Documentation

A. Videos of the insemination sample with FAST

Aliquots of the insemination sample (**NOT** the raw sample) should be taken for imaging with FAST following the instructions below.

1. Smear

A sperm smear should be made of the insemination sample:

a. Clean both surfaces of two frosted slides by wiping off with ethanol.

b. Label the frosted portion with the UNiTY couple ID and the cycle number (IVF/IUI1/IUI2/IUI3)

c. Apply a 5-10 µl aliquot of prepared sample, to the end of the slide. Use the second slide to pull the drop of sample along the surface of the slide (see figure).

d. Allow the slides to dry in air. Place in slide box and keep in the fridge until transfer to Birmingham.

To get the feel for the motion, place the dragging slide at an angle of 45° and move it into contact with the aliquot of semen *(a)*, which runs along the edge of the slide *(b)*. Bring the dragging slide slowly back (over approximately 1 second) along the length of the slide to produce the smear (c).

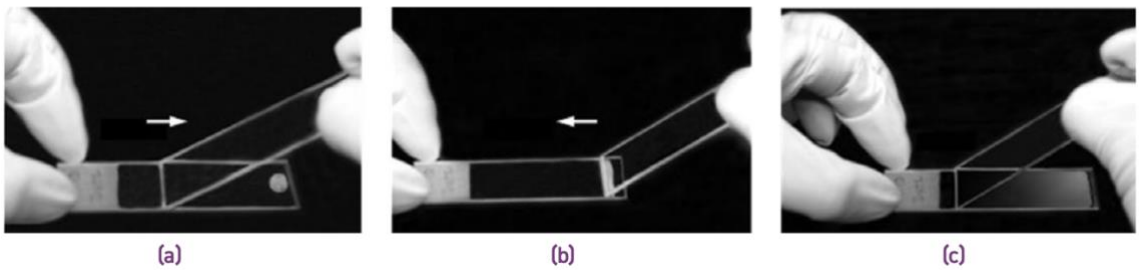


Image from WHO6 Manual

2. Concentration

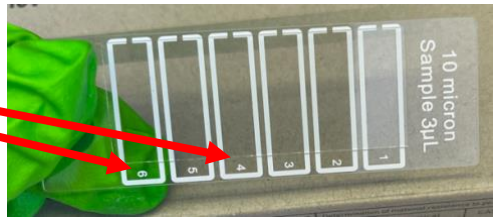
If the insemination sample has been prepared at a concentration > 15 M/ml then it must be diluted down below this threshold using additional sperm preparation media.

3. Imaging chambers

Place 3µl of the insemination sample in two chambers of the fixed-depth slide by pipetting onto the open numbered section.

Connect the sample with the slide opening by drawing the pipette tip so that they touch.

You **do not** need to 'push' the sample into the slide by pipetting directly under the coverslip.



4. Heated stage

Place the filled sample in the **pre-warmed** heated stage for imaging.

The heated stage should then be placed under the objective on the microscope.

5. FAST – Start Experiment

Now prepare FAST for imaging.

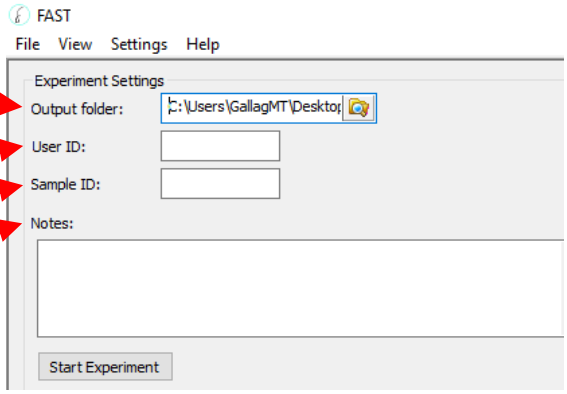
The Output folder should be prepopulated with your hard drive location.

Your initials go in the 'User ID' field.

The UNiTY couple ID goes in 'Sample ID'

In 'Notes' put which cycle this is for (IVF / IUI1 / IUI2 / IUI3)

When you have completed these fields, click 'Start Experiment' to record videos.



After clicking 'Start Experiment' you will see the video feed of the sperm, and you can focus the microscope. Make sure there is enough light that you can clearly make out the beating tails of the sperm.

If there is a background flow, wait 5 minutes to allow it to settle down. If after ~5 minutes there is still background flow, fill another chamber and image in that.

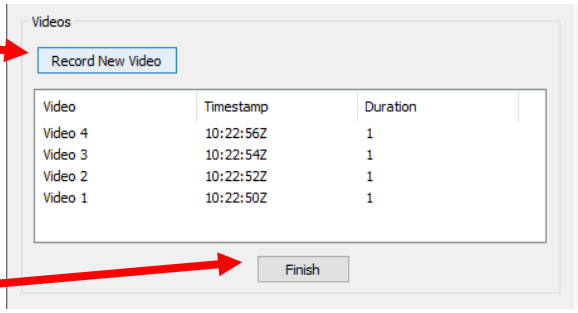
6. Capture videos

Click 'Record New Video' to capture a video of the swimming sperm.

