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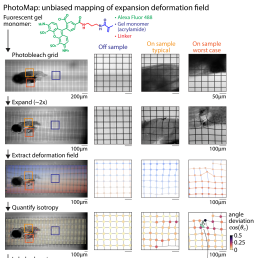
PhotoMap: unbiased mapping of Expansion Microscopy deformation fields V.(g7b4bziqx)



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Abstract

To quantify deformation introduced by expansion in a less biased manner, we developed PhotoMap, a method that measures the gel's deformation field from a pre-imposed reference pattern. A regular grid is photobleached into the sample prior to expansion and re-imaged afterwards, enabling straightforward visualization and quantification of spatial distortions.

Image Attribution

Virginia M. S. Ruetten

Protocol materials

- PBS, pH 7.4 **Thermo Fisher** **Catalog #**10010001
- Triton™ X-100 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**X100-5ML
- PBS - Phosphate-Buffered Saline (10X) pH 7.4, RNase-free **Thermo Fisher Scientific** **Catalog #**AM9625
- Agarose, low gelling temperature **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A9414-100G
- Acryloyl-X, SE (6-((acryloyl)amino)hexanoic acid, succinimidyl ester) **Thermo Fisher Scientific** **Catalog #**A20770
- DMSO, Anhydrous **Thermo Fisher** **Catalog #**D12345
- Press-to-Seal™ Silicone Isolator with Adhesive, eight wells, 9 mm diameter, 0.5 mm deep **Thermo Fisher Scientific** **Catalog #**P24743
- Fisherbrand™ Superfrost™ Disposable Microscope Slides **Fisher Scientific** **Catalog #**Catalog No.12-550-123
- Press-to-Seal™ Silicone Isolator with Adhesive, eight wells, 9 mm diameter, 0.5 mm deep **Thermo Fisher Scientific** **Catalog #**P24743
- Fisherbrand™ Superfrost™ Disposable Microscope Slides **Fisher Scientific** **Catalog #**Catalog No.12-550-123
- Poly-L-lysine hydrobromide **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**Poly-L-lysine hydrobromide
- Photo Flo 200 Solution **Electron Microscopy Sciences** **Catalog #**74257
- NaCl (5 M), RNase-free **Thermo Fisher Scientific** **Catalog #**AM9760G
- UltraPure™ SDS Solution, 10% **Thermo Fisher Scientific** **Catalog #**15553027
- UltraPure™ DNase/RNase-Free Distilled Water **Thermo Fisher Scientific** **Catalog #**10977023
- Sodium Hydroxide Solution (10N/Certified) **Fisher Scientific** **Catalog #**SS255-1
- UltraPure™ DNase/RNase-Free Distilled Water **Thermo Fisher Scientific** **Catalog #**10977023
- Acrylic acid **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**147230-5G
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- Sodium Hydroxide Solution (10N/Certified) **Fisher Scientific** **Catalog #**SS255-1
- UltraPure™ DNase/RNase-Free Distilled Water **Thermo Fisher Scientific** **Catalog #**10977023
- Ammonium persulfate (APS) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A3678-100G
- 4-Hydroxy-TEMPO (4HT) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**4-Hydroxy-TEMPO
- N,N,N′,N′-Tetramethylethylenediamine (TEMED) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**T7024-25ML
- 40% Acrylamide Solution **Bio-Rad Laboratories** **Catalog #**1610140
- 2% bis-acrylamide solution **Bio-Rad Laboratories** **Catalog #**1610142
- N-(3-Aminopropyl)methacrylamide hydrochloride **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**731099
- Triethylamine **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**471283

Alexa Fluor™ 488 NHS Ester (Succinimidyl Ester) **Thermo Fisher Scientific** **Catalog #**A20000

Troubleshooting

Before start

Make sure you have all the reagents at hand.

Reagents

1 **PBST-0.5**

Can be prepared in advance and stored at room temperature.
1x PBS with 0.5% or 0.1% Triton
PBST-0.5 and PBST-0.1 respectively
Triton dissolves terribly. It hardens upon making contact with water and takes time to dissolve fully. Prepare a 10% stock solution in 1x PBS and use that for subsequent rounds.
Keep at RT.

