EMBO Practical Course on Light Sheet Microscopy

Registration Techniques

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Motivation

Registration, that you have seen so far (in Stephans talk this morning)

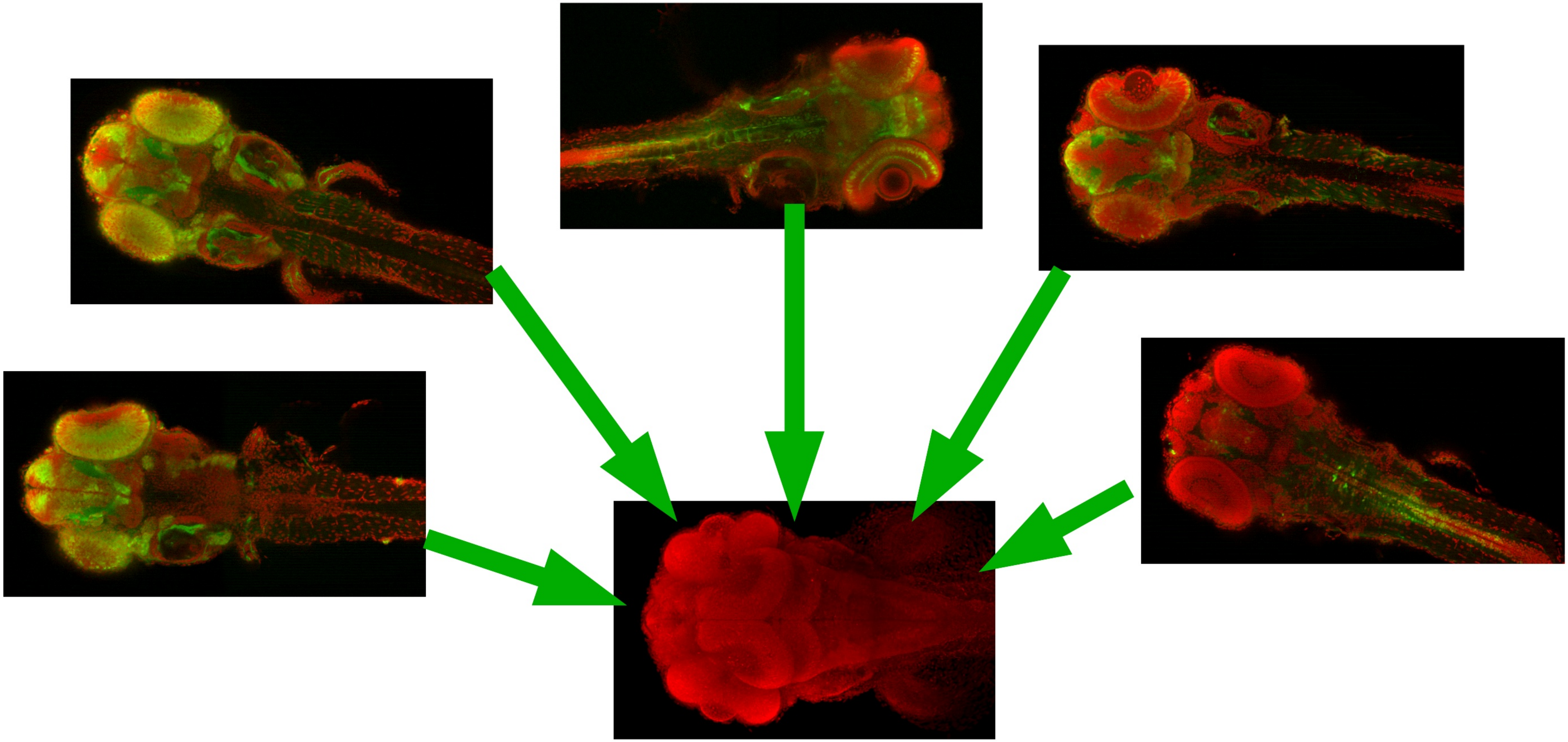
- All images taken from the **same individual**
- **Feature**-based registration using **point-to-point correspondences** (bead or nuclei positions)
- **Rigid/Affine** transformation

What I will show

- Images from **different individuals**
- Mainly **intensity-based** registration
- **Elastic** transformation

Elastic Registration

Map Patterns from Many Individuals to a Common Atlas

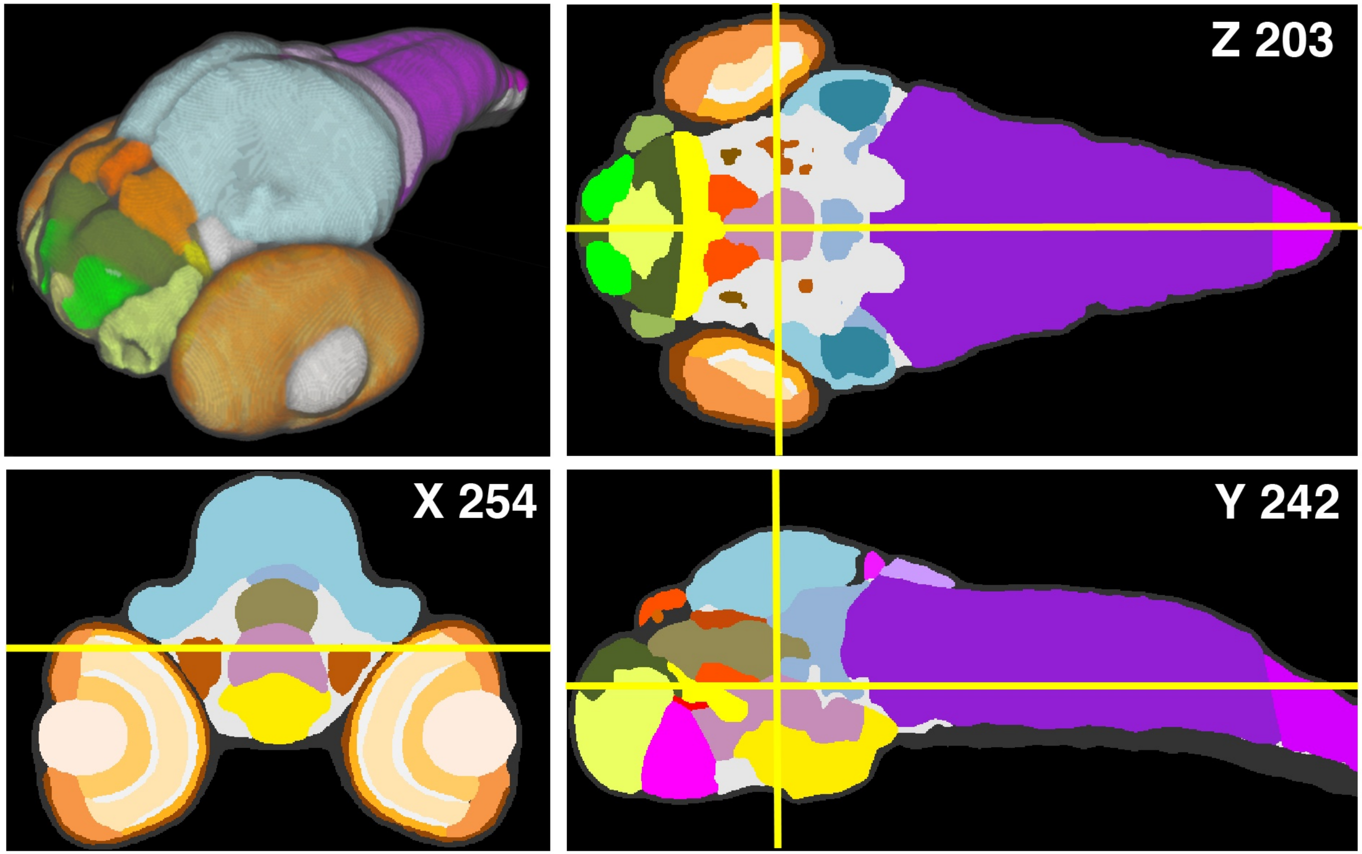


Standard Brain

O. Ronneberger, K. Liu, M. Rath, D. Rueß, T. Mueller, H. Skibbe, B. Drayer, T. Schmidt, A. Filippi, R. Nitschke, T. Brox, H. Burkhardt, W. Driever:
ViBE-Z: A Framework for 3D Virtual Colocalization Analysis in Zebrafish Larval Brains. **Nature Methods, 9(7): 735-742, 2012**

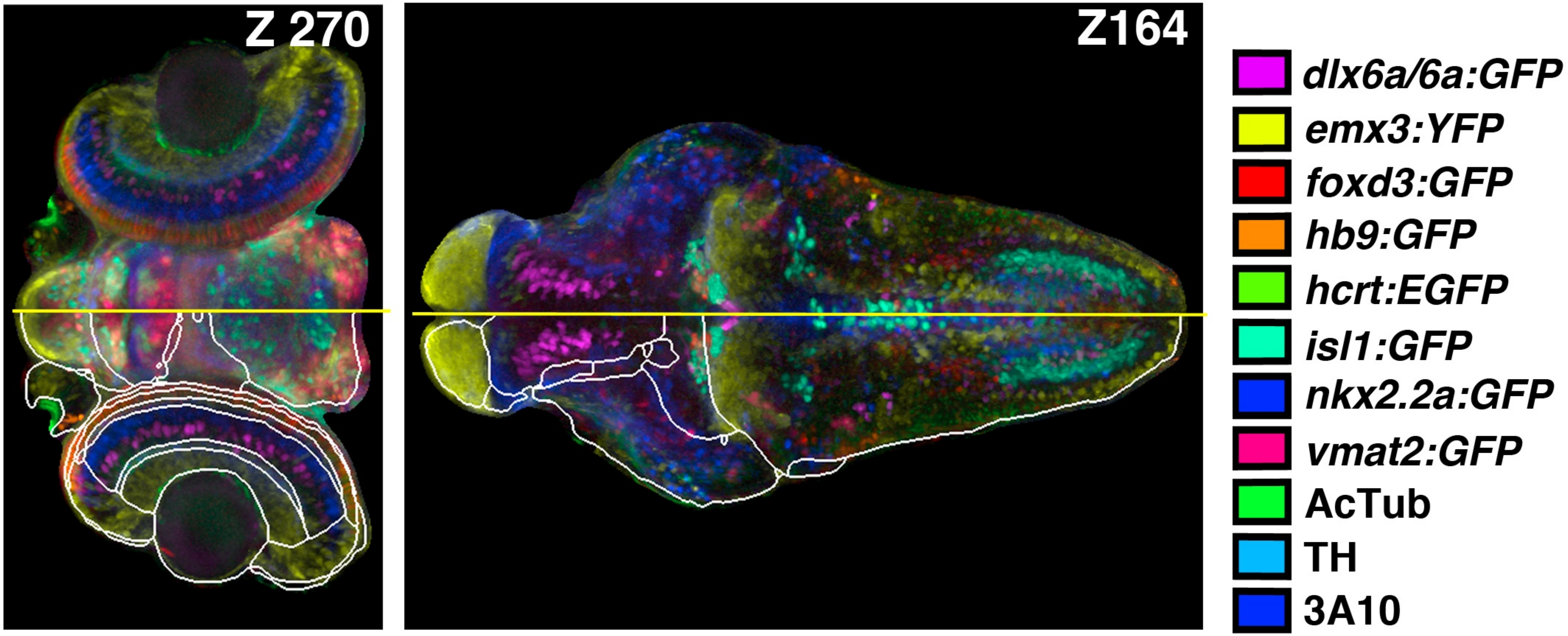
Virtual Colocalization Studies

Manual Segmentation of Reference Brain

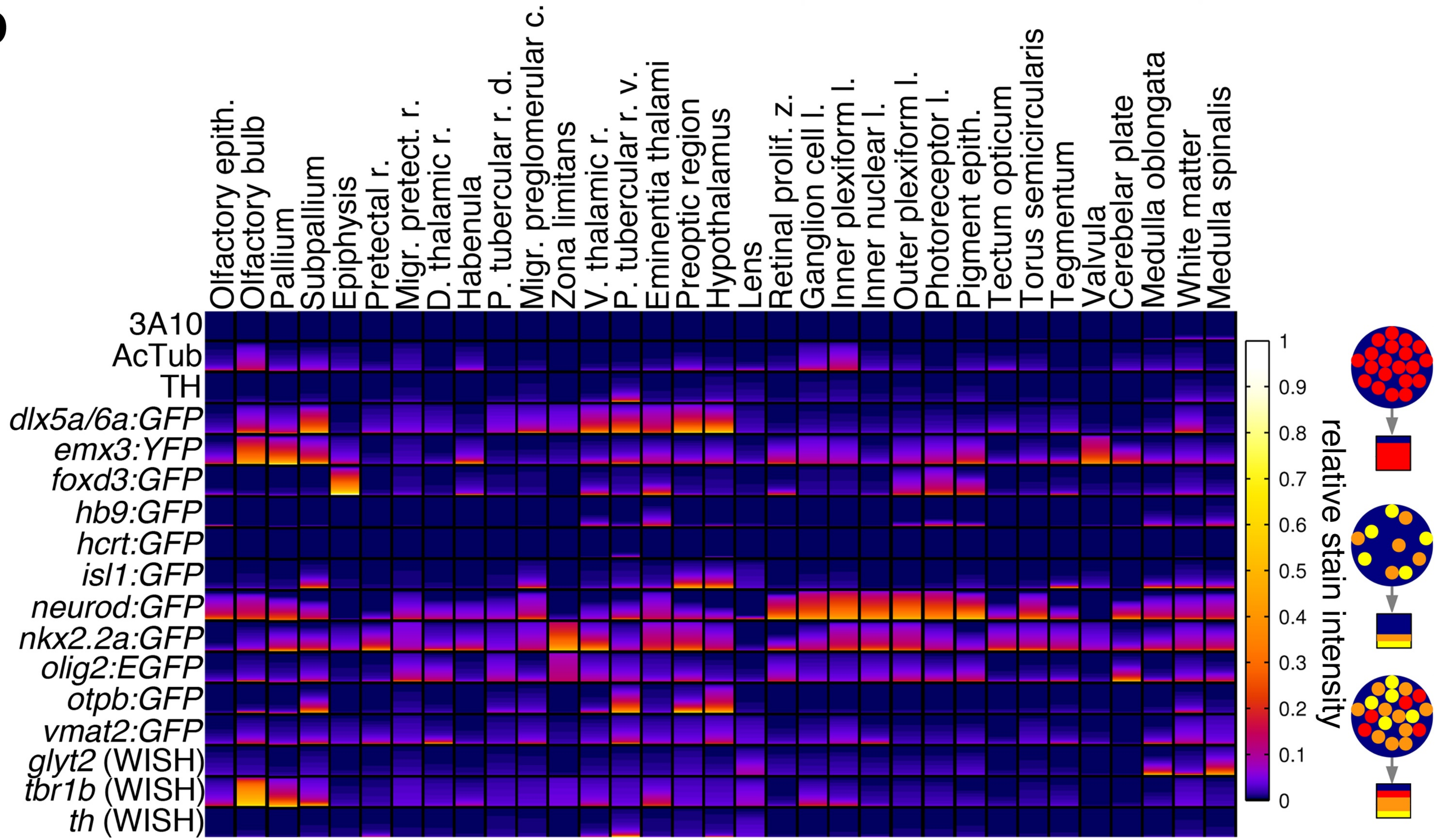


- olfactory epithelium
- olfactory bulb
- pallium
- subpallium
- epiphysis
- habenula
- pretectal region
- dorsal thalamic region
- posterior tubercular region dorsal
- posterior tubercular region ventral
- preglomerular complex
- zona limitans intrathalamica
- ventral thalamic region
- eminentia thalami
- preoptic region
- hypothalamus
- tectum opticum
- torus semicircularis
- tegmentum
- lens
- retinal profiferative zone
- ganglion cell layer
- inner plexiform layer
- inner nuclear layer
- outer plexiform layer
- photoreceptor layer
- pigment epithelium
- valvula
- cerebellar plate
- medulla oblongata
- medulla spinalis
- white matter

Automated Anatomical Assignment



Automated Anatomical Assignment



Elastic Registration

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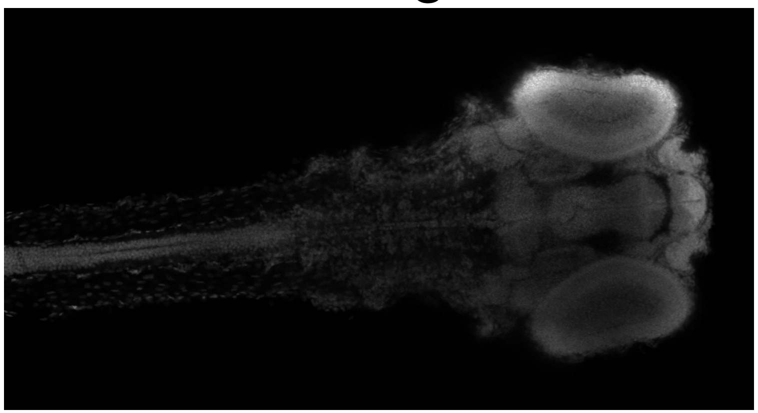
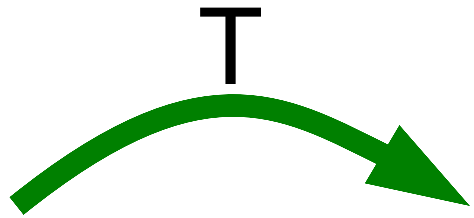
CENTRE FOR BIOLOGICAL SIGNALLING STUDIES

- Use the anatomy channel to **find the transformation**
- Use found transformation to **transform the pattern channel**

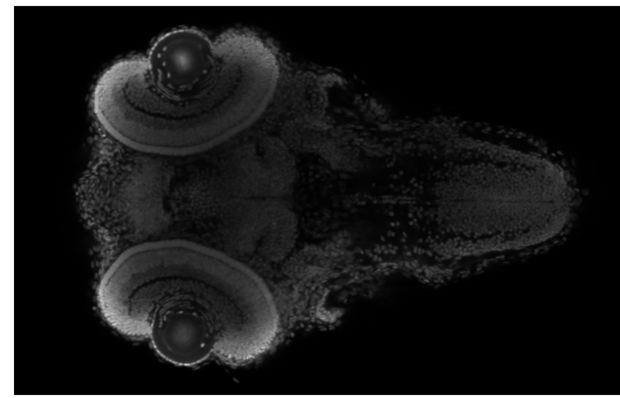
New Recording



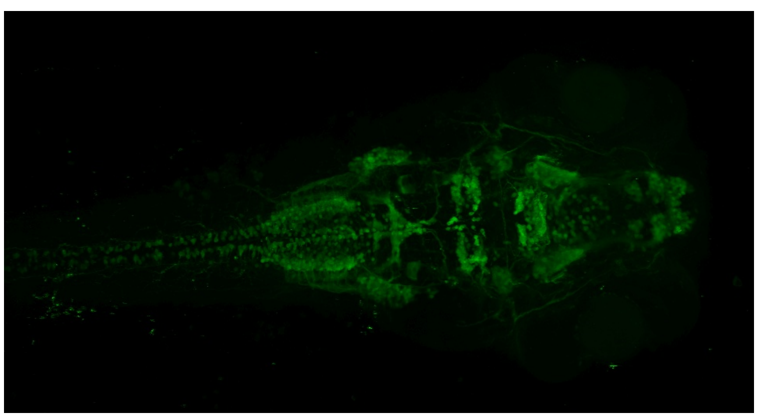
Anatomy channel



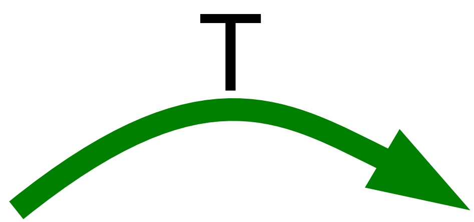
Reference (Atlas)

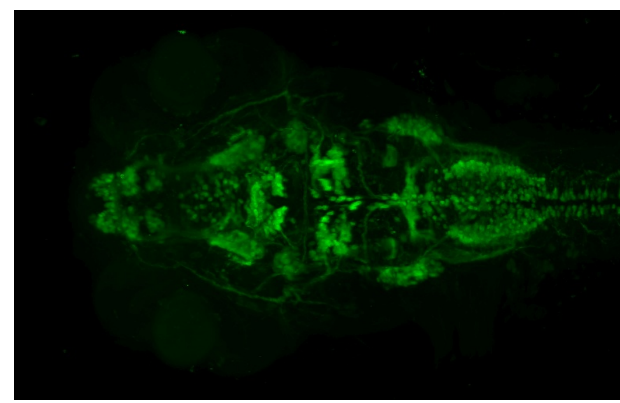


Anatomy channel



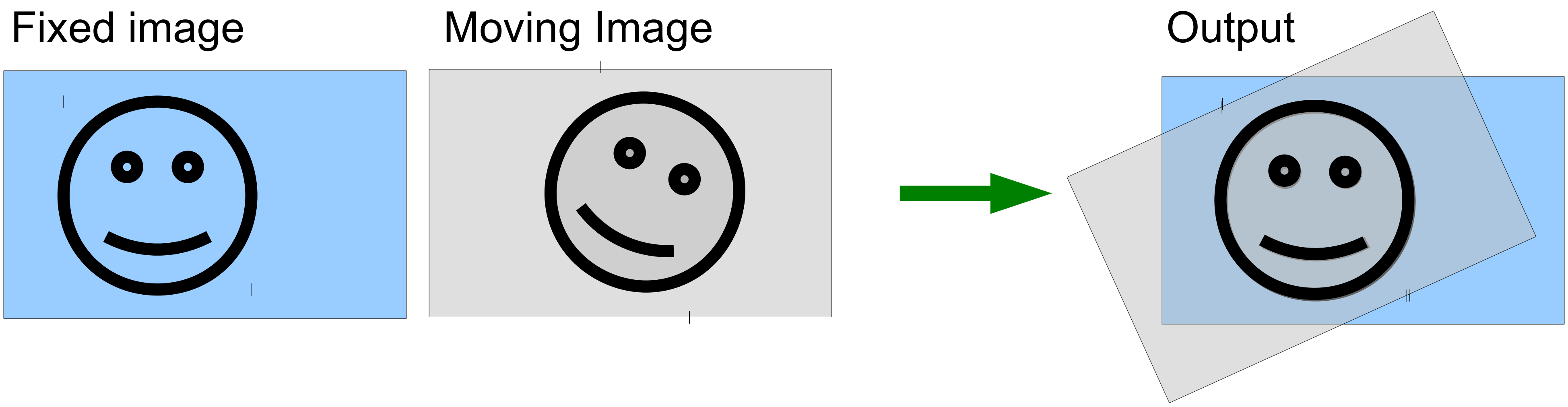
Pattern channel





Pattern channel (aligned to atlas)

