PerkinElmer®
For the Better

„Hilfe, ich muss ...

... replace an iMIC„

Version 1.3

Lars Oelerich
Technical Support Specialist Opera

PerkinElmer Cellular Technologies Germany GmbH
Schnackenburgallee 114
22525 Hamburg
Germany

Tel. : +49 - (0)40 - 560 81 - 564
Fax : +49 - (0)40 - 560 81 - 222
Mob. : +49 - (0)173 - 67 46 642
eMail : lars.oelerich@perkinelmer.com

History:

Version	date	author	changes
1.0	02.12.08	Oelerich	created the first version
1.1	05.01.08	Oelerich	more pictures
1.2	21.01.09	Kessler	missing pictures added, UV adjustment added
1.3	20.07.09	Kessler	"broken" document repaired

Responsible for this manual is:
If you have questions or comments regarding this manual please contact:

Lars Oelerich
lars.oelerich@perkinelmer.com
x - 564

Table of contents:

1. Why does the world need this manual?

Sometimes an issue with some component inside the iMIC occurs. Even if it might be only a small issue, in order to access anything in the iMIC, you have to get it out of the Opera. Then it is easier to install a new one and let the old one be fixed by the manufacturer. This manual should guide you through the exchange process. However, this job is a very advanced Opera service task, and should only be executed by very well trained Opera service personal. Also a broken iMIC always needs to be confirmed by the TSS for the Opera. Therefore please open a *Service Alert* for all iMIC replacements. Please follow all steeps carefully, as there are some small steps that may have a dramatic effect if you miss them …

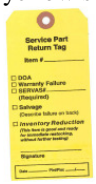
!!!

Please read (and understand) the whole manual before starting any work!

!!!

2. What parts are necessary?

- new iMIC
  → HH10051045 MODT iMIC
  → HH10051045R MODT iMIC Refurbished
  ■ Please check always with your customer care centre if a refurbished iMIC is available and use this if so
- yellow service part return tag



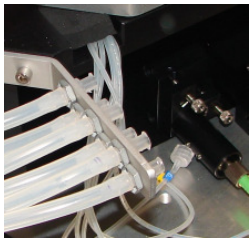
- JOE (1.25x Olympus objective, brass tube, brass lid with cross hair)
- iMIC tilt measurement set
- Opera adjustment plate
- Opera service adjustment plate

3. What tools do you need?

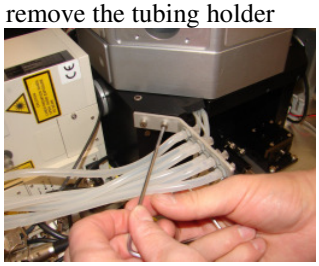
- set of allen keys
- set of flat screw drivers
- all the other stuff you have anyway, when working with an Opera
- torque wrench (for allen screws)

4. Preparation

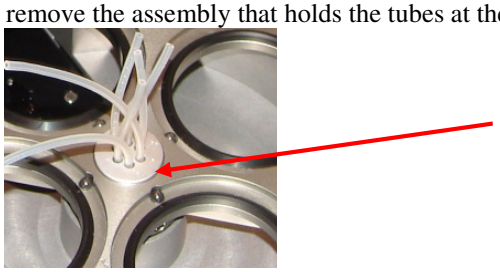
- let the customer perform a decontamination of the instrument
- let the customer fill out the decontamination certificate (from iMIC manufacturer)
- check the table leveling, it should be better than 100 µm, otherwise the stack experiment will not give conclusive results
- check the iMIC status
  → record a bead stack (-10 to + 10 with 0,5 µm distance)
  → safe the stack as **.flex* file
  → zip the file
  → send the file to lars.oelerich@perkinelmer.com
  → measure the tilt of the objective lever
  ■ select the air objective
  ■ move the *z*-axis up (somewhere between 15 and 20 mm absolute position)
  ■ remove the air objective cup and replace it with the angle measurement adapter
  ■ use the level box the measure the offset angle of the table in *x* and *y*
  ■ use the level box to measure the angle of the objective lever in *x* and *y*
  ■ enter those values into the protocol
- write down serial number of new iMIC
- remove all objectives from objective revolver
- shutdown the Opera software
- shutdown the Opera control PC
- switch off the Opera
- disconnect the immersion water tubes, watch out that leaking water is not damaging anything



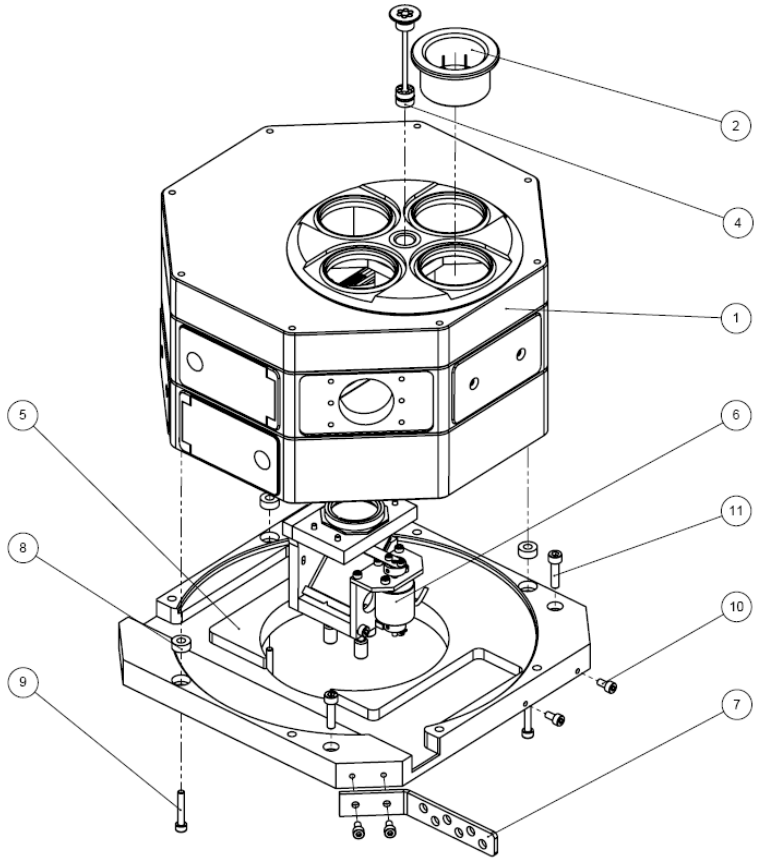
- remove the tubing holder



- remove the assembly that holds the tubes at the iMIC base plate

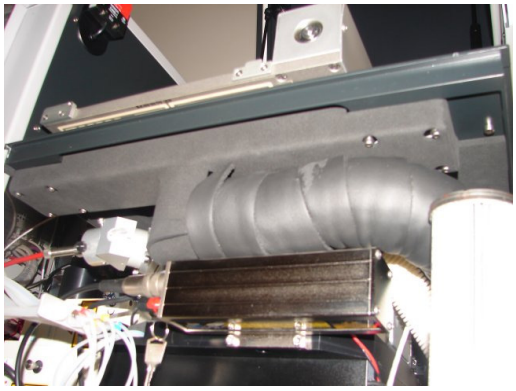


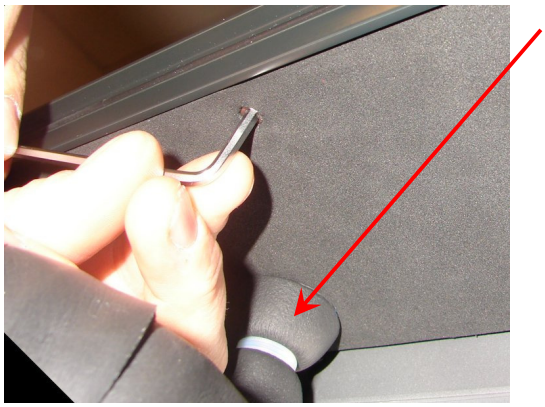
5. Doing the job

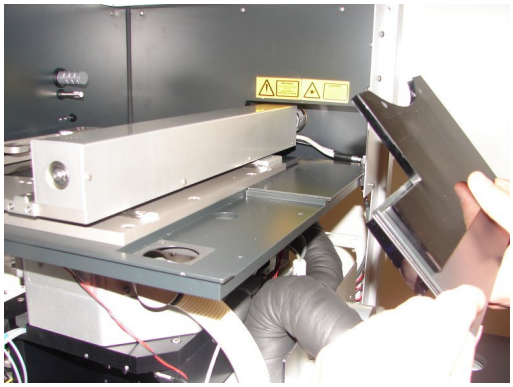
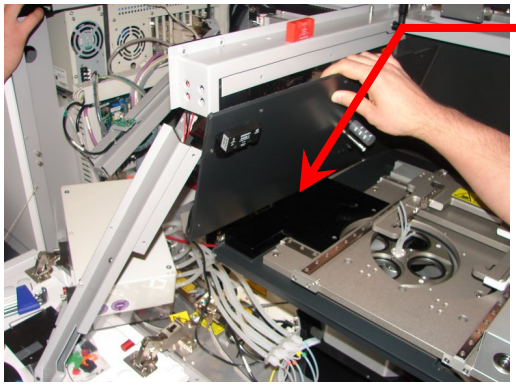


POS. -NR.	QUANTITY / Menge	TITLE / Benennung	DRAWING NUMBER (Zeichnungsnummer) / PDM-Nr.	MATERIAL / Werkstoff	FINISH / Oberfläche
1	1	MODT imic	HH10051045		
2	1	Tygon Carrier iMIC	HH10051037	AlMgSi1	anodized / eloxiert
3	1	Revolver Tab iMIC	HH10051038	AlMgSi1	anodized nature / natur eloxiert
4	1	Assy Imic2 Schlauchfuehrung	HH10050003		
5	1	Grundplatte I-Mic	HH10051001	C250 (Company Gleich) - PL 25	schwarz eloxiert / anodized black
6	1	Assy Bottom port	HH10050002	-	-
7	1	Luer Halter IMIC	HH10051006	AlMgSi1	anodized nature / natur eloxiert
8	3	Auflagerbuchse i-MIC	HH10051004	brass/Messing	
9	3	Zylinderschraube_DIN912_ISO4762 M4x25 V2A_V4A	HHN0100052	V2A/V4A	
10	4	Zylinderschraube_DIN912_ISO4762 M4x8 V2A_V4A	HHN0100043	V2A/V4A	
11	3	Zylinderschraube_DIN912_ISO4762 M5x20 V2A_V4A	HHN0100065	V2A/V4A	

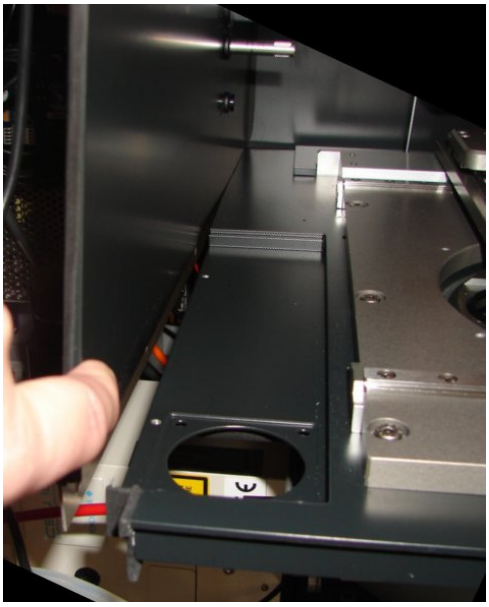
- remove the cover parts in the stage area

