Stampwell

PROTOCOL & SPECIFICATIONS

idylle

STAMPWELL PROTOCOL

The material you need

- ✔ Hydrogel (agarose, alginate, phytagel, methylcellulose ...)
- ✔ 35 mm petri dish, with or without glass bottom
- ✔ A Pipetman or a Pasteur pipette to load samples in wells
- ✔ A cutter blade
- ✔ A non-wounding spatula
- ✔ A stereomicroscope ideally

1. Cleaning

- The first time you use Stampwell, soak it in water overnight under stirring.
- Next time, if you need to work in sterile conditions, clean your stamp with Surfanios™ disinfectant detergent.
- Rinse with water.
- Moreover if bits of dirt are stuck in the grooves, you can use a sonicator.

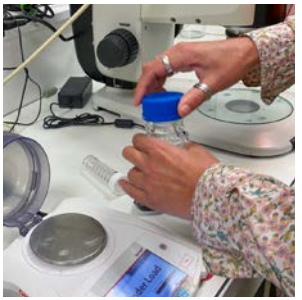
2. Agarose gel preparation

It is suggested to prepare a 2% normal gelling temperature agarose. You can use any other hydrogel of your choice, it only has to be stiff enough once reticulated.

- Ideally, prepare small amounts. 50 mL is more than enough.
- Use a bottle twice as big as the desired volume.
- In a 100 mL glass bottle, weight 1 gram of agarose.

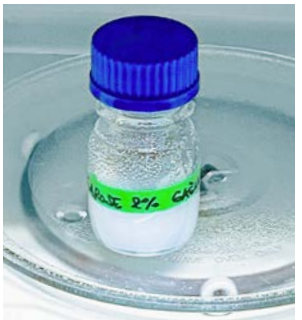
- Add 50 mL of distilled water.

- Close the bottle.



- Autoclave.

- Warm the agarose preparation in a microwave, full power.



- Slightly loosen the cap.
- Place the bottle on the periphery of the microwave plate (not on the center, this is where warming is the less efficient).
- Open the door and gently rotate the solution to spread the liquid agarose on top of the one that is still jellified.
- Repeat every 10 seconds without opening the cap until the agarose is fully dissolved.
- Stop it right away as soon as it boils (before it spills over!).

- Use your agarose gel right away when it is still liquid.

3. Wells imprinting

Pour liquid agarose directly onto the pins of Stampwell.

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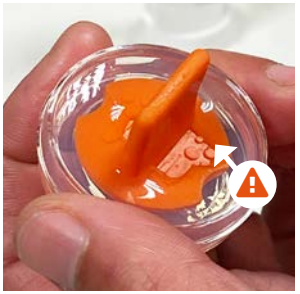
This will prevent the formation of bubbles.

- Pour liquid agarose in the dish.

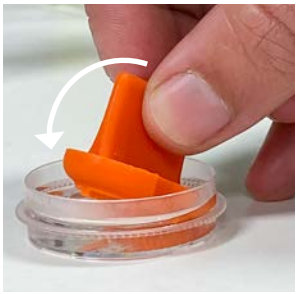
The level of agarose should reach half of the height of the lateral wall of the stamp, but never flow over it.



- Immediately place the stamp in the dish.

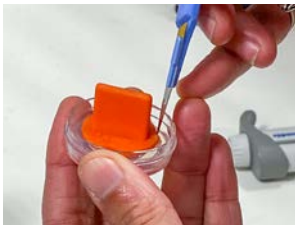
Avoid placing the stamp with a downward pressure.

Prefer a tilting movement (from the edge to the vertical position) until the whole surface of the stamp is in contact with the dish, to avoid the formation of air bubbles.



- If you are using a 6-well plate, you can align the stamps in each well to automatize more easily image acquisition with the microscope software.
- Reticulate the hydrogel.

- For an agarose gel, allow the whole set up to cool down: to speed up the process, you can leave it at 4 °C for 5 minutes.
- A neat result is obtained by passing a scalpel blade all around the stamp to make sure it is well detached from the gel pad.