Room temperature

Reagents

PBS, pH 7.4 **Thermo Fisher Catalog #**10010001

Triton™ X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**X100-5ML

2 **1% low-melting temperature agarose**

Can be prepared in advance.
Dissolve 1 g of low-melting point agarose in 100 ml of 1x PBS.
Add 1 g of power to 100 ml 1x PBS at RT. Stir with magnetic stirrer.
Bring to a boil using a microwave. Stir with magnetic stirrer until full dissolved.
Repeat boil and stirring until solution is clear.

Reagents

Agarose, low gelling temperature **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A9414-100G

PBS - Phosphate-Buffered Saline (10X) pH 7.4, RNase-free **Thermo Fisher Scientific Catalog #**AM9625

3 **Acryloyl-X Solution (AcX)**

Cannot be prepared in advance.
Stock concentration: 10 mg/ml
Dissolve to 10 mg/ml in anhydrous DMSO. Aliquot in 20 µl batches.
Store in a desiccated environment at -20ºC. Don't re-use AcX after thawing.
Working solution will be: 20 µg/ml, dilution 1:500 in 1x PBS

Reagents

Acryloyl-X, SE (6-((acryloyl)amino)hexanoic acid, succinimidyl ester) **Thermo Fisher Scientific Catalog #**A20770

DMSO, Anhydrous **Thermo Fisher Catalog #**D12345

4 **Na Acrylate Solution**

Fill a 500 ml beaker with ~300 ml of water for a water bath.
Place an open 50 ml Eppendorf tube into the bath and bring the setup to a fume hood.

The purpose of the water bath is to provide a highly conductive medium to cool the solution.
Add 9.0 ml of water to the Eppendorf tube.
Add 11 ml of acrylic acid to the Eppendorf tube. (Note that this is a flammable and reactive compound).
Add 14.4 ml of 10 M NaOH to the Eppendorf tube. This should be done dropwise to prevent excessive heating and boiling.
A yellow precipitate should be observable. Leave it to cool.
Calibrate a pH meter.
Remove the acrylate-filled tube from the fume hood.
By now, most of the acrylic acid will have been converted to non-volatile sodium acrylate.
Measure the pH of the solution.
Add NaOH to adjust the pH gradually to between 7.5-8 using a pH meter. Do NOT use pH test strips.
We recommend starting by adding 10 M NaOH solution, and when the pH gets close to the desired pH adjusting via the use of 1 M NaOH solution.
(As a general guidance: Add about 500 µl 10 M NaOH, 250 µl 10 M NaOH, 120 µL 10 M NaOH, then some 1 M NaOH)
Add water up to a final volume of 40 ml.
(Note: Acrylic acid has a pKa of 4.76 at pH 7.75 - this solution has about 4 mM remaining buffering capacity)

Acrylic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**147230-5G

Sodium Hydroxide Solution (10N/Certified) **Fisher Scientific Catalog #**SS255-1

UltraPure™ DNase/RNase-Free Distilled Water **Thermo Fisher Scientific Catalog #**10977023

5 **Fluorescent Monomer**

To make the fluorescent monomer, conjugate AF488-NHS to 3-aminopropyl methacrylamide (AmPAc) in the presence of triethylamine (TEA).

	A	B	C	D	E	F
	Name	Units	Stock conc.	Final conc.	Fraction	Vol (to make 50 µl)
	AF488-NHS	mg/ml	20 (31 mM)	15 mM	0.48	24.2
	AmPAc	mg/ml	10 (56 mM)	25 mM	0.45	22.3
	TEA	%	10 (720 mM)	50.7 mM	0.07	3.5

AF488-AmPAc

Combine AF488-NHS, AmPAc, and TEA as indicated by table above. Incubate reaction overnight at room temperature, protected from light.

Note

Perform all steps involving AF488 under low-light conditions.

