

Integration of the LEADseeker Multimodality Imaging System with automated plate loading and incubation using Overlord Instrument software

Key words: LEADseeker • imaging • automation • integration

The LEADseeker™ Multimodality Imaging System is a CCD-based optical imaging instrument that efficiently detects and quantitates light from a range of radiometric, luminescence, and fluorescence assays. Throughput of over 100 plates per hour is possible due to the combination of format-free imaging (fluorescence assays can be read in as little as 10 s) and the instrument's precise and rapid plate delivery mechanism. The instrument can be integrated with most automation devices via its open architecture software.

Here, we describe the integration of a LEADseeker instrument with a Kendro Heraeus™ Cytomat™ Incubator and a Zymark™ Twister™ II Robot, using Overlord™ Instrument software to control all the instruments.

Materials

Products used

LEADseeker Multimodality Imaging System 18-1140-71

[¹⁴C]Uniform 384-Well Reference Plate,

0.148 MBq, 4 µCi

CFQ11387

Other material required

Overlord Instrument software (Process Analysis and Automation Ltd.)

Zymark Twister II Robot (Caliper Life Sciences Ltd.)

Cytomat 6001 Incubator (Kendro Laboratory Products PLC.)

Method

Overlord communication settings

Overlord Instrument software was installed on a PC and used to control a Zymark Twister II Robot, Cytomat 6001 Incubator, and the LEADseeker instrument. Connections to the robot and the incubator were made by conventional 9-pin D connector RS-232 serial cables. The connection between the Overlord PC and the LEADseeker instrument PC was by a null modem cable* (Fig 1).

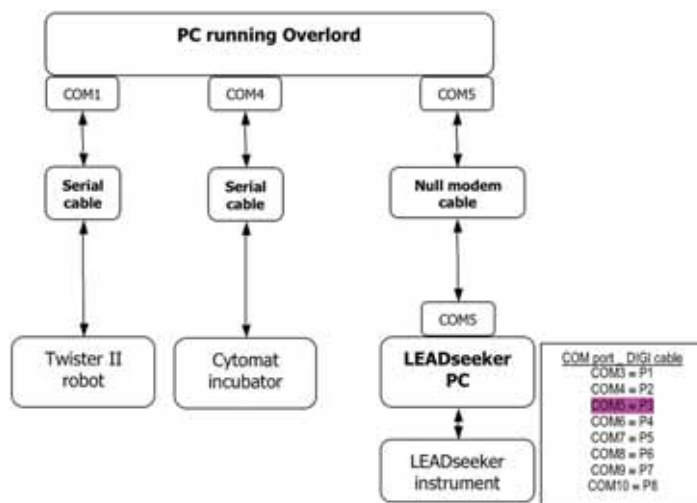


Fig 1. Schematic representation of data communication links between components where Overlord and LEADseeker instrument software are installed on separate PCs. The Overlord and LEADseeker PCs were connected by a null modem cable from Overlord PC COM5 to LEADseeker PC COM5 = P3.

* In situations where the LEADseeker instrument PC is required to drive the robot and any other equipment, a null modem cable is required to connect COM ports 5 and 6 together on the LEADseeker instrument PC. This is because the LEADseeker instrument can only accept instructions from an outside source via a serial cable, even though Overlord Instrument software is resident on the same PC.



The LEADseeker instrument PC is supplied with a DIGI™ multiport serial card to allow connection of up to eight serial devices, although the LEADseeker instrument is controlled through COM1 and COM2 so these ports cannot be used for other equipment. The cables from the DIGI card are labeled P1 to P8 and their COM port equivalents are shown in Figure 1 and Figure 2.

