Bruker BioSpin

CryoFit

Handling of Flow Cell and Capillaries

User Guide

Version 002

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1. Overview

This document describes the installation and removal instructions required for a flow cell in a CryoFit.

This is necessary for:

- **New CryoFit's**. For protection during transport the flow cell is shipped together with the CryoFit, inside the CryoFit box, but is ***not mounted***. In this case the user should proceed to Chapter 3 of this document.
- **Repairs*:*** In case a flow cell needs to be replaced for repair.

Note that:

- The flow cell comes with the in/outlet **capillaries connected**.
- All flow cells that are shipped are **NMR tested**!

Material requirements:

- 2 x latex-type **gloves**, powder free and lint-free **paper towels** (e.g. Kim wipes).
- 10 ml **acetone** for cleaning.
- Small **screwdriver** size (3 mm), 2 x **wrenches 3.2 mm.**
- Space on a **table/workbench** (60 x 200 cm).

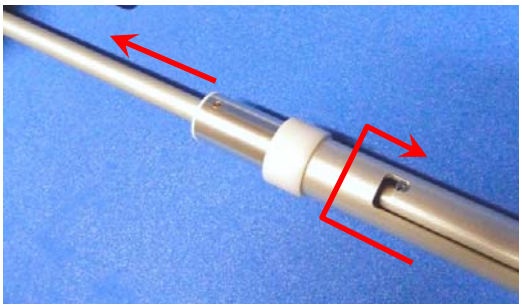
WARNING: Under no circumstances should a damaged flow cell be used, or should you attempt to repair a broken flow cell. If the flow cell is not correctly positioned it may damage the CryoProbe during the insertion procedure.

2. Removing the Flow Cell

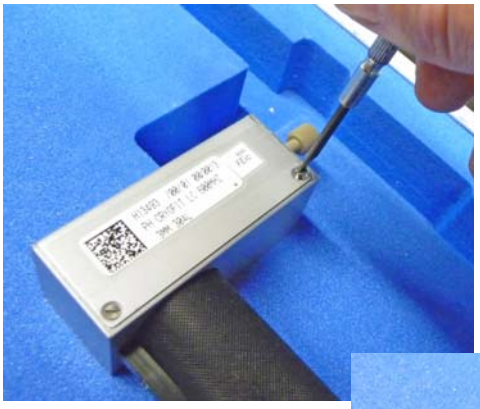
2.1 Preparation

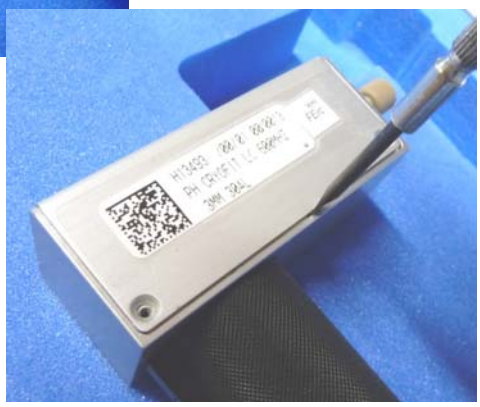
Use the CryoFit storage case with the **blue foam** insert as **support**.

Slide the flow cell **completely into** the CryoFit and **lock it**.

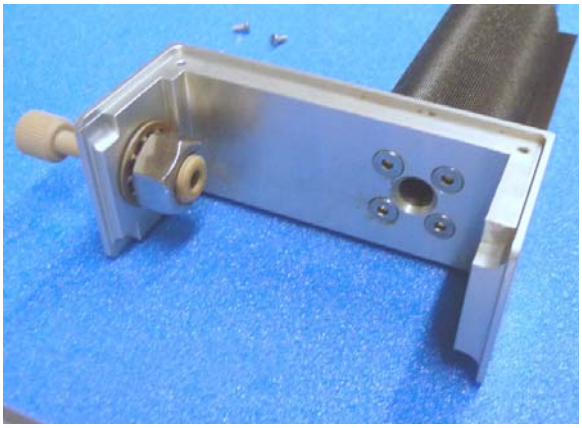


Open the **four screws** that hold the **U-shaped cover** of the top part of the CryoFit.



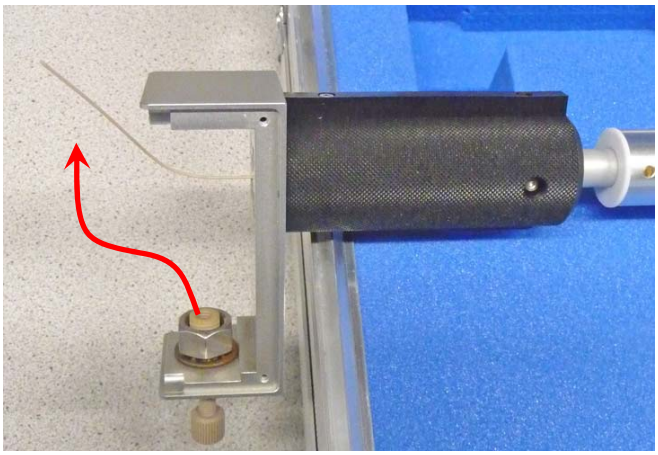


Remove the U-shaped **cover**.

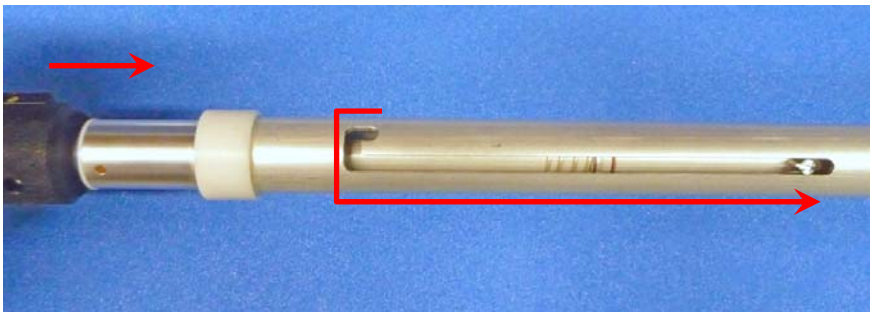


2.2 Removing the Current Flow Cell and Capillary

1. **Unscrew** the fitting and **disconnect capillary** from the fitting.

