From Eye to Insight



OPERATING MANUAL EM CPD300

167180032 Version 12/18



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Issued by:

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Leica EM CPD300

Operating Manual

Leica EM CPD300 Serial Number:

Date of purchase:

For the instrument serial number, please refer to the name type label on the back of the instrument!



Please read this instruction manual carefully before operating the instrument. For Research use only!

Foreword

This technical documentation is intended to provide essential information about the proper operation and servicing of the EM CPD300 critical point dryer.

Service and operating staff must familiarize themselves with all components of the system before commissioning. Particular attention must be paid to the aspect of safety.

Please retain this operating manual for future reference.

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In addition, all generally applicable legal and otherwise binding regulations for preventing accidents and protecting the environment must be observed by the user and communicated to all users.

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1. Introduction

In order to ensure the safety of service technicians and operators, and to prevent any damage to the EM CPD300 critical point dryer, it is essential to carefully read this manual before beginning any work with the system.

This Operating Manual is intended to help the user understand the system more completely, to use it within the specified limits of its working capabilities, and to maintain in accordance with its physical parameters.

This user manual includes important information regarding proper installation, operation, troubleshooting and repair. Following these instructions will help to prevent hazards, reduce repair and downtime costs, and prolong the system's service life.

Symbols in this manual and their meaning:



Danger!

All paragraphs in the Technical Documentation that contain instructions regarding possible hazards are identified with this symbol. Non-observance these alerts may result in serious injury! Users of the instrument must comply with instructions at all times.



Caution!

This symbol alerts the user to important information which may endanger staff or result in damage to the system if it is ignored.



Note!

This symbol indicates further information relating to a previous explanation, which does not have a safety-critical function. However, it is important to follow this information to ensure that the system functions optimally.

2. Safety

Warning!

STOP

The EM CPD300 critical point dryer can be operated simply and safely, if the system is operated according to the operating manual.

2.1 Safety concept of Leica Mikrosysteme GmbH

The use in accordance with regulations of the EM CPD300 critical point dryer consists of critical point drying of biological or industrial samples with minimal deformation of the specimens for subsequent SEM analysis.

Any other application without written approval of the manufacturer can lead to damages and injuries of instrument and user. The manufacturer refuses to take any responsibility for damage caused thereby.

The sample pressure chamber with a maximum working pressure of 150bar is secured with a Software and Hardware Cut Off function.

Hardware controlled cut off

Bursting membrane at 105bar at 20 °C (+/-10%)

Software controlled cut off

Pressure at 80bar and temperature at 45°C

For safe operation it is absolutely necessary to set up and connect the device according to the instructions in Chapter 3.

General safety regulations

Generally, the following safety regulations apply to the handling of the EM CPD300 critical point dryer:

- Every user is responsible for her / his own health.
- Only instructed users, authorized by the customer are allowed to work at or with the system.
- For all interaction with the EM CPD300 the user must wear protective clothing prescribed in the respective environment.
- It is strictly prohibited to alter or remove protective equipment or covers of any kind.
- Every user must be trained in safe handling of the process gases and fluids that are utilized in the EM CPD300 unit.
- After every repair the user (technician) must verify a flawless state of the system by a test run.
- Leica can guarantee full operation of the system only if original spare parts according to the part lists are used.
- Careful operation and preventive maintenance of the EM CPD300 reduces maintenance costs and assures reliable operation.
- By means of frequent checks and prompt rectification of even small damages, considerable damage can often be avoided.
- Observation of this operating manual and all notes installed on the system serves your own safety.

3. Installation and Warranty

3.1 Installation

Required space for setting up the unit: width 340 mm, depth 583 mm.

Operating room temperature:	$20 - 35C^{\circ}$ at 1bar (CO ₂ bottle limit)
Relative humidity:	5 – 90%

To allow adequate intake of cooling air required for the refrigeration unit and due to the inflexibility of the high-pressure gas hose, a free space of 150 mm has to be provided on the backside of the instrument.

Connection of CO₂ Container

It is absolutely **necessary** to use a pressurized gas container with a pipe (feed pipe), so that the medium (CO_2) can reach the specimen pressure chamber in liquid form. It is important here to ensure that the CO_2 -Container is not equipped with a residual pressure valve.



In order to prevent accidents, the CO_2 -pressurized gas container must be securely fastened in an upright position.

Screw the high-pressure gas hose onto the CO₂ IN connection.



Connect the other end of the high-pressure gas hose to the gas pressure container.



The high-pressure gas hose and seal are included in the accessory set of the unit.

Electrical Connection

Check assigned connecting voltage of the unit.



Connect power cable to wall socket.

3.2 Warranty

The EM CPD300 is covered by a WARRANTY in accordance with the conditions of sale. If functional errors should occur or if the components of the system sustain damage that is subject to warranty coverage during the warranty period, the manufacturer will repair or replace the faulty components following examination thereof.

The manufacturer warrants for the system in its original configuration.

Only original replacement parts may be used. The manufacturer accepts no liability for damage caused by use of other replacement parts.

Caution!



The environmental conditions that were agreed contractually and determined at the time of installation must be maintained.