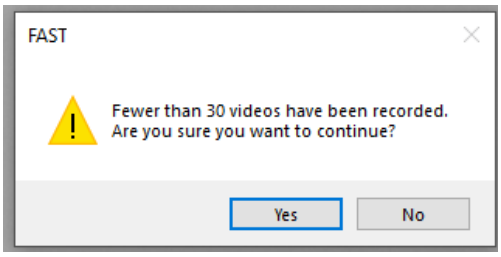
After each video, move the field so you are imaging new sperm.

Once you have taken 15 videos in a single chamber, move to the other chamber and take 15 more videos.

After you have taken 30 videos across two chambers, click 'Finish'.



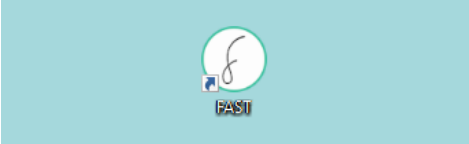
If you click 'Finish' before you capture 30 videos you will get a warning. Click 'no' to go back and continue to take videos until you reach 30 fields.



B. Launching FAST

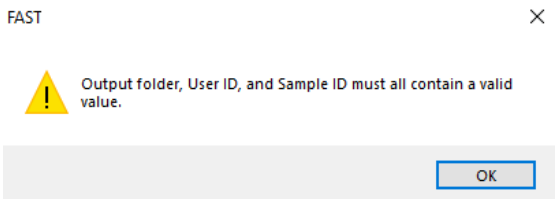
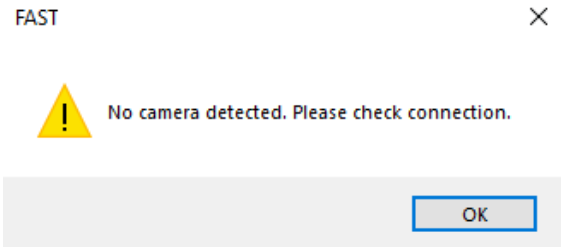
1. Launch FAST

To launch FAST double click the FAST logo on the desktop.



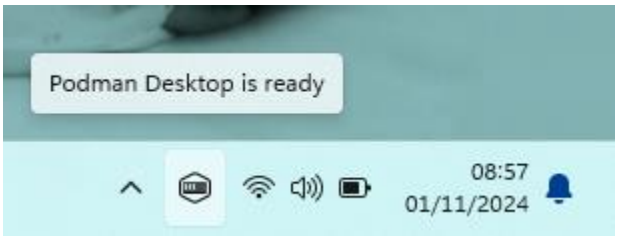
C. Trouble Shooting

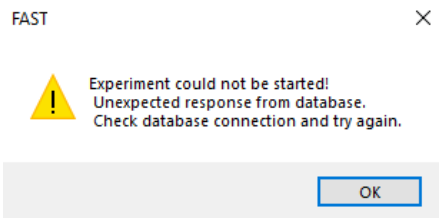
Occasionally you may come across an error. The below should help you troubleshoot.

"Background flow in the imaging chambers does not stop"	Occasionally a chamber doesn't fill properly, meaning that there is a background flow that doesn't go away. If you find this, first try filling a new chamber and imaging in that. If this is a repeated problem then try 'overfilling' by pipetting 4 µl instead of 3 µl onto the slide.
	Make sure you have filled in the Output Folder, User ID, Sample ID, and Notes boxes as in A.5. above.
	Unplug the USB cable connecting the camera and replug it back in, making sure that there is a green light showing on top of the camera. If this does not fix the problem, check the connection that the cable makes with the camera.

This indicates that a program called 'Podman desktop is not running correctly.

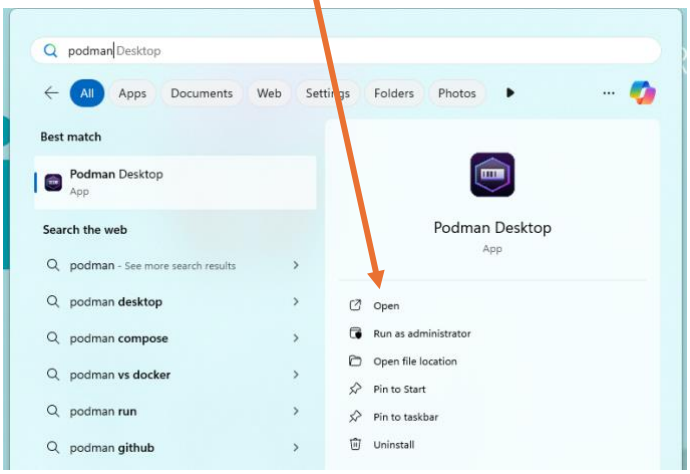
First check to see if this icon is in the taskbar:





If it is, right click and click quit.

Next, press the windows key and type podman desktop, click to open.



For any other problems please try turning the machine off and on again, following Section B.

For all other problems please get in touch with Dr Meurig Gallagher at m.t.gallagher@bham.ac.uk or by phone on 07474 403 387.