

MetaXpress External Control Protocol rev. C

1. Overview

1.1. General Purpose

This specification describes the communication protocol between the ImageXpress® System controlled by the MetaXpress® (MX) software and a central plate facility (CPF). The CPF consists of scheduling software controlling a robotic plate handler. The CPF may reside on the same computer as MX, or on a separate computer, but commands must be passed to MX using Serial communication. This communication protocol describes how MX indicates that it is ready to accept a plate; how the CPF indicates that it has loaded a plate; how the CPF indicates that the plate has a barcode and should be acquired using a specific protocol file; how MX reports back error and status information; and how MX reports that it has completed acquisition of the plate. Multiple MX instances may be communicating with the CPF, but the MX instances are not aware of one another.

1.2. Overview of use

Specific commands and codes are in tables in Appendices 1-5. Examples of use are presented in Appendix 6.

1.3. Products:

- ImageXpress System
- MetaXpress software
- External Control module. Consult the ImageXpress user guide for instructions on enabling the External Control module.

1.4 Abbreviations:

MX	MetaXpress Software. Software that controls the ImageXpress instrument
CPF	Central Plate Facility. The CPF consist of scheduling software and robotic plate handler
ID	System ID. System identification number as indicated on the MetaXpress software license key.
MX system	ImageXpress system with the MetaXpress software
CRLF	Carriage Return and Linefeed pair

2. Protocol:

2.1. General Format

The general format of commands is as follows:

ID, COMMAND [, DATA, DATA, ...] Terminator
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- Commands must be typed in ALL CAPS.

- The command is terminated by the command terminator, see section 2.2.
- ID is the identifier of the device sending the command. This is defined in the ID section (2.3). The ID must be present.
- Command is the command to be done, or an indicator of what the data is. This is an ASCII word, defined in the Command section (2.4). The Command must be present.
- Data is zero or more pieces of data associated with the command. This is an ASCII string, defined in the Data section (2.5). This is optional and may be absent.
- The ID, Command, and zero or more Data fields are separated by a comma character. Do not put a space after the comma.

2.2. Command Terminator

All commands are terminated by a Carriage Return and Linefeed pair (CRLF). This is ASCII 13 followed by ASCII 10.

2.3. ID

The ID is the identifier of the device sending the command. It will be either CPF for the Central Plate Facility, or the system ID number for the MX system, e.g. 20111.

***NOTE:** System IDs are typically 5 digits long, starting with 2.*

2.4. Command

The Command is a single word in ASCII that represents the purpose of this communication. The commands are described in Appendices 1 and 2 of this document.

2.5. Data

Zero or more comma-separated strings represent the Data. A Data string is a standard ASCII string which may contain any characters in the range of ASCII 32 to 126, except it may not contain the comma character (ASCII 44). This is because commas are used to separate the ID, Command, and Data fields. Data strings are defined in Appendices 1, 2, and 5 of this document.

2.5.1. Barcodes

Alphanumeric characters, spaces, and hyphens are acceptable in the barcode. Other characters may cause an error in MX.

3. Notes

3.1. Protocol Files

Protocol files (.HTS files) are text files whose format is defined by the MetaXpress software. The protocol file that is to be used by the MetaXpress software should be specified by the CPF. All lines in the file will be loaded into the MetaXpress software. Some lines within the protocol file may be removed for properly dealing with a multi-station system. When a line is removed from the protocol file, the last known value for the variable will be used. For example, the image storage location (stImageLocLabel) may be machine-dependent and can possibly be left

out of the file. Other items such as plate name ("stPlateType") are machine-dependent and plate-dependent. In this case, the plate name should be set the same for all machines so that the protocol can be safely sent to any machine.

Note: In earlier software versions, these files were called State files or Settings files.

3.2. Operating Modes

The system has 4 operating modes. These are "offline", "online", "running" and "paused". Certain commands are available only in certain modes. The modes are defined as:

3.2.1. Offline

MX is in interactive (normal) mode.

3.2.2. Online

MX is being controlled by the CPF, but an experiment is not running.

3.2.3. Running

MX is being controlled by the CPF, an experiment is running, and it is not paused.