Room temperature

Reagents

Alexa Fluor™ 488 NHS Ester (Succinimidyl Ester) **Thermo Fisher Scientific Catalog #**A20000

N-(3-Aminopropyl)methacrylamide hydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**731099

Triethylamine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**471283

6 **Monomer and Gelation Solutions #1 (Medium Density Gel with high Bis)**

	A	B	C	D	E	F
	name	units	stock conc.	final conc.	vol (ul)	vol (ml)
	Acrylamide	%	40	10	250	0.25
	Na Acrylate	M	4	0.5	125	0.125
	Bis	%	1	0.1	100	0.1
	10x PBS	x	10	1	100	0.1
	AF488-AmPAc	mM	10	0.1	10	0.01
	Water				355	0.355

Monomer Solution #1 (with fluorescent monomer)

	A	B	C	D	E
	name (units)	units	stock conc.	final conc.	vol (ul)
	Monomer Solution #1				940
	APS	%	10	0.2	20
	TEMED	%	10	0.2	20

	A	B	C	D	E
	4HT	%	0.5	0.01	20

Gelation Solution #1 (with fluorescent monomer)

Reagents

- 40% Acrylamide Solution **Bio-Rad Laboratories Catalog #**1610140
- 2% bis-acrylamide solution **Bio-Rad Laboratories Catalog #**1610142
- Acrylic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**147230-5G
- Sodium Hydroxide Solution (10N/Certified) **Fisher Scientific Catalog #**SS255-1
- UltraPure™ DNase/RNase-Free Distilled Water **Thermo Fisher Scientific Catalog #**10977023
- Ammonium persulfate (APS) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A3678-100G
- 4-Hydroxy-TEMPO (4HT) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**4-Hydroxy-TEMPO
- N,N,N′,N′-Tetramethylethylenediamine (TEMED) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T7024-25ML

7 **Disruption Buffer**

	A	B	C	D	E	F
					to make 1ml	to make 100ml
	component	units	stock conc.	final conc.	vol (ul)	vol
	SDS	%	10	5	0.5	50
	Tris pH 7.5	mM	1000	50	0.05	5
	NaCl	M	5	0.2	0.04	4
	MilliQ water				0.41	41

Disruption Buffer

Stock **Disruption Buffer** can be prepared in advanced and stored at RT.

- NaCl (5 M), RNase-free **Thermo Fisher Scientific Catalog #**AM9760G
- UltraPure™ SDS Solution, 10% **Thermo Fisher Scientific Catalog #**15553027
- UltraPure™ DNase/RNase-Free Distilled Water **Thermo Fisher Scientific Catalog #**10977023

8 **Gelation Chambers**
Silicone gaskets (Invitrogen P24743)
Glass slides (SuperFrost 12550123)
Scotch tape
Poly-l-lysine
Shaker (nutator - 75 RPM)
Reagents
Press-to-Seal™ Silicone Isolator with Adhesive, eight wells, 9 mm diameter, 0.5 mm deep **Thermo Fisher Scientific Catalog #**P24743
Fisherbrand™ Superfrost™ Disposable Microscope Slides **Fisher Scientific Catalog #**Catalog No.12-550-123
Poly-L-lysine hydrobromide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**Poly-L-lysine hydrobromide
Photo Flo 200 Solution **Electron Microscopy Sciences Catalog #**74257

9 **General note for sample handling**
Soak plastic transfer pipettes in PBST-0.5 before manipulating samples - otherwise, the fish will inevitably get stuck.
Never use forceps to handle or move the fish as this inevitably damages the sample.

Fixation and Permeabilization 10h

10 Euthanize samples with an overdose of MS-222 (a.k.a. tricaine) (200-300 mg/L).

11 Prepare fresh 4% PFA.

12 Place samples in 1 ml of 4% PFA.
Room temperature

13 Keep overnight in 4% PFA at 4ºC on a shaker. 9h
09:00:00
4 °C

14 Rinse samples in 4 × 15 min 1 ml 1x PBS. 1h
01:00:00
Room temperature

Permeabilization 5h 10m

15 For specimens that have not been stained with antibodies, permeabilize (at least 5 hr or overnight) in **PBST-0.5**. 5h

