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## Development of Microfluidic Chips To Immobilize *C.elegans* For Nervous System Irradiation With Proton Microbeam

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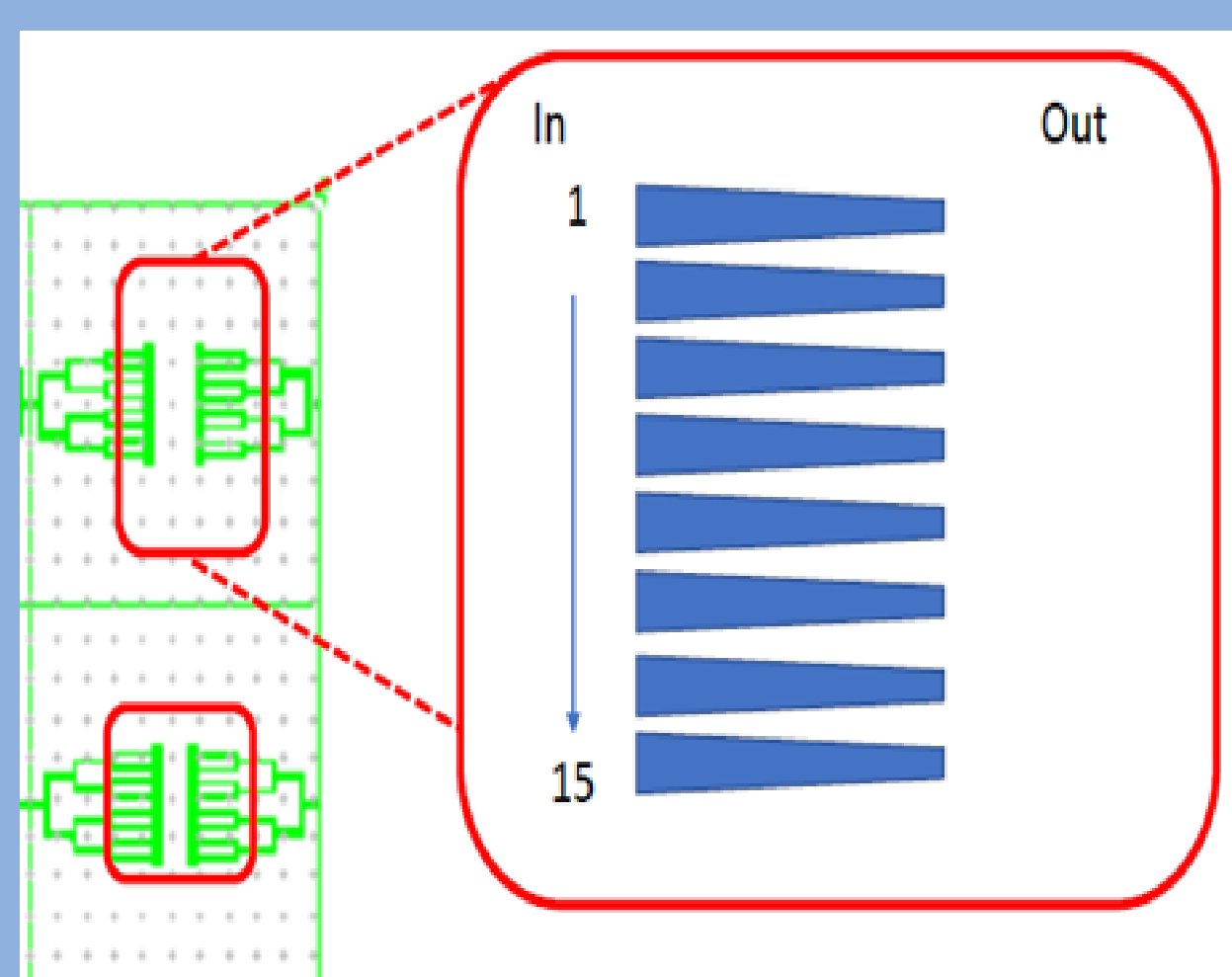
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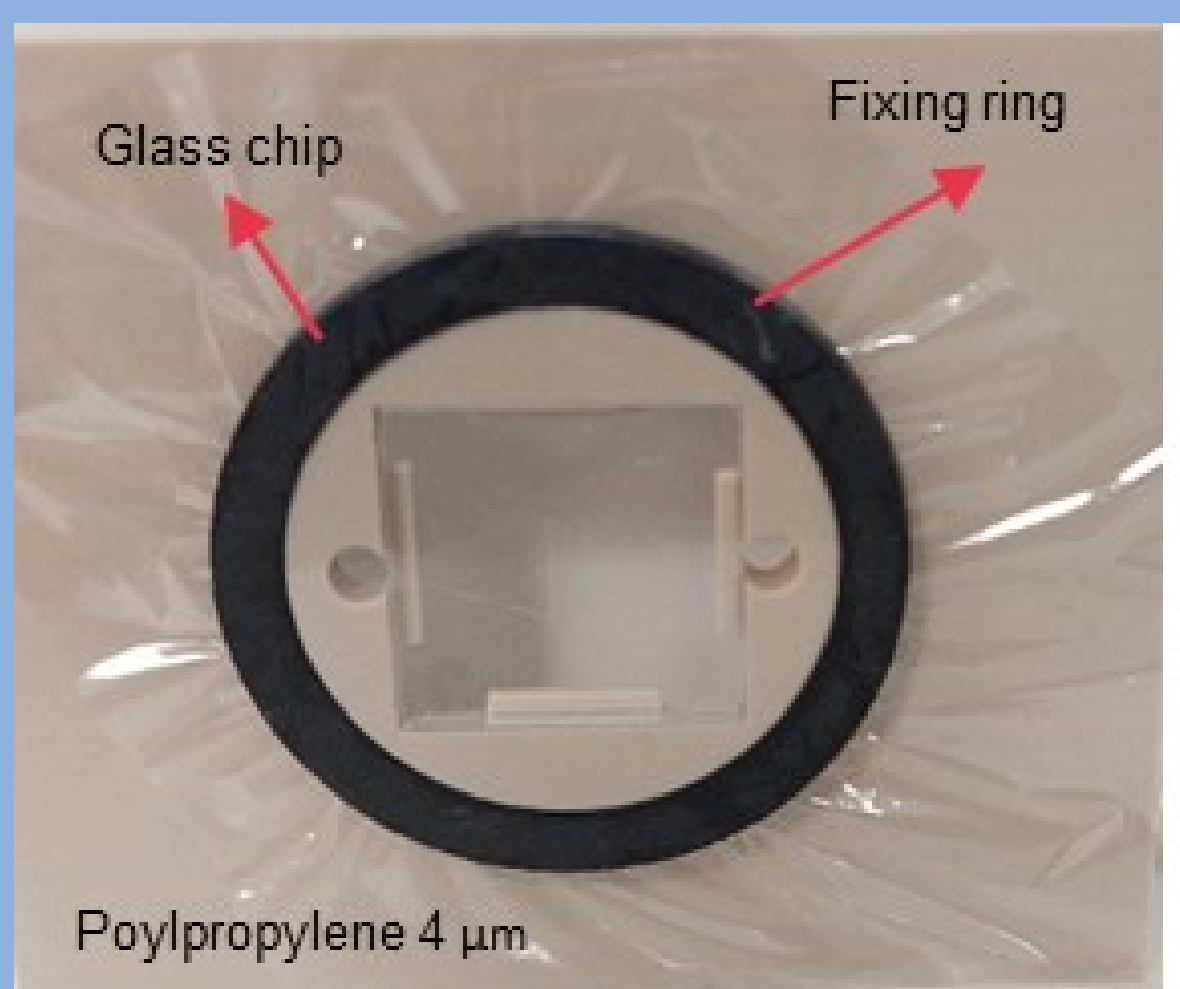
Regardless of its source (space, environment, medical, nuclear accident), all forms of ionizing radiation, from massless photons (x rays and  $\gamma$ ) to heavy charged particles (protons or carbon ions), can induce toxicity in the central nervous system. Despite the growth of the scientific and clinical literature production giving better understanding of how radiation causes brain injury, the precise mechanisms of neurotoxicity and neurodegeneration following ionizing radiation exposure remains poorly understood from a biological perspective, in particular regarding mitochondrial and DNA damage. Since targeted microbeam irradiation allows the effective knockdown of specific regions,

