Agilent Chip Cube Interface Assembly

User's Guide

PRELIMINARY

Agilent Technologies

Notices

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In This Guide...

This guide contains information to install the Chip Cube Interface Assembly on the Agilent 1100 Series LC/MSD Trap XCT, XCT Plus or XCT Ultra.

1 Installation of the chip cube adapter

In this chapter you install the Chip Cube adapter to the LC/MSD Trap instrument.

2 Verifying Performance

In this chapter, you verify the performance of your installed system.

3 Processing the MS/MS results of the BSA digest

In this chapter, you process the results of the BSA digest with Spectrum Mill, with MASCOT, or by spectral interpretation in the Trap Data Analysis application.

4 Verifying Performance on an Upgrade System

In this chapter, you verify the performance of your upgraded system.

5 Maintenance

This chapter contains basic maintenance tasks.

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1
Installation of the chip cube adapter

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The following steps are required for the proper installation and operation of the Chip Cube Interface Assembly These steps should be completed in conjunction with the steps outlined in the *G4240A Chip Cube Interface Service Manual* (p/n G4240-90100).

Step 1. Prepare the desolvation assembly for the Chip Cube Interface Assembly

1 Remove the existing plumbing and cables from both the nebulizer assembly and mass spectrometer.

   Cables and plumbing are dependant upon which source is attached to the ion trap at time of HPLC-Chip MS Interface installation.

2 Remove the spray shield and end plate from the desolvation assembly.

3 Remove the capillary cap from the end of the dielectric capillary. This is not necessary for the nanospray source as the spray shield and capillary cap are one piece.

4 Remove the two wires from the high voltage connection posts in the end cap mount of the spray chamber.

5 Remove the retaining ring for the end cap mount on the desolvation assembly.

6 Carefully remove the end plate mount (p/n G1946-20101) by sliding it off the end of the capillary.

CAUTION Do not break the vacuum seal at the back end of the desolvation assembly or damage the plating on the dielectric capillary.

See Figure 1.

You do not need to vent the mass spectrometer.

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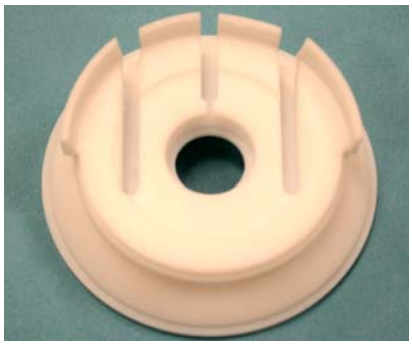

Figure 1 G1946-20101 End Plate Mount

7 Move the capillary electrode, capillary electrode insulator and the high voltage connector posts to the new Chip Cube end plate mount.

8 Place the assembled Chip Cube end plate mount into the desolvation assembly.

The Chip Cube end plate mount (see Figure 2) is included in the ship kit of the Chip Cube interface assembly.

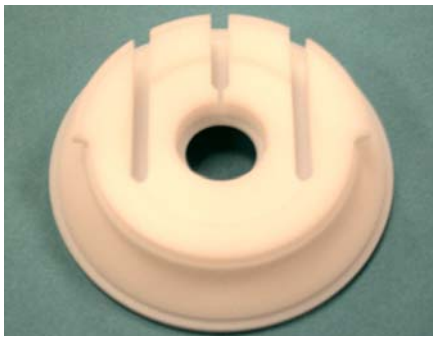

Figure 2 G1982-20101 Chip Cube End Plate Mount

9 Attach the Chip Cube end plate mount to the desolvation assembly with the clamp ring.

10 Modify the end plate with the nanospray drying gas diverter. This will not be necessary for systems currently running a nanospray spray shield.

11 Mount the end plate on to the Chip Cube end plate mount.

12 Position the hole in the drying gas diverter in the down position.

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13 Inset the PTFE tube included in the Chip Cube interface assembly into the port of the drying gas diverter.

This PTFE tube should easily fit into the port and act as a means to direct the drying gas out of the interface assembly.

14 Install the gold canted coil spring is in the nanospray shield. The groove is located inside the inner cone (see Figure 3).

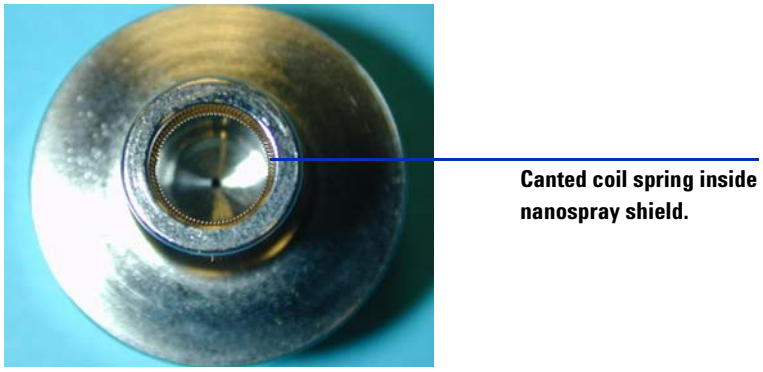

Figure 3 Nanospray shield

15 Insert the nanospray shield onto the end of the capillary (see Figure 4).

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CAUTION Do not twist or turn the nanospray shield when you insert the end of the capillary. You can damage the plating on the capillary, which causes poor ion transmission

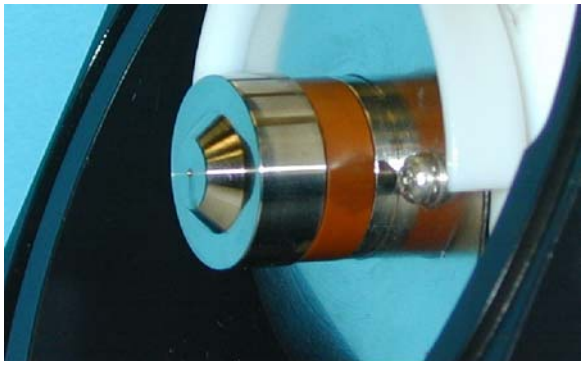

Figure 4 Nanospray shield assembly without gas diverter tube

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Step 2. Install the Chip Cube Interface Assembly

1 Mount the Chip Cube Interface Assembly to the ion trap desolvation assembly of the ion trap as you would any other Agilent ion source.

Two hinge pins on one side engages the two receivers of the desolvation assembly and the typical clasp mechanism holds the adapter tight to the instrument (see Figure 5).

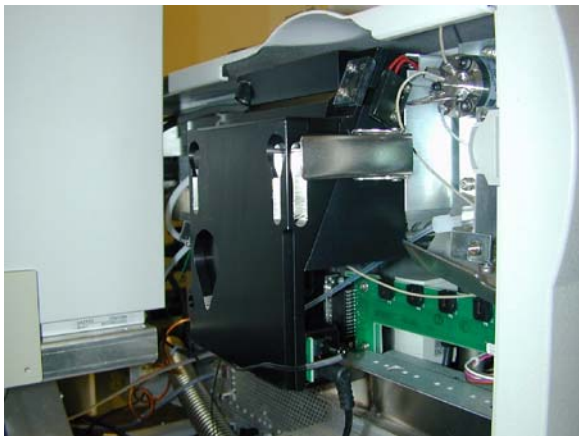

Figure 5 Chip Cube interface assembly closed without nano needle holder

2 Remove the PicoTip emitter holder from the HPLC-Chip/MS interface.

- Remove the T10 screw from the nano needle holder
- Lift the assembly off the plate. See Figure 7.