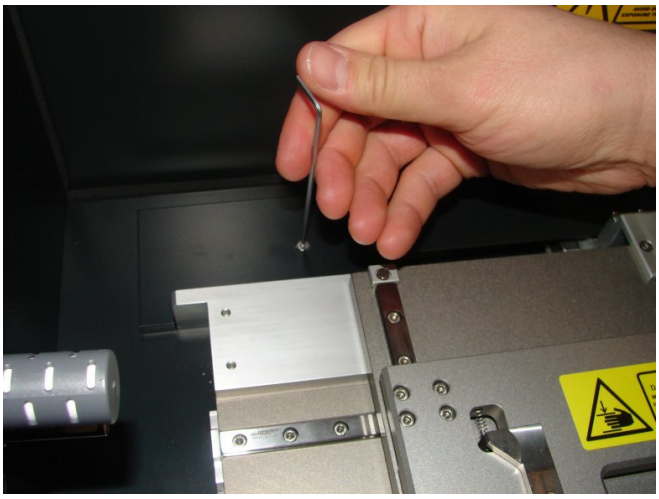


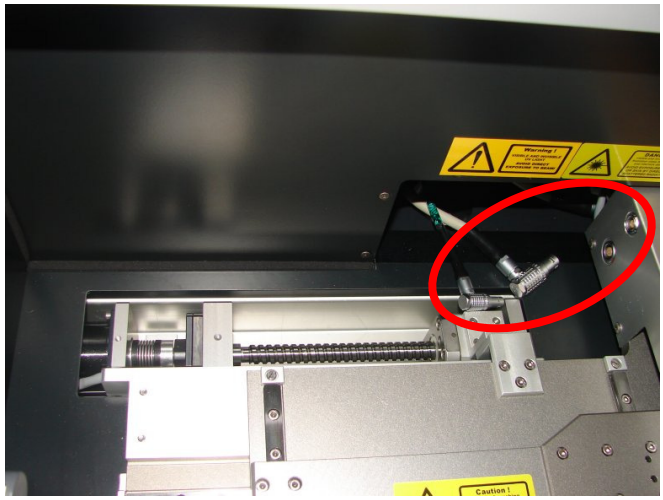


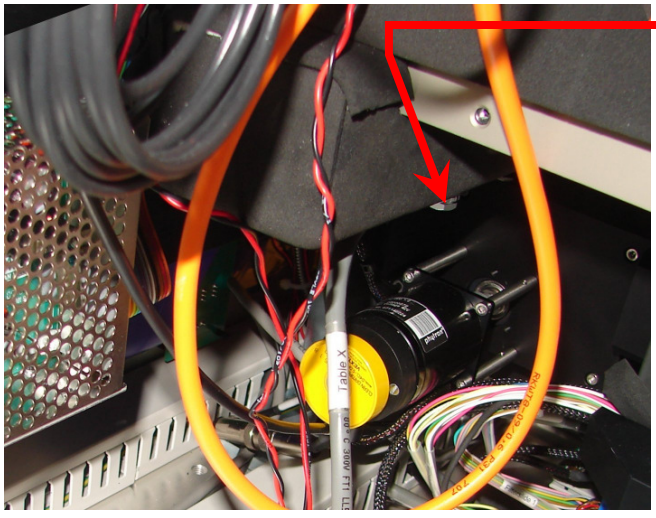



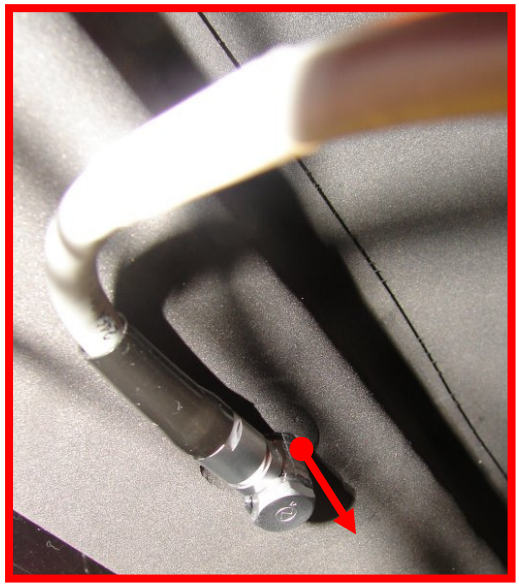
2x

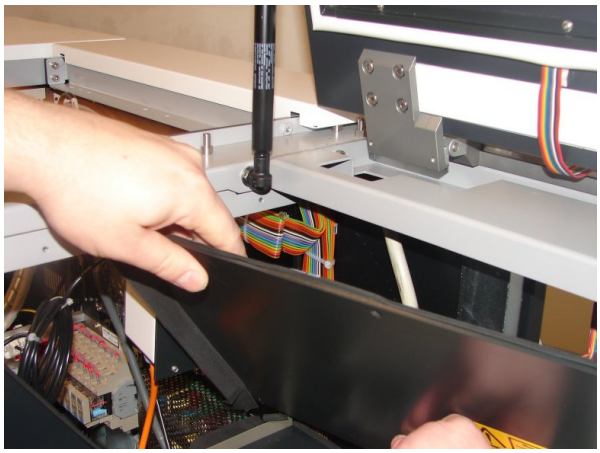


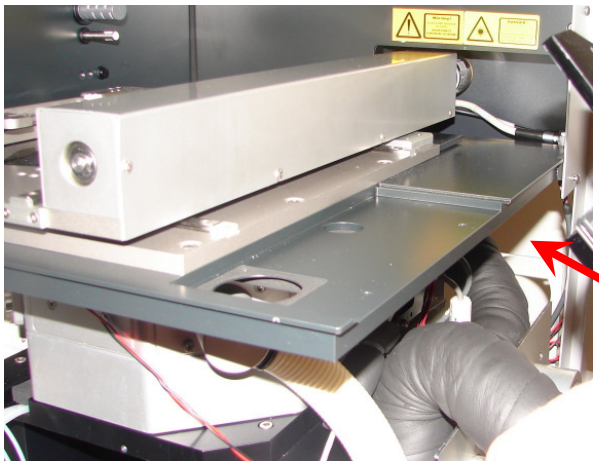


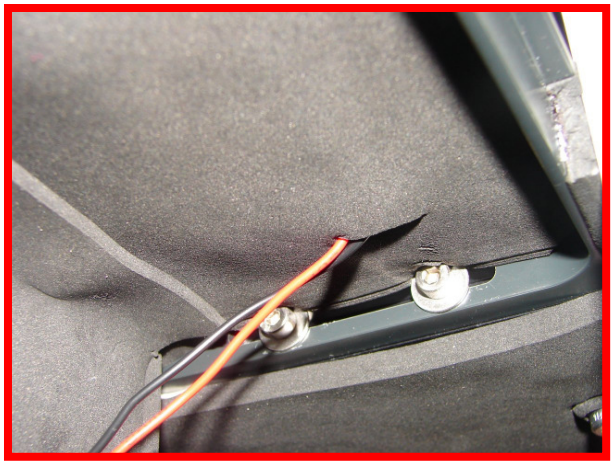




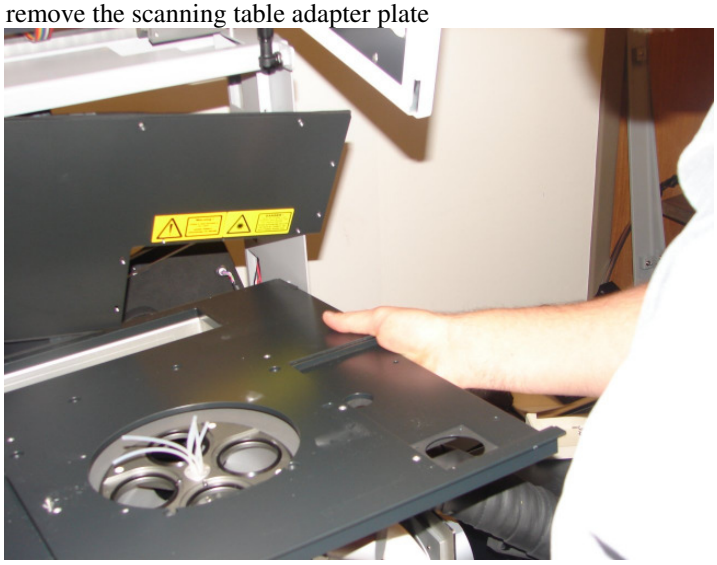




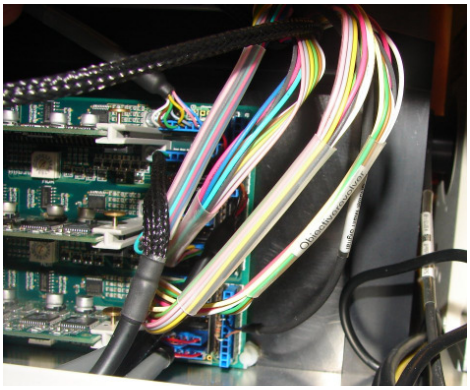




- remove the scanning table
- remove the scanning table adapter plate



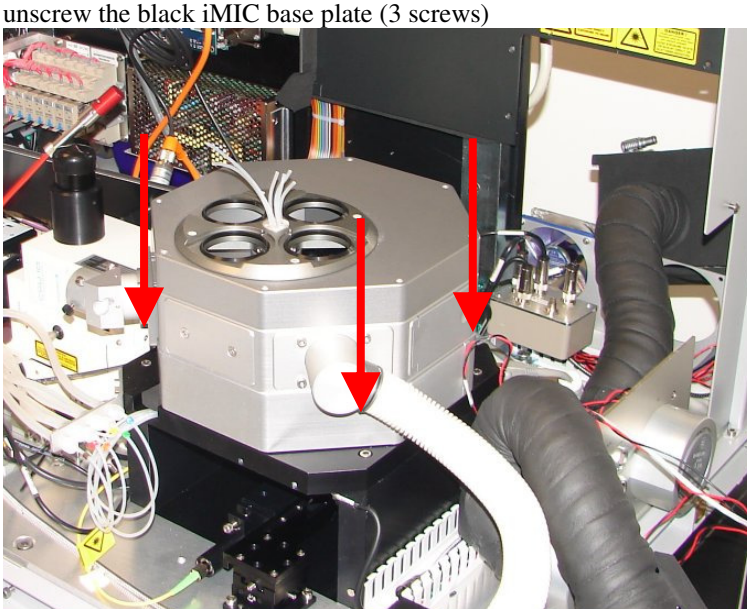
- if the Opera is equipped with an UV upgrade:
  → remove the UV dichros from the iMIC
  → remove the UV camera
  → remove the UV band pass wheel
  → remove the UV fiber from the excitation collimator
  → remove the UV excitation collimator
- unplug the following cables:
  → TMCM stack (behind iMIC)
  ■ objective carrousel
  ■ focus drive
  ■ shutter LED



→ pifoc (under PC)
■ Target
■ Probe
■ PZT out

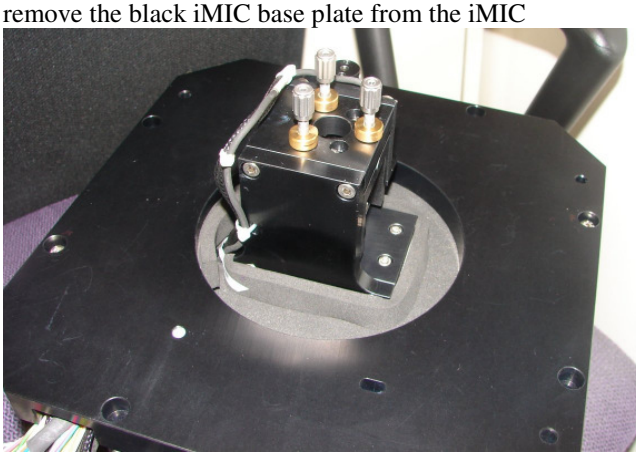
→ shutter box (somewhere behind iMIC)
■ P2 – Shutter

- unscrew the black iMIC base plate (3 screws)

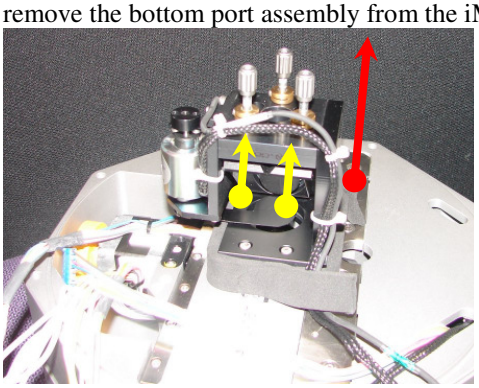


- lift the base plate together with the iMIC out of the Opera; make sure that no tube or cable is damaged

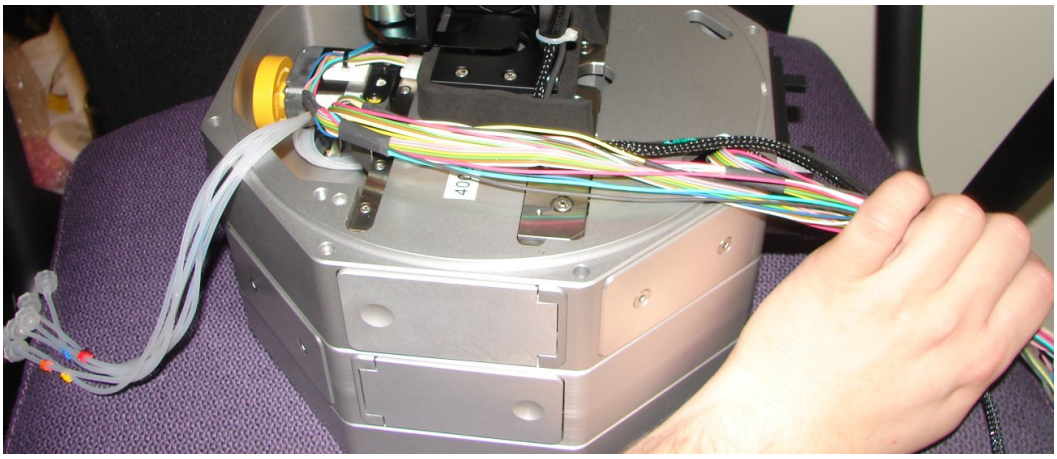
- remove the black iMIC base plate from the iMIC