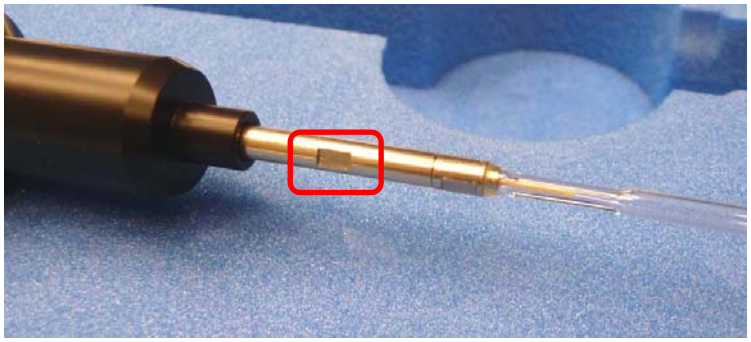


2. Slide the holder **completely out** of the CryoFit.



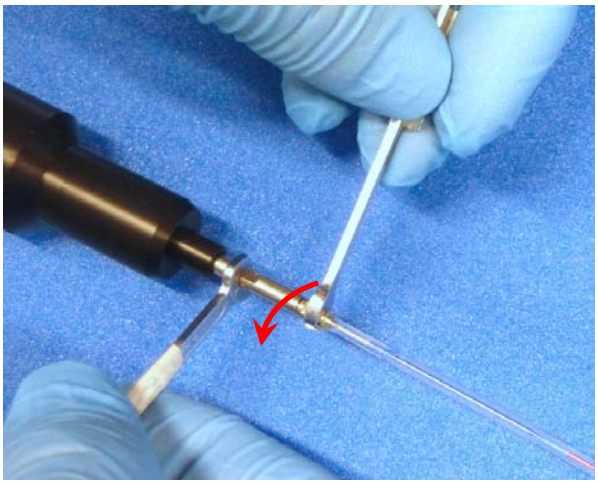
Removing the Flow Cell

3. The **flat part** of the **metal rod** for the wrench must be **outside**.

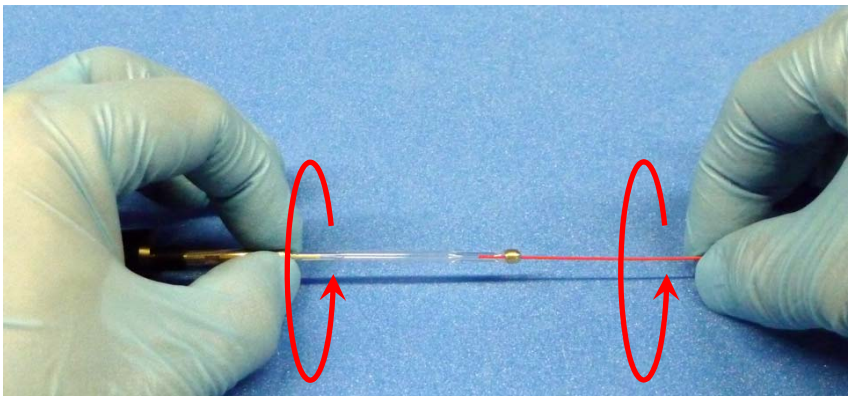


4. Put the **gloves** on.

5. Use the **3.2mm wrenches** to loose the connection of flow cell and metal rod by **½ turn**.



6. **Unscrew** the flow cell completely **by turning** it with your fingers.



7. While rotating the cell the **inlet** and **outlet** capillary **must freely rotate**, otherwise they will **disconnect** them from the cell!

8. **Pull** flow **cell and capillary** out of the CryoFit.

9. Do **not disconnect** the capillaries!

10. **Pack** it in **lint free paper** and store it dust free place.

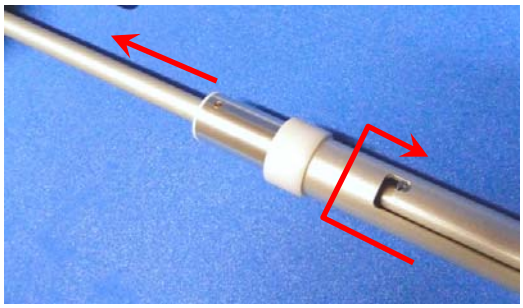
11. Avoid any contact with **sharp parts** that can scratch the surface of the glass.

3. Installing a New Flow Cell

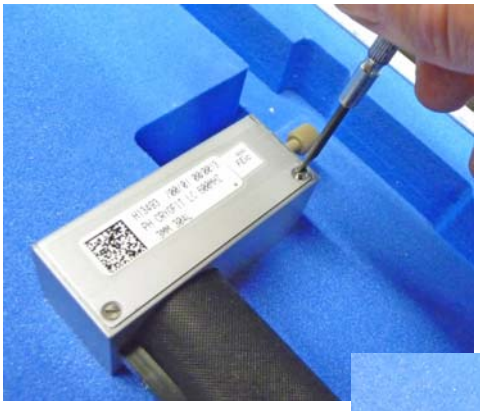
3.1 Preparation

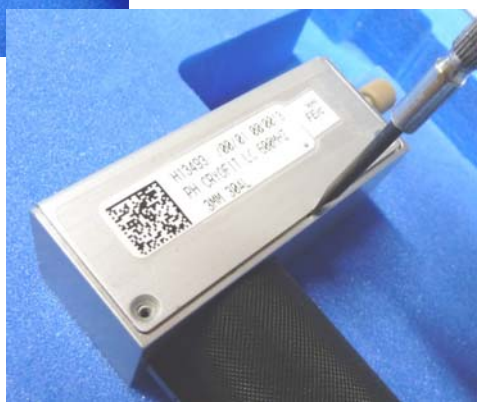
1. Use the CryoFit storage case with the **blue foam** insert as **support**.

