Leica SP5 Mid UV-Vis (Irchel) - Part 1: Start-up

How to start up and mounting sample at the SP5 Mid UV-VIS confocal laser scanning microscope located at Irchel, room Y34-E-36.

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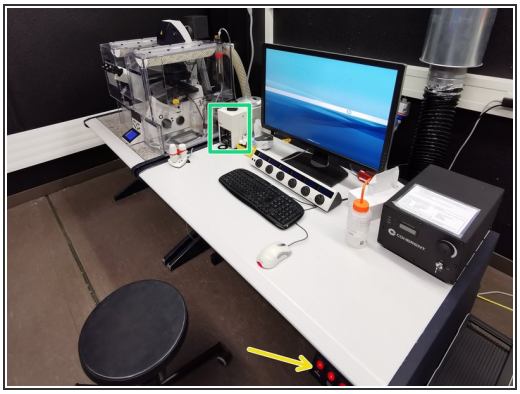
INTRODUCTION

How to start up and mount your first sample on the SP5 Mid UV-Vis confocal laser scanning microscope located at Irchel, room Y34-E-36.

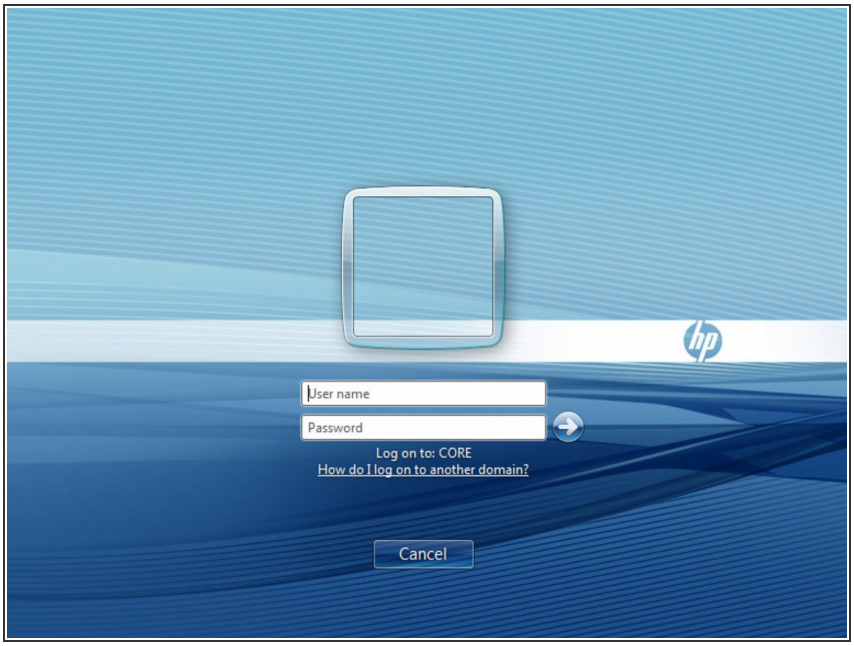
Please find detailed information about the system setup here.

Step 1 — Switching ON hardware



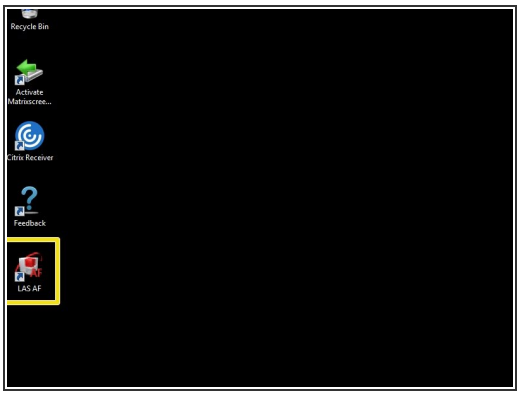
- Switch ON the **fluorescence lamp**.
- Switch ON the "**PC /Microscope**", **"Scanner Power"** and **"Laser Power"** and turn the **"Laser Emission" key** to "On-1" (main switch board underneath table right hand side).

