NANO CHANNEL CHIPS

Technical Brochure

Insight Chips

Revolutionizing liquid cell TEM

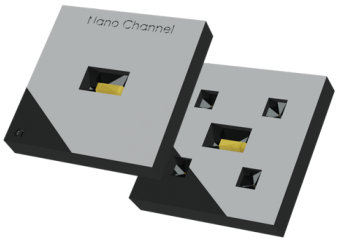
July 2025

1. INTRODUCTION

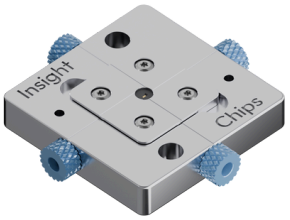
All you need to know about the Nano Channel Chip The world's first monolithic chip – for use in TEM, SEM and optical microscopy.

The Nano Channel Chip is based on 10 years of research and development at the Technical University of Denmark before being introduced as a commercial solution in 2022. Insight Chips aims to introduce a higher level of reproducibility, resolution, flow control and ease-of-use than any other solution. The Nano Channel Chip fits in our holders for TEM (JEOL and TFS), SEM and optical microscopes.

The Nano Channel Chip

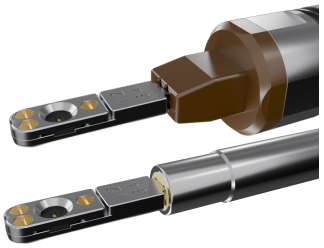


Optical / SEM flow holder



Optical / SEM static and storage holder

JEOL and Termo Fischer TEM holders



1.1. TOP UNIQUE FEATURES OF THE NANO CHANNEL CHIP

1. **No assembly needed**
   The chip is made in one piece so there is nothing to assemble. If your sample is ready, the holder can be prepared with a chip, ready to be inserted in the TEM in less than 5 minutes!

2. **Well-defined liquid thickness down to 20 nm!**
   Membrane bulging is also not an issue. Each channel is 1-2 µm wide and therefore typically bulges less than 10 nm inside the TEM. This ensures reliable and reproducible high resolution. This makes EELS and holography reliable techniques with the Nano Channel Chips.
3. **Silicon-nitride membranes down to 10 nm!**
   This aids in producing consistent high resolution in the TEM.
4. **Four individually controlled in/outlets in the chip**
   This creates two bypass channels inside the chip. Two of them can be used as inlets, the other two as outlets. Being very large – 100 µm wide and 15 µm deep, liquids can be flushed into the chip in a second and exchanged in just a few seconds. Several inlets can also be used to insert different liquids into the chip, both visible at once in the TEM.
5. **Unprecedented flow control and mixing directly in the field of view**

Two liquids from each bypass channel can be brought together in the field of view inside nano channels. The Nano Channel Chip is the first ever commercially available liquid cell that can mix directly in the field of view.

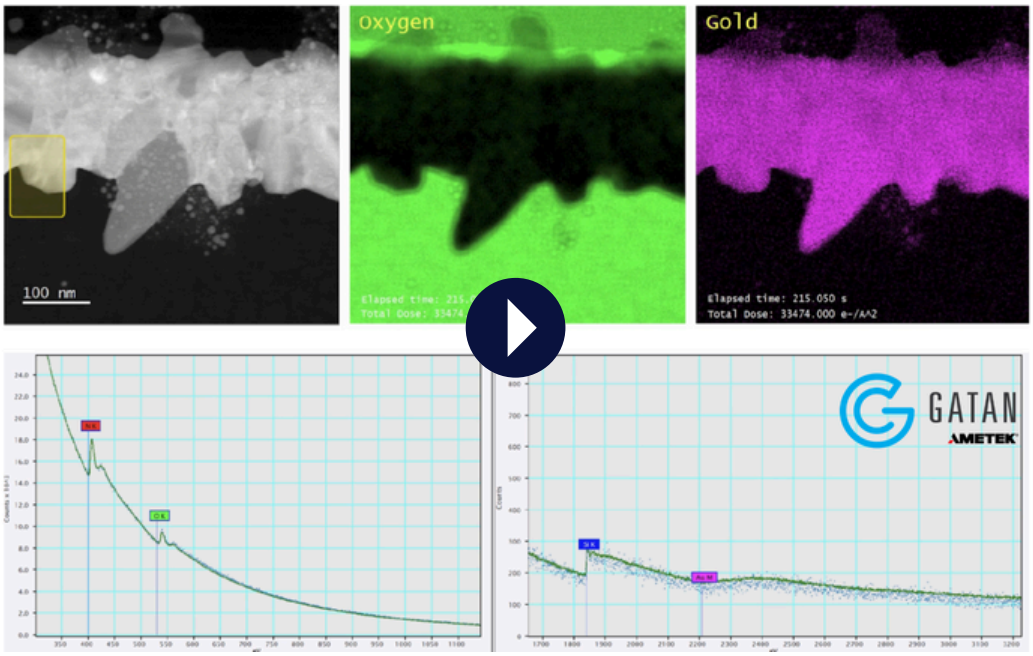
6. **Robust and reliable**

   Because the chips are already assembled with a firm wafer-bond, the suspended membrane can withstand a lot. In fact, a chip can be dropped on the floor and easily survive. Also, you will not have to worry about tightening the lid on top of the chip too much, or unevenly. Finally, the vacuum is the best of any liquid-cell holder. Because the chip is so robust, the chip is pushed very hard against sealing O-rings, creating a firm and reliable seal, without any danger of damaging the chip. We've tested our chips up to 12 bar in our TEM holder in vacuum without any pressure change, and the chips alone up to 38 bar before it gave out!

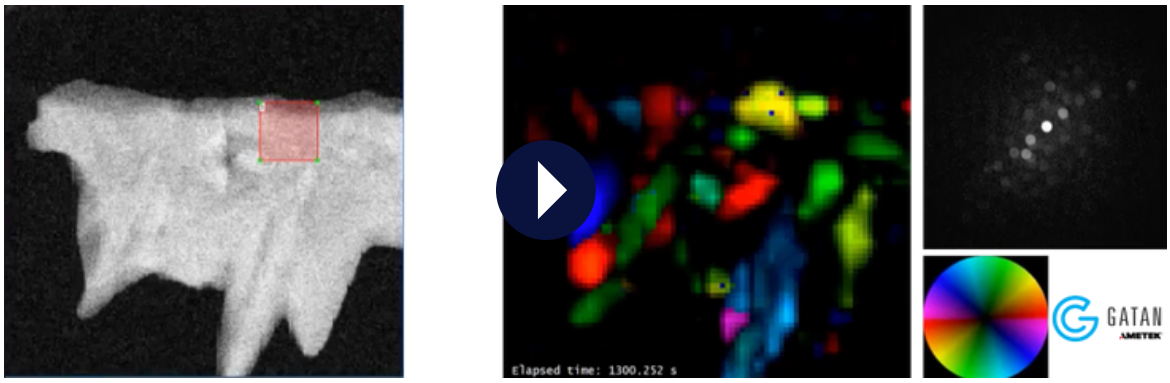
1.2. TOP HIGHLIGHT #1: THE WORLD'S ONLY RELIABLE EELS AND 4D-STEM IN LIQUIDS!

Due to reproducible and reliable liquid thickness down to 20 nm, Insight Chips delivers an EELS capable system with ease of use.

These results show Au crystal growth in 40 nm liquids, captured in collaboration with Gatan using the GIF Continuum K3 on a JEOL F200 TEM.