- remove the bottom port assembly from the iMIC

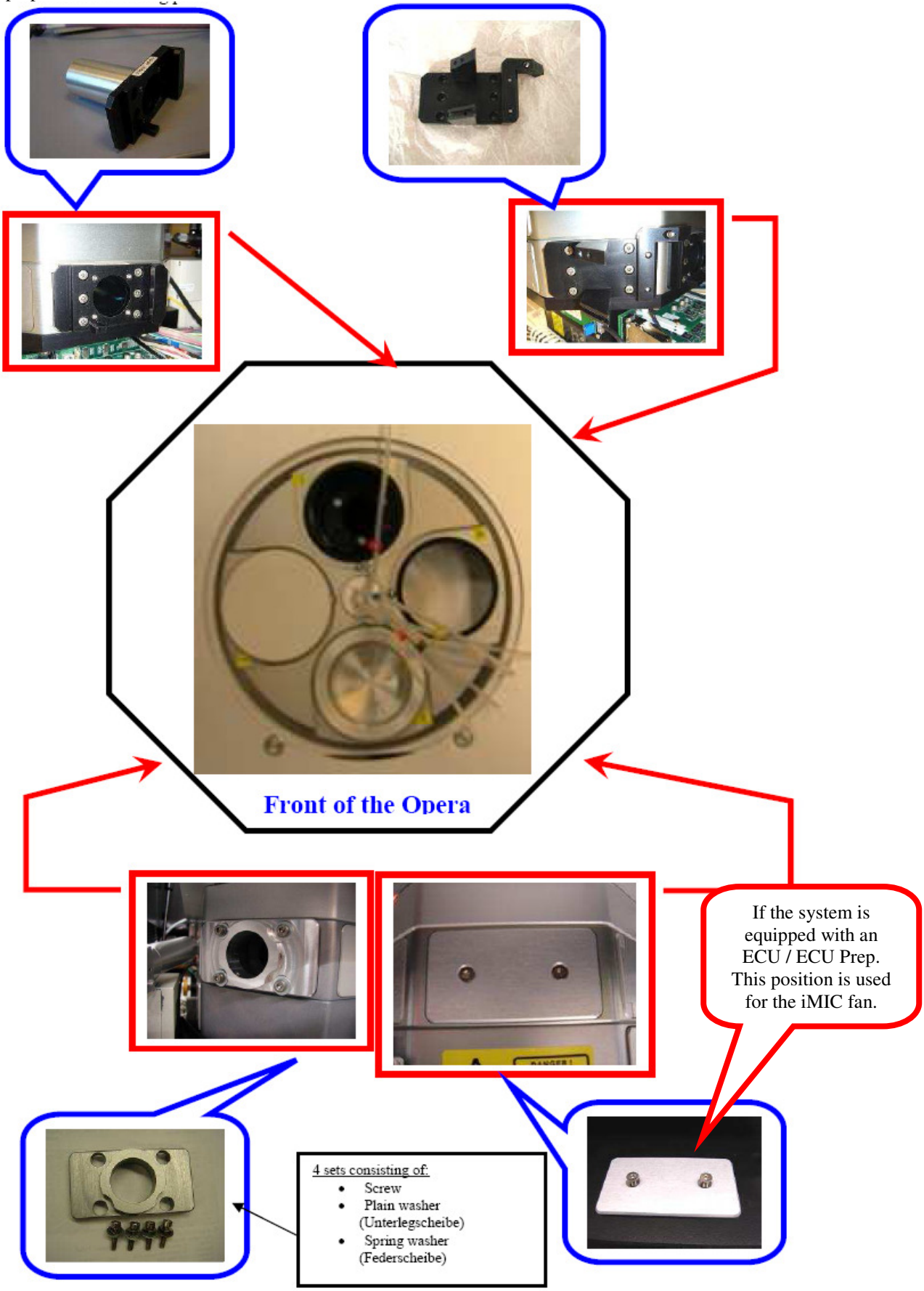


- check the bottom port lens and mirror for any dirt, clean if necessary
- install bottom port assembly to new iMIC
- install the black iMIC base plate to the iMIC
  → make sure all cables and tubes leave the base plate at the right location
  → make sure **no** cable or tube is pinched

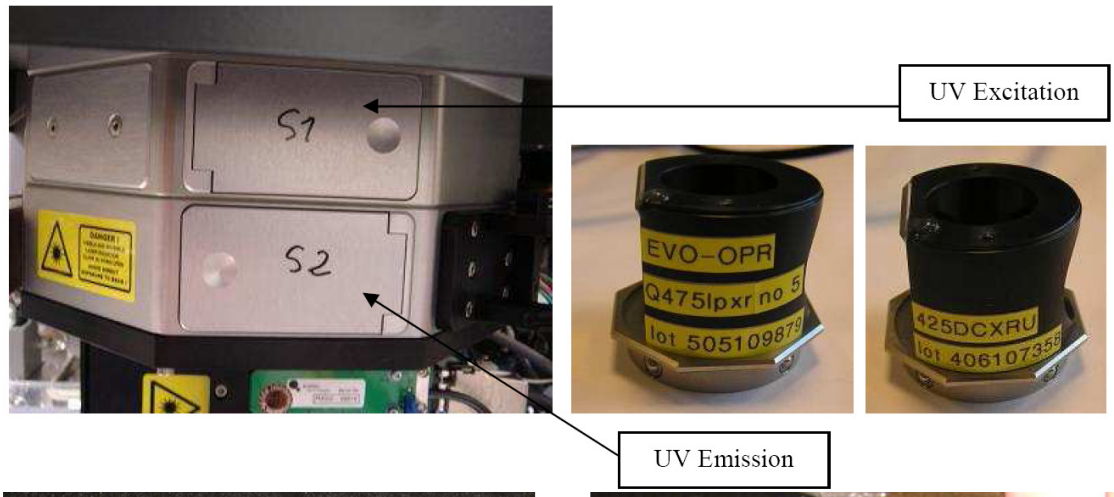


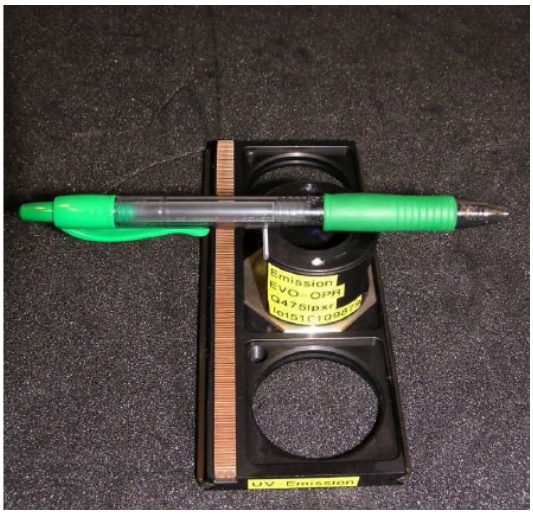
  → use only the 3 screw positions where the brass distance rings are located in the base plate
  → tighten the screws **only** wit a torque of **1 Nm**
- install the black iMIC base plate together with the iMIC in the Opera
- install the table base plate
- install the scanning table
- install all the covers

- if the Opera is equipped with an UV upgrade:
  → prepare the iMIC



→ install the UV dichros from the iMIC

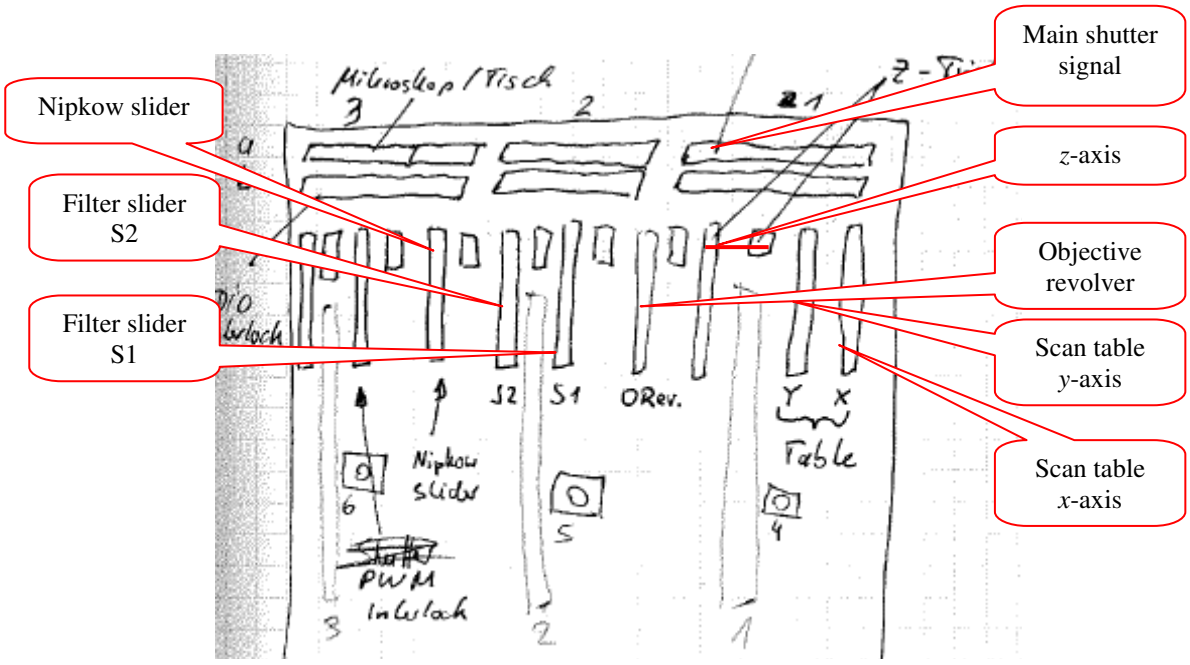




→ install the UV band pass wheel
→ install the UV camera
→ install the UV excitation collimator
→ install the UV fiber into the excitation collimator

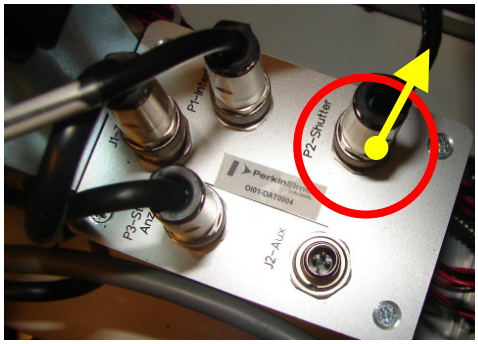
- install the cover parts in the stage area
- exchange the pifoc controller with the one from the new iMIC
- reconnect the following cables:
  → TMCM stack (behind iMIC)
  ■ objective carrousel
  ■ focus drive
  ■ shutter LED

TMCM no.	CAN ID	TMCM slot	plug pos: description	motor/PWM/DIO	usage
3	0x04	1	1: table *x*	motor 1	table *x*-axis
			2: table *y*	motor 2	table *y*-axis
			3: *z*-axis	motor 3	objective *z*-drive
			1a: shutter main signal	DI0	shutter main signal
4	0x05	2	4: objective revolver	motor 1	objective revolver
			5: filter slider 1	motor 2	filter slider 1
			6: filter slider 2	motor 3	filter slider 2
			2a: table sensors	DI0	screening sensor "TablePos"
				DI2	screening sensor "Plate"
			2b: door / cover sensors	DI1	screening sensor "Cover"
				DI3	screening sensor "Safety Door"
5	0x06	3	7: Nipkow slider	motor 1	Nipkow slider
			8: shutter main	PWM 2A	main shutter
			3a: NI-cable	DO0	dig. mod. AF laser
				DO1	servoON/OFF pifoc
			3b: DIO interlock	DO3	Scarina GetOK
				DO4	Scarina PutOK
				DI1	InterlockStatus



→ pifoc (under PC)
■ Target
■ Probe
■ PZT out

→ shutter box (somewhere behind iMIC)
■ P2 – Shutter



6. What to do after the job is done

6.1. Install and test critical components

- install the immersion water tubing holder
- install the objectives
- check the z-axis resolution
  → start the Opera software
  → [c] select the 20x water objective
  → measure the height of the objective
  → [s] move the objective 5 mm up
  → measure the height of the objective
  → the second position should be 5 mm higher than the first one. If this is not the case, the value for the resolution of the *z*-axis must be changed. This value is named "*StepsPerUOM*" and can be found under:
  ■ HKEY_LOCAL_MACHINE\SOFTWARE\Evotec\OperaPI\2.0\Units\OperaUnitMgr\MetaUnitFTable\FocusTable_1\MetaUnitManager\TableT\Table\Axis_Z\AxisConfig
  ■ only the values "*6400*"and "*3200*" are allowed
- check the movement of all filter sliders
- check the immersion water supply and removal for all 3 water objective positions. If one tube is pinched you may have to remove the iMIC again and pay more attention to the tubing routing.