The manufacturer will not accept liability for damage caused by misuse of the system or its use for purposes other than the intended use, nor for damage caused by work on the system that is not described in this manual.

4. Method

4.1 Critical Point Drying Method

Drying water-containing biological and material specimens in air or under vacuum can drastically alter their structures or even destroy them completely by tangential forces, caused by surface tension of the water (A). The surface tension is a quality of surfaces between a liquid and a gas and the tangential forces appears by boundary crossing from liquid to gaseous phase.

Phase diagram



 $P_c = Critical Point$

 $P_t = Triple Point$

A = Air drying (phase boundary crossing)

B and C = Critical point drying (no phase boundary crossing)

For biological and most micro-electro-mechanical (MEM) samples, critical point drying cannot be performed with water. Water's critical point is at 374°C and 228.5bar. Every biological and MEM sample would be destroyed at this temperature and pressure. Therefore, the use of CO₂ as transitional fluid is the first choice for critical point drying. It is liquid at room temperature at a comparative low pressure of 60bar. Its critical point is at 31°C and 73.8bar. Technically these temperature and pressure requirements of the CO₂ can be implemented relatively easily. Hence, the water in the cell is replaced by an exchange medium like acetone or ethanol, which unlike water, are very soluble with liquid CO₂. After the replacement of the water by an exchange fluid, this exchange medium is substituted with liquid CO₂ through serial dilution steps. Thereafter by increasing the temperature, the pressure is increased proportionally and will transfer the CO₂ through its critical point into a supercritical state (B). At constant temperature the controlled and carful depressurization converts the supercritical CO₂ into its gaseous phase without crossing the phase boundary between liquid and gas (C). After coating, the dried sample can be analyzed in a scanning electron microscope (SEM).

Comparison between air and critical point drying:



Critical point dried sample (Water flea)



Air dried sample (Water flea)

5. Description and Specification

Introduction

Biological specimens intended for investigation in a scanning electron microscope must be dried before being introduced into the microscope to allow the specimen to be imaged. If specimens with water content are dried, tangential forces, caused by surface tension of the water, destroys the structures of the specimen as mentioned in the previous chapter.

Critical point drying with the EM CPD300 is the optimal method to prevent the damaging effects of the above-mentioned forces by bringing the liquid to the gas phase without boundary crossing.

5.1 Technical specifications

Dimensions:



Cooling Cooling capacity Refrigerant Quantity	90 W at -5°C (2500 1/min) R134a 120 g
Cooling range	adjustable 5 °C to 25 °C
Cool down time	2.0 °C/min.
Electrical Connection:	
Voltage	100 V -230 V
Frequency	50 / 60 Hz
Power consumption	170 VA
Gas Connection:	
Gas inlet	M 12 x 1.5
Gas outlet	Ø 8 mm (inner diameter)
-	

Requirements

Transitional fluid: Carbon Dioxide (chemical formula CO₂) CO₂ pressure bottle with feed pipe Exchange fluid: Acetone, Ethanol

6. Preparation of Specimens

Wash in a physiological salt solution.

Fix chemically with a suitable fixing agent.

Wash out fixing agent with a suitable buffer solution.

Dehydrate the chemically fixed specimen with acetone or ethanol.

(Protocol examples are shown in the EM CPD300 Application booklet).

7. Automatic Operation of EM CPD300

7.1 Screen descriptions

Initialization screen: Touch screen after initialization.





Main Screen:



- 1 Version of the EM CPD300.
- 2 Switch to program panel (see page 15).
- 3 Status display of fillers and holder in the sample chamber. Programmable under programs.
- 4 Status display temperature, pressure and time to finish the process (last point only with auto function).
- 5 Switch to settings (see page 37).
- 6 Light on/off
- 7 Status display of programmed process. In auto version buttons have no function.
- 8 Cooling temperature to keep CO₂ fluid (can be changed under settings, page 37).
- 9 CO₂ influx speed in pressure chamber. Programmable under programs.
- 10 Exchange speed (1-10) and status of finished exchange cycles. Programmable under programs.
- 11 Heating speed and heating temperature for critical point. Programmable under programs.
- 12 Status display gas out speed. Programmable under programs.
- 13 Process start. (after defining program).
- 14 Timer function (see page 27).
- 15 Program name of activated program (see page 15).



- 1 Activates key pad to enter program name.
- 2 Activated program is marked green.
- 3 Stirrer on / off with speed control.
- 4 Activation of auto version. If not highlighted manual version is active. Only selectable in automated version.
- 5 Sets speed of CO₂ influx in pressure chamber. Three possibilities: slow, medium, fast.
- 6 Switch to filler and holder panel. Display of filler and holder status.
- 7 Sets delay time after influx of CO₂ and before starting exchange process.
- 8 Sets exchange speed from 1-10. The number relates to the setting of the needle valve during exchange. 1 means a small opening and staying open for longer (slow flowing speed), 10 means a big opening for a short amount of time (fast flowing speed). The parameters where set in a way that after a minimum of 12 exchange cycles only liquid CO2 is left in the chamber.
- 9 Sets exchange cycles. 12 cycles means one chamber volume is completely exchanged. Minimum is 12 cycles.
- 10 Sets heating speed for critical point. Three possibilities: slow, medium, fast.
- 11 Sets gas out speed. Possibilities: slow, medium, fast.