thus helping to identify their roles in processes such as neurodegeneration, we decided to study the consequences of proton exposure, using the IRSN ion microbeam facility MIRCUM, at the molecular and tissue level in the nervous system of *C.elegans*. This new facility, introduced by IRSN in 2018, allows to target cellular or sub-cellular components and therefore the fraction of the cell targeted by the particles, the number of particles for each target, and their location can be known accurately. In order to immobilize *C.elegans* worms without anesthesia, we developed ultra-thin, ion-penetrable, PDMS microfluidic chips and glass chips

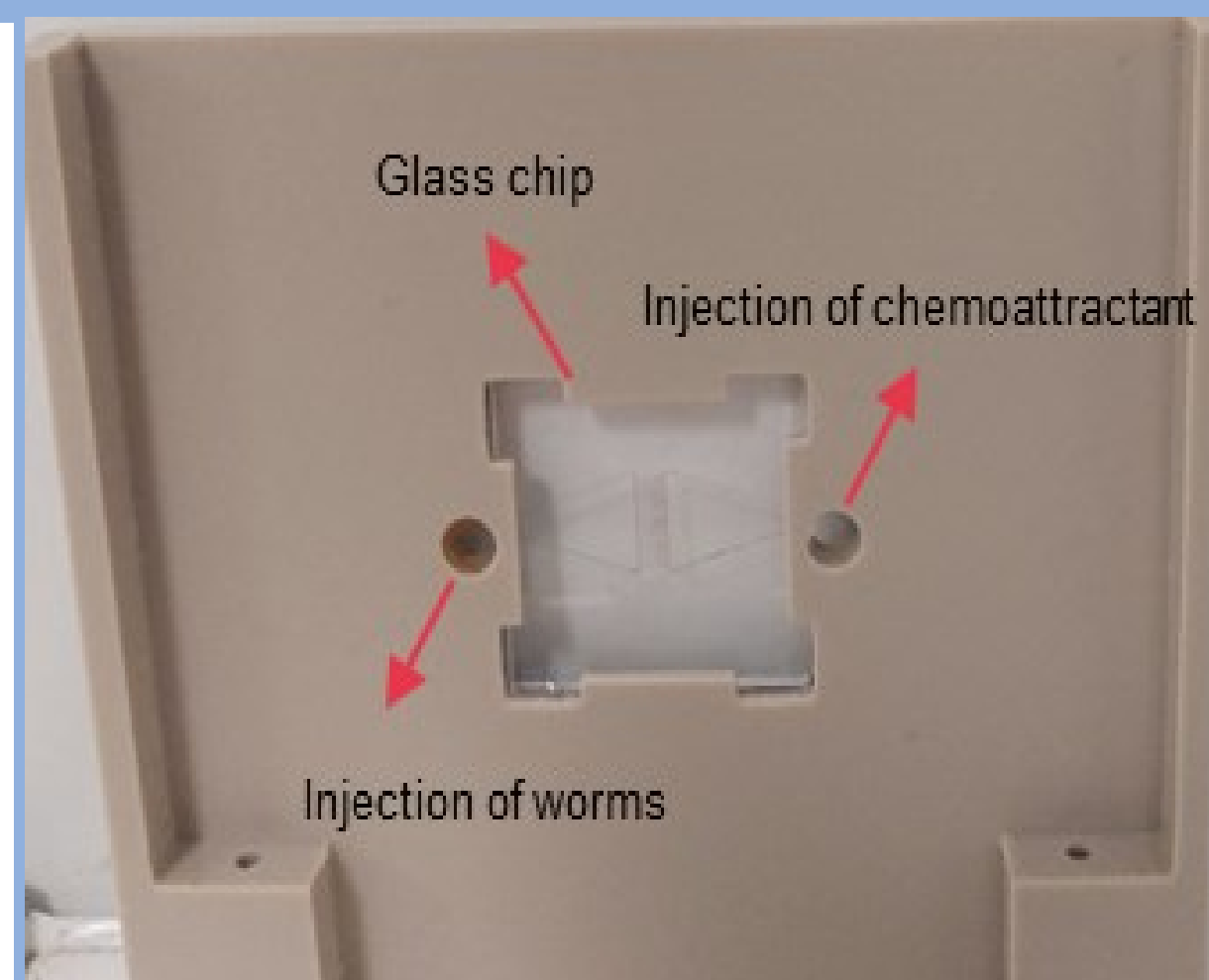
### Development of microfluidic glass chips to immobilize nematodes