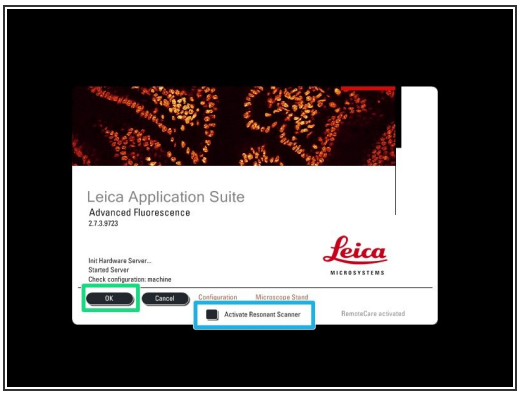
Step 2 — Sign-In



- **Sign-in** with your **ZMB core** credentials.

Step 3 — Starting up the "LAS AF" software

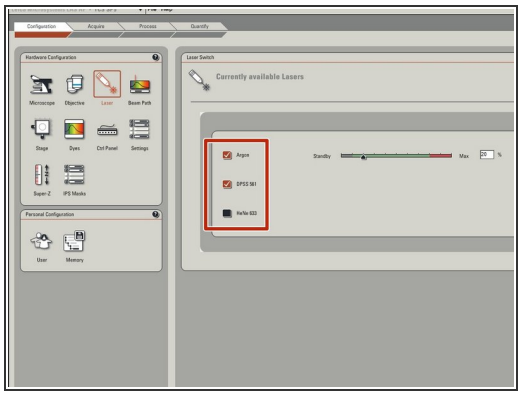




- Start the **"LAS AF"** software.
- If the **"Resonant Scanner"** is needed check "Activate Resonant Scanner".
- Click "**OK**".
- Select "**Yes**" in order to **initialize the x/y stage.** *Please make sure that nothing is placed on the stage.*

  An x/y stage initialization is necessary in order to use the "Tilescanning", and "Mark and Find" function.

Step 4 — Switch ON the lasers in the software



- Go to "**Configuration**".
- Select "**Laser**".
- **Switch ON** the lasers you will need.
  - Adjust the **Argon** laser to **20%**.
  - The **405 nm** laser has to be switched ON via the **external** button.

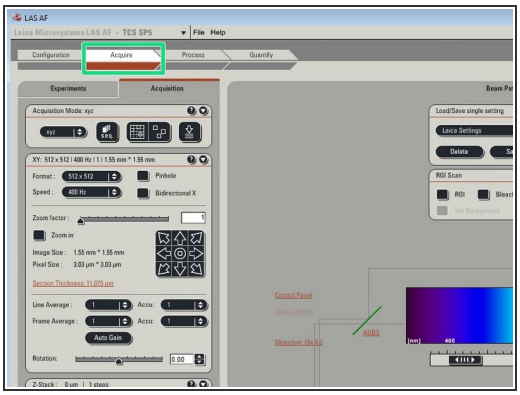
ⓘ Follow next step, if in addition the **355 nm laser** is needed, otherwise continue with step 6.

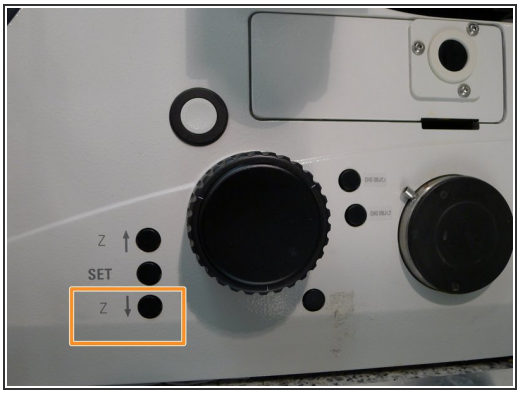
Step 5 — Optional - 355 nm UV-Laser

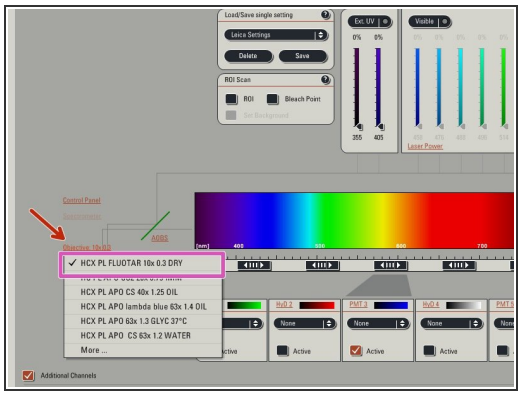
Optional step: follow this step only if the 355 nm laser is needed for your experiment, otherwise continue with step 6.