The needle holder is pre-installed onto the HPLC-Chip/MS interface at the factory. See Figure 6 .

Make sure that you do not remove the top plate of the Chip Cube interface assembly. The PicoTip emitter holder is attached to this plate at the top of the HPLC-Chip/MS interface.

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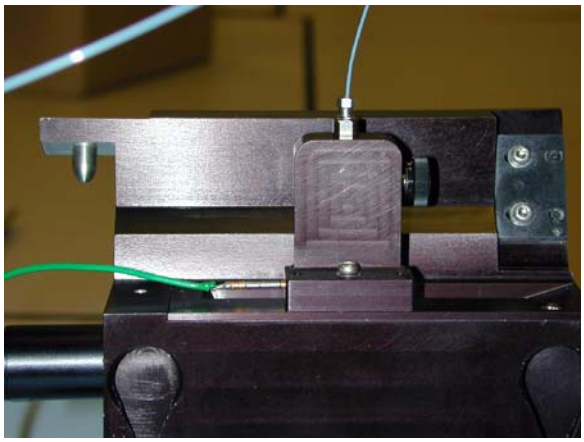

Figure 6 PicoTip emitter holder with ground connection.

Figure 7 HPLC-Chip/MS interface top plate

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3 Locate the grounding cable attached to the base of the HPLC-Chip/MS interface (see Figure 8).

Figure 8 Grounding pin on the base of interface

4 Attach the other end of this grounding cable to one of the high voltage connections on the mass spectrometer (see Figure 9).

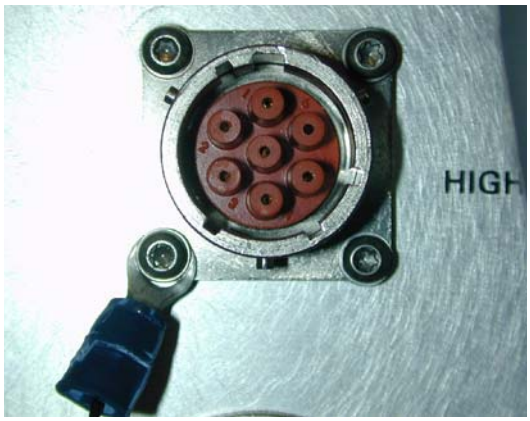

Figure 9 Ground wire attached to high voltage feed through.

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Step 3. Connect the monitor, camera, and illumination kit

1. Install power cord into the back of the monitor.
2. Attach the BNC adapter to the back of the monitor on video input “one”.
3. Attach the video cable from the camera to the BNC adapter on the monitor.
4. Connect the 9 volt power supply to the plug box at the bottom of the HPLC-Chip/MS interface (see Figure 10).

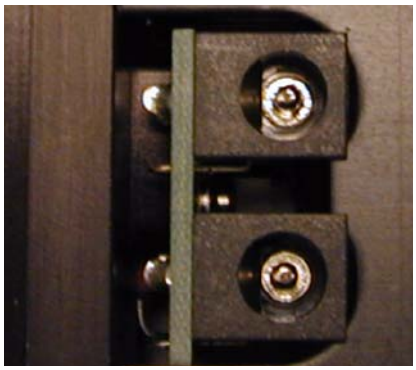

Figure 10 Plug box at base of HPLC-Chip/MS interface.

The power lead for the camera is attached to the remaining open port on this box. There is no particular orientation for either plug top or bottom port (see Figure 10).

At this point. with the monitor power on and the correct channel selected on the monitor. an image of the inside of the source should be visible.

The red illumination LED inside the HPLC-Chip/MS interface should be lit.

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Step 4. Install the Chip Cube interface

You attach the HPLC-Chip/MS interface to the Chip Cube when the PicoTip emitter holder is removed from the adapter.

1 Align the two pins on the interface with the assembly.

The pins fit into the keyhole-style slots to allow the interface to lower into the correct position on the interface assembly and to lock it in place.

2 Follow the instructions in the service manual that is included with the Chip Cube for proper connection to the nanoLC and Agilent Trap system.

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Verifying Performance

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In this chapter, you verify the performance of the Chip Cube with the Agilent 1100 Series LC/MSD Ion Trap.

Use the steps in this chapter to complete the checkout process of the Chip Cube Interface with the Agilent 1100 Series LC/MSD Ion Trap on new installs or bundles. For upgrade systems, see "Verifying Performance on an Upgrade System" on page 27.

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Step 1. Calibrate the Trap

Use the MS calibration and diagnostic chip (p/n G4240-61001) to calibrate the system.

1. Set the source type in the Trap Control Software to **On-line nano ESI**.
2. Insert the MS calibration and diagnostic chip into the HPLC Chip/MS interface, and select operate from the ChemStation interface.
3. Load the following method:
   - **STD_NOR.M** for SL
   - **ULT_SCN.M** for XCT, XCT Plus and XCT Ultra
4. After the method is loaded into the trap control software, change the tune tab view in trap control to “expert”.
5. Set the capillary voltage in the range of 1800 to 2000 volts.
6. Set the drying gas temperature to 200^0C and flow to be 3.0 to 3.5 L/min.
7. If necessary, save the modified calibration methods to a different name that is easily understood (i.e. **chpcal.m**).
8. With the chip loaded, infuse the calibrant via the infusion capillary (p/n G4240-87302) that is attached to port 3 of the stator assembly.
9. Infuse the ESI calibrant with the included 100 µL syringe (p/n 5183-4545) on the standard ion trap infusion pump (p/n 3162-0178). This is infused through the blue line on the Chip Cube.

   Use a dilution of 1:5 in 100% acetonitrile.

   Flow rate for the calibrant is 20 µL/hr .

   The dilution of the calibrant should be made fresh each time of use due to lack of stability.

   Detector calibration will be necessary to ensure proper detection and ion trap filling. This requires an accurate scan calibration.
10. Verify that the scan calibration is within acceptable amu range ($\pm$ 0.2 u).
11. If the mass axis for ultra scan mode is within 0.2 u of the target masses in the calibrant, run the detector calibration.
12. If the masses are off significantly run the scan calibration and then perform the detector calibration.

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Do a scan calibration for all modes. Do an isolation and fragmentation calibration for the Ultra scan mode.

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Step 2.Create method for BSA digest sample

- Create the following LC/MSD method for analysis of the BSA digest sample.
  - Use the specified solvents for the mobile phase on the Nano and Capillary flow pumps.
  - The needle wash solvent of 20% methanol/0.1% formic (methanol) should be made of HPLC grade methanol. Solvents are not supplied with Chip Cubes for upgrade customers.

