Assembling Models of Embryo Development: Image Analysis and the Construction of Digital Atlases

Carlos Castro-González, María J. Ledesma-Carbayo, Nadine Peyriéras, and Andrés Santos*

Digital atlases of animal development provide a quantitative description of morphogenesis, opening the path toward processes modeling. Prototypic atlases offer a data integration framework where to gather information from cohorts of individuals with phenotypic variability. Relevant information for further theoretical reconstruction includes measurements in time and space for cell behaviors and gene expression. The latter as well as data integration in a prototypic model, rely on image processing strategies. Developing the tools to integrate and analyze biological multidimensional data are highly relevant for assessing chemical toxicity or performing drugs preclinical testing. This article surveys some of the most prominent efforts to assemble these prototypes, categorizes them according to salient criteria and discusses the key questions in the field and the future challenges toward the reconstruction of multiscale dynamics in model organisms.

INTRODUCTION

Studying how the known genomes relate to the spatiotemporal behavior of cell dynamics, tissue patterning, and embryo morphogenesis is one of the key questions for developmental biology in the postgenomic era. Despite completion of the genome sequence of many organisms (McPherson et al., 2001), we are still far from understanding, modeling, and predicting how organisms develop from one single cell into an organized, multicellular individual.

However, comprehensive understanding of biological mechanisms is a fundamental issue for efficient preclinical testing of potential new drugs (Goldsmith, 2004; Sipes et al., 2011). Potential applications include treatment of heart diseases (Milan et al., 2003; Barros et al., 2008), leukemia (North et al., 2007), bone disorders (Paul et al., 2008), cancer (Amatruda et al., 2002; Lu et al., 2011), schizophrenia, Parkinson's, Alzheimer's, and other dementia (Martone et al., 2008).

Many fundamental challenges pave the way toward the long term goal of reconstructing living systems multiscale dynamics. The quantitative assessment of the temporal and spatial gene expression distribution in multicellular organisms is required for building and modeling gene regulatory networks underlying morphogenesis (Davidson and Erwin, 2006; Li and Davidson, 2009).

Recent advances in labeling techniques (Vonesch et al., 2006; Choi et al., 2010) and microscopic imaging (Megason and Fraser, 2007) have steered this field from a static, "omics"-like approach (Walter et al., 2002) toward image-based strategies providing spatial and temporal quantitative information (Fernandez-Gonzalez et al., 2006; Gorfinkiel et al., 2011). Hence, the current trend toward automatic, high-content, high-throughput screening brings new bottlenecks in the domain of image analysis (Baker, 2010; Truong and Supatto, 2011): The unprecedented rise in complexity and size of data have favored the blossoming of a new discipline, bioimage informatics (Peng, 2008), or the science of organizing distributed and heterogeneous biological image data into typed data and categorized quantitative information.

In particular, this review deals with the recent strategies developed to achieve the reconstruction of digital anatomy and gene expression atlases for different model organisms (Table 1). The reconstruction

TABLE 1. Overview of Recent Strategies for Reconstructing Digital Anatomy and Gene Expression Atlases for Animal Organisms

Reference	Animal model	Imaging modality	Spatial scope and resolution		Developmental period, age and # of steps			Data type: # of specimens, template type, # of genes and gene product quantification				Matching type and transformation	
Murray et al. (2008)	*C. elegans*	In vivo microscopy	Whole organism	Cellular	Early	4–350 cells	5 hr[c]	20	Individual	4	Yes	Object-based	Nonrigid
Long et al. (2009)	*C. elegans*	Microscopy	Whole organism	Cellular	Early	L1	1	15	Individual	0	No	Object-based	Affine
Liu et al. (2009)	*C. elegans*	Microscopy	Whole organism	Cellular	Early	L1	1	324	Synthetic	93	Yes	Object-based	Nonrigid
Tomer et al. (2010)	*Platynereis*	Microscopy	Brain	Cellular	Late	48 hr	1	171	Average	8	No	Intensity-based	Affine + nonrigid
Fowlkes et al. (2008)	*Drosophila*	Microscopy	Whole organism	Cellular	Early	0–100%	6	1822	Average	95	Yes	Object-based	Nonrigid
Frise et al. (2010)[a]	*Drosophila*	Microscopy	Whole organism	Multicellular	Early	5%	1	2693	Synthetic	1881	Yes	Object-based	Nonrigid
Peng et al. (2011)	*Drosophila*	Microscopy	Brain	Tissular	Adulthood	Adult	1	2945	Average	470	No	Object-based	Affine + nonrigid
Potikanond et al. (2011)	Zebrafish	Microscopy	Whole organism	Tissular	Late	24–72 hr	4	≈75	Individual	≈20	No	Semantic	-
Rittscher et al. (2011)	Zebrafish	Microscopy	Whole organism	Organular	Late	120 hr	1	11	Average	0	No	Object-based	Rigid + nonrigid
Ullman et al. (2010)	Zebrafish	MRI	Brain	Tissular	Late	4 months	1	1[b]	Individual	0	No	None	None
Castro et al. (2009)	Zebrafish	Microscopy	Whole organism	Cellular	Early	6 hr	1	6	Individual	5	Yes	Intensity-based	Rigid
Ruffins et al. (2007)	Quail	MRI	Whole organism	Organular	Late	5–10 days	6	6[b]	Individual	0	No	None	None
Fisher et al. (2011)	Chicken	OPT	Wing bud	Tissular	Late	3–5 days	3	45	Individual	5	No	Object-based	Nonrigid
Baldock et al. (2003)[a]	Mouse	OPT	Whole organism	Organular	Late	2–17 days	22	23,484	Individual	14927	No	Object-based	Nonrigid
Carson et al. (2005)	Mouse	Microscopy	Brain	Cellular	Late	7 days	1	200	Synthetic	200	Yes	Object-based	Affine + nonrigid
Kovacevic et al. (2005)	Mouse	MRI	Brain	Tissular	Late	56 days	1	9	Average	0	No	Intensity-based	Affine + nonrigid
Ma et al. (2005)	Mouse	MRI	Brain	Tissular	Late	56 days	1	10	Probabilistic	0	No	Intensity-based	Affine + nonrigid
Johnson et al. (2010)	Mouse	MRI	Brain	Tissular	Late	56 days	1	14	Probabilistic	0	No	Intensity-based	Affine
Lein et al. (2007)[a]	Mouse	Microscopy + MRI	Brain	Cellular	Late	56 days	1	≈20,000	Average	≈20000	Yes	Intensity-based	Rigid + nonrigid
Woods et al. (1999)	Human	MRI	Brain	Organular	Adulthood	Adult	1	22	Average	0	No	Intensity-based	Nonrigid
Rex et al. (2003)	Human	MRI	Brain	Tissular	Adulthood	Young adult	1	452	Average	0	No	Intensity-based	Affine + nonrigid
Smith et al. (2004)	Human	MRI	Brain	Tissular	Adulthood	Adult	1	58	Average	0	No	Intensity-based	Affine
Kerwin et al. (2010)	Human	OPT	Brain	Tissular	Late	47–55 days	2	5	Individual	3	No	Object-based	Nonrigid

