![Vitrolife logo]

Guided Annotation™ tool
Detailed guidelines
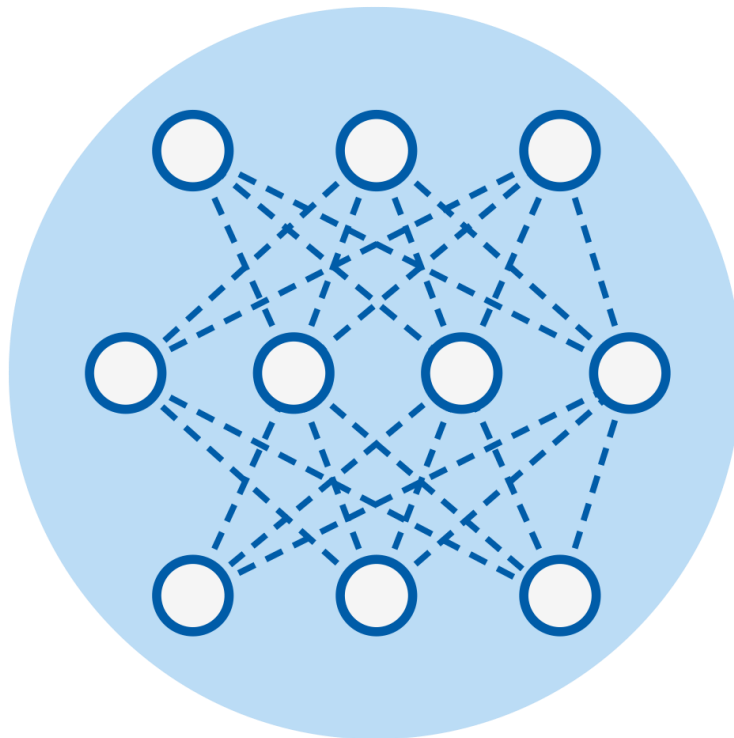
CE

MD

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1 Introduction

These guidelines provide detailed information on how to use the Guided Annotation tool. The tool is an add-on to the EmbryoViewer software. This manual should therefore be used in conjunction with the complete user manual for the EmbryoViewer software.

1.1 Conventions used in these guidelines

RESTRICTIONS ON USE: Functions or actions restricted to certain identified persons or purposes or regulatory restrictions that MUST be observed when using the Guided Annotation tool.

WARNINGS: Conditions or actions that – if the instructions are ignored – may potentially result in erroneous data or non-recoverable loss of data.

CAUTIONS: Important information needed before or during usage of the Guided Annotation tool.

NOTES: Additional information provided for the purpose of clarification.

1.2 Important warnings

The following warnings will ensure the correct use of the Guided Annotation tool by qualified clinic personnel. Users must be qualified to operate the tool and qualified to perform procedures associated with tool use in accordance with local qualification standards.

All users of the tool must agree to read and understand these guidelines and read the following warnings.

Users should contact Vitrolife immediately to report any incident and/or injury to a patient, operator or maintenance employee that occurred as a direct or indirect result of operating the tool and associated hardware. Any serious incident that has occurred in relation to the tool should be reported to the competent authority of the Member State in which the user is established.

WARNING
• For variables with a confidence estimate below the user-defined threshold, users must manually verify the estimates.

WARNING
• For non-timing variables, the time points suggested by the Guided Annotation tool should ONLY be used as a starting point. Embryo developments and characteristics are often dynamic, and their final annotation should therefore be based on a period rather than a single point in time.

Please also refer to the user manual for the EmbryoViewer software for a complete list of the restrictions that apply when the software is used.

1.3   General cyber security recommendations

Users are advised and expected to take the following measures to reduce cyber security risk in order to ensure that the device will work as designed in the intended user environment:

- Ensure that personnel are properly trained in cyber security awareness

- Prevent physical access to the equipment by unauthorised users

- Use strong passwords (at least eight characters including both uppercase and lowercase letters, numbers and at least one special character).

Users must inform Vitrolife A/S without any undue delay upon becoming aware of a cyber security vulnerability incident or any suspected security events.

For details about how to reduce cyber security risk, please refer to the separate guide on this subject provided by Vitrolife.

1.4   Purpose of the Guided Annotation tool

The Guided Annotation tool is designed to provide a simplified and consistent annotation workflow.

At the core of the tool lies an annotation strategy. This annotation strategy determines which variables users should annotate and in which order.

Once an annotation strategy has been defined, the tool guides users through the process of performing the actual annotations:

1) All variables included in the selected annotation strategy are automatically presented to the user together with an image of the current embryo.

2) The image series is automatically forwarded to an estimated point in time where the annotation process may start.

3) The user completes the workflow either by evaluating the embryo and performing the annotation or by confirming the annotations suggested by the tool.

The **Annotate** page integrates an automatic image analysis feature. The feature automatically detects cell division and morphological events and inserts estimates of division timings and morphological parameters (PN, ICM and TE). The automatic image analysis will estimate cell division timings and morphological parameters with varying degrees of certainty (confidence levels). When setting up your annotation strategies, you can specify which confidence level you require for annotation estimates that can be automatically confirmed. If you set a high confidence threshold, the estimated timings and morphological parameters will most often be correct. However, you have to check more estimates manually. If you set a low confidence threshold, the

estimates will more often be less precise, but you will have fewer estimates to check. The threshold should be based on the clinic's own tolerance.

1.5   Intended users

Embryologists, other laboratory personnel and clinic staff at IVF clinics trained by Vitrolife A/S-certified instructors.

1.6   Clinical benefit

As an accessory to a medical device, the EmbryoViewer incl. Guided Annotation software provides the indirect clinical benefit of efficient evaluation and improved selection of embryos incubated in the incubator(s) connected to the system, thereby supporting:

- Improved implantation/pregnancy rate
- Reduced pregnancy loss rate.

1.7   Requirements for using the Guided Annotation tool

To be able to use the Guided Annotation tool, you need to have installed:

- EmbryoViewer software with the **Compare & Select** feature included, version 7.9 or later
- ES server with a valid licence file installed, version 7.9 or later.

2 General workflow overview

ROLE	FUNCTION	TASK	
Administrator *(non-repetitive task)*	**Annotations** tab (**Settings** page) Settings	Define an annotation strategy, or determine which pre-defined annotation strategy to use. Set an active annotation strategy as default. Enter which confidence threshold you want the automatic image analysis to meet, and set the **Auto Forward** check box depending on whether or not you want the automatic workflow to stop at annotation estimates on or above the threshold. Select the **Skip avoided embryos** check box if you want to exclude all avoided embryos from the annotation workflow.	See sections 3.2 and 3.3.
All users *(once per treatment)*	**Annotate** page Annotate	Mark all non-viable embryos with ❌ before performing any annotations for the viable embryos.	See sections 3.3 and 4.3.
All users *(daily or once per embryo before transfer)*	**Annotate** page Annotate	Select an annotation strategy and annotate the variables that the tool automatically prompts you to annotate.	See section 4.3.
All users *(once per treatment)*	**Compare & Select** page Compare & Select	On the **Compare & Select** page, calculate a score for the annotated embryos by applying a model to them.	See the complete user manual for the Embryo-Viewer software.

3 Annotations tab

The **Annotations** tab is available from the **Settings** page. It is only accessible to users with administrator rights*.*

To be able to use the Guided Annotation tool, you first need to set up an annotation strategy (see section 3.3). Alternatively, you may decide to use one of the pre-defined annotation strategies delivered with the tool (see section 3.2).

The annotation strategy determines which variables should be annotated and in which order.

Annotation strategies are defined on the **Annotations** tab of the **Settings** page.

NOTE
• The Guided Annotation tool cannot function without an annotation strategy. Annotation strategies are defined on the **Settings** page of the EmbryoViewer software. • Only users with administrator rights can create, edit, delete and activate/inactivate annotation strategies.