Registration Definition: Bring two images into spatial alignment



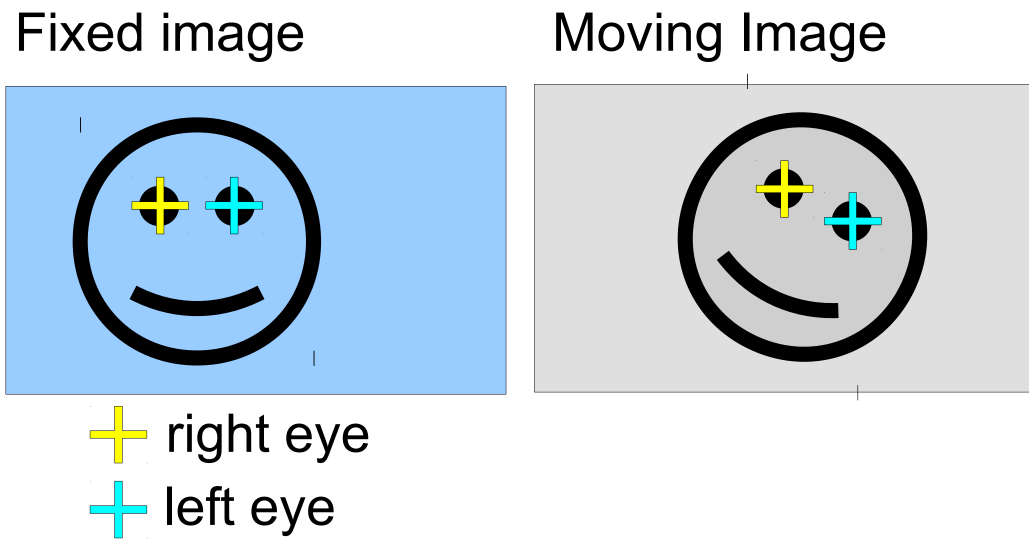
More technical:

- One **"fixed image"** and one **"moving image"**
- Find optimal **transformation parameters**, such that the "moving image" matches best the "fixed image"
- Output:
  - ➢ **Transformation parameters**
  - ➢ **Transformed moving image**

Registration Categories

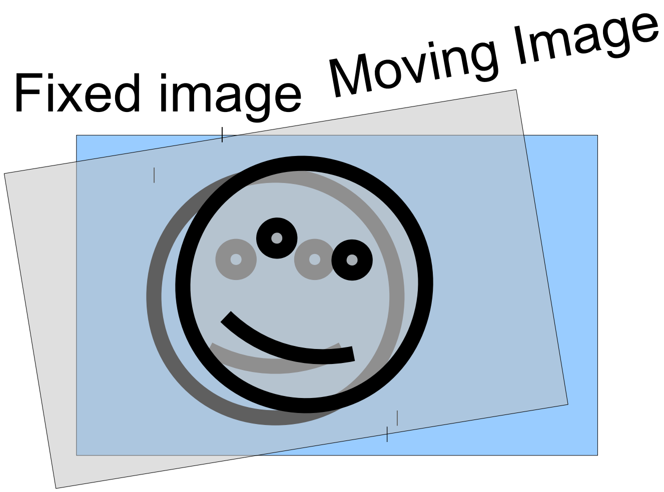
Feature based Registration

- use manually or automatically detected **landmarks**
- compute transformation, such that "moving" and "fixed" **landmarks are placed onto each other**

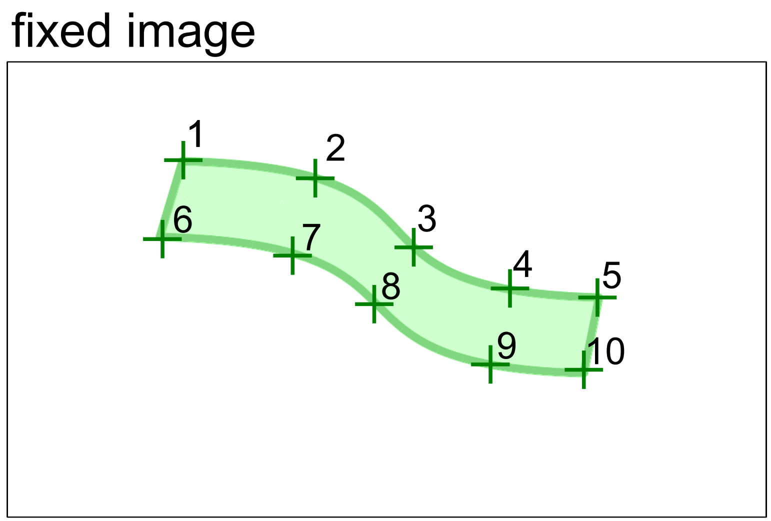


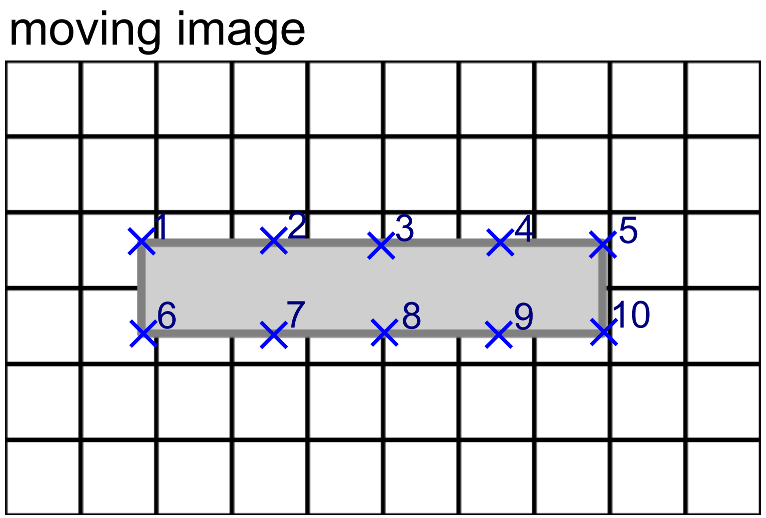
Intensity based Registration

- Iteratively transform moving image and **compare image intensities** to find optimal alignment

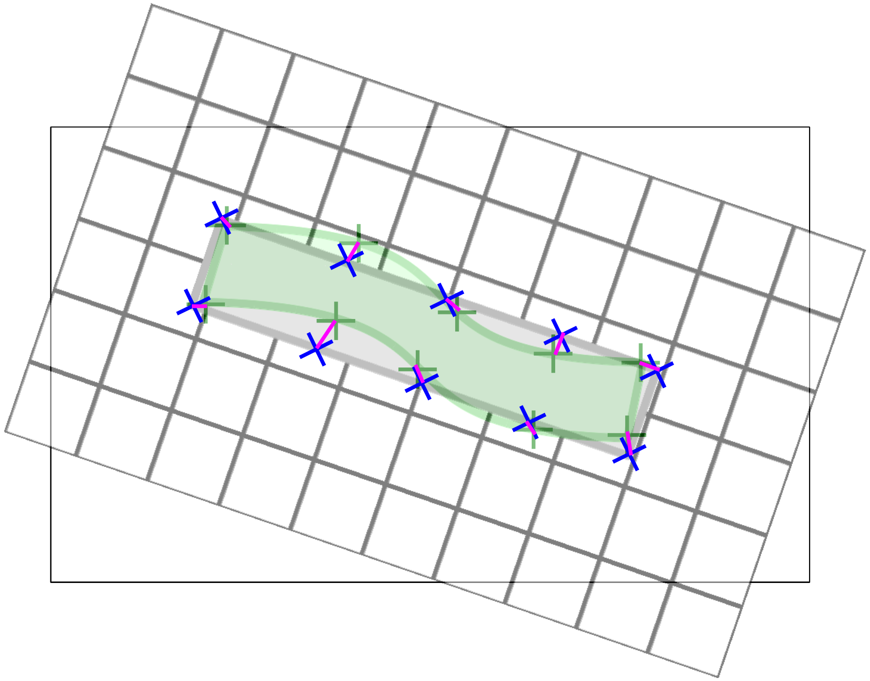


Landmark Based Registration



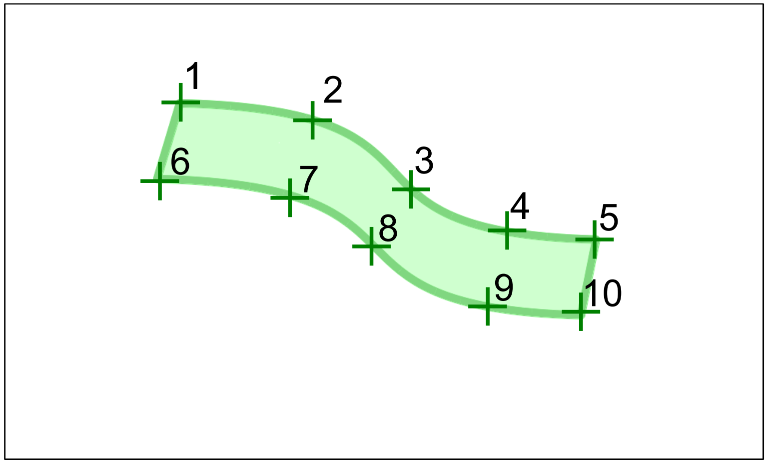


Rigid transformation:
minimize the **squared distance** between corresponding landmarks

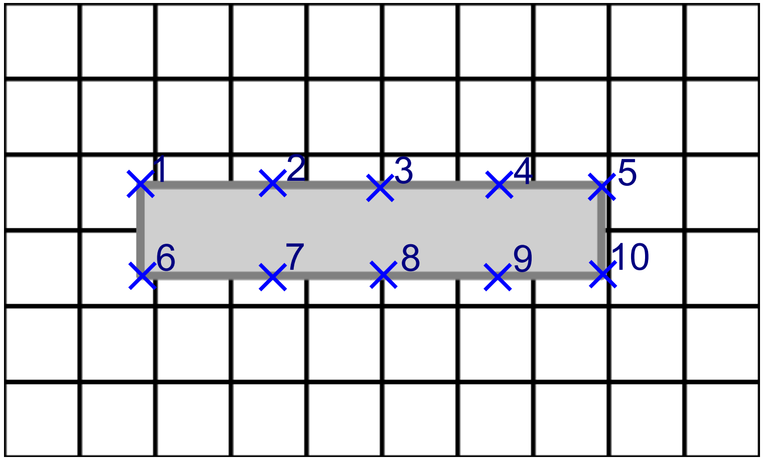


Landmark Based Registration

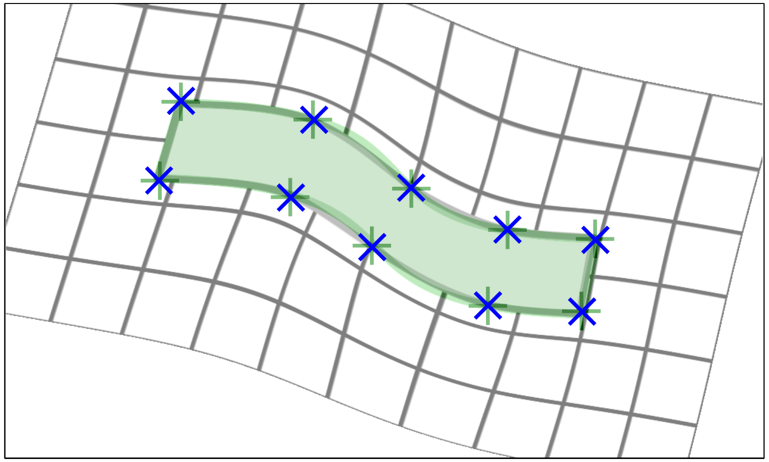
fixed image



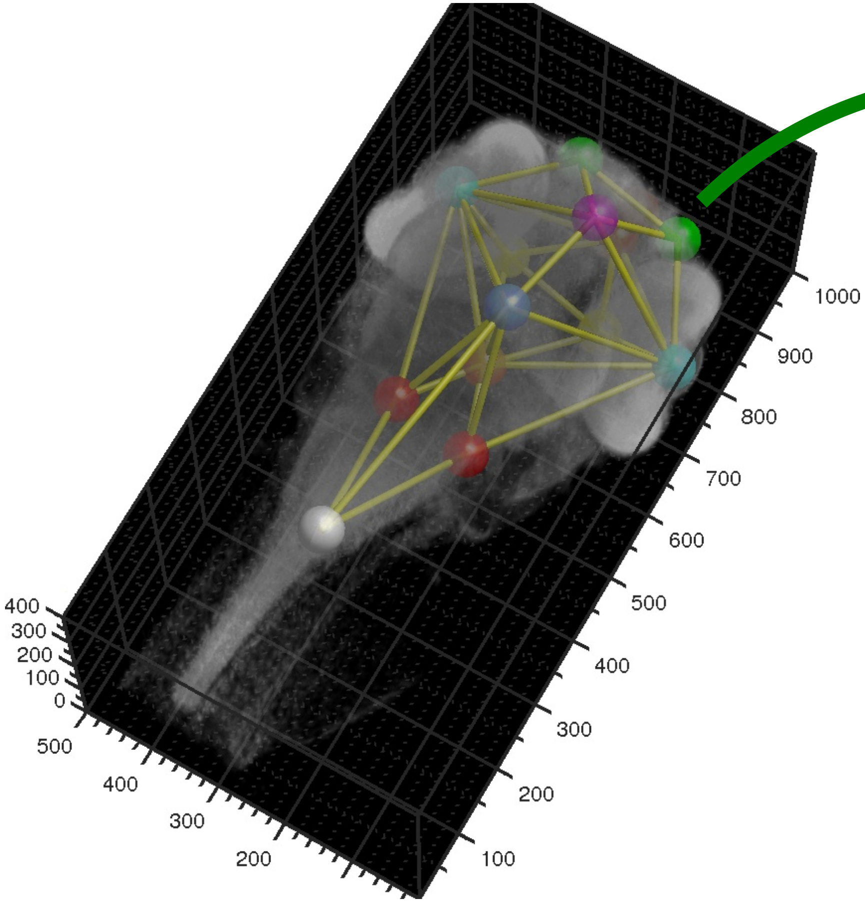
moving image



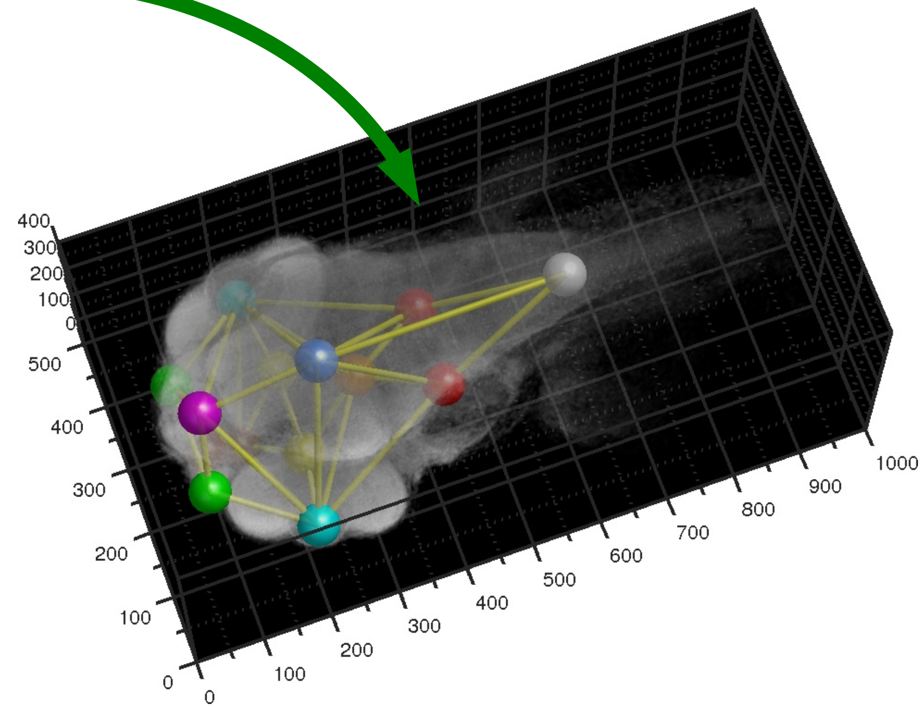
Elastic Transformation (here Thin-plate Splines):
deform image, such that
landmarks match exactly