hr = hours, % = percentage of membrane invagination.
[a]2D gene expressions.
[b]No matching procedure: Each specimen was directly employed as the template of its corresponding developmental stage.
[c]3D+t live imaging.

of a digital atlas requires a series of image processing steps ("Proposed image processing pipeline" section) to map a cohort of individuals onto a common reference space. These operations allow the investigator to combine unrelated data and to provide a single representation to visualize, mine, correlate, and interpret information at different scales.

The result is the assembly of a digital prototypic model of a "standard" individual which constitutes the essential scaffold where to make the accurate, repeatable, consistent, and quantitative measures required for comparative studies (Oates et al., 2009). Atlases can be compared to geographic information systems (GIS): "spatial databases to which diverse data, primarily but not restricted to imaging data, can be registered and queried" (Martone et al., 2008). For example, atlases are used to identify and categorize anatomic and genetic differences between cohorts of individuals, such as different mutant strains (Warga and Kane, 2003) and constitute an essential tool that allows relating genotypes and phenotypes. This review is organized as follows: "Classification of digital atlases, Animal models, Imaging modalities, Spatial scopes and resolutions, Developmental stages, Data types, and Matching procedures" sections discuss recent trends in the field and propose a classification of anatomy and gene expression atlases for model organisms and human based on various criteria. "Proposed image processing pipeline and Visualization and validation" sections describe a generic image processing framework to reconstruct, validate, visualize, and interact with a digital model of embryo development. "Biological insights" section surveys some of the biological insights that can be derived from such atlases. Finally, "Perspectives" section deals with the discussion and perspectives on the subject.

CLASSIFICATION OF DIGITAL ATLASES

We propose a classification of digital anatomy and gene expression atlases for animal organisms based on the following ontology (Table 1): (1) Animal models, (2) imaging modalities, (3) spatial scopes and resolutions, (4) developmental stages, (5) data types, and (6) matching procedures. In the following sections, we will discuss these separate criteria in more detail.

The construction of anatomy atlases and the development of appropriate computation strategies is a major issue in the medical field (Park et al., 2003; Aljabar et al., 2009; Fonseca et al., 2011). The construction of human brain atlases received special attention and many algorithmic reconstruction and visualization methods and tools come from this field (Mazziotta et al., 2001; Toga et al., 2006). The study of model organisms allowed exploring fine spatial and temporal scales and aimed at gathering an increasing amount of information including gene expression data. We focus here on many model organisms ("Animal models" section) imaged with three different image modalities ("Imaging modalities" section). We distinguish between atlases limited to the brain and atlases encompassing the whole organism ("Spatial scopes and resolutions" section), either at the adult stage or throughout embryonic stages ("Developmental stages" section) and focusing either on anatomical structures or gathering gene expression data ("Data types" section). We also consider different strategies to match individuals into the atlas model ("Matching procedures" section).

ANIMAL MODELS

Model organisms are chosen for their small size, good properties in terms of phylogenetic position (Fig. 1), transparency, and/or relevance for studies related to human health.

The nematode *Coenorhabditis elegans*, having the most ancient evolutionary emergence among the considered animal models, has a largely invariant cell lineage and stereotyped development which greatly facilitates comparisons between different individuals (Murray et al., 2008; Liu et al., 2009; Long et al., 2009).

The worm *Platynereis* kept several ancestral traits (Tomer et al., 2010) and proved being insightful for comparative studies.

The fruit fly *Drosophila melanogaster* has been extensively studied in the field of genetics and developmental biology (Fowlkes et al., 2008; Frise et al., 2010; Peng et al., 2011). Sixty percent of so called genetic diseases in humans have their counterpart in the *Drosophila* genome.

The zebrafish (*Danio rerio*) has more recently emerged as a model for developmental biology research because of its amenability to genetic investigations and the transparency of its tissues. In addition, its closer phylogenetic position to human makes it a valuable model for toxicology and pharmacology studies (Hill et al., 2005; Yang et al., 2009). Anatomy and gene expression atlases for the zebrafish brain or whole organism at different developmental stages are underway (Castro et al., 2009; Ullmann et al., 2010; Potikanond and Verbeek, 2011; Rittscher et al., 2011).