3.1 The layout of the Annotations tab

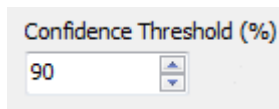Below is an overview of the various parts of the **Annotations** tab:

1. Annotation strategies
2. Description of the annotation strategy
3. Order of annotation variables
4. Logical groups of variables
5. Annotation variables included in the selected group
6. Set the confidence level (between 50% and 100%)
7. Switch on/off automatic confirmation of estimates
8. Add or remove variables
9. Description of the variable
10. Add or delete comments

1. List of available annotation strategies with indication of which ones are active.

2. Name and description of the annotation strategy.

3. **Annotation order**: variables included in this annotation strategy.

4. **Variable groups**: logical groups of variables, e.g. timing variables or stage-specific variables.

5. **Variables** box: overview of annotation variables included in the selected variable group.

6. Set the desired confidence level for automatic annotation estimates (applies to all strategies).

7. Switch on/off automatic confirmation of annotation estimates that are on or above the confidence threshold (applied on the **Annotate** page).

8. Buttons to add/remove variables or to change their order.

9. Description of the variable currently selected in the **Variables** box.

10. Add or delete user-defined comments. Can be used on the **Annotate** page.

The following is a further description of some of the elements on the page.

- **Confidence Threshold***:*

    Confidence Threshold (%)
    90

    The automatic image analysis will analyse the images and suggest annotations with varying degrees of certainty. The confidence threshold is the degree of certainty that you have decided will be the tolerance level for automatic confirmation of annotations in your specific clinic (see the description of the **Auto Forward** check box below). When the program presents the annotation estimates on the **Annotate** page, there will be a visible difference between estimates that are on or above the confidence threshold and estimates that are below the threshold.

    The estimate is *below the confidence threshold* (i.e. less confident than the entered level):

    t3   33.9 h   *(italics without boldface)*

    The estimate is *on or above the confidence threshold* (i.e. as confident as or more confident than the entered level):

    t2   24.2 h   **(italics in boldface)**

    Estimates for one or more variables may not be displayed for a number of reasons, e.g.:

    - The variables have not yet occurred.
    - The Guided Annotation tool is not set to estimate the variables.
    - The Guided Annotation tool is unable to automatically estimate the variables.

    tB

    When the clinic first starts working with the automatic image analysis, Vitrolife recommends to set the confidence threshold to a high percentage until you have been working with the automatic image analysis for some time. As you gain more experience with the feature, you may gradually set the confidence threshold to a lower percentage.

- **Auto Forward**:

If the **Auto Forward** check box has been selected on the **Settings** page, the automatic workflow will not stop at variables with estimates on or above the confidence threshold. You still need to confirm these estimates when the workflow is complete.

Confirmed annotation:

 (boldface)

When you first start working with the automatic image analysis, Vitrolife recommends not to select the **Auto Forward** check box until you have gained some experience with the feature.

- **Annotation order**:

When you apply the annotation strategy on the **Annotate** page, you will be prompted to annotate the variables in their order of appearance on this list.

Example:
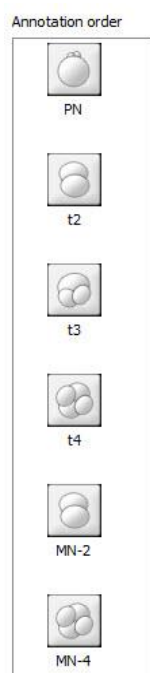
In this example, you will first be prompted to annotate PN, then t2, etc.

- **Add/remove variables from the annotation strategy and change the annotation order**:

  When you are defining an annotation strategy, you can include or exclude variables in the annotation strategy and move included variables up or down on the prioritised list. Use the available buttons for this purpose:

◄■	Add variable – adds the selected variable to the annotation strategy.
■►	Remove variable – removes the selected variable from the annotation strategy.
▫↰	Move up – moves the selected variable one step up on the list.
■↳	Move down – moves the selected variable one step down on the list.

- **Variable groups and the Variables box**:

  When you select a group, the variables included in the group are displayed in the **Variables** box to the right. The **All** group contains all possible values.

  The **Most used** variable group is selected:
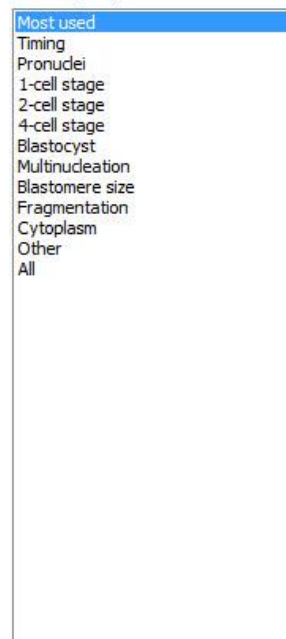
The contents of the **Most used** variable group are displayed:
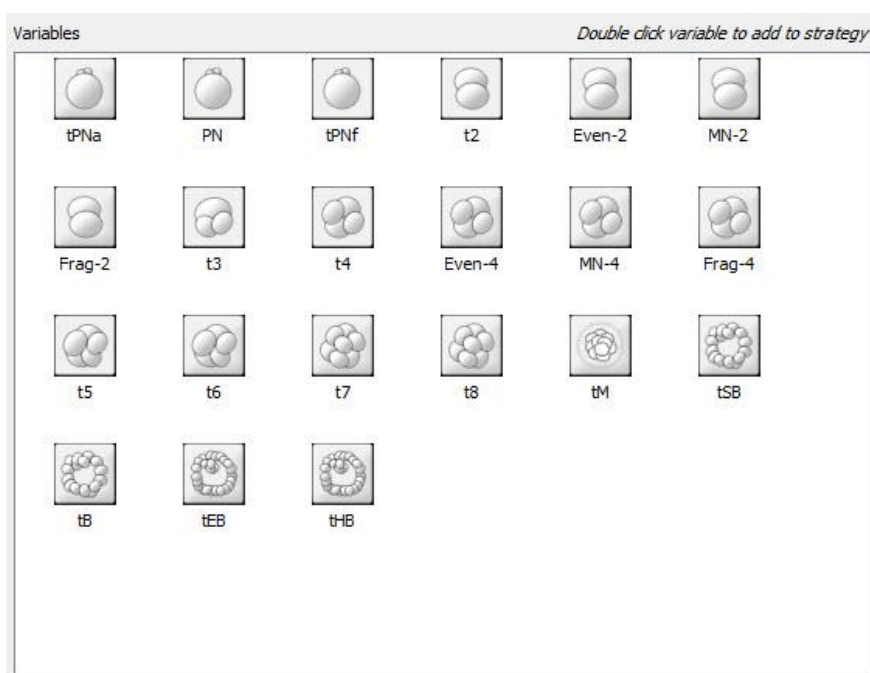
3.2 Pre-defined annotation strategies

The Guided Annotation tool is delivered with two pre-defined annotation strategies. The pre-defined annotation strategies are locked, which means that they can neither be deleted, nor modified. They can, however, be inactivated or used as the basis for a new, customised strategy with a different name (see section 3.4).

- KIDScore D3 Annotation
- KIDScore D5 v3 Annotation (not available in the US).

3.3 Defining a customised annotation strategy

The Guided Annotation tool allows you to define your own annotation strategies. You can define an unlimited number of strategies for different purposes.

The annotation strategy determines which variables the user will be prompted to annotate and in which order when this annotation strategy is applied.

To define an annotation strategy:

1. Go to the **Settings** page and select the **Annotations** tab.
2. In the **Name** field, enter the name of your strategy.

3. *Optional step:*

   Enter a description of your strategy. This is particularly useful if you define more than one strategy.