- Start the 355 nm laser.
  - Turn ON **main switch** (on the back). The LED "SYSTEM FAULT" lights up. *Please note, key switch in the front must be off.*
  - Wait until laser has powered up and the indicator "SYSTEM FAULT" has turned off. *This takes approx. 1min.*
  - The "INTLK OK" should light up now.
  - Turn the **laser key** to "ON".
  - **Set** the power with the control knob. *Turn for fine adjustment, press and turn for coarse adjustment. The LED "LASER ON" lights up.*

Step 6 — Choosing an objective





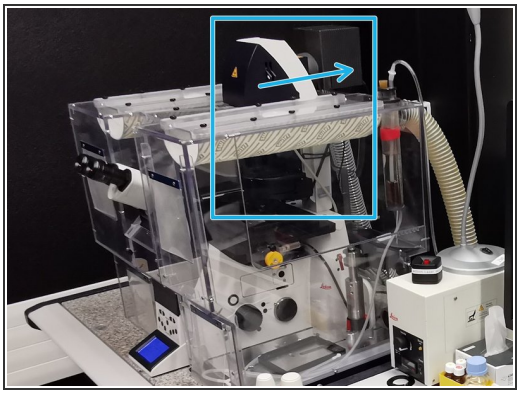


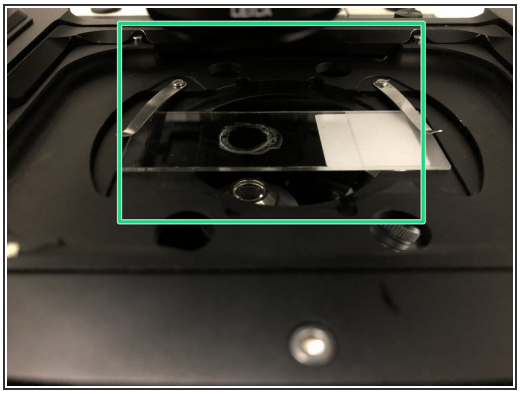
- Go back to **"Acquire"**.
- **Lower** the **objective turret** by pressing the downwards "Z" button on the right side of the microscope.

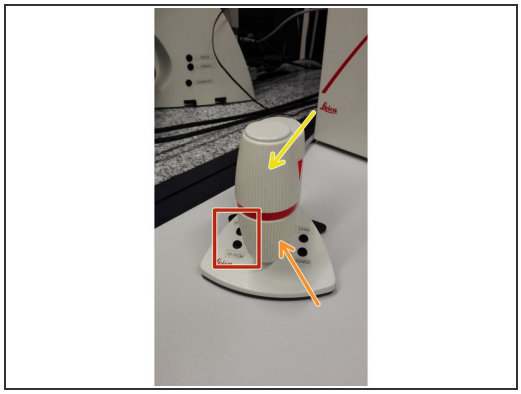
  ⚠ This is a **mandatory step** as it avoids possible collision of the objectives and stage during exchange of inserts and/or samples.
- You can now toggle between objectives within the software (drop-down menu).
- Select the **10x dry objective**.

  ⓘ *In order to facilitate the focusing process it is recommended to start with the 10x dry objective.*

Step 7 — Mount and position your sample





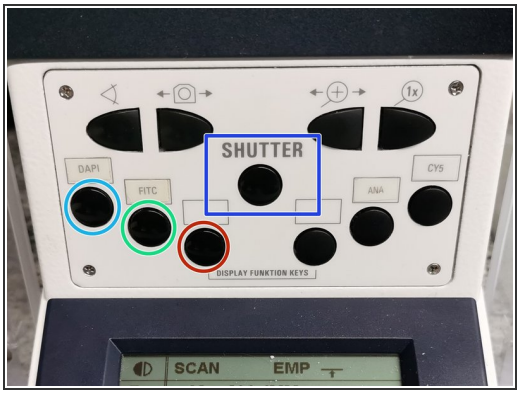


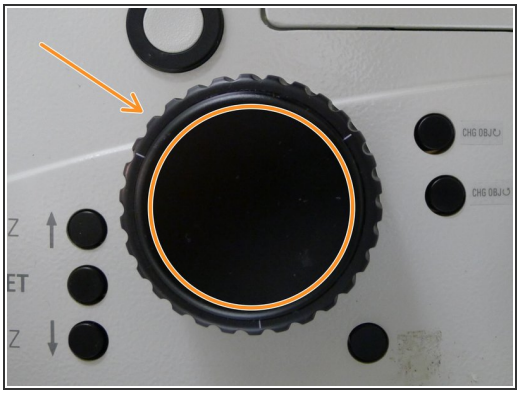
- **Push** the condenser arm of the microscope to the **back**.
- Insert your sample with the **coverslip facing down** and fix it with the two springs.