Thin-Plate Splines (TPS) Example

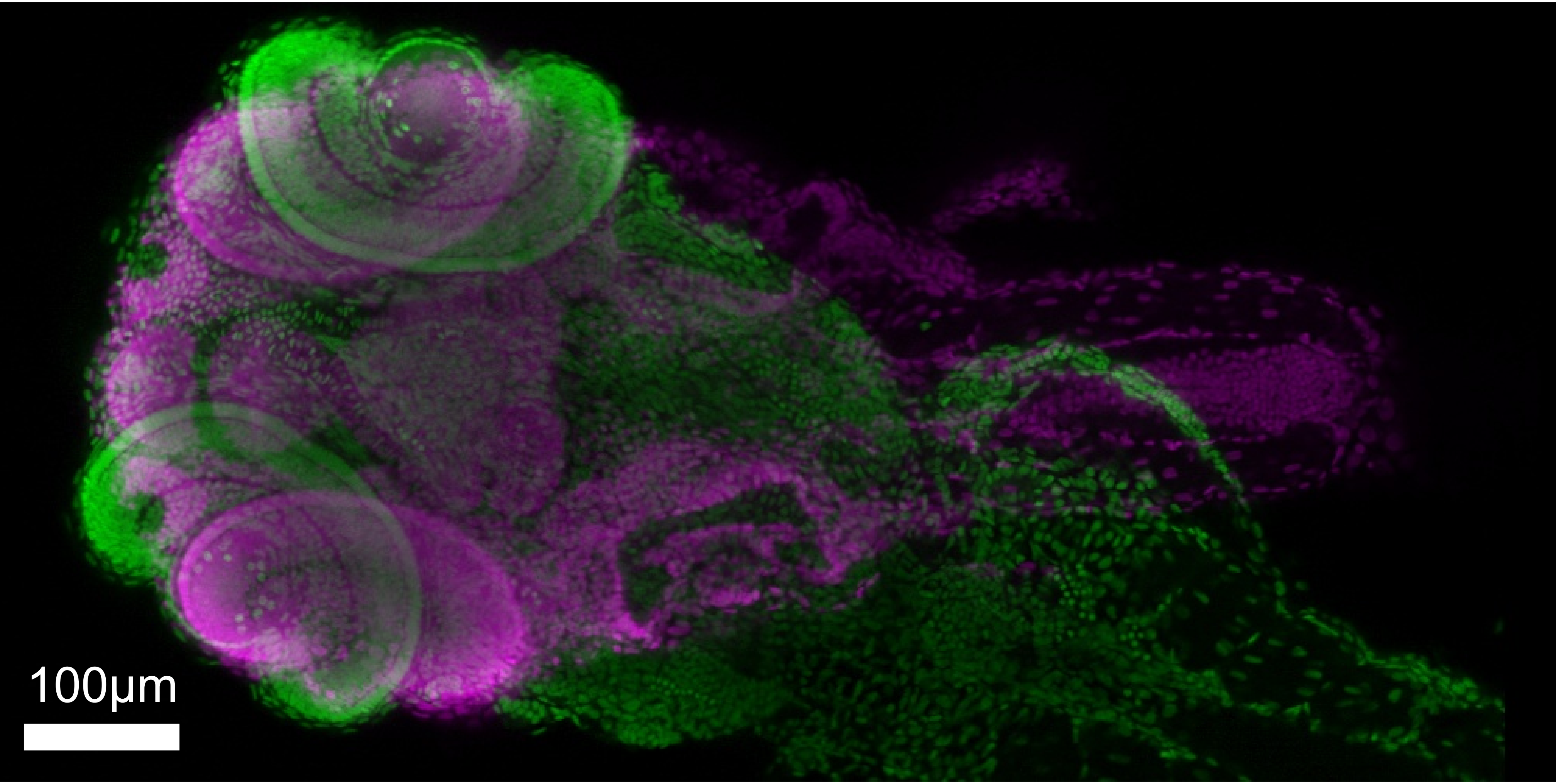


new larva with landmarks



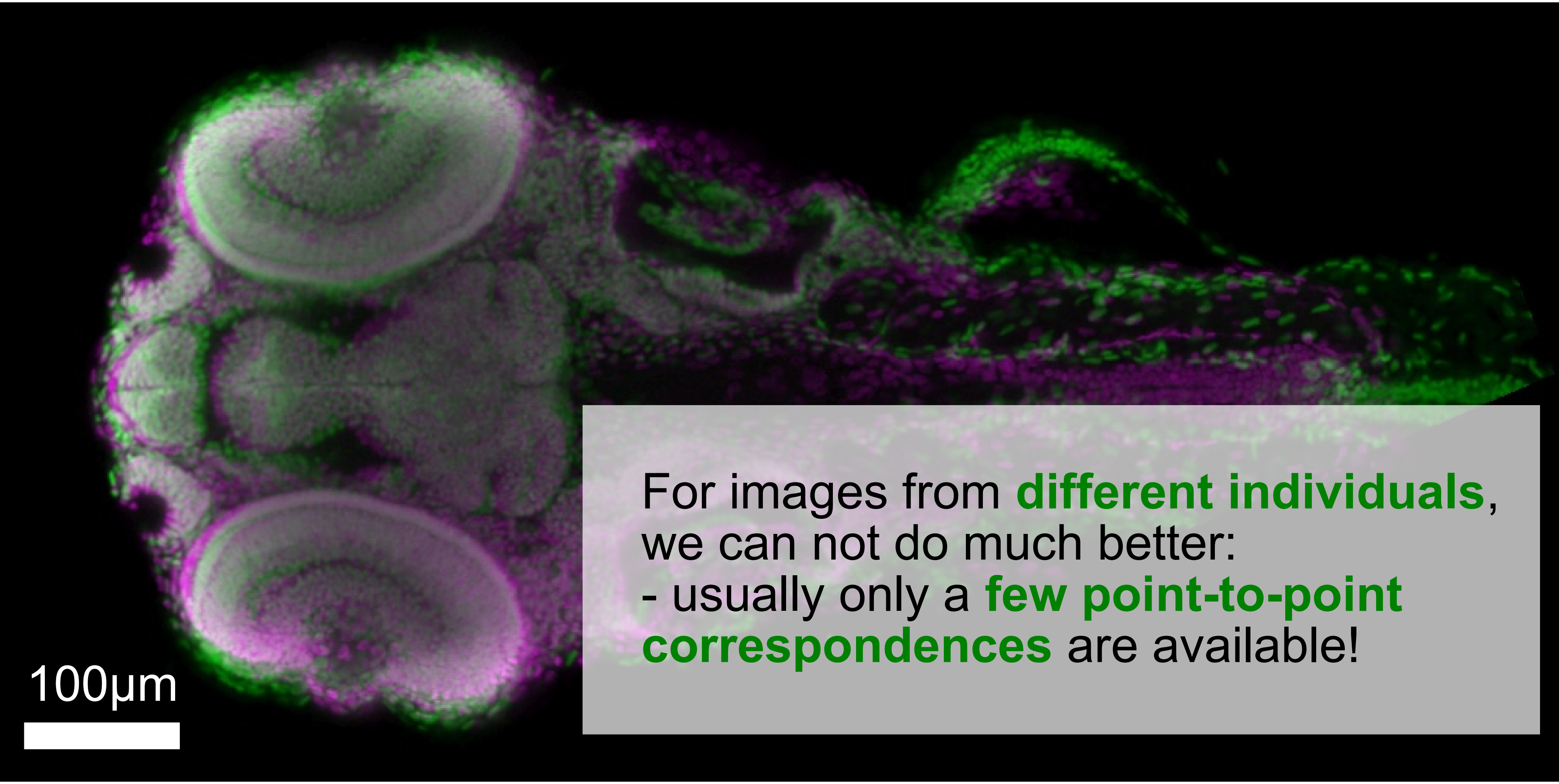
reference larva with landmarks

Raw Overlay



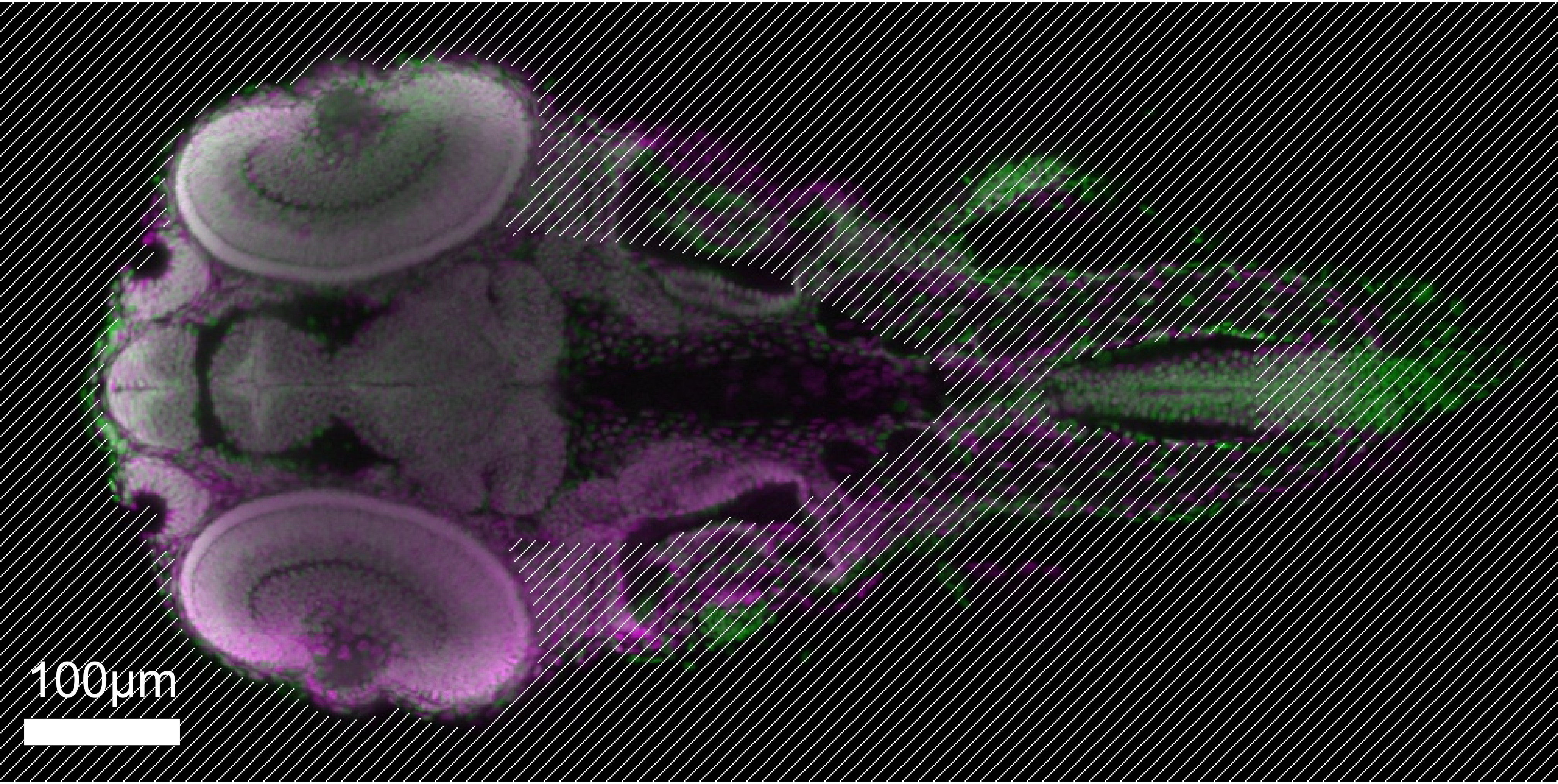
Central slice. magenta: reference larva, green: new larva

After Landmark-Based Elastic Registration



Central slice. magenta: reference larva, green: new larva

After Intensity Based Elastic Registration

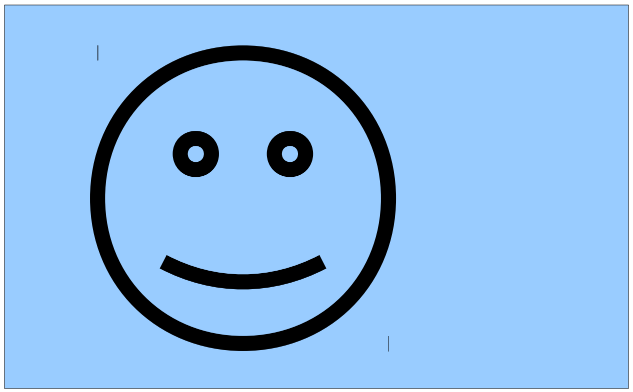


Central slice. magenta: reference larva, green: new larva
only brain region is considered

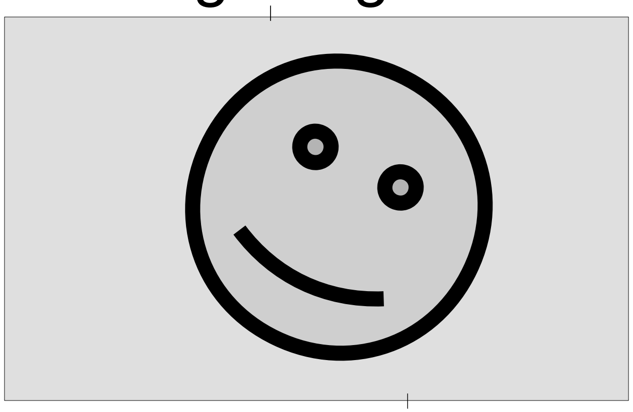
Intensity based Registration

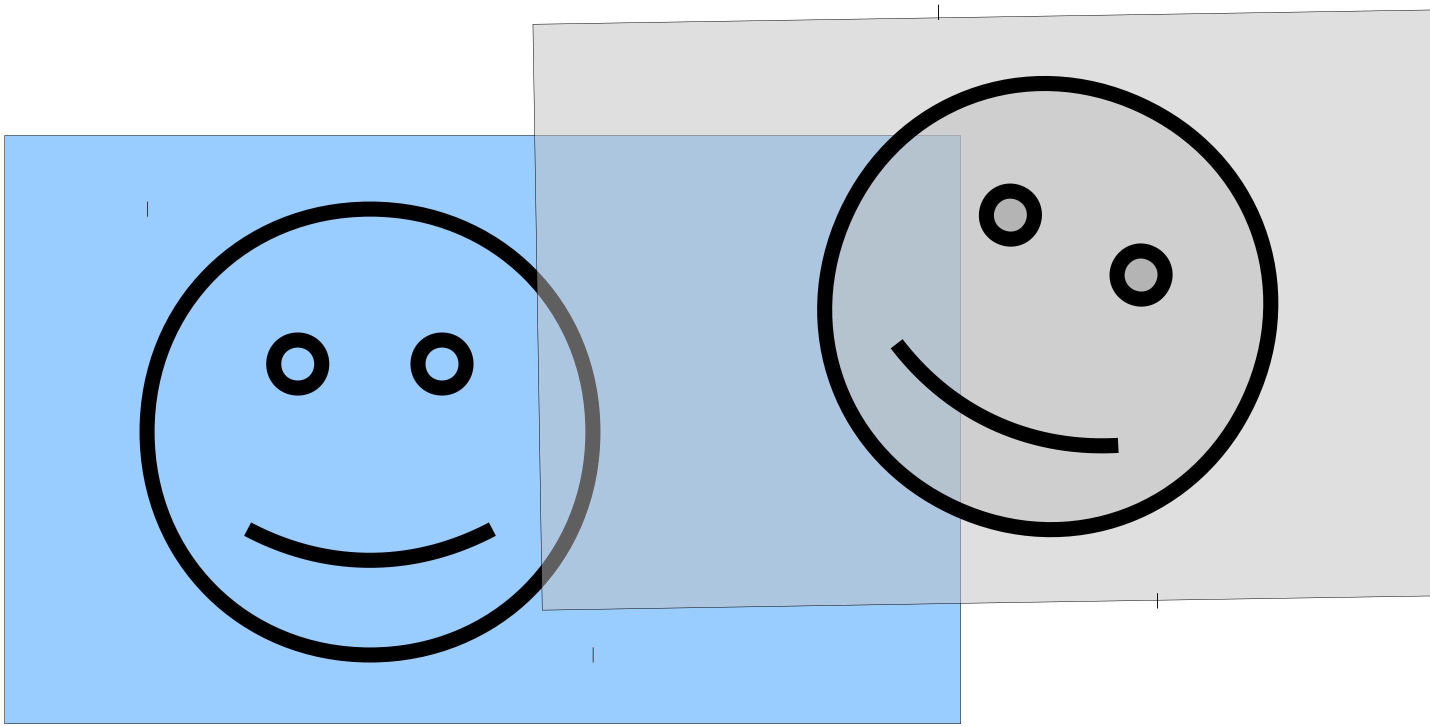
How does a human register two images?