6.2. Readjust the system

6.2.1. Adjust offset of objective revolver

- start the Opera software
- [c] use the 20x air objective
- [m] move the table to the middle of the plate
- [s] move the z-axis to 20 mm (not 20 mm up!)
- check whether the plastic cup with the objective stands concentric in the middle of the iMIC revolver hole
- if not change the value of the registry key "PosOffset"
  (see HKEY_LOCAL_MACHINE\SOFTWARE\Evotec
  \OperaPI\2.0\Units\OperaUnitMgr\MetaUnitFTable\Focustable_1\MetaUnitManager
  \GActuator\ObjectiveRevolver)
  → value in degrees, a positive number will produce a clockwise shift of the objective revolver
- **ATTENTION:** Only change the value in a step width of 1 or smaller in order to avoid damages!
- Change the value in the registry only if the Opera software is **not** running anymore!

6.2.2. Bottom port adjustment

Only a fine adjustment of the bottom port should be necessary, as the unit was already adjusted. So you can start the adjustment at the point "Fine adjustment". However, the full adjustment procedure is included here, just in case …

- old design (up to Opera 36, 3615, Merck Rahway Opera): loose the 4 screws that fix the Nipkow module to the ground plate, the 4 screws that connect the base of the iMIC and the Nipkow module and the fasteners of the cameras (see *Figure 1* and *2*)

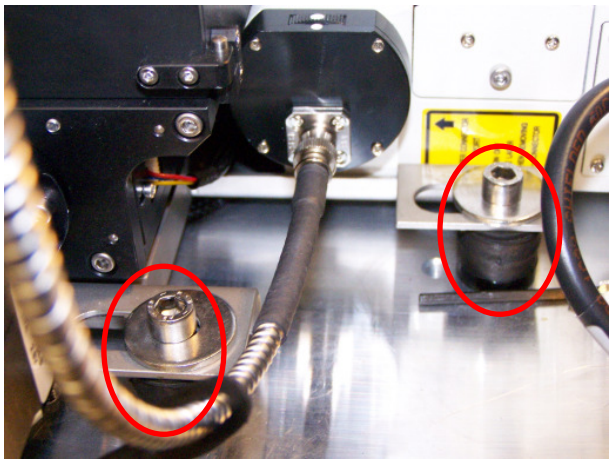


Figure 1: 2 of the 4 screws that fix the Nipkow module to the ground plate; the other 2 screws are on the back of the Nipkow module

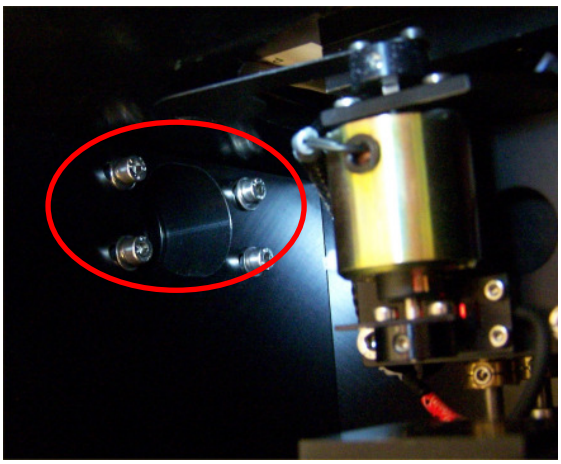


Figure 2: 4 screws that connect the base of the iMIC and the Nipkow module

- new design (from Opera 37, 3616, RWTH Aachen Opera on): the Nipkow module is now mounted an a slide → loose the screw that fixes the slide to the ground plate; there are now no more screws that connect the base of the iMIC and the Nipkow module and the fasteners of the cameras (see *Figure 3* and *4*)

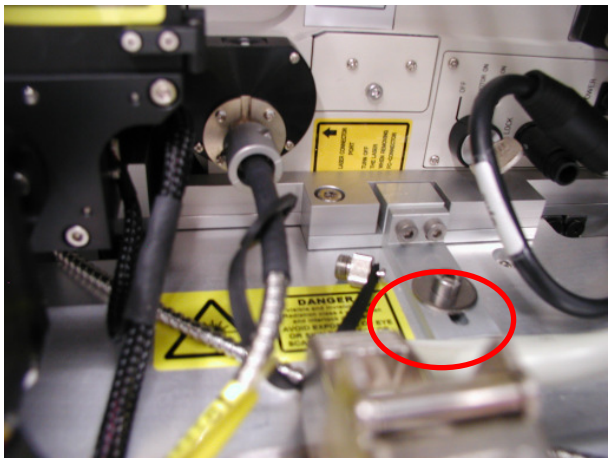


Figure 3: screw that fixes the slide of the Nipkow module to the ground plate

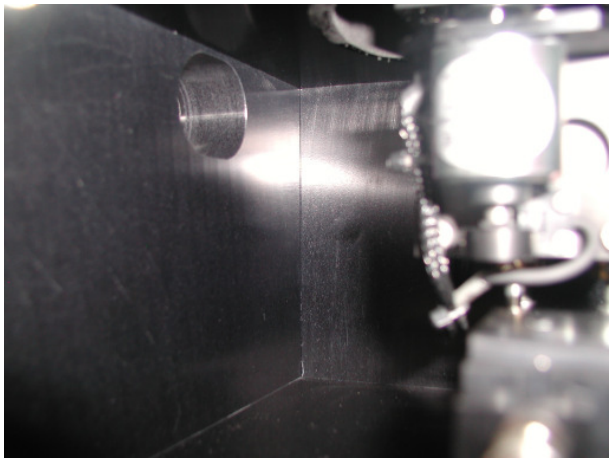
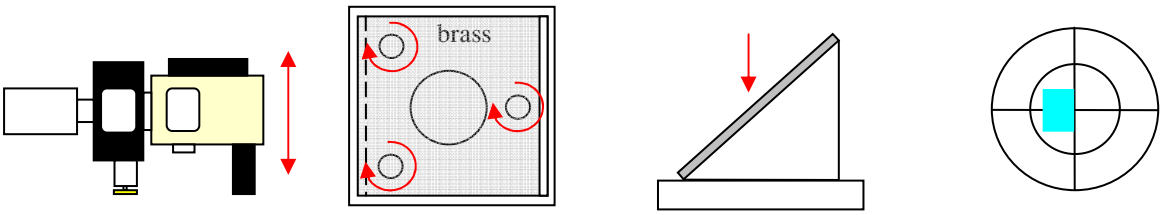


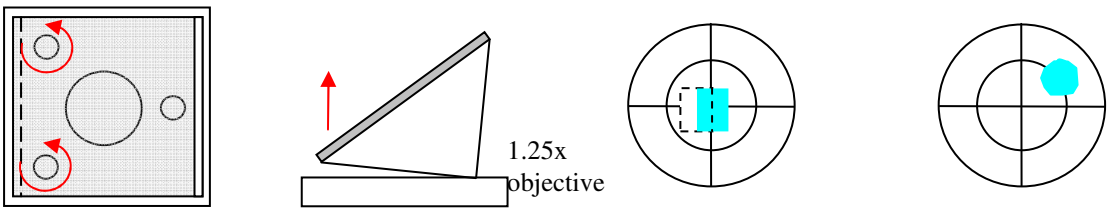
Figure 4: in the new design there are no more screws that connect the base and Nipkow module

Raw adjustment *(Should not be necessary in this case!)*:

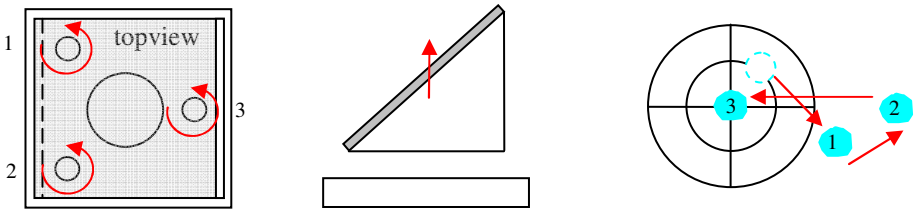
- screw down all fine adjustment screws of the bottom port mirror
- the mirror is on the lowest position now → screws should be lightly engaged
- move the Nipkow module parallel to bring the image area in a central position
  ATTENTION: this is only possible if the iMIC base has long holes in connection base – Nipkow module!
  For details refer to the file "*opera QEHS V1 modification timeline bis KST 3638.xls*" to be found on \\lashamf01\evotec technologies\Cell Handling and Analysis\OPERA\Delivered Instruments\Enwicklungsstand der ausgelieferten Operas
- control the position of the image area with the 1.25x objective and the cross-hair (JOE)



- turn both screws on the left counterclockwise to tilt the mirror
- the image area is moving to the center of the cross-hair
- on the brass tube tool the spot is somewhere outside the center

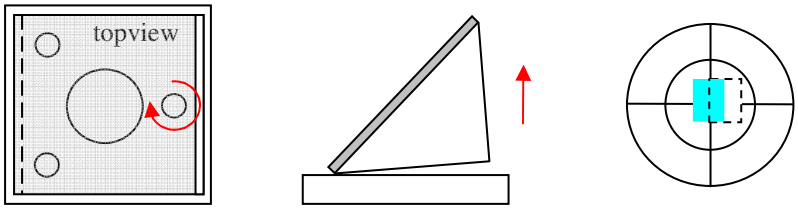


- (Repeat)
  - turn all three screws (nearly) equally clockwise
  - start with screw no.1, follow with no.2 und at last screw no. 3
  - move the mirror (parallel) upwards, the aim is to move the spot into the center (≈ 4 – 5 turns)



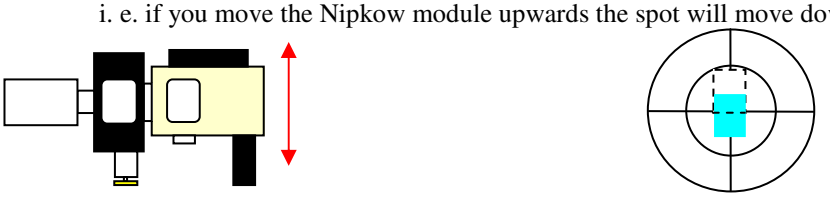
Control Step 1:

- control the position of the image area with the 1.25x objective
- if necessary, correct the position of the image area to the center of the cross-hair by turning the third screw on the right side



Control step 2:

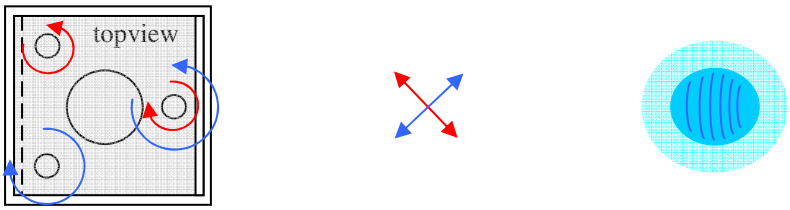
- control the position of the image area with the 1.25x objective
- if necessary, correct the position of the image area to the center of the cross-hair by moving the Nipkow module parallel → the spot will move inversely to the movement of the Nipkow module, i. e. if you move the Nipkow module upwards the spot will move downwards



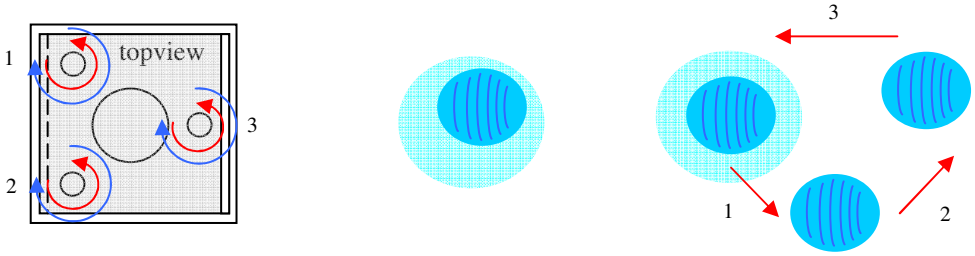
- (Until) the laser spot is in the middle using both the 1.25x objective and the brass tube tool

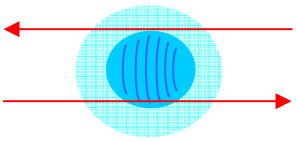
Fine adjustment ***(Start here after iMIC exchange)*****:**