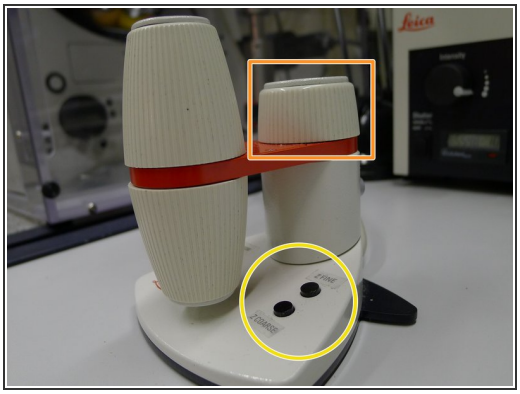
  Special stage inserts/adapters are available for other samples than regular slides (please see last step of this guide).
- Move your sample over the objective with the help of the external controller **"Smart Move"**.
  - Movement in y-direction.
  - Movement in x-direction.
  - Toggle between coarse movement "XY Fast" and slow movement "XY Precise".

Bring back condenser arm to its straight position.

Step 8 — Focus your sample





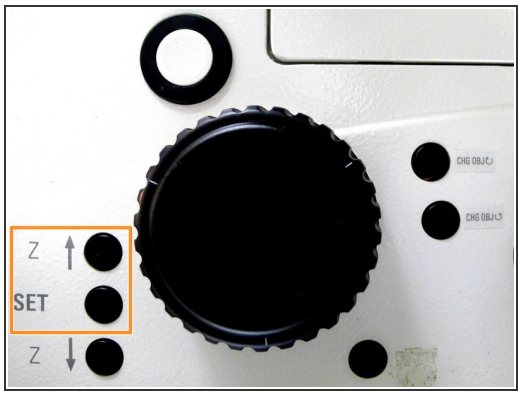


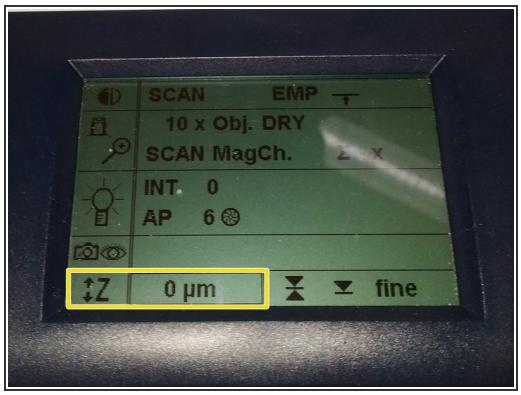
- On the front panel of the microscope select an appropriate **fluorescence filter**:
  - filter for UV dyes like DAPI,
  - filter for green dyes like FITC or Alexa 488,
  - filter for red dyes like TRITC or Alexa 568.
- Press the **"SHUTTER"** in order to illuminate your sample.
- Look through the oculars and **focus** your **sample** by using the **focus wheel** on the microscope stand or the **external controller (Smart Move)**.

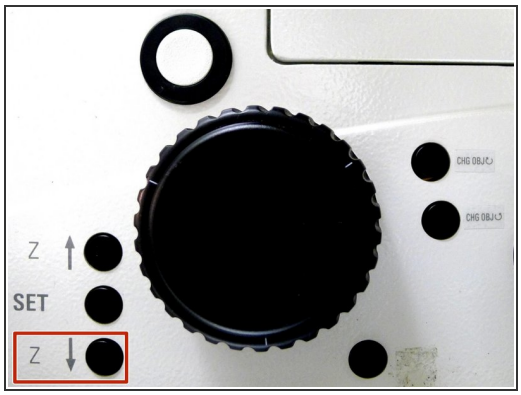
  Moving objectives upwards (towards sample) turn z-wheels clockwise/away from you. Moving objectives downwards (away from sample) turn z-wheels counter-clockwise/towards you.