Slow speed can be decreased up to 20% of its normal speed.

- 12 Scrolls programs from 1-10.
- 13 Confirms activated program. Switch to main screen.

Filler/Holder Panel:

Leica	Program I	Name	Programs
MICROSYSTEMS		Exchange	Tiograms
Nr. Auto	Holder 1/1	0 🕨	Holder d
1 50%	🕗 Holder 1/2	0 🕨	Filler 50%
2 🍱 🗸	C Holder 1/3	0 🕨]]]]
3 🕹 🗸	🥭 Holder 1/6	0 🕨	2
4 50%	Filler 1/3 ┥	1 🕨	
5 50%	🕳 Filler 1/6 ◀	1 3	
	<u> </u>		
ок			

- 1 Filler and holder panel.
- 2 Status display of fillers and holders.
- 3 Sets specific holder and fillers. Combination of holders and fillers depends on their volumes.
- 4 Confirms filler and holder setting.

7.2 Programming by example



Press Programs

Leica		Pi		Programs					
MICROSYSTEMS		CO ₂ IN		Exch	ange	Gas OL	UT		
Nr. Au	to Speed	Fillers	Dela _{mi}	_{n]} Speed	Cycles	Heat	Speed		
1 50%	slow		2	5	12	med.	med.		
2 50%	fast			5		slow	fast		
3 50%	fast			5		slow	fast		
4 50%	fast			5		slow	fast		
5 50%	fast			5		slow	fast		
ок		_	_	_	_	_	_		

Press Program Name

Leica MICROSYSTEMS			Р	rograms	Type Program
	CO ₂ IN	Exchange	Gas OUT		name
Nr. Auto	Program Name		78	OK 🔺	
2 0	i o p a	s d f	4 5	6	
3 🕹 🗸	g h j k	b z x	12	3	
4 50%	C I V N) m	0		
5 50%	Shift		BS		
ОК					

Leica			Progr	ams
MICROSYSTEMS	CO ₂ IN	Exchange	Gas OUT	
Nr. Auto	Test		OK	
	qwer	t y u	78	
2 🕹 🗸	i o p a	s d f	4 5 🔓	
3 🕹 🗸	g h j k	bzx	123	
4 50%	C I V N	m	0	
5 50%	Shift		BS CLR	
ОК				-

Press OK to confirm program name

Leica		P		Programs				
MICROSYSTEMS		CO₂ IN		Exch	ange	Gas Ol	л Л	anis
Nr. Au	to Speed	Fillers	Delay _{[min}	Speed	Cycles	Heat	Speed	
1 50%	slow		2	5	12	med.	med.	
2 🌆	fast			5		slow	fast	
3 5	fast			5		slow	fast	
4 50%	fast			5		slow	fast	
5 50%	fast			5		slow	fast	
	_							
ОК		_	_	_	_	_	_	_

Activate stirrer

feica				Brograms						
MICROSYSTEMS			co2	IN	Exch	ange	Gas OL	Frograms		
Nr.	Au	to Spee	d Fille	rs Delay	(_{min]} Speed	Cycles	Heat	Speed		
1		50%		2	5	12	med.	med.		
2 50%		fast	:		5		slow	fast		
3 50%		fast	t		5		slow	fast		
4 50%		fast	t 📃		5		slow	fast		
5 50%		fast	t		5		slow	fast		
Oł	<									

Select stirrer speed

Speed display will disappear after 4 sec.

ſ

Leica		P	rogram N	lame		_	Prog	rams	Activated stirrer
MICROSYSTEMS	S	CO2 IN		Exch	ange	Gas OL	JT	anno	symbol becomes
Nr. A	uto Speed	Fillers	Delay _{[min}	Speed	Cycles	Heat	Speed		green
1 50%	✓ slow		2	5	12	med.	med.		Set CO ₂₋ IN speed
2 50%				5		slow	fast		
3 50%	fast			5		slow	fast		
4 50%	fast			5		slow	fast		
5 50%	fast			5		slow	fast		
OK		_	_	_	_	_	_		

Leica			Pi	rogram	Name		Produ	ame	Press Fillers	
MICROSYSTEMS			CO ₂ IN		Exch	nange	Gas OL	л	ams	
Nr.	Auto	Speed	Fillers	Delay _{[mi}	_{n]} Speed	Cycles	Heat	Speed		
1 50%	√	slow		2	5	12	med.	med.		
2 50%	5	fast			5		slow	fast		
3 50%	5	fast			5		slow	fast		
4 50%	5	fast			5		slow	fast		
5 50%	5	fast			5		slow	fast		
0	к		_	_	_	_	_	_		

Leica	Test		Programs
MICROSYSTEMS		Exchange	Con OUT
Nr. Auto	🥙 Holder 1/1	0 🕨	Holder
1 50%	Holder 1/2	0 🕨	Filler 0%
2 🕹 🗸	🥭 Holder 1/3	0 🕨	
3 0	🦢 Holder 1/6	0 🕨	
4 50%	Filler 1/3	0 🕨	
5 50%	e Filler 1/6	0 🕨	
ОК		_ T.	