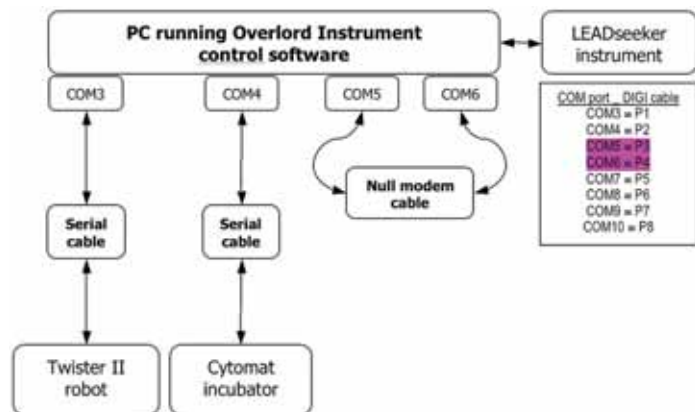


Fig 2. Schematic representation of data communication links between components where Overlord and LEADseeker software are installed on the same PC. Within the Overlord Instrument software, LEADseeker is connected to COM6[†] = P4.

Overlord program

Overlord Instrument software has a simple drag and drop interface for setting up communication between instruments. The software also permits a range of programming elements such as numeric and string variables, conditional statements (e.g., IF... THEN, ELSE), and control loops (e.g., FOR...NEXT). A simple program typically used in this work is summarized in Figure 3.

The purpose of the Overlord program illustrated in Figure 3 was to take a number of microplates from storage in the Cytomat Incubator, use the Zymark Twister II Robot to transfer them to the LEADseeker instrument for data acquisition, and then return the microplates to the Cytomat Incubator for storage or further incubation. The program allows the user to enter the number of plates to be processed and the number of the plate with which the run starts.

Following data acquisition, the numeric file associated with the processed LEADseeker image was stored on the Overlord computer by transfer across the serial connection. The filename assigned for each file was generated by string concatenation shown in Figure 3.

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MANUAL DATA INPUT: Store number into #A      "Enter first Cytomat position"
MANUAL DATA INPUT: Store number into #B      "Enter number of plates to run"
SET VARIABLE: #C = #A + #B - 1

Amersham LEADseeker on COM5: Select Method

FOR #A = #A TO #C STEP 1
    SET VARIABLE: $AB = "c:\ls\" + STR$(#A) + ".txt"
    Kendro Cytomat on COM4: Get Plate from position #A
    Zymark Twister II on COM1: Run procedure: GetPlateFromCytomat
    Zymark Twister II on COM1: Run procedure: PutPlateOnLEADseeker
    Amersham LEADseeker on COM5: Load plate
    Amersham LEADseeker on COM5: Read Plate and save results to $AB,
        wait until finished
    Amersham LEADseeker on COM5: Unload plate
    Zymark Twister II on COM1: Run procedure: GetPlateFromLEADseeker
    Zymark Twister II on COM1: Run procedure: PutPlateOnCytomat
    Kendro Cytomat on COM4: Put Plate into position #A
NEXT refers to FOR...LOOP above

END of procedure

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Fig 3. Typical Overlord program used to supply microplates to the LEADseeker instrument for imaging.

[†] The LEADseeker instrument usually expects to receive input signals through COM5; this is configured in the MS Windows registry and can be confirmed using REGEDIT.EXE.

Zymark Twister II Robot settings

The Zymark Twister II Robot was taught its positions and procedures using Overlord Instrument software. Robot procedures were given self-explanatory names such as **GetPlateFromCytomat**, **PutPlateOnLEADseeker** etc. Full descriptions of the process of robot position teaching and procedure development are beyond the scope of this application note. Further information can be obtained from the instrument suppliers (Caliper Technologies Inc) or from Process and Automation Analysis Ltd.

Kendro Heraeus Cytomat 6001 Incubator settings

A Cytomat 6001 Incubator was set to run at 30 °C. No changes were made to any other settings. The overall size of the Twister II grippers and the robot head is such that it cannot access the plate transfer station of the incubator. To facilitate entry of the robot to this position, the casing over the plate entry door was modified (raised) to give the necessary clearance. Similar modifications should be confirmed first with the instrument supplier.

LEADseeker instrument settings

The LEADseeker instrument was used in radiometric imaging mode to test reproducibility of a range of 384-well ¹⁴C Uniform 384-Well Reference Plates. For convenience, the exposure time on the system was set to 10 s. During acquisition, the LEADseeker computer displays the communication dialogue between the LEADseeker instrument and the Overlord computer in the **Connect** window (Fig 4).