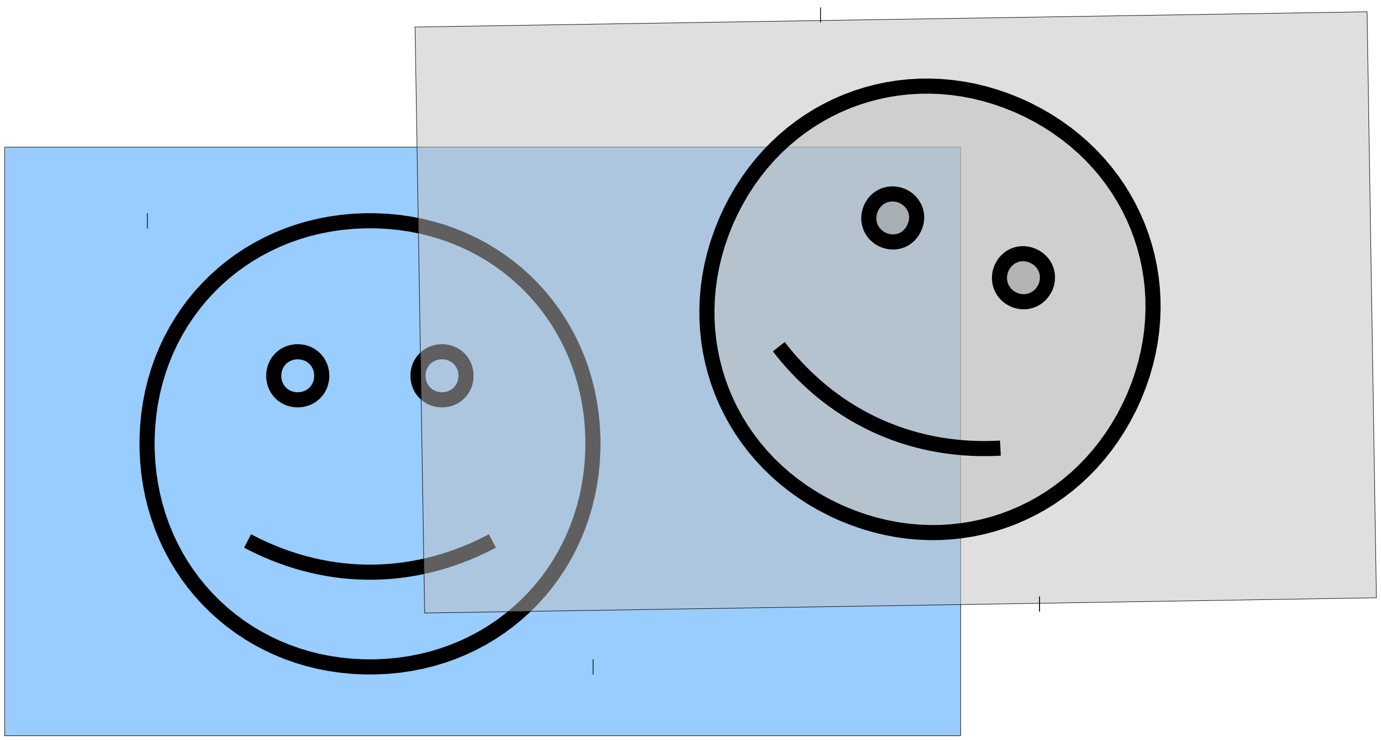
Fixed image

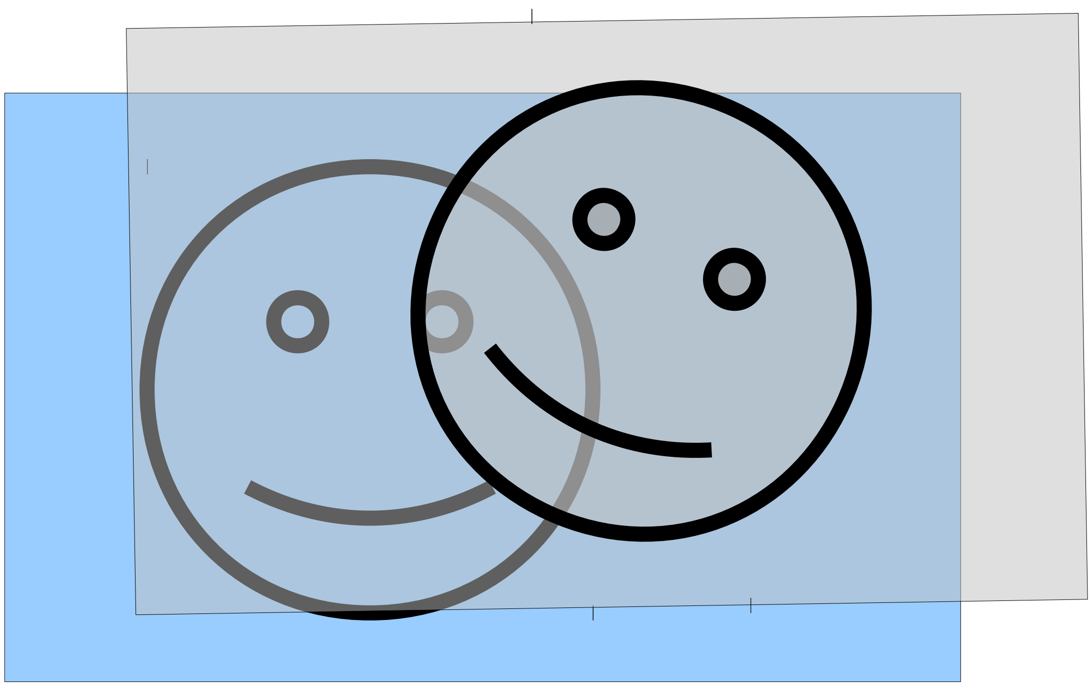


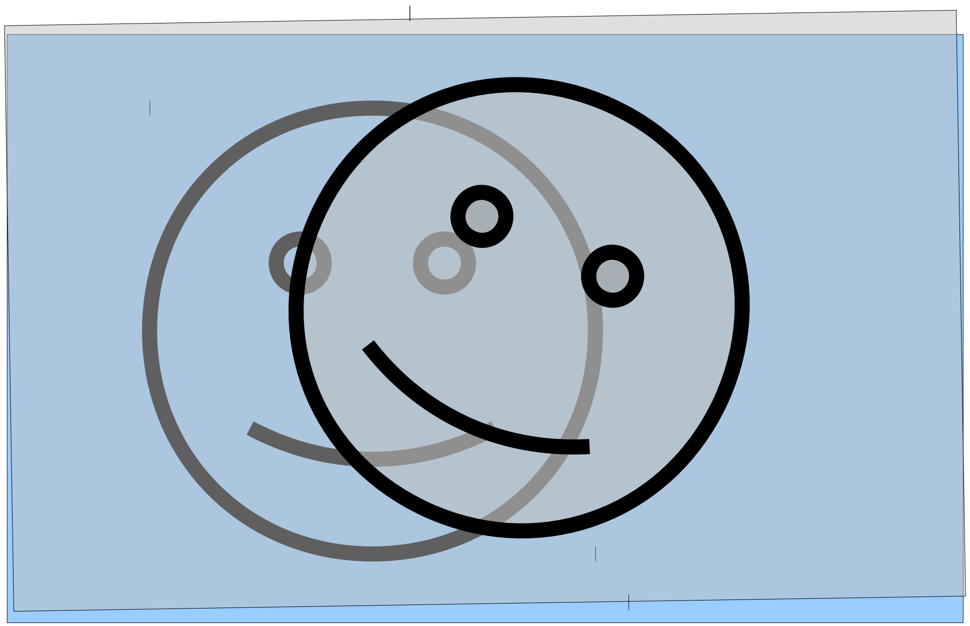
Moving Image on transparent slide

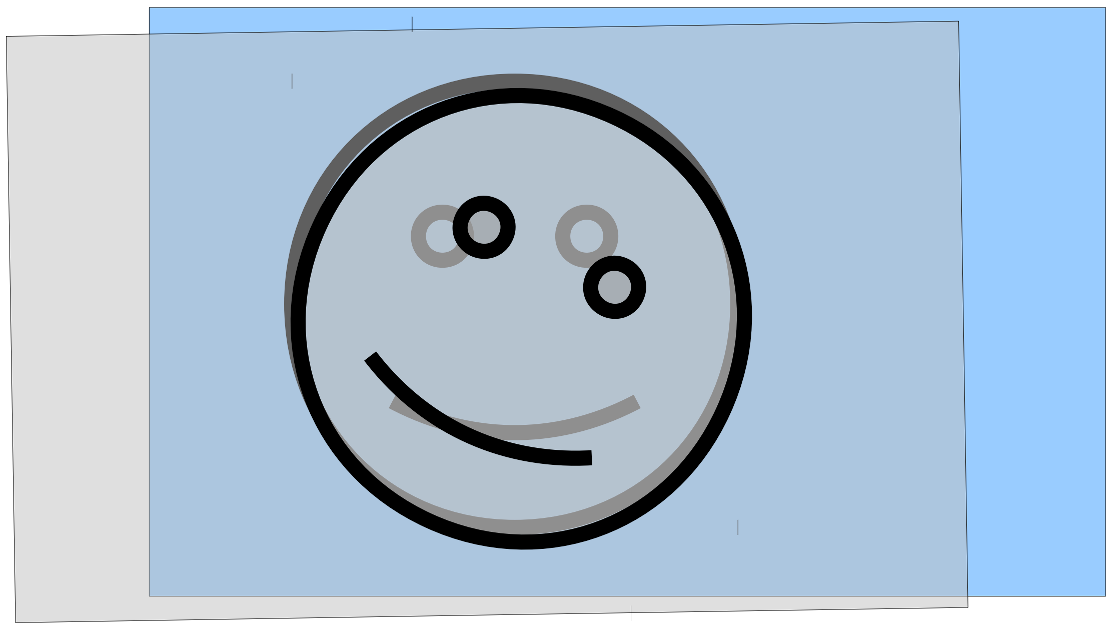


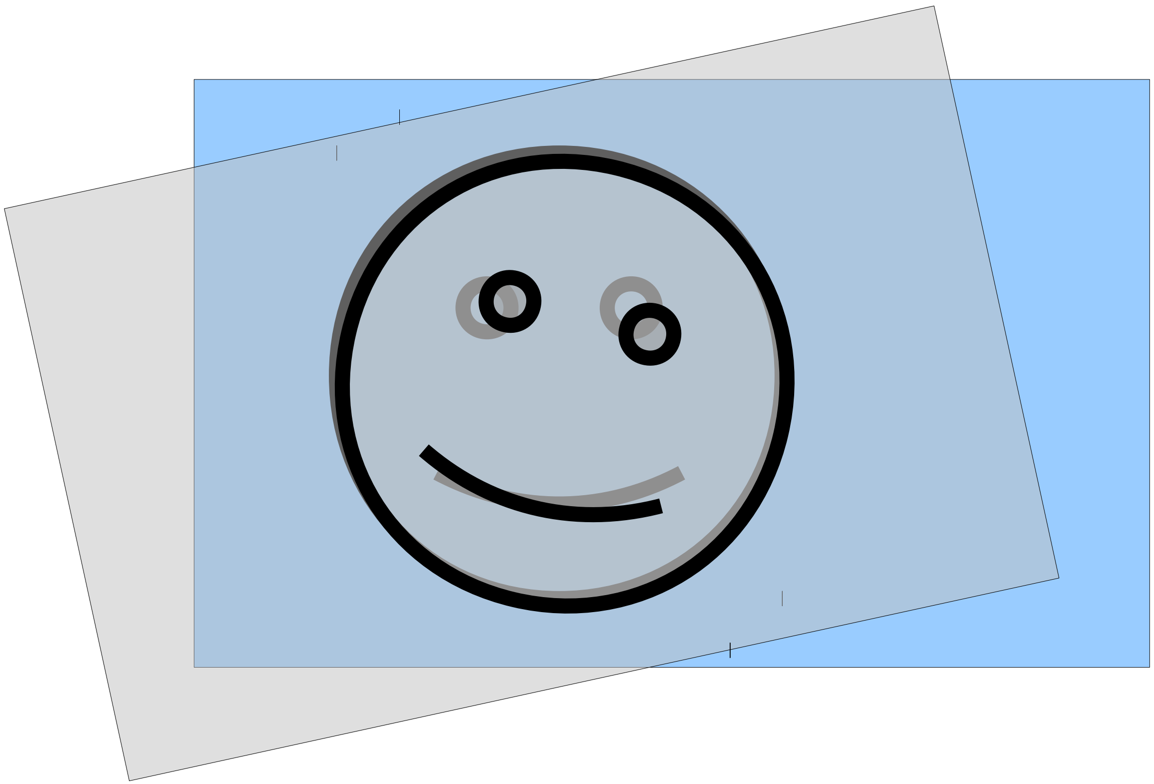


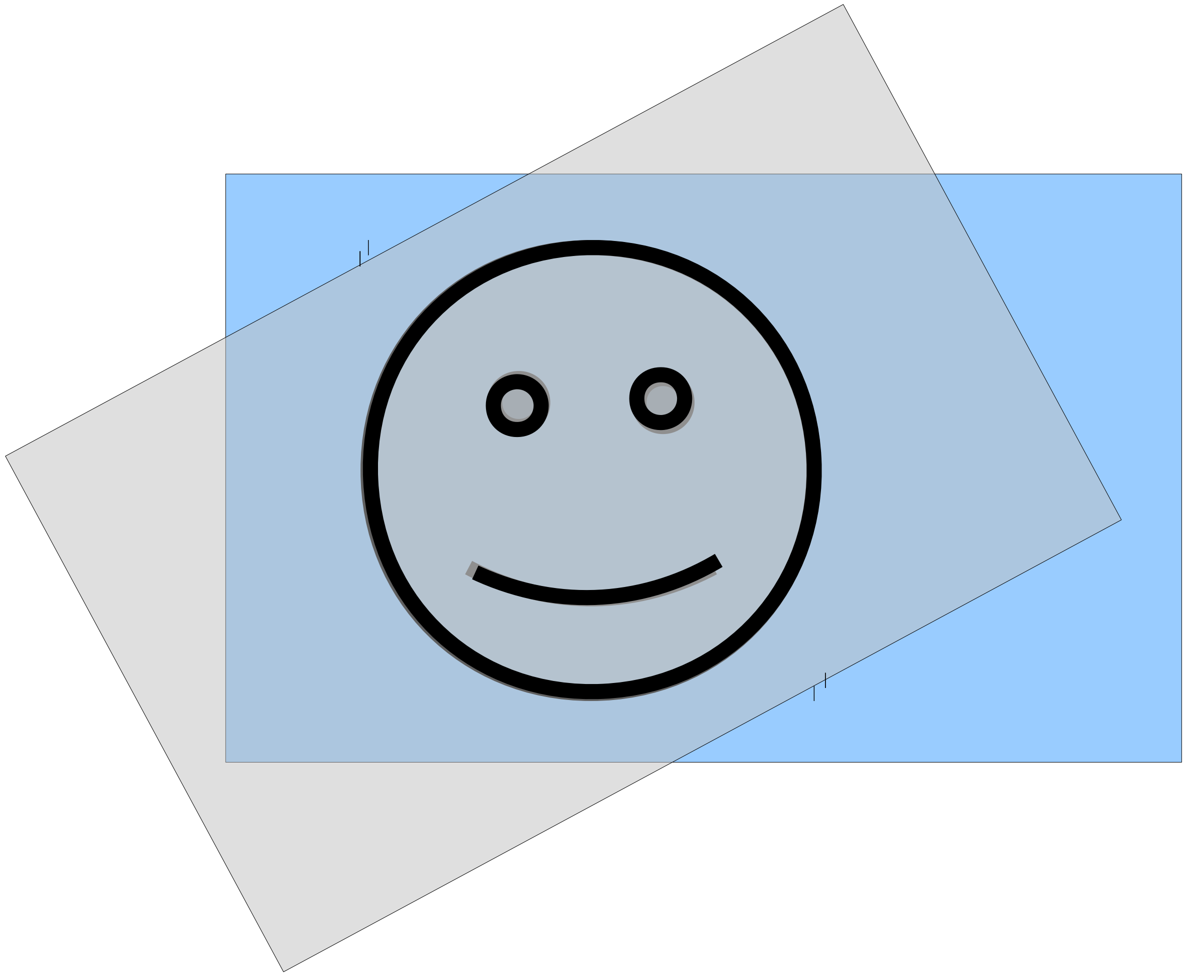








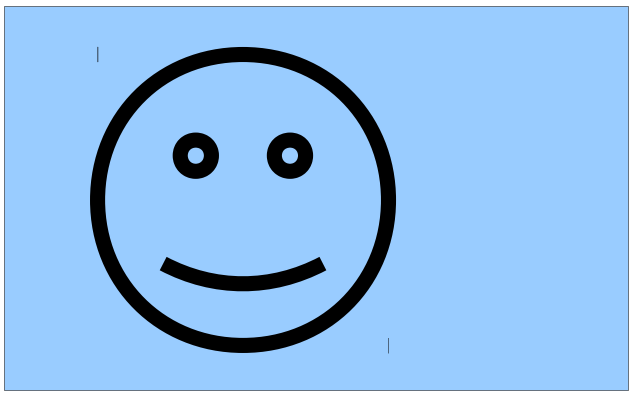




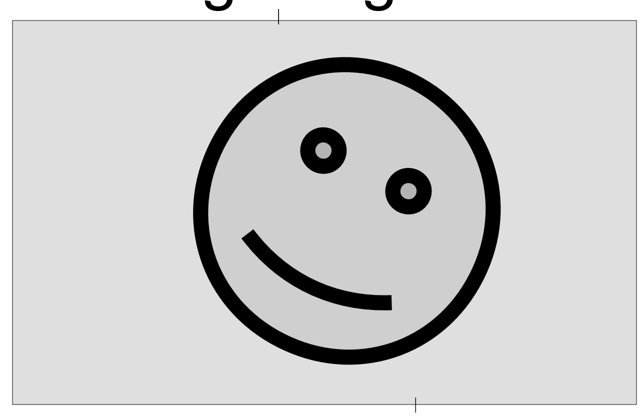
Parts of a Registration Algorithm

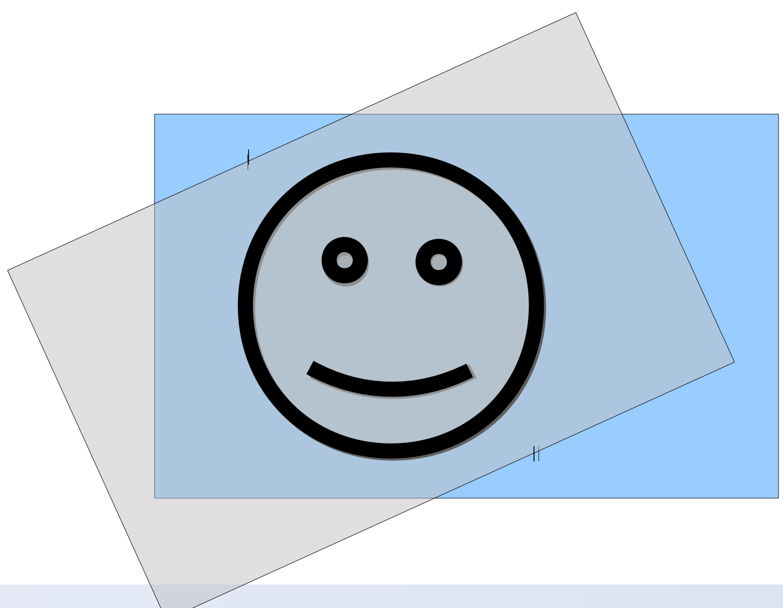
How does a human register two images?

Fixed image



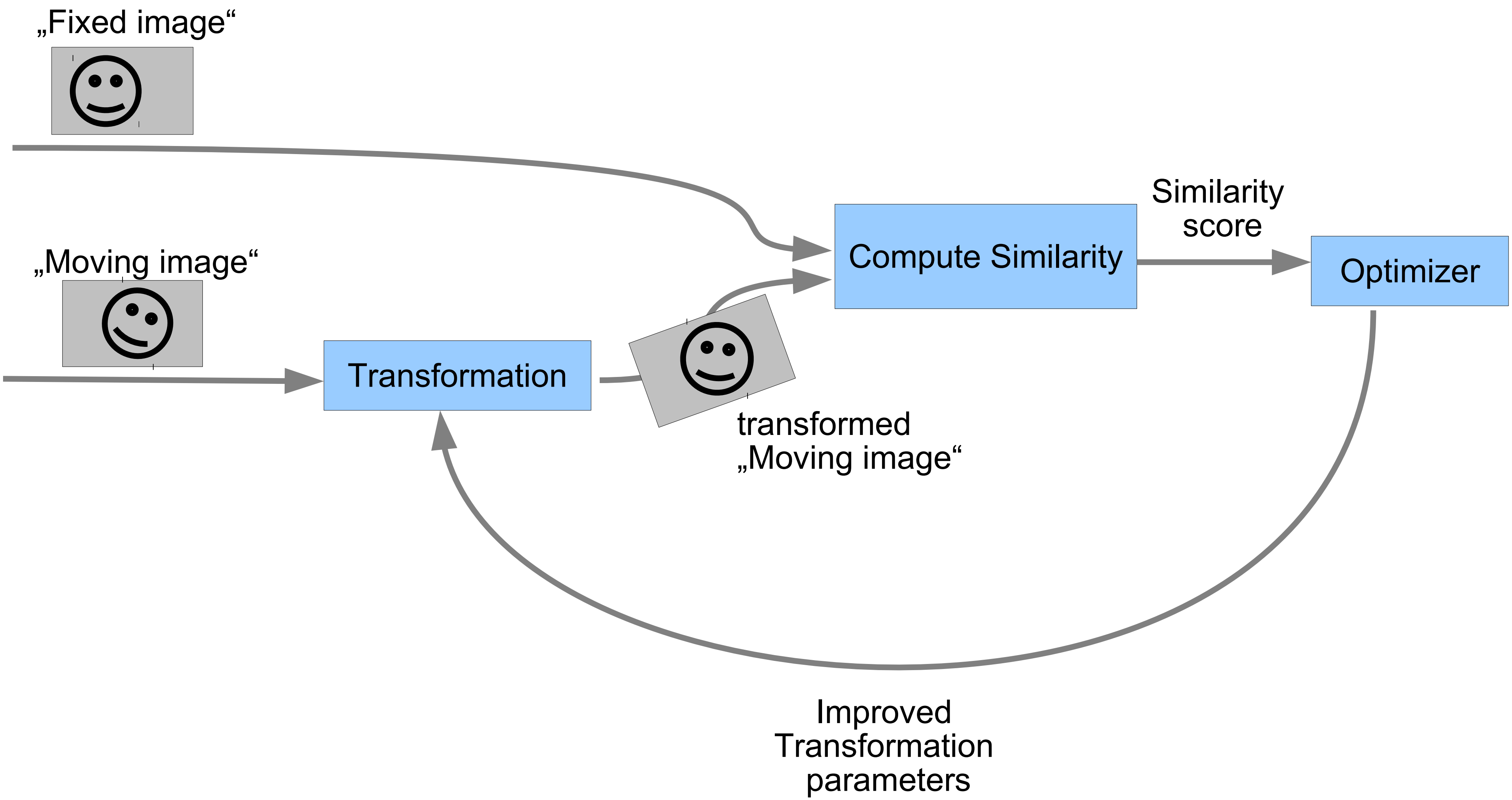
Moving Image on transparent slide





Final registration

General Flow Chart for a Registration



The 3 Parts of a Registration Algorithm

A transformation model

- What can you do with the moving image.

  e.g. **rigid transformation** (corresponds to our slide) or **elastic transformation** (put moving image on a rubber skin)

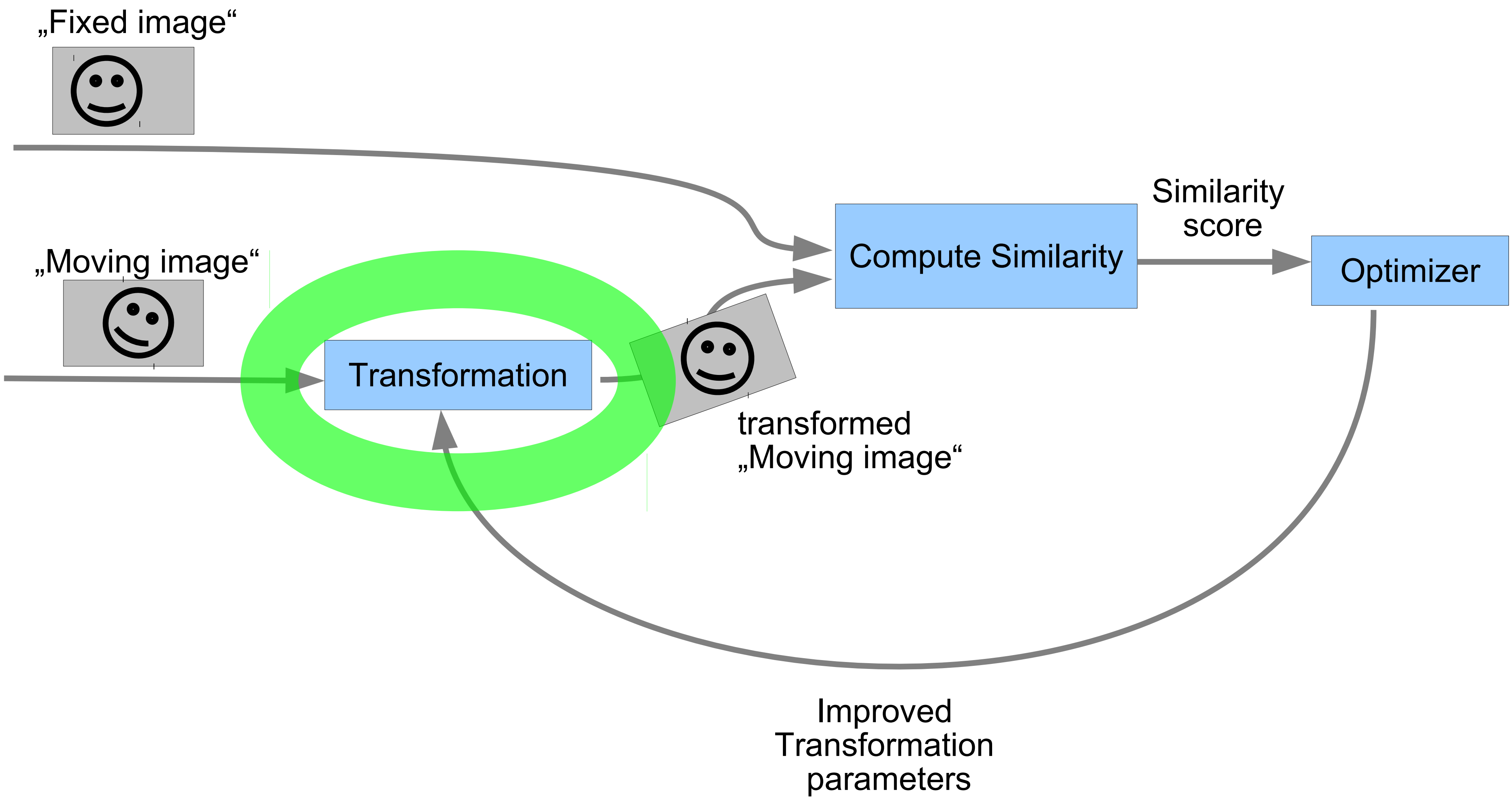
A similarity measure

- **Compare the gray values** of fixed and moving image and output a score

An optimizer

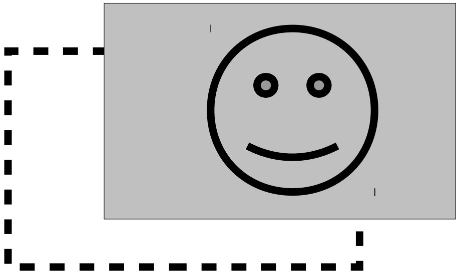
- Algorithm to **find the transformation parameters**

Component 1: The Transformation



Typical Transformation Models

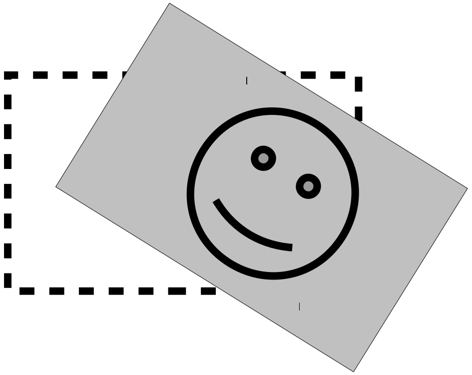
Translation only



- 2D images: **2 parameters** (shift in x and shift in y direction, denoted as u,v)
- 3D images: **3 parameters** (shift in x, y, and z direction)

$$B(x, y) = A(x + u, y + v)$$

Rigid Transformation (shift and rotation)



- 2D images: **3 parameters** (shift x, shift y, rotation angle)
- 3D images: **6 parameters** (shift x, y, and z, rotation around x-, y- and z-axis)

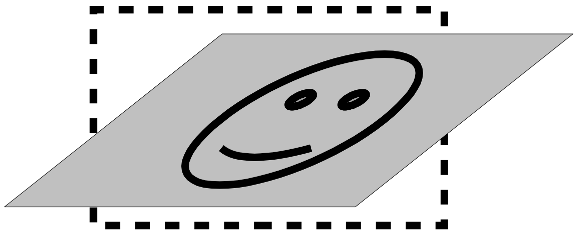
$$B(x, y) = A(x \cdot \cos\alpha - y \cdot \sin\alpha + u, \\ x \cdot \sin\alpha + y \cdot \cos\alpha + v)$$

Typical Transformation Models

Affine Transformation (shift, rotation, scale and skew)