Quail (Ruffins et al., 2007) and chicken (Fisher et al., 2008; Fisher et al., 2011) are also used as vertebrate models and have interesting features for experimental embryology. The embryo can develop outside the egg, is quite well amenable to *in vivo* imaging ("Imaging modalities" section) and the construction of atlases with cellular resolution (see "Spatial scopes and resolutions" section).

Mouse is the major mammalian model organism for biomedical investigations and much effort has been devoted to the reconstruction of their development (MacKenzie-Graham et al., 2004; Carson et al., 2005; Kovać;ević; et al., 2005; Ma et al., 2005; Lein et al., 2007; Johnson et al., 2010; Richardson et al., 2010; Hawrylycz et al., 2011). The relatively large size of the mouse embryo makes it difficult to capture the whole specimen in a single-shot, in-toto imaging strategy ("Imaging modalities" section) with sufficient spatial resolution ("Spatial scopes and resolutions" section).

The same difficulty applies to fixed human embryos (Woods et al., 1999; Rex et al., 2003; Smith et al., 2004; Kerwin et al.,

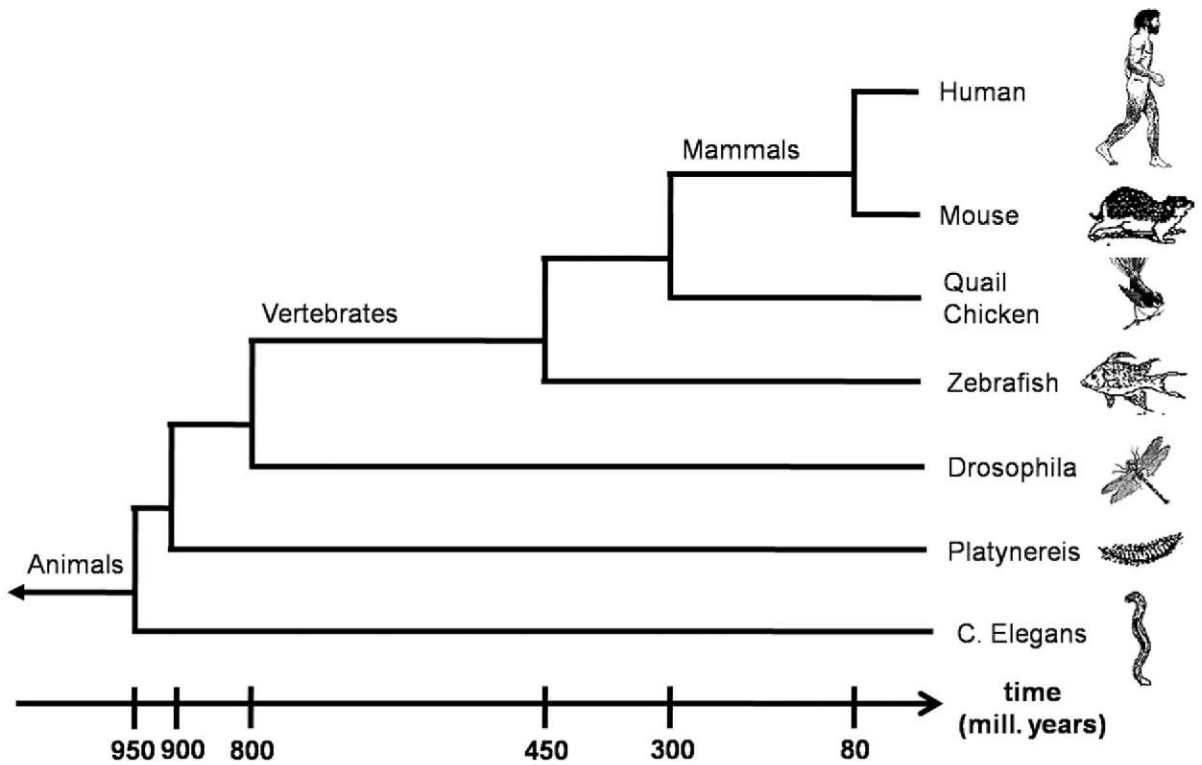

Figure 1. Timing of evolutionary emergence and phylogenetic relationships of different model organisms. Time estimations were extracted from Hedges et al. (2006).

2010), where the creation of a standard cartography of human brains is of fundamental importance in medical studies.

IMAGING MODALITIES

Three main image modalities have been employed in the assembly of digital atlases: Fluorescence microscopy, magnetic resonance imaging (MRI) and optical projection tomography (OPT).

Each of these modalities has different optical resolutions and lead to different types of atlases (Table 1). The choice depends on the specimen thickness and its optical transparency. For each animal model ("Animal models" section) these properties vary with the age of the specimen ("Developmental stages" section).

Recent advances in photonic microscopy imaging (Fig. 2A) include multiharmonic (Evanko et al., 2010) and fluorescence imaging by confocal, multiphoton laser scanning microscopy (Abbott, 2009; Pardo-Martin et al., 2010) or light-sheet fluorescence microscopy (LSFM) (Huisken and Stainier, 2009; Keller et al., 2010), combined with newly developed fluorescent proteins and biological sensors (Chudakov et al., 2005; Giepmans et al., 2006) and in situ hybridization (ISH) techniques (Welten et al., 2006; Brend and Holley, 2009). These advances opened new perspectives for the construction of high resolution anatomical and gene expression atlases. Spatial resolution of hundreds of nanometers and temporal resolution of minutes have been achieved for the observation of entire organisms at different levels of organization. However, photonic microscopy imaging is still limited to small model organisms with good optical properties.