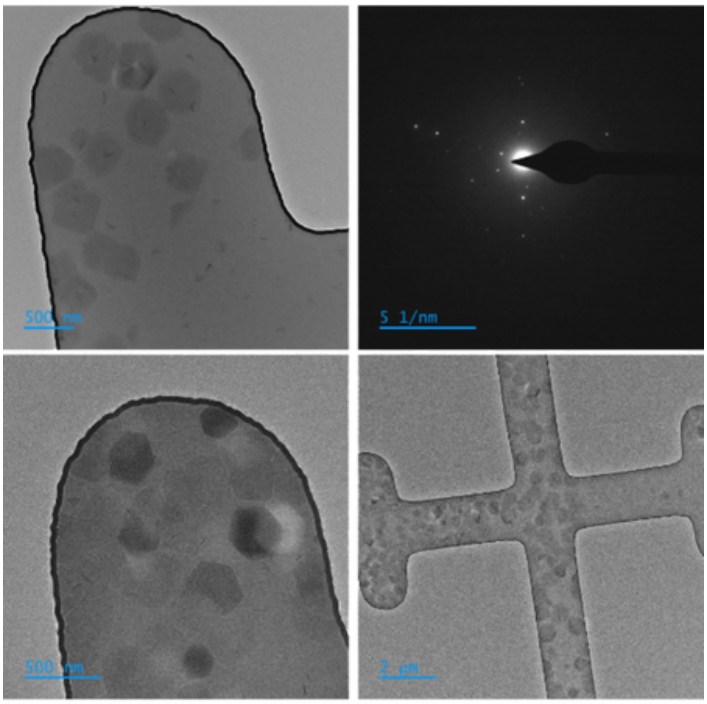
Using the same chips and the same detectors, 4D-STEM results were also gathered in collaboration with Gatan.



See the video here

1.3. TOP HIGHLIGHT #2: ATOMIC RESOLUTION READILY AVAILABLE

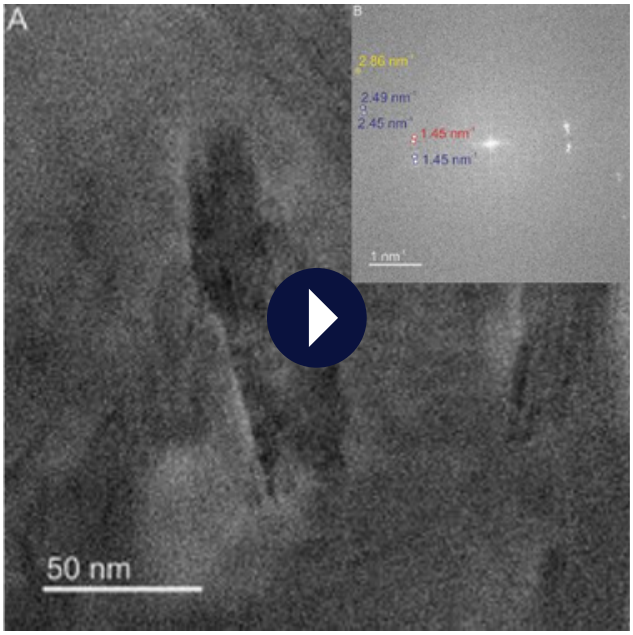
1.3.1. ZIF-8



Grown in the channels by mixing two liquids together in the chip. Images by: Joakim Lajer (DTU), Justin Mulvey (UCI), Joe Patterson (UCI)

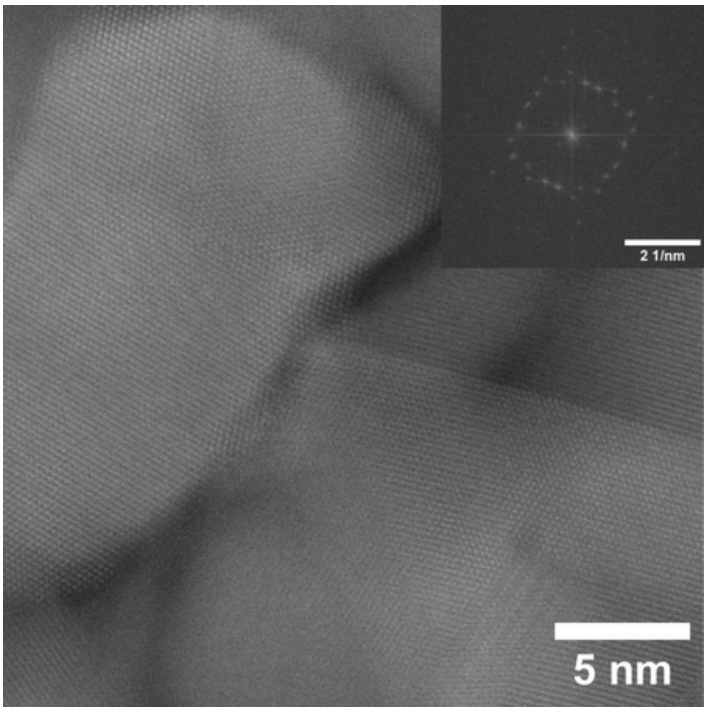
1.3.2. Copper Benzenehexathiol

Paper by David Mücke et al (Ulm University) showing atomic resolution in situ growth of this unique MOF.



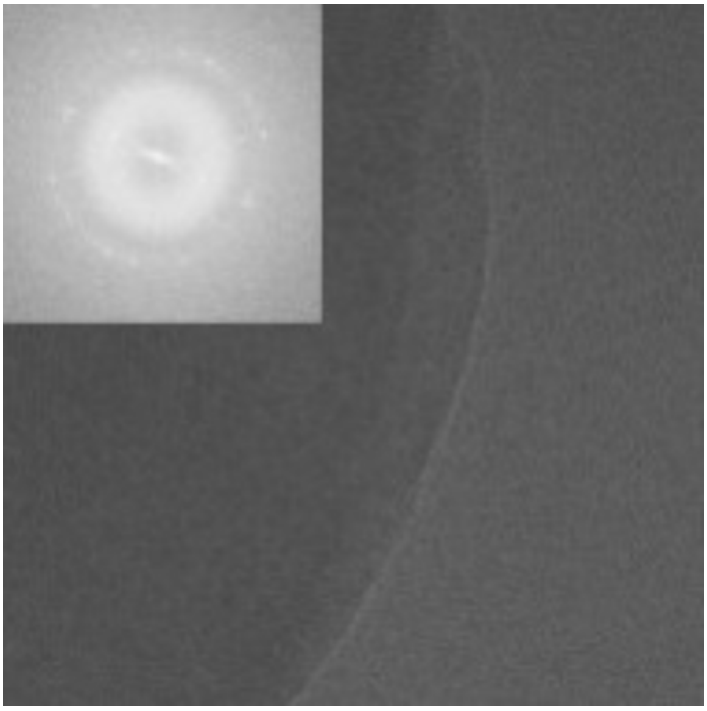
1.3.3. Palladium

Beam-induced in situ growth of palladium with atomic resolution captured by Prof. Rolf Erni, Dr. Marta Rossel and Dr. Walid Dachraoui (EMPA).



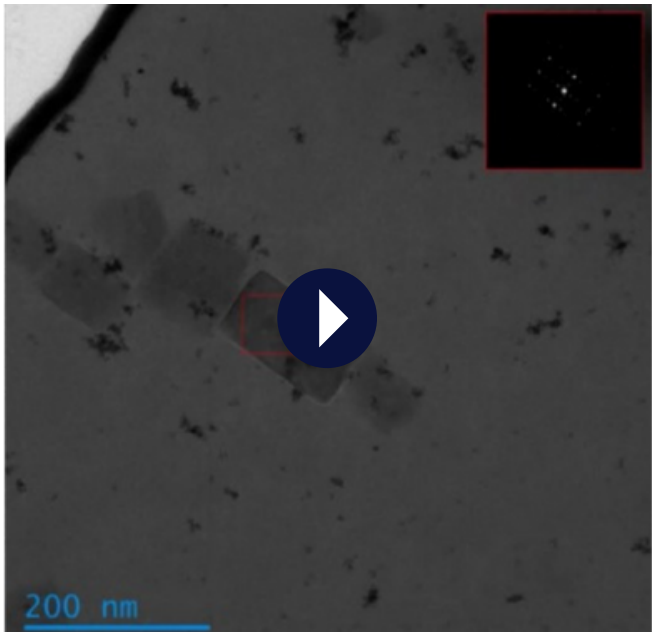
1.3.4. CaCO3

Calcium carbonate growth captured by heating up supersaturated solution to 80 C in the TEM using our patent-pending solution for a temperature controlled holder. Images captured by Assist. Prof. Murat Yesibolati (DTU).