- 2D images: **6 parameters**
- 3D images: **12 parameters**

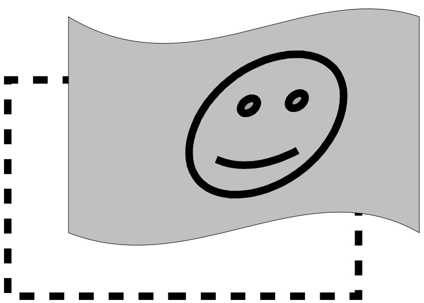
$$B(x,y) = A(a_1 x + a_2 y + u,\\ a_3 x + a_4 y + v)$$



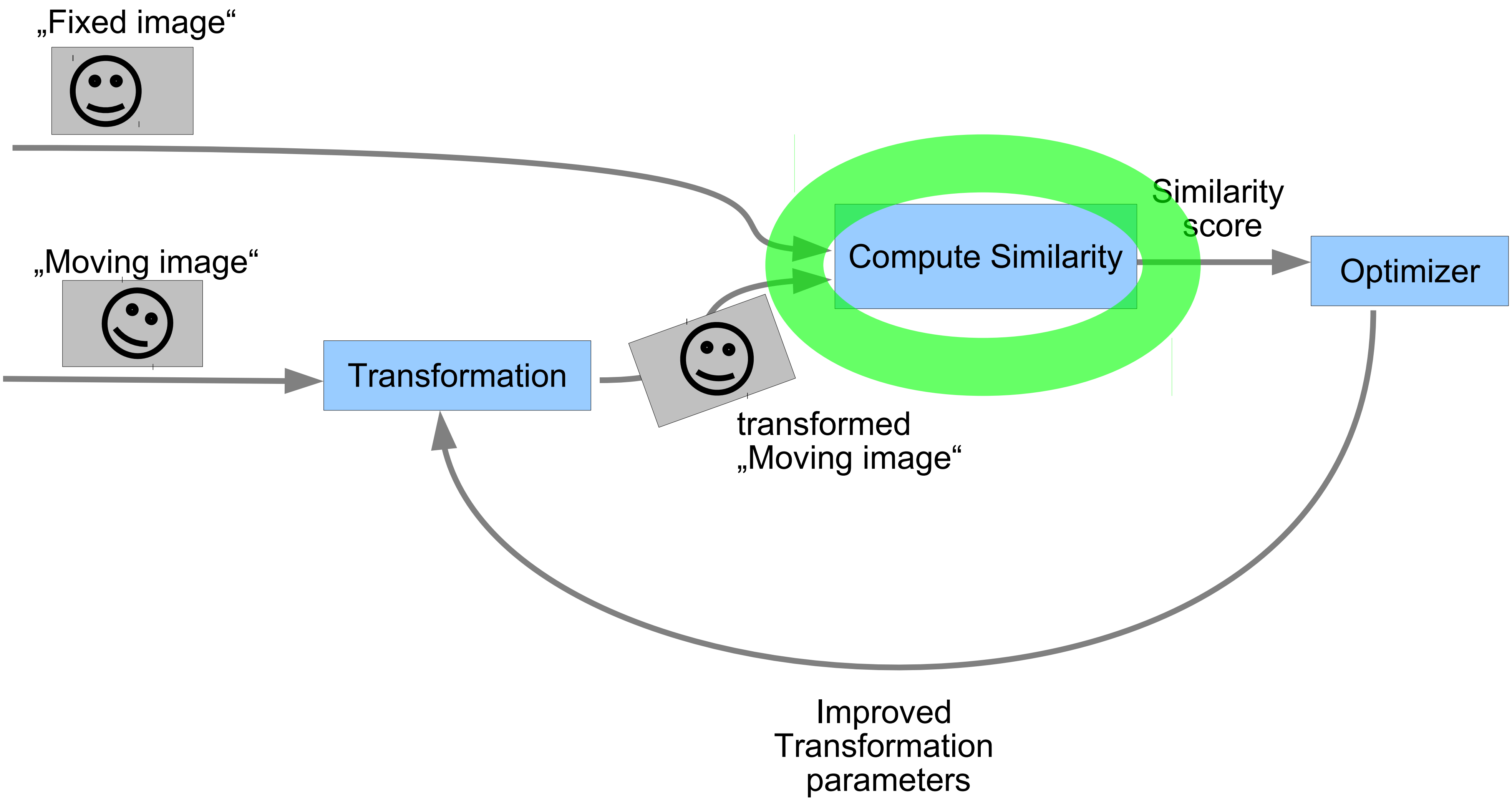
Elastic Registration

- 2D images: **2 times the number of pixels**
- 3D images: **3 times the number of voxels**

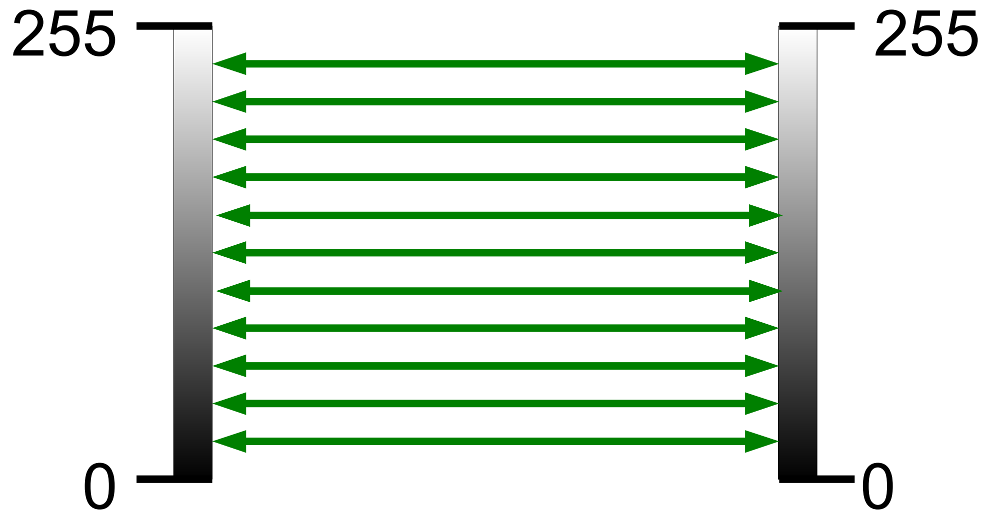
$$B(x,y) = A(x + u(x,y),\\ y + v(x,y))$$

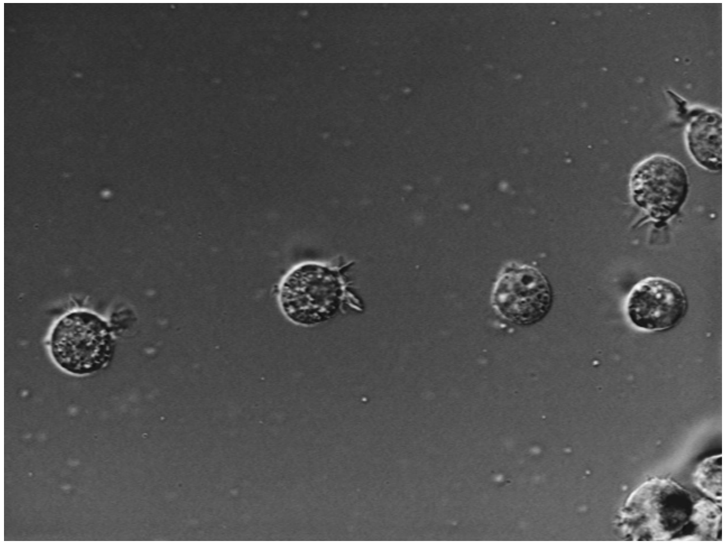


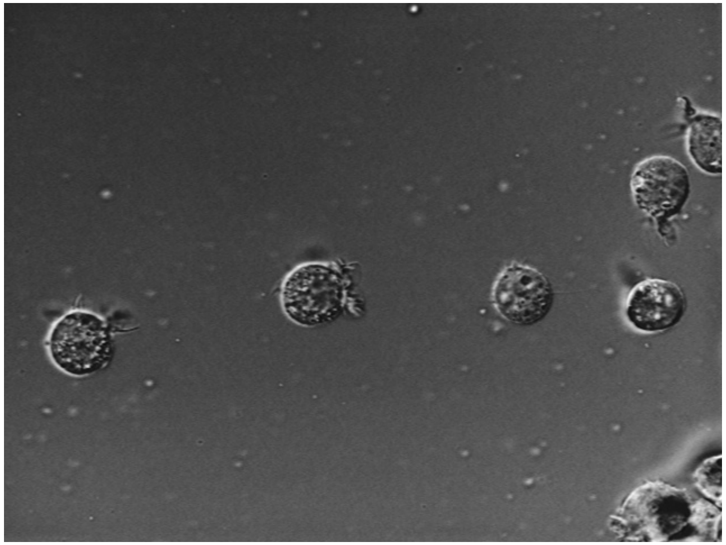
Component 2: The Similarity Measure



Similarity Measures
Case 1: Identical Gray Values







e.g., transmitted light images of moving cells

Similarity Measures for Intensity Based Registration

a_0

	A	B	C	D	E	F	G	H	I	J	K	L	M	
1	$\boxed{5}$	5	5	6	10	8	9	12	10	10	10	14	11	1
2	4	5	7	7	9	9	11	12	11	12	13	13	13	12
3	5	6	7	6	8	8	11	14	14	13	14	16	13	11
4	6	4	7	6	6	8	11	14	15	13	12	13	11	10
5	7	6	7	6	7	10	14	14	16	13	11	10	10	10
6	6	7	6	8	10	11	16	14	15	14	11	10	11	10
7	6	8	10	10	9	12	15	15	13	12	12	9	10	8
8	7	8	10	13	13	14	15	15	13	12	8	8	9	9
9	7	10	11	12	15	16	15	12	15	10	10	8	10	9
10	9	11	13	17	14	16	16	14	16	14	12	10	10	8
11	14	15	14	16	17	17	17	18	19	15	13	12	10	11
12	13	15	15	16	16	19	19	21	20	19	15	14	17	1
13	14	16	17	19	17	20	20	21	23	20	20	18	17	
14	14	15	16	20	19	23	21	21	22	23	23	25	27	
15	15	18	17	16	22	20	22	23	25	26	26	26	27	
16	17	14	14	16	17	22	21	23	24	27	27	28	2	
17	13	15	17	16	15	23	24	26	23	28	26	31	2	
18	10	11	12	13	16	19	23	22	24	24	30	31	3	
19	9	10	7	11	15	16	18	22	21	25	32	32	3	
20	9	8	10	10	14	16	17	21	24	29	31	34	38	
21	7	7	9	8	12	11	19	20	25	29	30	32	37	
22	7				10	13	15	17	25	30	32	32	38	4
								14	23	29	32	3		

Image A

b_0

	A	B	C	D	E	F	G	H	I	J	K	L	M	
1	$\boxed{5}$	5	5	6	10	8	9	12	10	10	10	14	11	1
2	4	5	7	7	9	9	11	12	11	12	13	13	13	12
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7	6	8	10	10	9	12	15	15	13	12	12	9	10	8
8	7	8	10	13	13	14	15	15	13	12	8	8	9	9
9	7	10	11	12	15	16	15	12	15	10	10	8	10	9
10	9	11	13	17	14	16	16	14	16	14	12	10	10	8
11	14	15	14	16	17	17	17	18	19	15	13	12	10	11
12	13	15	15	16	16	19	19	21	20	19	15	14	17	1
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17	13	15	17	16	15	23	24	26	23	28	26	31	2	
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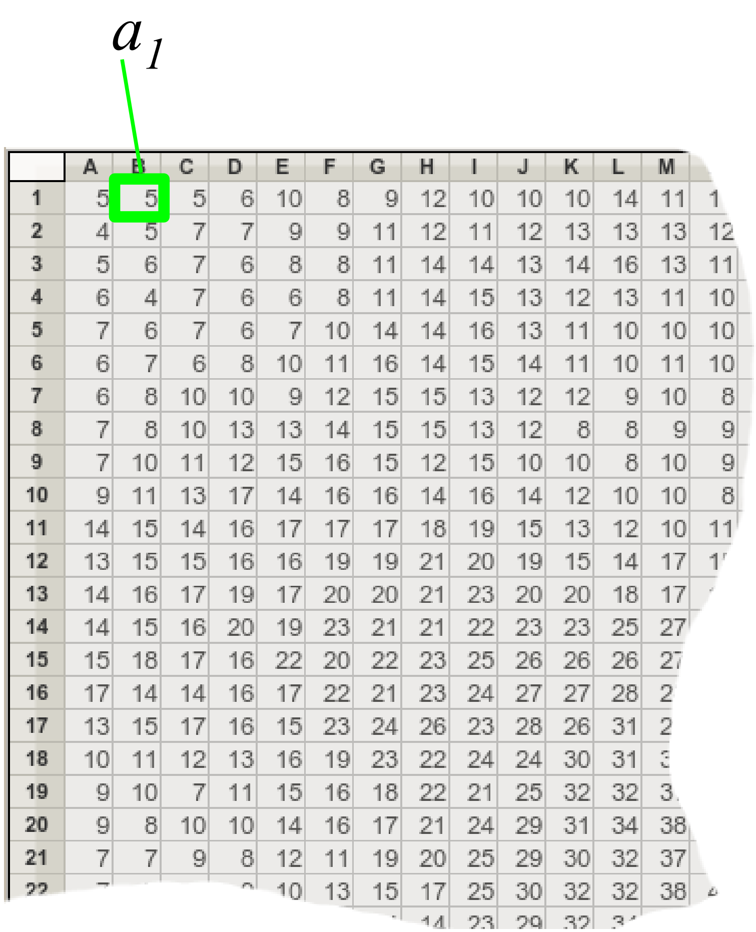
21	7	7	9	8	12	11	19	20	25	29	30	32	37	
22	7				10	13	15	17	25	30	32	32	38	4
								14	23	29	32	3		

Image B

Similarity Measures for Intensity Based Registration



	A	B	C	D	E	F	G	H	I	J	K	L	M	
1	5	5	5	6	10	8	9	12	10	10	10	14	11	1
2	4	5	7	7	9	9	11	12	11	12	13	13	13	12
3	5	6	7	6	8	8	11	14	14	13	14	16	13	11
4	6	4	7	6	6	8	11	14	15	13	12	13	11	10
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								14	23	29	32	3		

Image A

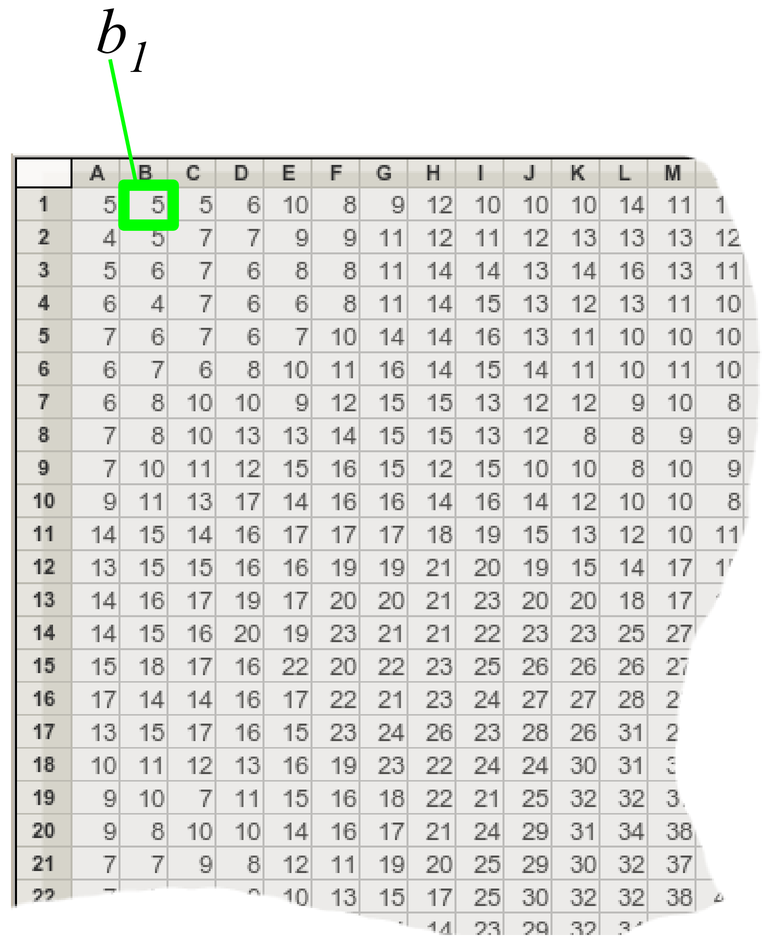

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Image B

Similarity Measures for Intensity Based Registration

a_2

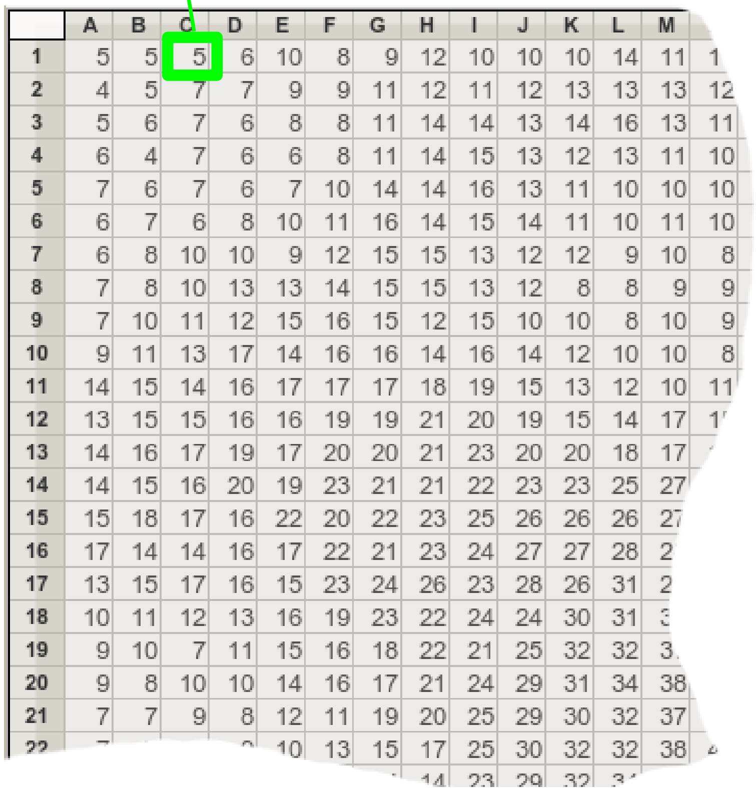

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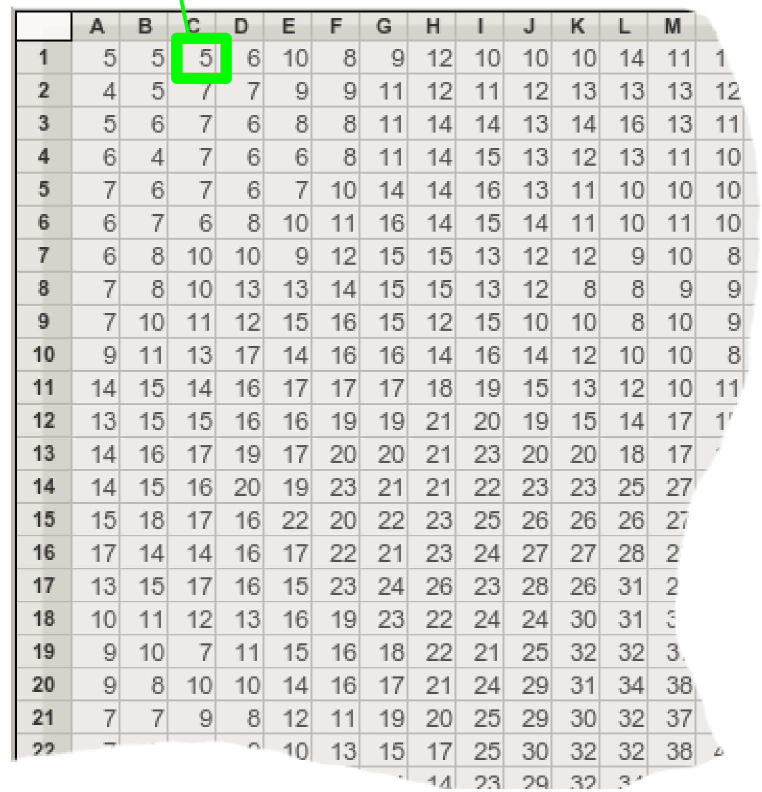
Image A

b_2



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22					10	13	15	17	25	30	32	32	38	
								14	23	29	32			

Image B

Similarity Measures
Case 1: Identical Gray Values

Sum of Squared Differences (SSD)

- Compute **squared difference** between intensity of each **pixel a_i** in the “fixed image” and intensity of the corresponding **pixel b_i** in the transformed “moving image”
- **Sum up** for all pixels

$$SSD = \frac{1}{N} \sum_i (a_i - b_i)^2$$

- Standard Error model (assumes **Gaussian distribution of errors**)
- Allows **effective optimization** in certain algorithms

Similarity Measures
Case 1: Identical Gray Values

Sum of Absolute Differences (SAD)

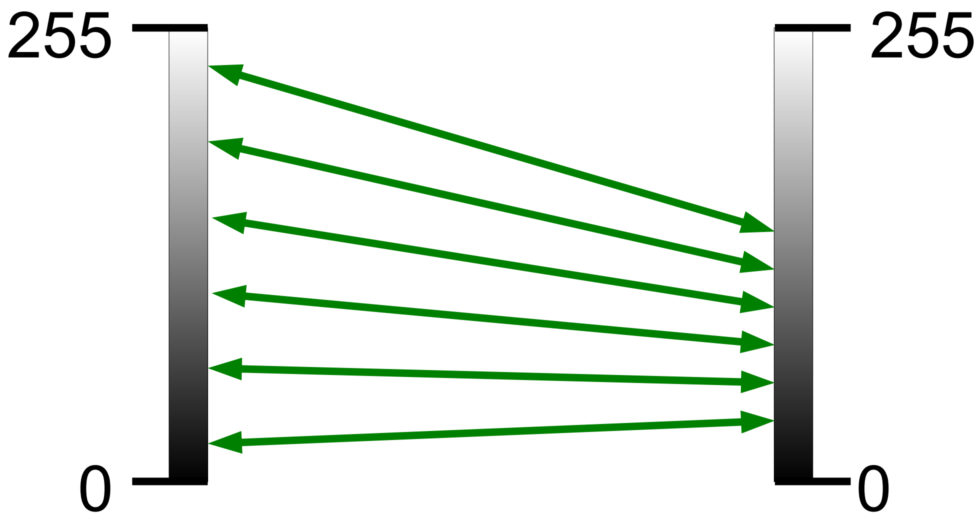
- Compute **absolute difference** between intensity of each **pixel a_i** in the “fixed image” and intensity of the corresponding **pixel b_i** in the transformed “moving image”
- **Sum up** for all pixels

$$SAD = \frac{1}{N}\sum_i |a_i - b_i|$$

- Less sensitive to **outlier intensities** than SSD

Both methods only for images recorded under identical conditions!