4. Indicate your desired confidence threshold for the automatic image analysis. Then indicate whether you want the automatic workflow to stop at annotation variables where the estimates are on or above the confidence threshold. If you do *not* want the workflow to stop at these variables, select the **Auto Forward** check box:

   Automatic Annotation Estimates
   Confidence Threshold (%)
   90   ▲▼   ☐ Auto Forward

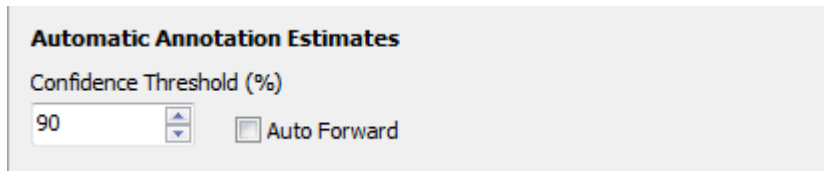   The selections made in the **Automatic Annotation Estimates** box apply to all available strategies. You can change the settings in this box without affecting the contents of the strategies. The settings in the box are independent of the strategies and only define how users can work with the automatic image analysis.

5. *Optional step:*
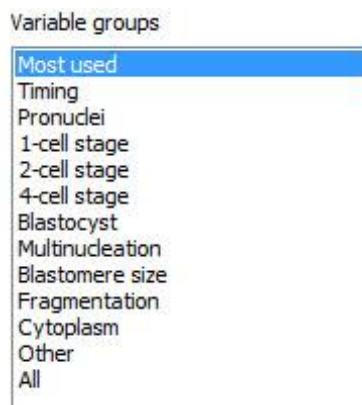
   Indicate whether you want to disregard the embryos marked to be avoided ☒ when you apply the current annotation strategy:

   ☑ Skip avoided embryos

   This will speed up your workflow when the annotation strategy is applied on the **Annotate** page.

   If you do not select this check box, the annotation strategy will prompt you to annotate ALL embryos in a given treatment, even the ones marked to be avoided for transfer ☒.

6. From the list of variable groups, select the logical group you want to pick your variables from, e.g. most used variables or variables related to a specific cell stage:

   Variable groups

   | Most used |
   | Timing |
   | Pronuclei |
   | 1-cell stage |
   | 2-cell stage |
   | 4-cell stage |
   | Blastocyst |
   | Multinucleation |
   | Blastomere size |
   | Fragmentation |
   | Cytoplasm |
   | Other |
   | All |

   You can choose from as many groups as you like and include an unlimited number of variables.

7. From the **Variables** group box, select the variables you want to include in your strategy:
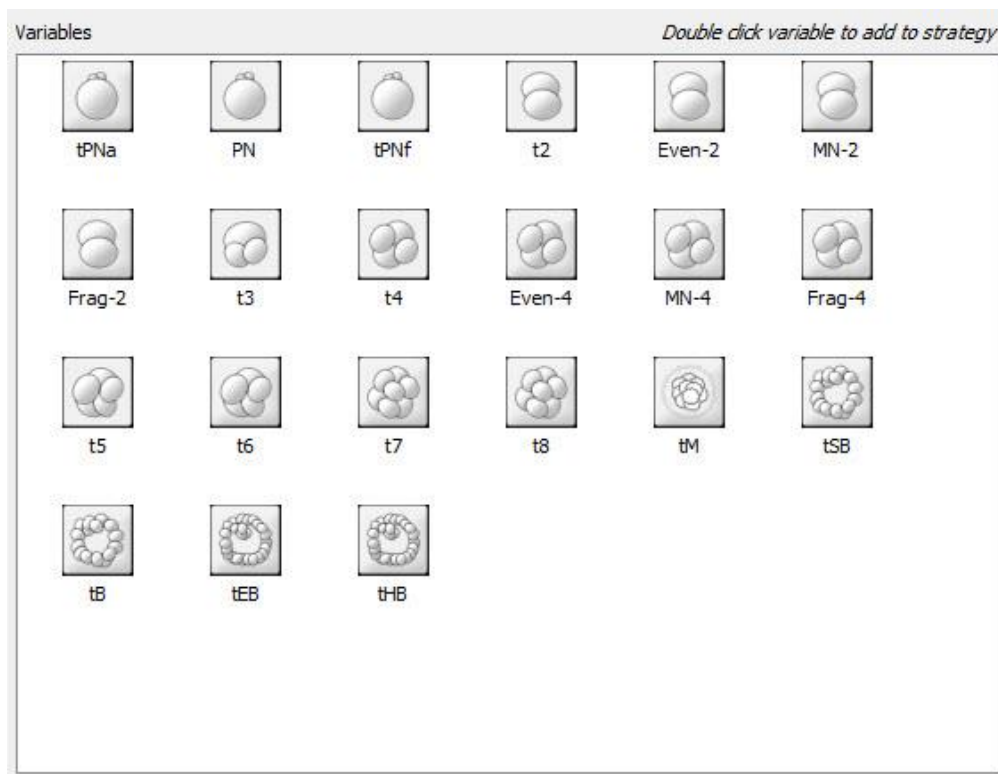
Either double-click on an icon to include the corresponding variable, or click once on the icon and then on the add variable button:

When you click on an icon once, the tool displays a description of the corresponding variable:

The t2 variable is active

Description of the t2 variable
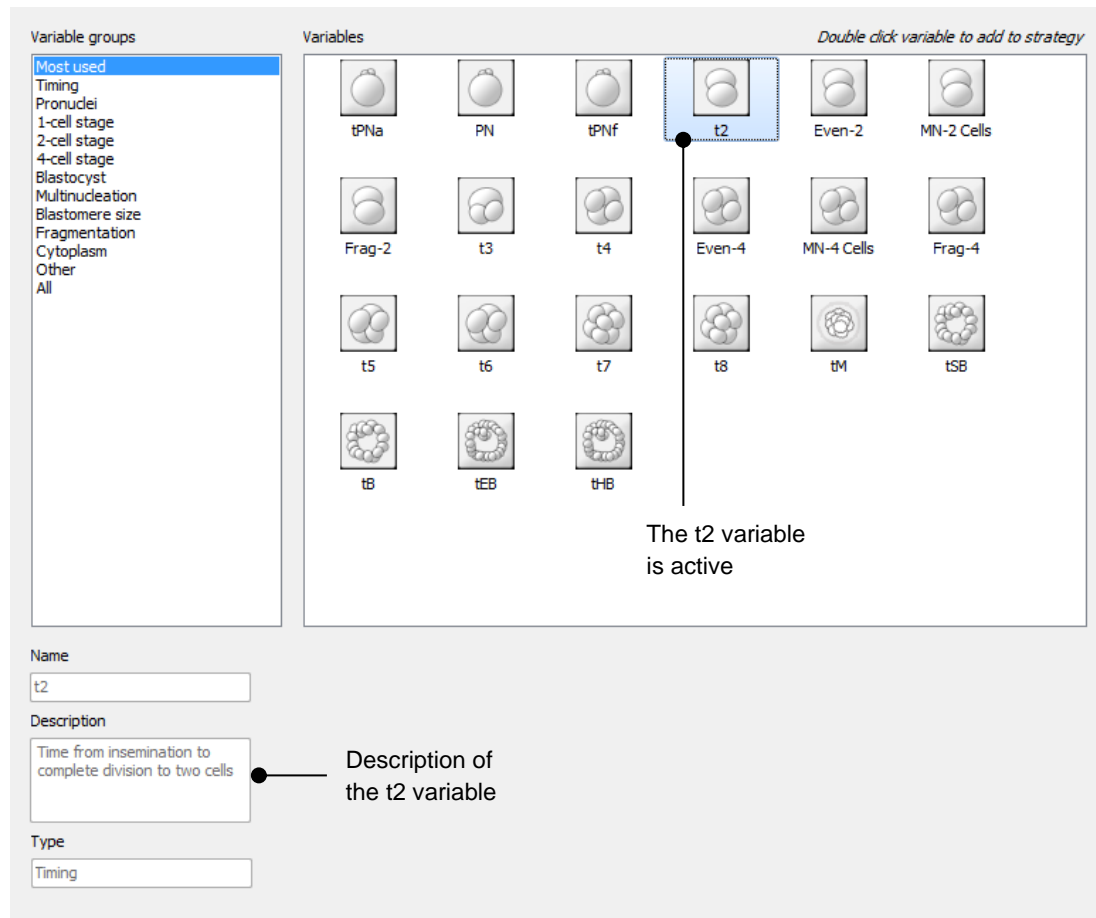
8. *Optional step:*

To remove an unwanted variable from the strategy, select it in the **Annotation order** list and click the remove variable button:

9. Repeat steps 6 and 7 until you have included all desired variables.

The variables will appear in the **Annotation order** list. The first selected variable is placed at the top of the list, the last selected variable is placed at the bottom, etc.