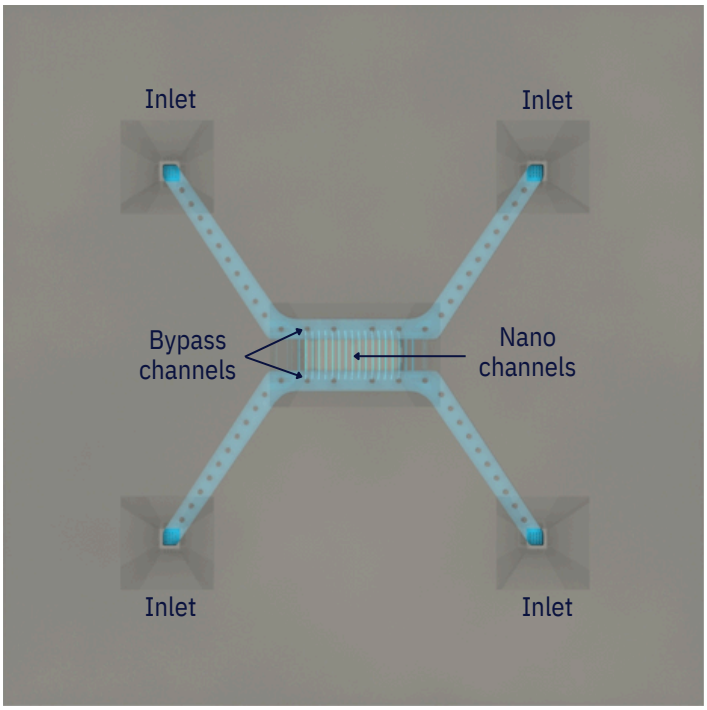
1.3.5. Ettringite

Ettringite has never been imaged in TEM before because it breaks down in vacuum. The reason is, over 50% of the structure is water and so it must remain wet. **O. Rindle et at from TUM published a paper**.

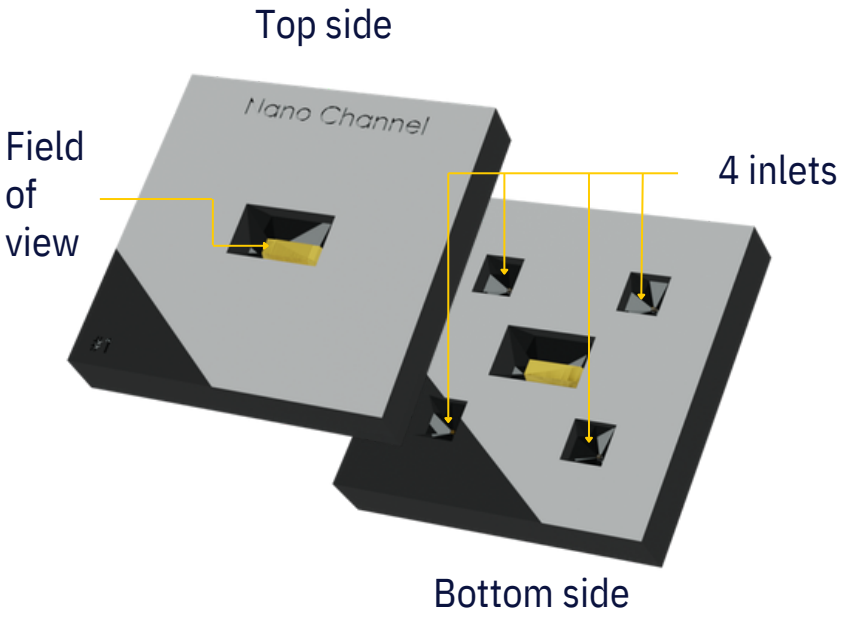


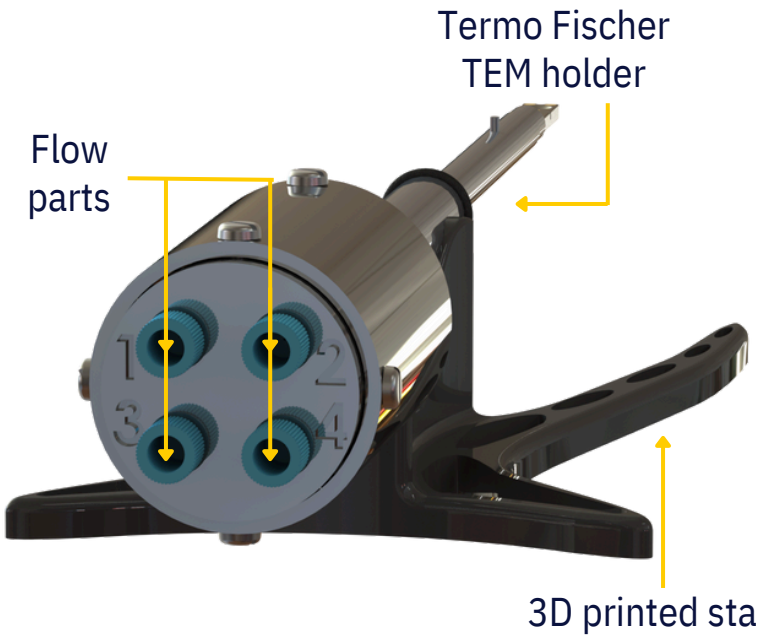
2. A TRUE NANO-FLUIDIC FLOW CELL FOR TEM AND SEM

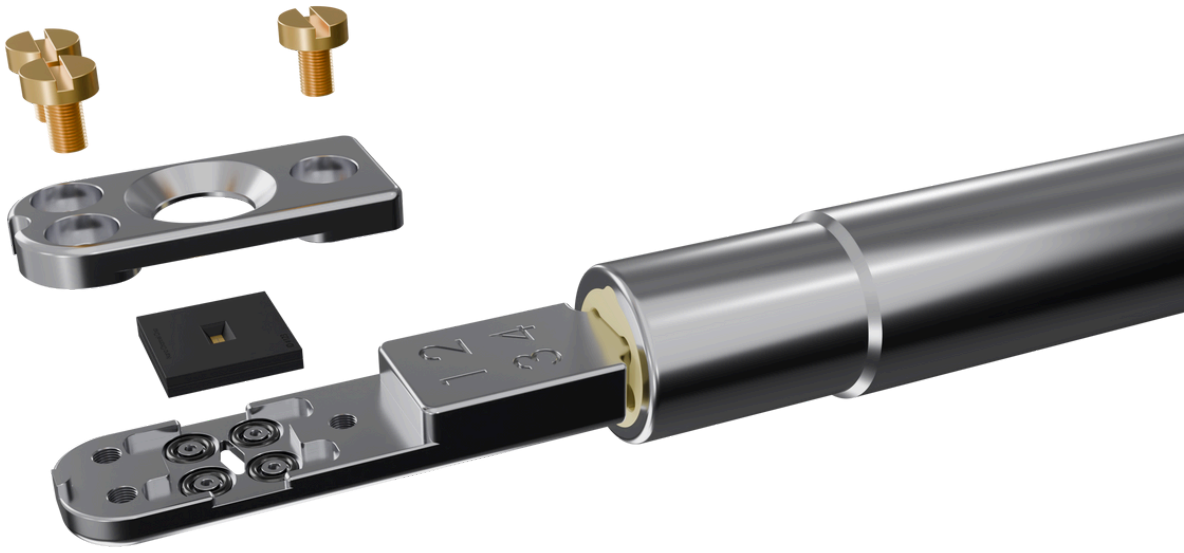
The Nano Channel Chip turns a common microfluidic flow-cell structure into a truly nanofluidic one and makes it adaptable with electron microscopes. The chip has four inlets and an internal flow structure with two micron-sized bypass channels with nano channels connecting them. When a higher pressure is applied to one bypass channel, the liquid flows through the nano channels into the other bypass channel, and vice versa. Only the nano channels are visible in the field of view, whereas the larger micro/bypass channels are hidden away inside the chip to protect the TEM from leakage of a larger volume of liquid. The volume of a nano channel is on the order of femtoliters and poses no risk of breaking the vacuum in the column on its own. Even when a nano channel fractures during a TEM experiment, the column vacuum remains intact. The four inlets are sealed with O-rings in the tip of the holder and held in place with a lid. Tubes connect inlet ports in the backside of the holder to each of the four inlets in the chip. From there, the holder can be controlled with syringes or a pressure-based pump system. For this, we recommend using **Fluigent's EZ Flow** which can be used to control each inlet separately and precisely.