- map the image of the 1.25x objective **<u>without cross-hair</u>** on the ceiling of the room



- (Repeat)
  - turn all three screws
  - start with screw no.1, follow with no.2 und at last screw no. 3
  - move the mirror parallel upwards or downwards



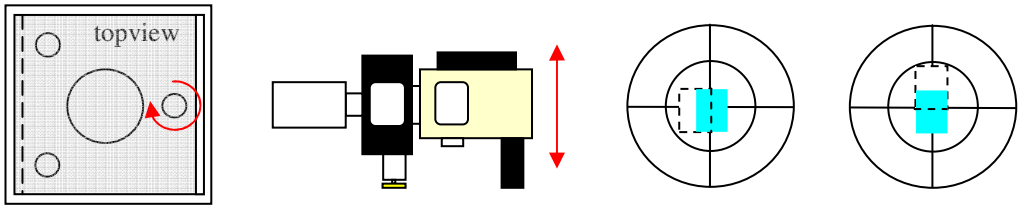


movement of the spot by turning the screw **counterclockwise**, i. e. the mirror moves up

movement of the spot by turning the screw **clockwise**, i. e. the mirror moves down

Control step:

- control the position of the image area with the 1.25x objective and cross-hair
- if necessary, correct the position of the image area to the center of the cross-hair → turning screw 3 and moving the Nipkow module



- (Until) **Finished:**
- near and far criteria of the image areas are both laid in the center
- fix **all** screws **very carefully** (fine adjustment screws, Nipkow module)

6.2.3. AF area (*zMax*, *zMin*)

- [c] select "*Service_Adjustment_Plate*" as used plate
- select the 20x water objective
- put the service adjustment plate on the reader
- [m] move to well (26|16)
- [f] select the *562/40* filter for camera 1, the *568* or *580* detection dichro and the *(405)/488/635(640)* primary dichro (if it is an Opera with UV option, make sure, that both the UV emission and excitation are set to "*empty*")
- move the *z*-drive 8.5 mm up (to 18.5 mm) → make sure that you **do not** push the plate up → there should be a clearly visible distance between the plate and the objective
- [l] use the "*Immersion*" button for objective 1 to put water on the objective (switch on "*Waste*" as well)
- if the water drop does not stay between glass plate and objective move z in 0.2 mm steps up until the drop stays
- close the cover or bypass the interlock system
- start the program *FourChannelPlotter*
- [s] (Repeat)
  - activate the checkbox "*Online*" next to the "*Rescan*" button (focus height = 0)
  - move *z* 0.1 mm up
- (Until) you see the bottom reflex of the glass plate in the *FourChannelPlotter*
- move the peak to the middle of the pifoc range (≈ at 125 µm) by moving *z* further up(with smaller steps) and watching the signal in the *FourChannelPlotter*
- deactivate the "*Online*" checkbox next to the "*Rescan*" button
- [m] click into the "*Focused*" field and note down the value of "*z*-position" in the maintenance checklist
- "*zMax*" = *z*-position + 0.6 mm
- "*zMin*" = *z*-position – 0.8 mm
- close the software and set the values for "*zMax*" and "*zMin*" in the registry to the evaluated ones (see HKEY_LOCAL_MACHINE\SOFTWARE\Evotec\OperaPI\2.0\Units\OperaUnitMgr\MetaUnitFTable\FocusTable_1

6.2.4. Pixel jump test

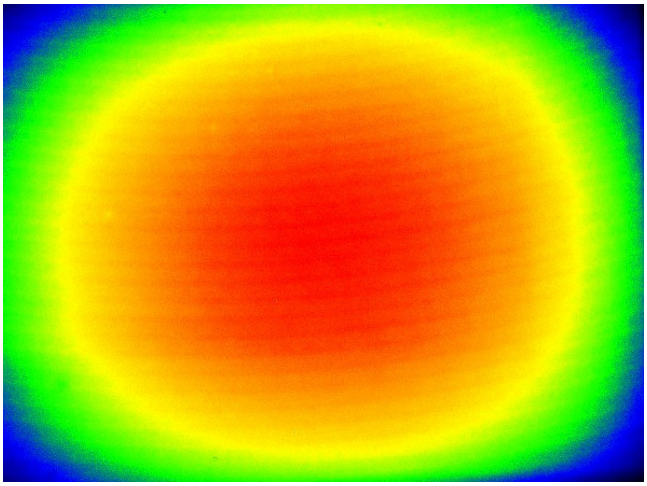
- start the Opera software
- [c] select "*96_Opera_Adjustment_Plate*" as used plate (put the Opera adjustment plate on the reader)
- use the 20x water objective
- [m] move to a well with beads (∅ = 5 µm)
- select the *540/75* filter for camera 1, the *585/40* filter for camera 2, the *568* or *580* as detection dichro and the *(405)/488/635(640)* as primary dichro
- push the "*Focus*" button, switch on the blue 488 laser, set camera 2 to "*Live*" mode
- switch to detection dichro *510* and watch how many pixel the center of a bead moves in *x*- and *y*-direction
- if it is less than 15 pixel do a normal camera adjustment
- if it more than 15 pixel but less than 30 pixel adjust camera 2 to the average position of both detection dichros
- if is more than 30 pixel the detection dichro slider needs to be replaced
- **ATTENTION**: Make sure that you watch the picture of **camera 2** for this test!

6.2.5. Camera 1 angle adjustment

- [c] select "*Service_Adjustment_Plate*" as used plate and put it on the reader
- select the 20x water objective
- [m] move to well (1|1)
- [f] select the *562/40* filter for camera 1, the *568* or *580* as detection dichro and the *(405)/488/635(640)* as primary dichro
- activate the "*Live*" mode for camera 1
- use a flashlight as transmission light
- if not already done, move the focus drive up until the picture (cross hair) is focused
- adjust only the rotation of CCD 1 to the cross hair

6.2.6. Camera 1 dye

- [c] select "*96_Opera_Adjustment_Plate*" as used plate and put it on the reader
- select the 20x water objective
- [m] move to well with the right dye for camera 1 (typical the 488 dye)
- [s] select the *562/40* filter for camera 1, the *568* or *580* detection dichro and the *(405)/488/635(640)* as primary dichro
- activate the "*Live*" mode for camera 1
- activate and use the 488 laser
- focus the well with a focus height of 50 µm
- adjust the camera position in a way that the illumination is as centric as possible and no shadows at the edges are visible. (use the center cross function of the service mode)



- fix CCD 1 completely (all screws and fasteners)

6.2.7. Camera 2 *x*-,*y*-adjustment

- [c] select "*Service_Adjustment_Plate*" as used plate and put it on the reader
- select the 20x water objective
- [m] move to well (1|1)
- [f] select the *562/40* filter for camera 1, the *585/40* filter for camera 2, the *568* or *580* detection dichro and the *(405)/488/635(640)* primary dichro
- use a flashlight as transmission light
- if not already done, move the focus drive up until the picture is focused
- make a (live) picture with camera 1, activate the "*Live*" mode for camera 2
- use the "*Overlay*" button to overlay both pictures
- evaluate the picture: the pictures should match each other quite good. A mismatch of a few pixels is allowed. The mismatch could bee seen at the edges of the cross (see *Figure 8*).
- whenever the pictures do not match as well as they should, a mechanical *x*-,*y*-adjustment is necessary
- save the overlaid picture
- do this as well for camera 3
- camera 1 is always the reference (for non climate chamber Operas)

Adjusting and fixing of the cameras:

- during first adjusting (during production) all screws and fasteners are normally loose
- activate the "*Live*" mode for camera 2 (3)
- after matching the crosshair of camera 2 (3) to camera 1 (reference) tighten all three grub screws (see *Number 1* in *Figure 5* and *Figure 6*)
- use the two verniers for further (fine) adjustment (see *Number 3* in *Figure 5* and *Figure 6*)
- if a vernier does not reach the flange any more move the flange manually towards the flange until the vernier reaches it again
- fasten all four hexagon bolts carefully (see *Number 2* in *Figure 5* and *Figure 6*)
- fix the camera's fastener (first tighten them only "handwarm"), begin with screws (1), end with screws (3) (see *Figure 7*)
- **ATTENTION**: Make sure that each part of the fastener is planar to its corresponding part! Tilted parts may ruin your adjustments!
- if after fixing the fastener the overlay is no longer optimal loose the hexagon screws a little bit and do the fine adjustment again
- tighten all screws completely
- do this as well with camera 3
- camera 1 is always the reference (for non climate chamber Operas)

- during maintenance it is normally not necessary to loose the fastener and the grub screws → sufficient to only loose the hexagon bolts and do the fine adjustment with the verniers

By the way:
For the adjustment of camera 2 (3) it can be easier to use a bead plate instead of the service adjustment plate. Go to a well with small beads (∅ = 2.5 µm) and try to optimize the overlay of camera 1 and 2 (3) by watching the beads in "*Live*" mode of camera 2 (3). The cameras should be adjusted in that way that the best overlay of the beads is in the middle of the picture (see *Figure 9*).

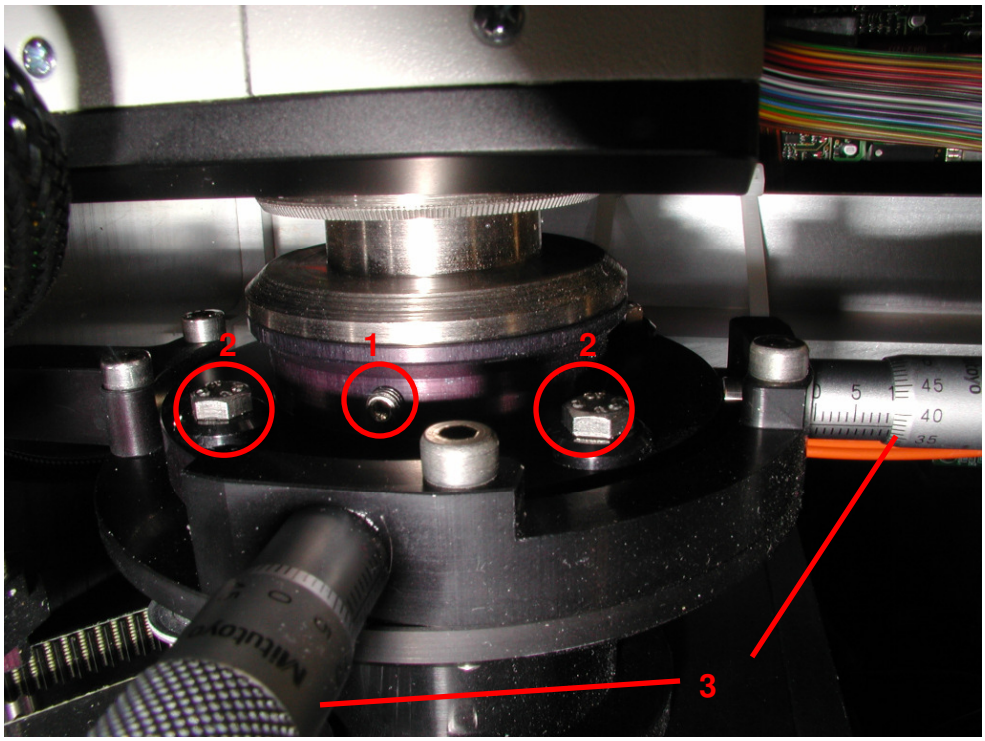


Figure 5: camera flange (old design) with all relevant screws: (1) grub screw; (2) hexagon bolts; (3) verniers

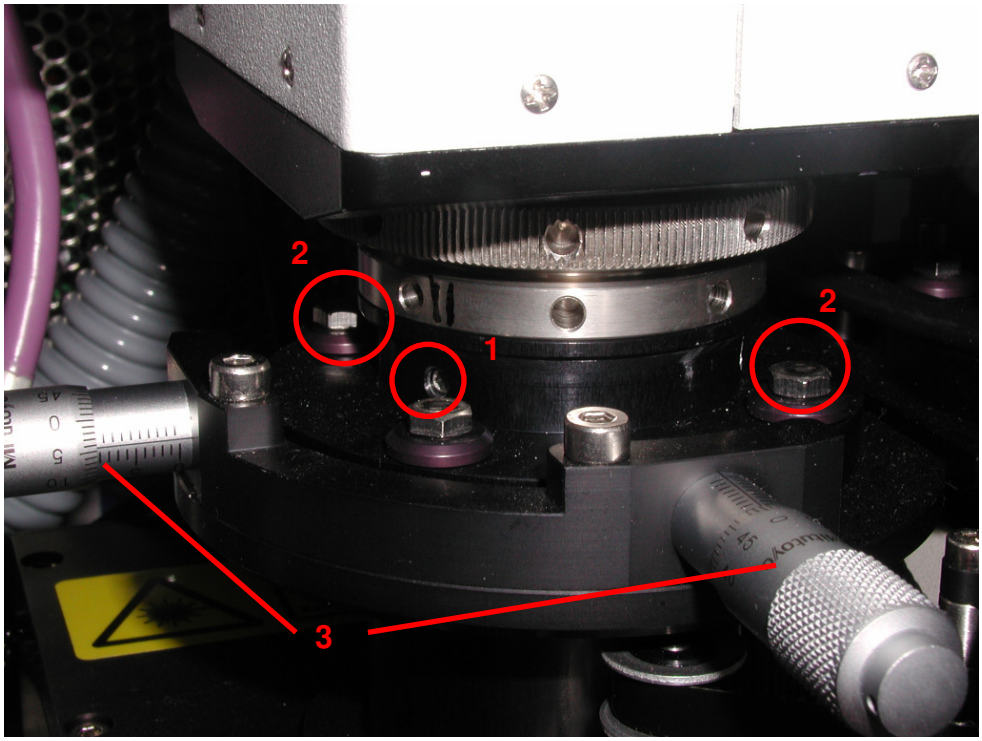


Figure 6: camera flange (new design) with all relevant screws: (1) grub screw; (2) hexagon bolts; (3) verniers

