Understanding your ISO 16232 report: Calibration Factor or Scale or Pixel Resolution

There is a lot of information on an ISO 16232 report, some of it is very easy to understand like the sample ID which likely will include the part number of the part or parts being tested. Other items on your ISO 16232 report may lie outside your experience – so today let's tackle one of those and try to take the mystery out of it.

Particles are found, counted and measured via visual analysis software which is fed images from a light microscope outfitted with a very precise automated stage and a high resolution digital camera. The potential accuracy of the analysis is greatly impacted by several different factors one of which is the calibration factor or scale. So what does that "calibration factor" or "scale" mean and why does it impact accuracy? Additionally what minimum level of accuracy potential is required by the ISO 16232 standard itself – since that is critical to decisions regarding what to purchase or what lab to use.

The calibration factor or scale is the size of pixel which that particular camera and lens system has in the analysis image itself - it is impacted by several factors including the lens system and the camera. The pixel is the increment of measurement which is being used - so the smaller the pixel then the more accuracy potential the system has – the better job it can do at accurately measuring small particles (within the resolution limits of the lens system, etc..) Think about a tape measure – if it is marked only down to one-quarter inch increments then it's accuracy potential is much less than a tape measure marked down to one-sixteenth inch. With the one-quarter inch increments you can perhaps do some rough work but you definitely are going to want 1/16th inch increments or even 1/32nd inch increments if you are doing fine work and you likely will want the smaller increments of measure even when doing most rough work. It's the same with particle measuring - if you are measuring only very large particles then a large pixel size is workable but if you are measuring particles down to 5µm as required in a full blown ISO 16232 report then you need a very small pixel to get accurate measurements.

ISO 16232 specifies that for most particles you must be able to fit a minimum of ten pixels into the longest axis of the particle – it's length. Then due to the requirement to measure very small particles – particles actually smaller than a human red blood cell – it allows very small particles to be measured by fitting 5 pixels into the longest axis. See Figure 3 from ISO 16232-7:2007(E) to help you visualize what I am trying to explain with words.

NOTE 1 The pixel resolution of the analysis image (µm/pixel) shall be ⩽ 1/10 of the smallest particle size to be measured or 1/5 for small particles, as is the case for the resolution of the objective lens or the camera of the light microscope (see 5.2.2.1.2).

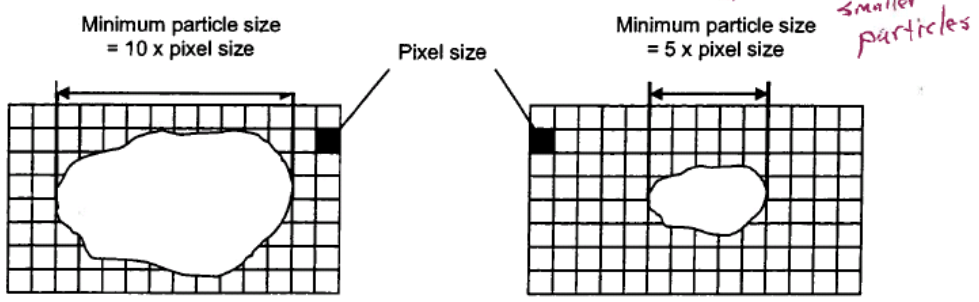

Figure 3 — Pixel resolution of the analysis image

For our discussion today "small particles" means particles which are 20 microns (µm) or smaller - to give that a reference point human red blood cells typically range between six and eight microns (µm) and there are 1,000µm in a millimeter. So let's consider the minimum pixel size to be in compliance with ISO 16232 relative to calibration factor or scale. If you are reporting measurements for a full blown ISO 16232 report you must be able to accurately count and measure particles slightly smaller than a human red blood cell – particles starting at 5µm – since these qualify as "small particles" you then need a minimum of five pixels to fit in the longest axis which means the very minimum pixel size required would be 1µm/pixel. Any pixel size larger than that would not qualify under ISO 16232 to be considered accurate enough for counting particles as small as 5µm. --- Let's say you only need to start counting and reporting at 15µm – then what is the minimum calibration factor or scale you must use to be in compliance with ISO 16232 requirements? That turns out to be trickier than it looks at first glance – 15µm quailifies as a "small particle" so it fits into the 5 pixel per particle length "small particle" requirement which means that a minimum calibration factor or scale of 3µm/pixel would comply but once you start counting and measuring particles 20µm or larger you need to comply with ISO 16232's minimum of ten pixels per particle length – which means a minimum calibration factor or scale of 2µm/pixel would be required. So to qualify under ISO 16232 to count and measure particles starting at 15µm the system you need to purchase or the lab you use must use a calibration factor or scale no larger than 2µm/pixel. Yes you do need to ask – yes you do need to check your reports - because systems which don't comply can regrettably still kick out reports showing counts and measurements of particles smaller than they are qualified to measure – much like you could use an exactly one yard long stick with only "one foot" markings on it to measure the size of picture frame you need. But with marks at only each foot what happens if you are trying to frame a 4"x6" or 5"x7" or 8" x 11" photo – it's going to take some serious luck to either purchase or make the right size picture frame when your unit of measure is actually larger than what you are trying to accurately measure. The key is to ensure your unit of measure is up to doing the job required – and being able to fit five or ten or more of the units of measure into the length you are measuring definitely helps ensure more accurate measurement.

So hopefully that takes the mystery out of calibration factor or scale or pixel resoluton – which every ISO 16232 report is required to report. So if you are shopping for a microscope system or a lab capable of doing your ISO 16232 testing work keep the required calibration factor or scale in mind. Remember to ask and remember to check your report because unfortunately I have seen far too many reports generated by labs in North American and Europe which violate the ISO 16232 requirement for calibration factor or scale – sometimes by a large margin such as displaying particle counts starting at 5µm when their pixel size is actually larger than 5µm.

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Here at the Paul Hutchison Clean Technology Laboratory we use ONLY compound microscopes for ISO 16232 related particle counting so we have both the lens resolution and the camera resolution to qualify to count particles in compliance with ISO 16232 requirements. When your reporting needs require starting counts at 5µm our calibration factor or scale is 0.8789µm/pixel or smaller.

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Example reports – showing both compliance and non-compliance regarding Calibration Factor or Scale