 and 5
1100   your instruction                case of greater than 5
1200   your instruction                case of less than 3
```

| User Variables<br><br>User Variables are identified by their Index, a numeric name.<br>You must explicitly execute some instruction to assign values to User Variables. | |
|---|---|
| Index | Description |
| 1...100 | General purpose numeric variables assigned by user |
| 101 | Black level in image |
| 102 | White level in image |
| 103 | Sharpness (similar to focus) value of image |
| 104 | Contact angle |
| 105 | Left side contact angle |
| 106 | Right side contact angle |
| 107 | Sessile drop volume |
| 108 | Sessile base width (diameter) |
| 109 | Sessile drop height |
| 110 | Drop baseline tilt angle |
| 111 | Instrument tilt angle |
| 112 | Tip width (diameter) |
| 113 | Interfacial tension |
| 114 | Pendant drop volume |
| 115 | Pendant drop surface area |
| 116 | Pendant drop major diameter |
| 117 | Laplace-Young radius at apex |
| 118 | Laplace-Young beta |
| 119 | Laplace-Young RMS drop profile fit error |
| 120 | Measured temperature in chamber |

## Frequently Asked Questions

1.If you do not see the Sequencer tab in Analysis on the Video window, make sure the hardware you selected on the LogOn screen supports the Sequencer. The FTA1000 and FTA2000 do support it.

2.If the script “crashes”, use Single Step mode to determine when some strangeness first appears. Inspect that carefully. The fault that starts here may not be fatal until several steps later.

3.See the FTA website for examples of Sequencer programs. One link is

http://www.firsttenangstroms.com/pdfdocs/SequencerExamples.pdf

4.See the FTA website for updated FAQ's discussing problems and solutions. For example:

http://www.firsttenangstroms.com/faq/DebuggingSequencerPrograms.html

File: SequencerDescription.doc