05:00:00

16 Wash samples (2× 5 min at RT) in 1x PBS. 10m

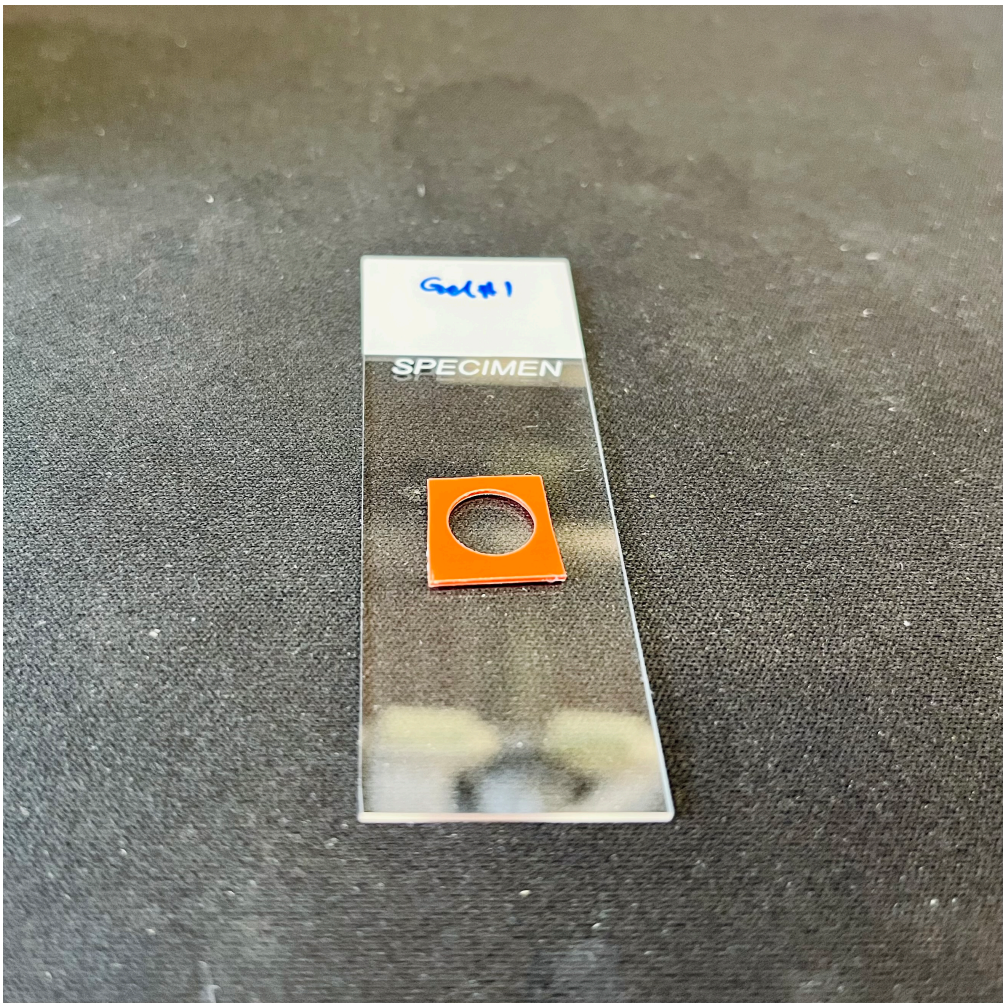
Room temperature

00:10:00

Agarose Embedding

17 Heat agarose in microwave until it liquifies.
Cool agarose to 50ºC (by placing in 50ºC incubator).

18 Adhere silicone gasket (Invitrogen P24743 or Invitrogen P24740) on a glass slide (Superfrost Microscope Slides #12550123) to form a **Mounting Chamber**.



Mounting Chamber

Reagents

Press-to-Seal™ Silicone Isolator with Adhesive, eight wells, 9 mm diameter, 0.5 mm deep **Thermo Fisher Scientific Catalog #**P24743

Fisherbrand™ Superfrost™ Disposable Microscope Slides **Fisher Scientific Catalog #**Catalog No.12-550-123

19 Place fish in **Mounting Chamber**, remove any access PBS, and cover with ~70 µl of 1% low melting point agarose. Orientate as desired (on side or dorsal side up) and leave to solidify.
Additional notes:
Use sharp and clean utensil to nudge the fish into the desired orientation.
If more time needed, the mounting chamber can be placed on a heating block to ensure that the agarose doesn't solidify pre-emptively.
Always begin by nudging the fish to the very bottom of the agarose drop to avoid the sample being held by surface tension to the top of the drop. Make sure that the fish is fully submersed in agarose.