10. *Optional step:*

Reorganise the list to change the order of the variables. Use the move up and move down buttons for this purpose:

NOTE
• When you perform annotations based on this annotation strategy, the Guided Annotation tool will automatically guide you through the variables in their order of appearance on the **Annotation order** list.

11. Click **Save** to save the new strategy.

3.4 Editing an existing annotation strategy

To edit an existing annotation strategy:

1. Go to the **Settings** page and select the **Annotations** tab.

2. From the list of defined annotation strategies, select the annotation strategy you want to edit.

3. Make the required adjustments to the annotation strategy, e.g. by removing, adding or reorganising one or more variables.

4. Click **Save**.

5. Click **OK**.

NOTE
• The pre-defined annotation strategies delivered together with Guided Annotation are locked for editing. They are displayed with a padlock icon on the **Annotations** tab:

Active		Name	Default	Creator	Date
☑	🔒	KIDScore D3 Annotation		Vitrolife	2017-02-27
☑	🔒	KIDScore D5 v3 Annotation	Default	Vitrolife	2019-07-03

It is, however, still possible to create a new annotation strategy based on a pre-defined strategy (see section 3.5).

NOTE
• Existing annotations will not be deleted if the administrator edits an annotation strategy that has already been used.

3.5   Creating a new strategy based on an existing one

Follow this procedure to create an additional annotation strategy based on an existing one:

1. Go to the **Settings** page and select the **Annotations** tab.

2. From the list of defined annotation strategies, select the annotation strategy you want to use as the basis for the new one.

3. Make the required adjustments to the annotation strategy, e.g. by removing or adding one or more variables.

4. Click **Save as…** and enter a new name for the annotation strategy.

5. Click **OK**.

   Both the original and the new annotation strategy now appear in the list of annotation strategies.

3.6   Importing/exporting annotation strategies

You can import an annotation strategy or export an annotation strategy you want to share with another clinic:

1. Go to the **Settings** page and select the **Annotations** tab.

2. Click the **Import Strategy**/**Export Strategy** button, depending on which operation you want to perform.

   Windows Explorer opens.

   a. To import: Select the *.fts file that contains the annotation strategy you want to import and click **Open**.

   If another annotation strategy with an identical name already exists, the Guided Annotation tool prompts you to enter a different name for the imported annotation strategy.

   b. To export: Select a location for the *.fts file you want to export and click **Save**.

   The file containing the annotation strategy is exported to the selected file location. The file can now be imported to an ES server via the EmbryoViewer software. All users who have access to the same server will also have access to the exported annotation strategy.

3.7 Pre-defined variables

The Guided Annotation tool is delivered with a number of pre-defined variables. The variables fall into three categories:

- Binary: Variables that are either true or false.

- Range: Numerical variables that can be set up with minimum and maximum values in models.

- Info: Variables that carry no weight and do not affect the scores assigned by a model. Binary variables and variables of the range type can be used as information variables if they are set up with no weight and no minimum and maximum values.

The following pre-defined variables are available:

Variable	Description	Values	Usage
NOT2PN	Maximum number of pronuclei differs from two	TRUE/FALSE	Binary
UNEVEN2	Uneven size of blastomeres at the 2-cell stage	TRUE/FALSE	Binary
UNEVEN4	Uneven size of blastomeres at the 4-cell stage	TRUE/FALSE	Binary
MN2	Multinuclearity occurs at the 2-cell stage	TRUE/FALSE	Binary
MN4	Multinuclearity occurs at the 4-cell stage	TRUE/FALSE	Binary
tPB2	Time from insemination until second polar body is extruded	Hours	Range
tPNa	Time from insemination until pronuclei have appeared	Hours	Range
tPNf	Time from insemination until pronuclei have faded	Hours	Range
t2	Time from insemination to complete division to two cells	Hours	Range
t3	Time from insemination to complete division to three cells	Hours	Range
t4	Time from insemination to complete division to four cells	Hours	Range
t5	Time from insemination to complete division to five cells	Hours	Range
t6	Time from insemination to complete division to six cells	Hours	Range
t7	Time from insemination to complete division to seven cells	Hours	Range
t8	Time from insemination to complete division to eight cells	Hours	Range
t9+	Time from insemination to complete division to nine or more cells	Hours	Range
tSC	Time from insemination to start of compaction	Hours	Range
tM	Time from insemination to formation of morula	Hours	Range
tSB	Time from insemination to start of blastulation	Hours	Range
tB	Time from insemination to formation of blastocyst	Hours	Range

Variable	Description	Values	Usage
tEB	Time from insemination to formation of expanded blastocyst	Hours	Range
tHB	Time from insemination to hatching blastocyst	Hours	Range
tDead	Time from insemination to arrested embryo development	Hours	Range
TE	Trophectoderm evaluation	A, B, C	Info
ICM	Inner cell mass	A, B, C	Info
TE-Last	Assessment of trophectoderm at the last image	A, B, C	Info
ICM-Last	Assessment of inner cell mass at the last image	A, B, C	Info
PN	Number of pronuclei	0, 1, 2, 3, 4, 4 or more	Range
Even-2	Symmetry of blastomere sizes at the 2-cell stage	Even/Uneven	Info
Even-4	Symmetry of blastomere sizes at the 4-cell stage	Even/Uneven	Info
MN-2 Cells	Assessment of the number of blastomeres with multinucleation at the 2-cell stage	0-2	Range
MN-4 Cells	Assessment of the number of blastomeres with multinucleation at the 4-cell stage	0-4	Range
Frag-2	Percentage of fragmentation at the 2-cell stage	0-100%	Range
Frag-4	Percentage of fragmentation at the 4-cell stage	0-100%	Range
Frag-8	Percentage of fragmentation at the 8-cell stage	0-100%	Range
Frag-2 Cat.	Percentage of fragmentation at the 2-cell stage	0-10%, 10-20%, 20-50%, 50-100%	Info
Frag-4 Cat.	Percentage of fragmentation at the 4-cell stage	0-10%, 10-20%, 20-50%, 50-100%	Info
Frag-8 Cat.	Percentage of fragmentation at the 8-cell stage	0-10%, 10-20%, 20-50%, 50-100%	Info
Frag-2 Type	Assessment of type of fragmentation at the 2-cell stage	Cluster, Spread, Small, Large	Info
Frag-4 Type	Assessment of type of fragmentation at the 4-cell stage	Cluster, Spread, Small, Large	Info
Frag-8 Type	Assessment of type of fragmentation at the 8-cell stage	Cluster, Spread, Small, Large	Info
Morph Grade	Morphological grade	Text	Info
Morph G. D2	Morphological grade D2	Text	Info
Morph G. D3	Morphological grade D3	Text	Info
Morph G. D5	Morphological grade D5	Text	Info
Dyn Score	Dynamic score	Text	Info
Z Score	Z score	Text	Info

