The DuaLink chips: Improved fluidic isolation in microfluidic devices designed for neurons culture

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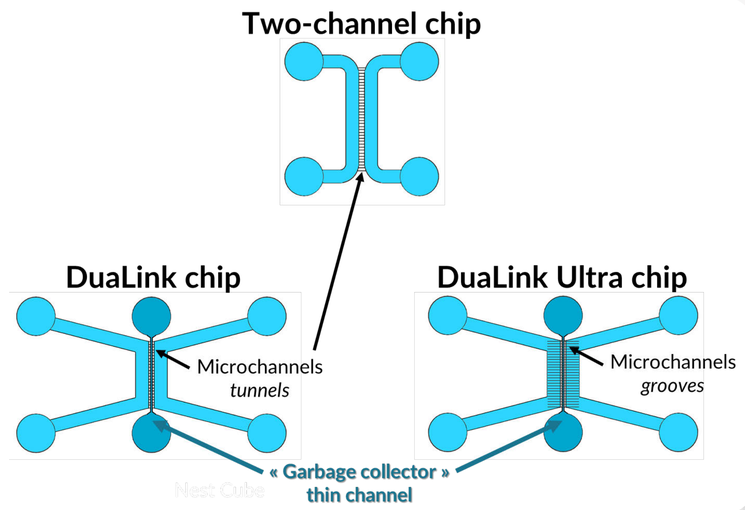
Background

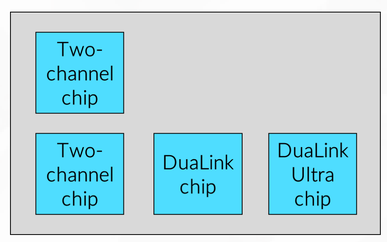
- NETRI develops microfluidic chips for neurons culture allowing compartmentalization of somas and axonal endings using microchannels between channels
- **Fluidic isolation between the main channels is essential** to allow drug testing on either soma or axonal compartment, and to co-cultivate another cell type in axonal channel with its specific medium
- **Problematic: manual handling of microfluidic chips can lead to medium leakage** from one compartment to the other through microchannels, a phenomenon which can also greatly differ between operators, leading to lower reproducibility of results.

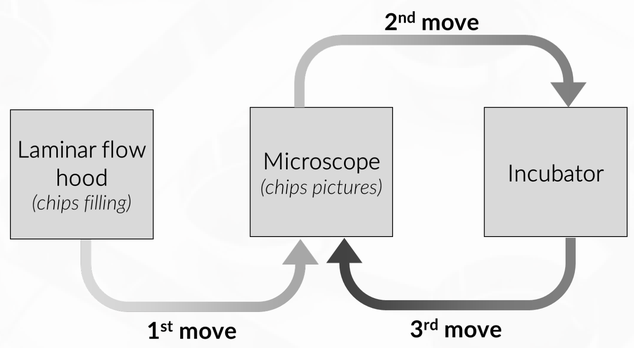
NETRI developed new chip architectures with the addition of a thin central channel between the two main channels, called DuaLink chips, to enhance fluidic isolation
Here we analyzed the fluid isolation of the conventional two-channel and the DuaLink chips



Materials and methods

- The different chip architectures to compare were bonded on the same microplate
- The chips were filled under laminar flow hood with fluorescent compound (either Red or FITC-Dextran 3-5 kDa) or PBS to follow the fluids leakage between channels
- **To reproduce classical handling** of *in vitro* cultured microfluidic devices, the microplates were moved between several lab equipment's, and pictures were taken to analyze fluid leakage between channels
- **To be representative of the end-users' profile heterogeneity**, the experiments were done by trained and non-trained operators for the use of microfluidic devices





Results

1 Performance of the conventional two-channel chip under hydrostatic pressure

- Hydrostatic pressure technique is created by filling different volumes between channels, and is commonly used to isolate fluids[1]
- Here we demonstrated that this technique can only partially thwart the fluid leakage:
  - Red-dextran (in the channel under pressure) is well confined to channel 1, even with moves
  - But **up to 48 % of leakage of fluid** from channel 2 to channel 1 after 3 moves

The hydrostatic pressure technique leads large and highly variable fluid leakage and compound dilution

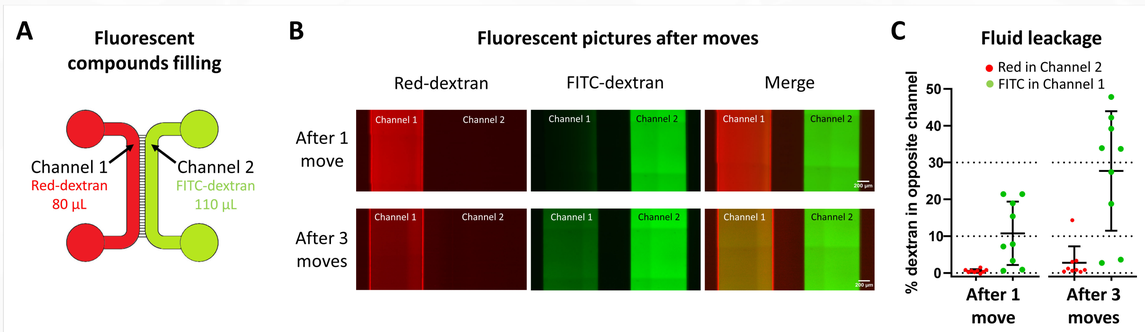


Fig. 1: Analysis of fluidic leakage between channels under hydrostatic pressure in the two-channel chip. (A) At T0, channel 1 of the two-channel chip was filled with 80 µL of Red-dextran and channel 2 with 110 µL of FITC-dextran, thus creating a hydrostatic pressure between channels. (B) Pictures of the devices after 1 move or 3 moves. (C) Quantification of the percentage of each compound (dextran) in the opposite channel after 1 and 3 moves.

