Simplified Refeyn TwoMP protocol

STOP This protocol is for qualified users operating TwoMP in IBC 402 only. Dr. Jao accepts no responsibility for actions taken as a result of using this protocol. Reading the manufacturer's User's Manual is highly recommended.

I. Warm UP Instrument

A. Open the lid on the TwoMP. Ensure that the objective and sample carrier are clean.

B. Turn ON the switch that is located at the back of the electronics unit **at least one hour** before starting your experiments.

C. Turn ON the "PC-1" and open "AcquireMP" 

(software should be on during warm up)

D. Turn ON the vibration-isolation table by pressing the buttons "Power" and "Isolation".

E. Click "Open Project Folder", set Desktop:/Temporary Data as your project folder.

II. Preparing and Mounting Coverslips

A. Clean both sides of coverslip by "ddH2O → isopropanol → ddH2O → isopropanol → ddH2O" and dry it with compressed air or N2. Pre-cleaned coverslip can be stored in coverslip box for a short time.

B. Using soft-tipped tweezers, place a gasket in the middle of the aliment tool, and then place the pre-cleaned coverslip slide on top.

C. Press gently on the coverslip between the wells with soft-tipped tweezers.

D. Open the lid on the TwoMP unit and place a small drop of immersion oil on the objective lens.

E. Flip and mount the prepared coverslip (with the gasket facing the top), and place two magnets diagonally at each end of the coverslip.

F. Select a well (on the gasket) you want to start your experiment on. Use lateral control at speed 50x to move the stage so that the red laser beam is focused on the middle of the selected well. Once this is done, you may now set the well position on the software. This will allow for automatic stage positioning to another well when you run your next sample.

III. Data Collection

Before you start running your samples, please ensure that your buffer, samples and calibration standards are ready and at **room temperature.** Concentration calculator in AquireMP is helpful when you need to dilute your samples. The first sample you measured should be a calibration standard, appropriate to your experiment type (measure Calibrants in your sample buffer).

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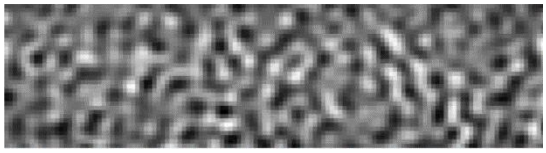
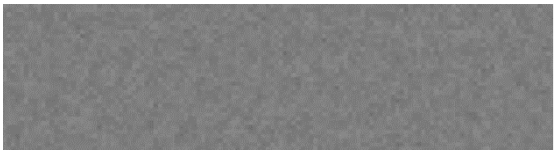
A. Open the lid and add 18 µl of buffer to the selected well.

B. Lower the lid of TwoMP and click “Droplet-dilution” to find focus.

C. Switch between native and ratiometric views to assess image quality.

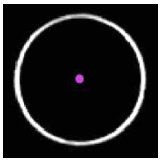


Native image of clean slide and buffer



ratiometric image of clean slide and buffer

D. Select “Manual Focus” and examine the autofocus ring. It should be a thin, clean outline with no interruptions.



autofocus ring

E. Click “enable analysis preview” to watch data collection in real time.

F. Inject 2 µl of your diluted sample (100 nM-200 nM) into the buffer droplet used to find focus. Mix thoroughly by pipetting up and down.

G. Close the lid. Press “Record” button. The software will now record data for 1min. Do not touch or shake the table and the instrument during recording.

H. Click “Next well” to automatically move to next well.

I. Repeat finding focus and collecting sample data.

IV. Data Analysis

AquireMP and DiscoverMP 

cannot be opened at the same time. Analysis your data on “PC-2”.

A. Turn on “PC-2” and ensure “Warmhole switch”,  which can transfer files between PCs, is being active.

B. Copy your files from “PC-1” and paste to your folder in “PC-2”.

C. Open the DiscoverMP software.

D. Upload your data files by click “+” button. Select all files you would like to analyze.

E. Adjust the contrast limits on a particular region of the data.

1. Set the upper contrast to 0.
2. Shorten the lower contrast limit to hide rare aggregates.

F. Take a look at info to make sure the number of counts is within the optical range for your sample (1500-3000 for Calibration standards).

G. Create a calibration curve.

1. Choose your mass calibration standard file.
2. Clear the fitting curve which software automatically done on the histogram.
3. Double click on each population to create a Gaussian fit (or click and drag over a peak).

4. In Mass Calibration Section (bottom left), click “Create”.
5. Select the Calibration standard file(s) and enter the mass of your calibrants.
6. Saving the calibration file and it will be added to Mass Calibrations Section.

H. Apply the calibration curve to other data files by double click the file of interest and then double click the calibration curve file in Mass Calibrations Section.

I. Create Figures in DiscoverMP.
1. Click the “Figures” tab and select “Add Figure”
2. Choose 2D histogram, 3D histogram or Vertical histogram series.
3. Drag the measurement file(s) and drop it in the figure set-up box.
4. Deselect any aspects of the data you don’t wish to include.
5. Use the formatting option to change the graph title, axes, units and style.

J. Save the workspace to preserve your analysis. [File -> Save Workspace]

K. Export your figure(s).

V. Shutdown

A. Close the AquireMP by clicking [File -> Quit]. The stage will return to the center position.

B. Turn off the electronics unit and vibration isolation bench (Press “Isolation” and then “Power”).

C. Clean the objective with Lens Cleaning Tissue **(Not Kimwipe!)** and isopropanol when data collection is complete.

D. Close the lid to avoid dust accumulation.

E. Delete all your files in PC-1 and shutdown PC-1 after transfer them into PC-2

F. Shut down PC-2 when you finish your analysis.