2. Slide the flow cell **completely into** the CryoFit and **lock it**.

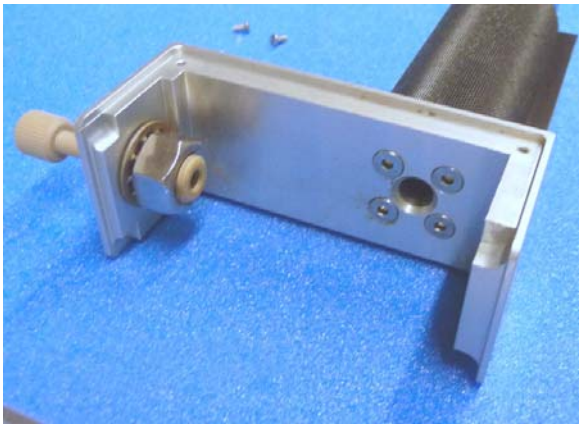


3. Open the **four screws** that hold the **U-shaped cover** of the top part of the CryoFit.



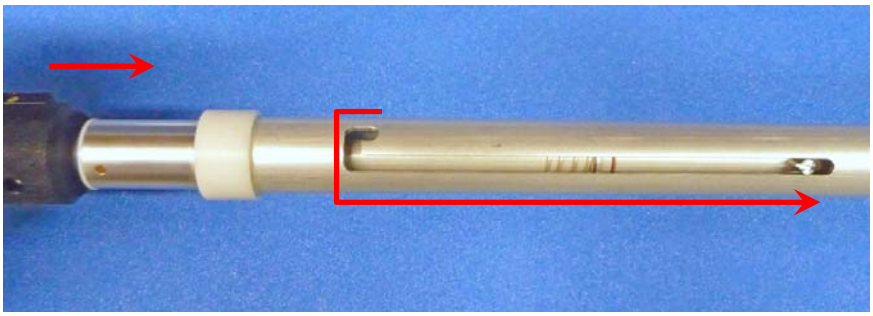


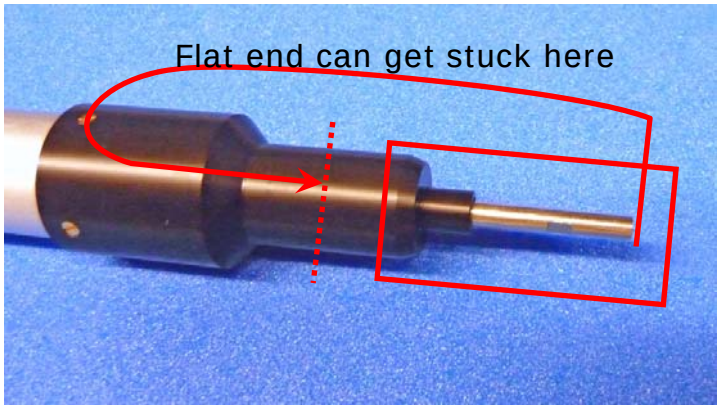
4. **Remove** the U-shaped **cover**.



3.2 Additional Preparations

1. Slide the holder **completely out** of the CryoFit. If the holder gets stuck halfway, hold the complete CryoFit vertically and move the holder up/down until it slides out.





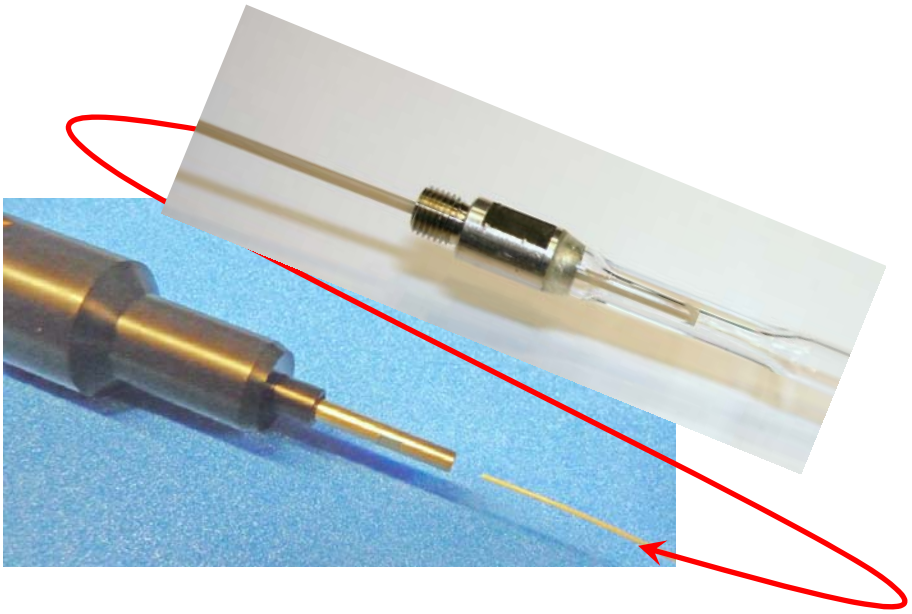
2. You must see the **metal rod** at the **bottom** of the CryoFit.

3. Put the **gloves** on and remove the new flow cell from the packing.
   - Do **not touch** the flow cell with **bare fingers**!
   - Do **not pull** on the capillaries.

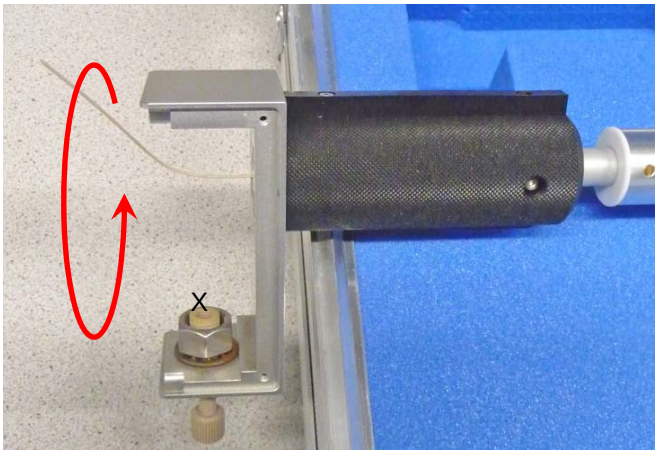
3.3 Inserting the Capillary

1. Slide outlet capillary into the metal rod.

   Note: The outlet capillary is a brown peek capillary with a 0.4 mm inner diameter:

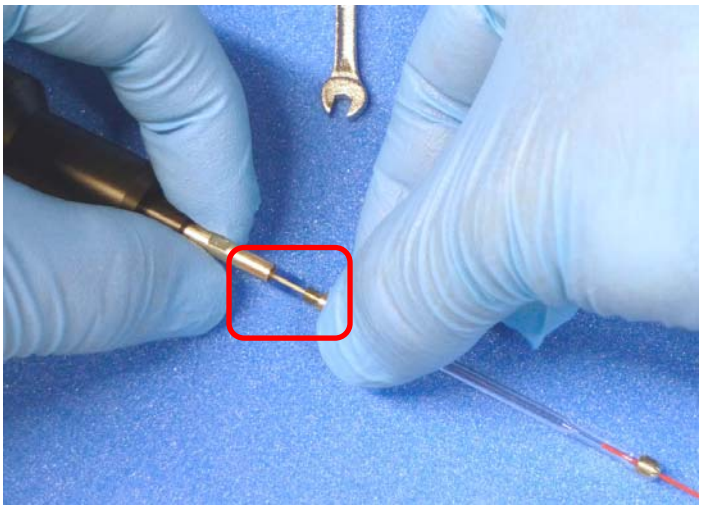


2. The outlet capillary is connected to the side of the flow cell where the metal **thread** is visible.
3. **Push** the capillary **completely through** the CryoFit until is comes out at the top.
4. Do **not connect** the capillary yet!
5. Place the upper part over the rim of the CryoFit box. Ensure the capillary **can rotate** freely (especially if the end is bent) as it will spin during the connection of the flow cell.



3.4 Mounting the Flow Cell

1. **Connect** the metal thread to the rod.



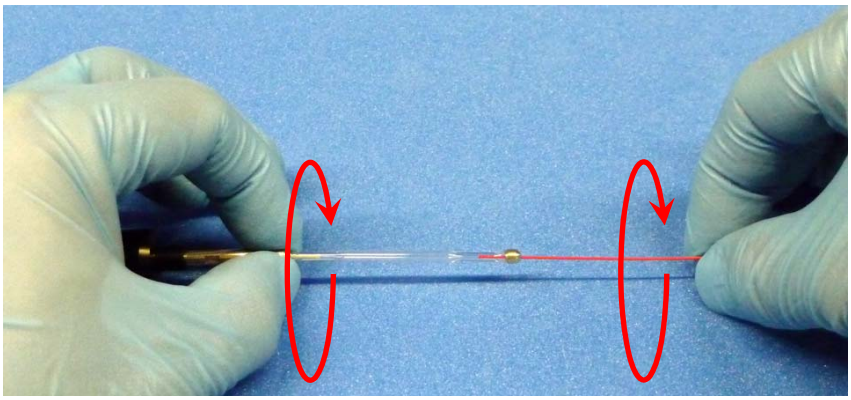
2. Screw the flow cell into the rod, by **turning** cell and inlet capillary **simultaneously**!

   Note: Three different inlet capillaries are in use:

   Red: LC-SPE-NMR (0.13 mm inner diameter)

   Blue: LC-NMR or LC-NMR and LC-SPE-NMR (0.25 mm inner diameter)

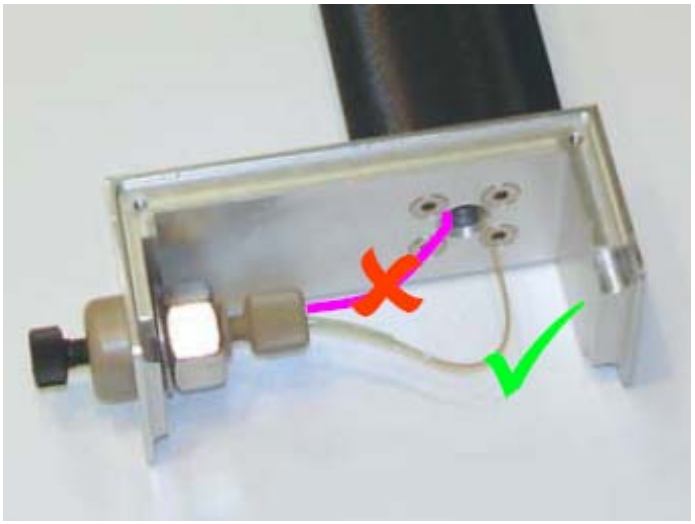
   Brown: BEST-NMR (0.40 mm inner diameter)



3. Check that the **outlet capillary** at the top can also **turn without restriction**!
4. After a finger tight connection is reached, **tighten** with the 3.2 mm wrenches by another ¼ **turn**.

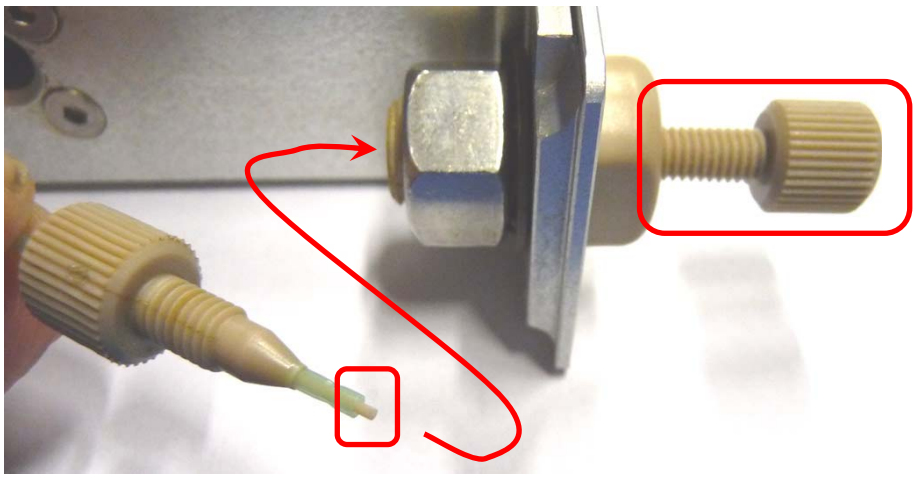
3.5 Connecting the Output Capillary

1. Slide the flow cell **completely** into the CryoFit and **lock it**.
2. Cut the capillary to the required length. It must be **3-5 cm longer** than the distance to the connector, so it **forms a curve**. This additional length is **required** when the CryoFit is **bent**!



3. On the top part, **close** the connector with a **stopper**.
4. Connect the outlet capillary using a **sleeve** and **fitting**.

5. Push the outlet capillary **2-3 mm through** the fitting; it will be pushed back to the correct position when it is mounted.