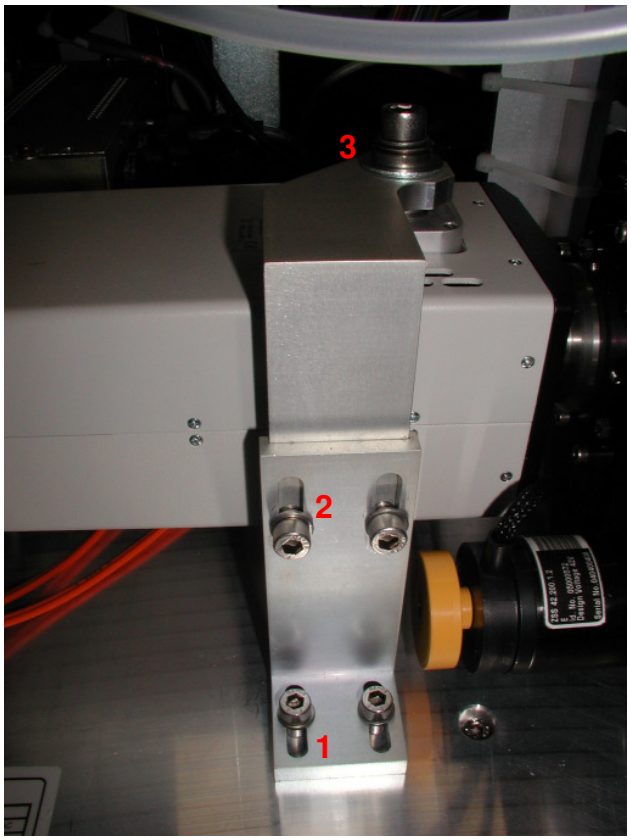


Figure 7: camera fastener with its according screws (1), (2), (3)

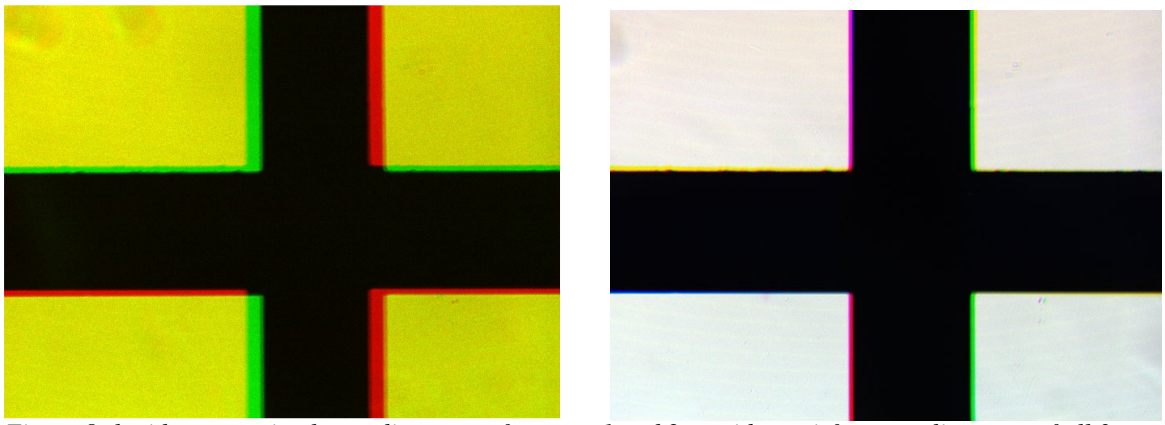


Figure 8: l: side: not optimal x,y-adjustment of camera 1 and 2; r: side: satisfactory adjustment of all 3 cameras

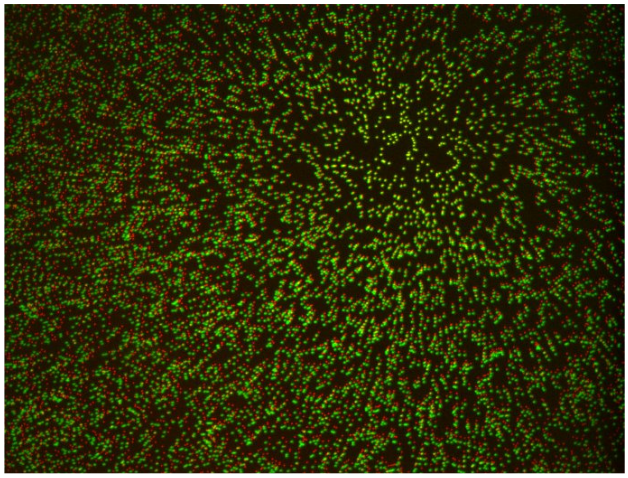


Figure 9: overlay (2.5 beads) of camera 1 and 2 (3)

6.2.8. Camera 3 *x*-,*y*-adjustment

Same procedure as with camera 2!

By the way:

The magnification of camera 3 is slightly different therefore a 100 % match is not possible.

6.2.9. Well (1|1) adjustment (*PlateOrigin*)

- start the Opera software
- [c] select "*Service_Adjustment_Plate*" as used plate and put it on the reader
- select the 20x water objective
- [s] from the drop down menu choose *PlateOrigin* and push the *Move To Pos* button → table will move to well (1|1)
- [f] select the *562/40* filter for camera 1, the *568* or *580* detection dichro and the *(405)/488/635(640)* primary dichro (if it is an Opera with UV option, make sure, that both the UV emission and excitation are set to "*empty*")
- Focus with a focus height of 0
- activate the "*Live*" mode for camera 1
- use a flashlight as transmission light
- if not already done, move the focus drive up until the picture is focused
- (Repeat)
  - move x and y (≈ 0.01 steps)
  - have a look at the picture
- (Until) the cross is in the middle of the picture. If the cross is so far away, that it could not be seen, you can approach the right position by looking direct at the glass plate and moving the table with the software. Be sure that you adjust the table to a cross of two straight lines, and not to the place where a straight line crosses the circle.
- for adjustment help it is possible to mark the checkbox *Add center cross to picture*
- if you have found the right position of the cross push the *Save Pos* button → the software will automatically calculate the new values for "*PlateOffsetX*" and "*PlateOffsetY*" and write them to the registry
- Check this for all used Objective Positions.
- (See also "*Opera QEHS Adjustment Guide*" for further information!)

6.2.10. Table orientation

This test is to ensure that both table axes are perpendicular

- start the Opera software
- [c] select "*Service_Adjustment_Plate*" as used plate and put it on the reader
- select the 20x water objective
- [s] move to absolute x = 80.7 | y = 58.5
- focus on the cross hair
- center the cross hair and note the position in the protocol
- move **relatively** y - 36 mm
- center the cross hair and note the position in the protocol
- move **relatively** x - 54 mm
- center the cross hair and note the position in the protocol
- move **relatively** y + 36 mm
- center the cross hair and note the position in the protocol

6.2.11. Table leveling

With this adjustment it is made sure that the plate lies horizontally on the table

- start the Opera software
- [c] select "*Service_Adjustment_Plate*" as used plate and put it on the reader
- select the 20x water objective
- [m] move to well (2|2)
- [f] select the *562/40* filter for camera 1, the *568* or *580* detection dichro and the *(405)/488/635(640)* primary dichro (if it is an Opera with UV option, make sure, that both the UV emission and excitation are set to "*empty*")
- [s] push the "*Focus*" button (focus height 0)
- click into the green "*focused*" field and write down the shown value e. g. 20.758 µm
- repeat the procedure in wells (2|50), (30|50) and (30|2)

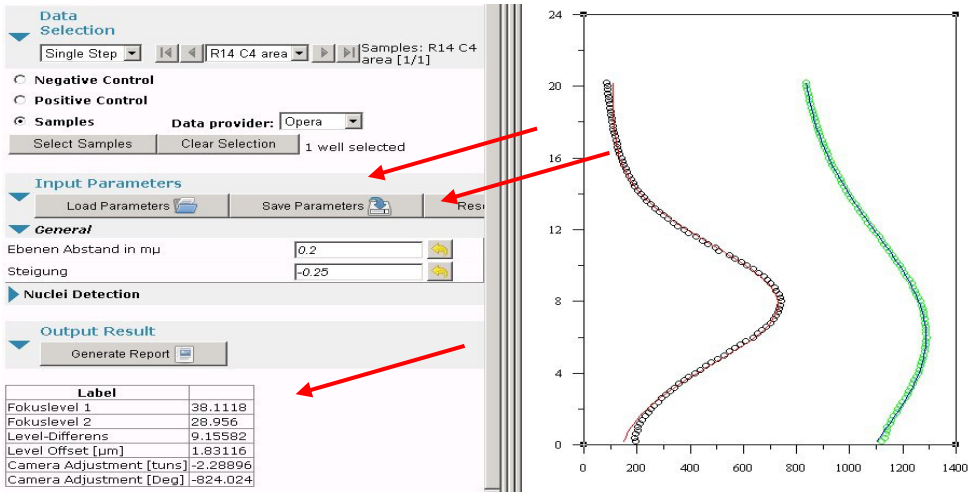
- have a look to all 4 heights → the difference between the lowest and highest value should **not** exceed 50 µm otherwise an adjustment of the 3 holding brackets needs to be done

ATTENTION: If you have done major changes to the table please check the AF area again (zMax, zMin).

6.3. Readjustments of Operas with UV upgrade

6.3.1. Camera 4 *z*-adjustment

- start the Opera software
- [c] select "*96_Opera_Adjustment_Plate*" as used plate and put it on the reader
- select the 20x water objective
- [m] move to a well containing 5 µm beads
- set the wavelength of the UV Lamp to 365 nm
- select the *540/75* filter for camera 1, the *568* or *580* detection dichro and the *(405)/488/635(640)* primary dichro (make sure, that both the UV emission and excitation are set to the filter positions)
- [s] push the "*Focus*" button (focus height ≈ 3)
- switch camera 1 to "*Live*" mode
- activate the 488 laser
- find the optimal focal plane (reduce laser power if necessary)
- activate the UV lamp
- switch camera 4 to "*Live*" mode
- move the *z*-adjustment screw until you can see the beads as sharp as possible (you may notice that the beads bleach very fast, if you do not get a good enough signal any more, please start all over at a different position)
- prepare a stack with this parameter : -50 / +50 / 0.2
- select the "*Use*" box for camera 1 and 4
- select the 488 laser and the UV lamp
- press the "*Take height*" button
- press the "*Focus Exposure 1*" button
- press the "*Expose All*" button and check if the beads are clearly visible in both camera channels (you may have to adapt the exposure times and / or the laser power to get a good intensity of both images → the intensity should be between 2000 and 3000)
- click on the "*Condition Test Run*" button to load the prepared stack
- (Repeat)
  - click on "*Test Run*"
  - click on the "*Analysis*" button to send the images to the Acapella player
  - only on the first run → load the Acapella script "*z Justage Script UV Camera Version 2x0.script*" → to be found on the Service DVD (\FAT + SAT\Scripts)
  - enter the right input parameter (Ebenen = 0.2, Steigung = -0.25) if necessary
  - click on the "*Eval*" button
  - look at the results (if the beads are bleached too much, you will see, that the displayed curves do not have a clear maximum anymore → in this case move to a different position in the well)
- (Until) the difference of the focus planes ("*Level Offset [µm]*") is smaller than ≤ 0.5 µm



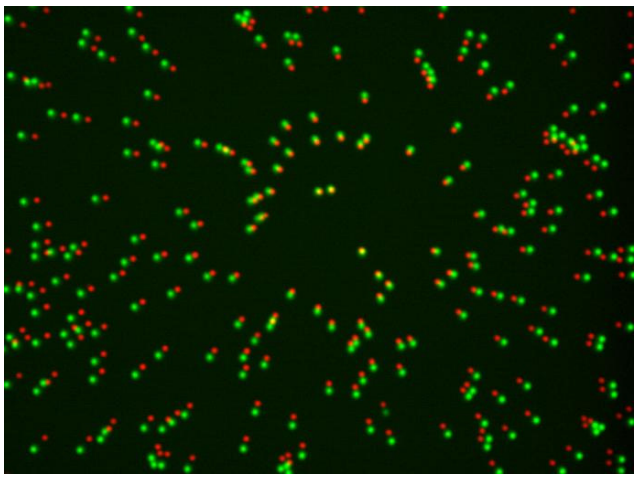
6.3.2. Camera 4 dirt check

- start the Opera software
- [c] select "*96_Opera_Adjustment_Plate*" as used plate and put it on the reader
- select the 20x water objective
- [m] move to a well containing the UV dye
- set the wavelength of the UV Lamp to 365 nm
- select the *450/50* filter for camera 4 and the *425* and *475* dichro
- [s] push the "*Focus*" button (focus height ≈ 50)
- switch camera 4 to "*Live*" mode
- have a look to the dye picture, there should be no sign of major dust / dirt particles → if there is remove the camera and clean it (with compressed air and / or ethanol)

6.3.3. Camera 4 *x*-,*y*-adjustment

- adjust the camera until the bead images for camera 1 and 4 match in the middle of the image
- secure the fixing screw for the *x*- and *y*-direction at the *xyz*-manipulator for camera 4 using the special "Nubsie" (look for the small lock picture)

By the way:
The image of camera 4 is slightly different therefore a 100 % match is not possible.