Method name chpbsams2.m

Column / Chip Protein I.D. chip (p.n. G4240-62001)

Nanaflow pump: A1 = 0.1% formic acid in water

B1 = 90% Acetonitrile / 0.1% formic acid in water

Flow= 600 nL/min

Table 1 Nanoflow pump solvent gradient

Time	%B
7.0	80.0
7.01	3.0

Max pressure 150 bar

Stop time 9 minutes

Post time 0 minutes

Capillary pump: A = 0.1% formic acid in water (Solvents can be mixed in one reservoir for ease of use.)

B = 90% acetonitrile + 0.1% formic acid in water

Flow = 4.00 µL/min

Max pressure 150 bar

Needle flush solvent: 20% methanol + 0.1 % formic acid in water

ALS Therm On, 4^0C

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Injector

Inj Flush Vol = 0.05 µL

Inject with needle wash in flush port for 5.0 sec.

Inj Flush Vol = 8 µL

Loading Pump = G1376

Analytical Pump = G2262

Chip Cube

Inner value = Enrich

Outer Value = Not Used

Time Table = 7.00 min Enrich

MS/MS Method:

Table 2 Source and ion optic conditions

Parameter	Value
Ionization mode	positive nanospray
Scan mode	standard enhanced
Dry gas flow	4.0 L/min.
Dry gas temp	300 ^{0} C
V cap	1800 to 1900 V
Sk 1	30 V
Cap exit offset	100 V

Table 3 Settings for MS

Parameter	Value
Trap drive	85 V
Averages	2
ICC	On
Acc time	150 ms
Smart target ICC	125,000 for XCT 500,000 for XCT Plus and XCT Ultra
Mass range	300 to 2000

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Table 4 Settings for MS/MS

Parameter	Value
Scan mode	Ultra Scan
Number of precursors	5
Averages	2
Frag Amp	1.3 V
Smart Frag	On, 30 to 200 %
Active exclusion	On, 2 spectra, 1 minute
Prefer 2+	On
MS/MS range	100 to 2000
Smart Target ICC	125,000 for XCT 500,000 for XCT Plus and XCT Ultra

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Step 3. Prepare sample for performance checkout

1 Prepare the carboxymethylated BSA tryptic digest standard (p/n G1990-85000) by adding 500 µL of 97% water / 3% acetonitrile with 0.1% formic acid to the BSA standard.

2 If you use a pre-mixed solvent on the capillary pump of the HPLC stack, take the aliquot from this solvent to dilute the BSA sample.

3 Use a vortex mixer to dissolve the BSA sample.

This provides a final working concentration of 1 pmol / µL.

4 Place approximately 50 µL of the 1 pmol / µL BSA sample into a conical bottom vial (p/n 5188-2788) and top with the appropriate caps (p/n 5182-0541).

These vials and caps are included in the shipment.

5 Create a blank vial of 97% water / 3% acetonitrile with 0.1% formic acid using the same conical vial and cap used for the BSA sample.

Step 4. Prepare the system for checkout

1. Create a sequence in ChemStation to make 2 50-fmole injections of on-column (0.05 µL) BSA digest.
2. Prepare the HPLC stack with the appropriate reagents as listed in the method for analysis.
3. Prime the nano and cap LC pumps with their respective solvents, and establish stable flow as per specifications for each pump.
4. Install a Protein ID chip into the Chip Cube in order to establish pump flow.

   Flow should start on the chip once the air from the capillaries and chip is displaced.
5. Prime the µWPS flush port with the needle wash solvent. The needle wash solvent reduces the amount of carryover.
6. Run the sequence.

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3 Processing the MS/MS results of the BSA digest

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In this section, you process the results of the BSA digest in one of three ways:

- with Spectrum Mill
- with MASCOT
- by spectral intepretation in the Trap Data Analysis application

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To process results with Spectrum Mill

1 After acquisition of the data move the data file to the Spectrum Mill server under the folder **E:\SpectrumMill\msdataSM.**

2 (optinoal) Create an indexed data base for faster searching.

a Open the Spectrum Mill web page (see Figure 11).

Figure 11 Spectrum Mill web page.

b Select the **Protein Databases** option under **Utilities.**

c Click the radio button for **Create species subset database** and create a suffix for the subset of **Bovine**.

d Use the **NCBInr** database.

e Select the species **BOS TAURUS.**

f Click the **Create** button.

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3 Click the **Data Extractor** option under **Mass Spectral Interpretation Tools**.

4 In the Data Extractor screen, click **Data Directory**.

A new window opens showing the file structure of the **msdataSM** directory.

5 Select the appropriate data directory from this window and click **OK**.

6 In the Data Extractor screen, select the appropriate cysteine modification (**carboxymethylation**), then **extract**.

7 Once the data extraction is complete, click the **MS/MS Search** tab at the top of the Data Extractor screen.

8 In the MS/MS Search screen select the appropriate data directory if not specified.

9 Select the correct data base of (**NCBInr Bovine**), enzyme (**trypsin**), instrument (**ion trap**), and the cysteine modification (**carboxymethylation**).

10 Click the **Start Search** button at the top of the MS/MS Search screen.

11 To tabulate the results of the MS/MS search, click the **Protein/Peptide Summary** button at the top of the screen.

12 In Protein/Peptide Summary, click the **Summarize** button.

13 Examine the results aseess the quality of the data. BSA should be correctly identified by multiple peptides. See Figure 12 for an example of results.

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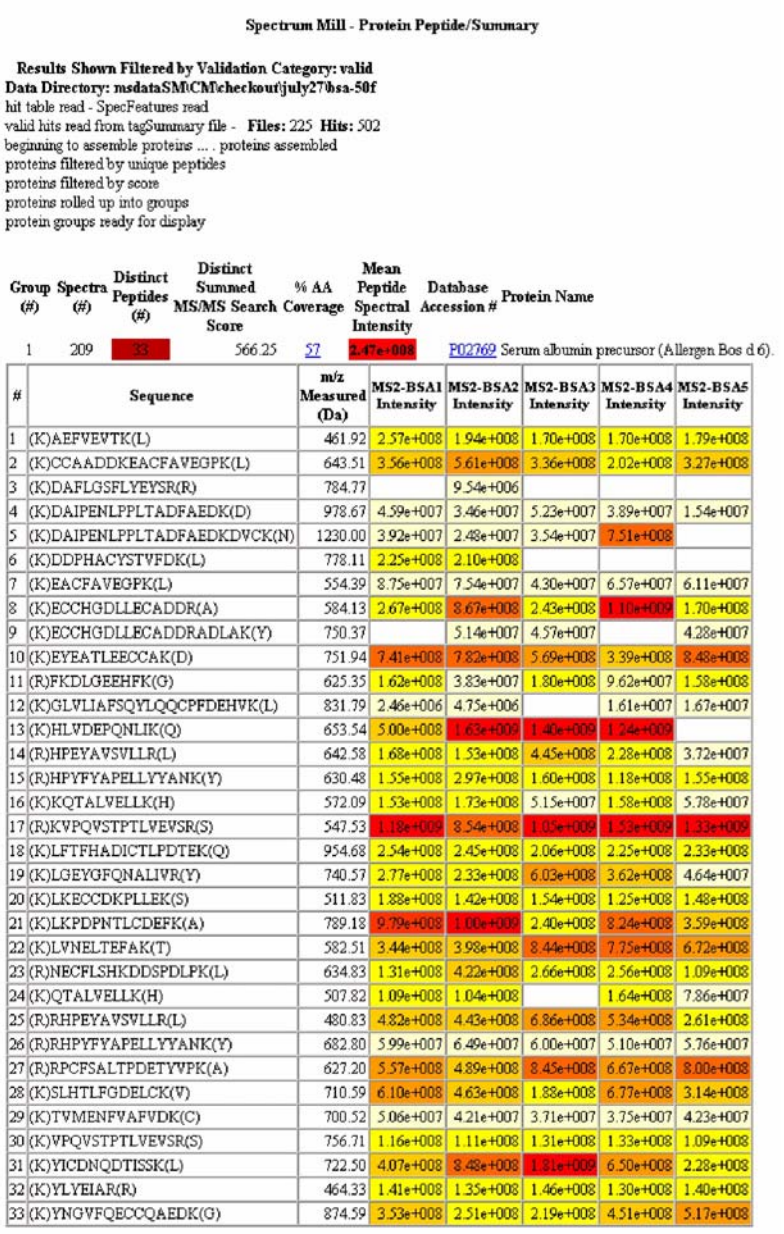