Program Filler/Holder combination

Example: 1/2 Holder with 1/6 and 1/3 Filler

Leica	Test		Programs
MICROSYSTEMS		Evohando	200 OUT
Nr. Auto	🥭 Holder 1/1	0 🕨	Holder
1 50%	🕗 Holder 1/2 ◀	1 🕨	Filler 100%
2 🔶 🗸	C Holder 1/3	0 🕨	
3 🔶 🗸	👝 Holder 1/6	0 🕨	
4 50%	ᆕ Filler 1/3 ◀	1 🕨	
5 50%	🕳 Filler 1/6 ◀	1	
ОК			T

Press OK to confirm Filler/Holder settings

Leica		Pi	rogram N	lame			Produ	rame
MICROSYSTEMS	5	CO2 IN		Exch	ange	Gas Ol	л	anns
Nr. A	uto Speed	Fillers	Delay _{[min}	_] Speed	Cycles	Heat	Speed	
1 50%	✓ slow		2	5	12	med.	med.	
2 50%	fast			5		slow	fast	
3 50%	fast			5		slow	fast	
4 50%	fast			5		slow	fast	
5 50%	fast			5		slow	fast	
ОК								_

Press Delay

leica	Program N	ame	
Nr. Au	CO ₂ IN to Speed Fillers Delay _{im}	Clear	Tams
1 50%	slow 😑 2	1 2 3	
2 50	Delay:	4 5 6	
3 50%	2 min	7 8 9	
4 50%		+/-	
5 509	Concel		
OK	Cancer		

Set Delay time

Example: Set to 2min



Leic	a			P	rogram N	lame			Produ	rame
MICROSYST	EMS	5				Exch	ange	Gas O	UT	anis
Nr.	Α	uto	Speed	Fillers	Delay _{[min}	Speed	Cycles	Heat	Speed	
1 0	•%	\checkmark	slow		2	5	12	med.	med.	
2 Č	%		fast					slow	fast	
3 ⁵	%		fast			5		slow	fast	
4 c	%		fast			5		slow	fast	
5 c	%		fast			5		slow	fast	
	ок			_	_	_	_	_	_	_

Press Exchange Speed

leica	Progra	m Name		
MICROSYSTEMS	CO ₂ IN	Exchange	Gas OUT	ams
Nr. Au	to Speed Fillers Delay	y _{rmin1} Speed Cycles	Heat Speed	
1 50% 🗸	slow 😑 🖪	5 🕨	med. med.	
2 50%	fast		slow fast	
3 50%	fast	5	slow fast	
4 50%	fast	5	slow fast	
5 50%	fast	5	slow fast	
ок				_

Set Exchange Speed

Example: set to 5

Exchange Speed display will disappear after 4 sec.



Press Exchange Cycles



Set Exchange Cycles

Example: set to 12



	<u>feica</u>			P	rogram I	Varne			Drog	ame
j	MICROSYSTEMS					Exch	ange	Gas OU	Flogi	ams
	Nr.	Auto	Speed	Fillers	Delay _{[min}	Speed	Cycles	Heat	Speed	
	1 50%	✓	slow		2	5	12	med.	med.	
	2 50%		fast			5		<u>s</u> v	fast	
	3 50%		fast			5		slow	fast	
	4 50%		fast			5		slow	fast	
	5 50%		fast			5		slow	fast	
	0	K		_	_	_	_	_	_	_

Set heating speed

Example: from medium to fast

Leica		Pi	rogram N	ame			Drogu	
MICROSYSTEMS				Exch	ange	Gas OL	Frogr	ams
Nr. A	uto Speed	Fillers	Delay _[min]	Speed	Cycles	Heat	Speed	
1 50%	✓ slow	8	2	5	12	med.	med.	
2 50%	fast			5		slow		
3 50%	fast			5		slow	fast	
4 50%	fast			5		slow	fast	
5 50%	fast			5		slow	fast	
ОК								

Set Gas out speed

Example: from fast to slow

Leica		Program	Name		Progr	ame
MICROSYSTEMS		CO ₂ IN	Exchange	Gas OL	т л	ams
Nr. Aut	o Speed	Fillers Delay _{[mi}	Speed Cycles	Heat	Speed	
1 50%	slow	2	100%		slow 100%	
2 50%	fast		5	slow	fast	
3 50%	fast		5	slow	fast	
4 50%	fast		5	slow	fast	
5 50%	fast		5	slow	fast	
ОК						

Select slow speed setting

Example: Set to 100% slow

Slow Gas out Speed display will disappear after 4 sec.

Leica			P	rogram I	Varne			Produ	rame
MICROSYSTEMS					Exch	ange	Gas OL	T T	anis
Nr.	Auto	Speed	Fillers	Delay _{[mir}	Speed	Cycles	Heat	Speed	
1 50%		slow		2	5	12	med.	med.	
2 50%		fast			5		slow	fast	
3 50%		fast			5		slow	fast	
4 50%		fast			5		slow	fast	
5 50%		fast			5		slow	fast	
0	ĸ								

Press OK to confirm



Press Start to activate program Test

7.3 Programming the EM CPD300 procedure

Before placing the sample into the pressure chamber, program the EM CPD300 protocol. Switch to the program screen panel and follow the instructions under Program Panel (see page 17-25). Do not forget adjustment of the pressure threshold for the bottle empty function (see page 38-39).