Transparent view of the Nano Channel Chip, showing the internal bypass channels

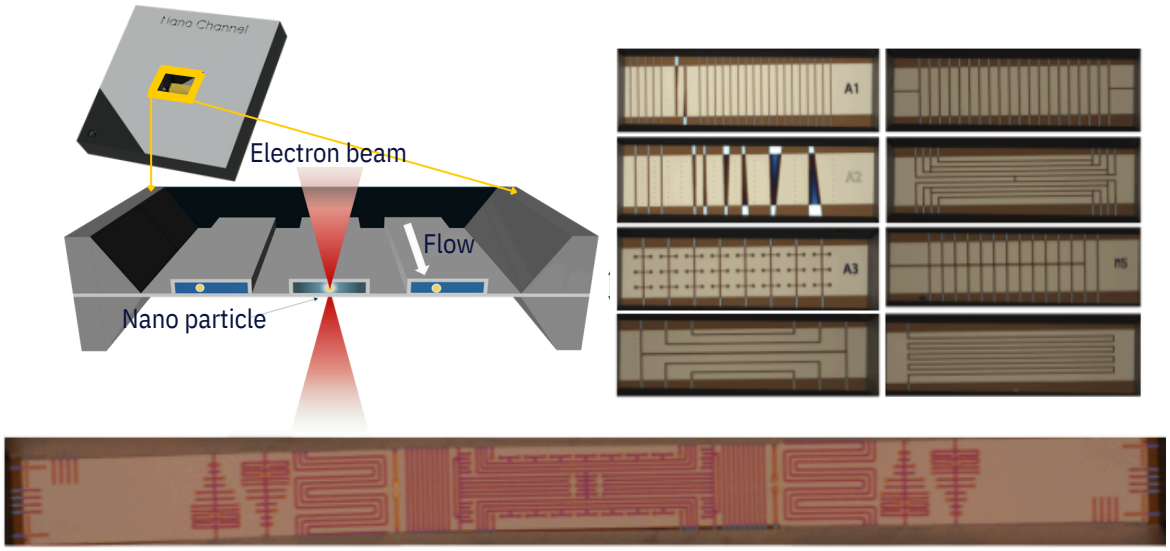

Top side
Nano Channel
Field of view
4 inlets
Bottom side


Termo Fischer TEM holder
Flow parts
1
2
3
4
3D printed stand


1
2
3
4

2.1. MANY NANO CHANNELS IN A LARGE FIELD OF VIEW

The chip has a large, suspended membrane, up to 1,250 x 200 µm, made of Silicon-Nitride. The entire membrane is electron transparent and visible in the TEM. Across this membrane are as many nano channels as we want, in any pattern, down to 1 µ resolution. We use maskless lithography to make the channel patterns, so design changes are easy from batch to batch, and we can make any number of different designs in one batch. Different channel designs yield different types of flow in the channels, suitable for flushing out radiolysis, trapping particles, or mixing two liquids together in the field of view.

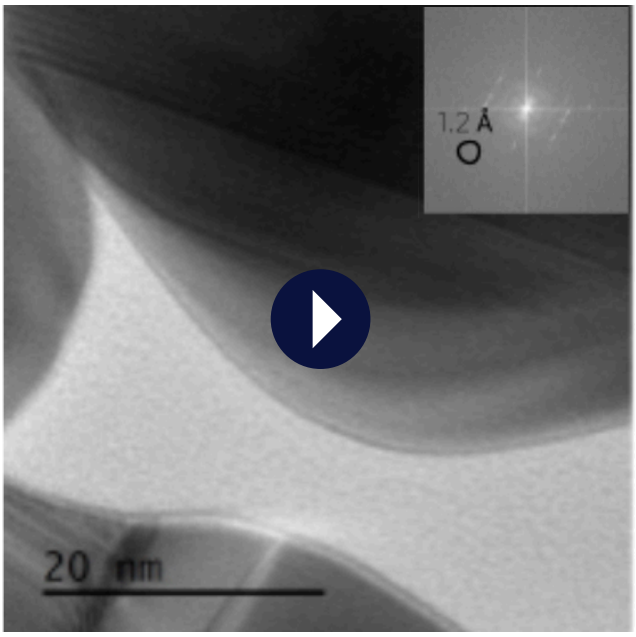


Top left: Sketch of the field of view. It consists of two membranes bonded together. One has channels etched into it, the other is completely flat, creating the passages for the liquid between bypass channels in the chip. **Top right:** 20x optical microscope images of 8 different channel designs from one batch, indicating the wide variety of channel patterns that can be made in the Nano Channel Chip. **Bottom:** a 1,250 x 100 µm field of view, packed with various types of nano channel patterns for flow and mixing.

2.2. HIGH RESOLUTION ENSURED WITH WELL-DEFINED LIQUID THICKNESS AND MEMBRANES

The nano channels are well-defined from the fabrication stage. It is therefore always possible to use a chip with the right liquid thickness, for each experiment. For example, if one wishes to study particles that are 10 nm large, it is possible to use Nano Channel Chips with channels that are only 20 nm thin, making enough room for the particle to flow into the channel, but without compromising on the resolution with a much thicker than needed liquid. If one wishes to study larger particles, say 100 nm, one simply uses a chip with a liquid thickness of 120 nm. We can control liquid thickness in this way with each batch of chips, with 5-10 nm precision.

The membranes of the Nano Channel Chip are made of silicon-nitride (SiN) and we have successfully made chips that are down to 10 nm thin!
Ute Kaiser's group at Ulm University in Germany have repeatedly achieved about 1Å resolution while imaging gold. An image and video of a growing particle can be seen below.

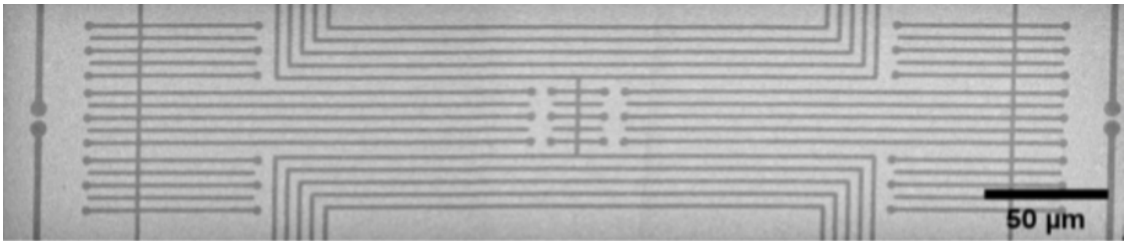


1M magnification image of gold particles nucleating inside a nano channel with 60 nm liquid and 10 nm SiN membranes. Image credit: David Mücke, Ute Kaiser's group at Ulm University, Germany.