3.2.4. Paused

MX is being controlled by the CPF, an experiment is running, and that experiment is paused.

3.3. HTSResult Variable

Custom errors can be generated using a MetaXpress journal to assign a value to the MetaXpress variable HTSResult. Any non-zero value assigned by a journal is considered an error. Use a negative value, as MX reserves all positive error values. The non-zero error value will be returned back to the CPF. If no journal is used, the variable will default to a value of zero, which will return as success. For example, a protocol file can specify a custom journal to run after the plate is loaded and the initial autofocus (Find Sample) is performed. The journal can analyze an image for cell count or intensity, and determine success based on the results of that measurement.

3.4. Commands and Responses

Appendix 1 describes commands that the CPF can send to MX. Each command may only be valid in certain operating modes. Appendix 2 describes responses that MX can send to the CPF. Appendices 3, 4, and 5 describe codes used in the various commands or responses. Appendix 6 contains some examples of communication.

Appendix 1 – Commands and Data sent by CPF

Command	Mode	Data	Description
ONLINE	Offline	None	ONLINE is sent to MX by the CPF when the CPF wants MX to go online (be able to receive commands). The only commands MX will respond to when it is not online are the ONLINE, EXIT, and STATUS commands. MX will return either the OK or ERROR command. Errors are defined in Appendix 3.
OFFLINE	Online	None	CPF can take MX offline by sending the OFFLINE command. MX will return either OK or ERROR.
EXIT	* All *	None	CPF can cause MX to exit by sending the EXIT command. MX will return either OK or ERROR.
STATUS	* All *	None	CPF sends the STATUS command to find out the state of MX. MX will respond to a status command as indicated in Appendix 4.
GOTO	Online	Position code, see Appendix 5.	CPF can move the stage on MX by using the GOTO command. The Data field contains a position code, which will cause MX to move to a pre-defined position. Once the position is reached, MX will send a response to the CPF. Appendix 5 details the position and response codes. If MX cannot perform the GOTO it will return ERROR.
RUN	Online	Barcode, complete path to protocol file ¹	RUN is sent by the CPF to MX when a plate has been loaded and needs to be acquired. One or two Data parameters are sent. The first one is the barcode of the plate. MX will use that to identify this plate in subsequent commands. The second Data value is the complete path to the protocol file that will be loaded and used to control image acquisition. If the second data value is missing, the current state will be used. MX will return OK once it validates and successfully loads the protocol file. From that point, the CPF needs to poll with the STATUS command to make sure the run of the plate is successful and to determine when the plate has completed. MX will return ERROR if it cannot execute the RUN command or if the protocol file is invalid.
PAUSE	Running	None	CPF can cause MX to pause a running experiment by sending the PAUSE command. MX will return either OK or ERROR. When in the paused state, the only valid commands CPF can send MX are RESUME, CANCEL, EXIT, and STATUS.
RESUME	Paused	None	CPF can cause MX to resume from a paused state by sending the RESUME command. MX will return either OK or ERROR.
CANCEL	Running Paused	None	CPF can cause MX to cancel the run of a plate by sending the CANCEL command. MX will return either OK or

¹ See discussion of Protocol Files in section 3, Notes, above.

			ERROR.
PLAYJOURNAL	Online	Complete path of journal file to be run	CPF can cause MX to run a journal by sending the PLAYJOURNAL command. MX will return either OK or ERROR. ERROR will be returned if the journal file can not be located or the journal was cancelled for any reason. The barcode can optionally be added. Format is: CPF,PLAYJOURNAL,BARCODESTRING,JOURNALFILE or CPF,PLAYJOURNAL,JOURNALFILE
MARKPOSITION	Online	Position code, see Appendix 5.	CPF can mark the current stage position for later use by a GOTO command. The Data field contains a position code. Valid positions are LOAD, and UNLOAD. MX will record the current position of the stage as the location to be used for the position specified. MX will send a response to the CPF. MX will return either OK or ERROR.
VERSION	Offline, Online	None	Returns version #. VERSION 0 [anything prior to MM 6.1.6] VERSION will return error of an unknown command: 42,ERROR,0,10 VERSION 1.1 Added with MM 6.1.6. Adds option barcode to PLAYJOURNAL function.