Spectrum Mill - Protein Peptide/Summary

Results Shown Filtered by Validation Category: valid
Data Directory: msdataSM\CM\checkout\july27\bsa-50f
hit table read - SpecFeatures read
valid hits read from tagSummary file - **Files:** 225 **Hits:** 502
beginning to assemble proteins ... . proteins assembled
proteins filtered by unique peptides
proteins filtered by score
proteins rolled up into groups
protein groups ready for display

Group (#)	Spectra (#)	Distinct Peptides (#)	Distinct Summed MS/MS Search Score	% AA Coverage	Mean Peptide Spectral Intensity	Database Accession #	Protein Name
1	209	33	566.25	57	2.47e+008	P02769	Serum albumin precursor (Allergen Bos d 6).

#	Sequence	m/z Measured (Da)	MS2-BSA1 Intensity	MS2-BSA2 Intensity	MS2-BSA3 Intensity	MS2-BSA4 Intensity	MS2-BSA5 Intensity
1	(K)AEFVEVTK(L)	461.92	2.57e+008	1.94e+008	1.70e+008	1.70e+008	1.79e+008
2	(K)CCAADDKEACFAVEGPK(L)	643.51	3.56e+008	5.61e+008	3.36e+008	2.02e+008	3.27e+008
3	(K)DAFLGSFLYEYSR(R)	784.77		9.54e+006			
4	(K)DAIPENLPPLTADFAEDK(D)	978.67	4.59e+007	3.46e+007	5.23e+007	3.89e+007	1.54e+007
5	(K)DAIPENLPPLTADFAEDKDVCK(N)	1230.00	3.92e+007	2.48e+007	3.54e+007	7.51e+008	
6	(K)DDPHACYSTVFDK(L)	778.11	2.25e+008	2.10e+008			
7	(K)EACFAVEGPK(L)	554.39	8.75e+007	7.54e+007	4.30e+007	6.57e+007	6.11e+007
8	(K)ECCHGDLLECADDR(A)	584.13	2.67e+008	8.67e+008	2.43e+008	1.10e+009	1.70e+008
9	(K)ECCHGDLLECADDRADLAK(Y)	750.37		5.14e+007	4.57e+007		4.28e+007
10	(K)EYEATLEECCAK(D)	751.94	7.41e+008	7.82e+008	5.69e+008	3.39e+008	8.48e+008
11	(R)FKDLGEEHFK(G)	625.35	1.62e+008	3.83e+007	1.80e+008	9.62e+007	1.58e+008
12	(K)GLVLIAFSQYLQQCPFDEHVK(L)	831.79	2.46e+006	4.75e+006		1.61e+007	1.67e+007
13	(K)HLVDEPQNLIK(Q)	653.54	5.00e+008	1.63e+009	1.40e+009	1.24e+009	
14	(R)HPEYAVSVLLR(L)	642.58	1.68e+008	1.53e+008	4.45e+008	2.28e+008	3.72e+007
15	(R)HPYFYAPELLYYANK(Y)	630.48	1.55e+008	2.97e+008	1.60e+008	1.18e+008	1.55e+008
16	(K)KQTALVELLK(H)	572.09	1.53e+008	1.73e+008	5.15e+007	1.58e+008	5.78e+007
17	(R)KVPQVSTPTLVEVSR(S)	547.53	1.18e+009	8.54e+008	1.05e+009	1.53e+009	1.33e+009
18	(K)LFTFHADICTLPDTEK(Q)	954.68	2.54e+008	2.45e+008	2.06e+008	2.25e+008	2.33e+008
19	(K)LGEYGFQNALIVR(Y)	740.57	2.77e+008	2.33e+008	6.03e+008	3.62e+008	4.64e+007
20	(K)LKECCDKPLLEK(S)	511.83	1.88e+008	1.42e+008	1.54e+008	1.25e+008	1.48e+008
21	(K)LKPDPNTLCDEFK(A)	789.18	9.79e+008	1.00e+009	2.40e+008	8.24e+008	3.59e+008
22	(K)LVNELTEFAK(T)	582.51	3.44e+008	3.98e+008	8.44e+008	7.75e+008	6.72e+008
23	(R)NECFLSHKDDSPDLPK(L)	634.83	1.31e+008	4.22e+008	2.66e+008	2.56e+008	1.09e+008
24	(K)QTALVELLK(H)	507.82	1.09e+008	1.04e+008		1.64e+008	7.86e+007
25	(R)RHPEYAVSVLLR(L)	480.83	4.82e+008	4.43e+008	6.86e+008	5.34e+008	2.61e+008
26	(R)RHPYFYAPELLYYANK(Y)	682.80	5.99e+007	6.49e+007	6.00e+007	5.10e+007	5.76e+007
27	(R)RPCFSALTPDETYVPK(A)	627.20	5.57e+008	4.89e+008	8.45e+008	6.67e+008	8.00e+008
28	(K)SLHTLFGDELCK(V)	710.59	6.10e+008	4.63e+008	1.88e+008	6.77e+008	3.14e+008
29	(K)TVMENFVAFVDK(C)	700.52	5.06e+007	4.21e+007	3.71e+007	3.75e+007	4.23e+007
30	(K)VPQVSTPTLVEVSR(S)	756.71	1.16e+008	1.11e+008	1.31e+008	1.33e+008	1.09e+008
31	(K)YICDNQDTISSK(L)	722.50	4.07e+008	8.48e+008	1.81e+009	6.50e+008	2.28e+008
32	(K)YLYEIAR(R)	464.33	1.41e+008	1.35e+008	1.46e+008	1.30e+008	1.40e+008
33	(K)YNGVFQECCQAEDK(G)	874.59	3.53e+008	2.51e+008	2.19e+008	4.51e+008	5.17e+008

PRELIMINARY

Figure 12 An example of a results file

To process results with MASCOT

1 Check that the Deconvolution license is installed.

2 Open the data file in DataAnalysis.

The TIC is displayed.