OPT (Sharpe et al., 2002) was introduced as an alternative optical method to fluorescence microscopy and overcomes the limitation of the specimen thickness. OPT generates data by acquiring many views of the same specimen at different rotation angles then assembled to create a 3D volume (Fig. 2C). OPT resolution in the range of millimeters does not however allow working at the single cell level.

Alternatively, MRI (Jacobs et al., 2003) does not use fluorescent staining and has thus a broad range of applications (Fig. 2B). Indeed, MRI contrast does not depend on the penetration of photons but on the voxel-to-voxel variations in water content leading to diverging spins when submitted to magnetic fields. MRI achieves a spatial resolution of about tens of microns only, and although more and more intense magnetic fields are used, single cell resolution is barely achieved.

SPATIAL SCOPES AND RESOLUTIONS

Constructing a prototypic model for an organism can achieve different scopes, from particular organs to the whole organism, which can be resolved at either the organ, tissue, multicellular, or eventually cellular resolutions (Table 1). The reconstruction of atlases with resolution at the cellular level (Fig. 2D) focused on the species more phylogenetically distant from human ("Animal

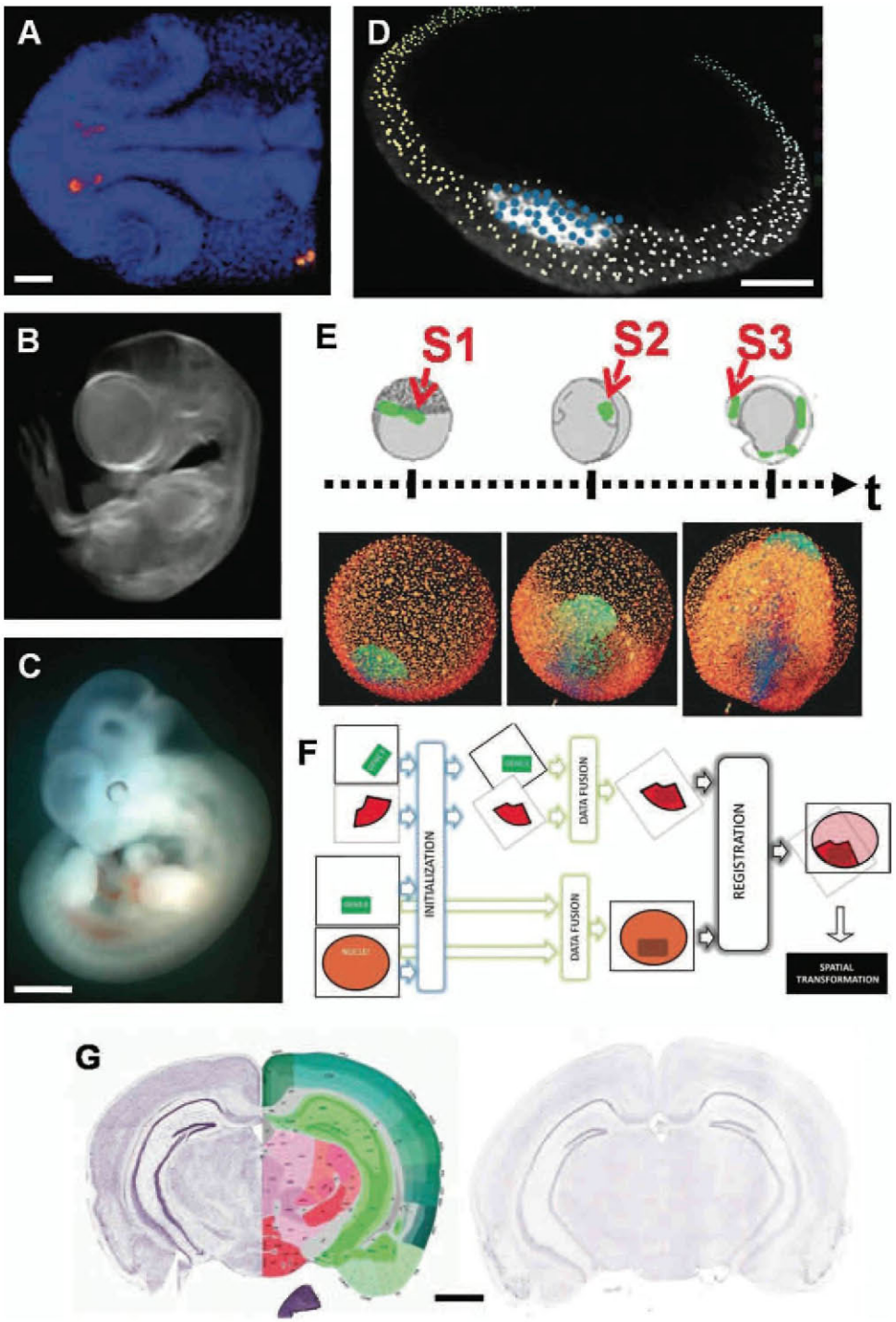

Figure 2. Examples of components involved in an atlas model construction. **A**: Confocal microscopy acquisition of a 24 hours post fertilization (hpf) zebrafish brain labeled by fluorescent ISH (tyrosine hydroxylase RNA probe) and DAPI staining of cell nuclei. Scale bar 100 microns. **B**: MRI of a quail extracted from Caltech's "Quail Developmental Atlas." Available from: http://131.215.15.121/. **C**: OPT of a late mouse embryo extracted from the "EMAP eMouse Atlas Project," http://www.emouseatlas.org. Scale bar: 1000 microns. **D**: Orthoslice showing the nuclei of a zebrafish early embryo where the raw gene expression from another specimen has been integrated. Cells positive for the expression of the gene are highlighted in blue. Scale bar: 100 microns. **E**: Zebrafish templates for three different developmental stages where individuals can be mapped using a reference gene pattern. **F**: Reconstruction of a mosaic-like atlas: Guided by a reference pattern, partial views of different individuals are mapped into a complete template. **G**: Left panel, coronal section of an averaged 3D template showing organ-level anatomical annotations of an adult mouse brain at a given developmental stage. Right panel, an ISH slice warped into the atlas template through deformable models. Extracted from the "Allen Mouse Brain Atlas [Internet]. Seattle (WA): Allen Institute for Brain Science. 2009." Available from: http://mouse.brain-map.org. Scale bar: 1300 microns.

models" section), and addressed rather early developmental stages ("Developmental stages" section). Identifying every single cell position in the whole imaged specimen (Long et al., 2009) requires advanced image processing methods ("Proposed image processing pipeline" section). Algorithmic strategies for the approximate detection of the cell nuclei center in 3D volumes encompassing several thousands of cells have been described (Drblikova et al., 2007; Frolkovic et al., 2007; Krivá et al., 2010). In addition, the identification of cell contours helps assigning RNAs or protein expression to the cell. Voronoi geometries have been proposed as a simple approach to determine cellular boundaries (Luengo-Oroz et al., 2008). The cell shape can better be obtained by the algorithmic segmentation of cell membranes in 3D when the latter is available (Zanella et al., 2010; Mikula et al., 2011).

Working at the mesoscopic scale of the multicellular structure is less demanding and has already provided useful information (Fisher et al., 2008; Frise et al., 2010).

Annotating and segmenting the different anatomical structures of interest at the tissue level is required to reconstruct prototypic models of organs. Examples of such methods can be found in Ma et al. (2005), Kovaćević et al. (2005), Dorr et al. (2008), Johnson et al. (2010), and Ullmann et al. (2010).