Variable	Description	Values	Usage
Halo	Presence of cytoplasmic halo	No, Yes	Info
MN-2 Type	Nuclear morphology at the 2-cell stage	Mono, Bi, Multi, Micro, Cluster, Unknown	Info
MN-4 Type	Nuclear morphology at the 4-cell stage	Mono, Bi, Multi, Micro, Cluster, Unknown	Info
Collapse	Blastocyst collapse	No, Yes	Info
Pulsing	Blastocyst collapse and re-expansion	No, Yes	Info
Coll. Count	Number of blastocyst collapses	Number	Range
Re-exp Count	Number of blastocyst re-expansions	Number	Range
Strings	Presence of cytoplasmic strings	No, Yes	Info
t2Roll(i)	Time of initiation of embryo rolling without divisions	Hours	Range
t2Roll(end)	Time of end of embryo rolling without divisions	Hours	Range
SER	Existence of smooth endoplasmic reticulum (SER) discs	No, Yes	Info
EVEN PN	Relative pronuclei size assessment	Even/Uneven	Info
PN1a	Time from insemination until appearance of the first pronucleus	Hours	Range
PN2a	Time from insemination until appearance of the second pronucleus	Hours	Range
PN3a	Time from insemination until appearance of the third pronucleus	Hours	Range
PN4a	Time from insemination until appearance of the fourth pronucleus	Hours	Range
PN1f	Time from insemination until fading of the first pronucleus	Hours	Range
PN2f	Time from insemination until fading of the second pronucleus	Hours	Range
PN3f	Time from insemination until fading of the third pronucleus	Hours	Range
PN4f	Time from insemination until fading of the fourth pronucleus	Hours	Range
NPB	Assessment of nuclear precursor bodies	Few, Many, Even no., Uneven no.	Info
ZP	Assessment of zona pellucida	Firm, Loose, Rough, Smooth, Thin, Thick, Uniform, Non-uniform	Info

Variable	Description	Values	Usage
Cumulus	Assessment of attached cumulus cells	None, Few, Moderate, Many	Info
PB	Assessment of the polar bodies' relative proximity	Proximate, Distant	Info
Cytoplasm-1	Assessment of cytoplasm at the 1-cell stage	Granular, Homogeneous, Dark, Inclusion, Other	Info
Cytoplasm-2	Assessment of cytoplasm at the 2-cell stage	Granular, Homogeneous, Dark, Inclusion, Other	Info
Cytoplasm-4	Assessment of cytoplasm at the 4-cell stage	Granular, Homogeneous, Dark, Inclusion, Other	Info
Cytoplasm-8	Assessment of cytoplasm at the 8-cell stage	Granular, Homogeneous, Dark, Inclusion, Other	Info
Blast Expand	Assessment of blastocyst expansion	0-6	Range
BS Exp.-Last	Assessment of blastocyst expansion at the last image	0-6	Range
Cells Last	Number of cells at the last image	Number	Range
Maturity	Grade of oocyte maturity	GV, MF1, MF2, Unknown	Info

3.8 Importing new variables

If you need additional variables to be available, please contact support.embryoscope@vitrolife.com. It is not possible to define additional variables directly in the Guided Annotation tool.

Once Vitrolife has created the requested variable, it will be available in a file:

1. Go to the **Settings** page and select the **Annotations** tab.
2. Click the **Import Variable** button.

   Windows Explorer opens.
3. Select the *.ftv file containing the variable and click **Open**.

   The variable is now imported and available from the **Annotations** tab.

3.9 Adding user-defined comments

On the **Annotations** tab, you can add one or more user-defined comments. These comments can afterwards be selected and automatically inserted in the **Comments** field of the **Annotate** page (see section 4.3).

We recommend that events that only occur occasionally are set up as user-defined comments and not as variables in an annotation strategy.

Only users with administrator rights can add and delete user-defined comments.

To create a user-defined comment:

1.   Go to the **Settings** page and select the **Annotations** tab.

2.   Next to the **User Defined Comments** field, click **Add** and enter a comment.

If a user-defined comment is included in a model, the comment will automatically be displayed in the **User Defined Comments used in model** field on the right. When the user-defined comment is displayed in this field, it can no longer be modified or deleted. This ensures that information used in evaluation models cannot be deleted.

3.   Repeat until you have added all desired comments.

4.   Click **Save**.

On the **Annotate** page, the user will now automatically be presented with the user-defined comment if there is a (partial) match with the text entered in the **Comments** field:

See section 4.3.

NOTE
• The entered user-defined comments are global and thus available regardless of which annotation strategy is selected on the **Annotate** page.

4 Annotate page

NOTE
• If the Guided Annotation tool is installed, this section replaces the description of the **Annotate** page as provided in the user manual for the EmbryoViewer software.

On the **Annotate** page, the Guided Annotation tool guides you through the process of annotating the variables that are part of the selected annotation strategy.

4.1 The layout of the Annotate page

Below is an overview of the various elements of the **Annotate** page. The page consists of two parts: the time-lapse part, which displays images of the embryos, and the analysis part, which lists all variables in the current annotation strategy as well as all culture dishes belonging to the selected treatment. The number of available wells in each culture dish depends on the incubator used.

Time-lapse part of page   Analysis part of page

An explanation of each part is provided in the following sections.

4.1.1 Time-lapse part of the Annotate page

Comments icon. Only active when you have inserted one or more annotation comments. Click the icon to display the comment(s).

Change focal plane by using the up or down arrows on the keyboard or by dragging the bullet with the mouse.

Adjust brightness and contrast. To activate the tool, click on either the contrast or the brightness icon. Adjust by dragging the slider with the mouse. The setting only takes effect for the current well. Once you proceed to another well, the images are displayed with the default settings.

The video has automatically been forwarded to the estimated time when the embryo cleaved into two cells. At this time, the blastomere activity also peaks as illustrated by the chart.

When you open the **Annotate** page, the division chart is based on estimated timings for embryo development stages. Once the annotations have been confirmed, the division chart also becomes available on the **View Slide** page and the **Compare & Select** page.

4.1.2   Analysis part of the Annotate page

Percentages display how much of the selected annotation strategy has been completed for each well.

Description of the currently active variable.

t2
Time from insemination to complete division to two cells

Annotate ↵

Same as t3 3

Same as t4 4

Same as t5 5

Shortcut buttons for the current variable (indicated in the small squares).

Anno. Tool	Value
PN	**2**
t2	**27.7 h**
t3	*40.5 h*
t4	*40.7 h*
t5	*53.9 h*
tB	*101.2 h*
ICM	*A*
TE	*A*

List of variables included in the annotation strategy. Next to the variables, the tool displays the estimated (italics or boldface italics) or actually annotated (boldface) value of the variable.

Well	Dec.	Progress
AB-1		100 %
AB-2		100 %
AB-3		25 %
AB-4		12 %
AB-5		
AB-6		
AB-7		
AB-8		
AB-9		
AB-10		
AB-11		
AB-12		
AB-13		
AB-14		
AB-15		
AB-16		

List of all wells in the treatment. The number of available wells depends on the incubator used.

Enter a new comment or insert a pre-defined comment.

Comments *

Confirm Estimates ,

Not Applicable /

Prev -   Next +

Strategy
KIDScore D5 v3 Annotation ⌄ — Select an annotation strategy.

Step by
◉ Variable   ○ Well — Progress by variable or by well.

The Guided Annotation tool is focused on complete treatments. The page thus displays all wells in all culture dishes that are part of the current treatment. The wells are identified by two letters (AA, AB, AC …) that indicate which culture dish this well belongs to and the number of the well in this culture dish. For example, AA-1 is the first well in the first culture dish, and AB-3 is the third well in the second culture dish.

The wells in the list are marked with different colours depending on their status:

- Light blue: currently being annotated

- Dark blue: not currently being annotated

- Grey: empty wells.

4.1.3 Measuring and decision tools on the Annotate page

For a description of how to use the tools available at the bottom of the **Annotate** page (measuring tools, decision tool, etc.), please refer to the complete user manual for the EmbryoViewer software:

4.2 Navigation on the Annotate page

Navigation in the Guided Annotation tool is partly automatic and partly manual.

The tool automatically activates the variables and wells to be annotated based on a selected treatment and annotation strategy. In addition, the tool automatically forwards the embryo image series to estimated time points where users can start their evaluation of the currently active variable.

For the manual part of the workflow, the tool is designed for keyboard navigation.

4.2.1 Shortcut buttons on the Annotate page

When a variable is activated on the **Annotate** page, a number of shortcut buttons become available at the top part of the page.