Appendix 2 – Responses returned by MX

Command	Data	Description
OK	Barcode	OK is sent as a response to acknowledge a command has successfully occurred. MX will send OK when the ONLINE, OFFLINE, EXIT, GOTO, RUN, PAUSE, or RESUME commands are successfully executed. If a barcode is known, that will be returned in the data field. If no barcode is known, the data field will have a "0" for the barcode.
ERROR	Barcode, Error Code	ERROR is sent as a response when a command has failed, or as a response to STATUS. MX will send ERROR when the ONLINE, OFFLINE, EXIT, GOTO, RUN, PAUSE, RESUME, or CANCEL commands have failed. If a barcode is known, that will be returned as the first data field. If no barcode is known, the first data field will have a "0" for the barcode. The second data field will have an error code as defined in Appendix 3.
OFFLINE	None	Response to STATUS. MX is offline.
EXITING	None	Response to STATUS. MX is exiting.
PAUSED	Barcode,R,C,S	Response to STATUS. An experiment being run (by the RUN command) is paused. Barcode is the barcode of the plate being run. R,C,S indicate the row, column, and site where the experiment is paused at. Row is a letter and Column is a number. Site will be 1 if multiple sites are not enabled in the current protocol file.
RUNNING	Barcode,R,C,S	Response to STATUS. An experiment is being run (by the RUN command). Barcode is the barcode of the plate being run. R,C,S indicate the row, column, and site where the experiment is currently at. Row is a letter and Column is a number. Site will be 1 if multiple sites are not enabled in the current protocol file. Note: Row, Column, and Site will all be 0 during the Find Sample phase, because a well has not yet been acquired. After that, the status will return the last well / site that was moved to.
DONE	Barcode,R,C,S	Response to STATUS. An experiment that was run (by the RUN command) has completed successfully. Barcode is the barcode of the completed experiment plate. R,C,S indicate the row, column, and site where the stage is now at. Row is a letter and Column is a number. Site will be 1 if multiple sites are not enabled in the current protocol file. Note: The next OFFLINE, EXIT, GOTO, or RUN command will reset the DONE state.
READY	Position code, from Appendix 5, or UNKNOWN.	Response to STATUS. MX is online and is not running an experiment – it is ready to act on commands. The data field will contain a position code from Appendix 5 which indicates where the stage is. If the position is not known, then UNKNOWN is returned in the data field.

Appendix 3 – Error Codes

Error Code	Description
Negative value	A user-defined error code, stored in the HTSError variable from a journal
0	Not Defined
1	MX is in Offline mode, command cannot be completed
2	MX is in Online mode, command cannot be completed
3	MX is in Running mode, command cannot be completed
4	MX is in Paused mode, command cannot be completed
5	MX is busy, command cannot be completed
6	Timeout error occurred waiting for response from MX
7	Error occurred moving to desired stage position
8	Protocol file is invalid
9	Invalid parameter specified
10	Unexpected Command (sent to MX from the CPF)
11	Error running a journal (if a journal fails to run properly)
12	Error connecting to the Database
13	Append Time Point plate validation failed (MetaXpress version 6.5 and above)
14	Initial Plate Find Sample failed (MetaXpress version 6.5 and above)
15	Water Immersion Source Bottle is Empty (MetaXpress version 6.6 and above, for systems with Water Immersion option)
16	Water Immersion Waste Bottle is Full (MetaXpress version 6.6 and above, for systems with Water Immersion option)
17	Water Immersion System Leak Detected (MetaXpress version 6.6 and above, for systems with Water Immersion option)
18	Water Immersion Pressure Test Failed (MetaXpress version 6.6 and above, for systems with Water Immersion option)
19	Water Immersion Vacuum Test Failed (MetaXpress version 6.6 and above, for systems with Water Immersion option)
20	Water Immersion Timeout with WI module (MetaXpress version 6.6 and above, for systems with Water Immersion option)
21	Camera Timeout (MetaXpress version 6.5.5 and above)
22	User Canceled Acquisition (MetaXpress version 6.6 and above)
23	Failed to Find A01 Centerpoint for Round Bottom Plates (MetaXpress version 6.6 and above)
24 and higher	MetaXpress specific error code