Finally, large organisms with a huge number of cells and high complexity in terms of organization led to organ-level atlases that do not resolve the single cell level (Fig. 2G). This strategy has been used for vertebrates at late developmental stages ("Developmental stages" section) when the specimen's size and lack of optical transparency do not allow imaging with resolution at the single cell level (Baldock et al., 2003; Ruffins et al., 2007; Rittscher et al., 2011).

DEVELOPMENTAL STAGES

The construction of anatomical and gene expression atlases focused on early developmental stages as well as adulthood (Table 1). At early developmental stages, the whole organism is more easily amenable to *in toto* imaging with resolution at the single cell level, Figure 2D, E (Fowlkes et al., 2008; Castro et al., 2009; Long et al., 2009).

At later developmental stages or in the adult, it can be more relevant to focus on specific organs (Fig. 2B, C) such as the brain (Woods et al., 1999; Lein et al., 2007; Peng et al., 2011) or appendages (Fisher et al., 2011).

Most studies targeted a single developmental stage (Fig. 2G). However, the temporal scale is essential to the understanding of biological mechanisms and gathering atlases with the relevant kinetics is a major issue in the field (Fig. 2E). Fisher et al. (2011) reconstructed atlases combining fate mapping data and gene expression patterns for three consecutive developmental stages of the chick wing bud. Murray et al. (2008) took advantage of the largely invariant lineage of *C. elegans* to build the first 3D+time atlas of transgenic reporters' expression patterns in *C. elegans* from the 4-cell stage to the 350-cell stage.

DATA TYPES

Many different specimens are assembled in the construction of atlases models that can just carry anatomical information or multilevel, genomewide data.

Anatomical atlases providing a scaffold with the morphological and histological landmarks characteristic of a cohort (Rex et al., 2003; Ruffins et al., 2007; Ullmann et al., 2010) constitute a reference shape or template to integrate further information coming from other individuals and reflect the intrinsic multilevel of morphogenesis processes. Genomewide atlases (Fisher et al., 2008; Richardson et al., 2010) integrate gene expression patterns and multilevel information from various sources into anatomical atlases (Fig. 2D). This approach emulates a virtual multiplexing and overcomes the restrictions in the number of gene products and/or functional patterns that can be simultaneously assessed. As a consequence, they are becoming a major tool for making spatiotemporal correlations between the different levels of biological organization, comparing individuals, building prototypic models, and deciphering the relationship between genotypes and phenotypes.

Building an anatomical atlas requires defining a common scaffold, frequently called template, where to gather all the information collected from different specimens. There are diverging criteria in the literature about how an atlas template should be built. Several studies (Ruffins et al., 2007; Castro et al., 2009; Ullmann et al., 2010) employed one single individual to match all the rest of the population (Fig. 2D). This individual is chosen for its "standard" appearance and the corresponding data should be of the highest quality. Alternatively, an iterative method has been used to identify the median individual within a population and select it as the template (Long et al., 2009). Other projects (Frise et al., 2010) used a synthetic template to map all the data from a cohort of specimens. This template consists in an engineered "virtual specimen" which retains the essential features of a species. The use of an average template (Fig. 2G) is widely spread (Rex et al., 2003; Fowlkes et al., 2008; Peng et al., 2011). Ma et al. (2005) constructed a "minimal deformation average template" as an idealized specimen minimizing the deformation required to fit any specimen of the cohort. Although average templates usually imply a better signal-to-noise ratio than individual specimens and exhibit a better definition in very similar regions between specimens, they fail to faithfully model fine features and regions with a high variability, lowering their definition (Kovaćević et al., 2005; Dorr et al., 2008). Finally, some approaches used a probabilistic template (Johnson et al., 2010) where specimens' variability is represented by statistical confidence limits.