Do not remove the stamp vertically.

Option 1: take out the stamp by tilting it a bit at first, then pull firmly.

Option 2: hold the gel pad with a non-wounding spatula to avoid the gel detaching itself from the dish. Then pull the stamp firmly.

4. Samples loading

- Wash the gel.

- For live samples: If you have prepared your gel from water, it is recommended to wash the whole gel with culture medium thrice.
- Keep it at the desired temperature for a few hours, in the incubator for example.
- For fixed samples, operate the same pre-washing step but use the mounting medium.
- Prepare the material to place samples: either a 100-200 µL yellow tip or an embryologist mouth pipette using the following protocol.

HOW TO MAKE AN EMBRYOLOGIST MOUTH PIPETTE

A. Bring the center of a glass capillary tube into a medium height flame.

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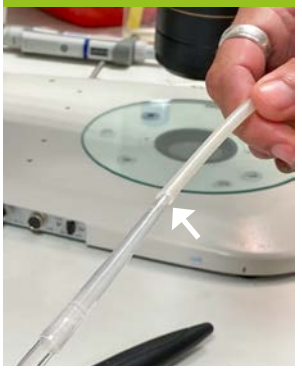
B. As the glass capillary tube begins to melt, quickly lift the tube out of the flame and then pull the tube outward about a few centimeters.

C. The pulled tube is then snapped in the middle, producing two glass transfer pipettes.

D. The glass transfer pipette is then connected to a Tygon® tube with a blue tip at one end a yellow one at the other end to hold it (then, indeed it is on you to decide!).

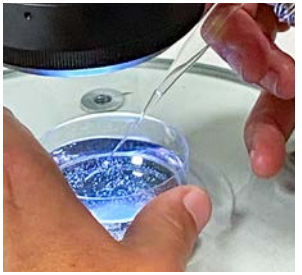


To control where and how each sample is placed in each well, it is highly recommended to use a stereomicroscope.

- Train yourself before with random samples to be familiar with this step.
- Before pipetting your sample, pre-rinse your tip or pipette with serum or medium. It prevents the samples to stick to the tip (or the glass pipette).

Make sure you have pipetted enough medium before taking the samples, so that it will not stick to the air-liquid interface.

- Take one sample at the time when you start. Once you feel comfortable enough, you can load up to tens of samples. It depends on the length of the straight part of the pipette. Do not forget to space the samples enough in the pipette to avoid mixing them.



If you use a Sampwell V-Shape: the conical tip of Stampwell imprints a shape of well which includes a slope. It allows to slide the tip on it. Left-handed people: beware, you might want to rotate the dish so that the slope is on your left side.

- Do not touch the gel with the tip.
- Once the tip is at the bottom of the well, release the pressure very gently.
- If the flow is too strong, the sample will pop out of the well and will float around in the dish. The sample should almost fall in the well by gravity rather than being pipetted out.

5. Imaging

- If possible, automatize the acquisition: the spacing between wells is standard (See Stampwell specifications). If your samples are homogenous in size, they should be all at the same distance from the bottom of the dish.
- Upon labelling the positions while looking through the eyepieces, you can easily read the number of the well. Hence, you can keep track of the specimen and proceed to further analysis (fixing and staining, or snap freeze and sequence...).
- You can reiterate imaging for several weeks. You may re-use the saved position parameters on the microscope. See "after acquisition" to take care of your samples during these weeks.

6. Manipulating samples

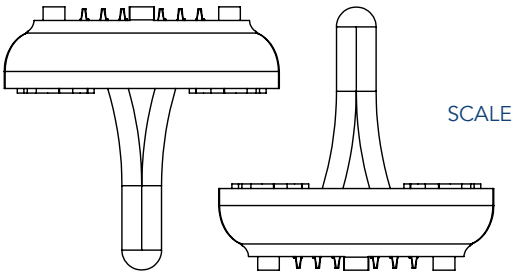
- Operate with a stereomicroscope.
- Gently place the tip to the bottom of the well (with already a small amount of liquid) and suck the sample out.
- For the fish/tissue explants wells, make sure you use smooth forceps and tools not to scratch the gel pad.