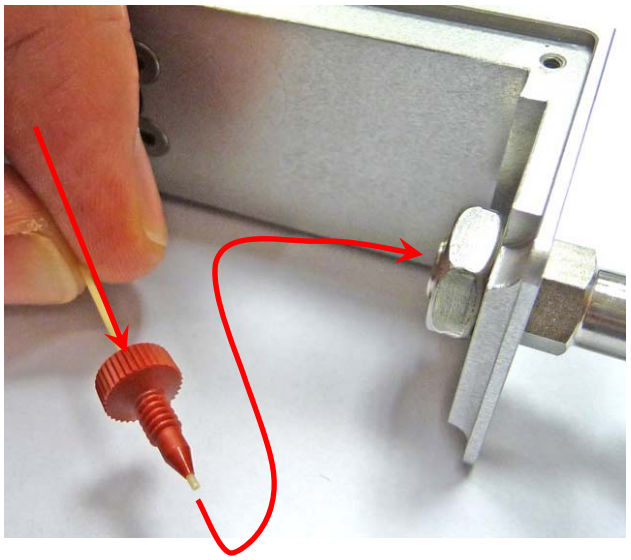


6. Leave the **upper part** of the CryoFit **open**.

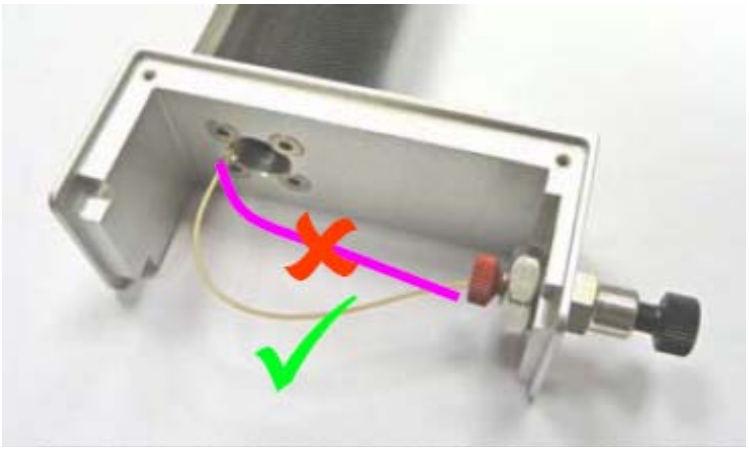
3.5.1. Connecting the Micro Connector

Two different connectors are used. The following describes the **micro connector**.

1. Slide the flow cell **completely into the CryoFit** and **lock it**.



2. Cut the capillary to the required length. It must be **3-5cm longer** than the distance to the connector, so it **forms a curve**. This additional length is **required** when the CryoFit is **bent**!

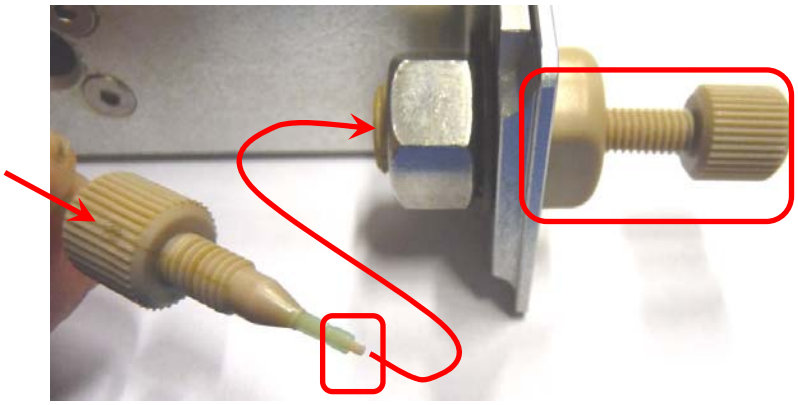


3. Connect the capillary **directly**.
4. Leave the **upper part** of the CryoFit **open**.

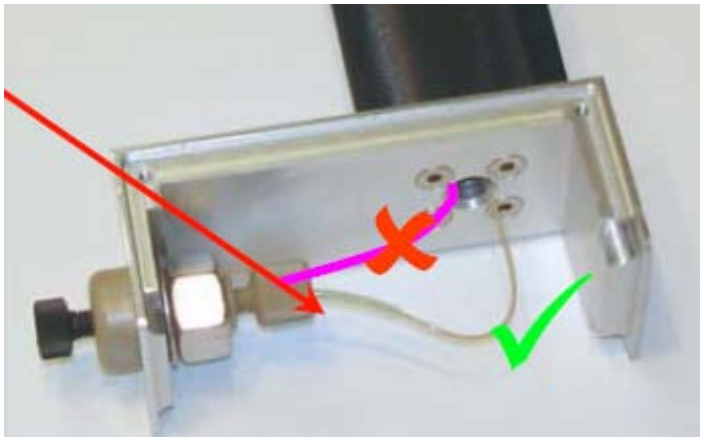
3.5.2. Connecting the Standard Connector

Two different connectors are used. This page describes the **standard connector** 1/16” capillaries.

1. Slide the flow cell **completely** into the CryoFit and **lock it**.



2. Cut the capillary to the required length. It must be **3-5 cm longer** than the distance to the connector, so it **forms a curve**. This additional length is **required** when the CryoFit is bent!



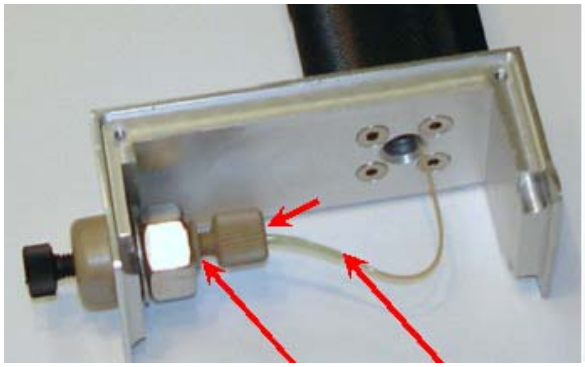
3. Close the outlet with a **stopper**.
4. Use a fitting **and sleeve** for the connection.
5. Push the capillary **2-3 mm through** the sleeve. The front end will is automatically aligned.
6. Leave the **upper part** of the CryoFit **open**.