  - Toggle between "Z FINE" and "Z COARSE" directly on the Smart Move.

Step 9 — Optional - Save your focus position







The storage of the focal plane is helpful in order to find the focus back if the sample or objective will be changed.

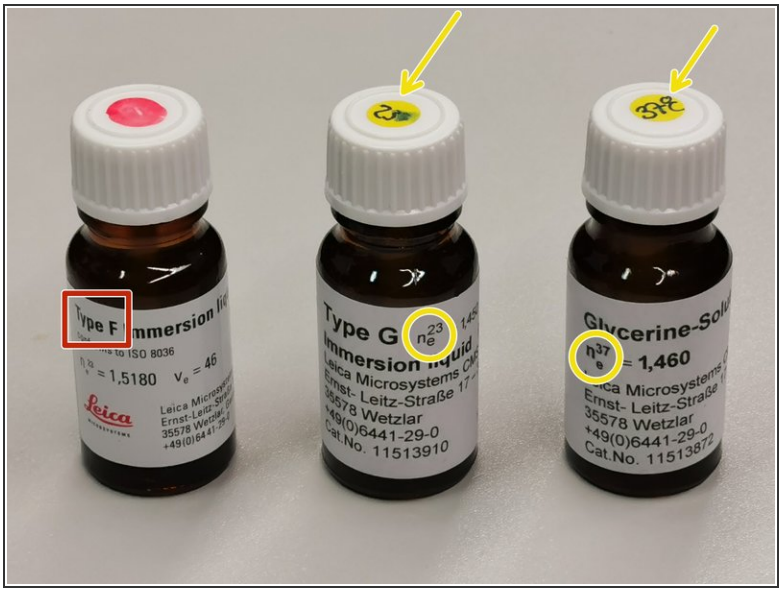
- **Jointly** press the "SET" and upper " Z" button (right side of the microscope stand) in order to set the current z-positon to zero.

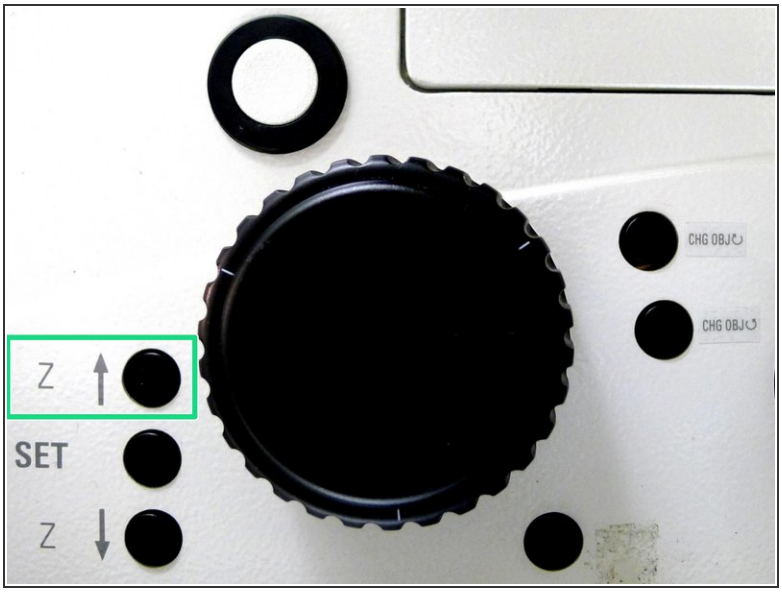
  Depending if already a focus was saved by a previous user, you have to do that "once" (nothing was saved) or "twice" (different focus was saved and needs to be first deleted).

  - The z-position on the display should now show "**0 mm**".

- Press the lower **"Z"** button in order to move down *(for safe change of the objective or the sample)*.

Step 10 — Switching to a higher magnification





- Remove your sample and toggle within the software to the objective of choice.

