

**Topic**

This note describes a problem with surface area measurements made in Volocity 2.6.2 and earlier. It also discusses more generally how surface area measurements are made in Volocity.

**Discussion****Measurement error in Volocity 2.6.2 and earlier**

In July 2004, Improvision discovered a flaw in Volocity which caused inaccurate calibrated surface area measurements to be calculated. This was due to an error in the algorithm that converted un-calibrated areas to calibrated ones.

The error is a scaling error. If users were comparing the relative areas of two objects, this comparison will still be valid.

This problem was fixed in Volocity 3.0 and Volocity 2.6.3. Surface area measurements made in the new versions of Volocity correctly take calibrations into account. Additionally, Volocity 3.0 and Volocity 2.6.3 will automatically correct inaccurate calculations made in existing sessions. This will happen when each measurement session is opened – no user intervention is required.

Improvision considers this problem to be critical, and for this reason we have released Volocity 2.6.3. This version of Volocity can be used by customers who do not have a valid Software Maintenance Agreement for Volocity 3.0.

**General information about surface area measurements**

Selecting a region of interest to measure can be performed in several ways in Volocity:

- By using the selection tools (e.g. magic wand)
- By using a Classifier

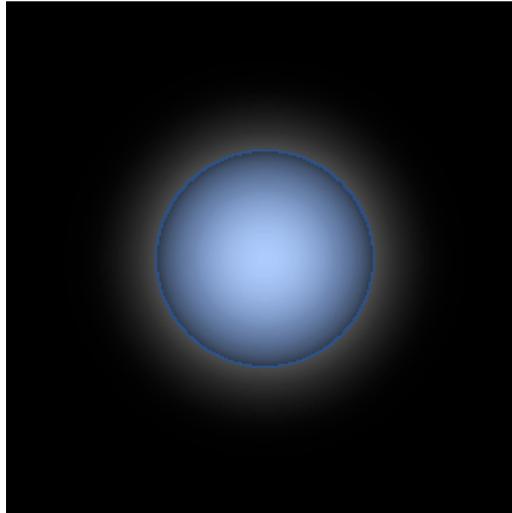
Internally, each object is represented by a 3D binary mask. Volocity measures the surface area of objects by fitting a “skin” to the surface of the mask. It does this at the voxel level.

This can cause results that might not be expected. For instance, the surface area of a sphere can be calculated as:

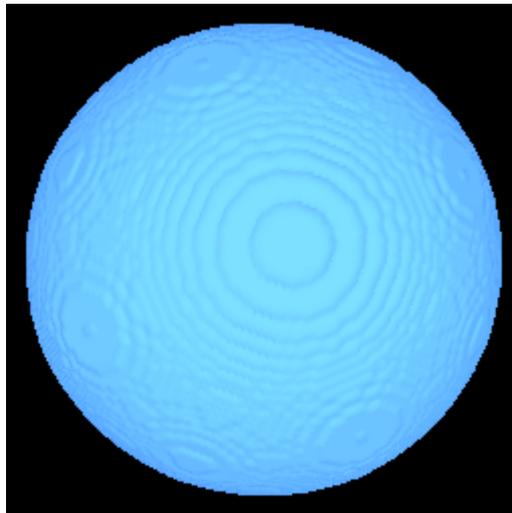
$$\text{area} = 4\pi r^2$$

We would expect that a sphere in Volocity (e.g. a perfect calibration bead) would have this surface area. In practice, the measured surface area tends to be higher. The reason for this is that at the voxel level the sphere is quite bumpy – these bumps add to the overall surface area of the object.

For example, this is an X-Y image of a synthetic sphere in Volocity:



This is the rendered surface of the sphere:



In the rendered image, the surface “bumpiness” is clearly visible. In this particular case, the measured surface area is about 25% higher than the surface area as calculated from the radius of the sphere. This is due to the “bumpiness” at the voxel level.

This effect would be more pronounced for a real image generated from a microscope, where noise from the detector would add to the roughness of any surface.

### **Conclusion**

It is important when using any measurement to ensure that results are statistically significant compared to any measurement error or noise. This is especially important with surface area measurements where noise and surface features can have a considerable impact on the measurement.