Similarity Measures
Case 2: Gray Value Offset and Scale



e.g. fluorescent cells, over time with bleaching

Normalized Cross Correlation

- **Subtract the mean value** of each image
- Compute the **product** of each **pixel a_i** in the “fixed image” and the corresponding **pixel b_i** in the transformed “moving image” and **sum up** for all pixels
- **Normalize with standard deviation** of each Image

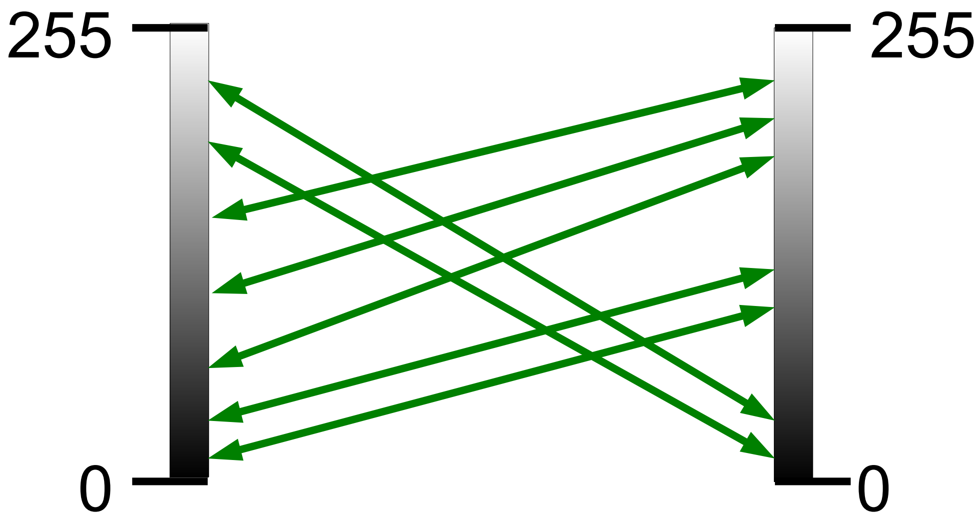
$$NCC = \frac{1}{\sigma_a \cdot \sigma_b} \sum_i (a_i - \bar{a}) \cdot (b_i - \bar{b})$$

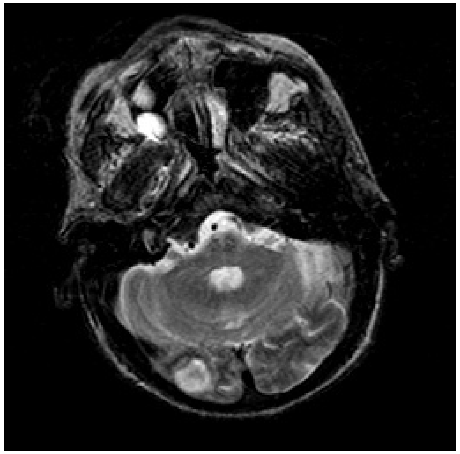
standard deviation of intensities

mean value of intensities

- different intensity offsets (e.g. **background intensities**) are compensated
- different intensity scales (**contrast changes**) are compensated
- **Extension**: Compute mean and stddev only in a **local surrounding**: High **robustness**, even for very different contrast within the same image!

Similarity Measures
Case 3: Different Modalities





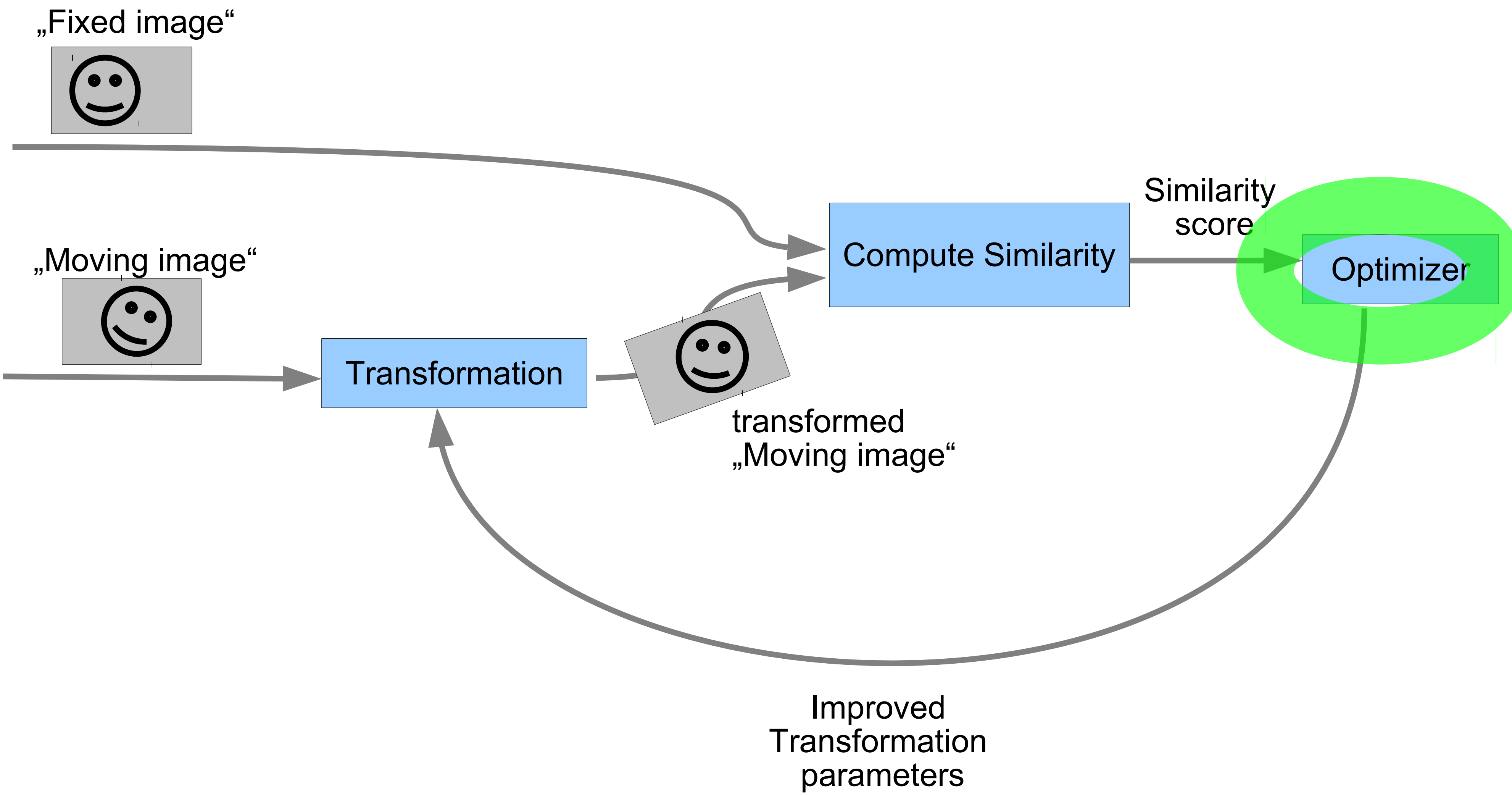
e.g. CT scan (X-Ray) and MRI scan (Magnetic Resonance)

Similarity Measures
Case 3: Different Modalities

Mutual Information

- Standard measure in **medical image registration**, when registering images from different modalities (e.g. CT and MRI)
- Allows **any dependencies of intensities** in the images (e.g. bones are white in CT and black in MRI)
- (we don't go into detail here)

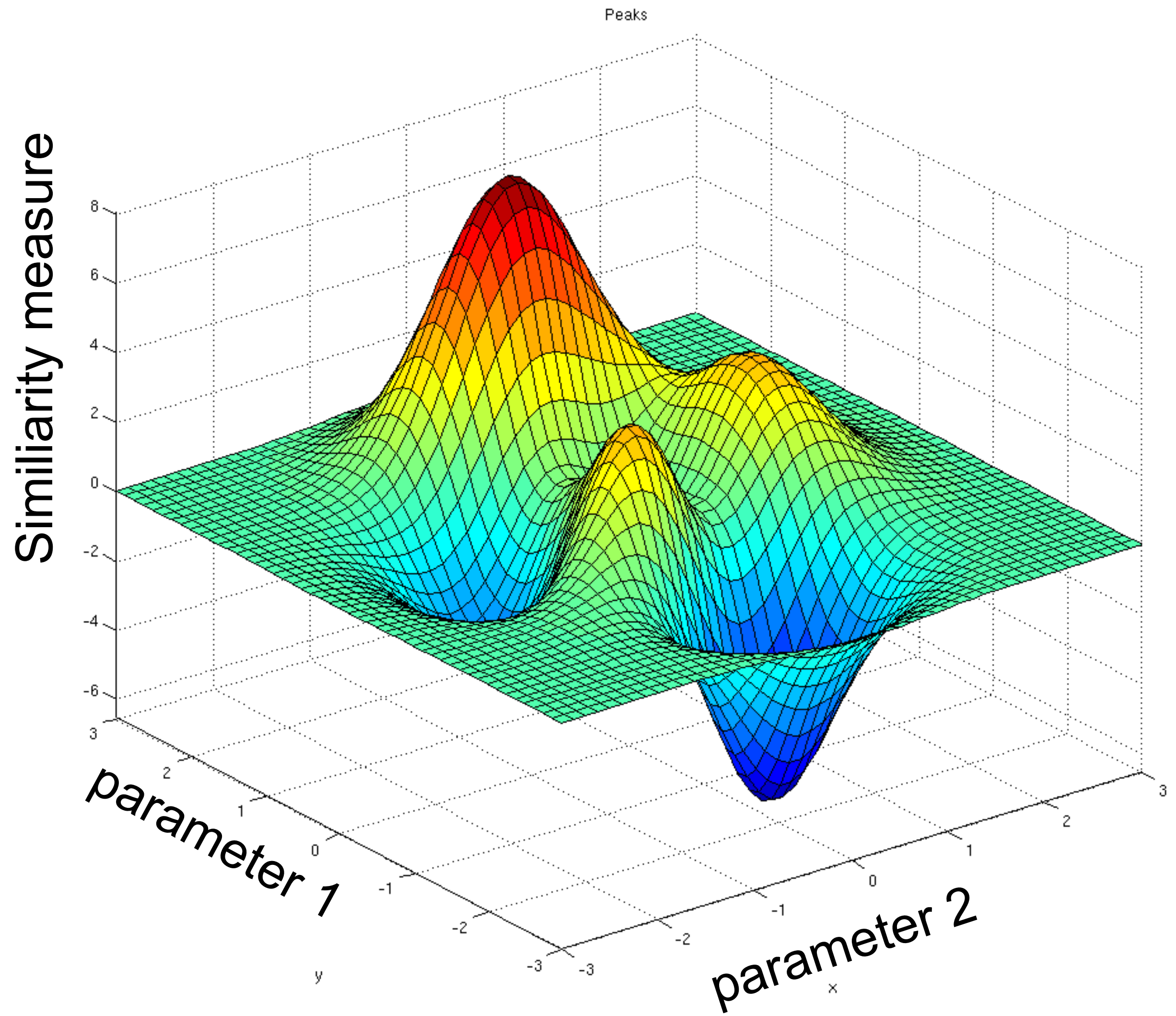
Component 3: The Optimizer



Optimiziers

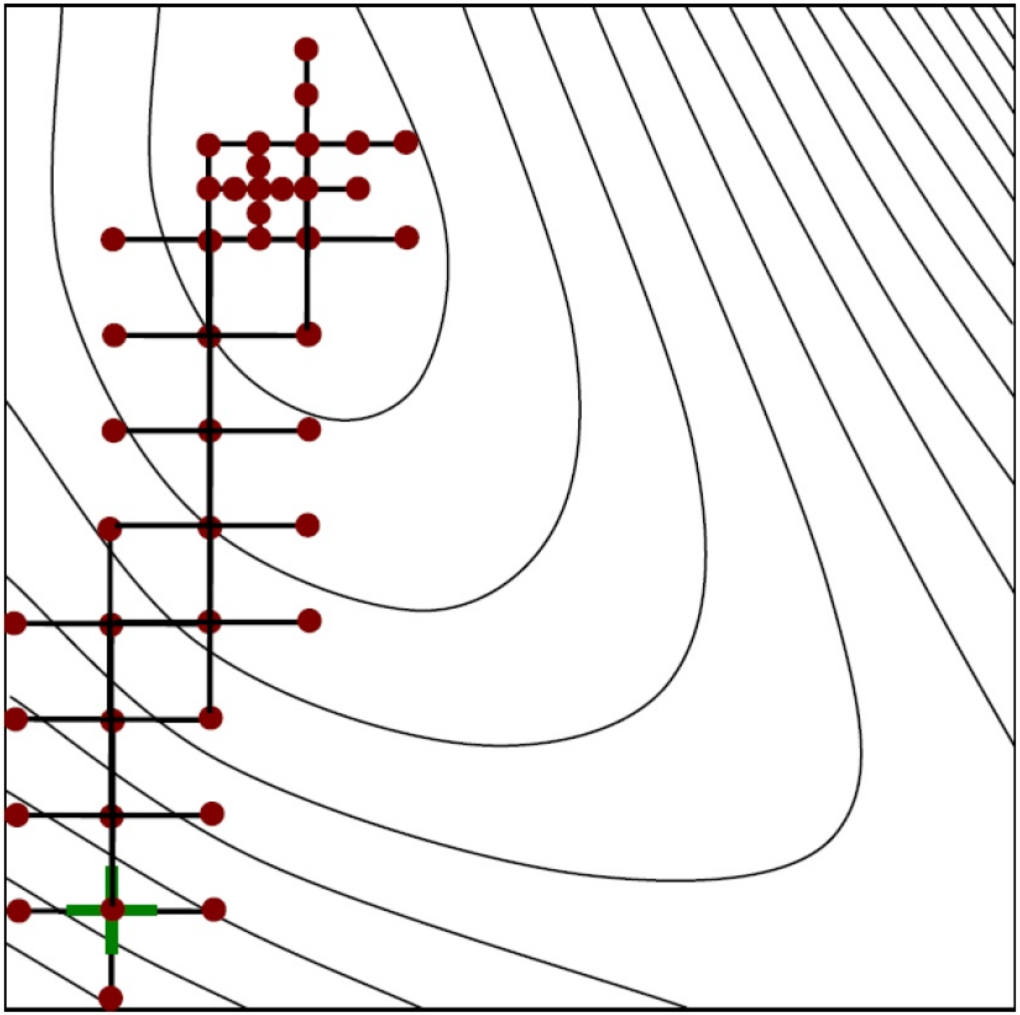
The Optimizier searches the best Parameters.

- Intuitive visualisation of optimizer by drawing the **similarity measure as function** of the parameters
- For two parameters, you can think of **mountains**.
- Your job is to **find the peak**.
- But you are **blind**, you can explore the surrounding by feeling with your feet



Best Neighbor Optimizer

1. Evaluate all **2n neighbors** of the current location
2. If **better than the current** value, set best neighbor as next location, repeat 1.
3. Otherwise **half the step size** and try again
4. **Abort** if step size or cost function difference **below limit**

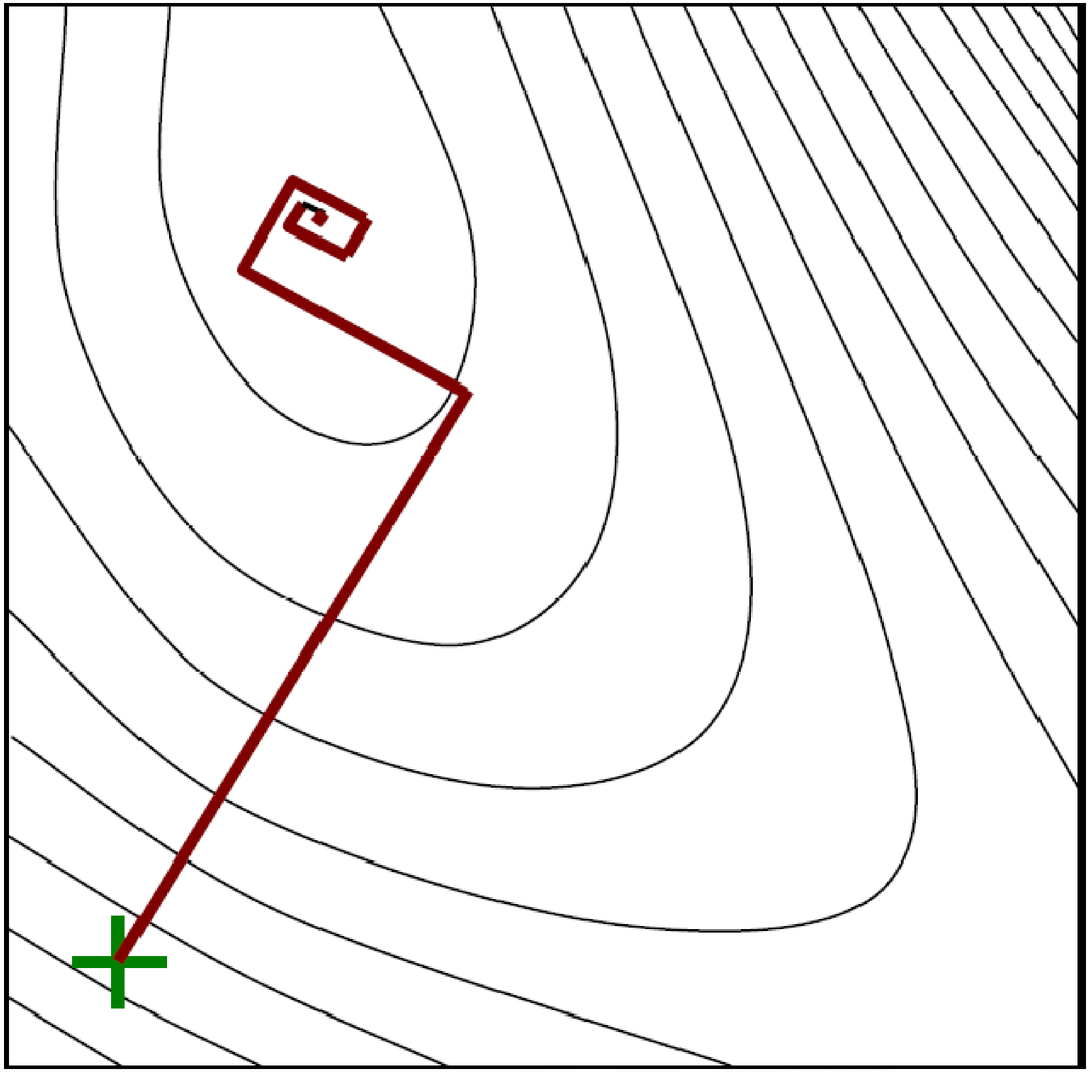


Slow, but works very stable – only locations are chosen that have been evaluated

Gradient Descent Optimizer

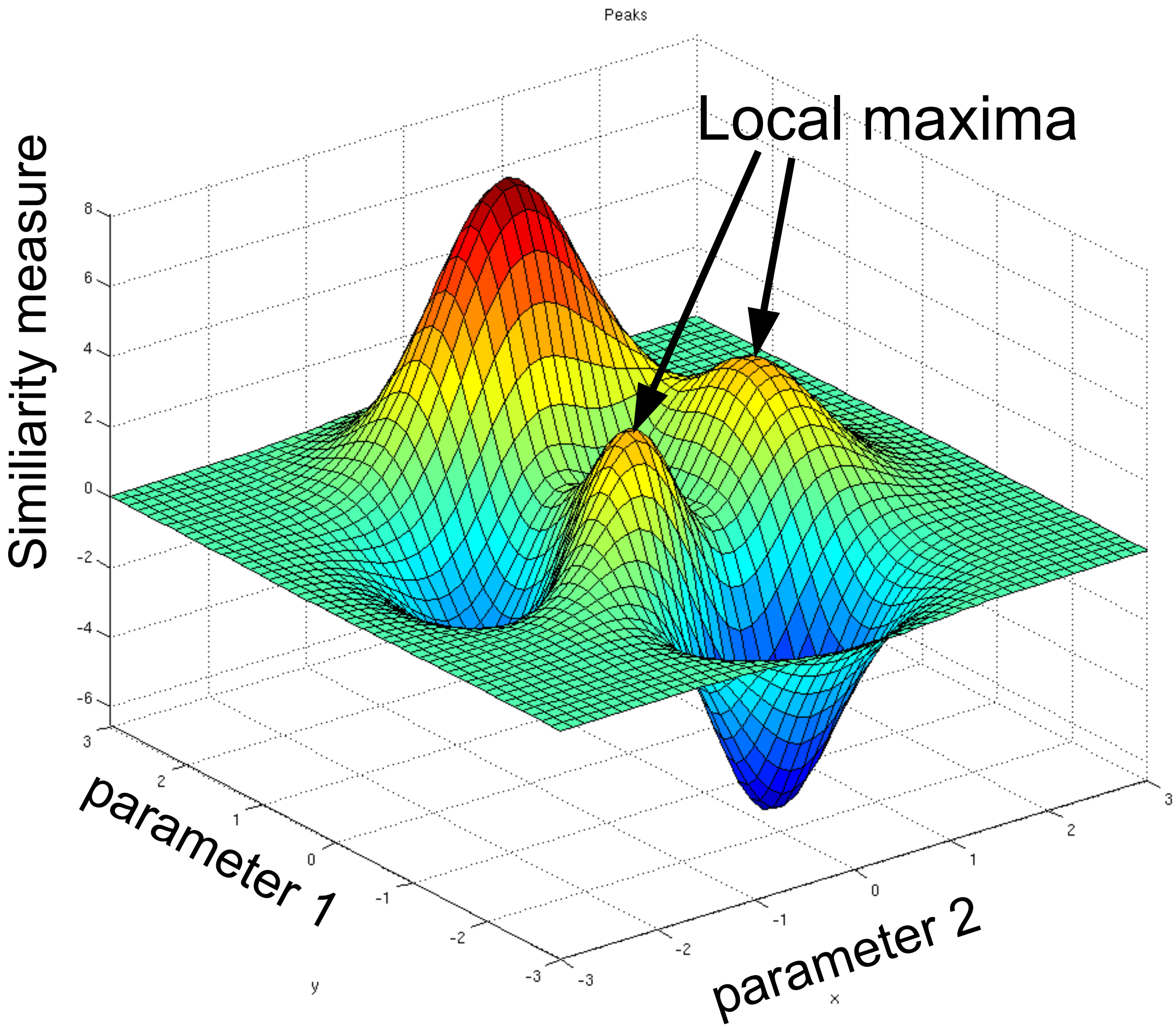
If you can compute the gradient at your current position

1. determine **gradient** (that is, in with direction the mountain raises)
2. go as far **in that direction,** until it does not increase anymore
3. repeat steps 1,2 **until convergence**



Problem of these optimizers

Do you see the problem of all these optimizers?