The construction of prototypical genomewide atlases implies imag-

ing gene expression patterns in 3D with resolution at the cellular level (Hendriks et al., 2006). Image processing methods are required to achieve the automated segmentation of gene expression domains and the quantification of gene products to allow for example the description of expression domain borders. A simple quantification strategy is based on the assumption of a linear relationship between fluorescence intensity and gene expression level (Wu and Pollard, 2005; Frise et al., 2010). The obtained measurements are often normalized with respect to the nuclei channel fluorescence (Liu et al., 2009), which is considered to be constant, to compensate for thickness-dependent signal detection. Normalization with respect to the background intensity (Murray et al., 2008) is also a common strategy. Another possibility consists in clustering a population of cells into discrete levels (e.g. strong, moderate, weak, and none) depending on the gene expression signal intensity (Carson et al., 2005). Although the three different methods yielded correlated measurements across different individuals, the relevance of the obtained quantitative measurements to compare different specimens is questionable and this issue remains a challenge. Current efforts to achieve the quantitative comparison of gene expression levels in different individuals include the minimization of variability within a population (Fowlkes et al., 2008) and the conversion of fluorescence signal into fluorescent proteins number in transgenic specimens (Damle et al., 2006).

MATCHING PROCEDURES

A matching procedure is required to import each specimen (the source) into the template according to the maximization of a likelihood criteria. Repeating this operation is the core of digital atlases construction. For the same purpose, medical imaging makes extensive use of registration techniques (Maintz and Viergever, 1998; Zitova and Flusser, 2003). Three main registration techniques to build digital atlases can be distinguished according to the information used to assemble the data and the minimization criteria chosen accordingly: Intensity-based, object-based, and semantic-based registrations ("Intensity-based registration, Object-based registration, and Semantic-based registration" subsections). We can also distinguish three different transformation types between the source and the template space: Rigid, affine or nonrigid ("Transformation categories" subsection).

Before the registration step, an initialization scheme is generally applied to get a rough alignment between source and template. The initialization scheme helps the registration to reach an accurate solution. Two common initialization techniques consist of coarsely aligning anatomical landmarks (Lein et al., 2007) or the major orientation axis of an organism such as the anterior–posterior or dorsal–ventral axis (Blanchoud et al., 2010). Qu and Peng (2010) developed an original skeleton standardization technique to rule out part of the geometrical variability between *Drosophila* embryos. In the same line, Peng et al. (2008) designed a method to straighten *C. elegans* worms into the same canonical space.

The populations of individuals to be registered are normally composed of complete specimens imaged similarly. Accurately matching cohorts of partial specimens (Fig. 2F) is one of the current challenges in the field (Peng et al., 2011) and very few strategies addressed this case (Castro et al., 2009).

Transformation Categories

Rigid transformations are applied when the mapping between the source and template spaces consists of spatial translations and rotations (Castro et al., 2009). Rigid registration has the advantage of keeping the original raw data unaltered, allowing faithful measurements and validation of the true volumes in the final atlas representation. Affine transformations (Rex et al., 2003; Smith et al., 2004) also include a scaling factor in addition to translations and rotations. Both rigid and affine transformations are linear and globally applied to all voxels.

On the contrary, nonrigid transformations are nonlinear and locally warp the source image to fit into the template (Woods et al., 1998; Ng et al., 2007; Ng et al., 2009; Rittscher et al., 2010). This typically results in an alteration of the original raw data.

Intensity-Based Registration

Intensity-based registration procedures align the source and template by trying to maximize a similarity metric (typically mutual information or cross correlation) between the gray level values in the voxels of both images.

The most common approaches (Lein et al., 2007; Tomer et al., 2010) include an initialization performed by a global, intensity-based affine or rigid registration, followed by local deformable warps (Fig. 2G). Multiresolution approaches are also employed to optimize the mapping procedure in a coarse-to-fine strategy (Smith et al., 2004; Kovaćević et al., 2005; Tomer et al., 2010). Finally, multimodal approaches combine information coming from different imaging modalities, merging, for instance, histology and MRI (MacKenzie-Graham et al., 2004; Johnson et al., 2010). Such approaches provide multiple entry points to match different individuals and heterogeneous populations into the same coordinate system.

Object-Based Registration

Object-based transformations attempt to bring into alignment equivalent sets of characteristic points or landmarks present in both the source and template images (Liu et al., 2009). These transformations are local and nonlinear and typically produce an alteration of the data shapes and volumes. Peng et al. (2011) developed an automatic pattern recognition system to identify and match visual anatomic references with certain geometric properties such as high local curvature, and Fowlkes et al. (2008) cre-

ated a method to unequivocally identify the cell to cell correspondence in *C. elegans* embryos.

Semantic-Based Registration

Unlike intensity-based and object-based registrations, semantic-based registration does not operate on the geometrical space and is based on the use of standardized ontologies (Ashburner et al., 2000) and web queries (Zaslavsky et al., 2004; Potikanond and Verbeek, 2011).

After following an annotation procedure for anatomy and gene expression data with a controlled, standard vocabulary, the mapping procedure is reduced to just linking names to positions or domains (Baldock et al., 2003; Boline et al., 2008). Given the difficulty of geometric registration across greatly variable resources, this strategy is useful to guarantee interoperability and can bring together data coming from different laboratories, resources, developmental stages, or even different species.