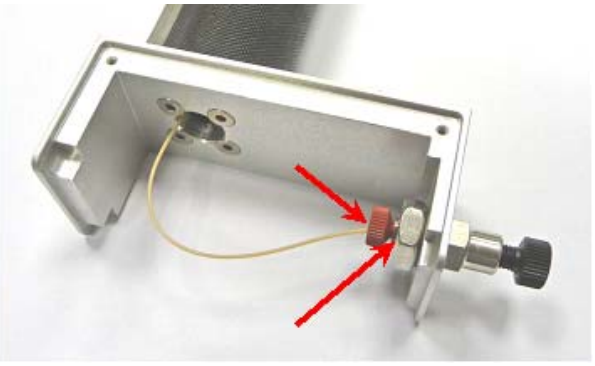
3.6 Checking the Liquid Connections

You ***must*** perform a **liquid test** as described in the following steps before you use the CryoFit in a CryoProbe. The reason for doing this is:

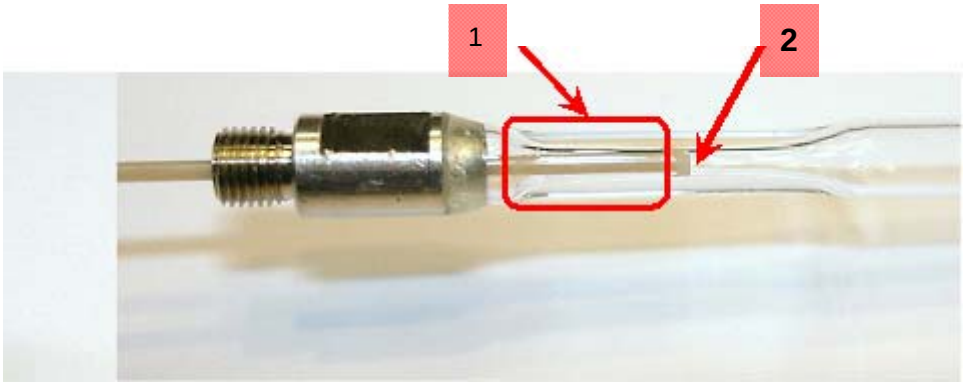
- Leaks are not visible inside the probe.
- Solvent or sample may **contaminate** or **damage** the probe.
- Connection of capillaries to the flow cell may lead to blockages.

1. To find leaks at the top of the CryoFit, check for drops forming at the following positions:
   - **Around the fitting** of the outlet capillary.
   - **Inside the fitting** of the outlet capillary.
   - Between the **sleeve** and outlet capillary.





2. Leaks at the **outlet** are difficult to detect.
   - You must check for liquid inside the flow cell in the compartment (2) behind the contact point (1) of capillary and glass wall.



   - Observe this area during the first filling of the flow cell.

Do **not unscrew** the flow cell to check for leaks. **Rotating** the flow cell will **disconnect** it from the outlet capillary.

3. For leaks at the **inlet** check for:

- Drops forming at the bottom of the flow cell, where the inlet capillary passes through the metal connector (3).

Wear ***safety glasses*** and ***gloves***!

Take a **5 ml plastic** syringe and fill it with 2ml of the liquid.

- H2O/D2O for BEST-Systems.
- CH3CN (acetonitrile) for LC-NMR and LC-SPE-NMR systems.

Connect the syringe to the **inlet** capillary and open the **outlet**.

Push the liquid into the CryoFit

- When you see it in the flow cell, **stop the flow**. The liquid must stop. If it is **moving backwards** you have a counter pressure (blockage) in the outlet capillary.
- Continue until **several drops** form at the outlet.

Close the outlet with a **stopper**, leave the **syringe connected**.

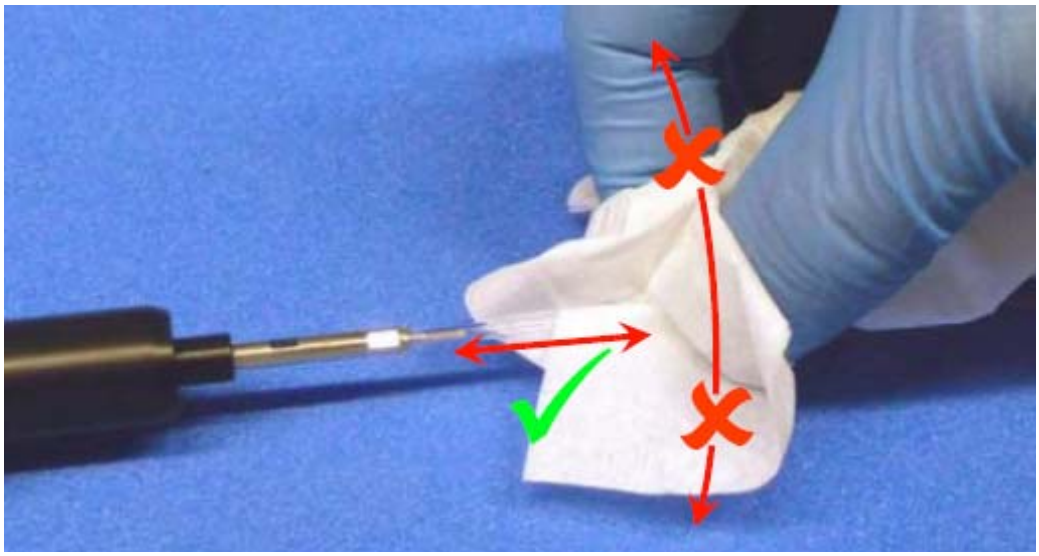
Compress the liquid in the syringe. With a 5 ml **plastic** syringe and one hand you will reach a pressure of **10-15 bar** only.

Maintain the pressure for **1 minute** and check for leaks.

Empty the CryoFit before storage or usage with the NMR.

3.7 Final Steps

1. After successful leak test **close the top** of the CryoFit.
2. **Slide out** the flow cell completely.
3. Clean it with acetone and **lint free** paper.
4. Do not apply any force **perpendicular** to the flow cell.