7.4 Filling the Sample Chamber with exchange fluid

Open the screw-on cover of the sample chamber by turning cover counter clockwise.





Sample Container with quick release pin

Before filling the sample chamber with exchange fluid, place the magnetic stirrer rod in bottom of the chamber.

Fill exchange fluid into the sample chamber until the stirrer is covered. Then fill the sample container with exchange fluid to such a level that the sample holder and specimen will be completely submerged when inserted.

7.5 Inserting Specimen into Sample Container

Open lid and transfer prepared specimen into a suitable sample holder. The sample holder must be filled with exchange fluid prior to transfer.

Insert the sample holder into the sample container and fill sample container with exchange fluid until the sample holder is covered. Transfer the sample container into the sample chamber and check if sample holder is still covered with exchange fluid.

Adjust container volume if required with fillers or additional sample holders. If sample holders with the volume of 33 - 50% are used, more sample holders or fillers can be added to the pressure chamber (up to 100%).

Fully open the shut-off valve of the CO₂ bottle.

7.6 Preset of Cooling Temperature of the Sample Chamber

The pressure chamber cools up to the preset cooling temperature $(15^{\circ}C \text{ factory setting})$. The cooling temperature depends on the bottle temperature. Please see chapter "7.6.1. CO₂-Bottle Temperature / Pressure Function" to select the appropriate cooling temperature. The cooling unit automatically keeps the temperature at the preset cooling temperature or below (see subchapter 7.6.1 below).

7.6.1 CO₂-Bottle Temperature / Pressure Function

For correct filling of the pressure chamber with CO_2 a temperature difference of 4 °C minimum and a pressure difference of 5 bar is essential. Therefore, the pressure chamber has always to be minimum 4 °C cooler than the CO_2 -Bottle (see list bellow). You can find the adjustment of pressure chamber temperature under "settings" (see operating manual).

The factory preset cooling temperature of the pressure chamber is 15 °C. If the CO_2 does not fill the chamber within a certain time, "Timeout CO_2 -IN" shows in the yellow box. If the poral filter is clean and the bottle is not empty the reason for the warning is the CO_2 temperature bottle which is cooler than the chamber temperature. This means, due to the low temperature difference, the pressure of the CO_2 in the bottle is not sufficient to fill-up the chamber.

The temperature of the bottle can be estimated by measuring the bottle surface with a thermometer. The CO_2 temperature is then about 2 °C cooler than the bottle surface. Decrease the chamber temperature according to the list below and fill again. The green marked values indicate the optimal working temperature and pressure range.

CO ₂ -Temperature (°C)	Recommended pressure chamber cooling temperature (°C)
14	9
15	10
16	11
18	13
20	15
22	17
24	19
25	20
26	21
28	23

Example: If the bottle surface temperature is 22 °C the estimated CO_2 temperature is 20 °C, the cooling temperature of the chamber should be set to 15 °C.

Fully open the shut-off valve of the CO₂ bottle.

7.7 Starting the EM CPD300 auto procedure

After programming go back to the main menu and press start.

7.8 Time and timer function

Press timer function (1).



The timer display pops up (2).

Leica MICROSYSTEMS	System Time	1 2 3	gram 1
CPD	Hour Minute Second 13: 31: 05 Year Month Day	4 5 6	15 °C
P	2011 : 07 : 19	789	
Тс	Start Time	0 <	0/16
P c Pror	Hour Minute	2	35 °C
	2011 : 07 : 20	Cancel OK	
Settir	igs 💦	Start)

Now you can change the local time (3)...



...or set the timer with the keypad (4). The timer function is activated if watch symbol is green (5).

CPD:	System Time Hour Minute Second [13]: 31]: 05 Year Month Day [2011]: 07]: 19	1 2 3 4 5 6 7 8 9	gram 1 15 °C
T c P c Proc	Start Time 4 Image: Start Time Image: Start Time 4 Image: Start	0 < Cancel OK	0/16 35 °C
Settir	ngs 🖟	Start	

Activated time fields become blue (6).

Leica	System Time		gram 1
CPD	Hour Minute Second 13 Year Year Month Day	4 5 6	15 °C
T c	2011 : 07 : 19 Start Time	7 8 9	0/16
P c Proc	Hour Minute 6 : 3 6 Year Month Day		35 °C
	2011 : 07 : 20	Cancel OK	
Setti	ngs 🔊	Start	

Press OK and settings will be saved (7).



In the main screen the green watch symbol beside the local time indication shows that time is activated (8). By activating the timer no programming or other changes are possible.

Leica 8 🗉 13	31 Set		Test Program 1
CPD300 AUTO	Holder	Cool	15 °C
Programs	Filler 100%	CO ₂ IN	fast
T c 26 °C		Exchange	7 0/12
P c 1.0 bar	0	Heat	fast 35 °C
Process time 1:08:39	0	Gas OUT	slow 75%
Settings		Stop	9

To cancel timer settings, press stop (9) ...