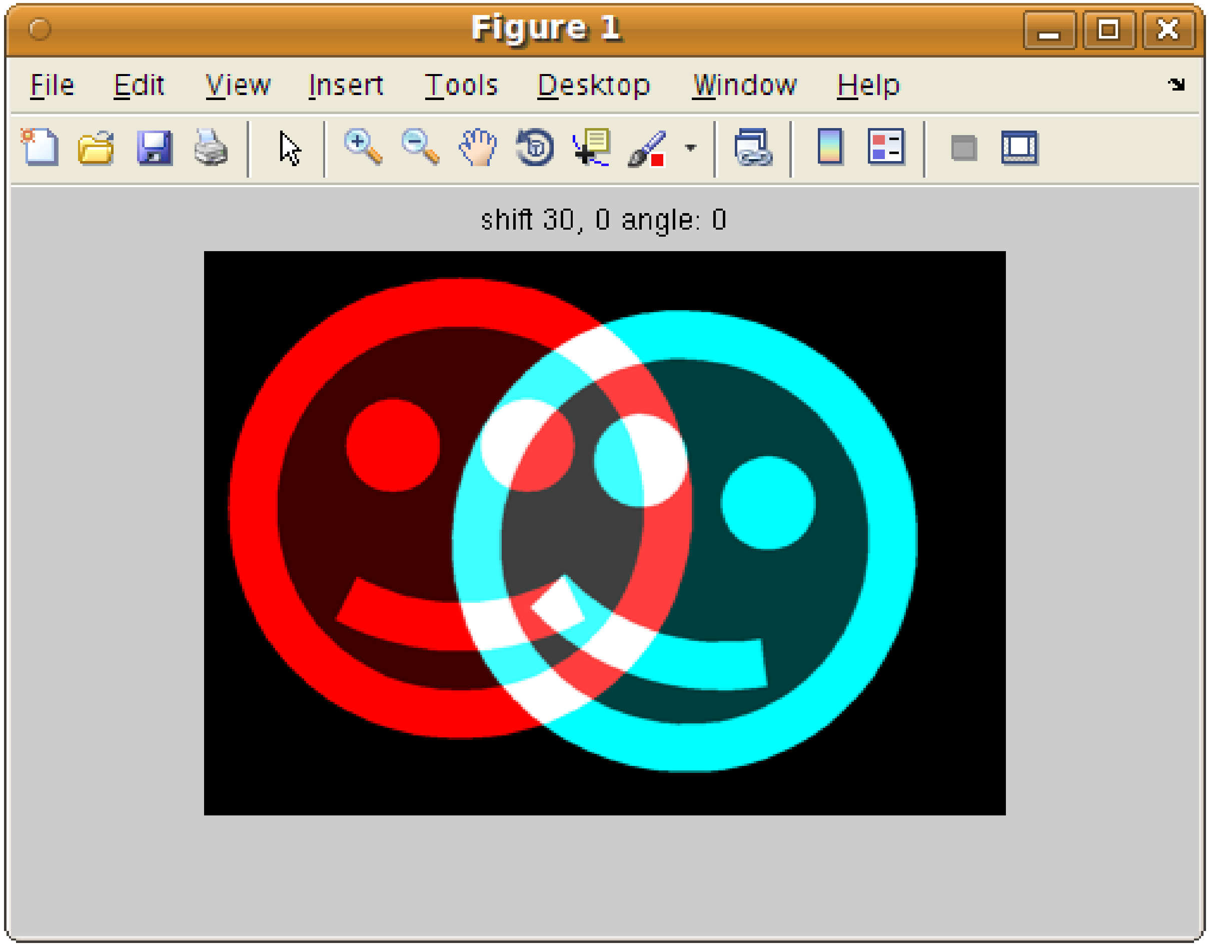
Higher Dimensional Parameter Spaces

- In reality there are usually **much more** than only **2 parameters** (like in the **mountain metaphor**)
- Metaphor for **3 parameters**: You **dive in the ocean**, and you have to find the **warmest spot**.

[image from wikipedia: “Taucher”]

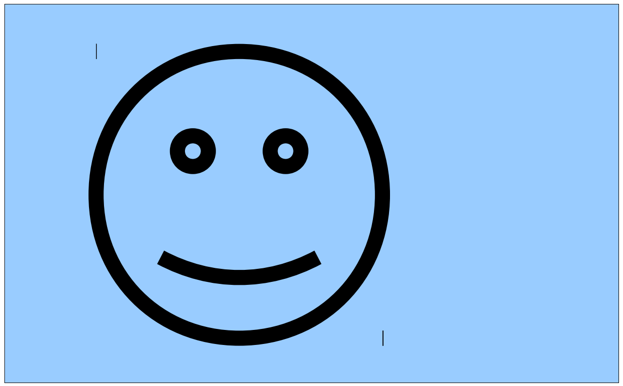
Put all together for 2D smiley registration

matlab demo:

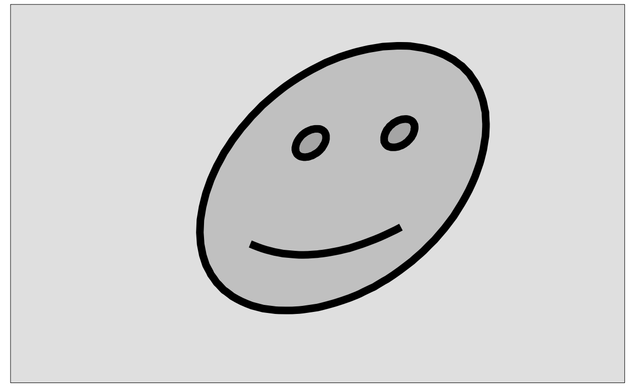


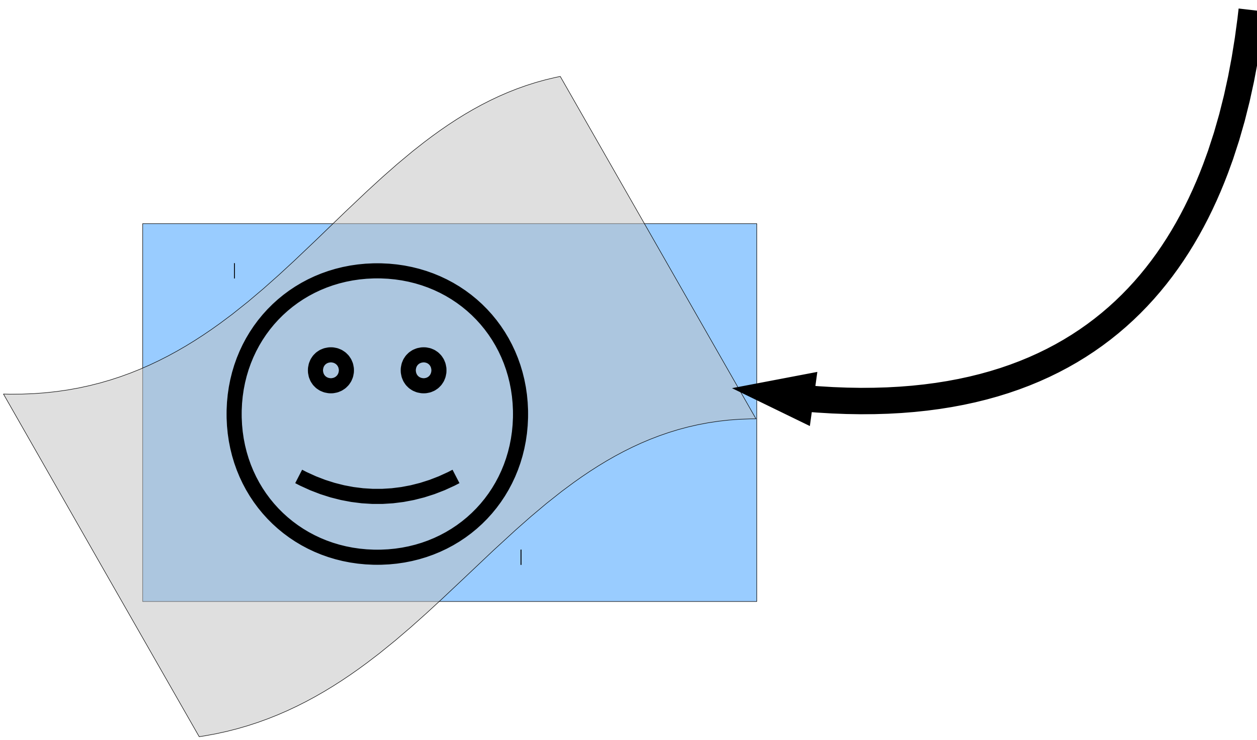
Elastic Registration

“Fixed” image I

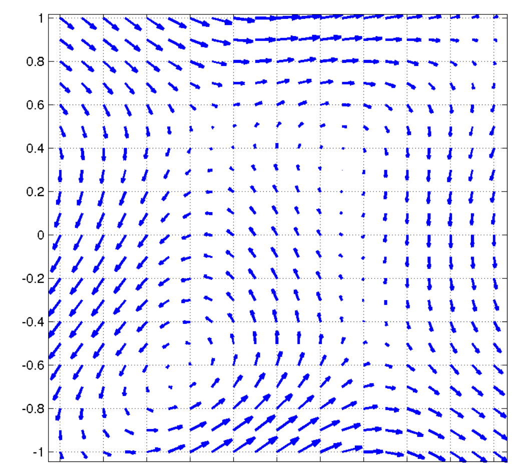


“Moving” Image J



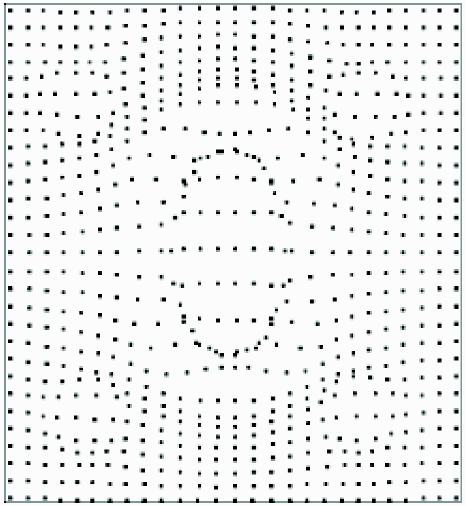


Transformation T

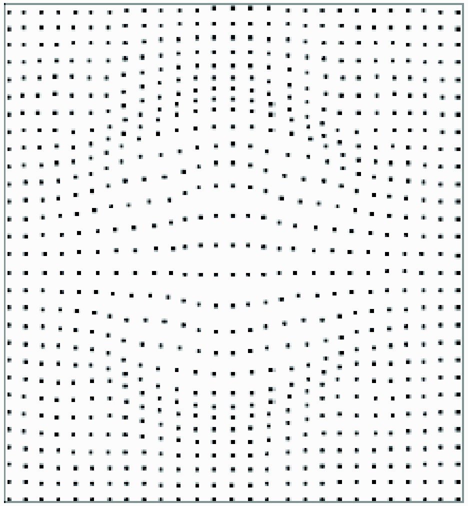


Smoothness Term

- Transformation is described by a **vector field** (individual displacement vector for each voxel)
- Not all vector fields describe **reasonable deformations**
- **Neighboring points** should have **similar displacement**
- We need a **regularization,** that enforces a **smooth deformation**



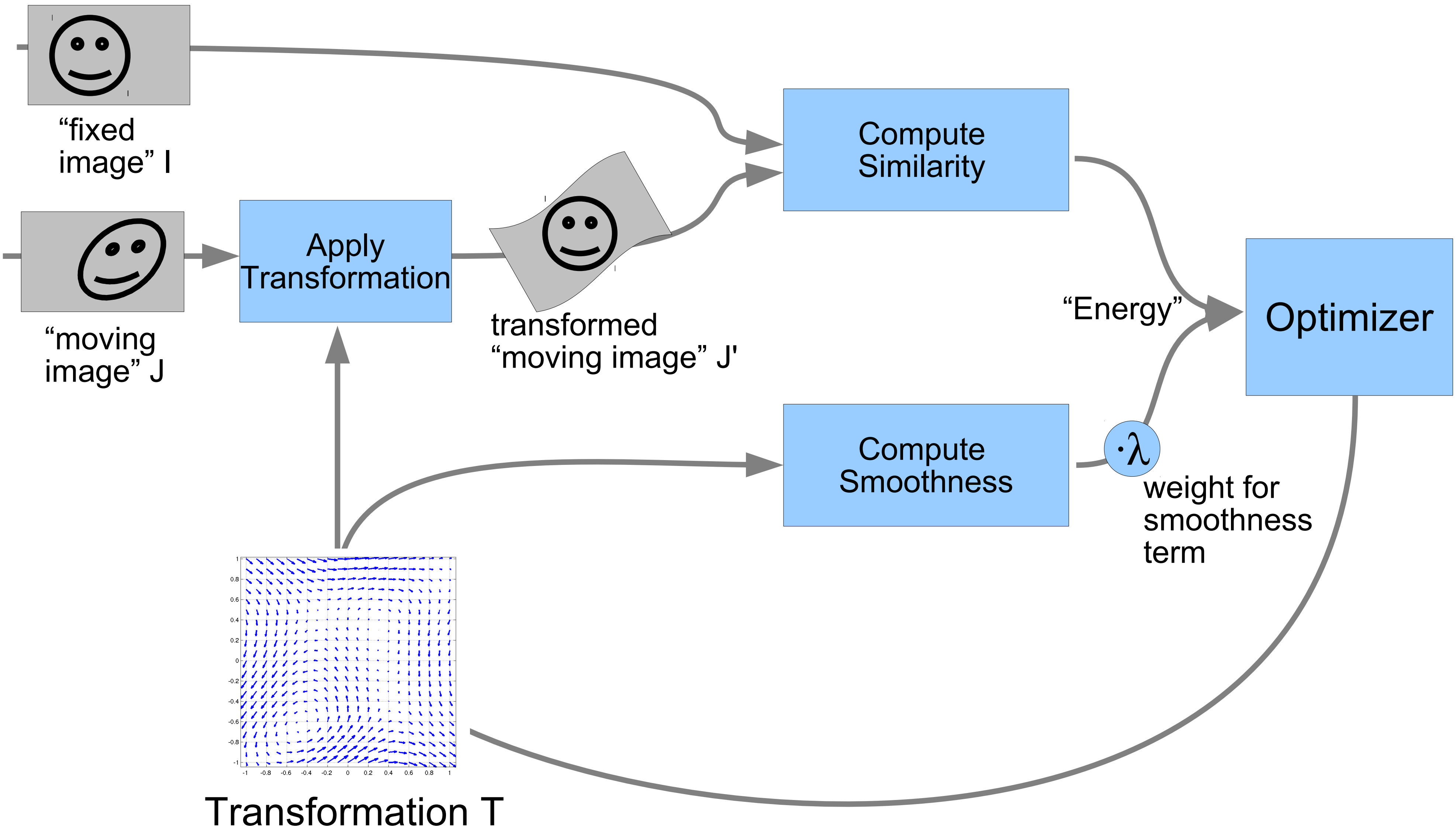
Un-smooth deformation



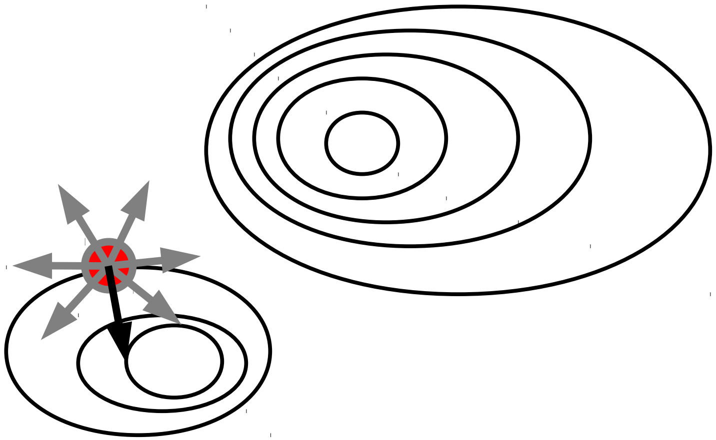
Smoother deformation

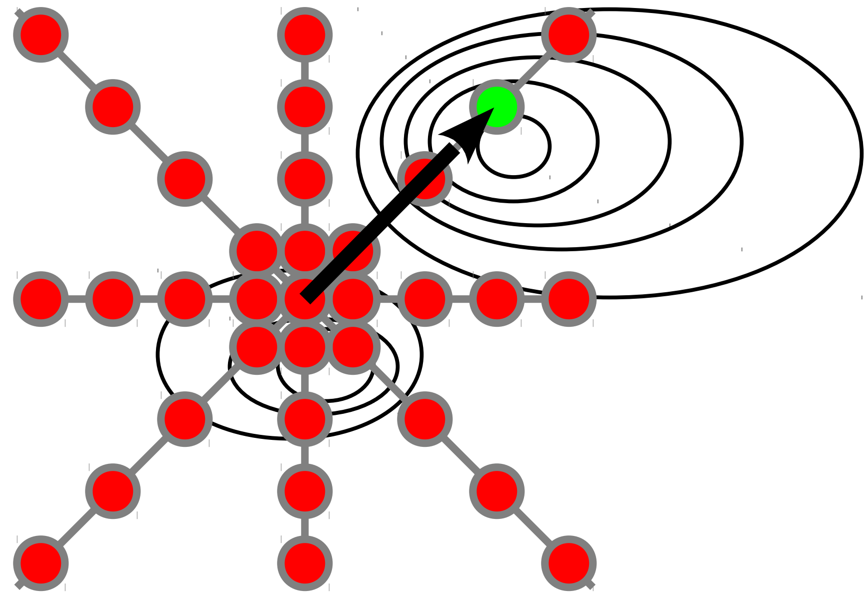
- Very important: **Optimize smoothness and image similarity simultaneously!** Many available software packages do this wrong.