2.3. FILLING, FLOWING, AND MIXING IN THE NANO CHANNEL CHIP

2.3.1. Filling

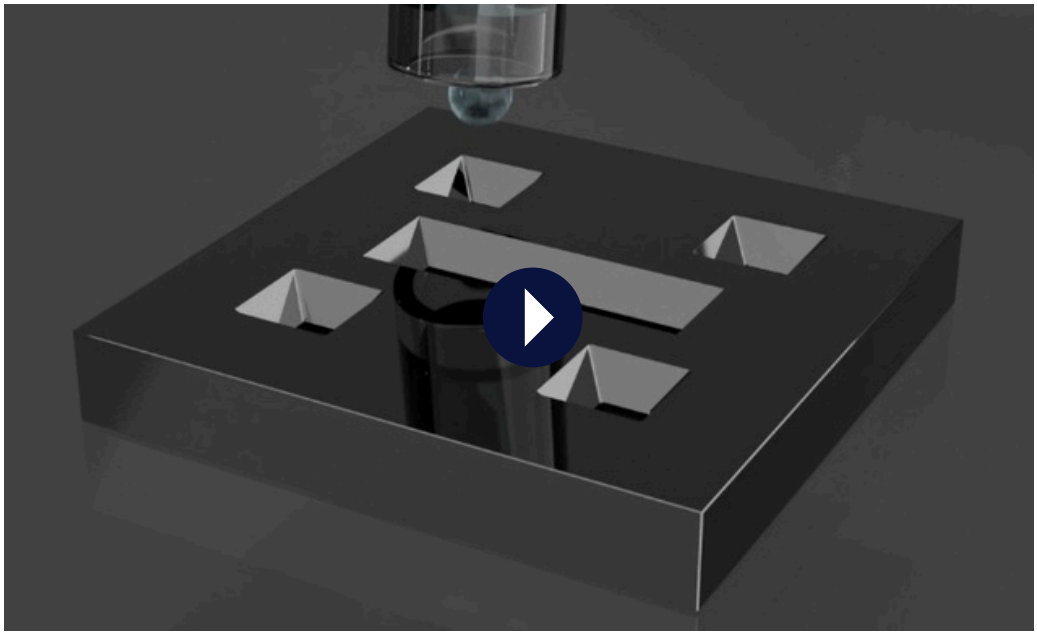
The Nano Channel Chip is mounted in a TEM or SEM holder in less than five minutes. From here, the holder can be placed in the microscope, tubes can be attached to the backside of it and liquids can be flown into the system. The channels are filled almost instantaneously as liquid comes into contact with the highly hydrophilic channel surfaces. Capillary forces on the order of 10 bar 'suck' in the liquid forcefully, ensuring a complete filling.



Low-magnification TEM image of the full field of view. The nano channels are fully filled with water.

Before inserting the chip into the holder, the four inlets need to be broken as a 10-25 nm thin SiN membrane is sealing the channels. This seal is a natural part of the clean room fabrication process and has the positive effect of keeping the channels protected from contamination and even air which over time will turn the channels inside the chip hydrophobic. By fracturing these membranes right before an experiment, the channels are ensured to stay chemically clean and hydrophilic membranes right before an experiment, the channels are ensured to stay chemically clean and hydrophilic.

Animation video of chip filling can be seen below:



2.3.2. What about bubbles?

This is a question we get a lot and luckily the answer is positive! Because bubbles are usually ‘squeezed’ out of the liquid and dissolve into it due to high capillary forces in the channels. A video of the filling of channels, slowed down, can be seen here. In this video, you can see ‘dead-end’ channels being filled with water, trapping air/bubbles. Within about 10 seconds, most of these bubbles have disappeared.

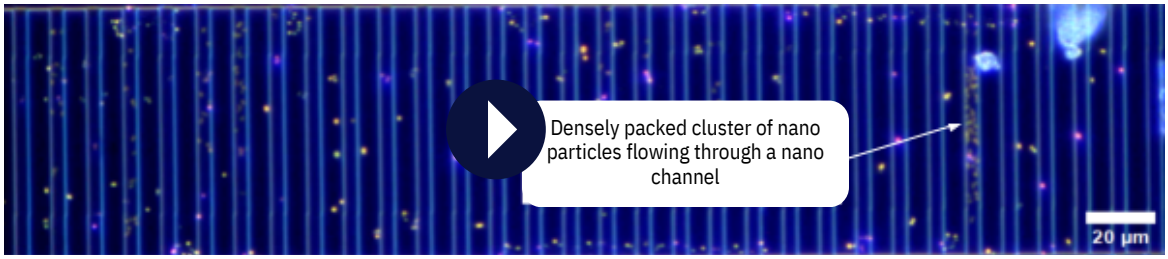
Radiolytic bubbles may be created again if the electron beam is too intense. But in this case, simply move to another nano channel and continue the experiment in fresh conditions.

2.3.3. Flushing out radiolytic byproducts

In a TEM experiment, it may be beneficial to have a constant flow through the channels to avoid the buildup of radiolytic byproducts. With a Fluigent pressure setup, the pressure can either be set to a constant value, typically 100-500 mbar, or even programmed if different flow rates are desired throughout the experiment.

2.4. FLOW IN THE NANO CHANNELS

After the liquid has filled up the chip, the capillary forces are no longer acting on the liquid, it is now in a completely balanced, steady state. Now, the pressure applied to the micro/bypass channels will determine the flow inside the channels: A larger pressure on one bypass channel will push the liquid through to the other bypass channel, and vice versa.

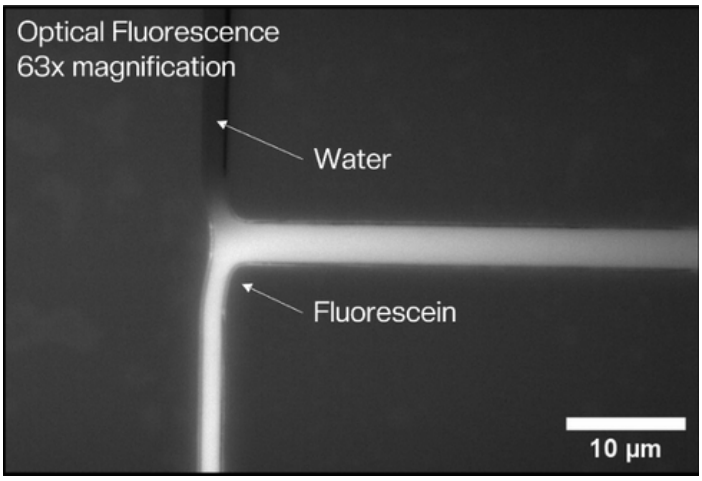


It is impossible to actually see the flow in the channels if there is nothing moving in them. Therefore, to verify flow in the channels and to establish flow procedures, we have often used nano particles which can be assumed to move precisely along with the liquid. In the video above, you can see an example of how nano particles can be tracked in the channels. In this case, we used a syringe to adjust the position of nano particles in the channels. They are controllably flown in one direction in the channels until they are centered, after which the applied pressure is stopped, leaving the particles diffusing around in the field of view.

2.5. MIXING IN THE FIELD OF VIEW

2.5.1. Bringing two liquids together in one channel

One of the most unique features of the Nano Channel Chip is its ability to bring two channels, containing different reagents, together directly in the field of view so their chemical reactions can be observed in real time. The basic principle is beautifully illustrated with this below image of fluorescent fluorescein and water coming together from two nanochannels, into one:



The above image clearly verifies the concept of mixing in the field of view. However, it is a challenge to mix liquids reliably in the TEM using this method since it requires very precise balancing of pressures in both micro/bypass channels at once. Even tiny differences in viscosities or potential micro-agglomerations of particles will have a big effect when it comes to nanofluidic channels containing only femtoliters of fluid!

2.5.2. The ”Butterfly Mixer”

To create more robust mixing methods, we’ve created a design that is less sensitive to having exactly matching pressures in both micro/bypass channels. We call it the “Butterfly

Mixer" due to some internal channel structures that look like the wings of a butterfly. This design is more dominated by diffusion, which is very reliable compared to pressure control in a nanofluidic flow cell.