Appendix 4 – Status Codes

Status Code	Description
OFFLINE	MX is offline. The OFFLINE command will be returned, see Appendix 2 for details.
EXITING	MX is exiting. The EXITING command will be returned, see Appendix 2 for details.
PAUSED	MX is paused. The PAUSED command will be returned, see Appendix 2 for details.
RUNNING	MX is running (in response to a RUN command). The RUNNING command will be returned, see Appendix 2 for details.
DONE	MX has completed running an experiment (in response to a RUN command). The DONE command will be returned, see Appendix 2 for details. Note: The next OFFLINE, EXIT, GOTO, or RUN command will reset the DONE state.
READY	MX is online and is ready to execute commands. The READY command will be returned, see Appendix 2 for details.
ERROR	An error occurred in a previous command. The ERROR command will be returned, see Appendix 2 for details.

Appendix 5 – Position Codes

Position Code	Description
LOAD	MX moves to the LOAD position. MX will reply OK once the LOAD position is reached, or ERROR if an error occurs.
UNLOAD	MX moves to the UNLOAD position. MX will reply OK once the UNLOAD position is reached, or ERROR if an error occurs.
SAMPLE	MX moves to the well specified in the current protocol as the “Find Sample” well; if multiple sites per well are enabled in the current protocol, it will move to the first enabled site for that well. MX will reply OK once the position is reached, or ERROR if an error occurs.

Appendix 6 – Overview of Use – Example 1:

This example illustrates the communication exchange for a typical session where an ImageXpress system with the MetaXpress software (both referred to as MX system below) starts up, processes some plates, and then is shut down by the CPF.

In this example, no errors occur. ID 20111 represents an example system ID for the MX system.

Time	ID	CPF	MX	DATA fields	Discussion
0					MX system has been started
1	CPF	ONLINE			CPF tells MX system to go online.
2	20111		OK	0	MX system enters online mode. Data field is 0 because no barcode is applicable.
3	CPF	STATUS			CPF asks MX for status.
4	20111		READY	UNKNOWN	MX is ready for commands, but the stage is not in a ready position.
5	CPF	GOTO		LOAD	CPF tells MX to go to the plate loading position.
6	20111		OK	0	MX replies OK as soon as the stage has successfully reached the load position. Again, the data field is 0 because no barcode is applicable.
7					CPF robot deposits a plate onto the MX system.
8	CPF	STATUS			CPF asks MX for status.
9	20111		READY	LOAD	MX is ready and knows that its stage is in the load position. This step is important because it allows the CPF to verify that a user has not taken MX offline in between the last status and the deposit of the plate.
10	CPF	RUN		8675309,n:\cpf\jenny.hts	CPF tells MX to run an experiment on the just-loaded plate, the barcode of the plate is 8675309 and the protocol file to use is located in n:\cpf\jenny.hts.
11					MX loads and validates the protocol file.
12	20111		OK	8675309	MX returns OK in response to the RUN command, indicating that the protocol file has been processed and the plate will now be run.
13	CPF	STATUS			CPF asks for status.
14	20111		RUNNING	8675309,0,0,0	MX is in the middle of doing the initial find sample, so the well / site information is undefined.
15	CPF	STATUS			CPF asks for status

16	20111		RUNNING	8675309,B,2,0	MX is either acquiring at Well B2, or else it just finished acquiring at Well B2 but has not yet reached the next well on the list.
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Appendix 6 – Overview of Use – Example 1 (Continued...):

Time	ID	CPF	MX	DATA fields	Discussion
17	CPF	STATUS			CPF asks for status
18	20111		DONE	8675309 ,F,7,0	MX has completed the experiment for the plate that was loaded. The stage is resting at Well F7, as that was the last well specified to be acquired by the protocol file for this plate.
19	CPF	GOTO		UNLOAD	CPF asks MX to move to the unload position.
20	20111		OK	8675309	MX moves to the unload position and then returns OK.
21					CPF removes the plate
22	CPF	STATUS			CPF asks MX for status
23	20111		READY	UNLOAD	MX is online and ready to run another plate. It knows it is in the UNLOAD position.
24					(at this point, CPF can run other plates, go to step 5)
25	CPF	EXIT			CPF tells MX to shutdown.
26	20111		OK	0	MX begins the exiting process. 0 is returned for Data because no barcode is applicable.
27	CPF	STATUS			CPF asks for status
28	20111		EXITING		MX is in the middle of exiting
29	CPF	STATUS			CPF asks for status
30					(CPF never gets a response because MX has exited. When the timeout period has been reached, CPF can assume that the MX has exited).

