The reference system

The reference system must be specified using the command options --ref1, --ref2 and --ref3, according to the following conventions.

The physical acquisition space is assumed to have a Cartesian reference system. The two dimensions of the X-Y plane are conventionally named as 'V' the vertical one, i.e. that commonly labeled as 'y', and as 'H' the horizontal one, i.e. that commonly labeled as 'x'.
The third dimension, i.e. that commonly labeled as 'z', is conventionally named as 'D', for depth.
These three dimensions are ordered, being V the first dimension, H the second, and D the third. Indeed, if we see a 3D image as a series of slices, and each slice as a 2D matrix, the first index V is the index of rows of slices, the second index H is the index of columns of slices, and the third index D is the slice index.
When tiles are saved during acquisition they have a nominal physical position in the reference space, i.e. they are associated to three real numbers that specify the position of some fixed point of the tile, usually the left-most, upper vertex of the first slice. The third number is assumed to be always the coordinate along dimension D, but the first two may refer to either V and H, or to H and V, respectively. In the first case, the first coordinate refers to the first dimension and the second coordinate to the second dimension of the conventional space, whereas in the second case the opposite happens. Moreover, considering the relations between physical coordinates and indices in the 3D image matrix, for any dimension physical coordinates can increase or decrease with the corresponding index.
To precisely specify the relations between physical acquisition space and indices in the 3D image matrix we use the command options --ref1, --ref2 and --ref3, associated respectively to dimensions V, H, and D, to specify which is the correspondent coordinate provided by the acquisition system. A positive number is then used to associate a coordinate with a dimension if the coordinate grows with increasing indices, whereas a negative number is used in the opposite case.

For instance the command options

--ref1=1 --ref2=2 --ref3=3

mean that the first coordinate refers to V, the second to H and the third to D, and that all grow with increasing indices. Conversely the command options:

--ref1=2 --ref2=-1 --ref3=3

mean that the first coordinate refers to H, the second to V and the third to D, and that coordinates along H decrease with increasing indices.

With this convention, assuming that the third coordinate (the one identifying the slice) is always associated to D, there are 16 different possible reference systems, which are listed in Table I. It is worth noting however that TeraStitcher currently does not support reference systems in which coordinates along D decrease with increasing indices. Of course, in the import step the actual reference system used by the motorized microscope must be specified otherwise tiles are stitched in flipped positions.

ref1	ref2	ref3	Description
1	2	3	First coordinate is associate to V and increases with indices Second coordinate is associated to H and increases with indices Third coordinate is associated to D and increases with indices
-1	2	3	First coordinate is associate to V and decreases with indices Second coordinate is associated to H and increases with indices Third coordinate is associated to D and increases with indices
1	-2	3	First coordinate is associate to V and increases with indices Second coordinate is associated to H and decreases with indices Third coordinate is associated to D and increases with indices
-1	-2	3	First coordinate is associate to V and decreases with indices Second coordinate is associated to H and decreases with indices Third coordinate is associated to D and increases with indices
2	1	3	First coordinate is associate to H and increases with indices Second coordinate is associated to V and increases with indices Third coordinate is associated to D and increases with indices
-2	1	3	First coordinate is associate to H and decreases with indices Second coordinate is associated to V and increases with indices Third coordinate is associated to D and increases with indices
2	-1	3	First coordinate is associate to H and increases with indices Second coordinate is associated to V and decreases with indices Third coordinate is associated to D and increases with indices
-2	-1	3	First coordinate is associate to H and decreases with indices Second coordinate is associated to V and decreases with indices Third coordinate is associated to D and increases with indices
1	2	-3	First coordinate is associate to V and increases with indices Second coordinate is associated to H and increases with indices Third coordinate is associated to D and decreases with indices
-1	2	-3	First coordinate is associate to V and decreases with indices Second coordinate is associated to H and increases with indices Third coordinate is associated to D and decreases with indices
1	-2	-3	First coordinate is associate to V and increases with indices Second coordinate is associated to H and decreases with indices Third coordinate is associated to D and decreases with indices
-1	-2	-3	First coordinate is associate to V and decreases with indices Second coordinate is associated to H and decreases with indices Third coordinate is associated to D and decreases with indices
2	1	-3	First coordinate is associate to H and increases with indices Second coordinate is associated to V and increases with indices Third coordinate is associated to D and decreases with indices
-2	1	-3	First coordinate is associate to H and decreases with indices Second coordinate is associated to V and increases with indices Third coordinate is associated to D and decreases with indices
2	-1	-3	First coordinate is associate to H and increases with indices Second coordinate is associated to V and decreases with indices Third coordinate is associated to D and decreases with indices
-2	-1	-3	First coordinate is associate to H and decreases with indices Second coordinate is associated to V and decreases with indices Third coordinate is associated to D and decreases with indices

Table I: The 16 possible reference systems for the acquisition instrument space.