Supplementary File	Title
Supplementary Figure 1	Correlation between the automatically and manually generated alignment quality scores Q_i and Q_v of 805 alignments. The central brain, left and right optic lobes were scored separately to best assess the consistency of the two alignment quality scores in different brain areas.
Supplementary Figure 2	Choosing the target brain. 295 images were aligned to an initial target brain T_R. The mean image of all aligned brains, T_A, was chosen as the new target brain. Initially all brains had different orientations and sizes, and thus overlapping them did not yield a meaningful pattern. Through the global alignment these brains were transformed to have the same center of mass, similar orientations and sizes. However, overlapping them did not yet produce a bright consensus and sharp border in the average brain. Once the local alignment was performed, we obtained an average brain (T_A) that was much brighter and less fuzzy than that of the globally registered brains. The correlation (= 0.940) between T_A and T_R was substantially higher than that (= 0.467) between the mean of the globally aligned brains and T_R, because of the good local alignment.
Supplementary Figure 3	Improvement of alignment of 496 brain images by replacing T_R using T_A.
Supplementary Figure 4	Comparison of computational and biological methods to determine expression pattern overlap of the aligned and co-expressed patterns of *a156-GAL4; UAS-mCD8-GFP* and *LexAP078; lexAop-CD2-GFP*. Yellow arrows, the most overlapping regions.
Supplementary Figure 5	Comparison of obligate subsets with parent expression patterns. (**a**) Original GAL4 pattern (magenta) of *CG8916_1-3-X-GAL4; UAS-mCD8-GFP*. (**b-c**) Two example Flp-Out clones (yellow and green) of this GAL4 line. Red arrows and P_1, P_2, P_3: three obligate subset-patterns. (**d**) Aligned original pattern and obligate Flp-Out subsets show substantial overlap. F: the parent pattern. CC: the regional correlation coefficients of patterns P_1, P_2, and P_3 and the parent pattern. All p-values of calculated correlation statistics are less than 10^{-5}. P_S: a location for zoom-in inspection. (**e**) Cross-sectional view the region around P_S in (d) shows co-localization of the obligate subset and the parent GAL4 pattern.
Supplementary Figure 6	Analysis of a 3D neuronal expression pattern atlas of a large collection of aligned GAL4 lines. (**a**) Distribution of

	GAL4 expressions in different brain compartments. The volume percentages of compartments in the target brain are shown in the brackets following the compartment names. (**b**) Compartment-volume normalized distribution of GAL4 expressions. The statistic was rescaled to have a range of [0, 1], for better visualization. (**c**) The volume of different compartments in our target brain. In the bracket the number of image voxels in a compartment is also displayed.
Supplementary Note	Comparison of several different aligners on LSM images of Drosophila brains
Supplementary Video 1	Aligned and overlaid neuronal patterns of *a64-GAL4* and *a74-GAL4*. Magenta, *a64-GAL4*; green, *a74-GAL4*.
Supplementary Video 2	Six aligned and overlaid GAL4 patterns in Fig. 1d. Color schema: see Fig. 1d.
Supplementary Video 3	3D reconstructed neurite tracts from 20 aligned *a278-GAL4* images, along with their mean tract model (red).
Supplementary Video 4a	The aligned patterns of *a156-GAL4; UAS-mCD8-GFP* and *LexAP078; lexAop-mCD2-GFP*. Magenta, *a156-GAL4*; green, LexAP078.
Supplementary Video 4b	The co-expressed patterns of *a156-GAL4; UAS-mCD8-GFP* and *LexAP078; lexAop-mCD2-GFP*. Magenta, *a156-GAL4*; green, LexAP078.
Supplementary Video 5	Aligned and overlaid patterns of *CG8916_1-3-X-GAL4* along with its two Flp-Out segments. Magenta, the original pattern; yellow and green, the Flp-Out patterns.
Supplementary Video 6a	Maximal intensity project view of six aligned GAL4 patterns in the central complex. Color schema: see Fig. 1d.
Supplementary Video 6b	Cross-sectional view of six aligned GAL4 patterns in the central complex. Color schema: see Fig. 1d.
Supplementary Video 7	A database of 269 stereotyped neurite tracts throughout the *Drosophila* brain. The width of each tract equals the respective spatial deviation. The tracts are color-coded randomly for better visualization.