Dimension: L x l x H: 10 x 10 x 1 mm  
Channel: 200  $\mu\text{m}$  — > 34  $\mu\text{m}$ , Depth z: 80  $\mu\text{m}$



PEEK container: 5.4 cm  
The chip in front of the microbeam

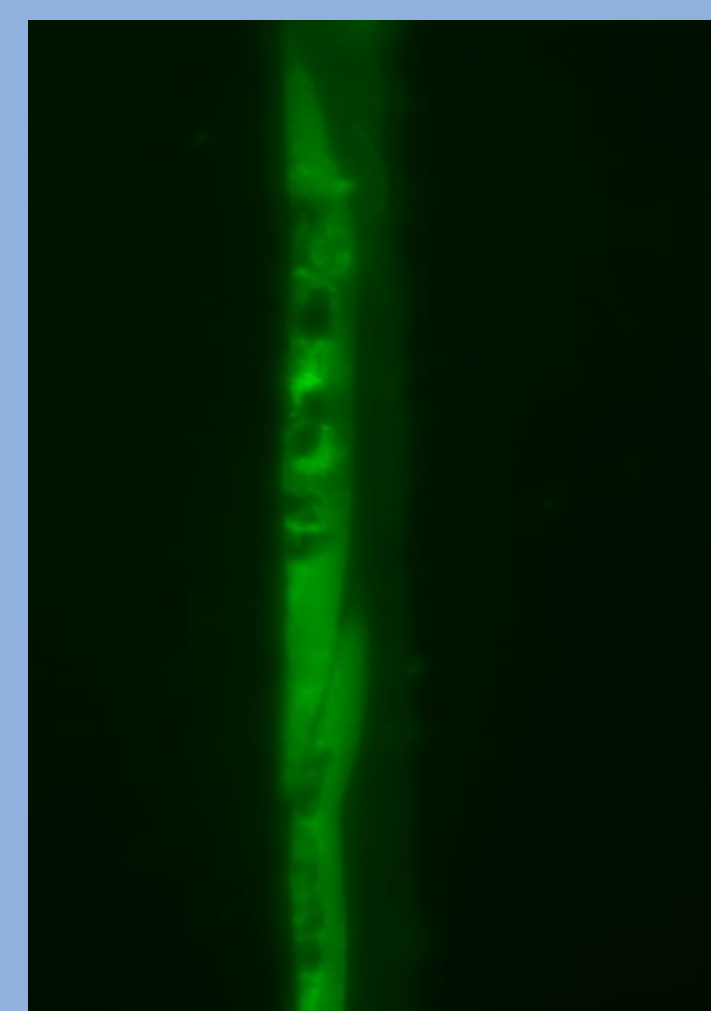
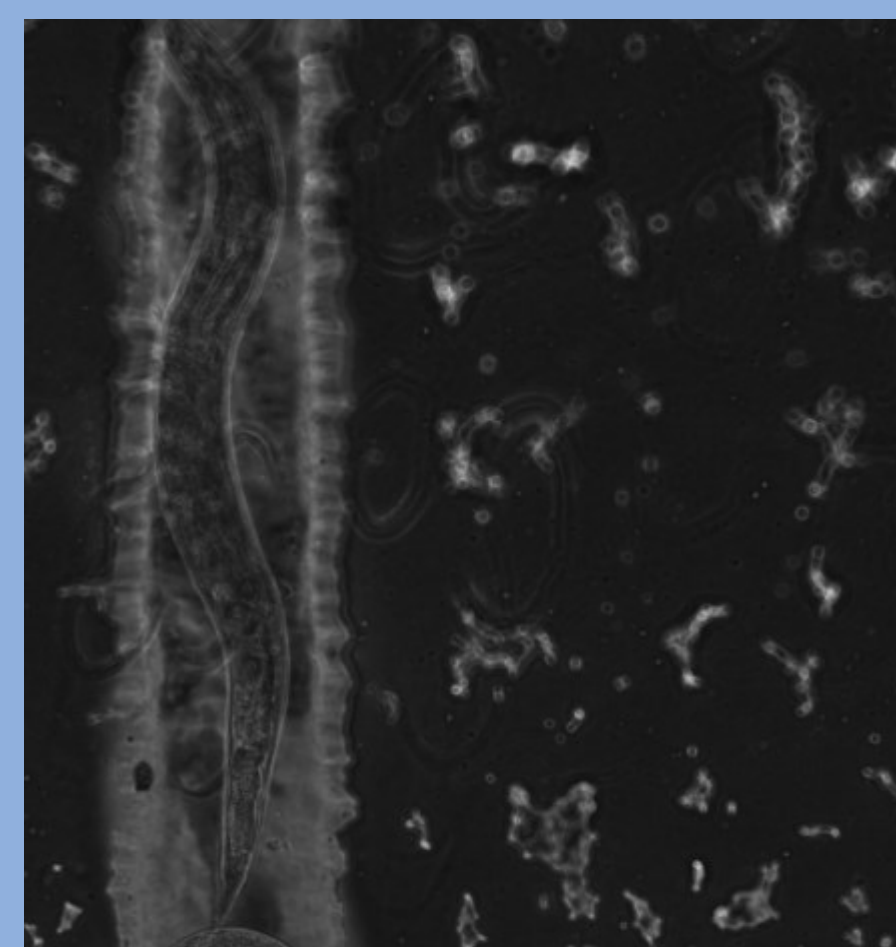
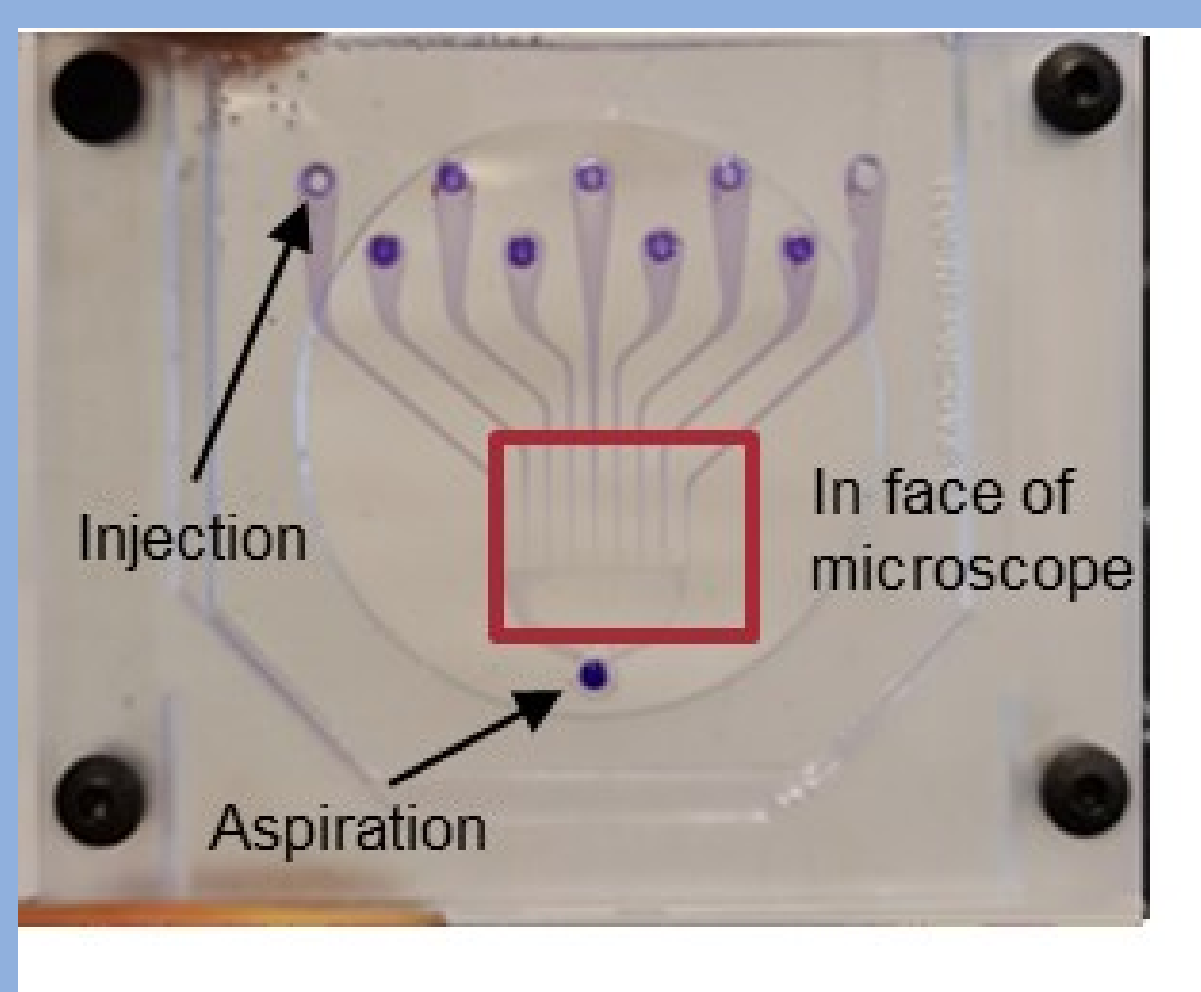