PROPOSED IMAGE PROCESSING PIPELINE

The construction of atlases or digital representations of anatomic and genetic features from an increasing amount of more and more complex data, requires sophisticated image analysis algorithms (Khairy and Keller, 2011) replacing nonefficient and time consuming processing performed manually or through generic imaging software.

We describe a generalized image processing pipeline to gather quantitative, genomewide data from a cohort of individuals in a prototype with resolution at the cellular level (Fig. 3). This pipeline can achieve a complete (x, y, z, G) model, including quantitative data for gene or protein expression level (G) in each cell position (x, y, z) in a developing model organism (Castro et al., 2011).

This process can involve preprocessing steps, such as image enhancement or multiviews fusion algorithms (Rubio-Guivernau et al., 2012). Then, cell nuclei detection and cell segmentation techniques ("Spatial scopes and resolutions" section) are applied to one embryo, i, to extract cell position, (xi, yi, zi), and volume. Next, signal quantification procedures ("Data type" section) are applied on gene expression, gi, to identify positive cells, (xi, yi, zi, gi). Repeating this procedure for all the individuals of a cohort, 1 to N, yields measurements for relevant patterns, (x1, y1, z1, g1)...(xN, yN, zN, gN), which are finally combined through a registration procedure ("Matching procedures" section). The anatomical information extracted during the cell detection step and automatically segmented or identified landmarks guide the registration process. The final result is a single, quantitative model of the specimen development, (x, y, z, g1 ...gN). Validation of the model and further analysis use a dedicated, custom-made interactive visualization interface ("Visualization and validation" section).

VISUALIZATION AND VALIDATION

The reconstruction of digital atlases relies on automatic algorithms that can handle the enormous amount of large 3D images providing multilevel data for cohorts of individuals at different developmental stages. The lack of gold standards in the field requires the manual curation and correction of the results (Long et al., 2009).

Several indirect validation techniques have been exploited: Fowlkes et al. (2008) and Peng et al. (2011) showed that the gene expression variability in their atlas model was comparable to that shown by individuals, implying that the experimental errors introduced in the model could be considered negligible. Fisher et al. (2011) applied hierarchical clustering (Pearson Correlation) to replicates coming from different specimens and found that they segregated as expected. In addition to these indirect validation measures, visual assessment is the common validation standard for virtually all the previously described strategies (Table 1). Consequently, many sophisticated visualization platforms have been developed to display the multidimensional input data and output results, interactively run the previously described methods on request while providing the necessary tools to correct, annotate, quantify, and mine their outcomes. These platforms represent the necessary trade-off between the automated, high-throughput, fast computer algorithms and the manual, low-throughput but accurate human interactions.

A comprehensive review of such visualization tools can be found in Walter et al. (2010). Some relevant instances include: FlyEx (Pisarev et al., 2009), GoFigure (Gouaillard et al., 2007), PointCloudExplore (Weber et al., 2009; Rúbel et al., 2010), Mov-IT (Olivier et al., 2010), BrainExplorer (Lau et al., 2008), BrainGazer (Bruckner et al., 2009), CellProfiler (Jones et al., 2008), and V3D (Peng et al., 2010).

BIOLOGICAL INSIGHTS

The application of image processing tools (see "Proposed image processing pipeline and Visualization and validation" sections) to prototypes construction (see "Animal models, Imaging modalities, Spatial scopes and resolutions, Developmental stages, Data types, and Matching procedures" sections) paved the way for biological insights in developmental processes (Luengo-Oroz et al., 2011). Below, we comment some of the most prominent results derived from anatomical and gene expression atlases.

Kovaćević et al. (2005) used an atlas model to perform genetic and anatomic phenotyping, achieving the automated detection of mutant strains. Atlases also had major implications in evolutionary studies and Tomer et al. (2010) identified related parts of the brain in phylogenetically distant animals. Chiang et al. (2011) created a comprehensive brain wiring map of the adult Drosophila brain which