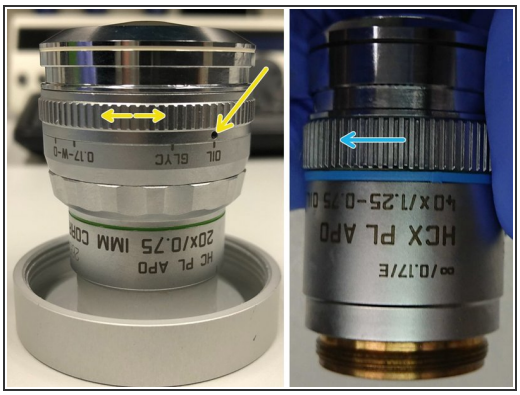
ⓘ Depending on the objective different **immersion media** will be used. Apply either on the sample or directly to the objective.

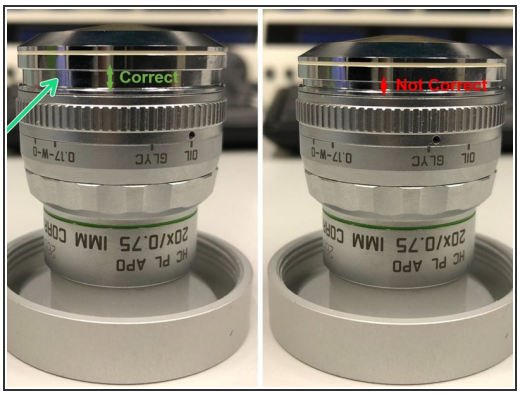
- Oil objectives: *"Type-F" immersion liquid*.
- "Glycerin" objectives: either *"Type-G" immersion liquid* (for RT measurements) or *"Glycerin" immersion liquid* (for measurements at 37°C).
- "Water" objectives: *ddH2O* (always use fresh).

⚠ Please further consider the additional information in the next step to guaranty proper image acquisition.

- **Mount** your sample again and press the upper **"Z"** button.
- Focus your sample as described previously.

Step 11 — Additional information - Immersion objectives





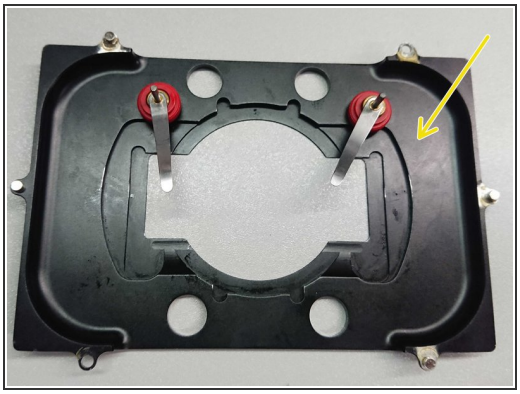
⚠ For some objectives the **correction collar** has to be adjusted.

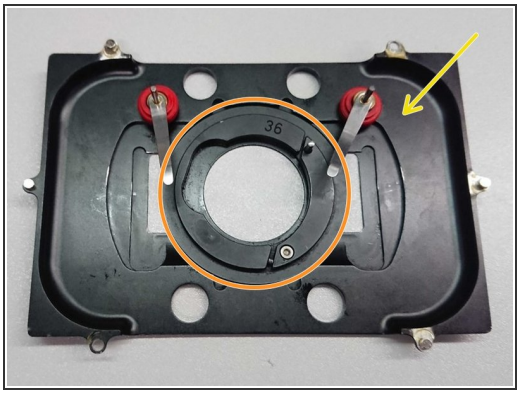
- **20x IMM** (multi-immersion - Oil, Glycerin or Water) needs to be set to the corresponding **immersion media** ("OIL", "GLYC" or "0.17-W" (with cover glass) or "W-0" (without cover glass)).
- **40x OIL** fully open the aperture (NA 1.25) of the lens by turning the correction collar in clockwise direction.
- You can correct for the **cover glass thickness** and temperature at the **63x Glyc** (0.14-0.20) and **63x water** (0.14-0.18). Standard is usually 0.17 mm

- Make sure that the cap of the **spring-loaded front lens** is released (working position).

⚠ Please, DO NOT remove the objectives for adjustment. They can be also accessed on the system.

Step 12 — Additional information - Stage inserts





Stage inserts can be easily exchanged or adapted for other samples than regular slides. You can find additional inserts and adapters in the box on the table behind the microscope.

- Standard insert for slides, including chamber slides.
- Standard insert with petri dish adapter (for 36 mm diameter).
- Insert for multiwell plates.