7. After acquisition

- Clean Stampwell as previously described. See Cleaning, section 1.
- Either single shot or time lapses, you can take your samples back to the incubator. They will keep on growing in the wells. They will not be constrained.

Stampwell | Specifications

STAMPWELL SPECIFICATIONS



SCALE 1.5 : 1

All shapes

Manufacturing process: we produce countertyped silicone molds to manufacture polyurethane stamps.

External diameter: 26 mm. It is compatible with almost all 35-mm dishes.

Distance between the wells bottom and the dish bottom: 100 µm. Three feet have been designed so that Stampwell can lay on the glass of almost all glass bottom dishes, whatever their size. It ensures that the bottom of each well is at the very same distance from the bottom of the dish.

Demolding: is made easy thanks to an ergonomic handle and curvature of the stamp's base, as well as a notch allowing an object with a rounded tip to pass through to hold the hydrogel during demolding.

Lifetime: each stamp can be used dozens of times.

V-shape 300 µm - for the imaging and culture of medium-size rounded 3D objects such as spheroids and organoids

Number of pins: 42

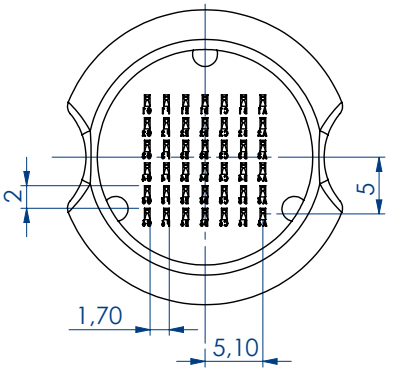
Pins imprint wells with the following dimensions:

- **Depth of wells:** 1 mm
- **Wells bottom diameter:** 300 µm - **Shape:** circular
- **Wells upper side:** 1 mm * 0,5 mm - **Shape:** rectangular
- **Spacing between 2 wells:** 1,70 mm on the lines and 2 mm on the columns

Spotting: each well is identified with both a letter and a number. It allows the localization of the sample you want to further study.

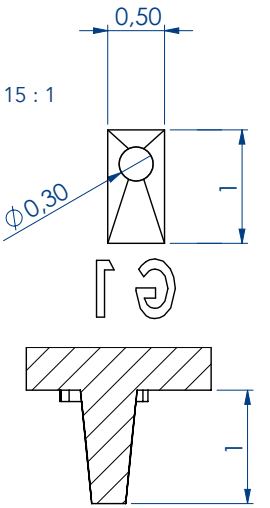
The right edge of the wells provides a slope to support and guide the pipette cone when loading the samples.

V-shape 300 µm



SCALE 1,5 : 1

SCALE 15 : 1



SCALE 15 : 1

V-shape 500 µm - for the imaging and culture of large-size rounded 3D objects such as spheroids and organoids

Number of pins: 42

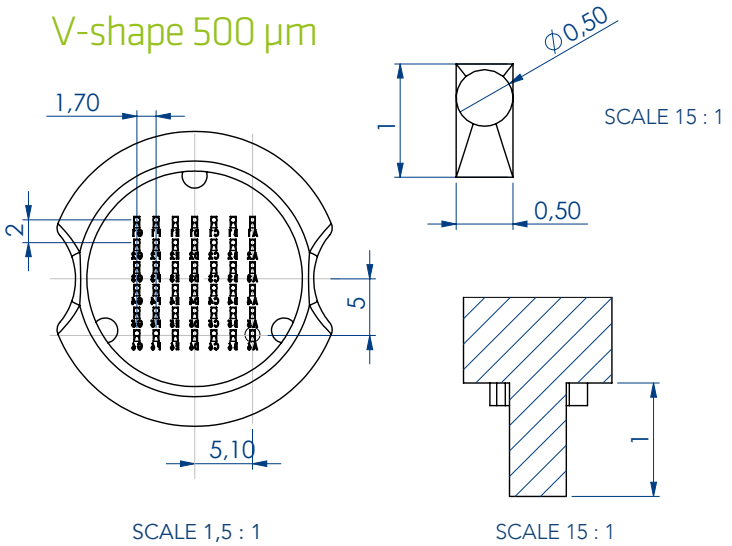
Pins imprint wells with the following dimensions:

- **Depth of wells:** 1 mm
- **Wells bottom diameter:** 500 µm - **Shape:** circular
- **Wells upper side:** 1 mm * 0,5 mm - **Shape:** rectangular
- **Spacing between 2 wells:** 1,70 mm on the lines and 2 mm on the columns

Spotting: each well is identified with both a letter and a number. It allows the localization of the sample you want to further study.

The right edge of the wells provides a slope to support and guide the pipette cone when loading the samples.

V-shape 500 µm



Embryo 1 - for the imaging of embryos such as 1 DPF zebrafish, Medaka or Astyanax embryos

Number of pins: 35

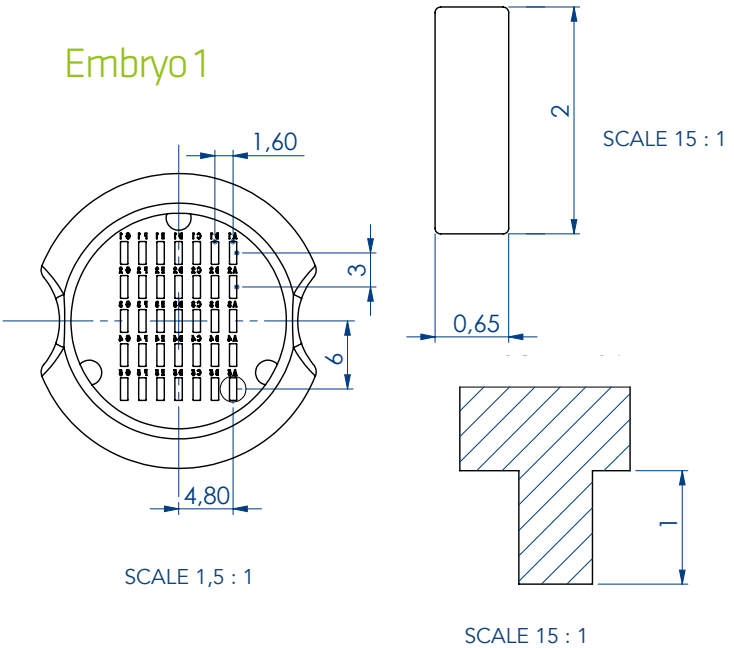
Pins imprint wells with the following dimensions:

- **Depth of wells:** 1 mm
- **Length * width:** 2 mm * 0,65 mm
- **Spacing between 2 wells:** 1,60 mm on the lines and 3 mm on the columns

Position of embryos in the wells: laying face downwards

Spotting: each well is identified with both a letter and a number. It allows the localization of the sample you want to further study.

Embryo 1



Embryo 2 - for the imaging of 2 DPF zebrafish embryos or 9 DPF (stage 39) medaka embryos

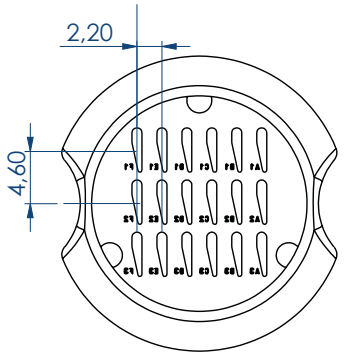
Number of pins: 18

Pins imprint wells with the following features:

- **Depht of wells:** 0.50 mm
- **Length of wells:** 3.90 mm
- **Width of wells:** 0.88 mm
- **Horizontal pitch:** 2.20 mm
- **Vertical pitch:** 4.6 mm

Position of embryos in the wells: laying on the side

Spotting: each well is identified with both a letter and a number. It allows the localization of the sample you want to further study.