The shortcut buttons at the lower part of the page are permanent and not dependent on the selection of a specific variable.

The t2 annotation variable is active.

t2

Time from insemination to complete division to two cells

Anno. Tool	Value		Well	Dec.	Progress
PN	2		AB-1		100 %
t2	27.7 h		AB-2		100 %
t3	40.5 h		AB-3		25 %
t4	40.7 h		AB-4		12 %
t5	53.9 h		AB-5		
tB	101.2 h		AB-6		
ICM	A		AB-7		
TE	A		AB-8		
			AB-9		
			AB-10		
			AB-11		
			AB-12		
			AB-13		
			AB-14		
			AB-15		
			AB-16		

Annotate [↵]

Same as t3 [3]

Same as t4 [4]

Same as t5 [5]

Shortcut buttons related to the t2 variable. The keyboard shortcuts are displayed in the small squares next to the descriptive text on the buttons.

Comments [*]

Confirm Estimates [,]

Not Applicable [/]

Shortcut buttons. The keyboard shortcuts are displayed in the small squares.

Prev [-]   Next [+]
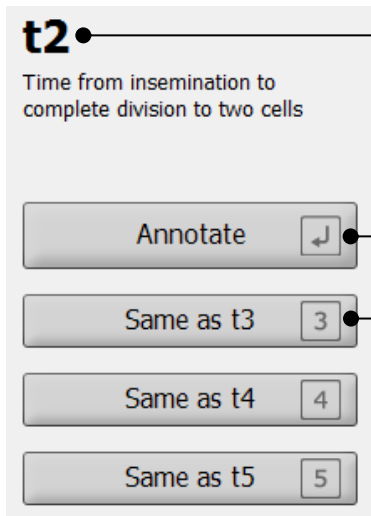
Strategy

KIDScore D5 v3 Annotation ⌄

Step by
◉ Variable   ○ Well

Each button has a keyboard shortcut, which is displayed directly on the button:

 — Currently active annotation variable.

Press Enter on the keyboard to annotate the timing currently displayed in the time-lapse video.

Press 3 on the keyboard to annotate the **Same as t3** value if the embryo divided directly from one to three cells and there is thus no separate time-lapse image of t2.

You can use the keyboard shortcuts to speed up the annotation process. All estimates and annotations can be confirmed with the Enter key.

4.2.2 Same as … shortcut buttons

Some of the variables in the annotation strategy may be equal because one of the cell stages is not verifiable. You will then not be able to annotate all of the cell stages that are part of the selected annotation strategy. In such cases, you have an option to annotate **Same as t3**, **Same as t4**, etc. depending on the development pattern of the embryo.

If you e.g. annotate **Same as t4** for the t3 variable, the Guided Annotation tool will annotate the selected timing for both t3 and t4 at the same time:

The t3 variable has been annotated as **Same as t4** as the embryo divided synchronously from three to four cells. The t5 variable is next in the annotation strategy and activated (indicated by the blue colour).

4.2.3   Confirm Estimates shortcut button

The **Confirm Estimates** shortcut button allows you to manually confirm all estimates performed by the automatic image analysis.

Confirm Estimates [,]
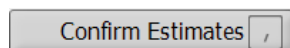
Use this button when you have checked and, if relevant, adjusted the estimates.

4.2.4   Not Applicable shortcut button

The **Not Applicable** shortcut button is available for all variables in the strategy:

Not Applicable [/] ——— Press / on the keyboard to annotate NA for
the currently active variable

Use this shortcut button if it is not possible to annotate a specific variable. In this case, you can press **/** on the keyboard to insert the value NA for this variable.

4.2.5   Prev and Next shortcut buttons

Use the **Prev** and **Next** shortcut buttons if you need to manually activate either the next or the previous well in the treatment.
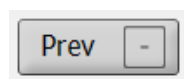
Prev [-] ——— Press - on the keyboard to activate the
previous well in the treatment
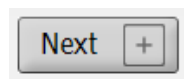
Next [+] ——— Press + on the keyboard to activate the next
well in the treatment

When you go to either the previous or the next well, the tool automatically activates the first variable that has not yet been annotated.

### 4.2.6   Step by Variable and Step by Well radio buttons

Select **Step by Variable** if you want to annotate *all* variables in the annotation strategy for one of the wells before proceeding to the next well in the treatment (recommended if you annotate complete treatments either on the day of transfer or retrospectively).

Select **Step by Well** if you want to annotate one of the variables for *all* wells in the treatment before proceeding to the next variable in the annotation strategy (recommended for performing PN annotations).

4.2.7   Arrow and Page Up/Down keys on the keyboard

The arrow keys and the Page Up/Down keys on the keyboard can be used for:

- Adjusting the focal plane of the images. Press arrow up ▲ or arrow down ▼ to adjust the focal plane of the image.

- Stopping the image series from being automatically forwarded and instead manually rewinding/forwarding the image series. Press either the right arrow ► or the left arrow ◄. Alternatively, slightly turn the jog wheel to stop the ongoing automatic forward operation.

- Rewinding and forwarding the image series. Press the right arrow ► or the Page Up key to forward to the next image in the series. Press the left arrow ◄ or the Page Down key to rewind to the preceding image in the series.

- Playing the video fast forward or backwards. Press and hold down the Page Down key to play the video fast forward. Press and hold down the Page Up key to play the video fast backwards.

Adjust the image focal plane

Rewind or forward the time-lapse video
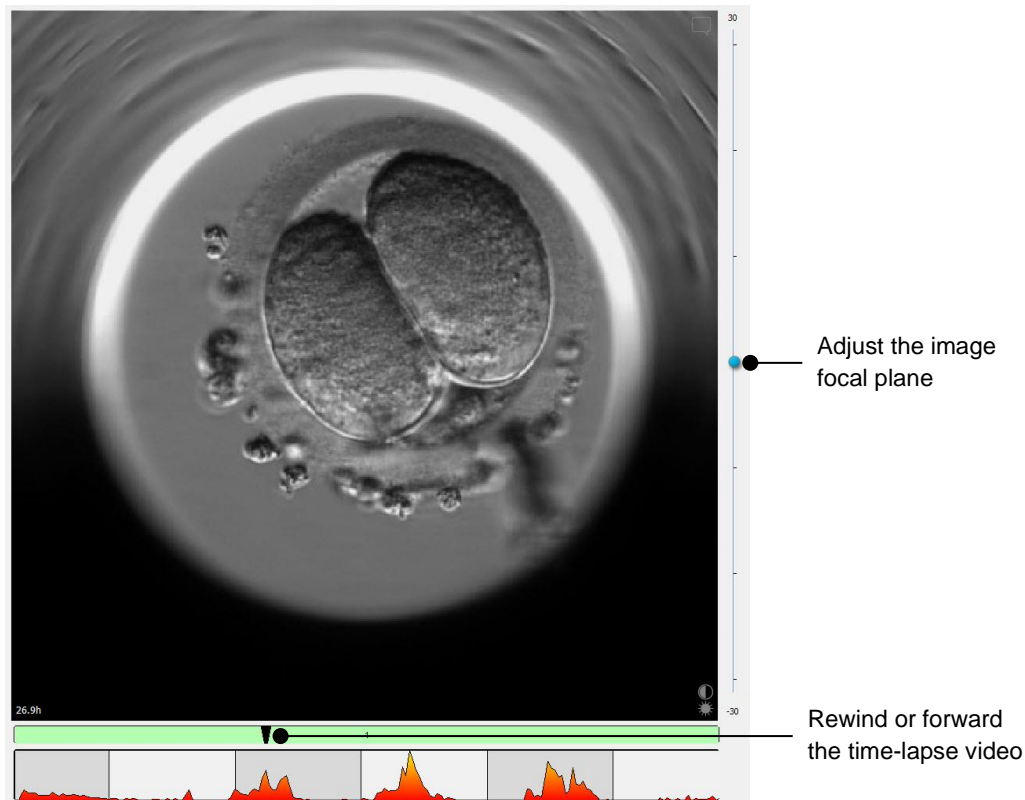
4.3   Performing annotations

Recommended workflow to annotate variables for embryos:

1. On the **View Running** page, select a running culture dish.

2. Go to the **Annotate** page.

   The page lists all culture dishes that are part of the selected treatment. The automatic image analysis will be active. Thus, for most cell division variables, the page will display estimates of timing and morphological parameters.