20 With a razor blade, cut out a rectangle around the agarose-embedded sample and delicately lift the sample and transfer it to 12 well-plate with 1 ml of 1x PBS/well.
Cut as close to the fish as possible with some safety margin.

Protein Anchoring

1h

21 Prepare **Acryloyl-X Solution** (stock solution 10 mg/ml, working solution: 20 µg/ml, dilution 1:500 in 1x PBS).
1 ml per sample is needed.
Do not prepare in advance.

22 Incubate each sample in 1 ml of **Acryloyl-X Solution** (1 hr at RT) shaking in 12-well plate. 1h

Room temperature

01:00:00

Preparation of Gelation Chamber #1

23 Prepare chambers for gelation, one for each sample.
Layer 11 pieces of Scotch tape together to create ~0.6 mm thick spacers.
Cut two strips of spacer material, about 2 cm long and 0.5 cm wide.
Stick two strips to a glass slide ~15 mm apart to form the side walls of the **Gelation Chamber #1**.

Gelation Chamber #1

Incubation with Gelation Solution 1

45m

24 Rinse samples with 1x PBS (3× 5 min). 15m

00:15:00

25 Thaw or prepare **Monomer Solution #1 (initially without the AF488-AmPAc)**, as well as 4HT, TEMED and APS. Vortex well and keep on ice.
Just before use add AF488-AmPAc to the monomer solution (1:100).

26 Mix **Monomer Solution #1** and 4HT, TEMED and APS at a ratio of 94:2:2:2 to produce **Gelation Solution #1**.
Vortex.
Each sample needs ~3 ml of **Gelation Solution #1**..
~1 ml per incubation round.

27 Incubate samples in **Gelation Solution #1** on ice (3× 10 min at 4ºC) with 1 ml of **Gelation Solution #1** on a shaker in a 12-well plate. 30m

00:30:00

Gelation 1

2h

28 With spatula, transfer agarose block containing fish into the **Gelation Chamber #1**.

29 Gently place cover slip over the agarose block lying on the walls of **Gelation Chamber #1** (made of scotch tape).
The cover slip should lay flat on the agarose block.
Gently press to seal.

30 Slowly pipette **Gelation Solution #1** into **Gelation Chamber #1** until full. Avoid any air bubbles.
Solution will hold by water tension.
One can use **Gelation Solution #1** that was used during the previous incubation (no need to prepare fresh one).

31 Once **Gelation Chamber #1** is filled, place the chambers at 37ºC for 2 hr to induce polymerization. 2h
Ensure incubator is humidified.
37 °C
02:00:00

Photobleaching

32 Keep sample in **Gelation Chamber #1** and mount sample in a 2 photon microscope.
Photobleach a 3D grid into the sample (e.g.: 50 × 50 × 50 µm grid).

Disruption 9h 5m

33 Turn on the 100ºC heat block (for later disruption step).
Prepare **Disruption Buffer** (5 ml/sample).
Stock **Disruption Buffer** can be prepared in advanced and stored at RT (e.g.: 50-100 ml stock).

34 Place gels at RT, and allow them to cool on the bench for > 5 min. 5m
Room temperature
00:05:00

35 Take off coverslip lid with a razor blade.
Under a stereomicroscope, trim the gels into a rectangle with a ~2-3 mm border on either side of the sample.
Add a nick on the top right corner to be able to track orientation of the samples.
The gel should be about 9 mm x 4 mm.

36 Transfer each gel to a 2 ml Eppendorf tube and add 1.8 ml of **Disruption Buffer.**

37 Incubate each samples in **Disruption Buffer** (overnight at 100ºC). 9h
Make sure that lid is closed tightly to avoid evaporation.

100 °C

09:00:00

After disruption, the gel should be about 12.5 mm x 6 mm.

Washing

2h 5m

38 Place gels at RT, and allow them to cool on the bench for > 5 min. 5m
Take off coverslip lid with a razor blade.

Room temperature

00:05:00

39 Rinse samples with 1x PBS (3× 5 min).

Imaging

40 Image sample using a 2 photon microscope.

41 For long term storage, keep gels in 1x PBS at 4ºC.

Protocol references

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