#### Supplemental file 1: Analysis of spheroid size and volume

The following protocol assumes access to a phase-contrast microscope capable of image acquisition and a basic user knowledge of ImageJ. Proficient users can automate the following steps by creating an analysis script.

1. Collect photomicrographs of spheroids by phase contrast microscopy, ensuring the same objective lens is used throughout. Images can also be used to confirm stability of spheroids throughout the XFe assay, e.g., spheroids dislodged during the assay may yield erroneous data (see **Figure 7**).

NOTE: Typically, a 2–4X objective lens is suitable for most 100–1,000 mM diameter spheroids.

1. Collect photomicrographs of a known image scale.

NOTE: A simple cell culture haemocytometer is suitable as it is manufactured to known dimensions.

1. Open ImageJ and import the hemocytometer image.
2. Using the **Straight** annotation tool, draw a line between two known points on the hemocytometer image, e.g., each of the 16 squares within the 4 hemocytometer counting areas measures 250 µm x 250 µm (1 mm2total quadrant) on the typical Neubauer-type hemocytometer.
3. Go to **Analyse/Set scale** and set **Known distance: 250** and **Unit of length: µm.** Click to set to **Global.**
4. Import spheroid images into ImageJ.
5. Using the **Freehand selection** tool, draw around the circumference of each spheroid and collect data by going to **Analyse/Measure** for each spheroid.
6. Copy **Measurement table** to clipboard and import into R’, Microsoft excel, or equivalent.
7. Use **Feret diameter** to estimate spheroid volume (mm3) using **Feret** and **MinFeret** values according to the following equation:

Feret diameter = ((0.5) × (Feret value) × (MinFeret value2))

NOTE: A true spheroid would have Feret/MinFeret value equal to 1; thus, these values can also be used to estimate ‘roundness’ of spheroids.