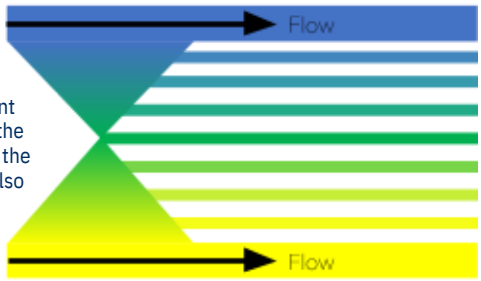
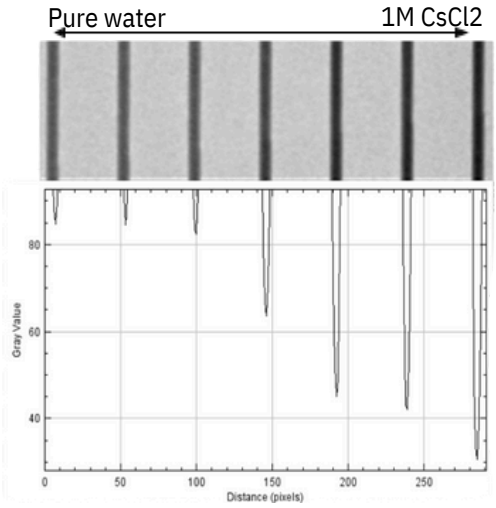
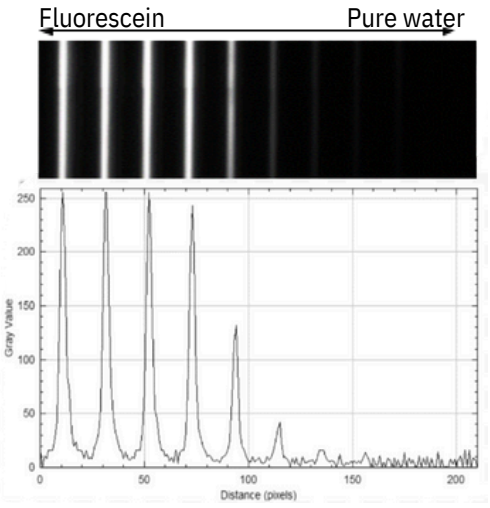


Illustration of the Butterfly Mixer: Two liquids are flown in in each micro/bypass channel and mix very little through a small connection point between them. The point is so small that pressure differences between the bypass channels never drive a very large flow between them. Therefore, the mixing in this area is primarily driven by diffusion, which is slower, and also very stable in nanofluidic channels.

We have verified the Butterfly Mixer chip both in optical microscope and TEM. In optical, we've again utilized the fluorescent agent fluorescein to record images of the channels with varying degrees of mixing with pure water. In the TEM, we have used a solution of 1M CsCl2, a heavy compound solution showing up as almost black in the TEM, against the more light-gray pure water. We have seen repeatedly that we get an array of varying mixing conditions in the array of nano channels.



TEM image of the Butterfly Mixer in action. To the left, pure water is coming in through one bypass/microchannel. To the right, 1M CsCl2 is coming in through the other bypass/micro channel. The intensity plot in the bottom shows how the intensity is roughly 50% of max right in the middle. Image credit: Tayyaba Malik.

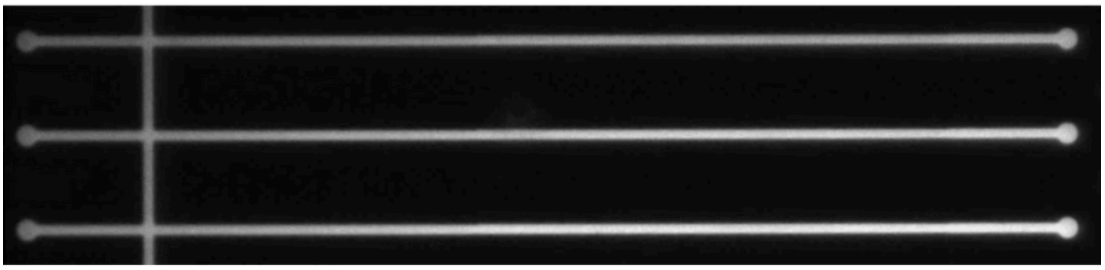


Fluorescent optical microscope image of the Butterfly Mixer with fluorescein coming in from the left and pure water coming in from the right. Image credit: Tayyaba Malik.

2.5.3. The sequential diffusion mixing method

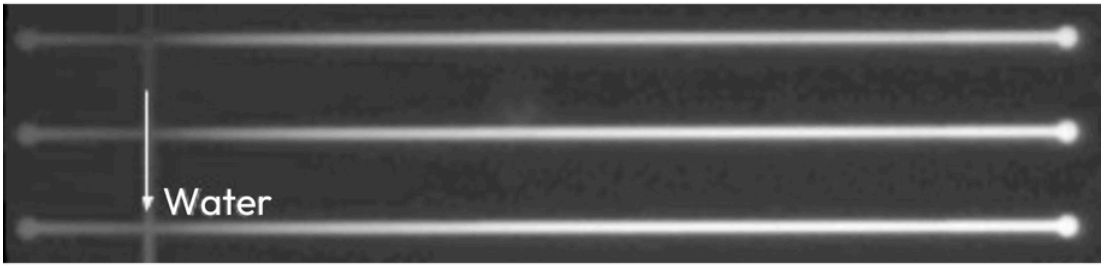
As you can see above, the Butterfly Mixer does not guarantee a perfect 50-50 mixing right in the central channel. To create a fool-proof mixing method that works every time and guarantees stable and well-known initial conditions, we also utilize dead-end channels (which have zero flow) to mix, relying 100% of diffusion and less on the precision of applied pressures to the system. This method utilizes the guaranteed no-flow conditions of dead-end channels and works as follows:

1. The first reagent is injected into the nano channels and fills up every available space, also the dead-end channels. Even bubbles are squeezed out of the liquid due to the high capillary pressure.



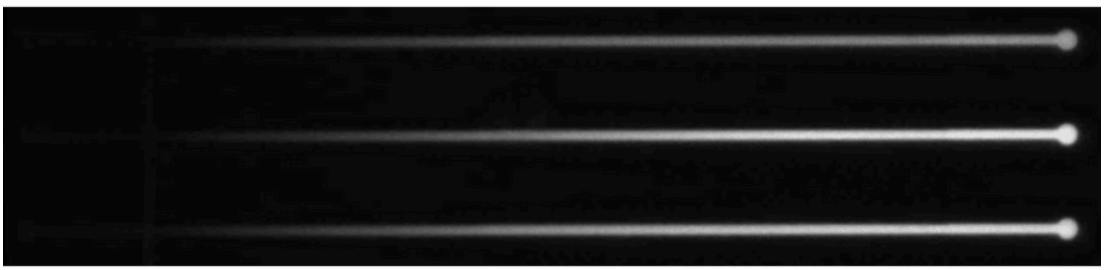
That first liquid could be fluorescein as is shown above, evenly filling all parts of the nano channels, both short and longer dead-end channels. The concentration is known with 100% accuracy.

2. A second reagent is flushed in, pushing the first reagent out of the channel which is connecting the two bypass channels. The first reagent stays stuck in the dead-end channels.

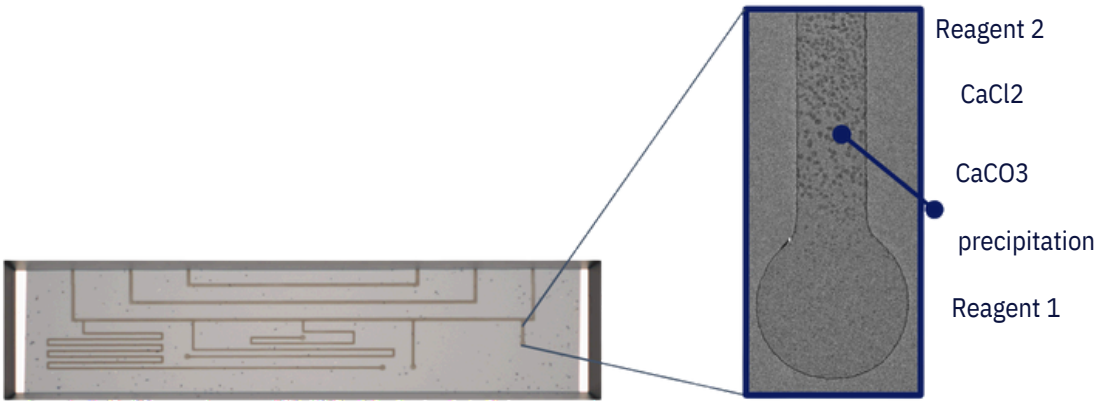


In this example, water can be seen pushing out the fluorescein from the channel coming down through the flow channel. Almost immediately, the fluorescein in the short dead-end channels (to the left) diffuses out and gets carried away with the ongoing water flow. In the longer dead-end channels, however, plenty of fluorescein remains, slowly diffuse together with the fresh supply of pure water.