Results

Typical data from reading a group of ¹⁴C Uniform 384-Well Reference Plates (23 separate plates) is shown in Figure 5. The data shows that each plate was successfully loaded from the incubator into the LEADseeker instrument via the Zymark Twister II Robot and subsequently read using the protocol requested via the Overlord Instrument software. The exported data was then used to plot the mean signal ± 3 SD (n = 384) from each 384-well plate.

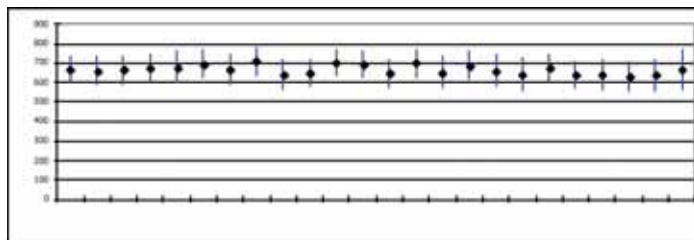


Fig 5. Variation in the LEADseeker instrument output of 23 ¹⁴C Uniform 384-Well Reference Plates. The black diamond represents the mean IOD value for each plate and the top and bottom of the blue line shows the mean ± 3 SD of the plate IOD value.

Conclusion

The LEADseeker Multimodality Imaging System can be successfully integrated with the Zymark Twister II Robot and the Kendro Heraeus Cytomat Incubator and controlled using Overlord Instrument software.

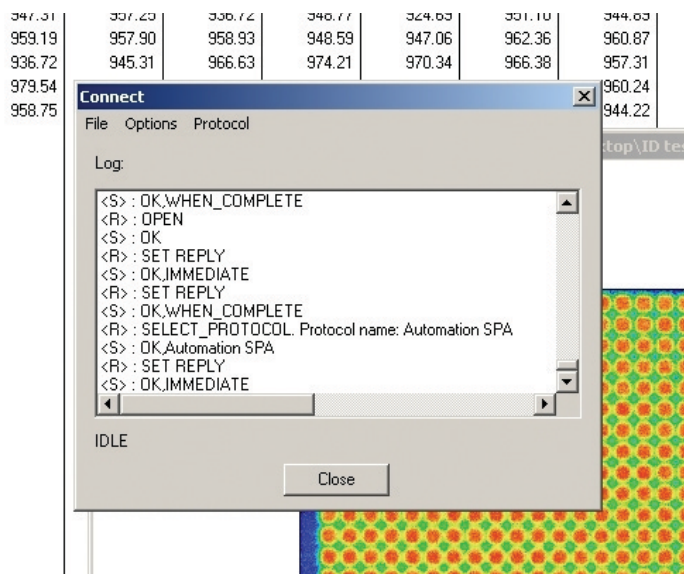


Fig 4. Example of LEADseeker automation screen output. During operation, commands sent by the LEADseeker instrument and received from Overlord control software are displayed in the **Connect** window.

Asia Pacific	Tel: +852 2811 8693	Fax: +852 2811 5251
Australasia	Tel: + 61 2 9899 0999	Fax: +61 2 9899 7511
Austria	Tel: 01/57606-1619	Fax: 01/57606-1627
Belgium	Tel: 0800 73 888	Fax: 03 272 1637
Canada	Tel: 1 800 463 5800	Fax: 1 800 567 1008
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Japan	Tel: +81 3 5331 9336	Fax: +81 3 5331 9370

Latin America	Tel: +55 11 3933 7300	Fax: + 55 11 3933 7304
Middle East & Africa	Tel: +30 210 9600 687	Fax: +30 210 9600 693
Netherlands	Tel: 0165 580 410	Fax: 0165 580 401
Norway	Tel: 815 65 555	Fax: 815 65 666
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UK	Tel: 0800 616928	Fax: 0800 616927
USA	Tel: +1 800 526 3593	Fax: +1 877 295 8102

www.amershambiosciences.com

GE Healthcare Ltd

Amersham Place
Little Chalfont
Buckinghamshire
HP7 9NA
UK

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