...then pause (10)...

1	eica	🕎 13:	31 Set		Test Pro	gram 1
C	:PD3	OTUA 00	Heldor	Cool)	15 °C
	Pr	ograms	Filler 100%	processir	ıg	
	Τc	28 °C				0/16
	Рc	1.0 bar	10	Pause C	ontinue	35 °C
	Proce	ess time 1:17:16		Gas OUT	slow 75%	
	Setti	ngs 🕋		Stop		

...and cancel (11).

Leica 🔯 13:	31 Set	Test Pro g	ıram 1
CPD300 AUTO	Holder	Cool	15 °C
Programs	Filler 100%	Process paused	_
T c 28 °C			0/16
Pc 1.0 bar		Continue Cancel	11
Process time 1:17:16		Gas OUT slow 75%	
Settings		Stop	

Touch Process time field to check (in the main screen) the timer starting time (12)...

Leica 🔯 13:31 Set	Test Program 1
CPD300 AUTO Holder	Cool 15 °C
Filler 100	CO ₂ IN fast
T c 28 °C	Exchange 7 0/16
P c 1.0 bar	C Heat fast 35 °C
Starting time 07-20 06:30:00 12	Gas OUT slow 75%
Settings	Stop

Leica MICROSYSTEMS 🛅 13 31 Set Test Program 1 15 °C Cool CPD300 AUTO Holder Filler 100% CO₂ IN fast Programs Exchange 7_ 0/16 28 °C Τc P c 1.0 bar 35 °C Heat fast (Finishing time 13 07-20 07:47:00 Gas OUT slow 75% -Settings Stop

...and touch starting time to check finishing time (13).

8. Manual Operation of EM CPD300

8.1 Screen descriptions

Initialization screen: Touch screen after initialization



Main Screen before start:



Dark gray buttons can be activated, light gray buttons are inactive!

Main Screen after start:



- 1 Version of the EM CPD300.
- 2 Status display temperature, pressure.
- 3 Switch to Settings (see Page 37).
- 4 Light on/off.
- 5 Process Start / Stop.
- 6 Stirrer on / off. Is controlled in setting panel (see Page 37).
- 7 Cooling temperature to keep CO₂ fluid.
- 8 Sets speed of CO₂ influx in pressure chamber. Three possibilities: slow, medium, fast. To inactivate press again.
- 9 Sets exchange speed. Speed from 1-10. Shows opening status of the needle valve. To inactivate press again.
- 10 Sets heating speed for critical point. Three possibilities: slow, medium, fast. To inactivate press again.
- 11 Sets Gas out speed. Possibilities: slow, medium, fast. Slow speed can be decreased up to 20% of its normal speed. Adjustment under Settings / advanced slow Gas Out (see page 37).
- 12 Sets Time (see page 27).

8.2 Filling the chamber with exchange fluid

Do not forget adjustment of the pressure threshold for the bottle empty function (See page 38-39)!

Open the screw-on cover of the sample chamber by turning counter clockwise.





Spacer with magnetic Stirrer

Before filling the sample chamber with exchange fluid, place the magnetic stirrer rod together with the spacer into bottom of the sample chamber. Fill exchange fluid into the sample chamber until the stirrer and spacer is covered.

Fill the sample chamber with exchange fluid to such a level that the specimen holder with specimen will be completely submerged when inserted.

8.3 Inserting of Specimen into Sample Chamber

Open lid and transfer prepared specimen into a suitable sample holder. Quickly insert the sample holder with the specimen into the sample chamber filled with exchange fluid. Check if sample holder is still covered with exchange fluid.

8.4 Cooling of the Sample Chamber

After tightly closing the screw-on cover of the sample pressure chamber, press Start. Press the cooling button and wait until pressure chamber cools up to the preset cooling temperature (15°C factory setting). The cooling temperature depends on the bottle temperature. Please see sub chapter "7.6.1 CO2-Bottle Temperature / Pressure Function" to select the appropriate cooling temperature. The cooling unit automatically keeps the temperature at the preset cooling temperature or below.

8.5 Filling the CO₂ into Sample Chamber

Wait until the sample chamber temperature has reached 15° C. Once this temperature is reached, completely open the shut-off valve of the CO₂ bottle. Press CO₂ in with the selected speed (slow, medium, or fast).

The pressure display shows the pressure in the sample chamber (identical to the pressure in the CO_2 bottle).

Fill the sample chamber up to the upper edge of the front viewing glass with liquid CO_2 .



Press the CO_2 -In button again. CO_2 goes off. The CO_2 level in the sample chamber may rise slightly.

8.6 Mixing the two liquid media

Press the STIRRER key. The magnetic stirrer is switched on. The magnetic stirrer should not be used if the specimens are very delicate.

8.7 Exchange media mixture from Sample Chamber

Press the EXCHANGE button. Drain the media mixture from the sample chamber until the specimen is barely covered by the liquid.

Press the EXCHANGE button again to close.

This procedure of filling, mixing, and draining needs to be repeated several times (minimum 12 times) before heating to remove all the exchange fluid from the specimens. Multiple and, or larger specimens require more changes.