Appendix 6 – Overview of Use – Example 2:

This example illustrates the communication exchange for a session where an ImageXpress system with the MetaXpress software (both referred to as MX system below) starts up, then is put under manual control so that a user can manipulate images or data on the MX system, and then the MX is put back under CPF control. ID 20222 represents an example system ID for the MX system.

Time	ID	CPF	MX	DATA fields	Discussion
0					MX system is started
1	CPF	ONLINE			CPF tells MX system to go online.
2	20222		OK	0	MX system enters online mode. Data field is 0 because no barcode is applicable.
3	CPF	STATUS			CPF asks MX for status
4	20222		READY	UNKNOWN	MX is ready for commands, but the stage is not in a ready position.
5					(At this point a user decides to do something on the MX system. Choosing the menu item “Go Offline” will take MX out of online mode and back into offline mode).
6					(User does things on the MX system)
7	CPF	STATUS			CPF asks MX for status
8	20222		OFFLINE		MX informs CPF that it is offline.
9					(Since CPF knows that it put MX online, and that it is now offline, it can assume that a user took MX offline. CPF may want to just poll MX repeatedly until it goes back online).
10	CPF	STATUS			CPF asks MX for status
11	20222		OFFLINE		MX is still offline.
12					(User is done, and chooses the menu item “Go Online” which puts the MX back online).
13	CPF	STATUS			CPF asks MX for status
14	20222		READY	UNKNOWN	MX is back online, it is ready to run a plate but does not know where the stage is.
15					At this point, refer back to Example 1, step 5, to resume running an experiment.

Appendix 6 – Overview of Use – Example 3:

This example illustrates the communication exchange for a session where an ImageXpress system with the MetaXpress software (both referred to as MX system below) starts up, and an error occurs because the plate was not deposited correctly. The exchange illustrates how to recover from this type of error. ID 20333 represents an example system ID for the MX system.

Time	ID	CPF	MX	DATA fields	Discussion
0					MX system is started
1	CPF	ONLINE			CPF tells MX system to go online.
2	20333		OK	0	MX system enters online mode. Data field is 0 because no barcode is applicable.
3	CPF	STATUS			CPF asks MX for status.
4	20333		READY	UNKNOWN	MX is ready for commands, but the stage is not in a ready position.
5	CPF	GOTO		LOAD	CPF tells MX to go to the plate loading position.
6	20333		OK	0	MX replies OK as soon as the stage has successfully reached the load position. Again, the data field is 0 because no barcode is applicable.
7					CPF robot deposits a plate onto the MX system.
8	CPF	STATUS			CPF asks MX for status.
9	20333		READY	LOAD	MX is ready and knows that its stage is in the load position. This step is important because it allows the CPF to verify that a user has not taken MX offline in between the last status and the deposit of the plate.
10	CPF	RUN		8675309,n:\cpf\jenny.hts	CPF tells MX to run an experiment on the just-loaded plate, the barcode of the plate is 8675309 and the protocol file to use is located in n:\cpf\jenny.hts.
11					MX loads and validates the protocol file.
12	20333		OK	8675309	MX returns OK in response to the RUN command, indicating that the protocol file has been processed and the plate will now be run.
13	CPF	STATUS			CPF asks MX for status
14	20333		RUNNING	8675309,0,0,0	MX is in the middle of doing the

					initial find sample, so the well / site information is undefined.
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Appendix 6 – Overview of Use – Example 3 (Continued...):