3. Mixing occurs between the two points of well-known conditions, via diffusion which can be modelled precisely.



In this case, the left side is now 100% pure water, and the right side is 100% of the fluorescein solution. In between the two points, diffusion will be responsible for a steady reaction site where each point can be modelled precisely in terms of mixing concentrations over time.

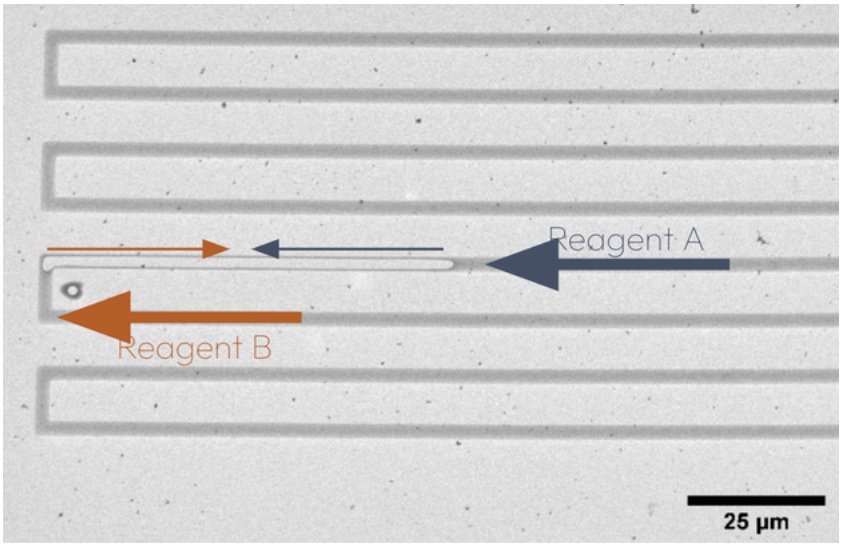


Optical image of the chip design containing many dead-end channels

Low-mag TEM

2.5.4. Seeing the first milli-second of two liquids coming into contact

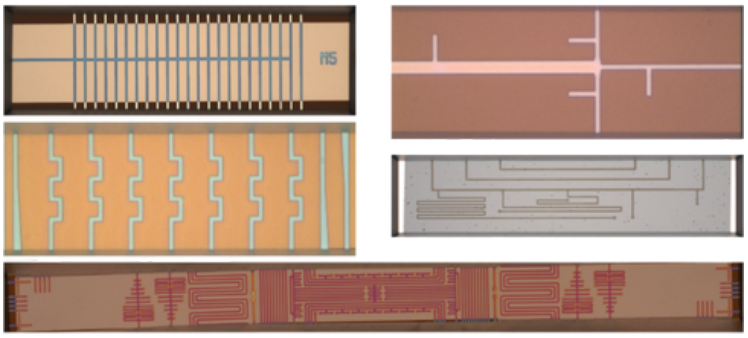
A recent discovery about mixing two liquids together in the field of view was discovered by Jeffrey George, PhD student at Nanyang Technological University in Singapore. He found that our long meander channels - which are over 1 mm long! – could be used to introduce two liquids at the same time from either side. The two liquids would naturally trap air in between them, but due to the capillary pressure, the trapped air would dissolve into the liquids, bringing them closer and closer to each other until they finally touch. We have yet to see the results of chemical reactions happening in this way, but the method is very exciting and undoubtedly will be useful at some point going forward.



Low-mag TEM image. Credit: Jeffrey George, Nanyang Technical University

2.6. WHY ARE THE CHIPS DIFFERENT COLORS?

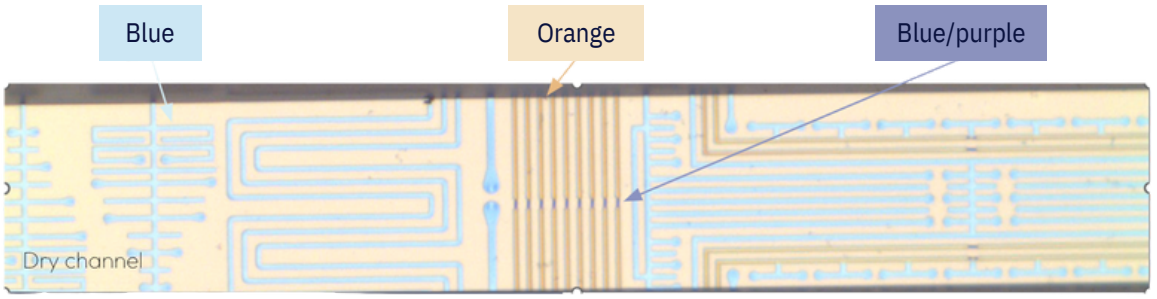
The Nano Channel Chips all look the same to the naked eye, but when put under an optical microscope, the channels can be seen in great detail. The channel patterns obviously are different, but so is the color of the channels, as well as the entire membrane.



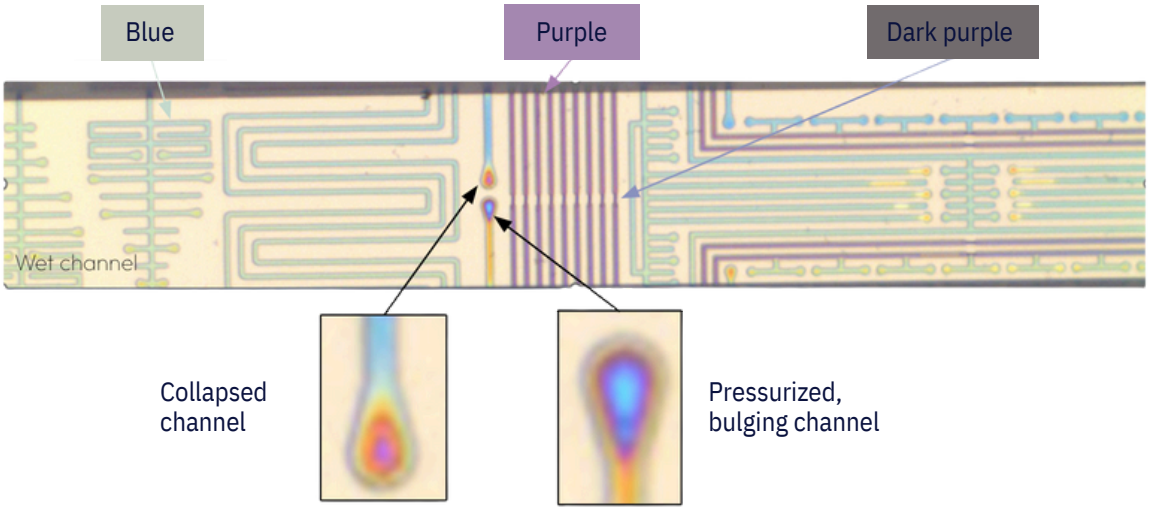
The field of view of different batches of nano channels as seen with a 20x objective.

The reason for this is the change in refractive index as visible light travel through, and reflects, on the membrane. The membrane without any channels will change color based on its thickness and so will the channels based on their thickness, as well as the nitride surrounding them. Additionally, as liquids enter the channels, the refractive index will again be different than when the channels are full of air, which agian will give different color of the channel.

As an example, that shows a lot of color dynamics in one chip, this one below has three different depths of channels which comes out as three different colors. When the chip is dry/filled with air, the turquoise channel are around 150 nm, the orange are about 200nm, and the blue/purple are about 59 nm. These 50 nm parts create traps, which means that particles under 200 nm can be flown into the channels but get stuck at the 50 nm thin part.