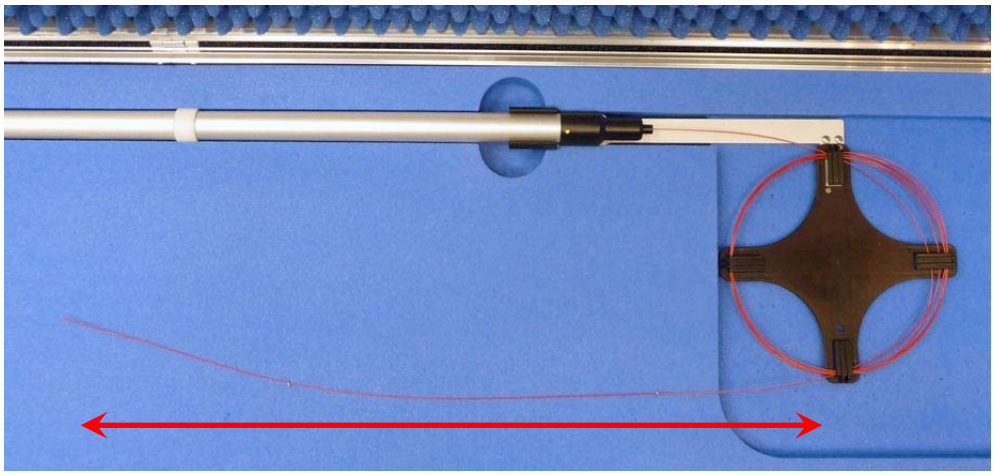


5. Let the completely acetone dry.
6. Check for **particles on the glass**.
7. Slide the flow cell into the CryoFit and **lock it**.
8. Connect the **Capillary holder** wind up the capillary.
9. Leave the **last 50 cm** straight, do **not wind** it up! Insertion into the CryoProbe may otherwise get difficult.

The CryoFit is ready for use!

3.8 Packing the CryoFit

1. **Wash** and **dry** the CryoFit before packing.
2. **Close** the **outlet port** with a stopper.
3. Pack **it in the box** delivered with the CryoFit if it is not in use.
4. Coil up the capillary for storage, however leave the **last 50 cm straight**, to facilitate insertion into the magnet.

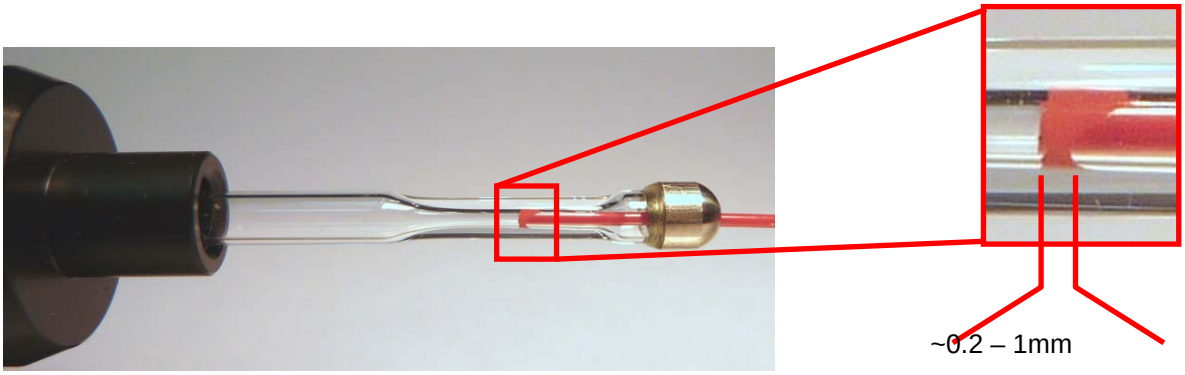


4. Capillary Connections

4.1 General Capillary Connections

1. The inlet and outlet of the flow cell form a **cone**. The connection is made by **pressing** the capillary into this cone.

2. With a correctly inserted capillary a **contact area** of glass and capillary should be visible as a ring of approximately **0.2 – 1 mm** depth.



3. A correctly inserted capillary withstands pressures of **up to 50 bar**!

4. For testing you can **pull** at the capillaries. A correctly inserted capillary will hold a force of **more then 1 kg**.

!!! Attention – handle the flow cell delicately !!!

5. The connection of the flow cell and metal holder is **very fragile**.
   - Relatively **strong forces** are required to establish proper connections.
   - **Preferably** connect/disconnect the capillaries while the flow cell is dismounted from the CryoFit.

6. The end of the capillary must have a **clean, square cut**.

7. Before the capillary is pushed into the flow cell, **clean** the **outside** of the capillary end with acetone and **dry it**.

8. Ensure that the inside of the flow cell **is clean and dry**.

9. Always **wear gloves** when handling the flow cell.

4.2 Selection of Capillary Type

The capillary diameter depends on the type of applications for which the CryoFit is used.

The outlet capillary always has a 0.4 mm inner diameter capillary (light brown). This minimizes the risk of clogging and reduces the backpressure that is created when liquid is flowing through the flow cell. For the inlet capillary three different diameters are available:

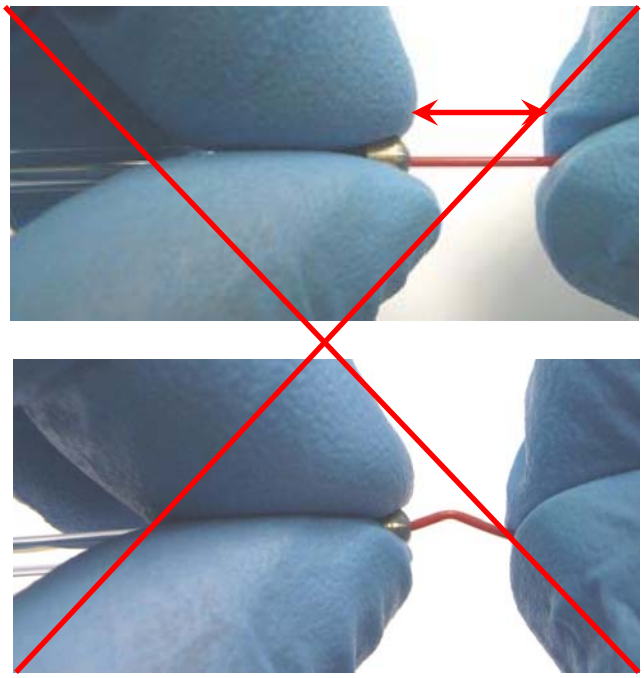
- For pure LC-SPE-NMR systems use a 0.13 mm inner diameter capillary (red) to minimize the void volume of the system.
- For LC-NMR systems, and systems that use a combination of LC-NMR and LC-SPE-NMR, use a 0.25 mm inner diameter (blue) inlet capillary.
- For BEST-NMR systems use a 0.4 mm inner diameter (light brown) inlet capillary to allow fast sample transfer.

4.3 Connecting the Standard Capillary

If the flow cell **is already mounted** to the CryoFit, go to the next section.

