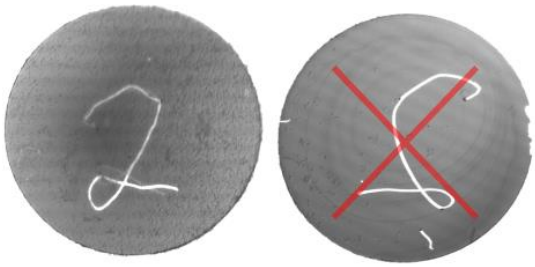
# Handling of sapphire disks

**General tips to handle sapphire disks:**

- Disks should be glow discharged to make them hydrophilic; please coordinate with EMC to get the required amount of disks just before you seed the cells
- Disks are coated with a gold layer, in which the figure 2 is inscribed to keep track of the orientation; make sure the 2 faces up before and after the cells are plated on the disk
- Handle disks using very fine forceps: do not touch the coated surface, it will easily scratch
- Consider limiting the total number of sapphire disks per plate, especially when using multi-well plates, to minimize the handling time outside the incubator when freezing
- Disks are expensive, please return all unused disks



*Left: Correct orientation, confluent monolayer. Right: Incorrect orientation, cell density too low.*

**Protocol:**

1. Sterilize disks before seeding cells:
    - Disks and forceps should be sterilized (e.g. UV light, heat, microwave)
    - Coating of disks with collagen, poly L lysine, gelatin, or similar is optional, but recommended especially with less adherent cell types
2. Transfer disks into medium:
    - Use wells larger than 96-well to allow easy removal of disks
    - To prevent disks from floating: add some medium to the culture well first, then push the disk under the surface of the medium all the way to the bottom; make sure no air bubbles are attached to the disks
    - Use two or more disks per well
3. Add cell suspension:
    - Ideally the cells will reach ~80% confluency by the day of HPF
    - Ensure that the disks are oriented correctly before adding the cell suspension
    - Cell suspension should be added very gently to avoid flipping the disks
    - Shake well gently to spread cells evenly
4. Transfer to incubator:
    - Ensure that the disks are still oriented correctly before placing into incubator
5. If cells are frozen alive, transfer to EMC at least one day before processing:
    - Coordinate transfer with EMC to make sure the incubator is available
    - Minimize temperature change by transporting cells in a thermo/styrofoam box during transport
    - Keep transport times brief, and be careful not to disturb the cells, flip the disks etc
6. If cells are chemically fixed before HPF:
    - Get EM grade fixative from EMC
    - Aspirate most of the medium (it is not necessary to wash cells), carefully add warm (!!) fixative and incubate at room temperature for 30 min
    - Carefully replace fixative with PBS