Elastic Registration Flow Diagram



Problem with Gradient-based Optimization



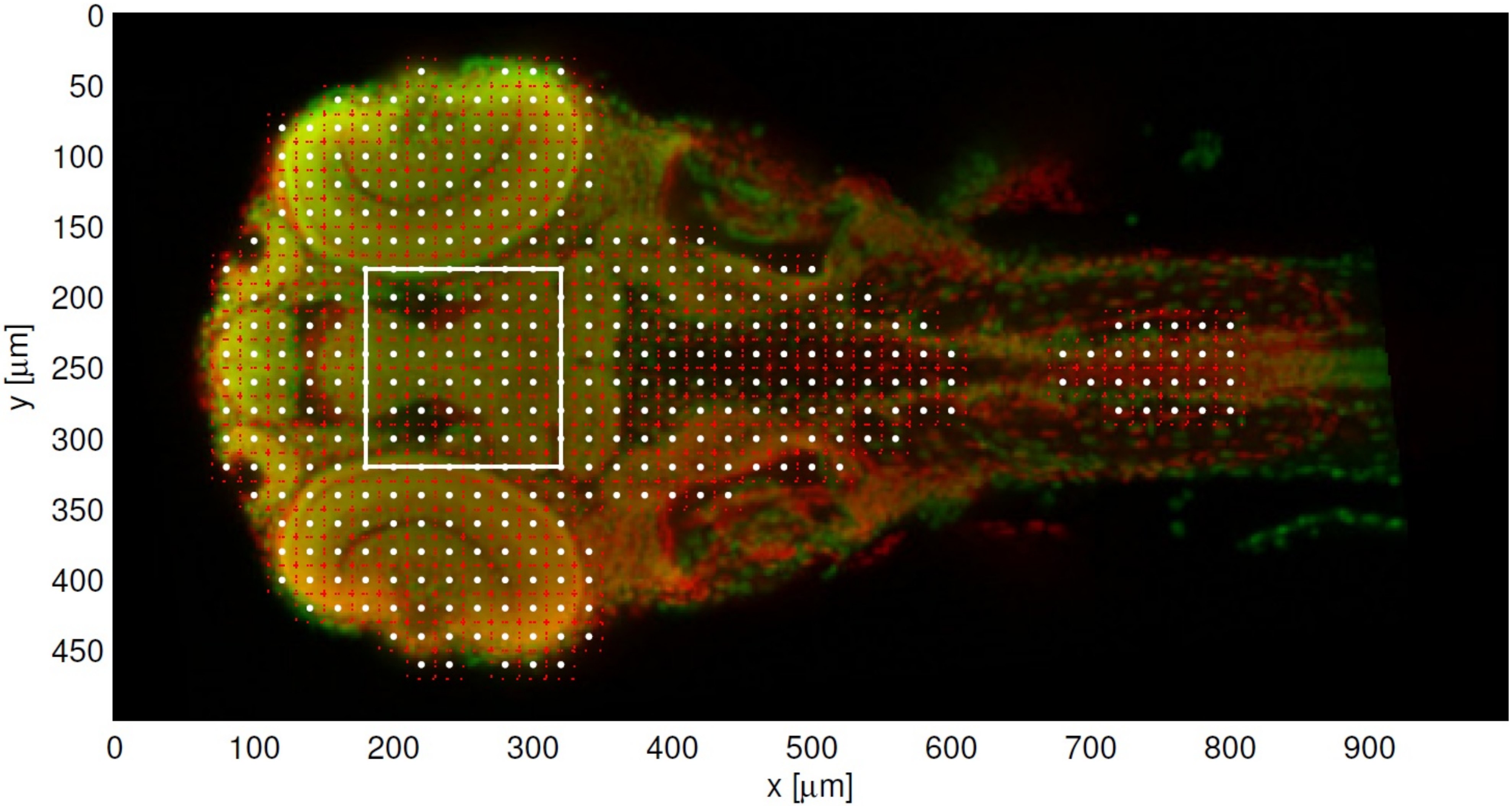


Standard approach:
Optimize per position
1 continuous variable (3 comp)
- runs into **"best" direction**

Combinatorial approach:
Optimize per position
N binary variables
(with constraint: sum=1)
- checks **many hypotheses**

- Problem: In 3D not possible to **store all these variables** for each voxel: Number of voxels times number of displacement hypotheses
- Use **grid based** registration to reduce number of variables.

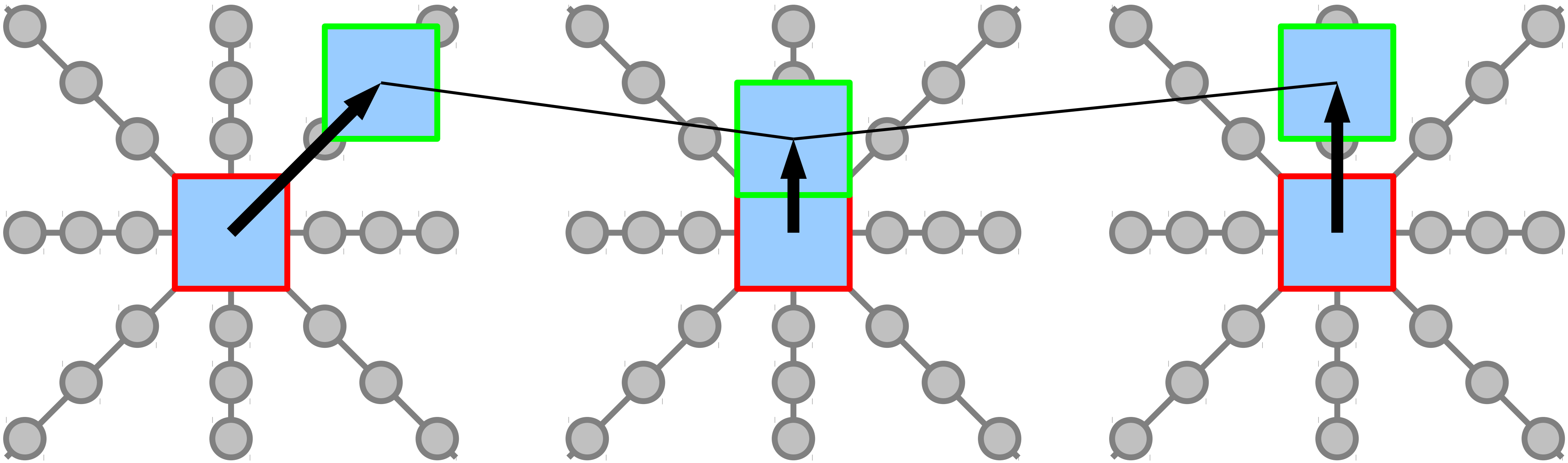
Control Point Grid



(c) Control points $\mathbf{p}_i$ (white) and corresponding regions Ω_i (red)

Combinatorial Optimization

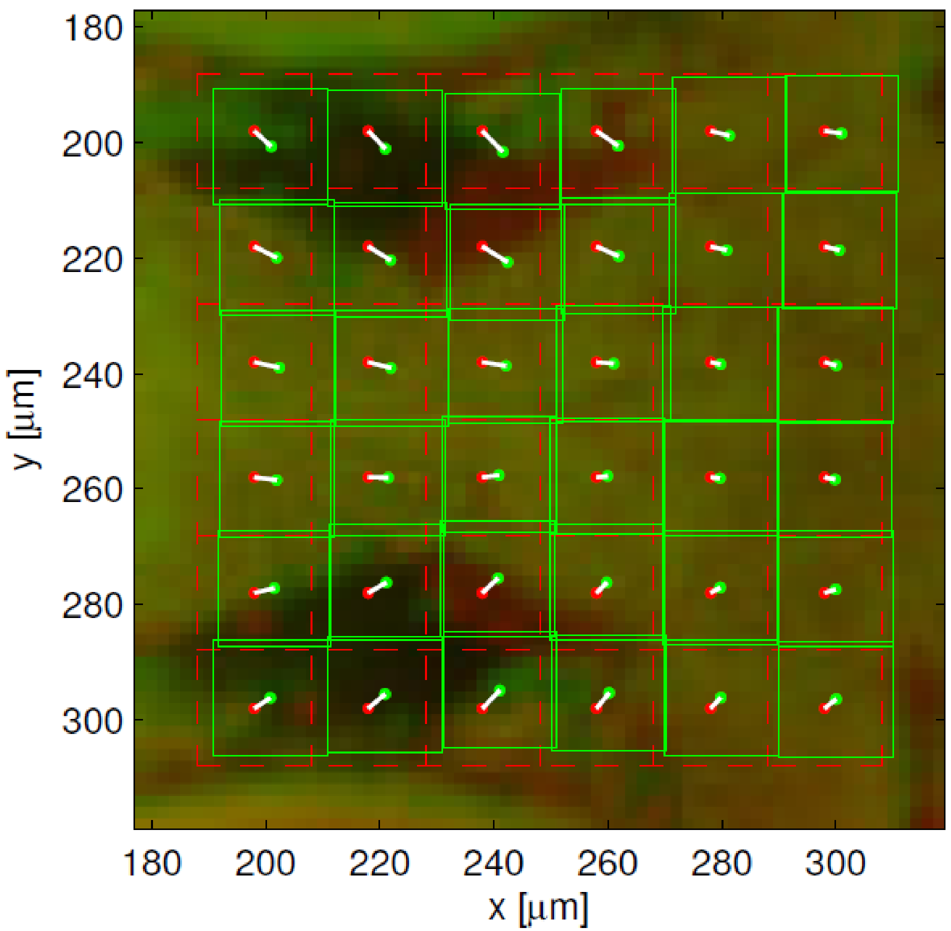
CENTRE FOR BIOLOGICAL SIGNALLING STUDIES

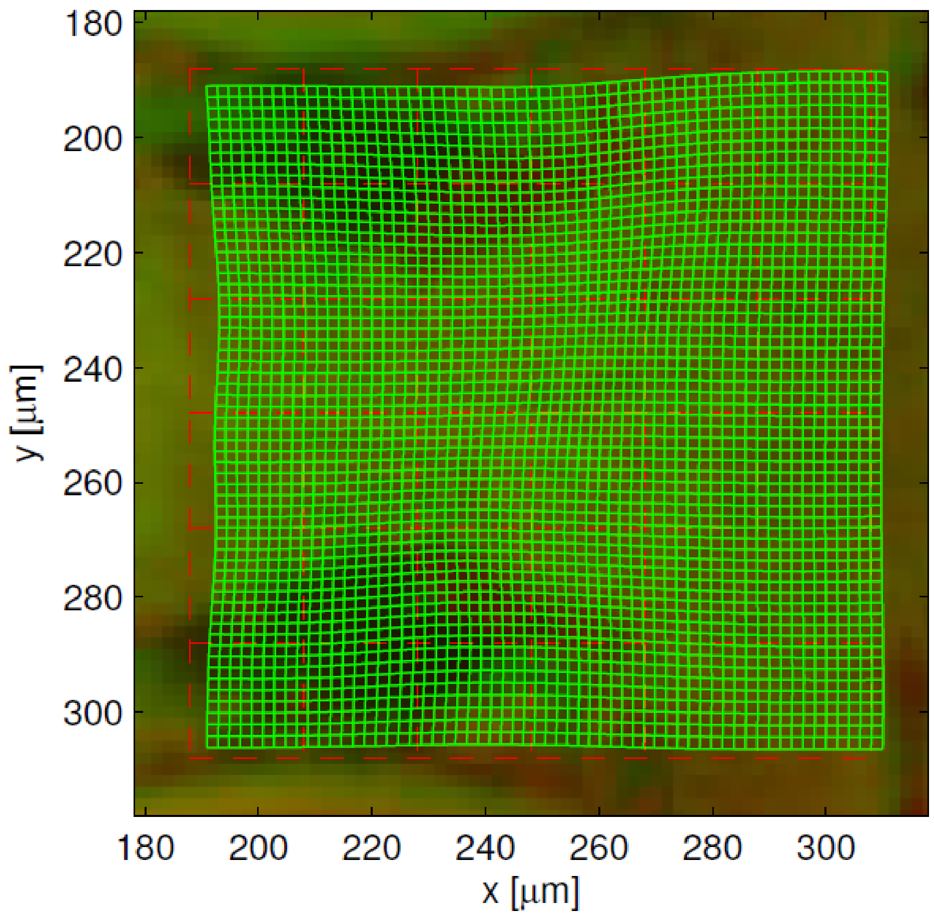


Discretization:

- Regular grid of **control points** (**nodes**)
- At each control point discrete number of **displacement hypotheses** (**labels**)
- **Data term** → **unary term**: similarity measure of fixed image (red patch) and moving image (green patch) for the given displacement
- **Smoothness term** → **pairwise term**: difference of two neighboring displacement vectors

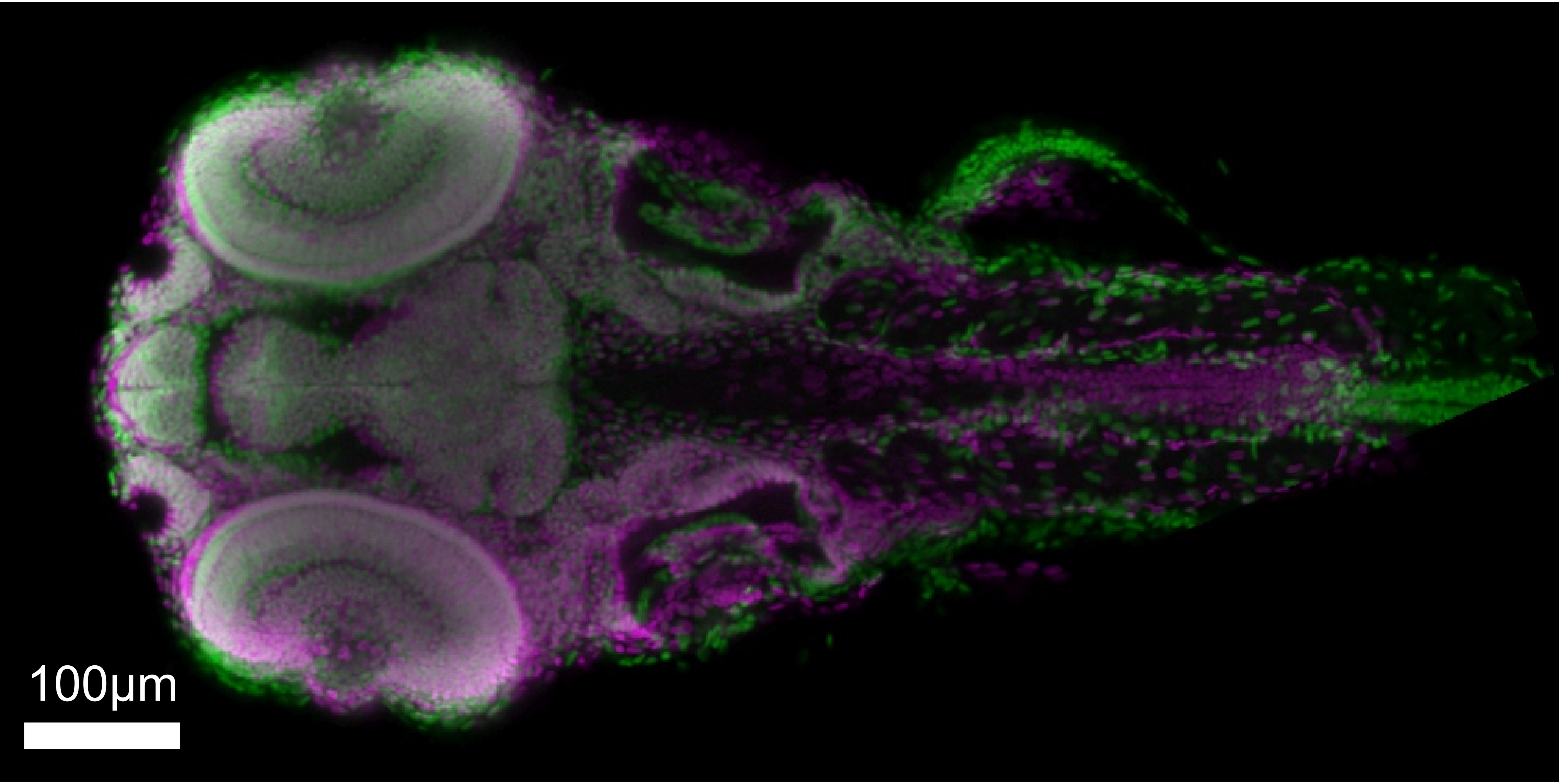
Dense displacement field





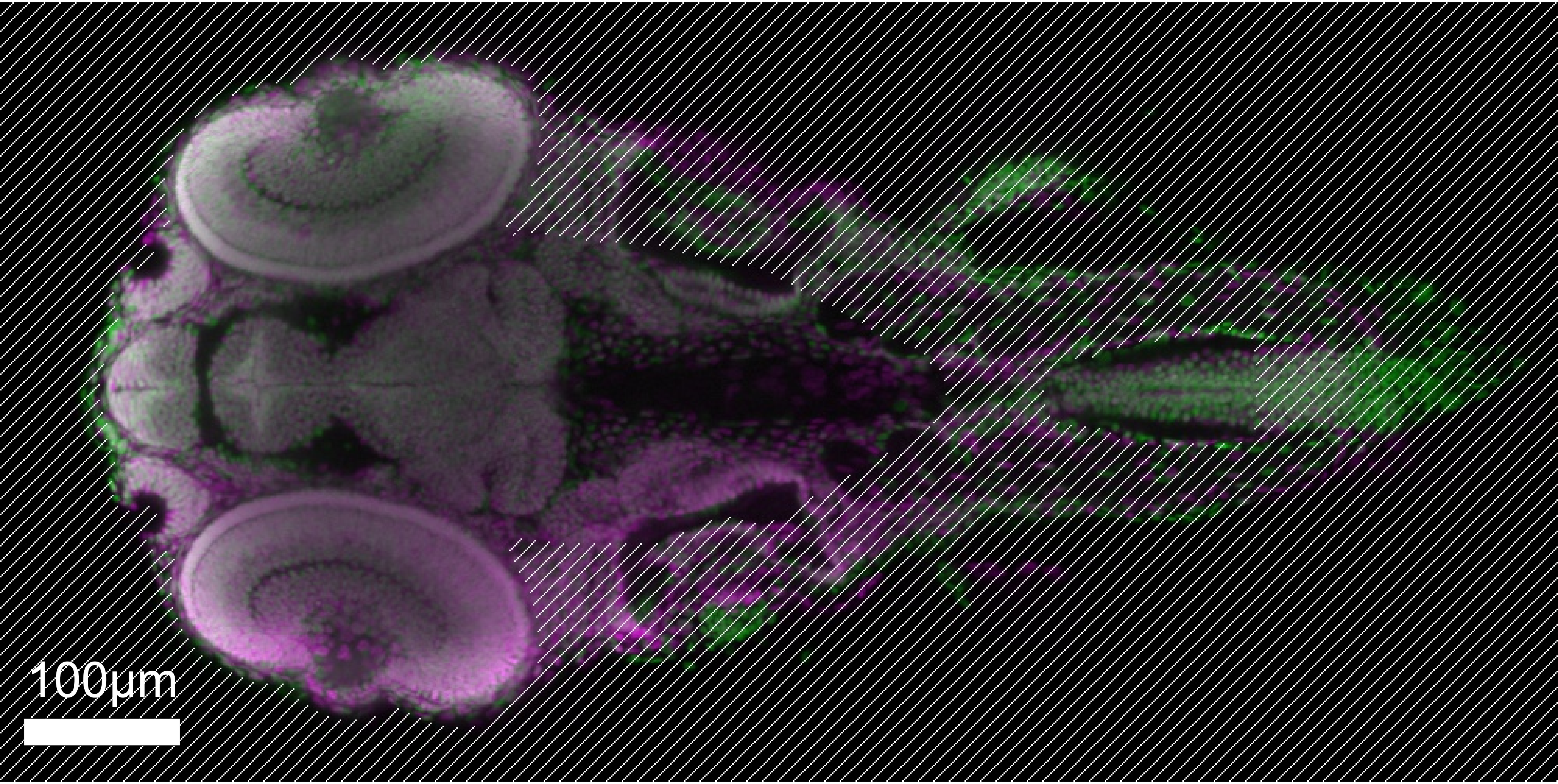
(e) Dense displacement field **U** by cubic spline interpolation

Landmark-Based Pre-Registration



Central slice. magenta: reference larva, green: new larva

After Intensity Based Elastic Registration



Central slice. magenta: reference larva, green: new larva
only brain region is considered

Results

- Use **FastPD solver** for combinatorial optimization (Komodakis et al. 2008)
  - Primal-Dual strategy, updates use fast graph-cut algorithm
- Very fast algorithm. In zebrafish setup **solver only takes a few seconds**. Most time is spent on computing the cross-correlations
- Only **two iterations** needed
- Overall computation time approx. **3 minutes** on a 6core Xeon
  - dataset with **1000x500x500 voxels** (3.6 million voxels after downscaling and masking)

Nikos Komodakis, Georgios Tziritas, and Nikos Paragios. Performance vs computational efficiency for optimizing single and dynamic mrfs: Setting the state of the art with primal-dual strategies. Comput. Vis. Image Underst., 112:14–29, October 2008.

Limitations

- Only **binary potentials possible** – i.e. in current implementation rotation is more expensive than shear
- Problem becomes **non-submodular** from second iteration on (i.e. same label on neighbouring control points has not a pairwise energy zero)
  - work-around used by Glocker et al: “fluid-like” registration...
- **More complex smoothness terms** make problem non-submodular also in first iteration
- Solution: Don't use FastPD (with graph cuts), but alpha expansion with **QPBO** --> much slower.

Conclusions

- Alignment of **different individuals** needs **intensity-based** registration with **elastic transformation**
- **Combinatorial optimization** makes elastic registration less susceptible for **local optima.**
- **Building block** for many further image analysis applications
- You can try the “vibez_elastic_registration” on saturday or sunday afternoon

Thanks to Pavel, Jan, Jan, Lars and Emmanuel for organizing this great event

Thank you for your attention.