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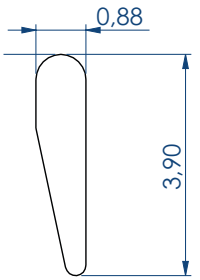
Embryo 2



SCALE 15 : 1

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Larvae 1 - for the imaging and culture of 3 – 14 DPF zebrafish or stage 39 - 41 medaka larvae

Number of pins: 15

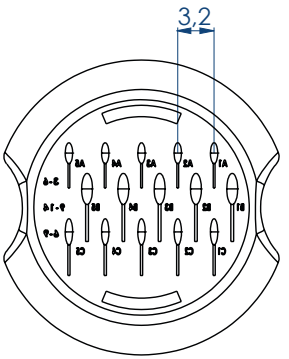
Pins imprint wells with the following features:

- **Wells shapes:** cuvette
- **Depth:** 0.6 – 1.4 mm
- **Dimensions of the smaller wells:** 4 x 0.65 mm
- **Dimensions of the intermediate wells:** 5 x 0.8 mm
- **Dimensions of the largest wells:** 6 x 0.96 mm

Position of larvaes in the wells: laying face downwards

Spotting: each well is identified with both a letter and a number. It allows the localization of the sample you want to further study.

Larvae 1



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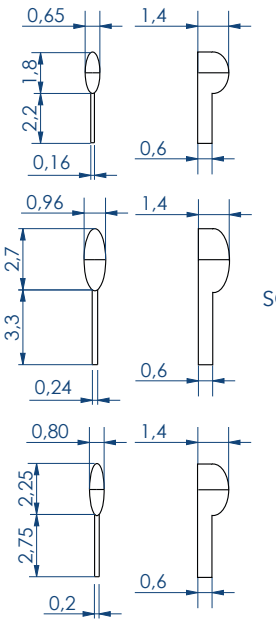


SCALE 3 : 1

Larvae 2 - for the imaging and culture of zebrafish or stage 39 - 42 medaka larvae imaging

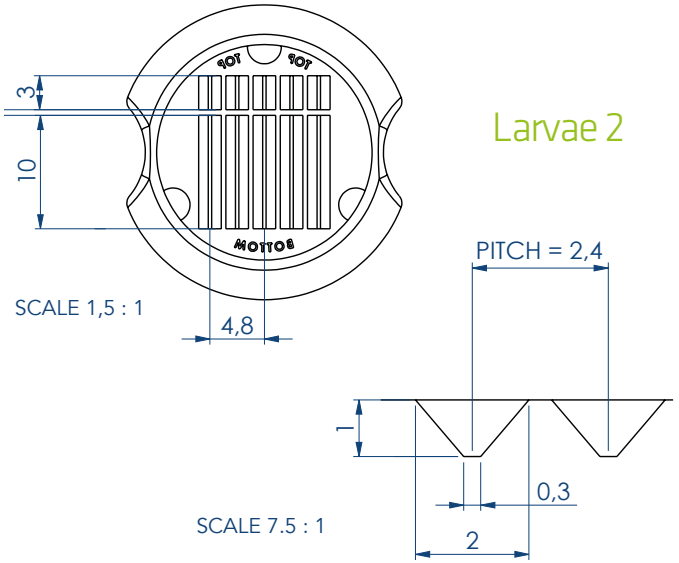
Number of pins: 10

Pins imprint wells with the following features:

- **Wells shapes:** Prism
- **Depth:** 1 mm
- **Length for big wells:** 10 mm
- **Length for small wells:** 3 mm
- **Width of the bottom of the wells:** 0.3 mm
- **Width of the upper side of the wells:** 2 mm
- **Pitch:** 2.4 mm

Position of larvaes in the wells: laying face downwards

Spotting: simple coding (“top” / “bottom”)



Larvae 2

Check videos of protocol, examples
of results and much more on:
idylle-labs.com/stampwell-by-mountn

A protocol first designed in December 2020
and updated in January 2023 with the Embryo 1, Embryo 2,
Larvae 1 and Larvae 2 new shapes.

Thank you to all the early-users who shared their feedback
on the Stampwell they tried during their Test Program:
their contribution also helped to finetune this protocol of use!

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