3 Right-click the Chromatogram window and from the context menu, click **Add Base Peak Chromatogram > All MS**, and for **All MS(n).**

Three traces are available in the Chromatogram window.

4 Highlight the range in the BPC MS(n) that shows data peaks.

5 From the drop down menus click **Find > Compounds > AutoMS(n)** using default method parameters.

6 Select the **Compound Mass Spectra** option in the Analysis List window and click **Deconvolute > Mass Spectra.**

7 Select the **Compound Mass Spectra** option in the Analysis List window and click **File > Export > Compounds**.

The Export Compound List window appears.

8 Export the data to this folder and save the data as a ***.mgf** file (MASCOT Generic File).

9 Access the MASCOT web site at www.matrixscience.com.

10 If an internal MASCOT server is available use it for your search.

11 Use the settings shown in Figure 13.

PRELIMINARY

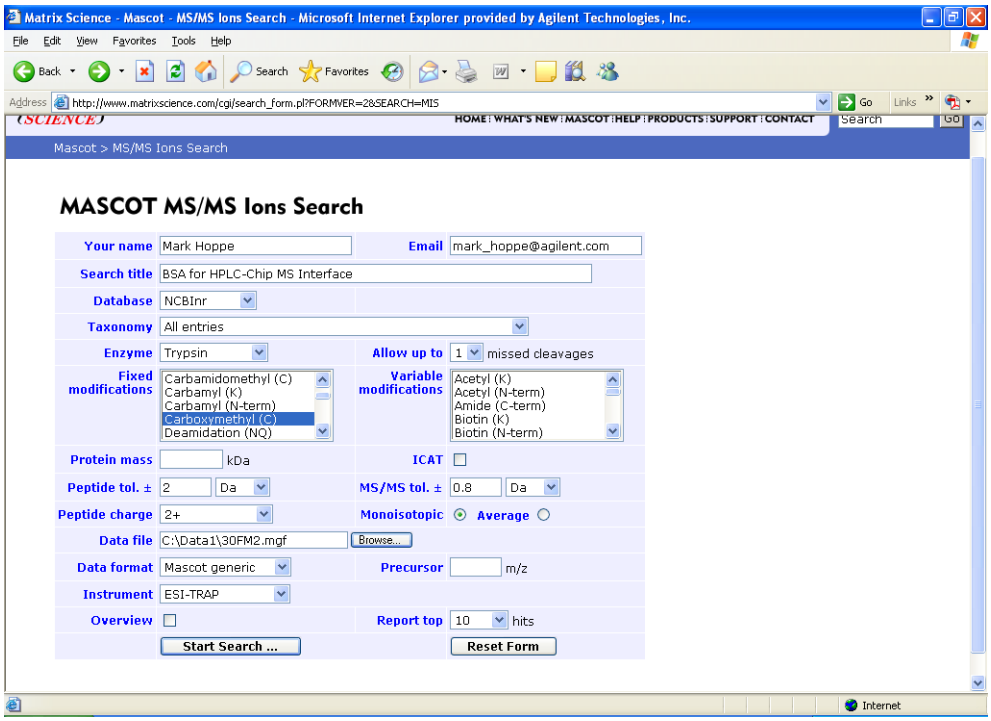

Figure 13 MASCOT MS/MS Ions Search web site.

12 Click **Start Search** at the bottom of the screen.

Passing results are those that are a significant match (above the “green”) for BSA.

To process the results by spectral interpretation

This step does not require Spectrum Mill or MASCOT.