8.8 Heating the Sample Chamber

Press the HEAT button and wait until sample chamber is heated up to 35 °C. The cooling unit automatically keeps the temperature of the pressure chamber at 35 °C. With the rising temperature the pressure in the sample chamber also increases as shown on the display.

At the time the critical temperature and the critical pressure (31 °C and 73,8 bar for CO_2) have been passed, the liquid CO_2 is transformed into the supercritical status. The system will stop at 35 °C and 79 bar to be safe on the supercritical state. The change of the physical state can be observed through the front viewing glass.

8.9 Releasing gaseous CO₂ from Sample Chamber

After reaching the supercritical status, choose the GAS-OUT speed (slow, medium or fast) and press the GAS-OUT button. The temperature will be constant at 35 °C only when the valve is opening to release the CO_2 , which will change the physical status from supercritical to gaseous due to the pressure decrease under constant temperature.

9. Removing Dried Specimen



Close the shut-off valve of the CO₂ bottle.

Open the screw-on cover of specimen sample chamber. Remove specimen holder with dried specimen from sample chamber for further processing.

Since the dried specimen is highly hygroscopic, it has to be coated as quickly as possible with a thin metal- or carbon film to protect it from atmospheric humidity.

If this is not possible, it is recommended to keep the specimen in a desiccator until it is processed further.

10. Switching off the Unit



Leave the sample chamber open. Leave the CO_2 bottle closed.

Switch off the main switch located on the unit.

11. Setting Panel

Dark gray buttons can be activated, light gray buttons are inactive!

	Leica MICROSYSTEMS	10	Settings
	Cooling temperature [°C]	Units	Volume
1		°C K	- - ()) 9 +
	Heat temperature [°C]	bar psi	Performed RUNS
2			0 8
	Stirrer speed	Advance	ed SLOW Gasout
3	52.0 %		100.0 %
	Main 4	Upda	te Service 6

- 1 Sets cooling temperature to keep CO₂ fluid. (Not recommended to change).
- 2 Sets heating temperature to reach the critical point. (Not recommended to change).
- 3 Sets stirrer speed, (recommended adjusting to sample sensitivity).
- 4 Press Main to switch to main screen.
- 5 Press update for software updates (USB).
- 6 Service Panel just for Service technician (see Service Manual).
- 7 Adjustment of Gas-out speed at slow. Function under settings only in the manual version accessible. In the auto version the advanced slow Gas-out speed adjustment is accessible under program display (see page 15).
- 8 System counts every complete run for maintenance advices (see page 40).
- 9 Volume change.
- 10 Physical unit change.

12. CO₂-Bottle Temperature / Pressure Function

For correct filling of the pressure chamber with CO_2 a temperature difference of 4 °C minimum and a pressure difference of 5 bar is essential. Therefore, the pressure chamber has always to be minimum 4 °C cooler than the CO_2 -Bottle (see list bellow). You can find the adjustment of pressure chamber temperature under "settings" (see operating manual).

The factory preset cooling temperature of the pressure chamber is 15 °C. If the CO_2 does not fill the chamber within a certain time, "Timeout CO_2 -IN" shows in the yellow box. If the poral filter is clean and the bottle is not empty the reason for the warning is the CO_2 temperature bottle which is cooler than the chamber temperature. This means, due to the low temperature difference, the pressure of the CO_2 in the bottle is not sufficient to fill-up the chamber.

The temperature of the bottle can be estimated by measuring the bottle surface with a thermometer. The CO_2 temperature is then about 2 °C cooler than the bottle surface. Decrease the chamber temperature according to the list below and fill again. The green marked values indicate the optimal working temperature and pressure range.

CO ₂ -Temperature (°C)	Recommended pressure chamber cooling temperature (°C)
14	9
15	10
16	11
18	13
20	15
22	17
24	19
25	20
26	21
28	23

Example: If the bottle surface temperature is 22 °C the estimated CO_2 temperature is 20 °C, the cooling temperature of the chamber should be set to 15 °C.

13. Adjustments of Pressure Threshold for Bottle Empty Function

The bottle empty function was developed to protect the samples if the CO_2 bottle becomes empty during a run. When the warning occurs, all valves will be closed so that the pressure chamber is sealed, and the empty bottle can be exchanged with reduced possibility of sample damage. The threshold for this function has to be adapted to the CO_2 temperature. See list below. Green marked values indicate optimal working temperature and pressure range.

CO ₂ -Temperature (°C)	Recommended threshold for pressure (bar)	Pressure of full CO ₂ - Bottle (bar)
14	47	50
15	48	51
16	49	52
18	52	55
20	54	57
22	57	60
24	60	63
25	61	64
26	63	66
28	66	69

Adjustments of Pressure Threshold:

Go to settings and set the desired empty pressure threshold (see table above) under the " CO_2 bottle empty pressure" area.

<u>Leica</u>		Settings
Cooling temperature [°C]	Units °C K bar psi	Volume ()) +
Heat temperature [°C]	Performed RUNS	Set 52.0 bar CO2 bottle empty pressure
Stirrer speed	Advanced	SLOW Gas OUT
Main	Update	e Service

Change CO_2 bottle empty pressure threshold value according to the list on page 45. The CO_2 temperature can be estimated by measuring bottle surface with thermometer. CO_2 temperature is then about 1-2 °C cooler then the bottle surface.