6.3.4. Camera 4 excitation beam / illumination

There a three criteria to align the emission beam path. The angle is aligned with the adjustment objective (1.25x), the position with the brass adjustment tube (you need JOE for these two adjustments) and the illumination is aligned with the Opera adjustment plate using the UV dye well. Adjust the angle first, then the position, check the angle again and as last step adjust the illumination.

- alignment of the angle
  → put the adjustment objective on the Opera
  → put the brass lid with cross hair on top of the objective
  → adjust the angle by moving the silver screws on the condenser (see *Figure* 10) until the beam profile is in the middle of the cross hair

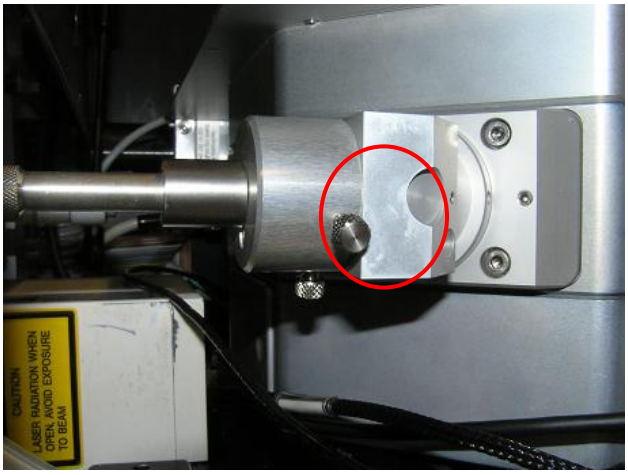


Figure 10: Adjustment screws for excitation (angle)

- alignment of the position
  → put the brass adjustment tube on the Opera
  → put the brass lid with cross hair on top of the tube
  → move the whole excitation flange in *x*- and *y*-direction until the beam profile is in the middle of the cross hair

- alignment of the illumination
  → start the Opera software
  → [c] select "*96_Opera_Adjustment_Plate*" as used plate and put it on the reader
  → select the 20x water objective
  → [m] move to a well containing the UV dye
  → set the wavelength of the UV Lamp to 365 nm
  → select the *450/50* filter for camera 4 and the *425* and *475* dichro
  → [s] push the "*Focus*" button (focus height ≈ 50.0)
  → switch camera 4 to "*Live*" mode
  → have a look to the dye picture, adjust the illumination by moving the silver screws on the condenser (see *Figure* 10) until the illumination is centric (see *Figure* 11)

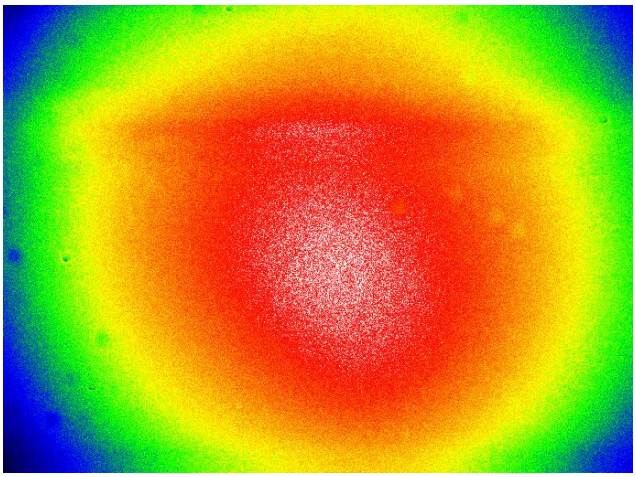


Figure 11: satisfactory adjustment of UV excitation illumination

6.4. Test the system

- check water supply and removal for all installed water objectives
- check auto focus for all objectives with the Opera adjustment plate
- check auto focus for all objectives with the plate the customer uses most
- check the iMIC status
  → record a bead stack (-10 to +10 with 0.5 µm distance)
  → safe the stack as **.flex* file
  → zip the file
  → send the file to lars.oelerich@perkinelmer.com
  → measure the tilt of the objective lever
  ■ select the air objective
  ■ move the *z*-axis up (somewhere between 15 and 20 mm absolute position)
  ■ remove the air objective cup and replace it with the angle measurement adapter
  ■ use the level box the measure the offset angle of the table in *x* and *y*
  ■ use the level box to measure the angle of the objective lever in *x* and *y*
  ■ enter those values in the protocol
- do an automatic test run to prove that the auto focus system is working reliably

6.5. Prepare old iMIC for shipping

- write down serial number of old iMIC in protocol
- prepare the iMIC for shipping:
  → make sure the decontamination certificate is packed together with the iMIC and a copy of it is attached outside of its shipping box
  → fill out yellow service part return tag (salvage)
  → attach yellow return tag on box
  → ship iMIC (it is important to ship the old iMICs as soon as possible as, we need them for the refurbishment process)
  ■ US / Canada: ship to Shelton, CT, USA; Attn. John Spagnola
  ■ ROW: ship to Hamburg, Germany; Attn. Heike Ahrens
- sent a copy of the "*iMIC Replacement Checklist*" to lars.oelerich@perkinelmer.com

7. Known problems

- reduced power after recalibration of laser(s) → readjust laser(s), recalibrate power meter
- no camera and laser shown → check if you have plugged in the power cable for the UV band pass filter wheel and restart the software

8. General things of interest

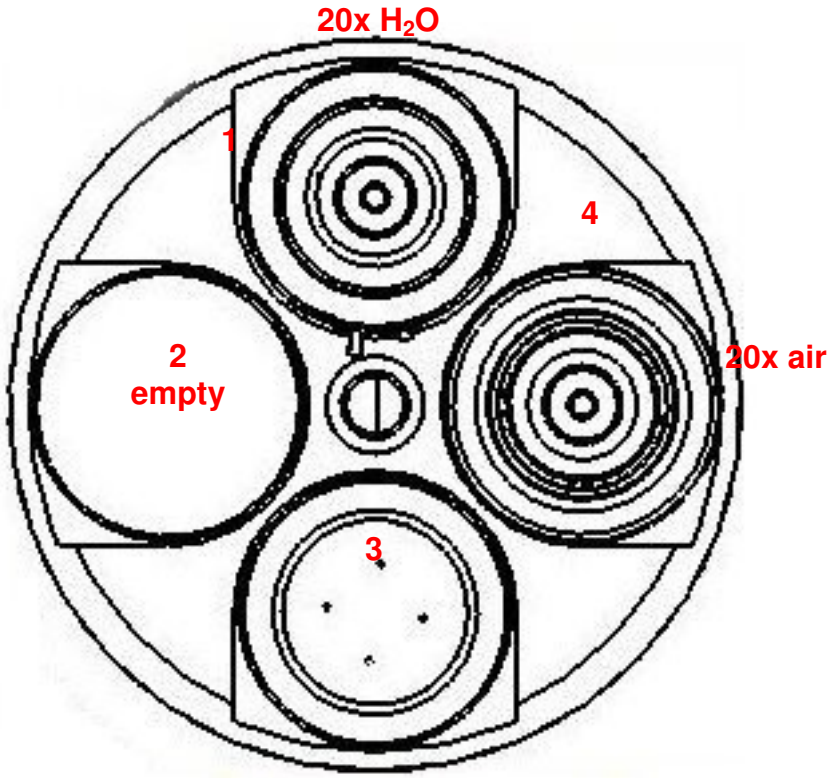


*Figure 12: Sketch of the objective revolver, positions after homing procedure; standard set of objectives -> positions 2 and 3 can be used for additional water objectives, position 4 is **only** to be used with air objectives*

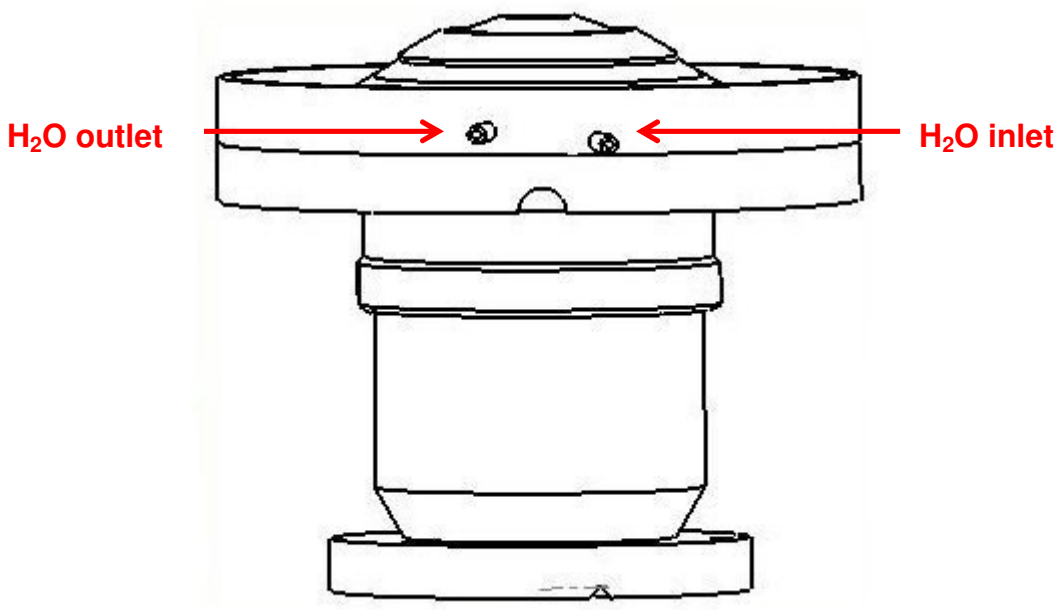


Figure 13: Sketch of an objective with its water collar

The lower small tube applies to be always the inlet for all water collars.
The inlet is always connected to tubes with uneven numbers (1, 3, 5 → "*objective 1 – 3*" in the fluidic box), the outlet is always connected to tubes with even number (2, 4, 6 → "*collar 1 – 3*" in the fluidic box).