1 Do step 1 through step 5 of “To process results with MASCOT” on page 23 to create a BPC similar to Figure 14.

2 Examine the spectra and look for ions that match ones in Figure 14.

PRELIMINARY

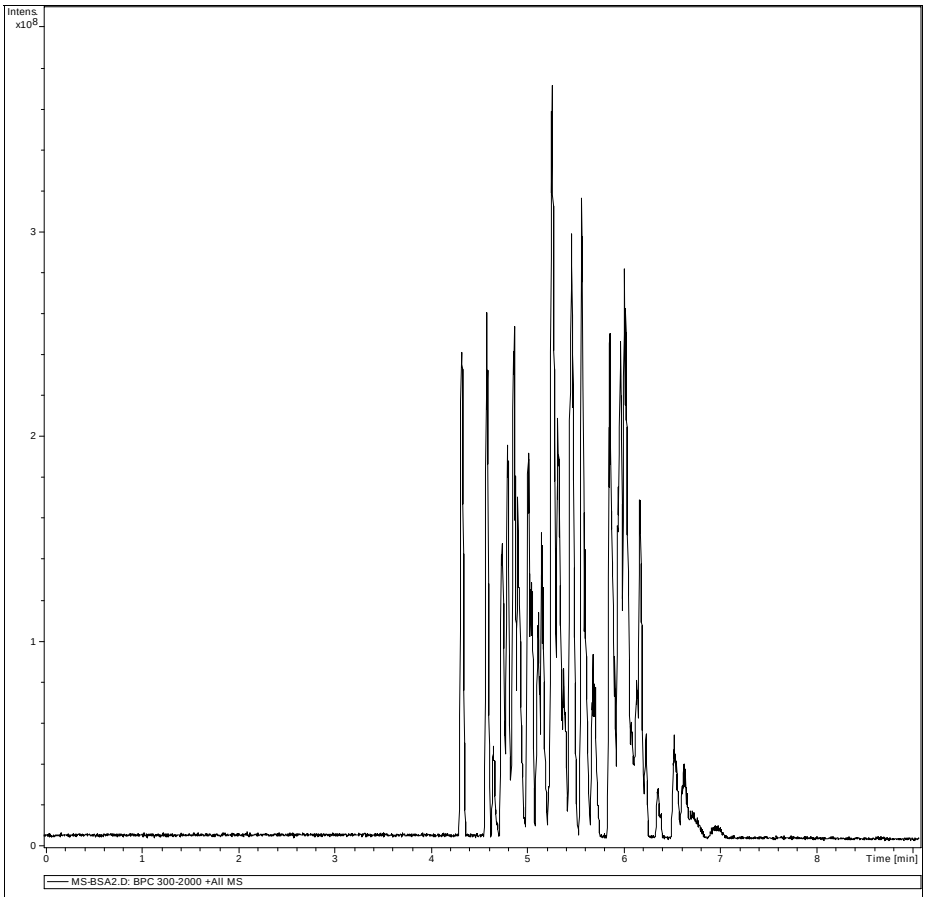

Figure 14 BSA spectra

PRELIMINARY

PRELIMINARY

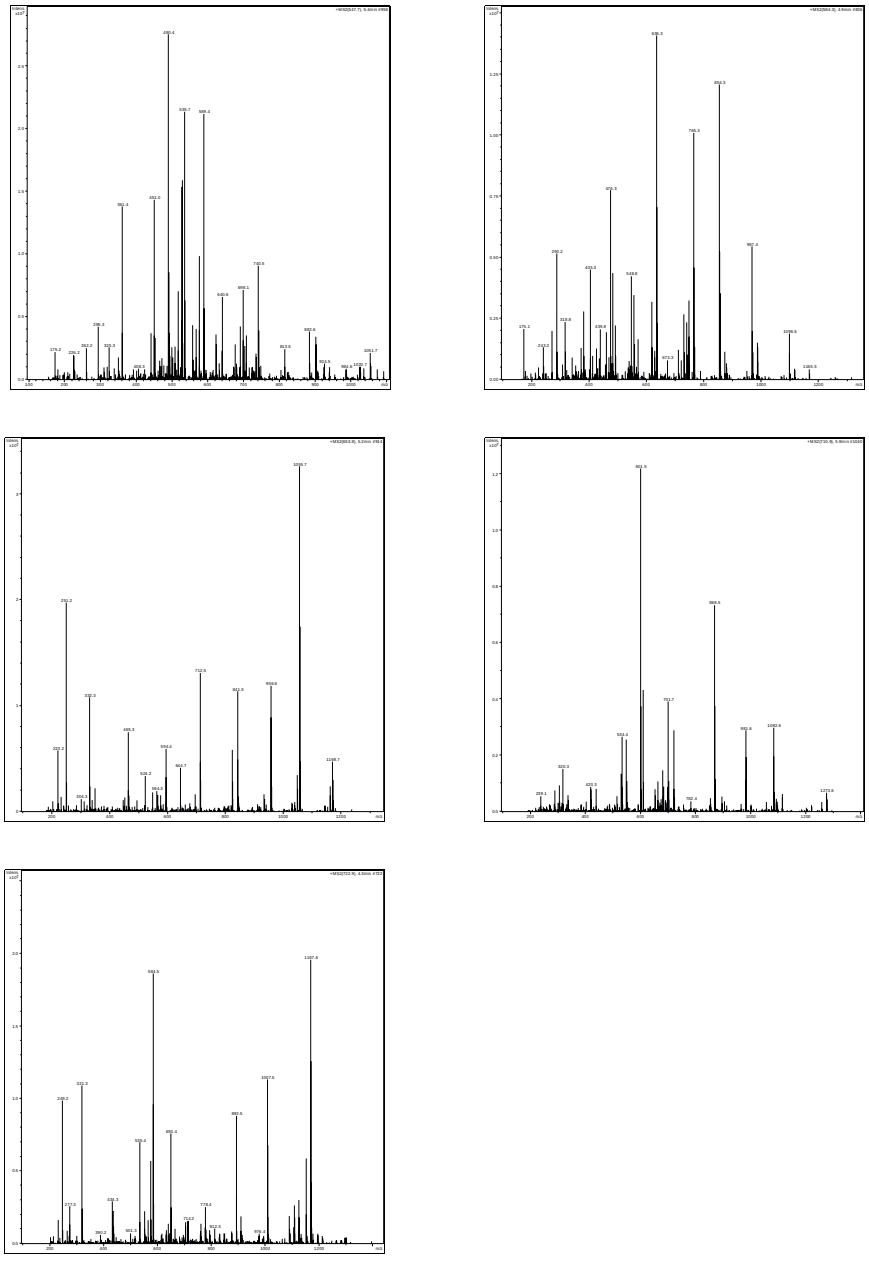

Figure 15 MS spectra

4 Verifying Performance on an Upgrade System

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PRELIMINARY

In this chapter, you verify the performance of the Chip Cube with the Agilent 1100 Series LC/MSD Ion Trap on an upgrade system.

An upgrade system is one in which:

- The ion trap is already installed.
- The Chip Cube with necessary components is being added after the initial mass spectrometer install has been completed.

Sensitivity checkout will not be performed on this system due to prior execution of sensitivity checkout at time of mass spectrometer installation. Customers are responsible for running BSA digest standard on their system before installation to demonstrate the functionality on the existing components.

The BSA tryptic digest standard (part number G1990-85000) is available from Agilent Technologies, Inc. The customer is responsible for running 50 fmol of on-column BSA digest with a standard method to show instrument functionality and adequate sensitivity.

Step 1. Create the method

- Create the following check out method.

This will run an MS-only method to prove chromatography of the Chip and performance of the LC coupled with the Chip Cube and mass spectrometer.

Method name chpbsams.m

Column / Chip Protein I.D. chip (p/n G4240-62001)

Nanaflow pump: A1 = 0.1% formic acid in water

B1 = 90% Acetonitrile / 0.1% formic acid in water

Flow= 600 nL/min

Table 5 Nanoflow pump solvent gradient

Time	%B
7.0	80.0
7.01	3.0

Max pressure 150 bar

Stop time 9 minutes

Post time 0 minutes

Capillary pump: A1 = 0.1% formic acid in water

B1 = 90% Acetonitrile / 0.1% formic acid in water

Flow = 4.00 µL/min

Max pressure 150 bar

Needle flush solvent: 20% methanol / 80% H_2O with 0.1 % formic acid

ALS Therm On, 4^0C

PRELIMINARY

Injector

Inj Flush Vol = 0.05 µL

Inject with needle wash in flush port for 5.0 sec.

Inj Flush Vol = 8 µL

Loading Pump = G1376

Analytical Pump = G2262

Chip Cube

Inner value = Enrich

Outer Value = Not Used

Time Table = 7.00 min Enrich

MS/MS Method:

Table 6 Source and ion optic conditions

Parameter	Value
Ionization mode	positive nanospray
Scan mode	standard enhanced
Dry gas flow	4.0 L/min.
Dry gas temp	300 ^{0} C
V cap	1900 V

Table 7 Settings for MS

Parameter	Value
Ion trap conditions	MS
Trap drive	85 V
Averages	2
ICC	On
Acc time	150 ms
Smart target ICC	125,000 for XCT 500,000 for XCT Plus and XCT Ultra
Mass range	300 to 2000

PRELIMINARY

Table 8 Settings for MS/MS

Parameter	Value
Ion trap conditions	MS/MS
Scan mode	Ultra Scan
Number of precursors	5
Averages	2
Frag Amp	1.3 V
Smart Frag	On, 30 to 200 %
Active exclusion	On, 2 spectra, 1 minute
Prefer 2+	On
MS/MS range	100 to 1800
Smart Target ICC	125,000 for XCT 500,000 for XCT Plus and XCT Ultra

PRELIMINARY

Step 2. Prepare sample for performance checkout

1. Prepare the carboxymethylated BSA tryptic digest standard (p/n G1990-85000) by adding 500 µL of 97% water / 3% acetonitrile with 0.1% formic acid to the BSA standard.
2. If you use a pre-mixed solvent on the capillary pump of the HPLC stack, take the aliquot from this solvent to dilute the BSA sample.
3. Use a vortex mixer to dissolve the BSA sample.

   This provides a final working concentration of 1 pmol / µL.
4. Place approximately 50 µL of the 1 pmol / µL BSA sample into a conical bottom vial (p/n 5188-2788) and top with the appropriate caps (p/n 5182-0541).

   These vials and caps are included in the shipment.
5. Create a blank vial of 97% water / 3% acetonitrile with 0.1% formic acid using the same conical vial and cap used for the BSA sample.

PRELIMINARY

Step 3. Prepare the system for checkout

1. Create a sequence in ChemStation to make 2 50-fmole injections of on-column (0.05 µL) BSA digest.
2. Prepare the HPLC stack with the appropriate reagents as listed in the method for analysis.
3. Prime the nano and cap LC pumps with their respective solvents, and establish stable flow as per specifications for each pump.
4. Install a Protein ID chip into the Chip Cube in order to establish pump flow.