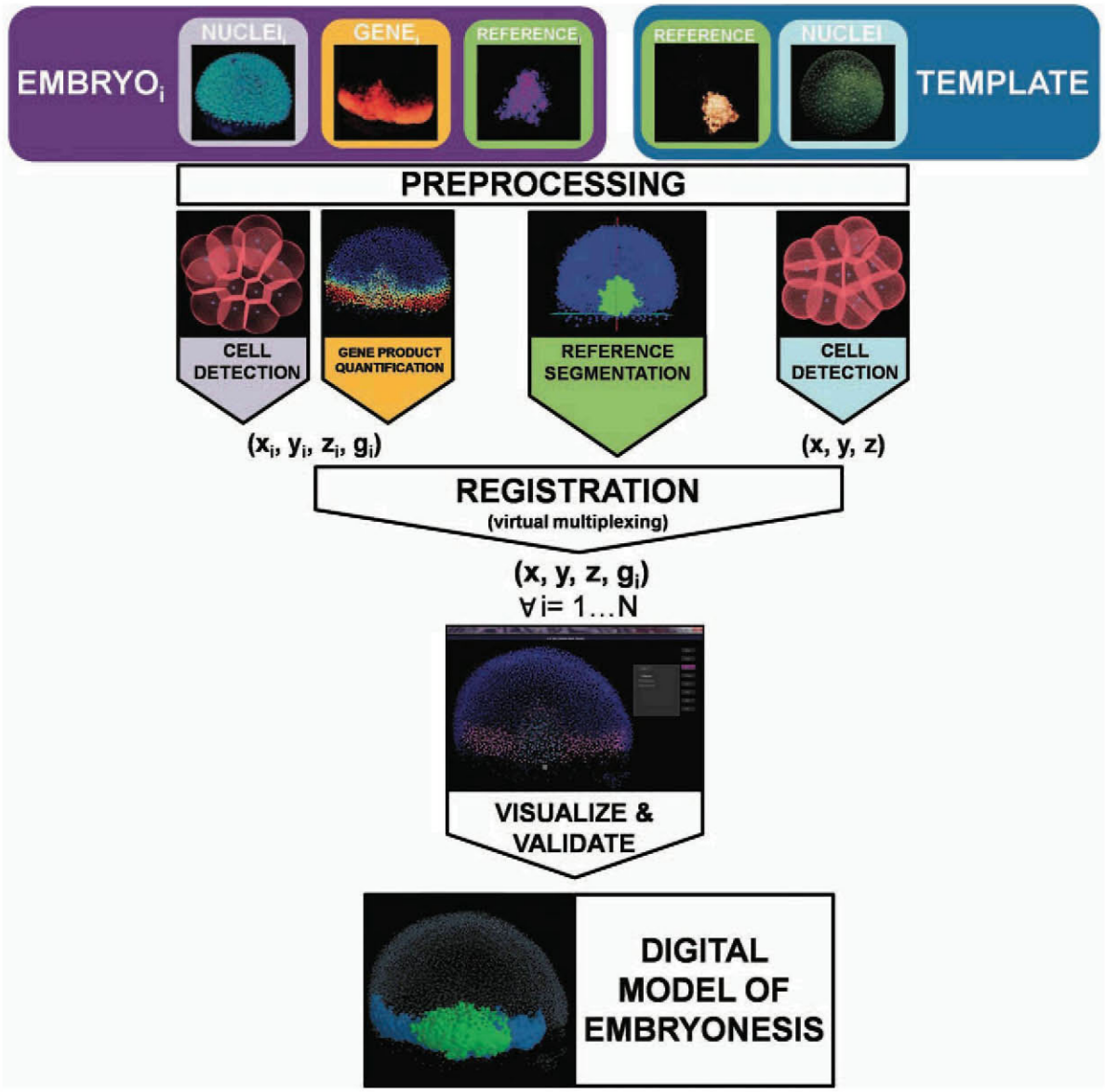

Figure 3. Image processing pipeline to build a digital atlas model: After preprocessing, nuclei detection and cell segmentation algorithms are applied to extract cell position and volume. This information is combined with quantification schemes. These operations, iterated throughout a cohort of individuals, yield cellular-level, quantitative measurements of many genetic and/or functional patterns. A common reference and/or landmarks highlighted in all individuals are automatically segmented and identified to steer a registration procedure. The latter multiplex all measured patterns into a single, digital template. The resulting atlas can be validated and mined through dedicated, interactive visualization tools.

provides a crucial tool to analyze information processing within and between neurons.

Using all the genetic information gathered in their model, Frise et al. (2010) clustered genes coexpression domains to elucidate previously unknown genetic functions and molecular and genetic interactions. Lein et al. (2007) detected highly specific cellular markers and deciphered cellular heterogeneity previously unidentified in the adult mouse brain with a gene expression atlas of more than 20,000 genes. Carson et al. (2005) discovered gene expression possibly related to Parkinson disease.

Liu et al. (2009) showed in *C. elegans* that different gene regulatory pathways can correlate with identical cell fates. However, cell fate modules with specific molecular signatures repeatedly occurred along the cell lineage, revealing bifurcations toward cell differentiation. Fisher et al. (2011) identified the digits identity in chick embryos by the computational analysis of genes expression and cell fate. The association of cell tracking techniques (McMahon et al., 2008; Pastor et al., 2009; Suppatto et al., 2009) together with gene expression atlases lead to fate maps, which are promising tools for stem cell studies and regenerative medicine.

PERSPECTIVES

The next generation of in situ hybridization techniques is expected to overcome the current limitations in the number of gene patterns that can be simultaneously labeled in a single specimen. Choi et al. (2010) recently developed a new multiplexing technique that allows to fluorescently tag up to five different mRNA targets at a time. Compared to present double or triple in situ hybridization techniques, this scheme will drastically facilitate the acquisition of data and matching operations.

A long anticipated goal (Megason and Fraser, 2007) in computational biology consists in the reconstruction of continuous spatiotemporal prototypes where gene expression can be determined for every cell in the embryo not only at certain, discrete developmental stages, but at any possible developmental time: (x, y, z, t, G). Achieving this goal depends on the proper integration of gene expression atlases with the reconstructed cell lineage tree (Supatto et al., 2009; Olivier et al., 2010; Luengo-Oroz et al., 2012b). Recent work in this direction (Castro-González et al., 2010) indicates that 3D+ time atlases toward an integrated model of living systems and multiscale dynamics, require the use of image processing techniques operating directly in the 4D space (Luengo-Oroz et al., 2012a).

Finally, a key future challenge revolves around achieving standards and making databases coming from different laboratories interoperable. As data acquisition goes on at an accelerated pace, it becomes crucial to achieve systems helping to contribute, organize, and find relevant data. As an example, machine learning has been recently applied to the automatic recognition and ontological annotation of gene expression patterns in the mouse embryo with anatomical terms (Han et al., 2011). Following this trend, there has been a series of recent standardization efforts to the crossplatform integration of multimodal data through the use of controlled terminologies, or ontologies (Diez-Roux et al., 2011) that can be accessed by query-based web systems (Hawrylycz et al., 2011; Milyaev et al., 2012). This will ultimately allow the systematic comparison of individuals within and even between species.

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