2 NETRI's DuaLink architectures rise fluidic isolation and intra- and inter-operators' reproducibility

- Significant **decreased fluid leakage** between channels in the DuaLink chips, compared to the conventional two-channel chip
- Maximal leakage value **divided by 2 to 3** in the DuaLink chips

The addition of the thin "garbage collector" channel improves fluidic isolation

- Significant **decreased variance** of the results (F test) with the DuaLink chips compared to the two-channel one (p<0,001 for 1 move and p<0,01 for 3 moves, whether for *tunnels* or *grooves*), **with reduced intra- and inter-operators' variance**.

The fall in variance between operators and between replicates enables a better reproducibility in the results.

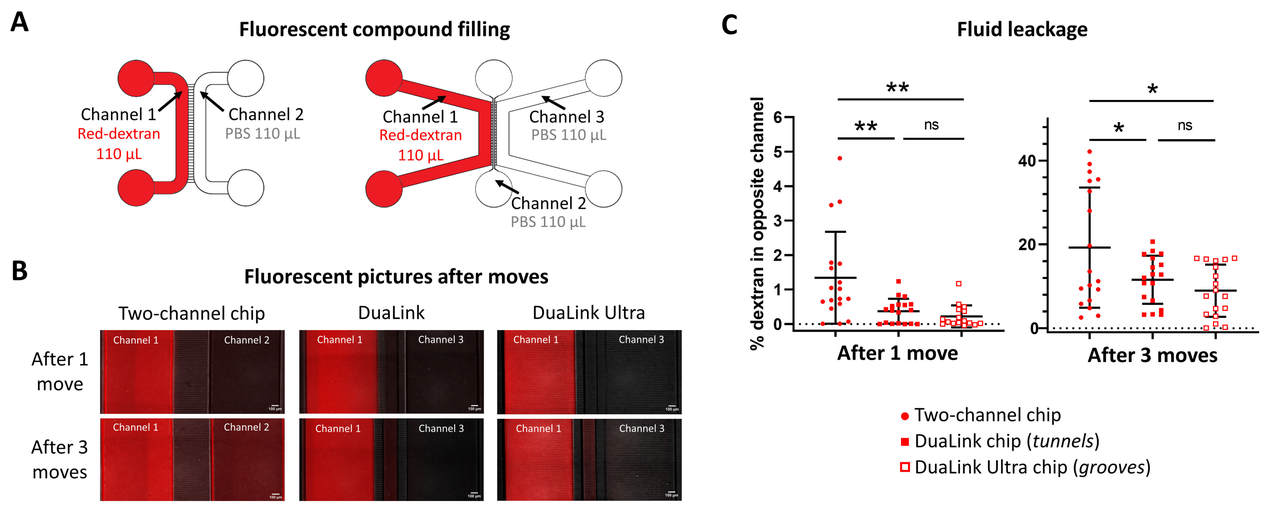
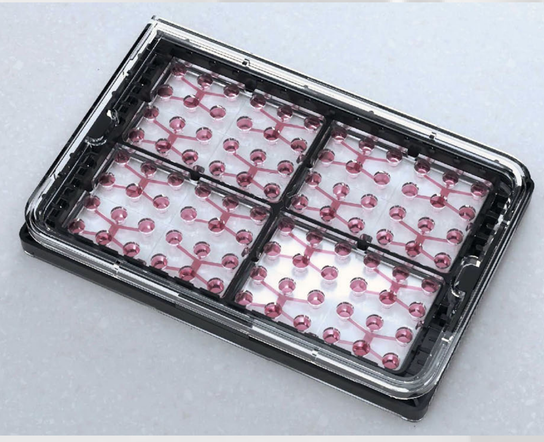


Fig. 2: Improved fluidic isolation and reproducibility with the NETRI's DuaLink and DuaLink Ultra chips, compared to the two-channel device. (A) At T0, channels 1 were filled with 110 µL of Red-dextran and channels 2 and 3 with 110 µL of PBS. (B) Pictures of the devices after 1 move or 3 moves. (C) Quantification of the percentage of red-dextran in the opposite channel after 1 and 3 moves.

Conclusions

- ✓ Handling of microfluidic chips leads to high and variable fluidic leakage between channels
- ✓ NETRI's DuaLink and DuaLink Ultra microfluidic chips are designed with a thin central channel which acts as a "garbage collector"
- ✓ **Less than 1 % of leakage between opposite channels in the NETRI's DuaLink chips** with a typical move like from the laminar flow hood to the incubator
- ✓ **Increased results reproducibility in the NETRI's DuaLink chips** with reduced variance between replicates and operators
- ✓ **With optimal fluidic isolation, NETRI's DuaLink chips allow compartiment-specific compound testing and co-culture of different cell types with respective optimal cell culture medium**



References

1. Taylor AM, Blurton-Jones M, Rhee SW, Cribbs DH, Cotman CW, Jeon NL. A microfluidic culture platform for CNS axonal injury, regeneration and transport. Nature Methods. 2005;2(8):599-605.

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