   Flow should start on the chip once the air from the capillaries and chip is displaced.
5. Prime the µWPS flush port with the needle wash solvent. The needle wash solvent reduces the amount of carryover.
6. Run the sequence.
7. Create the BPC for MS on the samples. See Figure 16 for an example.
8. Examine the data for the presence of BSA ions. See Table 9.

Table 9 BSA ions

$[m+2N]^{+2}$
751.9
653.5
547.5
789.2
722.5

PRELIMINARY

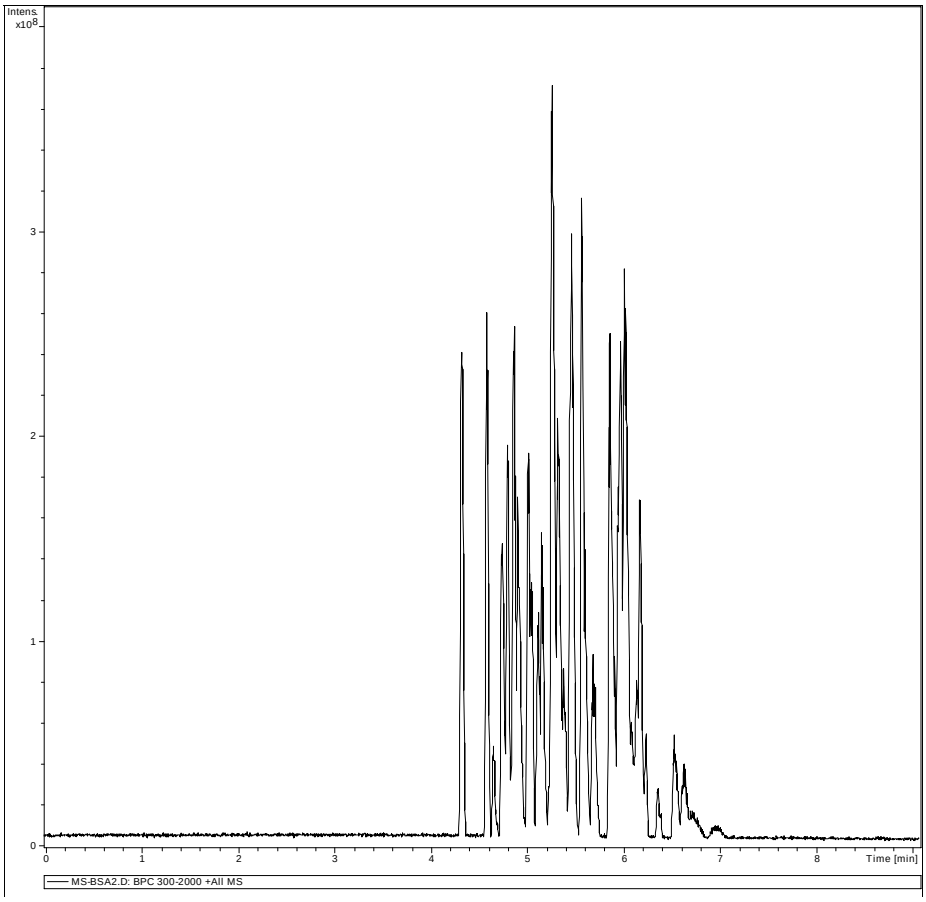

Figure 16 BSA spectra

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5 Maintenance

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To create ions in the Chip Cube Interface Assembly 40

This chapter contains maintenance tasks for the Chip Cube Interface Assembly.

PRELIMINARY

To replace the camera

1 Loosen the set screw located at the top of the adapter above the camera.

This screw is a 1.5 mm Allen head screw.

2 Slide the camera out of its mount (see Figure 17).

Figure 17 Camera removed from adapter mount.

3 Install the new camera using the end cap, PicoTip emitter, and counter electrode as focal points.

The camera should slide into the adapter and about 12 to 15 mm of the camera should stick into the adapter with all objects in good focus.

If the camera is not in focus, see "To focus the camera" on page 37.

PRELIMINARY

To focus the camera

New cameras ordered as replacement parts will come pre-focused in the factory. You only need to focus the camera if the camera is not correctly focused upon shipment.

1 Insert camera into the adapter port to a depth between 12 to 15 mm (see Figure 18).

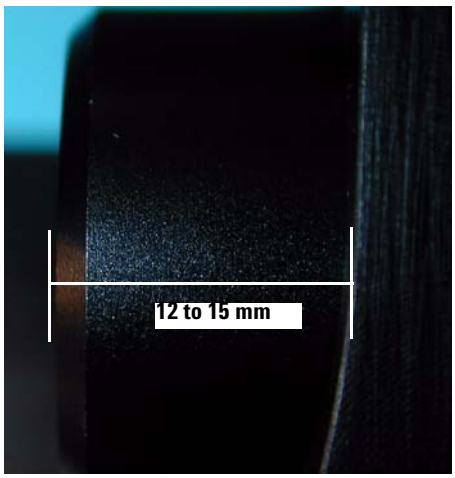

Figure 18 Distance the camera protrudes into the adapter is 12-15 mm. The red line shows how the depth is measured.

2 Check that the camera does not focus over the 12 to 15 mm range of movement allowed.

If the focus still cannot be achieved, remove the front cover of the camera while it is located in the adapter.

At this point the focusing ring will be exposed.

3 Turn the ring until you have a clear focus on the offset electrode and cone set.

4 Remove the camera from the adapter and screw the cap back on tightly.

5 Re-insert the camera into the adapter and try to achieve the same focus by adjusting the depth in the 12 to 15mm range.

PRELIMINARY

To replace the LED post assembly.

1 Loosen the 0.8 mm Allen head screw from the post assembly with the included 0.8 mm Allen wrench (p/n 8710-1225).

2 Remove the cover from the plug box.

3 Disconnect the mini plug on the board in the plug box (see Figure 19)

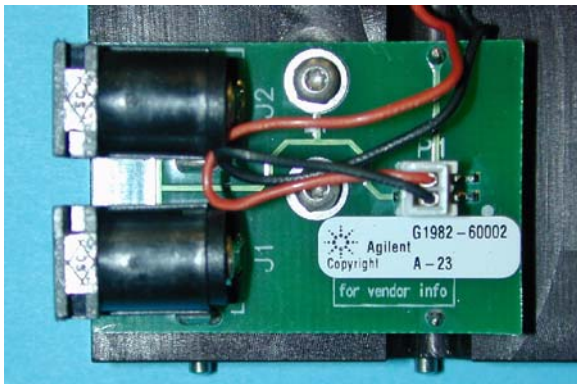

Figure 19 Plug box with cover removed and mini plug for LED showing.

4 Slide the post assembly out of the top of the adapter (see Figure 20).

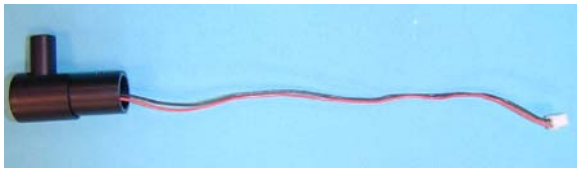

Figure 20 Post assembly.