9. Checklists

PerkinElmer For the Better

Opera QEHS
iMIC Replacement

SN :		intern SN :		Date :
Customer :				
Location/ Country :		Done by :		

☐ **Decontamination of instrument performed**

☐ **Decontamination Certificate prepared and signed**

☐ **Table leveling checked** *(Limit : 0.1 mm)*

please adjust [*mm*]

well 2|2		well 2|50	<	Up
fix point >	z_pos [mm]			
well 2|30		well 30|50	<	Up

Diff:
Mean
Mean left

Status :

☐ **Do stack measurement and send it to TSS Opera (Lars Oelerich)**

☐ **iMIC exchange confirmed by TSS**

☐ **Check *z*-drive for tilt**

	measured on *z*-drive	measured on iMIC	result for *z*-drive
x	°	°	°
y	°	°	°

☐ **Serial numbers of the new iMIC**

iMIC :	
Pifoc controler :	
Pifoc actor :	

☐ **Serial numbers of the old iMIC**

iMIC :	
Pifoc controler :	
Pifoc actor :	

PerkinElmer For the Better

Opera QEHS

iMIC Replacement

SN :		intern SN :		Date :
Customer :				
Location/ Country :		Done by :		

☐ **z-axis resolution checked**

☐ **Check offset of objective revolver**

PosOffset :	

clockwise = +

☐ **Check imersion water system**

	Objective 1	2	3
	20x Water	**40x Water**	**60x Water**
water supply is working	☐	☐	☐
water removal is working	☐	☐	☐
flush all water objectives	☐	☐	☐
no leakage	☐	☐	☐

☐ **Bottom port adjusted**

☐ **Check the AF scan area**

QEHS : zMin = height of layer - 800 µm zMax = height of layer + 600 µm

z-position	

(all values in mm)

zMin :

zMax :

Put the *Service_Adjustment_Plate* on the Opera
Select a well in the midle of the plate (16|26)
Focus with focus height 0.0
Click on the green box saying "*Focused*"
Read the *z*-position

■ Values entered in registry

☐ **Pixel jump test**

☐ **Camera 1 angle adjusted**

Opera QEHS

iMIC Replacement

SN :		intern SN :		Date :
Customer :				
Location/ Country :		Done by :		

Pixel jump

x		-		=		==>	
y		-		=			

☐ < 15 normal cam 2 adjustment
☐ < 30 average cam 2 adjustment
☐ > 30 new detection dichro slider necessary

detection dichro :

filter settings

primary dichro	detection dichro

Cam 1 (dye pic)

☐ adjustment ok (no edges visible)

☐ camera bracket fixed before adjusting camera 2

insert picture of dye well here measure approx. **50 µm** above plate bottom

☐ pic saved **(dye_cam1.jpg)**

Cam 1 + 2 *(cross-hair on service adjustment plate)*

☐ adjustment ok

☐ camera bracket fixed before adjusting camera 3

insert overlay picture 1+ 2 here

(overlay_cam1+2.jpg)

☐ pic saved

Cam 1 + 3 *(cross-hair on service adjustment plate)*

☐ adjustment ok

☐ all camera brackets fixed

insert overlay picture 1+ 3 here

(overlay_cam1+3.jpg)

☐ pic saved

Cam 1 + 2 + 3

Service adjustment plate

☐ pic saved

(overlay_cam1+2+3.jpg)

Beads

☐ pic saved

(overlay_beads.jpg)

PerkinElmer For the Better

Opera QEHS

iMIC Replacement

SN :		intern SN :		Date :
Customer :				
Location/ Country :		Done by :		

Position	1	Water	x	Magnification	20x	NA	0,7
		Air					

PlateOrigin adjusted via service mode *(all values in mm)*

Position of well (1|1)	
x	
y	

==>

PlateOffset (from registry)	
x	
y	

Offsets:

(positions taken from service mode; cross-hair moved back into center of image for according objective)

Objektive	Position x	*x*-offset to 20xW	Position y	*y*-offset to 20xW
Ref. (20x Water)				
10x Air				
10x Water				
20x Air				
40x Water				
60x Water				

Table orientation

Instruction

[s] Move to

x	80,7
y	58,5

Focus
Center cross ==> x, y in 1)
Move relative y : -36 mm
Center cross ==> x, y in 2)
Move relative x : -54 mm
Center cross ==> x, y in 3)
Move relative y : +36 mm

Limit :	0,500

Measured positions :

1)	x		4)	x		Delta
	y			y		
2)	x		3)	x		
	y			y		

Opera QEHS

iMIC Replacement

SN :		intern SN :		Date :
Customer :				
Location/ Country :		Done by :		

As it is : please adjust [*mm*]

well 2|2 — well 2|50 < — Up

fix point > z_pos [mm]

well 2|30 — well 30|50 < — Up

Diff:
Mean
Mean left

Status :

Approach: 1 please adjust [*mm*]

well 2|2 — well 2|50 < — Up

fix point > z_pos [mm]

well 2|30 — well 30|50 < — Up

Diff:
Mean
Mean left

Status :

Approach: 2 please adjust [*mm*]

well 2|2 — well 2|50 < — Up

fix point > z_pos [mm]

well 2|30 — well 30|50 < — Up

Diff:
Mean
Mean left

Status :

Manual

Put the *Service_Adjustment_Plate* on the reader
Move to well (2|2)
Hit the focus button
Click into the green "*Focused*" field and note the height information
Do the same for wells (2|30), (2|50) & (30|50)
If neccessary move the plate holding brackets
Repeat the whole measurement
Repeat the process until the "*Status*" turns green (*ok*)
Check AF area (zMax, zMin)

Limit : 0.05 mm

Opera QEHS

iMIC Replacement

SN :		intern SN :		Date :
Customer :				
Location/ Country :		Done by :		

☐ ***z*-offset (measured by script)** µm *(-> must be ? 0.5 µm)*

☐ **Camera 4 dirt check / camera 4 illumination**

illumination of UV channel (dye)
■ pic saved **(dye_cam4.jpg)**

☐ **Camera 4 *x*,*y*-adjustment**

overlay of UV channel (with camera 1)
■ pic saved **(overlay_cam1+4.jpg)**

PerkinElmer For the Better

Opera QEHS

iMIC Replacement

SN :		intern SN :		Date :
Customer :				
Location/ Country :		Done by :		

	fine	fixed	part	remark
E1. Wear parts				
E1.1 check bottle for dirt	☐	☐	☐	☐
E1.2 check external tubing for dirt	☐	☐	☐	☐
E1.3 exchange water filter (HH10900102)	☐	☐	☐	☐
E1.4 exchange Hepa filter (once a year) (HH10900107)	☐	☐	☐	☐
E1.5 check ACU for water damages	☐	☐	☐	☐
E2. Opera hood adjustment				
E2.1 no visible gaps	☐	☐	☐	☐
E2.2 easy to open and to close	☐	☐	☐	☐
E2.3 metall positioning pins adjusted	☐	☐	☐	☐
E3. Heating foils				
E3.1 all heating foils are connected (getting warm)	☐	☐	☐	☐
E3.2 all available sensor signals plausible	☐	☐	☐	☐
E3.3 after 0.5 h operating time all 6 / 12 heating foils are at the range of 37 °C +/- 0.2 °C, foil "*Scantable*" may deviate +/- 0.2-0.6 °C	☐	☐	☐	☐
E4. Opera plate nest door				
E4.1 use Opera SW to eject the scan table 5 times, door must close immediately	☐	☐	☐	☐
E5. Interlock switch Opera hood				
E5.1 clicking sound of the CO2 valve stopps immediately after the cover is opened (interlock working)	☐	☐	☐	☐
E6. Performance	☐	☐	☐	☐

Criteria	Start time	Stop time	Duration	Limit	Status
Set temperature to 37 °C					
Temperature is stable (+/- 0.5 °C)				1:00	
Wait 1 hour for the system to heat up					
Set humidity to 80 %					
Humidity is stable (+/- 2.0 %)				1:00	
Set CO2 to 5.0 %					
CO2 is stable (+/- 0.5 %)				0:30	
Temperature, humidity, CO2 are stable (37 °C, 80 % rH, 8 % CO2)					
Set CO2 to 0.0 %, write down start time; end time is when CO2 is < 4 %					
CO2 is below 4 %				0:10	
Temperature, humidity, CO2 are stable (37 °C, 80 % rH, 5 % CO2)					
Use "Table In/Out", leave the table outside for 30 seconds					
all values are stable				0:10	
Temperature, humidity, CO2 are stable (set as above)					
all values are stable				0:30	

PerkinElmer For the Better

Opera QEHS

iMIC Replacement

SN :		intern SN :		Date :
Customer :				
Location/ Country :		Done by :		

E7. **Sensor functionality**

		fine	fixed	part	remark
E7.1	temperature sensor is in place and undamaged	☐	☐	☐	☐
E7.2	humidity sensor is in place and undamaged	☐	☐	☐	☐
E7.3	CO2 sensor is in place and undamaged	☐	☐	☐	☐

E7.4 values of the sensors

Insert a handheld temperature/humidity sensor and a CO2 handheld sensor through the hood flap onto a sample carrier. The sensors sensitve parts should be located in the air without any contact to the plate or other chamber parts. Use tape to make the flap region air tight.

	Temperature (°C)		**Relative humidty (%)**		**CO_2 concentration (%)**	
Set Value	**37,0**		**80,0**		**5,0**	
measured value (software)						
measured value (reference)						
deviation from set value		**Limit : 0,2**		**Limit : 2,0**		**Limit : 0,2**
deviation from reference		**Limit : 0,2**		**Limit : 2,0**		**Limit : 0,2**
status						

E8. **Condensation**

		fine	fixed	part	remark
E8.1	run the ECU for 16 hours at 37 °C, 80 % rH, 5 % CO2	☐			☐
E8.2	check for condensation				
E8.21	hood	☐	☐	☐	☐
E8.22	chamber	☐	☐	☐	☐
E8.23	scan table	☐	☐	☐	☐
E8.24	iMIC	☐	☐	☐	☐
E8.25	Opera_In connector	☐	☐	☐	☐
E8.26	Opera_Out connector	☐	☐	☐	☐
E8.27	_____	☐	☐	☐	☐

E9. **Software versions**

E9.1	**ClimatisationControl**		☐	☐	☐
E9.2	**Firmware ACU**		☐	☐	☐
E9.3	**Firmware ACUx(xl)**		☐	☐	☐

Opera QEHS

iMIC Replacement

SN :		intern SN :		Date :
Customer :				
Location/ Country :		Done by :		

☐ **Check water supply and removal for all used objectives**

☐ **Check AF system for all objectives with the Opera adjustment plate**

☐ **Check AF system for all objectives with the plates the customer uses most**

☐ **Do stack measurement and send it to TSS Opera (Lars Oelerich)**

☐ **Check *z*-drive for tilt**

	measured on *z*-drive	measured on iMIC	result for *z*-drive
x	°	°	°
y	°	°	°

☐ **Prepare the iMIC for shipping**

☐ Make sure that the serial numbers are in this protocol

☐ Fill out the fields :

Customer name	
Customer address	
Customer city	
Customer fax no.	
Customer country	
Decon sheet signed by	
Position	
Substances that had been used on the Opera :	

☐ Describe iMIC issue :

☐ Pifoc lever bent

☐ Pifoc not working : ☐ no action at all ☐ range < 250 µm

☐ Homing not working : ☐ Objective revolver ☐ FS 1 ☐ FS 2

☐ Other : _____
(please specify !!!)

☐ Send this protocol to TSS Opera (Lars Oelerich; lars.oelerich@perkinelmer.com)

PerkinElmer For the Better

Opera QEHS

iMIC Replacement

SN :		intern SN :		Date :
Customer :				
Location/ Country :		Done by :		

iMIC S/N	
Piezo controler S/N	
Piezo actor S/N	

TILL PHOTONICS

Health and Safety declaration

Please enclose this form (e.g. in the shipping envelope) so that it is accessible from the outside of the packaging (in urgent cases first of all by fax).

Address:

Fax:

We want to protect our employees from contaminated equipment (bacteriological, virological, chemical or radioactive). We therefore ask for your understanding that we can only execute calibrations/repairs if this declaration has been submitted to us filled out correctly and signed.

Equipment shipment from / to RMA No.:

The signing person ensures:

- that the equipment was carefully cleaned and decontaminated before dispatch.
- that no contamination can eminate from the equipment at a bacteriological, virological, chemical or radioactive danger.
- that she/ he is authorized to be able to give such statements for the represented company/ laboratory.

University/ Institute/ Company/ Laboratory (stamp)

Fon/ Fax/ email:

Name .....

Position .....

Date, Signature .....

Please inform us with which substances you have worked or with which of these your device has been in contact:

.....

iMIC issue :

- ☐ Pifoc lever bent
- ☐ Pifoc not working : ☐ no action at all ☐ range < 250 µm
- ☐ Homing not working : ☐ Objective revolver ☐ FS 1 ☐ FS 2
- ☐ Other : _____

10. Decontamination Certificate

Health and Safety declaration

TILL PHOTONICS

Please enclose this form (e.g. in the shipping envelope) so that it is accessible from the outside of the packaging (in urgent cases first of all by fax).

Address:

Fax:

We want to protect our employees from contaminated equipment (bacteriological, virological, chemical or radioactive). We therefore ask for your understanding that we can only execute calibrations/repairs if this declaration has been submitted to us filled out correctly and signed.

Equipment shipment from / to RMA No.:

The signing person ensures:

- that the equipment was carefully cleaned and decontaminated before dispatch.
- that no contamination can eminate from the equipment at a bacteriological, virological, chemical or radioactive danger.
- that she/ he is authorized to be able to give such statements for the represented company/ laboratory.

University/ Institute/ Company/ Laboratory (stamp)

Fon/ Fax/ email:

.....
Name

.....
Position

.....
Date, Signature

Please inform us with which substances you have worked or with which of these your device has been in contact:

.....