Press " Update " to confirm.

14. Software Update

ROSYSTEMS CON	nect service d	ata stick to USB	port.	Upda
Software	Part ID	Cur. Version	New Version	
User Interface	6613 31-910	01.01.09	-	Update
Operating System	6613 31-940	01.01.01	-	Update
CPD - Board	6613 31-901	01.02.03	-	Update
Controller	????-??	??.??.??	-	Update
				0.0%
Back				

Insert USB stick with EM CPD300 software.

Software modules will be automatically recognized by the system. Choose the module you want to update.

Software	Part ID	Cur. Version	New Version	
User Interface	6613 31-910	01.01.10	01.01.10	Update
Operating System	6574 31-911	04.01.05	??.??.??	Update
CPD - Board	6613 31-901	01.02.03	01.02.03	Update
Controller	????-??	??.??.??	-	Update
				0.0

After software update, press back.

Leica MICROSYSTEMS	Update			
Software	Part ID	Cur. Version	New Version	
User Interface	6613 31-910	01.01.09	01.01.09	Update
Operating System	6613 31-940	01.01.01	??.??.??	Update
💠 CPD - Board	6613 31-901	01.02.03	01.02.04	Update
Controller	????-??	??.??.??	-	Update
				20.8%
Back				

15. Service Notes

15.1 Gas-Out filter maintenance

Leica NICROSYSTEMS	Settings
Cooling temperature [°C]	Units C K bar psi Set 52.0 bar
	Performed RUNS CO2 bottle empty pressure
Stirrer speed	Advanced SLOW Gas OUT
Main	Update Service

After 100 runs are performed, the following note appears:



Under Chapter 14 (Maintenance) the poral filter replacement is described.

15.2 General maintenance

After 500 runs a general maintenance is recommended!

Leica	10:39 Set	Program 2
CPD300	Service Note	15 °C
Progr	Recommended Maintenance	ow 0/12
T c P c	after 500 runs performed	əd. 35 °C
Process	Please contact Leica Technical Service.	st
Settings	Start	

16. User Maintenance



Before starting the maintenance, the pressure has to be released in the sample chamber, the CO_2 -bottle has to be closed, and the CO_2 connection to the EM CPD300 has to be disconnected.

16.1 Poral filter replacement

Remove front cover



Use a 17 size wrench to open the nut in the valve block.



Remove nut, which includes porous filter, from the valve block.



Remove poral filter with spring from the nut.



When exchanging the poral filters the filters must be inserted into the filter holder so that the hole in the poral filter is pointing to the filter holder (the flat surface of the poral filter must point to the springs)



Exchange poral filter with the correct size (Poral filter (0.5 μ m) for CO₂ Inlet (left) and Poral filter (75 - 100 μ m) for CO₂ Outlet (right). Reassemble poral filter in to the nut and screw nut in valve block.

16.2 Cleaning

All surfaces can be cleaned with aqueous reagents or 60% ethanol and a clean cloth.

17. Warnings and Troubleshooting

The list below summarizes Warnings (W...).

Regarding most of the displayed information, just follow the instructions; in the case of error messages, contacting your local Leica Service is required.

Code	Warning	Cause	Action
10E11	Com connection default		Call Leica service
10E12	Lid open	Process can not start if lid open	Close lid
10E13	Separator missing	Process can not start or continue if separator is missing	Insert separator
10E14	Separator full	Process can not start or continue if separator is full	Empty separator
10E16	Heatup error	Pressure too low	Check CO ₂ -bottle
10E17	Timeout CO ₂ -IN	CO ₂ -bottle empty or poral inlet filter is clogged	Check CO ₂ -bottle or exchange poral inlet filter
100E3	Pressure sensor failure		Call Leica service
100E4	Pressure fault	Poral outlet filter blocked	Exchange poral outlet filter
100E5	Temperature sensor failure		Call Leica service



EC Declaration of Conformity

EG Konformitätserklärung

Déclaration CE de Conformité

Leica Mikrosysteme GmbH Hernalser Hauptstrasse 219 A-1170 Vienna/Wien/Vienne, Austria/Österreich/Autriche

declares in exclusive responsibility that the product erklärt in alleiniger Verantwortung, dass das Produkt déclare sous sa responsabilité que le produit			
Model/Modell/modèle	Leica EM CPD300		
Type/Typenbezeichnung/type	EM CPD300		
to which this declaration relates is in conformity with the following standards: auf das sich diese Erklärung bezieht, mit den folgenden Normen übereinstimmt: auquel se réfère cette déclaration est conforme aux normes:	EN 61010-1 EN 61326-1		
following the provisions of directive gemäß den Bestimmungen der Richtlinie conformément aux dispositions de directive			
(Electromagnetic compatibility) (Elektromagnetische Verträglichkeit)	2014/30EU		
(Low Voltage Equipment) (Niederspannungsrichtlinie)	2014/35EU		
(RoHS directive) (RoHS Richtlinie)	2011/65/EU		

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Vienna/Wien/Vienna, 21/07/2017





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