Requirements:

- **Microscope** (phase contrast with 20X objective recommended) fitted with KR406B 10x10 eyepiece reticle (reticles.com)
- **Scaling Factor** for objective and microscope (FSI Technical Bulletin “Scaling Factor Determination” available on our website)
- **Micropipetor** with 6µL capacity and tips
- Semen sample or QC

**Loading the SPERMOCYTOMETER®:**

1. Mix semen well using vortex mixer, 2-3 pulses of 3-5 seconds each.
2. Slowly dispense ~6µL of sample into a chamber loading port (A or B) and observe filling. The chamber is filled when the sample reaches the air outlet. A small amount of seminal fluid volume may remain in loading area.
3. Position the SPERMOCYTOMETER® on the microscope stage so chamber is under the objective. Wait 1-2 minutes for the sample to settle until no drifting is observed.
4. Scan the chamber to verify the absence of clumping and air bubbles.
Counting Sperm:

1. Position the center of the chamber under the microscope objective.
2. Count the sperm in all 100 squares of the reticle grid using Sperm Counting Rules. Do not count partial grids.
3. Record the number of sperm in the grid.
4. Repeat steps 2 & 3 in four adjacent fields.

Sperm Counting Rules

- Analyze each square of the grid individually.
- Analyze all 100 squares in the grid and a minimum of 5 grids.
- Count only intact sperm with head and tail.
- Count sperm whose heads lie completely within the square and any heads that lie on the upper and left lines. Do not count tail-less heads, head-less tails or sperm whose heads lie on the lower and right lines.
- If the total number of sperm in 5 grids is less than 200, consider counting another aliquot to confirm.
- If a high sperm concentration makes counting difficult, dilute the semen and reload a new chamber.
- For post-vasectomy samples, examine entire chamber using your laboratory’s procedure.

Calculate Sperm Concentration:

\[
\text{Grid 1} + \text{Grid 2} + \text{Grid 3} + \text{Grid 4} + \text{Grid 5} = \text{Total Sperm}
\]

\[
\frac{\text{Total Sperm}}{500 \text{ (squares counted)}} = \text{Average # of sperm per square}
\]

\[
\text{Average # of sperm per square} \times \text{Scaling Factor} = \text{Sample Concentration in million/mL}
\]

Troubleshooting:

- **Incomplete Chamber Load**
  - Sample volume may be insufficient
  - Solution: check pipettor settings & calibration

- **Semen may have high viscosity or incomplete liquefaction**
  - Solution: add chymotrypsin to semen, incubate and load new chamber

- **Clumping**
  - Can result from incomplete mixing
  - Solution: mix thoroughly before loading

- **Sperm aggregation or agglutination may indicate an abnormal pathology.**

- **Field suddenly shifts**
  - Air may have been introduced in loading
  - Solution: load new chamber

Additional Notes:

- For calculation examples, visit our website.
- Round cells and aggregation/agglutination also can be evaluated using this chamber.
- For semen analysis procedure including motility, call Technical Service: (216) 491-0030
- Mark each chamber after use with permanent marker to indicate it was used.