5 Reverse the process to install the new post assembly, but do not tighten the 0.8 mm set screw.

6 Adjust the position of the LED on the post assembly by using the monitor as reference, and rotating the post assembly until the light from the LED is just behind the counter electrode.

7 Tighten the 0.8 mm set screw to hold the post assembly in place.

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To replace the plug box

1. Remove the T-10 Torx screw from the plug box.
2. Remove the cover from the plug box.
3. Disconnect the mini plug from the board.

   The mini plug for the post assembly is located under the cover (see Figure 19).
4. Remove the two screws that hold the board down.
5. The two screws are T-6 Torx head (will be changed to Phillips head screws by production).
6. Replace the board with a new part and reassemble.

To clean the adapter

The adapter will need to be cleaned on an as needed basis. You use solvents to wash out the area around the offset electrode.

- Spray an acceptable solvent into the adapter with a squeeze bottle. Use sparingly.

  Acceptable solvents methanol, isopropanol, water, and a mixture of 50/50 isopropanol and acetonitrile.

CAUTION Do not submerge the adapter into any solvents due to the electrical components that are attached.

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To create ions in the Chip Cube Interface Assembly

For basic troubleshooting of the HPLC-Chip MS Interface, Nanoflow HPLC, and ion trap, you need to break the system down into sub components. The PicoTip emitter holder and assembly allows you to create ions in the HPLC-Chip MS Interface adapter separate from the HPLC-Chip MS Interface or Nanoflow HPLC.

1 Assemble the PicoTip emitter assembly.

- The PicoTip emitter assembly includes one finger tight nut (p/n G1982-60023), a conductive ferrule (p/n 0100-2262), zero dead volume fitting (p/n 5022-2184), 100 µL manual syringe (p/n 5183-4545) and a 0.75 mm i.d. capillary (p/n 5042-1379).
- This emitter assembly is assembled as a normal PicoTip emitter used for infusion on the Agilent Nanospray source. (see Figure 21).

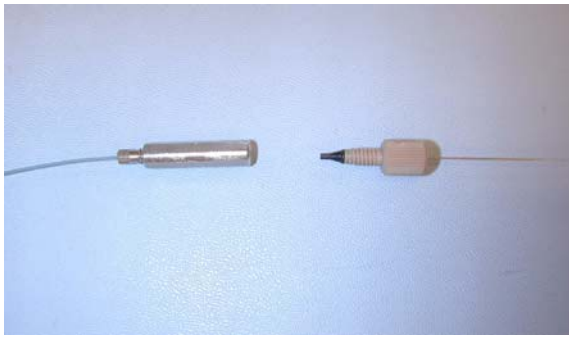

Figure 21 PicoTip assembly.

2 Connect the PicoTip emitter holder to the HPLC-Chip MS Interface adapter.

- The PicoTip emitter holder can be mounted onto the HPLC-Chip MS adapter via a T-10 Torx head screw mounted onto the top plate of the adapter (see Figure 22).

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Figure 22 Top plate of adapter.

3 Connect the grounding wire from the gold pin on the PicoTip emitter holder to the grounding pin at the base of the HPLC-Chip MS interface adapter (see Figure 23 and Figure 24)

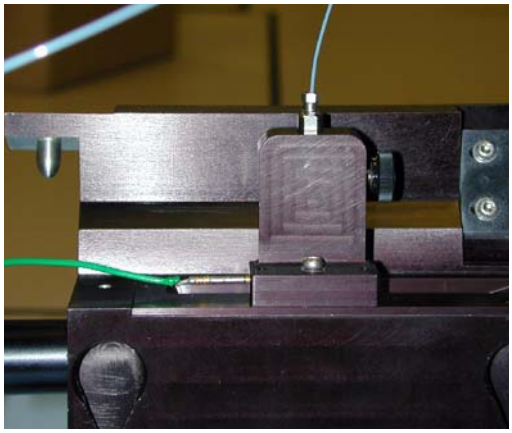

Figure 23 Ground lead attached to gold pin on PicoTip emitter holder.

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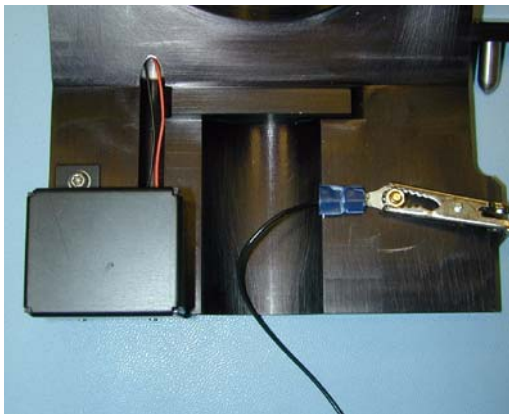

Figure 24 Grounding clip on the adapter ground pin.

4 To use the PicoTip assembly and holder.

a Insert the PicoTip assembly into the holder and align emitter as seen with the picture below (see Figure 25).

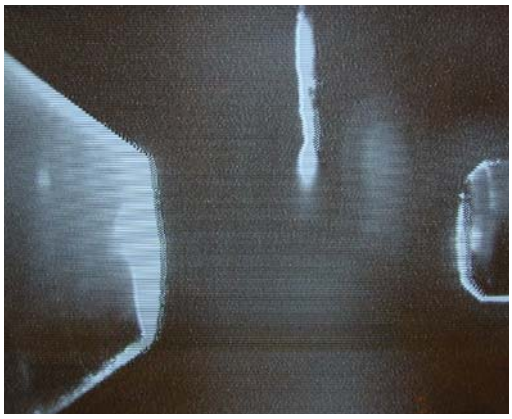

Figure 25 Alignment of PicoTip emitter

b Infuse ESI calibrant at a rate of 20 µL/hr.

- You may need to make a 1:5 dilution in ACN of the ESI calibrant due to the high sensitivity of nanospray.

c Load the appropriate method for the particular ion trap, and make the changes listed below. (SL = stdnor.m, XCT & XCT Plus = ultscn.m)

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d Start the capillary voltage at 1400 volts, and increase until a stable spray is initiated.

e Typical range of voltage for optimal spray is 1800 to 2000 volts.

The accumulation time will be very low (0.2 to 0.1 ms).

Drying gas flow rates should be around 4 L/min at 200^{0}C

f Observe the ions in the ion trap control portion of the software, and verify that the ions are at the correct mass assignment.

g Verify the resolution of the peaks in the calibrant.

You should see clear isotope peaks next to each mass and good resolution on the main C^{12} peaks in standard enhanced mode.

h When you are done with the PicoTip emitter assembly and holder, remove it from the adapter.

Remove the PicoTip emitter assembly first, followed by the grounding cable, and finally the T-10 Torx head screw that holds the adapter on.

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5 Maintenance

PRELIMINARY

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PRELIMINARY

PRELIMINARY

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In This Book

This book includes information that describes how to install, verify and maintain the Agilent Chip Cube Interface Assembly with your Agilent Trap system.

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