A great difference is seen when the channels are filled with water. All the channels change color due to being filled with liquid:

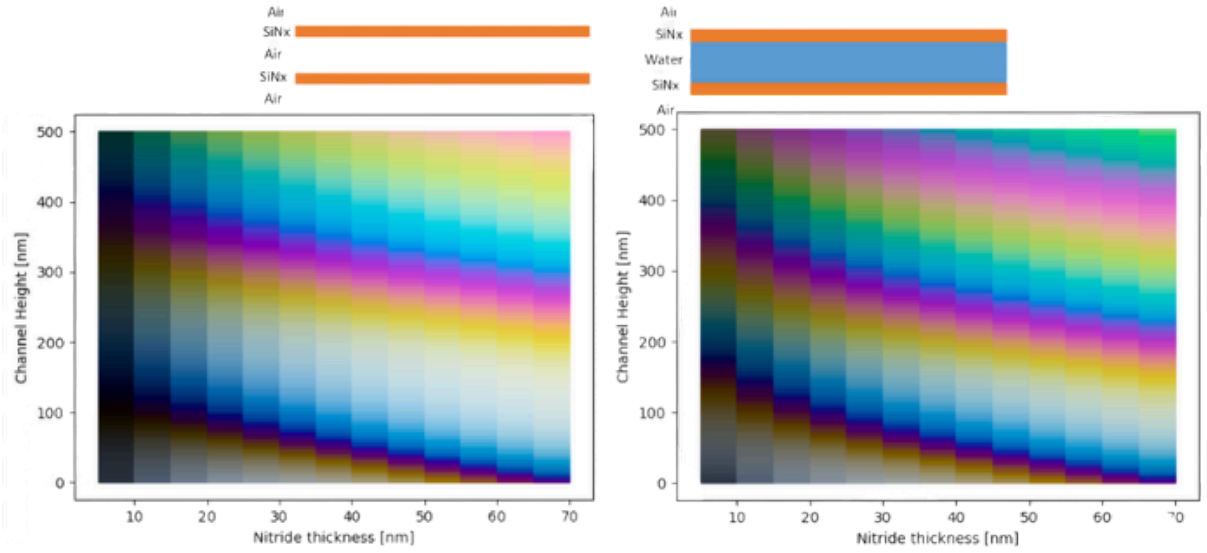


Further, the dead-end channels around the middle of the image reveals collapsing and pressurization of decoupled channels, which show a gradient of different colors as the channel varies in thickness. The bottom-channel is the pressurized one, and the top channel

is still mostly dry (turquoise) but some moisture must have reached the channel via the other nano channels and creates capillary forces that makes the channel collapse.

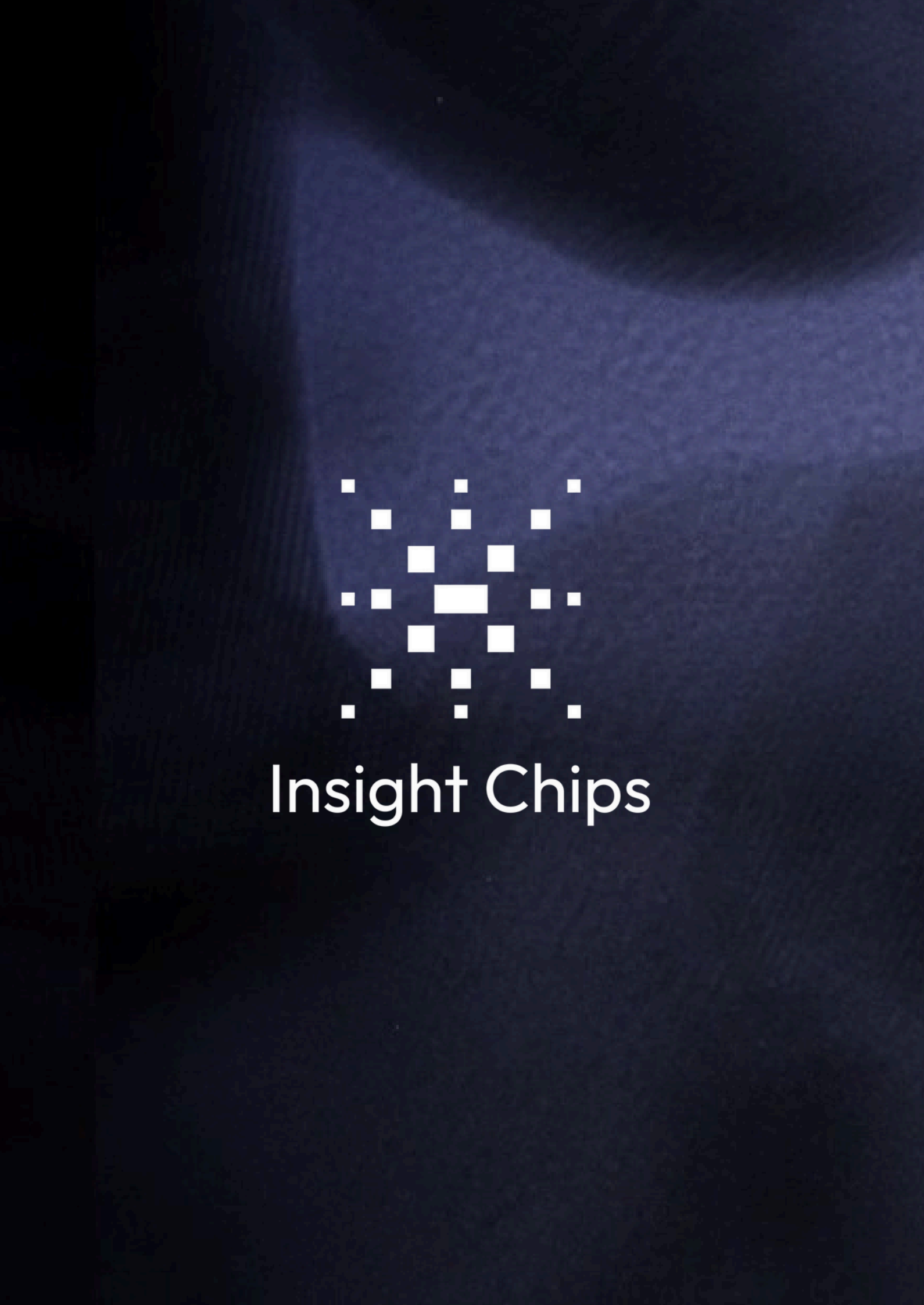
2.6.1. Color charts

We often use the colors of the chips to verify that they are the expected thickness. Below diagram hangs in our lab at DTU by the optical microscope so that we can always look up and confirm we have the right chip for our experiment – whether it's dry or already filled with water. It only requires prior knowledge of the nitride thickness to read these charts and we'll make sure to provide you with that knowledge with all the chips you receive from us.



3. NANO CHANNEL CHIP GENERAL SPECIFICATIONS

SPECIFICATIONS	NANO CHANNEL CHIP
Chip dimensions	4.2x4.2x0.7 mm
Nano Channel width	1-3 µm
Nano Channel depth	Down to 20 nm
Nano Channel length	Up to 1 mm
Silicon nitride membrane thickness	Down to 10 nm
Al2O3 coating on membranes	Down to 3 nm
Bypass/Micro channel height	10-15 µm
Typical bypass flow rate at 100 mbar	0.1 µL/min
Typical volume of a single nano channel	0.1x1x100 µm3; 10−17 m3; 10 fL
Typical volume of a single bypass /micro channel	1x10x1000 µm3; 10−14 m3; 10 pL
Nano Channel chip material	Si, SiO2 in inlets, Silicon nitride in all flow channels, coated with Al2O3 in all flow channels
Pressure tolerance	Not recommended to use over 2 bar in any SEM or TEM, although some chips can tolerate well over 20 bar
Allowed sample chemistry	The membranes of silicon nitride and Al2O3 appear stable with most dilute acids and bases. Avoid pH lower than 2 and higher than 10. Be cautious with higher concentrations of HNO3, H2SO4, H2O2.


Insight Chips