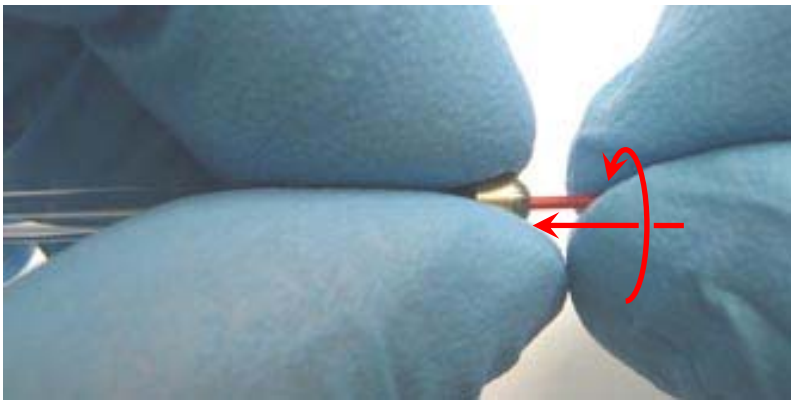
1. Hold the flow cell at the **metal end** part.
2. **Gently slide** the capillary into the cell.

➢ IMPORTANT: Follow the steps exactly or the capillary may bend:



3. Hold the capillary **close to the flow cell**.

4. **Push** and simultaneously turn the capillary into the flow cell.



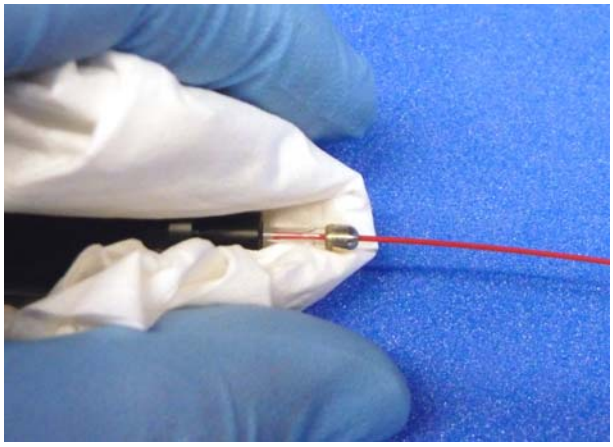
5. **Pull slightly** at the capillary; a good connection holds more then **1 kg**!

6. Perform a **liquid test** (see section 3.6).

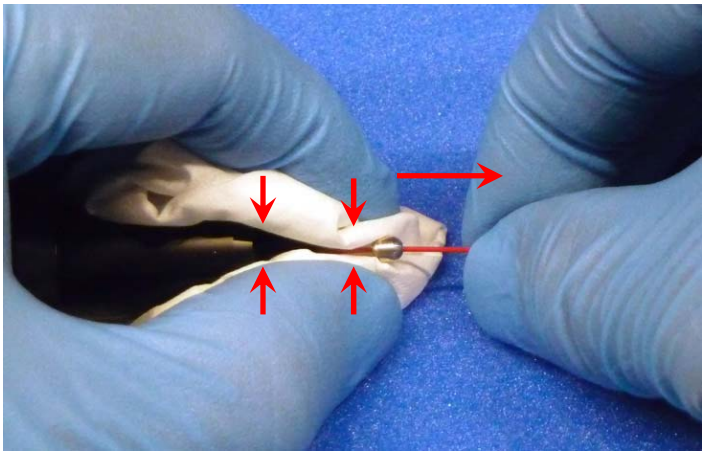
4.4 Replacing the Capillary While the Cell is Mounted

1. Replacing the inlet capillary **while the cell is mounted** in the CryoFit requires certain **precautions**.
2. Use the blue foam of the CryoFit box as support.

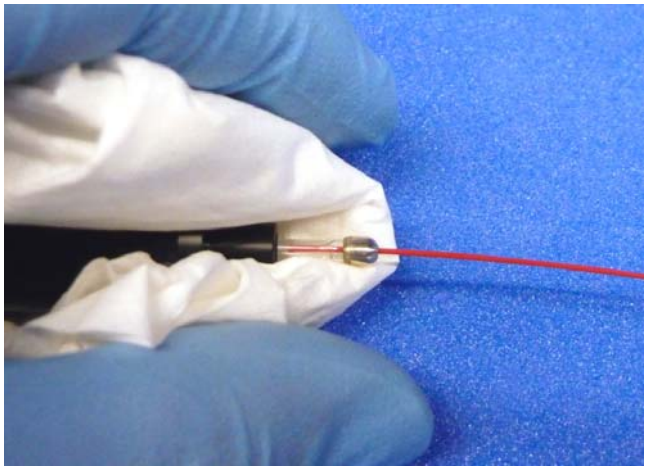
3. Slide the flow cell **partially** out, so that **only 1cm** is visible.
4. Take **several layers** of paper towels and hold the flow cell **together** with the **bottom part** of the CryoFit – to **prevent side movement** of cell.



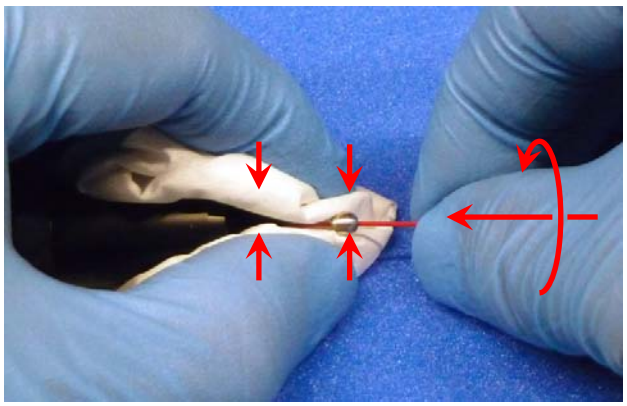
5. **Pull** the capillary out of the flow cell (required force = **2-3 kg**!).



6. The new capillary end **must**:
   - Have a clean rectangular cut.
   - Be **cleaned** with acetone and **dried**.
7. The **inlet** of the glass cell must be **dry**.
8. Hold flow cell and **bottom part** of the CryoFit together to **prevent side movement** of cell.



9. Hold the capillary **close to the flow cell** so that it cannot bend!
10. Simultaneously **push** and **turn** the capillary into the flow cell.



11. Pull **slightly** at the capillary; a good connection holds more then **1 kg**.
12. Perform a **liquid test** (see section 3.6).

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