Time	ID	CPF	MX	DATA fields	Discussion
15					The Find Sample fails because the plate is misloaded, or because an inappropriate protocol file was selected. MX stops the experiment after the find sample operation, because of this error.
16	CPF	STATUS			CPF asks MX for status.
17	20333		ERROR	14	MX reports back the error of the initial plate find sample failure.
18					(CPF interprets the error and determines to retrieve the plate.)
19	CPF	GOTO		UNLOAD	CPF tells MX to move the stage to the unload position (this is so the robot can attempt to pick up and re-load the plate).
20	20333		OK	8675309	MX replies OK once the stage has completed moving to the UNLOAD position.
21					(CPF picks up the plate and then re-loads it).
22	CPF	RUN		8675309,n:\cpf\jenny.hts	CPF tells MX to run the experiment again.
23					MX loads and validates the protocol file.
24	20333		OK	8675309	MX returns OK in response to the RUN command, indicating that the protocol file has been processed and the plate will now be run.
25	CPF	STATUS			CPF asks MX for status
26	20333		RUNNING	8675309,0,0,0	MX is in the middle of doing the initial find sample, so the well / site information is undefined.
27	CPF	STATUS			CPF asks MX for status
28	20333		RUNNING	8675309,A,1,2	MX returns that the plate is running, and is at well A1, site #2. (This means the pickup and deposit of the plate succeeded,

					and the experiment is now running properly).
29					(Continue with Example 1, step 17).

Appendix 6 – Overview of Use – Example 4:

This example illustrates the communication exchange for a session where an ImageXpress system with the MetaXpress software (both referred to as MX system below) starts up, runs a plate, and during the run of the plate one of the system components (such as the camera) fails. ID 20444 represents an example system ID for the MX system.

Time	ID	CPF	MX	DATA fields	Discussion
0					MX system is started
1	CPF	ONLINE			CPF tells MX system to go online.
2	20444		OK	0	MX system enters online mode. Data field is 0 because no barcode is applicable.
3	CPF	STATUS			CPF asks MX for status.
4	20444		READY	UNKNOWN	MX is ready for commands, but the stage is not in a ready position.
5	CPF	GOTO		LOAD	CPF tells MX to go to the plate loading position.
6	20444		OK	0	MX replies OK as soon as the stage has successfully reached the load position. Again, the data field is 0 because no barcode is applicable.
7					CPF robot deposits a plate onto the MX system.
8	CPF	STATUS			CPF asks MX for status.
9	20444		READY	LOAD	MX is ready and knows that its stage is in the load position. This step is important because it allows the CPF to verify that a user has not taken MX offline in between the last status and the deposit of the plate.
10	CPF	RUN		8675309,n:\cpf\jenny.hts	CPF tells MX to run an experiment on the just-loaded plate, the barcode of the plate is 8675309 and the protocol file to use is located in n:\cpf\jenny.hts.
11					MX loads and validates the protocol file.
12	20444		OK	8675309	MX returns OK in response to the RUN command, indicating that the protocol file has been processed and the plate will now be run.
13	CPF	STATUS			CPF asks for status.
14	20444		RUNNING	8675309,0,0,0	MX is in the middle of doing the initial find sample, so the well / site information is undefined.

15	CPF	STATUS			CPF asks for status
16	20444		RUNNING	8675309,B,2,0	MX is either acquiring at Well B2, or else it just finished acquiring at Well B2 but has not yet reached the next well on the list.

Appendix 6 – Overview of Use – Example 4 (Continued...):

Time	ID	CPF	MX	DATA fields	Discussion
17	CPF	STATUS			CPF asks for status
18	20444		ERROR	8675309 ,23	MX returns an error code for the plate with this barcode. The error code is 23, which is not one of the defined errors in the table. Since the error is positive, it means it is a MX-specific error.
19					(Errors like this are not recoverable. The CPF decides to remove the plate and send it to another MX station for analysis).
20	CPF	GOTO		UNLOAD	CPF asks MX to move to the unload position.
21	20444		OK	8675309	MX moves to the unload position and then returns OK.
22					CPF removes the plate
23	CPF	STATUS			CPF asks MX for status
24	20444		ERROR	0,23	Because a previous unrecoverable error occurred, that error is still returned.
25					CPF removes this MX system from its list of stations.