R&D SYSTEMS
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Luminex® FLEXMAP 3D® Quick Guide

(For full details, reference the software manual)

1. **Turn on instrument – the power switch is on the right side of the instrument**
   a. Warm up takes 30 minutes
   b. System will shut down the lasers after 4 hours of inactivity
2. **Turn on computer**
3. **Open xPonent® software**
4. **Adjust probe height using the Probe Height Adjustment Tool. If the adjustment tool is not available, use a 96-well plate.** (This technique may cause the probe to punch through the bottom of the plate. It may take multiple attempts to adjust properly.)
   a. Go to Maintenance Tab > Probe & Heater
   b. For 96-well plate: use well F8 (2.1 mm) on the Probe Height Adjustment Tool or use 2 discs in 96-well plate
   c. For 384-well plate: use well E1 (1.4 mm) on the Probe Height Adjustment Tool
   d. Do not use any discs in Calibration/Verification strip
5. **Run daily instrument startup routine**
   a. Go to Maintenance Tab > Cmds & Routines > Daily Instrument Startup (dropdown menu)
   b. Fill reservoirs with appropriate reagents
      i. diH_2O
      ii. 70% EtOH

   NOTE: Startup routine takes <10 minutes

6. **If needed, run calibration and verification**
    a. Go to Maintenance > Auto Maint > System Initialization
    b. Warm reagents to room temperature
    c. Vortex vigorously before dropping into strip wells
    d. 5 drops per reagent

    NOTE: Full calibration/verification takes approximately 20 minutes

7. **Create protocol**
    a. SETTINGS / STEP 1:
        i. Follow instrument setting instructions in assay protocol for sample volume, bead type, and gates
        ii. Volume: This is how much of the sample/well will be aspirated
        iii. Analysis Type: Quantitative Protocol
        iv. Enter number of standards (do not include the blank)
        v. Unless required in the PDF result file, leave controls as zero
    b. ANALYTES / STEP 2:
        i. Select bead regions
        ii. Name analytes
        iii. Count = 50
        iv. Units = pg/mL
        v. Click Apply All
    c. PLATE LAYOUT / STEP 3:
        i. Change Replicate Count
        ii. Assign Background and Standards - this can be changed in the batch

    NOTE: All users can share a protocol if running the exact same analytes and bead regions

8. **Create standard curve for protocol**
    a. Go to Protocols Tab > Stds & Ctrls > Create New Std/Ctrl Lots
    b. Choose protocol from dropdown menu
    c. Enter highest concentration
        i. This information is on the Certificate of Analysis or Standard Value Card
    d. Enter dilution as a whole number
        i. Not sample dilution! Dilution factor of the standard curve.
    e. Highlight and apply all using arrows

9. **Create batch**
    a. Select Protocol
    b. Associate Std Curve
    c. Assign Unknown Sample Wells
    d. Program any options specific to your batch
        i. Add multiple plates
    e. Name Samples (optional)
        i. Can Import List – comma delineated list of Sample ID and Dilution Factor
    f. Add Sample Dilution Factor
        i. If you add sample names after sample dilution factor, you will need to add sample dilution factor again
10. **Results**
    a. Go to Results > Saved Batches > Select Batch to Analyze
    b. Check box for all analytes
    c. Generate report
    d. SAVE AS .PDF: Save All Button
    e. SAVE AS EXCEL: Save Button for each different analyte
11. **System shutdown**
    a. Remove Assay Plate
    b. Go to Maintenance > Auto Maint > Shutdown
    c. Fill off-plate reagent reservoir block with appropriate reagents and run Shutdown
    d. Remove off-plate reagent reservoir block, empty it and replace it in the instrument
    e. Exit xPonent software and shut down the instrument and computer

    NOTE: System shutdown takes approximately 5 minutes

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