   The estimates may have different confidence levels in relation to the confidence threshold defined on the **Settings** page. The confidence level of each estimate is displayed as follows:

   The estimate is *below the confidence threshold* (i.e. less confident than the entered level):

   | t3 | *33.9 h* |

   (italics without boldface)

   The estimate is *on or above the confidence threshold* (i.e. as confident as or more confident than the entered level):

   | t2 | *24.2 h* |

   (italics in boldface)

   Estimates for one or more variables may not be displayed for a number of reasons, e.g.:

   - The variables have not yet occurred.
   - The Guided Annotation tool is not set to estimate the variables.
   - The Guided Annotation tool is unable to automatically estimate the variables.

   | tB | |

When you open the **Annotate** page, the division chart is based on estimated timings for embryo development stages. Once the annotations have been confirmed, the division chart also becomes available on the **View Slide** page and the **Compare & Select** page.

Confirm Estimates button to confirm all automatic estimates that are on or above the confidence threshold.

Automatic estimated timing for t2 on or above the confidence threshold

Prev and **Next** buttons to jump to the previous or next embryo in the selected culture dish.

3. From the **Strategy** field, select the desired annotation strategy:
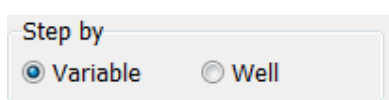

Select an annotation strategy

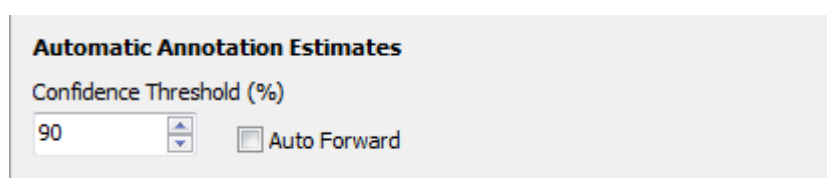| NOTE |
| --- |
| • Only the strategies that are marked as **Active** on the **Annotations** tab of the **Settings** page are available. |

4.   On day 1, select **Step by Well**:

5.   Then annotate PN for all wells in the treatment. Mark all embryos that are *not* 2PN to be avoided: .

6.   On the day of transfer/cryopreservation, go to the **View Slide** page and mark all embryos that have not developed as desired to be avoided: .

7.   Go to the **Annotate** page and select **Step by Variable**.

If the **Auto Forward** check box has been selected on the **Settings** page, the automatic workflow will not stop at estimates on or above the confidence threshold. You still need to confirm these estimates when the workflow is complete.

We recommend that you do not select the **Auto Forward** check box until you have gained some experience of working with the automatic image analysis.

8.   Annotate all remaining unconfirmed variables in the annotation strategy for one well at a time.

The Guided Annotation tool automatically activates the first well where the annotation is missing or has not been confirmed. Confirmed annotations appear in boldface:

9.   Confirm the estimates or, if required, adjust the automatically estimated value for each variable. Check whether the estimates are correct by forwarding and rewinding the time-lapse video and by changing the focal plane up and down.

WARNING
• For variables with a confidence estimate below the user-defined threshold, users must manually verify the estimates.

10. Use the relevant buttons or keyboard shortcuts to perform the annotations, or enter a numerical value if you are prompted to do so. For timing variables, you can press the Enter key to insert the timing represented by the current image.

WARNING
• For non-timing variables, the time points suggested by the Guided Annotation tool should ONLY be used as a starting point. Embryo developments and characteristics are often dynamic, and their final annotation should therefore be based on a period rather than a single point in time.

11. Click the **Confirm** button when you have evaluated the annotations performed for the current embryo:
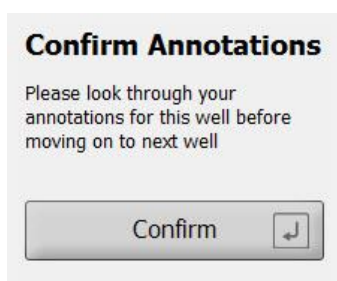

The Guided Annotation tool now automatically proceeds to the next non-annotated well and forwards the video to the estimated timing of the first variable in the annotation strategy.

12. Continue annotating all wells. The progress is displayed next to each well:

All annotations have been completed for well AA-1

12% of the annotations have been completed for wells AA-3 – AA-5

Well AA-6 is marked to be avoided for transfer

Empty wells

13.　　*Optional step:*

If one of the variables included in the annotation strategy is irrelevant to a specific embryo or you are unable to annotate the variable, click the **Not Applicable** button:
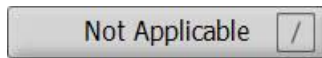

14.　　*Optional step:*

a. Press * on the keyboard to go to the **Comments** field and add a comment to an image.

b. If the comment has already been created as a user-defined comment, click the text and press Enter to insert it.


The comment will be displayed together with the associated image when you click the comments icon in the top right corner of the image:

Comments icon. Activated when you have entered a comment on the image. Click to display the comment.


Comment indicator　　　Open comment
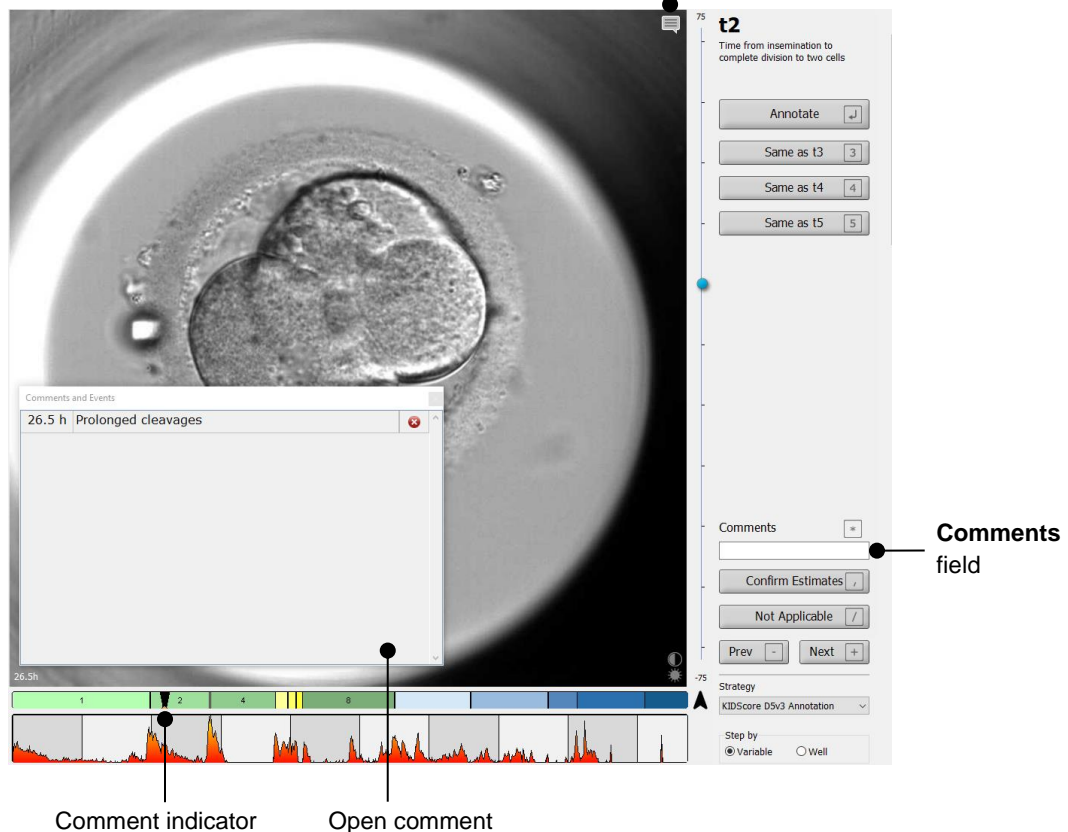
All comments are represented by a red indicator on the division chart. When you select a comment in the **Comments and Events** box, the video will automatically jump to the time point in question.