PEEK container: 5.4 cm  
The chip in front of the microbeam

The first chip is made of glass, in a 1 mm square shape and contained in a PEEK container. On the microbeam side, the chip is covered with a 4  $\mu\text{m}$  thick polypropylene membrane allowing the penetration of protons. On the other side, the nematodes will be observed in real time with the microscope. Two injection chambers allow the fluid containing the nematodes to be injected on one side and the chemoattractant on the other side. The 2 fluids will connect via a network of 15 channels with a reduction in size, by funnel effect, from 200  $\mu\text{m}$  down to 40  $\mu\text{m}$ . The channel is 80  $\mu\text{m}$  deep in (z).

**Advantages: Reusable and Optimizable: possibility of automating the injection of fluid with a longer maintenance of the worms in the chip.**

### Development of PDMS microfluidic chips to immobilize nematodes



Sod-1::gfp transgene observed in the chip  
Sod-1 : an anti-oxidative stress protein

The second model is the PDMS chip. Using the same PEEK container, the PDMS chip is placed in front of the microbeam with 9 channels covered with polypropylene. On the other side the injection is performed in each entrance door and then the aspiration is done at the level of the outlet of the connection network. The liquid aspirates carries with it the nematodes which will be trapped at the end of the channel in front of the microbeam and the microscope.

**Advantages:**

Consumable and low price

Hydrophobic and hydrophilic PDMS: possibility to compare the behaviour of the nematode with respect to the material

### Application

The chips are thin, allowing ions such as proton of 4 MeV to pass through the polypropylene membrane containing the worms. As an example of the application of those chips, we targeted neurons and analysed the mitochondrial activities of immobilized animals using OH441 transgene and mitochondrial dyes. As a conclusion,

compared to actual techniques, those improved chips will become a powerful tool for prolonged immobilizing of *C. elegans* and microbeam irradiation without the use of anaesthesia and will help to target specific neurological pathways and study the related mitochondrial dysfunction.

**PROTONS INDUCE A SIGNIFICANT LOSS OF MITOCHONDRIAL MEMBRANE POTENTIAL IN TMRE-STAINED *C. ELEGANS* NEURONS.**

**IRRADIATION OF THE NERVE RING OF THE HEAD OF THE NEMATODE: AREA WHERE THE MAJORITY OF NEURONS ARE ACCUMULATED**