When you have opened the comment, it will not automatically close. To close the comment box, click the close icon in the top right corner:

Close comment
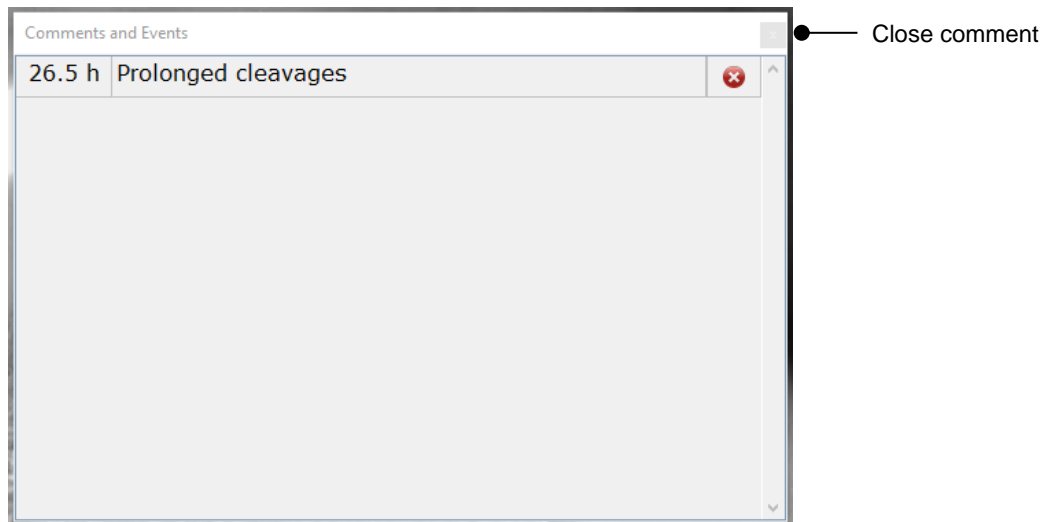
15. *Optional step:*

    Delete a comment by clicking the delete icon next to the selected comment:

Delete comment
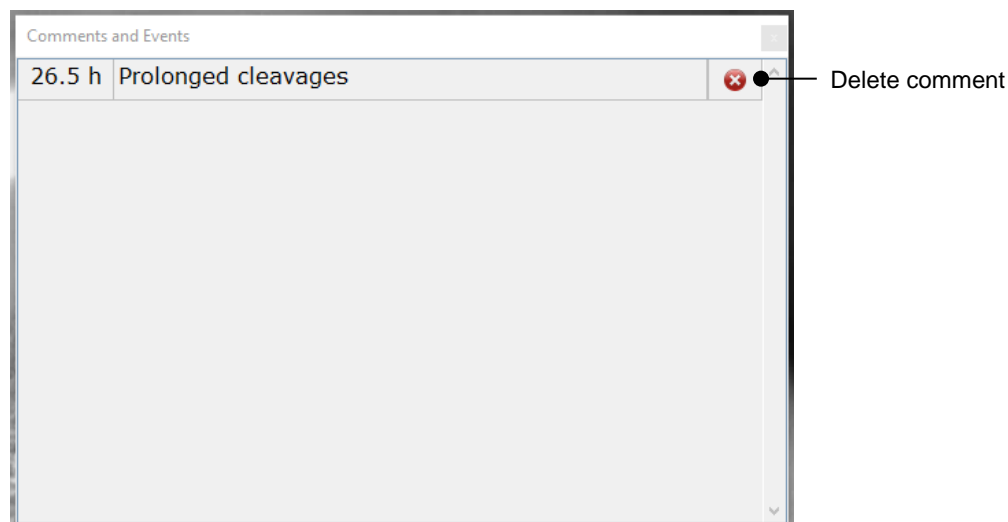
16. *Optional step:*

    Go to the **Compare & Select** page and apply the desired model to the annotated embryos in order to score the embryos and decide which ones to freeze and transfer:


17. Click **Save**.

4.3.1 Deactivating automatic forwarding of the time-lapse video to estimated timings

In some cases, you may find it easier to annotate the embryos by manually forwarding the time-lapse video to a certain point in time. This could e.g. be the case when an embryo does not follow a normal development pattern.

To stop automatically forwarding the video to estimated timings, either press ESC on the keyboard or click the forward video icon once:
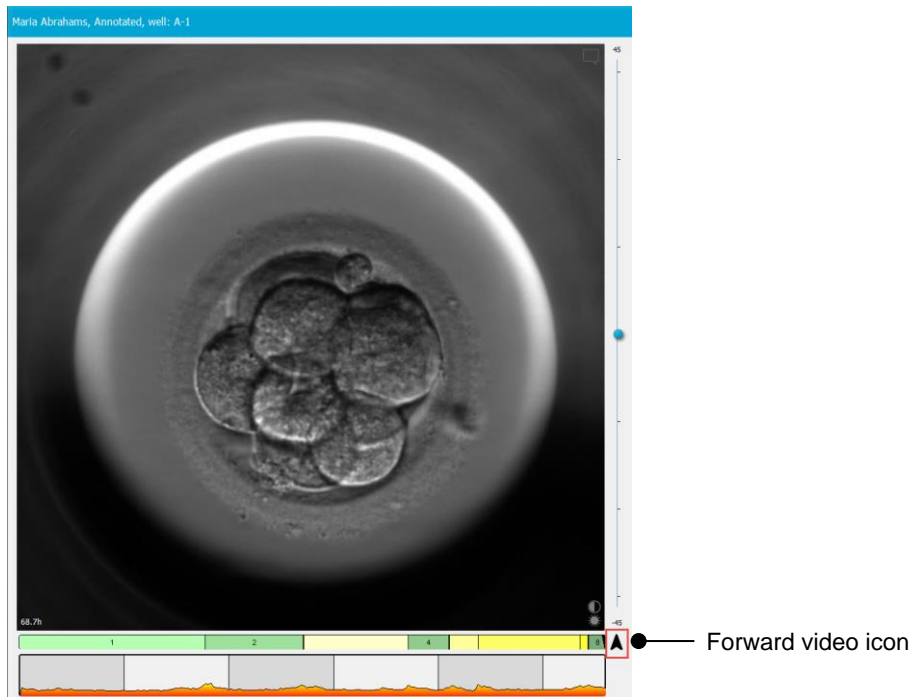
Forward video icon

When the forward video function has been deactivated, the icon will appear with a red cross, and an information message will temporarily appear on the screen:

Time suggestions disabled

The forward video function will automatically be re-activated when you continue to the next embryo.

5 Symbols and labels

Label	Description	Note
CE	Declaration by the manufacturer that the device meets all of the applicable requirements in the Medical Device Regulation (EU) 2017/745	-
MD	Medical device	-
UDI	Unique device identifier	-
(manufacturer symbol)	Manufacturer name and address	See section 7.

6 Disposal of waste

In order to minimise the waste of electrical and electronic equipment, waste must be disposed in accordance with the Directive 2012/19/EU on waste electrical and electronic equipment (WEEE) as amended by Directive (EU) 2018/849. This includes: PCBs (lead-free HASL), switches, PC batteries, printed circuit boards and external electrical cables. All components are in accordance with the RoHS 2 Directive 2011/65/EU, which states that new electrical and electronic components do not contain lead, mercury, cadmium, hexavalent chromium, polybrominated biphenyls (PBB) or polybrominated diphenyl ethers.

7 Contact information

Urgently need help? Call our service hotline for support:

+45 7023 0500

(available 24 hours a day, 7 days a week)

E-mail support: support.embryoscope@vitrolife.com

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